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Potent human glutaminyl cyclase inhibitors as potential anti-Alzheimer's agents: Structure-activity relationship study of Arg-mimetic region

Van T.H. Ngo [a], Van-Hai Hoang [a], Phuong-Thao Tran [b], Jihyae Ann [a], Minghua Cui [c], Gyungseo Park [c], Sun Choi [c], Jiyoun Lee [d], Hee Kim [e], Hee-Jin Ha [e], Kwanghyun Choi [e], Young-Ho Kim [e], Jeewoo Lee [a,*]

[a] Laboratory of Medicinal Chemistry, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea
[b] Department of Medicinal Chemistry, Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Hoan Kiem, Hanoi, Viet Nam
[c] National Leading Research Laboratory of Molecular Modeling & Drug Design, College of Pharmacy and Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul 03760, Republic of Korea
[d] Department of Global Medical Science, Sungshin University, Seoul 01133, Republic of Korea
[e] Medifron DBT, Sandanro 349, Danwon-Gu, Ansan-City, Gyeonggi-Do 15426, Republic of Korea

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ABSTRACT

Pyroglutamate-modified amyloid β peptides (pGlu-Aβ) are highly neurotoxic and promote the formation of amyloid plaques. The pGlu-Aβ peptides are generated by glutaminyl cyclase (QC), and recent clinical studies indicate that QC represents an alternative therapeutic target to treat Alzheimer's disease (AD). We have previously developed a series of QC inhibitors with an extended pharmacophoric scaffold, termed the Arg-mimetic D-region. In the present study, we focused on the structure activity relationship (SAR) of analogues with modifications in the D-region and evaluated their biological activity. Most compounds in this series exhibited potent activity in vitro, and our SAR analysis and the molecular docking studies identified compound **202** as a potential candidate because it forms an additional hydrophobic interaction in the *h*QC active site. Overall, our study provides valuable insights into the Arg-mimetic pharmacophore that will guide the design of novel QC inhibitors as potential treatments for AD.

1. Introduction

β-Amyloid (Aβ) peptides are produced by the sequential cleavage of the amyloid precursor protein (APP), generating a heterogeneous mixture of peptides of various sizes. These Aβ peptides not only exhibit distinct structural and functional features[1,2] but also participate in a wide range of physiological processes, including neurogenesis,[3] neuronal survival,[4–6] oxidative stress,[7,8] and innate immunity.[9] Although elevated levels of Aβ peptides and amyloid plaques are thought to be the hallmarks of Alzheimer's disease (AD) pathology, given the diverse roles of Aβ peptides, general blockade of Aβ production may not result in effective therapeutic outcomes.[10,11]

The brain Aβ pool consists of a mixture of peptides containing 30–51 amino acid residues. $Aβ_{1–40}$ and $Aβ_{1–42}$ are the most abundant isoforms and are considered the major initiators of AD pathogenesis. In addition, a significant amount of the *N*-terminally truncated species, such as $Aβ_{n-40/42}$, where n ranges from 2 to 11, is also present in the brains of patients with AD.[12–14] These *N*-terminally truncated species undergo enzyme-catalyzed cyclization to form pyroglutamate (pGlu)-Aβ products that are prone to rapid aggregation and are much more resistant to proteolytic degradation due to their increased hydrophobicity. Moreover, pGlu-Aβ peptides are more neurotoxic than $Aβ_{1–40}$ and $Aβ_{1–42}$ and promote the formation of amyloid and tau plaques.[15–17] Considering the prion-like behavior and high neurotoxicity of these cyclized peptides, strategies that specifically target pGlu-Aβ peptides may offer an efficient alternative to conventional anti-Aβ strategies.

The pGlu-Aβ peptides are produced by glutaminyl cyclase (QC), which is mainly located in the pituitary, hypothalamus, and brain.[18] Human QC is implicated in various pathological conditions, including inflammation,[19] septic arthritis,[20] Huntington's disease,[21] and Alzheimer's disease.[22,23] In particular, QC is upregulated in the brains of patients and animal models with AD,[22,23] and the enzymatic activity of QC correlates with high brain levels of $Aβ_{1–8}$ and $Aβ_{1–40}$ in patients with AD.[24] Treatment with small molecule QC inhibitors reduces brain pGlu-Aβ levels and Aβ plaques[25,26] and

* Corresponding author.
E-mail address: jeewoo@snu.ac.kr (J. Lee).

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decreases gliosis and recovered memory deficits in AD mice.[22] In a recently completed phase IIa clinical trial,[27] the QC inhibitor PQ912 exhibited favorable safety and tolerability but also showed a slight improvement in synaptic and neurological functions in patients with AD,[28] supporting the hypothesis that QC is a promising therapeutic target that represents a novel treatment strategy for AD.

Previously reported QC inhibitors have three pharmacophores designated the A-, B- and C-regions, as shown in Fig. 1.[29,30] 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.[31] The newly developed QC inhibitors display improved potency, 5–40-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. 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. Results 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.

For the synthesis of the 4-phenylpiperidine D-region fragments (Scheme 1), the protected piperdin-4-one (**5**) was converted to the corresponding triflate **6**,[32] which underwent the Suzuki coupling reaction with phenylboronic acid derivatives to afford compounds **7** and **8**, respectively.[33] The phenol **7** was reacted with 2-bromo-5-nitroanisole through the Ullmann reaction to generate the 4-phenyltetrahydropyridine **9**.[34] 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**.

For the synthesis of 4-phenylpiperazine fragments (Scheme 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 con-

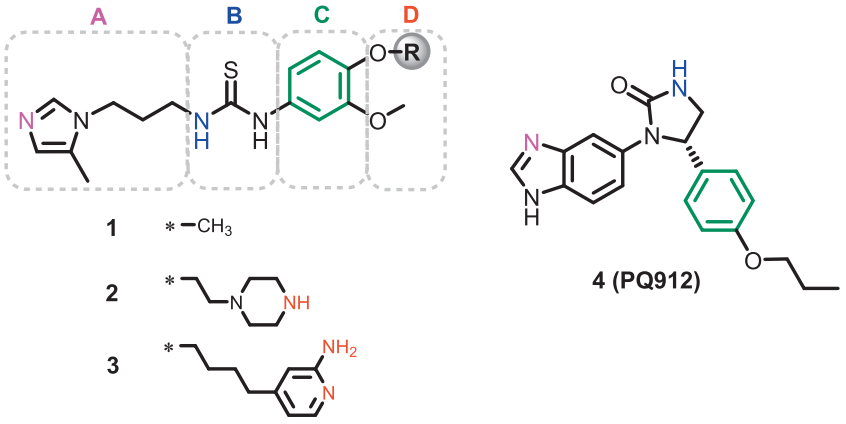
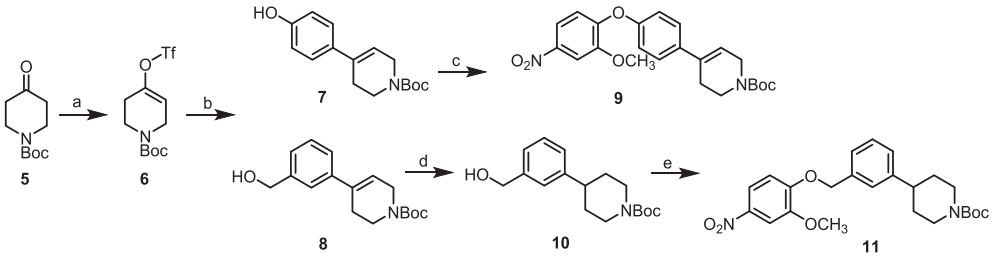
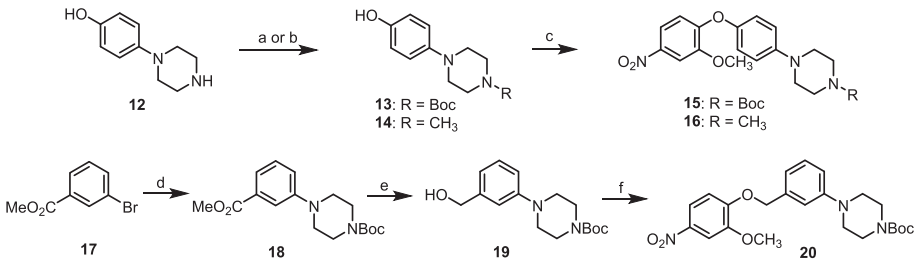


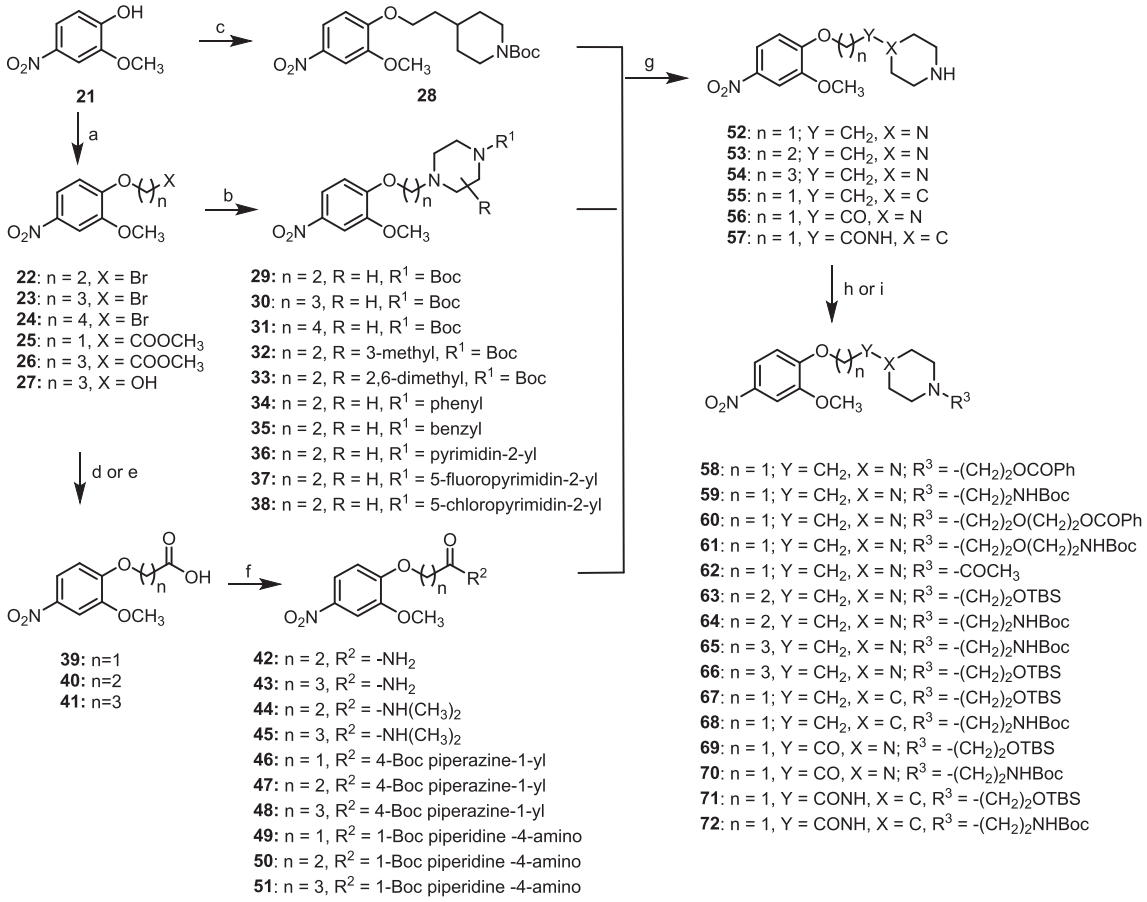
Fig. 1. Representative structures of QC inhibitors.



Scheme 1. Reagents and conditions: (a) Tf_2NPh, 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, r.t., 2 h; (e) 4-nitroguaiacol, DEAD, PPh_3, DCM, r.t., 3 h.



Scheme 2. Reagents and conditions: (a) Boc_2O, MC, 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, toluene, $Pd(OAc)_2$, NaOtBu, 100 °C, 15 min; (e) $LiAlH_4$, THF, 0 °C, 1 h; (f) 4-nitroguaiacol, DEAD, PPh_3, DCM, r.t., 3 h.

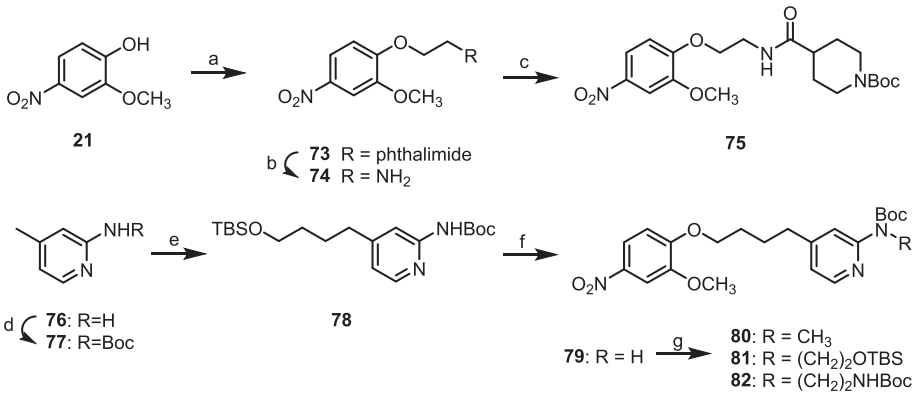


Scheme 3. Reagents and conditions: (a) $Br(CH_2)_nBr$ or $Br(CH_2)_3OH$ or $Br(CH_2)_nCOOCH_3$, Cs_2CO_3, DMF, 100 °C, 30 min; (b) piperazine derivatives, Cs_2CO_3, DMF, 70 °C, 30 min; (c) 1-Boc-4-(2-hydroxyethyl)piperazine, DEAD, PPh_3, MC, r.t., overnight; (d) NaOH, MeOH, reflux, 1 h; (e) $K_2Cr_2O_7$, 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), DCM, 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**).

ditions[35] 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 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.

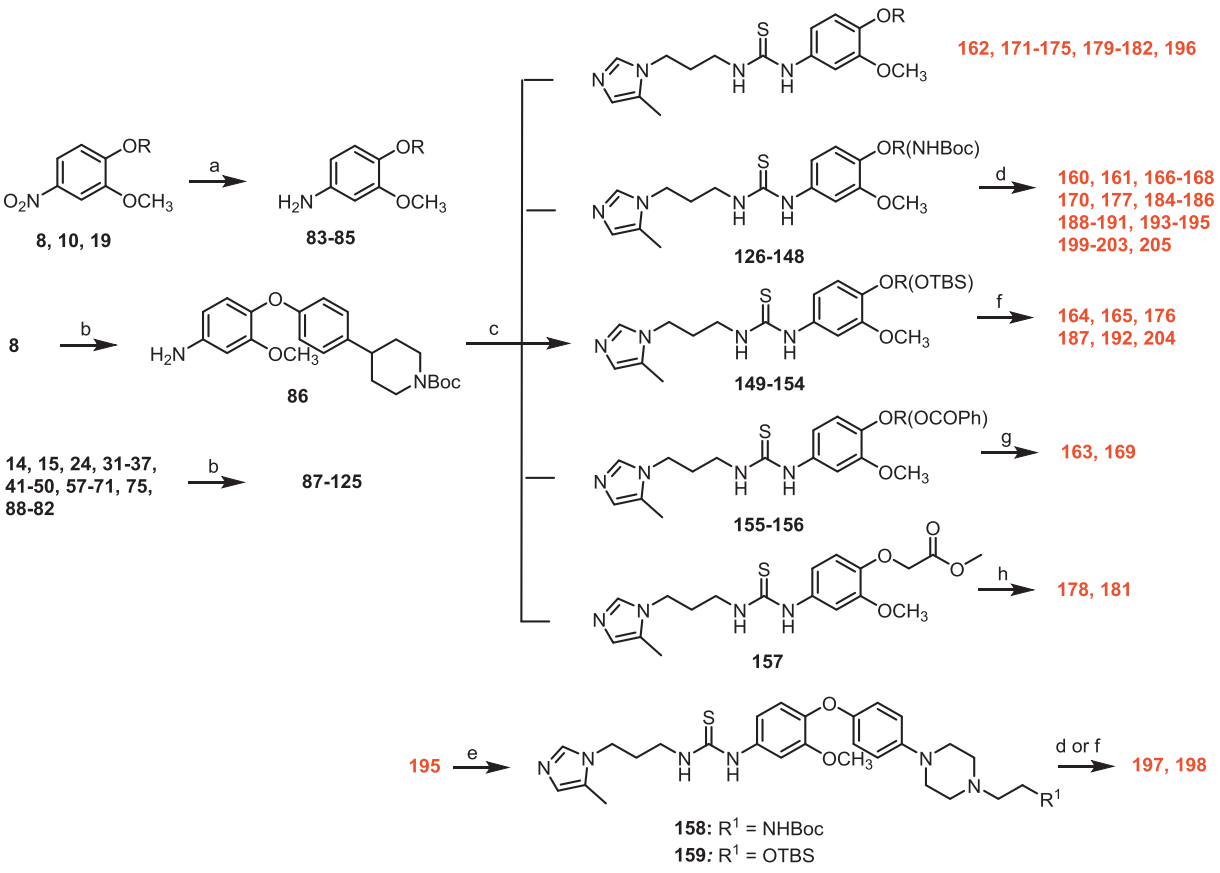
The 4-carboxamidopiperidine fragment **75** was synthesized from compound **21** in 3 steps (Scheme 4). For the syntheses of 4-alkyl-2-aminopyridine fragments (Scheme 4), 2-amino-4-picoline **76** was protected and then alkylated with *O*-TBS 3-bromopropanol



Scheme 4. 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, MC; (g) RI, Cs_2CO_3 (or NaH), DMF, heat.

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.

The synthesis of the final compounds is described in Scheme 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-1*H*-imidazol-1-yl)propan-1-amine[36] 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 5. 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, r.t., overnight.

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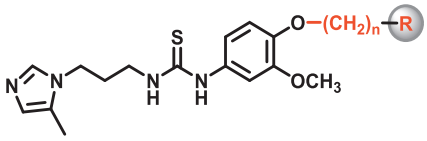
2.2. In vitro QC inhibition

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[37] 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 = 1–3), 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} = 12–21.6 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**).

Next, we examined the amidoalkyl derivatives in the D-region since the aminoalkyl derivatives showed potent inhibitory activity

Table 1
IC_{50} values for the inhibition of *h*QC by Group I compounds.



Compound	R	n	IC_{50} (nM)[a]	
1	*–Me	0	29.2[b]	
2	*–N⟨NH⟩	2	0.7[c]	
160	*–N⟨NH⟩	2	5.8	(±1.0)
161	*–N⟨NH⟩	2	9.9	(±0.7)
162	*–N⟨N–C(=O)–⟩	2	30.8	(±4.2)
163	*–N⟨N–CH2CH2OH⟩	2	7.4	(±1.2)
164		3	23.5	(±9.8)
165		4	4.8	(±1.0)
166	*–N⟨N–CH2CH2NH2⟩	2	7.5	(±6.8)
167		3	3.8	(±1.9)
168		4	8.6	(±0.6)
169	*–N⟨N–CH2CH2O–CH2CH2OH⟩	2	17.3	(±3.1)
170	*–N⟨N–CH2CH2O–CH2CH2NH2⟩	2	3.6	(±1.6)
171	*–N⟨N–Ph⟩	2	21.6	(±5.0)
172	*–N⟨N–CH2–Ph⟩	2	12.7	(±2.9)
173	*–N⟨N–pyrimidin-2-yl⟩	2	12	(±3.2)
174	*–N⟨N–(5-F-pyrimidin-2-yl)⟩	2	40.3	(±24)
175	*–N⟨N–(5-Cl-pyrimidin-2-yl)⟩	2	66.8	(±45.4)
176	*–N⟨CH2CH2OH⟩	2	9.0	(±3.7)
177	*–N⟨CH2CH2NH2⟩	2	10.6	(±2.2)

[a] Values indicate the means of at least three experiments.
[b] Ref. [30].
[c] Ref. [31].

in our previous study.[31] As described in Table 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 = 1–3) 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.

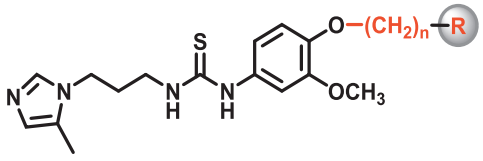
Next, we modified the linker that connects the C- and the D-regions to confer a rigid conformation and evaluated the inhibitory activity of these derivatives (Table 3). In general, compounds in this series displayed slightly decreased activity compared to the compounds with a flexible linker. The piperidine 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.

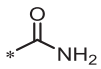
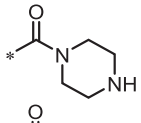
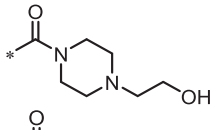
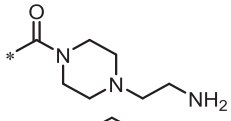
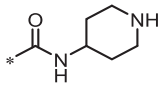
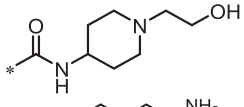
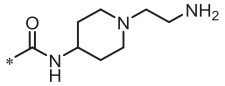
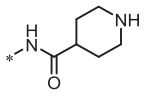
Finally, we evaluated the inhibitory activity of analogues containing the 2-aminopyridine group containing and summarized these results in Table 4. The 2-aminoethylamino substituent in compound **205** improved the inhibitory activity (IC_{50} = 1.8 nM) compared to its parent compound **3** (IC_{50} = 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**.

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

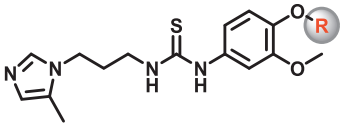
Table 2
IC_{50} values for the inhibition of *h*QC by Group II compounds.

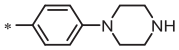
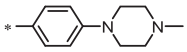
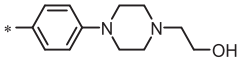
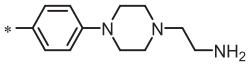
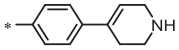
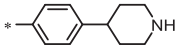
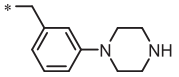
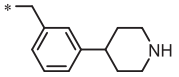


Compound		n	IC_{50} (nM)[a]	
178		1	89.7	(±17)
179		2	19.0	(±11.0)
180		3	53.8	(±5.7)
181		1	117	(±19.4)
182		2	35.4	(±4.0)
183		3	51.0	(±4.0)
184		1	15.0	(±2.5)
185		2	7.7	(±1.8)
186		3	7.0	(±1.1)
187		1	48.7	(±3.5)
188		1	7.8	(±4.1)
189		1	10.3	(±1.5)
190		2	5.5	(±0.2)
191		3	16.7	(±1.1)
192		1	17.7	(±6.0)
193		1	4.5	(±4.8)
194		2	15.7	(±2.0)

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

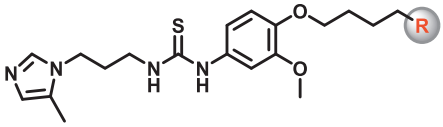
Table 3
IC_{50} values for the inhibition of *h*QC by Group III compounds.

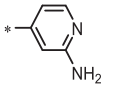
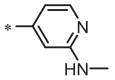
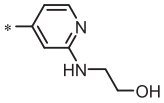
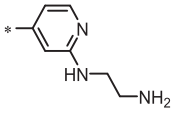


Compound	R	IC_{50} (nM)[a]	
195		8.5	(±0.5)
196		17.0	(±11.4)
197		16.1	(±8.3)
198		12.0	(±6.4)
199		9.4	(±3.4)
200		9.9	(±1.7)
201		11.5	(±4.1)
202		6.2	(±2.9)

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

Table 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].

(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 $A\beta_{3-40}$ (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 $A\beta_{N3pE-40}$ in the brain extracts of these mice on the next day to determine the QC inhibitory activity. As described in Table 5, compounds **185**, **190**, **199** and **202** appeared to suppress the formation of $A\beta_{N3pE-40}$ by 13.5–30% 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 $A\beta_{N3pE-40}$-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)[38] 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

Table 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.

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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**.

2.4. Molecular modeling

We performed sequential molecular modeling studies using the X-ray crystal structure of *h*QC (PDB id: 3PBB)[39] 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 (Fig. 2**A**).

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 (Fig. 2**B**). The local optimization refinement further shifted the Glu327 side chain toward the piperidine ring of compound **202** (Fig. 2**C**). We conducted Monte Carlo minimization to identify the global minimum (Fig. 2**D**). 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 (Fig. 3).

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 = 4.9–5.8) displayed good in vivo activity. In particular, compound **202** reduced $A\beta_{N3pE\text{-}40}$ 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 an 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:

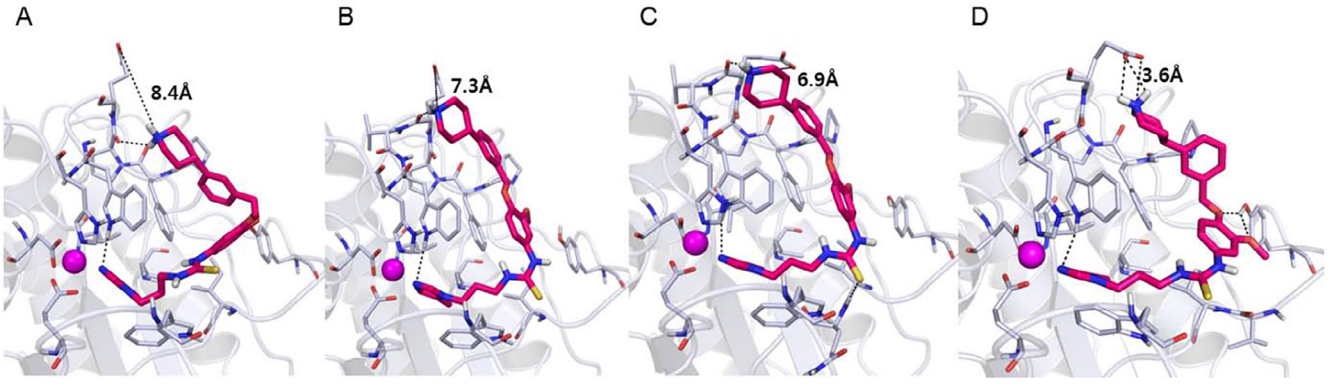

Fig. 2. Binding modes of compound **202** in *h*QC after (A) Glide SP docking, (B) QPLD, (C) local optimization, and (D) Monte Carlo minimization. Binding modes of the protonated form of compound **202** are shown in each step. Interactions with Glu327 are highlighted in red-dotted boxes, and the distances between Glu327 and the terminal N from the D-region of the ligands are marked with black dashed lines.

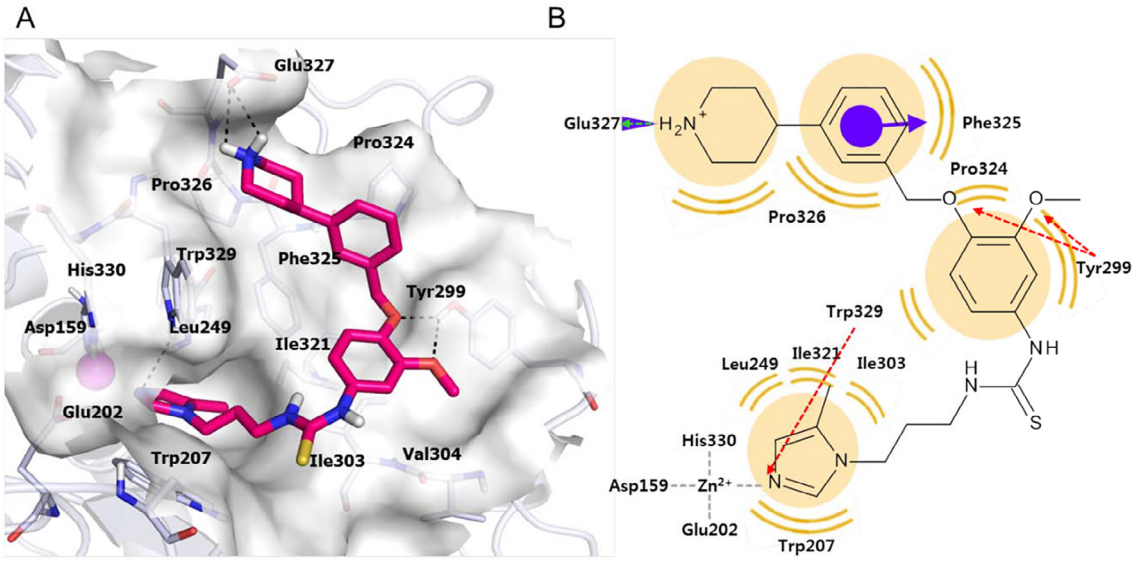

Fig. 3. Refined structure of compound **202** docked with *h*QC. (A) Binding interactions of compound **202** at the active site of the *h*QC. Compound **202** is displayed as sticks with magenta carbon atoms, and Zn^{2+} is depicted as a purple ball. The interacting residues are depicted as light blue 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.

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 (2 × 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 (2 × 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 min 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 min 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 × 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 h and then cooled to room temperature before being quenched with water. The mixture was extracted with EtOAc (2 × 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 DCM 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 h 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. Final compounds

4.1.3.1. 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 °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).

4.1.3.2. 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).

4.1.3.3. 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).

4.1.3.4. 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 min, 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).

4.1.3.5. 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).

4.1.3.6. 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).

4.1.3.7. 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).

4.1.3.8. 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).

4.1.3.9. 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).

4.1.3.10. 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, 10H), 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).

4.1.3.11. 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).

4.1.3.12. 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).

4.1.3.13. 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).

4.1.3.14. 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).

4.1.3.15. 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).

4.1.3.16. 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).

4.1.3.17. 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).

*4.1.3.18. 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).

*4.1.3.19. 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).

*4.1.3.20. 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).

*4.1.3.21. 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).

*4.1.3.22. 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).

*4.1.3.23. 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).

*4.1.3.24. 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).

*4.1.3.25. 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).

*4.1.3.26. 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).

*4.1.3.27. 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).

*4.1.3.28. 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).

4.1.3.29. 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).

4.1.3.30. 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).

4.1.3.31. 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).

4.1.3.32. 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).

4.1.3.33. 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).

4.1.3.34. 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).

4.1.3.35. 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).

4.1.3.36. 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).

4.1.3.37. 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).

4.1.3.38. 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).

4.1.3.39. 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).

4.1.3.40. 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).

4.1.3.41. 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).

4.1.3.42. 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).

4.1.3.43. 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).

4.1.3.44. 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).

4.1.3.45. 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).

4.1.3.46. 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.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.01.015.

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