Non-nucleoside HIV Reverse Transcriptase Inhibitors, Part 6[1]: Synthesis and Anti-HIV Activity of Novel 2-[(Arylcarbonylmethyl)thio]-6-arylthio DABO Analogues

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2-(Arylcarbonylmethyl)thio-6α-naphthylmethyl derivatives of dihydro-alkoxy-benzyl-oxopyrimidines (DABO) were newly found to exhibit activity against both HIV-1 and HIV-2. To further explore their structure-activity relationship, the modified *S*-DABO analogues (**5a−g** and **6e−f**) with a 1-naphthylthio or phenylthio group at the C-6 position were synthesized. *S*-Alkylation of 5-ethyl-2-thiouracil with substituted 2-bromo-acetophenones provided crude 2-[(arylcarbonylmethyl)thio]-5-ethyl-(3H)-uracil **2a−e**, which was directly subjected to toluenesulfonylation with TsCl to afford disulfonate **4a−e**. Substitution of **4a−e** with arylthiol afforded the desired *S*-DABO analogues **5a−g** and **6e−f**. The compounds were evaluated for their *in vitro* anti-HIV activity in MT-4 cells. The IC_{50} values for anti-HIV-1 activity fall into the range 0.37−29.50 μM, and the IC_{50} values for anti-HIV-2 activity fall into the range 23.11−181.07 μM. The results indicated that these compounds are moderately active against HIV-1 and HIV-2.

Keywords: NNRTI; *S*-DABO analogues; Antiviral activity; HIV-1; HIV-2

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Introduction

Since the discovery of Zidovudine (AZT), reverse transcriptase (RT) inhibitors of human immunodeficiency virus (HIV) have played an important role in the treatment of AIDS in the past two decades [1, 2]. As we know, RT inhibitors are divided into two functionally distinct classes [3−5]: (A) nucleoside RT inhibitors (NRTI) such as AZT, which bind to the polymerase site and thereby act as competitive inhibitors and exhibit equal activity against HIV-1 and HIV-2; (B) non-nucleoside RT inhibitors (NNRTI) as exemplified by Nevirapine, Efavirenz, HEPT and others, which bind to a specific allosteric site close to the polymerase site of HIV-1 RT, lead to a non-competitive inhibition mechanism and generally are highly potent against HIV-1. To date, 14 RT inhibitors have been successfully approved as drugs for the treatment of AIDS, but their clinical use is limited because of the severe adverse side effects or drug resistance [2, 6]. Therefore, researchers in the RT inhibitor field now focus on finding novel compounds with different mechanisms of action and strong inhibition of the resistant mutants.

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Dihydro-alkoxy-benzyl-oxopyrimidine (DABO) (Figure 1) analogues are known as a typical class of NNRTI with similar structures related to HEPT (Figure 1). A series of DABO derivatives has been found to have excellent anti-HIV-1 activities [7, 8]. Very recently, when introducing a β-carbonyl group into the C-2 side chain of 6α-naphthylmethyl *S*-DABO, we found that the resulting 2-(arylcarbonylmethyl)-thio-6α-naphthylmethyl *S*-DABO derivatives (Figure 1) exhibited marked activity against both HIV-1 and HIV-2 [9]. Among them, the candidates bearing an ethyl group at the C-5 position of the pyrimidin-4-one ring were more effective against HIV-2 than those bearing a methyl or isopropyl group at the same position. These interesting results prompted us to further explore the structure-activity relationship of *S*−DABO. Herein, a series of 6-arylthio-substituted *S*-DABO derivatives were designed, synthesized and evaluated for their anti-HIV activity.

Results and discussion

Chemistry

The synthetic route to the target compounds **5a−f** and **6e−f** is outlined in Scheme 1. According to the previously reported procedure [10], 5-ethyl-2-thiouracil **1** was easily prepared by the condensation of diethyl ethylmalonate with

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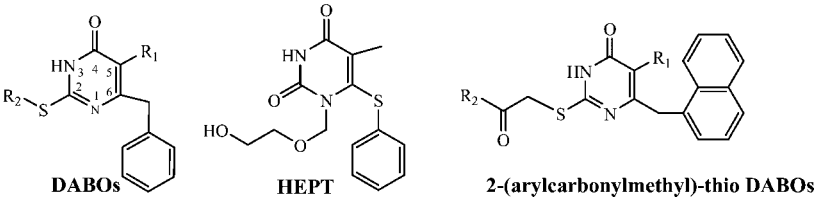
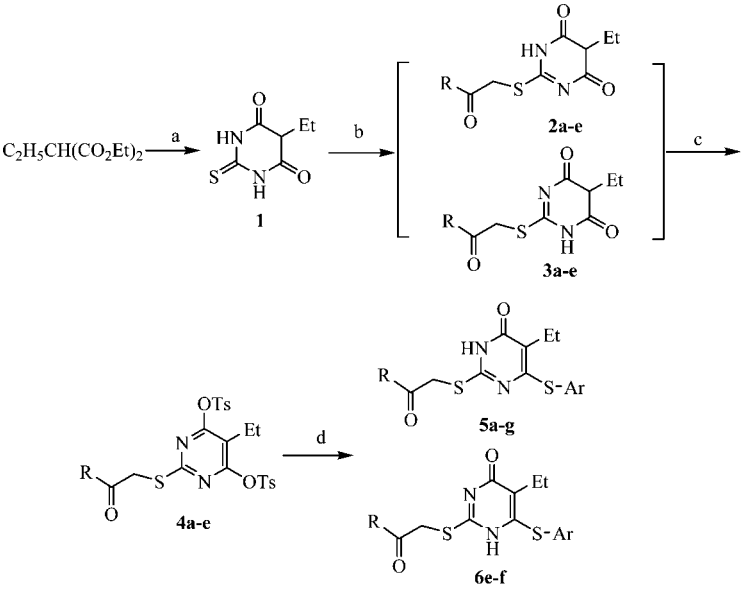


Figure 1. Chemical structures of DABO derivatives and HEPT.



Scheme 1. Synthesis of the DABO analogues **5a**−**g** and **6e**−**f**. Reagents and conditions: (a) NH_2CSNH_2, $NaOCH_3/CH_3OH$, reflux, 6 h; (b) $RCOCH_2Br$, K_2CO_3/DMF, r. t., 12 h; (c) TsCl, Et_3N/CH_2Cl_2, 0 °C, 6 h; (d) ArSH, EtOH/0.5N NaOH-EtOH, r. t., 12 h, then 10% NaOH-H_2O, 3 h.

thiourea in the presence of CH_3ONa/CH_3OH with 83% yield. *S*-Alkylation of **1** with various substituted 2-bromoacetophenones in the presence of K_2CO_3 in anhydrous DMF solution provided a mixture of 2-[(arylcarbonylmethyl)thio]-5-ethyl-(3*H*)-uracils **2a**−**e** and 2-[(arylcarbonylmethyl)thio]-5-ethyl-(1*H*)-uracils **3a**−**e** with 81−97% yields. Without separation, the mixture was directly subjected to toluenesulfonylation with TsCl in the Et_3N/CH_2Cl_2 system to afford disulfonates **4a**−**e** with 37−70% yields, which reacted with 1-naphthalenethiol or benzenethiol under alkaline conditions to afford **5a**−**g** with 39−58% yields, along with their isomers **6e**−**f** with 8−24% yields. The structures of two isomers were determined by comparing the spectral data with similar compounds [8, 11].

Biological properties

The novel *S*-DABO derivatives (**5a**−**g** and **6e**−**f**) were evaluated for their cytotoxicity and anti-HIV activity in MT-4 cells using the MTT method [12]. The used virus strains are HIV-1 strain IIIB and HIV-2 strain ROD. The results were summarized in Table 1.

As can be seen from the anti-HIV-1 activity data listed in Table 1, the compounds (**5c**, **5e**) with a 6-(1-naphthylthio) substituent were 1.1-fold and 14.9-fold, respectively, more active than their counterparts with a 6-benzenethiol substituent (**5f**, **5g**), and these results suggested that the 6-(1-naphthylthio) group contributed more to improve the biological activity than the 6-benzenethiol group. Moreover, the pyrimidinone derivatives **5a**−**g** inhibited HIV-1 replication in a range of micromolar concentrations (IC_{50} = 0.37−6.72 μM), and compounds **6e**−**f** showed less activity (IC_{50} = 16.8 and 29.5 μM, respectively). It has been deduced that the 3-*NH* function enhances anti-HIV activity by donation of a hydrogen bond to the carbonyl oxygen of Lys101 in RT [8]; our results further confirmed that the 3-*NH* function of DABO was essential for the inhibition of HIV RT.

Table 1. Biological activities of compounds **5a**–**h** and **6e**–**f** against HIV-1 and HIV-2.

Compound	Ar	R	IC_{50} μM		CC_{50} μM	SI (HIV-1)
			HIV-1	HIV-2		
5a	1-Naphthyl	Ph	0.37	> 26.39	36.74	101
5b	1-Naphthyl	4-Cl-Ph	5.94	> 25.5	22.44	4
5c	1-Naphthyl	4-F-Ph	3.33	> 23.11	26.04	8
5d	1-Naphthyl	2,4-F_2-Ph	0.38	> 31.20	41.03	107
5e	1-Naphthyl	4-H_3CO-Ph	0.45	> 157.58	160.6	359
5f	Phenyl	4-F-Ph	3.53	> 37.5	36.5	10
5g	Phenyl	4-H_3CO-Ph	6.72	> 181.07	181.48	27
6e	1-Naphthyl	4-H_3CO-Ph	16.77	36.15	74.09	4
6f	Phenyl	4-F-Ph	29.50	> 27.50	29.58	< 1
HEPT			5.06	> 405.8	399	79
DDI			5.37	2.71	529	98

It was also found that all compounds showed less activity against HIV-2 in comparison with that against HIV-1, and the replacement of the 6α-naphthylthio group by the 6-phenylthio group or the replacement of the 3-*NH* function by the 1-*NH* function in target compounds had no influence on the anti-HIV-2 activity. Moreover, the compounds with a 4-methoxyphenyl group at the C-2 side chain (**5e**, **5g** and **6e**) showed less cytotoxicity.

In conclusion, the novel 2-[(arylcarbonylmethyl)thio]-6-arylthio *S*-DABO were synthesized and evaluated for their anti-HIV activity. The results revealed that the target compounds had moderate activities against HIV-1 and HIV-2, but were less active in comparison with the previously reported 2-(arylcarbonylmethyl)thio *S*-DABO derivatives [9].

Experimental

General

Melting points (mp) were measured on a WRS-1B digital melting point apparatus and are uncorrected. IR spectra were recorded on an Avvatar 360 FT-IR instrument. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL EX-400 (400 MHz) spectrometer. Chemical shifts (δ) were expressed in ppm with the protonated solvent as reference. Mass spectra (MS) were recorded on MAT95 and for the electronic impacts (EI) at 70 eV. Column chromatography was performed with silica gel G (300–400 mesh); TLC was performed on plates coated with silica gel GF_{254}. Solvents were purified using standard procedures [13].

Chemistry

*General procedure for the synthesis of **2a–e and 3a–e***

A mixture of 5-ethyl-2-thiouracil (**1**) (0.624 g, 3.6 mmol), 2-bromoacetophenone or substituted 2-bromo-acetophenone (4.0 mmol) and anhydrous K_2CO_3 (0.556 g, 4 mmol) in anhydrous DMF (10 mL) was stirred at room temperature for 12 h, then was poured into cold H_2O (40 mL) and neutralized with 10% aq. HCl to pH 5–6. The white precipitate was collected by filtration, successively washed with cold H_2O (3 × 15 mL) and ether (2 × 20 mL), and dried *in vacuo* to afford the mixture of **2a**–**e** and **3a**–**e** with 84–97% yields, which were directly used in the next step without separation.

*General procedure for the synthesis of **4a–e***

To a stirred mixture of crude 5-ethyl-2-[(arylcarbonylmethyl)thio]-uracils **3a**–**e** (3.0 mmol), Et_3N (5.0 mL) and CH_2Cl_2 (20 mL) was added dropwise a solution of TsCl (1.2 g, 6.2 mmol) in CH_2Cl_2 (10 mL) at 0–5°C. The reaction mixture was stirred at room temperature for an additional 6 h; then H_2O (20 mL) was added dropwise and the organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic phases were washed with H_2O (2 × 20 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated *in vacuo*, and the residue was purified by column chromatography on silica gel (eluent AcOEt/petroleum ether, 1 : 2 vol/vol, 60–90°C) to afford **4a–e** with 37–70% yields.

*5-Ethyl-2-(phenacylmethyl)thio-4,6-di(p-tolylsulfonyloxy)primidine (**4a**)*

Yield 1.14 g (64%) as a yellow powder. mp 91–93°C. ^{1}H-NMR ($CDCl_3$): δ = 1.01 (t, 3H, J = 7.7 Hz, CH_3), 2.40 (s, 6H, × 2 CH_3), 2.45 (q, 2H, J = 7.7 Hz, CH_2), 4.45 (s, 2H, SCH_2), 6.95–7.96 (m, 13H, H_{arom}).

*5-Ethyl-2-[(4′-chlorophenacylmethyl)thio]-4,6-di(p-tolylsulfonyloxy)primidine (**4b**)*

Yield 1.32 g (70%) as a yellow powder. mp 127–129°C. ^{1}H-NMR ($CDCl_3$): δ = 1.09 (t, 3H, J = 7.4 Hz, CH_3), 2.46 (s, 6H, × 2 CH_3), 2.53 (q, 2H, J = 7.4 Hz, CH_2), 4.55 (s, 2H, SCH_2), 7.36 (d, 4H, J = 8.2 Hz, H_{arom}), 7.52 (d, 2H, J = 8.8 Hz, H_{arom}), 7.93 (d, 4H, J = 8.2 Hz, H_{arom}), 7.97 (d, 2H, J = 8.8 Hz, H_{arom}).

*5-Ethyl-2-[(4′-methoxyphenacylmethyl)thio]-4,6-di(p-tolylsulfonyloxy)primidine (**4c**)*

Yield 1.17 g (62%) as a yellow powder. mp 123–124°C. ^{1}H-NMR ($CDCl_3$): δ = 1.10 (t, 3H, J = 7.7 Hz, CH_3), 2.41 (s, 6H, × 2 CH_3), 2.54 (q, 2H, J = 7.7 Hz, CH_2), 3.89 (s, 3H, OCH_3), 4.46 (s, 2H, SCH_2), 6.98 (d, 2H, J = 9.2 Hz, H_{arom}), 7.32 (d, 4H, J = 8.3 Hz, H_{arom}), 7.92 (d, 4H, J = 8.3 Hz, H_{arom}), 7.96 (d, 2H, J = 9.2 Hz, H_{arom}).

*5-Ethyl-2-[(4′-fluorophenacylmethyl)thio]-4,6-di(p-tolylsulfonyloxy)primidine (**4d**)*

Yield 0.99 g (54%) as a yellow powder. mp 128–131°C. ^{1}H-NMR ($CDCl_3$): δ = 1.06 (t, 3H, J = 7.8 Hz, CH_3), 2.41 (s, 6H, × 2 CH_3), 2.47 (q, 2H, J = 7.8 Hz, CH_2), 4.51 (s, 2H, SCH_2), 7.16 (m, 2H,

H_{arom}), 7.32 (d, 4H, J = 8.2 Hz, H_{arom}), 7.89 (d, 4H, J = 8.2 Hz, H_{arom}), 8.03 (m, 2H, H_{arom}).

*5-Ethyl-2-[(2′,4′-difluorophenacylmethyl)thio]-4,6-di(p-tolylsulfonyloxy)primidine (**4e**)*

Yield 0.71 g (37%) as a yellow powder. mp 136–138 °C. ^{1}H-NMR ($CDCl_3$): δ = 1.07 (t, 3H, J = 7.8 Hz, CH_3), 2.41 (s, 6H, × 2 CH_3), 2.46 (q, 2H, J = 7.8 Hz, CH_2), 4.61 (s, 2H, SCH_2), 6.97 (m, 2H, H_{arom}), 7.27 (d, 4H, J = 8.2 Hz, H_{arom}), 7.52 (m, 1H, H_{arom}), 7.87 (d, 4H, J = 8.2 Hz, H_{arom}).

*General procedure for the synthesis of **5a–g** and **6e–f***

To a stirred mixture of 1-naphthalenethiol or benzenethiol (0.5 mmol) and 0.5 M ethanolic NaOH solution (1.0 mL) in EtOH (4 mL) under a nitrogen atmosphere was added **4a–e** (0.5 mmol) in one portion; the resulting mixture was stirred overnight at room temperature until **4a–e** had disappeared (monitored by TLC). Then, 10% NaOH solution (2 mL) was added and the mixture was continuously stirred for an additional 3 h at room temperature. The solvent was evaporated *in vacuo*, and the residue was suspended in H_2O (5 mL), acidified to pH 5–6 with 1 M aq. HCl and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated to dryness; the residue was purified by column chromatography on silica gel (eluent AcOEt/petroleum ether, 1 : 1 vol/vol, 60–90 °C) to afford **5a–g** and **6e–f**.

*2-[(Phenacylmethyl)thio]-5-ethyl-6α-naphthylthio pyrimidin-4(3H)-one (**5a**)*

Yield: 0.10 g (46%) as a white powder. mp 178–181 °C. ^{1}H-NMR ($CDCl_3$): δ = 1.01 (t, 3H, J = 7.3 Hz, CH_3), 2.38 (q, 2H, J = 7.3 Hz, CH_2), 4.53 (s, 2H, SCH_2), 7.09–7.98 (m, 12H, H_{arom}), 8.67 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ = 13.8, 18.4, 37.6, 122.7, 124.4 (d) 124.8, 125.1, 125.3, 126.3, 126.8, 127.0, 127.4, 127.7, 127.9, 128.8, 130.6, 134.1 (d), 136.1, 151.2, 159.9, 163.0, 192.8. MS: *m/z* (%): 432 (24.6), 327 (74.1), 313 (62.7), 105 (100).

*2-[(4′-Chlorophenacylmethyl)thio]-5-ethyl-6α-naphthylthio pyrimidin-4(3H)-one (**5b**)*

Yield: 0.18 g (58%) as a white solid. mp 182–184 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.94 (t, 3H, J = 7.4 Hz, CH_3), 2.42 (q, 2H, J = 7.4 Hz, CH_2), 4.45 (s, 2H, SCH_2), 7.15–8.05 (m, 11H, H_{arom}), 9.73 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ = 13.7, 22.3, 37.9, 122.4, 123.4 (d), 125.1, 125.6, 126.3, 126.8, 127.3, 127.9, 128.4, 128.7, 129.3, 129.7, 131.8, 134.4, 137.7, 154.2, 158.1, 165.3, 192.6. MS: *m/z* (%): 466 (6.0), 327 (21.3), 313 (24.5), 139(100).

2-[(4′-Fluorophenacylmethyl)thio]-5-ethyl-6α-naphthylthio pyrimidin-4(3H)-one (**5c**)

Yield: 0.15 g (48%) as a white solid. mp 175–177 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.98 (t, 3H, J = 7.4 Hz, CH_3), 2.41 (q, 2H, J = 7.4 Hz, CH_2), 4.50 (s, 2H, SCH_2), 7.10–7.94 (m, 11H, H_{arom}), 9.16 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ = 13.5, 21.7, 37.9, 116.8 (d), 123.1, 125.0, 126.1, 126.5, 127.1, 127.4, 127.7, 127.9, 128.7, 129.3, 129.7, 130.8, 134.1, 151.3, 153.1, 165.3, 166.7, 192.5. MS: *m/z* (%): 450 (17.7), 327 (100), 313 (78.6), 123 (80.1).

*2-[(2′,4′-Difluorophenacylmethyl)thio]-5-ethyl-6α-naphthylthio pyrimidin-4(3H)-one (**5d**)*

Yield: 0.13 g (42%) as a yellow solid. mp 166–168 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.99 (t, 3H, J = 7.3 Hz, CH_3), 2.45 (q, 2H, J = 7.3 Hz, CH_2), 4.53 (s, 2H, SCH_2), 6.92–7.99 (m, 10H, H_{arom}), 9.42 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ = 14.1, 21.9, 38.6, 106.1, 113.3, 123.3, 123.9, 125.0, 126.2, 126.7, 126.9, 127.1, 127.5, 128.3, 129.4, 129.6, 138.4, 143.4, 152.6, 154.2, 165.6, 166.1, 168.4, 191.9. MS: *m/z* (%): 468 (20.3), 327 (43.7), 313 (32), 141 (100).

*5-Ethyl-2-[(4′-methoxyphenacylmethyl)thio]-6-1-naphthylthiopyrimidin-4(3H)-one (**5e**)*

Yield: 0.12 g (52%) as a white powder. mp 197–198 °C. ^{1}H-NMR ($CDCl_3$): δ = 1.04 (t, 3H, J = 7.3 Hz, CH_3), 2.43 (q, 2H, J = 7.3 Hz, CH_2), 3.90 (s, 3H, OCH_3), 4.51 (s, 2H, SCH_2), 6.97–7.99 (m, 11H, H_{arom}), 10.01 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ = 13.4, 21.8, 37.7, 55.6, 114.2, 121.5, 123.9, 124.3, 124.6, 125.6, 125.9, 126.4, 127.5, 127.8, 128.4, 129.8, 130.4, 134.0, 158.2, 161.7, 163.1, 163.4, 192.8. MS: *m/z* (%): 462 (16.2), 327 (28.3), 313 (36.8), 135 (100).

*2-[(4′-Fluorophenacylmethyl)thio]-5-ethyl-6-phenylthio pyrimidin-4(3H)-one (**5f**)*

Yield: 0.094 g (47%) as a white solid. mp 171–174 °C. ^{1}H-NMR ($CDCl_3$): δ = 1.07 (t, 3H, J = 7.3 Hz, CH_3), 2.61 (q, 2H, J = 7.3 Hz, CH_2), 4.52 (s, 2H, SCH_2), 6.92–7.45 (m, 9H, H_{arom}), 9.81 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): δ = 13.7, 21.8, 38.1, 118.3 (d), 124.6, 125.6, 127.6, 128.8, 129.6, 132.8, 134.1, 152.9, 153.5, 165.7, 166.9, 193.2. MS: *m/z* (%): 400 (23.4), 277 (68.1), 263 (52.3), 123 (100).

*2-[(4′-Methoxyphenacylmethyl)thio]-5-ethyl-6-phenylthio pyrimidin-4(3H)-one (**5g**)*

Yield: 0.081 g (39%) as a yellow solid. mp 153–155 °C. ^{1}H-NMR ($CDCl_3$): δ = 1.05 (t, 3H, J = 7.3 Hz, CH_3), 2.58 (q, 2H, J = 7.3 Hz, CH_2), 3.89 (s, 3H, OCH_3), 4.53 (s, 2H, SCH_2), 6.89–7.38 (m, 11H, H_{arom}). 10.12 (br, 1H, NH), ^{13}C-NMR ($CDCl_3$): δ = 13.3, 21.2, 36.8, 55.3, 114.3 (d), 116.7, 123.9, 127.5, 128.6, 129.8, 132.6, 133.5, 152.2, 153.1, 162.7, 165.3, 192.7. MS: *m/z* (%): 412 (26.7), 277 (100), 263 (49.2), 135 (83.2).

*5-Ethyl-2-[(4′-methoxyphenacylmethyl)thio]-6-1-naphthylthiopyrimidin-4(1H)-one (**6e**)*

Yield: 0.075 g (24%) as a yellow powder. mp 105–108 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.89 (t, 3H, J = 7.3 Hz, CH_3), 2.38 (q, 2H, J = 7.3 Hz, CH_2), 3.49 (s, 1H, NH), 3.84 (s, 3H, OCH_3), 4.41 (s, 2H, SCH_2), 6.84–8.02 (m, 11H, H_{arom}). ^{13}C-NMR ($CDCl_3$): δ = 13.1, 20.7, 36.5, 55.3, 114.2 (d), 118.3, 122.3, 123.7, 123.9, 124.5, 125.8, 126.1, 126.6, 127.8, 128.1, 128.3, 129.9, 133.6, 151.7, 153.4, 162.8, 167.3, 191.3. MS: *m/z* (%): 462 (5.1), 327 (63.6), 154 (23.1), 135 (100).

*2-[(4′-Fluorophenacylmethyl)thio]-5-ethyl-6-phenylthio pyrimidin-4(1H)-one (**6f**)*

Yield: 0.042 g (21%) as a yellow solid. mp 93–95 °C. ^{1}H-NMR ($CDCl_3$): δ = 0.86 (t, 3H, J = 7.3 Hz, CH_3), 2.41 (q, 2H, J = 7.3 Hz, CH_2), 3.51 (s, 1H, NH), 4.40 (s, 2H, SCH_2), 7.09–7.99 (m, 11H, H_{arom}). ^{13}C-NMR ($CDCl_3$): δ = 13.3, 20.9, 37.1, 115.2 (d), 118.7, 124.3, 125.4, 126.9, 127.5, 131.9, 133.7, 148.3, 149.1, 165.2, 168.5, 191.7. MS: *m/z* (%): 400 (4.9), 291 (35.3), 277 (54.8), 123 (100).

Anti-HIV-1 activity assays

The activity of the compounds against HIV-1 (HTLV-IIIB strain) and the Y181C+K103N mutant strain was based on the inhibition of the virus-induced cytopathic effect on MT-4 cells using the MTT method. Briefly, virus stocks were titrated in MT-4 cells and expressed as 50% cell culture-infective dose (CCID50). MT-4 cells

were suspended in culture medium at 1×10^5 cells/mL and infected with HIV at a multiplicity of infection of 0.02. Immediately after virus infection, 100 μL of the cell suspension was brought into each well of a flat-bottom microtiter tray containing various concentrations of the test compounds. The test compounds were dissolved in dimethyl sulfoxide at 50 mM or higher. After a 4-day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Compounds were tested in parallel for their cytotoxic effect on uninfected MT-4 cells.

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