Synthesis and Biological Properties of Novel 2-Aminopyrimidin-4(3*H*)-ones Highly Potent against HIV-1 Mutant Strains

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Following the disclosure of dihydro-alkoxy-, dihydro-alkylthio-, and dihydro-alkylamino-benzyl-oxopyrimidines (DABOs, *S*-DABOs, and *NH*-DABOs) as potent and selective anti-HIV-1 agents belonging to the non-nucleoside reverse transcriptase inhibitor (NNRTI) class, we report here the synthesis and biological evaluation of a novel series of DABOs bearing a *N*,*N*-disubstituted amino group or a cyclic amine at the pyrimidine-C_2 position, a hydrogen atom or a small alkyl group at C_5 and/or at the benzylic position, and the favorable 2,6-difluorobenzyl moiety at the C_6 position (F_2-*N*,*N*-DABOs). The new compounds were highly active up to the subnanomolar level against both wt HIV-1 and the Y181C mutant and at the submicromolar to nanomolar range against the K103N and Y188L mutant strains. Such derivatives were more potent than *S*-DABOs, *NH*-DABOs, and nevirapine and efavirenz were chosen as reference drugs. The higher inhibitor adaptability to the HIV-1 RT non-nucleoside binding site (NNBS) may account for the higher inhibitory effect exerted by the new molecules against the mutated RTs.

Introduction

Human immunodeficiency virus (HIV)[a1] is the etiological agent of acquired immunodeficiency syndrome (AIDS), an infection characterized by loss of helper T lymphocytes and heavy damage of lymphatic tissues.[1] The current therapy against AIDS is based on four classes of anti-HIV drugs: the nucleoside and nucleotide reverse transcriptase (RT) inhibitors (indicated as NRTIs and NtRTIs, respectively), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), the protease inhibitors (PIs), and the fusion inhibitors (FIs).[2,3] NRTIs, NNRTIs, and PIs are actually mixed in the highly active antiretroviral therapy (HAART), which dramatically reduces the incidence of HIV infection and death. Despite HAART combination regimens that have significantly decreased the morbidity and mortality among patients with HIV infections, slowing viral replication to very low levels, they are still unable to eradicate the retroviral infection.[4] Thus, the needed long-term or permanent use of anti-AIDS drugs induces the selection of drug-resistant viral mutants and the emergence of undesired metabolic side effects.[4] Therefore, although the HIV treating physician has a multitude of agents to choose from, rapid development of drug resistance, long-term reduced compliance, and toxicity problems demand the discovery of novel anti-HIV agents effective against resistant mutants without unpleasant side effects. Actually, the search for novel anti-HIV drugs is pursued either by improvement of the existing drug classes (NRTIs, NtRTIs, NNRTIs, PIs, and FIs) or by the discovery of new agents showing different mechanisms of action (such as inhibition of integrase, Rnase-H, and viral entry).[5]

The NNRTIs identified so far include more than fifty structurally quite different classes of molecules.[6] The marketed drugs nevirapine, delavirdine, and efavirenz are significant examples of such inhibitors (Chart 1). Despite their low toxicity and favorable pharmacokinetic properties, the use of first-generation NNRTIs both in monotherapy and in multidrug anti-AIDS cocktails led to unsuccessful results due to the rapid emergence of drug resistance.[7] As a consequence, in the NNRTI field, the efforts are now focused on the development of novel compounds endowed with higher anti-HIV-1 activity and a resistance profile different from that of known drugs (second generation NNRTIs). Continuous efforts in this field are documented by the wide number of NNRTIs described in the recent literature,[2,5,8−10] some of which, such as capravirine[11] and etravirine (TMC125)[12] (Chart 1) are under clinical trials.

Dihydro-alkoxy-benzyl-oxopyrimidines (DABOs) were disclosed by our group in 1992 as a novel NNRTI class.[13−15] Since then, a great number of oxopyrimidines were synthesized and tested as anti-HIV-1 agents to obtain more potent and selective compounds.[16−28] In addition, the pyrimidin-4(3*H*)-one scaffold attracted the interest of other authors for the design of new anti-

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[a] Abbreviations: DABOs, dihydro-alkoxy-benzyl-oxopyrimidines; HIV, human immunodeficiency virus; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NNBS, non-nucleoside binding site; RT, reverse transcriptase; AIDS, acquired immunodeficiency syndrome; NRTIs, nucleoside reverse transcriptase inhibitors; NtRTIs, nucleotide reverse transcriptase inhibitors; PIs, protease inhibitors; FIs, fusion inhibitors; HAART, highly active antiretroviral therapy; SAR, structure-activity relationship; *S*-DABOs, dihydro-alkylthio-benzyl-oxopyrimidines; *NH*-DABOs, dihydro-alkylamino-benzyl-oxopyrimidines; F_2-*N*,*N*-DABOs, 5-alkyl-2-(*N*,*N*-disubstituted)-amino-6-(2,6-difluorophenylalkyl)-3,4-dihydropyrimidin-4(3*H*)ones; NVP, nevirapine; EFV, efavirenz; EC_{50}, effective concentration able to protect 50% of cells from the HIV-1 induced cytopathogenicity; CC_{50}, compound concentration toxic for 50% of cells; WT, wild type; RCSB, research collaboratory for structural bioinformatics; PDB, protein data bank; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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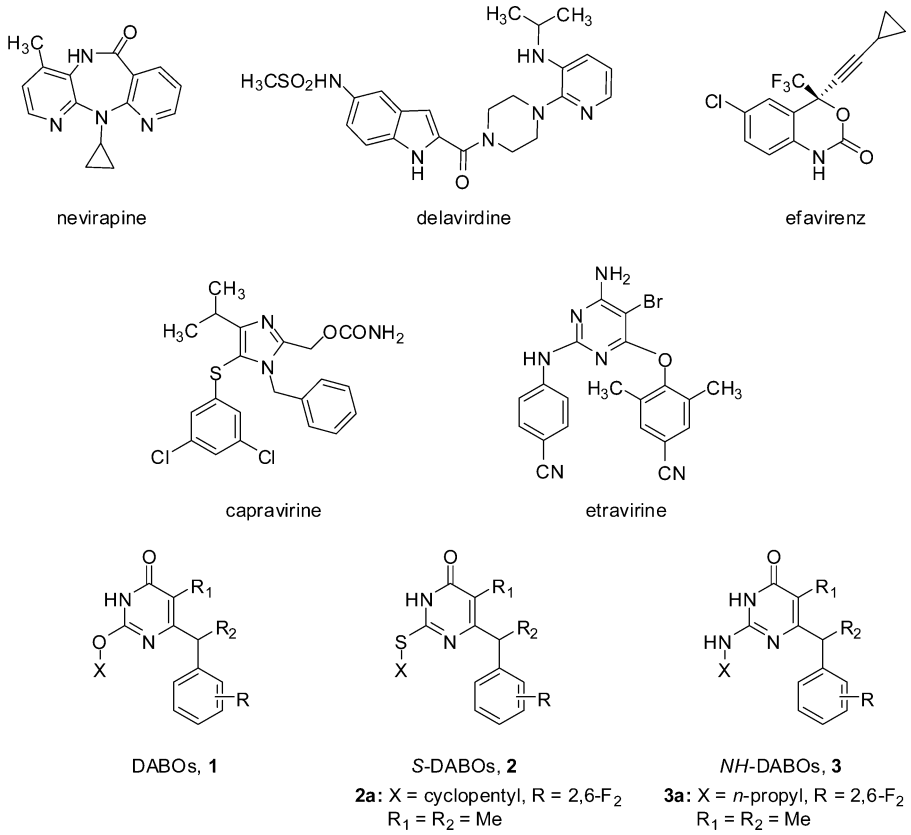
Chart 1. Known Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors



HIV agents,[29−35] and some DABO-related triazines and pyrimidines were described by the Janssen group as highly potent, wide spectrum NNRTIs, including the clinical candidate etravirine.[36−40]

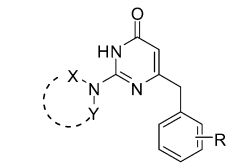
A structure−activity relationship (SAR) profile of DABOs together with molecular modeling investigation on their putative binding mode have shown that the presence of a C_2-alkoxy (DABOs, **1**), C_2-alkylthio (*S*-DABOs, **2**), or C_2-alkylamino (*NH*-DABOs, **3**) side chain is a structural determinant for the antiviral activity of these derivatives, with the length and size of the C_2 side chain having only modulator effects on potency (Chart 1).[16−19,22,26,28] The 2,6-difluoro substitution at the C_6-benzyl moiety of *S*-DABOs and *NH*-DABOs produced favorable π-stacking interactions with the Tyr188 side chain into the non-nucleoside binding site (NNBS), leading to compounds (F_2-*S*-DABOs and F_2-*NH*-DABOs) active in the nanomolar range.[22,26−28] Moreover, the insertion of two methyl groups, one at the pyrimidine C_5 position, and the second one on the methylene connecting pyrimidine and phenyl rings (benzylic position), furnished new conformationally restricted compounds exhibiting nanomolar activity against wild-type HIV-1 and submicromolar activity against the Y181C mutant strain.[26] Docking experiments performed on F_2-*NH*-DABOs confirmed that their further anchor point (hydrogen bond of C_2−NH function with Lys101) into the NNBS produced a tight ligand binding that may compensate the incoming lack of positive hydrophobic ligand/NNBS interactions due to Tyr181 to Cys181 mutation.[27,28]

On the other hand, the F_2-*NH*-DABO further anchor point is a source of some strictness in the ligand/enzyme interaction; moreover, cross-docking experiments[41] performed on 68 different NNRTI/RT complexes suggested that a double *N*,*N* substitution on the 2-amino-DABO template, though preventing the capability of the C_2−NH group to establish a further hydrogen bond into the NNBS, can confer an implicit increased capacity to re-adapt the obtained molecules into different experimental NNBS conformations. This implies that such a flexibility could lead to a broader range of RT inhibitors endowed with high activity against both wild-type HIV-1 and resistant mutants.

Prompted by these computational hypotheses, and to establish further SAR on 2-amino-DABO derivatives, we prepared a novel series of *N*,*N*-DABOs (**4**−**7**) with a C_2-dialkylamino or -aza(hetero)cycloalkyl side chain, with a hydrogen, methyl, or ethyl substituent at the pyrimidine C_5 and/or the C_6-benzylic position and with the highly favorable 2,6-difluoro substitution at the C_6-phenylalkyl group (Chart 2).

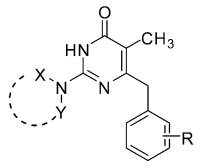
Chemistry. Title compounds were routinely synthesized by nucleophilic displacement of the methylthio group from the 5-alkyl-6-(2,6-difluorophenylalkyl)-2-methylthiopyrimidin-4(*3H*)-ones **8a**−**d**[22,26] (a: $R_1 = R_2 = H$; b: $R_1 = Me$, $R_2 = H$; c: $R_1 = H$, $R_2 = Me$; d: $R_1 = R_2 = Me$) with the appropriate secondary or cyclic amines, by heating the two starting materials in a sealed tube at 170 °C for 8 h. Alternatively, with low-boiling amines (*N*-methyl-*N*-ethylamine, *N*-methyl-*N*-*n*-propylamine, *N*-methyl-*N*-*iso*-propylamine), some *N*,*N*-DABOs were obtained by heating the proper 2-methylthiopyrimidinone with the amine as an acetate salt in an oil bath at 160 °C for 12 h (Scheme 1, A). Condensation of *N*,*N*-dimethylguanidine sulfate with the ethyl 2,4-dialkyl-3-oxo-4-substitutedphenylbutyrates **9a**−**h** (a: R = 2,6-Cl_2, $R_1 = R_2 = Me$; b: R = 2-F, $R_1 = R_2 = Me$; c: R = 2,6-F_2, $R_1 = Me$, $R_2 = Et$; d: R = 2,6-F_2, $R_1 = Et$, $R_2 = Me$; e: R = 2,6-F_2, $R_1 = R_2 = H$; f: R = 2,6-F_2, $R_1 = Me$, $R_2 = H$; g: R = 2,6-F_2, $R_1 = H$, $R_2 = Me$; h: R = 2,6-F_2, $R_1 = R_2 = Me$) in the presence of sodium ethoxide furnished the 2-*N*,*N*-dimethylaminopyrimidinones **4a**, **5a**, **6a**, **6c**, and **7a**−**e**. The unknown β-oxoesters **9a**−**d** were prepared by reaction of the 2-(2,6-dichlorophenyl)propionic acid

Chart 2. Novel 5-Alkyl-2-(*N*,*N*-disubstituted)amino-6-(2,6-difluorophenylalkyl)-3,4-dihydropyrimidin-4(3*H*)-ones (F_2-*N*,*N*-DABOs) and Related Derivatives **4**−**7**



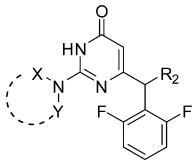
4a-q

X, Y = alkyl chains, (hetero)cycloalkane
R = 2,6-F_2, 2-F-6-pyrrolidinyl



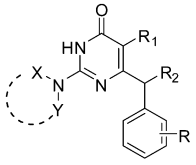
5a-u

X, Y = alkyl chains, (hetero)cycloalkane
R = 2,6-F_2, 2-F-6-pyrrolidinyl



6a-c

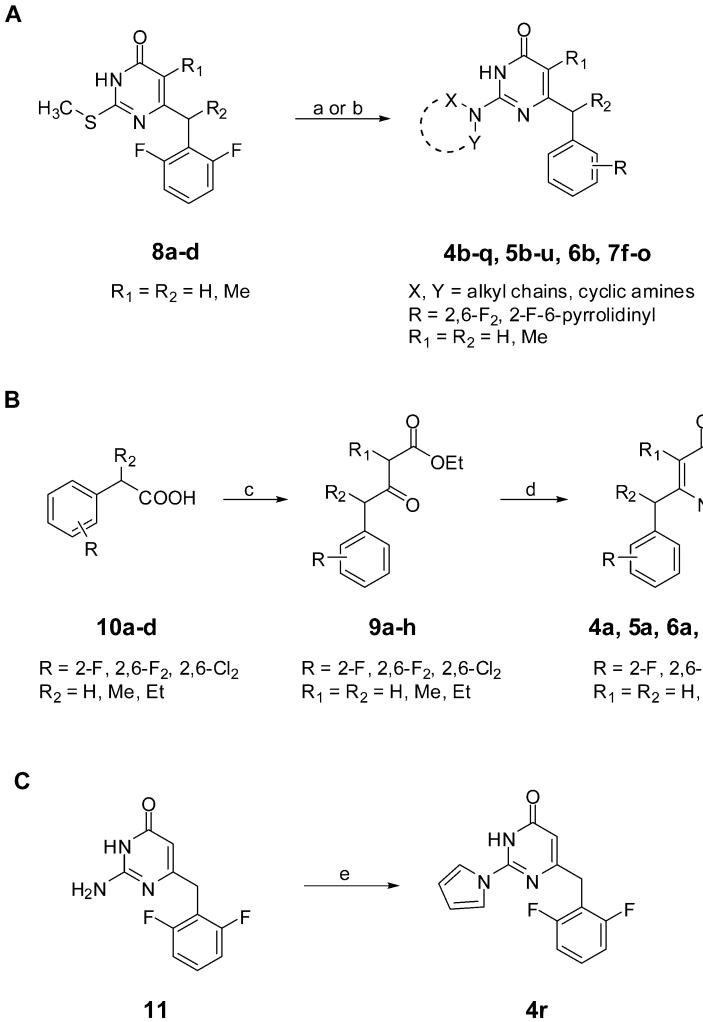
X, Y = alkyl chains
R_2 = Me, Et



7a-o

X, Y = alkyl chains, (hetero)cycloalkane
R = 2,6-F_2, 2,6-Cl_2, 2-F; R_1 = R_2 = Me, Et

Scheme 1[a]



[a] (a) XYNH, 170 °C, sealed tube; (b) XYNH·CH_3COOH, 160 °C, oil bath; (c) (1) $SOCl_2$, room temperature, (2) $EtOCOCHR_1COOK$, $(Et)_3N$, $MgCl_2$, MeCN, room temperature, (3) 13% HCl, room temperature; (d) *N*,*N*-dimethylguanidine sulfate, EtONa, EtOH, reflux; (e) 2,5-dimethoxytetrahydrofuran, CH_3COOH, reflux.

(**10a**),[26] or 2-(2-fluorophenyl)propionic acid (**10b**), or 2-(2,6-difluorophenyl)propionic acid (**10c**),[26] or 2-(2,6-difluorophenyl)-butyric acid (**10d**)[26] with (*i*) thionyl chloride, (*ii*) potassium ethyl malonate or potassium ethyl 2-methyl- or 2-ethylmalonate in the presence of triethylamine and magnesium dichloride, and (*iii*) acidic hydrolysis with 13% HCl (part B of Scheme 1).

Table 1. Physical and Chemical Data for **4**−**7**

cpd	R	R_1	R_2	X, Y	mp, °C	recryst solvent[a]	synth way[b]	% yield	formula[c]
4a	2,6-F_2	H	H	Me, Me	210−211	A	d	85	$C_{13}H_{13}F_2N_3O$
4b	2,6-F_2	H	H	Me, Et	150−151	B	b	72	$C_{14}H_{15}F_2N_3O$
4c	2,6-F_2	H	H	Me, *i*-Pr	153−154	C	b	64	$C_{15}H_{17}F_2N_3O$
4d	2,6-F_2	H	H	Me, *n*-Pr	150−151	A	b	41	$C_{15}H_{17}F_2N_3O$
4e	2,6-F_2	H	H	Et, Et	159−160	B	a	87	$C_{15}H_{17}F_2N_3O$
4f	2,6-F_2	H	H	*n*-Pr, *n*-Pr	111−112	C	a	86	$C_{17}H_{21}F_2N_3O$
4g	2,6-F_2	H	H	pyrrolidin-1-yl	238−239	B	a	23	$C_{15}H_{15}F_2N_3O$
4h	2-F-6-pyrrolidin-1-yl	H	H	pyrrolidin-1-yl	233−234	B	a	39	$C_{19}H_{23}FN_4O$
4i	2,6-F_2	H	H	piperidin-1-yl	209−210	B	a	51	$C_{16}H_{17}F_2N_3O$
4j	2,6-F_2	H	H	2-Me-piperidin-1-yl	215−216	D	a	49	$C_{17}H_{19}F_2N_3O$
4k	2,6-F_2	H	H	3-Me-piperidin-1-yl	181−182	B	a	53	$C_{17}H_{19}F_2N_3O$
4l	2,6-F_2	H	H	4-Me-piperidin-1-yl	183−184	E	a	55	$C_{17}H_{19}F_2N_3O$
4m	2,6-F_2	H	H	hexahydroazepin-1-yl	174−175	B	a	48	$C_{17}H_{19}F_2N_3O$
4n	2,6-F_2	H	H	morpholin-4-yl	215−216	B	a	63	$C_{15}H_{15}F_2N_3O_2$
4o	2,6-F_2	H	H	thiomorpholin-4-yl	233−234	B	a	57	$C_{15}H_{15}F_2N_3OS$
4p	2,6-F_2	H	H	4-Me-piperazin-1-yl	195−196	B	a	40	$C_{16}H_{18}F_2N_4O$
4q	2,6-F_2	H	H	1,4-dioxa-8-aza spiro[4.5]decan-8-yl	202−203	B	a	54	$C_{18}H_{19}F_2N_3O_3$
4r	2,6-F_2	H	H	1*H*-pyrrol-1-yl	235−236	B	e	43	$C_{15}H_{11}F_2N_3O$
5a	2,6-F_2	Me	H	Me, Me	237−238	B	d	68	$C_{14}H_{15}F_2N_3O$
5b	2,6-F_2	Me	H	Me, Et	191−192	B	b	51	$C_{15}H_{17}F_2N_3O$
5c	2,6-F_2	Me	H	Me, *i*-Pr	176−178	B	b	86	$C_{16}H_{19}F_2N_3O$
5d	2,6-F_2	Me	H	Me, *n*-Pr	173−174	B	b	68	$C_{16}H_{19}F_2N_3O$
5e	2,6-F_2	Me	H	Me, *c*-hex	170−171	B	a	44	$C_{19}H_{23}F_2N_3O$
5f	2,6-F_2	Me	H	Me, Ph	188−189	B	a	39	$C_{19}H_{17}F_2N_3O$
5g	2,6-F_2	Me	H	Et, Et	168−169	B	a	82	$C_{16}H_{19}F_2N_3O$
5h	2,6-F_2	Me	H	*n*-Pr, *n*-Pr	142−143	F	a	75	$C_{18}H_{23}F_2N_3O$
5i	2,6-F_2	Me	H	pyrrolidin-1-yl	229−230	B	a	8	$C_{16}H_{17}F_2N_3O$
5j	2-F-6-pyrrolidin-1-yl	Me	H	pyrrolidin-1-yl	219−220	B	a	35	$C_{20}H_{25}FN_4O$
5k	2,6-F_2	Me	H	piperidin-1-yl	210−211	B	a	53	$C_{17}H_{19}F_2N_3O$
5l	2,6-F_2	Me	H	2-Me-piperidin-1-yl	134−135	A	a	51	$C_{18}H_{21}F_2N_3O$
5m	2,6-F_2	Me	H	3-Me-piperidin-1-yl	201−202	B	a	47	$C_{18}H_{21}F_2N_3O$
5n	2,6-F_2	Me	H	4-Me-piperidin-1-yl	261−262	B	a	52	$C_{18}H_{21}F_2N_3O$
5o	2,6-F_2	Me	H	hexahydroazepin-1-yl	224−225	B	a	56	$C_{18}H_{21}F_2N_3O$
5p	2,6-F_2	Me	H	morpholin-4-yl	244−245	B	a	49	$C_{16}H_{17}F_2N_3O_2$
5q	2,6-F_2	Me	H	thiomorpholin-4-yl	255−256	B	a	56	$C_{16}H_{17}F_2N_3OS$
5r	2,6-F_2	Me	H	4-Me-piperazin-1-yl	235−236	B	a	25	$C_{17}H_{20}F_2N_4O$
5s	2,6-F_2	Me	H	1,4-dioxa-8-aza spiro[4.5]decan-8-yl	232−233	B	a	48	$C_{19}H_{21}F_2N_3O_3$
5t	2,6-F_2	Me	H	4-(2-MeO−Ph)-1-piperazinyl	197−198	B	a	51	$C_{23}H_{24}F_2N_4O_2$
5u	2,6-F_2	Me	H	4-(2-pyridinyl)-1-piperazinyl	255−256	G	a	46	$C_{21}H_{21}F_2N_5O$
6a	2,6-F_2	H	Me	Me, Me	198−199	A	d	78	$C_{14}H_{15}F_2N_3O$
6b	2,6-F_2	H	Me	Me, *n*-Pr	137−138	H	b	31	$C_{16}H_{19}F_2N_3O$
6c	2,6-F_2	H	Et	Me, Me	136−138	B	d	72	$C_{15}H_{17}F_2N_3O$
7a	2,6-F_2	Me	Me	Me, Me	174−175	H	d	73	$C_{15}H_{17}F_2N_3O$
7b	2,6-Cl_2	Me	Me	Me, Me	>200	B	d	61	$C_{15}H_{17}Cl_2N_3O$
7c	2-F	Me	Me	Me, Me	>200	B	d	65	$C_{15}H_{18}FN_3O$
7d	2,6-F_2	Me	Et	Me, Me	148−149	C	d	72	$C_{16}H_{19}F_2N_3O$
7e	2,6-F_2	Et	Me	Me, Me	145−146	I	d	84	$C_{16}H_{19}F_2N_3O$
7f	2,6-F_2	Me	Me	Me, Et	166−167	C	b	81	$C_{16}H_{19}F_2N_3O$
7g	2,6-F_2	Me	Me	Me, *i*-Pr	156.5−157.0	D	b	58	$C_{17}H_{21}F_2N_3O$
7h	2,6-F_2	Me	Me	Me, *n*-Pr	128−130	J	b	38	$C_{17}H_{21}F_2N_3O$
7i	2,6-F_2	Me	Me	pyrrolidin-1-yl	188−189	B	a	32	$C_{17}H_{19}F_2N_3O$
7j	2,6-F_2	Me	Me	piperidin-1-yl	151−152	B	a	57	$C_{18}H_{21}F_2N_3O$
7k	2,6-F_2	Me	Me	3-Me-piperidin-1-yl	152−153	C	a	48	$C_{19}H_{23}F_2N_3O$
7l	2,6-F_2	Me	Me	4-Me-piperidin-1-yl	165−166	C	a	53	$C_{19}H_{23}F_2N_3O$
7m	2,6-F_2	Me	Me	hexahydroazepin-1-yl	188−189	B	a	55	$C_{19}H_{23}F_2N_3O$
7n	2,6-F_2	Me	Me	morpholin-4-yl	193−194	B	a	51	$C_{17}H_{19}F_2N_3O_2$
7o	2,6-F_2	Me	Me	thiomorpholin-4-yl	219−220	B	a	55	$C_{17}H_{19}F_2N_3OS$

[a] A: cyclohexane/benzene; B: acetonitrile; C: *n*-hexane; D: benzene/acetonitrile; E: acetonitrile/diethyl ether; F: petroleum ether; G: ethanol/water; H: cyclohexane; I: diethyl ether; J: dichloromethane/diethyl ether. [b] See Scheme 1. [c] Analytic results were within ±0.40% of the theoretical values.

Finally, the 1*H*-pyrrol-1-yl derivative **4r** was prepared from the 2-amino-6-(2,6-difluorobenzyl)pyrimidin-4(3*H*)-one **11**[28] with 2,5-dimethoxytetrahydrofuran and glacial acetic acid under Clauson−Kaas conditions (part C of Scheme 1).

Compounds **7** sharing a chiral center at the benzylic position were prepared and tested as racemates. Chemical and physical data of **4**−**7** are listed in Table 1. Chemical and physical data of the intermediate compounds **9a**−**d** and **10b** are listed in Table 2.

Table 2. Physical and Chemical Data for **9a**−**d** and **10b**

cpd	R	R_1	R_2	mp, °C	% yield	formula[a]
9a	2,6-Cl_2	Me	Me	oil	69	$C_{14}H_{16}Cl_2O_3$
9b	2-F	Me	Me	oil	81	$C_{14}H_{17}FO_3$
9c	2,6-F_2	Me	Et	oil	75	$C_{15}H_{18}F_2O_3$
9d	2,6-F_2	Et	Me	oil	79	$C_{15}H_{18}F_2O_3$
10b	2-F		Me	oil	76	$C_9H_9FO_2$

[a] Analytic results were within ±0.40% of the theoretical values.

Results and Discussion

Wild-Type HIV-1 Inhibiting Activity. The novel *N*,*N*-DABOs **4**−**7** were tested against wild-type (wt) HIV-1 NL4−3 strain to evaluate their in-cell anti-HIV-1 activity in parallel with cytotoxicity. Nevirapine (NVP) and efavirenz (EFV) were included in the test as reference drugs. The results, expressed as EC_{50} (effective concentration able to protect 50% of cells

Table 3. Cytotoxicity and Anti-HIV-1 Activity of **4**−**7**[a]

compound	R	R_1	R_2	X, Y	CC_{50},[b] μM	EC_{50},[c] μM	SI[d]
4a	2,6-F_2	H	H	Me, Me	>94[e]	0.023	>4087
4b	2,6-F_2	H	H	Me, Et	>89	0.013	>6846
4c	2,6-F_2	H	H	Me, *i*-Pr	85	0.008	10 625
4d	2,6-F_2	H	H	Me, *n*-Pr	85	0.009	9444
4e	2,6-F_2	H	H	Et, Et	>85	0.133	>639
4f	2,6-F_2	H	H	*n*-Pr, *n*-Pr	>78	1.78	>44
4g	2,6-F_2	H	H	pyrrolidin-1-yl	>86	0.093	>925
4h	2-F-6-pyrrolidin-1-yl	H	H	pyrrolidin-1-yl	>73	0.71	>103
4i	2,6-F_2	H	H	piperidin-1-yl	>82	0.013	>6308
4j	2,6-F_2	H	H	2-Me-piperidin-1-yl	>78	0.107	>729
4k	2,6-F_2	H	H	3-Me-piperidin-1-yl	>78	0.032	>2437
4l	2,6-F_2	H	H	4-Me-piperidin-1-yl	>78	0.035	>2229
4m	2,6-F_2	H	H	hexahydroazepin-1-yl	>78	0.022	>3545
4n	2,6-F_2	H	H	morpholin-4-yl	>81	0.211	>384
4o	2,6-F_2	H	H	thiomorpholin-4-yl	>77	0.035	>2200
4p	2,6-F_2	H	H	4-Me-piperazin-1-yl	>78	6.09	>13
4q	2,6-F_2	H	H	1,4-dioxa-8-aza spiro[4.5]decan-8-yl	>69	2.22	>31
4r	2,6-F_2	H	H	1*H*-pyrrol-1-yl	>87	17.32	>5
5a	2,6-F_2	Me	H	Me, Me	>89	0.005	>17 800
5b	2,6-F_2	Me	H	Me, Et	>85	0.018	>4722
5c	2,6-F_2	Me	H	Me, *i*-Pr	79	0.002	39 500
5d	2,6-F_2	Me	H	Me, *n*-Pr	>81	0.002	>40 500
5e	2,6-F_2	Me	H	Me, *c*-hex	>72	0.009	>8000
5f	2,6-F_2	Me	H	Me, Ph	>73	0.086	>849
5g	2,6-F_2	Me	H	Et, Et	>81	0.030	>2700
5h	2,6-F_2	Me	H	*n*-Pr, *n*-Pr	>74	2.09	>35
5i	2,6-F_2	Me	H	pyrrolidin-1-yl	>82	0.032	>2562
5j	2-F-6-pyrrolidin-1-yl	Me	H	pyrrolidin-1-yl	>70	0.62	>113
5k	2,6-F_2	Me	H	piperidin-1-yl	>78	0.003	>26 000
5l	2,6-F_2	Me	H	2-Me-piperidin-1-yl	75	0.009	8333
5m	2,6-F_2	Me	H	3-Me-piperidin-1-yl	>75	0.018	>4167
5n	2,6-F_2	Me	H	4-Me-piperidin-1-yl	>75	0.100	>750
5o	2,6-F_2	Me	H	hexahydroazepin-1-yl	>75	0.05	>1500
5p	2,6-F_2	Me	H	morpholin-4-yl	>78	0.026	>3000
5q	2,6-F_2	Me	H	thiomorpholin-4-yl	>74	0.004	>18 500
5r	2,6-F_2	Me	H	4-Me-piperazin-1-yl	>75	0.598	>125
5s	2,6-F_2	Me	H	1,4-dioxa-8-aza spiro[4.5]decan-8-yl	>66	0.65	>101
5t	2,6-F_2	Me	H	4-(2-MeO−Ph)-1-piperazinyl	37	0.218	170
5u	2,6-F_2	Me	H	4-(2-pyridinyl)-1-piperazinyl	>63	0.012	>5250
6a	2,6-F_2	H	Me	Me, Me	89	0.001	89 000
6b	2,6-F_2	H	Me	Me, *n*-Pr	>81	0.0006	>135 000
6c	2,6-F_2	H	Et	Me, Me	73	0.002	36 500
7a	2,6-F_2	Me	Me	Me, Me	>17	0.0003	>56 667
7b	2,6-Cl_2	Me	Me	Me, Me	35	0.03	1167
7c	2-F	Me	Me	Me, Me	44	0.006	7333
7d	2,6-F_2	Me	Et	Me, Me	>81	0.007	>11 571
7e	2,6-F_2	Et	Me	Me, Me	>81	0.002	>40 500
7f	2,6-F_2	Me	Me	Me, Et	81	0.0003	270 000
7g	2,6-F_2	Me	Me	Me, *i*-Pr	>81	0.0005	>162 000
7h	2,6-F_2	Me	Me	Me, *n*-Pr	>78	0.0006	>130 000
7i	2,6-F_2	Me	Me	pyrrolidin-1-yl	>78	0.002	>39 000
7j	2,6-F_2	Me	Me	piperidin-1-yl	>75	0.007	>10 714
7k	2,6-F_2	Me	Me	3-Me-piperidin-1-yl	>72	0.003	>24 000
7l	2,6-F_2	Me	Me	4-Me-piperidin-1-yl	54	0.002	27 000
7m	2,6-F_2	Me	Me	hexahydroazepin-1-yl	>72	0.009	>8000
7n	2,6-F_2	Me	Me	morpholin-4-yl	>74	0.002	>37 000
7o	2,6-F_2	Me	Me	thiomorpholin-4-yl	>71	0.003	>23 667
2a					>3	0.001	>3000
3a					35	0.003	11 667
NVP					>7	0.08	>87
EFV					>0.3	0.004	>75

[a] Values are means ± SD determined from at least two experiments. [b] Cytotoxic concentration 50, concentration to induce 50% death of noninfected cells, evaluated with the MTT method in MT-4 cells. [c] Effective concentration 50, concentration needed to inhibit 50% HIV-induced cytopathic effect, evaluated with the MTT method in MT-4 cells (HIV-1 strain: NL4−3). [d] Selectivity index, CC_{50}/EC_{50} ratio. [e] Higher concentrations could not be achieved for the precipitation of compounds in the culture medium.

from the HIV-1-induced cytopathogenicity) and CC_{50} (compound concentration toxic for 50% of cells) values, are reported in Table 3. As the virus strain used in this article differs from the virus strain used by us in our earlier publications, the measured activities of the *S*-DABO and *NH*-DABO prototypes **2a** and **3a** as well as those of the reference compounds NVP and EFV differ in this and earlier articles.

From inhibitory data, we can draw the following structure−activity relationship (SAR).

Amine Substituent at the Pyrimidine-C_2 Position. In the **4**, **5**, and **7** series, both the F_2-*N*,*N*-DABOs carrying a *N*,*N*-disubstituted amine and a cyclic amine at the pyrimidine-C_2 generally displayed similar HIV-1 inhibiting activity. In some cases, compounds bearing a pyrrolidine, 2- or 4-methylpiperidine, and morpholine at C_2 showed a drop of activity with a corresponding lowering of the selectivity index (for instance, **4g**, **4j**, **4n**, and **5n**). To reach the highest potency, among the two alkyl moieties at the C_2-amino group, the first should be a

Table 4. Anti-HIV-1 Activity of **4**−**7** against HIV-1 Clinical Isolate and Mutant Strains[a]

					EC_{50},[b] μM *(fold-resistance)*[c]			
cpd	R	R_1	R_2	X, Y	IRLL98[d]	K103N	Y181C	Y188L
4a	2,6-F_2	H	H	Me, Me	2.0 *(87)*	>10	3.6 *(156)*	>10
4b	2,6-F_2	H	H	Me, Et	2.0 *(154)*	>10	0.50 *(38)*	>10
4c	2,6-F_2	H	H	Me, *i*-Pr	0.18 *(22)*	>10	0.03 *(4)*	>10
4g	2,6-F_2	H	H	pyrrolidin-1-yl	>10	>10	>10	>10
4i	2,6-F_2	H	H	piperidin-1-yl	2.2 *(169)*	>10	0.71 *(55)*	8 *(615)*
4j	2,6-F_2	H	H	2-Me-piperidin-1-yl	3 *(28)*	>10	1.14 *(11)*	>10
4k	2,6-F_2	H	H	3-Me-piperidin-1-yl	1.8 *(56)*	>10	0.43 *(13)*	>10
4l	2,6-F_2	H	H	4-Me-piperidin-1-yl	4.6 *(131)*	>10	1.29 *(37)*	>10
4m	2,6-F_2	H	H	hexahydroazepin-1-yl	1.4 *(64)*	>10	0.43 *(19)*	>10
4n	2,6-F_2	H	H	morpholin-4-yl	>10	>10	>10	>10
4o	2,6-F_2	H	H	thiomorpholin-4-yl	>10	>10	>10	>10
5a	2,6-F_2	Me	H	Me, Me	0.5 *(100)*	2.4 *(480)*	1.82 *(364)*	>10
5b	2,6-F_2	Me	H	Me, Et	0.42 *(23)*	4.6 *(256)*	0.71 *(39)*	8 *(444)*
5c	2,6-F_2	Me	H	Me, *i*-Pr	0.08 *(40)*	1.2 *(600)*	0.03 *(15)*	2.3 *(1150)*
5d	2,6-F_2	Me	H	Me, *n*-Pr	0.055 *(27)*	0.75 *(375)*	0.039 *(19)*	1.6 *(800)*
5e	2,6-F_2	Me	H	Me, *c*-hexyl	2 *(222)*	4.4 *(489)*	0.14 *(16)*	6.2 *(689)*
5i	2,6-F_2	Me	H	pyrrolidin-1-yl	>10	>10	5.15 *(161)*	>10
5k	2,6-F_2	Me	H	piperidin-1-yl	0.82 *(273)*	>10	0.14 *(47)*	10 *(3333)*
5l	2,6-F_2	Me	H	2-Me-piperidin-1-yl	1.4 *(156)*	>10	0.14 *(16)*	>10
5m	2,6-F_2	Me	H	3-Me-piperidin-1-yl	2.1 *(117)*	>10	0.57 *(32)*	>10
5n	2,6-F_2	Me	H	4-Me-piperidin-1-yl	>10	>10	>10	>10
5o	2,6-F_2	Me	H	hexahydroazepin-1-yl	1.7 *(34)*	>10	0.57 *(11)*	>10
5p	2,6-F_2	Me	H	morpholin-4-yl	>10	>10	>10	>10
5q	2,6-F_2	Me	H	thiomorpholin-4-yl	>10	>10	>10	>10
6a	2,6-F_2	H	Me	Me, Me	0.67 *(670)*	0.12 *(120)*	0.26 *(260)*	>10
6b	2,6-F_2	H	Me	Me, *n*-Pr	0.15 *(250)*	0.08 *(133)*	0.05 *(83)*	>10
6c	2,6-F_2	H	Et	Me, Me	1.6 *(800)*	0.18 *(90)*	0.43 *(215)*	>10
7a	2,6-F_2	Me	Me	Me, Me	0.005 *(17)*	0.05 *(167)*	0.014 *(47)*	0.05 *(167)*
7d	2,6-F_2	Me	Et	Me, Me	1.18 *(169)*	1.83 *(261)*	0.29 *(41)*	0.48 *(69)*
7e	2,6-F_2	Et	Me	Me, Me	0.88 *(440)*	0.25 *(125)*	0.10 *(50)*	0.55 *(275)*
7f	2,6-F_2	Me	Me	Me, Et	0.003 *(10)*	0.02 *(67)*	0.002 *(7)*	0.03 *(100)*
7g	2,6-F_2	Me	Me	Me, *i*-Pr	0.001 *(2)*	0.006 *(12)*	0.0007 *(1.4)*	0.04 *(80)*
7h	2,6-F_2	Me	Me	Me, *n*-Pr	0.001 *(1.7)*	0.01 *(17)*	0.0006 *(1)*	0.03 *(50)*
7i	2,6-F_2	Me	Me	pyrrolidin-1-yl	0.2 *(100)*	0.155 *(77)*	0.071 *(35)*	>10
7j	2,6-F_2	Me	Me	piperidin-1-yl	0.06 *(9)*	0.52 *(74)*	0.07 *(10)*	0.39 *(56)*
7k	2,6-F_2	Me	Me	3-Me-piperidin-1-yl	0.2 *(67)*	0.193 *(64)*	0.014 *(5)*	0.12 *(40)*
7l	2,6-F_2	Me	Me	4-Me-piperidin-1-yl	0.3 *(150)*	0.322 *(161)*	0.01 *(5)*	0.22 *(110)*
7n	2,6-F_2	Me	Me	morpholin-4-yl	0.05 *(25)*	0.39 *(195)*	0.09 *(45)*	0.56 *(280)*
7o	2,6-F_2	Me	Me	thiomorpholin-4-yl	0.06 *(20)*	1.24 *(413)*	0.03 *(10)*	0.86 *(287)*
2a					0.12 *(120)*	0.225 *(225)*	0.097 *(97)*	>3
3a					0.09 *(30)*	0.37 *(125)*	0.06 *(20)*	>10
NVP					9.9 *(124)*	1.8 *(23)*	0.87 *(11)*	5.6 *(70)*
EFV					0.08 *(20)*	0.09 *(22)*	0.006 *(1.5)*	0.23 *(57)*

[a] Values are means ± SD determined from at least two experiments. [b] Effective concentration 50, concentration needed to inhibit 50% HIV-induced cytopathic 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 wild-type NL4−3 strain (Table 3). [d] Clinical isolate resistant to NRTIs (lamivudine, emtricitabine) and NNRTIs (NVP, delavirdine, EFV).

methyl group, and the latter a C2- or better a C3-carbon unit chain (compare **4a** with **4d**, and **5a** with **5d**). The presence of a first alkyl group bigger than methyl (i.e., ethyl (**4e**, **5g**) or, to a further extent, *n*-propyl (**4f**, **5h**)) strongly decreased the anti-HIV-1 activity, as well as the introduction, in addition to the methyl group, of a phenyl ring as a second amine substituent (**5f**). Among the cyclic amines inserted at the F_2-*N*,*N*-DABOs' pyrimidine-C_2, the piperidine, thiomorpholine, and, to a lesser extent, pyrrolidine and morpholine furnished the most-active derivatives in inhibiting wt HIV-1. The introduction of less-hydrophobic amines, such as 1,4-dioxa-8-azaspiro[4.5]decane and 4-methylpiperazine, as well as the insertion of the aromatic, planar pyrrole ring, abated the inhibiting activity of the F_2-*N*,*N*-DABOs, whereas the activity was restored when the C_2-piperazine residue was substituted at N_4 with a 2-pyridinyl ring (**5u**, EC_{50} = 12 nM).

Pyrimidine-C_5 Substituent. As already reported for the previous series of DABOs, thymine derivatives (**5**,**7**) were, in general, more efficient than the corresponding uracils (**4**,**6**) in inhibiting wt HIV-1.

Substitution at the C_6-Benzylic Position. The real increase of activity in the F_2-*N*,*N*-DABO series was obtained with the introduction of small alkyl (methyl, ethyl) groups at the C_6-benzylic position of the pyrimidine ring and was better if together with a C_5-methyl/ethyl substitution. By inserting the above substitutions, highly active at subnanomolar level anti-HIV-1 compounds (**6b**, **7a**,**f**−**h**) were obtained, with an increase of up to 30-fold in potency, with respect to the uracil counterparts. Moreover, the selectivity indexes of the above derivatives reached very high values such as 270 000 (**7f**), >162 000 (**7g**), and >130 000 (**7h**).

Substituents at the Benzene Ring. The reaction of 6-(2,6-difluorobenzyl)-2-methylthiopyrimidin-4(3*H*)-ones **8a**,**b** with pyrrolidine to synthesize the 2-pyrrolidino analogues **4g** and **5i** also yielded 6-[2-fluoro-6-(pyrrolidin-1-yl)benzyl]-2-(pyrrolidin-1-yl)pyrimidin-4(3*H*)-ones **4h** and **5j** as side-products. Tested against wt HIV-1 NL4−3 strain, **4h** and **5j** were 10- to 40-fold less potent than the corresponding 2,6-difluoroderivatives **4g** and **5i**. **7b** and **7c** were synthesized, respectively, as the 6-[1-(2,6-dichlorophenyl- and 2-fluorophenyl)ethyl] analogues of the 6-[1-(2,6-difluorophenyl)ethyl]-2-dimethylamino-5-methylpyrimidin-4(*3H*)-one **7a**, one of the most-active derivatives. When tested, they were 7−30 times less potent than **7a**.

Activity Against a Clinical Isolate and Clinically Relevant HIV-1 Mutant Strains. Most of the reported F_2-*N*,*N*-DABOs were tested against the HIV-1 clinical isolate IRLL98, containing

Table 5. Inhibitory Potencies of Selected **4**−**7** in the Presence of HIV-1 RT Wild-Type and NNRTI-resistant Mutants[a]

	K_i,[b] μM *(fold-resistance)*[c]						
cpd	WT_{IIIB}	K103N	L100I	V106A	V179D	Y181I	Y188L
4a	0.08	3 *(37)*	10 *(125)*	0.5 *(6)*	5 *(62)*	3 *(37)*	30 *(375)*
4i	2.5	>20	10 *(4)*	0.2 *(0.08)*	>20	>20	>20
4n	0.7	>20	15 *(21)*	2.9 *(4)*	>40	>20	>20
4o	0.2	1.5 *(7)*	0.6 *(3)*	3 *(15)*	1 *(5)*	0.4 *(2)*	1 *(5)*
4p	11.23	>20	>20	>40	>40	>20	>20
5a	0.1	9 *(90)*	10 *(100)*	0.7 *(7)*	1.6 *(16)*	>20	11 *(110)*
5d	0.1	>20	18 *(180)*	0.008 *(0.08)*	0.2 *(2)*	>20	>20
5p	0.05	>20	0.9 *(18)*	0.4 *(8)*	8.3 *(166)*	>20	>20
5q	0.15	>20	5 *(33)*	0.09 *(0.6)*	40 *(267)*	>20	>20
5r	2	ND[d]	ND	0.9 *(0.45)*	>40	ND	ND
5t	0.25	>20	0.22 *(0.9)*	4.1 *(16)*	8.5 *(34)*	>20	25.7 *(103)*
5u	0.05	>20	>20	0.01 *(0.2)*	0.01 *(0.2)*	>20	0.49 *(10)*
6c	0.1	10 *(100)*	3.3 *(33)*	0.14 *(1.4)*	0.03 *(0.3)*	>20	5 *(50)*
7a	0.1	0.5 *(5)*	1 *(10)*	0.1 *(1)*	2 *(20)*	4 *(40)*	0.5 *(5)*
7d	1	>20	>20	>40	>40	>20	>20
7e	0.05	2 *(40)*	4 *(80)*	0.06 *(1.2)*	0.03 *(0.6)*	5 *(100)*	10 *(200)*
7f	0.04	0.5 *(12)*	2.5 *(62)*	0.02 *(0.5)*	0.02 *(0.5)*	10 *(250)*	4 *(100)*
7g	0.04	0.08 *(2)*	0.12 *(3)*	0.03 *(0.7)*	ND	0.85 *(21)*	1.19 *(30)*
7h	0.03	0.17 *(6)*	0.18 *(6)*	0.02 *(0.7)*	ND	1.26 *(42)*	1.43 *(48)*
7j	0.07	0.6 *(9)*	2 *(29)*	0.02 *(0.3)*	0.01 *(0.14)*	>20	3.5 *(50)*
7n	0.003	3.3 *(1100)*	10 *(3333)*	>20	>20	>20	5.5 *(1833)*
7o	0.06	10 *(167)*	5 *(83)*	0.03 *(0.5)*	0.02 *(0.33)*	>20	4 *(67)*
2a	0.005	0.03 *(6)*	0.3 *(60)*	0.5 *(100)*	0.25 *(50)*	0.9 *(180)*	1.4 *(280)*
3a	0.04	0.47 *(12)*	0.31 *(8)*	0.04 *(1)*	ND	5.02 *(125)*	3.11 *(78)*
NVP	0.4	7 *(17)*	9 *(22)*	10 *(25)*	0.3 *(0.8)*	35 *(87)*	18 *(45)*
EFV	0.03	3 *(100)*	ND	ND	ND	0.08 *(3)*	ND

[a] Values are means ± SD determined from at least three experiments. [b] Compound concentration required to inhibit the HIV-1 rRT activity by 50%. [c] Fold-resistance: ratio of $K_i$mut/$K_i$wt values. [d] ND, not determined.

multiple drug-resistance mutations (to both NRTIs (lamivudine, emtricitabine) and NNRTIs (NVP, delavirdine, EFV)), as well as against a panel of clinically relevant HIV-1 mutant strains (K103N, Y181C, and Y188L), and the results are summarized in Table 4. To define the resistance profile of these inhibitors, both the absolute activity against the HIV-1 clinical isolate and mutants (EC_{50} values) and the relative activity compared with the activity against wt HIV-1 (fold-resistance) need to be considered. In particular, in this assay, the uracil derivatives **4a**−**c**,**i**−**m** retained in part their activities against the clinical isolate IRLL98 and the Y181C mutant, with EC_{50} values at the low micromolar (IRLL98) or submicromolar (Y181C) level, whereas they were inactive against the HIV-1 K103N and Y188L mutant strains. Nevertheless, their fold-resistance values ranged from 22 to 169 (IRLL98), and from 4 to 156 (Y181C), so that the pyrimidin-4(3*H*)-one **4c**, carrying at the C_2 position a *N*-methyl-*N*-*iso*-propylamine residue, was the only real highly active compound (EC_{50}^{IRLL98} = 0.18 μM with a 22-fold resistance ratio with respect to wt, and EC_{50}^{Y181C} = 0.03 μM with a 4-fold resistance ratio).

The introduction of a methyl group at the C_5 position (thymine derivatives) conferred to the C_2-*N*-methyl-*N*-alkylamino-substituted compounds (**5a**−**e**) an additional inhibitory activity against the K103N and Y188L mutant strains (at the micromolar range) besides that against the clinical isolate IRLL98 and the mutant Y181C (at the submicromolar/nanomolar level). Differently, the thymine derivatives having at C_2 a cyclic amine remained inactive against the K103N and Y188L mutants. In this series also, the fold-resistance values of the derivatives showed moderate to high values. The most-active compounds were **5c** and **5d**, bearing an *N*-methyl-*N*-*iso*-propylamino (**5c**) or an *N*-methyl-*N*-propylamino (**5d**) substituent at C_2 and endowed with nanomolar activity against IRLL98 and Y181C mutants [$EC_{50}s^{IRLL98}$ (nM)/*fold resistance*: 80/*40* (**5c**) and 55/*27* (**5d**); $EC_{50}s^{Y181C}$ (nM)/*fold resistance*: 30/*15* (**5c**) and 39/*19* (**5d**)], and micromolar inhibiting activity against the K103N and Y188L mutant strains ($EC_{50}s^{K103N}$ = 1.2 (**5c**) and 0.75 μM (**5d**); $EC_{50}s^{Y188L}$ = 2.3 (**5c**) and 1.6 μM (**5d**)), but joined to high fold-resistance values.

The introduction of a methyl/ethyl substituent at the C_6 benzylic position of the pyrimidine ring in the uracil series of the F_2-*N*,*N*-DABOs (**6a**−**c**) increased the inhibitory potency of the derivatives against the clinical isolate IRLL98 and the K103N mutant. However, **6a**−**c** were not active against the Y188L mutant strain. Differently, the introduction at the same time of methyl groups at the pyrimidine C_5 and at the benzylic position (**7a**,**d**−**l**,**n**,**o**) produced the most interesting derivatives, highly active against all of the HIV-1 mutant strains tested. In particular, **7a**, **7f**, **7g**, and **7h**, carrying *N*-methyl-*N*-alkylamino substituents at the C_2 pyrimidine ring showed single-digit nanomolar inhibition against the clinical isolate IRLL98 containing multiple drug-resistance mutations (EC_{50}s: 5 (**7a**), 3 (**7f**), 1 (**7g**), and 1 (**7h**) nM) and nanomolar to subnanomolar inhibiting activity against the Y181C mutant (EC_{50}s: 14 (**7a**), 2 (**7f**), 0.7 (**7g**), and 0.6 (**7h**) nM). The same derivatives were able to inhibit the K103N and Y188L HIV-1 mutant strains in the range of 50 to 6 nM. Moreover, **7g** and **7h** displayed very low or no fold-resistance values (1 to 17) against the IRLL98, K103N, and Y181C variants. When compared with the prototypes of F_2-*S*-DABOs (**2a**) and F_2-*NH*-DABOs (**3a**) as well as with EFV, **7a**, **7f**, **7g**, and **7h**, they were one or 2 orders of magnitude more potent than the reference drugs against the tested clinical isolate and mutant strains. The F_2-*N*,*N*-DABOs of series **7** and those bearing a cyclic amine at C_2 (**7i**−**l**,**n**,**o**) were less potent than the corresponding derivatives with a *N*-methyl-*N*-alkylamino moiety at C_2, and they were active at the submicromolar to nanomolar level against the tested HIV-1 mutant strains. **7i**−**l**,**n**,**o** displayed activities similar to those of **2a** and **3a** and lower than that of EFV. Differently from **2a** and **3a**, inactive against the Y188L mutant strain, the F_2-*N*,*N*-DABOs **7j**−**l**,**n**,**o** showed similar activity as EFV against the above HIV-1 mutant. The replacement of one of the two C_5/benzylic methyl groups in the **7a** structure with an ethyl group (**7d**, with the ethyl group at the benzylic position, and **7e**, carrying a C_5-

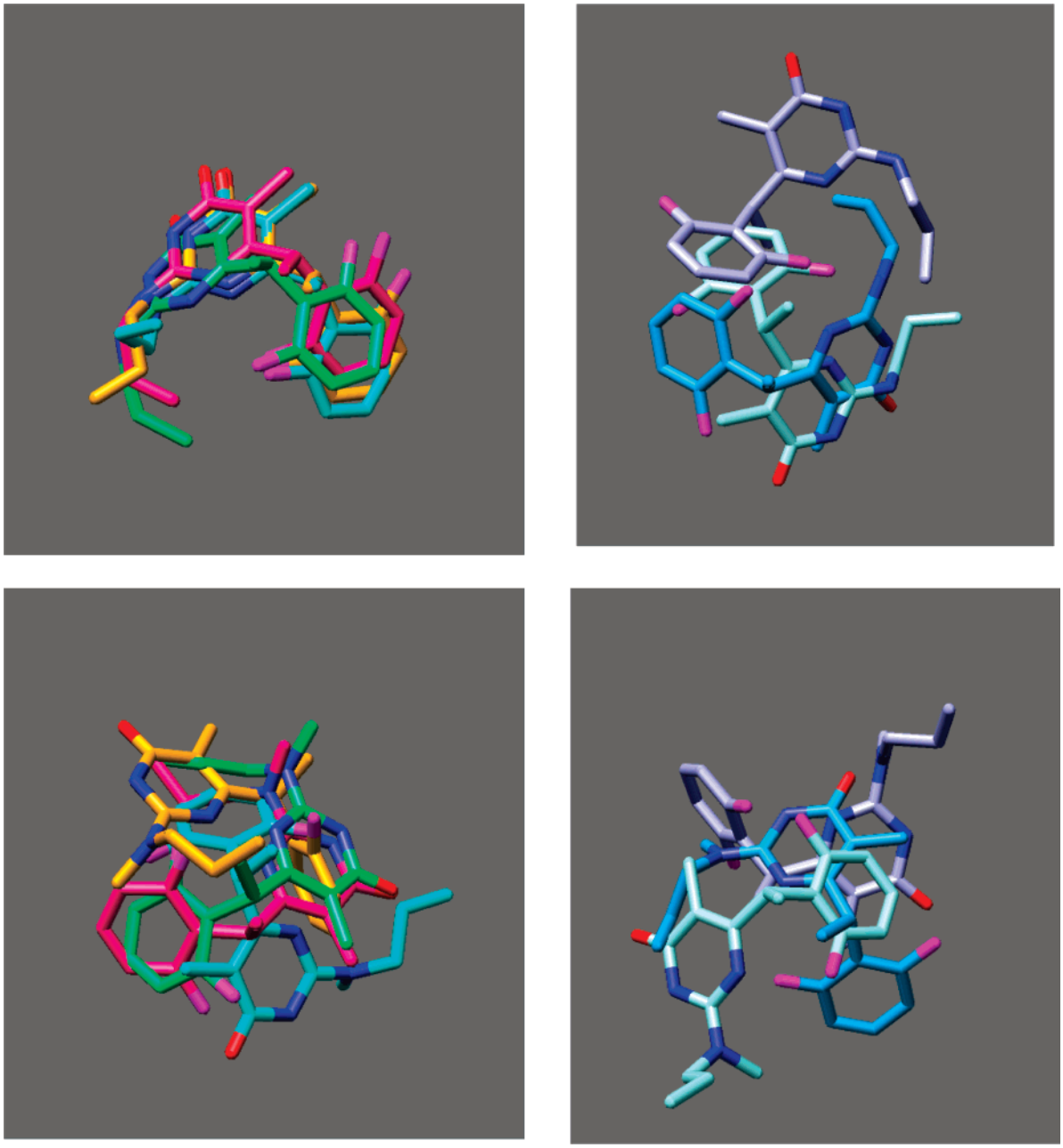


Figure 1. Upper left: **3a** conformations as docked into WT (cyan), L100I (orange), Y181C (deep pink), and Y188C (spring green). Upper right: **3a** conformations as docked into V106A (cyan), Y188L (light purple) and K103N (light green). Lower left: **7h** conformations as docked into WT (cyan), L100I (orange), Y181C (deep pink), Y188C (spring green). Lower right: **7h** conformations as docked into V106A (cyan), Y188L (light purple) and K103N (light green). All of the conformations are readily comparable because the corresponding RTs were superimposed before any docking experiments. Figures were generated with the Chimera program,[51] and color names are taken directly from the all-color grid within the program. Hydrogen atoms are not shown for sake of clarity.

ethyl substitution) was detrimental for the inhibitory potencies of the derivatives: indeed, the EC_{50} values of **7d**,**e** were 5- to 236-fold lower than that of **7a**.

Enzyme Inhibiting Activity against HIV-1 RT Wild-Type and NNRTI-Resistant Mutants. Selected F_2-*N*,*N*-DABOs **4**−**7** were tested against a panel of recombinant HIV-1 RTs either wild type (WT_{IIIB}) or carrying known NNRTI-resistance mutations (K103N, L100I, V106A, V179D, Y181I, and Y188L) (Table 5). As in a kinetic study,[42] a number of *N*,*N*-DABOs belonging to the **7** series (i.e., **7a**, **7f**, **7j**, and **7n**) displayed lower association rates (K_{on}) and much slower dissociation rates (K_{off}) than EFV and UC781,[43] used as reference compounds (thus behaving as slow-, tight-binding inhibitors of HIV-1 RT), the inhibitory potencies of DABO derivatives were determined with 10 min preincubations in the presence of both wt and NNRTI-resistant mutant RTs.

Against K103N RT, among the tested uracil and thymine derivatives, only 2-thiomorpholin-4-yl uracil analogue **4o** retained to some extent a limited inhibitory activity. Differently, the compounds carrying the double-methyl substitution at the C_5/benzylic positions of the pyrimidine ring and a *N*,*N*-dimethylamino (**7a**), *N*-methyl-*N*-alkylamino (**7f**−**h**), or piperidin-1-yl (**7j**) substituent at C_2 showed a submicromolar potency, lower than that of **2a**, similar or slightly higher than that of **3a**, and up to 87- and 37-fold higher than the ones exhibited by EFV and NVP, respectively, used as reference drugs.

The L100I mutant RT was inhibited at submicromolar level by the uracil **4o**, the thymine derivatives **5p** and **5t**, and **7g** and

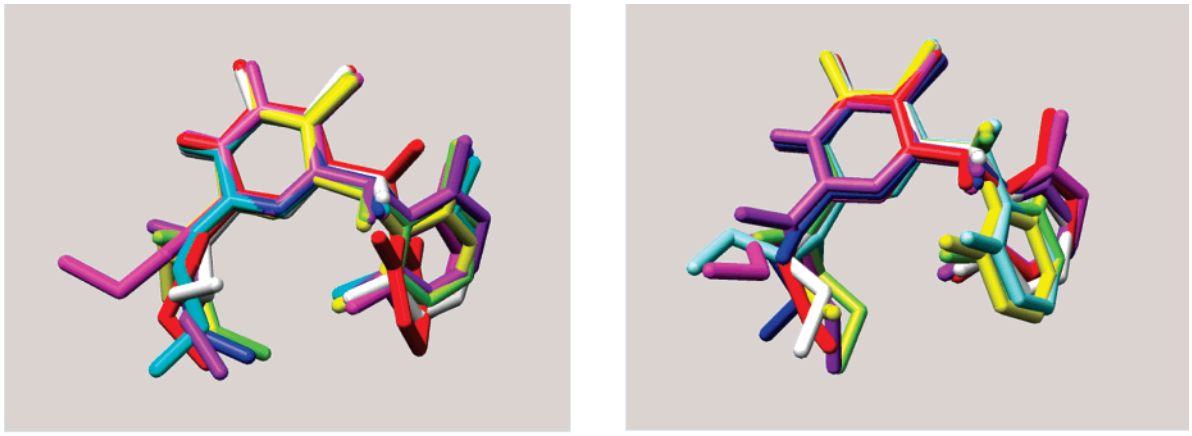


Figure 2. **3a** (left) and **7h** (right) rigidly superimposed by means of surflex.[50]

7h carrying the two methyl groups at pyrimidine-C_5 and at the benzylic position, in addition to the *S*-DABO **2a** and the *NH*-DABO **3a.** In the same assay, the thymine **5q**, the ethyl-substituted at linker position **6c**, and most of the compounds belonging to the **7** series (**7a**, **7e**, **7f**, **7i**, **7n**) were active at a low micromolar range.

Against the mutant V106A and V179D RTs, an unexpected range of activity with *N*,*N*-DABOs was observed: such mutant RTs seem to be more susceptible toward a lot of the tested compounds than the WT RT (for example **4i**, **5d**,**q**,**r**,**u**, and **7f**−**h**,**j**,**o**, that were more potent against V106A than against the RT wt, and **6c** and **7a**,**e** that, similarly to **3a**, showed the same activities against the two cited RTs). This behavior is totally different from that of the *S*-DABO prototype **2a**. More intriguingly, V179D has been reported as a RT mutation elicited from cellular chronic treatment with *S*-DABOs.[21]

Against the Y181I mutant RT, the DABOs **4o**, and **7g**, as well as **2a** and the reference drug EFV retained inhibitory activity at the submicromolar level, and **4a**, **7a**, **7e**, **7f**, and **7h** together with **3a** were shown to be active at the low micromolar range. In the presence of Y188L, the uracil derivative **4o**, the thymine **5u**, and all of the tested compounds **7** except for **7d** (in addition to the *S*-DABO **2a** and the *NH*-DABO **3a**) displayed micromolar/submicromolar inhibitory activity.

Binding Mode Studies. On several occasions, we have described the ability of the Autodock[44] program as a useful tool both to investigate the binding mode and to design new enzyme inhibitors.[27,41,45−47] In particular, when used in cross-docking experiments, Autodock proved to be a valuable docking program to include the ligand-induced fit in molecular-modeling-assisted drug design.[41] Therefore, we decided to employ the Autodock suite to look into the binding mode of the newly synthesized F_2-*N*,*N*-DABOs. Derivative **7h** was highly active against a panel of NNRTI-resistant RT mutants [K_i values, μM: 0.03 (WT), 0.17 (K103N), 0.18 (L100I), 0.02 (V106A), 1.26 (Y181I), and 1.43 (Y188L)] and showed the highest inhibitory action against HIV-1 clinical isolate and some mutant strains [EC_{50} values, μM: 0.0006 (WT), 0.001 (IRLL98), 0.01 (K103N), 0.0006 (Y181C), and 0.03 (Y188L)]. Thus, **7h** was taken as a representative compound to study the F_2-*N*,*N*-DABOs' binding mode into the experimental RT non-nucleoside binding site (NNBS) by the means of cross-docking studies.[41] **7h** was modeled in its *R* conformation, because it was expected to be the most-active enantiomer in analogy with that reported with *S*-DABOs and *NH*-DABOs.[26−28,48] The experimental 3D coordinates of 65 HIV-RT/ligand complexes (including wt and K103N, Y181C, Y188L, Y188C, V106A, V108I, and L100I-mutated NNBSs; Table A in the Supporting Information) were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB).[49] To check the structural role of either the single or double substitution at the C_2-*N* DABO nitrogen atom, beyond the above selected **7h**, a structurally related F_2-*NH*-DABO (**3a**) was also cross-docked in the 65 NNBSs. The analyses of the docking results revealed that monosubstituted F_2-*NH*-DABO **3a** has a common binding mode to wt, Y181C, Y188C, and L100I NNBSs, similar to those earlier described for other F_2-*NH*-DABOs[28] in wt and Y181C RTs (Figure 1). These observations confirm that the introduction of an extra anchor point in the *S*-DABO → *NH*-DABO conversion was an important improvement[28] for the anti-RT activity against Y181C, Y188C, and L100I mutated forms. Regarding the **3a** binding mode into the V106A, Y188L, and K103N RTs, the docked conformations display different arrangements into the NNBSs (Figure A, in the Supporting Information). This could reflect that, for such mutations, the introduced anchor point of the F_2-*NH*-DABO[28] would not be any more sufficient to contrast the NNBS geometry variations, and a sort of adaptation to the mutated RTs occurred (Figure 1).

Interestingly, in the cross-docking studies, **7h** (the *N*-methyl F_2-*N*,*N*-DABO analogue of **3a**) did not display any common binding mode within the seven docked conformations. This higher adaptable behavior of **7h** resembles that described by Das et al.[38] for the experimental conformations of etravirine and analogues. In the cross-docking studies performed here, **7h**, when compared to **3a**, seems to have a higher level of adaptability into the NNBS binding pocket that involves a combination of conformational adjustments ("wiggling") and rotational and translational shifts ("jiggling") of the inhibitor within the binding pocket.[38] In particular, the rigidly surflex[50] superimposed **3a** and **7h** docked conformations (Figure 2) suggested that the conformational variations of F_2-*N*,*N*-DABO derivatives would mainly result from changes in torsion angles τ_3 and τ_4 (Figure 3) because τ_1 and τ_2 are preset by internal energies.[26] Moreover, the **3a** and **7h** compact structures also permit rotational and translational shifts within the binding pocket. Apparently, the F_2-*N*,*N*-DABO analogues can change conformation (wiggle) and reorient and reposition (jiggle) in response to changes in the NNBS caused by mutations, and this adaptability may help to explain their enhanced ability to inhibit mutated HIV-1 RTs.

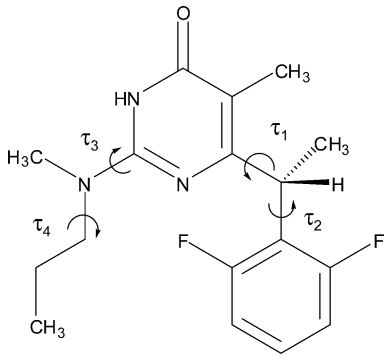


Figure 3. **7h** and the torsional angles influencing the compound flexibility.

Conclusion

In this article, a number of 5-alkyl-2-(*N*,*N*-disubstituted)-amino-6-(2,6-difluorophenylalkyl)-3,4-dihydropyrimidin-4(3*H*)-ones (F_2-*N*,*N*-DABOs) and related derivatives **4**−**7** belonging to the DABO family[14] have been reported as novel anti-HIV-1 agents. Such compounds were tested against wt HIV-1 NL4−3-infected MT-4 cells as well as against MT-4 cells infected with three different HIV-1 mutant strains (K103N, Y181C, and Y188L). In addition, the effect of the F_2-*N*,*N*-DABOs **4**−**7** against IRLL98, an HIV-1 clinical isolate resistant to NRTIs (lamivudine, emtricitabine) and NNRTIs (NVP, delavirdine, EFV) was determined. Cytotoxicity data of **4**−**7** were evaluated on MT-4 cells with the mock-infected method. Enzyme inhibitory assays were performed with selected derivatives against a panel of recombinant HIV-1 RTs, either wild type or carrying known NNRTI-resistance mutations (K103N, L100I, V106A, V179D, Y181I, and Y188L).

In cellular assays, the majority of the synthesized F_2-*N*,*N*-DABOs proved to be highly active against both wt and mutant HIV-1 strains, at the submicromolar to nanomolar range. In some cases (**6b**, **7a,f**−**h**), they showed subnanomolar potency against wt HIV-1 and, more interestingly, against the Y181C mutant strain (**7g**,**h**). The highest HIV-1 inhibitory activity is associated with the presence of two methyl groups at the pyrimidine-C_5 position and at the benzylic position, together with the insertion of a *N*-methyl-*N*-alkylamino chain at the pyrimidine-C2, in addition to the highly favorable 2,6-difluorobenzyl substitution at the C_6 position of the pyrimidine ring. Compounds bearing all such chemical features were more potent than the F_2-*S*-DABO and F_2-*NH*-DABO prototypes **2a** and **3a** and than NVP and EFV used as reference drugs. The introduction of a cyclic amine (pyrrolidine, piperidine, morpholine, thiomorpholine, etc.) at the C2 position of the pyrimidine ring instead of the *N*-methyl-*N*-alkylamino moiety led to derivatives active at the nanomolar (against HIV-1 wt) or submicromolar (HIV-1 mutant strains) level, they being more potent than NVP and active at the same range of **2a**, **3a**, and EFV. Enzyme inhibitory data fully agree with cellular evaluation. Binding mode analyses performed on F_2-*NH*-DABO **3a** and F_2-*N*,*N*-DABO **7h** suggest that the higher adaptability showed by **7h** in comparison with **3a**, by changing conformation (wiggle) and reorienting and repositioning (jiggle) in response to changes in the HIV-1 RT NNBS may almost in part explain its higher inhibitory effect against the mutated RTs.

Experimental Section

Chemistry. Melting points were determined on a Buchi 530 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AC 400 spectrometer; chemical shifts are reported in δ units relative to the internal reference tetramethylsilane (Me_4Si). All of the 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. All of the 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 a reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results are within ±0.40% of the theoretical values. All of the chemicals were purchased from Aldrich Chimica, Milan (Italy), or from Lancaster Synthesis GmbH, Milan (Italy), and were of the highest purity.

General Procedure for the Synthesis of Ethyl β-Oxoesters (9). Example: Ethyl 4-(2,6-Difluorophenyl)-2-ethyl-3-oxopentanoate (9d). Triethylamine (12.6 mL, 90.2 mmol) and magnesium dichloride (6.71 g, 70.5 mmol) were added to a stirred suspension of potassium 2-ethylmalonate monoethyl ester[24] (11.74 g, 59.2 mmol) in dry acetonitrile (90 mL), and stirring was continued at room temperature for 2 h. Then, a solution of the 2-(2,6-difluorophenyl)propionic imidazolide in the same solvent (28 mL), prepared 15 min before by reaction a between 2-(2,6-difluorophenyl)propionic acid[26] (**10c**, 5.25 g, 28.2 mmol) and *N*,*N*′-carbonyldiimidazole (5.49 g, 33.8 mmol) in acetonitrile (28 mL), was added. The reaction mixture was stirred overnight at room temperature and then was heated at reflux for 2 h. After the mixture was cooled, 13% HCl (235 mL) was cautiously added while the temperature was kept below 25 °C, and the resulting clear mixture was stirred for a further 15 min. The organic layer was separated and evaporated, and the residue was treated with ethyl acetate (100 mL). The aqueous layer was extracted with ethyl acetate (3 × 100 mL), and the organic phases were collected, washed with a sodium hydrogen carbonate saturated solution (3 × 100 mL) and brine (100 mL), dried, and concentrated to give pure **9d** (6.00 g) as a yellow oil, which was directly used in the following step. ^{1}H NMR ($CDCl_3$) δ 0.83 (t, 3H, $CHCH_2CH_3$), 1.20 (t, 3H, $COCH_2CH_3$), 1.48 (d, 3H, $CHCH_3$), 1.85 (m, 2H, CH_2CH_3), 3.39 (m, 1H, $CHCH_2CH_3$), 4.12 (m, 3H, $COCH_2CH_3$ and $CHCH_3$), 6.91 (m, 2H, $C_{3,5}$-H Ar), 7.24 (m, 1H, C_4−H Ar). Anal. C, H, F.

General Procedure for the Preparation of 6-[(2,6-Difluorophenyl)alkyl]-3,4-dihydro-2-dimethylamino-5-(un)substitutedpyrimidin-4(3*H*)-ones (4a, 5a, 6a, 6c, and 7a−e). Example: 6-[1-(2,6-Difluorophenyl)ethyl]-3,4-dihydro-2-dimethylamino-5-methylpyrimidin-4(3*H*)-one (7a). Sodium metal (0.68 g, 29.75 mmol) was dissolved in 100 mL of absolute ethanol, and 1,1-dimethylguanidine sulfate (2.70 g, 9.92 mmol) and ethyl 4-(2,6-difluorophenyl)-2-methyl-3-oxopentanoate[26] (2.68 g, 9.92 mmol) were added to the clear solution. The mixture was heated at reflux for 5 h. After cooling, the solvent was evaporated in a vacuum at 40−50 °C until dry, and the residue was dissolved in a small amount of water (20 mL), neutralized with 1 N HCl, and extracted with ethyl acetate (3 × 25 mL). The organic extracts were washed with brine (3 × 50 mL), dried, and evaporated in a vacuum, and the residue was purified by chromatography on silica gel eluting with ethyl acetate/chloroform 1:1. ^{1}H NMR (DMSO-d_6): δ 1.65−1.66 (d, 3H, ArCHC*H*3), 1.92 (s, 3H, C_5−CH_3), 3.11 (s, 6H, NCH_3), 4.53−4.58 (q, 1H, ArCHCH$_3$), 6.77−6.85 (m, 2H, $C_{3,5}$-Ar−*H*), 7.12−7.20 (m, 1H, C_4−Ar−*H*). Anal. C, H, F, N.

General Procedure for the Preparation of 2-*N*,*N*-Disubstituted- (or cyclic) -amino-6-[1-(2,6-difluorophenyl)alkyl]-3,4-dihydro-5-(un)substitutedpyrimidin-4(3*H*)-ones (4e−q, 5e−u, and 7i−o). Example: 6-[1-(2,6-Difluorophenyl)ethyl]-3,4-dihydro-5-methyl-2-(morpholin-4-yl)pyrimidin-4(3*H*)-one (7n). A mixture of 6-(2,6-difluorophenylmethyl)-3,4-dihydro-2-methylthio-5-methylpyrimidin-4(3*H*)-one[24] (0.3 g, 1.01 mmol) and morpholine (5 mL) was heated in a sealed tube at 170 °C for 8 h. After cooling, the crude residue was dissolved in ethyl acetate (60 mL) and water (60 mL). The aqueous phase was extracted with ethyl acetate (2 × 50 mL). The organic extracts were washed with brine (3 × 50 mL), dried, evaporated under reduced pressure, and purified by column chromatography (silica gel, ethyl acetate/chloroform 1:1). ^{1}H NMR

(DMSO-d_6): δ 1.51−1.55 (d, 3H, ArCHC*H*3), 1.80 (s, 3H, C_5−C*H*$_3$), 3.55−65 (m, 8H, morpholine ring), 4.50−4.53 (q, 1H, ArC*H*CH$_3$), 6.95−7.05 (m, 2H, $C_{3,5}$-Ar−*H*), 7.31−7.41 (m, 1H, C_4−Ar−*H*). Anal. C, H, F, N.

In the same conditions, the reaction of pyrrolidine with 6-(2,6-difluorophenylmethyl)-3,4-dihydro-2-methylthiopyrimidin-4(3*H*)-one[26] and 6-(2,6-difluorophenylmethyl)-3,4-dihydro-2-methylthio-5-methylpyrimidin-4(3*H*)-one[26] afforded a mixture of two compounds (**4g**,**h** and **5i**,**j**, respectively), that were separated by column chromatography (silica gel, ethyl acetate/chloroform 3:1). Example: 6-(2,6-Difluorophenylmethyl)-5-methyl-2-(pyrrolidin-1-yl)-pyrimidin-4(3*H*)-one (**5i**): ^{1}H NMR (CDCl$_3$): δ 1.90 (m, 4H, NCH$_2$C*H*$_2$C*H*$_2$), 2.04 (s, 3H, C_5−C*H*$_3$), 3.35 (m, 4H, C*H*$_2$NC*H*$_2$), 4.14 (s, 2H, C_6−C*H*$_2$), 6.84 (m, 2H, $C_{3,5}$-Ar−*H*), 7.12 (m, 1H, C_4−Ar−*H*), 10.74 (s, 1H, N*H*). Anal. C, H, F, N.

6-[2-Fluoro-6-(pyrrolidin-1-yl)-phenylmethyl]-5-methyl-2-(pyrrolidin-1-yl)pyrimidin-4(3*H*)-one (**5j**): ^{1}H NMR (CDCl$_3$): δ 1.87 (m, 8H, 2 × NCH$_2$C*H*$_2$C*H*$_2$), 2.03 (s, 3H, C_5−C*H*$_3$), 3.10−3.38 (m, 8H, 2 × C*H*$_2$NC*H*$_2$), 4.12 (s, 2H, C_6−C*H*$_2$), 6.67 (m, 2H, $C_{3,5}$-Ar−*H*), 7.05 (m, 1H, C_4−Ar−*H*), 10.96 (s, 1H, N*H*). Anal. C, H, F, N.

General Procedure for the Preparation of 2-*N*,*N*-dialkylamino-6-[1-(2,6-difluorophenyl)alkyl]-3,4-dihydro-5-(un)substitutedpyrimidin-4(3*H*)-ones (4b−d, 5b−d, 6b, and 7f−h). Example: 6-[1-(2,6-Difluorophenyl)methyl]-3,4-dihydro-2-(*N*-ethyl-*N*-methylamino)-5-methylpyrimidin-4(3*H*)-one (5b). *N*-Ethyl-*N*-methylammonium acetate salt was prepared by slowly the addition of 2.1 mL (0.036 mol) of glacial acetic acid to 3.1 mL (0.036 mol) of *N*-ethyl-*N*-methylamine in an ice bath. Afterward, 6-(2,6-difluorophenylmethyl)-3,4-dihydro-5-methyl-2-methylthiopyrimidin-4(3*H*)-one[26] (0.53 g, 1.8 mmol) was added, and the mixture was fused for 5 h at 160 °C. After cooling, the residue was partitioned between water (60 mL) and ethyl acetate (60 mL), and the aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with brine (3 × 50 mL) and dried. Evaporation of the solvent furnished a crude residue, which was purified by column chromatography (silica gel, ethyl acetate/chloroform 2:1). ^{1}H NMR (CDCl$_3$): δ 0.98 (t, 3H, NCH$_2$C*H*3), 2.04 (s, 3H, C_5−C*H*$_3$), 2.96 (s, 3H, NC*H*$_3$), 3.37 (q, 2H, NC*H*$_2$CH$_3$), 3.87 (s, 2H, C_6−C*H*$_2$), 6.85 (m, 2H, $C_{3,5}$-Ar−*H*), 7.15 (m, 1H, C_4−Ar−*H*), 11.26 (s, 1H, N*H*). Anal. C, H, F, N.

Synthesis of 6-(2,6-Difluorophenylmethyl)-2-(1*H*-pyrrol-1-yl) pyrimidin-4(3*H*)-one (4r). 2,5-Dimethoxytetrahydrofuran (1.96 g, 14.8 mmol) was added to a solution of 2-amino-6-(2,6-difluorophenylmethyl)pyrimidin-4(3*H*)-one **11**[28] (3.00 g, 13.5 mmol) in glacial acetic acid (50 mL), and the resulting mixture was stirred at reflux for 5 min. After the completion of the reaction, the mixture was poured into crushed ice and stored at 0−5 °C overnight. The resulting solid was then filtered, washed with water, dried, and purified by recrystallization from acetonitrile. ^{1}H NMR (CDCl$_3$−CD$_3$OD): δ 4.01 (s, 2H, C_6−C*H*$_2$), 6.08 (d, 2H, $C_{3,4}$-pyrrole−*H*), 6.29 (s, 1H, C_5-*H*), 6.93 (m, 2H, $C_{3,5}$-Ar−*H*), 7.28 (m, 1H, C_4−Ar−*H*), 7.62 (d, 2H, $C_{2,5}$-pyrrole−*H*). Anal. C, H, F, N.

Synthesis of 2-(2-Fluorophenyl)propionic Acid (10b). A diethylamine (2.5 mL, 24.2 mmol) solution in dry THF (5 mL) was added dropwise at −78 °C to a buthyllithium (19.4 mL of 2.5 M solution in *n*-hexane, 48.4 mmol) solution in dry THF (10 mL), and the resulting mixture was stirred for 0.5 h at 0 °C. A solution of 2-fluorophenylacetic acid (3.39 g, 22.0 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 methyl iodide (3.43 g, 1.5 mL, 24.2 mmol) in dry THF (5 mL) was added at −78 °C. The mixture was stirred and gradually warmed from −78 °C to room temperature over 1 h and then poured into water (150 mL) and extracted with ethyl acetate (3 × 50 mL). The aqueous layer was acidified with concentrated hydrochloric acid at 0 °C and then extracted with ethyl acetate (4 × 50 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, which was purified by column chromatography (silica gel, ethyl acetate/chloroform 1:5). ^{1}H NMR (CDCl$_3$): δ 1.50 (d, 3H, CHC*H*$_3$), 4.11 (q, 1H, C*H*CH$_3$), 6.87 (m, 3H, C_{3-5}-Ar-*H*), 7.21 (m, 2H, C_{2-4}-Ar-*H*). Anal. C, H, F.

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.[52] 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 IRLL98 HIV-1 strain contains the following mutations in the RT coding sequence:[53] M41L, D67N, Y181C, M184V, R211K, T215Y (conferring resistance to NRTIs) and mutations K101Q, Y181C, G190A (conferring resistance to NNRTIs). The HIV-1 strains containing the multi-NNRTI mutation, K103N, or the Y188L mutant were received from the Medical Research Council Centralized Facility for AIDS Reagents, Herfordshire, UK.

Anti-HIV Reverse Transcriptase Assays. RNA-dependent DNA polymerase activity was assayed as described[54] 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 an 8% final concentration of DMSO.

Reagents. [^{3}H]-dTTP (40 Ci/mmol) was from Amersham and unlabeled dNTP's from Boehringer. Whatman was the supplier of the GF/C filters. All of the 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.[54] All of the 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 (K_i) determination, an interval of inhibitor concentrations between 0.2 and 5 K_i was used in the inhibition assays. Data were then plotted according to Lineweaver−Burke and Dixon.

Molecular Modeling Studies. RT Structures Preparation. Sixty-five publicly available X-ray crystal structures of reverse transcriptase complexed with a NNRTI were retrieved from the Protein Data Bank[55−57] (Supporting Information). Of the 65 complexes, 45 belonged to the wild-type enzyme (because the long distance from the NNBS, the tetra mutant 1RT3, and the C280S mutated forms were considered as wild-type RT) and 20 belonged to RT mutated forms. All of the complexes were superimposed onto each other arbitrarily using nevirapine/RT (1VRT) as a reference complex. The superimposition of the RT complexes was performed by the means of the program ProFit version 2.2,[58] using the implemented McLachlan algorithm.[59] All of the residue's backbone atoms comprised in a 20 Å core from nevirapine (1VRT) were selected with the Chimera program[60] (182 residues) and used as a reference for the fitting, the same number of residues (Trp88A-Tyr115A, Ser156A-Leu210A, Trp212A, Leu214A-Ile244A, Lys263A, Asn265A-Tyr271A, Glu312A-Ile326A, Tyr339A-Thr351A, Ile375A, Thr377A-Lys385A, Gln23B, Pro25B-Glu28B, Ile31B-Lys32B, Val35B, Thr131B-Arg143B) was used for all of the complexes' superimpositions.

Then, using the AMBER 8.0 suite, the complexes were minimized to alleviate steric contacts, which usually arise due to the random way in which hydrogen atoms are added to the heavy atoms. Hydrogen atoms were added to the receptors with the tLeap module. Protonation states were assumed to be those most common at pH 7, that is, lysines, arginines, histidines, aspartates, and glutamates were considered in the ionized form. Each complex was solvated (SOLVATEOCT command) with water molecules (TIP3 model) in a box extending 10 Å, and counterions were added to neutraliza-

tion. The solvated complex was then refined by minimization using the SANDER module of AMBER. The parameters for the cocrystallized NNRTI were calculated using the antechamber module of AMBER and the atomic charges were calculated using the AM1 Hamiltonian. Once the minimizations were complete, the ligands (NNRTIs) and the receptors (RTs) were extracted into separate files (locks and keys) to be used for the subsequent docking set up.

Docking Set-Up. Autodock 3.0.5[44] was used for all of the docking calculations. The AutodockTools package version 1.4.4 was employed to generate the docking input files and to analyze the docking results, the same procedure as described in the manual was followed. All of the nonpolar hydrogen atoms and the water molecules were removed. The Kollmann charges were loaded for the proteins, whereas the Gasteiger charges were used for the ligands. A grid box size of 61 × 81 × 61 points with a spacing of 0.375 Å between the grid points was implemented and covered more than 8 Å of the NNBS. The grid was centered on the mass center of the experimental bound nevirapine (1VRT) coordinates taken as a NNRTI representative. For all of the inhibitors, the single bonds including the amide bonds were treated as active torsional bonds. One hundred docked structures, that is, 100 runs, were generated by using genetic algorithm searches. A default protocol was applied, with an initial population of 50 randomly placed individuals, a maximum number of 2.5 × 105 energy evaluations, and a maximum number of 2.7 × 104 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were used. Results differing by less than 2.0 Å in a positional root-mean-square deviation (rmsd) were clustered together and represented by the result with the most-favorable free energy of binding. As previously reported, Autodock proved to reproduce the experimental structure of the NNRTIs with low rmsd values. The representative F_2-*NH*-DABO (**3a**) and F_2-*N*,*N*-DABO (**7h**) structures were built by means of the stand-alone version of the program PRODRG[61] in conjunction with GROMACS[62] and then cross-docked in the 66 RTs using the above settings. The cross-docking results for each of the seven enzyme isoforms (WT, L100I, V106A, K103N, Y181C, Y188C, and Y188L) and were then reclustered using a rmsd tolerance value of 2.0. In each cluster, the best cluster[41] conformation coincided with the best docked conformation,[41] therefore only seven conformations for both **3a** and **7h** were used for the binding mode analyses.

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Supporting Information Available: Molecular modeling details; elemental analyses of compounds **4**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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