Ph.D. Dissertation of Pharmacy

Structure-Activity Relationship Investigation of Potent Human Glutaminyl Cyclase Inhibitor Based on Pharmacophoric Regions

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College of Pharmacy

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Structure-Activity Relationship Investigation of Potent Human Glutaminyl Cyclase Inhibitor Based on Pharmacophoric Regions

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Abstract

Alzheimer's disease (AD) is consider as a disorder of progressives, unremitting, neurodegenerative which cause loss of memory and severely causing cognitive ability. Until now, reduce the level of the formation of neurotoxic Aβ species in brain is consider as priority pathology of AD treatment. Recent studies show Pyroglutamate-Abeta (pE-Aβ) which express highly abundant in the brains of AD is rapid aggregation and more toxicity than Aβ. pE-Aβ is product of a substrate amino-terminally truncated Aβ beginning at glutamate 3 or 11 which catalyzed by Glutaminyl Cyclase (QC).

Recent clinical studies show that QC could be an alternative therapeutic target to treat AD. Our research group focus on investigation and development a series of QC inhibitors with an extended pharmacophoric scaffold, modification in B and C region. In this work, firstly we researched on the structure activity relationship (SAR) of analogues mimic Arg region. Most compounds in this series exhibited potent activity *in vitro*. The selected compound with $IC_{50} < 10$ nM was subjected to *in vivo* test. The result show that addition of aminoethyl group to 2-aminopyridine ring slightly improved the *in vitro* activity up to 2.5-fold. And the molecular docking studies present compound **202** as a potential candidate since it forms an additional hydrophobic interaction in the *h*QC active site.

From the aforementioned result, in part 2, we continued to develop novel QC inhibitors that contain 3-aminoaklyloxy-4-methoxyphenyl and 4-aminoalkyloxyphenyl group to replace the above designed pharmacophore. Some novel inhibitors were identified based on the IC_{50} value. They were further studied for *in vitro* toxicity and *in vivo* activity. The result showed that inhibitors **51** & **53** displayed the most potent $A\beta_{N3pE-40}$-lowering effect *in vivo* acute model with reasonable BBB penetration, without showing cytotoxicity and *h*ERG inhibition. Among two compound, we chosen compound **53** as subject of modeling analysis. The salt bridge interaction and hydrogen bonding in the active site explain its high potency. This compound having favorable BBB penetration could be serve as potential candidate for anti-Alzheimer's agents.

Last but not least, we find out that the dimethoxy group in C region is not stable and cause toxicity to liver metabolism and the modification in B region increase the *in vitro* activity. Hence, we continued to introduce novel heterocyclic in C region and the linker between B & C region together. However, compare with the parent

compound, heterocyclic in C region reduced remarkable the affect biological activity, especially the ring of indole, benzofuran, benzooxazole, benzothiazole from 2.0 to 6.0 fold. The addition of some ring to B region interestingly slightly increase the *in vitro* affect. This result suggests us valuable information for our effort in lead optimization to identify QC inhibitors with better penetration and *in vivo* activity.

Keyword: Alzheimer Disease, Glutaminyl Cyclase, Aβ, *h*QC, pyroglutamate;

Table of Contents

List of figure

List of Scheme

List of Table

1. Introduction

1. Alzheimer's Disease

Alzheimer's disease (AD) is a progressives, unremitting, neurodegenerative disorder that affects wide areas of the cerebral cortex and hippocampus[(1)]. It was firstly reported by Dr. Alzherimer in German in 1907 in 51-year-old woman with strong feeling of jealousy toward her husband and other person[(2)]. up to now, It is estimated that there were 46.8 million people worldwide living with dementia in 2015 and will be reach 3 times greater in 2050 [(3)]. It is worsen over time.

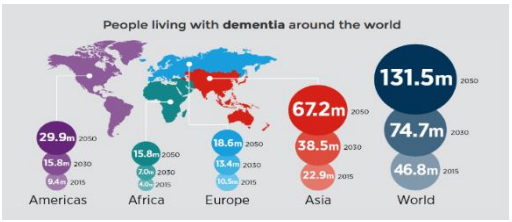


Figure 1: Estimated number of AD in 2050[(3)]

AD is officially listed as the 6th leading cause of death in US [(4)]. It is also estimated that the number of people age 65 and older with AD will nearly triple from 5.3 million to nearly 13.8 million by 2050 all over the world [(5)]. For example, Indonesia, the world's 4th most populated country, estimated in 2015 over 550,000 people with AD and will rise quarter to nearly 2.3 million in 2030. The Republic of Korea, a high income country, between 2000 and 2015, was the 4th fastest rate of ageing in the world, and have nearly 480,000 people with AD in 2015 and will be increase about triple in 2030[(3)].

1.1. Stage of AD

In its early stages, memory loss is mild, but with late-stage Alzheimer's, individuals lose the ability to carry on a conversation and respond to their environment.

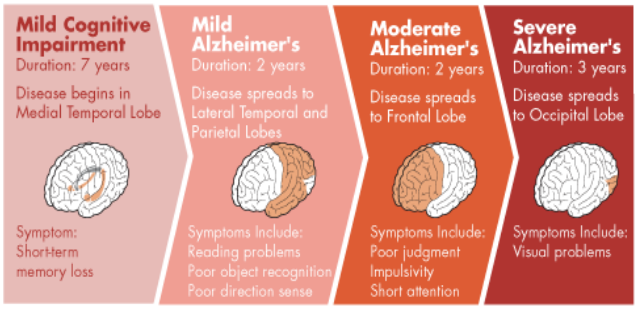


Figure 2: The progression of AD pathology and symptoms[(6)]

1.2. The risk factors of AD

There are two kind of risk factors:

- The 1st is the non-genetic risk factor such as: age, sex, alcohol consumption, depression, etc. Most AD patient are 65 and older-third of people age 85 and older have AD [7,8].
- The second factor related to the genetic one, i.e. APP, PS-1, PS-2, APOE, CTNNA3, GAB2,PVRL2,TOMM40, APOC1 which are considered as reason of AD[9]

AD treatment in present

Until now, it has no current cure, but treatments for symptoms are available, or delay its onset, and prevent it from developing. Hence, AD is the most expensive disease, take America as example, in 2017, the direct costs to American society of caring for AD patients and other dementias are nearly $259 billion. Estimating that Medicare spending on AD patients will increase about quarter in 2050, nearly $570 billion. It means one in every $3 of total Medicare spending.

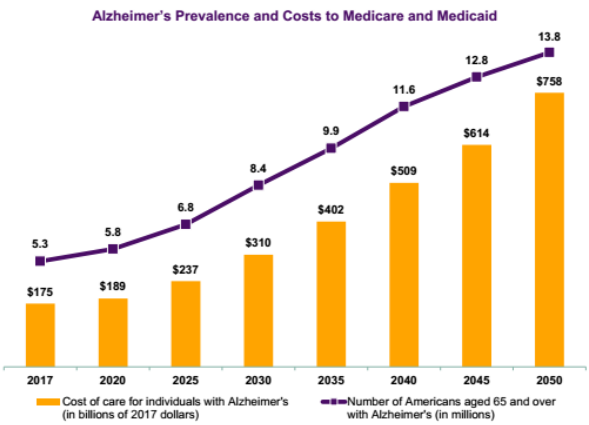


Figure 3: Alzheimer's prevalence and cost to medicare and mediaid in USA 2017 [10]

In the base opinion, the global costs of dementia increase 35.4% from $ 604 billion to $ 818 billion in 2015, nearly 1.01% of global GDP. But the most proportion of costs incurred in high income contries (nearly 80%) and other low and middle income countries is too small. The main reason for expensive spending is that there is currently no cure for AD, thus AD should be suggest to use medical support and medication in order to reduce and slow down the progression of the AD developing.

Unfortunately, up to day, in order to face with AD, it is used drug and non-drug treatment to improve cognitive and behavioral symptoms.

- For treating cognitive - FDA has approved two type of medications:

  Cholinesterase inhibitors (Donepezil (Aricept),

Rivastigmine (Exelon), Galatamine (RazadyneMenantine (Namenda)[11]

- For treating behaviors and sleep changes: mostly base on non-drug approaches before introducing medication since up to now no drug are specially approved by FDA to treat behavioral dementia symptoms.

In recent research, there is supposed that AD is thought to be related with two-prolonged attach on the brain, adding to degeneration of nature neurons, disruption of the neurogenic niches in the brain.[12]

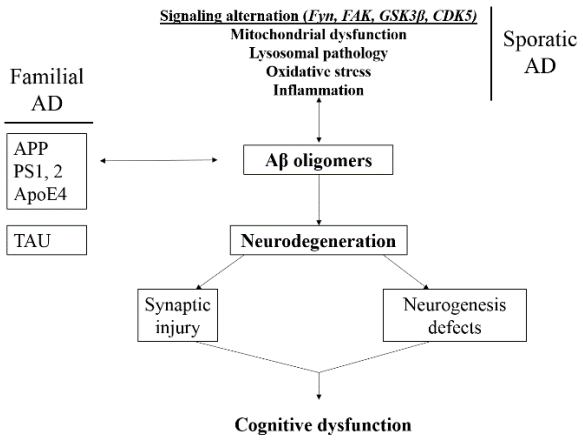


Figure 4: Factors of AD

Most of studies agreed that the accumulation of insoluble forms of amyloid-β (Aβ) in plaques in extracellular spaces, walls of bloods vessels[13, 14] and aggregation of the microtubule protein tau in neurofibrillary tangles in neurons are main factors of AD.[15,16] Microtubule-associated protein tau is a major antigenic component of paired helical filaments in Alzheimer disease[17]

2. Genetic factors in AD

2.1. Aβ in AD

Step 1: Amyloid‐β (Aβ) is cleaved from amyloid precursor protein (APP; step 1) and is released into the extracellular milieu — by a process that is unclear — as diffusible oligomers (Aβo).

Step 2: Aβo can be cleared by mechanisms that involve APOE or can be taken up by astrocytes via low‐density lipoprotein receptor‐related protein 1 (LRP1; step 2).

Step 3: Aβo can also aggregate in the intercellular space to form fibrillary constructs, which in turn assemble into plaques (step 3).

Step 4: Aβ plaques can be cleared from the brain via degradation by endocytic or phagocytic clearance (in macrophages and microglia), or by endoproteases from astrocytes (such as insulin‐degrading enzyme (IDE), neprolysin (NEP) and matrix metalloproteinase (MMP); step 4).

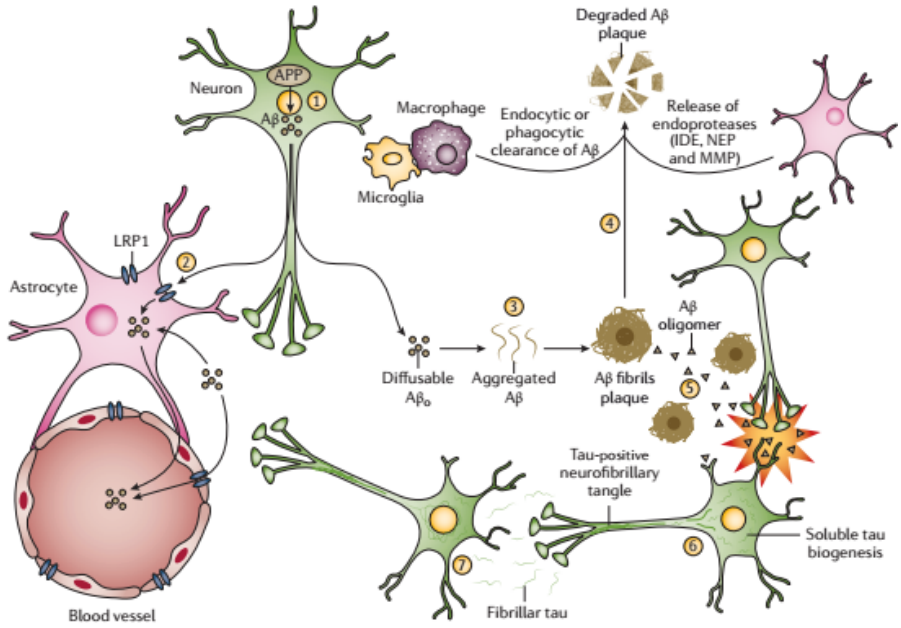


Figure 5: Pathways leading to plaques and tangles form the basis of the amyloid-β theory of Alzheimer's disease[18].

Step 5: However, some conformational oligomers that dissociate from Aβ fibrils and plaques may not be cleared and are toxic to adjacent synapses (step 5), it disrupt the signal transmit from one synapse to the other as well as immune the surrounding neuron and around blood vessels in the brain cause blood loss call CAA

Step 6: Tau damage occurs in neurons and is mediated by the development of tau- positive neurofibrillary tangles (which extend into the dendrites; step 6).

Step 7: Fibrillar tau can be released and taken up by healthy neurons, triggering tau damage in the uptaking cell (step 7). In addition, Aβ oligomers might drive α- synuclein aggregation in the plaques. Besides Aβ oligomers, mitochondrial damage or dysfunction might also be involved in the neurodegenerative process.

Hence, it is important to monitor and reduce the level of Ab in brain to cure AD.

2.2. APP in AD

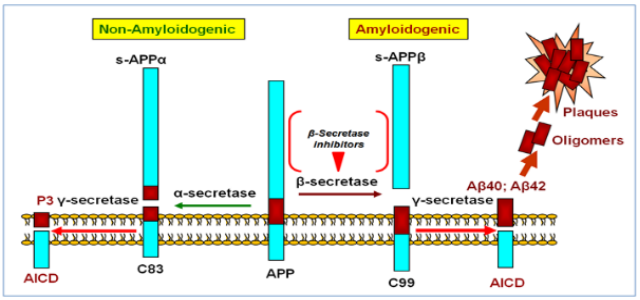


Figure 6: The process of APP[23]

APP gene is located on chromosome 21 in humans with 3 major isoforms[19]. It is suggested that protein and mRNA levels of KPI (Kunitz Protease Inhibitor)-containing APP isoforms are elevated in AD brain and associated with increase Aβ deposition[20]. APP belongs to a protein family that includes APP-like protein 1 (APLP1) and 2 (APLP2) in mammals[21, 22.]

APP, type I transmembrane protein, is synthesized in the endoplasmic reticulum (ER), transported through Golgi apparatus to the TGN (trans-Golgi-networkn)[24, 25.]

<u>α-secretase & sAPPα</u>

APP in the cell surface is cleavage by α-secretase at cleavage site (at the Lys16-Leu17 bond) to generate sAPPα – a large soluble ectodomain or αCTF[26.] or reinternalized via and endosomal lysosomal degradation[27,28]. Most of APP's normal function is mediated by sAPPα. sAPPα have role in neuronal plasticity and for the early CNS development, protective against excitotoxicity, regulate neural stem cell proliferation, inhibit stress-induced CDK5 activation, join in neuroprotective reagent-mediated excitoprotection[29, 30.]

<u>B-secretase & sAPPβ</u>

APP is cleavage at β-site location, Asp1 and Glu 11, by β-secretase, BACE1 to release sAPPβ and βCTF[31.] sAPPβ differs from sAPPα by lacking Aβ1-16 region at its carboxyl-terminus, have function as death receptor 6 ligand, mediate axonal pruning, neuronal cell death, rescue gene expression of thrasthyretin and Klotho[32, 33.]

γ-secretase & its fragments

In APP process, APP αCTF and βCTF are further cleaved by γ-secretase complex at one of several sites varying from +40 to +44 to generate Aβ peptides (1-40 & 1-42: most common)

γ-secretase & γ-processing

APP αCTF and βCTF are then cleaved by γ-secretase to obtain p83 and Aβ.

p83 is believed have no important role since degraded rapidly.

Meanwhile, overproduction of Aβ is suggest as result in neurodegenerative cascade, synaptic dysfunction, neuron loss in affected area of brain will be discussed later[34]. Meanwhile, γ-secretase activity to reside in a high molecular weight complex consisting of at least four compnents: Presenilin (PS, PS1, PS2), Niscastrin, anterior pharynx-defective1 (APH-1), Presenilin enhancer-2 (PEN-2)[35, 36.]

PS1:

+ located on chromosome 14 (14q24.3);

+ mutation in PS1 count for greater percentage of EOAD case about 18-50%[37]

+ can be substantial variation in age of on set (mean 45.5 years old)

+ severity of disease (after diagnosis: live about 8.4 year more)

+ mutation in PS1 could cause the secretase activity of γ-secretase and increase the ratio of Aβ42 to Aβ40.

PSEN 2:

+ located on chromosome 1(1q31-q42)

+ PSEN2-related AD is rare compared PSEN1

+ higher age of onset (about 53.7 year old), live longer after diagnosis (10.6 years)

2.3. TAU IN AD

Tau is a cytosolic protein encoded by a gene on chromosome 17 (17q21),have > 100kb and 16 exons

Normally, Tau proteins interact with tubulin, make microtubules stable and promote the assembly of tubulin into microtubules. They are the product of alternative splicing from a single gene call "MAPT" (microtubule-associated protein tau). When tau proteins are defective, they no longer stabilize microtubules properly. They are capable of aggregating and fibrillating to form NFTs, another pathological hallmark of AD

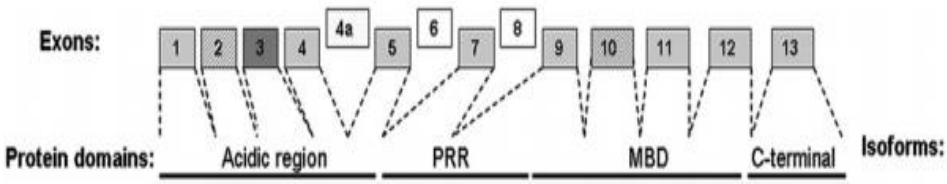


Figure 7: Structure of TAU[39]

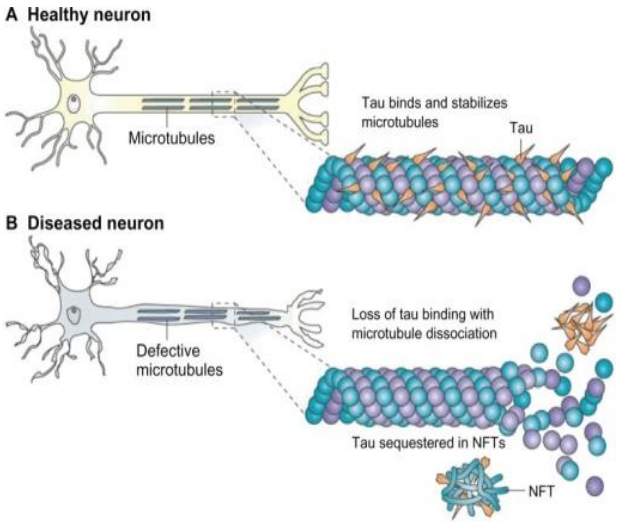


Figure 8: TAU in AD[40]

Moreover, amyloid fibril formation alters the phosphorylation state of tau, induced the concomitant activation of MAP kinase and GSK3 beta, resulting in the loss of microtubule binding capacity and somatodendritic accumulation, properties also exhibited by tau in the AD brain. Hence, it may therefore be an abnormal phosphorylation of tau and neuritic degeneration in AD[41, 42.]

3. QC in AD

3.1. Pyrutamate in AD

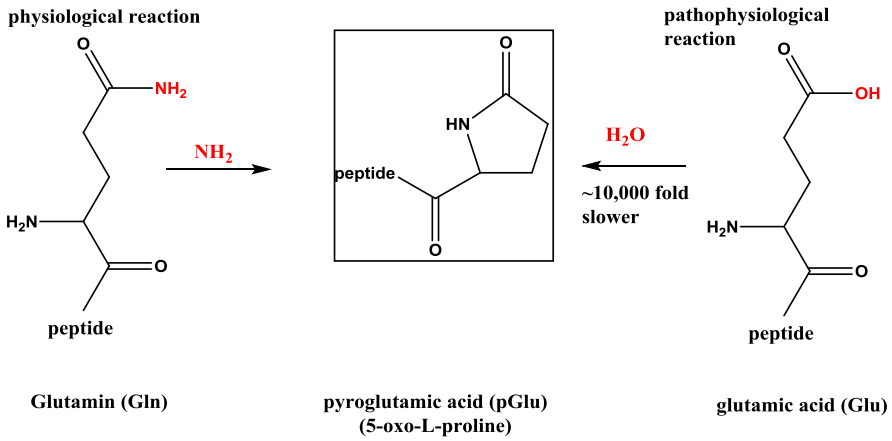


Figure 9: Pyrogulatame formation under enzyme Glutaminyl Cyclase[43]

Pyroglutamate-Abeta (pE-Aβ), hightly abundant in the brains of AD are type of *N*-truncated Aβ forms containing an *N*-terminal pyroglutamate at position 3 or 11 in Aβ. Due to their increase hydrophobicity, pE-Aβ product are prone to rapid aggregation

and are much more resistant to proteolytic degradation and causing exacerbated neurotoxicity. Moreover, pE-Aβ is considered more neurotoxic than $Aβ_{1-40}$ and $Aβ_{1-42}$ and promote the formation of amyloid and tau plaques. The formation of the pyroglutamate from the *N*-terminal glutamate of Aβ is catalyzed by glutaminyl cyclase (QC)

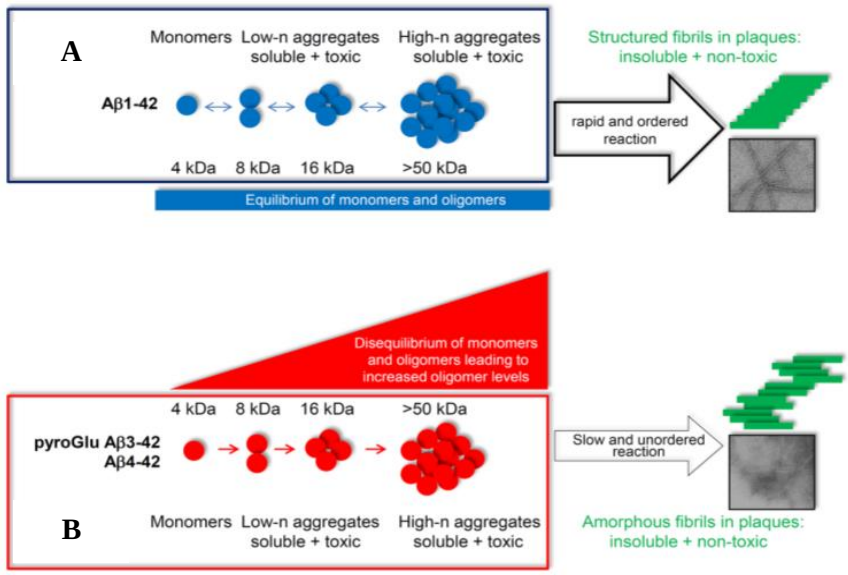


Figure 10: Toxicity of soluble pE-Aβ and Aβ

N-truncated pyroglutamate Aβ3–42 are more toxic as compared to full-length Aβ1–42 due to reduced neutralization via plaque formation (figure 10)[44]. Figure 10A Monomers and low- and high-molecular weight aggregates of Aβ1–42 (blue) are in equilibrium and are toxic as long as they stay soluble Once high-molecular weight aggregates are formed, they rapidly react into highly ordered and insoluble, non-toxic fibrils Aβ found in plaques. Therefore, although soluble low- and high molecular weight oligomers are toxic, it can be non-toxicity by forming monomers and/or fibrils. As Aβ1–42 is a physiological peptide, which is continuously generated also in healthy individuals, plaque formation may be one way to neutralize full-length Aβ during the prodromal stage of the disease. Meanwhile, figure 10B displayed soluble monomers, low- and high-molecular weight aggregates of N-truncated pyroglutamate Aβ3–42 are in disequilibrium and are toxic. High-molecular weight aggregates also can be neutralized by plaque formation to be nontoxic. However, the process is significant

slower tendency as compared to full-length Aβ, because the fibrillation process is unordered forming only amorphous fibrils. As a result, the level of soluble low- and high-molecular weight aggregates of N-truncated Aβ variants increase over time.

Hence, the novel pE-Aβ has altered biochemical properties with severe pathological consequences. Such as increase hydrophobicity; neurological deficits as show in figure 11; thereby pE-Aβ playing a major role in AD. Therefore, if GC was inhibited, the pE-Ab formation will be decreased

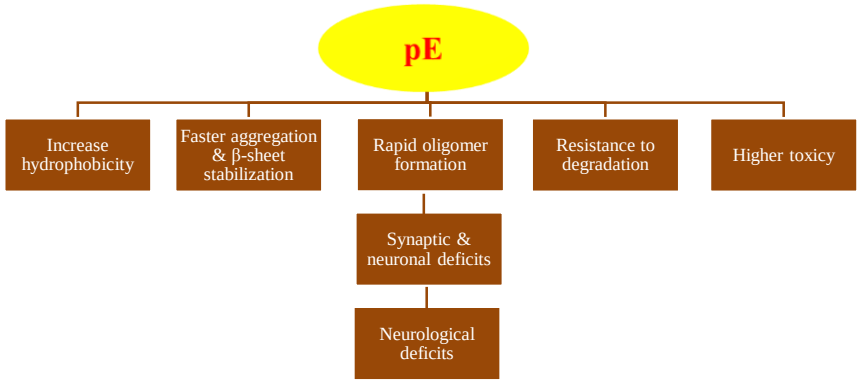


Figure 11: Severe pathological consequence of Aβ

3.2. Glutaminyl Cyclase (QC)

QC (QCs, EC 2.3.2.5) have been identified in both animal and plant such as[45-47]. GC is widely distributed in mammalian brain with expression in hippocampus and cortex[48-49].

Two types of QCs have been defined thus far. Type I QCs were found in plants and in several pathogenic bacteria and human parasites, while type II QCs were mainly identified in the neuroendocrine tissues of mammals[50-52].

Papaya QC (pQC) is the best-known type I QC. This enzyme was first discovered in the latex of the tropical plant Carica papaya[53].

Structure of human QC

Overall Structure. The mature domain (residues 33–361) of human QC was shown to possess glutaminyl and glutamyl cyclase in physiological substrate of *h*QC. **Structure of *h*QC**. (*A*) A ribbon diagram of the overall structure of human QC. The central six -strands are colored orange. The helices located on the top, bottom, and

edge are colored cyan, magenta, and yellow, respectively. The zinc ion is shown as a yellow sphere. The zinc-coordinated residues, Arg-54 (genetic mutation to Trp residue occurred frequently in adult women with osteoporosis), and a sulfate ion are depicted with a ball-and-stick model. The coils and loops adjacent to the catalytic center are painted green, whereas those distant from the active site are colored gray. Gray dots represent the disordered region of residues 183–188. (*B*) A topology diagram of the human QC structure. The color codes for secondary structural elements are identical to those in *A*. (*C*) A stereo-view of the human QC catalytic region. The active-site residues in conformation-A are shown and labeled. Possible hydrogen and coordination bonds are represented with dotted lines colored cyan and yellow, respectively. The green dotted lines depict the possibly unusual hydrogen bonds between D305 and E201 (3.06 Å) and between D305 and D248 (2.53 Å).[54]

In the past, there are two possible mechanism of *h*QC catalysis.

First, the catalysis of the formation of a covalent intermediate could happen. At the beginning, a nucleophilic residue attacks the γ-amide group of N-terminal glutaminyl form acyl-enzyme intermediate and release ammonia. Then α-amino group of glutamine attack nucleophile the γ-carbonyl.

The second proposal ones is based on non-covalent reaction catalyzied by *h*QC.

Catalytic cycle of *h*QC catalyzing N-terminal glutaminyl substrates. First step is the formation of the Michaelis-Menten complex via binding of the substrate. Thereby it showes the coordinated water molecule and occupies the fourth coordination site of the catalytic zinc. The catalytic Zn ion acts as a Lewis acid, pulls out electrons from the γ-carbonyl moiety of the N-terminal glutamine, hence activating the γ-carbonyl carbon electrophile. Moreover, Glu201 activates via acid-base catalysis the α-amino group, which in turn gets more nucleophilic. Afterwards, the α-amino group performs a nucleophilic attack on the γ-carbonyl carbon, forming to a short-lived tetrahedral intermediate. Then, an intrinsic proton transfer to the potential leaving group via a conserved hydrogen bond network is performed to subsequently release ammonia and the product.

More detail, Huang et al. also propose detail substrate specificity of *h*QC and its structural relationship.

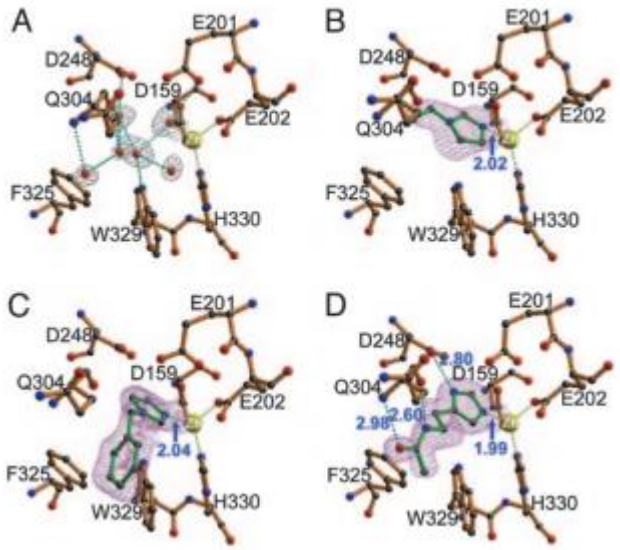


Figure 12: Structures of human QC bound to imidazole-derived inhibitors.

The Zn-binding environment of the free-form human QC. The 2*Fo*-*Fc* electron density maps (contoured at 1.0) (gray) corresponding to the water molecules inside the active-site pocket are shown. Representations of the models, hydrogen bonds, and coordination bonds are identical to those in Fig. 1*C*. (*B–D*) Structures of *h*QC bound to 1-vinylimidazole (1.68-Å resolution), 1-benzylimidazole (1.64-Å resolution), and *N*--acetylhistamine (1.56-Å resolution), respectively. The 2*Fo*-*Fc* maps (contoured at 1.0) (magenta) for the inhibitors are overlaid with the final refined models. Distances for enzyme–inhibitor interaction are indicated in Å.

The conserved Glu-201 plays as the general base and acid to transfer a proton from the -amino group of the substrate (blue) to the leaving amino group on the scissile -amide. The zinc ion polarizes the -amide carbonyl group of the substrate and simultaneously

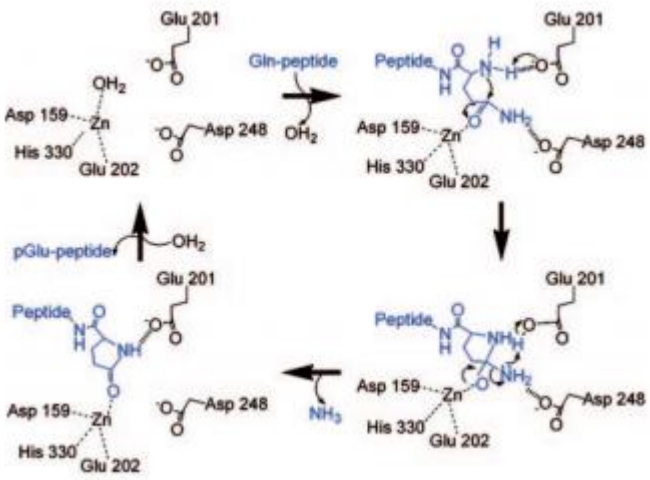


Figure 13. Proposed catalysis mechanism of human QC.

stabilizes the oxyanion formed by the nucleophilic attack of the -nitrogen. Asp-248 probably stabilizes the leaving -amide amino group during the catalysis process. In the mechanism of glutamyl cyclase activity, this leaving amino group is replaced by a hydroxyl group, and the reaction is good at pH 6.0 (Figure 13).

QC have important role in the production of $A\beta_{pE3}$, i.e: injection of $A\beta_{3-40}$ cause to significant level of $A\beta_{pE3-40}$[50]. Beside, in order to study the effect of ectopic *h*QC overexpression, 5XFAD mice was crossed with the transgenic mice expressing *h*QC 5XFAD/*h*QC bigenic mice showed remarkable increased levels of TBS-, SDS- and formic acid soluble $A\beta_{pE3-42}$. Effect of endogenous QC was verified by generating 5XFAD/QC-KO mice. 5XFAD/QC-KO mice showed a worthy reduction in $A\beta_{pE3-42}$ levels[55, 56].

Involvement of hQC activity in the formation of pyroglutamyl peptides. By the inhibition of QC in neuronal cellsof the central nervous system of AD patients would suppress the formation of N-terminal of N-terminal glutamyl cyclization. Thus, QC is a key factor in monitor $A\beta_{pE3-x}$ level *in vivo*.

3.3. Some QC inhibitors – promising treatment strategy

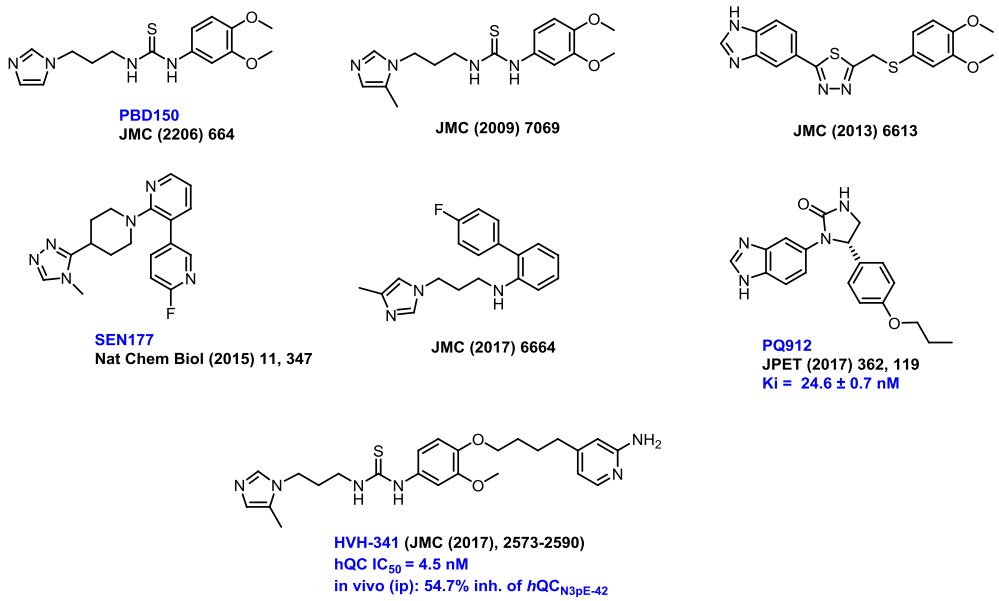


Figure 14: Reported QC inhibitors

As mentioned, in present QC is becoming promising strategy in treatment of AD. Take PQ-912 as example, the product is developed by Probiodrug, has well tolerated and metabolically stability for patients[57]. Base on the good result, it is now in phase II of clinical[58]. Also our previous researched compound HVH 341[59] has potent *in vitro* activity. The two different transgenic model mice of AD, APP/PS1, and 5xFAD showed that it not only reduced the brain concentration of pE-Aβ and total Aβ in APP/PS1 mice but also restored cognitive function in 5xFAD mice.

1. Design and Pharmacophore

Previously reported QC inhibitors have three pharmacophores designated the A-, B- and C-regions, as shown in **Figure 1.1**.[60, 61] The A-region contains a zinc-binding motif (ZBM), the B-region contains a hydrogen bond donor, and the C-region contains an aromatic ring that mimics the Phe side chain at the penultimate position to the *N*-terminus of the substrate $A\beta_{3E\text{-}42}$. Inspired by these findings, our group previously investigated a series of QC inhibitors with an extended scaffold based on the *N*-terminal tripeptide (Glu-Phe-Arg) of $A\beta_{3E\text{-}42}$, and identified an additional pharmacophore, the D-region, which mimics the binding interaction of the guanidine moiety of Arg.[59] The newly developed QC inhibitors display improved potency, 5 to 40-fold increases, compared to the previously reported inhibitor **1**. According to our molecular modeling studies, the Arg mimetic D-region forms strong interactions with the carboxylate group of Glu327, supporting our hypothesis that the additional pharmacophore provides an extra binding interaction.

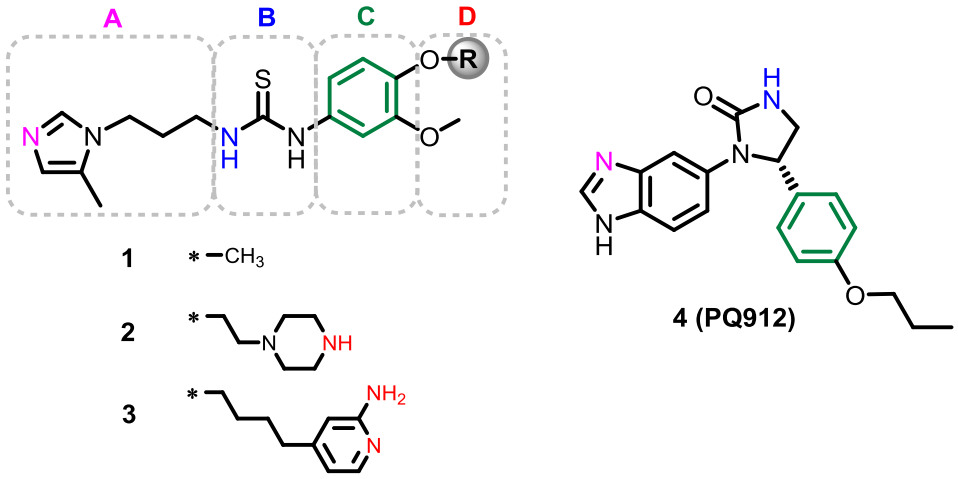


Figure 1.1: Representative structures of QC inhibitors

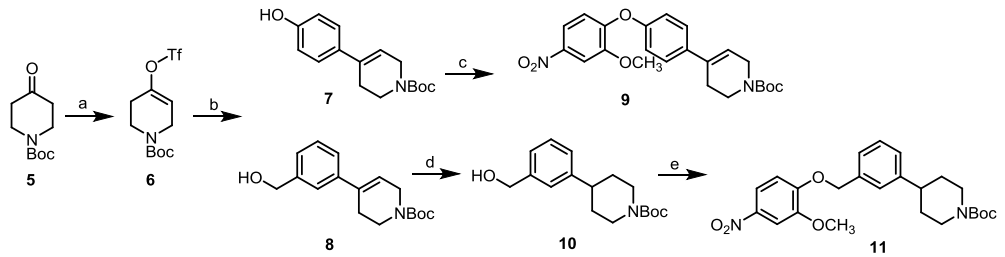
Although compound **2** was the most potent inhibitor in our previous study (IC_{50} = 0.7 nM for *h*QC), it displayed moderate efficacy in an in vivo model, likely due to the low blood-brain barrier (BBB) penetration, whereas compound **3** showed a better in vivo efficacy, reducing the brain concentrations of Aβ and restoring cognitive functions in AD mice. Based on these findings, we aim to develop a library of D-region-modified analogues with improved potency and BBB penetration. All compounds in this series have the same scaffolds in the A-, B-, and C-regions, but they contain various moieties, including substituted piperazines, 2-aminopyridines,

anilines, and phenyl group derivatives, in the D-region. We also synthesized a group of compounds that contain a phenyl and a benzyl linker group between the C- and D-regions to study the conformational effect of modifications at this specific position. We evaluated the QC inhibitory activity in vitro and the in vivo activity of several selected compounds; we also analyzed the specific binding interactions between the selected inhibitor and the QC active site by performing molecular docking studies.

2. Result and discussion

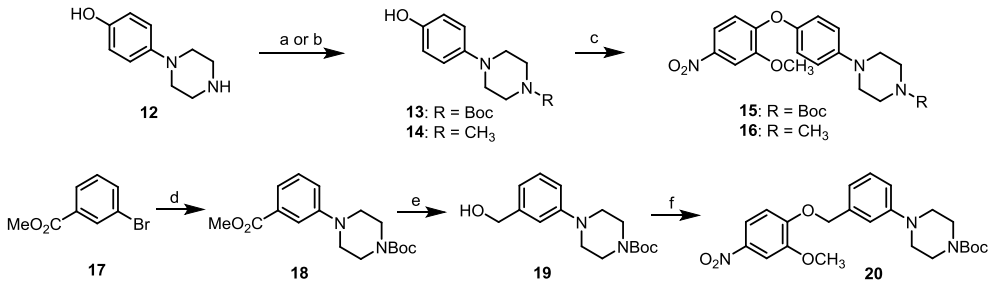
2.1. Chemistry

A library of 46 compounds with modifications in the D-region was synthesized. We first synthesized the C/D-region, 4-alkyl(or aryl)oxy-3-methoxyaniline fragment, and then coupled them to 3-(5-methyl-1*H*-imidazol-1-yl)propan-1-amine, which represents the A-region, to obtain the final compounds.



Scheme 1.1. Synthesis of piperidine derivatives. Reagents and conditions: (a) $PhTf_2$, LDA, THF, -78 °C, overnight; (b) phenylboronic acids, $Pd(PPh_3)_4$, MeCN, Na_2CO_3, reflux, overnight; (c) 2-bromo-5-nitroanisole, Cs_2CO_3, TMEDA, CuI, DMF, 90 °C, 24 h; (d) H_2, Pd/C, MeOH, rt, 2 h; (e) 4-nitroguaiacol, DEAD, PPh_3, DCM, r.t., 3 h.

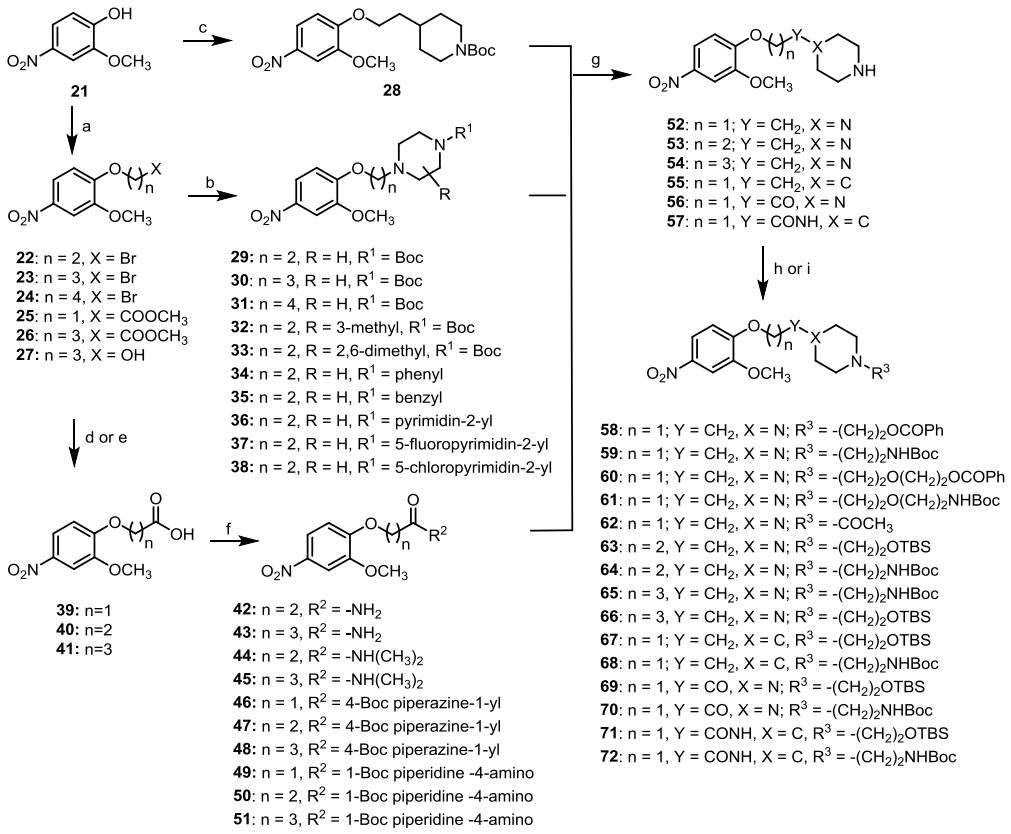
For the synthesis of the 4-phenylpiperidine D-region fragments (**Scheme 1.1**), the protected piperdin-4-one (**5**) was converted to the corresponding triflate **6**,[62] which underwent the Suzuki coupling reaction with phenylboronic acid derivatives to afford compounds **7** and **8**, respectively.[63] The phenol **7** was reacted with 2-bromo-5-nitroanisole through the Ullmann reaction to generate the 4-phenyltetrahydropyridine **9**.[64] The double bond of compound **8** was carefully reduced to piperidine **10** and then coupled with 4-nitroguaiacol in the Mitsunobu reaction to afford 4-phenylpiperidine **11**.



Scheme 1.2. Synthesis of piperazine derivatives. Reagents and conditions: (a) Boc_2O, DCM, r.t., overnight; (b) HCHO, HCOOH, reflux, overnight; (c) 2-bromo-5-nitroanisole, Cs_2CO_3, TMEDA, CuI, DMF, 90 °C, 24 h; (d) *t*-butyl piperazine-1-carboxylate, BINAP, $PhCH_3$, $Pd(OAc)_2$, NaOtBu, 100 °C, 15 min; (e) $LiAlH_4$, THF, 0 °C, 1 h; (f) 4-nitroguaiacol, DEAD, PPh_3, DCM, r.t., 3 h.

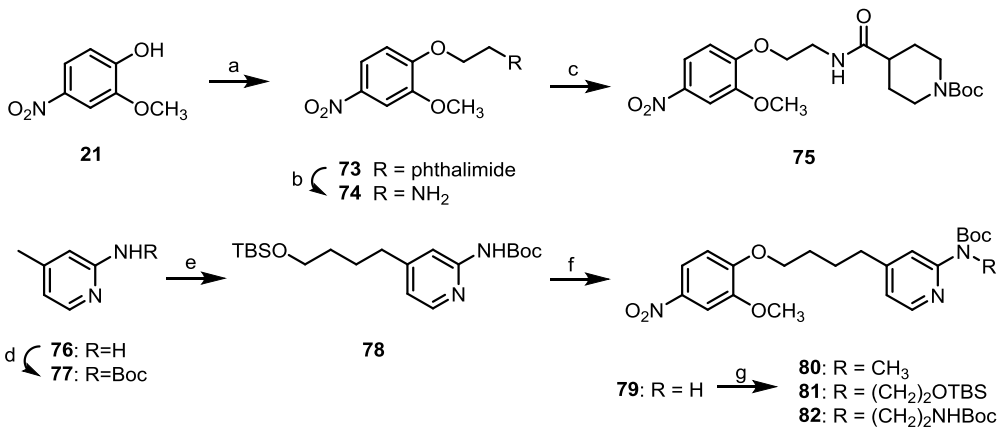
For the synthesis of 4-phenylpiperazine fragments (**Scheme 1.2**), 4-(piperazin-1-yl)phenol **12** was protected by a Boc group or reductively methylated to yield compound **13** or **14**, respectively, which underwent the Ullmann coupling reaction with 2-bromo-5-nitroanisole to afford compounds **15** and **16**. Methyl 3-bromobenzoate **17** was reacted with *N*-Boc piperazine under Buchwald-Hartwig conditions[65] to yield compound **18**, whose ester underwent reduction followed by the Mitsunobu reaction with 4-nitroguaiacol to produce compound **20**.

For the synthesis of 4-alkylpiperidine, 4-alkylpiperazine and 4-amidoalkyl D-region fragments (**Schemes 1.3**), 4-nitroguaiacol **21** was condensed with 1-Boc-4-(2-hydroxyethylpiperidine) using the Mitsunobu reaction to yield 4-ethylpiperazine derivative **28**. The Williamson reaction of compound **21** with dibromoalkanes followed by *N*-alkylation with the corresponding piperazine derivatives produced 4-alkylpiperazine derivatives **29**-**38**, respectively. For 4-oxopiperidine and piperazine derivatives, the acids (**39** and **41**) were obtained by the hydrolysis of corresponding esters (**25** and **26**), which were prepared by the O-alkylation of compound **21**. Meanwhile, acid **40** was synthesized from the corresponding alcohol **27** through the unwanted β-elimination of 3-bromopropanoate during the O-alkylation with compound **21**. The acids (**39**-**41**) were converted to acyclic and cyclic amides (**42**-**51**) by coupling with the corresponding amines, respectively. The *N*-Boc piperidine and piperazine derivatives (**28**-**31**, **46**, and **49**) were deprotected to provide the corresponding amines (**52**-**57**), which underwent *N*-alkylation or *N*-acetylation to afford compounds **58**-**72**, respectively.



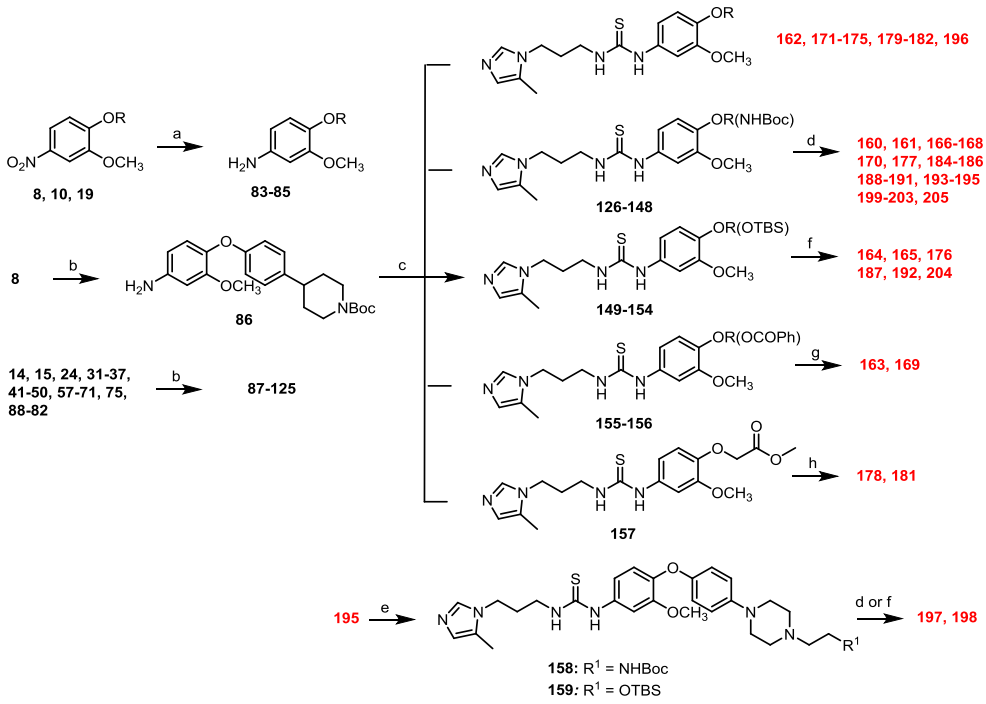
Scheme 1.3. Synthesis of 4-alkylpiperidine, 4-alkylpiperazine and 4-amidoalkyl D-region fragments Reagents and conditions: (a) $Br(CH_2)_nBr$ or $Br(CH_2)_3OH$ or $Br(CH_2)_nCOOCH_3$, Cs_2CO_3, DMF, 100 °C, 30 min; (b) piperazine derivatives, Cs_2CO_3, DMF, 70 °C, 30 min; (c) 1-Boc-4-(2-hydroxyethyl)piperazine, DEAD, PPh_3, DCM, r.t., overnight; (d) NaOH, MeOH, reflux, 1 h; (e) $K_2Cr_2O_7$, H_2SO_4, acetone, H_2O, r.t., overnight; (f) EDC·HCl, HOBt, DCM, NH_3 (or $NH(CH_3)_2$·HCl, 1-Boc-4-piperidinamine, 1-Boc piperazine), MC, r.t., overnight; (g) TFA, DCM, r.t., overnight; (h) RBr, Cs_2CO_3, DMF, 100 °C, 30 min; (i) acetyl chloride, TEA, DCM, r.t., 1 h (for compound **62**).

The 4-carboxamidopiperidine fragment **75** was synthesized from compound **21** in 3 steps (**Scheme 1.4**). For the syntheses of 4-alkyl-2-aminopyridine fragments (**Scheme 4**), 2-amino-4-picoline **76** was protected and then alkylated with *O*-TBS 3-bromopropanol to generate compound **78**. After deprotection of compound **78**, the Mitsunobu reaction was performed with 4-nitroguaiacol followed by *N*-alkylation with the corresponding alkyl iodides to generate compounds **80-82**, respectively.



Scheme 1.4. Synthesis of 4-carboxamidopiperidine fragment and 2-aminopyridyl moiety. Reagents and conditions: (a) 2-(2-bromoethyl)isoindoline-1,3-dione, K_2CO_3, DMF, 100 °C, 30 min; (b) $N_2H_4 \cdot H_2O$, EtOH, r.t., overnight; (c) 1-Boc piperidine-4-carboxylic acid, EDC·HCl, HOBt, DCM, r.t., overnight; (d) Boc_2O, t-BuOH, r.t., overnight; (e) $Br(CH_2)_3OTBS$, n-BuLi, THF, -78 °C; (f) TBAF, THF, r.t., 2 h, then 4-nitroguaiacol, DEAD, PPh_3, DCM; (g) RI, Cs_2CO_3 (or NaH), DMF, heat.

The synthesis of the final compounds is described in **Scheme 1.5**. The 4-nitroguaiacol fragments prepared above were reduced by either reacting them with zinc powder in acidic medium or hydrogenation to yield the corresponding anilines **83-125**, respectively. All synthesized anilines were coupled *in situ* with 3-(5-methyl-1H-imidazol-1-yl)propan-1-amine[66] via isothiocyanate to provide the final thioureas **162, 171-175, 179-182,** and **196**, as well as the precursors that were converted to the final compounds **160-161, 166-168, 170, 177, 184-186, 188-191, 193-195, 199-203,** and **205** by Boc deprotection, **164-165, 176, 187, 192,** and **204** by TBS deprotection and **163** and **169** by benzoyl deprotection, respectively. The amide final compounds **178** and **181** were synthesized from ester **157** by condensation with the corresponding amines. The 1-alkyl-4-phenylpiperazines containing the final compounds **197-198** were prepared from compound **195** by *N*-alkylation followed by deprotection.



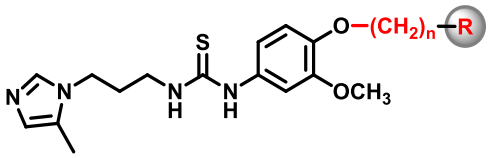
Scheme 1.5. Synthesis of final compounds. Reagents and conditions: (a) Zn, MeOH, AcOH, r.t., 2 h; (b) Pd/C, H_2, MeOH, r.t., 3 h; (c) 3-(5-methyl-1*H*-imidazol-1-yl)propan-1-amine, TCDI, TEA, DCM, r.t., overnight; (d) TFA, DCM, r.t., overnight; (e) RBr, NaH, DMF, 0 °C to 100 °C, 30 min; (f) HCl, MeOH, r.t., overnight or TFA:H_2O (9:1), DCM, r.t., overnight; (g) NaOH, MeOH, H_2O, reflux, 30 min; (h) NH_3 or $NH(CH_3)_2$, MeOH, rt, overnight.

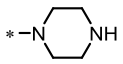
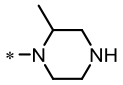
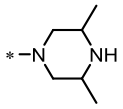
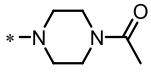
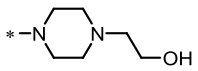
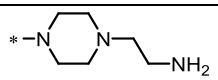
2.2. *In vitro* assay

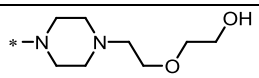
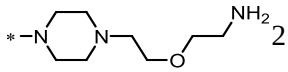
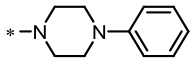
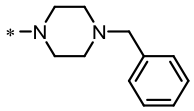
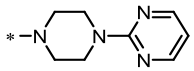
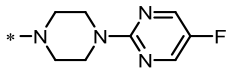
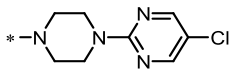
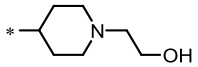
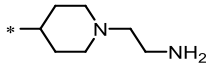
We performed QC activity assays using a fluorogenic substrate, Gln-AMC (L-glutamine 7-amido-4-methylcoumarin), and pyroglutamyl peptidase (pGAP) as an auxiliary enzyme[67] to evaluate the ability of the D-region-modified library to inhibit QC. We first investigated a group of compounds containing the modified piperazine ring in the D-region and summarized their structures and *in vitro* inhibition as Group I in **Table 1**. The incorporation of a methyl group at the 2- or 3-position of the piperazine (**160** and **161**) led to a slight reduction in activity, probably due to steric hindrance. Among the 4-*N* substituted piperazine analogues (**162-175**), compounds with a relatively small sized substituent displayed slightly better activity than

compounds with aromatic and heteroaromatic rings. Specifically, compounds with an alkylamine substituent (**167** and **170**) appeared to be the most potent of the Group I compounds, with IC_{50} values of 3.8 and 3.6 nM, respectively, suggesting that their terminal amino groups may be involved in an additional ionic interaction. When we varied the length of the spacer between the piperazine substituent and the C-ring oxygen (compounds **163-168**, n = 1 to 3), we did not observe any particular trend in the inhibitory effect, suggesting that specific interactions, such as the ionic interaction inside the binding pocket, may be more important than steric effects. The 4-phenyl (**171**), benzyl (**172**) and pyrimidine (**173-175**) derivatives showed moderate inhibition (IC_{50} = 12 to 21.6 nM), and the introduction of a halogen at the 5-position of the pyrimidine further reduced the activity. The two piperidine surrogates (**176** and **177**) were slightly less active than the corresponding piperazine derivatives (**163** and **166**).

Table 1.1. IC_{50} values for the inhibition of *h*QC by Group I (piperazine and piperidine) compounds



Compound	R	n	IC_{50} (nM)[a]	
1	*–Me	0	29.2[b]	
2		2	0.7[c]	
160		2	5.8	(±1.0)
161		2	9.9	(±0.7)
162		2	30.8	(±4.2)
163		2	7.4	(±1.2)
164		3	23.5	(±9.8)
165		4	4.8	(±1.0)
166		2	7.5	(±6.8)
167		3	3.8	(±1.9)

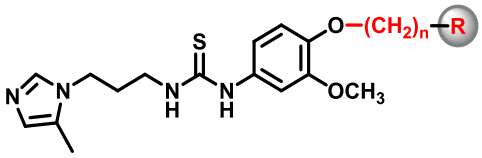
168		4	8.6	(±0.6)
169		2	17.3	(±3.1)
170		2	3.6	(±1.6)
171		2	21.6	(±5.0)
172		2	12.7	(±2.9)
173		2	12	(±3.2)
174		2	40.3	(±24)
175		2	66.8	(±45.4)
176		2	9.0	(±3.7)
177		2	10.6	(±2.2)

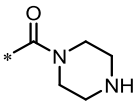
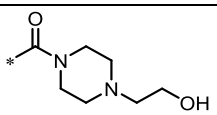
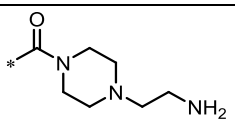
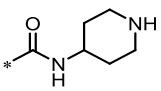
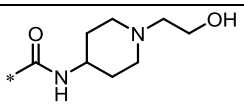
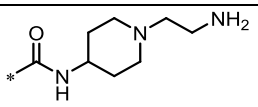
[a] Values indicate the means of at least three experiments; [b]

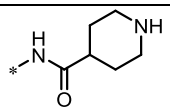
Next, we examined the amidoalkyl derivatives in the D-region since the aminoalkyl derivatives showed potent inhibitory activity in our previous study.[59] As described in **Table 1.2**, compounds with a primary amide (**178**-**180**), tertiary amide (**181**-**183**) and piperazinyl amide (**184**-**186**) appeared to be less potent than the previously reported aminoalkyl derivatives, probably due to the decreased basicity of the amide nitrogen that is thought to be involved in the salt bridge interaction with the enzyme. The amide surrogate of 4-aminoethylpiperazinyl derivative (**188**) exhibited a comparable activity to the compound without the amide group (**166)** because both compounds contain the terminal amine. Among the *N*-(piperidin-4-yl)amido derivatives (**189**-**194**), the *N*-(aminoethyl)piperidinyl derivative (**193**) was the most potent in the series, with an IC_{50} of 4.5 nM. In addition, (piperidin-4-yl)carbamoyl derivative **190** exhibited comparable activity, even without the 4-aminoethyl group (IC_{50} = 5.5 nM), whereas its reverse amide (**194**) showed reduced inhibition (IC_{50} = 15.7 nM). Interestingly, the length of the spacer between the C-

region oxygen and the D-region (n = 1 to 3) appears to affect the inhibitory activity of compounds within this series; compounds with the ethylene spacer (n = 2) generally displayed better potency, likely due to the location of the amide nitrogen.

Table 1.2. IC_{50} values for the inhibition of *h*QC by Group II (amido compounds)



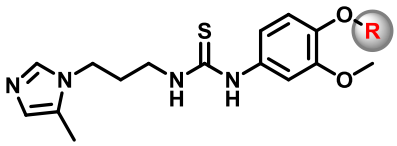
Compound		n	IC_{50} (nM)[a]	
178		1	89.7	(±17)
179		2	19.0	(±11.0)
180		3	53.8	(±5.7)
181		1	117	(±19.4)
182		2	35.4	(±4.0)
183		3	51.0	(±4.0)
184		1	15.0	(±2.5)
185		2	7.7	(±1.8)
186		3	7.0	(±1.1)
187		1	48.7	(±3.5)
188		1	7.8	(±4.1)
189		1	10.3	(±1.5)
190		2	5.5	(±0.2)
191		3	16.7	(±1.1)
192		1	17.7	(±6.0)
193		1	4.5	(±4.8)

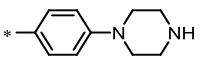
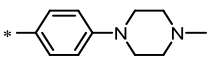
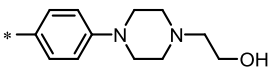
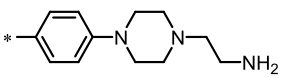
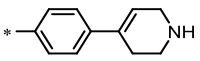
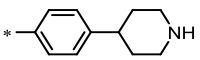
194		2	15.7	(±2.0)

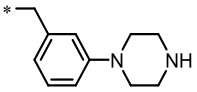
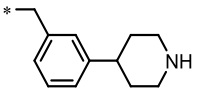
[a] Values indicate the means of at least three experiments.

Next, we modified the linker that connects the C- and the D-regions to confer a rigid conformation and evaluated the inhibitory activity of these derivatives (**Table 1.3**). In general, compounds in this series displayed slightly decreased activity compared to the compounds with a flexible linker. The piperidine analogue **195** and the piperazine analogues **199**, **200**, and **202** displayed similar inhibitory activities. Compounds with a benzyl linker (**201** and **202**) showed a comparable activity to the compounds with a phenyl linker. Overall, the conformational rigidity between the two regions did not appear to have a significant impact on QC inhibition.

Table 3. IC_{50} values for the inhibition of *h*QC by Group III compounds



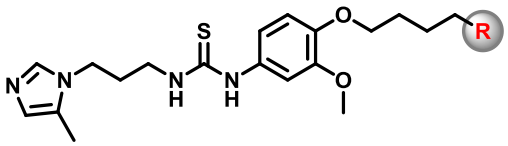
Compound	R	IC_{50} (nM)[a]	
195		8.5	(±0.5)
196		17.0	(±11.4)
197		16.1	(±8.3)
198		12.0	(±6.4)
199		9.4	(±3.4)
200		9.9	(±1.7)

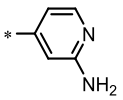
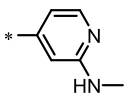
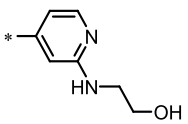
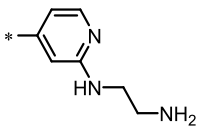
201		11.5	(±4.1)
202		6.2	(±2.9)

[a] Values indicate the means of at least three experiments.

Finally, we evaluated the inhibitory activity of analogues containing the 2-aminopyridine group containing and summarized these results in **Table 1.4**. The 2-aminoethylamino substituent in compound **205** improved the inhibitory activity (IC_{50} = 1.8 nM) compared to its parent compound **3** (IC_{50} = 4.2 nM), which was also observed with the piperidine derivatives. In contrast, the 2-methylamino pyridine derivative (**203**) and the 2-hydroxyethylamino pyridine derivative (**204**) exhibited slightly decreased activity compared to compound **3**.

Table 1.4. IC_{50} values for the inhibition of *h*QC by Group IV compounds



Compound	R	IC_{50} (nM)[a]	
3		4.2[b]	
203		13.4	(±5.5)
204		8.7	(±3.4)
205		1.8	(±0.7)

[a] Values indicate the means of at least three experiments; [b] Ref.[31]

2.3. In vivo activity

Based on the *in vitro* QC inhibition data, we selected 20 compounds with IC_{50} values less than 10 nM for further *in vivo* studies. We first screened these compounds at one fixed concentration (10 μM) in an immortalized hippocampal neuronal cell line (HT-22) to evaluate cytotoxicity, and found that none of the compounds, with the exception of compound **170**, were cytotoxic. We successively injected human $A\beta_{3\text{-}40}$ (5 μg) and each compound (25 mg/kg) into deep cortical/hippocampal tissues of ICR mice (male, six weeks old) by intracerebroventricular (icv) administration to assess the *in vivo* activity of the selected compounds. We measured the levels of human $A\beta_{N3pE\text{-}40}$ in the brain extracts of these mice on the next day to determine the QC inhibitory activity. As described in **Table 1.5**, compounds **185**, **190**, **199** and **202** appeared to suppress the formation of $A\beta_{N3pE\text{-}40}$ by 13.5% to 30% compared to the vehicle control. In particular, compound **202**, which showed the potent inhibition *in vitro* with an IC_{50} value of 6.2 nM, exhibited the most potent $A\beta_{N3pE\text{-}40\text{-}42}$ lowering effects (30%). Because compound **202** contains a benzylic linker with a piperidine moiety, this potent *in vivo* activity may be attributed to its high BBB penetration.

We performed a parallel artificial membrane permeability assay (PAMPA)[68] that can be translated to the ability of the compounds to penetrate the blood-brain barrier (BBB). The four most active compounds *in vivo* **185**, **190**, **199**, and **202**, showed reasonable permeability, with a range of 4.9-5.8 for –logPe, supporting the hypothesis that the *in vivo* activity of these compounds resulted from good BBB penetration and QC inhibition. In contrast, the compounds that showed potent *in vitro* activity but were ineffective *in vivo*, such as **167**, **168**, **188**, **204** and **205**, exhibited very low permeability (–logPe = 10).

We performed an *h*ERG channel assay for all compounds to assess potential drug toxicity. Although compounds **185** and **190** slightly inhibited the *h*ERG channel by less than 5%, compounds **199** and **202** moderately inhibited the *h*ERG channel by 52.8% and 40.1%, respectively, at 10 μM. Although compound **202** moderately inhibited the *h*ERG channel, overall, this compound exhibited potent *in vitro* and *in vivo* activities and good brain penetration; therefore, we decided to perform a molecular docking study with compound **202**.

Table 1.5. QC inhibition in acute model-based studies *in vivo*[a]

	In vitro IC_{50} (nM)	Cytotoxicity at 10 µM (% of control)	% inhibition of human $A\beta_{N3pE-40}$ formation (icv)	PAMPA (-logPe)	hERG FP at 10 µM (% inhibition)
160	5.8	~100	3.01	5.8	33.4
161	9.9	~100	NE[b]	5.3	2.5
163	7.4	~100	NE	6.0	9.7
165	4.8	~100	NT[c]	8.7	NT
166	7.5	~100	5.43	5.7	2.5
167	3.8	~100	NE	10.0	28.9
168	9.7	~100	NE	10.0	21.1
170	3.6	51.1	NT	NT	NT
176	9.0	~100	0.19	6.7	8.2
185	7.7	~100	18.7	5.7	1.7
186	7.0	~100	8.07	5.7	1.9
188	7.8	~100	NE	10.0	14.0
190	5.5	~100	13.5	5.6	5.0
193	4.5	~100	NE	6.4	17.8
195	8.5	~100	NE	5.6	39.7
199	9.4	~100	16.1	4.9	52.8
200	9.9	~100	NE	5.6	72.8
202	6.2	~100	30.0	5.8	40.1
204	8.7	~100	NE	10.0	44.4
205	1.8	~100	NE	10.0	31.5

[a] Five microliters of human $A\beta_{3-40}$ in PBS (1 µg/µL) were injected into the deep cortical/hippocampal tissues of 5-week-old ICR mice (25 g, n = 4, males) using a stereotaxic frame to induce acute Aβ toxicity. Test compounds were administered via an icv. A sandwich ELISA was performed to quantify the brain $A\beta_{N3pE-40}$ level; [b]NE = not effective; [c]NT = not tested.

2.4. Molecular modeling

We performed sequential molecular modeling studies using the X-ray crystal structure of *h*QC (PDB id: 3PBB)[69] to investigate the interactions between *h*QC and compound **202**. The initial docking study was conducted using the piperidine

protonated form of compound **202** at pH 7.4, utilizing Glide SP (Standard Precision). The presence of the 5-methyl imidazole in the A-region chelated zinc and formed an H-bond with the indole NH of Trp329, as well as several hydrophobic interactions with Leu249, Trp207, and Ile321. The thiourea group in the B-region contributed to the appropriate positioning of the C-region phenyl ring for the hydrophobic interaction with Tyr299. Interestingly, the phenyl ring located between the C- and D-regions participated in a hydrophobic interaction with Pro324. The piperidine ring of the D-region participated in a hydrophobic interaction with Pro326 (**Figure 1.2A**).

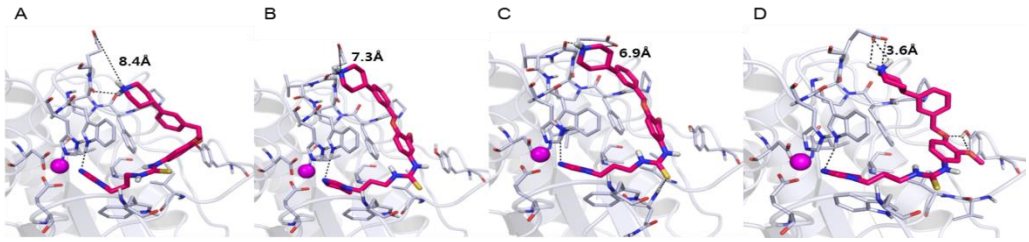


Figure 1.2. Binding modes of compound **202** in *h*QC after (A) Glide SP docking, (B) QPLD, (C) local optimization, and (D) Monte Carlo minimization. Binding modes of the protonated form of compound **202** are shown in each step. Interactions with Glu327 are highlighted in red-dotted boxes, and the distances between Glu327 and the terminal N from the D-region of the ligands are marked with black dashed lines.

Subsequently, we performed Glide QM-Polarized Ligand Docking (QPLD) in Maestro. The piperidine ring in the D-region moved toward Glu327 of the *h*QC active site (**Figure 1.2B**). The local optimization refinement further shifted the Glu327 side chain toward the piperidine ring of compound **202** (**Figure 1.2C**). We conducted Monte Carlo minimization to identify the global minimum (**Figure 1.2D**). This type of sequential optimization of the protein-ligand complexes induced a remarkable change in the orientation of the Glu327 side chain, leading to the formation of a salt bridge interaction, along with the H-bond with the D-region of compound **202**. Overall, the A-region maintained its binding position and interactions throughout the optimization procedure, whereas the phenyl ring in the C-region formed an additional H-bond with Tyr299. Moreover, the phenyl ring located between the C- and D-regions showed additional π- π interactions with Phe325 (**Figure 1.3**).

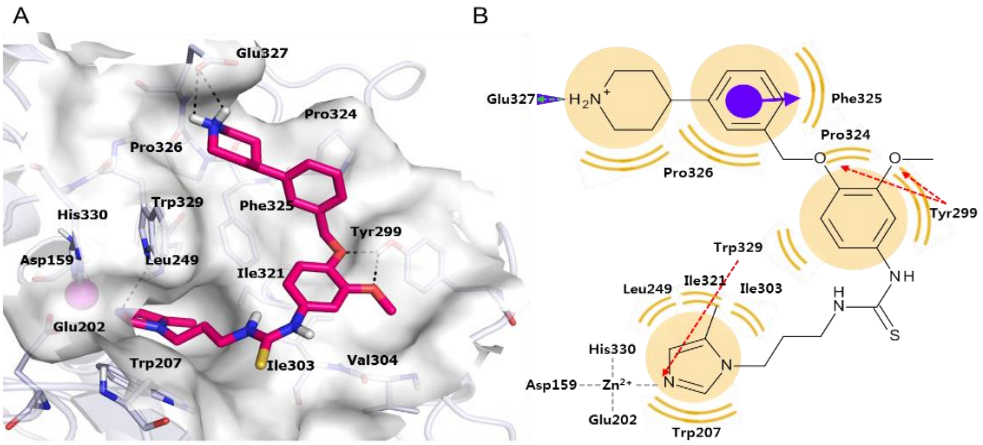


Figure 1.3. Refined structure of compound **202** docked with *h*QC. (A) Binding interactions of compound **202** at the active site of the *h*QC. Compound **202** is displayed as sticks with magenta carbon atoms, and Zn^{2+} is depicted as a purple ball. The interacting residues are depicted as light blue sticks. Hydrogen bonds are depicted as black dashed lines. (B) A 2D representation of the interactions of compound **202** with the active site residues of *h*QC. Hydrophobic interactions are marked in light brown. Hydrogen bonds are shown as red-dotted arrows with the indicated directionality. The π-π stacking interaction is marked as a blue disc and arrow, and the salt bridge interaction is displayed as blue wedged line.

3. Conclusion

In the present study, we synthesized and evaluated the biological activity of the D-region-modified analogues based on the previously developed lead compounds **2** and **3**. In general, the modification of the piperazinyl group maintained or slightly reduced QC inhibition *in vitro* compared to its parent compound **2**, and the rigidification of the linker between the C- and D-regions did not appear to affect biological activity. Compared to the lead compound **3**, the addition of an aminoethyl group to the 2-aminopyridine ring of the D-region slightly improved the *in vitro* activity up to 2.5-fold. When we tested compounds with low IC_{50} values (<10 nM) in mice, four compounds with high membrane penetration (-logPe = 4.9 to 5.8) displayed good in vivo activity. In particular, compound **202** reduced $A\beta_{N3pE-40}$ formation in the brain by 30% compared to the vehicle-treated control. According to the molecular docking study of compound **202**, the benzyl linker between the C- and

D-regions participated in an additional hydrophobic interaction with Phe325 in the active site. We believe that our SAR studies added valuable information regarding the D-region pharmacophore, and we will continue our efforts in lead optimization to identify QC inhibitors with better penetration and *in vivo* activity without any potential toxicity.

4. Experimental

4.1. Chemistry

4.1.1. General

All chemical reagents were commercially available. Silica gel column chromatography was performed on silica gel 60, 230-400 mesh, Merck. ^{1}H NMR spectra were recorded on a a JEOL JNM-LA 300 at 300 MHz, Bruker Analytik, DE/AVANCE Digital 400 at 400 MHz, a Bruker Analytik, DE/AVANCE Digital 500 at 500 MHz, and a JEOL JNM-ECA-600 at 600 MHz. Mass spectra were recorded on a VG Trio-2 GC−MS instrument and a 6460 Triple Quad LC−MS instrument. Melting points were determined on a melting point Buchi B-540 apparatus and are uncorrected. All final compounds were assessed for purity by high performance liquid chromatography (HPLC) on Agilent 1120 Compact LC (G4288A) system via the following conditions. Column: Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm). Mobile phase A: MeOH, Mobile phase B: 0.1% TFA in water (v/v) in 30 min. Wavelength: 254 nM. Flow: 0.7 mL/min. According to the HPLC analyses, all final compounds showed a purity of ≥95%.

4.1.2. General procedure

4.1.2.1. Suzuki coupling (Procedure 1)

A solution containing the triflate compound (1.0 equiv) and boronic compound (1.0 equiv) in acetonitrile and sodium carbonate (1.5 equiv) was added to a dried two-neck flask. Then, the mixture was degassed and back-filled with dry nitrogen before a suspension of tetrakis(triphenylphosphine)palladium(0) (5% mol) in acetonitrile was added. The reaction was refluxed overnight, then cooled to room temperature, quenched with water, extracted with EtOAc (2x 50 mL), dried over $MgSO_4$, and concentrated. The concentrate was purified by silica gel chromatography with EA:*n*-hexane to obtain the product.

4.1.2.2. Ullmann reaction (Procedure 2)

A dried two-neck flask was charged with aryl halide (1 equiv), phenol compound (1 equiv), cesium carbonate (2 equiv) and *N,N'*-dimethylethylenediamine (0.2 equiv) in anhydrous DMF. The reaction was degassed and back-filled with dry nitrogen before CuI (0.1 equiv) in DMF was added. The reaction was stirred at 90-100 °C for 24 h, cooled to room temperature, quenched with $NaHCO_3$ and extracted with EtOAc (2x 50 mL). The organic layer was washed with water 3 times, dried over $MgSO_4$, and concentrated. The concentrate was purified by silica gel chromatography with EtOAc:*n*-hexane to obtain the desired product.

4.1.2.3. Mitsunobu reaction (Procedure 3)

Triphenylphosphine (1.3 equiv) was added to a solution of 4-nitroguanicol (1.0 equiv) in DCM under a nitrogen atmosphere, followed by the addition of a primary alcohol (1.2 equiv) and a solution of diethyl azodicarboxylate (1.3 equiv) in DCM. After the solution was stirred for 30 minutes at room temperature, the reaction was poured onto a column of silica and was eluted with EtOAc:*n*-hexane to yield the desired product.

4.1.2.4. Reduction (Procedure 4)

Procedure 4.1: AcOH (5 equiv) and Zn dust (5 equiv) were added to a solution of a nitro compound in MeOH (10 mL) at room temperature. The reaction mixture was stirred at room temperature for 30 minutes and then filtered through a celite filter. The filtrate was portioned between H_2O (10 mL) and DCM (30 mL). The organic layer was separated, dried over $MgSO_4$, concentrated, and purified by column chromatography to provide the product.

Procedure 4.2: The nitro compound or alkene derivative was dissolved in MeOH (or mixture of MeOH and THF) and then 10% Pd/C was added. The mixture was stirred at room temperature under hydrogen gas until all starting material was consumed (confirmed by TCL). The crude mixture was filtered through celite filter, washed with MeOH (3 x 50 mL) and then concentrated. The product was subjected to the next step without further purification.

4.1.2.5. Williamson reaction (Procedure 5)

Alkyl halide (4.0 equiv) was added to a suspension of 4-nitroguanicol (1.0 equiv) and cesium carbonate (2.0 equiv) in anhydrous DMF. The reaction mixture was heated to 100 °C for 1 hour and then cooled to room temperature before being quenched with

water. The mixture was extracted with EtOAc (2 x 50 mL). The organic layer was washed with water 3 times, dried with $MgSO_4$ and concentrated. The concentrate was purified by column chromatography to obtain the product.

4.1.2.6. Boc protection and deprotection

Procedure 6.1: Triethylamine (1.2 equiv) and di-*tert*-butyl dicarbonate (2.5 equiv) in DCM were added to a suspension of the starting amine material (1.0 equiv) in DCM in an ice bath. The mixture was stirred at room temperature until starting material was consumed (confirmed by TLC). Water was added to the mixture and subsequently extracted with DCM. The organic layer was washed with a 10% aqueous $NaHCO_3$ solution, water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography to obtain the desired product.

Procedure 6.2: Trifluoroacetic acid (10 equiv) was added to the solution of the *boc*-protected compound (1.0 equiv) in DCM (DCM:TFA = 1:1 (v/v)). Then, the mixture was stirred at room temperature until the starting material was consumed and evaporated. The residue was dissolved in MeOH and purified on an ion-exchange column to obtain the desired product or subjected to the next step without further purification.

4.1.2.7. TBDMS deprotection (Procedure 7)

A solution of conc. HCl (0.1 mL) was added to the solution of *t*-butyl dimethyl silyl ether dissolved in MeOH (10 mL). The mixture was stirred at room temperature overnight and then concentrated. The concentrate was dissolved in MC and washed with water. The organic layer was concentrated to obtain the desired product or purified by flash chromatography.

4.1.2.8. Thiourea coupling (Procedure 8)

A solution of the amine (1.0 equiv) in anhydrous DCM was added to a solution of 1,1'-thiocarbonyldiimidazole (1.02 equiv) in anhydrous DCM in a dropwise manner under nitrogen gas at room temperature. The reaction mixture was stirred at room temperature until the starting material was consumed. Then, the solution of 3-(5-methyl-1*H*-imidazol-1-yl)propan-1-amine (1.1 equiv) in anhydrous DCM was added

dropwise, followed by the addition of triethylamine (3.0 equiv), and stirred at room temperature until the reaction was complete (monitored with TLC). The mixture was washed with water 2 times, the combined organic layer was dried over $MgSO_4$, concentrated, and purified by column chromatography.

4.1.2.9. EDC coupling (Procedure 9)

EDC.HCl (1.1 equiv) and *N,N*-diisopropylethylamine (2.2 equiv) were added to a solution of the amine compound (1.0 equiv), acid compound (1.0 equiv) and HOBt (1.1 equiv) in DCM. The mixture was stirred for 24 hours at room temperature under nitrogen. The solvent was removed in vacuo and the residue purified by column chromatography on silica gel eluted with DCM:MeOH to produce the desired compound.

4.1.2.10. N-Alkylation (Procedure 10)

A mixture of the alkyl halide, nitrogen-containing compound and excess base (NaH for compounds **80** and **81,** Cs_2CO_3 for other compounds) in DMF was stirred at 60 °C for 30 min. The reaction was quenched with water and extracted with EA. The organic layer was washed with water and brine, concentrated and purified by column chromatography.

4.1.3. Intermediate compound

*4.1.3.1. tert-Butyl 4-(((trifluoromethyl)sulfonyl)oxy)-3,6-dihydropyridine-1(2H)-carboxylate (**6**).* *tert*-Butyl 4-oxopiperidine-1-carboxylate (1 eq) was added slowly to solution of LDA (1.1 eq) in THF at -78 ^oC. The resulting solution was warmed to room temperature and stirred for 30 min. The solution was cooled to -78 ^oC again and a solution *N*-phenyltrifluromethanesulfonimide (1.05 eq) in THF was added slowly. The solution was warmed to room temperature and allowed to stir for 2 more hours. The reaction was quenched with water (50 mL). Aqueous extraction was performed with EA (2 x 50 mL), dried over $MgSO_4$ and dried under vacuum rotation to get crude oil (51%). ^1H NMR (300 MHz, $CDCl_3$) δ 5.80-5.72 (m, 1H), 4.06-4.02 (m, 2H), 3.63 (t, J = 5.60 Hz, 2H), 2.48-2.40 (m, 2H), 1.47 (s, 9H).

*4.1.3.2. tert-Butyl 4-(4-hydroxyphenyl)-3,6-dihydropyridine-1(2H)-carboxylate (**7**).* Prepare from compound **6** following the general procedure **1** to get product (86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, J = 8.61 Hz, 2H), 6.83 (d, J = 8.61 Hz, 2H), 5.89 (s, 1H), 4.06 (d, J = 2.73 Hz, 2H), 3.63 (t, J = 5.67 Hz, 2H), 2.47 (t, J = 5.67 Hz, 2H), 1.48 (s, 9H).

4.1.3.3. *tert-Butyl 4-(3-(hydroxymethyl)phenyl)-5,6-dihydropyridine-1(2H)-carboxylate (**8**)*. From compound **6**, procedure **1**; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.27 (m, 4H), 6.03 (s, 1H), 4.69 (d, J = 5.55 Hz, 2H), 4.08 (br, 2H), 3.61 (t, J = 5.40 Hz, 2H), 2.51 (br, 2H), 1.69 (t, J = 5.85 Hz, 1H), 1.47 (s, 9H).

*4.1.3.4. tert-Butyl 4-(4-(2-methoxy-4-nitrophenoxy)phenyl)-3,6-dihydropyridine-1(2H)-carboxylate (**9**).* Prepare from compound **7** following the general procedure **2** to get product (75%).

*4.1.3.5. tert-Butyl 4-(3-(hydroxymethyl)phenyl)piperidine-1-carboxylate **(10)***. From compound **8**, procedure **4.2**; yield 99%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22-7.12 (m, 4H), 4.69 (s, 2H), 4.23 (br, 2H), 2.80 (t, J = 11.73 Hz, 2H), 2.66 (tt, J = 12.09, 3.87 Hz, 1H), 1.83-1.80 (m, 2H), 1.70-1.56 (m, 2H), 1.48 (s, 9H).

*4.1.3.6. tert-Butyl 4-(3-((2-methoxy-4-nitrophenoxy)methyl)phenyl)piperidine-1-*carboxylate ***(11)***. From compound **10**, procedure **3**; yield 64%, light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (dd, J = 8.79, 2.55 Hz, 1H), 7.77 (d, J = 2.55 Hz, 1H), 7.36-7.26 (m, 2H), 7.18 (d, J = 8.52 Hz, 2H), 6.92 (d, J = 8.97 Hz, 1H), 5.22 (s, 2H), 4.23 (br, 2H), 3.97 (s, 3H), 2.80 (t, J = 12.09 Hz, 2H), 2.66 (t, J = 12.27 Hz, 1H), 1.84-1.79 (m, 2H), 1.68-1.57 (m, 2H), 1.48 (s, 9H).

*4.1.3.7. tert-Butyl 4-(4-hydroxyphenyl)piperazine-1-carboxylate **(13)***. Prepare from commercially available 4-(1-piperazinyl)phenol **12** following the general procedure **6.1** to collect desired product (98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86-6.82 (m, 2H), 6.79-6.75 (m, 2H), 3.58 (t, J = 4.95 Hz, 4H), 3.00 (t, J = 4.95 Hz, 4H), 1.48 (s, 9H).

*4.1.3.8. 4-(4-Methylpiperazin-1-yl)phenol **(14)***. A solution of 4-(1-piperazinyl)phenol **12** in formic acid and formaldehyde (1:1, v/v) was refluxed overnight. Then mixture was basified by dilute bicarbonate solution. DCM work-up to get crude white solid (49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86-6.83 (m, 2H), 6.77-6.74 (m, 2H), 3.11 (t, J = 4.95 Hz, 4H), 2.60 (t, J = 4.95 Hz, 4H), 2.35 (s, 3H).

*4.1.3.9. tert-Butyl 4-(4-(2-methoxy-4-nitrophenoxy)phenyl)piperazine-1-carboxylate **(15)***. Prepared from compound **13** following the general procedure **2** to afford yellow solid (63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 2.55 Hz, 1H), 7.79 (dd, J = 2.76, 8.97 Hz, 1H), 7.02-6.93 (m, 4H), 6.76 (d, J = 8.79 Hz, 1H), 4.01 (s, 3H), 3.61 (t, J = 4.92 Hz, 4H), 3.14 (t, J = 4.95 Hz, 4H), 1.49 (s, 9H).

*4.1.3.10. 1-(4-(2-Methoxy-4-nitrophenoxy)phenyl)-4-methylpiperazine **(16)***. Prepare from compound **14** following the general procedure **2** to get titled product 125 mg (43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 2.55 Hz, 1H), 7.78 (dd, J = 2.67, 8.79 Hz, 1H), 6.99-6.96 (m, 4H), 6.75 (d, J = 8.97 Hz, 1H), 4.01 (s, 3H), 3.20 (t, J = 4.95 Hz, 4H), 2.60 (t, J = 4.95 Hz, 4H), 2.37 (s, 3H).

*4.1.3.11. tert-Butyl 4-(3-(methoxycarbonyl)phenyl)piperazine-1-carboxylate **(18)***. A mixture of *tert*-butyl piperazine-1-carboxylate (1.0 eq), **17** (1.0 eq.), BINAP (5% mol) and sodium *tert*-butoxide (3.0 eq) were placed in two neck round bottom flask. Then anhydrous toluene was added to the mixture. The mixture was degassed and back-filled with dry nitrogen gas before suspension of palladium (II) acetate (5% mol) in dry toluene was added. The mixture reaction was stirred at 100 ^{o}C for 15 min, cooled to room temperature, quenched by water. Normal extraction and purification was applied to get white solid, yield 30%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.54 (d, J = 7.68 Hz, 1H), 7.33 (t, J = 8.04 Hz, 1H), 7.10 (d, J = 8.25 Hz, 1H), 3.91 (s, 3H), 3.59 (t, J = 4.95 Hz, 4H), 3.18 (t, J = 5.31 Hz, 4H), 1.49 (s, 9H).

*4.1.3.12. tert-Butyl 4-(3-(hydroxymethyl)phenyl)piperazine-1-carboxylate **(19)***. The solution of **18** (1.0 eq.) in THF was cooled in ice bath. Then LAH (2.0 eq) was added slowly in the solution. After stirring 30 min the mixture reaction was applied Fisher work-up, concentrated to get white solid, yield 88%. ^{1}H NMR (300 MHz, $CDCl_3$) δ

7.25 (t, *J* = 7.89 Hz, 1H), 6.96 (s. 1H), 6.89-6.85 (m, 2H), 4.66 (s, 2H), 3.58 (t, *J* = 4.77 Hz, 4H), 3.15 (t, *J* = 5.13 Hz, 4H), 1.48 (s, 9H).

*4.1.3.13. tert-Butyl 4-(3-((2-methoxy-4-nitrophenoxy)methyl)phenyl)piperazine-1-carboxylate **(20)***. From compound **19**, procedure **3**, yield 66%, light yellow. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 8.79, 2.58 Hz, 1H), 7.76 (d, *J* = 2.37 Hz, 1H), 7.29 (t, *J* = 7.71 Hz, 1H), 6.98-6.88 (m, 4H), 5.21 (s, 2H), 3.97 (s, 3H), 3.58 (t, *J* = 4.41 Hz, 4H), 3.15 (t, *J* = 4.20 Hz, 4H), 1.48 (s, 9H).

*4.1.3.14. 1-(2-Bromoethoxy)-2-methoxy-4-nitrobenzene **(22)***. From 1,2-dibromoethane and **21**, procedure **5**, yield 73%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 8.97, 2.58 Hz, 1H), 7.78 (d, *J* = 2.73 Hz, 1H), 6.94 (d, *J* = 8.97 Hz, 1H), 4.44 (t, *J* = 6.39 Hz, 2H), 3.96 (s, 3H), 3.73 (t, *J* = 6.39 Hz, 2H).

*4.1.3.15. 1-(3-Bromopropoxy)-2-methoxy-4-nitrobenzene **(23)***. Prepare from commercially available **21** following the general procedure **5** to get product as pale solid (67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 2.55, 8.61 Hz, 1H), 7.75 (d, *J* = 2.37 Hz, 1H), 6.96 (d, *J* = 8.97 Hz, 1H), 4.28 (t, *J* = 5.85 Hz, 2H), 3.94 (s, 3H), 3.66 (2H, 6.21 Hz, 2H), 2.44 (quintet, *J* = 7.14 Hz, 2H).

*4.1.3.16. 1-(4-Bromobutoxy)-2-methoxy-4-nitrobenzene **(24)***. Prepare from commercially available **21** and dibromobutane following the general procedure **5** to get product (85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 2.76, 8.97 Hz, 1H), 7.52 (d, *J* = 2.55 Hz, 1H), 6.91 (d, *J* = 8.97 Hz, 1H), 4.17 (t, *J* = 5.88 Hz, 2H), 3.94 (s, 3H), 3.53 (t, *J* = 6.39 Hz, 2H), 2.08-2.03 (m, 4H)

*4.1.3.17. Methyl 2-(2-methoxy-4-nitrophenoxy)acetate **(25)***. Prepare from commercial compound 4-nitro guaiacol **21** following the general procedure **5** to get product as a pale yellow solid (90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, *J* = 2.55, 8.79 Hz, 1H), 7.79 (d, *J* = 2.58 Hz, 1H), 6.83 (d, *J* = 8.79 Hz, 1H), 4.81 (s, 2H), 3.98 (s, 3H), 3.82 (s, 3H).

*4.1.3.18. Methyl 4-(2-methoxy-4-nitrophenoxy)butanoate **(26)***. Prepare from 4-nitroguaiacol **21** following the general procedure **5** to afford product as a pale yellow solid (99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.58, 8.97 Hz, 1H), 7.74 (d, J = 2.58 Hz, 1H), 6.93 (d, J = 8.97 Hz, 1H), 4.19 (t, J = 6.21 Hz, 2H), 3.94 (s, 3H), 3.70 (s, 3H), 2.59 (t, J = 7.14 Hz, 2H), 2.25 (quintet, J = 6.57 Hz, 2H).

*4.1.3.19. 3-(2-Methoxy-4-nitrophenoxy)propan-1-ol **(27)***. From compound **21**, procedure **5**; yield 74%, light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 8.97, 2.55 Hz, 1H), 7.75 (d, J = 2.58 Hz, 1H), 6.93 (d, J = 8.79 Hz, 1H), 4.29 (t, J = 6.06 Hz, 2H), 3.95 (s, 3H), 3.90 (q, J = 7.14 Hz, 2H), 2.14 (quintet, J = 5.67 Hz, 2H).

*4.1.3.20. t-Butyl 4-(2-(2-methoxy-4-nitrophenoxy)ethyl)piperidine-1-carboxylate **(28)***. Prepare from ~~4-~~nitroguaiacol **21** following the general procedure 3 to get a yellow oil (90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.58, 8.79 Hz, 1H), 7.75 (d, J = 2.55 Hz, 1H), 6.90 (d, J = 8.97 Hz, 1H), 4.18-4.08 (m, 4H), 3.94 (s, 3H), 2.72 (t, J = 12.45 Hz, 2H), 1.87 (q, J = 6.42 Hz, 2H), 1.75-1.66 (m, 3H), 1.45 (s, 9H), 1.23-1.14 (m, 2H).

*4.1.3.21. tert-Butyl 4-(2-(2-methoxy-4-nitrophenoxy)ethyl)piperazine-1-carboxylate **(29)***. From *tert*-butyl piperazine-1-carboxylate and compound **22**, procedure **10**, yield 92%, orange solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 8.97, 2.55 Hz, 1H), 7.75 (d, J = 2.58 Hz, 1H), 6.93 (d, J = 8.97 Hz, 1H), 4.26 (t, J = 6.03 Hz, 2H), 3.94 (s, 3H), 3.46 (t, J = 4.77 Hz, 4H), 2.91 (t, J = 5.85 Hz, 2H), 2.56 (t, J = 4.95 Hz, 4H), 1.46 (s, 9H).

*4.1.3.22. tert-Butyl 4-(3-(2-methoxy-4-nitrophenoxy)propyl)piperazine-1-carboxylate **(30)***. Prepare from **23** following the general procedure **10** to afford white solid (70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.55, 8.97 Hz, 1H), 7.74 (d, J = 2.55 Hz, 1H), 6.95 (d, J = 8.97 Hz, 1H), 4.21 (t, J = 6.60 Hz, 2H), 3.94 (s, 3H), 3.45 (t, J = 4.77 Hz, 4H), 2.56 (t, J = 7.14 Hz, 2H), 2.42 (t, J = 4.59 Hz, 4H), 2.09 (quintet, J = 6.60 Hz, 2H), 1.46 (s, 9H).

4.1.3.23. tert-Butyl 4-(4-(2-methoxy-4-nitrophenoxy)butyl)piperazine-1-carboxylate ***(31)***. Prepare from **24** following the general procedure **5** to get product (76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.58, 8.79 Hz, 1H), 7.74 (d, J = 3.18 Hz, 1H), 6.91 (d, J = 8.79 Hz, 1H), 4.15 (t, J = 6.42 Hz, 2H), 3.94 (s, 3H), 3.44 (t, J = 4.95 Hz, 4H), 2.42-2.37 (m, 6H), 1.95 (quintet, J = 6.57 Hz, 2H), 1.71 (quintet, J = 6.96 Hz, 2H), 1.45 (s, 9H).

4.1.3.24. tert-Butyl 4-(2-(2-methoxy-4-nitrophenoxy)ethyl)-3-methylpiperazine-1-carboxylate ***(32)***. From *tert*-butyl-3-methylpiperazine-1-carboxylate and compound **22**, procedure **10**, yield 63%, light yellow oil.

4.1.3.25. tert-Butyl 4-(2-(2-methoxy-4-nitrophenoxy)ethyl)-2,6-dimethylpiperazine-1-carboxylate ***(33)***. From compound **22**, procedure **10**, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, J = 8.97, 2.55 Hz, 1H), 7.75 (d, J = 2.55 Hz, 1H), 6.96 (d, J = 8.79 Hz, 1H), 4.25 (t, J = 5.85 Hz, 2H), 4.13-4.05 (m, 2H), 3.94 (s, 3H), 2.85 (t, J = 6.03 Hz, 2H), 2.71 (d, J = 11.16 Hz, 2H), 2.32 (dd, J = 11.34, 4.38 Hz, 2H), 1.46 (s, 9H), 1.26 (d, J = 6.96 Hz, 6H).

4.1.3.26. 1-(2-(2-Methoxy-4-nitrophenoxy)ethyl)-4-phenylpiperazine ***(34)***. From 1-phenylpiperazine and compound **22**, procedure **10**, yield 99%, light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, J = 8.97, 2.76 Hz, 1H), 7.75 (d, J = 2.55 Hz, 1H), 7.30-7.25 (m, 2H), 6.96-6.93 (m, 3H), 6.87 (t, J = 7.32 Hz, 1H), 4.29 (t, J = 6.06 Hz, 2H), 3.95 (s, 3H), 3.23 (t, J = 4.95 Hz, 4H), 2.96 (t, J = 6.24 Hz, 2H), 2.78 (t, J = 4.95 Hz, 4H).

4.1.3.27. 1-Benzyl-4-(2-(2-methoxy-4-nitrophenoxy)ethyl)piperazine ***(35)***. Prepare from **22** following the general procedure **5** to give product as yellow solid (70%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.91 (dd, J = 2.58, 8.97 Hz, 1H), 7.73 (d, J = 2.73 Hz, 1H), 7.32-7.23 (m, 5H), 6.92 (d, J = 8.97 Hz, 1H), 4.25 (t, J = 6.24 Hz, 2H), 3.92 (s, 3H), 3.51 (s, 2H), 2.91 (t, J = 6.21 Hz, 2H), 2.63 (br, 4H), 2.50 (br, 4H).

4.1.3.28. 2-(4-(2-(2-Methoxy-4-nitrophenoxy)ethyl)piperazin-1-yl)pyrimidine ***(36)***. From 2-(piperazin-1-yl)pyrimidine and compound **22**, procedure **10**, yield 70%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, J = 4.8 Hz, 2H), 7.90 (dd, J = 8.8, 2.6 Hz, 1H),

7.75 (d, *J* = 2.6 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.50 (d, *J* = 4.8 Hz, 1H), 4.29 (t, *J* = 6.0 Hz, 2H), 3.95 (s, 3H), 3.86 (t, *J* = 5.0 Hz, 4H), 2.94 (t, *J* = 6.0 Hz, 2H), 2.67 (t, *J* = 5.0 Hz, 4H).

*4.1.3.29. 5-Fluoro-2-(4-(2-(2-methoxy-4-nitrophenoxy)ethyl)piperazin-1-yl)pyrimidine **(37)***. From compound **22**, procedure **10**

*4.1.3.30. 5-Chloro-2-(4-(2-(2-methoxy-4-nitrophenoxy)ethyl)piperazin-1-yl)pyrimidine **(38)***. From 5-chloro-2-(piperazin-1-yl)pyrimidine and compound **22**, procedure **10**, yield 57%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 2H), 7.90 (dd, *J* = 8.80, 2.6 Hz, 1H), 7.75 (d, *J* = 2.60 Hz, 1H), 6.94 (d, *J* = 8.80 Hz, 1H), 4.29 (t, *J* = 6.00 Hz, 2H), 3.95 (s, 3H), 3.80 (t, *J* = 5.00 Hz, 4H), 2.94 (t, *J* = 6.00 Hz, 2H), 2.67 (t, *J* = 5.00 Hz, 4H).

*4.1.3.31. 2-(2-Methoxy-4-nitrophenoxy)acetic acid **(39)***. Prepare from **25** following hydrolysis reaction in solution of NaOH to get a yellow oil as product (88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, *J* = 2.76, 8.97 Hz, 1H), 7.80 (d, *J* = 2.58 Hz, 1H), 6.89 (d, *J* = 9.00 Hz, 1H), 4.82 (s, 2H), 3.49 (s, 3H).

*4.1.3.32. 3-(2-Methoxy-4-nitrophenoxy)propanoic acid **(40)***. The cooled solution of **27** in acetone was added slow excess of solution $K_2Cr_2O_7$ in sulfuric acid. The mixture reaction was stirred room temperature overnight, extraced by EA. The organic layer was collected, washed by dilute bicarbonate solution, concentrated and purified by silica gel to get light yellow solid, yield 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, *J* = 8.97, 2.73 Hz, 1H), 7.75 (d, *J* = 2.55 Hz, 1H), 6.94 (d, *J* = 8.79 Hz, 1H), 4.39 (t, *J* = 6.39 Hz, 2H), 3.94 (s, 3H), 2.98 (t, *J* = 6.21 Hz, 2H).

*4.1.3.33. 4-(2-Methoxy-4-nitrophenoxy)butanoic acid **(41)***. Prepare from compound **26** following the hydrolysis reactin to give product as light yellow solid (58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 2.55, 8.97 Hz, 1H), 7.74 (d, *J* = 2.55 Hz, 1H), 6.92 (d, *J* = 8.79 Hz, 1H), 4.20 (t, *J* = 6.24 Hz, 2H), 3.93 (s, 3H), 2.65 (t, *J* = 7.14 Hz, 2H), 2.32 (quintet, *J* = 6.78 Hz, 2H).

*4.1.3.34. 3-(2-Methoxy-4-nitrophenoxy)propanamide **(42)***. From compound **40** and solution of NH_3 in THF, procedure **9**, yield 42%, light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 8.79, 2.58 Hz, 1H), 7.56 (d, J = 2.55 Hz, 1H), 6.97 (8.97 Hz, 1H), 4.40 (t, J = 6.03 Hz, 2H), 3.94 (s, 3H), 2.81 (t, J = 6.03 Hz, 2H).

*4.1.3.35. 4-(2-Methoxy-4-nitrophenoxy)butanamide **(43)***. Prepare from compound **41** following the general procedure **9** to afford the desired product (70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.73, 8.97 Hz, 1H), 7.75 (d, J = 2.73 Hz, 1H), 6.95 (d, J = 8.97 Hz, 1H), 5.62-5.38 (br, 2H), 4.21 (t, J = 6.06 Hz, 2H), 3.94 (s, 3H), 2.51 (t, J = 7.32 Hz, 2H), 2.26 (quintet, J = 6.24 Hz, 2H).

*4.1.3.36. 3-(2-Methoxy-4-nitrophenoxy)-N,N-dimethylpropanamide **(44)***. From compound **40** and dimethylamine, procedure **9**, yield 45%, light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 8.97, 2.76 Hz, 1H), 7.74 (d, J = 2.58 Hz, 1H), 7.00 (d, J = 8.97 Hz, 1H), 4.46 (t, J = 7.14 Hz, 2H), 3.94 (s, 3H), 3.07 (s, 3H), 2.98 (s, 3H), 2.95 (t, J = 7.14 Hz, 2H).

*4.1.3.37. 4-(2-Methoxy-4-nitrophenoxy)-N,N-dimethylbutanamide **(45)***. Prepare from compound **41** following the general procedure **9** to give desired product (38% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.73, 8.97 Hz, 1H), 7.75 (d, J = 2.73 Hz, 1H), 6.95 (d, J = 8.97 Hz, 1H), 4.21 (t, J = 6.24 Hz, 2H), 3.94 (s, 3H), 3.02 (s, 3H), 2.95 (s, 3H), 2.56 (t, J = 7.32 Hz, 2H), 2.25 (quintet, J = 6.27 Hz, 2H).

*4.1.3.38. tert-Butyl 4-(2-(2-methoxy-4-nitrophenoxy)acetyl)piperazine-1-carboxylate **(46)***. Prepare from **39** following the general procedure **9** to furnish the product as white foam (87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, J = 2.55, 8.79 Hz, 1H), 7.77 (d, J = 2.58 Hz, 1H), 7.00 (d, J = 8.97 Hz, 1H), 4.87 (s, 2H), 3.96 (s, 3H), 3.58-3.36 (m, 4H), 3.44-3.40 (m, 4H), 1.46 (s, 9H).

*4.1.3.39. tert-Butyl 4-(3-(2-methoxy-4-nitrophenoxy)propanoyl)piperazine-1-carboxylate **(47)***. From compound **40** and N-Bocpiperazine, procedure **9**; yield 76%, light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 8.79, 2.55 Hz, 1H), 7.74 (d, J = 2.55 Hz, 1H), 6.98 (d, J = 8.97 Hz, 1H), 4.46 (t, J = 6.75 Hz, 2H), 3.93 (s, 3H), 3.52 (br, 4H), 3.45-3.41 (m, 4H), 2.93 (t, J = 6.78 Hz, 2H), 1.48 (s, 9H).

*4.1.3.40. tert-Butyl 4-(4-(2-methoxy-4-nitrophenoxy)butanoyl)piperazine-1-carboxylate **(48)***. Prepare from compound **41** following the general procedure **9** to give desired product (32% yield) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.55, 8.79 Hz, 1H), 7.74 (d, J = 2.58 Hz, 1H), 6.97 (d, J = 8.97 Hz, 1H), 4.22 (t, J = 6.21 Hz, 2H), 3.94 (s, 3H), 3.60 (t, J = 7.32 Hz, 2H), 3.45 (m, 6H), 2.59 (t, J = 6.96 Hz, 2H), 2.25 (quintet, J = 6.60 Hz, 2H), 1.47 (s, 9H).

*4.1.3.41. tert-Butyl 4-(2-(2-methoxy-4-nitrophenoxy)acetamido)piperidine-1-carboxylate **(49)***. Prepare from **39** following the general procedure **9** to get the product as a white foam (84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, J = 2.55, 8.76 Hz, 1H), 7.20 (d, J = 2.55 Hz, 1H), 6.94 (d, J = 8.97 Hz, 1H), 6.70 (br, 1H), 4.58 (s, 2H), 4.03-4.00 (m, 3H), 3.98 (s, 3H), 2.92 (t, J = 11.34 Hz, 2H), 1.95-1.92 (m, 2H), 1.46 (s, 9H), 1.41-1.37 (m, 2H).

*4.1.3.42. tert-Butyl 4-(3-(2-methoxy-4-nitrophenoxy)propanamido)piperidine-1-carboxylate **(50)***. Prepare from **40** following the general procedure **9** to give desired product (76%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, J = 2.55, 8.79 Hz, 1H), 7.76 (d, J = 2.55 Hz, 1H), 6.96 (d, J = 8.79 Hz, 1H), 6.00 (br, 1H), 4.39 (t, J = 5.85 Hz, 2H), 3.93-3.92 (m, 3H), 3.93 (s, 3H), 2.89 (t, J = 7.32 Hz, 2H), 2.75 (t, J = 5.67 Hz, 2H), 1.89-1.87 (m, 2H), 1.45 (s, 9H), 1.33-1.32 (m, 2H).

*4.1.3.43. tert-Butyl 4-(4-(2-methoxy-4-nitrophenoxy)butanamido)piperidine-1-carboxylate **(51)***. From compound **41** and 1-Bocpiperidin-4-amine, procedure **9**, yield 94%, light yellow solid.

*4.1.3.44. 1-(2-(2-Methoxy-4-nitrophenoxy)ethyl)piperazine **(52)***. From compound **29**, procedure **6.2**, yield 85%.

*4.1.3.45. 1-(3-(2-Methoxy-4-nitrophenoxy)propyl)piperazine.trifluoroacetic acid **(53)***. Prepare from **30** following the general procedure **6.2** to give a pale yellow oil (93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, J = 2.58, 8.79 Hz, 1H), 7.82 (d, J = 2.58 Hz, 1H), 7.14 (d, J = 8.97 Hz, 1H), 4.31 (t, J = 5.49 Hz, 2H), 3.94 (s, 3H), 3.64-3.49 (m, 8H), 3.53 (t, J = 7.32 Hz, 2H), 2.37 (quintet, J = 6.60 Hz, 2H).

*4.1.3.46. 1-(4-(2-Methoxy-4-nitrophenoxy)butyl)piperazine **(54)***. Prepare from **31** following the general procedure **6.2** to give a pale yellow oil (95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 2.22, 8.79 Hz, 1H), 7.74 (d, *J* = 2.19 Hz, 1H), 6.90 (d, *J* = 8.97 Hz, 1H), 4.14 (t, *J* = 6.06 Hz, 2H), 3.93 (s, 3H), 3.25-3.22 (m, 4H), 2.84-2.81 (m, 4H), 2.64 (t, *J* = 6.96 Hz, 2H), 1.95-1.90 (quintet, *J* = 4.95 Hz, 2H), 1.75 (quintet, *J* = 6.96 Hz, 2H).

*4.1.3.47. 4-(2-(2-Methoxy-4-nitrophenoxy) ethyl) piperidine **(55)***. Prepare from **28** following the general procedure **6.2** to give a pale yellow oil (90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, *J* = 2.76, 8.97 Hz, 1H), 7.74 (d, *J* = 2.76 Hz, 1H), 6.90 (d, *J* = 8.97 Hz, 1H), 4.15 (t, *J* = 6.75 Hz, 2H), 3.94 (s, 3H), 3.17 (d, *J* = 12.27 Hz, 2H), 2.72-2.62 (m, 2H), 1.87 (q, *J* = 6.57 Hz, 2H), 1.81-1.73 (m, 3H), 1.45 (s, 9H), 1.23-1.14 (m, 2H).

*4.1.3.48. 2-(2-Methoxy-4-nitrophenoxy)-1-(piperazin-1-yl)ethan-1-one **(56)***. Prepare from **46** following the general procedure **6.2** to get desired product as a pale yellow semi liquid (98%). ^{1}H NMR (300 MHz, DMSO) δ 8.98 (br, 1H), 7.87 (dd, *J* = 2.73, 8.97 Hz, 1H), 7.76 (d, *J* = 2.55 Hz, 1H), 7.10 (d, *J* = 8.97 Hz, 1H), 5.11 (s, 2H), 3.90 (s, 3H), 3.66-3.64 (m, 4H), 3.20-3.16 (m, 4H)

*4.1.3.49. 2-(2-Methoxy-4-nitrophenoxy)-N-(piperidin-4-yl)acetamide **(57)***. Prepare from **49** following the general procedure **6.2** to get desired product as a pale yellow semi liquid (95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 2.55, 8.97 Hz, 1H), 7.80 (d, *J* = 2.55 Hz, 1H), 6.94 (d, *J* = 8.79 Hz, 1H), 6.70 (br, 1H), 4.58 (s, 2H), 3.98 (s, 3H), 3.95-3.93 (m, 1H), 3.09-3.05 (m, 2H), 2.75 (t, *J* = 11.52 Hz, 2H), 1.96-1.92 (m, 2H), 1.44-1.35 (m, 2H).

*4.1.3.50. 2-(4-(2-(2-Methoxy-4-nitrophenoxy)ethyl)piperazin-1-yl)ethyl 4-methoxybenzoate **(58)***. From 2-bromoethyl 4-methoxybenzoate and compound **52**, procedure **10**, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.97 Hz, 2H), 7.89 (dd, *J* = 8.79, 2.58 Hz, 1H), 7.74 (d, *J* = 2.55 Hz, 1H), 6.94-6.90 (m, 3H), 4.43 (t, *J* = 6.03 Hz, 2H), 4.24 (t, *J* = 6.03 Hz, 2H), 3.94 (s, 3H), 3.86 (s, 3H), 2.89 (t, *J* = 6.06 Hz, 2H), 2.79 (t, *J* = 6.06 Hz, 2H), 2.64 (br, 8H).

4.1.3.51. tert-Butyl 2-(4-(2-(2-methoxy-4-nitrophenoxy)ethyl)piperazin-1-yl)ethylcarbamate ***(59)***. From 2-(boc-amino)ethyl bromide and compound **52**, procedure **10**, yield 33%, white solid.

4.1.3.52. 2-(2-(4-(2-(2-Methoxy-4-nitrophenoxy)ethyl)piperazin-1-yl)ethoxy)ethyl 4-methoxybenzoate ***(60)***. From 2-(2-bromoethoxy)ethyl 4-methoxybenzoate and compound **52**, procedure **10**, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (d, J = 8.79 Hz, 2H), 7.89 (dd, J = 9.00, 2.58 Hz, 1H), 7.74 (d, J = 2.55 Hz, 1H), 6.93-6.89 (m, 3H), 4.44 (t, J = 4.92 Hz, 2H), 4.21 (t, J = 6.03 Hz, 2H), 3.93 (s, 3H), 3.85 (s, 3H), 3.78 (t, J = 4.95 Hz, 2H), 3.67 (t, J = 5.67 Hz, 2H), 2.87 (t, J = 6.21 Hz, 2H), 2.62 (br, 10H).

4.1.3.53. tert-Butyl 2-(2-(4-(2-(2-methoxy-4-nitrophenoxy)ethyl)piperazin-1-yl)ethoxy)ethylcarbamate ***(61)***. From *tert*-butyl *N*-(2-(2-bromoethoxy)ethyl)carbamate and compound **52**, procedure **10**, light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, J = 8.79, 2.58 Hz, 1H), 7.74 (d, J = 2.76 Hz, 1H), 6.91 (d, J = 8.79 Hz, 1H), 5.19 (br, 1H), 4.24 (t, J = 6.21 Hz, 2H), 3.91 (s, 3H), 3.58 (t, J = 5.67 Hz, 2H), 3.51 (t, J = 4.95 Hz, 2H), 3.30 (q, J = 4.92 Hz, 2H), 2.90 (t, J = 6.03 Hz, 2H), 2.66 (br, 4H), 2.59 (t, J = 5.70 Hz, 6H), 1.45 (s, 9H).

4.1.3.54. 1-(4-(2-(2-Methoxy-4-nitrophenoxy)ethyl)piperazin-1-yl)ethanone ***(62)***. The solution of **52** in DCM was added excess acetyl chloride and TEA at 0-5 ^{o}C. The mixture reaction was stirred at room temperature for 1 h, then diluted with DCM and quenched by water. The organic layer was washed with water, concentrated and purified by silica gel to get light yellow solid, yield 93%.

4.1.3.55. 1-(2-((tert-Butyldimethylsilyl)oxy)ethyl)-4-(3-(2-methoxy-4-nitrophenoxy)propyl)piperazine ***(63)***. Prepare from **53** following the general procedure **10** to afford red solid (65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, J = 2.60, 8.96 Hz, 1H), 7.72 (d, J = 2.56 Hz, 1H), 6.92 (d, J = 8.96 Hz, 1H), 4.17 (t, J = 6.56 Hz, 2H), 3.91 (s, 3H), 3.75 (t, J = 6.36 Hz, 2H), 2.53-2.49 (m, 12H), 2.06 (quintet, J = 6.80 Hz, 2H), 0.86 (s, 9H), 0.03 (s, 6H).

4.1.3.56. tert-Butyl (2-(4-(3-(2-methoxy-4-nitrophenoxy)propyl)piperazin-1-yl)ethyl)carbamate ***(64)***. Prepare from **53** following the general procedure **10** to afford red solid (47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, J = 2.58, 8.79 Hz, 1H), 7.74 (d, J = 2.58 Hz, 1H), 6.95 (d, J = 8.97 Hz, 1H), 4.97 (br, 1H), 4.20 (t, J = 6.39 Hz, 2H), 3.94 (s, 3H), 3.24 (q, J = 6.96 Hz, 2H), 2.58-2.51 (m, 12H), 2.10 (quintet, 2H), 1.45 (s, 9H).

4.1.3.57. tert-Butyl (2-(4-(4-(2-methoxy-4-nitrophenoxy)butyl)piperazin-1-yl)ethyl)carbamate ***(65)***. Prepare from **54** following the general procedure 10 to afford yellow semi solid (38%).

4.1.3.58. 1-(2-((tert-Butyldimethylsilyl)oxy)ethyl)-4-(4-(2-methoxy-4-nitrophenoxy)butyl)piperazine ***(66).*** Prepare from **54** following the general procedure **10** to afford yellow semi solid (40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, J = 2.52, 8.96 Hz, 1H), 7.71 (d, J = 2.52 Hz, 1H), 6.88 (d, J = 8.92 Hz, 1H), 4.12 (t, J = 6.64 Hz, 2H), 3.91 (s, 3H), 3.74 (t, J = 6.40 Hz, 2H), 2.52-2.49 (m, 10H), 2.40 (t, J = 7.16 Hz, 2H), 1.92 (quintet, J = 4.95 Hz, 2H), 1.69 (quintet, J = 6.36 Hz, 2H), 0.86 (s, 9H), 0.03 (s, 6H).

4.1.3.59. 1-(2-(tert-Butyldimethylsilyloxy) ethyl)-4-(2-(2-methoxy-4-nitrophenoxy) ethyl) piperidine ***(67)***. Prepare from **55** following the general procedure **10** to afford a yellow semi solid (42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, J = 2.73, 8.97 Hz, 1H), 7.68 (d, J = 2.55 Hz, 1H), 6.83 (d, J = 8.97 Hz, 1H), 4.11-4.06 (m, 4H), 3.88 (s, 3H), 3.74 (t, J = 4.77 Hz, 2H) 2.72-2.62 (m, 2H), 1.87 (q, J = 6.6 Hz, 2H), 1.71-1.67 (m, 3H), 1.21-1.13 (m, 4H), 0.82 (s, 9H), 0.03 (s, 6H)

4.1.3.60. tert-Butyl 2-(4-(2-(2-methoxy-4-nitrophenoxy)ethyl)piperidin-1-yl)ethylcarbamate ***(68)***. Prepare from **55** following the general procedure **10** to afford yellow semi solid (43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, J = 2.55, 8.79 Hz, 1H), 7.75 (d, J = 2.55 Hz, 1H), 6.93 (d, J = 8.97 Hz, 1H), 5.04 (s, NH), 4.16 (t, J = 6.24 Hz, 2H), 3.94 (s, 3H), 3.24 (br, 2H), 2.47 (t, J = 6.06 Hz, 2H), 2.03-1.99 (m, 2H), 1.87 (quintet, J = 6.42 Hz, 4H), 1.77-1.73 (m, 3H), 1.45 (s, 9H), 1.40-1.35 (m, 2H).

4.1.3.61. 1-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-2-(2-methoxy-4-nitrophenoxy)ethan-1-one ***(69)***. Prepare from **56** following the general procedure **10** to get 353 mg (78%) of white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (dd, J = 2.55, 9.00 Hz, 1H), 7.71 (d, J = 2.76 Hz, 1H), 6.92 (d, J = 8.79 Hz, 1H), 4.81 (s, 2H), 3.90 (s, 3H), 3.71 (t, J = 5.88 Hz, 2H,), 3.57-3.49 (m, 2H), 3.53-3.50 (m, 2H), 2.50-2.42 (m, 6H), 0.80 (s, 9H), 0.02 (s, 6H).

4.1.3.62. tert-Butyl (2-(4-(2-(2-methoxy-4-nitrophenoxy)acetyl)piperazin-1-yl)ethyl)carbamate ***(70)***. Prepare from **56** following the general procedure **10** to give a white solid (31%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, J = 2.58, 8.97 Hz, 1H), 7.77 (d, J = 2.55 Hz, 1H), 6.98 (d, J = 8.97 Hz, 1H), 4.86 (s, 2H), 3.96 (s, 3H), 3.61-3.58 (m, 4H), 3.24-3.20 (m, 2H), 2.46-2.43 (m, 6H), 1.45 (s, 9H)

4.1.3.63. N-(1-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperidin-4-yl)-2-(2-methoxy-4-nitrophenoxy)acetamide ***(71)***. Prepare from **57** following the general procedure **10** to give an opaque semi liquid (45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (dd, J = 2.40, 8.79 Hz, 1H), 7.74 (d, J = 2.37 Hz, 1H), 6.87 (d, J = 8.79 Hz, 1H), 6.65 (br, 1H), 4.51 (s, 2H), 3.92 (s, 3H), 3.88-3.84 (m, 1H), 3.71 (t, J = 6.21 Hz, 2H), 2.82-2.78 (m, 2H), 2.51 (t, J = 6.24 Hz, 2H), 1.87-1.82 (m, 4H), 1.49-1.46 (m, 2H), 0.83 (s, 9H), 0.02 (s, 6H)

4.1.3.64. tert-Butyl (2-(4-(2-(2-methoxy-4-nitrophenoxy)acetamido)piperidin-1-yl)ethyl)carbamate ***(72)***. Prepare from **57** following the general procedure **5** to get pale semi solid (51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, J = 2.37, 8.97 Hz, 1H), 7.80 (d, J = 2.40 Hz, 1H), 6.93 (d, J = 8.79 Hz, 1H), 6.68 (br, 1H), 4.93 (br, 1H), 4.57 (s, 2H), 3.99 (s, 3H), 3.94-3.91 (m, 1H), 3.21 (q, J = 6.96 Hz, 2H), 2.83-2.79 (m, 2H), 2.47 (t, J = 6.06 Hz, 2H), 2.19 (t, J = 10.80 Hz, 2H), 1.96-1.92 (m, 2H), 1.52-1.46 (m, 2H), 1.45 (s, 9H).

4.1.3.65. 2-(2-(2-methoxy-4-nitrophenoxy)ethyl)isoindoline-1,3-dione ***(73)***. Prepare from 4-nitroguaiacol **21** following the general procedure **5** to afford product as a white solid (87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85-7.83 (m, 3H), 7.73-7.71 (m, 2H), 7.68 (d, J = 2.76 Hz, 1H), 6.93 (d, J = 8.7 Hz, 1H), 4.36 (t, J = 6.0 Hz, 2H), 4.15 (d, J = 6.42 Hz, 2H), 3.84 (s, 3H).

*4.1.3.66. 2-(2-methoxy-4-nitrophenoxy)ethanamine **(74)***. Prepare from **73** (530 mg, 1.55 mmol) was dissolved in 5 mL mixture of ethanol and dichloromethane (4/1 = v/v), $N_2H_4.H_2O$ (0.3 mL, 6.2 mmol) was added dropwise. The mixture was stirred at room temperature for 2 hours. A white precipitate formed. The precipitation was removed by vacuum filtration through a filter. The solid was wash with EtOH (20 mL x 3). The filtrate was collected and concentrated by rotary evaporation and purified by column chromatography ($MeOH/CH_2Cl_2$ = 1/9) to give desired product as yellow solid (78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (dd, J = 2.80, 8.72 Hz, 1H), 7.80 (d, J = 2.60 Hz, 1H), 7.11 (d, J = 8.92 Hz, 1H), 4.14 (t, J = 5.32 Hz, 2H), 3.93 (s, 3H), 3.06 (t, J = 5.32 Hz, 2H).

*4.1.3.67. t-Butyl 4-(2-(2-methoxy-4-nitrophenoxy)ethylcarbamoyl)piperidine-1-carboxylate **(75)***. Prepare from **74** following the general procedure **9** to afforded the desired amide (84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (dd, J = 2.56, 8.92 Hz, 1H), 7.74 (d, J = 2.56 Hz, 1H), 6.91 (d, J = 8.96 Hz, 1H), 6.04 (br, NH) 4.19 (t, J = 5.08 Hz, 2H), 4.16 (br, 2H), 3.93 (s, 3H), 3.73 (q, J = 5.36 Hz, 2H), 2.75 (t, J = 11.72 Hz, 2H), 2.27-2.20 (m, 1H), 1.82-1.79 (m, 2H), 1.65-1.56 (m, 2H), 1.42 (s, 9H).

*4.1.3.68. tert-Butyl 4-methylpyridin-2-ylcarbamate **(77)***. From 2-amino-4-picoline **76**, procedure **6.1**, yield 77%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.14 (d, J = 5.13 Hz, 1H), 7.82 (s, 1H), 6.78 (d, J = 5.13 Hz, 1H), 2.35 (s, 3H), 1.54 (s, 9H).

*4.1.3.69. tert-Butyl 4-(4-(tert-butyldimethylsilyloxy)butyl)pyridin-2-ylcarbamate **(78)***. From compound **77**, yield 94%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, J = 5.13 Hz, 1H), 7.75 (s, 1H), 7.70 (br, 1H), 6.75 (dd, J = 5.31, 1.47 Hz, 1H), 3.58 (t, J = 6.42 Hz, 2H), 2.57 (t, J = 7.32 Hz, 2H), 1.73-1.62 (m, 2H), 1.59-1.46 (m, 2H), 1.49 (s, 9 H), 0.85 (s, 9H), 0.06 (s, 6H).

*4.1.3.70. tert-Butyl 4-(4-(2-methoxy-4-nitrophenoxy)butyl)pyridin-2-ylcarbamate **(79)***. Compound **78** was first de protected group by tetra-n-butylammonium fluoride in THF then following the general procedure **3** to get light yellow solid product, yield 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, J = 5.13 Hz, 1H), 7.89 (dd, J = 8.79, 2.58 Hz, 1H), 7.82 (s, 1H), 7.74 (d, J = 2.55 Hz, 1H), 7.72 (br, 1H), 6.87 (d, J = 8.97

Hz, 1H), 6.81 (dd, *J* = 5.13, 1.47 Hz, 1H), 4.12 (t, *J* = 6.03 Hz, 2H), 3.94 (s, 3H), 2.70 (t, *J* = 7.32 Hz, 2H), 1.95-1.84 (m, 4H), 1.53 (s, 9H).

*4.1.3.71. tert-Butyl (4-(4-(2-methoxy-4-nitrophenoxy)butyl)pyridin-2-yl)(methyl)carbamate **(80)***. Prepare from **79** following the general procedure **10** to afford pale yellow solid (64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, *J* = 5.13 Hz, 1H), 7.91 (dd, *J* = 2.76, 9.15 Hz, 1H), 7.74 (d, *J* = 2.85 Hz, 1H), 7.55 (s, 1H), 6.89-6.86 (m, 3H), 4.11 (t, *J* = 6.42 Hz, 2H), 3.94 (s, 3H), 3.41 (s, 3H), 2.72 (t, *J* = 7.71 Hz, 2H), 1.93-1.84 (m, 4H), 1.52 (s, 9H).

*4.1.3.72. tert-Butyl (4-(4-(2-methoxy-4-nitrophenoxy)butyl)pyridin-2-yl)(methyl)carbamate **(81)***. Prepare from **79** following the general procedure **10** to afford pale yellow solid (53%).

*4.1.3.73. tert-Butyl (2-((tert-butoxycarbonyl)amino)ethyl)(4-(4-(2-methoxy-4-nitrophenoxy)butyl)pyridin-2-yl)carbamate **(82)***. Prepare from **79** following the general procedure **10** to afford red solid product (52%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, *J* = 4.95 Hz, 1H), 7.41 (s, 1H), 6.87 (dd, *J* = 1.47, 5.13 Hz, 1H), 6.72 (d, *J* = 8.40 Hz, 1H), 6.31 (d, *J* = 2.58 Hz, 1H), 6.23 (dd, *J* = 2.55, 8.43 Hz, 1H), 4.08 (t, *J* = 6.60 Hz, 2H), 3.95 (t, *J* = 6.03 Hz, 2H), 3.93 (s, 3H), 3.79 (t, *J* = 6.42 Hz, 2H), 2.68 (t, *J* = 7.14 Hz, 2H), 1.83-1.79 (m, 4H), 1.51 (s, 9H), 1.41 (s, 9H).

*4.1.3.74. tert-Butyl 4-(4-(4-amino-2-methoxyphenoxy)phenyl)-3,6-dihydropyridine-1(2H)-carboxylate **(83)***. Prepare from compound **9** following the general procedure **4.1** to get product as opaque solid (65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.61 Hz, 2H), 6.85 (d, *J* = 8.61 Hz, 2H), 6.36 (d, *J* = 2.55 Hz, 1H), 6.28 (dd, *J* = 2.55, 8.43 Hz, 1H), 6.25 (s, 1H), 5.93-5.90 (m, 1H), 4.05-4.02 (m, 2H), 3.75 (s, 3H), 3.64 (t, *J* = 6.69 Hz, 2H), 2.48-2.45 (m, 2H), 1.48 (s, 9H).

*4.1.3.75. tert-Butyl 4-(3-((4-amino-2-methoxyphenoxy)methyl)phenyl)piperidine-1-carboxylate **(84)***. From compound **11**, procedure **4.1**; yield 93%, red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29-7.26 (m, 2H), 7.13-7.11 (m, 2H), 6.70 (d, *J* = 8.43 Hz, 1H), 6.32 (d, *J* = 2.55 Hz, 1H), 6.16 (dd, *J* = 8.40, 2.55 Hz, 1H), 5.01 (s, 2H), 4.22 (br,

2H), 3.84 (s, 3H), 2.75 (t, *J* = 13.17 Hz, 2H), 2.65 (tt, *J* = 12.06, 1H), 1.83-1.79 (m, 2H), 1.69-1.53 (m, 2H), 1.42 (s, 9H).

*4.1.3.76. tert-Butyl 4-(3-((4-amino-2-methoxyphenoxy)methyl)phenyl)piperazine-1-carboxylate **(85)***. From compound **20**, procedure 4.1, yield 78%, red solid.

*4.1.3.77. tert-Butyl 4-(4-(4-amino-2-methoxyphenoxy)phenyl)piperidine-1-carboxylate **(86)***. Prepare from compound **9** following the general procedure **4.2** to get product as red semi solid (81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08-7.05 (m, 3H), 6.85 (d, *J* = 8.64 Hz, 2H), 6.36 (d, *J* = 2.58 Hz, 1H), 6.27 (dd, *J* = 2.76, 8.43 Hz, 1H), 4.20 (t, *J* = 10.08 Hz, 2H), 3.76 (s, 3H), 2.77 (t, *J* = 11.52 Hz, 2H), 2.58-2.53 (m, 1H), 1.81 (m, 2H), 1.54 (m, 2H), 1.45 (s, 9H).

*4.1.3.78. tert-Butyl 4-(4-(4-amino-2-methoxyphenoxy)phenyl)piperazine-1-carboxylate **(87)***. Prepared from compound **15** following the general producer **4.2** to afford a red solid (99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84-6.80 (m, 4H), 6.79 (d, *J* = 8.43 Hz, 1H), 6.36 (d, *J* = 2.40 Hz, 1H), 6.25 (dd, *J* = 2.55, 8.40 Hz, 1H), 3.77 (s, 3H), 3.58 (t, *J* = 4.95 Hz, 4H), 3.03 (t, *J* = 4.56 Hz, 4H), 1.47 (s, 9H).

*4.1.3.79. 3- Methoxy-4-(4-(4-methylpiperazin-1-yl)phenoxy)aniline **(88)***. Prepare from compound **16** following the general procedure **4.2** to get titled product as red solid (96%).

*4.1.3.80. Methyl 2-(4-amino-2-methoxyphenoxy)acetate **(89)***. Prepare from compound **25** following the general procedure **4.2** to to afford desired product as yellow solid (99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (d, *J* = 8.40 Hz, 1H), 6.29 (s, 1H), 6.20 (d, *J* = 8.43 Hz, 1H), 4.59 (s, 2H), 3.82 (s, 3H), 3.78 (s, 3H).

*4.1.3.81. tert-Butyl 4-(2-(4-amino-2-methoxyphenoxy)ethyl)-3-methylpiperazine-1-carboxylate **(90)***. From compound **32**, procedure **4.2**, yield 99%, pink oil.

*4.1.3.82. tert-Butyl 4-(2-(4-amino-2-methoxyphenoxy)ethyl)-2,6-dimethylpiperazine-1-carboxylate **(91)***. From compound **33**, procedure **4.2**; yield 99%, pink solid.

4.1.3.83. 3-Methoxy-4-(2-(4-phenylpiperazin-1-yl)ethoxy)aniline ***(92)***. From compound **34**, procedure **4.2**, yield 92%, red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (t, J = 7.97 Hz, 2H), 6.94 (d, J = 7.86 Hz, 2H), 6.86 (t, J = 7.32 Hz, 1H), 6.77 (d, J = 8.25 Hz, 1H), 6.30 (d, J = 2.55 Hz, 1H), 6.21 (dd, J = 8.25, 2.37 Hz, 1H), 4.11 (t, J = 6.03 Hz, 2H), 3.82 (s, 3H), 3.24 (t, J = 4.95 Hz, 4H), 2.86 (t, J = 6.21 Hz, 2H), 2.75 (t, J = 4.95 Hz, 4H).

4.1.3.84. 4-(2-(4-Benzylpiperazin-1-yl)ethoxy)-3-methoxyaniline ***(93)***. Prepare from **35** following the general procedure **4.2** to afford desired product (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.30 (m, 5H), 6.74 (d, J = 8.25 Hz, 1H), 6.28 (s, 1H), 6.21 (d, J = 8.25 Hz, 1H), 4.07 (t, J = 5.85 Hz, 2H), 3.79 (s, 3H), 3.51 (s, 2H), 2.81 (t, J = 6.21 Hz, 2H), 2.60 (br, 4H), 2.51 (br, 4H).

4.1.3.85. 3-Methoxy-4-(2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethoxy)aniline ***(94)***. From compound **36**, procedure **4.2**, yield 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 4.80 Hz, 2H), 6.77 (d, J = 8.40 Hz, 1H), 6.48 (t, J = 4.80 Hz, 1H), 6.30 (d, J = 4.60 Hz, 1H), 6.21 (dd, J = 8.40, 2.60 Hz, 1H), 4.11 (t, J = 6.00 Hz, 2H), 3.86 (t, J = 5.00 Hz, 4H), 3.81 (s, 3H), 2.84 (t, J = 6.00 Hz, 2H), 2.64 (t, J = 5.00 Hz, 4H).

4.1.3.86. 4-(2-(4-(5-Fluoropyrimidin-2-yl)piperazin-1-yl)ethoxy)-3-methoxyaniline ***(95)***. From compound **37**, procedure **4.2**, yield 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 2H), 6.77 (d, J = 8.40 Hz, 1H), 6.30 (d, J = 2.64 Hz, 1H), 6.21 (dd, J = 8.20, 2.60 Hz, 1H), 4.10 (t, J = 6.00 Hz, 2H), 3.81-3.77 (m, 7H), 2.83 (t, J = 6.00 Hz, 2H), 2.64 (t, J = 5.04 Hz, 4H).

4.1.3.87. 4-(2-(4-(5-Chloropyrimidin-2-yl)piperazin-1-yl)ethoxy)-3-methoxyaniline ***(96).*** From compound **38**, procedure **4.2**, yield 36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 2H), 6.76 (d, J = 8.40 Hz, 1H), 6.30 (d, J = 2.64 Hz, 1H), 6.21 (dd, J = 8.24, 2.60 Hz, 1H), 4.10 (t, J = 6.00 Hz, 2H), 3.81-3.77 (m, 7H), 2.83 (t, J = 6.00 Hz, 2H), 2.64 (t, J = 5.00 Hz, 4H).

4.1.3.88. 3-(4-Amino-2-methoxyphenoxy)propanamide ***(97)***. From compound **42**, procedure **4.2**, yield 66%, red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (d, J = 8.43

Hz, 1H), 6.30 (d, *J* = 2.55 Hz, 1H), 6.22 (dd, *J* = 8.25, 2.58 Hz, 1H), 5.43 (br, 2H), 4.15 (t, *J* = 5.49 Hz, 2H), 3.81 (s, 3H), 2.68 (t, *J* = 5.49 Hz, 2H).

*4.1.3.89. 4-(4-Amino-2-methoxyphenoxy)butanamide **(98)***. Prepare from compound **43** and solution of NH_3 in THF following the general procedure **4.2**, (96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (d, *J* = 8.40 Hz, 1H), 6.30 (d, *J* = 2.55 Hz, 1H), 6.23 (dd, *J* = 2.73, 8.40 Hz, 1H), 5.96 (br, NH), 5.27 (br, NH), 4.00 (t, *J* = 6.03 Hz, 2H), 3.80 (s, 3H), 2.49 (t, *J* = 6.93 Hz, 2H), 2.14 (quintet, *J* = 5.85 Hz, 2H).

*4.1.3.90. 3-(4-Amino-2-methoxyphenoxy)-N,N-dimethylpropanamide **(99)***. From compound **44**, procedure **4.2**, yield 88%, red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (d, *J* = 8.43 Hz, 1H), 6.30 (d, *J* = 2.55 Hz, 1H), 6.22 (dd, *J* = 8.25, 2.55 Hz, 1H), 4.27 (t, *J* = 7.32 Hz, 2H), 3.81 (s, 3H), 3.04 (s, 3H), 3.96 (s, 3H), 2.85 (t, *J* = 7.35 Hz, 2H).

*4.1.3.91. 4-(4-Amino-2-methoxyphenoxy)-N,N-dimethylbutanamide **(100)***. Prepare from compound **45** following the general procedure **4.2** to afford product (97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (d, *J* = 8.22 Hz, 1H), 6.30 (d, *J* = 2.55 Hz, 1H), 6.22 (dd, *J* = 2.73, 8.40 Hz, 1H), 4.01 (t, *J* = 6.21 Hz, 2H), 3.80 (s, 3H), 3.01 (s, 3H), 2.94 (s, 3H), 2.56 (t, *J* = 7.50 Hz, 2H), 2.12 (quintet, *J* = 7.68 Hz, 2H).

*4.1.3.92. tert-Butyl 4-(2-(4-amino-2-methoxyphenoxy)acetyl)piperazine-1-carboxylate **(101)***. From compound **46**, procedure **4.2**, yield 95% as red solid.

*4.1.3.93. tert-Butyl 4-(3-(4-amino-2-methoxyphenoxy)propanoyl)piperazine-1-carboxylate **(102)***. From compound **47**, procedure **4.2**, yield 88%, red solid.

*4.1.3.94. tert-Butyl 4-(4-(4-amino-2-methoxyphenoxy)butanoyl)piperazine-1-carboxylate **(103)***. Prepare from compound **48** following the general procedure **4.2** to afford product (99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (d, *J* = 8.43 Hz, 1H), 6.30 (d, *J* = 2.58 Hz, 1H), 6.22 (dd, *J* = 2.58 Hz, 1H), 4.01 (t, *J* = 5.85 Hz, 2H), 3.80 (s, 3H), 3.59-3.51 (m, 4H), 3.45-3.41 (m, 4H), 2.58 (t, *J* = 7.14 Hz, 2H), 2.12 (quintet, *J* = 7.32 Hz, 2H), 1.47 (s, 9H).

*4.1.3.95. tert-Butyl 4-(2-(4-amino-2-methoxyphenoxy)acetamido)piperidine-1-carboxylate **(104)***. Prepare from **49** following the general procedure **4.2** to afford a red solid (86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (br, 1H), 6.76 (d, J = 8.43 Hz, 1H), 6.29 (d, J = 2.55 Hz, 1H), 6.23 (dd, J = 2.37, 8.25 Hz, 1H), 4.43 (s, 2H), 3.98-3.88 (m, 3H), 3.84 (s, 3H), 2.93 (t, J = 12.63 Hz, 2H), 1.92-1.88 (m, 2H), 1.46 (s, 9H), 1.36-1.33 (m, 2H)

*4.1.3.96. tert-Butyl 4-(3-(4-amino-2-methoxyphenoxy)propanamido)piperidine-1-carboxylate **(105)***. Prepare from **50** following the general procedure **4.2** to afford product, yield (89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (d, J = 8.40 Hz, 1H), 6.30 (d, J = 2.58 Hz, 1H), 6.23 (dd, J = 2.37, 8.22 Hz, 1H), 4.15 (t, J = 5.67 Hz, 2H), 4.01-3.98 (m, 3H), 3.80 (s, 3H), 2.93 (t, J = 11.55 Hz, 2H), 2.65 (t, J = 5.67 Hz, 2H), 1.92-1.87 (m, 2H), 1.45 (s, 9H), 1.35-1.31 (m, 2H).

*4.1.3.97. tert-Butyl 4-(4-(4-amino-2-methoxyphenoxy)butanamido)piperidine-1-carboxylate **(106)***. From compound **51**, procedure **4.2**, yield 90%, red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (d, J = 8.32 Hz, 1H), 6.28 (d, J = 2.48 Hz, 1H), 6.19 (dd, J = 8.40, 2.52 Hz, 1H), 5.82 (d, J = 7.68 Hz, 1H), 3.95-3.87 (m, 5H), 3.79 (s, 3H), 2.80 (t, J = 11.92 Hz, 2H), 2.37 (t, J = 7.08 Hz, 2H), 2.06 (quintet, J = 6.40 Hz, 2H), 1.81 (br, 2H), 1.43 (s, 9H), 1.23-1.17 (m, 2H).

*4.1.3.98. 2-(4-(2-(4-Amino-2-methoxyphenoxy)ethyl)piperazin-1-yl)ethyl 4-methoxybenzoate **(107)***. From compound **52**, procedure **4.2**, yield 62%, pink oil.

*4.1.3.99. tert-Butyl 2-(4-(2-(4-amino-2-methoxyphenoxy)ethyl)piperazin-1-yl)ethylcarbamate **(108)***. From compound **59**, procedure **4.2**, yield 99%, pink solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (d, J = 8.43 Hz, 1H), 6.30 (d, J = 2.58 Hz, 1H), 6.20 (dd, J = 8.43, 2.58 Hz, 1H), 4.99 (s, 1H), 4.06 (t, J = 6.03 Hz, 2H), 3.81 (s, 3H), 3.22 (br, 2H), 2.80 (t, J = 6.03 Hz, 2H), 2.61 (br, 4H), 2.47 (br, 6H), 1.45 (s, 9H).

*4.1.3.100. 2-(2-(4-(2-(4-Amino-2-methoxyphenoxy)ethyl)piperazin-1-yl)ethoxy)ethyl 4-methoxybenzoate **(109)***. From compound **60**, procedure **4.2**, pink oil.

4.1.3.101. tert-Butyl 2-(2-(4-(2-(4-amino-2-methoxyphenoxy)ethyl)piperazin-1-yl)ethoxy)ethylcarbamate ***(110)***. From compound **61**, procedure **4.2**, yield 80%, pink oil.

4.1.3.102. 1-(4-(2-(4-Amino-2-methoxyphenoxy)ethyl)piperazin-1-yl)ethanone ***(111)***. From compound **62**, procedure **4.2**, yield 99%, red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (d, J = 8.43 Hz, 1H), 6.30 (d, J = 2.37 Hz, 1H), 6.21 (dd, J = 8.34, 2.37 Hz, 1H), 4.06 (t, J = 5.67 Hz, 2H), 3.80 (s, 3H), 3.65 (t, J = 4.56 Hz, 4H), 3.49 (t, J = 4.95 Hz, 4H), 2.80 (t, J = 5.85 Hz, 2H), 2.61-2.53 (m, 4H), 2.09 (s, 3H).

4.1.3.103. 4-(3-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)propoxy)-3-methoxyaniline ***(112)***. Prepare from **63** following the general procedure **4.2** to afford red solid (80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.69 (d, J = 8.22 Hz, 1H), 6.24 (d, J = 2.58 Hz, 1H), 6.17 (dd, J = 2.04, 7.99 Hz, 1H), 3.93 (t, J = 5.57 Hz, 2H), 3.75 (s, 3H), 3.72 (t, J = 6.57 Hz, 2H), 2.49-2.45 (m, 12H), 1.95 (q, J = 7.86 Hz, 2H), 0.83 (s, 9H), 0.02 (s, 6H).

4.1.3.104. tert-Butyl (2-(4-(3-(4-amino-2-methoxyphenoxy)propyl)piperazin-1-yl)ethyl)carbamate ***(113)***. Prepare from **64** following the general procedure **4.2** to afford red solid (92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (d, J = 6.57 Hz, 1H), 6.29 (d, J = 2.55 Hz, 1H), 6.22 (dd, J = 2.61, 8.85 Hz, 1H), 4.96 (br, 1H), 3.97 (t, J = 6.39 Hz, 2H), 3.82 (s, 3H), 3.21-3.18 (m, 2H), 2.46-2.40 (m, 12H), 1.96 (quintet, J = 7.14 Hz, 2H), 1.45 (s, 9H).

4.1.3.105. tert-Butyl (2-(4-(4-(4-amino-2-methoxyphenoxy)butyl)piperazin-1-yl)ethyl)carbamate ***(114)***. Prepare from **65** following the general procedure **4.2** to afford red solid (91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.71 (d, J = 8.43 Hz, 1H), 6.28 (d, J = 2.37 Hz, 1H), 6.20 (dd, J = 2.55, 8.43 Hz, 1H), 4.94 (br, NH), 3.93 (t, J = 6.60 Hz, 2H), 3.78 (s, 3H), 3.18 (q, J = 6.66 Hz, 2H), 2.44-2.34 (m, 12H), 1.76 (quintet, J = 7.14 Hz, 2H), 1.67 (quintet, J = 6.96 Hz, 2H), 1.43 (s, 9H).

4.1.3.106. 4-(4-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)butoxy)-3-methoxyaniline ***(115)***. Prepare from **66** following the general procedure **4.2** to afford red solid (91%).

*4.1.3.107. 4-(2-(1-(2-(tert-Butyldimethylsilyloxy)ethyl)piperidin-4-yl)ethoxy)-3-methoxyaniline **(116)***. Prepare from **67** following the general procedure **4.2** to afford a red solid (73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.72 (d, J = 8.40 Hz, 1H), 6.30 (d, J = 2.55 Hz, 1H), 6.22 (dd, J = 2.55, 8.40 Hz, 1H), 3.97 (t, J = 6.78 Hz, 2H), 3.80 (s, 3H), 3.78 (t, J = 7.14 Hz, 2H), 2.51 (br, 2H), 2.07 (br, 2H), 1.73-1.63 (m, 6H), 1.20-1.13 (m, 3H), 0.88 (s, 9H), 0.05 (s, 6H)

*4.1.3.108. tert-Butyl 2-(4-(2-(4-amino-2-methoxyphenoxy)ethyl)piperidin-1-yl)ethylcarbamate **(117)***. Prepare from **68** following the general procedure **4.2** to afford a red solid (75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.73 (d, J = 8.97 Hz, 1H), 6.29 (d, J = 2.55 Hz, 1H), 6.22 (dd, J = 2.40, 8.40 Hz, 1H), 3.95 (t, J = 7.14 Hz, 2H), 3.80 (s, 3H), 3.25 (br, 2H), 2.90 (br, 2H), 2.46 (br, 2H), 2.05 (br, 2H), 1.73-1.63 (m, 5H), 1.45 (s, 9H), 1.25-1.14 (m, 2H)

*4.1.3.109. 2-(4-Amino-2-methoxyphenoxy)-1-(4-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)ethan-1-one **(118)***. Prepare from **69** following the general procedure **4.2** to afford as red solid (91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.75 (d, J = 8.43 Hz, 1H), 6.23 (d, J = 2.55 Hz, 1H), 6.15 (dd, J = 2.58, 8.43 Hz, 1H), 4.56 (s, 2H), 3.75 (s, 3H), 3.71 (t, J = 6.06 Hz, 2H), 3.57-3.54 (m, 4H), 2.48-2.41 (m, 6H), 0.83 (s, 9H), 0.02 (s, 6H).

*4.1.3.110. tert-Butyl (2-(4-(2-(4-amino-2-methoxyphenoxy)acetyl)piperazin-1-yl)ethyl)carbamate **(119)***. Prepare from **70** following the general procedure **4.2** to afford as an red solid (61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (d, J = 8.40 Hz, 1H), 6.27 (d, J = 2.48 Hz, 1H), 6.18 (dd, J = 2.64, 8.48 Hz, 1H), 4.89 (br, 1H), 4.59 (s, 2H), 3.78 (s, 3H), 3.64-3.60 (m, 4H), 3.21-3.19 (m, 2H), 2.44-2.41 (m, 6H), 1.43 (s, 9H)

*4.1.3.111. 2-(4-Amino-2-methoxyphenoxy)-N-(1-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperidin-4-yl)acetamide **(120)***. Prepare from **71** following the general procedure **4.2** to afford red solid (86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (d, J = 8.48 Hz, 1H), 6.26 (d, J = 2.52 Hz, 1H), 6.17 (dd, J = 2.52, 8.48 Hz, 1H), 4.59 (s, 2H), 3.81-3.79 (m, 1H), 3.78 (s, 3H), 3.70 (t, J = 5.32 Hz, 2H), 2.74-2.67 (m, 4H), 1.88-1.85 (m, 4H), 1.32-1.25 (m, 2H), 0.86 (s, 9H), 0.03 (s, 6H).

4.1.3.112. tert-Butyl (2-(4-(2-(4-amino-2-methoxyphenoxy)acetamido)piperidin-1-yl)ethyl)carbamate ***(121)***. Prepare from **72** following the general procedure **4.2** to afford red solid (96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (br, 1H), 6.76 (d, *J* = 8.40 Hz, 1H), 6.30 (d, *J* = 2.55 Hz, 1H), 6.23 (dd, *J* = 2.58, 8.43 Hz, 1H), 4.96 (br, 1H), 4.43 (s, 2H), 3.84 (s, 3H), 3.82-3.80 (m, 1H), 3.20 (q, *J* = 6.66 Hz, 2H), 2.82-2.79 (m, 2H), 2.46 (t, *J* = 5.88 Hz, 2H), 2.17(t, *J* = 10.80 Hz, 2H), 1.94-1.90 (m, 2H), 1.49-1.46 (m, 2H), 1.45 (s, 9H).

4.1.3.113. tert-Butyl 4-(2-(4-amino-2-methoxyphenoxy)ethylcarbamoyl)piperidine-1-carboxylate ***(122)***. Prepare from **75** following the general procedure **4.2** to give product (99%). ^{1}H NMR (500 MHz, CD_3OD) δ 6.74 (d, *J* = 8.40 Hz, 1H), 6.44 (d, *J* = 2.45 Hz, 1H), 6.26 (dd, *J* = 2.55, 8.52 Hz, 1H), 4.09 (d, *J* = 13.25 Hz, 2H), 3.93 (t, *J* = 5.4 Hz, 2H), 3.78 (s, 3H), 3.48 (t, *J* = 5.4 Hz, 2H), 2.78 (br, 2H), 2.41-2.35 (m, 1H), 1.74 (d, *J* = 12 Hz, 2H), 1.59-1.51 (m, 2H), 1.44 (s, 9H).

4.1.3.114. tert-Butyl (4-(4-(4-amino-2-methoxyphenoxy)butyl)pyridin-2-yl)(methyl)carbamate ***(123)***. Prepare from **80** following the general procedure **4.2** to afford a red solid (93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, *J* = 5.13 Hz, 1H), 7.49 (s, 1H), 6.87 (d, *J* = 5.13 Hz, 1H), 6.71 (d, *J* = 8.25 Hz, 1H), 6.30 (d, *J* = 2.58 Hz, 1H), 6.22 (dd, *J* = 2.76, 8.43 Hz, 1H), 3.93 (t, *J* = 6.45 Hz, 2H), 3.80 (s, 3H), 3.38 (s, 3H), 2.66 (t, *J* = 7.53 Hz, 2H), 1.82-1.80 (m, 4H), 1.51 (s, 9H).

4.1.3.115. tert-Butyl (4-(4-(4-amino-2-methoxyphenoxy)butyl)pyridin-2-yl)(2-((tert-butyldimethylsilyl)oxy)ethyl)carbamate ***(124)***. Prepare from **81** following the general procedure **4.2** to afford a red solid (91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, 1H, *J* = 5.10 Hz), 7.41 (s, 1H), 6.87 (d, *J* = 5.13 Hz, 1H), 6.72 (d, *J* = 8.43 Hz, 1H), 6.31 (d, *J* = 2.55 Hz, 1H), 6.23 (dd, *J* = 2.55, 8.40 Hz, 1H), 4.08 (t, *J* = 6.39 Hz, 2H), 3.94 (t, *J* = 5.67 Hz, 2H), 3.81 (s, 3H), 3.79 (t, *J* = 6.42 Hz, 2H), 2.68-2.65 (m, 2H), 1.83-1.79 (m, 4H), 1.50 (s, 9H), 0.82 (s, 9H), 0.01 (s, 6H).

4.1.3.116. tert-Butyl (4-(4-(4-amino-2-methoxyphenoxy)butyl)pyridin-2-yl)(2-((tert-butoxycarbonyl)amino)ethyl)carbamate ***(125)***. Prepare from **82** following the general procedure **4.2** to afford 60 mg as red solid (90%).

4.1.3.117. tert-Butyl 4-(2-(4-(3-(3-(1H-imidazol-1-yl)propyl)thioureido)-2-methoxyphenoxy)ethyl)-3-methylpiperazine-1-carboxylate ***(126)***. From compound **90**, procedure **8**, yield 51%, pink oil.

4.1.3.118. tert-Butyl 4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)ethyl)-2,6-dimethylpiperazine-1-carboxylate ***(127)***. From compound **91**, procedure **8**, yield 50%, white solid.

4.1.3.119. tert-Butyl 2-(4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)ethyl)piperazin-1-yl)ethylcarbamate ***(128)***. From compound **108**, procedure **8**, yield 68%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.37 (d, J = 0.93 Hz, 1H), 6.89 (d, J = 8.25 Hz, 1H), 6.75-6.69 (m, 3H), 5.90 (s, 1H), 4.97 (s, 1H), 4.15 (t, J = 6.03 Hz, 2H), 3.89 (t, J = 7.14 Hz, 2H), 3.84 (s, 3H), 3.66 (q, J = 6.39 Hz, 2H), 3.22 (br, 2H), 2.86 (t, J = 6.03 Hz, 2H), 2.62 (br, 4H), 2.46 (br, 6H), 2.18 (d, J = 1.11 Hz, 3H), 2.05 (quintet, J = 7.32 Hz, 2H), 1.45 (s, 9H).

4.1.3.120. tert-Butyl (2-(4-(3-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)propyl)piperazin-1-yl)ethyl)carbamate ***(129)***. Prepare from **113** following the general procedure **8** to afford the desired product as off white solid (63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.45 (s, 1H), 6.83 (d, J = 2.55 Hz, 1H), 6.67-6.63 (m, 3H), 6.09 (br, 1H), 4.92 (br, 1H), 4.04 (t, J = 6.57 Hz, 2H), 3.87 (t, J = 7.14 Hz, 2H), 3.76 (s, 3H), 3.62-3.60 (m, 2H), 3.17-3.13 (m, 2H), 2.53-2.49 (m, 12H), 2.12 (s, 3H), 1.99-1.97 (m, 4H), 1.38 (s, 9H).

4.1.3.121. tert-Butyl (2-(4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)butyl)piperazin-1-yl)ethyl)carbamate ***(130)***. Prepare from **114** following the general procedure **8** to afford the desired product as off white solid (40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.35 (s, 1H), 6.86 (d, J = 8.43 Hz, 1H), 6.71-6.66 (m, 3H), 4.94 (br, NH), 4.04 (t, J = 6.42 Hz, 2H), 3.89 (t, J = 6.96 Hz, 2H), 3.81 (s, 3H), 3.65 (q, J = 7.53 Hz, 2H), 3.18 (q, J = 7.14 Hz, 2H), 2.45-2.37 (m, 12H), 2.15 (s, 3H), 2.04 (quintet, J = 7.32 Hz, 2H), 1.83 (quintet, J = 6.96 Hz, 2H), 1.67-1.62 (quintet, J = 7.14 Hz, 2H), 1.42 (s, 9H).

4.1.3.122. tert-Butyl 2-(2-(4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)ethyl)piperazin-1-yl)ethoxy)ethylcarbamate ***(131)***. From compound **110**, procedure **8**, yield 50%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.35 (s, 1H), 6.87 (d, J = 8.1 Hz, 1H), 6.71-6.66 (m, 3H), 5.87 (s, 1H), 5.16 (br, 1H), 4.12 (t, J = 6.0 Hz, 2H), 3.67 (t, J = 6.9 Hz, 2H), 3.8 (s, 3H), 3.63 (q, J = 6.3 Hz, 2H), 3.54 (t, J = 6.0 Hz, 2H), 3.49 (t, J = 4.8 Hz, 2H), 3.27 (q, J = 5.7 Hz, 2H), 2.85 (t, J = 6.3 Hz, 2H), 2.62 (br, 4H), 2.56 (t, J = 5.7 Hz, 6H), 2.15 (d, J = 0.9 Hz, 3H), 2.02 (quintet, J = 6.9 Hz, 2H), 1.42 (s, 9H).

4.1.3.123. tert-Butyl 2-(4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)ethyl)piperidin-1-yl)ethylcarbamate ***(132)***. Prepare from **117** following the general procedure **8** to afford the desired product as an off white solid (62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (s, NH), 7.42 (s, 1H), 6.88 (d, J = 8.22 Hz, 1H), 6.73-6.71 (m, 3H), 5.58 (br, NH), 5.18 (br, NH), 4.05 (t, J = 6.60 Hz, 2H), 3.90 (t, J = 7.14 Hz, 2H), 3.83 (s, 3H), 3.64 (q, J = 6.42 Hz, 2H), 3.28 (br, 2H), 3.03 (br, 2H), 2.95 (br, 2H), 2.52 (br, 2H), 2.17 (d, J = 0.93 Hz, 3H), 2.09-2.02 (m, 4H), 1.81-1.70 (m, 3H), 1.69-1.63 (m, 2H), 1.44 (s, 9H).

4.1.3.124. tert-Butyl 4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)acetyl)piperazine-1-carboxylate ***(133)***. From compound **101**, procedure **8**; yield 74%, white solid.

4.1.3.125. tert-Butyl 4-(3-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)propanoyl)piperazine-1-carboxylate ***(134)***. From compound **102**, procedure **8**, yield 71%, white solid.

4.1.3.126. tert-Butyl 4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)butanoyl)piperazine-1-carboxylate ***(135)***. Prepare from compound **103** following the general procedure **8** to afford product (54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.36 (s, 1H), 6.94 (d, J = 8.22 Hz, 1H), 6.74-6.68 (m, 3H), 5.87 (s, 1H), 4.13 (t, J = 6.03 Hz, 2H), 3.91 (t, J = 7.32 Hz, 2H), 3.83 (s, 3H), 3.69 (q, J = 6.39 Hz, 2H), 3.63-3.59 (m, 2H), 3.46-3.41 (m, 6H), 2.58 (t, J = 6.96 Hz, 2H), 2.20 (t, J = 7.50 Hz, 2H), 2.17 (d, J = 0.90 Hz, 3H), 2.07 (quintet, J = 6.96 Hz, 2H), 1.47 (s, 9H).

4.1.3.127. tert-Butyl (2-(4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)acetyl)piperazin-1-yl)ethyl)carbamate ***(136)***. Prepare from **119** following the general procedure **8** to afford the desired product as an off white solid (44%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.43 (s, 1H), 6.93 (d, J = 8.40 Hz, 1H), 6.74-6.70 (m, 3H), 6.08 (br, 1H), 4.90 (br, 1H), 4.77 (s, 2H), 3.92 (t, J = 7.14 Hz, 2H), 3.84 (s, 3H), 3.67-3.49 (m, 6H), 3.22 (q, J = 6.36 Hz, 2H), 2.49-2.45 (m, 6H), 2.18 (d, J = 0.90 Hz, 3H), 2.07 (quintet, J = 7.14 Hz, 2H), 1.45 (s, 9H).

4.1.3.128. tert-Butyl 4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)acetamido)piperidine-1-carboxylate ***(137).*** Prepare from **104** following the general procedure **8** to afford afford the desired product as off white solid (64%).

4.1.3.129. tert-Butyl 4-(3-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)propanamido)piperidine-1-carboxylate ***(138)***. Prepare from **105** following the general procedure **8** to afforded product (76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.65 (s, 1H), 6.88-6.85 (m, 2H), 6.77-6.74 (m, 2H), 6.64 (d, J = 7.68 Hz, 1H), 6.47 (br, 1H), 4.29 (t, J = 5.70 Hz, 2H), 3.94-3.93 (m, 5H), 3.80 (s, 3H), 3.65 (q, J = 5.85 Hz, 2H), 2.92 (t, J = 12.81 Hz, 2H), 2.69 (t, J = 5.88 Hz, 2H), 2.20 (s, 3H), 2.12 (quintet, J = 6.75 Hz, 2H), 1.92 (t, J = 12.63 Hz, 2H), 1.45 (s, 9H), 1.30-1.25 (m, 2H).

4.1.3.130. tert-Butyl 4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)butanamido)piperidine-1-carboxylate ***(139)***. From compound **106**, procedure **8**, yield 30%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.35 (s, 1H), 6.90 (d, J = 8.07 Hz, 1H), 6.74-6.70 (m, 3H), 5.93 (t, J = 6.42 Hz, 1H), 5.78 (d, J = 7.89 Hz, 1H), 4.09 (t, J = 5.87 Hz, 2H), 4.07-3.98 (m, 3H), 3.90 (t, J = 7.14 Hz, 2H), 3.84 (s, 3H), 3.66 (q, J = 6.42 Hz, 2H), 2.83 (t, J = 11.92 Hz, 2H), 2.39 (t, J = 7.14 Hz, 2H), 2.17-2.13 (m, 5H), 2.06 (quintet, J = 6.96 Hz, 2H), 1.87 (br, 2H), 1.45 (s, 9H), 1.25-1,21 (m, 2H).

4.1.3.131. tert-Butyl (2-(4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)acetamido)piperidin-1-yl)ethyl)carbamate ***(140)***. Prepare from **121** following the general procedure **8** to afford the desired product as

an off white solid (28%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.35 (s, 1H), 6.89 (d, J = 8.97 Hz, 1H), 6.76 (s, 1H), 6.72-6.70 (m, 3H), 5.93 (br, 1H), 4.93 (br, 1H), 4.50 (s, 2H), 3.94 (t, J = 7.14 Hz, 2H), 3.90 (s, 3H), 3.87-3.85 (m, 1H), 3.65 (q, J = 6.66 Hz, 2H), 3.20-3.17 (m, 2H), 2.78-2.75 (m, 2H), 2.44 (t, J = 5.97 Hz, 2H), 2.18 (s, 3H), 2.06-2.04 (m, 2H), 1.88-1.80 (m, 2H) 1.45 (s, 9H), 1.41-1.37 (m, 2H).

4.1.3.132. tert-Butyl 4-(2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)-phenoxy)ethylcarbamoyl)piperidine-1-carboxylate ***(141)***. Prepare from **122** following the general procedure **8** to afford the desired product as an off white solid (48%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (d, J = 0.90 Hz, 1H), 6.97 (d, J = 6.00 Hz, 1H), 6.94 (s, 1H), 6.77 (dd, J = 2.10, 8.10 Hz, 1H), 6.66 (s, 1H), 4.12-4.02 (m, 2H), 4.00 (t, J = 7.50 Hz, 2H), 3.82 (s, 3H), 3.61 (t, J = 6.90 Hz, 2H), 3.56 (t, J = 5.43 Hz, 2H), 2.78 (br, 2H), 2.42-2.33 (m, 3H), 2.22 (d, J = 0.93 Hz, 3H), 2.08 (quintet, J = 7.50 Hz, 2H), 1.75-1.71 (m, 2H), 1.65-1.48 (m, 2H), 1.44 (s, 9H).

4.1.3.133. tert-Butyl 4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl) propyl) thioureido) phenoxy)phenyl)piperazine-1-carboxylate ***(142)***. Prepared from compound **87** following the general producer **8** to afford an off white solid (72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.37 (s, 1H), 6.97-6.89 (m, 4H), 6.82-6.79 (m, 2H), 6.73 (s, 1H), 6.69 (dd, J = 2.19, 8.19 Hz, 1H), 6.02 (br, 1H), 3.92 (t, J = 6.96 Hz, 2H), 3.87 (s, 3H), 3.70 (q, J = 7.68 Hz, 2H), 3.60 (t, J = 4.95 Hz, 4H), 3.09 (t, J = 4.92 Hz, 4H), 2.18 (s, 3H), 2.08 (quintet, J = 6.96 Hz, 2H), 1.48 (s, 9H)

4.1.3.134. tert-Butyl 4-(4-(2-methoxy-4-(2-((3-(5-methyl-1H-imidazol-1-yl)propyl)amino)-2-thioxoethyl)phenoxy)phenyl)-3,6-dihydropyridine-1(2H)-carboxylate ***(143)***. Prepare from compound **83** following the general procedure **8** to get desired product as off white solid (42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.39 (s, 1H), 7.35 (d, J = 8.64 Hz, 2H), 6.95-6.87 (m, 4H), 6.73 (d, J = 8.61 Hz, 2H), 6.16 (br, 1H), 5.99 (s, 1H), 4.07-4.05 (m, 2H), 3.94 (t, J = 6.96 Hz, 2H), 3.83 (s, 3H), 3.69 (t, J = 7.14 Hz, 2H), 3.63 (t, J = 6.96 Hz, 2H), 2.50-2.47 (m, 2H), 2.19 (s, 3H), 2.10-2.05 (quintet, J = 7.50 Hz, 2H), 1.49 (s, 9H)

4.1.3.135. tert-Butyl 4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)phenyl)piperidine-1-carboxylate ***(144).*** Prepare from compound **86** following the general procedure **8** to get desired product as an off white solid (44%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.39 (s, 1H), 7.17 (d, J = 8.61 Hz, 2H), 6.93-6.89 (m, 3H), 6.84 (d, J = 2.22 Hz, 1H), 6.73 (d, J = 8.58 Hz, 2H), 6.07 (br, 1H), 4.25-4.21 (m, 2H), 3.93 (t, J = 7.14 Hz, 2H), 3.84 (s, 3H), 3.71-3.65 (m, 2H), 2.79-2.74 (m, 2H), 2.62-2.58 (m, 1H), 2.18 (s, 3H), 2.10-2.05 (m, 2H), 1.83-1.79 (m, 2H), 1.57-1.51 (m, 2H), 1.48 (s, 9H).

4.1.3.136. tert-Butyl 4-(3-((2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)methyl)phenyl)piperazine-1-carboxylate ***(145)***. From compound **85**, procedure **8**, yield 56%, white solid.

4.1.3.137. tert-Butyl 4-(3-((2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)methyl)phenyl)piperidine-1-carboxylate ***(146)***. From compound **84**, procedure **8**; yield 72%, white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.37 (s, 1H), 7.32-7.26 (m, 3H), 7.16 (t, J = 7.14 Hz, 1H), 6.90 (d, J = 8.25 Hz, 1H), 6.73-6.67 (m, 3H), 5.93 (brt, 1H), 5.12 (s, 2H), 4.22 (br, 2H), 3.89 (t, J = 7.14 Hz, 2H), 3.86 (s, 3H), 3.66 (q, J = 7.68 Hz, 2H), 2.80 (t, J = 11.52 Hz, 2H), 2.66 (tt, J = 12.09, 3.48, 1H), 2.16 (d, 0.93 Hz, 3H), 2.04 (quintet, J = 7.14 Hz, 2H), 1.83-1.79 (m, 2H), 1.66-1.55 (m, 2H), 1.48 (s, 9H).

4.1.3.138. tert-Butyl (4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)butyl)pyridin-2-yl)(methyl)carbamate ***(147)***. Prepare from **123** following the general procedure **8** to afford the desired product as off white solid (58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, J = 5.13 Hz, 1H), 7.60 (s, 1H), 7.53 (s, 1H), 7.37 (s, 1H), 6.86-6.82 (m, 2H), 6.72-6.70 (m, 3H), 5.97 (br, 1H), 4.04 (t, J = 5.61 Hz, 2H), 3.91 (t, J =7.14 Hz, 2H), 3.83 (s, 3H), 3.69 (q, J = 6.60 Hz, 2H), 3.38 (s, 3H), 2.71 (t, J = 7.32 Hz, 2H), 2.17 (s, 3H), 2.09 (quintet, J = 7.32 Hz, 2H), 1.87-1.82 (m, 4H), 1.52 (s, 9H).

4.1.3.139. tert-Butyl (2-((tert-butoxycarbonyl)amino)ethyl)(4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)butyl)pyridin-2-yl)carbamate ***(148)***. Prepare from **125** following the general procedure **8** to afford the

desired product as off white solid (56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, 1H, J = 5.13 Hz), 7.61 (s, 1H), 7.46 (s, 1H), 7.32 (s, 1H), 6.90 (m, 2H), 6.72 (m, 3H), 6.21 (br, 1H), 5.95 (br, 1H), 4.06 (t, 2H, J = 6.21 Hz), 4.00 (t, 2H, J = 4.23 Hz), 3.91 (t, 2H, J = 7.14 Hz), 3.83 (s, 3H), 3.69 (q, 2H, J = 6.42 Hz), 3.40 (q, 2H, J = 5.31 Hz), 2.71 (t, 2H, J = 7.32 Hz), 2.17 (s, 3H), 2.07 (quintet, 2H, J = 7.32 Hz), 1.87-1.83 (m, 4H), 1.52 (s, 9H), 1.42 (s, 9H).

4.1.3.140. 1-(4-(3-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)propoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(149)***. Prepare from **112** following the general procedure **8** to afford the desired product as off white solid (37%).^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.45 (s, 1H), 6.90 (d, J = 8.79 Hz, 1H), 6.74 (m, 3H), 6.06 (br, 1H), 4.11 (t, J = 6.24 Hz, 2H), 3.93 (t, J = 7.14 Hz, 2H), 3.83 (s, 3H), 3.80 (t, J = 6.03 Hz, 2H), 3.69 (q, J = 6.03 Hz, 2H), 2.61-2.55 (m, 8H), 2.08-2.01 (m, 8H), 0.88 (s, 9H), 0.01 (s, 6H).

4.1.3.141. 1-(4-(4-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)butoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(150)***. Prepare from **115** following the general procedure **8** to afford the desired product as off white solid (50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.34 (s, 1H), 6.82 (d, J = 8.22 Hz, 1H), 6.68-6.65 (m, 3H), 5.91 (br, 1H), 4.00 (t, J = 6.42 Hz, 2H), 3.86 (t, J = 7.14 Hz, 2H), 3.77 (s, 3H), 3.72 (t, J = 6.39 Hz. 2H), 3.64-3.57 (m, 2H), 2.51-2.35 (m, 12H), 2.12 (s, 3H), 2.04 (quintet, J = 4.95 Hz, 2H), 1.83 (quintet, J = 6.36 Hz, 2H), 1.65-1.62 (m, 2H), 0.83 (s, 9H), 0.01 (s, 6H)

4.1.3.142. 1-(4-(2-(1-(2-(tert-Butyldimethylsilyloxy)ethyl)piperidin-4-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(151)***. Prepare from **116** following the general procedure **8** to afford the desired product as off white solid (41%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (br, NH), 7.39 (s, 1H), 6.87 (d, J = 8.25 Hz, 1H) 6.73-6.70 (m, 3H), 5.93 (br, NH), 4.05 (t, J = 6.60 Hz, 2H), 3.91 (t, J = 7.14 Hz, 2H), 3.83 (s, 3H), 3.85-3.78 (m, 4H), 3.67 (q, J = 7.68 Hz, 2H), 3.03 (br, 2H), 2.60 (br, 2H), 2.17 (s, 3H), 2.09-2.02 (m, 3H), 1.81-1.70 (m, 6H), 0.88 (s, 9H), 0.05 (s, 6H).

4.1.3.143. 1-(4-(2-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-2-oxoethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(152)***. Prepare from **118** following the general procedure **8** to afford the desired product as an off white solid (70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.28 (s, 1H), 6.86 (d, J = 8.43 Hz, 1H), 6.71-6.63 (m, 3H), 6.13 (br, 1H), 4.71 (s, 2H), 3.86 (t, J = 7.14 Hz, 2H), 3.78 (s, 3H), 3.69 (t, J = 5.85 Hz, 2H), 3.59-3.49 (m, 6H), 2.50-2.45 (m, 6H), 2.12 (s, 3H), 2.04 (quintet, J = 6.96 Hz, 2H), 0.83 (s, 9H), 0.02 (s, 6H).

4.1.3.144. N-(1-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperidin-4-yl)-2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)acetamiden ***(153).*** Prepare from **120** following the general procedure **8** to afford the desired product as off white solid (38%).

4.1.3.145. tert-Butyl (2-((tert-butyldimethylsilyl)oxy)ethyl)(4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)butyl)pyridin-2-yl)carbamate ***(154)***. Prepare from **124** following the general procedure **8** to afford the desired product as an off white solid (56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, J = 5.13 Hz, 1H), 7.50 (s, 1H), 7.45 (s, 1H), 7.38 (s, 1H), 6.86-6.85 (m, 2H), 6.73-6.69 (m, 3H), 5.90 (br, 1H), 4.08 (t, J = 6.57 Hz, 2H), 4.04 (t, J = 6.03 Hz, 2H), 3.91 (t, J = 7.14 Hz, 2H), 3.83 (s, 3H), 3.81 (t, J = 6.21 Hz, 2H), 3.70 (q, J = 6.87 Hz, 2H), 2.69 (t, J = 7.68 Hz, 2H), 2.17 (s, 3H), 2.07 (quintet, J = 7.14 Hz, 2H), 1.88-1.81 (m, 4H), 1.51 (s, 9H), 0.81 (s, 9H), 0.01 (s, 6H).

4.1.3.146. 2-(4-(2-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)ethyl)piperazin-1-yl)ethyl4-methoxybenzoate ***(155)***. From compound **107**, procedure **8**, yield 68%, white solid.

4.1.3.147. 2-(2-(4-(2-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)ethyl) piperazin-1-yl)ethoxy)ethyl 4-methoxybenzoate ***(156)***. From compound **109**, procedure **8**, white solid.

4.1.3.148. Methyl 2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)-phenoxy)acetate ***(157)***. Prepare from compound **89** following the general procedure **8** afford the desired product as white solid (60%). ^{1}H NMR

(300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.36 (s, 1H), 6.83-6.77 (m, 2H), 6.73-6.70 (m, 2H), 6.15 (br, 1H), 4.71 (s, 2H), 3.92 (t, *J* = 6.96 Hz, 2H), 3.86 (s, 3H), 3.80 (s, 3H), 3.70 (q, *J* = 6.42 Hz, 2H), 2.18 (d, *J* = 0.75 Hz, 3H), 2.10 (quint, *J* = 7.14 Hz, 2H).

4.1.3.149. tert-Butyl (2-(4-(4-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)phenyl)piperazin-1-yl)ethyl)carbamate ***(158)***. Prepared from compound **195** following the general producer **10** to afford a white solid (45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.47 (s, 1H), 6.96-6.88 (m, 5H), 6.83 (s, 1H), 6.80 (d, *J* = 8.43 Hz, 1H), 6.74 (s, 1H), 6.68 (dd, *J* = 2.55, 8.58 Hz, 1H), 6.14 (br, 1H), 3.93 (t, *J* = 6.78 Hz, 2H), 3.86 (s, 3H), 3.70 (q, *J* = 6.78 Hz, 2H), 3.26-3.24 (t, *J* = 5.97 Hz, 2H), 3.15 (t, *J* = 4.77 Hz, 4H), 2.63 (t, *J* = 4.77 Hz, 4H), 2.54 (t, *J* = 5.85 Hz, 2H), 2.18 (s, 3H), 2.09 (quintet, *J* = 6.96 Hz, 2H), 1.45 (s, 9H)

4.1.3.150. 1-(4-(4-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)phenoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(159)***. Prepared from compound **195** following the general producer **10** to afford a white solid (34%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.46 (s, 1H), 6.92-6.88 (m, 4H), 6.82-6.74 (m, 3H), 6.68 (dd, , *J* = 2.22, 8.07 Hz, 1H), 6.08 (br, 1H), 3.93 (t, *J* = 7.14 Hz, 2H), 3.86 (s, 3H), 3.82 (t, *J* = 6.21 Hz, 2H), 3.68 (q, *J* = 6.96 Hz, 2H), 3.17 (t, *J* = 4.56 Hz, 4H), 2.70 (t, *J* = 4.77 Hz, 4H), 2.60 (t, *J* = 6.39 Hz, 2H), 2.18 (s, 3H), 2.09 (quintet, *J* = 7.32 Hz, 2H), 0.90 (s, 9H), 0.07 (s, 6H)

4.1.4. Final compounds

4.1.4.1. 1-(3-Methoxy-4-(2-(2-methylpiperazin-1-yl)ethoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(160)***. Starting with compound **126** as following the general procedure **6.2**, compound **160** was obtained as a white solid, 42% yield, mp = 77-78 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.37 (s, 1H), 6.89 (d, *J* = 8.43 Hz, 1H), 6.75-6.73 (m, 3H), 5.92 (s, 1H), 4.13 (t, *J* = 6.42 Hz, 2H), 3.89 (t, *J* = 6.96 Hz, 2H), 3.83 (s, 3H), 3.66 (q, *J* = 7.53 Hz, 2H), 3.22-3.13 (m, 1H), 2.93-2.78 (m, 5H), 2.56-2.41 (m, 3H), 2.18 (d, *J* = 0.93 Hz, 3H), 2.05 (p, *J* = 7.32 Hz, 2H), 1.08 (d, *J* = 5.88 Hz, 3H). MS (ESI) *m/z* 447 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{22}H_{34}N_6O_2S$ $[M+H]^+$ 447.2542, found: 447.2535. Anal. HPLC 97.7% (R_t = 3.45 min).

4.1.4.2. 1-(4-(2-(3,5-Dimethylpiperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(161)***. Starting with compound **127** as following the general procedure **6.2,** compound **161** was obtained as white solid, 78% yield, mp = 58-59 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (s, 1H), 6.95 (d, J = 8.61 Hz, 1H), 6.94 (d, J = 2.37 Hz, 1H), 6.75 (dd, J = 8.43, 2.40 Hz, 1H), 6.67 (s, 1H), 4.14 (t, J = 5.13 Hz, 2H), 3.98 (t, J = 6.96 Hz, 2H), 3.81 (s, 3H), 3.59 (t, J = 6.96 Hz, 2H), 3.11 (br, 4H), 2.84 (t, J = 5.31 Hz, 2H), 2.22 (d, J = 0.90 Hz, 3H), 2.08-1.94 (m, 4H), 1.16 (d, J = 6.21 Hz, 6H). MS (ESI) *m/z* 461 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{23}H_{36}N_6O_2S$ $[M+H]^+$ 461.2699, found: 461.2705. Anal. HPLC 95.7% (R_t = 3.48 min).

4.1.4.3. 1-(4-(2-(4-Acetylpiperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(162)***. Starting with compound **111** as following the general procedure **8**, compound **162** was obtained as white solid, 87% yield, mp = 55-56 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.39 (s, 1H), 6.89 (d, J = 9.15 Hz, 1H), 6.75-6.72 (m, 3H), 5.97 (s, 1H), 4.15 (t, J = 5.89 Hz, 2H), 3.90 (t, J = 7.14 Hz, 2H), 3.81 (s, 3H), 3.69-3.62 (m, 4H), 3.49 (t, J = 4.95 Hz, 2H), 2.87 (t, J = 5.67 Hz, 2H), 2.60 (t, J = 5.1 Hz, 2H), 2.56 (t, J = 5.10 Hz, 2H), 2.18 (s, 3H), 2.09 (s, 3H), 2.05 (p, J = 7.14 Hz, 2H). MS (ESI) *m/z* 475 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{23}H_{34}N_6O_3S$ $[M+H]^+$ 475.2492, found: 475.2495. Anal. HPLC 96.7% (R_t = 3.76 min).

4.1.4.4. 1-(4-(2-(4-(2-Hydroxyethyl)piperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(163)***. An excess solution of 10% NaOH was added in solution of **155** in MeOH (10 mL). The mixture was heated to reflux for 30 minutes, diluted with DCM, washed with water. The organic layer was concentrated, purified by column chromatography to afford compound **163** as a white solid, 35% yield, mp = 64-65 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.36 (s, 1H), 6.89 (d, J = 8.79 Hz, 1H), 6.75-6.72 (m, 3H), 5.99 (s, 1H), 4.15 (t, J = 6.06 Hz, 2H), 3.89 (t, J = 7.14 Hz, 2H), 3.83 (s, 3H), 3.69-3.60 (m, 4H), 2.87 (t, J = 5.85 Hz, 2H), 2.63-2.54 (m, 10H), 2.18 (s, 3H), 2.05 (p, J = 7.32 Hz, 2H). MS (ESI) *m/z* 477 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{23}H_{36}N_6O_3S$ $[M+H]^+$ 477.2647, found: 477.2633. Anal. HPLC 98.5% (R_t = 3.63 min).

*4.1.4.5. 1-(4-(3-(4-(2-Hydroxyethyl)piperazin-1-yl)propoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(164)***. Starting with compound **149** as following the general procedure **7**, compound **164** was obtained as off white solid, 47% yield, mp = 78-79 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 7.58 (s, 1H), 6.95 (d, J = 8.50 Hz, 1H), 6.90 (s, 1H), 6.75 (dd, J = 2.15, 8.50 Hz, 1H), 6.66 (s, 1H), 4.05 (t, J = 6.05 Hz, 2H), 3.98 (t, J = 7.20 Hz, 2H), 3.80 (s, 3H), 3.68 (t, J = 6.00 Hz, 2H), 3.60 (t, J = 6.35 Hz, 2H), 2.58-2.55 (m, 8H), 2.54 (t, J = 6.00 Hz, 4H), 2.21 (s, 3H), 2.06 (p, J = 7.10 Hz, 2H), 1.97 (p, J = 6.95 Hz, 2H). MS (ESI) *m/z* 491 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{38}N_6O_3S$ $[M+H]^+$ 491.2799, found 491.2795. Anal. HPLC 100% (R_t = 2.95 min).

*4.1.4.6. 1-(4-(4-(4-(2-Hydroxyethyl)piperazin-1-yl)butoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(165)***. Starting with compound **150** as following the general procedure **7**, compound **165** was obtained desired product as a white solid, 84% yield, mp = 58-59 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 6.95 (d, J = 8.43 Hz, 1H), 6.89 (d, J = 2.4 Hz, 1H), 6.76 (dd, J = 2.22, 8.43 Hz, 1H), 6.66 (s, 1H), 4.03 (t, J = 6.45 Hz, 2H), 3.97 (t, J = 7.14 Hz, 2H), 3.80 (s, 3H), 3.68 (t, J = 6.03 Hz, 2H), 3.61 (t, J = 6.78 Hz, 2H), 2.54-2.41 (m, 12H), 2.22 (d, J = 0.90 Hz, 3H), 2.05 (p, J = 4.95 Hz, 2H), 1.79-1.73 (m, 4H). MS (ESI) *m/z* 505 $[M+H]^+$. HRMS (ESI) calc. for $C_{25}H_{40}N_6O_3S$ $[M+H]^+$ 505.2955, found 505.2957. Anal. HPLC 98.4% (R_t = 2.99 min).

*4.1.4.7. 1-(4-(2-(4-(2-Aminoethyl)piperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(166)***. Starting with compound **128** as the general procedure **6.2**, compound 166 was obtained as white solid, 85% yield, mp = 81-82 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (d, J = 0.93 Hz, 1H), 6.96 (d, J = 8.43 Hz, 1H), 6.92 (d, J = 2.40 Hz, 1H), 6.75 (dd, J = 8.40, 2.37 Hz, 1H), 6.66 (s, 1H), 4.14 (t, J = 5.49 Hz, 2H), 3.97 (t, J = 6.96 Hz, 2H), 3.81 (s, 3H), 3.60 (t, J = 6.75 Hz, 2H), 2.82 (t, J = 5.49 Hz, 2H), 2.79 (t, J = 6.60 Hz, 2H), 2.68 (br, 4H), 2.55 (br, 4H), 2.47 (t, J = 6.42 Hz, 2H), 2.22 (d, J = 0.93 Hz, 3H), 2.03 (p, J = 6.96 Hz, 2H). MS (ESI) *m/z* 476 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{23}H_{37}N_7O_2S$ $[M+H]^+$ 476.2808, found: 476.2823. Anal. HPLC 96.5% (R_t = 3.52 min).

*4.1.4.8. 1-(4-(3-(4-(2-Aminoethyl)piperazin-1-yl)propoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(167)***. Starting with compound **129** as

following the general procedure **6.**2, compound **167** was obtained as a red solid, 65% yield, mp = 107-108 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 7.58 (s, 1H), 6.95 (d, *J* = 8.55 Hz, 1H), 6.91 (d, *J* = 2.05 Hz, 1H), 6.75 (dd, *J* = 2.25, 8.35 Hz, 1H), 6.66 (s, 1H), 4.03 (t, 2H, *J* = 6.20 Hz), 3.98 (t, *J* = 7.20 Hz, 2H), 3.80 (s, 3H), 3.60 (t, *J* = 6.60 Hz, 2H), 2.74 (t, *J* = 6.70 Hz, 2H), 2.59-2.55 (m, 8H), 2.48-2.44 (m, 4H), 2.23 (s, 3H), 2.09 (p, *J* = 7.10 Hz, 2H), 1.99 (p, *J* = 6.95 Hz, 2H). MS (ESI) *m/z* 490 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{39}N_7O_2S$ $[M+H]^+$ 490.2959, found 490.2968. Anal. HPLC 100% (R_t = 2.87 min).

4.1.4.9. 1-(4-(4-(4-(2-Aminoethyl)piperazin-1-yl)butoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(168)***. Starting from compound **130** as following the general procedure **6.2**, compound **168** was obtained as a white solid, 71% yield, mp = 131-132 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (d, *J* = 0.90 Hz, 1H), 6.95 (d, *J* = 8.31 Hz, 1H), 6.89 (d, *J* = 2.40 Hz, 1H), 6.76 (dd, *J* = 2.58, 6.03 Hz, 1H), 6.66 (s, 1H), 4.03 (t, *J* = 5.88 Hz, 2H), 3.97 (t, *J* = 7.14 Hz, 2H), 3.80 (s, 3H), 3.59 (q, *J* = 6.42 Hz, 2H), 2.75 (t, *J* = 6.60 Hz, 2H), 2.53-2.47 (br, 12H), 2.22 (d, *J* = 1.11 Hz, 3H), 2.03 (p, *J* = 7.50 Hz, 2H), 1.77-1.67 (m, 4H). MS (ESI) *m/z* 504 $[M+H]^+$. HRMS (ESI) calc. for $C_{25}H_{41}N_7O_2S$ $[M+H]^+$ 504.3115, found 504.3127. Anal. HPLC 99.5% (R_t = 2.99 min).

4.1.4.10. 1-(4-(2-(4-(2-(2-Hydroxyethoxy)ethyl)piperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(169)***. Starting with compound **156** as following the experiment procedure used for compound **163** to obtained compound **169** as a white solid, 50% yield, mp = 64-65 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.66 (s, 1H), 6.96 (d, *J* = 8.43 Hz, 1H), 6.93 (d, *J* = 2.19 Hz, 1H), 6.75 (dd, *J* = 8.43, 2.4 Hz, 1H), 6.70 (s, 1H), 4.18 (t, *J* = 5.31 Hz, 2H), 3.99 (t, *J* = 7.14 Hz, 2H), 3.81 (s, 3H), 3.66 (t, *J* = 4.92 Hz, 4H), 3.62-3.52 (m, 4H), 2.87 (t, *J* = 5.31 Hz, 2H), 2.75 (br, 10 H), 2.23 (s, 3H), 2.04 (p, *J* = 7.14 Hz, 2H). MS (ESI) *m/z* 521 $[M+H]^+$. HRMS (FAB) *m*/*z* calc. for $C_{25}H_{40}N_6O_4S$ $[M + H]^+$ 521.2905, found: 521.2896. Anal. HPLC 99.3% (R_t = 3.09 min).

4.1.4.11. 1-(4-(2-(4-(2-(2-Aminoethoxy)ethyl)piperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(170)***. Starting with compound **131** as following the general procedure **6.2**, compound **170** was

obtained as white solid, 73% yield, mp = 57-58 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (s, 1H), 6.96 (d, *J* = 8.43 Hz, 1H), 6.92 (d, *J* = 2.73 Hz, 1H), 6.75 (dd, *J* = 8.43, 2.37 Hz, 1H), 6.66 (s, 1H), 4.14 (t, *J* = 5.49 Hz, 2H), 3.97 (t, *J* = 7.14 Hz, 2H), 3.81 (s, 3H), 3.63-3.57 (m, 4H), 3.50 (t, *J* = 5.13 Hz, 2H), 2.84-2.80 (m, 4H), 2.61 (br, 10H), 2.22 (d, *J* = 0.93 Hz, 3H), 2.03 (p, *J* = 6.96 Hz, 2H). MS (ESI) *m/z* 520 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{25}H_{41}N_7O_3S$ $[M + H]^+$ 520.3070, found: 520.3076. Anal. HPLC 96.1% (R_t = 3.35 min).

*4.1.4.12. 1-(3-Methoxy-4-(2-(4-phenylpiperazin-1-yl)ethoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(171)***. Starting with compound **92** as following the general procedure **8**, compound **171** was obtained as white solid, 50 % yield, mp = 64-65°C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (s, 1H), 7.22 (t, *J* = 7.97 Hz, 2H), 7.00-6.92 (m, 4H), 6.83 (t, *J* = 6.15 Hz, 1H), 6.76 (dd, *J* = 8.40, 2.73 Hz, 1H), 6.67 (s, 1H), 4.19 (t, *J* = 5.31 Hz, 2H), 3.97 (t, *J* = 7.14 Hz, 2H), 3.82 (s, 3H), 3.59 (t, *J* = 6.57 Hz, 2H), 3.20 (t, *J* = 4.95 Hz, 4H), 2.88 (t, *J* = 5.52 Hz, 2H), 2.80 (t, *J* = 4.95 Hz, 4H), 2.22 (d, *J* = 0.93 Hz, 3H), 2.03 (p, *J* = 6.96 Hz, 2H). MS (ESI) *m/z* 509 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{27}H_{37}N_6O_2S$ $[M + H]^+$ 509.2699, found: 509.2693. Anal. HPLC 96.9% (R_t = 3.81 min).

*4.1.4.13. 1-(4-(2-(4-Benzylpiperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(172)***. Starting with compound **93** as following the general procedure **8**, compound **172** was obtained as a white solid, 47% yield, mp = 78-80 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (s, 1H), 7.32-7.26 (m, 5H), 6.96 (d, *J* = 8.58 Hz, 1H), 6.90 (d, *J* = 0.75 Hz, 1H), 6.76 (dd, *J* = 2.37, 8.40 Hz, 1H), 6.66 (s, 1H), 4.14 (t, *J* = 5.49 Hz, 2H), 3.99 (t, *J* = 7.14 Hz, 2H), 3.79 (s, 3H), 3.59 (t, *J* = 5.49 Hz, 2H), 3.53 (s, 2H), 2.83 (t, *J* = 5.49 Hz, 2H), 2.66-2.53 (m, 8H), 2.21 (s, 3H), 2.05 (p, *J* = 7.14 Hz, 2H). MS (FAB) *m/z* 523 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{28}H_{38}N_6O_2S$ $[M + H]^+$ 523.2855, found: 523.2861. Anal. HPLC 95.7% (R_t = 3.32 min).

*4.1.4.14. 1-(3-Methoxy-4-(2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(173)***. Starting with compound **94** as following the general procedure **8**, compound **173** was obtained as white solid, 94% yield, mp = 71-72 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, *J* = 4.74 Hz, 2H), 7.77

(s, 1H), 7.42 (s, 1H), 6.91 (d, *J* = 8.97 Hz, 1H), 6.76-6.74 (m, 3H), 6.50 (t, *J* = 4.74 Hz, 1H), 6.08 (br, 1H), 4.25-4.15 (m, 2H), 3.95-3.80 (m, 9H), 3.66 (dd, *J* = 13.74, 6.60 Hz, 2H), 2.91 (t, *J* = 5.85 Hz, 2H), 2.75-2.60 (m, 4H), 2.18 (s, 3H), 2.13-1.97 (m, 2H). MS (ESI) *m/z* 511 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{25}H_{34}N_8O_2S$ $[M+H]^+$ 511.2604, found: 511.2609. Anal. HPLC 97.5% (R_t = 3.58 min).

*4.1.4.15. 1-(4-(2-(4-(5-Fluoropyrimidin-2-yl)piperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(174)***. Starting with compound **95** as following the general procedure **8**, compound **174** was obtained as white solid, 78% yield, mp = 71-72 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 2H), 7.79 (s, 1H), 7.42 (s, 1H), 6.95-6.87 (m, 1H), 6.80-6.68 (m, 3H), 6.10 (br, 1H), 4.25-4.13 (m, 2H), 3.95-3.86 (m, 2H), 3.83 (s, 3H), 3.82-3.75 (m, 4H), 3.72-3.60 (m, 2H), 2.95-2.85 (m, 2H), 2.70-2.60 (m, 4H), 2.18 (s, 3H), 2.13-1.97 (m, 2H). MS (ESI) *m/z* 529 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{25}H_{33}FN_8O_2S$ $[M+H]^+$ 529.2509, found: 529.2508. Anal. HPLC 97.3% (R_t = 3.56 min).

*4.1.4.16. 1-(4-(2-(4-(5-Chloropyrimidin-2-yl)piperazin-1-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(175)***. Starting with compound **96** as following the general procedure **8**, compound **175** was obtained as white solid, 35% yield, mp = 87-88 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 2H), 7.72 (s, 1H), 7.40 (s, 1H), 6.90 (d, *J* = 9.0 Hz, 1H), 6.76-6.72 (m, 3H), 6.04 (br, 1H), 4.18 (t, *J* = 5.9 Hz, 2H), 3.89 (m, 2H), 3.83-3.80 (m, 7H), 3.65 (m, 2H), 2.89 (t, *J* = 5.9 Hz, 2H), 2.65 (t, *J* = 5.1 Hz, 2H), 2.18 (s, 3H), 2.05 (m, 2H). MS (ESI) *m/z* 545 $[M+H]^+$. HRMS (FAB) *m/z* calc. for $C_{25}H_{33}ClN_8O_2S$ $[M + H]^+$ 545.2214, found: 545.2220. Anal. HPLC 99.0% (R_t = 3.73 min).

*4.1.4.17. 1-(4-(2-(1-(2-Hydroxyethyl)piperidin-4-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(176)***. Starting with compound **151** as following the general procedure **7**, compound **176** was obtained as a pale red solid, 62% yield, mp = 62-63 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.58 (d, *J* = 1.11 Hz, 1H), 6.95 (d, *J* = 8.61 Hz, 1H), 6.90 (d, *J* = 2.19 Hz, 1H), 6.75 (dd, *J* = 2.40, 8.43 Hz, 1H), 6.66 (s, 1H), 4.05 (t, *J* = 6.21 Hz, 2H), 3.99 (t, *J* = 7.32 Hz, 2H), 3.81 (s, 3H), 3.67 (t, *J* = 6.24 Hz, 2H), 3.59 (t, *J* = 6.78 Hz, 2H), 2.98-2.95 (m, 2H), 2.53 (t, *J* = 6.21 Hz, 2H), 2.21 (d, *J* = 1.08 Hz, 3H), 2.14-1.98 (m, 5H), 1.79-1.68 (m, 4H), 1.37-1.28

(m, 2H). MS (ESI) *m/z* 476 $[M+H]^+$. HRMS (ESI) *m/z* calcd for $C_{24}H_{37}N_5O_3S$ $[M+H]^+$ 476.2690, found: 476.2677. Anal. HPLC 100.0% (R_t = 2.96 min).

*4.1.4.18. 1-(4-(2-(1-(2-Aminoethyl)piperidin-4-yl)ethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(177)***. Starting with compound **132** as following the general procedure **6.2**, compound **177** was obtained as an off white solid, 76% yield, mp = 76-77 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.58 (d, *J* = 1.08 Hz, 1H), 6.95 (d, *J* = 8.43 Hz, 1H), 6.90 (d, *J* = 2.19 Hz, 1H), 6.75 (dd, *J* = 2.40, 8.43 Hz, 1H), 6.66 (t, *J* = 2.55 Hz, 1H), 4.05 (t, *J* = 6.42 Hz, 2H), 3.99 (t, *J* = 7.14 Hz, 2H), 3.81 (s, 3H), 3.59 (t, *J* = 7.14 Hz, 2H), 2.94 (br, 2H), 2.78 (t, *J* = 6.42 Hz, 2H), 2.45 (t, *J* = 7.32 Hz, 2H), 2.21 (d, *J* = 1.11 Hz, 3H), 2.04-1.98 (m, 5H), 1.79-1.68 (m, 4H), 1.37-1.28 (m, 2H). MS (ESI) *m/z* 475 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{38}N_6O_2S$ $[M+H]^+$ 475.2850, found 475.2837. Anal. HPLC 99.5% (R_t = 2.89 min).

*4.1.4.19. 2-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)-phenoxy)acetamide **(178)***. Starting with compound **157** as following the reaction with NH_3 to obtained compound **178**, 62% yield, mp = 147-149 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 7.02 (d, *J* = 2.37 Hz, 1H), 7.00 (d, *J* = 8.43 Hz, 1H), 6.79 (dd, *J* = 2.40, 8.61 Hz, 1H), 6.66 (s, 1H), 4.48 (s, 2H), 4.00 (t, *J* = 7.32 Hz, 2H), 3.85 (s, 3H), 3.61 (t, *J* = 6.78 Hz, 2H), 2.22 (d, *J* = 0.90 Hz, 3H), 2.08 (p, *J* = 7.14 Hz, 2H). MS (FAB) *m/z* 378 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{17}H_{23}N_5O_3S$ $[M+H]^+$ 378.1600 found: 378.1604. Anal. HPLC 97.0% (R_t = 3.99 min).

*4.1.4.20. 3-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)propanamide **(179)***. Starting with compound **97** as following the general procedure **8**, compound **179** was obtained as white solid, 78% yield, mp = 115-117 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.64 (s, 1H), 6.98 (d, *J* = 8.58 Hz, 1H), 6.92 (d, *J* = 2.37 Hz, 1H), 6.75 (dd, *J* = 8.43, 2.40 Hz, 1H), 6.69 (s, 1H), 4.24 (t, *J* = 6.24 Hz, 2H), 3.98 (t, *J* = 7.14 Hz, 2H), 3.80 (s, 3H), 3.59 (t, *J* = 7.14 Hz, 2H), 2.67 (t, *J* = 6.24 Hz, 2H), 2.22 (d, *J* = 0.93 Hz, 3H), 2.03 (p, *J* = 7.14 Hz, 2H). MS (ESI) *m/z* 392 $[M+H]^+$. MS (HRMS) calc. for $C_{18}H_{26}N_5O_2S$ $[M + H]^+$ 392.1751, found 392.1750. Anal. HPLC 99.2% (R_t = 3.22 min).

4.1.4.21. 4-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)butanamide ***(180)***. Starting with compound **98** as following the general procedure **8**, compound **180** was obtained as white solid, 20% yield, mp = 158-160 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.56 (s, 1H), 6.92 (d, *J* = 8.61 Hz, 1H), 6.86 (s, 1H), 6.71 (d, *J* = 8.61 Hz, 1H), 6.63 (s, 1H), 4.00 (t, *J* = 6.24 Hz, 2H), 3.96 (t, *J* = 7.14 Hz, 2H), 3.77 (s, 3H), 3.57 (t, *J* = 6.78 Hz, 2H), 2.39 (t, *J* = 7.32 Hz, 2H), 2.17 (d, *J* = 0.9 Hz, 3H), 2.04 (p, *J* = 7.32 Hz, 4H). MS (FAB) *m/z* 406 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{19}H_{27}N_5O_3S$ $[M + H]^+$ 406.1913, found: 406.1907. Anal. HPLC 99.6% (R_t = 3.32 min).

4.1.4.22. 2-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)-N,N-dimethylacetamide ***(181)***. Starting with compound **157** as following the reaction with dimethylamine, compound **181** was obtained as a white solid, 78% yield, mp = 120-122 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 (s, 1H), 6.95 (d, *J* = 2.16 Hz, 1H), 6.91 (dd, *J* = 8.56 Hz, 1H), 6.81 (s, 1H), 6.73 (dd, *J* = 8.52, 2.40 Hz, 1H), 4.79 (s, 2H), 4.03 (t, *J* = 7.20 Hz, 2H), 3.84 (s, 3H), 3.61 (t, *J* = 6.88 Hz, 2H), 3.09 (s, 3H), 2.96 (s, 3H), 2.25 (d, *J* = 0.76 Hz, 3H), 2.07 (p, *J* = 7.00 Hz, 2H). MS (ESI) *m/z* 406 $[M + H]^+$. HRMS (FAB) for $C_{19}H_{28}N_5O_3S$ $[M + H]^+$ 406.1907, found 406.1908. Anal. HPLC 95.0% (R_t = 4.06 min).

4.1.4.23. 3-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)-N,N-dimethylpropanamide ***(182)***. Starting with compound **99** as following the general procedure **8**, compound **182** was obtained as white solid, 63% yield, mp = 181-183 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.36 (s, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.71 (dd, *J* = 7.8, 3.0 Hz, 1H), 6.70 (s, 1H), 6.68 (d, *J* = 2.1 Hz, 1H), 5.97 (t, *J* = 7.5 Hz, 1H), 4.33 (t, *J* = 7.2 Hz, 2H), 3.87 (t, *J* = 7.5 Hz, 2H), 3.80 (s, 3H), 3.64 (q, *J* = 7.5 Hz, 2H), 3.04 (s, 3H), 2.95 (s, 3H), 2.88 (t, *J* = 6.9 Hz, 2H), 2.15 (d, *J* = 0.6 Hz, 3H), 2.02 (p, *J* = 6.9 Hz, 2H). MS (ESI) *m/z* 420 $[M + H]^+$. HRMS (ESI) calc. for $C_{20}H_{30}N_5O_3S$ $[M+H]^+$ 420.2064, found 420.2065. Anal. HPLC 97.4% (R_t = 4.56 min).

4.1.4.24. 4-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)-N,N-dimethylbutanamide ***(183)***. Starting with compound **100** as following the general procedure **8**, compound **183** was obtained as

white solid, 60% yield, mp = 70-72 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.38 (s, 1H), 6.96 (d, *J* = 8.25 Hz, 1H), 6.74-6.69 (m, 3H), 5.98 (s, 1H), 4.14 (t, *J* = 6.24 Hz, 2H), 3.91 (t, *J* = 7.14 Hz, 2H), 3.82 (s, 3H), 3.69 (q, *J* = 6.21 Hz, 2H), 3.02 (s, 3H), 2.95 (s, 3H), 2.55 (t, *J* = 6.96 Hz, 2H), 2.17 (d, *J* = 0.90 Hz, 3H), 2.14 (p, *J* = 6.78 Hz, 2H), 2.09 (p, *J* = 7.32 Hz, 2H). MS (FAB) *m/z* 434 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{21}H_{31}N_5O_3S$ $[M+H]^+$ 434.2226, found: 434.2228. Anal. HPLC 98.6% (R_t = 3.40 min).

4.1.4.25. 1-(3-Methoxy-4-(2-oxo-2-(piperazin-1-yl)ethoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(184).*** Starting with compound **133** as following the general procedure **6.2**, compound **184** was obtained as white solid, 34% yield, mp = 124-125 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.40 (s, 1H), 6.91 (d, *J* = 8.43 Hz, 1H), 6.77 (d, *J* = 2.22 Hz, 1H), 6.70 (s, 1H), 6.69 (dd, *J* = 8.07, 2.37 Hz, 1H), 6.21 (t, *J* = 7.14 Hz, 1H), 4.77 (s, 2H), 3.90 (t, *J* = 7.35 Hz, 2H), 3.82 (s, 3H), 3.65 (t, *J* = 7.14 Hz, 2H), 3.39-3.55 (m, 4H), 2.88-2.84 (m, 4H), 2.18 (d, *J* = 0.75 Hz, 3H), 2.03 (p, *J* = 7.14 Hz, 2H). MS (ESI) *m/z* 447 $[M + H]^+$. HRMS (FAB) calc. for $C_{21}H_{31}N_6O_3S$ $[M+H]^+$ 447.2173, found 447.2165. Anal. HPLC 95.7% (R_t = 4.34 min).

4.1.4.26. 1-(3-Methoxy-4-(3-oxo-3-(piperazin-1-yl)propoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(185)***. Starting with compound **134** as following the general procedure **6.2**, compound **185** was obtained as white solid, 31% yield, mp = 155-157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1H), 6.97 (d, *J* = 8.61 Hz, 1H), 6.93 (d, *J* = 3.00 Hz, 1H), 6.74 (dd, *J* = 8.40, 2.37 Hz, 1H), 6.68 (s, 1H), 4.27 (t, *J* = 6.24 Hz, 2H), 3.98 (t, *J* = 7.14 Hz, 2H), 3.80 (s, 3H), 3.71-3.57 (m, 6H), 2.97 (t, *J* = 4.95 Hz, 2H), 2.88 (t, *J* = 5.85 Hz, 4H), 2.22 (d, 1.11 Hz, 3H), 2.04 (p, *J* = 7.14 Hz, 2H). MS (ESI) *m/z* 461 $[M + H]^+$. HRMS (FAB) calc. for $C_{22}H_{33}N_6O_3S$ $[M+H]^+$ 461.2329, found 461.2323. Anal. HPLC 95.0% (R_t = 3.15 min).

4.1.4.27. 1-(3-Methoxy-4-(4-oxo-4-(piperazin-1-yl)butoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(186)***. Starting with compound **135** as following the general procedure **6.2**, compound **186** was obtained as a white solid, 39% yield, mp = 91-93 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 6.96 (d, *J* = 8.61 Hz, 1H), 6.92 (d, *J* = 2.37 Hz, 1H), 6.76 (dd, *J* = 2.37, 8.61 Hz, 1H), 6.66 (s, 1H), 4.06 (t, *J* = 6.21 Hz, 2H), 3.99 (t, *J* = 7.50 Hz, 2H), 3.81 (s, 3H), 3.61-3.55 (m, 6H), 2.80-

2.77 (m, 4H), 2.61 (t, *J* = 7.32 Hz, 2H), 2.22 (d, *J* = 0.93 Hz, 3H), 2.08 (p, *J* = 6.78 Hz, 4H). MS (FAB) *m/z* 475 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{34}N_6O_3S$ $[M+H]^+$ 475.2491, found: 475.2483. Anal. HPLC 95.5% (R_t = 3.43 min).

*4.1.4.28. 1-(4-(2-(4-(2-Hydroxyethyl)piperazin-1-yl)-2-oxoethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(187)***. Starting with compound **152** as following the general procedure **7**, compound **187** was obtained as a white solid, 44% yield, mp = 90-91 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 6.87-6.84 (m, 2H), 6.71 (dd, *J* = 2.52, 9.03 Hz, 1H), 6.68 (s, 1H), 4.41 (s, 2H), 3.98 (t, *J* = 7.23 Hz, 2H), 3.82 (s, 3H), 3.68 (t, *J* = 6.03 Hz, 2H), 3.59-3.55 (m, 2H), 2.87 (t, *J* = 5.13 Hz, 4H), 2.52-2.48 (m, 6H), 2.21 (d, *J* = 1.2 Hz, 3H), 2.02 (p, *J* = 6.96 Hz, 2H), MS (ESI) *m/z* 491 $[M+H]^+$. HRMS (ESI) calc. for $C_{23}H_{34}N_6O_4S$ $[M+H]^+$ 491.2435, found 491.2456. Anal. HPLC 97.5% (R_t = 2.99 min).

*4.1.4.29. 1-(4-(2-(4-(2-Aminoethyl)piperazin-1-yl)-2-oxoethoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(188)***. Starting with compound **136** as following the general procedure **6.2**, compound **188** was obtained as a white solid, 24% yield, mp = 79-81 °C .^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (s, 1H), 6.97 (d, *J* = 2.28 Hz, 1H), 6.94 (d, *J* = 8.52 Hz, 1H), 6.75 (dd, *J* = 2.28, 8.48 Hz, 1H), 6.66 (s, 1H), 4.78 (s, 2H), 3.99 (t, *J* = 7.16 Hz, 2H), 3.82 (s, 3H), 3.61-3.59 (m, 6H), 2.76 (t, *J* = 6.44 Hz, 2H), 2.51 (t, *J* = 3.16 Hz, 2H), 2.47 (t, *J* = 4.68 Hz, 4H), 2.21 (d, *J* = 0.96 Hz, 3H), 2.07 (p, *J* = 5.44 Hz, 2H). MS (ESI) *m/z* 490 $[M+H]^+$. HRMS (ESI) calc. for $C_{23}H_{35}N_7O_3S$ $[M+H]^+$ 490.2595, found 490.2595. Anal. HPLC 98.8% (R_t = 2.87 min).

*4.1.4.30. 2-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)-N-(piperidin-4-yl)acetamide **(189)***. Starting with compound **177** as following the general procedure **6.2**, compound **189** was obtained as a white solid, 41% yield, mp = 57-58 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (d, *J* = 1.11 Hz, 1H), 7.07 (d, *J* = 2.40 Hz, 1H), 7.01 (d, *J* = 8.61 Hz, 1H), 6.80 (dd, *J* = 2.40, 8.43 Hz, 1H), 6.66 (s, 1H), 4.49 (s, 2H), 4.00 (t, *J* = 7.32 Hz, 2H), 3.87 (s, 3H), 3.84-3.81 (m, 1H), 3.62 (t, *J* = 6.78 Hz, 2H), 3.05 (d, *J* = 12.81 Hz, 2H), 2.68 (t, *J* = 11.91 Hz, 2H), 2.22 (d, *J* = 1.08 Hz), 2.06 (p, *J* = 6.96 Hz, 2H), 1.88-1.84 (m, 2H), 1.50-1.42 (m, 2H). MS (ESI) *m/z* 461 $[M+H]^+$. HRMS (ESI) calc.

for $C_{22}H_{32}N_6O_3S$ $[M+H]^+$ 461.2329, found 461.2318. Anal. HPLC 98.5% (R_t = 2.96 min).

4.1.4.31. 3-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)-N-(piperidin-4-yl)propanamide ***(190)***. Starting with compound **138** as following the general procedure **6.2**, compound **190** was obtained as a white solid, 24% yield, mp = 89-90 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.58 (s, 1H), 6.97 (d, *J* = 8.43 Hz, 1H), 6.92 (d, *J* = 2.19 Hz, 1H), 6.77 (dd, *J* = 2.37, 8.61 Hz, 1H), 6.66 (s, 1H), 4.25 (t, *J* = 6.03 Hz, 2H), 4.06-4.04 (m, 1H), 3.99 (t, *J* = 7.32 Hz, 2H), 3.81 (s, 3H), 3.61 (t, *J* = 7.14 Hz, 2H), 3.08-3.03 (m, 2H), 2.71-2.60 (m, 4H), 2.21 (d, *J* = 0.93 Hz, 3H), 2.05 (p, *J* = 6.96 Hz, 2H), 1.88-1.85 (m, 2H), 1.46-1.41 (m, 2H). MS (FAB) *m/z* 475 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{34}N_6O_3S$ $[M + H]^+$ 475.2491, found: 475.2477. Anal. HPLC 97.8% (R_t = 3.91 min).

4.1.4.32. 4-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)-N-(piperidin-4-yl)butanamide ***(191)***. Starting with compound **139** as following the general procedure **6.2**, compound **191** was obtained as white solid, 87% yield, mp = 97-98 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.58 (d, *J* = 0.90 Hz, 1H), 6.93 (d, *J* = 8.61 Hz, 1H), 6.92 (s, 1H), 6.74 (dd, *J* = 8.43, 2.40 Hz, 1H), 6.66 (s, 1H), 4.01 (t, *J* = 6.06 Hz, 2H), 3.97 (t, *J* = 7.32 Hz, 2H), 3.81 (s, 3H), 3.79-3.74 (m, 1H), 3.59 (t, *J* = 6.96 Hz, 2H), 3.08 (dt, *J* = 9.90, 2.94 Hz, 2H), 2.72 (td, *J* = 12.09, 2.04 Hz, 2H), 2.38 (t, *J* = 7.14 Hz, 2H), 2.21 (d, *J* = 0.9 Hz, 3H), 2.10-1.99 (m, 4H), 1.88 (d, *J* = 10.08 Hz, 2H), 1.41 (qt, *J* = 11.70, 2.91 Hz, 2H). MS (ESI) *m/z* 489 $[M + H]^+$. HRMS (FAB) calc. for $C_{24}H_{37}N_6O_3S$ $[M+H]^+$ 489.2642, found 489.2659. Anal. HPLC 98.5% (R_t = 3.52 min).

4.1.4.33. N-(1-(2-Hydroxyethyl)piperidin-4-yl)-2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)acetamide ***(192)***. Starting with compound **153** as following the general procedure **7**, compound **192** was obtained as a white solid, 41% yield, mp = 104-106 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.05 (d, *J* = 2.19 Hz, 1H), 7.01 (d, *J* = 8.40 Hz, 1H), 6.80 (dd, *J* = 2.37, 8.43 Hz, 1H), 6.66 (s, 1H), 4.49 (s, 2H), 4.00 (t, *J* = 7.32 Hz, 2H), 3.87 (s, 1H), 3.85-3.82 (m, 1H), 3.69 (t, *J* = 6.06 Hz, 2H), 3.61 (t, *J* = 6.96 Hz, 2H), 2.95 (d, *J* = 12.09 Hz), 2.54 (t, *J* = 6.06 Hz, 2H), 2.22 (d, *J* = 0.72 Hz, 3H), 2.16-2.13 (m, 2H), 2.08 (p, *J* = 7.32 Hz), 1.88-

1.82 (m, 2H), 1.66-1.54 (m, 2H). MS (ESI) *m/z* 505 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{36}N_6O_4S$ $[M+H]^+$ 505.2592, found 505.2583. Anal. HPLC 100.0% (R_t = 2.97 min).

*4.1.4.34. N-(1-(2-Aminoethyl)piperidin-4-yl)-2-(2-methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)acetamide **(193)***. Starting with compound **140** as following the general procedure **6.2**, compound **193** was obtained as a white solid, 77% yield, mp = 125-126 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 7.59 (s, 1H), 7.06 (d, *J* = 1.75 Hz, 1H), 7.00 (d, *J* = 8.55 Hz, 1H), 6.79 (dd, *J* = 2.15, 8.50 Hz, 1H), 6.66 (s, 1H), 4.49 (s, 2H), 3.99 (t, *J* = 7.20 Hz, 2H), 3.87 (s, 3H), 3.79-3.76 (m, 1H), 3.59 (t, *J* = 6.60 Hz, 2H), 2.90 (d, *J* = 11.35 Hz, 2H), 2.82 (t, *J* = 6.55 Hz, 2H), 2.49 (t, *J* = 6.60 Hz, 2H), 2.22 (s, 3H), 2.19-2.14 (m, 2H), 2.07 (p, *J* = 7.10 Hz, 2H), 1.89-1.87 (m, 2H), 1.62 (q, *J* = 8.95 Hz, 2H). MS (ESI) *m/z* 504 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{37}N_7O_3S$ $[M+H]^+$ 504.2751, found 504.2750. Anal. HPLC 100.0% (R_t = 2.80 min).

*4.1.4.35. N-(2-(2-Methoxy-4-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thioureido)phenoxy)-ethyl)piperidine-4-carboxamide **(194)***. Starting with compound **141** as following the general procedure **6.2**, compound **194** was obtained as a white solid, 54% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 7.51 (d, *J* = 1.29 Hz, 1H), 6.89 (d, *J* = 2.40 Hz, 1H), 6.87 (d, *J* = 8.61 Hz, 1H), 6.69 (dd, *J* = 2.40, 8.61 Hz, 1H), 6.57 (s, 1H), 3.98 (t, *J* = 5.67 Hz, 2H), 3.91 (t, *J* = 7.32 Hz, 2H), 3.71 (s, 3H), 3.52-3.41 (m, 4H), 3.28-3.24 (m, 2H), 2.89 (td, *J* = 12.27 Hz, 3.66, Hz, 2H), 2.41-2.38 (m, 1H), 2.13 (d, *J* = 0.90 Hz, 3H), 1.99 (quint, *J* = 6.75 Hz, 2H), 1.83-1.78 (m, 2H), 1.76-1.71 (m, 2H). MS (FAB) *m/z* 475 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{22}H_{34}N_6O_3S$ $[M+H]^+$ 475.2491, found: 475.2491. Anal. HPLC 99.6% (R_t = 4.29 min).

*4.1.4.36. 1-(3-Methoxy-4-(4-(piperazin-1-yl)phenoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(195)***. Starting with compound **142** as following the general producer **6.2**, compound **195** was obtained as a white solid, 69% yield, mp = 88-90 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (s, 1H), 7.09 (d, *J* = 2.37 Hz, 1H), 6.92-6.89 (m, 2H), 6.86-6.81 (m, 3H), 6.76 (dd, *J* = 2.55, 8.58 Hz, 1H) 6.66 (s, 1H), 4.01 (t, *J* = 7.14 Hz, 2H), 3.78 (s, 3H), 3.63 (t, *J* = 6.54 Hz, 2H), 3.06-3.02 (m, 4H), 2.97-2.85 (m, 4H), 2.22 (s, 3H), 2.07 (p, *J* = 6.96 Hz, 2H).

MS (ESI) m/z 481 $[M+H]^+$. HRMS (ESI) calc. for $C_{25}H_{32}N_6O_2S$ $[M+H]^+$ 481.2380, found 481.2394. Anal. HPLC 96.6% (R_t = 2.91 min).

4.1.4.37. 1-(3-Methoxy-4-(4-(4-methylpiperazin-1-yl)phenoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(196)***. Starting with compound **88** as following the general producer **8**, compound **196** was obtained as a white solid, 49% yield, mp = 104-106 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.63 (s, 1H), 7.10 (d, *J* = 2.37 Hz, 1H), 6.95-6.92 (m, 2H), 6.89 (s, 1H), 6.86 (s, 1H), 6.84-6.81 (m, 2H), 6.79 (dd, *J* = 2.37, 8.43 Hz, 1H), 4.02 (t, *J* = 7.23 Hz, 2H), 3.78 (s, 3H), 3.63 (t, *J* = 6.93 Hz, 2H), 3.14 (t, *J* = 4.95 Hz, 4H), 2.66 (t, *J* = 4.95 Hz, 4H), 2.36 (s, 3H), 2.23 (d, *J* = 0.90 Hz, 3H), 2.10 (p, *J* = 6.78 Hz, 2H). MS (ESI) *m/z* 495 $[M+H]^+$. HRMS (ESI) calc. for $C_{26}H_{34}N_6O_2$ $[M+H]^+$ 495.2537, found 495.2532. Anal. HPLC 99.8% (R_t = 2.99 min).

4.1.4.38. 1-(4-(4-(4-(2-Hydroxyethyl)piperazin-1-yl)phenoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(197)***. Starting with compound **159** as following the general producer **7**, compound **197** was obtained as a white solid, 89% yield, mp = 66-67 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.92 (s, 1H), 7.12 (d, *J* = 2.19 Hz, 1H), 6.97-6.94 (m, 2H), 6.87-6.84 (m, 2H), 6.82-6.77 (m, 3H), 4.07 (t, *J* = 7.14 Hz, 2H), 3.82 (t, *J* = 5.52 Hz, 2H), 3.75 (s, 3H), 3.24-3.22 (m, 6H), 3.04 (t, *J* = 4.56 Hz, 4H), 2.91 (t, *J* = 5.67 Hz, 2H), 2.26 (d, *J* = 0.90 Hz, 3H), 2.11 (p, *J* = 7.32 Hz, 2H). MS (ESI) *m/z* 525 $[M+H]^+$. HRMS (ESI) calc. for $C_{27}H_{36}N_6O_3S$ $[M+H]^+$ 525.2642, found 525.2633. Anal. HPLC 96.3% (R_t = 2.92 min).

4.1.4.39. 1-(4-(4-(4-(2-Aminoethyl)piperazin-1-yl)phenoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(198)***. Starting with compound **158** as following the general producer **6.2**, compound **198** was obtained as a white solid, 75% yield, m.p = 75-77 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 7.09 (d, *J* = 2.37 Hz, 1H), 6.96-6.93 (m, 2H), 6.84-6.81 (m, 3H), 6.79 (dd, *J* = 2.22, 8.43 Hz, 1H), 6.66 (s, 1H), 4.01 (t, *J* = 7.50 Hz, 2H), 3.78 (s, 3H), 3.61 (t, *J* = 7.14 Hz, 2H), 3.13 (t, *J* = 5.10 Hz, 4H), 2.82 (t, *J* = 6.39 Hz, 2H), 2.66 (t, *J* = 5.13 Hz, 4H), 2.53 (t, *J* = 6.60 Hz, 2H), 2.22 (d, *J* = 0.90 Hz, 3H), 2.07 (p, *J* = 7.14 Hz, 2H). MS (ESI) *m/z* 524 $[M+H]^+$. HRMS (ESI) calc. for

$C_{27}H_{37}N_7O_2S$ $[M+H]^+$ 524.2802, found 524.2808. Anal. HPLC 99.5% (R_t = 2.75 min).

4.1.4.40. 1-(3-Methoxy-4-(4-(1,2,3,6-tetrahydropyridin-4-yl)phenoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(199)***. Starting with compound **143** as following the general procedure **6.2**, compound **199** was obtained as a white solid, 91% yield, mp = 95-97 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (s, 1H), 7.34 (d, J = 8.79 Hz, 2H), 7.15 (d, J = 2.40 Hz, 1H), 6.99 (d, J = 8.61 Hz, 1H), 6.84-6.80 (m, 3H), 6.67 (s, 1H), 6.08 (s, 1H), 4.02 (t, J = 7.14 Hz, 2H), 3.76 (s, 3H), 3.64 (t, J = 6.78 Hz, 2H), 3.44 (q, J = 3.12 Hz, 2H), 3.04 (t, J = 5.88 Hz, 2H), 2.47-2.43 (m, 2H), 2.23 (d, J = 0.93 Hz, 3H), 2.08 (p, J = 7.14 Hz, 2H). MS (ESI) *m/z* 478 $[M+H]^+$. HRMS (ESI) calc. for $C_{26}H_{31}N_5O_2S$ $[M+H]^+$ 478.2271, found 478.2288. Anal. HPLC 98.6% (R_t = 2.91 min).

4.1.4.41. 1-(3-Methoxy-4-(4-(piperidin-4-yl)phenoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(200)***. Starting with compound **144** as following the general procedure **6.2**, compound **200** was obtained as a white solid, 61% yield, mp = 87-89 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.59 (s, 1H), 7.15-7.12 (m, 3H), 6.94 (d, J = 8.40 Hz, 1H), 6.82-6.80 (m, 3H), 6.66 (s, 1H), 4.01 (t, J = 7.28 Hz, 2H), 3.78 (s, 3H) 3.63 (t, J = 6.96 Hz, 2H), 3.14-3.11 (m, 2H), 2.74-2.71 (m, 2H), 2.64-2.62 (m, 1H), 2.23 (d, J = 0.64 Hz, 3H), 2.14-2.00 (m, 4H), 1.63-1.57 (m, 2H). MS (ESI) *m/z* 480 $[M+H]^+$. HRMS (ESI) calc. for $C_{26}H_{33}N_5O_2S$ $[M+H]^+$ 480.2428, found 480.2424. Anal. HPLC 97.7% (R_t = 2.90 min).

4.1.4.42. 1-(3-Methoxy-4-(3-(piperazin-1-yl)benzyloxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(201)***. Starting with compound **145** as following the general procedure **6.2**, compound **201** was obtained as a white solid, 75% yield, mp = 106-108 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 7.23 (t, J = 7.86 Hz, 1H), 7.06 (s, 1H), 6.97 (d, J = 7.89 Hz, 1H), 6.93-6.90 (m, 3H), 6.71 (dd, J = 7.89, 1.83 Hz, 1H), 6.66 (s, 1H), 5.07 (s, 2H), 3.96 (t, J = 7.14 Hz, 2H), 3.84 (s, 3H), 3.59 (t, J = 6.96 Hz, 2H), 3.13 (t, J = 4.38, 4H), 2.96 (t, J = 5.31 Hz, 4H), 2.21 (s, 3H), 2.02 (p, J = 7.14 Hz, 2H). MS (ESI) *m/z* 495 $[M + H]^+$. HRMS (FAB) calc. for $C_{26}H_{35}N_6O_2S$ $[M+H]^+$ 495.2537 found 495.2542. Anal. HPLC 95.0% (R_t = 3.15 min).

4.1.4.43. 1-(3-Methoxy-4-(3-(piperidin-4-yl)benzyloxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(202)***. Starting with compound **146** as following the general procedure **6.2**, compound **202** was obtained as a white solid, 68% yield, mp = 166-167 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.58 (d, *J* = 1.08 Hz, 1H), 7.33-7.26 (m, 3H), 7.19 (tt, *J* = 6.24 Hz, 1H), 6.97 (d, *J* = 8.61 Hz, 1H), 6.94 (d, *J* = 2.37 Hz, 1H), 6.72 (dd, *J* = 8.43, 2.37 Hz, 1H), 6.66 (s, 1H), 3.96 (t, *J* = 7.14 Hz, 2H), 3.83 (s, 3H), 3.59 (t, *J* = 6.78 Hz, 2H), 3.25 (br, 1H), 3.21 (br, 1H), 2.84 (td, *J* = 12.45, 2.94 Hz, 2H), 2.74 (tt, *J* = 11.91, 3.66 Hz, 1H), 2.21 (d, *J* = 0.90 Hz, 3H), 2.03 (p, *J* = 7.14 Hz, 2H), 1.90-1.86 (br, 2H), 1.75 (p, *J* = 8.40, 3.84 Hz, 2H). MS (ESI) *m/z* 494 [M + H]$^+$. HRMS (FAB) calc. for $C_{27}H_{36}N_5O_2S$ [M+H]$^+$ 494.2584, found 494.2595. Anal. HPLC 97.7% (R_t = 3.18 min).

4.1.4.44. 1-(3-Methoxy-4-(4-(2-(methylamino)pyridin-4-yl)butoxy)phenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(203)***. Starting with compound **147** as following the general procedure **6.2**, compound **203** was obtained as a white solid, 83% yield, mp = 56-58 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.79 (d, *J* = 4.95 Hz, 1H), 7.59 (d, *J* = 0.90 Hz, 1H), 6.93 (d, *J* = 8.79 Hz, 1H), 6.89 (d, *J* = 2.55 Hz, 1H), 6.75 (dd, *J* = 2.37, 8.01 Hz, 1H), 6.66 (s, 1H), 6.45 (dd, *J* = 1.29, 5.31 Hz, 1H), 6.35 (s, 1H), 4.04 (t, *J* = 5.64 Hz, 2H), 3.94 (t, *J* = 7.14 Hz, 2H), 3.80 (s, 3H), 3.61 (t, *J* = 6.57 Hz, 2H), 2.82 (s, 3H), 2.57 (t, *J* = 7.29 Hz, 2H), 2.21 (d, *J* = 0.90 Hz, 3H), 2.07 (p, *J* = 7.32 Hz, 2H), 1.80-1.78 (m, 4H). MS (ESI) *m/z* 483 [M+H]$^+$. HRMS (ESI) calc. for $C_{25}H_{34}N_6O_2S$ [M+H]$^+$ 483.2537, found 483.2534. Anal. HPLC 99.2% (R_t = 2.96 min).

4.1.4.45. 1-(4-(4-(2-((2-Hydroxyethyl)amino)pyridin-4-yl)butoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(204)***. Starting with compound **154** as following the general procedure **7**, compound **204** was obtained as a white solid, 68% yield, mp = 103-104 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.79 (d, *J* = 5.28 Hz, 1H), 7.59 (s, 1H), 6.93-6.90 (m, 2H), 6.75 (dd, *J* = 2.55, 9.12 Hz, 1H), 6.66 (s, 1H), 6.46 (d, *J* = 5.31 Hz, 1H), 6.41 (s, 1H), 4.00 (t, *J* = 5.97 Hz, 2H), 3.97 (t, *J* = 7.14 Hz, 2H), 3.80 (s, 3H), 3.70 (t, *J* = 5.49 Hz, 2H), 3.59 (q, *J* = 6.87 Hz, 2H), 3.37 (t, *J* = 5.49 Hz, 2H), 2.57 (m, 2H), 2.21 (d, *J* = 0.90 Hz, 3H), 2.02 (p, *J* = 6.96 Hz, 2H), 1.79 (m, 4H). MS (ESI) *m/z* 513 [M+H]$^+$. HRMS (ESI) calc. for $C_{26}H_{36}N_6O_3S$ [M+H]$^+$ 513.2642, found 513.2633. Anal. HPLC 98.1% (R_t = 2.95 min).

*4.1.4.46. 1-(4-(4-(2-((2-Aminoethyl)amino)pyridin-4-yl)butoxy)-3-methoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(205)***. Starting with compound as **148** following the general procedure **6.2**, compound **205** was obtained as a white solid, 46% yield, mp = 100-102 °C. ^{1}H NMR (500 MHz, DMSO) δ 9.51 (br, 1H), 7.82 (d, 1H, *J* = 5.05 Hz), 7.76 (br, 1H), 7.53 (s, 1H), 6.97 (s, 1H), 6.89 (d, 1H, *J* = 9.05 Hz), 6.76 (d, 1H, *J* = 8.15 Hz), 6.60 (s, 1H), 6.33-6.27 (m, 3H), 3.93 (t, 2H, *J* = 5.55 Hz), 3.89 (t, 2H, *J* = 7.05 Hz), 3.71 (s, 3H), 3.43 (q, *J* = 5.34 Hz, 2H), 3.19 (q, 2H, *J* = 6.20 Hz), 2.66 (t, 2H, *J* = 6.25 Hz), 2.47-2.45 (m, 2H), 2.14 (s, 3H), 1.92 (p, 2H, *J* = 7.00 Hz), 1.68-1.61 (m, 4H). MS (ESI) *m/z* 512 $[M+H]^+$. HRMS (ESI) calc. for $C_{26}H_{37}N_7O_2S$ $[M+H]^+$ 512.2802, found 512.2800. Anal. HPLC 97.1% (R_t = 2.80 min).

4.2. Molecular Modeling

The X-ray crystal structure of human glutaminyl cyclase (PDB ID: 3PBB) [39] was prepared using the Protein Preparation Wizard in Maestro v.10.2 (Schrödinger, LLC, New York, NY, USA). During the preparation process, bond orders were assigned, zero-order bonds to Zn^{2+} were created, and hydrogen atoms were added. All hydrogen atoms were energy minimized with the optimized potential for liquid simulation (OPLS) 2005 force field. The protonation states of the ligand molecules were predicted using the pKa prediction module in ACD/I-Lab web server (ACD/Labs, Toronto, ON, Canada). The 3D structure of compound **202** was generated by LigPrep v.3.4 in Maestro and the resulting structure was energy minimized in implicit solvent with OPLS 2005 force field in Maestro. The prepared ligand molecules were docked to hQC using Glide v.6.7 in Maestro. The grid for the active site was generated using the centroid of the co-crystallized ligand, PBD150, and the grid box size was set as default. The metal coordination constraint was set to the tetrahedral geometry for Zn^{2+}. Glide SP docking was performed with the maximum number of 30 poses per ligand. The resulting top 5 poses of compound **202** were selected and used for the following QM-Polarized Ligand Docking (QPLD) process. The partial charges of the docked ligands were calculated using Jaguar with the option of accurate QM level. Then, the ligands with the updated charges were re-docked using Glide extra precision (XP). The protein-ligand complex obtained from QPLD was used for further optimization by the Refine Protein-Ligand Complex module in Prime v.4.0 in Maestro. Protein

residues within 5 Å of the docked ligand were minimized by local optimization refinement. The side chain conformations of the selected protein residues were predicted and minimized along with the docked ligand during this process. The resulting structures were further energy minimized using Monte Carlo sampling algorithm in Maestro in 2500 steps. All figures of the molecular structures were generated using PyMOL software (http://www.pymol.org). All computational studies were performed on an Intel Xeon Octa-Core 2.67 GHz workstation with Linux CentOS release 6.7.

1. Design and Pharmacophore

Previously reported QC inhibitors were developed by a pharmacophore design based on the *N*-terminal structure of its substrate $A\beta_{3\text{-}42}$. The representative inhibitors developed by Probiodrug (**1**, **2**),[60, 61, 70,] Wu and colleagues (**3**)[71] and our research group (**4**)[59, 66, 72] are shown in **Figure 2.1**. Naturally occurring inhibitors[73, 74] and other small molecule inhibitors developed by the fragment-based approach[75] were also reported; however, these inhibitors demonstrated only modest activity. Currently, PQ912, developed by Probiodrug, is undergoing a clinical trial, and it has exhibited favorable safety and tolerability. More importantly, PQ912 demonstrated a slight improvement in synaptic and neurological functions in patients with AD in a recently completed phase IIa clinical trial [76, 77] supporting that QC is a potential therapeutic target for the treatment of AD.

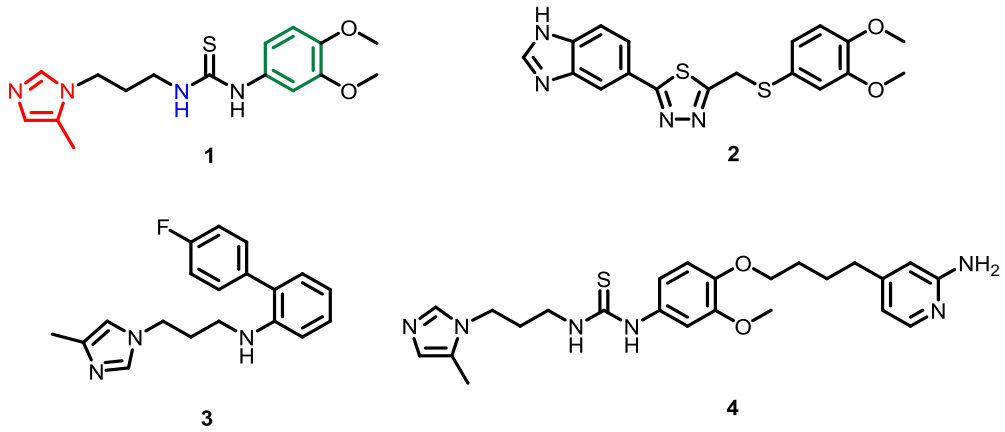


Figure 2.1. Representative QC inhibitors.

The Probiodrug compound **1** contains three key pharmacophores derived from the *N*-terminal Glu-Phe moiety of $A\beta_{3E\text{-}42}$.[60, 61] The 5-methylimidazole ring (red) mimics the *N*-terminal carboxylic acid as a zinc-binding motif. The distal NH of thiourea (blue) serves as a hydrogen bond donor, mimicking the first peptide bond from the *N*-terminus. The phenyl ring (green) mimics the Phe side chain at the penultimate position to the *N*-terminus. Inspired by this approach, we had previously developed a series of QC inhibitors (template **A**), with an extended scaffold as described in **Figure 2.2**.[59, 72] The scaffold contains an additional pharmacophore that mimics the binding interaction of the guanidine side chain of Arg at the

antepenultimate position to the *N*-terminus. The newly developed QC inhibitors displayed much improved potency with a range of 5 to 40-fold more potent inhibition than **1**. Specifically, compound **4** not only exhibited potent inhibition without cytotoxicity but also significantly reduced the brain concentrations of pE-Aβ and total Aβ while restoring cognitive functions in an AD animal model. The molecular modeling analysis of **4** indicated that the 2-aminopyridine moiety showed strong interactions with the carboxylate group of Glu327 in the QC binding site.

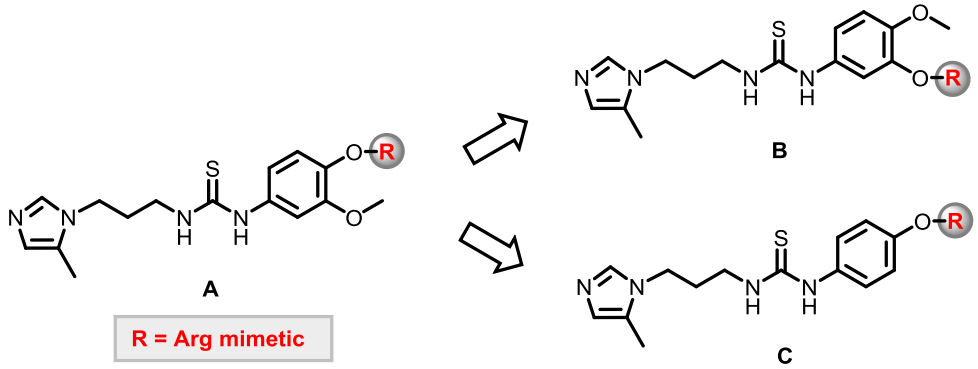


Figure 2.2. Design rationale for QC inhibitors with novel templates

In this study, we investigated a series of 3-aminoalkyloxy-4-methoxyphenyl (template B) and 4-aminoalkyloxyphenyl (template C) surrogates as variants of template A. We anticipate that these templates would provide useful information to optimize the position of Arg-mimetic region (from template B) and to identify the significance of the 3-methoxy group (from template C) for QC inhibition. We evaluated the human QC inhibitory activity of the synthesized compounds *in vitro* and selected several potent inhibitors (IC_{50} < 10 nM). We further tested these compounds for *in vitro* toxicity/permeability and *in vivo* activity in acute AD model mice. Finally, a molecular modeling study was performed to analyze the specific binding interactions in the QC active site.

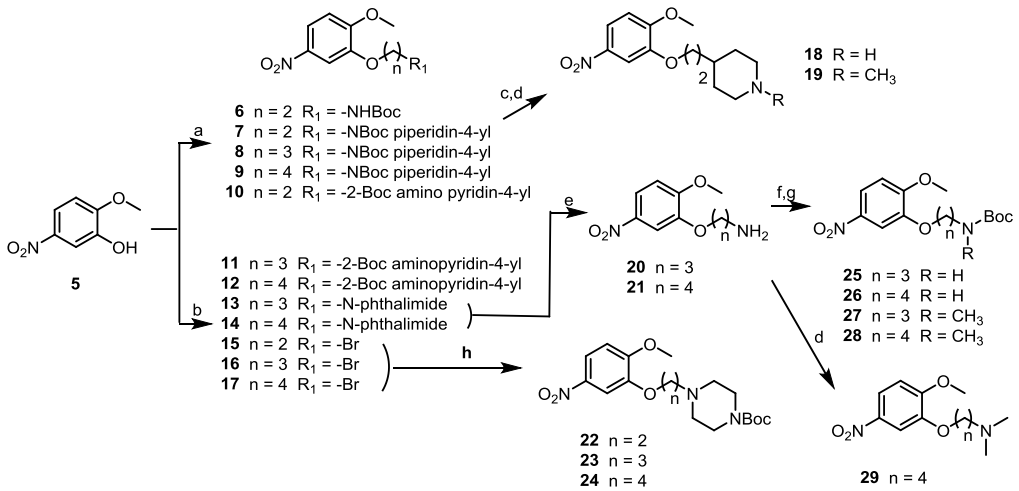
2. Results and discussion

2.1. Chemistry

In general, the final thiourea compounds were synthesized by the coupling reaction between 3-(5-methyl-1*H*-imidazol-1-yl)propan-1-amine and aniline

intermediates obtained by the reduction of nitro fragments prepared in **Schemes 2.1** and **2.2**.

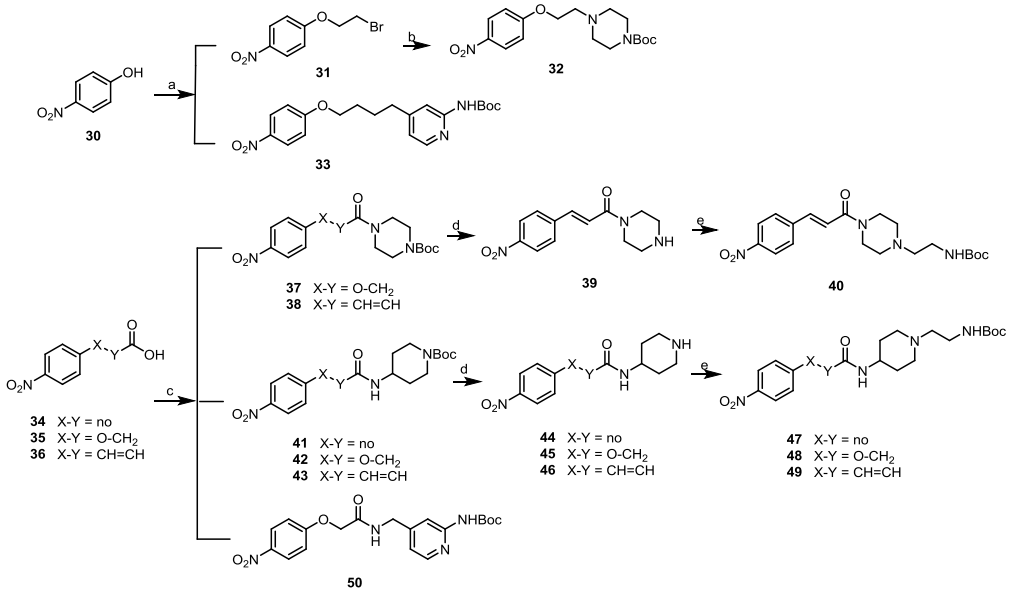
The synthesis of the 3-aminoalkyloxy-4-methoxy-1-nitrobenzene fragments is described in **Scheme 2.1**. Starting from 5-nitroguaiacol (**5**), the Mitsunobu reaction or Williamson reaction incorporated *N*-protected aminoalkyl moieties into the phenolic position to provide **6**-**17**. Among them, the *N*-Boc protected amino (**6**-**12**) and phthalimide protected amino (**13**, **14**) intermediates were directly employed for the thiourea coupling. The bromides **15**-**17** were reacted with *N*-Boc piperazine to give **22**-**24**. After deprotection, the free amines (**18**, **21**) were converted to the *N*-methylpiperidine (**19**) and dimethylamino (**29**) analogues by reductive amination, respectively. Meanwhile, the amines (**20**, **21**) were protected with a Boc group and then *N*-methylated to afford the corresponding *N*-Boc methylamino (**27**, **28**) analogues.



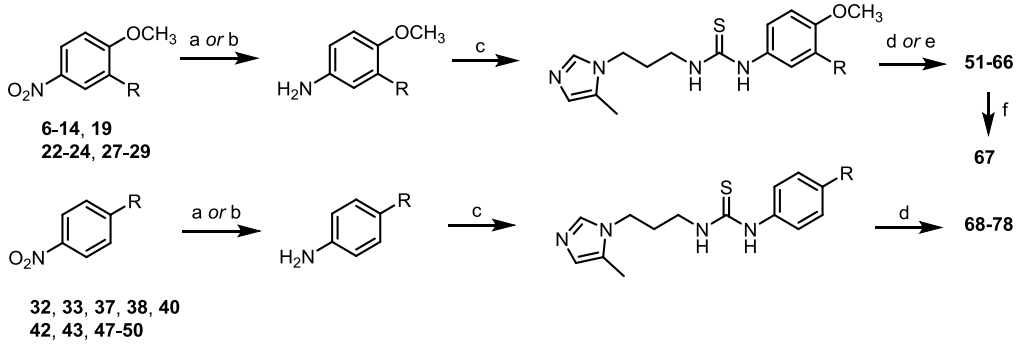
Scheme 2.1. 3-aminoalkyloxy-4-methoxy-1-nitrobenzene fragments. Reagents and conditions: (a) *N*-Boc ethanolamine, *N*-Boc piperidine derivatives or *tert*-butyl (4-(2-hydroxyethyl)pyridin-2-yl)carbamate, DEAD, PPh_3, DCM, r.t., overnight; (b) alkyl halides, K_2CO_3, DMF, 100 °C, 1 h; (c) TFA, DCM; (d) $ZnCl_2$, HCHO, $NaBH_3CN$, MeOH, r.t., overnight; (e) $N_2H_4.H_2O$, EtOH, r.t., overnight; (f) Boc_2O, TEA, DCM; (g) CH_3I, NaH, THF, 0 °C for **27**, **28**; (h) *N*-Boc piperazine, DMF, TEA, 60 °C, 2 h.

The synthesis of 4-aminoalkyloxy-1-nitrobenzene fragment is shown in **Scheme 2.2**. The *N*-Boc aminoalkyloxy analogues (**32**, **33**) were prepared from 4-nitrophenol (**30**) by alkylation reactions. The *N*-Boc amido analogues (**37**, **38**, **42**, **43**,

50) were synthesized from 4-nitrobenzoic acid (**34**), 2-(4-nitrophenoxy)acetic acid, (**35**) or 4-nitro cinnamic acid (**36**), by coupling with the corresponding amines. The *N*-aminoethyl piperazinyl (**40**) and piperidinyl (**47-49**) analogues were prepared from the corresponding piperazine (**39**) and piperazine (**44-46**) precursors by alkylation with *tert*-butyl (2-iodoethyl)carbamate.



Scheme 2.2. 4-aminoalkyloxy-1-nitrobenzene fragment. Reagents and conditions: (a) alkyl halide, Cs_2CO_3, DMF, heat; (b) *N*-Boc piperazine, Cs_2CO_3, DMF, heat; (c) RNH_2, EDC, HOBt, DCM; (d) TFA, DCM; (e) *tert*-Butyl (2-iodoethyl)carbamate, NaH, DMF, 0 °C to r.t., 2 h.



Scheme 2.3. Synthesis of final compound of Phe-Arg mimetic region. Reagents and conditions: (a) Pd, H_2, MeOH; (b) Zn, AcOH, MeOH; (c) 3-(5-methyl-1H-imidazol-1-yl)propan-1-amine, TCDI, Et_3N, DCM, r.t., overnight; (d) TFA, DCM, 0 °C to r.t.,

overnight; (e) $N_2H_4.H_2O$, EtOH, r.t., overnight; (f) 2-chloropyrimidine, TEA, EtOH, reflux, 2 days.

The synthesis of the final thiourea compounds is illustrated in **Scheme 2.3**. The synthesized anilines were reduced either by hydrogenation or by zinc in acetic acid to provide the corresponding amines, which were then coupled with 3-(5-methyl-1*H*-imidazol-1-yl)propan-1-amine by previous method[59] to afford the corresponding thioureas. Finally, the deprotection of *N*-Boc and *N*-phthalimide provided the final 3-aminoalkyloxy-4-methoxyphenyl (**51**-**66**) and 4-aminoalkyloxyphenyl (**68**-**78**) derivatives, respectively. The pyrimidine **67** was synthesized from amine **53** by reacting with 2-chloropyrimidine.

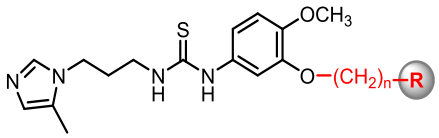
2.2. In vitro QC Inhibition

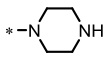
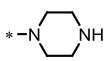
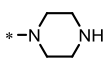
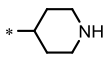
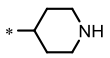
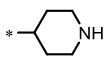
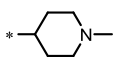
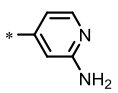
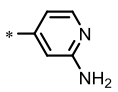
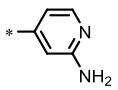
As previously reported, to evaluate the human QC inhibition of the synthesized compounds we performed the QC activity assays employing a fluorogenic substrate, Gln-AMC (L-glutamine 7-amido-4-methylcoumarin), and pyroglutamyl peptidase (pGAP) as an auxiliary enzyme. [67]

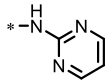
First, we investigated a series of 3-aminoalkyloxy-4-methoxyphenyl analogues represented as template B (**Table 2.1**). The primary amine derivatives (**51**-**53**) exhibited similarly potent inhibition regardless of the length of linkers with a range of IC_{50} = 7.9-9.0 nM, which were 3.5-fold more potent than the parent **1**. The secondary amine derivatives (**54**, **55**) were found approximately 3-fold less active than the corresponding primary amines (**52**, **53**). The tertiary amine derivative (**56**) showed poor inhibition compared to the corresponding secondary derivative (**55**). Next, we examined the cyclic amine derivatives. The piperazine derivatives (**57**-**59**) also displayed reasonable inhibitory effect regardless of the linker length. The piperidine derivatives (**60**-**62**) exhibited better activity than those of piperazine derivatives, and they showed similar IC_{50} values compared to the primary amine derivatives (**51**-**53**). However, the *N*-methylation of the piperidine (**63**) resulted in the loss of activity. Because the previously developed 2-aminopyridine derivatives (template A, **Figure 2**) showed promising activities both in *in vitro* (IC_{50} = 4.5 nM for **4**) and *in vivo*, we also examined the 2-aminopyridine analogues (**64**-**66**). Unfortunately, these compounds were found to be less potent than their 3-methoxyphenyl-4-aminoalkyloxy counter parts (template A). When the

aminopyridine group was replaced with a 2-aminopyrimidine (**67**), the compound showed similar inhibition as the aminopyridine analogue **66**.

Table 2.1. IC_{50} values for the inhibition of *h*QC by 3-aminoalkyloxy-4-methoxy-1-nitrobenzene compounds



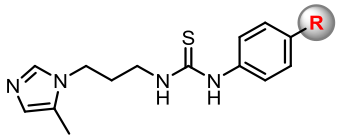
Cpd#	R	n	IC_{50} (nM)[a]	
1			29.2[b]	
51		2	7.9	(±0.7)
52		3	9.0	(±2.3)
53		4	8.8	(±0.6)
54		3	26.8	(±4.7)
55		4	22.0	(±6.8)
56		4	46.8	(±10.8)
57		2	18.4	(±1.9)
58		3	15.8	(±2.8)
59		4	15.0	(±0.5)
60		2	7.3	(±6.3)
61		3	8.8	(±2.2)
62		4	7.9	(±2.1)
63		2	20.6	(±7.4)
64		2	26.4	(±8.8)
65		3	15.0	(±2.9)
66		4	15.9	(±7.4)

67		4	15.2	(±2.0)

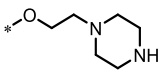
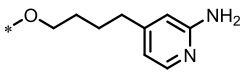
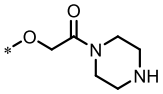
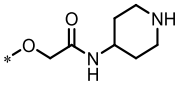
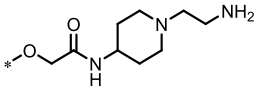
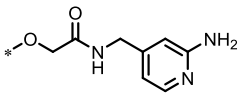
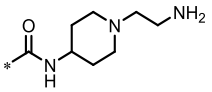
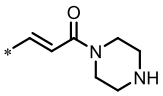
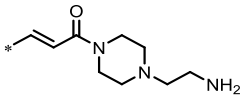
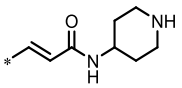
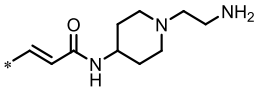
[a] The values indicate the mean of at least three experiments. [b] Refs. 14 and 18

Next, we examined a series of 4-aminoalkyloxyphenyl analogues, in which the 3-methoxy group was removed and only contained the substituent at the 4-position and was represented as template C (**Table 2.2**). We first tested compounds **68** and **69**, which were most potent when they contained the 3-methoxy group.[59] However, it was found that compounds **68** and **69** were much less active, by 27- and 5.5-fold respectively, than their previously reported 3-methoxy counterparts, suggesting that the 3-methoxy group represents an important binding interaction for inhibitory effect. We also examined inhibition of compounds **70-72** with an amido linker to compare the structure-activity relationship of the previously developed derivatives containing a 3-methoxy group.[71] These compounds appeared to maintain similar activity compared to the previously reported compounds,[71] while the *N*-aminoethyl piperidine derivative (**72**) was found to be more potent than that of **70**, suggesting that the presence of an additional amide group may aid extra binding interactions and that the terminal amino group may serve as an Arg-mimetic. However, shortening or rigidifying the linker group mostly resulted in the loss of activity, as demonstrated in **73** and **74** (a 2-fold reduction than compounds **69** and **72**, respectively). As a bioisostere of methyleneoxy group in the linker, we also tested the cinnamic linker surrogates (**75-78**). Although compounds containing the cinnamic linker appeared to be slightly less active than the corresponding alkyloxy derivatives, the addition of an *N*-aminoethyl group to compound **75** increased the activity 3-fold to give potent inhibitor **76** with an IC_{50} = 6.4 nM, again suggesting that the terminal amino group likely served as an Arg-mimetic group to interact with QC.

Table 2.2. IC_{50} values for inhibition of *h*QC by 4-aminoalkyloxy-1-nitrobenzene compounds



Cpd#	R	IC_{50} (nM)[a]

68		18.7	(±5.8)
69		24.7	(±6.5)
70		11.4	(±2.0)
71		12.9	(±5.9)
72		7.9	(±7.3)
73		56.5	(±12)
74		16.2	(±4.0)
75		19.4	(±9.9)
76		6.4	(±0.6)
77		26.0	(±9.6)
78		11.2	(±3.2)

[a] The values indicate the mean of at least three experiments.

2.3. In vivo activity

Based on the *in vitro* QC inhibition of the synthesized compounds, we selected eight of the most potent inhibitors with IC_{50} values less than 10 nM for further evaluation. We first examined cytotoxicity by incubating HT-22 cells, an immortalized hippocampal neuronal cell line, with 10 μM of each compound, and performed MTT assays. All tested compounds were not cytotoxic, demonstrating normal cell viability. We also evaluated the ability of *h*ERG channel blocking for all

compounds to assess drug toxicity. All compounds showed moderate (35.1%, **62**) to low inhibition (2.8%, **53**) at 10 µM, indicating that they pose a marginal to low risk for cardiotoxicity. To evaluate the ability of the compounds to penetrate the blood-brain barrier (BBB), we carried out a parallel artificial membrane permeability assay (PAMPA). Six compounds, **51**-**53** and **60**-**62**, showed reasonable permeability, with a range of 5.0-5.9 for –logPe. Interestingly, all of the primary amine (**51**-**53**) and piperidine (**60**-**62**) derivatives showed reasonable values for BBB penetration regardless of the length of the carbon linker, whereas the *N*-aminoethyl derivatives, **72** and **76**, exhibited very low permeability (–logPe = 10 and 9.0).

Table 2.3. Studies of *in vitro* toxicity, permeability, and *in vivo* QC inhibition in acute model

	in vitro IC_{50} (nM)	cytotoxicity at 10 µM (% of control)	*h*ERG assay (10 µM, % inhibition)	PAMPA (-logPe)	% inhibition of human $A\beta_{N3pE-40}$ formation (icv)[a]
51	7.9	~100	15.3	5.7	35.3
52	9.0	~100	8.8	5.6	12.8
53	8.8	~100	2.8	5.9	36.6
60	7.3	~100	14.9	5.3	1.8
61	8.8	~100	25.6	5.0	5.5
62	7.9	~100	35.1	5.7	14.4
72	7.9	~100	19.9	10.0	NE
76	6.4	~100	7.2	9.0	22.9

[a] 5 µL of human $A\beta_{3-40}$ in PBS (1 µg/µL) was injected into the deep cortical/hippocampus to 5 weeks old ICR mice (25 g, n = 4, male) using a stereotaxic frame to induce acute Aβ toxicity. Test compounds were administrated via icv injection. Sandwich ELISA was performed for the quantification of the brain $A\beta_{N3pE-40}$

Finally, we tested each compound in acute AD model mice to evaluate QC inhibition *in vivo*. We first injected human $A\beta_{3-40}$ (5 µg) and each compound (25 mg/kg) successively into deep cortical/hippocampal tissues of ICR mice (male, six weeks old) by intracerebroventricular (icv) administration. On the next day, we measured the levels of human $A\beta_{N3pE-40}$ in the brain extracts of each mouse to determine the QC inhibitory activity. As described in **Table 2.3**, compounds **51**-**53**,

62 and **76** suppressed the formation of $A\beta_{N3pE-40}$ by 12.8% to 36.6% compared to the vehicle control. In particular, compounds **51** and **53**, with *in vitro* IC_{50} values of 7.9 and 8.8 nM, exhibited the most potent $A\beta_{N3pE-40}$-lowering effects by 35.3 and 36.6% reduction, respectively, indicating that the specific inhibition of QC resulted in the reduced brain levels of $A\beta_{N3pE-40}$. Overall, these two compounds exhibited potent *in vitro* and *in vivo* QC inhibitory activities and good brain penetration without potential toxicity.

2.4. Molecular modeling

To evaluate the binding interactions between the *h*QC and the potent inhibitor **53**, we carried out the sequential docking studies using X-ray crystal structure of *h*QC (PDB id: 3PBB).[69] For the initial docking study, the protonated amine form of **53** at pH 7.4 was used and placed into a Glide SP (Standard Precision).

The result exhibited that the *N*-3 nitrogen of the 5-methyl imidazole chelated with zinc and formed a hydrogen bonding with Trp329. Additionally, the 5-methyl group occupied a hydrophobic pocket composed of Trp207, Leu249, Ile303, Ile321, and Phe325. The thiourea group caused the appropriate positioning of the phenyl ring for the hydrophobic interaction with Ile303, while the methoxy oxygen on the phenyl ring formed a hydrogen bond with Tyr299 (**Figure 2.3**).

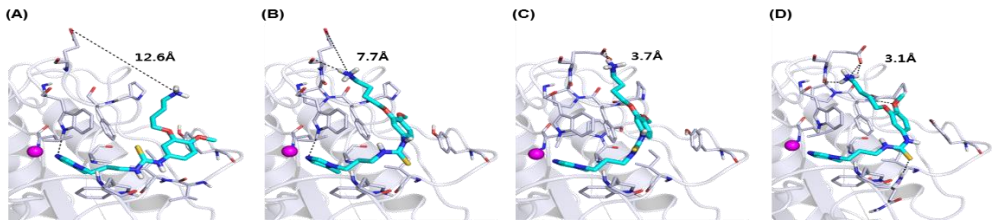


Figure 2.3. Sequential docking and refinement of **53** in *h*QC.

(A) Glide SP docking, (B) QPLD, (C) Local optimization, and (D) Monte Carlo minimization. Binding modes of **53** in protonated form were shown in each step. The distances between Glu327 and the terminal *N* from D-region of the ligands were revealed in black dashed lines.

Afterwards, Glide QM-Polarized Ligand Docking (QPLD) in Maestro was implemented, and the result showed the amino group in the side chain shifted toward Glu327 of the *h*QC active site (**Figure 2.3B**). The local optimization refinement was

conducted, and the result displayed the bended Glu327 side chain, and constituted a salt bridge with the amino group of **53** (**Figure 2.3C**). To search for the global minimum, we performed a Monte Carlo minimization (**Figure 2.3D**). This type of sequential optimization for the protein-ligand complexes formed hydrogen bonding as well as salt bridge interactions, along with the H-bonding with the phenyl ring of **53** (**Figure 2.3**).

Accordingly, the imidazole ring formed π-π interactions with Trp329, and the thiourea group showed a hydrogen bond with Gln304 while the dimethoxyphenyl group formed a H-bonding interaction and π-π interaction with Phe325. Moreover, the protonated amine group located in the side chain displayed a salt bridge interaction with Glu327 and H-bonding with Pro326 and Glu327. Overall, we believe that switching the substituents in the 3-and 4-positions did not alter the binding interactions significantly, partly due to the flexibility of the 4-aminoalkoxy chain, which is also supported by the SAR found in the analogues with rigid and short linkers.

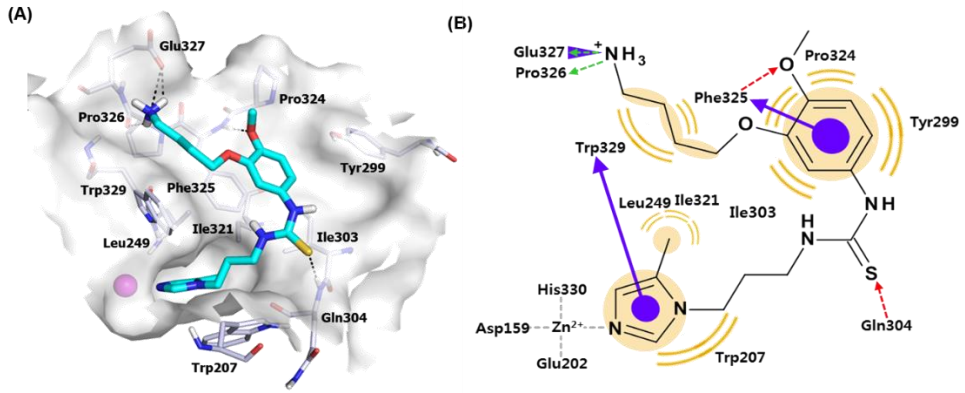


Figure 2.4. Docked and refined structure of **53** in *h*QC.

(A) Binding interactions of **53** at the active site of the *h*QC. Ligand is displayed as sticks with cyan carbon atoms, and Zn^{2+} is in purple ball. The interacted residues are shown in light blue sticks. Hydrogen bonds are described as black dashed lines. (B) 2D illustration of the binding interactions between **53** with *h*QC. Hydrophobic interactions are indicated in light brown. Hydrogen bonds are exposed as red- and green-dotted point with the directionality. The π-π stacking interaction is signified in purple disc and arrow, and the salt bridge interaction is displayed as purple wedge line.

3. Conclusion

In this study, we investigated a series of QC inhibitors containing 4-aminoalkyloxy-3-methoxyphenyl and 3-aminoalkyloxyphenyl groups as Phe-Arg mimetics of $A\beta_{3-42}$. The primary amines (**51**-**53**) and 4-piperidinyl (**57**-**59**) derivatives exhibited potent QC inhibition, demonstrating 3-4 fold more potent activity than the parent inhibitor **1** by Probiodrug. Further *in vivo* studies revealed that inhibitors **51** and **53** displayed the most potent $A\beta_{N3pE-40}$-lowering effects with 35.3 and 36.6% *in vivo*, respectively, with reasonable BBB penetration, which also corresponded to their *in vitro* potency. The molecular modeling analysis of **53** indicated that the salt bridge interaction and the hydrogen bonding of the protonated amine group with Glu327 and Pro326 provided a high potency compared to the parent inhibitor **1**. Given the potent QC inhibitory effect, favorable BBB penetration, and the toxicity profile, we believe that compound **53** may serve as a potential candidate for anti-Alzheimer's agents.

4. Experimental

4.1. Chemistry

4.1.1. General

All chemical reagents were commercially available. Melting points were determined on a melting point Buchi B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230-400 mesh, Merck. ^{1}H NMR spectra were recorded on a a JEOL JNM-LA 300 at 300 MHz, Bruker Analytik, DE/AVANCE Digital 400 at 400 MHz, a Bruker Analytik, DE/AVANCE Digital 500 at 500 MHz, and a JEOL JNM-ECA-600 at 600 MHz. Mass spectra were recorded on a VG Trio-2 GC−MS instrument and a 6460 Triple Quad LC−MS instrument. All final compounds were assessed for purity by high performance liquid chromatography (HPLC) on Agilent 1120 Compact LC (G4288A) system via the following conditions. Column: Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm). Mobile phase A: MeOH, Mobile phase B: 0.1% TFA in water (v/v) in 30 min. Wavelength: 254 nM. Flow: 0.7 mL/min. According to the HPLC analyses, all final compounds showed a purity of ≥95%.

4.1.2. General procedure

4.1.2.1. Mitsunobu reaction (Procedure 1)

Triphenylphosphine (1.3 eq) was added under nitrogen to a solution of 5-nitroguaiacol (1.0 eq) in CH_2Cl_2, followed by adding alcohol (1.2 eq) and a solution of diethyl azodicarboxylate (1.3 eq) in CH_2Cl_2. After the solution was stirred for 30 min at room temperature, the reaction was poured onto a column of silica and was eluted with EtOAc/*n*-hexane to give desired product.

4.1.2.2. Williamson reaction (Procedure 2)

Alkyl halide (4.0 eq) was added to a suspension of 5-nitroguaiacol or 4-nitrophenol (1.0 eq) and Cs_2CO_3 (2.0 eq) in anhydrous DMF. The reaction mixture was heated to 100 °C for 1 h and then cooled to room temperature before quenched by H_2O. The mixture was extracted with EtOAc (2 x 50 mL). The organic layer was washed by H_2O three times, dried by $MgSO_4$ and concentrated. This concentration was then purified by column chromatography to get product.

4.1.2.3. Deprotection of phthalimide group (Procedure 3)

Hydrazine monohydrate was added to a solution of phthalimide compound in ethanol and stirred at room temperature overnight. The precipitate was filtered and washed with EtOH. The filtrate was collected and concentrated in vacuo. The residue was then purified by PLC ($MeOH/CH_2Cl_2$) to give product.

4.1.2.4. N-Alkylation (Procedure 4)

A mixture of alkyl halide, amine, and excess base in DMF was stirred at 60 °C for 30 min. The mixture reaction was quenched by H_2O and extracted by EtOAc several times. The combined organic layer was washed with H_2O and brine and concentrated in vacuo. The residue was purified by column chromatography.

4.1.2.5. Boc protection (Procedure 5)

To a suspension of starting material amine (1.0 eq) in CH_2Cl_2 was added triethylamine (1.2 eq) and di-*tert*-butyl dicarbonate (2.5 eq) in CH_2Cl_2 under ice bath. The mixture was stirred at room temperature until starting material was consumed, by checking with TLC. The mixture was diluted with H_2O and extracted with CH_2Cl_2 several times. The combined organic layers were washed with 10% aqueous $NaHCO_3$

solution, H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography to get the desired product.

4.1.2.6. Boc deprotection (Procedure 6)

Trifluoroacetic acid (10 eq) was added to the solution of *Boc*-protected compound (1.0 eq) in CH_2Cl_2 (DCM:TFA = 1:1 (v/v)). Then, the mixture was stirred at room temperature until the starting material consumed and evaporated. The residue was dissolved in MeOH and purified by ion-exchange column to get desired product or carried to the next step without further purification.

4.1.2.7. Reductive methylation of amine (Procedure 7)

To a stirred solution of amine (1 eq) in MeOH (5 mL) containing 37% aqueous formaldehyde (3 eq) at room temperature was added a solution of sodium cyanoborohydride (1 eq) and zinc chloride (0.5 eq) in MeOH (5 mL). After the reaction mixture was stirred at room temperature for overnight, the solution was taken up in 0.1 N NaOH (10 mL), and most of the MeOH was evaporated under reduced pressure. After the aqueous solution was extracted with EtOAc (20 mL x 3), the combined extracts were washed with H_2O and brine, dried over $MgSO_4$ and evaporated until dry . The residue was distilled in vacuo to give the desired product.

4.1.2.8. EDC coupling (Procedure 8)

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide HCl (EDC-HCl, 1.1 eq) and *N,N*-diisopropylethylamine (2.2 eq) were added to a solution of amine (1.0 eq), acid (1.0 eq) and 1-hydroxybenzotriazole (HOBt, 1.1 eq) in CH_2Cl_2. The mixture was stirred for 24 h at room temperature under nitrogen. The solvent was removed in vacuo and the residue purified by column chromatography on silica gel, eluting with CH_2Cl_2/MeOH to provide the desired compound.

4.1.3. Intermediate compounds

4.1.3.1. tert-Butyl (2-(2-methoxy-5-nitrophenoxy)ethyl)carbamate ***(6)***. The title compound was prepared from 5-nitroguaiacol (**5**) according to procedure **1** as a yellow solid in 99% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (dd, J = 8.97, 2.55 Hz,

1H), 7.76 (d, *J* = 2.58 Hz, 1H), 6.93 (d, *J* = 8.97 Hz, 1H), 5.07 (br, NH), 4.15 (t, *J* = 5.31 Hz, 2H), 3.96 (s, 3H), 3.61 (q, *J* = 5.49 Hz, 2H), 1.45 (s, 9H).

4.1.3.2. tert-Butyl 4-(2-(2-methoxy-5-nitrophenoxy)ethyl)piperidine-1-carboxylate ***(7)***. The title compound was prepared from compound **5** according to procedure **1** as a yellow oil in 63% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 8.97, 2.58 Hz, 1H), 7.73 (d, *J* = 2.55 Hz, 1H), 6.92 (d, *J* = 8.97 Hz, 1H), 4.35-4.17 (m, 3H), 3.96 (s, 3H), 2.72 (t, *J* = 11.73 Hz, 2H), 1.85-1.80 (m, 2H), 1.75-1.72 (m, 2H), 1.46 (s, 9H), 1.46-1.45 (m, 2H), 1.36-1.30 (m, 2H).

4.1.3.3. tert-Butyl 4-(3-(2-methoxy-5-nitrophenoxy)propyl)piperidine-1-carboxylate ***(8)***. The title compound was prepared from compound **5** according to procedure **1** as a yellow oil in 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 8.97, 2.73 Hz, 1H), 7.73 (d, *J* = 2.73 Hz, 1H), 6.92 (d, *J* = 8.97 Hz, 1H), 4.15-4.05 (m, 4H), 3.96 (s, 3H), 3.48 (m, 1H), 2.72 (t, *J* = 12.06 Hz, 2H), 1.95 (p, *J* = 6.60 Hz, 2H), 1.72 (d, *J* = 13.53 Hz, 2H), 1.45 (s, 9H), 1.45-1.39 (m, 2H), 1.17-1.11 (m, 2H).

4.1.3.4. tert-Butyl 4-(4-(2-methoxy-5-nitrophenoxy)butyl)piperidine-1-carboxylate ***(9)***. The title compound was prepared from compound **5** according to procedure **1** as a brown oil in 63% yield. ^{1}H NMR (300 MHz, CDCl3) δ 7.92 (dd, *J* = 8.79, 2.55 Hz, 1H), 7.73 (d, *J* = 2.76 Hz, 1H), 6.91 (d, *J* = 8.79 Hz, 1H), 4.15-4.06 (m, 4H), 3.96 (s, 3H), 2.71 (t, *J* = 12.09 Hz, 2H), 1.91 (p, *J* = 6.96 Hz, 2H), 1.68-1.64 (m, 2H), 1.51-1.47 (m, 3H), 1.45 (s, 9H), 1.35-1.28 (m, 2H), 1.15-1.02 (m, 2H).

4.1.3.5. tert-Butyl (4-(2-(2-methoxy-5-nitrophenoxy)ethyl)pyridin-2-yl)carbamate ***(10)***. The title compound was prepared from compound **5** according to procedure **1** as a yellow solid in 59% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 5.13 Hz, 1H), 7.94-7.90 (m, 2H), 7.73 (d, *J* = 2.55 Hz, 1H), 7.43 (br, NH), 6.94 (dd, *J* = 5.13, 1.47 Hz, 1H), 6.92 (d, *J* = 8.97 Hz, 1H), 4.33 (t, *J* = 6.78 Hz, 2H), 3.96 (s, 3H), 3.19 (t, *J* = 6.78 Hz, 2H), 1.53 (s, 9H).

4.1.3.6. tert-Butyl (4-(3-(2-methoxy-5-nitrophenoxy)propyl)pyridin-2-yl)carbamate ***(11)***. The title compound was prepared from compound **5** according to procedure **2** as a yellow solid in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, *J* = 5.10 Hz,

1H), 7.93 (dd, *J* = 8.79, 2.55 Hz, 1H), 7.84 (s, 1H), 7.70 (d, *J* = 2.58 Hz, 1H), 7.64 (s, 1H), 6.92 (d, *J* = 8.79 Hz, 1H), 6.84 (dd, *J* = 5.13, 2.28 Hz, 1H), 4.11 (t, *J* = 6.24 Hz, 2H), 3.97 (s, 3H), 2.86 (t, *J* = 7.14 Hz, 2H), 2.27 (p, *J* = 6.24 Hz, 2H), 1.52 (s, 9H).

4.1.3.7. tert-Butyl (4-(4-(2-methoxy-5-nitrophenoxy)butyl)pyridin-2-yl)carbamate ***(12)***. The title compound was prepared from compound **5** according to procedure **2** as a yellow solid in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, *J* = 5.31 Hz, 1H), 7.92 (dd, *J* = 8.97, 2.76 Hz, 1H), 7.80 (br, NH), 7.72 (d, *J* = 2.55 Hz, 1H), 7.21 (br, NH), 6.91 (d, *J* = 8.79 Hz, 1H), 6.83 (dd, *J* = 4.95, 1.47 Hz, 1H), 4.11 (t, *J* = 6.06 Hz, 2H), 3.96 (s, 3H), 2.72 (t, *J* = 7.68 Hz, 2H), 1.89-1.81 (m, 4H), 1.52 (s, 9H).

4.1.3.8. 2-(3-(2-Methoxy-5-nitrophenoxy)propyl)isoindoline-1,3-dione ***(13)***. The title compound was prepared from compound **5** according to procedure **2** as a yellow solid in 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90-7.81 (m, 3H), 7.75-7.61 (m, 3H), 6.84 (d, *J* = 8.97 Hz, 1H), 4.18 (t, *J* = 6.03 Hz, 2H), 3.96 (d, *J* = 6.60 Hz, 2H), 3.73 (s, 3H), 2.31 (p, *J* = 6.24 Hz, 2H).

4.1.3.9. 2-(4-(2-Methoxy-5-nitrophenoxy)butyl)isoindoline-1,3-dione ***(14)***. The title compound was prepared from compound **5** according to procedure **2** as a yellow solid in 94% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 8.97, 2.55 Hz, 1H), 7.86-7.81 (m, 2H), 7.75-7.69 (m, 3H), 6.90 (d, *J* = 8.97 Hz, 1H), 4.14-4.10 (m, 2H), 3.94 (s, 3H), 3.81-3.77 (m, 2H), 1.94-1.91 (m, 4H).

4.1.3.10. 2-(2-Bromoethoxy)-1-methoxy-4-nitrobenzene ***(15)***. The title compound was prepared from compound **5** according to procedure **2** as a yellow solid in 73% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (dd, *J* = 8.76, 2.55 Hz, 1H), 7.77 (d, *J* = 2.58 Hz, 1H), 6.95 (d, *J* = 8.97 Hz, 1H), 4.42 (t, *J* = 6.42 Hz, 2H), 3.98 (s, 3H), 3.72 (t, *J* = 6.21 Hz, 2H).

4.1.3.11. 2-(3-Bromopropoxy)-1-methoxy-4-nitrobenzene ***(16)***. The title compound was prepared from compound **5** according to procedure **2** as a yellow solid in 71% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 8.97, 2.58 Hz, 1H), 7.78 (d, *J* = 2.58 Hz, 1H), 6.93 (d, *J* = 8.76 Hz, 1H), 4.28 (t, *J* = 7.14 Hz, 2H), 3.96 (s, 3H), 3.80 (t, *J* = 6.21 Hz, 2H), 2.37 (p, *J* = 6.06 Hz, 2H).

4.1.3.12. 2-(4-Bromobutoxy)-1-methoxy-4-nitrobenzene ***(17)***. The title compound was prepared from compound **5** according to procedure **2** as a yellow solid in 63% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, J = 8.97, 2.55 Hz, 1H), 7.74 (d, J = 2.58 Hz, 1H), 6.92 (d, J = 8.97 Hz, 1H), 4.14 (t, J = 5.85 Hz, 2H), 3.96 (s, 3H) 3.53 (t, J = 6.24 Hz, 2H), 2.10-2.05 (m, 4H).

4.1.3.13. 4-(4-(2-Methoxy-5-nitrophenoxy)butyl)piperidine ***(18).*** The title compound was prepared from compound **7** according to procedure **6** as a red solid in 75% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 7.91 (dd, J = 8.97, 2.76 Hz, 1H), 7.76 (d, J = 2.58 Hz, 1H), 7.09 (d, J = 8.97 Hz, 1H), 4.14 (t, J = 5.85 Hz, 2H), 3.93 (s, 3H), 3.06-3.02 (m, 2H), 2.65-2.56 (m, 2H), 1.80-1.74 (m, 5H), 1.29-1.18 (m, 2H).

4.1.3.14. 4-(2-(2-Methoxy-5-nitrophenoxy)ethyl)-1-methylpiperidine ***(19)***. The title compound was prepared from compound **18** according to procedure **7** as a white solid in 45% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, J = 8.40, 2.58 Hz, 1H), 7.73 (d, J = 2.73 Hz, 1H), 6.92 (d, J = 8.97 Hz, 1H), 4.14 (t, J = 6.60 Hz, 2H), 3.96 (s, 3H), 3.12 (d, J = 11.88 Hz, 2H), 2.45 (s, 3H), 2.26 (t, J = 9.90 Hz, 2H), 1.89-1.83 (m, 4H), 1.56-1.47 (m, 3H)

4.1.3.15. 3-(2-Methoxy-5-nitrophenoxy)propan-1-amine ***(20)***. The title compound was prepared from compound **13** according to procedure **3** as a light yellow solid in 94% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (dd, J = 8.43, 2.43 Hz, 1H), 7.72 (d, J = 2.43 Hz, 1H), 6.88 (d, J = 8.46 Hz, 1H), 4.13 (t, J = 5.82 Hz, 2H), 3.93 (s, 3H), 2.62 (t, J = 6.12 Hz, 2H), 1.98-1.88 (m, 2H).

4.1.3.16. 4-(2-Methoxy-5-nitrophenoxy)butan-1-amine ***(21)***. The title compound was prepared from compound **14** according to procedure **3** as a white solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, J = 8.40, 2.40 Hz, 1H), 7.71 (d, J = 2.40 Hz, 1H), 6.88 (d, J = 8.88 Hz, 1H), 4.13 (t, J = 5.80 Hz, 2H), 3.93 (s, 3H), 2.79 (t, J = 6.68 Hz, 2H), 2.00-1.83 (m, 4H)

4.1.3.17. tert-Butyl 4-(2-(2-methoxy-5-nitrophenoxy)ethyl)piperazine-1-carboxylate ***(22)***. The title compound was prepared from compound **15** according to procedure **4** as a white solid in 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (dd, J = 8.40, 2.58

Hz, 1H), 7.80 (d, *J* = 2.58 Hz, 1H), 6.92 (d, *J* = 8.97 Hz, 1H), 4.24 (t, *J* = 5.85 Hz, 2H), 3.95 (s, 3H), 3.47 (t, *J* = 4.95 Hz, 4H), 2.90 (t, *J* = 5.85 Hz, 2H), 2.56 (t, *J* = 4.74 Hz, 4H), 1.46 (s, 9H).

4.1.3.18. tert-Butyl 4-(3-(2-methoxy-5-nitrophenoxy)propyl)piperazine-1-carboxylate ***(23)***. The title compound was prepared from compound **16** according to procedure **4** as a white solid in 89% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 9.15, 2.55 Hz, 1H), 7.77 (d, *J* = 2.19 Hz, 1H), 6.91 (d, *J* = 9.15 Hz, 1H), 4.18 (t, *J* = 6.57 Hz, 2H), 3.95 (s, 3H), 3.44 (t, *J* = 4.95 Hz, 2H), 2.57 (t, *J* = 6.60 Hz, 4H), 2.41 (t, *J* = 4.95 Hz, 4H), 2.08 (p, *J* = 6.78 Hz, 2H), 1.46 (s, 9H).

4.1.3.19. tert-Butyl 4-(4-(2-methoxy-5-nitrophenoxy)butyl)piperazine-1-carboxylate ***(24)***. The title compound was prepared from compound **17** according to procedure **4** as a white solid in 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 8.97, 2.55 Hz, 1H), 7.73 (d, *J* = 2.55 Hz, 1H), 6.92 (d, *J* = 8.79 Hz, 1H), 4.15 (d, *J* = 6.96 Hz, 2H), 3.95 (s, 3H), 3.44 (t, *J* = 5.13 Hz, 4H), 2.45-2.37 (m, 6H), 1.96 (p, *J* = 6.75 Hz, 2H), 1.74 (p, *J* = 7.89 Hz, 2H), 1.46 (s, 9H).

4.1.3.20. tert-Butyl (2-(2-methoxy-5-nitrophenoxy)ethyl)carbamate ***(25)***. The title compound was prepared from compound **20** according to procedure **5** as an opaque semi-solid in 72% yield, which was used for the next step without further purification.

4.1.3.21. tert-Butyl (3-(2-methoxy-5-nitrophenoxy)propyl)carbamate ***(26)***. The title compound was prepared from compound **21** according to procedure **5** as a white solid in 62% yield, which was used for the next step without further purification.

4.1.3.22. tert-Butyl (2-(2-methoxy-5-nitrophenoxy)ethyl)(methyl)carbamate ***(27)***. The title compound was prepared from compound **25** according to procedure **4** as a light yellow solid in 51% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 8.46, 2.40 Hz, 1H), 7.72 (d, *J* = 2.43 Hz, 1H), 6.87 (d, *J* = 8.61 Hz, 1H), 4.14 (t, *J* = 6.03 Hz, 2H), 3.93 (s, 3H), 2.92 (t, *J* = 6.12 Hz, 2H), 2.78 (s, 3H), 2.01-1.90 (m, 2H), 1.44 (s, 9H).

4.1.3.23. tert-Butyl (3-(2-methoxy-5-nitrophenoxy)propyl)(methyl)carbamate ***(28)***. The title compound was prepared from compound **26** according to procedure **4** as a light yellow solid in 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, J = 8.79, 2.73 Hz, 1H), 7.72 (d, J = 2.73 Hz, 1H), 6.89 (d, J = 8.97 Hz, 1H), 4.14 (t, J = 6.21 Hz, 2H), 3.94 (s, 3H), 2.65 (t, J = 6.12 Hz, 2H), 2.80 (s, 3H), 2.01-1.87 (m, 4H), 1.44 (s, 9H).

4.1.3.24. 4-(2-Methoxy-5-nitrophenoxy)-N,N-dimethylbutan-1-amine ***(29)***. The title compound was prepared from compound **21** according to procedure **7** as a red semi-solid in 21% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, J = 8.97, 2.73 Hz, 1H), 7.74 (d, J = 2.58 Hz, 1H), 6.92 (d, J = 8.97 Hz, 1H), 4.14 (t, J = 6.24 Hz, 2H), 3.96 (s, 3H), 2.62 (t, J = 8.61 Hz, 2H), 2.40 (s, 6H), 1.95 (p, J = 6.60 Hz, 2H), 1.82 (p, J = 7.32 Hz, 2H).

4.1.3.25. 1-(2-Bromoethoxy)-4-nitrobenzene ***(31).*** The title compound was prepared from compound **30** according to procedure **2** as a yellow solid in 64% yield. ^{1}H NMR (300 MHz, CDCl3) δ 8.20 (m, 2H), 6.97 (m, 2H), 4.36 (d, J = 6.03 Hz, 2H), 3.68 (d, J = 6.24 Hz, 2H).

4.1.3.26. tert-Butyl 4-(2-(4-nitrophenoxy)ethyl)piperazine-1-carboxylate ***(32)***. The title compound was prepared from compound **31** according to procedure **4** as a white solid in 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (2H, m), 6.97 (2H, m), 4.19 (t, J = 5.67 Hz, 2H), 3.45 (m, 4H), 2.85 (t, J = 5.70 Hz, 2H), 2.52 (m, 4H), 1.45 (s, 9H).

4.1.3.27. tert-Butyl (4-(4-(4-nitrophenoxy)butyl)pyridin-2-yl)carbamate ***(33)***. The title compound was prepared from compound **30** according to procedure **2** as a white solid in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, J = 9.15 Hz, 2H), 8.11-8.09 (m, 2H), 7.86 (s, 1H), 6.94 (d, J = 9.15 Hz, 2H), 6.82 (dd, J = 5.73, 1.47 Hz, 1H), 4.06 (t, J = 5.67 Hz, 2H), 2.68 (t, J = 6.06 Hz, 2H), 1.86-1.81 (m, 4H), 1.51 (s, 9H).

4.1.3.28. tert-Butyl 4-(2-(4-nitrophenoxy)acetyl)piperazine-1-carboxylate ***(37)***. The title compound was prepared from compound **35** according to procedure **8** as a yellow solid in 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, J = 9.33 Hz, 2H), 6.99 (d,

J = 9.33 Hz, 2H), 4.75 (s, 2H), 3.53-3.49 (m, 2H), 3.46-3.43 (m, 2H), 3.40-3.34 (m, 4H), 1.39 (s, 9H).

4.1.3.29. tert-Butyl (E)-4-(3-(4-nitrophenyl)acryloyl)piperazine-1-carboxylate ***(38)***. The title compound was prepared from commercial available **36** according to procedure **8** as a white solid in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8.79 Hz, 2H), 7.70 (d, *J* = 15.36 Hz, 1H), 7.65 (d, *J* = 8.61 Hz, 2H), 7.02 (d, *J* = 15.57 Hz, 1H), 3.72-3.64 (m, 4H), 3.42-3.36 (m, 4H), 1.48 (s, 9H).

4.1.3.30. (E)-3-(4-Nitrophenyl)-1-(piperazin-1-yl)prop-2-en-1-one ***(39)***. The title compound was prepared from compound **38** according to procedure **6** as a yellow oil in 94% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (d, *J* = 8.79 Hz, 2H), 7.89 (d, *J* = 8.58 Hz, 2H), 7.71 (d, *J* = 15.57 Hz, 1H), 7.39 (d, *J* = 15.57 Hz, 1H), 4.00-3.95 (m, 4H), 3.39-3.22 (m, 4H).

4.1.3.31. tert-Butyl (E)-(2-(4-(3-(4-nitrophenyl)acryloyl)piperazin-1-yl)ethyl)carbamate ***(40)***. The title compound was prepared from compound **39** according to procedure **4** as a pale yellow solid in 54% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.79 Hz, 2H), 7.72-7.65 (m, 3H), 7.03 (d, *J* = 15.57 Hz, 1H), 3.76-3.66 (m, 4H), 3.27 (q, *J* = 5.31 Hz, 2H), 2.53-2.47 (m, 6H), 1.46 (s, 9H).

4.1.3.32. tert-Butyl 4-(4-nitrobenzamido)piperidine-1-carboxylate ***(41)***. The title compound was prepared from compound **34** according to procedure **8** as a white solid in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, *J* = 8.79 Hz, 2H), 7.95 (d, *J* = 8.79 Hz, 2H), 6.18 (d, *J* = 7.50 Hz, NH), 4.15-4.10 (m, 3H), 2.94 (t, *J* = 12.24 Hz, 2H), 2.06 (t, *J* = 4.59 Hz, 2H), 1.46 (s, 9H), 1.42-1.39 (m, 2H).

4.1.3.33. tert-Butyl 4-(2-(4-nitrophenoxy)acetamido)piperidine-1-carboxylate ***(42)***. The title compound was prepared from compound **35** according to procedure **8** as a white solid in 82% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, *J* = 9.15 Hz, 2H), 7.02 (d, *J* = 9.33 Hz, 2H), 6.32 (br, NH), 4.54 (s, 2H), 4.13-4.09 (m, 3H), 2.88 (t, *J* = 12.09 Hz, 2H), 1.93-1.90 (m, 2H), 1.43 (s, 9H), 1.38-1.33 (m, 2H).

4.1.3.34. tert-Butyl (E)-4-(3-(4-Nitrophenyl)acrylamido)piperidine-1-carboxylate ***(43)***. The title compound was prepared from compound **36** according to procedure **8** as a white solid in 83% yield. ^{1}H NMR (300 MHz, $CDCl_3$) 8.25 (d, *J* = 8.79 Hz, 2H), 7.70 (d, *J* = 15.36 Hz, 1H), 6.65 (d, *J* = 8.61 Hz, 2H), 6.54 (d, *J* = 15.57 Hz, 1H), 5.76 (d, *J* = 7.89 Hz, NH), 4.13 (m, 3H), 2.93 (t, *J* = 11.73 Hz, 2H). 2.05-1.98 (m, 2H), 1.46 (s, 9H), 1.36-1.32 (m, 2H).

4.1.3.35. 4-Nitro-N-(piperidin-4-yl)benzamide ***(44)***. The title compound was prepared from compound **41** according to procedure **6** as a pale yellow oil in 89% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, *J* = 8.79 Hz, 2H), 7.94 (d, *J* = 8.79 Hz, 2H), 6.05 (d, *J* = 7.89 Hz, NH), 4.14-4.04 (m, 1H), 3.16 (td, *J* = 12.45, 2.55 Hz, 2H), 2.81 (dt, *J* = 12.27, 2.55 Hz, 2H), 2.08-2.08 (m, 2H), 1.51-1.38 (m, 2H).

4.1.3.36. 2-(4-Nitrophenoxy)-N-(piperidin-4-yl)acetamide ***(45)***. The title compound was prepared from compound **42** according to procedure **6** as a a brown semi-solid in 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8.97 Hz, 2H), 7.02 (d, *J* = 9.03 Hz, 2H), 6.32 (br, NH), 4.54 (s, 2H), 4.13-4.09 (m, 1H), 3.16-3.10 (m, 2H), 2.78 (t, *J* = 12.09 Hz, 2H), 2.00-1.95 (m, 2H), 1.48-1.39 (m, 2H).

4.1.3.37. (E)-3-(4-Nitrophenyl)-N-(piperidin-4-yl)acrylamide ***(46)***. The title compound was prepared from compound **43** according to procedure **6** as a yellow solid in 94% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (d, *J* = 8.79 Hz, 2H), 7.80 (d, *J* = 8.79 Hz, 2H), 7.65 (d, *J* =15.93 Hz, 1H), 6.77 (d, *J* = 15.90 Hz, 1H), 4.08-4.01 (m, 1H), 3.53-3.43 (m, 2H), 3.19 (dt, *J* = 12.99, 3.12 Hz , 2H), 2.18-2.14 (m, 2H), 1.79-1.65 (m, 2H).

4.1.3.38. tert-Butyl (2-(4-(4-nitrobenzamido)piperidin-1-yl)ethyl)carbamate ***(47)***. The title compound was prepared from compound **44** according to procedure **4** as an opaque semi-solid in 51% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, *J* = 8.61 Hz, 2H), 7.93 (d, *J* = 8.79 Hz, 2H), 6.07 (d, *J* = 8.07 Hz, NH), 4.98 (br, NH), 4.02-3.95 (m, 1H), 3.24 (q, *J* = 6.69 Hz, 2H), 2.93-2.90 (m, 2H), 2.51 (t, *J* = 6.06 Hz, 2H), 2.26 (t, *J* = 11.55 Hz, 2H), 2.08-2.04 (m, 2H), 1.63-1.59 (m, 2H), 1.45 (s, 9H).

*4.1.3.39. tert-Butyl (2-(4-(2-(4-nitrophenoxy)acetamido)piperidin-1-yl)ethyl)carbamate **(48)***. The title compound was prepared from compound **45** according to procedure **4** as a red semi-solid in 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, J = 9.15 Hz, 2H), 7.01 (d, J = 9.15 Hz, 2H), 6.38 (br, NH), 5.12 (br, NH), 4.53 (s, 2H), 3.94-3.90 (m, 1H), 3.25-3.23 (m, 2H), 2.93-2.91 (m, 2H), 2.54-2.51 (m, 2H), 2.24-2.20 (m, 2H), 1.96-1.94 (m, 2H), 1.62-1.58 (m, 2H), 1.42 (s, 9H).

*4.1.3.40. tert-Butyl (E)-(2-(4-(3-(4-nitrophenyl)acrylamido)piperidin-1-yl)ethyl)carbamate **(49)***. The title compound was prepared from compound **46** according to procedure **4** as a pale yellow solid in 21% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (d, J = 8.97 Hz, 2H), 7.80 (d, J = 8.79 Hz, 2H), 7.60 (d, J =15.90 Hz, 1H), 6.79 (d, J = 15.72 Hz, 1H), 4.08-4.01 (m, 1H), 3.53-3.43 (m, 2H), 2.93-2.87 (m, 2H), 2.55-2.51 (m, 2H), 2.42-2.39 (m, 2H), 1.96-1.89 (m, 2H), 1.65-1.55 (m, 2H), 1.42 (s, 9H).

*4.1.3.41. tert-Butyl (4-((2-(4-nitrophenoxy)acetamido)methyl)pyridin-2-yl)carbamate **(50)***. The title compound was prepared from compound **35** according to procedure **8** as a white solid in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, J = 9.15 Hz, 2H), 8.19 (d, J = 5.31 Hz, 1H), 7.88 (s, 1H), 7.06 (d, J = 9.15 Hz, 2H), 6.87 (dd, J = 5.31, 1.47 Hz, 1H), 4.64 (s, 2H), 4.53 (d, J = 6.03 Hz, 2H), 1.50 (s, 9H).

4.1.4. Final compounds

4.1.4.1. General procedure for final compound

All nitro compounds were reduced by either hydrogenation using 10% Pd/C or zinc powder in acidic medium to obtain the corresponding amines, respectively. The amines were converted into the isothiocyanates by 1,1'-thiocarbonyldiimidazole (1.02 eq) in anhydrous CH_2Cl_2, and then coupled with 3-(5-methyl-1*H*-imidazol-1-yl)propan-1-amine (1.1 eq) to afford the corresponding thiourea, respectively. The Boc deprotection by following the general procedure 6 provided the final compounds.

*4.1.4.2. N-(3-(2-Aminoethoxy)-4-methoxyphenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(51)***. mp = 58-59 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.37 (s, 1H), 6.91 (d, J = 8.43 Hz, 1H), 6.78-6.72 (m, 3H), 5.93 (br, 1H), 4.02 (t, J = 5.13 Hz, 2H), 3.91 (t, J = 7.14 Hz, 2H), 3.88 (s, 3H), 3.69 (q, J = 6.24 Hz, 2H), 3.14 (t, J = 5.13 Hz, 2H), 2.17 (d, J = 0.93 Hz, 3H), 2.09 (p, J = 7.14 Hz, 2H). MS (FAB)

m/z 364 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{17}H_{25}N_5O_2S$ $[M + H]^+$ 364.1807, found: 364.1818. Anal. HPLC 99.33% (R_t = 3.723 min).

4.1.4.3. N-(3-(3-Aminopropoxy)-4-methoxyphenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(52)***. mp = 154-155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (br, 1H), 7.38 (s, 1H), 6.88-6.85 (m, 1H), 6.76-6.72 (m, 3H), 6.10 (br, 1H), 4.09 (t, *J* = 6.03 Hz, 2H), 3.91 (t, *J* = 7.14 Hz, 2H), 3.86 (s, 3H), 3.68 (q, *J* = 6.24 Hz, 2H), 2.93 (t, *J* = 6.60 Hz, 2H), 2.17 (d, *J* = 0.90 Hz, 3H), 2.09-1.92 (m, 4H). MS (FAB) *m/z* 378 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{18}H_{27}N_5O_2S$ $[M + H]^+$ 378.1964, found: 378.1972. Anal. HPLC 99.53% (R_t = 3.641 min)

4.1.4.4. N-(3-(4-Aminobutoxy)-4-methoxyphenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(53)***. mp = 55-56 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (br, 1H), 7.43 (s, 1H), 6.80 (d, *J* = 8.43 Hz, 1H), 6.76-6.70 (m, 3H), 6.00 (br, 1H), 4.00 (t, *J* = 6.60 Hz, 2H), 3.91 (t, *J* = 7.14 Hz, 2H), 3.87 (s, 3H), 3.68 (q, *J* = 6.42 Hz, 2H), 2.79 (t, *J* = 6.78 Hz, 2H), 2.17 (d, *J* = 0.90 Hz, 3H), 2.09 (p, *J* = 7.14 Hz, 2H), 1.92 (p, *J* = 6.78 Hz, 2H), 1.66-1.59 (m, 2H). MS (FAB) *m/z* 392 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{19}H_{29}N_5O_2S$ $[M + H]^+$ 392.2120, found: 392.2127. Anal. HPLC 100.00% (R_t = 3.199 min).

4.1.4.5. N-(4-Methoxy-3-(3-(methylamino)propoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(54)***. mp = 88-89 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (s, 1H), 6.86 (d, *J* = 8.43 Hz, 1H), 6.78 (dd, *J* = 8.43, 2.37 Hz, 1H), 6.73 (d, *J* = 2.19 Hz, 1H), 6.68 (s, 1H), 6.09 (br, 1H), 4.07 (t, *J* = 6.24 Hz, 2H), 3.89 (t, *J* = 6.96 Hz, 2H), 3.87 (s, 3H), 3.66 (q, *J* = 6.60 Hz, 2H), 2.83 (t, *J* = 6.57 Hz, 2H), 2.47 (s, 3H), 2.17 (s, 3H), 2.09-2.02 (m, 4H). MS (ESI) *m/z* 392 $[M+H]^+$. HRMS (ESI) calcd for $C_{19}H_{29}N_5O_2S$ $[M + H]^+$ 392.2115, found 392.2097. Anal. HPLC 98.5% (R_t = 3.222 min).

4.1.4.6. N-(4-Methoxy-3-(4-(methylamino)butoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(55)***. mp = 76-77 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.43 (d, *J* = 0.93 Hz, 1H), 6.85 (d, *J* = 8.43 Hz, 1 H), 6.84 (d, *J* = 2.40 Hz, 1H), 6.69 (dd, *J* = 8.61, 2.37 Hz, 1H), 6.57 (s, 1H), 3.92-3.85 (m, 4H), 3.73 (s, 3H), 3.49 (t, *J* = 6.96 Hz, 2H), 2.67 (t, *J* = 7.32 Hz, 2H), 2.37 (s, 3H), 2.12 (d, *J* = 0.90 Hz, 3H), 1.93

(p, *J* = 7.14 Hz, 2H), 1.75-1.58 (m, 4H). MS (ESI) *m/z* 406 $[M+H]^+$. HRMS (ESI) calcd for $C_{20}H_{31}N_5O_2S$ $[M + H]^+$ 406.2271, found 406.2262. Anal. HPLC 97.6% (R_t = 3.012 min).

4.1.4.7. N-(3-(4-(Dimethylamino)butoxy)-4-methoxyphenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(56)***. mp = 51-52 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.58 (s, 1H), 6.96 (d, *J* = 8.61 Hz, 1H), 6.91 (d, *J* = 2.40 Hz, 1H), 6.77 (dd, *J* = 8.58, 2.55 Hz, 1H). 6.66 (s, 1H), 4.01 (t, *J* = 6.06 Hz, 2H), 3.97 (t, *J* = 7.32 Hz, 2H), 3.82 (s, 3H), 3.61 (t, *J* = 6.96 Hz, 2H), 2.42 (t, *J* = 7.71 Hz, 2H), 2.25 (s, 6H), 2.21 (d, *J* = 0.90 Hz, 3H), 2.05 (p, *J* = 7.14 Hz, 2H), 1.81-1.75 (m, 2H), 1.72-1.67 (m, 2H). MS (FAB) *m/z* 420 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{21}H_{33}N_5O_2S$ $[M + H]^+$ 420.2428, found: 420.2438. Anal. HPLC 98.23% (R_t = 3.454 min).

4.1.4.8. N-(4-Methoxy-3-(2-(piperazin-1-yl)ethoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(57)***. mp = 71-72 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 6.79-6.94 (m, 2H), 6.80 (dd, *J* = 8.43, 2.37 Hz, 1H), 6.66 (s, 1H), 4.14 (t, *J* = 5.49 Hz, 2H), 3.99 (t, *J* = 7.14 Hz, 2H), 3.81 (s, 3H), 3.59 (q, *J* = 7.14 Hz, 2H), 2.88 (t, *J* = 4.92 Hz, 4H), 2.82 (t, *J* = 5.49 Hz, 2H), 2.61 (t, *J* = 4.95 Hz, 4H), 2.22 (d, *J* = 1.08 Hz, 3H), 2.05 (p, *J* = 6.78 Hz, 2H). MS (FAB) *m/z* 433 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{21}H_{32}N_6O_2S$ $[M + H]^+$ 433.2380, found: 433.2370. Anal. HPLC 99.30% (R_t = 4.101 min).

4.1.4.9. N-(4-Methoxy-3-(3-(piperazin-1-yl)propoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(58)***. mp = 58-59 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (d, *J* = 0.90 Hz, 1H), 6.96 (d, *J* = 8.58 Hz, 1H), 6.91 (d, *J* = 2.37 Hz, 1H), 6.77 (dd, *J* = 8.40, 2.37 Hz, 1H), 6.66 (s, 1H), 4.05 (t, *J* = 6.03 Hz, 2H), 3.99 (t, *J* = 6.96 Hz, 2H), 3.81 (s, 3H), 3.61 (t, *J* = 6.75 Hz, 2H), 2.87 (t, *J* = 4.95 Hz, 4H), 2.57-2.49 (m, 6H), 2.22 (d, *J* = 1.11 Hz, 3H), 2.07-1.96 (m, 4H). MS (ESI) *m/z* 447 $[M+H]^+$. HRMS (ESI) *m/z* calcd for $C_{22}H_{34}N_6O_2S$ $[M + H]^+$ 447.2537, found: 447.2534. Anal. HPLC 99.32% (R_t = 3.799 min).

4.1.4.10. N-(4-Methoxy-3-(4-(piperazin-1-yl)butoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(59)***. mp = 82-83 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 6.95-6.92 (m, 2H), 6.77 (dd, *J* = 8.40, 2.37 Hz, 1H), 6.66 (s, 1H), 4.03

(t, *J* = 6.03 Hz, 2H), 3.99 (t, *J* = 7.32 Hz, 2H), 3.82 (s, 3H), 3.61 (t, *J* = 7.14 Hz, 2H), 2.92 (t, *J* = 4.92 Hz, 4H), 2.50-2.41 (m, 6H), 2.22 (d, *J* = 1.11 Hz, 2H), 2.08 (p, *J* = 7.50 Hz, 2H), 1.80 (p, *J* = 6.21 Hz, 2H), 1.69-1.61 (m, 2H). MS (FAB) *m/z* 461 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{36}N_6O_2S$ $[M + H]^+$ 461.2693, found: 461.2705. Anal. HPLC 99.69% (R_t = 3.701 min).

4.1.4.11. N-(4-Methoxy-3-(2-(piperidin-4-yl)ethoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(60)***. mp = 83-84 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (d, *J* = 1.11 Hz, 1H), 6.96 (d, *J* = 8.61 Hz, 1H), 6.91 (d, *J* = 2.19 Hz, 1H), 6.77 (dd, *J* = 8.43, 2.40 Hz, 1H), 6.67 (s, 1H), 4.04 (t, *J* = 5.85 Hz, 2H), 3.99 (t, *J* = 7.14 Hz, 2H), 3.82 (s, 3H), 3.58 (t, *J* = 6.96 Hz, 2H), 3.07-3.02 (m, 2H), 2.65 (td, *J* = 10.08, 3.84 Hz, 2H), 2.22 (d, *J* = 0.93 Hz, 3H), 2.07 (p, *J* = 7.14 Hz, 2H), 1.80-1.74 (m, 4H), 1.73-1.72 (m, 1H), 1.23-1.11 (m, 2H). MS (FAB) *m/z* 432 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{22}H_{33}N_5O_2S$ $[M + H]^+$ 432.2433, found: 432.2426. Anal. HPLC 95.47% (R_t = 4.070 min).

4.1.4.12. N-(4-Methoxy-3-(3-(piperidin-4-yl)propoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(61)***. mp = 49-50 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.60 (d, *J* = 1.11 Hz, 1H), 6.95 (d, *J* = 8.58 Hz, 1H), 6.91 (d, *J* = 2.40 Hz, 1H), 6.76 (dd, *J* = 8.43, 2.40 Hz, 1H), 6.66 (s, 1H), 3.98 (t, *J* = 6.39 Hz, 4H), 3.82 (s, 3H), 3.61 (t, *J* = 6.96 Hz, 2H), 3.11-3.07 (m, 2H), 2.69 (td, *J* = 12.27, 2.58 Hz, 2H), 2.22 (d, *J* = 0.93 Hz, 3H), 2.07 (p, *J* = 7.14 Hz, 2H), 1.86-1.76 (m, 5H), 1.44-1.37 (m, 2H), 1.25-1.12 (m, 2H). MS (FAB) *m/z* 446 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{35}N_5O_2S$ $[M + H]^+$ 446.2589, found: 446.2595. Anal. HPLC 96.06% (R_t = 4.066 min).

4.1.4.13. N-(4-Methoxy-3-(4-(piperidin-4-yl)butoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(62)***. mp = 64-65 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.50 (s, 1H), 6.87 (d, *J* = 8.61 Hz, 1H), 6.81 (d, *J* = 2.19 Hz, 1H), 6.67 (dd, *J* = 8.40, 2.37 Hz, 1H), 6.57 (s, 1H), 3.90 (t, *J* = 6.21 Hz, 4H), 3.73 (s, 3H), 3.52 (t, *J* = 6.96 Hz, 2H), 2.99-2.95 (m, 2H), 2.57 (t, *J* = 12.45 Hz, 2H), 2.14 (d, *J* = 0.93 Hz, 3H), 1.98 (p, *J* = 7.14 Hz, 2H), 1.67-1.63 (m, 5H), 1.40-1.35 (m, 2H), 1.25-1.18 (m, 2H), 1.11-1.03 (m, 2H). MS (FAB) *m/z* 460 $[M+H]^+$. HRMS (FAB) *m/z* calcd for

$C_{24}H_{37}N_5O_2S$ $[M + H]^+$ 460.2746, found: 460.2744. Anal. HPLC 98.64% (R_t = 3.851 min).

4.1.4.14. N-(4-Methoxy-3-(2-(1-methylpiperidin-4-yl)ethoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(63)***. mp = 71-72 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (s, 1H), 6.95-6.91 (m, 2H), 6.77 (dd, *J* = 8.40, 2.55 Hz, 1H), 6.66 (s, 1H), 4.04 (t, *J* = 6.06 Hz, 2H), 3.96 (t, *J* = 7.50 Hz, 2H), 3.81 (s, 3H), 3.58 (t, *J* = 7.14 Hz, 2H), 2.88 (d, *J* = 10.26 Hz, 2H), 2.26 (s, 3H), 2.21 (d, *J* = 0.90 Hz, 3H), 2.08-2.00 (m, 4H), 1.80-1.71 (m, 4H), 1.33-1.29 (m, 3H). MS (FAB) *m/z* 446 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{35}N_5O_2S$ $[M + H]^+$ 446.2584, found: 446.2581. Anal. HPLC 98.99% (R_t = 4.109 min).

4.1.4.15. N-(3-(2-(2-Aminopyridin-4-yl)ethoxy)-4-methoxyphenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(64)***. mp = 66-67 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.77 (d, *J* = 5.31 Hz, 1H), 7.51 (s, 1H), 6.96-6.91 (m, 2H), 6.78 (dd, *J* = 8.61, 2.37 Hz, 1H), 6.65 (s, 1H), 6.60 (dd, *J* = 5.52, 1.47 Hz, 1H), 6.53 (s, 1H), 4.21 (t, *J* = 6.60 Hz, 2H), 3.98 (t, *J* = 7.14 Hz, 2H), 3.80 (s, 3H), 3.60 (t, *J* = 6.21 Hz, 2H), 2.98 (t, *J* = 6.39 Hz, 2H), 2.20 (d, *J* = 0.75 Hz, 3H), 2.06 (p, *J* = 6.93 Hz, 2H). MS (FAB) *m/z* 441 $[M+H]^+$. HRMS (FAB) *m/* calcd for $C_{22}H_{28}N_6O_2S$ *z* $[M + H]^+$ 441.2067, found: 441.2067. Anal. HPLC 99.52% (R_t = 3.967 min).

4.1.4.16. N-(3-(3-(2-Aminopyridin-4-yl)propoxy)-4-methoxyphenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(65)***. mp = 76-77 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.75 (d, *J* = 5.24 Hz, 1H), 7.58 (s, 1H). 6.97 (d, *J* = 8.56 Hz, 1H), 6.88 (d, *J* = 2.16 Hz, 1H), 6.78 (dd, *J* = 8.52, 2.36 Hz, 1H), 6.65 (s, 1H), 6.50 (d, *J* = 4.28 Hz, 1H), 6.45 (s, 1H), 3.99-3.94 (m, 4H), 3.84 (s, 3H), 3.60 (t, *J* = 6.88 Hz, 2H), 2.70 (t, *J* = 7.43 Hz, 2H), 2.20 (d, *J* = 0.72 Hz, 3H), 2.08-2.00 (m, 4H). MS (FAB) *m/z* 455 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{30}N_6O_2S$ $[M + H]^+$ 455.2224, found: 455.2223. Anal. HPLC 96.06% (R_t = 3.979 min).

4.1.4.17. N-(3-(4-(2-Aminopyridin-4-yl)butoxy)-4-methoxyphenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(66)***. mp = 63-64 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, *J* = 5.49 Hz, 1H), 7.60 (s, 1H), 7.39 (s, 1H), 6.89 (d, *J* = 8.43 Hz, 1H), 6.76-6.73 (m, 2H), 6.66 (d, *J* = 2.19 Hz, 1H), 6.51 (d, *J* = 5.49 Hz, 1H), 6.36 (s, 1H), 5.94 (br, NH), 3.98 (t, *J* = 6.06 Hz, 2H), 3.91 (t, *J* = 7.14 Hz, 2H), 3.87 (s, 3H), 3.69 (q, *J*

= 6.60 Hz, 2H), 2.60 (t, *J* = 7.32 Hz, 2H), 2.17 (d, *J* = 0.72 Hz, 3H), 2.09 (p, *J* = 7.32 Hz, 2H), 1.85-1.80 (m, 4H). MS (FAB) *m/z* 469 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{24}H_{32}N_6O_2S$ $[M + H]^+$ 469.2380, found: 469.2385. Anal. HPLC 98.26% (R_t = 4.017 min).

*4.1.4.18. N-(4-Methoxy-3-(4-(pyrimidin-2-ylamino)butoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(67)***. To a solution of **53** (1 eq) in EtOH was added 2-chloropyrimidine (2 eq) and triethylamine (2.5 eq). The mixture was refluxed for 2 days, then solvent was removed by evaporation. The residue was purified by column chromatography (MeOH : CH_2Cl_2) to give white solid, 35% yield. mp = 50-51 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, *J* = 4.74 Hz, 2H), 7.60 (s, 1H), 7.37 (s, 1H), 6.88 (d, *J* = 8.61 Hz, 1H), 6.76-6.68 (m, 3H), 6.52 (t, *J* = 4.77 Hz, 1H), 5.94 (br, 1H), 5.35 (br, 1H), 4.03 (t, *J* = 6.21 Hz, 2H), 3.91 (t, *J* = 7.14 Hz, 2H), 3.87 (s, 3H), 3.69 (q, *J* = 6.42 Hz, 2H), 3.52 (q, *J* = 6.60 Hz, 2H), 2.17 (d, *J* = 0.93 Hz, 3H), 2.09 (p, *J* = 7.32 Hz, 2H), 1.99 (p, *J* = 7.86 Hz, 2H), 1.85 (p, *J* = 6.78 Hz, 2H). MS (FAB) *m/z* 470 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{31}N_7O_2S$ $[M + H]^+$ 470.2338, found: 470.2340. Anal. HPLC 96.60% (R_t = 4.414 min).

*4.1.4.19. N-(4-(2-(Piperazin-1-yl)ethoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(68)***. mp = 85-86 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.58 (s, 1H), 7.17 (m, 2H), 6.97 (m, 2H), 6.66 (s, 1H), 4.15 (t, *J* = 5.31 Hz, 2H), 3.98 (t, *J* = 6.96 Hz, 2H), 3.60 (t, *J* = 6.96 Hz, 2H), 2.87 (m, 4H), 2.81 (t, *J* = 5.70 Hz, 2H), 2.57 (m, 4H), 2.21 (s, 3H), 2.04 (m, 2H). MS (FAB) *m/z* 403 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{20}H_{30}N_6OS$ $[M + H]^+$ 403.2275, found: 403.2282. Anal. HPLC 98.22% (R_t = 2.893 min).

*4.1.4.20. N-(4-(4-(2-Aminopyridin-4-yl)butoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(69)***. mp = 58-59 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.75 (d, *J* = 5.49 Hz, 1H), 7.59 (s, 1H), 7.14 (d, *J* = 8.79 Hz, 2H), 6.93 (d, *J* = 8.97 Hz, 2H), 6.66 (s, 1H), 6.49 (dd, *J* = 5.31, 1.47 Hz, 1H), 6.44 (s, 1H), 3.99-3.97 (m, 4H), 3.60 (t, *J* = 6.39 Hz, 2H), 2.56 (t, *J* = 6.96 Hz, 2H), 2.21 (s, 3H), 2.04 (p, *J* = 6.96 Hz, 2H), 1.78-1.75 (m, 4H). MS (FAB) *m/z* 439 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{30}N_6OS$ $[M + H]^+$ 439.2275, found: 439.2268. Anal. HPLC 99.67% (R_t = 2.957 min).

4.1.4.21. N-(4-(2-Oxo-2-(piperazin-1-yl)ethoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(70)***. mp = 95-96 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 7.18 (d, *J* = 8.97 Hz, 2H), 6.69 (d, *J* = 8.97 Hz, 2H), 6.66 (s, 1H), 4.82 (s, 2H), 3.99 (t, *J* = 7.32 Hz, 2H), 3.60-3.50 (m, 6H), 2.85-2.77 (m, 4H), 2.21 (s, 3H), 2.07 (p, *J* = 6.96 Hz, 2H). MS (FAB) *m/z* 417 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{20}H_{28}N_6O_2S$ $[M + H]^+$ 417.2067, found: 417.2066. Anal. HPLC 96.16% (R_t = 2.936 min).

4.1.4.22. N-(4-(2-Oxo-2-(piperidin-4-ylamino)ethoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(71)***. mp = 98-99 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.21 (d, *J* = 8.79 Hz, 2H), 7.01 (d, *J* = 8.97 Hz, 2H), 6.66 (s, 1H), 4.50 (s, 2H), 3.99 (t, *J* = 7.32 Hz, 2H), 3.91-3.84 (m, 1H), 3.58 (t, *J* = 6.06 Hz, 2H), 3.08-3.04 (m, 2H), 2.71 (t, *J* = 12.27 Hz, 2H), 2.22 (s, 3H), 2.07 (p, *J* = 6.96 Hz, 2H), 1.87-1.83 (m, 2H), 1.50-1.45 (m, 2H). MS (FAB) *m/z* 431 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{21}H_{30}N_6O_2S$ $[M + H]^+$ 431.2224, found: 431.2246. Anal. HPLC 100.00 % (R_t = 2.947 min).

4.1.4.23. N-(4-(2-((1-(2-Aminoethyl)piperidin-4-yl)amino)-2-oxoethoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(72)***. mp = 73-74 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 7.21 (d, *J* = 8.76 Hz, 2H), 7.01 (d, *J* = 8.76 Hz, 2H), 6.66 (s, 1H), 4.49 (s, 2H), 3.99 (t, *J* = 7.32 Hz, 2H), 3.79-3.74 (m, 1H), 3.58 (t, *J* = 6.66 Hz, 2H), 2.88-2.85 (m, 2H), 2.75-2.71 (t, *J* = 6.78 Hz, 2H), 2.45 (t, *J* = 6.75 Hz, 2H), 2.22 (s, 3H), 2.14-2.11 (m, 2H), 2.05-2.01 (p, *J* = 6.93 Hz, 2H), 1.86-1.82 (m, 2H), 1.65 (m, 2H). MS (FAB) *m/z* 474 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{35}N_7O_2S$ $[M + H]^+$ 474.2646, found: 474.2662. Anal. HPLC 98.75% (R_t = 2.779 min).

4.1.4.24. N-(4-(2-(((2-Aminopyridin-4-yl)methyl)amino)-2-oxoethoxy)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(73)***. mp = 90-91 °C.^{1}H NMR (300 MHz, CD_3OD) δ 7.79 (d, *J* = 5.49 Hz, 1H), 7.58 (s, 1H), 7.20 (d, *J* = 8.98 Hz, 2H), 7.03 (d, *J* = 8.97 Hz, 2H), 6.66 (s, 1H), 6.50 (dd, *J* = 5.31, 1.53 Hz, 1H), 6.45 (s, 1H), 4.60 (s, 2H), 4.34 (s, 2H), 4.00 (t, *J* = 6.96 Hz, 2H), 3.59 (t, *J* = 6.06 Hz, 2H), 2.22 (d, *J* = 0.93 Hz, 3H), 2.05 (p, *J* = 6.66 Hz, 2H). MS (FAB) *m/z* 454 $[M+H]^+$. HRMS (FAB)

m/z calcd for $C_{22}H_{27}N_7O_2S$ $[M + H]^+$ 454.2020, found: 454.2046. Anal. HPLC 98.24% (R_t = 4.023 min)

4.1.4.25. N-(4-((1-(2-Aminoethyl)piperidin-4-yl)carbamoyl)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(74)***. mp = 91-92 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.81 (d, *J* = 8.61 Hz, 2H), 7.60 (s, 1H), 7.50 (d, *J* = 8.43 Hz, 2H), 6.67 (s, 1H), 4.01 (t, *J* = 7.32 Hz, 2H), 3.87-3.84 (m, 1H), 3.62 (t, *J* = 7.14 Hz, 2H), 2.99-2.95 (m, 2H), 2.75 (t, *J* = 6.96 Hz, 2H), 2.49 (t, *J* = 6.87 Hz, 2H), 2.23 (s, 3H), 2.16-2.14 (m, 2H), 2.10 (t, *J* = 6.93 Hz, 2H), 1.95-1.88 (m, 2H), 1.72-1.65 (m, 2H). MS (FAB) *m/z* 444 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{22}H_{33}N_7OS$ $[M + H]^+$ 444.2540, found: 444.2557. Anal. HPLC 99.61% (R_t = 3.661 min).

4.1.4.26. N-((E)-4-(3-oxo-3-(piperazin-1-yl)prop-1-en-1-yl)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(75)***. mp = 112-113 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.63-7.60 (m, 3H), 7.57 (d, *J* = 15.36 Hz, 1H), 7.41-7.35 (m, 2H), 7.11 (d, *J* = 15.39 Hz, 1H), 6.67 (s, 1H), 4.02 (t, *J* = 7.14 Hz, 2H), 3.68-3.59 (m, 6H), 2.84-2.80 (m, 4H), 2.23 (d, *J* = 0.93 Hz, 3H), 2.09 (p, *J* = 6.75 Hz, 2H), MS (FAB) *m/z* 413 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{21}H_{28}N_6OS$ $[M + H]^+$ 413.2118, found: 413.2111. Anal. HPLC 96.61% (R_t = 2.938 min).

4.1.4.27. N-((E)-4-(3-(4-(2-aminoethyl)piperazin-1-yl)-3-oxoprop-1-en-1-yl)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(76)***. mp = 66-67 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.63 (m, 3H), 7.57 (d, *J* = 15.36 Hz, 1H), 7.41 (d, *J* = 8.61 Hz, 2H), 7.11 (d, *J* = 15.39 Hz, 1H), 6.67 (s, 1H), 4.02 (t, *J* = 7.14 Hz, 2H), 3.68-3.64 (m, 4H), 3.61 (t, *J* = 6.57 Hz, 2H), 2.84 -2.81 (m, 4H), 2.52-2.48 (m, 4H), 2.23 (s, 3H), 2.09 (p, *J* = 6.96 Hz, 2H). MS (FAB) *m/z* 456 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{33}N_7OS$ $[M + H]^+$ 456.2540, found: 456.2518. Anal. HPLC 98.74% (R_t = 2.943 min).

4.1.4.28. N-((E)-4-(3-oxo-3-(piperidin-4-ylamino)prop-1-en-1-yl)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea ***(77)***. mp = 76-77 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (s, 1H), 7.55-7.46 (m, 3H), 7.40 (d, *J* = 8.43 Hz, 2H), 6.67 (s,1H), 6.57 (d, *J* = 15.70 Hz, 1H), 4.02 (t, *J* = 7.50 Hz, 2H), 3.78-3.72 (m, 1H), 3.61 (t, *J* = 6.69 Hz, 2H), 3.07-3.03 (m, 2H), 2.70-2.63 (m, 2H), 2.23 (d, *J* = 0.93 Hz, 3H), 2.09 (p, *J* = 6.96 Hz, 2H), 1.93-1.90 (m, 2H), 1.59-1.55 (m, 2H)**.** MS (FAB) *m/z* 427

$[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{22}H_{30}N_5OS$ $[M + H]^+$ 427.2275, found: 427.2277. Anal. HPLC 98.19% (R_t = 2.602 min).

*4.1.4.29. N-(E)-(4-(3-(4-(2-Aminoethyl)piperazin-1-yl)-3-oxoprop-1-en-1-yl)phenyl)-N'-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea **(78)***. mp = 80-81 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (d, *J* = 8.45 Hz, 2H), 7.59 (s, 1H), 7.56 (d, *J* = 15.40 Hz, 1H), 7.42 (d, *J* = 8.50 Hz, 2H), 7.11 (d, *J* = 15.40 Hz, 1H), 6.67 (s, 1H), 4.02 (t, *J* = 7.15 Hz, 2H), 3.76-3.72 (m, 5H), 3.62-3.55 (m, 2H), 2.83 (t, *J* = 6.25 Hz, 2H), 2.52-2.40 (m, 6H), 2.23 (d, *J* = 1.00 Hz, 3H), 2.10 (p, *J* = 7.05 Hz, 2H). MS (FAB) *m/z* 456 $[M+H]^+$. HRMS (FAB) *m/z* calcd for $C_{23}H_{33}N_7OS$ $[M + H]^+$ 456.2540, found: 456.2518. Anal. HPLC 97.30% (R_t = 2.863 min).

4.2. Molecular Modeling

The X-ray crystal structure of the human glutaminyl cyclase (PDB ID: 3PBB)[26] was prepared via the Protein Preparation Wizard in Maestro v.10.2 (Schrödinger, LLC, New York, NY, USA). During the preparation process, bond orders were assigned, zero-order bonds to Zn^{2+} were generated, and hydrogen atoms were added. The entire hydrogen atoms were energy minimized with the optimized potential for liquid simulation (OPLS) 2005 force field. The protonation states of the ligand molecules were forecasted by the pKa prediction module in ADMET Predictor™ (Simulations Plus, Lancaster, CA, USA). The 3D structure of **53** was created by LigPrep v.3.4 in Maestro and the resulting structure was energy minimized in implicit solvent with OPLS 2005 force field in Maestro. The prepared ligand molecules were docked to the *h*QC with Glide v.6.7 in Maestro. The grid for the active site was generated through the centroid of the co-crystallized ligand, PBD150, and the grid box size was selected as default. Metal coordination constraint was set as tetrahedral geometry for the Zn^{2+}. Glide SP docking was completed with the maximum number of 30 poses per ligand. The resulting best pose of **53** were chosen and conducted for the subsequent QM-Polarized Ligand Docking (QPLD) procedure. The partial charges of the docked ligands were analyzed by Jaguar with the option of accurate QM level. Then, the ligands accompanied with the updated charges were re-docked using Glide extra precision (XP). The protein-ligand complex obtained from the QPLD was taken for further optimization by Refine Protein-Ligand Complex module in Prime v.4.0 in Maestro. Protein residues within 5 Å of the docked ligand were minimized by local

optimization refinement. The side chain conformations of the selected protein residues were predicted and minimized along with the docked ligand during this process. The results were further energy minimized using Monte Carlo sampling algorithm in 2500 steps in Maestro.

All the molecular graphic figures were generated by PyMOL software (http://www.pymol.org). All computational studies were undertaken on an Intel Xeon Octa-Core 2.67 GHz workstation with Linux CentOS release 6.7.

1. Design and Pharmacophore

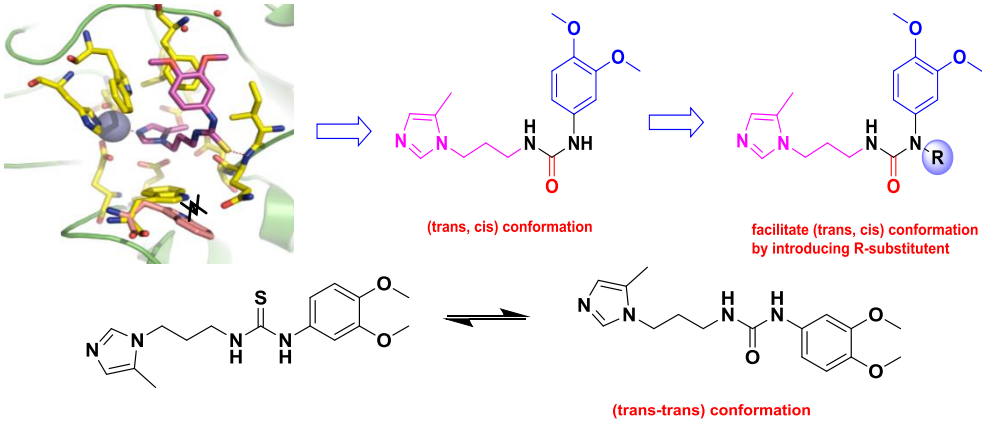


Figure 3.1: Binding complex of compound **1** and *h*QC acitive site

The parent compound **1** may have 4 conformations: cis-cis; trans-trans; trans-cis and cis-trans in order to interact with *h*QC active site. Base of the binding complex of compound **1** with hQC active site (**Figure 3.1**), we know that it interacts with *h*QC by trans-cis conformation; Hence, to make easy to have trans-cis conformation, we introduced a substituent group at B region that produce the compound with potent *in vitro* activity from 3-22 fold better then parent compound 1. Unfortunately, their *in vivo* assays exhibited less potent due to low brain permeability.

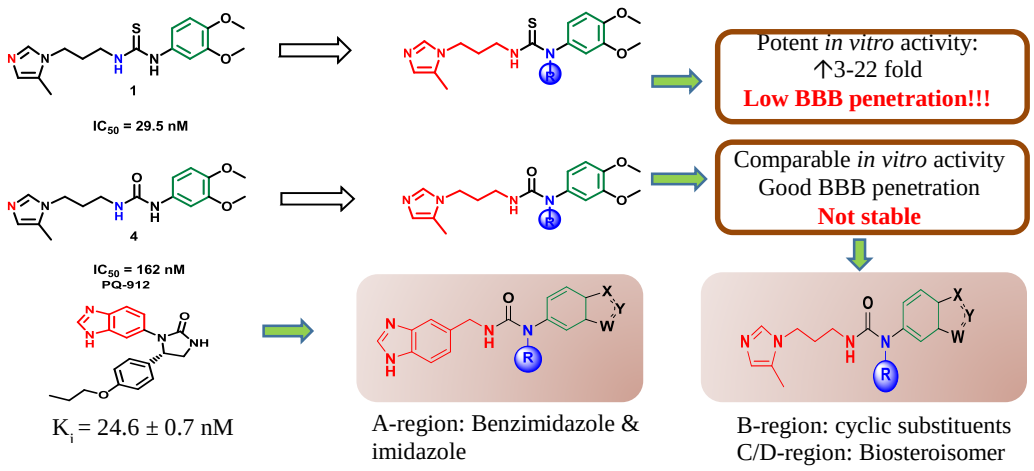


Figure 3.2: Urea and Thiurea QC inhibitors effects

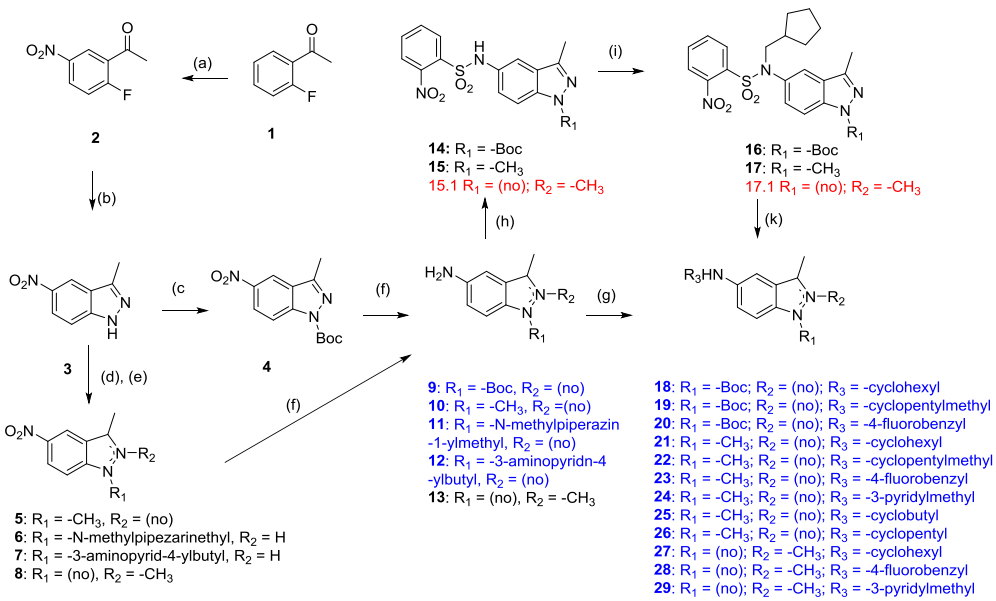
So we changed from thiourea type to Urea type in order to improve BBB penetration (**Figure 3.2**). The *in vitro* results prove that these compounds had

comparable activity as well as good BBB penetration. However, they are not stable in metabolism due to the dimethoxy group at C region[78]. In the fact, Oxygen in heterocyclic at C/D region have hydrogen bonding with *h*QC active site, having an important function in pharmacophore. So in this series we anticipate that bioisomer in C region would maintain the hydrogen bonding also keep compound stable in metabolism.

Based on our previous study, compound **4** have IC_{50} = 162 nM, decrease nearly 5.5-fold less than the compound **1**. However, when B region was substituted by different group R, it showed significantly increase the QC inhibition activity. Also, the docking study of PQ912[79] which was developed by Probiodrug exhibited the Zinc binding motif between benzimidazole and Zinc of *h*QC active site. Hence, we continued to survey the modification of C/D region with heterocyclic ring with different substituted group at B region as well as the A region with normal 5-methyl imidazole and novel benzimidazole. We anticipated that these modifications could provide useful information for the investigation of Phe-Agr mimetic region.

2. Result and discussion

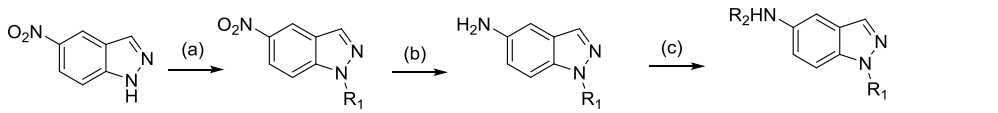
2.1. Chemistry



Scheme 3.1. Synthesis of 3-methyl-5-nitroindazole derivatives. Reagents and conditions : (a) HNO_3, c.H_2SO_4,-15 ^oC, 20 mins; (b) $N_2H_4.H_2O$, EtOH, reflux, o.n.;

(c) Boc_2O, DMAP, DCM; (d) halide alkyl, Cs_2CO_3, DMF; (e) N-methylpiperazine, Cs_2CO_3, DMF (for **7**); (f) Pd/C, H2, MeOH; (g) aldehyde or ketone derivatives, $NaBH_3CN$, AcOH, MeOH, o.n; (h) 2-nitrobenzenesulfonyl chloride, TEA, DCM, 0 ^oC-r.t., 4h; (i) cyclopentylmethanol, DEAD, Ph_3P, DCM, r.t, o.n.; (k) thiophenol, K_2CO_3, ACN, r.t., o.n.

First for synthesis the 3-methyl-5-nitroindazole fragments which was showed in **scheme 3.1**, 2'-fluoro-acetophenone was nitration via electrophile substitution in fuming nitric acid and conc. H_2SO_4 at -42 ^oC to obtain compound **2**[80]. Nitro compound was intramolecular cyclized in excess hydrazine to produce 5-indazole derivative **3**[81]. Compound **3** was protected by Boc-group then reduce to obtain intermediate **9**. Meanwhile it also underwent Williamson reaction and N-alkylation to achieve intermediate **5-8**. All of these intermediates were subjected for reduction to get the corresponding amine **10-13**. The primary amine **9-13** underwent directly reductive amination with different aldehyde or ketone to obtain secondary amine[82] **18, 20-21, 23-27, 29**. In order to obtain intermediate **19, 22, 28** with cyclopentylmethyl substituent, their corresponding primary amine were firstly protected with 2-nitrobenzensulfonyl chloride[81], then alkylation amine group by Mitsunobu reaction to obtaine **16-17.1.** the nitrobenzensulfomide removed easily with soft nucleophiles via Meisenheimer complexes to give the corresponding secondary amines[83].



30

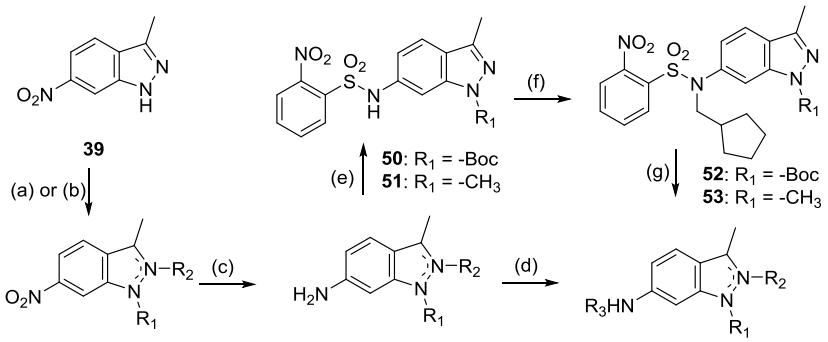
31: R_1 = -propyl
32: R_1 = -isopropyl

33: R_1 = -propyl
34: R_1 = -isopropyl

35: R_1 = -propyl; R_2 = -cyclohexyl
36: R_1 = -propyl; R_2 = -4-fluorobenzyl
37: R_1 = -isopropyl; R_2 = -cyclohexyl
38: R_1 = -isopropyl; R_2 = -4-fluorobenzyl

Scheme 3.2. Synthesis of 5-nitro indazole derivatives. Reagents and conditions: (a) halide alkyl, Cs_2CO_3, DMF; (b) Pd/C, H_2, MeOH; (c) aldehyde or ketone derivatives, $NaBH_3CN$, AcOH, MeOH, o.n.

Meanwhile, 5-indazole without the 3-methyl group derivatives were synthesized as in **scheme 3.2**. N-alkylation of 5-nitro-indazole to achieve intermediate **31, 32** which were reduced to get corresponding amine **33, 34**. The primary amines were reacted with 4-fluorobenzyaldehyde and cyclohexanone to produce **35-38**.



40: R_1 = -Boc, R_2 = (no)
41: R_1 = -CH_3, R_2 = (no)
42: R_1 = -N-methylpiperazyl, R_2 = (no)
43: R_1 = -3-aminopyrid-4-ylbutyl, R_2 = (no)
44: R_1 = (no), R_2 = -CH_3

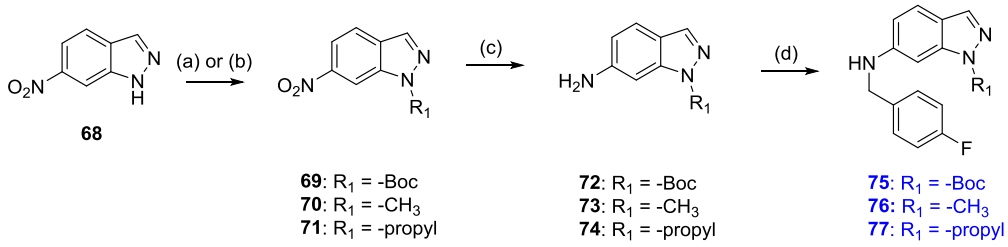
45: R_1 = -Boc, R_2 = (no)
46: R_1 = -CH_3, R_2 = (no)
47: R_1 = -N-methylpiperazyl, R_2 = (no)
48: R_1 = -3-aminopyrid-4-ylbutyl, R_2 = (no)
49: R_1 = (no), R_2 = -CH_3

54: R_1 = -Boc, R_2 = (no), R_3 = -cyclohexyl
55: R_1 = -Boc, R_2 = (no), R_3 = -cyclopentylmethyl
56: R_1 = -Boc, R_2 = (no), R_3 = -4-fluorobenzyl
57: R_1 = -Boc, R_2 = (no), R_3 = -3-pyridylmethyl
58: R_1 = -Boc, R_2 = (no), R_3 = -N-methylpiperazyl
59: R_1 = -CH_3, R_2 = (no), R_3 = -cyclohexyl
60: R_1 = -CH_3, R_2 = (no), R_3 = -cyclopentylmethyl
61: R_1 = -CH_3, R_2 = (no), R_3 = -4-fluorobenzyl
62: R_1 = -CH_3, R_2 = (no), R_3 = -3-pyridylmethyl
63: R_1 = -CH_3, R_2 = (no), R_3 = -N-methylpiperazyl
64: R_1 = -CH_3, R_2 = (no), R_3 = -cyclobutyl
65: R_1 = -CH_3, R_2 = (no), R_3 = -cyclopentylmethyl
66: R_1 = (no), R_2 = -CH_3, R_3 = -4-fluorobenzyl
67: R_1 = (no), R_2 = -CH_3, R_3 = -3-pyridylmethyl

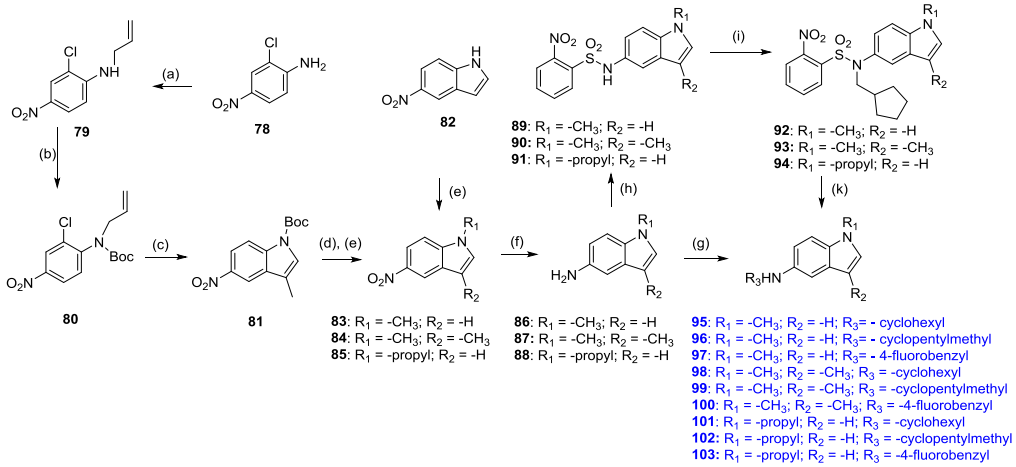
Scheme 3.3. Synthesis of 3-methyl-6-nitroindazole derivatives. Reagents and conditions: (a) Boc_2O, DMAP, DCM; (b) alkyl halide, Cs_2CO_3, DMF; (c) Pd/C, H_2, MeOH; (d) aldehyde or ketone derivatives, $NaBH_3CN$, AcOH, MeOH, o.n.; (e) 2-nitrobenzenesulfonyl chloride, TEA, DCM, 0 ^oC-r.t., 4h; (f) cyclopentylmethanol, DEAD, Ph_3P, DCM, r.t, o.n.; (g) thiophenol, K_2CO_3, ACN, r.t., o.n.

Scheme 3.3 showed the synthesis of 3-methylindazole-6-yl derivative. 3-methyl-6-nitroindazole **39** reacted with Boc_2O or alkyl halide to achieve **40-44** which was reduced in hydrogenation to obtain primary amine of **45-49**. The primary amine underwent the reduction amination with aldehyde or ketone to produce secondary amine of **54, 56-59, 61-64, 66-67**. Meanwhile, primary amine **45-46** was reacted with 2-nitrobenzensulfonyl chloride then Mitsunobu reaction with cyclopentylmethanol to produce **52-53**. The protected group of nitrobenzensulfonyl was removed by thiol to obtain the corresponding secondary amine **54, 60**.

N-(4-fluorobenzyl)-indazol-6-amine-1-yl derivatives were synthesized as **scheme 3.4**. 6-nitroindazole reacted with Boc_2O or N-alkylation of alkyl halide, then reduced in hydrogenation to obtain corresponding primary amine **72-74**. The amine then underwent reductive amination with 4-fluorobenzaldehyde to get the secondary amine of **75-77**

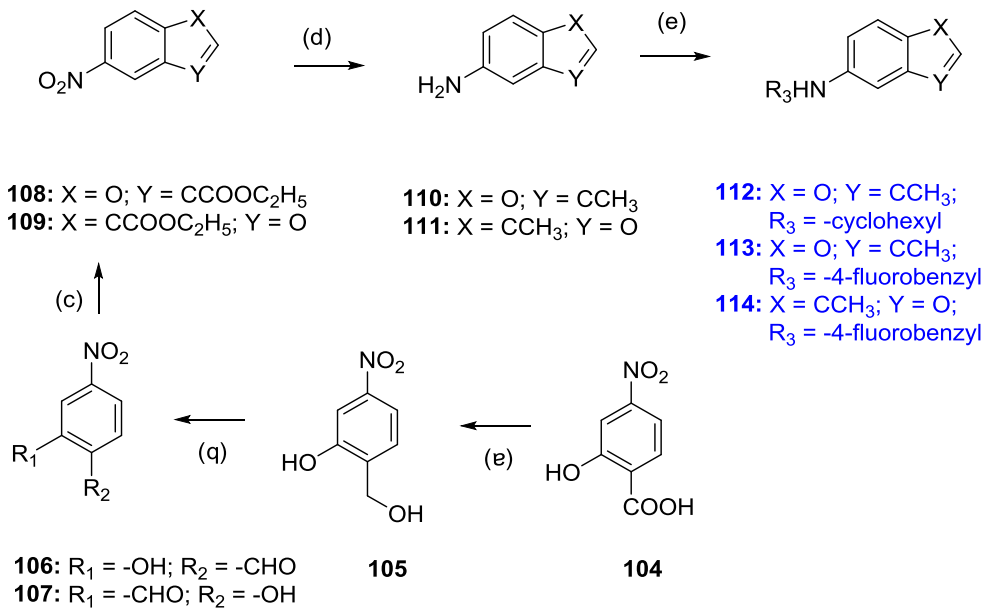


Scheme 3.4. Synthesis of 6-nitroindazole derivatives. Reagents and conditions: (a) Boc_2O, DCM; (b) alkyl halide, Cs_2CO_3, DMF; (c) Pd/C, H_2, MeOH; (d) 4-fluorobenzaldehyde, $NaBH_3CN$, AcOH, MeOH, o.n.



Scheme 3.5. Synthesis of 5-nitroindole derivatives. Reagent and conditions: (a) allyl bromide, t-BuOK, DMF, 30 min, 0 °C-r.t, 18 h; (b) Boc_2O, DMAP, DCM; (c) t-Bu_4NBr, TEA, $Pd(OAc)_2$, xantphos, DMF, reflux, 48h; (d) TFA, MC; (e) alkyl halide, Cs_2CO_3, DMF; (f) Pd/C, H_2, MeOH; (g) aldehyde or ketone derivatives, $NaBH_3CN$, AcOH, MeOH, o.n.; (h) 2-nitrobenzenesulfonyl chloride, TEA, DCM, 0 ^oC-r.t., 4h; (i) cyclopentylmethanol, DEAD, Ph_3P, DCM, r.t, o.n.; (k) thiophenol, K_2CO_3, ACN, r.t., o.n.

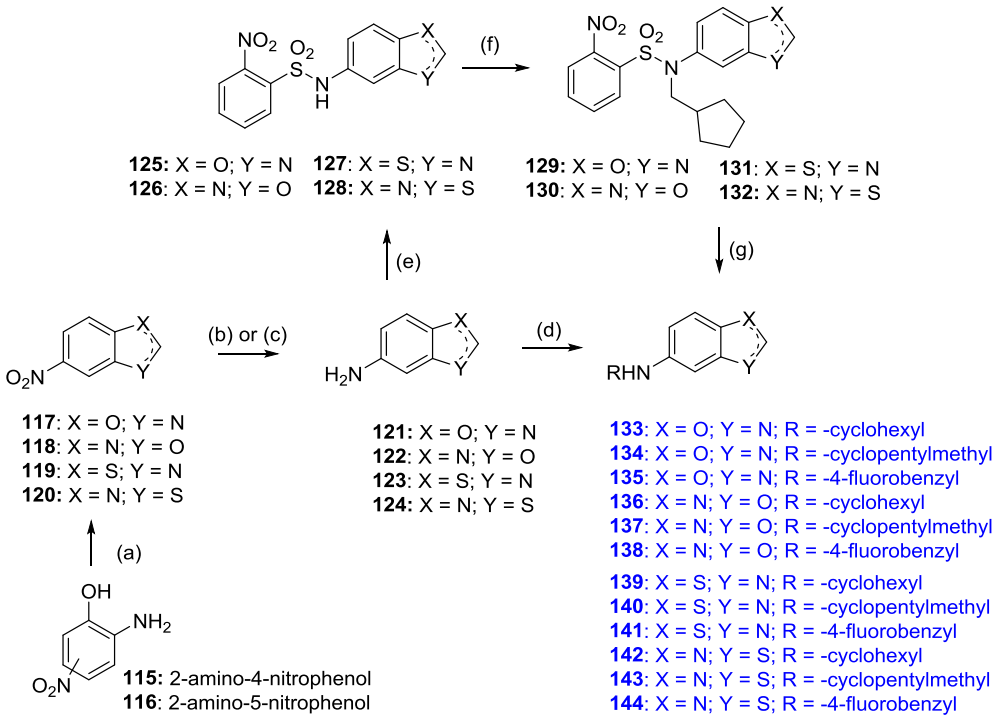
5-indole derivatives were produced as in **scheme 3.5**. 2-chloro-4-nitroaniline **78** firstly was alkylated and Boc protection to achieve intermediate **80**. The compound was intramolecular cyclized via Buchward Hardwig reaction to get 5-indole **81** [84].



Scheme 3.6. Synthesis of benzofuran derivatives. Reagents and conditions: (a) BH_3-THF, 0 ^oC-rt; (b) MnO_2, DCM; (c) N_2CHCO_2Et, $HBF_4.Et_2O$, DCM, 0 ^oC, 1h, c. H_2SO_4; (d) i. DIBAL, toluene; ii. I_2, Ph_3P, Imidazole, DCM, r.t., 1h; iii. $NaBH_4$, diglyme, r.t., 2h; (d) $SnCl_2$, c.HCl, EtOH, reflux, 2h; (e) aldehyde or ketone derivatives, $NaBH_3CN$, AcOH, MeOH, o.n.

The Boc group of compound was removed by TFA in MC then followed by N-alkylate to achieve **83-84**. Also, **85** was obtained from N-alkylation of 5-nitroindole **82**. These nitro intermediates were reduced in hydrogenation to get the primary amine of **86-88** which were not only protected with 2-nitrobenzensulfonyl before substituted with cyclopentylmethyl, but also underwent the reductive amination with aldehyde or ketone derivative to introduce intermediate **95-103**.

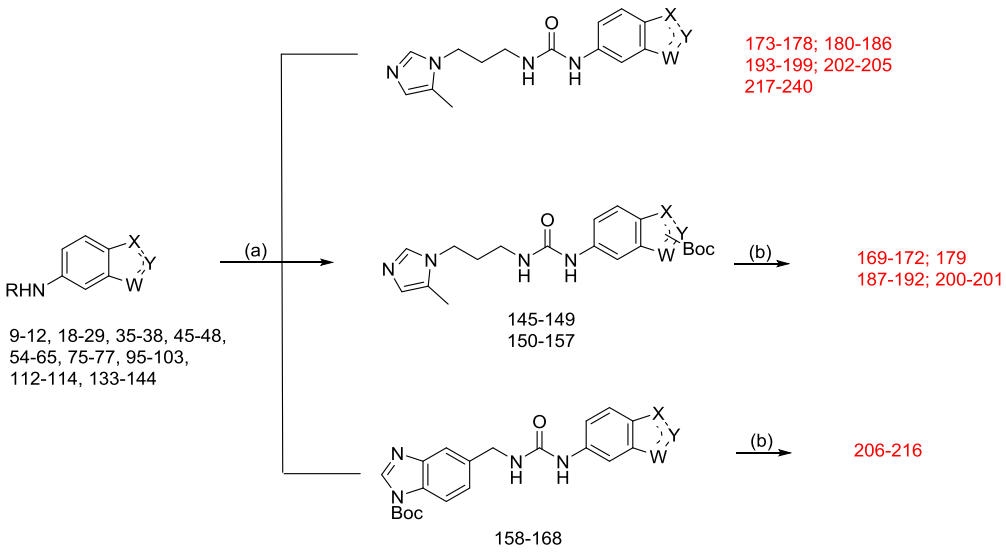
For synthesis of 5/6-benzofuran derivatives as in **scheme 3.6**, 4/5-nitrobenzoic acid was reduced in BH_3 then oxidized by MnO_2 to achieve intermediate **106-107**. The benzaldehyde intermediates were intra molecular cyclized by diazo derivatives[85], then underwent the reduction and Appel reaction to provide primary amine **110-111**. These primary amines were subjected for reductive amination to achieve secondary amine of **112-114**.



Scheme 3.7. Synthesis of benzooxazole and benzothiazole derivatives. Reagents and conditions: (a) triethyl orthoformate, 80 ^{o}C, 1h; (b) Pd/C, H2, MeOH; (c) $SnCl_2$, c.HCl, EtOH, reflux, 2h; (d) aldehyde or ketone derivatives, $NaBH_3CN$, AcOH, MeOH, o.n.; (e) 2-nitrobenzenesulfonyl chloride, TEA, DCM, 0 ^{o}C-r.t., 4h; (f) cyclopentylmethanol, DEAD, Ph_3P, DCM, r.t, o.n.; (g) thiophenol, K_2CO_3, ACN, r.t., o.n.

Benzothiazole and benzoxazole were synthesized as in **scheme 3.7**, nitrophenol derivatives **115-116** were cyclized intra molecular in triethyl orthoformate to achieve heterocyclic **117-120**. These intermediates were reduced by hydrogenation or tinc chloride to obtain corresponding amine **121-124**. The primary amine underwent reductive amination with aldehyde or ketone or via Mitsunobu reaction for cyclopentylmethyl to produce secondary amine of **133-144**.

For achieve the final compound as showed in **scheme 3.8**, secondary amines were coupled with Azide derivative via Aza Wittig coupling[86] to have final compounds of **173-178; 180-186; 193-199; 202-205; 217-240** and other precursors of **145-168**. All of the precursors were removed the protected group to have to obtain the remaining final compounds of **169-172; 179; 187-192; 200-201; 206-216**.

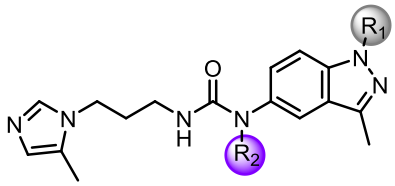


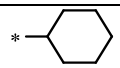
Scheme 3.8. Synthesis of final compounds. Reagents and conditions: (a) azide derivatives, Ph_3P, CO_2, toluene, reflux, o.n.; (b) TFA, DCM, r.t., o.n.

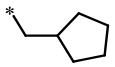
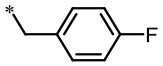
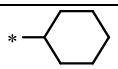
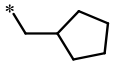
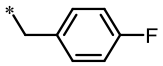
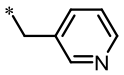
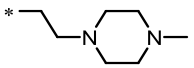
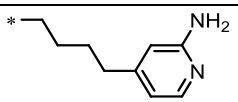
2.2. *In vitro* assay

We performed QC activity assays using a fluorogenic substrate, Gln-AMC (L-glutamine 7-amido-4-methylcoumarin), and pyroglutamyl peptidase (pGAP) as an auxiliary enzyme[67] to evaluate the ability of the D-region-modified library to inhibit QC. We first investigated a group of compounds containing 5-amino-3-methylindazole derivatives in D-region **Table 3.1**.

Table 3.1. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of 5-amino-3-methylindazole derivatives.



Compound	R_1	R_2	IC_{50} (nM)[a]	
Parent compound			162	
169	H	H	107	(±37.8)
170	H		2.3	(±0.7)

171	H		18.2	(±0.3)
172	H		14.0	(±2.5)
173	Me	H	5.6	(±2.1)
174	Me		4.4	(±1.0)
175	Me		13.3	(±5.0)
176	Me		3.0	(±2.7)
177	Me		13.3	(±0.6)
178		H	29.0	(±0.6)
179		H	11.4	(±1.6)

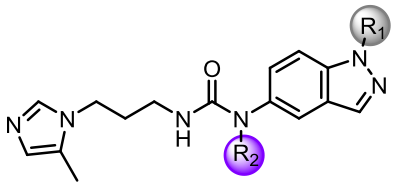
[a] The values indicate the mean of at least three experiments.

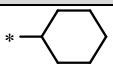
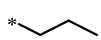
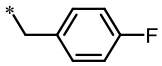
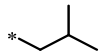
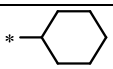
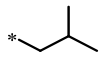
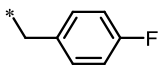
This is result of *in vitro* of 3-methyl-5-indazole derivatives, It showed that biosteroisomer derivatives increase *in vitro* activity. When B region was substituted by different group to have a trans-cis conformation, the compound activity increases significantly about 9-70 fold. Especially, compound **170** with cyclohexyl at B region is the most potent in this series. It suggested that substituent groups at B region made compound easier to having binding complex with *h*QC active side and nitrogen on heterocyclic had H-bond also. When addition methyl group at position 1 of indazole, compound **173-179** also have comparable activity. Furthermore, when B region was substituent ground and D region was moiety of methyl-piperazylethyl and 2-amino-pyridylbutyl, which moiety in our previous study displayed potent activity due to terminally amine position, compounds less activity compare with the above-mentioned compound.

However, in table 2, 5-indazole without methyl group at position 3, and position 1 was propyl or isobutyl and B region was substituted by cyclohexyl and 4-fluorobenzyl. The compounds showed less comparable with compounds in previous table. May be the steric bulk at position 1 affect to H-bonding between Nitrogen and *h*QC active site. However, interestingly compound **181** with 4-fluorobenzyl at B-

region and isopropyl at position 1 have better activity than compound **183** with isobutyl at position 1 about 3.8 fold.

Table 3.2. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of 5-aminoindazole derivatives



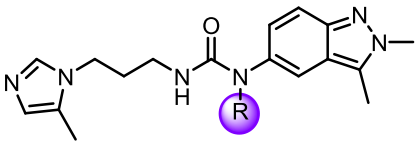
Compound	R_1	R_2	IC_{50} (nM)[a]	
180			39.7	(±7.6)
181			8.6	(±2.0)
182			31.8	(±3.9)
183			32.4	(±6.0)

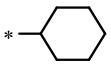
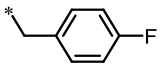
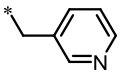
[a] The values indicate the mean of at least three experiments.

We also consider the effect of Nitrogen at position 2 by survey 2,3-dimethylindazole-5-yl derivatives in **table 3.3**. Unfortunately, these compound also exhibited less activity. Even when, 3-aminopyridylmethyl-substitued B region compound **186** displayed weak activity with IC_{50} = 210 nM.

Next, we examined series of 6-amino-3-methylindazole as shown in **table 3.4**. Firstly, the compounds in this series also showed good activity as compound of 1-methyl-indazole-5-yl derivatives. When substituted B region with different cyclic group, their activity increase from 3-50 fold compared with compound **4**. Also, addition methyl group at position 1, these compound **193-198** had comparable activity with IC_{50} of 2.3 to 9.8 nM, except compound **197** with B region substituted by 3-pyridymethyl displayed weak activity with IC_{50} of 65.9 nM. Moreover, when surveyed D-region with moieties of N-methylpiperazylethyl and 2-aminopyridyl-4yl-butyl, the compound **199-200** showed less activity compare with compound of methyl substituents with IC_{50} of 71.2 and 20.4 nM, respectively.

Table 3.3. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of 5-amino-2,3-dimethylindazole derivative

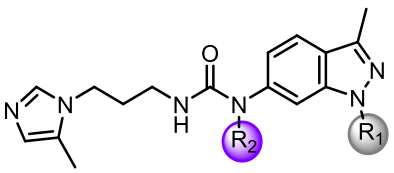


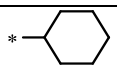
Compound	R_2	IC_{50} (nM)[a]	
184		31.7	(±3.8)
185		22.7	(±2.4)
186		210	(±161)

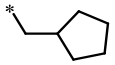
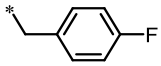
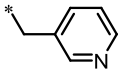
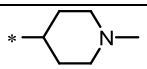
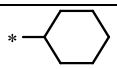
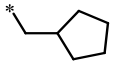
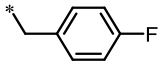
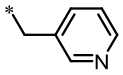
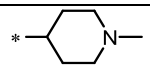
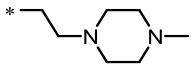
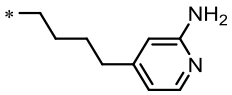
[a] The values indicate the mean of at least three experiments.

In previous series, compound **190** and **196** with 4-fluorobenzyl at B region showed comperable potent in indazole series with IC_{50} of 11.1 and 2.3 nM, respectively. Hence, in **table 3.**5, we examined the compound with B region is 4-fluorobenzyl and modified the C-linker of 1-substitued groups. Unfortunately, the activity of these compounds **201-203** showed moderate potent of IC_{50} from 29.0 to 66.2 nM.

Table 3.4. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds 6-amino-3-methylindazole derivatives

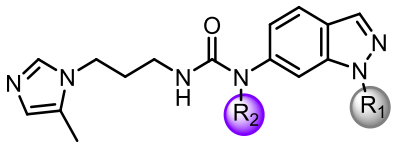


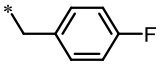
Compound	R_1	R_2	IC_{50} (nM)[a]	
187	H	H	141	(±55.1)
188	H		3.2	(±2.4)

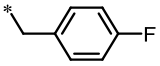
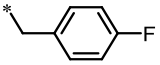
189	H		14.5	(±2.8)
190	H		11.1	(±1.7)
191	H		25.4	(±2.6)
192	H		53.8	(±20.0)
193	Me	H	87.4	(±13.2)
194	Me		5.3	(±3.2)
195	Me		3.4	(±1.1)
196	Me		2.3	(±2.0)
197	Me		65.9	(±2.7)
198	Me		9.8	(±3.0)
199		H	71.2	(±0.6)
200		H	20.4	(±1.9)

[a] The values indicate the mean of at least three experiments.

Table 3.5. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of 6-aminoindazole derivatives



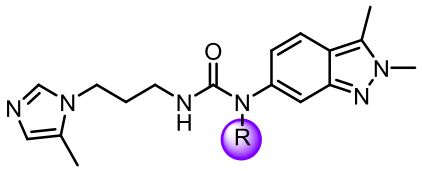
Compound	R_1	R_2	IC_{50} (nM)[a]	
201	H		66.2	(±16.4)

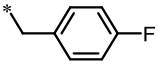
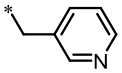
202	Me		29.0	(±6.8)
203			62.4	(±8.2)

[a] The values indicate the mean of at least three experiments.

For survey the 2,3-dimethyl derivative with B region is 4-fluorobenzyl and 3-pyridylmethyl, IC_{50} of these compound showed less active as corresponding compound of 5-indazole as showed in table 3.6. Interestingly, compound 204 with 4-fluorobenzyl at B-region is more active 3-times than compound 205 with 3-pyridylmethyl at B region.

Table 3.6. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of 6-amino-2,3-dimethylindazole derivatives



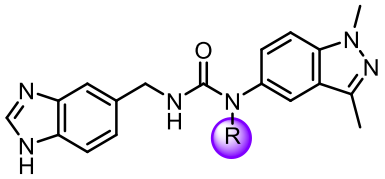
Compound	R	IC_{50} (nM)[a]	
204		15.5	(±4.5)
205		43.8	(±18.4)

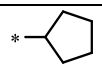
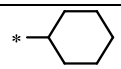
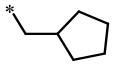
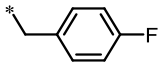
[a] The values indicate the mean of at least three experiments.

In order to optimize A region with benzimidazole, **table 3.7** and **table 3.8** displayed QC inhibitory activity of compounds of 5/6-indazole with different group at B region. The IC_{50} results display less activity compare with those of imidazole in A region, regardless of the steric hindrance with IC_{50} of 24.6-76.1 nM.

Meanwhile, 6-indazole derivatives compound **211-216**, **table 3.8**, also have the similar weak activity as 5-indazole derivatives. Compound **215** with 4-fluorobenzyl at B region once again displayed potent activity than others compound in this series.

Table 3.7. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of 6-amino-3-methylindazole derivative with benzimidazole at A region

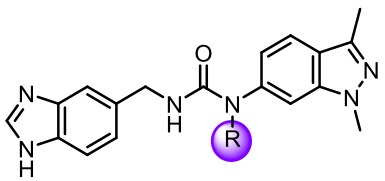


Compound	R	IC_{50} (nM)[a]	
206		24.6	(±1.1)
207		54.9	(±2.4)
208		76.1	(±17.0)
209		35.3	(±3.3)
210		31.1	(±0.8)

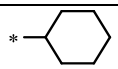
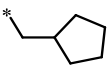
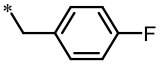
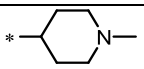
[a] The values indicate the mean of at least three experiments.

We also surveyed C-region with indole derivatives as in **table 3.9**, in which there was only one Nitrogen atom on heterocyclic ring. Unfortunately, although all of these compound **217-225** showed increased inhibitory activity 2-4 fold compare with leading compound **4**, their activity much weaker compare with compound of indazole derivatives. This result could exhibited the important of hydrogen bonding between C-region and *h*QC active site.

Table 3.8. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of 6-amino-3-methylindazole derivatives with benzimidazole at A region

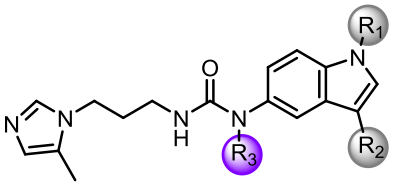


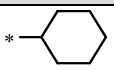
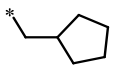
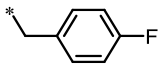
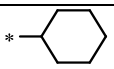
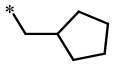
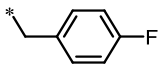
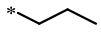
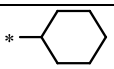
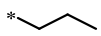
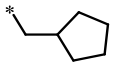
Compound	R	IC_{50} (nM)[a]	
211		35.4	(±8.0)

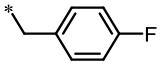
212		53.5	(±5.9)
213		57.2	(±7.9)
214		30.0	(±3.2)
215		18.8	(±6.9)
216		74.6	(±1.3)

[a] The values indicate the mean of at least three experiments.

Table 3.9. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of 5-aminoindole derivatives



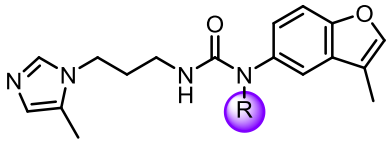
Cpd.	R_1	R_2	R_3	IC_{50} (nM)[a]	
217	Me	H		49.5	(±5.9)
218	Me	H		74.6	(±14.9)
219	Me	H		61.2	(±4.2)
220	Me	Me		48.1	(±7.2)
221	Me	Me		41.9	(±10.0)
222	Me	Me		41.7	(±7.7)
223		H		35.9	(±8.4)
224		H		60.3	(±5.8)

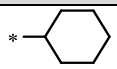
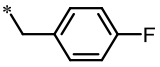
225		H		60.0	(±6.5)

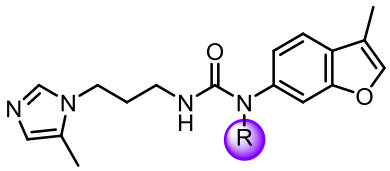
[a] The values indicate the mean of at least three experiments.

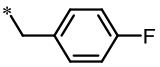
Finally we also considered C region with benzofuran, benzooxazole, benzothiazole derivatives **226-228** as showed in **table 3.10** in order to continuously evaluate the important of biosteroisomer in this series. The *in vitro* result displayed the weak activity compared with those of indazole derivative. Even, compound with 4-fluorobenzyl at B region also decreased activity significantly, especially compound 228 of 6-aminobenzofuran derivative was the weakest inhibitory activity with IC_{50} of 113 nM.

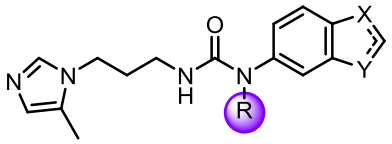
Table 3.10. IC_{50} values for inhibition of *h*QC by N-substituted urea compounds of benzofuran, benzooxazole and benzothiazole derivatives

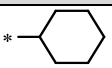
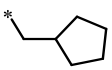
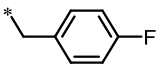
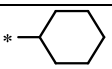
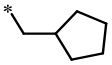
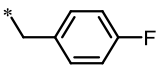
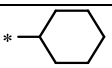
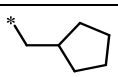
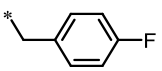
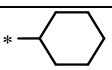
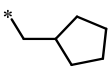
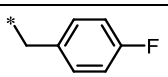


Compound	R_2	IC_{50} (nM)[a]	
226		81.0	(±22.7)
227		48.3	(±22.4)



228		113	(±26.1)



	X	Y	R	IC_{50} (nM)[a]	
229	O	N		33.5	(±1.2)
230	O	N		59.4	(±12.4)
231	O	N		26.9	(±3.4)
232	N	O		47.7	(±13.4)
233	N	O		32.9	(±1.9)
234	N	O		39.5	(±6.9)
235	S	N		50.2	(±2.9)
236	S	N		47.3	(±6.2)
237	S	N		37.5	(±0.7)
238	N	S		47.1	(±4.9)
239	N	S		35.6	(±0.7)
240	N	S		38.2	(±0.8)

[a] The values indicate the mean of at least three experiments.

3. Conclusion

In this part, we continued to synthesize and evaluate the biological activity of C-region with heterocyclic ring base on the binding complex of parent compound 1 and *h*QC active site of **72 compounds.** In general, most of them were showed less activity compare with our previous study derivative. Compound with indazole at C region

had better activity than those of indole, benzooxazole, benzothiazole, benzofuran fragment. This suggested that the C region interact with *h*QC active site by H-bond play an important role. Furthermore, when we changed the A region from imidazole derivatives to benzimidazole derivates, *h*QC inhibitory activity reduced remarkably. We needed to evaluate *in vivo* and acute AD model test of 10 potent compound for further discovery.

4. Experimental

4.1. Chemistry

4.1.1. General

All chemical reagents were commercially available. Melting points were determined on a melting point Buchi B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230-400 mesh, Merck. ^{1}H NMR spectra were recorded on a a JEOL JNM-LA 300 at 300 MHz, Bruker Analytik, DE/AVANCE Digital 400 at 400 MHz, a Bruker Analytik, DE/AVANCE Digital 500 at 500 MHz, and a JEOL JNM-ECA-600 at 600 MHz. Mass spectra were recorded on a VG Trio-2 GC−MS instrument and a 6460 Triple Quad LC−MS instrument. All final compounds were assessed for purity by high performance liquid chromatography (HPLC) on Agilent 1120 Compact LC (G4288A) system via the following conditions. Column: Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm). Mobile phase A: MeOH, Mobile phase B: 0.1% TFA in water (v/v) in 30 min. Wavelength: 254 nM. Flow: 0.7 mL/min. According to the HPLC analyses, all final compounds showed a purity of $\geq$ 95%.

4.1.2. General procedure

4.1.2.1. Produce 1- Nitration

To a mechanically stirred slurry of conc. H_2SO_4 (93-98%, 360 mL) at -42 °C were added dropwise 2'-fluoro-acetophenone 45 (90.0 g, 652 mmol) and a solution of fuming nitric acid (53.1 mL) in conc. H_2SO_4 (129 mL). The slurry was stirred for 30 min at -42 °C. The mixture was slowly poured onto 1.3 kg of ice. To the mixture was added water (1 L). The product precipitated out of solution. After all of the ice melted, the product was collected via filtration. The solid was dissolved with EtOAc. The organic layer was washed with 5% Na_2CO_3 (2 x 300 mL), water (300 mL), and brine

(300 mL), and dried over Na_2SO_4. It was filtered, the filtrate was concentrated to give compound

4.1.2.2. Procedure 2- indazole cyclisation

To a solution of 2-fluoroacetophenone derivatives (14.48 mmol, 1.0 equiv) in ethylene glycol (10 mL) was added hydrazine monohydrate (15.06 mmol, 1.04 equiv) at 25 °C. After 2 h at 25 °C, the reaction was heated to 165 °C and stirred until complete by TLC. After cooled to 25 °C, the mixture was extracted with CH_2Cl_2 (3 x). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. After filtration and concentration in vacuo, the residue was purified via flash column chromatography on silica gel to indazole analogs.

4.1.2.3. Procedure 3 – Reductive amination

To a solution of 10% AcOH in MeOH was added the SM (1 equiv) and dry acetone or aldehyde (0.90 equiv). The solution was stirred at RT 1 h, after which time it was cooled to 0 ^oC and treated with $NaCNBH_3$ (1.5 equiv). The reaction was stirred at RT for 5 h. The mixture was concentrated and the residue brought to pH = 10 using Na_2CO_3. The mixture was extracted with EtOAc and the organic layer was washed with H_2O, brine, dried ($MgSO_4$), and concentrated to dryness. The crude material was purified by silica gel column chromatography (2% MeOH/DCM) to provide the product as a yellow solid.

4.1.2.4. Procedure 4

Procedure 4.1. The primary amine in DMC was all nitrobenzensulfonyl chloride (1.3 equiv) and TEA (2.0 equiv). Then the mixture was stirred at r.t until SM was consumed (by TCL). The mixture was pour into water and extracted by DCM (50 mL x 3 times). The combined organics were wash with brine, dried ($MgSO_4$), and concentrated to dryness and purified by silia gel column chromatography to provide the product.

Procedure 4.2. Solution of nitrobenzensulfonyl compound (1 equiv.) in DCM (10 mL) was add thiophenol (1.3 equiv.) and potassium carbonate (3 equiv.). The mixture was stirred at r.t. overnight then quenched with brine and extracted with DCM (3 time x

50 mL). The combined organics were dried ($MgSO_4$) and concentrated and purified by silica gel column chromatography to get the desired product.

4.1.2.5. Procedure 5 - Reduction

Procedure 5.1. BH3 reduction: To a 0.24M THF suspension of the SM (1 equiv) at 0 ^oC was added BH_3-THF (3 equiv). The reaction mixture was stirred under Ar at r.t for 66 h then quenched by addition of EtOH (15 mL) at 0 ^oC and stirred an additional 15 min. The mixture was poured into H_2O and extracted with DCM. The combined organics were washed with brine, dried (Na_2SO_4), and concentrated to provide the crude product as a white solid.

Procedure 5.2: Hydrogenation

The nitro compound or alkene derivative was dissolved in MeOH (or mixture of MeOH and THF) and then 10% Pd/C was added. The mixture was stirred at room temperature under hydrogen gas until all starting material was consumed (confirmed by TCL). The crude mixture was filtered through celite filter, washed with MeOH (3 x 50 mL) and then concentrated. The product was subjected to the next step without further purification.

Procedure 5.3: Zinc powder reduction

AcOH (5 equiv) and Zn dust (5 equiv) were added to a solution of a nitro compound in MeOH (10 mL) at room temperature. The reaction mixture was stirred at room temperature for 30 minutes and then filtered through a celite filter. The filtrate was portioned between H_2O (10 mL) and DCM (30 mL). The organic layer was separated, dried over $MgSO_4$, concentrated, and purified by column chromatography to provide the product.

4.1.2.6. Procedure 6

The entire two-step, one-pot reaction can be accomplished in less than 1 h. For each experiment, $HBF_4 \cdot OEt_2$ was added to the aldehyde (1 g) in CH_2Cl_2 (2 mL). A solution of ethyl diazoacetate in CH_2Cl_2 was introduced into the reaction mixture as the evolution of N_2 gas permitted (ca. 3-6 min addition time) and the reaction was not allowed to go above 38 °C. Once gas evolution ceased, the reaction mixture was

concentrated by rotary evaporator and H_2SO_4 (0.3 to 0.5 mL) was added to the mixture while stirring. After 5 to 10 min, the mixture was diluted with CH_2Cl_2 (5 to 10 mL) and the H_2SO_4 was quenched with solid $NaHCO_3$. The mixture was then filtered through silica gel (1 g), and concentrated by rotary evaporator to give an oil, which slowly crystallized under high vacuum.

4.1.2.7. Procedure 7 - Benzooxazole

According to a procedure, the corresponding 2-aminophenol (5 mmol) and triethyl orthoformate (15 mL) was refluxed for 4 h. After cooling to room temperature, the remaining triethyl orthoformate was removed under reduced pressure and the residue was purified by column chromatography to yield the desired substituted benzoxazole

4.1.2.8. Mitsunobu reaction (Procedure 8)

Triphenylphosphine (1.3 equiv) was added to a solution of 4-nitroguanicol (1.0 equiv) in DCM under a nitrogen atmosphere, followed by the addition of a primary alcohol (1.2 equiv) and a solution of diethyl azodicarboxylate (1.3 equiv) in DCM. After the solution was stirred for 30 minutes at room temperature, the reaction was poured onto a column of silica and was eluted with EtOAc:*n*-hexane to yield the desired product.

4.1.2.9. Boc protection and deprotection

Procedure 9.1: Triethylamine (1.2 equiv) and di-*tert*-butyl dicarbonate (2.5 equiv) in DCM were added to a suspension of the starting amine material (1.0 equiv) in DCM in an ice bath. The mixture was stirred at room temperature until starting material was consumed (confirmed by TLC). Water was added to the mixture and subsequently extracted with DCM. The organic layer was washed with a 10% aqueous $NaHCO_3$ solution, water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography to obtain the desired product.

Procedure 9.2: Trifluoroacetic acid (10 equiv) was added to the solution of the *boc*-protected compound (1.0 equiv) in DCM (DCM:TFA = 1:1 (v/v)). Then, the mixture was stirred at room temperature until the starting material was consumed and evaporated. The residue was dissolved in MeOH and purified on an ion-exchange

column to obtain the desired product or subjected to the next step without further purification.

4.1.2.10 Procedure 10-Azide coupling reaction

Procedure 10: Amine derivative (1 equiv), triphenylphosphine (1.1 equiv), 1-(3-azidopropyl)-5-methyl-1H-imidazole (1.1 equiv) were dissolve in toluene (5 mL). The solution was degas then refluxed under CO_2 condition overnight. The mixture was cooled down and evaporated to remove toluene. The residue was purified by DCM/ MeOH to get the product

4.1.2.11. Procedure 11 – N-alkylation

A mixture of the alkyl halide, nitrogen-containing compound and excess base in DMF was stirred at 60 °C for 30 min. The reaction was quenched with water and extracted with EA. The organic layer was washed with water and brine, concentrated and purified by column chromatography.

4.2. Compound

4.2.1. Intermediate

*4.2.1.1. 1-(2-Fluoro-5-nitrophenyl)ethan-1-one **(02)***. Starting with compound **01** following the general procedure **01**, compound **02** was obtained as yellow solid, yield 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (dd, J = 2.91, 6.03 Hz, 1H), 8.44-8.38 (m, 1H), 7.37 (t, J = 9.33 Hz, 1H), 2.71 (d, J = 4.77 Hz, 3H).

*4.2.1.2. 3-Methyl-5-nitro-1H-indazole **(03)***. Starting with compound **02** following the general procedure **02**, compound **03** was obtained as yellow solid, yield 84%. ^{1}H NMR (300 MHz, DMSO) δ 13.30 (s, NH), 8.76 (s, 1H), 8.18 (dd, J = 2.01, 9.15 Hz, 1H), 7.63 (d, J = 9.15 Hz, 1H), 2.56 (s, 3H).

*4.2.1.3. tert-Butyl 3-methyl-5-nitro-1H-indazole-1-carboxylate **(04)***. Starting with compound **03** following the general procedure **9.1**, compound **04** was obtained as yellow solid, yield 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (d, J = 2.01 Hz, 1H), 8.40 (dd, J = 9.36, 2.22 Hz, 1H), 8.24 (d, J = 9.36 Hz, 1H), 2.67 (s, 3H), 1.74 (s, 9H).

4.2.1.4. 1,3-Dimethyl-5-nitro-1H-indazole ***(05)***. Starting with compound **03** following the general procedure **11**, compound **05** was obtained as pale solid, yield 77%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, J = 2.04 Hz, 1H), 8.28 (dd, J = 2.19, 9.15 Hz, 1H), 7.38 (d, J = 9.15 Hz, 1H), 4.06 (s, 3H), 2.62 (s, 3H), 1.56 (s, 9H).

4.2.1.5. 3-Methyl-1-(2-(4-methylpiperazin-1-yl)ethyl)-5-nitro-1H-indazole ***(06)*****.** Starting with compound **03** following the general procedure **11**, compound **06** was obtained as yellow solid, yield 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (d, J = 2.01 Hz, 1H), 8.24 (dd, J = 9.15, 2.19 Hz, 1H), 7.41 (d, J = 9.15 Hz, 1H), 4.45 (t, J = 6.87 Hz, 2H), 2.86 (t, J = 6.78 Hz, 2H), 2.62 (s, 3H), 2.55 (br, 4H) , 2.41 (br, 4H), 2.26 (s, 3H).

4.2.1.6. tert-Butyl (4-(4-(3-methyl-5-nitro-1H-indazol-1-yl)butyl)pyridin-2-yl)carbamate ***(07)*****.** Starting with compound **03** following the general procedure **11**, compound **07** was obtained as yellow solid, yield 88%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (d, J = 2.01 Hz, 1H), 8.24 (dd, J = 9.15, 1.32 Hz, 1H), 8.11 (d, J = 4.74 Hz, 1H), 7.95 (s, 1H), 7.77 (s, 1H), 7.35 (d, J = 9.15 Hz, 1H), 6.72 (dd, J = 5.13, 1.47 Hz, 1H), 4.35 (t, J = 6.93 Hz, 2H), 2.62 (s, 3H), 2,62 (t, J = 7.62 Hz, 2H), 1.96 (quintet, J = 7.86 Hz, 2H), 1.74-1.58 (m, 2H).1.53 (s, 9H).

4.2.1.7. 2,3-Dimethyl-5-nitro-2H-indazole ***(08)*****.** Starting with compound **03** following the general procedure **11**, compound **08** was obtained as yellow solid, yield 20%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (dd, J = 2.01, 0.57 Hz, 1H), 8.05 (dd, J = 9.33, 2.19 Hz, 1H), 7.47 (dd, J = 9.15, 0.54 Hz, 1H), 4.13 (s, 3H), 2.64 (s, 3H).

4.2.1.8. tert-Butyl 5-amino-3-methyl-1H-indazole-1-carboxylate ***(09)*****.** Starting with compound **04** following the general procedure **5.2**, compound **09** was obtained as red solid, yield 96%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 8.97 Hz, 1H), 6.92 (dd, J = 8.79, 2.19 Hz, 1H), 6.84 (d, J = 1.83 Hz, 1H), 3.73 (s, 2H), 2.51 (s, 3H), 1.70 (s, 9H).

4.2.1.9. 1,3-Dimethyl-1H-indazol-5-amine ***(10)*****.** Starting with compound **05** following the general procedure **5.2**, compound **10** was obtained as pink solid, yield 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (d, J = 9.33 Hz, 1H), 6.87-6.83 (m, 2H), 3.92 (s, 3H), 2.46 (s, 3H).

4.2.1.10. 3-Methyl-1-(2-(4-methylpiperazin-1-yl)ethyl)-1H-indazol-5-amine ***(11).*** Starting with compound **06** following the general procedure **5.2**, compound **11** was obtained as red solid, yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, J = 9.33 Hz, 1H), 6.86-6.84 (m, 2H), 4.37 (t, J = 7.35 Hz, 2H), 3.36 (br, 2H), 2.82 (t, J = 7.35 Hz, 2H), 2.56 (br, 4H), 2.47 (s, 3H), 2.44 (br, 4H), 2.27 (s, 3H).

4.2.1.11. tert-Butyl (4-(4-(5-amino-3-methyl-1H-indazol-1-yl)butyl)pyridin-2-yl)carbamate ***(12).*** Starting with compound **07** following the general procedure **5.2**, compound **12** was obtained as red solid, yield 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.11 (d, J = 4.74 Hz, 1H), 7.13 (d, J = 9.45 Hz, 1H), 6.98-6.95 (m, 2H), 6.86-6.82 (m, 2H), 4.26 (t, J = 6.78 Hz, 2H), 3.86 (br, 2H), 2.59 (t, J = 7.86 Hz, 2H), 2.48 (s, 3H), 1.93-1.86 (m, 2H), 1.42 (s, 9H).

4.2.1.12. 2,3-Dimethyl-2H-indazol-5-amine **(13).** Starting with compound **08** following the general procedure **5.2**, compound **13** was obtained as red solid, yield 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 8.97 Hz, 1H), 7.33 (s, 1H), 7.04 (d, J = 8.97 Hz, 1H), 3.98 (s, 3H), 2.49 (s, 3H).

4.2.1.13. tert-Butyl 3-methyl-5-((2-nitrophenyl)sulfonamido)-1H-indazole-1-carboxylate **(14)**. Starting with compound **09** following the general procedure **4.1**, compound **14** was obtained as pale solid, yield 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (d, J = 7.89 Hz, 1H), 7.89-7.79 (m, 2H), 7.75-7.65 (m, 2H), 7.55-7.51 (m, 2H), 7.26 (d, J = 8.61 Hz, 1H), 2.54 (s, 3H), 1.70 (s, 9H).

4.2.1.14. N-(2,3-Dimethyl-2H-indazol-5-yl)-2-nitrobenzenesulfonamide ***(15).*** Starting with compound **10** following the general procedure **4.1**, compound **15** was obtained as pale solid, yield 71%.

4.2.1.15. tert-Butyl 5-((N-(cyclopentylmethyl)-2-nitrophenyl)sulfonamido)-3-methyl-1H-indazole-1-carboxylate ***(16).*** Starting with compound **14** following the general procedure **8**, compound **16** was obtained as red solid, yield 53%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 8.80 Hz, 1H), 7.62-7.58 (m, 2H), 7.53 (s, 1H), 7.41-7.36 (m, 2H), 7.28 (dd, J = 1.60, 8.85 Hz, 1H), 6.37 (s, 1H), 3.74 (d, J = 7.70 Hz, 2H), 2.52 (s, 3H), 1.87-1.83 (m, 1H), 1.68 (s, 9H), 1.65-1.58 (m, 4H), 1.51-1.47 (m, 2H), 1.33-1.23 (m, 4H).

4.2.1.16. N-(cyclopentylmethyl)-N-(2,3-dimethyl-2H-indazol-5-yl)-2-nitrobenzenesulfonamide ***(17).*** Starting with compound **15** following the general procedure **08**, compound **17** was obtained as pale red solid, yield 64%.

4.2.1.17. tert-Butyl 5-(cyclohexylamino)-3-methyl-1H-indazole-1-carboxylate ***(18).*** Starting with compound **09** following the general procedure **03**, compound **18** was obtained as pale red solid, yield 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 8.40 Hz, 1H), 6.85 (dd, J = 2.40, 8.97 Hz, 1H), 6.65 (d, J = 2.19 Hz, 1H), 3.34-3.27 (m, 1H), 2.51 (s, 3H), 2.10-2.07 (m, 2H), 1.80-1.76 (m, 2H0, 1.67 (s, 9H), 1.65-1.60 (m, 2H), 1.48-1.30 (m, 3H), 1.26-1.16 (m, 3H).

4.2.1.18. tert-Butyl 5-((cyclopentylmethyl)amino)-3-methyl-1H-indazole-1-carboxylate ***(19).*** Starting with compound **16** following the general procedure **4.2**, compound **19** was obtained as opaque solid, yield 77%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, J = 8.79 Hz, 1H), 6.87 (dd, J = 2.40, 8.97 Hz, 1H), 6.65 (d, J = 2.19 Hz, 1H), 3.73 (br, NH), 3.08 (d, J = 7.32 Hz, 2H), 2.52 (s, 3H), 2.24-2.12 (m, 1H), 1.90-1.80 (m, 2H), 1.70 (s, 9H), 1.67-1.60 (m, 4H), 1.33-1.23 (m, 2H)

4.2.1.19. tert-Butyl 5-(4-fluorobenzylamino)-3-methyl-1H-indazole-1-carboxylate ***(20).*** Starting with compound **09** following the general procedure **03**, compound **20** was obtained as red crude solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 8.43 Hz, 1H), 7.39-7.32 (m, 2H), 7.08-7.02 (m, 2H), 6.91-6.87 (dd, J = 8.76, 2.19 Hz, 1H), 6.68 (d, J = 2.04 Hz, 1H), 4.35 (s, 2H), 4.08 (s, 1H), 2.49 (s, 3H), 1.70 (s, 9H).

4.2.1.20. N-Cyclohexyl-1,3-dimethyl-1H-indazol-5-amine ***(21).*** Starting with compound **10** following the general procedure **03**, compound **21** was obtained as red solid, yield 66%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (d, J = 8.79 Hz, 1H), 6.81 (dd, J = 2.19, 8.97 Hz, 1H), 6.69 (d, J = 1.83 Hz, 1H), 3.92 (s, 3H), 3.31-3.25 (m, 1H), 2.49 (s, 3H), 2.11-2.08 (m, 2H), 1.80-1.76 (m, 2H), 1.65-1.56 (m, 3H), 1.46-1.34 (m, 2H), 1.29-1.15 (m, 3H).

4.2.1.21. N-(Cyclopentylmethyl)-2,3-dimethyl-2H-indazol-5-amine ***(22).*** Starting with compound **17** following the general procedure **4.2**, compound **22** was obtained as opaque solid, yield 75%.

4.2.1.22. N-(4-Fluorobenzyl)-1,3-dimethyl-1H-indazol-5-amine ***(23).*** Starting with compound **10** following the general procedure **03**, compound **23** was obtained as pink solid, yield 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (dd, J = 8.43, 5.49 Hz, 2H), 7.14 (d, J = 8.79 Hz, 1H), 7.04-6.99 (m, 2H), 6.82 (dd, J = 8.97, 2.19 Hz, 1H), 6.68 (d, J = 2.01 Hz, 1H), 4.31 (s, 2H), 3.92 (s, 3H), 2.45 (s, 3H).

4.2.1.23. 1,3-Dimethyl-N-(pyridin-3-ylmethyl)-1H-indazol-5-amine ***(24).*** Starting with compound **10** following the general procedure **03**, compound **24** was obtained as pink solid, yield 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.48 (s, 1H), 7.68 (d, J = 7.89 Hz, 1H), 7.23-7.19 (m, 1H), 7.11 (d, J = 8.79 Hz, 1H), 6.78 (d, J = 8.97 Hz, 1H), 6.62 (s, 1H), 4.34 (s, 2H), 3.87 (s, 3H), 2.39 (s, 3H).

4.2.1.24. N-Cyclobutyl-1,3-dimethyl-1H-indazol-5-amine ***(25).*** Starting with compound **10** following the general procedure **03**, compound **25** was obtained as opaque solid, yield 57%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, J = 8.85 Hz, 1H), 6.76 (d, J = 8.80 Hz, 1H), 6.59 (d, J = 1.55 Hz, 1H), 3.97-3.93 (m, 1H), 3.90 (s, 3H), 2.47 (s, 3H), 2.45-2.39 (m, 2H), 1.85-1.76 (m, 4H).

4.2.1.25. N-Cyclopentyl-1,3-dimethyl-1H-indazol-5-amine ***(26).*** Starting with compound **10** following the general procedure **03**, compound **26** was obtained as pale red solid, yield 77%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (d, J = 8.80 Hz, 1H), 6.78 (dd, J = 1.55, 8.85 Hz, 1H), 6.66 (s, 1H), 3.90 (s, 3H), 3.84-3.79 (m, 1H), 3.48 (s, 3H), 2.07-2.01 (m, 2H), 1.73-1.58 (m, 4H), 1.52-1.47 (m, 2H).

4.2.1.26. N-Cyclohexyl-2,3-dimethyl-2H-indazol-5-amine ***(27).*** Starting with compound **13** following the general procedure **03**, compound **27** was obtained as red solid, yield 35%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, J = 9.10 Hz, 1H), 6.71 (d, J = 8.75 Hz, 1H), 6.44 (s, 1H), 4.00 (s, 3H), 3.28-3.22 (m, 1H), 2.49 (s, 3H), 2.10-2.07 (m, 2H), 1.77-1.74 (m, 2H), 1.66-1.63 (m, 1H), 1.41-1.24 (m, 2H), 1.21-1.14 (m, 3H).

4.2.1.27. N-(4-Fluorobenzyl)-2,3-dimethyl-2H-indazol-5-amine ***(28).*** Starting with compound **13** following the general procedure **03**, compound **28** was obtained as red solid, yield 50%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, J = 8.97 Hz, 1H), 7.31 (dd, J = 8.61, 5.67 Hz, 1H), 6.96-6.94 (m, 2H), 6.71 (dd, J = 9.15, 2.19 Hz, 1H), 6.35 (d, J = 2.01 Hz, 1H), 4.24 (s, 2H), 3.96 (s, 3H), 2.42 (s, 3H).

4.2.1.28. 2,3-Dimethyl-N-(pyridin-3-ylmethyl)-2H-indazol-5-amine ***(29).*** Starting with compound **13** following the general procedure **03**, compound **29** was obtained as dark solid, yield 29%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (s, 1H), 8.47 (s, 1H), 8.01 (d, J = 8.43 Hz, 1H), 7.58-7.49 (m, 1H), 7.32 (d, J = 7.26 Hz, 1H), 6.74 (d, J = 9.18, 2.40 Hz, 1H), 6.14 (d, J = 2.01 Hz, 1H), 4.41 (s, 2H), 3.88 (s, 3H), 2.35 (s, 3H).

4.2.1.29. 5-Nitro-1-propyl-1H-indazole ***(31).*** Starting with compound **30** following the general procedure **11**, compound **31** was obtained as yellow solid, yield 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J = 1.96 Hz, 1H), 8.26 (dd, J = 2.08, 9.20 Hz, 1H), 8.18 (s, 1H), 7.45 (d, J = 9.24 Hz, 1H), 4.39 (t, J = 7.00 Hz, 2H), 2.01-.91 (p, m, 2H), 0.93 (t, J = 7.48 Hz, 3H).

4.2.1.30. 1-Isobutyl-5-nitro-1H-indazole ***(32).*** Starting with compound **30** following the general procedure **11**, compound **32** was obtained as yellow solid, yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (d, J = 2.01 Hz, 1H), 8.24 (dd, J = 8.52, 2.01 Hz, 1H), 8.19 (d, J = 0.72 Hz, 1H), 7.43 (d, J = 9.15 Hz, 1H), 4.20 (d, J = 7.32 Hz, 2H), 2.34 (hept, J = 6.78 Hz, 1H), 0.92 (d, J = 6.60 Hz, 6H).

4.2.1.31. 1-Propyl-1H-indazol-5-amine ***(33).*** Starting with compound **31** following the general procedure **5.2**, compound **33** was obtained as red solid, yield 88%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 0.75 Hz, 1H), 7.22 (d, J = 8.79 Hz, 1H), 6.91 (dd, J = 0.75, 2.01 Hz, 1H), 6.85 (dd, J = 2.19, 8.79 Hz, 1H), 4.27 (t, J = 6.93 Hz, 2H), 1.96-.84 (m, 2H), 0.91 (t, J = 7.32 Hz, 3H).

4.2.1.32. 1-Isobutyl-1H-indazol-5-amine ***(34).*** Starting with compound **32** following the general procedure **5.2**, compound **34** was obtained as red solid, yield 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 0.72 Hz, 1H), 7.20 (d, J = 8.79 Hz, 1H), 6.91 (d, J = 1.47 Hz, 1H), 6.83 (dd, J = 8.79, 2.19 Hz, 1H), 4.08 (d, J = 7.35 Hz, 2H), 3.57 (br, 2H), 2.29 (hept, J = 6.96 Hz, 1H), 0.88 (d, J = 6.78 Hz, 6H).

4.2.1.33. N-Cyclohexyl-1-propyl-1H-indazol-5-amine ***(35).*** Starting with compound **33** following the general procedure **3**, compound **35** was obtained as red solid, yield 59%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 0.72 Hz, 1H), 7.20 (d, J = 9.51 Hz, 1H), 6.78-6.75 (m, 2H), 4.26 (t, J = 7.14 Hz, 2H), 3.37 (br, NH), 3.27-

3.20 (m, 1H), 2.10-2.02 (m, 2H), 1.95 (p, *J* = 7.32 Hz, 2H), 1.78-1.73 (m, 2H), 1.67-1.62 (m, 1H), 1.43-1.31 (m, 2H), 1.27-1.09 (m, 3H), 0.91 (t, *J* = 7.32 Hz, 3H).

4.2.1.34. N-(4-Fluorobenzyl)-1-propyl-1H-indazol-5-amine ***(36).*** Starting with compound **33** following the general procedure **3**, compound **36** was obtained as red solid, yield: 84%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 0.93 Hz, 1H), 7.40-7.35 (m, 2H), 7.23 (s, 1H), 7.07-7.00 (m, 2H), 6.84 (dd, *J* = 8.79, 2.19 Hz, 1H), 6.76 (d, *J* = 1.65 Hz, 1H), 4.32 (s, 2H), 4.27 (t, *J* = 7.14 Hz, 2H), 1.92 (hex, *J* = 6.96 Hz, 2H), 0.91 (t, *J* = 7.32 Hz, 3H).

4.2.1.35. N-Cyclohexyl-1-isobutyl-1H-indazol-5-amine ***(37).*** Starting with compound **34** following the general procedure **3**, compound **37** was obtained as red solid, yield 87%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, *J* = 0.72 Hz, 1H), 7.13 (d, *J* = 8.61 Hz, 1H), 6.76 (s, 1H), 6.75 (d, *J* = 8.97 Hz, 1H), 4.01 (d, *J* = 7.32 Hz, 2H), 3.21-3.14 (m, 1H), 2.23 (hept, *J* = 6.78 Hz, 1H), 2.02 (d, *J* = 12.45 Hz, 2H), 1.71-1.56 (m, 3H), 1.36-1.06 (m, 5H), 0.83 (d, *J* = 6.60 Hz, 6H).

4.2.1.36. N-(4-Fluorobenzyl)-1-isobutyl-1H-indazol-5-amine ***(38).*** Starting with compound **34** following the general procedure **3**, compound **38** was obtained as red solid, yield 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 0.93 Hz, 1H), 7.37-7.33 (m, 2H), 7.21 (d, *J* = 8.97 Hz, 1H), 7.06-6.99 (m, 2H), 6.81 (dd, *J* = 8.79, 2.22 Hz, 1H), 6.74 (d, *J* = 1.65 Hz, 1H), 4.30 (s, 2H), 4.08 (d, *J* = 7.32 Hz, 2H), 3.92 (br, 1H), 2.29 (hept, *J* = 6.78 Hz, 1H), 0.69 (d, *J* = 6.78 Hz, 6H).

4.2.1.37. tert-Butyl 3-methyl-6-nitro-1H-indazole-1-carboxylate ***(40).*** Starting with compound **39** following the general procedure **9.1**, compound **40** was obtained as yellow solid, yield 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (d, *J* = 1.62 Hz, 1H), 8.18 (dd, *J* = 8.61, 2.01 Hz, 1H), 7.78 (dd, *J* = 8.79, 0.72 Hz, 1H), 2.66 (s, 3H), 1.76 (s, 9H).

4.2.1.38. 1,3-Dimethyl-6-nitro-1H-indazole ***(41).*** Starting with compound **39** following the general procedure **11**, compound **41** was obtained as yellow solid, yield 78%.

4.2.1.39. 3-Methyl-1-(2-(4-methylpiperazin-1-yl)ethyl)-6-nitro-1H-indazole ***(42).*** Starting with compound **39** following the general procedure **11**, compound **42**

was obtained as yellow solid, yield 54%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (d, J = 1.47 Hz, 1H), 7.96 (dd, J = 8.79, 1.83 Hz, 1H), 7.72 (d, J = 8.79 Hz, 1H), 4.50 (t, J = 6.57 Hz, 2H), 2.85 (t, J = 6.42 Hz, 2H), 2.61 (s, 3H), 2.56 (br, 4H), 2.41 (br, 4H), 2.67 (s, 3H).

*4.2.1.40. tert-Butyl (4-(4-(3-methyl-6-nitro-1H-indazol-1-yl)butyl)pyridin-2-yl)carbamate **(43)*.** Starting with compound **39** following the general procedure **11**, compound **42** was obtained as yellow solid, yield 76%.

*4.2.1.41. tert-Butyl (4-(4-(3-methyl-6-nitro-1H-indazol-1-yl)butyl)pyridin-2-yl)carbamate **(43)*.** Starting with compound **39** following the general procedure **11**, compound **43** was obtained as yellow solid, yield 57%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.25 (d, J = 1.50 Hz, 1H), 8.11 (d, J = 5.10 Hz, 1H), 7.93 (dd, J = 8.80, 1.80 Hz, 1H), 7.75 (s, 1H), 7.70 (d, J = 8.80 Hz, 1H), 6.71 (d, J = 5.10 Hz, 1H), 4.37 (t, J = 7.05 Hz, 2H), 2.61 (t, J = 7.90 Hz, 2H), 2.58 (s, 3H), 1.95 (quintet, J = 7.50 Hz, 2H), 1.65 (quintet, J = 7.50 hz, 2H), 1.50 (s, 9H).

*4.2.1.42. 2,3-Dimethyl-6-nitro-2H-indazole **(44)*.** Starting with compound **39** following the general procedure **11**, compound **43** was obtained as yellow solid, yield 59%.

*4.2.1.43. tert-Butyl 6-amino-3-methyl-1H-indazole-1-carboxylate **(45)*.** Starting with compound **40** following the general procedure **5.2**, compound **45** was obtained as red solid, yield 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 8.43 Hz, 1H), 7.37 (s, 1H), 6.65 (dd, J = 8.43, 2.01 Hz, 1H), 3.99 (s, 2H), 2.50 (s, 3H), 1.70 (s, 9H).

*4.2.1.44. 1,3-Dimethyl-1H-indazol-6-amine **(46)*.** Starting with compound **41** following the general procedure **5.2**, compound **46** was obtained as red solid, yield 93%.

*4.2.1.45. 3-Methyl-1-(2-(4-methylpiperazin-1-yl)ethyl)-1H-indazol-6-amine **(47)*.** Starting with compound **42** following the general procedure **5.2**, compound **47** was obtained as red solid, yield 83%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 9.15 Hz, 1H), 6.54-6.51 (m, 2H), 4.03 (t, J = 7.32 Hz, 2H), 3.84 (br, 2H), 2.81 (t, J = 7.68 Hz, 2H), 2.58 (br, 4h), 2.47 (br, 7H), 2.82 (s, 3H).

4.2.1.46. tert-Butyl (4-(4-(6-amino-3-methyl-1H-indazol-1-yl)butyl)pyridin-2-yl)carbamate ***(48).*** Starting with compound **43** following the general procedure **5.2**, compound **48** was obtained as red solid, yield 78%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.11 (d, J = 4.95 Hz, 1H), 7.36 (d, J = 8.43 Hz, 1H), 7.76 (s, 1H), 6.72 (d, J = 5.13 Hz, 1H), 6.51 (dd, J = 8.61, 1.83 Hz, 1H), 6.42 (d, J = 1.80 Hz, 1H), 4.17 (t, J = 6.96 Hz, 2H), 3.85 (br, 2H), 2.64 (t, J = 7.50 Hz, 2H), 2.47 (s, 3H), 1.89 (quintet, J = 7.32 Hz, 2H), 1.70-1.60 (m, 4H), 1.43 (s, 9H).

4.2.1.47. 2,3-Dimethyl-2H-indazol-6-amine ***(49).*** Starting with compound **44** following the general procedure **5.2**, compound **49** was obtained as red solid, yield 89%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (dd, J = 8.79, 0.75 Hz, 1H), 6.72 (dd, J = 1.83, 0.57 Hz, 1H), 6.53 (dd, J = 8.79, 2.01 Hz, 1H), 3.99 (s, 3H), 3.72 (br, 2H), 2.52 (s, 3H).

4.2.1.48. tert-Butyl 3-methyl-6-((2-nitrophenyl)sulfonamido)-1H-indazole-1-carboxylate ***(50).*** Starting with compound **45** following the general procedure **4.1**, compound **50** was obtained as pale semi solid, yield 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, J = 1.47 Hz, 1H), 7.88-7.85 (m, 2H), 7.71-7.51 (m, 3H), 7.46 (s, 1H), 7.20 (dd, J = 1.83, 8.61 Hz, 1H), 2.53 (s, 3H), 1.69 (m, 9H).

4.2.1.49. N-(1,3-Dimethyl-1H-indazol-6-yl)-2-nitrobenzenesulfonamide ***(51).*** Starting with compound **46** following the general procedure **4.1**, compound **51** was obtained as pale red solid, yield 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88-7.79 (m, 2H), 7.70-7.64 (m, 1H), 7.56-7.45 (m, 2H), 7.38-7.30 (m, 1H), 7.28 (d, J = 1.11 Hz, 1H), 6.84 (d, J = 1.83 Hz, 1H), 8.43 Hz, 1H), 3.95 (s, 3H), 2.49 (s, 3H)

4.2.1.50. tert-Butyl 6-((N-(cyclopentylmethyl)-2-nitrophenyl)sulfonamido)-3-methyl-1H-indazole-1-carboxylate ***(52).*** Starting with compound **50** following the general procedure **08**, compound **52** was obtained as yellow solid, yield 63%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.62-7.55 (m, 3H), 7.48-7.37 (m, 2H), 7.22 (dd, J = 1.83, 8.43 Hz, 1H), 3.80 (d, J = 7.68 Hz, 2H), 2.57 (s, 3H), 1.94-1.89 (m, 1H), 1.75-1.70 (m, 2H), 1.66 (s, 9H), 1.59-1.49 (m, 4H), 1.36-1.30 (m, 2H).

4.2.1.51. N-(Cyclopentylmethyl)-N-(1,3-dimethyl-1H-indazol-6-yl)-2-nitrobenzenesulfonamide ***(53).*** Starting with compound **51** following the general procedure **08**, compound **53** was obtained as yellow solid, yield 56%. ^{1}H NMR (300

MHz, $CDCl_3$) δ 7.61-7.59 (m, 2H), 7.51 (d, J = 8.43 Hz, 1H), 7.46-7.32 (m, 2H), 6.85 (dd, J = 1.65, 8.58 Hz, 1H), 6.36 (br, 1H), 3.96 (s, 3H), 3.79 (d, J = 7.68 Hz, 2H), 2.53 (s, 3H), 1.93-1.88 (m, 1H), 1.75-1.60 (m, 4H), 1.55 (s, 9H), 1.52-1.42 (m, 2H), 1.40-1.30 (m, 2H).

4.2.1.52. tert-Butyl 6-(cyclohexylamino)-3-methyl-1H-indazole-1-carboxylate ***(54).*** Starting with compound **45** following the general procedure **3**, compound **54** was obtained as red solid, yield 59%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J = 8.43 Hz, 1H), 7.18 (s, 1H), 6.55 (dd, J = 2.01, 8.58 Hz, 1H), 3.93 (br, NH), 3.37-3.30 (m, 1H), 2.47 (s, 3H), 2.12-2.09 (m, 2H), 1.80-1.76 (m, 2H), 1.70 (s, 9H), 1.68-1.61 (m, 1H), 1.42-1.30 (m, 2H), 1.25-1.18 (m, 3H).

4.2.1.53. tert-Butyl 6-((cyclopentylmethyl)amino)-3-methyl-1H-indazole-1-carboxylate ***(55).*** Starting with compound **52** following the general procedure **4.2**, compound **55** was obtained as pale yellow solid, yield 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 8.61 Hz, 1H), 7.22 (s, 1H), 6.59 (dd, J = 2.01, 8.61 Hz, 1H), 4.05 (s, NH), 3.12 (d, J = 5.13 Hz, 2H), 2.48 (s, 3H), 2.25-2.15 (m, 1H), 1.99-1.83 (m, 2H), 1.70 (s, 9H), 1.68-1.60 (m, 4H), 1.32-1.23 (m, 2H).

4.2.1.54. tert-Butyl 6-(4-fluorobenzylamino)-3-methyl-1H-indazole-1-carboxylate ***(56).*** Starting with compound **45** following the general procedure **3**, compound **56** was obtained as red solid, yield 75%. ^{1}H NMR (300MHz, $CDCl_3$) δ 7.38 (d, J = 8.43 Hz, 1H), 7.37-7.32 (m, 2H), 7.28 (s, 1H), 7.08-7.01 (m, 2H), 6.01 (dd, J = 8.91, 2.31 Hz, 1H), 4.38 (s, 2H), 2.49 (s, 3H), 1.66 (s, 9H).

4.2.1.55. tert-Butyl 3-methyl-6-(pyridin-3-ylmethylamino)-1H-indazole-1-carboxylate ***(57).*** Starting with compound **45** following the general procedure **3**, compound **57** was obtained as red solid, yield 61%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, J = 2.01 Hz, 1H), 8.56 (dd, J = 4.74, 1.65 Hz, 1H), 7.71 (td, J = 8.07 Hz, 1H), 7.39 (d, J = 8.43 Hz, 1H), 7.31-7.28 (m, 1H), 6.63 (dd, J = 8.43, 2.01 Hz, 1H), 4.46 (s, 2H), 4.41 (br, 1H), 2.50 (s, 3H), 1.65 (s, 9H).

4.2.1.56. tert-Butyl 3-methyl-6-((1-methylpiperidin-4-yl)amino)-1H-indazole-1-carboxylate ***(58).*** Starting with compound **45** following the general procedure **3**, compound **58** was obtained as red solid, yield 61%.

4.2.1.57. N-Cyclohexyl-1,3-dimethyl-1H-indazol-6-amine ***(59).*** Starting with compound **46** following the general procedure **3**, compound **59** was obtained as red solid, yield 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 8.60 Hz, 1H), 6.41 (dd, J = 1.84, 8.60 Hz, 1H), 6.23 (d, J = 1.60 Hz, 1H), 3.84 (s, 3H), 3.35-3.28 (m, 1H), 2.43 (s, 3H), 2.11-2.07 (m, 2H), 1.79-1.74 (m, 2H), 1.68-1.63 (m, 1H), 1.45-1.34 (m, 2H), 1.28-1.13 (m, 3H).

4.2.1.58. N-(Cyclopentylmethyl)-1,3-dimethyl-1H-indazol-6-amine ***(60).*** Starting with compound **53** following the general procedure **4.2**, compound **60** was obtained as pale yellow solid, yield 86%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (d, J = 8.61 Hz, 1H), 6.47 (dd, J = 1.83, 8.61 Hz, 1H), 6.25 (d, J = 1.65 Hz, 1H), 3.87 (s, 3H), 3.10 (d, J = 7.32 Hz, 2H), 2.46 (s, 3H), 2.26-2.16 (m, 1H), 1.89-1.81 (m, 2H), 1.69-1.58 (m, 4H), 1.34-1.23 (m, 2H).

4.2.1.59. N-(4-Fluorobenzyl)-1,3-dimethyl-1H-indazol-6-amine ***(61).*** Starting with compound **46** following the general procedure **3**, compound **61** was obtained as red solid, yield 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40-7.34 (m, 3H), 7.08-7.01 (m, 2H), 6.49 (dd, J = 8.61, 1.83 Hz, 1H), 6.27 (d, J = 1.65 Hz, 1H), 4.39 (s, 2H), 4.23 (s, 1H), 3.84 (s, 3H), 2.47 (s, 3H).

4.2.1.60. 1,3-Dimethyl-N-(pyridin-3-ylmethyl)-1H-indazol-6-amine ***(62).*** Starting with compound **46** following the general procedure **3**, compound **62** was obtained as red solid, yield 88%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, J = 1.83 Hz, 1H), 8.55 (dd, J = 4.95, 1.65 Hz, 1H), 7.73 (dt-like, J = 7.68 Hz, 1H), 7.40 (d, J = 8.61 Hz, 1H), 7.29 (dd, J = 8.43, 4.77 Hz, 1H), 6.51 (dd, J = 8.41, 2.04 Hz, 1H), 6.27 (d, J = 1.83 Hz, 1H), 4.44 (d, J = 4.59 Hz, 1H), 4.27 (s, 1H), 3.83 (s, 3H), 2.47 (s, 3H).

4.2.1.61. 1,3-Dimethyl-N-(1-methylpiperidin-4-yl)-1H-indazol-6-amine ***(63).*** Starting with compound **46** following the general procedure **3**, compound **63** was obtained as red solid, yield 71%.

4.2.1.62. N-Cyclobutyl-1,3-dimethyl-1H-indazol-6-amine ***(64).*** Starting with compound **46** following the general procedure **3**, compound **64** was obtained as red solid, yield 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 8.61 Hz, 1H), 6.41 (dd,

J = 8.61, 1.65 Hz, 1H), 6.18 (d, *J* = 1.65 Hz, 1H), 4.02-3.95 (m, 1H), 3.87 (s, 3H), 2.52-2.46 (m, 2H), 2.46 (s, 3H), 1.90-1.82 (m, 2H).

4.2.1.63. N-Cyclopentyl-1,3-dimethyl-1H-indazol-6-amine ***(65).*** Starting with compound **46** following the general procedure **3**, compound **65** was obtained as red solid, yield 81%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, *J* = 8.61 Hz, 1H), 6.42 (dd, *J* = 8.58, 1.83 Hz, 1H), 6.26 (d, *J* = 1.65 Hz, 1H), 3.92-3.81 (m, 1H), 3.87 (s, 3H), 2.46 (s, 3H), 2.11-2.03 (m, 2H), 1.81-1.43 (m, 6H).

4.2.1.64. N-(4-Fluorobenzyl)-2,3-dimethyl-2H-indazol-6-amine ***(66).*** Starting with compound **49** following the general procedure **3**, compound **66** was obtained as red solid, yield: 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-34 (m, 2H), 7.32 (dd, *J* = 8.79, 0.54 Hz, 1H), 7.04-7.69 (m, 2H), 6.52 (s, 1H), 6.49 (dd, *J* = 8.61, 1.83 Hz, 1H), 4.33 (s, 2H), 4.07 (s, 1H), 3.97 (s, 3H), 2.51 (s, 3H).

4.2.1.65. 2,3-Dimethyl-N-(pyridin-3-ylmethyl)-2H-indazol-6-amine ***(67).*** Starting with compound **49** following the general procedure **3**, compound **67** was obtained as white solid, yield 87%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.51 (d, *J* = 4.77 Hz, 1H), 7.73 (d, *J* = 7.68 Hz, 1H), 7.33 (d, *J* = 8.61 Hz, 1H), 7.26-7.23 (m, 1H), 6.53-6.50 (m, 2H), 4.40 (s, 2H), 4.12 (br, 1H), 3.97 (s, 3H), 2.51 (s, 3H).

4.2.1.66. tert-Butyl 6-nitro-1H-indazole-1-carboxylate ***(69).*** Starting with compound **68** following the general procedure **9.1**, compound **69** was obtained as white solid, yield 78%.

4.2.1.67. 1-Methyl-6-nitro-1H-indazole ***(70).*** Starting with compound **68** following the general procedure **11**, compound **70** was obtained as white solid, yield 61%.

4.2.1.68. 6-Nitro-1-propyl-1H-indazole ***(71).*** Starting with compound **68** following the general procedure **11**, compound **71** was obtained as yellow solid, yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.10 (d, *J* = 0.72 Hz, 1H), 8.00 (dd, *J* = 1.88, 8.96 Hz, 1H), 7.82 (d, *J* = 9.04 Hz, 1H), 4.43 (t, *J* = 7.08 Hz, 2H), 2.03-1.94 (m, 2H), 0.95 (t, *J* = 7.44 Hz, 3H).

4.2.1.69. *tert-Butyl 6-amino-1H-indazole-1-carboxylate* ***(72).*** Starting with compound **69** following the general procedure **5.2**, compound **72** was obtained as red solid, yield 88%.

4.2.1.70. *1-Methyl-1H-indazol-6-amine* ***(73).*** Starting with compound **70** following the general procedure **5.2**, compound **73** was obtained as red solid, yield 85%.

4.2.1.71. *1-Propyl-1H-indazol-6-amine* ***(74).*** Starting with compound **71** following the general procedure **5.2**, compound **74** was obtained as red solid, yield 83%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 0.75 Hz, 1H), 7.42 (d, J = 9.18 Hz, 1H), 6.50-6.4 (m, 2H), 4.15 (t, J = 6.96 Hz, 2H), 1.90-1.78 (m, 2H), 0.87 (t, J = 7.32 Hz, 3H).

4.2.1.72. *tert-Butyl 6-(4-fluorobenzylamino)-1H-indazole-1-carboxylate* ***(75).*** Starting with compound **72** following the general procedure **3**, compound **75** was obtained as red solid, yield 84%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 0.54 Hz, 1H), 7.46 (d, J = 8.61 Hz, 1H), 7.37-7.33 (m, 3H), 7.07-7.02 (m, 2H), 6.63 (dd, J = 8.58, 2.01 Hz, 1H), 4.39 (s, 2H), 1.67 (s, 9H).

4.2.1.73. *N-(4-Fluorobenzyl)-1-methyl-1H-indazol-6-amine* ***(76).*** Starting with compound **73** following the general procedure **3**, compound **76** was obtained as red solid, yield 57%.

4.2.1.74. *N-(4-Fluorobenzyl)-1-propyl-1H-indazol-6-amine* ***(77).*** Starting with compound **74** following the general procedure **3**, compound **77** was obtained as red solid, yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, J = 0.72 Hz, 1H), 7.46 (d, J = 8.61 Hz, 1H), 7.37 (dd, J = 5.49, 8.79 Hz, 2H), 7.05 (t, J = 8.61 Hz, 2H), 6.52 (dd, J = 2.04, 8.61 Hz, 1H), 6.3 (s, 1H), 4.34 (s, 2H), 4.17 (t, J = 6.96 Hz, 2H), 1.88-1.79 (m, 2H), 0.90 (t, J = 7.32 Hz, 3H).

4.2.1.75. *N-Allyl-2-chloro-4-nitroaniline* ***(79).*** Starting with compound **78** following the general procedure **3**, compound **79** was obtained as yellow solid, yield 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, J = 2.58 Hz, 1H), 8.06 (dd, J = 2.55, 9.15 Hz, 1H), 6.60 (d, J = 8.97 Hz, 1H), 5.96-5.84 (m, 1H), 5.30-5.23 (m, 2H), 3.96-3.91 (m, 2H).

4.2.1.76. tert-Butyl allyl(2-chloro-4-nitrophenyl)carbamate ***(80).*** Starting with compound **79** following the general procedure **9.1**, compound **80** was obtained as white solid, yield 69%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (d, J = 2.35 Hz, 1H), 8.10 (dd, J = 2.40, 8.65 Hz, 1H), 7.35 (s, 1H), 5.89-5.81 (m, 1H), 5.10-5.04 (m, 2H), 4.36 (br, 1H), 4.02 (br, 1H), 1.34 (s, 9H).

4.2.1.77. tert-Butyl 3-methyl-5-nitro-1H-indole-1-carboxylate ***(81).*** Starting with compound **80** following the general procedure **6**, compound **81** was obtained as yellow solid, yield 24%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (s, Hz, 1H), 8.08 (dd, J = 2.04, 8.40 Hz, 1H), 7.31 (d, J = 8.97 Hz, 1H), 7.05 (s, 1H), 2.31 (d, J = 0.90 Hz, 3H), 1.97 (s, 9H).

4.2.1.78. 1-Methyl-5-nitro-1H-benzo[d]imidazole ***(83).*** Starting with compound **82** following the general procedure **3**, compound **83** was obtained as yellow solid, yield 35%.

4.2.1.79. 1,3-Dimethyl-5-nitro-1H-indole ***(84).*** Starting with compound **81** following the general procedure **9.2** and **11**, compound **84** was obtained as yellow solid, yield 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, J = 2.19 Hz, 1H), 8.11 (dd, J = 2.22 Hz, 1H), 7.26 (d, J = 8.40 Hz, 1H), 6.94 (s, 1H), 3.77 (s, 3H), 2.33 (d, J= 1.08 Hz, 3H).

4.2.1.80. 5-Nitro-1-propyl-1H-indole ***(85).*** Starting with compound **82** following the general procedure **11**, compound **85** was obtained as white solid, yield 73%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (d, J = 2.19 Hz, 1H), 8.13 (dd, J = 2.19, 8.97 Hz, 1H), 7.37 (d, J = 8.97 Hz, 1H), 7.25 (d, J = 3.30 Hz, 1H), 6.68 (dd, J = 0.90, 3.30 Hz, 1H), 4.16 (t, J = 7.14 Hz, 2H), 1.95-1.83 (m, 2H), 0.96 (t, J = 7.32 Hz, 3H)

4.2.1.81. 1-Methyl-1H-indol-5-amine ***(86).*** Starting with compound **83** following the general procedure **5.2**, compound **86** was obtained as red solid, yield 93%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, J = 8.43 Hz, 1H), 6.95 (dd, J = 3.12 Hz, 1H), 6.69 (dd, J = 2.19, 8.61 Hz, 1H), 6.27 (dd, J = 0.72, 2.91 Hz, 1H)

4.2.1.82. 1,3-Dimethyl-1H-indol-5-amine ***(87).*** Starting with compound **84** following the general procedure **5.2**, compound **87** was obtained as red solid, yield

91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (d, J = 8.43 Hz, 1H), 6.84 (d, J = 2.19 Hz, 1H), 6.72 (s, 1H), 6.68 (dd, J = 2.01, 8.43 Hz, 1H), 3.64 (s, 3H), 2.22 (s, 3H).

4.2.1.83. 1-Propyl-1H-indol-5-amine ***(88).*** Starting with compound **85** following the general procedure **5.2**, compound **88** was obtained as red semi solid, yield 95%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (d, J = 8.61 Hz, 1H), 7.01 (d, J = 3.12 Hz, 1H), 6.93 (dd, J = 3.60, 1.83 Hz, 1H), 6.68 (dd, J = 2.19, 8.61 Hz, 1H), 6.29 (dd, J = 0.72, 3.12 Hz, 1H), 4.03 (t, J = 7.14 Hz, 2H), 1.89-1.77 (m, 2H), 0.93 (t, J = 7.32 Hz, 3H).

4.2.1.84. N-(1-Methyl-1H-indol-5-yl)-2-nitrobenzenesulfonamide ***(89).*** Starting with compound **86** following the general procedure **4.1**, compound **89** was obtained as red solid, yield 81%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (dd, J = 1.08, 8.04 Hz, 1H), 7.64 (dd, J = 1.29, 7.71 Hz, 1H), 7.60 (td, J_d = 1.47, J_t = 7.86 Hz, 1H), 7.42 (td, J_d = 1.29, J_t = 7.68 Hz, 1H), 7.34 (d, J = 2.19 Hz, 1H), 7.15 (br, NH), 7.12 (d, J = 8.61 Hz, 1H), 9.99-6.93 (m, 2H), 6.33 (d, J = 3.12 Hz, 1H), 3.76 (s, 3H).

4.2.1.85. N-(1,3-Dimethyl-1H-indol-5-yl)-2-nitrobenzenesulfonamide ***(90).*** Starting with compound **87** following the general procedure **4.1**, compound **90** was obtained as pale solid, yield 78%. ^{1}H (300 MHz, $CDCl_3$) δ 7.85 (dd, J = 1.29, 8.07 Hz, 1H), 7.70 (td, J_d = 1.47 Hz, J_t = 7.89 Hz, 1H), 7.63 (dd, J = 1.47, 7.71 Hz, 1H), 7.48 (td, J_d = 1.26 Hz, J_t = 7.68 Hz, 1H), 7.32 (d, J = 2.01 Hz, 1H), 7.20 (s, 1H), 7.10 (d, J = 8.58 Hz, 1H), 6.96 (dd, J = 2.19, 8.61 Hz, 1H), 6.80 (s, 1H), 3.66 (s, 3H), 2.20 (s, 3H).

4.2.1.86. 2-Nitro-N-(1-propyl-1H-indol-5-yl)benzenesulfonamide ***(91).*** Starting with compound **88** following the general procedure **4.1**, compound **91** was obtained as yellow solid, yield 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (dd, J = 1.29, 7.86 Hz, 1H), 7.74 (dd, J = 1.44, 7.86 Hz, 1H), 7.67 (td, J_d = 1.47 Hz, J_t = 7.71 Hz, 1H), 7.51 (td, J_d = 1.29 Hz, J_t = 7.71 Hz, 1H), 7.40 (d, J = 2.01 Hz, 1H), 7.21 (d, J = 8.79 Hz, 1H), 7.10 (d, J = 3.12 Hz, 1H), 7.02 (dd, J = 2.01, 8.61 Hz, 1H), 6.40 (dd, J = 0.72, 3.09 Hz, 1H), 4.03 (t, J = 7.14 Hz, 2H), 1.89-1.77 (m, 2H), 0.93 (t, J = 7.32 Hz, 3H).

4.2.1.87. N-(Cyclopentylmethyl)-N-(1-methyl-1H-indol-5-yl)-2-nitrobenzenesulfonamide ***(92).*** Starting with compound **89** following the general

procedure **08**, compound **92** was obtained as yellow solid, yield 63%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56-7.51 (m, 2H), 7.44 (d, *J* = 2.04 Hz, 1H), 7.39-7.29 (m, 2H), 7.20 (d, *J* = 8.79 Hz, 1H), 7.06 (d, *J* = 3.12 Hz, 1H), 7.01 (dd, *J* = 2.01, 8.79 Hz, 1H), 6.4 (d, *J* = 2.94 Hz, 1H), 3.76 (s, 3H), 3.72 (d, *J* = 7.68 Hz, 2H), 1.94-1.84 (m, 1H), 1.68-1.57 (m, 4H), .47-1.42 (m, 2H), 1.36-1.26 (m, 2H)**.**

4.2.1.88. N-(Cyclopentylmethyl)-N-(1,3-dimethyl-1H-indol-5-yl)-2-nitrobenzenesulfonamide ***(93).*** Starting with compound **90** following the general procedure **08**, compound **93** was obtained asred solid, yield 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57-7.55 (m, 2H), 7.39-7.29 (m, 3H), 7.14 (d, *J* = 8.58 Hz, 1H), 6.97 (dd, *J* = 2.01, 8.79 Hz, 1H), 6.82 (s, 1H), 3.72 (d, *J* = 7.68 Hz, 2H), 3.69 (s, 3H), 2.20 (d, *J* = 0.90 Hz, 3H), 1.92-1.85 (m, 1H), 1.68-1.58 (m, 4H), 1.48-1.44 (m, 2H), 1.35-1.25 (m, 2H)

4.2.1.89. N-(Cyclopentylmethyl)-2-nitro-N-(1-propyl-1H-indol-5-yl)benzenesulfonamide ***(94).*** Starting with compound **91** following the general procedure **08**, compound **94** was obtained as white solid, yield 49%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51-7.47 (m, 2H), 7.37-7.24 (m, 3H), 7.16 (d, *J* = 8.61 Hz, 1H), 7.06 (d, *J* = 3.12 Hz, 1H), 6.93 (dd, *J* = 2.01, 8.61 Hz, 1H), 6.35 (d, *J* = 3.09 Hz, 1H), 4.00 (t, *J* = 6.96 Hz, 2H), 3.66 (d, *J* = 7.68 Hz, 2H), 1.86-1.73 (m, 1H), 1.63-1.53 (m, 4H), 1.43-1.39 (m, 2H), .29-1.26 (m, 2H), 1.21 (t, *J* = 7.14 Hz, 2H), 0.89 (t, *J* = 7.32 Hz, 3H).

4.2.1.90. N-Cyclohexyl-1-methyl-1H-indol-5-amine ***(95).*** Starting with compound **86** following the general procedure **3**, compound **95** was obtained as red solid, yield 41%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (d, *J* = 8.61 Hz, 1H), 6.87 (d, *J* = 2.94 Hz, 1H), 6.77 (d, *J* = 2.19 Hz, 1H), 6.59 (dd, *J* = 2.22, 8.91 Hz, 1H), 6.22 (d, *J* = 2.37 Hz, 1H), 3.64 (s, 3H), 3.23-3.14 (m, 1H), 2.05-1.97 (m, 2H), .72-.67 (m, 2H0, 1.60-1.56 (m, 1H), 1.37-1.24 (m, 2H), 1.20-1.01 (m, 3H).

4.2.1.91. N-(Cyclopentylmethyl)-1-methyl-1H-indol-5-amine ***(96).*** Starting with compound **92**following the general procedure **4.2**, compound **96** was obtained as white solid, yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, *J* = 8.72 Hz, 1H), 6.92 (d, *J* = 3.0 Hz, 1H), 6.82 (d, *J* = 2.12 Hz, 2H), 6.65 (dd, *J* = 2.12, 10.80 Hz, 1H), 6.28 (d, *J* = 2.88 Hz, 1H), 3.70 (s, 3H), 3.06 (d, *J* = 7.16 Hz, 2H), 2.21-2.14 (m, 1H), .86-.78 (m, 2H), 1.66-.49 (m, 4H), 1.31-1.22 (m, 2H).

4.2.1.92. N-(4-Fluorobenzyl)-1-methyl-1H-indol-5-amine ***(97).*** Starting with compound **86** following the general procedure **3**, compound **97** was obtained as red solid, yield 45%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (dd, J = 5.52, 8.61 Hz, 2H), 6.97 (t, J = 8.58 Hz, 2H), 6.67 (s, NH), 6.52-6.46 (m, 2H), 6.40-6.33 (m, 2H), 4.15 (s, 2H), 3.66 (s, 3H).

4.2.1.93. N-Cyclohexyl-1,3-dimethyl-1H-indol-5-amine ***(98).*** Starting with compound **87** following the general procedure **3**, compound **98** was obtained as red solid, yield 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (d, J = 8.61 Hz, 1H), 6.74 (d, J = 2.04 Hz, 1H), 6.70 (s, 1H), 6.64 (dd, J = 2.22, 8.61 Hz, 1H), 3.64 (s, 3H), 3.31-3.23 (m, 1H), 2.23 (d, J = 0.93 Hz, 3H), 2.10-2.07 (m, 2H), 1.76-1.72 (m, 2H), 1.65-1.60 (m, 1H), 1.42-1.30 (m, 2H), 1.19-1.07 (m, 3H)

4.2.1.94. N-(Cyclopentylmethyl)-1,3-dimethyl-1H-indol-5-amine ***(99).*** Starting with compound **93** following the general procedure **4.2**, compound **99** was obtained as **w**hite solid, yield 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (d, J = 8.61 Hz, 1H), 6.75 (d, J = 2.01 Hz, 1H), 6.70 (s, 1H), 6.67 (dd, J = 2.01, 8.61 Hz, 1H), 3.64 (s, 3H), 3.09 (d, J = 7.14 Hz, 2H), 2.24 (s, 3H), 2.21-2.13 (m, 1H), 1.84-1.78 (m, 2H), 1.66-1.52 (m, 4H), 1.31-1.23 (m, 2H).

4.2.1.95. N-(4-Fluorobenzyl)-1,3-dimethyl-1H-indol-5-amine ***(100).*** Starting with compound **87** following the general procedure **3**, compound **100** was obtained as red solid, yield 63%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (dd, J = 5.49, 8.61 Hz, 2H), 7.09 (d, J = 8.61 Hz, 1H), 6.99 (t, J = 8.64 Hz, 2H), 6.86 (s, 1H), 6.73 (s, 1H), 4.33 (s, 2H), 3.65 (s, 3H), 2.20 (s, 3H).

4.2.1.96. N-Cyclohexyl-1-propyl-1H-indol-5-amine ***(101)***. Starting with compound **88** following the general procedure **3**, compound **101** was obtained as red solid, yield 54%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (d, J = 8.61 Hz, 1H), 6.99 (d, J = 3.09 Hz, 1H), 6.84 (d, J = 2.19 Hz, 1H), 6.63 (dd, J = 2.40, 8.79 Hz, 1H), 6.29 (d, J = 3.12 Hz, 1H), 4.03 (t, J = 7.14 Hz, 2H), 3.28-3.21 (m, 1H), 2.69 (t, J = 4.56 Hz, 2H), 2.12-2.04 (m, 2H), 1.89-1.77 (m, 2H), 1.67-1.59 (m, 2H), 1.40-1.23 (m, 2H), 1.19-1.09 (m, 3H), 0.96-0.86 (m, 5H).

4.2.1.97. N-(Cyclopentylmethyl)-1-propyl-1H-indol-5-amine ***(102).*** Starting with compound **94** following the general procedure **4.2**, compound **102** was obtained as white solid, yield 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J = 8.58 Hz, 1H), 6.93 (d, J = 2.97 Hz, 1H), 6.76 (d, J = 2.01 Hz, 1H), 6.58 (dd, J = 2.19, 8.79 Hz, 1H), 6.23 (dd, J = 0.75, 2.94 Hz, 1H), 3.95 (t, J = 6.96 Hz, 2H), 3.00 (d, J = 7.14 Hz, 2H), 2.17-2.05 (m, 1H), 1.82-1.70 (m, 4H), 1.60-1.45 (m, 4H), 1.24-1.16 (m, 2H), 0.86 (t, J = 7.32 Hz, 3H).

4.2.1.98. N-(4-Fluorobenzyl)-1-propyl-1H-indol-5-amine ***(103).*** Starting with compound **88** following the general procedure **3**, compound **103** was obtained as red solid, yield 70%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (dd, J = 5.67, 8.61 Hz, 2H), 7.10 (d, J = 8.79 Hz, 1H), 6.97-6.92 (m, 3H), 6.76 (d, J = 2.19 Hz, 1H), 6.60 (dd, J = 2.37, 8.61 Hz, 1H), 6.22 (d, J = 2.37 Hz, 1H), 4.26 (s, 2H), 3.96 (t, J = 6.96 Hz, 2H), 1.80-1.73 (m, 2H), 0.86 (t, J = 7.32 Hz, 3H).

4.2.1.99. 2-(Hydroxymethyl)-5-nitrophenol ***(105).*** Starting with compound **104** following the general procedure **5.1**, compound **105** was obtained as yellow solid, yield 77%. ^{1}H NMR (300MHz, $CDCl_3$) δ 7.63 (dd, J = 8.43, 2.19 Hz, 1H), 7.55 (d, J = 2.40 Hz, 1H), 7.32 (d, J = 8.43 Hz, 1H), 4.69 (s, 2H).

4.2.1.100. 2-Hydroxy-4-nitrobenzaldehyde ***(106)***. Starting with compound **105** in DCM was added five times molar MnO_2. The mixture reaction was stirred overnight at room temperature. Then, the solid was filtered off, the filtrate was concentrated and purified by silica gel column to get aldehyde derivative **106** was obtained as yellow solid, yield 84%. ^{1}H NMR (300MHz, $CDCl_3$) δ 11.13 (s, 1H), 10.03 (s, 1H), 7.85-7.76 (m, 3H).

4.2.1.101. Ethyl 5-nitrobenzofuran-3-carboxylate ***(108).*** Starting with compound **106** (1.0 equiv) in DCM was added solution $HBF_4{\cdot}OEt_2$ (1.0 equiv) under nitrogen atmosphere at 0 ^{o}C, followed by adding dropwise solution of ethyl diazoacetate (1.3 equiv). Once gas evolution ceased, the reaction mixture was concentrated and added concentrated H_2SO_4 (about 0.5 mL). After 10 min stirring, the mixture was diluted with DCM, quenched with solution bicarbonate. The organic layer was collected, dried, concentrated and purified by silica gel column to afford ester benzofuran compound as borrow solid, yield: 60%. ^{1}H NMR (500 MHz, $CDCl_3$)

δ 8.95 (d, *J* = 2.50 Hz, 1H), 8.37 (s, 1H), 8.27 (dd, *J* = 9.10, 2.35 Hz, 1H), 7.62 (d, *J* = 9.10 Hz, 1H), 4.44 (q, *J* = 7.15 Hz, 2H), 1.44 (t, *J* = 7.10 Hz, 3H).

4.2.1.102. *Ethyl 6-nitrobenzofuran-3-carboxylate* ***(109)***. Starting with compound **107** following the procedure as compound **108** to get product as yellow solid, yield 48%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.44 (dd, *J* = 1.83, 0.36 Hz, 1H), 8.27 (dd, *J* = 8.61, 2.01 Hz, 1H), 8.18 (d, *J* = 8.79 Hz, 1H), 4.42 (q, *J* = 6.96 Hz, 2H), 1.42 (t, *J* = 7.14 Hz, 3H).

4.2.1.103. *3-Methylbenzofuran-5-amine* ***(110).*** Starting with compound **108** (1.0 equiv) in toluene was added dropwise solution 1M DIBAL (1.05 equiv) in hexane under nitrogen atmosphere at 0 ^{o}C. After 2h of stirring at room temperature, the mixture was cooled again to 0 ^{o}C and taken play Fisher work-up. After work up, the solution was concentrated to have alcohol compound and used for next step without purification.

A mixture of alcohol, Ph_3P, imidazole and I_2 in DCM was stirred for 1 h. Then the mixture was loaded directly in silica gel column to get designed iodine derivative.

The iodine derivative in diethylene glycol was added slowly $NaBH_4$ while stirring. Then the mixture was risen up to 40 ^{o}C for 2h. After that, the mixture was cooled to room temperature, quenched with water, extracted with DCM. The organic layer was washed with water and brine, dried and concentrated to get designed product.

These nitro derivative (1.0 equiv) in ethanol was added 1-2 drops concentrated HCl and $SnCl_2.2H_2O$ (5.0 equiv). The mixture reaction was refluxed for 2h, quenched with concentrated carbonate and extracted by EA. The organic layer was washed with water, dried with $MgSO_4$, concentrated and used for next step without purification as yellow solid, yield 48%.

4.2.1.104. *3-Methylbenzofuran-6-amine* ***(111)***. Starting with compound **109** following the procedure of compound **110** to get product as red solid, yield 42%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.07 Hz, 1H), 7.20 (q, *J* = 1.29 Hz, 1H), 6.75 (d, *J* = 1.83 Hz, 1H), 6.62 (dd, *J* = 8.04, 1.83 Hz, 1H), 3.69 (brs, 2H), 2.16 (d, *J* = 1.29 Hz, 3H).

4.2.1.105. *N-Cyclohexyl-3-methylbenzofuran-5-amine* ***(112).*** Starting with compound **110** following the general procedure **03**, compound **112** was obtained to get product as red solid, yield 42%.

4.2.1.106. N-(4-Fluorobenzyl)-3-methylbenzofuran-5-amine ***(113).*** Starting with compound **110** following the general procedure **03**, compound **113** was obtained as red solid, yield 53%.

4.2.1.107. N-(4-Fluorobenzyl)-3-methylbenzofuran-6-amine ***(114).*** Starting with compound **111** following the general procedure **03**, compound **114** was obtained as red solid, yield 40%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.31 (m, 3H), 7.19 (q, J = 1.29 Hz, 1H), 7.04-6.98 (m, 2H), 6.64 (d, J = 2.04 Hz, 1H), 6.61-6.56 (m, 1H), 4.32 (s, 2H), 4.09 (s, 1H), 2,15 (d, J = 1.29 Hz, 3H).

4.2.1.108. 5-Nitrobenzo[d]oxazolem ***(117)***, Starting with compound **115** (5 mmol) and triethyl orthoformate (15 mL) was refluxed for 4 h. After cooling to room temperature, the remaining triethyl orthoformate was removed under reduced pressure and the residue was purified by columnchromatography to yield the desired substituted benzoxazole as white solid, yield 61%. ^{1}H (300 MHz, DMSO) δ 9.01 (s, 1H), 8.68 (d, J = 2.19 Hz, 1H), 8.37 (dd, J = 2.19, 8.97 Hz, 1H), 8.04 (d, J = 8.97 Hz, 1H).

4.2.1.109. 6-Nitrobenzo[d]oxazole ***(118)***. Starting with compound **116** (5 mmol) and triethyl orthoformate (15 mL) was refluxed for 4 h. After cooling to room temperature, the remaining triethyl orthoformate was removed under reduced pressure and the residue was purified by columnchromatography to yield the desired substituted benzoxazole as white solid, yield 63%.

4.2.1.110. Benzo[d]oxazol-5-amine ***(121).*** Starting with compound **117** following the general procedure **5.2**, compound **121** was obtained to get product as red solid, yield 95%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H), 1.04 (d, J = 8.55 Hz, 1H), 7.03 (s, 1H), 6.73 (d, J = 8.50 Hz, 1H).

4.2.1.111. Benzo[d]oxazol-6-amine ***(122)***. Starting with compound **118** following the general procedure **5.2**, compound **122** was obtained to get product as red semi solid, yield 95%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.51 (d, J = 8.50 Hz, 1H), 6.83 (d, J = 2.0 Hz, 1H), 6.70 (dd, J = 2.05, 8.40 Hz, 1H), 3.81 (br, 2NH)

4.2.1.112. *Benzo[d]oxazol-5-amine* ***(123)***. Starting with compound **119** (1.0 equiv) in ethanol was added 1-2 drops concentrated HCl and $SnCl_2.2H_2O$ (5.0 equiv). The mixture reaction was refluxed for 2h, quenched with concentrated carbonate and extracted by EA. The organic layer was washed with water, dried with $MgSO_4$, concentrated to obtanined compound **123** as red solid, yield 87%.

4.2.1.113. *Benzo[d]thiazol-6-amine* ***(124).*** Starting with compound **120** (1.0 equiv) in ethanol was added 1-2 drops concentrated HCl and $SnCl_2.2H_2O$ (5.0 equiv). The mixture reaction was refluxed for 2h, quenched with concentrated carbonate and extracted by EA. The organic layer was washed with water, dried with $MgSO_4$, concentrated to obtanined compound **124** as red solid, yield 91%. ^{1}H NMR (300MHz, DMSO) δ 8.88 (s, 1H), 7.71 (d, J = 8.25 Hz, 1H), 7.12 (s, 1H), 6.81 (d, J = 8.61 Hz, 1H), 5.40 (s, 2H).

4.2.1.114. *N-(Benzo[d]oxazol-5-yl)-2-nitrobenzenesulfonamide* ***(125)***. Starting with compound **121** following the general procedure **4.1**, compound **125** was obtained as pale semi solid, yield 71%.

4.2.1.115. *N-(Benzo[d]oxazol-6-yl)-2-nitrobenzenesulfonamide* ***(126).*** Starting with compound **122** following the general procedure **4.1**, compound **126** was obtained as pale semi solid, yield 65%.

4.2.1.116. *N-(Benzo[d]thiazol-5-yl)-2-nitrobenzenesulfonamide* ***(127).*** Starting with compound **123** following the general procedure **4.1**, compound **127** was obtained as red solid, yield 43%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (s, 1H), 7.94-7.82 (m, 4H), 7.68 (td, J = 7.68, 1.44 Hz, 1H), 7.57-7.51 (m, 2H), 7.42-7.38 (dd, J = 8.61, 2.19 Hz, 1H),

4.2.1.117. *N-(Benzo[d]thiazol-6-yl)-2-nitrobenzenesulfonamide* ***(128).*** Starting with compound **123** following the general procedure **4.1**, compound **127** was obtained as red crude solid. ^{1}H NMR (300 MHz, DMSO) δ 10.96 (br, 1H), 9.29 (s, 1H), 8.30-8.26 (m, 1H), 8.14-7.76 (m, 5H), 7.29 (dd, J = 8.97. 2.19 Hz, 1H).

4.2.1.118. *N-(Benzo[d]oxazol-5-yl)-N-(cyclopentylmethyl)-2-nitrobenzenesulfonamide* ***(129).*** Starting with compound **125** following the general procedure **8**, compound **129** was obtained as white solid, yield 75%.

4.2.1.119. N-(Benzo[d]oxazol-6-yl)-N-(cyclopentylmethyl)-2-nitrobenzenesulfonamide ***(130).*** Starting with compound **126** following the general procedure **8**, compound **130** was obtained as white solid, yield 65%.

4.2.1.120. N-(Benzo[d]thiazol-5-yl)-N-(cyclopentylmethyl)-2-nitrobenzenesulfonamide ***(131).*** Starting with compound **127** following the general procedure **8**, compound **131** was obtained as red solid, yield 89%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (s, 1H), 7.94 (d, J = 2.37, 1H), 9.13 (d, J = 8.79 Hz, 1H), 7.65-7.58 (m, 2H), 7.51-7.39 (m, 3H), 3.79 (d, J = 7.68 Hz, 2H), 1.90 (quintet, J = 7.35 Hz, 1H), 1.66-1.58 (m, 4H), 1.52-1.49 (m, 2H), 1.34-1.23 (m, 2H).

4.2.1.121. N-(Benzo[d]thiazol-6-yl)-N-(cyclopentylmethyl)-2-nitrobenzenesulfonamide ***(132).*** Starting with compound **128** following the general procedure **8**, compound **132** was obtained as red solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.04 (d, J = 8.64 Hz, 1H), 7.95 (d, J = 2.01 Hz, 1H), 7.66-7.59 (m, 2H), 7.46-7.38 (m, 2H), 7.30 (dd, J = 8.49, 2.01 Hz, 1H), 3.79 (d, J = 7.68 Hz, 2H), 1.96-1.86 (m, 1H), 1.76-1.65 (m, 4H), 1.52-1.47 (m, 2H), 1.38-1.25 (m, 2H).

4.2.1.122. N-Cyclohexylbenzo[d]oxazol-5-amine ***(133).*** Starting with compound **121** following the general procedure **3**, compound **133** was obtained as red solid, yield 43%. ^{1}H (300 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.27 (d, J = 8.79 Hz, 1H), 6.87 (d, J = 2.37 Hz, 1H), 6.59 (dd, J = 2.19, 8.61 Hz, 1H), 3.50 (br, NH), 3.23-3.16 (m, 1H), 2.04-2.00 (m, 2H), 1.73-1.58 (m, 3H), 1.38-1.26 (m, 2H), 1.18-1.02 (m, 3H).

4.2.1.123. N-(Fyclopentylmethyl)benzo[d]oxazol-5-amine ***(134).*** Starting with compound **129** following the general procedure **4.2**, compound **134** was obtained as white solid, yield 43%.

4.2.1.124. N-(4-Fluorobenzyl)benzo[d]oxazol-5-amine ***(135).*** Starting with compound **121** following the general procedure **3**, compound **135** was obtained as red solid, yield 56%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.36-7.31 (m, 3H), 7.04 (tt, J = 2.01, 8.79 Hz, 2H), 6.92 (d, J = 2.40 Hz, 1H), 6.70 (dd, J = 2.40, 8.79 Hz, 1H), 4.31 (s, 2H).

4.2.1.125. N-Cyclohexylbenzo[d]oxazol-6-amine ***(136)***. Starting with compound **122** following the general procedure **3**, compound **136** was obtained as

red solid, yield 56%. ^{1}H (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.48 (d, J = 8.61 Hz, 1H), 6.70 (d, J = 2.19 Hz, 1H), 6.59 (dd, J = 2.19, 8.58 Hz, 1H), 3.28-3.17 (m, 1H), 2.08-2.00 (m, 2H), 1.79-.63 (m, 3H), 1.43-1.31 (m, 2H), 1.27-1.09 (m, 3H).

4.2.1.126. N-(Cyclopentylmethyl)benzo[d]oxazol-6-amine ***(137).*** Starting with compound **130** following the general procedure **4.2**, compound **137** was obtained as white solid, yield 43%.

4.2.1.127. N-(4-Fluorobenzyl)benzo[d]oxazol-6-amine ***(138)***, Starting with compound **122** following the general procedure **3**, compound **138** was obtained as pale solid, yield 56%. ^{1}H (300 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.52 (d, J = 8.61 Hz, 1H), 7.35 (dd, J = 5.49, 8.61 Hz, 2H), 7.05 (tt, J = 2.01, 8.61 Hz, 2H), 6.71-6.6 (m, 2H), 4.23 (s, 2H).

4.2.1.128. N-Cyclohexylbenzo[d]thiazol-5-amine ***(139).*** Starting with compound **123** following the general procedure **3**, compound **139** was obtained as red solid, yield: 91%. ^{1}H NMR (300MHz, $CDCl_3$) δ 8.88 (s, 1H), 7.64 (d, J = 8.76 Hz, 1H), 7.29 (d, J = 2.40 Hz, 1H), 6.76 (dd, J = 8.58, 2.19 Hz, 1H), 3.38-3.29 (m, 1H), 2.15-2.10 (m, 2H), 1.81-1.62 (m, 4H), 1.48-1.12 (m, 4H).

4.2.1.129. N-(Cyclopentylmethyl)benzo[d]thiazol-5-amine ***(140).*** Starting with compound **131** following the general procedure **4.2**, compound **140** was obtained as red solid, yield 47%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.89 (s, 1H), 7.66 (d, J = 8.58 Hz, 1H), 7.29 (d, J = 2.37 Hz, 1H), 6.79 (dd, J = 8.61, 2.40 Hz, 1H), 3.87 (s, 1H), 3.11 (d, J = 7.14 Hz, 2H), 2.28-2.15 (m, 1H), 1.89-1.80 (m, 2H), 1.69-1.54 (m, 4H), 1.33-1.25 (m, 2H).

4.2.1.130. N-(4-Fluorobenzyl)benzo[d]thiazol-5-amine ***(141).*** Starting with compound **123** following the general procedure **3**, compound **141** was obtained as red solid, yield 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.89 (s, 1H), 7.68 (d, J = 8.61 Hz, 1H), 7.39-7.35 (m, 2H), 7.29 (d, J = 2.19 Hz, 1H), 7.07-7.00 (m, 2H), 6.82 (dd, J = 8.61, 2.37 Hz, 1H), 4.39 (s, 2H), 4.25 (s, 1H).

4.2.1.131. N-Cyclohexylbenzo[d]thiazol-6-amine ***(142).*** Starting with compound **124** following the general procedure **3**, compound **142** was obtained as red solid, yield 18%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.85 (d, J = 8.97

Hz, 1H), 7.02 (d, *J* = 2.22 Hz, 1H), 6.76 (dd, *J* = 8.79, 2.4 Hz, 1H), 3.35-3.26 (m, 1H), 2.11-2.07 (m, 2H), 1.80-1.13 (m, 8H).

4.2.1.132. N-(Cyclopentylmethyl)benzo[d]thiazol-6-amine ***(143).*** Starting with compound **132** following the general procedure **4.2**, compound **143** was obtained as red solid, yield 70%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.84 (d, *J* = 8.79 Hz, 1H), 7.00 (d, *J* = 2.37 Hz, 1H), 6.77 (dd, *J* = 8.76, 2.37 Hz, 1H), 3.88 (s, 1H), 3.06 (d, *J* = 7.14 Hz, 2H), 2.18 (hept, *J* = 7.50 Hz, 1H), 1.88-1.78 (m, 2H), 1.67-1.51 (m, 4H), 1.30-1.22 (m, 2H).

4.2.1.133. N-(4-Fluorobenzyl)benzo[d]thiazol-6-amine ***(144).*** Starting with compound **124** following the general procedure **3**, compound **144** was obtained as red solid, yield 27%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.89 (d, *J* = 8.79 Hz, 1H), 7.38-7.32 (m, 2H), 7.08-7.02 (m, 3H), 6.84 (dd, *J* = 8.79, 2.40 Hz, 1H), 4.37 (s, 2H), 4.28 (s, 1H).

4.2.1.134. tert-Butyl 3-methyl-5-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-1H-indazole-1-carboxylate ***(145)***. Starting with compound **09** following the general procedure **10**, compound **145** was obtained as white solid, yield 50%. ^{1}H (300 MHz, $CDCl_3$) δ 7.98-7.90 (m, 3H), 7.45 (s, 1H), 7.22 (dd, *J* = 1.83, 8.97 Hz, 1H), 6.79 (s, 1H), 5.84 (t, *J* = 6.03 Hz, NH), 4.00 (t, *J* = 6.75 Hz, 2H), 3.26 (q, *J* = 6.24 Hz, 2H), 2.53 (s, 3H), 2.22 (s, 3H), 2.03 (p, *J* = 6.42 Hz, 2H), 1.70 (s, 9H).

4.2.1.135. tert-Butyl 5-(1-cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-3-methyl-1H-indazole-1-carboxylate ***(146).*** Starting with compound **18** following the general procedure **10**, compound **146** was obtained as white semi solid, yield 56%. ^{1}H (500 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.65 Hz, 1H), 7.37 (s, 1H), 7.30 (s, 1H), 7.23 (s, 1H), 6.68 (s, 1H), 4.48-4.43 (m, 1H), 3.83 (t, *J* = 5.55 Hz, NH), 3.79 (t, *J* = 7.10 Hz, 2H), 3.14 (q, *J* = 6.40 Hz, 2H), 2.59 (s, 3H), 2.10 (s, 3H), 1.89-1.87 (m, 2H), 1.87 (p, *J* = 6.80 Hz, 2H), 1.70 (s, 9H), 1.57-1.53 (m, 3H), 1.42-1.34 (m, 2H), 1.00-0.93 (m, 2H), 0.85-0.82 (m, 1H).

4.2.1.136. tert-Butyl 5-(1-(cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-3-methyl-1H-indazole-1-carboxylate ***(147).*** Starting with compound **19** following the general procedure **10**, compound **147** was obtained as

white semid solid, yield 55%. ^{1}H (500 MHz, $CDCl_3$) δ 8.16 (d, J = 8.65 Hz, 1H), 7.46 (d, J = 1.45 Hz, 1H), 7.33-7.31 (m, 2H), 6.68 (s, 1H), 4.10 (t, J = 5.55 Hz, NH), 3.80 (t, J = 7.15 Hz, 2H), 3.67 (d, J = 7.70 Hz, 2H), 3.17 (q, J = 6.50 Hz, 2H), 2.58 (s, 3H), 2.11 (s, 3H), 1.97-1.94 (m, 1H), 1.85 (p, J = 6.90 Hz, 2H), 1.70 (s, 9H), 1.63-1.59 (m, 4H), 1.48-1.46 (m, 2H), 1.27-1.22 (m, 4H).

4.2.1.137. tert-Butyl 5-(1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-3-methyl-1H-indazole-1-carboxylate ***(148).*** Starting with compound **20** following the general procedure **10**, compound **148** was obtained as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 8.79 Hz, 1H), 7.35 (s, 1H), 7.20-7.15 (m, 2H), 7.12 (dd, J = 8.79, 1.83 Hz, 1H), 6.95 (t, J = 8.61 Hz, 2H), 6.57 (s, 1H), 4.85 (s, 2H), 4.34 (t, J = 5.85 Hz, 1H), 3.83 (t, J = 7.50 Hz, 2H), 3.22 (q, J = 6.39 Hz, 2H), 2.50 (s, 3H), 2.13 (s, 3H), 1.83 (quintet, J = 7.14 Hz, 2H), 1.71 (s, 9H).

4.2.1.138. tert-Butyl (4-(4-(3-methyl-5-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-1H-indazol-1-yl)butyl)pyridin-2-yl)carbamate ***(149).*** Starting with compound **12** following the general procedure **10**, compound **149** was obtained as white solid, yield 56%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J = 5.13 Hz, 1H), 7.72 (s, 1H), 7.65 (s, 1H), 7.44 (s, 1H), 7.24-7.15 (m, 3H), 7.12 (s, 1H), 6.95 (d, J = 5.13 Hz, 1H), 6.92 (s, 1H), 6.76 (s, 1H), 5.32 (t, J = 6.96 Hz, 1H), 4.29 (t, J = 6.96 Hz, 2H), 3.93 (t, J = 6.96 Hz, 2H), 3.26 (q, J = 6.42 Hz, 2H), 2.60 (t, J = 7.50 Hz, 2H), 2.52 (s, 3H), 2.19 (s, 3H), 1.99-1.89 (m, 4H), 1.66-1.61 (m, 2H), 1.40 (s, 1H).

4.2.1.139. tert-Butyl 3-methyl-6-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-1H-indazole-1-carboxylate ***(150).*** Starting with compound **45** following the general procedure **10**, compound **150** was obtained as white solid, yield 66%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (s, 1H), 8.08 (s, 1h), 7.48-7.45 (M, 2h), 7.31-7.28 (m, 1H), 6.79 (s, 1H), 5.98 (t, J = 4.95 Hz, NH), 4.00 (t, J = 6.96 Hz, 2H), 3.27 (q, J = 5.31 Hz, 2H), 2.52 (s, 3H), 2.21 (s, 3H), 2.02 (p, J = 6.24 Hz, 2H), 1.67 (s, 9H).

4.2.1.140. tert-Butyl 6-(1-cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-3-methyl-1H-indazole-1-carboxylate ***(151).*** Starting with compound **54** following the general procedure **10**, compound **151** was obtained as white solid, yield 60%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.70 (d, J = 8.61 Hz, 1H), 7.31 (s, 1H), 7.08 (dd, J = 1.62, 8.22 Hz, 1H), 6.69 (s, 1H), 4.52-4.44 (m,

1H), 3.91 (t, *J* = 6.21 Hz, NH), 3.82 (t, *J* = 7.14 Hz, 2H), 3.18 (q, *J* = 6.78 Hz, 2H), 2.61 (s, 3H), 2.11 (s, 3H), 1.92-1.88 (m, 2H), 1.83 (p, *J* = 6.96 Hz, 2H), 1.75-1.72 (m, 2H), 1.71 (s, 9H), 1.68-1.60 (m, 1H), 1.48-1.34 (m, 2H), 1.11-1.00 (m, 2H), 0.90-0.82 (m, 1H).

4.2.1.141. tert-Butyl 6-(1-(cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-3-methyl-1H-indazole-1-carboxylate ***(152).*** Starting with compound **55** following the general procedure **10**, compound **152** was obtained as white semi solid, yield 59%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.67 (d, *J* = 8.30 Hz, 1H), 7.31 (s, 1H), 7.14 (d, *J* = 8.25 Hz, 1H), 6.67 (s, 1H), 4.23 (t, *J* = 5.55 Hz, 1H), 3.82 (t, *J* = 7.20 Hz, 2H), 3.71 (d, *J* = 7.65 Hz, 2H), 3.18 (q, *J* = 6.45 Hz, 2H), 2.58 (s, 3H), 3.12 (s, 3H), 2.02-1.97 (m, 2H), 1.87 (p, *J* = 6.95 Hz, 2H), 1.68 (s, 9H), 1.65-1.55 (m, 4H), 1.48-1.46 (m, 2H), 1.32-1.22 (m, 4H).

4.2.1.142. tert-Butyl 6-(1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-3-methyl-1H-indazole-1-carboxylate ***(153).*** Starting with compound **56** following the general procedure **10**, compound **153** was obtained as white semi solid, yield 51%.

4.2.1.143. tert-Butyl 3-methyl-6-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(pyridin-3-ylmethyl)ureido)-1H-indazole-1-carboxylate ***(154).*** Starting with compound **57** following the general procedure **10**, compound **154** was obtained as white semi solid, yield 53%.

4.2.1.144. tert-Butyl 3-methyl-6-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-methylpiperidin-4-yl)ureido)-1H-indazole-1-carboxylate ***(155).*** Starting with compound **58** following the general procedure **10**, compound **155** was obtained as off white solid, yield 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.67 (d, *J* = 8.40 Hz, 1H), 7.30 (s, 1H), 7.04 (dd, *J* = 2.01, 8.40 Hz, 1H), 6.67 (s, 1H), 4.56-4.49 (m, 1H), 4.07 (t, *J* = 4.95 Hz, NH), 3.80 (t, *J* = 7.14 Hz, 2H), 3.14 (q, *J* = 6.60 Hz, 2H), 2.87-2.80 (m, 2H), 2.58 (s, 3H), 2.23 (s, 3H), 2.09 (s, 3H), 1.85-1.79 (m, 4H), 1.68 (s, 9H), 1.50-1.43 (m, 2H).

4.2.1.145. tert-Butyl (4-(4-(3-methyl-6-(3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-1H-indazol-1-yl)butyl)pyridin-2-yl)carbamate ***(156).*** Starting with compound **58** following the general procedure **10**, compound **156** was obtained as

white solid, yield 44%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.05 (d, J = 8.85 Hz, 1H), 7.86 (s, 1H), 7.71 (d, 1H), 7.49 (s, 1H), 7.43 (d, J = 8.58 Hz, 1H), 6.98-6.96 (m, 2H), 6.80 (s, 1H), 6.76 dd, J = 8.61 Hz, 1H), 5.87 (t, 1H), 4.25 (t, J = 6.96 Hz, 2H), 3.98 (t, J = 6.75 Hz, 2H), 3.26 (q, J = 5.31 Hz, 2H), 2.63 (t, J = 7.68 Hz, 2H), 2.49 (s, 3H), 2.22 (s, 3H), 2.02-1.90 (m, 4H), 1.69-2.58 (m, 2H), 1.43 (s, 9H).

4.2.1.146. tert-Butyl 6-(1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)ureido)-1H-indazole-1-carboxylate ***(157).*** Starting with compound **75** following the general procedure **10**, compound **157** was obtained as white solid, yield 22%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, J = 0.75 Hz, 1H), 7.97 (s, 1H), 7.71 (d, J = 7.41 Hz, 1H), 7.33 (s, 1H), 7.22-7.18 (m, 2H), 6.98 (dd, J = 8.43, 1.86 Hz, 1H), 6.97-6.92 (m, 2H), 6.69 (s, 1H), 4.90 (s, 2H), 4.35 (t, J = 5.67 Hz, 1H), 3.84 (t, J = 7.32 Hz, 2H), 3.23 (q, J = 6.21 Hz, 2H), 2.31 (s, 3H), 1.89 (quintet, J = 6.96 Hz, 2H), 1.67 (s, 9H).

4.2.1.147. tert-Butyl 5(6)-((3-cyclobutyl-3-(1,3-dimethyl-1H-indazol-5-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(158).*** Starting with compound **25** following the general procedure **10**, compound **158** was obtained as white solid, yield 52%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35, 7.84 (d, J = 6.90 Hz, 1H), 8.32, 7.82 (s, 1H), 7.54-7.51 (m, 2H), 7.36, 7.34 (d, J = 2.25 Hz, 1H), 7.24-7.21 (m, 1H), 7.17-7.10 (m, 1H), 5.13-5.05 (m, 1H), 4.45 (d, J = 6.05 Hz, 2H), 4.37 (t, J = 4.75 Hz, NH), 3.98 (s, 3H), 2.54 (s, 3H), 2.15-2.09 (s, 2H0, 1.79-1.74 (m, 2H), 1.66 (s, 9H), 1.59-1.51 (m, 2H), 1.44-1.34 (m, 2H).

4.2.1.148. tert-Butyl 5(6)-((3-cyclopentyl-3-(1,3-dimethyl-1H-indazol-5-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(159).*** Starting with compound **26** following the general procedure **10**, compound **159** was obtained as white solid, yield 55%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35, 7.82 (s, 1H), 8.32, 7.84 (d, J = 8.55 Hz, 1H), 7.54-7.51 (m, 2H), 7.33, 7.31 (d, J = 2.22 Hz, 1H), 7.22 (d, J = 5.07 Hz, 1H), 7.17-7.12 (m, H), 4.92-4.88 (m, 1H), 4.46 (d, J = 7.0 Hz, 2H), 4.35 (t, J = 5.70 Hz, NH), 3.96 (s, 3H), 2.52 (s, 3H0, 1.90-1.88 (m, 2H), 1.65 (s, 9H), 1.52-1.43 (m, 4H), 1.28-1.22 (m, 2H)

4.2.1.149. tert-Butyl 5(6)-((3-cyclohexyl-3-(1,3-dimethyl-1H-indazol-5-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(160).*** Starting with compound **21** following the general procedure **10**, compound **160** was obtained as

white solid, yield 54%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35, 7.84 (s, 1H), 8.34, 7.87 (d, J = 7.89 Hz, 1H), 7.54-7.50 (m, 2H), 7.35-7.31 (m, 1H), 7.26-7.24 (m, 1H), 7.19-7.13 (m, 2H), 4.59-4.51 (m, 1H), 4.47 (d, J = 5.70 Hz, 2H), 4.33 (t, J = 4.95 Hz, NH), 3.98 (s, 3H), 2.55, 2.52 (s, 3H), 1.95-1.91 (m, 2H), 1.75-1.71 (m, 2H), 1.68, 1.65 (s, 9H), 1.53-1.34 (m, 3H), 1.09-1.00 (m, 2H), 0.93-0.81 (m, 1H).

4.2.1.150. tert-Butyl 5-((3-(cyclopentylmethyl)-3-(1,3-dimethyl-1H-indazol-5-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(161).*** Starting with compound **22** following the general procedure **10**, compound **161** was obtained as white solid, yield 54%.

4.2.1.151. tert-Butyl 5-((3-(1,3-dimethyl-1H-indazol-5-yl)-3-(4-fluorobenzyl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(162).*** Starting with compound **23** following the general procedure **10**, compound **162** was obtained as white solid, yield 45%.

4.2.1.152. tert-Butyl 5(6)-((3-cyclobutyl-3-(1,3-dimethyl-1H-indazol-6-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(163).*** Starting with compound **64** following the general procedure **10**, compound **163** was obtained as white solid, yield 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38, 8.35 (s, 1H), 7.87-7.85 (m, 1H), 7.71-7.64 (m, 2H), 7.55-7.43 (m, 1H), 7.17-7.13 (m, 1H), 6.93-6.87 (m, 1H), 5.11-5.05 (m, 1H), 4.47-4.43 (m, 3H), 3.99, 3.99 (s, 3H), 2.56, 2.56 (s, 3H), 2.17-2.09 (m, 2H), 1.83-1.74 (m, 2H), 1.69-1.68 (s, 9H), 1.49-1.42 (m, 2H).

4.2.1.153. tert-Butyl 5(6)-((3-cyclopentyl-3-(1,3-dimethyl-1H-indazol-6-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(164).*** Starting with compound **65** following the general procedure **10**, compound **164** was obtained as off-white solid, yield 52%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38, 8.34 (s, 1H), 7.85 (s, 1H), 7.70-7.63 (m, 2H), 7.55-7.43 (m, 1H), 7.24-7.13 (m, 1H), 6.93-6.87 (m, 1H), 5.11-5.05 (m, 1), 4.47-4.43 (m, 3H), 4.00, 3.99 (s, 3H), 2.56 (s, 3H), 2.17-2.09 (m, 2H), 1.83-1.74 (m, 2H), 1.69, 1.68 (s, 9H), 1.61-1.54 (m, 2H), 1.49-1.42 (m, 2H).

4.2.1.154. tert-Butyl 5(6)-((3-cyclohexyl-3-(1,3-dimethyl-1H-indazol-6-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(165).*** Starting with compound **59** following the general procedure **10**, compound **165** was obtained as white solid, yield 42%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38, 7.70 (s, 1H), 8.34, 7.85

(d, *J* = 11.16 Hz, 1H), 7.57-7.51 (m, 1H), 7.45-7.39 (m, 1H), 7.30-7.26 (m, 1H), 7.17-7.12 (m, 1H), 6.95-6.92 (m, 1H), 4.46, 4.44 (d, *J* = 5.31 Hz, 2H), 4.40-4.32 (m, 1H), 3.99, 3.98 (s, 3H), 2.55 (s, 3H), 1.93-1.86 (m, 2H), 1.75-1.70 (m, 2H), 1.68 (s, 9H), 1.65-1.59 (m, 1H), 1.47-1.35 (m, 2H), 1.14-1.06 (m, 2H), 0.97-0.88 (m, 1H).

4.2.1.155. tert-Butyl 5 (6)-((3-(cyclopentylmethyl)-3-(1,3-dimethyl-1H-indazol-6-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(166).*** Starting with compound **60** following the general procedure **10**, compound **166** was obtained as white solid, yield 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38, 8.35 (s, 1H), 7.88-7.86 (m, 1H), 7.28-7.26 (m, 2H), 7.24-7.20 (m, 2H), 7.03-6.97 (m, 1H), 4.71-4.61 (m, NH), 4.51 (d, *J* = 5.31 Hz, 2H), 3.97 (s, 3H), 3.77 (d, *J* = 7.86 Hz, 2H), 2.54 (s, 3H), 2.10-2.04 (m, 1H), 1.0-1.68 (m, 4H), 1.61 (s, 9H), 1.49-1.45 (m, 2H), 1.35-1.25 (m, 4H).

4.2.1.156. tert-Butyl 5(6)-((3-(1,3-dimethyl-1H-indazol-6-yl)-3-(4-fluorobenzyl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(167).*** Starting with compound **61** following the general procedure **10**, compound **167** was obtained as white crude solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39, 8.35 (s, 1H), 7.91-7.87 (m, 1H), 7.70-7.47 (m, 3H), 7.29-7.21 (m, 2H), 7.02-6.92 (m, 3H), 6.85-6.80 (m, 1H), 4.92 (s, 2H), 4.72-4.66 (m, 1H), 4.54, 4.52 (d, *J* = 5.7 Hz, 2H), 3.88 (s, 3H), 2.51 (s, 3H), 1.69 (s, 9H).

4.2.1.157. tert-Butyl 5 (6)-((3-(1,3-dimethyl-1H-indazol-6-yl)-3-(1-methylpiperidin-4-yl)ureido)methyl)-1H-benzo[d]imidazole-1-carboxylate ***(168).*** Starting with compound **63** following the general procedure **10**, compound **168** was obtained as white solid, yield 48%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (7.83) (s, 1H), 8.32 (7.85) (d, *J* = 8.40 Hz, 1H), 7.66-7.58 (m, 2H), 7.19-7.10 (m, 3H), 6.91 (6.84) (dd, *J* = 1.65, 8.43 Hz, 1H), 4.59-4.51 (m, 1H), 4.44 (4.42) (s, 2H), 3.95 (3.94) (s, 3H), 2.82-2.79 (m, 2H), 2.51 (s, 3H), 2.19 (s, 3H), 2.11-2.02 (m, 2H), 1.88-1.80 (m, 2H), 1.66 (s, 9H), 1.50-1.41 (m, 2H).

4.2.2. Final

4.2.2.1. 1-(3-(5-Methyl-1H-imidazol-1-yl)propyl)-3-(3-methyl-1H-indazol-5-yl)urea ***(169).*** Starting with compound **145** following the general procedure **9.2**, compound **169** was obtained as white solid, yield 70%, mp = 90-91 ^{o}C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.72 (d, *J* = 1.11 Hz, 1H), 7.59 (s, 1H), 7.38 (dd, *J* = 0.75, 8.79 Hz, 1H), 7.27 (dd, *J* = 1.83, 8.79 Hz, 1H), 6.67 (s, 1H), 4.03 (t, *J* = 7.14 Hz, 2H), 3.25 (t, *J* =

6.78 Hz, 2H), 2.50 (s, 3H), 2.23 (d, *J* = 0.93 Hz, 3H), 2.00 (p, *J* = 6.78 Hz, 2H). MS (ESI) *m/z* 313 $[M+H]^+$. HRMS (ESI) calc. for $C16H_{20}N_6O$ $[M+H]^+$ 313.1771, found 313.1776. Anal. HPLC 100.0% (R_t = 3.222 min).

4.2.2.2. 1-Cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methyl-1H-indazol-5-yl)urea ***(170).*** Starting with compound **146** following the general procedure **9.2**, compound **170** was obtained as white solid, yield 71%, mp = 103-104 ^oC. ^1H NMR (300 MHz, MeOD) δ 7.54-7.49 (m, 3H), 7.14 (d, *J* = 10.44 Hz, 1H), 6.61 (s, 1H), 4.44-4.35 (m, 1H), 3.86 (t, *J* = 7.32 Hz, 2H), 3.11 (t, *J* = 6.60 Hz, 2H), 2.55 (s, 3H), 2.14 (d, *J* = 0.93 Hz, 3H), 1.96-1.86 (m, 2H), 1.82-1.72 (m, 4H), 1.58-1.53 (m, 1H), 1.41-1.28 (m, 2H), 1.12-1.00 (m, 2H), 0.93-0.85 (m, 1H). MS (ESI) *m/z* 395 $[M+H]^+$. HRMS (ESI) calc. for $C_{22}H_{30}N_6O$ $[M+H]^+$ 395.2554, found 395.2544. Anal. HPLC 95.1% (R_t = 4.161 min).

4.2.2.3. 1-(Cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methyl-1H-indazol-5-yl)urea ***(171).*** Starting with compound **147** following the general procedure **9.2**, compound **171** was obtained as white solid, yield 75%, mp = 80-81 ^oC. ^1H (500 MHz, MeOD) δ 7.61 (d, *J* = 1.25 Hz, 1H), 7.55 (d, *J* = 8.75 Hz, 1H), 7.50 (s, 1H), 7.23 (dd, *J* = 1.75 Hz, 1H), 6.62 (s, 1H), 3.88 (t, *J* = 7.15 Hz, 2H), 3.65 (d, *J* = 7.65 Hz, 2H), 3.12 (t, *J* = 6.70 Hz, 2H), 2.55 (s, 3H), 2.16 (s, 3H), 2.02 (p, *J* = 7.50 Hz, 2H), 1.98 (p, *J* = 6.70 Hz, 2H), 1.71-1.60 (m, 4H), 1.53-1.50 (m, 2H), 1.28-1.20 (m, 4H). MS (ESI) *m/z* 395 $[M+H]^+$. HRMS (ESI) calc. for $C_{22}H_{30}N_6O$ $[M+H]^+$ 395.2554, found 395.2567. Anal. HPLC 97.6% (R_t = 4.548 min).

4.2.2.4. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methyl-1H-indazol-5-yl)urea ***(172).*** Starting with compound **147** following the general procedure **9.2**, compound **172** was obtained as white solid, yield 45%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.79 Hz, 1H), 7.34 (s, 1H), 7.30 (d, *J* = 1.29 Hz, 1H), 7.21-7.17 (m, 2H), 6.97-6.90 (m, 3H), 6.69 (s, 1H), 4.84 (s, 2H), 4.34 (t, *J* = 5.85 Hz, 1H), 3.83 (t, *J* = 7.14 Hz, 2H), 3.21 (q, *J* = 6.60 Hz, 2H), 2.51 (s, 3H), 2.12 (d, *J* = 0.90 Hz, 3H), 1.88 (quintet, *J* = 6.60 Hz, 2H). HRMS (ESI) calc. for $C_{23}H_{26}FN_6O$ $[M + H]^+$ 421.2147, found 421.2161.

*4.2.2.5. 1-(1,3-Dimethyl-1H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(173).*** Starting with compound **10** following the general procedure **10**, compound **173** was obtained as white solid, yield 63%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.47 (s, 1H), 7.40 (d, J = 0.90 Hz, 1H), 7.21 (d, J = 8.25 Hz, 1H), 7.15 (dd, J = 8.79, 1.83 Hz, 1H), 6.76 (s, 1H), 5.59 (t-like, 1H), 3.95 (s, 3H), 3.92 (t, J = 6.96 Hz, 2H), 3.23 (q, J = 6.39 Hz, 2H), 2.50 (s, 3H), 2.19 (d, J = 0.75 Hz, 3H), 1.95 (quintet, J = 6.78 Hz, 2H). HRMS (ESI) calc. for $C_{17}H_{23}N_6O$ $[M + H]^+$ 327.1928, found 327.1934.

*4.2.2.6. 1-Cyclohexyl-1-(1,3-dimethyl-1H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(174).*** Starting with compound **21** following the general procedure **10**, compound **174** was obtained as white solid, yield 41%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (d, J = 1.65 Hz, 1H), 7.34 (d, J = 8.79 Hz, 1H), 7.29 (s, 1H), 7.07 (dd, J = 8.79, 1.83 Hz, 1H), 6.67 (s, 1H), 4.50 (tt, J = 12.09, 3.48 Hz, 1H), 4.01 (s, 3H), 3.90 (t, J = 5.85 Hz, 1H), 3.77 (t, J = 7.32 Hz, 2H), 3.11 (q, J = 6.21 Hz, 2H), 2.55 (s, 3H), 2.09 (s, 3H), 1.89-1.82 (m, 2H), 1.79 (quintet, J = 6.96 Hz, 2H), 1.69 (d, J = 12.99 Hz, 2H), 1.53 (d, J = 12.65 Hz, 1H), 1.45-1.32 (m, 2H), 1.04-0.97 (m, 2H), 0.90-0.81 (m, 1H). HRMS (ESI) calc. for $C_{23}H_{23}N_6O$ $[M + H]^+$ 409.2710, found 409.2711.

*4.2.2.7. 1-(Cyclopentylmethyl)-1-(1,3-dimethyl-1H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(175).*** Starting with compound **22** following the general procedure **10**, compound **175** was obtained as white solid, yield 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 1.83 Hz, 1H), 7.38 (d, J = 8.79 Hz, 1H), 7.31 (s, 1H), 7.18 (dd, J = 8.79, 1.83 Hz, 1H), 6.69 (s, 1H), 4.16 (t, J = 5.85 Hz, 1H), 4.03 (s, 3H), 3.81 (t, J = 7.14 Hz, 2H), 3.67 (d, J = 7.71 Hz, 2H), 3.16 (q, J = 6.39 Hz, 2H), 2.57 (s, 3H), 2.12 (s, 3H), 2.05-1.95 (m, 1H), 1.84 (quintet, J = 7.14 Hz, 2H), 1.70-1.65 (m, 4H), 1.55-1.45 (m, 2H), 1.38-1.24 (m, 2H). HRMS (ESI) calc. for $C_{23}H_{33}N_6O$ $[M + H]^+$ 410.2739, found 410.2755.

*4.2.2.8. 1-(1,3-Dimethyl-1H-indazol-5-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(176).*** Starting with compound **23** following the general procedure **10**, compound **176** was obtained as white solid, yield 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.27 (d, J = 6.03 Hz, 1H), 7.25 (s, 1H), 7.16 (dd, J = 8.43, 5.49 Hz, 1H), 6.95-6.89 (m, 3H), 6.69 (s, 1H), 4.81 (s, 2H), 4.20 (t, J = 6.06 Hz, 1H), 3.97 (s, 3H), 3.81 (t, J = 7.32 Hz, 2H), 3.18 (q, J = 6.42 Hz, 2H), 2.47 (s, 3H),

2.11 (s, 3H), 1.88-1.82 (m, 2H). HRMS (ESI) calc. for $C_{24}H_{28}FN_6O$ $[M + H]^+$ 435.2303, found 435.2314.

4.2.2.9. 1-(1,3-Dimethyl-1H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(pyridin-3-ylmethyl)urea ***(177).*** Starting with compound **24** following the general procedure **10**, compound **177** was obtained as white solid, yield 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, J = 3.09 Hz, 1H), 8.37 (s, 1H), 7.64 (d, J = 7.68 Hz, 1H), 7.35 (s, 1H), 7.29-7.26 (m, 2H), 7.21 (dd, J = 8.04, 4.92 Hz, 1H), 6.93 (dd, J = 8.79, 1.83 Hz, 1H), 6.68 (s, 1H), 4.86 (s, 2H), 4.25 (t-like, 1H), 3.81(t, J = 7.14 Hz, 2H), 3.17 (q, J = 6.39 Hz, 2H), 2.47 (s, 3H), 2.11 (s, 3H), 1.87-1.82 (m, 2H). HRMS (ESI) calc. for $C_{23}H_{28}N_7O$ $[M + H]^+$ 418.2350, found 418.2344.

4.2.2.10. 1-(3-Methyl-1-(2-(4-methylpiperazin-1-yl)ethyl)-1H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(178).*** Starting with compound **11** following the general procedure **10**, compound **178** was obtained as white solid, yield 59%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, J = 1.47 Hz, 1H), 7.40 (s, 1H), 7.28 (d, J = 8.97 Hz, 1H), 7.17 (dd, J = 8.97, 2.04 Hz, 1H), 7.15 (s, 1H), 6.76 (s, 1H), 5.33 (t, 1H), 4.40 (t, J = 6.96 Hz, 2H), 3.93 (t, J = 6.96 Hz, 2H), 3.24 (q, J = 6.03 Hz, 2H), 2.83 (t, J = 7.32 Hz. 2H), 2.55 (br, 4H), 2.52 (s, 3H), 2.43 (br, 4H), 2.27 (s, 3H), 2.19 (s, 3H), 1.96 (quintet, J = 6.60 Hz, 2H).). HRMS (ESI) calc. for $C_{23}H_{35}N_8O$ $[M + H]^+$ 439.2928, found 439.2925.

4.2.2.11. 1-(1-(4-(2-Aminopyridin-4-yl)butyl)-3-methyl-1H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(179).*** Starting with compound **149** following the general procedure **9.2**, compound **179** was obtained as white solid, yield 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, J = 5.85 Hz, 1H), 7.67 (s, 1H), 7.44 (s, 1H), 7.33 (s, 1H), 7.20-7.14 (m, 2H), 6.75 (s, 1H), 6.39 (dd, J = 5.31, 1.47 Hz, 1H), 6.15 (s, 1H), 5.46 (brt, 1H), 4.27 (t, J = 6.75 Hz, 2H), 3.92 (t, J = 6.78 Hz, 2H), 3.24 (q, J = 6.06 Hz, 2H), 2.51 (s, 3H), 2.44 (t, J = 7.50 Hz, 2H), 1.99-1.83 (m, 4H), 1.61-1.51 (m, 2H). HRMS (ESI) calc. for $C_{25}H_{33}N_8O$ $[M + H]^+$ 461.2772, found 461.2789.

4.2.2.12. 1-Cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-propyl-1H-indazol-5-yl)urea ***(180)***. Starting with compound **35** following the general procedure **10**, compound **180** was obtained as white solid, yield 51%, mp = 53-54 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, J = 0.90 Hz, 1H), 7.47 (d, J = 1.29 Hz, 1H), 7.43 (d, J = 8.79 Hz, 1H), 7.27 (s, 1H), 7.08 (dd, J = 1.83, 8.79 Hz, 1H), 6.66 (s, 1H),

4.49-4.41 (m, 1H), 4.36 (t, *J* = 6.96 Hz, 2H), 3.90 (t, *J* = 5.94 Hz, NH), 3.79 (t, *J* = 7.14 Hz, 2H), 3.15 (q, *J* = 6.45 Hz, 2H), 2.08 (d, *J* = 0.90 Hz, 3H), 2.00 (p, *J* = 7.14 Hz, 2H), 1.88-1.84 (m, 2H), 1.82 (p, *J* = 7.14 Hz, 2H), 1.73-.65 (m, 2H), .54-1.50 (m, 1H), 1.44-1.31 (m, 2H), 1.06-0.98 (m, 2H), 0.89-0.77(m, 1H). MS (ESI) *m/z* 423 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{34}N_6O$ $[M+H]^+$ 423.2867, found 423.2880. Anal. HPLC 95.4% (R_t = 7.379 min).

4.2.2.13. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-propyl-1H-indazol-5-yl)urea ***(181).*** Starting with compound **36** following the general procedure **10**, compound **181** was obtained as white solid, yield 54%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, *J* = 0.93 Hz, 1H), 7.38 (d, *J* = 8.79 Hz, 1H), 7.36 (d, *J* = 1.29 Hz, 1H), 7.32 (s, 1H), 7.22-1.17 (m, 2H), 6.98 (dd, *J* = 8.79, 2.04 Hz, 1H), 6.97-6.92 (m, 2H), 6.69 (s, 1H), 4.84 (s, 2H), 4.33 (t, *J* = 6.96 Hz, 2H), 4.23 (t, *J* = 5.88 Hz, 1H), 3.82 (t, *J* = 7.14 Hz, 2H), 3.20 (q, *J* = 6.21 Hz, 2H), 2.12 (d, *J* = 0.90 Hz, 3H), 1.98 (hex, *J* = 7.32 Hz, 2H), 1.87 (quintet, *J* = 6.96 Hz, 2H), 0.95 (t, *J* = 7.32 Hz, 3H). HRMS (ESI) calc. for $C_{25}H_{30}FN_6O$ $[M + H]^+$ 449.2460, found 449.2471.

4.2.2.14. 1-Cyclohexyl-1-(1-isobutyl-1H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(182).*** Starting with compound **37** following the general procedure **10**, compound **182** was obtained as white solid, yield 78%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, *J* = 0.93 Hz, 1H), 7.47 (d, *J* = 1.29 Hz, 1H), 7.40 (d, *J* = 8.79 Hz, 1H), 7.27 (s, 1H), 7.06 (dd, *J* = 8.61, 1.83 Hz, 1H), 6.66 (s, 1H), 4.44 (tt, *J* = 12.09, 3.66 Hz, 1H), 4.16 (d, *J* = 5.34 Hz, 2H), 3.91 (t, *J* = 5.67 Hz, 2H), 3.76 (t, *J* = 7.14 Hz, 2H), 3.11 (q, *J* = 6.78 Hz, 2H), 2.35 (hept, *J* = 6.60 Hz, 1H), 2.08 (d, *J* = 0.72 Hz, 3H), 1.88-1.75 (m, 4H), 1.69 (d, *J* = 13.38 Hz, 2H), 1.52 (d, *J* = 12.84 Hz, 1H), 1.44-1.31 (qt-like, 2H), 1.05-0.94 (m, 2H), 0.95 (d, *J* = 6.60 Hz, 6H), 0.90-0.77 (dt-like, 1H). HRMS (ESI) calc. for $C_{25}H_{37}N_6O$ $[M + H]^+$ 437.3023, found 437.3036.

4.2.2.15. 1-(4-Fluorobenzyl)-1-(1-isobutyl-1H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(183).*** Starting with compound **38** following the general procedure **10**, compound **183** was obtained as white solid, yield: 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 0.72 Hz, 1H), 7.32-7.25 (m, 2H), 7.15-7.10 (m, 2H), 6.93-6.85 (m, 3H), 6.62 (s, 1H), 4.76 (s, 2H), 4.18 (t, *J* = 6.33 Hz, 1H), 4.09 (t, *J* = 7.32 Hz, 2H), 3.75 (t, *J* = 7.14 Hz, 2H), 3.13 (q, *J* = 6.42 Hz, 2H), 2.26 (hept, *J* = 6.60 Hz, 1H), 2.04 (d, *J* = 0.72 Hz, 3H), 1.80 (quintet, *J* = 6.96 Hz, 2H), 0.87 (d,

J = 6.60 Hz, 6H). HRMS (ESI) calc. for $C_{26}H_{32}FN_6O$ $[M + H]^+$ 436.2616, found 436.2612.

4.2.2.16. 1-Ccyclohexyl-1-(2,3-dimethyl-2H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(184).*** Starting with compound **27** following the general procedure **10**, compound **184** was obtained as white solid, yield 56%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.97 Hz, 1H), 7.31 (s, 1H), 7.28 (d, *J* = 1.29 Hz, 1H), 6.93 (dd, *J* = 8.97, 1.83 hz, 1H), 6.69 (s, 1H), 4.43 (tt, *J* = 12.06, 3.48 Hz, 1H), 4.11 (s, 3H), 4.01 (t, *J* = 5.67 Hz, 1H), 3.77 (t, *J* = 7.32 Hz, 2H), 3.11 (q, *J* = 6.21 Hz, 2H), 2.61 (s, 3H), 2.09 (s, 3H), 1.86-1.78 (m, 2H), 1.77 (quintet, *J* = 7.14 Hz, 2H), 1.72-1.67 (m, 2H), 1.53 (d, *J* = 12.99 Hz, 1H), 1.44-1.31 (m, 2H), 1.06-1.03 (m, 2H), 0.90-0.82 (m, 1H). HRMS (ESI) calc. for $C_{23}H_{23}N_6O$ $[M + H]^+$ 409.2710, found 409.2723.

4.2.2.17. 1-(2,3-Dimethyl-2H-indazol-5-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(185).*** Starting with compound **28** following the general procedure **10**, compound **185** was obtained as white solid, yield 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.97 Hz, 1H), 7.29 (s, 1H), 7.18 (dd, *J* = 8.40, 5.49 Hz, 2H), 7.13 (d, *J* = 1.47 Hz, 1H), 6.91 (t, *J* = 8.61 Hz, 2H), 6.84 (dd, *J* = 8.79, 2.01 Hz, 1H), 6.67 (s, 1H), 4.80 (s, 2H), 4.29 (t, *J* = 6.06 Hz, 1H), 4.08 (s, 3H), 3.79 (t, *J* = 7.14 Hz, 2H), 3.17 (q, *J* = 6.60 Hz, 2H), 2.52 (s, 3H), 2.10 (d, *J* = 0.72 Hz, 3H), 1.83 (quintet, *J* = 7.32 Hz, 2H). HRMS (ESI) calc. for $C_{24}H_{28}FN_6O$ $[M + H]^+$ 425.2303, found 425.2304.

4.2.2.18. 1-(2,3-Dimethyl-2H-indazol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(pyridin-3-ylmethyl)urea ***(186).*** Starting with compound **29** following the general procedure **10**, compound **186** was obtained as white solid, yield 28%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, *J* = 3.30 Hz, 1H), 8.44 (s, 1H), 7.91 (d, *J* = 1.26 Hz, 1H), 7.64 (d, 1H), 7.61 (d, *J* = 8.97 Hz, 1H), 7.25-7.21 (m, 2H), 6.88 (dd, *J* = 8.97, 2.04 Hz, 1H), 6.83 (s, 1H), 4.88 (s, 2H), 4.56 (t, *J* = 5.85 Hz, 1H), 4.09 (s, 3H), 3.96 (t, *J* = 6.93 Hz, 2H), 3.24 (q, *J* = 6.21 Hz, 2H), 2.55 (s, 3H), 2.20 (d, *J* = 0.90 Hz, 3H), 1.90 (quintet, *J* = 6.96 Hz, 2H).

4.2.2.19. 1-(3-(5-Methyl-1H-imidazol-1-yl)propyl)-3-(3-methyl-1H-indazol-6-yl)urea ***(187).*** Starting with compound **150** following the general procedure **9.2**, compound **187** was obtained as white solid, yield 69%, mp = over 200 ^{o}C. ^{1}H NMR

(300 MHz, MeOD) δ 7.76 (d, J = 1.08 Hz, 1H), 7.59 (s, 1H), 7.56 (d, J = 8.79 Hz, 1H), 6.88 (dd, J = 1.83, 8.61 Hz, 1H), 6.67 (s, 1H), 4.03 (t, J = 7.14 Hz, 2H), 3.26 (t, J = 6.78 Hz, 2H), 2.48 (s, 3H), 2.23 (d, J = 0.93 Hz, 3H), 2.01 (p, J = 6.78 Hz, 2H). MS (ESI) *m/z* 313 $[M+H]^+$. HRMS (ESI) calc. for $C_{16}H_{20}N_6O$ $[M+H]^+$ 313.1771, found 313.1792. Anal. HPLC 100.0% (R_t = 3.339 min).

4.2.2.20. 1-Cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methyl-1H-indazol-6-yl)urea ***(188).*** Starting with compound **151** following the general procedure **9.2**, compound **188** was obtained as white solid, yield 71%, mp = 73-74 ^oC. ^1H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, J = 8.43 Hz, 1H), 7.29 (s, 1H), 7.14 (s, 1H), 6.88 (dd, J = 1.47, 8.22 Hz, 1H), 6.68 (s, 1H), 4.50-4.41 (m, 1H), 3.91 (t, J = 6.42 Hz, NH), 3.82 (t, J = 6.96 Hz, 2H), 3.20 (q, J = 6.60 Hz, 2H), 2.61 (s, 3H), 2.11 (d, J = 0.75 Hz, 3H), 1.90-1.81 (m, 4H), 1.72-1.64 (m, 2H), 1.57-1.53 (m, 1H), 1.44-1.36 (m, 2H), 1.10-1.03 (m, 2H), 0.92-0.83 (m, 1H). MS (ESI) *m/z* 395 $[M+H]^+$. HRMS (ESI) calc. for $C_{22}H_{30}N_6O$ $[M+H]^+$ 395.2554, found 395.2577. Anal. HPLC 96.6% (R_t = 4.163 min).

4.2.2.21. 1-(Cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methyl-1H-indazol-6-yl)urea ***(189).*** Starting with compound **152** following the general procedure **9.2**, compound **189** was obtained as white solid, yield 77%, mp = 80-81 ^oC. ^1H NMR (500 MHz, MeOD) δ 7.79 (d, J = 8.45 Hz, 1H), 7.50 (s, 1H), 7.34 (s, 1H), 6.99 (d, J = 8.35 Hz, 1H), 6.62 (s, 1H), 3.88 (t, J = 7.15 Hz, 2H), 3.67 (d, J = 7.65 Hz, 2H), 3.13 (t, J = 6.55 Hz, 2H), 2.55 (s, 3H), 2.16 (s, 3H), 2.04 (p, J = 5.70 Hz, 2H), 1.86 (p, J = 6.70 Hz, 2H),, 1.86-1.60 (m, 4H), 1.51-1.49 (m, 2H), 1.31-1.23 (m, 4H). MS (ESI) *m/z* 395 $[M+H]^+$. HRMS (ESI) calc. for $C_{22}H_{30}N_6O$ $[M+H]^+$ 395.2554, found 395.2554. Anal. HPLC 95.2% (R_t = 4.726 min).

4.2.2.22. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methyl-1H-indazol-6-yl)urea ***(190).*** Starting with compound **153** following the general procedure **9.2**, compound **190** was obtained as white solid, yield 93%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, J = 8.43 Hz, 1H), 7.33 (s, 1H), 7.16 (t, J = 8.25 Hz, 2H), 6.94-6.88 (m, 3H), 6.78 (d, J = 8.61 Hz, 1H), 6.65 (s, 1H), 4.83 (s, 2H), 4.37

(br, 1H), 3.84 (t, *J* = 6.96 Hz, 2H), 3.25 (q, *J* = 5.88 Hz, 2H), 2.54 (s, 3H), 2.18 (s, 3H), 2.02-1.92 (m, 2H).

*4.2.2.23. tert-Butyl 3-methyl-6-(pyridin-3-ylmethylamino)-1H-indazole-1-carboxylate **(191).*** Starting with compound **154** following the general procedure **9.2**, compound **191** was obtained as white solid, yield: 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (br, 2H), 7.62-7.56 (m, 3H), 7.19 (br, 1H), 7.03 (s, 1H), 6.78 (d, *J* = 8.43 Hz, 1H), 6.70 (br, 1H), 4.88 (s, 3H), 3.91 (t, *J* = 6.78 Hz, 2H), 3.29 (q, *J* = 6.03 Hz, 2H), 2.46 (s, 3H), 2.15 (s, 3H), 1.95 (quintet, *J* = 6.78 Hz, 2H). HRMS (ESI) calc. for $C_{22}H_{26}N_7O$ $[M + H]^+$ 404.2193, found 404.2210.

*4.2.2.24. 3-(3-(5-Methyl-1H-imidazol-1-yl)propyl)-1-(3-methyl-1H-indazol-6-yl)-1-(1-methylpiperidin-4-yl)urea **(192).*** Starting with compound **155** following the general procedure **9.2**, compound **192** was obtained as white solid, yield 91%, mp = 182-183 ^{o}C. ^{1}H NMR (300 MHz, MeOH) δ 7.80 (d, *J* = 8.43 Hz, 1H), 7.50 (d, *J* = 1.11 Hz, 1H), 7.28 (d, *J* = 0.90 Hz, 1H), 6.91 (dd, *J* = 1.62, 8.58 Hz, 1H), 6.62 (s, 1H), 4.43-4.35 (m, 1H), 3.87 (t, *J* = 6.96 Hz, 2H), 3.12 (t, *J* = 6.39 Hz, 2H), 2.86-2.82 (m, 2H), 2.17 (s, 3H), 2.15 (d, *J* = 0.93 Hz, 3H), 2.12-2.08 (m, 2H), 1.86-1.75 (m, 4H), 1.49-1.41 (m, 2H). MS (ESI) *m/z* 410 $[M+H]^+$. HRMS (ESI) calc. for $C_{22}H_{31}N_7O$ $[M+H]^+$ 410.2663, found 410.2686. Anal. HPLC 97.5% (R_t = 3.055 min).

*4.2.2.25. 1-(1,3-Dimethyl-1H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(193).*** Starting with compound **46** following the general procedure **10**, compound **193** was obtained as white solid, yield 73%, mp = 77-78 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.84 (s, 1H), 7.46 (s, 1H), 7.44 (d, *J* = 8.61 Hz, 1H), 6.80 (s, 1H), 6.66 (dd, *J* = 1.47, 8.40 Hz, 1H), 6.04 (t, *J* = 4.95 Hz, NH), 4.00 (t, *J* = 6.57 Hz, 2H), 3.92 (s, 3H), 3.25 (q, *J* = 5.67 Hz, 2H), 2.49 (s, 3H), 2.22 (s, 3H), 2.02 (p, *J* = 6.21 Hz, 2H). MS (ESI) *m/z* 327 $[M+H]^+$. HRMS (ESI) calc. for $C_{17}H_{22}N_6O$ $[M+H]^+$ 327.1928, found 327.1949. Anal. HPLC 100.0% (R_t = 3.459 min).

*4.2.2.26. 1-Cyclohexyl-1-(1,3-dimethyl-1H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(194).*** Starting with compound **59** following the general procedure **10**, compound **194** was obtained as white solid, yield 65%, mp = 91-92^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.43 Hz, 1H), 7.39 (s, 1H), 7.08 (d, *J* =

1.11 Hz, 1H), 6.86 (dd, *J* = 1.65, 8.43 Hz, 1H), 6.68 (s, 1H), 4.51-4.43 (m, 1H), 4.01 (s, 3H), 3.94 (t, *J* = 6.03 Hz, NH), 3.81 (t, *J* = 7.14 Hz, 2H), 3.18 (q, *J* = 6.78 Hz, 2H), 2.58 (s, 3H), 2.11 (d, *J* = 0.90 Hz, 3H), 1.93-1.89 (m, 2H), 1.84 (p, *J* = 7.14 Hz, 2H), 1.77-1.70 (m, 2H), 1.58-1.54 (m, 1H), 1.47-1.34 (m, 2H), 1.11-1.00 (m, 2H), 0.94-0.85 (m, 1H). MS (ESI) *m/z* 409 $[M+H]^+$. HRMS (ESI) calc. for $C_{23}H_{32}N_6O$ $[M+H]^+$ 409.2710, found 409.2718. Anal. HPLC 95.9% (R_t = 5.209 min).

4.2.2.27. 1-(Cyclopentylmethyl)-1-(1,3-dimethyl-1H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(195).*** Starting with compound **60** following the general procedure **10**, compound **195** was obtained as white solid, yield 75%, mp = 71-72 ^oC. ^1H NMR (300 MHz, CDCl3) δ 7.69 (d, *J* = 8.22 Hz, 1H), 7.31 (s, 1H), 7.16 (s, 1H), 6.95 (dd, *J* = 1.65, 8.43 Hz, 1H), 6.69 (s, 1H), 4.22 (t, *J* = 4.59 Hz, NH), 4.00 (s, 3H), 3.83 (t, *J* = 7.14 Hz, 2H), 3.72 (d, *J* = 7.71 Hz, 2H), 3.21 (q, *J* = 6.78 Hz, 2H), 2.57 (s, 3H), 2.12 (d, *J* = 0.72 Hz, 3H), 2.03-1.95 (m, 1H), 1.89 (p, *J* = 7.14 Hz, 2H), 1.69-1.66 (m, 2H), 1.64-1.58 (m, 2H), 1.53-1.48 (m, 2H), 1.35-1.25 (m, 2H). MS (ESI) *m/z* 409 $[M+H]^+$. HRMS (ESI) calc. for $C_{23}H_{32}N_6O$ $[M+H]^+$ 409.2710, found 409.2726. Anal. HPLC 97.4% (R_t = 5.281 min).

4.2.2.28. 1-(1,3-Dimethyl-1H-indazol-6-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(196).*** Starting with compound **61** following the general procedure **10**, compound **196** was obtained as white solid, yield 30%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.61 (dd, *J* = 8.43, 0.54 Hz, 1H), 7.32 (s, 1H), 7.23-7.18 (m, 2H), 6.98-6.92 (m, 3H), 6.75 (dd, *J* = 8.43, 1.83 Hz, 1H), 6.69 (s, 1H), 4.87 (s, 2H), 4.28 (t, *J* = 5.85 Hz, 1H), 3.92 (s, 3H), 3.83 (t, *J* = 7.14 Hz, 2H), 3.21 (q, *J* = 6.24 Hz, 2H), 2.55 (s, 3H), 2.12 (d, *J* = 0.90 Hz, 3H), 1.87 (quintet, *J* = 7.14 Hz, 2H). HRMS (ESI) calc. for $C_{24}H_{28}FN_6O$ $[M + H]^+$ 435.2303, found 435.2313.

4.2.2.29. 1-(1,3-Dimethyl-1H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(pyridin-3-ylmethyl)urea ***(197).*** Starting with compound **62** following the general procedure **10**, compound **197** was obtained as white solid, yield 24%. ^1H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, *J* = 4.95 Hz, 1H), 8.41 (s, 1H), 7.67 (dt-like, 8.01 Hz, 1H), 7.63 (d, *J* = 8.43 Hz, 1H), 7.32 (s, 1H), 7.23 (dd, *J* = 7.89, 2.76 Hz, 1H), 6.97 (s, 1H), 6.76 (dd, *J* = 8.40, 1.44 Hz, 1H), 6.69 (s, 1H), 4.92 (s, 2H), 4.32 (t-like, 1H), 3.92 (s, 3H), 3.83 (t, *J* = 6.96 Hz, 2H), 3.21 (q, *J* = 6.60 Hz, 2H), 2.55 (ds, 3H),

2.12 (s, 3H), 1.87 (quintet, *J* = 6.96 Hz, 2H). HRMS (ESI) calc. for $C_{23}H_{28}N_7O$ [M + H]$^+$ 418.2350, found 418.2365.

4.2.2.30. 1-(1,3-Dimethyl-1H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-methylpiperidin-4-yl)urea ***(198).*** Starting with compound **63** following the general procedure **10**, compound **198** was obtained as white solid, yield 35%, mp > 200 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.43 Hz, 1H), 7.23 (s, 1H), 7.01 (s, 1H), 6.77 (d, *J* = 8.43 Hz, 1H), 6.62 (s, 1H), 4.48-4.40 (m, 1H), 3.92 (s, 1H), 3.84 (t, *J* = 4.95 Hz, NH), 3.75 (t, *J* = 7.32 Hz, 2H), 3.09 (q, *J* = 6.60 Hz, 2H), 2.77-2.73 (m, 2H), 2.50 (s, 3H), 2.13 (s, 3H), 2.04-1.97 (m, 5H), 1.78-1.73 (m, 4H), 1.42-1.34 (m, 2H). HRMS (ESI) calc. for $C_{23}H_{33}N_7O$ [M + H]$^+$ 423.2747.

4.2.2.31. 1-(3-Methyl-1-(2-(4-methylpiperazin-1-yl)ethyl)-1H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(199).*** Starting with compound **47** following the general procedure **10**, compound **199** was obtained as white solid, yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.48-7.45 (m, 2H), 7.43 (s, 1H), 6.79 (s, 1H), 6.72 (dd, *J* = 8.40, 2.13 Hz, 1H), 5.51 (t, 1H), 4.38 (t, *J* = 7.14 Hz, 2H), 3.98 (t, *J* = 6.78 Hz, 2H), 3.26 (q, *J* = 5.85 Hz, 2H), 2.84 (t, *J* = 6.93 Hz, 2H), 2.57 (br, 4H), 2.50 (s, 3H), 2.42 (br, 4H), 2.27 (s, 3H), 2.20 (s, 3H), 2.05-1.99 (m, 2H).). HRMS (ESI) calc. for $C_{23}H_{35}N_8O$ [M + H]$^+$ 439.2928, found 439.2949.

4.2.2.32. 1-(1-(4-(2-Aminopyridin-4-yl)butyl)-3-methyl-1H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(200).*** Starting with compound **156** following the general procedure **9.2**, compound **200** was obtained as white solid, yield 97%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.88 (d, *J* = 5.31 Hz, 1H), 7.82 (s, 1H), 7.42 (s, 1H), 7.41 (d, *J* = 8.43 Hz, 1H), 6.79 (s, 1H), 6.65 (dd, *J* = 8.43, 1.47 Hz, 1H), 6.41 (d, *J* = 4.32 Hz, 1H), 6.27 (s, 1H), 6.06 (t, 1H), 4.50 (s, 2H), 4.23 (t, *J* = 7.32 Hz, 2H), 3.94 (t, *J* = 6.60 Hz, 2H), 3.23 (q, *J* = 5.88 Hz, 2H), 2.49 (s, 3H), 2.46 (t, *J* = 7.71 Hz, 2H), 2.19 (s, 3H), 1.97 (quintet, *J* = 6.21 Hz, 2H), 1.90-1.83 (m, 2H), 1.59 (quintet, *J* = 6.96 Hz, 2H).). HRMS (ESI) calc. for $C_{25}H_{33}N_8O$ [M + H]$^+$ 461.2772, found 461.2765.

4.2.2.33. 1-(4-Fluorobenzyl)-1-(1H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(201).*** Starting with compound **157** following the general procedure **9.2**, compound **201** was obtained as white solid, yield 93%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.73 (d, *J* = 8.61 Hz, 1H), 7.35 (s, 1H), 7.20-7.15 (m,

2H), 6.98 (s, 1H), 6.96-6.89 (m, 2H), 6.82 (dd, *J* = 8.61, 1.83 Hz, 1H), 6.66 (s, 1H), 4.84 (s, 2H), 4.33 (br, 1H), 3.86 (t, *J* = 6.78 Hz, 2H), 3.26 (q, *J* = 6.39 Hz, 2H), 2.13 (s, 3H), 1.91 (quintet, *J* = 6.57 Hz, 2H).). HRMS (ESI) calc. for $C_{22}H_{24}FN_6O$ $[M + H]^+$ 407.1990, found 407.2004.

4.2.2.34. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-methyl-1H-indazol-6-yl)urea ***(202)*****.** Starting with compound **76** following the general procedure **10**, compound **202** was obtained as white solid, yield 43%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 0.75 Hz, 1H), 7.70 (d, *J* = 8.43 Hz, 1H), 7.32 (s, 1H), 7.23-7.18 (m, 2H), 7.02 (s, 1H), 6.98-6.92 (m, 2H), 6.80 (d, *J* = 8.40, 1.62 Hz, 1H), 6.68 (s, 1H), 4.87 (s, 2H), 4.30 (t, *J* = 5.85 Hz, 1H), 3.99 (s, 3H), 3.83 (t, *J* = 7.14 Hz, 2H), 3.22 (q, *J* = 6.78 Hz, 2H), 2.12 (s, 3H), 1.88 (quintet, *J* = 6.96 Hz, 2H).). HRMS (ESI) calc. for $C_{23}H_{26}FN_6O$ $[M + H]^+$ 421.2147, found 421.2159.

4.2.2.35. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-propyl-1H-indazol-6-yl)urea ***(203)***. Starting with compound **77** following the general procedure **10**, compound **203** was obtained as white solid, yield 57%. ^{1}H (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 0.93 Hz, 1H), 7.71 (d, *J* = 8.43 Hz, 1H), 7.30 (s, 1H), 7.19 (dd, *J* = 5.49, 8.79 Hz, 2H), 9.94-6.88 (m, 3H), 6.81 (dd, *J* = 1.83, 8.61 Hz, 1H), 6.67 (s, 1H), 4.83 (s, 1H), 4.25 (t, *J* = 5.16 Hz, NH), 4.20 (t, *J* = 6.78 Hz, 2H), 3.83 (t, *J* = 7.14 Hz, 2H), 3.23 (q, *J* = 6.78 Hz, 2H)2.10 (d, *J* = 0.93 Hz, 3H), 1.87-1.78 (m, 4H0, 0.84 (t, *J* = 7.32 Hz, 3H). MS (ESI) *m/z* 449 $[M+H]^+$. HRMS (ESI) calc. for $C_{25}H_{29}FN_6O$ $[M+H]^+$ 449.2460, found 449.2471. Anal. HPLC 95.6% (R_t = 4.317 min).

4.2.2.36. 1-(2,3-Dimethyl-2H-indazol-6-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(204)*****.** Starting with compound **66** following the general procedure **10**, compound **204** was obtained as white solid, yield 41%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.61 Hz, 1H), 7.30 (s, 1H), 7.23-7.18 (m, 2H), 6.94-6.88 (m, 2H), 6.69 (s, 1H), 6.67 (d, *J* = 8.79, 1.65 Hz, 1H), 4.86 (s, 2H), 4.45 (t, *J* = 5.49 Hz, 1H), 4.08 (s, 3H), 3.81 (t, *J* = 7.32 Hz, 2H), 3.19 (q, *J* = 6.24 Hz, 2H), 2.60 (s, 3H), 1.86 (quintet, *J* = 7.14 Hz, 2H). HRMS (ESI) calc. for $C_{24}H_{28}FN_6O$ $[M + H]^+$ 435.2303, found 435.2314.

4.2.2.37. 1-(2,3-Dimethyl-2H-indazol-6-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(pyridin-3-ylmethyl)urea ***(205)*****.** Starting with compound **67** following

the general procedure **10**, compound **205** was obtained as white solid, yield 37%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (br, 1H), 8.40 (br, 1H), 7.67 (d, *J* = 7.68 Hz, 1H), 7.56 (d, *J* = 8.61 Hz, 1H), 7.30 (s, 1H), 7.27 (s, 1H), 7.23-7.19 (m, 1H), 7.69 (s, 1H), 6.67 (d, *J* = 8.79 Hz, 1H), 4.91 (s, 2H), 4.49 (t, *J* = 5.67 Hz, 1H), 4.09 (s, 3H), 3.83 (t, *J* = 7.32 Hz, 2H), 3.20 (q, *J* = 6.21 Hz, 2H), 2.61 (s, 3H), 1.88 (quintet, *J* = 6.96 Hz, 2H).

4.2.2.38. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-cyclobutyl-1-(1,3-dimethyl-1H-indazol-5-yl)urea ***(206).*** Starting with compound **158** following the general procedure **9.2**, compound **206** was obtained as white solid, yield 88%, mp = 140-11 ^{o}C. ^{1}H NMR (300 MHz, MeOD) δ 8.09 (s, 1H0, 7.57-7.53 (m, 2H), 7.50 (d, *J* = 7.89 Hz, 1H), 7.43 (s, 1H), 7.19 (dd, *J* = 2.01, 8.79 Hz, 1H), 7.13 (d, *J* = 8.43 Hz, 1H), 4.95-4.92 (m, 1H), 4.37 (s, 2H), 3.99 (m, 3H), 2.52 (s, 3H0, 2.12-2.09 (m, 2H), 1.82-1.75 (m, 2H), 1.59-1.42 (m, 2H). MS (ESI) *m/z* 389 $[M+H]^+$. HRMS (ESI) calc. for $C_{22}H_{24}N_6O$ $[M+H]^+$ 389.2084, found
389.2099. Anal. HPLC 100.0% (R_t = 7.708 min).

4.2.2.39. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-cyclopentyl-1-(1,3-dimethyl-1H-indazol-5-yl)urea ***(207).*** Starting with compound **159** following the general procedure **9.2**, compound **207** was obtained as white solid, yield 71%, mp = 137-138 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.45-7.42 (m, 3H), 7.35 (d, *J* = 8.58 Hz, 1H), 7.17 (dd, *J* = 1.83, 8.79 Hz, 1H), 7.06 (d, *J* = 8.25 Hz, 1H), 4.97-4.86 (m, 1H), 4.44 (s, 2H), 3.99 (s, 3H), 2.54 (s, 3H), 1.99-1.89 (m, 2H), 1.50-1.46 (m, 4H), 1.29-1.25 (m, 2H). MS (ESI) *m/z* 403 $[M+H]^+$. HRMS (ESI) calc. for $C_{23}H_{26}N_6O$ $[M+H]^+$ 403.2241, found 403.2263. Anal. HPLC 100.0% (R_t = 4.268 min).

4.2.2.40. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-cyclohexyl-1-(1,3-dimethyl-1H-indazol-5-yl)urea ***(208).*** Starting with compound **160** following the general procedure **9.2**, compound **208** was obtained as white solid, yield 72%, mp = 150-151 ^{o}C. ^{1}H NMR (300 MHz, MeOD) δ 8.09 (s, 1H), 7.54-7.51 (m, 2H), 7.48 (s, 1H), 7.42 (s, 1H), 7.21 (dd, *J* = 1.65, 8.79 Hz, 1H), 7.12 (d, *J* = 8.43 Hz, 1H), 4.37 (s, 2H), 4.35-4.31 (m, 1H), 3.98 (s, 3H), 2.51 (s, 3H), 1.93-1.89 (m, 2H), 1.76-1.72 (m, 2H), 1.55-1.51 (m, 1H), 1.41-1.27 (m, 2H), 1.08-1.03 (m, 2H), 0.90-0.86 (m, 1H). MS (ESI) *m/z* 417 $[M+H]^+$. HRMS (ESI) calc.

for $C_{24}H_{28}N_6O$ $[M+H]^+$ 417.2397, found 417.2409. Anal. HPLC 100.0% (R_t = 4.851 min).

4.2.2.41. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-(cyclopentylmethyl)-1-(1,3-dimethyl-1H-indazol-5-yl)urea ***(209).*** Starting with compound **161** following the general procedure **9.2**, compound **209** was obtained as white solid**.** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.49 (d, *J* = 1.38 Hz, 1H), 7.43 (br, 2H), 7.32 (d, *J* = 8.70 Hz, 1H), 7.21 (dd, *J* = 8.70, 1.80 Hz, 1H), 7.05 (d, *J* = 8.22 Hz, 1H), 4.61 (t, *J* = 6.0 Hz, 1H), 4.43 (d, *J* = 5.46 Hz, 2H), 3.96 (s, 3H), 3.70 (d, *J* = 7.80 Hz, 2H), 2.52 (s, 3H), 2.03-1.98 (m, 1H), 1.67-1.62 (m, 2H), 1.59-1.55 (m, 2H), 1.46-1.44 (m, 2H), 1.28-1.24 (m, 2H). HRMS (ESI) calc. for $C_{24}H_{29}N_6O$ $[M + H]^+$ 417.2397, found 417.2399.

4.2.2.42. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-(1,3-dimethyl-1H-indazol-5-yl)-1-(4-fluorobenzyl)urea ***(210).*** Starting with compound **162** following the general procedure **9.2**, compound **210** was obtained as white solid, yield 79%**.** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.45 (br, 2H), 7.30 (d, *J* = 1.32 Hz, 1H), 7.22 (d, *J* = 8.70 Hz, 1H), 7.18 (dd, *J* = 8.22, 5.46 Hz, 2H), 7.08 (d, *J* = 8.28 Hz, 1H), 6.97 (dd, *J* = 8.70, 1.86 Hz, 1H), 6.91 (t, *J* = 8.58 Hz, 2H), 4.86 (s, 2H), 4.66 (t, *J* = 6.00 Hz, 1H), 4.47 (d, *J* = 5.94 Hz, 2H), 3.93 (s, 3H), 2.44 (s, 3H). HRMS (ESI) calc. for $C_{25}H_{24}FN_6O$ $[M + H]^+$ 443.1990, found 443.1999.

4.2.2.43. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-cyclobutyl-1-(1,3-dimethyl-1H-indazol-6-yl)urea ***(211).*** Starting with compound **163** following the general procedure **9.2**, compound **211** was obtained as white solid, yield 89%. ^{1}H NMR (300MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.66 (d, *J* = 8.25 Hz, 1H), 7.47-7.43 (m, 2H), 7.14 (s, 1H), 7.02 (d, *J* = 8.22 Hz, 1H), 6.88 (dd, *J* = 8.43, 1.47 Hz, 1H), 5.10-4.98 (m, 1H), 4.66 (t, *J* = 5.85 Hz, 1H), 4.43 (d, *J* = 5.67 Hz, 2H), 3.96 (s, 3H), 2.54 (s, 3H), 2.17-2.09 (m, 2H), 1.87-1.73 (m, 2H), 1.63-1.38 (m, 2H). HRMS (ESI) calc. for $C_{22}H_{25}N_6O$ $[M + H]^+$ 389.2084, found 389.2100.

4.2.2.44. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-cyclopentyl-1-(1,3-dimethyl-1H-indazol-6-yl)urea ***(212).*** Starting with compound **164** following the general procedure **9.2**, compound **212** was obtained as white solid, yield 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.64 (d, *J* = 8.25 Hz, 1H), 7.45 (d, *J* = 9.15 Hz, 1H), 7.44 (s, 1H), 7.15 (d, *J* = 0.93 Hz, 1H), 7.02 (dd, *J* = 8.22, 1.2 Hz, 1H), 6.91

(dd, *J* = 8.25, 1.47 Hz, 1H), 4.94-4.83 (m, 1H). 4.62 (t, *J* = 5.49 Hz, 1H), 4.44 (d, *J* = 5.85 Hz, 2H), 3.95 (s, 3H), 2.53 (s, 3H), 1.92-1.88 (m, 2H), 1.49-1.46 (m, 4H), 1.40-1.26 (m, 2H). HRMS (ESI) calc. for $C_{23}H_{27}N_6O$ $[M + H]^+$ 403.2241, found 403.2259.

4.2.2.45. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-cyclohexyl-1-(1,3-dimethyl-1H-indazol-6-yl)urea ***(213).*** Starting with compound **165** following the general procedure **9.2**, compound **213** was obtained as white solid, yield 75%, mp = 133-134 ^oC. ^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.65 (d, *J* = 8.25 Hz, 1H), 7.51 (d, *J* = 8.61 Hz, 1H), 7.45 (s, 1H), 7.12 (s, 1H), 7.07 (d, *J* = 8.25 Hz, 1H), 6.91 (dd, *J* = 1.65, 8.40 Hz, 1H), 4.56-4.48 (m, 1H), 4.43 (s, 2H), 3.97 (s, 3H), 2.55 (s, 3H), 1.95-1.91 (m, 2H), 1.73-1.69 (m, 2H), 1.56-1.52 (m, 1H), 1.44-1.30 (m, 2H), 1.13-1.02 (m, 2H), 0.89-0.84 (m, 1H). MS (ESI) *m/z* 417 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{28}N_6O$ $[M+H]^+$ 417.2397, found 417.2428. Anal. HPLC 95.4% (R_t = 5.061 min).

4.2.2.46. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-(cyclopentylmethyl)-1-(1,3-dimethyl-1H-indazol-6-yl)urea ***(214).*** Starting with compound **166** following the general procedure **9.2**, compound **214** was obtained as white solid, yield 70%, mp = 122-123 ^oC. ^1H NMR (300 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.66 (d, *J* = 8.43 Hz, 1H), 7.55-7.48 (m, 2H), 7.19 (s, 1H), 7.12 (d, *J* = 8.25 Hz, 1H), 7.00 (d, *J* = 8.40 Hz, 1H), 4.68 (t, *J* = 4.59 Hz, NH), 4.48 (d, *J* = 5.88 Hz, 2H), 3.96 (s, 3H), 3.77 (d, *J* = 7.50 Hz, 2H), 2.54 (s, 3H), 2.10-2.01 (m, 1H), 1.75-1.62 (m, 4H), 1.52-1.48 (m, 2H), 1.30-1.26 (m, 4H). MS (ESI) *m/z* 417 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{28}N_6O$ $[M+H]^+$ 417.2397, found 417.2424. Anal. HPLC 100.0% (R_t = 5.173 min).

4.2.2.47. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-(1,3-dimethyl-1H-indazol-6-yl)-1-(4-fluorobenzyl)urea ***(215).*** Starting with compound **167** following the general procedure **9.2**, compound **215** was obtained as white solid**.** ^1H NMR (300 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.58 (d, *J* = 8.97 Hz, 1H), 7.53-7.49 (m, 2H), 7.22 (dd, *J* = 8.25, 5.49 Hz, 1H), 7.11 (d, *J* = 8.25 Hz, 1H), 6.98 (s, 1H), 6.94 (t, *J* = 8.61 Hz, 2H), 6.81 (d, *J* = 8.40 Hz, 1H), 4.92 (s, 2H), 4.79 (t, *J* = 5.67 Hz, 1H), 4.50 (d, *J* = 5.67 Hz, 2H), 3.87 (s, 3H), 2.51 (s, 3H).

4.2.2.48. 3-((1H-Benzo[d]imidazol-5-yl)methyl)-1-(1,3-dimethyl-1H-indazol-6-yl)-1-(1-methylpiperidin-4-yl)urea ***(216)***. Starting with compound **168** following

the general procedure **9.2**, compound **216** was obtained as white solid, yield 51%, mp = 74-75 ^{o}C. ^{1}H NMR (300 MHz, MeOH) δ 8.10 (s, 1H), 7.77 (d, *J* = 8.43 Hz, 1H), 7.50 (d, *J* = 8.25 Hz, 1H), 7.44 (s, 1H), 7.37 (d, *J* = 0.90 Hz, 1H), 7.14 (dd, *J* = 1.26, 8.40 Hz, 1H), 6.95 (dd, *J* = 1.65, 8.40 Hz, 1H), 4.47-4.42 (m, 1H), 4.37 (s, 2H), 3.94 (s, 3H), 2.88-2.84 (m, 2H), 2.50 (s, 3H), 2.19 (s, 3H), 2.15-2.12 (m, 2H), 1.92-1.87 (m, 2H), 1.53-1.41 (m, 2H). MS (ESI) *m/z* 432 $[M+H]^{+}$. HRMS (ESI) calc. for $C_{24}H_{29}N_{7}O$ $[M+H]^{+}$ 432.2506, found 432.2540. Anal. HPLC 97.5% (R_t = 3.055 min).

*4.2.2.49. 1-Cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-methyl-1H-indol-5-yl)urea **(217).*** Starting with compound **95** following the general procedure **10**, compound **217** was obtained as white solid, yield 56%, mp = 51-52 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (d, *J* = 1.65 Hz, 1H), 7.33 (d, *J* = 8.61 Hz, 1H), 7.12 (d, *J* = 3.12 Hz, 1H), 6.94 (dd, *J* = 2.01, 8.61 Hz, 1H), 6.66 (s, 1H), 6.49 (dd, *J* = 0.72, 3.12 Hz, 1H), .4.48-4.40 (m, 1H), 3.99 (t, *J* = 5.70 Hz, NH), 3.81 (s, 3H), 3.77 (t, *J* = 7.32 Hz, 2H), 3.12 (q, *J* = 6.60 Hz, 2H), 2.08 (d, *J* = 0.93 Hz, 3H), 1.89-1.85 (m, 2H), 1.82 (p, *J* = 6.78 Hz, 2H), 1.72-1.66 (m, 2H), 1.53-1.49 (m, 2H), 1.43-1.30 (m, 2H0, 1.09-0.97 (m, 2H), 0.88-0.80 (m, 1H). MS (ESI) *m/z* 394 $[M+H]^{+}$. HRMS (ESI) calc. for $C_{23}H_{31}N_{5}O$ $[M+H]^{+}$ 394.2601, found 394.2613. Anal. HPLC 99.5% (R_t = 5.339 min).

*4.2.2.50. 1-(Cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-methyl-1H-indol-5-yl)urea **(218)).*** Starting with compound **96** following the general procedure **10**, compound **218** was obtained as white solid, yield 56%, mp = 66-67 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 1.65 Hz, 1H), 7.35 (d, *J* = 8.61 Hz, 1H), 7.25 (s, 1H), 7.12 (d, *J* = 3.12 Hz, 1H), 7.03 (dd, *J* = 1.83, 8.40 Hz, 1H), 6.67 (s, 1H), 6.49 (d, *J* = 3.09 Hz, 1H), 4.21 (t, *J* = 5.67 Hz, NH), 3.81 (s, 3H), 3.79 (t, *J* = 7.35 Hz, 2h), 3.66 (d, *J* = 7.50 Hz, 2H), 3.15 (q, *J* = 6.60 Hz, 2H), 2.09 (d, *J* = 0.72 Hz, 3H), 2.04-1.94 (m, 1H), 1.83 (p, *J* = 6.75 Hz, 2H0, 1.65-1.57 (m, 4H), 1.48-1.42 (m, 2H), 1.30-1.20 (m, 2H). MS (ESI) *m/z* 394 $[M+H]^{+}$. HRMS (ESI) calc. for $C_{23}H_{31}N_{5}O$ $[M+H]^{+}$ 394.2601, found 394.2596. Anal. HPLC 100.0% (R_t = 5.480 min).

*4.2.2.51. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-methyl-1H-indol-5-yl)urea **(219).*** Starting with compound **97** following the general procedure **10**, compound **219** was obtained as white solid, yield 51%, mp = 55-56 ^{o}C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27-7.24 (m, 2H), 7.20 (dd, *J* = 5.49, 8.58 Hz, 2H), 7.10 (d, *J* = 3.12 Hz, 1H), 6.94 (t, *J* = 8.79 Hz, 2H), 6.81 (dd, *J* = 2.01 Hz, 1H), 6.67 (s, 1H), 6.43 (d, *J* = 3.12 Hz, 1H), 4.82 (s, 2H), 4.27 (t, *J* = 5.94 Hz, NH), 3.78 (s, 3H0, 3.75 (t, *J* = 7.32 Hz, 2H), 3.18 (q, *J* = 6.57 Hz, 2H), 2.09 (d, *J* = 0.90 Hz, 3H), 1.86 (p, *J* = 7.50 Hz, 2H). MS (ESI) *m/z* 420 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{26}FN_5O$ $[M+H]^+$ 420.2194, found 420.2195. Anal. HPLC 95.6% (R_t = 4.317 min

4.2.2.52. 1-Cyclohexyl-1-(1,3-dimethyl-1H-indol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(220).*** Starting with compound **98** following the general procedure **10**, compound **220** was obtained as white solid, yield 55%, mp = 79-80 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.28-7.25 (m, 2H), 6.92-6.89 (m, 2H), 6.71 (s, 1H), 4.48-4.40 (m, 1H), 4.00 (t, *J* = 5.04 Hz, NH), 3.81 (t, *J* = 7.32 Hz, 2H), 3.75 (s, 3H), 3.11 (q, *J* = 6.96 Hz, 2H), 2.29 (s, 3H), 2.10 (s, 3H), 1.90-1.86 (m, 2H), 1.80 (p, *J* = 6.96 Hz, 2H), 1.71-1.67 (m, 2H), 1.55-1.49 (m, 1H), 1.40-1.36 (m, 2H), 1.16-1.07 (m, 2H), 0.85-0.81 (m, 1H). MS (ESI) *m/z* 408 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{33}N_5O$ $[M+H]^+$ 408.2758, found 408.2764. Anal. HPLC 96.9% (R_t = 6.637 min).

4.2.2.53. 1-(Cyclopentylmethyl)-1-(1,3-dimethyl-1H-indol-5-yl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(221).*** Starting with compound **99** following the general procedure **10**, compound **221** was obtained as white solid, yield 51%, mp = 53-53 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.35 (m, 2H), 7.29 (d, *J* = 8.55 Hz, 1H), 7.00 (dd, *J* = 1.70, 8.45 Hz, 1H), 6.89 (s, 1H), 6.70 (s, 1H), 4.24 (t, *J* = 5.65 Hz, NH), 3.80 (t, *J* = 7.25 Hz, 2H), 3.74 (s, 3H), 3.67 (d, *J* = 7.70 Hz, 2H), 3.14 (q, *J* = 6.35 Hz, 2H), 2.29 (s, 3H), 2.10 (s, 3H), 2.03-1.99 (m, 1H), 1.82 (p, *J* = 6.75 Hz, 2H), 1.65-1.59 (m, 4H), 1.47-1.45 (m, 2H), 1.30-1.23 (m, 2H). MS (ESI) *m/z* 408 $[M+H]^+$. HRMS (ESI) calc. for $C_{24}H_{33}N_5O$ $[M+H]^+$ 408.2758, found 408.2765. Anal. HPLC 98.6% (R_t = 6.982 min).

4.2.2.54. 1-(1,3-Dimethyl-1H-indol-5-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(222).*** Starting with compound **100** following the general procedure **10**, compound **222** was obtained as white solid, yield 51%, mp = 71-72 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 1H), 7.16-7.12 (m, 4H), 6.89 (t, *J* = 8.79 Hz, 2H), 6.81 (s, 1H), 6.72 (dd, *J* = 1.83, 8.40 Hz, 1H), 6.66 (s, 1H), 4.77 (s, 2H), 4.25 (t, *J* = 5.67 Hz, NH), 3.79 (t, *J* = 7.14 Hz, 2H), 3.66 (s, 3H), 3.14 (q, *J* =

6.39 Hz, 2H), 2.17 (s, 3H), 2.06 (s, 3H), 1.81 (p, *J* = 6.60 Hz, 2H). MS (ESI) *m/z* 434 $[M+H]^+$. HRMS (ESI) calc. for $C_{25}H_{28}FN_5O$ $[M+H]^+$ 434.2351, found 434,2363. Anal. HPLC 99.6% (R_t = 5.012 min).

4.2.2.55. 1-Cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-propyl-1H-indol-5-yl)urea ***(223).*** Starting with compound **101** following the general procedure **10**, compound **223** was obtained as white solid, yield 51%, mp = 44-45 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 1.76 Hz, 1H), 7.33 (d, *J* = 8.60 Hz, 1H), 7.25 (s, 1H), 7.16 (d, *J* = 3.08 Hz, 1H), 6.90 (dd, *J* = 1.88, 8.56 Hz, 1H), 6.66 (s, 1H), 6.49 (d, *J* = 3.08 Hz, 1H), 4.48-4.40 (m, 1H), 4.10 (t, *J* = 7.12 Hz, 2H), 4.01 (t, *J* = 5.76 Hz, NH), 3.77 (t, *J* = 7.36 Hz, 2H), 3.12 (q, *J* = 6.52 Hz, 2H), 2.07 (s, 3H), 1.92-1.85 (m, 4H), 1.81 (p, *J* = 6.76 Hz, 2H), 1.69-1.66 (m, 2H), 1.52-1.49 (m, 1H), 1.43-1.32 (m, 2H), 1.10-1.00 (m, 2H), 0.98 (t, *J* = 7.44 Hz, 3H), 0.87-0.81 (m, 1H). MS (ESI) *m/z* 422 $[M+H]^+$. HRMS (ESI) calc. for $C_{25}H_{35}N_5O$ $[M+H]^+$ 422.2914, found 422.2929. Anal. HPLC 98.7% (R_t = 7.266 min).

4.2.2.56. 1-(Cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-propyl-1H-indol-5-yl)urea ***(224).*** Starting with compound **102** following the general procedure **10**, compound **224** was obtained as white solid, yield 56%, mp = 52-53 ^oC. ^1H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, *J* = 1.83 Hz, 1H), 7.36 (d, *J* = 8.61 Hz, 1H), 7.27 (s, 1H), 7.17 (d, *J* = 3.09 Hz, 1H), 7.00 (dd, *J* = 2.01, 8.61 Hz, 1H), 6.67 (s, 1H), 6.48 (d, *J* = 3.12 Hz, 1H), 4.26 (t, *J* = 5.67 Hz, NH), 4.10 (t, *J* = 6.96 Hz, 2H), 3.79 (t, *J* = 7.14 Hz, 2H), 3.66 (d, *J* = 7.68 Hz, 2H), 3.16 (q, *J* = 6.60 Hz, 2H), 2.09 (d, *J* = 1.11 Hz, 3H), 1.88-1.75 (m, 4H), 1.68-1.58 (m, 4H), 1.60-1.42 (m, 2H), 1.27 (t, *J* = 7.14 Hz, 2H), 0.97 (t, *J* = 7.32 Hz, 3H). MS (ESI) *m/z* 422 $[M+H]^+$. HRMS (ESI) calc. for $C_{25}H_{35}N_5O$ $[M+H]^+$ 422.2914, found 422.2930. Anal. HPLC 100.0% (R_t = 7.708 min).

4.2.2.57. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(1-propyl-1H-indol-5-yl)urea ***(225).*** Starting with compound **103** following the general procedure **10**, compound **225** was obtained as white solid, yield 58%, mp = 73-74 ^oC. ^1H NMR (300 MHz, $CDCl_3$) δ 7.28 (s, 1H), 7.24 (s, 1H), 7.22-7.17 (m, 2H), 7.14 (d, *J* = 3.12 Hz, 1H), 6.94 (t, *J* = 8.76 Hz, 2H), 6.76 (dd, *J* = 2.01, 8.58 Hz, 1H), 6.67 (s,

1H), 6.42 (d, *J* = 2.37 Hz, 1H), 4.81 (s, 2H), 4.31 (t, *J* = 4.59 Hz, NH), 4.07 (t, *J* = 7.14 Hz, 2H), 3.80 (t, *J* = 7.14 Hz, 2H), 3.19 (q, *J* = 6.57 Hz, 2H), 2.08 (d, *J* = 0.90 hz, 3H0, 1.88-1.77 (m, 4H), 0.95 (t, *J* = 7.35 Hz, 3H). MS (ESI) *m/z* 448 $[M+H]^+$. HRMS (ESI) calc. for $C_{26}H_{30}FN_5O$ $[M+H]^+$ 448.2507, found 448.2520. Anal. HPLC 100.0% (R_t = 4.851 min).

4.2.2.58. 1-Cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methylbenzofuran-5-yl)urea ***(226).*** Starting with compound **112** following the general procedure **10**, compound **226** was obtained as white solid, yield 42%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47-7.45 (m, 2H), 7.27 (m, 1H), 7.00 (dd, *J* = 8.61, 2.19 Hz, 1H), 6.67 (s, 1H), 4.49-4.41 (tt-like, 1H), 3.89 (t-like, 1H), 3.77 (t, *J* = 7.32 Hz, 2H), 3.12 (q, *J* = 6.42 Hz, 2H), 2.23 (d, *J* = 1.11 Hz, 3H), 2.09 (d, *J* = 0.72 Hz, 3H), 1.89-1.78 (m, 4H), 1.70 (d, *J* = 12.27 Hz, 2H), 1.54 (d-like, 1H), 1.45-1.32 (m, 2H), 1.07-0.99 (m, 2H), 0.92-0.78 (m, 1H). HRMS (ESI) calc. for $C_{23}H_{31}N_4O_2$ $[M + H]^+$ 395.2442, found 395.2448.

4.2.2.59. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methylbenzofuran-5-yl)urea ***(227).*** Starting with compound **113** following the general procedure **10**, compound **227** was obtained as white solid, yield 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 1.08 Hz, 1H), 7.38 (d, *J* = 8.58 Hz, 1H), 7.29 (s, 1H), 7.20-7.13 (m, 3H), 6.92 (t, *J* = 8.61 Hz, 2H), 6.85 (dd, *J* = 8.61, 2.19 Hz, 1H), 6.68 (s, 1H), 4.81 (s, 2H), 4.19 (t, *J* = 5.70 Hz, 1H), 3.79 (t, *J* = 7.14 Hz, 2H), 3.17 (q, *J* = 6.21 Hz, 2H), 2.16 (d, *J* = 1.29 Hz, 3H), 2.10 (d, *J* = 0.90 Hz, 3H), 1.84 (quintet, *J* = 7.14 Hz, 2H). HRMS (ESI) calc. for $C_{24}H_{26}FN_4O_2$ $[M + H]^+$ 421.2034, found 421.2048.

4.2.2.60. 1-(4-Fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-1-(3-methylbenzofuran-6-yl)urea ***(228).*** Starting with compound **114** following the general procedure **10**, compound **228** was obtained as white solid, yield 22%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.22 Hz, 1H), 7.38 (q, *J* = 1.11 Hz, 1H), 7.15-7.10 (m, 3H), 7.05 (d, *J* = 1.47 Hz, 1H), 6.90-6.79 (m, 4H), 4.76 (s, 2H), 4.23 (t-like, 1H), 3.77 (t-like, 2H), 3.13 (q, *J* = 5.88 Hz, 2H), 2.16 (d, *J* = 1.29 Hz, 3H), 2.06 (s, 3H), 1.81-1.74 (m, 2H). HRMS (ESI) calc. for $C_{24}H_{26}FN_4O_2$ $[M + H]^+$ 421.2034, found 421.2043.

*4.2.2.61. 1-(Benzo[d]oxazol-5-yl)-1-cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(229).*** Starting with compound **133** following the general procedure **10**, compound **229** was obtained as white solid, yield 55%, mp = 84-85 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.61 (d, *J* = 8.61 Hz, 1H), 7.55 (d, *J* = 1.83 Hz, 1H), (s, 1H), 7.14 (dd, *J* = 2.01, 8.58 Hz, 1H), 6.64 (s, 1H), 4.47-4.396 (m, 1H), 3.80 (t, *J* = 4.95 Hz, NH), 3.77 (t, *J* = 7.14 Hz, 2H), 3.13 (q, *J* = 6.57 Hz, 2H), 2.07 (d, *J* = 0.93 Hz, 3H), 1.86-1.83 (m, 2H), 1.81 (p, *J* = 7.14 Hz, 2H), 1.70-1.65 (m, 2H), 1.53-1.49 (m, 1H), 1.42-1.29 (m, 2H), 1.01-0.93 (m, 2H), 0.88-0.78 (m, 1H). MS (ESI) *m/z* 382 $[M+H]^+$. HRMS (ESI) calc. for $C_{21}H_{27}N_5O$ $[M+H]^+$ 328.2238, found 382.2251. Anal. HPLC 96.1% (R_t = 4.279 min).

*4.2.2.62. 1-(Benzo[d]oxazol-5-yl)-1-(cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(230).*** Starting with compound **134** following the general procedure **10**, compound **230** was obtained as white solid, yield 57%, mp = 60-61 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.65-7.62 (m, 2H), 7.28 (s, 1H), 7.25 (dd, *J* = 2.01, 8.40 Hz, 1H), 6.67 (s, 1H), 4.14 (t, *J* = 5.04 Hz, NH), 3.81 (t, *J* = 7.14 Hz, 2H), 3.67 (d, *J* = 7.68 Hz, 2H), 3.18 (q, *J* = 6.78 Hz, 2H), 2.10 (s, 3H), 1.98-1.93 (m, 1H), .87 (p, *J* = 7.14 Hz, 2H), 1.65-1.57 (m, 4H), 1.50-1.43 (m, 2H), 1.30-1.19 (m, 2H). MS (ESI) *m/z* 382 $[M+H]^+$. HRMS (ESI) calc. for $C_{21}H_{27}N_5O_2$ $[M+H]^+$ 382.2238, found 382.2250. Anal. HPLC 95.6% (R_t = 4.337 min).

*4.2.2.63. 1-(Benzo[d]oxazol-5-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(231).*** Starting with compound **135** following the general procedure **10**, compound **231** was obtained as white solid, yield 51%, mp = 74-75 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.56 (d, *J* = 8.61 Hz, 1H), 7.45 (d, *J* = 2.01 Hz, 1H), 7.28 (s, 1H), 7.17 (dd, *J* = 5.31, 8.61 Hz, 1H), 7.04 (dd, *J* = 2.01, 8.40 Hz, 1H), 6.94 (tt, *J* = 2.19, 8.61 Hz, 1H), 6.67 (s, 1H), 4.83 (s, 2H), 4.16 (t, *J* = 4.95 Hz, NH), 3.82 (t, *J* = 7.14 Hz, 2H), 3.22 (q, *J* = 6.78 Hz, 2H), 2.10 (d, *J* = 0.90 Hz, 3H), 1.90 (p, *J* = 6.96 Hz, 2H). MS (ESI) *m/z* 408 $[M+H]^+$. HRMS (ESI) calc. for $C_{22}H_{22}FN_5O_2$ $[M+H]^+$ 408.1830, found 408.1841. Anal. HPLC 96.1% (R_t = 4.279 min).

*4.2.2.64. 1-(Benzo[d]oxazol-6-yl)-1-cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(232)***. Starting with compound **136** following the general procedure

10, compound **232** was obtained as white solid, yield 45%, mp = 96-97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.78 (d, *J* = 8.43 Hz, 1H), 7.33 (d, *J* = 1.65 Hz, 1H), 7.23 (s, 1H), 7.10 (dd, *J* = 1.83, 8.25 Hz, 1H), 6.62 (s, 1H), 4.46-4.35 (m, 1H), 3.81 (t, *J* = 6.03 Hz, NH), 3.75 (t, *J* = 7.14 Hz, 2H), 3.12 (q, *J* = 6.57 Hz, 2H), 2.05 (s, 3H), 1.84-1.81 (m, 2H), 1.78 (p, *J* = 7.14 Hz, 2H), 1.68-1.63 (m, 2H), 1.51-1.47 (m, 1H), 1.39-.26 (m, 2H), 1.01-0.92 (m, 2H), 0.89-0.73 (m, 1H). MS (ESI) *m/z* 382 $[M+H]^+$. HRMS (ESI) calc. for $C_{21}H_{27}N_5O_2$ $[M+H]^+$ 382.2238, found 382.2248. Anal. HPLC 97.1% (R_t = 4.270 min).

*4.2.2.65. 1-(Benzo[d]oxazol-6-yl)-1-(cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(233).*** Starting with compound **137** following the general procedure **10**, compound **233** was obtained as white solid, yield 55%, mp = 130-131 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.78 (d, *J* = 8.40 Hz, 1H), 7.41 (d, *J* = 1.83 Hz, 1H), 7.25 (s, 1H), 7.18 (dd, *J* = 2.01, 8.43 Hz, 1H), 6.62 (s, 1H), 4.12 (t, *j* = 5.85 Hz, NH), 3.77 (t, *j* = 7.14 Hz, 2H), 3.63 (d, *j* = 7.68 Hz, 2H), 3.15 (q, *J* = 6.78 Hz, 2H), 2.06 (s, 3H), 1.97-1.86 (m, 1H), 1.83 (p, *J* = 6.75 Hz, 2H), 1.61-1.53 (m, 4H), 1.48-1.42 (m, 2H), 1.21-1.16 (m, 2H). MS (ESI) *m/z* 382 $[M+H]^+$. HRMS (ESI) calc. for $C_{21}H_{27}N_5O_2$ $[M+H]^+$ 382.2238, found 382.2250. Anal. HPLC 95.6% (R_t = 4.334 min).

*4.2.2.66. 1-(Benzo[d]oxazol-6-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(234).*** Starting with compound **138** following the general procedure **10**, compound **234** was obtained as white solid, yield 55%, mp = 48-49 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.76 (d, *J* = 8.43 Hz, 1H), 7.30 (s, 1H), 7.18 (dd, *J* = 5.49, 8.43 Hz, 2H), 7.06 (dd, *J* = 1.83, 8.22 Hz, 1H), 6.67 (s, 1H), 4.83 (s, 2H0, 4.18 (t, *J* = 6.03 Hz, NH), 3.83 (t, *J* = 7.14 Hz, 2H), 3.12 (q, *J* = 6.42 Hz, 2H), 2.11 (d, *J* = 0.72 Hz, 3H), 1.88 (p, *J* = 6.57 Hz, 2H). MS (ESI) *m/z* 408 $[M+H]^+$. HRMS (ESI) calc. for $C_{22}H_{22}FN_5O_2$ $[M+H]^+$ 408.1830, found 408.1837.

*4.2.2.67. 1-(Benzo[d]thiazol-5-yl)-1-cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea **(235).*** Starting with compound **139** following the general procedure **10**, compound **235** was obtained as white solid, yield 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.02 (d, *J* = 8.43 Hz, 1H), 7.93 (d, *J* = 1.83 Hz, 1H), 7.29 (s, 1H), 7.22 (dd, *J* = 8.43, 2.01 Hz, 1H), 6.68 (s, 1H), 4.50 (tt, *J* = 12.06, 3.69 Hz, 1H),

3.92 (t, *J* = 5.49 Hz, 1H), 3.80 (t, *J* = 7.32 Hz, 2H), 3.15 (q, *J* = 6.21 Hz, 2H), 2.11 (s, 3H), 1.92 (d, *J* = 12.99 Hz, 2H), 1.88-1.79 (m, 2H), 1.73 (d, *J* = 13.17 Hz, 2H), 1.56 (d, *J* = 12.45 Hz, 1H), 1.47-1.34 (m, 2H), 1.04 (qd, *J* = 12.27, 3.66 Hz, 2H), 0.90-0.80 (m, 1H).

4.2.2.68. 1-(Benzo[d]thiazol-5-yl)-1-(cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(236).*** Starting with compound **140** following the general procedure **10**, compound **236** was obtained as white solid, yield 71%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.01 (d, *J* = 8.58 Hz, 1H), 7.98 (d, *J* = 1.83 Hz, 1H), 7.31-7.28 (m, 2H), 6.66 (s, 1H), 4.19 (t-like, 1H), 3.79 (t, *J* = 7.14 Hz, 2H), 3.70 (d, *J* = 7.68 Hz, 2H), 3.16 (q, *J* = 6.06 Hz, 2H), 2.10 (d, *J* = 0.57 Hz, 3H), 2.01-1.94 (m, 1H), 1.83 (quintet, *J* = 7.14 Hz, 2H), 1.70-1.55 (m, 4H), 1.50-1.45 (m, 2H), 1.27-1.23 (m, 2H). HRMS (ESI) calc. for $C_{21}H_{28}N_5OS$ $[M + H]^+$ 398.2009, found 398.2020.

4.2.2.69. 1-(Benzo[d]thiazol-5-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(237).*** Starting with compound **141** following the general procedure **10**, compound **237** was obtained as white solid, yield 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.94 (d, *J* = 8.40 Hz, 1H), 7.84 (d, *J* = 2.01 Hz, 1H), 7.31 (s, 1H), 7.22-7.18 (dd, *J* = 8.43, 7.29 Hz, 2H), 7.10 (dd, *J* = 8.40, 2.04 Hz, 2H), 6.68 (s, 1H), 4.89 (s, 2H), 4.29 (t, *J* = 5.88 Hz, 1H), 3.83 (t, *J* = 7.14 Hz, 2H), 3.22 (q, *J* = 6.60 Hz, 2H), 2.12 (s, 3H), 1.88 (quintet, *J* = 7.14 Hz, 2H). HRMS (ESI) calc. for $C_{22}H_{23}FN_5OS$ $[M + H]^+$ 424.1602, found 424.1609.

4.2.2.70. 1-(Benzo[d]thiazol-6-yl)-1-cyclohexyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(238).*** Starting with compound **142** following the general procedure **10**, compound **238** was obtained as white solid, yield 64%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.09 9s, 1H), 8.18 (d, *J* = 8.40 Hz, 1H), 7.75 (d, *J* = 1.83 Hz, 1H), 7.33 (s, 1H), 7.30-7.27 (m, 1H), 6.69 (s, 1H), 4.49 (tt, *J* = 8.22, 3.27 Hz, 1H), 3.91 (t, *J* = 6.60 Hz, 1H), 3.80 (t, *J* = 7.14 Hz, 2H), 3.16 (q, *J* = 6.57 Hz, 2H), 2.11 (s, 3H), 1.92-1.71 (m, 6H), 1.56 (d, *J* = 13.2 Hz, 1H), 1.47-1.34 (m, 2H), 1.10-0.97 (m, 2H), 0.89-0.80 (m, 1H). HRMS (ESI) calc. for $C_{21}H_{28}N_5OS$ $[M + H]^+$ 398.2009, found 398.2023.

4.2.2.71. 1-(Benzo[d]thiazol-6-yl)-1-(cyclopentylmethyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(239).*** Starting with compound **143** following the general procedure **10**, compound **239** was obtained as off-white solid, yield 73%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.15 (d, *J* = 8.61 Hz, 1H), 7.79 (d, *J* = 2.01 Hz,

1H), 7.34 (dd, *J* = 8.61, 2.19 Hz, 1H), 7.29 (s, 1H), 6.66 (s, 1H), 4.17 (t, *J* = 5.85 Hz, 1H), 3.79 (t, *J* = 7.32 Hz, 2H), 3.68 (d, *J* = 7.77 Hz, 2H), 3.16 (q, *J* = 6.24 Hz, 2H), 2.10 (s, 3H), 1.99-1.92 (m, 2H), 1.83 (quintet, *J* = 6.96 Hz, 2H), 1.62-1.54 (m, 4H), 1.50-1.47 (m, 2H), 1.27-1.24 (m, 2H). HRMS (ESI) calc. for $C_{21}H_{28}N_5OS$ $[M + H]^+$ 398.2009, found 398.2020.

4.2.2.72. 1-(Benzo[d]thiazol-6-yl)-1-(4-fluorobenzyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)urea ***(240)*****.** Starting with compound **144** following the general procedure **10**, compound **240** was obtained as white solid, yield 49%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.09 (d, *J* = 8.58 Hz, 1H), 7.62 (d, *J* = 2.01 Hz, 1H), 7.32 (s, 1H), 7.22-7.17 (m, 3H), 6.99-6.92 (m, 2H), 6.67 (s, 1H), 4.87 (s, 2H), 4.34 (t, *J* = 5.67 Hz, 2H), 3.83 (t, *J* = 7.20 Hz, 2H), 3.23 (q, *J* = 6.78 Hz, 2H), 2.12 (s, 3H), 1.88 (quintet, *J* = 6.96 Hz, 2H). HRMS (ESI) calc. for $C_{22}H_{23}FN_5OS$ $[M + H]^+$ 424.1602, found 424.1614.

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알츠하이머 병 (Alzheimer 's disease, AD)은 기억력 상실과 인지력을 심각하게 저하시키는 지속적이고, 끊임 없는 신경 퇴행성의 장애로 간주된다. 지금까지 뇌에서 신경 독성 Aβ 종의 형성 수준을 낮추는 것이 AD 치료의 우선적인 병리학으로 간주되고 있다. 최근 연구사례들에 따르면 AD 환자의 뇌에서 과도하게 발현되어 농도가 높아진 pyroglutamate (pE-Aβ)는 Aβ 보다 빠르게 응집되고 더 큰 독성을 갖고 있다고 한다. pE-Aβ 는 Glutaminyl Cyclase(QC) 라는 효소에 의해 Amino-terminally 절단된 Aβ 의 glutamate 3 또는 11 을 기질로 하여 cyclization 이 촉매작용이 일어나서 만들어진 생성물이다.

최근 임상 연구에 따르면 QC 는 AD 치료에 대한 대체적 치료표적이 될 수 있음을 보여주고 있다. 우리 연구 그룹에서는 일련의 QC 저해제의 연구 개발, 특히 기존의 저해제를 기준으로 B-region 과 C-region 에서의 구조적 변형을 통해 좀더 확장된 구조에 초점을 맞추고 있다. 이 연구에는 저희는 우선 Arg region 을 모방한 유사체의 구조 활성 관계 (SAR)에 대해 연구했다. Arg region 시리즈에서 대부분의 화합물들은 *in-vitro* 실험에서 우수한 활성을 보여주었다. 그중 $IC_{50} < 10$ nM 의 우수한 활성을 화합물들을 선별하여 in-vivo 실험을 진행하였다. 그 결과 2-aminopyridi 에 aminoethyl 을 도입한 경우 in-vitro 실험에서 약 2.5 배의 활성이 증가됨을 보였다. 그리고 분자 도킹 모델 연구를 통해 화합물 **202** 가 hQC 의 active site 에 추가적인 hydrophobic interation 을 보여줌으로써 QC 저해제로서 유력한 후보로 기대된다.

앞서 언급된 결과들을 바탕으로 part2 에서는 위에서 설계한 약리활성 구조를 대체하기 위해 3-alinoalaklyloxy-4-methoxyphele 및 4-aminoalkoxheyphel 을 포함한 새로운 QC 저해제를 계속해서 개발하였다. IC50 값을 기반으로 활성이 우수한 화합물들을 식별하였다. 그렇게 선별된 화합물들은 *in vitro* 독성과 *in*

vivo 활성을 추가로 진행하였다. 그 결과 QC 저해제 51 과 53 은 BBB 투과가 가능한 *in vivo* 급성 모델에서 우수한 $A\beta_{N3pE-40}$-Lowering Effect 를 나타냈고, cytotoxicity 와 hERG 저해를 나타내지 않았다. 두 화합물 중 모델 분석의 대상으로 화합물 **53** 을 선택했다. 모델 결과 **53** 의 우수한 효과는 활성부위에서의 염다리효과와 수소결합이라고 설명할 수 있다. BBB 의 투과가 더 양호한 이 화합물은 Anti-Alzheimer 에이전트의 잠재적 후보가 될 수 있을 것이다

마지막으로 중요한 것은 C 지역의 dimethoxy 이 안정적이지 않고 간 신진대사에 독성을 일으키며 B-region 의 변형은 *in-vitro* 활성을 증가시킨다는 것을 발견했다. 따라서, 새로운 heterocyclic 을 C-region 과 B-region 과 C-region 의 연결 고리에 계속 도입했다. 그러나, 모 화합물과 비교하여, C 영역의 헤테로 고리, 특히 인돌, 벤조 퓨란, 벤조 옥사졸, 벤조 티아졸의 고리들은 활성을 2.0 ~ 6.0 배 감소시킨 다는 것을 보였다. 흥미롭게도 일부의 고리 구조들은 B 영역에 도입되었을 때 *in vitro* 활성이 약간 증가함을 보였다. 이러한 결과들은 잠재적인 독성이 없고 우수한 투과력과 활성을 갖는 QC 저해제의 개발에 있어 선도물질 최적화에 귀중한 정보를 줄것이라고 생각한다.

키워드: Alzheimer Disease, Glutaminyl Cyclase, Aβ, *h*QC, pyroglutamate;

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