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Synthesis and anti-HIV-1 activity of *S*-dihydro(alkyloxy)benzyloxypyrimidine derivatives

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Abstract Several 2-heteroaryl-, 2-heteroarylcarbonylmethyl-, 2-arylcarbonylmethyl, and 2-arylethyl derivatives of *S*-dihydro(alkyloxy)benzyloxypyrimidines have been synthesized and the anti-HIV activities of these compounds were tested in C8166 cell and against RT enzyme. It was found that some of these compounds showed good activity against HIV-1 ($EC_{50} = 0.014–0.8\,\mu M$) with low toxicity ($CC_{50}$ value of $222–564\,\mu M$) and high selectivity (*SI* value of 278–37743). The structure-activity relationships (SAR) of these compounds have also been discussed.

Keywords NNRTIs; *S*-DABOs; Antiviral activity; HIV-1.

Introduction

Acquired immunodeficiency syndrome (AIDS) was identified by Centers for Disease Control and Prevention (CDC) in June 1981. Since then, it has been widely spread in the world and seriously affect healthy conditions of human being, HIV-1 reverse transcriptase (RT) [1] played a critical role in the process of HIV replication and has been identified as one of the main targets for anti-HIV drug discovery [2]. Two classifications of RT inhibitors have been studied: nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside transcriptase inhibitors (NNRTIs). NNRTIs, being less toxic than NRTIs, had received great attention [3–5]. Three NNRTIs nevirapine [6], delavirdine [7], and efavirenz [8] have been approved by FDA. Not only being effective RT inhibitors, these three NNRTIs also acted as the key components of the combination therapy [9–11]. However, NNRTIs can easily induce drug-resistant variants of HIV-1, which have also been seen in the treatment with other chemotherapeutic agents. New anti-HIV agents with novel structures or mechanism(s) of action are still in demand [12, 13].

We have previously developed a series of 6-naphthylmethyl substituted 2-(alkylthio)-5-alkyl-6-arylmethyl-3,4-dihydro-4-oxopyrimidines (*S*-dihydro(alkyloxy)benzyloxypyrimidines) [14], characterized by the presence of a β-carbonyl group on the C-2 side chain (**1**, Fig. 1) [15, 16]. These compounds exhibited significant anti-HIV activity and more in-

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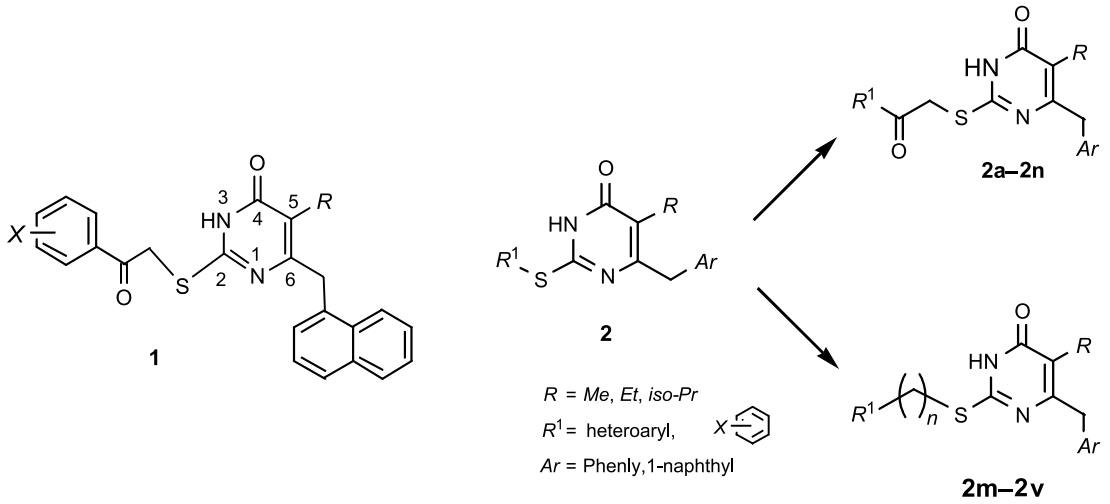


Fig. 1 Structures of *S*-DABOs

teresting, they might interfere with a target that differed from HIV RT, or act on RT in a way that is different from typical NNRTIs. To further optimize the structure of *S*-DABO derivatives and improve the anti-HIV functionality, we designed two series of oxopyrimidine derivatives **2** based on previously investigated scaffold **1** (Fig. 1). These novel chemical entities were characterized by (i) introduction of a heteroaromatic ring at the end of C-2 side chain (**2a**–**2n**); (ii) the presence of an arylalkylthio substituent at position 2 (**2m**–**2v**) to investigate the importance of the β-carbonyl group; (iii) replacement of the fused naphthylmethyl at the C-6 position present in **1** with the phenylmethyl moiety, which might favor the entrance of intact molecular structure to the binding pocket of the target due to the increasing flexibility. Herein, we report the synthesis, antiviral activity evaluation, and the structure activity relationships of these compounds.

Results and discussion

Chemistry

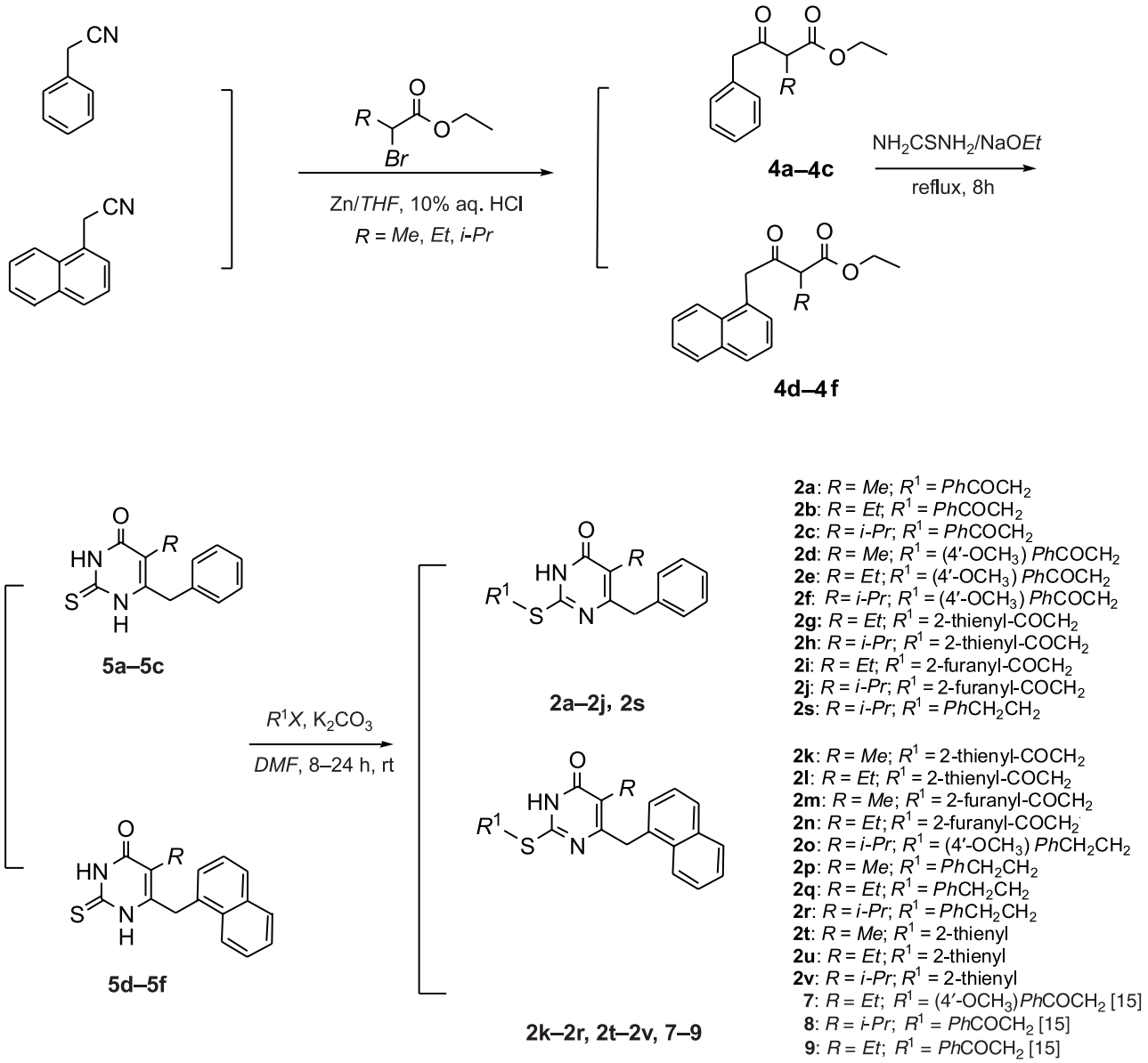
The synthetic route of novel *S*-DABO derivatives **2a**–**2v** is outlined in Scheme 1. Following the procedure described previously [17, 18], the 3-oxo ester **4a**–**4f** was prepared by reacting 1-naphthylacetonitrile or phenylacetonitrile with ethyl-2-bromoalkanoates **3a**–**3c** in the presence of activated zinc dust in refluxing *THF* for 8 h. Condensation of **4a**–**4f** with thiourea in the presence of sodium ethoxide in refluxing ethanol afforded the key intermediate 2-thiouracil **5a**–**5f**, which were subjected to S-alkylation in anhydrous *DMF* with appropriate halide(R^1X) in the presence of K_2CO_3 to afford the desirable *S*-DABO analogues **2a**–**2v** (Scheme 1). Structures of these compounds were determined by mass spectral, ^{1}H NMR and ^{13}C NMR data. A single crystal X-ray structure analysis was also performed for **2j** (Fig. 2).

Antiviral activity

The novel *S*-DABO derivatives (**2a**–**2v**) were tested for their cytotoxicities and anti-HIV-1 activities in C8166 cells infected by the HIV-1_{IIIB} and compared with AZT. The capability of these *S*-DABO derivatives to inhibit HIV-1 RT was also measured by using homopolymeric template primers. The activity data were interpreted in CC_{50} values (cytotoxicities), EC_{50} (anti-HIV-1 activities), *SI* (selectivity, given by the CC_{50}/EC_{50} ratio) and IC_{50} (RT inhibitory activity) values (Table 1). In addition, the previously reported **7**–**9** [15] were included in our assays for comparison.

The results of cellular assays showed (Table 1) that the majority of the *S*-DABO derivatives, except for **2o**, **2t**, **2u**, and **2v**, exhibited anti-HIV-1 activities in the low micromolar to submicromolar ranges. The cytotoxicity of most of these compounds against C8166 cells was at the dose of 194 μM. In comparision, **2g**, **2o**, and **2t** displayed slightly higher toxicity with the CC_{50} value ranging from 88 to 112 μM.

In the series, 6-benzyl-5-isopropyl-2-[(phenylcarbonylmethyl)thio]pyrimidin-4(*3H*)-one (**2c**) was



Scheme 1

the most promising compound. It exhibited extremely potent inhibitory activity against HIV-1 replication ($EC_{50} = 0.014\ \mu M$) and highest selectivity ($SI > 37743$), which is somewhat better than those found for AZT. Besides **2c**, **2b**, **2e**, and **2j** also prevented the cytopathic effect of HIV-1_{IIIB} at low concentrations ($EC_{50} = 0.08$, 0.048, and $0.095\ \mu M$, respectively) and were minimally toxic to C8166 cells ($CC_{50} > 549$, 507, and $543\ \mu M$) resulting in a remarkably high selectivity indices ($SI > 6859$, 10560, and 5714, respectively).

Using poly(r*C*) ligo(d*G*) as the template primer, many of these analogues exhibited inhibitory activity against HIV-1RT. Compounds with high activities in cell assay also endowed with favorable activity in enzyme analysis. For example, **2c**, **2b**, **2i**, and **2j** displayed anti-HIV-1 activity in the range of micromolar concentration ($IC_{50} = 0.35$–$0.66\ \mu M$). However, as showed in Table 1, the IC_{50} values in enzyme assay were 1–2 orders higher than the EC_{50} values in cell assay. This might because these compounds also interfered with another target except for HIV-1 RT.

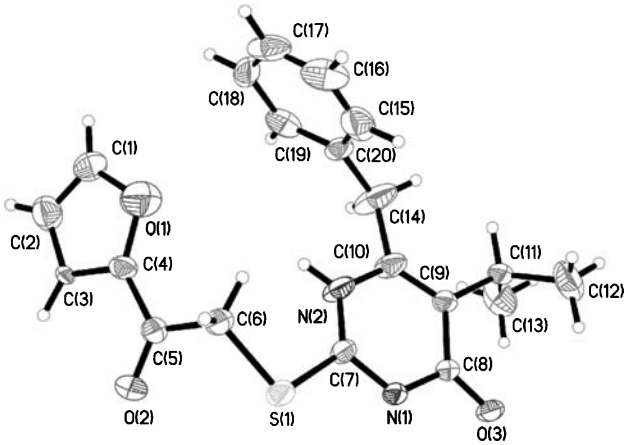


Fig. 2 X-Ray crystal structure of **2j**

Table 1 Cytotoxicity and anti-HIV-1 activity of target compounds[a]

Comp.	$EC_{50}/\mu M$[b]	$CC_{50}/\mu M$[c]	SI[d]	$IC_{50}/\mu M$[e]
2a	0.80	222	278	1.85
2b	0.08	>549	>6859	0.35
2c	0.014	>528	>37743	0.66
2d	1.47	>526	>357	1.10
2e	0.048	>507	>10560	1.06
2f	0.144	>489	>3399	1.46
2g	5.39	95.7	17.7	17.8
2h	0.28	194	679	37.8
2i	0.16	>564	>3399	0.45
2j	0.095	>543	>5714	0.43
2k	1.92	>492	>256	1.86
2l	0.62	227	367	9.67
2m	1.82	>512	>281	5.99
2n	1.43	201	140	17.5
2o	20.9	112	5.37	449
2p	5.40	>517	>95	517
2q	1.79	321	179	499
2r	7.11	>482	>68	482
2s	1.04	>549	>5268	549
2t	43.3	88.3	2.04	509
2u	14.1	280	19.9	548
2v	24.1	230	9.55	528
7	0.427	>450	>1052	
8	0.280	>467	>1666	
9	0.579	>482	>833	
AZT	0.017	4413	260243	

[a] All data represent mean values for three separate experiments
[b] Concentration required to protect the cell against viral cytopathogenicity by 50% in C8166 cells
[c] Concentration that reduces the C8166 cell viability by 50%
[d] Selectivity index ratio CC_{50}/EC_{50}
[e] Concentration required to inhibit the HIV-1 rRT activity by 50%

The biological activity data (Table 1) indicated that the anti-HIV-1 activity was strongly dependent on the nature of the substituent at C-2, C-5, and C-6 of the pyrimidin ring. In general, the 6-benzyl substituted derivatives (**2c**, **2b**, **2e**, and **2i**) were 10–20 fold more active than the 6-napthylmethyl counterparts (**8**, **9**, **7**, and **2n**) with the only exception of **2l**, which was about 9-fold more active than the corresponding 6-benzyl derivative **2g**.

It was also observed that the activities of these novel *S*-DABO derivatives were closely related to the length of the C-2 side chain. These C-2 substituent groups having suitable length, such as *Ph*$COCH_2$, (4′-OCH_3)*Ph*$COCH_2$, and 2-furanyl-$COCH_2$, were beneficial to the anti-HIV-1 activity. In contrast, the 2-thiophen derivatives were almost inactive (**2t**, **2u**, and **2v**). Concerning the SAR of C-2 side chain was concerned, the *Ph*$COCH_2$ derivatives (**2b**, **2c**, and **9**) were more potent than their 2-furanyl-$COCH_2$ counterparts (**2i**, **2j**, and **2n**). In the 6-benzyl series, the 2-furanyl-$COCH_2-$ derivative **2i** was 3.3 folds more potent than its 2-thienyl-$COCH_2$ counterpart **2g**. Otherwise, in the 6-napthylmethyl series, the 2-furanyl-$COCH_2$ derivative **2k** and its 2-thienyl-$COCH_2-$ counterpart **2m** exhibited similar activities. When the *C-2-β*-carbonyl was replaced with a methylene, the activity decreased significantly (**2q** was 3-fold less active than **9** and **2r** was 25-fold less active than **8**). These results showed that the C-2-*β*–carbonyl is a crucial factor to improve the activity. Based on a molecular modeling study, the C-2 side chain was adjacent to amino acid residue *Try*318 of HIV RT enzyme. We speculated that the enhanced activity might be related to the formation of hydrogen bond between C-2-*β*-carbonyl and the amide of the main chain of *Try*318. The detailed SAR analysis will be reported elsewhere.

Finally, the activity of *S*-DABO derivatives increased proportionally to the modication of the C-5 substitution in the order of $Me \rightarrow Et \rightarrow i\text{-}Pr$, which was also shown in previous studies [15]. One exception here was the pairs **2e**, **2f**, and **2q**, **2r**, wherein the 5-ethyl derivatives were 3–4 fold more active.

In conclusion, we discovered a series of HIV inhibitors with high activities. The EC_{50} values to HIV-1 of four compounds in the series, **2b**, **2c**, **2e**, and **2j**, were at the range of submicromolar and all showed low toxicity and high selectivity exponents. To explore the use of these compounds as anti-HIV-1 candidates, further biological studies are still in progress.

Experimental

Melting points were determined on a XT-4 binocular microscope melting point instrument. Infrared (IR) spectra were recorded on an AVATAR FT-IR spectrometer as KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were obtained on a *Bruker* AM-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 an Agilent LC/MSD TOF mass spectrometer. Regents and solvents were all analytical grade and were purified and dried by stands methods before use. All air-sensitive reactions were run under an atmosphere of N_2.

General procedure for preparation of derivatives ***2a–2v***
To a solution of 2-thiouracil **5** [17, 18] (2 mmol) in anhydrous *DMF* (8 cm^3) were added K_2CO_3 (2.2 mmol) and halide (R^1X) (2.2 mmol). The mixture was stirred at room temperature for 8–24 h. After TLC (*EtOAc*:PE) revealed the disappearance of the starting material, the reaction mixture was filtered. The filtrate was concentrated *in vacuo*, and CH_2Cl_2 (60–80 cm^3) was added to the residue. The organic phase was washed with H_2O (2×50 cm^3), dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give the crude products **2a**–**2v**. The crude products were purified by column chromatography (eluent: CH_2Cl_2:*Et*OA*c*:hexane). Selected analytical data for compounds **2a**–**2v** are presented below.

6-Benzyl-5-methyl-2-[(phenylcarbonylmethyl)thio]-pyrimidin-4(3H)-one (**2a**, $C_{20}H_{18}N_2O_2S$)
Yield 27%; mp 166–167°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2970, 1673, 1654 cm^{-1}; ^{1}H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.89$ (s, 3H, CH_3), 3.57 (s, 2H, SCH_2), 4.78 (s, 2H, CH_2Ph), 6.96–8.03 (m, 10H, H_{armo}), 12.75 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 10.7$ (CH_3), 37.9 (CH_2Ph), 39.4 (SCH_2), 116.2 (C-5), 126.5–138.3 (12C, 2*Ph*), 159.8 (C-6), 160.3 (C-4), 161.5 (C-2), 193.5 (C=O) ppm; MS (EI): $m/z =$ 350 (M^+).

6-Benzyl-5-ethyl-2-[(phenylcarbonylmethyl)thio]pyrimidin-4(3H)-one (**2b**, $C_{21}H_{20}N_2O_2S$)
Yield 21%; mp 158–159°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2924, 1679, 1628 cm^{-1}; ^{1}H NMR (500 MHz, *DMSO*-d_6): $\delta = 0.88$ (t, 3H, $J = 7.05$ Hz, CH_3), 2.40 (q, $J = 7.20$ Hz, 2H, CH_2), 3.59 (s, 2H, SCH_2), 4.70 (s, 2H, CH_2Ph), 6.99–7.99 (m, 10H, H_{armo}), 12.64 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 13.4$ (CH_3), 18.5 (CH_2), 37.9 (CH_2Ph), 39.4 (SCH_2), 116.2 (C-5), 126.4–134.4 (12C, 2*Ph*), 156.5 (C-6), 160.8 (C-2), 163.6 (C-4), 193.5 (C=O) ppm; MS (EI): $m/z =$ 364 (M^+).

6-Benzyl-5-isopropyl-2-[(phenylcarbonylmethyl)thio]-pyrimidin-4(3H)-one (**2c**, $C_{22}H_{22}N_2O_2S$)
Yield 50%; mp 187.1–187.8°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2924, 1696, 1641 cm^{-1}; ^{1}H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.09$ (d, 6H, $J = 6.75$ Hz, $2CH_3$), 3.01 (m, 1H, $J = 6.75$ Hz, CH), 3.61 (s, 2H, SCH_2), 4.69 (s, 2H, CH_2Ph), 6.95–8.01 (m, 10H, H_{armo}), 12.62 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 19.9$ ($2CH_3$), 27.4 (CH), 37.4 (CH_2Ph), 37.9 (SCH_2), 123.7 (C-5), 126.4–138.8 (12C, 2*Ph*), 156.1 (C-6), 159.2 (C-2), 163.0 (C-4), 193.60 (C=O) ppm; MS (EI): $m/z = 378$ (M^+).

6-Benzyl-5-methyl-2-[(4′-methoxyphenylcarbonylmethyl)-thio]pyrimidin-4(3H)-one (**2d**, $C_{21}H_{20}N_2O_3S$)
Yield 45%; mp 162–163°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2925, 1674, 1643 cm^{-1}; ^{1}H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.89$ (s, 3H, CH_3), 3.61 (s, 2H, SCH_2), 3.83 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2Ph), 7.01–7.99 (m, 9H, H_{armo}), 12.73 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 10.6$ (CH_3), 37.6 (CH_2Ph), 38.6 (SCH_2), 55.9 (OCH_3), 110.2 (C-5), 114.3–138.3 (12C, 2*Ph*), 59.8 (C-6), 161.3 (C-4), 163.8 (C-2), 191.8 (C=O) ppm; MS (EI): $m/z = 380$ (M^+).

6-Benzyl-5-ethyl-2-[(4′-methoxyphenylcarbonylmethyl)-thio]pyrimidin-4(3H)-one (**2e**, $C_{22}H_{22}N_2O_3S$)
Yield 29%; mp 190–191°C; FT-IR (KBr): $\bar{\nu} = 3424$, 2924, 1683, 1644 cm^{-1}; ^{1}H NMR (500 MHz, *DMSO*-d_6): $\delta = 0.88$ (t, 3H, $J = 7.15$ Hz, CH_3), 2.40 (q, $J = 7.25$ Hz, 2H, CH_2), 3.62 (s, 2H, SCH_2), 3.83 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2Ph), 7.04–7.99 (m, 9H, H_{armo}), 12.68 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 13.4$ (CH_3), 18.5 (CH_2), 37.6 ($PhCH_2$), 39.4 (SCH_2), 56.0 (OCH_3), 116.2 (C-5), 114.3–138.6 (12C, 2*Ph*), 156.5 (C-6), 160.8 (C-2), 163.8 (C-4), 193.5 (C=O) ppm; HRMS: $m/z = 394.1366$, calcd. 394.1351.

6-Benzyl-5-isopropyl-2-[(4′-methoxyphenylcarbonylmethyl)-thio]pyrimidin-4(3H)-one (**2f**, $C_{23}H_{24}N_2O_3S$)
Yield 51%; mp 185.5–186.5°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2924, 1696, 1641 cm^{-1}; ^{1}H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.11$ (d, 6H, $J = 6.60$ Hz, $2CH_3$), 3.02 (m, 1H, $J = 6.60$ Hz, CH), 3.64 (s, 2H, SCH_2), 3.83 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2Ph), 6.99–8.00 (m, 9H, H_{armo}), 12.59 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 19.9$ ($2CH_3$), 27.4 (CH), 37.5 ($PhCH_2$), 39.5 (SCH_2), 55.9 (OCH_3), 110.7 (C-5), 114.3–138.9 (12C, 2*Ph*), 156.1 (C-6), 159.2 (C-2), 163.7 (C-4), 193.6 (C=O) ppm; MS (EI): $m/z = 408$ (M^+).

6-Benzyl-5-ethyl-2-[(thiophen-2-ylcarbonylmethyl)thio]pyrimidin-4(3H)-one (**2g**, $C_{19}H_{18}N_2O_2S_2$)
Yield 57%; mp 195–196°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2972, 1679, 1663 cm^{-1}; ^{1}H NMR (500 MHz, *DMSO*-d_6): $\delta = 0.89$ (t, 3H, $J = 7.20$ Hz, CH_3), 1.17 (q, $J = 7.10$ Hz, 2H, CH_2), 3.19 (s, 2H, SCH_2), 4.02 (s, 2H, CH_2, phenyl), 7.02–8.26 (m, 8H, H_{armo}), 12.65 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 13.4$ (CH_3), 18.8 (CH_2), 37.2 (CH_2Ph), 37.8 (SCH_2), 116.2 (C-5), 124.4–144.4 (6C, *Ph*, 4C, thienyl), 156.5 (C-6), 160.8 (C-2) ppm; MS (EI): $m/z = 370$ (M^+).

6-Benzyl-5-isopropyl-2-[(thiophen-2-ylcarbonylmethyl)thio]-pyrimidin-4(3H)-one (**2h**, $C_{22}H_{20}N_2O_2S_2$)
Yield 20%; mp 187.1–187.8°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2953, 1675, 1641 cm^{-1}; ^{1}H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.19$ (d, 6H, $J = 7.35$ Hz, $2CH_3$), 3.11 (m, 1H, $J = 6.85$ Hz, CH), 3.28 (s, 2H, SCH_2), 3.95 (s, 2H, CH_2Ph), 7.14–7.33 (m, 8H,

H_{armo}), 12.69 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 19.4$ ($2CH_3$), 27.0 (CH), 37.0 (CH_2*Ph*), 37.2 (SCH_2), 116.2 (C-5), 123.6–134.3 (6C, *Ph*, 4C, thienyl), 156.2 (C-6), 160.4 (C-2), 162.8 (C-4), 192.7 (C=O) ppm; MS (EI): $m/z = 384$ (M^+).

6-Benzyl-5-ethyl-2-[(furan-2-ylcarbonylmethyl)thio]pyrimidin-4(3H)-one (**2i**, $C_{19}H_{18}N_2O_3S$)
Yield 56%; mp 165–166°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2924, 1675, 1642 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 0.87$ (t, 3H, $J = 7.40$ Hz, CH_3), 2.40 (q, $J = 6.95$ Hz, 2H, CH_2), 3.65 (s, 2H, SCH_2), 4.49 (s, 2H, CH_2*Ph*), 6.72–8.01 (m, 8H, H_{armo}), 12.70 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 13.3$ (CH_3), 18.5 (CH_2), 36.6 (CH_2*Ph*), 37.8 (SCH_2), 110.2 (C-5), 113.0–151.5 (6C, *Ph*, 4C, furanyl), 159.9 (C-6), 160.39 (C-4), 161.6 (C-2), 182.0 (C=O) ppm; HRMS: $m/z = 354.1042$, calcd. 354.1038.

6-Benzyl-5-isopropyl-2-[(furan-2-ylcarbonylmethyl)thio]-pyrimidin-4(3H)-one (**2j**, $C_{20}H_{20}N_2O_3S$)
Yield 31%; mp 163–164.8°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2924, 1675, 1642 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.03$ (d, 6H, $J = 7.35$ Hz, $2CH_3$), 3.01 (m, 1H, $J = 6.85$ Hz, CH), 3.64 (s, 2H, SCH_2), 4.49 (s, 2H, CH_2*Ph*), 6.71–8.26 (m, 8H, H_{armo}), 12.71 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 19.82$ ($2CH_3$), 27.38 (CH), 36.54 (CH_2*Ph*), 39.42 (SCH_2), 109.54 (C-5), 113.0–151.6 (6C, *Ph*, 4C, furanyl), 156.1 (C-6), 159.2 (C-2), 163.0 (C-4), 182.1 (C=O) ppm; HRMS: $m/z = 368.1204$, calcd. 368.1195.

5-Methyl-2-[(thiophen-2-ylcarbonylmethyl)thio]-6-(1-naphthylmethyl)pyrimidin-4(3H)-one (**2k**, $C_{22}H_{18}N_2O_2S_2$)
Yield 40%; mp 210–211°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2953, 1675, 1635 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.94$ (s, 3H, CH_3), 4.14 (s, 2H, SCH_2), 4.42 (s, 2H, CH_2, naphthyl), 7.09–8.27 (m, 10H, H_{armo}), 12.55 (s, brs, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 10.7$ (CH_3), 37.3 (*Ph*CH_2), 37.7 (SCH_2), 122.8 (C-5), 124.3–142.4 (10C, naphthyl, 4C, thienyl), 159.9 (C-6), 160.3 (C-2), 161.6 (C-4), 186.4 (C=O) ppm; MS (EI): $m/z = 406$ (M^+).

5-Ethyl-2-[(thiophen-2-ylcarbonylmethyl)thio]-6-(1-naphthylmethyl)pyrimidin-4(3H)-one (**2l**, $C_{23}H_{20}N_2O_2S_2$)
Yield 38%; mp 191–192°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2953, 1675, 1641 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 0.85$ (t, 3H, $J = 7.10$ Hz, CH_3), 2.42 (q, $J = 6.90$ Hz, 2H, CH_2), 4.14 (s, 2H, SCH_2), 4.41 (s, 2H, CH_2, naphthyl), 7.04–8.30 (m, 10H, H_{armo}), 12.75 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 13.1$ (CH_3), 18.6 (CH_2), 36.4 (*Ph*CH_2), 37.3 (SCH_2), 110.8 (C-5), 112.8–151.1 (10C, naphthyl, 4C, thienyl), 159.9 (C-6), 160.3 (C-2), 161.6 (C-4), 181.8 (C=O) ppm; MS (EI): $m/z = 420$ (M^+).

5-Methyl-2-[(furan-2-ylcarbonylmethyl)thio]-6-(1-naphthylmethyl)pyrimidin-4(3H)-one (**2m**, $C_{22}H_{18}N_2O_3S$)
Yield 41%; mp 206–207°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2845, 1674, 1642 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.95$ (s, 3H, CH_3), 4.15 (s, 2H, SCH_2), 4.32 (s, 2H, CH_2, naphthyl), 6.58–7.99 (m, 10H, H_{armo}), 12.78 (s, brs, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 12.59$ (CH_3), 36.6 (CH_2, naphthyl), 37.8 (SCH_2), 110.2 (C-5), 113.0–151.5 (10C, naphthyl, 4C, furanyl), 159.9 (C-6), 160.3 (C-4), 161.6 (C-2), 182.0 (C=O) ppm; MS (EI): $m/z = 390$ (M^+).

5-Ethyl-2-[(furan-2-ylcarbonylmethyl)thio]-6-(1-naphthylmethyl)pyrimidin-4(3H)-one (**2n**, $C_{23}H_{20}N_2O_3S$)
Yield 64%; mp 164.5–165.5°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2953, 1683, 1639 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 0.85$ (t, 3H, $J = 7.35$ Hz, CH_3), 2.42 (q, $J = 7.35$ Hz, 2H, CH_2), 4.15 (s, 2H, SCH_2), 4.28 (s, 2H, CH_2, naphthyl), 6.51–7.98 (m, 10H, H_{armo}), 12.72 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 13.3$ (CH_3), 18.5 (CH_2), 36.6 (CH_2, naphthyl), 37.8 (SCH_2), 110.2 (C-5), 113.0–151.5 (10C, naphthyl, 4C, furanyl), 159.3 (C-6), 160.1 (C-4), 160.9 (C-2), 182.2 (C=O) ppm; MS (EI): $m/z = 404$ (M^+).

5-Isopropyl-2-[(4′-methoxy-phenylethyl)thio]-6-(1-naphthylmethyl)pyrimidin-4(3H)-one (**2o**, $C_{27}H_{28}N_2O_2S$)
Yield 13%; mp 137–138°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2953, 1641 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.15$ (d, 6H, $J = 6.85$ Hz, $2CH_3$), 2.94 (m, 1H, $J = 6.85$ Hz, CH), 3.48 (t, 2H, $J = 7.45$ Hz, *Ph*CH_2), 3.83 (s, 3H, OCH_3), 4.30 (s, 2H, CH_2, naphthyl), 4.36 (t, 2H, $J = 7.45$ Hz, SCH_2), 7.10–8.15 (m, 11H, H_{armo}), 12.71 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 19.9$ ($2CH_3$), 27.9 (CH), 31.3 (*Ph*CH_2), 37.7 (CH_2, naphthyl), 39.9 (SCH_2), 55.9 (OCH_3, 123.70 (C-5), 124.0–134.8 (10C, naphthyl, 6C, *Ph*), 159.8 (C-6), 160.3 (C-2), 161.6 (C-4) ppm; MS (EI): $m/z = 444$ (M^+).

5-Methyl-2-[(phenylethyl)thio]-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one (**2p**, $C_{24}H_{22}N_2OS$)
Yield 21%; mp 175–176°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2953, 1654 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 2.05$ (s, 3H, CH_3), 2.56 (q, $J = 7.35$ Hz, 2H, *Ph*CH_2), 2.99 (t, 2H, $J = 7.35$ Hz, SCH_2), 4.37 (s, 2H, CH_2, naphthyl), 6.92–8.16 (m, 12H, H_{armo}), 12.58 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 10.8$ (CH_3), 31.0 (*Ph*CH_2), 34.87 (napthyl, CH_2), 38.73 (SCH_2), 122.7 (C-5), 124.6–140.1 (10C, naphthyl, 6C, *Ph*), 159.9 (C-6), 160.39 (C-2), 161.56 (C-4) ppm; MS (EI): $m/z = 386$ (M^+).

5-Ethyl-2-[(phenylethyl)thio]-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one (**2q**, $C_{25}H_{24}N_2OS$)
Yield 27%; mp 123.5–124.5°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2965, 1649 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 0.93$ (t, 3H, $J = 7.40$ Hz, CH_3), 2.08 (q, 2H, $J = 7.40$ Hz, CH_2), 2.56 (q, 2H, $J = 7.35$ Hz, *Ph*CH_2), 2.97 (q, 2H, $J = 7.35$ Hz, SCH_2), 4.48 (s, 2H, CH_2, naphthyl), 6.86–8.30 (m, 12H, H_{armo}), 12.56 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 13.4$ (CH_3), 18.6 (CH_2), 30.9 (*Ph*CH_2), 34.9 (CH_2, naphthyl), 37.1 (SCH_2), 122.8 (C-5), 124.6–140.1 (10C, naphthyl, 6C, *Ph*), 159.9 (C-6), 160.3 (C-2), 161.6 (C-4) ppm; MS (EI): $m/z = 400$ (M^+).

5-Isopropyl-2-[(phenylethyl)thio]-6-(1-naphthylmethyl)-pyrimidin-4(3H)-one (**2r**, $C_{26}H_{26}N_2OS$)
Yield 25%; mp 95–96°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2924, 1638 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.23$ (d, 6H, $J = 6.85$ Hz, $2CH_3$), 2.58 (t, 2H, $J = 7.35$ Hz, *Ph*CH_2), 3.01 (t, 2H, $J = 7.35$ Hz, SCH_2), 3.13 (m, 1H, $J = 6.80$ Hz, CH), 4.01 (s, 2H, CH_2, naphthyl), 6.90–8.31 (m, 12H, H_{armo}), 12.46 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 19.90$ ($2CH_3$), 27.4 (CH), 30.4 (*Ph*CH_2), 37.4 (CH_2-naphthyl), 37.9 (SCH_2), 123.7 (C-5), 126.4–138.8 (10C, naphthyl, 6C, *Ph*), 156.1 (C-6), 159.2 (C-2), 163.0 (C-4) ppm; MS (EI): $m/z = 414$ (M^+).

6-Benzyl-5-isopropyl-2-[(phenylethyl)thio]-pyrimidin-4(3H)-one (**2s**, $C_{22}H_{24}N_2OS$)
Yield 29%; mp 130–131°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2953, 1642 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.18$ (d, 6H, $J = 6.80$ Hz, $2CH_3$), 2.86 (t, 2H, $J = 7.45$ Hz, *Ph*CH_2), 3.11 (m, 1H, $J = 6.80$ Hz, CH), 3.27 (t, 2H, $J = 7.45$ Hz, SCH_2), 3.94 (s, 2H, CH_2*Ph*), 7.15–7.33 (m, 10H, H_{armo}), 12.71 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 19.4$ ($2CH_3$), 27.0 (CH), 30.2 (*Ph*CH_2), 35.3 (CH_2*Ph*), 37.2 (SCH_2), 123.7 (C-5), 113.5–154.3 (12C, 2*Ph*), 156.1 (C-6), 159.2 (C-2), 163.0 (C-4) ppm; MS (EI): $m/z = 364$ (M^+).

5-Methyl-6-(1-naphthylmethyl)-2-[(thiophen-2-yl)thio]-pyrimidin-4(3H)-one (**2t**, $C_{20}H_{16}N_2OS_2$)
Yield 46%; mp 218–219°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2953, 1669 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 2.12$ (s, 3H, CH_3), 4.26 (s, 2H, CH_2, naphthyl), 7.26–9.48 (m, 10H, H_{armo}), 12.78 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 12.8$ (CH_3), 32.1 (CH_2, naphthyl), 112.5 (C-5), 118.7–133.6 (10C, naphthyl, 4C, thienyl), 149.30 (C-6), 161.85 (C-2), 174.66 (C-4) ppm; MS (EI): $m/z = 364$ (M^+).

5-Ethyl-6-(1-naphthylmethyl)-2-[(thiophen-2-yl)thio]-pyrimidin-4(3H)-one (**2u**, $C_{21}H_{18}N_2OS_2$)
Yield 44%; mp 184–185°C; FT-IR (KBr): $\bar{\nu} = 3425$, 2952, 1649 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 0.81$ (t, 3H, $J = 7.35$ Hz, CH_3), 1.18 (q, $J = 7.35$ Hz, 2H, CH_2), 4.32 (s, 2H, CH_2, naphthyl), 7.08–8.14 (m, 10H, H_{armo}), 12.49 (s, brs, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 13.2$ (CH_3), 18.1 (CH_2), 32.2 (CH_2, naphthyl), 112.8 (C-5), 118.7–133.6 (10C, naphthyl, 4C, thienyl), 149.3 (C-6), 161.9 (C-2), 174.7 (C-4) ppm; MS (EI): $m/z = 378$ (M^+).

5-Isopropyl-6-(1-naphthylmethyl)-2-[(thiophen-2-yl)thio]-pyrimidin-4(3H)-one (**2v**, $C_{22}H_{20}N_2OS_2$)
Yield 56%; mp 219–220°C; FT-IR (KBr): $\bar{\nu} = 3541$, 2965, 1650 cm^{-1}; ^1H NMR (500 MHz, *DMSO*-d_6): $\delta = 1.33$ (d, 6H, $J = 7.35$ Hz, $2CH_3$), 3.04 (m, 1H, J = 6.85, CH), 4.30 (s, 2H, CH_2, naphthyl), 6.84–8.92 (m, 10H, H_{armo}), 12.46 (s, br, s, 1H, NH) ppm; ^{13}C NMR (500 MHz, *DMSO*-d_6): $\delta = 19.8$ ($2CH_3$), 27.4 (CH), 32.2 (CH_2, naphthyl), 112.8 (C-5), 118.7–133.6 (10C, naphthyl, 4C, thienyl), 149.31 (C-6), 161.88 (C-2), 174.67 (C-4) ppm; HRMS: $m/z = 392.1025$, calcd. 392.1017.

Table 2 Crystal data and structure refinement for X-ray crystal structures of compounds **2j**

Parameter	Compound **2j**
Crystal size	$0.30 \times 0.19 \times 0.08\ mm^3$
Unit cell dimensions	$a = 12.5785$ (17) Å $\alpha = 90°$, $b = 9.6073$ (13) Å $\beta = 102.653$ (2)°, $c = 15.676$ (2) Å $\gamma = 90°$.
F (*000*)	776
Space group	Monoclinic, *P*2 (1)/*c*
Cell volume	1848.4 (4)$Å^3$
Z, Calculated density	4, 1.324 mg/m^3
θ range for data collection	1.66 to 26.43°
Limiting indices	$-15 \le h \le 14$, $-10 \le k \le 12$, $-19 \le l \le 19$.
Reflections collected/unique	10773/3770 [R(int) = 0.0348]
Data/restraints/parameters	3770/0/235
Goodness-of-fit on F^2	1.071
Final *R* indices [$I > 2\sigma(I)$]	$R1 = 0.0910$, $wR2 = 0.2691$
R indices (all data)	$R1 = 0.1293$, $wR2 = 0.3056$
Absorption coefficient	0.197 mm^{-1}
Largest diff. peak and hole	0.935 and −0.621 $eÅ^{-3}$

X-Ray crystallography
Colorless cubic crystals of **2j** 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 ($0.30 \times 0.19 \times 0.08$ mm) of **2j** 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 2). Crystallographic data were deposited at the Cambridge Crystallographic Data Centre with CCDC No. 689793 and may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk, or http://www.ccdc.cam.ac.uk).

Antiviral assay procedures

Cells and viruses
Human T-cell lines (C8166, H9) and HIV-1_{IIIB} were kindly donated by Medical Research Council, AIDS Regent Project. The cells were maintained at 37°C in 5% CO_2 in RPMI-1640 medium supplemented with 10% heat-inactivating fetal calf serum (Gibco). HIV-1_{IIIB} was prepared from the supernatants of H9/HIV-1_{IIIB} cells. The 50% HIV-1 tissue culture in-

fectious dose ($TCID_{50}$) in C8166 cells was determined and calculated by *Reed* and *Muench* method. Virus stocks were stored in small aliquots at −70°C. The titer of virus stock was 6.0×10^5 $TCID_{50}$ per cm.

Quantification of the inhibitory effect of compounds on reverse transcriptase

HIV-1 RT activity was measured by ELISA RT kit using a commercially available kit (Roche) according to the instructions of the manufacturer. The compounds **2a–2v** and **7–9** were dissolved in *DMSO* and stored at −4°C. The compounds were incubated with DIG-labeled reaction mixture at 37°C for 2 h, then anti-DIG-POD solution was added, followed by substrate ABTS. Foscarnet was used as a positive control. The absorbency at 405 nm/490 nm (A405/490) was read on Bio-Tek ELx 800 ELISA reader [19].

Cytotoxicity assay

The cellular toxicity of compounds on C8166 cells was assessed by MTT colorimetric assay as described previously [20]. Briefly, 100 mm^3 of 4×10^5 cells $\cdot$ cm^3 were plated into 96-well microtiter plates, 100 mm^3 of various concentrations of compounds was added and incubated at 37°C in a humidifed atmosphere of 5% CO_2 for 72 h. Discard 100 mm^3 supernatant, MTT reagent was added and incubated for 4 h, 100 mm^3 50% *DMF*-10% *SDS* was added. After the formazum was dissolved completely, the plates were read on a Bio-Tek ELx 800 ELISA reader at 595 nm/630 nm. The results were shown by absorbance values. The minimum toxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was determined from dose-response curve.

Inhibition of syncytium formation

C8166 cells (4×10^5 cells $\cdot$ cm^3) were treated with different concentrations of the compounds and HIV-1_{IIIB} (M.O.I. = 0.02), and incubated in a humidified incubator at 37°C in final volume of 200 mm^3. *AZT* was used for positive drug control. After 3 days incubation of culture, the cytopathic effect (CPE) was measured by counting the number of syncytium (multinucleated giant cell) in each well under an inverted microscope [21]. The Percentage inhibition of syncytial cell formation (EC_{50}) was calculated by the percentage of syncytial cell number in compounds treated culture to that in infected control culture.

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