Design, Synthesis, SAR, and Molecular Modeling Studies of Acylthiocarbamates: A Novel Series of Potent Non-nucleoside HIV-1 Reverse Transcriptase Inhibitors Structurally Related to Phenethylthiazolylthiourea Derivatives

Angelo Ranise,*,§ Andrea Spallarossa,§ Silvia Schenone,§ Olga Bruno,§ Francesco Bondavalli,§ Laura Vargiu,† Tiziana Marceddu,† Massimo Mura,† Paolo La Colla,*,‡ and Alessandra Pani‡

Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV 3, I-16132 Genova, Italy, Cooperative Laboratory Idenix—University of Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato (Cagliari), Italy, and Dipartimento di Biologia Sperimentale, Università di Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato (Cagliari), Italy

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A novel series of potent, selective HIV-1 *N*-acylthiocarbamate (ATC) nonnucleoside reverse transcriptase inhibitors (NNRTIs) is described. The title compounds were synthesized through a highly convergent, one-pot procedure. In cell-based assays, the lead compound (**17c**) prevented the HIV-1 multiplication with an EC_{50} of 8 μM. The lead optimization strategy was developed by single or multiple modifications of the three molecular portions, in which **17c** was notionally divided. Molecular modeling studies led to the synthesis of *O*-(2-phthalimidoethyl)-*N*-(*p*-substituted phenyl)-*N*-acylthiocarbamates, which showed in vitro activities against HIV-1 in the low nanomolar range. Nevertheless, the title compounds retained low potency against HIV-1 strains carrying mutations (K103R, Y181C, and K103N/Y181C) responsible for NNRTI resistance. The hypothetical docking model of RT/**17c** and RT/**25c**, derived from X-ray crystallographic structure of a PETT derivative in complex with HIV-1 RT, revealed that the model structures of ATCs do not approximate the NNRTI butterfly-like conformation. Analysis of these hypotetical complexes helps to rationalize some SARs and resistance data.

Introduction

Despite the considerable success in Western Countries of highly active antiretroviral therapy (HAART), AIDS remains one of the most urgent world health problems, being the first cause of death in Africa and the fourth leading cause of death worldwide.[1] Moreover, rapid emergence of drug resistant HIV variants and severe side effects related to both short- and long-term treatments limit the efficacy of existing therapies.[2−5]

The above considerations, together with the knowledge that HIV cannot be eradicated from the infected body because of its persistence in reservoirs with low turnover rates,[6,7] highlight the need of new drugs which are less toxic, active against the drug resistant mutants selected by current therapies, or addressed toward novel targets in the viral replicative cycle.[2,8]

Chemotherapy of HIV infections/AIDS currently employs inhibitors of two products of the viral *pol* gene, the enzymes reverse transcriptase (RT) and protease. RT-targeting drugs[9−11] can be grouped into two classes: nucleoside analogues (nucleoside reverse transcriptase inhibitors, NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs). NRTIs mechanistically act as DNA chain terminators. On the other hand, NNRTIs bind to a hydrophobic pocket close to, but distinct from, the RT active site in the p66 subunit and inhibit the enzyme activity by mediating allosteric changes in the RT, thus causing a distortion of the catalytic active site aspartyl residues.[12] Despite the favorable toxicity and adherence properties, resistance to NNRTIs develops rapidly, mostly as a consequence of single mutations.[13−16]

Despite the NNRTI chemical diversity (Chart 1),[10,17−20] X-ray HIV-1 RT/NNRTI complexes[15,21−30] show a common configuration resembling a butterfly-like shape, where the wings are usually occupied by π-electron systems that can interact with aromatic amino acid residues within the NNRTI binding site (NNIBS).[10,30]

A few years ago we reported the highly convergent "one-pot" synthesis of *O*-substituted-*N*-acyl-*N*-phenylthiocarbamates (ATCs),[31] accomplished through a three-step sequence by combining three different building blocks: alcohols, isothiocyanates, and acyl chlorides.

Thus, with the aim at identifying novel antiviral agents, we planned the synthesis of a number of ATCs and their evaluation in cell-based assays has been performed.[32] Among the above derivatives, 2-phenoxyethyl benzoyl(phenyl)thiocarbamate **17c** (Chart 2), sharing some structural features with PETT derivatives (Chart 1c), showed anti-HIV-1 activity and low cytotoxicity (CC_{50}: 122 μM, EC_{50}: 8 μM, selectivity index: 15.2).

* Corresponding authors: Prof. A. Ranise. Phone: +39 010 353 8370. Fax: +39 010 353 8358. E-mail: ranise@unige.it. Prof. P. La Colla. ′ Phone: +39 070 675 4148. Fax: +39 070 675 4210. E-mail: placolla@unica.it.
§ Università di Genova.
† Cittadella Universitaria di Monserrato.
‡ Università di Cagliari.

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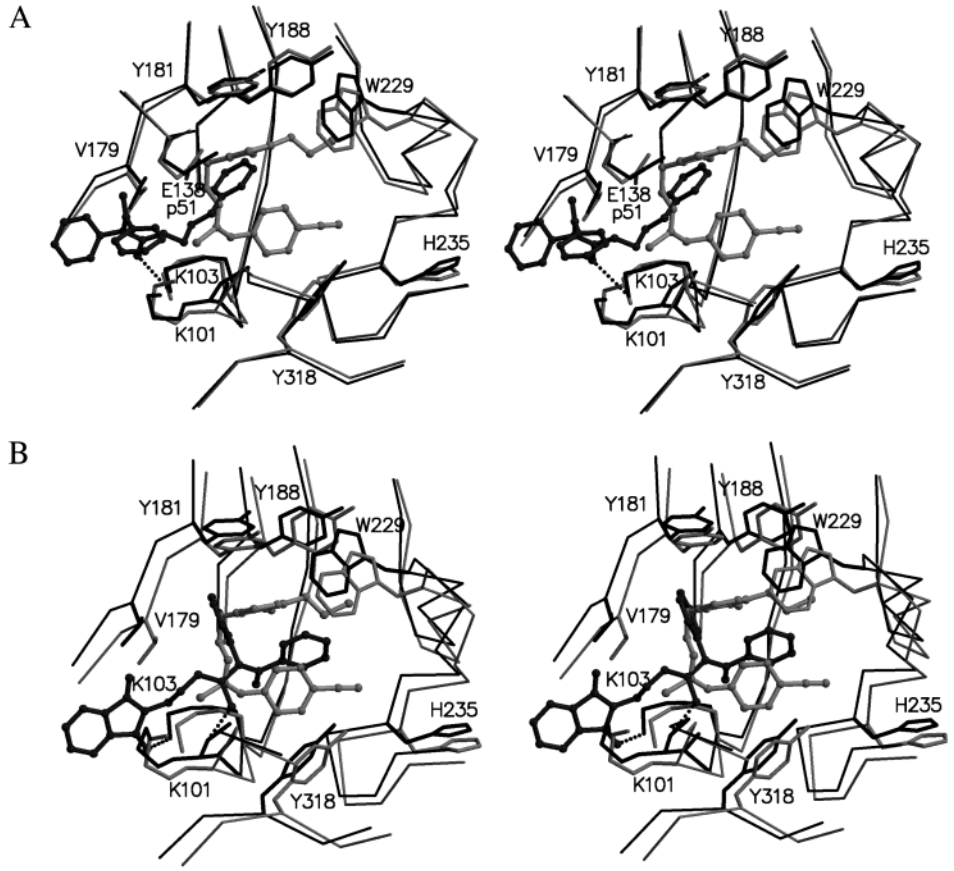


Figure 1. Positioning of PETT-1, **17c**, and **25c** in the NNIBS of HIV-1 RT. Stereodiagrams showing the relative positions and orientations of PETT-1 (grey ball-and-stick, X-ray structure model), **17c** (A), and **25c** (B) (black ball-and-stick, model structures). The surrounding RT side chains that contribute to form the NNIBS (as described by Ren et al.[30]) are shown as black sticks for the model structure and gray for the X-ray structure, respectively. The H-bonds are indicated by dotted lines.

Chart 1. Structures Representative of NNRTIs

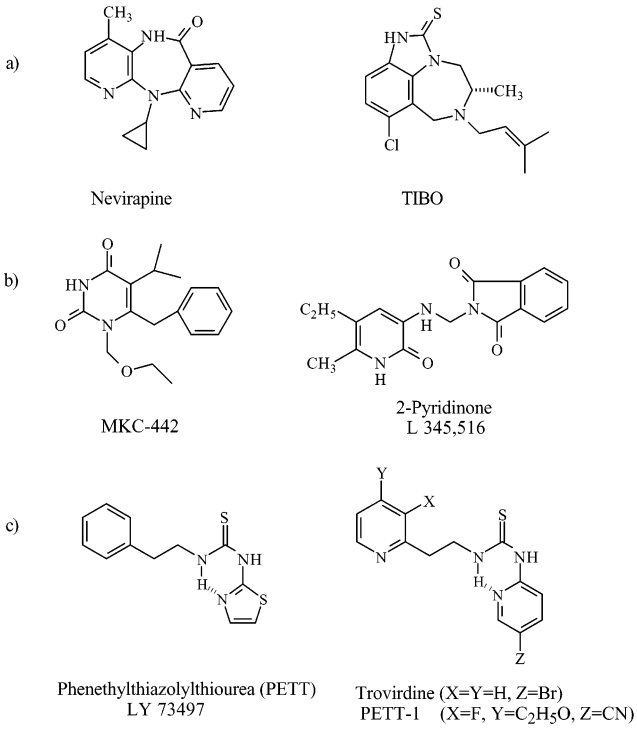
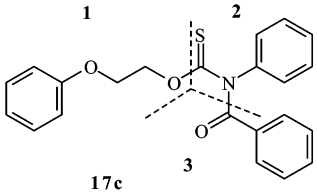


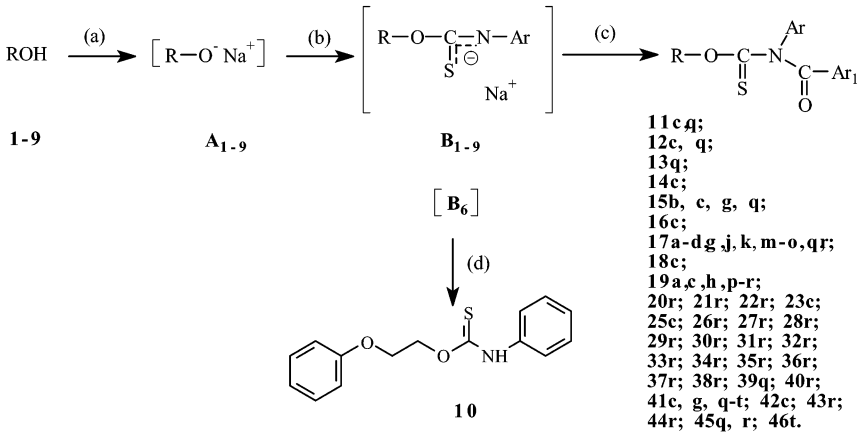
Chart 2. Structure of Lead Compound **17c**, Segmented in Three Molecular Portions



The ATC Design. The goal of the present investigation was the optimization of the potency of lead **17c**. To rationalize SAR strategy, the chemical structure of **17c** was retrosynthetically divided into three molecular portions (Chart 2).

In the first phase we evaluated the dependence of the antiviral activity on the nature of the acyl moiety (portion 3, compounds **17**). Then, we modified portion 2 either by introducing a fluorine atom at position 4 of the phenyl ring (**18c**) or by replacing the latter with a cyclohexyl nucleus (**16c**). The study of portion 1 was performed as follows: (i) by introducing a methyl group on the oxyethyl linker (**19**) and, as in the case of **20r**, **21r**, and **22r**, electron-withdrawing groups on the *N*-phenyl ring at positions meta (Cl) and para (Cl, NO_2); (ii) by reducing the length of the oxyethyl spacer (compounds **13q**, **14c**, and **15**); (iii) by conformationally constraining entire portion 1 with the cagelike structure of the adamantane nucleus (compounds **12**), this framework being employed in the synthesis of potential HIV-1 agents;[33−35] (iv) by eliminating the phenyl moiety (compounds **11**). Finally, we prepared thioisostere **23c** and bioisosteres **24u**−**w**.

Scheme 1. Convergent One-Pot Synthesis of *O*-Substituted-*N*-substituted-*N*-acylthiocarbamates **11c**−**23c** and **25c**−**46t**[a]



[a] Reaction conditions: (a) NaH, dry THF or dry DMF or dry toluene, various temperature conditions; (b) Ar−N=C=S, cooling or room temperature or heating; (c) dry pyridine (and TMEDA only for **25c**−**46t**), then Ar_1CO−Cl, room temperature or heating; (d) 1 M HCl. For ROH, ArNCS, Ar_1COCl structure list, see Chart 3.

To better understand the RT/**17c** interaction, we derived a hypothetical model for docking of **17c** in the NNIBS (Figure 1A). A polar interaction between the ether oxygen and the Glu138 side chain (from the p51 subunit) suggested the selection of aryl−alkyl alcoholic building blocks bearing hydrogen bond acceptor group(s) adjacent to the phenyl ring. Thus, taking into account that the phthalimide moiety is present in both 2-pyridinone compound L-345,516 [36] (Chart 1b) and some PETT derivatives,[20,37] we constructed a docking model for compound **25c** (Figure 1B). This model indicated a better interaction between RT and the compound (vide infra) which therefore was proposed for synthesis. The computer-assisted lead optimization strategy of **25c** was focused on substituent variation on the *N*-phenyl ring and/or modification of the acyl moety (**26r**−**46t**). In derivatives **26r**−**38r**, **40r**, **41r**, **43r**, **44r**, **45r**, the acyl moiety was kept constant (2-thenoyl) to better evaluate the effects determined by exclusive modification of the *N*-phenyl ring.

Chemistry

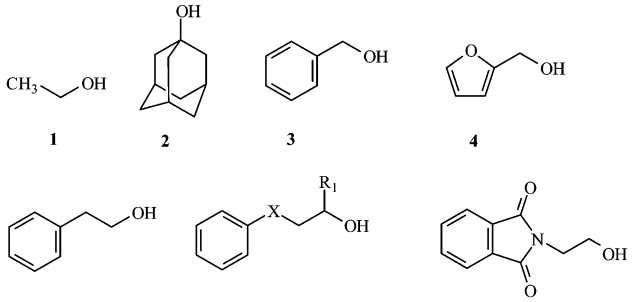
The *O*-substituted-*N*-substituted-*N*-acylthiocarbamates (**11c**−**23c, 25c**−**46t**) were prepared according to general Scheme 1. Briefly, starting alcohols **1**−**9** (Chart 3a) were first transformed into their corresponding salts in the presence of 60% sodium hydride dispersion in mineral oil in anhydrous aprotic solvents (toluene, THF, DMF, Py) having different polarity. Then, alcoholates $\mathbf{A_{1-9}}$ were condensed in situ with isothiocyanates at varied temperatures to give saline adducts $\mathbf{B_{1-9}}$ that were subsequently coupled with acyl chlorides to afford the desired products (for the isothiocyanate and acyl chloride building blocks employed, see Chart 3b,c). To obviate the dissimilar reactivity of **A** and/or **B** toward isothiocyanates and acylating reagents, respectively, different reaction conditions were adopted according to procedures P_{1-4} (see the Experimental Section).

Compounds **11c**,**q**, **13q**, **14c**, **15b**,**c**,**g**,**q**, **16c**, **17a**−**d**,**g**,**j**,**k**,**m**,**n**,**o**,**q**,**r**, **18c**, **19a**,**c**,**h**,**p**,**q**,**r**, **20r**, **21r**, **22r**, **23c** were prepared according to procedure P_1 (**1**, **3**-**8**, dry toluene or THF, room temperature). To render **B** more soluble (toluene) and/or to enhance their reactivity toward the acyl reagents, dry pyridine was added to the reaction mixture.

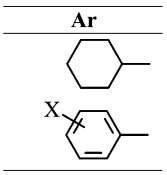
Compounds **12c**,**q** were obtained by procedure P_2, (**2**, dry DMF/pyridine). The reaction mixture needed to be warmed at 90−95 °C, owing to the poor reactivity of 1-adamantanol and the corresponding alcoholate.

Chart 3. The Building Blocks Used

a) Alcohls **1-9**



b) Isothiocyanates **Ar-NCS**

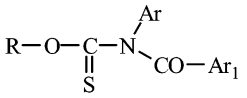


X = H; *o*-, *m*-, *p*-CH_3; *o*-, *p*-C_2H_5; *m*-CF_3; *m*-$COCH_3$; *m*-, *p*-F; *o*-, *m*-, *p*-Cl; *m*-, *p*-Br; *p*-I; *p*-$N(C_2H_5)_2$; *m*-, *p*-NO_2; *o*-, *m*-OCH_3; *p*-O C_2H_5

c) Acyl chlorides **Ar_1CO-Cl**

	Ar_1CO
a	phenoxyacetyl
b	trans-cinnamoyl
c	benzoyl
d	4-toluoyl
e	4-phenylbenzoyl
f	4-fluorobenzoyl
g	4-chlorobenzoyl
h	3-nitrobenzoyl
i	4-anisoyl
j	2-acetoxybenzoyl
k	2,4-dichlorobenzoyl
l	3,4-dichlorobenzoyl
m	3,5-dichlorobenzoyl
n	4-chloro-3-nitrobenzoyl
o	3,4,5-trimethoxybenzoyl
p	1-naphthoyl
q	2-furoyl
r	2-thenoyl
s	2-chloronicotinoyl
t	6-chloronicotinoyl

Table 1. Physical and Chemical Data of *O*-Substituted-*N*-substituted-*N*-acylthiocarbamates **11c**−**23c** and **25c**−**46t**

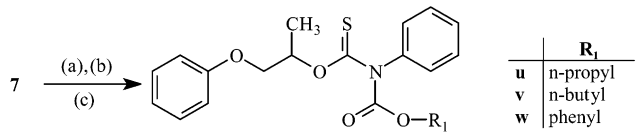


compd	R	Ar	Ar_1-CO	mp, °C[a]	% yield	formula[b]
11c	ethyl	C_6H_5	benzoyl	85−86[c]	60	$C_{16}H_{15}NO_2S$
11q	ethyl	C_6H_5	2-furoyl	67−69[c]	67	$C_{14}H_{13}NO_3S$
12c	1-adamantyl	C_6H_5	benzoyl	199−200[d]	56	$C_{24}H_{25}NO_2S$
12q	1-adamantyl	C_6H_5	2-furoyl	133−134[d]	58	$C_{22}H_{23}NO_3S$
13q	benzyl	C_6H_5	2-furoyl	117−119[e]	79	$C_{19}H_{15}NO_3S$
14c	furfuryl	C_6H_5	benzoyl	167−169[f]	60	$C_{19}H_{15}NO_3S$
15b	2-(phenyl)ethyl	C_6H_5	*trans*-cinnamoyl	89−91[d]	72	$C_{24}H_{21}NO_2S$
15c	2-(phenyl)ethyl	C_6H_5	benzoyl	67−68[d]	76	$C_{22}H_{19}NO_2S$
15g	2-(phenyl)ethyl	C_6H_5	4-chlorobenzoyl	55−57[c]	71	$C_{22}H_{18}ClNO_2S$
15q	2-(phenyl)ethyl	C_6H_5	2-furoyl	83−84[d]	74	$C_{20}H_{17}NO_3S$
16c	2-(phenoxy)ethyl	C_6H_{11}	benzoyl	91−93[c]	59	$C_{22}H_{25}NO_3S$
17a	2-(phenoxy)ethyl	C_6H_5	phenoxyacetyl	111−113[g]	55	$C_{23}H_{21}NO_4S$
17b	2-(phenoxy)ethyl	C_6H_5	*trans*-cinnamoyl	117−118[c]	95	$C_{24}H_{21}NO_3S$
17c	2-(phenoxy)ethyl	C_6H_5	benzoyl	67−68[c]	86	$C_{22}H_{19}NO_3S$
17d	2-(phenoxy)ethyl	C_6H_5	4-toluoyl	89−91[g]	70	$C_{23}H_{21}NO_3S$
17g	2-(phenoxy)ethyl	C_6H_5	4-chlorobenzoyl	101−103[g]	74	$C_{22}H_{18}ClNO_3S$
17j	2-(phenoxy)ethyl	C_6H_5	2-acetoxybenzoyl	105−107[d]	55	$C_{24}H_{21}NO_5S$
17k	2-(phenoxy)ethyl	C_6H_5	2,4-dichlorobenzoyl	106−108[g]	50	$C_{22}H_{17}Cl_2NO_3S$
17m	2-(phenoxy)ethyl	C_6H_5	3,5-dichlorobenzoyl	83−84[c]	81	$C_{22}H_{17}Cl_2NO_3S$
17n	2-(phenoxy)ethyl	C_6H_5	4-chloro-3-nitrobenzoyl	92−94[d]	65	$C_{22}H_{17}ClN_2O_5S$
17o	2-(phenoxy)ethyl	C_6H_5	3,4,5-trimethoxy benzoyl	102−104[g]	58	$C_{25}H_{25}NO_6S$
17q	2-(phenoxy)ethyl	C_6H_5	2-furoyl	98−99[g]	85	$C_{20}H_{17}NO_3S$
17r	2-(phenoxy)ethyl	C_6H_5	2-thenoyl	91−92[g]	85	$C_{20}H_{17}NO_3S_2$
18c	2-(phenoxy)ethyl	4-F-C_6H_4	benzoyl	85−87[h]	65	$C_{22}H_{18}FNO_3S$
19a	1-(methyl)−2-(phenoxy)ethyl	C_6H_5	phenoxyacetyl	85−87[c]	61	$C_{24}H_{23}NO_4S$
19c	1-(methyl)−2-(phenoxy)ethyl	C_6H_5	benzoyl	105−106[d]	84	$C_{23}H_{21}NO_3S$
19h	1-(methyl)−2-(phenoxy)ethyl	C_6H_5	3-nitrobenzoyl	136−138[d]	80	$C_{23}H_{20}N_2O_5S$
19p	1-(methyl)−2-(phenoxy)ethyl	C_6H_5	1-naphthoyl	122−124[g]	68	$C_{27}H_{23}NO_3S$
19q	1-(methyl)−2-(phenoxy)ethyl	C_6H_5	2-furoyl	108−110[c]	82	$C_{21}H_{19}NO_4S$
19r	1-(methyl)−2-(phenoxy)ethyl	C_6H_5	2-thenoyl	118−119[g]	93	$C_{21}H_{19}NO_3S_2$
20r	1-(methyl)−2-(phenoxy)ethyl	3-Cl-C_6H_4	2-thenoyl	126−128[i]	52	$C_{21}H_{18}ClNO_3S_2$
21r	1-(methyl)−2-(phenoxy)ethyl	4-Cl-C_6H_4	2-thenoyl	124−126[i]	58	$C_{21}H_{18}ClNO_3S_2$
22r	1-(methyl)−2-(phenoxy)ethyl	4-NO_2-C_6H_4	2-thenoyl	108−110[i]	54	$C_{21}H_{18}ClN_2O_5S_2$
23c	2-(phenylthio)ethyl	C_6H_5	benzoyl	74−76[d]	70	$C_{22}H_{19}NO_2S_2$
25c	2-(phthalimido)ethyl	C_6H_5	benzoyl	153−155[j]	50	$C_{24}H_{18}N_2O_4S$
26r	2-(phthalimido)ethyl	2-CH_3-C_6H_4	2-thenoyl	143−144[j]	48	$C_{23}H_{18}N_2O_4S_2$
27r	2-(phthalimido)ethyl	2-C_2H_5-C_6H_4	2-thenoyl	131−133[j]	56	$C_{24}H_{20}N_2O_4S_2$
28r	2-(phthalimido)ethyl	2-Cl-C_6H_4	2-thenoyl	133−134[j]	47	$C_{22}H_{15}ClN_2O_4S_2$
29r	2-(phthalimido)ethyl	2-CH_3O-C_6H_4	2-thenoyl	185−186[j]	60	$C_{23}H_{18}N_2O_5S_2$
30r	2-(phthalimido)ethyl	3-CH_3-C_6H_4	2-thenoyl	137−139[j]	44	$C_{22}H_{18}N_2O_4S_2$
31r	2-(phthalimido)ethyl	3-CF_3-C_6H_4	2-thenoyl	144−145[j]	57	$C_{23}H_{15}F_3N_2O_4S_2$
32r	2-(phthalimido)ethyl	3-CH_3CO-C_6H_4	2-thenoyl	156−158[j]	58	$C_{24}H_{18}N_2O_5S_2$
33r	2-(phthalimido)ethyl	3-F− C_6H_4	2-thenoyl	135−137[j]	52	$C_{22}H_{15}FN_2O_4S_2$
34r	2-(phthalimido)ethyl	3-Cl-C_6H_4	2-thenoyl	102−104[j]	41	$C_{22}H_{15}ClN_2O_4S_2$
35r	2-(phthalimido)ethyl	3-Br-C_6H_4	2-thenoyl	116−118[j]	31	$C_{22}H_{15}BrN_2O_4S_2$
36r	2-(phthalimido)ethyl	3-NO_2-C_6H_4	2-thenoyl	169−171[j]	46	$C_{22}H_{15}N_3O_6S_2$
37r	2-(phthalimido)ethyl	3-CH_3O-C_6H_4	2-thenoyl	124−125[j]	67	$C_{23}H_{18}N_2O_5S_2$
38r	2-(phthalimido)ethyl	4-CH_3-C_6H_4	2-thenoyl	136−138[j]	71	$C_{23}H_{18}N_2O_4S_2$
39q	2-(phthalimido)ethyl	4-C_2H_5-C_6H_4	2-furoyl	145−146[j]	58	$C_{24}H_{20}N_2O_5S$
40r	2-(phthalimido)ethyl	4-F-C_6H_4	2-thenoyl	147−149[j]	43	$C_{22}H_{15}FN_2O_4S_2$
41c	2-(phthalimido)ethyl	4-Cl-C_6H_4	benzoyl	183−185[j]	57	$C_{24}H_{17}ClN_2O_4S$
41g	2-(phthalimido)ethyl	4-Cl-C_6H_4	4-chlorobenzoyl	163−165[j]	54	$C_{24}H_{16}Cl_2N_2O_4S$
41q	2-(phthalimido)ethyl	4-Cl-C_6H_4	2-furoyl	154−155[j]	61	$C_{22}H_{15}ClN_2O_5S$
41r	2-(phthalimido)ethyl	4-Cl-C_6H_4	2-thenoyl	151−152[j]	65	$C_{22}H_{15}ClN_2O_4S_2$
41s	2-(phthalimido)ethyl	4-Cl-C_6H_4	2-chloro nicotinoyl	158−160[i]	62	$C_{23}H_{15}Cl_2N_3O_4S$
41t	2-(phthalimido)ethyl	4-Cl-C_6H_4	6-chloro nicotinoyl	161−162[j]	66	$C_{23}H_{15}Cl_2N_3O_4S$
42c	2-(phthalimido)ethyl	4-Br-C_6H_4	benzoyl	175−176[j]	56	$C_{24}H_{17}BrN_2O_4S$
43r	2-(phthalimido)ethyl	4-I-C_6H_4	2-thenoyl	149−151[j]	68	$C_{22}H_{15}IN_2O_4S_2$
44r	2-(phthalimido)ethyl	4-$(C_2H_5)_2N$-C_6H_4	2-thenoyl	154−156[k]	12	$C_{26}H_{25}N_3O_4S_2$
45q	2-(phthalimido)ethyl	4-NO_2-C_6H_4	2-furoyl	169−171[j]	68	$C_{22}H_{15}N_3O_7S$
45r	2-(phthalimido)ethyl	4-NO_2-C_6H_4	2-thenoyl	163−164[j]	55	$C_{22}H_{15}N_3O_6S_2$
46t	2-(phthalimido)ethyl	4-C_2H_5O-C_6H_4	6-chloronicotinoyl	138−139[j]	33	$C_{25}H_{20}ClN_3O_5S$

[a] Crystallization solvent(s): DE = diethyl ether, DM = dichloromethane, E = ethanol, M = methanol, P = petroleum ether. [b] All compounds were analyzed for C, H, N, and S; analytical results were within ±0.4% of the theoretical values. [c] DE−P. [d] DE−M. [e] DM−P. [f] DE−DM. [g] DE. [h] DE−M−P. [i] DE−E. [j] DM−E. [k] E.

In procedure P_3 (**9**, dry pyridine, 0−5 °C to room temperature), to facilitate the acylation step and increase overall yield and purity of compounds **25c**, **26r**−**38r**, **39q**, **40r**, **41c**,**g**,**q**−**t**, **42c**, **43r**, **44r**, **45q**,**r**,**t**, the reaction mixture was treated with TMEDA (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine), before adding the acyl chloride. In the preparation of ortho-derivatives **26r**−**29r**, the reaction mixture was heated at 55−60 °C for 3 h, before workup. Physical and chemical data of the title compounds are given in Table 1. In procedure P_4 (synthetic variant), $\mathbf{B_7}$ was reacted with prolyl, butyl, and phenyl chloroformates in dry THF in the absence of pyridine to give *N*-alkoxycarbonyl-*N*-phenyl thiocarbamates **24u**,**v**,**w** (Scheme 2).

Scheme 2. One-Pot Synthesis of *O*-Substituted *N*-(Propoxy- or Butoxy- or Phenoxycarbonyl)-*N*-phenylthiocarbamates **24u**,**v**,**w**[a]



	R_1
u	n-propyl
v	n-butyl
w	phenyl

[a] Reaction conditions: (a) NaH, dry THF, room temperature; (b) $C_6H_5-N=C=S$; (c) $R_1-O-CO-Cl$, heat.

The entire procedure deserves comments. Alcohols needed to be transformed into the respective salts, owing to their well-established low reactivity toward isothiocyanates.[38] A great variety of (hetero)aromatic acyl chlorides could be employed, but, surprisingly, the aliphatic ones were generally unreactive (only 2-phenoxyacetyl chloride could successfully be used). Probably, the excessive basicity of adducts **B** and the presence of pyridine (it is noteworthy that in the absence of pyridine the reaction did not take place) favored the elimination of hydrogen chloride from acyl chlorides to afford ketenes[39] rather than the nucleophilic displacement of chloride anion. Unlike aliphatic acyl chlorides, aroyl chlorides cannot undergo dehydroalogenation. Furthermore, we hypothesized that also the absence of reactivity of **B** toward ketenes, which are well-known acylating agents, could be explained according to the HSAB principle.[40] From this point of view, **B** (owing to the presence of the sulfur atom) would be soft bases unable to react with ketenes, due to the hardness of the latter reagents. Interestingly, compound **11c** was prepared also by reacting the corresponding parent thiocarbamate[41] with benzoyl chloride in pyridine, so demonstrating that acylation can occur starting from preformed thiocarbamate in basic medium.

These procedures paralleled the one-pot method, developed by some of us,[42] for preparation of *N*,*N*-disubstituted-*N*′-acyl-*N*′-phenylthioureas starting from weakly basic amines. In this case, the adduct intermediates, obtained from sodium salts of starting amines and arylisothiocyanates, were reacted with acyl chlorides to afford *N*-acylthioureas. Nevertheless, the two types of saline adducts showed different stability. Thus, for example, treatment of $\mathbf{B_6}$ with acids (HCl, CH_3COOH) afforded the corresponding thiocarbamate **10** (Scheme 1), whereas, under the same condition, the thiourea salts decomposed, restoring starting materials.

As for acylated thioureas,[43] to make possible unambiguous assignment of the acyl group position and to confirm the sulfur or nitrogen regioisomer, we recorded ^{13}C NMR spectra of lead compound **17c** and of its parent thiocarbamate **10**, which exhibited the thione carbon signals at δ 192.13 and 188.62, respectively. It is apparent that the thione carbon signal cannot be consistent with the acyl chloride attack on the sulfur atom. In addition, introduction of an acyl (benzoyl) group on the nitrogen atom caused a modest downfield effect on the resonance of the thione carbon atom in comparison with that of **10**. Unfortunately, the above procedures were not suitable to synthesize acylcarbamate isosteres, owing to minor nucleophilicity of the carbamate sodium salts (prepared from the corresponding alcoholates and phenyl isocyanate) toward acyl reagents. These adducts, under the same or even more drastic reaction conditions than **B**, did not react with acyl chlorides, the isolated products being the unreacted carbamates (data not shown). Also the reaction of $\mathbf{B_{7,9}}$ with mesyl and tosyl chlorides, to afford the corresponding sulfonyl(phenyl)thiocarbamate bioisosteres, was unsuccessful.

Biological Results and Discussion

The test products were evaluated for their cytotoxicity and anti-HIV-1 activity in MT-4 cells (Tables 2 and 3) using trovirdine as reference compound. The most potent derivatives were tested in enzyme assays against virion-associated RT (vRT) (Table 4) and in cell-based assays against wt III_B virus (Table 5) and some NNRTI resistant strains[16,44] carrying clinically relevant mutations (K103R, Y181C, and K103N plus Y181C, Table 6).

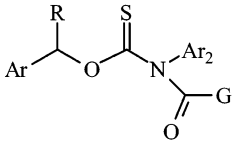
Table 2 shows the effects of the above-mentioned SAR strategy on lead compound **17c**. Modification of the acyl moiety (**17a**,**b**,**d**,**g**,**j**,**k**,**m**,**n**,**o**,**q**,**r**) and introduction of a fluorine atom at position para of the *N*-phenyl ring (**18c**) failed to improve the antiretroviral potency of **17c** (EC_{50} value range: 4−11.6 μM). Replacement of the phenyl moiety with a cyclohexyl ring (**16c)** caused loss of activity, probably due to the missing hydrophobic aromatic contacts, which represent one of the most important interactions in the RT/NNRTI complexes.[10,30] Introduction of a methyl group on the carbon adjacent to the thiocarbamic oxygen (**19a**,**c**,**h**,**p**,**q**,**r**, tested as racemic mixtures), led to a 4−6-fold increase in potency with respect to the unbranched derivatives (compare **17a** with **19a**, **17c** with **19c**, **17q** with **19q**, and **17r** with **19r**). Further introduction of electron-withdrawing groups at position meta (Cl) or para (Cl, NO_2) of the *N*-phenyl ring resulted in derivatives (**20r**, **21r**, **22r**) as potent as **17c**. Replacement of the ether oxygen with sulfur diminished activity (**23c**), whereas introduction of the propoxy-, butoxy-, and phenoxycarbonyl groups instead of the benzoyl moiety gave poorly active (**25u**, **25v**) or inactive (**25w**) compounds.

Removal of the ether oxygen (2-phenethyl derivatives **15b**,**c**,**g**,**q**) led to a 2-fold increase of potency (compare **15b** with **17b**, **15c** with **17c**, **15g** with **17g,** and **15q** with **17q**), whereas the shortening of the spacer to a methylene bridge caused decrease (**13q**) or loss (**14c**) of activity. Interestingly, similar modifications greatly influenced activity of PETT derivatives, where the ethyl linker gave the best results and shortening this chain by one carbon caused a significant decrease of potency.[19]

Removal of the phenyl ring of portion 1 (*O*-ethyl derivatives **11c**,**q**) as well as the conformational restriction of entire portion 1 (*O*-(1-adamantyl) derivatives **12c**,**q**), led to the loss of activity (data not shown).

Replacement of portion 1 with the 2-(*N*-phthalimido)-ethyl moiety led to compound **25c**, which resulted 20-fold more potent than **17c** (Table 3). This finding was in agreement with docking studies, which suggested the existence, in the case of **25c**, of an additional H-bond (see below). Table 3 summarizes the results obtained with this second lead compound and with its analogues designed to improve antiretroviral activity. Introduction of a chlorine atom at position 4 of the *N*-phenyl ring (**41c**,**g**,**q**−**t**) determined a 4−80-fold increase of the potency (EC_{50} value range: 0.1−0.005 μM) with respect

Table 2. Cytotoxicity and Anti-HIV-1 Activity of Lead Compound **17c**, Its Analogues, and (Bio)isosteres[a]



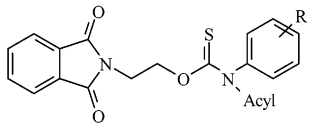
compd	Ar	R	Ar_2	G-CO	CC_{50}[b] (μM)	EC_{50}[c] (μM)	SI[d]
13q	phenyl	H	C_6H_5	2-furoyl	111	58	1.9
14c	2-furyl	H	C_6H_5	benzoyl	38.3	>38.3	-
15b	benzyl	H	C_6H_5	trans-cinnamoyl	40.4	4.5	9
15c	benzyl	H	C_6H_5	benzoyl	91	4.2	22
15 g	benzyl	H	C_6H_5	4-chlorobenzoyl	43	4	10.7
15q	benzyl	H	C_6H_5	2-furoyl	102	4.3	24
16c	phenoxymethyl	H	C_6H_{11}	benzoyl	>200	200	-
17a	phenoxymethyl	H	C_6H_5	phenoxyacetyl	103	6	17.1
17b	phenoxymethyl	H	C_6H_5	*trans*-cinnamoyl	66.6	7.7	8.65
17c	phenoxymethyl	H	C_6H_5	benzoyl	122	8	15.2
17d	phenoxymethyl	H	C_6H_5	4-toluoyl	>200	9.9	>20.2
17g	phenoxymethyl	H	C_6H_5	4-chlorobenzoyl	133	10.3	12.9
17j	phenoxymethyl	H	C_6H_5	2-acetoxybenzoyl	>200	9.5	>21
17k	phenoxymethyl	H	C_6H_5	2,4-dichlorobenzoyl	43	11.6	3.7
17m	phenoxymethyl	H	C_6H_5	3,5-dichlorobenzoyl	43	8.8	4.8
17n	phenoxymethyl	H	C_6H_5	4-chloro-3-nitrobenzoyl	80	7.6	10.5
17o	phenoxymethyl	H	C_6H_5	3,4,5-trimethoxybenzoyl	>200	9.6	>20.8
17q	phenoxymethyl	H	C_6H_5	2-furoyl	82	8.4	9.7
17r	phenoxymethyl	H	C_6H_5	2-thenoyl	125.2	8.6	14.5
18c	phenoxymethyl	H	4-F-C_6H_5	benzoyl	70.7	4	17.6
19a	phenoxymethyl	CH_3	C_6H_5	phenoxyacetyl	122.4	1.4	87.4
19c	phenoxymethyl	CH_3	C_6H_5	benzoyl	>200	1.3	>153.8
19h	phenoxymethyl	CH_3	C_6H_5	3-nitrobenzoyl	>200	3.6	>55.5
19p	phenoxymethyl	CH_3	C_6H_5	1-naphthoyl	>200	6	>33.3
19q	phenoxymethyl	CH_3	C_6H_5	2-furoyl	51.5	1.3	39.6
19r	phenoxymethyl	CH_3	C_6H_5	2-thenoyl	>200	2	>100
20r	phenoxymethyl	CH_3	3-Cl-C_6H_5	2-thenoyl	>100	11	>9
21r	phenoxymethyl	CH_3	4-Cl-C_6H_5	2-thenoyl	>100	11	>9
22r	phenoxymethyl	CH_3	4-NO_2-C_6H_5	2-thenoyl	63	6	10.5
23c	phenylthiomethyl	H	C_6H_5	benzoyl	44	>44	-
24u	phenoxymethyl	CH_3	C_6H_5	*n*-propoxycarbonyl	>200	131	>1.5
24v	phenoxymethyl	CH_3	C_6H_5	*n*-butoxycarbonyl	>200	47	>4
24w	phenoxymethyl	CH_3	C_6H_5	phenoxycarbonyl	>200	>200	-
Trovirdine					60	0.02	3000

[a] The data mean represent 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 the HIV-1 induced cytopathogenicity, as determined by the MTT method. [d] Selectivity index: CC_{50}/EC_{50} ratio.

to **25c**. Replacement of the chlorine atom with bromine (**42c**) or the nitro group (**45q**,**r**) still led to nanomolar inhibitors. With the exception of **44r** (4-diethylamino), also the other *N*-(*p*-substituted)phenyl analogues tested (4-methyl **38r**, 4-ethyl **39q**, 4-fluoro **40r**, 4-iodo **43r**, 4-ethoxy **46t**) were endowed with high potency and they were more potent than meta- (**30r**−**37r**) and ortho-derivatives (**26r**−**29r**). This trend is well exemplified by positional isomers **26r**, **30r**, **38r** and **28r**, **34r**, **41r**; in fact, when compared to para counterparts, all the *N*-(ortho- and meta-substituted)phenyl analogues showed a dramatic decrease of activity (EC_{50} value range: 6−11 μM). At the same time, the data indicate that activity is affected not only by the substitution pattern on the *N*-phenyl ring, but also by electronic nature of the para substituents. Thus, the analogues bearing electron-withdrawing groups (fluoro **40r**, chloro **41**, bromo **42c**, iodo **43r**, nitro **45q**,**r**) and, to a lesser extent, those bearing weak electron-donating groups (ethyl, **39q** > methyl, **38r**) are more potent than the para-congeners with electron-donating groups (ethoxy, **46t** > diethylamino, **44r**). On the other hand, the greater activity of the para-derivatives bearing the more sterically demanding chloro (**41c**−**t**), bromo (**42c**), iodo (**43r**) and ethyl (**39q**) groups (compare with fluoro (**40r**) and methyl (**38r**) analogues), underlines the importance of the size of the substituents at position para, to more efficiently interact with the amino acid residues lining the hydrophobic NNIBS. Interestingly, *N*-(4-chloro- and 4-nitro)phenyl derivatives **41c**,**g**,**q**−**t** and **45q**,**r** show that, unlike what was found with analogues of **17c**, the optimization of antiviral activity of the phthalimidyl derivatives, can also depend on modifications of the acyl moiety (compare the potency of **41c** with those of **41g**, **41q**−**t**, and the cytotoxicity of **41q** and **45q** with those of **41r** and **45r**, respectively). Thus, the trend of the potency is in the order: heteroaroyl > 4-chlorobenzoyl > benzoyl, having the 2-furoyl, 2-thenoyl, and 2- and 6-chloro-substituted nicotinoyl derivatives approximately the same range of EC_{50} values (0.005−0.01 μM).

To determine whether the title compounds targeted HIV-1 RT, derivatives **41q**,**r**,**s** were tested in enzyme assays against the HIV-1 virionic RT (vRT). Trovirdine and Nevirapine were used as reference drugs (Table 4). All test compounds showed IC_{50}s in the micromolar range. In particular, Trovirdine and Nevirapine resulted 200-fold and 40-fold less active than in enzyme assays using recombinant RT (rRT),[18] thus making more

Table 3. Cytotoxicity and HIV-1 Activity of *O*-(2-Phthalimidoethyl) ATCs[a]



compd	R	acyl	CC_{50}[b]	EC_{50}[c]	SI[d]
25c	H	benzoyl	>200	0.4	>500
26r	2-CH_3	2-thenoyl	>100	11	>9
27r	2-C_2H_5	2-thenoyl	≥100	11	≥9
28r	2-Cl	2-thenoyl	>100	11	9
29r	2-OCH_3	2-thenoyl	>100	11	>9
30r	3-CH_3	2-thenoyl	>100	10	>10
31r	3-CF_3	2-thenoyl	>100	11	>9
32r	3-$COCH_3$	2-thenoyl	>100	6	>17
33r	3-F	2-thenoyl	>100	11	>9
34r	3-Cl	2-thenoyl	>100	6	>17
35r	3-Br	2-thenoyl	53	1.2	44
36r	3-NO_2	2-thenoyl	100	0.38	263
37r	3-OCH_3	2-thenoyl	>100	3.5	>28
38r	4-CH_3	2-thenoyl	>100	0.4	>250
39q	4-C_2H_5	2-furoyl	>100	0.03	>3333
40r	4-F	2-thenoyl	>100	0.1	>1000
41c	4-Cl	benzoyl	≥200	0.1	≥2000
41g	4-Cl	4-chlorobenzoyl	>100	0.025	>4000
41q	4-Cl	2-furoyl	41	0.007	5857
41r	4-Cl	2-thenoyl	>200	0.008	>25000
41s	4-Cl	2-chloronicotinoyl	>200	0.005	>40000
41t	4-Cl	6-chloronicotinoyl	>200	0.006	>33333
42c	4-Br	benzoyl	>200	0.035	>5714
43r	4-I	2-thenoyl	18	0.01	1800
44r	4-N(C_2H_5)$_2$	2-thenoyl	>100	11	9
45q	4-NO_2	2-furoyl	18	0.008	2250
45r	4-NO_2	2-thenoyl	168	0.01	16800
46t	4-O C_2H_5	6-chloronicotinoyl	>100	0.5	>200
Trovirdine			60	0.02	3000

[a−d] See legend to Table 2.

Table 4. Activity of Selected ATCs in Enzyme Assays against Virion-Associated RT (vRT)

compd	IC_{50}[a]
41q	2.3
41r	2.5
41s	2.0
Nevirapine	6.0
Trovirdine	4.2

[a] Compound concentration [μM] required to inhibit the HIV-1 virion-associated RT (vRT) activity by 50%.

Table 5. EC_{90} Values of Selected ATCs against wt HIV-1

compd	EC_{90}[a]
25c	0.8
41c	0.13
41g	0.02
41q	0.005
41r	0.008
41s	0.005
41t	0.005
45q	0.005
Trovirdine	0.015

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

evident the difference between the potency of the compounds in enzyme and cell-based assays. Several factors could account for this result. The first one is the profound difference between vRT and rRT. The former is a p51-p66 dimer engaged in complex interactions with the viral genome and core proteins that may not become totally disrupted during virion lysis, thus affecting binding of NNRTI to the NNIBS. The latter is generally a p66-p66 dimer that may be far from adequately mimicking the native enzyme.

Table 6. Anti HIV-1 Acitivity of Selected ATCs against Y181C Resistant Mutant

compd	EC_{50}[c]
25c	>100
41c	>100
41g	11.6
41q	1.7
41r	1.0
41s	1.4
41t	1.4
45q	14
45r	58
Trovirdine	1.2

[c] See legend to Table 2.

But, a second explanation could also account for the considerable difference between the EC_{50}s and IC_{50}s of Trovirdine and the title compounds. That is, the possibility that they are slow binding RT inhibitors.[45] As a matter of fact, unlike what happened with Nevirapine, a 30 min preincubation of Trovirdine and ATCs with RT reduced the IC_{50}s of the latter by 8−15-fold, thus suggesting slow binding kinetics of these inhibitors to vRT (results not shown). Further studies are in progress to determine association/dissociation kinetics of test compounds to RT. Nevertheless, taken together with those related to the lack of inhibitory effect of title derivatives against HIV-2 (data not shown), data in Table 4 suggest that ATCs target the HIV-1 RT.

The most active derivatives, whose potency was confirmed by EC_{90} values (Table 5), were also tested in cell-based assays against HIV-1 strains carrying some clinically relevant NNRTI mutations[16,44] (K103R, Y181C, and K103N+Y181C). While the K103R and K103N+Y181C mutant strains proved unsusceptible to ATC derivatives and Trovirdine (results not shown), the Y181C mutant was inhibited by derivatives **41q−t** (Table 6). The potency of ATCs against this mutant was in the same range (micromolar) as that of Trovirdine and correlates with the presence of the heteroaryl moieties in the acyl portion.

On one hand, these results highlight the low capability of phthalimidyl ATCs to adapt to the shape of the NNIBS of HIV-1 strains carrying crucial NNRTI resistance mutations (K103R/K103N). On the other hand, their capability to inhibit the Y181C mutant suggests that a suitable design of the acyl moiety could further improve the potency and, hopefully, the spectrum of ATCs.

Computer-Aided Ligand Design. A computational simulation of the binding of **17c** and **25c** into the allosteric binding site of HIV-1 RT was performed by a molecular docking procedure. The models were derived as follows. The low energy conformers of **17c** and **25c**, obtained by Monte Carlo random search, were docked (Dock 4.0) in the NNIBS. The coordinates of the crystal structure of PETT-1 (Chart 1, c) with HIV-1 RT (pdb code 1DTQ)[30] were employed after erasing PETT-1 from the complex. The resulting ATC/RT complexes were energy minimized by simulated annealing, followed by Powell minimization. Some features of these models (Figure 1A and 1B) are worthy to be outlined.

Figure 1A shows that the phenyl ring of the portion 1 of **17c**, located at the top of the binding site, makes van der Waals interactions with the Gly190, Tyr188, and Val106 side chains, while the linker region is in

close contact with Lys101. Hydrophobic contacts are likely established between: (i) the phenyl ring of portion 2 and Pro176 and Lys101; (ii) the phenyl ring of portion 3 and Lys101, Glu138, and Ile135 (from the p51 subdomain). The sulfur atom is probably involved in a hydrogen bond with the Lys103 side chain, whereas the ether oxygen seems to have a polar interaction with the Glu138 (p51) carboxyl group.

Interestingly, Figure 1B shows that **25c** lies along the NNIBS in opposite direction to that of **17c**, with the phthalimide moiety that locks access to the pocket. The *N*-phenyl ring is likely involved in van der Waals interactions with Val179, Tyr181, Tyr188, and Gly190, whereas the phenyl ring of the benzoyl group makes hydrophobic contacts with Val106 and Lys103. Also the ethyl linker would be in close contact with the latter residue. Notably, the model shows the formation of two potential intermolecular hydrogen bonds: the former between the sulfur and the Lys101 main chain amido group, the latter between one of the imidic oxygen atoms and the side chain of Lys103.

This additional H-bond might explain not only the higher antiviral activity of **25c** with respect to **17c** through a more stable interaction with RT, but also its reverse binding mode to the NNIBS. In fact, if their binding modes were similar, the phthalimide scaffold should be located at the top of the RT pocket, as above said for the phenyl ring of portion 1 of **17c**. As a consequence, this moiety would not be suitably situated to form the H-bond with Lys103. Comparison of the conformations of the RT-ATC complexes with that of PETT-1[30] (Figure 1) reveals that the title compounds do not approximate the butterfly-like structure commonly observed with other NNRTI.[15,23−30] Thus, while the heteroaromatic rings of PETT-1 are positioned approximately at right angles with the ethyl spacer folded in a constrained, nonextended conformation, in the ATC derivatives the ethyl spacer would seem to be completely extended with the *N*-phenyl and *N*-benzoyl moieties positioned at nearly 90°. Likely, the uncommon binding mode of ATCs depends on the presence of the acyl moiety, as it can be inferred from molecular modeling studies performed on the parent thiocarbamates (such as **10**), whose modeled structures more closely resemble PETT derivatives by assuming the typical butterfly-like conformation (the data will be reported in a subsequent paper). Support for some of the interactions suggested by the two docking models can be derived from the reported SARs.

As above said, the model of **17c** clarifies the role of the ether oxygen, whose removal does not affect the anti-HIV-1 activity (see derivatives **15**). In the NNRTI field, it has been observed that analogues containing minor structural changes can interact with RT in similar ways, although the specific interactions with protein and the shape of the allosteric site may be different.[26,46,47] This might be the case in the ATC series.

Interestingly, the docking model offers an explanation of the lack of activity of thioisostere **23c**: in that, it is unable to assume the hypothesized extended conformation of the lead compound **17c** (data not shown).

Inspection of the RT/**25c** complex revealed that the *N*-phenyl ring is hosted in a subpocket, made up by the side chains of Val179, Ile180, Tyr181, and Tyr188. Notably, these residues are involved through prominent van der Waals and π-π stacking interactions, with the π-electron systems located in the butterfly wings of NNRTI conformation.[10,30] We hypothesized that an efficient occupancy of this region by strategically designed aromatic substituents, preferably at position para, should yield more potent anti HIV-1 agents with higher affinity for the RT binding pocket. Thus, a chlorine atom at position para of the *N*-phenyl ring (**41c**) would give additional contacts with Val179, Ile180, Tyr181, and Tyr188. As a matter of fact, the model shows that most of the above interactions are lost when the chlorine atom is shifted from position para to position meta or ortho of the *N*-phenyl ring. On the other hand, if an additional chlorine is introduced at the para position of the phenyl ring of the benzoyl group (**41g**), it would provide further hydrophobic contacts with Phe227, Trp229, and Leu234. Actually, the analogues **41c** and **41g** were 4- and 16-fold more active than **25c**, respectively, while **28r** and **34r** were less active (Table 3). Moreover, the high potency of *p*-bromo **(42c)**, *p*-iodo **(43r)**, *p*-nitro **(45q**,**r)**, *p*-ethyl **(39q)**, and 2- and 6-chloronicotinoyl analogues **(41s**,**t)** suggests that there is a wide sterically allowed usable space in the regions hosting the *N*-phenyl and the (hetero)aroyl moieties. These observations could provide the basis for further structural modifications of portions 2 and 3.

Notably, the reverse binding mode of **25c** compared to **17c**, can well justify the remarkable difference in potency between the phenoxyethyl- and phthalimidoethyl-ATCs.

Finally, the interaction model proposed for **25c** helps also to rationalize the dramatic activity decrease of ATCs against NNRTI resistant HIV-1 strains. Thus, the resistance of ATCs to the K103R mutant is consistent with the hydrogen bond donor role proposed for the Lys103 side chain to one of the imidic oxygens. Even if the mutation of Lys to Arg does not prevent the possibility of hydrogen bonding, lengthening of the side chain to that of bulkier Arg is likely to reduce this interaction.

The significant activity still shown by derivatives **41r**,**s**,**t** against the Y181C mutant (although it is 125-, 280-, 233-fold lower than that against wt HIV-1, respectively) emphasizes the role of Tyr181 in the binding to phthalimidyl ATCs. According to the model, one of the most significant hydrophobic contacts may be through the $\pi-\pi$ stacking interactions of Tyr181 and the *N*-phenyl ring. Mutation of Tyr181 to smaller nonaromatic Cys would abolish most of these interactions.

Conclusion

In conclusion, in this preliminary study, we described a novel, very potent, and selective HIV-1 NNRTI class, which however, is poorly effective against Y181C mutant and totally ineffective against K103N mutant strains. Extension of the illustrated SAR strategy to identify additional activity parameters in the ATC series to overcome resistance, is ongoing.

Experimental Section

All the building blocks used are commercially available. Alcohols (ethanol, 1-adamantanol, benzyl alcohol, 1-phenethyl alcohol, 2-phenoxyethanol, (±)-1-phenoxy-2-propanol, 2-phenylthioethanol, *N*-(2-hydroxyethyl)phthalimide), isothiocyan-

ates, acyl chlorides, chloroformates, and reagents (60% sodium hydride dispersion in mineral oil, TMEDA) were purchased by Chiminord and Aldrich Chemical, Milan (Italy). Solvents were reagent grade (toluene, THF, DMF, pyridine). DMF was dried on molecular sieves (5 Å, 1/16 in. pellets). Unless otherwise stated, all commercial reagents were used without further purification.

Organic solutions were dried over anhydrous sodium sulfate and evaporated using a rotatory evaporator operating at reduced pressure of about 10−20 Torr.

Thin-layer chromatography system for routine monitoring the course of reactions and confirming the purity of analytical samples employed aluminum-backed silica gel plates (Merck DC−Alufolien Kieselgel 60 F_{254}): chloroform was used as a developing solvent and detection of spots was made by UV light and/or by iodine vapors.

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 398 spectrometer as KBr disks.

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or $DMSO_{6d}$ on a Varian Gemini 200 instrument, chemical shifts were reported in δ (ppm) units relative to the internal reference tetramethylsilane. Coupling constant values were given in hertz. Elemental analyses were performed by an EA1110 Analyzer, Fison Instruments (Milan), and were within ±0.4% of theoretical values.

The synthesis of Trovirdine was accomplished according to the published procedure.[19]

General One-Pot Procedure P_1 for the Preparation of *N*-Acylthiocarbamates 11c,q; 13q; 14c; 15b,c,g,q; 16c; 17a−d,g,j,k,m,n,o,q,r; 18c; 19a,c,h,p,q,r; 20r; 21r; 22r; 23c. Sodium hydride dispersion (60%) in mineral oil (0.44 g, ∼10 mmol) was added in a single portion to a stirred solution (15 mL) of starting alcohol (**1**, **5**, **6**, **7**, **8**) (10 mmol) in anhydrous toluene or in THF (for **3** and **4**) at room temperature. As soon as the hydrogen evolution ceased, isothiocyanate (phenyl-, cyclohexyl-, *p*-fluorophenyl isothiocyanate, 10 mmol) and anhydrous pyridine (5 mL) were added to the reaction mixture, after 5 and 15 min, respectively. Then, proper freshly distilled or recrystallized acyl chloride (11 mmol) was added in a single portion to the reaction mixture after 15 min, prolonging stirring for 6 h. After dilution with water (200 mL), the mixture was extracted with diethyl ether. The combined extracts were washed with water (30 mL × 2), 2 M HCl (30 mL × 2), and 1 M $NaHCO_3$ (30 mL), dried, and filtered through a pad of Florisil (diameter 5 × 2 cm). Evaporating in vacuo gave a residue which was crystallized from suitable solvent or solvent mixture.

Isolation of *O*-(2-Phenoxyethyl)phenylthiocarbamate Intermediate 10. Sodium hydride dispersion (60%) in mineral oil (0.44 g,∼10 mmol) was added in a single portion to a stirred solution (15 mL) of **6** (1.38 g, 10 mmol) in anhydrous THF. As soon as the hydrogen evolution is ceased, phenyl isothiocyanate (1.35 g, 10 mmol) was added to the mixture at room temperature, prolonging stirring for 30 min. Evaporation in vacuo gave a residue which was treated with water (20 mL) and 1 M HCl (10 mL). Then, the mixture was extracted with diethyl ether, and the combined organic layers were washed with water and 5% $NaHCO_3$ (20 mL × 2) and dried. The organic layer was evaporated under reduced pressure, and the oily residue was crystallized from methanol-diethyl ether to afford **10** (2.05 g, 75%), mp 104−106 °C; IR (KBr) cm^{-1} 3170, 1550, 1345; ^{1}H NMR ($CDCl_3$) δ 4.27 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.91 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.68−7.55 (m, 10H, arom H), 8.82 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 66.19, 115.23, 121.85, 129.55, 130.13, 158.92 (C−N), 188.62 (C═S). Anal. ($C_{15}H_{15}NO_2S$) C, H, N, S.

***O*-(Ethyl) benzoyl(phenyl)thiocarbamate (11c):** IR (KBr) cm^{-1} 1690; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7 Hz, 3H, CH_3), 4.32 (q, J = 7 Hz, 2H, CH_2), 7.10−8.10 (m, 10H, arom H).

***O*-(Ethyl) 2-furoyl(phenyl)thiocarbamate (11q):** IR (KBr) cm^{-1} 1690; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, J = 7 Hz, 3H, CH_3), 4.51 (q, J = 7 Hz, 2H, CH_2), 6.48−6.67 (m, 1H, H-4 fur.), 7.11−7.70 (m, 7H, 5 arom H and H-3, H-5 fur.).

***O*-(Benzyl) 2-furoyl(phenyl)thiocarbamate (13q):** IR (KBr) cm^{-1} 1695; ^{1}H NMR ($CDCl_3$) δ 5.52 (s, 2H, CH_2), 6.37−6.65 (m, 1H, H-4 fur.), 7.04−7.53 (m, 13H, 10 arom H and H-3, H-5 fur.).

***O*-(2-Furylmethyl) benzoyl(phenyl)thiocarbamate (14c):** IR (KBr) cm^{-1} 1685; ^{1}H NMR ($CDCl_3$) δ 5.30 (s, 2H, CH_2), 6.09−6.40 (m, 2H, H-3 and H-4 fur.), 7.18−8.15 (m, 11H, 10 arom H and H-5 fur.).

***O*-(2-Phenethyl) (*E*)-cinnamoyl(phenyl)thiocarbamate (15b):** IR (KBr) cm^{-1} 1685, 1615; ^{1}H NMR ($CDCl_3$) δ 2.98 (t, J = 6.5 Hz, 2H, CH_2), 4.74 (t, J = 6.5 Hz, 2H, CH_2O), 6.80−7.95 (m, 17H, 15 arom H and 2H/cinnamoyl).

***O*-(2-Phenethyl) benzoyl(phenyl)thiocarbamate (15c):** IR (KBr) cm^{-1} 1680; ^{1}H NMR ($CDCl_3$) δ 2.53 (t, J = 6.5 Hz, 2H, CH_2O), 4.48 (t, J = 6.5 Hz, 2H, CH_2O), 6.79−8.06 (m, 15H, arom H).

***O*-(2-Phenethyl) 4-chlorobenzoyl(phenyl)thiocarbamate (15g):** IR (KBr) cm^{-1} 1685; ^{1}H NMR ($CDCl_3$) δ 2.68 (t, J = 6.5 Hz, 2H, CH_2), 4.55 (t, J = 5 Hz, 2H, CH_2O), 6.59−7.98 (m, 14H, arom H).

***O*-(2-Phenethyl) 2-furoyl(phenyl)thiocarbamate (15q):** IR (KBr) cm^{-1} 1678; ^{1}H NMR ($CDCl_3$) δ 2.84 (t, J = 6.5 Hz, 2H, CH_2), 4.66 (t, J = 6.5 Hz, 2H, CH_2O), 6.42 (m, 1H, H-4 fur.), 6.80−7.65 (m, 12H, 10 arom H and H-3, H-5 fur.).

***O*-(2-Phenoxyethyl) benzoyl(cyclohexyl)thiocarbamate (16c):** IR (KBr) cm^{-1} 1700; ^{1}H NMR ($CDCl_3$) δ 0.71−2.26 (m, 10H, 5 CH_2/cyclohexyl), 3.72 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.62 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 4.60−5.13 (m, 1H, CH/cyclohexyl), 6.63−7.98 (m, 10H, arom H).

***O*-(2-Phenoxyethyl) 2-phenoxyacetyl(phenyl)thiocarbamate (17a):** IR (KBr) cm^{-1} 1730; ^{1}H NMR ($CDCl_3$) δ 4.30 (t, J = 5.6 Hz, 2H, CH_2/β-H phenoxyethyl), 4.88 (t, J = 5.6 Hz, 2H, CH_2/α-H phenoxyethyl), 6.70−7.60 (m, 15H, arom H).

***O*-(2-Phenoxyethyl) (E)-Cinnamoyl(phenyl)thiocarbamate (17b):** IR (KBr) cm^{-1} 1690, 1615; ^{1}H NMR ($CDCl_3$) δ 4.26 (t, J = 5.6 Hz, 2H, CH_2/β-H phenoxyethyl), 4.95 (t, J = 5.6 Hz, 2H, CH_2/α-H phenoxyethyl), 6.65−8.03 (m, 17H, 15 arom H and 2H/cinnamoyl).

***O*-(2-Phenoxyethyl) benzoyl(phenyl)thiocarbamate (17c):** IR (KBr) cm^{-1} 1695; ^{1}H NMR ($CDCl_3$) δ 3.73 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.63 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.63−8.05 (m, 15H, arom H); ^{13}C NMR ($CDCl_3$) δ 65.19, 70.73, 115.07, 121.69, 128.67, 128.88, 129.19, 129.89, 129.95, 133.18, 135.86, 141.86, 141.61, 158.66 (C−N), 172.33 (C═O), 192.13 (C═S).

***O*-(2-Phenoxyethyl) 4-methylbenzoyl(phenyl)thiocarbamate (17d):** IR (KBr) cm^{-1} 1700; ^{1}H NMR ($CDCl_3$) δ 2.29 (s, 3H, CH_3), 3.80 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.70 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.60−7.97 (m, 14H, arom H).

***O*-(2-Phenoxyethyl) 4-chlorobenzoyl(phenyl)thiocarbamate (17g):** IR (KBr) cm^{-1} 1685; ^{1}H NMR ($CDCl_3$) δ 3.85 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.70 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.60−7.95 (m, 14H, arom H).

***O*-(2-Phenoxyethyl) 2-acetoxybenzoyl(phenyl)thiocarbamate (17j):** IR (KBr) cm^{-1} 1765, 1695; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H, acetyl), 3.62 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.66 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.57−7.83 (m, 14H, arom H).

***O*-(2-Phenoxyethyl) 2,4-dichlorobenzoyl(phenyl)thiocarbamate (17k):** IR (KBr) cm^{-1} 1700; ^{1}H NMR ($CDCl_3$) δ 3.68 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.67 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.68−7.62 (m, 13H, arom H).

***O*-(2-Phenoxyethyl) 3,5-dichlorobenzoyl(phenyl)thiocarbamate (17m):** IR (KBr) cm^{-1} 1700; ^{1}H NMR ($CDCl_3$) δ 3.90 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.71 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.60−7.85 (m, 13H, arom H).

***O*-(2-Phenoxyethyl) 4-chloro-3-nitrobenzoyl(phenyl)-thiocarbamate (17n):** IR (KBr) cm^{-1} 1690; ^{1}H NMR ($CDCl_3$) δ 4.15 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.78 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.58−8.46 (m, 13H, arom H).

***O*-(2-Phenoxyethyl) 3,4,5-trimethoxybenzoyl(phenyl)-thiocarbamate (17o):** IR (KBr) cm^{-1} 1695; ^{1}H NMR ($CDCl_3$)

δ 3.70−4.10 (m, 11H, 9H/trimethoxy and 2H/β-H phenoxyethyl), 4.70 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.70−7.60 (m, 12H, arom H).

***O*-(2-Phenoxyethyl) 2-furoyl(phenyl)thiocarbamate (17q):** IR (KBr) cm^{-1} 1675; ^1H NMR ($CDCl_3$) δ 4.09 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.85 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.40−6.59 (m, 1H, H-4 fur.), 6.70−8.15 (m, 12H, 10 arom H and H-3, H-5 fur.).

***O*-(2-Phenoxyethyl) phenyl(thien-2-ylcarbonyl)thiocarbamate (17r):** IR (KBr) cm^{-1} 1675; ^1H NMR ($CDCl_3$) δ 4.09 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.82 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.67−7.84 (m, 13H, 10 arom H and 3H thioph).

***O*-(2-Phenoxyethyl) benzoyl(4-fluorophenyl)thiocarbamate (18c):** IR (KBr) cm^{-1} 1695; ^1H NMR ($CDCl_3$) δ 3.74 (t, J = 5 Hz, 2H, CH_2/β-H phenoxyethyl), 4.64 (t, J = 5 Hz, 2H, CH_2/α-H phenoxyethyl), 6.58−8.10 (m, 14 H, arom H).

(±) *O*-(1-Methyl-2-phenoxyethyl) phenoxyacetyl(phenyl)thiocarbamate (19a): IR (KBr) cm^{-1} 1725; ^1H NMR ($CDCl_3$) δ 1.59 (d, J = 6 Hz, 3H, CH_3), 4.25 (d, J = 5 Hz, 2H, CH_2), 5.15 (s, 2H, OCH_2CO), 5.65−6.22 (m, 1H, CH), 6.35−8.10 (m, 15H, arom H).

(±) *O*-(1-Methyl-2-phenoxyethyl) benzoyl(phenyl)-thiocarbamate (19c): IR(KBr) cm^{-1} 1690; ^1H NMR ($CDCl_3$) δ 1.26 (d, J = 6 Hz, 3H, CH_3), 3.71 (d, J = 5 Hz, 2H, CH_2), 5.42−6.93 (m, 1H, CH), 6.66−8.11 (m, 15H, arom H).

(±) *O*-(1-Methyl-2-phenoxyethyl) 3-nitrobenzoyl(phenyl)thiocarbamate (19h): IR (KBr) cm^{-1} 1705; ^1H NMR ($CDCl_3$) δ 1.25 (d, J = 6 Hz, 3H, CH_3), 3.80 (d, J = 5 Hz, 2H, CH_2), 5.45−6.03 (m, 1H, CH), 6.09−7.78 and 7.94−8.65 (m, 13H, arom H).

(±) *O*-(1-Methyl-2-phenoxyethyl) 1-naphthoyl(phenyl)-thiocarbamate (19p): IR (KBr) cm^{-1} 1680; ^1H NMR ($CDCl_3$) δ 0.74 (d, J = 6 Hz, 3H, CH_3), 3.19 (d, J = 6 Hz, 2H, CH_2), 5.24−5.75 (m, 1H, CH), 6.45−8.55 (m, 17 H, arom H).

(±) *O*-(1-Methyl-2-phenoxyethyl) 2-furoyl(phenyl)-thiocarbamate (19q): IR (KBr) cm^{-1} 1705; ^1H NMR ($CDCl_3$) δ 1.33 (d, J = 6 Hz, 3H, CH_3), 3.98 (d, J = 6 Hz, 2H, CH_2), 5.68−5.99 (m, 1H, CH), 6.41−6.60 (m, 1H, H-4 fur.), 6.71−7.65 (m, 12 H, 10 arom H and H-3, H-5 fur.).

(±) *O*-(1-Methyl-2-phenoxyethyl) phenyl(thien-2-ylcarbonyl)thiocarbamate (19r): IR (KBr) cm^{-1} 1690; ^1H NMR ($CDCl_3$) δ 1.35 (d, J = 6 Hz, 3H, CH_3), 3.95 (d, J = 6 Hz, 2H, CH_2), 5.60−6.08 (m, 1H, CH), 6.70−8.05 (m, 13 H, arom H).

(±) *O*-(1-Methyl-2-phenoxyethyl) 3-chlorophenyl-(thien-2-ylcarbonyl)thiocarbamate (20r): IR (KBr) cm^{-1} 1687; ^1H NMR ($CDCl_3$) δ 1.33 (d, J = 6 Hz, 3H, CH_3), 3.95 (d, J = 6 Hz, 2H, CH_2), 5.62−5.97 (m, 1H, CH), 6.50−7,85 (m, 12 H, 9 arom H and 3H thioph).

(±) *O*-(1-Methyl-2-phenoxyethyl) 4-chlorophenyl-(thien-2-ylcarbonyl)thiocarbamate (21r): IR (KBr) cm^{-1} 1685; ^1H NMR ($CDCl_3$) δ 1.32 (d, J = 6 Hz, 3H, CH_3), 3.95 (d, J = 6 Hz, 2H, CH_2), 5.55−5.96 (m, 1H, CH), 6.68−7,82 (m, 12 H, 9 arom H and 3H thioph).

(±) *O*-(1-Methyl-2-phenoxyethyl) 4-nitrophenyl(thien-2-ylcarbonyl)thiocarbamate (22r): IR (KBr) cm^{-1} 1697; ^1H NMR ($CDCl_3$) δ 1.33 (d, J = 6 Hz, 3H, CH_3), 3.98 (d, J = 6 Hz, 2H, CH_2), 5.70−6.06 (m, 1H, CH), 6.73−7.88 and 7.98−8.43 (m, 12 H, 9 arom H and 3H thioph).

***O*-(2-Phenylsulfanylethyl) benzoyl(phenyl)thiocarbamate (23c):** IR (KBr) cm^{-1} 1695; ^1H NMR ($CDCl_3$) δ 2.69 (t, J = 7 Hz, 2H, CH_2/β-H phenoxyethyl), 4.42 (t, J = 7 Hz, 2H, CH_2/α-H phenoxyethyl), 7.08−8.05 (m, 15 H, arom H).

Synthesis of *O*-(Ethyl) Benzoyl(phenyl)thiocarbamate 11c Starting from *O*-(Ethyl) Phenylthiocarbamate. Neat benzoyl chloride (1.54 g, 11 mmol) was added in a single portion to a solution of *O*-(ethyl) phenylthiocarbamate[41] (1.81 g, 10 mmol) in pyridine (15 mL) at room temperature. The resulting mixture was stirred overnight at room temperature and treated with water (50 mL) and 4 M HCl (80 mL). The solid precipitated was extracted with ether, and the combined extracts were washed with water. After drying, solvent was evaporated under reduced pressure. The residue was crystallized from diethyl ether−petroleum ether to yield **11c** (1.71 g, 60%).

General One-Pot Procedure P_2 for the Preparation of *O*-(1-Adamantyl) Acyl(phenyl)thiocarbamates 12c,q. Sodium hydride dispersion (60%) in mineral oil (0.44 g, ∼10 mmol) was added to a stirred anhydrous DMF (20 mL) solution of 1-adamantanol (1.53 g, 10 mmol) at room temperature. After heating at 90−95 °C for 20 min, an anhydrous DMF (5 mL) solution of phenyl isothiocyanate (1.35 g, 10 mmol) was added to the reaction mixture, prolonging heating and stirring for 30 min. Then, anhydrous pyridine (5 mL) and proper acyl chloride (12 mmol) were added in a single portion to the reaction mixture, cooled at room temperature. The resulting mixture was stirred for 6 h at room temperature and then heated at 55 °C for 1 h. The solid or liquid phase, separated upon dilution with water (200 mL), was extracted with dichloromethane. The organic layer was washed with water (30 mL × 5), dried, filtered through a pad of Florisil (diameter 5 × 2 cm), and evaporated in vacuo to yield a residue which was crystallized from suitable solvent mixture.

***O*-(1-Adamantyl) benzoyl(phenyl)thiocarbamate (12c):** IR (KBr) cm^{-1} 1690; ^1H NMR ($CDCl_3$) δ 1.30−1.71 (m, 6H, $3CH_2$), 1.82−2.25 (m, 9H, $3CH_2$ and 3CH), 7.18−8.16 (m, 10H, arom H).

***O*-(1-Adamantyl) 2-furoyl(phenyl)thiocarbamate (12q):** IR (KBr) cm^{-1} 1685; ^1H NMR ($CDCl_3$) δ 1.48−1.85 (m, 6H, $3CH_2$), 2.00−2.40 (m, 9H, $3CH_2$ and 3CH), 6.58−6.67 (m, 1H, H-4 fur.), 6.90−7.98 (m, 7H, 5 arom H, H-3 and H-5 fur.).

General Procedure P_3 for the preparation of 2-phthalimidoethyl acyl(aryl)thiocarbamates 25c, 26r; 27r; 28r; 29r; 30r; 31r; 32r; 33r; 34r; 35r; 36r; 37r; 38r; 39q; 40r; 41c,g,q−t, 42c, 43r; 44r; 45q,r; 46t. Sodium hydride dispersion (60%) in mineral oil (0.44 g, ∼10 mmol) was added in a single portion to a stirred, ice-cooled solution of **9** (1.91 g, 10 mmol) and of the proper isothiocyanate (10 mmol) in anhydrous pyridine (25 mL). The reaction mixture was allowed to react for 4 h under stirring, and then TMEDA (1.74 g, 15 mmol) and proper acyl chloride (12 mmol) were added. The resulting mixture was stirred at room temperature for 6 h, treated with water (150 mL), and extracted with dichloromethane. (In the preparation of ortho-derivatives **26r**−**29r**, the reaction mixture was heated at 55−60 °C for 3 h, before workup). The organic layer was washed with water (four times) and 1 M HCL (20 mL × 4), dried, filtered through a plug of Florisil, and evaporated under reduced pressure to give a residue which was purified by crystallization.

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] benzoyl(phenyl)thiocarbamate (25c):** IR (KBr) cm^{-1} 1775, 1710, 1685; ^1H NMR ($CDCl_3$) δ 3.81 (t, J = 6 Hz, 2H, CH_2N), 4.62 (t, J = 6 Hz, 2H, CH_2O), 7.23−7.56 and 7.63−8.03 (m, 14H, arom H).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] thien-2-ylcarbonyl(2-tolyl)thiocarbamate (26r):** IR (KBr) cm^{-1} 1770, 1712; ^1H NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.97 (t, J = 6 Hz, 2H, CH_2N), 4.76 (t, J = 6 Hz, 2H, CH_2O), 6.95−7.98 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-ethylphenyl(thien-2-ylcarbonyl)thiocarbamate (27r):** IR (KBr) cm^{-1} 1770, 1714, 1671; ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7 Hz, 3H, CH_3/ethyl), 2.62 (q, 2H, CH_2/ethyl), 3.93 (t, J = 6 Hz, 2H, CH_2N), 4.72 (t, J = 6 Hz, 2H, CH_2O), 6.25−7.95 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-chlorophenyl(thien-2-ylcarbonyl)thiocarbamate (28r):** IR (KBr) cm^{-1} 1770, 1715, 1678; ^1H NMR ($CDCl_3$) δ 3.99 (t, J = 6 Hz, 2H, CH_2N), 4.77 (t, J = 6 Hz, 2H, CH_2O), 6.90−7.10 (m, 1H, H-4 thioph) 7.25−8.05 (m, 10H, 8 arom H, H-3 and H-5 thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-methoxyphenyl(thien-2-ylcarbonyl)thiocarbamate (29r):** IR (KBr) cm^{-1} 1770, 1715, 1678; ^1H NMR ($CDCl_3$) δ 3.77 (s, 3H,

CH_3O), 3.95 (t, J = 6 Hz, 2H, CH_2N), 4.75 (t, J = 6 Hz, 2H, CH_2O), 6.76−7.97 (m, 11H, 8 arom H and 3H thioph).

O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]thien-2-ylcarbonyl(3-tolyl)thiocarbamate (30r): IR (KBr) cm^{-1} 1775, 1714, 1693; ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H, CH_3), 3.98 (t, J = 6 Hz, 2H, CH_2N), 4.78 (t, J = 6 Hz, 2H, CH_2O), 6.85−7.33 and 7.40−7.98 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3-trifluoromethylphenyl(thien-2-ylcarbonyl)thiocarbamate (31r):** IR (KBr) cm^{-1} 1777, 1713 1698; ^1H NMR ($CDCl_3$) δ 3.97 (t, J = 6 Hz, 2H, CH_2N), 4.73 (t, J = 6 Hz, 2H, CH_2O), 6.96−7.07 (m, 1H, H-4 thioph), 7.42−7.88 (m, 10H, 8 arom H, H-3 and H-5 thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]3-acetylphenyl(thien-2-ylcarbonyl)thiocarbamate (32r):** IR (KBr) cm^{-1} 1775, 1713 1684, 1670; ^1H NMR ($CDCl_3$) δ 2.59 (s, 3H, CH_3), 4.02 (t, J = 6 Hz, 2H, CH_2N), 4.78 (t, J = 6 Hz, 2H, CH_2O), 6.90−7.12 (m, 1H, H-4 thioph), 7.45−8.11 (m, 10H, 8 arom H, H-3 and H-5 thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3-fluorophenyl(thien-2-ylcarbonyl)thiocarbamate (33r):** IR (KBr) cm^{-1} 1770, 1710; ^1H NMR ($CDCl_3$) δ 3.98 (t, J = 6 Hz, 2H, CH_2N), 4.78 (t, J = 6 Hz, 2H, CH_2O), 6.87−7.95 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]3-chlorophenyl(thien-2-ylcarbonyl)thiocarbamate (34r):** IR (KBr) cm^{-1} 1770, 1711; ^1H NMR ($CDCl_3$) δ 3.97 (t, J = 6 Hz, 2H, CH_2N), 4.73 (t, J = 6 Hz, 2H, CH_2O), 6.98−7.04 and 7.51−7.59 (m, 2H, H-4 and H-5 thioph), 7.23−7.42 and 7.70−7.88 (m, 9H, 8 arom H and H-3 thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]3-bromophenyl(thien-2-ylcarbonyl)thiocarbamate (35r):** IR (KBr) cm^{-1} 1778, 1713, 1695; ^1H NMR ($CDCl_3$) δ 4.00 (t, J = 6 Hz, 2H, CH_2N), 4.78 (t, J = 6 Hz, 2H, CH_2O), 6.85−7.97 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 3-nitrophenyl(thien-2-ylcarbonyl)thiocarbamate (36r):** IR (KBr) cm^{-1} 1774, 1709; ^1H NMR ($CDCl_3$) δ 3.97 (t, J = 6 Hz, 2H, CH_2N), 4.77 (t, J = 6 Hz, 2H, CH_2O), 6.96−8.32 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]3-methoxyphenyl(thien-2-ylcarbonyl)thiocarbamate (37r):** IR (KBr) cm^{-1} 1776, 1714, 1677; ^1H NMR ($CDCl_3$) δ 3.76 (s, 3H, CH_3O), 4.00 (t, J = 6 Hz, 2H, CH_2N), 4.78 (t, J = 6 Hz, 2H, CH_2O), 6.70−7.89 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]thien-2-ylcarbonyl(4-tolyl)thiocarbamate (38r):** IR (KBr) cm^{-1} 1775, 1716, 1687; ^1H NMR ($CDCl_3$) δ 3.99 (t, J = 6 Hz, 2H, CH_2N), 4.77 (t, J = 6 Hz, 2H, CH_2O), 6.85−8.01 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]4-ethylphenyl(2-furoyl)thiocarbamate (39q):** IR (KBr) cm^{-1} 1770, 1714, 1682; ^1H NMR ($CDCl_3$) δ 1.21 (t, J = 7 Hz, 3H, CH_3/ethyl), 2.59 (q, J = 7 Hz, 2H, CH_2/ethyl), 3.98 (t, J = 6 Hz, 2H, CH_2N), 4.77 (t, J = 6 Hz, 2H, CH_2O), 6.25−6.50 (m, 1H, H-4 fur.), 7.05−8.01 (m, 10H, 8 arom H, H-3 and H-5 fur.).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-fluorophenyl(thien-2-ylcarbonyl)thiocarbamate (40r):** IR (KBr) cm^{-1} 1777, 1718; ^1H NMR ($CDCl_3$) δ 4.08 (t, J = 6 Hz, 2H, CH_2N), 4.78 (t, J = 6 Hz, 2H, CH_2O), 6.80−7.98 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] benzoyl(4-chlorophenyl)thiocarbamate (41c):** IR (KBr) cm^{-1} 1778, 1710; ^1H NMR ($CDCl_3$) δ 3.80 (t, J = 6 Hz, 2H, CH_2N), 4.65 (t, J = 6 Hz, 2H, CH_2O), 7.17−7.57 and 7.66−8.02 (m, 13H, arom H).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]4-chlorobenzoyl(4-chlorophenyl)thiocarbamate (41g):** IR (KBr) cm^{-1} 1775, 1710; ^1H NMR ($CDCl_3$) δ 3.88 (t, J = 6 Hz, 2H, CH_2N), 4.65 (t, J = 6 Hz, 2H, CH_2O), 7.20−8.26 (m, 12H, arom H).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]4-chlorophenyl(2-furoyl)thiocarbamate (41q):** IR (KBr) cm^{-1} 1780, 1710; ^1H NMR ($CDCl_3$) δ 3.95 (t, J = 6 Hz, 2H, CH_2N), 4.63 (t, J = 6 Hz, 2H, CH_2O), 6.30−6.50 (m, 1H, H-4 fur.), 7.01−7.97 (m, 10H, 8 arom H, H-3 and H-5 fur.).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]4-chlorophenyl(thien-2-ylcarbonyl)thiocarbamate (41r):** IR (KBr) cm^{-1} 1775, 1710; ^1H NMR ($CDCl_3$) δ 3.98 (t, J = 6 Hz, 2H, CH_2N), 4.76 (t, J = 6 Hz, 2H, CH_2O), 6.90−7.98 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]2-chloronicotinoyl(4-chlorophenyl)thiocarbamate (41s):** IR (KBr) cm^{-1} 1778, 1710, 1667; ^1H NMR ($DMSO_{6d}$) δ 3.73 (t, J = 6 Hz, 2H, CH_2N), 4.57 (t, J = 6 Hz, 2H, CH_2O), 7.10−8.62 (m, 11H, 8 arom H and 3H pyr).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]6-chloronicotinoyl(4-chlorophenyl)thiocarbamate (41t):** IR (KBr) cm^{-1} 1777, 1709; ^1H NMR ($CDCl_3$) δ 3.81 (t, J = 6 Hz, 2H, CH2N), 4.64 (t, J = 6 Hz, 2H, CH2O), 7.20−8.92 (m, 11H, 8 arom H and 3H pyr).

O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] benzoyl(4-bromophenyl)thiocarbamate (42c): IR (KBr) cm-1 1775, 1710; 1H NMR (CDCl3) δ 3.77 (t, J = 6 Hz, 2H, CH_2N), 4.61 (t, J = 6 Hz, 2H, CH2O), 7.05−8.00 (m, 13H, 8 arom H).

O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-iodophenyl(thien-2-ylcarbonyl)thiocarbamate (43r): IR (KBr) cm-1 1776, 1711; 1H NMR (CDCl3) δ 3.95 (t, J = 6 Hz, 2H, CH2N), 4.71 (t, J = 6 Hz, 2H, CH2O), 6.85−7.22 and 7.45−7.98 (m, 11H, 8 arom H and 3H thioph).

O-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-diethylaminophenyl(thien-2-ylcarbonyl)thiocarbamate (44r): IR (KBr) cm^{-1} 1775, 1710; ^1H NMR ($CDCl_3$) δ 1.12 (t, J = 6 Hz, 6H, $2CH_3$/diethyl), 3.34 (q, J = 6 Hz, 4H, $2CH_2$/diethyl), 4.08 (t, J = 6 Hz, 2H, CH_2N), 4.79 (t, J = 6 Hz, 2H, CH_2O), 6.40−7.95 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 2-furoyl(4-nitrophenyl)thiocarbamate (45q):** IR (KBr) cm^{-1} 1780, 1710; ^1H NMR ($CDCl_3$) δ 3.95 (t, J = 6 Hz, 2H, CH_2N), 4.63 (t, J = 6 Hz, 2H, CH_2O), 6.30−6.50 (m, 1H, H-3 fur.), 7.01−7.97 (m 10 H, 8 arom H, H-2 and H-4 fur.).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl] 4-nitrophenyl(thien-2-ylcarbonyl)thiocarbamate (45r):** IR (KBr) cm^{-1} 1778; ^1H NMR ($CDCl_3$) δ 4.00 (t, J = 6 Hz, 2H, CH_2N), 4.76 (t, J = 6 Hz, 2 H, CH_2O), 6.87−8.35 (m, 11H, 8 arom H and 3H thioph).

***O*-[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethyl]6-chloronicotinoyl(4-ethoxyphenyl)thiocarbamate (46t):** IR (KBr) cm^{-1} 1773, 1713; ^1H NMR ($CDCl_3$) δ 1.37 (t, J = 7 Hz, 3H, CH_3/ethyl), 3.80−4.08 (m, 4H, CH_2N and CH_2O/ethyl), 4.66 (t, J = 6 Hz, 2H, CH_2O), 6.65−7.40 and 7.52−8.14 (m, 11H, 8 arom H and 3H pyr.).

General One-Pot Procedure P_4 for the Preparation of Compounds 24u,v,w. Sodium hydride dispersion (60%) in mineral oil (0.50 g, ∼12.5 mmol) was added in a single portion to a stirred ice-cooled solution of **7** (1.52 g, 10 mmol) in dry THF. When hydrogen evolution subsided, neat phenyl isothiocyanate (1.35 g, 10 mmol) was added to the resulting mixture at room temperature. After 20 min, *n*-propyl (1.35 g, 11 mmol) or *n*-butyl (1.50 g, 11 mmol) or phenyl (1.72 g, 11 mmol) chloroformate was added to the reaction mixture which was heated at 60 °C for 3 h. After removing THF by evaporation in vacuo, the residue was treated with water (100 mL) and extracted with diethyl ether. The organic layer was dried, filtered through a plug of silica gel, and evaporated under reduced pressure to give an oily residue which was purified by crystallization.

(±) *O*-(1-Methyl-2-phenoxyethyl) phenyl(propoxycarbonyl)thiocarbamate (24u): 2.5 g (67%), mp 81−83 °C (from dichloromethane-ether); IR (KBr) cm-1 1725; 1H NMR ($CDCl_3$) d 0.78 (t, J = 6.5 Hz, 3H, CH_3/propyl), 1.06−2.00 (m, 5H, CH_3 and CH_2/propyl), 3.93−4.32 (m, 4H, $2CH_2O$), 5.71−6.13 (m, 10H, arom H). Anal. ($C_{20}H_{23}NO_4S$) C, H, N, S.

(±) *O*-(1-Methyl-2-phenoxyethyl) butoxycarbonyl(phenyl)thiocarbamate (24v): 2.17 g (56%), mp 78−79 °C (from dichloromethane-ether); IR (KBr) cm^{-1} 1730; ^1H NMR ($CDCl_3$) δ 0.85 (t, J = 6.5 Hz, 3H, CH_3/butyl), 1.05−1.70 (m, 7H, CH_3

and $2CH_2$), 3.95−4.32 (m, 4H, $2CH_2O$), 6.75−7.10 (m, 10H, arom H). Anal. ($C_{21}H_{25}NO_4S$) C, H, N, S.

(±) *O*-(1-Methyl-2-phenoxyethyl) phenoxycarbonyl-(phenyl)thiocarbamate (24w): 1.3 g (64%), mp 96−98 °C; IR (KBr) cm^{-1} 1738; ^1H NMR ($CDCl_3$) δ 1.49 (d, J = 6.5 Hz, 3H, CH_3), 4.12 (d, J = 6.5 Hz, 2H, CH_2), 5.70−6.13 (m, 1H, CH), 6.72−7.60 (m, 15H, arom H). Anal.($C_{23}H_{21}NO_4S$) C, H, N, S.

Molecular Modeling. The structures of **17c** and **25c** were generated and energy minimized using the MM2 force field included in MacroModel.[48] Random conformational analysis was carried out using the Monte Carlo (MC) option (Macromodel). All the rotatable bonds were allowed to freely rotate according to the MM2 parametrization. Starting from a randomly generated **17c** conformer, an MC search was performed obtaining 503 conformations that were clustered into 10 groups using Xcluster program. To explore as well as possible the **17c** conformational space, the less populated cluster, i.e., the high energy conformation, was selected as starting point for a new MC cycle. This strategy was repeated for five times examining a total number of 2447 conformations. The cluster-leaders generated from the fifth MC cycle were superimposed (Macromodel RigidSA option) to the previously found conformers. Visual inspection of the superimposed structures demonstrated that the last conformers have been already found in other MC runs clearly indicating that the random search could be stopped. For each MC cycle the following parameters were defined: automatic setting, the maximum number of search iteractions was 1000, the energy cut off was set to 5 kcal/mol. The 50 lead conformations, representative of each cluster generated for the five MC runs, were searched by cluster analysis to identify the 10 clusters out of which the three low energy conformers were analyzed in the following docking study.

A similar protocol was applied for **25c** generating 4896 conformers in seven MC runs. The final cluster analysis lead to four low energy conformers.

The geometries of **17c** and **25c** low energy conformers were optimized with semiempirical quantum mechanic calculation, using the Hamiltonian AM1 as implemented in MOPAC package version 6.0.[49] The same program was used to assign atom centered partial charges to the obtained conformations. This step drove the conformations to the minimum and allowed an accurate estimation of charge distribution useful for docking studies.

The selected conformers were docked in the NNRTI binding pocket as it was described in the crystal structure of PETT-RT complex solved by Ren et al.[30] at 2.8 Å resolution (PDB code 1DTQ). The docking models were obtained with the DOCK 4.0 program[50] which, through a topological description of the solvent accessible surface of the two interacting entities, provides a series of orientations of the ligands in the receptor RT binding site ranked according to the free energy of the complexes. In particular, starting from the RT-PETT-1 crystal structure, the inhibitor was removed and the region formed by residues located within 10 Å from any non-hydrogen atom of the PETT-1 derivative was topologically described. Hydrogen atoms were added to the ligand-free enzyme (InsightII, biopolymer module), and partial charges were calculated according to CVFF force field.[51] All **17c** and **25c** low energy conformers were docked in the NNRTI binding pocket. Among the tested conformers only one conformation for **17c** and **25c** fit the shape and the volume of the binding cavity. The orientation which provide the low energy complex was selected. DOCK 4.0 is able to perform a rigid body docking, omitting therefore ligand and side chain flexibility in complex formation. To obtain a more reliable model, DOCK4.0 low energy complexes were energy minimized by CNS,[52] performing a cycle of simulated annealing (starting temperature 1000 K) followed by 120 cycles of Powell minimization.

All the calculations were performed in vacuo on Silicon Graphic Indigo2 and Origin 200 workstations. Model analysis were carried out using CCP4 (Collaborative Computational Project Number 4) program suite.

Virology: Materials and Methods

Compounds. The compounds were solubilized in DMSO at an initial concentration of 200 mM and then were diluted in culture medium.

Cells and Viruses. MT-4, C8166, H9/IIIB and CEM cells were grown at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G and 100 μg/mL streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with a Myco Tect (Gibco). HIV-1 strain IIIB and HIV-2 strain ROD, kindly provided by L Montagnier (Paris, France), were obtained from supernatants of persistently infected H9/IIIB and CEM cells, respectively. HIV-1 and HIV-2 stock solutions had titers of 4.5×10^6 and 1.4×10^5 50% cell culture infectious doses ($CCID_{50}$)/mL, respectively. The K103R mutant (which also contains the mutations V179D and P225H) was derived from an III_B strain passaged in C8166 cells in the presence of Efavirenz (up to 2 μM). The Y181C mutant (NIH N119) derives from an AZT-sensitive clinical isolate passaged initially in CEM, and then in MT-4 cells, in the presence of nevirapine (10 μM). The K103N−Y181C (NIH A17) derives from the III_B strain passaged in H9 cells in the presence of BI−RG 587 (1 μM). K103R, Y181C and K103N−Y181C stock solutions had titers of 3.0×10^5 $CCID_{50}$/mL, 1.3×10^6 $CCID_{50}$/mL, and 2.5×10^5 $CCID_{50}$/mL, respectively.

HIV Titration. Titration of HIV was performed in C8166 cells by the standard limiting dilution method (dilution 1:2, four replica wells/dilution) in 96-well plates. The infectious virus titer was determined by light microscope scoring of cytopathogenicity after 4 days of incubation, and the virus titers were expressed as $CCID_{50}$/mL.

Anti-HIV Assays. The activity of the test compounds against multiplication of wt HIV-1, Y181C, and K103N−Y181C in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells. The activity of the compounds against HIV-2 and K103R multiplication in acutely infected cells was based on inhibition of p24 antigen in C8166 cells. Briefly, 50 μL of culture medium containing 1×10^4 cells were added to each well of flat-bottom microtiter trays containing 50 μL of culture medium with or without various concentrations of the test compounds. Then, 20 μL of HIV suspensions (containing the appropriate amount of $CCID_{50}$ to cause complete cytopathogenicity at day 4) were added. After incubation at 37 °C, cell viability was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[53,54] Alternatively, p24 levels were determined by an immunoenzymatic kit (Abbott). The cytotoxicity of compounds was evaluated in parallel with their antiviral activity. It was based on the viability of mock-infected cells, as monitored by the MTT method.

RT Assays. Assays of virion-associated RT (vRT) were performed on detergent-disrupted (0.5% NP-40) preparations of stock virus.

Assays were performed as previously described.[55] Briefly, vRT was assayed for its RNA-dependent polymerase-associated activity in a 50 μL volume containing 50 mM Tris-HCl pH 8.5, 80 mM KCl, 6 mM $MgCl_2$, 10 mM DTT, 250 μg/mL BSA, 0.5 A_{260} units/mL template:primer [poly(rA):oligo$(dT)_{12-18}$] and 6.25 μM [^{3}H]dTTP (30 Ci/mmol). After incubation for 60 min at 37 °C, the samples were spotted on glass fiber filters (Whatman GF/A), and the acid-insoluble radioactivity was determined.

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