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# Thiocarbamates as non-nucleoside HIV-1 reverse transcriptase inhibitors. Part 1: Parallel synthesis, molecular modelling and structure–activity relationship studies on *O*-[2-(hetero)arylethyl]-*N*-phenylthiocarbamates

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**Abstract**—In order to expand the structure–activity relationship (SAR) studies on Thiocarbamates (TCs), a recently discovered class of potent non-nucleoside HIV-1 reverse transcriptase inhibitors, 38 analogues of the lead *O*-[2-(2-pyridyl)ethyl]-*N*-phenylthiocarbamate **1** were prepared by parallel solution-phase synthesis. The SAR strategy was focused on the variation (mono- and disubstitution) of the *N*-phenyl ring and the replacement of the 2-pyridyl with 4-pyridyl, 2-thienyl and phenyl rings. The majority of the new TCs proved to prevent the wild-type HIV-1 multiplication in MT-4 cell culture and the most potent congeners displayed an $EC_{50}$ value of 100 nM. Two TCs were active also at micromolar concentrations against the Y181C- and/or K103N/Y181C-resistant mutants. Docking simulations helped to rationalize the SARs.


## 1. Introduction

Non-nucleoside inhibitors (NNRTIs) targeting the HIV-1-encoded reverse transcriptase (RT) have proved to be effective in treating the HIV infection and AIDS.[1,2] NNRTIs[3–15] bind to the non-nucleoside binding site (NNBS), an allosteric hydrophobic pocket located about 10 Å far from the polymerase active site. First generation NNRTIs (e.g. nevirapine, delavirdine, tivirapine[16] and loviride[17]) are effective against wild-type HIV-1 but show significantly lower potency against common NNRTI-resistant mutants. In contrast, second generation NNRTIs, such as efavirenz,[18] some thiocarboxanilide,[19] quinoxaline,[20] imidoylthiourea (ITU), diaryltriazine (DATA) and diarylpyrimidine (DAPY) derivatives[21,22] retain activity against variants carrying either single or double NNRTI resistance mutations. Despite their chemical diversity,[23] most of the first generation NNRTIs bind in a superimposable 'butterfly-like' mode, as assessed by X-ray crystallography.[24–31] On the basis of this binding mode, Wing I, Wing II and body-linker have been identified as modular segments susceptible of modification to afford new classes of NNRTI.[24] Wing I and Wing II generally contain aromatic rings that have π–π interactions with aromatic amino acids within the NNBS (Tyr181, Tyr188, Trp229 and Tyr318). The fact that cross-resistance extends to the whole NNRTI class calls for development of new agents capable of inhibiting clinically relevant NNRTI-resistant mutants.[32,33]

We have previously described a novel class of potent NNRTIs, i.e. *O*-(2-phthalimidoethyl)–*N*-arylthiocarbamates (C-TCs) and their corresponding ring-opened analogues (O-TCs) (Fig. 1a shows the leads C-TC 12 and O-TC 51).[34] In particular, the *N-para*-substituted-phenyl C-TCs proved to be potent inhibitors of the multiplication of wild type HIV-1, significantly active against the Y181C mutant, but ineffective against the

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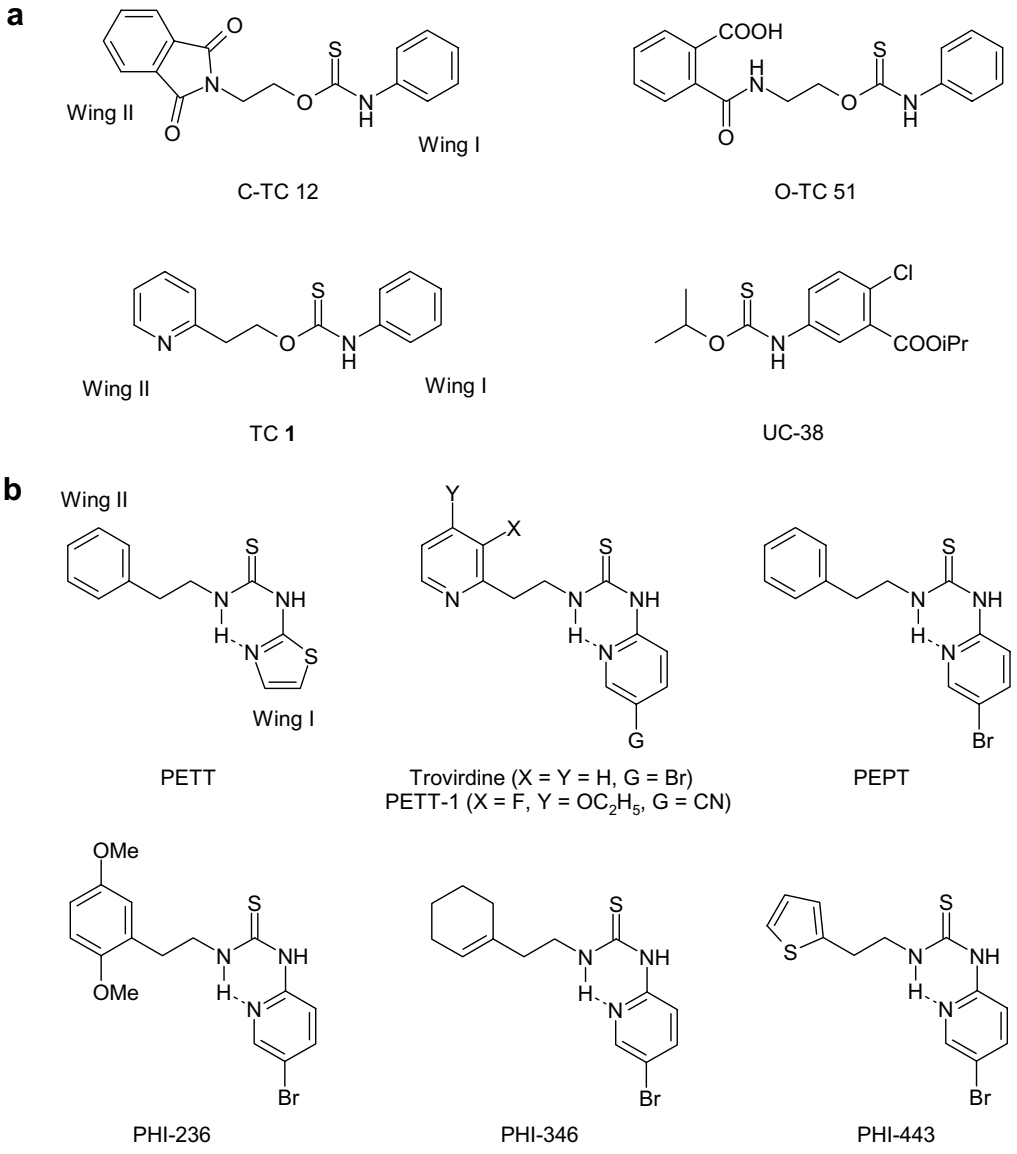


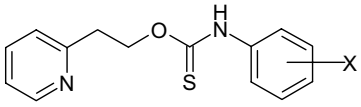

**Figure 1.** Chemical structure of C-TC 12,[34] O-TC 51,[34] lead compound **1**, UC-38, PETT, Trovirdine, PETT-1, PEPT, PHI-236, PHI-346 and PHI-443. (a) TCs and (b) PETT derivatives.

K103R- and K103N/Y181C-resistant mutants. Thiocarbamates (TCs) are isosterically related to *N*-phenethyl-*N*′-thiazolylthiourea (PETT) NNRTIs (Fig. 1b).[30,35–49] In PETT derivatives, Wing I is represented by an intramolecular H-bonded heterocyclic ring, generally a 2-aminothiazolyl group or a 5-bromopyridyl group (Fig. 1b). Wing II often consists of a 2-pyridyl, 2-thienyl, 1-cycloesenyl or phenyl ring, separated from the thiourea function by an ethyl linker. Docking studies on C-TCs suggested that also these inhibitors assume a 'butterfly-like' bioactive conformation.[34] In C-TCs, Wing I is a phenyl and Wing II is a phthalimide scaffold separated from the thiocarbamate function by an ethyl linker (Fig. 1a). In consequence of the isosteric NH/O replacement, TCs have no internal H-bond, which is instead essential for PETT anti-HIV activity.[35] The lack of this conformational constrain makes TCs more flexible than thiourea NNRTIs. In this regard, it was speculated that a greater conformational freedom of inhibitors might be a useful design feature for reducing drug resistance.[21,22,50,51] In the field of NNRTIs, structure-based design of analogues of Trovirdine and PETT is an attractive research area for medicinal chemists, as documented by papers recently published.[34,42,44–49,52] In particular, some of PETT derivatives, such as PHI-236, PHI-346 and PHI-443 (Fig. 1b),[41] are under development as vaginal and rectal microbicides for curbing mucosal HIV transmission via semen.[46,47] In addition, *N*-phenethyl-*N*′-5-bromopyridylthiourea (PEPT) (Fig. 1b) analogues possess dual function as anti-HIV agents with antioxidant properties.[43] Notably, the thiocarbamate UC-38 (Fig. 1a) was selected as an anti HIV-1 agent in the early 1990s for preclinical development.[53]

In order to further explore the potential of TCs as anti-HIV-1 agents, expand the structure–activity relationship (SAR) studies on this class and investigate the effects on antiretroviral activity due to the replacement of the

phthalimide moiety in C-TCs, we wanted to prepare a new series of TCs. Therefore, we initially designed, synthesized and tested *O*-[2-(2-pyridyl)ethyl]-*N*-phenylthiocarbamate **1** (Fig. 1a), which shares the heteroaryl-ethyl portion with Trovirdine and the *N*-phenylthiocarbamic moiety with C-TC 12. TC **1** proved to be active with an $EC_{50}$ value of 7.4 μM and was selected as a lead compound. Since the lead structure can incorporate a large number of diversity points and a variety of substitution patterns, a simple, parallel, convergent solution-phase method was set up for rapid analogueing. We focused our SAR strategy on structural modifications of **1** by keeping constant the 2-pyridylethyl substructure and varying the *N*-phenyl portion (**1**–**28**, Tables 1 and 2). These modifications concern the monosubstitution with various groups at position *ortho*, *meta* and *para* (**1**–**20**) and the disubstitution with equal (**21**–**25**) or different (**26**–**28**) groups. Then, we synthesized a number of analogues with the 2-pyridyl replaced by a phenyl (**29**–**33**), 2-thienyl (**34**), or 4-pyridyl ring (**35**–**38**) (Table 3), and the *N*-phenyl unsubstituted or monosubstituted at *para* position with functional groups with various electronic, steric and lipophilic properties ($CH_3$, Cl, $NO_2$ and $OCH_3$).

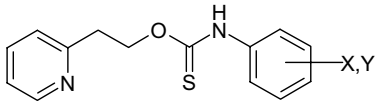
**Table 1.** Cytotoxicity and anti HIV-1 activity of *O*-[2-(2-pyridyl)ethyl]*N*-monosubstituted-phenylthiocarbamates **1–20**[a]



| Compound | X | $CC_{50}$[b] | $EC_{50}$[c] | SI[d] |
|---|---|---|---|---|
| **1** | H | >100 | 7.4 | >14 |
| **2** | 2-$CH_3$ | >100 | 69 | >1.4 |
| **3** | 2-Cl | >100 | 43 | >2.3 |
| **4** | 3-$CH_3$ | >100 | 7.7 | >13 |
| **5** | 3-F | ⩾100 | 1.1 | ⩾91 |
| **6** | 3-Cl | 47 | 1.4 | 34 |
| **7** | 3-$NO_2$ | 100 | 1.6 | 63 |
| **8** | 4-$CH_3$ | 75 | 1.7 | 44 |
| **9** | 4-$C_2H_5$ | 44 | 10 | 4.4 |
| **10** | 4-$CH(CH_3)_2$ | 45 | >45 | <1.0 |
| **11** | 4-$CF_3$ | 50 | 13 | 3.8 |
| **12** | 4-CN | 45 | 0.2 | 225 |
| **13** | 4-$COCH_3$ | ⩾100 | >100 | — |
| **14** | 4-F | >100 | 1.2 | >83 |
| **15** | 4-Cl | 52 | 0.1 | 520 |
| **16** | 4-Br | 37 | 0.2 | 185 |
| **17** | 4-I | 34 | 0.5 | 68 |
| **18** | 4-$N(CH_3)_2$ | 60 | >60 | <1.0 |
| **19** | 4-$NO_2$ | >100 | 0.7 | >143 |
| **20** | 4-$OCH_3$ | 48 | 5.6 | 8.6 |
| Trovirdine | | 60 | 0.02 | 3000 |

[a] Data mean values for three separate experiments. Variation among triplicate samples was less than 10%.
[b] Compound concentration (μM) required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[c] Compound concentration (μM) required to achieve 50% protection of MT-4 cell from HIV-1 induced cytopathogenicity, as determined by the MTT method.
[d] Selectivity index: $CC_{50}/EC_{50}$ ratio.

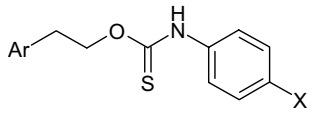
**Table 2.** Cytotoxicity and anti HIV-1 activity of *O*-[2-(2-pyridyl)ethyl]*N*-disubstituted-phenylthiocarbamates **21**–**28**[a]



| Compound | X,Y | $CC_{50}$[b] | $EC_{50}$[c] | SI[d] |
|---|---|---|---|---|
| **21** | 2,5-F,F | >100 | 9.0 | >11 |
| **22** | 2,6-F,F | >100 | 38 | >2.6 |
| **23** | 3,5-F,F | >100 | 9.0 | >11 |
| **24** | 2,4-Cl,Cl | >100 | 2.9 | >34 |
| **25** | 3,4-Cl,Cl | 40 | 0.2 | 200 |
| **26** | 3-Cl,4-$CH_3$ | 59 | 0.7 | 84 |
| **27** | 4-Cl,3-$CF_3$ | 42 | 5.5 | 7.6 |
| **28** | 4-Cl,3-$NO_2$ | 41 | 0.1 | 410 |
| Trovirdine | | 60 | 0.02 | 3000 |

[a,b,c,d] See legend to Table 1.

**Table 3.** Cytotoxicity and anti HIV-1 activity of *O*-[2-(4-pyridyl)- or -(2-thienyl)- or (phenyl)-ethyl]*N*-phenylthiocarbamates **29**–**38**[a]



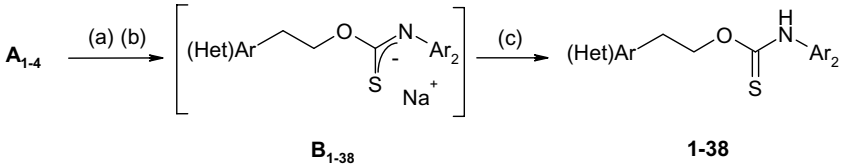
| Compound | Ar | X | $CC_{50}$[b] | $EC_{50}$[c] | SI[d] |
|---|---|---|---|---|---|
| **29** | phenyl | H | 100 | 6.0 | 17 |
| **30** | phenyl | $CH_3$ | >100 | 4.3 | >23 |
| **31** | phenyl | Cl | >100 | 0.5 | >200 |
| **32** | phenyl | $NO_2$ | >100 | 0.9 | >111 |
| **33** | phenyl | $OCH_3$ | 36 | 17 | 2.1 |
| **34** | 2-thienyl | H | >100 | 14 | >7.1 |
| **35** | 4-pyridyl | $CH_3$ | >100 | 63 | >1.6 |
| **36** | 4-pyridyl | Cl | 70 | >70 | <1.0 |
| **37** | 4-pyridyl | $NO_2$ | >100 | >100 | — |
| **38** | 4-pyridyl | $OCH_3$ | 59 | >59 | <1.0 |
| Trovirdine | | | 60 | 0.02 | 3000 |

[a,b,c,d] See legend to Table 1.

## 2. Chemistry

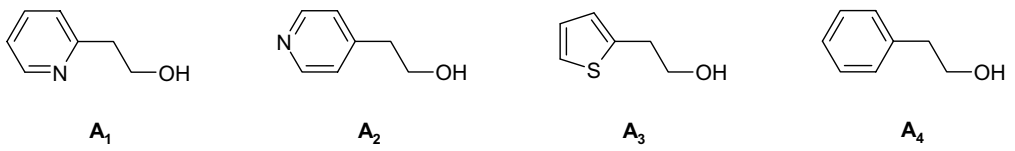
In the modern drug discovery process,[54] and particularly in lead identification and optimisation, parallel synthesis plays an important role as it allows to produce a (large) number of compounds in short times, by using simple and rapid purification methods. Recently, solution-phase chemistry has largely supplanted solid-phase chemistry as the method of choice for parallel synthesis of small organic molecules.[55]

TC **1**–**38** were prepared by a convergent solution-phase parallel synthesis (Scheme 1), by using ordered arrays of spatially separated reaction vessels (Carousel reaction station™). This two-step procedure combines two building blocks (alcohols and isothiocyanates, functionalised according to the planned SARs). Starting alcohols $\mathbf{A_{1-4}}$ (Fig. 2a) were first transformed into their corresponding salts in the presence of sodium hydride in dry THF (emerged during the synthetic method set-up as a better solvent than DMF and pyridine). Then, the alcoholates




**Scheme 1.** Reagents and conditions: (a) NaH, dry THF, rt, 30 min; (b) $Ar_2$—N=C=S ($\mathbf{I}_{1-28}$), (for **2** and **4**, pre-heating at 60 °C for 2 h), rt, 18 h; (c) $NH_4Cl_{(aq)}$. For the structure list of alcohols $\mathbf{A}_{1-4}$ and isothiocyanates $\mathbf{I}_{1-28}$, see Figure 2.

**a**




$\mathbf{A}_1$ $\mathbf{A}_2$ $\mathbf{A}_3$ $\mathbf{A}_4$

**b**

| | $Ar_2$ | | $Ar_2$ |
|---|---|---|---|
| $\mathbf{I}_1$ | phenyl | $\mathbf{I}_{15}$ | 4-chlorophenyl |
| $\mathbf{I}_2$ | 2-tolyl | $\mathbf{I}_{16}$ | 4-bromophenyl |
| $\mathbf{I}_3$ | 2-chlorophenyl | $\mathbf{I}_{17}$ | 4-iodophenyl |
| $\mathbf{I}_4$ | 3-tolyl | $\mathbf{I}_{18}$ | 4-dimethylaminophenyl |
| $\mathbf{I}_5$ | 3-fluorophenyl | $\mathbf{I}_{19}$ | 4-nitrophenyl |
| $\mathbf{I}_6$ | 3-chlorophenyl | $\mathbf{I}_{20}$ | 4-methoxyphenyl |
| $\mathbf{I}_7$ | 3-nitrophenyl | $\mathbf{I}_{21}$ | 2,5-difluorophenyl |
| $\mathbf{I}_8$ | 4-tolyl | $\mathbf{I}_{22}$ | 2,6-difluorophenyl |
| $\mathbf{I}_9$ | 4-ethylphenyl | $\mathbf{I}_{23}$ | 3,5-difluorophenyl |
| $\mathbf{I}_{10}$ | 4-*i*-propylphenyl | $\mathbf{I}_{24}$ | 2,4-dichlorophenyl |
| $\mathbf{I}_{11}$ | 4-trifluoromethylphenyl | $\mathbf{I}_{25}$ | 3,4-dichlorophenyl |
| $\mathbf{I}_{12}$ | 4-cyanophenyl | $\mathbf{I}_{26}$ | 3-chloro-4-methylphenyl |
| $\mathbf{I}_{13}$ | 4-acetylphenyl | $\mathbf{I}_{27}$ | 4-chloro-3-trifluoromethylphenyl |
| $\mathbf{I}_{14}$ | 4-fluorophenyl | $\mathbf{I}_{28}$ | 4-chloro-3-nitrophenyl |

**Figure 2.** Building blocks used. (a) Alcohols $\mathbf{A}_{1-4}$ and (b) isothiocyanates $Ar_2NCS$ ($\mathbf{I}_{1-28}$).

($\mathbf{B}_{1-38}$) condensed in situ with the suitable isothiocyanate ($\mathbf{I}_{1-28}$, Fig. 2b) to give the corresponding thiocarbamate sodium salts, which were converted into the desired products by treatment with a solution of $NH_4Cl$ in water. The work-up simply required filtrations or extractions and the final products were purified by crystallization (only **2** and **4** were purified by chromatography). The yields (not optimized) ranged from 15% to 96%.

## 3. Biological results and discussion

The antiretroviral activity of TC **1**–**38** was evaluated in MT-4 cell-based assays by assessing the reduction of the HIV-1 induced cytopathogenicity. The results are expressed as $EC_{50}$ values. In parallel with antiretroviral activity, the TC-induced cytotoxicity was evaluated in mock-infected MT-4 cells and the results are expressed as $CC_{50}$ values. Trovirdine was used as the reference compound (Tables 1–3). The most potent derivatives were also tested against the clinically relevant K103R-, Y181C- and K103N/Y181C-resistant mutants,[56,57] employing Efavirenz as reference molecule (Table 4).

Most of the new TCs resulted to prevent the wild-type HIV-1 multiplication and 10 out of 38 turned out to be submicromolar inhibitors. Notably, PETT derivatives possessing a phenyl ring as Wing I were almost inactive,[35] as they could not assume the 'high-activity rigid conformation', because of the lack of the intramolecular H bond (Fig. 1b). This evidence points out a substantial difference between the TC and PETT series.

**Table 4.** Anti HIV-1 activity of **7** and **16** against Y181C and K103N/Y181C resistant mutants

| Compound | $EC_{50}$ (μM)[a] | |
|---|---|---|
| | Y181C | K103N/Y181C |
| **7** | 87 | 91 |
| **16** | 20 | n.a.[b] |
| Efavirenz | 0.01 | 0.04 |

[a] See Footnote 'c' of legend to Table 1.
[b] Not active.

Congeners **1**–**20** (Table 1) bear *N*-phenyl ring *ortho*, *meta* and *para* substituents with various electronic (inductive and/or mesomeric), lipophilic and steric properties. The *ortho* derivatives **2** and **3** showed low activity ($EC_{50}$ = 69 and 43 μM, respectively). The *meta* analogues (**4**–**7**) proved to be active in the low micromolar concentration range ($EC_{50}$ = 1.1–7.7 μM). Most of the *para*-substituted TCs (**8**–**20**) showed $EC_{50}$ values in the 0.1–13 μM concentration range, except for 4-isopropyl **10**, 4-acetyl **13** and 4-dimethylamino **18** (the last compound was synthesized to enhance water solubility of TCs), which were inactive. The most potent derivatives ($EC_{50}$ = 0.1–0.7 μM) were **12** (4-cyano), **15** (4-chloro), **16** (4-bromo), **17** (4-iodo) and **19** (4-nitro), which were

respectively 37-, 74-, 37-, 15- and 11-fold more active than the lead compound **1**. As previously observed for C-TCs,[34] the activity was strongly affected by the substitution pattern of the *N*-phenyl ring with the following potency trend: *para* > *meta* > *ortho*, as clearly exemplified by positional isomers **2**, **4**, **8** (2-, 3-, 4-tolyl); **3**, **6**, **15** (2-, 3-, 4-chloro) and **7**, **19** (3-, 4-nitro). However, differently from C-TCs,[34] also the electronic properties of the substituents seemed to affect activity. In general, the analogues bearing an electron-withdrawing group were more potent than the congeners with an electron-donating group in the same position, as it appears comparing **3** (2-chloro) with **2** (2-methyl); **5** (3-fluoro), **6** (3-chloro) and **7** (3-nitro) with **4** (3-methyl); **12** (4-cyano), **14** (4-fluoro), **15** (4-chloro), **16** (4-bromo), **17** (4-iodo) and **19** (4-nitro) with **8** (4-methyl), **9** (4-ethyl), **10** (4-isopropyl), **18** (4-dimethylamino) and **20** (4-methoxy). Regarding the size of the *para* substituents, data in Table 1 indicate that activity decreases with the steric demand of the substituents. Thus, the potency is in the order chloro **15** > bromo **16** > iodo **17** and methyl **8** > ethyl **9** > isopropyl **10**, the last compound being inactive like the other analogues bearing a sterically demanding group **13** (acetyl) and **18** (dimethylamino). Notably, the potency order of the *para*-halo-substituted derivatives of **1** is inverse when compared to the corresponding analogues of C-TC 12.[34]

Given the positive results obtained with the above-mentioned *para*-halo-substituted TCs, we wanted to evaluate the effects of the disubstitution with halogen atoms (**21**–**25**) and the combination of halogens with electron-donating (**26**) or electron-withdrawing (**27** and **28**) groups (Table 2). All the analogues synthesized were active ($EC_{50}$ = 0.1–38 μM). The most potent compounds shared the 3,4-disubstitution pattern, and out of them, TC **28** (4-chloro and 3-nitro) proved to be as active as **15** (4-chloro) ($EC_{50}$ = 0.1 μM), and more potent than **25** (3,4-dichloro) and **26** (3-chloro, 4-methyl) which displayed sub-micromolar activities ($EC_{50}$ = 0.2 and 0.7 μM, respectively). The presence of a trifluoromethyl group at position 3 (**27**) or a chlorine atom at position 2 (**24**) of the 4-chloro-phenyl moiety caused a 55- or a 29-fold decrease in potency, respectively. The difluoro-TCs (**21**–**23**) showed activities in the micromolar concentration range ($EC_{50}$ = 9–38 μM), and in particular **21** (2,5-difluoro) and **23** (3,5-difluoro) were only slightly less active than the lead compound **1**.

Successively, to evaluate the effects of the replacement of the 2-pyridyl of the lead **1** with other (hetero)aryl groups, TC **29**–**38** (Table 3) were prepared. The *N*-phenyl was unsubstituted or monosubstituted at the *para* position (according to the previous results) with groups with various electronic, steric and lipophilic properties. The 2-phenylethyl derivatives (**29**–**33**) were similarly or slightly less active than the corresponding 2-(2-pyridyl)ethyl TCs (compare **29** with **1**, **30** with **8**, **31** with **15**, **32** with **19** and **33** with **20**), and particularly the congeners bearing an electron-withdrawing group (**31**: 4-chloro; **32**: 4-nitro) showed sub-micromolar activity ($EC_{50}$ = 0.5 and 0.9 μM, respectively). On the contrary, the replacement of the 2-pyridyl ring with a 4-pyridyl (**35**–**38**) caused a decrease or loss in activity (compare **35** with **8**; **36** with **15**; **37** with **19**; **38** with **20**), while with a 2-thienyl led to the potency halving (**34** vs **1**). This contrasted with the SARs of PETT derivatives, according to which the replacement of the 2-pyridyl with a 2-thienyl ring diminished the activity only slightly,[41c] and the replacement with a phenyl caused a more significant potency decrease.[35,36] Conversely, the drop in activity due to the pyridine nitrogen shift to the 4-position was in accordance with the PETT SARs.[35]

The activity of TC **19** was confirmed also by means of the determination of the dose required to reduce HIV-1 p24 antigen levels by 90% in virus infected C8166 cultures ($EC_{90}$ = 0.8μM) in comparison with Trovirdine ($EC_{90}$ = 0.015 μM). In cell-based assays, the Y181C, K103R and K103N + Y181C mutated strains proved to be unsusceptible to the TCs, with the exception of **7** and **16** (Table 4), which turned out to be weakly active against Y181C- and/or K103N + Y181C-resistant mutants.

All compounds, except the 2-pyridyl derivatives **10** and **18** and the 4-pyridyl derivatives **36** and **38**, showed values of $CC_{50}$ higher than $EC_{50}$ ($CC_{50}$ in many cases superior to 100 μM).

## 4. Molecular modeling

To rationalize the most relevant SARs, computational studies were performed to construct a docking model of pyridylethyl- and phenylethyl-TCs bound into the HIV-1 RT NNBS. Briefly, TC **1**, **12**, **15**, **19**, **25**, **28**, **29**, **31**, **32** and **36** were docked (Autodock 3.05) into the NNBS using the X-ray coordinates of RT–PETT-1 complex (PDB code 1DTQ)[30] as template structure. The resulting RT–TC complexes were energy-minimized by a combined protocol of simulated annealing and Powell minimization. As shown in Figure 3a, the RT-**1** complex is stabilized by a hydrogen bond between the thiocarbamic NH group and the Lys101 main chain carbonyl (the same hydrogen bond was present in the complexes between RT and C-TCs[34] and between RT and PETT derivatives[38]) and van der Waals contacts between the thiocarbonyl sulfur atom and the Lys101 backbone NH group. Moreover, the 2-pyridyl ring (positioned at the 'top' of the NNRTI pocket) establishes π–π interactions with Tyr181 and its nitrogen atom is engaged in a polar interaction with the carboxyl group of Glu138(p51 subunit). The *N*-phenyl is involved in hydrophobic contacts with the side chain of Leu100, Lys103, Val106, Pro236 and Tyr318. Figure 4 shows that the bioactive conformation of **1** resembles the 'butterfly-like' conformation of C-TCs and PETT derivatives, with the 2-pyridyl and phenyl rings representing Wing 2 and Wing 1, respectively (Fig. 1a).

Docking simulations suggest that the increase in activity of the *N*-*para*-substituted-phenyl derivatives **15** and **19** compared to **1** is due to the further stabilizing effect

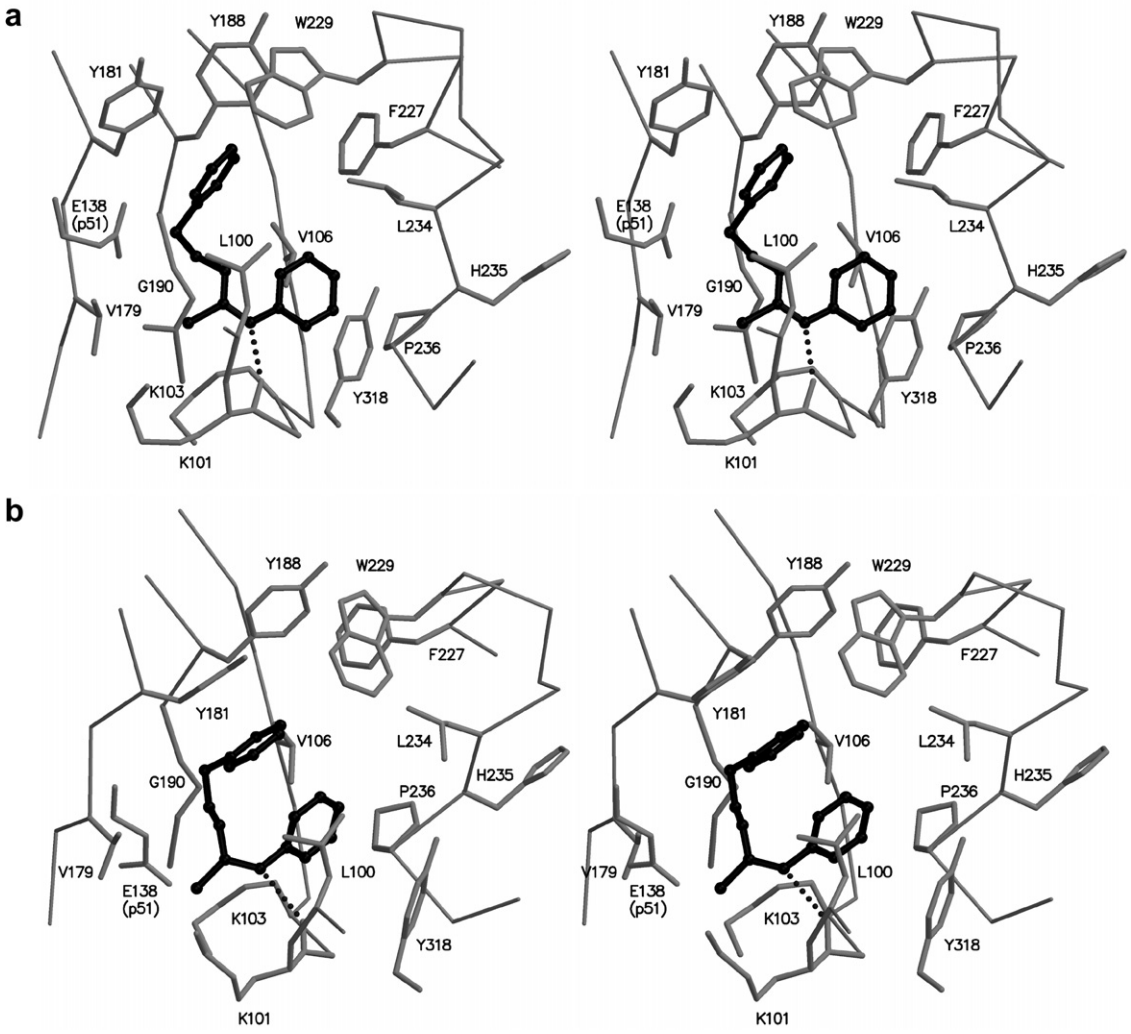



**Figure 3.** Stereoview showing the position and orientation of TC **1** (a) and **29** (b). The ligands are represented as black balls-and-sticks, while the residues lining RT non-nucleoside binding site are shown as gray sticks. Hydrogen bonds are depicted as dotted lines. The drawing was realized by the programmes MolScript[62] and Raster 3D.[63]

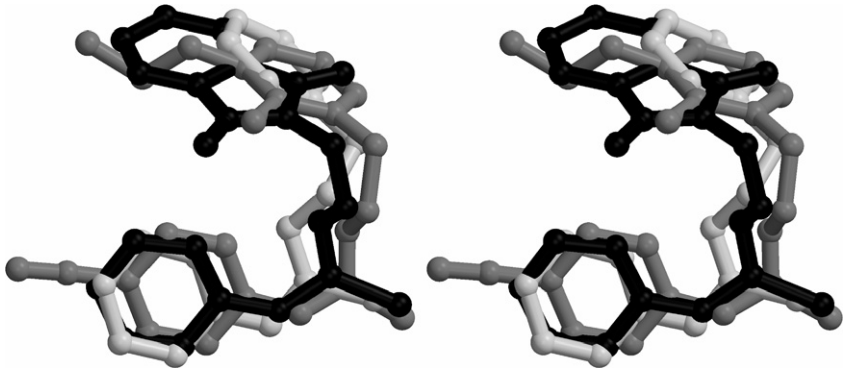


**Figure 4.** Stereodiagram showing superposition of TC **1**, C-TC 12[34] and PETT-1 (Fig. 1) within the NNBS. The relative positions and conformations for TC **1** (model structure, white ball-and-stick), C-TC 12 (model structure, black ball-and-stick) and PETT-1 (X-ray structure, gray ball-and-stick) are shown in their respective complexes with RT. The structures were superimposed on the basis of the amino acid residues surrounding the RT pocket, as described by Ren et al.[30] Despite the lack of the internal H-bond typical of PETT derivatives, TC **1** and C-TC 12 assume the 'butterfly-like' bioactive conformation, strongly mimicking the RT-bound PETT-1 conformation and orientation. The programmes MolScript[62] and Raster 3D[63] were used to draw the Figure.

of the chlorine atom (**15**) or the nitro group (**19**), which make extensive interactions with Leu234, His235 and Pro236. In **19** the nitro group establishes also contacts with Phe227 and a polar interaction with the Pro236

nitrogen. The cyano group of **12** compensates the lack of interactions with Leu234, making a number of contacts with Val106 and Phe227. The higher potency of **28** in comparison with **1** would be due to the formation of a hydrogen bond between the *meta*-nitro group and the Tyr318 hydroxyl, and to hydrophobic contacts involving the *para*-chlorine atom, Val106 and Phe227. In the 3,4-dichloro-derivative **25**, the chlorine atoms make extensive interactions with Val106, Lys103 and Pro236. The shift of the polar nitrogen atom of the pyridyl ring from position 2 to 4 (**36**) causes loss in activity because the nitrogen atom can establish neither the polar interaction with Glu138(p51) nor hydrophobic contacts with the ring plane of the Trp229 side-chain.

In the modelled RT-**29** complex (Fig. 3b), the phenyl replacing the 2-pyridyl establishes hydrophobic contacts with Tyr181 and Tyr188. The hydrogen bond between the thiocarbamic NH group and the Lys101 main chain carbonyl is conserved. The ethyl linker is involved in hydrophobic contacts with the Val179 and Gly190 main chains, while the *N*-phenyl establishes van der Waals interactions with the Lys101 carbonyl oxygen and hydrophobic contacts with the side chains of Leu100, Lys103, Val106 and Pro236. TC **29** is able to keep the same level of antiviral activity of **1** in spite of the lack of the polar interaction with Glu138(p51) and of the π–π interactions, presumably because these are compensated by the number of hydrophobic contacts in which **29** is engaged. The increase in activity of **31** and **32** compared to **29** would be due to the further stabilizing effect of a number of van der Waals contacts between the thiocarbonyl sulfur atom and the NH group of the Lys101 backbone. In addition, in the RT-**31** complex the phenyl of the phenylethyl moiety establishes π–π stacking interactions with Tyr181 and the chlorine atom makes van der Waals contacts with Phe227 and Leu234. In the RT-**32** complex, the π–π stacking are missing, but the nitro group is involved in van der Waals interactions with Val106, Phe227, Leu234, His235, Pro236 and Tyr318.

## 5. Conclusions

Our SAR expansion strategy has highlighted that the replacement of the phthalimide with a 2-pyridyl or a phenyl ring led to TCs with anti-HIV-1 activity in the micro- and sub-micromolar concentration range. The most potent TCs **15** and **28** displayed an $EC_{50}$ value of 100 nM. The *N*-phenyl *para*-substitution with electron-withdrawing groups was found to be beneficial. TC **7** and **16** also resulted weakly effective against Y181C and/or K103N + Y181C mutant strains. However, the easy synthetic accessibility of TCs prompted us to continue in the design and synthesis of new molecules with the aim at identifying additional activity parameters in the TC series and improving potency and resistance profile. Further SAR studies on *O*-(2-phenylethyl)-*N*-arylthiocarbamates, keeping constant the *N*-phenyl substitution patterns emerged as the best in the present work, will be the object of the following paper.

## 6. Experimental

### 6.1. Chemistry

**6.1.1. General.** All chemicals were purchased by Chiminord and Aldrich Chemical, Milan (Italy). Solvents were reagent grade. THF was distilled in the presence of sodium. Unless otherwise stated, all commercial reagents were used without further purification. Organic solutions were dried over anhydrous sodium sulphate.

Thin layer chromatography (TLC) system for routine monitoring the course of reactions and confirming the purity of analytical samples employed aluminium-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 $F_{254}$): $CHCl_3$ or $CHCl_3$/methanol or petroleum ether/ethyl acetate were used as developing solvents and detection of spots was made by UV light and/or by iodine vapours. Merck silica gel, 230–400 mesh, was used for chromatography.

The parallel solution-phase chemistry was performed by using a Carousel Reaction Station™ (Radleys Discovery Technologies, Italian distributor: StepBio, Bologna). The evaporation of solutions in parallel fashion was performed with an Evaposel™ apparatus (Radleys Discovery Technologies, Italian distributor: StepBio, Bologna) operating at reduced pressure of about 15–20 Torr. Yields were not optimized. Melting points were determined on a Fisher–Johns apparatus and are uncorrected.

IR spectra were recorded on a Perkin-Elmer 398 spectrometer as KBr discs. $^1$H NMR spectra were recorded in $CDCl_3$ or DMSO-$d_6$ on a Varian Gemini 200 instrument. Chemical shifts were reported in $\delta$ (ppm) units relative to the internal standard tetramethylsilane, and the splitting patterns were described as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), m (multiplet) and br s (broad singlet). The first order values reported for coupling constants *J* were given in Hertz. Elemental analyses were performed by an EA1110 Elemental Analyser (Fison-Instruments, Milan) and were within ±0.4% of the theoretical values. The synthesis of Trovirdine was accomplished according to the published procedure.[35]

**6.1.2. Parallel synthesis of *O*-[2-(2-pyridyl)ethyl]/*N*-phenylthiocarbamates 1–28; *O*-(2-phenylethyl)*N*-phenylthiocarbamates 29–33; *O*-[2-(2-thienyl)ethyl]/*N*-phenylthiocarbamate 34 and *O*-[2-(4-pyridyl)ethyl]/*N*-phenylthiocarbamates 35–38.** Sodium hydride (60%) dispersion in mineral oil (0.40 g, 10 mmol) was added in a single portion at rt to each numbered reaction tube of a 12-Carousel Reaction Station™, containing a stirred solution of the starting alcohol $\mathbf{A}_{1-4}$ (10 mmol) in dry THF (30 mL). After stirring for 30 min, the proper isothiocyanate $\mathbf{I}_{1-28}$ (10 mmol) was added to each reaction mixture, which was then stirred for 18 h at rt (for **2** and **4**, pre-heating at 60 °C for 2 h). Two different types of work-up were carried out. Work-up (i) (**1**–**6**, **8**–**21**, **25**, **28**, **29** and **34**): after parallel evaporation of THF in vacuo using an Evaposel™ apparatus, a solution of $NH_4Cl$ (1 g in

40 mL of water) was added into each tube. The contents of the tubes were then transferred into a set of separating funnels. Further solution of $NH_4Cl$ (3 g in 120 mL of water) was added into each funnel. After parallel extraction with diethyl ether ($CH_2Cl_2$ for **13**), the combined extracts of each reaction were washed with water, dried over anhydrous $Na_2SO_4$ and filtered in parallel through pads of Florisil (diameter 5 × 2 cm) by an in-house device. Evaporation in parallel under reduced pressure using an Evaposel™ apparatus gave residues which were purified by crystallization from the suitable solvents or solvent mixtures. For **2** and **4**, the residue was purified by chromatography (eluents: petroleum ether/ethyl acetate 1:1 for **2** and $CH_2Cl_2$/methanol 98:2 for **4**). Work-up (ii) (**7**, **22**–**24**, **26**, **27**, **30**–**33** and **35**–**38**): after parallel evaporation of THF in vacuo using an Evaposel™ apparatus, a solution of $NH_4Cl$ (1 g in 40 mL of water) was added into each tube. The contents of the tubes were then transferred into a set of beakers. Further solution of $NH_4Cl$ (3 g in 120 mL of water) was added into each beaker. The precipitates obtained were filtered off in parallel by an in-house device and dissolved in $CH_2Cl_2$ (diethyl ether for **22**, **24**, **26** and **27**). The solutions were washed with water, dried over anhydrous $Na_2SO_4$ and filtered in parallel through pads of Florisil (diameter 5 × 2 cm) by an in-house device. Evaporation in parallel under reduced pressure using an Evaposel™ apparatus gave residues which were purified by crystallization from the suitable solvents or solvent mixtures.

**6.1.2.1. *O*-[2-(2-pyridyl)ethyl]*N*-phenylthiocarbamate (1).** Mp 168–170 °C; yield: 68% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3220. $^1H$ NMR ($CDCl_3$) $\delta$: 3.26 (t, $J$ = 6.6 Hz, 2H, $CH_2Py$), 4.98 (t, $J$ = 6.6 Hz, 2H, $CH_2O$), 6.86–8.77 (m, 9H, arom. H), 9.15 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{14}N_2OS$: C, 65.09; H, 5.46; N, 10.84; S, 12.41. Found: C, 64.87; H, 5.46; N, 10.71; S, 12.12.

**6.1.2.2. *O*-[2-(2-pyridyl)ethyl]*N*-(2-tolyl)thiocarbamate (2).** Mp 59–61 °C; yield: 29% from petroleum ether/ethyl acetate. IR (KBr) $cm^{-1}$: 3134, 2872; $^1H$ NMR ($CDCl_3$) $\delta$: 2.19 (s, 3H, $CH_3$), 3.16 (t, $J$ = 6.6 Hz, 2H, $CH_2Py$), 4.87 (t, $J$ = 6.6 Hz, 2H, $CH_2O$), 6.65–7.78 (m, 7H, arom. H), 8.45–8.83 (m, 2H, py H + NH, exchangeable). Anal. Calcd for $C_{15}H_{16}N_2OS$: C, 66.15; H, 5.92; N, 10.29; S, 11.77. Found: C, 66.24; H, 5.99; N, 10.38; S, 11.74.

**6.1.2.3. *O*-[2-(2-pyridyl)ethyl]*N*-(2-chlorophenyl)thiocarbamate (3).** Mp 64–65 °C; yield: 38% from acetone. IR (KBr) $cm^{-1}$: 3103. $^1H$ NMR ($CDCl_3$) $\delta$: 3.18 (t, $J$ = 6.6 Hz, 2H, $CH_2Py$), 4.88 (t, $J$ = 6.6 Hz, 2H, $CH_2O$), 6.93–7.32 (m, 6H, arom. H), 7.47–7.58 (m, 1H, arom. H),8.42 (br s, 1H, NH, exchangeable), 8.48–8.55 (m, 1H, py H). Anal. Calcd for $C_{14}H_{13}ClN_2OS$: C, 57.43; H, 4.48; N, 9.57; S, 10.95. Found: C, 57.25; H, 4.53; N, 9.52; S, 10.89.

**6.1.2.4. *O*-[2-(2-pyridyl)ethyl]*N*-(3-tolyl)thiocarbamate 4.** Mp 47–49 °C; yield: 63% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3124, 2947; $^1H$ NMR ($CDCl_3$) $\delta$: 2.27 (s, 3H, $CH_3$), 3.26 (t, $J$ = 6.6 Hz, 2H, $CH_2Py$), 4.98 (t, $J$ = 6.6 Hz, 2H, $CH_2O$), 6.50–7.83 (m, 7H, arom. H), 8.45–8.83 (m, 2H, py H + NH, exchangeable). Anal. Calcd for $C_{15}H_{16}N_2OS$: C, 66.15; H, 5.92; N, 10.29; S, 11.77. Found: C, 66.20; H, 6.04; N, 10.45; S, 11.87.

**6.1.2.5. *O*-[2-(2-pyridyl)ethyl]*N*-(3-fluorophenyl)thiocarbamate (5).** Mp 87–88 °C; yield: 21% from acetone/methanol. IR (KBr) $cm^{-1}$: 3185, 3127. $^1H$ NMR ($CDCl_3$) $\delta$: 3.08–3.27 (m, 2H, $CH_2Py$), 4.74–5.01 (m, 2H, $CH_2O$), 6.58–7.12 (m, 6H, arom. H), 7.44–7.62 (m, 1H, arom. H), 8.39–8.54 (m, 1H, py H), 9.16 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}FN_2OS$: C, 60.85; H, 4.74; N, 10.14; S, 11.60. Found: C, 60.75; H, 4.72; N, 10.13; S, 11.50.

**6.1.2.6. *O*-[2-(2-pyridyl)ethyl]*N*-(3-chlorophenyl)thiocarbamate (6).** Mp 65–66 °C; yield: 15% from diethyl ether. IR (KBr) $cm^{-1}$: 3222, 3171. $^1H$ NMR ($CDCl_3$) $\delta$: 3.20 (t, $J$ = 6.4 Hz, 2H, $CH_2Py$), 4.83–4.98 (m, 2H, $CH_2O$), 6.95–7.23 (m, 7H, arom. H), 7.49–7.62 (m, 1H, py H), 8.78 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}ClN_2OS$: C, 57.43; H, 4.48; N, 9.57; S, 10.95. Found: C, 57.53; H, 4.52; N, 9.29; S, 10.93.

**6.1.2.7. *O*-[2-(2-pyridyl)ethyl]*N*-(3-nitrophenyl)thiocarbamate (7).** Mp 142–144 °C; yield: 48% from acetone. IR (KBr) $cm^{-1}$: 3177, 3123, 1526, 1351. $^1H$ NMR ($CDCl_3$) $\delta$: 3.32 (t, $J$ = 6 Hz, 2H, $CH_2Py$), 5.04 (t, $J$ = 6 Hz, 2H, $CH_2O$), 7.03–8.40 (m, 7H, arom. H), 8.52–8.72 (m, 1H, py H), 8.98 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}N_3O_3S$: C, 55.43; H, 4.32; N, 13.85; S, 10.57. Found: C, 55.54; H, 4.54; N, 13.73; S, 10.51.

**6.1.2.8. *O*-[2-(2-pyridyl)ethyl]*N*-(4-tolyl)thiocarbamate (8).** Mp 110–111 °C; yield: 38% from diethyl ether/methanol. IR (KBr) $cm^{-1}$: 3228, 3178. $^1H$ NMR ($CDCl_3$) $\delta$: 2.24 (s, 3H, $CH_3$), 3.18 (t, $J$ = 6.6 Hz, 2H, $CH_2Py$), 4.79–4.96 (m, 2H, $CH_2O$), 6.77–7.22 (m, 6H, arom. H), 7.47–7.59 (m, 1H, arom. H), 8.40 (br s, 1H, NH, exchangeable), 8.47–8.53 (m, 1H, py H). Anal. Calcd for $C_{15}H_{16}N_2OS$: C, 66.15; H, 5.92; N, 10.29; S, 11.77. Found: C, 66.15; H, 5.95; N, 10.28; S, 11.70.

**6.1.2.9. *O*-[2-(2-pyridyl)ethyl]*N*-(4-ethylphenyl)thiocarbamate (9).** Mp 71–73 °C; yield: 27% from methanol. IR (KBr) $cm^{-1}$: 3171, 2964. $^1H$ NMR ($CDCl_3$) $\delta$: 1.14 (t, $J$ = 7 Hz, 3H, $CH_3$), 2.53 (q, $J$ = 7 Hz, 2H, $CH_2Ph$), 3.18 (t, $J$ = 6.6 Hz, 2H, $CH_2Py$), 4.76–4.97 (m, 2H, $CH_2O$), 6.81–7.22 (m, 6H, arom. H), 7.47–7.58 (m, 1H, arom. H), 8.46–8.57 (m, 1H, py H), 8.67 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{16}H_{18}N_2OS$: C, 67.10; H, 6.34; N, 9.78; S, 11.19. Found: C, 67.14; H, 6.43; N, 9.87; S, 11.00.

**6.1.2.10. *O*-[2-(2-pyridyl)ethyl]*N*-(4-*i*-propylphenyl)thiocarbamate (10).** Mp 66–67 °C; yield: 48% from diethyl ether/methanol. IR (KBr) $cm^{-1}$: 3171, 2954. $^1H$ NMR ($CDCl_3$) $\delta$: 1.22 (d, $J$ = 7 Hz, 6H, $2CH_3$), 2.70–3.07 (m, 1H, CHPh), 3.24 (t, $J$ = 6.6 Hz, 2H, $CH_2Py$), 4.98 (t, $J$ = 6.6 Hz, 2H, $CH_2O$), 6.99–7.35 (m, 6H, arom. H), 7.46–7.70 (m, 1H, arom. H), 8.49–8.71 (m, 1H, py

H), 9.00 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{20}N_2OS$: C, 67.97; H, 6.71; N, 9.32; S, 10.67. Found: C, 67.71; H, 6.67; N, 9.31; S, 10.66.

**6.1.2.11. *O*-[2-(2-pyridyl)ethyl]*N*-(4-trifluoromethylphenyl)thiocarbamate (11).** Mp 112–114 °C; yield: 19% from diethyl ether/methanol. IR (KBr) $cm^{-1}$: 3180, 3116; $^1H$ NMR ($CDCl_3$) $\delta$: 3.21 (t, $J = 6.4$ Hz, 2H, $CH_2Py$), 4.93 (t, $J = 6.4$ Hz, 2H, $CH_2O$), 7.03–7.62 (m, 7H, arom. H), 8.47–8.53 (m, 1H, py H), 8.81 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{15}H_{13}F_3N_2OS$: C, 55.21; H, 4.02; N, 8.58; S, 9.82. Found: C, 55.02; H, 3.93; N, 8.55; S, 9.72.

**6.1.2.12. *O*-[2-(2-pyridyl)ethyl]*N*-(4-cyanophenyl)thiocarbamate (12).** Mp 156–157 °C; yield: 36% from diethyl ether/methanol. IR (KBr) $cm^{-1}$: 3170, 2226. $^1H$ NMR (DMSO-$d_6$) $\delta$: 3.29 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 5.00 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 7.05–7.80 (m, 7H, arom. H), 8.50–8.73 (m, 1H, py H), 10.61 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83; S, 11.31. Found: C, 63.79; H, 4.63; N, 14.81; S, 11.29.

**6.1.2.13. *O*-[2-(2-pyridyl)ethyl]*N*-(4-acetylphenyl)thiocarbamate (13).** Mp 135–137 °C; yield: 17% from diethyl ether/methanol. IR (KBr) $cm^{-1}$: 3342, 3286, 3162, 1675. $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.56 (s, 3H, $CH_3$), 3.14–3.48 (m, 2H, $CH_2Py$), 4.78–4.99 (m, 2H, $CH_2O$), 7.14–8.81 (m, 8H, arom. H), 11.38 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{16}H_{16}N_2O_2S$: C, 63.98; H, 5.37; N, 9.33; S, 10.67. Found: C, 64.10; H, 5.59; N, 9.10; S, 10.66.

**6.1.2.14. *O*-[2-(2-pyridyl)ethyl]*N*-(4-fluorophenyl)thiocarbamate (14).** Mp 95–96 °C; yield: 55% from diethyl ether/methanol. IR (KBr) $cm^{-1}$: 3179, 3126. $^1H$ NMR ($CDCl_3$) $\delta$: 3.24 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.96 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.76–7.39 (m, 6H, arom. H), 7.46–7.73 (m, 1H, arom. H), 8.48–8.68 (m, 1H, py H), 9.12 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}FN_2OS$: C, 60.85; H, 4.74; N, 10.14; S, 11.60. Found: C, 60.95; H, 4.62; N, 10.03; S, 11.62.

**6.1.2.15. *O*-[2-(2-pyridyl)ethyl]*N*-(4-chlorophenyl)thiocarbamate (15).** Mp 114–115 °C; yield: 33% from diethyl ether. IR (KBr) $cm^{-1}$: 3200, 3158. $^1H$ NMR ($CDCl_3$) $\delta$: 3.27 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 5.00 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 7.02–7.41 (m, 6H, arom. H), 7.50–7.78 (m, 1H, arom. H), 8.51–8.73 (m, 1H, py H), 9.08 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}ClN_2OS$: C, 57.43; H, 4.48; N, 9.57; S, 10.95. Found: C, 57.76; H, 4.67; N, 9.49; S, 10.85.

**6.1.2.16. *O*-[2-(2-pyridyl)ethyl]*N*-(4-bromophenyl)thiocarbamate (16).** Mp 115–117 °C; yield: 37% from diethyl ether/methanol. IR (KBr) $cm^{-1}$: 3154; $^1H$ NMR ($CDCl_3$) $\delta$: 3.25 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.97 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.91–7.74 (m, 7H, arom. H), 8.47–8.74 (m, 1H, py H), 9.15 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}BrN_2OS$: C, 49.86; H, 3.89; N, 8.31; S, 9.51. Found: C, 50.11; H, 4.11; N, 8.23; S, 9.49.

**6.1.2.17. *O*-[2-(2-pyridyl)ethyl]*N*-(4-iodophenyl)thiocarbamate (17).** Mp 108–109 °C; yield: 70% from diethyl ether. IR (KBr) $cm^{-1}$: 3206, 3158. $^1H$ NMR ($CDCl_3$) $\delta$: 3.25 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.97 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.77–7.76 (m, 7H, arom. H), 8.45–8.71 (m, 1H, py H), 8.92 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}IN_2OS$: C, 43.76; H, 3.41; N, 7.29; S, 8.34. Found: C, 43.75; H, 3.46; N, 7.23; S, 8.33.

**6.1.2.18. *O*-[2-(2-pyridyl)ethyl]*N*-(4-dimethylaminophenyl)thiocarbamate (18).** Mp 108–110 °C; yield: 28% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3193, 3155, 2924. $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.76 (s, 3H, $CH_3$), 2.79 (s, 3H, $CH_3$), 3.02–3.14 (m, 2H, $CH_2Py$), 4.64–4.78 (m, 2H, $CH_2O$), 6.38–6.50 (m, 1H, arom. H), 6.53–6.66 (m, 1H, arom. H), 6.77–6.88 (m, 1H, arom. H), 7.13–7.33 (m, 3H, arom. H), 7.59–7.73 (m, 1H, py H), 8.44–8.50 (m, 1H, py H), 10.73 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{16}H_{19}N_3OS$: C, 63.76; H, 6.35; N, 13.94; S, 10.64. Found: C, 63.57; H, 6.54; N, 13.91; S, 10.60.

**6.1.2.19. *O*-[2-(2-pyridyl)ethyl]*N*-(4-nitrophenyl)thiocarbamate (19).** Mp 172–174 °C; yield: 66% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3200. $^1H$ NMR (DMSO-$d_6$) $\delta$: 3.28 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.96 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 7.08–8.40 (m, 8H, arom. H), 8.65 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}N_3O_3S$: C, 55.43; H, 4.32; N, 13.85; S, 10.57. Found: C, 55.70; H, 4.27; N, 13.55; S, 10.21.

**6.1.2.20. *O*-[2-(2-pyridyl)ethyl]*N*-(4-methoxyphenyl)thiocarbamate (20).** Mp 90–91 °C; yield: 58% from diethyl ether/petroleum ether. IR (KBr) $cm^{-1}$: 3198, 2955. $^1H$ NMR ($CDCl_3$) $\delta$: 3.24 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 3.79 (s, 3H, $CH_3$), 4.97 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.63–7.73 (m, 7H, arom. H), 8.50–8.72 (m, 1H, py H), 9.32 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 9.71; S, 11.12. Found: C, 62.59; H, 5.60; N, 9.66; S, 11.16.

**6.1.2.21. *O*-[2-(2-pyridyl)ethyl]*N*-(2,5-difluorophenyl)thiocarbamate (21).** Mp 69–71 °C; yield: 37% from diethyl ether/ethanol. IR (KBr) $cm^{-1}$: 3107. $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.93–3.23 (m, 2H, $CH_2Py$), 4.60–4.94 (m, 2H, $CH_2O$), 6.82–7.81 (m, 6H, arom. H), 8.29–8.60 (m, 1H, arom. H); 10.86 (s, 1H, NH, exchangeable), 8.48–8.55 (m, 1H, py H). Anal. Calcd for $C_{14}H_{12}F_2N_2OS$: C, 57.13; H, 4.11; N, 9.52; S, 10.89. Found: C, 57.20; H, 4.31; N, 9.40; S, 10.82.

**6.1.2.22. *O*-[2-(2-pyridyl)ethyl]*N*-(2,6-difluorophenyl)thiocarbamate (22).** Mp 92–94 °C; yield: 40% from diethyl ether/ethanol. IR (KBr) $cm^{-1}$: 3100. $^1H$ NMR ($CDCl_3$) $\delta$: 3.18 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.87 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.67–7.79 (m, 6H, arom. H), 8.38–8.67 (m, 1H, py H), 8.88 (br s, 1H, NH, exchangeable). Calcd for $C_{14}H_{12}F_2N_2OS$: C, 57.13; H, 4.11; N, 9.52; S, 10.89. Found: C, 57.34; H, 4.18; N, 9.49; S, 10.88.

**6.1.2.23. *O*-[2-(2-pyridyl)ethyl]*N*-(3,5-difluorophenyl) thiocarbamate (23).** Mp 122–124 °C; yield: 79% from $CH_2Cl_2$/diethyl ether. IR (KBr) $cm^{-1}$: 3197, 3118. $^1H$

NMR ($CDCl_3$) $\delta$: 3.28 (t, $J = 6$ Hz, 2H, $CH_2Py$), 5.01 (t, $J = 6$ Hz, 2H, $CH_2O$), 6.36–7.38 (m, 5H, arom. H), 7.48–7.78 (m, 1H, arom. H), 8.50–8.71 (m, 1H, py H), 9.36 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{12}F_2N_2OS$: C, 57.13; H, 4.11; N, 9.52; S, 10.89. Found: C, 57.19; H, 4.19; N, 9.25; S, 11.03.

**6.1.2.24. *O*-[2-(2-pyridyl)ethyl]*N*-(2,4-dichlorophenyl) thiocarbamate (24).** Mp 96–97 °C; yield: 41% from diethyl ether/ethanol. IR (KBr) $cm^{-1}$: 3138. $^1H$ NMR ($CDCl_3$) $\delta$: 3.26 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.98 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.99–7.90 (m, 6H, arom. H), 8.42–8.76 (m, 2H, py H + NH, exchangeable). Anal. Calcd for $C_{14}H_{12}Cl_2N_2OS$: C, 51.39; H, 3.7; N, 8.56; S, 9.8. Found: C, 51.39; H, 3.79; N, 8.45; S, 9.83.

**6.1.2.25. *O*-[2-(2-pyridyl)ethyl]*N*-(3,4-dichlorophenyl) thiocarbamate (25).** Mp 106–108 °C; yield: 26% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3158. $^1H$ NMR ($CDCl_3$) $\delta$: 3.27 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 5.00 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.97–7.76 (m, 6H, arom. H), 8.52–8.71 (m, 1H, py H), 8.94 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{12}Cl_2N_2OS$: C, 51.39; H, 3.70; N, 8.56; S, 9.80. Found: C, 51.41; H, 3.72; N, 8.28; S, 9.77.

**6.1.2.26. *O*-[2-(2-pyridyl)ethyl]*N*-(3-chloro-4-methylphenyl)thiocarbamate (26).** Mp 84–86 °C; yield: 54% from diethyl ether/ethanol. IR (KBr) $cm^{-1}$: 3191, 3152. $^1H$ NMR ($CDCl_3$) $\delta$: 2.22 (s, 3H, $CH_3$), 3.16 (t, $J = 6.4$ Hz, 2H, $CH_2Py$), 4.75–4.97 (m, 2H, $CH_2O$), 6.95–7.23 (m, 5H, arom. H), 7.45–7.58 (m, 1H, py H), 8.42–8.53 (m, 1H, py H), 8.95 (br s, 1H, NH, exchangeable). Calcd for $C_{15}H_{15}ClN_2OS$: C, 58.72; H, 4.93; N, 9.13; S, 10.45. Found: C, 58.77; H, 5.01; N, 9.09; S, 10.48.

**6.1.2.27. *O*-[2-(2-pyridyl)ethyl]*N*-(4-chloro-3-trifluoromethylphenyl)thiocarbamate (27).** Mp 115 °C; yield: 37% from diethyl ether/methanol. IR (KBr) $cm^{-1}$: 3178. $^1H$ NMR ($CDCl_3$) $\delta$: 3.26 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.98 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 7.00–7.88 (m, 6H, arom. H), 8.44–8.68 (m, 1H, py H), 9.42 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{15}H_{12}ClF_3N_2OS$: C, 49.94; H, 3.35; N, 7.76; S, 8.89. Found: C, 49.97; H, 3.31; N, 7.68; S, 8.85.

**6.1.2.28. *O*-[2-(2-pyridyl)ethyl]*N*-(4-chloro-3-nitrophenyl)thiocarbamate (28).** Mp 148–150 °C; yield: 24% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3157, 1534, 1339. $^1H$ NMR ($CDCl_3$) $\delta$: 3.21 (t, $J = 6$ Hz, 2H, $CH_2Py$), 4.84–4.97 (m, 2H, $CH_2O$), 7.09–7.21 (m, 3H, arom. H), 7.28–7.38 (m, 2H, arom. H), 7.53–7.67 (m, 1H, py H), 8.47–8.53 (m, 1H, pyr H), 8.68 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{12}ClN_3O_3S$: C, 49.78; H, 3.58; N, 12.44; S, 9.49. Found: C, 49.76; H, 3.62; N, 12.31; S, 9.53.

**6.1.2.29. *O*-(2-phenylethyl)*N*-phenylthiocarbamate (29).** Mp 91–93 °C; yield: 60% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3170. $^1H$ NMR ($CDCl_3$) $\delta$: 4.27 (t, $J = 5.0$ Hz, 2H, $CH_2Ph$), 4.91 (t, $J = 5.0$ Hz, 2H, $CH_2O$), 6.68–7.55 (m, 10H, arom. H), 8.82 (br s, 1H, NH, exchangeable). $^{13}C$ NMR ($CDCl_3$) $\delta$: 66.19, 115.23, 121.85, 129.55, 130.13, 158.92 (C–N), 188.62 (C=S). Anal. Calcd for $C_{15}H_{15}NOS$: C, 65.91; H, 5.53; N, 5.12; S, 11.73. Found: C, 66.12; H, 5.60; N, 5.13; S, 11.59.

**6.1.2.30. *O*-(2-phenylethyl)*N*-(4-tolyl)thiocarbamate (30).** Mp 97–99 °C; yield: 91% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3209, 2922. $^1H$ NMR ($CDCl_3$) $\delta$: 2.27 (s, 3H, $CH_3$), 3.03 (t, $J = 6.6$ Hz, 2H, $CH_2Ph$), 4.76 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.74–7.57 (m, 9H, arom. H), 8.85 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{16}H_{17}NOS$: C, 70.82; H, 6.31; N, 5.16; S, 11.81. Found: C, 70.86; H, 6.66; N, 5.14; S, 11.75.

**6.1.2.31. *O*-(2-phenylethyl)*N*-(4-chlorophenyl)thiocarbamate (31).** Mp 117–118 °C; yield: 82% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3208. $^1H$ NMR ($CDCl_3$) $\delta$: 3.05 (t, $J = 6.6$ Hz, 2H, $CH_2Ph$), 4.80 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 7.05–7.50 (m, 9H, arom. H), 9.14 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{15}H_{14}ClNOS$: C, 61.74; H, 4.84; N, 4.80; S, 10.99. Found: C, 61.99; H, 4.85; N, 4.84; S, 11.00.

**6.1.2.32. *O*-(2-phenylethyl)*N*-(4-nitrophenyl)thiocarbamate (32).** Mp 142–143 °C; yield: 92% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3266, 1551, 1335. $^1H$ NMR ($CDCl_3$) $\delta$: 3.14 (t, $J = 6.6$ Hz, 2H, $CH_2Ph$), 4.39 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 7.14–7.54 (m, 7H, arom. H), 7.98–8.30 (m, 2H, arom. H), 8.90 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{15}H_{14}N_2O_3S$: C, 59.59; H, 4.67; N, 9.27; S, 10.60. Found: C, 59.54; H, 4.67; N, 9.23; S, 10.53.

**6.1.2.33. *O*-(2-phenylethyl)*N*-(4-methoxyphenyl)thiocarbamate (33).** Mp 83–85 °C; yield: 96% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3228, 2926. $^1H$ NMR ($CDCl_3$) $\delta$: 3.75 (s, 3H, $CH_3$), 4.17–4.42 (m, 2H, $CH_2Ph$), 4.75–5.05 (m, 2H, $CH_2O$), 6.72–7.56 (m, 9H, arom. H), 8.90 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{16}H_{17}NO_2S$: C, 66.87; H, 5.96; N, 4.87; S, 11.16. Found: C, 66.76; H, 6.17; N, 4.62; S, 11.13.

**6.1.2.34. *O*-[2-(2-thienyl)ethyl]*N*-phenylthiocarbamate (34).** Mp 93–94 °C; yield: 76% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3210. $^1H$ NMR ($CDCl_3$) $\delta$: 3.28 (t, $J = 6$ Hz, 2H, $CH_2$Thioph.), 4.80 (t, $J = 6$ Hz, 2H, $CH_2O$), 6.53–7.12 (m, 8H, arom. H), 8.83 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{13}H_{13}NOS_2$: C, 59.29; H, 4.98; N, 5.32; S, 24.35. Found: C, 59.51; H, 5.11; N, 5.30; S, 24.04.

**6.1.2.35. *O*-[2-(4-pyridyl)ethyl]*N*-(4-tolyl)thiocarbamate (35).** Mp 130–132 °C; yield: 91% from $CH_2Cl_2$/ether. IR (KBr) $cm^{-1}$: 3117, 2956. $^1H$ NMR ($CDCl_3$) $\delta$: 2.31 (s, 3H, $CH_3$), 3.05 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.83 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.93–7.34 (m, 6H, arom. H), 8.33–8.67 (m, 2H, py H), 9.57 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{15}H_{16}N_2OS$: C, 66.15; H, 5.92; N, 10.29; S, 11.77. Found: C, 66.03; H, 6.11; N, 10.41; S, 11.78.

**6.1.2.36. *O*-[2-(4-pyridyl)ethyl]*N*-(4-chlorophenyl)thiocarbamate (36).** Mp 129–131 °C; yield: 79% from diethyl

ether. IR (KBr) $cm^{-1}$: 3164, 3101. $^1H$ NMR ($CDCl_3$) $\delta$: 3.08 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 4.85 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 7.05–7.48 (m, 6H, arom. H), 8.46–8.69 (m, 2H, py H), 8.94 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}ClN_2OS$: C, 57.43; H, 4.48; N, 9.57; S, 10.95. Found: C, 57.28; H, 4.51; N, 9.63; S, 10.92.

**6.1.2.37. *O*-[2-(4-pyridyl)ethyl]*N*-(4-nitrophenyl)thiocarbamate (37).** Mp 141–144 °C; yield: 93% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3102, 1501, 1307; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.92–3.08 (m, 2H, $CH_2Py$), 4.57–4.76 (m, 2H, $CH_2O$), 7.17–7.78 (m, 4H, arom. H), 7.95–8.12 (m, 2H, arom. H), 8.32–8.47 (m, 2H, py H), 11.57 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{14}H_{13}N_3O_3S$: C, 55.43; H, 4.32; N, 13.85; S, 10.57. Found: C, 55.44; H, 4.34; N, 13.99; S, 10.60.

**6.1.2.38. *O*-[2-(4-pyridyl)ethyl]*N*-(4-methoxyphenyl) thiocarbamate (38).** Mp 118–120 °C; yield: 88% from $CH_2Cl_2$/methanol. IR (KBr) $cm^{-1}$: 3152, 2923. $^1H$ NMR ($CDCl_3$) $\delta$: 3.04 (t, $J = 6.6$ Hz, 2H, $CH_2Py$), 3.79 (s, 3H, $CH_3$), 4.81 (t, $J = 6.6$ Hz, 2H, $CH_2O$), 6.69–7.53 (m, 6H, arom H), 8.41–8.66 (m, 2H, py H), 9.56 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 9.71; S, 11.12. Found: C, 62.59; H, 5.66; N, 9.68; S, 11.01.

### 6.2. Virology: Materials and methods

The biological evaluation of the synthesized compounds was performed according to the previously reported procedures.[34,52]

### 6.3. Molecular modeling

The molecular structures of **1** and **29** were built by Insight II (Builder module), parameterized according to CVFF force field[58] and their RT complexes were calculated using Autodock 3.05.[59] After the removal of the PETT inhibitor from the crystal structure solved by Ren et al.[30] (PDB code 1DTQ), hydrogens were added and partial charges were assigned according to CVFF force field (Insight II, biopolymer module). The ligand 'root' was defined automatically and all bonds were allowed to freely rotate. A $50 \times 50 \times 50$-grid (grid spacing 0.375 Å) was centred on the NNBS and electrostatic and affinity maps for each atom type of the ligand were calculated. The docking search was performed over 256 conformers using the Genetic Algorithm Local Search protocol as implemented in AutoDock (Population size: 100; Rate of gene mutation: 0.02; Rate of Crossover: 0.8). The docking poses were clustered (rmsd 2.0 Å) and the best conformation of the low energy, highest populated cluster was select as the binding conformation. The resulting models were energy-minimized by CNS,[60] performing a cycle of simulated annealing (starting temperature 1000 K) followed by 120 cycles of Powell minimization.

The minimized structures of RT-**1** and RT-**29** complexes were used as starting structures for the calculation of **12**, **15**, **19**, **25**, **28**, **31**, **32** and **36** binding modes.

All the calculations were carried out on Silicon Graphic Indigo2 and Origin 200 workstations. Model analyses were performed using the CCP4 program suite.[61] The programs Molscript[62]and Raster3D[63] were used to draw the figures.

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