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Dedicated to Prof. Marino Artico in the occasion of his 80° birthday.

Abstract

A comparison of the effects of the 6-(2-chloro-6-fluorobenzyl)-2-(alkylthio)pyrimidin-4(3*H*)-ones (2-Cl-6-F-*S*-DABOs) **7-12** and the related 6-(2,6-difluorobenzyl) counterparts **13-15** in HIV-1 infected cells and in the HIV-1 reverse transcriptase (RT) assays is here described. The new 2-Cl-6-F-*S*-DABOs showed up to picomolar activity against wt HIV-1. Against clinically relevant HIV-1 mutants and in enzyme assays, the simultaneous C5(methyl)/C6(methyl/ethyl) substitution in the 2-Cl-6-F- and 2,6-F_2-benzyl series furnished compounds with the highest, wide-spectrum inhibitory activity against HIV-1. Three representative 2-Cl-6-F-*S*-DABOs carrying two (**9c**, **10c**) or one (**10a**) stereogenic centres were resolved into their individual stereoisomers, and showed a significant diastereo- and enantioselectivity in HIV-1 inhibition, the highest antiviral activity well correlating with the *R* absolute configuration to the stereogenic centre of the C6-benzylic position in both cellular and enzymatic tests. Application of previously reported COMBINEr protocol on **9c** and **10c** confirmed the influence of the stereogenic centres on their binding modes in the HIV-1 RT.

Introduction

The dihydro-alkyloxy-benzyl-oxopyrimidines (DABOs)[1-3] were disclosed as non-nucleoside reverse transcriptase inhibitors (NNRTIs)[4,5] by our group in 1992.[6-8] Since then, many structural modifications have been performed on the pyrimidine ring with the aim to obtain more potent and selective compounds. These efforts led to the discovery by us and other groups of some series of excellent DABO and DABO-related compounds such as *S*-DABOs,[9-19] *NH*-DABOs,[20] *N,N*-DABOs,[21-23] and DAPY-DABO hybrids[24,25] that are endowed with inhibitory potencies in the low nanomolar or subnanomolar range against wild-type and mutant strains without a significant cytotoxicity at higher concentrations (up to >100 μM) (Figure 1).

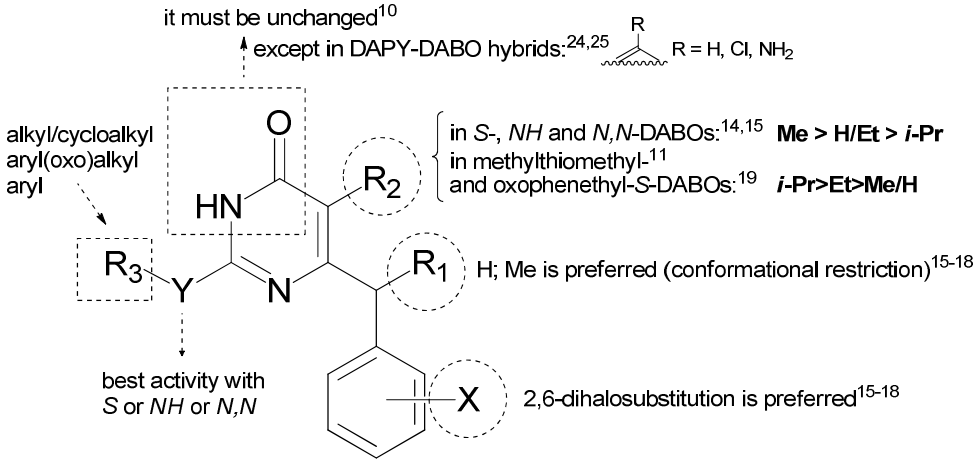


Figure 1. SAR graphical summary for the various DABO derivatives.

In previous works we have reported that, in the series of alkyl-*S*-DABOs, the 2,6-difluorobenzyl/1-(2,6-difluorophenyl)ethyl substitution at the C6 position (R_1 = H/Me in **1**, Figure 2) of the pyrimidin-4(3*H*)-one ring is better than the 2,6-dichloro counterpart in terms of HIV-1 inhibiting activity,[14,15] independently on the substitution at C5 (R_2 in **1**, Figure 2) and C2 (S-R_3 in **1**, Figure 2) positions.

In 2005, some properly substituted 6-(2,6-dichlorobenzyl)-2-(benzylthio)pyrimidin-4(3*H*)-ones (Cl_2-*S*-DABOs) **2** (Figure 2) were reported to be more potent than their 2,6-difluorobenzyl analogues,[17] but this trend was inverted when a methyl group was inserted at the benzylic position of such derivatives (F_2-*S*-DABOs **3**, Figure 2).[18]

In 2008, we reported the first series of DABO-related compounds bearing a 2-chloro-6-fuorobenzyl substitution at C6, the oxophenethyl-*S*-DABOs **4** (Figure 2).[19] Such compounds showed a peculiar SAR profile, deeply different from the (cyclo)alkyl-*S*-DABOs, with the 2,6-dichlorobenzyl/2-chloro-6-fluorobenzyl moiety at C6 and the ethyl/*iso*-propyl group at C5 being crucial for the broadest spectrum anti-HIV-1 activity (see also Figure 1).[19] Also in the structurally related 2-((4-benzyl-6-oxo-1,6-dihydropyrimidin-2-yl)thio)-*N*-phenylacetamide **5** (Figure 2), the 2-chloro-6-fluorobenzyl substitution afforded higher anti-HIV-1 activity than the 2,6-difluorobenzyl one.[26]

In a comparative study between 2,6-difluoro- (F_2-) and 2-chloro-6-fluoro- (2-Cl-6-F-) *N,N*-DABO derivatives, we observed that the latter are endowed with an improved activity against wild-type and mutated HIV-RT forms and display a peculiar mechanism of action especially toward mutated (K103N and Y181I) RT enzymes.[23] In contrast to F_2-*N,N*-DABOs which behave as slow-binding/high-affinity RT inhibitors,[22] the 2-Cl-6-F-*N,N*-DABOs show highly improved association rates toward wild-type/mutated HIV-1 RT enzymes coupled with an high-affinity binding to the same forms and with a peculiar higher-affinity binding to mutated RT forms than to the wild-type enzyme (**6**, Figure 2).[23]

Thus, in some contexts, in addition to the F_2-, also the 2-Cl-6-F-benzyl substitution at the C6 position of the *S*-DABOs afforded potent and selective anti-HIV-1 agents. Nevertheless, a systematic study on the effect of the 2-Cl-6-F substitution on the "classical" alkyl/cycloalkyl-*S*-DABOs was still missing.

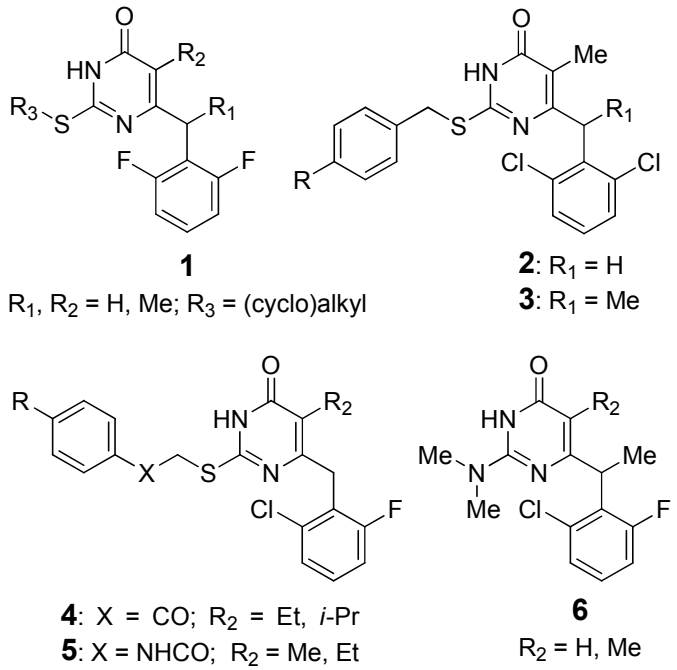


Figure 2. Previously reported 2,6-dihalosubstituted DABO compounds (**1-6**).

Therefore, we prepared a new series of *S*-DABOs characterized by the best (cyclo)alkylthio substituents at C2 (S-R_3 in Figure 2), as resulted from previous SAR studies,[1-3,14,15] by a 2-Cl-6-F-benzyl group at C6 position, and by an hydrogen, methyl or ethyl substituent at C5 (R_2 in Figure 2) and/or at the C6-benzylic (R_1 in Figure 2) moiety (**7-12**, Figure 2). For comparison purposes, we also included in the study some previously reported[15,27,28] and newly synthesized F_2-benzyl analogues characterized by the same substitutions at C2, and by a methyl or ethyl substituent at both C5 and C6-benzylic group (**13-15**, Figure 2).

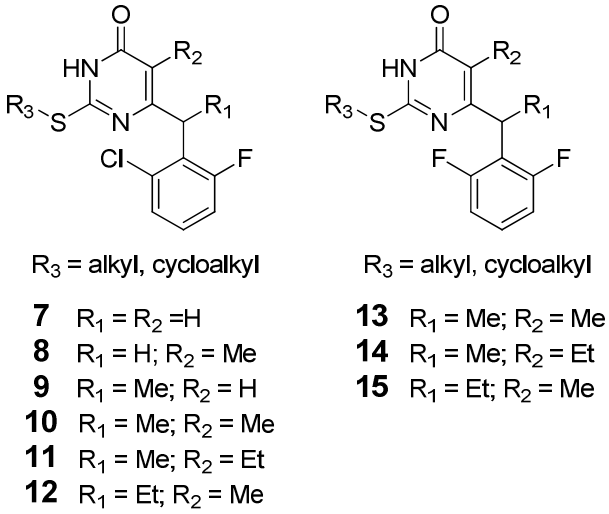


Figure 2. 2,6-Dihalosubstituted alkyl-*S*-DABO derivatives (**7-15**) object of the present study.

Since many derivatives contain one or two stereogenic centres and, therefore, exist as racemic or stereoisomers' mixtures, respectively, we decided to perform a systematic investigation on their potential enantio-/diastereoselective anti-HIV-1 activity. Focusing our attention on three of the most potent compounds (**9c**, **10a**, **10c**), we performed the enantio-/diastereoseparation, the chiroptical characterization, the absolute configuration assignation (where it was possible), and the enzymatic and cellular evaluation of the HIV-1 inhibitory activity of the single enantiomers/stereoisomers in comparison with the corresponding racemic/stereoisomers' mixtures.

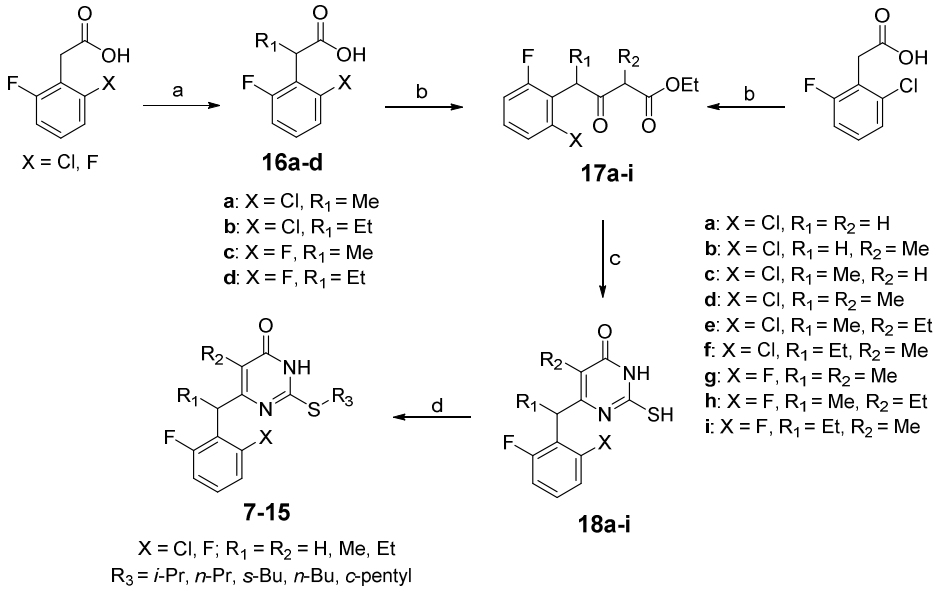
Finally, a structure-based studies by the means of a previously reported COMBINEr protocol was undertaken on **9c** and **10c** to gather the influence of the stereogenic centers on their binding modes in the HIV-1 RT.

Chemistry

The required 2-(2,6-dihalophenyl)propionic/butanoic acids **16a-d** were obtained by alkylation of the corresponding commercially available (2,6-dihalo)phenylacetic acids with the proper alkyl iodides in the presence of *n*-butyllithium in anhydrous THF first at -78 °C and then at room

temperature.[15,23,28] The imidazolides obtained from the acids **16a-d** and from the commercial 2-chloro-6-fluorophenylacetic acid by treatment with *N,N*-carbonyldiimidazole (CDI) in dry acetonitrile at room temperature were then treated with potassium ethylmalonate, potassium ethyl-2-methylmalonate or potassium ethyl-2-ethylmalonate in the presence of the magnesium dichloride/triethylamine system to yield, after decarboxylation with 12% hydrochloric acid, the required ethyl β-oxoesters **17a-i**.[14,15,18,19,21,23,27,28] Condensation of these β-ketoesters with thiourea in the presence of sodium ethoxide in dry ethanol under reflux conditions gave the intermediates 6-[(2,6-dihalophenyl)alkyl]-5-alkyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)ones **18a-i**,[15,18,19,28] which were finally *S*-alkylated in dry DMF with the proper commercially available alkyl/cycloalkyl halides in the presence of anhydrous potassium carbonate to yield the final compounds **7-15** (Scheme 1).[15,28] Chemical and physical data for compounds **7-18** (only for the novel compounds) are listed in Table S1 in Supporting Information.

Scheme 1. Synthesis of Compounds 7-15.[a]



[a](a) (1) *n*-BuLi, $(Et)_2NH$, dry THF, -78 °C; (2) proper alkyl iodides, dry THF, -78 °C then room temp; (b) (1) CDI, dry CH_3CN, room temp; (2) $KOOCCHR_2COOEt$, $MgCl_2$, Et_3N, dry CH_3CN,

reflux; (3) 12% HCl, room temp; (c) NH_2CSNH_2, EtONa, reflux; (d) alkyl/cycloalkyl iodide or bromide, anhydrous K_2CO_3, dry DMF, room temp.

Results and Discussion

Cytotoxicity and Wild Type HIV-1 Inhibiting Activity. The new alkyl-*S*-DABOs **7-15** were tested in MT-4 cells to evaluate their cytotoxicity and their capability to inhibit by 50% the HIV-1-induced cytopathic effect in MT-4 cells (HIV-1 strain: NL4-3). Nevirapine (NVP) and efavirenz (EFV) were also tested as reference drugs (Table 1).

Table 1. Cytotoxicity and Anti-HIV-1 Activity (wt HIV-1 Strain: NL4-3) of **7-15**[a]

compound	R_1	R_2	R_3	EC_{50},[b] μM	CC_{50},[c] μM	selectivity index[d]
7a	H	H	*i*-Pr[e]	0.38	>80[f]	>210
7b	H	H	*n*-Pr	0.26	>80	>308
7c	H	H	*s*-Bu	0.16	>76	>475
7d	H	H	*n*-Bu	0.29	>76	>262
7e	H	H	cyclopentyl	0.15	>74	>493
8a	H	Me	*i*-Pr	0.12	>76	>633
8b	H	Me	*n*-Pr	0.086	>76	>884
8c	H	Me	*s*-Bu	0.047	>73	>1,553
8d	H	Me	*n*-Bu	0.11	>73	>664
8e	H	Me	cyclopentyl	0.18	>71	>394
9a	Me	H	*i*-Pr	0.002	>75	>37,500
9b	Me	H	*n*-Pr	0.003	>75	>25,000
9c	Me	H	*s*-Bu	0.001	>73	>73,000
9d	Me	H	*n*-Bu	0.0003	>73	>243,333
9e	Me	H	cyclopentyl	0.0003	>71	>236,667

10a	Me	Me	*i*-Pr	0.00004	>15	>375,000
10b	Me	Me	*n*-Pr	0.00005	>1.5	>30,000
10c	Me	Me	*s*-Bu	0.0003	>1.5	>5,000
10d	Me	Me	*n*-Bu	0.0003	>1.5	>5,000
10e	Me	Me	cyclopentyl	0.00003	>15	>500,000
11a	Me	Et	*i*-Pr	0.96	>3	>3
11b	Me	Et	*n*-Pr	0.028	>3	>107
11c	Me	Et	*s*-Bu	0.27	>3	>11
11d	Me	Et	*n*-Bu	0.064	>3	>47
11e	Me	Et	cyclopentyl	>3	>3	
12a	Et	Me	*i*-Pr	0.084	>3	>36
12b	Et	Me	*n*-Pr	0.026	>3	>115
12c	Et	Me	*s*-Bu	0.054	>3	>56
12d	Et	Me	*n*-Bu	0.11	>3	>27
12e	Et	Me	cyclopentyl	0.58	>3	>5
13a[g]	Me	Me	*i*-Pr	0.001	>3	>3,000
13b	Me	Me	*n*-Pr	0.028	>3	>107
13c[g]	Me	Me	*s*-Bu	0.0009	>3	>3,333
13d[g]	Me	Me	*n*-Bu	0.050	>3	>60
13e[g]	Me	Me	cyclopentyl	0.001	>3	>3,000
14a	Me	Et	*i*-Pr	0.089	>3	>34
14b	Me	Et	*n*-Pr	0.24	>3	>12
14c	Me	Et	*s*-Bu	0.14	>3	>21
14d	Me	Et	*n*-Bu	0.057	>3	>53
14e	Me	Et	cyclopentyl	0.16	>3	>19
15a[h]	Et	Me	*i*-Pr	0.004	>30	>7,500
15b	Et	Me	*n*-Pr	0.012	>3	>250
15c[h]	Et	Me	*s*-Bu	0.0008	>3	>3,750

15d	Et	Me	*n*-Bu	0.023	>3	>130
15e[h]	Et	Me	cyclopentyl	0.011	30	2,727
NVP				0.12	>7	>62
EFV				0.007	>3	>429

[a]Values are means determined from at least two experiments. [b]Effective concentration 50, concentration needed to inhibit by 50% the HIV-1-induced cytopatic effect, evaluated with the MTT method in MT-4 cells (HIV-1 strain: NL4-3). [c]Cytotoxic concentration 50, concentration needed to induce 50% death of non-infected cells, evaluated with the MTT method in MT-4 cells. [d]Selectivity index (SI), CC_{50}/EC_{50}. [e]Abbreviations: *i*-Pr, *iso*-propyl; *n*-Pr, *normal*-propyl; *s*-Bu, *sec*-butyl; *n*-Bu, *normal*-butyl. [f]Higher concentrations could not be tested due to precipitation of compounds in the culture medium. [g]Ref. 15. [h]Ref. 28.

All the tested compounds without solubility problems (**7-9**) were not cytotoxic up to 71 μM. Some compounds (**10-15**) showed >1.5 or >3.0 μM values of CC_{50} (compound concentration toxic for 50% of cells) because higher concentration could not be achieved for the precipitation of the compounds in the culture medium.

From the analysis of inhibition data for wt HIV-1 in MT-4 cells (Table 1), it is clear that an alkyl substituent at the benzylic portion of the molecule is crucial to increase the anti-HIV-1 potency of the new 2-Cl-6-F-*S*-DABOs (compare the EC_{50} values of the benzyl-unsubstituted uracils and thymines **7** and **8** with the corresponding analogues **9** and **10** carrying a methyl group at the benzylic position). In analogy to what observed in other DABO and DABO-related series,[15,16,18,21,24] also among the 2-Cl-6-F-*S*-DABOs **7-12** the double methyl substitution at both C6(R_1) and C5(R_2) positions was the most productive in terms of wt HIV-1 inhibition, and led to a set of derivatives (**10a-e**) active in the picomolar range (300 to 30 pM), from 23- to 230-fold more potent than efavirenz, and in some cases (**10a** and **10e**) with impressive selectivity indexes (SI, CC_{50}/EC_{50}) > 375,000.

Despite the presence of the C6-benzylic methyl substituent, the replacement of the C5-methyl with a C5-ethyl group (compounds **11a-e**) gave up to 5-magnitude orders decrease of potency of the derivatives (compare **10a** with **11a**). Keeping fixed the C5-methyl group, the change of the C6-methyl with a C6-ethyl group (compounds **12a-e**) again led to less potent compounds, also if in this case the drop of potency seems to be less pronounced (3 or 4-magnitude orders).

As reported in previous *S*-DABO series,[9,10,12-15] the alkyl chain inserted at C2 (branched, such as *iso*-propyl and *sec*-butyl, linear, such as *n*-propyl and *n*-butyl, and cyclic, such as cyclopentyl) has modulating effects on the anti-HIV-1 activity. In particular, in the C6-methyl uracil (**9a-e**) and thymine (**10a-e**) derivatives the C2-alkyl chain gave up to 10-fold differences in EC_{50} values of the compounds, the best substituent being the cyclopentyl (**9e** and **10e**) and the *iso*-propyl ones (**9a** and **10a**), and up to 100-fold differences in EC_{50} values in the series **11a-e** [C5(Et)/C6(Me)] and **12a-e** [C5(Me)/C6(Et)] where, on the contrary, the cyclopentyl substituent was the worst in terms of potency (**11e** and **12e**, respectively), whereas the *n*-propyl was the best (**11b** and **12b**, respectively).

The F_2-*S*-DABOs **13-15** never reached the extremely high potency and selectivity of the best 2-chloro-6-fluoro compounds (**10a-e**), and showed a trend in the wt HIV-1 inhibiting activity partially different from what observed in the 2-chloro-6-fluoro analogues (**10-12**). Indeed, in the F_2-*S*-DABO series there were no significant differences between thymine compounds substituted with the C6-methyl (**13a-e**) or C6-ethyl (**15a-e**) group, all showing EC_{50} values in the low nanomolar/picomolar range. Differently, the potency significantly decreased in case of C5-methyl to C5-ethyl replacement and simultaneous C6-methyl substitution (**14a-e**, EC_{50}s in the high nanomolar range).

Inhibitory Activity Against Clinically Relevant HIV-1 Mutant Strains. The alkyl-*S*-DABOs **7-15** were also tested against a panel of clinically relevant HIV-1 mutants (K103N, Y181C, and Y188L) (Table 2). In analogy to what observed against wt HIV-1, among the 2-chloro-6-fluoro alkyl-*S*-DABOs **7-12** the inhibitory activity decreased according to the order C6(R_1)/C5(R_2) Me/Me

> Me/H > H /Me > H/H ≅ Et/Me ≅ Me/Et. Also in this case, the double methyl substitution at both C6(R_1) and C5(R_2) positions led to the most potent derivatives of the 2-Cl-6-F series (**10a-e**), that were active in the submicromolar range against the mutants K103N (EC_{50} values ranging from 0.093 μM for **10e** to 0.66 μM for **10d**) and Y181C (EC_{50} values ranging from 0.23 μM for **10e** to 0.62 μM for **10d**) and in the single digit micromolar range against Y188L, resulting more potent than nevirapine against all three mutants, and more (vs K103N), equal (vs Y188L), or less (vs Y181C) potent than efavirenz depending on the HIV-1 mutant strain. The C6-methyl uracil derivatives **9a-e** were in general 1-magnitude order less potent than the corresponding thymines **10a-e**, whereas the remaining 2-Cl-6-F-*S*-DABOs displayed a more severe drop of inhibiting potency. Since a concentration higher than 3 μM could not be tested due to precipitation of compounds in the culture medium, it was impossible (with the only exception of compound **12b**) to determine reliable EC_{50} values for the series **11a-e** and **12a-e**, and, for this reason, any comparison with the series **7a-e** and **8a-e** was precluded. The F_2-*S*-DABOs **13-15** substantially confirmed against the mutants the same SAR observed against the wt HIV-1. The two most potent series (EC_{50} values in the submicromolar range) were **13a-e** [C6(Me)/C5(Me)] and **15a-e** [C6(Et)/C5(Me)], with a slight predominance of the first (**13a-e**) against K103N and Y181C, and of the second (**15a-e**) against Y188L. At the C2 position, a branched *S*-alkyl chain (*iso*-propyl, *sec*-butyl and cyclopentyl) assured the highest potency. On the other hand, the C2 linear alkyl (*n*-propyl and *n*-butyl) -substituted derivatives (**13b**,**d** and **15b**,**d**) together with all the derivatives of the series **14a-e** [C6(Me)/C5(Et)] were markedly less potent against all of the three tested mutant strains (EC_{50} > 3 μM).

Table 2. Anti-HIV-1 Activity of **7-15** against Clinically Relevant HIV-1 Mutant Strains[a]

compd	R_1	R_2	R_3	EC_{50},[b] μM (*fold-resistance*)[c]		
				K103N	Y181C	Y188L
7a	H	H	*i*-Pr[d]	39.2 *(103)*	17.8 *(47)*	>80[e]
7b	H	H	*n*-Pr	18.8 *(72)*	8.2 *(31)*	>80
7c	H	H	*s*-Bu	25.2 *(157)*	8.3 *(52)*	>76
7d	H	H	*n*-Bu	29.2 *(101)*	16.7 *(58)*	>76
7e	H	H	cyclopentyl	42.1 *(281)*	18.3 *(122)*	>74
8a	H	Me	*i*-Pr	10.6 *(88)*	8.4 *(70)*	>76
8b	H	Me	*n*-Pr	9.2 *(107)*	2.3 *(27)*	>76
8c	H	Me	*s*-Bu	5.4 *(115)*	5.2 *(111)*	>73
8d	H	Me	*n*-Bu	20.0 *(182)*	2.3 *(21)*	>73
8e	H	Me	cyclopentyl	>71	>71	>71
9a	Me	H	*i*-Pr	1.1 *(550)*	7.5 *(3,750)*	30.7 *(15,350)*
9b	Me	H	*n*-Pr	0.89 *(297)*	2.3 *(767)*	7.9 *(2,633)*
9c	Me	H	*s*-Bu	1.0 *(1,000)*	3.6 *(3,600)*	7.5 *(7,500)*
9d	Me	H	*n*-Bu	1.1 *(3,667)*	3.9 *(13,000)*	5.6 *(18,667)*
9e	Me	H	cyclopentyl	0.9 *(3,000)*	3.0 *(10,000)*	>71
10a	Me	Me	*i*-Pr	0.14 *(4,667)*	0.25 *(8,333)*	3.4 *(113,333)*
10b	Me	Me	*n*-Pr	0.35 *(7,000)*	0.26 *(5,200)*	>1.5
10c	Me	Me	*s*-Bu	0.32 *(1,067)*	0.39 *(1,300)*	>1.5
10d	Me	Me	*n*-Bu	0.66 *(2,200)*	0.62 *(2,067)*	>1.5
10e	Me	Me	cyclopentyl	0.093 *(3,100)*	0.23 *(7,667)*	2.9 *(96,667)*
11a	Me	Et	*i*-Pr	>3	>3	>3
11b	Me	Et	*n*-Pr	>3	>3	>3
11c	Me	Et	*s*-Bu	>3	>3	>3
11d	Me	Et	*n*-Bu	>3	>3	>3
11e	Me	Et	cyclopentyl	>3	>3	>3

12a	Et	Me	*i*-Pr	>3	>3	>3
12b	Et	Me	*n*-Pr	2.1 *(81)*	2.8 *(108)*	>3
12c	Et	Me	*s*-Bu	>3	>3	>3
12d	Et	Me	*n*-Bu	>3	>3	>3
12e	Et	Me	cyclopentyl	>3	>3	>3
13a[f]	Me	Me	*i*-Pr	0.27 *(270)*	0.12 *(120)*	>3
13b	Me	Me	*n*-Pr	>3	>3	>3
13c[f]	Me	Me	*s*-Bu	0.11 *(122)*	0.071 *(79)*	>3
13d[f]	Me	Me	*n*-Bu	>3	>3	>3
13e[f]	Me	Me	cyclopentyl	0.23 *(230)*	0.1 *(100)*	>3
14a	Me	Et	*i*-Pr	>3	>3	>3
14b	Me	Et	*n*-Pr	>3	>3	>3
14c	Me	Et	*s*-Bu	>3	>3	>3
14d	Me	Et	*n*-Bu	>3	>3	>3
14e	Me	Et	cyclopentyl	>3	>3	>3
15a[g]	Et	Me	*i*-Pr	0.53 *(132)*	0.75 *(187)*	0.27 *(67)*
15b	Et	Me	*n*-Pr	>3	>3	>3
15c[g]	Et	Me	*s*-Bu	0.2 *(250)*	0.3 *(375)*	0.9 *(1,125)*
15d	Et	Me	*n*-Bu	>3	>3	>3
15e[g]	Et	Me	cyclopentyl	1.0 *(91)*	1.1 *(100)*	1.6 *(145)*
NVP				5.6 *(47)*	>7	>7
EFV				0.34 *(49)*	0.013 *(2)*	1.97 *(281)*

[a]Values are means determined from at least two experiments. [b]Effective concentration 50, concentration needed to inhibit by 50% the HIV-1-induced cytopatic effect, evaluated with the MTT method in MT-4 cells. [c]Fold resistance: ratio of EC_{50} value against drug-resistant strain and EC_{50} of the wt NL4-3 strain (Table 2). [d]Abbreviations: *i*-Pr, *iso*-propyl; *n*-Pr, *normal*-propyl; *s*-Bu, *sec*-butyl; *n*-Bu, *normal*-butyl. [e]Higher concentrations could not be tested due to precipitation of compounds in the culture medium. [f]Ref. 15. [g]Ref. 28.

Enzyme Inhibiting Activity: Effect on HIV-1 RT Wild Type and NNRTI-resistant Mutants. The most potent 2-Cl-6-F-*S*-DABOs in cell assays **9-12** as well as their 2,6-difluoro counterparts **13-15** were tested against recombinant wt HIV-1 RT and against a panel of recombinant RTs carrying known NNRTI-resistance mutations (K103N, Y181I, and L100I) (Table 3). Similarly to what observed in cellular assays, the double C6/C5-methyl-substituted analogs **10a-e** displayed the highest - from nanomolar (**10a**,**d**,**e**) to picomolar (**10b** and **10c**) - inhibition of wt HIV-1 RT. Unfortunately, they suffered from a great decrease of potency against the mutant RTs, in particular against the Y181I mutant. The related compounds **9a-e** sharing the sole C6-methyl substitution showed in general less potency than **10a-e** against wt RT, but higher inhibition towards the Y181I mutant, with lower fold-resistance values respect to **10a-e**. The introduction of a C5-ethyl group in addition to the C6-methyl group (compounds **11a-e**) further decreased the potency of derivatives against wt RT, but maintained the same potency against the mutated RTs as the uracil counterparts **9a-e** (single-digit micromolar against K103N, micromolar against Y181I, and submicromolar against L100I). All considering, in the enzyme assays the 2-Cl-6-F-*S*-DABOs bearing an ethyl group at C6 and a methyl group at C5 (**12a-e**) showed the most valuable anti-RT activity, because they displayed a slightly reduced potency against wt RT with respect to **10a-e** (no compounds with picomolar activity among **12a-e**), but showed submicromolar inhibitory potencies against the K103N and L100I mutants, and single-digit micromolar activity against Y181I, the less sensitive RT mutant.

The tested F_2-*S*-DABOs **13-15**, apart their C6/C5 methyl or ethyl substitution, generally exerted nanomolar inhibition of wt RT and submicromolar (K103N and L100I) or micromolar (Y181I) potency against mutant RTs. Similarly as the 2-Cl-6-F-*S*-DABOs **12a-e**, also with the 2,6-difluoro compounds the C6-ethyl/C5-methyl substitution (compounds **15a-e**) assured the highest potency against the Y181I RT mutant, at single digit micromolar level. Globally, both the most

active 2-Cl-6-F- and F_2-*S*-DABOs **12** and **15** were more potent than the reference drug efavirenz against the wt RT and the K103N RT mutant, and less effective against the Y181I RT mutant.

Table 3. Inhibitory Activity of **9-15** against HIV-1 RT Wild Type and NNRTI-resistant Mutants[a]

compd	R_1	R_2	R_3	ID_{50},[b] μM (*fold-resistance*)[c]			
				wt	K103N	Y181I	L100I
9a	Me	H	*i*-Pr[d]	0.008	1.3 *(162)*	10.0 *(1,250)*	0.34 *(42)*
9b	Me	H	*n*-Pr	0.008	1.3 *(162)*	15.0 *(1,875)*	0.5 *(62)*
9c	Me	H	*s*-Bu	0.008	3.0 *(375)*	21.1 *(2,638)*	0.2 *(25)*
9d	Me	H	*n*-Bu	0.01	2.2 *(220)*	15.2 *(1,520)*	0.6 *(60)*
9e	Me	H	cyclopentyl	0.015	1.8 *(120)*	16.6 *(1,107)*	1.1 *(73)*
10a	Me	Me	*i*-Pr	0.015	1.4 *(93)*	200.0 *(13,333)*	0.29 *(19)*
10b	Me	Me	*n*-Pr	0.0009	1.5 *(1,667)*	80.0 *(88,889)*	0.09 *(100)*
10c	Me	Me	*s*-Bu	0.0003	1.75 *(5,833)*	35.0 *(116,667)*	0.075 *(250)*
10d	Me	Me	*n*-Bu	0.003	0.93 *(310)*	60.0 *(20,000)*	0.156 *(52)*
10e	Me	Me	cyclopentyl	0.011	1.3 *(118)*	32.0 *(2,909)*	0.12 *(11)*
11a	Me	Et	*i*-Pr	0.048	2.6 *(54)*	27.0 *(562)*	1.1 *(23)*
11b	Me	Et	*n*-Pr	0.010	2.6 *(260)*	4.7 *(470)*	0.23 *(23)*
11c	Me	Et	*s*-Bu	0.008	2.4 *(300)*	16.2 *(2,025)*	0.4 *(50)*
11d	Me	Et	*n*-Bu	0.022	2.3 *(104)*	13.1 *(595)*	0.33 *(15)*
11e	Me	Et	cyclopentyl	0.024	1.8 *(75)*	15.6 *(650)*	0.53 *(22)*
12a	Et	Me	*i*-Pr	0.006	0.42 *(70)*	7.7 *(1,283)*	0.12 *(20)*
12b	Et	Me	*n*-Pr	0.005	0.63 *(126)*	3.5 *(700)*	0.24 *(48)*
12c	Et	Me	*s*-Bu	0.002	0.45 *(225)*	5.9 *(2,950)*	0.12 *(60)*
12d	Et	Me	*n*-Bu	0.003	1.0 *(333)*	6.1 *(2,033)*	0.26 *(87)*
12e	Et	Me	cyclopentyl	0.004	0.34 *(85)*	6.0 *(1,500)*	0.068 *(17)*
13a[e]	Me	Me	*i*-Pr	0.003	0.58 *(193)*	47.0 *(15,667)*	0.28 *(93)*

13b	Me	Me	*n*-Pr	0.001	0.38 *(380)*	5.4 *(5,400)*	0.23 *(230)*
13c[e]	Me	Me	*s*-Bu	0.031	1.7 *(55)*	96.0 *(3,097)*	0.67 *(22)*
13d[e]	Me	Me	*n*-Bu	0.003	0.45 *(150)*	4.6 *(1,533)*	0.23 *(77)*
13e[e]	Me	Me	cyclopentyl	0.005	0.03 *(6)*	0.9 *(180)*	0.3 *(60)*
14a	Me	Et	*i*-Pr	0.012	0.83 *(69)*	129.4 *(10,783)*	27.2 *(2,267)*
14b	Me	Et	*n*-Pr	0.003	0.43 *(143)*	2.8 *(933)*	0.27 *(90)*
14c	Me	Et	*s*-Bu	0.011	0.45 *(41)*	4.6 *(418)*	0.11 *(10)*
14d	Me	Et	*n*-Bu	0.003	0.39 *(130)*	3.0 *(1,000)*	0.28 *(93)*
14e	Me	Et	cyclopentyl	0.002	0.2 *(100)*	1.8 *(900)*	0.13 *(65)*
15a[f]	Et	Me	*i*-Pr	0.004	0.31 *(78)*	4.1 *(1,025)*	0.21 *(53)*
15b	Et	Me	*n*-Pr	0.006	0.42 *(70)*	3.6 *(600)*	0.22 *(37)*
15c[f]	Et	Me	*s*-Bu	0.006	0.14 *(23)*	4.0 *(667)*	0.048 *(8)*
15d	Et	Me	*n*-Bu	0.003	0.28 *(93)*	4.6 *(1,533)*	0.23 *(77)*
15e[f]	Et	Me	cyclopentyl	0.005	0.38 *(76)*	4.9 *(980)*	0.11 *(22)*
NVP				0.4	7.0 *(17)*	35.0 *(87)*	9.0 *(22)*
EFV				0.03	3.0 *(100)*	0.08 *(3)*	ND[d]

[a]Values are means determined from at least three experiments. [b]Inhibitory dose 50, compound dose required to inhibit the HIV-1 rRT activity by 50%. [c]Fold resistance: ratio of ID_{50}mut/ID_{50}wt values. [d]Abbreviations: *i*-Pr, *iso*-propyl; *n*-Pr, *normal*-propyl; *s*-Bu, *sec*-butyl; *n*-Bu, *normal*-butyl. [e]Ref. 15. [f]Ref. 28.

Separation and Chiroptical Characterization of 9c, 10a and 10c Single Stereoisomers

Three 2-Cl-6-F-*S*-DABOs, namely the 6-[1-(2-chloro-6-fluorophenyl)ethyl]-2-(*sec*-butylsulfanyl)pyrimidin-4(3*H*)-one (**9c**), the 6-[1-(2-chloro-6-fluorophenyl)ethyl]-2-(*iso*-propylsulfanyl)-5-methylpyrimidin-4(3*H*)-one (**10a**), and the 6-[1-(2-chloro-6-fluorophenyl)ethyl]-2-(*sec*-butylsulfanyl)-5-methylpyrimidin-4(3*H*)-one (**10c**), were resolved by stereoselective HPLC and the collected single stereoisomers screened in cellular and enzyme assays, to assess the influence of chirality in their antiviral activity (see below). The direct chromatographic resolution

was achieved by using the immobilized-type Chiralpak IA chiral stationary phase (CSP) under normal-phase and polar organic conditions.[29] As an example, Figure 3 shows the simultaneous enantio- and diastereoseparation of **9c** and the analytical check of the enantiomeric (ee) and diastereomeric (de) excess of the stereoisomers collected on a semipreparative scale. By analysis of the sign of the chiroptical properties and relative peak areas (Figures 3 and 4) it was possible to identify the first and fourth eluted stereoisomer of compound **9c**, and the first and third of compound **10c**, as belonging to the same enantiomeric pairs (compounds **9c′** and **10c′**, respectively), and the second and third one of **9c** and the second and fourth one of **10c** to the diastereomeric pairs of enantiomers (compounds **9c″** and **10c″**, respectively). The absolute configuration of the enantiomers of **10a** and of the stereogenic centre of the C6-benzylic moiety of **9c** and **10c** was achieved by circular dichroism (CD) correlation method[28,30] and is in full agreement with docking studies (see below). The absolute configuration of the stereogenic centre for the C2-*sec*-butylthio chain of **9c** and **10c** (*R* or *S*) was suggested by docking studies (see below).

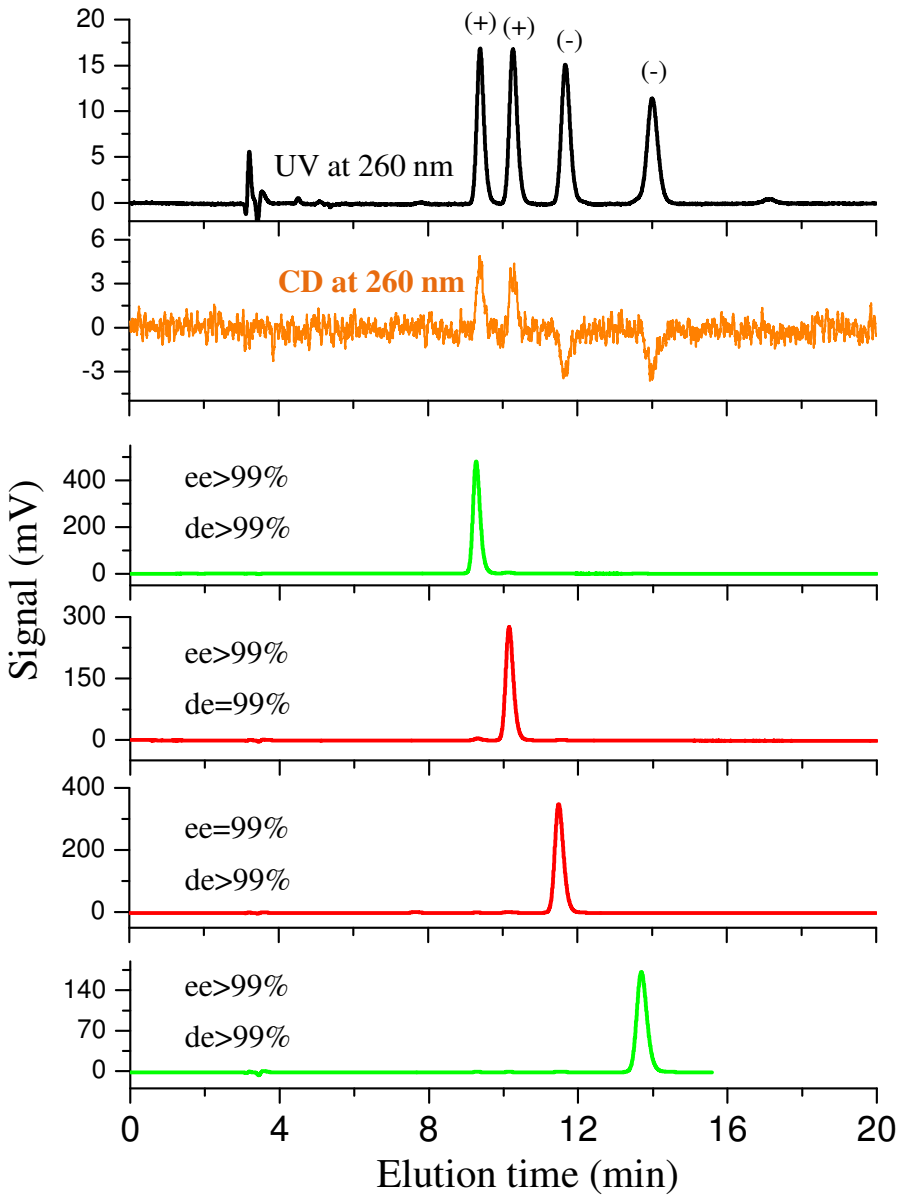


Figure 3. From top to bottom: typical chromatogram illustrating the resolution of **9c** using simultaneous UV (black) and CD (orange) detection; purity control of the single fractions collected at semipreparative scale; ee, enantiomeric excess; de, diastereomeric excess. Column: Chiralpak IA

250 mm × 4.6 mm I.D.; detection: UV at 260 nm; eluent: *n*-hexane-ethanol 95:5 (v/v); flow-rate: 1.0 mL min^{-1}; column temperature: 25 °C.

As previously demonstrated,[28,30] the CD properties of 2-(*sec*-butylthio)-6-[1-(2,6-dichlorophenyl)propyl]-5-methylpyrimidin-4(3*H*)-one (Figure 4), which is structurally similar to the compounds studied in this work, depend only on the absolute configuration of the stereogenic centre at C6 and they are not greatly influenced by the configuration at the alkyl group linked to the sulfur atom at C2 position of the pyrimidine ring. As a result, the diastereomers with the same stereochemistry at the C6 stereogenic centre adjacent to the 2,6-dihalophenyl moiety showed similar CD profile and the same sign of the diagnostic CD band located at around 245 nm. On this basis, the CD spectra of the single stereoisomers of **9c**, **10a** and **10c** were recorded in the same solvent (ethanol) and compared with those of the diastereomers (*S*,*R*)- and (*R*,*R*)-2-(*sec*-butylthio)-6-[1-(2,6-dichlorophenyl)propyl]-5-methylpyrimidin-4(3*H*)-one.[28,30]

As shown in Figure 4, the (-)-**10a** enantiomer and the couples of the diastereomers (-)-**9c′**/(-)-**9c″** and (-)-**10c′**/(-)-**10c″** displayed bisigned CD curves between 300 and 210 nm with a positive Cotton effect strikingly similar to those of the reference stereoisomers with *R* absolute configuration at C6-benzyl methylene unit. Therefore, as a result of the CD study, the *R* configuration was empirically assigned to the stereogenic centre of the benzyl fragment of the levorotatory stereoisomers of all the three chiral compounds. Differently, the CD analysis not allowed us to define the absolute configuration of the stereogenic centre of the alkylthio group at C2 pyrimidine ring position of **9c** and **10c**, and the reported configuration (*R* or *S*) was suggested by molecular modelling studies (see below).

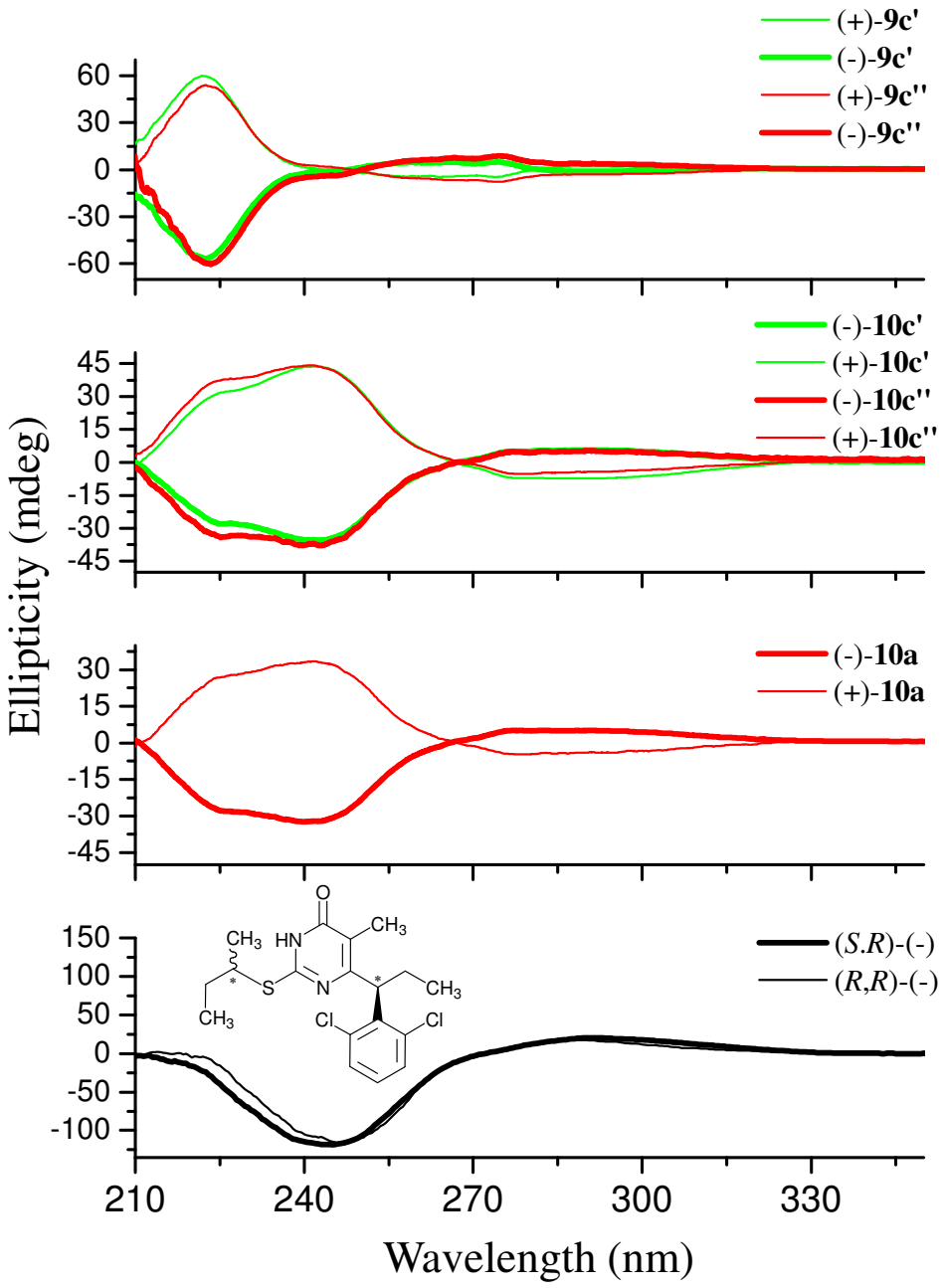


Figure 4. Comparison between the CD spectra of the stereoisomers of **9c**, **10a** and **10c**, and the *S,R* and *R,R* diastereoisomers of 2-(*sec*-butylthio)-6-[1-(2,6-dichlorophenyl)propyl]-5-methylpyrimidin-4(3*H*)-one recorded in ethanol.

Anti-HIV-1 Activity of 9c, 10a and 10c Individual Stereoisomers. After separation, the four stereoisomers of **9c** [**(+)-** and **(-)-9c′**, and **(+)**- and **(-)-9c′′**], the two enantiomers of **10a** [**(+)**- and **(-)**-**10a**], and the four stereoisomers of **10c** [**(+)**- and **(-)-10c′**, and **(+)**- and **(-)-10c′′**] were individually tested in a separate sequence of cellular and enzymatic experiments, in comparison with the corresponding mixtures (hereafter simply referred to as **9c**, **10a**, and **10c**). Nevirapine and efavirenz were added and tested as reference drugs (Tables 4 and 5).

Table 4. Cytotoxicity and Anti-HIV-1 Activity against WT (NL4-3) and Clinically Relevant HIV-1 Mutant Strains of **9c**, **10a** and **10c** and their Individual Stereoisomers[a]

compd[b]	R_1	R_2	R_3	EC_{50},[c] μM (*fold-resistance*)[d]				CC_{50},[e] μM	SI[f]
				NL4-3	K103N	Y181C	Y188L		
9c	Me	H	*s*-Bu[g]	0.004	1.3 *(325)*	2.3 *(575)*	19.2 *(4800)*	>73[h]	>18250
(*R*,*S*)-(+)-9c′	Me	H	*s*-Bu	0.011	1.22 *(111)*	1.24 *(113)*	7.4 *(673)*	>39	>3545
(*S*,*S*)-(+)-9c′′	Me	H	*s*-Bu	0.144	7.64 *(53)*	>73.3	>73.3	>73	>507
(*R*,*R*)-(-)-9c′′	Me	H	*s*-Bu	0.0003	0.2 *(610)*	0.58 *(1924)*	0.86 *(2859)*	>31	>103333
(*S*,*R*)-(-)-9c′	Me	H	*s*-Bu	0.047	5.56 *(118)*	13.2 *(281)*	>51.6	>52	>1106
10a	Me	Me	*i*-Pr	0.0001	0.22 *(2200)*	0.99 *(9900)*	6.3*(63000)*	>73	>730000
(*R*)-(-)-10a	Me	Me	*i*-Pr	0.00003	0.010 *(333)*	0.15 *(5000)*	0.35 *(11667)*	>23.5	>783333
(*S*)-(+)-10a	Me	Me	*i*-Pr	0.22	>36.9	>36.9	>36.9	>36.9	>168.7
10c	Me	Me	*s*-Bu	0.0009	0.06 *(67)*	0.41 *(456)*	2.1 *(2333)*	>70.4	>78222
(*R*,*R*)-(-)-10c′	Me	Me	*s*-Bu	0.00006	0.015 *(14)*	0.087 *(79)*	0.39 *(351)*	>70.4	>1173333
(*S*,*R*)-(-)-10c′′	Me	Me	*s*-Bu	0.0014	0.15 *(135)*	0.89 *(811)*	3.3 *(3006)*	>70.4	>50285
(*S*,*S*)-(+)-10c′	Me	Me	*s*-Bu	3.66	>47	>47	>47	>47	>12.8
(*R*,*S*)-(+)-10c′′	Me	Me	*s*-Bu	43.9	>70	64 *(58407)*	>70	>70.4	>1.6
NVP				0.12	5.6 *(47)*	>7	>7	>7	>62

EFV	0.007	0.34 *(49)*	0.013 *(2)*	1.97 *(281)*	>3	>429

[a]Values are means determined from at least two experiments. [b]Compounds were listed according to their elution order. The underlined *R* or *S* indicates the absolute configuration of the *sec*-butyl chiral centre, as suggested by modelling studies (see below). [c]Effective concentration 50, concentration needed to inhibit by 50% the HIV-1-induced cytophatic effect, evaluated with the MTT method in MT-4 cells. [d]Fold resistance: ratio of EC_{50} value against drug-resistant strain and EC_{50} of the wt NL4-3 strain. [e]Cytotoxic concentration 50, concentration needed to induce 50% death of non-infected cells, evaluated with the MTT method in MT-4 cells. [f]Selectivity index, CC_{50}/EC_{50}. [g]Abbreviations: *i*-Pr, *iso*-propyl; *s*-Bu, *sec*-butyl. [h]Higher concentrations could not be tested due to precipitation of compounds in the culture medium.

In general, for all the three investigated compounds a marked diastereo-/enantioselective effect in the anti-HIV-1 activity has been observed, with the results and the order of relative potency against wt and mutant strains always consistent among the different stereoisomers at both cellular (Table 4) and enzymatic levels (Table 5).

Among the four stereoisomers of **9c**, the highest antiviral activity was displayed by the third eluted stereoisomer **(*R*,*R*)-(-)-9c″** (the absolute configuration of the *sec*-butyl chiral centre is underlined because it was suggested by modelling studies, see below): it was active at picomolar level against wt HIV-1 (EC_{50} = 300 pM), and at submicromolar level against the clinically relevant mutants. Globally, **(*R*,*R*)-(-)-9c″** was up to 13- and 480-fold more potent and selective than **9c** (mixture of four stereoisomers) and **(*S*,*S*)-(+)-9c″** (the less active stereoisomer, belonging to the same enantiomeric pair), respectively (Table 4). In enzyme assays, **(*R*,*R*)-(-)-9c″** showed the highest potency against wt RT as well as against the mutant RTs (Table 5).

The second best-scoring stereoisomer of the **9c** mixture, in terms of potency and selectivity both in cellular (wt and mutant HIV-1 strains) and enzyme (wt and mutant RTs) assays, was the first eluted one **(*R*,*S*)-(+)-9c′**. This suggests that in addition to the C6 chiral centre, that with the *R* configuration drives the highest antiviral potency (see **(*R*,*R*)-(-)-9c″** above), also the chiral centre

due to the C2 *sec*-butylthio chain plays a role for determining the anti-HIV-1 effects of the derivatives. Indeed, if the C2 sec-butylthio substituent would display no stereoselective antiviral activity, the second more potent stereoisomer should be expected to be the diastereoisomer **(*S*,*R*)-(-)-9c′**, that instead is the third of the **9c** series for antiviral potency and selectivity.

The different anti-HIV-1 potency observed between the two enantiomers of compound **10a** further confirmed the crucial role of the *R* stereochemistry at the chiral centre at the C6-benzylic position of DABOs. Indeed, the eutomer **(*R*)-(-)-10a** was active at picomolar level against wt HIV-1 and at submicromolar level against the three tested mutants (Table 4), and showed nanomolar and (sub)micromolar inhibition against the wt RT and the mutant RTs (Table 5), with improved potency respect to both the other enantiomer **(*S*)-(+)-10a** and the corresponding racemic mixture.

About the four stereoisomers of compound **10c**, as expected the two dextrorotatory stereoisomers **(*S*,*S*)-(+)-10c′** and **(*R*,*S*)-(+)-10c″**, with the *S* configuration at the C6-benzylic chiral centre, were practically devoid of any significant anti-HIV-1 activity both in cell and in enzyme assays (Tables 4 and 5). On the other hand, the two levorotatory isomers **(*R*,*R*)-(-)-10c′** and **(*S*,*R*)-(-)-10c″** were potent anti-HIV-1 agents in cells up to picomolar (wt) and nanomolar (mutant strains) concentration (Table 4), and displayed nanomolar and (sub)micromolar inhibition against RT and the mutant RTs, respectively, in enzyme assays (Table 5).

Table 5. Inhibitory Activity of Derivatives **9c, 10a** and **10c** and their Individual Stereoisomers against HIV-1 RT Wild Type and NNRTI-Resistant Mutants[a]

compd[b]	R_1	R_2	R_3	ID_{50},[c] μM (*fold-resistance*)[d]			
				wt	K103N	Y181I	L100I
9c	Me	H	*s*-Bu[e]	0.01269	10.2 *(804)*	>20[f]	0.1294 *(10)*
(*R*,*S*)-(+)-9c′	Me	H	*s*-Bu	0.02689	6.157 *(229)*	>20	0.6491 *(24)*
(*S*,*S*)-(+)-9c″	Me	H	*s*-Bu	0.5467	>20	>20	2.218 *(4)*

(*R*,*R*)-(-)-9c″	Me	H	*s*-Bu	0.003759	1.613 *(488)*	14.11 *(3753)*	0.06871 *(18)*
(*S*,*R*)-(-)-9c′	Me	H	*s*-Bu	0.04052	14.8 *(365)*	>20	0.1481 *(4)*
10a	Me	Me	*i*-Pr	0.015	3.204 *(214)*	>40	0.498 *(33)*
(*R*)-(-)-10a	Me	Me	*i*-Pr	0.009	0.610 *(67.7)*	21.19 *(2354)*	0.177 *(19.7)*
(*S*)-(+)-10a	Me	Me	*i*-Pr	3.348	>40	>40	1.486 *(0.4)*
10c	Me	Me	*s*-Bu	0.011	12.43 *(1130)*	>40	0.377 *(34)*
(*R*,*R*)-(-)-10c′	Me	Me	*s*-Bu	0.001	0.101 *(101)*	3.55 *(3550)*	0.041 *(41)*
(*S*,*R*)-(-)-10c″	Me	Me	*s*-Bu	0.014	0.972 *(69)*	>40	0.524 *(37)*
(*S*,*S*)-(+)-10c′	Me	Me	*s*-Bu	4.664	>40	>40	0.913 *(0.2)*
(*R*,*S*)-(+)-10c″	Me	Me	*s*-Bu	5.918	>40	>40	0.596 *(0.1)*
NVP				0.4	7.0 *(17)*	35.0 *(87)*	9.0 *(22)*
EFV				0.03	3.0 *(100)*	0.08 *(3)*	ND[f]

[a]Values are means determined from at least three experiments. [b]Compounds were listed according to their elution order. The underlined *R* or *S* indicates the absolute configuration of the *sec*-butyl chiral centre, as suggested by modelling studies (see below). [c]Inhibitory dose 50, compound dose required to inhibit the HIV-1 rRT activity by 50%. [d]Fold resistance: ratio of ID_{50}mut/ID_{50}wt values. [d]Abbreviation: *s*-Bu, *sec*-butyl. [e]Higher concentrations could not be tested due to precipitation of compounds in the culture medium.[f]ND, not determined.

Molecular Modeling, Docking Calculations and COMBINEr predictions. A structure-based study by the means of a previously reported COMBINEr protocol[31] was undertaken to further support experimental evidences on the absolute **9c** and **10c** stereoisomer configurations. For comparison purposes, calculations were performed also on the F_2-S-DABO **13e**.[13] The statistically most robust COMBINEr model CM4 was used to verify the mutation role on the activity profiles of **9c** and **10c** stereoisomers. The binding modes of the *S*-DABO derivatives were analyzed by the means of the Vina program. Similarly as reported in previous studies, the *R* configuration at the C6-benzylic chiral centre displays an overall binding profile similar to that described for *NH*-DABOs,[25]

while the S configuration prevents such behavior (compare binding modes in Figures S1A, S1B and S1C in Supporting Information).

Interestingly, the role of the C5 methyl group is somehow controversial. On one hand its presence exerts a conformation constraint[13,15] pre-selecting a bioactive-like conformation for *(R)*-C6 stereoisomers (Figure S1B), thus leading to more potent derivatives[13] in particular for wt, L100I and K103N RT isoforms. On the contrary, the enhanced rigidity is undesired for the *(R)*-C6 stereoisomers when binding to Y181I mutated RT, where the replacement of the Tyr181 with an isoleucine prevent the binding mode maintenance as for the other RT isoforms (compare conformations for ***(R,R)*-10c'** and ***(S,R)*-10c''** stereoisomers in the last column in Figure S1B). In fact, for the ***(R,R)*-9c''** and ***(S,R)*-9c'** stereoisomers, the lack of the C5 methyl allows the compounds to adopt all similar binding modes (Figure S1A), although not being always more potent than their cognate **10c** derivatives. Regarding the *(S)*-C6 configuration, the above described behavior (presence of C5 methyl group) is even more stressed: in no case ***(S,S)*-10c'** and ***(R,S)*-10c''** showed a putative binding conformation close to those of ***(R,R)*-10c'** and ***(S,R)*-10c''** configurations. Differently, for ***(S,S)*-9c''** and ***(R,S)*-9c'** four out of eight binding modes are close to those of ***(R,R)*-9c''** and ***(S,R)*-9c'** stereoisomers. This scenario is in good agreement with the experimental activities reported in Table 5. Interestingly, in the **9c** stereoisomers the decrease of IC_{50} values due to the *S* configuration at the C6-benzylic position is at certain level alleviated by the higher conformational flexibility due to the lack of the C5 methyl group. This is correctly recognized by the subsequent application of the COMBINEr model by predicting, in most cases, ***(S,S)*-9c''** and ***(R,S)*-9c'** more potent than their C5-methyl homologues ***(S,S)*-10c'** and ***(R,S)*-10c''**, respectively (Table 6).

For comparison purposes, the docking and binding mode analysis with the same procedure were also performed on the **13e** enantiomers.[13] Experimental activities for ***(R)*-13e** and ***(S)*-13e** were only determined against wild type RT,[13] nevertheless their binding modes (Figure S1C) against the

four considered isoforms were fully overlapping with those of the **9c** and **10c** stereoisomers (compare Figures S1A, B and C).

Once the binding mode of **9c** and **10c** had been estimated, as above anticipated, the previously reported COMBINEr model CM4 was readily applied to the eight stereoisomers. Apart the exact numerical values, the model successfully reproduced the experimental eudismic and RT isoform selectivity ratio (not shown) of most (*R*) and (*S*) enantiomers as well as the general activity trend (Table 6 and Figure S2 in Supporting Information). In general, the COMBINEr model is slightly over predictive: the greater error of prediction is obtained for ***(R,S)*-9c'**, likely due also to some inaccuracy in the binding mode proposed by Autodock Vina. As reported in Table 6, the experimental/predicted pIC_{50} matching supported the indirect assigned C6-benzyl configurations and aided to hypothesize the full absolute configurations for all the **9c** and **10c** stereogenic centres. In particular, regarding the *sec*-butyl absolute configuration and focusing on the two ***(R,R)*-10c'** and ***(S,R)*-10c''** diastereomers, from the Autodock Vina proposed binding modes into the wild type RT non-nucleoside binding site (NNBS), the slightly bulkier ethyl group seems to force the *sec*-butyl moiety to point towards a wider zone delimited by residues Val106, Phe227, Leu234, His235 and Pro236 (Figure 5). This zone identifies the opening of the channel that was found to be occupied by the delavirdine indole group[32] (Figure 5). In this context, the ***(R,R)*-10c'** *(R)-sec*-butyl configuration in the NNBS adopts a relaxed conformation without any particular repulsive interaction. On the other hand, the ***(S,R)*-10c''** *(S)-sec*-butyl configuration, to avoid steric clashes with the NNBS, forces a slight molecular rearrangement (Figure 5) leading to a minor loss of steric interactions (not shown). Furthermore, considering the ligand binding strain energies[13,33] listed in Table 6, the latter scenario is of particular advantage for the *(R)-sec*-butyl over the *(S)-sec*-butyl compound, and is in good agreement with the fact that *(R,R)* configurations are predicted more potent than the *(S,R)* ones (Table 6 and Table S3 in Supporting Information).

Table 6. COMBINEr model CM4 predicted activities (pIC_{50}) for **9c**, **10c** and **13e** stereoisomers. For direct comparison the experimental pID_{50}s are also reported.

compd[a]	wt		K103N		Y181I		L100I	
	expermtl	predct	expermtl	predct	expermtl	predct	expermtl	predct
***(R,S)*-9c'**	6.262	9.035	<4.699	4.531	<4.699	5.847	5.654	4.822
***(S,S)*-9c''**	7.570	9.029	5.211	4.690	<4.699	6.004	6.188	5.138
***(R,R)*-9c''**	8.425	9.277	5.792	5.362	4.850	5.793	7.163	6.932
***(S,R)*-9c'**	7.392	9.029	4.830	4.690	<4.699	6.596	6.829	7.134
***(R,R)*-10c'**	9.000	9.299	6.996	7.182	5.450	5.358	7.387	8.514
***(S,R)*-10c''**	7.854	9.323	6.012	6.001	<4.398	5.665	6.281	6.840
***(S,S)*-10c'**	5.331	5.623	<4.398	6.000	<4.398	5.355	6.040	5.137
***(R,S)*-10c''**	5.228	5.204	<4.398	5.457	<4.398	5.570	6.225	5.357
***(R)*-13e**	8.097[b]	9.194	ND[c]	ND	ND	ND	ND	ND
***(S)*-13e**	6.000[b]	7.775	ND	ND	ND	ND	ND	ND

[a]Compounds were listed according to their elution order. The underlined *R* or *S* indicates the absolute configuration of the *sec*-butyl chiral centre as suggested by modelling studies; [b]Ref. 13; [c]ND, not determined.

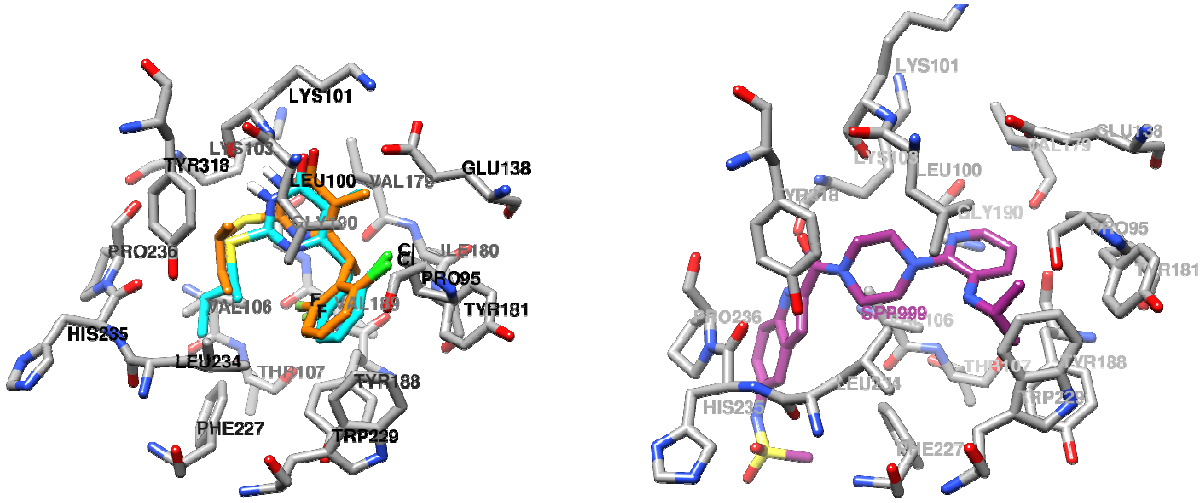


Figure 5. Left: ***(R,R)*-10c'** (orange) and ***(S,R)*-10c''** (cyan) binding mode comparison in wild type RT (gray). Right: Delavirdine in complex with RT (PDB entry code 1KLM).

Conclusions

New alkyl-*S*-DABOs bearing 2-chloro-6-fluoro substitution at the C6-benzyl position (2-Cl-6-F-*S*-DABOs, **7-12**) have been reported as NNRTIs active against wt HIV-1 (NL4-3 strain) as well as against a panel of clinically relevant HIV-1 mutant strains (K103N, Y181C, and Y188L). In addition, some previously reported[15,28] and newly synthesized F_2-*S*-DABOs (**13-15**) have been included in the study for comparison purposes and in order to evaluate the effects of the simultaneous alkyl substitution (Me, Et) at both C5 and C6-benzyl moiety of the pyrimidinone ring. In general terms, the cellular and enzyme assays here presented confirmed and significantly expanded the SAR information already known about the *S*-DABOs.[1-3,14,15,17,18] Indeed, among the four different substitutions studied in the present work (at the C6-benzyl moiety, at the methylene unit of the C6-benzyl moiety (R_1); at C5 position (R_2); at the C2-alkylthio chain (R_3)), the simultaneous double Me substitution at C5 and C6 in the 2-chloro-6-fluoro- and 2,6-difluoro-benzyl series, and the C5(Me)/C6(Et) disubstitution in the 2,6-difluoro-benzyl series joined to branched (*iso*-propyl and *sec*-butyl) and cyclic (cyclopentyl) alkyl substituents at C2 seem to be suitable to obtain the compounds with the highest, wide-spectrum inhibitory activity against HIV-1, with a prevalence of the 2-chloro-6-fluoro derivatives **10a**,**c**,**e** against wt virus and the mutant K103N, and a slight predominance of 2,6-difluoro compounds against Y181C (**13a**,**c**,**e**) and Y188L (**15a**,**c**,**e**) mutant strains. The 6-[1-(2-chloro-6-fluorophenyl)ethyl]-5-methyl-2-(cyclopentylthio)pyrimidin-4(3*H*)-one **10e** and the 6-[1-(2,6-difluorophenyl)propyl]-5-methyl-2-(*sec*-butylthio)pyrimidin-4(3*H*)-one **15c**, with EC_{50} values in the picomolar range against the wt virus [$EC_{50}s^{WT}$ (pM): 30 (**10e**) and 800 (**15c**)], and at nanomolar level versus the mutant strains [$EC_{50}s^{K103N}$ (nM): 93 (**10e**) and 200 (**15c**); $EC_{50}s^{Y181C}$: 230 (**10e**) and 300 (**15c**); $EC_{50}s^{Y188L}$: 2900 (**10e**) and 900 (**15c**)], were two of the compounds endowed with the highest, broad spectrum HIV-1 inhibitory activity, that

resulted much more potent than EFV against wt HIV-1, more active against the K103N variant, and similarly and less potent against the mutants Y188L and Y181C, respectively.

The enzymatic data were substantially in agreement with the cellular results highlighting the capability of the C5(Me)/C6(Et) substitution pattern to confer a very high activity against mutated RTs (K103N, Y181I, and L100I), in particular in the 2,6-difluoro-benzyl series (**15a**,**c**,**e**). Three of the most potent 2-chloro-6-fluoro alkyl-*S*-DABOs, carrying two (**9c**, **10c**) and one (**10a**) stereogenic centres in their structure, were resolved and characterized for their diastereo- and enantioselective activities in HIV-1-infected cells as well as in enzyme-based assays. Interestingly, in both cellular and enzymatic tests, it was possible to observe a significant diastereo- and enantioselectivity in HIV-1 inhibition, with the best inhibitors being the isomers **(*R*,*R*)-(-)-9c″**, **(*R*)-(-)-10a** and **(*R*,*R*)-(-)-10c′**, and it was possible to correlate the highest antiviral activity with the *R* absolute configuration to the stereogenic centre of the 2-chloro-6-fluoro C6-benzylic moiety. The *R* or *S* configuration at the C6-benzylic chiral centre of **9c** and **10c** was fully confirmed by the docking/COMBINEr calculations. Finally, further analyses led to the full **9c** and **10c** stereochemical characterization of both the *sec*-butyl and the C6-benzyl chiral atoms.

Experimental Section

Chemistry. Melting points were determined on a Buchi 530 melting point apparatus. ^{1}H-NMR spectra were recorded at 400 MHz using a Bruker AC 400 spectrometer; chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (Me_4Si). Mass spectra were recorded on a API-TOF Mariner by Perspective Biosystem (Stratford, Texas, USA), samples were injected by an Harvard pump using a flow rate of 5−10 μL/min, infused in the Electrospray system. All compounds were routinely checked by TLC and ^{1}H NMR. TLC was performed on aluminum-backed silica gel plates (Merck DC, Alufolien Kieselgel 60 F_{254}) with spots visualized by

UV light or using a $KMnO_4$ alkaline solution. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at reduced pressure of ~ 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Elemental analysis has been used to determine purity of the described compounds, that is >95%. Analytical results are within 0.40% of the theoretical values. All chemicals were purchased from Sigma Aldrich s.r.l., Milan (Italy) or from TCI Europe N.V., Zwijndrecht (Belgium), and were of the highest purity. As a rule, samples prepared for physical and biological studies were dried in high vacuum over P_2O_5 for 20h at temperatures ranging from 25 to 40 °C, depending on the sample melting point.

General Procedure for the Preparation of 2-(2,6-Dihalophenyl)alkanoic Acids (16a-d). Example: 2-(2-Chloro-6-fluorophenyl)butanoic acid (16b). A diethylamine (2.75 mL, 26.4 mmol) solution in dry THF (5.5 mL) was added dropwise at -78 °C to a buthyllithium (21.2 mL of 2.5 M solution in *n*-hexane, 52.8 mmol) solution in dry THF (11 mL) under nitrogen, and the resulting mixture was stirred at 0 °C for 0.5 h. A solution of 2-chloro-6-fluorophenylacetic acid (4.149 g, 22 mmol) in anhydrous THF (5 mL) was then added dropwise at -78 °C to the mixture. After 0.5 h of stirring at 0 °C, a solution of ethyl iodide (2.1 mL, 26.4 mmol) in dry THF (5.5 mL) was added at -78 °C. The mixture was gradually warmed from -78 °C to room temperature and stirred overnight. After completion the mixture was poured into water (120 mL) and extracted with ethyl acetate (3 × 40 mL). The aqueous layer was acidified with concentrated hydrochloric acid at 0 °C and then extracted with ethyl acetate (4 × 40 mL). The combined organic layers were washed with brine to neutral pH and dried with anhydrous sodium sulfate. Evaporation of the solvent gave the crude acidic fraction as an orange oil, which was purified by column chromatography (silica gel, ethyl acetate/chloroform 1/5) giving the expected product as a white powder. Yield: 80%. ^{1}H-NMR ($CDCl_3$): δ 0.89 (t, 3H, CH_2CH_3), 1.92 (m, 1H, $CHHCH_3$), 2.25 (m, 1H, $CHHCH_3$), 4.18 (m, 1H,

C*H*CH_2CH_3), 6.97-7.02 (m, 1H, C_4-H phenyl ring), 7.19-7.21 (m, 2H, $C_{3\text{-}5}$-H phenyl ring), 9.45 (br, 1H, COO*H*). MS**,** m/z: 215 $[M\text{-}H]^+$.

General Procedure for the Preparation of Ethyl 4-(2,6-Dihalophenyl)-3-oxoalkanoates and 2-Alkyl-4-(2,6-dihalophenyl)-3-oxoalkanoates (17a-i). Example : Ethyl 4-(2-Chloro-6-fluorophenyl)-2-methyl-3-oxohexanoate (17f). Triethylamine (10.7 mL, 76.8 mmol) and magnesium dichloride (5.71 g, 60.0 mmol) were added to a stirred suspension of potassium methylmalonate monoethyl ester (9.29 g, 50.4 mmol) in dry acetonitrile (76 mL) and stirring was continued at room temperature for 2 h. Then, a solution of the 2-(2-chloro-6-fluorophenyl)butanoic imidazolide in the same solvent [prepared 15 minutes before use by reaction between 2-(2-chloro-6-fluorophenyl)butanoic acid (5.2 g, 24 mmol) and *N,N′*-carbonyldiimidazole (4.67 g, 29 mmol) in dry acetonitrile (25 mL)] was added. The reaction mixture was then stirred overnight at room temperature and finally heated at reflux for 2 h. After the completion of the reaction the mixture was cooled to room temperature and the organic layer removed by rotary evaporation, 12% HCl (200 mL) was cautiously added, keeping the temperature below 25 °C, and the resulting clear mixture was stirred for a further 15 min. The aqueous layer was extracted with ethyl acetate (4 × 70 mL), and the combined organic layers were washed with a sodium hydrogen carbonate saturated solution (3 × 70 mL) and brine (3 × 70 mL), dried and concentrated to give pure **17f** as an oil, which was directly used in the following step without further purification. Yield: 78%. ^{1}H-NMR ($CDCl_3$) δ 0.84 (t, 3H, $CHCH_2CH_3$), 1.18-1.33 (m, 6H, $COOCH_2CH_3$ and $CHCH_3$), 1.75-1.80 (m, 1H, $CHCHHCH_3$), 2.26-2.30 (m, 1H, $CHCHHCH_3$), 3.44-3.46 (m, 1H, $CHCH_3$), 4.06-4.21 (m, 3H, $CHCH_2CH_3$ and $COOCH_2CH_3$), 6.99-7.03 (m, 1H, C_4-H phenyl ring), 7.18-7.25 (m, 2H, $C_{3,5}$-H phenyl ring). MS**,** m/z: 301 $[M+H]^+$.

General Procedure for the Preparation of 6-[1-(2,6-Dihalophenyl)alkyl]-2-thioxo-2,3-dihydropyrimidin-4(1*H*)ones and 6-[1-(2,6-Dihalophenyl)alkyl]- 5-alkyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)ones (18a-i). Example: 6-(2-Chloro-6-fluorobenzyl)-5-ethyl-2-thioxo-

2,3-dihydropyrimidin-4(1*H*)-one (18e). Sodium metal (1.08 g, 47.1 mmol) was dissolved in 25 mL of absolute ethanol, then thiourea (2.69 g, 35.3 mmol) and 2-ethyl-4-(2-chloro-6-fluorophenyl)-3-oxopentanoic acid ethyl ester (5.9 g, 19.6 mmol) were added to the clear solution. The resulting mixture was heated at reflux overnight. After cooling, the solvent was removed in vacuo at 40-50 °C and the residue was dissolved in water (20 mL) and made acidic with 2 N HCl, keeping the temperature below 25 °C. The aqueous layer was extracted with ethyl acetate (4 × 30 mL), then the organic phase was washed with brine (2 × 15 mL), dried and concentrated under reduced pressure to give crude **18e**, which was purified by recrystallization from ethyl acetate. Yield: 55%. ^{1}H-NMR (DMSO) δ 3.89 0.42 (t, 2H, CH_2CH_3), 1.60 (d, 3H, $CHCH_3$), 2.00-2.07 (m, 2H, CH_2CH_3), 4.55 (q, 1H, $CHCH_3$), 7.21-7.26 (m, 1H, C_4-H phenyl ring), 7.35-7.37 (m, 2H, $C_{3,5}$-H phenyl ring), 11,40 (s, 1H, *NH*), 12.51 (s, 1H, *NH*). MS**,** m/z: 313 $[M+H]^+$.

General Procedure for the Preparation of 6-[1-(2,6-Dihalophenyl)alkyl]-2-alkylthiopyrimidin-4(3*H*)ones and 6-[1-(2,6-Dihalophenyl)alkyl]-5-alkyl-2-alkylthiopyrimidin-4(3*H*)ones (7-15). Example: 6-[1-(2-Chloro-6-fluorophenyl)ethyl]-5-methyl-2-*sec*-butylthio-pyrimidin-4(3*H*)-one (10c). Anhydrous potassium carbonate (117 mg, 0.85 mmol) and 2-iodobutane (1.1 eq, 0.097 mL, 0.85 mmol) were added in sequence to a suspension of 6-[1-(2-chloro-6-fluorophenyl)ethyl]-5-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **18d** (230 mg, 0.77 mmol) in dry DMF (1.0 mL). After stirring for 5 h at room temperature, the mixture was quenched with cold water (10 mL), stirred for 1 h and filtered. The obtained solid residue was recrystallized from acetonitrile to provide pure **10c** as a white solid. Yield: 71%. ^{1}H-NMR ($CDCl_3$) δ 0.91-0.95 and 1.00-1.04 (2t, 3H, $SCH(CH_3)CH_2CH_3$ first enantiomeric pair + $SCH(CH_3)CH_2CH_3$ second enantiomeric pair), 1.27-1.28 and 1.39-1.41 (2d, 3H, $SCH(CH_3)CH_2CH_3$ first enantiomeric pair + $SCH(CH_3)CH_2CH_3$ second enantiomeric pair), 1.59-1.75 (m, 5H, Ar-$CHCH_3$ and $SCH(CH_3)CH_2CH_3$), 1.90 (s, 3H, CH_3 at C_5 pyrimidine ring), 3.84-3.86 (m, 1H,

SC*H*(CH_3)CH_2CH_3), 4.67-4.68 (q, 1H, Ar-C*H*CH3), 6.90-6.93 (m, 1H, C_4-H phenyl ring), 7.12-7.16 (m, 2H, $C_{3,5}$-H phenyl ring), 12.35 (s, 1H, *NH*). MS, m/z: 355 $[M+H]^+$.

HPLC Stereoseparation. HPLC stereoseparations were performed by using the stainless-steel Chiralpak IA (250 mm x 4.6 mm i.d. and 250 x 10 mm i.d.) (Chiral Technologies Europe, Illkirch, France) columns. All chemicals solvents for HPLC, synthesis and spectral grade solvents were purchased from Aldrich (Italy) and used without further purification. The analytical HPLC apparatus consisted of a Perkin-Elmer (Norwalk, CT, USA) 200 LC pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 20-µl sample loop, a HPLC Dionex CC-100 oven (Sunnyvale, CA, USA) and a Jasco (Jasco, Tokyo, Japan) Model CD 2095 Plus UV/CD detector. For semipreparative separations a Perkin-Elmer 200 LC pump equipped with a Rheodyne injector, a 500 µL sample loop, a Perkin-Elmer LC 101 oven and Waters 484 detector (Waters Corporation, Milford, MA, USA) were used. The signal was acquired and processed by Clarity software (DataApex, Prague, The Czech Republic). The CSP/mobile phase system and the corresponding analytical chromatographic data for each resolved compound are summarized as follows: **9c**: Chiralpak IA/*n*-hexane-ethanol 95:5 (v/v), k_a = 2.14, k_b = 2.41 k_c = 2.83 k_d = 3.55; **10a**: Chiralpak IA/ethyl acetate, k_a = 1.10, k_b = 1.72;**10c**: Chiralpak IA/ethyl acetate, k_a = 0.95, k_b = 0.95, k_c = 1.86 k_d = 2.22; the mixture **10c**-a+b was separated using *n*-hexane-THF 90:10 (v/v) as mobile phase (k_a = 9.55, k_b = 9.85). k_a: retention factor of the first eluted stereoisomer, defined as $(t_a - t_0)/t_0$ where t_0 is the void time of the column.

Chiroptical Characterization. Specific rotations of stereoisomers of **9c**, **10a** and **10c**, dissolved in ethanol, were measured at 589 nm by a Perkin-Elmer polarimeter model 241 equipped with a Na/Hg lamp. The volume of the cell was 1 ml and the optical path was 10 cm. The system was set at a temperature of 20 °C. The specific rotation values were: (+)-**9c'**: $[\alpha]_D^{20}$ +94 (0.14, EtOH) (first eluted stereoisomer); (-)-**9c'**: $[\alpha]_D^{20}$ -95, (0.13, EtOH) (fourth eluted stereoisomer); (+)-

9c'': $[\alpha]_D^{20}$ +59 (0.12, EtOH) (second eluted stereoisomer); (-)-**9c''**: $[\alpha]_D^{20}$ -60 (0.12, EtOH) (third eluted stereoisomer); (-)-**10a**: $[\alpha]_D^{20}$ -86 (0.12, EtOH) (first eluted enantiomer); (+)-**10a** : $[\alpha]_D^{20}$ +87 (0.12, EtOH) (second eluted enantiomer); (-)-**10c'**: $[\alpha]_D^{20}$ -81 (0.12, EtOH) (first eluted stereoisomer); (+)-**10c'**: $[\alpha]_D^{20}$ +81 (0.12, EtOH) (third eluted stereoisomer); (-)-**10c''**: $[\alpha]_D^{20}$ -133 (0.12, EtOH) (second eluted stereoisomer); (+)-**10c''**: $[\alpha]_D^{20}$ +134 (0.12, EtOH) (fourth eluted stereoisomer). The CD spectra were recorded in ethanol solution (concentration about 0.3 mg/mL), in a quartz cell (0.1 cm-path length) at 25 °C by using a Jasco Model J-700 spectropolarimeter. The spectra are average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg).

Biology. Anti-HIV Activity in Lymphoid Cells. Biological activity of the compounds was tested in the lymphoid MT-4 cell line (received from the NIH AIDS Reagent Program) against the wt HIV-1 NL4-3 strain and three different HIV-1 strains, as described before.[34-36] Briefly, MT-4 cells were infected with the appropriate HIV-1 strain (or mock-infected to determine cytotoxicity) in the presence of different drug concentrations. At day five post-infection, a tetrazolium-based colorimetric method (MTT method) was used to evaluate the number of viable cells. The HIV-1 K103N, Y181C, or Y188L mutant were received from the Medical Research Council Centralised Facility for AIDS Reagents, Herefordshire, UK.

Anti-HIV Reverse Transcriptase Assays. RNA-dependent DNA polymerase activity was assayed as described[37] in the presence of 0.5 µg of poly(rA)/oligo(dT)$_{10:1}$ (0.3 µM 3'-OH ends), 10 µM [3H]-dTTP (1 Ci/mmol) and 2-4 nM RT in the presence of 8% final concentration of DMSO.

Reagents. [^{3}H]-dTTP (40 Ci/mmol) was from Amersham and unlabelled dNTP's from Boehringer. Whatman was the supplier of the GF/C filters. All other reagents were of analytical grade and purchased from Merck or Fluka. The homopolymer poly(rA) (Pharmacia) was mixed at

weight ratios in nucleotides of 10:1, to the oligomer oligo(dT)$_{12-18}$ (Pharmacia) in 20 mM Tris-HCl (pH 8.0), containing 20 mM KCl and 1 mM EDTA, heated at 65 °C for 5 min and then slowly cooled at room temperature.

Proteins. Recombinant proteins expression and purification was as described.[37] All enzymes were purified to > 95% purity.

RT Inhibition Assays. Time-dependent incorporation of radioactive nucleotides into poly(rA)/oligo(dT)$_{10:1}$ at different nucleotide substrate concentrations was monitored by removing 25 µL-aliquots at 2-min time intervals. Initial velocities of the reaction were then plotted against the corresponding substrate concentrations. For inhibition constant (ID_{50}) determination, an interval of inhibitor concentrations between 0.2 ID_{50} and 5 ID_{50} was used in the inhibition assays. ID_{50} values were determined with computer-aided curve fitting of the experimental data to a fully non-competitive model. Curve fitting was performed with the program GraphPad Prism 3.0.

Molecular Modeling, Docking Calculations and COMBINEr Predictions. All molecular modeling and docking calculations were performed as previously reported[31] on a 6 blades (8 Intel-Xeon E5520 2.27 GHz CPU and 24 GB DDR3 RAM each) cluster (48 CPU total) running the Debian GNU/Linux 6.03 operating system. The reported COMBINERr CM4 model[31] was used directly to predict the pIC_{50} of the **9c**, **10c** and **13e** stereoisomers. Starting **9c**, **10c** and **13e** conformations were modeled with the UCSF Chimera build module using as starting template the previous reported *NH*-DABOs.[25] The strain energy referred in Table 6 is defined as the energy difference between the docked conformation and the calculated global minima. Conformational energies were calculated using the obenergy command (MMFF94s force field) of the openbabel opensource utilities.[38] Conformational searches on isolated ***(R,R)*-10c** and ***(S,R)*-10c** were conducted with the TINKER Molecular Modeling Package[39] by a simulated annealing procedure using 200 iterations and 1000 steps. As control the conformational energies calculated by TINKER and obenergy were in very good agreement (not shown).

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Supporting Information Available: Chemical and physical data of compounds **7-15**. Elemental analyses of compounds **7-15**. ^{1}H-NMR and MS data for compounds **7-15**. Molecular modelling supplemental figures. This material is available free of charge via the Internet at http://pubs.acs.org.

Abbreviations used: CC_{50}, compound concentration toxic for 50% of cells; CD, circular dichroism; CSP, chiral stationary phase; DABOs, dihydro-alkoxy-benzyl-oxopyrimidines; DAPYs, diarylpyrimidines; EC_{50}, effective concentration 50, concentration needed to protect 50% of cells from the HIV-1 induced cytopathogenicity; EFV, efavirenz; F_2-*N,N*-DABOs, 5-alkyl-2-(*N,N*-disubstituted)amino-6-(2,6-difluorophenylalkyl)pyrimidin-4(3*H*)ones; HIV, human immunodeficiency virus; ID_{50}, inhibitory dose 50, compound dose required to inhibit HIV-1 rRT activity by 50%; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *NH*-DABOs, dihydro-alkylamino-benzyl-oxopyrimidines; NNBS, non-nucleoside binding site; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NVP, nevirapine; RT, reverse transcriptase; SAR, structure-activity relationship; *S*-DABOs, dihydro-alkylthio-benzyl-oxopyrimidines; wt, wild type.

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Table of Contents graphic

Exploring the Role of 2-Chloro-6-Fluoro Substitution in 2-Alkylthio-6-benzyl-5-alkylpyrimidin-4(3*H*)-ones: Effects in HIV-1-Infected Cells and in HIV-1 Reverse Transcriptase Enzymes

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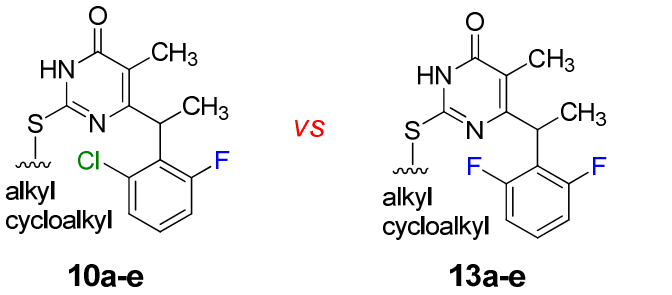

10a-e **13a-e**

inhibition range in HIV-1 infected cells

wt: pM
K103N or Y181C: sub-μM

wt: nM
K103N or Y181C: (sub)μM

inhibition range of HIV-1 RTs

wt: nM/pM
K103N: single-digit or sub-μM
L100I: sub-μM

wt: nM
K103N or L100I: sub-μM