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Enantioselective binding of second generation pyrrolobenzoxazepinones to the catalytic ternary complex of HIV-1 RT wild-type and L100I and K103N drug resistant mutants

Stefania Butini [a,b], Sandra Gemma [a,b], Margherita Brindisi [a,b], Giuseppe Borrelli [a,b], Isabella Fiorini [a,b], Alberta Samuele [a,c], Aristotele Karytinos [a,c], Marcella Facchini [a,c], Andrea Lossani [a,c], Samantha Zanoli [a,c], Giuseppe Campiani [a,b,*], Ettore Novellino [a,d], Federico Focher [a,c], Giovanni Maga [a,c,*]

[a] European Research Centre for Drug Discovery and Development (NatSynDrugs), via Aldo Moro, 2–Università di Siena, 53100 Siena, Italy
[b] Dipartimento Farmaco Chimico Tecnologico, via Aldo Moro, 2–Università di Siena, 53100 Siena, Italy
[c] DNA Enzymology and Molecular Virology, Institute of Molecular Genetics IGM-CNR, National Research Council, 27100 Pavia, Italy
[d] Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli Federico II, via D. Montesano 49, 80131 Napoli, Italy

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ABSTRACT

We investigated some pyrrolobenzoxazepinone (PBOs, **3e**–**i**) analogues of early described effective non-nucleoside inhibitors of HIV-1 reverse transcriptase (RT). Enzymological studies of **3e**–**i** enantiomers, with wild type (wt) RT and some drug-resistant mutants, revealed a stereoselective mode of action and selectivity for RT ternary complex. Unexpectedly (+)-**3g** was found more potent towards the L100I mutant than towards the wt RT, whereas (+)-**3h** inhibited the K103N mutant and RT wt with comparable potency.

The virus-encoded human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) is one major target for antiviral chemotherapy of AIDS/HIV. Two classes of inhibitors target the viral RT: nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs). The NNRTIs nevirapine (**1**, Fig. 1), delavirdine, efavirenz, and the recently licensed etravirine (**2**, Fig. 1) are used in clinical practice and are characterized by less severe adverse effects than NRTIs or protease inhibitors (PIs).[1] Combinations of NNRTIs with NRTIs and PI, have been shown to produce more prolonged suppression than monotherapy or dual therapy, allowing CD4T cells restoration, immune system function restoration, and clinical improvement.[2] However, the occurrence of just a few single amino acid substitutions in the RT gene may confer resistance to most of the NNRTIs.[3] Therefore, the development of novel NNRTIs with improved pharmacological, pharmacokinetic, and drug resistance mutation profiles, is critical for a more successful application of NNRTIs in combination therapy.[4,5]

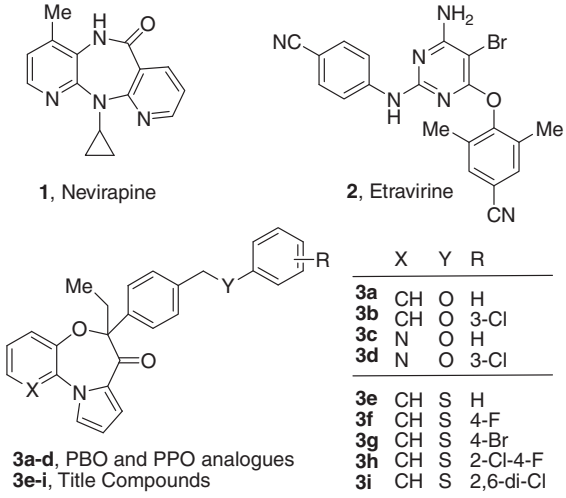

Figure 1. Reference and Title Compounds

* Corresponding authors. Tel.: +039 0577 234172; fax: +39 0577 234333 (G.C.); tel.: +39 0382 546355; fax: +39 0382 422286 (G.M.).
E-mail addresses: campiani@unisi.it (G. Campiani), maga@igm.cnr.it (G. Maga).

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Table 1
Inhibition (IC_{50}, μM) of HIV-1 wt and drug resistant mutant (K103N, L100I, and V179D) RT enzymes

Compds	IC_{50} (μM)			
	wt	K103N	L100I	V179D
1	0.40	7.0	9.0	2.0
(±)-**3a**	0.15	0.4	0.20	NT[a]
(±)-**3b**	0.10	0.30	0.25	0.3
(±)-**3c**[b]	0.25	1.50	NT[a]	3.6
(±)-**3d**[b]	0.11	0.80	0.80	2.0
(±)-**3e**	0.06	0.25	0.99	0.15
(±)-**3f**	0.25	3.35	>400	>40
(±)-**3g**	0.06	1.16	>400	0.29
(±)-**3h**	0.61	20.51	>400	>40
(±)-**3i**	7.36	>400	>400	>40

Each value is the mean of at least three experiments (SD are within 10%)
[a] NT, not tested
[b] Ki μM from Ref. 7.

We have previously described a novel class of NNRTIs based on pyrrolobenzo(pyrido)oxazepinone structure, (PBO, PPO **3a**,**d** Fig. 1),[6,7] which were effective against either wild type (wt) RT or carrying known drug resistance mutations. PBOs selectively targeted the catalytic ternary complex formed by the viral RT with its substrates nucleic acid and nucleotide, suggesting novel strategies for drug combinations.[8]

Due to the potential interest of PBOs as novel NNRTIs, we sought to further investigate their mechanism of action, by focusing on second generation chiral PBOs. For this purpose, we selected the racemic mixtures of compounds (±)-**3e**–**i**, and we compared their inhibition potencies and drug resistance profiles with those of their corresponding (+)- and (−)-enantiomers.

Derivatives (±)-**3e**–**i** were synthesized following a standard procedure[7] and we resolved the racemic mixtures, into the corresponding pure (+)- and (−)-enantiomers by means of chiral HPLC.

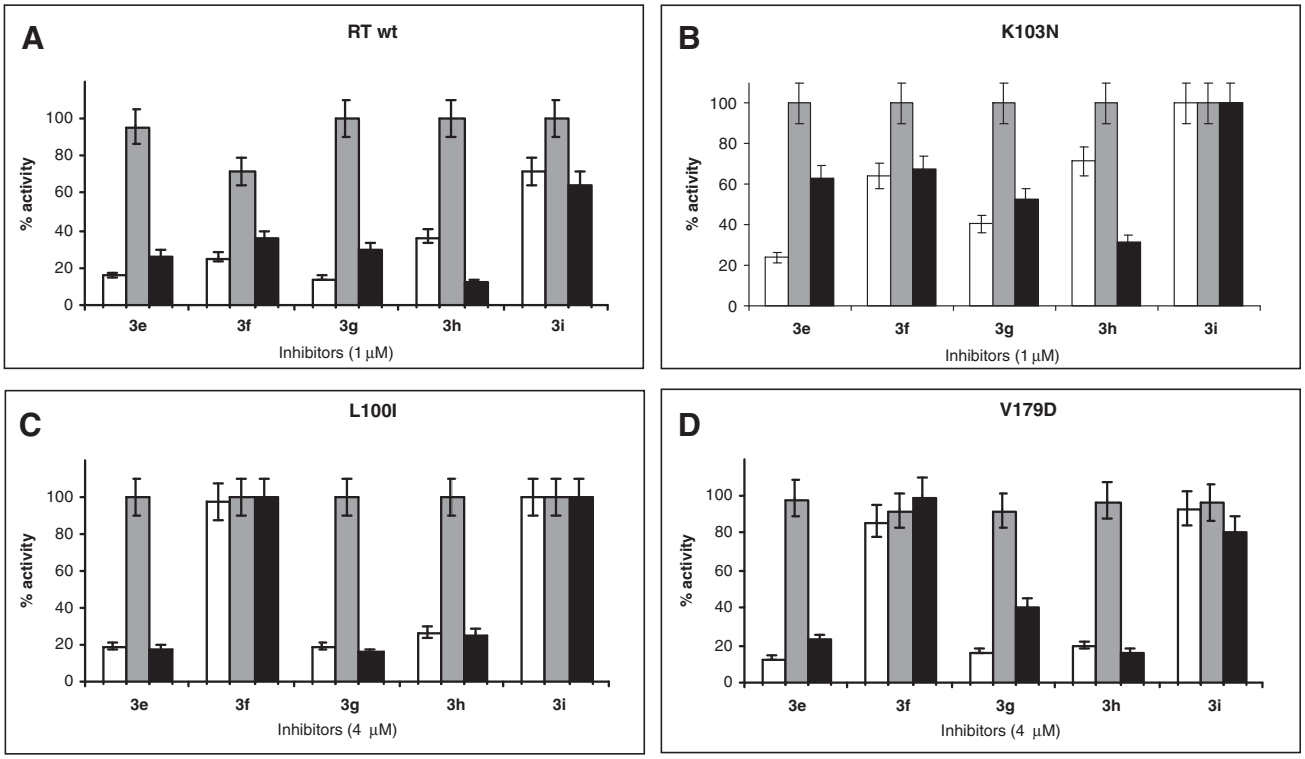

Figure 2. Effects of (±)-**3e-i** and their (−)- and (+)- enantiomers on HIV-1 RT wt (panel A) and drug resistant mutants (panels B–D). HIV-1 RT RNA-dependent DNA polymerase activity was tested in the presence of the indicated concentrations of inhibitors. Data obtained were then plotted as the relative (% ±SD) catalytic activity for each enzyme. Bars: white for racemic mixture; grey for (−)-enantiomers; black for (+)-enantiomers. Error bars indicate the standard deviations (±SD).

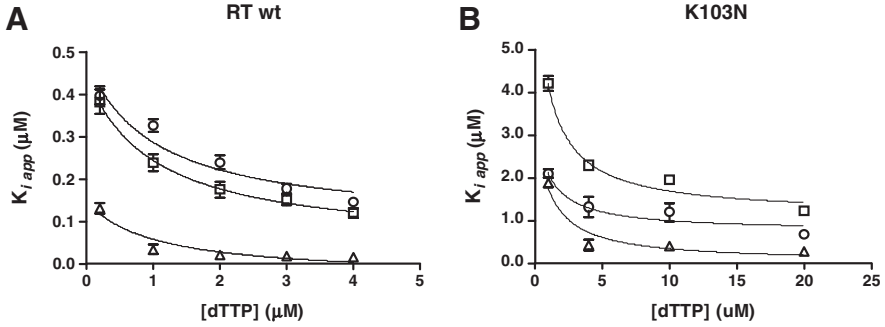

Figure 3. Variations of the apparent inhibition constant ($K_{i\ app}$) for (+)-**3e**,**g**,**h** against RT wt (A) and K103N (B), as a function of increasing dTTP concentrations. All points represent the mean values of three independent experiments. Error bars indicate the standard deviations (±SD). Symbols: ○, (+)-**3e**; □, (+)-**3g**; Δ, (+)-**3h**.