Structure-Based Design, Parallel Synthesis, Structure−Activity Relationship, and Molecular Modeling Studies of Thiocarbamates, New Potent Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitor Isosteres of Phenethylthiazolylthiourea Derivatives

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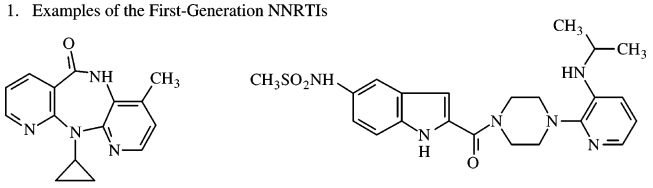
In this paper we describe our structure-based ligand design, synthetic strategy, and structure−activity relationship (SAR) studies that led to the identification of thiocarbamates (TCs), a novel class of non-nucleoside reverse transcriptase inhibitors (NNRTIs), isosteres of phenethylthiazolylthiourea (PETT) derivatives. Assuming as a lead compound *O*-[2-(phthalimido)ethyl]-phenylthiocarbamate **12**, one of the precursors of the previously described acylthiocarbamates (Ranise, A.; et al. *J. Med. Chem*. **2003**, 46, 768−781), two targeted solution-phase TC libraries were prepared by parallel synthesis. The lead optimization strategy led to para-substituted TCs **31**, **33**, **34**, **39**, **40**, **41**, **44**, **45**, and **50**, which were active against wild-type HIV-1 in MT-4-based assays at nanomolar concentrations (EC_{50} range: 0.04−0.01 μM). The most potent congener **50** (EC_{50} = 0.01 μM) bears a methyl group at position 4 of the phthalimide moiety and a nitro group at the para position of the *N*-phenyl ring. Most of the TCs showed good selectivity indices, since no cytotoxic effect was detected at concentrations as high as 100 μM. TCs **31**, **37**, **39**, **40**, and **44** significantly reduced the multiplication of the Y181C mutant, but they were inactive against K103R and K103N + Y181C mutants. Nevertheless, the fold increase in resistance of **41** was not greater than that of efavirenz against the K103R mutant in enzyme assays. The docking model predictions were consistent with in vitro biological assays of the anti-HIV-1 activity of the TCs and related compounds synthesized.

Introduction

Nucleoside (NRTIs) and non-nucleoside inhibitors (NNRTIs) targeting the HIV-1 encoded reverse transcriptase (RT)[1] have to be proved effective in treating the HIV infection and AIDS.[2] NNRTIs[3−13] bind to an allosteric site (non-nucleoside binding site, NNBS) largely contained within the RT p66 subunit, some 10 Å from the polymerase active site. Despite their chemical diversity, NNRTIs interact with the NNBS, showing a similar three-dimensional arrangement, the so-called "butterfly-like conformation" typical of first-generation NNRTIs,[14] as demonstrated by X-ray crystallography of HIV-1 RT−NNRTI complexes.[15−23] However, the relatively unconserved amino acid sequence of the NNBS favors the rapid selection of NNRTI-resistant viruses, both in vitro and in vivo.[24] As a result of single-point mutations in the NNBS,[25] first-generation NNRTIs, e.g., nevirapine and delavirdine (Chart 1.1) show a loss of potency of several orders of magnitude. In contrast, second-generation NNRTIs, such as efavirenz (Chart 1.2)[26] and some thiocarboxanilide[27] and quinoxaline[28] derivatives, result in minor losses of activity against variants carrying either single or double NNRTI resistance mutations. Nevertheless, the fact that cross-resistance extends to the whole NNRTI class calls for development of new agents capable of inhibiting clinically relevant NNRTI-resistant mutants.

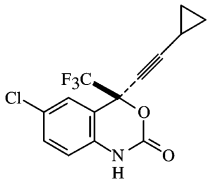
Chart 1

1. Examples of the First-Generation NNRTIs



Nevirapine Delavirdine

2. Example of the Second-Generation NNRTIs



(-) Efavirenz

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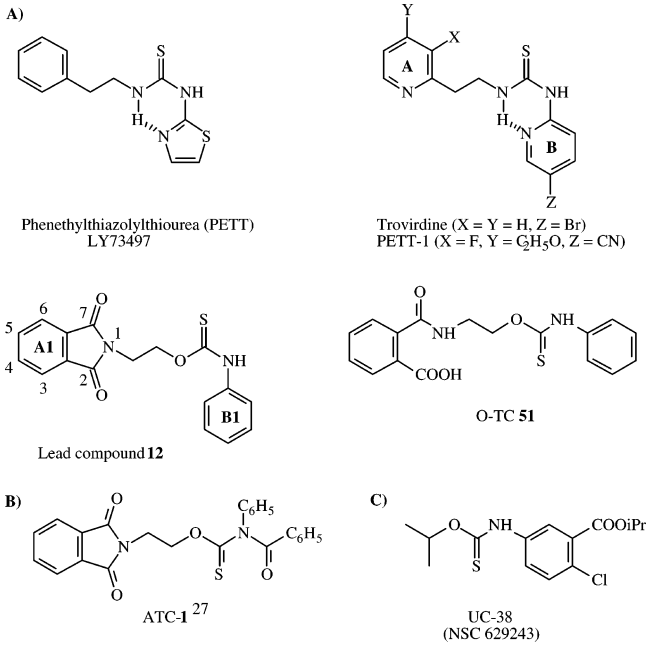
Chart 2. Chemical Structures of PETT, Trovirdine, PETT-1, Lead Compound **12**, O-TC **51**, ATC-**1**, and UC-38



We have previously described a novel class of NNRTIs, i.e., O-substituted *N*-acyl-*N*-arylthiocarbamates (ATCs)[29] structurally related to *N*-phenethyl-*N*′-thiazolylthiourea (PETT) derivatives[30,31] (Chart 2A). Among the ATCs, the phthalimidoethyl-ATCs proved to be potent inhibitors of the multiplication of wild-type HIV-1, significantly active against Y181C mutants but ineffective against K103R mutants.

Figure 1 shows the relative positions and orientations of the modeled structures of ATC-**1**, one of the lead compounds of the ATC series (Chart 2B), and of its intermediary thiocarbamate (TC) **12** (Chart 2A) superimposed on the X-ray structure model of PETT-1 (Chart 2A) within the NNBS. It is apparent that the conformation of **12**, unlike that of ATC-**1**, more closely resembles the conformation of PETT-1. Furthermore, the thiocarbamate UC-38 (NSC 629243) (Chart 2C) was selected as an anti-HIV-1 agent in the early 1990s for preclinical development.[32]

Starting from these preliminary remarks and taking into account that screening of synthesis intermediates represents one of the strategies in the search for new lead compounds,[33] **12** was proposed for synthesis and tested both in enzyme assays against HIV-1 virion RT (vRT) and in cell-based assays for cytotoxicity and anti-HIV-1 activity.[29] As anticipated by its docking model, TC **12** proved to be able to target vRT ($IC_{50} = 0.6\ \mu M$) and to selectively inhibit the HIV-1 induced cytopathogenicity in MT-4 cells ($CC_{50} > 100\ \mu M$, $EC_{50} = 1.2\ \mu M$, Table 3), even if it was 3-fold less potent than ATC-**1** ($EC_{50} = 0.4\ \mu M$).[29] Therefore, to further explore the potential of TCs as anti-HIV-1 agents, we designed two small libraries of analogues of the lead compound **12**. An additional scope of our work was to compare some relevant SARs of the TC and PETT derivatives owing to their isosteric relationship. In the field of NNRTIs, structure-based design of analogues of trovirdine[31] (Chart 2A), the most representative of PETT derivatives, is an attractive research area for medicinal chemists, as documented by recent, published papers.[34−45]

However, PETT isostere TCs have not been hitherto reported in the literature. Notably, TCs, unlike PETT derivatives such as PETT LY73497, PETT-1 (Chart 2A), and trovirdine, have no internal H-bond, and therefore, the lack of this conformational constraint makes them more flexible than PETT derivatives. In this regard, it was speculated that a greater conformational freedom of inhibitors might be a useful design feature for reducing drug resistance.[46−49]

With the aim to establish the key structural requirements of TCs for anti-HIV-1 activity, our lead optimization strategy was centered on (1) substituent variation on the *N*-phenyl ring (B1), (2) modification of the benzene ring (A1) of the phthalimide moiety, (3) shortening, lengthening, and branching the ethyl spacer, (4) modification of the thiocarbamate scaffold through isosteric replacements (S/O and NH/O), and (5) opening of the imide ring. Indeed, the last modification, planned for future SAR strategies, was anticipated because in the TC parallel synthesis several ring-opened analogues (O-TCs) (see Chemistry) were concomitantly obtained along with their corresponding ring-closed congeners (C-TCs). Since the overall conformation of enzyme-bound **51** (Chart 2A), the corresponding O-TC analogue of lead **12**, approximates the X-ray structure of PETT-1 within the NNBS (Figure 2B), the O-TCs were also tested for HIV-1 activity. Finally, some structural variations were performed with the aim not only to expand the SARs but also to validate the TC docking model (for representative examples, see derivatives **77**−**81** and **92**−**95**).

Chemistry

Combinatorial library methodologies[50] play a pivotal role in the modern drug discovery process, in particular in lead identification and optimization efforts. Library synthesis of small, druglike molecules is carried out by means of manual, semiautomated, or automated synthesizers. Among the combinatorial approaches, parallel synthesis is aimed at fast preparation of a (large) number of compounds in short times, using simple and rapid purification methods.[51]

Since the new highly convergent synthesis of ATCs was performed through a one-pot three-step procedure by sequentially combining three types of building blocks (alcohols, isothiocyanates, acyl chlorides),[29] in principle, TCs, being precursors of ATCs, could be prepared with the above procedure by omitting the acylation step. As previously reported,[29] one of the intermediary TCs was actually isolated by acidifying the reaction medium, before acylating its corresponding sodium salt. Because the synthetic protocol can be easily adapted to parallel solution-phase synthesis (and nowadays the use of solution-phase techniques has been accepted as a valuable alternative to solid-supported chemistry approaches[52]), we designed and synthesized two small, targeted TC libraries. Preliminarily, the syntheses of alcohol building blocks **3**, **4**, (±)-**5**, (±)-**6**, **8**, and **9** (Chart 3), not commercially available, were carried out starting from anhydrides **I**−**V** and amino alcohols (Scheme 1). Thus, according to literature procedures,[53] fusion of anhydrides **I**, **II**, **IV**, and **V** with

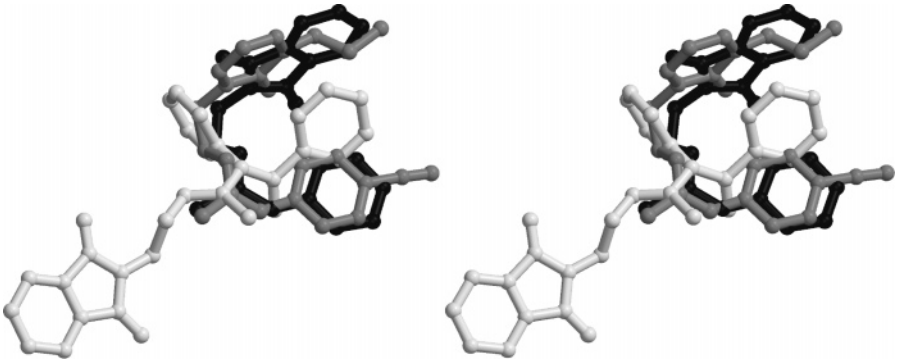


Figure 1. Stereodiagram showing superposition of TC **12**, ATC-**1**, and PETT-1 (see Chart 1) within the NNBS. The relative positions and conformations for TC **12** (model structure, black ball-and-stick), ATC-**1** (model structure, white ball-and-stick), and PETT-1 (X-ray structure, gray ball-and-stick) are shown in their respective complexes with RT. The structures were superimposed on the basis of the amino acid residues surrounding the RT pocket, as described by Ren et al.[16] Figure 1 indicates the different binding mode of ATC-**1** and its TC intermediate **12**. Despite the lack of the internal H-bond typical of PETT derivatives, **12** assumes the butterfly-shaped bioactive conformation of NNRTIs, strongly mimicking the RT-bound PETT-1 conformation and orientation. The programs MolScript[61] and Raster3D[62] were used for drawing the figure.

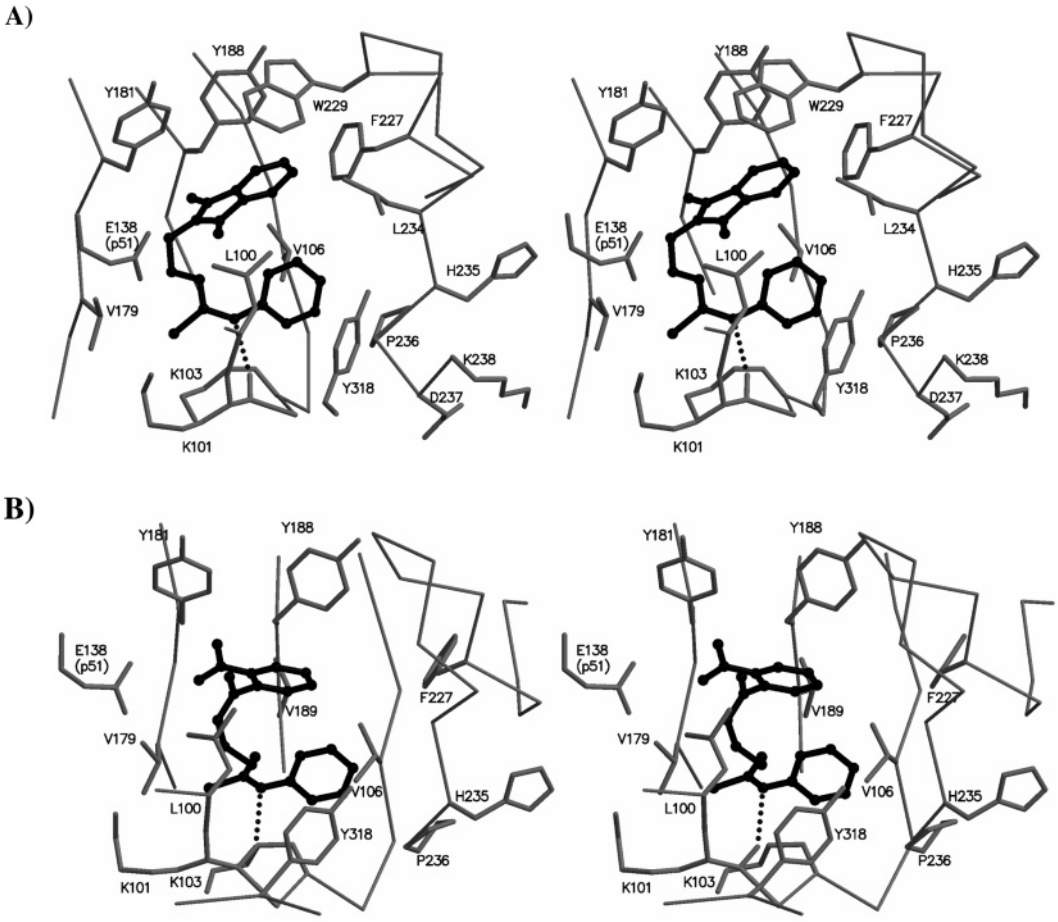
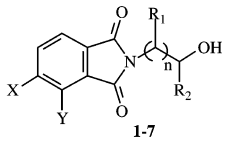


Figure 2. Stereoview showing the position and orientation of C-TC **12** (A) and its corresponding O-TC **51** (B). The ligands are represented as black balls-and-sticks, while the residues lining RT non-nucleoside binding site are shown as gray sticks. Hydrogen bonds are depicted as dotted lines. The drawing was realized by the programs MolScript[61] and Raster3D.[62]

2-aminoethanol at 175−180 °C for 2 h gave **3**, **4**, **8**, and **9**, respectively. Similarly, **III** condensed both with *d*,*l*-2-amino-1-propanol at 140−150 °C and with *d*,*l*-1-amino-2-propanol at 160−175 °C to afford the respective alcohols **5** and **6** as racemic mixtures. Scheme 2 summarizes the general procedure for the preparation of libraries 1 and 2. The parallel analogue synthesis was carried out by using a manual synthesizer (Carousel reaction station). Typically, in DMF, dried on molecular sieves (library 1), or in anhydrous pyridine (library 2), alcohols **1**−**7** (Chart 3A), due to their low nucleophilicity, were transformed in the presence of a 60% NaH dispersion in mineral oil into their corresponding alcoholates $\mathbf{A}_{1-7}$, which condensed in situ with appropriate isothiocyanates (Chart 3B) to afford either adducts **B** (sodium salts of the C-TCs) or **B** and their ring-opened counterparts **SS** (di-sodium salts of the O-TCs), according to the experimental results (see below). To improve the

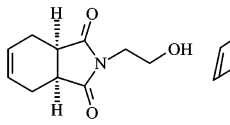
Chart 3. Alcohols **1**−**10** and Isothiocyanates (Ar_1NCS) Used as Synthetic Building Blocks

A)



	n	X	Y	R_1	R_2
1	0	H	H	-	H
2	1	H	H	H	H
3	1	H	CH_3	H	H
4	1	CH_3	H	H	H
5	1	H	H	CH_3	H
6	1	H	H	H	CH_3
7	2	H	H	H	H

1-7



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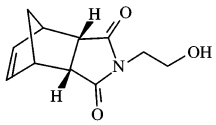

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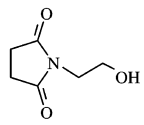
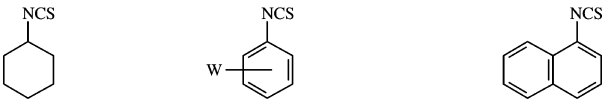


10

B)



W	
H	*o*-, *m*-, *p*-Cl
o-, *m*-, *p*-CH_3	*o*-, *p*-Br
p-C_2H_5	*p*-I
o-, *p*-$CH(CH_3)_2$	*p*-$N(CH_3)_2$
o-, *m*-, *p*-CF_3	*p*-$N(C_2H_5)_2$
m-, *p*-$COCH_3$	*o*-, *m*-, *p*-NO_2
o-, *m*-, *p*-$COOCH_3$	*o*-, *m*-, *p*-OCH_3
p-$COOC_2H_5$	*p*-OC_2H_5
p-CN	*p*-$OCH_2C_6H_5$
o-, *p*-F	*m*-SCH_3

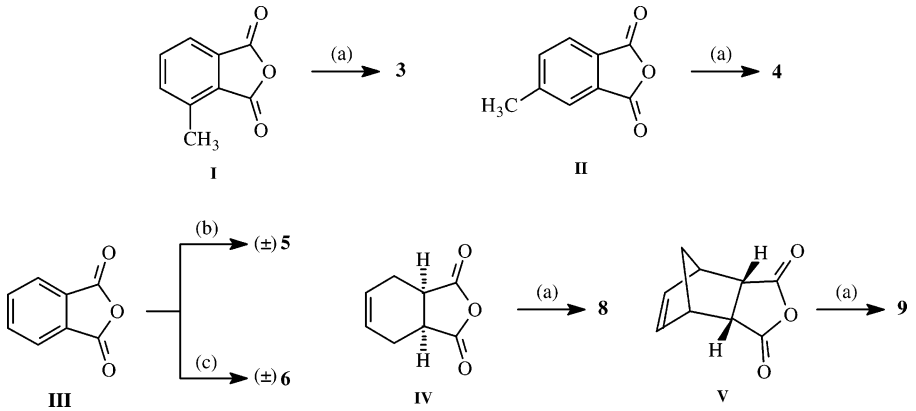
yield and purity of TCs of library 2, isothiocyanates were made more reactive by means of pyridine.[54] Sodium sand,[55] currently employed in the synthesis of thiocarbamates, was less effective than sodium hydride (data not shown). Upon treatment of the reaction medium with water and ammonium chloride, C-TCs **11**−**22**, **24**−**50**, and **82**−**91** precipitated, while ring-opened adducts **SS** were transformed into **S** (monosodium salts of the O-TCs). Eventually, the subsequent acidification of the reaction mother liquors with 2 M HCl caused separation of O-TCs **51**−**76** as solids from the solution. Notably, in the synthesis of C-TCs **11**, **13**, **24**, **25**, **29**, **34**−**36**, **42**, **43**, **47**−**50**, **82**, **86**−**91** (library 1) and C-TCs **15**, **16**, **19**, **20** (library 2), it was impossible to isolate workable amounts of the corresponding O-TCs (yields were less than 0.5−1%; data not shown). In contrast, reaction of alcohol **2** with 3-tolyl isothiocyanate and 4-benzyloxyphenyl isothiocyanate afforded only O-TCs **53** and **73** (library 2), respectively. It is noted that this anomalous ring opening of the phthalimide moiety was also found in hydrochlorides of basically substituted phthalimidyl derivatives, which unexpectedly gave the corresponding ring-opened products on careful neutralization with alkali in cold water.[56] In addition, in the synthesis of C-TCs **47**, **48**, **49**, **50** (library 1), we isolated their corresponding O-TCs, but these derivatives have not been fully characterized yet (the ring opening of the asymmetric 3-methyl- and 4-methyl phthalimmide moieties might give a mixture of two regioisomers). Their elucidated structures will be reported in due course. Purification of TCs was carried out by crystallization. The physicochemical constants of C-TCs **11**−**22**, **24**−**50**, **82**−**91** and of O-TCs **51**−**76** are reported Tables 1 and 2, respectively.

The dehydrative cyclization of O-TC **57** in the presence of excess P_2O_5 in DMF (Scheme 3) afforded C-TC **23**, not directly obtainable with the above procedures. TCs **77**−**81** were synthesized in parallel by reacting **10**, **8** in DMF, or **9** in anhydrous THF with the proper isothiocyanate in the presence of sodium hydride (Scheme 4). Starting from alcohol **2**, Scheme 5 illustrates the synthesis of compounds **92**−**95**, structurally related to the title compounds. Derivatives **92** and **93** were prepared according to a literature-reported method[57] by reacting **2**, 4-tolyl chlorothionoformate, and 4-chlorophenyl chlorothionoformate in the presence of DMAP. Carbamate **94**, unlike sulfur isostere **44**, was synthesized by heating a mixture of 1:1 pyridine/DMF of **2** and 4-nitrophenylisocyanate at 140 °C because the procedure via alcoholate was unsuccessful. Finally, **95** was obtained by grounding a mixture of **2** and thiocarbonyldiimidazole in an agate mortar through a solvent-free procedure[58] until the reaction was complete (TLC monitoring).

The formation of O-TCs and their separation from C-TCs deserve further comment. Scheme 6a illustrates that the **SS** salts can be generated either directly from **B** (route b) and or indirectly from ring-opened intermediates **2′**, **5′**, **6′**, and $\mathbf{A_{2',5',6'}}$ (route c). Clearly, these species, derived from **2**, **5**, **6**, and $\mathbf{A_{2,5,6}}$ (Scheme 6b), are expected to react like the corresponding ring-closed counterparts according to route a. Scheme 6b depicts the common mechanism hypothesized for the imide ring opening of **B**, **2**, **5**, **6**, and $\mathbf{A_{2,5,6}}$, due to the attack of the hydroxide ion on the carbonyls of the phthalimide moiety. The presence of hydroxide ions in the reaction medium can be influenced by the solvents and base employed. Thus, DMF, dried on molecular sieves may still contain trace water that would react with sodium hydride to give sodium hydroxide ($NaH + H_2O \rightarrow NaOH + H_2$). On the other hand, anhydrous pyridine, stored on sodium hydroxide pellets, as well as sodium hydride dispersion in mineral oil could release alkali impurities within the reaction medium.

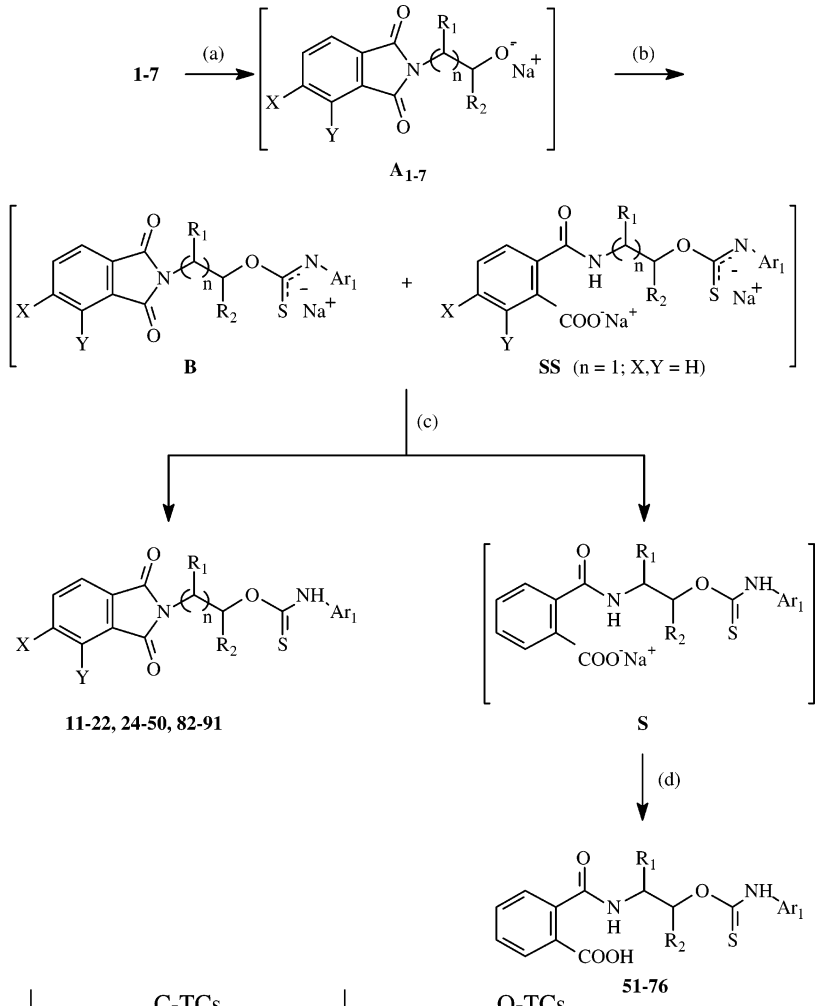
As for the easy separation of the C-TCs from the O-TCs, it is mainly due to protolysis of basic salts **B**, **SS**, and **S**, occurring under different pH conditions (Scheme 6c). Thus, upon the weakly acidic treatment (water and ammonium chloride) of the reaction mixture, the ionized thiocarbamic moieties of **B** and **SS** are protonated. This process affords their relative conjugate acids (i.e., C-TCs and **S**), and sodium hydroxide as a byproduct. Consequently, C-TCs **12**, **14**, **17**, **18**, **21**, **22**, **26**−**28**, **30**−**33**, **37**−**41**, **44**−**46**, **83**−**85** precipitate, while salts **S** are kept in solution (indeed, it is not ruled out that ammonium chloride salting-out effect contributes to the complete separation of the C-TCs from **S**). In turn, sodium hydroxide liberates ammonia from ammonium chloride, and therefore, an NH_3/NH_4Cl buffer is established, as proved by the measured pH value (about 9) of the final mixture. The protonation of the carboxylate group of **SS** cannot concomitantly occur at this pH value because its basicity is much lower than that of the ionized thiocarbamic moiety, as inferred indirectly from the estimated acidity of the thiocarbamic group of, for example, O-TC **51**, which is about 8 pK_a units higher than that of the carboxylic group (see Chart 4). Therefore, according to the Brönsted−Lowry acid−base theory, a similar difference also has to exist in the basic strength of their corresponding ionized forms. For protonation of the carboxylate group of **S** to occur, the

Scheme 1. Synthesis of Alcohols **3**−**6**, **8**, and **9**[a]



[a] Reaction conditions: (a) 2-aminoethanol, 175−180 °C, 2 h; (b) (±)-2-amino-1-propanol, 140−150 °C, 2 h; (c) (±)-1-amino-2-propanol, 160−175 °C, 2 h.

Scheme 2. General Procedure for Preparation of Libraries 1 and 2 by Parallel Analogue Synthesis[a]



	C-TCs	O-TCs
Library 1	**11-13, 21, 24-26, 28, 29, 31, 32, 34-44, 46-50, 79, 82-91**	**51, 55, 58, 61-63, 65-70, 72, 74-76**
Library 2	**14-20, 22, 27, 30, 33, 45**	**52-54, 56, 57, 59, 60, 64, 71, 73**

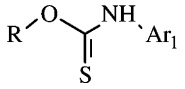
[a] Reaction conditions. Library 1: (a, b) isothiocyanate, NaH, dried DMF, 0−5 °C, then room temp for 24 h. Library 2: (a, b) isothiocyanate, NaH, anhydrous pyridine, room temp for 15 min, then 60−65 °C for 3 h; (c) H_2O, NH_4Cl; (d) 2 M HCl.

pH is adjusted at low acidic values. Thus, by acidification of the reaction mother liquors with 2 M HCl, O-TCs **51**−**56**, **58**−**72**, and **74**−**76** are separated from the solution.

Biological Results and Discussion

The antiretroviral activity of the TCs and thionocarbonates **92** and **93**, carbamate **94** (listed in Tables 3−7),

Table 1. Physicochemical Constants of C-TCs **11**−**22**, **24**−**50**, **82**−**91**



compd	R	Ar_1	mp (°C), in crystallization solvent	yield (%)	formula[k]
11	2-(phthalimido)ethyl	cyclohexyl	132−133[a]	40	$C_{17}H_{20}N_2O_3S$
12	2-(phthalimido)ethyl	phenyl	118−119[a]	41	$C_{17}H_{14}N_2O_3S$
13	2-(phthalimido)ethyl	1-naphthyl	148−150[b]	24	$C_{21}H_{16}N_2O_3S$
14	2-(phthalimido)ethyl	2-tolyl	128−129[a]	44	$C_{18}H_{16}N_2O_3S$
15	2-(phthalimido)ethyl	2-isopropylphenyl	135−136[c]	37	$C_{20}H_{20}N_2O_3S$
16	2-(phthalimido)ethyl	2-trifluoromethylphenyl	128−130[a]	51	$C_{18}H_{13}F_3N_2O_3S$
17	2-(phthalimido)ethyl	2-metoxycarbonylphenyl	122−124[a]	53	$C_{19}H_{16}N_2O_5S$
18	2-(phthalimido)ethyl	2-fluorophenyl	135−137[a]	44	$C_{17}H_{13}FN_2O_3S$
19	2-(phthalimido)ethyl	2-chlorophenyl	138−140[d]	40	$C_{17}H_{13}ClN_2O_3S$
20	2-(phthalimido)ethyl	2-bromophenyl	123−125[e]	27	$C_{17}H_{13}BrN_2O_3S$
21	2-(phthalimido)ethyl	2-nitrophenyl	145−146[a]	27	$C_{17}H_{13}N_3O_5S$
22	2-(phthalimido)ethyl	2-methoxyphenyl	134−135[f]	37	$C_{18}H_{16}N_2O_4S$
24	2-(phthalimido)ethyl	3-trifluoromethylphenyl	165−167[a]	46	$C_{18}H_{13}F_3N_2O_3S$
25	2-(phthalimido)ethyl	3-acetylphenyl	116−117[a]	32	$C_{19}H_{16}N_2O_4S$
26	2-(phthalimido)ethyl	3-methoxycarbonylphenyl	80−81[c]	15	$C_{19}H_{16}N_2O_5S$
27	2-(phthalimido)ethyl	3-chlorophenyl	159−161[f]	41	$C_{17}H_{13}ClN_2O_3S$
28	2-(phthalimido)ethyl	3-methylsulfanylphenyl	102−104[a]	43	$C_{18}H_{16}N_2O_3S_2$
29	2-(phthalimido)ethyl	3-nitrophenyl	190−191[a]	25	$C_{17}H_{13}N_3O_5S$
30	2-(phthalimido)ethyl	3-methoxyphenyl	98−100[a]	38	$C_{18}H_{16}N_2O_4S$
31	2-(phthalimido)ethyl	4-tolyl	133−134[a]	66	$C_{18}H_{16}N_2O_3S$
32	2-(phthalimido)ethyl	4-ethylphenyl	149−151[b]	11	$C_{19}H_{18}N_2O_3S$
33	2-(phthalimido)ethyl	4-isopropylphenyl	160−162[a]	32	$C_{20}H_{20}N_2O_3S$
34	2-(phthalimido)ethyl	4-trifluoromethylphenyl	169−171[a]	18	$C_{18}H_{13}F_3N_2O_3S$
35	2-(phthalimido)ethyl	4-ethoxycarbonylphenyl	166−168[a]	33	$C_{20}H_{18}N_2O_5S$
36	2-(phthalimido)ethyl	4-acetylphenyl	179−180[h]	26	$C_{19}H_{16}N_2O_4S$
37	2-(phthalimido)ethyl	4-cyanophenyl	174−176[a]	98	$C_{18}H_{13}N_3O_3S$
38	2-(phthalimido)ethyl	4-fluorophenyl	150−152[a]	32	$C_{17}H_{13}FN_2O_3S$
39	2-(phthalimido)ethyl	4-chlorophenyl	157−159[a]	24	$C_{17}H_{13}ClN_2O_3S$
40	2-(phthalimido)ethyl	4-bromophenyl	156−158[a]	36	$C_{17}H_{13}BrN_2O_3S$
41	2-(phthalimido)ethyl	4-iodophenyl	177−179[a]	25	$C_{17}H_{13}IN_2O_3S$
42	2-(phthalimido)ethyl	4-dimethylaminophenyl	194−196[f]	29	$C_{19}H_{19}N_3O_3S$
43	2-(phthalimido)ethyl	4-diethylaminophenyl	166−168[h]	13	$C_{21}H_{23}N_3O_3S$
44	2-(phthalimido)ethyl	4-nitrophenyl	196−197[a]	45	$C_{17}H_{13}N_3O_5S$
45	2-(phthalimido)ethyl	4-methoxypheny	125−127[a]	12	$C_{18}H_{16}N_2O_4S$
46	2-(phthalimido)ethyl	4-ethoxyphenyl	114−115[a]	26	$C_{19}H_{18}N_2O_4S$
47	2-(3-methylphthalimido)ethyl	4-chlorophenyl	169−171[f]	27	$C_{18}H_{15}ClN_2O_3S$
48	2-(3-methylphthalimido)ethyl	4-nitrophenyl	215−217[h]	35	$C_{18}H_{15}N_3O_5S$
49	2-(4-methylphthalimido)ethyl	4-chlorophenyl	182−184[i]	30	$C_{18}H_{15}ClN_2O_3S$
50	2-(4-methylphthalimido)ethyl	4-nitrophenyl	202−204[i]	62	$C_{18}H_{15}N_3O_5S$
82	phthalimidomethyl	4-nitrophenyl	187−189[g]	59	$C_{16}H_{11}N_3O_5S$
83	2-methyl-2-phthalimidoethyl	4-chlorophenyl	130−131[a]	44	$C_{18}H_{15}ClN_2O_3S$
84	2-methyl-2-phthalimidoethyl	4-nitrophenyl	143−144[b]	37	$C_{18}H_{15}N_3O_5S$
85	1-methyl-2-phthalimidoethyl	4-tolyl	176−178[a]	16	$C_{19}H_{18}N_2O_3S$
86	1-methyl-2-phthalimidoethyl	4-cyanophenyl	155−157[a]	80	$C_{19}H_{15}N_3O_3S$
87	1-methyl-2-phthalimidoethyl	4-chlorophenyl	172−174[a]	24	$C_{18}H_{15}ClN_2O_3S$
88	1-methyl-2-phthalimidoethyl	4-bromophenyl	168−169[a]	31	$C_{18}H_{15}BrN_2O_3S$
89	1-methyl-2-phthalimidoethyl	4-nitrophenyl	184−185[a]	39	$C_{18}H_{15}N_3O_5S$
90	3-phthalimidopropyl	phenyl	133−135[a]	41	$C_{18}H_{16}N_2O_3S$
91	3-phthalimidopropyl	4-nitrophenyl	205−207[j]	55	$C_{18}H_{15}N_3O_5S$

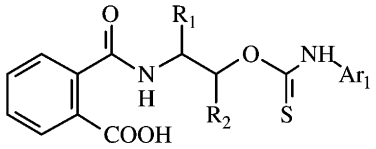
[a] In dichloromethane−methanol. [b] In diethyl ether−ethanol. [c] In diethyl ether−methanol. [d] In acetone−diethyl ether. [e] In acetone−methanol. [f] In diethyl ether−dichloromethane. [g] In dichloromethane−ethanol. [h] In acetone−ethanol. [i] In acetone. [j] In dichloromethane−ethanol−acetone. [k] All compounds were analyzed for C, H, N, and S. Analytical results were within ±0.4% of the theoretical values.

and imidazole-1-carbothioate **95** was evaluated in cell-based assays by assessing the reduction of the HIV-1 induced cytopathogenicity in MT-4 cells. The results are expressed as EC_{50} values. At the same time, the TC-induced cytotoxicity was evaluated in parallel with the antiretroviral activity in mock-infected MT-4 cells, and the results are expressed as CC_{50} values. Trovirdine was used as the reference compound.

C-TC **41** and its O-TC **69** counterpart were also assayed for inhibitory activity against HIV-1 recombinant wild-type (wt) RT (rRT) and rRTs carrying clinically relevant single and double mutations such as Y181C, K103R, K103N plus Y181C.[57,58] Trovirdine and efavirenz were used as reference compounds (Table 8). C-TCs **31**, **37**−**40**, and **44**, whose potency was confirmed in HIV-1 infected C8166 cells (Table 9) along with **41**, were also tested against the Y181C resistant strain (Table 10).

Table 3 summarizes the cytotoxic and antiretroviral activities of the lead compound **12** and its analogues modified in ring B1 (**11** and **13**−**46**). It is worth nothing that while **12** was active in the low-micromolar concentration range, PETT isosteres (ring B = phenyl), not having the internal H bond, were almost inactive[29] likely because such derivatives cannot assume the "high-activity rigid conformation" of PETT-1 (Chart 2A), as illustrated in Figure 1. This evidence points out a substantial difference between the TC and PETT series.

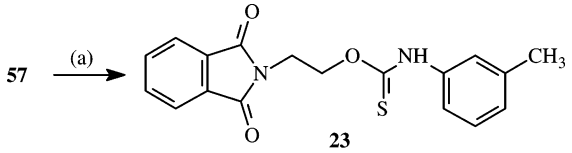
Table 2. Physicochemical Constants of O-TCs **51**−**76**



compd	R_1	R_2	Ar_1	mp (°C), in crystallization solvent	yield (%)	formula[g]
51	H	H	phenyl	114−116[a]	10	$C_{17}H_{16}N_2O_4S$
52	H	H	2-tolyl	167−169[a]	28	$C_{18}H_{18}N_2O_4S$
53	H	H	2-methoxycarbonylphenyl	149−151[b]	47	$C_{19}H_{18}N_2O_6S$
54	H	H	2-fluorophenyl	154−156[c]	36	$C_{17}H_{15}FN_2O_4S$
55	H	H	2-nitrophenyl	159−161[d]	26	$C_{17}H_{15}N_3O_6S$
56	H	H	2-methoxyphenyl	150−151[d]	15	$C_{18}H_{18}N_2O_5S$
57	H	H	3-tolyl	162−164[d]	48	$C_{18}H_{18}N_2O_4S$
58	H	H	3-methoxycarbonylphenyl	151−152[e]	15	$C_{19}H_{18}N_2O_6S$
59	H	H	3-chlorophenyl	136−138[c]	9	$C_{17}H_{15}ClN_2O_4S$
60	H	H	3-methoxyphenyl	139−141[d]	15	$C_{18}H_{18}N_2O_5S$
61	H	H	3-methylsulfanylphenyl	138−139[a]	41	$C_{18}H_{18}N_2O_4S_2$
62	H	H	4-tolyl	153−155[a]	18	$C_{18}H_{18}N_2O_4S$
63	H	H	4-ethylphenyl	158−159[f]	35	$C_{19}H_{20}N_2O_4S$
64	H	H	4-isopropylphenyl	162−163[a]	14	$C_{20}H_{22}N_2O_4S$
65	H	H	4-cyanophenyl	123−125[d]	30	$C_{18}H_{15}N_3O_4S$
66	H	H	4-fluorophenyl	172−173[a]	30	$C_{17}H_{15}FN_2O_4S$
67	H	H	4-chlorophenyl	156−158[a]	14	$C_{17}H_{15}ClN_2O_4S$
68	H	H	4-bromophenyl	248−250[a]	22	$C_{17}H_{15}BrN_2O_4S$
69	H	H	4-iodophenyl	175−177[a]	27	$C_{17}H_{15}IN_2O_4S$
70	H	H	4-nitrophenyl	167−168[a]	21	$C_{17}H_{15}N_3O_6S$
71	H	H	4-methoxyphenyl	164−165[b]	19	$C_{18}H_{18}N_2O_5S$
72	H	H	4-ethoxyphenyl	170−171[a]	22	$C_{19}H_{20}N_2O_5S$
73	H	H	4-benzyloxyphenyl	141−143[f]	44	$C_{24}H_{22}N_2O_5S$
74	H	CH_3	4-tolyl	173−175[a]	27	$C_{19}H_{20}N_2O_4S$
75	CH_3	H	4-chlorophenyl	184−186[d]	39	$C_{18}H_{17}ClN_2O_4S$
76	CH_3	H	4-nitrophenyl	194−196[d]	15	$C_{18}H_{17}N_3O_6S$

[a] In dichloromethane−methanol. [b] In acetone−ethanol. [c] In diethyl ether−ethanol. [d] In acetone−methanol. [e] In diethyl ether−acetone. [f] In dichloromethane−diethyl ether. [g] All compounds were analyzed for C, H, N, and S. Analytical results were within ±0.4% of the theoretical values.

Scheme 3. Synthesis of C-TCs **23**[a]

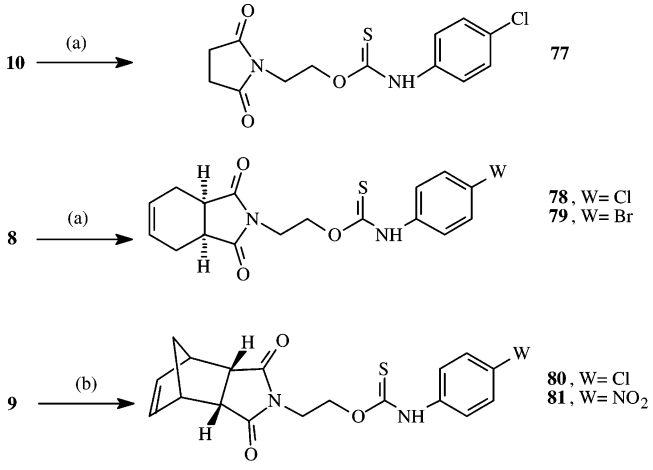


[a] Reaction conditions: (a) dried DMF, P_2O_5, 65 °C for 5 h, Na_2CO_3.

To increase flexibility of the lead, we replaced aromatic ring B1 with a cyclohexyl nucleus. This modification enhanced the potency because TC **11** resulted in being 4-fold more potent than **12**. To further probe spatial requirements, the B1 ring was replaced with the 1-naphthyl nucleus, but the activity of **13** was diminished by about 5-fold in comparison to **12**. Therefore, the hydrophobic interactions of ring B1 could not be improved by synthesizing an analogue where an extra aromatic ring is fused onto the original ring of the lead.

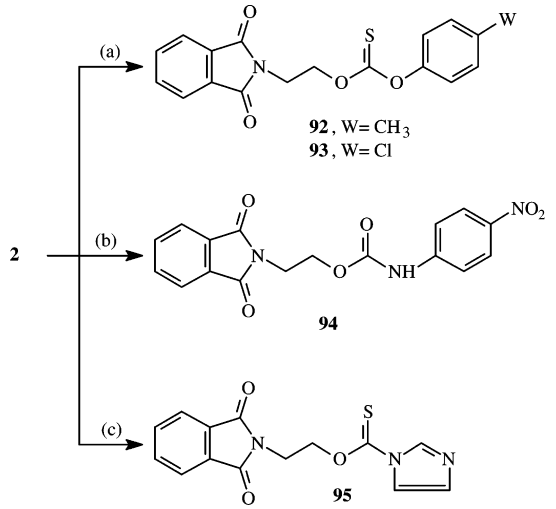
Congeners **14**−**22**, **23**−**30**, and **31**−**46** bear ortho, meta, and para substituents, respectively, having a wide range of electronic (inductive and/or mesomeric), lipophilic, and steric properties. The ortho derivatives (**14**−**22**) showed EC_{50} values in the range 3.0−65 μM. Exceptions were 2-methoxycarbonyl **17** and 2-nitro **21**, which were inactive. Apart from 3-nitro **29** (EC_{50} = 0.6 μM), most of the meta derivatives (**23**−**30**) were also micromolar inhibitors, even if they were slightly more active (EC_{50} = 1.5−21 μM) than the ortho-substituted C-TCs. In contrast, most of the para-substituted TCs (**31**−**46**) proved to be active at low-nanomolar concentrations (EC_{50} = 20−140 nM), with the exception of 4-ethoxycarbonyl **35**, 4-ethoxy **46**, 4-dimethylamino **42**, and 4-diethylamino **43** (those last two compounds were synthesized to enhance water solubility of TCs), which were weak inhibitors. Among the most potent C-TCs, 4-methyl **31** and 4-iodo **41** (EC_{50} = 0.02 μM) were equipotent to trovirdine, followed by 4-bromo **40**, 4-methoxy **45** (EC_{50} = 0.03 μM), 4-isopropyl **33**, 4-trifluromethyl **34**, 4-chloro **39**, and 4-nitro **44** (EC_{50} = 0.04 μM). Therefore, the activity was strongly affected by the substitution pattern of the *N*-phenyl ring with the

Scheme 4. Parallel Synthesis of TCs **77**−**81**[a]



[a] Reaction conditions: (a) dried DMF, NaH, isothiocyanate, room temp for 20 h; (b) anhydrous THF, NaH, isothiocyanate, room temp for 20 h.

Scheme 5. Synthesis of Thionocarbonates **92**, **93**, Carbamate **94**, and Phthalimidoethyl Imidazole-1-carbothioate **95**[a]



[a] Reaction conditions: (a) acetonitrile, DMAP, chlorothionoformate, room temp for 24 h; (b) DMF/Py (1:1), *p*-nitrophenyl isocyanate, 140 °C for 5 h; (c) mixture of **2** and thiocarbonyldiimidazole ground in a mortar for 15 min.

following potency trend: para ≫ meta > ortho, as well exemplified by positional isomers **14**, **23**, **31** (2-, 3-, 4-tolyl), **22**, **30**, **45** (2-, 3-, 4-methoxy), **19**, **27**, **39** (2-, 3-, 4-chloro), **21**, **29**, **44** (2-, 3-, 4-nitro), **16**, **24**, **34** (2-, 3-, 4-trifluromethyl), **15**, **33** (2-, 4-isopropyl), **18**, **38** (2-, 4-fluro), and **20**, **40** (2-, 4-bromo). Furthermore, the data indicate that the electronic properties of the para substituents do not seem to affect activity; in fact, the most potent para-substituted derivatives bear both electron-withdrawing groups, such as nitro (**44**), trifluoromethyl (**34**), chloro (**39**), bromo (**40**), and iodo (**41**), and electron-donating groups, such as methoxy (**45**), methyl (**31**), and isopropyl (**33**).

As far as the size of the para substituens is concerned, the relative potency of the C-TCs bearing halogens and cognate functions (such as the cyano and trifluromethyl groups) is in the order iodo **41** > bromo **40** > chloro **39** = trifluromethyl **34** > cyano **37** > fluoro **38**, making apparent that the potency correlates with the presence of more sterically demanding groups (in this context, the same activity shown by **34** and **39** correlates with the comparable size of the chlorine atom and the trifluromethyl group). It is intriguing that the *p*-chloroanilino moiety of **39** is also peculiar to other potent NNRTIs, such as TIBO (R-82913, R-86183), the PETT derivative MSC-127, dihydroquinazolinethiones, quinoxalines (S-2720), oxathiin carboxanilide (NSC 615985), and UC-38 (Chart 2C).[3] Conversely, the results concerning the *p*-dialkylamino-, *p*-alkyl-, and *p*-alkoxy-C-TCs suggest that the activity decreases with the steric demand of the substituents. Thus, diethylamino **43** was 2.3-fold less active than dimethylamino **42**, and ethyl **32** and isopropyl **33** were 4- and 2-fold less potent than methyl **31**, respectively. An even more dramatic decrease in activity was observed in the alkoxy series, in which ethoxy **46** was about 233-fold less active than methoxy **45**. These differences in activity can be rationalized by the docking models of some of the above para-substituted C-TCs (see below).

Table 4 shows the effect of the opening of the phthalimide ring and of modification of ring B1 on cytotoxicity and antiviral activity. Apart from 2-methoxycarbonyl **53**, 2-nitro **55**, and 3-methoxycarbonyl **58**, which were inactive, O-TCs **51**, **52**, **54**, **56**, **57**, **59**−**73** were selectively active against HIV-1. As in the case of the C-TCs, the potency of ring-opened congeners depends on the position of the substituent on the *N*-phenyl ring. In fact, the EC_{50} values of ortho (**52**, **53**, **55**, and **56**) and meta congeners (**57**, **59**−**61**) are in the micromolar range (EC_{50} = 65−3 μM) except 2-fluoro O-TC **54**, which is 6 times more potent (EC_{50} = 0.5 μM) than 2-fluoro C-TC **18**. The para-substituted O-TCs **62**, **63**, **65**−**71**, and **73** were active at submicromolar concentrations (EC_{50} = 0.1−0.5 μM), with the exception of 4-ethoxy **72** and 4-isopropyl **64**, which showed EC_{50} values of 2 and 3.7 μM, respectively. Therefore, even though the general pattern of activity of the O-TCs mirrored that of the C-TCs (para > meta and ortho), overall the para-substituted O-TCs were less potent than the corresponding para-substituted C-TCs. For example, O-TCs 4-methyl **62** and 4-iodo **69** were 20- and 6.5-fold less potent than C-TCs 4-methyl **31** and 4-iodo **41**, respectively. Inspection of Tables 3 and 4 reveals that the potency order of the para-substituted C-TCs (4-Me = 4-I > 4-Br = 4-MeO > 4-Cl = 4-NO_2 > 4-CN > 4-Et > 4-F) is different from that of the O-TCs bearing the same para substituents (4-Cl = 4-Br = 4-CN ∼ 4-I > 4-MeO ∼ 4-F > 4-NO_2 = 4-Et > 4-Me). Moreover, the activity data of the O-TCs are not affected by the electronic nature and the steric demand of the para substituents. Thus, 4-cyano **65** was equipotent to 4-chloro **67**, 4-bromo **68**, and 4-iodo **69** (EC_{50} = 0.1 μM), and 4-methoxy **71** and 4-fluoro **66** were nearly equally active (EC_{50} = 0.20 and 0.25 μM, respectively). On the other hand, 4-nitro **70** showed the same activity as 4-ethyl **63** (EC_{50} = 0.30 μM), which in turn was 1.3- and 12.3-fold more active than 4-methyl **62** and 4-isopropyl **64**, respectively. After identification of some para-substituted rings B1 as suitable moieties, all derivatives (except **90**) reported in Tables 5−7 carry the more promising groups, such as nitro, chloro, bromo, cyano, and methyl at position 4 of ring B1.

To enhance the hydrophobic interactions within the highly hydrophobic RT pocket, C-TCs **47**−**50** having a methyl group at the 3- and 4-position of ring A1 were prepared and tested (Table 5). Introduction of a methyl group at position 3 of the phthalimide scaffold diminished the activity because 4-chloro **47** and 4-nitro **48** were 0.5- and 1.8-fold less potent than the corresponding 4-chloro **39** and 4-nitro **44**, respectively. Conversely, positional isomers **49** and **50**, carrying the methyl group at position 4 of the phthalimide moiety, were 1.6- and 7-fold more potent than **39** and **44**, respectively. Congener **50** was the most active C-TC (EC_{50} = 0.01 μM), being 2-fold more potent than trovirdine.

Furthermore, to evaluate the importance of the aromatic ring A1 in eliciting hydrophobic interactions and to probe the spatial relationship of the fused rings, succinimido TC **77**, lacking ring A1, and bicyclic TCs **78**−**81** were synthesized. As expected, **77** showed a 60-fold lower activity with respect to **39** likely because of the loss of hydrophobic aromatic interactions of ring A1. The change of ring A1 with a cyclenic equivalent of the

Scheme 6. (a, b) Hypothesized Mechanism for Formation of **SS** (Routes b and c) and (c) Separation of the C-TCs from Their Corresponding O-TCs for Protolysis of Salts **B**, **SS**, and **S** under Different pH Conditions

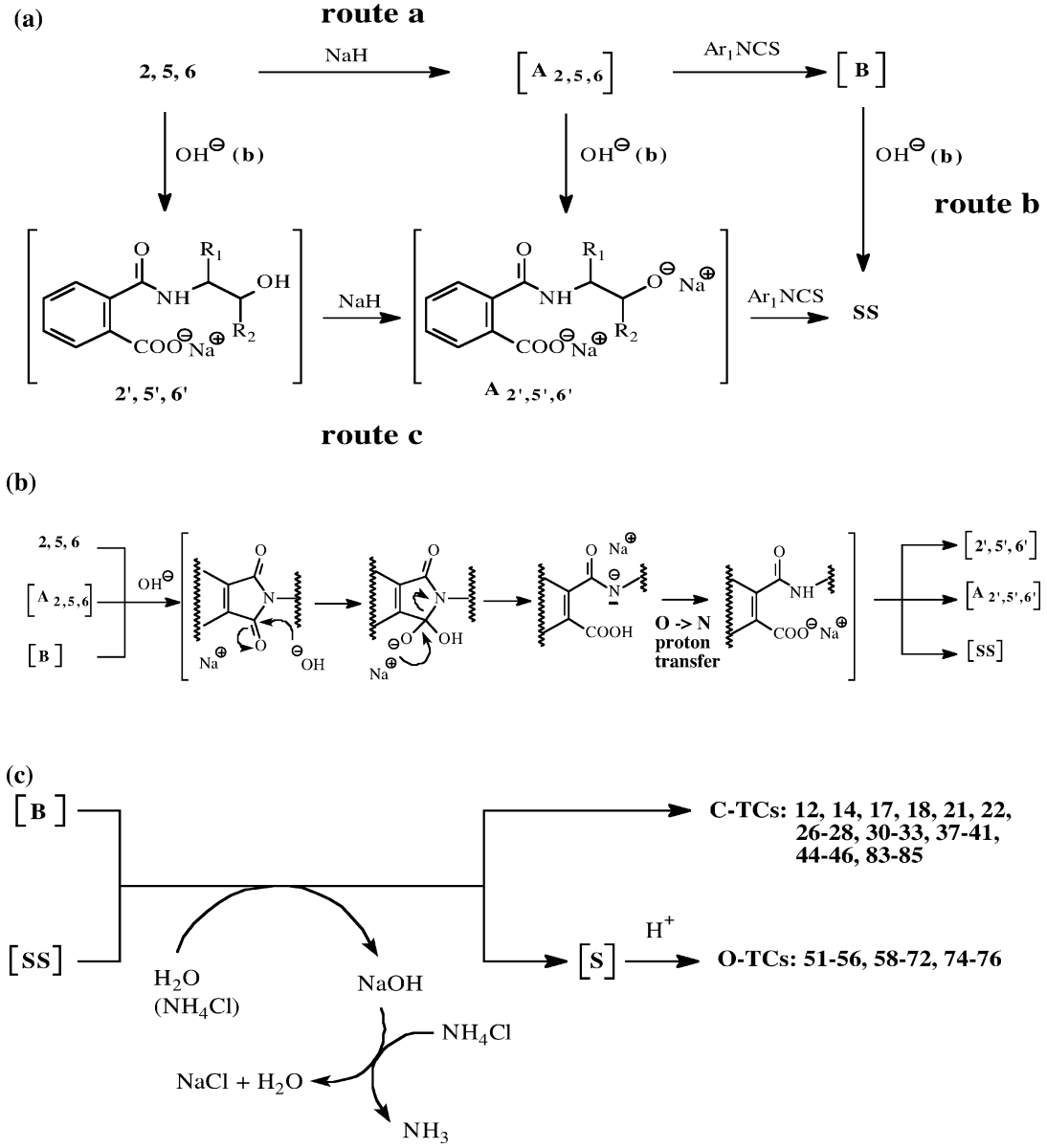


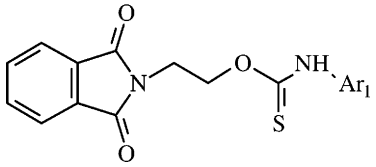
Chart 4. Calculated pK_a of C-TC **44**, Carbamate **94**, and O-TC **51**[a]

	pKa[a]	
Compounds	**-CX-NH-Ar$_1$**	**-COOH**
44 (X=S)	10.27 ± 0.70	-
94 (X=O)	12.51 ± 0.70	-
51 (X=S)	11.68 ± 0.70	3.47 ± 0.36

[a] These values have been obtained through ACD Labs 7.0 software.

phenyl ring, such as the cyclohexenyl ring, or with a more rigid bridged system, such as the norbornene nucleus, caused a drastic decrease of activity. Thus, 4-chloro **78** and 4-bromo **79** were about 235- and 77-fold less active than **39** and **40**, respectively. Similarly, 4-chloro **80** and 4-nitro **81** were less active than **39** and **44**. The lower activity of **78**−**81** with respect to **39, 40**, and **44** is probably due not only to loss of aromatic interactions but also to nonplanar spatial rearrangement of the fused rings (cis and cis-endo, respectively). Table 6 summarizes the results obtained by shortening (**82**), lengthening (**90**, **91**), and branching the ethyl spacer (**83**−**89**) with the aim of evaluating both the optimal A1−B1 distance and the effects on the overall bioactive conformations of TCs. Shortening or extending the spacer by only one methylene unit completely abolished the activity or caused a significant drop in the anti-HIV-1 potency (TC **82**, the lower homologue of **44**, was inactive, whereas **90** and **91**, the superior homologues of **12** and **44**, respectively, were inactive or poorly active). Addition of a methyl group on the spacer carbon atom adjacent to the thiocarbamic oxygen resulted in a strong decrease in activity: 4-methyl **85**, 4-cyano **86**, 4-cloro **87**, 4-bromo **88**, and 4-nitro **89** were much less active than the corresponding unbranched C-TC derivatives **31**, **37**, **39**, **40**, and **44**, respectively. When the methyl group is located on the carbon adjacent to the imide nitrogen, a lesser decrease of activity was observed, depending on the nature of the para substituent. Thus, 4-chloro **83** and 4-nitro **84** were 1.2- and 15-fold less potent than unbranched 4-chloro **39** and 4-nitro **44**, respectively. The same effect, due to methyl-branching,

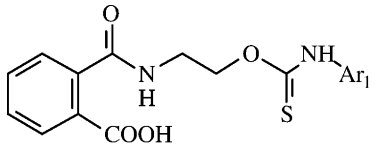
Table 3. Effects of Modification of the *N*-Phenyl Ring on Cytotoxicity and Anti-HIV-1 Activity (C-TCs **11**−**46**)[a]



compd	Ar_1	CC_{50} [b] (μM)	EC_{50} [c] (μM)	SI [d]
11	cyclohexyl	>100	0.3	>333
12	phenyl	>100	1.2	>83.3
13	1-naphthyl	>100	6.5	>15.4
14	2-tolyl	>100	3.7	>27
15	2-isopropylphenyl	>100	43	>2.3
16	2-trifluoromethylphenyl	>100	65	>1.5
17	2-metoxycarbonylphenyl	45	>45	
18	2-fluorophenyl	>100	3.0	>33
19	2-chlorophenyl	>100	17	>5.9
20	2-bromophenyl	>100	14	>7.1
21	2-nitrophenyl	>100	>100	
22	2-methoxyphenyl	>100	5.6	>17.9
23	3-tolyl	>100	1.5	>66.7
24	3-trifluoromethylphenyl	>100	13	>7.7
25	3-acetylphenyl	>100	4.3	>23
26	3-methoxycarbonylphenyl	>100	21	>4.8
27	3-chlorophenyl	>100	2.0	>50
28	3-methylsulfanylphenyl	>100	7.0	>14.3
29	3-nitrophenyl	>100	0.6	>167
30	3-methoxyphenyl	>100	4.0	>25
31	4-tolyl	>100	0.02	>5000
32	4-ethylphenyl	>100	0.08	>1250
33	4-isopropylphenyl	94	0.04	2350
34	4-trifluoromethylphenyl	>100	0.04	>2500
35	4-ethoxycarbonylphenyl	>100	23	>4.3
36	4-acetylphenyl	>100	0.14	>714
37	4-cyanophenyl	>100	0.07	>1429
38	4-fluorophenyl	>100	0.10	>1000
39	4-chlorophenyl	>100	0.04	>2500
40	4-bromophenyl	>100	0.03	>3333
41	4-iodophenyl	>100	0.02	>5000
42	4-dimethylaminophenyl	>100	0.60	>167
43	4-diethylaminophenyl	>100	1.40	>71
44	4-nitrophenyl	65	0.04	1625
45	4-methoxyphenyl	>100	0.03	>3333
46	4-ethoxyphenyl	>100	7.0	>14.3
trovirdine		60	0.02	3000

[a] Data represent mean values for three separate experiments. Variation among triplicate samples was less than 10%. [b] Compound concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. [c] Compound concentration 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.

Table 4. Effects of the Opening of the Imide Ring and of Modification of the *N*-Phenyl Ring on Cytotoxicity and Anti-HIV-1 Activity (O-TCs **51**−**73**)[a]



compd	Ar_1	CC_{50} [b] (μM)	EC_{50} [c] (μM)	SI [d]
51	phenyl	>100	3	>33.3
52	2-tolyl	>100	27	>3.7
53	2-methoxycarbonylphenyl	>100	>100	
54	2-fluorophenyl	>100	0.5	>200
55	2-nitrophenyl	>100	>100	
56	2-methoxyphenyl	>100	65	>1.5
57	3-tolyl	>100	5.0	>20
58	3-methoxycarbonylphenyl	>100	>100	
59	3-chlorophenyl	>100	3.0	>33.3
60	3-methoxyphenyl	>100	2.6	>38.5
61	3-methylsulfanylphenyl	>100	11	>9.1
62	4-tolyl	>100	0.4	>250
63	4-ethylphenyl	>100	0.3	>333
64	4-isopropylphenyl	>100	3.7	>27
65	4-cyanophenyl	>100	0.1	>1000
66	4-fluorophenyl	>100	0.25	>400
67	4-chlorophenyl	>100	0.1	>1000
68	4-bromophenyl	>100	0.1	>1000
69	4-iodophenyl	>100	0.13	>769
70	4-nitrophenyl	>100	0.30	>333
71	4-methoxyphenyl	>100	0.2	>500
72	4-ethoxyphenyl	100	2.0	50
73	4-benzyloxyphenyl	>100	0.5	>200
trovirdine		60	0.02	3000

[a] Data represent mean values for three separate experiments. Variation among triplicate samples was less than 10%. [b] Compound concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. [c] Compound concentration 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.

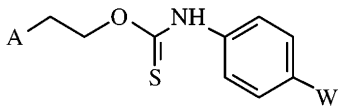
was found in O-TCs 4-methyl **74**, 4-chloro **75**, and 4-nitro **76**, even though their decrease in activity was greater when compared to the ring-closed counterparts.

C-TCs **83**−**89** and O-TCs **74**−**76** were tested as racemates, and no attempt was made to resolve them. As a matter of fact, judging from the range of antiviral potency, resolution of the racemates might not result in a significant activity increase of the resultant enantiomers. Interestingly, the activity trend due to modification of the ethyl linker of TCs is similar to the activity trend obtained in the PETT series.[31] Therefore, the unbranched ethyl linker is optimal for the activity of both TC and PETT series.

Table 7 indicates that isosteric replacement of the NH group or thione sulfur of the thiocarbamic moiety with oxygen (**92**, **93**, and **94** are isosteres of **31**, **39**, and **44**, respectively) was not beneficial for activity. Thus, 4-methyl **92** and 4-chloro **93** thionocarbonates were practically inactive (EC_{50} > 50 μM), whereas 4-nitro carbamate **94** (EC_{50} = 0.5 μM) was 12.5 less potent than 4-nitro C-TC **44**. The lack of activity of **92**, **93**, and **95** (data not shown), the last embodying the thiocarbamic nitrogen into an imidazole nucleus, emphasizes the crucial role played by the NH group and, to a lesser extent, by the thione sulfur in binding to the RT (see Molecular Modeling).

C-TC 4-iodo **41** and O-TC 4-iodo **69** were also tested in enzyme assays against recombinant wt RT (rRT) and rRTs carrying clinically relevant NNRTI resistance mutations (K103R, Y181C, and K103N + Y181C); evafirenz and trovirdine were used as reference compounds (Table 8). Assays against both wt and mutant enzymes proved that TCs, like efavirenz and trovirdine, targeted the HIV-1-RT. The IC_{50} values of **41** and **69** were 4.5- and 13-fold lower than the respective EC_{50} values (Tables 3 and 4), thus making more evident the different potency of the test compounds in enzyme- and cell-based assays. Perhaps the principal factor that can account for these results is influenced by the nature of the rRT; it would be unable to adequately mimic the native enzyme, being generally a p66−p66 dimer. Notably, similar differences between enzymatic and cell culture activity have also been observed for ATC derivatives.[27]

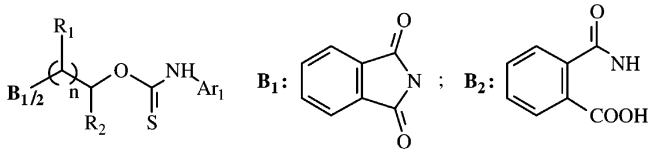
Table 5. Effects of Modification and Omission of the Phthalimide Phenyl Ring on Cytotoxicity and Anti-HIV-1 Activity (TCs **47**−**50**, **77**−**81**)[a]



compd	A	W	CC_{50} [b] (μM)	EC_{50} [c] (μM)	SI [d]
47	3-methylphthalimido	Cl	>100	0.05	>2000
48	3-methylphthalimido	NO_2	71	0.07	1014
49	4-methylphthalimido	Cl	>100	0.025	>4000
50	4-methylphthalimido	NO_2	80	0.01	8000
77	succinimido	Cl	>100	2.4	>42
78	*cis*-1,2,3,6-tetrahydrophthalimido	Cl	>100	9.4	>10.6
79	*cis*-1,2,3,6-tetrahydrophthalimido	Br	>100	2.3	>43
80	*cis*-5-norbornene-*endo*-2,3-dicarboximido	Cl	>100	68	>1.5
81	*cis*-5-norbornene-*endo*-2,3-dicarboximido	NO_2	>100	>100	
trovirdine			60	0.02	3000

[a] Data represent mean values for three separate experiments. Variation among triplicate samples was less than 10%. [b] Compound concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. [c] Compound concentration 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.

Table 6. Effect of Modification of the Aliphatic Spacer on Cytotoxicity and Anti-HIV-1 Activity (C-TCs **82**−**87**, O-TCs **74**−**76**)[a]



compd	$B_{1/2}$	*n*	R_1	R_2	Ar_1	CC_{50} [b] (μM)	EC_{50} [c] (μM)	SI [d]
82	B_1	0		H	4-nitrophenyl	23	>23	
83[e]	B_1	1	CH_3	H	4-chlorophenyl	44	0.05	880
84[e]	B_1	1	CH_3	H	4-nitrophenyl	>100	0.6	>167
85[e]	B_1	1	H	CH_3	4-tolyl	>100	48	>2.1
86[e]	B_1	1	H	CH_3	4-cyanophenyl	>100	36	>2.7
87[e]	B_1	1	H	CH_3	4-chlorophenyl	>100	1.7	>58.8
88[e]	B_1	1	H	CH_3	4-bromophenyl	>100	2.3	>43.5
89[e]	B_1	1	H	CH_3	4-nitrophenyl	>100	8.6	>11.6
90	B_1	2	H	H	phenyl	>100	>100	
91	B_1	2	H	H	4-nitrophenyl	≥100	19	≥5.3
74[e]	B_2	1	H	CH_3	4-tolyl	>100	41	>2.4
75[e]	B_2	1	CH_3	H	4-chlorophenyl	>100	5.0	>20
76[e]	B_2	1	CH_3	H	4-nitrophenyl	>100	13	>7.7
trovirdine						60	0.02	3000

[a] Data represent mean values for three separate experiments. Variation among triplicate samples was less than 10%. [b] Compound concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. [c] Compound concentration 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] TCs tested as racemic mixtures.

Unlike efavirenz, **41**, **69**, and trovirdine were inactive against the double mutant. However, **41** showed the same level of activity as efavirenz against the K103R mutant (IC_{50} = 2.3 vs 2.1 μM, respectively), while **69** and trovirdine were inactive (IC_{50} > 20 μM). Conversely, **69** was 3-fold more active than trovirdine against Y181C, while **41** was inactive. Probably the different resistance profile of **41** and **69** against the above single mutations correlates with the preservation or opening of the imide ring.

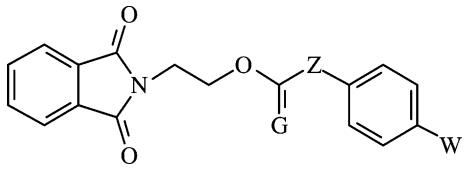
The doses required to reduce HIV-1 p24 antigen levels by 90% were determined for para-substituted C-TCs **31**, **37**−**41**, and **44** in comparison with trovirdine (Table 9). The EC_{90} values of **31**, **39**, **40**, and **44** confirmed their highest activity. Finally, **31**, **37**−**40**, and **44** were tested in cell-based assays against HIV-1 strains, carrying the clinically relevant Y181C mutation in comparison with the reference compound trovirdine (Table 10). The data indicate that the Y181C mutant was significantly inhibited by C-TCs **31**, **37**, **39**, **40**, **44**. In contrast, the K103R and the K103N + Y181C double mutant strains turned out to be generally unsusceptible to the test compounds (results not shown), with the exception of **44** that showed a weak activity against the K103N mutant with an EC_{50} value of 20 μM.

Molecular Modeling

To guide the lead optimization strategy and rationalize the SARs, computational studies were performed to construct a docking model of the TCs into the HIV-1 RT NNBS according to the procedure previously described for the ATCs.[29] Briefly, low-energy conformers of the TCs, obtained by Monte Carlo random search, were docked (Dock 4.0) in the NNBS. The X-ray coordinates of the crystal complex of PETT-1 (Chart 2A) with HIV-1 RT (PDB code 1DTQ)[16] were employed after erasing the PETT derivative from the complex. The resulting RT−TC complexes were energy-minimized by simulated annealing, followed by Powell minimization. The following features of the TC docking models are worthy to be outlined.

As shown in Figure 2A, the RT−**12** complex is stabilized by a hydrogen bond, involving the Lys101 main chain carbonyl and the NH group. Another polar interaction occurs between one of the imidic oxygen atoms and the carboxylic group of Glu138 (distance of 3.2 Å) from the p51 subunit [thereafter Glu138 (B)]. This interaction prompted selection of alcohol building blocks (see Chart 3A) featured by a five-membered imide ring. Moreover, ring A1, like ring A of PETT-1,[16] establishes hydrophobic contacts with Val106 and $\pi-\pi$ interactions with Tyr181, Tyr188, and Phe227, while ring B1 interacts with the Leu100, Lys103, Pro236, and Tyr318 side chains. Furthermore, the ethyl linker is involved in hydrophobic contacts with the Val179 side chain and the sulfur atom has van der Waals contacts with the Lys101 backbone. The docking model of **11** explains why the replacement of ring B1 with a cyclohexyl nucleus caused a 4-fold increase of activity in comparison with **12**. In the RT−**11** complex the larger volume of the cyclohexyl nucleus would better fill the spacious region hosting ring B1. This ring is likely to be engaged in extensive hydrophobic interactions with Leu100, Lys101, Val106, His235, Pro236, and Tyr318. Moreover, the complex is stabilized by a number of van der Waals contacts between the thiocarbonyl sulfur atom and the NH group of the Lys101 main chain. Another strategy to efficiently fill the region hosting ring B1 is to introduce substituents onto the para position of this ring. Therefore, a greater number of para-substituted isothiocyanates were selected as building blocks in the TC synthesis. The models of the RT−**39** and RT−**44** complexes show that the 4-chloro and 4-nitro substituents strongly interact with Phe227, Leu234, His235, Pro236, and Tyr318 and that van der Waals contacts between the thiocarbonyl group and the Lys101 backbone are strengthened. Congener 4-iodo **41** was 2-fold more potent than 4-chloro **39** probably because in the RT−**41** complex the bulkier iodine atom makes better contacts with the above amino acid residues. Notably, the other para substituents such as methyl, ethyl, methoxy, and ethoxy groups cause different positions of the inhibitor within the NNBS, due to rotation of ring B1, relative to its orientation in the hypothetical model of **12**. Thus, in the RT−**45** complex, rotation of ring B1 of about 54° provides strong interactions between the methoxy group and Phe227 and His235, with the ether oxygen establishing a polar interaction with the Tyr318 OH group. Conversely, in the RT−**46** complex, because the rotation of ring B1 is about 78°, the NH function is unable to form a hydrogen bond to the Lys101 main chain carbonyl and the 4-ethoxy group, unlike the 4-methoxy group, does not interact with Phe227, His235, and Tyr318. The docking models of several ortho- and meta-substituted C-TCs, such as **17**, **19**, **21**, **27**, predicted that both the ortho and the meta groups are not in proximity to amino acid residues suitable for additional interactions (data not shown). The effects on antiviral activity due to the introduction of a methyl group at the 3- and 4-position of the phthalimide ring are readily explained by the docking model of TCs **47**−**50**. The minor potency of **47** and **48** is explained by the steric clash of the 3-methyl group with the Trp229 C^{α} atom, which forces the inhibitors in an orientation that weakens the polar interaction between one of the imidic oxygens and the Glu138(B) carboxylic group. Conversely, in the RT−**49** and RT−**50** complexes the 4-methyl group is in proximity to be engaged in hydrophobic interactions with the highly conserved Trp229. Conformational analyses offered a possible explanation for the great difference in activity between **83**, **84** and **85**−**89**, carrying a methyl group in positions 2 and 1 of

Table 7. Effects of Isosteric Modifications (NH/O and O/S) on Cytotoxicity and Anti-HIV-1 Activity[a]



compd	G	Z	W	CC_{50} [b] (μM)	EC_{50} [c] (μM)	SI [d]
92[e]	S	O	CH_3	>100	>100	
93[f]	S	O	Cl	>100	>100	
94[g]	O	NH	NO_2	>100	0.5	>200
trovirdine				60	0.02	3000

[a] Data represent mean values for three separate experiments. Variation among triplicate samples was less than 10%. [b] Compound concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method. [c] Compound concentration 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] Isostere of C-TC **31**. [f] Isostere of C-TC **39**. [g] Isostere of C-TC **44**.

Table 8. IC_{50} of C-TCs **41** and O-TC **69** against Wild Type and Single and Double Mutant Recombinant RTs

	IC_{50} [a] (μM)			
compd	wt_{IIIB}	K103R	Y181C	K103N + Y181C
41	0.09	2.3	>20	>20
69	1.7	>20	4.8	>20
efavirenz	0.014	2.1	0.02	0.5
trovirdine	1.03	>20	15	>20

[a] Compound concentration required to inhibit in enzyme assays the HIV-1 recombinant RT activities by 50%.

Table 9. EC_{90} Values of Selected C-TCs against wt HIV-1

compd	EC_{90} [a] (μM)
31	0.015
37	0.1
38	0.1
39	0.04
40	0.015
41	0.04
44	0.03
trovirdine	0.015

[a] Compound concentration required to reduce the amount of p24 by 90% in virus-infected C8166 cultures.

Table 10. EC_{50} Values of Selected C-TCs against the Y181C Mutant in Cell-Based Assays[a]

compd	EC_{50} [b] (μM)
31	1.8
37	5.9
38	38
39	2.3
40	2.4
44	6.0
trovirdine	1.2

[a] Data represent mean values for three separate experiments. Variation among triplicate samples was less than 10%. [b] Compound concentration required to achieve 50% protection of MT-4 cell from HIV-1 induced cytopathogenecity, as determined by the MTT method.

the ethyl spacer, respectively. The Macromodel program (DRIVE module) predicted **83** and **87**, selected as the representatives of the above TCs, to exist in three low-energy conformations, i.e., one anti and two gauche (data not shown). Calculations indicated the anti conformer of **83** to be about 0.5 kcal/mol lower in energy with respect to the anti conformer of **87**. There is a correlation between the more potent **83** and the preference for the anti conformation. The lack of activity of **92** and **93** is probably due to the replacement of the NH group with oxygen, causing the loss of the H-bond involving the NH group and the Lys101 main chain carbonyl. The lower activity of carbamate **94** compared with **44** can be explained by the replacement of the sulfur with a less polarizable oxygen, which would be expected to create less favorable dispersion interactions. Furthermore, the energy required to desolvate a carbonyl will be greater than for a thione group, which cannot form such strong interactions with water.[19] On the other hand, the NH group of **44**, adjacent to a thiocarbonyl group, is more acidic than the NH group of **94**, close to a carbonyl (the calculated pK_a of **44** is about 100 times greater than the pK_a of **94**; see Chart 4). Therefore, the thiocarbamic NH group is a better H-bond donor group than the carbamic NH group, and as consequence, it will form stronger hydrogen bonds. These observations are consistent with the H-bond donor role proposed for the NH group to the Lys101 carbonyl (Figure 2A). Interestingly, also in the docking model of **51** (Figure 2B), the NH group forms an H-bond with the Lys101 carbonyl and the sulfur atom has van der Waals contacts with the Lys101 backbone. The *N*-phenyl ring makes hydrophobic interactions with Val106, His235, Pro236, and Tyr318, while the phthalyl moiety is in contact with Leu100, Tyr181, and Tyr188. The carbamoyl−ethyl linker has a number of van der Waals interactions with Val179, Tyr181, Tyr188, Val189, and Gly190, while the carboxylic group interacts with the Leu100 and Tyr181 side chains.

Finally, the binding model proposed for **12** also helps to partially rationalize the activity decrease of the TCs tested against NNRTI resistant strains. The decrease of activity of C-TCs **31**, **37**−**41**, **44** in cell-based assays and of O-TC **69** in an enzyme-based assay against the Y181C mutant emphasizes the role of Tyr181 in the binding to the TCs. This mutation causes a dramatic change from a hydrophobic environment to a hydrophilic environment. According to the model, one of the most favorable hydrophobic contacts is due to the $\pi-\pi$ stacking interactions of Tyr181 and ring A1. Mutation of Tyr181 to nonaromatic cysteine would abolish most of these hydrophobic contacts. Overall, information regarding the hypothetical TC binding mode proved to be useful in selecting alcohol and isothiocyanate building blocks for the TC library synthesis. Moreover, the relevant predictions of the TC models are supported by the reported SARs.

Conclusion

In this study we have described a new class of NNRTI TC isosteres of PETT derivatives. The biological results show that the C-TCs bearing para substituents on the *N*-phenyl ring, such as methyl, iodo, chloro, bromo, nitro, methoxy, were very potent inhibitors, but maximum potency was reached by introducing an additional methyl group at the 4-position of the phthalimide framework in the *p*-nitro C-TC **44**. In terms of resistance against the clinically relevant mutations, the major molecular flexibility of the TCs with respect to PETT derivatives did not give the eagerly awaited results. Nevertheless, the significant activity of **41** (IC_{50} = 2.3 μM) against the K103R mutant in enzyme assays and of **31**, **37**, **39**, **40**, and **44** against the Y181C in cell-based assays offers a stimulus for the design of new TC analogues with better resistance profile.

Experimental Section

Chemistry. Starting alcohols **1**, **2**, **7**, **10**, anhydrides **I**−**V**, and other reagents such as 60% sodium hydride dispersion in mineral oil, 2-aminoethanol, (±)-2-amino-1-propanol, (±)-1-amino-2-propanol, isothiocyanates, *p*-chloro thionoformates, and *p*-tolyl thionoformates, phenyl isocyanate, 4-nitrophenyl isocyanate, and thiocarbonyldiimidazole were purchased from Chiminord and Aldrich Chemical, Milan (Italy). Solvents (THF, pyridine, DMF) were reagent grade. DMF was dried on molecular sieves (5Å, $^1/_{16}$ in. pellets). Organic solutions were dried over anhydrous sodium sulfate and concentrated using a rotatory evaporator operating at reduced pressure of about 15−20 Torr. TLC systems for routine monitoring of reaction mixtures and for confirming the purity of analytical samples employed aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}) with chloroform or chloroform−methanol as developing solvents. Developed plates were visualized by UV light and iodine. The parallel solution-phase chemistry was performed by using a Carousel Reaction Station (Radleys Discovery Technologies, Italian distributor: StepBio, Bologna).

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Microanalyses were performed on an EA 1110 elemental analyzer from FISON Instruments (Milan, Italy). The IR spectra were recorded on a Perkin-Elmer 398 spectrometer, using KBr disks. The ^{1}H NMR spectra were recorded in $CDCl_3$ 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), m (multiplet), and bs (broad singlet). The first-order values reported for coupling constants were given in hertz. The synthesis of trovirdine was performed according to the published procedure.[31] All the new compounds reported in this paper gave satisfactory spectral and microanalysis data.

General Procedure for the Preparation of Starting Alcohols 3, 4, 8, 9. A mixture of 2-aminoethanol (0.7 g, 12 mmol) and the proper anhydride (3-methylphthalic anhydride **I**, 4-methylphthalic anhydride **II**, *cis*-1,2,3,6-tetrahydrophthalic anhydride **IV**, or *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride **V**; 10 mmol) was heated at 175−180 °C under stirring for 2 h. After cooling, the reaction mixture was dissolved in CH_2Cl_2 and washed with water (3 × 50 mL). The organic layer was dried and evaporated under reduced pressure to give the desired product, which was purified by crystallization from diethyl ether−dichloromethane (6:1).

2-(2-Hydroxyethyl)-4-methyl-1*H*-isoindole-1,3(2*H*)-dione (3): 1.83 g (89%), mp 109−110 °C.

2-(2-Hydroxyethyl)-5-methyl-1*H*-isoindole-1,3(2*H*)-dione (4): 1.39 g (68%), mp 94−96 °C.

(3a*R*,7a*S*)-2-(2-Hydroxyethyl)-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione (8): 1.19 g (61%), mp 78−80 °C.

(3a*R*,4*S*,7*S*,7a*S*)-2-(2-Hydroxyethyl)-3a,4,7,7a-tetrahydro-1*H*-4,7-methanoisoindole-1,3-dione (9): 0.97 g (47%), mp 91−92 °C.

Synthesis of (±)-2-(2-Hydroxy-1-methylethyl)-1*H*-isoindole-1,3(2*H*)-dione(5). A mixture of **III** (1.48 g, 10 mmol) and *d,l*-alanilol (0.7 g, 12 mmol) was heated at 140−150 °C under stirring for 2 h. After cooling, the mixture was dissolved in methylene chloride and washed with water (3 × 50 mL). The

organic layer was dried and evaporated under reduced pressure to yield **5**, which was crystallized from $Et_2O-CH_2Cl_2$ (6:1): 1.27 g (62%), mp 104−106 °C.

Synthesis of (±)-2-(2-Hydroxypropyl)-1*H*-isoindole-1,3-(2*H*)-dione (6). A mixture of **III** (1.48 g, 10 mmol) and 1-amino-2-propanol (0.7 g, 12 mmol) was heated at 160−170 °C under stirring for 2 h. After cooling, the mixture was dissolved in methylene chloride and washed with water (3 × 50 mL). The organic layer was dried and evaporated under reduced pressure to yield **6**, which was crystallized from chloroform: 1.03 g (50%), mp 90−92 °C.

General Procedure for the Preparation of Library 1. To an ice-cooled, stirred solution of the proper alcohol (**1**−**7**, 10 mmol) and isothiocyanate (10 mmol) in dried DMF (15 mL), a 60% sodium hydride dispersion in mineral oil (0.45 g, ∼10 mmol) was added in a single portion. After 1 h, the mixture was allowed to stand at room temperature and stirring was prolonged for 24 h. On addition of water (50 mL) and ammonium chloride (7 g) as a powder, the C-TCs precipitated were filtered off. The crude solids were dissolved in dichloromethane, and the resulting solutions were filtered through a pad of Florisil (5 cm × 2 cm). After the solutions were evaporated in vacuo, the residues were crystallized from suitable solvents (Table 1) to afford TCs **11**−**13**, **21**, **24**−**26**, **28**, **29**, **31**, **32**, **34**−**44**, **46**−**50**, **79**, **82**−**91**. Upon acidification (pH 0) of the reaction mother liquors with 2 M HCl (20 mL), the O-TCs precipitated as solids or separated as oils, which crystallized on standing. The crude solids were then filtered off and purified by crystallization from proper solvents (Table 2) to afford O-TCs **51**, **55**, **58**, **61**−**63**, **65**−**70**, **72**, **74**−**76**.

General Procedure for the Preparation of Library 2. A 60% sodium hydride dispersion in mineral oil (0.44 g, 10 mmol) was added in a single portion to a solution of alcohol **2** (1.91, 10 mmol) and proper isothiocyanate (10 mmol) in anhydrous pyridine (15 mL). The reaction mixture was stirred at room temperature for 15 min and then heated at 60−65 °C for 3 h. After addition of water (50 mL) and NH_4Cl (7 g) as a powder, the C-TCs precipitated as solids or, occasionally, separated as oils. The solids were filtered off and dissolved in dichloromethane, and the resulting solutions were dried (solution a). The oils were extracted with dichloromethane (30 mL × 2), and the combined extracts were washed with 2 N HCl (20 mL × 4), dried, and filtered through a pad of Florisil (5 cm × 2 cm) (solution b). Then, the dichloromethane solutions a and b were evaporated under reduced pressure, and the resulting residues were crystallized from the suitable solvents to afford C-TCs **14**−**20**, **22**, **27**, **30**, **33**, **45** (Table 1). On treatment of the reaction mother liquors with 2 N HCl (80 mL), the products, precipitated as solids, were worked up according to the procedure for library 1 to give O-TCs **52**−**54**, **56**, **57**, **59**, **60**, **64**, **71**, **73** (Table 2).

Synthesis of *O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3-Tolylthiocarbamate (23). Phosphorus pentoxide (2.84 g, 20 mmol) was added to a solution of **57** (3.58 g, 10 mmol) in dried DMF (40 mL). The resulting suspension was heated at 65 °C for 5 h and then treated with 1 M Na_2CO_3 (50 mL). The solid precipitated was filtered, washed with water, and crystallized from a mixture of dichloromethane and methanol to yield **23**: 0.71 g (21%), mp 102−104 °C.

General Procedure for Preparation of TCs 77−81. A 60% sodium hydride dispersion in mineral oil (0.44 g, 10 mmol) was added to an iced-cooled solution of **8** (1.95 g, 10 mmol) or **10** (1.43 g, 10 mmol) in dried DMF (15 mL) or of **9** (2.07 g, 10 mmol) in anhydrous THF (15 mL). As soon as hydrogen evolution ceased, the proper isothiocyanate (10 mmol) was added to the mixture, which was allowed to react at room temperature for 20 h. After treatment with water (50 mL) and NH_4Cl (7 g) added as a powder, the resulting mixture was extracted with dichloromethane, washed with water (7 × 20 mL), dried, and evaporated under reduced pressure. The oily or solid residue was crystallized from dichloromethane−methanol to yield TCs **77**−**81**.

***O*-[2-(Succinimido)ethyl] 4-chlorophenylthiocarbamate (77):** 1.12 g (36%), mp 143−144 °C.

***O*-{2-[(3a*R*,7a*S*)-1,3-Dioxo-1,3,3a,4,7,7a-hexahydro-2*H*-isoindol-2-yl]ethyl} 4-chlorophenylthiocarbamate (78):** 1.46 g (40%), mp 118−119 °C.

***O*-{2-[(3a*R*,7a*S*)-1,3-Dioxo-1,3,3a,4,7,7a-hexahydro-2*H*-isoindol-2-yl]ethyl} 4-bromophenylthiocarbamate (79):** 2.0 g (49%), mp 155−156 °C.

***O*-{2-[(3a*R*,4*S*,7*S*,7a*S*)-1,3-Dioxo-1,3,3a,4,7a-hexahydro-2*H*-4,7-methanoisoindol-2-yl]ethyl} 4-chlorophenylthiocarbamate (80):** 2.56 g (68%), mp 156−158 °C.

***O*-{2-[(3a*R*,4*S*,7*S*,7a*S*)-1,3-Dioxo-1,3,3a,4,7a-hexahydro-2*H*-4,7-methanoisoindol-2-yl]ethyl} 4-nitrophenylthiocarbamate (81):** 3.10 g (80%), mp 199−201 °C.

General Procedure for the Preparation of Compounds 92 and 93. To a solution of **2** (1.91 g, 10 mmol) and DMAP (2.2 g, 18 mmol) in acetonitrile (25 mL), *p*-chlorophenyl chlorothionoformate or *p*-tolyl chlorothionoformate (10 mmol) was added in a single portion. The mixture was stirred at room temperature for 24 h. Following addition of water (50 mL), the precipitate was filtered off and dissolved in chloroform. The organic solution was then washed with water (3 × 20 mL), dried, and evaporated under reduced pressure. The oily residue was crystallized from a mixture of diethyl ether and petroleum ether to afford the desired product.

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] *O*-(4-tolyl)thiocarbonate (92):** 3.11 g (91%), mp 106−108 °C.

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] *O*-(4-chlorophenyl)thiocarbonate (93):** 2.54 g (78%), mp 124−126 °C.

Synthesis of *O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-Nitrophenylcarbamate (94). **2** (1.0 g, 5 mmol) and 4-nitrophenyl isocyanate (0.9 g, 5 mmol) were dissolved in a 1:1 mixture of pyridine (15 mL) and DMF (15 mL) and then heated at 140 °C for 5 h. Following addition of water (150 mL), the precipitate was filtered and dissolved in a hot methanol−acetone mixture (1:3) from which **94** crystallized as a yellow solid: 0.30 g (17%), mp 234−236 °C.

Synthesis of *O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 1*H*-Imidazole-1-carbothioate (95). **2** (1.52 g, 8 mmol) and thiocarbonyldiimidazole (1.8 g, 10 mmol) were mixed and ground in a mortar, prolonging the manual mixing for 15 min. Then, the paste was washed with water and dissolved in chloroform. The solution was dried and evaporated under reduced pressure. The oily residue was crystallized from ethyl ether to give **95**: 2.17 g (72%), mp 143−145 °C.

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Supporting Information Available: Spectroscopic (IR and ^1H NMR) and microanalytical data for all synthesized compounds described in the Experimental Section and experimental procedures for molecular modeling and biological evaluation. This material is available free of charge via the Internet at http://pubs.acs.org.

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