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Structure–activity relationship studies of 1-(4-chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl)thiourea, a non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus type-1

Michal Weitman [a], Keti Lerman [a], Abraham Nudelman [a,*], Dan Thomas Major [a], Amnon Hizi [b,*], Alon Herschhorn [b,1]

[a] Chemistry Department, Bar Ilan University, Ramat Gan 52900, Israel
[b] Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

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ABSTRACT

The reverse transcriptase (RT) of the human immunodeficiency virus type-1 (HIV-1) is still a prime target for drug development due to the continuing need to block drug-resistant RT mutants by new inhibitors. We have previously identified 1-(4-chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl)thiourea, compound **1**, as a potent RT inhibitor from an available chemical library. Here, we further modified this compound to study structure–activity relationships when replacing various groups in the molecule. Different functional groups were systematically introduced on the aromatic ring and the aliphatic chain of the compound was modified. The effect of these modifications on viral infectivity was then evaluated. The most potent compound found was propyl 4-(amino-*N*-(4-chloro-2,5-dimethoxyphenyl)methanethioamino)butanoate, **45c**, which inhibited infectivity with a calculated IC_{50} of about 1.1 μM. Docking studies identified potential important interactions between the top scoring ligands and HIV-1 RT, and the predicted relative affinity of the ligands was found to be in agreement with the experimental results.

1. Introduction

The reverse transcriptase of the human immunodeficiency virus type-1 (HIV-1) which causes acquired immunodeficiency syndrome (AIDS) in humans, is an essential enzyme for the life cycle of the virus [1,2]. RT synthesizes a double-stranded DNA by copying the viral single-stranded genomic (+) RNA in a complex process that requires three distinct RT activities. The first activity is the RNA-dependent DNA polymerase (RDDP) that synthesizes the (−) strand complementary DNA from the viral (+) strand RNA template. Concurrently, this RNA strand in the nascent RNA–DNA heteroduplex is hydrolyzed by the ribonuclease H (RNase H) activity of RT. This is followed by DNA-dependent DNA polymerase (DDDP) RT activity that synthesizes the second (+) DNA strand, using the already synthesized (−) DNA strand as a template. These three interconnected activities of RT collectively generate the double-stranded DNA that is subsequently integrated into the cellular genomic DNA by a second retroviral enzyme, the HIV-1 integrase [1]. Blocking each of the RT activities has proven to protect target cells from infection by HIV-1. Accordingly, HIV-1 RT has become a prime target for drug development, leading so far to more than ten RT inhibitors that are currently used for HIV-1 therapy as part of a highly active antiretroviral therapy for HIV-1/AIDS.

Non-nucleoside RT inhibitors (NNRTIs) are a variety of hydrophobic compounds that are potent noncompetitive inhibitors of the DNA polymerase activity of HIV-1 RT. These compounds are presumed to bind specifically the hydrophobic pocket that is located in the proximity of the DNA polymerase active site of the RT [3]. Most NNRTIs are highly specific against HIV-1 RT and, therefore, they are usually not toxic to human cells. However, this high specificity also creates a significant obstacle for their systematic use in HIV-1/AIDS patients, due to their reduced efficacy against mutated variants of RT that emerge quite rapidly in the infected HIV-1 population [4]. Consequently, intensive efforts have been invested in recent years to find novel broad-spectrum NNRTIs that inhibit both wild-type and variant HIV-1 RTs, which are resistant to the currently-used antiretroviral drugs. This search has recently led to the discovery of

* Corresponding authors.
E-mail address: nudelman@mail.biu.ac.il (A. Nudelman).
[1] Current address: Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute and Department of Pathology, Division of AIDS, Harvard Medical School, Boston, MA.

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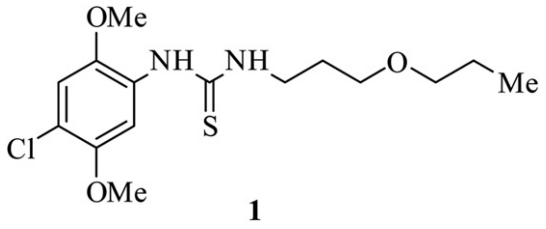


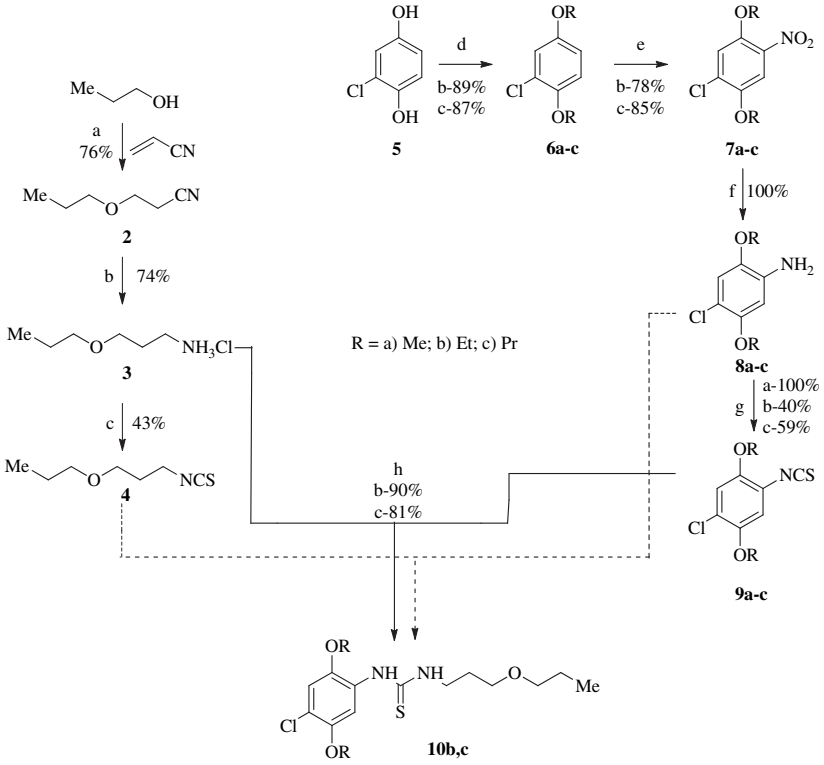
Fig. 1. Lead compound **1**.

several novel and highly-potent HIV-1 RT inhibitors, including 4-({6-amino-5-bromo-2->[(4-cyanophenyl)amino]-4-pyrimidinyl} oxy)-3,5-dimethylbenzonitrile (etravirine), *N*-[4-(aminosulfonyl)-2-methylphenyl]-2-[4-chloro-2-(3-chloro-5-cyanobenzoyl)phenoxy] acetamide (GW-678248), 5-bromo-*N*-[4-chloro-5-isopropyl-3-methyl-1,3-thiazol-2(3H)-ylidene]-2-hydroxybenzene-sulfonamide (YM-215389), 4-({4-[(2,4,6-trimethylphenyl)amino]pyrimidin-2-yl} amino)benzenecarbonitrile (dapivirine), and 4-{[4-({4-[(1E)-2-cyanoethenyl]-2,6-dimethylphenyl}amino)-2-pyrimidinyl]amino}benzonitrile (rilpivirine) [5–8]. Unfortunately, HIV-1 RT is a flexible protein with an outstanding ability to tolerate mutations (that develop quite rapidly in virions in AIDS patients treated with anti HIV-1 drugs), while still remaining functionally active. As a result, many of these RT mutants are resistant to the commonly used anti-RT drugs. Consequently, new inhibitors are constantly required to increase the available arsenal against HIV-1 and to fight AIDS.

In previous investigations, we have identified novel inhibitors of HIV-1 RT, by virtually screening a commercially-available library of compounds against two crystal structures of HIV-1 RT [9]. This approach resulted in the identification and characterization of two potent RT inhibitors. One of them, 1-(4-chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl)thiourea, inhibited in vitro the RDDP activity of recombinant HIV-1 RT with an IC_{50} value (apparent concentration that inhibits 50% of the initial RT activity) of ~510 nM. This compound also inhibited the infection of human lymphocytes by HIV-1 pseudovirus (that is equivalent to HIV-1 infectivity) with an IC_{50} value of ~168 nM. Our data suggested that this inhibitor probably interacts primarily with the NNRTI-binding pocket of HIV-1 RT, despite inhibiting an NNRTI-resistant RT mutant better than nevirapine [9]. Moreover, 1-(4-chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl)thiourea has also shown a partial inhibition of HIV-2 RT that is fully resistant to most NNRTIs, including nevirapine. In order to try improving this novel HIV-1 RT inhibitor, we have extended in the present investigation our previous study with 1-(4-chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl)thiourea and analyzed the effects of the different functional groups on the inhibitory capacity of the compound in a structure–activity relationship (SAR) study [10].

2. Results and discussion

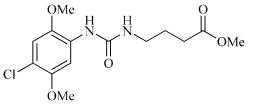
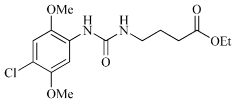
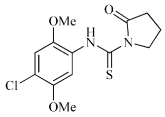
Our aim in this study was to synthesize and evaluate biologically a series of analogs of 1-(4-chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl)thiourea (compound **1**, Fig. 1) [10] by modifying systematically the molecule, in order to explore the SAR of these derivatives. Accordingly, the following modifications were introduced: a) Elongation of the alkoxy moiety b) Replacement of the chlorine atom by other electron-withdrawing groups c) Replacing the thiourea with isosteric groups d) Shortening the aliphatic chain between the thiourea and the ether and elongating the alkoxy group e) Replacing the ether with isosteric groups.



a) triton B; b) borane-DMS, THF, reflux. than $HCl_{(aq)}$; c) thiophosgene, EtOAc; d) R-I, K_2CO_3, acetone; e) HNO_3 -SiO_2, CH_2Cl_2, sonication; f) H_2, Pd/C, MeOH; g) thiophosgene, EtOAc; h) Et_3N/EtOH

Scheme 1. Synthesis of compounds **10b,c**.

Table 1
Compounds and by-products related to Scheme 10.

Entry	Substrate (RH)	%Yield (step I)	%Yield (step II)	%Yield (step III)	By-products
		43	**44**	**45**	**46**
a	MeOH	90	90	20	
b	EtOH	87	98	59	
c	*n*-PrOH	81	98	49	–
d	*n*-BuOH	89	100	56	–
e	*n*-PrNH$_2$	86	100	41	–
f	*n*-PrSH	94	89	10	

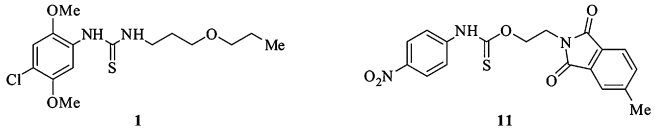


Fig. 2. Structural resemblance of compounds **1** and **11**.

2.1. Isosteric modifications and synthetic approaches

Examination of the lead compound **1** reveals two parts: an aromatic ring and an aliphatic chain, linked through a thiourea functionality.

2.1.1. Elongation of the alkoxy moiety

The first approach was to synthesize the corresponding aniline **8** and to couple it with the appropriate aliphatic isothiocyanate **4** (Scheme 1). The synthesis of aniline **8** involved etherification of the hydroquinones **5** to give compounds **6a**–**c**, which were selectively nitrated at the desired position under electrophilic conditions to give compounds **7a**–**c**. This reaction took place conveniently on silica gel as an acidic support under ultrasound conditions [11]. The work-up of this reaction required only filtration and solvent evaporation leading to the desired nitro derivatives in quantitative yield. Catalytic hydrogenation of the nitro group gave the corresponding anilines **8** (Scheme 1). The synthesis of the aliphatic chain fragment involved the preparation of the isothiocyanate **4** through a Michael addition of *n*-propanol to acrylonitrile to give the β-alkoxynitrile **2** [12], followed by reduction of the nitrile into the corresponding amine **3** and final conversion of the amine into an isothiocyanate **4** (Scheme 1).

Surprisingly, the attempted coupling of anilines **8** with isothiocyanates **4** did not succeed. Although several studies [13–15] of this type of reaction (using different compounds) have been reported with satisfactory results, in our laboratory (even under harsher conditions such as reflux, solvent-free microwave irradiation, irradiation using acetonitrile as a solvent or sonication), only the starting materials were identified (Scheme 1). A subsequent manipulation involved switching the components whereby the aniline was converted into isothiocyanate **9** and the aliphatic chain was added in the form of amines **3**. Under these conditions, involving stirring at room temperature for several hours, the desired target molecules of type **10** were obtained. Initially, we tested the effect of elongation the alkoxy side chain on the inhibitory capacity of the compounds. Several compounds were prepared with diverse longer groups but this modification resulted in a dramatic decrease in the inhibition of HIV-1 pseudovirus infection (Table 1). Therefore, in subsequent syntheses we only used methoxy derivatives.

Recently, Ranise et al. [16] described new types of novel NNRTI isosters of PETT derivatives [17,18]. The most potent compound **11** had a resemblance to our general structure **1**. In both cases, an electron withdrawing group (chloride, nitro) is found at the *para*-position to a thiourea or a thiocarbamate attached to an aliphatic chain followed by a polar ether or phthalimido group (Fig. 2). Based on these reports, we replaced the chlorine with a nitro group.

The commercially-available aniline **12** was converted into the corresponding isothiocyanate **13**, followed by coupling with the appropriate amine to give the desired thiourea **14** (Scheme 2). Compound **14** was found to be less active than **12** (Table 2).

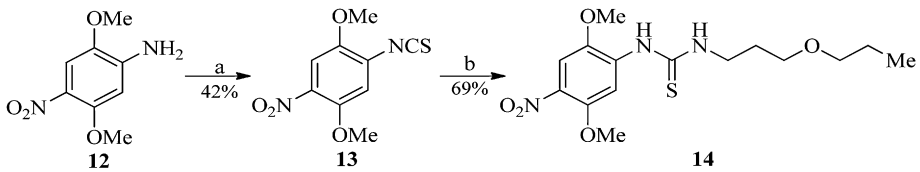
2.1.2. Isosteric thiourea replacements

The first isosteric replacement of the thiourea functionality was a urea. For this purpose, compound **8a** was treated with triphosgene to give **15** [10], which was coupled with a series of amines, leading to their corresponding ureas **16a**–**c** (Scheme 3), which displayed poor activity (Table 2).

2.1.3. Modifications in the aliphatic chain

2.1.3.1. Change of the length of the bridge between the thiourea and the ether. Next, we examined the influence of the length of the aliphatic chain on the binding affinity of the derivatives. As indicated, in the model compound **11**, the thiocarbamate function is linked via a bridge of two methylenes to the polar phthalimido group. In comparison, in our leading compound **1** the thiourea is linked via a bridge of three methylenes to the polar ether group. Thus, by analogy with **11**, compound **17** (Scheme 4) was found to be much less potent than compound **1**, indicating that the length of the bridge was important for the inhibitory activity (Table 2).

2.1.3.2. Modification of the alkoxy group. As shown in Scheme 4, thioureas **18a,b** that have ethoxy and methoxy groups instead of a propoxy group were synthesized. Compound **18b** inhibited about



a) thiophosgene, EtOAc; b) **4,** EtOH

Scheme 2. Synthesis of compound **14**.

Table 2
Antiviral activity of the synthesized compounds against HIV-1 pseudovirus infection.[a]

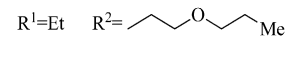
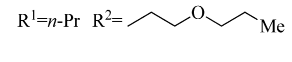
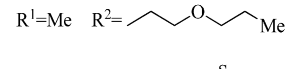
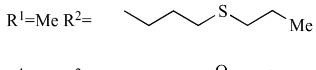
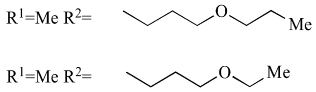
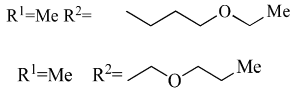
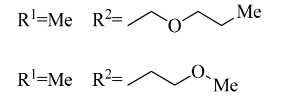
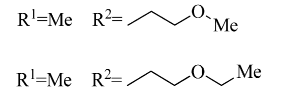
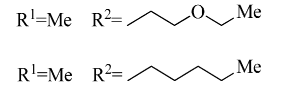
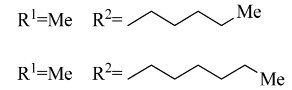
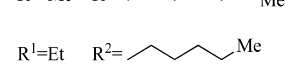
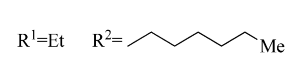
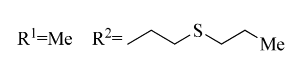
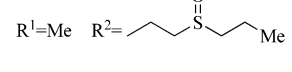
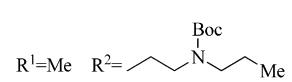
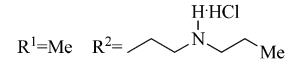
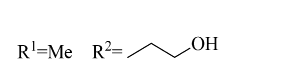
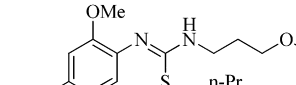
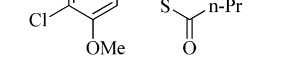
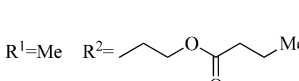
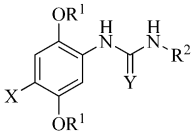
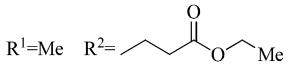
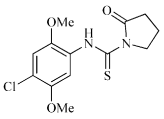
Compound	Structure	% Infectivity of HIV-1 pseudovirus [at 5 μM of inhibitor]
10b-I		58 ± 7
10c-I		107 ± 11
14		107 ± 3
16a		Toxic[b]
16b		47 ± 6
16c		89 ± 12
17		46 ± 4
18a		105 ± 4
18b		66 ± 8
19a-I		87 ± 2
19a-II		87 ± 15
19b-I		107 ± 2
19b-II		69 ± 10
22		24 ± 1
24		91 ± 6
29		Toxic[b]
30		83 ± 8
31		92 ± 9
32a		87 ± 6
36a		65 ± 14

Table 2 (*continued*)

Compound	Structure	% Infectivity of HIV-1 pseudovirus [at 5 μM of inhibitor]
36b		86 ± 3
36c		123 ± 14
40a		43 ± 9
40b		66 ± 8
42a		56 ± 2
42a′		66 ± 12
42b		91 ± 9
45a		73 ± 7
45b		24 ± 5
45c		17 ± 2
45d		29 ± 4
45e		69 ± 7
45f		79 ± 8
46a		87 ± 0

(*continued on next page*)

Table 2 (*continued*)

Compound	Structure	% Infectivity of HIV-1 pseudovirus [at 5 μM of inhibitor]
		
46b		65 ± 4
46f		104 ± 4

[a] Inhibition activity was measured by infecting B cells with HIV-1 based pseudovirus in the presence or absence of the specified inhibitor as previously described [9]. In the presence of several compounds, the residual infection was slightly higher than 100%, which can be attributed to either statistical variations or to a minor enhancement of the viral infection by the compound.

[b] HIV-1 pseudovirus infection, in the presence or absence of specific compounds, was assessed from the fluorescence of live cells, which were gated according to their side and forward scatter. Accordingly, any decrease in infectivity was related to the specific inhibition activity of each compound and not to any cytotoxic effect of these compounds. Compounds **16a** and **29** have lead to a massive cell death (based on number of cells that could be measured with the typical side and forward parameters values); therefore, they were defined as toxic.

66% of the viral infection whereas **18a** showed a lower inhibitory potency (Table 2).

2.1.4. Isosteric ether group modifications

2.1.4.1. Replacement of oxygen by a methylene group. The polarity of the ether part of the aliphatic chain was lowered by the replacement of the oxygen with a methylene group. In one case, using heptylamine the length of the chain was retained to give a seven-atom side chain, whereas in another case, the chain length was reduced to six atoms by using *n*-hexylamine (Scheme 5), to give compounds **19**, all of which were found to possess a lower inhibitory profile than compound **1** (Table 2).

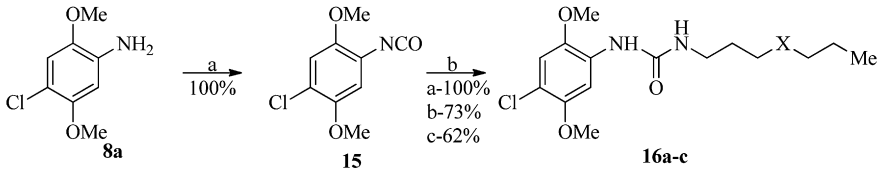
2.1.4.2. Replacement of oxygen by sulfur. Michael addition of *n*-propylthiol to acrylonitrile took place efficiently under ultrasonic conditions in the presence of KF/basic alumina [19] to give **20** [20] in quantitative yield. Subsequent reduction of the nitrile gave amine **21**, which upon coupling with isothiocyanate **9a** gave the thiourea **22**. Oxidation of the sulfide led to the corresponding water-soluble sulfoxide **23** which was used in the synthesis of **24** (Scheme 6). Compound **22** was found to be one of the most active derivatives, while the sulfoxide **24** was inactive (Table 2).

2.1.4.3. Replacement of O by an NH group. Addition of *n*-propylamine to acrylonitrile [21] gave amine **25** [22] along with traces of **26** [23] identified by NMR and HRMS. Protection of **25** with *tert*-Boc [24], followed by reduction of the nitrile functionality yielded the primary amine **28** [25], which without further purification, was reacted with isothiocyanate **9a** to give **29** [26]. Acidic removal of the *tert*-Boc protective group of **29** gave the target amine **30** (Scheme 7). Compound **30** was less active than **1** but still retained some inhibitory activity (Table 2).

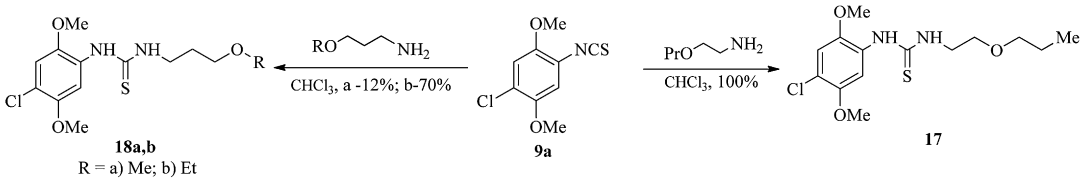
2.1.4.4. Replacement of the ether by an ester group. The initial synthetic route explored in this study involved the reaction of 3-amino-1-propanol with isothiocyanate **9a** to construct the thiourea moiety [26] of compound **31**, followed by esterification with a suitably activated carboxylic acid [27]. Esterification with butyryl chloride provided **32a**, the product of double esterification. Alternatively, the amino group of 3-amino-1-propanol was protected with a *tert*-Boc group [24] and the protected derivative **33** [28] was treated with butyryl chloride [27] to provide **34a** or with propionic acid activated in situ by Bop/DMAP to give **34b** [29]. To remove the *tert*-Boc group, compound **34a** was added to a 1:1 solution of AcCl/MeOH (in situ generation of an anhydrous HCl solution), to give a mixture of **35a** [30] and the by-product **35c** formed by transesterification in the presence of AcCl/MeOH. To avoid the formation of **35c**, a freshly prepared solution of 4 N HCl in EtOAc was added to **34b**, and the hydrochloride salt of **35b** was isolated [29]. The last step of the sequence involved the reaction of the mixture of **35a** and **35c** with isothiocyanate **9a**, to provide a mixture of **36a** and **36c** or **35b** to yield **36b**. All three compounds **36a**–**c** were found to be less potent than **1** in inhibiting HIV-1 infection (Table 2) (Scheme 8).

Additional thiourea derivatives were synthesized as shown in Scheme 9. Oxidation of **33** with $KMnO_4$ [31] gave the carboxylic acid **37** [32], which underwent esterification with *n*-butanol or *n*-propanol, catalyzed by Bop and DMAP [33] to provide **38a** and **38b**, respectively [34]. Removal of the protecting group upon acidification with 4 N HCl [29] gave **39a** and **39b** which were coupled with isothiocyanate **9a**, to give **40a** and **40b** [26]. Alternatively, compound **37** was deprotected using two approaches: a) Stirring of **37** in the presence of AcCl/MeOH provided **41a** and **41a′** [35] as unexpected by-products, whereas, b) stirring in 4 N HCl gave the desired **41b** [36]. It is assumed that the methyl ester **41a** was the product of esterification with the MeOH, used to generate the HCl upon reaction with the AcCl, while the ethyl ester **41a′** was the by-product of transesterification with EtOAc. Compounds **41a**, **41a′** and **41b** when reacted with **9a** led to the thioureas [26] **42a**, **42a′** and **42b**. The mixture of **42a**/**42a′** was separated by chromatography (Scheme 9). Thioureas **40a** and **40b** displayed some inhibitory activity while **42a**, **42a′** and **42b** were found to be much less potent inhibitors (Table 2).

Compound **45c** (Table 2) was found to be the most potent inhibitor and efficiently suppressed the replication of HIV-1 pseudovirus. This molecule was prepared by an analogous sequence of reactions to those described in Scheme 10. In addition, using Boc-GABA as starting



Scheme 3. Synthesis of compounds **16a–c**.

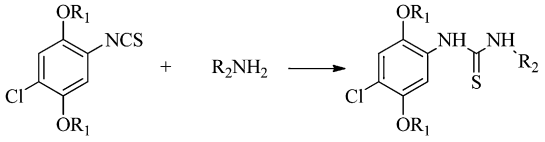


18a,b
R = a) Me; b) Et

9a

17

Scheme 4. Synthesis of compound **17**.



9a,b

19aI (26%), **19aII** (100%), **19bI** (51%), **19bII** (55%)
R_1 = a) Me; b) Et
R_2 = I) hexyl; II) heptyl

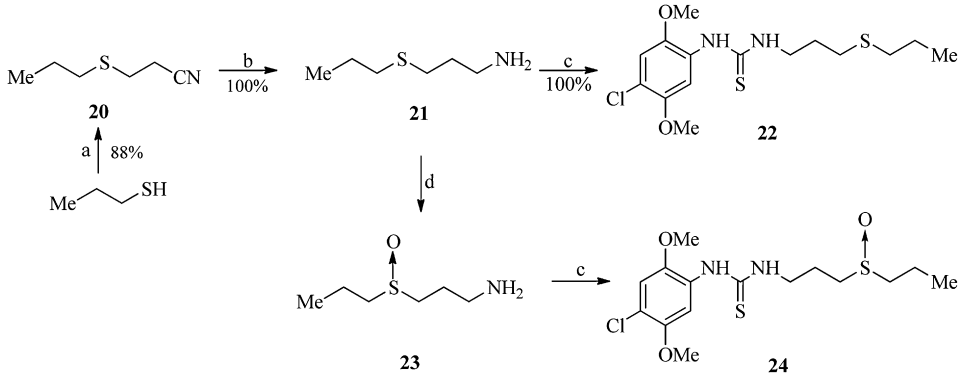
Scheme 5. Synthesis of compounds **19aI–II** and **19bI–II**.

material the analogs **45a,b,d,e,f** of **45c** were prepared (Scheme 10, Table 1).

The by-products **46a,b,f** were isolated and identified by NMR and HRMS. The suggested mechanism of the formation of **46f** formation is shown in Scheme 11.

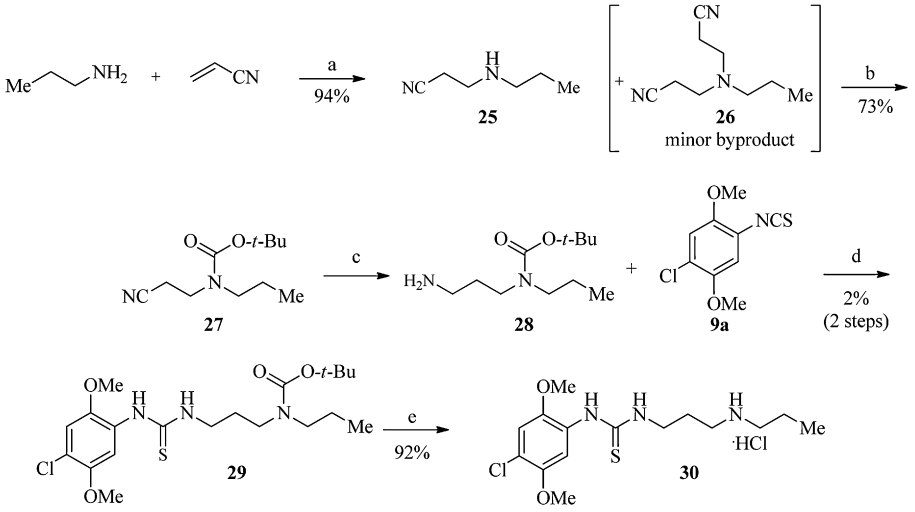
2.2. Biological evaluations

The prepared compounds and their inhibitory activities of HIV-1 infectivity are summarized in Table 2.



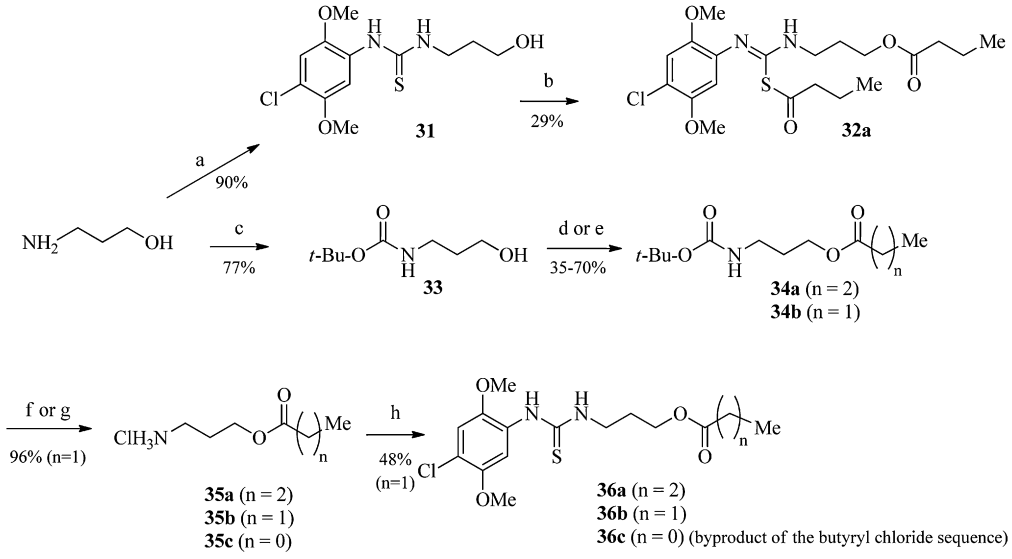
a) KF/basic alumina. acrylonitrile, CH_2Cl_2, sonication; b) borane-DMS, THF, reflux; c) **9a**, $CHCl_3$ (yield of **24** was not determined since the compound underwent decomposition upon attempted purification); d) $NaIO_4$, H_2O (yield of **23** was not calculated since product contained salts and was used without further purioficiton)

Scheme 6. Synthesis of compound **24**.



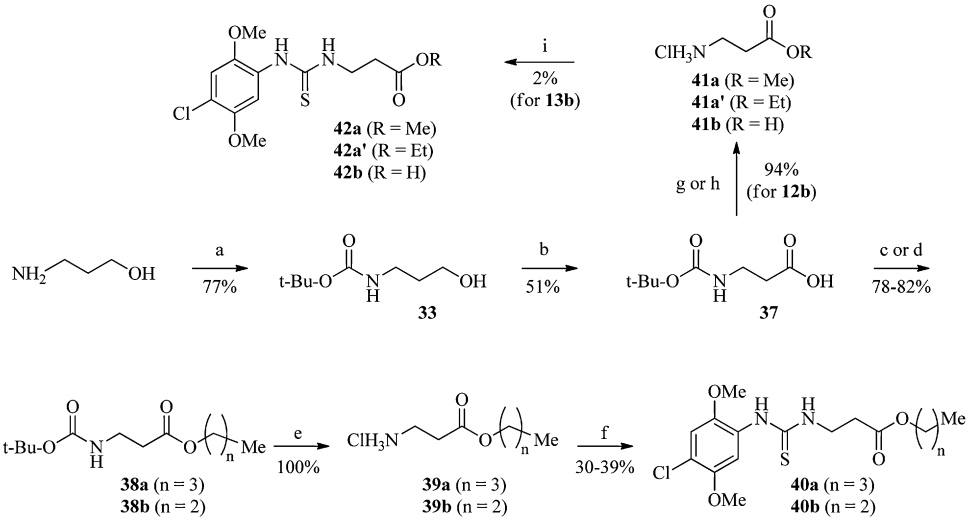
a) MeOH, 3 h, rt; b) $(Boc)_2O$, $NaHCO_3$, MeOH, 6 h, sonication; c) $BH_3{\cdot}SMe_2$ (2M), THF (dry), 4 h, reflux; d) Et_3N, $CHCl_3$, overnight, rt; e) 37% HCl (1 mL), ether, 15 min.

Scheme 7. Synthesis of compound **30**.



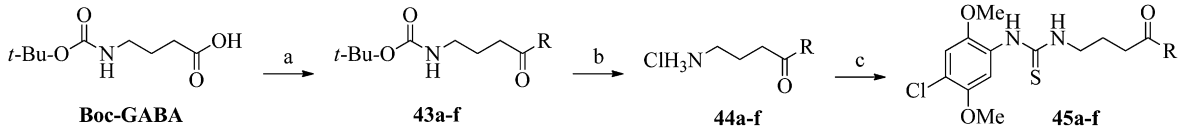
a) **9a**, $CHCl_3$, 2 h, rt; b) Butyryl chloride, DMAP, Et_3N, CH_2Cl_2; c) $(Boc)_2O$, $NaHCO_3$, MeOH, 6 h, sonication; d) Butyryl chloride, DMAP, Et_3N, CH_2Cl_2; e) Propionic acid, DMAP, Et_3N, Bop, CH_2Cl_2, overnight, rt; **f)** AcCl (20 eq), MeOH (20 eq); g) EtOAc, b. 4N HCl, EtOAc; h) **9a,** $CHCl_3$, overnight, rt.

Scheme 8. Synthesis of compounds **35a–c** and **36a–c**.



a) $(Boc)_2O$, $NaHCO_3$, MeOH, 6 h, sonication; b) $KMnO_4$, NaOH, H_2O, rt; c) n-Butanol, DMAP, Et_3N, Bop, CH_2Cl_2, overnight, rt; d) n-Propanol, DMAP, Et_3N, Bop, CH_2Cl_2, overnight, rt; e) 4N HCl, EtOAc; f) **9a,** $CHCl_3$, overnight, rt; g) AcCl (20 eq), MeOH (20 eq), EtOAc: h) 4N HCl, EtOAc; i) $CHCl_3$, overnight, rt.

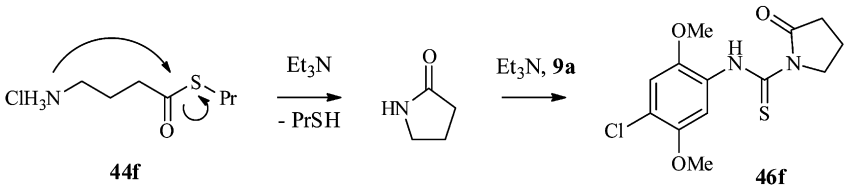
Scheme 9. Synthesis of compounds **40a,b**.



a) BOP, DMAP, Et_3N, CH_2Cl_2, rt, RH (see Table 1); b) 4N HCl, EtOAc ; c) **9a,** Et_3N, $CHCl_3$, rt.

R= a) MeO; b) EtO; d) *n*-BuO; e) *n*-PrNH; f) *n*-PrS

Scheme 10. Synthesis of compounds **45a–f**.



Scheme 11. Synthesis of compound **46f**.

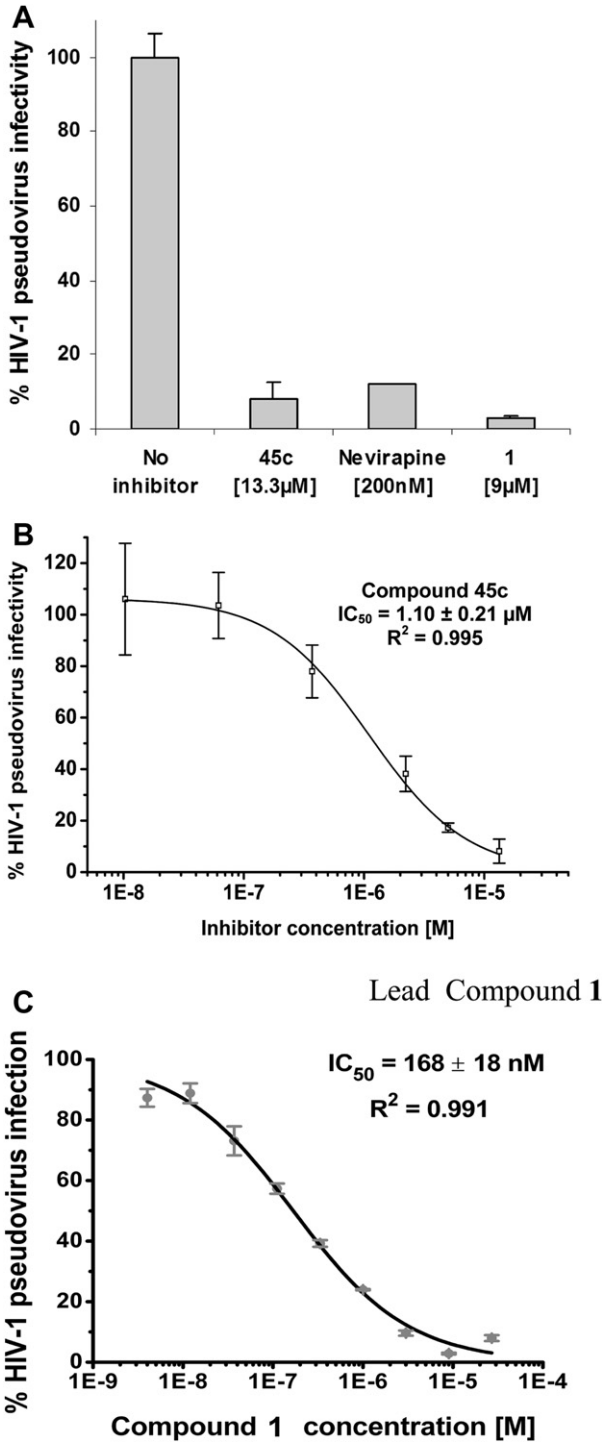


Fig. 3. Antiviral activity against HIV-1 pseudovirus infection. (A), Inhibition activity of compound **45c**. The concentrations used are indicated for each compound. (B), Dose–response curve of compound **45c**. (C) Dose–response curve of compound **1** (adapted, with permission, from our previous published study [9]).

Out of 36 compounds tested, sixteen inhibited less than 20% of viral infectivity and these modifications seem to render the molecules inactive toward HIV-1 RT. Other compounds were more potent with different degrees of inhibition (Table 2). Most notable was compound **45c** that inhibited about 83% of infectivity at a 5 μM concentration. A shorter version of the aliphatic chain, in which the propylene was replaced by an ethylene group in compound **45b**, was still active, albeit showing a little less inhibitory capacity with about 76% inhibition of infectivity at the same concentration. The most potent inhibitor (**45c**) was further evaluated and tested at different concentrations to assess its IC_{50} value. Using flow cytometry based assay we calculated its IC_{50} to be about 1.1 μM (Fig. 3B).

In the experimental system employed herein to test HIV-1 infectivity (in the experiments described in both Table 2 and Fig. 3), a successful single cycle of infection results in the expression of the green fluorescent protein (GFP) in the target cells that can be measured by flow cytometry. Infection was tested in the absence or the presence of compound **45c** and the average fluorescence that was measured for each concentration of the compound was compared to the fluorescence obtained in the absence of the inhibitor. Consequently, a dose–responses curve for suppressing viral infectivity were calculated from the flow cytometry results and the corresponding IC_{50} values were calculated using the four-parameter equation, as previously described [9]. The results are the average of at least three independent measurements (Fig. 3A). They are shown together with the positive control of the original lead compound **1** in Fig. 3C.

3. Computational chemistry – docking

To better understand the nature of the interactions of the thiourea derivatives with the RT binding pocket, we examined the

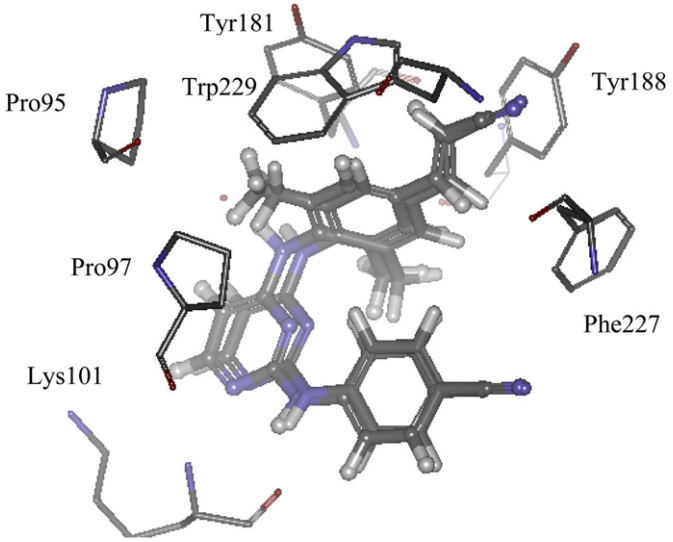


Fig. 4. Structural superimposition of the docked rilpivirine (stick) and rilpivirine from the X-ray structure (ball and stick).

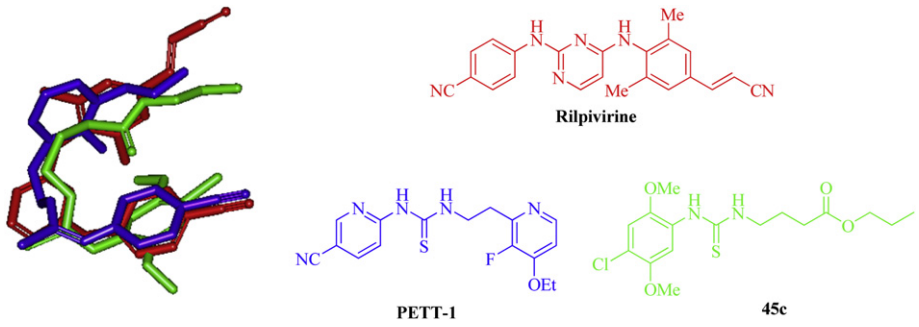


Fig. 5. Structural superimposition of PETT (blue), rilpivirine (red) and **45c** (green) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

corresponding contacts of three known NNRTIs: Efavirenz [37], phenylethylthiazolylthiourea PETT [38], and rilpivirine [39] with HIV-1 RT thus revealing the spatial recognition in the binding site, along with conserved interactions. The procedure was carried out by superimposing three crystal structures of the wild-type HIV-1 RT enzyme containing these inhibitors. The superimposition revealed that the orientations of most of the RT residues wrapping the inhibitors were similar. The most significant change was in the pocket that contained the PETT derivative that is found as a pyridinium salt at physiological pH. Because of the positive charge on the inhibitor, the ionized Glu138 is oriented facing the pyridinium nitrogen. In the two other complexes, the ligands are neutral inhibitors and the Glu138 is oriented towards Lys101. Despite the differences in their chemical structures, the three inhibitors occupy almost identical positions inside the hydrophobic pocket of RT. This structure similarity may also apply to our inhibitors and is important for elimination of potential erroneous poses, produced during the docking process. Our compounds may be considered as isosteric PETT derivatives (phenyl ring instead of pyridine, chlorine instead of nitrile, and ester/thioether instead of pyridine). However the PETTs are charged positively at physiological pH, whereas our inhibitors are neutral. Based on this rationale, we compared in this analysis compounds **22**, **45c** and **45d** to rilpivirine. Initially, we docked rilpivrine to HIV-1 RT to validate the CDocker approach for the current project. Indeed, CDocker predicted the correct pose for rilpivirine (Fig. 4) with an RMSD relative to the crystal structure of 0.494 Å. The predicted docking energy was −53.7 kcal/mol. This result suggests that CDocker is suitable for the current system. Rilpivirine, which is a neutral inhibitor analogous to our inhibitors, contains an aromatic ring, substituted with a nitrile group at the *para*-position to a 2-amino-pyrimidine moiety. Our inhibitors contain an aromatic ring, substituted with chlorine at the *para*-position to a thiourea moiety, which is isosteric to a 2-amino-pyrimidine (Fig. 5). Rilpivirine is a potent inhibitor, and its X-ray structure, when complexed with HIV-1 RT, has been solved at a good resolution (1.8 Å). It is important to emphasize that the inhibitor can induce and influence the hydrophobic pocket of HIV-1 RT, which is not seen in structures of HIV-1 RT without an inhibitor. Therefore, our analysis is given under the limitations of the available crystal structures.

Based on these guidelines, we docked structures of **22**, **45c**, and **45d**, computed the 50 best poses for each candidate, and examined in detail the top 25 for each compound. Among them, the poses that best fit the rilpivirine binding pose were chosen. For each inhibitor, typical interactions were searched and the CDocker [40] energies were compared. The best inhibitor **45c** (Table 2), appears to be wrapped in a rilpivirine-like pose (Fig. 6), and the thiourea moiety is found in the same orientation as the 2-amino-pyrimidine group of the rilpivirine (Fig. 7). The most important observed interactions involve an H-bond between the NH of the thiourea and the carbonyl of Lys101. The thiocarbonyl is held by the amines of Lys101 and Lys103, and by a water molecule. The other NH of the thiourea has an intramolecular H-bond with the carbonyl of the ester. In addition, the aromatic ring has hydrophobic interactions with Leu100 and Val106, and the aliphatic chain is stabilized by Tyr181, Tyr183, Tyr188, Phe227, and Trp229 of HIV-1 RT. The calculated CDocker energy is −50.55 kcal/mol. The second best inhibitor is **22**, which also fits the rilpivirine pose. Here, the phenyl ring superimposes one of the rilpivirine rings, the electron withdrawing chlorine group is in the same orientation as the acrylonitrile moiety, and the thiourea is again in the same direction as the 2-amino-pyrimidine of rilpivirine. Interesting interactions include a π-interaction between the substrate phenyl and Tyr181 and a T-stacking interaction with Trp229, while this ring also interacts with Tyr188. One of the NH groups has an H-bond to a water molecule and the thiocarbonyl interacts with another water molecule. The aliphatic chain forms hydrophobic interactions with Lys103, Pro225, Phe227, Leu234, Pro236, and Tyr318 of HIV-1 RT (Fig. 8). The apparent calculated CDocker energy is −49.94 kcal/mol. The last compound examined was **45d**, where a resemblance to bound rilpivirine was observed, with almost the same orientation as that found for compound **22**. Nonetheless, the thiourea is not oriented similarly to the 2-amino-pyrimidine and, therefore, no H-bond with the thiourea was observed. The aromatic ring has a π-interaction with Tyr181, a T-stacking interaction with the RT's Trp229 and hydrophobic interactions with Tyr188. One of the methoxy groups interacts with Pro95, and the other one with Val106 and Phe227, whereas the aliphatic chain interacts with Lys103, Val179, Leu234, Pro236, and Tyr318 of the RT (Fig. 9). The calculated CDocker energy is −37.01 kcal/mol.

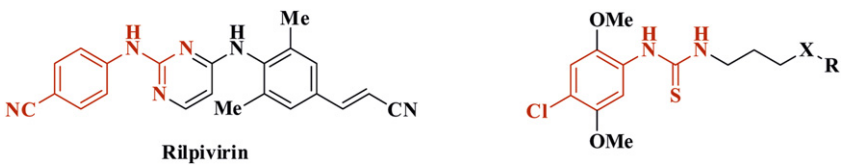


Fig. 6. Structural resemblance between rilpivirine and the thiourea derivatives.

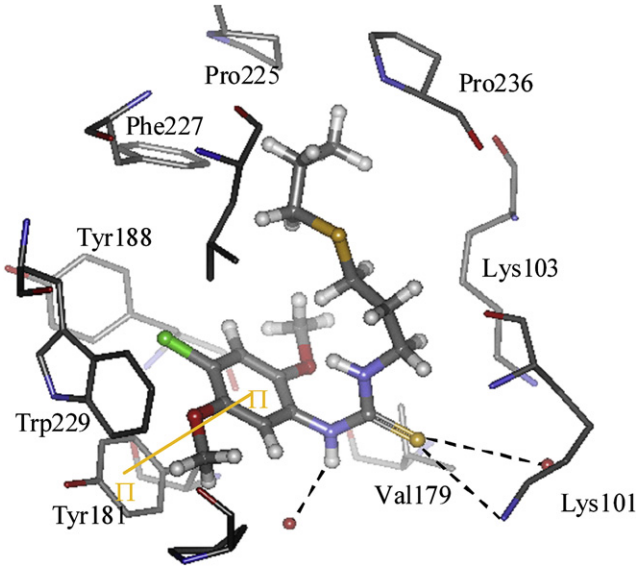


Fig. 7. Compound **45c** in the NNRTI-binding pocket.

Finally, we docked **45f**, which is an isoster of **45c**, but a much less potent inhibitor than **45c**, in order to examine the differences between the two. The aromatic ring of **45f** has almost the same orientation, but the conformation of the aliphatic chain is different in **45f**, most likely due to its sulfur atom, which has a greater vdW radius than oxygen. This suttle change leads to the loss of the important H bond interaction between the thiouea moiety to the backbone amide of Lys101 (Fig. 10). Indeed, its calculated CDocker energy is −33.3 kcal/mol, which is the least favorable score of the docked compounds.

It has been suggested that two main interactions are required for inhibition of HIV-1 RT [39]. These are the H-bond between the carbonyl moiety of Lys101 and an H-donor of the substrate (where typically an NH serves as the H-bond donor) and the π-stacking interaction between Trp229 and an aromatic ring of the substrate. In order for both of these interactions to be utilized, the substrate's pharmacophoric H-bond donor and the aromatic ring must be separated by a distance of ∼5 Å. In the case of the compounds tested here, these functional groups are connected through a single bond and, therefore, both of these interactions cannot be accomplished simultaneously. Compound **45c** satisfies the H-bond with Lys101, while compounds **22** and **45d** do not form H-bonds with this residue. On the other hand, compounds **22** and **45d** display π-stacking interactions with Trp229 (see Figs. 8 and 9, respectively), while **45c** does not (Fig. 7). One might have expected that **45c** and **45d** should bind in a similar mode due to their similarity; however, this is not suggested by the docking studies. A rationale for this observation might be attributed to the slightly longer alkyl chain in **45d** that prevents it from fitting into the aromatic pocket in the allosteric site. Compound **22** contains a sulfur atom that is conceivably too large to fit into the pocket that encapsulates **45c** and therefore it binds the RT in a mode more similar to that of **45d**.

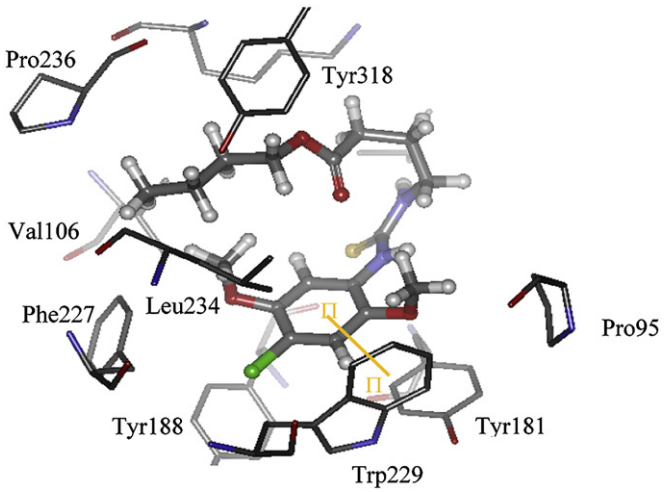


Fig. 9. Compound **45d** in the NNRTI-binding pocket.

In conclusion, rilpivirine may be a good template for neutral inhibitors. In this study, binding of our three leading compounds to poses similar to that of rilpivirine is supported by the relative CDocker energy scores for the three compounds that match their biological evaluations.

4. Conclusions

A variety of new analogs of compound **1**, modified at six positions of the basic molecule were synthesized, purified and tested. Based on our SAR study, it was found that the aromatic residue

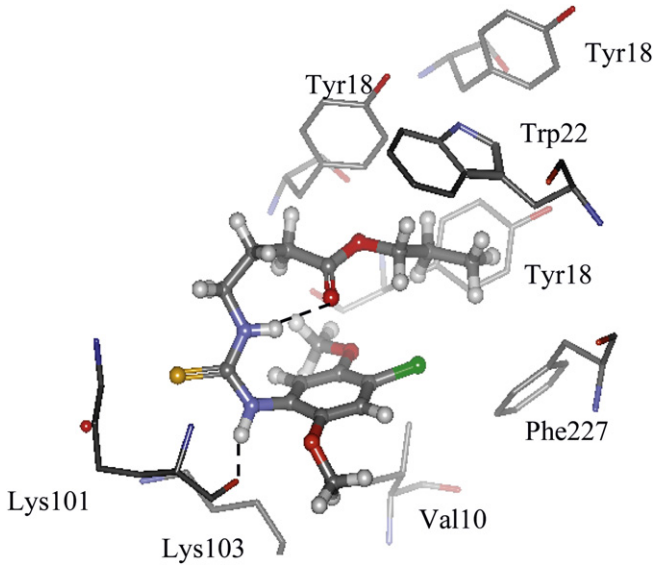


Fig. 8. Compound **22** in the NNRTI-binding pocket.

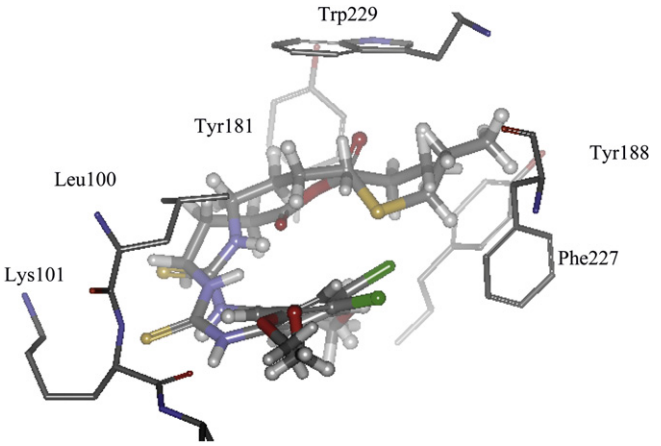


Fig. 10. Compounds **45c** and **45f** in the NNRTI-binding pocket.

could not be changed and the methoxy groups were essential and could not be lengthened. Replacement of the chloride by a nitro group led to a less potent inhibitor (but still an active one). The thiourea group could be isosterically replaced with urea, and the ether with sulfide and ester functionalities, but not with a methylene, amine, thioester or an amide. The derivatives of compound **17** that possess a shortened aliphatic chain between the thiourea and the ether were inactive. Several compounds showed an inhibitory capacity comparable to that of the original compound **1** and highlighted the importance of the functional groups necessary for preservation of this activity.

5. Experimental protocols

5.1. Chemistry

5.1.1. General remarks

^{1}H and ^{13}C NMR spectra 200, 300 and 600 MHz were obtained on Bruker AC-200 and AM-300 spectrometers, respectively. Chemical shifts are expressed in ppm downfield from Me_4Si used as internal standard. The values are given in δ scale. ^{1}H and/or ^{13}C NMR spectra are shown for compounds that have been reported in the literature but for which no ^{1}H and/or ^{13}C NMR spectra were found. In the NMR spectra of thioureas, when taken in acetone, we often observe the presence of distinct peaks corresponding to two rotamers. The rotation barrier for thioureas is known to be much higher than for ureas, and a more polar solvent will stabilize the ground state, increasing the rotation barrier [41]. Mass spectra were obtained on a Varian Mat 731 spectrometer. HRMS were obtained on a AutoSpec Premier (Waters UK) spectrometer in CI, CH_4. LRMS were obtained on a QToF micro (Waters UK) spectrometer in ESI.

5.1.2. Procedure 1 – reduction of nitriles [42]

To a solution of borane-DMS complex (12 mL 2 M in THF, 24.1 mmol) in dry THF (25 ml) was slowly added a nitrile (1.36 g, 12.1 mmol) dissolved in dry THF (15 ml) at 0 °C. The solution was stirred for 30 min at 0 °C, after which it was heated to reflux for 4 h. The mixture was cooled to room temperature, and MeOH (8 ml) was added dropwise (Caution: hydrogen-gas evolution) and was subsequently evaporated. Trimethyl borate was removed by co-evaporations with MeOH (3 × 10 ml). Concentrated HCl (0.4 mL) was added slowly, and the mixture was stirred for 15 min. Diethyl ether and water were then added to the viscous liquid obtained. The aqueous layers were combined and evaporated on a rotary evaporator.

5.1.3. Procedure 2 – synthesis of aromatic ethers [43]

A mixture of a hydroquinone **6** (1.0 mmol), an alkyl iodide (2.2 mmol) and anhydrous K_2CO_3 (1.0 mmol) in dry acetone was refluxed for 48 h, evaporated and the residue was extracted with EtOAc–water. The organic phase was dried ($MgSO_4$), filtered and evaporated.

5.1.4. Procedure 3 – synthesis of nitro phenyls

The nitro derivatives were prepared as described [11].

5.1.5. Procedure 4 – synthesis of anilines

a) A solution of an aromatic nitro compound (1.0 mmol) in MeOH was stirred vigorously under an atmosphere of hydrogen in the presence of 10% w/w Pd/C (0.1% w) at room temperature for 16 h. The mixture was filtered through celite to remove the catalyst and the filtrate was evaporated under reduced pressure, the product was purified by flash chromatography [44]; b) An aromatic nitro (10.0 mmol) compound was dissolved in EtOH (50 mL), $FeSO_4$ heptahydrate (30 mmol), water (9 mL) and NH_4Cl (80 mmol) were added subsequently with efficient stirring. Zn powder (30 mmol) was then added and the mixture was heated to an internal temperature of 50 °C, stirred for an additional 1–3 h, cooled to room temperature and suction-filtered through a pad of celite. The filter cake was washed with EtOH (70 mL), and the filtrate was concentrated. The residue was extracted with EtOAc (70 mL) and 25% aqueous NH_4Cl (30 mL) solution. The biphasic mixture was stirred at room temperature for 5 min and the organic layer was separated, and washed with water (30 mL), saturated $NaHCO_3$ (30 mL), brine (30 mL), and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by flash chromatography [45].

5.1.6. Procedure 5 – synthesis of isothiocyanates [46]

A solution of an aniline (1.0 mmol) in EtOAc (mL) was treated with thiophosgene (2.0 mmol) and stirred at room temperature overnight. The solvent was evaporated to remove excess of thiophosgene and the residue was extracted EtOAc–water. The organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was purified as indicated.

5.1.7. Procedure 6 – synthesis of thioureas and ureas [47]

To a solution of an isothiocyanate or an isocyanate in the indicated solvent, an appropriate amine was added and stirred at room temperature for the indicated time (If the amine hydrochloride was used, an equimolar amount of triethylamine was added). The solvent was evaporated, and the product was purified in flash chromatography.

5.1.8. Procedure 7 – protection with N-tert-Boc group [24]

A mixture of 1.0 mmol of the amine salt, 1 mmol $(Boc)_2O$ and 2.0 mmol of $NaHCO_3$ in 25 mL of dry MeOH, under N_2, was sonicated for 6 h. The solid was filtered and the solvent evaporated. The residue is then dissolved in distilled water and acidified with saturated solution of $KHSO_4$ to pH 2, extracted with two portions of EtOAc, the organic phase was washed with brine, dried over Na_2SO_4 and evaporated to give the pure protected amine.

5.1.9. Procedure 8 – construction of ester functionality [33]

The *N-t*-Boc protected amino acid/alcohol (5.7 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL), under N_2, followed by addition of a suitable alcohol/acid (5.7 mmol), DMAP (5.7 mmol), Bop (5.7 mmol) and Et_3N (17 mmol). The mixture was stirred at room temperature overnight. The solvent was then evaporated and the residue was dissolved in EtOAc/H_2O. The organic phase was washed with concentrated $KHSO_4$ and with saturated $NaHCO_3$ solution. The organic phase was separated, washed with brine, dried over Na_2SO_4 and evaporated.

5.1.10. 3-Propoxypropanenitrile, ***2***

Michael addition – To a stirred mixture of *n*-PrOH (1.0 mmol) and aqueous 40% trimethylbenzylammonium hydroxide ("Triton B," 1 drop), acrylonitrile (1.0 mmol) was added dropwise and the mixture was stirred 1 h after. Acetic acid was added till pH ~6, and water–EtOAc were added. The organic phase was dried (Na_2SO_4), filtered and evaporated [48]. Compound **3** was obtained as a colorless oil in 76% yield from. ^{1}H NMR (300 MHz, acetone-d_6) δ 0.91 (t, J = 7.5 Hz, 3H), 1.56 (sext, J = 7.5 Hz, 2H), 2.68 (t, J = 6 Hz, 2H), 3.43 (t, J = 7.6 Hz, 2H), 3.63 (t, J = 6.3 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.8, 19.0, 23.4, 66.1, 73.1, 119.0. MS (CI^+) m/z 114.10 (MH^+, 39.4). HRMS: calcd. for $C_6H_{12}NO$ 114.0919 found 114.0955.

5.1.11. 3-Propoxypropan-1-amomium hydrochloride, ***3***

Compound **2** was obtained as a white hygroscopic solid in 74% yield from **3** (Procedure 1). ^{1}H NMR (200 MHz, D_2O) δ 0.89 (t,

J = 7.5 Hz, 3H), 1.58 (sext, J = 7.2 Hz, 2H), 1.94 (quint, J = 6 Hz, 2H), 3.10 (br t, J = 7.5 Hz, 2H), 3.48, 3.59 (two t, J = 6.3 Hz, 2H). ^{13}C NMR (75 MHz, D_2O) δ 10.3, 22.6, 27.2, 38.2, 68.2, 73.3. MS (CI^+) m/z (%): 118.13 (MH^+, 100). HRMS: calcd. for $C_6H_{16}NO$ 118.1232 found 118.1279.

5.1.12. 1-Isothiocyanato-3-propoxypropane, ***4***

Compound **4** was obtained as an orange oil in 48% yield from **3** (Procedure 5). NMR data assignment was aided by several two-dimensional spectra including COSY, HMQC and HMBC analyses. ^{1}H NMR (600 MHz, acetone-d_6) δ 0.79 (t, J = 7.2 Hz, 3H), 1.44 (sext, J = 7.2 Hz, 2H), 1.83 (quint, J = 6 Hz, 2H), 3.26 (t, J = 6.6 Hz, 2H), 3.40 (t, J = 6 Hz, 2H), 3.59 (t, J = 6.6 Hz, 2H). ^{13}C NMR (150 MHz, acetone-d_6) δ 10.8, 23.4, 30.7, 42.7, 67.2, 73.0, 130.0.

5.1.13. 2-Chloro-1,4-diethoxybenzene, ***6b*** *[49]*

Compound **6b** was obtained as a brown oil in 89% yield from **5b** (Procedure 2, solvent acetone). ^{1}H NMR (300 MHz, acetone-d_6) δ 1.33, 1.38 (two t, J = 6.9 Hz, 3H), 3.84, 4.00 (two sext, J = 6.9 Hz, 2H), 6.76 (dd, J = 6.9 Hz, 3 Hz, 1H), 6.91 (d, J = 9 Hz, 1H), 6.95 (d, J = 9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 15.11, 64.5, 65.8, 114.2, 115.6, 117.1, 123.7, 149.3, 153.9. MS (CI^+) m/z 201.07 (MH^+, 52.1), 200.06 (M^+, 85.8), 173.04 ($MH^+ - C_2H_4$, 43.4), 172.03 ($M^+ - C_2H_4$, 54.2), 144.00 ($M^+ - 2C_2H_4$, 100). HRMS: calcd. for $C_{10}H_{13}O_2Cl$ 200.0604 found 200.0610.

5.1.14. 2-Chloro-1,4-dipropoxybenzene, ***6c***

Compound **6c** was obtained as a brown oil in 87% yield from **5c** (Procedure 2, solvent acetone). ^{1}H NMR (300 MHz, acetone-d_6) δ 1.00, 1.04 (two t, J = 7.5 Hz, 3H), 1.77 (m, 4H), 3.88, 3.93 (two t, J = 6.6 Hz, 2H), 6.82 (m, 1H), 6.97 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.73, 10.8, 23.22, 23.3, 70.7, 71.8, 114.5, 115, 117.2, 123.8, 149.6, 154.28. MS (CI^+) m/z 228.09 (M^+, 42.9), 186.1 (M^+-Pr^+, 25.5), 144.0 ($M^+ - 2Pr^+$, 100), HRMS: calcd. for $C_{12}H_{17}O_2Cl$ 228.0917 found 228.0942.

5.1.15. 1-Chloro-2,5-diethoxy-4-nitrobenzene, ***7b*** *[44]*

Compound **7b** was obtained as a yellow-brown solid in 78% yield from **6b** (Procedure 3, solvent CH_2Cl_2), mp 96–98 °C. NMR data assignment was aided by several two-dimensional spectra including COSY, HMQC and HMBC analyses. ^{1}H NMR (600 MHz, acetone-d_6) δ 1.38, 1.42 (two t, J = 7.5 Hz, 3H), 4.18, 4.22 (two t, J = 7.2 Hz, 2H), 7.40 (s, 1H), 7.56 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.8, 66.4, 67.0, 110.7, 118.2, 128.7, 139.6, 146.8, 148.8. MS (CI^+) m/z 246.1 (MH^+, 50.9), 245.1 (M^+, 68.49), 215.1 (M^+ – Et, 25.2), 189.0 (M^+ – 2Et, 100). HRMS: calcd. for $C_{10}H_{12}NO_4Cl$ 245.455 found 245.0478.

5.1.16. 1-Chloro-4-nitro-2,5-dipropoxybenzene, ***7c*** *[50]*

Compound **7c** was obtained as a yellow solid in 85% yield from **6c** (Procedure 3, solvent CH_2Cl_2, chromatography eluent CH_2Cl_2), mp 79–83 °C ^{1}H NMR (300 MHz, acetone-d_6) δ 1.03, 1.06 (two t, J = 7.5 Hz, 3H), 1.83 (m, 4H), 4.09, 4.12 (two t, J = 6.6 Hz, 2H), 7.4 (s, 1H), 7.57 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.6, 10.65, 23.1, 72.3, 72.7, 110.8, 118.0, 128.9, 134.2, 147.1 (4^{ary}), 148.9 (4^{ary}). MS (CI^+) m/z 247.08 (MH^+, 82.1). HRMS: calcd. for $C_{12}H_{17}NO_4^{35}Cl$ 274.0846 found 274.0819, calcd. for $C_{12}H_{17}NO_4^{37}Cl$ 276.0817 found 276.0786.

5.1.17. 4-Chloro-2,5-diethoxyaniline, ***8b*** *[51]*

Compound **8b** was obtained as a white solid from **7b** (Procedure 4, solvent MeOH, chromatography eluent 2:1 hexane:CH_2Cl_2), mp 69–72 °C ^{1}H NMR (300 MHz, acetone-d_6) δ 1.38, 1.42 (two t, J = 7.2 Hz, 3H), 3.96, 3.99 (two sext, J = 6.9 Hz, 2H), 4.51 (br t, 2H), 6.52 (s, 1H), 6.82 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.2, 65.4, 65.7, 102.3, 113.4, 114.8, 138.7, 141.3, 149.9.

5.1.18. 4-Chloro-2,5-dipropoxyaniline, ***8c*** *[52]*

Compound **8c** was obtained as a brown oil in quantitative yield from **7c** (Procedure 4, solvent MeOH). ^{1}H NMR (300 MHz, acetone-d_6) δ 1.022, 1.025 (two t, J = 8 Hz, 3H), 1.763, 1.768 (two sext, J = 7.2 Hz, 2H), 3.87, 3.90 (two t, J = 6.3 Hz, 2H), 5.8 (br t, 2H), 6.69 (s, 1H), 6.82 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.8, 23.1, 23.3, 71.7, 71.9, 103.4, 113.5, 114.7, 136.5, 141.9, 149.8.

5.1.19. 1-Chloro-4-isothiocyanato-2,5-dimethoxybenzene, ***9a***

Compound **9a** was obtained as a bright yellow solid in quantitative yield from **8a** (Procedure 5, reflux in $CHCl_3$ 4.5 h, recrystallized from EtOAc:hexane), mp 88-87 °C, ^{1}H NMR (300 MHz, acetone-d_6) δ 3.86, 3.93 (two s, 3H) 7.03 (s, 1H), 7.19 (s, 1H). ^{13}C NMR (50 MHz, acetone-d_6) δ 56.5, 56.6, 109.2, 113.7, 119.3, 121.2, 141.1, 148.7, 150.1. MS (CI^+) m/z 232.00 (MH^+, 35.585), 230.00 (MH^+, 100). HRMS: calcd. for $C_9H_9NO_2S^{35}Cl$ 230.0043 found 230.0032, calcd. for $C_9H_9NO_2S^{37}Cl$ 232.0013 found 232.0007.

5.1.20. 1-Chloro-2,5-diethoxy-4-isothiocyanatobenzene, ***9b***

Compound **9b** was obtained as a white solid in 40% yield from **8b** (Procedure 5, solvent EtOAc, chromatography eluent 2.5:1 hexane:CH_2Cl_2), mp 69–72 °C ^{1}H NMR (300 MHz, acetone-d_6) δ 1.38, 1.42 (two t, J = 6.9 Hz, 3H), 4.04 4.10 (two sext, J = 6.9 Hz, 2H), 6.85, 7.07 (s, 1H, s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.87, 14.9, 66.0, 66.2, 111.1, 115.4, 120.1, 122.4, 142.5, 149.0, 150.56. MS (CI^+) m/z 257.03 (M^+, 100), HRMS: calcd. for $C_{11}H_{12}NO_2S^{35}Cl$ 257.0277 found 257.0266, calcd. for $C_{11}H_{12}NO_2S^{37}Cl$ 259.0248 found 259.0243.

5.1.21. 1-Chloro-4-isothiocyanato-2,5-dipropoxybenzene, ***9c***

Compound **9c** was obtained as a white-pink solid in 59% yield from **7c** (Procedure 5, solvent EtOAc, chromatography eluent hexane:CH_2Cl_2 2:1), mp 36–39 °C, ^{1}H NMR (300 MHz, acetone-d_6) δ 1.03, 1.08 (two t, J = 7. 5 Hz, 3H), 1.81 (m, 4H), 3.97, 4.05 (two t, J = 6.3 Hz, 2H), 6.39 (s, 1H), 7.14 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.9, 10.0, 22.3, 71.16, 71.18, 110.5, 114, 121.8 (4^{ary}), 148.4 (4^{ary}), 150.1 (4^{ary}). At the concentration of the sample, the 4^{ary} carbons were not observed. MS (CI^+) m/z 286.07 (MH^+, 66.2), 285.06 (M^+, 77.8), 244.02 ($MH^+ - C_3H_6$, 100), 200.96 ($M^+ - 2C_3H_6$, 49.6), HRMS: calcd. for $C_{13}H_{16}NO_2SCl$ 285.059 found 285.0599.

5.1.22. 1-(4-Chloro-2,5-diethoxyphenyl)-3-(3-propoxypropyl) thiourea, ***10b***

Compound **10b-I** was obtained as a colorless oil in 81% yield from **8b** (Procedure 6, solvent EtOH, chromatography eluent 2.5:1 hexane:CH_2Cl_2). ^{1}H NMR (300 MHz, acetone-d_6) δ 0.84 (t, J = 7 Hz, 3H), 1.35, 1.39 (two t, J = 7.2 Hz, 3H), 1.46 (sext, J = 7 Hz, 2H), 1.86 (quint, J = 6.3 Hz, 2H), 3.30 (t, J = 6.6 Hz, 2H), 3.48 (t, J = 6 Hz, 2H), 3.67 (sext, J = 6.6 Hz, 2H), 4.07, 4.08 (two sext, J = 7.0 Hz, 2H), 7.04 (s, 1H), 7.50 (br t, 1H), 7.85 (br t, 1H), 8.25 (br t, 1H). ^{13}C NMR (50 MHz, acetone-d_6) δ 10.8, 15.0, 23.4, 28.6, 29.6, 43.5, 65.7, 69.5, 73.0, 111.9, 115.4, 118.6, 128.5, 145.9, 148.8, 181.7. MS (CI^+) m/z 357.15 (MH^+, 100), 329.11 (MH^+ – E1tOH, 29.8). HRMS: calcd. for $C_{17}H_{28}N_2O_3S^{35}Cl$ 357.1509 found 357.1528, calcd. for $C_{17}H_{28}N_2O_3S^{37}Cl$ 377.1480 found 377.1497.

5.1.23. 1-(4-Chloro-2,5-dipropoxyphenyl)-3-(3-propoxypropyl) thiourea, ***10c-I***

Compound **10c-I** was obtained as a colorless oil in 81% yield from **8c** (Procedure 6, solvent EtOH, chromatography eluent 1:1 CH_2Cl_2:hexane till all the isothiocyanate was eluted, then pure CH_2Cl_2). ^{1}H NMR (300 MHz, acetone-d_6) δ 0.67 (t, J = 7.35 Hz, 3H), 0.97, 1.00 (two, J = 7.5 Hz, 3H), 1.20 (sext, J = 6.3 Hz, 2H), 1.76 (m, 6H), 3.15 (t, J = 6.6 Hz, 2H), 3.45 (t, J = 5.4 Hz, 2H), 3.67 (br sext, J = 5.4 Hz, 2H), 3.84, 3.86 (two t, J = 6.6 Hz, 2H), 6.90 (s, 1H), 7.11 (br q, 1H), 7.55 (br t, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.3, 10.5, 22.3, 22.5, 28.4, 45.4, 70.3, 71.2, 71.3, 73.0, 110.8, 115, 120.2, 125.0, 145.6,

148.6, 180.0. MS (CI^+) *m/z* 403.18 (MH^+, 42.5). HRMS: calcd. for $C_{19}H_{32}N_2O_3SCl$ 403.1822 found 403.1801.

5.1.24. 1-Isothiocyanato-2,5-dimethoxy-4-nitrobenzene, ***13***

A solution of corresponding aniline (0.2 g, 1 mmol) in $CHCl_3$ was treated with thiophosgene (0.11 g, 2 mmol) and was refluxed overnight. The solvent was evaporated to remove excess of thiophosgene and the residue was extracted EtOAc–water. The organic phase was dried (Na_2SO_4), filtered and evaporated. The residue was purified by flash chromatography 3:1hexane:$CHCl_3$. Compound **13** was obtained as a yellow solid in 42% yield. ^1H NMR (200 MHz, acetone-d_6) δ 3.95, 4.01 (two s 3H), 7.23, 7.61 (two s, 1H). ^{13}C NMR (50 MHz, acetone-d_6) δ 57.5, 57.7, 109.5, 112.2, 126.2, 147.9, 150.3 (three 4^{ary} Cs). The 4^{ary} carbons could not be detected due to the instability of the compound when left in the NMR solution overnight. MS (CI^+) *m/z* 241.02 (MH^+, 12.3), 240.02 (M^+, 25.6), 210.04 ($MH^+ - MeO$, 65.1), 195.02 ($MH^+ - NO_2$, 27.6). HRMS: calcd. for $C_9H_8N_2O_4S$ 240.0205 found 240.0191.

5.1.25. 1-(2,5-Dimethoxy-4-nitrophenyl)-3-(3-propoxypropyl) thiourea, ***14***

Compound **14** was obtained as a yellow solid in 69% yield from **13** (Procedure 6, solvent EtOH, chromatography eluent CH_2Cl_2), mp 116–119 °C ^1H NMR (300 MHz, acetone-d_6 + CD_3OD) δ 0.85 (t, $J = 7.5$ Hz, 3H), 1.51 (sext, $J = 7.5$ Hz, 2H), 1.84 (quint, $J = 6.6$ Hz, 2H), 3.33 (t, $J = 6.6$ Hz, 2H), 3.47 (t, $J = 6.3$ Hz, 2H), 3.61 (t, $J = 6.9$ Hz, 2H), 3.85, 3.88 (two s, 3H), 7.50 (s, 1H), 8.93 (s, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ 10.8, 23.7, 28.6, 42.7, 57.0, 57.4, 69.3, 73.5, 107.2, 108.5, 133.4, 136.5, 143.4, 149.5, 181.34. MS (CI^+) *m/z* 358.15 (MH^+, 10), HRMS: calcd. for $C_{15}H_{24}N_3O_5S$ 358.1437 found 35.1457.

5.1.26. 1-Chloro-4-isocyanato-2,5-dimethoxybenzene, ***15***

A solution of **9a** (0.5 g, 2.66 mmol) in $CHCl_3$ (40 mL) was treated with triphosgene (0.88 mmol) was reflux overnight (till the solution became clear). The solvent was evaporated. **16** was obtained as a white solid in quantitative yield, mp 78–79 °C ^1H NMR (200 MHz, acetone-d_6) δ 3.80, 3.81 (two s, 3H) 6.63, 6.97 (two s, 1H). ^{13}C NMR (50 MHz, acetone-d_6) δ 56.8, 57.0, 108.9, 113.6, 119.3, 123.1, 131.3, 148.2, 149.9. MS (CI^+) *m/z* 213.02 (M^+, 100), 198.00 ($M^+ - Me$, 48.6), 172.03 ($M^+ - NCO$, 26.7), HRMS: calcd. for $C_9H_3NO_3Cl$ 213.0193 found 213.0211.

5.1.27. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-(propylthio)propyl) urea, ***16a***

Compound **16a** was obtained as a colorless oil in quantitative yield from **15** (Procedure 6, solvent $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, $J = 7.5$ Hz, 3H), 1.58 (sext, $J = 7.2$ Hz, 2H, 1), 1.80 (quint, $J = 6.9$ Hz, 2H), 2.44 (br t, 2H, bt), 3.36 (t, $J = 6.6$ Hz, 2H), 3.75, 3.84 (two s, 3H), 6.83 (s, 1H), 7.20 (br t, 1H), 7.97 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 22.9, 29.4, 29.7, 34.2, 39.4, 56.4, 56.6, 104.4, 112.4, 114.0, 128.2, 141.8, 149.2, 155.6. MS (CI^+) *m/z* 347.12 (MH^+, 27.9). HRMS: calcd. for $C_{15}H_{23}N_2O_3S^{35}Cl$ 347.1196 found 347.1227, calcd. for $C_{15}H_{23}N_2O_3S^{37}Cl$ 348.1088 found 348.1137.

5.1.28. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl) urea, ***16b***

Compound **16b** was obtained as a white solid oil in 73% yield from **15** (Procedure 6, solvent $CHCl_3$, chromatography eluent CH_2Cl_2), mp 90–92 °C ^1H NMR (200 MHz, acetone-d_6) δ 0.87 (t, $J = 7$ Hz, 3H), 1.50 (sext, $J = 6.4$ Hz, 2H, 1), 1.72 (quint, $J = 7.4$ Hz, 2H), 3.29 (sext, $J = 5.6$ Hz, 2H), 3.32 (t, $J = 5.6$ Hz, 2H), 3.45 (t, $J = 6.1$ Hz, 2H), 3.79 (s, 6H), 3.43 (t, $J = 6.6$ Hz, 2H), 3.66 (br sext, $J = 6.6$ Hz, 2H), 3.83 (two s, 3H), 6.52 (br t, 1H), 6.92 (s, 1H), 7.74 (br t, 1H), 8.24 (s, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ 10.9, 23.6, 31.1, 37.7, 56.7, 56.9, 68.7, 73.0, 109.6, 113.1, 130.6, 142.5, 149.9, 156.1. MS (CI^+) *m/z* 331.14 (MH^+, 94.1), 330.14 (M^+, 83.0). HRMS: calcd. for $C_{15}H_{23}N_2O_4Cl$ 330.1346 found 330.1361.

5.1.29. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-heptylurea, ***16c***

Compound **16c** was obtained as a white solid in 62% yield from **15** (Procedure 6, solvent $CHCl_3$, chromatography eluent CH_2Cl_2), mp 87–89 °C ^1H NMR (200 MHz, acetone-d_6) δ 0.88 (m, 3H), 1.31 (m, 8H), 1.52 (m, 2H), 3.23 (sext, $J = 6.7$ Hz, 2H), 3.81, 3.82 (two s, 3H), 6.46 (br t, $J = 6.7$ Hz, 1H), 6.94 (s, 1H), 7.70 (br t, 1H), 8.27 (s, 1H). ^{13}C NMR (50 MHz, acetone-d_6) δ 14.3, 23.3, 27.6, 29.9, 28.0, 32.6, 40.4, 56.7, 56.9, 104.5, 113.0, 113.1, 130.7, 142.5, 150.0, 155.9. MS (CI^+) *m/z* 329.16 (MH^+, 49.3), 328.16 (M^+, 39.7). HRMS: calcd. for $C_{16}H_{25}N_2O_3Cl$ 328.1554 found 328.1567.

5.1.30. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(2-propoxyethyl) thiourea, ***17***

Compound **17** was obtained as a white solid in quantitative yield from **10a** (Procedure 6, solvent $CHCl_3$, recrystallized from hexane:CH_2Cl_2), mp 95–97 °C ^1H NMR (200 MHz, acetone-d_6) δ 0.80 (t, $J = 7.35$ Hz, 3H), 1.53 (sext, $J = 7$ Hz, 2H), 3.39 (t, $J = 5.8$ Hz, 2H), 3.59 (t, $J = 5.2$ Hz, 2H), 3.77 (td, $J = 5$ Hz, 1.6 Hz, 2H), 3.83 (two s, 3H), 7.06 (s, 1H), 7.55 (br t, 1H), 8.1, 8.13 (s, 1H, s, 1H), 8.52 (br t, 1H). ^{13}C NMR (50 MHz, acetone-d_6) δ 10.8, 23.5, 45.1, 45.2, 57.0, 69.4, 73.1, 110.3, 110.5, 114.1, 117.7, 128.3, 146.4, 149.4, 182.0, 182.1. MS (CI^+) *m/z* 333.10 (MH^+, 98.33), 301.07 ($MH^+ - MeOH$, 98.40). HRMS: calcd. for $C_{14}H_{22}N_2O_3S^{35}Cl$ 333.1040 found 333.1032, calcd. for $C_{14}H_{22}N_2O_3S^{37}Cl$ 335.1010 found 335.1018.

5.1.31. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-methoxypropyl) thiourea, ***18a***

Compound **18a** was obtained as a colorless oil in 12% yield from **10a** (Procedure 6, solvent $CHCl_3$), ^1H NMR (300 MHz, acetone-d_6) δ 1.85 (quint, $J = 6.6$ Hz, 2H), 3.21 (s, 3H), 3.43 (t, $J = 6.6$ Hz, 2H), 3.66 (br sext, $J = 6.6$ Hz, 2H), 3.83, 3.84 (two s, 3H), 7.06 (s, 1H), 7.51 (br t, $J = 6.6$ Hz, 1H), 7.89 (br t, 1H), 8.34 (br t, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ 29.5, 43.4, 56.7, 58.5, 71.5, 110.9, 114, 118.2, 127.8, 146.8, 149.4, 181.7. MS (CI^+) *m/z* 319.08 (MH^+, 13.6). HRMS: calcd. for $C_{13}H_{20}N_2O_3S$ 319.0883 found 319.0837.

5.1.32. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-ethoxypropyl) thiourea, ***18b***

Compound **18b** was obtained as a colorless oil in 70% yield from **10a** (Procedure 6, solvent $CHCl_3$). NMR data assignment was aided by several two-dimensional spectra including COSY, HMQC and HMBC analysis. ^1H NMR (600 MHz, acetone-d_6) δ 1.04 (t, $J = 7.0$ Hz, 3H), 1.86 (quint, $J = 6.0$ Hz, 2H), 3.39 (sext, $J = 7.0$ Hz, 2H), 3.48 (t, $J = 6.0$ Hz, 2H), 3.67 (t, 2H), 3.84, 3.85 (two s, 3H), 7.09 (s, 1H), 7.65 (br t, 1H), 7.91 (br t, 1H), 8.47 (br t, 1H). ^{13}C NMR (150 MHz, acetone-d_6) δ 15.3, 29.8, 43.2, 56.6, 66, 69.2, 110.1, 113.9, 117.7, 127.3, 146.3, 149.1, 181.1. MS (CI^+) *m/z* 333.11 (MH^+, 87.8), 301.08 ($MH^+ - MeOH$, 100). HRMS: calcd. for $C_{14}H_{22}N_2O_3SCl$ 333.1040 found 333.1081.

5.1.33. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-hexylthiourea, ***19a-I***

Compound **19a-I** was obtained as a white solid in 26% yield from **10a** (Procedure 6, solvent $CHCl_3$, chromatography eluent hexane:CH_2Cl_2), mp 84–86 °C ^{13}C NMR (50 MHz, acetone-d_6) δ 14.2, 23.2, 27.3, 29.5, 32.3, 45.2, 56.9, 110.4, 114.1, 117.6, 128.3, 146.3, 149.4, 181.7.

5.1.34. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-heptylthiourea, ***19a-II***

Compound **19a-II** was obtained as a white solid in quantitative yield from **10a** (Procedure 6, solvent $CHCl_3$, chromatography eluent CH_2Cl_2), mp 70–72 °C, ^1H NMR (200 MHz, acetone-d_6) ppm δ 0.84 (m, 3H), 0.84 (m, 8H), 1.61 (br t, $J = 6.8$ Hz, 2H), 3.57 (sext, $J = 6.8$ Hz, 2H), 3.81 (two s, 6H), 7.02 (s, 1H), 7.74 (br t, $J = 6.8$ Hz, 1H), 8.02 (s, 1H), 8.47 (br t, 1H). ^{13}C NMR (50 MHz, acetone-d_6) δ 13.1, 21.9, 26.3, 28.3,

28.5, 31.2, 43.9, 55.6, 109.0, 112.7, 117.0, 127.1, 144.9, 148.0, 180.3. MS (CI^+) *m/z* 345.19 (MH^+, 100). HRMS: calcd. for $C_{16}H_{26}N_2O_3S^{35}Cl$ 345.1404 found 345.1387, calcd. for $C_{16}H_{26}N_2O_3S^{37}Cl$ 347.1359 found 347.1369.

5.1.35. 1-(4-Chloro-2,5-diethoxyphenyl)-3-hexylthiourea, ***19b-I***

Compound **19b-I** was obtained as a white solid in 51% yield from **9b** (Procedure 6, solvent $CHCl_3$, chromatography eluent 1:1 hexane:CH_2Cl_2), mp 82–84 °C, ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 0.88 (m, 3H), 1.31 (m, 6H), 1.41, 1.44 (two t, J = 7 Hz), 1.60 (br sext, 2H), 3.51 (br t, 2H), 4.02, 4.05 (two sext, 2H, J = 7 Hz), 6.24 (br t, 1H), 6.97 (s, 1H), 7.66 (br t, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ 14.0, 14.8, 22.5, 26.7, 28.1, 31.4, 45.4, 65.4, 65.7, 110.7, 115.5, 120.3, 125.2, 145.3, 148.5, 180.1. MS (CI^+) *m/z* 159.15 (MH^+, 2.1). HRMS: calcd. for $C_{17}H_{28}N_2O_2ClS$ 359.1560 found 359.1530.

5.1.36. 11-(4-Chloro-2,5-diethoxyphenyl)-3-heptylthiourea, ***19b-II***

Compound **19b-II** was obtained as a white solid in 55% yield from **9b** (Procedure 6, solvent EtOH, chromatography eluent 1.5:1 CH_2Cl_2:hexane), mp 76–78 °C ^{1}H NMR (300 MHz, acetone-d_6) δ 0.89 (m, 3H), 1.30 (m, 8H), 1.40, 1.44 (two t, J = 7 Hz), 1.60 (br sext, 2H), 3.57 (br t, 2H), 4.02, 4.05 (two sext, 2H, J = 7 Hz), 6.28 (br t, 1H), 6.96 (s, 1H), 7.71(br t, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ 14.0, 14.75, 14.78, 22.5, 26.9, 28.9, 29.6, 31.7, 45.3, 65.4, 65.6, 110.7, 115.5. At the concentration of the sample, the 4^{ary} carbon was not observed 125.3, 145.3, 148.5, 180.1. MS (CI^+) *m/z* 373.17 (MH^+, 100). HRMS: calcd. for $C_{18}H_{30}N_2O_2SCl$ 373.1717 found 373.1695.

5.1.37. 3-(Propylthio)propanenitrile, ***20*** *[20]*

Compound **20** was prepared from propanethiol and acrylonitrile as described [19], and was isolated in 88% yield upon filtration and evaporation of the solvent and was used without further purification. ^{1}H NMR (300 MHz, acetone-d_6) δ 1.02 (t, J = 7.3 Hz, 3H), 1.63 (sext, J = 7.3 Hz, 2H), 2.05 (t, J = 7.3 Hz, 2H), 2.54–2.8 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.1, 18.7, 22.6, 27.3, 33.9, 118.4. $C_6H_{11}NS$ 129.2. MS (CI^+) *m/z* 130.07 (MH^+, 100). HRMS: calcd. for $C_6H_{12}NS$ 130.069 found 130.0649.

5.1.38. 3-(Propylthio)propan-1-amine, ***21*** *[53]*

Compound **21** was obtained as a colorless oil in quantitative yield from **20** (Procedure 1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 7.5 Hz, 3H), 1.47 (sext, J = 7.2 Hz, 2H), 1.6 (quint, J = 7.2 Hz, 2H), 2.36, 2.45 (t, J = 7.5 Hz, 2H, and t, J = 7.2 Hz, 2H), 2.68 (br t, J = 6.6 Hz, 2H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.3, 22.8, 29.6, 33.0, 34.1, 41.0.

5.1.39. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-(propylthio)propyl) thiourea, ***22***

Compound **22** was obtained as a colorless oil in quantitative yield from **21** (Procedure 6, solvent $CHCl_3$). ^{1}H NMR (200 MHz, acetone-d_6) δ 1.06 (t, J = 7.4 Hz, 3H), 1.66 (sext, J = 7.2 Hz, 2H), 2.01 (quint, J = 7.2 Hz, 2H), 2.59 (t, J = 7.4 Hz, 2H), 2.67 (t, J = 7.0 Hz, 2H), 3.80 (sext, J = 5.6 Hz, 2H), 3.93, 3.94 (two s, 3H), 7.41 (s, 1H), 7.72 (br t, 1H), 8.09 (s, 1H), 8.53 (br t, 1H). ^{13}C NMR (50 MHz, acetone-d_6) δ 13.6, 23.5, 29.6, 29.7, 30.6, 40.3, 56.9, 110.7, 114.2, 118.1, 128.0, 146.6, 149.4, 181.9. MS (CI^+) *m/z* 363.10 (MH^+, 11.6). HRMS: calcd. for $C_{15}H_{24}N_2O_2S_2Cl$ 363.0968 found 363.0992.

5.1.40. 3-(Propylsulfinyl)propan-1-amine, ***23*** *[54]*

In the work up, a slight modification of the literature procedure was used, whereby the filter cake of sodium iodate was washed with water and three portions of CH_2Cl_2. The aqueous layer was separated, and evaporated to obtain white solid. The latter was washed with MeOH and evaporated. Compound **23** was obtained as colorless oil (which contained salts). ^{1}H NMR (600 MHz, CD_3OD) δ 1.21 (t, J = 7.2 Hz, 3H), 1.81 (m, 2H), 2.13 (m, 2H), 2.81 (t, J = 7.5, Hz, 2H), 2.84 (dt, J = 13.5, 7.5 Hz, 2H), 2.95 (dt, J = 13.5, 7.5 Hz, 2H), 3.08 (t, J = 7.5, Hz, 2H). ^{13}C NMR (150 MHz, D_2O) δ 13.5, 17.5, 22.7, 39.7, 49.3, 54.7.

5.1.41. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-(propylsulfinyl) propyl)thiourea, ***24***

Compound **24** was obtained from **9a** and **23** (Procedure 6, solvent $CHCl_3$). Attempted chromatographic purification led to decomposition, therefore the crude product was evaluated biologically. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 1.58 (sext, J = 7.2 Hz, 2H), 1.80 (quint, J = 6.9 Hz, 2H), 2.44, 2.50 (two bt, 2H), 3.36 (t, J = 6.6 Hz, 2H), 3.75, 3.84 (two s, 3H), 6.83 (s, 1H), 7.20 (br t, 1H), 7.97 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 22.9, 29.4, 29.7, 34.2, 39.4, 56.4, 56.6, 104.4, 112.4, 114.0, 128.2, 141.8, 149.2, 155.59.

5.1.42. 3-(Propylamino)propanenitrile, ***25***

To a solution of propylamine (8.47 mmol) in 10 mL of MeOH was added acrylonitrile (8.47 mmol), the solution was stirred at room temperature for 3 h and concentrated. The residue was purified by chromatography (3:1 hexane:EtOAc). After separation of the first compound the solvent was changed to (1:1 MeOH/EtOAc) and the desired product was isolated from the subsequent fractions as a yellow oil in 94% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 2.83 (t, 2H, J = 6.9 Hz), 2.56 (t, 2H, J = 6.9 Hz), 2.53 (t, 2H, J = 7.5 Hz), 1.45 (sext, 2H, J = 7.5 Hz), 0.87 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 119.79, 51.46, 45.75, 23.57, 18.8, 11.85. MS (CI^+): *m/z* 113.11 (MH^+, 17.37), 83.06 ([M^+ – CH_2Me], 100), 72.082 ([MH^+ – MeCN], 72.67). HRMS: calcd. for $C_6H_{13}N_2$ (MH^+, CI^+) 113.1079 found 113.1103.

5.1.43. 3,3′-(Propylazanediyl)dipropanenitrile, ***26***

To a solution of *n*-$PrNH_2$ (8.47 mmol) in 10 mL of MeOH was added acrylonitrile (8.47 mmol). The reaction was stirred at ambient temperature for 3 h and evaporated to afford compound **26** (3% yield) which was purified by chromatography (3:1 hexane:EtOAc), together with **25** as a major product. ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 2.87 (t, 4H, J = 6.4 Hz), 2.62–2.5 (m, 6H), 1.49 (sext, 2H, J = 7.2 Hz), 0.90 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 119.9, 55.8, 50.1, 21.3, 16.8, 11.81. MS (CI^+): *m/z* 166.13 (MH^+, 0.91), 165.13 (M^+, 1.05), 136.09 ([M^+ – CH_2Me], 16.47), 125.10 ([MH^+ – MeCN], 100). HRMS: calcd. for $C_9H_{15}N_3$ (MH^+, CI^+) 165.1266 found 165.1277.

5.1.44. tert-Butyl 2-cyanoethylpropylcarbamate, ***27***

Compound **27** was obtained from **26** (Procedure 7) as a yellowish oil in 73% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 3.49 (t, 2H, J = 6.6 Hz), 3.23 (t, 2H, J = 7.2 Hz), 2.67 (t, 2H, J = 6.6 Hz), 1.56 (sext, 2H, J = 7.2 Hz), 1.54 (s, 9H), 0.87 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 155.6, 119.0, 79.8, 50.2, 49.3, 44.16, 43.91, 28.41, 22.54, 21.93, 17.69, 16.98, 11.38. MS (CI^+): *m/z* 212.15 (M^+, 1.53), 157.10 ([MH^+ – CH_2CMe_2, 100). HRMS: calcd. for $C_{11}H_{20}N_2O_2$ (M^+, CI^+) 212.1525 found 212.1514.

5.1.45. tert-Butyl 3-(3-(4-chloro-2,5-dimethoxyphenyl)thioureido) propylpropylcarbamate, ***29***

To a solution of $BH_3{\cdot}SMe_2$ (2 M in THF, 49 mmol) was added slowly **27** (11 mmol) dissolved in freshly distilled THF (10 mL) at 0 °C. The solution was stirred for 30 min at 0 °C, after which it was heated to reflux for 4 h. The mixture was cooled to 0 °C, and MeOH was added dropwise until no hydrogen evolution was detected. The solvent was evaporated. Trimethyl borate was removed by three co-evaporations with MeOH. Without further purification the product **28** was reacted with **9a** (Procedure 6) to give **29**, which was chromatographed (5:1 hexane:EtOAc). The product was obtained as a white solid in 2% yield (2 steps), mp 86–87 °C ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.32 (br t, 1H), 7.69 (br t, 1H), 7.61 (br t, 1H), 7.06 (s, 1H), 3.84 (s, 3H, *H*-C8), 3.82 (s, 3H), 3.59 (br sext, 2H, J = 5.4 Hz), 3.27

(t, 2H, $J = 6.9$ Hz), 3.14 (t, 2H, $J = 7.5$ Hz), 1.8 (br t, 2H), 1.54 (sext, 2H, $J = 7.5$ Hz), 1.41 (s, 9H), 0.85 (t, 3H, $J = 7.5$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 181.8, 156.9, 149.6, 147.5, 127.7, 119.0, 114.4, 111.6, 79.5, 57.0, 49.33, 44.54, 42.3, 28.5, 28.2, 22.6, 11.52. MS (CI^+): *m/z* 447.18 (M^+ (^{37}Cl), 12.96), 446.18 (MH^+, 16.59), 445.18 (M^+, 24.47), 414.16 ([MH^+ – MeOH], 40.89), 346 ([MH^+ – CH_2CMe_2 – CO_2], 44.14), 348 ([MH^+(^{37}Cl) – CH_2CMe_2 – CO_2], 17.84), 230.00 ([MH^+ – $H_2N_3N(COOCMe_3)_2Me$], 61.22), 228.10 ([MH^+ – $H_3N_3N(COOCMe_3)_2Me$], 100.02), 187.04 ([MH^+ – $C(S)NH_3N(COOCMe_3)_2Me$], 92.98). HRMS: calcd. for $C_{20}H_{32}N_3O_4S^{37}Cl$ (M^+, CI^+) 447.1773 found 447.1796, calcd. for $C_{20}H_{32}N_3O_4S^{35}Cl$ (M^+, CI^+) 445.1802 found 445.1780.

5.1.46. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-(propylamino) propyl)thiourea hydrochloride, **30**

Compound **29** was dissolved in Et_2O and 1 mL of concentrated HCl 37% was added. The solution was stirred for 15 min then additional HCl was added. The aqueous phase was separated and concentrated to afford **30** as a white solid in 92% yield, mp 116–117 °C ^{1}H NMR (300 MHz, MeOD) ppm δ 7.58 (br t, 1H), 7.09 (s, 1H), 3.83, 3.82 (two s, 3H), 3.74 (t, 2H, $J = 6.6$ Hz), 3.07 (t, 2H, $J = 6.9$ Hz), 2.99 (t, 2H, $J = 7.8$ Hz), 1.99 (quint, 2H, $J = 6.6$ Hz), 1.75 (sext, 2H, $J = 7.8$ Hz), 1.03 (t, 3H, $J = 7.5$ Hz). ^{13}C NMR (50 MHz, MeOD) ppm δ 182.5, 150.2, 148.4, 127.2, 120.8, 115.0, 112.37, 57.3, 57.1, 50.8, 46.0, 41.8, 27.6, 20.8, 11.3. MS (CI^+): *m/z* 348.14 (MH^+(^{37}Cl), 1.29), 346.13 (MH^+, 6.75), 314.12 ([MH^+ – MeOH], 24.53), 229.10 ([MH^+ – $H_2N_3NH_2Me$], 85.89), 228.99 ([MH^+ – $H_3N_3NH_2Me$], 100), 188.06 ([M^+(^{37}Cl) – $C(S)NH_3NH_2Me$], 25.24), 187.06 ([MH^+ – $C(S)NH_3NH_2Me$], 78.74). HRMS: calcd. for $C_{15}H_{25}N_3O_2S^{35}Cl$ (MH^+, CI^+) 346.1356 found 346.1341.

5.1.47. 1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-hydroxypropyl) thiourea, **31**

Compound **31** was obtained from **9a** and 3-amino-1-propanol (Procedure 6). The residue was purified by silica gel chromatography eluted with CH_2Cl_2/EtOAc (4:1) to obtain **31** in 90% yield, mp 100–102 °C ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 8.39 (br t, 1H), 7.80 (br t, 1H), 7.58 (br t, 1H), 7.06 (s, 1H), 3.84, 3.83 (two s, 3H), 3.72 (sext, 2H, $J = 6.4$ Hz), 3.63 (t, 2H, $J = 6$ Hz), 2.99 (br t, 1H), 1.77 (quint, 2H, $J = 6.4$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 181.7, 149.4, 146.9, 127.5, 118.0, 114.3, 110.9, 60.1, 56.9, 56.9, 42.9, 32.6. MS (CI^+): *m/z* 305.08 (MH^+, 7.85), 230.01 ([MH^+ – $H_2CH_2CH_2OH$], 100), 230.01 ([MH^+ – $H_3CH_2CH_2OH$], 56.34). HRMS: calcd. for $C_{12}H_{18}O_3N_2SCl$ (MH^+, CI^+) 305.0725 found 305.075, calcd. for $C_9H_9NO_2SCl$ (MH^+, CI^+) 230.0043 found 230.0061.

5.1.48. Butyric (Z)-N-(3-(butyryl oxy)propyl)-N-(4-chloro-2,5-dimethoxyphenyl)carbamimidic thioanhydride, **32a**

To a solution of **31** (0.19 mmol) in CH_2Cl_2 (20 mL) at 0 °C, under N_2, DMAP (0.19 mmol) and Et_3N (0.19 mmol) were added. The resulting mixture was stirred for a few minutes and butyryl chloride (0.19 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The solution was brought to room temperature and stirred overnight. The mixture was quenched with water and extracted with CH_2Cl_2. The organic phase was washed with 1 N HCl, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated. The residue was purified by chromatography (6:1 hexane:EtOAc) to give **32a** as a colorless oil in 29% yield. ^{1}H NMR (600 MHz, acetone-d_6) ppm δ 11.58 (br t, 1H), 7.19 (s, 1H), 7.1 (s, 1H), 4.18 (t, 2H, $J = 6.6$ Hz), 3.84, 3.83 (two s, 3H), 3.76 (td, 2H, $J = 6.6$ Hz, $J = 1.8$ Hz), 2.33 (t, 2H, $J = 7.2$ Hz), 2.09–2.03 (m, 4H), 1.63 (sext, 2H, $J = 7.2$ Hz), 1.53 (sext, 2H, $J = 7.2$ Hz), 0.93 (t, 3H, $J = 7.2$ Hz), 0.81 (t, 3H, $J = 7.2$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 184.6, 178.8, 173.6, 150.7, 150.13, 130.3, 123.5, 117.0, 115.1, 62.8, 57.2, 57.0, 44.23, 36.4, 30.4, 27.5, 19.0, 18.4, 13.9, 13.7. MS (CI^+): *m/z* 447.15 (MH^+(^{37}Cl), 28.39), 446.15 (M^+ (^{37}Cl), 33.69), 445.15 (MH^+, 69.65), 444.15 (M^+, 40.55), 187.03 ([M^+ – $Me(CH_2)_2COO(CH_2)_3NHCSCO(CH_2)_2Me$], 100), 172.01 ([$M^+$ – $NC(SC_2)(HN_3OC_2Me)$], 55.57), 128.10 ([M^+ – $C_6H_2Cl(OMe)_2N{=}C(NH_2)(SC)(O)(CH_2)_2Me$], 49.81), 128.10 ([$M^+$ – $C_6H_2Cl_2N{=}C(SC_2)$], 81.82). HRMS: calcd. for $C_{20}H_{29}N_2O_5S^{35}Cl$ (M^+, CI^+) 444.1486 found 444.1455 calcd. for $C_{20}H_{29}N_2O_5S^{37}Cl$ (M^+, CI^+) 446.1456 found 446.1493.

5.1.49. tert-Butyl 3-hydroxypropylcarbamate, **33**

Compound **33** was obtained as a colorless oil in 77% yield from 3-amino-1-propanol (Procedure 7). No starting material (detected by TLC, 3:1 hexane:EtOAc) was observed. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 6.1 (br t, 1H), 3.9 (br t, 1H), 3.57 (t, 2H, $J = 6$ Hz), 3.16 (s, 2H, $J = 6.3$ Hz), 1.64 (quint, 2H, $J = 6.3$ Hz), 1.39 (s, 9H). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 157.2, 78.6, 59.9, 38.0, 33.2, 28.58. MS (CI^+): *m/z* 176.13 (MH^+, 7.97), 120.06 ([MH^+ – CH_2CMe_2], 61.87), 102.05 ([M^+ – $OCMe_3$], 32.06), 76.06 ([MH^+ – CH_2CMe_2 – CO_2], 100). HRMS: calcd. for $C_8H_{18}N\,O_3$ (MH^+, CI^+) 176.1287 found 176.1281.

5.1.50. tert-Butyl 3-(butyryloxy)propylcarbamate, **34a**

To a solution of **33** (0.84 mmol) in CH_2Cl_2 (30 mL) at 0 °C, under N_2, DMAP (0.84 mmol) and Et_3N (0.84 mmol) were added. The resulting mixture was stirred for a few minutes and butyryl chloride (0.84 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The solution was brought to room temperature and stirred overnight. It was then quenched with diluted water and extracted with CH_2Cl_2. The organic phase was washed with concentrated $KHSO_4$, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated, to give **34a** as a yellowish oil in 79% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 6.01 (br t, 1H), 4.08 (t, 2H, $J = 6.6$ Hz), 3.14 (sext, 2H, $J = 6.6$ Hz), 2.26 (t, 2H, $J = 7.2$ Hz), 1.81 (quint, 2H, $J = 6.6$ Hz), 1.57 (sext, 2H, $J = 7.2$ Hz), 1.39 (s, 9H), 0.91 (t, 3H, $J = 7.2$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.42, 156.52, 78.40, 62.22, 37.83, 36.32, 29.85, 28.61, 18.89, 13.85. MS (CI^+): *m/z* 246.17 (MH^+, 22.62), 190.107 ([MH^+ – CH_2CMe_2], 100), 89.03 ([MH^+ + H^+ – $(CH_2)_3NHCOOCMe$], 98.39). HRMS: calcd. for $C_{12}H_{24}NO_4$ (MH^+, CI^+) 246.1705 found 246.1715, calcd. for $C_8H_{16}NO_4$ 190.1079 found 190.1070.

5.1.51. tert-Butyl 3-(Propionyloxy)propylcarbamate, **34b**

Compound **33** was reacted with propionic acid (Procedure 8) to give **34b** as a yellowish oil in 35% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 6.04 (br t, 1H), 4.07 (t, 2H, $J = 6.6$ Hz), 3.14 (sext, 2H, $J = 6.6$ Hz), 2.29 (sext, 2H, $J = 7.5$ Hz), 1.79 (quint, 2H, $J = 6.6$ Hz), 1.38 (s, 9H), 1.06 (t, 3H, $J = 7.5$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.6, 155.9, 77.7, 61.6, 37.1, 29.3, 28.3, 26.9, 8.6. MS (CI^+): *m/z* 232.15 (MH^+, 1.02), 176.09 ([MH^+ – CH_2CMe_2], 16.22), 132.1 ([MH^+ – CH_2CMe_2 – CO_2], 44.84), 102.06 ([MH^+ – CH_2CMe_2 – $HOOCCH_2Me$], 100). HRMS: calcd. for $C_{11}H_{22}NO_4$ (MH^+, CI^+) 232.1549 found 232.1509.

5.1.52. 3-Aminopropyl propionate, **35b**

Compound **35b** obtained from **34b** upon treatment with HCl/EtOAc [29], as a yellow semi-solid in 96% yield. The reaction stirred for 2 h and was stopped when no starting material was detected by TLC (4:1 hexane:EtOAc). ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 8.54 (br t), 4.36 (t, 2H, $J = 6.2$ Hz), 3.33 (br sext, 2H, $J = 6.4$ Hz), 2.49 (sext, 2H, $J = 7.4$ Hz), 2.32 (quint, 2H, $J = 7.6$ Hz), 1.2 (t, 3H, $J = 7.4$ Hz). ^{13}C NMR (50 MHz, acetone-d_6) ppm δ 174.6, 62.2, 37.9, 27.8, 27.4, 9.3. MS (CI^+): *m/z* 132.10 (MH^+, 80.05), 115.06 ([MH^+ – NH_3], 45.44). HRMS: calcd. for $C_6H_{14}NO_2$ (MH^+, CI^+) 132.1025 found 132.0977.

5.1.53. 3-(3-(4-Chloro-2,5-dimethoxyphenyl)thioureido)propyl butyrate, **36a**

The *N*-*t*-Boc protected **34a** was converted to the corresponding amine hydrochloric by reaction with 4 N HCl in EtOAc [29]. The

reaction was stopped when no starting material was remained (detected by TLC 4:1 hexane:EtOAc). The crude compound was reacted without further purification with **9a** (Procedure 6) and the product was chromatographed (2:1 hexane:EtOAc) to give **36a** as a colorless oil. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.38 (br t, 1H), 7.91 (br t, 1H), 7.53 (br t, 1H), 7.06 (s, 1H), 4.13 (t, 2H, J = 6.6 Hz), 3.84, 3.83 (two s, 3H), 3.7 (sext, 2H, J = 6.6 Hz), 2.25 (t, 2H, J = 7.2 Hz), 1.96 (quint, 2H, J = 6.6 Hz), 1.59 (sext, 2H, J = 7.2 Hz), 0.91 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 181.9, 173.7, 149.5, 146.8, 127.7, 118.3, 114.3, 110.8, 62.5, 57.0, 42.1, 36.4, 29.0, 19.0, 13.88. MS (CI$^+$): m/z 375.11 (MH$^+$, 27.16), 230 ([MH$^+$ − $H_2N(CH_2)_3OCO(CH_2)_2Me$], 100), 188.05 ([MH(^{37}Cl)$^+$ − $C(S)NH(CH_2)_3OC(O)CH_2CH_2Me$], 29.68), 187.04 ([MH$^+$ − $C(S)NH(CH_2)_3OC(O)CH_2CH_2Me$], 35.27), 129.08 ([M$^+$ − $C_6H_2(Cl)(OMe)_2(NHC(S)NH)$, 69.93). HRMS: calcd. for $C_{16}H_{24}N_2O_4S^{35}Cl$ (MH$^+$, CI$^+$) 375.1145 found 375.1109.

5.1.54. 3-(3-(4-Chloro-2,5-dimethoxyphenyl)thioureido)propyl propionate, **36b**

Compound **36b** was obtained from **35b** and isothiocyanate **9a** (Procedure 6). The residue was purified by silica gel chromatography eluted with 3:1 hexane:EtOAc to give **36b** in 48% yield as a colorless oil. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.38 (br t, 1H), 7.91 (br t, 1H), 7.53 (br t, 1H, J = 4.8 Hz), 7.06 (s, 1H), 4.12 (t, 2H, J = 6.3 Hz), 3.84, 3.83 (two s, 3H), 3.7 (sext, 2H, J = 6.6 Hz), 2.29 (2H, J = 7.5 Hz), 1.94 (quint, 2H, J = 6.6 Hz), 1.06 (t, 3H, J = 7.5 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 182.0, 174.5, 149.5, 146.8, 127.7, 118.3, 114.3, 110.8, 62.5, 57.0, 42.1, 29.0, 27.8, 9.4. MS (CI$^+$): m/z 361.10 (MH$^+$, 50.52), 360.10 (M$^+$, 29.47), 329.08 ([MH$^+$ − MeOH], 53.54), 287.06 ([MH$^+$ − $HOOCCH_2Me$], 38.19), 230.01 ([MH$^+$ − $H_2N(CH_2)_3OCOCH_2Me$], 75.8), 229.01 ([MH$^+$ − $H_3N(CH_2)_3OCOCH_2Me$], 99.99), 187.05 ([MH$^+$ − $C(S)NH(CH_2)_3OCOCH_2Me$], 55.11). HRMS: calcd. for $C_{15}H_{22}N_2O_4S^{35}Cl$ (MH$^+$, CI$^+$) 361.0989 found 361.1035, calcd. for $C_{14}H_{18}N_2O_3S^{35}Cl$ 329.0727 found 329.0770.

5.1.55. 3-(3-(4-Chloro-2,5-dimethoxyphenyl)thioureido)propyl acetate, **36c**

A mixture of **36c** (major product) and **36a** (major product) were obtained from **34a** upon treatment with HCl/EtOAc [29], followed by Procedure 6 and were separated by chromatography (2:1 hexane:EtOAc). Compound **36c** mp 74–76 °C ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 8.41 (br t, 1H), 7.87 (br t, 1H), 7.5 (br t, 1H), 7.06 (s, 1H), 4.1 (t, 2H, J = 6.4 Hz), 3.83, 3.82 (two s, 3H), 3.69 (sext, 2H, J = 6.4 Hz), 1.98 (s, 3H), 1.96 (quint, 2H, J = 6.6 Hz). ^{13}C NMR (50 MHz, acetone-d_6) ppm δ 181.8, 171.1, 149.3, 146.8, 127.5, 118.3, 114.2, 110.8, 62.6, 56.9, 42.0, 28.3, 20.8. MS (CI$^+$): m/z 347.08 (MH$^+$, 46.09), 230.01 ([MH$^+$ − H_2N_3OCOMe], 92.21), 187.04 ([MH$^+$ − $C(S)NH_3OCMe$], 56.08), 101.05 ([M$^+$ − $C_6H_2Cl_2(NHC(S)NH)$], 100). HRMS: calcd. for $C_{14}H_{20}N_2O_4S^{35}Cl$ (MH$^+$, CI$^+$) 347.0832 found 347.0844.

5.1.56. 3-(tert-Butoxycarbonylamino)propanoic acid, **37**

To a solution of NaOH (1.71 mmol) in H_2O (20 mL) were added **33** and $KMnO_4$ (1.89 mmol). The purple mixture was stirred at room temperature for 7 h. The solution was then acidified with concentrated $KHSO_4$ and extracted with two portions of EtOAc. The organic phase was separated, washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by chromatography (1:1 hexane:EtOAc) and the product **37** was isolated as a white solid in 51% yield, mp 71–72 °C ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 10.54 (br t, 1H), 6.04 (br t, 1H), 3.31 (sext, 2H, J = 6.6 Hz), 2.5 (t, 2H, J = 6.6 Hz), 1.38 (s, 9H). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 174.0, 156.7, 78.9, 37.0, 34.7, 28.5. MS (CI$^+$): m/z 190.11 (MH$^+$, 1.79), 134.04 ([MH$^+$ − $HCMe_3$], 99.99), 116.04 ([MH$^+$ − $HOCMe_3$], 63.18), 90.06 ([MH$^+$ + H − $COOCMe_3$], 70.27). HRMS: calcd. for $C_8H_{16}NO_4$ (MH$^+$, CI$^+$) 190.1079 found 190.1103, calcd. for $C_4H_8NO_4$ 134.0453 found 134.0436.

5.1.57. tert-Butyl 2-(butoxycarbonyl)ethylcarbamate, **38a**

Compound **37** was reacted with n-BuOH (Procedure 8) to give **38a** as a yellowish oil in 78% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 5.99 (br t, 1H), 4.05 (t, 2H, J = 6.6 Hz), 3.32 (sext, 2H, J = 6.6 Hz), 2.5 (t, 2H, J = 6.9 Hz), 1.6 (quint, 2H, J = 6.6 Hz), 1.47–1.31 (m, 11H), 0.91 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 172.2, 156.4, 78.7, 64.5, 37.1, 35.2, 31.3, 28.6, 19.7, 14.0. MS (CI$^+$): m/z 246.17 (MH$^+$, 1.33), 190.11 ([MH$^+$ − CH_2CMe_2], 40.03), 146.12 ([MH$^+$ − CH_2CMe_2 − CO_2], 98.39). HRMS: calcd. for ($C_{12}H_{24}NO_4$ (MH$^+$, CI$^+$)) 246.1705 found 246.168, calcd. for $C_8H_{16}NO_4$ 190.1079 found 190.1101, calc. for $C_7H_{16}NO_2$ 146.1181 found 146.1168.

5.1.58. Propyl 3-(3,3-dimethylbutanamido)propanoate, **38b**

Compound **37** was reacted with n-PrOH (Procedure 8) to gives **38b** as a yellowish oil in 82% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 6.01 (br t, 1H), 4.00 (t, 2H, J = 6.6 Hz), 3.32 (sext, 2H, J = 6.6 Hz), 2.5 (t, 2H, J = 6.9 Hz), 1.62 (sext, 2H, J = 6.9 Hz), 1.39 (s, 9H), 0.92 (t, 3H, J = 7.5 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 171.5, 155.6, 77.9, 65.5, 36.3, 34.4, 28.5, 21.8, 9.8. MS (CI$^+$): m/z 232.16 (MH$^+$, 0.31), 230.10 (M, 0.5), 176.09 ([MH$^+$ − CH_2CMe_2], 25.67), 132.10 ([MH$^+$ − CH_2CMe_2 − CO_2], 100). HRMS: calcd. for $C_{11}H_{22}NO_4$ (MH$^+$, CI$^+$) 232.1549 found 232.1588.

5.1.59. Butyl 3-aminopropanoate, **39a** [55]

Compound **39a** was obtained as a yellowish oil in quantitative yield from **38a** upon treatment with HCl/EtOAc [29]. No starting material was detected by TLC (4:1 hexane:EtOAc) after 1.5 h of stirring. ^{1}H NMR (300 MHz, MeOD) ppm δ 8.00 (br t, 2H), 4.15 (t, 2H, J = 6.6 Hz), 3.22 (br t, 2H, J = 6 Hz), 2.79 (t, 2H, J = 6.3 Hz), 1.65 (quint, 2H, J = 6.6 Hz), 1.41 (sext, 2H, J = 7.2 Hz), 0.95 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, MeOD) ppm δ 172.2, 66.0, 36.5, 32.2, 31.6, 20.1, 14.0. MS (CI$^+$): m/z 146.12 (MH$^+$, 100), 90.06 ([MH$^+$ + H$^+$ − $CH_2CH_2CH_2Me$], 33.05), 72.04 ([MH$^+$ − $HOCH_2CH_2CH_2Me$], 55.45). HRMS: calcd. for $C_7H_{16}NO_2$ (MH$^+$, CI$^+$) 146.1181 found 146.12.

5.1.60. Propyl 3-aminopropanoate, **39b** [56]

Compound **39b** was obtained as a yellowish semi-solid in quantitative yield from **38b** upon treatment with HCl/EtOAc [29]. No starting material was detected by TLC (3:1 hexane:EtOAc) after 2 h of stirring. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.51 (br t, 2H), 4.08 (t, 2H, J = 6.6 Hz), 3.36 (br t, 2H), 3.01 (t, 2H, J = 6.9 Hz), 1.66 (sext, 2H, J = 7.2 Hz), 0.93 (t, 3H, J = 7.5 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 170.8, 66.2, 35.6, 31.3, 21.7, 9.8. MS (CI$^+$): m/z 132.10 (MH$^+$, 100), 90.06 ([MH$^+$ + H$^+$ − CH_2CH_2Me], 17.47), 72.05 ([MH$^+$ − $HOCH_2CH_2CH_2Me$], 67.94). HRMS: calcd. for $C_6H_{14}NO_2$ (MH$^+$, CI$^+$) 132.1025 found 132.1026, calcd. for C_3H_6NO 72.0449 found 72.0467.

5.1.61. Butyl 3-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioami)propanoate, **40a**

Compound **40a** was obtained from **39a** and isothiocyanate **9a** (Procedure 6). The residue was purified by silica gel chromatography eluted with 4:1 hexane:EtOAc to give **40a** in 39% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) ppm δ 7.81 (br t, 1H), 7.13 (br t, 1H), 7.07 (br t, 1H), 6.92 (s, 1H), 3.99 (t, 2H, J = 6.6 Hz), 3.88–3.82 (m, 5H), 3.73 (s, 3H), 2.65 (t, 2H, J = 5.7 Hz), 1.52 (quint, 2H, J = 6.6 Hz), 1.29 (sext, 2H, J = 7.2 Hz), 0.86 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) ppm δ 179.9, 173.1, 149.1, 146.0, 124.5, 119.7, 114.2, 109.3, 64.7, 56.7, 56.4, 40.6, 33.1, 30.5, 19.1, 13.7. MS (CI$^+$): m/z 377.11 (MH$^+$(^{37}Cl), 25.84), 375.11 (MH$^+$(^{35}Cl), 64.91), 374.11 (M$^+$, 23.41), 229.10 ([MH$^+$ − $H_2N_2COO_3Me$], 100), 228.99 ([MH$^+$ − $H_3N_2COO_3Me$], 80.44), 187.04 ([MH$^+$ − $C(S)NH_2COO_3Me$], 52.73). HRMS: calcd. for

$C_{16}H_{24}N_2O_4S^{35}Cl$ (MH^+, CI^+) 375.1145 found 375.1127, calcd. for $C_{16}H_{24}N_2O_4S^{37}Cl$ (MH^+, CI^+) 377.1116 found 377.1104.

5.1.62. Propyl 3-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioami)propanoate, **40b**

Compound **40b**, obtained from amine hydrochloride **39b** and isothiocyanate **9a** (Procedure 6), was purified by column chromatography (3:1 hexane:EtOAc) and was isolated as a yellowish oil in 30% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.47 (br t, 1H), 7.93 (br t, 1H), 7.6 (br t, 1H, $J = 4.2$ Hz), 7.07 (s, 1H), 4.01 (t, 2H, $J = 6.6$ Hz), 3.87 (sext, 2H, $J = 6.6$ Hz), 3.85, 3.82 (two s, 3H), 2.71 (t, 2H, $J = 6.6$ Hz), 1.62 (sext, 2H, $J = 7.5$ Hz), 0.91 (t, 3H, $J = 7.5$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 181.8, 172.7, 149.5, 146.6, 127.8, 118.2, 114.3, 110.6, 66.5, 57.0, 40.9, 34.0, 22.6, 10.6. MS (CI^+): *m/z* 363.10 ($MH^+(^{37}Cl)$, 37.54), 361.10 ($MH^+(^{35}Cl)$, 99.99), 360.09 (M, 45.07), 329.07 ([MH^+ – MeOH], 53.75), 301.04 ([MH^+ – HO_2Me], 12.65), 230.0 ([MH^+ – $H_2N_2COO_2Me$], 44.97), 187.04 ([MH^+ – C(S)NH_2COO_2Me], 41.76). HRMS: calcd. for $C_{15}H_{22}N_2O_4S^{35}Cl$ (MH^+, CI^+) 361.0989 found 361.0983, calcd. for $C_{15}H_{22}N_2O_4S^{37}Cl$ (MH^+, CI^+) 363.0959 found 363.0964.

5.1.63. Methyl 3-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioami)propanoate, **42a**

To remove the *N-tert*-Boc group, compound **37** was added to a freshly-prepared solution of HCl in EtOAc. Methyl 3-aminopropanoate was obtained as a second by-product and reacted with **9a** (Procedure 6) to give **42a,** which was purified by column chromatography (2:1 hexane:EtOAc) and isolated as a white solid, mp 103–104 °C ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.47 (br t, 1H), 7.94 (br t, 1H), 7.6 (br t, 1H), 7.07 (s, 1H), 3.87 (sext, 2H, $J = 6.6$ Hz), 3.85, 3.83 (two s, 3H), 3.64 (s, 3H, CO), 2.71 (t, 2H, $J = 6.6$ Hz). ^{13}C NMR (50 MHz, acetone-d_6) ppm δ 181.9, 173.0, 149.0, 146.6, 127.8, 118.1, 114.2, 110.6, 56.9, 51.7, 40.8, 33.7. MS (CI^+): *m/z* 35.06 ($MH^+(^{37}Cl)$, 14.55), 333.07 ($MH^+(^{35}Cl)$, 35.51), 332.06 (M, 10.43), 301.05 ([MH^+ – MeOH], 14.10), 231.10 ([$M(^{37}Cl)$ – HN$(CH_2)_2$COOMe], 36.11), 221.0 ([M – $NH(CH_2)_2$COOMe], 99.99), 228.99 ([$M(^{35}Cl)$ – $H_2N(CH_2)_2$COOMe], 45.58). HRMS: calcd. for $C_{13}H_{18}N_2O_4S^{35}Cl$ (MH^+, CI^+) 333.0676 found 333.0684, calcd. for $C_{13}H_{18}N_2O_4S^{37}Cl$ (MH^+, CI^+) 335.0646 found 335.0632.

5.1.64. Ethyl 3-(3-(4-chloro-2,5-dimethoxyphenyl)thioureido) propanoate, **42a′**

To remove the *N-tert*-Boc group, compound **37** was added to a freshly-prepared solution of HCl in EtOAc. Ethyl 3-aminopropanoate was obtained as a byproduct and reacted with **9a** (Procedure 6) to give **42a′**, which was purified by column chromatography (2:1 hexane:EtOAc) and isolated as a white solid, mp 84–85 °C ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.48 (br t, 1H), 7.96 (br t, 1H), 7.62 (br t, 1H), 7.09 (s, 1H), 4.12 (sext, 2H, $J = 7.2$ Hz), 3.91–3.83 (sext, 2H, $J = 6.6$ Hz), 3.86, 3.84 (two s, 3H), 2.71 (t, 2H, $J = 6.6$ Hz), 1.23 (t, 3H, $J = 6.9$ Hz). ^{13}C NMR (50 MHz, acetone-d_6) ppm δ 181.8, 172.6, 149.4, 146.6, 127.8, 118.1, 114.2, 110.6, 60.9, 56.9, 40.8, 34.0, 14.4. MS (CI^+): *m/z* 349.08 ($MH^+(^{37}Cl)$, 37.62), 347.08 ($MH^+(^{35}Cl)$, 100), 346.08 (M^+, 53.76), 315.01 ([MH^+ – MeOH], 72.43), 228.99 ([MH^+ – H_3N_2CO Me], 67.1), 187.02 ([MH^+ – C(S)NH_2CO Me], 66.67). HRMS: calcd. for $C_{14}H_{20}N_2O_4S^{35}Cl$ (MH^+, CI^+) 347.0832 found 347.0834, calcd. for $C_{14}H_{20}N_2O_4S^{37}Cl$ (MH^+, CI^+) 349.0803 found 349.0804.

5.1.65. 3-(Amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioami)propanoic acid, **42b**

Compound **42b**, obtained from **41b** and isothiocyanate **9a** (Procedure 6), was purified by column chromatography (20:1 CH_2Cl_2:MeOH) and was isolated as a white solid in 2% yield, mp 157–159 °C ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 8.49 (br t, 1H), 7.91 (br t, 1H), 7.6 (br t, 1H), 7.06 (s, 1H), 3.86 (sext, 2H, $J = 6.4$ Hz), 3.84, 3.82 (two s, 3H), 2.71 (t, 2H, $J = 6.4$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 181.7, 173.7, 149.4, 146.4, 127.9, 118.1, 114.2, 110.4, 56.9, 40.7, 33.5. MS (CI^+): *m/z* 347.08 ([$M^+ + C_2H_5$], 5.63), 232.01 ([$MH^+(^{37}Cl)$ – $H_2N(CH_2)_2$COOH], 35.86), 230.01 ([MH^+ – $H_2N(CH_2)_2$COOH], 100), 229.01 ([MH^+ – $H_3N(CH_2)_2$COOH], 57.96), 188.05 ([$M^+(^{37}Cl)$ – C(S)$NH(CH_2)_2$COOH], 54.7), 187.05 ([MH^+ – C(S)$NH(CH_2)_2$COOH], 62.13). HRMS: calcd. for $C_{14}H_{20}N_2O_4S^{35}Cl$ (MH^+, CI^+) 347.0832 found 347.0832.

5.1.66. tert-Butyl 3-(methoxycarbonyl)propylcarbamate, **43a** [57]

Boc-Gaba was reacted with MeOH (Procedure 8) to provide **43a** as a yellowish oil in 90% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 6.04 (br t, 1H), 3.6 (s, 3H, *H*-C9), 3.09 (sext, 2H, $J = 6.9$ Hz), 2.33 (t, 2H, $J = 7.5$ Hz), 1.75 (quint, 2H, $J = 7.5$ Hz), 1.38 (s, 9H). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.8, 156.6, 78.4, 51.5, 40.3, 31.5, 28.6, 26.0. MS (CI^+): *m/z* 218.14 (MH^+, 71.89), 162.07 ([MH^+ – CH_2CMe_2], 96.36), 161.07 ([MH^+ – CMe_3], 61.20), 144.07 ([MH^+ – $HOCMe_3$], 54.12), 118.08 ([MH^+ – CH_2CMe_2 – CO_2], 100). HRMS: calcd. for $C_{10}H_{20}NO_4$ (MH^+, CI^+) 218.1392 found 218.1377.

5.1.67. tert-Butyl 3-(ethoxycarbonyl)propylcarbamate, **43b** [46]

Boc-Gaba was reacted with EtOH (Procedure 8) to provide **43b** as a yellowish oil in 87% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 6.04 (br t, 1H), 4.07 (sext, 2H, $J = 7.2$ Hz), 3.09 (sext, 2H, $J = 6.6$ Hz), 2.31 (t, 2H, $J = 7.5$ Hz), 1.75 (quint, 2H, $J = 7.5$ Hz), 1.39 (s, 9H), 1.2 (t, 3H, $J = 7.2$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.4, 156.6, 78.4, 60.5, 40.3, 31.8, 28.6, 26.0, 14.5. MS (CI^+): *m/z* 232.16 (MH^+, 74.57), 176.09 ([MH^+ – CH_2CMe_2], 99.34), 175.09 ([MH^+ – CMe_3], 63.49), 158.08 ([MH^+ – $HOCMe_3$], 57.84), 132.10 ([MH^+ – CH_2CMe_2 – CO_2], 100), 130.08 ([M^+ – Me_3COOC], 86.46). HRMS: calcd. for $C_{11}H_{22}NO_4$ (MH^+, CI^+) 232.1549 found 232.157.

5.1.68. tert-Butyl 3-(propoxycarbonyl)propylcarbamate, **43c**

Boc-Gaba was reacted with *n*-PrOH (Procedure 7) to give **43c** as a yellowish oil in 81% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 6.02 (br t, 1H), 3.99 (t, 2H), $J = 6.9$ Hz, 3.1 (sext, 2H, $J = 6.6$ Hz), 2.33 (t, 2H, $J = 7.5$ Hz), 1.76 (quint, 2H, $J = 7.5$ Hz), 1.61 (sext, 2H, $J = 6.9$ Hz), 1.39 (s, 9H), 0.91 (t, 3H, $J = 7.5$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.5, 156.7, 78.4, 66.1, 40.3, 31.8, 28.6, 26.1, 22.6, 10.6. MS (CI^+): *m/z* 231.15 ([M^+ – CH_2], 29.72), 190.09 ([MH^+ – CH_2CMe_2], 38.12), 146.12 ([MH^+ – CH_2CMe_2 – CO_2], 100.01). HRMS: calcd. for $C_{11}H_{21}NO_4$ (MH^+, CI^+) 231.1471 found 231.15049.

5.1.69. tert-Butyl 3-(butoxycarbonyl)propylcarbamate, **43d**

Boc-Gaba reacted with *n*-BuOH (Procedure 8) to give **43d** as yellowish oil in 89% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 5.98 (br t, 1H), 4.04 (t, 2H, $J = 6.6$ Hz), 3.1 (sext, 2H, $J = 6.6$ Hz), 2.32 (t, 2H, $J = 7.5$ Hz), 1.76 (quint, 2H, $J = 7.2$ Hz), 1.59 (quint, 2H, $J = 6.9$ Hz), 1.43–1.31 (m, 11H), 0.92 (t, 3H, $J = 7.2$ Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.4, 156.6, 78.4, 64.3, 40.4, 31.9, 31.4, 28.6, 26.1, 19.7, 14.0. MS (CI^+): *m/z* 260.19 (MH^+, 16.41), 204.12 ([$MH^+ + H^+ - (CH_2)_3$Me], 53.48), 160.13 ([MH^+ – CH_2CMe_2 – CO_2], 100), 158.12 ([M^+ – CMe_3 – CO_2], 39.24). HRMS: calcd. for $C_{13}H_{26}NO_4$ (MH^+, CI^+) 260.1862 found 260.1852.

5.1.70. tert-Butyl 3-(propylcarbamoyl)propylcarbamate, **43e**

Boc-Gaba was reacted with *n*-$PrNH_2$ (Procedure 8) to provide **43e** as a pure white solid in 86% yield, mp 44–45 °C ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 7.42 (br t, 1H), 6.2 (br t, 1H), 3.15–3.03 (m, 4H), 2.2 (t, 2H, $J = 7.2$ Hz), 1.76 (quint, 2H, $J = 7.2$ Hz), 1.48 (sext, 2H, $J = 7.2$ Hz), 1.39 (s, 9H), 0.87 (t, 3H, $J = 7.4$ Hz). ^{13}C NMR (50 MHz, acetone-d_6) ppm δ 173.1, 156.8, 78.4, 41.5, 40.7, 34.0, 28.6, 27.0, 23.4, 11.7. MS (CI^+): *m/z* 245.18 (MH^+, 44.45), 189.12 ([MH^+ – CH_2CMe_2], 33.29), 145.13 ([MH^+ – CH_2CMe_2 – CO_2], 100), 128.10

([MH^+ – $H_2NCOOCMe_3$], 57.68). HRMS: calcd. for $C_{12}H_{25}N_2O_3$ (MH^+, CI^+) 245.1865 found 245.1830.

5.1.71. tert-Butyl 3-((propylthio)carbonyl)propylcarbamate, ***43f***

Boc-Gaba was reacted with *n*-PrSH (Procedure 8) to provide **43f** as a colorless oil in 94% yield. ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 5.99 (br t, 1H), 3.1 (sext, 2H, J = 6.8 Hz), 2.83 (t, 2H, J = 7.2 Hz), 2.6 (t, 2H, J = 7.2 Hz), 1.8 (quint, 2H, J = 6.8 Hz), 1.56 (sext, 2H, J = 7.2 Hz), 1.39 (s, 9H), 0.93 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 198.7, 156.6, 78.5, 41.8, 40.2, 30.9, 28.6, 26.6, 23.6, 13.4. MS (CI^+): m/z 262.15 (MH^+, 94.95), 206.10 ([MH^+ – CH_2CMe_2], 62.12), 188.08 ([MH^+ – $HOCMe_3$], 47.57), 162.11 ([MH^+ – CH_2CMe_2-CO_2], 58.49), 130.06 ([M^+ – $(CH_2)_2COS(CH_2)_2Me$], 59.16). HRMS: calcd. for $C_{12}H_{24}NO_3S$ (MH^+, CI^+) 262.1477 found 262.1495.

5.1.72. Methyl 4-aminobutanoate, ***44a*** *[58]*

Compound **44a** was obtained as a white solid in 90% yield from **43a** upon treatment with HCl/EtOAc [29]. No starting material was detected by TLC (1:3 hexane:EtOAc) after 3.5 h of stirring, mp 103 °C ^{1}H NMR (300 MHz, MeOD) ppm δ 3.71 (s, 2H), 3.06 (t, 2H, J = 7.5 Hz), 2.54 (t, 2H, J = 7.2 Hz), 2.03 (quint, 2H, J = 7.5 Hz). ^{13}C NMR (75 MHz, MeOD) ppm δ 174.5, 52.4, 40.1, 31.5, 23.67. MS (CI^+): m/z 118.09 (MH^+, 85.36), 102.07 ([MH^+-CH_4], 39.19), 101.06 ([M^+ – CH_4], 82.96), 87.06 ([MH^+ – OMe], 100), 86.06 ([MH^+ – HOMe], 76.79). HRMS: calcd. for $C_5H_{12}NO_2$ (MH^+, CI^+) 118.0868 found 118.0883.

5.1.73. Ethyl 4-aminobutanoate, ***44b*** *[59]*

Compound **44b** was obtained as white solid in 98% yield from **43b** upon treatment with HCl/EtOAc [29]. No starting material was detected by TLC (1:3 hexane:EtOAc) after 3.5 h of stirring, mp 38 °C ^{1}H NMR (300 MHz, MeOD) ppm δ 4.14 (sext, 2H, J = 7.2 Hz), 3.03 (t, 2H, J = 7.2 Hz), 2.5 (t, 2H, J = 7.2 Hz), 1.99 (quint, 2H, J = 7.5 Hz), 1.25 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, MeOD) ppm δ 174.0, 61.7, 40.1, 31.8, 23.7, 14.5. MS (CI^+): m/z 133.11 (MD^+, 54.01), 132.10 (MH^+, 88.99), 86.07 ([MH^+ – $MeCH_2O$], 55.44). HRMS: calcd. for $C_6H_{14}NO_2$ (MH^+, CI^+) 132.1025 found 132.0992, calcd. for $C_6H_{15}NO_2$ (MH^+, CI^+) 133.1103 found 133.1098.

5.1.74. Propyl 4-aminobutanoate, ***44c***

Compound **44c** was obtained as a colorless semi-solid oil in 98% yield from **43c** upon treatment with HCl/EtOAc [29]. No starting material was detected by TLC (3:1 hexane:EtOAc) after 4 h of stirring. ^{1}H NMR (300 MHz, MeOD) ppm δ 8.01 (br t), 4.05 (t, 2H, J = 6.6 Hz), 3.02 (t, 2H, J = 7.2 Hz), 2.51 (t, 2H, J = 7.2 Hz), 1.99 (quint, 2H, J = 7.2 Hz), 1.65 (sext, 2H, J = 6.6 Hz), 0.95 (t, 3H, J = 7.5 Hz). ^{13}C NMR (75 MHz, MeOD) ppm δ 174.1, 67.3, 40.1, 31.7, 23.7, 22.9, 10.7. MS (CI^+): m/z 147.13 (MD^+, 28.63), 146.12 (MH^+, 64.96), 87.06 ([MH^+ – OCH_2CH_2Me], 100), 86.07 ([MH^+ – $HOCH_2CH_2Me$], 69.19). HRMS: calcd. for $C_7H_{16}NO_2$ (MH^+, CI^+) 146.1181 found 146.1193.

5.1.75. Butyl 4-aminobutanoate, ***44d*** *[60]*

Compound **44d** was obtained as a yellow-brown semi-solid in quantitative yield from **43d** upon treatment with HCl/EtOAc [29]. No starting material was detected by TLC (3:1, hexane:EtOAc) after 5 h of stirring. ^{1}H NMR (200 MHz, MeOD) ppm δ 4.09 (t, 2H, J = 6.4 Hz), 3.03 (br t, 2H, J = 7.2 Hz), 2.5 (t, 2H, J = 7.2 Hz), 2.00 (quint, 2H, J = 6.8 Hz), 1.62 (quint, 2H, J = 7.4 Hz), 1.39 (sext, 2H, J = 7.4 Hz), 0.94 (t, 3H, J = 7.2 Hz). ^{13}C NMR (50 MHz, MeOD) ppm δ 174.1, 65.5, 40.1, 31.8, 31.7, 23.7, 20.08, 14.0. MS (CI^+): m/z 389.13 (MH^+, 35.56), 388.12 (M^+, 21.41), 357.08 ([MH^+ – MeOH], 54.21), 230.97 ([MH^+ – $HN(CH_2)_3COO(CH_2)_3Me$], 68.66), 189.02 ([MH^+(^{37}Cl) – $C(S)NH(CH_2)_3COO(CH_2)_3Me$], 33.31), 187.02 ([$MH^+$ – $C(S)NH(CH_2)_3COO(CH_2)_3Me$], 100). HRMS: calcd. for $C_8H_{18}NO_2$ (MH^+, CI^+) 160.1338 found 160.1299.

5.1.76. 4-Amino-N-propylbutanamide, ***44e***

Compound **44e** was obtained as a white solid in quantitative yield from **43e** upon treatment with HCl/EtOAc [29]. No starting material was detected by TLC after 4.5 h of stirring, mp 48–49 °C ^{1}H NMR (300 MHz, MeOD) ppm δ 7.09 (br t, 1H), 3.22 (t, 2H, J = 7.2 Hz), 3.02 (t, 2H, J = 7.2 Hz), 2.53 (t, 2H, J = 7.2 Hz), 2.02 (quint, 2H, J = 7.2 Hz), 1.58 (sext, 2H, J = 7.2 Hz), 0.94 (t, 3H, J = 7.5 Hz). ^{13}C NMR (75 MHz, MeOD) ppm δ 175.6, 43.1, 40.1, 33.1, 24.7, 23.1, 11.7. MS (CI^+): m/z 145.14 (MH^+, 21.48), 128.11 ([MH^+ – NH_3], 100), 86.25 ([MH^+ – H_2N_2Me], 25.73). HRMS: calcd. for $C_7H_{17}N_2O$ (MH^+, CI^+) 145.1341 found 145.1352, calcd. for $C_7H_{14}NO$ 128.1075 found 128.1072.

5.1.77. S-Propyl 4-aminobutanethioate, ***44f***

Compound **44f** was obtained as a white solid in 89% yield from **43f** upon treatment with HCl/EtOAc [29]. No starting material was detected by TLC (3:1 hexane:EtOAc) after 3.5 h of stirring, mp 49–50 °C ^{1}H NMR (300 MHz, MeOD) ppm δ 3.02 (t, 2H, J = 7.5 Hz), 2.9 (t, 2H, J = 7.5 Hz), 2.78 (t, 2H, J = 7.2 Hz), 2.04 (quint, 2H, J = 7.2 Hz), 1.61 (sext, 2H, J = 7.5 Hz), 0.99 (t, 3H, J = 7.5 Hz). ^{13}C NMR (75 MHz, MeOD) ppm δ 199.7, 41.3, 39.9, 31.6, 24.2, 24.0, 13.6. MS (CI^+): m/z 162.09 (MH^+, 100), 145.07 ([MH^+ – NH_3], 34.65), 144.09 ([M^+ – NH_3], 37.13). HRMS: calcd. for $C_7H_{16}NOS$ (MH^+, CI^+) 162.0953 found 162.0927.

5.1.78. Methyl 4-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioamino)butanoate, ***45a***

Compound **45a**, obtained from **44a** and isothiocyanate **9a** (Procedure 6), was purified by column chromatography (3:1 hexane:EtOAc) and was isolated as a yellowish solid in 20% yield, mp 77 °C. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.38 (br t, 1H), 7.91 (br t, 1H), 7.54 (br t, 1H, J = 4.8 Hz), 7.05 (s, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.64 (sext, 2H, J = 6.6 Hz), 3.61 (s, 3H), 2.4 (t, 2H, J = 7.5 Hz), 1.89 (quint, 2H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 181.9, 173.8, 149.4, 146.7, 127.7, 118.2, 114.2, 110.8, 56.9, 51.6, 44.4, 31.6, 25.0. MS (CI^+): m/z 349.08 (MH^+(^{37}Cl), 10.74), 348.08 (M^+ (^{37}Cl), 14.61), 347.08 (MH^+, 32.55), 346.08 (M^+, 25.20), 317.06 ([MH^+(^{37}Cl) – MeOH], 30.86), 315.06 ([MH^+ – MeOH], 85.08), 231.00 ([MH^+(^{37}Cl) – H_3N_3COOMe], 37.03), 230.01 ([MH^+ – H_2N_3COOMe], 40.03), 229.00 ([MH^+-H_3N_3COOMe], 100), 187.05 ([MH^+ – $C(S)NH_3COOMe$], 59.1). HRMS: calcd. for $C_{14}H_{20}N_2O_4S^{35}Cl$ (MH^+, CI^+) 347.0832 found 347.0845, calcd. for $C_{14}H_{20}N_2O_4S^{37}Cl$ (MH^+, CI^+) 349.0803 found 349.0770.

5.1.79. Ethyl 4-(3-(4-chloro-2,5-dimethoxyphenyl)thioureido) butanoate, ***45b***

Compound **45b**, obtained from **44b** and isothiocyanate **9a** (Procedure 6), was purified by column chromatography (2:1 hexane:EtOAc) and was isolated as a yellowish oil in 59% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.39 (br t, 1H), 7.88 (br t, 1H), 7.53 (br t, 1H, J = 4.8 Hz), 7.04 (s, 1H), 4.07 (sext, 2H, J = 7.2 Hz), 3.83, 3.82 (two s, 3H), 3.65 (sext, 2H, J = 6.6 Hz), 2.38 (t, 2H, J = 7.2 Hz), 1.89 (quint, 2H, J = 7.2 Hz), 1.2 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 181.7, 173.4, 149.3, 146.7, 127.5, 118.2, 114.1, 110.7, 60.6, 56.9, 44.4, 31.9, 25.0, 14.5. MS (CI^+): m/z 363.10 (MH^+(^{37}Cl), 4.1), 361.10 (MH^+, 19.15), 329.08 ([MH^+ – MeOH], 69.46), 228.99 ([MH^+ – $H_3N(CH_2)_3COOCH_2Me$], 100), 189.03 ([MH^+(^{37}Cl) – $C(S)NH(CH_2)_3COOCH_2Me$], 17), 187.03 ([MH^+ – $C(S)NH(CH_2)_3COOCH_2Me$], 68.42). HRMS: calcd. for $C_{15}H_{21}N_2O_4S^{35}Cl$ (M^+, CI^+) 360.0911 found 360.0920, calcd. for $C_{15}H_{22}N_2O_4S^{35}Cl$ (MH^+, CI^+) 361.0989 found 361.0995, calcd. for $C_{15}H_{22}N_2O_4S^{37}Cl$ (MH^+, CI^+) 363.0959 found 363.0988.

5.1.80. Propyl 4-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioami)butanoate, ***45c***

Compound **45c**, obtained from amine hydrochloride **44c** and isothiocyanate **9a** (Procedure 6), was purified by column

chromatography (3:1 hexane:EtOAc) and was isolated as a yellow oil in 49% yield. ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 8.38 (br t, 1H), 7.92 (br t, 1H), 7.54 (br t, 1H, J = 4.6 Hz), 7.05 (s, 1H), 3.99 (t, 2H, J = 6.8 Hz), 3.83 (s, 6H), 3.65 (sext, 2H, J = 6.8 Hz), 2.4 (t, 2H, J = 7.4 Hz), 1.92 (quint, 2H, J = 7.4 Hz), 1.61 (sext, 2H, J = 6.8 Hz), 0.91 (t, 3H, J = 7.4 Hz). ^{13}C NMR (50 MHz, acetone-d_6) ppm δ 181.9, 173.5, 149.4, 146.7, 127.7, 118.2, 114.2, 110.7, 66.2, 56.9, 44.4, 31.9, 25.1, 22.6, 10.6. MS (CI$^+$): m/z 377.12 (MH$^+$(^{37}Cl), 33.83), 375.12 (MH$^+$, 87.86), 374.11 (M$^+$, 21.21), 232.00 ([MH$^+$(^{37}Cl) − $H_2N_3COO_2Me$], 29.84), 230.01 ([MH$^+$ − $H_2N_3COO_2Me$], 78.74), 228.10 ([MH$^+$ − $H_3N_3COO_2Me$], 49.73), 188.05 ([M$^+$(^{37}Cl) − C(S)NH_3COO_2Me], 38.41), 187.05 ([MH$^+$ − C(S)NH_3COO_2Me], 33.01). HRMS: calcd. for $C_{16}H_{24}N_2O_4S^{35}Cl$ (MH$^+$, CI$^+$) 375.1145 found 375.1187, $C_{16}H_{24}N_2O_4S^{37}Cl$ (MH$^+$, CI$^+$) 377.1116 found 377.1158.

5.1.81. Butyl 4-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioamino)butanoate, **45d**

Compound **45d**, obtained from **44d** and isothiocyanate **9a** (Procedure 6), was purified by column chromatography (3:1 hexane:EtOAc) and was isolated as a colorless oil in 56% yield. ^{1}H NMR (200 MHz, acetone-d_6) ppm δ 8.36 (br t, 1H), 7.93 (br t, 1H), 7.52 (br t, 1H, J = 4.8 Hz), 7.05 (s, 1H), 4.04 (t, 2H, J = 6.6 Hz), 3.84, 3.83 (two s, 3H), 3.66 (sext, 2H, J = 7.2 Hz), 2.4 (t, 2H, J = 7.4 Hz), 1.92 (quint, 2H, J = 7.2 Hz), 1.58 (quint, 2H, J = 6.6 Hz), 1.37 (sext, 2H, J = 7 Hz), 0.91 (t, 3H, J = 7.2 Hz). ^{13}C NMR (50 MHz, acetone-d_6) ppm δ 181.9, 173.4, 149.4, 146.7, 127.8, 118.2, 114.2, 110.8, 64.5, 57.0, 44.5, 32.0, 31.4, 25.1, 19.7, 13.9. MS (CI$^+$): m/z 389.13 (MH$^+$, 35.56), 388.12 (M$^+$, 21.41), 357.08 ([MH$^+$ − MeOH], 54.21), 230.97 ([MH$^+$ − HN_3COO_3Me], 68.66), 189.02 ([MH$^+$(^{37}Cl) − C(S)NH_3COO_3Me], 33.31), 187.02 ([MH$^+$ − C(S)NH_3COO_3Me], 100). HRMS: calcd. for $C_{17}H_{26}N_2O_4S^{35}Cl$ (MH$^+$, CI$^+$) 389.1302 found 389.1277, calcd. for $C_{17}H_{26}N_2O_4S^{37}Cl$ (MH$^+$, CI$^+$) 391.1272 found 391.1272.

5.1.82. 1-(3-(Propylcarbamoyl)propyl)-3-(4-chloro-2,5-dimethoxyphenyl)thiourea, **45e**

Compound **45e**, obtained from **44e** and isothiocyanate **9a** (Procedure 6), was purified by column chromatography (1:5 hexane:EtOAc) and was isolated as a colorless oil in 41% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.44 (br t, 1H), 8.00 (br t, 1H), 7.86 (br t, 1H, J = 4.8 Hz), 7.26 (br t, 1H), 7.04 (s, 1H), 3.83, 3.825 (two s, 3H), 3.63 (sext, 2H, J = 6.9 Hz), 3.12 (sext, 2H, J = 6.9 Hz), 2.28 (t, 2H, J = 7.2 Hz), 1.91 (quint, 2H, J = 6.9 Hz), 1.47 (sext, 2H, J = 6.9 Hz), 0.86 (t, 3H, J = 7.5 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 181.9, 173.2, 149.4, 146.5, 128.2, 117.9, 114.1, 110.7, 57.0, 45.0, 41.7, 34.1, 25.8, 23.5, 11.7. MS (CI$^+$): m/z 373.13 (M$^+$, 1.06), 232.00 ([MH$^+$(^{37}Cl) − $H_2N_3CONH_2Me$], 11.37), 230.10 ([MH$^+$ − HN_3CONH_2Me], 36.69), 228.99 ([MH$^+$ − $H_3N_3CONH_2Me$], 100). HRMS: calcd. for $C_{16}H_{24}N_3O_3S^{35}Cl$ (M$^+$, CI$^+$) 373.1227 found 373.1274.

5.1.83. S-Propyl 4-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioamino)butanethioate, **45f**

Compound **45f**, obtained from **44f** and isothiocyanate **9a** (Procedure 6), was purified by column chromatography (4:1 hexane:EtOAc) and was isolated as a colorless oil in 10% yield. ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.36 (br t, 1H), 7.93 (br t, 1H), 7.53 (br t, 1H), 7.06 (s, 1H), 3.84, 3.835 (two s, 3H), 3.66 (sext, 2H, J = 6.9 Hz), 2.84 (t, 2H, J = 7.2 Hz, S), 2.67 (t, 2H, J = 7.2 Hz), 1.94 (quint, 2H, J = 7.2 Hz), 1.56 (sext, 2H, J = 7.2 Hz), 0.94 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 198.9, 182.1, 149.5, 146.8, 127.9, 118.3, 114.3, 110.9, 57.0, 44.3, 41.9, 31.1, 25.7, 23.8, 13.5. MS (solid insertion, CI$^+$): m/z 391.09 (MH$^+$, 3.85), 390.08 (M$^+$, 6.61), 359.06 ([MH$^+$ − MeOH], 9.21), 316.05 ([MH$^+$ − S$(CH_2)_2$Me], 24.03), 314.05 ([M$^+$ − HS$(CH_2)_2$Me], 58.83), 285.03 ([M$^+$ − H$^+$ − COS$(CH_2)_2$Me], 33.83), 228.99 ([MH$^+$ − HN$(CH_2)_3$COS$(CH_2)_2$Me], 100). HRMS: calcd. for $C_{16}H_{24}N_2O_3S_2^{35}Cl$ (MH$^+$, CI$^+$) 391.0917 found 391.0897 calcd. for $C_{16}H_{24}N_2O_3S_2^{37}Cl$ (MH$^+$, CI$^+$) 393.0887 found 393.0876.

5.1.84. N-(4-Chloro-2,5-dimethoxyphenyl)-2-oxopyrrolidine-1-carbothioamide, **46f**

Compound **46f**, obtained from **44f** (Procedure 6) was purified by chromatography (2:1 hexane:EtOAc), together with **45f** as a major product. Compound **46f** was isolated as a white solid in 16% yield, mp 189 °C ^{1}H NMR (300 MHz, $CDCl_3$) ppm δ 12.91 (br t, 1H), 8.73 (s, 1H), 6.93 (s, 1H), 4.23 (t, 2H, J = 7.2 Hz), 3.87, 3.85 (two s, 3H), 2.79 (t, 2H, J = 8.1 Hz), 2.06 (quint, 2H, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) ppm δ 177.1, 176.5, 148.4, 144.9, 127.1, 118.2, 112.9, 107.9, 56.9, 56.8, 51.2, 34.7, 16.7. MS (CI$^+$): m/z 317.05 (MH$^+$(^{37}Cl), 15.14), 316.05 (M$^+$ (^{37}Cl), 26.8), 315.05 (MH$^+$, 40.58), 314.05 (M$^+$, 57.75), 282.96 ([MH$^+$ − MeOH], 100), 228.99 ([M$^+$ − $HNCO_3$], 66.22), 83.96 ([MH$^+$ − C_6H_{22}Cl(NHC(S)), 37.59). HRMS: calcd. for $C_{13}H_{16}N_2O_3S^{35}Cl$ (MH$^+$, CI$^+$) 315.057 found 315.0535, calcd. for $C_{13}H_{16}N_2O_3S^{37}Cl$ (MH$^+$, CI$^+$) 317.0541 found 317.0524.

5.1.85. Methyl 4-(3-(4-chloro-2,5-dimethoxyphenyl)ureido) butanoate, **46a**

Compound **46a** together with **45a** as a major product were obtained from **44a** and isothiocyanate **9a** (Procedure 6), and were separated by chromatography (3:1 hexane:EtOAc). Compound **46a** was isolated as a white-yellow solid, mp 92 °C ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.24 (s, 1H), 7.7 (br t, 1H), 6.93 (s, 1H), 6.49 (br t, 1H, J = 4.8 Hz), 3.81 (s, 3H), 3.8 (s, 3H), 3.61 (s, 3H), 3.26 (sext, 2H, J = 6.6 Hz), 2.38 (t, 2H, J = 7.5 Hz), 1.81 (quint, 2H, J = 7.5 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.9, 156.0, 150.0, 142.5, 130.6, 113.1, 104.6, 56.9, 56.8, 51.6, 39.7, 31.7, 26.3. MS (CI$^+$): m/z 333.11 (MH$^+$(^{37}Cl), 4.54), 332.10 (M$^+$(^{37}Cl), 16.68), 331.11 (MH$^+$, 12.86), 330.1 (M$^+$, 47.69), 299.08 ([MH$^+$ − MeOH], 13.05), 298.07 ([M$^+$ − MeOH], 23.79), 215.01 ([MH$^+$ − HN$(CH_2)_3$COOMe], 31.86), 212.10 ([MH$^+$ − H_3N$(CH_2)_3$COOMe], 100), 187.02 ([MH$^+$ − C(S)NH$(CH_2)_3$COOMe], 94.48). HRMS: calcd. for $C_{14}H_{19}N_2O_5^{35}Cl$ (M$^+$, CI$^+$) 330.0982 found 330.1, calcd. for $C_{14}H_{19}N_2O_5^{37}Cl$ (M$^+$, CI$^+$) 332.0953 found 332.0982.

5.1.86. Ethyl 4-(3-(4-chloro-2,5-dimethoxyphenyl)ureido) butanoate, **46b**

Compound **46b**, was obtained together with **45b** as a major product, from **44b** and isothiocyanate **9a** (Procedure 6), and were separated by chromatography (2:1 hexane:EtOAc). Compound **46b** was isolated as a white-yellow solid, mp 103 °C ^{1}H NMR (300 MHz, acetone-d_6) ppm δ 8.23 (s, 1H), 7.7 (br t, 1H), 6.93 (s, 1H), 6.48 (br t, 1H, J = 5.1 Hz), 4.08 (sext, 2H, J = 7.2 Hz), 3.81, 3.8 (two s, 3H), 3.27 (sext, 2H, J = 6.9 Hz), 2.36 (t, 2H, J = 7.2 Hz), 1.81 (quint, 2H, J = 6.9 Hz), 1.2 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, acetone-d_6) ppm δ 173.4, 155.9, 149.9, 142.5, 130.6, 113.1, 113.0, 104.5, 60.6, 56.9, 56.7, 39.6, 31.9, 26.3, 14.5. MS (CI$^+$): m/z 344.12 (M$^+$, 19.58), 299.107 ([MH$^+$ − EtOH], 3.82), 298.09 ([M$^+$ − EtOH], 8.7), 258.08 ([MH$^+$ − CH_2COOCH_2Me], 74.50), 213.03 ([M$^+$ − H$^+$ − HN$(CH_2)_3$COOCH$_2$Me], 88.92), 187.05 ([MH$^+$ − COHN$(CH_2)_3$COOCH$_2$Me], 44.48). HRMS: calcd. for $C_{20}H_{21}N_2O_5^{35}Cl$ 344.1139 found 344.1183.

5.2. Biology

5.2.1. Evaluation of the antiviral activity against HIV-1 pseudovirus infection

In the HIV-1 pseudovirus infection system, a productive single cycle infection by HIV-1 results in the expression of the GFP in the target cells that can be measured by flow cytometry. Infectious virions were prepared as described previously in detail by us [9]. The virions were produced by transfecting 293 T cells with three plasmids: pCMVΔ8.2Gagpol [61], which encodes for HIV-1 proteins (except for Env), pHRCMVGFP, which is transcribed to mRNA containing

encapsidation signal and the GFP coding sequences, and pVSV-G that supplies an envelope protein capacitated to infect a wide variety of cells. Chloroquine, to a final concentration of 25 μM, was added to the medium prior to transfection, and 48 h post transfection the supernatant was collected, filtered through 0.45 μM filter, equilibrated with 50 mM HEPES, pH 7.2 and stored at −80 °C. Virions were thawed at 37 °C and allowed to infect B lymphocytes (721.221 cells) in the presence of 5.6 μg/mL polybrene and specific inhibitor, each at a final concentration of 5 μM in 1% DMSO in RPMI medium. Between 48 and 72 h post infection, the cells were collected, fixed with 1% paraformaldehyde and analyzed by flow cytometry (BD biosciences) or fluorescent microscopy.

In the case of compound **45c**, dose–response curve (Fig. 3) for suppressing viral infectivity was calculated from the flow cytometry results and the corresponding IC_{50} value was calculated using the four-parameter equation as previously described [9].

5.3. Docking experiments

To explore the HIV-1 RT binding mode of selected active compounds, docking studies were performed using the CDocker program implemented in the Discovery Studio 2.5 package (Accelrys Inc.) [40]. CDocker randomly generates ligand seeds in the binding site. These initial structures are subjected to high-temperature molecular dynamics, where the van der Waals interactions are scaled down to allow rapid barrier crossings. Subsequently, further simulated annealing molecular dynamics followed by minimization steps yield the final pose. Specifically, the crystal structure of HIV-1 RT with bound rilpivirine (pdb code: 2ZD1) was used as a starting point, as rilpivirine is a neutral inhibitor similar to the compounds studied herein. The CHARMm force field was employed with Momany-Rone partial charges. The hydrogen atoms of the enzyme were added, using the Discovery Studio 2.5 interface and then minimized. The complete ligands were also geometry optimized. Initially, a sphere of radius 10 Å was superimposed on the active site, and each ligand was docked inside this sphere using 50 initial random conformations. These random conformations were subjected to random dynamics with 1000 steps and a time step of 1fs. The target temperature for the random conformation stage was 1000 K. The 25 top scoring poses for each ligand were chosen for further simulated annealing, minimization and inspection. The simulated annealing used 2000 heating steps to a temperature of 700 K followed by 5000 cooling steps to a final temperature of 300 K. The final minimization employed the complete force-field expression, while the final score of the docked poses used the CDocker score function [40].

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