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Original article

Parallel synthesis, molecular modelling and further structure–activity relationship studies of new acylthiocarbamates as potent non-nucleoside HIV-1 reverse transcriptase inhibitors

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ABSTRACT

The structure–activity relationships (SARs) of acylthiocarbamates (ATCs), a new class of non-nucleoside HIV-1 reverse transcriptase inhibitors, have been expanded. Sixty-six new analogues were prepared by parallel solution-phase synthesis. In general, the potency of new ATCs was better than that of the first series and *O*-[2-phthalimidoethyl] 4-chlorophenyl(3-nitrobenzoyl) thiocarbamate turned out to be the most potent ATC so far synthesized ($EC_{50} = 1.5$ nM). Several ATCs were active at micromolar concentrations against HIV-1 strains carrying the RT Y181C mutation and one of them was also moderately active against the K103R variant. Docking simulations were carried out to rationalize the most relevant SARs.

1. Introduction

HIV-1 reverse transcriptase (RT) catalyses the conversion of a single-stranded RNA into a double-stranded DNA that is then integrated into the host cells' genome. Due to its essential role in the HIV-1 life-cycle, RT is a primary target for the highly active antiretroviral therapy (HAART). Non-nucleoside inhibitors (NNRTIs) [1–9] are chemically diverse and selective RT targeting agents that lock this enzyme in an inactive form [4] by binding to an allosteric hydrophobic pocket (namely, non–nucleoside inhibitor binding site, NNBS) located about 10 Å far from the polymerase active site.

O-(2-Phthalimidoethyl)-*N*-aryl-*N*-acylthiocarbamates (ATCs) have been recently identified [10] as a new class of HIV-1 NNRTIs, structurally related to *N*-phenethyl-*N*′-thiazolylthiourea (PETT) [11–19] and thiocarbamate (TC) [20–23] derivatives (Fig. 1). ATCs can be retrosynthetically fragmented into three portions (namely, **a**–**c**. Fig. 1B). Previous structure–activity relationship (SAR) studies [10], mainly based on modifications of the **b** substructure of lead **I**, had identified the *N*-phenyl *para* substitution as a key feature to gain potent inhibitors (Fig. 1B).

With the aim at acquiring additional key elements to maximize the activity against wild-type RT and some clinically relevant RT mutations, a new series of ATCs was designed. Initially, the SAR strategy was focused on the variation of the substructure **c** [mono- (**1**–**16**), di-substituted (hetero)aroyl (**19**–**47**) and bicyclic (hetero)aroyl (**48**–**50**) groups] by keeping constant portion **a** (2-phthalimidoethyl) and the *para*-substitution pattern (halide, methyl) of portion **b**. Analogues **17** and **18** were synthesized to evaluate the effect of portion **c** polysubstitution on the activity of *N*-unsubstituted phenyl ATCs. Then, some of the most favourable *N*-phenyl(4-chlorophenyl, 4-nitrophenyl) and acyl(3-nitrobenzoyl, 4-chlorobenzoyl, 2-thenoyl, 2-chloronicotinoyl) substructures were assembled with portion **a** carrying modifications on the ethyl linker (**51**–**54**) and on the phthalimide phenyl ring (**55**–**60**). Finally, we evaluated the influence of the *N*-phenyl ring di- and tri-chlorosubstitution on the anti-HIV-1 activity by keeping constant portion **a** (2-phthalimidoethyl) and embedding two of the most promising acyl moieties (2-furoyl, 3-nitrobenzoyl) (**61**–**66**).

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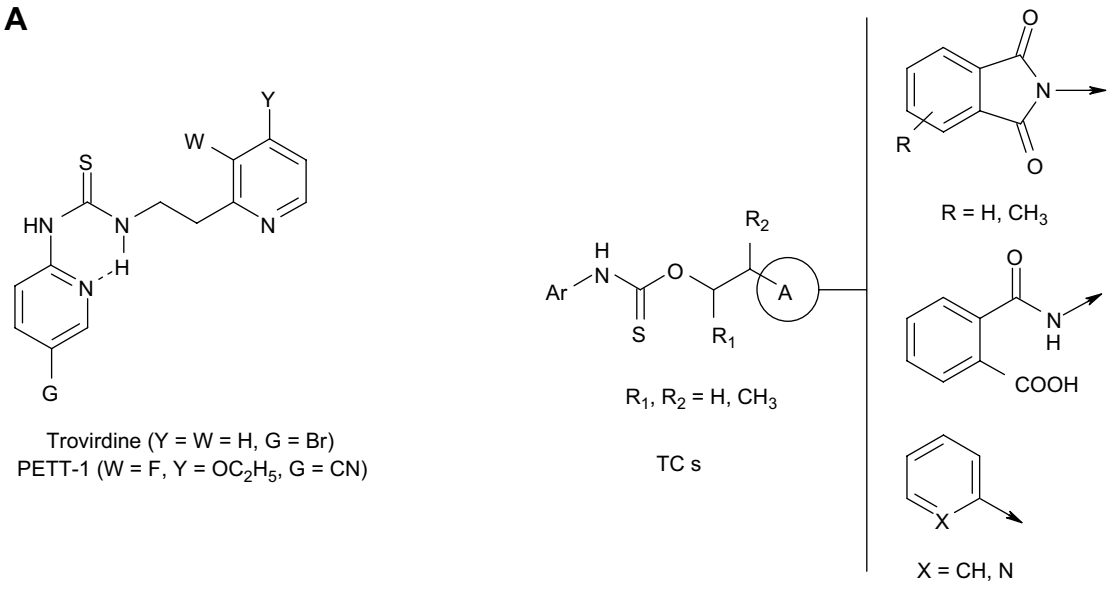


	X	(Het)Ar-CO	EC_{50} (μM)
I	H	benzoyl	0.4
II	Cl	benzoyl	0.1
III	Cl	4-chlorobenzoyl	0.025
IV	Cl	2-furoyl	0.007
V	Cl	2-thenoyl	0.008
VI	Cl	2-chloronicotinoyl	0.005
VII	Cl	6-chloronicotinoyl	0.006

Fig. 1. A] PETT and TC [20–23] derivatives structurally related to ATCs. B] Molecular portions, chemical structure and antiretroviral activity of previously prepared ATCs **I–VII** [10].

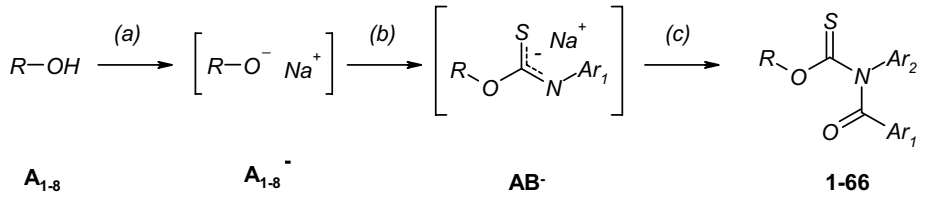
2. Chemistry

The synthesis of ATCs **1–66** was carried out by the parallelization of the previously reported highly convergent three-step one-pot solution-phase protocol [10]. As shown in Scheme 1, starting alcohols $\mathbf{A_{1-8}}$ (Fig. 2A) were transformed into the corresponding alcoholates $\mathbf{A}^-$ in the presence of sodium hydride in anhydrous aprotic solvents (DMF or pyridine) and condensed *in situ* with the proper isothiocyanate **B** (Fig. 2B). The sodium thiocarbamate adducts $\mathbf{AB}^-$ were subsequently coupled with acyl chlorides **C** (Fig. 2C) in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) to afford the desired products. Two synthetic variants (namely, procedures P_1 and P_2, see Section 6) were adopted to obviate the different reactivity of key intermediates $\mathbf{A}^-$ and $\mathbf{AB}^-$ vs building blocks **B** and **C**. Thus, ATCs **1–49**, **52–57** and **59** were prepared in anhydrous pyridine stirring the mixtures overnight at rt (procedure P_1). For derivatives **50**, **51**, **58**, **60–66** (procedure P_2), dry DMF was used as reaction medium and different temperatures and times were employed to increase the product yields. The overall yields for ATCs **1–66** ranged from 12 to 88% (see Section 6).

The most relevant features of this parallel methodology are: i) no intermediate needs to be isolated; ii) high atom economy is realized, as only one molecule of HCl is formally lost in the whole process (this allows the incorporation into the products of all the chemical features of the commercially available or readily accessible building blocks used); iii) minimal sample handling is required during the purification protocol; iv) the modular character of the synthesis allows the independent variation of portions **a–c**.

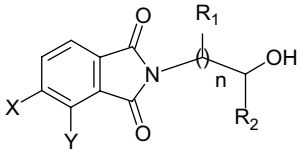
3. Biological results and discussion

ATCs **1–66** were evaluated for their cytotoxicity and anti-HIV-1 activity in MT-4 cells using **Trovirdine** as the reference molecule (Tables 1–5). Selected analogues were screened in enzymatic assays against HIV-1 wt RT, using **Efavirenz** as control compound (Table 6). The most potent derivatives were also tested against the clinically

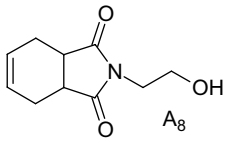


Scheme 1. General procedure for the preparation of ATCs **1–66** by parallel, convergent, one-pot solution-phase synthesis. *Reaction conditions*: (a) NaH, dry DMF or dry pyridine, 0 °C or rt; (b) Ar_1–NCS ($\mathbf{B_{1-12}}$), 0 °C or rt; (c) TMEDA then Ar_2COCl ($\mathbf{C_{1-33}}$), rt or heating. The structures of alcohols $\mathbf{A_{1-8}}$, isothiocyanates $\mathbf{B_{1-12}}$ and acyl chlorides $\mathbf{C_{1-33}}$ are listed in Fig. 2.

A] ROH A_1-A_8



	n	X	Y	R_1	R_2
A_1	0	H	H	-	H
A_2	1	H	H	H	H
A_3	1	H	CH_3	H	H
A_4	1	CH_3	H	H	H
A_5	1	H	H	CH_3	H
A_6	1	H	H	H	CH_3
A_7	2	H	H	H	H



B] Ar_1-NCS B_1-B_{12}

	Ar_1
B_1	phenyl
B_2	4-tolyl
B_3	4-fluorophenyl
B_4	4-chlorophenyl
B_5	4-bromophenyl
B_6	4-nitrophenyl
B_7	2,3-dichlorophenyl
B_8	2,4-dichlorophenyl
B_9	2,6-dichlorophenyl
B_{10}	3,4-dichlorophenyl
B_{11}	3,5-dichlorophenyl
B_{12}	2,4,6-trichlorophenyl

C] Ar_2-CO-Cl C_1-C_{33}

	Ar_2-CO
C_1	2-toluoyl
C_2	2-nitrobenzoyl
C_3	3-toluoyl
C_4	3-nitrobenzoyl
C_5	4-toluoyl
C_6	4-trifluoromethylbenzoyl
C_7	4-chlorobenzoyl
C_8	4-bromobenzoyl
C_9	4-pentyloxybenzoyl
C_{10}	4-nitrobenzoyl
C_{11}	2,3-difluorobenzoyl
C_{12}	2,4-difluorobenzoyl
C_{13}	2,5-difluorobenzoyl
C_{14}	2,6-difluorobenzoyl
C_{15}	3,4-difluorobenzoyl
C_{16}	3,5-difluorobenzoyl
C_{17}	2,3-dichlorobenzoyl

	Ar_2-CO
C_{18}	2,4-dichlorobenzoyl
C_{19}	2,6-dichlorobenzoyl
C_{20}	3,4-dichlorobenzoyl
C_{21}	3,5-dichlorobenzoyl
C_{22}	2,6-dimethoxybenzoyl
C_{23}	2-chloro-4-nitrobenzoyl
C_{24}	2-chloro-5-nitrobenzoyl
C_{25}	4-chloro-3-nitrobenzoyl
C_{26}	4-bromo-3-methylbenzoyl
C_{27}	1-naphthoyl
C_{28}	2-naphthoyl
C_{29}	2-furoyl
C_{30}	2,5-dimethyl-3-furoyl
C_{31}	2-thenoyl
C_{32}	2-chloronicotinoyl
C_{33}	thianaphthene-2-carbonyl

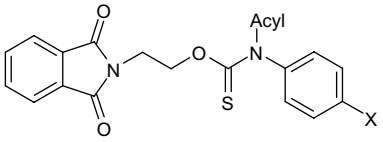
Fig. 2. Building blocks used for the ATC parallel synthesis.

relevant K103R, Y181C (Table 7) and K103 N/Y181C resistant mutants [24,25].

With the exception of **1**, ATCs bearing *ortho*-, *meta*- and *para*-monosubstituted (hetero)aroyl moieties (**1**–**16**, Table 1) emerged as potent NNRTIs, active in the sub-micromolar or nanomolar concentration range. In particular, **4**, **5** and **7** were 2-, 13- and 4-fold more active than **Trovirdine**, respectively, whereas **14** and **15** were equipotent to the reference compound. Analogue **5** turned out to be the most potent ATC so far synthesized ($EC_{50} = 1.5$ nM). The substitution pattern as well as the electronic properties of the acyl substituent significantly affected the activity. Thus, the *meta*- and *para*-substituted ATCs showed higher potency than the corresponding *ortho* congeners (**4** and **6** vs **2**; **5** and **8** vs **3**; **13** and **15** vs **11**; **14** and **16** vs **12**). The toluoyl derivatives were more active than the nitrobenzoyl analogues among the *ortho* and *para* series (**2** vs **3**; **6** vs **8**; **11** vs **12**; **15** vs **16**); this trend was reversed within the *meta*-substituted ATCs (**4** vs **5**; **13** vs **14**). Furthermore, the ATCs' potency is concomitantly influenced by the acyl substitution pattern and the electronic nature of the portion **b** substituents. Thus, among the *ortho*- and *meta*-substituted aroyl ATCs, the *N*-4-chlorophenyl analogues were more active than the *N*-4-tolyl congeners (**2** vs **11**; **3** vs **12**; **4** vs **13**; **5** vs **14**), whereas the *para* derivatives showed an opposite trend (compare **6** with **15**; **8** with **16**).

The activities of ATCs featured by di-substituted (hetero)aroyl groups (**17**–**47**) are shown in Table 2. The most potent derivatives shared the *N*-4-chlorophenyl substructure and showed EC_{50} values lower than (**19**, **20**, **23**, **24**, **28**, **29**, **31**, **34**) or equal to (**21**, **22**, **33**, **35**) that of **Trovirdine**. The *N*-phenyl *para* substitution as well as the electronic properties of the substituent on portion **b** proved to play a pivotal role on anti-HIV-1 activity. Thus, the *N*-unsubstituted

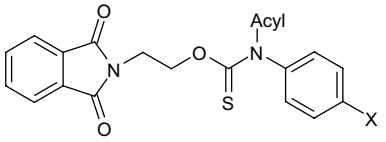
Table 1
Cytotoxicity and anti-HIV-1 activity of ATCs **1**–**16**.[a]



Cpd	X	Acyl	CC_{50}[b]	EC_{50}[c]	SI[d]
1	F	4-pentyloxybenzoyl	>100	8.5	>11.8
2	Cl	2-toluoyl	21	0.04	525
3	Cl	2-nitrobenzoyl	21	0.07	300
4	Cl	3-toluoyl	58	0.01	5800
5	Cl	3-nitrobenzoyl	18	0.0015	12,000
6	Cl	4-toluoyl	>100	0.05	>2000
7	Cl	4-trifluoromethylbenzoyl	10	0.005	2000
8	Cl	4-nitrobenzoyl	>100	0.06	1667
9	Br	4-bromobenzoyl	73	0.035	2086
10	Br	2-chloronicotinoyl	>100	0.2	>500
11	CH_3	2-toluoyl	>100	0.20	>500
12	CH_3	2-nitrobenzoyl	100	0.40	250
13	CH_3	3-toluoyl	>100	0.04	>2500
14	CH_3	3-nitrobenzoyl	100	0.02	5000
15	CH_3	4-toluoyl	83	0.02	4150
16	CH_3	4-nitrobenzoyl	58	0.03	1933
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.

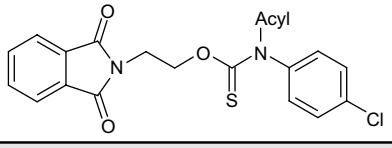
Table 2
Cytotoxicity and anti-HIV-1 activity of ATCs **17**–**47**.[a]



Cpd	X	Acyl	CC_{50}[b]	EC_{50}[c]	SI[d]
17	H	3,4-dichlorobenzoyl	52	0.5	104
18	H	4-chloro-3-nitrobenzoyl	51	0.35	146
19	Cl	2,3-difluorobenzoyl	58	0.008	7250
20	Cl	2,4-difluorobenzoyl	52	0.01	5200
21	Cl	2,5-difluorobenzoyl	54	0.02	2700
22	Cl	2,6-difluorobenzoyl	39	0.02	1950
23	Cl	3,4-difluorobenzoyl	52	0.008	6500
24	Cl	3,5-difluorobenzoyl	59	0.007	8429
25	Cl	2,3-dichlorobenzoyl	54	0.1	540
26	Cl	2,4-dichlorobenzoyl	18	0.03	600
27	Cl	2,6-dichlorobenzoyl	20	5.6	3.6
28	Cl	3,4-dichlorobenzoyl	35	0.009	3889
29	Cl	3,5-dichlorobenzoyl	32	0.008	4000
30	Cl	2,6-dimethoxybenzoyl	69	0.4	172
31	Cl	2-chloro-4-nitrobenzoyl	19	0.008	2375
32	Cl	2-chloro-5-nitrobenzoyl	45	0.07	643
33	Cl	4-chloro-3-nitrobenzoyl	48	0.02	2400
34	Cl	4-bromo-3-methylbenzoyl	18	0.007	2571
35	Cl	2,5-dimethyl-3-furoyl	40	0.02	2000
36	CH_3	2,4-difluorobenzoyl	20	0.03	667
37	CH_3	2,5-difluorobenzoyl	62	0.04	1,550
38	CH_3	2,6-difluorobenzoyl	65	1.0	65
39	CH_3	3,5-difluorobenzoyl	54	0.05	1080
40	CH_3	2,3-dichlorobenzoyl	35	0.2	175
41	CH_3	2,4-dichlorobenzoyl	49	0.3	163
42	CH_3	2,6-dichlorobenzoyl	22	9	2.4
43	CH_3	3,4-dichlorobenzoyl	17	0.05	340
44	CH_3	2,6-dimethoxybenzoyl	36	22	1.6
45	CH_3	2-chloro-4-nitrobenzoyl	17	0.09	189
46	CH_3	4-chloro-3-nitrobenzoyl	47	0.06	783
47	CH_3	4-bromo-3-methylbenzoyl	>100	0.05	>2000
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 3
Cytotoxicity and anti-HIV-1 activity of ATCs **48**–**50**.[a]

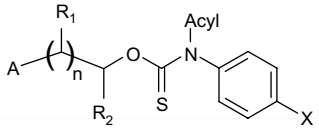


Cpd	Acyl	CC_{50}[b]	EC_{50}[c]	SI[d]
48	1-naphthoyl	6	0.03	200
49	2-naphthoyl	37	0.02	1850
50	Thianaphthene-2-carbonyl	56	0.2	280
Trovirdine		60	0.02	3000

[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.

phenyl derivatives were less active than their corresponding *N*-*para*-substituted phenyl analogues (compare **17** with **28** and **43**; **18** with **33** and **46**) and the *N*-4-chlorophenyl ATCs (bearing an electron-withdrawing substituent on portion **b**) resulted from 1.6- to 55-fold more active than those embedding the electron-donating methyl group (**20** vs **36**, **21** vs **37**, **22** vs **38**, **24** vs **39**, **25** vs **40**, **26** vs **41**, **27** vs **42**, **28** vs **43**, **30** vs **44**, **31** vs **45**, **33** vs **46**, **34** vs **47**). The difluorobenzoyl derivatives **19**–**24** were highly active in a narrow concentration range (EC_{50} range: 7–20 nM), thus suggesting that activity does not correlate with a specific position of the two aromatic fluorine atoms. The dichlorobenzoyl derivatives were less active than their difluoro isosters (**25** vs **19**; **26** vs **20**; **27** vs **22**; **28** vs **23**; **29** vs **24**; **41** vs **36**; **42** vs **38**) and showed a strong dependence of the antiretroviral activity on the acyl moiety substitution pattern. In particular, the 3,4- and 3,5-dichloro analogues were significantly more active than their 2,3-, 2,4- and 2,6-dichloro positional isomers (compare **28** and **29** with **25**–**27**; **43** with **40**–**42**). Noteworthy, **27**, **38**, **42** and **44** (sharing a 2,6-di-substituted acyl moiety) emerged as the weakest derivatives of the series indicating this pattern as particularly unfavourable for ATC antiviral activity. The chloro-nitrobenzoyl ATCs **31**–**33**, **45**, **46**

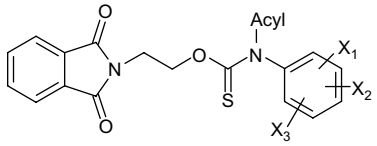
Table 4
Cytotoxicity and anti-HIV-1 activity of ATCs **51**–**60**.[a]



Cpd	A	*n*	R_1	R_2	Acyl	X	CC_{50}[b]	EC_{50}[c]	SI[d]
51	*N*-phthalimido	0	–	H	3-nitrobenzoyl	Cl	>100	>100	–
52[e]	*N*-phthalimido	1	CH_3	H	3-nitrobenzoyl	Cl	77	0.05	1540
53[e]	*N*-phthalimido	1	H	CH_3	3-nitrobenzoyl	Cl	31	0.7	44.3
54	*N*-phthalimido	2	H	H	3-nitrobenzoyl	Cl	53	29	1.8
55	*N*-(3-methyl)phthalimido	1	H	H	3-nitrobenzoyl	Cl	44	0.03	1467
56	*N*-(4-methyl)phthalimido	1	H	H	3-nitrobenzoyl	Cl	>100	0.05	>2000
57	*N*-(4-methyl)phthalimido	1	H	H	2-thenoyl	Cl	>100	0.02	>5000
58	*N*-(4-methyl)phthalimido	1	H	H	2-chloronicotinoyl	Cl	49	0.06	817
59	*N*-(4-methyl)phthalimido	1	H	H	4-chlorobenzoyl	NO_2	>100	0.04	>2500
60	*N*-(*cis*-1,2,3,6-tetrahydrophthalimido)	1	H	H	4-chlorobenzoyl	Cl	>100	83	>1.2
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.
[e] Tested as racemic mixture.

Table 5
Cytotoxicity and anti-HIV-1 activity of ATCs **61**–**66**.[a]



Cpd	Acyl	X_1	X_2	X_3	CC_{50}[b]	EC_{50}[c]	SI[d]
61	2-furoyl	2-Cl	3-Cl	H	100	>100	–
62	2-furoyl	2-Cl	4-Cl	H	>100	2.6	>38
63	3-nitrobenzoyl	2-Cl	6-Cl	H	58	17	3.4
64	2-furoyl	3-Cl	4-Cl	H	100	0.69	145
65	2-furoyl	3-Cl	5-Cl	H	>100	>100	–
66	3-nitrobenzoyl	2-Cl	4-Cl	6-Cl	20	>20	–
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.

proved to be potent derivatives within both the *N*-4-chlorophenyl and the *N*-4-tolyl series.

To further probe the spatial requirements of portion **c**, the bicyclic (hetero)aroyl compounds **48**–**50** were tested (Table 3). The naphthoyl derivatives **48** and **49** showed an EC_{50} comparable with that of **Trovirdine**, being more active than their less hindered benzoyl analogue **II**. Surprisingly, **50** showed a reduced potency in comparison with both its naphthoyl isosters and its thenoyl analogue (compare **50** with **48**, **49** and **V**).

The EC_{50} values of ATCs **51**–**60** (Table 4), embodying some of the most favourable **b** and **c** portions combined with variations of portion **a**, indicate that: i) the ethyl linker shortening or lengthening caused loss or drop in activity, respectively (**51** and **54** vs **5**); ii) the methyl branching of the ethyl linker caused a reduction of potency (compare **52** and **53** with **5**); however, when the methyl group was located on the C-atom adjacent to the phthalimide nitrogen, the activity was kept high, differently from the methylation on the carbon atom next to the thiocarbamic functionality (compare **52** with **53**); iii) the methyl substitution of the phthalimide system was well tolerated (compare **55** and **56** with **5**; **57** with **V**; **58** with **VI**). The partial saturation of the phthalimide benzene ring and the consequent planarity disruption of the bicyclic system caused a dramatic decrease in activity (compare **60** with **III**).

The *N*-dichloro- and *N*-trichloro-substituted phenyl ATCs (**61**–**66**, Table 5) were poorly active or inactive, differently from the corresponding *para* mono-chloro-substituted analogues (compare **63** and **66** with **5**; **61**, **62**, **64** and **65** with **IV**).

To determine whether the title compounds targeted HIV-1 RT, ATCs **10** and **59** were tested by enzyme assay (Table 6). Both derivatives showed good affinity for wt HIV-1 wt RT with IC_{50} values in the sub-micromolar concentration range. The considerable difference of potency in enzyme- and cell-based assays (observed also for TCs [21–23] and the previously reported analogues [10]) could be ascribed to the possibility that ATCs are slow binding kinetics inhibitors [26].

Table 6
Activity of selected ATCs in enzyme assays against the wt HIV-1 wt RT.[a]

Cpd	IC_{50}[b]
10	0.7 ± 0.08
59	0.3 ± 0.05
Efavirenz	0.0013 ± 0.0003

[a] Data mean values for three separate experiments. Variation among triplicate samples was less than 10%.
[b] IC_{50} [μM] ± standard deviation.

Table 7
Activity of selected ATCs against Y181C resistant mutant.[a]

Cpd	EC_{50}[b]		
	wt	Y181C	Ratio[c]
4	0.01	6.0	600
6	0.05	8.5	170
8	0.06	26	433
10	0.2	2.3	11
19	0.008	6.0	750
20	0.01	5.4	540
21	0.02	5.7	285
23	0.008	56	7000
25	0.1	4.2	42
29	0.008	8.2	1025
33	0.02	9.0	450
34	0.007	10.9	1557
35	0.02	7.5	375
49	0.02	11	550
50	0.2	12	60
52	0.05	5.0	100
55	0.03	4.2	140
58	0.06	6.7	112
Trovirdine	0.02	1.2	60
Efavirenz	n.d.[d]	0.01	n.d.[d]

[a] Data mean values for three separate experiments. Variation among triplicate samples was less than 10%.
[b] Compound concentration [μM] required to achieve 50% protection of MT-4 cell from HIV-1 induced cytopathogenicity, as determined by the MTT method.
[c] EC_{50} (Y181C)/EC_{50}(wt) ratio.
[d] Not determined.

A number of ATCs [sharing the *N*-4-(chloro or bromo)phenyl moiety] showed moderate activity against the Y181C strain (EC_{50} range = 2.3–56 μM, Table 7). The most interesting compounds (EC_{50} range 2.3–5.7 μM) bear the 2,4-, 2,5- and 3,5-difluorobenzoyl (**20**, **21** and **24**, respectively), 2,3-dichlorobenzoyl (**25**) or 2-chloronicotinoyl (**10**) moieties. Moreover, the methyl-branched **52** and 3-methyl phthalimidyl **55** (sharing the 3-nitrobenzoyl moiety) were active in the same micromolar concentration range. In addition, ATC **10** showed a modest activity also against the K103R mutation (EC_{50}: 58 μM), differently from all ATCs so far synthesized [10]. No activity against the K103 N/Y181C mutation was detected (data not shown).

4. Modelling studies

To rationalize the most relevant SARs, docking studies (Autodock 3.05) [27] were carried out using the X-ray coordinates of the RT/PETT complex (PDB code: 1DTQ) [28] as the template structure.

The docking model built for the most potent derivative **5** suggested for the inhibitor an extended conformation superimposable with that predicted for **I** [10]. The RT/**5** complex is mainly stabilized by two hydrogen bonds: the former involving the thiocarbonyl sulphur atom and the Lys101 amidic nitrogen (S–NH distance: 2.9 Å), the latter between one of the imidic carbonyls and the Lys103 side chain amino group (Fig. 3). The higher potency of **5**, in comparison with that of the lead **I**, would be explained by the hydrophobic contacts of the chlorine atom with Val179, Ile180, Tyr181 and Tyr188 and by the polar interactions of the 3-nitro group with the Phe227, Leu234, His235 and Pro236 main chain atoms. According to this model, the chlorine atom would be at halogen bond distance (Cl–O distance: 3.1 Å) [29] with the carbonyl oxygen of Val179 main chain, thus rationalizing the higher potency of the *N*-4-chlorophenyl derivatives in comparison with that of their *N*-4-tolyl analogues. It should be noted that Leu234 and

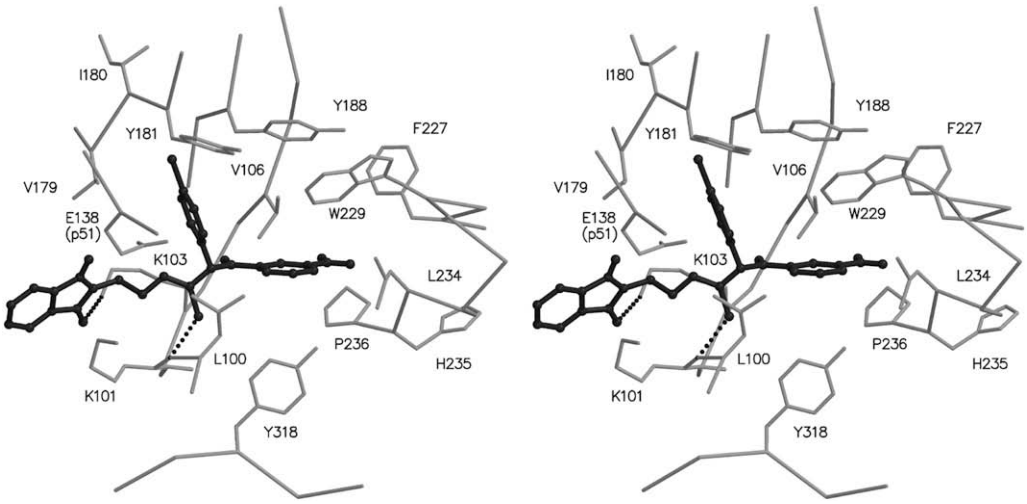


Fig. 3. Stereodiagram showing the position and orientation of **5** modelled structures inside the NNBS. Amino acid residues lining the NNBS are represented by lines and labelled according to the one-letter code. The ligand is displayed in ball-and-sticks model. The hydrogen bonds are shown as dotted lines.

Pro236 (along with Trp229) are highly conserved amino acids in the NNBS and therefore recognized of strategic relevance for the design of new NNRTIs more resilient to the effect of RT mutations in this site [6]. Interestingly, X-ray studies carried out on **14** (a close analogue of **5**) as free ligand [30] showed the acylthiocarbamic group in an *E*,*E*′ conformation, whereas docking calculation predicted a *Z*,*Z*′ arrangement. As a consequence, the modelled and X-ray structures show different torsional angles along the C(S)–N and C(O)–N bonds with an inversion of the positions of the *N*-phenyl and *N*-acyl moieties (Fig. 4).

The docking models of RT/**38**, RT/**42** and RT/**44** complexes rationalize the poor activity of this 2,6-di-substituted derivatives. Thus, the 2,6 pattern induces a conformation change responsible for the loss of one (for **38**) or both hydrogen bonds (for **42** and **44**) relative to the RT/**5** docking model.

The docking models of **49** and **50** showed the two inhibitors in an extended conformation with the naphthoyl moieties in contact with Val106, Gly190, Trp229, Leu234. In addition, the 1-naphthyl is in contact with Tyr188, Tyr318 and the 2-naphthyl interacts with Leu100 and Lys103. The 1-naphthyl and the 2-naphthyl would be rotated of about 129° and 135° relative to the 3-nitrophenyl fragment of **5**, respectively.

5. Conclusion

The parallel synthesis of sixty-six ATCs complemented our SAR strategy centred on molecular modifications of lead **I**. The new ATCs were, in general, more active than those previously described. In particular, **5** emerged as the most potent ATC so far synthesized. Besides the high potency against wild-type HIV-1, several ATCs inhibited the Y181C variant at micromolar concentrations and one of them showed a moderate activity against the K103R mutant, not found in the first ATC series. These results encourage further efforts to improve ATC resilience to the most important drug resistant RT mutations.

6. Experimental section

6.1. Chemistry

6.1.1. General

N-(Hydroxymethyl)phthalimide, *N*-(2-hydroxyethyl)phthalimide, *N*-(3-hydroxypropyl)phthalimide, isothiocyanates, acyl chlorides and reagents (60% sodium hydride dispersion in mineral oil, TMEDA) were purchased from Chiminord and Aldrich Chemical, Milan (Italy). Alcohols $\mathbf{A_{3-6}}$ and $\mathbf{A_8}$ were prepared according to the previously reported protocol [20]. The synthesis of **Trovirdine** was accomplished according to the published procedure [31]. Solvents were of reagent grade (DMF, pyridine). DMF was dried on molecular sieves (5 Å 1/16″ inch pellets). 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 parallel reactions and confirming the purity of analytical samples employed aluminium-packed silica gel plates (Merck DC-Alufolien Kieselgel 60 F254). $CHCl_3$ or $CHCl_3$/methanol was used as a developing solvent and detection of spots was made by UV light and/or by iodine vapours. The parallel solution-phase chemistry was performed by using a 12-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

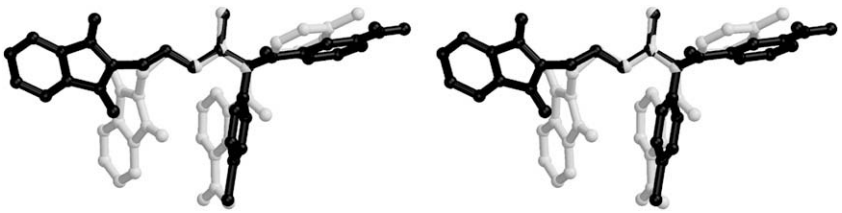


Fig. 4. Stereodiagram showing the superposition of **14** X-ray structure [30] (grey) and **5** docked conformation (black). The superposition has been obtained by overlying the N–C(S)–O atoms in the two structures.

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 δ (ppm) units relative to the internal reference tetramethylsilane, and the splitting patterns were described as follows: s (singlet), d (doublet), t (triplet) and m (multiplet). The first order values reported for coupling constants J were given in Hz. Elemental analyses were performed by an EA1110 Analyser, Fison Instruments (Milan).

*6.1.2. General parallel synthetic procedure P_1 for the preparation of ATCs **1–49**, **52–57**, **59***

Alcohols $\mathbf{A_{2-7}}$ (10 mmol) and isothiocyanates $\mathbf{B_{1-6}}$ (10 mmol) were added to each reaction tube of a Carusel Reaction Station™ and dissolved in anhydrous pyridine (25 mL) under stirring at rt. 60% sodium hydride dispersion in mineral oil (0.44 g, ~10 mmol) was poured in a single portion into each tube; as soon as the hydrogen evolution ceased, TMEDA (1.28 g, 11 mmol; for **10**: 15 mmol; and for **35**: 12 mmol) and the proper acyl chloride (11 mmol; for **10** and **35**: 12 mmol) were added. Each reaction mixture was stirred for 12 h at rt (for **35**: 60 °C for 2 h). Each reaction mixture was transferred into a set of separating funnels, diluted with water (150 mL) and extracted with dichloromethane (30 mL × 2). The combined extracts were washed with water (30 mL × 2), 1 N HCl (30 mL × 2, except for **10**), dried and filtered in parallel through pads of Florisil (diameter 5 × 2 cm). Evaporating in vacuo gave a residue that was purified by crystallization from the suitable solvent or solvent mixture.

6.1.2.1. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-fluorophenyl[4-(pentyloxy)benzoyl]thiocarbamate* ***1***. Mp 166–167 °C (from CH_2Cl_2/Et_2O); yield: 67%. IR (KBr) cm^{-1}: 2938, 1778, 1713, 1693. ^{1}H NMR (DMSO) δ 0.75–1.09 (m, 3H, CH_3), 3.67–4.21 (m, 6H, 3CH_2/pentyl), 3.67–4.21 (m, 4H, CH_2N and CH_2O/pentyl), 4.36–4.73 (m, 2H, CH_2O), 6.78–8.07 (m, 12H, arom H). Calcd for $C_{29}H_{27}FN_2O_5S$: C, 65.15; H, 5.09; N, 5.24; S, 6.00. Found: C, 65.28; H, 5.23; N, 5.29; S, 5.77.

6.1.2.2. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2-methylbenzoyl)thiocarbamate* ***2***. Mp 114–116 °C (from CH_2Cl_2/Et_2O); yield: 60%. IR (KBr) cm^{-1} 1776, 1708. ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 3.75 (t, J = 6.0 Hz, 2H, CH_2N), 4.49 (t, J = 6.0 Hz, 2H, CH_2O), 6.94–7.97 (m, 12H, arom H). Calcd for $C_{25}H_{19}ClN_2O_4S$: C, 62.69; H, 4.00; N, 5.85; S, 6.69. Found: C, 62.43; H, 4.22; N, 5.66; S, 6.82.

6.1.2.3. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2-nitrobenzoyl)thiocarbamate* ***3***. Mp 178–179 °C (from CH_2Cl_2/EtOH); yield: 69%. IR (KBr) cm^{-1} 1768, 1717. ^{1}H NMR ($CDCl_3$) δ 3.77 (t, J = 6.0 Hz, 2H, CH_2N), 4.53 (t, J = 6.0 Hz, 2H, CH_2O), 7.28–8.38 (m, 12H, arom H). Calcd for $C_{24}H_{16}ClN_3O_6S$: C, 56.53; H, 3.16; N, 8.24; S, 6.29. Found: C, 56.24; H, 3.14; N, 8.21; S, 6.19.

6.1.2.4. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(3-methylbenzoyl)thiocarbamate* ***4***. Mp 134–136 °C (from CH_2Cl_2/EtOH); yield: 88%. IR (KBr) cm^{-1} 1777, 1709. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3), 3.83 (t, J = 6.0 Hz, 2H, CH_2N), 4.63 (t, J = 6.0 Hz, 2H, CH_2O), 7.08–7.94 (m, 12H, arom H). Calcd for $C_{25}H_{19}ClN_2O_4S$: C, 62.69; H, 4.00; N, 5.85; S, 6.69. Found: C, 62.54; H, 4.03; N, 5.76; S, 6.42.

6.1.2.5. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(3-nitrobenzoyl)thiocarbamate* ***5***. Mp 167–169 °C (from CH_2Cl_2/EtOH); yield: 83%. IR (KBr) cm^{-1} 1777, 1711. ^{1}H NMR ($CDCl_3$) δ 4.00 (t, J = 6.0 Hz, 2H, CH_2N), 4.68 (t, J = 6.0 Hz, 2H, CH_2O), 7.21–8.76 (m, 12H, arom H). Calcd for $C_{24}H_{16}ClN_3O_6S$: C, 56.53; H, 3.16; N, 8.24; S, 6.29. Found: C, 56.42; H, 3.35; N, 8.26; S, 6.30.

6.1.2.6. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(4-methylbenzoyl)thiocarbamate* ***6***. Mp 175–176 °C (from CH_2Cl_2/Et_2O); yield: 71%. IR (KBr) cm^{-1} 1777, 1712. ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 3.86 (t, J = 6.0 Hz, 2H, CH_2N), 4.63 (t, J = 6.0 Hz, 2H, CH_2O), 6.99–7.39 and 7.62–7.95 (m, 12H, arom H). Calcd for $C_{25}H_{19}ClN_2O_4S$: C, 62.69; H, 4.00; N, 5.85; S, 6.69. Found: C, 62.54; H, 4.11; N, 5.84; S, 6.58.

6.1.2.7. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(4-trifluorometylbenzoyl)thiocarbamate* ***7***. Mp 166–168 °C (from CH_2Cl_2/EtOH); yield: 18%. IR (KBr) cm^{-1} 1777, 1712. ^{1}H NMR ($CDCl_3$) δ 3.88 (t, J = 6.0 Hz, 2H, CH_2N), 4.63 (t, J = 6.0 Hz, 2H, CH_2O), 7.07–8.14 (m, 12H, arom H). Calcd for $C_{25}H_{16}ClF_3N_2O_4S$: C, 56.35; H, 3.03; N, 5.26; S, 6.02. Found: C, 56.25; H, 3.18; N, 5.19; S, 6.13.

6.1.2.8. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(4-nitrobenzoyl)thiocarbamate* ***8***. Mp 190–192 °C (from CH_2Cl_2/EtOH); yield: 39%. IR (KBr) cm^{-1} 1775, 1711. ^{1}H NMR ($CDCl_3$) δ 3.89 (t, J = 6.0 Hz, CH_2N), 4.65 (t, J = 6.0 Hz, CH_2O), 7.15–7.38 and 7.60–8.40 (m, 12H, arom H). Calcd for $C_{24}H_{16}ClN_3O_6S$: C, 56.53; H, 3.16; N, 8.24; S, 6.29. Found: C, 56.82; H, 3.08; N, 7.94; S, 6.15.

6.1.2.9. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-bromobenzoyl(4-bromophenyl)thiocarbamate* ***9***. Mp 185–186 °C (from CH_2Cl_2/EtOH); yield: 57%. IR (KBr) cm^{-1} 1775, 1709. ^{1}H NMR ($CDCl_3$) δ 3.90 (t, J = 6.0 Hz, 2H, CH_2N), 4.66 (t, J = 6.0 Hz, 2H, CH_2O), 7.02–7.97 (m, 12H, arom H). Calcd for $C_{24}H_{16}Br_2N_2O_4S$: C, 49.00; H, 2.74; N, 4.76; S, 5.45. Found: C, 48.84; H, 3.04; N, 4.53; S, 5.52.

6.1.2.10. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-bromophenyl[(2-chloropyridin-3-yl)carbonyl]thiocarbamate* ***10***. Mp 165–167 °C (from MeOH); yield: 67%. IR (KBr) cm^{-1} 1778, 1710, 1668. ^{1}H NMR ($CDCl_3$) δ 3.80 (t, J = 6.0 Hz, 2H, CH_2N), 4.59 (t, J = 6.0 Hz, 2H, CH_2O), 7.16–7.29 and 7.76–7.89 (m, 8H, arom H), 7.46–7.50 and 8.41–8.44 (m, 3H, py H). Calcd for $C_{23}H_{15}BrClN_3O_4S$: C, 50.71; H, 2.78; N, 7.71; S, 5.89. Found: C, 50.36; H, 2.90; N, 7.57; S, 5.69.

6.1.2.11. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-methylbenzoyl(4-methylphenyl)thiocarbamate* ***11***. Mp 135–137 °C (from CH_2Cl_2/EtOH); yield: 27%. IR (KBr) cm^{-1} 1775, 1715, 1682. ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 2.42 (s, 3H, CH_3/acyl), 3.71 (t, J = 6.0 Hz, 2H, CH_2N), 4.48 (t, J = 6.0 Hz, 2H, CH_2O), 6.98–8.02 (m, 12H, arom H). Calcd for $C_{26}H_{22}N_2O_4S$: C, 68.11; H, 4.84; N, 6.11; S, 6.99. Found: C, 68.36; H, 4.86; N, 6.14; S, 6.79.

6.1.2.12. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-methylphenyl(2-nitrobenzoyl)thiocarbamate* ***12***. Mp 179–181 °C (from CH_2Cl_2/EtOH); yield: 43%. IR (KBr) cm^{-1} 1768, 1717. ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H, CH_3), 3.76 (t, J = 6.0 Hz, 2H, CH_2N), 4.54 (t, J = 6.0 Hz, 2H, CH_2O), 7.00–8.34 (m, 12H, arom H). Calcd for $C_{25}H_{19}N_3O_6S$: C, 61.34; H, 3.91; N, 8.58; S, 6.55. Found: C, 61.41; H, 3.89; N, 8.62; S, 6.50.

6.1.2.13. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3-methylbenzoyl(4-methylphenyl)thiocarbamate* ***13***. Mp 137–139 °C (from CH_2Cl_2/EtOH); yield: 42%. IR (KBr) cm^{-1} 1775, 1717. ^{1}H NMR ($CDCl_3$) δ 2.20–2.47 (m, 6H, 2CH_3), 3.77 (t, J = 6.0 Hz, 2H, CH_2N), 4.61 (t, J = 6.0 Hz, 2H, CH_2O), 7.11–8.19 (m, 12H, arom H). Calcd for $C_{26}H_{22}N_2O_4S$: C, 68.11; H, 4.84; N, 6.11; S, 6.99. Found: C, 68.12; H, 4.82; N, 6.10; S, 6.68.

6.1.2.14. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-methylphenyl(3-nitrobenzoyl)thiocarbamate* ***14***. Mp 178–179 °C (from CH_2Cl_2/EtOH); yield: 40%. IR (KBr) cm^{-1} 1771, 1712, 1688. ^1H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 3.87 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.67 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.07–8.77 (m, 12H, arom H). Calcd for $C_{25}H_{19}N_3O_6S$: C, 61.34; H, 3.91; N, 8.58; S, 6.55. Found: C, 61.31; H, 3.91; N, 8.52; S, 6.55.

6.1.2.15. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-methylbenzoyl(4-methylphenyl)thiocarbamate* ***15***. Mp 142–144 °C (from CH_2Cl_2/EtOH); yield: 48%. IR (KBr) cm^{-1} 1773, 1710. ^1H NMR ($CDCl_3$) δ 2.29 (s, 6H, $2CH_3$), 3.86 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.64 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.97–7.97 (m, 12H, arom H). Calcd for $C_{26}H_{22}N_2O_4S$: C, 68.11; H, 4.84; N, 6.11; S, 6.99. Found: C, 68.18; H, 4.91; N, 6.16; S, 6.77.

6.1.2.16. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-methylphenyl(4-nitrobenzoyl)thiocarbamate* ***16***. Mp 170–172 °C (from CH_2Cl_2/EtOH); yield: 25%. IR (KBr) cm^{-1} 1776, 1709. ^1H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 3.90 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.70 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.08–8.39 (m, 12H, arom H). Calcd for $C_{25}H_{19}N_3O_6S$: C, 61.34; H, 3.91; N, 8.58; S, 6.55. Found: C, 61.34; H, 3.91; N, 8.56; S, 6.47.

6.1.2.17. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3,4-dichlorobenzoyl(phenyl)thiocarbamate* ***17***. Mp 127–129 °C (from CH_2Cl_2/Et_2O); yield: 57%. IR (KBr) cm^{-1} 3088, 3071, 1777, 1715. ^1H NMR (DMSO) δ 3.85 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.61 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.20–8.10 (m, 12H, arom H). Calcd for $C_{24}H_{16}Cl_2N_2O_4S$: C, 57.73; H, 3.23; N, 5.61; S, 6.42. Found: C, 57.64; H, 3.10; N, 5.32; S, 6.12.

6.1.2.18. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chloro-3-nitrobenzoyl(phenyl)thiocarbamate* ***18***. Mp 120–121 °C (from CH_2Cl_2/Et_2O); yield: 12%. IR (KBr) cm^{-1} 1775, 1717. ^1H NMR (DMSO) δ 3.82 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.61 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.08–8.48 (m, 12H, arom H). Calcd for $C_{24}H_{16}ClN_3O_6S$: C, 56.53; H, 3.16; N, 8.24; S, 6.29. Found: C, 56.39; H, 3.00; N, 8.28; S, 6.10.

6.1.2.19. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,3-difluorobenzoyl)thiocarbamate* ***19***. Mp 149–150 °C (from CH_2Cl_2/EtOH); yield: 82%. IR (KBr) cm^{-1} 1776, 1708. ^1H NMR ($CDCl_3$) δ 3.86 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.65 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.81–8.02 (m, 11H, arom H). Calcd for $C_{24}H_{15}ClF_2N_2O_4S$: C, 57.55; H, 3.02; N, 5.59; S, 6.40. Found: C, 57.35; H, 2.98; N, 5.54; S, 6.62.

6.1.2.20. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,4-difluorobenzoyl)thiocarbamate* ***20***. Mp 121–123 °C (from Et_2O/EtOH); yield: 70%. IR (KBr) cm^{-1} 1777, 1713. ^1H NMR ($CDCl_3$) δ 3.87 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.58 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.13–8.01 (m, 11H, arom H). Calcd for $C_{24}H_{15}ClF_2N_2O_4S$: C, 57.55; H, 3.02; N, 5.59; S, 6.40. Found: C, 57.72; H, 3.27; N, 5.45; S, 6.29.

6.1.2.21. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,5-difluorobenzoyl)thiocarbamate* ***21***. Mp 148–149 °C (from CH_2Cl_2/EtOH); yield: 56%. IR (KBr) cm^{-1} 1778, 1711, 1683. ^1H NMR ($CDCl_3$) δ 3.84 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.65 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.89–8.10 (m, 11H, arom H). Calcd for $C_{24}H_{15}ClF_2N_2O_4S$: C, 57.55; H, 3.02; N, 5.59; S, 6.40. Found: C, 57.18; H, 3.07; N, 5.52; S, 6.37.

6.1.2.22. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,6-difluorobenzoyl)thiocarbamate* ***22***. Mp 135–137 °C (from CH_2Cl_2/EtOH); yield: 48%. IR (KBr) cm^{-1} 1775, 1709, 1692. ^1H NMR ($CDCl_3$) δ 3.87 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.63 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.61–8.02 (m, 11H, arom H). Calcd for $C_{24}H_{15}ClF_2N_2O_4S$: C, 57.55; H, 3.02; N, 5.59; S, 6.40. Found: C, 57.20; H, 3.13; N, 5.55; S, 6.18.

6.1.2.23. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(3,4-difluorobenzoyl)thiocarbamate* ***23***. Mp 188–190 °C (from CH_2Cl_2/EtOH); yield: 45%. IR (KBr) cm^{-1} 1777, 1710. ^1H NMR ($CDCl_3$) δ 3.78–4.08 (m, 2H, CH_2N), 4.50–4.81 (m, 2H, CH_2O), 7.05–8.06 (m, 11H, arom H). Calcd for $C_{24}H_{15}ClF_2N_2O_4S$: C, 57.55; H, 3.02; N, 5.59; S, 6.40. Found: C, 57.84; H, 2.90; N, 5.57; S, 6.38.

6.1.2.24. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(3,5-difluorobenzoyl)thiocarbamate* ***24***. Mp 148–149 °C (from CH_2Cl_2/EtOH); yield: 65%. IR (KBr) cm^{-1} 1776, 1715, 1695. ^1H NMR ($CDCl_3$) δ 3.85 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.65 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.91–8.01 (m, 11H, arom H). Calcd for $C_{24}H_{15}ClF_2N_2O_4S$: C, 57.55; H, 3.02; N, 5.59; S, 6.40. Found: C, 57.50; H, 3.19; N, 5.51; S, 6.32.

6.1.2.25. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,3-dichlorobenzoyl)thiocarbamate* ***25***. Mp 189–191 °C (from CH_2Cl_2/EtOH); yield: 37%. IR (KBr) cm^{-1} 1773, 1706. ^1H NMR ($CDCl_3$) δ 3.80 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.57 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.78–8.05 (m, 11H, arom H). Calcd for $C_{24}H_{15}Cl_3N_2O_4S$: C, 54.00; H, 2.83; N, 5.25; S, 6.01. Found: C, 53.93; H, 2.84; N, 5.18; S, 6.21.

6.1.2.26. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,4-dichlorobenzoyl)thiocarbamate* ***26***. Mp 123–125 °C (from CH_2Cl_2/EtOH); yield: 37%. IR (KBr) cm^{-1} 1769, 1713, 1698. ^1H NMR ($CDCl_3$) δ 3.81 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.60 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.00–8.10 (m, 11H, arom H). Calcd for $C_{24}H_{15}Cl_3N_2O_4S$: C, 54.00; H, 2.83; N, 5.25; S, 6.01. Found: C, 53.78; H, 3.00; N, 5.11; S, 5.88.

6.1.2.27. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,6-dichlorobenzoyl)thiocarbamate* ***27***. Mp 154–156 °C (from CH_2Cl_2/EtOH); yield: 43%. IR (KBr) cm^{-1} 1767, 1706. ^1H NMR ($CDCl_3$) δ 3.84 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.68 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.00–8.20 (m, 11H, arom H). Calcd for $C_{24}H_{15}Cl_3N_2O_4S$: C, 54.00; H, 2.83; N, 5.25; S, 6.01. Found: C, 53.78; H, 2.92; N, 5.18; S, 5.82.

6.1.2.28. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(3,4-dichlorobenzoyl)thiocarbamate* ***28***. Mp 162–164 °C (from CH_2Cl_2/EtOH); yield: 70%. IR (KBr) cm^{-1} 1777, 1710. ^1H NMR ($CDCl_3$) δ 3.97 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.65 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.01–8.21 (m, 11H, arom H). Calcd for $C_{24}H_{15}Cl_3N_2O_4S$: C, 54.00; H, 2.83; N, 5.25; S, 6.01. Found: C, 54.14; H, 2.65; N, 5.13; S, 6.06.

6.1.2.29. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(3,5-dichlorobenzoyl)thiocarbamate* ***29***. Mp 145–147 °C (from CH_2Cl_2/EtOH); yield: 73%. IR (KBr) cm^{-1} 1778, 1715. ^1H NMR ($CDCl_3$) δ 3.94 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.61 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.18–8.08 (m, 11H, arom H). Calcd for $C_{24}H_{15}Cl_3N_2O_4S$: C, 54.00; H, 2.83; N, 5.25; S, 6.01. Found: C, 54.28; H, 2.96; N, 5.02; S, 6.18.

6.1.2.30. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,6-dimethoxybenzoyl)thiocarbamate* ***30***. Mp 158–159 °C (from CH_2Cl_2/Et_2O); yield: 45%. IR (KBr) cm^{-1} 1777, 1716, 1597. ^1H NMR ($CDCl_3$) δ 3.50 (m, 8H, CH_2N and $2CH_3O$), 4.51 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.31–6.68 and 7.01–7.98 (m, 11H, arom H). Calcd for $C_{26}H_{21}ClN_2O_6S$: C, 59.49; H, 4.03; N, 5.34; S, 6.11. Found: C, 59.48; H, 4.23; N, 5.52; S, 5.96.

6.1.2.31. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-chloro-4-nitrobenzoyl(4-chlorophenyl)thiocarbamate* ***31***. Mp 168–170 °C (from CH_2Cl_2/EtOH); yield: 50%. IR (KBr) cm^{-1} 1772, 1715,

1695. ^{1}H NMR ($CDCl_3$) δ 3.62 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.60 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.10–8.32 (m, 11H, arom H). Calcd for $C_{24}H_{15}Cl_2N_3O_6S$: C, 52.95; H, 2.78; N, 7.72; S, 5.89. Found: C, 52.61; H, 2.83; N, 7.65; S, 5.74.

6.1.2.32. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-chloro-5-nitrobenzoyl(4-chlorophenyl)thiocarbamate* ***32***. Mp 109–110 °C (from CH_2Cl_2/EtOH); yield: 30%. IR (KBr) cm^{-1} 1776, 1710. ^{1}H NMR ($CDCl_3$) δ 3.68–4.00 (m, 2H, CH_2N), 4.41–4.79 (m, 2H, CH_2O), 7.21–8.48 (m, 11H, arom H). Calcd for $C_{24}H_{15}Cl_2N_3O_6S$: C, 52.95; H, 2.78; N, 7.72; S, 5.89. Found: C, 53.16; H, 2.88; N, 7.50; S, 5.84.

6.1.2.33. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chloro-3-nitrobenzoyl(4-chlorophenyl)thiocarbamate* ***33***. Mp 119–121 °C (from CH_2Cl_2/EtOH); yield: 41%. IR (KBr) cm^{-1} 1777, 1712. ^{1}H NMR ($CDCl_3$) δ 3.96 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.71 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.01–8.50 (m, 11H, arom H). Calcd for $C_{24}H_{15}Cl_2N_3O_6S$: C, 52.95; H, 2.78; N, 7.72; S, 5.89. Found: C, 52.77; H, 3.08; N, 7.54; S, 5.72.

6.1.2.34. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-bromo-3-methylbenzoyl(4-chlorophenyl) thiocarbamate* ***34***. Mp 138–140 °C (from CH_2Cl_2/Et_2O); yield: 44%. IR (KBr) cm^{-1} 1776, 1710. ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 3.88 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.67 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.20–7.94 (m, 11H, arom H). Calcd for $C_{25}H_{18}BrClN_2O_4S$: C, 53.83; H, 3.25; N, 5.02; S, 5.75. Found: C, 53.64; H, 3.23; N, 4.98; S, 5.85.

6.1.2.35. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2,5-dimethyl-3-furoyl)thiocarbamate* ***35***. Mp 118–119 °C (from Et_2O); yield: 39%. IR (KBr) cm^{-1} 1775, 1714. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.90 (t, $J = 5.6$ Hz, CH_2N), 4.63 (t, $J = 5.6$ Hz, CH_2O), 5.94 (s, 1H, CH fur.), 7.09–7.24 (m, 4H, arom H), 7.66–7.79 (m, 4 H, phtha. arom H). Calcd for $C_{24}H_{19}ClN_2O_5S$: C, 59.69; H, 3.97; N, 5.80; S, 6.64. Found: C, 59.35; H, 4.21; N, 5.86; S, 6.60.

6.1.2.36. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,4-difluorobenzoyl(4-methylphenyl)thiocarbamate* ***36***. Mp 135–137 °C (from Et_2O/EtOH); yield: 27%. IR (KBr) cm^{-1} 1773, 1715, 1691. ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 3.84 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.68 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.61–7.26 and 7.75–7.96 (m, 11H, arom H). Calcd for $C_{25}H_{18}F_2N_2O_4S$: C, 62.49; H, 3.78; N, 5.83; S, 6.67. Found: C, 62.21; H, 3.77; N, 5.55; S, 6.50.

6.1.2.37. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,5-difluorobenzoyl(4-methylphenyl)thiocarbamate* ***37***. Mp 144–146 °C (from CH_2Cl_2/EtOH); yield: 40%. IR (KBr) cm^{-1} 1772, 1715, 1681. ^{1}H NMR ($CDCl_3$) δ 2.29 (s, 3H, CH_3), 3.82 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.66 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.85–8.00 (m, 11H, arom H). Calcd for $C_{25}H_{18}F_2N_2O_4S$: C, 62.49; H, 3.78; N, 5.83; S, 6.67. Found: C, 62.35; H, 3.74; N, 5.82; S, 6.55.

6.1.2.38. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,6-difluorobenzoyl(4-methylphenyl)thiocarbamate* ***38***. Mp 146–148 °C (from CH_2Cl_2/Et_2O); yield: 32%. IR (KBr) cm^{-1} 1766, 1711. ^{1}H NMR ($CDCl_3$) δ 2.29 (s, 3H, CH_3), 3.84 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.63 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.65–8.01 (m, 11H, arom H). Calcd for $C_{25}H_{18}F_2N_2O_4S$: C, 62.49; H, 3.78; N, 5.83; S, 6.67. Found: C, 62.21; H, 3.63; N, 5.74; S, 6.94.

6.1.2.39. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3,5-difluorobenzoyl(4-methylphenyl)thiocarbamate* ***39***. Mp 132–134 °C (from CH_2Cl_2/EtOH); yield: 45%. IR (KBr) cm^{-1} 1779, 1713. ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 3.90 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.69 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.03–7.92 (m, 12H, arom H). Calcd for $C_{25}H_{18}F_2N_2O_4S$: C, 62.49; H, 3.78; N, 5.83; S, 6.67. Found: C, 62.22; H, 3.80; N, 5.70; S, 6.50.

6.1.2.40. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,3-dichlorobenzoyl(4-methylphenyl)thiocarbamate* ***40***. Mp 114–116 °C (from CH_2Cl_2/EtOH); yield: 32%. IR (KBr) cm^{-1} 1773, 1715, 1685. ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 3.78 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.54 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.09–8.01 (m, 11H, arom H). Calcd for $C_{25}H_{18}Cl_2N_2O_4S$: C, 58.49; H, 3.53; N, 5.46; S, 6.24. Found: C, 58.69; H, 3.62; N, 5.38; S, 6.26.

6.1.2.41. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,4-dichlorobenzoyl(4-methylphenyl)thiocarbamate* ***41***. Mp 112–114 °C (from CH_2Cl_2/EtOH); yield: 47%. IR (KBr) cm^{-1} 1777, 1711. ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 3.81 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.59 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.07–7.96 (m, 11H, arom H). Calcd for $C_{25}H_{18}Cl_2N_2O_4S$: C, 58.49; H, 3.53; N, 5.46; S, 6.24. Found: C, 58.39; H, 3.64; N, 5.38; S, 6.15.

6.1.2.42. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,6-dichlorobenzoyl(4-methylphenyl)thiocarbamate* ***42***. Mp 162–164 °C (from CH_2Cl_2/EtOH); yield: 20%. IR (KBr) cm^{-1} 1771, 1713. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3), 3.82 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.68 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.01–8.04 (m, 11H, arom H). Calcd for $C_{25}H_{18}Cl_2N_2O_4S$: C, 58.49; H, 3.53; N, 5.46; S, 6.24. Found: C, 58.34; H, 3.55; N, 5.40; S, 6.01.

6.1.2.43. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3,4-dichlorobenzoyl(4-methylphenyl)thiocarbamate* ***43***. Mp 126–128 °C (from CH_2Cl_2/EtOH); yield: 58%. IR (KBr) cm^{-1} 1774, 1709. ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 3.91 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.70 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.09–8.08 (m, 11H, arom H). Calcd for $C_{25}H_{18}Cl_2N_2O_4S$: C, 58.49; H, 3.53; N, 5.46; S, 6.24. Found: C, 58.17; H, 3.72; N, 5.17; S, 6.21.

6.1.2.44. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,6-dimethoxybenzoyl(4-methylphenyl)thiocarbamate* ***44***. Mp 115–117 °C (from CH_2Cl_2/EtOH); yield: 18%. IR (KBr) cm^{-1} 1770, 1709. ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 3.54–3.91 (m, 8H, CH_2N and $2CH_3O$), 4.50 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.36–6.63 and 7.01–7.97 (m, 11H, arom H). Calcd for $C_{27}H_{24}N_2O_6S$: C, 64.24; H, 4.79; N, 5.55; S, 6.35. Found: C, 64.12; H, 4.87; N, 5.58; S, 6.15.

6.1.2.45. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-chloro-4-nitrobenzoyl(4-methylphenyl)thiocarbamate* ***45***. Mp 141–143 °C (from CH_2Cl_2/EtOH); yield: 39%. IR (KBr) cm^{-1} 1772, 1715, 1691. ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 3.87 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.66 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.11–7.29 and 7.72–8.31 (m, 11H, arom H). Calcd for $C_{25}H_{18}ClN_3O_6S$: C, 57.31; H, 3.46; N, 8.02; S, 6.12. Found: C, 57.32; H, 3.26; N, 7.90; S, 6.23.

6.1.2.46. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chloro-3-nitrobenzoyl(4-methylphenyl)thiocarbamate* ***46***. Mp 135–137 °C (from CH_2Cl_2/EtOH); yield: 26%. IR (KBr) cm^{-1} 1772, 1717. ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 3.98 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.76 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.08–7.28 and 7.68–8.52 (m, 11H, arom H). Calcd for $C_{25}H_{18}ClN_3O_6S$: C, 57.31; H, 3.46; N, 8.02; S, 6.12. Found: C, 57.34; H, 3.39; N, 7.82; S, 6.15.

6.1.2.47. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-bromo-3-methylbenzoyl(4-methylphenyl)thiocarbamate* ***47***. Mp 174–176 °C (from CH_2Cl_2/EtOH); yield: 40%. IR (KBr) cm^{-1} 1772, 1710. ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 2.40 (s, 3H, CH_3/acyl), 3.87 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.68 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.05–7.97 (m, 12H, arom H). Calcd for $C_{26}H_{21}BrN_2O_4S$: C, 58.11; H, 3.94; N, 5.21; S, 5.97. Found: C, 58.38; H, 4.05; N, 5.19; S, 6.07.

6.1.2.48. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(1-naphthoyl)thiocarbamate* ***48***. Mp 139–140 °C (from CH_2Cl_2/EtOH); yield: 41%. IR (KBr) cm^{-1} 1776, 1710. ^1H NMR ($CDCl_3$) δ 3.60 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.36 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.27–8.42 (m, 15H, arom H). Calcd for $C_{28}H_{19}ClN_2O_4S$: C, 65.30; H, 3.72; N, 5.44; S, 6.23. Found: C, 63.05; H, 3.75; N, 5.40; S, 6.34.

6.1.2.49. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(2-naphthoyl)thiocarbamate* ***49***. Mp 163–165 °C (from CH_2Cl_2/EtOH); yield: 48%. IR (KBr) cm^{-1} 1777, 1709. ^1H NMR ($CDCl_3$) δ 3.84 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.66 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.28–8.16 (m, 15H, arom H). Calcd for $C_{28}H_{19}ClN_2O_4S$: C, 65.30; H, 3.72; N, 5.44; S, 6.23. Found: C, 65.05; H, 3.93; N, 5.40; S, 6.37.

6.1.2.50. (±)O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl] 4-chlorophenyl(3-nitrobenzoyl)thiocarbamate* ***52***. Mp 173–175 °C (from CH_2Cl_2/EtOH); yield: 39%. IR (KBr) cm^{-1} 1777, 1708. ^1H NMR ($CDCl_3$) δ 1.37 (d, $J = 6.0$ Hz, 3H, CH_3), 4.56–5.01 (m, 3H, CH and CH_2O), 7.06–7.90 (m, 12H, arom H). Calcd for $C_{25}H_{18}ClN_3O_6S$: C, 57.31; H, 3.46; N, 8.02; S, 6.12. Found: C, 57.64; H, 3.59; N, 7.93; S, 6.04.

6.1.2.51. (±)O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-methylethyl] 4-chlorophenyl(3-nitrobenzoyl)thiocarbamate* ***53***. Mp 159–161 °C (from CH_2Cl_2/Et_2O); yield: 13%. IR (KBr) cm^{-1} 1780, 1713. ^1H NMR ($CDCl_3$) δ 1.05 (d, $J = 6.0$ Hz, 3H, CH_3), 3.65–4.00 (m, 3H, CH and CH_2N), 7.20–8.74 (m, 12H, arom H). Calcd for $C_{25}H_{18}ClN_3O_6S$: C, 57.31; H, 3.46; N, 8.02; S, 6.12. Found: C, 57.02; H, 3.78; N, 7.93; S, 6.32.

6.1.2.52. O-*[3-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl] 4-chlorophenyl(3-nitrobenzoyl)thiocarbamate* ***54***. Mp 144–146 °C (from CH_2Cl_2/Et_2O); yield: 16%. IR (KBr) cm^{-1} 1772, 1718, 1699. ^1H NMR ($CDCl_3$) δ 1.53–2.17 (m, 2H, C–CH_2–C), 3.25–3.73 (m, 2H, CH_2N), 4.12–4.54 (m, 2H, CH_2O), 7.07–8.75 (m, 12H, arom H). Calcd for $C_{25}H_{18}ClN_3O_6S$: C, 57.31; H, 3.46; N, 8.02; S, 6.12. Found: C, 57.68; H, 3.56; N, 8.08; S, 6.25.

6.1.2.53. O-*[2-(4-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(3-nitrobenzoyl)thiocarbamate* ***55***. Mp 140–142 °C (from CH_2Cl_2/EtOH); yield: 36%. IR (KBr) cm^{-1} 1766, 1707, 1691. ^1H NMR ($CDCl_3$) δ 2.70 (s, 3H, CH_3), 3.88 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.69 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.02–8.68 (m, 11H, arom H). Calcd for $C_{25}H_{18}ClN_3O_6S$: C, 57.31; H, 3.46; N, 8.02; S, 6.12. Found: C, 57.18; H, 3.69; N, 7.89; S, 6.30.

6.1.2.54. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(3-nitrobenzoyl)thiocarbamate* ***56***. Mp 169–170 °C (from CH_2Cl_2/EtOH); yield: 47%. IR (KBr) cm^{-1} 1773, 1712. ^1H NMR ($CDCl_3$) δ 2.66 (s, 3H, CH_3), 3.88 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.62 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.10–7.80 (m, 11H, arom H). Calcd for $C_{25}H_{18}ClN_3O_6S$: C, 57.31; H, 3.46; N, 8.02; S, 6.12. Found: C, 57.13; H, 3.52; N, 8.00; S, 6.25.

6.1.2.55. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl(thien-2-ylcarbonyl)thiocarbamate* ***57***. Mp 142–143 °C (from CH_2Cl_2/EtOH); yield: 46%. IR (KBr) cm^{-1} 1775, 1716. ^1H NMR (DMSO) δ 2.51 (s, 3H, CH_3), 3.89 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.79 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.98–8.09 (m, 10H, 7 arom H and 3H thioph). Calcd for $C_{23}H_{17}ClN_2O_4S_2$: C, 56.96; H, 3.53; N, 5.78; S, 13.22. Found: C, 57.10; H, 3.31; N, 5.75; S, 13.00.

6.1.2.56. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorobenzoyl(4-nitrophenyl)thiocarbamate* ***59***. Mp 194–197 °C (from CH_2Cl_2/EtOH); yield: 34%. IR (KBr) cm^{-1} 1770, 1714. ^1H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 3.88 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.66 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.16–8.41 (m, 11H, arom H). Calcd for $C_{25}H_{18}ClN_3O_6S$: C, 57.31; H, 3.46; N, 8.02; S, 6.12. Found: C, 57.00; H, 3.24; N, 7.99; S, 5.96.

6.1.3. General parallel synthetic procedure P_2 for the preparation of ATCs ***50***, ***51***, ***58***, ***60–66***

60% sodium hydride dispersion in mineral oil (0.44 g, ~10 mmol) was poured in a single portion into each of the 12-Carousel Reaction Station™ tubes containing a stirred, dry DMF (25 mL), ice-cooled solution of starting alcohols $\mathbf{A_1}$, $\mathbf{A_2}$, $\mathbf{A_4}$, $\mathbf{A_8}$ (10 mmol) and isothiocyanates $\mathbf{B_4}$, $\mathbf{B_{7-12}}$ (10 mmol). Each reaction mixture was allowed to react for 1 h under stirring and then TMEDA (1.28 g, 11 mmol) and the proper acyl chloride (11 mmol) were added. The resulting mixtures were stirred at rt for 12 h [for **58**: 6 h. In the case of **60–62**, **64** and **65** the reaction mixtures were heated at 65–70 °C for 1.5 h and then kept under stirring at room temperature for 12 h. For **50** an extra aliquot of acyl chloride (11 mmol) and TMEDA (11 mmol) was added and then the mixture was heated at 75 °C for 15 min]. Each reaction mixture was transferred into a set of separating funnels, diluted with water (150 mL) and extracted with dichloromethane (30 mL × 2). The combined extracts were washed with water (20 mL × 4) and 1 M HCl (20 mL × 2) (except for **50** and **58**), dried, filtered through a plug of Florisil (diameter 5 × 2 cm) and evaporated under reduced pressure to give a residue that was purified by crystallization from the suitable solvent or solvent mixture.

6.1.3.1. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 1-benzothien-2-ylcarbonyl(4-chlorophenyl)thiocarbamate* ***50***. Mp 145–147 °C (from Et_2O); yield: 61%. IR (KBr) cm^{-1} 1777, 1713. ^1H NMR ($CDCl_3$) δ 3.80 (t, $J = 4.8$ Hz, 2H, CH_2N), 4.77 (t, $J = 4.8$ Hz, 2H, CH_2O), 7.20–7.50 and 7.60–8.08 (m, 13H, arom H). Calcd for $C_{26}H_{17}ClN_2O_4S_2$: C, 59.94; H, 3.29; N, 5.38; S, 12.31. Found: C, 60.32; H, 3.28; N, 5.26; S, 12.05.

6.1.3.2. O-*[(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl] 4-chlorophenyl(3-nitrobenzoyl)thiocarbamate* ***51***. Mp 135–137 °C (from CH_2Cl_2/EtOH); yield: 18%. IR (KBr) cm^{-1} 1782, 1722. ^1H NMR ($CDCl_3$) δ 5.82 (s, 2H, CH_2), 7.17–8.47 (m, 12H, arom H). Calcd for $C_{23}H_{14}ClN_3O_6S$: C, 55.71; H, 2.85; N, 8.47; S, 6.47. Found: C, 55.91; H, 3.03; N, 8.40; S, 6.44.

6.1.3.3. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chlorophenyl[(2-chloropyridin-3-yl)carbonyl]thiocarbamate* ***58***. Mp 156–158 °C (from CH_2Cl_2/EtOH); yield: 68%. IR (KBr) cm^{-1} 1773, 1710. ^1H NMR ($CDCl_3$) δ 2.56 (s, 3H, CH_3), 3.79 (t, $J = 5.2$ Hz, 2H, CH_2N), 4.58 (t, $J = 5.2$ Hz, 2H, CH_2O), 7.22–7.35 (m, 5H, arom H), 7.56–7.87 (m, 4H, arom H), 8.44 (m, 1H, arom H). Calcd for $C_{24}H_{17}Cl_2N_3O_4S$: C, 54.99; H, 3.41; N, 8.36; S, 6.38. Found: C, 54.90; H, 3.39; N, 7.99; S, 6.16.

6.1.3.4. O-*{2-[(3a*R*,7a*S*)-1,3-Dioxo-1*S*,3,3a,4,7,7a-hexahydro-2*H*-isoindol-2-yl]ethyl} 4-chlorobenzoyl(4-chlorophenyl)thiocarbamate* ***60***. Mp 155–157 °C (from CH_2Cl_2/EtOH); yield: 48%. IR (KBr) cm^{-1} 2950, 1787, 1695. ^1H NMR ($CDCl_3$) δ 1.94–2.58 (m, 4H, $2CH_2$), 2.88–3.17 (m, 2H, 2CH), 3.68 (t, $J = 6$ Hz, 2H, CH_2N), 4.54 (t, $J = 6$ Hz, 2H, CH_2O), 5.80–6.01 (m, 2H, 2CH = C), 7.08–8.29 (m, 8H, arom H). Calcd for $C_{24}H_{20}Cl_2N_2O_4S$: C, 57.25; H, 4.00; N, 5.56; S, 6.37. Found: C, 56.98; H, 3.90; N, 5.34; S, 6.05.

6.1.3.5. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,3-dichlorophenyl(2-furoyl)thiocarbamate* ***61***. Mp 163–165 °C (from CH_2Cl_2/EtOH); yield: 49%. IR (KBr) cm^{-1} 3128, 3068, 1776, 1716, 1684. ^1H NMR ($CDCl_3$) δ 3.92 (t, $J = 6$ Hz, 2H, CH_2N), 4.78 (t, $J = 6$ Hz, 2H, CH_2O), 6.30–6.45 (m, 1H, H-4, fur), 7.02–8.09 (m, 9H, 7 arom H and H-3 and H-5 fur). Calcd for $C_{22}H_{14}Cl_2N_2O_5S$: C, 54.00; H, 2.88; N, 5.72; S, 6.55. Found: C, 54.15; H, 2.87; N, 5.71; S, 6.25.

6.1.3.6. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 2,4-dichlorophenyl(2-furoyl)thiocarbamate ***62***. Mp 130–132 °C (from CH_2Cl_2/EtOH); yield: 74%. IR (KBr) cm^{-1} 1771, 1716, 1688. ^1H NMR ($CDCl_3$) δ 3.95 (t, $J = 6$ Hz, 2H, CH_2N), 4.70 (t, $J = 6$ Hz, 2H, CH_2O), 6.31–6.48 (m, 1H, H-4, fur), 7.05–7.98 (m, 9H, 7 arom H and H-3 and H-5 fur). Calcd for $C_{22}H_{14}Cl_2N_2O_5S$: C, 54.00; H, 2.88; N, 5.72; S, 6.55. Found: C, 53.88; H, 2.77; N, 5.70; S, 6.20.

6.1.3.7. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 2,6-dichlorophenyl(3-nitrobenzoyl)thiocarbamate ***63***. Mp 185–187 °C (from CH_2Cl_2/EtOH); yield: 45%. IR (KBr) cm^{-1} 1774, 1713, 1690. ^1H NMR ($CDCl_3$) δ 3.77 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.63 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.25–8.92 (m, 11H, arom H). Calcd for $C_{25}H_{15}Cl_2N_3O_6S$: C, 52.95; H, 2.78; N, 7.72; S, 5.89. Found: C, 52.72; H, 2.79; N, 7.62; S, 5.72.

6.1.3.8. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 3,4-dichlorophenyl(2-furoyl)thiocarbamate ***64***. Mp 150–152 °C (from CH_2Cl_2/EtOH); yield: 61%. IR (KBr) cm^{-1} 3091, 1772, 1712. ^1H NMR ($CDCl_3$) δ 3.97 (t, $J = 6$ Hz, 2H, CH_2N), 4.75 (t, $J = 6$ Hz, 2H, CH_2O), 6.37–6.60 (m, 1H, H-4, fur), 7.03–7.94 (m, 9H, 7 arom H and H-3 and H-5 fur). Calcd for $C_{22}H_{14}Cl_2N_2O_5S$: C, 54.00; H, 2.88; N, 5.72; S, 6.55. Found: C, 53.93; H, 2.78; N, 5.68; S, 6.35.

6.1.3.9. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 3,5-dichlorophenyl(2-furoyl)thiocarbamate ***65***. Mp 144–146 °C (from CH_2Cl_2/EtOH); yield: 71%. IR (KBr) cm^{-1} 3093, 1778, 1710. ^1H NMR ($CDCl_3$) δ 3.96 (t, $J = 6$ Hz, 2H, CH_2N), 4.74 (t, $J = 6$ Hz, 2H, CH_2O), 6.47–6.51 (m, 1H, H-4, fur), 7.20–7.96 (m, 9H, 7 arom H and H-3 and H-5 fur). Calcd for $C_{22}H_{14}Cl_2N_2O_5S$: C, 54.00; H, 2.88; N, 5.72; S, 6.55. Found: C, 54.06; H, 2.83; N, 5.74; S, 6.35.

6.1.3.10. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 3-nitrobenzoyl(2,4,6-trichlorophenyl)thiocarbamate ***66***. Mp 185–187 °C (from CH_2Cl_2/EtOH); yield: 45%. IR (KBr) cm^{-1} 1774, 1718, 1694. ^1H NMR ($CDCl_3$) δ 3.76 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.61 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.29–8.77 (m, 10H, arom H). Calcd for $C_{24}H_{14}Cl_3N_3O_6S$: C, 49.80; H, 2.44; N, 7.26; S, 5.54. Found: C, 49.93; H, 2.47; N, 7.24; S, 5.33.

6.2. Virology

6.2.1. Cell-based assays

The biological evaluation of the ATCs **1**–**66** was performed according to the previously reported procedures [10].

6.2.2. Enzyme assays

RT assays were carried out using a non-radioactive method. Purified HIV-RT was purchased from Ambion and assayed for its RNA-dependent polymerase-associated activity in a 20 μL volume containing 60 mM Tris–HCl (pH = 8.1), 60 mM KCl, 8 mM $MgCl_2$, 13 mM DTT, 2.5 ng μL^{-1} template: primer [poly(A)-oligo(dT)$_{16}$] and 100 μM dTTP. After incubation for 30 min at 37 °C, reactions were stopped by addiction of 2 μL of 200 mM EDTA. 138 μL of PicoGreen Quantitation Reagent (Molecular Probes), diluted 1/345 in TE, was added to each sample and incubated at room temperature, protected from ambient light, for 5 min. Sample fluorescence from each well of a 96-well *microtiter* plate was measured using a microplate VICTOR^3 Multilabel Plate Reader (PerkinElmer). Percent residual activity was plotted vs increasing concentration of compounds. The curve was fit with Kaleidagraph (Synergy Software) to obtain IC_{50} values.

6.3. Molecular modelling

The docking models of analogues **5**, **38**, **42**, **44**, **49** and **50** were calculated using Autodock 3.0.5 [27]. The crystal structure of RT/PETT complex solved by Ren et al. [28] (PDB code: 1DTQ) was used as starting structure. The ligands were built by Insight II (Builder module) and parameterized according to the CVFF force field [32]. The docking calculations were carried out as previously reported [21–23]. The RT–ATC complexes were energy-minimized by CNS [33], performing a cycle of simulated annealing (starting temperature 1000 K) followed by 120 cycles of Powell minimization. All the calculations were performed on Silicon Graphic Indigo2 and Origin 200 workstations. Model analyses were carried out using CCP4 (Collaborative Computational Project Number 4) program suite [34]. The programs Molscript [35] and Raster3D [36] were used for drawing the figures.

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