The First Potent Inhibitors for Human Glutaminyl Cyclase: Synthesis and Structure−Activity Relationship

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The first effective inhibitors for human glutaminyl cyclase (QC) are described. The structures are developed by applying a ligand-based optimization approach starting from imidazole. Screening of derivatives of that heterocycle led to compounds of the imidazol-1-yl-alkyl thiourea type as a lead scaffold. A library of thiourea derivatives was synthesized, resulting in an inhibitory improvement by 2 orders of magnitude, leading to 1-(3-(1*H*-imidazol-1-yl)propyl)-3-(3,4-dimethoxyphenyl)thiourea as a potent inhibitor. Systematic exploitation of the scaffold revealed a strong impact on the inhibitory efficacy and resulted in the development of imidazole−propyl−thioamides as another new class of potent inhibitors. A flexible alignment of the most potent compounds of the thioamide and thiourea class and a QC substrate revealed a good match of characteristic features of the molecules, which suggests a similar binding mode of both inhibitors and the substrate to the active site of QC.

Introduction

Glutaminyl cyclase (QC, EC 2.3.2.5) catalyzes the intramolecular cyclization of N-terminal glutamine residues to pyroglutamic acid (pGlu) under liberation of ammonia.[1−3] QC is located in mammalian pituitary, hypothalamus, other parts of the brain, adrenal medulla, and B lymphocytes.[1,4] Some peptide hormones, such as tyrotropin releasing hormone (TRH) and gonadotropin releasing hormone (GnRH), require the pGlu on the N-terminus for their biological activity.[5,6] The colocalization of QC and its putative products within the regulated secretory pathway suggests a potential involvement of the enzyme in the final maturation of these peptide hormones.[7] The recently described ability of human QC to convert the N-terminal glutamate of the peptide Glu^3-Aβ(3−21), of the amyloid precursor protein (APP), into the respective $pGlu^3$-Aβ(3−21) in an in vitro experiment suggests QC's possible involvement in the initiation of the neurotoxic plaque formation in Alzheimer's disease (AD).[8] It was shown that, preferentially, the core of diffuse plaques consists of peptides containing N-terminal pGlu that have an enhanced tendency to aggregate, originating from precursors bearing an N-terminal glutamate.[9−12] Hence, besides the crucial role in hormone maturation, human QC might be involved in the pathophysiological process of AD, evoking its qualification as a new potential drug target. Testing of that hypothesis requires the development of highly efficient inhibitors.

Recently, we have reported investigations of the substrate specificity demonstrating a preference of aromatic side chains in the penultimate position to the N-terminal glutamine in the case of small tripeptide substrates.[3] Moreover, the catalytic activity was zinc-dependent.[13] This finding is supported by the strong structural homology between *Aeromonas proteolytica* aminopeptidase (ApAP) and QC and a conservation of all residues that are necessary for an effective zinc coordination.[14,15]

We found imidazole to be a weak inhibitor of QC activity; therefore, screening results of imidazole derivatives leading to suggestions for inhibitory structures were presented.[13] Besides histidine (frequently found as a zinc-coordinating ligand in metalloenzymes), imidazoles are reported to interact with accessible zinc atoms in the active site of carboxypeptidase A or to form stable complexes with active site residues of serine proteases under the presence of zinc ions.[16−18] The imidazole derivatives emerging from the here presented QSAR study as powerful QC inhibitors are likely to act as such zinc-chelating agents.

Results and Discussion

Screening. On the basis of the finding that imidazole is a weak inhibitor of QC, an initial screening was performed with a set of five- and six-membered heterocycles. Partial results of this screening campaign were published earlier.[13] The exchange of nitrogen for other heteroatoms or a change of the ring size led to nonpotent compounds (Figure 1).

The substitution pattern of the imidazole derivatives had a strong influence on the inhibitory effect (Figure 2). 1-Methylation led to a 3-fold improvement and 1-benzylation led to a 14-fold improvement of inhibitory power compared to that of imidazole. Interestingly, a 1-phenylation or an additional methylation in the 2-position of 1-benzylimidazole decreased the inhibitory effect dramatically.

On the basis of these findings, a library of 1-alkylimidazoles was screened for inhibitory efficacy against human QC. The chemical structure of the obtained hits uncovered a preference for a longer alkyl spacer connected to a hydrophobic residue for effective binding to QC. The best results are displayed in Figure 3. Due to its good chemical accessibility, compound **2** was selected for further optimization and analysis of the inhibitory potency of its derivatives.

Chemistry. The preparation of the urea and thiourea type analogues of **2** was conducted according to Schemes 1 and 2. Thereby, the unbranched 1-aminoalkyl-imidazole precursors **8** and **10** were prepared by alkylation of imidazole with the bromophthalimides **4** and **5** followed by the conversion of the phthalimides into primary amines by hydrazinolysis.[19] The 2-methyl-branched derivatives **17** and **18** were generated starting from the 2(*R*)- or 2(*S*)-isomers of 3-bromo-2-methylpropanol,

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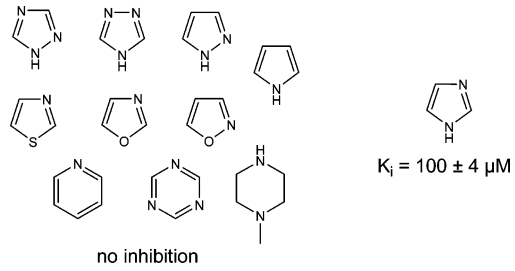


Figure 1. Screening results for different heterocycles.

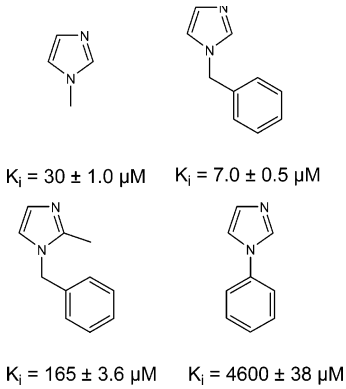


Figure 2. Screening results; 1-benzyl-1*H*-imidazole shows an improved inhibitory potency.

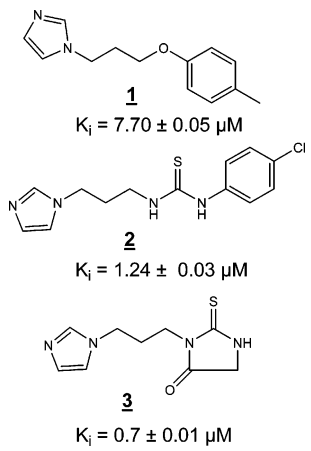


Figure 3. Screening results, **2** was selected for further optimization.

respectively. After protection of the alcohol by means of a THP-ether, followed by alkylation with phthalimide, the THP-protecting group was removed and the resulting alcohol was reacted with mesyl chloride to give the corresponding mesylates **15** and **16**. The alkylation of imidazole with these mesylates, followed by hydrazinolysis, led to the primary amines **17** and **18**. The 1-(3-amino-2-cyclopropylpropyl)imidazole **22** was generated from methyl 1-carbamoylcyclopropanecarboxylate **19**.[20] Reduction to the corresponding amino alcohol, followed by Boc-protection of the amino-function and subsequent introduction of imidazole after mesylation, led to the protected primary amine **21**, and deprotection led to **22**. The *N*-methylated precursor **25** resulted from the alkylation of imidazole with the N-Boc protected 1-methylamino-3-chloropropane **24** followed by deprotection of the methylamino function.

The urea and thiourea analogues **26**−**59** were obtained from the reaction of the 1-(aminoalkyl)imidazoles **8**−**10**, **17**, **18**, **22**, and **25** with the corresponding isocyanate and isothiocyanates.

The preparation of the benzothiazole analogues **66**−**68** was performed starting from the corresponding 2-chlorobenzothiazole derivatives and subsequent alkylation with 1-(3-aminopropyl)imidazole **9**.[21] The dimethoxy derivative **69** was prepared starting from 3,4-dimethoxyaniline **60** and subsequent formation of the 2-amino-5,6-dimethoxy-benzimidazole **61**[22] (Scheme 3). After conversion into the chloro derivative **62**,[23] 5,6-dimethoxy-2-imidazolylpropylaminobenzothiazole **69** was obtained after alkylation with **9**.

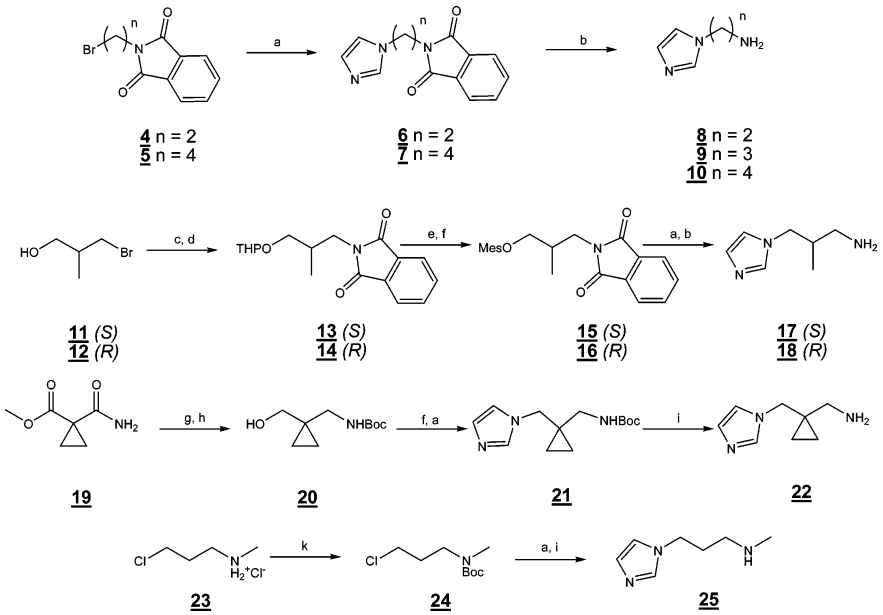
The thioamides **70**−**77** were prepared according to Scheme 4, by reacting the corresponding phenylacetyl chlorides with **9**. The resulting amide was converted into the thioamide by means of Lawesson's reagent. Compound **81** was prepared from 3,4-dimethoxyphenylacetonitrile **78**. Thereby, the cyclopropyl ring was introduced via a phase-transfer reaction using benzyltriethylammonium chloride (TEBA) as catalyst.[24] After hydrolysis of the nitrile,[25] the carboxylic acid **79** was reacted with **9** under mixed anhydride conditions. The resulting carboxamide **80** was then converted into the thioamide **81**. The inverse thioamide **85** (Scheme 4) resulted from the reaction of 5-bromopentanoyl chloride **82** with 3,4-dimethoxyaniline, followed by alkylation to imidazole. The formation of a cyclic side product led to a diminished yield for the alkylation step. The crude mixture was subjected to the reaction with Lawesson's reagent, and the byproduct **85a** was separated from the desired thioamide **85** by means of flash column chromatography.

Results. The data shown in Table 1 represent the results for the QSAR of thiourea analogues of **2**. Keeping the imidazole−propyl−thiourea part of the molecule constant, the variation of the aryl part revealed a big influence on the inhibitory potency. Thereby, the methyl derivative **26** and the branched alkyl derivatives **28** and **29** were found to be the inhibitors with the lowest potency. In contrast, the unbranched *n*-butyl derivative **27** was found to be of a 3-fold increased efficacy as compared to the branched homologues.

An increased potency compared to **26**, **28**, and **29** was found for the norbornyl, cyclohexyl, benzyl, and phenyl derivatives **30**−**33**. Thereby, the saturated cyclohexyl **31** and the phenyl derivative **33** were of similar potency. An elongation of the distance between the thiourea part and hydrophobic substituent as in the benzyl derivative **32** led to a decrease in potency compared to **33**. In contrast, an improvement was observed for the bulky norbornyl derivative **30** and the α-naphthyl derivative **34**. As the screening hit **2** was a *p*-chloro-substituted phenyl thiourea, further investigation was focused on the influence of substituents in the para-position of the phenyl residue. As a result, a *p*-methyl (**35**), *p*-ethyl (**36**), *p*-(dimethylamino) (**38**), *p*-nitro (**39**), *p*-acetyl (**40**), or *p*-methylthio (**41**) substitution led to a group of compounds with comparable inhibitory potency of around 2 μM. Interestingly, the electronical character of these substituents had only little impact on the potency. For instance, the basic *p*-dimethylamino function (**38**) had an influence comparable to a *p*-methyl (**35**) or *p*-nitro group (**39**). A slight potency improvement was achieved after the introduction of the *p*-acetyl substituent as in **40** or a methylthio substitution as in **41**. In contrast, a *p*-fluoro substitution as in **37** led to a 4-fold decreased potency compared to **2**.

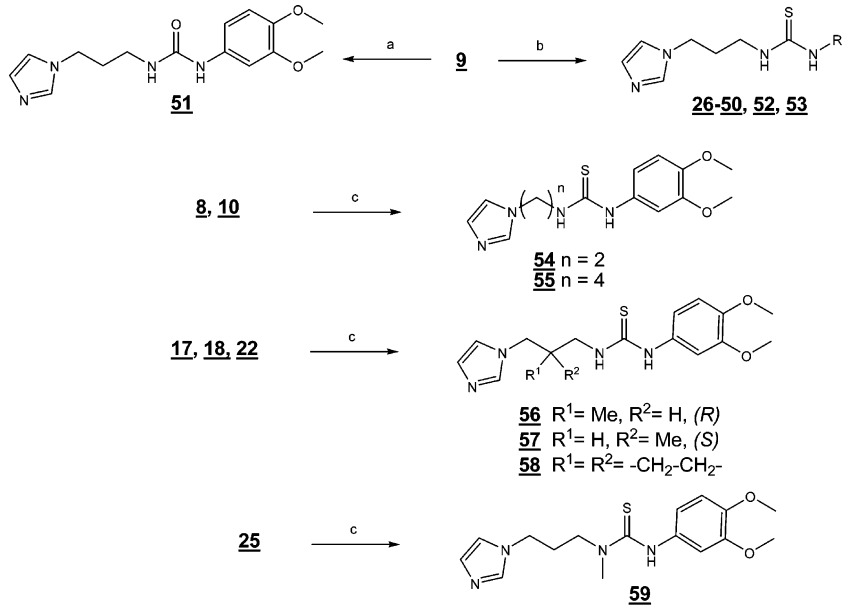
The introduction of an ether moiety as in **42**−**44** resulted in a submicromolar inhibitory activity. Because of the inhibitory activity improvement resulting from the introduction of a *p*-methoxy function, a closer look was taken at the influence of the number and position of methoxy substituents on the phenyl ring. The results are shown in Table 2. The shift of the methoxy group to position 3 as in **46** decreased the potency 2-fold as compared to **44**. In strong contrast to that, the combination of two methoxy substituents, leading to the 3,4-dimethoxyphe-

Scheme 1[a]



[a] Reagents and conditions: (a) Na-imidazolate, DMF, 8 h, 100 °C; (b) $H_2N-NH_2 \cdot H_2O$, EtOH, 8 h, reflux then 4 N HCl, 6 h, reflux; (c) 3,4-dihydro-2*H*-pyran, pyrTos, CH_2Cl_2, 12 h, rt; (d) K-phthalimide, DMF, 24 h, 80 °C; (e) pyrTos, EtOH, 3 h, 55 °C; (f) MsCl, NEt_3, CH_2Cl_2, 3 h, 0 °C, 12 h, rt; (g) $LiAlH_4$, THF, 4 h rt, 2 h reflux; (h) Boc_2O, NEt_3, CH_3OH, 12 h, 50 °C; (i) HCl in 1,4-dioxane (4 M), 1 h, 0 °C; (k) Boc_2O, NaOH, 1,4-dioxane/water (2:1), 20 h, rt.

Scheme 2[a]

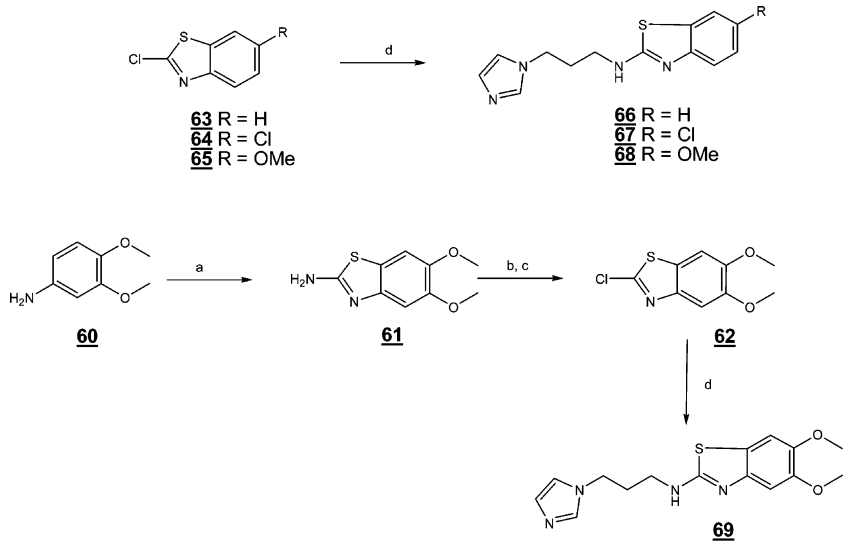


[a] Reagents and conditions: (a) 3,4-dimethoxyphenyl isocyanate, acetonitrile, 8 h, rt; (b) R−NCS, EtOH, 2 h, reflux; (c) 3,4-dimethoxyphenyl isothiocyanate, EtOH, 2 h, reflux.

nylthiourea **53**, resulted in a remarkable improvement of the inhibitory power, exhibiting a K_i value of 60 nM. As a result of that finding, the two methoxy functions were incorporated in a ring system as in **45** and **48**. The dioxolane-containing derivative **45** and the dioxane-derivative **48** both lost inhibitory power as compared to **53**. Moreover, neither the combination of the two methoxy functions in the 3,5- (**49**) or 2,4-positions (**50**) nor the incorporation of an additional methoxy function (**52**) improved the potency compared to **53**, but they did lead to more potent compounds than the screening hit **2**.

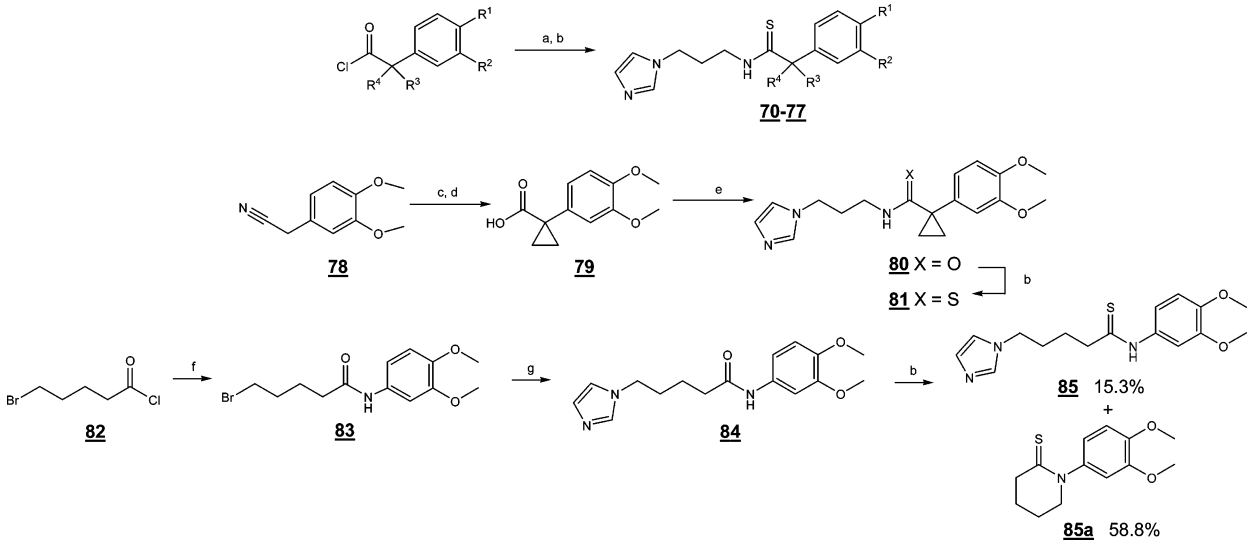
Taking the potent 3,4-dimethoxyphenyl derivative **53** as a starting point, more general changes to the molecule's scaffold have been performed. For example, the change of the thiourea moiety of **53** into an urea as for compound **51** led to a decreased potency, as did the prolongation of the distance between the phenyl core and the thiourea by one methylene unit in the case of compound **47**. The latter confirms the finding for the difference in potency in the case of compounds **32** and **33**, both lacking the two methoxy functions at the phenyl ring. The derivatives with a different distance between imidazole and thiourea, as well as branched propyl chains (Table 3), were prepared in order to investigate the influence of the chain length and flexibility of the propyl linker between imidazole and thiourea part of **53**. As a result, the reduction by one methylene unit, as in **54**, led to a dramatic decrease of the inhibitory power. On the other hand, extension of the linker by one methylene unit, as in **55**, resulted in only a moderate decrease in inhibitory activity. The introduction of branches at the 2-propyl position

Scheme 3[a]



[a] Reagents and conditions: (a) KSCN, acetic acid (96%), bromine, 10 h, 35 °C; (b) H_3PO_4 (85%), $NaNO_2$, H_2O, 2 h, −10 to 0 °C; (c) $CuSO_4 \cdot 5\ H_2O$, NaCl, 2 h, −5 °C; (d) **9**, NEt_3, 1-butanol, 24 h, reflux.

Scheme 4[a]

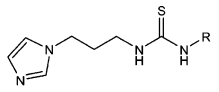


[a] Reagents and conditions: (a) **9**, NEt_3, CH_2Cl_2, 1 h, rt; (b) Lawesson's reagent, 1,4-dioxane, 8 h, reflux; (c) 2-bromo-1-chloroethane, TEBA, KOH, 2 d, rt; (d) KOH, ethylene glycol, 12 h, reflux; (e) **9**, CAIBE, NMM, THF, 5 min, −5 °C, 10 h, rt; (f) 3,4-dimethoxyaniline, NEt_3, 1,4-dioxane, 2 h, rt; (g) Na-imidazolate, 8 h, 100 °C.

again decreased the inhibitory efficacy in general. Therefore, the 2(*R*)-methyl derivative **56** was more effective compared to the (*S*)-configurated compound **57**. The most rigid cyclopropyl derivative **58** had a 40-fold higher K_i value compared to **53**. Moreover, a methyl group, introduced at the 1-nitrogen of the thiourea part of **53**, leading to compound **59**, resulted in a 80-fold decrease of inhibitory power. In the case of the derivatives **66**−**69**, the phenyl part and the thiourea moiety were incorporated in a benzothiazole ring system (Table 4). All compounds showed a decreased inhibitory potency as compared to **53**. Again, the substitution pattern influenced the inhibitory effect. Thereby, the introduction of a chloro substitutiuent in the 6-position (**67**) of the benzothiazole had no effect compared to the unsubstituted molecule **66**, but a methoxy group at this position (**68**) led to a 2-fold decreased K_i value. In contrast to compound **53**, the introduction of a second methoxy function at the 5-position led to a less potent derivative **69** as compared to the monomethoxy substituted equivalent **68**.

The thioamide derivative **70**, lacking one potential H-bond donor at the thiourea part, exhibited submicromolar inhibitory activity. Interestingly, the inversion of the thioamide as in **85** (see Figure 4) caused a potency loss. This finding led to a further change of the scaffold of **70** by substitution of the methylene protons of the benzyl residue (Table 5). Thereby, phenyl- (**71**, **72**), *p*-chlorophenyl- (**75**, **76**), and the 4-methoxy-substituted thioamides (**74**, **77**) were used to study the influence of the substitution pattern of the benzyl moiety. The introduction of a methyl group in the (*R*)- (**71**) or (*S*)-configuration (**72**) influenced the inhibitory potency compared to the analogue phenyl thiourea **33**. In the case of the (*S*)-enantiomer **72**, it led to a potency improvement. The phenyl-substituted derivative **73** was of comparable potency to compound **33**. An incorporation of an alkyl ring at the methylene group, leading to phenylcycloalkyl thioamides, influenced the inhibitory potency, depending on the ring size. A five-membered ring as in **76** was found to be less potent than the cyclobutyl derivative **75**, both featuring a strong

Table 1



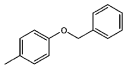
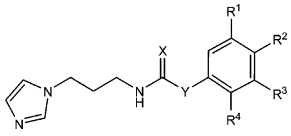
Compound	R	K_i (μM)
26	CH_3	13.02 ± 0.62
27	$(CH_2)_3CH_3$	4.65 ± 0.12
28	$C(CH_3)_3$	14.72 ± 1.70
29	$CH(CH_3)_2$	11.30 ± 0.21
30		3.66 ± 0.06
31		4.70 ± 0.01
32		5.67 ± 0.04
33		4.48 ± 0.13
34		2.79 ± 0.11
35		2.14 ± 0.05
36		2.78 ± 0.01
37		4.73 ± 0.15
38		2.03 ± 0.06
39		2.68 ± 0.07
40		1.79 ± 0.02
41		1.66 ±0.04
42		0.97 ± 0.04
43		0.89 ± 0.02
44		0.70 ± 0.02

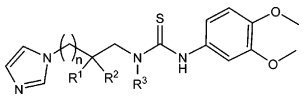
Table 2



compd	R^1	R^2	R^3	R^4	X	Y	K_i (μM)
45	H	$-OCH_2O-$		H	S	NH	5.66 ± 0.17
46	OMe	H	H	H	S	NH	1.86 ± 0.08
47	H	OMe	OMe	H	S	$NHCH_2$	1.55 ± 0.02
48	H	$-OCH_2CH_2O-$		H	S	NH	1.12 ± 0.03
49	OMe	H	OMe	H	S	NH	0.75 ± 0.02
50	H	OMe	H	OMe	S	NH	0.56 ± 0.02
51	H	OMe	OMe	H	O	NH	0.49 ± 0.01
52	OMe	OMe	OMe	H	S	NH	0.34 ± 0.01
53	H	OMe	OMe	H	S	NH	0.06 ± 0.0002

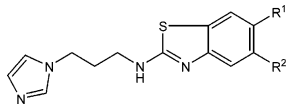
decreased potency compared to the *p*-chlorophenyl thiourea **2**. The bulky cyclohexyl thioamide **77** was 3 times less potent as compared to the 4-methoxyphenyl thiourea **44**. In contrast, the cyclopropyl-substituted derivative **74** was of comparable potency

Table 3



compd	R^1	R^2	R^3	*n*	K_i (μM)
54	H	H	H	0	17.66 ± 0.83
55	H	H	H	2	0.55 ± 0.02
56 (*R*)	Me	H	H	1	0.34 ± 0.007
57 (*S*)	H	Me	H	1	0.76 ± 0.004
58	$-(CH_2)_2-$		H	1	2.33 ± 0.50
59	H	H	Me	1	4.83 ± 0.11

Table 4



compd	R^1	R^2	K_i (μM)
66	H	H	3.73 ± 0.20
67	Cl	H	3.35 ± 0.01
68	OMe	H	1.57 ± 0.06
69	OMe	OMe	2.00 ± 0.01

to **44** and the introduction of the second methoxy group finally led to compound **81**, with a potency comparable to compound **53**.

Molecular Modeling. Since all compounds act as competitive inhibitors against a glutamine-containing QC substrate, investigations of the possible binding mode were undertaken. To focus on similarities of molecular features, a flexible alignment of the good binding substrate H-Gln-Phe-Ala-NH_2 and the most potent compounds **53** (Table 2) and **81** (Table 5) was performed. The resulting database consisted of 192 suggestions for possible alignments. The evaluation, with respect to their average strain energy (*U*) and the alignment score (*S*), led to a solution ranking on position 37 of the database. A good match of characteristic features necessary for an effective binding was observed. First, the acceptor nitrogen of imidazole matches with the acceptor carbonyl of the glutamine side chain as the probable zinc-binding site. The donor nitrogen of **53** (Figure 5A) matches the C-terminal amide nitrogen of glutamine. The finding that the second donor nitrogen of **53** is not overlaid with a donor of the substrate can support the result of the weaker binding of **85** (Figure 4), which lacks the first donor but features the second nitrogen donor. The preference of QC for hydrophobic side chains in penultimate position to the N-terminal glutamine of the substrates corresponds to the finding of the beneficial phenyl substitution for the thiourea-containing inhibitor molecules and the benzyl substitution in case of the thioamides. The phenyl side chain of the substrate and the phenyl part of the inhibitor molecules **53** and **81** are overlaid in the alignment.

Discussion. Lacking 3D structural information of the target enzyme QC, the ligand-based approach exemplified here led to potent inhibitors of the enzyme with enhanced activity as compared with the initial screening compounds. The most potent competitive inhibitors, **53** and **81**, were used in an alignment with the short peptide H-Gln-Phe-Ala-NH_2, exhibiting one of the highest specificity constants as QC substrate. As a result, similarities in their structure responsible for a productive binding to the active site could be identified. This finding has helped to shape a potential pharmacophore with respect to the binding features of the thiourea and thioamide class of inhibitors of QC.

Due to the structural homology of human QC to aminopeptidases and the conservation of the active site residues necessary

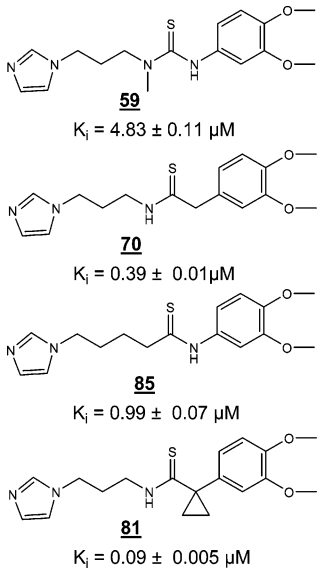
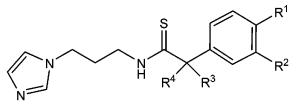


Figure 4. Comparison of the inhibitory efficacy of the *N*-methylthiourea **59**, the reverse thioamide **85**, and the thioamide compounds **70** and **81**.

Table 5



Compound	R^1	R^2	R^3	R^4	K_i (μM)
70	OMe	OMe	H	H	0.39 ± 0.01
71 *(R)*	H	H	Me	H	7.34 ± 0.26
72 *(S)*	H	H	H	Me	3.51 ± 0.09
73	H	H	H		4.48 ± 0.16
74	OMe	H	-$(CH_2)_2$-		0.40 ± 0.008
75	Cl	H	-$(CH_2)_3$-		4.88 ± 0.25
76	Cl	H	-$(CH_2)_4$-		7.33 ± 0.06
77	OMe	H	-$(CH_2)_5$-		2.22 ± 0.18
81	OMe	OMe	-$(CH_2)_2$-		0.09 ± 0.005

for a zinc binding, QC was determined to be a metalloenzyme.[13,14] With regard to that finding, we suggest an interaction of the basic imidazole nitrogen of the inhibitory structures with the catalytic zinc ion. This hypothesis is supported by the finding that the catalytic process of the pyroglutamyl formation was found to be zinc-dependent and that human QC, similar to metal-dependent aminopeptidases, is inhibited by imidazole, 1,10-phenanthroline, and dipicolinic acid.[13] In contrast, Bateman et al.[14] discuss a non-zinc-dependent catalysis by QC and the absence of zinc ions in the active site of the enzyme. However, we recently demonstrated stoichiometric Zn binding using two independent techniques.[26]

The mode of zinc binding of imidazole-containing inhibitors of QC is understood as a single coordination to the single zinc ion, since imidazole exhibits only one coordination site (see Figure 5). A proof for that hypothesis, however, should result from the solution of the 3D structure of the protein.

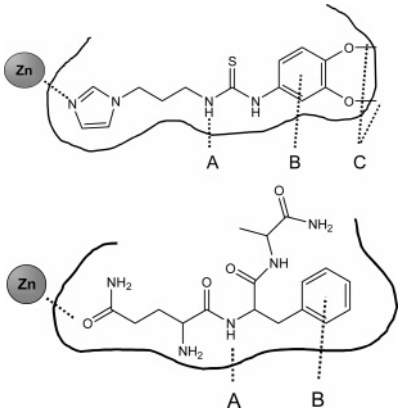


Figure 5. Proposed binding mode at the active site of human QC for **53** (above) and the substrate H-Gln-Phe-Ala-NH_2. The zinc atom is coordinated by the imidazole part of **53** or the glutamine side chain in case of the substrate. The essential H-bond donor function (A) was found in both structures, and so was the aromatic moiety (B), pointing toward an interaction with a hydrophobic pocket. The methoxy substituents (C) have a big impact on the inhibitory binding.

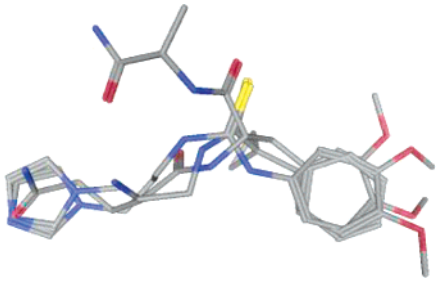


Figure 6. Flexible alignment of **53**, **81**, and the substrate H-Gln-Phe-Ala-NH_2. It was ranked at position 37 ($U = -4.32$ kcal/mol, $S = 97.91$ kcal/mol) of 192 solutions. A good match of the proposed zinc-coordination site, the H-bond donor nitrogens, and the aromatic substituents is recognizable.

For the *N*-alkyl spacer between the imidazole and thiourea moiety, a distance of three methylene units was found to be optimal. The prolongation by one methylene unit was tolerated (see compound **55**) but led to a decrease of inhibitory activity. A shortening by one methylene unit had a drastic decreasing effect (see compound **54**). The introduction of branches at the 2-propyl position reduces the inhibitory efficacy of all synthesized derivatives. However, a partial flexible chain as in the methyl-branched derivatives **56** and **57** was better tolerated as the fixation by means of a cyclopropyl ring as in **58**. This points toward the presence of a narrow space around the catalytic zinc ion, which is supported by the finding that only flat heterocycles, like the above-mentioned 1,10-phenanthroline and dipicolinic acid, are able to bind effectively to the active site of QC.

The thiourea moiety features two H-bond donor positions at the 1-N and 3-N atoms and one H-bond acceptor position at the sulfur atom of the thiocarbonyl moiety. An interesting finding was the higher efficacy of the respective thiourea-derivative **53** compared to the urea **51**. Obviously, the acceptor function of the thiocarbonyl moiety is less eminent for the interaction with the protein in terms of H-bonding, because sulfur is a weaker H-bond acceptor than oxygen. This points to a greater importance of the thioamide donor sites of the thiourea for an effective binding. Moreover, a calculation of the hydrogen-bonding energy between an amide- or thioamide-nitrogen H-bond donor and a carbonyl acceptor resulted in a stronger interaction between the thioamide donor and the carbonyl acceptor.[27,28] This could explain the better inhibitory efficacy of a thiourea over the respective urea analogue.

The thioamides (see Table 5), having only one potential H-bond donor site (see Figure 5A), show an inhibitory activity comparable to the respective thiourea compounds. On the other hand, the inverse thioamide **85** (see Figure 4) shows a reduced inhibitory activity compared to **70**. Moreover, in case of a change of the 1-N thioamide proton in compound **53** into a methyl group as in **59**, the inhibitory power drops by a factor of 80. This effect is likely due to the absence of the 1-N proton and could be amplified by a sterical hindrance when the methyl group is introduced at this position. All these investigations indicate that the 1-N thioamide position of the thiourea compounds, as well as the *N*-propyl thioamide functionality of thioamide derivatives, is a crucial point of interaction with the pharmacophore of QC.

The beneficial substitution of the thiourea by a phenyl group or a benzyl group in case of the thioamides, respectively, suggests an interaction with a hydrophobic pocket of the protein (Figure 5B). Thereby, the results regarding the influence of different kinds of alkyl substituents on the inhibitory potency are found to be difficult to link to certain structural features. The thiourea derivatives presented in Table 1 point toward a preference for space-filling hydrophobic substituents. The beneficial effect of the introduction of a norbornyl residue (see compound **30**) or α-naphthyl residue (see compound **34**) over small alkyl substituents (see compound **26**) and branched alkyl substituents (see compounds **28**, **29**) serves as an example. Moreover, an aromatic ring system seems to not be a requirement, probably ruling out a $\pi-\pi$-interaction with the protein. An example for that finding is compound **33**, wherein a phenyl substituent leads to a comparable potency as a cyclohexyl group (see compound **31**). A further hint for the hydrophobic character of the interaction with the protein is the improvement of the inhibitory power by the extension of the phenyl substituent by hydrophobic residues. Thereby, electron-enriching groups introduced in the para-position of the phenyl ring, like alkyl (see compound **35**, **36**), thioalkyl (see compound **41**), or alkyloxy (see compound **42**−**44**, **74**), always lead to an improved efficacy as compared to the unsubstituted phenyl residue.

In contrast, the electron-withdrawing fluorine as in compound **37** shows no difference in efficacy in comparison to **33**, whereas a chloro substitution (see compound **2**), probably due to the +M-effect of the chlorine, has a beneficial effect on the inhibitory power. The effect of the introduction of the electron-withdrawing *p*-nitro substituent (see compound **39**) or alkylcarbonyl (see compound **40**) remains difficult to interpret, as it has an improving effect on the inhibitory potency as compared to **33** and still is a more effective inhibitor than **37**. The basic *p*-dimethylamino compound **38** ranks within the class of derivatives with hydrophobic substitutents mentioned above, obviously lacking an additional hydrophobic interaction with the protein and featuring an increased effect on the inhibitory power compared to **33**. This as well is likely due to the higher electron density of the phenyl ring. The position of the methoxy group has a great influence on the inhibitory potency (see compound **46**, Table 2). Two methoxy groups lead to a great increase of the inhibitory power only when they were introduced in position 3 and 4 of the phenyl ring (see compound **53**). The strong decrease of the potency in the case of the incorporation of the 3,4-methoxy groups in a dioxane ring (see compound **48**), compared to that of **53**, and the loss of the potency in the case of the smaller and less flexible dioxolane (see compound **45**) are examples of the high sensitivity of the interactions between the hydrophobic pocket of the protein and the inhibitor molecule toward minor changes in the inhibitor structure.

The presented thiourea and urea class of compounds should feature a nearly planar geometry, due to the flat shape of the thiourea group and the partial hindrance of the free rotation of the phenyl ring caused by conjugative effects. In contrast to that, the phenyl ring as in the thioamide compound **70** is freely rotatable, which then leads to a loss of inhibitory activity as compared to **53**, probably resulting from a higher flexibility of the molecule. The stepwise reduction of rotatory freedom around the alkyl bonds of the benzyl residue (see compounds **70**−**77**, **81** Table 5) leads to the cyclopropyl-containing derivative **81** in which the inhibitory potency is reestablished. In addition, the spatial position of the phenyl ring, if influenced by the introduction a methyl group as in **71** and **72**, causes differences in the inhibitory efficacy depending on the configuration. The introduction of an additional bulky phenyl residue (see compound **73**), leading to a symmetrical substitution pattern, resulted in an efficacy comparable to the average activity of the two methyl analogues **71** and **72**.

In conflict with the binding mode of QC inhibitors evolved here is the finding that the screening compound **3**, lacking the 1-N H-bond donor site and the hydrophobic part of the molecule, features an inhibitory power comparable to **43** and **44**. An explanation for that effect could be that the molecule binds to different residues at the active site, probably involving its additional amide carbonyl function at the ring.

The incorporation of the thiourea and the phenyl moiety into a flat aromatic benzothiazole ring leads to another class of compounds with a different binding to the active site of QC (see Table 4). The unsubstituted derivative **66** was found to be of a comparable efficacy to **33**. In this context the introduction of hydrophobic, electron-enriching substituents did lead to an improvement of inhibitory efficacy in case of the methoxy derivative **68**, but not in case of the chloro derivative **67**. In contrast to the thiourea structures **44** and **53** and the thioamides **74** and **81**, the introduction of a second methoxy group in the 5-position of the benzimidazole ring did not lead to an improvement of inhibitory power (see compound **69**). Due to the change of sterical and electronical features of the molecule by creating a single bond between the thiourea-sulfur and the phenyl ring, obviously an optimal interaction with the hydrophobic pocket of the protein as with **53** and **81** is not possible. This leads to a drop of inhibitory activity.

Consequently, the compounds **53** and **81** appear to be the most powerful QC inhibitors so far. Moreover, the collection of the inhibitor molecules helped to identify crucial interaction points of the active site of QC. This will lead to further QSAR investigations involving also 3D pharmacophore searches and the 3D structure of QC.

Experimental Section

Screening. Screening compounds were purchased form Aldrich Co. The 1-alkylimidazole library was accomplished from the compound collections by VitasMLab Ltd., Interbioscreen Inc., World Molecules Inc., Maybridge Co., and ChemBridge Inc., summing up to 200 compounds selected for testing. Compound **1** was purchased from ChemBridge Inc., while **2** and **3** where purchased from Maybridge Co.

Chemistry. Starting materials and solvents were purchased from Aldrich and Maybridge Co. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. ESI-Mass spectra were obtained with a SCIEX API 365 spectrometer (Perkin-Elmer). The high-resolution positive ion ESI mass spectra were obtained from a Bruker Apex III 70e Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with an Infinity cell, a 7.0 T superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide, and

an external electrospray ion source (API Apollo) (voltages: endplate, −3.700 V; capillary, −4.400 V; capillary exit, 100 V; skimmer, 1.15 V; skimmer, 2.6 V). Nitrogen was used as drying gas at 150 °C. The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 μL h^{-1}. All data were acquired with 256 k data points and zero filled to 1024 k by averaging 32 scans. The ^{1}H NMR (500 MHz) data was recorded on a BRUKER AC 500, using DMSO-d_6 as solvent, unless otherwise specified. Chemical shifts are expressed as parts per million downfield from tetramethylsilane. Splitting patterns have been designated as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet), and br (broad signal). All solvents were dried and distilled prior to use. Aluminum oxide, basic type 5016A, was used for column chromatography. The purity of the compounds was determined by HPLC analysis. The system consisted of a Merck-Hitachi device (model LaChrom) utilizing a Li-Chrospher 100 RP 18 (5 μm), analytical column (length: 125 mm, diameter: 4 mm), and a diode array detector (DAD) with λ = 214 and 270 nm as the reporting wavelengths. The compounds were analyzed by applying the gradient 0−5 min 5% (A), 5−17 min 15% (A), 15−27 min 95% (A), and 27−30 min 95% (A) at a flow rate of 1 mL/min, where eluent A was acetonitrile and eluent B was water, both containing 0.1% (v/v) trifluoro acetic acid. The purities of all reported compounds were determined by the percentage of the peak area at 214 nm. Semipreparative HPLC was performed on a Merck-Hitachi device (model LaChrom) equipped with a SP250/21 Nucleosil 100−7 C18 semipreparative column (Machery-Nagel) (length, 250 mm; diameter, 21 mm). The compounds were eluted using the same solvent system as described above, applying a flow rate of 8 mL/min.

General Procedure for the Synthesis of the 1*H*-Imidazol-1-ylalkylamines 8, 10. The compounds were prepared from the corresponding ω-bromoalkylphthalimides according to the method described in ref 19 and used without further purification.

2-(1*H*-Imidazol-1-yl)ethanamine 8. Step A. 2-(2-(1*H*-Imidazol-1-yl)ethyl)isoindoline-1,3-dione 6. Imidazole (0.57 g, 8.4 mmol, 1.05 equiv), sodium hydride (60% in mineral oil, 0.34 g, 8.4 mmol, 1.05 equiv), and 2-(2-bromoethyl)isoindoline-1,3-dione **4** (2.0 g, 8.0 mmol, 1.0 equiv) yielded 1.5 g (75.0%): MS *m*/*z* 242.2 (M + H)$^+$, 174.3 ([M − $C_3H_3N_2$]•)$^+$.

Step B. 2-(1*H*-Imidazol-1-yl)ethanamine 8. 2-(2-(1*H*-Imidazol-1-yl)ethyl)isoindoline-1,3-dione **6** (1.5 g, 6.3 mmol, 1.0 equiv) and hydrazine monohydrate (0.35 g, 6.9 mmol, 1.1 equiv) yielded 0.27 g (38.5%): MS *m*/ *z* 112.1 (M + H)$^+$.

4-(1*H*-Imidazol-1-yl)butanamine 10. Step A. 2-(4-(1*H*-Imidazol-1-yl)butyl)isoindoline-1,3-dione 7. Imidazole (0.57 g, 8.4 mmol, 1.05 equiv), sodium hydride (60% in mineral oil, 0.34 g, 8.4 mmol, 1.05 equiv), and 2-(4-bromobutyl)isoindoline-1,3-dione **5** (2.2 g, 8.0 mmol, 1.0 equiv) yielded 1.7 g (75.0%): MS *m*/*z* 270.3 (M + H)$^+$, 202.2 ([M − $C_3H_3N_2$]•)$^+$, 160.1 ([M − $C_9H_6NO_2$]•)$^+$.

Step B. 4-(1*H*-Imidazol-1-yl)butanamine 10. 2-(4-(1*H*-Imidazol-1-yl)butyl)isoindoline-1,3-dione **7** (1.7 g, 6.3 mmol, 1.0 equiv) and hydrazine monohydrate (0.35 g, 6.9 mmol, 1.1 equiv) yielded 0.29 g (32.7%): MS *m*/ *z* 140.3 (M + H)$^+$.

3-(1*H*-Imidazol-1-yl)-2-methylpropan-1-amines 17, 18. Step A. 2-((*S*)- And 2-((*R*)-2-Methyl-3-(tetrahydro-2*H*-pyran-2-yloxy)-propyl)isoindoline-1,3-dione 13, **14**. The corresponding 3-bromo-2-methylpropan-1-ol (1.04 g, 6.8 mmol, 10 equiv) **11** and **12**, 3,4-dihydro-2*H*-pyran (0.93 mL, 10 mmol, 14.7 equiv), and pyridinium *p*-toluenesulfonate (0.17 g, 0.68 mmol, 1.0 equiv) were dissolved in dry CH_2Cl_2 (50 mL) and stirred at room temperature for 12 h. Et_2O (100 mL) was added; the organic layer was washed twice with brine, dried over Na_2SO_4, and filtered; and the solvent was evaporated. The crude products were used for the next step, whereby, the appropriate 2-(3-bromo-2-methylpropoxy)-tetrahydro-2*H*-pyran **13** and **14** (1.62 g, 6.8 mmol, 10 equiv) was dissolved in dry DMF (40 mL) and the mixture was heated to 80 °C. Potassium phthalimide (1.27 g, 6.8 mmol, 10 equiv) was added in one portion, and the solution was stirred for 24 h at 80 °C. The white precipitate was filtered off, and the organic solvent was evaporated. The resulting oil was used without further purification.

2-((*S*)-2-Methyl-3-(tetrahydro-2H-pyran-2-yloxy)propyl) isoindoline-1,3-dione 13: yield 1.91 g (92.6%); MS *m*/*z* 304.3 (M + H)$^+$, 321.3 (M + NH_4)$^+$, 220.3 ([M − $C_5H_9O_2$]•)$^+$.

2-((*R*)-2-Methyl-3-(tetrahydro-2H-pyran-2-yloxy)propyl) isoindoline-1,3-dione 14: yield 1.85 g (89.7%); MS *m*/*z* 304.3 (M + H)$^+$, 321.3 (M + NH_4)$^+$, 220.4 ([M − $C_5H_9O_2$]•)$^+$.

Step B. (*S*)- And (*R*)-2-Methyl-3-(1,3-dioxoisoindolin-2-yl)-propyl Methanesulfonate 15 and 16. The corresponding 2-methyl-3-(tetrahydro-2*H*-pyran-2-yloxy)propyl) isoindoline-1,3-dione **13** and **14** (1.82 g, 6.0 mmol, 10 equiv) and pyridinium *p*-toluenesulfonate (0.15 g, 0.60 mmol, 1.0 equiv) were dissolved in EtOH (30 mL), heated to 55 °C, and stirred for 3 h. The solvent was evaporated and the crude product was used for the further reaction without purification. The derived 2-(3-hydroxy-2-methylpropyl)-1*H*-isoindole-1,3(2*H*)diones (1.31 g, 5.9 mmol, 9.8 equiv) and NEt_3 (1.25 mL, 9.0 mmol, 15 equiv) were dissolved in dry CH_2Cl_2 (40 mL). The mixture was cooled to 0 °C and a solution of mesyl chloride (0.70 mL, 9.0 mmol, 15 equiv) in dry CH_2Cl_2 (10 mL) was added dropwise under an atmosphere of argon. The solution was stirred at 0 °C for 3 h, followed by an additional 12 h at room temperature. The organic layer was washed by means of water, dried over Na_2SO_4, and filtered, and the solvent was evaporated. The crude products were used for the next step without further purification.

(*S*)-2-Methyl-3-(1,3-dioxoisoindolin-2-yl)propyl methanesulfonate 15: yield 0.94 g (52.6%); MS *m*/*z* 298.2 (M + H)$^+$.

(*R*)-2-Methyl-3-(1,3-dioxoisoindolin-2-yl)propyl methanesulfonate 16: yield 0.95 g (53.5%); MS *m*/*z* 298.1 (M + H)$^+$.

Step C. (*S*)- And (*R*)-3-(1*H*-Imidazo-1-yl)-2-methylpropan-1-amine 17 and 18. Starting from the corresponding ω-methanesulfonate-2-methylpropyl-phthalimides **15** and **16**, the amines were synthesized as described for the amines **8**−**10**.[19] They were used without further purification.

(*S*)-3-(1*H*-Imidazol-1-yl)-2-methylpropan-1-amine 17 started from **15** (0.94 g, 3.2 mmol): yield 0.14 g (31.8%); MS *m*/*z* 140.2 (M + H)$^+$.

(*R*)-3-(1*H*-Imidazol-1-yl)-2-methylpropan-1-amine 18 started from **16** (0.95 g, 3.2 mmol): yield 0.15 g (34.4%); MS *m*/*z* 140.4 (M + H)$^+$.

(1-((1*H*-midazol-1-yl)methyl)cyclo-1-propyl)methanamine 22. (1-(Aminomethyl)cyclopropyl)methanol **19a** was prepared from 1-(aminocarbonyl)-1-cyclopropanecarboxylic acid methyl ester **19** in a manner similar to the method of ref 20. **19** (0.70 g, 4.9 mmol, 1.0 equiv) was dissolved in dry THF (20 mL), and $LiAlH_4$ (9.8 mL of a 1 M solution in THF, 9.8 mmol, 2.0 equiv) was added under ice cooling. After stirring for 4 h at room temperature, the mixture was heated and stirred under reflux for additional 2 h. The solvent was evaporated, and then water (5 mL) and THF (20 mL) were added. The solvent was removed under reduced pressure, the remaining precipitate was suspended in Et_2O (50 mL), and the suspension was filtered. The residue was washed three times by means of Et_2O (50 mL). After the organic phases were combined, dried over Na_2SO_4, and filtered, and the solvent was removed by reduced pressure: yield 0.40 g (79.6%); MS *m*/*z* 102.15 (M + H)$^+$.

Step A. *tert*-Butyl (1-(Hydroxymethyl)cyclo-1-propyl)methylcarbamate 20. **19a** (0.40 g, 3.9 mmol, 1.0 equiv), NEt_3 (1.1 mL, 7.9 mmol, 2.0 equiv), and di-*tert*-butyl dicarbonate (1.0 mL, 4.7 mmol, 1.2 equiv) were dissolved in dry CH_3OH (30 mL) and stirred at 50 °C for 12 h. The solvent was evaporated. The crude product was purified by chromatography using a Chromatotron device (Harrison Research Ltd.), utilizing silica gel plates of a layer thickness of 2 mm and $CHCl_3$ as eluent: yield 0.28 g (39.5%); ^{1}H NMR ($CDCl_3$) δ 0.38−0.50 (m, 4H), 1.42 (s, 9H), 3.09 (s, 2H), 3.36 (s, 2H), 4.85 (br s, 1H); MS *m*/*z* 202.2 (M + H)$^+$, 128.1 ([M − C_4H_9O]•)$^+$, 101.1 ([M − $C_5H_{10}NO$]•)$^+$.

Step B. *tert*-Butyl (1-((1*H*-Imidazol-1-yl)methyl)cyclo-1-propyl)methylcarbamate 21. **20** (0.28 g, 1.4 mmol, 1.0 equiv) and NEt_3 (0.210 mL, 1.54 mmol, 1.1 equiv) were dissolved in dry CH_2Cl_2 (20 mL). The mixture was cooled to 0 °C and a solution of

mesyl chloride (0.10 mL, 1.4 mmol, 1.0 equiv) in dry CH_2Cl_2 (5 mL) was added dropwise under an atmosphere of argon. The solution was stirred at 0 °C for 3 h and at room temperature for 12 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed once by means of saturated $NaHCO_3$, 1 N HCl, and water (20 mL for each washing step). The organic layer was dried over Na_2SO_4 and filtered, and the solvent was evaporated. The product was used without further purification for the next step. The alkylation was carried out according to the method described in ref 19 using the mesylated instead of the ω-bromoalkyl compound as the alkylating agent [imidazole (0.14 g, 2.1 mmol, 1.0 equiv), sodium hydride (60% in mineral oil, 0.08 g, 2.1 mmol, 1.0 equiv)]. The compound was purified by means of semipreparative HPLC: yield 0.15 g (43.5%); ^{1}H NMR ($CDCl_3$) δ 0.38−0.50 (m, 4H), 1.42 (s, 9H), 3.09 (s, 2H), 3.56 (s, 2H), 7.32−7.51 (m, 2H), 7.65 (s, 1H); MS *m*/*z* 252.1 $(M + H)^+$, 196.2 $([M - C_4H_9]^\bullet)^+$, 128.1 $([M - C_7H_{12}N_2]^\bullet)^+$.

Step C. (1-((1*H*-Imidazol-1-yl)methyl)cyclopropyl)methanamine 22. **21** (0.15 g, 0.59 mmol) was dissolved in a solution of HCl in 1,4-dioxane (4 M, 10 mL) and stirred at 0 °C for 1 h. After evaporation of the HCl/1,4-dioxane, the crude product was used without further purification for synthesis of thiourea **55**: yield 0.09 g (95.6%); MS *m*/*z* 152.2 $(M + H)^+$, 84.2 $([M - C_3H_3N_2]^\bullet)^+$.

3-(1*H*-Imidazol-1-yl)-*N*-methylpropan-1-amine 25. Step A. *tert*-Butyl 3-chloropropylmethylcarbamate 24. 3-Chloro-*N*-methylpropan-1-amine hydrochloride **23** (1.0 g, 6.9 mmol, 1.0 equiv) and di-*tert*-butyl dicarbonate (1.36 mL, 7.6 mmol, 1.1 equiv) were dissolved in a 2:1 mixture of 1,4-dioxane/water (30 mL). Aqueous NaOH (1 M, 10 mL) was added and the solution was stirred for 20 h at room temperature. The organic solvent was removed under reduced pressure and the remaining aqueous layer was washed by means of petroleum ether (2 × 80 mL). After the adjustment of the pH to a value between 8 and 10 by adding a solution of an aqueous solution of HCl (1 M), the aqueous layer was extracted by means of CH_2Cl_2 (3 × 50 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was used without further purification: yield 0.41 g (28.7%); MS *m*/*z* 208.2 $(M + H)^+$, 230.3 $(M + Na)^+$, 150.2 $([M - C_4H_9]^\bullet)^+$.

Step B. The alkylation was carried out according to the method described in ref 19 using the ω-chloro instead of the ω-bromoalkyl compound as the alkylating agent. The resulting *tert*-butyl-3-chloropropylmethylcarbamate **24** was reacted with a solution of HCl in 1,4-dioxane (4 M, 20 mL) at 0 °C. After the complete deprotection, the organic solvent was removed under vacuum and the resulting precipitate was used without further purification [imidazole (0.14 g, 2.0 mmol, 1.1 equiv), sodium hydride (60% in mineral oil, 0.07 g, 1.8 mmol, 1.0 equiv), *tert*-butyl-3-chloropropylmethylcarbamate (0.41 g, 2.0 mmol, 1.1 equiv)]: yield 0.22 g (99.8%); MS *m*/*z* 140.3 $(M + H)^+$.

General Procedure for the Synthesis of the 1-(1*H*-Imidazol-1-ylalkyl)-*n*-aryl/alkylthioureas 26−50, 52−59. All syntheses were performed using a Büchi Synchor parallel synthesizing device. Thereby, a typical reaction batch was performed by utilizing the isothiocyanate (4.0 mmol, 1.0 equiv) and the corresponding (1*H*-imidazol-1-yl)alkyl-1-amine (4.0 mmol, 1.0 equiv) in absolute EtOH (10 mL). After shaking for 2 h under reflux, the solvent was evaporated and the resulting solid was recrystallized from EtOH.

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-methylthiourea 26: yield 0.59 g (73.9%); mp 122−122.5 °C; ^{1}H NMR δ 1.85−1.95 (m, 2H), 2.84 (s, 3H), 3.31−3.35 (m, 2H), 3.95−3.97 (m, 2H), 6.85 (d, *J* = 1.0 Hz, 1H), 7.15 (s, 1H), 7.42 (br s, 2H), 7.65 (s, 1H); MS *m*/*z* 199.1 $(M + H)^+$, 221.3 $(M + Na)^+$, 131.0 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 221.08304 $([M + Na]^+$, calcd for $C_8H_{14}N_4SNa^+$ 221.08313); HPLC (214 nm) t_R 1.89 min (100%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-butylthiourea 27: yield 0.79 g (28.0%); mp 135.6−136.0 °C; ^{1}H NMR δ 0.82−0.90 (m, 3H), 1.21−1.51 (m, 4H), 1.84−1.98 (m, 2H), 3.17−3.33 (m, 4H), 3.92−3.99 (m, 2H), 6.87 (s, 1H), 7.15 (s, 1H), 7.36−7.38 (m, 2H), 7.65 (s, 1H); MS *m*/*z* 241.1 $(M + H)^+$, 173.1 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 241.14805 $([M + H]^+$, calcd for $C_{11}H_{21}N_4S^+$ 241.14814); HPLC (214 nm) t_R 15.15 min (98.6%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-*tert*-butylthiourea 28: yield 0.48 g (49.6%); mp 147.0−147.5 °C; ^{1}H NMR δ 1.36 (s, 9H), 1.85−1.95 (m, 2H), 3.22−3.32 (m, 2H), 3.88−3.95 (m, 2H), 6.85 (s, 1H), 7.11−7.13 (m, 1H), 7.21−7.29 (m, 2H), 7.65 (s, 1H); MS *m*/*z* 241.1 $(M + H)^+$, 173.1 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 263.13002 $([M + Na]^+$, calcd for $C_{12}H_{20}N_4SNa^+$ 263.13008); HPLC (214 nm) t_R 14.21 min (97.8%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-isopropylthiourea 29: yield 0.46 g (50.8%); mp 88.6−89.1 °C; ^{1}H NMR δ 1.00−1.06 (m, 6H), 1.85−1.89 (m, 2H), 3.28−3.41 (m, 3H), 3.90−3.94 (m, 2H), 6.85 (s, 1H), 7.15 (s, 1H), 7.16−7.25 (m, 2H), 7.65 (s, 1H); MS *m*/*z* 227.3 $(M + H)^+$, 159.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 249.11429 $([M + Na]^+$, calcd for $C_{10}H_{19}N_4SNa^+$ 249.11443); HPLC (214 nm) t_R 4.57 min (96.4%).

1-(Bicyclo[2.2.1]hept-5-en-2-yl)-3-(3-(1*H*-imidazol-1-yl)-propyl)thiourea 30: yield 0.44 g (39.8%); mp 141.5−142.1 °C; ^{1}H NMR δ 1.22−1.25 (m, 1H), 1.40−1.45 (m, 1H), 1.52−1.56 (m, 1H), 1.91−1.95 (m, 2H), 2.74 (s, 1H), 2.80 (s, 1H), 3.29−3.35 (m, 3H), 3.94−3.97 (m, 2H), 6.04−6.06 (m, 1H), 6.13−6.15 (m, 1H), 6.85 (s, 1H), 7.15 (s, 1H), 7.26 (br s, 1H), 7.53 (br s, 1H), 7.65 (s, 1H); MS *m*/*z* 277.0 $(M + H)^+$, 299.3 $(M + Na)^+$, 209.9 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 299.12991 $([M + Na]^+$, calcd for $C_{14}H_{20}N_4SNa^+$ 299.13008); HPLC (214 nm) t_R 21.04 min (98.3%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-cyclohexylthiourea 31: yield 1.05 g (98.5%); mp 95.4−96.3 °C; ^{1}H NMR δ 0.97−1.27 (m, 5H), 1.48−1.91 (m, 6H), 3.33−3.41 (m, 4H) 3.87−3.94 (m, 2H), 6.85 (s, 1H), 7.15 (s, 1H), 7.27−7.53 (m, 2H), 7.65 (s, 1H); MS *m*/*z* 267.2 $(M + H)^+$, 199.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 267.16389 $([M + H]^+$, calcd for $C_{13}H_{23}N_4^+$ 267.16379); HPLC (214 nm) t_R 18.78 min (98.5%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-benzylthiourea 32: yield 0.66 g (59.9%); mp 127.0−128.0 °C; ^{1}H NMR δ 1.85−1.95 (m, 2H), 3.28−3.40 (m, 2H), 3.92−3.93 (m, 2H), 4.61 (s, 2H), 6.85 (s, 1H), 7.15 (s, 1H), 7.19−7.35 (m, 5H), 7.52−7.57 (m, 2H), 7.85 (s, 1H); MS *m*/*z* 275.3 $(M + H)^+$, 207.1 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 297.11456 $([M + Na]^+$, calcd for $C_{14}H_{18}N_4SNa^+$ 297.11443); HPLC (214 nm) t_R 20.61 min (97.5%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-phenylthiourea 33: yield 0.78 g (75.0%); mp 166.5−167.0 °C; ^{1}H NMR δ 1.95−2.05 (m, 2H), 3.39−3.44 (m, 2H), 3.92−4.01 (m, 2H), 6.85 (s, 1H), 7.04−7.09 (m, 1H) 7.15 (s, 1H), 7.25−7.30 (m, 2H), 7.31−7.35 (m, 2H), 7.61 (s, 1H), 7.77 (br s, 1H), 9.46 (br s, 1H); MS *m*/*z* 261.1 $(M + H)^+$, 193.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 283.09865 $([M + Na]^+$, calcd for $C_{12}H_{16}N_4SNa^+$ 283.09861); HPLC (214 nm) t_R 14.00 min (97.4%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-naphthalen-1-ylthiourea 34: yield 1.06 g (85.4%); mp 143.2−143.9 °C; ^{1}H NMR δ 1.95−2.05 (m, 2H), 3.40−3.45 (m, 2H), 3.92−4.01 (m, 2H), 6.86 (s, 1H), 7.13 (s, 1H), 7.44−7.64 (m, 6H), 7.84−7.88 (m, 2H), 7.94−7.97 (m, 1H), 9.63 (br s, 1H); MS *m*/*z* 311.3 $(M + H)^+$, 243.4 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 333.11410 $([M + Na]^+$, calcd for $C_{17}H_{18}N_4SNa^+$ 333.11443); HPLC (214 nm) t_R 22.07 min (98.6%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(4-methylphenyl)thiourea 35: yield 1.07 g (97.5%); mp 159.4−160.3 °C; ^{1}H NMR δ 1.95−2.05 (m, 2H), 2.23 (s, 3H), 3.40−3.45 (m, 2H), 3.92−4.01 (m, 2H), 6.86 (s, 1H), 7.07−7.09 (m, 2H), 7.13 (s, 1H), 7.17−7.19 (m, 2H), 7.59 (s, 1H), 7.64 (br s, 1H), 9.35 (br s, 1H); MS *m*/*z* 275.2 $(M + H)^+$, 207.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 297.11419 $([M + Na]^+$, calcd for $C_{14}H_{18}N_4SNa^+$ 297.11443); HPLC (214 nm) t_R 19.72 min (97.4%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(4-ethylphenyl)thiourea 36: yield 0.83 g (72.2%); mp 100.0−100.5 °C; ^{1}H NMR δ 1.16−1.18 (m, 3H), 1.95−2.02 (m, 2H), 2.55−2.59 (m, 2H), 3.41−3.45 (m, 2H), 3.97−4.00 (m, 2H), 6.85 (s, 1H), 7.14−7.17 (m, 3H), 7.23−7.25 (m, 2H), 7.63 (s, 1H), 7.70 (br s, 1H), 9.40 (br s, 1H); MS *m*/*z* 289.3 $(M + H)^+$, 221.1 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 311.12976 $([M + Na]^+$, calcd for $C_{15}H_{20}N_4SNa^+$ 311.13008); HPLC (214 nm) t_R 22.62 min (99.1%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(4-fluorophenyl)thiourea 37: yield 0.79 g (71.1%); mp 147.0−148.0 °C; ^{1}H NMR δ 1.95−2.05

(m, 2H), 3.39−3.40 (m, 2H), 3.90−4.05 (m, 2H), 6.85 (s, 1H), 7.05−7.15 (m, 3H), 7.32−7.41 (m, 2H), 7.60 (s, 1H), 7.76 (br s, 1H), 9.41 (br s, 1H); MS *m/z* 279.3 $(M + H)^+$, 211.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 301.08915 $([M + Na]^+$, calcd for $C_{13}H_{15}N_4SFNa^+$ 301.08936); HPLC (214 nm) t_R 16.76 min (97.3%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(4-(dimethylamino)phenyl)-thiourea 38: yield 0.75 g (62.1%); mp 146.5−147.0 °C; ^{1}H NMR δ 1.91−2.02 (m, 2H), 2.93 (s, 6H), 3.39−3.42 (m, 2H), 3.93−4.01 (m, 2H), 6.67−6.70 (m, 2H), 6.92 (s, 1H), 7.05−7.10 (m, 2H), 7.15 (s, 1H), 7.40 (br s, 1H), 7.66 (s, 1H), 9.22 (s, 1H); MS *m/z* 304.2 $(M + H)^+$, 236.0 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 326.14070 $([M + Na]^+$, calcd for $C_{15}H_{21}N_5SNa^+$ 326.14098); HPLC (214 nm) t_R 9.15 min (98.2%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(4-nitrophenyl)thiourea 39: yield 0.86 g (70.2%); mp 165.0−166.0 °C; ^{1}H NMR δ 1.91−2.05 (m, 2H), 3.30−3.51 (m, 2H), 3.95−4.05 (m, 2H), 6.85 (s, 1H), 7.15 (s, 1H), 7.62 (s, 1H), 7.73−7.76 (m, 2H), 8.12−8.13 (m, 2H), 8.31 (br s, 1H), 10.12 (br s, 1H); MS *m/z* 306.2 $(M + H)^+$, 237.9 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 328.08369 $([M + Na]^+$, calcd for $C_{13}H_{15}N_5O_2SNa^+$ 328.08386); HPLC (214 nm) t_R 22.30 min (100%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(4-acetylphenyl)thiourea 40: yield 0.98 g (81.2%); mp 170.0−171.0 °C; ^{1}H NMR δ 1.91−2.12 (m, 2H), 2.48 (s, 3H), 3.20−3.52 (m, 2H), 3.92−4.11 (m, 2H), 6.85 (s, 1H), 7.15 (s, 1H), 7.51−7.65 (m, 3H), 7.81−7.92 (m, 2H), 8.02−8.09 (m, 1H), 9.81 (br s, 1H); MS *m/z* 303.2 $(M + H)^+$, 235.1 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 325.10906 $([M + Na]^+$, calcd for $C_{15}H_{18}N_4SNa^+$ 325.10935); HPLC (214 nm) t_R 18.58 min (99.8%).

1-(3-(1*H*-Imidazol-1-yl)-propyl)-3-(4-methylsulfanyl-phenyl)-thiourea 41: yield 1.22 g (99.5%); mp 140.0−140.5 °C; ^{1}H NMR δ 1.98−2.05 (m, 2H), 2.48 (s, 3H), 3.22−3.52 (m, 2H), 3.95−4.05 (m, 2H), 6.85 (s, 1H), 7.16−7.33 (m, 5H), 7.63 (s, 1H), 7.76 (br s, 1H), 9.44 (br s, 1H); MS *m/z* 307.2 $(M + H)^+$, 239.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 329.08653 $([M + Na]^+$, calcd for $C_{14}H_{18}N_4S_2Na^+$ 329.08650); HPLC (214 nm) t_R 22.18 min (97.8%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(4-(benzyloxy)phenyl)thiourea 42: yield 0.51 g (34.8%); mp 123.4−123.9 °C; ^{1}H NMR δ ($CDCl_3$) 2.03−2.10 (m, 2H), 3.58−3.63 (m, 2H), 3.94−3.97 (m, 2H), 5.04 (s, 2H), 6.01 (s, 1H), 6.81 (br s, 1H), 6.90−6.96 (m, 4H), 7.11−7.19 (m, 2H), 7.31−7.40 (m, 5H), 7.92 (s, 1H); MS *m/z* 367.2 $(M + H)^+$, 299.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 389.14028 $([M + Na]^+$, calcd for $C_{20}H_{22}ON_4SNa^+$ 389.14065); HPLC (214 nm) t_R 23.73 min (98.2%).

1-(4-Ethoxy-phenyl)-3-(3-(1*H*-imidazol-1-yl)-propyl)thiourea 43: yield 0.87 g (71.9%); mp 126.0−126.5 °C; ^{1}H NMR δ 1.28−1.32 (m, 3H), 1.94−2.01 (m, 2H), 3.41−3.45 (m, 2H), 3.93−4.02 (m, 4H), 6.85 (s, 1H), 7.16−7.33 (m, 5H), 7.63 (s, 1H), 7.76 (br s, 1H), 9.44 (br s, 1H); MS *m/z* 305.2 $(M + H)^+$, 237.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 327.12504 $([M + Na]^+$, calcd for $C_{15}H_{20}ON_4SNa^+$ 327.12500); HPLC (214 nm) t_R 20.64 min (98.4%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(4-methoxyphenyl)thiourea 44: yield 0.87 g (75.3%); mp 125.0−125.5 °C; ^{1}H NMR δ 1.87−2.00 (m, 2H), 3.29−3.44 (m, 2H), 3.70 (s, 3H), 3.91−4.02 (m, 2H), 6.72−6.93 (m, 3H), 7.11−7.21 (m, 3H), 7.50 (s, 1H), 7.61 (s, 1H), 9.21 (s, 1H); MS *m/z* 291.1 $(M + H)^+$, 223.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 313.10913 $([M + Na]^+$, calcd for $C_{14}H_{18}ON_4SNa^+$ 313.10935); HPLC (214 nm) t_R 22.83 min (97.3%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(benzo[*d*][1,3]dioxol-6-yl)-thiourea 45: yield 0.25 g (20.7%); mp 115.0−115.6 °C; ^{1}H NMR δ 1.99−2.03 (m, 2H), 3.42−3.44 (m, 2H), 4.08−4.15 (m, 2H), 6.01 (s, 2H), 6.67 (d, J = 6.6 Hz, 1H), 6.90 (d, J = 8.3 Hz, 1H), 6.95 (s, 1H), 7.25 (s, 1H), 7.45 (s, 1H), 7.68 (br s, 1H), 8.32 (br s, 1H), 9.38 (br s, 1H); MS *m/z* 305.2 $(M + H)^+$, 237.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 305.10642 $([M + H]^+$, calcd for $C_{14}H_{17}O_2N_4S^+$ 305.10667); HPLC (214 nm) t_R 13.71 min (96.6%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(3-methoxyphenyl)thiourea 46: yield 0.86 g (73.9%); mp 89.5−90.0 °C; ^{1}H NMR δ 1.99−2.05 (m, 2H), 3.42−3.46 (m, 2H), 3.71 (s, 3H), 3.97−4.01 (m, 2H), 6.67 (dd, J = 5.8 Hz, 2.5 Hz, 1H), 6.85−6.90 (m, 2H), 6.99−7.07 (m, 1H), 7.15−7.25 (m, 2H), 7.62 (s, 1H), 7.86 (br s, 1H), 9.51 (s, 1H); MS *m/z* 291.1 $(M + H)^+$, 223.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 313.10922 $([M + Na]^+$, calcd for $C_{14}H_{18}ON_4SNa^+$ 313.10935); HPLC (214 nm) t_R 16.40 min (98.5%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(3,4-dimethoxybenzyl)thiourea 47: yield 0.38 g (28.5%); mp:143.5−144.5 °C; ^{1}H NMR δ ^{1}H NMR δ 1.93−1.99 (m, 2H), 3.42−3.46 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 3.96−3.99 (m, 2H), 4.72 (s, 2H), 6.75−6.77 (m, 1H), 6.89−6.90 (m, 2H), 6.95 (s, 1H), 7.16 (s, 1H), 7.59 (br s, 1H), 7.62 (s, 1H), 9.33 (s, 1H); MS *m/z* 335.3 $(M + H)^+$, 267.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 357.113546 $([M + Na]^+$, calcd for $C_{16}H_{22}O_2N_4SNa^+$ 357.13556); HPLC (214 nm) t_R 19.41 min (97.3%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(2,3-dihydrobenzo[*b*][1,4]-dioxin-7-yl)thiourea 48: yield 0.17 g (13.1%); mp 103.0−103.5 °C; ^{1}H NMR δ 1.94−1.99 (m, 2H), 3.33−3.41 (m, 2H), 3.95−3.98 (m, 2H), 4.19−4.26 (m, 4H), 6.68−6.71 (m, 1H), 6.78−6.80 (m, 1H), 6.86−6.87 (m, 2H), 7.16 (s, 1H), 7.63 (s, 2H), 9.28 (s, 1H); MS *m/z* 319.3 $(M + H)^+$, 251.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 341.10400 $([M + Na]^+$, calcd for $C_{15}H_{18}O_2N_4SNa^+$ 341.10426); HPLC (214 nm) t_R 16.03 min (97.1%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(3,5-dimethoxyphenyl)thiourea 49: yield 1.18 g (92.4%); mp 142.0−143.0 °C; ^{1}H NMR δ 1.95−2.01 (m, 2H), 3.42−3.49 (m, 2H), 3.61 (s, 6H), 3.97−4.00 (m, 2H), 6.25 (s, 1H), 6.59 (s, 2H), 6.87 (s, 1H), 7.17 (s, 1H), 7.62 (s, 1H), 7.83 (s, 1H), 9.47 (s, 1H); MS *m/z* 321.2 $(M + H)^+$, 253.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 343.12010 $([M + Na]^+$, calcd for $C_{15}H_{20}O_2N_4SNa^+$ 343.11992); HPLC (214 nm) t_R 21.12 min (97.2%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(2,4-dimethoxyphenyl)thiourea 50: yield 1.25 g (97.6%); mp 120.0−120.5 °C; ^{1}H NMR δ 1.95−2.01 (m, 2H), 3.42−3.49 (m, 2H), 3.74 (s, 3H), 3.75 (s, 3H), 3.97−3.99 (m, 2H), 6.48 (dd, J = 6.1 Hz, 2.4 Hz, 1H), 6.60 (s, 1H), 6.87 (s, 1H), 7.15 (s, 1H), 7.31 (d, J = 6.1 Hz, 1H), 7.47 (br s, 1H), 7.61 (s, 1H), 8.73 (s, 1H); MS *m/z* 321.2 $(M + H)^+$, 253.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 343.12011 $([M + Na]^+$, calcd for $C_{15}H_{20}O_2N_4SNa^+$ 343.11992); HPLC (214 nm) t_R 17.71 min (99.0%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(3,4-dimethoxyphenyl)-urea 51. 3,4-Dimethoxyphenyl isocyanate (0.72 g, 4.0 mmol, 1.0 equiv) was added to a solution of 3-(1*H*-imidazol-1-yl)propan-1-amine (0.48 mL, 4.0 mmol, 1.0 equiv) in dry acetonitrile (50 mL). The mixture was stirred at room temperature for 8 h. After removing the solvent, the crude product was recrystallized from EtOH**:** yield 0.59 g (48.6%); mp 114.5−115.0 °C; ^{1}H NMR δ 1.81−1.86 (m, 2H), 3.00−3.05 (m, 2H), 3.66 (s, 3H), 3.69 (s, 3H), 3.95−3.98 (m, 2H), 6.09−6.12 (m, 1H), 6.79 (s, 2H), 6.88 (s, 1H), 7.14 (s, 1H), 7.17 (s, 1H), 7.62 (s, 1H), 8.24 (s, 1H); MS *m/z* 305.0 $(M + H)^+$, 237.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 327.14255 $([M + Na]^+$, calcd for $C_{15}H_{20}O_3N_4SNa^+$ 327.14276); HPLC (214 nm) t_R 11.73 min (97.1%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(3,4,5-trimethoxyphenyl)thiourea 52: yield 1.30 g (92.5%); mp 124.5−125.5 °C; ^{1}H NMR δ 1.94−2.01 (m, 2H), 3.41−3.52 (m, 2H), 3.62 (s, 3H), 3.73 (s, 6H), 3.97−4.00 (m, 2H), 6.65 (s, 2H), 6.87 (s, 1H), 7.17 (s, 1H), 7.63 (s, 1H), 7.73 (br s, 1H), 9.41 (s, 1H); MS *m/z* 351.3 $(M + H)^+$, 283.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 373.12977 $([M + Na]^+$, calcd for $C_{16}H_{22}O_3N_4SNa^+$ 373.13048); HPLC (214 nm) t_R 16.85 min (98.6%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(3,4-dimethoxyphenyl)thiourea 53: yield 0.66 g (51.3%); mp 160.0−161.0 °C; ^{1}H NMR δ 1.93−1.99 (m, 2H), 3.42−3.46 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 3.96−3.99 (m, 2H), 6.75−6.77 (m, 1H), 6.89−6.90 (m, 2H), 6.95 (s, 1H), 7.16 (s, 1H), 7.59 (br s, 1H), 7.62 (s, 1H), 9.33 (s, 1H); MS *m/ z* 321.2 $(M + H)^+$, 253.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 343.12009 $([M + Na]^+$, calcd for $C_{15}H_{20}O_2N_4SNa^+$ 343.11992); HPLC (214 nm) t_R 14.00 min (99.8%).

1-(2-(1*H*-Imidazol-1-yl)ethyl)-3-(3,4-dimethoxyphenyl)thiourea 54 started from **8** (0.27 g, 2.4 mmol) and 3,4-dimethoxyphenyl isothiocyanate (0.47 g, 2.4 mmol): yield 0.73 g (59.6%); mp

157.5−159.0 °C; ^{1}H NMR δ 3.69 (s, 3H), 3.72 (s, 3H), 3.74−3.78 (m, 2H), 4.16−4.19 (m, 2H), 6.68−6.70 (m, 1H), 6.85−6.89 (m, 3H), 7.13 (s, 1H), 7.47 (br s, 1H), 7.59 (s, 1H), 9.48 (s, 1H); MS *m/z* 307.2 $(M + H)^+$, 239.1 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 329.10398 ($[M + Na]^+$, calcd for $C_{14}H_{18}O_2N_4SNa^+$ 329.10426); HPLC (214 nm) t_R 11.12 min (99.4%).

1-(4-(1*H*-Imidazol-1-yl)butyl)-3-(3,4-dimethoxyphenyl)thiourea 55 started from **10** (0.29 g, 2.1 mmol) and 3,4-dimethoxyphenyl isothiocyanate (0.41 g, 2.1 mmol): yield 1.07 g (79.9%); mp 114.5−116.0 °C; ^{1}H NMR δ 1.42−1.51 (m, 2H), 1.63−1.71 (m, 2H), 3.42−3.51 (m, 2H), 3.69 (s, 3H), 3.72 (s, 3H), 3.95−4.01 (m, 2H), 6.68−6.70 (m, 1H), 6.85−6.89 (m, 1H), 6.91−6.98 (m, 2H), 7.13 (s, 1H), 7.47 (br s, 1H), 7.59 (s, 1H), 9.48 (s, 1H); MS *m/z* 335.3 $(M + H)^+$, 267.1 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 357.13563 ($[M + Na]^+$, calcd for $C_{16}H_{23}O_2N_4SNa^+$ 357.13556); HPLC (214 nm) t_R 16.47 min (99.1%).

1-((*R*)-3-(1*H*-Imidazol-1-yl)-2-methylpropyl)-3-(3,4-dimethoxyphenyl)thiourea 56 started from **18** (0.94 g, 3.2 mmol) and 3,4-dimethoxyphenyl isothiocyanate (0.63 g, 3.2 mmol): yield 0.57 g (53.8%); mp 155.0−157.5 °C; the purification was performed by means of semipreparative HPLC; ^{1}H NMR δ 0.83 (d, $J = 6.6$ Hz, 3H), 2.31−2.39 (m, 1H), 3.37−3.43 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 4.04−4.08 (m, 1H), 4.16−4.18 (m, 1H), 6.78 (d, $J = 8.5$ Hz, 1H), 6.89 (d, $J = 8.5$ Hz, 1H), 6.99 (s, 1H), 7.67 (s, 1 H), 7.75 (s, 2H), 9.11 (s, 1H), 9.50 (s, 1H); MS *m/z* 335.3 $(M + H)^+$, 267.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 335.15345 ($[M + H]^+$, calcd for $C_{16}H_{23}O_2N_4S^+$ 335.15362); HPLC (214 nm) t_R 19.13 min (97.9%).

1-((*S*)-3-(1*H*-Imidazol-1-yl)-2-methylpropyl)-3-(3,4-dimethoxyphenyl)thiourea 57 started from **17** (0.95 g, 3.2 mmol) and 3,4-dimethoxyphenyl isothiocyanate (0.63 g, 3.2 mmol): yield 0.53 g (49.4%); mp 150.5−151.5 °C; the purification was performed by means of semipreparative HPLC; ^{1}H NMR δ 0.83 (d, $J = 6.6$ Hz, 3H), 2.36−2.41 (m, 1H), 3.37−3.43 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 4.04−4.08 (m, 1H), 4.16−4.18 (m, 1H), 6.77 (d, $J = 8.5$ Hz, 1H), 6.89 (d, $J = 8.5$ Hz, 1H), 6.99 (s, 1H), 7.67 (s, 1 H), 7.75 (s, 2H), 9.11 (s, 1H), 9.50 (s, 1H); MS *m/z* 335.4 $(M + H)^+$, 267.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 335.15344 ($[M + H]^+$, calcd for $C_{16}H_{23}O_2N_4S^+$ 335.15362); HPLC (214 nm) t_R 17.93 min (98.1%).

1-((1-((1*H*-Imidazol-1-yl)methyl)cyclopropyl)methyl)-3-(3,4-dimethoxyphenyl)thiourea 58 started from **22** (0.09 g, 0.59 mmol) and 3,4-dimethoxyphenyl isothiocyanate (0.12 g, 0.59 mmol): yield 0.08 g (37.5%); mp 166.5−168.5 °C; the purification was performed by means of semipreparative HPLC; ^{1}H NMR (rotamers) δ 0.69−0.73 (m, 4H), 1.85−1.86 (m, 1.3H), 2.11−2.21 (m, 2.7H), 3.35−3.40 (m, 2H), 3.43−3.44 (m, 2H), 3.68−3.71 (4 × s, 12H), 4.13 (s, 2H), 4.94 (s, 2H), 6.75−6.78 (m, 2H), 6.84−6.89 (m, 2H), 6.98 (s, 2H), 7.48−7.49 (m, 2H), 7.60−7.61 (m, 2H), 7.65 (s, 1H), 7.78 (s, 1H), 8.82 (s, 1H), 9.07 (s, 1H), 9.36 (s, 1H), 9.43 (s, 1H); MS *m/z* 347.2 $(M + H)^+$, 279.2 $([M - C_3H_3N_2]^\bullet)^+$, 137.5 $([M - C_9H_{13}N_4S]^\bullet)^+$; ESI-FTICR-MS *m/z* 347.15352 ($[M + H]^+$, calcd for $C_{17}H_{23}O_2N_4S^+$ 347.15362); HPLC (214 nm) t_R 19.85 min (97.8%).

1-(3-(1*H*-Imidazol-1-yl)propyl)-3-(3,4-dimethoxyphenyl)-1-methylthiourea 59 started from **25** (0.220 g, 1.04 mmol, 1.0 equiv), 3,4-dimethoxyphenyl isothiocyanate (0.200 g, 1.04 mmol, 1.0 equiv), and NEt_3 (0.280 mL, 2.08 mmol, 2.0 equiv). The purification was performed by means of flash-chromatography using silica gel and a $CHCl_3/CH_3OH$ gradient as eluating system**:** yield 0.12 g (34.5%); mp 155.1−156.3 °C; ^{1}H NMR ($CDCl_3$) δ 2.17−2.21 (m, 2H), 3.08 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.94−3.98 (m, 2H), 4.03−4.07 (m, 2H), 6.76−6.80 (m, 2H), 6.81 (s, 1H), 6.89 (s, 1H), 7.04 (s, 1H), 7.14 (s, 1H), 7.52 (s, 1H); MS *m/z* 335.4 $(M + H)^+$, 267.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 357.13522 ($[M + Na]^+$, calcd for $C_{16}H_{22}O_2N_4SNa^+$ 357.13556); HPLC (214 nm) t_R 15.05 min (97.5%).

General Procedure for the Synthesis of the *N*-(3-(1*H*-Imidazol-1-yl)propyl)benzo[*d*]thiazol-2-amines 66−69. 1*H*-Imidazole-1-propanamine **9** was reacted with the corresponding 2-chlorobenzo[*d*]thiazoles in *n*-butanol for 24 h at reflux as described in ref 21. After removing the solvent and recrystallization from CH_3OH, **66**−**69** were yielded.

***N*-(3-(1*H*-imidazol-1-yl)propyl)benzo[*d*]thiazol-2-amine 66** started from **9** (1.50 mL, 12.6 mmol, 1.0 equiv) and 2-chlorobenzo[*d*]thiazole **63** (1.86 mL, 13.9 mmol, 1.1 equiv): yield 0.28 g (8.6%); mp 89.6−90.5 °C; ^{1}H NMR δ 1.98−2.02 (m, 2H), 3.27−3.34 (m, 2H), 4.03−4.06 (m, 2H), 6.89 (s, 1H), 6.98−7.00 (m, 1H), 7.18−7.21 (m, 2H), 7.35−7.37 (m, 1H), 7.65 (s, 2H), 8.06 (s, 1H); MS *m/z* 259.4 $(M + H)^+$, 191.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 281.08301 ($[M + Na]^+$, calcd for $C_{13}H_{14}N_4SNa^+$ 281.08313); HPLC (214 nm) t_R 9.71 min (99.1%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-6-chlorobenzo[*d*]thiazol-2-amine 67** started from **9** (0.26 mL, 2.2 mmol, 1.0 equiv) and 2,5-dichlorobenzo[*d*]thiazole **64** (0.500 g, 2.42 mmol, 1.1 equiv): yield 0.068 g (9.7%); mp 108.2−108.9 °C; ^{1}H NMR δ 1.98−2.02 (m, 2H), 3.27−3.34 (m, 2H), 4.03−4.06 (m, 2H), 6.90 (s, 1H), 7.20−7.22 (m, 2H), 7.32−7.37 (m, 1H), 7.65 (s, 1H), 7.77 (s, 1H), 8.20 (s, 1H); MS *m/z* 293.3 $(M + H)^+$; ESI-FTICR-MS *m/z* 293.06204 ($[M + H]^+$, calcd for $C_{13}H_{14}N_4SCl^+$ 293.06222); HPLC (214 nm) t_R 21.29 min (98.2%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-6-methoxybenzo[*d*]thiazol-2-amine 68** started from **9** (0.27 mL, 2.3 mmol, 1.0 equiv) and 2-chloro-5-methoxybenzo[*d*]thiazole **65** (0.50 g, 2.5 mmol, 1.1 equiv): yield 0.060 g (9.0%); mp 122.8−123.6 °C; ^{1}H NMR δ 1.98−2.02 (m, 2H), 3.27−3.34 (m, 2H), 3.69 (s, 3H), 4.03−4.06 (m, 2H), 6.77−6.78 (m, 1H), 6.87 (s, 1H), 7.17 (s, 1H), 7.23−7.26 (m, 2H), 7.63 (s, 1H), 7.81 (s, 1H); MS *m/z* 289.1 $(M + H)^+$, 221.4 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 289.11150 ($[M + H]^+$, calcd for $C_{14}H_{17}ON_4S^+$ 289.11175); HPLC (214 nm) t_R 14.77 min (97.7%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-5,6-dimethoxybenzo[*d*]thiazol-2-amine 69. Step A. 5,6-Dimethoxybenzo[*d*]thiazol-2-amine 61**.[22] 3,4-Dimethoxyaniline **60** (6.00 g, 39.2 mmol, 1.0 equiv) and KSCN (15.2 g, 157 mmol, 1.0 equiv) were dissolved in acetic acid (96%, 70 mL). Then a solution of bromine (6.23 g, 39.2 mmol, 1.0 equiv) in acetic acid (96%, 30 mL) was added dropwise and the solution was stirred for 10 h at 35 °C. The solution was filtered and neutralized by means of aqueous ammonia (33%). The precipitate was filtered off, dried over P_2O_5 under reduced pressure, and used without further purification**:** yield 0.92 g (11.5%).

Step B. 2-Chloro-5,6-dimethoxybenzo[*d*]thiazole 62.[23] **61** (0.950 g, 13.8 mmol, 1.0 equiv) was dissolved in aqueous H_3PO_4 (85%, 30 mL), and then a solution of $NaNO_2$ (0.950 g, 13.8 mmol, 1.0 equiv) in water (1.43 mL) was added dropwise over a period of 30 min. The solution was stirred for 30 min and then a solution of $CuSO_4 \cdot 5H_2O$ (4.56 g, 18.3 mmol, 1.33 equiv) and NaCl (5.65 g, 0.1 mol, 7.25 equiv) in water (18 mL) was added dropwise at −5 °C. The solution was stirred for 1 h at −5 °C and then extracted twice by means of Et_2O (50 mL). The organic phase was washed by means of aqueous $NaHCO_3$ followed by water, dried over Na_2SO_4, and filtered. The solvent was evaporated and the residue was used without further purification**:** yield 0.32 g (31.7%). **69** was prepared as described above [**9** (0.16 mL, 3.2 mmol), **62** (0.32 g, 1.4 mmol)]: yield 0.16 g (36.9%); mp 118.0−118.7 °C; ^{1}H NMR ($CDCl_3$) δ 1.98−2.02, (m, 2H), 3.27−3.34 (m, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 4.03−4.06 (m, 2H), 6.91 (s, 1H), 7.02 (s, 1H), 7.05 (s, 1H), 7.10 (s, 1H), 7.24 (s, 1H), 7.48 (s, 1H); MS *m/z* 319.2 $(M + H)^+$, 251.4 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m/z* 319.12219 ($[M + H]^+$, calcd for $C_{15}H_{19}O_2N_4S^+$ 319.12232); HPLC (214 nm) t_R 13.09 min (99.7%).

General Procedure for the Synthesis of the *N*-(3-(1*H*-Imidazol-1-yl)propyl)-2-phenylethanethioamides 70−77, 81. A mixture of NEt_3 (0.58 mL, 4.0 mmol, 1.0 equiv) and the corresponding primary amine (4.0 mmol, 1.0 equiv) in CH_2Cl_2 (20 mL) was added dropwise to an ice-cooled, stirred solution of the corresponding acid chloride (4.0 mmol, 1.0 equiv) in CH_2Cl_2 (30 mL). The mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was diluted by adding CH_2Cl_2 (20 mL). The organic layer was washed by means of a saturated aqueous solution of $NaHCO_3$ and water (30 mL per washing step). The organic solution was dried over Na_2SO_4 and filtered, and the solvent

was removed under reduced pressure. After redissolving in dry 1,4-dioxane (50 mL), Lawesson's reagent (0.89 g, 2.2 mmol, 0.55 equiv) was added, and the mixture was heated to reflux and stirred for 8 h. The solvent was removed under reduced pressure and the crude product was redissolved in CH_2Cl_2 (50 mL). The organic layer was washed three times by means of a saturated aqueous solution of $NaHCO_3$ followed three times by water (30 mL per washing step), dried over Na_2SO_4, and filtered, and the organic solvent was removed. The compounds were purified by chromatography using a Chromatotron device (Harrison Research Ltd.), utilizing silica gel plates of a layer thickness of 2 mm and a $CHCl_3$/MeOH gradient as eluting system.

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-2-(3,4-dimethoxyphenyl)-ethanethioamide 70:** yield 0.14 g (10.6%); mp 148.0−150.0 °C; ^{1}H NMR δ 2.06−2.09 (m, 2H), 3.45−3.47 (m, 2H), 3.70 (s, 6H), 3.77 (s, 2H), 4.11−4.15 (m, 2H), 6.80−6.86 (m, 3H), 7.66 (s, 1H), 7.72 (s, 1H), 8.92 (s, 1H), 9.09 (s, 1H); MS *m*/*z* 320.2 $(M + H)^+$, 252.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 342.12408 $([M + Na]^+$, calcd for $C_{16}H_{21}O_2N_3SNa^+$ 342.12466); HPLC (214 nm) t_R 21.25 min (100%).

(*R*)-*N*-(3-(1*H*-Imidazol-1-yl)propyl)-2-phenylpropanethioamide 71: yield 0.17 g (15.8%); mp 82.0−82.5 °C; ^{1}H NMR δ 1.48 (d, J = 7.3 Hz, 3H), 1.94−1.99 (m, 2H), 3.40−3.45 (m, 2H), 3.89−3.93 (m, 2H), 4.05 (q, J = 7.3 Hz, 1H), 6.88 (s, 1H), 7.12 (s, 1H), 7.15−7.23 (m, 1H), 7.24−7.35 (m, 2H), 7.35−7.41 (m, 2H), 7.55 (s, 1H), 10.09 (s, 1H); MS *m*/*z* 274.4 $(M + H)^+$, 206.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 296.11895 $([M + Na]^+$, calcd for $C_{15}H_{19}N_3SNa^+$ 296.11918); HPLC (214 nm) t_R 23.01 min (99.2%).

(*S*)-*N*-(3-(1*H*-Imidazol-1-yl)propyl)-2-phenylpropanethioamide 72: yield 0.15 g (13.3%); mp 82.5−83.5 °C; ^{1}H NMR δ identical with that of **71**; MS *m*/*z* 274.4 $(M + H)^+$, 206.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 296.11882 $([M + Na]^+$, calcd for $C_{15}H_{19}N_3SNa^+$ 296.11918); HPLC (214 nm) t_R 22.72 min (98.7%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-2,2-diphenylethanethioamide 73:** yield 0.015 g (1.62%); mp 91.0−92.5 °C; ^{1}H NMR δ ^{1}H NMR δ 1.94−1.99 (m, 2H), 3.40−3.45 (m, 2H), 3.89−3.93 (m, 2H), 4.73 (s, 1H), 6.88 (s, 1H), 6.91 (s, 1H), 7.09 (s, 1H), 7.15−7.23 (m, 4H), 7.24−7.35 (m, 2H), 7.35−7.45 (m, 4H), 10.13 (s, 1H); MS *m*/*z* 336.3 $(M + H)^+$, 268.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 336.15246 $([M + H]^+$, calcd for $C_{20}H_{22}N_3S^+$ 336.15289); HPLC (214 nm) t_R 32.93 min (97.9%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-1-(4-methoxyphenyl)cyclopropanecarbothioamide 74:** yield 0.45 g (35.5%); mp 129.0−129.5 °C; ^{1}H NMR δ 1.06−1.08 (m, 2H), 1.54−1.56 (m, 2H), 1.90−1.97 (m, 2H), 3.44−3.48 (m, 2H), 3.73 (s, 3H), 3.82−3.89 (m, 2H), 6.87−6.88 (m, 3H), 7.10 (s, 1H), 7.25−7.26 (m, 2H), 7.58 (s, 1H), 8.96 (br s, 1H); MS *m*/*z* 316.0 $(M + H)^+$, 248.4 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 338.12952 $([M + Na]^+$, calcd for $C_{17}H_{22}N_3OSNa^+$ 338.12975); HPLC (214 nm) t_R 14.96 min (98.2%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-1-(4-chlorophenyl)cyclobutanecarbothioamide 75:** yield 0.55 g (41.5%); mp 137.5−139.0 °C; ^{1}H NMR δ 1.55−1.75 (m, 2H), 1.85−1.95 (m, 2H), 2.48−2.53 (m, 2H), 2.77−2.83 (m, 2H), 3.41−3.45 (m, 2H), 3.79−3.81 (m, 2H), 6.85 (s, 1H), 7.03 (s, 1H), 7.37−7.39 (m, 2H), 7.48 (s, 1H), 7.52−7.54 (m, 2H), 9.62 (s, 1H); MS *m*/*z* 334.3 $(M + H)^+$, 266.1 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 334.12952 $([M + H]^+$, calcd for $C_{17}H_{21}N_3SCl^+$ 334.11392); HPLC (214 nm) t_R 14.67 min (100%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-1-(4-chlorophenyl)cyclopentanecarbothioamide 76:** yield 0.53 g (38.4%); mp 140.0−141.0 °C; ^{1}H NMR δ 1.51−1.63 (m, 4H), 1.86−1.92 (m, 2H), 2.00−2.05 (m, 2H), 2.57−2.62 (m, 2H), 3.44−3.48 (m, 2H), 3.76−3.79 (m, 2H), 6.85 (s, 1H), 7.03 (s, 1H), 7.34−7.37 (m, 2H), 7.40−7.42 (m, 2H), 7.48 (s, 1H), 9.34 (s, 1H); MS *m*/*z* 348.2 $(M + H)^+$, 280.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 348.12930 $([M + H]^+$, calcd for $C_{18}H_{23}N_3SCl^+$ 348.12957); HPLC (214 nm) t_R 15.67 min (99.3%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-1-(4-methoxyphenyl)cyclohexanecarbothioamide 77:** yield 0.49 g (33.9%); mp 162.5−164.0 °C; ^{1}H NMR δ 1.26−1.29 (m, 1H), 1.39−1.49 (m, 5H), 1.86−1.96 (m, 4H), 2.46−2.49 (m, 2H), 3.46−3.53 (m, 2H), 3.68 (s, 3H), 3.74−3.77 (m, 2H), 6.82−6.85 (m, 3H), 7.02 (s, 1H), 7.30−7.33 (m, 2H), 7.49 (s, 1H), 9.17 (s, 1H); MS *m*/*z* 358.3 $(M + H)^+$, 290.3 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 358.19456 $([M + H]^+$, calcd for $C_{20}H_{28}N_3OS^+$ 358.19475); HPLC (214 nm) t_R 12.61 min (99.2%).

***N*-(3-(1*H*-Imidazol-1-yl)propyl)-1-(3,4-dimethoxyphenyl)cyclopropanecarbothioamide 81. Step A. 1-(3,4-Dimethoxyphenyl)-cyclopropanecarboxylic acid 79** was prepared according to the method described in ref 24. Thereby, 3,4-dimethoxyphenylacetonitrile **78** (2.06 g, 11.6 mmol, 1.0 equiv), 2-bromo-1-chloroethane (2.90 mL, 34.8 mmol, 3.0 equiv), and TEBA (0.264 g, 1.16 mmol, 0.1 equiv) were dissolved in a solution of KOH (60% in water, 10 mL). The solution was vigorously stirred for 2 d at room temperature. The solution was diluted by means of water (50 mL) and extracted with CH_2Cl_2 (3 × 80 mL). The organic phase was separated, dried over Na_2SO_4, and filtered, and the solvent was evaporated. The residue was subjected to flash chromatography utilizing EtOAc/heptane as elution system. The resulting nitrile was dissolved in ethylene glycol (70 mL) and powdered KOH (1.85 g, 33.0 mmol, 2.8 equiv) was added. The solution was kept overnight at reflux. After cooling to room temperature, water (60 mL) was added and the solution was acidified by concentrated aqueous HCl. The solution was extracted by means of Et_2O (200 mL). The solvent was evaporated and the remaining oil was dried at 60 °C over KOH at reduced pressure**:** yield 0.76 g (29.6%); ^{1}H NMR δ 1.11 (dd, J = 3.7 Hz, 2.9 Hz, 2H), 1.58 (dd, J = 3.7 Hz, 2.9 Hz, 2H), 6.88−6.90 (m, 3H); MS (anion mode) *m*/*z* 221.2 $(M-H)^-$.

Step B. *N*-(3-(1*H*-Imidazol-1-yl)propyl)-1-(3,4-dimethoxyphenyl)cyclopropanecarboxamide 80. **79** (0.760 g, 3.44 mmol, 1.02 equiv) was dissolved dry THF (10 mL). Isobutyl chloroformate (CAIBE) (0.46 mL, 3.5 mmol) and *N*-methylmorpholine (NMM) (0.38 mL, 3.5 mmol, 1.02 equiv) were added. After stirring for 5 min at −5 °C, **9** (0.43 mL, 3.5 mmol, 1.02 equiv) was added and the mixture stirred for 10 h at room temperature. The solvent was evaporated and the residue was dissolved in $CHCl_3$ (20 mL). The organic layer was washed by means of a saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4, and filtered, and the solvent was evaporated. The remaining oil was subjected to purification by chromatography utilizing a Chromatotron device (Harrison Research Ltd.), utilizing silica gel plates of a layer thickness of 2 mm and $CHCl_3$ as eluent**:** yield 0.61 g (60.2%); ^{1}H NMR δ 1.11 (dd, J = 3.7 Hz, 2.9 Hz, 2H), 1.57 (dd, J = 3.7 Hz, 2.9 Hz, 2H), 2.02−2.05 (m, 2H), 3.48−3.55 (m, 2H), 3.73 (s, 6H), 4.09−4.23 (m, 2H), 6.88−6.90 (m, 3H), 7.65 (s, 1H), 7.75 (s, 1H), 8.15−8.27 (m, 1H), 9.09 (s, 1H); MS *m*/*z* 330.1 $(M + H)^+$, 262.1 $([M - C_3H_3N_2]^\bullet)^+$.

80 (0.67 g, 2.0 mmol, 1.4 equiv) was subjected to the method described above, utilizing Lawesson's reagent (0.580 g, 1.43 mmol, 1.0 equiv), generating **81:** yield 0.40 g (59.4%); mp 127.0−127.5 °C; ^{1}H NMR δ 1.12 (dd, J = 3.7 Hz, 2.9 Hz, 2H), 1.57 (dd, J = 3.7 Hz, 2.9 Hz, 2H), 2.02−2.05 (m, 2H), 3.48−3.55 (m, 2H), 3.73 (s, 6H), 4.09−4.23 (m, 2H), 6.88−6.90 (m, 3H), 7.65 (s, 1H), 7.75 (s, 1H), 8.92−9.05 (m, 1H), 9.09 (s, 1H); MS *m*/*z* 346.0 $(M + H)^+$, 278.2 $([M - C_3H_3N_2]^\bullet)^+$, 177.1 $([M - C_6H_8N_3S]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 346.15810 $([M + H]^+$, calcd for $C_{18}H_{24}N_3O_2S^+$ 346.15837); HPLC (214 nm) t_R 22.9 min (98.5%).

5-(1*H*-Imidazol-1-yl)-*N*-(3,4-dimethoxyphenyl)pentanethioamide 85. Step A. 6-Bromo-*N*-(3,4-dimethoxyphenyl)pentanamide 83. A mixture of NEt_3 (2.08 mL, 14.9 mmol, 1.0 equiv) and 3,4-dimethoxyaniline (2.08 g, 14.9 mmol, 1.0 equiv) in 1,4-dioxane (20 mL) was added dropwise to an ice-cooled, stirred solution of 5-bromopentanoyl chloride **82** (2.79 g, 14.9 mmol, 1.0 equiv) in 1,4-dioxane (30 mL). The solution was allowed to warm to room temperature and stirred for 2 h. The solvent was evaporated, and the remaining oil was redissolved in CH_2Cl_2. The organic layer was washed two times with water, dried over Na_2SO_4, and filtered, and the solvent was removed under reduced pressure. The resulting product was used for the next step without further purification**:** yield 3.80 g (80.5%); MS *m*/*z* 251.1 $(M + H)^+$.

Step B. 5-(1*H*-Imidazol-1-yl)-*N*-(3,4-dimethoxyphenyl)pentanamide 84. Imidazole (0.820 g, 12.0 mmol, 1.0 equiv) and

sodium hydride (60% in mineral oil, 0.480 g, 12.0 mmol, 1.0 equiv) were suspended in DMF (30 mL) and the mixture was stirred under argon atmosphere at room temperature for 3 h. **83** (3.80 g, 12.0 mmol, 1.0 equiv) was added and the mixture was heated to 100 °C and stirred for 8 h. The solvent was evaporated, hot toluene (20 mL) was added, and the solution was filtered. This procedure was repeated three times, and the filtrates were combined. The solvent was removed under reduced pressure and the remaining oil was used without further purification**:** yield 1.26 g (34.2%). **84** (1.26 g, 4.10 mmol) was subjected to the method described above, utilizing Lawesson's reagent (1.17 g, 2.90 mmol), yielding **85**. The resulting product was subjected to purification by chromatography utilizing a Chromatotron device (Harrison Research Ltd.), silica gel plates of a layer thickness of 2 mm, and a $CHCl_3$/MeOH as eluting system**:** yield 0.200 g (15.3%); mp 128.0−128.5 °C; ^{1}H NMR δ 1.65−1.70 (m, 2H), 1.75−1.80 (m, 2H), 2.71−2.72 (m, 2H), 3.71 (s, 3H), 3.74 (s, 3H), 4.02−4.05 (m, 2H), 6.87−7.13 (m, 2H), 7.19 (s, 1H), 7.28−7.29 (m, 1H), 7.52 (s, 1H), 7.72 (s, 1H), 11.40 (s, 1H); MS *m*/*z* 320.2 $(M + H)^+$, 252.2 $([M - C_3H_3N_2]^\bullet)^+$; ESI-FTICR-MS *m*/*z* 320.13550 ($[M + H]^+$, calcd for $C_{16}H_{22}N_3O_2S^+$ 320.13545); HPLC (214 nm) t_R 24.35 min (99.2%). side product **85a**: yield 0.610 g (58.8%); ^{1}H NMR δ 1.73−1.75 (m, 2H), 1.89−1.94 (m, 2H), 2.93−2.95 (m, 2H), 3.62−3.67 (m, 2H), 3.71 (s, 3H), 3.73 (s, 3H), 6.73 (d, J = 6.4 Hz, 1H), 6.84 (s, 1H), 6.96 (d, J = 6.4 Hz, 1H); MS *m*/*z* 252.1 $(M + H)^+$.

Inhibitor Testing. QC activity was evaluated fluorometrically in a coupled assay.[27] Thereby, Gln-AMC was used as substrate and pyroglutamyl peptidase as the auxiliary enzyme. After conversion of Gln-AMC into pGlu-AMC by QC, the pGlu-AMC was hydrolyzed by pyroglutamyl peptidase. The generated AMC was detected with excitation/emission wavelengths of 380/460 nm. All determinations were carried out at 30 °C using a BMG Novostar reader for microplates. The inhibition constants were evaluated by fitting the data of the obtained progress curves according to the general equation for competitive inhibition using GraFit software (Erithacus Software Ltd.).

Gln-AMC, pyroglutamyl peptidase, and QC were dissolved in 0.05 M Tris-HCl (pH 8.0). Depending on the solubility, inhibitor stock solutions (0.1 M) were prepared either in water or in DMSO. The final concentration of DMSO in the sample did not exceed 0.1%, which has shown to have no influence on determination of QC activity. Dilution series were prepared in 0.05 M Tris−HCl at pH 8.0.

A sample consists of varying concentrations of the substrate (100 μL, 0.25−4 × K_m), 100 μL of different concentrations of the inhibitor, 25 μL of pyroglutamyl peptidase (0.1 U/mL), and 25 μL of QC (0.12 μg/mL).

After incubation of 10 min, the reaction was started by addition of QC. The activity was determined from a standard curve of AMC under assay conditions for 10 min.

Molecular Modeling. The flex-alignment function of the MOE software package (ver. 2003.02, Chemical Computing Group, Montreal, Canada) was used by applying the following settings: mmff94s.ff force field, with all other tools were set following the instructions suggested in the tutorials. The results were sorted and ranked first concerning their values for the average strain energy (*U*), followed by the configuration score (*S*).

Note Added in Proof: During the editorial processing of this paper Huang et al.[30] published the 3D structure of human QC, containing also simple imidazole-derived inhibitors.[13] The results presented there prove the presence of one zinc-ion in the active site, as suggested by Schilling et al.[13,26] Moreover, the co-crystallized inhibitors were found to act as zinc-chelators utilizing one coordination site. This supports our assumptions in the present manuscript regarding the binding mode of the inhibitory compounds.

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Supporting Information Available: MS, HPLC, and NMR data and a zipped file containing a table with the solutions of the flexible alignment with the respective average strain energy (*U*) and configuration score (*S*) values in sdf format. This material is available free of charge via the Internet at http://pubs.acs.org.

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