Bioorganic & Medicinal Chemistry Letters 18 (2008) 5777–5780

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Towards novel *S*-DABOC inhibitors: Synthesis, biological investigation, and molecular modeling studies

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ARTICLE INFO

Article history:
Received 16 August 2008
Revised 16 September 2008
Accepted 17 September 2008
Available online 23 September 2008

Keywords:
HIV-1
Reverse Transcriptase
Mutant
NNRTIs
S-DABOCs
Molecular modeling

ABSTRACT

A small family of *S*-DABO cytosine analogs (*S*-DABOCs) has been synthesized and biologically evaluated as HIV-1 inhibitor both on wild type (wt) and drug-resistant mutants leading to the identification of an interesting compound (**5d**). Molecular modeling studies have been finally performed in order to rationalize the results.

In the fight against the AIDS plague, the selection of drug-resistant viruses represents a significant obstacle to the eradication of the HIV infection.[1] To overcome the therapeutic failures connected with the selection of mutant strains resistant to common Reverse Transcriptase inhibitors (RTIs) or Protease inhibitors (PIs), different molecular targets have been recently investigated. An arsenal of anti-HIV drugs belonging to six different classes, after the recent approval of maraviroc (entry inhibitor)[2] and raltegravir (integrase inhibitor), are currently on the market.[3] Although the discovery of the last two drugs represents an important success in the fight against drug resistant HIV-1 viruses, a few clinical studies demonstrated that therapies based on maraviroc and raltegravir have comparable efficacy to regimens based on the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz.[4] Reverse transcriptase (RT) represents therefore an old but still important target which is highly investigated for the identification of novel NNRTIs endowed with a better activity profile against clinically relevant mutant strains.[5] A number of potent second-generation NNRTIs are in fact currently in clinical trial (Fig. 1).[6] During the course of our long lasting studies on *S*-DABO RT inhibitors, we discovered very potent compounds active on HIV-1 (wt)-infected cells at picomolar concentration.[7] However, a pronounced loss of activity was commonly observed when the same compounds were tested against drug resistant HIV-1 mutants (K103N, Y181C, and Y188L). To overcome the loss of activity against the above-

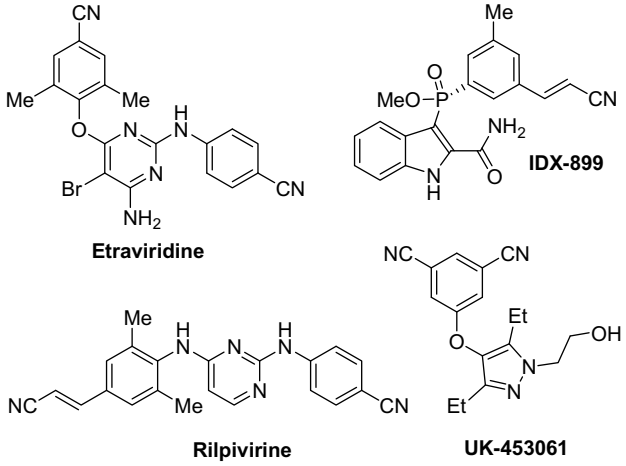

Figure 1. Etravirine and other selected NNRTIs in clinical trial.

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doi:10.1016/j.bmcl.2008.09.070

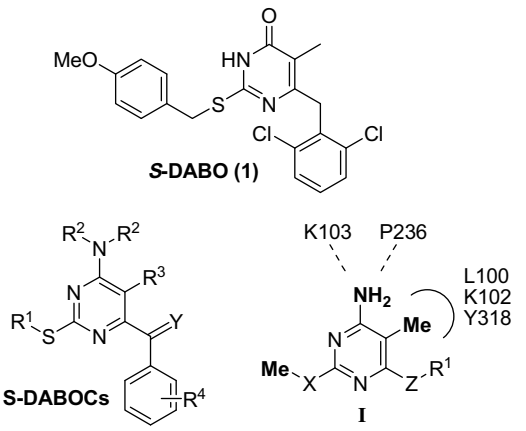
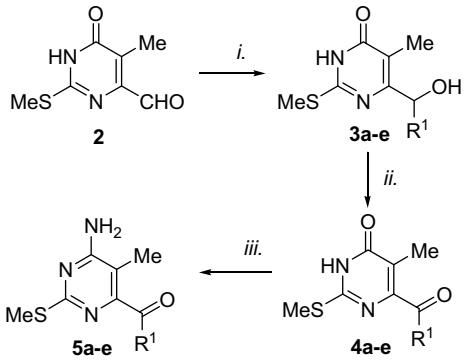


Figure 2. *S*-DABO precursor (**1**), *S*-DABOCs and new *S*-DABOC analogs with generic structure **I**.

mentioned mutants, we decided to synthesize simplified analogues of the *S*-DABO precursor **1** (Fig. 2) in order to identify a smaller molecule able to give specific interactions with residues of the allosteric site not associated with any drug-resistant mutation. Accordingly, the *S*-DABO cytosine analog family (*S*-DABOC) has been recently identified.[8] Based on the encouraging preliminary results on HIV inhibition, this study has been focused on the development of novel *S*-DABOC analogs with generic structure **I** in order to get additional information on the SAR for this new family of inhibitors (Fig. 2).

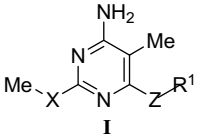
As starting point for this work, we decided to maintain invariate the functional groups which were previously found important for the RT:*S*-DABOCs interaction and to introduce additional variability at C2 and C6 positions (X, Z, and R_1): the target compounds (generic structure **I**) should therefore bear a C4 amino group (responsible for key hydrogen bond interaction with the backbone of Lys103 and Pro236) and a C5 methyl group (which seems to be accommodated in a hydrophobic region defined by Leu100, Lys102, and Tyr 318).[8b] Following a versatile synthetic approach previously reported by us,[8a] the key intermediate **2** was reacted with five different Grignard reagents to give the alcohols **3a–e** which were then oxidized to the corresponding C6-keto derivatives **4a–e** by reaction with Dess–Martin periodinane (Scheme 1). Finally, selective C4 chlorination in refluxing $POCl_3$ followed by nucleophilic substitution with methanolic ammonia gave the *S*-DABOC derivatives **5a–e** in which the X and Z moieties were kept fixed (and equal to S and CO, respectively) while introducing different R_1 groups on C6′ (see Table 1).

On the other hand, starting from the simpler derivative **5a**, R_1 was kept fixed (and equal to Ph) while additional functionalization was introduced in C6′ and C2 in order to select the most interesting substituents X and Z (for the antiviral activity) to be subsequently coupled with the best R_1 group. A series of more flexible derivatives was initially synthesized by reduction of the C6′ keto moiety with $NaBH_4$ to give the corresponding alcohol **6** which was further converted into the C6-chloromethyl phenyl and C6-fluoromethyl phenyl derivatives (**7a and 7b**) by reaction with DAST and PCl_5, respectively (Scheme 2).



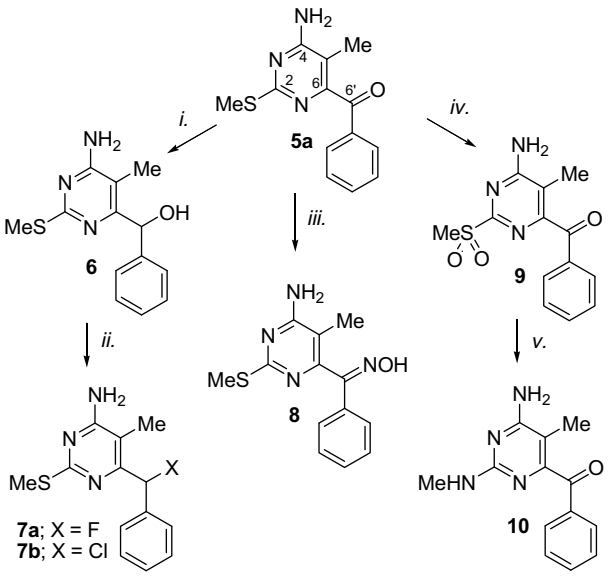
Scheme 1. Reagents and conditions: (i) RMgBr, THF, rt, 1 h; (ii) Dess–Martin periodinane, CH_2Cl_2, rt, 1 h; (iii) a—$POCl_3$, reflux, 1 h; b—$NH_{3(g)}$/MeOH, steal bomb, 100 °C, 2 h.

Table 1
Anti HIV-1 activity and cytotoxicity of *S*-DABOC derivatives



Entry	Compound	X	Z	R^1	ID_{50}[a,b] (μM)	ED_{50}[a,c] (μM)			CC_{50}[d]
					wt	NL4-3 wt	K103N	Y181C	
1	**5a**	S	CO	Ph	1.9	0.06	1.67 (28)[e]	1.71 (28)	>25
2	**6**	S	CHOH	Ph	7.7	>25	>25	>25	>25
3	**7a**	S	CHCl	Ph	>20	11.98	>25	>25	>25
4	**7b**	S	CHF	Ph	>20	>25	>25	>25	>25
5	**8**	S	CNOH	Ph	>20	10.45	>25	>25	>25
6	**9**	SO_2	CO	Ph	16	0.44	19.12 (43)	10.37 (23)	>25
7	**10**	NH	CO	Ph	>20	3.4	>25	>25	>25
8	**5b**	S	CO	CH_2Ph	>20	>25	>25	>25	>25
9	**5c**	S	CO	CH_2CH_2Ph	>20	>25	>25	>25	>25
10	**5d**	S	CO	3-F–Ph	0.2	0.034	0.695 (20)	0.779 (23)	>25
11	**5e**	S	CO	Naphthyl	2.7	>19.37	>19.37	>19.37	>19.37
12	**1**				0.004	0.007	4.7 (671)	nd[f]	>25
13	**NVP**	—	—	—	—	0.04	0.68 (17)	>2	>2
14	**EFV**	—	—	—	—	0.001	0.133 (133)	0.008 (8)	>1

[a] Data represent mean values of at least two experiments.
[b] ID_{50}: Inhibiting dose 50 or needed dose to inhibit 50% of the enzyme.
[c] EC_{50}: Effective concentration 50 or needed concentration to inhibit 50% HIV-induced cell death, evaluated with MTT method in MT-4 cells.
[d] CC_{50}: Cytotoxic concentration 50 or needed concentration to induce 50% death of noninfected cells evaluated with the MTT method in MT-4 cells.
[e] Fold-resistance is reported in parenthesis and expresses the ratio of EC_{50} value against drug-resistant strain and EC50 of the wild type NL4-3 strain.
[f] Nd, not determined.



Scheme 2. Reagents and conditions: (i) $NaBH_4$, MeOH, rt, 10 min; (ii) DAST, CH_2Cl_2, 0 °C, 1 h (for **7a**); $SOCl_2$, CH_2Cl_2, rt, 24 h (for **7b**); (iii) $NH_2OH?HCl$, AcONa, EtOH, reflux, 24 h; (iv) Oxone®, MeOH/H_2O 1:1, rt, 4 h; v. CH_3NH_2, MW, 170 °C, 30 min.

The oxime **8** was easily obtained reacting compound **5a** with hydroxylamine hydrochloride, while the functionalizations in C2 was accomplished by converting **5a** to the corresponding sulfone **9** and final nucleophilic substitution with methylamine under microwave assisted condition to give compound **10**. All the synthesized compounds were evaluated in enzymatic tests for their ability to inhibit wild type (wt) as well as on MT-4 cells for cytotoxicity and anti-HIV activity, in comparison with nevirapine, efavirenz and the *S*-DABO **1** used as reference drugs. In particular, the mutants K103N and Y181C were used for tests on cell lines. The biological results reported in Table 1 showed that among the Z substituents (entries 1–5), the best one was represented by the keto moiety (entry 1), while the introduction of a methylsulfonyl or a methylamino group on C2 in place of the methylthio determined a loss of activity (entries 6–7). Among the R_1 groups to be combined with the C6 keto moiety (entries 8–11), the best substituent was found to be the 3-fluorophenyl and the corresponding compound **5d** represents the most active derivative of the series both against HIV-1 wild type and resistant mutants (Table 1). As a general consideration, the increase of the C6 substituent flexibility either by introducing of a spacer between the C6′ keto moiety and the phenyl ring (**5b** and **5c**) or by converting **5a** into the C6-hydroxymethy phenyl, C6-chloromethy phenyl and C6-fluoromethyl phenyl derivatives (**6**, **7a**, and **7b** respectively), always led to a loss of activity (Table 1). In addition, the introduction of a polar moiety in C2 (X = SO_2, compound **9** and X = NH, compound **10**) always led to a loss of activity. The most important requirements for a good antiviral activity in the *S*-DABOC series seems to be the presence of a rigid structure characterized by a small substituted benzoyl group in C6: compound **5d** showed an activity profile better than that of both the *S*-DABO precursor **1** and nevirapine, exhibiting fold resistance values comparable to this commercial drug, EC_{50} values in the high-nanomolar range against all the studied mutants and low cytotoxicity. (see Fig. 3).

In order to have an idea of the druglikeness of our *S*-DABOC inhibitors, significant physicochemical properties were predicted using QikProp v2.5[9] and the results obtained were compared with that of common anti-HIV drugs on the market (Table 2). In a recently published review,[6] Sweeney and Klumpp reported the importance of pharmacokinetic factors in the success of first-line anti-HIV therapies based on NNRTIs. A head to head comparison between nevirapine and efavirenz, highlighted that despite the latter possesses higher in vitro antiviral activity, its poor pharmacokinetic profile make it clinically comparable to the less active nevirapine. Efavirenz has in fact shown low solubility (Log*S*), extensive binding to human serum proteins (Log*K*) and high incidence of CNS side effects (Log BB) that can be easily predicted with QikProp (Table 2). It was interesting to note that the predicted physicochemical properties for the most active *S*-DABOC **5d** seems to be similar to that of nevirapine thus making it a good candidate for further studies.

Docking simulations were finally conducted in order to get further insight on the SAR for the synthesized inhibitors starting from the crystal structure of the TNK-651:RT (wt) complex. A detailed analysis of the binding site was performed in order to identify the features responsible for the better activity of **5d** with respect

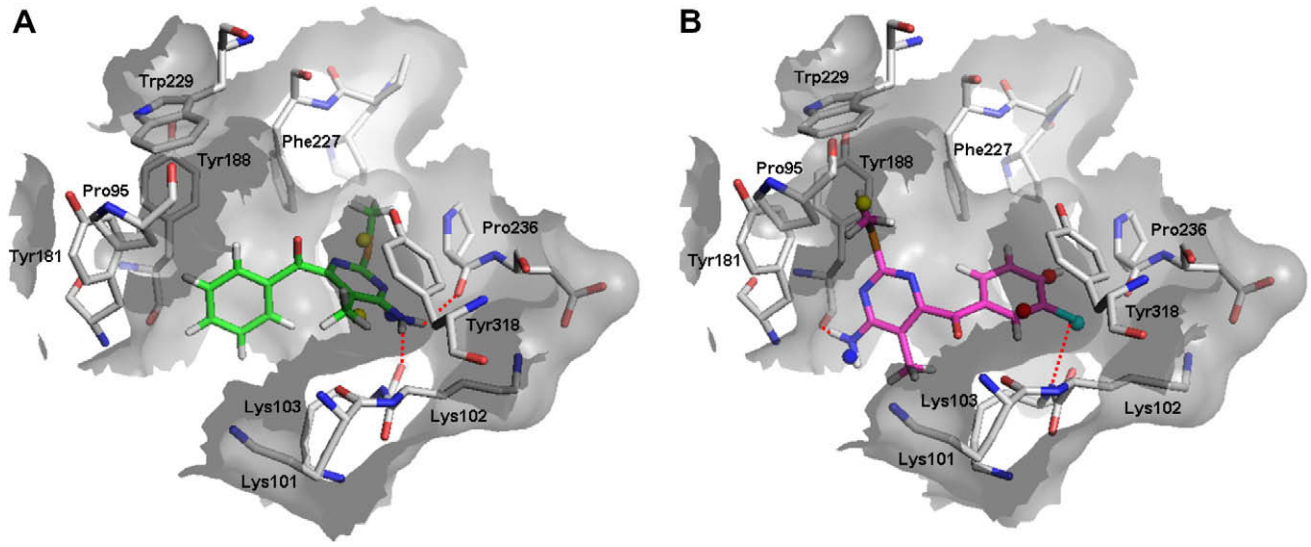


Figure 3. Graphical representation of the binding mode of the *S*-DABOCs **5a** and **5d** within the allosteric site of the TNK-651:RT complex (PDB code: 1RT2). Regions of minimum energy as derived from molecular interaction field calculations are also displayed for probes F (cyan spheres), CH_3 group (yellow spheres), CH aromatic or vinyl (red spheres), and neutral flat NH_2 (blue spheres). (A) Docked conformation of **5a** (sticks, green carbon atoms) and (B) docked conformation of **5d** (sticks, purple carbon atoms).

Table 2
Predicted physiochemical associated properties for synthesized *S*-DABOCs and commercial anti-HIV drugs

Compound	*QP*-Log *P*[a]	*QP*-Log *S*[b]	Number of metabolites[c]	*QP*-Log *K*[d]	*QP*-Log BB[e]	*QP*-Caco[f]
5a	2.28	−3.43	1	−0.12	−0.51	1039.53
6	2.24	−3.21	3	−0.17	−0.57	978.04
7a	3.57	−4.28	3	0.23	−0.06	2334.41
7b	3.26	−3.79	3	0.15	−0.12	2144.49
8	1.89	−3.05	1	−0.25	−0.9	509.9
5b	2.74	−3.9	2	0.01	−0.57	1098.97
9	0.27	−2.48	1	−0.6	−1.44	136.37
10	1.47	−2.91	1	−0.28	−0.88	539.46
5c	3.05	−4.23	3	0.09	−0.65	1138.96
5d	2.54	−3.87	1	−0.07	−0.41	1042.52
5e	3.45	−4.95	1	0.31	−0.6	997.79
1	5.5	−7.18	4	0.89	−0.36	1725.36
Nevirapine	2.32	−3.67	6	0.01	−0.1	1917.57
Efavirenz	3.51	−5.1	2	0.29	−0.02	1315.89
Delavirdine	2.61	−5.75	4	0.15	−1.6	243.63
Etravirine	2.67	−5.99	2	0.25	−2.1	55.64

[a] Predicted octanol/water partition coefficient; range of recommended values (−2.0)–(+6.5).
[b] Predicted aqueous solubility; range of recommended values: (−6.5)–(+0.5).
[c] Number of likely metabolic reactions; range of recommended values: 1–8.
[d] Prediction of binding to human serum albumin; range of recommended values: (−1.5)–(+1.5).
[e] Predicted brain/blood partition coefficient; range of recommended values: (−3.0)–(+1.2).
[f] Predicted apparent Caco-2 cell permeability in nm/s; range of recommended values: <25 poor; >500 great.

to **5a**. For this purpose, molecular interaction fields (MIFs) were calculated for the binding site using the software Grid[10] (for further details see Supplementary data). Details derived from both docking studies and Grid analysis allowed to rationalize the biological results. In summary, two different binding modes were found for the most active inhibitors: compound **5a** showed a binding mode similar to that previously found[8b] and characterized by key hydrogen bond interaction with the backbone of Lys103 and Pro236. This kind of interactions have been suggested to be very important for maintaining the activity against the RT mutants since are less likely to be disrupted as the result of a mutation.[11] Alternatively, the presence of a *meta*-fluoro substituent on the C6-benzoyl ring determine a 180° flip of the molecule along the CO axis in order to locate the fluoro atom and other important features on the corresponding minima calculated by GRID. In this orientation, compound **5d** gives two hydrogen bond contacts with the backbone of Lys103 and Tyr188, π–π interaction with Tyr318 and important hydrophobic interactions between the small C2-methylthio group and the hydrophobic pocket defined by Tyr181, Tyr188, Phe227 and Trp229 which could account for the better activity profile.

In conclusion, a SAR study on the synthesized *S*-DABOC analogues led to the identification of an interesting inhibitor (**5d**) endowed with nanomolar activity and predicted pharmacokinetic properties similar to that of anti-HIV drugs on the market. Molecular modeling studies suggested the important features responsible for the RT:*S*-DABOCs interaction and will be of help in the synthesis of more active analogues.

Acknowledgments

This study was supported by grants from the European THiNC Consortium. The University of Siena and the Spanish FIS PI060624 are also acknowledged for financial support. S.Z. is the recipient of a Buzzati-Traverso Fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.070.

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