European Journal of Medicinal Chemistry 44 (2009) 1650–1663

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Novel modifications in the series of *O*-(2-phthalimidoethyl)-*N*-substituted thiocarbamates and their ring-opened congeners as non-nucleoside HIV-1 reverse transcriptase inhibitors

Andrea Spallarossa [a,*], Sara Cesarini [a], Angelo Ranise [a], Olga Bruno [a], Silvia Schenone [a], Paolo La Colla [b,**], Gabriella Collu [b], Giuseppina Sanna [b], Barbara Secci [b], Roberta Loddo [b]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV 3, I-16132 Genova, Italy*
[b] *Dipartimento di Scienze e Tecnologie Biomediche, Università di Cagliari, Cittadella Universitaria, S.S. 554, Km 4,500, I-09042 Monserrato (Cagliari), Italy*

ARTICLE INFO

Article history:
Received 4 July 2008
Received in revised form 4 September 2008
Accepted 8 September 2008
Available online 30 September 2008

Keywords:
O-(2-Phthalimidoethyl)-*N*-arylthiocarbamates
HIV-1
Non-nucleoside reverse transcriptase inhibitors
Parallel synthesis
Molecular docking

ABSTRACT

The structure–activity relationships (SARs) of *N*-aryl-*O*-(2-phthalimidoethyl)thiocarbamates (C-TCs) and their imide ring-opened congeners (O-TCs) as non-nucleoside HIV-1 reverse transcriptase inhibitors were further investigated. The SAR strategy involved modifications of the *N*-phenyl ring followed by the hybridization of the most promising *N*-aryl and *O*-(2-phthalimidoethyl) substructures. The role of stereochemistry and *tert*-butyl substitution of the phthalimidoethyl moiety on activity was also investigated. Seventy-six analogues were prepared by parallel solution-phase synthesis. Twenty-two C-TCs displayed nanomolar activity against wild-type HIV-1 and a number of analogues were effective against the Y181C mutant. Compound **56** combined the highest activity so far identified against Y181C ($EC_{50} = 1.3$ μM) with good potency against the K103R mutant ($EC_{50} = 4.8$ μM). Docking simulations helped to rationalize the SARs.

1. Introduction

HIV-1 reverse transcriptase (RT) is a DNA-dependent polymerase responsible for the production of a double stranded DNA copy of a single stranded viral RNA. RT is essential for HIV-1 life cycle and therefore represents a privileged pharmacological target for anti-AIDS therapy [1]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a family of selective chemically diverse RT targeting agents. Crystallographic studies have shown that all NNRTIs bind to an allosteric binding pocket (the non-nucleoside binding site, NNBS), located in the vicinity of the active site in the p66 subunit [2–9]. The formation of RT–NNRTI complex results in short- and long-range conformational changes that lock the enzyme in an inactive form [6,10,11].

O-(2-Phthalimidoethyl)-*N*-arylthiocarbamates (C-TCs) and their imide ring-opened congeners (O-TCs) have been recently identified [12] as a novel class of HIV-1 NNRTIs that are isosterically related to *N*-phenethyl-*N*′-thiazolylthiourea (PETT) derivatives (Fig. 1A) [3,13–32]. The synthesis of thiocarbamates more structurally similar to PETT compounds (TC-1, TC-29, Fig. 1A) [33,34] has shed further light on the relationships between these two families of inhibitors. Preliminary efforts [12] to optimize the lead **I** (Fig. 1B) had been mainly based on independent variations of the C-TC portions **a** and **b** (Fig. 1A) and had led to the potent derivatives **II**–**XIII** (Fig. 1B) featured by the following substitution patterns: for portion **a**: (i) methyl substitution at position 4 of the phthalimide phenyl ring, (ii) methyl branching of the ethyl linker on the carbon atom adjacent to the imide nitrogen (hereinafter, β-carbon Fig. 1A); for portion **b**: (i) 4-monosubstitution of the phenyl ring, (ii) replacement of the phenyl ring with a cyclohexyl.

With an aim of acquiring additional elements to maximize the activity against wild-type and drug-resistant RT forms, a new series of C-TCs were designed and prepared by parallel synthesis. First, the *O*-(2-phthalimidoethyl) substructure was kept unmodified while portion **b** was varied by embodying (cyclo)alkyl, allyl and (hetero)arylalkyl groups (C-TCs **1**–**11**, Table 1) or poly-substituted phenyl moieties bearing atoms/groups endowed with different electronic, steric and lipophilic features (F, Cl, Br, CH_3, CF_3, OCH_3, NO_2) arranged in various patterns (C-TCs **12**–**39**, Table 2). Notably,

* Corresponding author. Tel.: +39 010 353 8370; fax: +39 010 353 8358.
** Corresponding author. Tel.: +39 070 675 4147; fax: +39 070 675 4210.
E-mail addresses: andrea.spallarossa@unige.it (A. Spallarossa), placolla@unica.it (P. La Colla).

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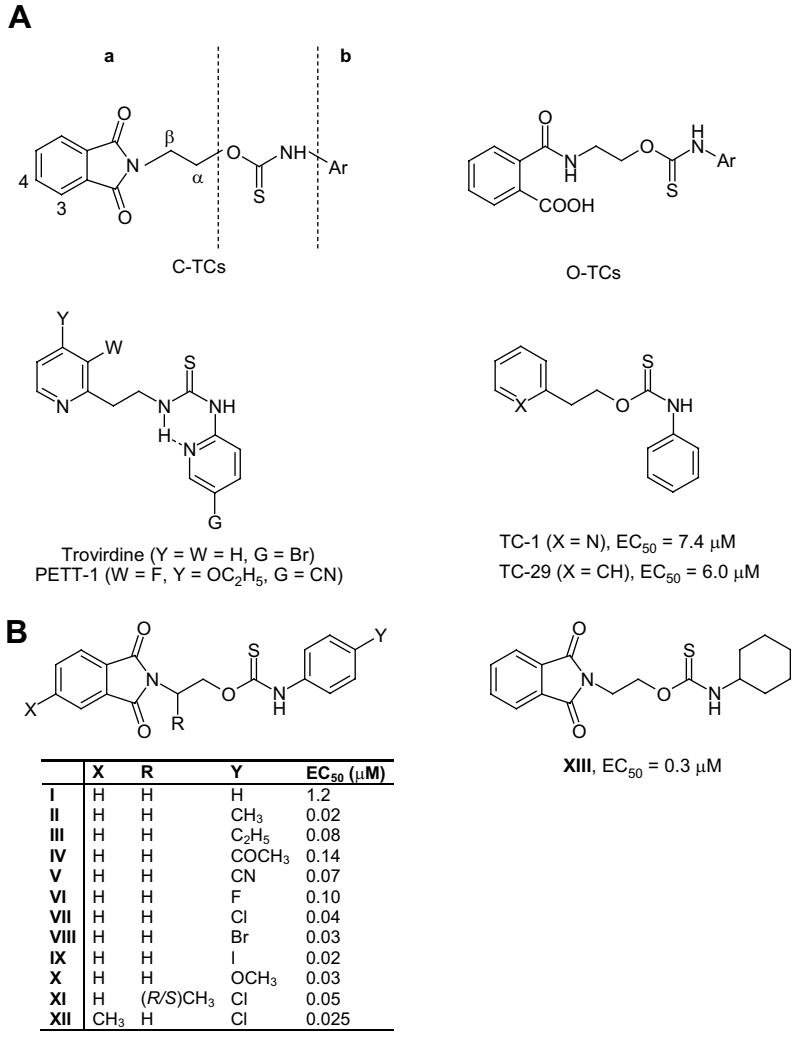
doi:10.1016/j.ejmech.2008.09.024



	X	R	Y	EC_{50} (μM)
I	H	H	H	1.2
II	H	H	CH_3	0.02
III	H	H	C_2H_5	0.08
IV	H	H	$COCH_3$	0.14
V	H	H	CN	0.07
VI	H	H	F	0.10
VII	H	H	Cl	0.04
VIII	H	H	Br	0.03
IX	H	H	I	0.02
X	H	H	OCH_3	0.03
XI	H	(*R*/*S*)CH_3	Cl	0.05
XII	CH_3	H	Cl	0.025

Fig. 1. (A) Chemical structures of thiocarbamates (C-TCs, O-TCs, TC-1, TC-29) [33,34] and PETT derivatives (Trovirdine and PETT-1). (B) Chemical structures and antiretroviral activity of previously synthesized C-TCs **I**–**XIII** [12].

the halosubstitution of portion **b** was carried out also in keeping with the paramount importance of aromatic halogen atoms observed for the activity of other class of NNRTIs structurally unrelated to thiocarbamates such as 2,3-diaryl-1,3-thiazolidin-4-(thi)ones [35,36] and indolyl aryl sulphones [37]. Then, the **a** and **b** substructures emerged as the most promising from the previous work were hybridized for further series expansion (Tables 3 and 4). Thus, the 4-methylphthalimidoethyl moiety was kept constant and combined with *N*-cyclohexyl (**40**) or *N*-4-monosubstitutedphenyl (CH_3, C_2H_5, $COCH_3$, CN, F, Br, I, OCH_3) (**41**–**48**) or *N*-3,4-disubstitutedphenyl (Cl,Cl; Cl,CH_3; Cl,CF_3; Cl,NO_2) (**49**–**52**) rings (Table 3). Moreover, the *N*-4-methyl- and *N*-4-chloro-phenyl moieties were coupled with portion **a** bearing: (i) a *tert*-butyl group at position 4 of the phthalimido phenyl ring (**53** and **54**, Table 3); (ii) a methyl branching on the linker β-carbon (pure enantiomers **55**–**57**, Table 4); (iii) methyl groups on both the phthalimide position 4 and the linker β-carbon (racemic **58** and **59**, Table 4).

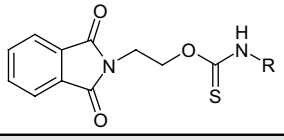
Finally, O-TCs **60**–**76** (Table 5) allowed us to evaluate the effects of imide ring opening concomitantly with *N*-phenyl ring modifications (**60**–**72**) and β-carbon methyl branching (**73**–**76**).

2. Chemistry

Preliminarily, the alcohol building blocks (*R*)-$\mathbf{A_2}$, (*S*)-$\mathbf{A_2}$, $\mathbf{A_4}$ and $\mathbf{A_5}$ (Fig. 2A) were synthesized by condensing the proper anhydride and aminoalcohol in solvent free conditions (Scheme 1), according to the general procedure previously detailed [12].

Compounds **1**–**76** were prepared by a convergent two-step solution-phase parallel procedure (Scheme 2) derived from our previously reported protocols [12,33,34]. Briefly, the starting alcohols $\mathbf{A_{1-5}}$ (Fig. 2A) were first transformed into their corresponding salts in the presence of sodium hydride in dry THF or DMF (procedures A and B, respectively. See Section 6). The alcoholate was then condensed in situ with isothiocyanates $\mathbf{I_{1-49}}$ (Fig. 2B) to afford the mono-sodium thiocarbamates **B** as single adducts or in mixture with the corresponding di-sodium ring-opened salts **SS**. After treatment with an aqueous ammonium chloride solution (pH = 8), **B** and **SS** were protonated to neutral C-TCs and carboxylate salts **S**, respectively. C-TCs were separated from the reaction mixture and extracted in parallel with diethyl ether, whereas **S** were kept in solution. Acidification of the reaction medium with a 2 N HCl solution (pH = 0) caused protonation of **S**, leading to O-TCs which precipitated as free acids and were collected by filtration. Notably, in the synthesis of C-TCs **1**–**10**, **13**, **14**, **19**–**25**, **27**, **29**–**32**, **34**, **37**, **38**, **40**–**54**, **58**, **59** the corresponding O-TCs were isolated in trace amounts (yields were less than 0.5–1%, data not shown). In contrast, reaction of $\mathbf{A_1}$ with $\mathbf{I_{44}}$ and (*S*)-$\mathbf{A_2}$ with $\mathbf{I_{13}}$ afforded only O-TCs **68** and **74**, respectively. Details on the hypothetical mechanism for the concomitant formation of C-TCs and O-TCs and the procedures of separation have been previously reported [12]. The final

Table 1
Cytotoxicity[a] and anti-HIV-1 activity of C-TCs **1**–**11**



Compound	R	CC_{50}[b]	EC_{50}[c]	SI[d]
1	*n*-Butyl	>100	7.2	>13.9
2	1,1,3,3-Tetramethylbutyl	>100	60	>1.7
3	Cyclopropyl	>100	>100	–
4	Cyclopentyl	>100	11	>9.1
5	Cyclooctyl	>100	0.6	>166.7
6	Cyclohexylmethyl	>100	1.1	>91
7	Allyl	>100	>100	–
8	Benzyl	>100	26	>3.8
9	2-Furylmethyl	>100	>100	–
10	2-Phenylethyl	>100	9.2	>10.9
11	2-(3,4-Dimethoxyphenyl)ethyl	>100	8	>12.5
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 cytopathogenecity, as determined by the MTT method.
[d] Selectivity index: CC_{50}/EC_{50} ratio.

products were purified by crystallization and the yields (not optimized) ranged from 9% to 73% (see Section 6).

3. Biological results and discussion

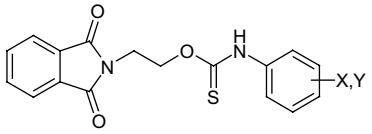
The antiretroviral activity of C-TCs (Tables 1–4) and O-TCs (Table 5) was evaluated in MT-4 cell-based assays by assessing the reduction of the HIV-1 induced cytopathogenicity. The results are expressed as EC_{50} values. In parallel with antiretroviral activity, the TC-induced cytotoxicity was evaluated in mock-infected MT-4 cells and the results are expressed as CC_{50} values. Trovirdine was used as the reference drug. The most potent derivatives were also tested against the clinically relevant K103R, Y181C and K103N/Y181C resistant mutants [38,39], employing Efavirenz as the reference molecule (Table 6).

Among C-TCs in which the *N*-phenyl ring of the lead **I** was replaced by allyl, (cyclo)alkyl or (hetero)arylalkyl groups (**1**–**10**, Table 1) only **5** (cyclooctyl) and **6** (cyclohexylmethyl) showed improved potency in comparison with **I** but resulted to be less active than the cyclohexyl derivative **XIII** (compare **5** and **6** with **XIII**).

A complex interplay between the poly-substitution patterns and the steric/electronic properties of the *N*-phenyl substituents affects the antiretroviral properties of **12**–**39** (Table 2). Thus, the activity trend of the dimethyl **12**–**14**, difluoro **15**–**18** and dichloro **19**–**24** derivatives [2,4-: F (**15**) > CH_3 (**12**) > Cl (**20**); 2,6-: F (**17**) > Cl (**22**) > CH_3 (**13**); 3,5-: CH_3 (**14**) > F (**18**) > Cl (**24**)] suggests that the relative positions of the *N*-phenyl substituents influence the antiretroviral activity more than how their electronic properties do. The difluoro analogues (**15**–**18**) were similarly effective in the low micromolar range except for **15** (2,4-diF, $EC_{50} = 0.5$ μM) that turned out to be 2.4-fold more potent than the lead **I**. The isosteric F/Cl replacement decreased activity, the bulkier dichloro derivatives being less effective than their difluoro counterparts (**20** vs **15**; **21** vs **16**; **22** vs **17**; **24** vs **18**).

Moreover, except for the difluoro derivatives, the disubstituted C-TCs bearing an *ortho*-substituent appeared to be less active than their 3,4- and 3,5-positional isomers (dimethyl: **14** vs **12**, **13**; dichloro: **23**, **24** vs **19**–**22**; chloro–methyl: **28** vs **25**–**27**; chloro–nitro: **30** vs **29**). The 3,4-pattern emerged as the most favourable; **23**, **28**, **30**, **33** and **34** were (sub)micromolar HIV-1 inhibitors and in particular **30** (4-Cl-3-NO_2) displayed an EC_{50} value of 50 nM. The tri-substituted C-TCs **35**–**38** and the tetrafluoro derivative **39** were active in a narrow micromolar concentration range (EC_{50} range: 9–11 μM), except for **35** that resulted to be more active.

Table 2
Cytotoxicity[a] and anti-HIV-1 activity of C-TCs **12**–**39**

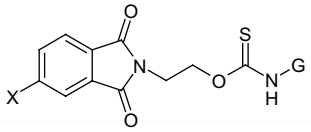


Compound	X,Y	CC_{50}[b]	EC_{50}[c]	SI[d]
12	2,4-Me_2	>100	6	16.7
13	2,6-Me_2	>100	75	>1.3
14	3,5-Me_2	>100	4	>25
15	2,4-F_2	>100	0.5	>200
16	2,5-F_2	>100	8	>12.5
17	2,6-F_2	>100	7	>14.3
18	3,5-F_2	>100	9	>11.1
19	2,3-Cl_2	>100	30	>3.3
20	2,4-Cl_2	>100	>100	–
21	2,5-Cl_2	>100	42	>2.4
22	2,6-Cl_2	>100	21	>4.8
23	3,4-Cl_2	>100	0.2	>500
24	3,5-Cl_2	>100	12	>8
25	4-Cl-2-Me	>100	27	>3.7
26	5-Cl-2-Me	>100	>100	–
27	2-Cl-6-Me	>100	23	>4.3
28	3-Cl-4-Me	>100	0.8	>125
29	2-Cl-4-NO_2	>100	>100	–
30	4-Cl-3-NO_2	>100	0.05	>2000
31	4-Br-2-Me	>100	19	>5.3
32	5-Cl-2-OMe	>100	48	>2.1
33	3,4-$(OMe)_2$	>100	3.5	>28.6
34	4-Cl-3-CF_3	84	8.0	10.5
35	2,4,6-F_3	>100	2	>50
36	2,3,4-Cl_3	>100	11	>9.1
37	2,4,6-Cl_3	>100	10	>10
38	4-Br-2,6-Me_2	>100	9	>11.1
39	2,3,5,6-F_4	>100	10	>10
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 cytopathogenecity, as determined by the MTT method.
[d] Selectivity index: CC_{50}/EC_{50} ratio.

The insertion of a methyl group at position 4 of the phthalimide substructure led to the highly active C-TCs **40**–**52** (Table 3) whose activities were influenced by the nature of portion **b**. Thus, the cyclohexyl derivative **40** displayed a reduced potency in comparison with the majority of its *N*-aryl substituted congeners. The *para*-substituted phenyl analogues **41**–**48** showed nanomolar potencies (EC_{50} range = 8–70 nM) and **44** (4-CN), **46** (4-Br) and **47** (4-I) resulted as more active than Trovirdine, while **41** (4-Me) and **42** (4-Et) were equipotent to the reference molecule. Compound **46** turned out to be the most active C-TC so far identified. The most potent *para*-substituted derivatives bear both electron-withdrawing (F, Br, I, CN) and electron-donating (Me, Et, MeO) groups thus confirming a marginal effect of the electronic properties of the *N*-phenyl substituent on antiretroviral activity. Among the 3,4-disubstituted analogues, **51** (4-Cl-3-NO_2) emerged as the most potent derivative ($EC_{50} = 50$ nM) and **49** (diCl) and **50** (3-Cl-4-CH_3) showed submicromolar activities. In comparison with their unsubstituted phthalimide-ring analogues, the 4-methyl substituted C-TCs **42**–**47**, **50**, and **52** were from 2- to 7-fold more potent whereas **40**, **41**, **48**, **49**, and **51** showed equal activity (**40** vs

Table 3
Cytotoxicity[a] and anti-HIV-1 activity of C-TCs **40–54**



Compound	X	G	CC_{50}[b]	EC_{50}[c]	SI[d]
40	CH_3	Cyclohexyl	>100	0.3	>333
41	CH_3	4-Tolyl	69	0.02	3450
42	CH_3	4-Ethylphenyl	43	0.02	2150
43	CH_3	4-Acetylphenyl	>100	0.07	>1429
44	CH_3	4-Cyanophenyl	57	0.01	5700
45	CH_3	4-Fluorophenyl	100	0.03	3333
46	CH_3	4-Bromophenyl	59	0.008	7375
47	CH_3	4-Iodophenyl	89	0.01	8900
48	CH_3	4-Methoxyphenyl	>100	0.03	>3333
49	CH_3	3,4-Dichlorophenyl	50	0.2	250
50	CH_3	3-Chloro-4-methylphenyl	66	0.4	165
51	CH_3	4-Chloro-3-nitrophenyl	>100	0.05	>2000
52	CH_3	4-Chloro-3-trifluoromethylphenyl	43	2.4	17.9
53	$C(CH_3)_3$	4-Tolyl	42	1.5	28
54	$C(CH_3)_3$	4-Chlorophenyl	55	1.2	45.8
Trovirdine			60	0.02	3000

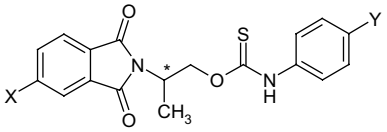
[a] Data mean values for three separate experiments. Variation among triplicate samples was less than 10%.
[b] Compound concentration (μM) required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[c] Compound concentration (μM) required to achieve 50% protection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

XIII; **41** vs **II**; **42** vs **III**; **43** vs **IV**; **44** vs **V**; **45** vs **VI**; **46** vs **VIII**; **47** vs **IX**; **48** vs **X**; **49** vs **23**; **50** vs **28**; **51** vs **30**; **52** vs **34**). The replacement of the methyl substituent with the more sterically demanding *tert*-butyl group caused a dramatic decrease in activity (**53** vs **41**; **54** vs **XII**).

As reported in Table 4, C-TC **56** ($EC_{50} = 10$ nM) was 2-fold more active than Trovirdine and showed higher potency in comparison with both the *S* isomer **57** and racemate **XI** (Fig. 1B) [12]. This evidence is consistent with the data reported for PETT derivatives for which the *R* isomers fit the NNRTI binding pocket much better than *S* enantiomers [27,28]. The 4-tolyl analogue **55** was 7-fold less active than **56** but still retained activity in the nanomolar concentration range. Moreover, the comparison of **55** and **56** with their corresponding unbranched congeners **II** and **VII** (Fig. 1B) showed opposite effects of the methyl branching on the activity. The double methyl substitution of the linker β-carbon and the phthalimide benzene ring (in an attempt to strengthen the hydrophobic contacts within the highly lipophilic NNRTI binding site) led to compounds **58** and **59**, active in the nanomolar concentration range and with a decreased activity in comparison with their analogues bearing single variation on portion **a** (compare **58** with **41**; **59** with **XI** and **XII**).

In accordance with the results of the previous studies [12], the imide ring opening (O-TCs **60–76**, Table 5) caused a decrease in activity (**60** vs **11**; **61** vs **12**; **62** vs **15**; **63** vs **16**; **64** vs **17**; **65** vs **18**; **66** vs **26**; **67** vs **28**; **69** vs **33**; **70** vs **35**; **71** vs **36**; **72** vs **39**; **73** vs **55**; **75** vs **56**; **76** vs **57**). O-TCs **62**, **67**, **70**, **73**, **75** and **76** emerged as the most

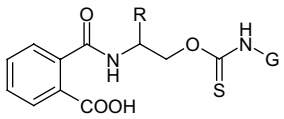
Table 4
Cytotoxicity[a] and anti-HIV-1 activity of branched C-TCs **55–59**



Compound	X	Isomer	Y	CC_{50}[b]	EC_{50}[c]	SI[d]
55	H	*R*	CH_3	76	0.07	1086
56	H	*R*	Cl	48	0.01	4800
57	H	*S*	Cl	84	0.2	420
58[e]	CH_3	*R*/*S*	CH_3	41	0.1	410
59[e]	CH_3	*R*/*S*	Cl	34	0.06	567
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 cytopathogenecity, as determined by the MTT method.
[d] Selectivity index: CC_{50}/EC_{50} ratio.
[e] Tested as a racemic mixture.

Table 5
Cytotoxicity[a] and anti-HIV-1 activity of O-TCs **60–76**

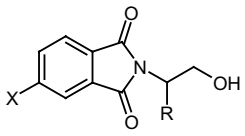


Compound	R	G	CC_{50}[b]	EC_{50}[c]	SI[d]
60	H	2-(3,4-Dimethoxyphenyl)ethyl	100	63	1.6
61	H	2,4-Dimethylphenyl	>100	53	>1.9
62	H	2,4-Difluorophenyl	>100	3.8	>26.3
63	H	2,5-Difluorophenyl	>100	51	>2.0
64	H	2,6-Difluorophenyl	>100	45	>2.2
65	H	3,5-Difluorophenyl	>100	62	>1.6
66	H	5-Chloro-2-methylphenyl	>100	30	>3.3
67	H	3-Chloro-4-methylphenyl	>100	4.3	>23.2
68	H	2-Methoxy-5-methylphenyl	>100	88	>1.1
69	H	3,4-Dimethoxyphenyl	>100	16	>6.2
70	H	2,4,6-Trifluorophenyl	>100	7.2	>13.9
71	H	2,3,4-Trichlorophenyl	>100	44	>2.3
72	H	2,3,5,6-Tetrafluorophenyl	>100	71	>1.4
73	(*R*)CH_3	4-Tolyl	>100	2.3	>43.5
74	(*S*)CH_3	4-Tolyl	>100	13.8	>7.2
75	(*R*)CH_3	4-Chlorophenyl	>100	0.3	>333
76	(*S*)CH_3	4-Chlorophenyl	>100	2.3	>43.5
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 cytopathogenecity, as determined by the MTT method.
[d] Selectivity index: CC_{50}/EC_{50} ratio.

A

Alcohols



	X	R
A1	H	H
(R)-**A2**	H	CH_3
(S)-**A2**	H	CH_3
A3	CH_3	H
A4	$C(CH_3)_3$	H
A5	CH_3	CH_3

B

Isothiocyanates (Ar-NCS)

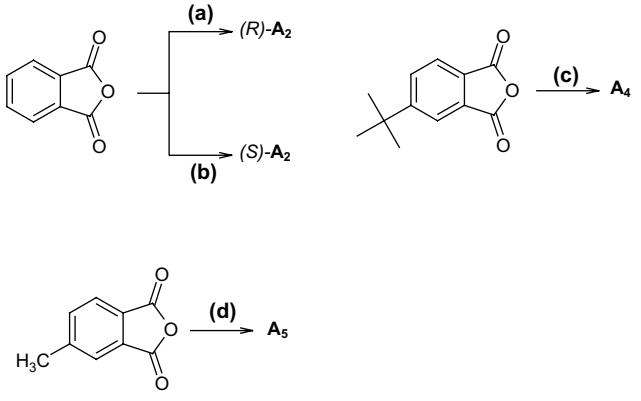
	Ar
I_1	*n*-butyl
I_2	1,1,3,3-tetramethylbutyl
I_3	cyclopropyl
I_4	cyclopentyl
I_5	cyclohexyl
I_6	cyclooctyl
I_7	cyclohexylmethyl
I_8	allyl
I_9	benzyl
I_{10}	2-furylmethyl
I_{11}	2-phenylethyl
I_{12}	2-(3,4-dimethoxyphenyl)ethyl
I_{13}	4-tolyl
I_{14}	4-ethylphenyl
I_{15}	4-acetylphenyl
I_{16}	4-cyanophenyl
I_{17}	4-fluorophenyl
I_{18}	4-chlorophenyl
I_{19}	4-bromophenyl
I_{20}	4-iodophenyl
I_{21}	4-methoxyphenyl
I_{22}	2,4-dimethylphenyl
I_{23}	2,6-dimethylphenyl
I_{24}	3,5-dimethylphenyl
I_{25}	2,4-difluorophenyl

	Ar
I_{26}	2,5-difluorophenyl
I_{27}	2,6-difluorophenyl
I_{28}	3,5-difluorophenyl
I_{29}	2,3-dichlorophenyl
I_{30}	2,4-dichlorophenyl
I_{31}	2,5-dichlorophenyl
I_{32}	2,6-dichlorophenyl
I_{33}	3,4-dichlorophenyl
I_{34}	3,5-dichlorophenyl
I_{35}	3,4-dimethoxyphenyl
I_{36}	4-chloro-2-methylphenyl
I_{37}	5-chloro-2-methylphenyl
I_{38}	2-chloro-6-methylphenyl
I_{39}	3-chloro-4-methylphenyl
I_{40}	2-chloro-4-nitrophenyl
I_{41}	4-chloro-3-nitrophenyl
I_{42}	4-chloro3-trifluoromethylphenyl
I_{43}	4-bromo-2-methylphenyl
I_{44}	2-methoxy-5-methylphenyl
I_{45}	2,4,6-trifluorophenyl
I_{46}	2,3,4-trichlorophenyl
I_{47}	2,4,6-trichlorophenyl
I_{48}	4-bromo-2,6-dimethylphenyl
I_{49}	2,3,5,6-tetrafluorophenyl

Fig. 2. Building blocks used.

active derivatives with EC_{50} values in the low micromolar or sub-micromolar concentration range. As observed for C-TCs, the *R* enantiomers **73** and **75** were from 6- to 7-fold more active than the corresponding *S* isomers **74** and **76**.

C-TCs **1**, **2**, **6**, **9**–**11**, **15**–**18**, **23**, **28**, **30**, **33**, **35**–**59** and O-TCs **60**, **62**–**65**, **70**–**76** were also screened against K103R, Y181C and Y181C + K103N resistant mutants. All compounds resulted to be inactive against the double mutant. The 12 derivatives listed in Table 6 were active against the Y181C variant. Compounds **30**, **44**, **47**, **55**, **56** and **59** displayed EC_{50} values lower than 10 μM. The *R* isomer **56** emerged as the most active analogue so far identified against the Y181C strain. Differently from all the previously prepared C-TCs, **56** showed micromolar activity against the K103R variant in a cell-based assay ($EC_{50} = 4.8$ μM). This observation coupled with the inactivity of **57** (the *S* enantiomer of **56**) suggests a strong effect of stereochemistry on C-TC resistance profile.

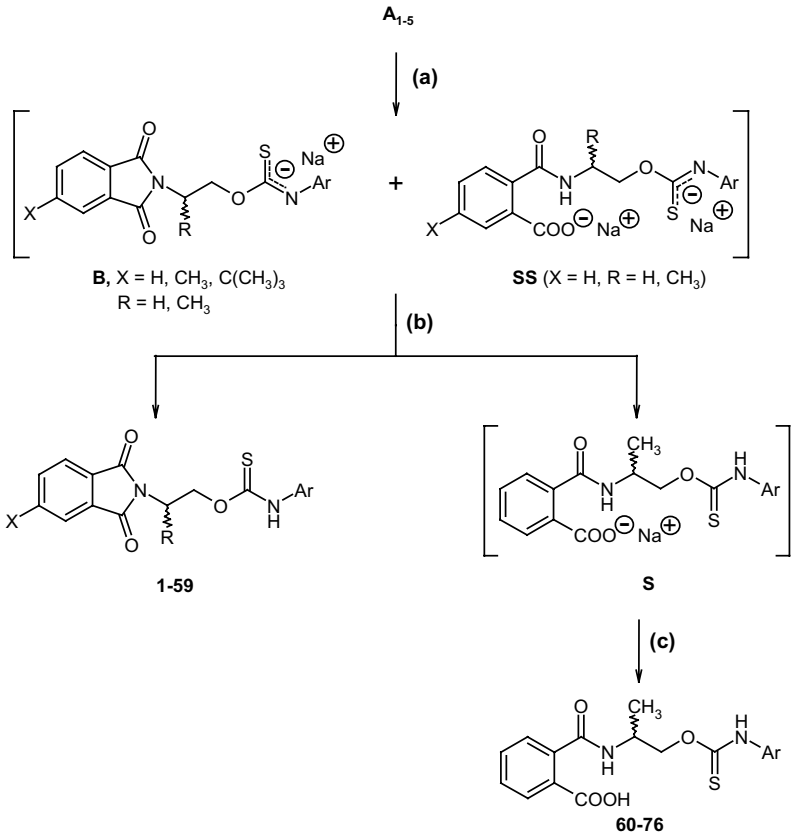


Scheme 1. Reagents and conditions: (a) (2*R*)-2-aminopropan-1-ol, 140–150 °C, 2 h; (b) (2*S*)-2-aminopropan-1-ol, 140–150 °C, 2 h; (c) 2-aminoethanol, 175–180 °C, 2 h; (d) (±) 2-aminopropan-1-ol, 140–150 °C, 2 h.

4. Molecular docking studies

To rationalize the most relevant SARs, docking studies (Autodock 3.05) [40] were carried out using the X-ray coordinates of RT/C-TC complex (PDB code: 2VG5) [9] as the template structure.

In the RT/**5** docking model, the ligand assumes a U-shaped bioactive conformation consistent with the binding mode of its *N*-aryl substituted analogues [9,12]. Nevertheless, the bulkiness of the cyclooctyl ring prevents the inhibitor from forming the hydrogen bond between the thiocarbamic NH group and Lys101 main chain carbonyl (C(S)N–OC estimated distance: 3.3 Å). This interaction is highly conserved in the C-TC class and in other thiocarbamic and thiourea NNRTIs, as assessed by X-ray crystallography [3,8,9,41]. The lack of this hydrogen bond is partially compensated by the



Scheme 2. Reagents and conditions: (a) Ar-NCS (**I_{1-49}**), NaH, dry THF r.t. for 24 h (procedure A) or dried DMF 0–5 °C then r.t. for 24 h; (b) aqueous NH_4Cl; (c) 2 N HCl. The structures of alcohols A1-5 and isothiocyanates I1-49 are listed in Fig. 2.

involvement of the phthalimide moiety in π–π stacking (Tyr181), hydrophobic (Tyr188, Trp229) and van der Waals [Pro95 and Glu138(p51)] interactions. Moreover, further stabilization to the complex is provided by the hydrophobic contacts between the cyclooctyl ring and the side chains of Lys103, Tyr318 and Val106 as well as by van der Waals interactions between the thiocarbamic functionality and the Lys101 backbone atoms.

The RT/**30** complex (Fig. 3A) is stabilized by a hydrogen bond between the NH group and the main chain carbonyl of Lys101 and van der Waals contacts involving the sulphur atom and the Val179 side chain. The oxyethyl linker interacts with the side chains of Leu100 and Glu138(p51), while the phthalimide moiety is involved in π–π stacking interactions with the Tyr181 side chain and in hydrophobic contacts with the Tyr188 and Trp229 residues. Furthermore, one of the imidic oxygen interacts with the Pro95 side chain. The *N*-phenyl ring hydrophobically contacts the Leu100, Lys103, Val106 and Tyr318 side chains. The chlorine atom establishes van der Waals contacts with the His235 main chain atoms and the nitro group is involved in a network of polar interactions with the Gly190 and Val189 main chains thus providing further stabilization to the complex. The impossibility of **23**, **28**, **33** and **34** (devoided of a 3-nitro substituent) to form polar interactions with Gly190 and Val189 backbones would structurally rationalize the higher potency of **30** in comparison with the other 3,4-disubstituted analogues. The lack of activity of **29** (positional isomer of **30**) would be ascribable to the *ortho* chlorine atom that prevents the ligand from assuming a bioactive U-shaped conformation within the NNBS and from establishing the hydrogen bond between the thiocarbamic NH group and the Lys101 backbone carbonyl.

Docking simulations carried out on **56** and **57** helped to rationalize the activity gap between the *R* and *S* stereoisomers. Thus, **56** would assume a bioactive conformation very similar to that observed in the X-ray structure of its unbranched analogue **VII** [9] (Fig. 3B). The RT/**56** complex would be mainly stabilized by the hydrogen bond between the NH thiocarbamic group and the Lys101 main chain carbonyl and the π–π stacking interactions between the phthalimide substructure and the Tyr181 side chain. Furthermore, van der Waals contacts would occur between the methyl on the β-carbon and the Val179, Tyr181 and Glu138(p51) side chains. To avoid steric clashes with Val179 side chain, **57** oxyethyl linker

Table 6
Anti-HIV-1[a] activity of C-TCs and O-TCs against the Y181C resistant mutant

Compound	EC_{50}[b]
30	2.8
42	16
44	4.5
45	68
47	9
48	44
51	30
55	9
56	1.3
58[c]	12
59[c]	4
75	18
Trovirdine	15
EFV	0.02

[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 cytopathogenecity, as determined by the MTT method.
[c] Tested as a racemic mixture.

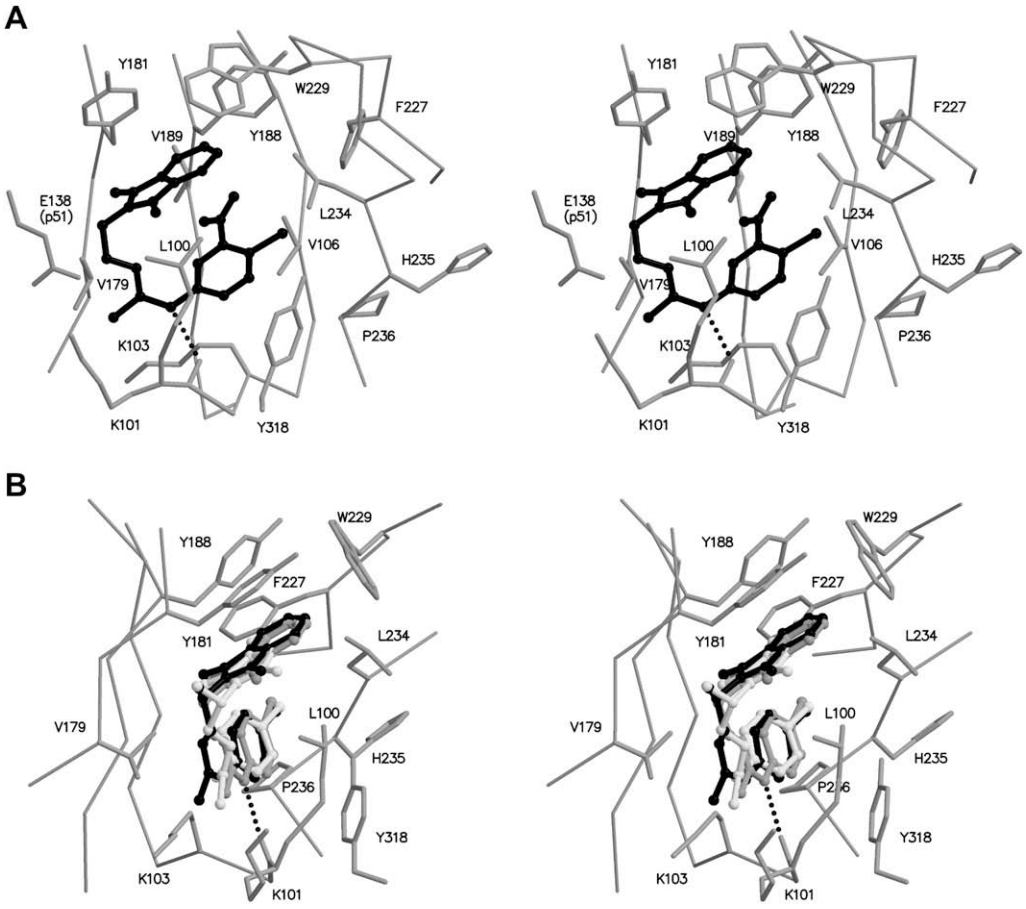


Fig. 3. (A) Stereoview of the RT/**30** modeled complex. The ligand is represented as black balls-and-sticks, while the amino acid residues lining the RT non-nucleoside binding site are shown as grey sticks. (B) Stereo diagram showing superposition of C-TCs **56** (modeled structure, black ball-and-stick) and **57** (modeled structure, white ball-and-stick) using RT/**VII** (grey ball-and-stick) crystallographic complex (PDB code: 2VG5) [9] as reference. Amino acid residues lining the non-nucleoside binding site are shown in sticks model, as observed in the crystallographic structure. Hydrogen bonds are depicted as dotted lines. The programs Molscript [47] and Raster3D [48] were used for drawing the figure.

would assume a different spatial arrangement (Fig. 3B). This would cause distortions of both the thiocarbamic functionality and the *N*-phenyl ring that weaken the hydrogen bond between the NH group and the Lys101 carbonyl thus decreasing the affinity of the inhibitor (C(S)N–OC estimated distances: 2.7 Å and 3.0 Å for **56** and **57**, respectively).

As observed in the RT/**XII** model [12], in the RT/**51** docking complex the methyl group at position 4 of the phthalimide substructure would further stabilize the complex by means of hydrophobic interactions with the side chains of Tyr188 and Trp229. The ability of the **51** nitro group to form polar interactions with the Gly190 and Val189 main chains would rationalize its higher activity in comparison with the other 3,4-disubstituted analogues **49**, **50** and **52**.

Finally, the structural comparison between RT/**54** and RT/**XII** modeled structures indicates that the *tert*-butyl group would cause a repositioning of the inhibitor within the binding site preventing the thiocarbamic functionality from forming the hydrogen bond with Lys101 and minimizing the interaction of the *N*-phenyl ring with RT.

5. Conclusions

Novel modifications on C-TCs and O-TCs were made to further expand the SAR studies on this class of NNRTIs. The replacement of the *N*-phenyl moiety with (aryl)alkyl portions led to weak anti-HIV-1 inhibitors, except for the cyclooctyl derivative. Among the *N*-phenyl ring poly-substituted patterns, the 3,4-arrangement gave the best results. The methyl substitution at position 4 of the phthalimide of the phthalimido phenyl ring as well as the *R* methyl branching of the β-carbon atom led to potent analogues with improved activity against wild-type HIV-1. Furthermore, some derivatives inhibited NNRTI-resistant Y181C variant at low micromolar concentrations and C-TCs **56** resulted effective against both Y181C and K103R mutants. The present study will provide the basis for the design and the synthesis of new analogues more effective against the clinically relevant HIV-1 mutated strains.

6. Experimental section

6.1. Chemistry

6.1.1. General

Alcohol $\mathbf{A_1}$, isothiocyanates $\mathbf{I_{1-49}}$ and the other chemicals were purchased from Chiminord and Aldrich Chemical, Milan (Italy). Syntheses of Trovirdine and $\mathbf{A_3}$ were accomplished according to the published procedures [12,13]. Solvents were of reagent grade. THF was distilled in the presence of sodium. DMF was dried on molecular sieves (5 Å 1/16″ pellets). Unless otherwise stated, all commercial reagents were used without further purification. Organic solutions were dried over anhydrous sodium sulphate. Thin layer chromatography (TLC) for routine monitoring of 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 was used as developing

solvent 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 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 determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured on a Perkin Elmer 241 MC spectropolarimeter; concentrations are expressed in g/100 mL and the cell length is 1 dm. 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 standard tetramethylsilane, and the splitting patterns were described as follows: s (singlet), d (doublet), t (triplet), m (multiplet) and br s (broad singlet). The first order values reported for coupling constants *J* were given in Hz. Elemental analyses were performed by an EA1110 Elemental Analyser (Fison-Instruments, Milan) and were within ± 0.4% of the theoretical values.

6.1.2. General procedure for the synthesis of starting alcohols ***A*****$_2$**, ***A*****$_4$**, *and* ***A*****$_5$**

A mixture of proper aminoalcohol (12 mmol) and proper anhydride (10 mmol) was heated (for **A_2**, **A_5**: 145–150 °C; for **A_4**: 175–180 °C) under stirring for 2 h. After cooling, the reaction mixture was dissolved in CH_2Cl_2 and washed with 2 N HCl (2 × 50 mL). The organic layer was dried and evaporated under reduced pressure to give (*R*)-**A_2**, (*S*)-**A_2**, **A_4**, **A_5** which were purified by crystallization from the proper solvent mixture.

*6.1.2.1. 2-[(1*R*)-2-Hydroxy-1-methylethyl]-1*H*-isoindole-1,3(2*H*)-dione, (R)-**A_2**.* Yield 69%; mp 84–86 °C (from CH_2Cl_2:Et_2O), lit. [42]: 84–85 °C (from EtOAc:petroleum ether). $[\alpha]_D^{20} = -14.7$ (*c* 1.03, $CHCl_3$), lit. [39]: −14.6 (*c* 3.2, $CHCl_3$). IR (KBr) 3490, 1772, 1689 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.44 (d, $J = 6.6$ Hz, 3H, CH_3), 2.67 (br s, 1H, OH, exchangeable), 3.86–4.10 (m, 2H, CH_2), 4.21–4.74 (m, 1H, CH), 7.61–8.01 (m, 4H, arom. H). Anal. Calcd for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.54; H, 5.16; N, 6.84.

*6.1.2.2. 2-[(1*S*)-2-Hydroxy-1-methylethyl]-1*H*-isoindole-1,3(2*H*)-dione, (S)-**A_2**.* Yield 73%; mp 84–86 °C (from CH_2Cl_2:Et_2O), lit. [42]: 84–85 °C (from EtOAc:petroleum ether). $[\alpha]_D^{20} = +15.5$ (*c* 1.05, $CHCl_3$), lit. [39]: +16.5 (*c* 2.0, $CHCl_3$). IR (KBr) 3490, 1772, 1689 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (d, $J = 6.6$ Hz, 3H, CH_3), 3.16 (br s, 1H, OH, exchangeable), 3.84–4.13 (m, 2H, CH_2), 4.21–4.74 (m, 1H, CH), 7.52–8.01 (m, 4H, arom. H). Anal. Calcd for $C_{11}H_{11}NO_3$: C, 64.38; H, 5.40; N, 6.83. Found: C, 64.19; H, 5.50; N, 7.01.

*6.1.2.3. 5-*tert*-Butyl-2-(2-hydroxyethyl)-1*H*-isoindole-1,3(2*H*)-dione,* ***A*****$_4$**.* Yield 82%; mp 60–62 °C (from Me_2CO). IR ($CHCl_3$) 3463, 2969, 1771, 1706 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, 3 CH_3), 3.47 (br s, 1H, OH, exchangeable), 3.62–4.15 (m, 4H, 2 CH_2), 7.55–8.04 (m, 3H, arom. H). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found: C, 68.26; H, 6.90; N, 5.72.

*6.1.2.4. (±) 2-(2-Hydroxy-1-methylethyl)-5-methyl-1*H*-isoindole-1,3(2*H*)-dione,* ***A*****$_5$**.* Yield 70%; mp 121–123 °C (from CH_2Cl_2:Et_2O), lit. [42]: 105–107 °C for the *S* isomer (from EtOAc:petroleum ether). IR (KBr) 3497, 2986, 2957, 1764, 1694 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 3H, CH_3-Phtha), 2.94 (br s, 1H, OH, exchangeable), 3.85–4.10 (m, 2H, CH_2), 4.20–4.63 (m, 1H, CH), 7.39–7.85 (m, 4H, arom. H). Anal. Calcd for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.70; H, 5.70; N, 6.37.

6.1.3. General procedure A for the parallel synthesis of C-TCs ***1*****–*****11*****,* ***15*****–*****17*****,* ***35*****–*****39*** *and O-TCs* ***60*****,* ***62*****–*****64*****,* ***70*****,* ***72***

A 60% sodium hydride dispersion in mineral oil (0.2 g, ∼5 mmol) was added in single portion at r.t. to a set of reaction tubes containing dry THF solution (15 mL) of **A_1** (0.45 g, 5 mmol). After stirring for 10 min, proper isothiocyanate (5 mmol) was added to each reaction mixture prolonging the stirring at r.t. for 24 h. The solvent of each reaction tube was evaporated by an Evaposel™ apparatus and the crude residue was treated with aqueous ammonium chloride (2.5 g dissolved in 100 mL of water). Each mixture was then transferred into a set of separating funnels and extracted with diethyl ether (2 × 40 mL). The combined extracts were dried, filtered through a pad of Florisil (diameter 5 × 2 cm) and evaporated in parallel by an Evaposel™ apparatus. The crude products were purified by crystallization to afford C-TCs. Following acidification (pH = 0) of the extracted aqueous phases with 2 N HCl, O-TCs separated as solids. The crude solid was filtered off and purified by crystallization.

*6.1.3.1. O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] butylthiocarbamate,* ***1*****.** Yield 28%; mp 64–65 °C (from Me_2CO). IR (KBr) 3249, 2953, 2924, 1772, 1709 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.68–0.88 (m, 3H, CH_3), 1.08–1.53 (m, 4H, CH_2CH_2Me), 3.05–3.18 and 3.32–3.47 (m, 4H, 2 CH_2N), 3.86–4.03 (m, 2H, CH_2O), 6.34 and 6.74 (br s, 1H, NH, exchangeable), 7.58–7.82 (m, 4H, arom. H). Anal. Calcd for $C_{15}H_{18}N_2O_3S$: C, 58.80; H, 5.92; N, 9.14; S, 10.47. Found: C, 58.88; H, 6.19; N, 8.97; S, 10.16.

*6.1.3.2. O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 1,1,3,3-tetramethylbutylthiocarbamate,* ***2*****.** Yield 11%; mp 112–113 °C (from Et_2O:MeOH). IR (KBr) 3232, 2951, 1775, 1716 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.81 (s, 9H, $C(CH_3)_3$), 1.24 (s, 6H, $C(CH_3)_2$), 1.48 (s, 2H, CH_2), 3.98–4.08 (m, 2H, CH_2N), 4.67–4.77 (m, 2H, CH_2O), 6.18 and 6.55 (br s, 1H, NH, exchangeable), 7.62–7.85 (m, 4H, arom. H). Anal. Calcd for $C_{19}H_{26}N_2O_3S$: C, 62.95; H, 7.23; N, 7.73; S, 8.85. Found: C, 62.88; H, 7.23; N, 7.77; S, 8.72.

*6.1.3.3. O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] cyclopropylthiocarbamate,* ***3*****.** Yield 49%; mp 144–145 °C (from CH_2Cl_2 :MeOH). IR (KBr) 3281, 1769, 1712 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.36–0.77 (m, 4H, 2 CH_2), 2.48–2.63 and 2.73–2.87 (m, 1H, CH), 3.85–4.06 (m, 2H, CH_2N), 4.53–4.72 (m, 2H, CH_2O), 6.47 and 6.89 (br s, 1H, NH, exchangeable), 7.56–7.83 (m, 4H, arom. H). Anal. Calcd for $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65; S, 11.04. Found: C, 57.71; H, 4.98; N, 9.53; S, 11.35.

*6.1.3.4. O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] cyclopentylthiocarbamate,* ***4*****.** Yield 52%; mp 115–116 °C (from CH_2Cl_2 :MeOH). IR (KBr) 3229, 2954, 2867, 1775, 1723 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.19–2.00 (m, 8H, 4 CH_2), 3.86–4.38 (m, 3H, CH and CH_2N), 4.55–4.68 (m, 2H, CH_2O), 6.38 and 6.68 (br s, 1H, NH, exchangeable), 7.58–7.88 (m, 4H, arom. H). Anal. Calcd for $C_{16}H_{18}N_2O_3S$: C, 60.36; H, 5.70; N, 8.80; S, 10.07. Found: C, 60.42; H, 5.95; N, 8.46; S, 9.94.

*6.1.3.5. O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] cyclooctylthiocarbamate,* ***5*****.** Yield 31%; mp 93–95 °C (from Et_2O:MeOH). IR (KBr) 3229, 2928, 1774, 1714 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25–1.85 (m, 14H, 7 CH_2), 3.70–4.17 (m, 3H, CH and CH_2N), 4.48–4.67 (m, 2H, CH_2O), 6.37 and 6.83 (br s, 1H, NH, exchangeable), 7.55–7.77 (m, 4H, arom. H). Anal. Calcd for $C_{19}H_{24}N_2O_3S$: C, 63.31; H, 6.71; N, 7.77; S, 8.90. Found: C, 63.11; H, 6.89; N, 7.70; S, 9.06.

*6.1.3.6. O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] cyclohexylmethylthiocarbamate,* ***6*****.** Yield 11%; mp 83–84 °C (from CH_2Cl_2:MeOH). IR (KBr) 3212, 2923, 1772, 1718 cm^{-1}; ^{1}H NMR

($CDCl_3$) δ 0.73–1.22 and 1.48–1.72 (m, 11H, 5 CH_2 and CH), 2.93–3.02 and 3.22–3.33 (m, 2H, CH_2-cyclohexyl), 3.88–4.04 (m, 2H, CH_2N), 4.58–4.68 (m, 2H, CH_2O), 6.31 and 6.67 (br s, 1H, NH, exchangeable), 7.59–7.83 (m, 4H, arom. H). Anal. Calcd for $C_{18}H_{22}N_2O_3S$: C, 62.40; H, 6.40; N, 8.09; S, 9.26. Found: C, 62.66; H, 6.47; N, 7.89; S, 8.94.

6.1.3.7. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] allylthiocarbamate,* ***7***. Yield 56%; mp 112–113 °C (from CH_2Cl_2:MeOH). IR (KBr) 3218, 3054, 2958, 1779, 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.71–3.81 and 3.88–4.11 (m, 4H, CH_2-allyl and CH_2N), 4.57–4.67 (m, 2H, CH_2O), 4.88–5.22 (m, 2H, CH_2=), 5.53–5.85 (m, 1H, CH=), 6.38 and 6.73 (br s, 1H, NH, exchangeable), 7.58–7.84 (m, 4H, arom. H). Anal. Calcd for $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65; S, 11.04. Found: C, 57.99; H, 5.03; N, 9.56; S, 11.07.

6.1.3.8. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] benzylthiocarbamate,* ***8***. Yield 18%; mp 96–98 °C (from Et_2O:MeOH). IR (KBr) 3336, 2960, 1771, 1708 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.89–4.02 (m, 2H, CH_2N), 4.31–4.36 and 4.58–4.70 (m, 4H, CH_2O and CH_2Ph), 6.63 and 6.96 (br s, 1H, NH, exchangeable), 7.07–7.27 (m, 5H, arom. H), 7.57–7.77 (m, 4H, arom. H). Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23; S, 9.42. Found: C, 63.50; H, 4.72; N, 8.17; S, 9.27.

6.1.3.9. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-furylmethylthiocarbamate,* ***9***. Yield 23%; mp 106–107 °C (from Me_2CO:MeOH). IR (KBr) 3340, 1770, 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88–4.05 (m, 2H, CH_2N), 4.27–4.36 and 4.54–4.69 (m, 4H, CH_2O and CH_2-Fur), 6.08–6.13 and 6.17–6.25 (m, 2H, $CH_{(3\text{-}fur)}$ and $CH_{(4\text{-}fur)}$), 6.74 and 6.95 (br s, 1H, NH, exchangeable), 7.06–7.09 and 7.23–7.27 (m, 1H, $CH_{(5\text{-}fur)}$), 7.58–7.79 (m, 4H, arom. H). Anal. Calcd for $C_{16}H_{14}N_2O_3S$: C, 58.17; H, 4.27; N, 8.48; S, 9.71. Found: C, 58.07; H, 4.46; N, 8.28; S, 9.51.

6.1.3.10. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-phenylethylthiocarbamate,* ***10***. Yield 41%; mp 92–92 °C (from CH_2Cl_2 :MeOH). IR (KBr) 3226, 1774, 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.60–2.88 (m, 2H, CH_2Ph), 3.33–3.47 and 3.58–3.74 (m, 2H, CH_2CH_2Ph), 3.84–4.03 (m, 2H, CH_2N), 4.55–4.68 (m, 2H, CH_2O), 6.43 and 6.83 (br s, 1H, NH, exchangeable), 7.00–7.27 (m, 5H, arom. H), 7.56–7.82 (m, 4H, arom. H). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90; S, 9.05. Found: C, 64.27; H, 5.25; N, 7.86; S, 8.70.

6.1.3.11. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-(3,4-dimethoxyphenyl)ethylthiocarbamate,* ***11***. Yield 73%; mp 121–123 °C (from CH_2Cl_2:MeOH). IR (KBr) 3222, 1776, 1721 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.57–2.66 and 2.69–2.80 (m, 2H, CH_2Ph), 3.31–3.43 and 3.58–3.73 (m, 2H, CH_2CH_2Ph), 3.77, 3.78, 3.80 (s, 6H, 2 CH_3O), 3.88–4.03 (m, 2H, CH_2N), 4.57–4.66 (m, 2H, CH_2O), 6.34 (br s, 1H, NH, exchangeable), 6.57–6.74 (m, 4H, arom. H and NH, exchangeable), 7.58–7.78 (m, 4H, arom. H). Anal. Calcd for $C_{21}H_{22}N_2O_5S$: C, 60.85; H, 5.35; N, 6.76; S, 7.74. Found: C, 60.79; H, 5.48; N, 6.67; S, 8.22.

6.1.3.12. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,4-difluorophenylthiocarbamate,* ***15***. Yield 36%; mp 130–132 °C (from CH_2Cl_2:MeOH). IR (KBr) 3260, 1772, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.98–4.25 (m, 2H, CH_2N), 4.71–4.99 (m, 2H, CH_2O), 6.64–7.07 and 7.51–8.06 (m, 7H, arom. H), 8.16 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}F_2N_2O_3S$: C, 56.35; H, 3.34; N, 7.73; S, 8.85. Found: C, 56.54; H, 3.34; N, 7.68; S, 8.54.

6.1.3.13. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,5-difluorophenylthiocarbamate,* ***16***. Yield 26%; mp 101–103 °C (from Et_2O:MeOH). IR (KBr) 3225, 1773, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.03–4.29 (m, 2H, CH_2N), 4.76–5.06 (m, 2H, CH_2O), 6.72–7.15 and 7.63–8.05 (m, 7H, arom. H), 8.32 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}F_2N_2O_3S$: C, 56.35; H, 3.34; N, 7.73; S, 8.85. Found: C, 56.05; H, 3.20; N, 7.70; S, 8.37.

6.1.3.14. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,6-difluorophenylthiocarbamate,* ***17***. Yield 47%; mp 204–205 °C (from CH_2Cl_2:MeOH). IR (KBr) 3320, 1771, 1717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.92–4.20 (m, 2H, CH_2N), 4.63–4.93 (m, 2H, CH_2O), 6.62–7.10 and 7.52–8.02 (m, 7H, arom. H), NH, not detected. Anal. Calcd for $C_{17}H_{12}F_2N_2O_3S$: C, 56.35; H, 3.34; N, 7.73; S, 8.85. Found: C, 56.69; H, 3.53; N, 7.51; S, 8.55.

6.1.3.15. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,4,6-trifluorophenylthiocarbamate,* ***35***. Yield 44%; mp 159–161 °C (from CH_2Cl_2:MeOH). IR (KBr) 3239, 1773, 1709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88–4.19 (m, 2H, CH_2N), 4.63–4.83 (m, 2H, CH_2O), 6.46–6.88 and 7.44–7.96 (m, 7H, arom. H and NH, exchangeable). Anal. Calcd for $C_{17}H_{11}F_3N_2O_3S$: C, 53.68; H, 2.92; N, 7.37; S, 8.43. Found: C, 53.99; H, 3.28; N, 7.15; S, 8.49.

6.1.3.16. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,3,4-trichlorophenylthiocarbamate,* ***36***. Yield 42%; mp 175–178 °C (from CH_2Cl_2:MeOH). IR (KBr) 3213, 1776, 1722 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.96–4.07 (m, 2H, CH_2N), 4.73–4.83 (m, 2H, CH_2O), 7.16–7.30 and 7.63–7.84 (m, 6H, arom. H), 8.17 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{11}Cl_3N_2O_3S$: C, 47.52; H, 2.58; N, 6.52; S, 7.46. Found: C, 47.50; H, 2.90; N, 6.81; S, 7.34.

6.1.3.17. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,4,6-trichlorophenylthiocarbamate,* ***37***. Yield 53%; mp 201–202 °C (from CH_2Cl_2:MeOH). IR (KBr) 3262, 1772, 1701 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.90–4.20 (m, 2H, CH_2N), 4.59–4.91 (m, 2H, CH_2O), 7.17–7.34 and 7.63–7.94 (m, 6H, arom. H), NH, not detected. Anal. Calcd for $C_{17}H_{11}Cl_3N_2O_3S$: C, 47.52; H, 2.58; N, 6.52; S, 7.46. Found: C, 47.80; H, 2.88; N, 6.33; S, 7.48.

6.1.3.18. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-bromo-2,6-dimethylphenylthiocarbamate,* ***38***. Yield 38%; mp 150–152 °C (from Et_2O:MeOH). IR (KBr) 3483, 3177, 1764, 1697 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.05 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 3.78–4.23 (m, 2H, CH_2N), 4.57–5.01 (m, 2H, CH_2O), 6.92–7.32 and 7.65–7.95 (m, 6H, arom. H), 8.13 (br s, 1H, NH exchangeable). Anal. Calcd for $C_{19}H_{17}BrN_2O_3S$: C, 52.66; H, 3.95; N, 6.46; S, 7.40. Found: C, 52.43; H, 4.28; N, 6.45; S, 7.30.

6.1.3.19. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2,3,5,6-tetrafluorophenylthiocarbamate,* ***39***. Yield 44%; mp 150–152 °C (from Et_2O:MeOH). IR (KBr) 3224, 1773, 1706 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88–4.01 (m, 2H, CH_2N), 4.63–4.95 (m, 2H, CH_2O), 6.87–7.28 and 7.69–7.96 (m, 5H, arom. H), 8.12 (br s, 1H, NH exchangeable). Anal. Calcd for $C_{17}H_{10}F_4N_2O_3S$: C, 51.26; H, 2.53; N, 7.03; S, 8.05. Found: C, 51.56; H, 2.94; N, 6.94; S, 7.85.

6.1.3.20. 2-[({2-[({[2-(3,4-Dimethoxyphenyl)ethyl]amino}carbonothioyl)oxy]ethyl}amino)carbonyl]benzoic acid, ***60***. Yield 23%; mp 166–168 °C (from Me_2CO:MeOH). IR (KBr) 3277, 1692, 1642 cm^{-1}; ^1H NMR (DMSO) δ 2.55–2.74 (m, 2H, CH_2Ph), 3.19–3.31 (m, 2H, CH_2CH_2Ph), 3.35–3.52 (m, 2H, CH_2N), 3.60 and 3.63 (s, 3H, OCH_3), 3.61 and 3.65 (s, 3H, OCH_3), 6.56–6.83, 7.27–7.53 and 7.63–7.72 (m, 7H, arom. H), 8.40 (br s, 1H, CONH, exchangeable), 9.15 (br s, 1H, CSNH, exchangeable), 12.76 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{21}H_{24}N_2O_6S$: C, 58.32; H, 5.59; N, 6.48; S, 7.41. Found: C, 58.08; H, 5.63; N, 6.59; S, 7.65.

6.1.3.21. 2-({[2-({[(2,4-Difluorophenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***62***. Yield 23%; mp 124–126 °C (from Me_2CO). IR (KBr) 3298, 1678, 1635 cm^{-1}; ^1H NMR (DMSO)

δ 3.13–3.62 (m, 2H, CH_2N), 4.37–4.57 (m, 2H, CH_2O), 6.84–7.81 (m, 7H, arom. H), 8.24 and 8.42 (br s, 1H, CONH, exchangeable), 10.67 and 10.93 (br s, 1H, CSNH, exchangeable), 12.88 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{17}H_{14}F_2N_2O_4S$: C, 53.68; H, 3.71; N, 7.36; S, 8.43. Found: C, 53.42; H, 3.79; N, 7.40; S, 8.45.

6.1.3.22. 2-({[2-({[(2,5-Difluorophenyl)amino]carbonothioyl}oxy)-ethyl]amino}carbonyl)benzoic acid, ***63****.* Yield 9%; mp 144–146 °C (from Me_2CO:MeOH). IR (KBr) 3341, 3178, 1722 cm^{-1}; ^1H NMR (DMSO) δ 3.61–4.02 (m, 2H, CH_2N), 4.15–4.39 (m, 2H, CH_2O), 7.37–8.08 (m, 7H, arom. H), 8.44 (br s, 1H, CONH, exchangeable), 10.92 (br s, 1H, CSNH, exchangeable), 12.56 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{17}H_{14}F_2N_2O_4S$: C, 53.68; H, 3.71; N, 7.36; S, 8.43. Found: C, 53.79; H, 3.79; N, 7.20; S, 8.45.

6.1.3.23. 2-({[2-({[(2,6-Difluorophenyl)amino]carbonothioyl}oxy)-ethyl]amino}carbonyl)benzoic acid, ***64****.* Yield 30%; mp 170–172 °C (from Me_2CO:MeOH). IR (KBr) 3345, 3180, 1723 cm^{-1}; ^1H NMR (DMSO) δ 3.22–3.62 (m, 2H, CH_2N), 4.34–4.57 (m, 2H, CH_2O), 7.01–7.78 (m, 7H, arom. H), 8.25 and 8.48 (br s, 1H, CSNH, exchangeable), 10.61 and 10.91 (br s, 1H, CONH, exchangeable), 12.90 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{17}H_{14}F_2N_2O_4S$: C, 53.68; H, 3.71; N, 7.36; S, 8.43. Found: C, 53.63; H, 3.51; N, 7.21; S, 8.21.

6.1.3.24. 2-({[2-({[(2,4,6-Trifluorophenyl)amino]carbonothioyl}oxy)-ethyl]amino}carbonyl)benzoic acid, ***70****.* Yield 22%; mp 143–145 °C (from Me_2CO:MeOH). IR (KBr) 3340, 3162, 1724 cm^{-1}; ^1H NMR (DMSO) δ 3.27–3.62 (m, 2H, CH_2N), 4.37–4.58 (m, 2H, CH_2O), 7.10–7.88 (m, 6H, arom. H), 8.26 and 8.48 (br s, 1H, CONH, exchangeable), 10.60 and 10.83 (br s, 1H, CSNH, exchangeable), 12.95 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{17}H_{13}F_3N_2O_4S$: C, 51.26; H, 3.29; N, 7.03; S, 8.05. Found: C, 51.42; H, 3.01; N, 6.93; S, 8.26.

6.1.3.25. 2-({[2-({[(2,3,5,6-Tetrafluorophenyl)amino]carbonothioyl}-oxy)ethyl]amino}carbonyl)benzoic acid, ***72****.* Yield 10%; mp 173–175 °C (from Me_2CO). IR (KBr) 3340, 3162, 1724 cm^{-1}; ^1H NMR (DMSO) δ 3.18–3.62 (m, 2H, CH_2N), 4.34–4.58 (m, 2H, CH_2O), 7.11–7.93 (m, 5H, arom. H), 8.38 (br s, 1H, CONH, exchangeable), 11.05 (br s, 1H, CSNH, exchangeable), 12.86 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{17}H_{12}F_4N_2O_4S$: C, 49.04; H, 2.91; N, 6.73; S, 7.70. Found: C, 49.17; H, 3.09; N, 6.80; S, 7.62.

6.1.4. General procedure B for the parallel synthesis of C-TCs ***12–14****,* ***18–34****,* ***40–59*** *and O-TCs* ***61****,* ***65–69****,* ***71****,* ***73–76***

To each ice-cooled reaction tube containing a stirred dried DMF solution (15 mL) of the proper alcohol (5 mmol) and the proper isothiocyanate (5 mmol), a 60% sodium hydride dispersion in mineral oil (0.2 g, ~5 mmol) was added in a single portion. Each reaction mixture was stirred at r.t. for 24 h. Upon treatment with aqueous ammonium chloride (2.5 g dissolved in 100 mL of water), C-TCs separated as oils or solids. Each reaction mixture was transferred into a set of separating funnels and extracted with diethyl ether (2 × 40 mL). The pooled organic phases were washed with water (4 × 20 mL), dried and evaporated in parallel by an Evapose™ apparatus. The crude products were purified by crystallization. Upon acidification (pH = 0) of the reaction mother liquors with 2 N HCl, O-TCs precipitated and were filtered off in parallel. Then, the crude solids were purified by crystallization.

6.1.4.1. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 2,4-dimethylphenylthiocarbamate, ***12****.* Yield 15%; mp 156–158 °C (from CH_2Cl_2:EtOH). IR (KBr) 3337, 1770, 1717, 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.18 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 4.05 (t, J = 6.0 Hz, 2H, CH_2N), 4.83 (t, J = 6.0 Hz, 2H, CH_2O), 6.81–7.16 and 7.71–7.93 (m, 7H, arom. H), 8.18 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90; S, 9.05. Found: C, 64.19; H, 5.32; N, 7.76; S, 8.81.

6.1.4.2. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 2,6-dimethylphenylthiocarbamate, ***13****.* Yield 23%; mp 173–175 °C (from CH_2Cl_2:EtOH). IR (KBr) 3291, 1769, 1713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 3.96–4.22 (m, 2H, CH_2N), 4.59–5.01 (m, 2H, CH_2O), 6.81–7.22 and 7.67–7.94 (m, 7H, arom. H), 8.12 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90; S, 9.05. Found: C, 64.56; H, 5.04; N, 7.91; S, 8.88.

6.1.4.3. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 3,5-dimethylphenylthiocarbamate, ***14****.* Yield 31%; mp 153–155 °C (from CH_2Cl_2:EtOH). IR (KBr) 3309, 1770, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.23 (s, 6H, 2 CH_3), 4.10 (t, J = 6.0 Hz, 2H, CH_2N), 4.86 (t, J = 6.0 Hz, 2H, CH_2O), 6.68–7.03 and 7.71–8.01 (m, 7H, arom. H), 8.39 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90; S, 9.05. Found: C, 64.36; H, 5.28; N, 7.87; S, 8.76.

6.1.4.4. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 3,5-difluorophenylthiocarbamate, ***18****.* Yield 47%; mp 156–158 °C (from Et_2O:EtOH). IR (KBr) 3308, 1777, 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.95–4.28 (m, 2H, CH_2N), 4.73–5.02 (m, 2H, CH_2O), 6.31–6.79, 6.93–7.00 and 7.62–7.99 (m, 7H, arom. H), 11.04 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}F_2N_2O_3S$: C, 56.35; H, 3.34; N, 7.73; S, 8.85. Found: C, 56.10; H, 3.58; N, 7.67; S, 8.93.

6.1.4.5. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 2,3-dichlorophenylthiocarbamate, ***19****.* Yield 36%; mp 119–121 °C (from CH_2Cl_2:MeOH). IR (KBr) 3342, 1774, 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.10 (t, J = 6.0 Hz, 2H, CH_2N), 4.87 (t, J = 6.0 Hz, 2H, CH_2O), 7.01–7.32 and 7.58–7.98 (m, 7H, arom. H), 8.15 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_3S$: C, 51.66; H, 3.06; N, 7.09; S, 8.11. Found: C, 51.51; H, 3.34; N, 6.95; S, 7.93.

6.1.4.6. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 2,4-dichlorophenylthiocarbamate, ***20****.* Yield 22%; mp 154–155 °C (from CH_2Cl_2:MeOH). IR (KBr) 3337, 1760, 1703 cm^{-1}; ^1H NMR (DMSO) δ 3.94 (t, J = 6.0 Hz, 2H, CH_2N), 4.73 (t, J = 6.0 Hz, 2H, CH_2O), 7.22–7.54 and 7.79–8.05 (m, 7H, arom. H), 10.38 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_3S$: C, 51.66; H, 3.06; N, 7.09; S, 8.11. Found: C, 51.66; H, 3.07; N, 7.11; S, 8.00.

6.1.4.7. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 2,5-dichlorophenylthiocarbamate, ***21****.* Yield 57%; mp 138–140 °C (from CH_2Cl_2:EtOH). IR (KBr) 3167, 1776, 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.11 (t, J = 6.0 Hz, 2H, CH_2N), 4.90 (t, J = 6.0 Hz, 2H, CH_2O), 7.01–7.46 and 7.71–8.11 (m, 7H, arom. H), 8.43 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_3S$: C, 51.66; H, 3.06; N, 7.09; S, 8.11. Found: C, 51.54; H, 3.35; N, 6.92; S, 7.98.

6.1.4.8. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 2,6-dichlorophenylthiocarbamate, ***22****.* Yield 37%; mp 200–202 °C (from CH_2Cl_2:EtOH). IR (KBr) 3308, 1770, 1713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.82–4.19 (m, 2H, CH_2N), 4.55–4.97 (m, 2H, CH_2O), 7.03–7.49 and 7.79–7.98 (m, 7H, arom. H), 9.31 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_3S$: C, 51.66; H, 3.06; N, 7.09; S, 8.11. Found: C, 51.49; H, 3.39; N, 6.79; S, 7.90.

6.1.4.9. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 3,4-dichlorophenylthiocarbamate, ***23****.* Yield 49%; mp 164–166 °C (from CH_2Cl_2:MeOH). IR (KBr) 3380, 1778, 1710 cm^{-1}; ^1H NMR (DMSO) δ 4.09 (t, J = 6.0 Hz, 2H, CH_2N), 4.89 (t, J = 6.0 Hz, 2H, CH_2O), 7.07–8.05 (m, 7H, arom. H), 10.37 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_3S$: C, 51.66; H, 3.06; N, 7.09; S, 8.11. Found: C, 51.74; H, 3.14; N, 7.13; S, 8.13.

6.1.4.10. O-[2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethyl] 3,5-dichlorophenylthiocarbamate, ***24****.* Yield 53%; mp 139–140 °C (from

CH_2Cl_2:MeOH). IR (KBr) 3180, 1775, 1710 cm^{-1}; ^1H NMR (DMSO) δ 3.97 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.76 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.03–7.50 and 7.65–8.13 (m, 7H, arom. H), 10.32 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_3S$: C, 51.66; H, 3.06; N, 7.09; S, 8.11. Found: C, 51.49; H, 3.25; N, 6.74; S, 7.87.

6.1.4.11. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chloro-2-methylphenylthiocarbamate,* ***25***. Yield 38%; mp 113–115 °C (from CH_2Cl_2:MeOH). IR (KBr) 3330, 1765, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.19 (s, 3H, CH_3), 4.06 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.80 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.88–7.39 and 7.64–8.28 (m, 7H, arom. H), 10.37 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}ClN_2O_3S$: C, 57.68; H, 4.03; N, 7.47; S, 8.55. Found: C, 57.64; H, 4.00; N, 7.51; S, 8.28.

6.1.4.12. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 5-chloro-2-methylphenylthiocarbamate,* ***26***. Yield 36%; mp 125–127 °C (from CH_2Cl_2:MeOH). IR (KBr) 3175, 1775, 1712 cm^{-1}; ^1H NMR (DMSO) δ 2.07 (s, 3H, CH_3), 3.95 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.71 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.03–7.32 and 7.78–8.10 (m, 7H, arom. H), 10.35 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}ClN_2O_3S$: C, 57.68; H, 4.03; N, 7.47; S, 8.55. Found: C, 57.98; H, 4.37; N, 7.15; S, 8.40.

6.1.4.13. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-chloro-6-methylphenylthiocarbamate,* ***27***. Yield 35%; mp 173–175 °C (from CH_2Cl_2:EtOH). IR (KBr) 3317, 1769, 1713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.09–2.41 (m, 3H, CH_3), 3.78–4.23 (m, 2H, CH_2N), 4.59–5.01 (m, 2H, CH_2O), 6.91–7.39 and 7.61–8.05 (m, 7H, arom. H), 8.34 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}ClN_2O_3S$: C, 57.68; H, 4.03; N, 7.47; S, 8.55. Found: C, 57.81; H, 4.29; N, 7.21; S, 8.26.

6.1.4.14. O-*[2-(1,3-Dioxo-1,3-dihydro-*2H*-isoindol-2-yl)ethyl] 3-chloro-4-methylphenylthiocarbamate,* ***28***. Yield 12%; mp 143–145 °C (from CH_2Cl_2:MeOH). IR (KBr) 3290, 1772, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.29 (s, 3H, CH_3), 4.11 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.88 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.93–8.47 (m, 7H, arom. H), 10.21 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}ClN_2O_3S$: C, 57.68; H, 4.03; N, 7.47; S, 8.55. Found: C, 57.98; H, 4.18; N, 7.33; S, 8.43.

6.1.4.15. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-chloro-4-nitrophenylthiocarbamate,* ***29***. Yield 45%; mp 142–143 °C (from CH_2Cl_2:MeOH). IR (KBr) 3387, 1770, 1705 cm^{-1}; ^1H NMR (DMSO) δ 3.94 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.20 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.44–8.27 (m, 7H, arom. H), 10.36 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}ClN_3O_5S$: C, 50.32; H, 2.98; N, 10.35; S, 7.90. Found: C, 50.43; H, 2.88; N, 10.32; S, 7.97.

6.1.4.16. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chloro-3-nitrophenylthiocarbamate,* ***30***. Yield 52%; mp 205–206 °C (from CH_2Cl_2:MeOH). IR (KBr) 3190, 1770, 1710 cm^{-1}; ^1H NMR (DMSO) δ 4.01 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.72 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.45–8.18 (m, 7H, arom. H), 10.35 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{17}H_{12}ClN_3O_5S$: C, 50.32; H, 2.98; N, 10.35; S, 7.90. Found: C, 50.55; H, 2.81; N, 10.45; S, 7.55.

6.1.4.17. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-bromo-2-methylphenylthiocarbamate,* ***31***. Yield 43%; mp 111–113 °C (from CH_2Cl_2:MeOH). IR (KBr) 3325, 1768, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H, CH_3), 4.41 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.84 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.99–7.38 and 7.97–8.08 (m, 7H, arom. H), 9.34 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}BrN_2O_3S$: C, 51.56; H, 3.61; N, 6.68; S, 7.65. Found: C, 51.27; H, 3.43; N, 6.73; S, 7.77.

6.1.4.18. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 5-chloro-2-methoxyphenylthiocarbamate,* ***32***. Yield 29%; mp 126–127 °C (from CH_2Cl_2:MeOH). IR (KBr) 3375, 1775, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.48 (s, 3H, CH_3O), 4.16 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.92 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.68–7.10 and 7.55–7.98 (m, 7H, arom. H), 10.12 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}ClN_2O_4S$: C, 55.32; H, 3.87; N, 7.17; S, 8.20. Found: C, 55.10; H, 3.85; N, 7.14; S, 8.21.

6.1.4.19. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3,4-dimethoxyphenylthiocarbamate,* ***33***. Yield 24%; mp 99–101 °C (from CH_2Cl_2:EtOH). IR (KBr) 3337, 1771, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.86 (s, 6H, 2 OCH_3), 4.08 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.84 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.63–6.96 and 7.68–7.96 (m, 7H, arom. H), 8.20 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{18}N_2O_5S$: C, 59.06; H, 4.70; N, 7.25; S, 8.30. Found: C, 59.32; H, 5.06; N, 7.23; S, 8.64.

6.1.4.20. O-*[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-chloro-3-trifluoromethylphenylthiocarbamate,* ***34***. Yield 43%; mp 163–165 °C (from CH_2Cl_2:EtOH). IR (KBr) 3193, 1773, 1718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.11 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.89 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.27–7.52 and 7.62–7.98 (m, 7H, arom. H), 8.14 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{12}F_3N_2O_3S$: C, 50.42; H, 2.82; N, 6.53; S, 7.48. Found: C, 50.42; H, 3.07; N, 6.66; S, 7.58.

6.1.4.21. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] cyclohexylthiocarbamate,* ***40***. Yield 20%; mp 146–147 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3230, 2926, 1776, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82–2.20 (m, 10H, 5 CH_2-cyclohexyl), 2.52 (s, 3H, CH_3), 3.21–3.31 (m, 1H, CH), 4.07 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.70 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.32 (br s, 1H, NH, exchangeable), 7.42–7.90 (m, 3H, arom. H). Anal. Calcd for $C_{18}H_{22}N_2O_3S$: C, 62.40; H, 6.40; N, 8.09; S, 9.25. Found: C, 62.47; H, 6.57; N, 7.97; S, 9.60.

6.1.4.22. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethyl] 4-methylphenylthiocarbamate,* ***41***. Yield 37%; mp 149–151 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3310, 1767, 1699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.04 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.85 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.88–7.27 and 7.50–7.84 (m, 7H, arom. H), 8.32 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90; S, 9.05. Found: C, 64.65; H, 5.25; N, 7.86; S, 9.31.

6.1.4.23. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-ethylphenylthiocarbamate,* ***42***. Yield 39%; mp 149–151 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3311, 1771, 1701 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (t, $J = 6.0$ Hz, 3H, CH_3-ethyl), 2.37–2.75 (m, 5H, CH_2 and CH_3), 4.04 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.85 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.07–7.86 (m, 7H, arom. H), 8.72 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{20}H_{20}N_2O_3S$: C, 65.20; H, 5.47; N, 7.60; S, 8.70. Found: C, 65.24; H, 5.37; N, 7.53; S, 8.95.

6.1.4.24. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethyl] 4-acetylphenylthiocarbamate,* ***43***. Yield 22%; mp 173–175 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3290, 1760, 1721, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H, $COCH_3$), 2.55 (s, 3H, CH_3), 4.09 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.89 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.17–8.02 (m, 7H, arom. H), 9.03 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{20}H_{18}N_2O_4S$: C, 62.81; H, 4.74; N, 7.33; S, 8.38. Found: C, 62.53; H, 4.86; N, 7.44; S, 8.07.

6.1.4.25. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-cyanophenylthiocarbamate,* ***44***. Yield 40%; mp 180–182 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3184, 2226, 1770, 1712, 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H, CH_3), 4.09 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.86 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.30–7.84 (m, 7H, arom. H), 10.32 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{15}N_3O_3S$: C, 62.45; H, 4.14; N, 11.50; S, 8.77. Found: C, 62.31; H, 4.40; N, 11.32; S, 8.57.

6.1.4.26. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) ethyl] 4-fluorophenylthiocarbamate,* ***45***. Yield 40%; mp 143–145 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3242, 1772, 1717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H, CH_3), 4.01 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.82 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.73–7.73 (m, 7H, arom. H), 8.55 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}FN_2O_3S$: C, 60.33; H, 4.22; N, 7.82; S, 8.95. Found: C, 60.42; H, 4.45; N, 7.83; S, 8.75.

6.1.4.27. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) ethyl] 4-bromophenylthiocarbamate,* ***46***. Yield 12%; mp 174–176 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3299, 1767, 1698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H, CH_3), 3.81–4.13 (m, 2H, CH_2N), 4.69–4.96 (m, 2H, CH_2O), 7.18–7.80 (m, 7H, arom. H), 11.13 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}BrN_2O_3S$: C, 51.56; H, 3.61; N, 6.68; S, 7.65. Found: C, 51.47; H, 3.89; N, 6.84; S, 7.31.

6.1.4.28. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-iodophenylthiocarbamate,* ***47***. Yield 37%; mp 183–185 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3202, 1772, 1718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 4.07 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.85 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.60–7.77 (m, 7H, arom. H), 8.32 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}IN_2O_3S$: C, 46.37; H, 3.24; N, 6.01; S, 6.88. Found: C, 46.10; H, 3.58; N, 5.95; S, 6.58.

6.1.4.29. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) ethyl] 4-methoxyphenylthiocarbamate,* ***48***. Yield 39%; mp 151–153 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3311, 1768, 1701 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 4.05 (t, $J = 6.0$ Hz, 2H, CH_2N), 4.84 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.59–7.76 (m, 7H, arom. H), 8.26 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{18}N_2O_4S$: C, 61.62; H, 4.90; N, 7.56; S, 8.65. Found: C, 61.87; H, 4.89; N, 7.58; S, 8.50.

6.1.4.30. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3,4-dichlorophenylthiocarbamate,* ***49***. Yield 64%; mp 164–166 °C (from CH_2Cl_2). IR (KBr) 3316, 1773, 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3); 3.92–4.23 (m, 2H, CH_2N), 4.68–5.03 (m, 2H, CH_2O), 7.10–7.88 (m, 6H, arom. H), 8.12 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{14}Cl_2N_2O_3S$: C, 52.82; H, 3.45; N, 6.84; S, 7.83. Found: C, 52.74; H, 3.48; N, 6.53; S, 7.54.

6.1.4.31. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) ethyl] 3-chloro-4-methylphenylthiocarbamate,* ***50***. Yield 30%; mp 136–138 °C (from CH_2Cl_2:MeOH). IR (KBr) 3307, 1770, 1699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.29 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.89–4.20 (m, 2H, CH_2N), 4.70–4.99 (m, 2H, CH_2O), 6.81–7.85 (m, 6H, arom. H), 8.39 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{17}ClN_2O_3S$: C, 58.68; H, 4.41; N, 7.20; S, 8.25. Found: C, 58.35; H, 4.68; N, 7.05; S, 8.56.

6.1.4.32. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) ethyl] 3-chloro-4-nitrophenylthiocarbamate,* ***51***. Yield 49%; mp 205–207 °C (from Me_2CO). IR (KBr) 3302, 1768, 1697 cm^{-1}; ^1H NMR (DMSO) δ 2.37 (s, 3H, CH_3), 3.80–3.92 (m, 2H, CH_2N), 4.64–4.76 (m, 2H, CH_2O), 7.42–7.68 (m, 6H, arom. H), 11.44 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{14}ClN_3O_5S$: C, 51.49; H, 3.36; N, 10.01; S, 7.64. Found: C, 51.72; H, 3.35; N, 9.98; S, 7.95.

6.1.4.33. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3-chloro-4-(trifluoromethyl)phenylthiocarbamate,* ***52***. Yield 33%; mp 133–135 °C (from Et_2O:MeOH). IR (KBr) 3307, 1772, 1698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H, CH_3), 3.93–4.24 (m, 2H, CH_2N), 3.72–4.98 (m, 2H, CH_2O), 7.23–7.85 (m, 6H, arom. H), 8.51 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{14}ClF_3N_2O_3S$: C, 51.53; H, 3.19; N, 6.33; S, 7.24. Found: C, 51.18; H, 3.27; N, 6.27; S, 7.44.

6.1.4.34. O-*[2-(5-*tert*-butyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) ethyl] 4-methylphenylthiocarbamate,* ***53***. Yield 22%; mp 118–120 °C (from Et_2O). IR (KBr) 3232, 1763, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (s, 9H, $C(CH_3)_3$), 2.24 (s, 3H, CH_3Ph), 3.88–4.19 (m, 2H, CH_2N), 4.63–4.92 (m, 2H, CH_2O), 6.89–7.27 and 7.61–7.99 (m, 7H, arom. H). Anal. Calcd for $C_{22}H_{24}N_2O_3S$: C, 66.64; H, 6.10; N, 7.07; S, 8.09. Found: C, 66.72; H, 6.13; N, 7.27; S, 7.91.

6.1.4.35. O-*[2-(5-*tert*-Butyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) ethyl] 4-chlorophenylthiocarbamate,* ***54***. Yield 15%; mp 101–103 °C (from Et_2O:MeOH). IR (KBr) 3253, 2963 1771, 1718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (s, 9H, $C(CH_3)_3$), 3.94–4.04 (m, 2H, CH_2N), 4.67–4.80 (m, 2H, CH_2O), 6.96–7.24, 7.63–7.67 and 7.75–7.81 (m, 7H, arom. H), 8.59 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{21}H_{21}ClN_2O_3S$: C, 60.50; H, 5.08; N, 6.72; S, 7.69. Found: C, 60.78; H, 5.21; N, 6.49; S, 7.44.

6.1.4.36. O-*[(2*R*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl] 4-methylphenylthiocarbamate,* ***55***. Yield 16%; mp 78–80 °C (from EtOH). $[\alpha]^{20}_D = -14.1$ (*c* 0.97, $CHCl_3$). IR (KBr) 3246, 1776, 1707 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (d, $J = 6.6$ Hz, 3H, CH_3), 2.09 (s, 3H, CH_3Ph), 4.63–4.79 (m, 1H, CH), 4.81–4.94 (m, 2H, CH_2O), 6.65–6.87 (m, 4H, arom. H), 7.60–7.84 (m, 4H, arom. H), 8.23 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90; S, 9.05. Found: C, 64.30; H, 5.44; N, 7.56; S, 8.81.

6.1.4.37. O-*[(2*R*)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl] 4-chlorophenylthiocarbamate,* ***56***. Yield 38%; mp 117–119 °C (from Me_2CO:EtOH). $[\alpha]^{20}_D = -23.2$ (*c* 0.99, $CHCl_3$). IR (KBr) 3244, 1776, 1707 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (d, $J = 6.6$ Hz, 3H, CH_3), 4.50–5.10 (m, 3H, CH and CH_2O), 6.87–7.28 and 7.68–7.91 (m, 8H, arom. H), 8.99 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}ClN_2O_3S$: C, 57.68; H, 4.03; N, 7.47; S, 8.55. Found: C, 57.92; H, 4.32; N, 7.20; S, 8.56.

6.1.4.38. O-*[(2S)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl] 4-chlorophenylthiocarbamate,* ***57***. Yield 22%; mp 118–120 °C (from Et_2O:MeOH). $[\alpha]^{20}_D = +22.1$ (*c* 1.09, $CHCl_3$). IR (KBr) 3244, 1776, 1707 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (d, $J = 6.6$ Hz, 3H, CH_3), 4.50–5.10 (m, 3H, CH and CH_2O), 6.87–7.28 and 7.68–7.91 (m, 8H, arom. H), 8.99 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{18}H_{15}ClN_2O_3S$: C, 57.68; H, 4.03; N, 7.47; S, 8.55. Found: C, 57.92; H, 4.32; N, 7.20; S, 8.56.

6.1.4.39. (±) O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)propyl] 4-methylphenylthiocarbamate,* ***58***. Yield 40%; mp 141–143 °C (from Et_2O:MeOH). IR (KBr) 3330, 1763, 1695 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.50 (d, $J = 6.6$ Hz, 3H, CH_3), 2.19 (s, 3H, CH_3Ph), 2.50 (s, 3H, CH_3Phtha), 4.39–5.03 (m, 3H, CH and CH_2O), 6.74–7.20 and 7.47–7.74 (m, 7H, arom. H), 8.52 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{20}H_{20}N_2O_3S$: C, 65.20; H, 5.47; N, 7.60; S, 8.70. Found: C, 65.48; H, 5.56; N, 7.52; S, 8.52.

6.1.4.40. O-*[2-(5-Methyl-1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl) propyl] 4-chlorophenylthiocarbamate,* ***59***. Yield 51%; mp 171–173 °C (from Me_2CO:CH_2Cl_2). IR (KBr) 3312, 1763, 1694 cm^{-1}; ^1H NMR (DMSO) δ 1.43 (d, $J = 6.6$ Hz, 3H, CH_3), 2.50 (s, 3H, CH_3Phtha), 4.29–4.94 (m, 3H, CH and CH_2O), 6.93–7.36 and 7.61–7.80 (m, 7H, arom. H), 11.31 (br s, 1H, NH, exchangeable). Anal. Calcd for $C_{19}H_{17}ClN_2O_3S$: C, 58.68; H, 4.41; N, 7.20; S, 8.25. Found: C, 58.65; H, 4.25; N, 7.08; S, 8.46.

6.1.4.41. 2-({[2-({[(2,4-Dimethylphenyl)amino]carbonothioyl}oxy) ethyl]amino}carbonyl)benzoic acid, ***61***. Yield 16%; mp 145–147 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3310, 3190, 1702, 1654 cm^{-1}; ^1H NMR (DMSO) δ 2.13 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.30–3.78 (m, 2H, CH_2N), 4.38–4.76 (m, 2H, CH_2O), 6.98–7.19 and 7.37–7.96 (m, 7H,

arom. H), 8.40 (br s, 1H, CSNH, exchangeable), 10.43 (br s, 1H, CONH, exchangeable), 12.81 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52; S, 8.61. Found: C, 61.33; H, 5.41; N, 7.50; S, 8.68.

6.1.4.42. 2-({[2-({[(3,5-Difluorophenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***65***. Yield 16%; mp 167–169 °C (from Me_2CO:MeOH). IR (KBr) 3331, 1698, 1650 cm^{-1}; ^1H NMR (DMSO) δ 3.46–3.84 (m, 2H, CH_2N), 4.50–4.84 (m, 2H, CH_2O), 6.74–7.98 (m, 8H, 7 arom. H and CONH), 8.57 (br s, 1H, CSNH, exchangeable), 11.62 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{17}H_{14}F_2N_2O_4S$: C, 53.68; H, 3.71; N, 7.36; S, 8.43. Found: C, 53.62; H, 4.05; N, 7.48; S, 8.41.

6.1.4.43. 2-({[2-({[(5-Chloro-2-methylphenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***66***. Yield 16%; mp 147–148 °C (from CH_2Cl_2:MeOH). IR (KBr) 3342, 3257, 1701 cm^{-1}; ^1H NMR (DMSO) δ 2.20 (s, 3H, CH_3), 3.35–3.80 (m, 2H, CH_2N), 4.50 (t, $J = 6.0$ Hz, 2H, CH_2O), 6.95–7.78 (m, 8H, 7 arom. H and CONH), 9.65 (br s, 1H, CSNH, exchangeable), 11.30 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{18}H_{17}ClN_2O_4S$: C,55.03; H, 4.36; N, 7.13; S, 8.16. Found: C, 54.90; H, 4.41; N, 7.15; S, 7.92.

6.1.4.44. 2-({[2-({[(3-Chloro-4-methylphenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***67***. Yield 61%; mp 166–168 °C (from CH_2Cl_2:MeOH). IR (KBr) 3340, 3250, 1688, 1581, 1530 cm^{-1}; ^1H NMR (DMSO) δ 2.30 (s, 3H, CH_3), 3.40–3.80 (m, 2H, CH_2N), 4.63 (t, $J = 6.0$ Hz, 2H, CH_2O), 7.05–7.95 (m, 8H, 7 arom. H and CONH), 8.78 (br s, 1H, CSNH, exchangeable), 11.80 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{18}H_{17}ClN_2O_4S$: C, 55.03; H, 4.36; N, 7.13; S, 8.16. Found: C, 55.04; H, 4.36; N, 7.10; S, 8.14.

6.1.4.45. 2-({[2-({[(2-Methoxy-5-methylphenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***68***. Yield 28%; mp 143–145 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3338, 3300, 1689, 1666 cm^{-1}; ^1H NMR (DMSO) δ 2.20 (s, 3H, CH_3), 3.29–3.87 (m, 5H, CH_2N and CH_3O), 4.32–4.73 (m, 2H, CH_2O), 6.83–7.98 (m, 7H, arom. H), 8.47 (br s, 1H, CSNH, exchangeable), 10.39 (br s, 1H, CONH, exchangeable), 13.07 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{19}H_{20}N_2O_5S$: C, 58.75; H, 5.19; N, 7.21; S, 8.25. Found: C, 58.77; H, 5.20; N, 7.24; S, 8.02.

6.1.4.46. 2-({[2-({[(3,4-Dimethoxyphenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***69***. Yield 31%; mp 147–149 °C (from Et_2O:CH_2Cl_2). IR (KBr) 3363, 3287, 1695, 1637 cm^{-1}; ^1H NMR (DMSO) δ 3.48–4.01 (m, 8H, CH_2N and 2 CH_3O), 4.34–4.80 (m, 2H, CH_2O), 6.65–7.96 (m, 7H, arom. H), 8.53 (br s, 1H, CSNH, exchangeable), 10.25 (br s, 1H, CONH, exchangeable), 13.01 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{19}H_{20}N_2O_6S$: C, 56.43; H, 4.98; N, 6.93; S, 7.93. Found: C, 56.67; H, 4.98; N, 6.91; S, 8.13.

6.1.4.47. 2-({[2-({[(2,3,4-Trichlorophenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***71***. Yield 35%; mp 81–82 °C (from Me_2CO). IR (KBr) 3142, 1712 cm^{-1}; ^1H NMR (DMSO) δ 3.32–3.78 (m, 2H, CH_2N), 4.43–4.75 (m, 2H, CH_2O), 7.08–7.98 (m, 6H, arom. H), 8.44 (br s, 1H, CONH, exchangeable), 11.24 (br s, 1H, CSNH, exchangeable), 13.05 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{17}H_{13}Cl_3N_2O_4S$: C, 45.60; H, 2.93; N, 6.26; S, 7.16. Found: C, 45.59; H, 3.02; N, 6.46; S, 7.41.

6.1.4.48. 2-({[(1R)-1-Methyl-2-({[(4-methylphenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***73***. Yield 16%; mp 157–159 °C (from Me_2CO:Et_2O). $[\alpha]_D^{20} = -11.9$ (*c* 0.91, Me_2CO). IR (KBr) 3322, 3237, 1696, 1650 cm^{-1}; ^1H NMR (DMSO) δ 0.99–1.19 (m, 3H, CH_3), 2.16 (s, 3H, CH_3Ph), 4.13–4.50 (m, 3H, CH_2O and CH), 6.97–7.23, 7.35–7.52 and 7.68–7.75 (m, 8H, arom. H), 8.26 (br s, 1H, CONH, exchangeable), 10.93 (br s, 1H, CSNH, exchangeable), 12.88 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52; S, 8.61. Found: C, 61.05; H, 5.16; N, 7.51; S, 8.71.

6.1.4.49. 2-({[(1S)-1-Methyl-2-({[(4-methylphenyl)amino]carbonothioyl}oxy)ethyl]amino}carbonyl)benzoic acid, ***74***. Yield 26%; mp 163–165 °C (from Me_2CO:Et_2O). $[\alpha]_D^{20} = +11.3$ (*c* 0.97, Me_2CO). IR (KBr) 3322, 3237, 1696, 1650 cm^{-1}; ^1H NMR (DMSO) δ 0.99–1.19 (m, 3H, CH_3), 2.16 (s, 3H, CH_3Ph), 4.13–4.50 (m, 3H, CH_2O and CH), 6.97–7.23, 7.35–7.52 and 7.68–7.75 (m, 8H, arom. H), 8.26 (br s, 1H, CONH, exchangeable), 10.93 (br s, 1H, CSNH, exchangeable), 12.88 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52; S, 8.61. Found: C, 61.05; H, 5.16; N, 7.51; S, 8.71.

6.1.4.50. 2-({[(1R)-2-({[(4-Chlorophenyl)amino]carbonothioyl}oxy)-1-methylethyl]amino}carbonyl)benzoic acid, ***75***. Yield 24%; mp 158–160 °C (from Me_2CO:Et_2O). $[\alpha]_D^{20} = -21.1$ (*c* 0.98, Me_2CO). IR (KBr) 3285, 1702, 1637 cm^{-1}; ^1H NMR (DMSO) δ 0.95–1.17 (m, 3H, CH_3), 4.10–4.52 (m, 3H, CH_2O and CH), 6.91–7.79 (m, 8H, arom. H), 8.29 (br s, 1H, CONH, exchangeable), 11.17 (br s, 1H, CSNH, exchangeable), 12.90 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{18}H_{18}ClN_2O_4S$: C, 55.03; H, 4.36; N, 7.13; S, 8.16. Found: C, 55.19; H, 4.65; N, 7.43; S, 8.26.

6.1.4.51. 2-({[(1S)-2-({[(4-Chlorophenyl)amino]carbonothioyl}oxy)-1-methylethyl]amino}carbonyl)benzoic acid, ***76***. Yield 21%; mp 147–149 °C (from Me_2CO). $[\alpha]_D^{20} = +20.1$ (*c* 1.39, Me_2CO). IR (KBr) 3285, 1702, 1637 cm^{-1}; ^1H NMR (DMSO) δ 0.95–1.17 (m, 3H, CH_3), 4.10–4.52 (m, 3H, CH_2O and CH), 6.91–7.79 (m, 8H, arom. H), 8.29 (br s, 1H, CONH, exchangeable), 11.17 (br s, 1H, CSNH, exchangeable), 12.90 (br s, 1H, COOH, exchangeable). Anal. Calcd for $C_{18}H_{18}ClN_2O_4S$: C, 55.03; H, 4.36; N, 7.13; S, 8.16. Found: C, 55.19; H, 4.65; N, 7.43; S, 8.26.

6.2. Virology: materials and methods

The biological evaluation of the synthesized compounds was performed according to the previously reported procedures [12,43].

6.3. Molecular modelling

The molecular structures of **5**, **23**, **28**–**30**, **33**, **34**, **49**–**52**, **54**, **56** and **57** were built by Insight II (Builder module), parameterized according to CVFF force field [44] and their RT complexes were calculated using Autodock 3.05 [40]. After the removal of the inhibitor from the crystal structure of RT/**VII** complex (PDB code 2VG5) [9], hydrogen atoms 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 selected as the binding conformation. The resulting models were energy-minimized by CNS [45], performing a cycle of simulated annealing (starting temperature 1000 K) followed by 120 cycles of Powell minimization. Model analyses were performed using the CCP4 program suite [46]. All the calculations were carried out on Silicon Graphic Indigo2 and Origin 200 workstations.

Acknowledgements

This work was supported by MURST (Cofinanziamento Nazionale), CNR (Rome) and Ministero Italiano della Salute – Istituto Superiore di Sanità (grant no 40D.46). Fondazione Banca Carige is gratefully acknowledged for financially supporting SC. The authors thank Mr. O. Gagliardo for the microanalyses, Mr. F. Tuberoni, Dr. C. Rossi and Dr. R. Raggio for the IR and NMR spectra.

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