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SYNTHESIS OF 8-AMINOQUINOLINES AS INHIBITORS OF TYROSINE KINASES

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ABSTRACT (ENGLISH VERSION)

Chronic myelogenous leukemia (CML), characterized by unregulated proliferation of myeloid cells in the bone marrow, accounts for 15 to 20 % of all adult leukemia cases in the Western population. The molecular cause for the disease is the characteristic translocation between chromosome 9 and 22 which results in the so called Philadelphia chromosome (Ph) and in the formation of the chimeric Bcr-Abl gene. In CML the protein product of this hybrid gene is a constitutively active protein kinase. Bcr-Abl kinase drives the pathogenesis of CML through the phosphorylation and activation of a broad range of downstream substrates playing a critical role in cellular signal transduction and cell transformation. ABL tyrosin kinase therefore is an interesting therapeutic target and many potent inhibitors have been developed and brought to the clinic in recent years, including Imatinib, Bosutinib, Nilotinib and Dasatinib. However, the T315I mutant form of Bcr-Abl, which is frequently found in CML patients, mediates complete resistance to Imatinib and all of the next generation Abl kinase inhibitors. Therefore, there is an eminent need for the development of drugs which are active against the T315I mutant of Bcr-Abl. Bosutinib and its quinoline scaffold have been chosen as a template for the construction of a new drug scaffold potentially able to inhibit the mutated Bcr-Abl. Starting from the model of Bosutinib bound to wild-type Abl kinase domain and the model of T315I Abl kinase domain it was apparent that in order to get potent inhibitors of Abl T315I the unfavorable interaction caused by the bulkiness of isoleucine had to be avoided and another strong favorable interaction should be added. The strategy to accomplish this goal was to remove the cyano group at 3 position of the quinoline and strengthen the interaction with the protein by adding an amino group at position 8 to establish an additional hydrogen bond with the backbone carbonyl of M318. Based on this rational, the scaffold of 8-aminoquinolines resulted to be promising for potential Bcr-Abl T315I inhibitors. Three main lines of compounds have been synthesized: 4-substituted-8-aminoquinolines, 3-substituted-8-aminoquinolines, 3,4-disubstituted-8-aminoquinolines. All of these molecules have an oxygen-linked alkyl group at position 7. Different groups have been employed to functionalize the position 7. 4-Substituted-8-aminoquinolines were synthesized starting from a 4,7-dihaloquinoline. Nitration of this molecule yielded the 8-nitro derivative which was then successfully functionalized firstly at position 7, and then at the position 4. Reduction of the nitro group allowed to obtain the desired 4-substituted-8-aminoquinolines. On the contrary, 3-substituted 8-aminoquinolines have been prepared starting from commercially available *m*-aminophenol. Nitration of this molecule yielded 2-nitro-3-aminophenol which was alkylated with an appropriate side chain at the phenolic oxygen and subsequently cyclized with α-bromoacrolein to give the 3-bromo-7-oxyalkyl-8-nitroquinoline. This precursor has been employed for functionalization at position 3. As for the 4-substituted derivatives, the last step was the transformation of nitro group into amino group. 3,4-Disubstituted-8-aminoquinolines, after a first attempt of synthesis from a substituted aniline, were synthesized starting from 4,7-dichloro-8-nitroquinoline. Position 4 was firstly functionalized with an electron donating group such as methoxy or pyrrolidino and bromine was successively introduced at position 3 by an electrophilic aromatic substitution. Attachment of the side chain at position 7 was followed by reduction from 8-nitro to 8-aminoquinoline. For all the synthesized molecules, different groups have been attached at the specific positions either via an oxygen or a nitrogen or a carbon linker. Oxygen and nitrogen linked groups have been introduced in the quinoline scaffold with an aromatic nucleophilic substitution using the corresponding alcoholate or amide. Carbon linked groups have been introduced through palladium catalyzed cross coupling reactions involving the corresponding aryl or alkyl boronic acids. Two alkynyl groups have been introduced under the classic Sonogashira reaction conditions coupling and then reduced to alkyl with hydrogen. All of the molecules synthesized have been tested in biological assays in order to verify their activity toward the enzyme in solution and toward cells expressing the oncogenic enzyme. It was discovered that as hydrochloride salt, the inhibitors were more active than as free base, therefore many inhibitors have been tested as water-soluble salts. Good results have been obtained, since the scaffold resulted

active in inhibiting the enzyme. Some of the compounds exhibited inhibitory activity in the nanomolar range. Seven of the most active and specific compounds have also been tested toward a panel of 85 protein kinases (Cohen Lab, Dundee) of different classes in order to asses their selectivity profile. Three compounds (CPD **131**, **106** and **148**) having nanomolar activity on T315I Abl resulted to have low selectivity on the panel, inhibiting respectively 10, 40 and 11 kinases by more of 50% at a concentration of 10 μM. In contrast, CPD **142**, **163**, **167** and **138** resulted very selective. Among them, CPD **138** appears to be the most selective one because it inhibits only the Aurora A kinases by more than 50% at the tested 10 μM concentration. All other compounds inhibited strongly PKBβ (AKT2) which is a Serine/Threonine kinase, while enzymes more related to Abl, e.g. LCK, SRC and FGFR1, are less targeted by the selected compounds. Insulin receptor kinase, a clear anti-target for protein kinase inhibitors, is not inhibited by these compounds. Interestingly there is an inverse correlation between the selectivity on cells (transduced cells versus non-transduced cells) and the PKBβ inhibitory activity. The higher the PKBβ inhibitory activity is the less selective are the compounds. This indicates that PKBβ is a target for substituted 8-aminoquinolines.

In conclusion, a novel active scaffold for the inhibition of Abl WT and Abl T315I has been developed. Very active compounds both on the cellular and enzymatic level have been found. The synthesized Abl T315 inhibitors allowed to asses the proposed binding mode and gave a consistent SAR. Based on the obtained results it is clear that the 8-aminoquinoline-based inhibitors are lead compounds which can be further developed in an optimization process to gain activity and better selectivity in order to be able to enter *in vivo* studies.

ABSTRACT (FRENCH VERSION)

La leucémie myéloïde chronique (LMC), caractérisée par une prolifération dérégulée des cellules myéloïdes dans la moelle osseuse, est responsable de 15-20% des cas de leucémie dans la population occidentale. La pathogénèse de cette maladie est liée à une translocation entre les chromosomes 9 et 22, résultante dans le bien connu chromosome de Philadelphie et la formation d'un gène chimérique, Bcr-Abl. Dans le cas de la LMC, le produit de ce gène hybride est une protéine avec activité tyrosine-kinase, la Bcr-Abl kinase qui est constitutivement active. Elle induit nombreuses phosphorylisations et l'activation d'une large gamme de substrats responsables de la transduction de signaux et de la transformation des cellules. De ce fait elle représente une cible thérapeutique intéressante. Ainsi, de nombreux inhibiteurs potentiels, notamment l'Imatinib, le Bosutinib, le Nilotinib et le Dasatinib, ont récemment été développés et testés en phase clinique. Néanmoins, la forme mutée T315I de Bcr-Abl fréquemment trouvée dans les patients de LMC montre une résistance absolue contre Imatinib et les inhibiteurs de la Abl-kinase de deuxième génération tels que Bosutinib, Nilotinib ou Dasatinib. Ceci rend imminent la nécessité de développer de nouveaux principes actifs contre cette forme mutée. En premier lieu une molécule contenant un squelette quinolinique, le Bosutinib, à été choisi comme modèle pour la construction d'une nouvelle structure moléculaire, potentiellement capable d'inhiber la forme mutée de Bcr-Abl. Partant du modèle 3D de Bosutinib lié au site de liaison du domaine "wild type" de la kinase Abl et du modèle du domaine de la kinase T315I kinase, il paraissait évident pour une inhibition de Bcr-Abl T315I, que, d'une part, l'interaction défavorable causée par la chaîne latérale de l'isoleucine devait être évitée et que d'autre part une nouvelle interaction favorable devait être ajoutée. Pour ce faire, la stratégie était d'éliminer le groupement nitrile en position 3 de la quinoline et de renforcer l'interaction de la molécule avec la protéine par l'introduction d'un groupement aminé en position 8, créant ainsi une nouvelle liaison d'hydrogène avec le methionine 318. Partant de ces hypothèses, le squelette 8-aminoquinolinique semblait être un candidat promettant de créer la base d'un inhibiteur de Bcr-Abl T315I. Ainsi, trois lignes principales de composés ont été synthétisées: 8-aminoquinoline substitué en position 4, 8-aminoquinoline substitué en position 3 et 8-

aminoquinoline di-substitué en positions 3 et 4. Tous ces molécules contiennent un groupement o-alkylique en position 7, divers groupement alkyliques ayant été utilisés. Les 8-aminoquinolines substituées en position 4 ont été synthétisés à partir d'une quinoline 4,7-di-halogénée. La nitration de cette molécule résulte dans un dérivé le 8-nitro, qui par suite à été fonctionnalisé en position 7, puis en position 4. Enfin, la réduction du groupement nitrique a permis la synthèse de la 8-aminoquinoline 4-substituées. Les 8-aminoquinolines substituées en position 3, en revanche, ont été préparées à partir de m-aminophénole. Par nitration de cette molécule on obtient la 2-nitro-3-aminophénole. Celle-ci était ensuite alkylée sur l'oxygène phénolique avec une chaîne latérale appropriée, et successivement cyclisée avec α-bromoacroléine donnant le précurseur 3-bromo-7-oxyalkyl-8-nitroquinoline qui peut être fonctionnalisée en position 3. De même que pour les dérivés substitués en position 4, le dernier pas consistait dans la réduction du groupement nitrique en amine. Les 8-aminoquinoline-3,4-disubstituées, après une première tentative à partir d'une aniline substituée, ont été synthétisées partant d'une base de 4,7dichloro-8-nitroquinoline. D'abord la position 4 était fonctionnalisée avec un groupement donateur d'électrons (méthoxy ou pyrrolidine), puis, le brome était successivement introduit en position 3 par moyen d'une substitution électrophile aromatique. L'attachement de la chaîne latérale en position 7 était suivi d'une réduction du groupe nitro en position 8 à amine pour donner la 8-aminoquinilone. Pour les molécules synthétisées, divers groupes ont été introduit dans les positions spécifiées à l'aide de linker d'oxygène, d'azote ou de carbone. Les groupements contenants un linker d'oxygène ou d'azote ont été introduit dans le squelette de quinoline par une substitution aromatique nucléophile en utilisant l'alcoolate ou l'amide correspondant. Les groupements carboniques ont été introduits à l'aide d'une réaction de couplage croisé (cross-coupling) des acides aryl- ou alkylboroniques avec palladium comme canalisateur. Deux groupements alkyliques ont été introduit par un couplement de Sonogashira avec une réduction successive du groupe alken en alkyl avec de l'hydrogène. Les molécules synthétisées ont été testées sur l'enzyme en solution ainsi que sur les cellules exprimant l'enzyme oncogénique afin de vérifier leurs activités. Il a été constaté que les sels hydrochloriques des inhibiteurs ont une activité plus importante comparé aux bases libres respectives. Pour cela la plupart des inhibiteurs a été testée entant que sels hydrosolubles. Le squelette 8-aminoquinolinique s'est avérée être actif dans l'inhibition de l'enzyme muté allant jusqu'à une activité inhibitrice dans les nanomolaires pour quelques uns des composés. Entre les composés les plus actifs et spécifiques, sept étaient testés sur une série de 85-protéine-kinases de types variés (Cohen labs, Dundee) avec le but d'identifier leur profiles de sélectivité. Trois composés (CPD **131**, **106** e **148**) avec une activité inhibitrice nanomolaire sur Abl T315I ont montré une basse sélectivité avec une inhibition de plus de 50% de 10, 40 et 11 kinases, respectivement, à une concentration de 10uM. En revanche, CPD **142**, **163**, **167** et **138** avaient une forte sélectivité avec CD **138** comme le plus sélectif avec comme seule activité inhibitrice à plus de 50% (concentration 10uM) une inhibition spécifique de la kinase Aurora A. Les autres composés avaient un grand effet inhibitoire sur la sérine/thréonine kinase, PKBβ (AKT2), tandis que les enzymes ressemblant à Abl, par ex. LCK, SRC e FGFR1, n'étaient quasiment pas inhibées par ces composés. Aucune inhibition n'a été détectée pour la kinase du récepteur de l'insuline qui est reconnu comme un «anti-target». Il est intéressant à noter qu'il existe une corrélation inverse entre la sélectivité sur les cellules (tranduites et non-tansduites) et l'activité inhibitrice des composés envers PKBβ: les composés avec la plus haute activité inhibitrice sur PKBβ sont les moins sélectifs au niveau cellulaire. Ceci indique clairement que PKBβ est une cible pour la 8-aminoquinoline.Pour conclure, un nouveau «scaffold» (8-aminoquinoliniques) avec activité inhibitrice envers Bcr-Abl T315I et Bcr-Abl WT a été développé. Des composés actifs au niveau cellulaire ainsi qu'enzymatique ont été trouvés. Les inhibiteurs synthétisés ont donné une relation structure activité (SAR) consistante et cohérente. Basé sur les résultats obtenus, il est évident que ces inhibiteurs sont des composés clés (lead compounds) qui ont le potentiel d'être optimisé à l'égard de leur activité et sélectivité avec pour but leur application dans des études in vivo.

CHAPTER 1

INTRODUCTION

1.1 DISEASE AND TARGET

1.1.1 Protein Kinases as targets for anticancer agents[1]

Most extracellular signals are amplified and transduced inside cells either by receptor activated tyrosine phosphorylations or by receptor coupled to GTP binding proteins.[2] A series of protein kinases in form of cascades helps propagating the signal within the cells. The perturbation of signaling pathways responsible for cell growth results usually in a deregulated, autonomous cell growth and differentiation[3]. In this context, protein kinases are key players. They catalyze the transfer of a phosphate group to peptidic substrates and are normally tightly regulated [2d,2e,2g,2f]. In case of mutations at the level of the protein kinase genes, the entire signaling networks is disturbed leading to changes in cell shape or motility, or deregulation of cell differentiation, division, and apoptosis[3a,3b,2d] that are typical for cancer. In the search for low-molecular weight therapeutic agents to treat cancers (and other disease processes), protein kinases have become attractive targets.[4] The complexity and number of protein kinases being used as molecular targets in drug discovery has greatly increased. Around 600 protein kinases and about 130 protein phosphatases genes have been discovered through the Human genome project[2d,5]. The protein kinases are subdivided in two categories based on their substrate specificity namely 1) enzymes specific for tyrosine and 2) enzymes specific for serine/threonine. While the substrate of each protein kinase is different, all the protein kinases use the co-substrate ATP as phosphate donor. Thus, inhibitors targeting the ATP binding site have to face the issue of selectivity and specificity. Many protein kinases can be found upstream or downstream of epidemiologically relevant oncogenes or tumor suppressors.[2d,2e,3d,6] Their activity in untransformed cells is normally tightly regulated, but they become transforming when mutated or overexpressed.[2d,2e,2g,4a,4c] The following alterations are associated with several human malignancies.[2d,2e,2g]

a. Genomic rearrangements, including chromosomal translocations that generate fusion proteins containing the catalytic kinase domain and part of an unrelated protein that

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[6] Sherr, C.J. *Cancer Res* **2000** , *60*, 3689.

usually provides the activation (dimerization) function. The classical example is Bcr-Abl which is causatively linked to chronic myelogenous leukemia (CML).[7]

b. Mutations of the protein leading to a constitutively activated kinase, which is associated with malignancies, such as acute myelogenous leukemia (Flt-3) and gastrointestinal tumors (c-Kit, the receptor of stem cell factor),[8] just to cite a few.
c. Deregulation of the kinase activity by activation of oncogenes or loss of tumor suppressor functions. Cancers carrying oncogenic Ras (about 30–50% of all human cancers) and deregulates Raf-kinase[2b] activity are examples of oncogene activation. Deregulation of cyclin-dependent kinase (CdK) activities by loss of tumor suppressor function has been associated with various malignancies.[6,9]
d. Deregulation of kinase activity by over-expression, as in the case of the epidermal growth factor-receptor (EGF-R)[10] and related RTPKs.[2d] The transforming effect is ascribed to enhanced kinase activity due, in part, to enforced dimerization, resulting in altered downstream signaling members.

The discovery of therapeutic agents, based on the inhibition of protein kinases, is very important. It has been tried to modulate the activity of protein kinases by low-molecular weight compounds capable to interfere with either ligand binding (in the case of RTPKs) or with protein substrate binding, but the task resulted to be very difficult.[11] Similarly, the approach to generate suitable non-competitive or allosteric inhibitors resulted difficult so far. Although the latter mechanism has received considerable attention and despite considerable efforts, the design of small molecule ligands to inhibit binding to Src homology-2 domains, and thereby regulate tyrosine kinase signaling, has failed.[11a,11c] Thus, targeting the catalytic site of kinases with ATP-competitive inhibitors remains a very promising approach for drug development. The two main problems of this approach, that have to be solved, are the access of the inhibitors to the intracellular targets and their selectivity. Considering the fact that there are around 600 human protein kinases, it is not surprising that selectivity has proven to be the more difficult of the two problems.[11d] The commonality as well as diversity among the ATP-binding sites of kinases has allowed building pharmacophore models for rational drug design. The recent progress made in the crystallization of protein kinases (more than 50 crystal structures of protein kinases, mostly complexed with ATP competitive inhibitors, are available in the PDB databank) has confirmed that the ATP-binding site of protein kinases is drugable and an attractive target for drug design.

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1.1.2 The ATP-binding site

ATP binds to protein kinases inside a deep cleft formed between the two lobes of the protein kinase. The interactions between the nucleotide and the protein are mainly of liphophilic nature[4c,12] but two important hydrogen-bonds are made with the hinge region of the protein (the region that connect the two lobes of the kinase) (see **Figure 1.1**)

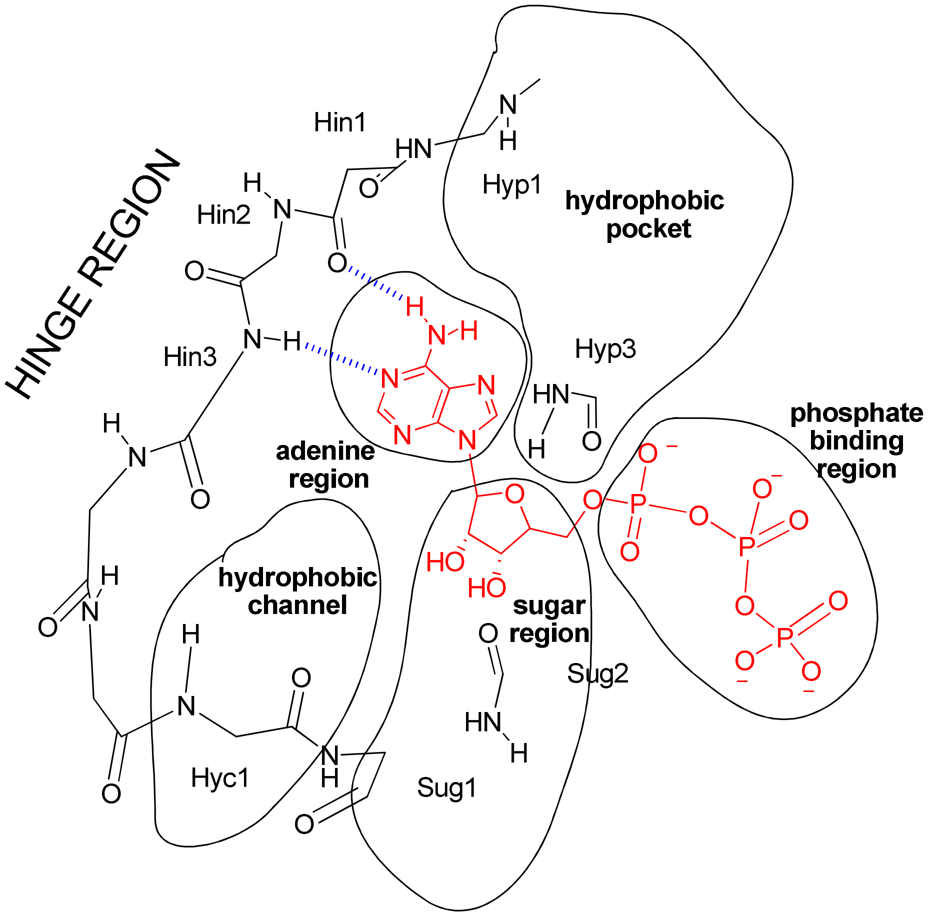

Figure 1.1[1]**:** *Schematic representation of the ATP binding site of protein kinases. Pharmacophore model of the ATP-binding site of protein kinases. ATP is in red. Sug1, Hyp1, and Hyc1 are residues lining the sugar region (Sug), hydrophobic pocket (Hyp), and hydrophobic channel (Hyc), respectively. Hin, hinge region.*

The ATP-binding site is very similar among different protein kinases. Nevertheless, the architecture in the regions proximal to the ATP-binding site shows important diversities which can become the keys to selectivity for a specific inhibitor. For practical drug discovery purposes, the binding site of ATP in protein kinases can be divided into the following main features (see **Figure 1.1**):

a. Adenine region. This region includes the two key hydrogen bonds formed by the interaction of the N-1 and N-6 amino groups of the adenine ring with the backbone NH and carbonyl groups of the hinge region of the protein kinase. Many potent inhibitors use at least one of these hydrogen bonds. Although not used by ATP, some of the backbone carbonyl of residues of the hinge region [position 123 in protein kinase A (PKA)] can also serve as a hydrogen bond acceptor for inhibitor binding.

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b. Sugar region. In most of the protein kinases, this region is hydrophilic and is the region where the ribose of the ATP accomodates.
c. Hydrophobic pocket (or selectivity pocket). This pocket is not used by ATP, but is exploited by most of the kinase inhibitors. It plays an important role for inhibitor selectivity, and its size is controlled mainly by two amino acid residues.
d. Hydrophobic channel. This channel is a spot that is open to the solvent. ATP doesn't use it but it can be exploited to gain binding affinity.
e. Phosphate binding region. This region appears to be the least important in terms of binding affinity, due to high solvent exposure. However, it is useful to improve selectivity or to gain some additional affinity in a lead optimization program.

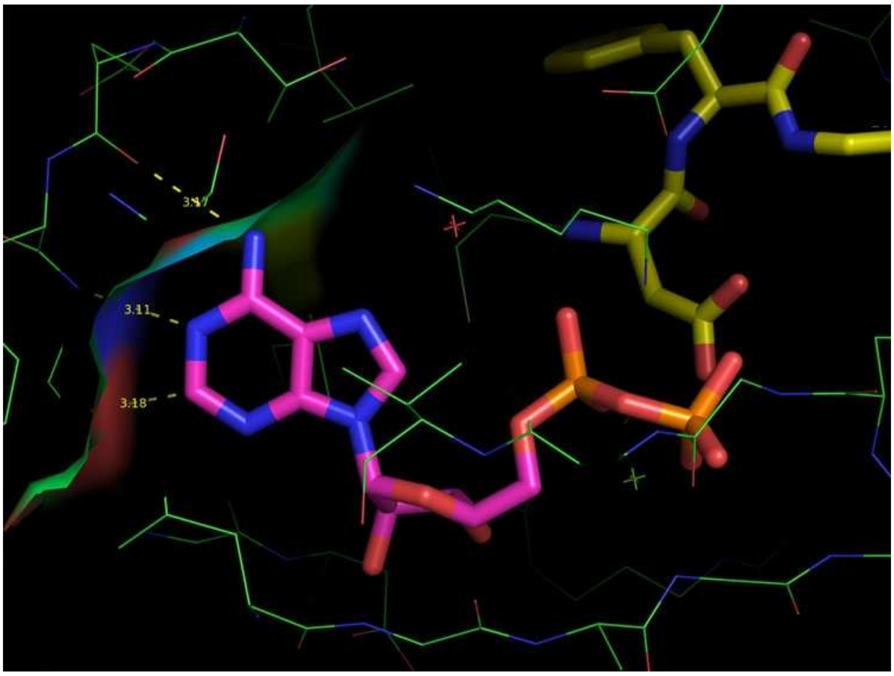

Figure 1.2 [13]: *Three-dimensional view of the ATP binding site of protein kinases.*

According to this pharmacophore model, the development of a drug that specifically targets a single kinase appears to be an almost impossible task. The main goal, therefore, should be the development of molecules possessing a ‘‘reasonable’’ inhibitory profile with sufficient selectivity, such that the inhibition of other often closely related kinases gives an ‘‘acceptable’’ side effect profile.

1.1.3 Chronic myeloid leukemia

Chronic myeloid leukemia (CML)[14] is a malignant clonal disorder of hematopoietic stem cells that results in an increase of myeloid cells accompained by an increase of erythroid cells and platelets in peripheral blood and marked myeloid hyperplasia in the bone marrow. The typical symptoms of CML are fatigue, anorexia, and weight loss. Around 40 percent of patients are asymptomatic, and in these patients, the diagnosis is based only on an abnormal blood count. The natural history of CML

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[13] Figure taken and modified from the site: www.kinasepro.wordpress.com/2009/05/25/allosterism-a-diatribe/

[14] For a review see: Sawyers, C.L. *New Engl. J. Med.***1999**, *40*, 1330.

is the progression from a benign chronic phase to a rapidly fatal blast crisis within three to five years. The blast crisis is often preceded by an accelerated phase. The acute leukemia phase is marked by the emergence within the clonal hematopoiesis of fully transformed cell clones arrested at an early stage of differentiation, either myeloid or lymphoid. The diagnosis of CML is usually based on detection of the Philadelphia (*Ph*) chromosome. This abnormality, first described as a shortened chromosome 22 in 1960[15] and then as a t(9;22) translocation in 1973[16], is present in 95% of patients. Another 5 percent have complex or variant translocations involving additional chromosomes that have the same end result, which is fusion of the Bcr (breakpoint cluster region) gene on chromosome 22 to the Abl (Abelson leukemia virus) gene on chromosome 9. The *Ph* chromosome is found in cells from the myeloid, erythroid, megakaryocytic, and B lymphoid lineages, indicating that CML is a stem-cell disease. The molecular consequence of the t(9;22) translocation[17] is the creation of the fusion protein Bcr-Abl which is a constitutively active cytoplasmic tyrosine kinase. Signal transduction pathways that are vital for the regulation of normal haematopoiesis are constitutively activated in cells that express Bcr-Abl, which leads to deregulated proliferation, differentiation and survival. However, despite the fact that the structural organization and the molecular biology of the Bcr-Abl gene as well as that of the normal Abl and Bcr genes have been subjects of intensive investigation in the last thirty years, many questions concerning the mechanisms by which the hybrid gene is formed and transforms the hemopoietic stem cells still remain unanswered.

Depending on the site of the breakpoint in the Bcr gene, the fusion protein can vary in size from 185 KDa to 230 KDa. Each fusion gene encodes the same portion of the Abl tyrosine kinase but differs in the length of Bcr sequence retained at the N terminus. This chimeric protein[18] has a causative role in neoplastic transformation of pluripotent stem cells, giving rise to the expansion of the myeloid compartment which characterizes the chronic phase of the disease. The *Ph*-chromosome may also be found in leukemias other than CML[19].

In the late 1980s, the data accumulated on the role of Bcr-Abl indicated Bcr-Abl as the most attractive target for molecularly targeted therapy approaches. Therefore, many attempts to inhibit the protein activity or to avoid its transcription have been done. This process finally ended with the discovery and the development of Imatinib mesylate[20]. This small, low molecular weight molecule, inhibits the kinase activity of Bcr-Abl at nanomolar concentrations (38 nM) and is able to stop cellular growth and to induce apoptosis of leukemic cells both *in vitro* and *in vivo*[21]. Discovery of Imatinib marked the beginning of the so called ”molecularly targeted therapy” .

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[15] Nowell, P.C.; Hungerford, D.A. *Science* **1960**, *132*, 1497.

[16] Rowley, J.D.. *Nature* **1973**, *243*, 290.

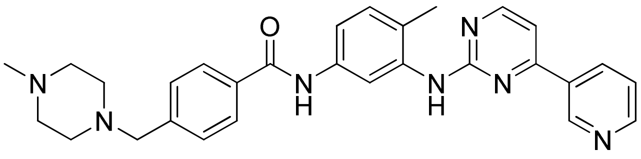
[17] (a)Bartram, C.R.; de Klein, A.; Hagemeijer, A.; Grosveld, G.; Heisterkamp, N.; Groffen. J. *Blood* **1984**, *63*, 223. (b) Groffen, J.; Stephenson, J.R.; Heisterkamp, N.; de Klein, A.; Bartram, C.R.; Grosveld, G. *Cell* **1984**, *36*, 93. (c) Stam, K.; Heisterkamp, N.; Grosveld, G.; de Klein, A.; Verma, R.S.; Coleman, M.; Dosik, H.; Groffen, J. *N. Engl. J. Med.* **1985**, *313*,1429.

[18] Ben-Neriah, Y., Daley, G.Q., Mes-Masson, A.M.; Witte, O.N.; Baltimore D. *Science* **1986**, *233*, 212.

[19](a) Melo, J.V. *Blood* **1996**, *88*, 2375. (b) Saglio, G.; Pane, F.; Martinelli, G.; Guerrasio, A.; *Leuk. Lymphoma* **1997**, *26*, 281.

[20] Buchdunger, E.; Zimmermann, J.; Mett, H.; Meyer, T.; Muller, M.; Druker, B.J. Lydon, N.B. *Cancer Res.* **1996**, *56*, 100.

[21](a) Druker, B.J.; Tamura, S.; Buchdunger, E.; Ohno, S.; Segal, G.M.; Fanning, S.; Zimmermann, J.; Lydon, N.B. *Nat. Med.* **1996**, *2*, 561. (b) Gambacorti-Passerini, C.; le Coutre, P.; Mologni, L.; Fanelli, M.; Bertazzoli, C.; Marchesi, E.; Di Nicola, M.; Biondi, A.; Corneo, G.M.; Belotti, D.; Pogliani, E.; Lydon, N.B. *Blood Cells Mol. Dis.* **1997**, *23*, 380. (c) Deininger, M.W.; Goldman, J.M.; Lydon, N.B.; Melo, J.V. *Blood* **1997**, *90*, 3691. (d) le Coutre, P.; Mologni, L.; Cleris,



Imatinib
IC_{50}= 38 nM

Figure 1.3: *Structural formula of Imatinib (Gleevec©)*

The X-ray structure of Abl in complex with Imatinib revealed its molecular mechanism of action[22].

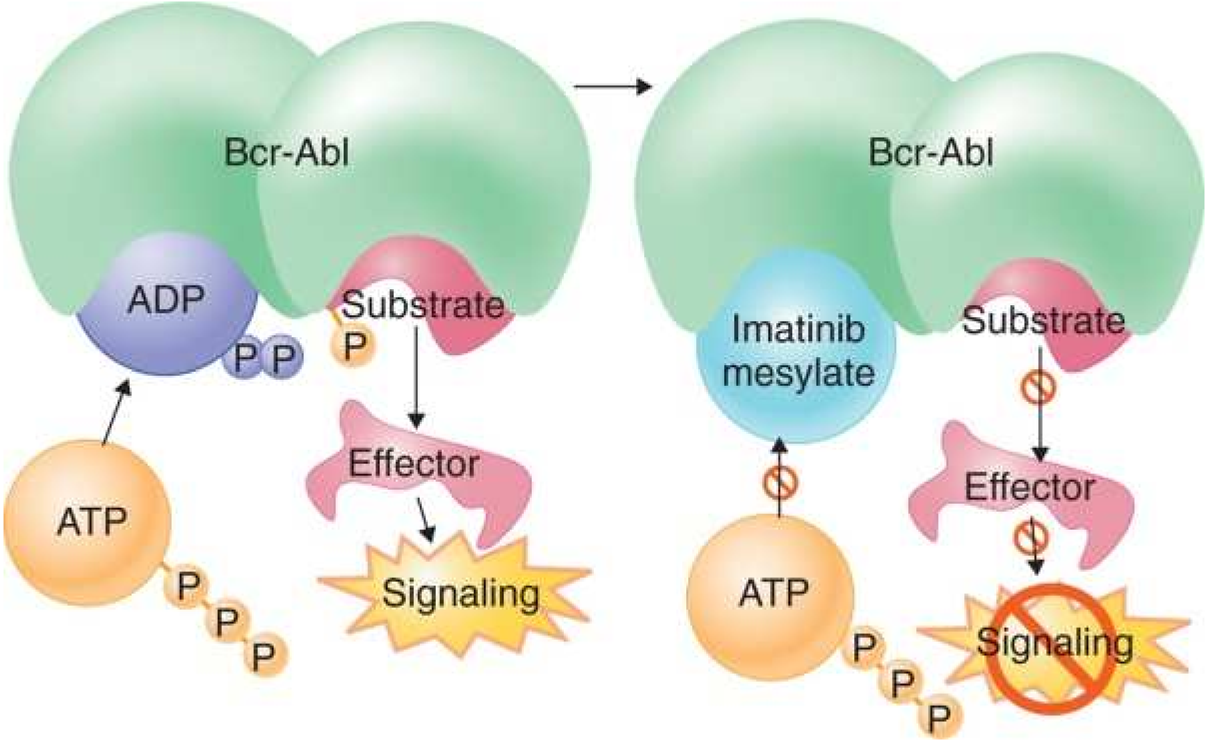


Figure 1.4[23]: *Principle of the Bcr-Abl based molecularly targeted therapy. A) The constitutively active Bcr-Abl tyrosine kinase transfers the phosphate from ATP to tyrosine residues of various substrates inducing excess proliferation and inhibition of apoptosis of myeloid cells characteristic of CML. B) Imatinib inhibits the kinase activity by blocking the binding of ATP to the protein, thus preventing CML.*

Imatinib was originally thought to act as a direct competitive inhibitor of ATP binding. However, structural resolution of Imatinib with a compound closely resembling Imatinib showed that it occupies only part of the ATP binding pocket[22,24] (**Figure 1.5**). Imatinib exploits the distinctive inactive conformation of the Bcr-Abl activation loop and consequently achieves high specificity. It

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L.; Marchesi, E.; Buchdunger, E.; Giardini, R.; Formelli, F.; Gambacorti-Passerini, C. *J. Natl. Cancer Inst.* **1999**, *91*, 163.

[22] Nagar, B.; Bornmann, W.G.; Pellicena, P.; Schindler, T.; Veach, D.R.; Miller, W.T.; Clarkson, B.; Kuriyan, J. *Cancer Res.* **2002**, *62*, 4236.

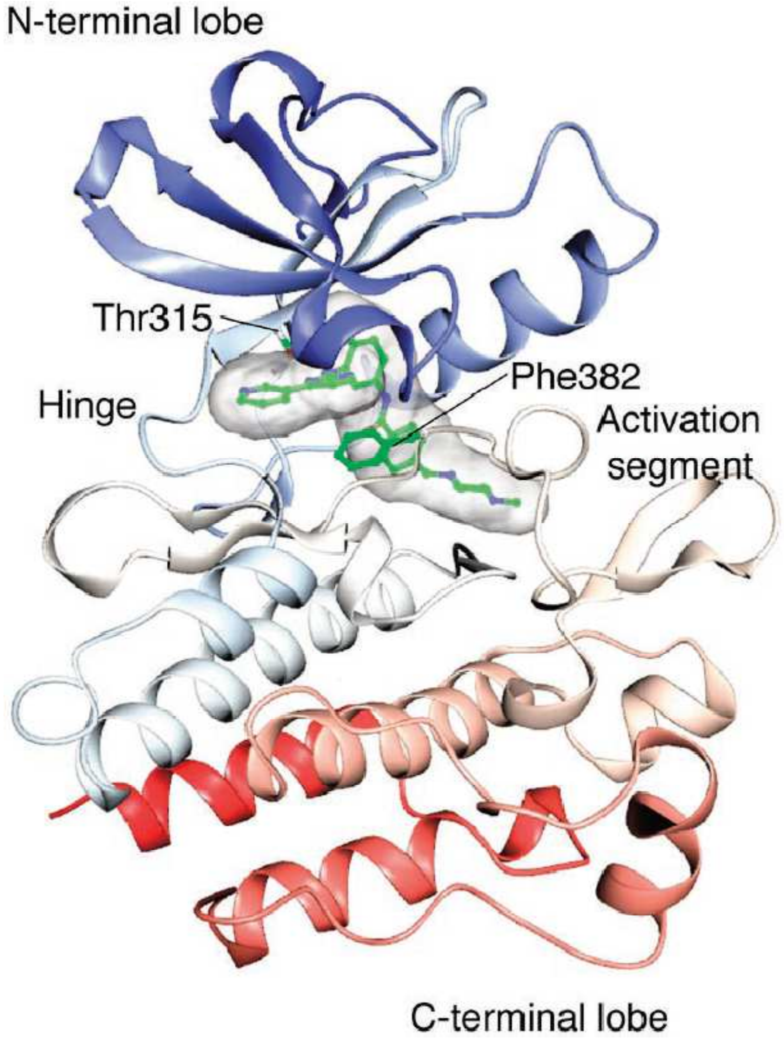
[23] Figure taken from: Kantarjian, H.; O'Brien, S. *Goldman: Cecil Medicine 23rd edition* chapter 195, Saunders ed. **2007**.

[24] Schindler, T.; Bornmann, W.; Pellicena, P.; Todd Miller, W.; Clarkson, B.; Kuriyan, J. *Science* **2000**, *289*, 1938.

contacts 21 amino acids within the ATP binding site and the activation loop. The protein is tightly held in the inactive state which prevents ATP binding and the phosphorylation of Bcr-Abl. Consequently the downstream effector's molecules are not phosphorylated and the signal transduction pathways are not activated.

Imatinib,4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamid, binds to a specific inactive conformation of Bcr-Abl in which the activation loop (A-loop) mimics the substrate and the phenylalanine of the DFG motif points towards the ATP binding site. Imatinib forms 6 hydrogen bonds with the protein and several hydrophobic contacts with amino acids forming an extended selectivity pocket favored by the closed conformation of the A-loop (**Figure 1.5**). The nitrogen of the pyridine moiety forms a hydrogen bond with the hinge region that is characteristic for tyrosine kinase inhibitors[25]. In addition, the amino group of the 2-phenylaminopyrimidine moiety builds a hydrogen bond with the gatekeeper amino acid Thr315.[22]

A



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[25] Moretti, L.; Tchernin, L.; Scapozza, L. *ARKIVOC* **2006**, *8*, 38.

B

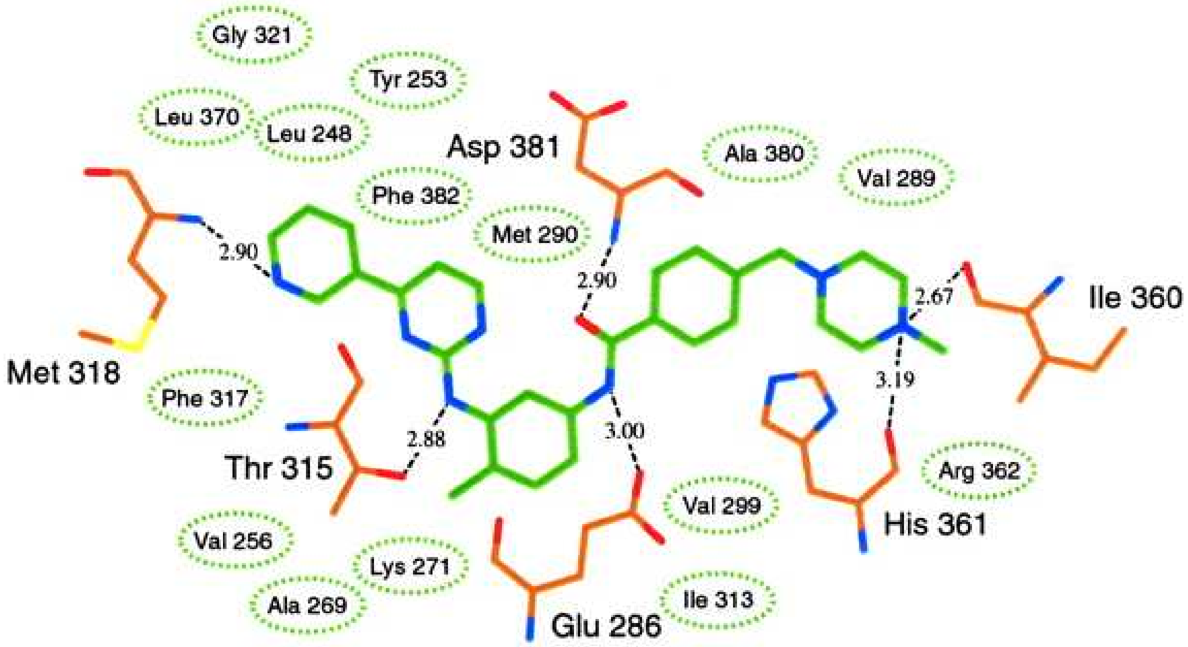

Figure 1.5: A)[26]*The structure of the catalytic domain of cAbl in complex with Imatinib The hinge region connects the two lobes. Imatinib is shown bound to the ATP–binding site. Thr*[315]*, the gatekeeper residue, and Phe*[382]*, the conserved phenylalanine of the DFG motif that marks the beginning of the activation segment, are marked.* **B)**[22] *Two-dimensions representation of the catalytic domain of cAbl in complex with Imatinib. Carbons are colored in green for Imatinib, and in orange for the protein. Nitrogens are colored in blue and oxygens in red. Hydrogen bonds between amino acids of Abl kinase and the inhibitor are indicated with dotted lines along with their distances. Residues forming Van der Waals interactions with Imatinib are circled with green dotted lines*.

The treatment of CML and other types of Philadelphia chromosome positive (*Ph*+) leukemias is entering a new phase, thanks to the development of Imatinib.[27] This molecule is specific and relatively non-toxic and is able to induce durable remissions in most patients with *Ph*+ leukemias, and cytogenetic responses in the majority of CML patients in chronic phase. No other CML drug had been capable to achieve such important results. During the 8 years of clinical investigation on Imatinib over 50.000 patients, mostly affected by CML or *Ph*+ALL (Acute Limphoblastic Leukemia), have received the treatment. Patients with acute leukemia (defined as the presence of more than 30% blasts in either the bone marrow or the peripheral blood) showed a high (>50%) rate of initial haematological responses: 52% of the patients with ”myeloid” BC-CML and 60% of the patients with *Ph*+ ALL or ”lymphoid” BC-CML showed reduction in blast values corresponding to a haematological response.[28] Patients included in this category present either ”de novo” acute

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[26] Figure taken from: Noble, M.E.M.; Endicott, J.A.; Johnson, L.N. *Science* **2004**, *303*, 1800.

[27] Goldman, J.M. Druker, B.J. *Blood* **2001**, *98*, 2039.

[28] (a) Ottmann, O.G.; Druker, B.J.; Sawyers, C.L.; Goldman, J.M.; Reiffers, J.; Silver, R.T.; Tura, S.; Fischer, T.; Deininger, M.W.; Schiffer, C.A.; Baccarani, M.; Gratwohl, A.; Hochhaus, A.; Hoelzer, D.; Fernandes-Reese, S.; Gathmann, I.; Capdeville, R.; O'Brien, S.G. *Blood* **2002**, *100*, 1965. (b) Sawyers, C.L.; Hochhaus, A.; Feldman, E.; Goldman, J.M.; Miller, C.B.; Ottmann, O.G.; Schiffer, C.A.; Talpaz, M.; Guilhot, F.; Deininger, M.W.; Fischer, T.; O'Brien, S.G.; Stone, R.M.; Gambacorti-Passerini, C.; Russell, N.H.; Reiffers, J.J.; Shea, T.C.; Chapuis, B.; Coutre, S.;

leukemias (*Ph*+ ALL or AML) or blast crisis CML. Unfortunately the effect of the treatment with Imatinib within this patient's population is quite short: after 3-6 months, almost all patients show relapses and resistance to the molecule. Although patients affected by the myeloid blast crisis CML seem to fare slightly better than the patients with ALL or lymphoid blast crisis CML, the present overall evaluation of Imatinib in this category of patients can be considered as a "qualified failure". Approximately 25% of patients with accelerated phase leukemia (AP, defined as blasts between 15 and 30%, basophiles > 20%, or platelet counts < 100 k/mm^3), show durable major cytogenetic responses although the treatment is probably able to prolong survival even in the absence of a cytogenetic response[29] . Patients affected by CML and treated with Imatinib in late chronic phase (defined as < 15% blasts, < 20% basophiles and with platelet count > 100 k/mm^3), who are intolerant, resistant or refractory to interferon treatment, respond better than the patients in more advanced phases. 64% of 450 patients who started Imatinib in late chronic phase obtained a major cytogenetic responses (MCyR), at a median time of 34 months after the diagnosis of CML[30]. The results of this trial have been reported after 5 year of median follow up[31]. After 60 months of treatment, 81% of patients who reached a MCyR are still in cytogenetic remission, and 92% are alive. Patients reach the cytogenetic remission quite early and the relapse rate (which is an indicator of the development of resistance to Imatinib) is ranging between 1 and 2% which if compared with a historical value of 20-25% clearly indicates that the treatment of CML with Imatinib changed the biological history of the disease. This statement is also supported by a subsequent trial on which Imatinib demonstrated a clear superiority when compared to the old treatments of Interferon beta/Ara-C[32]. These data indicate that, given the average age at which CML is diagnosed (45–50 years) and the average life expectation, and considering the present yearly risk of relapse, the majority of CML patients will die of other causes, and not of CML.

1.1.4 Resistance to Imatinib

The resistance to Imatinib can be defined as the lack of a complete haematological response in patients with chronic-phase disease[30], a return to chronic phase on patient in acute phase[29], or a partial response in patients with blast crisis CML or Ph-positive ALL[28b].

Resistance is common on patients in the acute phase, and occurs within 3-6 months of treatment in more than 70% of the cases. In contrast, resistance is less frequent on patients in the chronic phase. Generally a complete cytogenetic response is achieved in over 50% of patients within the first 3-6 months, and this answer is proved to be durable30. It remains to be seen if the mechanism of resistance for both patients in acute phase and chronic phase is the same. Therefore, although good

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Tura, S.; Morra, E.; Larson, R.A.; Saven, A.; Peschel, C.; Gratwohl, A.; Mandelli, F.; Ben-Am, M.; Gathmann, I.; Capdeville, R.; Paquette, R.L.; Druker, B.J. *Blood* **2002**, *99*, 3530.

[29] Talpaz, M.; Silver, R.T.; Druker, B.J.; Goldman, J.M.; Gambacorti-Passerini, C.; Guilhot, F.; Schiffer, C.A.; Fischer, T.; Deininger, M.W.; Lennard, A.L.; Hochhaus, A. Ottmann, O.G.; Gratwohl, A.; Baccarani, M.; Stone, R.; Tura, S.; Mahon, F.-X.; Fernandes-Reese, S.; Gathmann, I.; Capdeville, R.; Kantarjian, H.M.; Sawyers, C.L. *Blood* **2002**, *99*, 1928.

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[31] Gambacorti-Passerini, C.; Talpaz, M.; Sawyers, C.L.; Druker, B.J.; Hochhaus, A.; Schiffer, C.A.; Niederwieser, D.W.; Stone, R.M.; Goldman, J.M.; Mone, M.; Krahnke, T.; Gathmann, I.; Kantarjian, H.M. *ASH Annual Meeting Abstracts* **2005**, *106*, 1089.

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results have been obtained with Imatinib, the development of resistance remains a big problem in the treating of CML.

At the time of relapse, a fully active Bcr-Abl protein can be found again on patients,[33] indicating that the leukemic cells remains depending on the transforming activity of Bcr-Abl. Resistance therefore would develop because cells were able to maintain Bcr-Abl in an active form despite treatment with Imatinib, rather than becoming independent of Bcr-Abl-mediated signaling. It means that cells find ways to maintain sufficient amounts of Bcr-Abl signaling. There are three main different ways by which this result is achieved: Bcr-Abl gene amplification, Bcr-Abl gene mutation and incomplete Bcr-Abl inhibition.

When there is gene amplification, some cells express an increased amount of Bcr-Abl. Since Imatinib, produces a decrease in the degree of signaling, these cells, which maintain the minimum amount of signal transduction activity needed for cell survival, will be selected, surviving even in presence of Imatinib.

Since it is known that Imatinib binds Bcr-Abl not exactly in the ATP binding site, some mutation in the kinase domain of the enzyme could be able to disrupt Imatinib binding without interfering too much with its enzymatic activity. At least 90 different point mutations have been identified in leukemic cells from patients who have developed resistance to Imatinib.[34] The main position of the mutation are 250, 252, 253, 255, 289, 315, 317, 351, 396 and 486, the most frequent being those affecting E255 and T315. At position 255 glutamine is substituted by a lysine (E255K) or by a valine (E255V), while at position 315 threonine is substituted by an isoleucine (T315I). These mutations have not been found on patients before Imatinib treatment, but since mutations can be detected only when they account for at least 10% of whole cell population, it’s possible that the mutated cell were present in low amount before Imatinib therapy. These mutant, on which Imatinib is not active, have been selected because of the specific pressure exerted by the treatment.[35] Several studies confirmed this theory, suggesting that drug-resistant point mutation can arise during Imatinib treatment. This acquired resistance usually involves the re-appearance of the kinase activity of Bcr-Abl, suggesting that this protein is still a valuable target for inhibition in Imatinib-resistant patients.

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[35] Roche-Lestienne, C.; Soenen-Cornu, V.; Grardel-Duflos, N.;Laï, J.L.; Philippe, N.; Facon, T.; Fenaux, P.; Preudhomme, C. *Blood* **2002**, *100*, 1014.

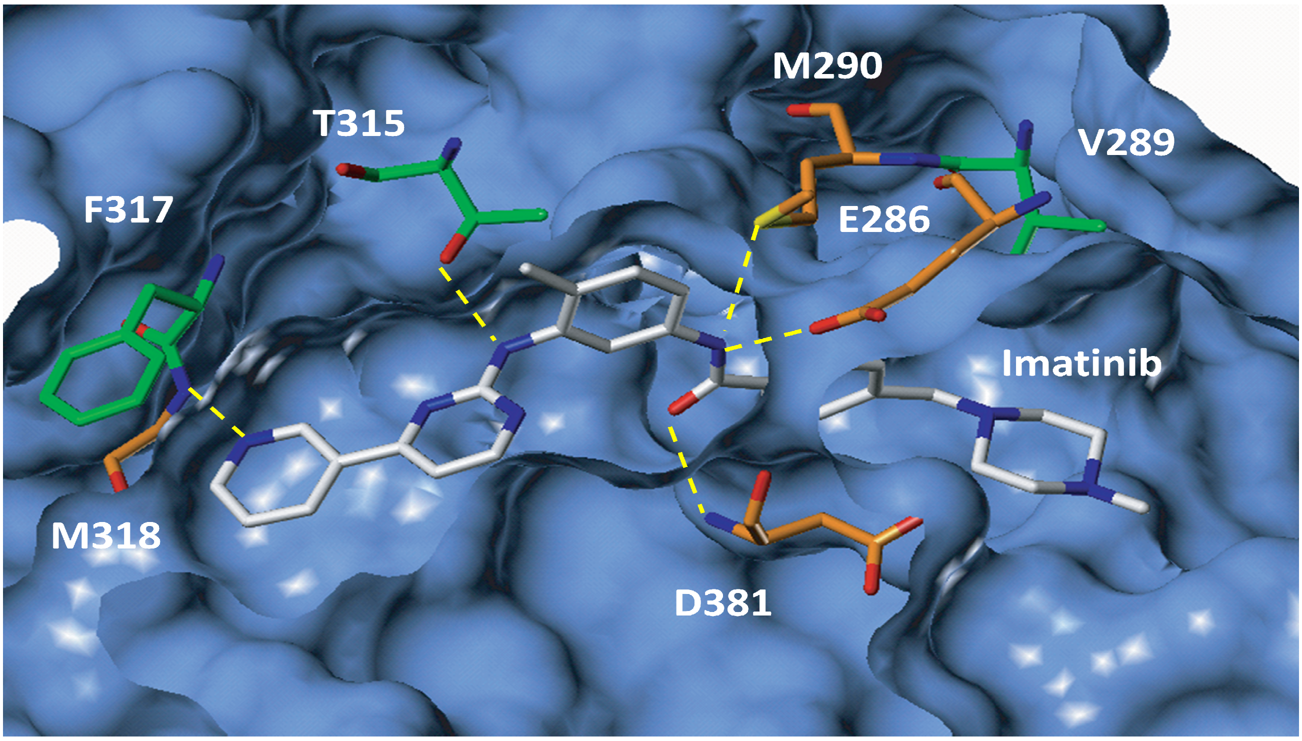

Figure 1.6[36]**:** *Mutations affecting the Imatinib-binding site in Abl. Imatinib, the aminoacids involved in direct hydrogen bonding, and the amino acids involved in some mutations are shown as color coded stick models. Nitrogen atoms are shown in blue, oxygen atoms in red, and sulphur atoms in yellow. The carbon atoms of the mutational positions are in green, those of Imatinib are in white, and those of the remaining amino acids are in orange. The molecular surface (blue) represents the van der Waals interactions between Imatinib and the protein. Hydrogen bonds are represented by dashed yellow lines. For clarity, the nucleotide-binding loop has been omitted.*

1.1.5 Second generation inhibitors of Bcr-Abl for the treatment of Imatinib-resistant CML[37]

The discovery of these mechanisms of Imatinib-resistances spurred the development of other inhibitors capable to override the resistance. Classes of these new molecules include selective Abl inhibitors, inhibitors of both Abl and Src-family kinases, Aurora kinase inhibitors and non-ATP competitive inhibitors of Bcr-Abl (**Figure 1.7**).

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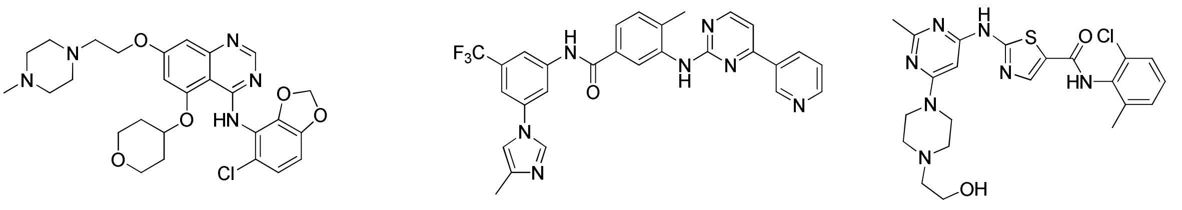
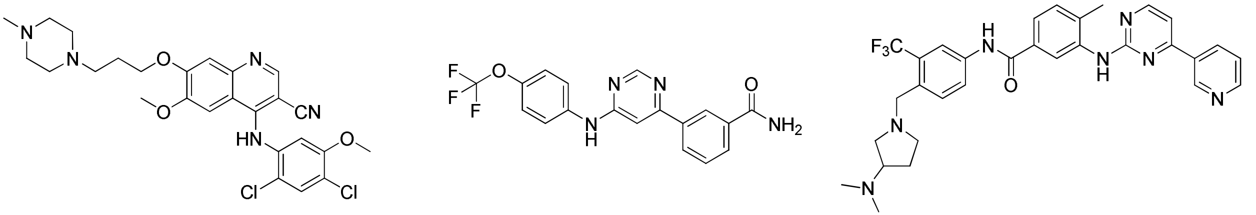
[36] Figure taken from: Gambacorti-Passerini, C.; Gunby, R.; Piazza, R.; Galietta, A.; Rostagno, R.; Scapozza, L. *Lancet Oncol.***2003**, *4*, 75.

[37] For a review see: Weisberg, E.; Manley, P.W.; Cowan-Jacobs, S. W.; Hochhaus, A.; Griffin, J.D. *Nat. Rev Cancer* **2007**, *7*, 345.



AZD0530 Nilotinib (AMN107) Dasatinib (BMS-354825)



Bosutinib (SKI-606) GNF-2 INNO-406 (NS-187)

Figure 1.7: *Molecular structures of second generation kinase inhibitors in clinical trials or approved for CML.*

Nilotinib (AMN107, Novartis). This compound has been synthesized after a rational drug design based on the crystal structure of Imatinib in complex with Abl.[38] It's about 30-fold more potent than Imatinib and it's more selective since it inhibits receptor tyrosine kinase KIT and PDGFRβ at the same level of Imatinib. The most important feature of this molecule is the inhibition of all cell lines bearing Bcr-Abl kinase domain mutation. The only mutation that is not inhibited by nilotinib is the T315I[39]. Also the Y235H and E459K mutant clone may also be relatively resistant to nilotinib[39b,40] The first approval for nilotinib came in Switzerland in July 2007, and was then approved in the US and European Union in the same year.

Dasatinib (BMS-354825, Bristol-Myers Squibb). Dasatinib is a highly potent orally active inhibitors of Src and Src-family kinases. It's also a potent Bcr-Abl inhibitor, and have also activity toward the KIT, PDGFR and ephrin receptor tyrosine kinases.[41] This lack of selectivity is probably due to the fact that Dasatinib, unlike Imatinib and nilotinib, binds to the active conformation of Bcr-Abl, which is very similar for Bcr-Abl and the Src family.[42] Dasatinib is able to inhibit 21 out of 22

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[38] Cowan-Jacob, S.W. *et al. Acta Cryst.* **2007**, *D63*, 803.

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[40] (a) Weisberg, D.; Manley, P.W.; Breitenstein, W. *Cancer Cell* **2005**, *7*, 129. (b) Kantarjian, H.M.; Giles, F.; Gattermann, N.; Bhalla, K.; Alimena, G.; Palandri, F.; Ossenkoppele, G.J.; Nicolini, F.E.; O'Brien, S.G; Litzow, M.; Bhatia, R.; Cervantes, F.; Haque, A.; Shou, Y.; Resta, D.J.; Weitzman, A.; Hochhaus, A.; le Coutre, P. *Blood* **2007**, *110*, 3540. (c) le Coutre, P.; Ottmann, O.G.; Giles, F.; Kim, D.W.; Cortes, J.; Gattermann, N.; Apperley, J.F.; Larson, R.A.; Abruzzese, E.; O'Brien, S.G.; Kuliczkowski, K.; Hochhaus, A.; Mahon, F.X.; Saglio, G.; Gobbi, G.; Kwong, Y.L.; Baccarani, M.; Hughes, T.; Martinelli, G.; Radich, J.P.; Zheng, M.; Shou, Y.; Kantarjian, H. *Blood* **2008**, *111*, 1834.

[41] Melnick, J. S.; Janes, J.; Kim, S.; Chang, J.Y.; Sipes, D.G.; Gunderson, D.; Jarnes, L.; Matzen, J.T.; Garcia, M.E.; Hood, T.L.; Beigi, R.; Xia, G.; Harig, R.A.; Asatryan, H.; Yan, S.F.; Zhou, Y.; Gu, X.-J.; Saadat, A.; Zhou, V.; King, F.J.; Shaw, C.M.; Su, A.I.; Downs, R.; Gray, N.S.; Schultz, P.G.; Warmuth, M.; Caldwell, J.S. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 3153.

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mutant forms of Bcr-Abl resistant to Imatinib,[43] but it does not have any effect on patients who carry the T315I Bcr-Abl mutation. It's also less effective against T315A and F317L/V.[44] This molecule passed successfully phase I and II trials, and received accelerate approval by the U.S. Food and Drug Administration in June 2006 for the treatment of adults at all stages of CML with resistance or intolerance to Imatinib therapy. Dasatinib represented a safe and effective therapy for chronic phase-CML patients who were resistant to conventional Imatinib doses, with improved cytogenetic and molecular response rates and progression-free survival relative to high-dose Imatinib.[45]

Bosutinib (SKI-606, Wyeth). Bosutinib has been developed as an inhibitor of Src family kinases, but it also inhibit Bcr-Abl with an IC_{50} of 1.4 nM. However, it is more specific than Imatinib and dasatinib since it doesn't inhibit KIT and PDGFR.[46] Bosutinib showed in vitro activity towards all the mutants except T315I. When tested in phase I and II clinical trials in Imatinib resistant CML and *Ph*-positive ALL, it showed excellent efficacy at well tolerated doses.[47]

INNO-406 (Innovive, originally developed by Nippon Shinyaku as NS-187). This molecule is a potent inhibitor of Bcr-Abl, chemically similar to Imatinib and nilotinib, which is in clinical development for the treatment of CML[48]. INNO-406 is more than 20-folds more potent than Imatinib in inhibiting the Bcr-Abl positive leukemia cell line K562 and KU812. It also inhibit the T315A and F317L/V mutants which were insensitive to dasatinib at physiological concentrations. It's able to prolonge the survival of mice injected with leukemic cells bearing all the point mutations but not the T315I.[49] A Phase I study on INNO-406 started in 2006 and the final results were reported at the 2007 Annual Meeting of the American Society of Hematology.[50] A Phase II study on INNO-406 is in preparation.[51]

AZD0530 (AstraZeneca). This compound inhibit very potently (nM range) all protein kinases of the family of Src kinases and more weakly Bcr-Abl. It has been developed for the treatment of solid

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tumors but it is useful also for leukemia. Currently it’s not clear if AZD0530 will be further developed for the treatment of CML.

Some inhibitors that bind to a non-ATP allosteric and competitive binding site of kinases have been recently developed. The advantage offered by this strategy is that the Imatinib-resistant mutants are not likely to resist to such inhibitors, owing to the different binding site. High throughput screening for inhibitors of Bcr-Abl found a compound that was subsequently modified to give 3-(6-((4-(trifluoromethoxy)phenyl)amino)-4-pyrimidinyl)benzamide (GNF-2) as a lead compound for the development of competitive allosteric inhibitors of Bcr-Abl.[52]

As for Imatinib, resistance could also develop for the second generation Bcr-Abl inhibitors, through the evolution of other point mutations that impair binding of the drug.
Therefore overriding these resistances could represent a future challenge in the treatment of Imatinib resistant patients. Nine point mutations affecting 8 amino acids have been discovered to be Nilotinib-resistant (G250E, Y253H, E255K/V, E292V, T315I, F359C, L384M and L387F). Excluding the T315I, all the other mutations have been inhibited when treated with a concentration of nilotinib higher than 2000 nM, which is usually achieved in patients treated with nilotinib at 400 mg twice a day. These mutations anyway are not selected to drive the disease, and the patients continue to respond to drug.
In a similar way, nine dasatinib-resistant mutants have been discovered (L248V, Q252H, E255K, V299L, T315I, F317I/C/L/V) affecting just six residues.[53] Mutations such as V299L and T315I, can emerge to drive disease during dasatinib therapy. This means that the inhibition of the Src-family kinases is not sufficient to stop the proliferation of these cells. This notion is further supported by the fact that dual Abl and Src-family kinase inhibitors are inactive *in vitro* against cells that express T315I Bcr-Abl. Therefore the inhibition of the Src family might not contribute towards efficacy in CML.
A potential strategy to circumvent resistance is given by the combination of different drugs. A combination of Imatinib and dasatinib is able to significantly decrease the occurrence of drug resistant mutants, and highlights the benefit to combine two inhibitors that binds to different conformations of Bcr-Abl. In fact Imatinib binds preferentially to the inactive conformation, while dasatinib can bind both active and inactive conformation. Nevertheless, T315I mutation is known to cause cross resistance for both compounds.

Although second generation Bcr-Abl inhibitors are able to significantly decrease the progression of CML, the development of resistances presents a challenge. Many progresses have been done toward the development of inhibitors able to overcome all the point mutations (but the danger of evolution of new drug-resistant point mutation is still high), but the development of a drug able to inhibit also T315I remains an open interesting challenge.

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1.1.6 Overriding resistance to T315I mutation: from second to third generation inhibitors[54]

As stated above, Imatinib and all second generation inhibitors are ineffective against T315I. Gorre and co-workers34[a] were the first to report that six out of nine patients showing resistance to Imatinib, had this point mutation. Some studies suggests that patients who carry this mutation have an average life expectation of 12.6 months from the start of the Imatinib therapy.24[,55] By contrast other studies suggest the opposite claiming that the survival of the patients remains dependent on the stage of the disease.[56] Despite the fact that the impact of this mutation on the disease is controversial, T315I remains one of the biggest obstacles in treatment of CML especially in the latest stages of the disease, even after the introduction of second generation inhibitors in the clinic.

Threonine 315 is an aminoacid located inside the Imatinib binding site. It directly interacts with the inhibitor via a hydrogen bond. At the beginning it was thought that the main reason for the resistance to Imatinib was due to the lack of this hydrogen bond, disruption given by the apolar and more bulkier isoleucine.[24,57] Recently it has been shown that the missing hydrogen bond is not the leading cause of the resistance, but instead it derives from a conformational change on the protein given by the new residue.[58] This change in the shape of the active site involves not only the isoleucine 315 but also many other amino acids that have important interactions with the drug. Recent molecular dynamics simulation studies[59] confirmed this theory, highlighting the fact that the T315I mutation decrease abruptly the Imatinib affinity because of some induced conformational changes that occur in the C-helix, in particular at aminoacids Glu286 and Met298. It is also possible that the same considerations can be done for the binding of nilotinib, since it’s proved that similarly to Imatinib, nilotinib has a close contact with E286.[60] It is still unclear whether this rational for the resistance can be applied also to dasatinib as dasatinib does not appear to have direct interaction with E286.

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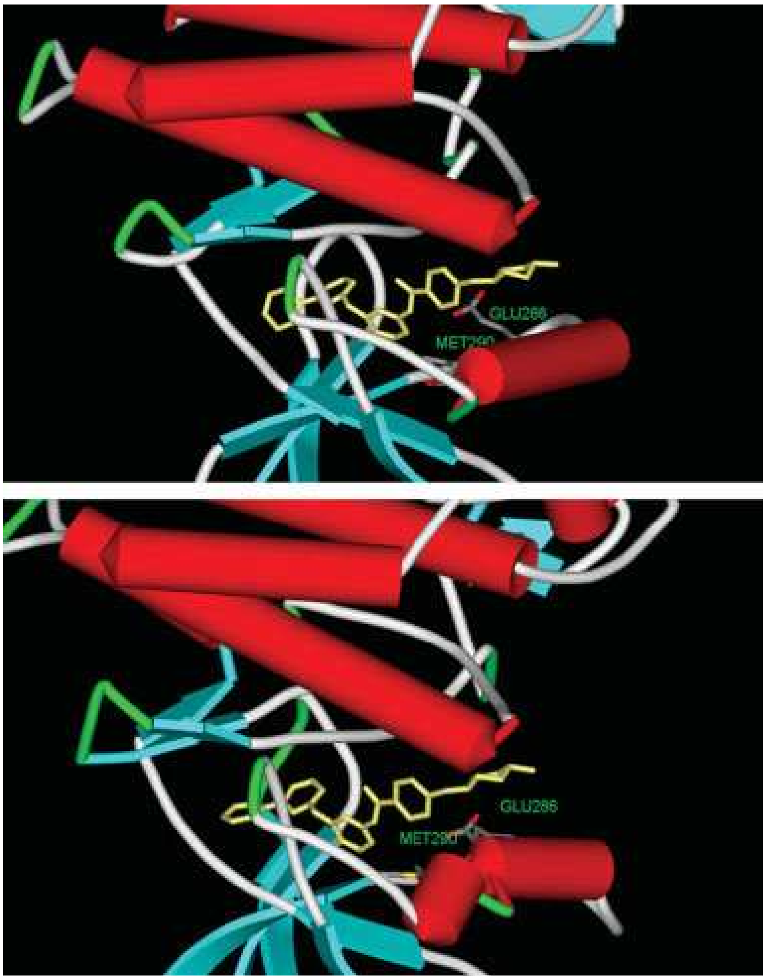
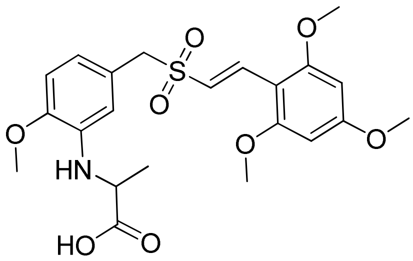


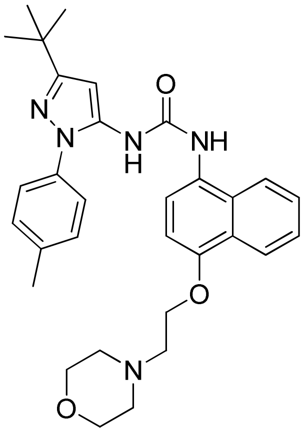
Figure1.859**:** *Representative schematic views of wild-type structures (top) and T315I structures (bottom) from a molecular dynamics simulations*[59]*. T315I clearly changed the conformation of the C-helix containing glutamate 286 (Glu286) and methionine 290 (Met290). The ‘‘head’’ of the C-helix moves away from the Imatinib molecule. Imatinib is shown in yellow.*

Many efforts have been profused to overcome the problem of resistance linked to the T315I mutation including the inhibition of other enzymes known to be strictly correlated to the proliferations of leukemic cells. The first approach aiming at developing substrate mimicking inhibitors led to the discovery of ON012380 which is a substrate competitive inhibitor of Bcr-Abl kinase.



ON012380

ON012380 (Onconova Therapeutics). This molecule is a substrate mimicking inhibitor of Bcr-Abl, and offers the possibility to overcome the problem of resistances to Imatinib and all second generation inhibitors, by targeting a non-ATP binding site. It inhibits the proliferation of Bcr-Abl and all Bcr-Abl mutants dependant cells (including T315I) at a concentration lower than 10 nM. In addition ON012380 caused a regression of leukemias in mice injected with cells expressing T315I, and works in combination with Imatinib to inhibit wild type Bcr-Abl, binding in two different binding sites.[61] Despite the promising *in vitro* and *in vivo* results, this molecule has not yet entered clinical trials.



BIRB-796

BIRB-796. It's a urea derivative and inhibits all of the p38 MAPK isoforms[62] and Carter and co-workers, reported that it was active against T315I *in vitro.*[63] In contrast, O' Hare and Drucker,[64] found that Carter's results were not consistent with the results they obtained, reporting that BIRB-796 failed to inhibit the proliferation of T315I dependant cells. The diatribe between the two groups ended with the conclusion that BIRB-796 itself is not of direct clinical utility, but can provide a good starting point in development of new drug with the same scaffold.[65]

Aurora kinases play a crucial role in the regulation of mitotic processes during cell division.[66] Overexpression of these enzymes may results in carcinogenesis[67], therefore targeting these enzymes became of great interest in cancer therapy.[68] Some Aurora Kinase inhibitors showed inhibitory activity on Imatinib resistant cells and thus they may be useful for the treatment of CML in patients resistant to Imatinib.

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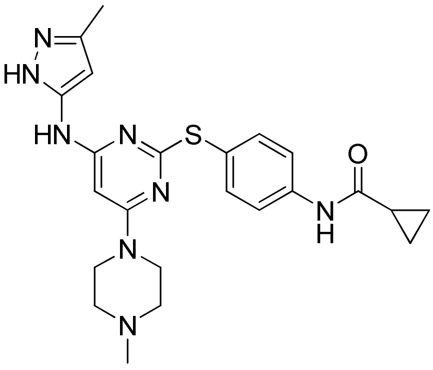
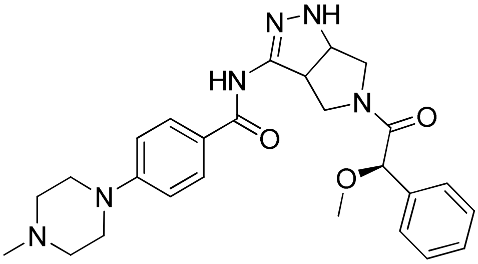


MK-0457 (VX-680)

MK-0457 (Merk, originally developed by Vertex Pharmaceuticals as VX-680). MK-0457 is an aurora kinase inhibitor[69] in clinical development for the treatment of tumors. It causes regression of leukemia and solid tumors like colon and pancreatic cancers. Other than inhibiting the three aurora kinases, this molecule weakly inhibits *ex vivo* cells of patients bearing the Bcr-Abl T315I mutation[70] with an IC_{50} of about 5 μM. MK-0457 inhibits also proliferation of parental non transformed cell in the submicromolar range. MK-0457 binds an inactive conformation of the Aurora-A kinase, exploiting an hydrophobic pocket that is generated when the protein closes from the active open conformation to the closed inactive conformation. The activity and selectivity of MK-0457 toward both Aurora and Bcr-Abl T315I is explainable by the fact that the two kinases have a very similar active site conformation that includes also the key hydrophobic pocket occupied by the inhibitor[71] (which is not the same exploited by Imatinib in Bcr-Abl). In contrast, MK-0457 binds Bcr-Abl T315I in an active conformation, with the key hydrophobic pocket conserved as shown by the X-ray structure[71]. It's not excluded that the inhibitor could also bind to the inactive conformation of Bcr-Abl T315I, but this hypothesis still remains to be demonstrated. Efficacy of MK-0547 in three *Ph*-positive patients bearing the T315I mutation have been reported.70[b] A phase II study had been initiated but these trials were halted owing to a potential heart safety issue.



PHA-739358

PHA-739358 (Nerviano Medical Science). *PHA-739358* is an orally bioavailable inhibitor of the three aurora kinases, which significantly decreases tumor growth in animals at well tolerated

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[69] Young, M.A.; Shah, N.P.; Chao, L.H.; Seeliger, M.; Milanov, Z.V.; Biggs III, W.H.; Treiber, D.K.; Patel, H.K.; Zarrinkar, P.P.; Lockhart, D.J.; Sawyers, C.L.; Kuriyan, J. *Cancer Res.* **2006**, *66*, 1007.

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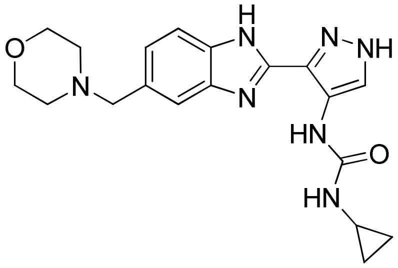
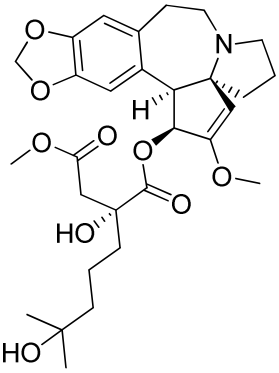
doses.[72] PHA-739358 exhibited strong antiproliferative and proapoptotic activity against a broad panel of human Bcr-Abl-positive and negative cell lines and against murine BaF3 cells ectopically expressing wild-type or Imatinib-resistant Bcr-Abl mutants, including T315I.[73] The inhibitor has been the first one crystallized in complex with Bcr-Abl T315I.[73a] The solved crystal structure provided the explanation for its activity. PHA-739358 binds to an open, active conformation of the kinase domain in the ATP-binding pocket and has no contacts with the more bulky mutated gatekeeper. This compound is being tested in phase II clinical trials for patients with CML who have relapsed after Imatinib treatment.[73b]



AT9283

AT9283. AT9283 is a potent inhibitor of many protein kinases in the low nanomolar range, including Bcr-Abl and its mutated form T315I.[74] Clinical studies to investigate the effect of AT9283 in patients with leukemia have been initiated and a number of CML patients have been treated.[74]



Homoarringtonine

Homoarringtonine (HHT). This cephalotaxine ester demonstrated clinical activity toward CML.[75] A possible combination of Imatinib and HHT for the treatment of CML has been observed *in vitro* and no cross resistance between the two molecules in Imatinib resistant cell lines have been observed. Good results have been obtained in treating patients that acquired resistance to Imatinib due to the

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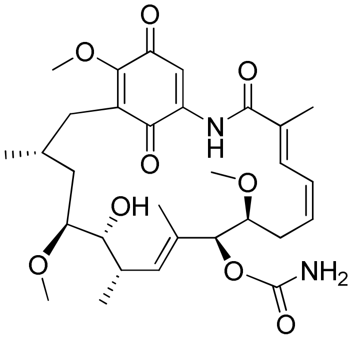
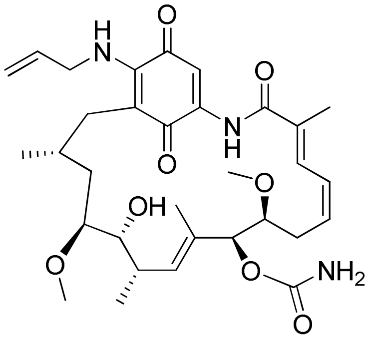
T315I mutation with HHT.[76] Patients can be treated with both natural HHT, or with omacetaxine mepesuccinate (a semisinthetic subcutaneously bioavailable form of HHT). An exhaustive review on the preclinical and clinical studies of HHT has been published by Quintàs-Cardana[77] in 2008.

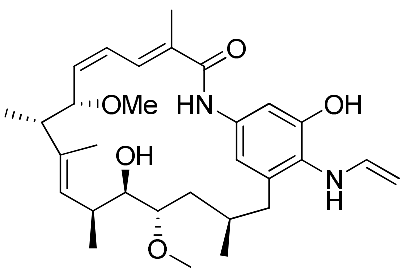
SGX393. SGX393 is a very potent inhibitor of Bcr-Abl wild type and of all its mutants, including T315I.[78] The structure is not reported in literature. Nevertheless, it belongs to the family of 3,5-disubstituted pyrrolopiridine. 2-amino-5-(3-(1-ethyl-1H-pyrazol-5-yl)-1H-pyrrolo[2,3-b]pyridin-5-yl)-N,N-dimethylbenzamide is a lead compound able to inhibit Bcr-Abl and Bcr-Abl T315I, and is structurally correlated to SGX393. Crystal structure of this molecule in complex with Bcr-Abl T315I shows that the molecule binds to the active conformation of the kinase domain. From this finding it can be inferred that also SGX393 binds to the active conformation of Bcr-Abl T315I. SGX393 blocks the growth of leukemic cell lines expressing T315I with only minimal toxicity toward Ph-negative cells. The combination of SGX393 with dasatinib or nilotinb prevented the emerging of resistant cells, including the T315I, giving rise to a very promising way of overcoming the drug resistance in CML.



Geldanamycine



17AAG



IPI 504

Geldanamycine, 17AAG and IPI504. Heat shock protein 90 (Hsp90) is a molecular chaperone which affects the stability and function of multiple oncogenic proteins including Bcr-Abl.[79] Disruption of Hsp90 function by Geldanamycine or its less toxic analog, 17-allylaminogeldanamycin (17-AAG), in Bcr-Abl-expressing leukemia cells has been shown to induce Bcr-Abl protein degradation and suppresses cell proliferation.[80] The same considerations can be done for leukemic cells expressing T315I and E255K mutations.79[a,81] In a similar way IPI-504 targets the Hsp90 and causes Bcr–Abl protein degradation, decreased numbers of leukemia stem cells, and the prolonged survival of mice with CML induced by Bcr–Abl–T315I.[82] Also heat-shock protein 32/heme oxygenase-1 (Hsp32/HO-1) has found to be a Bcr-Abl dependent survival protein

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[79] (a) An, W.G.; Schulte, T.W.; Neckers, L.M. *Cell Growth Differ*. **2000**, *11*, 355. (b) Shiotsu, Y.; Neckers, L.M.; Wortman, I.; An, W.G.; Schulte, T.W.; Soga, S.; Murakata, C.; Tamaoki, T.; Akinaga, S. *Blood* **2000**, *96*, 2284.

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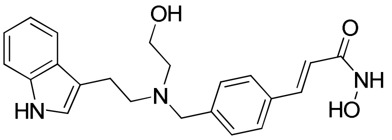
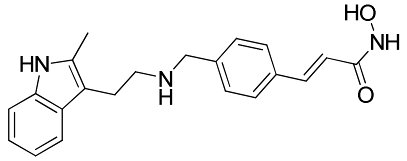
[82] Peng, C.; Brain, J.; Hu, Y.; Goodrich, A.; Kong, L.; Grayzel, D.; Pak, R.; Read, M.; Li, S. *Blood* **2007**, *110*, 678.

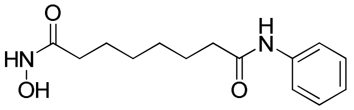
in CML cells. The inhibition of Hsp32/HO-1 with pegylated zinc proptoporphirine or styrene maleic acid-micelle-encapsulated ZnPP resulted in growth inhibition of Bcr-Abl transformed cells, including the T315I mutations.[83]



LAQ-824

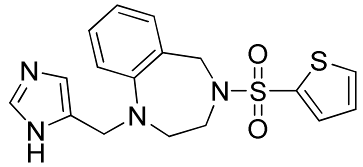


LBH589



SAHA

LAQ824, LBH589 and SAHA. Treatment with LAQ824, a histone deacetylase inhibitor results in downregulation of levels of Bcr-Abl T315I and induce apoptosis in CML-blast crisis cells that presented primary resistance to Imatinib.[84] A decrease of the number of these leukemic T315I expressing cells by apoptosis can also be obtained by treatment with a combination of LBH589, a novel cinnamic hydroxamic acid derivative histone deacetylase inhibitor, and 17-AAG, already reported to be a Hsp90 inhibitor.[85] LBH589 can also be given together with nilotinib: this combination is active against cultured or primary Imatinib-resistant CML cells, including those expressing Bcr-Abl T315I.[86] SAHA (Suberoylanilide Hydroxamic Acid) is another histone deacetylase that attenuates the levels of Bcr-Abl E255K and Bcr-Abl T315I and induced apoptosis of BaF3 cells with ectopic expression of the mutant forms of Bcr-Abl. If SAHA is used in combination with dasatinib better results can be obtained.[87] Effect of SAHA can further be amplified by treatment in combination with drugs that disrupts the autophagy pathway, e.g, the chloroquine.[88]



BMS-214662

BMS-214662. BMS-214662 is a cytotoxic farnesyl transferase inhibitor that was reported to kill non-proliferating tumor cells. BMS-214662, alone or in combination with Imatinib or dasatinib, potently induced apoptosis of both proliferating and quiescent CML stem/progenitor cells with less than 1% recovery of Philadelphia-positive long term culture-initiating cells. It's equally potent also on cells bearing point mutations including T315I. This is the first compound reported to selectively

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[83] (a) Mayerhofer, M.; Florian, S.; Krauth, M.T.; Aichberger, K.J.; Bilban, M.; Marculescu, R.; Printz, D.; Fritsch, G.; Wagner, O.; Selzer, E.; Sperr, W.R.; Valent, P.; Sillaber, C. *Cancer Res.* **2004**, 64, 3148. (b) Mayerhofer, M.; Gleixner, K.V.; Mayerhofer, J.; Hoermann, G.; Jaeger, E.; Aichberger, K.J.; Ott, R.G.; Greish, K.; Nakamura, H.; Derdak, S.; Samorapoompichit, P.; Pickl, W.F.; Sexl, V.; Esterbauer, H.; Schwarzinger, I.; Sillaber, C.; Maeda, H.; Valent, P. *Blood* **2008**, *111*, 2200.

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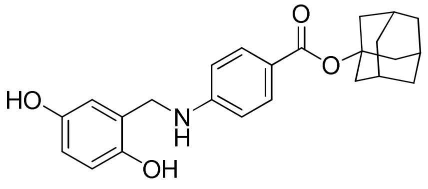
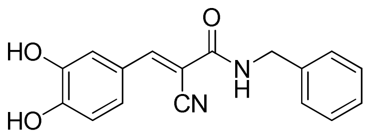
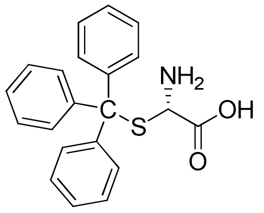
kill CML stem/progenitor cells through apoptosis, thus offers potential for eradication of chronic phase CML.[89]



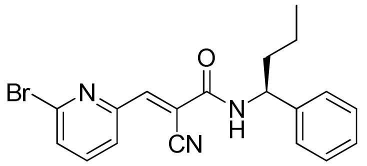
Adaphostin



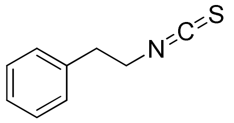
AG490



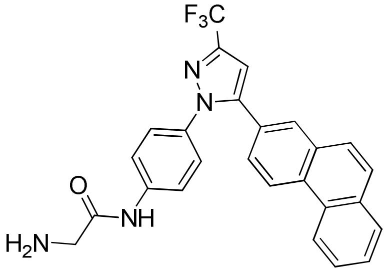
S-Trityl-L-cysteine



WP1130



β-phenylethyl isothiocyanate



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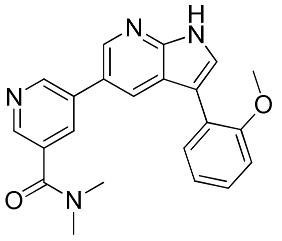
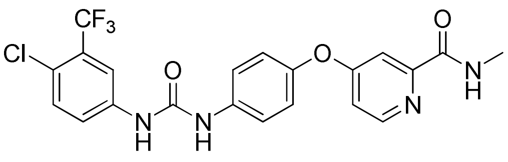
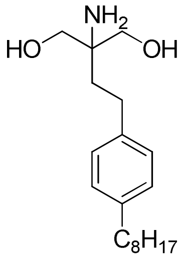


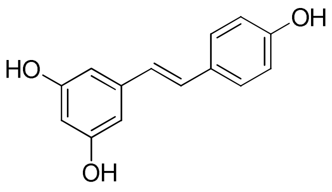
PPY-A



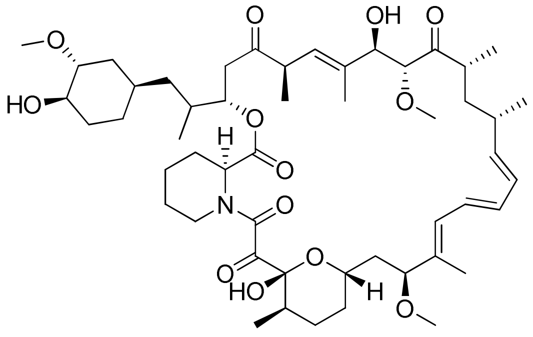
Sorafenib



FTY720



Resveratrol



Rapamycine

Other drugs. Other compounds have also been reported to be promising to override the problem of the T315I mutation. They are briefly reported here. Adaphostin, from tyrphostin family of tyrosine kinase inhibitors induces the generation of reactive oxygen species (ROS) that are lethal for the mutant cells.[90] AG490, is an inhibitor of the Janus kinase 2. Bcr-Abl is associated with a cluster of signaling proteins including the Janus kinase 2: inhibiting this enzyme with AG490 resulted in apoptosis of BaF3 cells expressing the T315I mutation.[91] *S*-trityl-L-cysteine, is able to inhibit the

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[89] Copland, M.; Pellicano, F.; Richmond, L.; Allan, E.K.; Hamilton, A.; Lee, F.Y.; Weinmann, R.; Holyoake, T.L. *Blood* **2008**, *111*, 2843.

[90] Dasmahapatra, G.; Nguyen, T.K.; Dent, P.; Grant, S. *Leuk. Res.* **2006**, *30*, 1263.

[91] Samanta, A.K.; Lin, H.; Sun, T.; Kantarjian, H.; Arlinghaus, R.B. *Cancer Res.* **2006**, *66*, 6468.

Eg5 enzyme, a microtubule-associated motor protein that is highly expressed in all *Ph*+ cell lines. Eg5 is located downstream of and regulated by Bcr-Abl, thus inhibition of its activity induces cell death also on cells resistant to Imatinib.[92] WP1130, is a second-generation tyrphostin derivative and is able to reduce wyld type and T315I Bcr-Abl protein levels in CML cells through the inhibition of a novel regulatory mechanism.[93] Symilarly to adaphostin, ß-phenylethyl isothiocyanate, a natural compound found in vegetables, degradates the Bcr-Abl proteins (mutated or not) by the action of reactive oxygen species.[94] OSU-03012 is a celecoxib-derived phosphoinositide dependent kinase-1 inhibitor that works in synergy with Imatinib targeting Akt, a protein that plays a pivotal role in Bcr-Abl mediated cell survival. The combined action of these two inhibitors is capable to overcome Imatinib resistance.[95] R18, a peptide-based 14–3–3 competitive antagonist, disrupts the 14-3-3 ligand association inducing apoptosis in in cells expressing the Bcr-Abl T315I mutation.[96] PPY-A, and related compounds, (structurarly similar to SGX3939) pyrrolopyridine inhibitors targets the ATP binding site of T315I but does not exploit the hydrophobic pocket behind the mutated gatekeeper, overcoming in this way the problem of resistance.[97] Sorafenib (Nexavar™), a potent oral multikinase inhibitor targets upstream tyrosine kinase receptors as well as downstream serine/threonine kinases, causing apoptosis in Imatinib resistant cells.[98] FTY720, plays its antitumoral role by the activation of PP2A, a protein that has tumor suppression activity.[99] Resveratrol, the naturally polyphenolic phytoalexin found in grapes and peanuts, induces apoptosis of T315I cells, caspase independent death, and differentiation that collectively contribute to the specific elimination of CML cells.[100] Rapamycin, inhibited the growth of Bcr-Abl T315I cells by targeting mTOR, an enzyme involved in the regulation of growth of neoplastic cells in CML.[101] DCC-2036; block the growth of T315I cells by inhibiting the switch pocket enzyme.[102]

In the table below all the inhibitors that are under preclinical and clinical investigation are resumed.

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[92] Carter, B.Z.; Mak, D.H.; Shi, Y. ; Schober, W.D.; Wang, R.Y.; Konopleva, M.; Koller, E.; Dean, N.M.; Andreef, M. *Cell Cycle* **2006**, *5*, 2223.

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[94] Zhang, H.; Trachootham, D.; Lu, W.; Carew, J.; Giles, F.J.; Keating, M.J.; Arlinghaus, R.B.; Huang, P. *Leukemia* **2008**, *22*, 1191.

[95] Tseng, P.H.; Lin, H.P.; Zhu, J.; Chen, K.F.; Hade, E.M.; Young, D.C.; Byrd, J.C.; Grever, M.; Johnson, K.; Druker, B.J.; Chen, C.S. *Blood* **2005**, *105*, 4021.

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[98] Rahmani, M.; Nguyen, T.K.; Dent, P.; Grant, S. *Mol. Pharmacol.* **2007**, *72*, 788.

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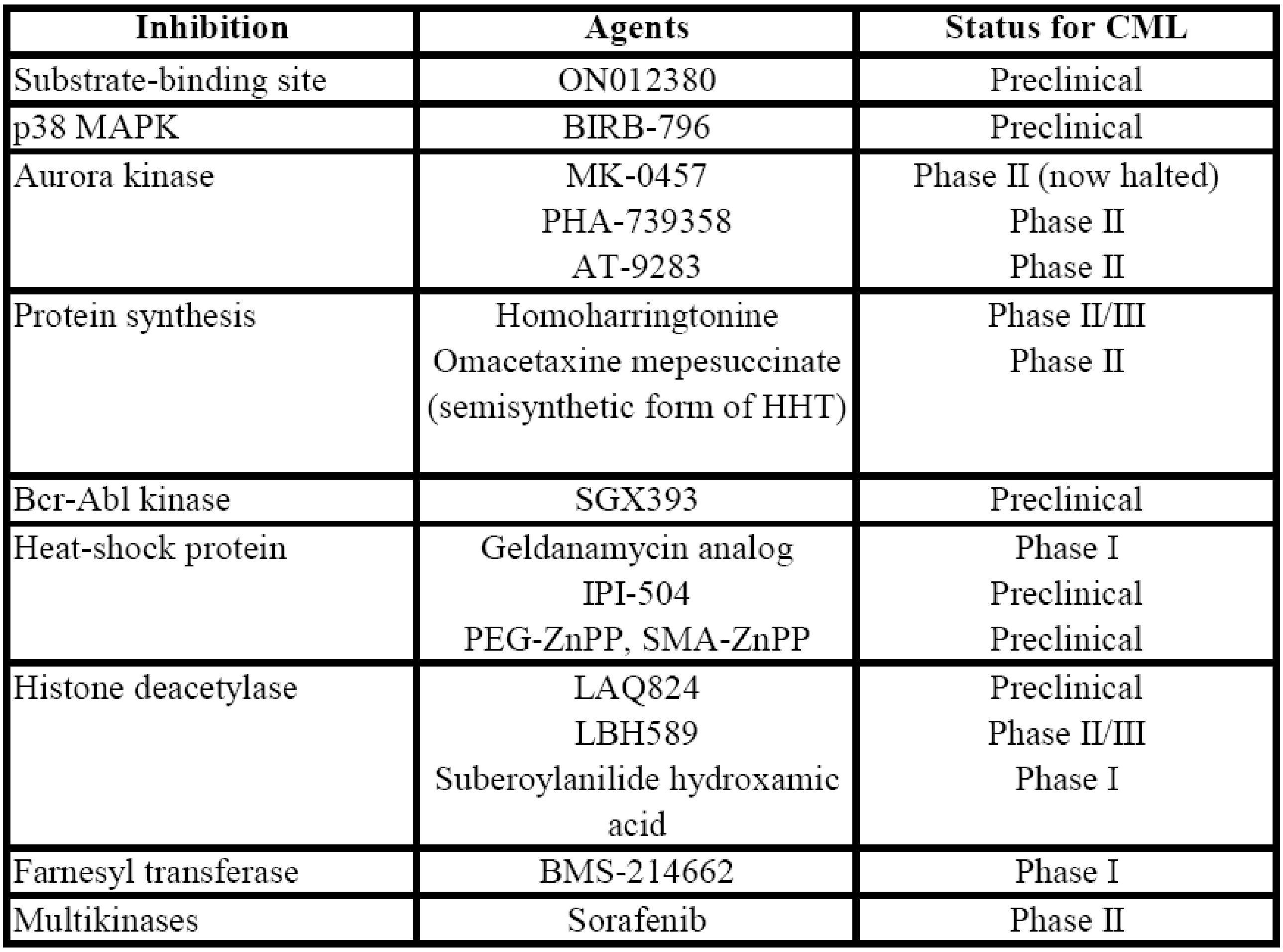
[101] Sillaber, C.; Mayerhofer, M.; Böhm, A.; Vales, A.; Gruze, A.; Aichberger, K. J.; Esterbauer, H.; Pfeilstöcker, M.; Sperr, W. R.; Pickl, W. F.; Haas, O. A.; Valent, P. *Eur. J. Clin. Invest.* **2008**, *38*, 43.

[102] Van Etten, R.A.; Chan, W.W.; Zaleskas, V.M.; *et al. Blood* **2007**, *110*, 142A.

Table 1.1: Inhibitors under preclinical and clinical investigation.



Inhibition	Agents	Status for CML
Substrate-binding site	ON012380	Preclinical
p38 MAPK	BIRB-796	Preclinical
Aurora kinase	MK-0457 PHA-739358 AT-9283	Phase II (now halted) Phase II Phase II
Protein synthesis	Homoharringtonine Omacetaxine mepesuccinate (semisynthetic form of HHT)	Phase II/III Phase II
Bcr-Abl kinase	SGX393	Preclinical
Heat-shock protein	Geldanamycin analog IPI-504 PEG-ZnPP, SMA-ZnPP	Phase I Preclinical Preclinical
Histone deacetylase	LAQ824 LBH589 Suberoylanilide hydroxamic acid	Preclinical Phase II/III Phase I
Farnesyl transferase	BMS-214662	Phase I
Multikinases	Sorafenib	Phase II

Most of the agents here reported are not targeting directly Bcr-Abl, but are broad spectrum agents for different kinases. It's not yet clear if targeting more than one kinase is more effective for CML. Komarova and co-workers[103] reported that multitarget therapeutics may be more potent and promising, although this approach could lead to more adverse effects than highly specific compounds.

Nevertheless targeting competitively the active site of Bcr-Abl T315I, remains an open challenge and a promising perspective to overcome the problem of Imatinib resistance.

1.2 BIOLOGICAL ASSAYS

While performing biological tests efficacy and potency have to be distinguished. Efficacy gives us the strength of the effect while potency gives us the concentration by which the compound is reaching its maximal effect. Nevertheless, in particular in the field of enzyme inhibition by small molecules, it's common to hear the potency reported as activity: a compound can be reported to be *more active* or *less active* than another one because they have different IC_{50} values. The IC_{50}, the half maximal inhibitory concentration, is a convenient way of measuring the potency of a

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[103] Komarova, N.L.; Wodarz, D. *Proc. Natl Acad. Sci. USA* **2005**, *102,* 9714.

compound in inhibiting biological or biochemical functions. The IC_{50} is the concentration of inhibitor required to produce 50% inhibition of an enzymatic reaction at a specific substrate concentration. In contrast to the inhibition constant (K_i) or dissociation constant (K_d), the IC_{50} value is dependent from the experimental conditions. The lower the IC_{50} value is, the more potent the compound is. When an inhibitor binds to a specific enzyme forming a complex, a binding affinity constant can be defined:

$$\mathbf{EI} \rightleftharpoons \mathbf{I} + \mathbf{E}$$

The strength of the binding affinity can be expressed as dissociation constant K_d which is equal the inhibition constant K_i. The dissociation constant K_d is expressed by the equation representing the equilibrium of the dissociation of the complex enzyme-inhibitor.

$$K_d = \frac{[Enzyme][Inhibitor]}{[EI_{complex}]}$$

K_d is the dissociation constant of the complex enzyme-inhibitor: the lower it is the higher the affinity of the inhibitor for the enzyme will be.

In an enzymatic reaction where an inhibitor is competitive to the specific substrate,

$$\begin{tikzcd}
\mathrm{E} \arrow[r, shift left, "\mathrm{S}"] \arrow[d, shift left, "\mathrm{I}"] & \mathrm{ES} \arrow[l, shift left] \arrow[r] & \mathrm{E} + \mathrm{P} \\
\mathrm{EI} \arrow[u, shift left] & &
\end{tikzcd}$$

the relation between the IC_{50} and the binding affinity constant K_d is given by the Cheng-Prusoff[104] equation:

$$IC_{50} = K_d\left(1 + \frac{S}{K_m}\right)$$

where S is the concentration of the substrate (that in the case of a tyrosine kinases is the ATP if the inhibitor is competing with ATP), and K_m is its Michaelis-Menten constant. IC_{50} values are dependent from the concentration of the substrate, hence values of IC_{50} determined under different assay condition are not comparable. On the contrary IC_{50} values of different inhibitors determined under the same experimental condition can be directly compared and the differences in IC_{50} values represent well the differences in binding affinities of the tested compounds: the lower the IC_{50} value, the higher the binding affinity of the inhibitor to its target.

In our specific case the IC_{50}values have been measured by our partners in the project at the University of Milano Bicocca. IC_{50} have been measured *in vitro*, using an ELISA-based target-based assay and a cell-based functional assay.

The ELISA-based inhibition assay has been performed on the recombinantly expressed kinase domain of Bcr-Abl and Bcr-Abl T315I. This assay allowed assessing the activity of newly

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[104] Cheng, Y.C.; Prusoff, W. *Biochem. Pharmacol.* **1973**, *22*, 3099.

synthesized inhibitors on the target and get molecular information on the interaction between inhibitors and targets *in vitro*, IC_{50} values have been measured using an ELISA-based assay on the isolated recombinantly expressed target[105] (**Figure 1.9**). ELISA is the acronym of Enzyme-linked immunosorbent assay, and is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. With the appropriate modifications it is also a good way for measuring the potency of inhibitors. In our specific case[106] a substrate peptide bearing a tyrosine is immobilized on the bottom of the well of a 96 wells-plate. The tyrosine kinase (wild type Bcr-Abl or Bcr-Abl T315I), ATP, the inhibitor and the buffer are then added and the mixture is incubated at 30°C for 15 minutes. In this step the inhibitor, if active, binds to the enzyme and impairs its activity. Thus the amount of phosphorylated immobilized peptide in presence of an inhibitor is reduced compared to the experiment in absence of inhibitor. The higher the potency of the inhibitor, the less phosphorylated will be the peptide. After the incubation time, everything that is in solution is washed off while the phosphorylated peptide remains on the bottom of the well. After the washing, a primary anti-phosphotyrosine antibody recognizing the phosphorilated peptide is added, and a complex primary antibody-phosphorilated peptide is formed. This complex is recognized by a secondary anti-mouse antibody which is conjugated with a peroxidase enzyme. Thus the new complex formed bears a peroxidase which transforms a specific substrate (TMB, tetramethylbenzidines) yielding a color that can be quantitatively detected by a spectrophotometer. Hence the more the inhibitor is active the less colored will be at the end the solution.

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[105] Gunby, R.H.; Tartari, C.J.; Porchia, F.; Donella-Deana, A .; Scapozza, L.; Gambacorti-Passerini, C. *Haematologica* **2005**, *90*, 988.

[106] For the detailed procedure see: Mologni, L.; Sala, E.; Riva, B.; Cesaro, L.; Cazzaniga, S.; Redaelli, S.; Marin, O.; Pasquato, N.; Donella-Deana, A.; Gambacorti-Passerini, C. *Protein Expr Purif.* **2005**, *41*, 177.

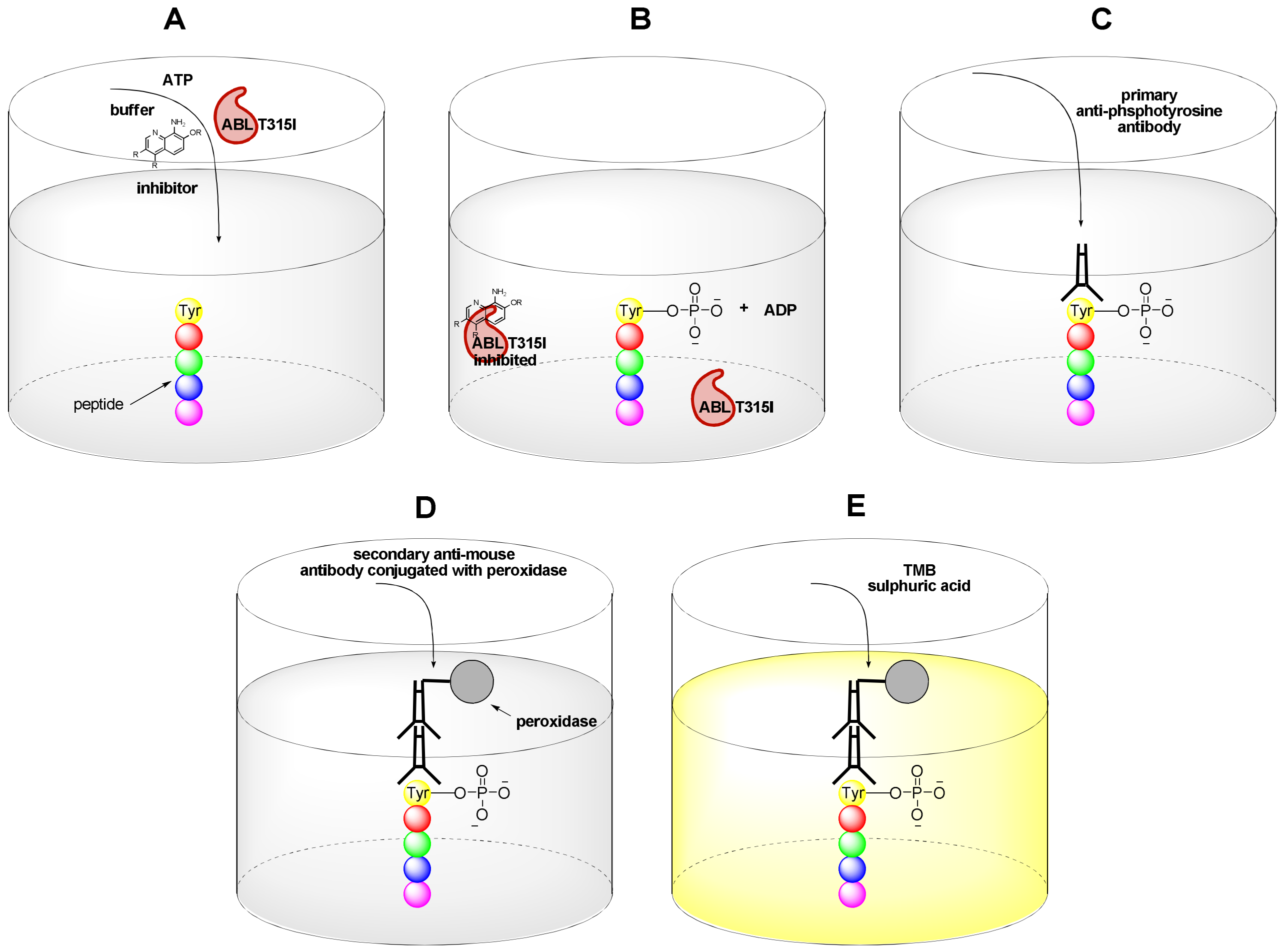

Figure 1.9: *ELISA-based kinase assay. A) A peptide is immobilized on the bottom of the well and Bcr-Abl, the inhibitor, ATP and the buffer are added. B)Uninhibited Bcr-Abl phosphorylates the peptide. C) Everything that is in solution is washed away and a primary anti-phosphotyrosine antibody is added. The antibody binds to the phosphorilated peptide. D) After a second washing step, the secondary antimouse antibody conjugated with a peroxidase recognizing the already present complex, is added. E) TMB, a substrate of the peroxidase, is added and transformed by the enzyme. The product is colored and the color is detected by a spectrophotometer.*

The cell-based functional assay is a proliferation inhibition assay. It has been carried out on transformed cells expressing the oncogenic fusion protein (Bcr-Abl and Bcr-Abl T315I) as well as on non-transformed cells. The latter have been used to assess the selectivity of the compounds at cellular level and indirectly their intrinsic toxicity. IC_{50} values for proliferation inhibition on Bcr-Abl (WT and T315I) transformed and non-transformed cells have been measured. The proliferation test allows evaluating the proliferation ability of cell lines subjected to treatment with inhibitors previously tested on the recombinantly expressed purified proteins. Different concentration of the inhibitors, were prepared directly in different wells of a 96 wells-plate. A concentration range around the IC_{50}value measured by the ELISA assay has been chosen for this assay. Untreated cells were used as control which the measurements in presence of inhibitors have been referred to. Cells have been incubated with different concentration of inhibitors and buffer for 72 hours. The cells have been pulsed with ^{3}H-T during the last 8 hours of culture and the cell-associated radioactivity was measured by liquid-scintillation. Percent inhibition of ^{3}H-T uptake at each inhibitor dose relative to control cells without treatment is then calculated. The concentration of the inhibitor at which the proliferation is reduced of 50% is the value of the IC_{50}.

The performed cellular assay whose results are reported on **Table 4.1**, **Table 4.2, Table 4.3** and **Table 4.4** (Chapter 4, **Appendix A**) have been performed using the following cell lines:

Ba/F IL3. The *Ba/F IL3* cell line is the non-transduced parental cell line also called wild type cell line that does not express any oncogenic fusion protein and grows only if Interleukin 3 (IL3) is added to the media. This cell line is used as negative control and indicates whether there is general cell toxicity. The ideal compound should not have any proliferation inhibition activity on this cell line.

Ba/F Bcr-Abl T315I. The *Ba/F Bcr-Abl T315I* cell line is the Bcr-AblT315I (mutant)-transduced cell line mimicking the CML cells. The growth of these cells is now dependent on the expression of the oncogenic fusion protein Bcr-AblT315I. The cell proliferation of this cell line is now IL3 independent. This cell line represents a state of the art cellular model used to mimic CMLs cells resistant to the Bcr-Abl tyrosine kinase inhibitors on the marked. The inhibitors should have a strong proliferation inhibitory effect on this cell line.

Ba/F Bcr-Abl. The *Ba/F Bcr-Abl* cell line is the Bcr-Abl-wild type-transduced cell line mimicking the CML cells.

1.3 AIM OF THE THESIS

Resistance to Imatinib and second generation Bcr-Abl inhibitors caused by the point mutation of the gatekeeper residue T315I is a clinical relevant problem. The exchange from the polar amino acid threonine with the apolar bulkier isoleucine impairs drug binding at the ATP binding site. Different strategies for circumventing resistance have been developed, e.g targeting other enzymes than Bcr-Abl involved in cell signaling cascade, targeting allosteric sites or oxydoreductive degradation of the oncogenic proteins. None of them led to a significant improvement of the clinical situation up to now. Thus, finding inhibitors able to efficiently inhibit the mutated form of Bcr-Abl remains an open challenge and a promising perspective to overcome the problem of Imatinib resistance.

Therefore on the basis of molecular modeling studies done within our research group in Geneva (explained in Chapter **1.4**)**, the aim of this thesis is the synthesis of a library of compounds with a novel scaffold, able to inhibit the T315I mutant of Bcr-Abl by binding on the ATP pocket.**

In the frame of a collaborative effort with Prof. Gambacorti-Passerini, the synthesized compounds have been then tested in biological assays in order to assess their efficacy and potencies on both wild-type Bcr-Abl and T315I Bcr-Abl .

1.4 RATIONAL OF THE SYNTHESIS OF 8-AMINOQUINOLINES

1.4.1 Rational

Bosutinib is known to be a strong inhibitor of Bcr-Abl wild type (IC_{50}=1.4 nM),[107] but it shows no activity on Bcr-Abl T315I mutant. In this project it has been thought to use this molecule as a template for the design of a novel scaffold being able to strongly inhibit the mutated form of the protein.

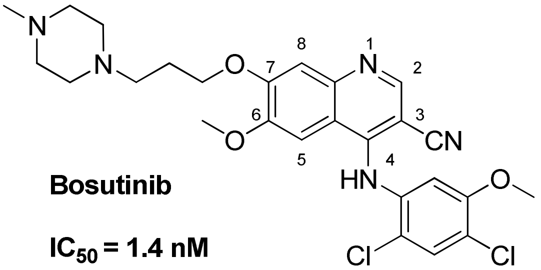

Figure 1.10: *Structure and activity of Bosutinib on wild type Bcr-Abl kinase domain.*

To date there are no crystal structure of Bosutinib bound to the kinase domain of Abl WT. However its binding mode can be inferred by the crystal structure of a related compound[108] to the kinase domain of the insulin-like growth factor receptor and also was predicted by means of molecular docking.[109] In this binding mode the nitrogen of the quinoline ring is making a hydrogen bond with the backbone NH of methionine 318 located at the hinge region, and the cyano group of Bosutinib

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[107] Boschelli D. H.; Ye, F.; Wang, Y. D.; Dutia, M.; Johnson, S. L.; Wu, B.; Miller, K.; Powell, D. W.; Yaczko, D.; Young, M.; Tischler, M.; Arndt, K.; Discafani, C.; Etienne, C.; Gibbons, J.; Grod, J.; Lucas, J.; Weber, J.M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 3965.

[108] Miller, L.M.; Mayer, S. C.; Berger, D.M.; Boschelli, D.H.; Boschelli, F.; Di, L.; Du, X.; Dutia, M.; Floyd, M.B.; Johnson, M.; Kenny,C.H.; Krishnamurthy, G.; Moy, F.; Petusky, S.; Tkach, D.; Torres, N.; Wu, B.; Xu, W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 62.

[109] Puttini, M.; Coluccia, A.M.L.; Boschelli, F.; Cleris, L.; Marchesi, E.; Donella-Deana, A.; Ahmed, S.; Redaelli, S.; Piazza, R.; Magistroni,V.; Andreoni, F.; Scapozza, L.; Formelli, F.; Gambacorti-Passerini, C. *Cancer Res.* **2006**, *66*, 11314.

is predicted to make a polar interaction with the hydroxyl group of the gatekeeper residue threonine 315. The 2,4-dichloro-5-methoxy-anilin substituent at position 4 of the quinoline ring instead, is interacting with the apolar residues in the hydrophobic pocket whereas the polar substituent at position 7 is predicted to point toward the solvent (see **Figure 1.11**).

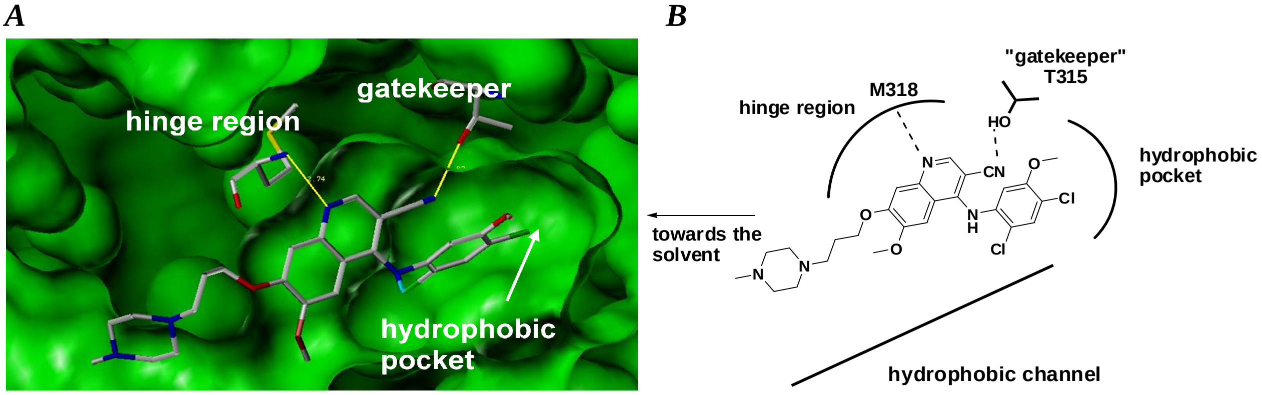

Figure 1.11: ***A)*** *Predicted binding mode of Bosutinib in the active site of the kinase domain of Abl WT.* ***B)*** *Schematic representation of its binding mode.*

The mutation T315I changes the gatekeeper from a polar residue to an apolar one. Therefore, in the binding mode shown in **Figure 1.11** the interaction of the cyano group with the isoleucine of the mutant would be unfavorable, which is in line with the missing activity of this compound in the mutated form of Abl. In addition the bulkiness of isoleucine compared to threonine leads to a clash with the cyano group of Bosutinib and hinders the entrance of the 2,4-dichloro-5-methoxy-anilin substituent into the hydrophobic pocket.

From these observations it was apparent that in order to get potent inhibitors of Abl T315I this unfavorable interaction needed to be avoided and another strong favorable interaction should be added. The strategy to accomplish this goal was to remove the cyano group at 3 position of the quinoline and strengthen the interaction with the hinge region by adding an amino group at position 8 to establish an additional hydrogen bond with the backbone carbonyl of M318. Furthermore, the substituent at position 4 was modified to optimize the entrance and fitting into the apolar pocket and the interaction with the mutated gatekeeper. From the docking studies of Bosutinib it was also seen that the methoxy group at position 6 of the quinoline moiety was not involved in strong interactions with the protein. Since three electron-donating groups on the aryl moiety of the quinoline could make the molecule oxidizable and thus unstable the methoxy group at position 6 of the quinoline has been removed. Studies on the role of the substituent at position 7 clearly showed that this substituent is needed for good cell activity but does not significantly influence the inhibitory activity on the isolated Abl kinase domain.[110] Thereof, the substituent in position 7 was kept unmodified compared to Bosutinib in the first series of synthesized compounds. The entire strategy outlined above is summarized in **Figure 1.12.**

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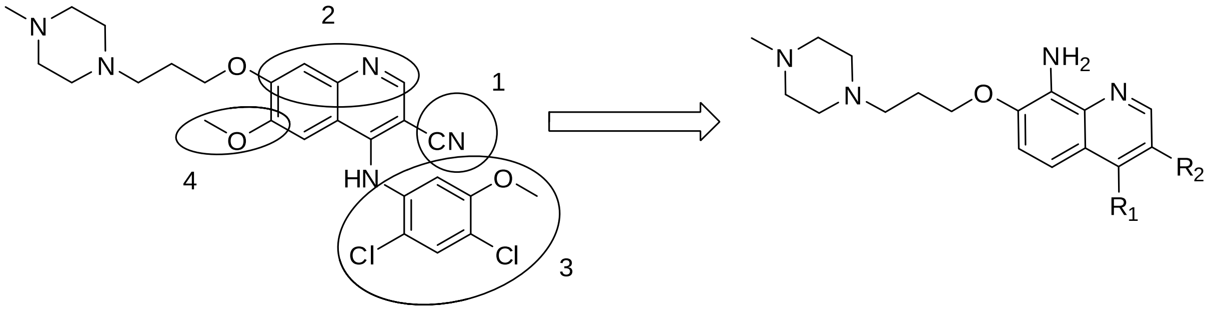
[110] Boschelli, D.H.; Wu, B.; Ye, F.; Wang, Y.; Golas, J.M.; Lucas, J.; Boschelli, F.; *J. Med. Chem.* **2006**, *49*, 7868.



1.Remove the CN group which is predicted to make polar interaction with the gatekeeper of ABL
2. Strenghten the interaction with hinge region in order to compensate the missing CN group
3. Optimize the apolar substituent pretended to interact with the mutated gatekeeper T315I
4. Remove the methoxy group for synthetic and stability reason

Figure 1.12: *Scheme for the conversion of Bosutinib into a new scaffold able to inhibit Bcr-Abl T315I.*

This strategy led to a compound with a novel 8-aminoquinoline scaffold which can be substituted at position 3 and/or 4.

The predicted binding mode of this scaffold is, according to docking studies, similar to the one of Bosutinib with an additional hydrogen bond to the backbone carbonyl of M318 formed with the newly introduced amino group (see **Figure 1.13**).

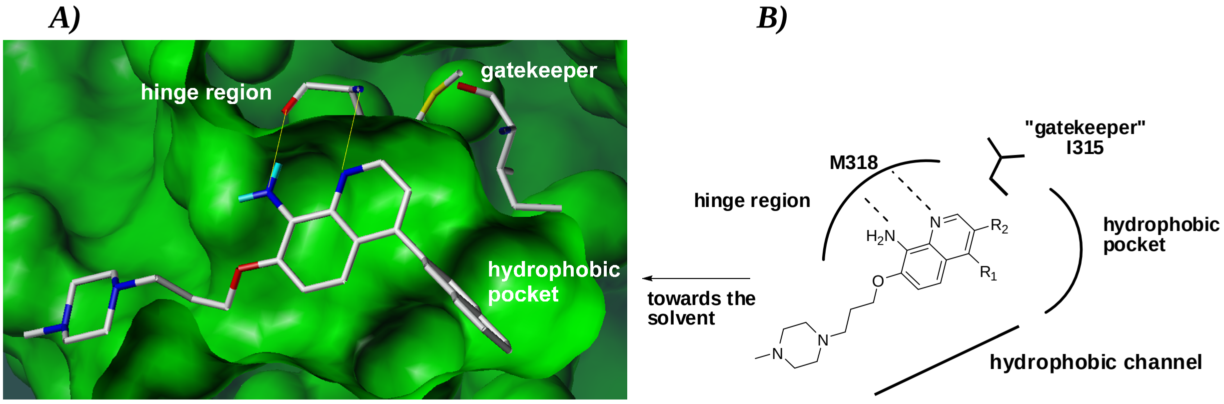


Figure 1.13: ***A)*** *Predicted binding mode of* ***105*** *in the active site of the kinase domain of Abl T315I (1M52 mutated, intermediate conformation).* ***B)*** *Schematic representation of the general binding mode of 8-aminoquinolines.*

1.4.2 Methods

Binding mode predictions and rational drug design was done using FlexX (BioSolveIT) and Sybyl (Trypos). FlexX allowed us to perform molecular docking of the compounds into the active site of Abl WT and Abl T315I. Sybyl was used for visualization and the analysis of the molecular docking results. The docking with FlexX was performed using default parameter if not stated otherwise. The crystal structures used for docking were 1M52 and 2F4J (both at intermediate conformation) in the case of the kinase domain of Abl WT and 2V7A[111] for the Abl T315I kinase domain. Since 2V7A is in an active conformation, and we cannot exclude that the 8-aminoquinolines bind to an intermediate conformation like the inhibitor PD173955 as reported in the crystal structure 1M52,

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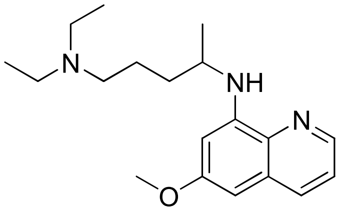
[111] These initials indicates the crystal structure of proteins stored in the protein database at the site www.pdb.org .

we generated a model of Abl T315I kinase domain in the intermediate conformation by changing the gatekeeper residue in 1M52. The model was submitted to a local minimization procedure 4.5 A around the mutation.

1.5 8-AMINOQUINOLINES AS DRUGS

The 8-aminoquinoline scaffold is known to be biologically active and it has been widely employed during the years to build new drugs. 8-aminoquinolines have been the first group of compounds synthesized specifically for antimalarial activity. Initially it has been tried to incorporate a diethylaminoalkylamino moiety (which was proved to enhance antimalarial activity of methylene blue) inside the 6-methoxyquinoline scaffold of quinine, resulting in the introduction of Plasmoquine later called pamaquine.[112] This compound was found to be effective in the prophylaxis against different strains of *Plasmodium vivax*[113].



Pamaquine

Figure1.14: *Structure of pamaquine.*

Unfortunately the side chain of pamaquine and other related N-alkyl-6-methoxy-8-aminoquinoline were found to cause severe, irreversible neurotoxicity.[114] In the forties a big research program was initiated in the USA to improve potency and decrease toxicity of antimalarial drugs. These effort resulted in the discovery of other four drugs, pentaquine,[115] isopentaquine, quinocide[116] and primaquine, the latter being the most promising[117] although all of these are shown to be neurotoxic in monkeys.[118]

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[112] Mühlens, P. *Naturwissenschaften* **1926**, *14*, 1162.

[113] (a) James, S. P. *Lancet*, **1931**, 2, 341. (b) Feldman, H.R.; Packer, H.; Murphy, F.D.; Watson, R.B. *Fed. Proc.* **1946**, 5, 244. (c) Jones, R.Jr.; Craige, B.Jr.; Alving, A.S.; Whorton, C.M.; Pullman, T.N.; Eichelberger, L. *J. Clin Invest.* **1948**, *27*, 6.

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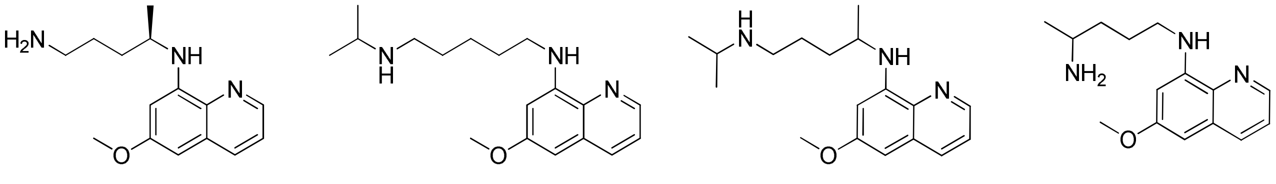
[115] Loeb, M.D. *J Am Med Assoc.* **1946**, *132*, 321.

[116] (a) Braude, M. B.; Stavrovskaya, V. *Z. Obshch. Khim.*, **1956**, *26*, 378. (b) Braude, M. B. ; Stavrovskaya, V. *Med. Prom. S.S.S.R.* **1957**, *7*, 19. (c) for a review see: Lisenko, A.Y. *Bull. World Healt Org.* **1960**, *22*, 641.

[117] (a) Edgcomb, J.H. et al. *J. Nat. Mal. Soc.***1950**, *9*, 285**.** **(**b) Alving, A.; Arnold, J.; Robinson, D.H.; *J. Am. Med. Ass.***1952**, *149*, 1558. (c) Cooper, W.C. *Am. J. Trop. Med. Hyg.***1953**, *2*, 949.

[118] (a) Moe, G.K.; Peralta, B.; Seevers, M.H.; *J. Pharm. Exp. Ther.*, **1949**, *95*, 407. (b) Schmidt, I.G.; Schmidt, L.H. *J. Neuropath. Exper. Neur.* **1951**, *10*, 231.



Primaquine **Pentaquine** **Isopentaquine** **Quinocide**

Figure 1.15: *Structure of primaquine, pentaquine, isopentaquine and quinocide.*

Primaquine is the drug of choice for the radical cure of relapsing malaria[119] caused by *Plasmodium vivax* and *Plasmodium ovale* and is also being used as a prophylactic against all major forms of human malaria[120]. In addition to its activity against the parasitic infections, primaquine in combination with clindamycin has been successfully used for the treatment as well as prophylaxis of *Pneumocystis carinii pneumonia* in AIDS patients.[121] This drug has also shown significant activity against other diseases-causing parasites such as *Trypanosoma*[122] and *Leishmania.*[123] There are also reports on the use of primaquine and other 8-aminoquinolines for the treatment of Trypanosoma cruzi infection in humans[122,124]. A set of 200 different 8-aminoquinolines has also been investigated for their effect radical cure of infections with sporozoites of Plasmodium cynomolgi.[125] 8-Aminoquinolines containing a stereogenic center, are usually administered as a racemic mixture. More recently it has been reported that in most of the cases one enantiomer is more potent and less toxic than the other, therefore a resolution of the racemate can be useful.[126]

Over the years, several chemical modification of primaquine structure have been made in order to improve the potency of primaquine against malaria and *Leishmania* and decrease the toxicity. Methyl group at position 4 and phenoxy group at position 5[127] or alkoxy groups[128] have been introduced obtaining inhibitors with much superior tissue and blood schizonticidal activity. However, toxicity studies have shown that these analogs also have a greater potential of producing methemoglobin[129] an hemoglobin form on which iron is oxidized to Fe^{3+} and is no more able to carry oxygen. The introduction of a methoxyl group at position 2 of the quinoline ring reduces the toxicity of some of these compounds, especially formation of methemoglobin, without losing

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[119] Panisko, D.M.; Keystone, J.S. *Drugs*, **1990**, *39*, 160. (b) Keystone, J.S. *Drugs*, **1990**, *39*, 337.

[120] Baird, J.K. Purnomo, H.B.; Bangs, M.J.; Andersen, E.M.; Jones, T.R.; Masbar, S.; Harjosuwarno, S.; Subianto, B.; Arbani, P.R. *Am. J. Trop. Med. Hyg.* **1993**, *49*, 707.

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[122] McCabe, R.E.; *J. Parasit.* **1988**, *74*, 748.

[123] Berman, D.J.; Lee, L.S. *Am. J. Trop. Med. Hyg.* **1983**, *32*, 753.

[124] Kinnamon, K.E. ; Poon,B.T.; Hanson, W.L.; Waits, V.B. *Ann. Trop. Med. Par.* **1997**, *91*, 147.

[125] Schmidt, L.H. *Antimic. Ag. Chemoter.***1983**, *24*, 615.

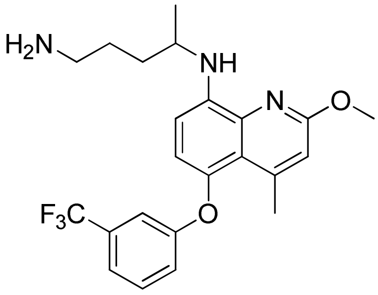
[126] Mc.Chesney, J.; Nanayakkara, D.; Martlett, M.; Ager, A.L. *US Patent* US6376511, **2001**.

[127] (a) LaMontagne, M.P. *J. Med. Chem.* **1982**, *25*, 1094. (b) Nodiff, E.A.; *J. Med. Chem.* **1982**, *25*, 1097.

[128] Chen, E.H. *J. Med. Chem.* **1987**, *30*, 1193.

[129] Anders, J.*et al. Fundam. Appl. Toxicol.* **1988**, *10*, 270.

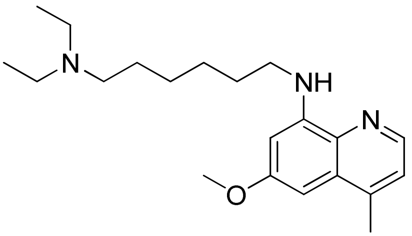
activity.[130] From these results an 8-aminoquinoline firstly called WR-238605 and later Tafenoquine emerged as a potential replacement for primaquine for the treatment of relapsing malaria.[131]



Tafenoquine

Figure 1.16: *Structure of Tafenoquine.*

The 8-aminoquinoline sitamaquine (WR-6026) 4-methylprimaquine analog was selected as a good candidate for the treatment of leishmaniasis.[132] A large number of 5-phenoxy or 5-alkoxy-4-sitamaquine analogs have been synthesized in order to decrease the toxicity. Some of these compounds have shown higher antileishmanial activity than WR-6026 in an *in vitro* assay.[123]



Sitamaquine

Figure 1.17: *Structure of Sitamaquine.*

Primaquine has also been found to have antipneumocystis activity in combination with clindamycin.[121d,133] Several other 8-aminoquinolines were evaluated for antipneumocystic activity[134] and some of them, even when used alone, were found to be superior to the primaquine/clindamycin combination.

Primaquine, pamaquine and pentaquine were also reported to be active against coccidiosis,[135] a devastating disease which causes severe economic losses in the poultry industry.

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[130] LaMontagne, M.P. *J. Med. Chem.* **1989**, *32*, 1728.

[131] (a) Brueckner, R.P.; Fleckenstein, L. *Pharm. Res.* **1991**, *8*, 1505. (b) Shanks, G.D.; Oloo, A.J.; Aleman, G.M.; Ohrt, C.; Klotz, F.W.; Braitman, D.;Horton, J.; Brueckner, R. *Clin Infect Dis* **2001**, *33*, 1968. (c) Crockett, M.; Kain, K.C. *Exp. Opin. Invest. Drugs* **2007**, *16*, 705. (d) Lell, B.; Faucher, J.F.; Missinou, M.A.; Borrmann, S.; Dangelmaier, O.; Horton, J.; Kremsner, P.G. *Lancet* **2000**, *355*, 2041.

[132] (a) Kinnamon, K.E.; Steck, E.A.; Loizeaux, P.S.; Hanson, W.L.; Chapman, Jr., W.L.; Waits, V.B. *Am. J. Trop. Med. Hyg.*, **1978**, *27*, 751. (b) Shipley, L.A. *Xenobiotica*, **1990**, *20*, 31.(c) Yeates, C. *Curr. Opin. Investig. Drugs* **2002**, *10*, 1446. (c) Sherwood, J.A.; Gachihi, G.S.; Muigai, R.K.; Skillman, D.R.; Mugo, M.; Rashid, J.R.; Wasunna, K.M.; Were, J.B.; Kasili, S.K.; Mbugua, J.M. *et al. Clin. Infect. Dis.* **1994**, *19*,1034.

[133] Queener, S.F. *et al. Antimicrob. Ag. Chemotherap.* **1988**, *32*, 807.

[134] (a) Bartlett, M.S. *Antimicrob. Ag. Chemotherap.* **1991**, *34*: 277. (b) Queener. S.F. *Antimicrob. Ag. Chemotherap.* **1993**, *37*, 2166.

[135] (a) Matsuno, T.; Hariguchi, F.; Okamoto, T.J. *Vet. Med. Sci.* **1991**, *53*, 13. (b) Arrner, R.E.; Barlow, J.S.; Dutton, C.J.; Greenway, D.H.J.; Greenwood, S.D.W.; Lad, N.; Tommasini, I. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2585. (c)

This brief report on the therapeutic use of 8-aminoquinolines, highlights the fact that all the 8-aminoquinolines employed as drug are N-alkylated, leaving therefore “free” the field for our 3,4,7-trisubstituted-8-NH_2-quinoline completely new scaffold.

1.6 CHEMISTRY OF QUINOLINES

In this chapter we describe the reactivity of quinolines and the main synthetic routes to obtain them, in order to offer a general overview on the different possibilities aimed to the synthesis and functionalization of this family of molecules.

In 1834 Runge described the isolation from coal tar of crude quinoline,[136] presumably also containing isoquinoline and the alkyl derivatives of both bases. Later, Gerhardt isolated quinoline from the alkaloids cinchonine and quinine by vigorous distillation with alkali.[137]

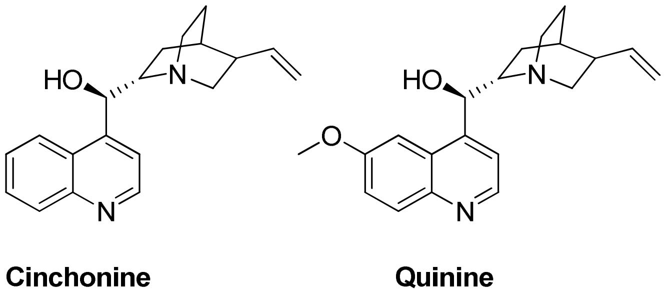

Figure 1.18: *Natural alkaloids cinchonine and quinine.*

Quinolines has also been obtained from crude petroleum, shale oil, and tobacco, by destructive distillation and by direct synthesis. The chemistry of quinolines have been extensively reviewed.[138]

Quinolines are usually synthesized from the readily available aromatic amines by completing and closing the heterocyclic ring. The problem presented by these syntheses is the ring closure between nitrogen and the ortho position of the benzene ring, further complicated by the requirement of the correct number of carbon atoms. As a consequence, only four fundamental synthetic processes **a**, **b**, **c**, and **d**, are possible; the other processes are mere variations of the former ones (see **Figure 1.19**).

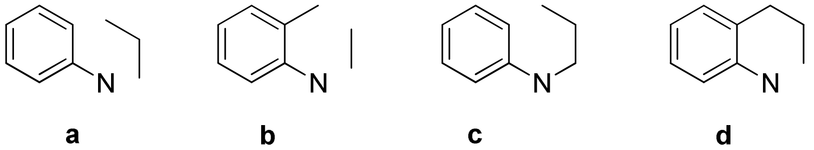

Figure 1.19: *Possible ways of quinoline ring closure.*

The Skraup reaction,[139] the Doebner-Von Miller reaction,[140] and their numerous variations: the Combes,[141] the Conrad-Limpach,[142] and the Knorr[143] reactions, use a three-carbon intermediate, as

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Armer, R.E.; Barlow, J.S.; Dutton, C.J.; Greenway, D.H.J.; Greenwood, S.D.W.; Lad, N.; Thompson, A.P.; Thong, K.-W.; Tommasini I. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1487. (d) Armer, R.E.; Barlow, J.S.; Chopra, N.; Dutton, C.J.; Greenway, D.H.J.; Greenwood, S.D.W.; Lad, N.; Shaw, J.;Thompson, A.P.; Thong, K.-W.; Tommasini I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2425.

[136] Runge, F.F. *Ann. Phys. Chem.* **1834**, *31*, 65.

[137] (a) Gerhardt, C. *Ann. Chim. Phys.***1842**, 7, 251. (b) Gerhardt, C. *Annalen* **1842**, *44*, 279.

[138] (a) Manske, R. H. F. *Chem. Rev.* **1942**, *30*, 113. (b) Bergstrom, F. W. *Chem. Rev.* **1944**, *35*, 77. (c) Elderfield, R. C. *Heterocyclic Compounds*, **1952**, Wiley, New York, Chapman and Hall, London, vol. 4, chapter 1. (d) Campbell, N. *Rodds Chemistry of Carbon Compounds*, ed. S. Coffey, Elsevier, Amsterdam, 2nd edition, **1976**, *4F*, 231.

[139] (a) Skraup, Z. H. *Ber.* **1880**, *13*, 2086. (b) Manske, R. H. F.; Kulka, M. *Organic Reactions* **1953**, *7*, 59.

[140] Doebner, O.; von Miller, W. *Ber.* **1881**, *14*, 2812.

illustrated by scheme **a** in **Figure 1.19.** The Friedlander[144] and Pfitzinger[145] syntheses are illustrated by scheme **b,** where a two carbon unit is used to complete the heterocyclic ring, by a final ring closure between carbon and carbon. Cyclization of cinnamanilides[146] and of derivatives of *o*-aminocinnamic acid[147] are illustrated by schemes **c** and **d** respectively.

In the Skraup synthesis, the aniline molecule with a vacant *ortho* position is heated with glycerol, sulphuric acid and an oxidizing agent, which may conveniently be the nitro compound corresponding to the aromatic amine. Other oxidizing agents, such as As_2O_5, H_3AsO_4, I_2, ferric salts, or *m*-nitrobenzenesulphonic acid can replace the aromatic nitro compound. The use of inorganic oxidizing agents reduces tar formation and gives cleaner products.

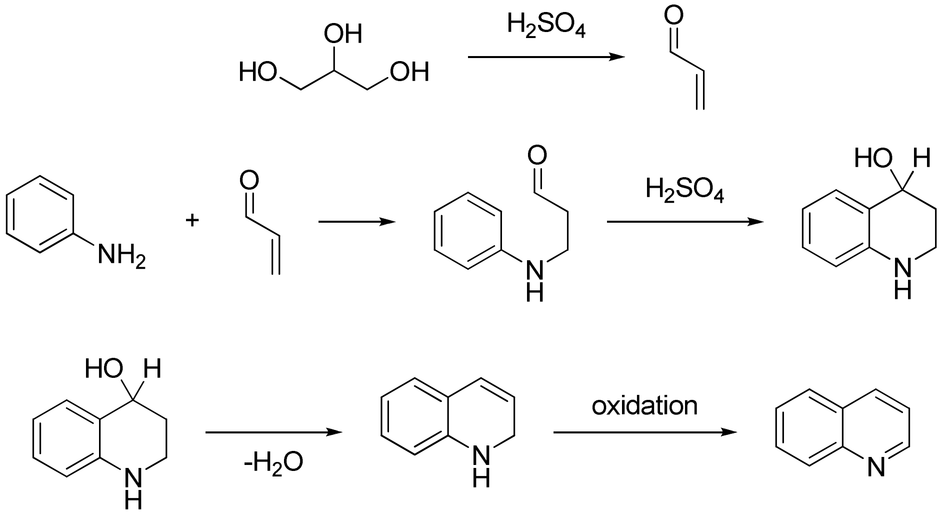

Figure 1.20: *Skraup synthesis of quinoline.*

The first step is the *in situ* dehydration of glycerol to acrolein, which then reacts with the aniline by Michael addition. The formed adduct closes intramolecularly by electrophilic attack of the carbonyl carbon to the ring; the newly formed ring is first dehydrated, and finally oxidized to quinoline.

In the Doebner von Miller synthesis, which is closely related to the Skraup synthesis, an aromatic amine is heated together with an aldehyde in the presence of chloridic acid.

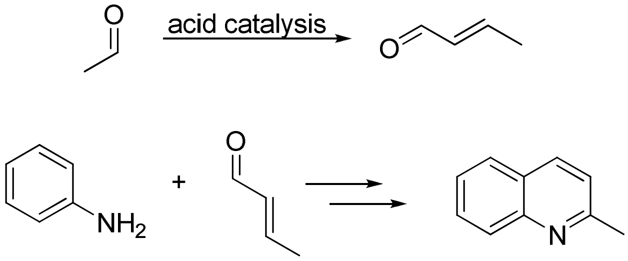

Figure 1.21: *Doebner-von Miller synthesis of quinolines.*

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[141] Combes, A. *Bull. Soc. Chim. France* **1888**, *49*, 89.
[142] Conrad, M.; Limpach, L.; *Ber.* **1888**, *21*, 523.
[143] Knorr, L. *Annalen* **1886**, *236*, 69.
[144] Friedlander, P. *Ber.* **1882**, *15*, 2572.
[145] Pfitzinger, W. *J. Prakt. Chem.* **1886,** *33*, 100.
[146] (a) Conley, R. T.; Knopka, W. N. *J. Org. Chem.* **1964**, *29*, 496. (b) Johnston, K. M.; Luker, R. M.; Williams, G. H. *J. Chem Soc. Perkin Trans. 1* **1972,** 1648.
[147] Chiozza, L.; *Annalen.* **1852**, *83*, 117.

The originally considered mechanism was supposed to involve the formation of an α,β–unsaturated carbonyl intermediate by an acid catalyzed aldol condensation and elimination of water. A Michael condensation with the aromatic amine followed by ring closure and oxidation, similarly to the Skraup reaction, will then complete the synthesis.

In the Combes reaction aniline and 1,3-diketones are heated together with sulphuric acid, to yield 2,4-disubstituted quinolines.

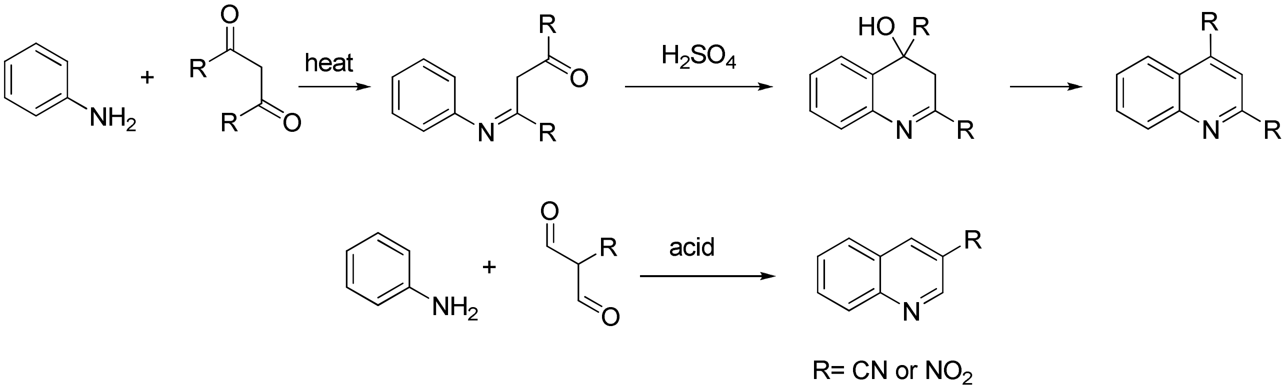

Figure 1.22: *Combes synthesis of quinolines.*

In the same way, 2-nitromalonodialdehydes or 2-cyanomalonodialdehydes give the 3-nitro and the 3-cyano quinolines. The mechanism involves firstly the imine formation between aniline and one of the two carbonyl groups, followed by acid catalyzed cyclization. Dehydration gives then the 2,4-disubstituted or the 3-substituted quinoline.

In the Conrad-Limpach and the Knorr synthesis, aromatic amines react with β-keto-esters to give 2 or 4-quinolones, which can be readily converted to chloroquinolines with phosphoryl chloride.[148]

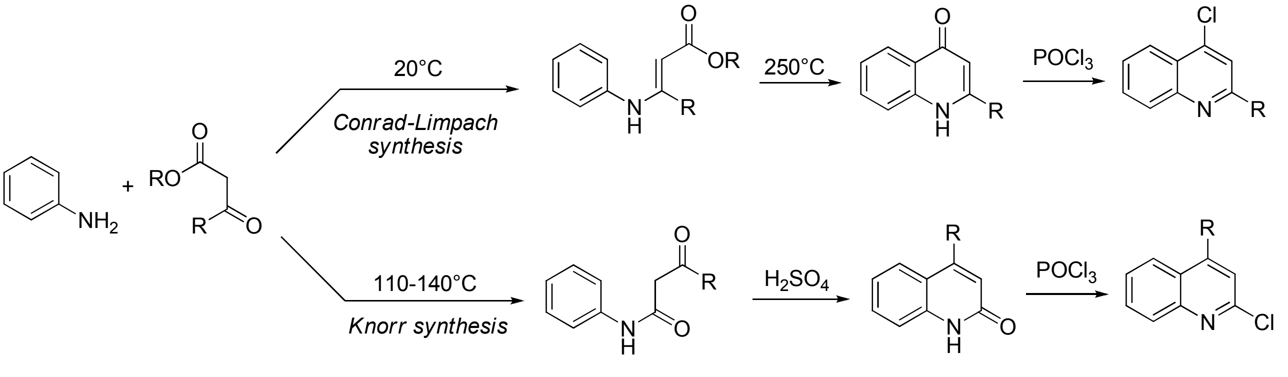

Figure 1.23: *Conrad-Limpach and Knorr synthesis of quinolines.*

The amine condenses at low temperatures with the more reactive keto group to give an ester, which on heating cyclizes to the 4-quinolone (Conrad-Limpach synthesis). At higher temperatures the initial product is the thermodynamically favored anilide, formed by the addition-elimination reaction of the amine with the carboxylic group of the ester. This anilide undergoes ring closure on heating, alone or with sulphuric acid, to give the 2-quinolone (Knorr synthesis).

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[148] Johnston, K. M.; Luker, R. M.; Williams, G. H. *J. Chem. Soc.* **1972**, 1648.

In the Friedlander synthesis quinolines are formed by the condensation of *o*-aminobenzaldehydes or *o*-aminoacetophenones with aldehydes, ketones or other compounds containing an activated hydrogen.

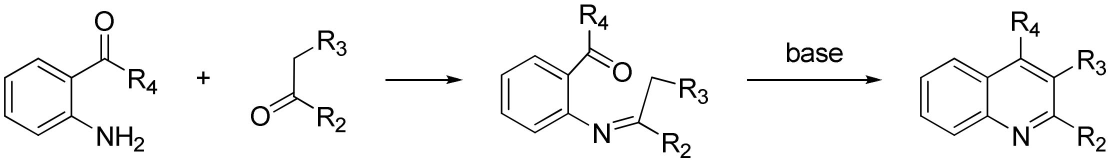

Figure 1.24: *Friedlander synthesis of quinolines.*

The first step is the formation of the Schiff base, followed by ring closure (a Knoevenagel condensation) between carbons 3 and 4 of the quinoline. The method suffers from the disadvantage that amino aldehydes present two functions, that can undergo self condensation. This fact can be avoided by the use of the Pfitzinger modification, where the *o*-aminobenzaldehyde is replaced by the anion of isatinic acid. The keto group β to the carboxylic group is less reactive than the aldehyde, so that autocondensation is avoided.

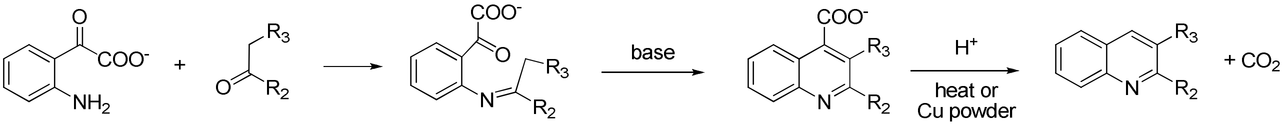

Figure 1.25: *Pfitzinger synthesis of quinolines.*

The quinoline 4-carboxylic acid can be decarboxylated by heating to melting point or by addition of Cu powder.

The *o*-amino derivatives of *cis*-cinnamic acid, obtained by reduction of the corresponding nitro compound, readily undergo ring closure to give 2 quinolones. Also the *trans* isomer gives the same product after rearrangement to the *cis* isomer by treatment with acetic anhydride or sulphuric acid.

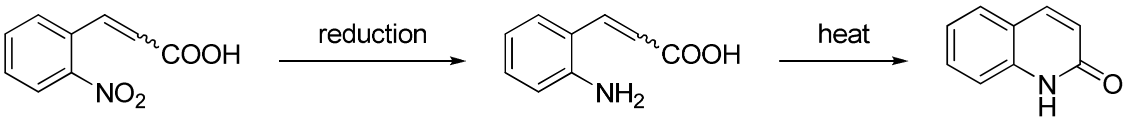

Figure 1.26: *Cyclization of ortho-amino derivatives of cinnamic acid.*

Another possibility for the synthesis of quinolines is offered by the cyclization of cinnamanilides. When these compounds are heated with polyphosphoric acid, 4-aryl-3,4-dihydro-2-quinolones are formed. On the contrary, the treatment with $AlCl_3$ gives 2-quinolones, with elimination of the aryl group.

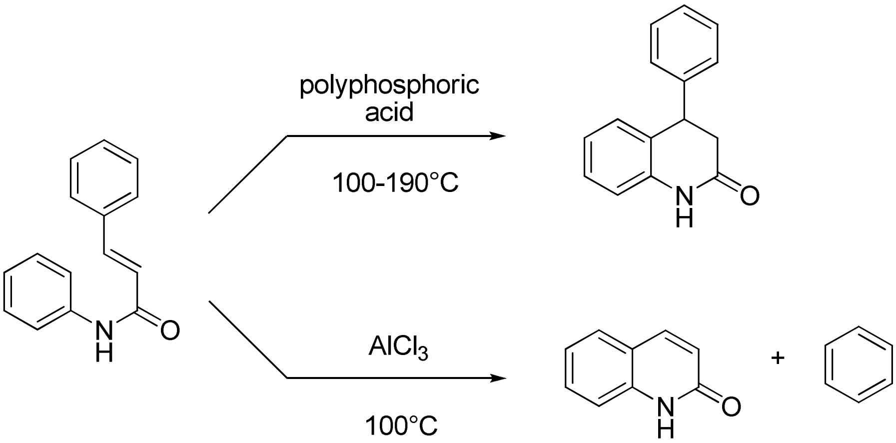

Figure 1.27: *Cyclization of cinnamanilides.*

These methods above described are the most common synthetic ways, leading to substituted quinolines. Some of them have been actually used for the preparation of the series of desired inhibitors.

As an aromatic compound, quinoline can undergo substitution reactions. The reaction outcome, and particularly the orientation of the substitution, can be rationalized in terms of chemistry of the pyridine moiety, and carbocyclic ring. The electron rich nitrogen atom of the free base is the main center of attack by electrophiles; however, when this is protonated or otherwise quaternarized, the main reaction is electrophilic substitution at the carbons of the carbocyclic ring. The number of canonical mesomeric structures describing the Wheland intermediate offers a rational for the substitution orientation. Thus, two mesomeric structures, not involving the destruction of the pyridine ring aromaticity, can be presented for the attack at the positions 5 or 8, but only one structure for the attack at the positions 6 and 7.

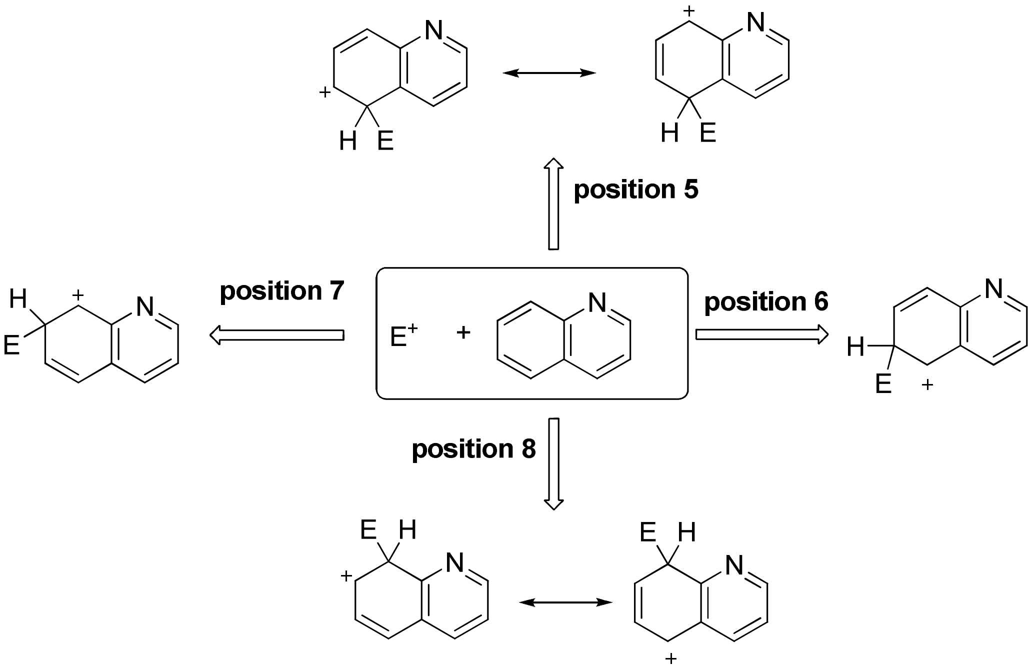

Figure 1.28: *Mesomeric structures of the Wheland intermediate in the electrophilic attack to quinoline.*

These considerations are also consistent with the calculated values of the electron delocalization energies for electrophilic attack on the quinolinium cation.[149]

As a π-deficient heterocycle, quinoline is less reactive towards electrophiles than benzene, particularly in acid solution. Conversely, nucleophilic attack is favored, and can occur particularly at the 2 and 4 electron-deficient positions in the heterocyclic ring. The quinolinium ion is even more reactive. In non-acidic media, nucleophilic substitution at 2 and 4 positions is observed, but also electrophilic substitution at position 3.

As an electrophilic process, oxidation of the pyridine ring requires stronger condition than that of benzene, and in general the heterocyclic ring is more resistant to degradation than carbocyclic ring, The effects of substituents may reverse this tendency. Reduction of the electron-deficient heterocyclic occurs readily, but again substituents can reverse the reactivity order.

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[149] Brown, R. D.; Harcourt, R.D. *J. Chem. Soc.* **1959**, 3451.

CHAPTER 2

RESULTS AND DISCUSSIONS

2.1 SYNTHESIS OF 8-AMINOQUINOLINES

The thorough investigation of the mutated protein requires the synthesis of a considerable number of substituted 8-aminoquinolines. At the beginning of the project, it was important to verify if the assumptions that had been presented for novel scaffold were correct. Therefore the priority was the quick synthesis and test of some specific compounds. The amino group and the group linked through oxygen are kept fixed at 8 and 7 positions respectively. It was required the insertion of a series of specific and different substituents at the 3 or 4 position, or at both positions.

A retrosynthetical analysis suggests that the 4 substituted molecules were easier and faster to synthesize, because there was no need to build the quinoline scaffold from substituted anilines. The steps of the synthesis will be discussed here in detail for every compound. The last step, the reduction to amines, is common for all synthetic projects, concerning either the 3,7 disubstituted molecules and the 3,4,7 trisubstituted ones, and will be discussed at the end of this section.

2.1.1 *Synthesisis of 4,7-disubstituted 8-aminoquinolines*

The target is shown in **Figure 2.1,** with the position numbering.

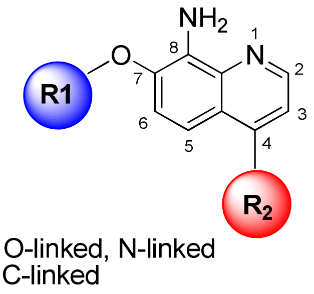

Figure 2.1:. *4,7-disubstituted-8-aminoquinolines.*

The retrosynthetic approach is useful to plan the best strategy of synthesis for the target. The amino group is easily obtainable from the reduction of the nitro group (**Figure 2.2**, step A). The nitro group is further necessary for the introduction of the oxygen-linked R_1 group. A different reaction will introduce the R_2 group. Both $R_1$and R_2 may replace halogen atoms, and the nature of the halogen will dictate the more appropriate reaction tipology (**Figure 2.2**, step B). Finally a nitro group is well known to be easy to introduce in a quinolinic ring (**Figure 2.2**, step C). As 4,7-dichloroquinoline is commercially available and relatively inexpensive, there is no need for the synthesis of the quinoline scaffold.

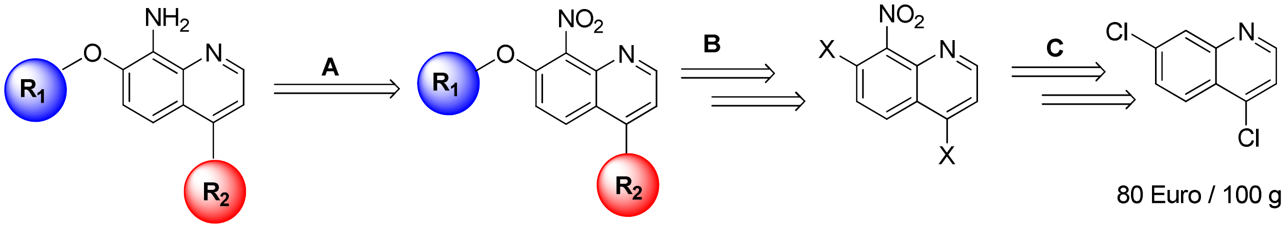

Figure 2.2. *Retrosynthetic approach to 4,7-disubstituted-8-aminoquinolines.*

From this retrosynthetic analysis, it is possible to plan the strategy for the syntheses of 4,7-disubstituted 8-aminoquinolines, starting from 4,7-dichloroquinoline. Different kinds of substituent were needed (C-linked, O-linked and N-linked) at position 4, and therefore different reaction typologies had to be considered, depending on the nature of the linking atom. When the linker is a carbon atom (both aromatic or aliphatic), the Suzuki or the Sonogashira cross coupling reactions are mandatory. As the most appropriate substituent for these reactions is iodine, the necessity of changing the halogen at 4 position from chlorine to iodine is here highlighted. The substitution must occur before the introduction of the nitro group, thus assuring that it will occur on the only ring which is activated for the nuclephilic substitution. On the contrary, when the linker is oxygen or nitrogen, the best synthetic choice is the nucleophilic aromatic substitution, and for this reaction the already present chlorine atom is the most appropriate. Therefore two different parallel ways of synthesis are needed, one starting from molecules bearing chlorine and the other with molecules bearing iodine at position 4 of quinoline.

For both 4,7-dichloroquinoline and 4-iodo-7-chloroquinoline the first step is the introduction of the nitro group at position 8, a strategy that also allows the subsequent nucleophilic substitution of the chlorine at position 7 with the oxygen linked side chain. At this point the halogen atom at position 4 can be replaced with the desired group. The last step is the reduction of the nitro group to amine.

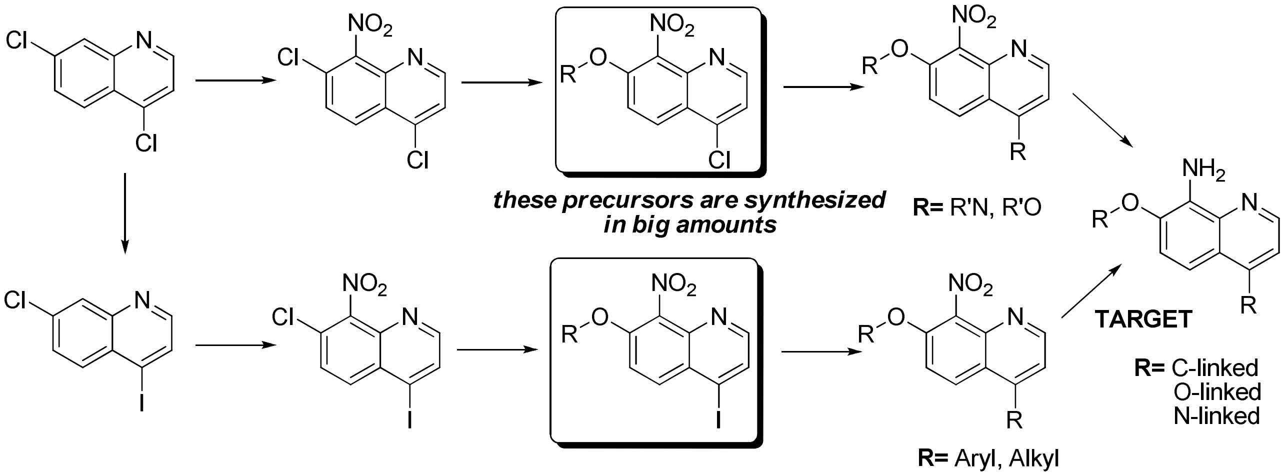

Figure 2.3: *Synthetic pathway to 4,7-disubstituted-8-aminoquinolines.*

The strategies were conceived in order to synthesize large amounts of the precursors, suitable for the different substitutions at the 4 position. This processes occur at the second-last step, so that the procedure allows the synthesis of a number of different compounds without the necessity of restarting anew the whole procedure for each final product. To further minimize the number of the reactions to perform, it had been thought to reduce the nitro group before the introduction of the substituent at position 4. Laboratory trials highlighted that this procedure was not workable. In the case of cross coupling reactions the starting materials was completely recovered: the most probably reason is the inactivation of the palladium catalyst by complexation with the amine moiety. On the other hand, in case of nucleophilic substitution, the free amino group could compete as a nucleophile.

2.1.1.1 Iodination of 4,7-dichloroquinoline

Following the procedure reported by Wolf,[150] which is based on nucleophilic aromatic substitution, the iodine atom can selectively replace the chlorine at position 4 in 4,7-dichloroquinoline.

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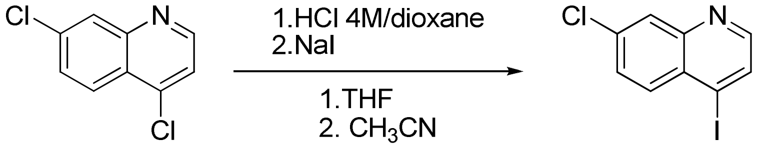
[150] Wolf,C.; Tumambac, G. E.; Villalobos, C. N.; *Synlett* **2003**, *12,* 1801.



1 **2**

Figure 2.4: *Iodination of 4,7-dichloroquinoline.*

The nucleophilic substitution requires a π–electron poor aromatic ring, which is assured by the presence of an electron widthdrawing group (EWG), which is, specifically, the nitrogen atom in pyridine, and an appropriate leaving group (LG), which in our case is the chlorine atom. The substitution is further facilitated when the leaving group is on an activated positions: the position 4 in compound **1**.

The mechanism involves the intermediacy of the Meisenheimer complex, where the nucleophile attacks at the LG position. The intermediate stability, and hence the reaction overall rate, is governed by the number of effectively contributing mesomeric structures that describe the complex. The subsequent departure of the LG regenerates the ring aromaticity, via an overall addition–elimination reaction.[151]

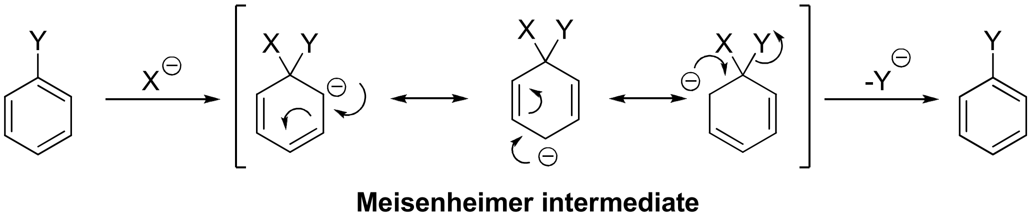


Figure 2.5: *Mechanism of SN$_{Ar}$.*

These considerations explain the regioselectivity of the nucleophilic substitution in 4,7-dichloroquinoline **1**, that is the reason because the nucleophile iodide ion substitutes the chlorine atom at position 4and not at position 7. Three mesomeric structures (see **Figure 2.6**) can be considered for the Meisenheimer intermediate in the substitution at position 7, while four can be drawn in the substitution at the 4 position. Furthermore, two of these latter structures show the negative charge onto the electronegative nitrogen atom. Thus mesomeric and inductive effects direct the observed regioselectivity.

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[151] March, J. *Advanced organic Chemistry, Reactions, mechanisms and structure* 5th Ed. Wiley Ed., Chapter *13*, 850.

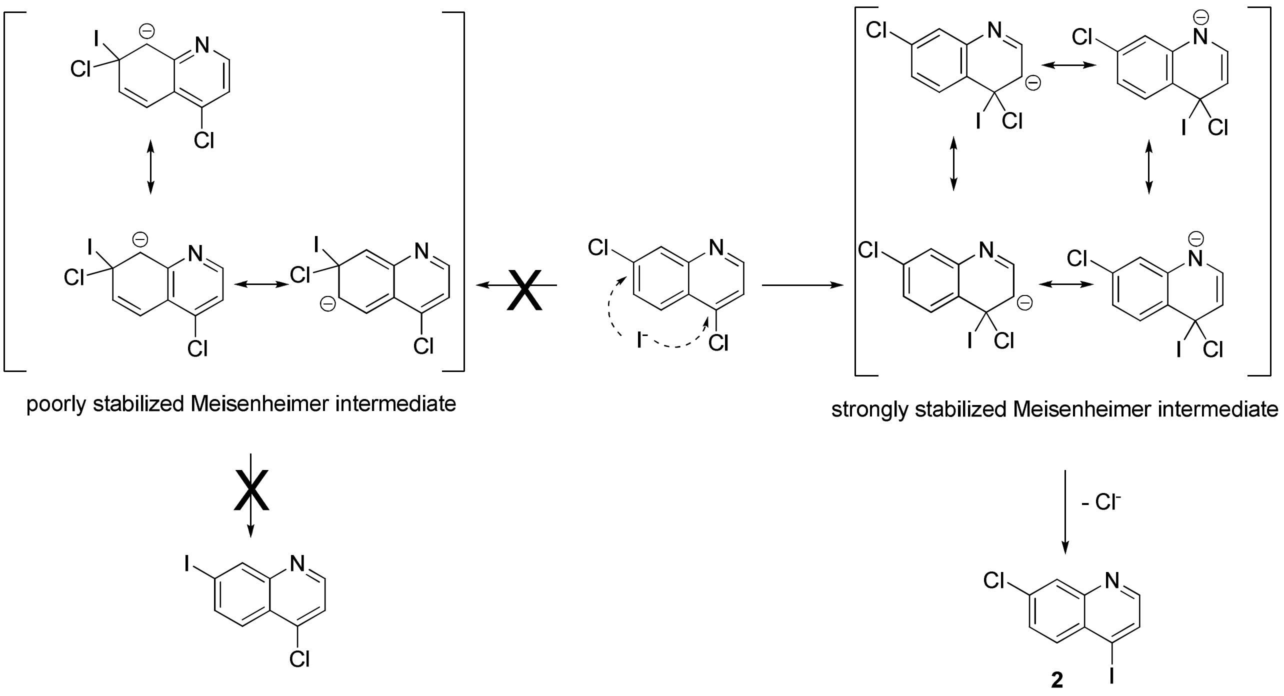

Figure 2.6: *Nucleophilic aromatic substitution on 4,7-dichloroquinoline: mesomeric structures and regioselectivity.*

When the nitrogen atom of pyridine is protonated, the reaction is even more favored: one mesomeric structure of the starting reagent brings a positive charge at the 4 positions, making this position more electrophilic.

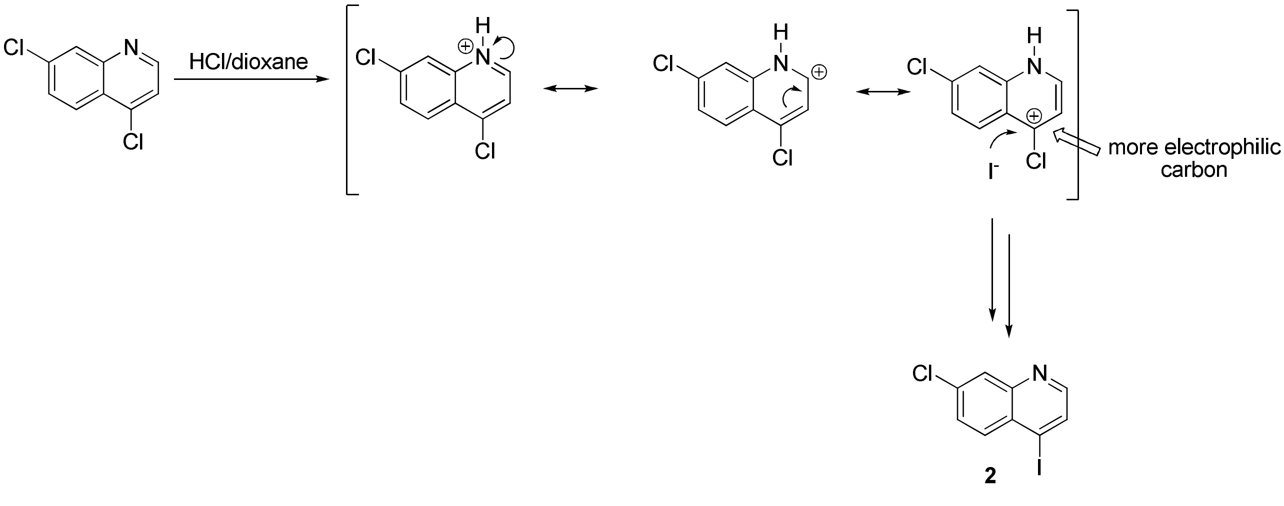

Figure 2.7: *Effect of protonation on the reaction.*

The reaction carried out in acid medium cleanly yielded the desired 4 substituted product exclusively,allowing to proceed in the synthetic sequence without purification.

2.1.1.2 Nitration of 4,7-dichloroquinoline and 7-chloro-4-iodoquinoline

As shown on the synthetic scheme, the first step of the synthesis involves the introduction of a nitro group at position 8 in the quinolininc ring. This nitro group plays a crucial role in the planned synthetic strategy:

a) it will be transformed in the final step of the process into the amino group, predicted to interact with the hinge region of the protein.
b) as a good electron withdrawing group, it will allow the substitution of the chlorine at position 7 with the oxygen linked group.

A literature report states that the nitro group can be selectively introduced at 8 position of 4,7-dichloroquinoline.[152] A similar reactivity can be predicted for 4-iodo-7-chloroquinoline. The reaction is a classic aromatic electrophilic substitution which uses concentrated sulphuric acid as solvent and dehydrating agent for the formation of nitronium ion. The conversion of the starting material was complete for both quinoline reagents, and totally selective at the 8 position.

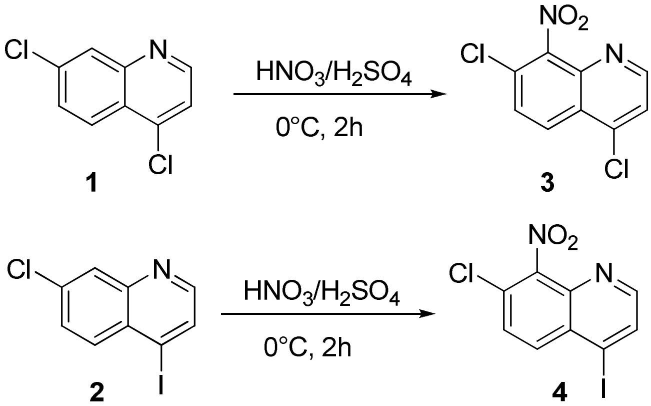

Figure 2.8: *Nitration of 4,7-dichloroquinoline and 7-chloro-4-iodoquinoline.*

The regioselectivity may be rationalized taking into account the relative stabilities of the Wheland intermediates arising from the nitronium ion attack at positions 8 and 6. Two mesomeric structures, which do not alter the aromaticity of the pyridine ring, can be written in the case of attack at position 8, differently a unique structure is possible for the attack at position 6.

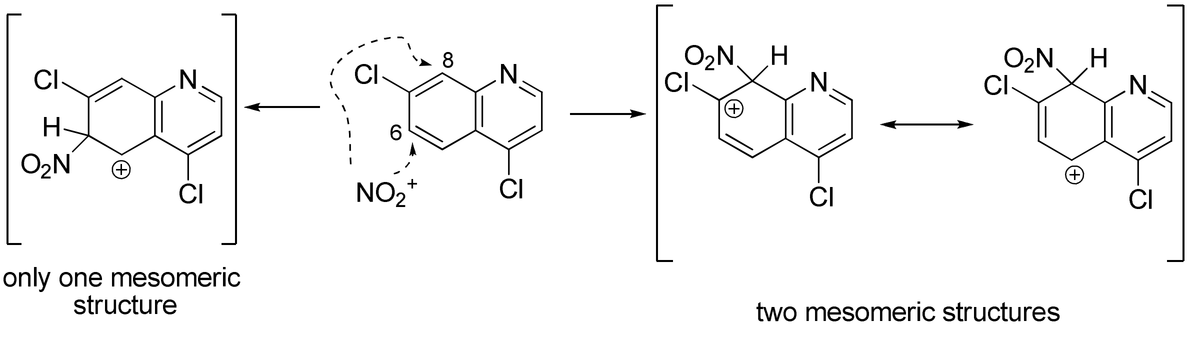

Figure 2.9: *Explanation for regioselectivity on nitration of 4,7-dichloroquinoline.*

2.1.1.3 Aromatic nucleophilic substitution at position 7

After the differentiation of the halogen atom at position 4, an oxygen linked side chain at position 7 must be introduced. If the hypothesized model describing the binding mode is correct, the side chain should point outside the protein pocket and toward the solvent, and therefore should not have important interactions with the active site. The main function of this side chain is to increase the solubility of the molecule, and in the first compounds synthesized the same dialkylpiperazinic

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[152] Surrey, A. R.; Hammer, H. F. *J. Am. Chem. Soc.* **1946**, *68*, 1244.

moiety of Bosutinib has been used. Being this not commercially available, it required to be synthesized.

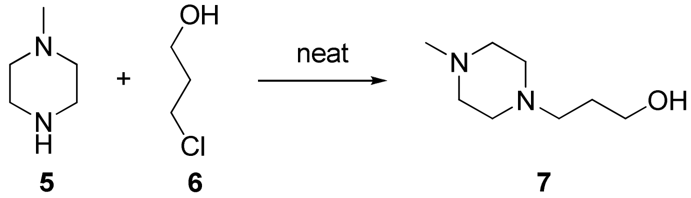

Figure 2.10: S*ynthesis of the piperazinic substituent at position 7.*

We used 2 equivalents of *N*-methylpiperazine, the first one as reactant and the second one as the base, for the salification of the HCl that evolves during the reaction.

The chlorine atom at position 7 has to be replaced by the oxygen linked chain. The only proposable reaction is the aromatic nucleophilic substitution with alcohol 7 or from the corresponding alcoholate. The regioselectivity problem presented by this choice is linked to the fact that two chlorine LG are present, at 4 and 7 positions, both on aromatic rings activated for nucleophilic substitution. Thus both products **8** (from 7 substitution) and **9** (from 4 substitution) can be generated. We had to resort to a series of empirical tests for establishing the best conditions for the desired selectivity.

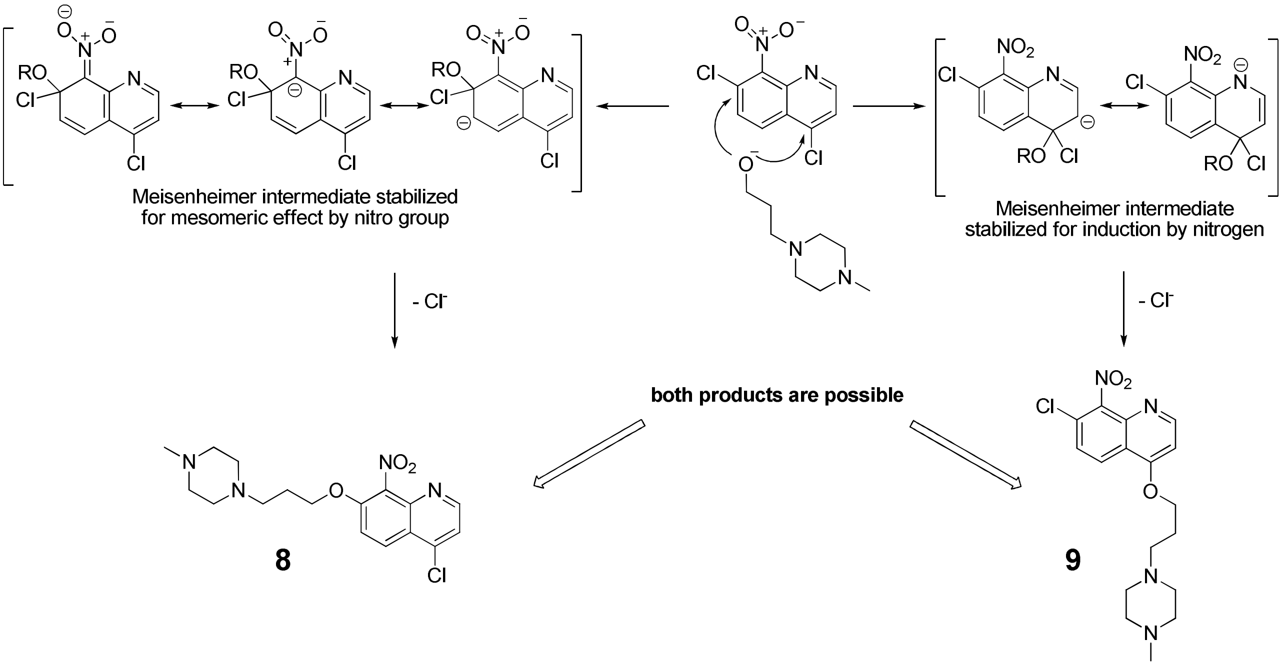

Figure 2.11: *Explanation for regioselectivity toward aromatic nucleophilc substitution on 4,7-dichloro-8-nitroquinoline* ***3***

The reaction kinetics can be improved if the alcoholate is adopted as nucleophile. Potassium *tert*-butoxyde has been used for this purpose, because it is heavily hindered , not nucleophilic and strong enough to deprotonate the alcohol function; also the generated *tert*-butanol can be easly removed from the reaction mixture.

When the reaction was performed in THF, product **8** was favored over product **9** in a 2:1 ratio. On the contrary, when the reaction is run in NMP, the only product obtained is **9**. This rational is offered: the NMP solvent, more polar than the THF solvent, presents stronger interactions with the polar nitro group, thus effectively hindering the position 7.

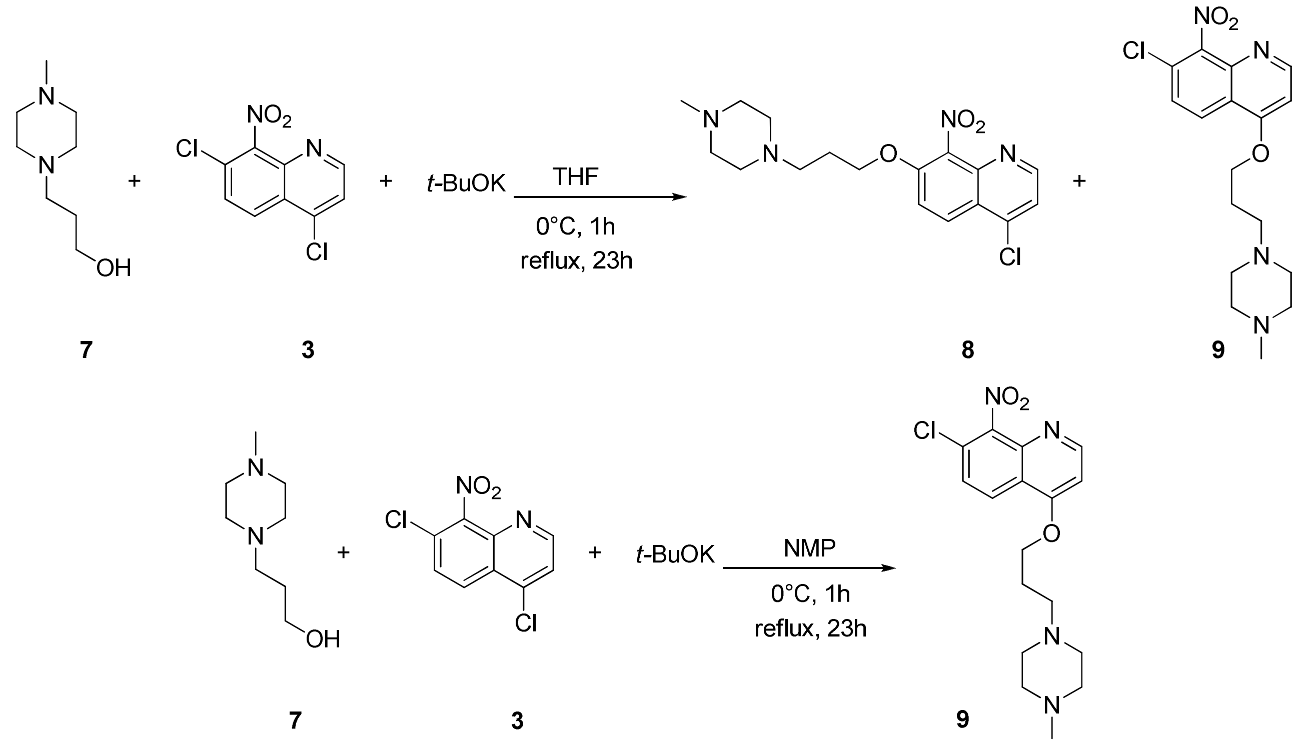

Figure 2.12: *Attachment of side chain on* ***3****.*

The regioselectivity toward the 7 position becomes almost selective when iodine is present at position 4 (molecule **4**). The rational is straighforward: because of the higher electronegativity, the clorine group is notoriously better at stabilizing the Meisenheimer intermediate than the iodine group. In our trial, the ratio between products **10** and **9** is 6:1.

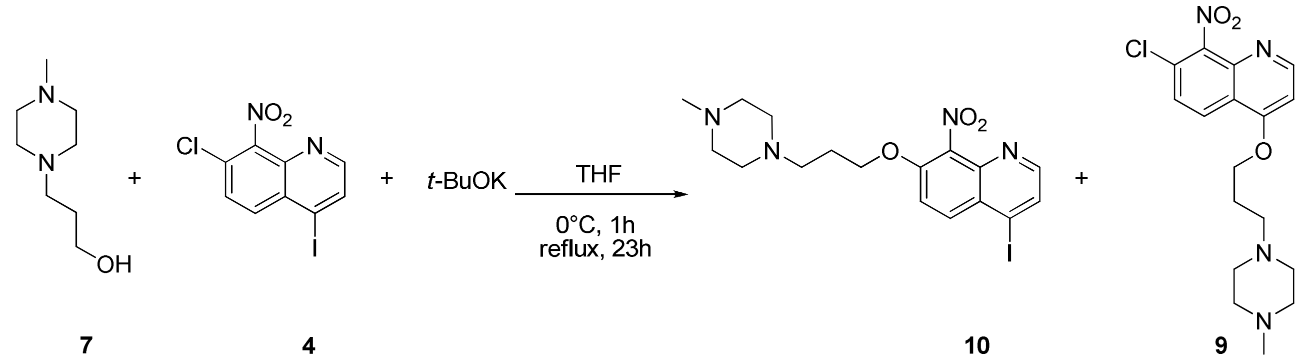

Figure 2.13: *Attachment of side chain on* ***4****.*

In all situations, the desired regioisomers **8** and **10** could be separated from the other regioisomer **9** via crystallization with ethyl acetate.

With the same reaction of nucleophilic substitution, other oxygen linked side chains have been attached on quinoline **3,** in order to have other precursors for the subsequent substitution at 4 position.

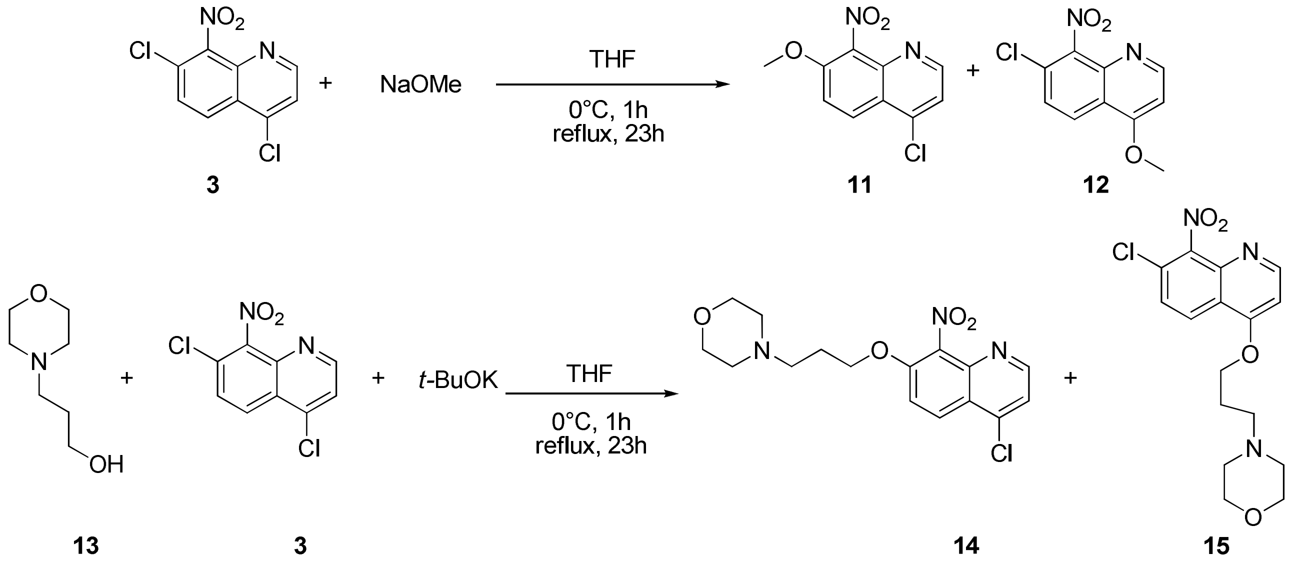

Figure 2.14: *Attachment of other side chains.*

Also in these cases crystallization from ethyl acetate allowed the separation of the regioisomers.[153]

The alcohol **13** has been synthesized similarly to the alcohol **7**, using morpholine rather than *N*-methylpiperazine.

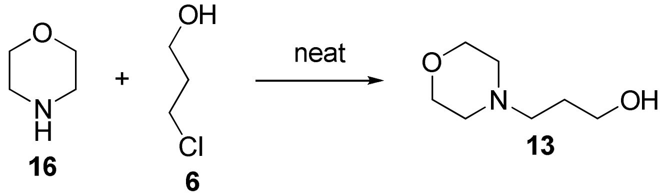

Figure 2.15: *SN$_2$ reaction for the synthesis of alcohol* **13**.

At this phase of the synthetic procedures, four different precursors (molecules **8**, **10, 11** and **14**) have been synthesized and purified in relevant quantities. The molecules **8**, **11** and **14**, bearing a chlorine at position 4, are suitable for the attachment of oxygen linked and nitrogen linked groups, via SN$_{Ar}$, while the molecule **10,** with iodine at position 4, can be subjected to Suzuki or Sonogashira cross coupling reactions.

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[153] Compound **15** has not been isolated and characterized.

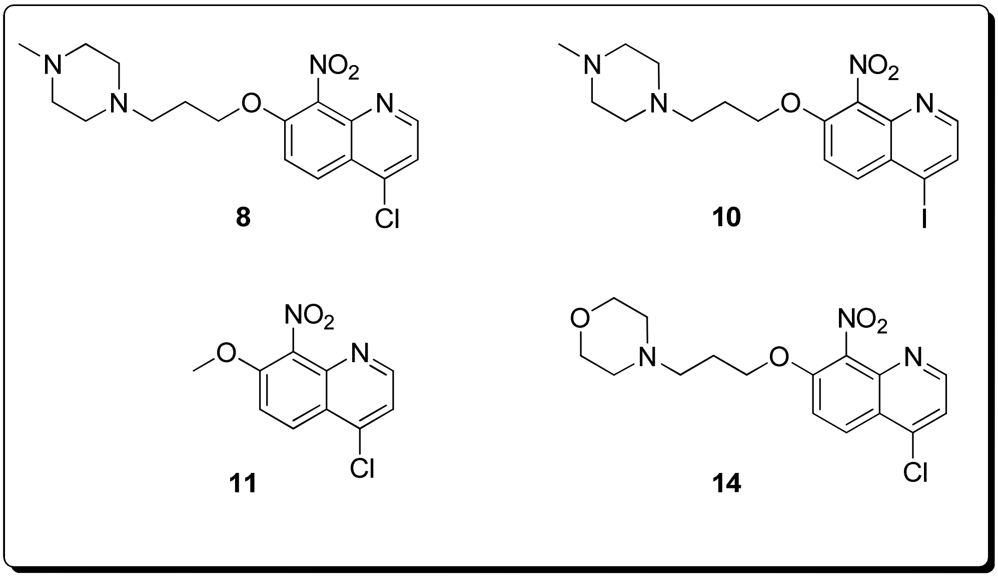

Figure 2.16: *4-substituted precursors synthesized.*

2.1.1.4 Substitution at position 4

2.1.1.4.1 Cross coupling reactions for 4-aryl and 4-alkyl substitution

The cross coupling reaction constitutes a fundamental synthetic methodology, where a carbon-carbon bond is generated from an organic halide and an organometallic compound, with the aid of a metal catalyst :

$$\text{R-M} \quad + \quad \text{R'-X} \xrightarrow{\text{Metal catalyst}} \text{R-R'} \quad + \quad \text{M-X}$$

For example, Kumada and Tamao[154] and Corriu[155] reported independently that the reaction of Grignard organomagnesium reagents with alkenyl or aryl halides could be catalyzed by Ni(II) complexes. Kochi[156] reported the efficiency of Fe(III) catalyst for the cross-coupling of Grignard reagents with 1-halo-1-alkenes and Li_2CuCl_4 catalyst for haloalkanes. The palladium catalyzed reaction of Grignard reagents was firstly reported by Murahashi.[157] The synthetic utility of this procedure was then amply demonstrated by Negishi[158] for the reactions of organic aluminum, zinc,

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[154] (a) Tamao, K.; Sumitani, K.; Kumada, M. *J. Am. Chem. Soc.* **1972**, *94*, 4374. (b) Tamao, K.; Kiso, Y.; Sumitani, K.; Kumada, M. *J. Am. Chem. Soc* **1972**, *94*, 9268. (c) Tamao, K.; Zembayashi, M.; Kiso, Y.; Kumada, M. *J. Organomet. Chem.* **1973**, *55*, C91. (d) Hayashi, T.; Konishi, M.; Fukushima, M.; Mise, T.; Kagotani, M.; Tajika, M.; Kumada, M. J. *Am. Chem. Soc*. **1982**, *104*, 180. (e) Hayashi, T.; Konishi, M.; Kobori, Y.; Kumada, M.; Higuchi, T.; Hirotsu, K. *J. Am. Chem. Soc*. **1984**, *106*, 158. (f) For a review, see: Kumada, M. *Pure Appl. Chem*. **1980**, *52*, 669.

[155] Corriu, R. J. P.; Masse, J. P. *J. Chem. Soc. Chem. Commun.* **1972,** 144**.**

[156] (a) Tamura, M.; Kochi, J. K. *J. Am. Chem. Soc.* **1971**, *93*, 1487**.** (b) Tamura, M.; Kochi, J.K. *Synthesis*, **1971**, 303**.** (c) Neumann, S. M.; Kochi, J. K. *J. Org. Chem.* **1975**, *40*, 599. (d) Kwan, C. L.;Kochi, J. K. *J. Am. Chem. Soc***. 1976**, *98*, 4903**.** (e) For a review,see: Kochi, J. K. *Acc. Chem. Res.* **1974**, *7*, 351.

[157] Yamamura, M.; Moritani, I.; Murahashi, S. *J. Organomet. Chem.***1975**, *91*, C39.

[158] (a) Aluminum: Negishi, E.; Baba, S. *J. Chem. Soc. Chem.Commun.* **1976**, 596**.** (b) Baba, S.; Negishi, E. *J. Am. Chem. Soc.* **1976**, *98*, 6729**.** (c) Zinc: Negishi, E.; King, A.; Okukado, N. *J. Org. Chem.* **1977**, *42*, 1821**.** (d) King, A.*;* Okukado, N.; Negishi, E. *J. Chem. Soc. Chem. Commun.* **1977**, 683. (e)Negishi, E.; King. A.; Okukado, N. *J. Org. Chem.* **1977**, *42*, 1821**.** (f) King, A.; Negishi, E. *J. Org. Chem.* **1978**, *43*, 358. (f) Zirconium: Negishi, E.; Van Horn, D. E. *J. Am. Chem. Soc.***1977**, *99*, 3168**.** (g) Van Horn, D.E.; Negishi, E. *J. Am. Chem. Soc***. 1978,** *100*, 2252. (h) Negishi, E.; Takahashi, T.; Baba, S.;Van Horn, D. E.; Okukado, N. *J. Am. Chem.* Soc**. 1987**, 109,2393. For reviews, see: (i) Negishi, E. *Aspects of Mechanism and Organometallic Chemistry;* Brewster, J.H., Ed.; Plenum Press: New York, **1978**, 285**.** (j) Negishi, E. *Acc. Chem. Res.* **1982**, *15*, 340. (k) Negishi, E**.** *Current Trends in Organic Synthesis* Nozaki, H., Ed. Pergamon: Oxford, **1983**, 269**.**

and zirconium reagents. After these initial discoveries, many other organometallic reagents have proven to be highly useful as nucleophiles for the cross-coupling reaction, e.g. organolithiums by Murahashi,[159] organostannans by Migita[160] and Stille,[161] 1-alkenylcopper(I) by Normant,[162] organosilicon compounds by Hiyama.[163] Organoboron compounds for cross coupling have been introduced by Suzuki and Miyaura.[164] The palladium-catalyzed cross coupling of aryl halides with boronic acids is an exceptionally versatile and highly utilized reaction for the construction of carbon-carbon bonds, and in particular for the preparation of heterobiaryls.[165]

A general catalytic cycle for the cross coupling reactions, shown in **Figure 2.17,** involves oxidative addition to a palladium atom, transmetalation and reductive elimination.

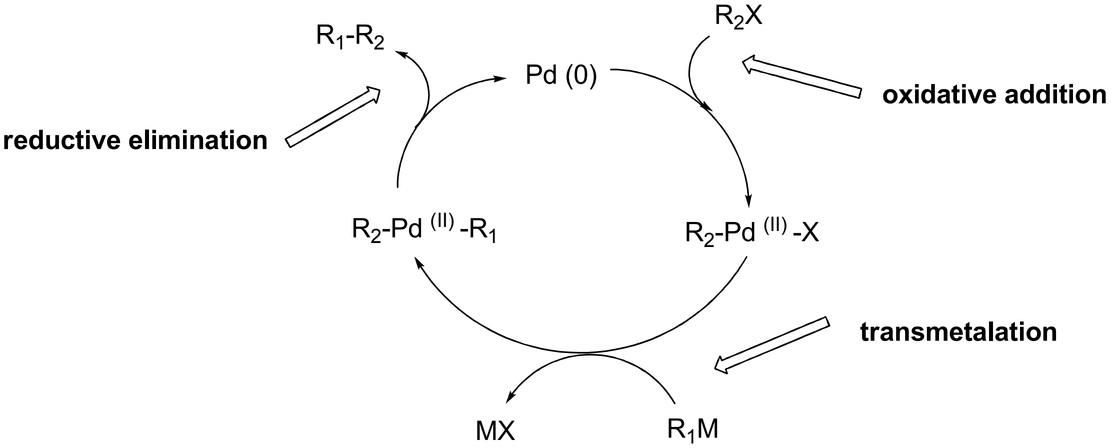

Figure 2.17: *General catalytic cycle for palladium catalyzed cross coupling reactions.*

Oxydative addition of 1-alkenyl, 1-alkynyl, allyl, benzyl, and aryl halides to a palladium (0) compound affords a stable *trans*-σ-palladium(II) complex. This is often the rate-determining step in the catalytic cycle. The relative reactivity decreases in the order of I > OTf > Br >>Cl. The most commonly used catalyst is $Pd(PPh_3)$, but also $PdCl_2(PPh_3)_2$, and $Pd(OAc)_2$ with PPh_3 or other phosphines, are also efficient, since they are stable to air and readily reduced to the active Pd(0) complex with the organometallics or the phosphines used for the cross coupling.[166]

Organoboron compounds are highly electrophilic, but the organic groups on boron are weakly nucleophilic, so that these compounds require some form of activation, in order to be utilized in the

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[159] Murahashi, S.; Yamamura, M.; Yanagisawa, K.; Mita, N.; Kondo,K. *J. Org. Chem:* **1979**, *44*, 2408**.**

[160] (a) Kosugi, M.; Simizu, Y.; Migita, T. *Chem. Lett.* **1977**, 1423**.** (b) Kosugi, M. Hagiwara, I.; Migita, T. *Chem. Lett.* **1983**, 839.

[161] (a) Milstein, D.; Stille, J.K. *J. Am. Chem. Soc.* **1979**, *101,* 4992. (b) Scott, W.J.; Crisp, G.T.; Stille, J.K. *J. Am. Chem.* Soc. **1984**, *106*, 4630. (c) Scott, W.J.; Stille, J.K. ***J.** Am. Chem. Soc.* **1986**, *108*, 3033. (d) Echavarren, A.M.; Stille, J.K. *J. Am. Chem. Soc.* **1987**, *109*, 5478. (e) For a review, see: Stille, B.J. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508.

[162] Alexakis, N. J.A.; Normant, J.F. *Tetrahedron Lett.* **1981**, *22*, 959.

[163] (a) Hatanaka, Y.; Hiyama, T. *J. Org. Chem.* **1988**, *53*, 918. (b) Hatanaka, Y.; Hiyama, T. *J. Org. Chem.* **1989**, *54*, 268. (c) Hatanaka, Y.; Matsui, K.; Hiyama, T. *Tetrahedron Lett.* **1989**, *30*, 2403. (d) Hatanaka, Y.; Hiyama, T. *J. Am. Chem. Soc.* **1990**, *112*, 7793. (e) For a review, see: Hatanaka, Y.; Hiyama, T. *Synlett* **1991**, 845.

[164] (a) Miyaura, N.; Yamada, K.; Suzuki, A. *Tetrahedron Lett.* **1979**, *20*, 3437. (b) Miyaura, N.; Suzuki, A. *Chem. Commun.* **1979**, 866.

[165] (a) Stanfort, S. P. *Tetrahedron* **1998**, *54*, 263. (b) Suzuki, A. *J. Organomet. Chem.* **2002**, *653*, 83. (c) Hassan, J.; Sévignon, M.; Gozzi, C.; Schulz, E.; Lemaire, M. *Chem. Rev. 2002*, *219*, 1359. (d) Miyaura, N. *Topics Curr. Chem.* **2002**, *219*, 5. For reviews see: (d) Suzuki, A. *Pure Appl. Chem.* **1991**, *63*, 419. (e) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95,* 2457. (f) Suzuki, A. *J. Organometallic Chem.* **1999**, *576*, 147.

[166] (a) Mc Crindle, R.; Ferguson, G.; Arsenault, G. J.; Mc Alees, A. J.;Stephanson D. K.; *J. Chem. Res. (S)*, **1984**, 360. (b) Amatore, C.; Jutand, A.; M'Barki, M. A *Organometallics* **1992**, *11,* 3009. (c) Ozawa, F.; Kubo, A.; Hayashi, T. *Chem. Lett.* **1992**, 2177. (d) Amatore, C.; Jutand, A.; Suarez, A. *J. Am. Chem. Soc.* **1993**, *115*, 9531. (e) Amatore, C.; Carrè, E.; Jutand, A.; M'Barki, M. A *Organometallics* **1989**, *8*, 180.

Suzuki-Miyaura cross coupling. It has been recognized that the coordination of a negatively charged base is an efficient method for increasing the nucleophilicity. The activation of the boron atom enhances the polarization of the organic ligand and facilitates transmetallation.

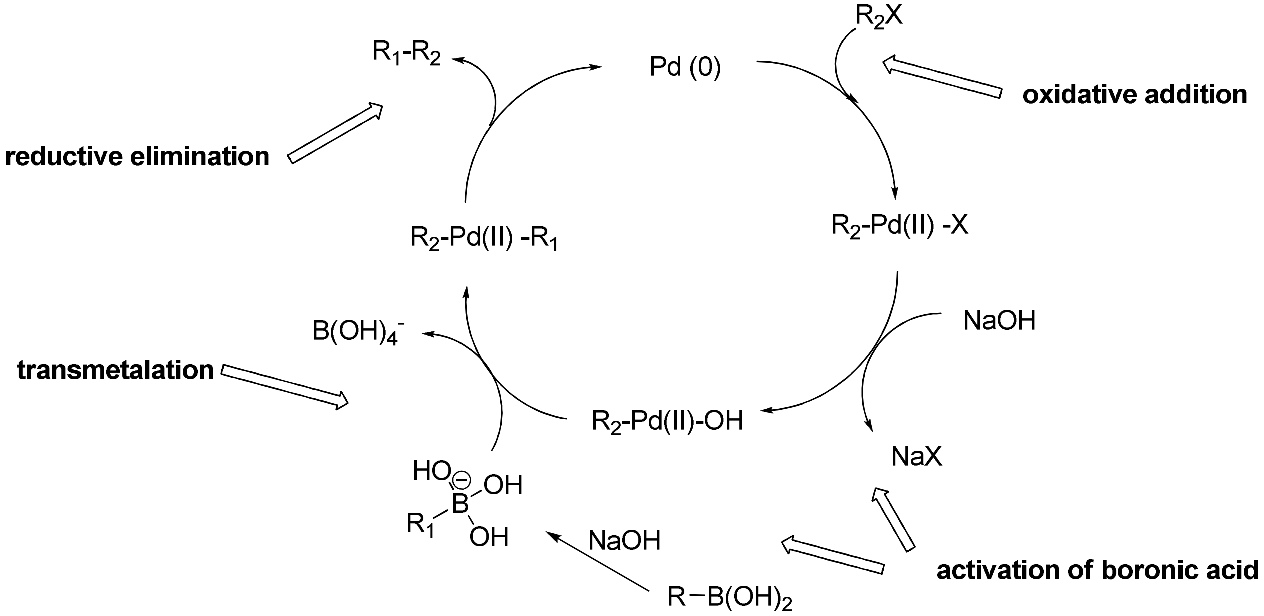
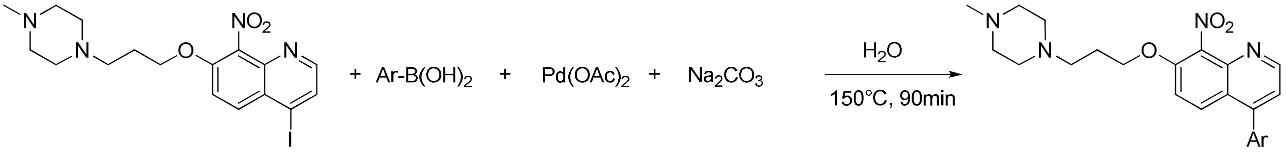


Figure 2.18: *Activation of boronic acids by bases in the catalytic cicle of palladium mediated cross coupling reactions.*

Friesen and Trimble reported a procedure where 4-iodoquinolines undergo Suzuki-Miyaura cross coupling with arylboronics acids catalyzed by phosphine-free palladium acetate in boiling water.[167] We tried the same procedure on precursor **10** with different boronic acids with excellent results. Thus we could prepare a library of 4-aryl-7-oxyalkyl-8-nitroquinolines. The yield for almost all compounds has been greater than 80% and in most cases the reaction raw was very clean, without the necessity of further purification.



10 **17-33**

Figure 2.19: *Suzuki-Miyaura cross coupling for the synthesis of 4-aryl-substituted-8-nitroquinolines.*

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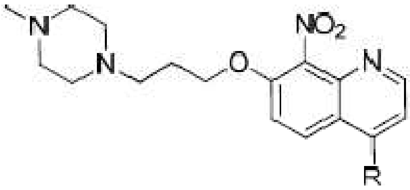
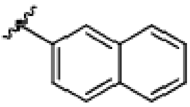
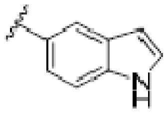
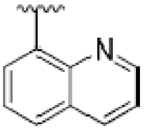
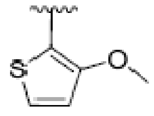
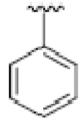
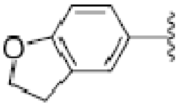
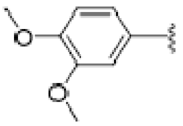
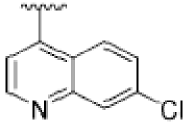
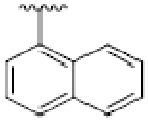
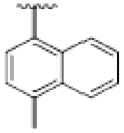
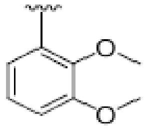
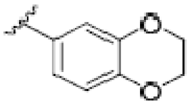
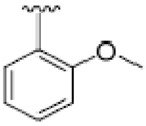
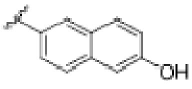
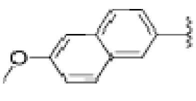
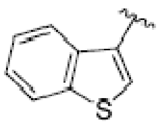
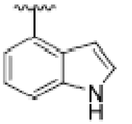
[167] Friesen, R. W.; Trimble, L. A.; *Can. J. Chem.* **2004**, *82*, 206.

Table 2.1: *4-Aryl-8-nitroquinolines synthesized with Suzuki-Miyaura cross coupling.*



N°	R	yield %	N°	R	yield %
17		90,0	**26**		90,0
18		90,0	**27**		65,0
19		86,0	**28**		74,0
20		98,0	**29**		65,0
21		99,0	**30**		88,0
22		59,0	**31**		80,0
23		94,0	**32**		55,0
24		90,0	**33**		90,0
25		90,0			

When the introduction of an alkyl group instead of aryl group was required, we adopted a different procedure. Wallace and Chen reported a protocol where cyclopropyl boronic acid is coupled with various aryl bromides, cytalyzed by palladium acetate in the presence of tricyclohexylphosphine.[168] In a similar way, Hof coupled aryl bromides with *n*-hexyl boronic acid, using the same metal but a

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[168] Wallace, D. J.; Chen, C.; *Tetrahedron Lett.* **2002**, *43*, 6987.

slightly different ligand (dicyclohexylphosphanyl)biphenyl).[169] The two phosphine ligands were not readily available in our laboratory, and therefore the reaction was carried out with dicyclohexyl(2',6'-dimethoxybiphenyl-2-yl)phosphine, very similar to those proposed in literature, with good results.

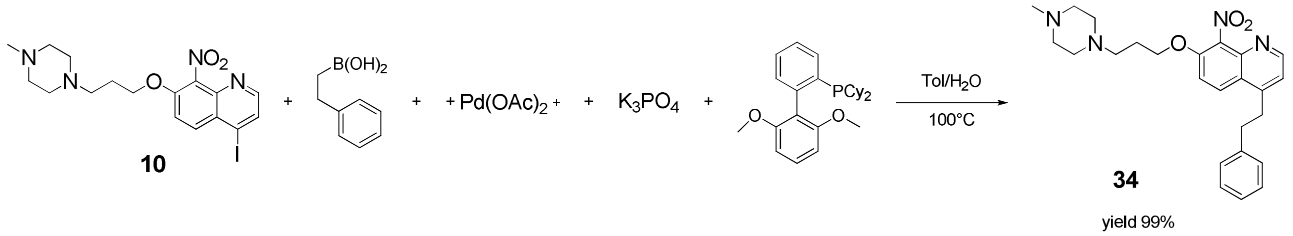

Figure 2.20: *Suzuki miyaura cross coupling.*

The other 3-substituted compound synthesized with this metodology will be reported in *chapter 2.1.2.4.1.*

Another possibility to have 4-alkyl substituted compounds from 4-iodoquinolines is represented by the Sonogashira cross coupling, followed by complete hydrogenation of the acetylenic function. Sonogashira cross-coupling is the palladium-catalyzed coupling reaction between aryl or alkenyl halides or triflates and terminal alkynes, in the presence of a copper(I) cocatalyst.

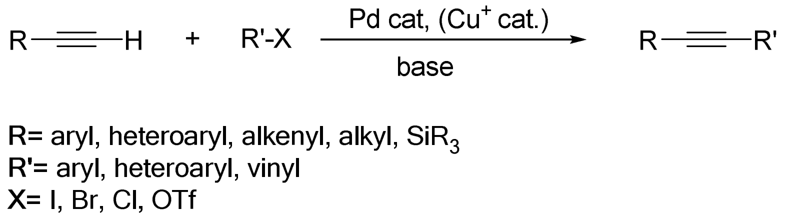

Figure 2.21: *General Sonogashira cross-coupling reaction.*

This reaction has become the most important method for preparing arylalkynes and conjugated enynes.[170] The reaction was first reported by Sonogashira, Tohda and Hagihara.[171] as an improvement of Heck[172] and Cassar[173] reactions. The Heck procedure, based on the palladium-catalyzed arylation or alkenylation of alkenes reported by Mizoroki and Heck[174], performs the coupling employing a phosphane-palladium complex as a catalyst and triethylamine or piperidine as base and solvent. The procedure of Cassar involves the use of a phosphane-palladium catalyst in combination with sodium methoxide as base and DMF as solvent. Both methods generally requirs high temperatures (up to 100 °C). Sonogashira and Hagihara reported that addition of a catalytic

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[169] Hof, F.; Schutz, A.; Fäh, C.; Meyer, S.; Bur, D.; Liu, J.; Goldberg, D. E.; Diedrich, F.; *Angewandte Chem. Int. Ed.* **2006**, *45*, 2138.

[170] (a) Brandsma, L. *Synthesis of Acetylenes, Allenes and Cumulenes: Methods and Techniques* Elsevier: Oxford, **2004**, p 293. (b) Sonogashira, K. In *Metal-Catalyzed Cross-Coupling Reactions* Diederich, F., de Meijera, A., Eds.; Wiley-VCH: Weinheim, **2004**, *Vol. 1*, p 319. (c) Tykwinski, R.R. A*ngew. Chem., Int. Ed.* **2003**, *42*, 1566. (d) Negishi, E.; Anastasia, L. *Chem. Rev.* **2003**, *103*, 1979. (e) Sonogashira, K. In *Handbook of Organopalladium Chemistry for Organic Synthesis*; Negishi, E.;de Meijere, A., Eds.; Wiley-Interscience: New York, **2002**, p 493. (f) Sonogashira, K. *J. Organomet. Chem.* **2002**, 653, 46. (g) Rossi, R.; Carpita, A.; Bellina, F. *Org. Prep. Proced. Int.* **1995**, 27, 127. (h) Sonogashira, K. In *Comprehensive Organic Synthesis*; Trost, B. M.; Fleming, I.; Eds.;Pergamon: Oxford, **1991**; *Vol. 3*, p 521. (i) For a review see: Chinchilla, R.; Najera, C. *Chem. Rev.* **2007**, *107*, 874.

[171] Sonogashira, K.; Tohda, Y.; Hagihara, N. Tetr. Lett. 1975, *16*, 4467.

[172] Diek, H. A.; Heck, F. R. *J. Organomet. Chem.* **1975**, *93*, 259.

[173] Cassar, L. *J. Organomet. Chem.* **1975**, *93*, 253.

[174] (a) Mori, K.; Mizoroki, T.; Ozaki, A. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 1505. (b) Ziegler Jr., C.B.; Heck, F. R. *J. Org. Chem.* **1978**, *43*, 2941.

amount of copper(I) iodide greatly accelerates the reaction, thus enabling alkynylation at room temperature, in accordance with the already known coupling between copper acetylides and phenyl or vinyl halides, the so-called Stephens-Castro reaction.[175]

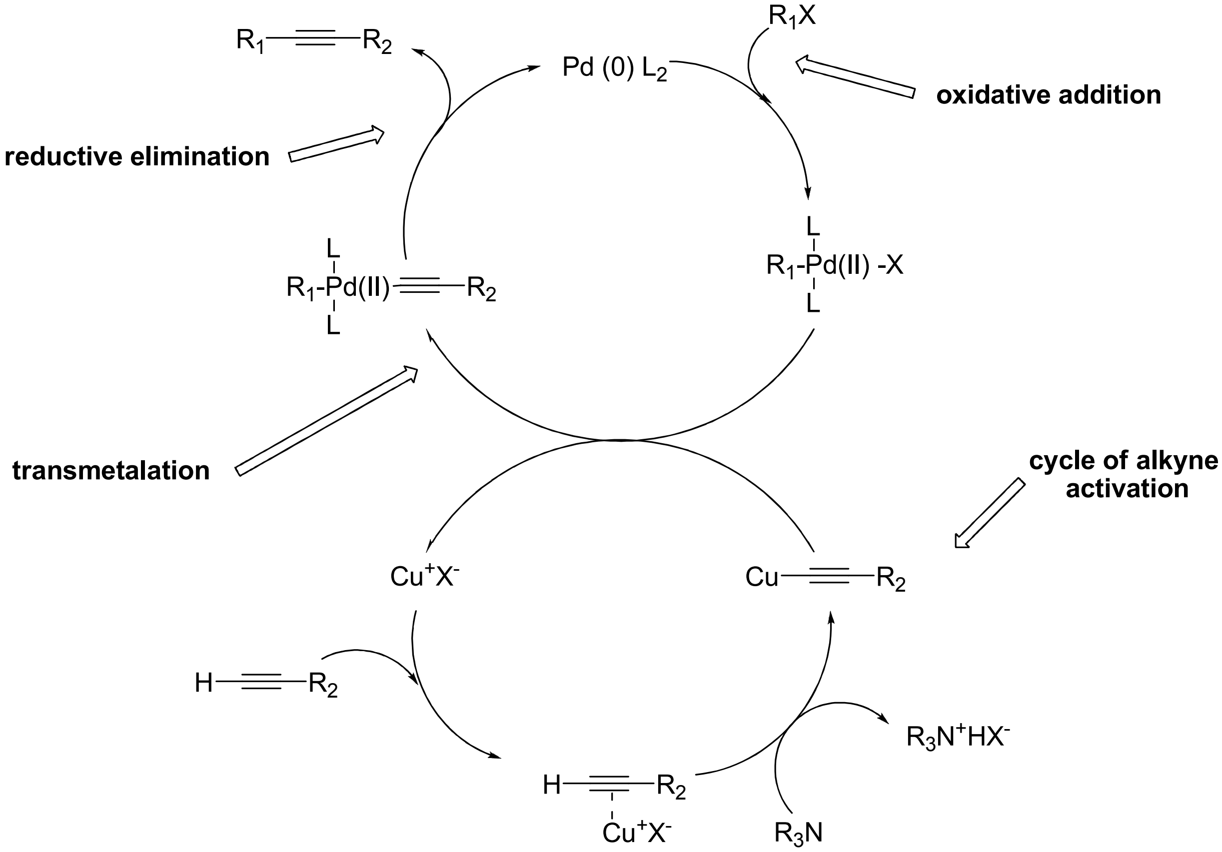

Figure 2.22: *Catalytic cycle for the copper-cocatalyzed Sonogashira reaction.*

The copper cocatalyzed Sonogashira reaction is supposed to take place through two independent catalytic cycles, as shown in **Figure 2.22**.The base action is exerted by the tertiary amine and by other amines and inorganic bases. The generally accepted cycle for the palladium catalysis (the Pd-cycle) is based on the usually fast oxidative addition of R_1-X (R_1 = aryl, hetaryl, vinyl; X = I, Br, Cl, OTf) to the real catalyst generated from the initial palladium complex. This specie is thought to be a classical 14-electron Pd(0)L_2, formed by reduction of different palladium-(II) complexes by s-electron donors, such as phosphanes, amines, and ethers, used as ligands and solvents, via a mechanism of σ-complexation-dehydropalladation-reductive elimination.[176]

The characteristics of the R_1-X substrate are crucial in the oxidative addition step, which is facilitated if X = I or OTf and if the electronic density is reduced on the C-X bond by the presence of electron-withdrawing groups. In the subsequent mechanistic step, the Pd cycle is connected with Cu cycle, the cycle of the copper cocatalyst. Thus, the usually rate-determining transmetalation from the copper acetylide formed in the Cu cycle generates the $R_1Pd(-C\equiv CR_2)L_2$ species, which in turn gives the final coupled alkyne after *trans/cis* isomerization, reductive elimination and regeneration of the catalyst. The second Cu cycle is still poorly understood. The base (generally the amine) is supposed to seize the acidic proton of the terminal alkyne, favouring, in the presence of

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[175] (a) Stephens, R. D.; Castro, C. E. *J. Org. Chem.* **1963**, *28*, 2163. (b) Stephens, R. D.; Castro, C. E. *J. Org. Chem.* **1963**, *28*, 3313.

[176] Negishi, E.; de Meijere, A. *Handbook of Organopalladium Chemistry for Organic Synthesis* Eds. Wiley: New York, **2002**.

the copper(I) salt, the formation of copper acetylide. It should be pointed out that the generally employed amines are not basic enough for deprotonating the alkyne. Therefore, a π-alkyne-Cu complex as shown in **Figure 2.22** could be involved in the cycle, thus making the alkyne proton more acidic for easier abstraction.

More recently, many procedures for a "copper free" Sonogashira coupling have been reported.[177] The absence of copper in the reaction mixture avoids the homocoupling of the intermediate copper acetylide occurrimg when it is exposed to oxidative agents or air.[178] The mechanism of the copper-free Sonogashira reaction is not well-known. While the first step is supposed to be the oxidative addition of R_1-X to the palladium(0) complex (**Figure 2.23**), the second step is still debated. As previously mentioned, the amines generally employed are usually not strong enough to deprotonate the alkyne. Also in this instance, the complex (η^2-RC≡CH)-$PdXL_2$ may display greater acidity and easier deprotonation to the complex $R_1Pd(-C{\equiv}CR_2)L_2$,[179] which will then give the coupling product R_1-C≡C-R_2 by reductive elimination.

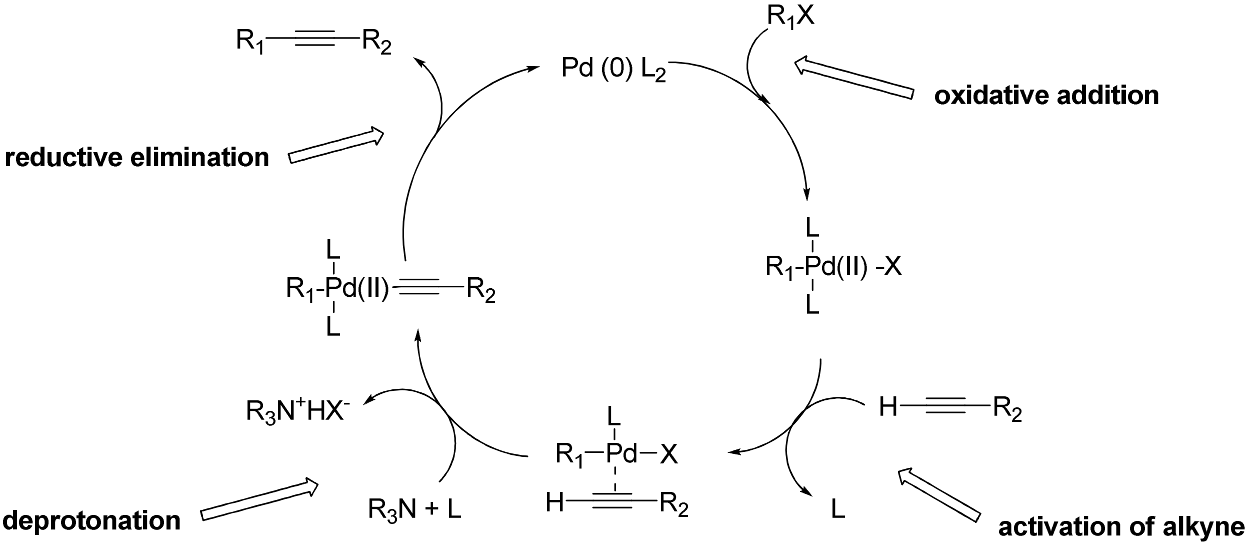

Figure 2.23: *Catalytic cycle for the "copper free" Sonogashira cross coupling.*

Liang reported a mild protocol for the copper free Sonogashira coupling of aryl iodides with terminal acetylenes in water under aerobic conditions.[180] We have adopted this procedure, obtaining the 4-alkynil quinolines **35** and **36** with good yields. The triple bond was subsequently reduced to the alkane together with nitro group in a single step.

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[177] (a) Alami, M.; Ferri, F.; Linstrumelle, G. *Tetrahedron Lett.* **1993**, *34*, 6403. (b) Nguefack, J.; Bolitt, V.; Sinou, D. *Tetrahedron Lett.* **1996**, *37*, 5527. (c) Herrmann, W. A.; Bohm Volker, P. W. *Eur. J. Org.Chem.* **2000**, *22*, 3679. (d) Fukuyama, T.; Shinmen, M.; Nishitani, S.; Sato, M.; Ryu, I. *Org. Lett.* **2002**, *4*, 1691. (e) Pal, M.; Parasuraman, K.; Gupta, S.; Yeleswarapu, K. R. *Synlett* **2002**, *12*, 1976. (f) Alonso, D.; Najera, C.; Pacheco, M. C. *Tetrahedron Lett.* **2002**, *43*, 9365. (g) Fu, X.; Zhang, S.; Yin, J.; Schumacher, D. P. *Tetrahedron Lett.* **2002**, *43*, 6673. (h) Uozumi, Y.; Kobayashi, Y. *Heterocycles* **2003**, *59*, 71. (i) Ma, Y.; Song, C.; Jiang, W.; Wu, Q.; Wang, Y.; Liu, X.; Andrus, M. B. *Org. Lett.* **2003**, *5*, 3317. (j) Soheili, A.; Albaneze-Walker, J.; Murry, J. A.; Dormer, P. G.; Hughes, D. L. *Org. Lett.* **2003**, *5*, 4191.

[178] (a) Glaser, C. *Ber. Dtsch. Chem. Ges.* **1869**, *2*, 42. (b) Hay, A. S. *J. Org. Chem.* **1962**, *27*, 3320. (c) For a review of alkyne coupling, see: Siemsen, P.; Livingston, R. C.; Diederich, F. *Angew. Chem. Int. Ed.* **2000**, *39*, 2632.

[179] Soheili, A.; Albaneze-Walker, J.; Murry, J. A.; Dormer, P. G.; Hughes, D. L. *Org. Lett.* **2003**, *5*, 4191.

[180] Liang,B.; Dai, M.; Jiahua, C.; Zhen, Y.; *J. Org. Chem.* **2005**, *70*, 391.

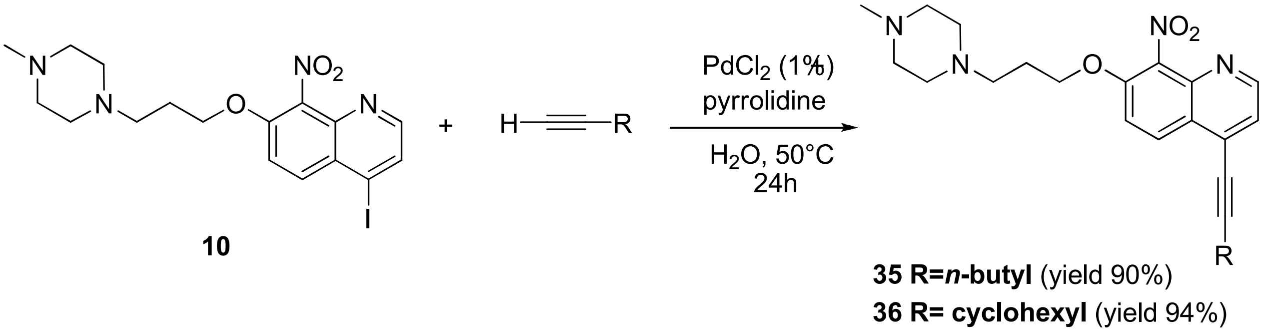

Figure 2.24: *Sonogashira cross coupling for the synthesis of 4-alkynyl-8-nitroquinolines.*

During a trial with an alkyne containg a free oxydrilic group, run under the basic catalysis of pyrrolidine, the reaction surprisingly yielded the 4-pyrrolidine substituted product **37**, and not the expected alkynyl compound. Compound **37**, obtained with low yield, has been purified and characterized. The reaction, repeated in the absence the alkyne, gave **37** in any case. It's possible that the reaction could take place even in absence of palladium, via an aromatic nucleophilic substitution, because of the high nucleophilicity of pyrrolidine, but this hypothesis has not been demonstrated. Although the investigation of this reaction could lead to really interesting and innovative results, we did not undertake further studies for a better characterization.

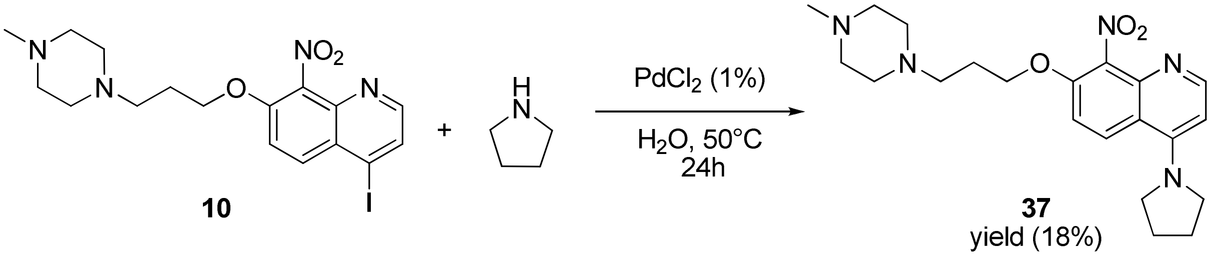

Figure 2.25: *Introduction of pyrrolidine moiety at the 4 position.*

The molecules synthesized with cross coupling methodologies have to be reduced to amines in order to be tested in biological assays.

2.1.1.4.2 Aromatic nucleophilc substitution for 4-Oxyaryl, 4-Oxyalkyl, 4-N-Alkyl, 4-N-Aryl quinolines

As already discussed in the section concerning functionalization of 7 position, aromatic nucleophilic substitution is the best methodology for the functionalization with oxygen linked or nitrogen linked organic moieties in 4 position. The precursors **8**, **11** and **14** have been synthesized in great quantities in order to insert an extended library of groups in 4 position. No regioselectivity problem is presented by these molecules. Both the pyridine ring and the all carbon ring are activated for nucleophuilic substutitiom, and both possess potential LG.[181] However, chlorine atom is notoriously a better LG then the nitro or the alcoholate groups.

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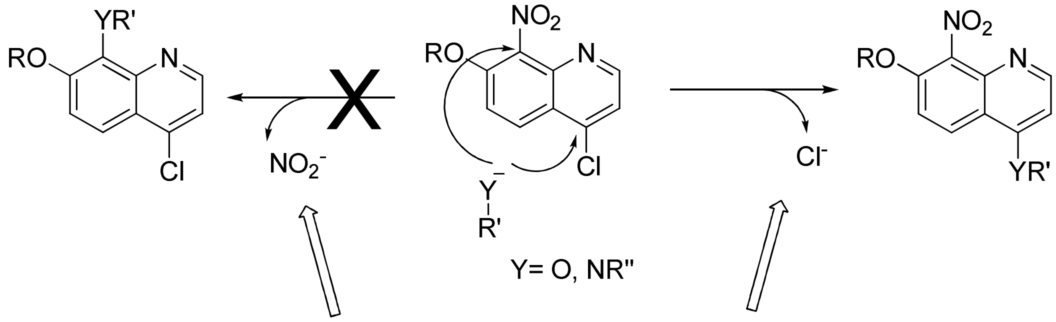
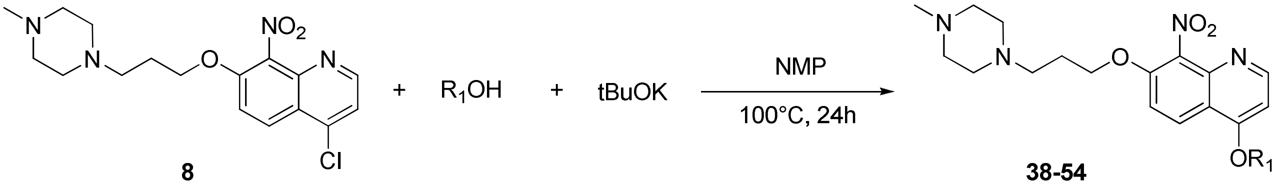
[181] (a) For a review see: Beck, J.R. *Tetrahedron* **1978**, *34*, 2057. (b) Effemberg; Koch; Stechemberg *Chem. Ber.* **1991**, *24*, 163.



nitrite worse leaving group than chloride

Figure 2.26: *Regioselectivity for position 4 in aromatic nucleophilic substitution.*

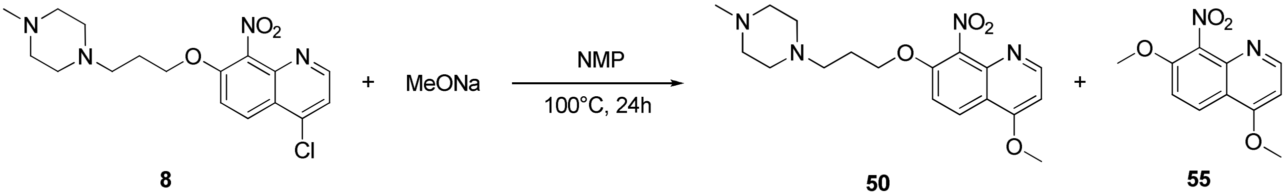
Furthermore, the solvent NMP proved to be totally selective toward the substitution at 4 positions, with no hint for products derived from 7 substitutions.



8 **38-54**

Figure:2.27: *Aromatic nucleophilc substitution for 4-oxyaryl and 4-alkyquinolines.*

Good yields have been been obtained in almost all reactions. Compounds **44** and **50** are two exceptions. Probably the yield of compound **44** was low because of the low nucleophilicity of the alcoholate induced by the presence of three electron withdrawing chlorine atoms. For the synthesis of compound **50**, we utilized a commercial 30% wt. solution of sodium methoxyde in methanol, and not the usual *t*-BuOK in methanol. Because of its strong nucleophilicity and the mass excess, NaOMe displaced also the side chain at 7 position, yielding compound **55** as major product.



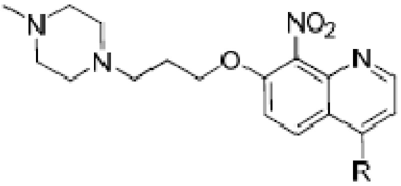
8 **50** **55**

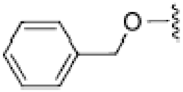
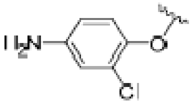
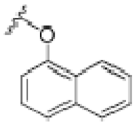
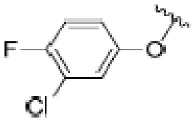
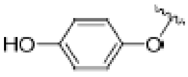
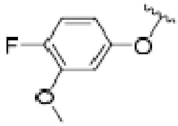
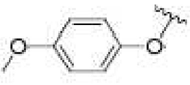
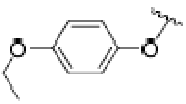
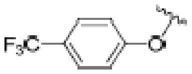
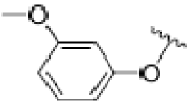
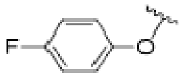
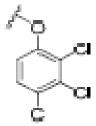
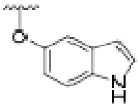
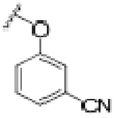
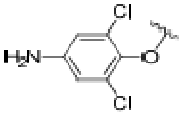
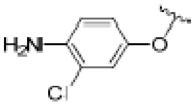
yield 15 % yield 75 %

Figure 2.28: *Byproduct in aromatic nucleophilic substitution*

The compound synthesized with this procedure are presented in **Table 2.2**

Table 2.2: *4-Oxyaryl and 4-oxyalkyl 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolines synthesized with* SN_{Ar}.



N°	R	yield %	N°	R	yield %
38		94,0	**47**		80,0
39		93,0	**48**		67,0
40		44,0	**49**		60,0
41		82,0	**50**		15,0
42		86,0	**51**		55,0
43		99,0	**52**		85,0
44		49,0	**53**		50,0
45		90,0	**54**		63,0
46		80,0			

The compounds derived from precursor **11** and **14** have been synthesized with the same procedure.

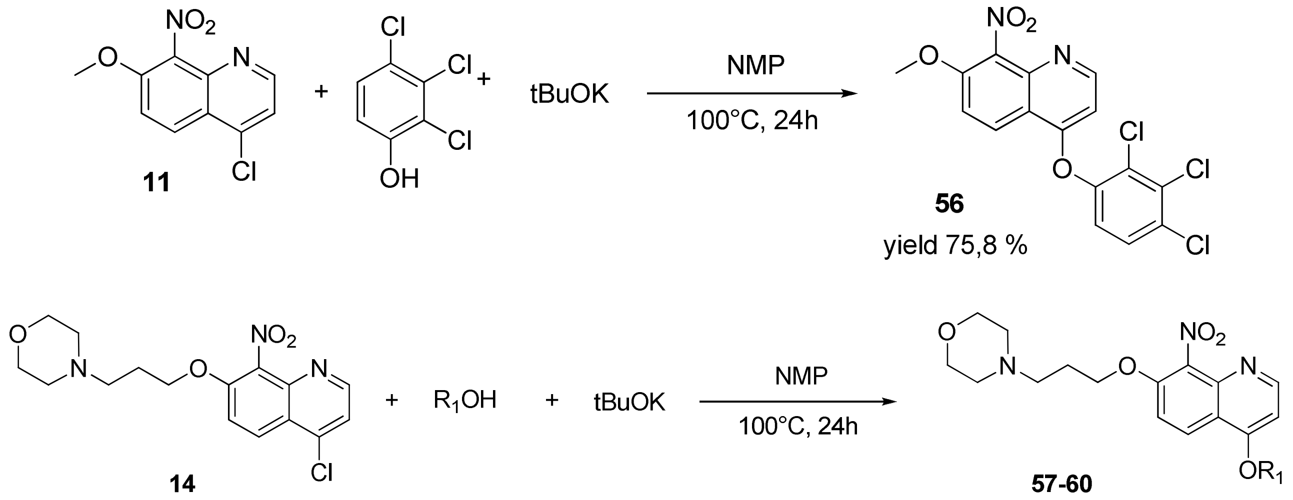


Figure 2.29: *Aromatic nucleophilic substitution for 4-Oxyaryl and alky quinolines.*

Compound **56** has also been tried to be synthesized by reacting 4,7-dichloroquinoline **3** first with the aromatic alcohol in NMP to functionalize position 4 and then with sodium methoxide to functionalize position 7 In the first step some disubstituted compound was found, thus we decided to push forth the reaction toward the complete difunctionalization with the 2,3,4-trichlorophenoxy moiety, gaining the compound **56a**.

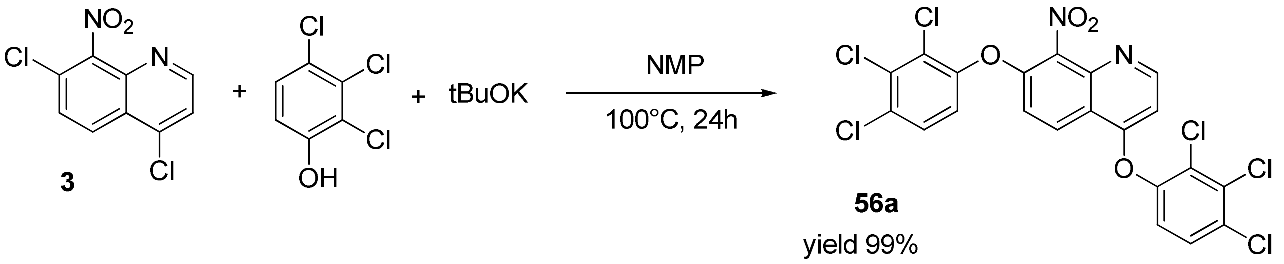


Figure 2.30: *SN$_{Ar}$ for disubstitution.*

In the case of the substitution reaction on **14** carried out in ethanol rather than in NMP, using one equivalent of KOH for the generation of the alcoholate, the compound **60a** was also obtained, arising from the displacement of propoxy-morpholino side chain (see **Figure 2.31**). This behavior may be attributed to the strong nucleophilicity of sodium ethoxyde.

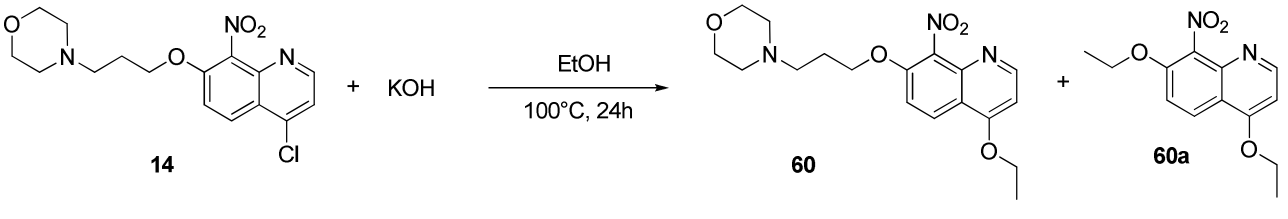
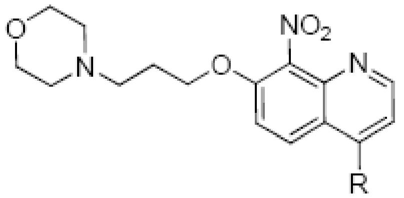


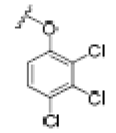
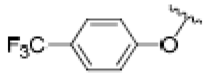
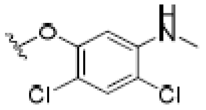
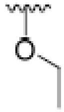
Figure 2.31: *Byproduct in SN$_{Ar}$.*

Compound **60a** can also be synthesized in 86% yield by the direct reaction of 4,7-dichloro-8-nitroquinoline **3** with two equivalents of KOH in ethanol .

The results are summarized in **Table 2.3**.

Table 2.3: *4-Oxyaryl and 4-oxyalkyl 7-(3-morpholinopropoxy)-8-Nitro quinolines synthesized with SN_{Ar}.*



N°	R	yield %	N°	R	yield %
57		74,4	**59**		74,4
58		16,5	**60**		80

Similarly to the alcohols that yields O-linked groups at position 4, amines can be introduced in the quinoline scaffold with an aromatic nucleophilic substitution to yield N-linked groups. Amines are known to be enough nucleophilic to give SN_{Ar} reactions[182] on activated aryl halides. However, in our case no reaction occurred. We therefore devised to increase the nucleophilicity of the amines by generating the corresponding amide anion with a strong base.[183] As a matter of fact, unactivated aryl halides react with KNH_2, with a mechanism involving formation of benzyne.[184]

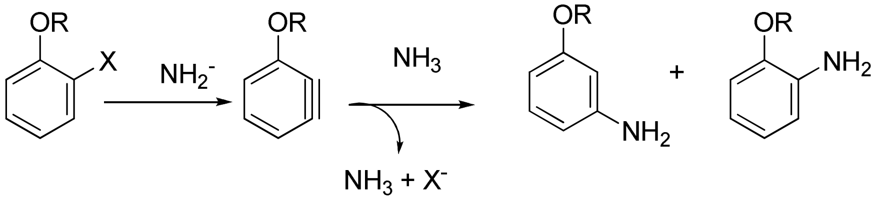


Figure 2.32: *Benzyne mechanism for aromatic nucleophilic substitution.*

In our case, because of the strength of the RNH^- nucleophile, both 3 and 4 amino substituted quinolines would be expected. We obtained only the 4 substituted product, either with the SN_{Ar} or the benzyne mechanism.

---

[182] March, J.; Smith, M. B. *Advanced Organic Chemistry*, Ed. Wiley **2001,** chapter *13-5*, 864.

[183] Nucleophilcity order is: $NH_2^- > Ph_3C^- > PhNH^- > ArS^- > RO^- > R_2NH > ArO^- > OH^- > ArNH_2 > NH_3 > I^- > Br^- > Cl^- > H_2O > ROH$**.** This list is compiled from data in Bunnet, J. F.; Zahler, R. E. Ref p. 340; Bunnet, J. F.; *Q. Rev. Chem. Soc.* **1958**, *12*, 1. See pag 13. (b) Sauer, J.; Huisgen, R. *Angew. Chem.* **1960**, *72*, 294. (c) Bunnett, J. F. *Annu. Rev. Phys. Chem.* **1963**, *14*, 271.

[184] Roberts, J. D.; Semenow, D. A.; Simmons, H. E.; Carlsmith, L. A. *J. Am. Chem. Soc.* **1965**, *78*, 601.

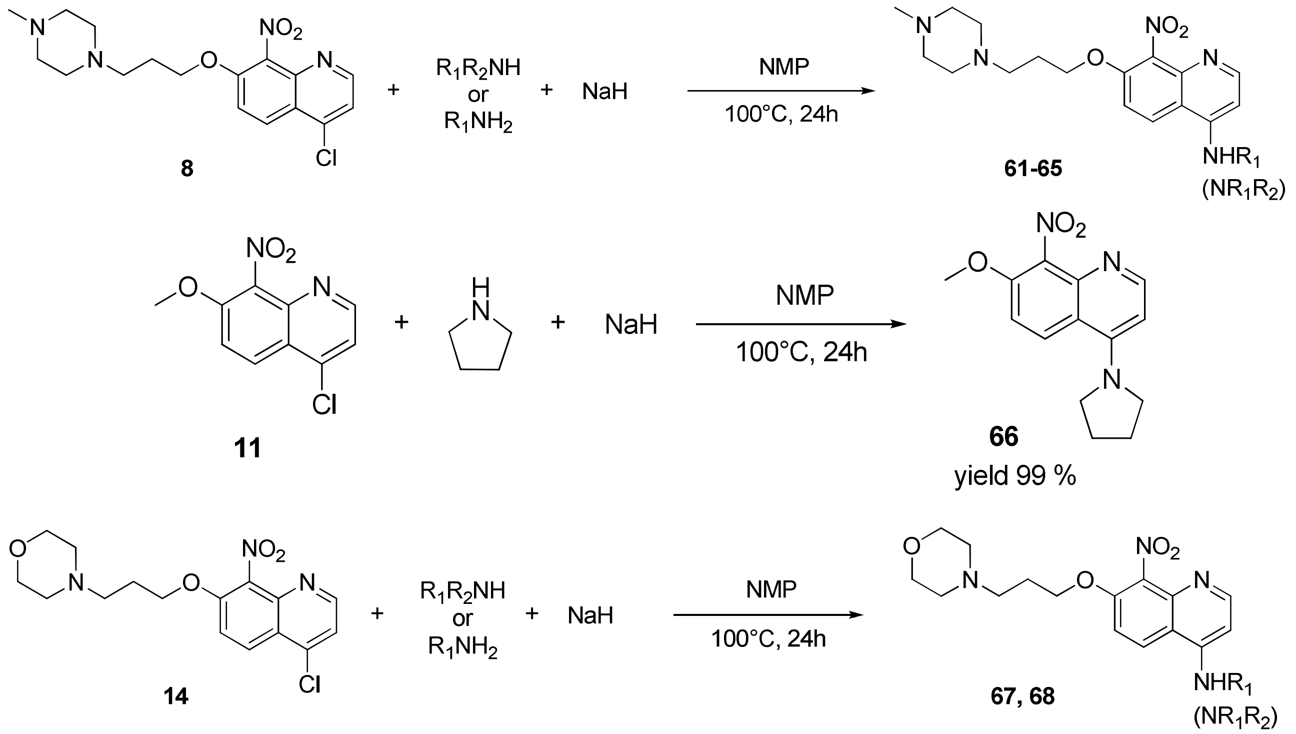
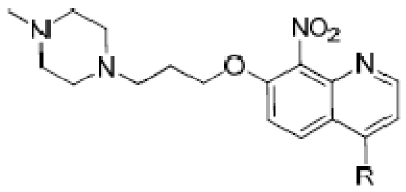


Figure 2.33: *Aromatic nucleophilc substitution for 4-N-aryl and alky quinolines.*

The base *t*-BuOK is not strong enough for generating the amide anion, and we had to resort to the stronger base NaH. Compound **66** is an exception, as *t*-BuOK can be utilized successfully. It is also possible that the reaction could also be carried out without the presence of a base, being cyclic amines in general highly nucleophilic. In fact compound **62** can be synthesized also using *N*-methylpiperazine as solvent and as a base without NaH.

Table 2.4: *4-N-aryl and 4-N-alkyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolines synthesized with aromatic nucleophilic substitution.*



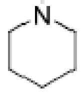
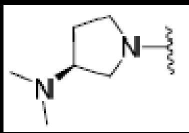
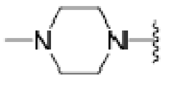
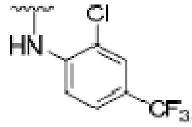
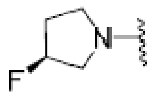
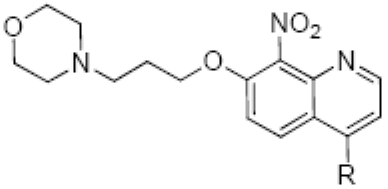
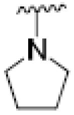
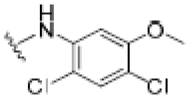
N°	R	yield %	N°	R	yield %
61		35,0	64		65,0
62		80,0	65		50,0
63		84,0			

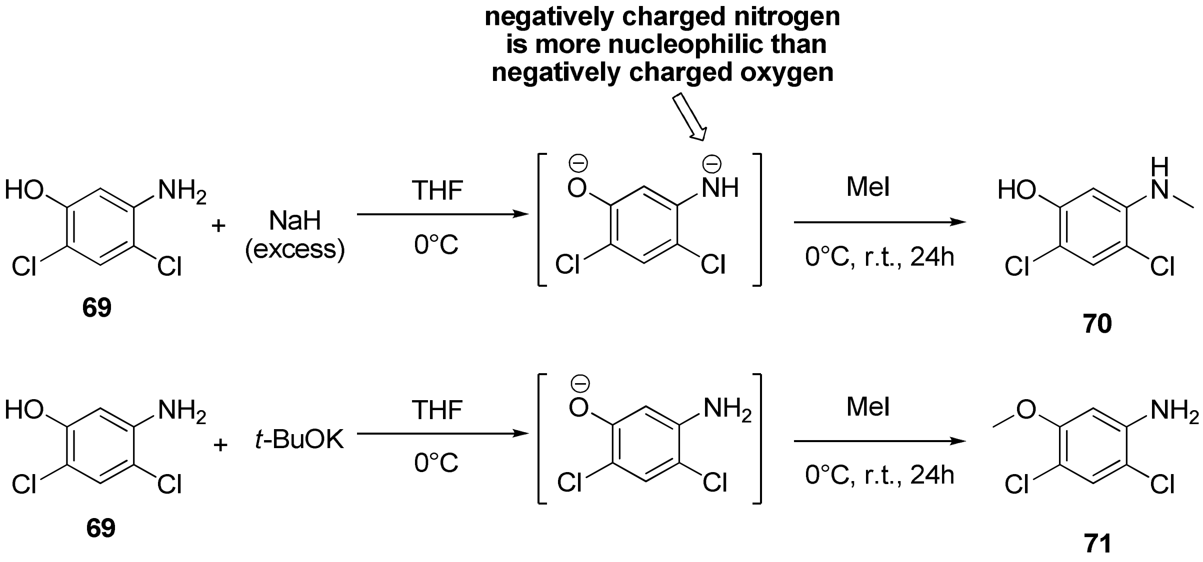
Table 2.5: *4-N-Aryl and 4-N-alkyl 7-(3-morpholinopropoxy)-8-nitroquinolines synthesized with aromatic nucleophilic substitution.*



N°	R	yield %
67		68,7
68		14,4

The alcohol necessary for the preparation of compound **58** was not commercially available, and was synthesized via nucleophilic substitution to methyl iodide by the correspondent primary amide anion. This anion is in fact a dianion, where also oxygen bears a negative charge: actually, the hydroxyl proton is far more acid then the amine proton, and two equivalents of a strong base are necessary to generate the dianion. At the same time, the amide group, as a stronger base than the alcoholate group, is also a stronger nucleophile. It is the former group, in fact, that displaces the electrophile MeI. After quench in water, the compound **70** is recovered.

Conversely, when the relatively milder base *t*-BuOK is used stoichiometrically, only the alcoholate is generated as nucleophile, and methylation occurs at this group, with generation of the methoxylated molecule **71**.



negatively charged nitrogen is more nucleophilic than negatively charged oxygen

Figure 2.34: *Methylation of* ***68*** *under different reactions conditions.*

The oxygen and nitrogen linked molecules at 4 position are to be subjected to reduction of the nitro group to the amine group, in order to be tested in the biological assays.

2.1.2 *Synthesis of 3,7-Disubstituted-8-Aminoquinolines*

The synthetic way so successfully utilized for the generation of the library of 4,7 disubstituted-8-aminoquinolines cannot be utilized for the generation of the corresponding library of 3,7 disubstituted 8-aminoquinolines. This unfortunate circumstance simply arises from the fact that 3,7-dihaloquinolines are not commercially available. Nevertheless the 3-haloquinoline remains a fundamental intermediate for the functionalization of position 3. We had therefore devised a novel synthetic strategy that allowed us to obtain the desired 3-halogenated precursor.

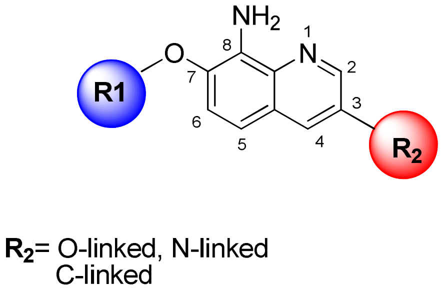


Figure 2.35: *3,7-Disubstituted-8-aminoquinolines.*

The strategy is, also in this case, based on the retrosynthetic analysis.

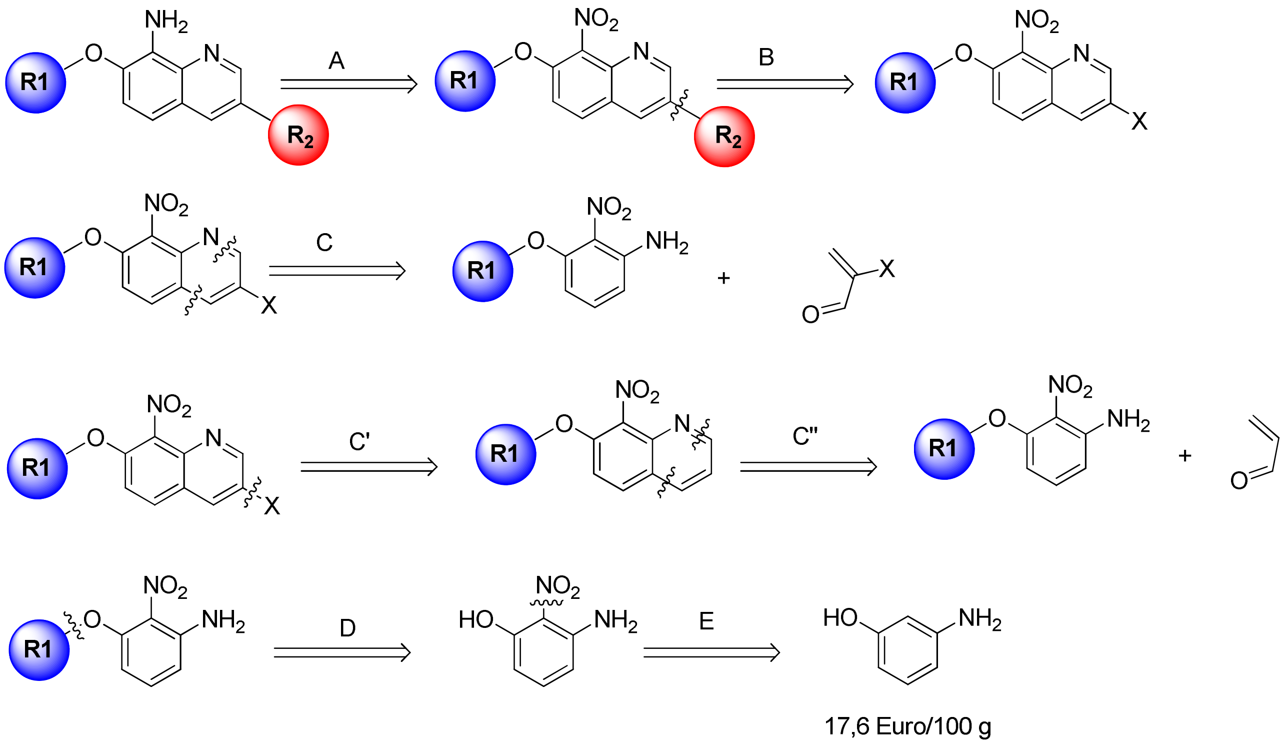


Figure 2.36: *Retrosynthetic approach to 3,7-disubstituted-8-aminoquinolines.*

The amino group at the position 8 could be obtained by reduction of nitro compounds (step A). The various typologies of substituents at 3 position (oxygen linked, nitrogen linked and carbon linked), could be sistematically obtained, with aromatic substitution or cross coupling reaction, when this position is properly activated with a halogen atom (step B). At this point the retrosynthetic analysis suggests two choices: (a) the direct construction of the quinoline scaffold from an halogenated three carbons moiety and aniline (step C); (b) the synthesis of the quinoline scaffold firstly, by reaction of aniline with a normal three carbons moiety. followed by the halogenations of the quinoline ring (steps C' and C''). In both cases, the synthesis of the quinoline scaffold was adjusted over the

Skraup and Doebner-Von Miller reactions. The steps D and E describe the usual attachment of the side chain and the introduction of the nitro group.

After this retrosynthetic analysis, it has been possible to plan a synthetic route that leads to 3,7-disubstituted-8-aminoquinolines.

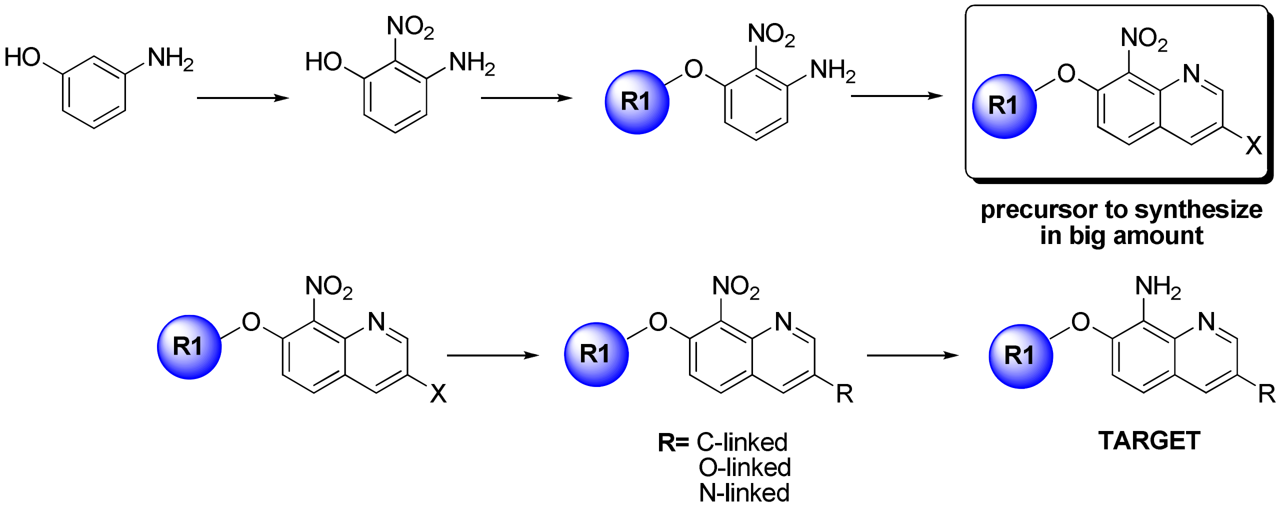


Figure 2.37: *Synthetic pathway to 3,7-disubstituted-8-aminoquinolines.*

In the actual synthetic project, 3-hydroxyaniline is firstly nitrated and then alkylated with the desired side chain. The target can be synthesized with either proposed way, the first one involving the ring closure followed by the selective halogenation at 3 position, the second one involving the direct ring closure with an halogenated three-carbon moiety. With the same strategy adopted in the case of the 4,7-disubstituted molecules, this synthetic plan was devised in order to gain a relevant amount of precursor, utilized for the creation of the whole library with different groups at the 3 position.

2.1.2.1 Nitration of 3-aminophenol

3-Amino-2-nitrophenol **72** can be obtained from the nitration of 3-aminophenol, strongly activated toward electrophilic substitution by the presence of two electron donating groups. In order to hinder polyfunctionalization, we attempted milder methods for the generation of the nitronium ion. A first attempt, where the nitronium ions was generated by molecular bromine, silver nitrate and triphenylphosphine,[185] was unsuccessful. Nor the method that utilizes nitric acid and nickel ammonium sulphate[186] in a series of different solvents (chloroform, dichloromethane, ether, DMSO), gave any result. After these unsuccessful trials, we turned to the same procedure that proved to be successful in the nitration of 4,7-dichloroquinoline,[187] namely the generation of the nitronium ion in the nitric acid/sulfuric acid mixture. The product **72** was obtained, together with the other two regioisomer **73** and **74**, *i.e.* all products derived from the functionalization of the activated *ortho* and *para* positions.

---

[185] Iranpoor, N.; Firouzabadi, H.; Nowrouzi, N.; Firouzabadi, D. *Tetrahedron Lett.* **2006**, *47*, 6879.
[186] Tasneem, M.; Ali, M.; Rajanna, K. C.; Saiparakash, P. K. *Synth. Commun.* **2001**, *31*, 1123.
[187] Surrey, A. R.; Hammer, H. F. *J. Am. Chem. Soc.* **1946**, *68*, 1244.

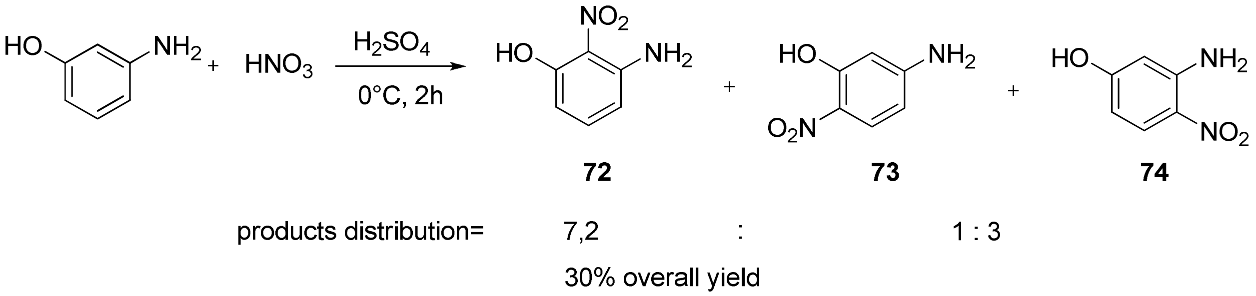


Figure 2.38: *Nitration of 3-aminophenol.*

The main product was the desired 3-amino-2-nitrophenol **72**, that could be separated from the other isomer, albeit with some difficulty. The separation of the mixture by crystallization with toluene gave mixed crystals of the major **72** and **74** isomers.[188] A series of other solvents have been tried, but always a mixture of both crystals was obtained. Because the crystals have different shape, they can be discriminated by sight and manually separated. The two resulting fractions are richer in one isomer, but still significantly contaminated by the other one. A second crystallization from toluene of the fraction richer in **72** yielded pure crystals of this isomer. Anyway, chromatographic separation on silica gel of the first precipitate with hexane:AcOEt (7:3) allows complete separation of the products and complete recover of **72**.

Any other attempt, with different reaction conditions (also the temperature lowering from 0°C to -15°C), did not change either regioselectivity or yield.

2.1.2.2 Side chain introduction

In order to have a comparison between activity of 3 and 4-substituted inhibitors, both kind of molecules needed to have the same side chain at position 7. In this case methoxy group like precursor **11** and *N*-propyl-morpholin group like precursor **14** were not used, but only the *N*-methylpiperazine “Bosutinib-like” side chain. In this case, and differently from the synthesis of 4-substituted molecules, the attachment of the O-linked side chain is an aliphatic nucleophilic substitution, where the “nucleophilic oxygen” is located on the aromatic ring, while the “electrophilic halide” is located on the side chain. Thus, the side chain has to be functionalized with an halogen leaving group. This molecule is not commercially available and must be synthesized.

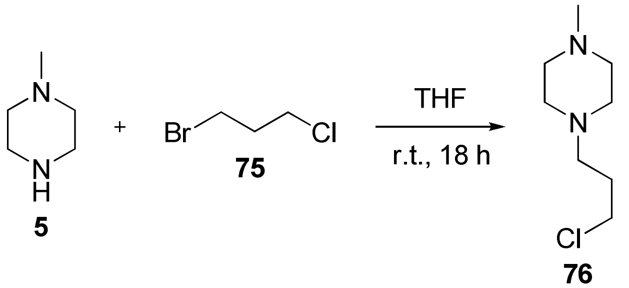


Figure 2.39: Synthesis of the *N*-methylpiperazine side chain.

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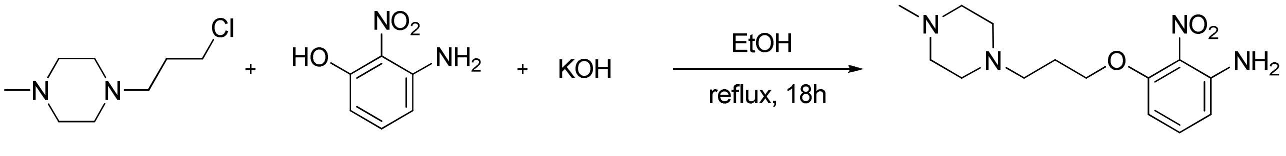
[188] Products **73** and **74** were not characterized and distinguished one from the other, because of the hydentical pattern of signal in ^{1}H-NMR spectroscopy.

The nucleophilic attack of *N*-methylpiperazine is regiospecifically directed toward the carbon bearing the bromine atom, which has a greater leaving group attitude than the chlorine atom. The nucleophile *N*-methylpiperazine acts also as a base, sequestering the proton liberated in the reaction.

The most nucleophilic centre of the aminophenol **72** is the nitrogen atom. As the most acidic center is instead the hydroxyl group, the proton remotion by a base, generates the alcoholate and the most nucleophilic center becomes the negatively charged oxygen. The utilized base is KOH, able at deprotonating the phenolic group, but not the the aniline group. Thus, when the reaction is carried out in alcoholic KOH, *N*-alkylation is avoided.

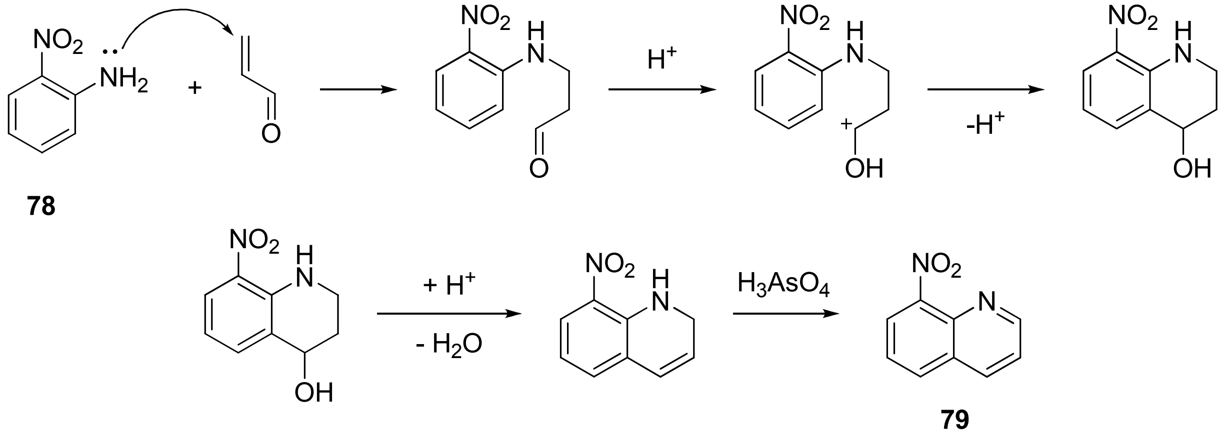


76 **72** **77**

Figure 2.40: *Introduction of the side chain.*

2.1.2.3 The quinoline ring closure

In our first attempt, we tried to build the quinoline scaffold in a two step procedure: the ring closure followed by halogenation. As anticipated in the retrosynthesis scheme, a modification of Skraup reaction can be used to transform an aniline derivative into a quinoline. Yale and Bernstein reported the synthesis of 8-nitroquinoline, involving the use of acrolein and arsenic acid as oxidizing agent.[189]



78

79

yield 43%

Figure 2.41: *Synthesis of 8-nitroquinoline.*

In this procedure, the aniline nitrogen undergoes firstly a Michael addition with acrolein. The acid behaviour of the solvent (H_3PO_4 80%) enhances the electrophilicity of carbonyl carbon, which closes the ring *ortho* to the aniline by aromatic electrophilic substitution. The hydroxylic intermediate is then dehydrated and finally oxidized by arsenic acid to full aromaticity, yielding the desired quinoline.

---

[189] Yale, H.L.; Bernstein, J. *J. Am. Chem. Soc.* **1948**, *70*, 254.

According to a procedure reported by Gershon,[190] 8-nitroquinoline can be halogenated with NBS or NCS to yield selectively the 3-halo-8-nitroquinoline.

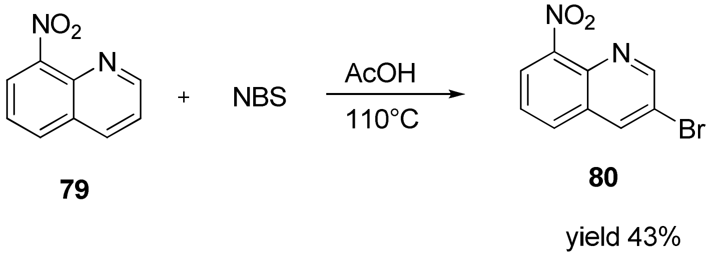


Figure 2.41: *Bromination of 8-nitroquinoline.*

In our hands, 3-bromo-8-nitroquinoline **80** was obtained in an acceptable yield by heating 8-nitroquinoline **79** at 110°C with NBS. This reactions has been investigated in order to establish whether the method was suitable for the preparation of our inhibitors.

We tried initially the Yale ring closure on compound **72,** but the corresponding ring closure product was detected only in traces in the reaction mixture, indicating that the hydroxyl group exherts a disruptive action.

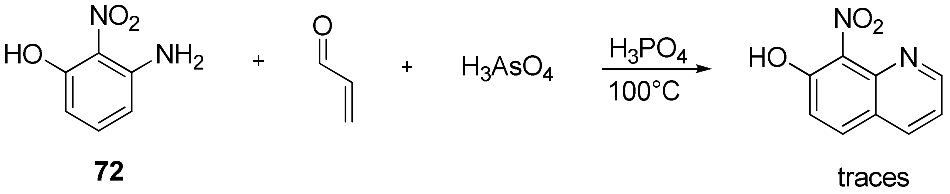


Figure 2.42: *Reaction of 72 with acrolein.*

Actually, when we tried the reaction with compound **77,** bearing an alkyl group at the phenolic oxygen , 7-oxyalkylated-8-nitroquinoline **81** was obtained in 75% yield.

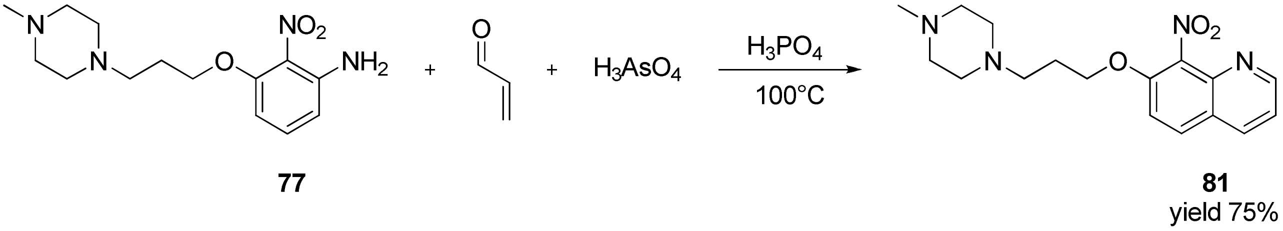


Figure 2.43: *Ring closure reaction.*

As already stated, we could easily brominate 8-nitroquinoline **79** on position 3 by using NBS in acetic acid. Nevertheless, the same reaction run on 7-alkoxylated-8-nitroquinoline **81,** using either NBS or NCS, was unsuccessfull, probably because of deactivation of the halogenating agent

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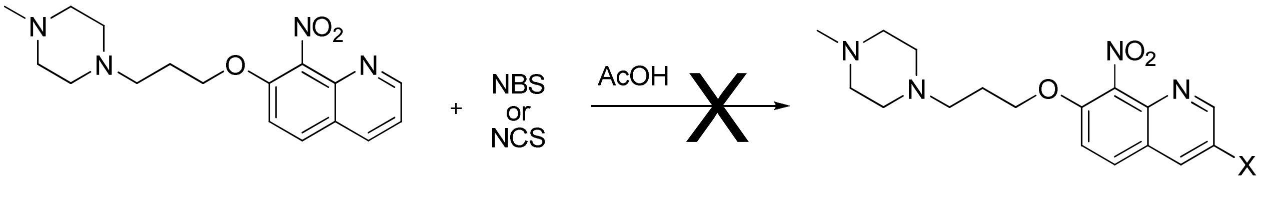
[190] Gershon, H.; Clarke, D. D. *Monatshefte für Chemie / Chemical Monthly* **1991**, *122*, 935.

exherted by the *N*-methylpiperazine moiety. Unchanged starting material have been recovered from the reaction mixture.

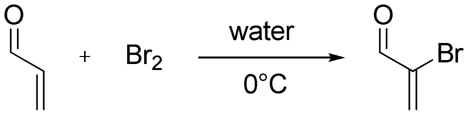


81 **X** = Br, Cl

Figure 1.44: *Halogenation attempt.*

This failure prompted us to the investigation of an alternative method of ring closure, namely that contemplating the closure of the ring with the halogen located at position 3 in a single step. Thus aniline **72** will be reacted with an α-haloacrolein, in a synthesis which is reminiscent of both the Skraup and Doebner-Von Miller reaction. The α-haloacrolein molecules are not commercially available and are to be synthesized. The firstly attempted synthesis regarded α-bromoacrolein **82**, with the aim of gaining 3-bromo-7-substituted-8-nitroquinolines, which are good substrates for coupling reactions.

For the synthesis of α-bromoacrolein **82** two reports are found in literature. The first one is the addition of bromine to acrolein in water, with steam distillation of the product.[191] In our hands, the yield was low (about 24%), probably because heating and acid environment promoted polymerization of the product, observed as tar in the distillation flask. Neutralization of the excess of Br_2 with $Na_2S_2O_3$ just before steam distillation increased the yield to 47%.



82

yield 47%

Figure 2.45: *First method for the synthesis of α-bromoacrolein.*

In the second method, reported by Corey,[192] the bromine addition to acrolein generates 2,3-dibromopropanal, which is then subjected to β-elimination by the action of a base. Acrolein was dissolved in dichloromethane at 0°C and bromine was added dropwise over 5 minutes. After the work-up, triethylamine (added in an amount equivalent to the quantity of 2,3-dibromopropanal recovered), promotes the precipitation of triethylamine hydrobromide. After filtration, the desired product could be recovered with good purity in 67% yield.

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[191] Smith, A. B.; Levenberg, P. A.; Jerris, P. J.; Scarborough, R. M.; Wovkulich, P. M. *J. Am. Chem. Soc.* **1981**, *103*, 1501.

[192] (a) Corey, E. J.; Loh, T.-P. *J. Am. Chem. Soc.* **1991**, *113*, 8966. (b) Paquette, L.; Huang Kuo, L.; Dogon, J. *J. Am. Chem. Soc.* **1997,** *119*, 3038.

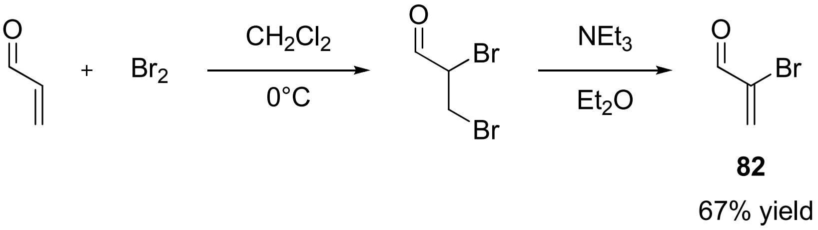

Figure 2.46: *Alternative method for the synthesis of α-bromoacrolein.*

The α-bromoacrolein **82** is a very reactive and unstable, ans must by freshly prepared and immediately utilized.

Tinsley[193] reported the possibility of obtaining 3-haloquinolines in good yields from aniline derivatives and 2,2,3-tribromopropanal **83**, when the *para* position to the amine group is protected. If the *para* position is free, the expected 3-bromoquinoline was obtained in 1% yield only and a considerable amounts of 6-bromoquinoline and 3,6-dibromoquinoline were found in the reaction mixture. The reagent 2,2,3-tribromopropanal **83** was prepared *in situ* by addition of bromine to α-bromoacrolein. More recently, Boger[194] and Gaillard[195] synthesized with similar substrates other 3-bromoquinolines from *para*-substituted anilines.

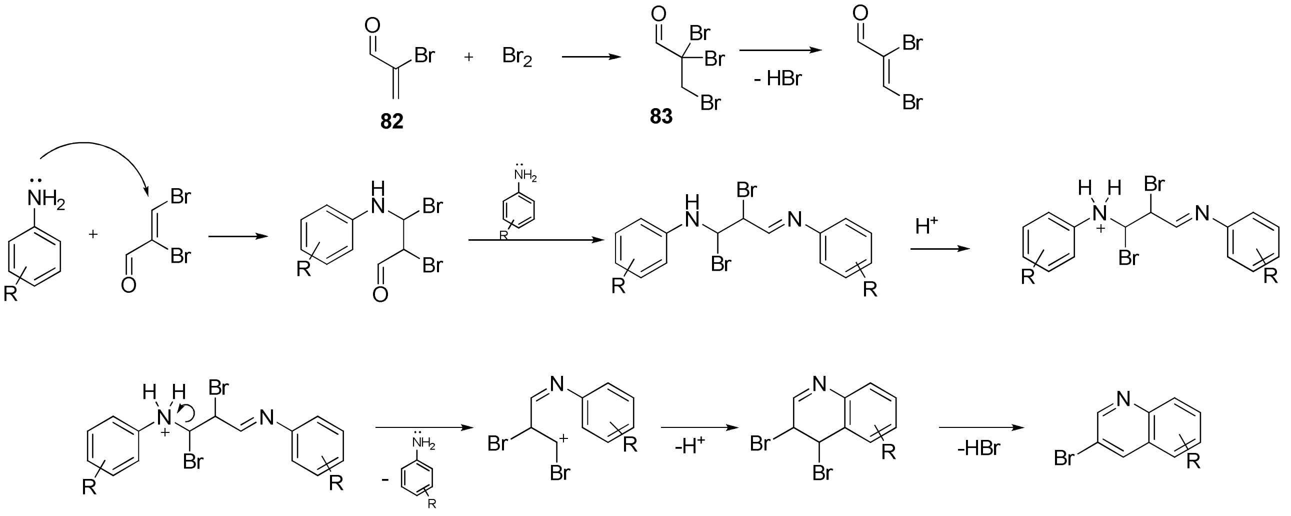

Figure 2.47: *Proposed mechanism for the ring closure reaction with α-bromoacrolein and bromine.*

The proposed mechanism is illustrated in **Figure 2.47**. In the first step, α-bromoacrolein is brominated to 2,2,3-tribromopropanal **83** and subsequently dehydrohalogenated to 2,3-dibromoacrolein. In the second step, the latter reagent is attacked by aniline with a Michael addition. A Schiff base is then formed with a second molecule of aniline. The protonation of the first aniline catalyzes its detachment and the formation of a bromocarbocation. Ring closure and dehydrohalogenation will lead to the final 3-brominated quinoline. Tha validity of this mechanism is supported by the isolation of the dibrominated intermediate, by reaction of 2,2,3-tribromoacrolein with the aromatic amine without solvent.

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[193] (a)Baker, R. H.; Tinsley, S. W.; Butler, D.; Riegel, B. *J. Am. Chem. Soc.* **1950**, *72*, 393. (b) Tinsley, S. W.; *J. Am. Chem. Soc.* **1955**, *77*, 4175.

[194] Boger, D. L.; Boyce, C. W. *J. Org. Chem.* **2000,** *65*, 4088.

[195] Gaillard, S.; Papamicaël, Marsais, F.; Dupas, G.; Levacher, V *Synlett*, **2005**, *3*, 441.

When this ring closure was tried with compound **72** and **77,** utilizing 2,2,3-tribromopropanal **83** generated *in situ* in acetic acid,[192a, 193, 194] which is reacted in neat conditions the aromatic amine.[192b] With both anilines **72** and **77** only yields of 3,6-dibrominated quinolines could be obtained, together with considerable amounts of tar, in accordance with the observations of Tinsley. It cannot be established whether the brominating agent at position 6 was the tribromopropanal **83**, a decomposition product or an intermediate of the reaction. It has been demonstrated[196] that α-bromoacrolein decomposes to a dimer and molecular bromine, so that the latter may be the likely brominating agent.

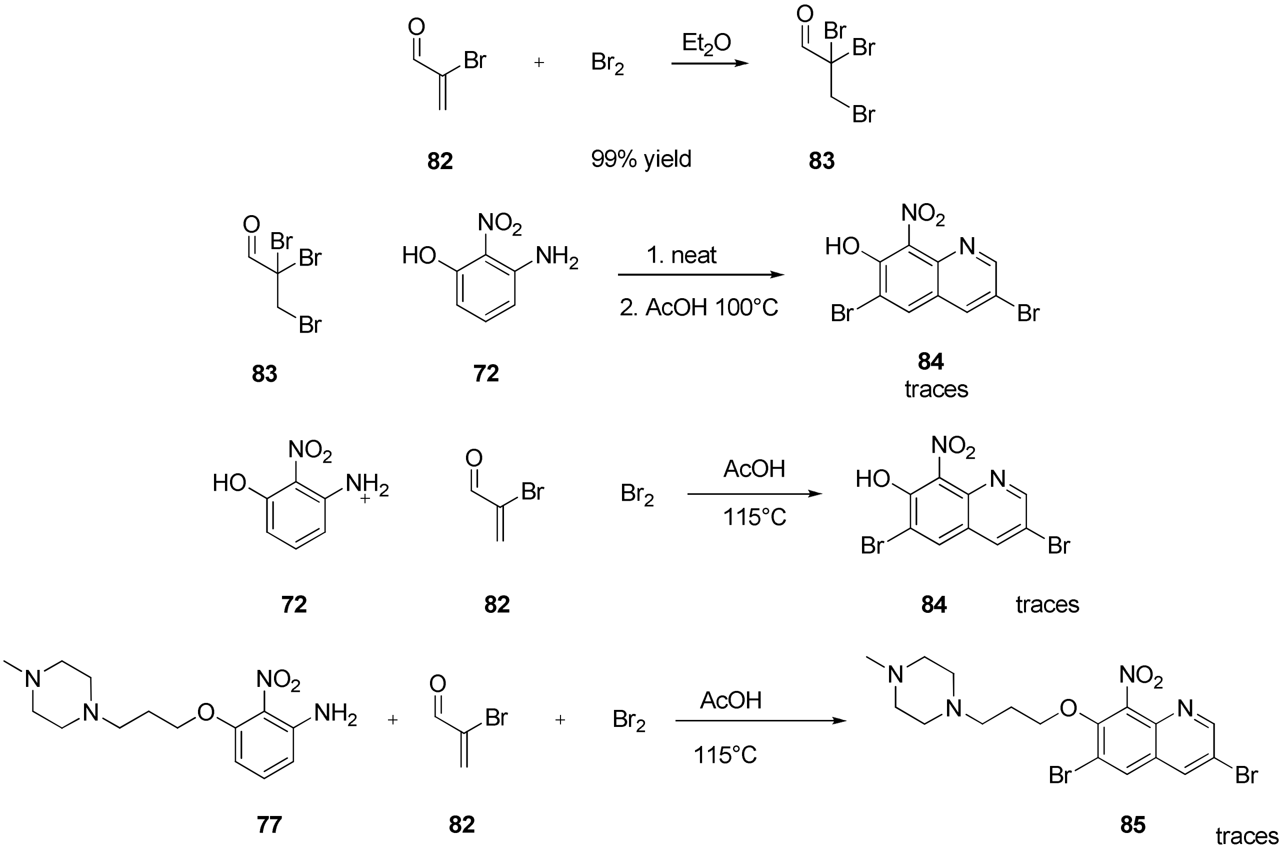

Figure 2.48: *Ring closure with brominated aldehydes.*

The desiders 3-brominated nitroquinoline **86** was finally obtained, albeit with a modest 10% yield, by using a modified version of Yale's procedure[189]. Nevertheless, if the reaction is performed at temperatures higher than 50°C, a bromination of the aniline ring occurs also in this case, even before the ring closure, yielding the byproducts **86a** and **86b**[197].

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[196] α-bromoacrolein has been reacted in a test tube with 1-hexene, and 1,2-dibromohexane has been found on the reaction mixture.

[197] Compounds **86a** and **86b** have been recognized by ^{1}H-NMR and ESI-MS, but have not been isolated.

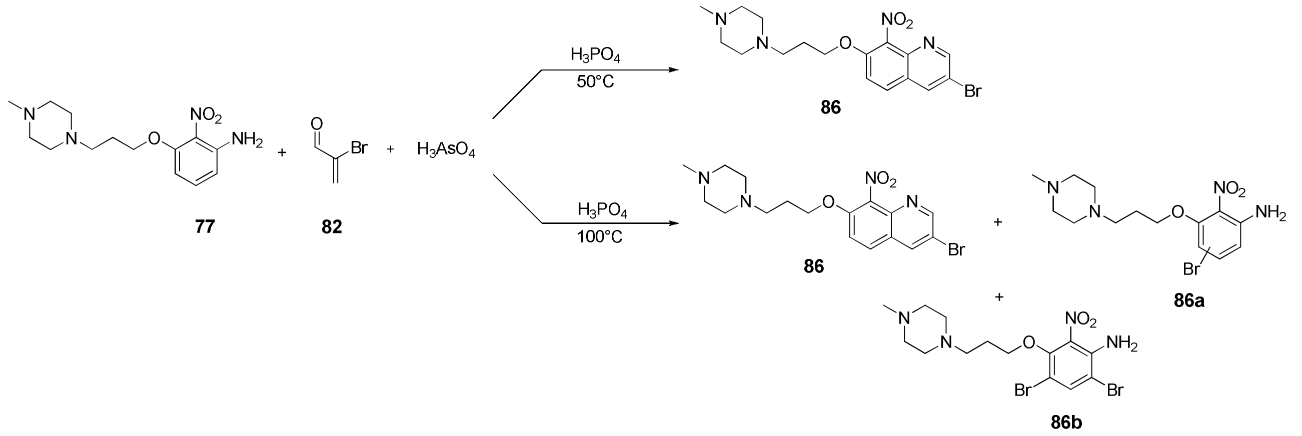


Figure 2.49: *Synthesis of precursor* ***86****.*

In conclusion, the precursor **86**, necessary for the cross-coupling reactions, could be obtained with only one procedure, and with a poor yield.

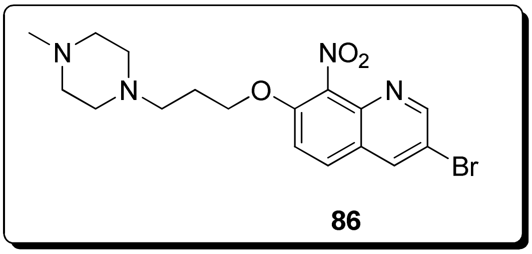


Figure 2.50: *Structure of precursor* ***86.***

2.1.2.4 Substitution at position 3

2.1.2.4.1 Cross coupling reactions for 3-aryl and 3-alkyl substitution

As already reported, aryl halides, and in particular iodides and bromides, can be easily coupled with alkyl or arylboronic acids. Using the same procedures described for the synthesis of 4,7-disubstituited quinolines[167-169], a library of 3-alkyl and 3-aryl substituted-8-nitroquinolines has been created.

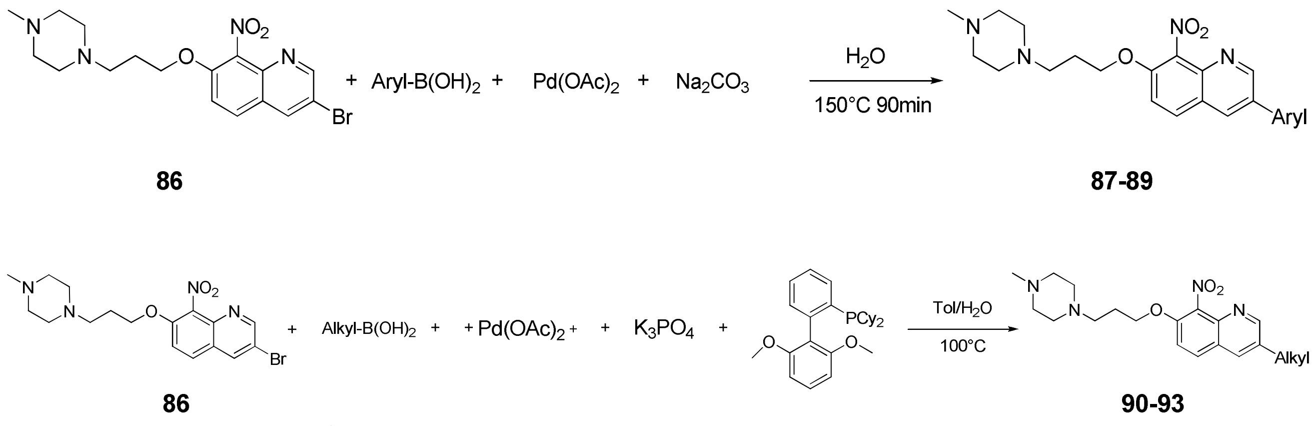
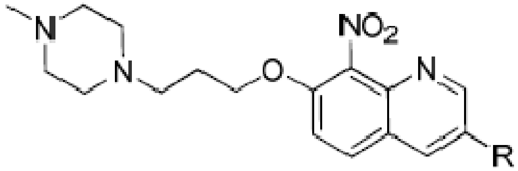
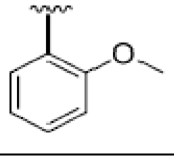
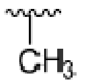
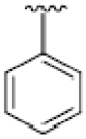
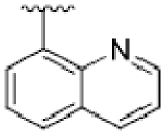


Figure 2.51: *Synthesis of 3,7-disubstituted-8-nitroquinolines.*

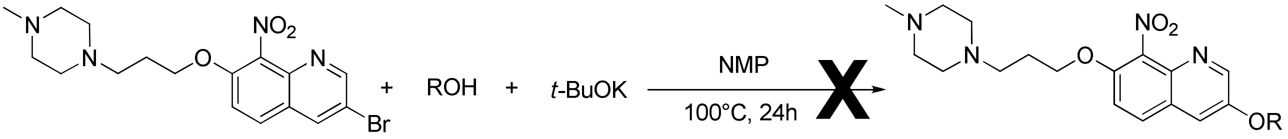
The synthesis of a limited library of 7 products was sufficient for the consumption of the modest quantity of the precursor **86** that we could isolate.

Table 2.6: *3-Aryl-8-nitroquinolines synthesized by Suzuki-Miyaura cross coupling.*



N°	R	yield %	N°	R	yield %
87		80,0	**91**		57,1
88		52,0	**92**		71,5
89		79,7	**93**		49,5
90		42,3			

The position 3 in a quinoline is known to be less electron poor than positions 2 and 4, and hence more prone to electrophilic attack rather than to nucleophilic attack. Nevertheless, we tried the nucleophilic substitution reaction at the 3 position with oxygen linked substituents. But our trials were unsuccessful.



86

Figure 2.52: *Attempt of SN_{Ar} reaction at position 3.*

Therefore, the library of 3,7-disubstituted-8-nitroquinolines is so far limited to carbon linked substituents at 3 position. As usual, they must be reduced to amines in order to be tested in biological assays.

2.1.3 Synthesisis of 3,4,7-trisubstituted 8-aminoquinolines

Also in the case of 3,4,7-trisubstituted-8-aminoquinolines, the quinoline bearing suitable functions at positions 3 and 4 is not commercially available, and we had to proceed to the synthesis of this scaffold anew.

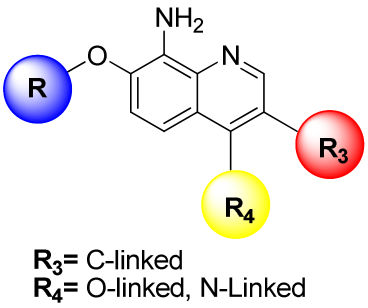


Figure 2.53: *3,4,7-disubstituted-8-aminoquinolines.*

Similarly to the other cases, the amino group could derive from a nitro group (step A) and both R_3 and R_4 could replace halogen atoms (step B). This 3,4-dihaloquinoline can be synthesized by halogenations of the correspondent 3-halo-4-quinolone (step C). In turn, the 4-quinolones could be made via Conrad-Limpach reaction, or with other methodologies that are modifications of this reactions (step D). The construction of the substituted aniline can be carried out with the same procedure used for 3,7-disubstituted quinolines (steps E and F).

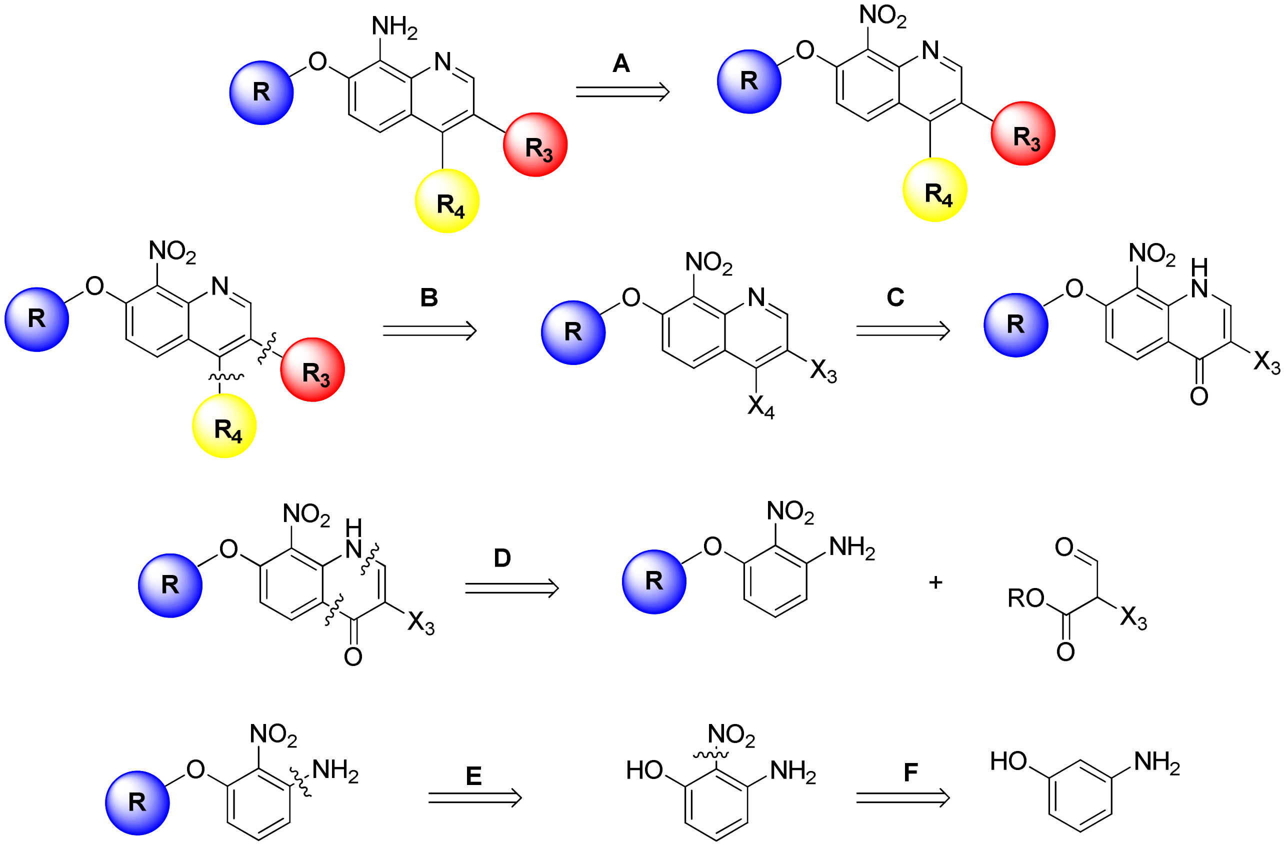


Figure 2.54: *Retrosynthetic approach for the synthesis of 3,4,7-trisubstituted-8-aminoquinolines.*

The actual synthetic route to 3,4,7-trisubstituted-8-aminoquinolines, devised from this retrosynthetic analysis, is presented in **Figure 2.55**.

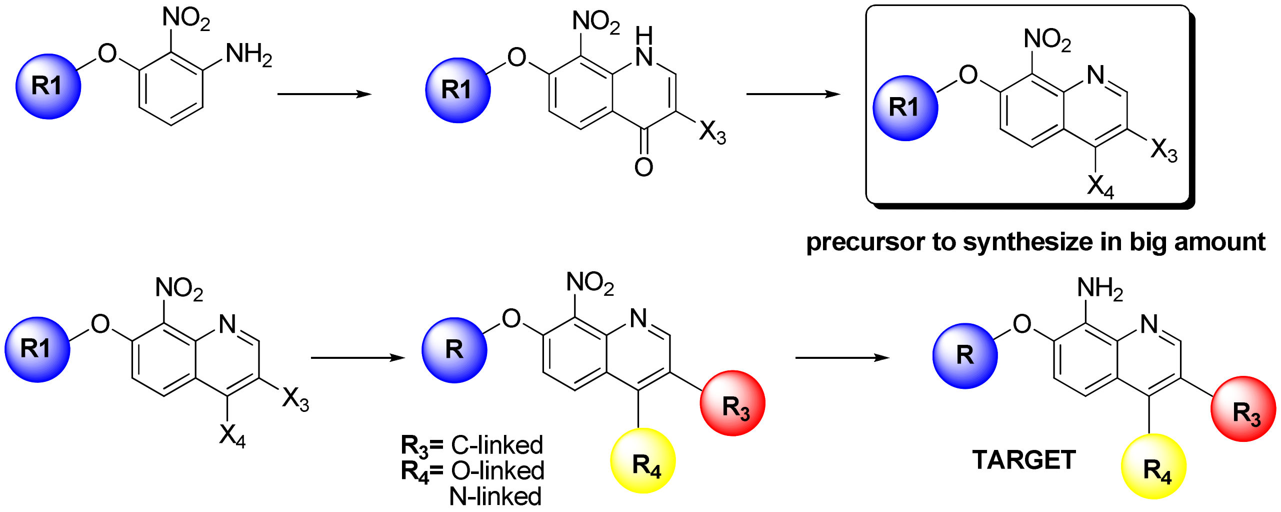


Figure 2.55: *Proposed synthetic route to 3,4,7-trisubstituted-8-aminoquinolines.*

The nitration and alkylation steps of the phenolic oxygen have already be dealt within the previous section and will not be illustrated here. The substituted aniline **72,** the starting point of the synthetic route, has been prepared with the procedures already described. The molecule will then be cyclized in a Conrad-Limpach-type ring closure with the α–halo-β-aldehyde-ester, or by a synthetic alternative, giving the 4-quinolone. This latter compound, treated with a halogenating agent such as $POCl_3$, $POBr_3$, $SOCl_2$, gives the corresponding 3,4 dihaloquinoline. With the procedures already described, the halogen atoms can be substituted with other carbon linked, oxygen linked or nitrogen linked groups. As usual, reduction is the last step that yields the target molecules.

2.1.3.1 Ring Closure and synthesis of ethyl-2-bromo-3-ethoxyacrylate.

The Conrad-Limpach synthesis was already described in the introduction. This reaction between an aniline and a β-keto-ester yields 2-alkyl-4-quinolones. If an aldehyde is present rather than a ketone, and if an R group is α to the ester, 3-substituted-4-quinolones should be formed. No example of these reactions are reported in literature. On the contrary, the Gould-Jacobs reaction,[198] a modification of the Conrad-Limpach synthesis, is largely employed.

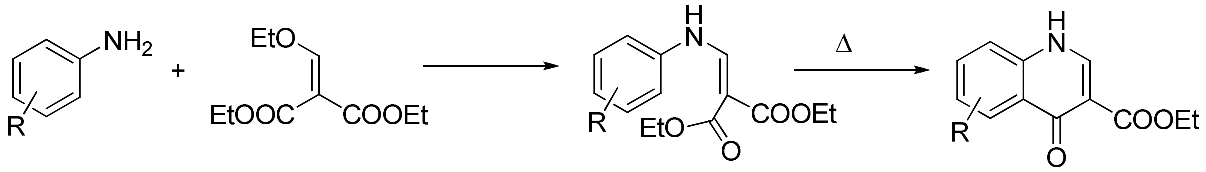


Figure2.56: *General mechanism for the Gould-Jacobs reaction.*

In this reaction the aniline reacts with diethyl 2-(ethoxymethylene)malonate to give the intermediate 2-(anilinomethylene)malonate, which then cyclizes to 3-carboxyethyl-4-quinolone. It's important to notice that the vinyl ether is a synthetic alternative to the unavailable aldehyde, because the intermediate product formed is the same that would arise from the reaction of the aniline with the corresponding aldehyde. A modification of the reaction involves the use of ethyl 2-cyano-3-

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[198] (a) Gould, R. G.; Jacobs, W. A. *J. Am.Chem. Soc.* **1939**, *61*, 2890. (b) Reitsema, R.H. *Chem. Rev.* **1948**, *43*, 53. (c) Elderfield, R.C. *Heterocyclic compounds* **1952**, *4*, 38. (d) Price, C.C.; Roberts, N.; *Org. Synth. Coll. Vol. III* **1955**, 272. (e) Markees, D. G.; Schwab, L. S. *Helv. Chim. Acta* **1972**, *55*, 1319. (f) Albrecht, R.; Hoyer, G.A. *Ber* **1972**, *105*, 3118. (g) Baker, J. M. *et al. J. Chem. Res.* (S) **1980**, 4. (h) Kim, J.N.; Lee, K.Y.; Kim, H.S.; Kim, T.Y. *Org. Lett.* **2000**, *2*, 343.

ethoxyacrylate, which is a derivative of diethyl 2-(ethoxymethylene)malonate[199]. This means that this reaction is versatile, so that it can be hopefully performed with other aldehyde analogues that will put a different subbstituent at the position 3.

Actually, our synthetic strategy is aimed at substituting one of the ester of diethyl 2-(ethoxymethylene)malonate with a bromine atom, thus leading to 3-bromo-4-quinolones as explained in **Figure 2.57**.

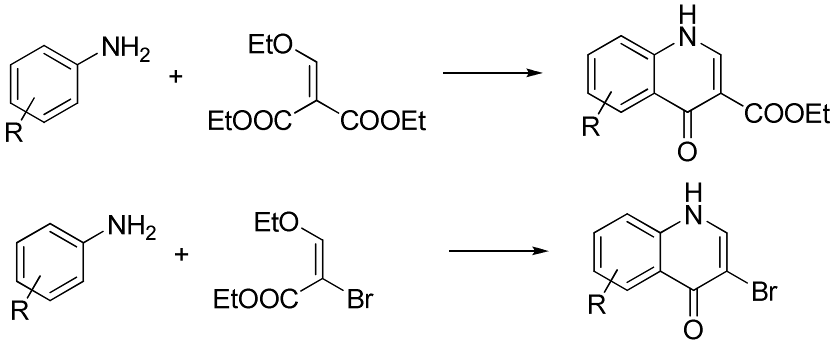
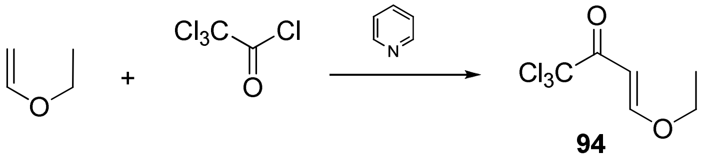


Figure 2.57: *Modification of Gould-Jacobs procedure.*

The compound necessary for this ring closure, 2-bromo-3-ethoxyacrylate, was not commercially available and had to be synthesized anew. The preparation of this molecule is described by Tietze,[200] and involves the first addition of ethylvinylether to trichloroacetylchloride, followed by bromination and alcoholysis of the CCl_3 moiety, which is a good leaving group.



94

Figure 2.58: *Synthesis of (E)-1,1,1-trichloro-4-ethoxybut-3-en-2-one.*

Only the (*E*) isomer of **94** is obtained in the first step. This selectivity is rationalized by the common knowledge that the E_1 elimination in the carbocationic intermediate requires that one bond with hydrogen must be eclipsed with the p orbital, and this occurs in two rotamers. The most stable rotamer, where the two bulkiest groups, the acetyl and the ethoxy groups, are antiperiplanar, leads to the (*E*) isomer.[201] The proof of the regioselectivity is given by the absence of interactions between the two vinylic protons in NOESY spectroscopy. This experimental evidence is supported by the ^3J coupling constant in ^{1}H-NMR spectrum between the two vinylic protons. The measured constant of 12.1 Hz, generally is typical of (*E*) isomers.

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[199] (a)Zhang, N.; Wu, B.; Powell, D.; Wissner, A.; Floyd, M.B.; Kovacs, E.D.; Toral-Barza, L.; Kohler, C. *Bioorg. Med. Chem. Lett.* **2000**, *24*, 2825. (b) Boschelli, D.H.; Wang, Y.D.; Johnson, S.; Wu, B.; Ye, F.; Sosa, A.C.B.; Golas, J.M.; Boschelli, F. *J. Med. Chem.* **2004**, *47*, 1599. (c) Osornio, Y.M.; Miranda, L.D.; Cruz-Almanza, R.; Muchowski, J.M. *Tetrahedron Lett.* **2004**, *45*, 2855. (d) Boschelli, D.H.; Wang, Y.D.; Ye, F.; Wu, B.; Zhang, N.; Dutia, M.; Powell, D.W.; Wissner, A.; Arndt, K.; Weber, J.M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 822.

[200] (c) Tietze L.F.; Meier, H.; Voss, E. *Synthesis* **1988**, 274. (b) For the synthesis of the first intermediate **87** see also: Colla, A.; Martins M.A.; Clar, G.; Krimmer, S.; Fischer, P. *Synthesis*, **1991**, 483.

[201] March, J.; Smith, M. B. *March's Advanced Organic Chemistry* **2001**, Wiley Ed., 5th edition, *chapter 17*, 1318.

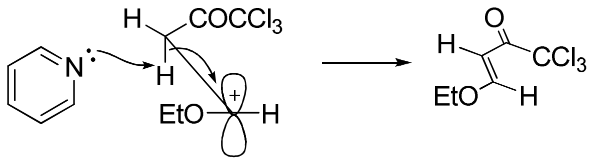

Figure 2.59: *Most stable conformation of the carbocation that leads to the (E) isomer.*

The addition of the vinylether to trichloroacetyl chloride must be done at low temperature and the yield is very good (90%). Bromination is also done at low temperature and gives selectively (*E*)-3-bromo-1,1,1-trichloro-4-ethoxybut-3-en-2-one **95**.

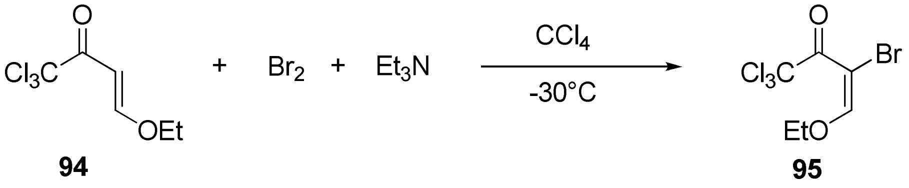

Figure 2.60: *Synthesis of (E)-3-bromo-1,1,1-trichloro-4-ethoxybut-3-en-2-one.*

The high stereospecifity of the reaction can be explained looking at its mechanism. The addition of bromine to the double bond of **95** gives two dibrominated enantiomers, which in the presence of a base undergo E_2 elimination (see **Figure 2.61**). This elimination only occurs when the C-Br bond and one C-H bond form a dihedral angle of 180°, *i.e.* when bromine and hydrogen are in anti-periplanar orientation. This orientation will be found only in those rotamers where the two bulkiest groups, the acetyl and the ethoxy groups, are syn oriented. Thus the elimination causes the generation of the alkene where these two groups are *cis* oriented.[202]

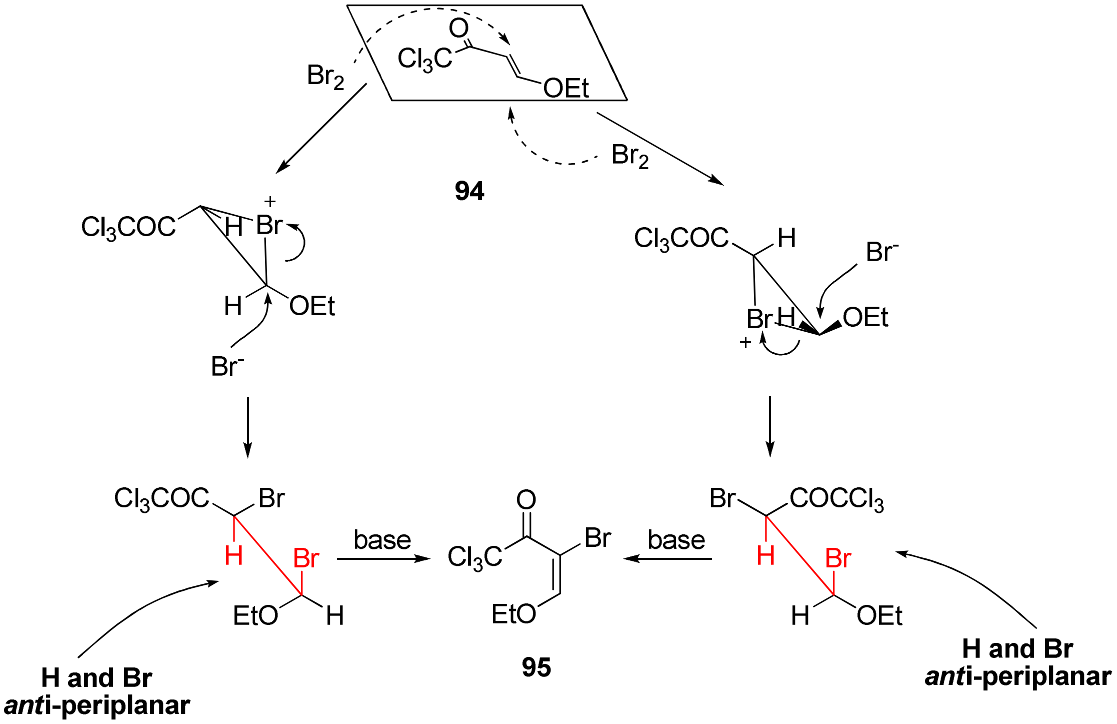

Figure 2.61: *Mechanism of formation of the (E) isomer.*

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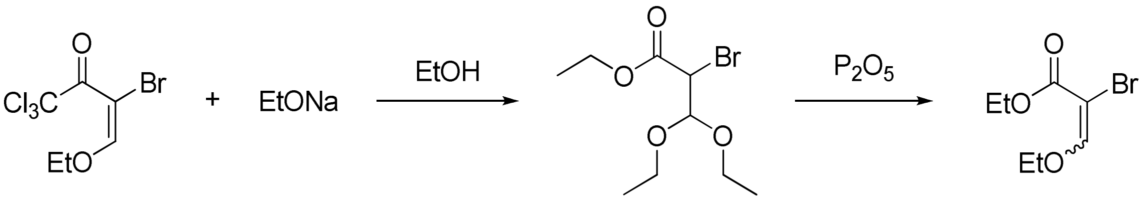
[202] March, J.; Smith, M. B. *March's Advanced Organic Chemistry* **2001**, Wiley ed., 5th edition, *chapter 17*, 1300.

According to Tietze,[200a] the treatment of **95** with catalytic K_2CO_3 in ethanol should afford a 1:1 mixture of ethyl 2-bromo-3,3-diethoxypropanoate **96** and of (*Z*)-ethyl-2-bromo-3-ethoxyacrylate. In our hands this reaction did not work. Therefore we resorted to the reaction of **95** with an excess of sodium ethoxyde in ethanol, gaining **96** as sole product, which in turn can be easily converted to the corresponding vinyl ether **97** with excellent yield.[203]



95 **96** **97**

Figure 2.62: *Synthesis of ethyl 2-bromo-3-ethoxyacrylate.*

We could not determine the configuration of **97**, either (*E*) or (*Z*) which is nevertheless not relevant for the course of following reaction.

Surprisingly, the reaction of **97** with **77** brings about the bromination of benzenic ring (the series of species **86a** and **86b**) and no ring closure, together with the formation of great amounts of tar.

In another trial, we tried the ring closure with the acetal **96**, that is a synthetic alternative to **97**. The use of a catalytic amount of PTSA should lead to the cleavage of the acetal and formation of the aldehyde expected to react in the same way of vinyl ether **97**. Only **86a** and tar have been recovered from the reaction mixture.

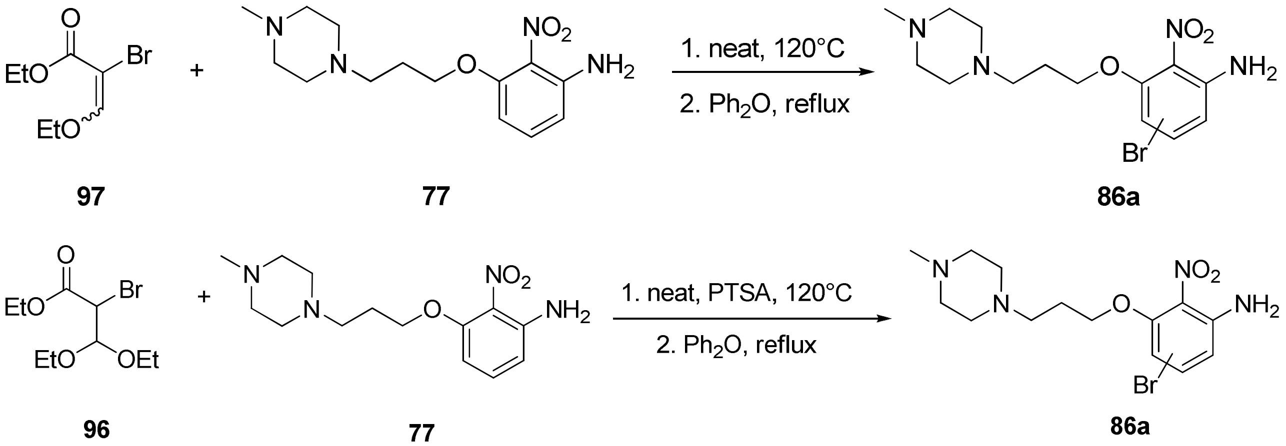


Figure 2.63: *Side-bromination of benzenic ring in the ring closure reaction.*

The behavior of **96** and **97** is similar to what reported for α-bromoacrolein **82**. The compounds **96** and **97** are not stable under the usual reaction conditions. We therefore devised an alternative route, where the bromination step occurs after the ring closure, in order to avoid the utilization of unstable reagent, as well as the easy bromination of the very active benzenic ring.

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[203] March, J.; Smith, M. B. *March's Advanced Organic Chemistry* **2001**, Wiley Ed., 5th edition, *chapter 17-2*, 1329.

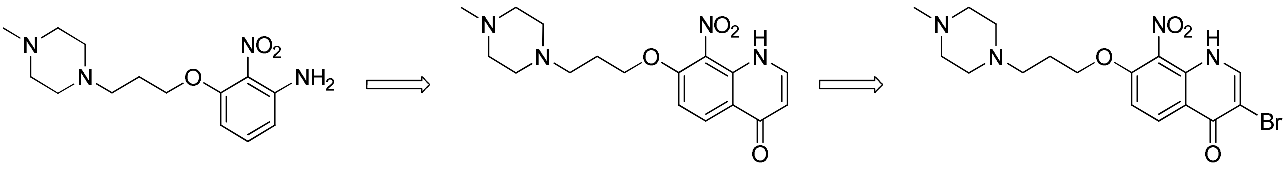

Figure 2.64: *Alternative synthesis of 3-substituted-4-quinolone.*

A methodology for the synthesis of 4-quinolones, reported by many authors,[204] involves the reaction of aniline with Meldrum's acid and ethylorthoformate. The first step of this reaction is a Knöevenagel condensation between ethylorthoformate and Meldrum's acid, generating a vinyl ether. This reagent is attacked by aniline nitrogen, giving a vinyl-aniline specie, which then undergoes thermal cyclization, liberating 4-quinolone, CO_2 and acetone.

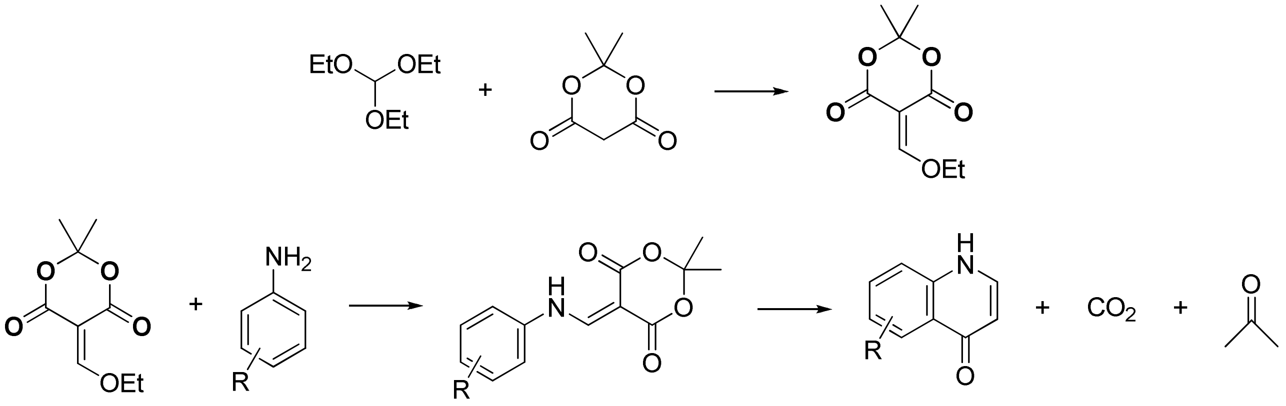

Figure2.65: *Ring closure with Meldrum's Acid.*

The proposed mechanism[204f] for this cyclization is illustrated in **Figure 2.66.** The vinyl-aniline loses one molecule of CO_2 and one of acetone, giving the vynileneketenic intermediate, which cyclizes to 4-quinolone.

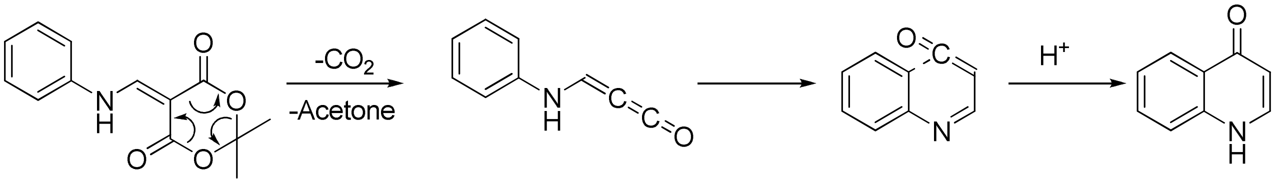

Figure 2.66: *Proposed mechanism for the cyclization of 2,2-dimethyl-5-((phenylamino)methylenyl)-1,3-dioxane-4,6-dione.*

In our trial, molecule **77** undergoes addition to Meldrum's acid vinyl ether but when heated in diphenyl ether for the cyclization, only insoluble tar could be recovered from the reaction mixture. The very high temperature needed for the thermal cyclization could lead to total decomposition of the molecule, we tried therefore the ring closure by heating the intermediate in a microwave oven, in order to reduce the heating time. However, no improvement was observed.

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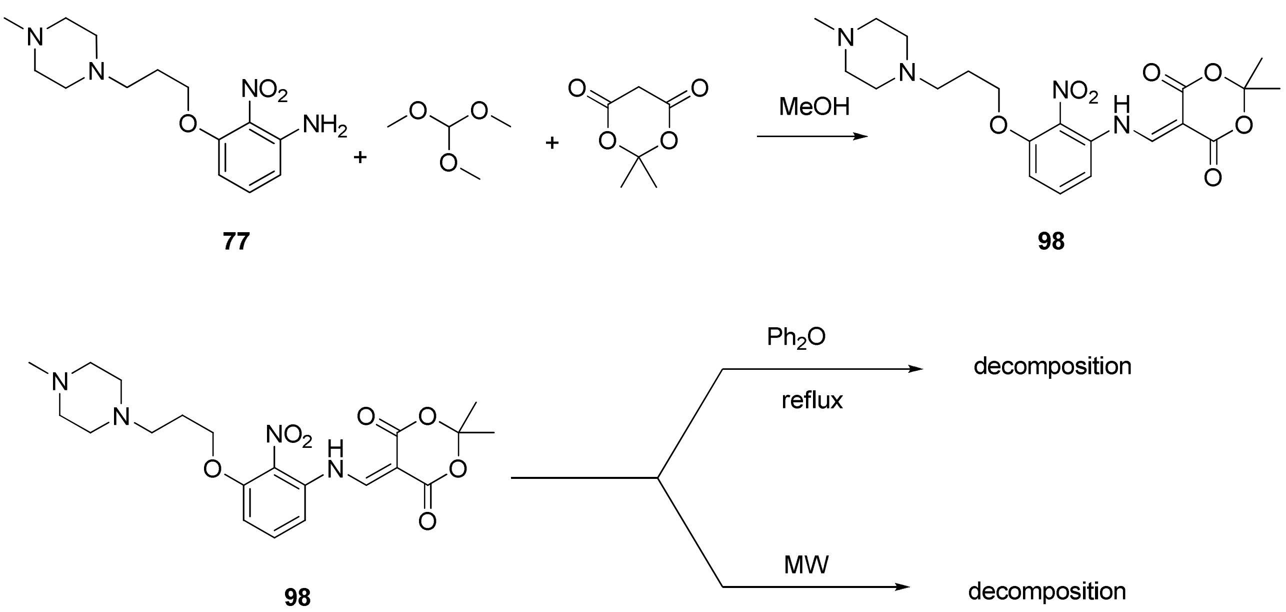
[204] (a) Beifuss, U.; Schniscke, U.; Feder, G. *Tetrahedron* **2001**, 57, 1005. (b)Huang, X.; Liu, Z. *J. Org. Chem.* **2002**, *67*, 6371. (c) Teague, S.J.; Barber, S.; King, S.; Stein, L. *Tetrahedron Lett.* **2005**, *46*, 4613. (d) Al-Awadi, N.A.; Abdelhamid, I.A.; Al-Etaibi, A.M.; Elnagdi, M.H. *Synlett* **2007**, 2205. (e) Peng, L.; Zhang, T.; Ying, L.; Li, Y.; *Synth. Commun.* **2002**, *32*, 785. (f) Walz, A.; Sundberg, R.J. *J. Org. Chem.* **2000**, *65*, 8001. (g) Kitahara, Y.; Tamura, F.; Nishimura, M.; Kubo, A. *Tetrahedron* **1998**, *54*, 8421.



77 **98** **98**

Figure 2.67: *Attempt of cyclization of **98.***

2.1.3.2 Bromination of 4-substituted quinolines

The search of an alternative synthetic route was dictated by the failure of our attempts to obtain 4-quinolones via Gould-Jacobs condensation and thermal cyclization.

The direct halogenation of the quinoline scaffold did not yield the 3-substituted quinoline (see paragraph **2.1.2**). This fact can be probably attributed to the pyridine ring of **81**, which is meta orientating but disactivating in the aromatic electrophilic substitution, and also to interferences of the N-methylpiperazine moiety toward NBS and NCS. We then thought at the possibility of activating the 3 position of quinoline by the introduction of an electron-donating group in 4 position, which is activating and ortho orientating. All the oxygen linked and nitrogen linked molecules synthesized for the 4,7-disubstituted library display this function.

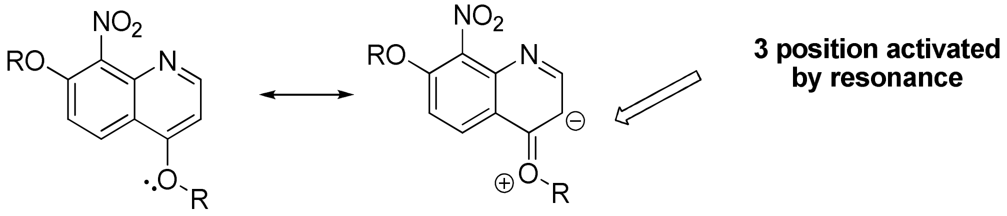


Figure 2.68: *Mesomeric structures showing the activation of position 3 toward aromatic electrophilic substitution in a quinoline subtituted at the 4 position by an EDG.*

The most readily available compound for testing this assumption was the product **12**, arising from the nuclephilic substitution with MeONa of one of the chloro groups of 3,6-dichloro-7-nitroquinoline **3** (the other product **11** is uninteresting). The treatment of **3** with MeONa in NMP as solvent gives **12** selectively. The same procedure, that yelded 3-bromo-8-nitroquinoline **80** from 8-nitroquinoloine **79**, was successful for the bromination of **12** to the desired 3,4,7-trisubstituted compound **99**[1900a].

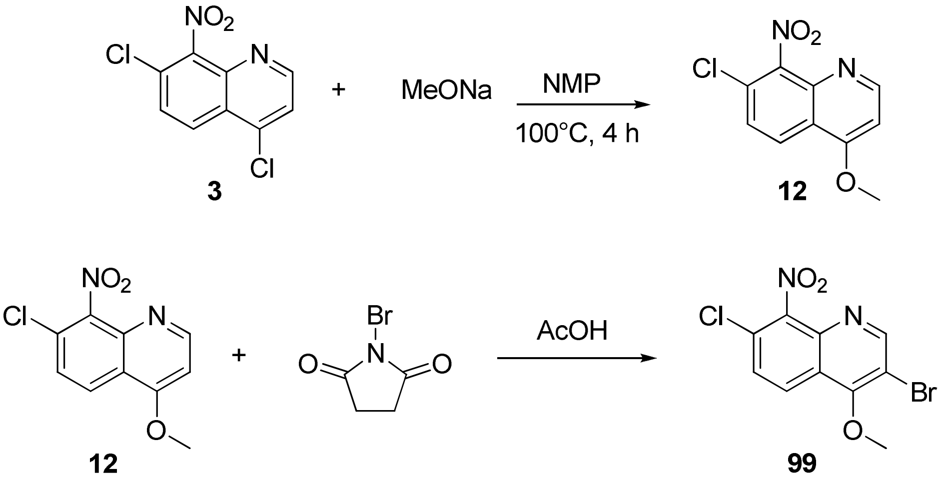


Figure 2.69: *Bromination of activated quinoline scaffold.*

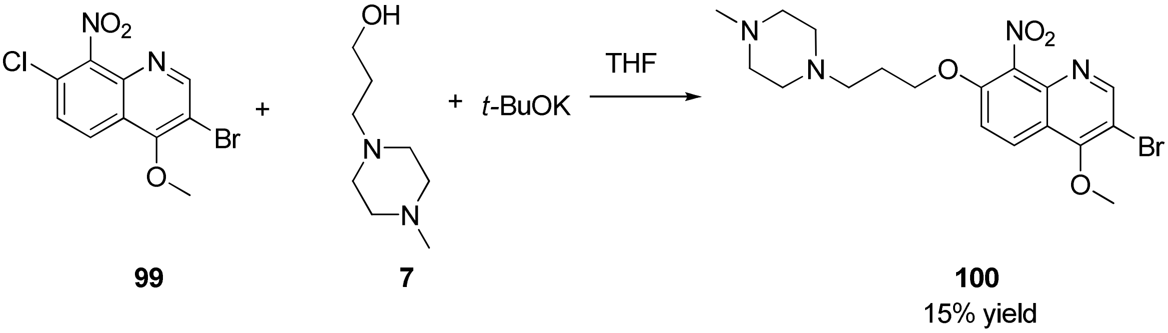


Figure 2.70: *Attachment of side chain at position 7.*

The following step is the attachment of the oxygen linked side chain at position **7**. This nucleophilic substitution occurred selectively, and compound **100** was obtained. Both the pyridine ring and the nitrobenzene ring are activated toward nucleophilic substitution, while the chlorine group and the bromine group are different but comparable leaving groups. The substitution occurs with this selectivity because position 7, *ortho* to the nitro group, is activated for this reaction, while position 3, *meta* to pyridine nitrogen, is not.

From the purification of compound **100**, some crystals of 3-bromo-4,7-dimethoxy-8-nitroquinoline **101** have been recovered. This compound derives from the bromination of 4,7-dimethoxy-8-nitroquinoline **55,** present as an impurity of compound **12**. This impurity can be further functionalized or directly reduced to amino compound in order to be tested. Thus a phenethyl group has been attached via Suzuki cross-coupling reaction,[168,169] with the procedure used for the other compounds.

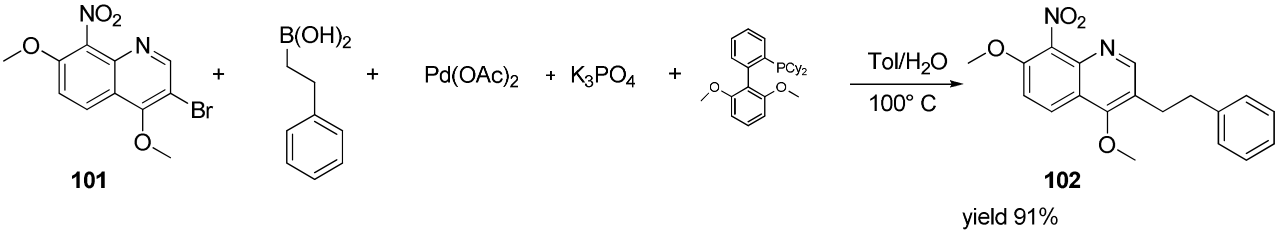
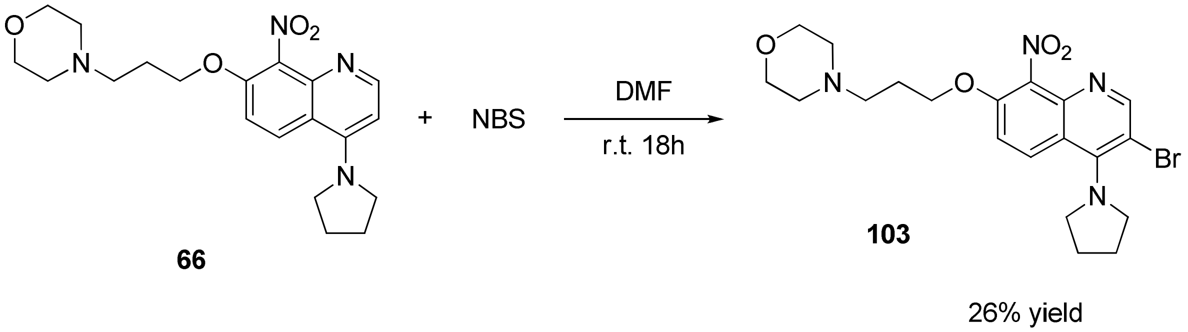


Figure 2.71: *Functionalization at position 3 of compound* **101**.

Compound **66,** with the activating pyrrolidine substituent at position 4, is another readily available molecule that can be tested for the electrophilic bromination. However, the activity of this group can be totally destroyed by the protonation of nitrogen. As a matter of fact, no reaction occurred when **66** was heated with NBS in glacial acetic acid. Mitchell reported a procedure for brominating activated aromatic compound in non acidic media.[205] We employed this procedure successfully, recovering the brominated product **103** in 26 % yield.



66

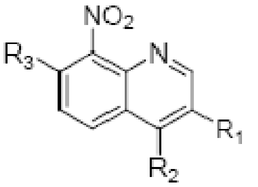
103

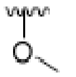
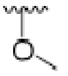
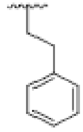
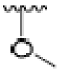
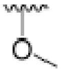
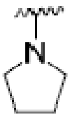
26% yield

Figure 2.72: *Bromination in non acidic solvent.*

The four 3,4,7-trisubstituted molecules **100**, **101**, **102** and **103** (summarized in **Table 2.7**) were reduced to amines in order to be tested in biological assays.

Table 2.7: *3,4,7-trisubstituted-8-aminoquinolines.*



N°	R_1	R_2	R_3	yield %
100				15,0
101				/
102				91,0
103				25,9

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[205] Mitchell, R.H.; Lai, Y.-H.; Williams, R.V. *J. Org. Chem.* **1979**, *44*, 4733.

2.1.4 Reduction of nitro group

From the library of 8-nitroquinoline derivatives, developed in these various synthetic procedures, a new library of 8-aminoquinoline has to be created, that will be tested as TK-inhibitors. Several methodologies are reported in literature for the reduction of nitro aromatic compound to anilines[206]. The most common reducing agents are metals as Zn, Sn, and Fe, but also catalytic hydrogenation is extensively employed[207]. Although the mechanism of these reactions have been scarcely studied, a radical mechanism is generally presented, which, at least with some reducing agents, postulate the presence of nitroso compounds and of hydroxylamines are intermediates. With metals and acids the following mechanism (see **Figure 2.73**)has been suggested.[208]

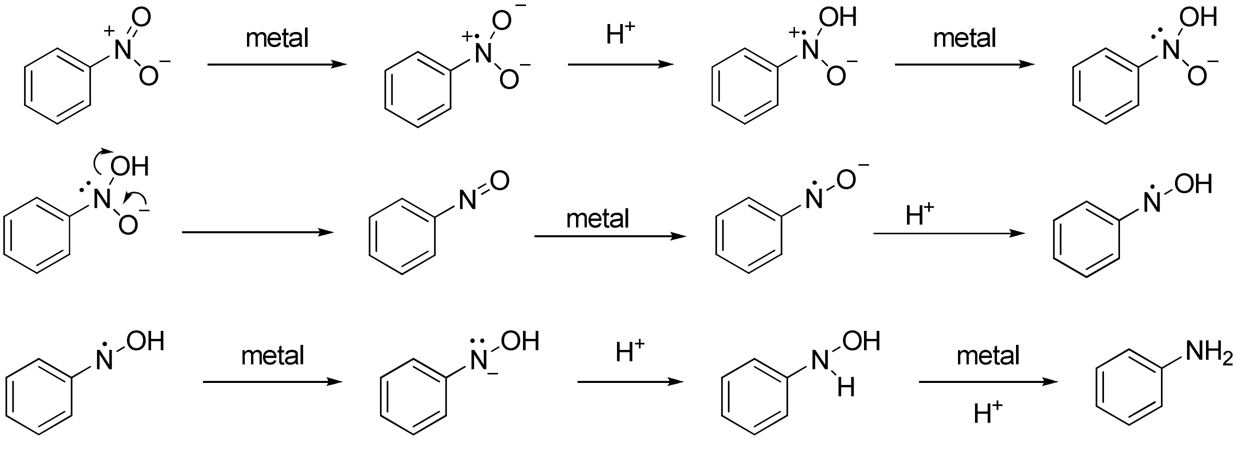

Figure 2.73: *Proposed mechanism for reduction of nitro groups to amines.*

Reduction of aromatic nitro compounds to anilines with tin (II) chloride dihydrate is a widely employed and effective method, [209] therefore we mainly employed this reactant to obtain the 8-aminoquinolines.[209c] Compounds **35** and **36** also require the reduction of the triple bond to a single bond, but $SnCl_2$ is not able to perform the reduction. On the other hand, molecular hydrogen and Pd/C are reported to be able to reduce both the nitro group[210] and triple bond[211]. We employed therefore the procedure reported by Hui-Chang with excellent results. This methodology, for compounds other than **35** and **36**, have been utilized. The reduction procedure specifically employed for every compound is not reported here, but in the Experimental Section (see Chapter **3.2**). We only report the % yields in **Table 2.8-Table 2.13**.

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[206] For an exhaustive review on the methodologies see: Larock, R.C. *Comprehensive Organic Transformations* 2nd Ed. Wiley, 823.

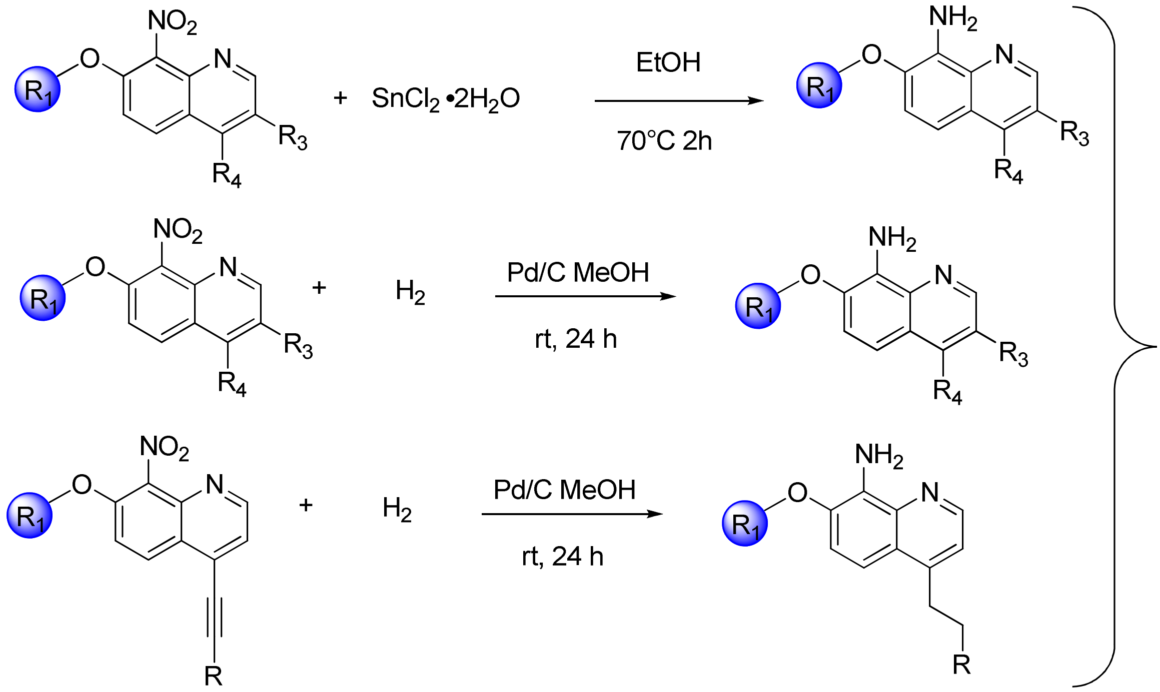
[207] Rylander, P. N. *Hydrogenation Methods* Ref. 497, p.104, *Catalytic Hydrogenation over Platinum Metal* Academic Press, NY, **1967**,168.

[208] March, J.; Smith, M. B. *March's Advanced Organic Chemistry* **2001**, Wiley ed., 5th edition, *chapter 19-12*, 1552.

[209] (a) Bellamy, F.D.; Ou, K. *Tetrahedron Lett.* **1984**, *25*, 839. (b) Bellamy, F.D.; Ou, K *Tetrahedron Lett.* **1984**, *26*, 1362. (c) Gilbert, A.M.; Katz, T.J.; Geiger, W.E.; Robben, M.P.; Rehingold, A.L. *J. Am. Chem. Soc.* **1993**, *115*, 3199. (d) Ling, C.; Lahti, P.M. *J. Am. Chem. Soc.* **1994**, *116*, 8784. (e) Eguchi, S.; Yamashita, K.; Matsushita, K.; Kakehi, A. *J. Org. Chem.* **1995**, *60*, 4006.

[210] (a) Secrist, J.A.; Logue M.W. *J. Org. Chem.* **1972**, *37*, 335. (b) Rogers, M.E.; Averill, B.A. *J. Org. Chem.* **1986**, *51*, 3308. (c) Lee, M.; Lown, J.W. *J. Org. Chem.* **1987**, *52*, 5717. (d) Manabe, K.; Okamura, K.; Date, T.; Koga, K.; *J. Org. Chem.* **1993**, *58*, 6692. (e) Mendenhall, G.D.; Smith, P.A.S. *Org. Syn. Coll. Vol. 5* **1973**, 829. (f) Burdeska, K. *Synthesis* **1982**, 940. (g) Sleath, P.R.; Noar, J.B.; Eberlein, G.A.; Bruice, T.C *J. Am. Chem. Soc.* **1985**, *107*, 3328.

[211] Hui-Chang, Z.; Wei-Sheng, H.; Lin, P.; *J. Org. Chem.* **2001**, *66*, 481.



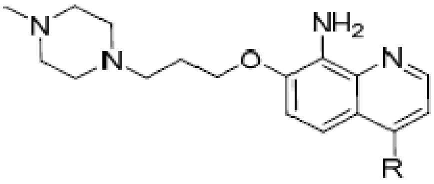
104-170

R_3= H or C-linked
R_4= C-linked, O-linked, N-linked

Figure 2.74: *Reduction of nitro groups.*

All reductions proceeded smoothly to the corresponding amines, but for compound **38,** presenting a benzyloxy subtituent at position 4, the reduction with hydrogen also induced, as expected, the hydrogenolysis of the benzyl group, with the generation of the 4-hydroxyl substituted compound **125** (see **Table 2.8)**.

Table 2.8: *4-Aryl and 4-alkyl 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-aminoquinolines.*



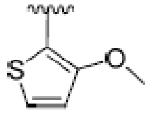
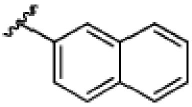
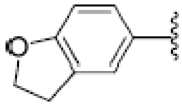
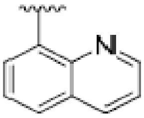
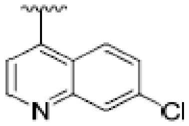
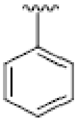
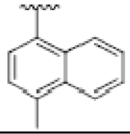
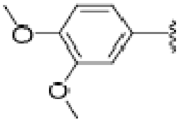
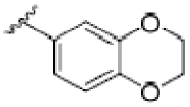
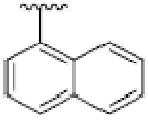
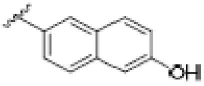
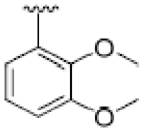
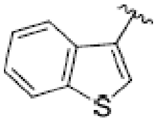
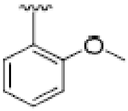
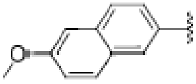
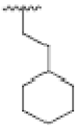
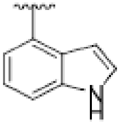
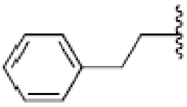
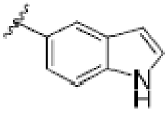
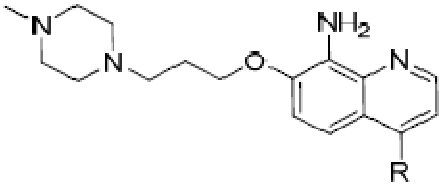
N°	R	yield %	N°	R	yield %
104		87,0	**115**		65,0
105		83,0	**116**		65,0
106		86,0	**117**		60,0
107		71,0	**118**		80,0
108		62,0	**119**		75,0
109		60,0	**120**		60,0
110		98,0	**121**		80,0
111		84,0	**122**		77,0
112		80,0	**123**		83,0
113		90,0	**124**		84,0
114		90,0			

Table 2.9: *4-Oxyaryl and 4-oxyalkyl 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-aminoquinolines.*



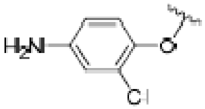
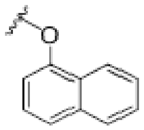
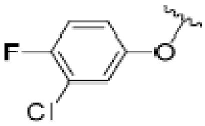
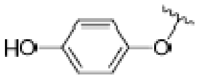
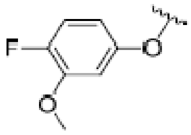
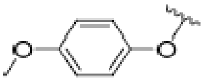
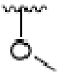
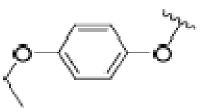
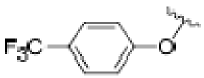
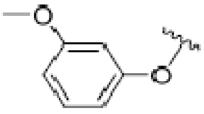
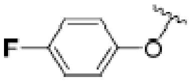
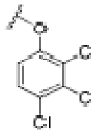
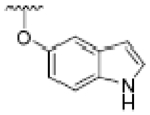
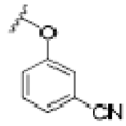
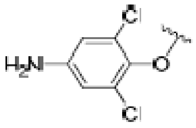
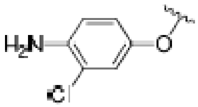
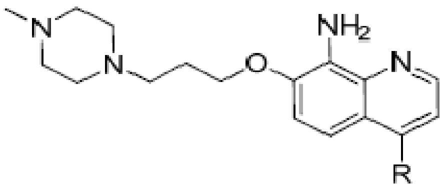
N°	R	yield %	N°	R	yield %
125		98,0	**134**		95,0
126		97,0	**135**		60,0
127		97,0	**136**		60,0
128		83,0	**137**		93,6
129		87,0	**138**		65,0
130		97,0	**139**		70,0
131		76,0	**140**		60,0
132		64,0	**141**		80,0
133		95,0			

Table 2.10: *4-N-aryl and4-N-alkyl 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-aminoquinolines.*



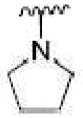
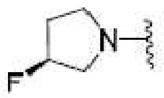
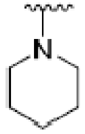
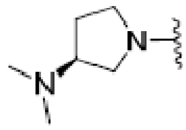
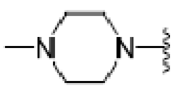
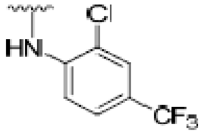
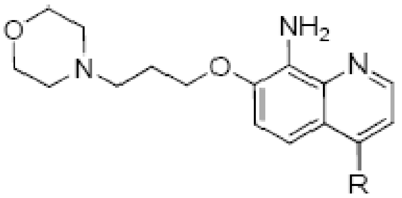
N°	R	yield %	N°	R	yield %
142		60,0	**145**		70,0
143		60,0	**146**		60,0
144		78,0	**147**		60,0

Table 2.11: *4-Oxyaryl and 4-Oxyalkyl 7-(3-morpholinopropoxy)-8-aminoquinolines and 4-N-aryl and 4-N-alkyl 7-(3-morpholinopropoxy)-8-aminoquinolines.*



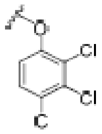
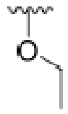
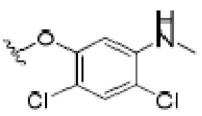
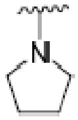
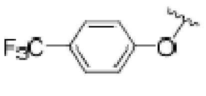
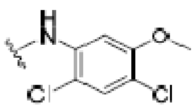
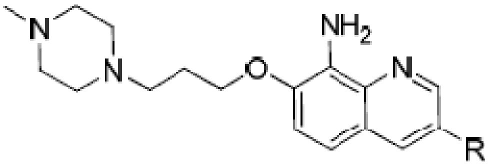
N°	R	yield %	N°	R	yield %
148		74,4	**151**		25,4
149		45,0	**152**		43,0
150		74,4	**153**		85,8

Table 2.12: *3-Aryl and alkyl 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-aminoquinolines.*



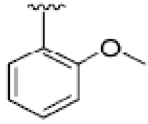
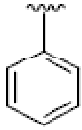
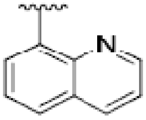
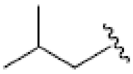
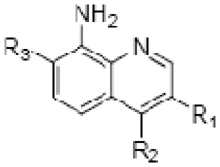
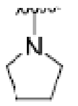
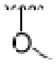
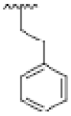
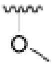
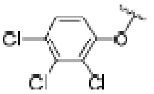
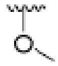
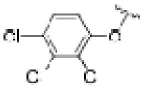
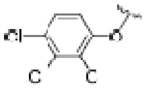
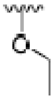
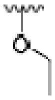
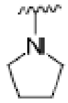
N°	R	yield %	N°	R	yield %
154		32,0	**158**		34,4
155	Br	68,6	**159**	CH_3	77,6
156		54	**160**		82,4
157		79,2	**161**		48,1

Table 2.13: *7-(Oxyalkyl)-3,4-disubstituted-8-aminoquinolines and 7-Oxygen linked-4-substituted-8aminoquinolines.*



N°	R_1	R_2	R_3	yield %	N°	R_1	R_2	R_3	yield %
162				79,4	**167**				39,2
163				79,4	**168**				99,0
164	Br			77,0	**169**				89,1
165	Br			66,0	**170**	Br			26,7
166				85,8					

At the beginning of this research project, only the free bases were subjected to the biological assays. It was later discovered that the proposed inhibitors are always more active in the form of hydrochloride than as free base. Some of these molecules have been therefore tested as salts.

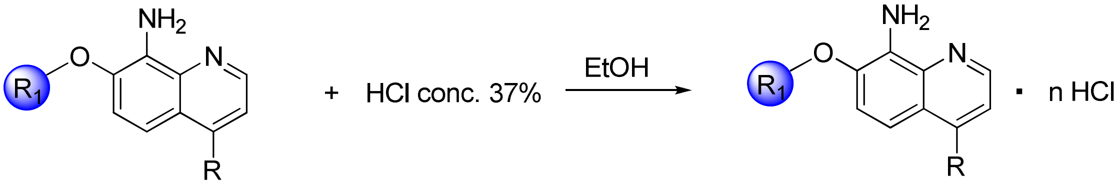


Figure 2.75: *Formation of 8-aminoquinolines hydrochloride salts.*

The molecules have been treated with an excess of chloridric acid. We did not investigate in detail the degree of protonation (mono or polyprotonation), nor which basic nitrogen underwent protonation. Nevertheless it can be assumed that the tertiary amine present at the substituent at position 7 (the nitrogen bearing the methyl group in the case of *N*-methyl piperazine) is the most basic and would be the first one to be protonated. In case of a double protonation, the second most basic center would be more likely the quinolinic nitrogen. Although we did not investigate the degree of protonation, the error in the molecular mass is, on average, no more than 10%, that is within the experimental error of the biological assays.

Potency date are reported on **Table 4.1**, **Table 4.2**, **Table 4.3**, and **Table 4.4**.

2.2 SAR OF THE SYNTHESIZED 8-AMINOQUINOLINES

2.2.1 4-Substituted-8-aminoquinolines

Initially only 4-substituted 8-aminoquinolines have been synthesized and tested (see **Table 4.1**). CPD **104**, the first compound synthesized at all, showed that the novel scaffold is indeed inhibiting the T315I mutant. This compound clearly indicated that the chlorine substituent at position 4 is not sufficient for a nM activity, suggesting the presence of reduced interactions of the atom with the protein. CPD **105,** containing the much bulkier β–naphtyl substituent, showed some improvement in activity compared to CPD **104** indicating stronger apolar interactions of this substituent with the protein. The third compound synthesized CPD **106** containing a quinoline substituent at position 4 was already active in the nM range showing an IC_{50} of 0.57 μM on the Abl T315I mutant and strong inhibition towards Abl WT. In contrast Imatinib and Bosutinib are only active against Abl WT. This compound has been tested as free base and also as hydrochloride salt. The activity of the salt improved by a factor of 3 (IC_{50} = 0.21 μM). The same behavior has been observed with other synthesized compounds. This phenomen was due to the amelioration of the solubility of the compounds under the biological assay conditions. In order to investigate the effect of the aromatic nitrogen of the quinoline substituent of CPD **106**, a 8-aminoquinoline having only a phenyl ring as substituent at position 4 (CPD **107**) was synthesized, and it showed a marked decrease in activity. This decrease and the model of CPD 106 bound to AblT315I let us assume the existence of a weak, most probably water mediated-hydrogen bond between the nitrogen atom of the quinoline and the protein. This assumption was also supported by CPD **109** in which the quinoline substituent was replaced by an α-naphtyl substituent and which shows a 1 log decrease in activity compared to CPD **106**. A reduced activity has been measured also with CPD **117.** This data supports the assumption of the water mediated-hydrogen bond: here the nitrogen of the substituent has been shifted and thus, it is no more able to do any water mediated interactions, leading to a decrease of the activity. Adding a methyl group at position 4 of the α-naphtyl substituent (CPD **118**) the activity can increases again by a factor of 4 compared to compound CPD **109**, because of better interaction with the hydrophobic surrounding. With the aim of further exploring the space around the phenyl substituent of CPD **107** to search for valuable alternative for the conjugated ring system, methoxy groups were added at the *para* and *meta* (CPD **108**) and at the *ortho* and *meta* position (CPD **110**) of the phenyl. CPD **108** was not active against the T315I mutant and only weakly active against Abl WT. This experimental value is in line with the modeling suggesting a possible unfavorable interaction between the sidechain of E286 and the methoxy group in para position. In contrast, CPD **110** showed some weak activity on the gatekeeper mutant indicating that a methoxy group at the *ortho* position is less detrimental compared to one in para. The docking experiment suggests that methoxy group in *ortho* points towards a small hydrophobic pocket lined by residues V299, L370, A380 and the gatekeeper residue. This hypothesis is proven with CPD **111** which has only a methoxy group at the ortho position of the phenyl substituent and which exhibited an activity which is 3 times better than that of compound CPD **110**. CPD **111** is very selective for Abl T315I because no inhibition was observed on the WT enzyme. Replacing the phenyl in CPD **111** with a thiophene (CPD **115**) the activity increase even further, namely by a factor of 4 compared to CPD **111**. In order to see whether an aromatic substituent at position 4 was essential for achieving submicromolar inhibition activity CPD **122** was synthesized. CPD **122** containing an aliphatic sidechain (*n*-hexyl) at position 4 had an activity in the low μM range on both Abl WT and Abl T315I. However this compound showed no cellular activity. Attaching an ethyl-cyclohexyl moiety to position 4 (CPD **123**) didn't lead to strong inhibition of Abl WT and Abl T315. With compound CPD **137** it could be shown that a methoxy group at position 4 is clearly not sufficient to yield good activity. In contrast, when a phenethyl group has been attached (CPD **124**), the activity increased to the nM range. The later data suggest that the 8-aminoquinolines might bind to an intermediate conformations of the protein where the phenyl ring of CPD **124** could establish favourable π–π interactions with the F381 of the DFG motif (see **Figure 2.75**). The next compound synthesized (CPD **143**) with a piperidine ring substituent was only moderately active against the T315I mutant (IC_{50} = 6.2 μM) but had some selectivity for it, as the activity on the WT is about 4 times lower. Interestingly, the very similar CPD **142** with a pyrrolidine substituent instead of the piperidine one was 1 log more active on Abl T315I (IC_{50} = 0.71 μM). From a structural point of view this difference is difficult to explain, however, the conformational analysis of the two compounds suggested that the preferred orientation of the 6-membered saturated ring differs from the one of the 5-membered one. The consequence of this difference is that the pyrrolidine moiety accomodates better in the active site of Abl than the piperidine one. Unfortunately, neither of the two compounds shows activity on cells. Surprisingly adding a fluorine (CPD **145**) or an N-dimethyl group (CPD **146**) to the 3 position of the pyrrolidine substituent, led to much less active molecules compared to the parent compound (CPD **142**). This might indicate some steric clashes of these groups. CPD **125** represents the first compound in which a polar substituent was added at position 4 to prove that apolar substituents in this position are mandatory. As expected this compound was inactive which clearly shows that the 4 position points towards a mainly hydrophobic region which does not tolerate polar substituents. By trying α- and β-indole at position 4 we investigated whether a hydrogen bond donor more distant to the 8-aminoquinoline than in CPD **125** would be tolerated. Compounds CPD **113** and CPD **114** showed that this is indeed the case. Modeling suggests that the NH group of the indole in CPD **114** could make favorable interactions with E286. This statement was further supported by CPD **116** (where an oxygen replaces the nitrogen of the indole and the 5-membered ring is saturated) which shows a 1 log decrease in activity compared to the β-indole substituent. None of the three compounds showed selectivity on cells. CPD **144** containing a *N*-methyl piperazine substituent directly linked to the quinoline ring at position 4 showed no relevant inhibitory activity. This is in line with the observation made with CPD **125** that attaching a polar substituent directly to the 4 position of the 8-aminoquinoline scaffold is not yielding active molecules.

In the following a series of compounds with an oxygen linker were synthesized as it was assumed that such kind of compounds might easier pass the gatekeeper residues and access the hydrophobic pocket. CPD **126** showed only moderate activity on Abl T315I and was weakly selective for Abl T315I while being unselective on cells. CPD **127** contains a *p*-hydroxy-phenyl attached to the oxygen linker. In contrast to what happened when adding a polar group directly to the 8-aminoquinoline scaffold (CPD **125**), this compound retained moderate activity on Abl T315I and showed no selectivity towards Abl WT. It exhibited only week activity on cells. The fact that the hydroxyl group was not detrimental in terms of inhibition supports the hypothesis that the elongated substituent is already pointing towards the phosphate binding site of the ATP binding pocket. Substituting the hydroxyl group with a methoxy group (CPD **128**) resulted in a compound that is still slightly more active on Abl T315I, is more selective towards T315I and more active on cells, though no selectivity was observed there. Shifting the methoxy group in *meta* position (CPD **130**) led to comparable results. However, at the level of the enzyme, CPD **130** is much more selective towards Abl T315I than CPD **128** indicating stronger interactions with the gatekeeper. The 2,3,4-trichloro-phenyl substituent (CPD **131**) is active in the nM range on both Abl variants and has a comparable activity on cells without major selectivity for the transduced cells compared to non-transduced ones. The most reasonable explanation for this observation is to assume that there is another target inhibited by this compound. CPD **133** (*m*-Cl-*p*-NH_2-phenyl), CPD **134** (*o*-Cl-*p*-NH_2-phenyl) and CPD **141** (*o*-di-Cl-*p*-NH_2) showed good activity which again indicates that a polar group at the *para* position of the phenyl is well tolerated. CPD **135** (*m*-Cl-*p*-F-phenyl), CPD **136** (*o*-methoxy-*p*-F-phenyl) and CPD **139** (*p*-F-phenyl) resulted to be less potent suggesting that a fluorine (in contrast to Cl) at the *para* position is lowering the inhibitory activity. The insignificant inhibitory activity of CPD **132** suggests that a cyano group at the meta position of the phenyl ring is not tolerated. Modeling suggests that the cyano group presumably gets too close to the gatekeeper similar to the cyano group in Bosutinib. CPD **140** which is very similar to CPD **113** and CPD **114** but has an oxygen linker showed better inhibitory activity than these compounds. This comparison suggests that the presence of the oxygen linker leads to more favorable distances for the formation of putative hydrogen bonds. Adding a trifluoromethyl to the *para* position of the phenyl substituent led to a moderately potent compound (CPD **138**) (IC_{50}= 9.6 μM) but it's the most selective for Abl T315I even in cells. Modeling suggests that the trifluoromethyl group is in close proximity to the mutated gatekeeper explaining the very different activities (see **Figure 2.75**). CPD **153** has the same substituent of Bosutinib at position 4. While it did not show great activity on the isolated enzymes, it was very active on cells. Nevertheless, CPD **153** did not show a good selectivity toward the Abl T315I transduced cell line, probably because it's also inhibiting another kinase.

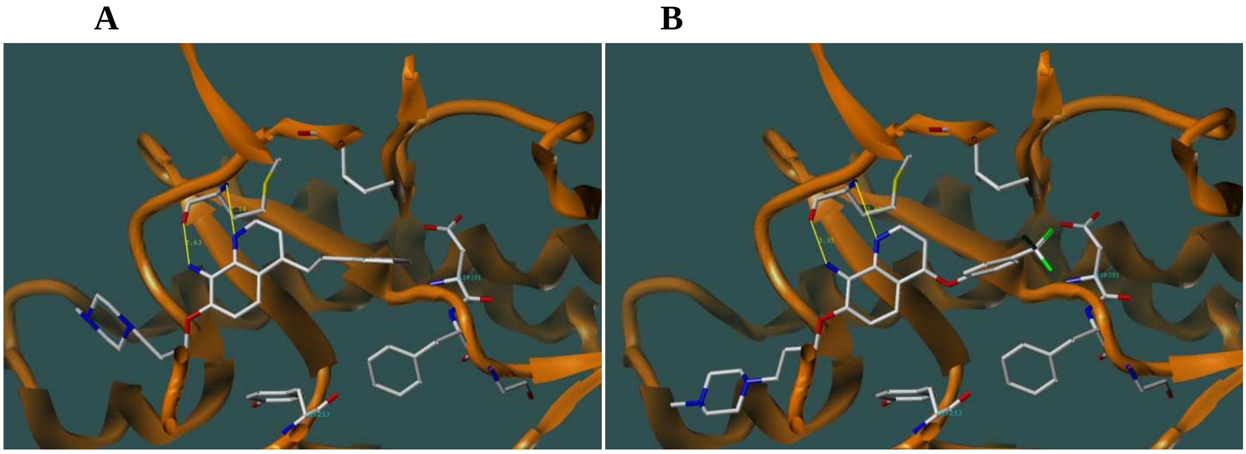


Figure 2.75. ***A)*** *Predicted binding mode of CPD* ***124*** *in the active site of T315I Abl intermediate conformation model.****B)*** *Same as A for CPD* ***138***

In an attempt to explore the importance of the substitution at position 7, two compounds bearing a methoxy group at 7 have been synthesized. These compounds CDP **163** and **162,** where the hydrophilic *N*-methylpiperazine tail was removed, are substructures of two of the most active compounds CPD **131** and **142** respectively. CPD **162** showed a much lower activity than the corresponding compound with *N*-methyl piperazine substituent (CPD **142**) at the level of the isolated kinase domain. This clearly indicates that this moiety of the molecule is making important interactions with the protein. Most probably it forms a hydrogen bond with its protonated nitrogen (the one bearing the methyl) with the backbone carbonyl of Y320. The same behavior was observed for CPD **163**. The cell activity of this compound was remarkable and always higher than the enzymatic one, like its analog CPD **131**, favoring the assumption that there is another target. To further explore the possibility of variation in substituent at position 7, the *N*-methylpiperazine moiety of compounds **131**, **142** and **138** was exchanged by morpholine leading to compounds CPD **148**, **152** and **150,** respectively. Exchanging the N-methylpiperazino substituent at position 7 of CPD **131** with a 2,3,4-trichlorophenoxy group (CPD **168**), the activity drops by a factor of 2-3, again showing that the *N*-methylpiperazine can have good interactions with the protein. No clear trend in terms of cell activity was observed. The activity of CPD **148** and **152** improved, while the one of CPD **150** was reduced. This inconsistency might derive from the fact that the substituent at position 4 and 7 may influence in a dependent manner the orientation of the 8-aminoquinoline scaffold at the hinge region.

2.2.2 3-substituted 8-aminoquinolines

To explore further the chemical space offered by the 8-aminoquinoline scaffold and its modified directionality for the substituents within the binding site compared to the aminoquinoline 3-substituted compounds were dsigned and synthesized. An increased interaction with the gatekeeper and with the the adjacent hydrophobic pocket was aimed by the 3-substitution, because no clear and strong interaction with these moieties of the binding site was observed for most of the 4-substituted 8-aminoquinolines (except for CPD **138**). The 3-position was the initial substitution point for the cyano group of Bosutinib which is involved in a polar interaction with the T315 in Abl WT, now because of the mutation in the gatekeeper, only apolar substituent have been tried (see **Table 4.3**). *Ortho*-methoxy-phenyl substituent (CPD **154**) was not as active as initially expected indicating that this bulky substituent exhibits some steric clashes with the gatekeeper residue. This observation is somehow supported by CPD **156** where the removing of the methoxy group leads to slightly better activity. Having a quinoline as substituent (CPD **157**) instead of a phenyl, does not improve activity in contrast to what has been observed for the 4-substituted compounds, so it’s clear that the 3 position is more constraint than the 4 position.

CPD **158**, CPD **160** and CPD **161** which have respectively *n*-butyl, *neo*-pentyl and *sec*-butyl groups are only weakly active against Abl T315I showing that aliphatic substitution is not favorable. Interestingly the bromo substituent (CPD **155**) is very potent on both the WT and the mutant but there is only moderate cell activity which is probably due to the hydrophobic nature of this compound. The good activity could originate from a putative water mediate interaction of the bromine with the backbone NH of D381 of the DFG motif (see **Figure 2.76**). This hypothesis could also explain the weak activity of the methyl substituent (CPD **159**) that is not able to do any water mediated interaction.

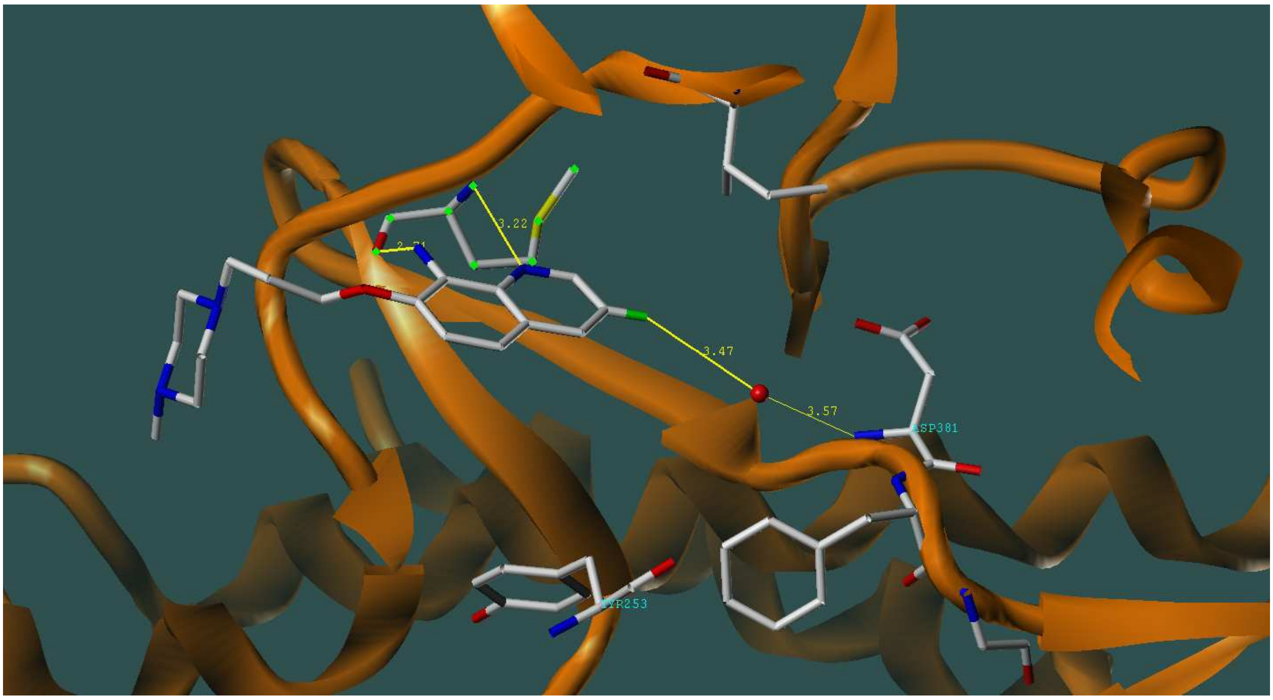


Figure 2.76: *Water mediated interaction between bromine of CPD* ***155*** *and the backbone NH of D381 in T315I Abl intermediate conformation model.*

2.2.3 3-4-substituted 8-aminoquinolines

Based on the results obtained with the single substitution and the derived structure activity relationship a small serie of synthetically accessible 3-4 disubstituted compounds was designed and synthesized (see **Table 4.4**). CPD **164** and **165** possess a methoxy group in position 4 and a Br in position 3 and two different substituent at position 7. The inhibitory activity of both compounds was dramatically decreased compared to the 3-bromine-substituted CPD **155**. Thus, it can be stated that the introduction of a methoxy group at position 4 (CPD **165** and CPD **164**) is detrimental for the inhibitory activity. A plausible explanation is that the methoxy group is displacing the hypothetized important water molecule that allows water-mediated hydrogen bond of the Br substituent with the protein. The direct comparison between CPD **164** and CPD **166** where the Br at position 3 was again removed and two methoxy substituents remained at position 4 and 7 show that bromine still has some favorable interaction besides the water-mediated interaction because the activity of CPD **166** is significantly lower than the one of CPD **164.** Exchanging the Br of CPD **164** with a phenethyl group (CPD **167**) turns out to be more favorable for the double substitution, most probably because of favorable interactions of the phenyl moiety with the gatekeeper residue and surrounding that is not filled by the small methoxy group in position 4. Nevertheless, the inhibitory activity of CPD **167** remained lower compared to the 3-Bromo compound CPD **155**. The 4,7-diethoxy substitution (CPD **169**) is as bad as the already discussed 4,7-dimethoxy one (CPD **167**).

In an attempt to combine the most active substituents at position 3 and 4, CPD **170** bearing a bromine at position 3 and a pyrrolidine at position 4. Unfortunately this combination led to a molecule less active compared to the respective monosubstituted compounds, probably for the same reason stated above for CDP **164** and **165**.

2.2.4 Selectivity profiles of most active compounds

To get a broader idea about the selectivity of the 8 aminoquinolines on kinase in general, 7 compounds (see **Table 4.5** and **Graph 4.1-Graph 4.7**) were subjected to a kinase selectivity panel (Cohen Lab, Dundee) containing 85 kinases of different classes. The 7 tested compounds were chosen according to highest activity data either on Abl WT/Abl T315I or on cells. Three very active compounds (CPD **131**, **106** and **148**) resulted not to be selective on the panel, inhibiting respectively 10, 40 and 11 kinases by more than 50% at a concentration of 10 μM. In contrast CPD **142**, **163**, **167** and **138** are selective, CPD **138** being the most selective one only inhibiting the Aurora A kinases by more than 50% at 10 μM concentration. All other compounds are strongly inhibiting PKBβ, which is a Serine/Threonine kinase, while enzymes more related to Abl, e.g. LCK, SRC and FGFR1, are less targeted by the selected compounds. Insuline receptor kinase that is a clear anti-target is not inhibited by these compounds. Interestingly there is an inverse correlation between the selectivity on cells (transduced cells versus non-transduced cells) and the PKBβ inhibitory activity. The higher PKBβ inhibitory activity is the less selective are the compounds. This indicates that PKBβ is a target for substituted 8-aminoquinolines and to reach higher specificity for Abl T315I substitution favorable for Abl T315I and disfavorable for PKBβ are needed.

In conclusion, a novel active scaffold for the inhibition of Abl WT and Abl T315I has been developed. Very active compounds both on the cellular and enzymatic level have been found. The proposed binding mode has been validated by a consistent SAR.

2.3 CONCLUSIONS AND PERSPECTIVES

All the inhibitors tested in the biological assays have been synthesized with methodologies inspired from literature and procedures set up in our lab. Since the priority in this work was the quick synthesis of a sufficient number of compounds to draw informative structure activity relationship, in general the reaction conditions have not been extensively studied for the optimization of yield and selectivity.

The synthesis of 4-substituted 8-aminoquinolines has been quite easy and straightforward, allowing therefore the obtaining of a large number of different compounds. The most problematic step is the attachment of the side chain at position 7 of the quinoline. The aromatic nucleophilic substitution, yielded the desired 7-Oxygen linked-8-nitroquinoline with a good regioselectivity (6:1) in the case of the 4-iodo substituted compound **10**. On the contrary, the regioselectivity of the synthesis of the 4-chloro substituted compound **8** was poor due to the comparable reactivity of the two chlorine atom of the precursor 4,7-dichloro8-nitroquinoline **3**. The change of reaction conditions (temperature, time, solvent, concentration) of this step of the synthesis could probably lead to better results in terms of regioselectivity, with an improvement of the overall yield of the synthesis.

The synthesis of 3-substituted-8-aminoquinolines instead has been much more complex and less straightforward. The increased difficulty of the synthesis together with the space constrain of the binding site of the target around this position led to the synthesis of a significant lower number of 3-substituted inhibitors. The nitration of *m*-aminophenol yielded as expected three different regioisomers that were quite difficult to separate due to the tendency of the molecules to form cocrystals. Even more difficult was the ring closure of the aromatic amine: the cyclization with acrolein gave the desired product together with considerable amounts of tar. Besides this we have not been able to halogenate the quinoline with the different pursued methods. Nevertheless, further efforts on this side could lead to the desired direct introduction of the halogen atom at position 3, (e.g. halogenating agents in non protic solvents[212]) opening therefore the way for the synthesis of a larger amount of 3-substituted inhibitors. The other synthetic route tried involved the ring closure of the aromatic amine with an already halogenated acrolein, and even if it allowed obtaining the desired 3-substituted-8-aminoquinolines, it needs to be improved. The main problem observed is that α-bromoacrolein thermally decomposes to bromine and polymers, brominating thus the not yet closed aromatic amine. The presence of these brominated byproducts, and most of all, the formation of big amounts of tar, lowered considerably the yield of the reaction. Similarly to the other ring closure, this reaction should be more extensively studied in order to improve yield and selectivity.

The synthesis of 3,4-disubstituted-8-aminoquinolines presented problems similar to those encountered in the synthesis of 3-substituted molecules. The nitration step is the same, and the ring closure, even if different presented the same bromination byproducts. Also in this case the reactant that should lead to the cyclisation of the aromatic amine is an α-bromo-α–β-unsaturated carbonyl compound. It's clear that this type of molecules are not stable under the reaction condition needed, therefore the closure strategy needed to be changed. In fact, the second strategy we pursued consisting in direct bromination of the already formed quinoline scaffold worked out better but only when a highly activating group was present at 4 position. The perspective for the synthesis of these molecules in the future is to find a synthetic route that allows halogenation of position 3 independently from the substituent in position4.

Since the first compound was tested on the Bcr-Abl T315I kinase domain, it emerged that the hypothesis done on the activity of the novel scaffold was correct. Many different type of substituents at position 3 and 4 were assessed to ameliorate the potency and specificity of the inhibitors while exploring the chemical space around the new scaffold. The groups that have been attached can be divided into three big categories: oxygen linked moieties, nitrogen linked moieties and carbon linked moieties. Also the side chain at position 7, that was supposed not to have strong interaction with the protein, has been varied in order to see the effect on the potency of the inhibitors. Activity and selectivity can be gained by exploring the chemical space at position 3 and 4 according to the predicted binding mode. The majority of the compounds were active in the micromolar range and some of them were not active at all. Some compounds resulted active in the nanomolar range, and 7 of them have also been tested toward a panel containing 85 kinases of different classes in order to verify their selectivity (Cohen Lab, Dundee). The 7 tested compounds were chosen according to highest inhibitory activity data either on Abl WT/Abl T315I kinase domain or on cells. Three very active compounds (CPD **131**, **106** and **148**) resulted not to be selective on the panel, inhibiting respectively 10, 40 and 11 kinases by more than 50% at a concentration of 10 μM. In contrast compounds **142**, **163**, **167** and **138** resulted to be more selective, compound **138** being the most selective one because it only inhibits the Aurora A kinases by more than 50% at the 10 μM concentration. All other compounds strongly inhibited PKBβ (also named AKT2), which is a key Serine/Threonine kinase for cell proliferation, while enzymes more related to Abl, e.g. LCK, SRC and FGFR1, are less targeted by the selected compounds. Furthermore anti-targets such as insulin receptor kinase was not inhibited by the compounds at 10 μM concentration. Interestingly there is an inverse correlation between the selectivity on cells (transduced cells versus non-transduced cells) and the PKBβ inhibitory activity. The higher the PKBβ inhibitory activity is the less selective are the compounds. This indicates that PKBβ is a target for substituted 8-aminoquinolines and to reach higher specificity for Abl T315I, substitutions favorable for Abl T315I and disfavorable for PKBβ are needed. The inhibition activity towards PKBβ of the 8-aminoquinoline derivatives may also be exploited for developing specific PKBβ

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[212] (a) Nickson, T.E.; Roche-Dolson, C.A. *Synthesis* **1985,** 663**.** (b) Mitchell, R.H.; Lai, Y-H.; Williams, R.V. *J. Org. Chem.* **1979**, *44*, 4733.

inhibitors to be used against cancers in which malfunctioning of PKBβ is responsible for oncogenesis[213].

Concluding, the novel 8-aminoquinoline scaffold resulted to be active on the T315I mutant form of Bcr-Abl. Compound **138** resulted to be the most selective among all the tested compounds, thus its structure must be used as a new starting point for the development of new molecules with improved potency and selectivity in order to be able to enter the preclinical *in vivo* tests.

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[213] (a) Cheng, J.Q.; Altomare, D.A.; Klein, M.A.; Lee, W.C.; Kruh, G.D.; Lissy, N.A.; Testa, J.R. *Oncogene.* **1997**, *14*, 2793. (b) Skeen, J.E.; Bhaskar, P.T.; Chen, C.C.; Chen, W.S.; Peng, X.D.; Nogueira, V.; Hahn-Windgassen, A.; Kiyokawa, H.; Hay, N. *Cancer Cell*. **2006**, *10*, 269. (c). Yoeli-Lerner, M.; Chin, Y.R.; Hansen, C.K.; Toker, A. *Mol. Cancer Res*. **2009**, *7*, 425.

CHAPTER 3

EXPERIMENTAL PART

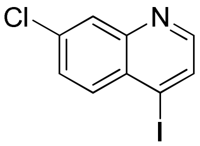
3.1 GENERAL PROCEDURES

Highly pure grade reagents and solvents, purchased directly from the supplier, have been employed without further purification. When required from the procedure, they have been purified according to the known procedures[214]. Anhydrous THF has been prepared just before use, by distillation on sodium in presence of benzophenone under argon atmosphere. Anhydrous NMP have been prepared just before use by distillation at reduced pressure on CaH_2 under argon atmosphere. Chromatographies have been performed on silica gel Merk 60, 230-400 mesh, or basic aluminum oxyde according to the methodologies indicated by Still[215]. Reactions have been monitored with TLC, ^1H-NMR and GC-MS.

^1H-NMR, ^{13}C-NMR, COSY, NOESY, HMQC and HMBC spectra have been recorded with a Varian UNITY 400 MHz or with a Varian UNITY 300 MHz. Resonance frequencies are referred to tetramethylsilane. Electron impact mass spectra EI-MS have been performed with a GC-MS Trace 2000 ThermoQuest, using capillary columns Rtx®-5MS (5% biphenyl/95% dimethylpolisiloxane) of 15 m length and 0.25 mm diameter. Electron spray mass spectra ESI-MS have been performed under negative or positive ionization mode on a API 150 EX Turbo ion spray Amplied Biosystem unit. High resolution mass spectra EI-HRMS analysis were performed under ESI positive ionization mode on a QSTAR XL-MS unit.

3.2 CHARACTERIZATION OF COMPOUNDS

7-Chloro-4-iodoquinoline



2

4M chloridric acid in dioxane (20 mL) was added to a solution of 4,7-dichloroquinoline (10g, 50.5 mmol) in THF (20 mL). After 5 minutes the solvent was removed at reduced pressure. The white solid residue, was dried overnight in a dessiccator containing P_2O_5, and then suspended in dry MeCN (260 mL). Freshly dried NaI (40g, 266 mmol) was added, and the yellow suspension was heated at reflux temperature for 24h. After cooling to r.t., CH_2Cl_2 (100 mL) and a solution of 10% K_2CO_3 and 5% $NaHSO_3$ (200 mL) were added. The acqueous phase was extracted with CH_2Cl_2 (3x50 mL) and the combined organic layer were dried over $MgSO_4$. The solvent was removed under reduced pressure, to give 14.43 g of colorless crystals of 7-chloro-4-iodoquinoline **2** (98% yield).

^1H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.45 (d, 1H, J = 4.5 Hz), 8.06 (d, 1H, J = 2.1 Hz), 7.99 (d, 1H, J = 4.5 Hz), 7.97 (d, 1H, J = 8.9 Hz), 7.57 (dd, 1H, J = 8.9, 2.1 Hz).

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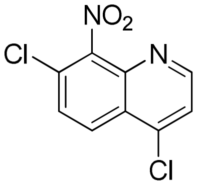
[214] Perrin, D.; Armarengo, W.L.F. *Purification of laboratory chemicals* Pergamon Press, Oxford, **1988.**

[215] Still, W.C.; Khan, M.; Mitra, A. *J. Org. Chem.* **1978**, *42*, 14.

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 150.69, 148.19, 136.46, 133.15, 132.72, 129.09, 128.99, 128.82, 111.42.

MS (70 eV) *m/z*: 289.1 [M^+](66), 162.2 (100), 135.2 (37), 127.2 (49), 99.2 (67) 74.2 (44).

4,7-Dichloro-8-nitroquinoline



3

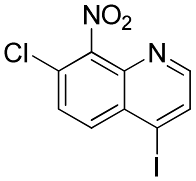
A solution of 65% nitric acid (5g, 3.6 mL, 51.6 mmol) in 97% sulphuric acid (50mL), was added dropwise at 0°C to a solution of 4,7-dichloroquinoline (10g, 50.5 mmol) in 97% sulphuric acid (50mL). The temperature was left to raise to r.t. during 5h. The reaction mixture was added to 200 mL of iced water and a pale yellow solid precipitated. The suspension was filtered and the solid was recrystalized in ethanol (180 mL) to give 8.4 g of 4,7-dichloro-8-nitroquinoline **3** as pale yellow crystals (68 % yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.87 (d, 1H, J = 4.8 Hz), 8.32 (d, 1H, J = 9.1 Hz), 7.72 (d, 1H, J = 9.1 Hz), 7.64 (d, 1H, J = 4.8 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 152.63, 143.01, 141.06, 136.64, 128.36, 126.98, 126.70, 125.60, 122.99.

MS (70 eV) *m/z*: 244.2 [M^+](26), 242.2 [M^+](40), 214.2 (20), 212.2 (32), 186.2 (63), 184.2 (100), 161.2 (48), 126.2 (22), 124.2 (36).

7-Chloro-4-iodo-8-nitroquinoline



4

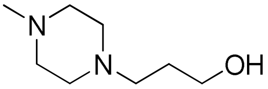
A solution of 65% nitric acid (1.54 g, 1.1 mL, 9.6 mmol) in 97% sulphuric acid (10mL), was added dropwise at 0°C to a solution of 7-chloro-4-iodoquinoline (2.78g, 9,6 mmol) in 97% sulphuric acid (10mL). The temperature was left to raise to r.t. during 5h. The reaction mixture was added to 100 mL of iced water and a pale yellow solid precipitated. The suspension was filtered and the solid was recrystalized in ethanol (40 mL) to give 2.74 g of 7-chloro-4-iodo-8-nitroquinoline **4** as yellow crystals (85%. yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.52 (d, 1H, *J* = 4.6 Hz), 8.13 (d, 1H, *J* = 9.1 Hz), 8.12 (d, 1H, *J* = 4.6 Hz), 7.69 (d, 1H, *J* = 9.1 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 152.18, 139.88, 134.30, 134.13, 129.50, 128.68, 126.88, 111.12, 110.07.

MS (70 eV) *m/z*: 335.9 [M^+](49), 333.9 [M^+](92), 305.9 (26), 303.9 (69), 277.9 (49), 275.9 (92), 163.0 (54), 160.9 (100), 151.2 (45): 148.9 (94), 133.9 (56), 126.1 (66.4), 113.9 (74), 99.0 (82), 74.0 (60.6).

3-(4-Methylpiperazin-1-yl)propan-1-ol



7

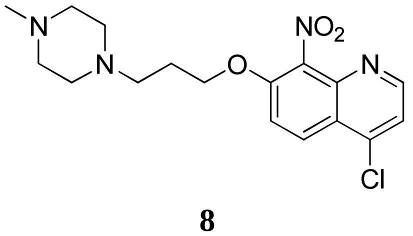
3-Chloropropan-1-ol (9.38 g, 8.3 mL, 99.8 mmol) was added to *N*-methylpiperazine (25 mL) while stirring, and this solution was heated at 90°C for 3h. After cooling to room temperature, 100 mL of ethyl acetate were added and a pale yellow solid precipitated. The suspension was filtered off and the solvent was removed under reduced pressure. The residue yellow dense oil, was distilled under reduced pressure, to give 14.1 g of 3-(4-methylpiperazin-1-yl)propan-1-ol **7** (89.2% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 5.36 (s, 1H), 3.80 (t, 2H, *J* = 5.2 Hz), 2.40 (bs, 8H), 2.62 (t, 2H, *J* = 5.7 Hz), 2.27 (s, 3H), 1.71 (“quintet”, 2H, “*J*” = 5.6 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 64.83, 58.94, 55.13 (2C), 53.32 (2C), 45,97, 26.94.

MS (70 eV) *m/z*: 158 [M^+](37), 113[M^+-C_2H_4OH](65), 70 (100), 56 (35), 43 (79), 42 (99).

4-Chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



8

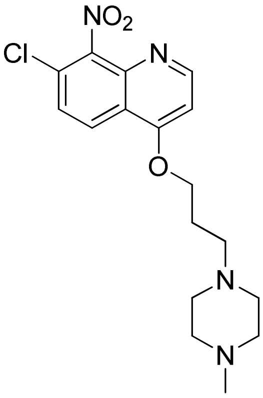
3-(4-Methylpiperazin-1-yl)propan-1-ol **7**(1g, 6.32 mmol) was added to a solution of *t*-BuOK (0.78 g, 6.95 mmol) in dry THF (5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4,7-dichloro-8-nitroquinoline (1.54 g, 6.32 mmol) in dry THF (5mL) at 0°C. The reaction mixture was refluxed overnight. After cooling to r.t. water (30 mL) was added, the acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized with AcOEt (15 mL) to obtain 1.3 g of yellow crystals of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8**. (56% yield)

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.80 (d, 1H, *J* = 4.8 Hz), 8.31 (d, 1H, *J* = 9.4 Hz), 7.52 (d, 1H, *J* = 9.4 Hz), 7.47 (d, 1H, *J* = 4.8 Hz), 4.36 (t, 2H, *J* = 6.2 Hz), 2.44 (bs, 8H), 2.52 (t, 2H, *J* = 7.0 Hz), 2.28 (s, 3H), 2.01 (“quintet”, 2H, “*J*” = 6.7 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 152.40, 150.88, 142.71, 141.55, 136.80, 127.03, 121.03, 120.63, 115.76, 68.53, 55.06 (2C), 54.06, 53.00 (2C), 45.91, 26.39.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 365.1374, observed, 365.1361 $[M+1]^+$

7-Chloro-4-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



9

Method A:

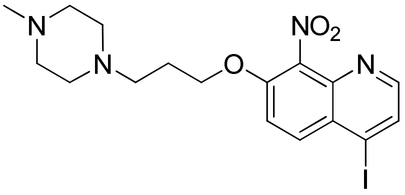
3-(4-Methylpiperazin-1-yl)propan-1-ol **7** (0.5 g, 3.16 mmol) was added to a solution of *t*-BuOK (0.43 g, 3.79 mmol) in dry NMP (5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4,7-dichloro-8-nitroquinoline **3** (0.77 g, 3.16 mmol) in NMP (5 mL) at 0°C. The temperature was left to raise to r.t. overnight. CH_2Cl_2 (20 mL) and water (30 mL) were added, the acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were washed with water (3x30 mL), brine and then dried over $MgSO_4$. The solvent was removed in vacuum, to give 0.41g of yellow crystals of 7-chloro-4-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **9** (36% yield).

Method B:

3-(4-methylpiperazin-1-yl)propan-1-ol **7** (3.3g, 20.60 mmol) was added to a solution of t-BuOK (2.5 g, 22.66 mmol) in dry THF (16 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4,7-dichloro-8-nitroquinoline **3** (5.0 g, 20.60 mmol) in dry THF (16 mL) at 0°C. The reaction mixture was refluxed overnight. After cooling to r.t. water (40 mL) was added, the acqueous phase was extracted with CH_2Cl_2 (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized with AcOEt (15 mL) to obtain 3.0 g of yellow crystals of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (40% yield). The solvent of the mother liquors was removed in vacuum and the residue recrystalized in AcOEt (10 mL) to give 1.0 g of yellow crystals of 7-chloro-4-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **9** (14% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.79 (d, 1H, *J* = 5.2 Hz), 8.24 (d, 1H, *J* = 9.1 Hz), 7.52 (d, 1H, *J* = 9.1 Hz), 6.86 (d, 1H, *J* = 5.3 Hz), 4.29 (t, 2H, *J* = 6.3 Hz), 2.49 (bs, 8H), 2.59 (t, 2H, *J* = 7.2 Hz), 2.29 (s, 3H), 2.13 ("quintet", 2H, "*J*" = 6.7 Hz).

4-Iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



10

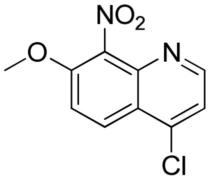
3-(4-Methylpiperazin-1-yl)propan-1-ol **7** (1.65g, 10.40 mmol) was added to a solution of t-BuOK (1.23 g, 10.92 mmol) in dry THF (35 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 7-chloro-4-iodo-8-nitroquinoline **4** (3.48 g, 10.40 mmol) in dry THF (35 mL) at 0°C. The temperature was left to raise to r.t. overnight, water (50 mL) was added, the acqueous phase was extracted with CH_2Cl_2 (3x35 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized with AcOEt (20 mL) to obtain 3.2 g of yellow crystals of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10**. (67% yield)

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.45 (d, 1H, *J* = 4.6 Hz), 8.11 (d, 1H, *J* = 9.4 Hz), 7.96 (d, 1H, *J* = 4.6 Hz), 7.49 (d, 1H, *J* = 9.4 Hz), 4.35 (t, 2H, *J* = 6.2 Hz), 2.46 (bs, 8H), 2.52 (t, 2H, *J* = 7.0 Hz), 2.28 (s, 3H), 2.01 ("quintet", 2H, "*J*" = 6.6 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 151.89, 150.76, 140.17, 136.43, 134.44, 131.88, 124.92, 116.08, 111.05, 68.51, 55.07 (2C), 54.06, 53.01 (2C), 45.92, 26.40.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 457.0731, observed, 457.0717.

4-Chloro-7-methoxy-8-nitroquinoline



11

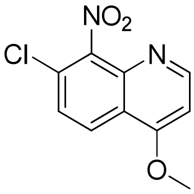
Sodium methoxyde (1.019 g, 3.59 mL of a 30% wt solution in MeOH, 18.87 mmol) was added slowly under argon atmosphere to a solution of 4,7-dichloro-8-nitroquinoline **3** (4.417 g, 18.87 mmol) in dry THF (60 mL). The reaction mixture was stirred for 4h. The solvent was evaporated and CH_2Cl_2 (50 mL) was added. The undissolved solid was filtered off and the CH_2Cl_2 was evaporated. The solid residue was separated from the other regioisomer (7-chloro-4-methoxy-8-nitroquinoline) via flash chromathography on silica gel (eluent AcOEt: hexane 4:6). 1.107 g of 4-chloro-7-methoxy-8-nitroquinoline **11** were obtained (24.6 % yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm):8.79 (d, 1H, *J* = 4.7 Hz), 8.31 (d, 1H, *J* = 9.5 Hz), 7.50 (d, 1H, *J* = 9.5 Hz), 7.46 (d, 1H, *J* = 4.7 Hz), 4.09 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 152.53, 151.24, 142.75, 141.42, 136.41, 128.06, 124.80, 120.66, 101.84, 56.24

MS (70 eV) *m/z*: 238 [M$^+$](100), 208 (72), 180 (90), 127 (40), 126 (42), 117 (53), 114 (84).

7-Chloro-4-methoxy-8-nitroquinoline



12

Method A:
Sodium methoxyde (1.019 g, 3.59 mL of a 30% wt solution in MeOH, 18.87 mmol) was added slowly under argon atmosphere to a solution of 4,7-dichloro-8-nitroquinoline **3** (4.417 g, 18.87 mmol) in dry THF (60 mL). The reaction mixture was stirred for 4h. The solvent was evaporated and CH_2Cl_2 (50 mL) was added. The undissolved solid was filtered off and the CH_2Cl_2 was evaporated. The solid residue was separated from the other regioisomer (4-chloro-7-methoxy-8-nitroquinoline) via flash chromathography on silica gel (eluent AcOEt: Hexane 4:6). 1.107 g of 4-chloro-7-methoxy-8-nitroquinoline **12** were obtained (44.9% yield).

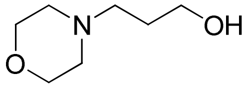
Method B:
Sodium (0.946 g, 41.14 mmol) was dissolved in dry methanol (20 mL) under argon atmosphere. The resulting clear solution was added dropwise to a solution of 4,7-dichloroquinoline **3** (10.0 g, 41.4 mmol) in dry NMP (30 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t., water (150 mL) was added, the acqueous phase was extracted with toluene (3x40 mL) and the combined organic layers were washed with water (3x50 mL), brine (50 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to obtain 8.214 g of 7-chloro-4-methoxy-8-nitroquinoline **12** (83% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.82 (d, 1H, *J* = 5.3 Hz), 8.26 (d, 1H, *J* = 9.0 Hz), 7.54 (d, 1H, *J* = 9.0 Hz), 6.86 (d, 1H, *J* = 5.3 Hz), 4, 09 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 162.16, 154.36, 146.76, 141.29, 126.07, 128.06, 124.80, 120.66, 101.84, 56.24.

MS (70 eV) *m/z*: 238.1 [M^+](79), 208.1 (58), 180.1 (41), 150.1 (100), 123.1 (44), 114.1 (55).

3-Morpholinopropan-1-ol



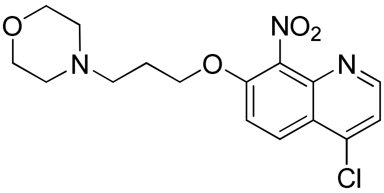
13

3-Chloropropan-1-ol (4.701 g, 8.3 mL, 50 mmol) was added to morpholine (8.72 mL) while stirring, and this solution was heated at 90°C for 10 minutes. After cooling to room temperature, 100 mL of diethyl ether were added and a white solid precipitated. The suspension was filtered off and the solvent was evaporated under reduced pressure to give 7.25 g of a dense oil of 3-(pyrrolidin-1-yl)propan-1-ol **13** (99.8% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 3.76 (t, 2H, *J* = 5.5 Hz), 3.67 (m, 4H), 2.57 (t, 2H, *J* = 5.8 Hz), 2.54 (m, 4H), 2.49 (bs, 4H), 1.69 (“quintet”, 2H, “*J*” = 5.9 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 66.87 (2C), 64.26, 59.01, 53.76 (2C), 26.93.

4-(3-(4-Chloro-8-nitroquinolin-7-yloxy)propyl)morpholine



14

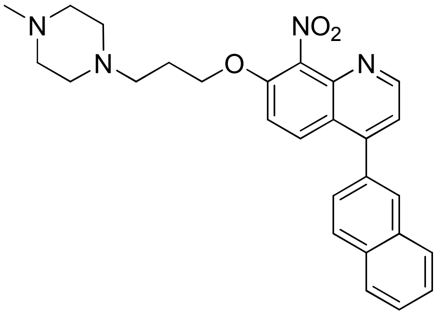
3-Morpholinopropan-1-ol **13** (1.79 g, 12.34 mmol) was added to a solution of *t*-BuOK (1.66 g, 14.80 mmol) in dry THF (37 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4,7dichloro-8-nitroquinoline **3** (3.0 g, 12.34 mmol) in dry THF (35 mL) at 0°C. The temperature was left to raise to r.t. and then the mixture was heated at reflux temperature for 18 h. After cooling to r.t. water (50 mL) was added, the acqueous phase was extracted with CH_2Cl_2 (3x35 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized with AcOEt (20 mL) to obtain 1.37 g of pale yellow crystals of 4-(3-(4-chloro-8-nitroquinolin-7-yloxy)propyl)morpholine **14** (31.6% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.79 (d, 1H, *J* = 4.8 Hz), 8.31 (d, 1H, *J* = 9.4 Hz), 7.51 (d, 1H, *J* = 9.5 Hz), 7.46 (d, 1H, *J* = 4.7 Hz), 4.36 (t, 2H, *J* = 6.2 Hz), 3.70 (t, 4H, *J* = 4.7 Hz), 2.53 (t, 2H, *J* = 7.0 Hz), 2.47 (bs, 4H), 2.02 (“quintet”, 2H, “*J*” = 6.5 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.44, 150.82, 142.74, 141.55, 136.83, 127.09, 121.09, 120.69, 115.71, 68.33, 66.80 (2C), 54.55, 53.58 (2C), 25.99.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 352.1058, observed, 352.1044 .

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(naphthalen-2-yl)-8-nitroquinoline

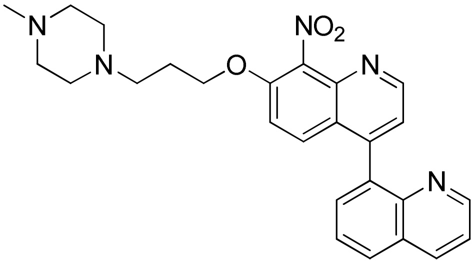


17

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (200 mg, 0.44mmol), naphthalen-2-ylboronic acid (77.4 mg, 0.45 mmol), $Pd(OAc)_2$ (14.8 mg, 0.066 mmol), Na_2CO_3 (0.23 mL of a 2M solution) and water (2 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 180 mg of dark solid of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-2-yl)-8-nitroquinoline **17** (90 % yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.00 (d, 1H, *J* = 4.4 Hz), 8.04 (d, 1H, *J* = 9.4 Hz), 8.02 (d, 1H, *J* = 8.5 Hz), 7.96 (m,3H), 7.61 (m,2H), 7.57 (dd, 1H, *J* = 8.9, 1.7 Hz), 7.42(d, 1H, *J* = 4.5 Hz), 7.37 (d, 1H, *J* = 9.5 Hz), 4.32 (t, 2H, *J* = 6.2 Hz), 2.50 (bs, 8H), 2.53 (t, 2H, *J* = 7.0 Hz), 2.28 (s, 3H), 2.01 (“quintet”, 2H, “*J*” = 6.7 Hz).

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-4-(8’-quinolyl)quinoline



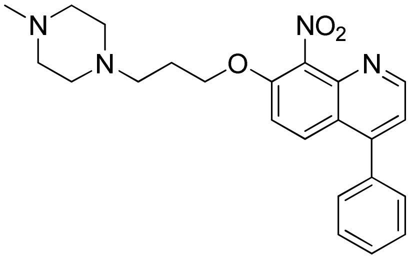
18

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (200 mg, 0.44mmol), quinoline-8-ylboronic acid (77.6 mg, 0.448 mmol), $Pd(OAc)_2$ (14.8 mg, 0.066 mmol), Na_2CO_3 (0.23 mL of a 2M solution) and water (2 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 2 hours the mixture was cooled to r.t., water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 175 mg of dark solid of 7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-4-(8-quinolyl)quinoline **18** (90% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.04 (d, 1H, *J* = 4.4 Hz), 8.80 (dd, 1H, *J* = 4.2, 1.8 Hz), 8.29 (dd, 1H, *J* = 8.3, 1.8 Hz), 8.02 (dd, 1H, *J* = 6.1, 3.5 Hz), 7.70 (d, 1H, *J* = 6.2 Hz), 7.70 (d, 1H, *J* = 3.5 Hz), 7.47(d, 1H, *J* = 9.4 Hz), 7.46 (dd, 1H, *J* = 8.3, 4.1 Hz), 7.43 (d, 1H, *J* = 4.4 Hz), 7.19 (d, 1H, *J* = 9.5 Hz), 4.25 (t, 2H, *J* = 6.2 Hz), 2.50 (t, 2H, *J* = 7.1 Hz), 2.48 (bs, 8H), 2.26 (s, 3H), 1.97 ("quintet", 2H, "*J*" = 6.7 Hz).

ESI-MS (*m/z*) $[M+H]^+$: 458.1.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-4-phenylquinoline

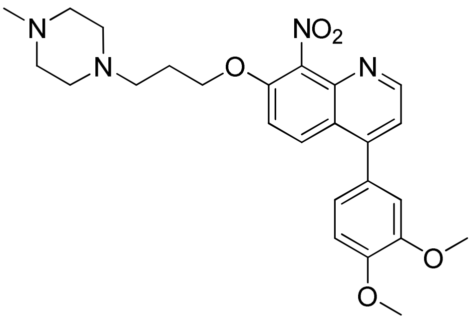


19

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (200 mg, 0.44 mmol), phenylboronic acid (54.7 mg, 0.448 mmol), $Pd(OAc)_2$ (15 mg, 0.066 mmol), Na_2CO_3 (0.23 mL of a 2M solution) and water (2 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 2 hours the mixture was cooled to r.t., water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 179 mg of dark solid of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-phenylquinoline **19** (86% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.96 (d, 1H, *J* = 4.5 Hz), 7.99 (d, 1H, *J* = 9.5 Hz), 7.54 (m, 3H), 7.47 (m, 2H), 7.36 (d, 1H, *J* = 9.5 Hz), 7.32 (d, 1H, *J* = 4.5 Hz), 4.31 (d, 2H, *J* = 6.2 Hz), 2.52 (t, 2H, *J* = 7.0 Hz), 2.47 (bs, 8H), 2.28 (s, 3H), 2.00 ("quintet", 2H, "*J*" = 6.7 Hz).

4-(3,4-Dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

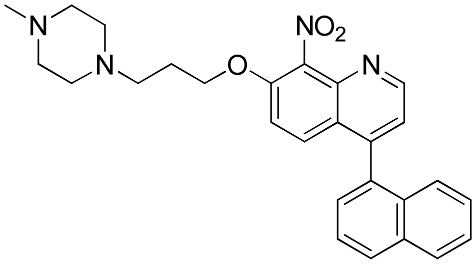


20

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (200 mg, 0.44 mmol), 3,4-dimethoxyphenylboronic acid (82 mg, 0.448 mmol), $Pd(OAc)_2$ (14.8 mg, 0.066 mmol), Na_2CO_3 (0.23 mL of a 2M solution) and water (2 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 6 hours the mixture was cooled to r.t., water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 205 mg of dark solid of 4-(3,4-dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **20** (98 % yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.94 (d, 1H, *J* = 4.5 Hz), 8.06 (d, 1H, *J* = 9.5 Hz), 7.36 (d, 1H, *J* = 9.5 Hz), 7.31 (d, 1H, *J* = 4.5 Hz), 7.04 (m, 2H), 6.97 (m, 1H), 4.32 (t, 2H, *J* = 6.2 Hz), 3.98 (s, 3H), 3.92 (s, 3H), 2.53 (t, 2H, *J* = 7.0 Hz), 2.46 (bs, 8H), 2.28 (s, 3H), 2.01 (“quintet”, 2H, “*J*” = 6.8 Hz).

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yl)-8-nitroquinoline

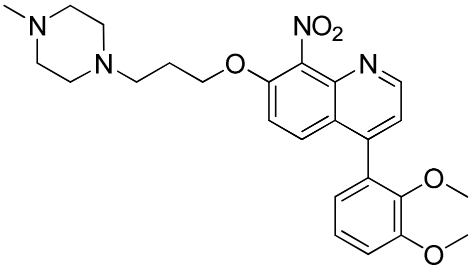


21

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (100 mg, 0.219 mmol), naphthalen-1-ylboronic acid (40 mg, 0.23 mmol), $Pd(OAc)_2$ (14.8 mg, 0.066 mmol), Na_2CO_3 (0.23 mL of a 2M solution) and water (1.5 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 99 mg of dark solid of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yl)-8-nitroquinoline **21** (99 % yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 9.02 (d, 1H, *J* = 4.4 Hz), 8.01 (d, 1H, *J* = 8.3 Hz), 7.96 (d, 1H, *J* = 8.2 Hz), 7.60 (dd, 1H, *J* = 8.3, 8.2 Hz), 7.52 (ddd, 1H, *J* = 8.1, 6.9, 1.3 Hz), 7.48 (d, 1H, *J* = 9.4 Hz), 7.42 (dd, 1H, *J* = 7.0, 1.2 Hz), 7.39 (d, 1H, *J* = 4.4 Hz), 7.36 (ddd, 1H, *J* = 8.1, 7.0, 1.3 Hz), 7.28 (dd, 1H, *J* = 8.5, 1.0 Hz), 7.19 (d, 1H. *J* = 9.5 Hz), 4.24 (t, 2H, *J* = 6.2 Hz), 2.45 (bs, 8H), 2.50 (t, 2H, *J* = 7.0 Hz), 2.25 (s, 3H), 1.97 (“quintet”, 2H, “*J*” = 6.3 Hz).

4-(2,3-Dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

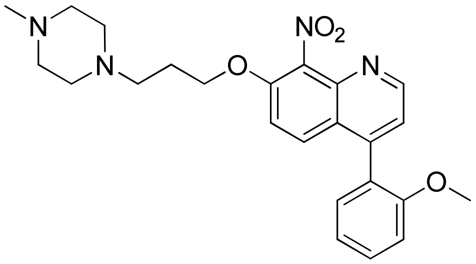


22

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (100 mg, 0.219 mmol), 2,3-dimethoxyphenylboronic acid (43.3 mg, 0.23 mmol), Pd(OAc)$_2$ (14.8 mg, 0.066 mmol), Na_2CO_3 (0.23 mL of a 2M solution) and water (2 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 6 hours the mixture was cooled to r.t., water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 60 mg of dark solid of 4-(2,3-dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **22** (59% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.94 (d, 1H, *J* = 4.5 Hz), 7.73 (d, 1H, *J* = 9.4 Hz), 7.30 (d, 1H, *J* = 4.5 Hz), 7.28 (d, 1H, *J* = 9.4 Hz), 7.19 (t, 1H, *J* = 7.9 Hz), 7.06 (dd, 1H, *J* = 8.3, 1.7 Hz), 6.91 (dd, 1H, *J* = 8.1, 1.2 Hz), 4.27 (t, 2H, *J* = 6.2 Hz), 3.63 (s, 3H), 3.45 (s, 3H), 2.50 (t, 2H, *J* = 7.0 Hz), 2.44 (bs, 8H), 2.25 (s, 3H), 1.97 (“quintet”, 2H, “*J*” = 6.7 Hz).

4-(2-Methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

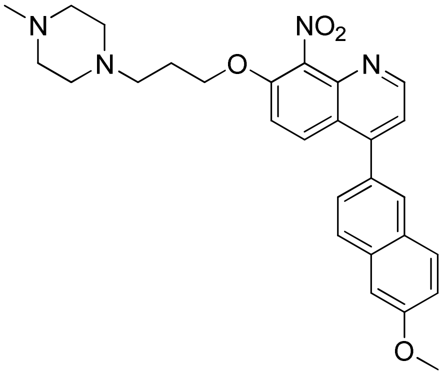


23

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (100 mg, 0.219 mmol), 3,4-dimethoxyphenylboronic acid (35.8 mg, 0.223 mmol, purity 95%), Pd(OAc)$_2$ (7.5 mg, 0.033 mmol), Na_2CO_3 (0.12 mL of a 2M solution) and water (1 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 3.5 hours the mixture was cooled to r.t., water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 90 mg of dark solid of 4-(2-methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **23** (94% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.94 (d, 1H, *J* = 4.4 Hz), 7.67 (d, 1H, *J* = 9.4 Hz), 7.49 (m, 1H), 7.29 (d, 1H, *J* = 4.5 Hz), 7.28 (d, 1H, *J* = 9.1 Hz), 7.22 (dd, 1H, *J* = 7.4, 1.7 Hz), 7.10 (dt, 1H, *J* = 7.4, 0.9 Hz), 7.05 (dd, 1H, *J* = 7.5, 0.9 Hz), 4.28 (t, 2H, *J* = 6.2 Hz), 3.69 (s, 3H), 2.50 (t, 2H, *J* = 7.1 Hz), 2.44 (bs, 8H), 2.25 (s, 3H), 1.98 (“quintet”, 2H, “*J*” = 6.8 Hz).

4-(6-Methoxynaphthalen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



24

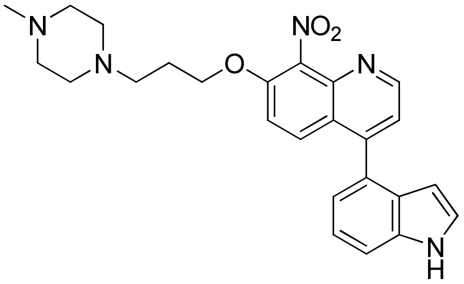
A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (1 g, 2.2 mmol), 6-methoxynaphthalen-2-ylboronic acid (450 mg, 2.2 mmol), Pd(OAc)$_2$ (74 mg, 0.3 mmol), Na_2CO_3 (1.1 mL of a 2M solution) and water (9.9 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (40 mL) was added and the mixture was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was recrystalized in CH_2Cl_2/*n*-hexane to give 963 mg of 4-(6-methoxynaphthalen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **24** as white solid (90 % yield).

^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm): 8.96 (d, 1H, *J* = 4.5), 8.05 (d, 1H, *J* = 9.5), 7.89 (d, 1H, *J* = 8.4), 7.86 (d, 1H, *J* = 1.7), 7.81 (d, 1H, *J* = 8.6), 7.52 (dd, 1H, *J* = 8.4, 1.8), 7.39 (d, 1H, *J* = 4.5), 7.34 (d, 1H, *J* = 9.6), 7.25 (d, 1H, *J* = 8.2), 7.24 (s, 1H), 4.30 (t, 2H, *J* = 6.3), 3.98 (s, 3H), 2.52 (t, 2H, *J* = 7.0), 2.46 (bs, 8H), 2.27 (s, 3H), 2.00 (“quintet”, 2H, “*J*” = 6.7).

^{13}C-NMR (CDCl$_3$, 100 MHz) δ (ppm): 158.59, 152.41, 149.93, 148.74, 141.26, 137.22, 134.55, 132.07, 129.73, 129.05, 128.66, 128.62, 127.41, 127.29, 121.75, 120.99, 119.87, 114.65, 105.71, 68.37, 55.43, 55.40, 55.11 (2C), 54.19, 53.07 (2C), 45.98, 26.48.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 487.2339, observed, 487.2340.

4-(1H-indol-4-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



25

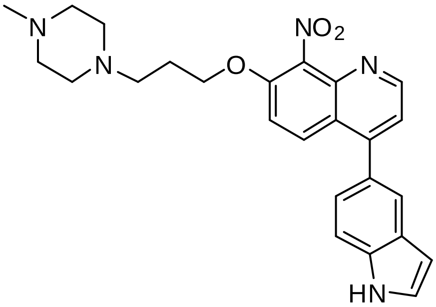
A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (1 g, 2.2 mmol), 1H-indol-4-ylboronic acid (360 mg, 2.2 mmol), Pd(OAc)$_2$ (74 mg, 0.3 mmol), Na_2CO_3 (1.1 mL of a 2M solution) and water (9.9 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (40 mL) was added and the mixture was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 882 mg of 4-(1H-indol-4-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **25** as yellow solid (90% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.99 (d, 1H, *J* = 4.5 Hz), 8.49 (bs, 1H), 7.91 (d, 1H, *J* = 9.5 Hz), 7.56 (dt, 1H, *J* = 8.2, 1.0 Hz), 7.47 (d, 1H, *J* = 4.4 Hz), 7.35 (dd, 1H, *J* = 8.3, 7.2 Hz), 7.26 (d, 1H, *J* = 9.5 Hz), 7.25 (d, 1H, *J* = 2.4 Hz), 7.15 (dd, 1H, *J* = 7.2, 1.0 Hz), 6.17 (ddd, 1H, *J* = 3.3, 2.0, 1.0 Hz), 4.28 (t, 2H, *J* = 6.2 Hz), 2.52 (t, 2H, *J* = 7.1 Hz), 2.47 (bs, 8H), 2.27 (s, 3H), 1.99 (“quintet”, 2H, “*J*” = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.53, 149.92, 147.97, 141.19, 135.91, 129.72, 129.09, 127.19, 125.14, 122.04, 121.92, 121.33, 121.28, 114.19, 111.83, 101.86, 100.22, 68.28, 55.07 (2C), 54.24, 53.04 (2C), 45.94, 26.47.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 446.2186, observed, 446.2181.

4-(1H-indol-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



26

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (1 g, 2.2 mmol), 1H-indol-5-ylboronic acid (360 mg, 2.2 mmol), Pd(OAc)$_2$ (74 mg, 0.3 mmol), Na_2CO_3 (1.1 mL of a

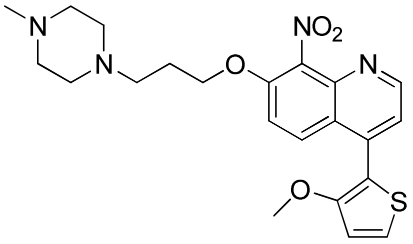
2M solution) and water (9.9 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (40 mL) was added and the mixture was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, the residue recrystalized in CH_2Cl_2/*n*-hexane to give 882 mg of 4-(1H-indol-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **26** as yellow solid (90 % yield).

^{1}H-NMR (DMSO, 400 MHz) δ (ppm): 8.90 (d, 1H, *J* = 4.2 Hz), 8.13 (d, 1H, *J* = 9.6 Hz), 7.74(d, 1H, *J* = 1.6 Hz), 7.72 (d, 1H, *J* = 9.6 Hz), 7.60 (d, 1H, *J* = 8.4 Hz), 7.56 (bs, 1H), 7.49 (d, 1H, *J* = 4.4 Hz), 7.48 (t, 1H, *J* = 2.7 Hz), 7.26 (dd, 1H, *J* = 8.4, 1.7 Hz), 6.55 (m, 1H), 4.34 (t, 2H, *J* = 6.2 Hz), 2.37 (t, 2H, *J* = 7.0 Hz), 2.30 (bs, 8H), 2.12 (s, 3H), 1.86 (“quintet”, 2H, “*J*” = 6.6 Hz).

^{13}C-NMR (DMSO, 100 MHz) δ (ppm): 152.35, 149.69, 149.24, 140.36, 136.13, 136.05, 129.49, 127.87, 127.04, 126.75, 122.58, 121.41, 121.20, 121.16, 115.41, 111.82, 101.68, 68.04, 54.67 (2C), 53.69, 52.59 (2C), 45.65, 26.00.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 446.2186, observed, 446.2191.

4-(3-Methoxythiophen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



27

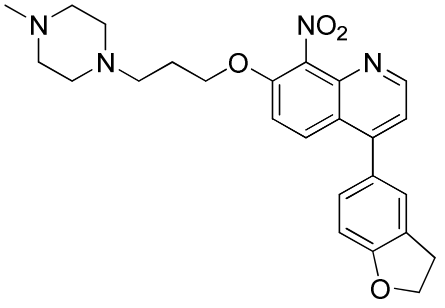
A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (314 mg, 0.7 mmol), 2-(3-methoxythiophen-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (360 mg, 2.2 mmol), $Pd(OAc)_2$ (23 mg, 0.1 mmol), Na_2CO_3 (0.4 mL of a 2 M solution) and water (3 mL) was placed in a 10 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x40 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, the residue recrystalized in CH_2Cl_2/n-hexane to give 201 mg of 4-(3-methoxythiophen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **27** as red solid (65 % yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.88 (d, 1H, *J* = 4.6 Hz), 8.12 (d, 1H, *J* = 9.5 Hz), 7.44 (d, 1H, *J* = 5.5 Hz), 7.38 (d, 1H, *J* = 4.5 Hz), 7.37 (d, 1H, *J* = 9.5 Hz), 7.01 (d, 1H, *J* = 5.5 Hz), 4.30 (t, 2H, *J* = 6.2 Hz), 3.81 (s, 3H), 2.52 (t, 2H, *J* = 7.1 Hz), 2.50 (bs, 8H), 2.29 (s, 3H), 1.99 (“quintet”, 2H, “*J*” = 6.7 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ (ppm): 155.33, 152.13, 149.95, 141.27, 139.70, 137.03, 129.80, 126.23, 122.21, 121.53, 116.86, 114.34, 113.92, 68.32, 58.81, 54.95 (2C), 54.17, 52.82 (2C), 45.78, 26.45.

ESI-HRMS (*m/z*) [M+H]$^+$ calculated, 443.1747, observed, 443.1772.

4-(2,3-Dihydrobenzofuran-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



28

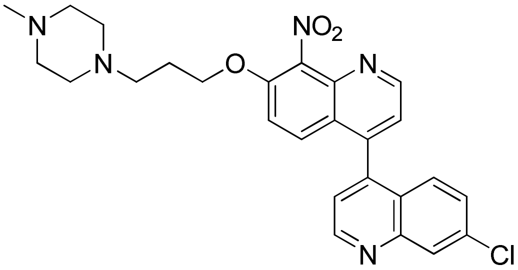
A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (700 mg, 1.5 mmol), 2,3-dihydrobenzofuran-5-ylboronic acid (256 mg, 1.5 mmol), Pd(OAc)$_2$ (50 mg, 0.2 mmol), Na_2CO_3 (0.8 mL of a 2M solution) and water (6.9 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (35 mL) was added and the mixture was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, the residue recrystalized in CH_2Cl_2 to give 500 mg of 4-(2,3-dihydrobenzofuran-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **28** as yellow solid (74 % yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.83 (d, 1H, *J* = 4.5 Hz), 8.01 (d, 1H, *J* = 9.5 Hz), 7.31 (d, 1H, *J* = 9.5 Hz), 7.25 (m, 1H), 7.22 (d, 1H, *J* = 4.5 Hz), 7.16 (dd, 1H, *J* = 8.2, 2.0 Hz), 6.88 (d, 1H, *J* = 8.2 Hz), 4.84 (t, 2H, *J* = 8.8 Hz), 4.26 (t, 2H, *J* = 6.2 Hz), 3.28 (t, 2H, *J* = 8.8 Hz), 2.47 (t, 2H, *J* = 7.2 Hz), 2.42 (bs, 8H), 2.23 (s, 3H), 1.94 (“quintet”, 2H, “*J*” = 6.7).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.94, 152.30, 149.79, 148.75, 141.18, 137.03, 129.64, 129.06, 129.03, 127.98, 126.10, 121.72, 120.62, 114.40, 109.51, 71.64, 68.28, 55.03 (2C), 54.16, 53.00 (2C), 45.92, 29.52, 26.41.

ESI-HRMS (*m/z*) [M+H]$^+$: calculated, 449.2183, observed, 449.2183.

7'-Chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4,4'-biquinoline



29

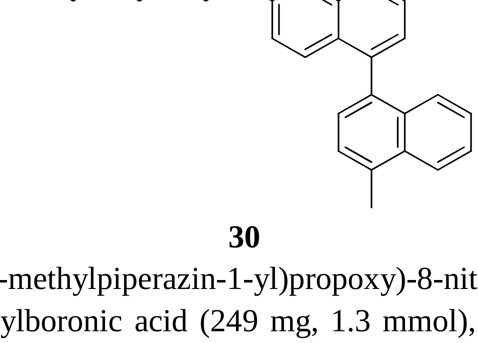
A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (800 mg, 1.7 mmol), 7-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (507 mg, 1.7 mmol), $Pd(OAc)_2$ (56 mg, 0.2 mmol), Na_2CO_3 (0.9 mL of a 2 M solution) and water (8 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (40 mL) was added and the mixture was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, the residue recrystalized in CH_2Cl_2/*n*-hexane to give 585 mg of 7'-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4,4'-biquinoline **29** as yellow solid (yield 65 %).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.04 (d, 1H, *J* = 4.4 Hz), 9.03 (d, 1H, *J* = 4.3 Hz), 8.21 (d, 1H, *J* = 2.2 Hz), 7.38 (d, 1H, *J* = 9.5 Hz), 7.37 (dd, 1H, *J* = 8.9, 2.1 Hz), 7.37 (d, 1H, *J* = 4.4 Hz), 7.36 (d, 1H, *J* = 4.4 Hz), 7.27 (d, 1H, *J* = 9.3 Hz), 7.24 (d, 1H, *J* = 8.9 Hz), 4.75 (bs, 2H), 4.26 (t, 2H, *J* = 6.1 Hz), 2.51 (t, 2H, *J* = 7.0 Hz), 2.51 (bs, 8H), 2.29 (s, 3H), 1.97 (“quintet”, 2H, “*J*” = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.40, 150.90, 150.37, 148.75, 143.79, 143.12, 140.84, 137.07, 136.10, 129.01, 128.52, 128.39, 126.67, 124.98, 121.82, 121.33, 121.18, 115.54, 68.40, 54.67 (2C), 53.99, 52.48 (2C), 52.44, 45.48, 26.27.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 492.1796, observed, 492.1809.

4-(4-Methylnaphthalen-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



30

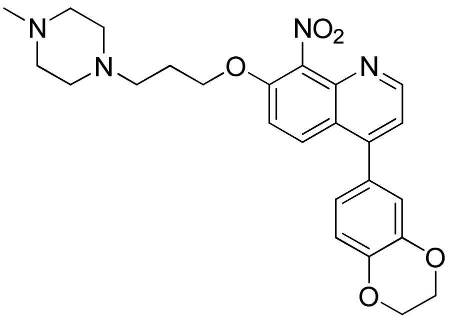
A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (600 mg, 1.3 mmol), 4-methylnaphthalen-1-ylboronic acid (249 mg, 1.3 mmol), $Pd(OAc)_2$ (44 mg, 0.2 mmol), Na_2CO_3 (0.5 mL of a 2M solution) and water (6 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (25 mL) was added and the mixture was extracted with CH_2Cl_2 (3x45 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was recrystalized in CH_2Cl_2/*n*-hexane to give 538 mg of 4-(4-methylnaphthalen-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **30** as grey solid (88 % yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.02 (d, 1H, *J* = 4.4Hz), 8.13 (ddd, 1H, *J* = 8.5, 1.2, 1.0 Hz), 7.57 (ddd, 1H, *J* = 8.5, 6.6, 1.4 Hz), 7.51 (d, 1H, *J* = 9.4 Hz), 7.46 (dq, 1H, *J* = 7.1, 1.0 Hz), 7.39 (d, 1H, *J* = 4.4 Hz), 7.37 (ddd, 1H, *J* = 8.3, 6.7, 1.2 Hz), 7.32 (d, 1H, *J* = 7.1 Hz), 7.31 (ddd, 1H, *J* = 8.5, 1.5, 0.7 Hz), 7.19 (d, 1H, *J* = 9.5 Hz), 4.26 (t, 2H, *J* = 6.2 Hz), 2.82 (d, 3H, *J* = 1.0 Hz), 2.51 (t, 2H, *J* = 7.1 Hz), 2.47 (bs, 8H), 2.27 (s, 3H), 1.98 ("quintet", 2H, "*J*" = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.46, 150.04, 147.81, 140.89, 137.08, 135.80, 132.72, 132.61, 131.71, 129.42, 127.13, 126.34, 126.17, 126.17, 125.99, 124.55, 122.93, 122.14, 114.69, 68.35, 55.08 (2C), 54.16, 53.04 (2C), 45.92, 26.46, 19.55.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 471.2390, observed, 471.2394.

4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



31

A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (600 mg, 1.3 mmol), 2,3-dihydrobenzo[b][1,4]dioxin-6-ylboronic acid (241 mg, 1.3 mmol), $Pd(OAc)_2$ (44 mg, 0.2 mmol), Na_2CO_3 (0.5 mL of a 2 M solution) and water (6 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (25 mL) was added and the mixture was extracted with CH_2Cl_2 (3x45 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was recrystalized in CH_2Cl_2 to give 483 mg of 4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **31** as yellow solid (80 % yield).

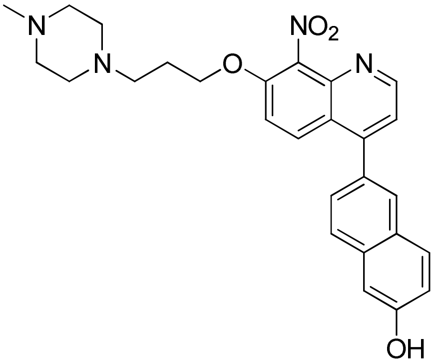
^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.90 (d, 1H, *J* = 4.5 Hz), 8.07 (d, 1H, *J* = 9.4 Hz), 7.34 (d, 1H, *J* = 9.6 Hz), 7.27 (d, 1H, *J* = 4.5 Hz), 7.01 (d, 1H, *J* = 8.2 Hz), 6.98 (d, 1H, *J* = 2.0 Hz), 6.93

(dd, 1H, *J* = 8.2, 2.1 Hz), 4.34 (m, 4H), 4.30 (t, 2H, *J* = 6.2 Hz), 2.51 (t, 2H, *J* = 7.1 Hz), 2.46 (bs, 8H), 2.27 (s, 3H), 1.99 ("quintet", 2H, "*J*" = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.35, 149.87, 148.04, 144.41, 143.75, 141.22, 137.17, 130.12, 129.00, 122.64, 121.58, 120.61, 118.40, 117.67, 114.54, 68.37, 64.48, 64.40, 55.08 (2C), 54.18, 53.03 (2C), 45.93, 26.47.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 465.2132, observed, 465.2122.

6-(7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yl)naphthalen-2-ol



32

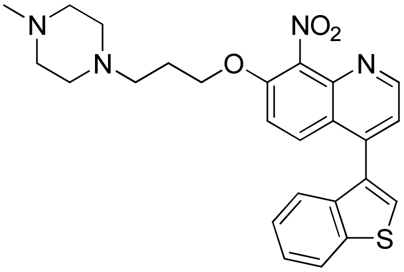
A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (600 mg, 1.3 mmol) 6-hydroxynaphthalen-2-ylboronic acid (252 mg, 1.3 mmol), $Pd(OAc)_2$ (44 mg, 0.2 mmol), Na_2CO_3 (0.5 mL of a 2M solution) and water (6 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (30 mL) was added and the mixture was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was recrystalized in CH_2Cl_2 to give 338 mg of 6-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yl)naphthalen-2-ol **32** as yellow crystals (55 % yield).

^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 9.99 (bs, 1H), 8.95 (d, 1H, *J* = 4.5 Hz), 8.10 (d, 1H, *J* = 5.5 Hz), 8.01 (d, 1H, *J* = 1.8 Hz), 7.89 (d, 1H, *J* = 8.7 Hz), 7.89 (d, 1H, *J* = 8.3 Hz), 7.75 (d, 1H, *J* = 9.7 Hz), 7.57 (dd, 1H, *J* = 8.5, 1.8 Hz), 7.56 (d, 1H, *J* = 4.5 Hz), 7.24 (d, 1H, *J* = 2.5 Hz), 7.18 (dd, 1H, *J* = 8.8, 2.5 Hz), 4.36 (t, 2H, *J* = 6.2 Hz), 2.37 (t, 2H, *J* = 7.0 Hz), 2.30 (bs, 8H), 2.11 (s, 3H), 1.87 ("quintet", 2H, "*J*" = 6.7 Hz).

^{13}C-NMR (DMSO-d_6, 100 MHz) δ (ppm): 156.41, 152.55, 149.41, 148.26, 140.34, 136.21, 134.61, 130.56, 130.09, 129.21, 128.81, 127.46, 127.23, 126.66, 121.20, 120.77, 119.58, 115.79, 108.64, 68.14, 54.74 (2C), 53.73, 52.68 (2C), 45.74, 26.03.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 473.2183, observed, 473.2191.

4-(Benzo[b]thiophen-3-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



33

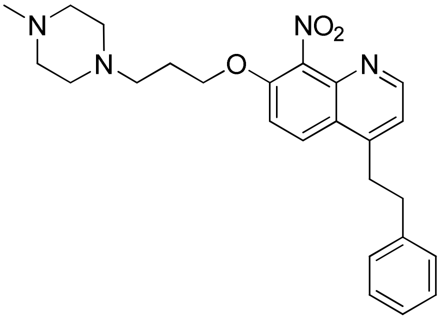
A mixture of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (600 mg, 1.3 mmol), benzo[b]thiophen-3-ylboronic acid (238 mg, 1.3 mmol), $Pd(OAc)_2$ (44 mg, 0.2 mmol), Na_2CO_3 (0.5 mL of a 2 M solution) and water (6 mL) was placed in a 25 mL round-bottom flask under argon atmosphere and immersed in an oil bath at 150°C. After stirring for 90 minutes the mixture was cooled to r.t., water (25 mL) was added and the mixture was extracted with CH_2Cl_2 (3x45 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was recrystalized in CH_2Cl_2/*n*-hexane to give 541 mg of 4-(benzo[b]thiophen-3-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **33** as brown solid (90 % yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.01 (d, 1H, *J* = 4.4 Hz), 7.99 (dt, 1H, *J* = 8.1, 1.0 Hz), 7.84 (d, 1H, *J* = 9.4 Hz), 7.57 (s, 1H), 7.44 (ddd, 1H, *J* = 8.0, 6.8, 1.5 Hz), 7.43 (d, 1H, *J* = 4.5 Hz), 7.40 (dm, 1H, *J* = 8.2 Hz), 7.35 (ddd, 1H, *J* = 8.0, 6.8, 1.2 Hz), 7.30 (d, 1H, *J* = 9.5 Hz), 4.29 (t, 2H, *J* = 6.2 Hz), 2.53 (t, 2H, *J* = 7.1 Hz), 2.49 (bs, 8H), 2.29 (s, 3H), 1.99 (“quintet”, 2H, “*J*” = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.58, 150.15, 142.58, 141.16, 140.19, 138.24, 136.99, 132.43, 128.98, 126.91, 125.09, 124.86, 122.94, 122.70, 122.00, 121.50, 114.73, 68.33, 55.02 (2C), 54.18, 52.99 (2C), 45.91, 26.40.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 463.1798, observed, 463.1795.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-4-phenethylquinoline



34

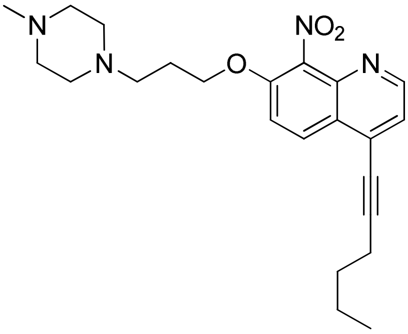
4-Iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (100 mg, 0.219 mmol), phenethylboronic acid (42.7 mg, 0.285 mmol), potassium phosphate (162.7 mg, 0.766 mmol), palladium acetate (2.5 mg, 0.011 mmol), dicyclohexyl(2',6'-dimethoxybiphenyl-2-yl)phosphine (9 mg, 0.022 mmol), toluene (1 mL) and water (47 μL), were placed in a sealed tube with stirring under argon atmosphere for 17 hours. After cooling to room temperature, water (20 mL) was added and the acqueous phase was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated in vacuum to give 94 mg (0.216 mmol) of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-phenethylquinoline **34** (99% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.80 (d, 1H, *J* = 4.5 Hz), 8.10 (d, 1H, *J* = 9.4 Hz), 7.38 (d, 1H, *J* = 9.5 Hz), 7.29 (m, 2H), 7.23 (m, 1H), 7.16 (d, 1H, *J* = 4.5 Hz), 7.15 (m, 2H), 4.35 (t, 2H, *J* = 5.7 Hz), 3.36 (t, 2H, *J* = 7.8 Hz), 3.05 (t, 2H, *J* = 7.8 Hz), 2.95 (bs, 8H), 2.74 (t, 2H, *J* = 6.8 Hz), 2.61 (s, 3H), 2.09 ("quintet", 2H, "*J*" = 6.3 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 152.82, 149.41, 147.81, 140.82, 140.23, 128.64, 128.33, 126.61, 126.55, 122.47, 120.69, 114.45, 107.66, 68.26, 54.10 (2C), 53.65 (2C), 50.56, 44.32, 36.10, 33.90, 25.88.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 435.2390, observed, 435.2401.

4-(Hex-1-ynyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

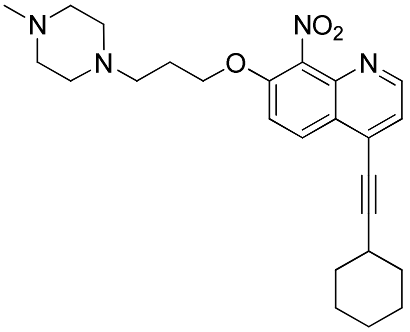


35

1-Hexyne (22mg, 31 μl, 0.26 mmol) was added while stirring to a suspension of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (100 mg, 0,219 mmol), pyrrolidine (77.9 mg, 91.4 μl, 1.095 mmol) and palladium chloride (0.4 mg, 0.002 mmol) in water (1 mL) at 50° C. The mixture was stirred at room temperature for 24 hours. Water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 81 mg of dark solid of 4-(hex-1-ynyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **35** (90% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.84(d, 1H, *J* = 4.5 Hz), 8.31 (d, 1H, *J* = 9.3 Hz), 7.45 (d, 1H, *J* = 9.4 Hz), 7.38 (d, 1H, *J* = 4.5 Hz), 4.32 (t, 2H, *J* = 6.2 Hz), 2.59 (t, 2H, *J* = 7.2 Hz), 2.52 (t, 2H, *J* = 7.0 Hz), 2.51 (bs, 8H), 2.28 (s, 3H), 2.02 ("quintet", 2H, "*J*" = 6.7 Hz), 1.71 ("quintet", 2H, "*J*" = 7.2 Hz), 1.55 ("quintet", 2H, "*J*" = 7.4 Hz), 1.00 (t, 3H, *J* = 7.3 Hz).

4-(Cyclohexylethynyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

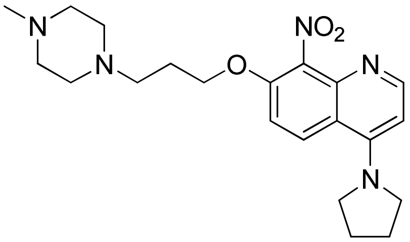


36

Ethynylcyclohexane (28.1 mg, 33.5 µl, 0.26 mmol) was added while stirring to a suspension of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (100 mg, 0,219 mmol), pyrrolidine (77.9 mg, 91.4 µl, 1.095 mmol) and palladium chloride (0.4 mg, 0.002 mmol) in water (1 mL) at 50°C. The mixture was stirred at room temperature for 24 hours. Water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 90 mg of dark solid of 4-(cyclohexylethynyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **36** (94% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.83 (d, 1H, *J* = 4.5 Hz), 8.31 (d, 1H, *J* = 9.3 Hz), 7.44 (d, 1H, *J* = 9.4 Hz), 7.38 (d, 1H, *J* = 4.5 Hz), 4.32 (t, 2H, *J* = 6.3 Hz), 2.77 (m, 1H), 2.52 (t, 2H, *J* = 6.9 Hz), 2.46 (bs, 8H), 2.27 (s, 3H), 2.00 ("quintet", 2H, "*J*" = 6.7 Hz), 1.97 (m, 2H), 1.79 (m. 2H), 1.63 (m, 3H), 1.42 (m, 3H).

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-4-(pyrrolidin-1-yl)quinoline

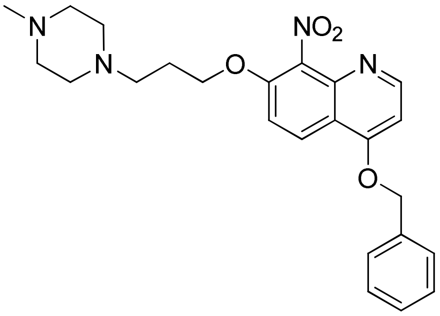


37

Pyrrolidine (77.9 mg, 91.4 µl, 1.095 mmol) was added while stirring to a suspension of 4-iodo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **10** (100 mg, 0,219 mmol) and palladium chloride (0.4 mg, 0.002 mmol) in water (1 mL) at 50°C. The mixture was stirred at 40°C for 2 days. Water (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3x15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum. The residue was purified by flash chromatography on basic alumina (eluent AcOEt) to give 16 mg of pale yellow oil of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-(pyrrolidin-1-yl)quinoline **37** (18% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.43(d, 1H, *J* = 5.6 Hz), 8.25 (d, 1H, *J* = 9.6 Hz), 7.09 (d, 1H, *J* = 9.6 Hz), 6.36 (d, 1H, *J* = 5.6 Hz), 4.25 (t, 2H, *J* = 6.2 Hz), 3.67 (m, 4H), 2.50 (t, 2H, *J* = 7.1 Hz), 2.45 (bs, 8H), 2.26 (s, 3H), 2.06 (m, 4H), 1.97 ("quintet", 2H, "*J*" = 6.9 Hz).

4-(Benzyloxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

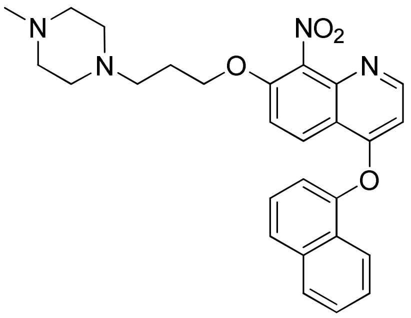


38

Benzyl alcohol (44.5 mg, 42.5 µl 0.41 mmol) was added to a solution of *t*-BuOK (46 mg, 0.41 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (100 mg, 0.274 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 100°C for 5 hours. After cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to obtain 112 mg of yellow crystals of 4-(benzyloxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **38** (94% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.73 (d, 1H, *J* = 5.3 Hz), 8.29 (d, 1H, *J* = 9.4 Hz), 7.50-7.38 (m, 5H), 7.31 (d, 1H, *J* = 9.4 Hz), 6.77 (d, 1H, *J* = 5.3 Hz), 5.29 (s, 2H), 4.29 (t, 2H, *J* = 6.2 Hz), 2.50 (t, 2H, *J* = 7.0 Hz), 2.45 (bs, 8H), 2.27 (s, 3H), 1.98 ("quintet", 2H, "*J*" = 6.7 Hz).

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yloxy)-8-nitroquinoline



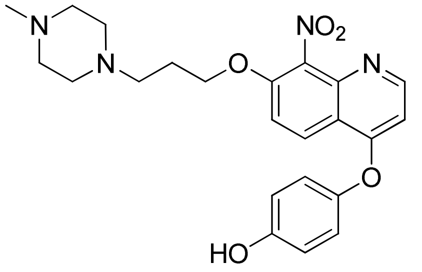
39

Naphthalen-1-ol (59.1 mg, 0.41 mmol,) was added to a solution of *t*-BuOK (46 mg, 0.41 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (100 mg, 0.274 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with toluene

(3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to obtain 120 mg of a yellow oil of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yloxy)-8-nitroquinoline **39** (93% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.58 (d, 1H, *J* = 5.2 Hz), 8.58 (d, 1H, *J* = 9.3 Hz), 7.95 (d, 1H, *J* = 8.3 Hz), 7.84 (d, 1H, *J* = 8.3 Hz), 7.79 (d, 1H, *J* = 8.4 Hz), 7.54 (m, 2H), 7.45 (d, 1H, *J* = 9.1 Hz), 7.44 (td, 2H, *J* = 7.7, 1.6 Hz), 7.29 (dd, 1H, *J* = 7.5, 0.9 Hz), 6.33 (d, 1H, *J* = 5.3 Hz), 4.35 (t, 2H, *J* = 6.2 Hz), 2.55 (t, 2H, *J* = 7.0 Hz), 2.52 (bs, 8H), 2.30 (s, 3H), 2.03 (“quintet”, 2H, “*J*” = 6.7 Hz).

4-(7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)phenol

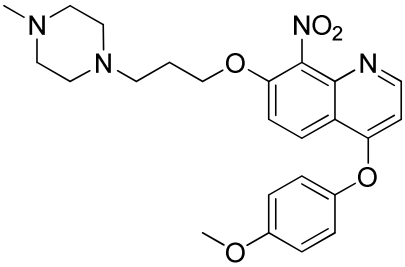


40

Hydroquinone (45 mg, 0.41 mmol) was added to a solution of *t*-BuOK (46 mg, 0.41 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (100 mg, 0.274 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 100°C for 2 hours. After cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue purified by chromatography on basic alumina (eluent AcOEt:hexane:MeOH 13:6:1) to obtain 53 mg of a yellow oil of 4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)phenol **40** (44% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.63 (d, 1H, *J* = 5.2 Hz), 8.37 (d, 1H, *J* = 9.4 Hz), 7.28 (d, 1H, *J* = 9.4 Hz), 6.99 (m, 2H, part of AA’BB’ system), 6.85 (m, 2H part of AA’BB’ system), 6.47 (d, 1H, *J* = 5.3 Hz), 4.32 (t, 2H, *J* = 6.2 Hz), 2.59 (t, 2H, *J* = 7.0 Hz), 2.55 (bs, 8H), 2.31 (s, 3H), 2.04 (“quintet”, 2H, “*J*” = 6.6 Hz).

4-(4-Methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

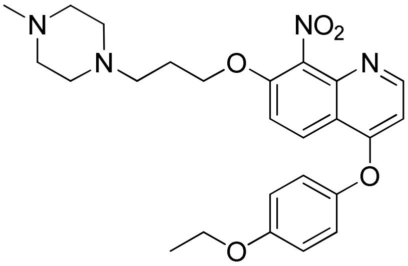


41

4-Methoxyphenol (51 mg, 0.41 mmol) was added to a solution of *t*-BuOK (46 mg, 0.41 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (100 mg, 0.274 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. toluene (20 mL) and water (30 mL) were added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the solid residue was recrystalized in diethyl ether (1 mL) to obtain 103 mg of yellow crystals of 4-(4-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **41** (82% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.68 (d, 1H, *J* = 5.3 Hz), 8.46 (d, 1H, *J* = 9.4 Hz), 7.44 (d, 1H, *J* = 9.4 Hz), 7.12 (m, 2H, part of AA’BB’ system), 7.01 (m, 2H, part of AA’BB’ system), 6.48 (d, 1H, *J* = 5.3 Hz), 4.36 (t, 2H, *J* = 6.2 Hz), 3.87 (s, 3H), 2.56 (t, 2H, *J* = 7.0 Hz), 2.51 (bs, 8H), 2.31 (s, 3H), 2.04 (“quintet”, 2H, *“J”* = 6.6 Hz).

4-(4-Ethoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

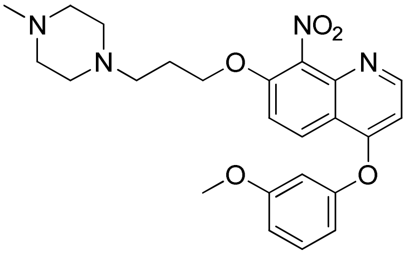


42

3-Ethoxyphenol (56.6 mg, 0.41 mmol) was added to a solution of *t*-BuOK (46 mg, 0.41 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (100 mg, 0.274 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the solid residue was recrystalized in diethyl ether (1 mL) to obtain 110 mg of yellow crystals of 4-(4-ethoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **42** (86% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.68 (d, 1H, *J* = 5.3 Hz), 8.46 (d, 1H, *J* = 9.4 Hz), 7.45 (d, 1H, *J* = 9.5 Hz), 7.11 (m, 2H, part of AA'BB' system), 7.00 (m, 2H, part of AA'BB' system), 6.48 (d, 1H, *J* = 5.2 Hz), 4.37 (t, 2H, *J* = 6.2 Hz), 4.09 (q, 2H, *J* = 6.9 Hz), 2.60 (t, 2H, *J* = 7.1 Hz), 2.50 (bs, 8H), 2.31 (s, 3H), 2.04 ("quintet", 2H, "*J*" = 6.9 Hz), 1.48 (t, 3H, *J* = 6.9 Hz).

4-(3-Methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline

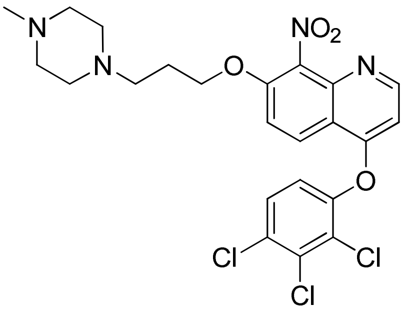


43

3-Methoxyphenol (51 mg, 44.5 μL, 0.41 mmol) was added to a solution of *t*-BuOK (46 mg, 0.41 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (100 mg, 0.274 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to obtain 122 mg of a yellow oil of 4-(3-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **43** (99% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.67 (d, 1H, *J* = 5.2 Hz), 8.40 (d, 1H, *J* = 9.4 Hz), 7.41 (d, 1H, *J* = 9.4 Hz), 7.37 (t, 1H, *J* = 8.1 Hz), 6.86 (ddd, 1H, *J* = 8.4, 2.4, 0.8 Hz), 6.75 (ddd, 1H, *J* = 8.0, 2.2, 0.8 Hz), 6.71 (t, 1H, *J* = 2.2 Hz), 6.55 (d, 1H, *J* = 5.2 Hz), 4.33 (t, 2H, *J* = 6.2 Hz), 3.82 (s, 3H), 2.53 (t, 2H, *J* = 7.0 Hz), 2.50 (bs, 8H), 2.29 (s, 3H), 2.01 ("quintet", 2H, "*J*" = 6.4 Hz).

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-4-(2,3,4-trichlorophenoxy)quinoline



44

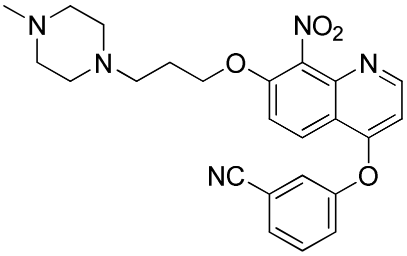
2,3,4-Trichlorophenol (59.5 mg, 0.30 mmol) was added to a solution of *t*-BuOK (37 mg, 0.33 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes, the suspension was heated at 100°C and then added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-

yl)propoxy)-8-nitroquinoline **8** (100 mg, 0.274 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the solid residue was recrystalized in ethyl acetate (1 mL) to obtain 70 mg of yellow crystals of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-(2,3,4-trichlorophenoxy)quinoline **44** (49% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.73 (d, 1H, *J* = 5.2 Hz), 8.46 (d, 1H, *J* = 9.4 Hz), 7.55 (d, 1H, *J* = 8.8 Hz), 7.50 (d, 1H, *J* = 9.4 Hz), 7.20 (d, 1H, *J* = 8.8 Hz), 6.40 (d, 1H, *J* = 5.2 Hz), 4.38 (t, 2H, *J* = 6.2 Hz), 2.57 (t, 2H, *J* = 7.0 Hz), 2.55 (bs, 8H), 2.33 (s, 3H), 2.05 (“quintet”, 2H, “*J*” = 6.5 Hz).

ESI-MS (*m/z*) $[M+H]^+$: 525.3, 527.1, 528.9.

3-(7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzonitrile



45

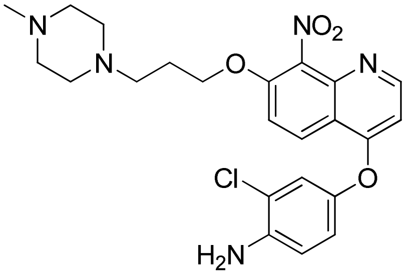
3-Hydroxybenzonitrile (319 mg, 3.3 mmol) was added to a solution of *t*-BuOK (368 mg, 3.3 mmol) in dry NMP (7 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (800 mg, 2.2 mmol) in dry NMP (7 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (100 mL) was added. The acqueous phase was extracted with toluene (3x80 mL) and the combined organic layers were washed with water (3x40 mL) and brine (40 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in CH_2Cl_2 to obtain 886 mg of 3-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzonitrile as pale yellow solid **45** (90% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.73 (d, 1H, *J* = 5.2 Hz), 8.36 (d, 1H, *J* = 9.4 Hz), 7.63 (m, 1H), 7.61 (m, 1H), 7.50 (m, 1H), 7.46 (d, 1H, *J* = 9.4 Hz), 7.43 (m, 1H), 6.52 (d, 1H, *J* = 5.2 Hz), 4.35 (t, 2H, *J* = 6.2 Hz), 2.54 (t, 2H, *J* =7.1 Hz), 2.50 (bs, 8H), 2.30 (s, 3H), 2.02 (“quintet”, 2H, “*J*” = 6.6 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ (ppm): 160.65, 154.31, 153.85, 151.08, 142.46, 136.78, 131.47, 129.61, 125.54, 124.68, 124.40, 117.38, 115.81, 114.60, 114.60, 104.19, 68.46, 55.00 (2C), 54.13, 52.91 (2C), 45.84, 26.39.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 448.1979, observed, 448.1977.

2-Chloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine



46

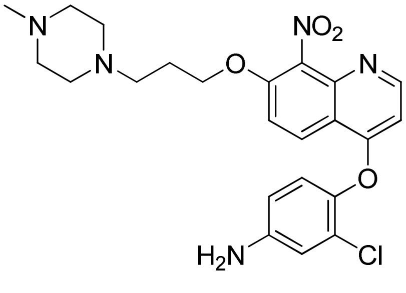
4-Amino-3-chlorophenol (492 mg, 3.4 mmol) was added to a solution of NaH (123 mg, 5 mmol) in dry NMP (6 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (760 mg, 2 mmol) in dry NMP (6 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (80 mL) was added. The acqueous phase was extracted with toluene (3x60 mL) and the combined organic layers were washed with water (3x40 mL) and brine (40 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in CH_2Cl_2 to obtain 755 mg of 2-chloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine **46** as yellow solid (80% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.66 (d, 1H, *J* = 5.3 Hz), 8.39 (d, 1H, *J* = 9.4 Hz), 7.41 (d, 1H, *J* = 9.5 Hz), 7.12 (d, 1H, *J* = 2.7 Hz), 6.91 (dd, 1H, *J* = 8.6, 2.6 Hz), 6.84 (d, 1H, *J* = 8.8 Hz), 6.49 (d, 1H, *J* = 5.3 Hz), 4.33 (t, 2H, *J* = 6.2 Hz), 4.12 (bs, 2H), 2.52 (t, 2H, *J* = 7.1 Hz), 2.46 (bs, 8H), 2.27 (s, 3H), 2.01 (“quintet”, 2H, “*J*” = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 162.26, 153.96, 150.82, 144.89, 142.22, 141.35, 136.73, 124.90, 122.34, 120.68, 119.62, 116.41, 115.83, 113.93, 103.18, 68.40, 55.15 (2C), 54.20, 53.12 (2C), 46.02, 26.48.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 472.1751, observed, 472.1742.

3-Chloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine



47

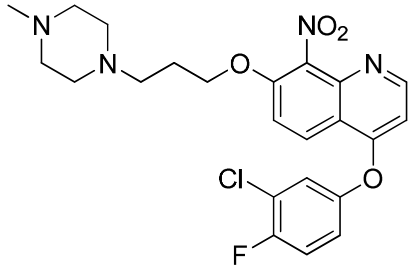
4-Amino-2-chlorophenol (492 mg, 3.4 mmol) was added to a solution of NaH (123 mg, 5 mmol) in dry NMP (6 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (760 mg, 2 mmol) in dry NMP (6 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (80 mL) was added. The acqueous phase was extracted with toluene (3x60 mL) and the combined organic layers were washed with water (3x40 mL) and brine (40 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in CH_2Cl_2 to obtain 755 mg of 3-chloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine **47** as yellow solid (80% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.66 (d, 1H, *J* = 5.3 Hz), 8.47 (d, 1H, *J* = 9.4 Hz), 7.43 (d, 1H, *J* = 9.4 Hz), 7.03 (d, 1H, *J* = 8.6 Hz), 6.82 (d, 1H, *J* = 2.7 Hz), 6.65 (dd, 1H, *J* = 8.6, 2.7 Hz), 6.38 (d, 1H, *J* = 5.3 Hz), 4.34 (t, 2H, *J* = 6.2 Hz), 3.82 (bs, 2H), 2.53 (t, 2H, *J* = 7.1 Hz), 2.50 (bs, 8H), 2.28 (s, 3H), 2.01 (“quintet”, 2H, “*J*” = 6.6 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.53, 153.95, 150.79, 145.65, 142.20, 140.76, 136.75, 127.50, 125.07, 123.88, 116.56, 115.62, 114.54, 113.94, 102.74, 68.39, 55.15 (2C), 54.19, 53.11 (2C), 46.00, 26.49.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 472.1751, observed, 472.1743

4-(3-Chloro-4-fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



48

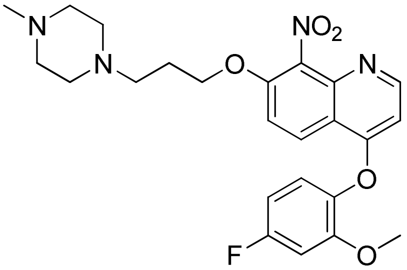
3-Chloro-4-fluorophenol (430 mg, 2.9 mmol) was added to a solution of NaH (104 mg, 4.3 mmol) in dry NMP (6 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (650 mg, 1.8 mmol) in dry NMP (6 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (70 mL) was added. The acqueous phase was extracted with toluene (3x50 mL) and the combined organic layers were washed with water (3x35 mL) and brine (35 mL) and then dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue recrystalized in CH_2Cl_2 to obtain 590 mg of 4-(3-chloro-4-fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **48** as white solid (67% yield).

1H-NMR (CDCl3, 400 MHz) δ(ppm): 8.69 (d, 1H, *J* = 5.2 Hz), 8.37 (d, 1H, *J* = 9.3 Hz), 7.44 (d, 1H, *J* = 9.4 Hz), 7.27 (m, 2H), 7.09 (ddd, 1H, *J* = 8.9, 3.8, 2.9 Hz), 6.52 (d, 1H, *J* = 5.2 Hz), 4.30 (t, 2H, *J* = 6.2 Hz), 2.52 (t, 2H, *J* = 7.1 Hz), 2.51 (bs, 8H), 2.29 (s, 3H), 2.00 (“quintet”, 2H, “*J*” = 6.6 Hz).

13C-NMR (CDCl3, 100 MHz) δ(ppm): 161.29, 156.04 (d, J_{CF} = 248.2 Hz), 153.84, 150.93, 149.61 (d, J_{CF} = 3.3), 142.29, 130.76, 124.72, 123.42, 122.51 (d, J_{CF} = 19.7 Hz), 120.71 (d, J_{CF} = 7.3 Hz), 117.81 (d, J_{CF} = 23.1 Hz), 115.73, 114.37, 103.65, 68.40, 54.88 (2C), 54.09, 52.72 (2C), 45.70, 26.35.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 475.1542, observed, 475.1556.

4-(4-Fluoro-2-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



49

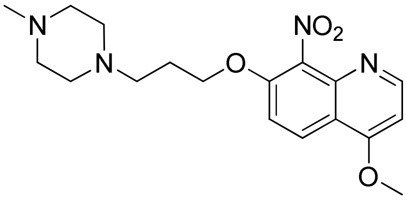
4-Fluoro-2-methoxyphenol (0.2 mL, 1.5 mmol) was added to a solution of NaH (56 mg, 2.3 mmol) in dry NMP (5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (390 mg, 1 mmol) in dry NMP (5 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (45 mL) was added. The acqueous phase was extracted with toluene (3x40 mL) and the combined organic layers were washed with water (3x30 mL) and brine (25 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in CH_2Cl_2/*n*-hexane to obtain 282 mg of 4-(4-fluoro-2-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **49** as white solid (60% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.60 (d, 1H, *J* = 5.2 Hz), 8.42 (d, 1H, *J* = 9.4 Hz), 7.40 (d, 1H, *J* = 9.4 Hz), 7.12 (dd, 1H, *J* = 8.8, 5.7 [HF] Hz), 6.77 (dd, 1H, *J* = 10.0 [HF], 2.8 Hz), 6.71 (ddd, 1H, *J* = 8.7, 7.8 [HF], 2.8 Hz), 6.31 (d, 1H, *J* = 5.3 Hz), 4.31 (t, 2H, *J* = 6.3 Hz), 3.69 (s, 3H), 2.49 (t, 2H, *J* = 7.0 Hz), 2.44 (bs, 8H), 2.25 (s, 3H), 1.98 (“quintet”, 2H, “*J*” = 6.9 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.59, 161.04 (J_{CF}= 246.0 Hz), 153.81, 152.36 (J_{CF}= 10.4 Hz), 150.65, 142.07, 137.68 (J_{CF}= 3.3 Hz), 136.62, 125.09, 123.37 (J_{CF}= 10.3 Hz), 115.56, 113.81, 107.26 (J_{CF}= 23.3 Hz), 102.50, 101.31 (J_{CF}= 27.4 Hz), 68.31, 55.95, 55.04 (2C), 54.12, 52.98 (2C), 45.90, 26.39.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 471.2038, observed, 471.2050.

4-Methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



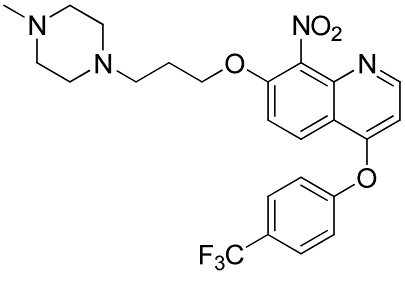
50

Sodium methoxyde (1.188 mL of a 30% wt. solution in MeOH, 6.24 mmol) was added under argon atmosphere to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (41.2 g, 0.170 mmol) in dry THF (1.5 mL) at 0°C. The temperature was left to raise to r.t. and then the mixture was refluxed for 18 h. After cooling to r.t. water (20 mL) was added. The acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum and the residue was filtered over a short pad of basic Al_2O_3 (eluent: AcOEt) to recover 365 mg of 4,7-dimethoxy-8-nitroquinoline **55** (yield 75.0%). Eluition with acetone afforded 83 mg of 4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **50** (14.7% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.68 (d, 1H, *J* = 5.3 Hz), 8.18 (d, 1H, *J* = 9.4 Hz), 7.29 (d, 1H, *J* = 9.4 Hz), 6.66 (d, 1H, *J* = 5.3 Hz), 4.25 (t, 2H, *J* = 6.3 Hz), 4.00 (s, 3H), 2.47 (t, 2H, *J* = 7.1 Hz), 2.41 (bs, 8H), 2.23 (s, 3H), 1.94 (“quintet”, 2H, “*J*” = 6.7 Hz)

^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 162.16, 154.13, 150.47, 141.69, 136.60, 125.00, 115.95, 113.28, 99.87, 68.23, 55.95, 55.04 (2C), 54.18, 53.01 (2C), 45.92, 26.42.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-4-(4-(trifluoromethyl)phenoxy)quinoline



51

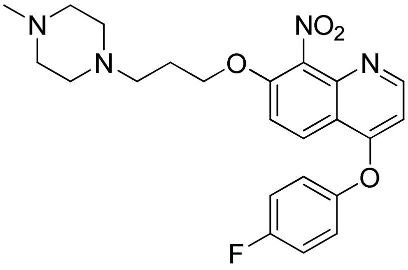
4-(Trifluoromethyl)phenol (398 mg, 2.4 mmol) was added to a solution of NaH (71 mg, 3 mmol) in dry NMP (5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (597 mg, 1.6 mmol) in dry NMP (5 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (50 mL) was added. The acqueous phase was extracted with toluene (3x50 mL) and the combined organic layers were washed with water (50 mL) and brine (40 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in ether to obtain 431 mg of a green crystals of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-(4-(trifluoromethyl)phenoxy)quinoline **51** (55% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.71 (d, 1H, *J* = 5.2 Hz), 8.37 (d, 1H, *J* = 9.4 Hz), 7.74 (m, 2H), 7.44 (d, 1H, *J* = 9.4 Hz), 7.28 (m, 2H), 6.56 (d, 1H, *J* = 5.2 Hz), 4.34 (t, 2H, *J* = 6.2 Hz), 2.53 (t, 2H, *J* = 7.0 Hz), 2.49 (bs, 8H), 2.29 (s, 3H), 2.01 ("quintet", 2H, "*J*" = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.73, 156.72, 153.84, 150.98, 142.44, 136.81, 128.17 (q, J_{CF}= 33.1 Hz), 127.80 (q, 2C, J_{CF}= 3.7 Hz), 124.77, 123.68 (q, J_{CF}= 272.4 Hz), 120.97 (2C), 116.01, 114.50, 104.56, 68.44, 54.99 (2C), 54.10, 52.87 (2C), 45.81, 26.40.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 491.1900, observed, 491.1882.

4-(4-Fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



52

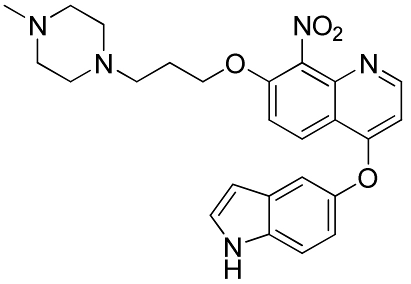
4-Fluorophenol (438 mg, 3.9 mmol) was added to a solution of NaH (120 mg, 5 mmol) in dry THF (10 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (1 g, 2.7 mmol) in dry THF (10 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with CH_2Cl_2 (3x50 mL) and the combined organic layers were washed with water (30 mL) and brine (25 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in CH_2Cl_2/*n*-hexane to obtain 1 g of 4-(4-fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **52** as yellow solid (85% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.65 (d, 1H, *J* = 5.2 Hz), 8.39 (d, 1H, *J* = 9.4 Hz), 7.42 (d, 1H, *J* = 9.4 Hz), 7.19-7.12 (m, 4H) 6.44 (d, 1H, *J* = 5.3 Hz), 4.32 (t, 2H, *J* = 6.3 Hz), 2.51 (t, 2H, *J* = 7.0 Hz), 2.45 (bs, 8H), 2.26 (s, 3H), 1.99 ("quintet", 2H, "*J*" = 6.6 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.84, 160.33 (d, J_{CF}= 245.5 Hz), 153.85, 150.87, 149.48 (d, J_{CF}= 3.0 Hz), 142.26, 136.79, 124.83, 122.60 (d, 2C, J_{CF}= 8.5 Hz), 117.10 (d, 2C, J_{CF}= 23.6 Hz), 115.86, 114.14, 103.36, 68.43, 55.12 (2C), 54.14, 53.07 (2C), 45.97, 26.46.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 441.1929, observed, 441.1917.

4-(1H-indol-5-yloxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



53

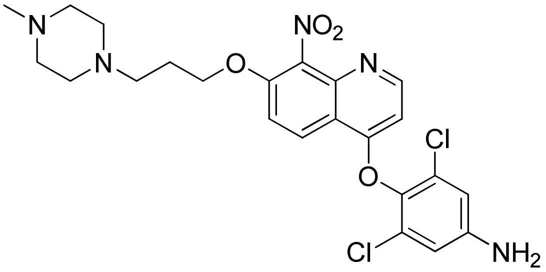
1H-indol-5-ol (186 mg, 1.4 mmol) was added to a solution of NaH (50 mg, 2.1 mmol) in dry NMP (4 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (350 mg, 0.9 mmol) in dry NMP (4 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (45 mL) was added. The acqueous phase was extracted with toluene (3x40 mL) and the combined organic layers were washed with water (3x30 mL) and brine (25 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in CH_2Cl_2 to obtain 207 mg of 4-(1H-indol-5-yloxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **53** as pale yellow solid (50% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.86 (bs, 1H), 8.60 (d, 1H, *J* = 5.3 Hz), 8.48 (d, 1H, *J* = 9.4 Hz), 7.45 (bd, 1H, *J* = 8.7 Hz), 7.41 (bd, 1H, *J* = 2.3 Hz), 7.39 (d, 1H, *J* = 9.5 Hz), 7.31 (dd, 1H, *J* = 3.0, 2.7 Hz), 6.96 (dd, 1H, *J* = 8.7, 2.3 Hz), 6.56 (m, 1H), 5.46 (d, 1H, *J* = 5.3 Hz), 4.32 (t, 2H, *J* = 6.2 Hz), 2.53 (t, 2H, *J* = 7.1Hz), 2.49 (bs, 8H), 2.28 (s, 3H), 2.01 (“quintet”, 2H, “*J*” = 6.6 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 163.10, 153.97, 150.70, 147.09, 142.13, 136.74, 133.82, 128.73, 126.18, 125.20, 116.17, 115.43, 113.70, 112.42, 112.38, 103.49, 102.84, 68.37, 55.10 (2C), 54.21, 53.06 (2C), 45.96, 26.47.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 462.2135, observed, 462.2139.

3,5-Dichloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine



54

4-Amino-2,6-dichlorophenol (268 mg, 1.5 mmol) was added to a solution of *t*-BuOK (186 mg, 1.6 mmol) in dry NMP (5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-

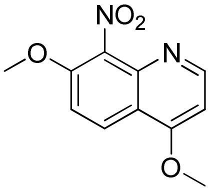
nitroquinoline **8** (500 mg, 1.4 mmol) in dry NMP (5 mL). The reaction mixture was heated at 100°C for 4 hours. After cooling to r.t. water (50 mL) was added. The acqueous phase was extracted with toluene (3x50 mL) and the combined organic layers were washed with water (40 mL) and brine (35 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in diethylether to obtain 446 mg of 3,5-dichloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine **54** as yellow crystals (63% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.68 (d, 1H, *J* = 5.2 Hz), 8.50 (d, 1H, *J* = 9.4 Hz), 7.45 (d, 1H, *J* = 9.5 Hz), 6.73 (s, 2H), 6.36 (d, 1H, *J* = 5.2 Hz), 4.35 (t, 2H, *J* = 6.2 Hz), 3.91 (bs, 2H), 2.54 (t, 2H, *J* = 7.0 Hz), 2.49 (bs, 8H), 2.30 (s, 3H), 2.02 (“quintet”, 2H, *“J”* = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.16, 153.87, 150.83, 145.62, 142.26, 136.98, 129.37, 125.09, 115.34, 114.86, 114.86, 114.17, 102.36, 68.42, 55.08 (3C), 54.13, 52.98 (2C), 45.90, 26.46.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 506.1356, observed, 506.1364.

4,7-Dimethoxy-8-nitroquinoline



55

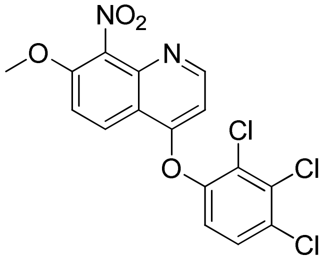
Sodium methoxyde (1.188 mL of a 30% wt. solution in MeOH, 6.24 mmol) was added under argon atmosphere to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline (41.2 g, 0.170 mmol) in dry THF (1.5 mL) at 0°C. The temperature was left to raise to r.t. and then the mixture was refluxed for 18 h. After cooling to r.t. water (20 mL) was added. The acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum and the residue was filtered over a short pad of basic Al_2O_3 (eluent: AcOEt) to recover 365 mg of 4,7-dimethoxy-8-nitroquinoline **55** (yield 75.0%). Eluition with acetone afforded 83 mg of 4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **50** (14.7% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.73 (d, 1H, *J* = 5.2 Hz), 8.25 (d, 1H, *J* = 9.3 Hz), 7.30 (d, 1H, *J* = 9.4 Hz), 6.69 (d, 1H, *J* = 5.4 Hz), 4.04 (s, 3H), 4.02 (s, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 162.20, 154.28, 150.93, 141.68, 125.21, 116.04, 112.09, 99.88, 56.91, 55.98.

MS (70eV) *m/z*: 233.8 [M^+] (100), 203.8 [M^+- CH_3-CH_3](57), 175.8 (43), 160.8 (22), 132.8 (24).

7-Methoxy-8-nitro-4-(2,3,4-trichlorophenoxy)quinoline



56

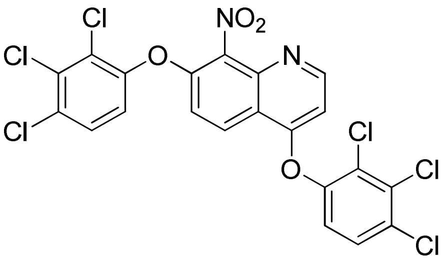
2,3,4-Trichlorophenol (91.0 mg, 0.466 mmol) was added to a solution of *t*-BuOK (56 mg, 0.502 mmol) in dry NMP (2.2 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-methoxy-8-nitroquinoline **11** (100 mg, 0.419 mmol) in dry NMP (2.2 mL). The reaction mixture was heated at 100°C for 16 hours. After cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to obtain 127 mg of a yellow oil of 7-methoxy-8-nitro-4-(2,3,4-trichlorophenoxy)quinoline **56** (75.8% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.71 (d, 1H, *J* = 5.2 Hz), 8.47 (d, 1H, *J* = 9.4 Hz), 7.53 (d, 1H, *J* = 8.8 Hz), 7.46 (d, 1H, *J* = 9.4 Hz), 7.18 (d, 1H, *J* = 8.8 Hz), 6.38 (d, 1H, *J* = 5.2 Hz), 4.10 (s, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.11, 153.96, 151.43, 148.94, 142.29, 131.96, 129.02, 128.76, 128.28, 125.02, 121.53, 115.31, 114.80, 113.26, 102.99, 57.08.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 398.9700, observed, 398.9725.

8-Nitro-4,7-bis(2,3,4-trichlorophenoxy)quinoline



56a

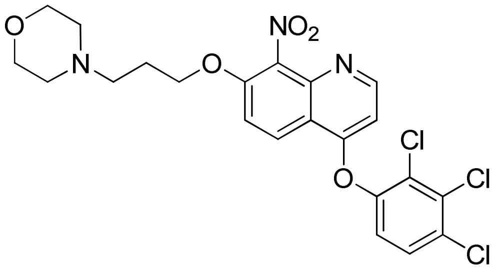
2,3,4-Trichlorophenol (60.7 mg, 0.308 mmol) was added to a solution of *t*-BuOK (37.6 mg, 0.356 mmol) in dry THF (1.5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4,7dichloro-8-nitroquinoline **3** (41.2 g, 0.170 mmol) in dry THF (1.5 mL) at 0°C. The temperature was left to raise to r.t. and then the mixture was refluxed for 18 h. After cooling to r.t. water (20 mL) was added, the acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum to obtain 96 mg of 8-nitro-4,7-bis(2,3,4-trichlorophenoxy)quinoline **56a** (99.9% yield). Reaction can also be performed in dry NMP. In this case toluene must be added instead of CH_2Cl_2 and the combined organic layers must be washed with water (3x20 mL).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.74 (d, 1H, *J* = 5.2 Hz), 8.41 (d, 1H, *J* = 9.4 Hz), 7.53 (d, 1H, *J* = 8.8 Hz), 7.44 (d, 1H, *J* = 8.9 Hz), 7.19 (d, 1H, *J* = 8.8 Hz), 7.09 (d, 1H, *J* = 8.9 Hz), 7.08 (d, 1H, *J* = 9.3 Hz), 6.47 (d, 1H, *J* = 5.2 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.12, 154.16, 150.15, 148.74, 148.18, 142.35, 138.00, 133.88, 133.62, 132.16, 131.19, 129.10, 128.78, 128.20, 127.56, 125.25, 121.49, 120.09, 117.43, 116.56, 104.10.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 562.8678 , observed, 562.8703.

4-(3-(8-Nitro-4-(2,3,4-trichlorophenoxy)quinolin-7-yloxy)propyl)morpholine



57

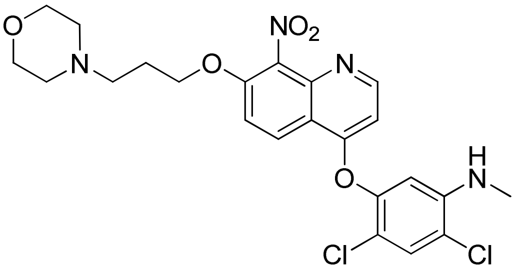
2,3,4-Trichlorophenol (61.7 mg, 0.312 mmol) was added to a solution of *t*-BuOK (38.3 mg, 0.341 mmol) in dry NMP (1.4 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-(3-(4-chloro-8-nitroquinolin-7-yloxy)propyl)morpholine **14** (100 mg, 0.284 mmol) in dry NMP (1.3 mL). The reaction mixture was heated at 100°C for 18 hours. After cooling to r.t. toluene (20 mL) and water (30 mL) were added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the solid residue recrystalized with Et_2O:AcOEt (1:1) to obtain 60 mg of a pale yellow crystals of 4-(3-(8-nitro-4-(2,3,4-trichlorophenoxy)quinolin-7-yloxy)propyl)morpholine **57** (74.4% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.70 (d, 1H, *J* = 5.2 Hz), 8.44 (d, 1H, *J* = 9.4 Hz), 7.53 (d, 1H, *J* = 8.8 Hz), 7.47 (d, 1H, *J* = 9.4 Hz), 7.17 (d, 1H, *J* = 8.8 Hz), 6.38 (d, 1H, *J* = 5.2 Hz), 4.37 (t, 2H, *J* = 6.1 Hz), 3.72 (t, 4H, *J* = 4.7 Hz), 2.55 (t, 2H, *J* = 6.9 Hz), 2.48 (bs, 4H), 2.04 ("quintet", 2H, "*J*" = 6.5 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.08, 153.80, 150.98, 149.03, 142.39, 136.84, 133.86, 131.94, 128.98, 128.26, 124.81, 121.41, 115.38, 114.53, 110.06, 103.10, 68.27, 66.81 (2C), 54.61, 53.59 (2C), 26.03.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 512.0546, observed, 512.0524.

2,4-Dichloro-*N*-methyl-5-(7-(3-morpholinopropoxy)-8-nitroquinolin-4-yloxy)aniline


58

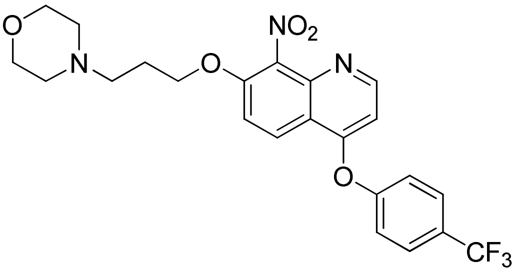
2,4-Dichloro-5-(methylamino)phenol **70** (164.0 mg, 0.854 mmol) was added to a suspension of NaH (57 mg, 1.432 mmol, 60% dispersion in mineral oil) in dry NMP (3.9 mL) under argon atmosphere. After 15 minutes, the suspension was added slowly via syringe, to a solution of 4-(3-(4-chloro-8-nitroquinolin-7-yloxy)propyl)morpholine **14** (250.3 mg, 0.712 mmol) in dry NMP (3.9 mL). The reaction mixture was heated at 100°C for 18 hours and after cooling to r.t., water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the solid residue was purified with cromathography on silica gel (eluent: ethyl acetate) to give 60 mg of 2,4-dichloro-*N*-methyl-5-(7-(3-morpholinopropoxy)-8-nitroquinolin-4-yloxy)aniline **58** (16.5% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.68 (d, 1H, *J* = 5.2 Hz), 8.46 (d, 1H, *J* = 9.4 Hz), 7.44 (d, 1H, *J* = 9.4 Hz), 7.40 (s, 1H), 6.48 (s, 1H), 6.43 (d, 1H, *J* = 5.2 Hz), 4.55 (q, 1H, *J* = 5.1 Hz), 4.36 (t, 2H, *J* = 6.1 Hz), 3.70 (t, 4H, *J* = 4.7 Hz), 2.86 (d, 3H, *J* = 5.1 Hz), 2.54 (t, 2H, *J* = 6.9 Hz), 2.46 (bs, 4H), 2.02 ("quintet", 2H, "*J*" = 6.6 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.68, 153.92, 150.78, 148.86, 145.43, 142.22, 136.75, 130.33, 125.01, 116.69, 115.54, 114.10, 112.94, 104.44, 103.13, 68.20, 66.84 (2C), 54.62, 53.59 (2C), 30.34, 26.06.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 507.1196, observed, 507.1186 [M+1]+

4-(3-(8-Nitro-4-(4-(trifluoromethyl)phenoxy)quinolin-7-yloxy)propyl)morpholine


60

4-(Trifluoromethyl)phenol (55.3 mg, 0.284 mmol) was added to a solution of *t*-BuOK (47.8 mg, 0.426 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-(3-(4-chloro-8-nitroquinolin-7-

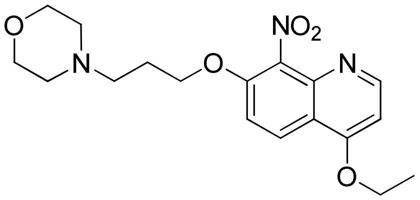
yloxy)propyl)morpholine **14** (100 mg, 0.284 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 100°C for 18 hours and after cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to obtain 101 mg of a yellow oil of 4-(3-(8-nitro-4-(4-(trifluoromethyl)phenoxy)quinolin-7-yloxy)propyl)morpholine **59** (74.4% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.67 (d, 1H, *J* = 5.2 Hz), 8.35 (d, 1H, *J* = 9.4 Hz), 7.72 (d, 2H, *J* = 8.6 Hz), 7.42 (d, 1H, *J* = 9.4 Hz), 7.27 (d, 2H, *J* = 8.6 Hz), 6.54 (d, 1H, *J* = 5.2 Hz), 4.34 (t, 2H, *J* = 6.1 Hz), 3.69 (t, 4H, *J* = 4.6 Hz), 2.53 (t, 2H, *J* = 7.0 Hz), 2.46 (m, 4H), 2.00 (“quintet”, 2H, “*J*” = 6.6 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.76, 153.84, 150.93, 142.37, 136.76, 128.16 (q, J_{CF} = 33.0 Hz), 127.80 (q, 2C, J_{CF} = 3.7 Hz), 124.85, 123.70 (q, 2C, J_{CF} = 271.5 Hz), 121.01 (2C), 116.02, 114.43, 104.54, 68.24, 66.82 (2C), 54.60, 53.59 (2C), 26.03.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 478.1589, observed, 478.1581.

4-(3-(4-Ethoxy-8-nitroquinolin-7-yloxy)propyl)morpholine



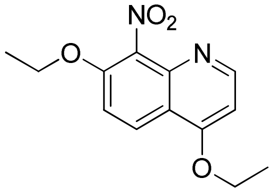
60

Potassium hydroxide (28.6 mg, 0.511 mmol) was dissolved in ethanol (2.5 mL) and the resulting clear solution was added to a solution of 4-(3-(4-chloro-8-nitroquinolin-7-yloxy)propyl)morpholine **14** (150 mg, 0.426 mmol) in dry ethanol (2.5 mL). The reaction mixture was heated at 100°C for 18 hours and after cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum to obtain 123 mg of a yellow oil of 4-(3-(4-ethoxy-8-nitroquinolin-7-yloxy)propyl)morpholine **60** (80.0% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.72 (d, 1H, *J* = 5.3 Hz), 8.27 (d, 1H, *J* = 9.4 Hz), 7.31 (d, 1H, *J* = 9.4 Hz), 6.68 (d, 1H, *J* = 5.3 Hz), 4.31 (t, 2H, *J* = 6.1 Hz), 4.27 (q, 2H, *J* = 7.0 Hz), 3.71 (t, 4H, *J* = 4.7 Hz), 2.53 (t, 2H, *J* = 7.0 Hz), 2.47 (m, 4H), 2.01 (“quintet”, 2H, “*J*” = 6.6 Hz), 1.57 (t, 3H, *J* = 7.0 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.49, 154.21, 150.44, 141.86, 136.79, 125.19, 125.18, 116.22, 113.15, 100.47, 68.10, 66.74 (2C), 64.60, 54.70, 53.60 (2C), 26.09, 14.35.

4,7-Diethoxy-8-nitroquinoline



60a

Potassium hydroxide (58.2 mg, 1.022 mmol) was dissolved in ethanol (2.5 mL) and the resulting clear solution was added to a solution of 4,7-dichloro-8-nitroquinoline **3** (103 mg, 0.426 mmol) in dry ethanol (2.5 mL). The reaction mixture was heated at 100°C for 18 hours. After cooling to r.t. CH_2Cl_2 (20 mL) and water (30 mL) were added. The acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum to obtain 96.1 mg of a yellow oil of 4,7-diethoxy-8-nitroquinoline **60a** (86.0% yield).

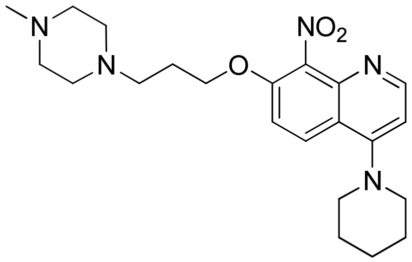
^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.70 (d, 1H, *J* = 5.3 Hz), 8.25 (d, 1H, *J* = 9.4 Hz), 7.26 (d, 1H, *J* = 9.4 Hz), 6.66 (d, 1H, *J* = 5.3 Hz), 4.28 (q, 2H, *J* = 7.0 Hz), 4.25 (q, 2H, *J* = 7.0 Hz), 1.55 (t, 3H, *J* = 7.0 Hz), 1.44 (t, 3H, *J* = 7.0 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.45, 154.13, 150.36, 141.86, 136.78, 125.12, 116.09, 113.07, 100.41, 65.87, 64.57, 14.62, 14.33.

MS (70 eV) *m/z*: 262.2 [M^+](71), 234.2 (32), 204.2 (32), 189.1 (34), 178.1 (42), 176.1 (63), 148.1 (100), 132.2 (55), 55.2 (60).

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 263.1026, observed, 263.1024.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-4-(piperidin-1-yl)quinoline

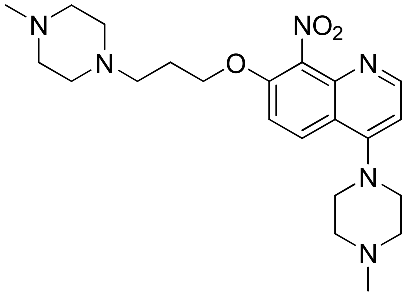


61

Piperidine (24.5 mg, 28.5 µL, 0.228 mmol) was added to a solution of *t*-BuOK (46 mg, 0.41 mmol) in dry NMP (1.5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (100 mg, 0.274 mmol) in dry NMP (1.5 mL). The reaction mixture was heated at 150°C for 18 hours and after cooling to r.t., water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL), brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue dissolved in AcOEt (2 mL). The resulting suspension was filtered and the solvent was evaporated in vacuum to obtain 40 mg of a yellow oil of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-(piperidin-1-yl)quinoline **61** (35% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.67 (d, 1H, *J* = 5.1 Hz), 8.02 (d, 1H, *J* = 9.4 Hz), 7.28 (d, 1H, *J* = 9.5 Hz), 6.76 (d, 1H, *J* = 5.2 Hz), 4.29 (t, 2H, *J* = 6.2 Hz), 3.17 (m, 4H), 2.51 (t, 2H, *J* = 7.0 Hz), 2.46 (bs, 8H), 2.27 (s, 3H), 1.98 ("quintet", 2H, "*J*" = 6.7 Hz), 1.83 (m, 4H), 1.71 (m, 2H).

4-(4-Methylpiperazin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



62

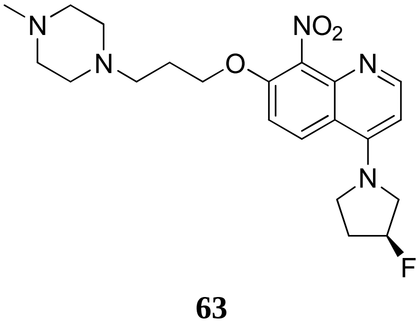
4-Chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (106 mg, 0.3 mmol) was added, at room temperature, to 1-methylpiperazine (3 mL). The mixture was stirred at 150°C for 12 h and concentrated in vacuum. to give 100 mg of 4-(4-methylpiperazin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **62** as brown oil (80% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.65 (d, 1H, *J* = 5.1 Hz), 8.00 (d, 1H, *J* = 9.4 Hz), 7.26 (d, 1H, *J* = 9.5 Hz), 6.75 (d, 1H, *J* = 5.2 Hz), 4.24 (t, 2H, *J* = 6.3 Hz), 3.21 (bt, 4H, *J* = 4.7 Hz), 2.64 (bt, 4H, *J* = 4.7 Hz), 2.46 (t, 2H, *J* = 7.1 Hz), 2.46 (bs, 8H), 2.88 (s, 3H), 2.23 (s, 3H), 1.94 ("quintet", 2H, "*J*" = 6.6 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 156.81, 153.29, 149.69, 142.16, 126.63, 118.03, 112.82, 108.32, 80.77, 68.18, 55.01 (2C), 54.99, 54.76 (2C), 52.97 (2C), 52.06 (2C), 45.97, 45.89, 26.38.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 429.2608, observed, 429.2612.

(S)-4-(3-fluoropyrrolidin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



63

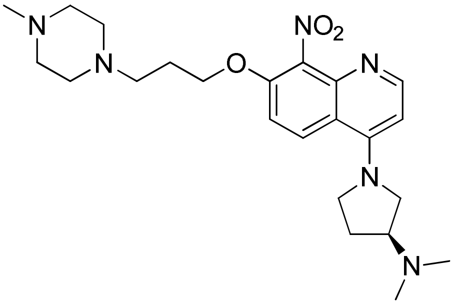
(S)-3-fluoropyrrolidine hydrochloride (73 mg, 0.6 mmol) was added to a solution of *t*-BuOK (102.4 mg, 0.9 mmol) in dry NMP (3 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (200 mg, 0.55 mmol) in dry NMP (3 mL). The reaction mixture was heated at 150°C for 5 hours. After cooling to r.t. water (60 mL) was added. The acqueous phase was extracted with toluene (3x30 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the residue recrystalized in CH_2Cl_2 to give 193 mg of (S)-4-(3-fluoropyrrolidin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **63** as yellow crystals (84% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.42 (d, 1H, *J* = 5.6 Hz), 8.16 (d, 1H, *J* = 9.6 Hz), 7.11 (d, 1H, *J* = 9.6 Hz), 6.36 (d, 1H, *J* = 5.6 Hz), 5.38 (dt, 1H. *J* = 52.4 [HF], 3.4 Hz), 4.15 (t, 2H, *J* = 6.3 Hz), 4.01-3.65 (m, 6H), 2.48 (t, 2H, *J* = 7.1 Hz), 2.43 (bs, 8H), 2.25 (s, 3H), 1.94 (“quintet”, 2H, “*J*” = 6.7 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ (ppm): 152.10, 151.67, 149.22, 143.10, 137.17, 127.52, 115.69, 110.54, 102.67, 91.80 (d, J_{CF}= 178.1), 68.02, 58.09, 57.87, 55.01 (2C), 54.22, 52.96 (2C), 49.41, 45.88, 26.41.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 418.2248, observed, 418.2238.

(S)-*N,N*-dimethyl-1-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yl)pyrrolidin-3-amine



64

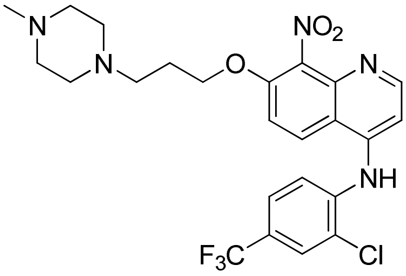
(S)-*N*,*N*-dimethylpyrrolidin-3-amine (0.25 mL, 1.9 mmol) was added to a solution of NaH (106 mg, 4.4 mmol) in dry NMP (5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (600 mg, 1.6 mmol) in dry NMP (5 mL). The reaction mixture was heated at 150°C for 5 hours. After cooling to r.t. water (80 mL) was added. The acqueous phase was extracted with toluene (3x40 mL) and the combined organic layers were washed with water (3x30 mL) and brine (30 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to obtain 471 mg of (S)-*N*,*N*-dimethyl-1-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yl)pyrrolidin-3-amine **64** as yellow crystals (65% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.46 (d, 1H, *J* = 5.6 Hz), 8.21 (d, 1H, *J* = 9.6 Hz), 7.13 (d, 1H, *J* = 9.6 Hz), 6.37 (d, 1H, *J* = 5.6 Hz), 4.25 (t, 2H, *J* = 6.3 Hz), 3.74 (m, 3H), 3.65 (t, 1H, *J* = 9.0 Hz), 2.81 (m, 1H), 2.50 (t, 2H, *J* = 7.1 Hz), 2.44 (bs, 8H), 2.34 (s, 6H), 2.27 (m, 1H), 2.27 (s, 3H), 1.98 (m, 1H), 1.95 (“quintet”, 2H, “*J*” = 6.9 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.03, 151.85, 149.15, 143.18, 137.16, 127.68, 115.69, 110.19, 102.24, 67.99, 65.33, 56.92, 55.08 (2C), 54.23, 53.05 (2C), 51.03, 45.95, 44.36 (2C), 30.33, 26.45.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 443.2765, observed, 443.2766.

N-(2-chloro-4-(trifluoromethyl)phenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-amine



65

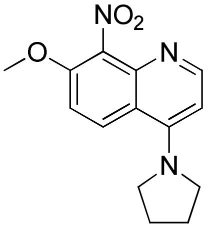
2-Chloro-4-(trifluoromethyl)benzenamine (0.23 mL, 1.7 mmol) was added to a solution of NaH (92 mg, 3.8 mmol) in dry NMP (5 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (580 mg, 1.6 mmol) in dry NMP (5 mL). The reaction mixture was heated at 150°C for 5 hours. After cooling to r.t. water (80 mL) was added. The acqueous phase was extracted with toluene (3x40 mL) and the combined organic layers were washed with water (3x30 mL) and brine (30 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to obtain 419 mg of *N*-(2-chloro-4-(trifluoromethyl)phenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-amine **65** as yellow solid (50% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.69 (d, 1H, J = 5.2 Hz), 8.05 (d, 1H, J = 9.4), 7.74 (d, 1H, J = 1.7 Hz), 7.57 and 7.49 (ABX system, 2H, J_{AB}= 8.6, J_{AX}= 1.8 Hz), 7.39 (d, 1H, J = 9.4 Hz), 7.11 (d, 1H, J = 5.2 Hz), 7.03 (bs, 1H), 4.31 (t, 2H, J = 6.2 Hz), 2.52 (t, 2H, J = 7.1 Hz), 2.49 (bs, 8H), 2.30 (s, 3H), 2.00 ("quintet", 2H, "J" = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 153.29, 150.43, 144.83, 142.09, 140.31, 127.47 (q, J_{CF}= 4.0 Hz), 125.84 (q, J_{CF}= 33.7 Hz), 124.94 (q, J_{CF}= 3.8 Hz), 124.47, 123.32, 123.23 (q, J_{CF}= 272.1 Hz), 119.32, 115.87, 114.06, 105.95, 68.42, 54.93 (2C), 54.08, 52.77 (2C), 45.73, 26.36.

ESI-HRMS (*m/z*) $[M+H]^+$ calculated, 524.1670, observed, 524.1654.

7-Methoxy-8-nitro-4-(pyrrolidin-1-yl)quinoline



66

Pyrrolidine (35.8 mg, 41.6 μL, 0.502 mmol) was added to a suspension of NaH (36.4 mg, 0.910 mmol, 60% dispersion in mineral oil) in dry THF (5 mL) under argon atmosphere. After 15 minutes, the suspension was added slowly via syringe, to a solution of 4-chloro-7-methoxy-8-nitroquinoline **11** (100.0 mg, 0.419 mmol) in dry THF (5 mL). The reaction mixture was heated at reflux for 18 hours and after cooling to r.t., water (30 mL) was added. The acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to give 129 mg of 7-methoxy-8-nitro-4-(pyrrolidin-1-yl)quinoline **66** (99.9% yield).

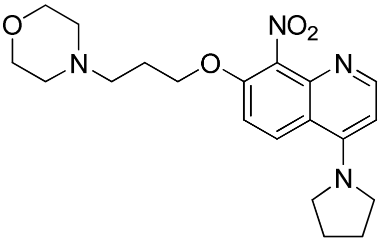
^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.44 (d, 1H, *J* = 5.7 Hz), 8.29 (d, 1H, *J* = 9.7 Hz), 7.09 (d, 1H, *J* = 9.7 Hz), 6.37 (d, 1H, *J* = 5.7 Hz), 4.00 (s, 3H), 3,68 (m, 4H), 2.07 (m, 4H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.14, 151.95, 149.60, 143.29, 128.12, 115.93, 108.66, 102.21, 56.67, 52.20 (2C), 25.93 (2C).

MS (70 eV) *m/z*: 273.2 [M^+](37).

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 274.1186, observed, 274.1174.

4-(3-(8-Nitro-4-(pyrrolidin-1-yl)quinolin-7-yloxy)propyl)morpholine



67

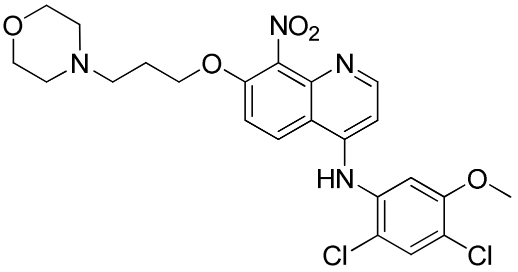
Pyrrolidine (60.6 mg, 70.5 μL, 0.852 mmol) was added to a suspension of NaH (36.4 mg, 0.910 mmol, 60% dispersion in mineral oil) in dry NMP (3.1 mL) under argon atmosphere. After 15 minutes, the suspension was added slowly via syringe, to a solution of 4-(3-(4-chloro-8-nitroquinolin-7-yloxy)propyl)morpholine **14** (200.0 mg, 0.569 mmol) in dry NMP (3.1 mL). The reaction mixture was heated at 100°C for 18 hours and after cooling to r.t., water (30 mL) was added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum to give 151 mg of 4-(3-(8-nitro-4-(pyrrolidin-1-yl)quinolin-7-yloxy)propyl)morpholine **67** (68.7% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.35 (d, 1H, *J* = 5.6 Hz), 8.21 (d, 1H, *J* = 9.6 Hz), 7.05 (d, 1H, *J* = 9.7 Hz), 6.29 (d, 1H, *J* = 5.7 Hz), 4.22 (t, 2H, *J* = 6.2 Hz), 3.65 (t, 4H, *J* = 4.8 Hz), 3.61 (m, 4H), 2.45 (t, 2H, *J* = 7.1 Hz), 2.40 (bs, 4H), 2.01 (m, 4H), 1.92 ("quintet", 2H, "*J*" = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 151.89, 151.89, 149.06, 143.24, 137.20, 128.03, 115.85, 109.87, 102.22, 67.84, 66.91 (2C), 54.62, 53.59 (2C), 52.14 (2C), 26.16, 25.84 (2C).

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 387.2026, observed, 387.2035.

N-(2,4-dichloro-5-methoxyphenyl)-7-(3-morpholinopropoxy)-8-nitroquinolin-4-amine



68

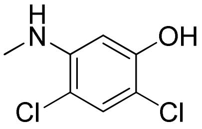
2,4-Dichloro-5-methoxyaniline **71** (140.0 mg, 0.781 mmol) was added to a suspension of NaH (37 mg, 0.923 mmol, 60% dispersion in mineral oil) in dry NMP (3.4 mL) under argon atmosphere. After 15 minutes, the suspension was added slowly via syringe, to a solution of 4-(3-(4-chloro-8-nitroquinolin-7-yloxy)propyl)morpholine **14** (249.8 mg, 0.710 mmol) in dry NMP (3.3 mL). The reaction mixture was heated at 100°C for 3 days. After cooling to r.t. toluene (20 mL) and water (30 mL) were added. The acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were washed with water (3x20 mL) and brine (20 mL) and then dried over $MgSO_4$. The solvent was removed in vacuum and the solid residue was filtered through a short pad of silica gel (eluent:diethyl ether) to remove the unreacted 2,4-dichloro-5-methoxyaniline **71**, and the mixture of *N*-(2,4-dichloro-5-methoxyphenyl)-7-(3-morpholinopropoxy)-8-nitroquinolin-4-amine and 4-(3-(4-chloro-8-nitroquinolin-7-yloxy)propyl)morpholine **68** was recoverd with acetone. The mixture was dissolved in a small amount of CH_2Cl_2 (about 0.5 mL) and then hexane (20 mL) was added. The precipitate was filtered and air dried to give 52 mg of *N*-(2,4-dichloro-5-methoxyphenyl)-7-(3-morpholinopropoxy)-8-nitroquinolin-4-amine **68** (14.4% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.53 (d, 1H, *J* = 5.3 Hz), 8.15 (d, 1H, *J* = 9.4 Hz), 7.45 (s, 1H), 7.29 (d, 1H, *J* = 9.4 Hz), 7.02 (s, 1H), 6.70 (d, 1H, *J* = 5.3 Hz), 4.31 (t, 2H, *J* = 5.8 Hz), 3.82 (s, 3H), 3.81 (t, 4H, *J* = 4.6 Hz), 2.74 (t, 2H, *J* = 7.2 Hz), 2.70 (bs, 4H), 2.12 ("quintet", 2H, "*J*" = 6.5 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm):154.56, 153.15, 149.88, 146.80, 141.71, 137.13, 135.71, 130.78, 123.84, 119.29, 118.94, 115.03, 113.09, 107.69, 103.33, 67.81, 65.63, 56.60, 54.74, 53.10, 25.13.

ESI-HRMS (*m/z*) [M+H]$^+$: calculated, 507.1196, observed, 507.1183.

2,4-Dichloro-5-(methylamino)phenol



70

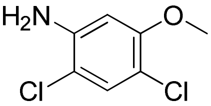
A solution of 5-amino-2,4-dichlorophenol (300.0 mg, 1.685 mmol) in dry THF (3.3 mL) was added dropwise at 0°C under argon atmosphere to a suspension of NaH (279.2 mg, 6.981 mmol, 60% dispersion in mineral oil) in dry THF (1.3 mL). After 15 minutes MeI (153 mg, 67.1 mL 1.08 mmol) was added, and the resulting suspension was left to stir at r.t for 16 h. The solvent was removed in vacuum and then water (30 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 164 mg of 2,4-dichloro-5-(methylamino)phenol **70** (50.7% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.16 (s, 1H), 6.28 (s, 1H), 3.88 (s, 1H), 2.82 (s, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 151.19, 145.15, 128.05, 110.70, 106.58, 98.17, 30.30.

MS (70 eV) *m/z*: 190.8 [M$^+$](100).

2,4-Dichloro-5-methoxyaniline



71

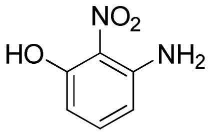
A solution of 5-amino-2,4-dichlorophenol (174.6 mg, 0.980 mmol) in dry THF (3 mL) was added dropwise at 0°C under argon atmosphere to a suspension of *t*-BuOK (164.9 mg, 1.470 mmol) in dry THF (3 mL). After 15 minutes MeI (153 mg, 67.1 mL 1.08 mmol) was added, and the resulting suspension was left to stir at r.t. for 16 h. The solvent was removed in vacuum and then water (30 mL) and CH_2Cl_2 (30 mL) were added. The acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 140 mg of 2,4-dichloro-5-methoxyaniline **71** (74.4% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.19 (s, 1H), 6.30 (s, 1H), 4.06 (bs, 2H), 3.79 (s, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 154.39, 142.48, 129.68, 111.30, 110.45, 99.67, 56.11.

MS (70 eV) *m/z*: 193.1 [M$^+$](61), 191.1[M$^+$](100), 150.1 (59), 148.0 (99).

3-Amino-2-nitrophenol



72

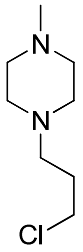
3-Aminophenol (20,0 g, 183 mmol) was dissolved in 96% sulphuric acid (370 mL) at 0°C. Nitric acid (11.5 g, 183 mmol, 13.7 mL of a 60% solution) in 96% sulphuric acid (41 mL) was then added dropwise over 20 min at 0°C. The mixture was left under stirring for 2h and then put on icy water (1 L). The red precipitate was air dried and the product was separated from other regioisomers by flash chromatography (eluent : hexane-ethyl acetate 7:3). 8.14 g (52.8 mmol) of 3-amino-2-nitrophenol **72** were recovered (29% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.26 (1H, t, J = 8.2 Hz), 6.32 (1H, dd, J =8.1, 1.2 Hz), 6.24 (1H, dd, J = 8.0, 1.2 Hz), 6.26 (3H, bs).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm) : 156.75, 146.95, 137.37, 137.35, 107.96, 105.22.

MS (70 eV) : *m/z*: 154 (M+, 100), 136 (26), 109 (31), 93 (32), 80 (18), 53 (35).

1-(3-Chloropropyl)-4-methylpiperazine



76

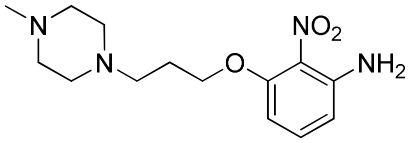
To a solution of 1-bromo-3-chloropropane (23.58 g, 149.7 mmol, 14.73 mL) in THF (50 mL) was added dropwise *N*-methylpiperazine (30.0 g, 299.5 mmol, 33.26 mL) over 5 min. The reaction was stirred overnight at room temperature and then filtered. The organic phase was evaporated under reduced pressure affording 26.45 g (149.7 mmol) of 1-(3-chloropropyl)-4-methylpiperazine **76** (100% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 3.62 (2H, t, J = 6.6 Hz), 2.51 (2H, t, J = 7.1 Hz), 2.48 (8H, bs), 2.31 (3H, s), 1.97 (2H, qui, J = 6.7 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm) : 55.30, 55.02, 53.10, 45.96, 43.10, 29.78.

ESI-MS (m/z) [M+H] $^+$: 177.4.

3-(3-(4-Methylpiperazin-1-yl)propoxy)-2-nitroaniline



77

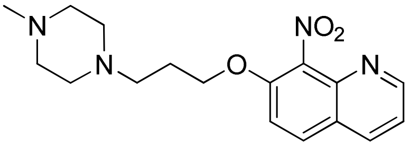
3-Amino-2-nitrophenol **72** (9.735 g, 63.17 mmol) was added to a solution of KOH (3.72 g, 66.33mmol) in ethanol (310 mL). The solution was heated at reflux temperature and then 1-(3-chloropropyl)-4-methylpiperazine **76** (11.16 g, 63.17 mmol) was added, and the resulting dark mixture was left to stir at reflux for 18 h. After cooling to room temperature, water (200 mL) was added. The acqueous phase was extracted with CH_2Cl_2 (3x60 mL), the combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuum. The product was separated from the unreacted starting material by flash cromatography in gradient of polarity (first eluent: acetone. Second eluent methanol and triethylamine 95:5). Unchanged 3-amino-2-nitrophenol **72** (1.816 g) was recovered with the first eluent, and 3-(3-(4-methylpiperazin-1-yl)propoxy)-2-nitroaniline **77** (12.914 g, 43.87 mmol) was recovered using the second eluent (85,4% yield calculated on converted reagent)

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.08 (1H, t, *J* = 8.3 Hz), 6.30 (1H, d, *J* = 8.3 Hz), 6.24 (1H, d, *J* = 8.2 Hz), 4.99 (2H, s), 4.02 (2H, t, *J* = 6.2 Hz), 2.48 (2H, t, *J* = 7.2 Hz), 2.41 (bs, 8H), 2.24 (3H, s), 1.91 (2H, qui, *J* = 6.9 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm) : 153.85, 143.02, 132.86 (2C), 109.31, 101.52, 67.43, 54.99, 54.60, 53.00, 45.90, 26.26.

ESI-MS (*m/z*) [M+H]$^+$: 295.4.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitroquinoline



81

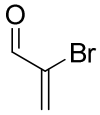
3-(3-(4-Methylpiperazin-1-yl)propoxy)-2-nitroaniline **77** (5.7, 19.36 mmol) and arsenic acid (5.495 g, 38.72 mmol, 2.7 mL) were dissolved in 85% phosphoric acid (3.0 mL) at 50°C. Acrolein (1.627 g, 2.904 mmol, 48.2 µL) was added and the mixture was heated at 50°C for 90 min. The mixture was then put in water (70 mL), basified with 25% NH_4OH until pH 13, and the acqueous phase was extracted with CH_2Cl_2 (3x30 mL). Combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure. The residue solid was then triturated with ethyl acetate (10 mL) and the resulting insoluble solid was filtered off and washed with ethyl acetate. Mother liquor was evaporated under reduced pressure to obtain 4.78 g of 37-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **81** (74.7 % yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.92 (dd, 1H, *J* = 4.3, 1.5 Hz), 8.13 (dd, 1H, *J* = 8.3, 1.5 Hz), 7.91 (d, 1H, *J* = 9.2 Hz), 7.42 (d, 1H, *J* = 9.2 Hz), 7.37 (dd, 1H, *J* = 8.3, 4.3 Hz), 4.31 (t, 2H, *J* = 6.2 Hz), 2.51 (t, 2H, *J* = 7.0 Hz), 2.45 (bs, 8H), 2.27 (s, 3H), 1.97 ("quintet", 2H, "*J*" = 6.5 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 152.84, 152.83, 150.16, 135.73, 130.55, 122.84, 120.56, 114.91, 114.89, 68.38, 55.08 (2C), 54.20, 53.06 (2C), 46.00, 45.97, 26.44.

ESI-MS (*m/z*) $[M+H]^+$: 331.5.

α-Bromoacrolein



82

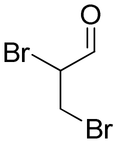
Method A:

Acrolein (15,0 g, 267.7 mmol, 17.76 mL (95% solution)) was suspended in water (135 mL) at 0°C. Bromine (44.90 g, 281.0 mmol, 14.40 mL) was added dropwise over 2h30'. After 30 minutes of additional stirring, a saturated solution of $Na_2S_2O_3$ (34 mL) was added and the solution was vigorously stirred for 10 min. The reaction mixture was steam distilled at atmospheric pressure and the organic layer afforded 16.98 g (125.8 mmol) of α-bromoacrolein **82** (47% yield).

Method B:

Acrolein (1,0 g, 17.8 mmol, 1.18 mL (95% solution)) was dissolved in CH_2Cl_2 (35 mL) at 0°C. Bromine (2.99 g, 18.7 mmol, 0.96 mL) was added dropwise over 5 min. A saturated solution of $Na_2S_2O_3$ (35 mL) was added and the resulting solution was stirred for 10 min at room temperature. The mixture was extracted with CH_2Cl_2 (2x15 mL) and dried over $MgSO_4$ and evaporated under reduced pressure (above 150 torr and at a temperature below 30°C). The resulting intermediate was then dissolved in ether (40 mL) and triethylamine (1.50 g, 15.7 mmol, 2.2 mL) was added dropwise over 5 min. The precipitate was filtered off and the organic phase was evaporated under reduced pressure (above 150 torr and at a temperature below 30°C) affording 1.55 g (11.5 mmol) of α-bromoacrolein **82**(67% yield).

2,3-dibromopropanal:



^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 9.40 (1H, d, *J* = 2.8 Hz), 4.55 (1H, ddd, *J* = 10.2, 4.7, 2.8 Hz), 3.90 (1H, t, *J* = 10.4 Hz), 3.75 (1H, dd, *J* = 10.5, 4.7 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm) : 188.99, 48.84, 26.79.

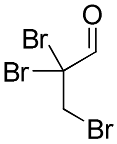
MS (70 eV) (*m/z)*: 217 (M^+, 3), 188 (M^+-CHO, 3), 106 (M^+-CHO-Br, 100).

α-bromoacrolein:
^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 9.26 (1H, s), 6.91 (1H, d, *J* = 2.2 Hz), 6.89 (1H, d, *J* = 2.2 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 186.0, 136.9, 132.8.

MS (70 eV): *m/z*: 136 (M+, 100), 134 (M+, 100), 108 (18), 107 (24), 106 (21), 105 (24), 55 (10), 54 (22).

2,2,3-Tribromopropanal



83

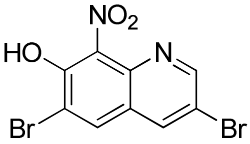
Bromine (1.184 g, 7.41 mmol) was added dropwise at 0°C to a stirred solution of α-bromoacrolein **82** (1.000 g, 7.41 mmol) in diethyl ether (20 mL). After the addition the solution was left to stir at 0°C for 30 minutes, then a saturated solution of $Na_2S_2O_3$ (30 mL) was added. The acqueous phase was extracted with diethyl ether (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give 2.184 g of 2,2,3-tribromopropanal **83** (99.9% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 9.15 (s, 1H), 4.23 (s, 1H).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 182.47, 63.89, 36.89.

MS(70 eV) *(m/z)*: 295.6 [M^+](3), 186.7 (100), 135 (3), 107 (32), 80 (6), 47 (7).

3,6-Dibromo-8-nitroquinolin-7-ol



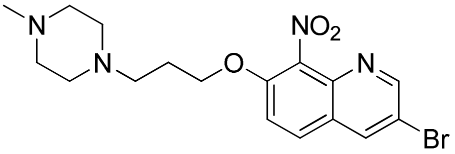
84

3-Amino-2-nitrophenol **72**(261 mg, 1.696 mmol) was added under stirring to 2,2,3-tribromopropanal (0.5 g, 1.696 mmol). The resulting slurry was heated at 100°C for 30 minutes. Acetic acid (5 mL) was added and the resulting suspension was heated at 110°C for 16 h. After cooling to r.t., a saturated solution of $NaHCO_3$ was added until the evolution of gas ceased. The acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined orgainic layers were dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give 84 mg of 3,6-dibromo-8-nitroquinolin-7-ol **84** (14% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 8.96 (d, 1H, *J* = 2.2 Hz), 8.68 (d, 1H, *J* = 2.2 Hz), 8.50 (s, 1H).

ESI-MS (*m/z*) $[M-H]^-$: 346.8-348.6-344.5.

3-Bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



86

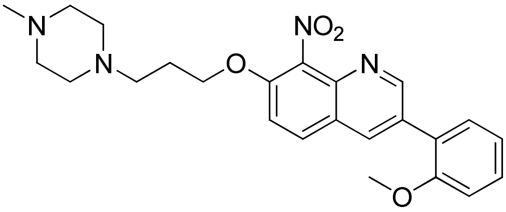
3-[3-(4-Methylpiperazin-1-yl)propoxy]-2-nitroaniline **77** (313 mg, 1.06 mmol) and arsenic acid (300 mg, 2.12 mmol, 0.15 mL) were dissolved in 85% phosphoric acid (3 mL) at 50°C. α-bromoacrolein **82** (138 mg, 1.020 mmol) was added and the mixture was heated at 50°C for 90 min. The mixture was then put in water (70 mL), basified with 25% NH_4OH until pH 13, and the acqueous phase was extracted with CH_2Cl_2 (3x30 mL). Combined organic phases were dried over $MgSO_4$ and evaporated under reduced pressure. The residue solid was then triturated with ethyl acetate (10 mL) and the resulting insoluble solid was filtered off and washed with AcOEt. Mother liquor was evaporated and the residue was recrystalized from ethyl acetate to afford 214 mg of 3-bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86** as pale yellow crystals (49% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.91 (d, 1H, *J* = 2.1 Hz), 8.30 (d,1H, *J* = 2.1 Hz), 7.83 (d,1H, *J* = 9.23 Hz), 7.46 (d,1H, *J* = 9.2 Hz), 4.32 (t, 2H, *J* = 6.2 Hz), 2.52 (t, 2H, *J* = 6.9 Hz), 2.50 (bs, 8H), 2.29 (s, 3H), 2.00 (“quintet”, 2H, “*J*” = 6.5 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 153.82, 150.35, 138.63, 138.60, 136.94, 135.65, 129.63, 123.53, 116.49, 116.10, 68.48, 67.75, 54.90 (2C), 54.10, 52.81 (2C), 52.71, 45.78, 26.33.

ESI-MS (*m/z*) $[M+H]^+$: 409.3-411.3.

3-(2-Methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



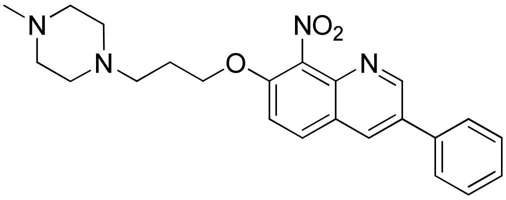
87

A mixture of 3-bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86** (13 mg, 0.031 mmol), 2-methoxyphenylboronic acid (5 mg, 0.033 mmol), Pd(OAc)$_2$ (1.05 mg, 0.004 mmol), Na$_2$CO$_3$ (0.016 mL of a 2M solution) and water (0.15 mL) was heathed at 150°C for 90 min. The mixture was then cooled to r.t., water (10 mL) was added and the acqueous layer was extracted with CH$_2$Cl$_2$ (3x10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated in vacuum, to give 11 mg (0.025 mmol) of 3-(2-methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **87** (80% yield).

^{1}H-NMR (300 MHz, CDCl$_3$) δ (ppm): 9.14 (d, 1H, *J* = 2.1 Hz), 8.23 (d, 1H, *J* = 2.1 Hz), 7.93 (d, 1H, *J* = 9.2 Hz), 7.43 (d, 1H, *J* = 9.1 Hz), 7.41 (m, 1H), 7.39 (m, 1H), 7.10 (td, 1H, *J* = 7.4, 1.2 Hz), 7.10 (dd, 1H, *J* = 7.7, 1.0 Hz), 4.33 (t, 2H, *J* = 6.2 Hz), 3.83 (s, 3H), 2.56 (t, 2H, *J* = 7.1 Hz), 2.53 (bs, 8H), 2.32 (s, 3H), 2.03 (“quintet”, 2H, “*J*” = 6.7 Hz).

ESI-MS (*m/z*) $[M+H]^+$: 437.0.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-8-nitro-3-phenylquinoline



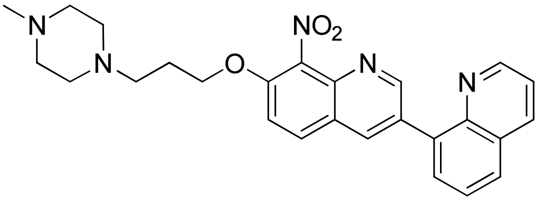
88

A mixture of 3-bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86**(50 mg, 0.122 mmol), phenylboronic acid (15.2 mg, 0.125 mmol), Pd(OAc)$_2$ (3.5 mg, 0.0157 mmol), Na$_2$CO$_3$ (0.06 mL of a 2M solution) and water (0.6 mL) was heathed at 150°C for 90 min. The mixture was then cooled to r.t., water (15 mL) was added and the acqueous layer was extracted with CH$_2$Cl$_2$ (3x10 mL). The combined organic layers were dried over MgSO$_4$, filtered and concentrated in vacuum, to give 26 mg (0.064 mmol) of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-3-phenylquinoline **88** (52% yield).

^{1}H-NMR (300 MHz, CDCl$_3$) δ (ppm): 9.20 (d,1H, *J* = 1.8 Hz), 8.28 (d,1H, *J* = 1.8 Hz), 7.96 (d,1H, *J* = 9.2 Hz), 7.66 (d, 2H, *J* = 7.1 Hz), 7.53 (t, 1H, *J* = 7.1 Hz), 7.45 (d,1H, *J* = 9.2 Hz), 7,56-7,35 (m, 2H), 4.33 (t, 2H, *J* = 6.0 Hz), 2.55 (t, 2H, *J* = 6.5 Hz), 2.53 (bs, 8H), 2.31 (s, 3H), 2.02 (qui, 2H, *J* = 5.7 Hz).

ESI-MS (*m/z*) $[M+H]^+$: 407.5.

7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-3-(8’-quinolyl)quinoline



89

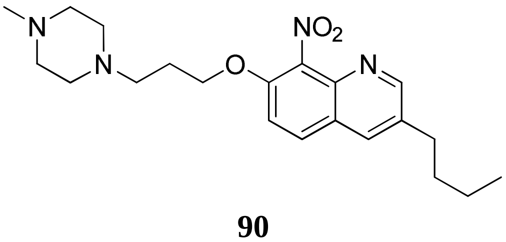
A mixture of 3-bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86** (100 mg, 0.244 mmol), 8-quinolineboronic acid (43.1 mg, 0.249 mmol), $Pd(OAc)_2$ (7.0 mg, 0.0314 mmol), Na_2CO_3 (0.12mL of a 2M solution) and water (1.5 mL) was heathed at 150°C for 2h. The mixture was then cooled to r.t., water (15 mL) was added and the acqueous layer was extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuum, to give 89 mg (0.194 mmol) of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-3-(8-quinolyl)quinoline **89** (79.7% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 9.32 (d, 1H, *J* = 2.1 Hz), 8.92 (dd, 1H, *J* = 4.3, 1.8 Hz), 8.48 (d, 1H, *J* = 2.1 Hz), 8.25 (dd, 1H, *J* = 8.3, 1.8 Hz), 7.98 (d, 1H, *J* = 9.2 Hz), 7.93 (dd, 1H, *J* = 8.1, 1.2 Hz), 7.81 (t, 1H, *J* = 7.2 Hz), 7.67 (t, 1H, *J* = 8.4 Hz), 7.47 (dd, 1H, *J* = 8.4, 4.2 Hz), 7.45 (d, 1H, *J* = 9.2 Hz), 4.34 (t, 2H, *J* = 6.2 Hz), 2.57 (t, 2H, *J* = 7.0 Hz), 2.54 (bs, 8H), 2.33 (s, 3H), 2.03 (“quintet”, 2H, *“J”* = 6.8 Hz)

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 155.30, 153.82, 150.58, 150.05, 139.44, 136.95, 136.39, 136.36, 132.26, 131.63, 130.85, 130.43, 129.63, 128.76, 126.47, 122.70, 121.57, 114.84, 68.40, 54.89 (2C), 54.21, 52.73 (2C), 45.75, 26.41.

ESI-MS (*m/z)* $[M+H]^+$: 457.3.

3-Butyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



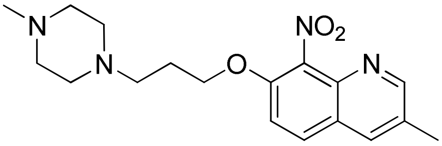
90

3-Bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86** (30 mg, 0.0733 mmol), *n*-butilboronic acid (12.3 mg, 0.121 mmol), potassium phosphate (31.1 mg, 0.145 mmol), bis(triphenylphosphine) palladium(II) dichloride (1.5 mg, 0.0021 mmol) and THF (1 mL) was placed in a sealed tube for three days. After cooling to room temperature, water (20 mL) was added and the acqueous phase was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were washed with acid water (pH<3) and extracted with diethyl ether (3x20 mL). The acqueous phase was then basified to pH>10 and extracted with CH_2Cl_2 (3x20 mL), the combined organic layers were dried over $MgSO_4$ and the solvent evaporated in vacuum. The residue was triturated with ethyl acetate and filtered. Mother liquors were evaporated in vacuum to give 12 mg (0.031 mmol) of 3-butyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **90** (42.3% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.80 (d, 1H, *J* = 2.0 Hz), 7.88 (d, 1H, *J* = 1.8 Hz), 7.83 (d, 1H, 9.1 Hz), 7.39 (d, 1H, *J* = 9.2 Hz), 4.30 (t, 2H, *J* = 6.4 Hz), 2.78 (t, 2H, *J* = 7.6 Hz), 2.52 (t, 2H, *J* = 7.0 Hz), 2.50 (bs, 8H), 2.29 (s, 3H), 2.00 (quintet ,2H, *J* = 6.7 Hz), 1.67 ("quintet", 2H, "*J*" = 7.7 Hz), 1.39 (ses, 2H, *J* = 7.6 Hz), 0.95 (t, 3H, *J* = 7.3 Hz).

ESI-MS (*m/z)* $[M+H]^+$: 387.3.

3-Methyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



91

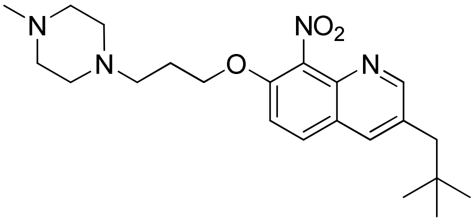
3-Bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86** (50 mg, 0.122 mmol), methylboronic acid (11.7 mg, 0.1952 mmol), potassium phosphate (51.0 mg, 0.240 mmol), bis(triphenylphosphine) palladium(II) dichloride (2.4 mg, 0.0034 mmol) and THF (0.43 mL) was placed in a sealed tube for 6 days. After cooling to room temperature, diluted HCl (20 mL) was added (pH<3) and the acqueous phase was extracted with diethyl ether (3x20 mL). The acqueous phase was then basified to pH>10 and extracted with CH_2Cl_2 (3x20 mL), the combined organic layers were dried over $MgSO_4$ and the solvent evaporated in vacuum. The residue was triturated with ethyl acetate and filtered. Mother liquor were evaporated in vacuum to give 24 mg (0.0697 mmol) of 3-methyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **91** (57.1% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.78 (d, 1H, *J* = 1.9 Hz), 7.89 (d, 1H, *J* = 1.9 Hz), 7.81 (d, 1H, 9.2 Hz), 7.38 (d, 1H, *J* = 9.2 Hz), 4.29 (t, 2H, *J* = 6.3 Hz), 2.51 (t, 2H, *J* = 7.1 Hz), 2.50 (s, 3H), 2.45 (bs, 8H), 2.28 (s, 3H), 1.99 ("quintet", 2H, "*J*" = 6.6 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm) : 154.73, 153.70, 138.73, 136.92, 134.30, 130.17, 129.78, 122.79, 114.91, 68.34, 55.03 (2C), 54.20, 53.00 (2C), 45.94, 26.44, 18.55.

ESI-MS (*m/z)* $[M+H]^+$: 345.3.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-3-neopentyl-8-nitroquinoline



92

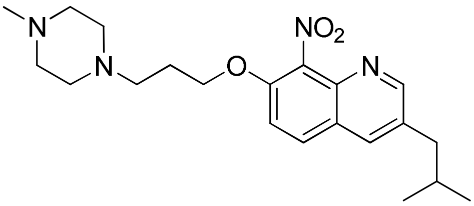
3-Bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86** (30 mg, 0.0733 mmol), neopentylboronic acid (11.1 mg, 0.0953 mmol), potassium phosphate (54.5 mg, 0.257 mmol), palladium acetate (0.8 mg, 0.00367 mmol), dicyclohexyl(2',6'-dimethoxybiphenyl-2-yl)phosphine (3 mg, 0.00733 mmol), toluene (0.33 mL) and water (16 μL), were placed in a sealed tube under argon atmosphere for 17 hours. After cooling to room temperature, water (20 mL) was added and the acqueous phase was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated in vacuum. The residue was triturated with ethyl acetate and filtered. Mother liquor were evaporated in vacuum to give 21 mg (0.0524 mmol) of 3-neopentyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **92** (71.5% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 8.73 (d, 1H, *J* = 1.8 Hz), 7.85 (d, 1H, *J* = 1.5 Hz), 7.84 (d, 1H, 9.0 Hz), 7.40 (d, 1H, *J* = 9.2 Hz), 4.30 (t, 2H, *J* = 6.0 Hz), 2.64 (s, 2H), 2.51 (t, 2H, *J* = 7.0 Hz), 2.47 (bs, 8H), 2.27 (s, 3H), 1.99 (“quintet”, 2H, “*J*” = 6.6 Hz), 0.94 (s, 9H).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 155.55, 152.82, 139.00, 135.72, 132.18, 130.10, 122.61, 120.55, 114.85, 68.40, 55.06 (2C) 55.06, 54.21, 53.02 (2C), 47.11, 32.09, 29.11 (3C), 26.46.

ESI-MS (*m/z)* $[M+H]^+$: 401.5.

3-Isobutyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



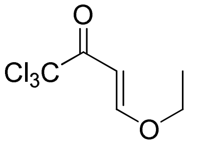
93

3-Bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86** (30 mg, 0.0733 mmol), isobutylboronic acid (14.9 mg, 0.1466 mmol), potassium phosphate (31.2 mg, 0.1466 mmol), palladium tetrakis triphenylphosphine (2.6 mg, 0.0022 mmol) and THF (0.30 mL) were placed in a sealed tube for 7 days. After cooling to room temperature, diluted HCl (20 mL) was added (pH<3) and the acqueous phase was extracted with diethyl ether (3x20 mL). The acqueous phase was then basified to pH>10 and extracted with CH_2Cl_2 (3x20 mL), the combined organic layers were dried over $MgSO_4$ and the solvent evaporated in vacuum. The residue was triturated with ethyl acetate and filtered. Mother liquor were evaporated in vacuum to give 14 mg (0.0362 mmol) of 3-isobutyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **93** (49.5% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.76 (d, 1H, J = 2.0 Hz), 7.86 (d, 1H, J = 2.5 Hz), 7.84 (d, 1H, 9.5 Hz), 7.39 (d, 1H, J = 9.2 Hz), 4.30 (t, 2H, J = 6.3 Hz), 2.64 (d, 2H, J = 7.1 Hz), 2.53 (t, 2H, J = 7.0 Hz), 2.50 (8H, bs), 2.28 (s, 3H), 2.00 ("quintet", 2H, "J" = 6.6 Hz), 1.93 (nonet, 1H, J = 7.1 Hz), 0.94 (d, 6H, J = 6.7 Hz).

ESI-MS (m/z) $[M+H]^+$: 387.3.

(*E*)-1,1,1-trichloro-4-ethoxybut-3-en-2-one



94

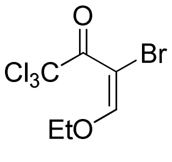
A solution of freshly distilled ethoxyethene (4.76 g, 66 mmol) in dry pyridine (4.85 mL) was slowly added dropwise to 2,2,2-trichloroacetyl chloride (10.91 g, 6.73 mL, 60 mmol) at -10°C . After the addition the mixture was left to stir at r.t. for 16h. The precipitate was filtered off and washed with diethyl ether (50 mL). The solvent was removed under reduced pressure to give 11.81 g of (*E*)-1,1,1-trichloro-4-ethoxybut-3-en-2-one **94** (90.5% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 7.86 (d, 1H, J = 12.1 Hz), 6.16 (d, 1H, J = 12.1 Hz), 4.10 (q, 2H, J = 7.1 Hz), 1.40 (t, 3H, J = 7.1 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 181.18, 167.49, 96.77, 92.22, 68.72, 14.45.

MS (70eV) *(m/z)*: 219.0 $[M^+]$ (26), 217.0 $[M^+]$ (28), 155.0 (58), 153.0 (67), 127.0 (44), 125.0 (60), 119.0 (50), 117.0 (52), 99.0 (100).

(*E*)-3-bromo-1,1,1-trichloro-4-ethoxybut-3-en-2-one



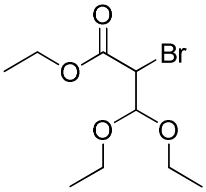
95

A solution of Br_2 (16.18 g, 5.19 mL, 104.5 mmol) in CCl_4 (18.4 mL) was added over 1 h to a solution of (*E*)-1,1,1-trichloro-4-ethoxybut-3-en-2-one **94** (22.07 g, 101.48 mmol) in CCl_4 (27.6 mL) at -30°C. After heating the solution to 0°C, NEt_3 (8.22 mL) was added and the mixture was left to stir at r.t. for 30 minutes. Diethyl ether (50 mL) was added and the precipitate was filtered off and washed with Et_2O (50 mL). The solvent was evaporated under reduced pressure to give 30.07 g of (*E*)-3-bromo-1,1,1-trichloro-4-ethoxybut-3-en-2-one **95** (99.9% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.38 (s, 1H), 4.36 (q, 2H, J = 7.1 Hz), 1.47 (t, 3H, J = 7.1 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 175.75, 171.42, 164.07, 95.17, 72.77, 15.45.

Ethyl 2-bromo-3,3-diethoxypropanoate



96

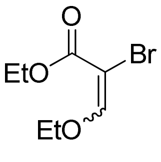
Sodium (4.667 g, 202.92 mmol) was dissolved in dry ethanol (80 mL)under argon atmosphere, and the solution was added dropwise at 0°C under argon atmosphere to a solution of (*E*)-3-bromo-1,1,1-trichloro-4-ethoxybut-3-en-2-one **96** (30.07 g, 101.46 mmol) in dry ethanol (29 mL). The mixture was left to stir at r.t for 18 h. The solvent was evaporated to give 13.7 g of ethyl 2-bromo-3,3-diethoxypropanoate **96** (50.1% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 4.86 (d, 1H, J = 8.3 Hz), 4.23 (q, 2H, J = 7.4 Hz), 4.21 (d, 1H, J = 8.6 Hz), 3.68 (m, 4H), 1.29 (t, 3H, J = 7.1 Hz), 1.25 (t, 3H, J = 7.1 Hz), 1.16 (t, 3H, J = 7.0 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 167.79, 101.90, 64.10, 62.74, 61.93, 44.99, 15.10, 15.08, 13.90.

MS (70 eV) *(m/z)*: 225.1 [M^+ -C_2H_5O](64), 223.1[M^+ -C_2H_5O](66), 197.0 (62), 195.0 (63), 169.0 (58), 166.9 (63), 151.0 (39), 149.0 (35), 125.0 (29), 124.0 (37), 123.0 (44), 122.0 (54), 103.1 (100), 75.0 (88).

Ethyl 2-bromo-3-ethoxyacrylate



97

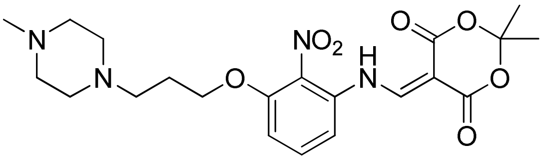
Phosphorous penthoxyde (1.7 g, 12 mmol) was added to a solution of ethyl 2-bromo-3,3-diethoxypropanoate **96** (860 mg, 2.90 mmol) in CH_2Cl_2 (6 mL). The mixture was refluxed for 4 hours. Water (20 mL) was added and the acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure to give 642 mg of ethyl 2-bromo-3-ethoxyacrylate **97** (99.2% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm):7.83 (s, 1H), 4.24 (q, 2H, *J* = 7.1 Hz), 4.20 (q, 2H, *J* = 7.1 Hz), 1.40 (t, 3H, *J* = 7.1 Hz), 1.31 (t, 3H, *J* = 7.1 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 163.62, 158.08, 91.87, 71.28, 61.62, 15.34, 14.27.

MS (70 eV) *(m/z)*: 224.1 [M^+](79), 222.1 [M^+](80), 196.1 (62), 194.1 (68), 179.1 (65), 177.0 (60), 168.0 (86), 166.0 (95), 150.1 (86), 148.0 (100), 103.1 (85), 75.1 (77), 47.1 (84).

2,2-Dimethyl-5-((3-(3-(4-methylpiperazin-1-yl)propoxy)-2-nitrophenylamino)methylene)-1,3-dioxane-4,6-dione



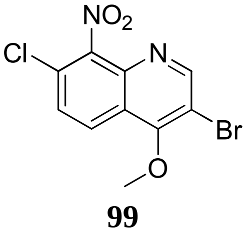
98

A solution of Meldrum's acid (709.8 mg, 4.925 mmol) in trimethoxymethane (4,49 g, 42,29 mmol, 4,6 mL) is heated at 100°C under argon atmosphere for 2 h. 3-(3-(4-Methylpiperazin-1-yl)propoxy)-2-nitroaniline **77** (1.0g, 3.397 mmol) is added and the resulting mixture is heated at 100°C for further 2 h. Solvent is removed in vacuum to give 1,08 g of 2,2-dimethyl-5-((3-(3-(4-methylpiperazin-1-yl)propoxy)-2-nitrophenylamino)methylene)-1,3-dioxane-4,6-dione **98** (70% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.54 (s, 1H), 7.51 (t, 1H, *J* = 8.4 Hz), 7.01 (d, 1H, *J* = 8.0 Hz), 6.97 (d, 1H, *J* = 8.6 Hz), 4.18 (t, 2H, *J* = 6.1 Hz), 2.58 (bs, 8H), 2.56 (t, 2H, *J* = 7.0 Hz), 2.37 (s, 3H), 1.99 (qui, 2H, *J* = 6.6 Hz), 1.74 (s, 6H).

ESI-MS *(m/z)* $[M+H]^+$: 449.3

3-Bromo-7-chloro-4-methoxy-8-nitroquinoline



99

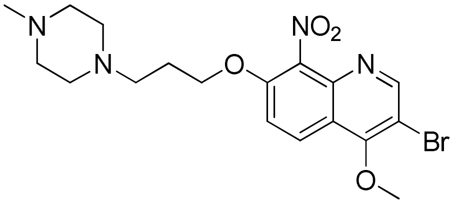
To a soloution of 7-chloro-4-methoxy-8-nitroquinoline **12** (200 mg, 0.838 mmol) at 110°C, NBS was added, and the mixture was brought to reflux and left to stir for 4 h without further heating. Water (40 mL) was added, and the acqueous phase was extracted with CH_2Cl_2 (3x30 mL). Combined organic layers were washed with water (3x30 mL) and brine (30 mL) and dried over $MgSO_4$. An attempt of crystalization of the residue in MeOH gave white crystals of 3-bromo-4,7-dimethoxy-8-nitroquinoline **101** because 4,7-dimethoxy-8-nitroquinoline **55** was present in the starting material as an impurity. Mother liquor was evaporated to give 210 mg of 3-bromo-7-chloro-4-methoxy-8-nitroquinoline **99** (79.8 % yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.95 (s, 1H), 8.20 (d, 1H, *J* = 9.1 Hz), 7.62 (d, 1H, *J* = 9.1 Hz), 4.19 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 160.41, 156.59, 146.99, 140.51, 128.00, 126.44, 124.60, 124.23, 110.06, 62.25.

MS (70 eV) *(m/z)*: 238 [M^+](94), 316 [M^+](73), 288 (62), 286 (49), 260 (46), 258 (36), 230 (100), 228 (79), 191 (70), 161 (58), 151 (57), 150 (72).

3-Bromo-4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline



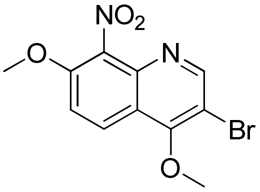
100

3-(4-Methylpiperazin-1-yl)propan-1-ol **7** (209.3 mg, 1.32 mmol) was added to a solution of *t*-BuOK (212 mg, 1.89 mmol) in dry THF (2 mL) under argon atmosphere. After 15 minutes, this solution was added slowly via syringe, to a solution of 3-bromo-7-chloro-4-methoxy-8-nitroquinoline **99** (400 mg, 1.26 mmol) in dry THF (2mL) at 0°C. The reaction mixture was refluxed overnight and after cooling to r.t. water (30 mL) was added. The acqueous phase was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum and the residue was purified via flash cromathograpy over basic alumina. Eluition was done in gradient of polarity. First eluent used was AcOEt, then acetone was employed. 83 mg of 3-bromo-4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **100** was recovered with the latter eluent (15% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.86 (s, 1H), 8.24 (d, 1H, *J* = 9.4 Hz), 7.38 (d, 1H, *J* = 9.4 Hz), 4.31 (t, 2H, *J* = 6.3 Hz), 4.05 (s, 3H), 2.62 (t, 2H, *J* = 7.2 Hz), 2.47 (bs, 8H), 2.28 (s, 3H), 2.10 (“quintet”, 2H, “*J*” = 6.8 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 159.70, 156.26, 150.96, 141.00, 136.48, 125.36, 119.94, 113.96, 108.08, 73.38, 57.05, 55.13 (2C), 54.41, 53.13 (2C), 46.03, 27.45.

3-Bromo-4,7-dimethoxy-8-nitroquinoline



101

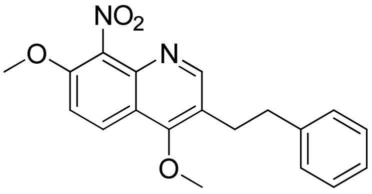
This product has been obtained as a byproduct of bromination of 7-chloro-4-methoxy-8-nitroquinoline **12**. An attempt of crystalization of the dried organic phase of product **100** in MeOH gave white crystals of 3-bromo-4,7-dimethoxy-8-nitroquinoline **101** because 4,7-dimethoxy-8-nitroquinoline **55** was present in the starting material as an impurity.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.90 (s, 1H), 8.23 (d, 1H, *J* = 9.4 Hz), 7.42 (d, 1H, *J* = 9.4 Hz), 4.16 (s, 3H),4.08 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 160.36, 156.32, 151.01, 140.94, 136.45, 125.14, 119.57, 114.10, 107.53, 62.09, 57.06.

MS (70 eV) (*m/z)*: 314.1 [M^+] (97), 312.1 [M^+] (100), 284.1 [M^+-CH_3-CH_3] (8857), 282.1 [M^+-CH_3-CH_3] (59), 269.1 (23), 267.1 (30), 256.1 (34), 254.1 (40), 241.1 (28), 239.1 (37), 157.1 (94), 127.1 (57), 114.1 (55).

4,7-Dimethoxy-8-nitro-3-phenethylquinoline



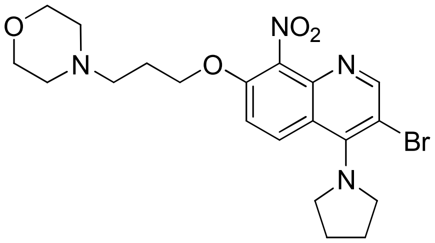
102

3-Bromo-4,7-dimethoxy-8-nitroquinoline **101** (71 mg, 0.227 mmol), phenethylboronic acid (44.2 mg, 0.295 mmol), potassium phosphate (168.5 mg, 0.794 mmol), palladium acetate (2.5 mg, 0.011 mmol), dicyclohexyl(2',6'-dimethoxybiphenyl-2-yl)phosphine (9.3 mg, 0.023 mmol), toluene (1 mL) and water (48 μL), were placed with stirring in a sealed tube under argon atmosphere for 17 hours. After cooling to room temperature, water (20 mL) was added and the acqueous phase was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated in vacuum to give 81 mg of 4,7-dimethoxy-8-nitro-3-phenethylquinoline **102** (91.0% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.55 (s, 1H), 7.39 (d, 1H, *J* = 9.0 Hz), 7.33-7.19 (m,6H), 4.89 (bs, 2H), 3.99 (s, 3H), 3.92 (s, 3H), 3.06 (m, 2H, part of AA'BB' system), 3.01 (m, 2H, part of AA'BB' system).

MS (70 eV) *(m/z)*: 338.3 [M^+](15), 247.2 (99), 201.3 (30), 200.2 (24), 91 (100).

4-(3-(3-Bromo-8-nitro-4-(pyrrolidin-1-yl)quinolin-7-yloxy)propyl)morpholine



103

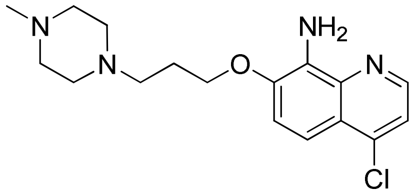
NBS (35.4 mg, 0.199 mmol) was added to a solution of 4-(3-(8-nitro-4-(pyrrolidin-1-yl)quinolin-7-yloxy)propyl)morpholine **67** (77 mg, 0.199 mmol) in DMF (2 mL) and the mixture was left to stir at r.t for 18 hours. Water (30 mL)was added, the acqueous phase was extracted with toluene (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 24 mg of 4-(3-(3-bromo-8-nitro-4-(pyrrolidin-1-yl)quinolin-7-yloxy)propyl)morpholine **103** (25.9% yield)

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.79 (s, 1H), 8.18 (d, 1H, *J* = 9.4 Hz), 7.32 (d, 1H, *J* = 9.4 Hz), 4.34 (t, 2H, *J* = 6.0 Hz), 3.79 (t, 4H, *J* = 4.6 Hz), 3.58 (m,4H), 2.67 (t, 2H, *J* = 7.1 Hz), 2.61 (bs, 4H), 2.10 (m, 6H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 156.61, 151.88, 149.71, 140.82, 137.08, 127.59, 123.74, 113.84, 112.30, 67.91, 66.16, 54.76, 53.32, 51.75, 26.14, 25.52.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 465.1127, observed, 465.1127.

4-Chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



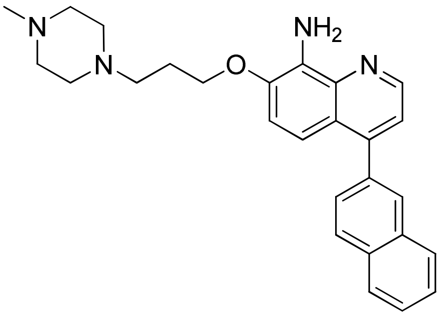
104

Tin chloride dihydrate (1.55g, 6.85 mmol) was added to a solution of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **8** (0.5 g, 1.37 mmol) in ethanol (10 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (25 mL) and AcOEt (20 mL) were added. The acqueous phase was exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 0.40 g of a dark solid of 4-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **104** (87% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.59 (d, 1H, *J* = 4.6 Hz), 7.50 (d, 1H, *J* = 9.1 Hz), 7.33 (d, 1H, *J* = 4.7 Hz), 7.33 (d, 1H, *J* = 8.9 Hz), 5.13 (s, 2H), 4.20 (t, 2H, *J* = 6.2 Hz), 2.61 (t, 2H, *J* = 7.2 Hz), 2.49 (bs, 8H), 2.31 (s, 3H), 2.06 (“quintet”, 2H, “*J*” = 7.1 Hz).

^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm): 147.11, 143.69, 142.42, 138.82, 132.63, 122.10, 119.30, 116.17, 111.31, 67.62, 54.94 (2C), 54.74, 52.76 (2C), 45.69, 26.75.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(naphthalen-2-yl)quinolin-8-amine



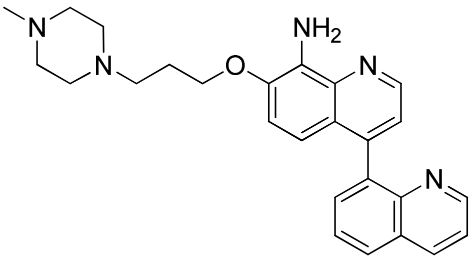
105

Tin chloride dihydrate (0.22 g, 0.986 mmol) was added to a solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-2-yl)-8-nitroquinoline **17** (0.090 g, 0.197 mmol) in ethanol (5 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (25 mL) and AcOEt (20 mL) were added. The acqueous phase was exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over MgSO$_4$. The solvent was removed in vacuum, to give 0.070 g of a dark solid of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-2-yl)quinolin-8-amine **105** (83% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ (ppm): 8.81 (d, 1H, *J* = 4.3 Hz), 7.97 (d, 1H. *J* = 2.2 Hz), 7.96 (d, 1H. *J* = 7.8 Hz), 7.92 (m, 2H), 7.62 (dd, 1H, *J* = 8.5, 1.7 Hz), 7.56 (m, 2H), 7.28 (d, 1H, *J* = 4.3 Hz), 7.22 (d, 1H, *J* = 9.3 Hz), 7.20 (d, 1H. *J* = 9.3 Hz), 5.17 (bs, 2H), 4.17 (t, 2H, *J* = 6.2 Hz), 2.61 (t, 2H, *J* = 7.1 Hz), 2.49 (bs, 8H), 2.30 (s, 3H), 2.05 ("quintet", 2H, *"J"* = 6.8 Hz).

^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm): 148.35, 147.72, 142.70, 138.83, 136.06, 133.21, 132.98, 132.59, 128.56, 128.19, 127.99, 127.80, 127.40, 126.60, 126.58, 122.78, 120.09, 115.41, 113.63, 67.15, 54.47, 53.53 (2C), 50.36 (2C), 44.10, 26.42.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(8'-quinolinyl)quinoline-8-amine



106

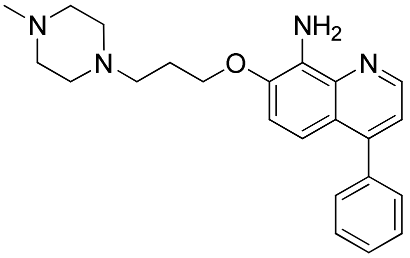
Tin chloride dihydrate (0.43 g, 1.9 mmol) was added to a solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4,8'-biquinoline **18** (0.175 g, 0.38 mmol) in ethanol (3 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (25 mL) and AcOEt (20 mL) were added. The acqueous phase was exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 0.14 g of a dark solid of 7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(8'-quinolinyl)quinoline-8-amine (86% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.86 (d, 1H, *J* = 4.3 Hz), 8.82 (dd, 1H, *J* = 4.2, 1.8 Hz), 8.25 (dd, 1H, *J* = 8.3, 1.8 Hz), 7.95 (dd, 1H, *J* = 7.9, 1.9 Hz), 7.69 (dd, 1H, *J* = 7.0, 1.8 Hz), 7.65 (dd, 1H, *J* = 7.8, 7.1 Hz), 7.42 (dd, 1H, *J* = 8.3, 4.2 Hz), 7.30 (d, 1H, *J* = 4.3 Hz), 7.05 (d, 1H, *J* = 9.1 Hz), 6.67 (d, 1H, *J* = 9.1 Hz) 5.14 (bs, 2H), 4.11 (t, 2H, *J* = 6.2 Hz), 2.59 (t, 2H, *J* = 7.1 Hz), 2.50 (bs, 8H), 2.29 (s, 3H), 2.01 ("quintet", 2H, "*J*" = 7.1 Hz)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 150.62, 147.34, 146.65, 146.63, 142.73, 138.77, 137.99, 136.18, 132.81, 130.94, 128.48, 128.38, 125.98, 124.02, 121.29, 120.99, 115.91, 113.88, 67.88, 55.10, 55.09 (2C), 53.19 (2C), 46.01, 26.98.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 428.2444 observed, 428.2461.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-phenylquinolin-8-amine



107

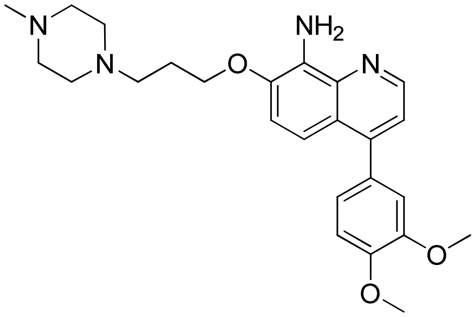
Tin chloride dihydrate (584 mg, 2.59 mmol) was added to a solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-phenylquinoline **19** (150 mg, 0.37 mmol) in ethanol (3 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (25 mL) and AcOEt (20 mL) were added. The acqueous phase was exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 100 mg of a dark oil of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-phenylquinolin-8-amine **107** (71% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.76 (d, 1H, *J* = 4.3 Hz), 7.48 (m, 5H), 7.19 (d, 1H, *J* = 9.3 Hz), 7.167 (d, 1H, *J* = 9.3 Hz), 7.165 (d, 1H, *J* = 4.3 Hz), 5.14 (bs, 2H), 4.16 (t, 2H, *J* = 6.2 Hz), 2.60 (t, 2H, *J* = 6.9 Hz), 2.49 (bs, 8H), 2.29 (s, 3H), 2.03 ("quintet", 2H, "*J*" = 7.0 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 148.31, 147.52, 142.90, 138.81, 138.69, 132.82, 129.48 (2C), 128.38 (2C), 128.15, 122.64, 119.67, 115.94, 113.28, 67.89, 55.11, 55.08 (2C), 53.17 (2C), 46.01, 26.98.

ESI-MS (*m/z*) $[M+H]^+$: 377.3.

4-(3,4-Dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



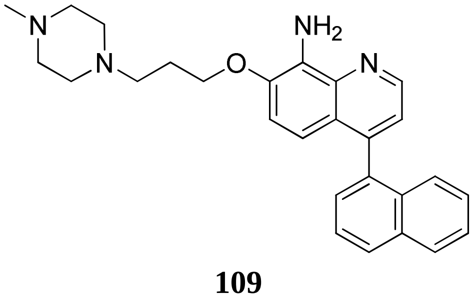
108

Tin chloride dihydrate (0.70 g, 3.05 mmol) was added to a solution of 4-(3,4-dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **20** (0.205 g, 0.44 mmol) in ethanol (4 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (25 mL) and AcOEt (20 mL) were added. The acqueous phase was exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 0.12 g of a dark solid of 4-(3,4-dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **108** (62% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.74 (d, 1H, *J* = 4.3 Hz), 7.24 (d, 1H, *J* = 9.1 Hz), 7.19 (d, 1H, *J* = 9.2 Hz), 7.16 (d, 1H, *J* = 4.3 Hz), 7.05 (dd, 1H, *J* = 8.1, 1.6 Hz), 7.01 (d, 1H, *J* = 1.7 Hz), 6.98 (d, 1H, *J* = 8.2 Hz),5.13 (bs, 2H), 4.16 (t, 2H, *J* = 6.2 Hz), 3.95 (s, 3H), 3.89 (s,3H), 2.60 (t, 2H, *J* = 7.2 Hz), 2.48 (bs, 8H), 2.28 (s, 3H), 2.03 (“quintet”, 2H, “*J*” = 6.9 Hz)

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 148.99, 148.68, 148.03, 147.47, 142.74, 138.77, 132.72, 131.21, 122.69, 121.90, 119.55, 115.78, 113.19, 112.66, 110.96, 67.78, 55.94, 55.92, 55.05, 55.01 (2C), 53.11 (2C), 45.96, 26.89.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yl)quinolin-8-amine



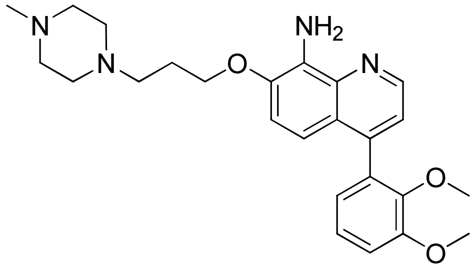
109

Palladium (5%) on activated carbon (10.5 mg) was added to a stirred solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yl)-8-nitroquinoline **21** (99 mg, 0,219 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 55 mg of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yl)quinolin-8-amine **109** as a yellow oil (yield 60%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.83 (d, 1H, *J* = 4.3 Hz), 7.95 (d, 1H, *J* = 9.1 Hz), 7.92 (d, 1H, *J* = 8.7 Hz), 7.56 (dd, 1H, *J* = 8.1, 7.1 Hz), 7.47 (ddd, 1H, *J* = 8.0, 6.7, 1.2 Hz), 7.43 (dd, 1H, *J* = 7.0, 1.0 Hz), 7.36 (d, 1H, *J* = 8.2 Hz), 7.29 (ddd, 1H, *J* = 8.1, 7.0, 1.2 Hz), 7.25 (d, 1H, *J* = 4.3 Hz), 7.04 (d, 1H, *J* = 9.1 Hz), 6.68 (d, 1H, *J* = 9.0 Hz), 5.16 (bs, 2H), 4.11 (t, 2H, *J* = 6.1 Hz), 2.60 (t, 2H, *J* = 7.2 Hz), 2.52 (bs, 8H), 2.32 (s, 3H), 2.02 ("quintet", 2H, "*J*" = 6.7 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 147.50, 147.06, 142.91, 138.58, 136.28, 133.40, 132.64, 131.91, 128.46, 128.18, 127.15, 126.22, 126.07, 126.00, 125.20, 123.91, 120.82, 115.85, 113.88, 67.72, 55.01, 54.90 (2C), 52.88 (2C), 45.79, 26.88.

4-(2,3-Dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



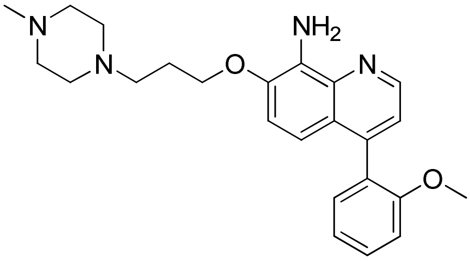
110

Palladium (5%) on activated carbon (9.3 mg) was added to a stirred solution of 4-(2,3-dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **22** (60 mg , 0.129 mmol) in methanol (2 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was filtered over silica with ethyl acetate, and then purified by flash chromatography on basic alumina (eluent AcOEt). The fractions containing the desired product were recristalyzed with hexane (1 mL) to give 55 mg of 4-(2,3-dimethoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **110** as a yellow solid (98% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.77 (d, 1H, *J* = 4.3 Hz), 7.19 (d, 1H, *J* = 4.3 Hz), 7.15 (d, 1H, *J* = 9.1 Hz), 7.149 (d, 1H, *J* = 7.9 Hz), 7.02 (dd, 1H, *J* = 8.2, 1.4 Hz), 6.94 (d, 1H, *J* = 9.0 Hz), 6.85 (dd, 1H, *J* = 7.7, 1.4 Hz), 5.12 (bs, 2H), 4.14 (t, 2H, *J* = 6.2 Hz), 3.94 (s, 3H), 3.46 (s,3H), 2.60 (t, 2H, *J* = 7.2 Hz), 2.48 (bs, 8H), 2.29 (s, 3H), 2.03 ("quintet", 2H, "*J*" = 6.9 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 150.85, 150.81, 147.92, 147.27, 142.84, 132.79, 132.57, 123.93, 123.74, 123.27, 122.95, 120.18, 115.89, 113.72, 112.34, 67.84, 60.97, 55.89, 55.10 (2C), 55.10, 53.19(2C), 46.00, 26.98.

4-(2-Methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



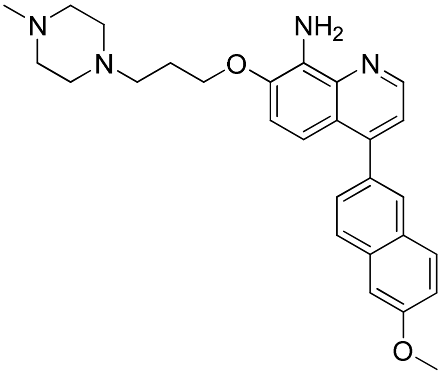
111

Palladium (5%) on activated carbon (9.3 mg) was added to a stirred solution of 4-(2-methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **23** (90 mg, 0,206 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was dissolved in ethyl acetate, filtered, and then purified with flash chromatography on basic alumina (eluent AcOEt:CH_2Cl_2 4:6) to give 70 mg of 4-(2-methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **111** as a yellow oil (84% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.77 (d, 1H, *J* = 4.3 Hz), 7.44 (ddd, 1H, *J* = 9.2, 7.4, 1.8 Hz), 7.23 (dd, 1H, *J* = 7.5, 1.8 Hz), 7.17 (d, 1H, *J* = 4.3 Hz), 7.14 (d, 1H, *J* = 9.1 Hz), 7.07 (dt, 1H, *J* = 7.4, 0.9 Hz), 7.04 (dd, 1H, *J* = 8.2, 0.9 Hz), 6.88 (d, 1H, *J* = 9.1 Hz), 5.11 (bs, 2H), 4.14 (t, 2H, *J* = 6.2 Hz), 3.70 (s, 3H), 2.60 (t, 2H, *J* = 7.2 Hz), 2.50 (bs, 8H), 2.29 (s, 3H), 2.03 (“quintet”, 2H, *“J”* = 6.6 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 156.78, 147.44, 145.50, 142.74, 138.63, 132.61, 131.18, 129.63, 127.42, 123.35, 120.48, 120.48, 115.79, 113.67, 111.13, 67.85, 55.57, 55.13, 55.08 (2C), 53.19 (2C), 46.02, 26.98**.**

4-(6-Methoxynaphthalen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



112

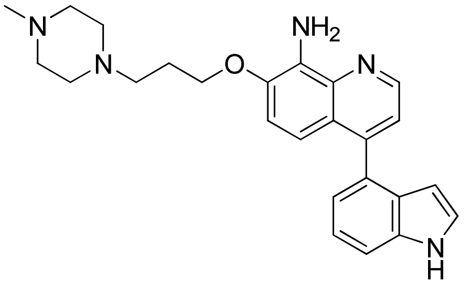
Palladium (5%) on activated carbon (80 mg) was added to a stirred solution of 4-(6-methoxynaphthalen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **24** (800 mg, 1.6 mmol) in methanol (25 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was dissolved in AcOEt, filtered, and then purified with flash chromatography (eluent CH_2Cl_2:methanol:Et_3N, 9.8:0.1:0.1) to give 584 mg of 4-(6-methoxynaphthalen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **112** as yellow solid (80% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.78 (d, 1H, *J* = 4.4 Hz), 7.89 (d, 1H, *J* = 1.8 Hz), 7.84 (d, 1H, *J* = 8.5 Hz), 7.79 (d, 1H, *J* = 9.7 Hz), 7.57 (dd, 1H, *J* = 8.4, 1.8 Hz), 7.25 (d, 1H, *J* = 4.4 Hz), 7.24 (d, 1H, *J* = 9.1 Hz), 7.21 (m, 2H), 7.18 (d, 1H, *J* = 9.2 Hz), 5.16 (bs, 2H), 4.16 (t, 2H, *J* = 6.2 Hz), 3.95 (s, 3H), 2.61 (t, 2H, *J* = 7.4 Hz), 2.50 (bs, 8H), 2.30 (s, 3H), 2.04 (“quintet”, 2H, “*J*” = 7.1 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 158.18, 148.34, 147.53, 142.87, 138.89, 134.20, 133.90, 132.85, 129.68, 128.68, 128.36, 127.95, 126.73, 122.83, 119.92, 119.38, 115.92, 113.37, 105.68, 67.86, 55.37, 55.06 (2C), 53.12 (2C), 45.95, 26.97.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 457.2598, observed, 457.2595.

4-(1H-indol-4-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



113

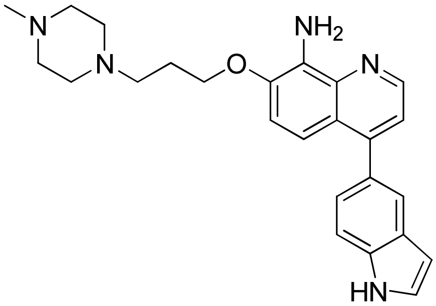
Tin chloride dihydrate (2 g, 9 mmol) was added to a solution of 4-(1H-indol-4-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **25** (800 mg, 1.8 mmol) in ethanol (25 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (40 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x50 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2/*n*-hexane to give 673 mg of 4-(1H-indol-4-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **113** as yellow solid (90% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.81 (d, 1H, *J* = 4.3 Hz), 8.56 (bs, 1H), 7.49 (dt, 1H, *J* = 8.2, 1.0 Hz), 7.34 (d, 1H, *J* = 4.3 Hz), 7.31 (dd, 1H, *J* = 8.2, 7.2 Hz), 7.19 (dd, 1H, *J* = 3.3, 2.3 Hz), 7.18 (dd, 1H, *J* = 7.2, 1.0 Hz), 7.12 (s, 2H) 6.22 (ddd, 1H, *J* = 3.3, 2.1, 1.0 Hz), 5.14 (bs, 2H), 4.15 (t, 2H, *J* = 6.2 Hz), 2.61 (t, 2H, *J* = 7.4 Hz), 2.50 (bs, 8H), 2.31 (s, 3H), 2.03 (“quintet”, 2H, “*J*” = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 147.56, 147.56, 142.87, 138.97, 135.89, 132.54, 130.93, 127.35, 124.56, 123.06, 121.82, 121.06, 120.21, 115.62, 114.22, 111.07, 102.32, 67.84, 55.10, 55.06 (2C), 53.13 (2C), 45.96, 26.99.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 416.2444, observed, 416.2433.

4-(1H-indol-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



114

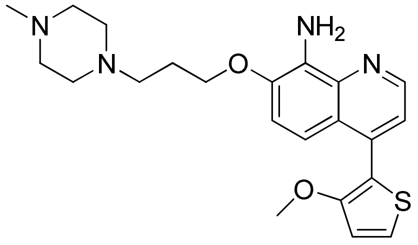
Tin chloride dihydrate (2 g, 9 mmol) was added to a solution of 4-(1H-indol-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **26** (800 mg, 1.8 mmol) in ethanol (25 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (40 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x50 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2/*n*-hexane to give 673 mg of 4-(1H-indol-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **114** as yellow solid (90% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.76 (d, 1H, *J* = 4.4 Hz), 8.50 (bs, 1H), 7.50 (dt, 1H, *J* = 8.3, 0.9 Hz), 7.33 (dd, 1H, *J* = 8.4, 1.7 Hz), 7.31 (d, 1H, *J* = 9.2 Hz), 7.30 (dd, 1H, *J* = 3.4, 2.3 Hz), 7.26 (d, 1H, *J* = 4.3 Hz), 7.16 (d, 1H, *J* = 9.1 Hz), 6.63 (ddd, 1H, *J* = 3.2, 2.0, 1.0 Hz), 4.98 (bs, 2H), 4.16 (t, 2H, *J* = 6.2 Hz), 2.63 (t, 2H, *J* = 7.1 Hz), 2.55 (bs, 8H), 2.32 (s, 3H), 2.04 (“quintet”, 2H, “*J*” = 6.7 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ (ppm): 149.70, 147.57, 142.79, 138.94, 135.60, 132.55, 130.34, 127.88, 125.07, 123.85, 123.27, 121.79, 120.13, 115.55, 114.01, 110.84, 102.98, 67.78, 54.99, 54.70 (2C), 52.74 (2C), 45.61, 26.89.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 416.2444, observed, 416.2427.

4-(3-Methoxythiophen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



115

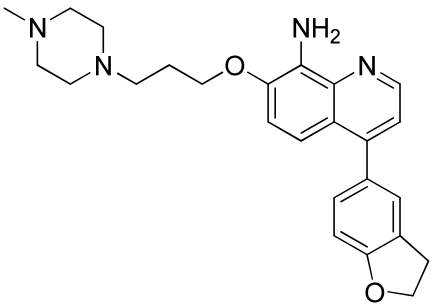
Tin chloride dihydrate (113 mg, 0.5 mmol) was added to a solution of 4-(3-methoxythiophen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **27** (58 mg, 0.1 mmol) in ethanol (8 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (15 mL) was added. The acqueous phase were exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue purified with flash chromatography (eluent CH_2Cl_2:EtOH:Et_3N, 9.6:0.3:0.1) to give 27 mg of 4-(3-methoxythiophen-2-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **115** as red solid (65% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.73 (d, 1H, *J* = 4.4 Hz), 7.38 (d, 1H, *J* = 5.5 Hz), 7.36 (d, 1H, *J* = 9.1 Hz), 7.31 (d, 1H, *J* = 4.4 Hz), 7.22 (d, 1H, *J* = 9.2 Hz), 7.00 (d, 1H, *J* = 5.5 Hz), 4.18 (t, 2H, *J* = 6.1 Hz), 3.79 (s, 3H), 2.69 (t, 2H, *J* = 7.2 Hz), 2.68 (bs, 8H), 2.43 (s, 3H), 2.07 ("quintet", 2H, "*J*" = 7.0 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 154.88, 147.26, 142.86, 139.05, 132.63, 124.94, 124.91, 122.87, 121.43, 117.00, 115.63, 113.84, 113.82, 67.55, 58.85 (2C), 54.82, 54.37, 54.35 (2C), 52.02, 29.65.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 413.2005, observed, 413.2044.

4-(2,3-Dihydrobenzofuran-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



116

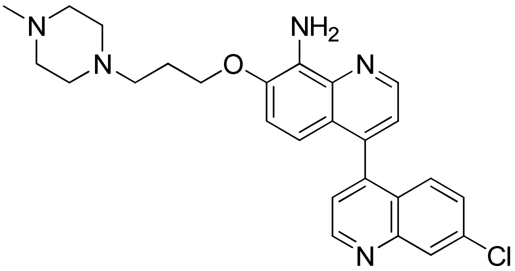
Tin chloride dihydrate (1 g, 5.5 mmol) was added to a solution of 4-(2,3-dihydrobenzofuran-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **28** (500 mg, 1.1 mmol) in ethanol (20 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (20 mL) was added. The acqueous phase were exctracted with CH_2Cl_2 (3x35 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue purified with flash chromatography (eluent CH_2Cl_2:EtOH:Et_3N, 9.4:0.5:0.1) to give 300 mg of 4-(2,3-dihydrobenzofuran-5-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **116** as yellow solid (65% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.71 (d, 1H, *J* = 4.4 Hz), 7.31 (m, 1H), 7.23 (d, 1H, *J* = 9.1 Hz), 7.23 (dm, 1H, *J* = 8.2 Hz), 7.17 (d, 1H, *J* = 9.2 Hz), 7.12 (d, 1H, *J* = 4.3 Hz), 6.88 (d, 1H, *J* = 8.2 Hz), 5.12 (bs, 2H), 4.64 (t, 2H, *J* = 8.7 Hz), 4.15 (t, 2H, *J* = 6.2 Hz), 3.27 (t, 2H, *J* = 8.8 Hz), 2.60 (t, 2H, *J* = 7.4 Hz), 2.48 (bs, 8H), 2.29 (s, 3H), 2.03 ("quintet", 2H, "*J*" = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.25, 148.39, 147.51, 142.78, 138.87, 132.74, 130.88, 129.52, 127.28, 126.12, 122.88, 119.61, 115.73, 113.36, 109.15, 71.49, 67.84, 55,07, 55.03, 53.12, 45.96, 29.63, 26.95.

ESI-HRMS (*m/z*) $[M+H]^+$:calculated, 419.2441, observed, 419.2434.

7'-Chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-4,4'-biquinolin-8-amine



117

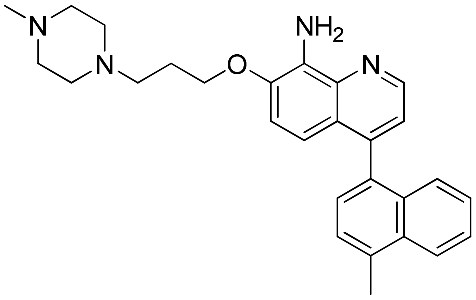
Tin chloride dihydrate (412 mg, 1.8 mmol) was added to a solution of 7'-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4,4'-biquinoline **29** (180 mg, 0.3 mmol) in ethanol (10 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (20 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x35 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue purified with flash chromatography (eluent CH_2Cl_2: ethanol:Et_3N, 9.4:0.5:0.1) to give 101 mg of 7'-chloro-7-(3-(4-methylpiperazin-1-yl)propoxy)-4,4'-biquinolin-8-amine **117** as yellow solid (60% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.03 (d, 1H, *J* = 4.4 Hz), 8.86 (d, 1H, *J* = 4.2 Hz), 8.21 (part of a deceptively simple system: pseudo t, 1H, "*J*" = 1.4 Hz), 7.39 (d, 1H, *J* = 4.3 Hz), 7.34 (part of a deceptively simple system: m, 2H), 7.21 (d, 1H, *J* = 4.3 Hz), 7.11 (d, 1H, *J* = 9.1 Hz), 6.57 (d, 1H, *J* = 9.1 Hz), 5.22 (bs, 2H), 4.14 (t, 2H, *J* = 8.2 Hz), 2.59 (t, 2H, *J* = 7.3 Hz), 2.49 (bs, 8H), 2.29 (s, 3H), 2.03 ("quintet", 2H, "*J*" = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 150.91, 148.69, 147.22, 145.16, 143.37, 143.15, 138.33, 135.64, 133.02, 128.71, 127.93, 127.33, 125.41, 122.54, 121.80, 119.92, 116.39, 112.73, 67.80, 55.07, 55.03, 53.17, 45.98, 26.88.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 462.1982, observed, 462.1982.

4-(4-Methylnaphthalen-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



118

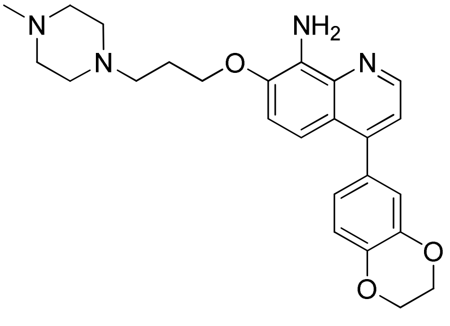
Tin chloride dihydrate (113 mg, 0.5 mmol) was added to a solution of 4-(4-methylnaphthalen-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **30** (458 mg, 1 mmol) in ethanol (15 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (25 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x45 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2 to give 352 mg of 4-(4-methylnaphthalen-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **118** as brown solid (80% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.83 (d, 1H, *J* = 4.3 Hz), 8.09 (dt, 1H, *J* = 8.1, 1.1 Hz), 7.52 (ddd, 1H, *J* = 8.2, 6.7, 1.4 Hz), 7.42 (dd, 1H, *J* = 7.1, 1.0 Hz), 7.39 (dm, 1H, *J* = 8.4 Hz), 7.33 (d, 1H, *J* = 6.7 Hz), 7.31 (ddd, 1H, *J* = 8.4, 7.0, 1.7 Hz), 7.25 (d, 1H, *J* = 4.3 Hz), 7.04 (d, 1H, *J* = 9.1 Hz), 6.70 (d, 1H, *J* = 9.1 Hz), 4.12 (t, 2H, *J* = 6.2 Hz), 2.79 (d, 3H, *J* = 0.8 Hz), 2.60 (t, 2H, *J* = 7.4 Hz), 2.52 (bs, 8H), 2.37 (s, 3H), 2.02 ("quintet", 2H, "*J*" = 6.7 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 147.51, 147.39, 142.90, 138.64, 134.83, 134.65, 132.68, 132.52, 131.98, 126.86, 126.75, 125.99, 125.83, 125.83, 124.30, 124.13, 120.99, 115.88, 113.98, 67.80, 55.05, 55.00 (2C), 53.03 (2C), 45.88, 29.67, 26.95, 19.56, 19.53.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 441.2648, observed, 441.2655.

4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



119

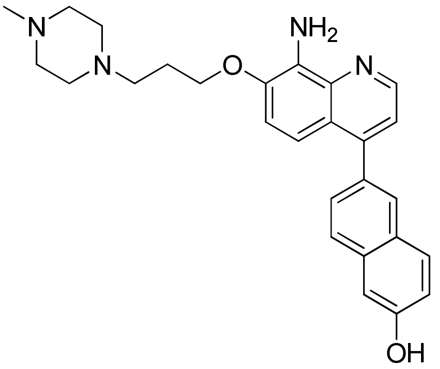
Tin chloride dihydrate (316 mg, 1.4 mmol) was added to a solution of 4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **31** (130 mg, 0.3 mmol) in ethanol (8 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (20 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x40 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2 to give 98 mg of 4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **119** as yellow solid (75% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.90 (d, 1H, *J* = 4.5 Hz), 8.07 (d, 1H, *J* = 9.4 Hz), 7.34 (d, 1H, *J* = 9.6 Hz), 7.27 (d, 1H, *J* = 4.5 Hz), 7.01 (d, 1H, *J* = 8.2 Hz), 6.98 (d, 1H, *J* = 2.0 Hz), 6.93 (dd, 1H, *J* = 8.2, 2.1Hz), 4.34 (m, 4H), 4.30 (t, 2H, *J* = 6.2 Hz), 2.51 (t, 2H, *J* = 7.1Hz), 2.46 (bs, 8H), 2.27 (s, 3H), 1.99 ("quintet", 2H, "*J*" = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 152.35, 149.87, 148.04, 144.41, 143.75, 141.22, 137.17, 130.12, 129.00, 122.64, 121.58, 120.61, 118.40, 117.67, 114.54, 68.37, 64.48, 64.40, 55.08 (2C), 54.18, 53.03 (2C), 45.93, 26.47.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 435.239, observed, 435.2383.

6-(8-Amino-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-4-yl)naphthalen-2-ol



120

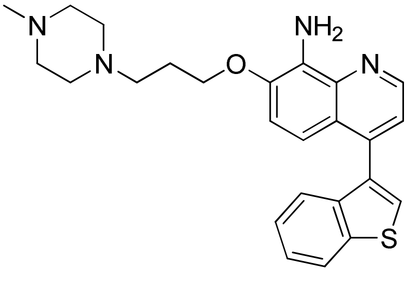
Tin chloride dihydrate (286 mg, 1.27 mmol) was added to a solution of 6-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yl)naphthalen-2-ol **32** (120 mg, 0.2 mmol) in ethanol (8 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (15 mL) was added. The acqueous phase were exctracted with CH_2Cl_2 (3x35 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in diethyl ether to give 53 mg of 6-(8-amino-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-4-yl)naphthalen-2-ol **120** as yellow solid (60% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.77 (d, 1H, *J* = 4.4 Hz), 7.82 (d, 1H, *J* = 1.8 Hz), 7.74 (d, 1H, *J* = 8.7 Hz), 7.70 (d, 1H, *J* = 8.6 Hz), 7.49 (dd, 1H, *J* = 8.4, 1.8 Hz), 7.23 (d, 1H, *J* = 4.3 Hz), 7.20 (d, 1H, *J* = 9.2 Hz), 7.16 (m, 1H), 7.15 (dd, 1H, *J* = 8.5, 2.5 Hz), 7.11 (d, 1H, *J* = 9.2 Hz), 5.10 (bs, 2H), 4.15 (t, 2H, *J* = 6.2 Hz), 2.66 (t, 2H, *J* = 7.1 Hz), 2.64 (bs, 8H), 2.37 (s, 3H), 2.06 ("quintet", 2H, "*J*" = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 154.91, 148.52, 147.59, 142.92, 138.86, 134.36, 133.44, 132.52, 129.97, 128.42, 128.32, 127.88, 126.25, 122.81, 119.89, 119.04, 115.61, 113.67, 109.45, 67.68, 65.82, 55.00, 54.67 (2C), 52.62 (2C), 45.63, 29.66, 26.76.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 443.2441, observed, 443.2461.

4-(Benzo[b]thiophen-3-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



121

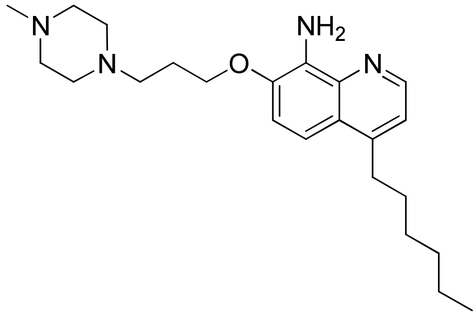
Tin chloride dihydrate (518 mg, 2.3 mmol) was added to a solution of 4-(4-methylnaphthalen-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **33** (212 mg, 0.4 mmol) in ethanol (14 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (25 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x40 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2 to give 138 mg of 4-(benzo[b]thiophen-3-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **121** as grey solid (80% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.81 (d, 1H, *J* = 4.3 Hz), 7.95 (dt, 1H, *J* = 8.1, 0.8 Hz), 7.52 (s, 1H), 7.45 (dt, 1H, *J* = 8.1, 0.7 Hz), 7.39 (ddd, 1H, *J* = 8.2, 7.1, 1.2 Hz), 7.29 (ddd, 1H, *J* = 8.2, 7.1, 1.2 Hz), 7.29 (d, 1H, *J* = 4.3 Hz), 7.13 (d, 1H, *J* = 9.2 Hz), 7.03 (d, 1H, *J* = 9.1 Hz), 5.15 (bs, 2H), 4.15 (t, 2H, *J* = 6.3 Hz), 2.61 (t, 2H, *J* = 7.4 Hz), 2.53 (bs, 8H), 2.31 (s, 3H), 2.03 ("quintet", 2H, "*J*" = 6.9 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 147.48, 143.02, 142.16, 140.06, 138.83, 138.72, 134.24, 132.84, 125.74, 124.64, 124.38, 123.25, 123.19, 122.68, 120.36, 115.92, 113.35, 67.80, 54.94 (2C), 52.93 (2C), 45.79, 29.64, 26.92.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 433.2056, observed, 433.2056.

4-Hexyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



122

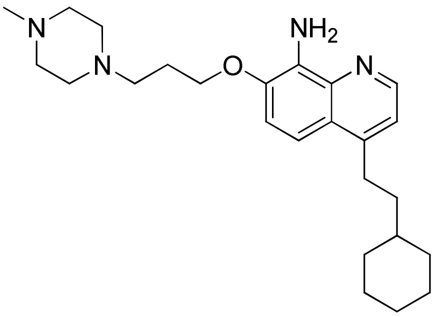
Palladium (5%) on activated carbon (9.3 mg) was added to a stirred solution of 4-(hex-1-ynyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **35** (81 mg , 0,197 mmol) in methanol (6 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 65 mg of 4-hexyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **122** as a yellow oil (77% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ (ppm): 8.62(d, 1H, *J* = 4.4 Hz), 7.31 (d, 1H, *J* = 9.2 Hz), 7.22 (d, 1H, *J* = 9.2 Hz), 7.07 (d, 1H, *J* = 4.4 Hz), 5.09 (bs, 2H), 4.17 (t, 2H, *J* = 6.0 Hz), 2.98 (t, 2H, *J* = 7.7 Hz), 2.61 (t, 2H, *J* = 7.2 Hz), 2.49 (bs, 8H), 2.28 (s, 3H), 2.03 (“quintet”, 2H, “*J*” = 6.6 Hz), 1.73 (“quintet”, 2H, “*J*” = 7.7 Hz), 1.41 (m, 2H), 1.31 (m, 4H,), 0.88 (m, 3H).

^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm): 148.60, 147.65, 142.64, 138.52, 133.10, 123.47, 119.06, 115.62, 110.88, 67.87, 55.10, 55.07 (2C), 53.18 (2C), 46.00, 32.35, 31.63, 29.95, 29.35, 26.97, 22.56, 14.05.

ESI-MS (*m/z*) [M+H]$^+$: 385.3.

4-(2-Cyclohexylethyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



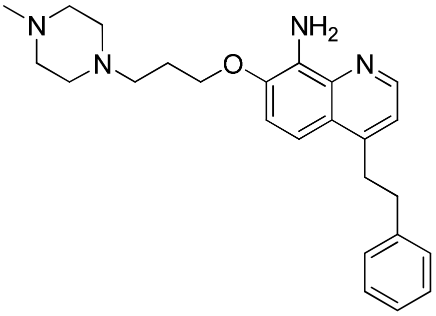
123

Palladium (5%) on activated carbon (11 mg) was added to a stirred solution of 4-(cyclohexylethynyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **36** (90 mg, 0,206 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 80 mg of 4-(2-cyclohexylethyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **123** as a yellow oil (83% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.62 (d, 1H, *J* = 4.4 Hz), 7.30 (d, 1H, *J* = 9.1 Hz), 7.23 (d, 1H, *J* = 9.2 Hz), 5.09 (bs, 2H), 4.16 (t, 2H, *J* = 6.2 Hz), 2.99 (m, 2H), 2.60 (t, 2H, *J* = 7.4 Hz), 2.49 (bs, 8H), 2.29 (s, 3H), 2.04 (“quintet”, 2H, “*J*” = 6.9 Hz), 1.81 (m, 2H), 1.75-1.58 (m, 5H), 1.27-1.12 (m, 4 H), 0.98 (m, 2H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 149.03, 147.70, 142.63, 138.51, 133.12, 123.49, 118.99, 115.62, 110.84, 67.87, 55.10, 55.07 (2C), 53.18 (2C), 46.00, 37.79, 33.24 (2C), 29.76, 29.67, 26.96, 26.60, 26.28 (2C).

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-phenethylquinolin-8-amine



124

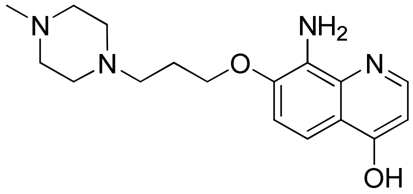
Palladium (5%) on activated carbon (15 mg) was added to a stirred solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-phenethylquinoline **34** (90 mg, 0,207 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. Na_2CO_3 (10 mL, 2M solution in water) was added, and the acqueous phase was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated to give 70 mg (0.173 mmol) of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-phenethylquinolin-8-amine **124** (84.0 % yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.61 (d, 1H, *J* = 4.3 Hz), 7.36 (d, 1H, *J* = 9.1 Hz), 7.29 (m, 2H), 7.23 (d, 1H, *J* = 9.1 Hz), 7.20 (m, 3H), 7.03 (d, 1H, *J* = 4.3 Hz), 5.02 (bs, 2H), 4.19 (t, 2H, *J* = 5.9 Hz), 3.30 (t, 2H, *J* = 8.0 Hz), 3.01 (t, 2H, *J* = 8.0 Hz), 2.92 (bs, 8H), 2.81 (t, 2H, *J* = 6.9 Hz), 2.61 (s, 3H), 2.12 (“quintet”, 2H, “*J*” = 6.2 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 147.70, 147.26, 142.48, 141.11, 138.40, 132.92, 128.42, 128.28, 126.15, 123.20, 119.23, 115.24, 110.82, 67.23, 54,52 (2C), 53.51 (2C), 50.64, 44.24, 36.06, 34.25, 26.38.

ESI-HRMS (*m/z*) $[M+H]^{+}$: calculated,405.2648, observed, 405.2646.

8-Amino-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-4-ol



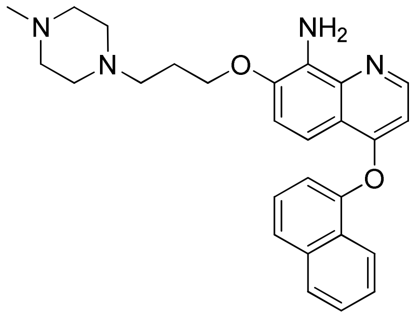
125

Palladium (5%) on activated carbon (9.3 mg) was added to a stirred solution of 4-(benzyloxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **38** (112 mg , 0,257 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 80 mg of 8-amino-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-4-ol **125** as a yellow oil (98% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ (ppm): 7.78 (d, 1H, *J* = 8.9 Hz), 7.68 (d, 1H, *J* = 6.7 Hz), 7.03 (d, 1H, *J* = 9.0 Hz), 6.21 (d, 1H, *J* = 6.9 Hz), 5.04 (bs, 2H), 4.16 (t, 2H, *J* = 6.1 Hz), 2.56 (t, 2H, *J* = 7.0 Hz), 2.48 (bs, 8H), 2.26 (s, 3H), 2.02 (“quintet”, 2H, “*J*” = 6.8 Hz).

^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm): 179.36, 147.13, 139.44, 130.32, 125.32, 121.22, 114.12, 110.80, 107.34, 67.47, 55.06, 55.00 (2C), 53.11 (2C), 45.93, 26.84.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yloxy)quinolin-8-amine



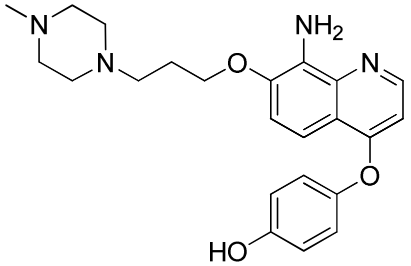
126

Palladium (5%) on activated carbon (11 mg) was added to a stirred solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yloxy)-8-nitroquinoline **39** (120 mg , 0,254 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 112 mg of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(naphthalen-1-yloxy)quinolin-8-amine **126** as pale yellow oil (97% yield).

^{1}H-NMR (400 MHz, CDCl$_3$) δ (ppm): 8.44 (d, 1H, *J* = 5.1 Hz), 7.93 (m, 2H), 7.82 (d, 1H, *J* = 9.0 Hz), 7.79 (m, 1H), 7.51 (m, 2H), 7.42 (m, 2H), 7.31 (d, 1H, *J* = 9.0 Hz), 7.26 (m, 1H), 6.29 (d, 1H, *J* = 5.1 Hz), 5.10 (bs, 2H), 4.21 (t, 2H, *J* = 6.2 Hz), 2.64 (t, 2H, *J* = 7.2 Hz), 2.55 (bs, 8H), 2.32 (s, 3H), 2.08 (“quintet”, 2H, “*J*” = 6.9 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 162.30, 150.26, 148.85, 143.90, 139.96, 135.13, 132.55, 128.07, 126.93, 126.81, 126.54, 125.82, 125.62, 119.24, 117.13, 116.89, 115.33, 109.09, 102.84, 67.85, 55.11, 54.99 (2C), 53.02 (2C), 45.93, 26.92.

4-(8-Amino-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-4-yloxy)phenol



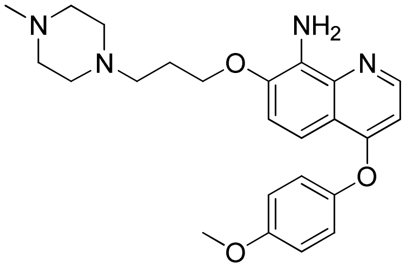
127

Palladium (5%) on activated carbon (11 mg) was added to a stirred solution of 4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)phenol **40** (53 mg, 0,121 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 40 mg of 4-(8-amino-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-4-yloxy)phenol **127** as pale yellow oil (97% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.47 (d, 1H, J = 5.1 Hz), 7.65 (d, 1H, J = 9.0 Hz), 7.19 (d, 1H, J = 9.0 Hz), 7.00 (m, 2H, part of a AA'BB' system), 6.85 (m, 2H, part of a AA'BB' system), 6.36 (d, 1H, J = 5.1 Hz), 5.01 (bs, 2H), 4.19 (t, 2H, J = 6.2 Hz), 2.64 (t, 2H, J = 7.2 Hz), 2.55 (bs, 8H), 2.32 (s, 3H), 2.07 ("quintet", 2H, "J" = 6.9 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 162.77, 154.08, 148.82, 147.11, 143.82, 139.77, 132.14, 122.39 (2C), 116.96, 116.84 (2C), 114.86, 109.31, 102.14, 67.74, 55.08, 54.87 (2C), 52.94 (2C), 45.88, 26.85.

4-(4-Methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



128

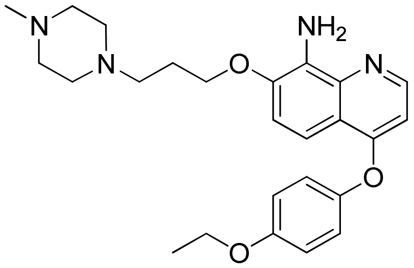
Palladium (5%) on activated carbon (11 mg) was added to a stirred solution of 4-(4-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **41** (103 mg, 0,228 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was recrystallized in diethyl ether (1 mL) to give 70 mg of 4-(4-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **128** as pale yellow crystals (83% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.48 (d, 1H, *J* = 5.1 Hz), 7.64 (d, 1H, *J* = 9.0 Hz), 7.26 (d, 1H, *J* = 9.0 Hz), 7.10 (m, 2H, part of AA'BB' system), 6.96 (m, 2H, part of AA'BB' system), 6.35 (d, 1H, *J* = 5.1 Hz), 5.06 (bs, 2H), 4.19 (t, 2H, *J* = 6.2 Hz), 3.84 (s, 3H), 2.62 (t, 2H, *J* = 7.2 Hz), 2.49 (bs, 8H), 2.30 (s, 3H), 2.06 ("quintet", 2H, "*J*" = 6.6 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 162.58, 157.07, 148.75, 147.76, 143.70, 139.92, 132.44, 122.20 (2C), 117.00, 115.10 (2C), 109.06, 102.25, 67.80, 55.65, 55.04, 54.94 (2C), 52.95 (2C), 45.83, 26.91.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 423.2390, observed, 423.2400.

4-(4-Ethoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



129

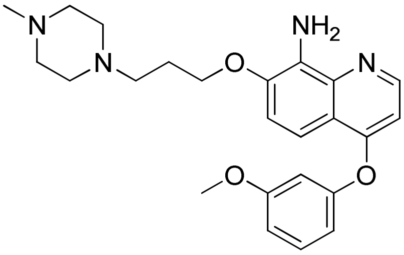
Palladium (5%) on activated carbon (11 mg) was added to a stirred solution of 4-(4-ethoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **42** (110 mg , 0,236 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was recrystallized in diethyl ether (1 mL) to give 70 mg of 4-(4-ethoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **129** as pale yellow crystals (yield 87%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.48 (d, 1H, *J* = 5.1 Hz), 7.65 (d, 1H, *J* = 9.0 Hz), 7.25 (d, 1H, *J* = 9.1 Hz), 7.08 (m, 2H, part of AA'BB' system), 6.95 (m, 2H, part of AA'BB' system), 6.36 (d, 1H, *J* = 5.1 Hz), 5.04 (bs, 2H), 4.20 (t, 2H, *J* = 6.2 Hz), 4.06 (q, 2H, *J* = 7.0 Hz), 2.65 (t, 2H, *J* = 7.2 Hz), 2.60 (bs, 8H), 2.36 (s, 3H), 2.07 ("quintet", 2H, "*J*" = 6.6 Hz), 1.45 (t, 3H, *J* = 7.0 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 162.61, 156.45, 148.78, 147.61, 143.67, 139.91, 132.40, 122.17 (2C), 117.01, 115.68 (2C), 115.03, 109.10, 102.26, 67.72, 63.90, 54.98, 54.78 (2C), 52.67 (2C), 45.64, 26.85, 14.84.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 437.2547, observed, 437.2562.

4-(3-Methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



130

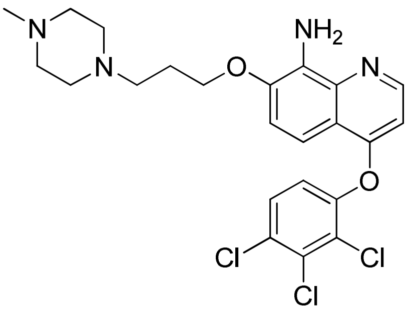
Palladium (5%) on activated carbon (11 mg) was added to a stirred solution of 4-(3-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **43** (122 mg, 0,268 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 110 mg of 4-(3-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **130** as dark yellow oil (97% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.55 (d, 1H, *J* = 5.0 Hz), 7.645 (d, 1H, *J* = 9.0 Hz), 7.36 (t, 1H, *J* = 8.2 Hz), 7.26 (d, 1H, *J* = 9.0 Hz), 6.83 (ddd, 1H, *J* = 8.3, 2.4, 0.9 Hz), 6.77 (ddd, 1H, *J* = 8.0, 2.2, 0.9 Hz), 6.74 (t, 1H, *J* = 2.2 Hz), 6.51 (d, 1H, *J* = 5.0 Hz), 5.09 (bs, 2H), 4.22 (t, 2H, *J* = 6.2 Hz), 3.83 (s, 3H), 2.65 (t, 2H, *J* = 7.2 Hz), 2.53 (bs, 8H), 2.33 (s, 3H), 2.08 (“quintet”, 2H, “*J*” = 6.6 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 161.66, 161.14, 155.81, 148.71, 143.71, 140.04, 132.54, 130.47, 117.21, 115.32, 112.94, 111.04, 109.00, 106.77, 103.31, 67.86, 55.44, 55.10, 55.09 (2C), 53.17 (2C), 45.99, 26.96.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 423.2390, observed, 423.2396.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(2,3,4-trichlorophenoxy)quinolin-8-amine



131

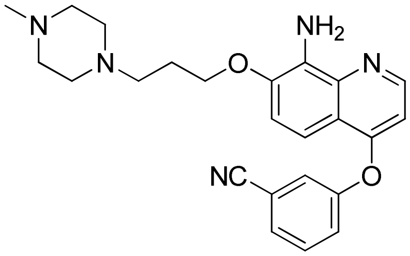
Palladium (5%) on activated carbon (11 mg) was added to a stirred solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-(2,3,4-trichlorophenoxy)quinoline **44** (70 mg, 0,097 mmol) in methanol (2 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was purified with chromatography on basic alumina (eluent AcOEt) to give 51 mg of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(2,3,4-trichlorophenoxy)quinolin-8-amine **131** as yellow oil (76% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.53 (d, 1H, *J* = 5.0 Hz), 7.60 (d, 1H, *J* = 9.1 Hz), 7.46 (d, 1H, *J* = 8.8 Hz), 7.30 (d, 1H, *J* = 9.2 Hz), 7.09 (d, 1H, *J* = 8.9 Hz), 6.31 (d, 1H, *J* = 5.0 Hz), 5.10 (bs, 2H), 4.21 (t, 2H, *J* = 6.2 Hz), 2.62 (t, 2H, *J* = 7.2 Hz), 2.50 (bs, 8H), 2.30 (s, 3H), 2.06 (“quintet”, 2H, “*J*” = 6.8 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 160.42, 148.53, 148.45, 143.89, 140.02, 132.70, 128.75, 128.32, 127.93, 127.35, 123.23, 121.08, 115.63, 108.75, 102.49, 67.87, 55.13 (2C), 55.13, 53.26 (2C), 46.09, 26.96.

ESI-MS (*m/z*) $[M+H]^+$: 494.9, 497.1, 499.3.

3-(8-Amino-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-4-yloxy)benzonitrile



132

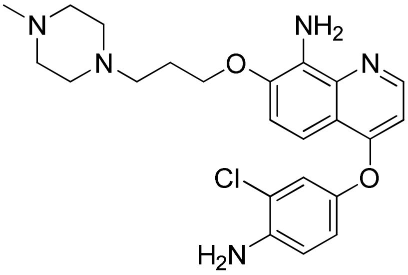
Tin chloride dihydrate (2 g, 9 mmol) was added to a solution of 3-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzonitrile **45** (585 mg, 1.3 mmol) in ethanol (25 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (25 mL) was added. The acqueous phase was exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2 to give 349 mg of 3-(8-amino-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-4-yloxy)benzonitrile **132** as yellow solid (64% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.57 (d, 1H, *J* = 5.0 Hz), 7.54 (m, 1H), 7.52 (m, 1H), 7.50 (d, 1H, *J* = 9.0 Hz), 7.43 (m, 1H), 7.39 (m, 1H), 7.26 (d, 1H, *J* = 9.1 Hz), 6.47 (d, 1H, *J* = 5.0 Hz), 5.10 (bs, 2H), 4.19 (t, 2H, *J* = 6.2 Hz), 2.63 (t, 2H, *J* = 7.1 Hz), 2.53 (bs, 8H), 2.32 (s, 3H), 2.02 (“quintet”, 2H, “*J*” = 6.5 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ (ppm): 160.41, 155.60, 148.52, 143.89, 140.14, 132.77, 131.10, 128.53, 125.05, 123.74, 117.78, 117.07, 115.67, 114.10, 108.64, 104.22, 67.79, 55.02, 54.95 (2C), 52.97 (2C), 45.85, 26.88.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 418.2243, observed, 418.2229.

4-(4-Amino-3-chlorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



133

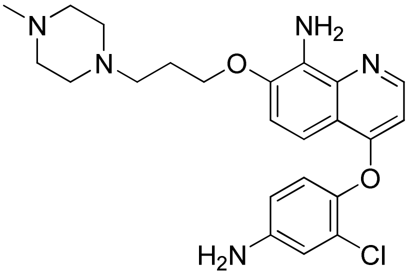
Palladium (5%) on activated carbon (42 mg) was added to a stirred solution of 2-chloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine **46** (430 mg, 0.9 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was dissolved in AcOEt, filtered, and then purified with flash chromatography (eluent CH_2Cl_2:MeOH:Et_3N, 5.9:4:0.1) to give 380 mg of 4-(4-amino-3-chlorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **133** as yellow solid (yield 95%).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.49 (d, 1H, *J* = 5.1 Hz), 7.60 (d, 1H, *J* = 9.1Hz), 7.23 (d, 1H, *J* = 9.1 Hz), 7.12 (d, 1H, *J* = 2.6 Hz), 6.90 (dd, 1H, *J* = 8.7, 2.6 Hz), 6.81 (d, 1H, *J* = 8.7 Hz), 6.38 (d, 1H, *J* = 5.1 Hz), 5.02 (bs, 2H), 4.19 (t, 2H, *J* = 6.2 Hz), 4.08 (bs, 2H), 2.66 (t, 2H, *J* = 7.4 Hz), 2.65 (bs, 8H), 2.38 (s, 3H), 2.06 (“quintet”, 2H, “*J*” = 7.0 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ (ppm): 162.40, 148.76, 145.88, 143.71, 140.76, 139.91, 132.39, 122.39, 120.81, 119.53, 116.88, 116.38, 115.03, 109.04, 102.28, 67.67, 54.91, 54.64 (2C), 52.44 (2C), 45.47, 26.80.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 442.2009, observed, 442.2015

4-(4-Amino-2-chlorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



134

Palladium (5%) on activated carbon (42 mg) was added to a stirred solution of 3-chloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine **47** (430 mg, 0.9 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for

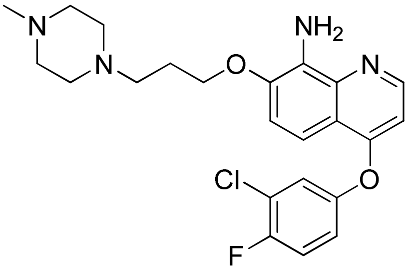
24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was dissolved in AcOEt, filtered, and then purified with flash chromatography (eluent CH_2Cl_2:MeOH:Et_3N, 5.9:4:0.1) to give 380 mg of 4-(4-amino-2-chlorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **134** as yellow solid (yield 95%).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.48 (d, 1H, *J* = 5.1 Hz), 7.68 (d, 1H, *J* = 9.0 Hz), 7.27 (d, 1H, *J* = 9.1 Hz), 7.01 (d, 1H, *J* = 5.1 Hz), 6.81 (d, 1H, *J* = 2.7 Hz), 6.62 (dd, 1H, *J* = 8.6, 2.7 Hz), 6.26 (d, 1H, *J* = 5.1 Hz), 5.03 (bs, 2H), 4.19 (t, 2H, *J* = 6.2 Hz), 3.77 (bs, 2H), 2.61 (t, 2H, *J* = 7.4 Hz), 2.50 (bs, 8H), 2.30 (s, 3H), 2.05 (“quintet”, 2H, “*J*” = 6.4 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ (ppm): 161.69, 148.70, 145.11, 143.76, 141.63, 139.93, 132.44, 127.65, 124.03, 116.60, 116.53, 115.18, 114.54, 109.16, 101.56, 67.87, 55.07, 55.06 (2C), 53.13 (2C), 45.94, 26.97.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 442.2009, observed, 442.2010.

4-(3-Chloro-4-fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



135

Tin chloride dihydrate (1.4 g, 6 mmol) was added to a solution of 4-(3-chloro-4-fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **48** (590 mg, 1.2 mmol) in ethanol (25 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (25 mL) was added. The acqueous phase were exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue purified with flash chromatography (eluent CH_2Cl_2:ethanol:Et_3N, 7.4:2.5:0.1) to give 320 mg of 4-(3-chloro-4-fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **135** as white solid (60% yield).

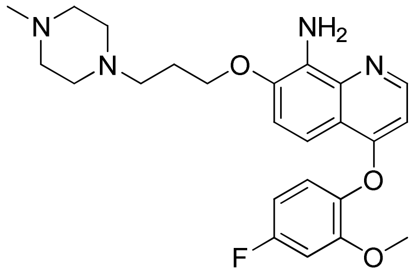
^{1}H-NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.54 (d, 1H, *J* = 5.0 Hz), 7.54 (d, 1H, *J* = 9.0 Hz), 7.26 (d, 1H, *J* = 9.1 Hz), 7.22 (m, 2H), 7.04 (ddd, 1H, *J* = 8.9, 3.9, 2.9 Hz), 6.41 (d, 1H, *J* = 5.0 Hz), 5.07 (bs, 2H), 4.19 (t, 2H, *J* = 6.2 Hz), 2.62 (t, 2H, *J* = 7.4 Hz), 2.53 (bs, 8H), 2.31 (s, 3H), 2.05 (“quintet”, 2H, “*J*” = 6.7 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ(ppm): 161.31, 155.50 (d, J_{CF} = 246.6 Hz), 150.76 (d, J_{CF} = 2.9 Hz), 148.53, 143.86, 140.02, 132.63 (d, J_{CF} = 1.8 Hz), 123.09, 122.08 (d, J_{CF} = 19.5 Hz), 120.44 (d, J_{CF}

= 7.2 Hz), 117.45 (d, J_{CF} = 28.0 Hz), 116.91, 115.47, 108.71, 103.15, 67.83, 55.02, 54.99 (2C), 53.01 (2C), 45.87, 26.91.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 445.1801, observed, 445.1814.

4-(4-Fluoro-2-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



136

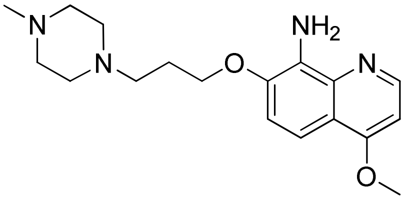
Tin chloride dihydrate (417 mg, 2 mmol) was added to a solution of 4-(4-fluoro-2-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **49** (160 mg, 0.4 mmol) in ethanol (10 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (15 mL) was added. The acqueous phase was exctracted with AcOEt (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue purified with flash chromatography (eluent CH_2Cl_2:ethanol:Et_3N, 7.4:2.5:0.1) to give 106 mg of yellow solid of 4-(4-fluoro-2-methoxyphenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **136** (60% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.47 (d, 1H, *J* = 5.1 Hz), 7.67 (d, 1H, *J* = 9.1 Hz), 7.26 (d, 1H, *J* = 9.1 Hz), 7.11 (dd, 1H, *J* = 8.8, 5.8 [HF] Hz), 6.78 (dd, 1H, *J* = 10.1 [HF], 2.8 Hz), 6.71 (ddd, 1H, *J* = 8.7, 7.8 [HF], 2.8 Hz), 6.23 (d, 1H, *J* = 5.1 Hz), 4.19 (t, 2H, *J* = 6.2 Hz), 3.73 (s, 3H), 2.62 (t, 2H, *J* = 7.3 Hz), 2.53 (bs, 8H), 2.32 (s, 3H), 2.05 (“quintet”, 2H, “*J*” = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.81, 160.80 (J_{CF}= 244.0 Hz), 152.67 (J_{CF}= 10.3), 148.66, 143.66, 139.90, 138.61, 132.41, 123.46 (J_{CF}= 10.4), 116.62, 115.14, 109.15, 107.16 (J_{CF}= 23.4 Hz), 101.39, 101.25 (J_{CF}= 27.3 Hz), 67.81, 56.04, 55.04, 54.97 (2C), 53.00 (2C), 45.85, 26.91.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 441.2296, observed, 441.2324.

4-Methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



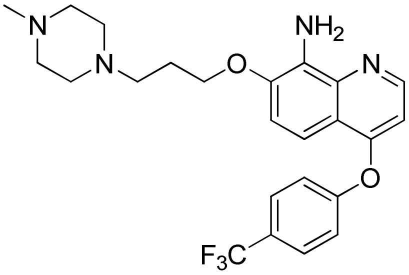
137

Tin chloride dihydrate (109 mg, 0.486 mmol) was added to a solution of 4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **50** (35 mg, 0.097 mmol) in ethanol (2 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 30 mg of 4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **137** (93.6% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.58 (d, 1H, J = 5.1 Hz), 7.46 (d, 1H, J = 9.1 Hz), 7.17 (d, 1H, J = 9.1 Hz), 6.59 (d, 1H, J = 5.1 Hz), 4.99 (s, 2H), 4.15 (t, 2H, J = 6.2 Hz), 3.99 (s, 3H), 2.59 (t, 2H, J = 7.3 Hz), 2.49 (bs, 8H), 2.29 (s, 3H), 2.02 (“quintet”, 2H, “J” = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 157.24, 143.82, 138.45, 134.20, 127.08, 111.77, 109.40, 103.89, 93.30, 62.57, 50.30, 49.90, 49.85 (2C), 47.92 (2C), 40.75, 21.76.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(4-(trifluoromethyl)phenoxy)quinolin-8-amine



138

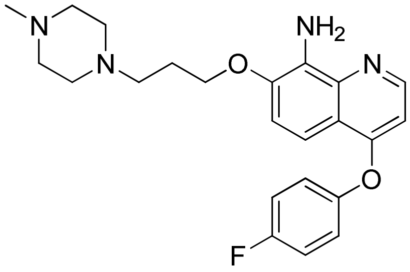
Tin chloride dihydrate (161 mg, 0.7 mmol) was added to a solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-(4-(trifluoromethyl)phenoxy)quinoline **51** (70 mg, 0.1 mmol) in ethanol (8 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (15 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x40 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue purified with flash chromatography (eluent CH_2Cl_2:ethanol:Et_3N, 5.9:4:0.1) to give 30 mg of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(4-(trifluoromethyl)phenoxy)quinolin-8-amine **138** as yellow solid (65% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.57 (d, 1H, *J* = 5.0 Hz), 7.68 (m, 2H), 7.52 (d, 1H, *J* = 9.0 Hz), 7.26 (d, 1H, *J* = 9.0 Hz), 7.23 (m, 2H), 6.53 (d, 1H, *J* = 5.0 Hz), 5.10 (bs, 2H), 4.19 (t, 2H, *J* = 6.2 Hz), 2.63 (t, 2H, *J* = 7.2 Hz), 2.53 (bs, 8H), 2.32 (s, 3H), 2.06 ("quintet", 2H, "*J*" = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.50, 158.09, 148.59, 148.54, 148.52, 143.85, 140.22, 132.79, 127.45 (q, 2C, J_{CF}= 3.7 Hz), 126.99 (q, J_{CF}= 32.6 Hz), 123.90 (q, J_{CF}= 272.0 Hz), 120.28, 117.31, 115.68, 108.77, 104.60, 67.86, 55.03 (2C), 53.08 (2C), 45.90, 29.64, 26.93.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 460.2086, observed, 461.2149.

4-(4-Fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



139

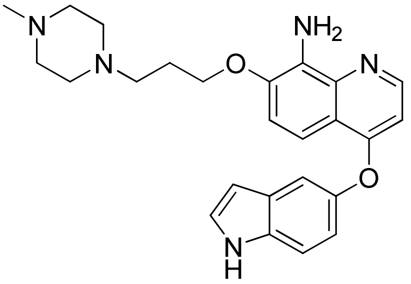
Tin chloride dihydrate (1.7 g, 7 mmol) was added to a solution of 4-(4-fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **52** (661 mg, 1.5 mmol) in ethanol (25 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (30 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x40 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2/*n*-hexane to obtain 1 g of 4-(4-fluorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **139** as yellow solid (yield 70%).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.49 (d, 1H, *J* = 5.1 Hz), 7.60 (d, 1H, *J* = 9.1 Hz), 7.24 (d, 1H, *J* = 9.1 Hz), 7.11 (m, 4H), 6.35 (d, 1H, *J* = 5.1 Hz), 5.05 (s, 2H), 4.18 (t, 2H, *J* = 6.2 Hz), 2.60 (t, 2H, *J* = 7.3 Hz), 2.49 (bs, 8H), 2.28 (s, 3H), 2.04 ("quintet", 2H, "*J*" = 7.0 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.97, 159.87 (d, J_{CF}= 244.1 Hz), 150.43, 148.60, 143.82, 139.97, 132.54, 122.45 (d, 2C, J_{CF}= 8.5 Hz), 116.98, 116.72 (d. 2C, J_{CF}= 23.5 Hz), 115.29, 108.90, 102.63, 67.85, 55.00 (2C), 53.07 (2C), 45.88, 26.94, 22.56.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 411.2190, observed, 411.2175.

4-(1H-indol-5-yloxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



140

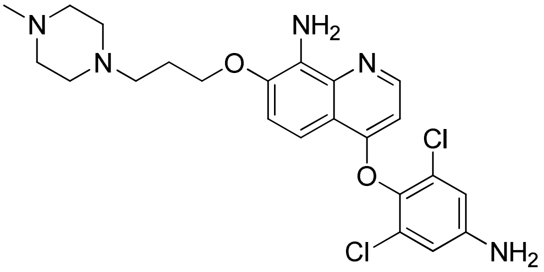
Tin chloride dihydrate (488 mg, 2.2 mmol) was added to a solution of 4-(1H-indol-5-yloxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **53** (200 mg, 0.4 mmol) in ethanol (12 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (15 mL) was added. The acqueous phase was exctracted with AcOEt (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue and the residue recrystalized in CH_2Cl_2 to give 103 mg of 4-(1H-indol-5-yloxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **140** as yellow solid (60% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.51 (bs, 1H), 8.46 (d, 1H, *J* = 5.1 Hz), 7.74 (d, 1H, *J* = 9.0 Hz), 7.45 (bd, 1H, *J* = 8.6 Hz), 7.30 (dd, 1H, *J* = 3.1, 2.7 Hz), 7.27 (d, 1H, *J* = 9.1 Hz), 7.01 (dd, 1H, *J* = 8.7, 2.3 Hz), 6.57 (m. 1H), 6.38 (d, 1H, *J* = 5.1 Hz), 5.03 (bs, 2H), 4.21 (t, 2H, *J* = 6.2 Hz), 2.67 (t, 2H, *J* = 7.4 Hz), 2.66 (bs, 8H), 2.39 (s, 3H), 2.08 ("quintet", 2H, "*J*" = 6.2 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 163.27, 148.88, 147.97, 143.66, 139.95, 133.54, 132.36, 128.67, 125.73, 117.20, 116.01, 114.97, 112.52, 112.11, 109.36, 102.92, 102.49, 67.73, 54.92, 54.66 (2C), 52.47 (2C), 45.47, 26.84.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 432.2394, observed, 432.2410.

4-(4-Amino-2,6-dichlorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



141

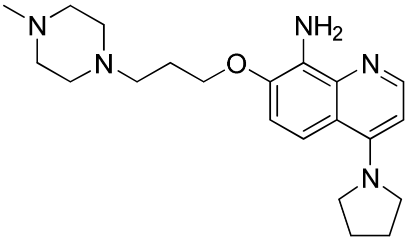
Tin chloride dihydrate (553 mg, 2 mmol) was added to a solution of 3,5-dichloro-4-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yloxy)benzenamine **54** (246 mg, 0.5 mmol) in ethanol (10 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (35 mL) was added. The acqueous phase was exctracted with CH_2Cl_2 (3x50 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2/*n*-hexane to give 187 mg of 4-(4-amino-2,6-dichlorophenoxy)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **141** as yellow solid (80% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.50 (d, 1H, *J* = 5.1 Hz), 7.72 (d, 1H, *J* = 9.1 Hz), 7.28 (d, 1H, *J* = 9.1 Hz), 6.69 (s, 2H), 6.23 (d, 1H, *J* = 5.1 Hz), 5.04 (bs, 2H), 4.19 (t, 2H, *J* = 6.2 Hz), 3.90 (bs, 2H), 2.60 (t, 2H, *J* = 7.3 Hz), 2.49 (bs, 8H), 2.29 (s, 3H), 2.04 ("quintet", 2H, "*J*" = 7.0 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.31, 148.63, 145.25, 143.84, 139.96, 137.67, 132.43, 129.56 (2C), 116.24, 115.27, 114.87 (2C), 109.28, 100.94, 67.87, 65.79, 55.05 (2C), 53.12 (2C), 45.94, 15.22.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 476.1614, observed, 476.1610.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(pyrrolidin-1-yl)quinolin-8-amine



142

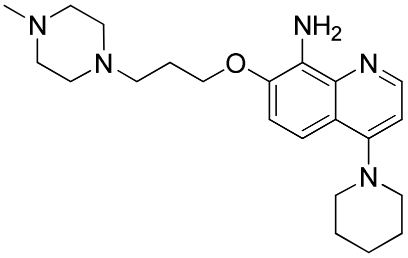
Palladium (5%) on activated carbon (9.3 mg) was added to a stirred solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-(pyrrolidin-1-yl)quinoline **37** (18 mg , 0,045 mmol) in methanol (2 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 10 mg of **7**-(3-(4-methylpiperazin-1-yl)propoxy)-4-(pyrrolidin-1-yl)quinolin-8-amine **142** as a yellow oil (60% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.32 (d, 1H, *J* = 5.7 Hz), 7.53 (d, 1H, *J* = 9.3 Hz), 7.03 (d, 1H, *J* = 9.3 Hz), 6.34 (d, 1H, *J* = 5.7 Hz), 5.19 (bs, 2H), 4.15 (t, 2H, *J* = 6.1 Hz), 3.71 (m, 4H), 2.60 (t, 2H, *J* = 7.2 Hz), 2.52 (bs, 8H), 2.31 (s, 3H), 2.04 (m, 6H).

^{13}C-NMR (100 MHz, DMSO-d6) δ (ppm): 152.04, 147.26, 141.52, 139.49, 132.15, 116.17, 112.03, 111.65, 101.27, 66.92, 54.85, 54.68, 54.38, 52.65, 51.65, 45.68, 26.42, 25.40.

ESI-MS (*m/z*) $[M+H]^+$: 370.1.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-4-(piperidin-1-yl)quinolin-8-amine



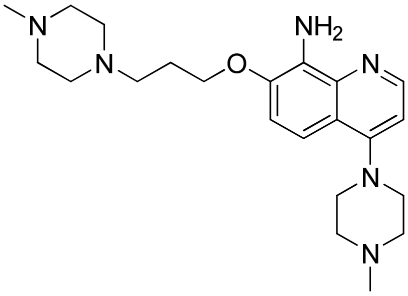
143

Palladium (5%) on activated carbon (11 mg) was added to a stirred solution of 3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-4-(piperidin-1-yl)quinoline **61** (40 mg, 0,097 mmol) in methanol (2 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was purified with chromatography on basic alumina (eluent AcOEt:hexane 1:1) to give 22 mg of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-(piperidin-1-yl)quinolin-8-amine **143** as pale yellow oil (60% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.55 (d, 1H, *J* = 5.0 Hz), 7.29 (d, 1H, *J* = 9.1 Hz), 7.15 (d, 1H, *J* = 9.1 Hz), 6.70 (d, 1H, *J* = 5.2 Hz), 5.03 (bs, 2H), 4.16 (t, 2H, *J* = 6.2 Hz), 3.16 (m, 4H), 2.61 (t, 2H, *J* = 7.2 Hz), 2.50 (bs, 8H), 2.30 (s, 3H), 2.04 ("quintet", 2H, "*J*" = 7.0 Hz), 1.82 (m, 4H), 1.69 (m, 2H).

^{13}C-NMR (100 MHz, DMSO-d6) δ (ppm): 158.04, 148.39, 139.80, 132.92, 122.84, 119.46, 114.29, 111.29, 107.13, 67.68, 54.96 (2C), 54.96, 53.45 (2C), 53.40 (2C), 45.87, 26.84, 25.98 (2C), 24.49.

4-(4-Methylpiperazin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



144

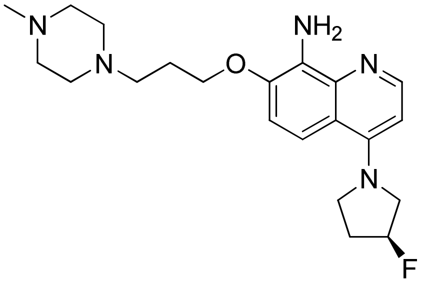
Palladium (5%) on activated carbon (10.4 mg) was added to a stirred solution of 4-(2-methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **62**(100 mg, 0,2 mmol) in methanol (7 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. The residue was dissolved in ethyl acetate, filtered, and then purified with flash chromatography (eluent CH_2Cl_2:EtOH:Et_3N, 6.9:3:0.1) to give 72 mg of 4-(4-methylpiperazin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine as brown oil **144** (78% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.57 (d, 1H, *J* = 4.9 Hz), 7.29 (d, 1H, *J* = 9.2 Hz), 7.16 (d, 1H, *J* = 9.2 Hz), 6.23 (d, 1H, *J* = 4.9 Hz), 5.05 (bs, 2H), 4.15 (t, 2H, *J* = 6.2 Hz), 3.25 (bs, 4H), 2.68 (bs, 4H), 2.60 (t, 2H, *J* = 7.3 Hz), 2.49 (bs, 8H), 2.40 (s, 3H), 2.29 (s, 3H), 2.03 ("quintet", 2H, "*J*" = 6.9 Hz).

^{13}C-NMR (CDCl3, 100 MHz) δ (ppm): 156.79, 148.43, 142.78, 139.72, 133.05, 119.12, 114.53, 111.00, 107.29, 67.79, 55.08 (2C), 55.05, 54.95 (2C), 53.01 (2C), 51.92 (2C), 46.08, 45.88, 26.90.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 399.2866, observed, 399.2860.

4-(3-Fluoropyrrolidin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



145

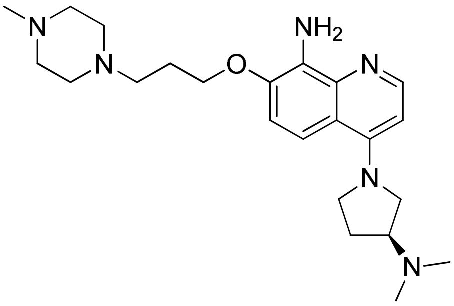
Tin chloride dihydrate (451 mg, 2 mmol) was added to a solution of 4-(3-fluoropyrrolidin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **63** (174 mg, 0.4 mmol) in ethanol (15 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (25 mL) was added. The acqueous phase was exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, the residue recrystalized in CH_2Cl_2 to give 108 mg of 4-(3-fluoropyrrolidin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **145** as pink crystals (70% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.42 (d, 1H, *J* = 5.3 Hz), 7.44 (d, 1H, *J* = 9.3 Hz), 7.07 (d, 1H, *J* = 9.3 Hz), 6.41 (d, 1H, *J* = 5.3 Hz), 5.37 (dt, 1H. *J* = 53.1 [HF], 3.4 Hz), 5.03 (bs, 2H), 4.16 (t, 2H, *J* = 6.2 Hz), 4.04-3.65 (m, 6H), 2.61 (t, 2H, *J* = 7.4 Hz), 2.50 (bs, 8H), 2.30 (s, 3H), 2.04 ("quintet", 2H, "*J*" = 6.5 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.44, 147.84, 142.57, 140.20, 132.45, 116.94, 112.57, 112.17, 102.16, 92.27 (d, J_{CF}= 177.5 Hz), 67.62, 58.18, 57.96, 55.08, 55.06 (2C), 53.17 (2C), 49.07, 45.98, 26.95.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 388.2507, observed, 388.2504.

(S)-4-(3-(dimethylamino)pyrrolidin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



146

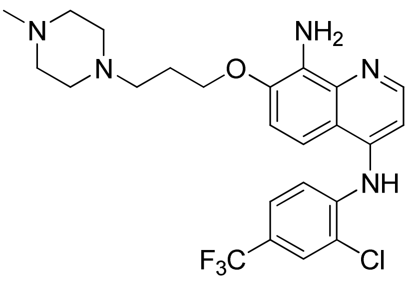
Tin chloride dihydrate (225 mg, 1 mmol) was added to a solution of (S)-*N*,*N*-dimethyl-1-(7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-yl)pyrrolidin-3-amine **64** (90 mg, 0.2 mmol) in ethanol (8 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (15 mL) was added. The acqueous phase was exctracted with AcOEt (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2 to give 49 mg of (S)-4-(3-(dimethylamino)pyrrolidin-1-yl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **146** as yellow crystals (60% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.37 (d, 1H, *J* = 5.3 Hz), 7.45 (d, 1H, *J* = 9.3 Hz), 7.04 (d, 1H, *J* = 9.4 Hz), 6.34 (d, 1H, *J* = 5.4 Hz), 4.14 (t, 2H, *J* = 6.3 Hz), 3.74 (m, 2H), 3.64 (m, 2H), 2.76 (m, 1H), 2.58 (t, 2H, *J* = 7.4 Hz), 2.48 (bs, 8H), 2.31(s, 6H), 2.28 (m, 1H), 2.21 (m, 1H), 2.02 (“quintet”, 2H, “*J*” = 6.7 Hz), 1.91 (m, 1H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.64, 147.81, 142.57, 140.23, 132.20, 116.78, 112.49, 112.17, 101.59, 67.57, 65.48, 56.83, 55.09, 55.02 (2C), 53.17 (2C), 53.12, 50.83, 45.94, 44.39, 30.40, 26.95.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 413.3023, observed, 413.3034.

N^4-(2-chloro-4-(trifluoromethyl)phenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinoline-4,8-diamine



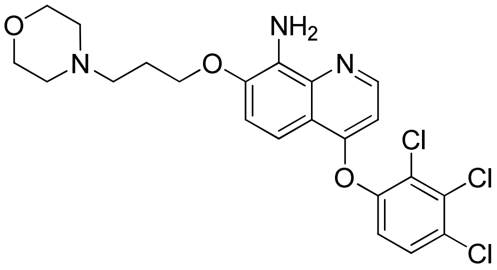
147

Tin chloride dihydrate (242 mg, 1.1 mmol) was added to a solution of N-(2-chloro-4-(trifluoromethyl)phenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinolin-4-amine **65** (113 mg, 0.2 mmol) in ethanol (8 mL). The resulting suspension was heated at 70°C for 3 h. A saturated solution of Na_2CO_3 (15 mL) was added. The acqueous phase were exctracted with CH_2Cl_2 (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue recrystalized in CH_2Cl_2 to give 60 mg of N^4-(2-chloro-4-(trifluoromethyl)phenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinoline-4,8-diamine **147** as green solid (60% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.58 (d, 1H, *J* = 5.1 Hz), 7.71 (d, 1H, *J* = 1.9 Hz), 7.53 (d, 1H, *J* = 8.5 Hz), 7.46 (dd, 1H, *J* = 8.6, 2.0 Hz), 7.23 (s, 2H), 7.12 (d, 1H, *J* = 5.0 Hz), 5.99 (bs, 1H), 5.20 (bs, 2H), 4.19 (t, 2H, *J* = 6.1 Hz), 2.80 (bs, 8H), 2.74 (t, 2H, *J* = 7.3 Hz), 2.50 (s, 3H), 2.09 ("quintet", 2H, "*J*" = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 148.03, 143.37, 141.13, 139.19, 133.32, 127.23 (q, J_{CF}= 3.7 Hz), 126.58 (q, J_{CF}= 34.3 Hz), 124.77 (q, J_{CF}= 3.7 Hz), 123.30, 120.73 (q, J_{CF}= 270.9 Hz), 118.00, 117.06, 115.14, 107.16, 105.28, 67.45, 54.63, 54.02 (2C), 51.43 (2C), 44.75, 26.51.

7-(3-Morpholinopropoxy)-4-(2,3,4-trichlorophenoxy)quinolin-8-amine



148

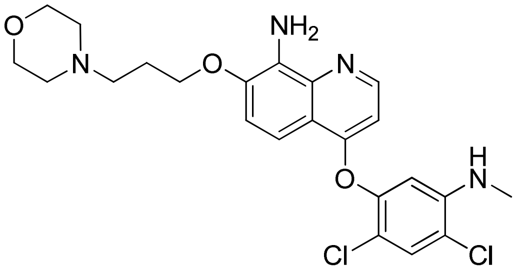
Tin chloride dihydrate (44.0 mg, 0.195 mmol) was added to a solution of 4-(3-(8-nitro-4-(2,3,4-trichlorophenoxy)quinolin-7-yloxy)propyl)morpholine **57** (20 mg, 0.039 mmol) in ethanol (2 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 14 mg of 7-(3-morpholinopropoxy)-4-(2,3,4-trichlorophenoxy)quinolin-8-amine **148** (74.4% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.53 (d, 1H, *J* = 5.0 Hz), 7.60 (d, 1H, *J* = 9.1 Hz), 7.45 (d, 1H, *J* = 8.8 Hz), 7.29 (d, 1H, *J* = 9.1 Hz), 7.09 (d, 1H, *J* = 8.8 Hz), 6.31 (d, 1H, *J* = 5.1 Hz), 4.22 (t, 2H, *J* = 6.1 Hz), 3.76 (t, 4H, *J* = 4.7 Hz), 2.64 (t, 2H, *J* = 7.2 Hz), 2.54 (bs, 4H), 2.09 ("quintet", 2H, "*J*" = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.11, 150.22, 148.45, 143.88, 140.06, 133.43, 132.66, 130.78, 128.66, 128.02, 120.95, 116.48, 115.45, 108.80, 102.62, 67.67, 66.79 (2C), 55.52, 53.68 (2C), 26.54.

ESI-HRMS (*m/z*) $[M+H]^+$:calculated, 482.0799, observed, 482.0817.

4-(2,4-Dichloro-5-(methylamino)phenoxy)-7-(3-morpholinopropoxy)quinolin-8-amine



149

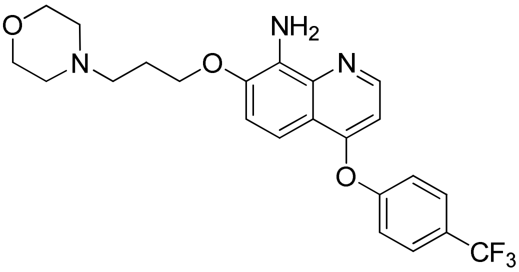
Tin chloride dihydrate (115.6 mg, 0.512 mmol) was added to a solution of 2,4-dichloro-*N*-methyl-5-(7-(3-morpholinopropoxy)-8-nitroquinolin-4-yloxy)aniline **58** (52 mg, 0.103 mmol) in ethanol (3 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 22 mg of 4-(2,4-dichloro-5-(methylamino)phenoxy)-7-(3-morpholinopropoxy)quinolin-8-amine **149** (45.0% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.51 (d, 1H, *J* = 5.1 Hz), 7.67 (d, 1H, *J* = 9.1 Hz), 7.39 (s, 1H), 7.28 (d, 1H, *J* = 9.1 Hz), 6.47 (s, 1H), 6.33 (d, 1H, *J* = 5.1 Hz), 5.04 (bs, 2H), 4.48 (q, 1H, *J* = 5.1 Hz), 4.21 (t, 2H, *J* = 6.2 Hz), 3.74 (t, 4H, *J* = 4.6 Hz), 2.83 (d, 3H, *J* = 5.1 Hz), 2.62 (t, 2H, *J* = 7.2 Hz), 2.51 (bs, 4H), 2.05 (“quintet”, 2H, “*J*” = 6.9 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.83, 149.76, 148.66, 145.24, 143.78, 139.97, 132.44, 130.14, 116.53, 116.01, 115.17, 113.34, 109.08, 104.68, 102.12, 67.68, 66.86 (2C), 55.54, 53.71 (2C), 30.38, 26.60.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 477.1454, observed, 477.1458.

7-(3-Morpholinopropoxy)-4-(4-(trifluoromethyl)phenoxy)quinolin-8-amine



150

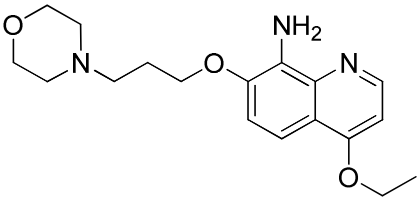
Tin chloride dihydrate (238.6 mg, 1.058 mmol) was added to a solution of 4-(3-(8-nitro-4-(4-(trifluoromethyl)phenoxy)quinolin-7-yloxy)propyl)morpholine **59** (101.0 mg, 0.211 mmol) in ethanol (6 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 101 mg of 7-(3-morpholinopropoxy)-4-(4-(trifluoromethyl)phenoxy)quinolin-8-amine **150** (74.4% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.56 (d, 1H, *J* = 5.0 Hz), 7.68 (d, 2H, *J* = 8.5 Hz), 7.54 (d, 1H, *J* = 9.0 Hz), 7.26 (d, 1H, *J* = 9.0 Hz), 7.22 (d, 2H, *J* = 8.5 Hz), 6.52 (d, 1H, *J* = 5.0 Hz), 5.07 (bs, 2H), 4.20 (t, 2H, *J* = 6.1 Hz), 3.75 (t, 4H, *J* = 4.6 Hz), 2.62 (t, 2H, *J* = 7.2 Hz), 2.52 (bs, 4H), 2.07 ("quintet", 2H, "*J*" = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.58, 148.62, 143.90, 140.12, 132.54, 127.47 (q, 2C, J_{CF} = 3.7 Hz), 127.04 (q, 2C, J_{CF} = 37.7 Hz), 123.90 (q, 2C, J_{CF} = 272.4 Hz), 122.27, 120.34 (2C), 117.27, 115.38, 108.95, 104.51, 67.63, 66.77 (2C), 66.70, 55.51, 53.66 (2C), 26.52.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 448.1842, observed, 448.1822.

4-Ethoxy-7-(3-morpholinopropoxy)quinolin-8-amine



151

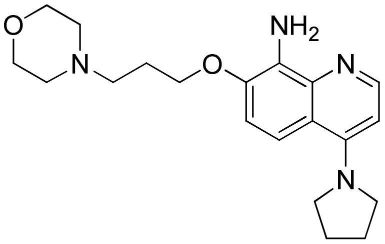
Tin chloride dihydrate (93.6 mg, 0.415 mmol) was added to a solution of 4-(3-(4-ethoxy-8-nitroquinolin-7-yloxy)propyl)morpholine **60** (30.0 mg, 0.083 mmol) in ethanol (2.4 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 7 mg of 7-(3-morpholinopropoxy)-4-(4-(trifluoromethyl)phenoxy)quinolin-8-amine **151** (25.4% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.58 (d, 1H, *J* = 5.2 Hz), 7.52 (d, 1H, *J* = 9.0 Hz), 7.18 (d, 1H, *J* = 9.0 Hz), 6.61 (d, 1H, *J* = 5.2 Hz), 4.25 (q, 2H, *J* = 7.0 Hz), 4.21 (t, 2H, *J* = 6.2 Hz), 3.84 (m, 4H), 2.78 (t, 2H, *J* = 7.3 Hz), 2.68 (bs, 4H), 2.16 ("quintet", 2H, "*J*" = 7.0 Hz), 1.55 (t, 3H, *J* = 7.0 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 162.49, 148.44, 143.80, 131.72, 117.04, 114.32, 109.51, 99.06, 67.33, 66.12 (2C), 64.25, 55.58, 53.35 (2C), 29.67, 14.45.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 332.1968, observed, 332.1966.

7-(3-Morpholinopropoxy)-4-(pyrrolidin-1-yl)quinolin-8-amine



152

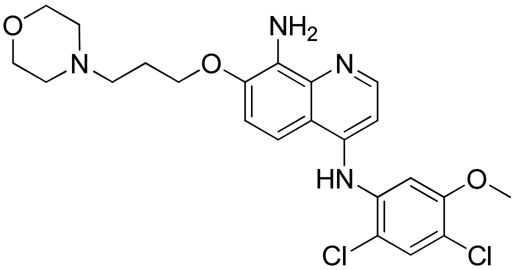
Tin chloride dihydrate (169.3 mg, 0.75 mmol) was added to a solution of 4-(3-(8-nitro-4-(pyrrolidin-1-yl)quinolin-7-yloxy)propyl)morpholine **67** (58 mg, 0.150 mmol) in ethanol (4 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 23 mg of 7-(3-morpholinopropoxy)-4-(pyrrolidin-1-yl)quinolin-8-amine **152** (43.0% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.35 (d, 1H, *J* = 5.6 Hz), 7.53 (d, 1H, *J* = 9.3 Hz), 7.04 (d, 1H, *J* = 9.3 Hz), 6.34 (d, 1H, *J* = 5.6 Hz), 5.09 (bs, 2H), 4.17 (t, 2H, *J* = 6.2 Hz), 3.73 (t, 4H, *J* = 4.8 Hz), 3.69 (m, 4H), 2.59 (t, 2H, *J* = 7.3 Hz), 2.49 (bs, 4H), 2.03 (m, 6H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 153.30, 149.02, 146.71, 142.98, 131.60, 116.53, 112.89, 111.81, 101.21, 67.49, 66.94 (2C), 55.53, 53.74 (2C), 52.19 (2C), 26.69, 25.86 (2C).

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 357.2285, observed, 357.2289.

N^4-(2,4-dichloro-5-methoxyphenyl)-7-(3-morpholinopropoxy)quinoline-4,8-diamine



153

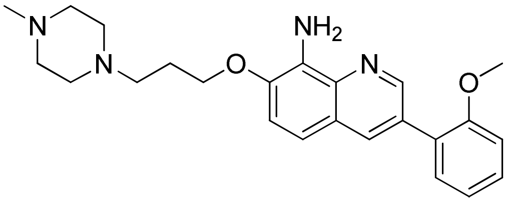
Tin chloride dihydrate (115 mg, 0.512 mmol) was added to a solution of *N*-(2,4-dichloro-5-methoxyphenyl)-7-(3-morpholinopropoxy)-8-nitroquinolin-4-amine **68** (52 mg, 0.1025 mmol) in ethanol (3 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 42 mg of *N*^{4}-(2,4-dichloro-5-methoxyphenyl)-7-(3-morpholinopropoxy)quinoline-4,8-diamine **153** (85.8% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.50 (d, 1H, *J* = 5.1 Hz), 7.45 (s, 1H), 7.23(bs, 2H), 7.05 (s, 1H), 6.92 (d, 1H, *J* = 5.1 Hz), 6.80 (bs, 1H), 5.14 (bs, 2H), 4.19 (t, 2H, *J* = 6.1 Hz), 3.82(s, 3H), 3.74 (t, 4H, *J* = 4.6 Hz), 2.61 (t, 2H, *J* = 7.2 Hz), 2.50 (bs, 4H), 2.05 (“quintet”, 2H, “*J*” = 6.8 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 154.57, 147.98, 145.81, 143.58, 139.09, 137.10, 133.47, 130.60, 117.26, 116.83, 115.09, 106.96, 105.29, 103.16, 67.89, 66.93, 56.61, 55.55 (2C), 53.81 (2C), 26.74.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 477.1454, observed, 477.1452.

3-(2-Methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



154

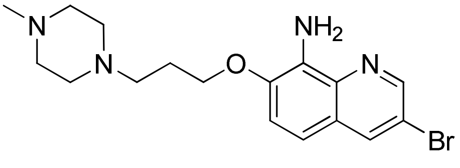
Palladium (5%) on activated carbon (5 mg) was added to a stirred solution of 3-(2-methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **87** (11 mg, 0,025 mmol) in methanol (3 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum to give 3.3 mg (0.0081 mmol) of 3-(2-methoxyphenyl)-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **154** (yield 32%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) 8.95 (d, 1H, *J* = 2.1 Hz), 8.35 (d, 1H, *J* = 2.2 Hz), 7.41 (m, 1H), 7.40 (m, 1H), 7.26 (d, 1H, *J* = 8.8 Hz), 7.17 (d, 1H, *J* = 8.9 Hz), 7.09 (td, 1H, *J* = 7.6, 1.0 Hz), 7.04 (dd, 1H, *J* = 7.7, 1.0 Hz), 5.07 (bs, 2H), 4.19 (t, 2H, *J* = 6.1 Hz), 3.84 (s, 3H), 2.63 (t, 2H, *J* = 7.1 Hz), 2.68 (bs, 8H), 2.41 (s, 3H), 2.08 (“quintet”, 2H, “*J*” = 6.6 Hz).

^{13}C-NMR ($CDCl_3$, 75.45 MHz) δ(ppm): 156.75, 149.57, 142.97, 136.96, 132.53, 131.43, 130.88, 129.82, 129.27, 127.59, 123.90, 121.09, 116.17, 115.33, 111.27, 67.79, 55.56, 54.93 (2C), 54.58 (2C), 52.35, 45.44, 26.77.

ESI-MS (*m/z*) $[M+H]^+$: 407.3.

3-Bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



155

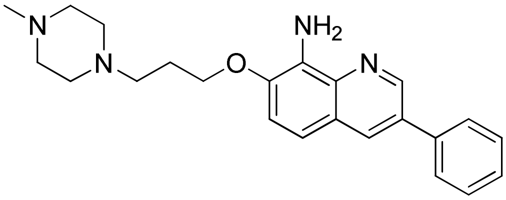
Tin chloride dihydrate (82 mg, 0.365 mmol) was added to a solution of 3-bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **86** (30 mg, 0.073 mmol) in ethanol (2.1 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (25 mL) was added, the acqueous phase was exctracted with AcOEt (3x30 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 19 mg of 7-bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **155** (68.6% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.70 (d, 1H, *J* = 2.2 Hz), 8.16 (d, 1H, *J* = 2.2 Hz), 7.26 (d, 1H, *J* = 8.8 Hz), 7.03 (d, 1H, *J* = 8.8 Hz), 5.08 (bs, 2H), 4.16 (t, 2H, *J* = 6.2 Hz), 2.60 (t, 2H, *J* = 7.1 Hz), 2.51 (bs, 8H), 2.30 (s, 3H), 2.04 (“quintet”, 2H, “*J*” = 6.8 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 148.61, 143.23, 136.97, 136.18, 132.93, 125.05, 117.04, 115.12, 113.96, 67.87, 55.00, 54.98 (2C), 53.06 (2C), 45.91, 26.88.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 379.1127, observed, 379.1121.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-3-phenylquinolin-8-amine



156

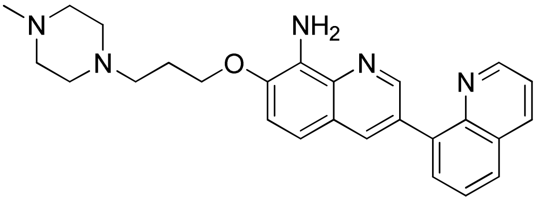
Tin chloride dihydrate (55.5 mg, 0.246 mmol) was added to a solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-3-phenylquinoline **88** (20 mg, 0.0492 mmol) in ethanol (1.5 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (20mL) was added, the acqueous phase was exctracted with AcOEt (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue was purified by flash cromathography on basic alumina (eluent: AcOEt) to give 10 mg of 7-bromo-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **156** (54.0% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 9.01 (d, 1H, *J* = 2.2 Hz), 8.18 (d, 1H, *J* = 2.3 Hz), 7.70 (m, 2H), 7.51 (m, 2H), 7.41 (m, 1H), 7.29 (d, 1H, *J* = 9.1 Hz), 7.19 (d, 1H, *J* = 8.9 Hz), 5.09 (bs, 2H), 4.19 (t, 2H, *J* = 6.1 Hz), 2.63 (t, 2H, *J* = 7.3 Hz), 2.54 (bs, 8H), 2.33 (s, 3H), 2.05 (“quintet”, 2H, “*J*” = 7.1 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 147.35, 143.11, 138.33, 135.87, 133.22, 132.59, 131.92, 129.06 (2C), 127.68, 127.21 (2C), 123.89, 119.23, 116.63, 67.93, 54.99 (2C), 53.08 (2C), 55.07, 45.91, 26.93.

ESI-MS (*m/z*) $[M+H]^+$: 377.4.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-3-(8'-quinolinyl)quinolin-8-amine



157

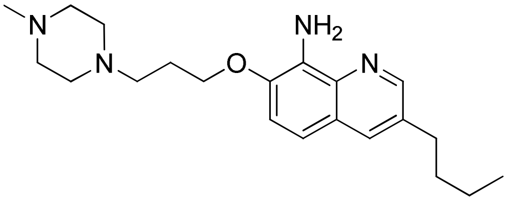
Tin chloride dihydrate (219.5 mg, 0.973 mmol) was added to a solution of 7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitro-3-(8-quinolyl)quinoline **89** (89 mg, 0.1945 mmol) in ethanol (5.9 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (20mL) was added, the acqueous phase was exctracted with AcOEt (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum to give 66 mg (0.154 mmol) of 7-(3-(4-Methylpiperazin-1-yl)propoxy)-3-(8'-quinolinyl)quinolin-8-amine **157** (79.2% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) 9.13 (d, 1H, *J* = 2.1 Hz), 8.92 (dd, 1H, *J* = 4.2, 1.6 Hz), 8.32 (d, 1H, *J* = 2.1 Hz), 8.25 (dd, 1H, *J* = 8.2, 1.7 Hz), 7.84 (m, 2H), 7.63 (m, 1H), 7.47 (dd, 1H, *J* = 8.0, 4.1 Hz), 7.26 (d, 1H, *J* = 8.9 Hz), 7.20 (d, 1H, *J* = 8.7 Hz), 5.11 (bs, 2H), 4.18 (t, 2H, *J* = 6.3 Hz), 2.62 (t, 2H, *J* = 7.5 Hz), 2.50 (bs, 8H), 2.30 (s, 3H), 2.05 ("quintet", 2H, "*J*" = 6.7 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm) : 150.31, 150.24, 150.00, 143.18, 137.19, 136.62, 136.24, 136.23, 132.50, 131.56, 130.34, 128.69, 128.00, 126.37, 123.81, 121.23, 116.10, 115.44, 67.85, 55.02, 54.92 (2C), 53.08 (2C), 45.86, 26.88.

ESI-HRMS (m/z) $[M+H]^+$: calculated, 429.2444, observed, 428.2459.

3-Butyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



158

Tin chloride dihydrate (64.2 mg, 0.285 mmol) was added to a solution of 3-butyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **90** (22 mg, 0.057 mmol) in ethanol (5.9 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (20mL) was added, the acqueous phase was exctracted with AcOEt (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum to give 7 mg (0.0196 mmol) of 3-butyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **158** (34.4% yield).

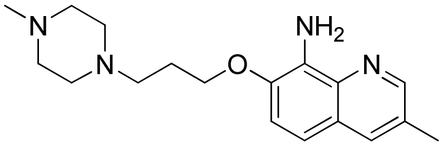
^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 8.59 (d, 1H, *J* = 2.1 Hz), 7.78 (d, 1H, *J* = 1.9 Hz), 7.23 (d, 1H, *J* = 8.8 Hz), 7.06 (d, 1H, *J* = 8.7 Hz), 5.06 (2H, bs), 4.15 (t, 2H, *J* = 6.3 Hz), 2.75 (t, 2H, *J* =

7.6 Hz), 2.62 (t, 2H, *J* = 7.1 Hz), 2.51(bs, 8H), 2.31 (s, 3H), 2.04 (“quintet”, 2H, *“J”* = 6.7 Hz), 1.67 (“quintet”, 2H, *“J”* = 7.6 Hz), 1.39 (sestet, 2H, *J* = 7.6 Hz), 0.94 (t, 3H, *J* = 7.3 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm) : 149.62, 142.37, 134.00, 133.32, 132.69, 124.20, 119.24, 116.55, 114.39, 68.07, 55.10, 55.06 (2C), 53.15 (2C), 46.01, 33.33, 32.75, 26.96, 22.20, 13.90.

ESI-HRMS (*m/z*) [M+H]$^+$: calculated, 357.2648, observed, 357.2640.

3-Methyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



159

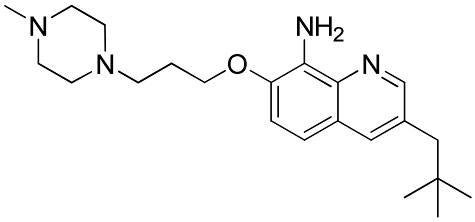
Palladium (5%) on activated carbon (7 mg) was added to a stirred solution of 3-methyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **91** (24mg, 0,0697 mmol) in methanol (2 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. Na_2CO_3 (10 mL, 2M solution in water) was added, and the acqueous phase was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated to give 17 mg (0.0541 mmol) of 3-methyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **159** (77.6% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 8.58 (d, 1H, *J* = 1.5 Hz), 7.77 (s, 1H), 7.21 (d, 1H, 8.7 Hz), 7.03 (d, 1H, *J* = 8.8 Hz), 5.05 (bs, 2H), 4.14 (t, 2H, *J* = 6.3 Hz), 2.60 (t, 2H, *J* = 7.1 Hz), 2.49 (bs, 8H), 2.46 (s, 3H), 2.30 (s, 3H), 2.03 (“quintet”, 2H, *“J”* = 6.7 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 149.82, 142.33, 136.81, 134.53, 132.65, 128.34, 124.12, 116.54, 114.21, 68.02, 55.10, 55.07 (2C), 53.18 (2C), 46.01, 26.96, 18.55.

ESI-HRMS (m/z) [M+H]$^+$: calculated, 315.2179, observed, 315.2172.

7-(3-(4-Methylpiperazin-1-yl)propoxy)-3-neopentylquinolin-8-amine



160

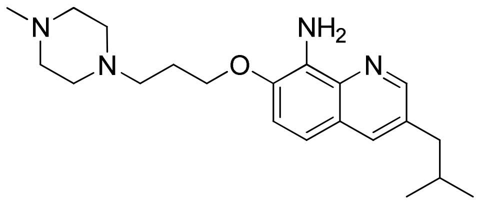
Tin chloride dihydrate (59.1 mg, 0.262 mmol) was added to a solution of 3-neopentyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **92** (21 mg, 0.0524 mmol) in ethanol (2 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of NaHCO (20mL) was added, the acqueous phase was exctracted with AcOEt (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum to give 16 mg (0.043 mmol) of 3-neopentyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **160** (82.4% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 8.55 (d, 1H, *J* = 1.8 Hz), 7.74 (d, 1H, *J* = 1.7 Hz), 7.23 (d, 1H, *J* = 8.6 Hz), 7.08 (d, 1H, *J* = 8.7 Hz), 5.08 (bs, 2H), 4.16 (t, 2H, *J* = 6.0 Hz), 2.62 (s, 2H), 2.61 (t, 2H, *J* = 7.0 Hz), 2.50 (bs, 8H), 2.30 (s, 3H), 2.04 (“quintet”, 2H, *“J”* = 6.6 Hz), 0.94 (s, 9H).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 150.76, 142.45, 136.07, 135.85, 132.66, 130.41, 119.22, 116.55, 114.54, 68.13, 55.08 (2C), 55.08, 55.04, 53.19 (2C), 47.23, 29.68, 29.21 (3C), 26.96.

ESI-HRMS (m/z) $[M+H]^+$: calculated, 371.2810, observed, 371371.2812.

3-Isobutyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



161

Palladium (5%) on activated carbon (7 mg) was added to a stirred solution of 3-isobutyl-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **93** (14mg, 0,0362 mmol) in methanol (2 mL). An hydrogen atmosphere was created in the reaction flask, and after stirring for 24 hours at room temperature, the mixture was filtered over celite, and then the solvent evaporated in vacuum. Na_2CO_3 (10 mL, 2M solution in water) was added, and the acqueous phase was extracted with CH_2Cl_2 (3x20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated to give 6.3 mg (0.0177 mmol) of 3-isobutyl-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **161** (48.1% yield).

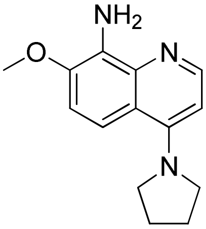
^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) : 8.59 (d, 1H, *J* = 2.2 Hz), 7.77 (d, 1H, *J* = 2.2 Hz), 7.25 (d, 1H, *J* = 9.2 Hz), 7.09 (d, 1H, *J* = 8.8 Hz), 5.05 (bs, 2H), 4.18 (t, 2H, *J* = 6.2 Hz), 2.64 (t, 2H, *J* = 7.1 Hz), 2.63 (d. 2H, *J* = 7.1 Hz), 2.54 (bs, 8H), 2.33 (s, 3H), 2.07 (“quintet”, 2H, *“J”* = 6.4 Hz), 1.97 (nonet, 1H, *J* = 6.6 Hz), 0.96 (d, 6H, J = 6.6 Hz).

^{13}C-NMR (75,45 MHz, $CDCl_3$) δ (ppm): 149.97, 142.40, 137.07, 135.86, 132.69, 132.13, 124.13, 116.28, 114.44, 68.08, 55.04, 55.04 (2C), 53.12 (2C), 45.99, 42.44, 30.17, 26.95, 22.24 (2C).

.

ESI-HRMS (m/z) $[M+H]^+$: calculated, 357.2654, observed, 357.2651.

7-Methoxy-4-(pyrrolidin-1-yl)quinolin-8-amine



162

Tin chloride dihydrate (503.6 mg, 2.23 mmol) was added to a solution 7-methoxy-8-nitro-4-(pyrrolidin-1-yl)quinoline **66** (122 mg, 0.440 mmol) in ethanol (10 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue was treated with hexane (5 mL). The precipitated white solid was collected by filtration, washed with diethyl ether and air dried to give 95 mg of 7-methoxy-4-(pyrrolidin-1-yl)quinolin-8-amine **162** (79.4% yield).

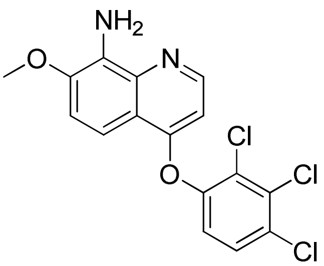
^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.37 (d, 1H, *J* = 5.4 Hz), 7.56 (d, 1H, *J* = 9.4 Hz), 7.04 (d, 1H, *J* = 9.4 Hz), 6.34 (d, 1H, *J* = 5.4 Hz), 4.87 (s, 2H), 3.95 (s, 3H), 3.67 (m, 4H), 2.01 (m, 4H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 147.71, 142.56, 138.39, 134.72, 126.01, 111.40, 107.90, 104.59, 96.08, 51.03, 46.87 (2C), 20.72 (2C).

MS (70 eV) *m/z*: 243.2 [M$^+$](100), 228.2 [M$^+$-CH_3] (63), 183.2 (34).

ESI-HRMS (*m/z*) [M+H]$^+$: calculated, 244.1444, observed, 244.1437.

7-Methoxy-4-(2,3,4-trichlorophenoxy)quinolin-8-amine



163

Tin chloride dihydrate (358.7 mg, 1.590 mmol) was added to a solution 7-methoxy-8-nitro-4-(2,3,4-trichlorophenoxy)quinoline **56** (127 mg, 0.317 mmol) in ethanol (8 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue was treated with diethyl ether (5 mL). The precipitated white solid was collected by filtration, washed with diethyl ether and air dried to give 93 mg of 7-methoxy-4-(2,3,4-trichlorophenoxy)quinolin-8-amine **163** (79.4% yield).

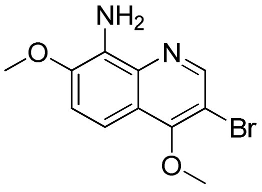
^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.54 (d, 1H, *J* = 5.0 Hz), 7.65 (d, 1H, *J* = 9.1 Hz), 7.46 (d, 1H, *J* = 8.8 Hz), 7.30 (d, 1H, *J* = 9.1 Hz), 7.10 (d, 1H, *J* = 8.8 Hz), 6.30 (d, 1H, *J* = 5.0 Hz), 4.93 (s, 2H), 4.02 (s, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.15, 150.13, 148.61, 144.82, 139.78, 133.40, 131.75, 130.81, 128.71, 128.05, 121.11, 116.09, 113.22, 110.05, 109.00, 102.34, 56.37.

MS (70 eV) *m/z*: 370.1 [M$^+$](53), 368.1[M$^+$](54), 355.1 [M$^+$ -CH_3](60), 353.1 [M$^+$ -CH_3](60), 305.1 (33), 304.1 (52), 303.1 (80), 302.1 (71), 301.1 (60), 145.1 (48), 130.1 (64), 103.1 (100).

ESI-HRMS (*m/z*) [M+H]$^+$: calculated, 368.9958, observed, 368.9953.

3-Bromo-4,7-dimethoxyquinolin-8-amine



164

Tin chloride dihydrate (176.5 mg, 0.782 mmol) was added to a solution 3-bromo-4,7-dimethoxy-8-nitroquinoline **101** (49 mg, 0.156 mmol) in ethanol (3 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue was purified with flash cromathography on silica gel (diethyl ether: hexane 4:6) to give 34 mg of 3-bromo-4,7-dimethoxyquinolin-8-amine **164** (77.0% yield).

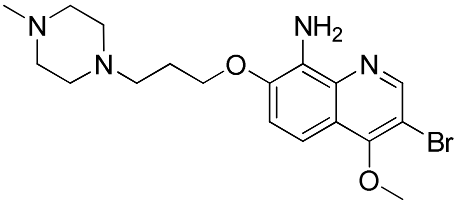
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.70 (s, 1H), 7.42 (d, 1H, *J* = 9.1 Hz), 7.25 (d, 1H, *J* = 9.1 Hz), 4.89 (bs, 2H), 4.08 (s, 3H), 3.99 (s, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 160.32, 150.82, 144.45, 138.61, 132.30, 120.31, 113.93, 109.16, 107.00, 61.60, 56.36.

MS (70 eV) (*m/z)*: 284.1 [M$^+$] (70), 282.1 [M$^+$] (75), 269.1 [M$^+$-CH_3] (88), 267.1 [M$^+$-CH_3] (100), 241.1 (39), 239.1 (45), 158.1 (29), 145 (37).

ESI-HRMS (*m/z*) [M+H]$^+$: calculated, 283.0076, observed, 283.0066.

3-Bromo-4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine



165

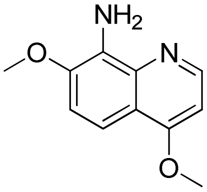
Tin chloride dihydrate (213.1 mg, 0.945 mmol) was added to a solution of 3-bromo-4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)-8-nitroquinoline **100** (83 mg, 0.189 mmol) in ethanol (3 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 30 mg of 3-bromo-4-methoxy-7-(3-(4-methylpiperazin-1-yl)propoxy)quinolin-8-amine **165** (66.0% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.67 (s, 1H), 7.42 (d, 1H, *J* = 9.1), 7.21 (d, 1H, *J* = 9.1), 4.87 (bs, 2H), 4.23 (t, 2H, *J* = 6.4), 3.96 (s, 3H), 2.64 (t, 2H, *J* = 7.7), 2.49 (bs, 8H), 2.28 (s, 3H), 2.09 ("quintet", 2H, "*J*" = 7.3).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 159.52, 150.77, 144.37, 138.59, 132.21, 120.61, 113.78, 109.31, 107.23, 72.75, 56.33, 55.12 (2C), 54.72, 53.10 (2C), 46.01, 27.60.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 409.1233, observed, 409.1221.

4,7-Dimethoxyquinolin-8-amine



166

Tin chloride dihydrate (400 mg, 1.77 mmol) was added to a solution of 4,7-dimethoxy-8-nitroquinoline **55** (83 mg, 0.354 mmol) in ethanol (2 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 62 mg of 4,7-dimethoxyquinolin-8-amine **166** (85.8% yield).

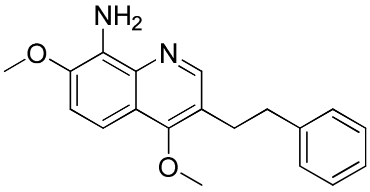
^{1}H-NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.59 (d, 1H, *J* = 5.1 Hz), 7.51 (d, 1H, *J* = 9.1 Hz), 7.18 (d, 1H, *J* = 9.1 Hz), 6.67 (d, 1H, *J* = 5.1 Hz), 3.99 (s, 3H), 3.96 (s, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 162.42, 149.16, 144.53, 139.20, 131.41, 116.65, 112.32, 109.29, 98.33, 56.30, 55.48.

MS (70eV) *(m/z)*: 203.8 [M$^+$] (83); 188.8 (100); 160.8 (52); 117.8 (19)

ESI-HRMS (*m/z*) [M+H]$^+$: calculated, 205.0971, observed, 205.0976.

4,7-Dimethoxy-3-phenethylquinolin-8-amine



167

Tin chloride dihydrate (280.0 mg, 1.241 mmol) was added to a solution of 4,7-dimethoxy-8-nitro-3-phenethylquinoline **102** (84 mg, 0.248 mmol) in ethanol (4 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, and the residue was purified with flash cromathography on silica gel (diethyl ether: hexane 4:6) to give 30 mg of 4,7-dimethoxy-3-phenethylquinolin-8-amine **167** (39.2% yield).

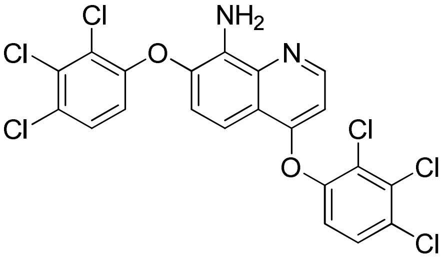
^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.54 (s, 1H), 7.38 (d, 1H, *J* = 9.1), 7.29 (m, 2H), 7.24 (d, 1H, *J* = 8.9), 7.21 (m, 3H), 4.89 (bs, 2H), 3.98 (s, 3H), 3.91 (s, 3H), 3.07 (m, 2H), 2.99 (m, 2H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ(ppm): 161.20, 151.34, 143.73, 141.39, 132.09, 129.00, 128.55, 128.46 (2C), 128.43 (2C), 126.11, 123.14, 113.45, 109.29, 62.15, 56.46, 36.94, 29.59.

MS (70 eV) *m/z*: 308.4 [M$^+$](37), 217.3 (36), 185.2 (38).

ESI-HRMS (*m/z*) [M+H]$^+$: calculated, 309.1597, observed, 309.1587.

4,7-Bis(2,3,4-trichlorophenoxy)quinolin-8-amine



168

Tin chloride dihydrate (90.0 mg, 0.395 mmol) was added to a solution of 8-nitro-4,7-bis(2,3,4-trichlorophenoxy)quinoline **56a** (45 mg, 0.0796 mmol) in ethanol (2 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$.

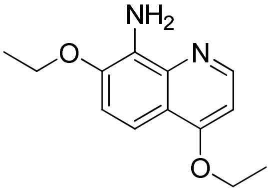
The solvent was removed in vacuum, to give 44 mg of 4,7-bis(2,3,4-trichlorophenoxy)quinolin-8-amine **168** (99.0% yield).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.58 (d, 1H, *J* = 5.1Hz), 7.64 (d, 1H, *J* = 9.1 Hz), 7.49 (d, 1H, *J* = 8.8 Hz), 7.25 (d, 1H, *J* = 9.0 Hz), 7.16 (d, 1H, *J* = 9.2 Hz), 7.13 (d, 1H, *J* = 8.7 Hz), 6.74 (d, 1H, *J* = 9.0 Hz), 6.42 (d, 1H, *J* = 5.1 Hz), 5.07 (bs, 2H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 160.39, 152.80, 149.74, 148.71, 140.69, 138.97, 135.01, 133.62, 132.91, 131.28, 128.82, 128.13, 127.72, 124.67, 121.18, 120.36, 118.73, 115.64, 110.05, 109.41, 103.77.

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 532.8946, observed, 532.8901.

4,7-Diethoxyquinolin-8-amine



169

Tin chloride dihydrate (163.5 mg, 0.724 mmol) was added to a solution of 4,7-diethoxy-8-nitroquinoline **60a** (38 mg, 0.145 mmol) in ethanol (4.2 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 30 mg of 7-(3-morpholinopropoxy)-4-(4-(trifluoromethyl)phenoxy)quinolin-8-amine **169** (89.1% yield).

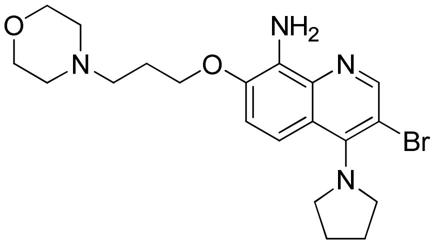
^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.57 (d, 1H, *J* = 5.1 Hz), 7.53 (d, 1H, *J* = 9.0 Hz), 7.16 (d, 1H, *J* = 9.0 Hz), 6.56 (d, 1H, *J* = 5.1 Hz), 4.87 (bs, 2H), 4.22 (q, 2H, *J* = 7.0 Hz), 4.20 (q, 2H, *J* = 7.0 Hz), 1.54 (t, 3H, *J* = 7.0 Hz), 1.47 (t, 3H, *J* = 7.0 Hz).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 161.78, 148.98, 143.78, 139.27, 131.72, 116.78, 113.64, 109.38, 98.93, 64.80, 63.89, 15.22, 14.47.

MS (70 eV) *(m/z)*: 232.2 $[M^+]$(55), 217.2 (24), 203.1 (65), 175.1 (100), 147.1 (53).

ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 233.1284, observed, 233.1286.

3-Bromo-7-(3-morpholinopropoxy)-4-(pyrrolidin-1-yl)quinolin-8-amine



170

Tin chloride dihydrate (48.5 mg, 0.215 mmol) was added to a solution of 4-(3-(3-bromo-8-nitro-4-(pyrrolidin-1-yl)quinolin-7-yloxy)propyl)morpholine **103** (20 mg, 0.043 mmol) in ethanol (1.5 mL). The resulting suspension was heated at 70°C for 1h. A saturated solution of Na_2CO_3 (20mL) was added, the acqueous phase was exctracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed in vacuum, to give 5 mg of 3-bromo-7-(3-morpholinopropoxy)-4-(pyrrolidin-1-yl)quinolin-8-amine **170** (yield 26.7%).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.65 (s, 1H), 7.39 (d, 1H, *J* = 9.1 Hz), 7.17 (d, 1H, *J* = 9.1 Hz), 4.19 (t, 2H, *J* = 6.0 Hz), 3.81 (bs, 4H), 3.53 (m,4H), 2.73 (t, 2H, *J* = 6.7 Hz), 2.63 (bs, 4H), 2.13 (m,2H), 2.08 (m, 4H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 151.57, 151.39, 142.95, 138.38, 132.88, 125.10, 115.07, 112.98, 111.83, 67.35, 66.31, 55.58, 53.43, 51.13, 29.68, 26.27.

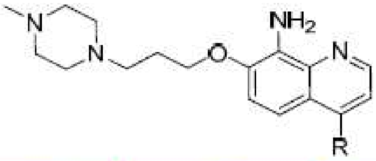
ESI-HRMS (*m/z*) $[M+H]^+$: calculated, 435.1390, observed, 435.1383.

CHAPTER 4

APPENDIX

APPENDIX A: POTENCY TABLES

Table 4.1: *Potency data of 7-(3-(4-methylpiperazin-1-yl)propoxy)-4-substituted 8-aminoquinolines. Assays have been performed with [ATP]= 300 µM. The relative standard deviation for every value is on average smaller than 20%.*



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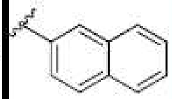
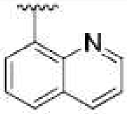
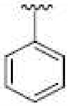
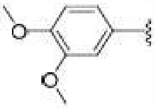
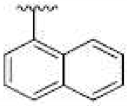
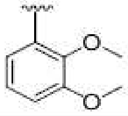
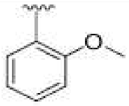
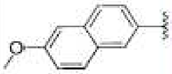
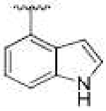
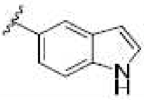
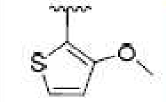
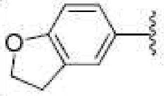
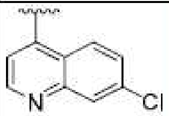
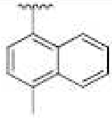
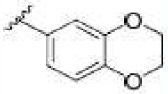
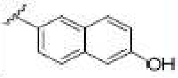
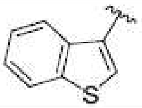
N°	R	free base	salt	free base	salt	free base	salt	free base	salt	free base	salt
104		17,0		13,0							
105		3,90		>10							
106		0,48	0,33	0,57	0,21	2,20	8,90	0,58	1,00	2,80	6,00
107		3,00		4,00		14,00		12,00		>30	
108		22,0		380							
109		17		13		>50		6,8		7,1	
110		>50		29		43		23		30	
111		>50		9,9		31		20		23	
112			2,3		1,6		14		12		13
113			5,2		4,3		>50		19		29
114			0,99		0,65		27		20		22

		Abl WT IC$_{50}$ (µM)		Abl T315I IC$_{50}$ (µM)		BaF3 IC$_{50}$ (µM)		BaF3 Bcr-Abl WT IC$_{50}$ (µM)		BaF3 Bcr-Abl T315I IC$_{50}$ (µM)	
N°	**R**	free base	salt	free base	salt	free base	salt	free base	salt	free base	salt
115			7,9		2,5		15		13		12
116					6,6		6,1		5,1		5,7
117					7,5		8		5,7		5,7
118					2,7		3,8		0,87		2,5
119					5,7		3,1		2,2		1,0
120					2,1		0,19		0,22		0,27
121					2,7		4,1		4,5		4,6
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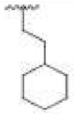
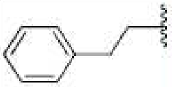
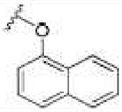
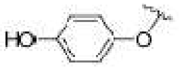
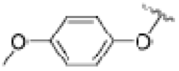
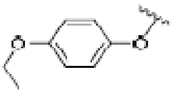
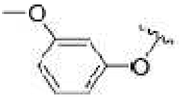
123		29		39							
124		1,1	0,36	0,64	0,34	13	14	3,6	5,2	8,2	12
125		152		63							
126		5,6		1,9		16		5,8		9,1	

		Abl WT IC$_{50}$ (µM)		Abl T315I IC$_{50}$ (µM)		BaF3 IC$_{50}$ (µM)		BaF3 Bcr-Abl WT IC$_{50}$ (µM)		BaF3 Bcr-Abl T315I IC$_{50}$ (µM)	
N°	**R**	free base	salt	free base	salt	free base	salt	free base	salt	free base	salt
127		6,6		5,9		45		26		29	
128		9,3		2,9		7,3		7,4		8,4	
129		2,1		2,7		6,3		6,5		6,5	
130		>50		5,6		11		9		10	
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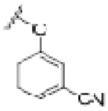
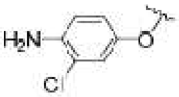
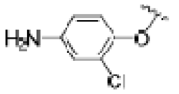
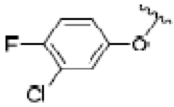
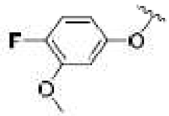
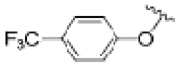
132			>10		22						
133			1,1		1,7		12		7		11
134			3,3		3,3		>50		23		29
135			25		4,1		7,9		3,8		4,4
136			85		16						
137					11		28		29		24
138			>100		9,6		15		4,5		0,9

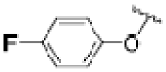
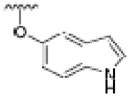
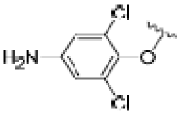
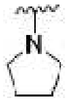
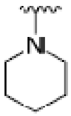
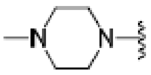
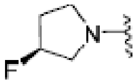
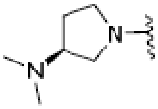
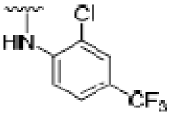
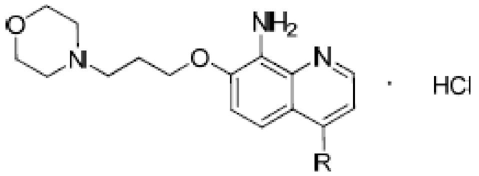
		Abl WT IC$_{50}$ (µM)		Abl T315I IC$_{50}$ (µM)		BaF3 IC$_{50}$ (µM)		BaF3 Bcr-Abl WT IC$_{50}$ (µM)		BaF3 Bcr-Abl T315I IC$_{50}$ (µM)	
N°	R	free base	salt	free base	salt	free base	salt	free base	salt	free base	salt
139					38						
140			0,96		0,29		73		42		37
141					1,7		5,4		2,7		2,7
142		2,6	1,8	1,1	0,71	61	57	35	48	40	49
143		27		6,2		>50		>50		>50	
144					17						
145					10		41		30		41
146					6,8						
147					2,2		2,2		1,1		1,2

Table 4.2 : *Potency data of 7-(3morpholinopropoxy)-4-substituted-8-aminoquinolines. Assays have been performed with [ATP]= 300 μM. The relative standard deviation for every value is on average smaller than 20%.*



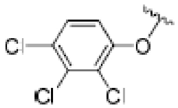
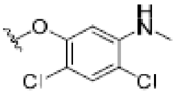
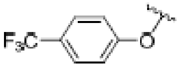
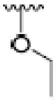
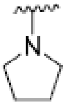
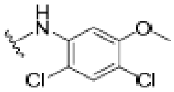
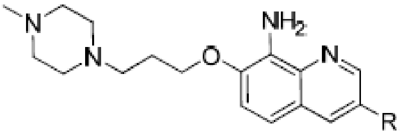
N°	R	Abl WT IC$_{50}$ (μM)	Abl T315I IC$_{50}$ (μM)	BaF3 IC$_{50}$ (μM)	BaF3 Bcr-Abl WT IC$_{50}$ (μM)	BaF3 Bcr-Abl T315I IC$_{50}$ (μM)
148		>100	3,8	9,00	1,50	0,92
149			6,60	4,1	2,6	3,3
150			9,10	4,9	1,8	3,4
151			32			
152			30	24	26	25
153			4,8	2,4	1,4	0,51

Table 4.3: *Potency data of 7-(3-(4-methylpiperazin-1-yl)propoxy)-3-substituted 8-aminoquinolines. Assays have been performed with [ATP] = 300 μM. The relative standard deviation for every value is on average smaller than 20%.*



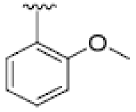
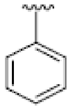
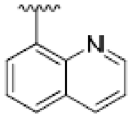
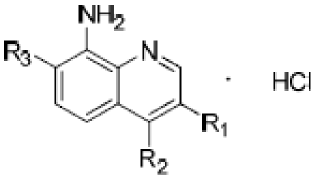
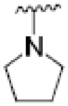
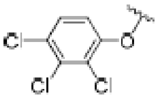
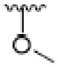
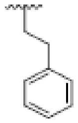
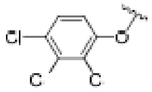
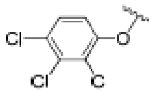
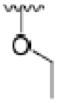
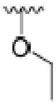
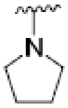
		Abl WT		Abl T315I		BaF3		BaF3 Bcr-Abl WT		BaF3 Bcr-Abl T315I	
		IC$_{50}$ (μM)		IC$_{50}$ (μM)		IC$_{50}$ (μM)		IC$_{50}$ (μM)		IC$_{50}$ (μM)	
N°	**R**	free base	salt	free base	salt	free base	salt	free base	salt	free base	salt
154		11,0		7,1							
155	 Br	5,30	0,37	4,10	0,30	41	34	13	33	34	35
156		6,80		2,70		25		13		18	
157		8,30	3,10	3,00	1,40	10	17	9	14	11	18
158		>10	278	88	148,00						
159	 CH$_3$	>10	28,00	>10	16,00						
160				5,5	4,6	13	14	4,1	7,6	10	13
161				26	15						

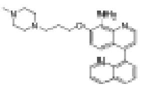
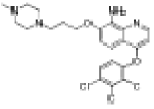
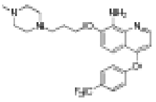
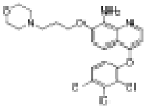
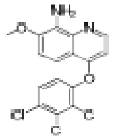
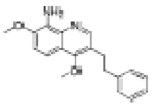
Table 4.4: *Potency data of 7-(Oxyalkyl)-3,4-disubstituted-8-aminoquinolines and 7-Oxygen linked-4-substituted-8aminoquinolines. Assays have been performed with [ATP]= 300 μM. The relative standard deviation for every value is on average smaller than 20%.*

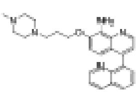
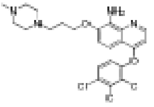
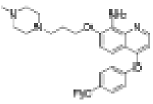
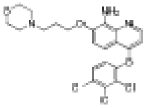
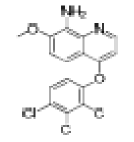
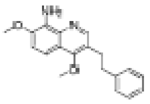


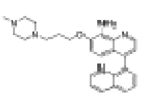
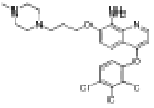
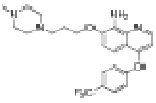
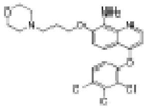
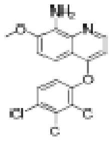
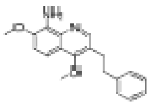
N°	R1	R2	R3	Abl WT IC50 (μM)	Abl T315I IC50 (μM)	BaF3 IC50 (μM)	BaF3 Bcr-Abl WT IC50 (μM)	BaF3 Bcr-Abl T315I IC50 (μM)
162					11,0	3,70	1,20	4,40
163					6,1	1,1	2,0	0,55
164					54	4,2	3,6	13
165					54	61	68	30
166					100			
167					15	13	10	7,1
168					1,9	26	15	5,5
169					66			
170					2,4	37	20	25

APPENDIX B: SELECTIVITY DATA

Table 4.5: *Percentage of reduction of the normal kinase activity by the inhibitors. All the compounds have been tested at 10 μM. Kinases strongly inhibited are highlighted***.**

N°	106	131	138	142	148	163	167
Structure Kinase							
MKK1	20	67	101	90	92	105	93
ERK1	81	105	90	90	108	112	98
ERK2	106	103	120	97	107	102	102
JNK1	101	98	86	111	92	113	98
JNK2	107	103	98	104	103	99	102
p38a MAPK	101	57	72	108	63	108	93
p38b MAPK	91	98	83	98	100	107	89
p38g MAPK	73	89	96	86	109	110	92
p38d MAPK	82	110	94	100	83	99	109
ERK8	42	81	89	89	72	76	79
RSK1	34	52	90	84	110	107	105
RSK2	15	58	88	44	61	52	66
PDK1	140	123	119	114	112	104	102
PKBa	20	90	62	83	76	93	93
PKBb	6	5	55	4	26	19	9
SGK1	3	64	76	94	70	97	100
S6K1	28	89	102	116	73	95	99
PKA	49	108	107	108	106	109	87
ROCK 2	84	103	98	101	92	93	101
PRK2	64	89	109	85	97	86	100
PKCa	26	98	100	92	95	107	91
PKCz	9	90	111	97	108	106	95
PKD1	30	68	83	89	72	58	82
MSK1	44	75	84	85	72	78	96
MNK1	106	95	90	113	94	97	102
MNK2	73	101	103	100	105	91	91
MAPKAP-K2	20	89	108	99	103	90	106
PRAK	61	102	129	89	94	63	86
CAMKKb	79	92	95	103	95	92	92
CAMK1	8	18	52	22	17	61	74
SmMLCK	104	61	75	114	84	101	87

N°	**106**	**131**	**138**	**142**	**148**	**163**	**167**
Structure							
Kinase							
PHK	30	47	114	46	123	45	41
CHK1	48	114	96	94	118	145	102
CHK2	5	70	85	79	58	71	87
GSK3b	80	106	116	98	102	92	93
PLK1	23	115	103	60	106	81	71
Aurora B	42	57	94	60	92	76	69
AMPK	100	105	102	96	99	99	101
MARK3	85	99	98	103	97	102	103
BRSK2	23	110	96	92	97	80	88
MELK	75	88	60	76	80	113	96
CK1	108	96	109	97	95	82	97
CK2	120	99	82	123	89	65	106
DYRK1A	62	102	105	98	79	72	81
DYRK2	71	136	113	102	103	71	91
DYRK3	37	75	79	89	37	87	90
NEK2a	3	86	108	89	58	95	101
NEK6	18	116	70	104	26	96	95
IKKb	10	47	75	52	29	76	67
PIM1	17	33	87	53	69	56	57
PIM2	37	79	101	81	82	100	95
PIM3	31	77	86	74	69	74	80
SRPK1	24	90	101	75	90	89	78
MST2	91	103	97	99	83	107	115
EF2K	49	93	109	100	120	101	92
HIPK2	20	97	86	86	68	103	101
PAK4	110	101	114	112	96	104	98
PAK5	98	107	107	107	81	94	90
PAK6	72	102	173	98	106	109	102
Src	40	46	89	82	79	35	68
Lck	140	115	154	158	108	99	99
CSK	47	77	109	112	102	99	99
FGF-R1	22	34	56	41	14	80	51
IRR	143	91	95	96	89	86	92
EPH A2	100	86	121	119	90	101	119
MST4	47	93	80	105	64	98	101
SYK	74	101	149	98	104	96	107
YES1	32	28	70	68	27	78	77

Compound Nº	106	131	138	142	148	163	167
Structure Kinase							
IGF-1R	78	113	99	97	97	100	112
VEG-FR	70	28	89	48	27	91	84
BTK	58	64	82	101	75	90	85
IR-HIS	125	87	76	84	70	84	87
EPH-B3	34	30	81	37	6	54	59
TBK1	59	123	88	91	85	90	116
IKKe	26	92	91	89	79	92	97
GCK	99	103	93	98	92	96	101
IRAK4	56	78	115	98	101	93	86
NUAK1	36	68	91	76	70	63	73
MLK1	37	79	100	90	78	82	80
MINK1	117	88	89	120	53	86	73
MLK3	30	95	97	104	66	81	80
LKB1	66	86	102	84	99	87	119
HER4	85	96	47	89	66	88	112
TTK	51	98	89	108	81	79	85
CDK2-Cyclin A	105	97	115	92	113	96	103
RIPK2	/	/	56	/	40	/	/
Aurora A	/	/	35	/	14	/	/
PAK2	/	/	82	/	77	/	/
BRSK1	/	/	108	/	107	/	/
HIPK3	/	/	93	/	94	/	/
HIPK1	/	/	91	/	77	/	/
JNK3	/	/	97	/	85	/	/
MAPKAP-K3	/	/	105	/	88	/	/
MARK2	/	/	108	/	98	/	/
MARK4	/	/	97	/	83	/	/

ATP concentration used is equal to the Km of ATP of each of the tested kinases. All assays are performed using a radioactive (^{33}P-ATP) filter-binding assay. This assay is the gold standard in terms of accuracy and sensitivity and avoids the false positives and negatives associated with fluorescent assay formats.[216]

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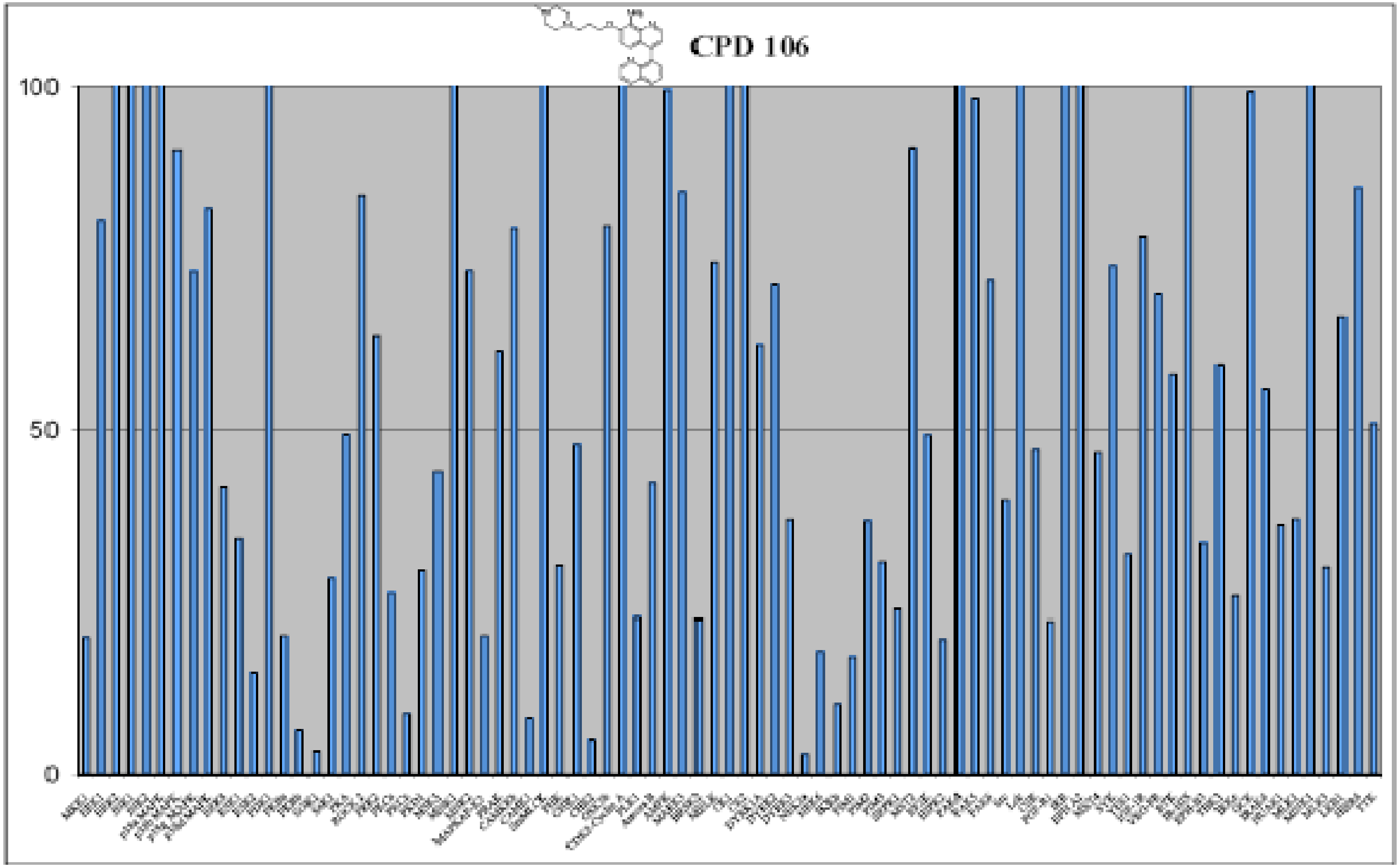
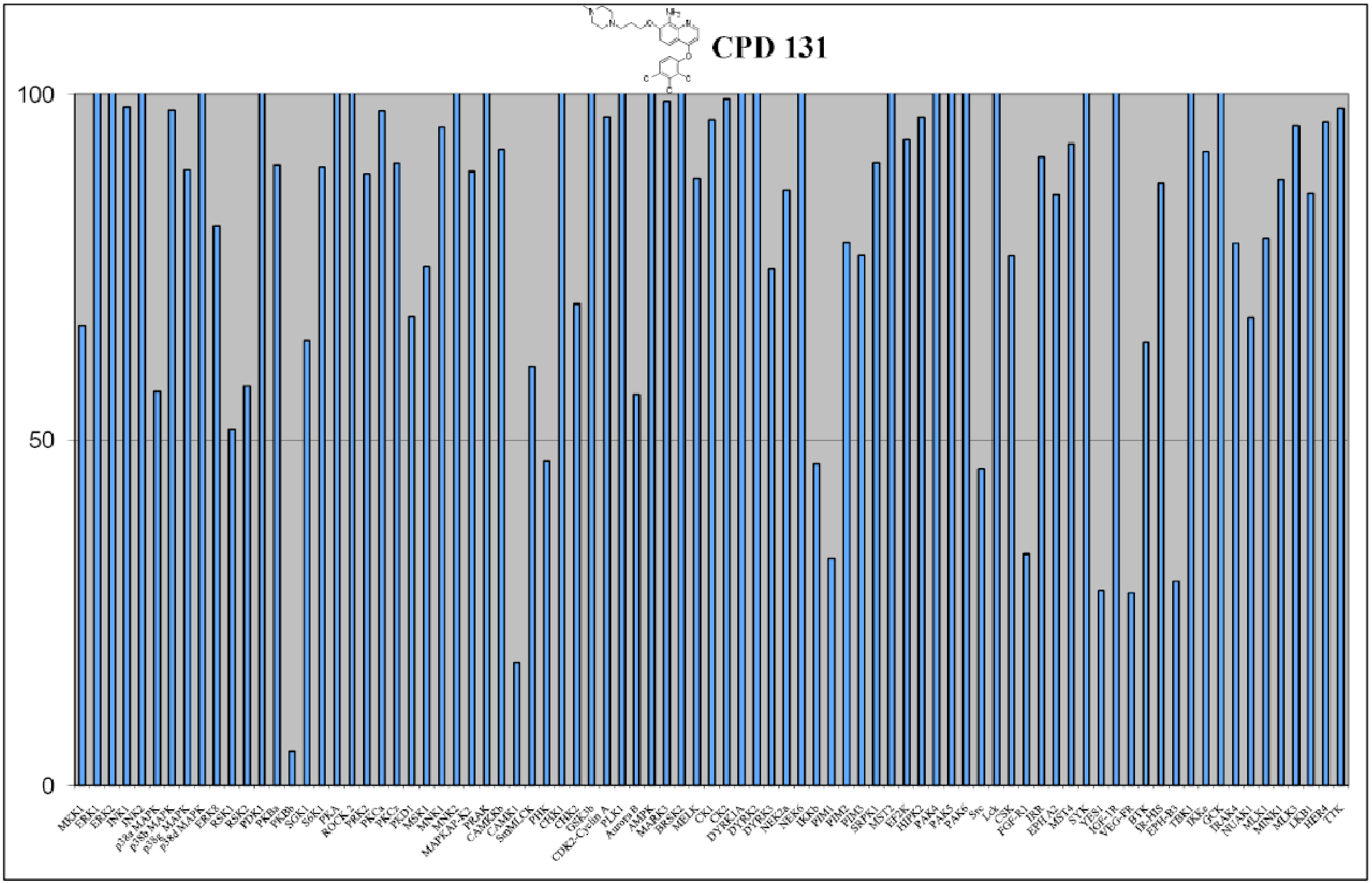
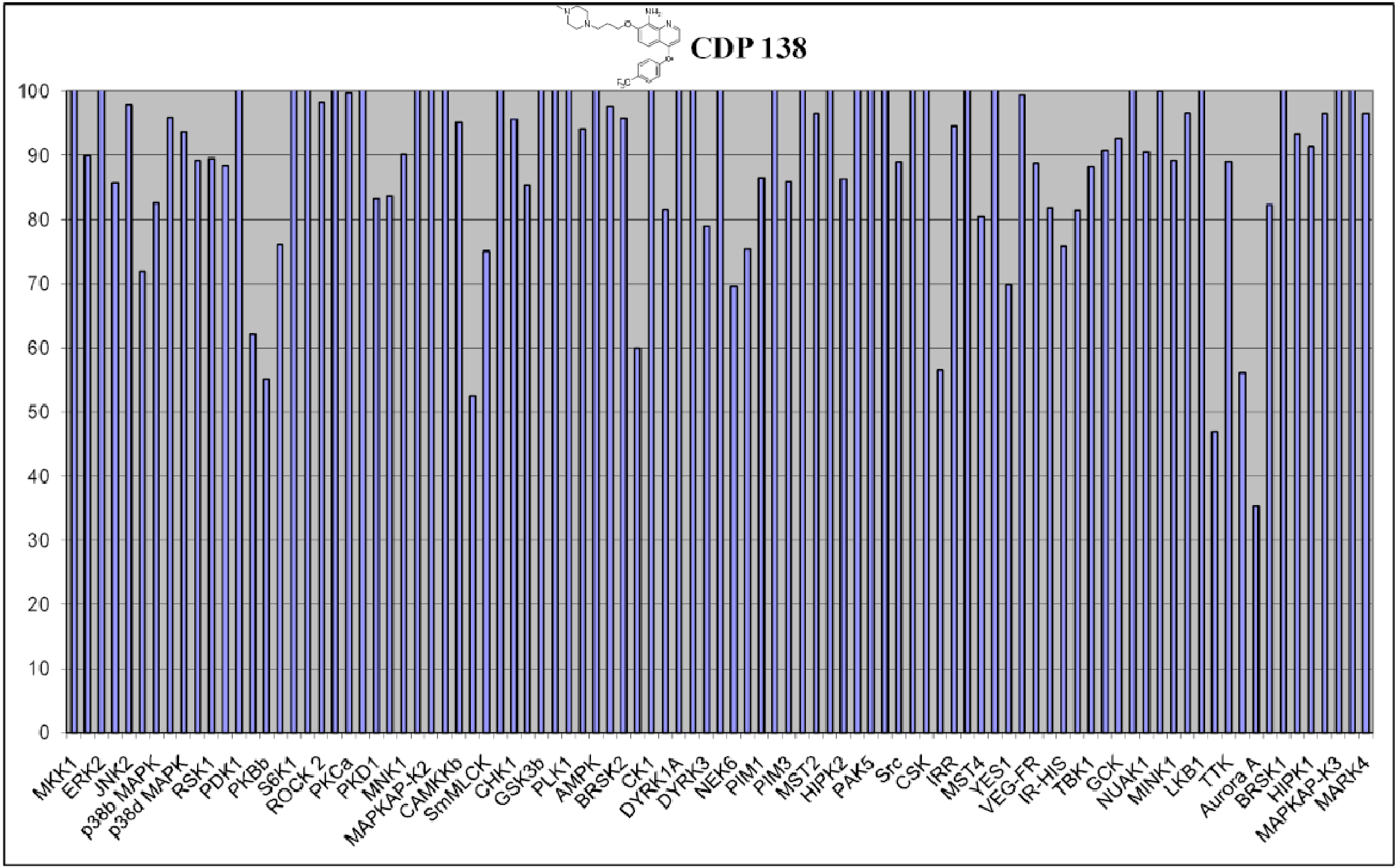
[216] http://www.kinase-screen.mrc.ac.uk/kinase-assay.htm



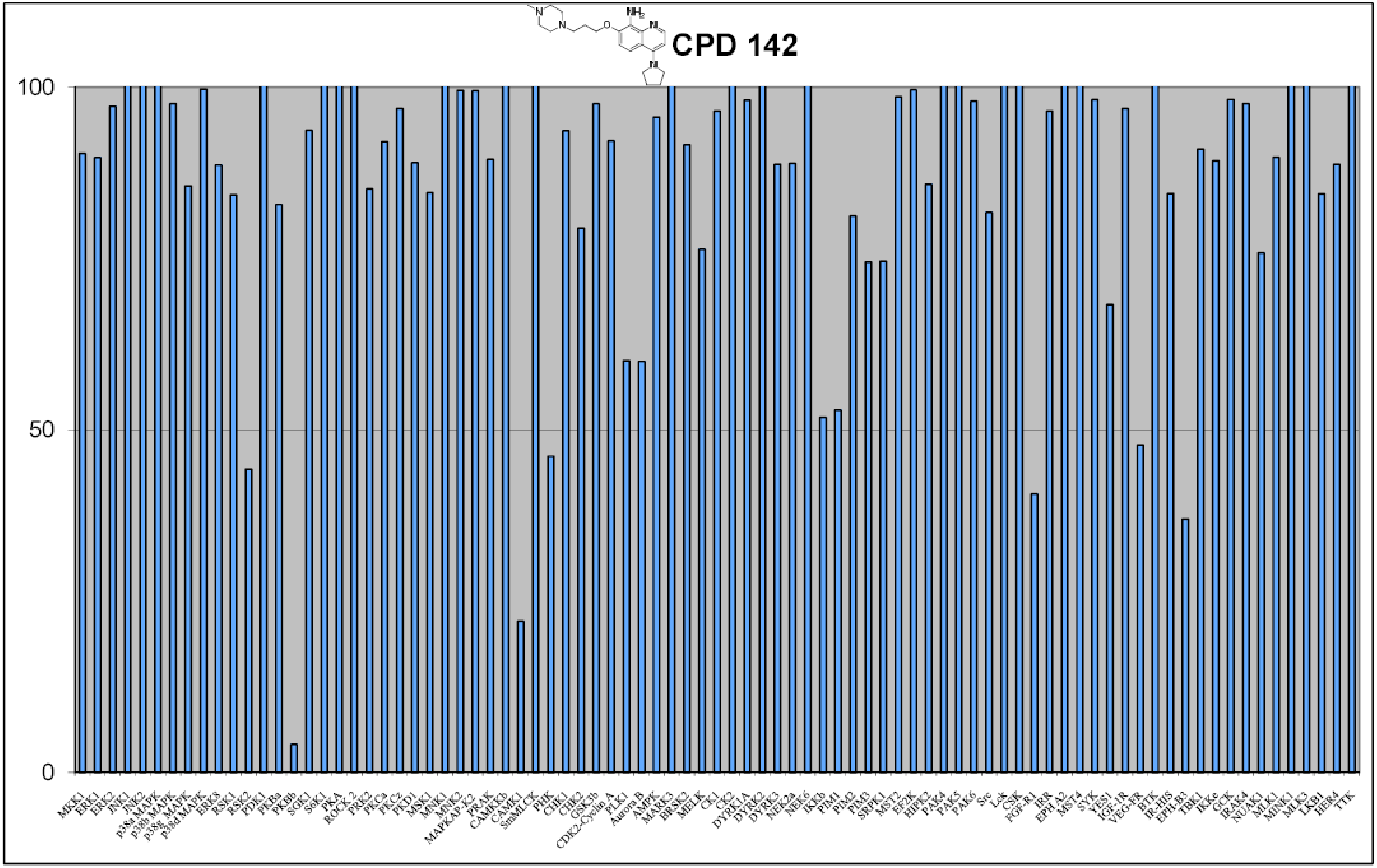
Graph 4.1: Istogram showing the *percentage of reduction of the normal kinase activity by compound 106, tested at 10 μM.*



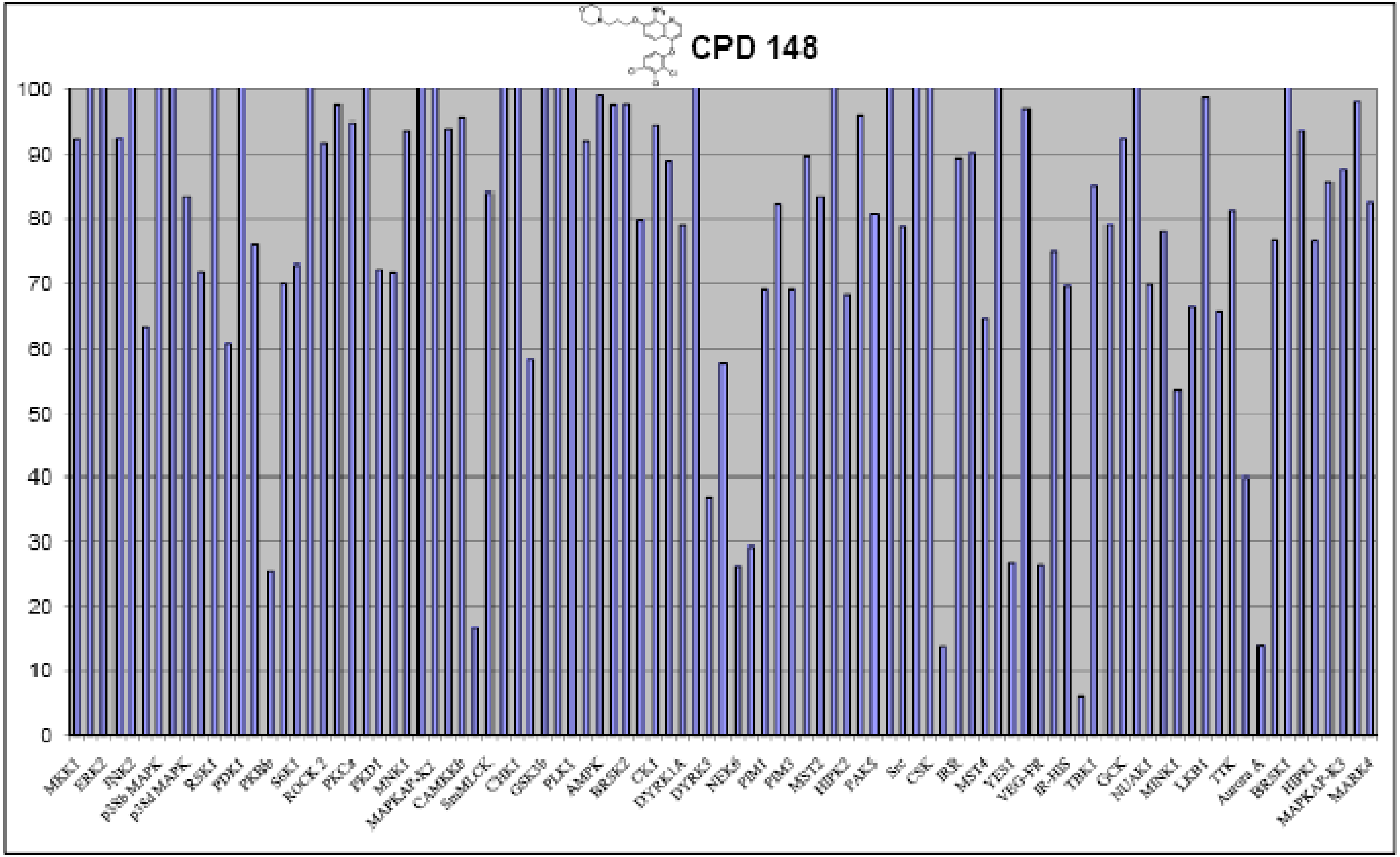
Graph 4.2: Istogram showing the *percentage of reduction of the normal kinase activity by compound 131, tested at 10 μM.*



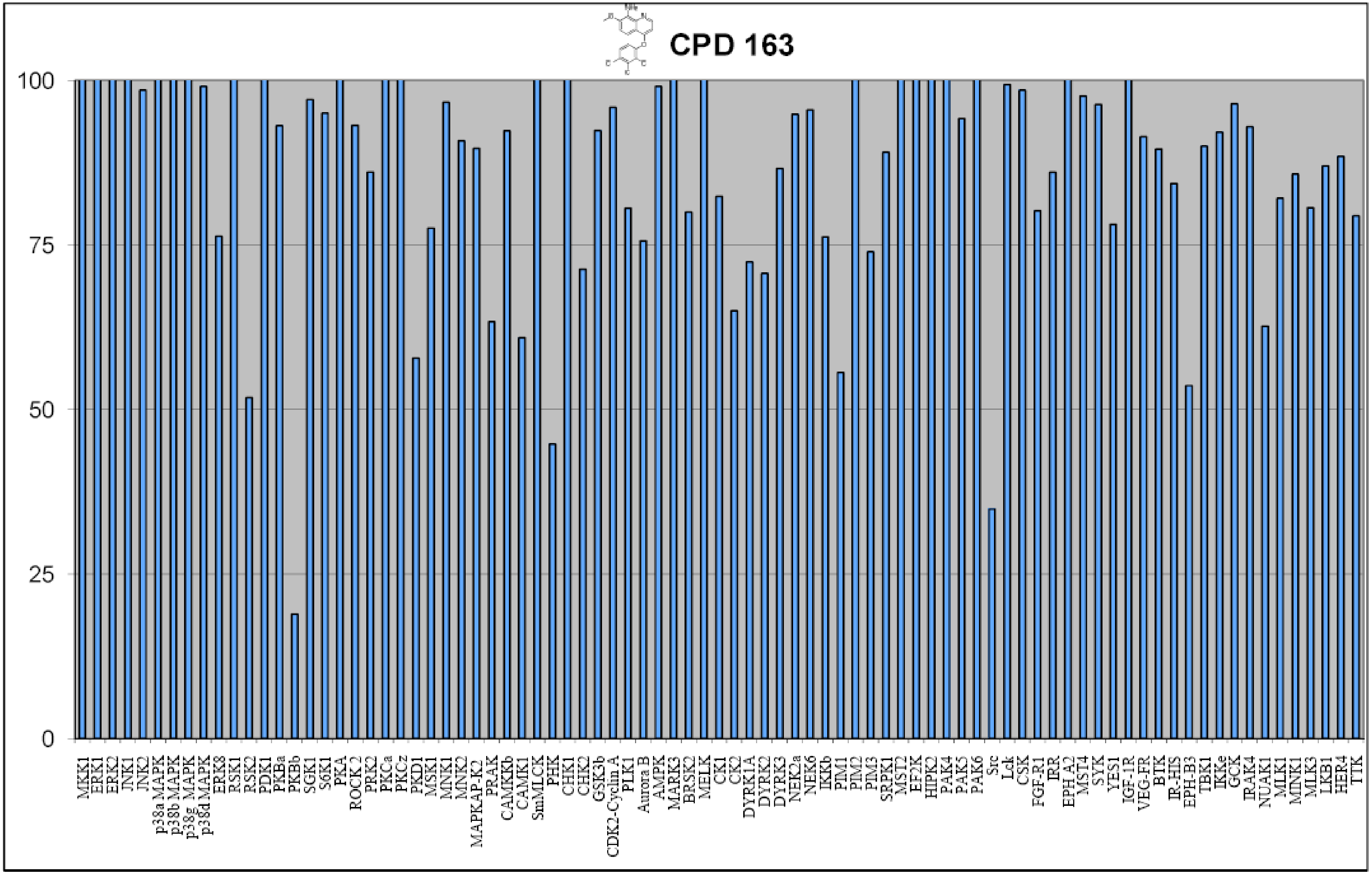
Graph 4.3: Istogram showing the *percentage of reduction of the normal kinase activity by compound **138**. tested at 10 μM.*



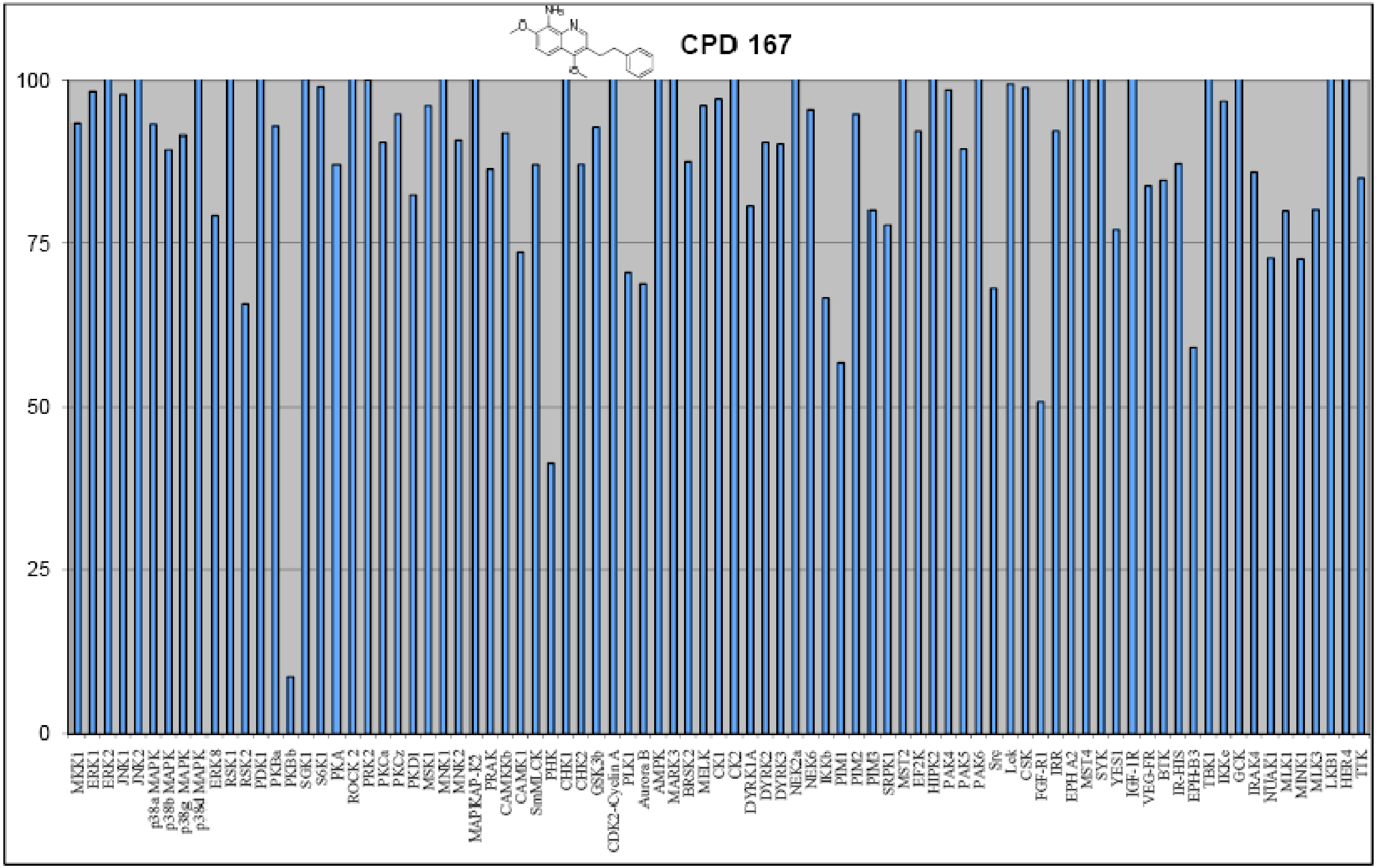
Graph 4.4: Istogram showing the *percentage of reduction of the normal kinase activity by compound* ***142****. tested at 10 μM.*



Graph 4.5: Istogram showing the p*ercentage of reduction of the normal kinase activity by compound 148. tested at 10 µM.*



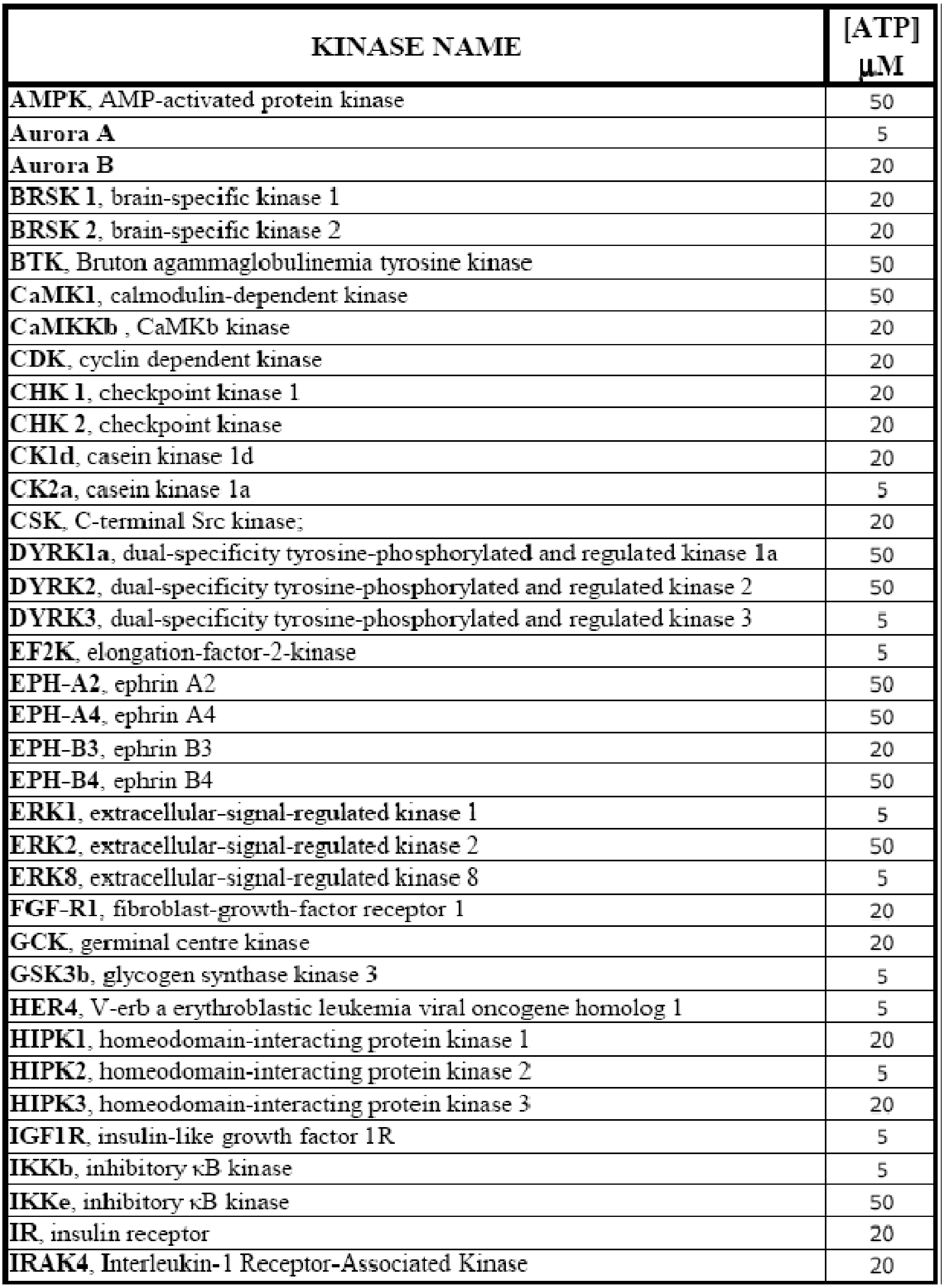
Graph 4.6: Istogram showing the p*ercentage of reduction of the normal kinase activity by compound* ***163*** *tested at 10 μM.*



Graph 4.7: Istogram showing the p*ercentage of reduction of the normal kinase activity by compound* ***167*** *tested at 10 μM.*

APPENDIX C: PROTEIN KINASES

Table 4.6: *Kinase panel for the selectivity profile with the ATP concentration employed for the test*[217].



KINASE NAME	[ATP] µM
AMPK, AMP-activated protein kinase	50
Aurora A	5
Aurora B	20
BRSK 1, brain-specific kinase 1	20
BRSK 2, brain-specific kinase 2	20
BTK, Bruton agammaglobulinemia tyrosine kinase	50
CaMK1, calmodulin-dependent kinase	50
CaMKKb , CaMKb kinase	20
CDK, cyclin dependent kinase	20
CHK 1, checkpoint kinase 1	20
CHK 2, checkpoint kinase	20
CK1d, casein kinase 1d	20
CK2a, casein kinase 1a	5
CSK, C-terminal Src kinase;	20
DYRK1a, dual-specificity tyrosine-phosphorylated and regulated kinase 1a	50
DYRK2, dual-specificity tyrosine-phosphorylated and regulated kinase 2	50
DYRK3, dual-specificity tyrosine-phosphorylated and regulated kinase 3	5
EF2K, elongation-factor-2-kinase	5
EPH-A2, ephrin A2	50
EPH-A4, ephrin A4	50
EPH-B3, ephrin B3	20
EPH-B4, ephrin B4	50
ERK1, extracellular-signal-regulated kinase 1	5
ERK2, extracellular-signal-regulated kinase 2	50
ERK8, extracellular-signal-regulated kinase 8	5
FGF-R1, fibroblast-growth-factor receptor 1	20
GCK, germinal centre kinase	20
GSK3b, glycogen synthase kinase 3	5
HER4, V-erb a erythroblastic leukemia viral oncogene homolog 1	5
HIPK1, homeodomain-interacting protein kinase 1	20
HIPK2, homeodomain-interacting protein kinase 2	5
HIPK3, homeodomain-interacting protein kinase 3	20
IGF1R, insulin-like growth factor 1R	5
IKKb, inhibitory κB kinase	5
IKKe, inhibitory κB kinase	50
IR, insulin receptor	20
IRAK4, Interleukin-1 Receptor-Associated Kinase	20

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[217] http://www.kinase-screen.mrc.ac.uk/kinase-panel.htm

KINASE NAME	[ATP] μM
IRR, insulin related receptor	5
JAK2, Janus Kinase2	5
JNK1a1, c-Jun N-terminal kinase a1	20
JNK2a2, c-Jun N-terminal kinase 2a2	20
JNK3a1, c-Jun N-terminal kinase 3a1	20
LCK, lymphocyte cell-specific protein tyrosine kinase	50
LKB1, **MO25**, **STRAD**, Ser/Thr Kinase 11	20
MAPKAP-K2, MAPK-activated protein kinase 2	20
MAPKAP-K3, MAPK-activated protein kinase 3	20
MARK1, microtubule-affinity-regulating kinase 1	20
MARK2, microtubule-affinity-regulating kinase 2	20
MARK3, microtubule-affinity-regulating kinase 3	5
MARK4, microtubule-affinity-regulating kinase 4	50
MEKK1, mitogen-activated protein kinase kinase kinase 1	20
MELK, maternal embryonic leucine-zipper kinase	50
MINK1, misshapen-like kinase 1	50
MKK1	5
MLCK, smooth-muscle myosin light-chain kinase	50
MLK1, mixed lineage kinase 1	20
MLK3, mixed lineage kinase 3	20
MNK1, MAPK-integrating protein kinase 1	50
MNK2a, MAPK-integrating protein kinase 2a	50
MSK1, mitogen- and stress-activated protein kinase 1	20
MST2, mammalian homologue Ste20-like kinase	20
MST4, mammalian homologue Ste20-like kinase 4	20
NEK2A, NIMA (never in mitosis in Aspergillus nidulans)-related kinase 2A	50
NEK6, NIMA (never in mitosis in Aspergillus nidulans)-related kinase 6	50
NUAK1, SnF1-like Kinase 1	20
p38a MAPK	50
p38b MAPK	20
p38g MAPK	5
p38d MAPK	5
PAK 2 T402E, p21-activated protein kinase 2	20
PAK 4, p21-activated protein kinase 4	5
PAK 5, p21-activated protein kinase 5	20
PAK 6 , p21-activated protein kinase 6	20
PHKy1, phosphorylase kinase y1	50
PDK1, 3-phosphoinositide-dependent protein kinase 1	20
PIM1, provirus integration site for Moloney murine leukaemia virus 1	20
PIM2, provirus integration site for Moloney murine leukaemia virus 2	5
PIM3, provirus integration site for Moloney murine leukaemia virus 3	20
PKA, cAMP-dependent protein kinase	20
PKBa, protein kinase B (also called Akt) a	5
PKBb, protein kinase B (also called Akt)b	50
PKCa, protein kinase C a	20

KINASE NAME	[ATP] µM
PKCz, protein kinase C z	5
PKD1, protein kinase D 1	50
PLK1, polo-like kinase 1	5
PRAK, p38-regulated activated kinase	20
PRK2, protein kinase C-related kinase 2	20
RIPK2, receptor interacting protein kinase 2	20
ROCKII, Rho-dependent protein kinase II	20
RSK1, p90 ribosomal S6 kinase 1	50
RSK2, p90 ribosomal S6 kinase 2	50
S6K1 T412E, S6 kinase 1	20
SGK1, serum- and glucocorticoid-induced kinase 1	20
Src, sarcoma kinase	50
SRPK1, serine-arginine protein kinase	50
SYK, spleen tyrosine kinase;	20
TAK1, Transforming growth factor beta activated kinase, **TAB1**, TAK1 binding subunit (FUSION)	5
TBK1, TANK-binding kinase 1	50
TrkA, Neurotrophic tyrosine kinase, receptor, type 1	20
TTK, Phosphotyrosine picked threonine kinase	20
VEGFR, vascular endothelial growth factor receptor	20
YES1, Yamaguchi sarcoma viral oncogene homologue.	20

APPENDIX D: ABBREVIATIONS

^{13}C-NMR = Carbon nuclear magnetic resonance
^{1}H-NMR = Protonic nuclear magnetic resonance
A = Alanine
Abl = Abelson Leukemia Virus
AcOEt = Ethyl acetate
ALL = acute lymphoblastic leukemia
AML = Acute Myeloid Leukemia
ATP = adenosine triphospate
BC = Blast crisis
Bcr = Breakpoint Cluster Region
Bcr- Abl = Breakpoint Cluster Region- Abelson Leukemia Virus (fusion protein)
C = Cysteine
CML = Chronic Myeloid Leukemia
COSY = Correlation spectroscopy
CPD = Compound
D = Aspartic acid
DMF = *N*-*N*-dimethylformamide
DMSO = Dimethylsulphoxyde
E = Glutammic Acid
EDG = Electron donating group
ELISA = Enzyme-Linked Immunosorbent Assay
ESI-HRMS = Electron spray ionization – high resolution mass spectroscopy
ESI-MS = Electron spray ionization – mass spectroscopy
Et$_3$N = Triethylamine
EtOH = Ethanol
EtONa = Sodium ethoxyde
EWG = Electron withdrawing group
F = Pheniylalanine
G = Glycine
GTP = guanosine triphosphate
H = Hystidine
HHT = Homoarringtonine
HMBC = Heteronuclear multiple bond coherence
HMQC = Heteronuclear multiple quantum coherence
I = Isoleucine
IC$_{50}$ = Half maximal inhibitory concentration
IL3 = Interleukin 3
K = Lysine
L = Leucine
LG = Leaving group
M = Methionine
MeCN = Acetonitrile
MeOH = Methanol
MeONa = Sodium methoxyde
MS = Mass spectroscopy
N = Asparagine
NBS = *N*-Bromosuccinimide
NCS = *N*-Chlorosuccinimide
NMP = *N-methylpirrolydinone*

NOESY = Nuclear Overhauser effect spectroscopy
P = Proline
PDB = Protein data bank
Ph = Philadelphia
PTSA = *Para*-toluensulfonic acid
Q = Glutammine
R = Arginine
S = Serine
SAR = Structure activity relationship
SN = Nucleophilc substitution
SN$_{\mathrm{Ar}}$ = Aromatic nucleophilic substitution
T = Threonine
***t*-BuOK** = Potassium *tert*-butoxide
THF = Tetrahydrofuran
TK = tyrosine kinase
TMB = Tetramethylbenzidines
V = Valine
W = Triptophane
WT = wild type
Y = Tyrosine

APPENDIX E: ACKNOWLEDGMENTS

Oh finalmente mi posso permettere di scrivere nella mia lingua… basta con ‘sto inglese. Anzi… quasi quasi comincio a scrivere in margherotto che mi viene molto piu naturale…Mamma ‘sto giro son tanti da ringraziare, in tre anni ne ho incontrate un sacco di persone… Da chi cominciamo? Cominciare sempre dalle sfere alte del potere mi suggeriscono dalla regia. Ma per primo voglio ringraziare Alfonso che ha creduto in me e mi ha voluto per questo dottorato. Grazie Alfonso! Grazie soprattutto perché mi avevi detto che saresti stato il mio tutor e il primo giorno di dottorato mi hai detto: “sbrigati che ho un ora per spiegarti quello che farai per i prossimi 3 anni, perché ho l’aereo per Londra e non torno piu!”…E adesso tocca a Lucchini che mi ha espressamente richiesto di non essere ringraziato, bensi di essere insultato. Ottimo prof., allora prima la ringrazio poi la insulto. Anzi, celo gli insulti tra i ringraziamenti, a lei capire gli uni e gli altri…Grazie prof. per essere riuscito a tirar fuori qualche chemical shift decente da spettri indecenti che ho fatto a Ginevra. Grazie per aver caratterizzato la tonnellata di composti che le portavo. Grazie per aver brontolato quando non le portavo composti perché la macchina non poteva rimanere ferma. Grazie per aver brontolato quando le portavo composti perché ero un rompipalle che la faceva lavorare troppo. Grazie perché nel periodo nero dello spike in centro spettro ogni pomeriggio veniva su in laboratorio a dire “la macchina funziona benissimo adesso!”. Grazie perché quando poi andavo giu’ a fare lo spettro, lo spike c’era ancora. Grazie perché ho imparato da lei alcuni dei molti segreti dell’NMR. Grazie per averci messo solamente 3 mesi ad imparare il mio nome e la mia faccia. Grazie perché non mi ha insegnato a fare l’omogeneità, bullandosi che lei era il migliore del mondo a farla.Grazie perché i refilling di elio erano sempre meglio di una puntata di Zelig grazie ai suoi:”ECCCHECCCAZZOOO! NON CI VEDOOOOO!” mentre l’elio liquido sgorgava copioso sui nostri piedi. Grazie prof. per aver messo su esperimenti per me anche alle 2 di notte di sabato. Grazie per la fiducia che mi ha dato nel portar avanti il progetto.Grazie perché nei primi tre mesi quando venivo a parlarle mi diceva: ”Chi è lei!?”. Grazie per essere sempre stato schietto e sincero! Grazie di tutto, prof…

Passiamo all’altro capo, Leonardo Scapozza. Grazie capo per avermi sopportato con le mie domande da prima elementare su come funzionano le proteine (…eeee ho studiato chimica io mica biologia… se poi sapessi che come prof. di chimica biologica ho avuto Stevanato e Bragadin, capiresti molte cose…). E’ stato bello averti come capo, da te ho imparato un sacco di cose. E’ stato bello perché hai sempre avuto totale fiducia in me.E’ stato bello perché immediatamente grazie alla ricca Svizzera mi è arrivato un computer nuovo. E’ stato bello anche quando appena arrivato a Ginevra, hai cercato di accoppiarmi con qualsiasi esemplare di sesso femminile di qualsiasi specie animale. E’ stato bello perché quando poi mi sono accoppiato senza seguire i tuoi consigli, hai continuato a cercare di accoppiarmi. E’ stato bello perché mi hai dato la possibilità di rimanere un anno a Ginevra. E’ stato bello perché tutta Ginevra sa che la mia alcoldeidrogenasi è un po fiacchetta. E’ stato bello perché quella volta che mi son fatto la barba da ubriaco togliendomi una fettina di faccia, hai guidato tu. E’ stato bello perché hai sempre dato molto valore al lato umano anche nel lavoro. Grazie Leonardo…

Bene, adesso mi sento in dovere anche di ringraziare Otto. E’ la prima volta che tu non sei il mio capo, pero’ alla fine i nostri laboratori sono comunicanti, e praticamente abbiamo convissuto per altri due anni… soprattutto a pranzo. Scusa se ho fatto entrare nel Tempio Della Chimica (il tuo studio) certe parole e certi argomenti… pero’ non è sempre colpa mia, alla fine anche tu sei diventato sboccato e volgare e davi la colpa a me perché sono io che ti ispiro… Ad ogni modo, grazie Otto! Grazie perché quando non sapevo come proseguire con la sintesi tu sei sempre stato la ad ascoltare le mie lagne… e grazie soprattutto della tua amicizia. Grazie anche perché ti tieni sempre la quel c*****o di Drambuie e non lo vuoi stappare…E’ il turno dei compagni di laboratorio! Partiamo da Venessia: grazie Fabrizio per i 379 consigli utili su come comportarsi in laboratorio e non sputtanarsi gli inibitori all'ultimo passaggio… grazie anche perché mi hai voluto come assistente di laboratorio per i laboratori studenti. SSSSSoreeeee…Posso scancellare? Ah.. dimenticavo, tu adesso ringrazia me per la corda e i moschettoni… Stefi…. Tocca a te adesso: un quarto degli inibitori della tesi escono dalla tua cappa.. soprattutto quello piu attivo di tutti… maledetta arrivi dopo nel progetto e attaccando un alcol a caso mi freghi anche il record di attività… Grazie davvero per avermi aiutato a domare i mille capricci della catena piperazinica, e per aver condiviso lo spazio moooooolto ristretto del nostro lab con un cazzone casinaro come me! Tocca a Riccardo, l'uomo in cui ragionamento ed istinto di riproduzione spesso si scambiano di posto, l'uomo la cui cappa potrebbe essere leccata da quanto pulita è, e le cui spruzzette sono perfettamente allineate … Grazie perché appena sono tornato da Ginevra hai subito iniziato a farti i c***i miei spudoratamente e allo stesso modo a raccontarmi i tuoi , riuscendo cosi ad arrivare a livello di amicizia che non pensavo…Grazie perché con i tuoi gialli fosforescenti dallo spettro di mmerda sei riuscito a ravvivare il laboratorio! Vuoi qualche consiglio? Cambia colore che hai rotto! Impara a parlare dalle quindicenni di MaRgheRa! E lascia un po piu di casino ogni tanto! Doveroso poi è il ringraziamento a quel menamaro' di Beppe per…….. si dai perrrr……daaaiiiiii avete capito no? Un po' di fantasia! Stavo facendo un'altra cosa! Grazie per lasciare tutte le frasi senza conclusione. Grazie per aver fatto funzionare l'HPLC a scapito della tua igiene mentale! Grazie per il tuo intestino di cristallo. Grazie per avermi usato come capro espiatorio per qualsiasi cosa. E grazie perché ti incazzi quando tua moglie ti dice che io sono bello! Sai Beppe cosa dovresti fare? Dovrestiiii….. si dai dovrestiiii….Un grazie sentito anche a Gianni, il chimico piu alto del mondo, la trasposizione in carne ed ossa di telespalla Bob, per averci fatto capire da vicino il significato di tiolo. Gianni, l'ottimismo è il profumo della vita! Scendiamo di almeno un metro e mezzo e troviamo la Cinzia la rompipalle piu simpatica di ca'Foscari! Simpatica come un riccio di mare nelle mutande! Cinzia coi tuoi percheeeeeeee ci hai veramente stressato, ma in fondo ti vogliamo bene cosi come sei. Tanto in fondo pero'…No dai.. grazie Cinzia per aver portato un po di femminilità all'interno di quello studio un po' troppo mascolino. 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Grazie per aver terrorizzato me e Leo a Sciandolen con quelle cacchio di storie sul demonio… ho ancora i brividi se ci penso… Loris e Anna, dirvi che siete splendidi è riduttivo…peccato che ve ne siate andati troppo presto…grazie Loris perché quella sera non hai mai hai fatto in modo che il mio bicchiere fosse vuoto, procurandomi la peggiore balla della mia vita… Grazie poi ad Anja che si è rivelata un ottima compagna di scalata e mi ha fatto scoprire le gioie del pesto rosso, grazie a Majdeline mia collega di laboratorio che ho sfruttato nel primo periodo per capire come muovermi, grazie a Carole che ha sempre compensato la mia totale incapacità burocratica, grazie ad Yvonne anche se non mi ha mai voluto parlare in italiano, grazie alla combriccola di indiani Yogesh, Sachin, Uttam, Dhabal, e Jennifer che allietavano il laboratorio col profumo delle orecchie di porco. Look at here. Ok you can go. Continuiamo poi con il ringraziare il resto del gruppone: Ralitza, Sabine, Remo, Chiara , Silvia chi? e Simone, il Robin

Hood all'incontrario. E dovrei aver finito. Ma non ho trovato solo colleghi d'università a Ginevra. Non posso non ringraziare Serge e Catherine i padroni di casa per essere due persone eccezionali. Grazie Serge per le infinite chiacchierate in cui hai cercato di farmi capire che l'omeopatia è la cura di tutti i mali e che come cerchiamo di curare noi il cancro non va bene. Grazie per Shinouk, che mi pisciava soprattutto in camera e sul tappeto della cucina, e grazie per avere una piscina sul tetto della casa! Una persona che voglio ringraziare di cuore è Gesine. Cara Gesine, sei proprio formaggio! E ricordati che piutosto che spanderghene un giosso xe meio beverghene un posso! Grazie Gesine per non avermi odiato quando ti insegnavo apposta le cose sbagliate in italiano... bucolico non vuol dire pieno di buchi, e non si dice fromaggio. Sei un'amica meravigliosa, diciamo pure la migliore amica che potessi trovare a Ginevra…ne avrei di cose da dire per ringraziarti, ma devo tagliare un po'… mi limito a ringraziarti per essere venuta con me molte volte a camminare in montagna! E dopo tocca a lui, Eugenio il mio moroso di Ginevra. Piu che un'amicizia la nostra è stata una storia d'amore omosessuale. Quanti filmini visti abbracciati sul divano! Quante cenette intime guardandoci negli occhi immaginando la scena di nostro figlio che ci presenta il fidanzato! Quante volte sul Saleve a tornar zo col scuro! Quante volte te go spetà che ti geri sempre in ritardo! Compagno, per fortuna che hai lasciato quel bigliettino in palestra senno' con chi andavo io ad arrampicare? Ma quanto ben me votu? Ma anche il suo indotto era notevole! Mi ha aperto un mondo di altra gente. Per prima la Gramiii! Ciao Topaa! Grazie per esser venuta con me ed Eugi in kayak (e ricordati che ti ho salvato la vita!) e a dormire all'aperto sotto le pareti! Poi mitica Christiane, 76 anni e 15 metri quadri di casa e lei dormiva in terrazza beveva latte giallo del mese prima e aveva lo chalet in montagna con le pantegane che facevano colazione assieme a noi! La migliore! Lei ha capito tutto della vita! Il resto mjidell'indotto sono in genere gnocche fotoniche e pure simpatiche che hanno allietato le nostre giornate! Non pensate male, diciamo che erano belle da vedere! Un grazie anche a tutti gli amici della Missione Italiana: Bea e Ceci prima di tutti visto che ci vediamo e ci sentiamo ancora, e poi un abbraccio pure a PG, Ale ed Umberto. Doverosissimo il ringraziamento poi a tutto il gruppo di Monza che ha fatto tutti i test biologici sui miei inibitori. Grazie Sara, Luca, Roberta e Carlo. Grazie oltre che per i test e per non avermi mandato a quel paese quando vi chiedevo ogni 3 secondi "allora sono arrivati i composti..?" anche e soprattutto per i meeting-grigliata a Valtournenche…

E adesso vien la parte piu importante: la mia famiglia e la morosa. Grazie babbo, grazie paggetto e grazie vecchietta perché a quasi trent'anni mi sopportate ancora e avete creduto in me quando vi ho detto che mollavo il posto fisso in BAF e andavo a fare il dottorato… I ringraziamenti per voi sarebbero sempre troppo pochi, ma è a voi che dedico la valanga di composti che spero un giorno possano curare la leucemia…un morso sulla cerega e un baap. Ah xe vero go anca un zemeo…Hmmm vaben dai, grazie anche a te Teo! Abooore tocca a te adesso! Non te lo meriteresti visto che non c'ero tra i tuoi ringraziamenti, ma io te li faccio lo stesso… Alla fine è merito di questo dottorato se io e te siamo assieme adesso..Grazie abore perché mi hai tenuto sveglio fino alle 2 di notte con punte delle 3, praticamente un giorno si e uno anche… grazie abore per aver contribuito a diminuire la mia produttività a Ginevra. Grazie abore per essere venuta a trovarmi ben due volte in 7 mesi, mentre io sono venuto giu almeno una decina di volte… grazie abore per avermi pettato la peste bubbonica quando sono sceso per il ponte del primo maggio! Grazie abore per tutto il resto che non scrivo qua senno' poi i ringraziamenti diventano zuccherosi come le urine di un diabetico… Ah un ultima cosa volevo dirti: Baaaaaaaaaaaaaaaaap! Concludo che avrete già preso il sonno tutti…. Mi sa che sono gli ultimi questi ringraziamenti che scrivero'… e mi sa tanto che la mia vita qui a S.Marta dopo una decina d'anni si avvia verso la conclusione… una lacrimuccia tende a rigarmi il volto, ma io tengo duro perché quelli da maRgheRa non devono piangere! Col cuore, davvero grazie a tutti…

Enrico

PS: MANGIÄMO?

Estratto per riassunto della tesi di dottorato

L’estratto (max. 1000 battute) deve essere redatto sia in lingua italiana che in lingua inglese e nella lingua straniera eventualmente indicata dal Collegio dei docenti.
L’estratto va firmato e rilegato come ultimo foglio della tesi.
Studente: __Enrico Rosso__________________________ matricola: ___ 955387________
Dottorato: ____Scienze Chimiche____
Ciclo: ____XXII°___
Titolo della tesi[1] : _Synthesis of 8-aminoquinoline as inhibitors of tyrosine kinases_______
Abstract:

ABSTRACT (ENGLISH VERSION)

Chronic myelogenous leukemia (CML), characterized by unregulated proliferation of myeloid cells in the bone marrow, accounts for 15 to 20 % of all adult leukemia cases in the Western population. The molecular cause for the disease is the characteristic translocation between chromosome 9 and 22 which results in the so called Philadelphia chromosome (Ph) and in the formation of the chimeric Bcr-Abl gene. In CML the protein product of this hybrid gene is a constitutively active protein kinase. Bcr-Abl kinase drives the pathogenesis of CML through the phosphorylation and activation of a broad range of downstream substrates playing a critical role in cellular signal transduction and cell transformation. ABL tyrosin kinase therefore is an interesting therapeutic target and many potent inhibitors have been developed and brought to the clinic in recent years, including Imatinib, Bosutinib, Nilotinib and Dasatinib. However, the T315I mutant form of Bcr-Abl, which is frequently found in CML patients, mediates complete resistance to Imatinib and all of the next generation Abl kinase inhibitors. Therefore, there is an eminent need for the development of drugs which are active against the T315I mutant of Bcr-Abl. Bosutinib and its quinoline scaffold have been chosen as a template for the construction of a new drug scaffold potentially able to inhibit the mutated Bcr-Abl. Starting from the model of Bosutinib bound to wild-type Abl kinase domain and the model of T315I Abl kinase domain it was apparent that in order to get potent inhibitors of Abl T315I the unfavorable interaction caused by the bulkiness of isoleucine had to be avoided and another strong favorable interaction should be added. The strategy to accomplish this goal was to remove the cyano group at 3 position of the quinoline and strengthen the interaction with the protein by adding an amino group at position 8 to establish an additional hydrogen bond with the backbone carbonyl of M318. Based on this rational, the scaffold of 8-aminoquinolines resulted to be promising for potential Bcr-Abl T315I inhibitors. Three main lines of compounds have been synthesized: 4-substituted-8-aminoquinolines, 3-substituted-8-aminoquinolines, 3,4-disubstituted-8-aminoquinolines. All of these molecules have an oxygen-linked alkyl group at position 7. Different groups have been employed to functionalize the position 7. 4-Substituted-8-aminoquinolines were synthesized starting from a 4,7-dihaloquinoline. Nitration of this molecule yielded the 8-nitro derivative which was then successfully functionalized firstly at position 7, and then at the position 4. Reduction of the nitro group allowed to obtain the desired 4-substituted-8-aminoquinolines. On the contrary, 3-substituted 8-aminoquinolines have been prepared starting from commercially available *m*-aminophenol. Nitration of this molecule yielded 2-nitro-3-aminophenol which was alkylated with an appropriate side chain at the phenolic oxygen and subsequently cyclized with α-bromoacrolein to give the 3-bromo-7-oxyalkyl-8-nitroquinoline. This precursor has been employed for functionalization at position 3. As for the 4-substituted derivatives, the last step was the transformation of nitro group into amino group. 3,4-Disubstituted-8-aminoquinolines, after a first attempt of synthesis from a substituted aniline, were synthesized starting from 4,7-dichloro-8-nitroquinoline. Position 4 was firstly functionalized with an electron donating group such as methoxy or pyrrolidino and bromine was successively introduced at position 3 by an electrophilic aromatic substitution. Attachment of the side chain at position 7 was followed by reduction from 8-nitro to 8-aminoquinoline. For all the synthesized molecules, different groups have been attached at the specific positions either via an oxygen or a nitrogen or a carbon linker. Oxygen and nitrogen linked groups have been introduced in the quinoline scaffold with an aromatic nucleophilic substitution using the corresponding alcoholate or amide. Carbon linked groups have been introduced through palladium catalyzed cross coupling reactions involving the corresponding aryl or alkyl boronic acids. Two alkynyl groups have been introduced under the classic Sonogashira reaction conditions coupling and then reduced to alkyl with hydrogen. All of the molecules synthesized have been tested in biological assays in order to verify their activity toward the enzyme in solution and toward cells expressing the oncogenic enzyme. It was discovered that as hydrochloride salt, the inhibitors were more active than as free base, therefore many inhibitors have been tested as water-soluble salts. Good results have been obtained, since the scaffold resulted active in inhibiting the enzyme. Some of the compounds exhibited inhibitory activity in the nanomolar range. Seven of the most active and specific compounds have also been tested toward a panel of 85 protein kinases (Cohen Lab, Dundee) of different classes in order to asses their selectivity profile. Three compounds (CPD **131**, **106** and **148**) having nanomolar activity on T315I Abl resulted to have low selectivity on the panel, inhibiting respectively 10, 40 and 11 kinases by more of 50% at a concentration of 10 μM. In contrast, CPD **142**, **163**, **167** and **138** resulted very selective. Among them, CPD **138** appears to be the most selective one because it inhibits only the Aurora A kinases by more than 50% at the tested 10 μM concentration. All other compounds inhibited strongly PKBβ (AKT2) which is a Serine/Threonine kinase, while enzymes more related to Abl, e.g. LCK, SRC and FGFR1, are less targeted by the selected compounds. Insulin receptor kinase, a clear anti-target for protein kinase inhibitors, is not inhibited by these compounds. Interestingly there is an inverse correlation between the selectivity on cells (transduced cells versus non-transduced cells) and the PKBβ inhibitory activity. The higher the PKBβ inhibitory activity is the less selective are the compounds. This indicates that PKBβ is a target for substituted 8-aminoquinolines.

In conclusion, a novel active scaffold for the inhibition of Abl WT and Abl T315I has been developed. Very active compounds both on the cellular and enzymatic level have been found. The synthesized Abl T315 inhibitors allowed to asses the proposed binding mode and gave a consistent SAR. Based on the obtained results it is clear that the 8-aminoquinoline-based inhibitors are lead compounds which can be further developed in an optimization process to gain activity and better selectivity in order to be able to enter *in vivo* studies.

ABSTRACT (FRENCH VERSION)

La leucémie myéloïde chronique (LMC), caractérisée par une prolifération dérégulée des cellules myéloïdes dans la moelle osseuse, est responsable de 15-20% des cas de leucémie dans la population occidentale. La pathogénèse de cette maladie est liée à une translocation entre les chromosomes 9 et 22, résultante dans le bien connu chromosome de Philadelphie et la formation d'un gène chimérique, Bcr-Abl. Dans le cas de la LMC, le produit de ce gène hybride est une protéine avec activité tyrosine-kinase, la Bcr-Abl kinase qui est constitutivement active. Elle induit nombreuses phosphorylisations et l'activation d'une large gamme de substrats responsables de la transduction de signaux et de la transformation des cellules. De ce fait elle représente une cible thérapeutique intéressante. Ainsi, de nombreux inhibiteurs potentiels, notamment l'Imatinib, le Bosutinib, le Nilotinib et le Dasatinib, ont récemment été développés et testés en phase clinique. Néanmoins, la forme mutée T315I de Bcr-Abl fréquemment trouvée dans les patients de LMC montre une résistance absolue contre Imatinib et les inhibiteurs de la Abl-kinase de deuxième génération tels que Bosutinib, Nilotinib ou Dasatinib. Ceci rend imminent la nécessité de développer de nouveaux principes actifs contre cette forme mutée. En premier lieu une molécule contenant un squelette quinolinique, le Bosutinib, à été choisi comme modèle pour la construction d'une nouvelle structure moléculaire, potentiellement capable d'inhiber la forme mutée de Bcr-Abl. Partant du modèle 3D de Bosutinib lié au site de liaison du domaine "wild type" de la kinase Abl et

du modèle du domaine de la kinase T315I kinase, il paraissait évident pour une inhibition de Bcr-Abl T315I, que, d'une part, l'interaction défavorable causée par la chaîne latérale de l'isoleucine devait être évitée et que d'autre part une nouvelle interaction favorable devait être ajoutée. Pour ce faire, la stratégie était d'éliminer le groupement nitrile en position 3 de la quinoline et de renforcer l'interaction de la molécule avec la protéine par l'introduction d'un groupement aminé en position 8, créant ainsi une nouvelle liaison d'hydrogène avec le methionine 318. Partant de ces hypothèses, le squelette 8-aminoquinolinique semblait être un candidat promettant de créer la base d'un inhibiteur de Bcr-Abl T315I. Ainsi, trois lignes principales de composés ont été synthétisées: 8-aminoquinoline substitué en position 4, 8-aminoquinoline substitué en position 3 et 8-aminoquinoline di-substitué en positions 3 et 4. Tous ces molécules contiennent un groupement o-alkylique en position 7, divers groupement alkyliques ayant été utilisés. Les 8-aminoquinolines substituées en position 4 ont été synthétisés à partir d'une quinoline 4,7-di-halogénée. La nitration de cette molécule résulte dans un dérivé le 8-nitro, qui par suite à été fonctionnalisé en position 7, puis en position 4. Enfin, la réduction du groupement nitrique a permis la synthèse de la 8-aminoquinoline 4-substituées. Les 8-aminoquinolines substituées en position 3, en revanche, ont été préparées à partir de m-aminophénole. Par nitration de cette molécule on obtient la 2-nitro-3-aminophénole. Celle-ci était ensuite alkylée sur l'oxygène phénolique avec une chaîne latérale appropriée, et successivement cyclisée avec α-bromoacroléine donnant le précurseur 3-bromo-7-oxyalkyl-8-nitroquinoline qui peut être fonctionnalisée en position 3. De même que pour les dérivés substitués en position 4, le dernier pas consistait dans la réduction du groupement nitrique en amine. Les 8-aminoquinoline-3,4-disubstituées, après une première tentative à partir d'une aniline substituée, ont été synthétisées partant d'une base de 4,7dichloro-8-nitroquinoline. D'abord la position 4 était fonctionnalisée avec un groupement donateur d'électrons (méthoxy ou pyrrolidine), puis, le brome était successivement introduit en position 3 par moyen d'une substitution électrophile aromatique. L'attachement de la chaîne latérale en position 7 était suivi d'une réduction du groupe nitro en position 8 à amine pour donner la 8-aminoquinilone. Pour les molécules synthétisées, divers groupes ont été introduit dans les positions spécifiées à l'aide de linker d'oxygène, d'azote ou de carbone. Les groupements contenants un linker d'oxygène ou d'azote ont été introduit dans le squelette de quinoline par une substitution aromatique nucléophile en utilisant l'alcoolate ou l'amide correspondant. Les groupements carboniques ont été introduits à l'aide d'une réaction de couplage croisé (cross-coupling) des acides aryl- ou alkylboroniques avec palladium comme canalisateur. Deux groupements alkyliques ont été introduit par un couplement de Sonogashira avec une réduction successive du groupe alken en alkyl avec de l'hydrogène. Les molécules synthétisées ont été testées sur l'enzyme en solution ainsi que sur les cellules exprimant l'enzyme oncogénique afin de vérifier leurs activités. Il a été constaté que les sels hydrochloriques des inhibiteurs ont une activité plus importante comparé aux bases libres respectives. Pour cela la plupart des inhibiteurs a été testée entant que sels hydrosolubles. Le squelette 8-aminoquinolinique s'est avérée être actif dans l'inhibition de l'enzyme muté allant jusqu'à une activité inhibitrice dans les nanomolaires pour quelques uns des composés. Entre les composés les plus actifs et spécifiques, sept étaient testés sur une série de 85-protéine-kinases de types variés (Cohen labs, Dundee) avec le but d'identifier leur profiles de sélectivité. Trois composés (CPD **131**, **106** e **148**) avec une activité inhibitrice nanomolaire sur Abl T315I ont montré une basse sélectivité avec une inhibition de plus de 50% de 10, 40 et 11 kinases, respectivement, à une concentration de 10uM. En revanche, CPD **142**, **163**, **167** et **138** avaient une forte sélectivité avec CD **138** comme le plus sélectif avec comme seule activité inhibitrice à plus de 50% (concentration 10uM) une inhibition spécifique de la kinase Aurora A. Les autres composés avaient un grand effet inhibitoire sur la sérine/thréonine kinase, PKBβ (AKT2), tandis que les enzymes ressemblant à Abl, par ex. LCK, SRC e FGFR1, n'étaient quasiment pas inhibées par ces composés. Aucune inhibition n'a été détectée pour la kinase du récepteur de l'insuline qui est reconnu comme un «anti-target». Il est intéressant à noter qu'il existe une corrélation inverse entre la sélectivité sur les cellules (transduites et non-tansduites) et l'activité inhibitrice des composés envers PKBβ: les composés avec la plus haute activité inhibitrice sur

PKBβ sont les moins sélectifs au niveau cellulaire. Ceci indique clairement que PKBβ est une cible pour la 8-aminoquinoline.Pour conclure, un nouveau «scaffold» (8-aminoquinoliniques) avec activité inhibitrice envers Bcr-Abl T315I et Bcr-Abl WT a été développé. Des composés actifs au niveau cellulaire ainsi qu'enzymatique ont été trouvés. Les inhibiteurs synthétisés ont donné une relation structure activité (SAR) consistante et cohérente. Basé sur les résultats obtenus, il est évident que ces inhibiteurs sont des composés clés (lead compounds) qui ont le potentiel d'être optimisé à l'égard de leur activité et sélectivité avec pour but leur application dans des études in vivo.

ABSTRACT (ITALIAN VERSION)

La leucemia mieloide cronica (CML), caratterizzata da una proliferazione deregolata delle cellule mieloidi nel midollo osseo, è causa del 15-20% di tutti i casi di leucemia nella popolazione. La causa molecolare della malattia è una caratteristica traslocazione tra i cromosomi 9 e 22, che genera il cosiddetto cromosoma Philadelphia (Ph) formando il gene chimericodi Bcr-Abl. Nella CML il prodotto proteico di questo gene ibrido è una protein-chinasi costitutivamente attiva. La tirosin-chinasi Bcr-Abl guida l'insorgere della malattia attraverso la fosforilazione e l'attivazione di un'ampia gamma di substrati localizzati a valle nella cascata dei segnali intracellulari, giocando un ruolo fondamentale nella loro trasmissione e nella trasformazione delle cellule. La tirosin-chinasi Bcr-Abl percio' è un interessante bersaglio terapeutico e molti suoi potenti inibitori come ad esempio Imatinib, Bosutinib e Dasatinib, sono stati sviluppati e portati alla sperimentazione clinica negli ultimi anni. Nonostante cio' la mutazione T315I di Bcr-Abl, che spesso viene riscontrata nei pazienti affetti da CML, risulta essere completamente refrattaria ad Imatinib ed a tutti gli inibitori di Bcr-Abl di seconda generazione. Per questo motivo lo sviluppo di farmaci in grado di inibire la forma mutata T315I di Bcr-Abl, è da considerarsi una priorità. Bosutinib, con il suo scheletro chinolinico, è stato scelto come modello per la costruzione di una nuova struttura molecolare potenzialmente capace di inbire la forma mutata di Bcr-Abl. Partendo dal modello computazionale di Bosutinib legato al domino chinasico di Bcr-Abl, e dal modello del dominio chinasico di Bcr-Abl T315I, risultava evidente che per ottenere inibitori di Bcr-Abl T315I, l'interazione sfavorevole causata dalla catena laterale dell'isoleucina doveva essere evitata, ed una nuova forte interazione favorevole doveva invece essere creata. La strategià percio' utilizzata prevedeva la rimozione del nitrile presente in posizione 3 della chinolina e il rinforzo delle interazioni con la proteina tramite l'introduzione di un gruppo amminico in posizione 8 in grado di formare un legame a idrogeno con la metionina 318. Basandosi su questi presupposti lo scheletro 8-amminoquinolinico che ne risulta, sembrava essere promettente per lo sviluppo di inibitori di Bcr-Abl T315I. Tre principali linee di composti sono state sintetizzate: 8-amminochinoline-4-sostituite, 8-amminochinoline-3-sostituite, 8-amminochinoline-3,4-disostituite. Tutte queste molecole hanno in posizione 7 un gruppo O-alchilico, e diversi gruppi alchilici sono stati utilizzati. Le 8-amminochinoline-4 sostituite sono state sintetizzarte partendo da una 4,7-dialogenochinolina. La nitrazione di questa molecol ha dato l'8-nitro derivato che è stato successivamente funzionalizzato prima alla posizione 7 e poi alla posizione 4. La riduzione del nitrogruppo ha permesso infine di arrivare alle 8-amminochinoline-4-sostituite. Le 8-amminochinoline-3-sostituite invece, sono state preparate partendo dal *m*-amminofenolo. La nitrazione di questsa molecola ha dato il 2-nitro-3-amminofenolo, il quale poi è stato alchilato sull'ossigeno con una particolare catena laterale, e successivamente ciclizzato con α-bromoacroleina per dare 3-bromo-7-ossialchil-8-nitrochinolina. Questo precursore è stato utilizzato per funzionalizzare la posizione 3. Come per le 4-sostituite, l'ultimo passaggio della sintesi era la riduzione del nitrogruppo ad ammina. Le 8-amminochinoline-3,4-disostituite dopo un primo tentativo di sintesi da un'anilina sostituita, sono state sintetizzate a partire dalla 4,7-dicloro-8nitrochinolina. La posizione 4 è stata funzionalizzata subito con un gruppo elettron-donatore (metossi o pirrolidina) e successivamente il bromo è stato introdotto nella posizione 3 con una sostituzione elettrofila aromatica. Il susseguente posizionamento della catena laterale in posizione 7

è stato seguito dalla riduzione da 8-nitro ad 8-amminochinolina. Per tutte le molecole sintetizzate gruppi diversi sono stati introdotti alle specifiche posizioni: gruppi legati tramite un atomo di carbonio, di ossigeno o di azoto. I gruppi legati tramite atomi di ossigeno o di azoto sono stati introdotti nella chinolina con reazioni di sostituzione elettrofila aromatica, usando il corrispondente alcolato o ammide. I gruppi legati tramite atomo di carbonio sono stati introdotti atteaverso reazioni di cross-coupling catalizzate da palladio, dei corrispondenti acidi boronici alchilici o arilici. Due gruppi alchinici sono stati introdotti con un coupling di Sonogashira, e successivamente ridotti ad alchili tramite idrogenazione. Tutte le molecole sintetizzate sono state testate in saggi biologici per verificarne l'attività verso l'enzima in soluzione e verso cellule che esprimono l'enzima oncogenico. Si è scoperto che il cloridrato degli inibitori è sempre piu attivo delle rispettive basi libere, percio' molti inibitori sono stati testati come cloridrati idrosolubili. Buoni risultati sono stati ottenuti, dal momento in cui lo scheletro 8-amminochinolinico è risultato attivo nell'inibire l'enzima mutato. Alcuni dei composti presentano attività nel nanomolare. Sette tra i composti piu' attivi e specifici sono anche stati testati su una serie di 85 protein-chinasi di diverso tipo (Cohen labs, Dundee) allo scopo di verificarne il loro profilo di selettività. Tre composti (CPD **131**, **106** e **148**) che posseggono attività nanomolare su Abl T315I sono scarsamente selettivi, inibendodi piu del 50% rispettivamente 10, 40 e 11 altre chinasi ad una concentrazione di 10μM. Al contrario CPD **142**, **163**, **167** and **138,** risultano essere molto selettivi, e tra i tre CPD **138** è il piu selettivo dato che inibisce solamente la chinasi Aurora A di piu del 50% alla concentrazione di 10μM. Tutti gli altri composti inibiscono pesantemente PKBβ (AKT2) che è una serin-treonin-chinasi, mentre enzimi piu simili ad Abl, come ad esempio LCK, SRC e FGFR1 sono meno inibiti da questi composti. L'Insulin receptor kinase, che è un enzima che non deve essere inibito dagli inibitori per le protein-chinasi, non è sensibile a questi composti. Interessante è la correlazione tra la selettività sulle cellule (trasdotte contro non trasdotte) e l'attività dei composti verso PKBβ : piu' elevata è l'attività su PKBβ e minore è la selettività del composto. Questo indica chiaramente che PKBβ è un target per le 8-amminochinoline.

Concludendo, un nuovo scaffold attivo per l'inbizione di Bcr-Abl WT e Bcr-Abl T315I è stato sviluppato. Composti molto attivi sia a livello enzimatico che cellulare sono stati trovati. Gli inibitori sintetizzati hanno permesso di accertare il binding-mode proposto e hanno dato una SAR coerente. Basandosi sui risultati ottenuti, è chiaro che gli inibitori 8-amminochinoloinici sono lead-compounds che possono essere ulteriormente sviluppati in un processo di ottimizzazione allo scopo di aumentarne selettività ed attività per poter iniziare degli studi *in vivo.*

Firma dello studente

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Università
Ca'Foscari
Venezia

DEPOSITO ELETTRONICO DELLA TESI DI DOTTORATO DICHIARAZIONE SOSTITUTIVA DELL'ATTO DI NOTORIETA'

(Art. 47 D.P.R. 445 del 28/12/2000 e relative modifiche)

Io sottoscritto ……Rosso Enrico…………………………………………………………………
nato a …………Mestre…………………… (prov. …VE) il ………17-08-1980…………
residente a ……Marghera……………………… in ………via d'Azeglio………………… n. …11
Matricola (se posseduta) …955387………… Autore della tesi di dottorato dal titolo:
……………………Synthesis of 8-aminoquinolines as inhibitors of tyrosine kinases…………
…………………………………………………………………………………………………
…………………………………………………………………………………………………
Dottorato di ricerca in ………Scienze Chimiche……………………………
(in cotutela con ……Università di Ginevra………………………)
Ciclo ……XXII…………
Anno di conseguimento del titolo ……2010…………

DICHIARO

di essere a conoscenza:

1) del fatto che in caso di dichiarazioni mendaci, oltre alle sanzioni previste dal codice penale e dalle Leggi speciali per l'ipotesi di falsità in atti ed uso di atti falsi, decado fin dall'inizio e senza necessità di nessuna formalità dai benefici conseguenti al provvedimento emanato sulla base di tali dichiarazioni;

2) dell'obbligo per l'Università di provvedere, per via telematica, al deposito di legge delle tesi di dottorato presso le Biblioteche Nazionali Centrali di Roma e di Firenze al fine di assicurarne la conservazione e la consultabilità da parte di terzi;

3) che l'Università si riserva i diritti di riproduzione per scopi didattici, con citazione della fonte;

4) del fatto che il testo integrale della tesi di dottorato di cui alla presente dichiarazione viene archiviato e reso consultabile via Internet attraverso l'Archivio Istituzionale ad Accesso Aperto dell'Università Ca' Foscari, oltre che attraverso i cataloghi delle Biblioteche Nazionali Centrali di Roma e Firenze;

5) del fatto che, ai sensi e per gli effetti di cui al D.Lgs. n. 196/2003, i dati personali raccolti saranno trattati, anche con strumenti informatici, esclusivamente nell'ambito del procedimento per il quale la presentazione viene resa;

6) del fatto che la copia della tesi in formato elettronico depositato nell'Archivio Istituzionale ad Accesso Aperto, ovvero depositato su supporto digitale presso la segreteria didattica del dipartimento di riferimento del corso di dottorato in due copie di cui una da trasmettere alle Biblioteche Nazionali Centrali di Roma e Firenze, è del tutto corrispondente alla tesi in formato cartaceo, controfirmata dal tutor, consegnata presso la segreteria didattica del dipartimento di riferimento del corso di dottorato ai fini del deposito

presso l’Archivio di Ateneo, e che di conseguenza va esclusa qualsiasi responsabilità dell’Ateneo stesso per quanto riguarda eventuali errori, imprecisioni o omissioni nei contenuti della tesi;

7) del fatto che la copia consegnata in formato cartaceo, controfirmata dal tutor, depositata nell’Archivio di Ateneo, è l’unica alla quale farà riferimento l’Università per rilasciare, a richiesta, la dichiarazione di conformità di eventuali copie;

Data ___4-12-2009____________ Firma ______________________________

Non Autorizzo

l’Università a riprodurre ai fini dell’immissione in rete e a comunicare al pubblico tramite servizio on line entro l’Archivio Istituzionale ad Accesso Aperto la tesi depositata per un periodo di 12 (dodici) mesi a partire dalla data di conseguimento del titolo di dottore di ricerca;

DICHIARO

1) che la tesi, in quanto caratterizzata da vincoli di segretezza, non dovrà essere consultabile on line da terzi per un periodo di 12 (dodici) mesi a partire dalla data di conseguimento del titolo di dottore di ricerca;

2) di essere a conoscenza del fatto che la versione elettronica della tesi dovrà altresì essere depositata a cura dell’Ateneo presso le Biblioteche Nazionali Centrali di Roma e Firenze dove sarà comunque consultabile su PC privi di periferiche; la tesi sarà inoltre consultabile in formato cartaceo presso l’Archivio Tesi di Ateneo;

3) di essere a conoscenza che allo scadere del dodicesimo mese a partire dalla data di conseguimento del titolo di dottore di ricerca la tesi sarà immessa in rete e comunicata al pubblico tramite servizio on line entro l’Archivio Istituzionale ad Accesso Aperto.

Specificare la motivazione:

☐ motivi di segretezza e/o di proprietà dei risultati e/o informazioni sensibili dell’Università Ca’ Foscari di Venezia.

☐ motivi di segretezza e/o di proprietà dei risultati e informazioni di enti esterni o aziende private che hanno partecipato alla realizzazione del lavoro di ricerca relativo alla tesi di dottorato.

☐ dichiaro che la tesi di dottorato presenta elementi di innovazione per i quali è già stata attivata / si intende attivare la seguente procedura di tutela:

...……………………………………….Brevetto……...…………………………………………;

☐ Altro (specificare):

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A tal fine:

- consegno la copia integrale della tesi in formato elettronico su supporto digitale presso la segreteria didattica del dipartimento di riferimento del corso di dottorato in due copie di cui una da trasmettere alle Biblioteche Nazionali di Roma e Firenze e l’altra da versare all’Archivio di Ateneo che si impegna al rispetto del periodo di embargo prima della sua pubblicazione on line nell’Archivio Istituzionale ad Accesso Aperto dell’Università Ca’ Foscari;

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- consegno la copia integrale della tesi in formato cartaceo presso la segreteria didattica del dipartimento di riferimento del corso di dottorato ai fini del deposito presso l’Archivio di Ateneo.

Data ……4-12-2009………………… Firma …………………………………………………………

La presente dichiarazione è sottoscritta dall’interessato in presenza del dipendente addetto, ovvero sottoscritta e inviata, unitamente a copia fotostatica non autenticata di un documento di identità del dichiarante, all’ufficio competente via fax, ovvero tramite un incaricato, oppure a mezzo posta

Firma del dipendente addetto …………………………………………………………