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Nonnucleoside HIV-1 reverse transcriptase inhibitors; part 3. Synthesis and antiviral activity of 5-alkyl-2-[(aryl and alkyloxyl-carbonylmethyl)thio]-6-(1-naphthylmethyl) pyrimidin-4(3*H*)-ones☆

Yanping He,[a] Fener Chen,[a,*] Xiongjie Yu,[a] Yueping Wang,[a] Erik De Clercq,[b] Jan Balzarini, and Christophe Pannecouque[b]

[a] *Department of Chemistry, Fudan University, Shanghai 200433, People's Republic of China*
[b] *Rega Institute for Medical Research, Katholieke Universiteit Leuven: 10 Minderbroedersstraat, B-3000 Leuven, Belgium*

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Abstract

A series of 6-naphthylmethyl substituted *S*-alkylated dihydroalkoxybenzyloxopyrimidine (*S*-DABO) analogues with a β-carbonyl group on the *C*-2 side chain were synthesized. All of the new compounds were evaluated for their anti-HIV activities in MT-4 cells. The most active compound, 5-isopropyl-2-[(4′-methoxyphenylcarbonyl-methyl)thio]-6-(1-naphthylmethyl)pyrimidin- 4(3*H*)-one showed activity against HIV-1 and against the double mutated strain of HIV(Y181C and K103N) in the micromolar range. Furthermore, some of the compounds are active against both HIV-1 and HIV-2 in cell culture. In view of the fact that the loss of antiviral activity of these compounds when tested against **S0561945** was much less pronounced than the loss of activity of typical NNRTIs, it is concluded that some of the compounds might interfere with another target or act on reverse transcriptase in a different way than the typical NNRTIs.

Keywords: NNRTIs; *S*-DABOs; Antiviral activity; HIV-1; HIV-2

☆ Parts 1 and 2 reported in references 18 and 19a, respectively.

* Corresponding author. Fax: +86-21-65641740.
E-mail address: rfchen@fudan.edu.cn (F. Chen).

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1. Introduction

Advances in acquired immune deficiency syndrome (AIDS) chemotherapy revealed several promising discoveries used to combat this disease. In particular, the FDA approvals of Nevirapine [1], Delavirdine [2], and Efavirenz [3] show that non-nucleoside inhibitors of reverse transcriptase (NNRTIs) are valid approaches to AIDS therapy. Although the therapeutic potential of this class of drugs has been compromised by the rapid development of resistance [4], their use in combination therapy has been encouraging and has revived interest in the search for novel and potent NNRTIs [5].

NNRTIs include many structurally distinct subclasses of compounds that bind to common allosteric site of HIV reverse transcriptase (RT) near the polymerase site and interfere with RT by altering either the conformation or the mobility of RT. This leads to noncompetitive inhibition of the enzyme [6]. This particular binding site corresponds to a flexible, highly hydrophobic pocket that is exclusively found in the RT of HIV-1. Hence, all NNRTIs are highly specific inhibitors for HIV-1 and have no efficacy against HIV-2 or other retroviruses [7]. Since the discovery of 1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine (HEPT) [8] (**1**, Fig. 1) and tetrahydro-imidazo[4,5,1-jkj][1,4]benzodiazepin-2(1H)-one and thione (TIBO) as NNRTIs in 1989, more than 30 different classes of NNRTIs have been reported [9]. Among them, dihydroalkoxybenzyloxopyrimidines (DABOs) [10] (**2**, Fig. 1) represented an intriguing class of NNRTIs developed in the past decade. From the chemical point of the view, DABOs belong to the 4-pyrimidinone series as well as HEPTs. Due to the structural similarities between them, DABOs and HEPTs share some chemical requirements for anti-HIV activity. Thus, the structure-activity relationship

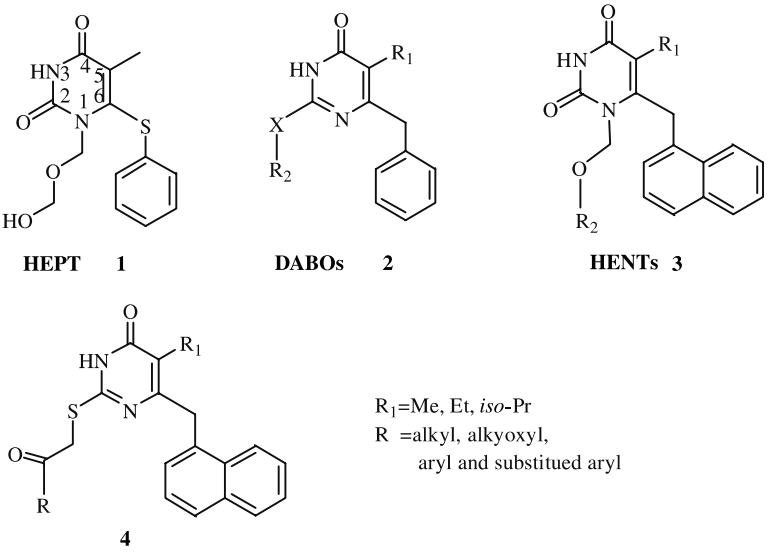


Fig. 1. Structures of HEPTs and DABOs.

(SAR) conclusions for HEPTs can be used to guide the design of DABO candidates [11]. In fact, a series of excellent *S*-DABO derivatives [12], including MTM-*S*-DABOs [13], DATNOs [14], and F_2-*S*-DABOs [15] have already been synthesized. The in vitro anti-HIV-1 potency and selectivity of the new *S*-DABO derivatives make them particularly attractive targets for further development as potential anti-AIDS drugs.

Recently, based on Hopkin's postulation [16] and 3D-QSAR studies of HEPT analogues [17], we successfully designed and synthesized a series of 6-(1-naphthylmethyl) substituted HEPT analogues (HENTs) [18] (**3**, Fig. 1) as potent HIV-1 inhibitors. Further SAR studies of these HEPT analogues indicated that in the RT nonnucleoside hydrophobic pocket, the *N*-1 side chain of the inhibitor was adjacent to residues Tyr318, Lys103, Pro236, and Pro225. Some enhancement of potency was attributed to the favourable hydrogen bonding interaction between the β-oxygen of the *N*-1 side chain and the main chain NH of Tyr318 residue [19]. Combined with the finding that the *C*-2 alkylthio chain, which is a major determinant for the anti-HIV-1 activity of *S*-DABOs, was locked in the same region of the binding site as the *N*1-substituted group on HEPT [11,20], we proposed that the introduction of a hydrogen bond acceptor to the corresponding site of *C*2 side chain of *S*-DABOs might enhance the interaction between the inhibitors and the RT. In order to examine our proposal and generate more potent NNRTIs, a series of 6-(1-naphthylmethyl) substituted *S*-DABO derivatives **4** (Fig. 1) with a β-carbonyl on the *C*2 side chain were designed. This paper describes the synthesis, antiviral activity evaluation, and the structure-activity relationships of these compounds.

2. Materials and methods

2.1. Chemical synthesis

2.1.1. Materials

Melting points were determined on a WRS-1 digital melting point instrument. Infrared (IR) spectra were recorded on a Nicolet FT-IR 360 spectrometer as KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DMX 500 MHz spectrometer using DMSO-d_6 as solvent. Chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (TMS). Mass spectra were obtained on a HP 5989A mass spectrometer. Regents and solvents were all analytical grade and were purified and dried by standards methods before use. All air-sensitive reactions were run under an atmosphere of N_2.

2.1.2. Synthesis

The synthesis of the *S*-DABO derivatives is shown in Fig. 2. The key intermediates thiourea **7a–c** were prepared from commercially available 1-naphthylacetonitrile and ethyl 2-bromoalkanoates following the procedure described previously [18]. Reaction of **7a–c** with various halo-esters or halo-ketones in dry DMF in the presence of K_2CO_3 afforded the target compounds **4a–u** in 31–69% yield. The structures of these

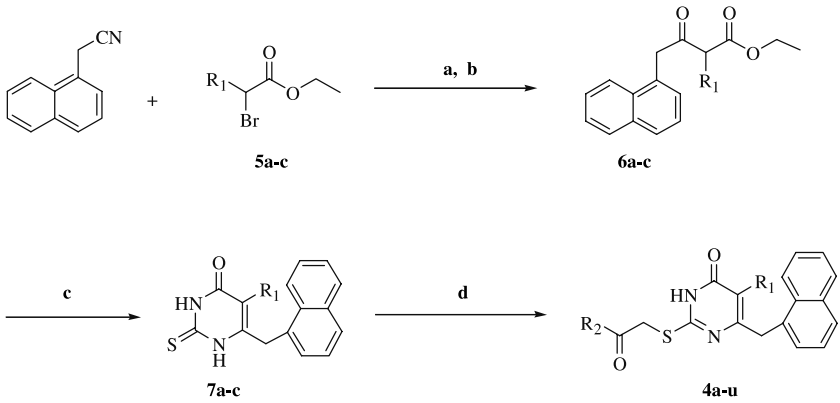


Fig. 2. Reagents and conditions: a, $R_1CHBrCOOEt/Zn/THF$; b, 10% aq. HCl; c, $NH_2CSNH_2/NaOEt$; d, R_2COCH_2Br, K_2CO_3, DMF, 14–24 h, rt.

compounds were determined by mass spectral, ^1H NMR, ^{13}C NMR data as well as a single crystal X-ray structure analysis for compound **4o** (Fig. 3).

*General procedure for preparation of derivatives **4a–u***. To a solution of thiouracil **7** (2 mmol) in anhydrous DMF (8 ml) were added K_2CO_3 (2.2 mmol) and $BrCH_2COR$ (2.2 mmol). The mixture was stirred at room temperature for 10–24 h. After TLC (EtOAc: PE, 1:1) revealed the disappearance of the starting material, the reaction mixture was filtered, the filtrate was concentrated in vacuo, and CH_2Cl_2 (50–80 mL) was added to the residue. The organic phase was washed with H_2O (2×50 mL), dried over anhydrous Na_2SO_4 and evaporated in vacuo to give the crude products **4a–u**. The crude products were purified by column chromatography (eluent: CH_2Cl_2: EtOAc: hexane). Selected analytical data for compounds **4a–u** are presented below.

*5-Methyl-6-(1-naphthylmethyl)-2-[(p-tolylethylcarbonylmethyl)thio]pyrimidin-4(3H)-one **(4a)***. Yield: 54%; mp: 187.1–187.8 °C; FT-IR (KBr): 3425, 2924, 1673,

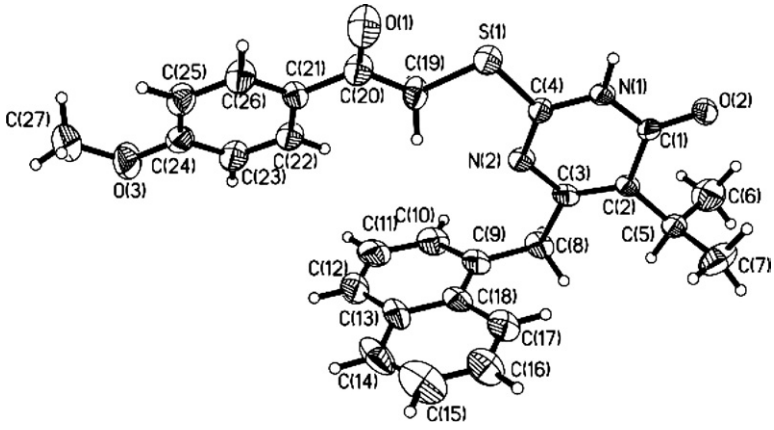


Fig. 3. X-ray crystal structure of **4o**.

1636 cm^{-1}; ^1H NMR δ 1.95 (s, 3H, CH_3), 2.30 (s, 3H, Ph-CH_3), 4.12 (s, 2H, SCH_2), 4.43 (s, 2H, CH_2naphthyl), 6.88–7.46 (m, 11H, ArH), 12.73 (s, br s, 1H, NH); ^{13}C NMR δ 10.87 (CH_3), 21.57 (Ph-CH_3), 37.7 (CH_2naphthyl), 37.8 (SCH_2), 116.2 (C-5), 124.4–134.4 (10C, naphthyl and 5C, Ph-C), 144.2 (Ph-C), 156.5 (C-6), 160.8 (C-2), 163.6 (C-4), 193.1 (C=O). HRMS *m/z* 414.1405 ($C_{25}H_{22}N_2O_2S$ requires 414.1402).

5-Methyl-2-[(4′-methoxy-phenylcarbonylmethyl) thio]-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4b)***. Yield: 34%; mp: 176.4–178.3 °C; FT-IR (KBr): 3421, 1673, 1644 cm^{-1}; ^1H NMR δ 1.96 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 4.16 (s, 2H, SCH_2), 4.40 (s, 2H, CH_2naphthyl), 6.88–7.96 (m, 11H, ArH), 12.73 (s, br s, 1H, NH); ^{13}C NMR δ 10.3 (CH_3), 37.0 (CH_2naphthyl), 37.2 (SCH_2), 55.4 (OCH_3), 113.6 (2C, Ph-C), 114.8 (C-5), 123.9–133.9 (10C, naphthyl and 3C, Ph-C), 156.5 (C-6), 160.8 (C-2), 162.9 (Ph-C), 163.1 (C-4), 193.1 (C=O); HRMS *m/z* 430.1362 (M^+, $C_{25}H_{22}N_2O_3S$ requires 430.1351).

5-Methyl-2-[(methylcarbonylmethyl) thio]-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4c)***. Yield: 63% mp: 149.6–150.8 °C; FT-IR (KBr): 3422, 2931, 1640 cm^{-1}; ^1H NMR δ 1.95 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 4.15 (s, 2H, SCH_2), 4.42 (s, 2H, CH_2naphthyl), 7.14–7.78 (m, 7H, ArH), 12.74 (s, br s, 1H, NH); ^{13}C NMR δ 10.8 (CH_3), 37.4 (CH_2naphthyl), 37.5 (SCH_2), 23.5 (CH_3), 115.3 (C-5), 123.9–134.0 (10C, naphthyl), 156.5 (C-6), 161.8 (C-2), 163.1 (C-4), 198.7 (C=O); HRMS *m/z* 338.1101 (M^+ $C_{19}H_{18}N_2O_2S$ requires 338.1089).

2-[(4′-Fluoro-phenylcarbonylmethyl) thio]-5-methyl-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4d)***. Yield: 60%; mp: 184.8–185.8 °C; FT-IR (KBr): 3422, 2926, 1689, 1632 cm^{-1}; ^1H NMR δ 1.95 (s, 3H, CH_3), 4.12 (s, 2H, SCH_2), 4.43 (s, 2H, CH_2naphthyl), 7.05–8.11 (m, 11H, ArH), 12.77 (s, br s, 1H, NH); ^{13}C NMR δ 10.8 (CH_3), 37.6 (CH_2naphthyl), 37.7 (SCH_2), 115.3 (C-5), 116.0 (2C, Ph-C), 123.7–134.4 (10C, naphthyl and 3C, Ph-C), 149.7 (C-6), 151.6 (C-2), 164.1 (C-4), 166.4 (Ph-C), 192.1 (C=O); HRMS *m/z* 418.1166 (M^+, $C_{24}H_{19}FN_2O_2S$ requires 418.1151).

2-[(4′-Chloro-phenylcarbonylmethyl) thio]-5-methyl-6-(1-naphthylmethyl)-pyrimidin-4-(3H)-one ***(4e)***. Yield: 58%; mp: 167.2–168.1 °C; FT-IR (KBr): 3415, 2912, 1693, 1643 cm^{-1}; ^1H NMR δ 1.94 (s, 3H, CH_3), 4.10 (s, 2H, SCH_2), 4.32 (s, 2H, CH_2naphthyl), 7.02–8.13 (m, 11H, ArH), 12.78 (s, br s, 1H, NH); ^{13}C NMR δ 10.8(CH_3), 37.6 (CH_2naphthyl), 37.7(SCH_2), 116.2 (C-5), 123.8–138.4 (10C, naphthyl and 6C, Ph), 156.1 (C-6), 158.7 (C-2), 163.4 (C-4), 192.1 (C=O); HRMS *m/z* 434.0859 (M^+, $C_{24}H_{19}ClN_2O_2S$ requires 434.0856).

2-[(Methoxycarbonylmethyl) thio]-5-methyl-6-(1-naphthylmethyl)- pyrimidin-4(3H)-one ***(4f)***. Yield: **69**%; mp: 183.0–183.4 °C; FT-IR (KBr): 3437, 2968, 1740, 1636 cm^{-1}; ^1H NMR δ 1.97 (s, 3H, CH_3), 3.42 (S, 3H, OCH_3), 3.75 (s, 2H, SCH_2), 4.28 (s, 2H, CH_2naphthyl), 7.20–8.14 (m, 7H, ArH), 12.78 (s, br s, 1H, NH); ^{13}C NMR δ 10.8 (CH_3), 32.1 (CH_2naphthyl), 37.8 (SCH_2), 52.5 (OCH_3), 124–134.5 (10C, naphthyl), 115.8 (C-5), 156.6 (C-6), 160.8 (C-2), 163.3 (C-4), 169.1 (C=O); HRMS *m/z* 354.1041 (M^+, $C_{19}H_{18}N_2O_3S$ requires 354.1038).

2-[(Ethoxycarbonylmethyl) thio]-5-methyl-6-(1-naphthylmethyl)- pyrimidin-4(3H)-one ***(4g)***. Yield: 52%; mp:163.2–163.8 °C; FT-IR (KBr): 3437, 2968, 1740, 1636 cm^{-1}; ^1H NMR δ 0.99 (t, *J* 7.0, 3H, CH_3), 1.96 (s, 3H, CH_3), 3.76 (s, 2H, SCH_2), 3.86 (q, *J* 7.0, 2H, OCH_2), 4.28 (s, 2H, CH_2naphthyl), 7.18–8.17 (m, 7H, ArH), 12.77 (s, br s, 1H,

NH); ^{13}C NMR δ 10.8 (CH_3), 13.6 (CH_3), 32.1 (CH_2naphthyl), 37.4 (SCH_2), 56.8 (OCH_2), 123.2–134.1 (10C, naphthyl), 116.1 (C-5), 154.3 (C-6), 161.8 (C-2), 164.2 (C-4), 170.1 (C=O); HRMS *m/z* 368.1205 (M^+, $C_{20}H_{20}N_2O_3S$ requires 368.1195).

5-Ethyl-6-(1-naphthylmethyl)-2-[(p-tolylethylcarbonylmethyl)thio]pyrimidin-4(3H)-one ***(4h)***. Yield: 42%; mp: 179.8–180.9 °C; FT-IR (KBr): 3425, 2924, 1740, 1673, 1636 cm^{-1}; ^{1}H NMR δ 0.89 (s, 3H, *J* 7.35, CH_3), 2.27 (s, 3H, Ph-CH_3), 2.49(q, *J* 7.35, 2H, CH_2), 4.13 (s, 2H, SCH_2), 4.41(s, 2H, CH_2naphthyl), 7.06–7.93 (m, 11H, ArH), 12.73 (s, br s, 1H, NH); ^{13}C NMR δ 13.4 (CH_3), 18.8 (CH_2), 21.2 (Ph-CH_3), 37.7 (CH_2naphthyl), 37.8 (SCH_2), 116.2 (C-5), 124.4–134.4 (10C, naphthyl; 5C, Ph-C), 144.2 (Ph-C), 156.5 (C-6), 160.8 (C-2), 163.6 (C-4), 192.6 (C=O); HRMS: *m/z* 428.1563 (M^+, $C_{26}H_{24}N_2O_2S$ requires 428.1558).

5-Ethyl-2-[(4′-methoxy-phenylcarbonylmethyl) thio]-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4i)***. Yield: 32 %; mp: 193.7–194.8 °C; ^{1}H NMR δ 0.90 (s, 3H, *J* 7.25, CH_3), 2.45 (q, *J* 7.25, 2H, CH_2), 3.77 (s, 3H, OCH_3), 4.16 (s, 2H, SCH_2), 4.39 (s, 2H, CH_2naphthyl), 6.84–7.96 (m, 11H, ArH), 12.73 (s, br s, 1H, NH); ^{13}C NMR δ 13.3 (CH_3), 18.8 (CH_2), 37.0 (CH_2naphthyl), 37.2 (SCH_2), 56.1 (OCH_3), 114.0 (2C, Ph-C), 115.1 (C-5), 123.9–134.0 (10C, naphthyl; 3C, Ph-C), 157.5 (C-6), 160.3 (C-2), 163.1 (C-4), 164.2 (Ph-C), 193.1 (C=O); HRMS *m/z* 444.1516 (M^+, $C_{26}H_{24}N_2O_3S$ requires 444.1508).

5-Ethyl-2-[(4′-fluoro-phenylcarbonylmethyl) thio]-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4j)***. Yield: 49%; mp:177.9-178.2 °C; FT-IR (KBr): 3446, 2965, 1677, 1636 cm^{-1}; ^{1}H NMR δ 0.91 (s, 3H, *J* 7.35, CH_3), 2.44 (q, *J* 7.35, 2H, CH_2), 4.12 (s, 2H, SCH_2), 4.41 (s, 2H, CH_2naphthyl), 7.03–7.91 (m, 11H, ArH), 12.79 (s, br s, 1H, NH); ^{13}C NMR δ 13.8 (CH_3), 18.5 (CH_2), 37.6 (CH_2naphthyl), 37.7 (SCH_2), 116.1 (C-5), 116.3 (2C, Ph-C), 123.7–134.0 (10C, naphthyl and 3C, Ph-C), 148.9 (C-6), 152.6 (C-2), 164.6 (C-4), 166.5 (Ph-C), 192.3 (C=O); HRMS *m/z* 432.1317 (M^+, $C_{25}H_{21}FN_2O_2S$ requires 432.1308).

2-[(4′- Chloro-phenylcarbonylmethyl) thio]-5-ethyl-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4k)***. Yield: 38%; mp:179.9–180.4 °C; FT-IR (KBr): 3415, 2932, 1677, 1636 cm^{-1} ^{1}H NMR δ 0.91 (s, 3H, *J* 7.35, CH_3), 2.43 (q, *J* 7.35, 2H, CH_2), 4.11(s, 2H, SCH_2), 4.40(s, 2H, CH_2naphthyl), 7.00–8.15 (m, 11H, ArH), 12.75 (s, br s, 1H, NH); ^{13}C NMR δ 13.8 (CH_3), 18.8 (CH_2),37.6 (CH_2naphthyl), 37.7 (SCH_2), 113.4 (C-5), 123.9–138.2 (10C, naphthyl and 6C, Ph), 152.1 (C-6), 156.7 (C-2), 163.1 (C-4), 192.4 (C=O); HRMS *m/z* 448.1008 (M^+, $C_{25}H_{21}ClN_2O_2S$ requires 448.1012).

5-Ethyl-2-[(methoxycarbonylmethyl) thio]-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4l)***. Yield: 51%; mp: 143.5–143.9 °C; FT-IR (KBr): 3415, 2940, 1743, 1640 cm^{-1}; ^{1}H NMR δ 0.89 (s, 3H, *J* 7.35, CH_3), 2.43(q, *J* 7.35, 2H, CH_2), 3.39 (s, 3H, OCH_3), 3.74 (s, 2H, SCH_2), 4.28 (s, 2H, CH_2naphthyl), 7.17–8.14 (m, 7H, ArH), 12.73 (s, br s, 1H, NH); ^{13}C NMR δ 13.4 (CH_3), 14.4 (CH_3), 19.1 (CH_2), 34.6 (CH_2naphthyl), 37.5 (SCH_2), 58.8 (OCH_2), 124.8–134.5 (10C, naphthyl), 113.8 (C-5), 155.4 (C-6), 161.1 (C-2), 162.4 (C-4), 169.5 (C=O); HRMS *m/z* 368.1120 (M^+, $C_{20}H_{20}N_2O_3S$ requires 368.1195).

5-Isopropyl-2-[(phenylcarbonylmethyl) thio]-6-(1-naphthylmethyl)pyrimidin-4(3H)-one ***(4m)***. Yield: 60%; mp: 181.0-182.0 °C; FT-IR (KBr): 3547, 2968, 1685, 1640 cm^{-1}; ^{1}H NMR δ 1.13 (d, 6H, *J* 6.60, 2CH_3), 2.98(m, 1H, *J* 6.6, CH_2), 4.13 (s, 2H, SCH_2), 4.49 (s, 2H, CH_2naphthyl), 7.02–7.93 (m, 12H, ArH), 12.66 (s, br s, 1H, NH);

^{13}C NMR δ 19.9 ($2CH_3$), 27.5 (CH), 37.6 (CH_2naphthyl), 37.8 (SCH_2), 117.9 (C-5), 124.2–136.2 (10C, naphthyl, and 6C, Ph), 157.2 (C-6), 160.1 (C-4), 163.1 (C-2), 193.6 (C=O); HRMS *m/z* 428.1570 (M^+, $C_{26}H_{24}N_2O_2S$ requires 428.1558).

5-Isopropyl-2-[(p-tolylcarbonylmethyl) thio]-6-(1-naphthylmethyl)pyrimidin-4(3H)-one ***(4n)***. Yield: 67%; mp: 186.9–188.5 °C; FT-IR (KBr): 3425, 1679, 1636, 2956 cm^{-1}; ^{1}H NMR δ 1.13 (*d*, 6H, *J* 6.60, $2CH_3$), 2.23 (s, 3H, Ph-CH_3), 2.95 (m, 1H, *J* 6.6, CH), 4.13 (s, 2H, SCH_2), 4.49 (s, 2H, CH_2naphthyl), 7.00–7.93 (m, 11H, ArH), 12.61 (s, br s, 1H, NH); ^{13}C NMR δ 19.4 ($2CH_3$), 20.9 (Ph-CH_3), 27.0 (CH), 37.0 (CH_2naphthyl), 37.2 (SCH_2), 116.2 (C-5), 123.6–134.3 (10C, naphthyl, and 5C, Ph-C), 143.5 (Ph-C), 156.2 (C-6), 160.4 (C-2), 162.8 (C-4), 192.7 (C=O); HRMS *m/z* 442.1726 (M^+, $C_{27}H_{26}N_2O_2S$ requires 442.1715).

5- Isopropyl-2-[(4'-methoxy-phenylcarbonylmethyl) thio]- 6 -(1-naphthylmethyl) pyrimidin-4(3H)one ***(4o)***. Yield: 51%; mp: 183.9–184.6 °C; FT-IR (KBr): 3415, 2958, 2870, 1670, 1639 cm^{-1}; ^{1}H NMR δ 1.15 (d, 6H, *J* 6.85, $2CH_3$), 2.97 (m, 1H, *J* 6.85, CH), 3.73 (s, 3H, OCH_3), 4.16 (s, 2H, SCH_2), 4.42 (s, 2H, CH_2naphthyl), 6.81–7.96 (m, 11H, ArH), 12.62 (s, br s, 1H, NH); ^{13}C NMR δ 19.45 ($2CH_3$), 27.0 (CH), 36.8 (CH_2naphthyl), 37.2 (SCH_2), 55.3 (OCH_3), 113.5 (Ph-C), 123.7 (C-5), 125.2 (10C, naphthyl, and 3C, Ph-C), 156.1 (C-6), 159.2 (C-2), 161.5 (Ph-C), 163.0 (C-4), 191.5 (C=O); HRMS *m/z* 458.1674 (M^+, $C_{27}H_{26}N_2O_3S$ requires 458.1664).

2-[(Methylcarbonylmethyl) thio]-5-isopropyl-6-(1-naphthylmethyl) -pyrimidin- 4(3H)-one ***(4p)***. Yield: 48 %; mp: 168.7–169.5 °C; FT-IR (KBr): 3439, 1635, 1564, 1511–1453 cm^{-1}; ^{1}H NMR δ 1.18 (d, 6H, *J* 6.8, $2CH_3$), 1.94 (s, 3H, CH_3), 2.97 (m, 1H, *J* 6.8, CH) 4.17 (s, 2H, SCH_2), 4.48 (s, 2H, CH_2naphthyl), 7.15–7.81 (m, 7H, ArH), 12.68 (s, br s, 1H, NH); ^{13}C NMR δ 19.9 ($2CH_3$), 26.0 (CH), 35.2 (CH_2naphthyl), 37.5 (SCH_2), 23.5 (CH_3), 116.4 (C-5), 123.4–134.2 (10C), naphthyl; 152.5 (C-6), 162.2 (C-2), 162.4 (C-4), 201.2 (C=O); HRMS *m/z* 366.1403 (M^+, $C_{21}H_{22}N_2O_2S$ requires 366.1402).

2-[(4'-Fluoro-phenylcarbonylmethyl)thio]-5-isopropyl-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4q)***. Yield: 62%; mp:187.1–187.4 °C; FT-IR (KBr): 3436, 2942, 1678, 1636 cm^{-1}; ^{1}H NMR δ 1.17 (d, 6H, J = 6.8, $2CH_3$), 2.96 (m, 1H, J = 6.8, CH), 4.11 (s, 2H, SCH_2), 4.44 (s, 2H, CH_2naphthyl), 6.96–7.90 (m, 11H, ArH), 12.65 (s, br s, 1H, NH); ^{13}C NMR δ 19.1 ($2CH_3$), 26.5 (CH), 35.6 (CH_2naphthyl), 37.5 (SCH_2), 115.4 (C-5), 116.3 (2C, Ph-C), 123.7–134.0 (10C, naphthyl, and 3C, Ph-C), 151.1 (C-6), 152.6 (C-2), 164.5 (C-4), 166.4 (Ph-C), 193.1 (C=O); HRMS *m/z* 446.5375 (M^+, $C_{26}H_{23}FN_2O_2S$ requires 446.1464).

2-[(4'Chloro-phenylcarbonylmethyl)thio]-5-isopropyl-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4r)***. Yield: 67%; mp: 178.4–179.0 °C; FT-IR (KBr): 3361, 2931, 1679, 1638 cm^{-1}; ^{1}H NMR δ 1.15 (d, 6H, *J* 6.85, $2CH_3$), 2.94m, 1H, *J* 6.85, CH, 4.09 (s, 2H, SCH_2), 4.43 (s, 2H, CH_2naphthyl), 6.94–7.88 (m, 11H, ArH), 12.67 (s, br s, 1H, NH); ^{13}C NMR δ 19.6 ($2CH_3$), 26.5 (CH), 37.6 (CH_2naphthyl), 37.8 (SCH_2), 115.1 (C-5), 123.9–138.2 (10C, naphthyl, and 6C, Ph), 151.5 (C-6), 154.9 (C-2), 162.6 (C-4), 191.3 (C=O); HRMS *m/z* 462.1175 (M^+, $C_{26}H_{23}ClN_2O_2S$ requires 462.1169).

2-[(Methoxycarbonylmethyl) thio]-5-isopropyl-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4s)***. Yield: 31%; mp: 138.5–141.7 °C; FT-IR (KBr): 3442, 2941, 1741, 1640 cm^{-1}; ^{1}H NMR δ 1.15 (d, 6H, *J* 6.85, $2CH_3$), 3.00 (m, 1H, *J* 6.85, CH), 3.39 (s, 3H, OCH_3), 3.78 (s, 2H, SCH_2), 4.31 (s, 2H, CH_2naphthyl), 7.10–8.15 (m, 7H, ArH),

12.60 (s, brs,1H, NH); ^{13}C NMR δ 19.6 ($2CH_3$), 27.5 (CH), 37.6 (CH_2naphthyl), 38.1 (SCH_2), 52.5 (OCH_2), 123.9–134.0 (10C, naphthyl), 115.1 (C-5), 151.9 (C-6), 161.5 (C-2), 162.6 (C-4), 170.1 (C=O); HRMS *m/z* 382.1359 (M^+, $C_{21}H_{22}N_2O_3S$ requires 382.1351).

2-[(Ethoxycarbonylmethyl) thio]-5-isopropyl-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one ***(4t)***. Yield: 37%; mp: 125.2–125.8 °C; FT-IR (KBr): 3448, 2957, 1740, 1640 cm^{-1}; ^{1}H NMR δ 0.93 (d, 6H, *J* 6.7, CH_3), 1.17 (m, 1H, *J* 6.85, CH), 2.96 (m, 1H, *J* 6.85, CH), 3.78 (s, 2H, SCH_2), 3.81 (q, 2H, *J* 6.7, OCH_2), 4.36 (s, 2H, CH_2naphthyl), 7.08–8.18 (m, 7H, ArH), 12.68 (s, br s, 1H, NH); ^{13}C NMR δ 13.8 (CH_3), 19.6 ($2CH_3$), 27.5 (CH), 37.5 (CH_2naphthyl), 37.7 (SCH_2), 56.8 (OCH_2), 123.9–134.0 (10C, naphthyl), 116.1 (C-5), 152.8 (C-6), 163.1 (C-2), 164.5 (C-4), 171.0 (C=O); HRMS *m/z* 396.1515 (M^+, $C_{22}H_{24}N_2O_3S$ requires 396.1508).

5- Isopropyl-2-[(2′, 4′-dimethylphenylcarbonylmethyl) thio]-6-(1-naphthylmethyl) pyrimidin-4(3H)-one ***(4u)***. Yield: 47%; mp: 176.7–178.0 °C; FT-IR (KBr): 3445, 2985, 1679, 1638 (C=O) cm^{-1}; ^{1}H NMR δ 1.16 (d, 6H, *J* 6.60, $2CH_3$), 2.21 (s, 3H, CH_3-Ph), 2.96 (m, 1H, *J* 6.6, CH), 4.19 (s, 2H, SCH_2), 4.37 (s, 2H, CH_2naphthyl), 6.76–7.98 (m, 10H, ArH), 12.64 (s, br s, 1H, NH); ^{13}C NMR δ 14.2 (Ph-CH_3), 21.6 (Ph-CH_3), 19.6 ($2CH_3$), 27.5 (CH), 36.2 (CH_2naphthyl), 36.8 (SCH_2), 115.5 (C-5), 123.9–135.1 (10C, naphthyl, and Ph-C), 137.8 (Ph-C), 142.2 (Ph-C), 152.5 (C-6), 161.1 (C-4), 163.4 (C-2), 192.3 (C=O); HRMS *m/z* 456.1876 (M^+, $C_{28}H_{28}N_2O_2S$ requires 456.1871).

2.2. X-ray crystallography

Colorless cubic crystals of **4o** were grown from ethyl acetate in separate experiments by slow evaporation at room temperature. The crystals were mounted on glass fibers by using epoxy, and X-ray diffraction data for a crystal ($0.30 \times 0.25 \times 0.10$ mm) of **4o** was collected at 22 °C by using a SMART charge-coupled device X-ray detector (Bruker Analytical X-Ray Systems, Madison, WI.). Structure solution and refinement were performed by using the SHELXTL suite of programs (Bruker Analytical X-Ray Systems). All nonhydrogen atoms were refined using anisotropic displacement parameters. Hydrogen atoms were placed at ideal positions and refined as riding atoms with relative isotropic displacement parameters Table 1.

2.3. Anti-HIV assays

Compounds were evaluated for possible antiviral activity against both strains of HIV-1 and HIV-2 using MT-4 cells as the target cells. As previously described [18], MT-4 cells were incubated with virus and growth medium containing dilutions of the test compounds for seven days. Noninfected control and virus infected cultures without compound added were grown in parallel. Expression of HIV in the cultures was quantified indirectly using the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenltetrazolium bromide (MTT) assay [21]. The inhibitory concentration of compounds was expressed as the concentration that caused 50% inhibition of viral cytopathogenicity (IC_{50}) without direct toxicity to the cells. Cytotoxicity of the compounds was evaluated in parallel with their anti-viral activity. The cytotoxic concentration (CC_{50}) of

Table 1
Crystal data and structure refinement for X-ray crystal structures of compounds **4o**

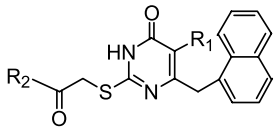
Parameter	Compound **4o**
Crystal size	$0.30 \times 0.25 \times 0.10$ mm
Unit cell dimensions	
a (Å)	11.329 (10)
b (Å)	11.936 (11)
c (Å)	12.374 (11)
α (°)	87.897 (16)
β (°)	76.980 (15)
γ (°)	63.155 (14)
F (000)	580
Space group	Triclinic, P-1
Cell volume (Å^3)	1450 (2)
Z	2
θ Range for data collection (°)	1.69–25.01
Limiting indices	$-13 \leqslant h \leqslant 12$
	$-14 \leqslant k \leqslant 13$
	$-14 \leqslant l \leqslant 14$
Reflections collected	6111
Independent reflection (R_{int})	5044 (0.0680)
Data/restraints/parameters	5044/6/355
Goodness-of-fit on F^2	0.929
Final R indices [$I > 2\sigma(I)$]	$R1$, 0.1193; $wR2$, 0.2796
R indices (all data)	$R1$, 0.2423; $wR2$, 0.3480
Absorption coefficient (mm^{-1})	0.153
Max. min. transmission	0.9848; 0.9555
Largest diff. Peaks (eÅ^{-3})	0.642, −0.469

the compounds was monitored based on the growth of noninfected cells by the trypan blue exclusion method and corresponded to the concentration required to cause 50% cell death. The test for activity against HIV-1 and HIV-2 was performed in MT-4 cell cultures infected with wild-type HIV-1 (strain IIIB), NNRTI resistant HIV-1 (strain SO561945), and wild-type HIV-2 (strain ROD).

3. Results and discussion

The novel *S*-DABO analogues were evaluated for their cytotoxicity and anti-HIV-1 activity in MT-4 cells infected with the wild-type HIV-1 strain III_B and compared with the same properties of HEPT and 2′,3′-dideoxyinosine (DDI). The results are summarized in Table 2. As shown in Table 2, all of the *S*-DABOs exhibited anti-HIV-1 activities and the majority of them were not cytotoxic to MT-4 cells at doses as high as 170 μM, except for five compounds, **4c**, **4h**, **4k**, **4p**, and **4u**, which show CC_{50} values ranging from 6 to 85 μM. 5-isopropyl-2-[(4′-methoxy-phenylcarbonyl-methyl)thio]-6-(1-naphthylmethyl)pyrimidin-(3*H*)-one (**4o**) was the most promising compound. It exhibited extremely potent inhibitory activity against HIV-1 replication with an IC_{50} value of 0.030 μM, and a CC_{50} value of 203 μM. The viral selectivity

Table 2
Antiviral activity of target compounds against HIV-1 in MT-4 cells[a]



4a-u

Compound	R_1	R_2	IC_{50} (μM)[b]	CC_{50} (μM)[c]	SI[d]
4a	Me	(4′-CH_3)Ph	0.67 ± 0.07	⩾251	⩾374
4b	Me	(4′-OCH_3)Ph	0.37 ± 0.07	>290	784
4c	Me	CH_3	1.12 ± 0.00	85 ± 10.41	76
4d	Me	(4′-F)Ph	0.180 ± 0.003	⩾275	⩾1527
4e	Me	(4′-Cl)Ph	0.55 ± 0.05	⩾198	⩾360
4f	Me	CH_3O	6.99 ± 0.10	⩾296	⩾42
4g	Me	CH_3 CH_2O	7.00 ± 0.47	⩾287	⩾41
4h	Et	(4′-CH_3)Ph	0.32 ± 0.07	6 ± 0.17	19
4i	Et	(4′-OCH_3)Ph	0.045 ± 0.003	178 ± 21.05	3955
4j	Et	(4′-F)Ph	0.078 ± 0.009	⩾240	⩾3076
4k	Et	(4′-Cl)Ph	0.26 ± 0.01	56 ± 7.87	215
4l	Et	CH_3O	4.72 ± 0.16	⩾196	⩾42
4m	i-Pr	Ph	0.046 ± 0.005	238 ± 7.15	5173
4n	i-Pr	(4′-CH_3)Ph	0.24 ± 0.01	225 ± 14.12	937
4o	i-Pr	(4′-OCH_3)Ph	0.030 ± 0.002	⩾203	⩾6766
4p	i-Pr	CH_3	0.410 ± 0.008	43 ± 1.41	105
4q	i-Pr	(4′-F)Ph	0.078 ± 0.001	186 ± 7.81	2384
4r	i-Pr	(4′-Cl)Ph	0.32 ± 0.02	>270	>843
4s	i-Pr	CH_3O	4.49 ± 0.39	179 ± 3.54	40
4t	i-Pr	CH_3CH_2O	1.41 ± 0.12	175 ± 9.09	>125
4u	i-Pr	(2′, 4′-CH_3)Ph	0.24 ± 0.03	27	112
HEPT			5.06 ± 0.06	405 ± 3.21	80
DDI			5.37 ± 0.1	⩾529	⩾98

[a] All data represent mean values for three separate experiments.
[b] Concentration required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells.
[c] Concentration that reduces the MT-4 cell viability by 50%.
[d] Selectivity index: ratio CC_{50}/IC_{50}, a higher SI means a more selective compound.

index was greater than 6766, which is much better than those found for HEPT, and DDI. Some compounds, (**4m**, **4q**, **4i**, and **4j**), also had high anti-HIV-1 potency (IC_{50} = 0.046, 0.078, 0.045, and 0.078 μM, respectively) and good selectivity indices (SI = 5173, 2384, 3955, and 3076, respectively).

In terms of the SARs, the antiviral activity of these analogues is very sensitive to the type of *C*2 substituent of the pyrimidine ring. The presence of an alkyloxyl group at the end of the *C*2 side chain diminished or eliminated the anti-HIV-1 activity. Compounds **4f**, **4g**, **4l**, **4s**, and **4t** were less active than the other compounds. However, the presence of bulky substituents at the *C*2 position of pyrimidine rings correlated with the higher antiviral activity. Optimal anti-HIV-1 activity was obtained with compounds having a terminal aryl moiety at the *C*2 side chain. Moreover, the

nature of the substituent on the 4′-position of the phenyl ring influenced the antiviral activity of these *S*-DABOs. In fact, the 4′-OCH_3 and 4′-F derivatives (compounds **4i**, **4j**, **4o**, and **4q**) were more potent than their 4′-CH_3 and 4′-Cl counterparts (compounds **4h**, **4k**, **4n**, and **4r**). Finally, as with the previous SARs for HEPTs, this series unambiguously showed that inhibitory activity increased proportionally with the modification of the *C*5 substituent in the order, Me → Et → *i*-Pr, where the influence of isopropyl substituent is only slightly greater than that of the ethyl substituent.

Interestingly, when these compounds were tested for their ability to inhibit HIV-2 multiplication in acutely infected MT-4 cells, it was found that some compounds exhibit anti-HIV-2 activity. This observation contrasts with the behaviour of other DABO and NNRT inhibitors. Recently, it has been suggested that NNRTI-mediated inhibition of HIV-2 is possible [22]. However, further investigation of the link between NNRTI cytotoxicity and inhibition of HIV-2 replication suggests that toxicity to the host cell may be the cause of the inhibition. Only one compound reported to date, SJ-3366 has significant anti-HIV-2 activity, without cellular toxicity. Two compounds, as shown in Table 3, **4b** and **4k** inhibit HIV-2 replication ($IC_{50} \geqslant 0.42$ and 0.26 μM, respectively) almost to the same extent as HIV-1 replication

Table 3
Anti-HIV-2 (ROD) or HIV-1 (SO561945[a] activity in MT-4 cells of *S*-DABO compounds

Compound	IC_{50} (μM)		CC_{50} (μM)
	HIV-2 (ROD)	SO561945[a]	
4a	⩾13.61	⩾251	⩾251
4b	⩾0.42	⩾290	>290
4c	⩾84.9	⩾58.2	85 ± 10.41
4d	⩾275	⩾275	⩾275
4e	19.8 ± 0.27	⩾198	⩾198
4f	⩾296	⩾296	⩾296
4g	⩾293	⩾317	⩾287
4h	⩾1.25	⩾5.62	6 ± 0.17
4i	⩾18.9	>178	178 ± 21.05
4j	16.3 ± 0.17	24.2 ± 0.88	⩾240
4k	⩾0.26	>56	56 ± 7.87
4l	⩾196	⩾196	⩾196
4m	22.0 ± 2.76	19.6 ± 0.42	238 ± 7.15
4n	⩾225	⩾225	225 ± 14.12
4o	13.4 ± 0.02	5.82 ± 0.09	⩾203
4p	>43.1	>43.1	43 ± 1.41
4q	⩾32.2	188	186 ± 7.81
4r	>270	>270	>270
4s	>179	>179	179 ± 3.54
4t	41.1	>175	175 ± 9.09
4u	4.00 ± 0.33	2.67	27
HEPT	NA[b]	>405	405 ± 3.21
DDI	2.71 ± 0.25	7.15 ± 0.26	⩾529

[a] **SO561945** is an HIV-1 $(III)_B$) strain with typical NNRTI-selected mutations in the RT (K103N and Y181C). These mutations confer resistance towards the classical NNRTIs (nevirapine, delavirdine, sustiva, and HEPT).

[b] NA, not active.

($IC_{50} = 0.36$ μM and 0.26 μM, respectively). Although compounds **4e**, **4i**, **4j**, **4m**, and **4o**, were only moderately active against HIV-2 ($IC_{50}s = 13$–22 μM), all of them had low cytotoxicities ($CC_{50} > 200$ μM).

Antiviral therapy with NNRTIs is usually compromised by the appearance of HIV-1 drug resistance. Improved activity against mutant enzymes may be beneficial in terms of suppressing the emergence of drug-resistant strains of virus. Therefore, these new compounds were tested in cell culture for their activity against the NNRT inhibitor resistant strain SO561945, which contains two mutations (Y181C and K103N). As a result, we found that some of the *S*-DABO compounds, (i.e., **4j** and **4m**), showed moderate activity against this double mutant HIV strain (Table 2). In particular, compound **4o** inhibits the mutant virus with the IC_{50} value of 5.82 μM, which was better than that observed for DDI ($IC_{50} = 7.15$ μM). Studies are in progress to find substituents that can be introduced to the **4o** skeleton in order to increase the *S*-DABO anti-HIV activity against clinically relevant HIV-1 mutants.

Since previous DABO derivatives targeted the HIV-1 RT, the title compounds were tested in enzyme assays against highly purified recombinant HIV-1 RT using poly(rC)-oligo(dG) as template primer. The results showed that these new compounds did inhibit HIV-1 reverse transcriptase. However, the EC_{50} values obtained in enzyme assays were 10–40 times higher than the IC_{50} values obtained in viral spread assays (results not shown). Thus, the possibility cannot be excluded that these new compounds target an additional step of the HIV-1 multiplication cycle.

In summary, the bioassay results show that our approach has led to the development of potent anti-HIV agents. It is clear that some of these *S*-DABO analogues act as NNRTIs but, they may also interfere with another target or act on RT in a different manner than typical NNRTIs. This conclusion is based on the observation that these compounds show activity against HIV-1 and HIV-2, and that the loss of antiviral activity for these compounds is much less pronounced when compared to the loss of activity of typical NNRTIs when tested against S0561945. Studies are in progress to determinate the exact mechanism for the anti-HIV activity of these compounds. In addition, further synthesis of analogues and QSAR studies are being carried out to determine the role of structural features on this activity.

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