A Versatile Route to C-6 Arylmethyl-Functionalized *S*-DABO and Related Analogues

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ABSTRACT



Since their discovery in 1992, 3,4-dihydro-2-alkoxy-6-benzyl-4-oxypyrimidines (DABOs) have been subjected to many structural modifications in order to obtain better non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of AIDS. Herein, we report a straightforward and versatile route for the synthesis of novel C-6 aryImethyl-functionalized *S*-DABO, a poorly explored class of derivatives. Finally, biological evaluation of the synthesized derivatives led to the identification of a promising anti-HIV-1 lead compound.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) represent essential components in first-line anti-HIV-1 therapy due to their good tolerability and lack of association with lipodystrophy generally associated with the administration of protease inhibitors.¹ NNRTIs include more than 30 structurally different classes of molecules, such as nevirapine, TIBO, HEPT, TNK-561, ITU, DATA, and DAPY.² These compounds bind to a specific allosteric site of HIV-1 RT near the polymerase site and interfere with reverse transcription by altering either the conformation or mobility of RT, thereby leading, with only one exception,³ to a noncompetitive inhibition of the enzyme. Among the NNRTIs reported to date, DABO analogues (Figure 1) have been the object

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(1) Barbaro, G. Barbini, G. Chemotherapy 2006, 52, 161-165.

(2) (a) Boone, L. R. Curr. Opin. Invest. Drugs 2006, 7, 128–135. (b) Barbaro, G.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. Curr. Pharm. Des. 2005, 11, 1805–1843.

of great interest since their discovery in 1992⁴ and have led to the identification of highly potent compounds against both HIV-1 RT wild type (wt) and drug-resistant mutants.⁵ In the



Figure 1. Common DABO analogues and target compound I.

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 ⁽³⁾ Maga, G.; Radi, M.; Zanoli, S.; Manetti, F.; Cancio, R.; Hübscher,
U.; Spadari, S.; Falciani, C.; Terrazas, M.; Vilarrasa, J.; Botta, M. Angew. Chem., Int. Ed. 2007, 46, 1810–1813.

Scheme 1. Retrosynthetic Approach for the Key Intermediates 3a,b



search for agents able to target the NNRTIs drug-resistant mutants, many different modifications have been performed on the pyrimidinone scaffold of the DABO-family during the past 25 years: (i) introduction of different chains at position C-2; (ii) substitution of the hydrogen in C-5 with bulkier groups; (iii) introduction of different substituents on the phenyl ring at position C-6; and (iv) substitution of the phenyl ring in C-6 with different aromatic or heteroaromatic moieties.⁶ However, few modifications of the arylmethyl carbon at the C-6 position have been reported so far, and it has been recently shown by Ji et al.7 that this kind of functionalization led to potent anti-HIV-1 DABOs, although only biological data for the HIV-1 (wt) infected MT-4 cells were disclosed.8

Herein, we report a straightforward and versatile approach for the synthesis of C-6 arylmethyl-functionalized S-DABO analogues (general structure I, Figure 1) accessible by consecutive functionalization of the C-6 hydroxy group in the key intermediates **3a**,**b** (Scheme 1). The identification of a lead compound belonging to a new family of S-DABO cytosine analogues is also disussed.

In our original idea, the key intermediate 3a could be obtained by two alternative pathways, namely the direct lithiation in C-6 and reaction with the appropriate aldheyde⁹ or passing through the C-6-formyl intermediate 2 (Scheme 2).10 However these approaches were unsuccessful even starting from the corresponding N³-benzyl- and O-benzyl-

(4) Botta, M.; Artico, M.; Massa, S.; Gambacorta, A.; Marongiu, M. E.; Pani, A.; La Colla, P. Eur. J. Med. Chem. 1992, 27, 251-257

(10) Megati, S.; Sodum, R.; Otter, G. M.; Klein, R. S.; Otter, B. A. Bioorg. Med. Chem. Lett. 1994, 4, 469-472.

protected derivatives of compound 1. Two different synthetic routes were then planned for the synthesis of the key intermediates **3a**,**b** (Scheme 1): according to route A, **3a**,**b** could be obtained after cyclization of S-methylisothiourea (SMT) with the β -ketoesters **7a**,**b** and final *O*-deacetylation. The intermediates **7a**,**b** could be achieved after condensation of potassium ethyl 2-methyl malonate with 6a,b, which could in turn be obtained via oxidation of 5a,b. According to route B, the key intermediates **3a**.**b** could be obtained via Grignard reaction on the aldehyde 2, which could in turn be obtained after deprotection of the acetal 12 resulting from the cyclization of SMT with the β -ketoester 11.



Following the approach described in route A, 4-fluorobenzaldehyde 4a was converted in compound 5a via reaction with ethynylmagnesium bromide and subsequent protection of the α -hydroxy group as acetyl derivative (Scheme 3). Compound 5a was then oxidized to the corresponding carboxylic acid **6a**, which was activated as imidazolide and then reacted with potassium ethyl 2-methylmalonate to give the β -ketoester **7a**. Unfortunately, the subsequent condensation of 7a with SMT did not afford the expected pyrimidinone 8a, while the lactone 9a was obtained as the only product. This synthetic pathway could represent, however, an alternative approach for the synthesis of 3,5-disubstituted

^{(5) (}a) Manetti, F.; Esté, J. A.; Clotet-Codina, I.; Armand-Ugón, M.; Maga, G.; Crespan, E.; Cancio, R.; Mugnaini, C.; Bernardini, C.; Togninelli, A.; Carmi, C.; Alongi, M.; Petricci, E.; Massa, S.; Corelli, F.; Botta, M. J. Med. Chem. 2005, 48, 8000-8008. (b) Cancio, R.; Mai, A.; Rotili, D.; Artico, M.; Sardella, G.; Clotet-Codina, I.; Esté, J. A.; Crespan, E.; Zanoli, S.; Hübscher, U.; Spadari, S.; Maga, G. ChemMedChem 2007, 2, 445-448

⁽⁶⁾ Artico, M. Drugs Future 2002, 27, 159-175.

⁽⁷⁾ Ji, L.; Chen, F.-E.; De Clercq, E.; Balzarini, J.; Pannecouque, C. J. Med. Chem. 2007, 50, 1778-1786.

⁽⁸⁾ The work cited in ref 7 was published during the preparation of the present manuscript whose content was already disclosed in the MD thesis of one of the authors: Contemori, L. MD thesis, University of Siena, Siena, Italy, 2006.

⁽⁹⁾ Petersen, L.; Jessen, C. H.; Pedersen, E. B.; Nielsen, C. Org. Biomol. Chem. 2003, 1, 3541-3545.



tetronic acid derivatives.¹¹ Different protecting groups were then used, in place of the acetyl, to mask the hydroxy moiety in order to overcome the intramolecular cyclization of compound **7a**, but the desired pyrimidinone **8a** was never obtained. Following route B (Scheme 4), mixed Claisen



condensation between the ethyl diethoxyacetate **10** (bearing a masked formyl group) and ethyl propionate gave quantitatively the β -ketoester **11** which was then submitted to cyclization reaction with SMT to give the pyrimidinone **12** in 70% yield. Deprotection of **12** in refluxing trifluoroacetic acid gave quantitatively the C-6 formyl derivative **2** which was then treated with the appropriate Grignard reagent to give the desired compounds **3a**,**b** in 60–70% yield. Route B therefore is a straightforward 4 step approach for the synthesis of the key intermediates **3a**,**b** in 42–49% overall yield. The significance of this approach is represented by the possibility of introducing two levels of functionalization: in the first level, different substituents can be introduced on the phenyl ring reacting the aldehyde 2 with different Grignard reagents while in the second level, the C-6 hydroxy group of the key intermediates can be additionally functionalized as shown in Scheme 5. Accordingly, compounds **3a**,**b** were oxidized with Dess-Martin periodinane affording the intermediates 13a,b which were then alternatively converted into C-6 functionalized pyrimidinones or cytosino analogues (Scheme 5). Reaction of 13a,b with methylmagnesium bromide afforded the alcohols 14a,b, which were then converted into the C-6 vinyl derivatives 15a,b after activation of the hydroxyl group as trifluoro acetate and subsequent acid-catalyzed elimination. On the other hand, selective C-4 chlorination of 13a,b using POCl₃ and subsequent nucleophilic substitution with methanolic ammonia, gave the cytosino analogues 16a,b. Reaction of the latter compounds with methylmagnesium bromide afforded 17a,b which were dehydrated by refluxing with concentrated HCl to give the cytosine derivatives **18a.b**. It should be mentioned that the dehydration of compounds 14a,b required the milder activation-elimination approach since reacting these compounds with refluxing HCl gave the S-demethylated product instead of the desired compounds 15a,b. No traces of the Sdemethylated side products were observed when the same reaction was carried out on cytosino analogues 17a,b: the desired compounds 18a,b were obtained in 20% yield together with easily removable decomposition products. The S-DABO analogues included in Scheme 5 were finally evaluated for cytotoxicity and anti-HIV-1-activity in comparison with nevirapine (NEV) and efavirenz (EFV), used as reference drugs (Table 1). The results of these assays allowed identification of an interesting lead compound (16a) possessing low cytotoxicity and submicromolar activity on MT-4 cells infected with both HIV-1 (wt) and clinically relevant mutants.

⁽¹¹⁾ Aragón, D. T.; López, G. V.; Garcia-Tellado, F.; Marrero-Tellado, J. J.; de Armas, P.; Terrero, D. J. Org. Chem. **2003**, 68, 3363–3365.



In summary, a straightforward and versatile approach for the synthesis of new C-6 arylmethyl functionalized *S*-DABO analogues has been developed. An efficient 4 step procedure

Table 1.	Cytotoxicity and Anti-HIV-1 Activity				
	$\mathrm{EC}_{50}(\mu\mathbf{M})^{a,b}$				
compd	NL4-3 (wt)	K103N	Y181C	Y188L	$\mathrm{CC}_{50}{}^{c}$
16a	0.58	5.96	0.21	>90.25	90.25
NVP	0.08	1.8	0.87	5.6	>100
EFV	0.004	0.09	0.006	0.23	>0.3

^{*a*} Data represent mean values of at least two experiments. ^{*b*} EC₅₀: effective concentration 50 or needed concentration to inhibit 50% HIV-induced cell death, evaluated with MTT method in MT-4 cells. ^{*c*} CC₅₀: cytotoxic concentration 50 or needed concentration to induce 50% death of noninfected cells evaluated with theMTT method in MT-4 cells.

led us to the synthesis of the key intermediates 3a,b (in 42–49% overall yield) while their further functionalization led to the identification of an interesting anti-HIV-1 lead compound (16a) belonging to a new family of *S*-DABO cytosine analogues. Exploitation of this procedure is underway in our laboratories for the synthesis of a large number of *S*-DABO cytosine analogues variously substituted on the C-6 arylmethyl moiety. An SAR study on this poorly explored class of derivatives might allow development of novel anti-HIV-1 inhibitors active on clinically relevant mutant strains.

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Supporting Information Available: Experimental procedures and spectral and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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