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Structure–activity relationship study of pyrimido[1,2-*c*][1,3]benzothiazin-6-imine derivatives for potent anti-HIV agents

Tsukasa Mizuhara [a], Shinya Oishi [a,*], Hiroaki Ohno [a], Kazuya Shimura [b], Masao Matsuoka [b], Nobutaka Fujii [a,*]

[a] *Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan*
[b] *Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan*

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ABSTRACT

3,4-Dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (PD 404182) is an antiretroviral agent with submicromolar inhibitory activity against human immunodeficiency virus-1 (HIV-1) and HIV-2 infection. In the current study, the structure–activity relationships of accessory groups at the 3- and 9-positions of pyrimido[1,2-*c*][1,3]benzothiazin-6-imine were investigated for the development of more potent anti-HIV agents. Several different derivatives containing a 9-aryl group were designed and synthesized using Suzuki–Miyaura cross-coupling and Ullmann coupling reactions. Modification of the *m*-methoxyphenyl or benzo[*d*][1,3]dioxol-5-yl group resulted in improved anti-HIV activity. In addition, the 2,4-diazaspiro[5.5]undec-2-ene-fused benzo[*e*][1,3]thiazine derivatives were designed and tested for their anti-HIV activities. The most potent 9-(benzo[*d*][1,3]dioxol-5-yl) derivative was two–threefold more effective against several strains of HIV-1 and HIV-2 than the parent compound, PD 404182.

1. Introduction

Highly active antiretroviral therapy, involving the co-administration of nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and/or protease inhibitors, is a standard treatment regimen for human immunodeficiency virus (HIV) infections. This regimen suppresses the replication of HIV and controls disease progression in HIV-infected patients.[1,2] Unfortunately, however, an increasing number of patients with HIV infection/AIDS have failed to respond to the current antiretroviral therapeutics because of serious problems including the emergence of drug-resistant HIV variants[3] and drug-related adverse effects.[4] With this in mind, there is therefore a continuous need to develop novel anti-HIV drugs that are more effective against drug-resistant viruses and produce fewer adverse effects. Recently, a series of extensive studies led to the development of a series of novel antiretrovirals with new mechanisms of action for anti-HIV therapy, including a fusion inhibitor (enfuvirtide),[5–7] an integrase inhibitor (raltegravir),[8,9] and a CC chemokine receptor type 5 (CCR5) antagonist (maraviroc).[10,11] CXC chemokine receptor type 4 (CXCR4) antagonists,[12–16] CD4 mimics,[17–20] gp41-binding peptides[21–23] and small molecules[24–26] represent promising alternative anti-HIV agents.

3,4-Dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (PD 404182) (**1**) was previously reported as an antimicrobial agent that inhibited 3-deoxy-D-manno-octulosonic acid 8-phosphate synthase[27] and phosphopantetheinyl transferase (Fig. 1).[28,29] Following a recent random screening program using a multinuclear activation of a galactosidase indicator (MAGI) assay, compound **1** was identified as a new antiretroviral candidate with a high therapeutic index (CC_{50}/EC_{50} >200). The MAGI assay allows for the inhibitory activity of an early-stage HIV infection, including inhibition of the virus attachment and membrane fusion to host cells, to be effectively evaluated.[30] Compound **1** showed a similar antiviral profile in HIV-1 infection to DS 5000[31] (adsorption inhibitor) and enfuvirtide (fusion inhibitor). The virucidal effects of compound **1** against the human hepatitis C virus, HIV, and simian immunodeficiency virus have also been reported.[32,33] The mechanism of action for compound **1**, however, has not yet been fully understood.

In our previous structure–activity relationship (SAR) study of compound **1**,[34] a number of PD 404182 derivatives were designed and synthesized according to a series of facile synthetic procedures,[35,36] in which the tricyclic heterocycles related to PD 404182 were easily obtained in a few steps from benzaldehydes via C–H functionalization or aromatic nucleophilic substitution.

Abbreviations: CCR5, CC chemokine receptor type 5; CXCR4, CXC chemokine receptor type 4; MAGI, Multinuclear activation of a galactosidase indicator; NNRTI, Non-nucleoside reverse transcriptase inhibitors; NRTI, Nucleoside reverse transcriptase inhibitors.

* Corresponding authors. Tel.: +81 75 753 4551; fax: +81 75 753 4570.
E-mail addresses: soishi@pharm.kyoto-u.ac.jp (S. Oishi), nfujii@pharm.kyoto-u.ac.jp (N. Fujii).

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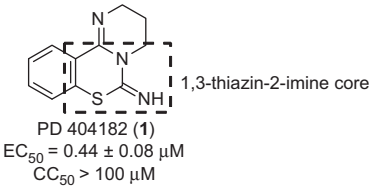


Figure 1. Structure of PD 404182.

The 6-6-6 fused pyrimido[1,2-*c*][1,3]benzothiazine scaffold and the heteroatom arrangement in the 1,3-thiazin-2-imine moiety are indispensable for the inhibitory activity of compound **1** against HIV infection (Fig. 1). Optimization studies indicated that the introduction of a hydrophobic group on the benzene ring and the cyclic amidine substructures effectively improved the antiviral activity by generating a potentially favorable interaction(s) with the target molecule(s). The most potent compounds identified were twofold more potent than PD 404182 and contained a phenyl group at 9-position of pyrimido[1,2-*c*][1,3]benzothiazine (compound **2**) or a geminal dimethyl group on the pyrimidine moiety (compound **3**) (Fig. 2).

In the current study, further structural optimization was conducted from the lead compounds **2** and **3** according to three approaches (Fig. 2), including the introduction of substituents on the 9-phenyl group (I), the substitution of the 9-phenyl group with fused arenes or heterocycles (II), and the modification of the cyclic amidine moiety (III). The anti-HIV profiles of the most potent derivative are also described.

2. Results and discussion

2.1. Synthesis of 9-aryl-3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine derivatives

The 9-aryl-3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine derivatives (**7** and **8**) were synthesized using a Suzuki–Miyaura cross-coupling reaction[37–39] of *N*-(*tert*-butyl)-protected bromide **4** with aryl boronic acid (pinacol ester) or by an Ullmann coupling[40] with pyrazole or imidazole (Scheme 1). Subsequent trifluoroacetic acid (TFA)-mediated deprotection of the *tert*-butyl groups afforded the desired biaryl-type derivatives.

2.2. Synthesis of spiropyrimidine-fused benzothiazinimine derivatives

The synthesis of the spiropyrimidine-fused derivatives started with the dialkylation of malononitrile with dihaloalkanes (**9**, **10**, or **12**, Scheme 2). BH_3-mediated reduction of the alkylated malononitriles (**13–15**) followed by oxidative amidination[41] with 4-bromo-2-fluorobenzaldehyde gave the 2-phenyl-1,4,5,6-tetrahydropyrimidine derivatives (**16–18**). Subsequent exposure of compounds **16–18** to *tert*-butylisothiocyanate provided the tetracyclic compounds **19**, **21**, and **23a**. Deprotection of the *tert*-butyl groups in compounds **19**, **21**, and **23a** afforded the desired spiropyrimidine-fused benzothiazinimine derivatives (**20**, **22**, and **24a**). The substitution of the *p*-methoxybenzyl (PMB) group in compound **24a** was also attempted (Scheme 3). The treatment of compound **23a** with methyl chloroformate or acetyl chloride directly provided derivatives **23b** and **23c**, respectively. A two-step procedure, including the removal of the PMB group by treatment with 1-chloroethyl chloroformate followed by modification with mesyl chloride (MsCl) or trimethylsilyl isocyanate (TMSNCO) was used for the synthesis of the derivatives **23d** and **23e**, respectively, because the reaction of compound **23a** with MsCl and TMSNCO failed. Deprotection of the *tert*-butyl group in **23b–e** afforded the respective N-substituted derivatives **24b–e**.

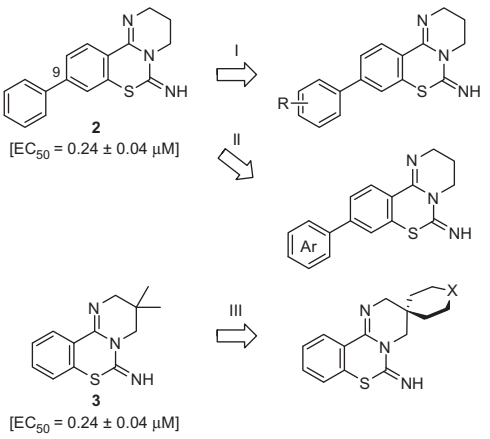
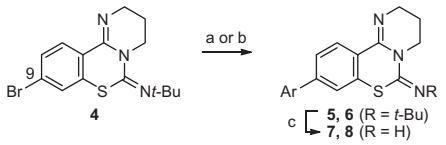
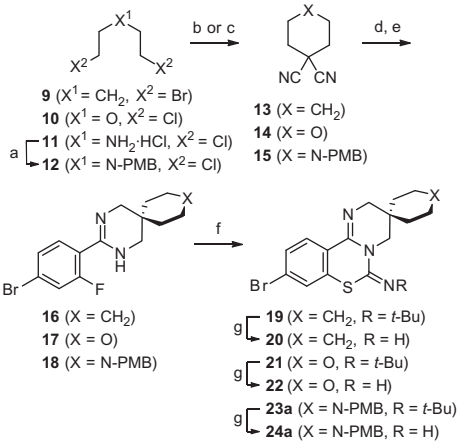


Figure 2. Strategy for the structural optimization of PD 404182 derivatives.



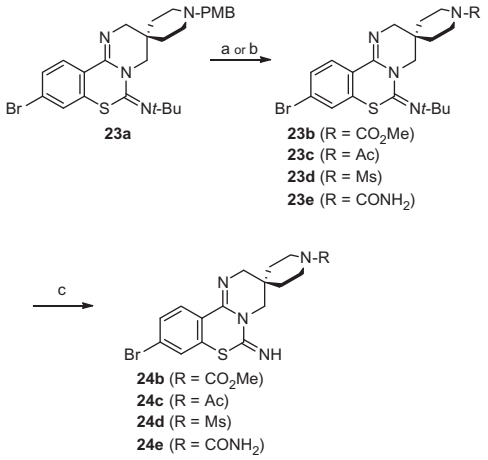
Scheme 1. Synthesis of 9-aryl-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine derivatives. Reagents and conditions: (a) R-B(OH)$_2$ or R-Bpin, Pd(PPh$_3$)$_4$, PdCl$_2$(dppf)·CH$_2$Cl$_2$, K$_2$CO$_3$, toluene or 1,4-dioxane, EtOH, H$_2$O, reflux, 29%-quant.; (b) pyrazole or imidazole, CuCl, K$_2$CO$_3$, acetylacetone, NMP, 130 °C, 51–71% (for **6n** or **6o**); (c) TFA, MS4Å, CHCl$_3$, (MeOH), reflux, 34–94%.



Scheme 2. Synthesis of spiropyrimidine-fused benzothiazinimine derivatives. Reagents and conditions: (a) (i) 4-methoxybenzoyl chloride, Et$_3$N, CH$_2$Cl$_2$, rt; (ii) LiAlH$_4$, Et$_2$O, rt, 75% (2 steps); (b) malononitrile, DBU, DMF, 50 °C, 8–60% (for **13** and **14**); (c) malononitrile, K$_2$CO$_3$, DMF, 65 °C, 85% (for **15**); (d) BH$_3$, THF, 0 °C to rt; (e) 4-bromo-2-fluorobenzaldehyde, I$_2$, K$_2$CO$_3$, *t*-BuOH, 70 °C, 11–62% [2 steps (d,e)]; (f) NaH, *t*-BuNCS DMF, rt −80 °C, 78–94%; (g) TFA, MS4Å, CHCl$_3$, reflux, 66–72%.

2.3. Structure–activity relationships of 9-phenylpyrimido[1,2-*c*][1,3]benzothiazine derivatives

We initially examined substituent effects at the *para*-position of the 9-phenyl group of compound **2** (Table 1). The introduction of methoxycarbonyl (**7a**), cyano (**7b**), nitro (**7c**), and trifluoromethyl (**7d**) groups slightly reduced the anti-HIV activity (EC$_{50}$ = 0.44–0.81 μM), whereas a significant decrease in the anti-HIV activity was observed following the introduction of a carbamoyl group (**7e**) with hydrogen-bond donor/acceptor properties



Scheme 3. Synthesis of derivatives **24b**–**e** from **23a**. Reagents and conditions: (a) $ClCO_2Me$ or AcCl, CH_2Cl_2, 0 °C, 81–96% (for **23b** or **23c**); (b) (i) 1-chloroethyl chloroformate, Et_3N, CH_2Cl_2, 0 °C, then MeOH, reflux, (ii) MsCl or TMSNCO, (Et_3N), CH_2Cl_2, rt, 29–82% (2 steps, for **23d** or **23e**); (c) TFA, MS4Å, $CHCl_3$, reflux, 65–94%.

(EC_{50} = 8.71 μM). Compounds containing a hydrophobic group, including methoxy (**7f**, EC_{50} = 0.24 μM), methylthio (**7g**, EC_{50} = 0.20 μM), and trifluoromethoxy (**7h**, EC_{50} = 0.38 μM) groups showed similar levels of anti-HIV activity to that of compound **2**. These results indicated that the hydrophobic and electron donating properties of these substituents had a positive impacts on improving the anti-HIV activity.

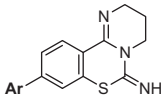
Similar SARs were observed following modifications at the *meta*-position of the 9-phenyl group (Table 1). For example, the addition of the electron-withdrawing methoxycarbonyl (**7i**), cyano (**7j**), and nitro (**7k**) groups resulted in a slight decrease in the anti-HIV activity (EC_{50} = 0.39–1.26 μM), whereas the hydrophilic (1-hydroxy)ethyl (**7l**, EC_{50} = 1.19 μM), acetamido (**7m**, EC_{50} >10 μM), mesylamido (**7n**, EC_{50} >10 μM), and hydroxy (**7o**, EC_{50} = 2.62 μM) groups led to a reduction or loss in the levels of anti-HIV activity. In contrast, the introduction of a methoxy group (**7p**) at the *meta*-position of the 9-phenyl group improved the inhibitory activity (EC_{50} = 0.15 μM). The more hydrophobic isopropoxy group (**7q**) maintained the anti-HIV activity of compound **2** (EC_{50} = 0.32 μM), whereas the introduction of a phenyl group (**7r**) led to a decrease in the inhibitory activity (EC_{50} = 1.35 μM).

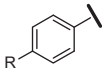
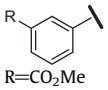
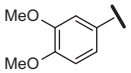
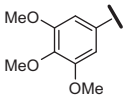
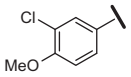
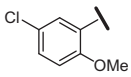
Similar anti-HIV activities to that of compound **2** were also exhibited by the *ortho*-methoxy (**7s**) and *ortho*-phenyl (**7t**) derivatives (EC_{50} = 0.41 and 0.32 μM, respectively), suggesting that the twisted conformation of the biaryl axis in the 9-aryl-modified PD 404182 derivatives can be tolerated and can interact with the target molecule(s).

To develop more potent anti-HIV agents, several compounds were designed with bis- and tris-modifications on the 9-phenyl group of compound **2** (Table 1). The introduction of the 3,4-dimethoxy (**7u**, EC_{50} = 0.27 μM) and 3,4,5-trimethoxy (**7v**, EC_{50} = 0.25 μM) groups did not alter the bioactivity. The Cl-modified derivatives **7w** and **7x** exhibited similar levels of potency to compound **2** (EC_{50} = 0.32 and 0.48 μM, respectively). Taken together, these results suggest that the hydrophobic property of the phenyl substituting group may provide the predominant contribution in any potential interaction with the target molecule(s).

We proceeded to investigate the impact of introducing a bicyclic aromatic group at the 9-position of the pyrimido[1,2-*c*][1,3]benzothiazine scaffold (Table 2). Modifications with a variety of 3,4-fused phenyl groups were investigated because the 2-naphthyl-modified analog (**8a**) exhibited slightly more potent

Table 1
Structure–activity relationships for biphenyl-type derivatives



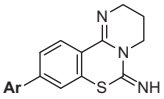
Compound	Ar	EC_{50}[a] (μM)
		
2	R=H	0.24 ± 0.04
7a	R=CO_2Me	0.81 ± 0.29
7b	R=CN	0.44 ± 0.10
7c	R=NO_2	0.46 ± 0.06
7d	R=CF_3	0.55 ± 0.16
7e	R=$CONH_2$	8.71 ± 0.82
7f	R=OMe	0.24 ± 0.04
7g	R=SMe	0.20 ± 0.06
7h	R=OCF_3	0.38 ± 0.06
		
7i	R=CO_2Me	0.39 ± 0.09
7j	R=CN	1.17 ± 0.27
7k	R=NO_2	1.26 ± 0.13
7l	R=$CH(OH)CH_3$	1.19 ± 0.19
7m	R=NHAc	>10
7n	R=NHMs	>10
7o	R=OH	2.62 ± 0.26
7p	R=OMe	0.15 ± 0.05
7q	R=O*i*-Pr	0.32 ± 0.10
7r	R=Ph	1.35 ± 0.26
		
7s	R=OMe	0.41 ± 0.10
7t	R=Ph	0.32 ± 0.12
7u		0.27 ± 0.04
7v		0.25 ± 0.03
7w		0.32 ± 0.04
7x		0.48 ± 0.06

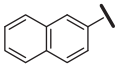
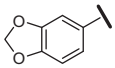
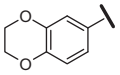
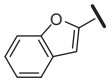
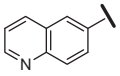
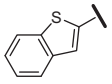
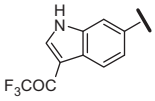
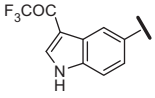
[a] EC_{50} values represent the concentration of compound required to inhibit the HIV-1 infection by 50% and were obtained from three independent experiments.

anti-HIV activity (EC_{50} = 0.20 μM) than that of the 1-naphthyl congener (**8b**, EC_{50} = 0.39 μM). Compound **8c**, which contained a benzo[*d*][1,3]dioxol-5-yl group, displayed inhibitory activity two-fold greater than that of compound **2** (EC_{50} = 0.15 μM), whereas the 2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl derivative **8d** and quinolin-6-yl derivative **8e** exhibited less favorable effects (EC_{50} = 0.26 μM and 0.25 μM, respectively). The introduction of trifluoroacetylindolyl groups (**8f** and **8g**) resulted in no anti-HIV activity, and the compounds also showed unexpected levels of cytotoxicity.

The substitution of the 9-phenyl group with a variety of different heterocyclic substructures was also investigated (Table 2). Pyridine substitution (**8h** and **8i**) led to a slight reduction in the anti-HIV activity (EC_{50} = 0.45 μM and 0.54 μM, respectively), whereas the introduction of a furan (**8j**), benzofuran (**8k**), thiophene (**8l**), benzothiophene (**8m**), and pyrazole (**8n**) was well

Table 2
Structure–activity relationships for biaryl-type derivatives



Compound	Ar	EC_{50}[a] (μM)	Compound	Ar	EC_{50}[a] (μM)
8a		0.20 ± 0.06	**8h**		0.45 ± 0.07
8b		0.39 ± 0.12	**8i**		0.54 ± 0.04
8c		0.15 ± 0.03	**8j**		0.26 ± 0.02
8d		0.26 ± 0.07	**8k**		0.20 ± 0.03
8e		0.25 ± 0.04	**8l**		0.22 ± 0.07
			8m		0.26 ± 0.06
8f		>1.00[b]			
			8n		0.42 ± 0.08
8g		>1.00[b]	**8o**		5.12 ± 1.02

[a] EC_{50} values represent the concentration of compound required to inhibit the HIV-1 infection by 50% and were obtained from three independent experiments.
[b] Cytotoxicity was observed at 10 μM.

tolerated and had little impact on the activity relative to compound **2** (EC_{50} = 0.20–0.42 μM). It is worthy of note that the substitution of the 9-phenyl group with a basic imidazole moiety led to a significant reduction in the anti-HIV (**8o**, EC_{50} = 5.12 μM).

Taken together, these data led to the identification of two highly potent compounds **7p** and **8c** (EC_{50} = 0.15 μM), which contained *m*-methoxyphenyl and benzo[*d*][1,3]dioxol-5-yl groups, respectively. Furthermore, no cytotoxic effects were observed for these derivatives at 10 μM in the MAGI assay.

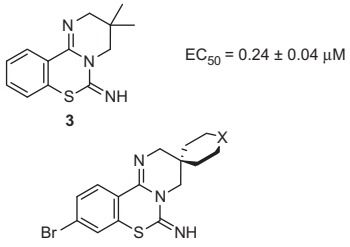
2.4. Structure–activity relationships of spiropyrimidine-fused benzothiazinimine derivatives

Several spiropyrimidine-fused derivatives were designed for the SAR study based on the geminal dimethylpyrimidine substructure **3** (Table 3).[42] Cyclohexane (**20**) and *N*-methoxycarbonylpiperidine (**24b**) derivatives exhibited the similar levels of anti-HIV activity to that of the parent dimethyl derivative **3**. In contrast, the tetrahydropyran (**22**) and *N*-(*p*-methoxybenzyl)piperidine (**24a**) derivatives exerted inhibitory activities that were five–sevenfold lower than that of the parent dimethyl derivative **3**. The *N*-acetyl- (**24c**), *N*-methanesulfonyl- (**24d**), and *N*-carbamoyl- (**24e**) piperidine derivatives also provided reduced levels of antiviral activity. With this in mind, the *N*-alkoxycarbonyl piperidine group was identified as a linkage for the introduction of additional functional group(s) to PD 404182 with potent anti-HIV activity (**24b**).

2.5. Anti-HIV profiles of the most potent derivative 8c

A time-of-drug addition study was carried out to further investigate the anti-HIV profile of the most potent derivative **8c** as an anti-HIV agent (Fig. 3). This assay has been used previously to

Table 3
Structure–activity relationships for spiropyrimidine-fused derivatives



Compound	X	EC_{50}[a] (μM)
20	CH_2	0.25 ± 0.01
22	O	1.73 ± 0.35
24a	N-PMB	1.45 ± 0.05
24b	N-CO_2Me	0.44 ± 0.02
24c	N-Ac	2.74 ± 0.15
24d	N-Ms	1.81 ± 0.43
24e	N-$CONH_2$	>10

[a] EC_{50} values represent the concentration of compound required to inhibit the HIV-1 infection by 50% and were obtained from three independent experiments.

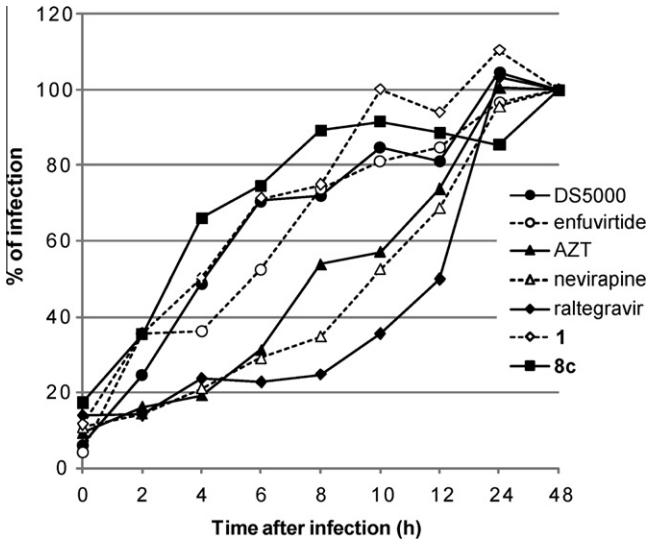


Figure 3. Time of drug addition profiles for infection by HIV-1_{IIIB} strain of HeLa-CD4/CCR5-LTR/β-gal cells.

Table 4
Anti-HIV activity of compounds **1** and **8c** against other HIV strains

Strains	EC_{50}[a] (μM)	
	1[34]	**8c**
HIV-1_{NL4-3}	0.38 ± 0.06	0.23 ± 0.09
HIV-1_{BaL}	0.37 ± 0.06	0.13 ± 0.05
HIV-2_{EHO}	0.31 ± 0.06	0.14 ± 0.02
HIV-2_{ROD}	0.30 ± 0.06	0.10 ± 0.04

[a] EC_{50} values represent the concentration of compound required to inhibit the HIV infection by 50% and were obtained from three independent experiments.

approximately determine which stage in the replication cycle of HIV-1 is inhibited by the compound. Two compounds (**1** and **8c**) were selected for testing in this assay together with five standard anti-HIV agents, including DS5000 (adsorption inhibitor),[31] enfuvirtide (fusion inhibitor),[6,7] AZT (NRTI),[43] nevirapine (NNRTI),[44,45] and raltegravir (integrase inhibitor).[8,9] The results revealed that the infection profile in the presence of compound **8c** was similar to that of DS5000 and enfuvirtide, suggesting that **8c** exerted its anti-HIV activity at the early stages of the viral infection, including the binding and fusion stage. This was similar to PD 404182, indicating that the bioactivity profile was not influenced by the newly appended functional group(s).

We also evaluated the antiviral activity of compounds **1** and **8c** against several HIV strains such as HIV-1_{NL4-3}, HIV-1_{BaL}, HIV-2_{EHO}, and HIV-2_{ROD}. This study enabled us to estimate the impact of the target molecules on the process of binding and fusion because these viruses have different susceptibilities[46] to different anti-HIV agents. These results implied that compounds **1** and **8c** exhibited their anti-HIV activity through different mechanisms from those of the known binding and fusion inhibitors including CCR5 antagonists, CXCR4 antagonists, and enfuvirtide. In addition, compound **8c** was two–threefold more effective against these HIV-1 and HIV-2 strains than PD 404182 (Table 4).

3. Conclusions

In conclusion, we have designed and synthesized a series of PD 404182 derivatives for the development of novel anti-HIV agents. The structural optimization study on the 9-position of pyrimido[1,2-*c*][1,3]benzothiazinimine identified two potent derivatives containing *m*-methoxyphenyl (**7p**) and benzo[*d*][1,3]dioxol-5-yl groups (**8c**) that exhibited threefold higher anti-HIV activity than that of PD 404182 (**1**). The common hydrophobic biaryl moiety is effective to improve the antiviral activity, providing potential interaction with the target molecule(s). In addition, we demonstrated that the most effective derivative, **8c**, inhibited viral infection against all of the HIV strains examined and acted at the early stage of the HIV infection. The design and synthesis of chemical probes based on these SAR data are being investigated to identify the target molecule(s).

4. Experimental

4.1. Synthesis

4.1.1. General methods

^{1}H NMR spectra were recorded using a JEOL AL-400 or a JEOL ECA-500 spectrometer. Chemical shifts are reported in δ (ppm) relative to Me_4Si ($CDCl_3$) or DMSO (DMSO-d_6) as internal standards. ^{13}C NMR spectra were referenced to the residual solvent signal. Exact mass (HRMS) spectra were recorded on a JMS-HX/HX 110A mass spectrometer. Melting points were measured by a hot stage melting point apparatus (uncorrected). For flash chromatography, Wakogel C-300E (Wako) or aluminium oxide 90 standardized (Merck) were employed. For preparative TLC, TLC silica gel 60 F_{254} (Merck) or TLC aluminium oxide 60 F_{254} basic (Merck) were employed. For analytical HPLC, a Cosmosil 5C18-ARII column (4.6 × 250 mm, Nacalai Tesque, Inc., Kyoto, Japan) was employed with method A [a linear gradient of CH_3CN containing 0.1% (v/v) TFA] or method B [a linear gradient of CH_3CN containing 0.1% (v/v) NH_3] at a flow rate of 1 mL/min on a Shimadzu LC-10ADvp (Shimadzu Corp., Ltd., Kyoto, Japan), and eluting products were detected by UV at 254 nm. The purity of the compounds was determined by combustion analysis or HPLC analysis as >95%.

4.1.2. General procedure of Suzuki–Miyaura cross coupling for 9-aryl pyrimido[1,2-*c*][1,3]thiazine derivatives 5 and 6: *N*-(*tert*-butyl)-3,4-dihydro-9-(4-methoxycarbonylphenyl)-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine 5a

To a solution of bromide **4** (52.8 mg, 0.15 mmol) and 4-(methoxycarbonyl)phenylboronic acid (32.4 mg, 0.18 mmol) in a mixture of toluene (1.5 mL), EtOH (0.9 mL) and 1 M aq K_2CO_3 (1.5 mL) was added $Pd(PPh_3)_4$ (6.9 mg, 4 mol %) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (3.7 mg, 3 mol %). After being stirred under reflux for 1 h, the mixture was extracted with $CHCl_3$. The organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography over aluminum oxide with *n*-hexane/EtOAc (10:0/9:1) to give the compound **5a** as colorless solid (47.3 mg, 77%): mp 201–202 °C (from $CHCl_3$–*n*-hexane); IR (neat) cm^{-1}: 1719 (C=O), 1593 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.40 (s, 9H, 3 × CH_3), 1.90–1.96 (m, 2H, CH_2), 3.65 (t, J = 5.5 Hz, 2H, CH_2), 3.89 (t, J = 6.1 Hz, 2H, CH_2), 3.94 (s, 3H, CH_3), 7.36 (d, J = 1.7 Hz, 1H, Ar), 7.44 (dd, J = 8.5, 1.7 Hz, 1H, Ar), 7.65 (d, J = 8.2 Hz, 2H, Ar), 8.10 (d, J = 8.2 Hz, 2H, Ar), 8.28 (d, J = 8.5 Hz, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.9, 30.0 (3C), 45.2, 45.4, 52.1, 54.2, 123.0, 124.8, 127.0 (2C), 127.3, 129.1, 129.6, 129.8, 130.2 (2C), 138.0, 141.7, 143.8, 147.5, 166.8; HRMS (FAB): *m*/*z* calcd for $C_{23}H_{26}N_3O_2S$ $[M+H]^+$ 408.1746; found: 408.1748.

4.1.3. *N*-(*tert*-Butyl)-3,4-dihydro-9-(1*H*-pyrazol-1-yl)-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (6n)

To a solution of bromide **4** (52.8 mg, 0.15 mmol), pyrazole (12.3 mg, 0.18 mmol), CuCl (1.5 mg, 0.015 mmol) and K_2CO_3 (21.8 mg, 0.16 mol) in *N*-methylpyrrolidone (0.3 mL) was added

acetylacetone (3.8 μL, 0.038 mmol) under an Ar atmosphere. After being stirred at 130 °C for 19 h, EtOAc and brine were added. The organic layers were washed with H_2O, and dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over aluminum oxide with *n*-hexane/EtOAc (7:3) to give the title compound **6n** as colorless solid (39.8 mg, 71%): mp 132–133 °C (from $CHCl_3$–*n*-hexane); IR (neat) cm^{-1}: 1597 (C=N); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.39 (s, 9H, 3 × CH_3), 1.90–1.96 (m, 2H, CH_2), 3.63 (t, J = 5.6 Hz, 2H, CH_2), 3.88 (t, J = 6.2 Hz, 2H, CH_2), 6.48 (dd, J = 2.7, 1.8 Hz, 1H, Ar), 7.47 (dd, J = 8.8, 2.2 Hz, 1H, Ar), 7.56 (d, J = 2.2 Hz, 1H, Ar), 7.73 (d, J = 1.8 Hz, 1H, Ar), 7.94 (d, J = 2.7 Hz, 1H, Ar), 8.28 (d, J = 8.8 Hz, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.8, 30.0 (3C), 45.0, 45.4, 54.2, 108.2, 114.3, 115.9, 125.4, 126.7, 129.9, 130.8, 137.7, 141.0, 141.7, 147.3; HRMS (FAB): *m*/*z* calcd for $C_{18}H_{22}N_5S$ $[M+H]^+$ 340.1596; found: 340.1598.

4.1.4. General procedure of *tert*-butyl deprotection for pyrimido[1,2-*c*][1,3]benzothiazin-6-imines (7, 8, 20, 22, and 24): 3,4-dihydro-9-(4-methoxycarbonylphenyl)-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (7a)

TFA (2.0 mL) was added to a mixture of *N*-(*tert*-butyl)-protected pyrimido[1,2-*c*][1,3]benzothiazin-6-imine **5a** (38.4 mg, 0.094 mmol) in small amount of $CHCl_3$ and MS4Å (300 mg, powder, activated by heating with Bunsen burner). After being stirred under reflux for 1 h, the mixture was concentrated. To a stirring mixture of the residue in $CHCl_3$ was added dropwise Et_3N at 0 °C to adjust pH to 8–9. The whole was extracted with EtOAc. The extract was washed with sat. $NaHCO_3$, brine, and dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over aluminum oxide with *n*-hexane/EtOAc (9:1/1:1) to give the title compound **7a** as colorless solid (27.3 mg, 83%): mp 185–186 °C (from $CHCl_3$–*n*-hexane); IR (neat) cm^{-1}: 1719 (C=O), 1619 (C=N), 1566 (C=N); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.97–2.02 (m, 2H, CH_2), 3.71 (t, J = 5.7 Hz, 2H, CH_2), 3.94 (s, 3H, CH_3), 4.04 (t, J = 6.0 Hz, 2H, CH_2), 7.27 (d, J = 1.7 Hz, 1H, Ar), 7.46 (dd, J = 8.0, 1.7 Hz, 1H, Ar), 7.63 (d, J = 8.6 Hz, 2H, Ar), 8.10 (d, J = 8.6 Hz, 2H, Ar), 8.30 (d, J = 8.0 Hz, 1H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 21.0, 43.8, 45.0, 52.2, 122.0, 125.1, 126.2, 126.9 (2C), 129.5, 129.6, 129.7, 130.2 (2C), 142.1, 143.4, 146.2, 153.0, 166.7; HRMS (FAB): *m*/*z* calcd for $C_{19}H_{18}N_3O_2S$ $[M+H]^+$ 352.1120; found: 352.1119

4.1.5. Bis(2-chloroethyl)-*N*-(4-methoxybenzyl)amine (12)

To a suspension of bis(2-chloroethyl)amine hydrochloride **11** (8.92 g, 50.0 mmol) in CH_2Cl_2 (300 mL) were added Et_3N (2.89 mL, 100.0 mmol) and 4-methoxybenzoyl chloride (6.77 mL, 50.0 mmol). After being stirred at rt for 2 h, the reaction mixture was washed with 1 N HCl, satd $NaHCO_3$, brine, and dried over $MgSO_4$. After concentration, the residue was dissolved in anhydrous Et_2O (250 mL) and $LiAlH_4$ (2.1 g, 55.0 mmol) was slowly added at 0 °C under an Ar atmosphere. After being stirred at rt overnight, the reaction mixture was quenched by addition of water, 2 N NaOH, and water. The mixture was dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over silica gel with *n*-hexane/EtOAc (19:1) to give the title compound **12** as colorless oil (9.88 g, 75%): ^1H NMR (400 MHz, $CDCl_3$) δ: 2.90 (t, J = 7.1 Hz, 4H, 2 × CH_2), 3.48 (t, J = 7.1 Hz, 4H, 2 × CH_2), 3.67 (s, 2H, CH_2), 3.80 (s, 3H, CH_3), 6.86 (d, J = 8.5 Hz, 2H, Ar), 7.24 (d, J = 8.5 Hz, 2H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 42.0 (2C), 55.2, 56.2 (2C), 58.6, 113.8 (2C), 129.7 (2C), 130.7, 158.9; LRMS (FAB): *m*/*z* $[M+H]^+$ 262.

4.1.6. 1-(4-Methoxybenzyl)piperidine-4,4-dicarbonitrile (15)

To a solution of malononitrile (2.49 g, 37.7 mmol) in DMF (94.3 mL) was added K_2CO_3 (5.73 mg, 41.5 mmol). After being stirred at 65 °C for 2 h, a solution of chloride **12** (9.88 mg, 37.7 mmol) in DMF (37.7 mL) was added. After being stirred at same temperature for 5 h, EtOAc was added. The mixture was washed with 5% aq $NaHCO_3$, and dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over silica gel with *n*-hexane/EtOAc (2:1) to give the title compound **15** as yellow oil (8.13 g, 85%): IR (neat) cm^{-1}: 2248 (C≡N); ^1H NMR (400 MHz, $CDCl_3$) δ: 2.22 (t, J = 5.4 Hz, 4H, 2 × CH_2), 2.61 (br s, 4H, 2 × CH_2), 3.48 (s, 2H, CH_2), 3.80 (s, 3H, CH_2), 6.86 (d, J = 8.5 Hz, 2H, Ar), 7.19 (d, J = 8.8 Hz, 2H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 31.1, 34.1 (2C), 48.5 (2C), 55.2, 61.9, 113.8 (2C), 115.4 (2C), 129.2, 130.1 (2C), 159.0; HRMS (FAB): *m*/*z* calcd for $C_{15}H_{18}N_3O$ $[M+H]^+$ 256.1450; found: 256.1454.

4.1.7. 3-(4-Bromo-2-fluorophenyl)-9-(4-methoxybenzyl)-2,4,9-triazaspiro[5.5]undec-2-ene (18)

To a solution of nitrile **15** (4.05 g, 15.9 mmol) in THF (39.8 mL) was added BH_3 in THF (79.5 mL, 79.5 mmol, 1.0 M) at 0 °C under an Ar atmosphere. The mixture was warmed to rt. After being stirred at 65 °C for 5 h, the reaction mixture was cooled to 0 °C, and 1 N HCl was added. After being stirred at rt for 1 h, the mixture was basified with 2 N NaOH. The whole was extracted with $CHCl_3$ and dried over $MgSO_4$. After concentration, the residue was dissolved in *t*-BuOH (159.0 mL) and 4-bromo-2-fluorobenzaldehyde (3.23 g, 15.9 mmol) was added. After being stirred at 70 °C for 30 min, K_2CO_3 (6.59 g, 47.7 mmol) and I_2 (5.05 g, 19.9 mmol) were added. After being stirred at same temperature for 3 h, the reaction mixture was quenched with sat. Na_2SO_3 until the iodine color almost disappeared. The reaction mixture was basified with 2 N NaOH. The whole was extracted with $CHCl_3$, and dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over aluminium oxide with EtOAc–MeOH (10:0/95:5) to give the title compound **18** as colorless solid (752 mg, 11%): mp 179–181 °C (from $CHCl_3$–*n*-hexane), IR (neat) cm^{-1}: 1630 (C=N); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.45 (t, J = 5.4 Hz, 4H, 2 × CH_2), 2.35 (t, J = 5.4 Hz, 4H, 2 × CH_2), 3.16 (s, 4H, 2 × CH_2), 3.40 (s, 2H, CH_2), 3.73 (s, 3H, CH_3), 4.63 (s, 1H, NH), 6.78 (d, J = 8.6 Hz, 2H, Ar), 7.14–7.23 (m, 4H, Ar), 7.62 (t, J = 8.3 Hz, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.3, 32.8 (2C), 49.1 (2C), 51.4 (2C), 55.2, 62.7, 113.5 (2C), 119.4 (d, J = 27.3 Hz), 122.7 (d, J = 12.4 Hz), 123.7 (d, J = 9.9 Hz), 127.8 (d, J = 3.3 Hz), 130.2, 130.3 (2C), 131.7 (d, J = 4.1 Hz), 150.3 (d, J = 1.7 Hz), 158.6, 159.7 (d, J = 251.6 Hz); ^{19}F NMR (500 MHz, $CDCl_3$) δ: −114.6. HRMS (FAB): *m*/*z* calcd for $C_{22}H_{26}BrFN_3O$ $[M+H]^+$ 446.1243; found: 446.1237.

4.1.8. General procedure for *t*-BuNCS mediated cyclization: 9-bromo-*N*-(*tert*-butyl)-1′-(4-methoxybenzyl)-2*H*-spiro[benzo[*e*]pyrimido[1,2-*c*][1,3]thiazine-3,4'-piperidin]-6(4*H*)-imine (23a)

To a mixture of fluoride **18** (2.0 g, 4.48 mmol) and NaH (358.4 mg, 8.96 mmol; 60% oil suspension) in DMF (14.8 mL) was added *t*-BuNCS (1.14 mL, 8.96 mmol) under an Ar atmosphere. After being stirred at rt overnight, the reaction mixture was warmed to 60 °C. After being stirred at this temperature for 1 h, EtOAc was added. The resulting solution was washed with sat. $NaHCO_3$, brine, and dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over aluminum oxide with *n*-hexane/EtOAc (10:0/9:1) to give the title compound **23a** as colorless solid (2.28 g, 94%): mp 89–91 °C (from $CHCl_3$–*n*-hexane); IR (neat) cm^{-1}: 1577 (C=N); ^1H NMR (500 MHz, $CDCl_3$) δ: 1.37 (s, 9H, 3 × CH_3), 1.49–1.52 (m, 4H, 2 × CH_2), 2.40–2.46 (m, 4H, 2 × CH_2), 3.41 (s, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.75 (s, 2H, CH_2), 3.80 (s, 3H, CH_3), 6.85 (d, J = 8.6 Hz, 2H, Ar), 7.22 (d, J = 8.6 Hz, 2H, Ar), 7.28–7.31 (m, 2H, Ar), 8.03 (d, J = 8.6 Hz, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.7, 29.9 (3C), 32.6 (2C), 49.2 (2C), 51.6, 54.3, 55.2, 55.5, 62.7, 113.6 (2C), 124.5, 126.3, 126.8, 129.2, 130.0, 130.1, 130.4 (2C), 130.9, 137.5, 146.3, 158.7; HRMS

(FAB): *m*/*z* calcd for $C_{27}H_{34}BrN_4OS$ $[M+H]^+$ 541.1637; found: 541.1633.

4.1.9. 9-Bromo-*N*-(*tert*-butyl)-1′-(methoxycarbonyl)-2*H*-spiro[benzo[*e*]pyrimido[1,2-*c*][1,3]thiazine-3,4'-piperidin]-6(4*H*)-imine (23b)

To the solution of *N*-(4-methoxybenzyl)piperidine **23a** (40.6 mg, 0.075 mmol) in CH_2Cl_2 (0.38 mL) was added methyl chloroformate (86.4 μL, 1.13 mmol) at 0 °C under an Ar atmosphere. After being stirred at same temperature for 30 min, the reaction mixture was concentrated. The residue was purified by flash chromatography over silica gel with *n*-hexane/EtOAc (1:1) to give compound **23b** as a colorless solid (29.2 mg, 81%): mp 157–158 °C (from *n*-hexane); IR (neat) cm^{-1}: 1699 (C=O), 1577 (C=N); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.37 (s, 9H, 3 × CH_3), 1.46 (t, J = 5.6 Hz, 4H, 2 × CH_2), 3.44 (br s, 4H, 2 × CH_2), 3.56 (br s, 2H, CH_2), 3.70 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 7.29–7.33 (m, 2H, Ar), 8.05 (d, J = 8.5 Hz, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.9 (3C), 30.1, 32.2 (2C), 39.9 (2C), 50.8, 52.5, 54.3, 55.2, 124.7, 126.1, 126.8, 129.3, 130.0, 130.9, 137.7, 146.3, 155.9; HRMS (FAB): *m*/*z* calcd for $C_{21}H_{28}BrN_4O_2S$ $[M+H]^+$ 479.1116; found: 479.1115.

4.1.10. 9-Bromo-*N*-(*tert*-butyl)-1′-(methanesulfonyl)-2*H*-spiro[benzo[*e*]pyrimido[1,2-*c*][1,3]thiazine-3,4'-piperidin]-6(4*H*)-imine (23d)

To the solution of *N*-(4-methoxybenzyl)piperidine **23a** (54.2 mg, 0.10 mmol) in CH_2Cl_2 (0.5 mL) were added Et_3N (28.9 μL, 0.20 mmol) and 1-chloroethyl chloroformate (21.8 μL, 0.20 mmol) at 0 °C under an Ar atmosphere. After being stirred at same temperature for 30 min, the reaction mixture was concentrated. The residue was dissolved in MeOH (2.0 mL). After being stirred under reflux for 10 min, the reaction mixture was concentrated. The residue was dissolved in $CHCl_3$, and was washed with sat. $NaHCO_3$, brine, and dried over $MgSO_4$. After concentration, the residue was dissolved in CH_2Cl_2 (1.0 mL) and Et_3N (28.9 μL, 0.20 mmol) and methanesulfonyl chloride (15.5 μL, 0.20 mmol) was added at rt under an Ar atmosphere. After being stirred at rt for 10 min, the reaction mixture was washed with sat. $NaHCO_3$, brine, and dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over aluminum oxide with *n*-hexane/EtOAc (6:4) to give compound **23d** as a colorless solid (40.9 mg, 82%): mp 177 °C (from $CHCl_3$–*n*-hexane); IR (neat) cm^{-1}: 1577 (C=N), 1331 (NSO_2), 1155 (NSO_2); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.38 (s, 9H, 3 × CH_3), 1.62 (t, J = 5.5 Hz, 4H, 2 × CH_2), 2.80 (s, 3H, CH_3), 3.21–3.27 (m, 2H, CH_2), 3.31–3.37 (m, 2H, CH_2), 3.46 (s, 2H, CH_2), 3.84 (s, 2H, CH_2), 7.29–7.33 (m, 2H, Ar), 8.05 (d, J = 8.5 Hz, 1H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.8, 29.9 (3C), 32.0 (2C), 34.7, 42.0 (2C), 50.1, 54.4, 55.1, 124.8, 125.9, 126.9, 129.4, 130.0, 130.8, 137.9, 146.3; HRMS (FAB): *m*/*z* calcd for $C_{20}H_{28}BrN_4O_2S_2$ $[M+H]^+$ 499.0837; found: 499.0840.

4.2. Determination of anti-HIV activity

The sensitivity of three HIV-1 strains and two HIV-2 strains was determined by the MAGI assay. The target cells (HeLa-CD4/CCR5-LTR/β-gal; 10^4 cells/well) were plated in 96-well flat microtiter culture plates. On the following day, the cells were inoculated with the HIV-1 (60 MAGI U/well, giving 60 blue cells after 48 h of incubation) and cultured in the presence of various concentrations of the test compounds in fresh medium. Forty-eight hours after viral exposure, all the blue cells stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) were counted in each well. The activity of test compounds was determined as the concentration that blocked HIV-1 infection by 50% (50% effective concentration [EC_{50}]). EC_{50} was determined by using the following formula:

$$EC_{50} = 10^{\wedge}[\log(A/B) \times (50 - C)/(D - C) + \log(B)], \quad (1)$$

wherein

A: of the two points on the graph which bracket 50% inhibition, the higher concentration of the test compound,
B: of the two points on the graph which bracket 50% inhibition, the lower concentration of the test compound,
C: inhibitory activity (%) at the concentration B,
D: inhibitory activity (%) at the concentration A.

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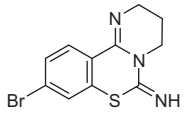
Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.030.

References and notes

1. Thompson, M. A.; Aberg, J. A.; Cahn, P.; Montaner, J. S. G.; Rizzardini, G.; Telenti, A.; Gatell, J. M.; Günthard, H. F.; Hammer, S. M.; Hirsch, M. S.; Jacobsen, D. M.; Reiss, P.; Richman, D. D.; Volberding, P. A.; Yeni, P.; Schooley, R. T. *J. Am. Med. Assoc.* **2010**, *304*, 321.
2. Rathbun, R. C.; Lockhart, S. M.; Stephens, J. R. *Curr. Pharm. Des.* **2006**, *12*, 1045.
3. Johnson, V. A.; Calvez, V.; Günthard, H. F.; Paredes, R.; Pillay, D.; Shafer, R.; Weinsing, A. M.; Richman, D. D. *Top. Antiviral Med.* **2011**, *19*, 156.
4. Carr, A.; Cooper, D. A. *Lancet* **2000**, *356*, 1423.
5. Kilby, J. M.; Eron, J. J. *N. Engl. J. Med.* **2003**, *348*, 2228.
6. Lalezari, J. P.; Henry, K.; O'Hearn, M.; Montaner, J. S.; Piliero, P. J.; Trottier, B.; Walmsley, S.; Cohen, C.; Kuritzkes, D. R.; Eron, J. J., Jr.; Chung, J.; DeMasi, R.; Donatacci, L.; Drobnes, C.; Delehanty, J.; Salgo, M. *N. Engl. J. Med.* **2003**, *348*, 2175.
7. Matthews, T.; Salgo, M.; Greenberg, M.; Chung, J.; DeMasi, R.; Bolognesi, D. *Nat. Rev. Drug Disc.* **2004**, *3*, 215.
8. Grinsztejn, B.; Nguyen, B.-Y.; Katlama, C.; Gatell, J. M.; Lazzarin, A.; Vittecoq, D.; Gonzalez, C. J.; Chen, J.; Harvey, C. M.; Isaacs, R. D. *Lancet* **2007**, *369*, 1261.
9. Steigbigel, R. T.; Cooper, D. A.; Kumar, P. N.; Eron, J. E.; Schechter, M.; Markowitz, M.; Loutfy, M. R.; Lennox, J. L.; Gatell, J. M.; Rockstroh, J. K.; Katlama, C.; Yeni, P.; Lazzarin, A.; Clotet, B.; Zhao, J.; Chen, J.; Ryan, D. M.; Rhodes, R. R.; Killar, J. A.; Gilde, L. R.; Strohmaier, K. M.; Meibohm, A. R.; Miller, M. D.; Hazuda, D. J.; Nessly, M. L.; DiNubile, M. J.; Isaacs, R. D.; Nguyen, B.-Y.; Teppler, H. *N. Engl. J. Med.* **2008**, *359*, 339.
10. Dorr, P.; Westby, M.; Dobbs, S.; Griffin, P.; Irvine, B.; Macartney, M.; Mori, J.; Rickett, G.; Smith-Burchnell, C.; Napier, C.; Webster, R.; Armour, D.; Price, D.; Stammen, B.; Wood, A.; Perros, M. *Antimicrob. Agents Chemother.* **2005**, *49*, 4721.
11. Fätkenheuer, G.; Pozniak, A. L.; Johnson, M. A.; Plettenberg, A.; Staszewski, S.; Hoepelman, A. I. M.; Saag, M. S.; Goebel, F. D.; Rockstroh, J. K.; Dezube, B. J.; Jenkins, T. M.; Medhurst, C.; Sullivan, J. F.; Ridgway, C.; Abel, S.; James, I. T.; Youle, M.; VanDerRyst, E. *Nat. Med.* **2005**, *11*, 1170–1172.
12. Bridger, G. J.; Skerlj, R. T.; Thornton, D.; Padmanabhan, S.; Martellucci, S. A.; Henson, G. W.; Abrams, M. J.; Yamamoto, N.; De Vreese, K.; Pauwels, R.; De Clercq, E. *J. Med. Chem.* **1995**, *38*, 366.
13. Bridger, G. J.; Skerlj, R. T.; Hernandez-Abad, P. E.; Bogucki, D. E.; Wang, Z.; Zhou, Y.; Nan, S.; Boehringer, E. M.; Wilson, T.; Crawford, J.; Metz, M.; Hatse, S.; Princen, K.; De Clercq, E.; Schols, D. *J. Med. Chem.* **2010**, *53*, 1250.
14. Fujii, N.; Oishi, S.; Hiramatsu, K.; Araki, T.; Ueda, S.; Tamamura, H.; Otaka, A.; Kusano, S.; Terakubo, S.; Nakashima, H.; Broach, J. A.; Trent, J. O.; Wang, Z.-X.; Peiper, S. C. *Angew. Chem., Int. Ed.* **2003**, *42*, 3251.
15. Ueda, S.; Oishi, S.; Wang, Z.-X.; Araki, T.; Tamamura, H.; Cluzeau, J.; Ohno, H.; Kusano, S.; Nakashima, H.; Trent, J. O.; Peiper, S. C.; Fujii, N. *J. Med. Chem.* **2007**, *50*, 192.
16. Inokuchi, E.; Oishi, S.; Kubo, T.; Ohno, H.; Shimura, K.; Matsuoka, M.; Fujii, N. *ACS Med. Chem. Lett.* **2011**, *2*, 477.
17. Zhao, Q.; Ma, L.; Jiang, S.; Lu, H.; Liu, S.; He, Y.; Strick, N.; Neamati, N.; Debnath, A. K. *Virology* **2005**, *339*, 213.

18. Lalonde, J. M.; Kwon, Y. D.; Jones, D. M.; Sun, A. W.; Courter, J. R.; Soeta, T.; Kobayashi, T.; Princiotto, A. M.; Wu, X.; Schön, A.; Freire, E.; Kwong, P. D.; Mascola, J. R.; Sodroski, J.; Madani, N.; Smith, A. B. *J. Med. Chem.* **2012**, *55*, 4382.
19. Curreli, F.; Choudhury, S.; Pyatkin, I.; Zagorodnikov, V. P.; Bulay, A. K.; Altieri, A.; Kwon, Y. D.; Kwong, P. D.; Debnath, A. K. *J. Med. Chem.* **2012**, *55*, 4764.
20. Wang, T.; Yin, Z.; Zhang, Z.; Bender, J. A.; Yang, Z.; Johnson, G.; Yang, Z.; Zadjura, L. M.; D'Arienzo, C. J.; Parker, D. D.; Gesenberg, C.; Yamanaka, G. A.; Gong, Y.-F.; Ho, H.-T.; Fang, H.; Zhou, N.; McAuliffe, B. V.; Eggers, B. J.; Fan, L.; Nowicka-Sans, B.; Dicker, I. B.; Gao, Q.; Colonno, R. J.; Lin, P.-F.; Meanwell, N. A.; Kadow, J. F. *J. Med. Chem.* **2009**, *52*, 7778.
21. Otaka, A.; Nakamura, M.; Nameki, D.; Kodama, E.; Uchiyama, S.; Nakamura, S.; Nakano, H.; Tamamura, H.; Kobayashi, Y.; Matsuoka, M.; Fujii, N. *Angew. Chem., Int. Ed.* **2002**, *41*, 2938.
22. Oishi, S.; Ito, S.; Nishikawa, H.; Watanabe, K.; Tanaka, M.; Ohno, H.; Izumi, K.; Sakagami, Y.; Kodama, E.; Matsuoka, M.; Fujii, N. *J. Med. Chem.* **2008**, *51*, 388.
23. Oishi, S.; Watanabe, K.; Ito, S.; Tanaka, M.; Nishikawa, H.; Ohno, H.; Shimane, K.; Izumi, K.; Sakagami, Y.; Kodama, E. N.; Matsuoka, M.; Asai, A.; Fujii, N. *Med. Chem. Commun.* **2010**, *1*, 276.
24. Katritzky, A. R.; Tala, S. R.; Lu, H.; Vakulenko, A. V.; Chen, Q.-Y.; Sivapackiam, J.; Pandya, K.; Jiang, S.; Debnath, A. K. *J. Med. Chem.* **2009**, *52*, 7631.
25. Jiang, S.; Tala, S. R.; Lu, H.; Abo-Dya, N. E.; Avan, I.; Gyanda, K.; Lu, L.; Katritzky, A. R.; Debnath, A. K. *J. Med. Chem.* **2011**, *54*, 572.
26. Zhou, G.; Wu, D.; Snyder, B.; Ptak, R. G.; Kaur, H.; Gochin, M. *J. Med. Chem.* **2011**, *54*, 7220.
27. Birck, M. R.; Holler, T. P.; Woodard, R. W. *J. Am. Chem. Soc.* **2000**, *122*, 9334.
28. Duckworth, B. P.; Aldrich, C. C. *Anal. Biochem.* **2010**, *403*, 13.
29. Foley, T. L.; Yasgar, A.; Garcia, C. J.; Jadhav, A.; Simeonov, A.; Burkart, M. D. *Org. Biomol. Chem.* **2010**, *8*, 4601.
30. Watanabe, K.; Negi, S.; Sugiura, Y.; Kiriyama, A.; Honbo, A.; Iga, K.; Kodama, E. N.; Naitoh, T.; Matsuoka, M.; Kano, K. *Chem. Asian J.* **2010**, *5*, 825.
31. Baba, M.; Scgols, D.; Pauwels, R.; Nakashima, H.; De Clercq, E. *J. Acquir. Immune. Defic. Syndr.* **1990**, *3*, 493.
32. Chockalingam, K.; Simeon, R. L.; Rice, C. M.; Chen, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 3764.
33. Chamoun, A. M.; Chockalingam, K.; Bobardt, M.; Simeon, R.; Chang, J.; Gallay, P.; Chen, Z. *Antimicrob. Agents Chemother.* **2012**, *56*, 672.
34. Mizuhara, T.; Oishi, S.; Ohno, H.; Shimura, K.; Matsuoka, M.; Fujii, N. *Org. Biomol. Chem.* **2012**, *10*, 6792.
35. Mizuhara, T.; Inuki, S.; Oishi, S.; Fujii, N.; Ohno, H. *Chem. Commun.* **2009**, 3413.
36. Mizuhara, T.; Oishi, S.; Fujii, N.; Ohno, H. *J. Org. Chem.* **2010**, *75*, 265.
37. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
38. Suzuki, A. *J. Organomet. Chem.* **1999**, *576*, 147.
39. Mitschb, A.; Altenkämpera, M.; Sattlerc, I.; Schlitzer, M. *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 9.
40. Kunz, K.; Scholz, U.; Ganzer, D. *Synlett* **2003**, 2428.
41. Ishihara, M.; Togo, H. *Tetrahedron* **2007**, *63*, 1474.
42. Because a 9-brominated derivative **25** exhibited comparable anti-HIV activity with compound **2** in our previous SAR study,[34] we employed compound **25** as a lead.



25

EC_{50} = 0.25 ± 0.09 μM

43. Fischl, M. A.; Richman, D. D.; Grieco, M. H. *N. Engl. J. Med.* **1987**, *317*, 185.
44. Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C.-K.; Eckner, K.; Hattox, S.; Adams, J.; Rosehthal, A. S.; Faanes, R.; Eckner, R. J.; Koup, R. A.; Sullivan, J. L. *Science* **1990**, *250*, 1411.
45. Skoog, M.; Hargrave, K. D.; Miglietta, J. J.; Kopp, E. B.; Merluzzi, V. J. *Med. Res. Rev.* **1992**, *12*, 27.
46. Witvrouw, M.; Pannecouque, C.; Switzer, W. M.; Folks, T. M.; De Clercq, E.; Heneine, W. *Antiviral Ther.* **2004**, *9*, 57.