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Identification of anti-HIV agents with a novel benzo[4,5]isothiazolo[2,3-*a*]pyrimidine scaffold

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ABSTRACT

3,4-Dihydro-2*H*-benzo[4,5]isothiazolo[2,3-*a*]pyrimidine is a newly identified antiviral agent against human immunodeficiency virus type 1 (HIV-1) infection, derived from 3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (PD 404182). The introduction of the hydrophobic 8-aryl substituent on the benzene substructure improved its anti-HIV activity, resulting in the identification of 6-fold more potent analogs. In addition, it was demonstrated that these isothiazolopyrimidine derivatives exert anti-HIV effects at an early stage of viral infection.

Keywords: anti-HIV agent, isothiazolopyrimidine, PD 404182, pyrimidobenzothiazine

Abbreviations: CCR5, CC chemokine receptor type 5; CXCR4, CXC chemokine receptor type 4; MAGI, multinuclear activation of a galactosidase indicator; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; INSTIs, integrase strand transfer inhibitors; PIDA, phenyliodine diacetate.

1. Introduction

A variety of anti-HIV drugs targeted to each stage of the infection have been developed for the treatment of human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS).¹ In particular, highly active antiretroviral therapy (HAART) by co-administration of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and integrase strand transfer inhibitors (INSTIs) is a standard treatment regimen for HIV-infected patients.² The HAART regimen strongly suppresses viral proliferation and has provided significant decline of morbidity and mortality,³ but a complete cure or eradication of HIV has not yet been achieved. Long-term administration of multiple antiretroviral agents causes the emergence of drug-resistant HIV variants and drug-related adverse effects,^{4,5} resulting in increased risk of virologic failure and/or limitation in the treatment strategy.² To overcome these problems, novel antiretroviral drugs with new mechanisms of action are now desired. Recently, a series of anti-HIV agents that inhibit an early stage of HIV infection are being explored, including the fusion inhibitor enfuvirtide,⁶ a CC chemokine receptor type 5 (CCR5) antagonist (maraviroc),⁷ CXC chemokine receptor type 4 (CXCR4) antagonists,^{8,9} and CD4 mimics.¹⁰

Previously, we and others reported 3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (**1**, PD 404182)¹¹⁻¹⁴ as a new antiviral agent against human hepatitis C virus (HCV),^{15,16} HIV,¹⁶⁻²⁰ simian immunodeficiency virus (SIV),¹⁶ and herpes simplex virus (HSV) (Figure 1).²⁰ A structure-activity relationship (SAR) study on compound **1** suggested that the 6-imino group and 7-sulfur atom were essential to interact with the potential target molecule(s), which was supported by a complete loss of its anti-HIV activity by substitution of these groups with other heteroatoms. Optimization of the benzene ring and the cyclic amidine moieties in the pyrimido[1,2-*c*][1,3]benzothiazin-6-imine scaffold demonstrated that the introduction of hydrophobic aryl moieties such as *m*-anisyl and 3,4-methylenedioxyphenyl groups into the 9-position improved the anti-HIV activity three-fold.^{17,18} The antiviral profile of **1** indicated that the pyrimido[1,2-*c*][1,3]benzothiazin-6-imine derivatives inhibited

early-stage HIV infection including virus attachment and membrane fusion to host cells as exemplified by DS 5000 (adsorption inhibitor)²¹ and enfuvirtide (fusion inhibitor)⁶. Although it was suggested that compound **1** exerts its virucidal effect against viral particles,^{16,20} its antiviral mechanism of action has not yet been sufficiently detailed.

During the course of our investigations of PD 404182 derivatives, 3,4-dihydro-2*H*-benzo[4,5]isothiazolo[2,3-*a*]pyrimidine **2** was found to exhibit potent anti-HIV activity (EC₅₀ = 0.29 μM) (Figure 1). The structure of this unprecedented scaffold was verified by X-ray crystal analysis. Although there have been several reports that similar isothiazolidine and thiadiazole derivatives exhibited anti-HIV activities as zinc finger inhibitors for HIV-1 nucleocapsid protein 7 (NCp7),^{22,23} this tetrahydropyrimidine-fused heterocyclic scaffold has not been explored as a potential anti-HIV agent candidate. In the current study, we investigated the synthesis of benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives and their structure-activity relationships as potent anti-HIV agents.

2. Results and Discussion

2.1. Synthesis of benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives

Our investigation began with the synthesis of benzo[4,5]isothiazolo[2,3-*a*]pyrimidine scaffold **9** having a characteristic S-N covalent bond, formed by oxidation of 2-(tetrahydropyrimidin-2-yl)thiophenol derivative **8** (Scheme 1). Previously we reported the synthesis of PD 404182 derivative **6** with the pyrimido[1,2-*c*][1,3]benzothiazin-6-imine core.^{17-19,24} Briefly, the oxidative amidination²⁵ of various benzaldehydes **3** gave the corresponding 2-phenyltetrahydropyrimidine derivative **4**. S_NAr-type C-S bond formation on **4** with *tert*-butyl isothiocyanate afforded *N*-(*tert*-butyl)-protected thiazinimine **5**, which was subjected to deprotection of the *tert*-butyl group with trifluoroacetic acid (TFA) to provide PD 404182 derivative **6**. The subsequent TFA-mediated ethanolysis of the imino group in **6c-i,k,l,n** generated the thiophenol precursor **8**. Without isolation of the thiophenol **8**, the desired benzo[4,5]isothiazolo[2,3-*a*]pyrimidines **9c-i,k,l,n** were obtained after linking the thiol group

and cyclic amidine NH group by phenyliodine diacetate (PIDA)²⁶-mediated oxidation. Alternatively, pyrimido[1,2-*c*][1,3]benzothiazin-6-thione derivatives **7j,m** were synthesized by addition of carbon disulfide to derivatives **4j,m** followed by S_NAr-type C-S bond formation.^{24,27} Alkaline hydrolysis of the thiocarbonyl group of **7j,m** followed by PIDA-mediated oxidation gave the compounds **9j,m**. The benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives **9c-n** were derivatized from benzothiazin-6-imine **6** and the precursor **7**,²⁴ which were employed for our previous structure-activity relationship studies of PD 404182 derivatives.^{17,18}

A series of 8-aryl benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives **9a,b,o-z** were also prepared by the identical protocol (Scheme 2).¹⁸ A Suzuki-Miyaura cross-coupling reaction of bromide **5d** with aryl boronic acid provided compounds **5a,b,o-z**. For the synthesis of 8-(pyridin-2-yl) derivative **9x**, 2-pyridinyltriolborate lithium salt was employed. Subsequent deprotection of the *tert*-butyl group of compound **5** gave the corresponding thiazinimine **6a,b,o-z**. After TFA-mediated alcoholysis of compound **6**, PIDA-mediated S-N bond formation afforded the desired 8-aryl benzo[4,5]isothiazolo[2,3-*a*]pyrimidines **9a,b,o-z**. For the preparation of *p*-hydroxyphenyl and *m*-hydroxyphenyl compounds **11a,b**, the TBS and *tert*-butyl protecting groups in the precursors **10a,b** were consecutively removed. Ullmann coupling of bromide **5d** with azoles followed by subsequent manipulations afforded pyrazole and triazole derivatives **14a,b**.

2.2. Structure–activity relationship study of the anti-HIV activity of benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives

Initially, we investigated the modifications of the benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives at the 7-, 8- and 9-positions (Table 1). The anti-HIV activity of a series of compounds was evaluated by the NCK assay,²⁸ in which the inhibition of virus attachment and membrane fusion to host cells during early-stage HIV infection is evaluated. The 8-phenyl modification (**9a**) maintained the potency of the lead compound **2** (EC₅₀ = 0.30 μM), while the *m*-anisyl (**9b**) and bromo (**9d**)

derivative exhibited two to three times greater anti-HIV activity (EC_{50} (**9b**) = 0.10 μ M, EC_{50} (**9d**) = 0.22 μ M). In contrast, the methoxy (**9c**), trifluoromethyl (**9e**) and nitro (**9f**) groups slightly decreased the anti-HIV activity, and the 8-acetamide group in **9g** remarkably attenuated the activity. Modifications at the 9-position of benzo[4,5]isothiazolo[2,3-*a*]pyrimidine were also unfavorable for anti-HIV activity. 9-Phenyl (**9i**), methyl (**9j**), methoxy (**9k**), bromo (**9l**) and trifluoromethyl (**9m**)-modified derivatives showed two- to seven-fold less anti-HIV activity compared with compound **2**. The 7,8-fused benzene (**9h**) (naphtho[2',1':4,5]isothiazolo[2,3-*a*]pyrimidine) and 7-bromo (**9n**) substituent exhibited cytotoxicity at 10 μ M with a loss of anti-HIV activity.

On the basis of the initial structure-activity relationships of the benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives, further modifications on the 8-aryl substituent were carried out (Table 2). First, we modified the *para*- and *meta*-positions of the 8-phenyl group in compound **9a**. The methoxy (**9o** and **9b**), methoxycarbonyl (**9p** and **9r**) and nitro (**9q** and **9s**) groups improved the anti-HIV activity (EC_{50} = 0.05–0.16 μ M), which was approximately two- to six-fold greater compared with compound **2**. Because the effects by introduction of electron-withdrawing and electron-donating substituents on the aromatic ring were similar, the electron density of the 8-phenyl group is unlikely to be the primary factor for the interaction with the target molecule(s). *Para*- and *meta*-hydroxy groups (**11a** and **11b**) reduced the anti-HIV activity (EC_{50} = 1.28 and 1.47 μ M, respectively). The substitution of the 8-phenyl group with a variety of hetero- and carbocyclic substructures was also investigated. The hydrophobic 3,4-methylenedioxyphenyl (**9t**), 2-naphthyl (**9u**), furan-2-yl (**9v**) and thiophen-3-yl (**9w**) groups increased the anti-HIV activity. In contrast, substitution with basic heterocycles such as pyridine (**9x-z**), pyrazole (**14a**) and triazole (**14b**) resulted in a slight decrease of the anti-HIV activity compared with compound **9a**, suggesting that nitrogen heterocycles are not favorable at this position. These results indicated that favorable hydrophobic interaction(s) of the 8-aryl groups with the potential target molecule(s) could play important roles for determining the anti-HIV activity. Cytotoxic effects were not observed at 10 μ M except for compound **9o**.

2.3. Mechanistic studies of anti-HIV isothiazolopyrimidine derivatives

We executed a time of drug addition study to estimate the mechanism of action of the isothiazolopyrimidine derivatives (Figure 2). In our previous studies, we determined that PD 404182 (**1**) and its derivatives inhibited an early stage of HIV infection.^{17,18} In this study, compound **2** and potent derivatives **9r** and **9v** were evaluated for their anti-HIV activity profiles compared with anti-HIV agents including DS 5000 (adsorption inhibitor),²¹ enfuvirtide (fusion inhibitor),⁶ AZT (NRTI),²⁹ nevirapine (NNRTI),³⁰ and raltegravir (integrase inhibitor).³¹ Isothiazolopyrimidine derivatives **2**, **9r**, and **9v** had similar inhibitory profiles to that of DS 5000 and compound **1**, which prevent an early stage of viral infection including the attachment and entry into the target cells. It should also be noted that both compounds **1** and **2** showed a broad spectrum of antiviral activity against RNA viruses and DNA viruses (data not shown). These results may suggest that these two series of compounds may have an identical or similar target molecule(s) or mechanism of action for the antiviral activities. Although a possible common viral target molecule(s) has not yet been identified,³² these derivatives could positively regulate the defense mechanism(s) of the host cells.

3. Conclusions

In this study, we identified a novel class of small-molecule anti-HIV agents with the benzo[4,5]isothiazolo[2,3-*a*]pyrimidine scaffold. A structure-activity relationship study of the lead compound **2** demonstrated that a hydrophobic aryl substituent at the 8-position of the scaffold was responsible for the potent anti-HIV activity. Although the improvement of the anti-HIV activity from **2** was not satisfactory, further SAR studies would provide promising candidates with higher potency and favorable antiviral profiles. The similar antiviral profiles between compounds **1** and **2** suggested that these could possibly binds with the common potential target molecule(s) in host cells. The

identification of the target molecule(s) and further optimization of potent derivatives should facilitate the development of novel antiviral agents.

4. Experimental Section

4.1. Synthesis

4.1.1. General synthesis

¹H NMR spectra were recorded using a JEOL AL-400 or a JEOL ECA-500 spectrometer. Chemical shifts are reported in δ (ppm) relative to Me₄Si as an internal standard. ¹³C NMR spectra were referenced to the residual solvent signal. Exact mass (HRMS) spectra were recorded on a JMS-HX/HX 110A mass spectrometer or Shimadzu LC-ESI-IT-TOF-MS equipment. For flash chromatography, Wakogel C-300E (Wako) was employed. For analytical HPLC, a Cosmosil 5C18-ARII column (4.6 \times 250 mm, Nacalai Tesque, Inc.) was employed with a linear gradient of CH₃CN containing 0.1% (v/v) TFA aq. at a flow rate of 1 mL/min, and eluting products were detected by UV at 254 nm. Preparative HPLC was performed using a Cosmosil 5C18-ARII preparative column (20 \times 250 mm, Nacalai Tesque, Inc.) with a linear gradient of CH₃CN containing 0.1% (v/v) TFA aq. at a flow rate of 8 mL/min. The purity of the compounds was determined as no less than 95% by combustion analysis or HPLC analysis.

4.1.2. General procedure for the synthesis of benzo[4,5]isothiazolo[2,3-*a*]pyrimidine from pyrimido[1,2-*c*][1,3]benzothiazin-6-imine: synthesis of 3,4-dihydro-2*H*-benzo[4,5]isothiazolo[2,3-*a*]pyrimidine (**2**)

TFA (171 μ L, 2.30 mmol) was added to a suspension of PD 404182 (**1**) (50.0 mg, 0.230 mmol) in CHCl₃ (2.30 mL) and EtOH (3.43 mL) dropwise. After being stirred at room temperature for 1 h, the mixture was quenched with Et₃N (321 μ L, 2.30 mmol), and PIDA (72.6 mg, 0.226 mmol) was added. After being stirred for 30 min at room temperature, the mixture was concentrated. Crude product was purified by HPLC to give the title compound **2** as colorless crystals (48.5 mg, 71%, TFA salt); mp 200–202 °C (from Et₂O–MeOH); IR (neat) cm⁻¹: 3117-3056 (OH), 1662 (C=O), 1630 (C=N); ¹H NMR (500 MHz, CD₃OD): δ 2.30-2.35 (m, 2H, CH₂), 3.67 (t, *J* = 5.4 Hz, 2H, CH₂), 4.16 (t, *J* = 5.7

Hz, 2H, CH₂), 7.63-7.66 (m, 1H, Ar), 7.84-7.87 (m, 1H, Ar), 8.04 (d, *J* = 8.6 Hz, 1H, Ar), 8.17 (d, *J* = 8.0 Hz, 1H, Ar); ¹³C NMR (125 MHz, CD₃OD): δ 20.7, 39.0, 46.3, 118.3 (q, *J* = 293.9 Hz), 122.5, 123.0, 125.1, 128.2, 134.7, 143.5, 156.0, 163.0 (q, *J* = 34.8 Hz); *Anal.* calcd for C₁₂H₁₁F₃N₂O₂S: C, 47.37; H, 3.64; N, 9.21. Found: C, 47.32; H, 3.80; N, 9.29.

4.1.3. 9-Methyl-3,4-Dihydro-2*H*-benzo[4,5]isothiazolo[2,3-*a*]pyrimidine (9j)

10-Methyl-3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazine-6-thione (7j) (60.0 mg, 0.242 mmol) was suspended in 0.1N NaOH in MeOH-H₂O (9:1, 4.83 mL). After being stirred under reflux for 12 h, the mixture was quenched with 1N HCl until pH was adjusted to 7. The whole was extracted with CHCl₃-MeOH (95:5), and dried over MgSO₄. After concentration, the residue in CHCl₃ (6.04 mL) was allowed to react with PIDA (76.3 mg, 0.237 mmol). After being stirred for 30 min at room temperature, the mixture was concentrated. Purification by HPLC gave the title compound 9j as colorless solid (4.5 mg, 6%, TFA salt); IR (neat) cm⁻¹: 3265-3141 (OH), 1669 (C=O), 1630 (C=N); ¹H NMR (500 MHz, CD₃OD): δ 2.29-2.34 (m, 2H, CH₂), 2.53 (s, 3H, CH₃), 3.66 (t, *J* = 5.7 Hz, 2H, CH₂), 4.14 (t, *J* = 5.7 Hz, 2H, CH₂), 7.71 (dd, *J* = 8.3, 1.4 Hz, 1H, Ar), 7.91 (d, *J* = 8.6 Hz, 1H, Ar), 7.96 (d, *J* = 1.7 Hz, 1H, Ar); ¹³C NMR (125 MHz, CD₃OD): δ 20.7, 21.1, 38.9, 46.3, 118.6 (q, *J* = 255.5 Hz), 122.1, 123.0, 124.6, 136.2, 139.0, 140.6, 155.7, 162.7 (q, *J* = 33.6 Hz); HRMS (FAB): *m/z* calcd for C₁₁H₁₃N₂S [M + H]⁺ 205.0799; found: 205.0795.

4.1.4. 8-(4-Hydroxyphenyl)-3,4-dihydro-2*H*-benzo[4,5]isothiazolo[2,3-*a*]pyrimidine (11a)

TFA (2.35 mL) was added to a mixture of *N*-(*tert*-butyl)-9-[4-(*tert*-butyldimethylsilyloxy)phenyl]-3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (10a) (112.6 mg, 0.235 mmol) in a few drops of CHCl₃ and MS4Å (528 mg, powder, activated by heating with Bunsen burner). After being stirred under reflux for 4 h, the mixture was added dropwise Et₃N at 0 °C to adjust pH to 8–9. The whole was extracted with EtOAc. The extract was washed with sat. NaHCO₃, brine, and dried over

MgSO₄. Concentration gave the crude imine as pale yellow solid (73.9 mg). By use of a procedure similar to that described for the preparation of the compound **2** from **1**, a part of the residue (20.0 mg) was converted to the title compound **11a** as colorless solid (11.0 mg, 44% in 2 steps, TFA salt); IR (neat) cm⁻¹: 3237-3066 (OH), 1684 (C=O), 1632 (C=N); ¹H NMR (500 MHz, CD₃OD): δ 2.31-2.35 (m, 2H, CH₂), 3.67 (t, *J* = 5.7 Hz, 2H, CH₂), 4.15 (t, *J* = 6.0 Hz, 2H, CH₂), 6.91-6.94 (m, 2H, Ar), 7.60-7.63 (m, 2H, Ar), 7.86 (dd, *J* = 8.6, 1.1 Hz, 1H, Ar), 8.15 (d, *J* = 8.6 Hz, 1H, Ar), 8.19 (d, *J* = 1.7 Hz, 1H, Ar); ¹³C NMR (125 MHz, CD₃OD): δ 20.7, 39.0, 46.2, 117.1 (2C), 118.2 (q, *J* = 238.7 Hz), 119.0, 120.8, 125.3, 126.7, 129.8 (2C), 130.8, 144.3, 147.7, 155.7, 160.0, 162.9 (q, *J* = 34.8 Hz); HRMS (ESI): *m/z* calcd for C₁₆H₁₅N₂OS [M + H]⁺ 283.0905; found: 283.0908.

4.2. Anti-HIV activity

The anti-HIV activity of a series of compounds against HIV-1_{IIIB} was determined by the NCK assay.²⁸ The target cells (NCK45-β-Gal; 10⁴ cells/well) were plated in 96-well flat microtiter culture plates. On the following day, the cells were inoculated with HIV-1 (60 NCK 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 [EC₅₀]). EC₅₀ was determined by using the following formula:

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

where

A: of the two points on the graph that bracket 50% inhibition, the higher concentration of the test compound,

B: of the two points on the graph that 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.2015.02.015>.

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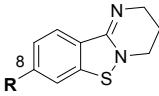
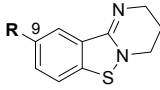
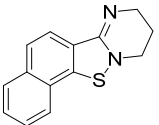
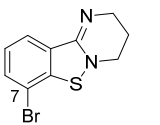
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was recently reported that PD 404182 (**1**) inhibits human dimethylarginine dimethylaminohydrolase isoform 1 (DDAH).¹⁴

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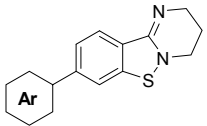
26. Among several reagents assessed for the oxidative cyclization including *m*CPBA, NBS, and iodosylbenzene, PIDA provided the most efficient S-N bond formation.
27. The synthetic process *via* 1,3-thiazine-2-thione intermediates **7j,m** was chosen for compounds **9j,m** because of the substrate availability.
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32. Of note, it was reported that PD 404182 showed the antiviral activities by virucidal effect via the physical disruption of virions, see refs.16 and 20.


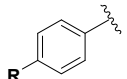




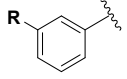



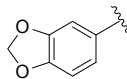
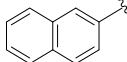
Table 1. Structure-activity relationships of benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives.

cmpd		EC ₅₀ ^a (μM)	cmpd		EC ₅₀ ^a (μM)
					
2	R = H	0.29 ± 0.09			
9a	R = Ph	0.30 ± 0.10	9i	R = Ph	0.56 ± 0.13
9b	R = <i>m</i> -anisyl	0.10 ± 0.03	9j	R = Me	0.85 ± 0.10
9c	R = OMe	0.58 ± 0.10	9k	R = OMe	2.11 ± 0.48
9d	R = Br	0.22 ± 0.07	9l	R = Br	1.01 ± 0.22
9e	R = CF ₃	1.28 ± 0.04	9m	R = CF ₃	1.78 ± 0.24
9f	R = NO ₂	1.79 ± 0.44			
9g	R = NHAc	>10			
9h		>1.0 ^{b,c}	9n		>10 ^c

^aEC₅₀ values represent the concentration of the compound required to inhibit the HIV-1 infection by 50%. The data were obtained from three independent experiments by the NCK assay. ^bThe compound inhibited the HIV-1 infection by 40% at 1.0 μM. ^cCytotoxicity was observed at 10 μM.

Table 2. Structure-activity relationship of 8-aryl benzo[4,5]isothiazolo[2,3-*a*]pyrimidines.


The image shows the general chemical structure of the compounds. It consists of a benzene ring fused to a 4,5-dihydroisothiazolo[2,3-a]pyrimidine ring system. An aryl group, labeled 'Ar', is attached to the 8-position of the benzene ring.

compd		EC ₅₀ ^a (μM)	compd	EC ₅₀ ^a (μM)	
9a		0.30 ± 0.10			
9o		R = OMe	9v		0.05 ± 0.02
9p		R = CO ₂ Me	9w		0.08 ± 0.01
9q		R = NO ₂	9x		0.34 ± 0.11
11a		R = OH	9y		0.56 ± 0.03
9b		R = OMe	9z		0.56 ± 0.17
9r		R = CO ₂ Me	14a		0.41 ± 0.07
9s		R = NO ₂	14b		0.42 ± 0.10
11b		R = OH			
9t		0.16 ± 0.02			
9u		0.12 ± 0.03			

^aEC₅₀ values represent the concentration of the compound required to inhibit the HIV-1 infection by 50%. The data were obtained from three independent experiments by the NCK assay. ^bCytotoxicity was observed at 10 μM.

Figure 1. Structures of PD 404182 (**1**) and benzo[4,5]isothiazolo[2,3-*a*]pyrimidine (**2**).

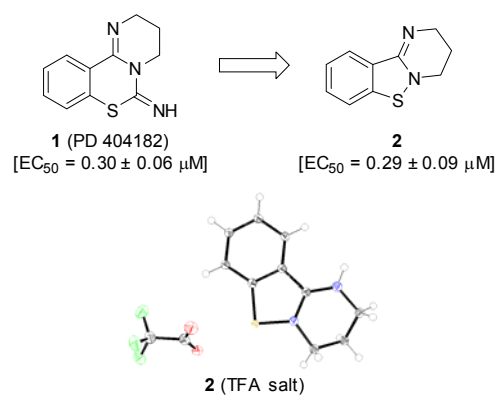
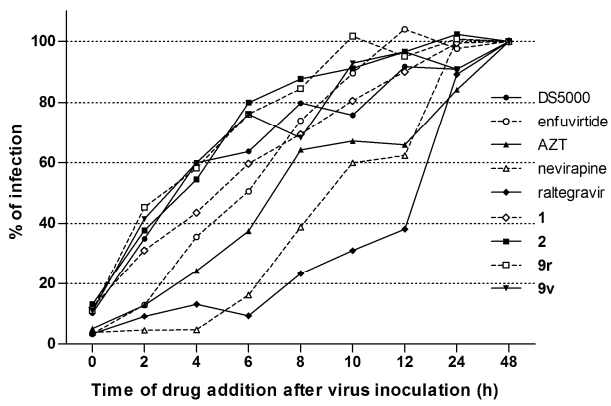
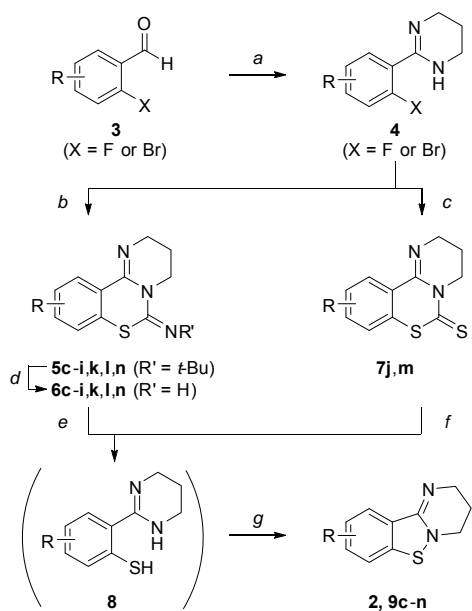


Figure 2. Time of drug addition profiles for HIV-1 infection.



Scheme 1. Synthesis of 7-, 8- and 9- substituted benzo[4,5]isothiazolo[2,3-*a*]pyrimidine derivatives.

Reagents and conditions: (a) 1,3-propanediamine, I₂, K₂CO₃, *t*-BuOH, 70 °C; (b) NaH or *t*-BuOK, *t*-BuNCS, DMF or DMAc, -20 – 80 °C; (c) NaH, CS₂, DMF, rt-80 °C; (d) TFA, MS4Å, CHCl₃, reflux; (e) TFA, CHCl₃/EtOH, rt; (f) NaOH, MeOH/H₂O, reflux; (g) PIDA, CHCl₃/EtOH or CHCl₃, rt.



Scheme 2. Synthesis of 8-aryl benzo[4,5]isothiazolo[2,3-*a*]pyrimidines. *Reagents and conditions:* (a) Ar-B(OH)₂, Pd(PPh₃)₄, Pd(dppf)Cl₂·CH₂Cl₂, K₂CO₃, toluene/EtOH/H₂O, reflux; (b) 2-pyridinyltriolborate lithium salt, Pd(OAc)₂, CuI, PPh₃, DMF, 80 °C; (c) TFA, MS4Å, CHCl₃, reflux; (d) TFA, CHCl₃/EtOH or EtOH, rt, then Et₃N, PIDA, CHCl₃/EtOH or EtOH, rt; (e) pyrazole or triazole, CuCl, K₂CO₃, acetylacetonate, NMP, 130 °C.

