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PAPER

Concise synthesis and anti-HIV activity of pyrimido[1,2-*c*][1,3]benzothiazin-6-imines and related tricyclic heterocycles†

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3,4-Dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (PD 404182) is a virucidal heterocyclic compound active against various viruses, including HCV, HIV, and simian immunodeficiency virus. Using facile synthetic approaches that we developed for the synthesis of pyrimido[1,2-*c*][1,3]-benzothiazin-6-imines and related tricyclic derivatives, the parallel structural optimizations of the central 1,3-thiazin-2-imine core, the benzene part, and the cyclic amidine part of PD 404182 were investigated. Replacement of the 6-6-6 pyrimido[1,2-*c*][1,3]benzothiazin-6-imine framework with 5-6-6 or 6-6-5 derivatives led to a significant loss of anti-HIV activity, and introduction of a hydrophobic group at the 9- or 10-positions improved the potency. In addition, we demonstrated that the PD 404182 derivative exerts anti-HIV effects at an early stage of viral infection.

Introduction

Since azidothymidine (AZT), a nucleoside reverse transcriptase inhibitor (NRTI), was approved for the treatment of HIV infections, a number of anti-HIV drugs have been launched. For example, saquinavir and nevirapine were the first protease inhibitor and non-nucleoside reverse transcriptase inhibitor (NNRTI), respectively.[1] Highly active antiretroviral therapy (HAART) using a combination of these antiretrovirals is a standard treatment regimen for HIV infections. The HAART regimen significantly reduces viral load in infected patients, leading to significant therapeutic gains and reductions in morbidity and mortality.[2] However, long-term administration of multiple antiretrovirals to maintain life-long latent infection triggers the emergence of drug-resistant variants[3] and drug-related adverse effects.[4] For example, high-level viral resistance to NRTI such as AZT, stavudine, and didanosine is conferred by mutations frequently observed in patients with virologic failure on an NRTI-containing regimen.[5] In addition, lipodystrophy and metabolic disorders are often observed in patients receiving HIV protease inhibitors.[6] To overcome these problems, several antiretrovirals with new mechanisms of action have been developed in this decade. A peptide-based fusion inhibitor (enfuvirtide),[7] an integrase inhibitor (raltegravir),[8] and a CC chemokine receptor type 5 (CCR5) antagonist (maraviroc)[9] are examples of new molecular entities used as anti-HIV agents.

Recently, highly potent small-molecule anti-HIV agents have been reported, which bind to viral envelope proteins (Fig. 1). 2-Thioxo-1,3-thiazolidine derivative **1** shows potent inhibition of HIV-1 replication at nanomolar levels,[10] which are directed at the deep hydrophobic pocket in the N-terminal heptad repeat trimer of the viral gp41. Compound **1** blocks HIV-1-mediated cell–cell fusion and the formation of gp41 six-helix bundles, as does enfuvirtide.[10b] The bisindole derivative **2** also exhibits submicromolar inhibition of HIV-1 replication by interaction with the gp41 hydrophobic pocket in which compound **1** binds.[11] Small-molecule CD4 mimics with oxalamide and related substructures are another series of anti-HIV agents.[12] The representative BMS-448043 (**3**) exhibits subnanomolar anti-HIV activity by interaction with the CD4 binding pocket in gp120.[12d] These small-molecule entry inhibitors with potential oral bioavailability will provide alternative combination regimen(s) of anti-HIV agents for the treatment of drug-resistant variants.

In our efforts to develop novel anti-HIV compounds,[13] we have carried out the random screening of small molecules using multinuclear activation of a galactosidase indicator (MAGI) assay, in which the inhibitory activity of early-stage HIV infection, including virus attachment and membrane fusion to host cells, is evaluated. Among more than 30 000 compounds screened, 3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine **4** (PD 404182) was identified as a potent anti-HIV agent lead (Fig. 1). Compound **4** was reported to be an enzyme inhibitor against 3-deoxy-D-manno-octulosonic acid 8-phosphate synthase[14] and phosphopantetheinyl transferase,[15] exerting

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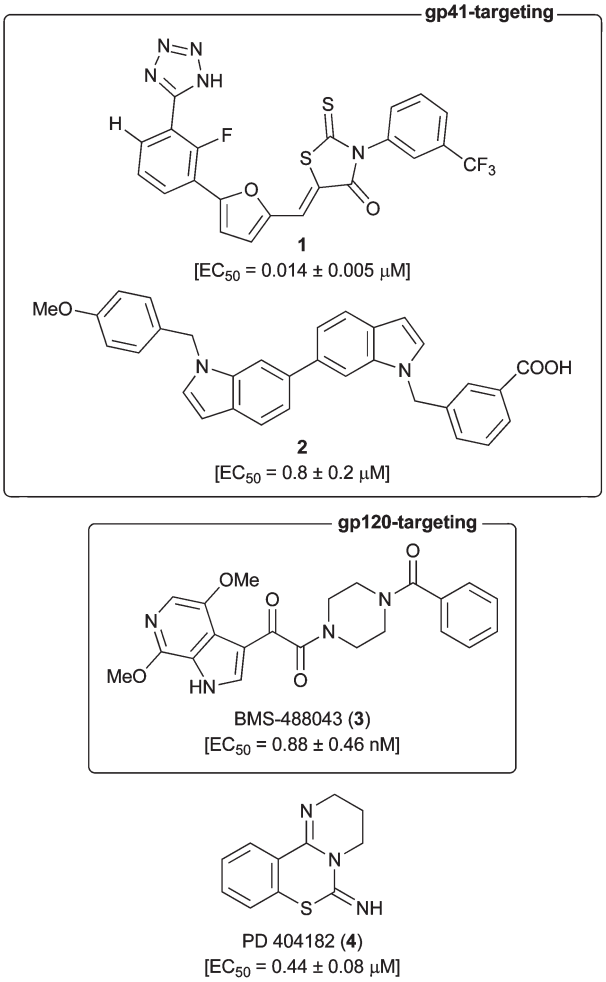
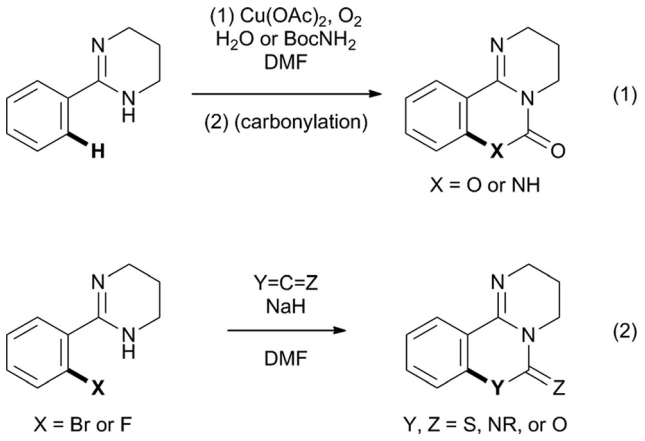


Fig. 1 Structures of newly reported anti-HIV compounds (**1–3**) targeting HIV-1 envelope proteins, and PD 404182 (**4**).

antimicrobial effects. In the course of our SAR investigations in this study, antiviral activities of **4** against HCV, HIV, and simian immunodeficiency virus were reported.[16,17] Although compound **4** exhibits virucidal effects at high concentrations, the mechanism of action and the target molecule remain ambiguous.[17]

Recently, we have established two independent approaches for the synthesis of PD 404182 derivatives (Scheme 1): C–H functionalization of 2-phenyl-1,4,5,6-tetrahydropyrimidine with water or *tert*-butylcarbamate in the presence of copper(II) acetate provides pyrimido[1,2-*c*][1,3]benzoxazine or pyrimido[1,2-*c*]-quinazoline in one or two step(s) (eqn (1), Scheme 1).[18] Alternatively, addition of 2-(2-halophenyl)-1,4,5,6-tetrahydropyrimidine to carbon disulfide, isocyanate, or isothiocyanate, and subsequent aromatic nucleophilic substitution (S_NAr) affords pyrimidobenzothiazines and -oxazines, and pyrimidoquinazolines (eqn (2), Scheme 1).[19] The derivatives obtained from these reactions were easily converted to the pyrimido[1,2-*c*][1,3]benzothiazin-6-imine scaffold. Our two synthetic methods provide a variety of PD 404182 derivatives from the corresponding benzaldehydes in a few steps and in good yields, facilitating the lead optimization process.[20] In this article, a SAR study of PD 404182 derivatives using these synthetic approaches is described.



Scheme 1 Our synthetic methods for PD 404182 derivatives.

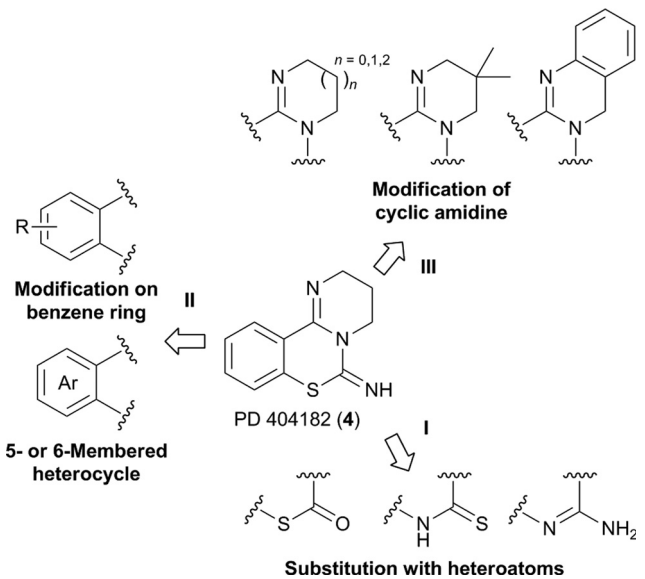


Fig. 2 Strategy for SAR study of PD 404182 (**4**).

Results and discussion

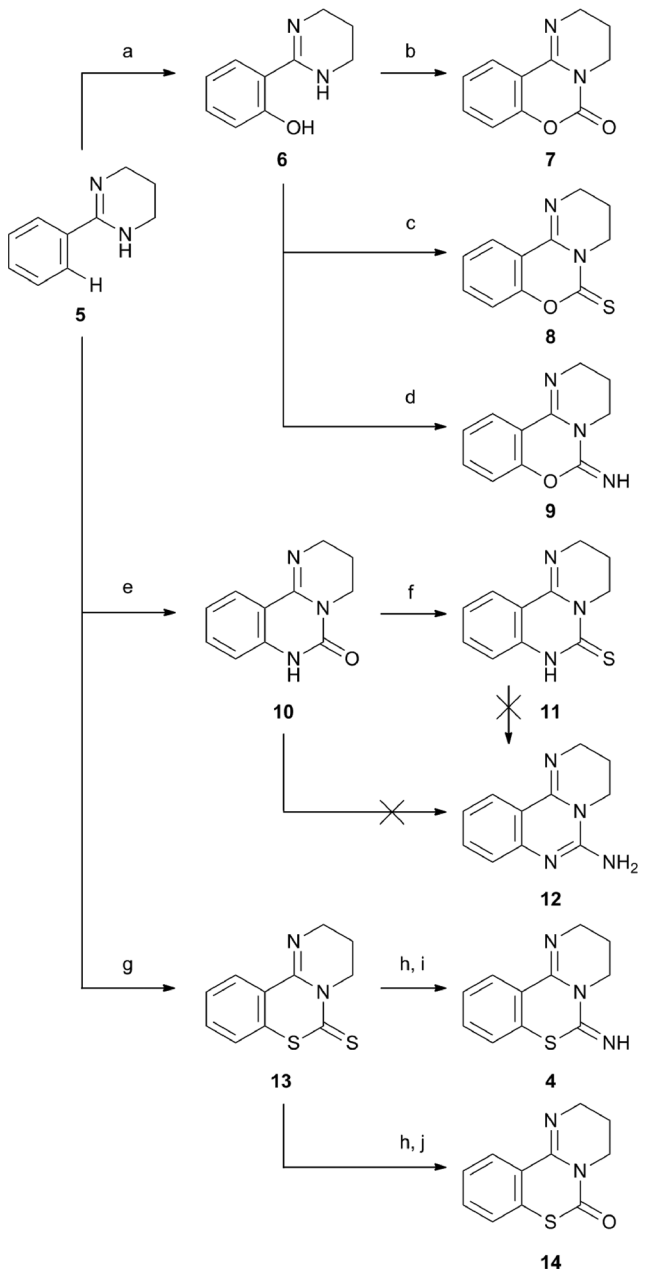
Strategy for the SAR study of PD 404182

PD 404182 consists of three components, namely a 1,3-thiazin-2-imine core, and left-fused benzene and cyclic amidine moieties (Fig. 2). In order to obtain detailed insights into the relationships between the compound structure and anti-HIV activity, we planned to investigate substituent effects on each component: (I) derivatives with various heteroatom (N, S, and O) arrangements on the 1,3-thiazin-2-imine core (Fig. 2); (II) pyrimido[1,2-*c*][1,3]-thiazin-6-imine derivatives fused with a substituted benzene ring or a five- or six-membered aromatic heterocycle; and (III) benzo-[*e*][1,3]thiazin-2-imine derivatives fused with a cyclic amidine ring with or without accessory alkyl or aryl groups.

Synthesis of pyrimido[1,2-*c*][1,3]benzothiazin-6-imines and related tricyclic heterocycles

Our investigation began with the synthesis of tricyclic heterocycles with different combinations of heteroatoms on the

1,3-thiazin-2-imine core. Previously, we reported syntheses of pyrimido[1,2-*c*][1,3]benzoxazine and pyrimido[1,2-*c*]quinazoline derivatives using copper(II)-mediated C–H functionalization;[18] this facilitates the introduction of oxygen or nitrogen functional groups at the *ortho*-position of 2-phenyl-1,4,5,6-tetrahydropyrimidine (**5**). Using compound **5** as a key starting material, a divergent approach was used for the preparation of a series of scaffolds (Scheme 2).



Scheme 2 Synthesis of PD 404182 derivatives with different combinations of heteroatoms. Reagents and conditions: (a) $Cu(OAc)_2$, H_2O, O_2, DMF, 130 °C, 69%; (b) triphosgene, TMEDA, CH_2Cl_2, 0 °C to rt, 70% [2 steps (a,b)]; (c) thiophosgene, Et_3N, CH_2Cl_2, 0 °C to rt, quant.; (d) BrCN, CH_2Cl_2, rt, 34%; (e) $Cu(OAc)_2$, $BocNH_2$, O_2, DMF, 130 °C, 53%; (f) Lawesson's reagent, xylene, reflux, 19%; (g) $Cu(OAc)_2$, CS_2, O_2, 1,4-dioxane, 130 °C, 11%; (h) NaOH, MeOH, H_2O, reflux; (i) BrCN, EtOH, reflux, 61% [2 steps (h,i)]; (j) triphosgene, Et_3N, CH_2Cl_2, 0 °C to rt, 65% [2 steps (h,j)].

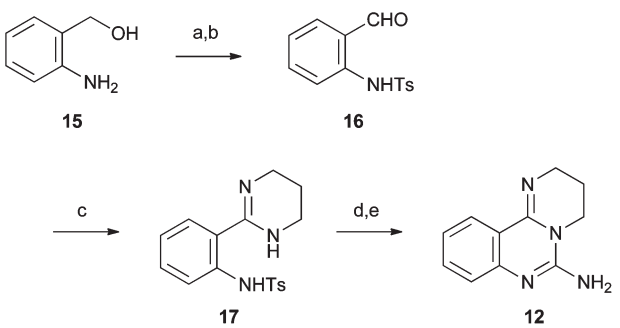
A one-pot reaction for $Cu(OAc)_2$-mediated C–H functionalization of **5** and subsequent treatment with triphosgene provided a 1,3-oxazin-2-one derivative **7** (Scheme 2). The same one-pot procedure using thiophosgene produced a trace amount of the desired thiocarbonyl derivative **8**; treatment of the purified intermediate **6** with thiophosgene provided the desired 1,3-oxazine-2-thione **8** in high yield. 1,3-Oxazin-2-imine **9** was obtained by the reaction of **6** with BrCN.

The copper-mediated C–N bond formation of compound **5** with *tert*-butylcarbamate followed by spontaneous intramolecular cyclization afforded a pyrimido[1,2-*c*]quinazolin-6-one scaffold **10**, as demonstrated in our previous report (Scheme 2).[18] Subsequent treatment with Lawesson's reagent led to formation of the thiocarbonyl derivative **11**. Since no hydrolysis of the carbonyl or thiocarbonyl group of compound **10** or **11** for construction of the 2-aminoquinazoline structure in **12** occurred, an alternative approach starting from 2-aminobenzyl alcohol **15** was used for the synthesis of the 2-aminoquinazoline derivative **12** (Scheme 3). After protection and PCC oxidation of **15**, oxidative amidination[21] provided 2-(*p*-tosylamino)phenyltetrahydropyrimidine **17**. Deprotection followed by BrCN-mediated cyclization of **17** provided the expected 2-aminoquinazoline derivative **12**.

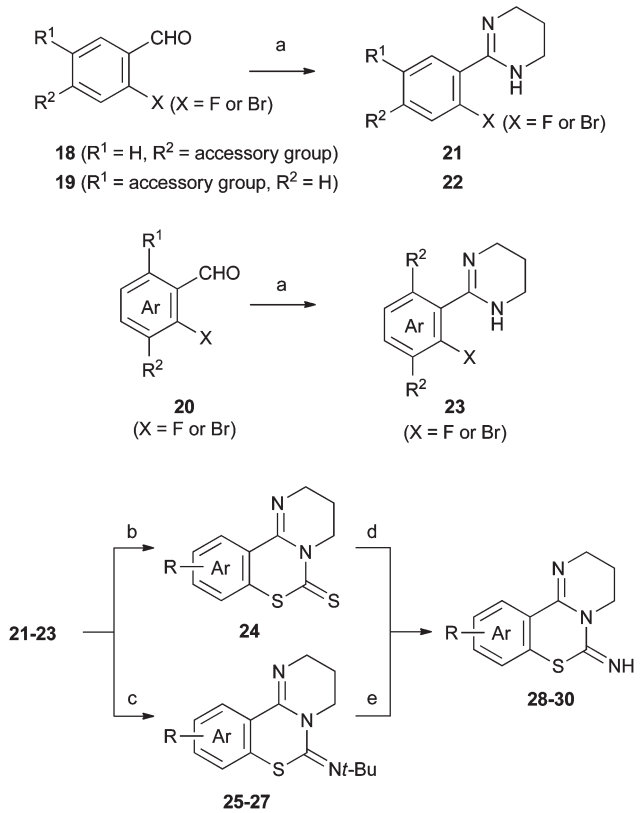
For the synthesis of pyrimido[1,2-*c*][1,3]benzothiazine derivatives, we adapted the C–H functionalization reaction for C–S bond formation (Scheme 2). After optimization of the reaction conditions, we found that exposure of compound **5** to CS_2 in the presence of $Cu(OAc)_2$ directly afforded a pyrimido[1,2-*c*][1,3]-benzothiazine-6-thione scaffold **13**. Hydrolysis of the thiocarbonyl group in **13** followed by treatment with BrCN or triphosgene provided 6-imino or 6-oxo derivatives (**4** or **14**), respectively.

Synthesis of pyrimido[1,2-*c*][1,3]thiazine derivatives with fused benzene and heterocycles

Pyrimido[1,2-*c*][1,3]thiazin-6-imine derivatives **28–30** with a series of fused ring systems were prepared by consecutive heterocumulene addition and S_NAr reactions (Scheme 4).[19] These reactions provide easy access to the construction of the 1,3-thiazin-2-imine derivatives and are more efficient than the diversity-oriented C–H functionalization approach. The oxidative amidination of aromatic aldehydes **18–20** with an accessory



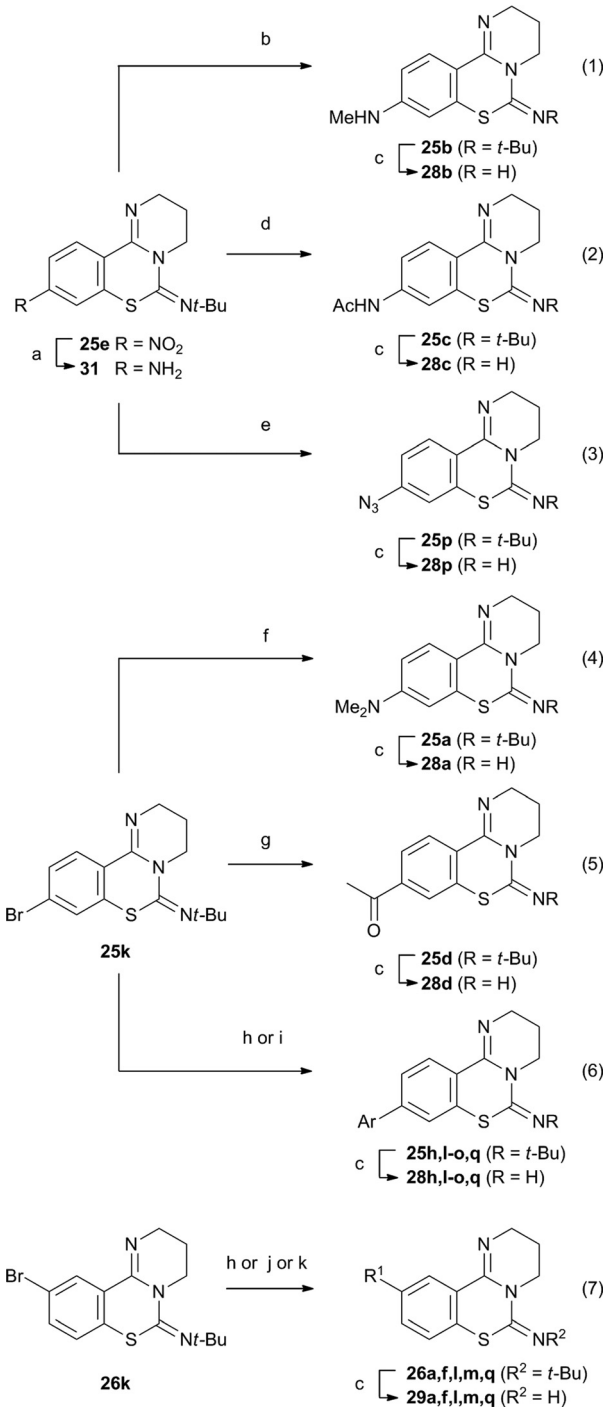
Scheme 3 Synthesis of 2-aminoquinazoline derivative **12**. Reagents and conditions: (a) *p*-TsCl, pyridine, $CHCl_3$, rt; (b) PCC, silica gel, CH_2Cl_2, rt, 80% [2 steps (a,b)]; (c) 1,3-propanediamine, I_2, K_2CO_3, *t*-BuOH, 70 °C, 98%; (d) conc. H_2SO_4, 100 °C, then NaOH, H_2O; (e) BrCN, EtOH, reflux, 66% [2 steps (d,e)].



Scheme 4 Synthesis of pyrimido[1,2-*c*][1,3]thiazin-6-imine derivatives fused with substituted benzene and heterocycles (**28–30**). Reagents and conditions: (a) 1,3-propanediamine, I_2, K_2CO_3, *t*-BuOH, 70 °C, 58–91%; (b) NaH, CS_2, DMF, 80 °C, 67%-quant.; (c) NaH or *t*-BuOK, *t*-BuNCS, DMF or DMAc, −20–80 °C, 28–95%; (d) (i) NaOH, MeOH, H_2O, reflux, (ii) BrCN, EtOH, reflux, 32–68%; (e) TFA, MS4Å, $CHCl_3$, reflux, 63–92%.

functional group afforded the corresponding 2-phenyltetrahydropyrimidine derivatives **21–23**. The pyrimido[1,2-*c*][1,3]thiazine-6-thione scaffold **24** was obtained by additions of **21f,g,i** or **23s,t,u** to carbon disulfide followed by S_NAr-type C–S bond formation. The desired 6-imino derivatives **28f,g,i** and **30s,t,u** were obtained *via* hydrolysis of the thiocarbonyl group of **24** followed by BrCN treatment. Alternatively, reactions of other 2-phenyltetrahydropyrimidines **21–23** with *tert*-butyl isothiocyanate afforded *N*-(*t*-Bu)-protected thiazinimine derivatives **25–27**, which were treated with TFA to provide the expected products **28–30**.

The intermediates **25e**, **25k**, and **26k** were subjected to further manipulations to obtain the functionalized derivatives (Scheme 5). The nitro group of **25e** was reduced by hydrogenation to form the 9-amino derivative **31**. Alkylation of **31** afforded the 9-(*N*-methylamino) derivative **25b** (eqn (1), Scheme 5). The 9-acetamide derivative **25c** was obtained by treatment of **31** with acetic anhydride (eqn (2), Scheme 5). Sandmeyer reaction of **31** gave the 9-azide derivative **25p** (eqn (3), Scheme 5). Me_2N- and MeO-substituted derivatives (**25a**, **26a**, and **26f**) were obtained by Me_2NH-mediated *N*-arylation[22] of the 9-bromo **25k** and 10-bromo derivatives **26k**, and NaOMe-mediated Ullmann coupling[23] of **26k**, respectively (eqn (4) and

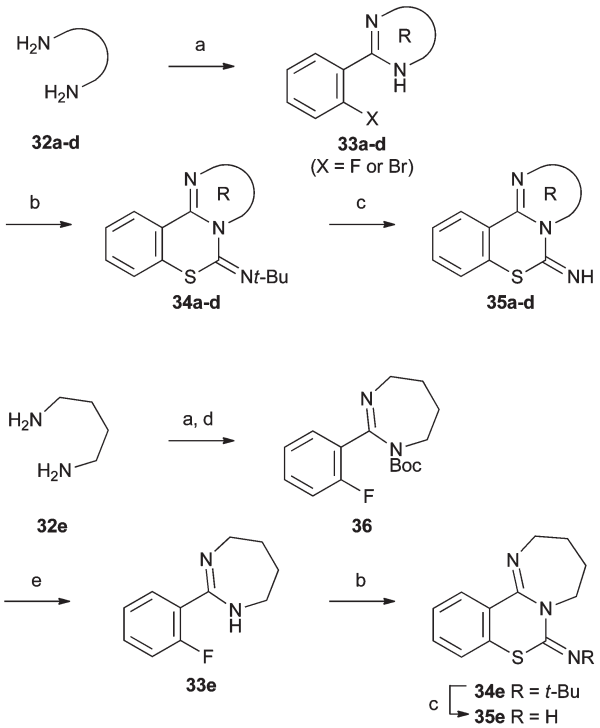


Scheme 5 Synthesis of 9- or 10-substituted pyrimido[1,2-*c*][1,3]benzothiazin-6-imine derivatives. Reagents and conditions: (a) H_2, Pd/C, EtOH, rt, 88%; (b) NaOMe, $(CH_2O)_n$, MeOH, reflux, then $NaBH_4$, 91%; (c) TFA, MS4Å, $CHCl_3$, reflux, 37–95%; (d) Ac_2O, DMAP, Et_3N, CH_2Cl_2, rt, quant.; (e) $NaNO_2$, AcOH, H_2O, 0 °C, then NaN_3, 70%; (f) $Pd(OAc)_2$, *t*-Bu_3P, $NHMe_2$, THF, KO*t*-Bu, toluene, reflux, quant.; (g) 2-hydroxyethylvinylether, $Pd(OAc)_2$, 1,3-bis(diphenylphosphino)-propane, K_2CO_3, H_2O, 90 °C, 13% [2 steps (g,c)]; (h) R-$B(OH)_2$ or R-Bpin, $Pd(PPh_3)_4$, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_2CO_3, toluene or 1,4-dioxane, EtOH, H_2O, reflux, 62–96%; (i) *n*-BuB$(OH)_2$, $Pd_2(dba)_3$, P(*t*-Bu)$_3$, $CsCO_3$, 1,4-dioxane, reflux, 6% (for **25h**); (j) Pd(P*t*-Bu)$_2$, $NHMe_2$, THF, KO*t*-Bu, toluene, 170 °C, 67% (for **26a**); (k) CuBr, NaOMe, MeOH, DMF, reflux, 40% (for **26f**).

(7), Scheme 5). The 9-acetyl derivative **25d** was obtained by Heck reaction[24] of **25k** with 2-hydroxyethyl vinyl ether (eqn (5), Scheme 5). Other derivatives with a variety of functional groups (**25h,l–o,q** and **26l,m,q**) were synthesized by Suzuki–Miyaura coupling reactions[25] of **25k** and **26k** with boronic acids or their pinacol esters (eqn (6) and (7), Scheme 5). Final deprotection of the *tert*-butyl group in **25** and **26** afforded the 9- or 10-substituted pyrimido[1,2-*c*][1,3]benzothiazine derivatives **28** and **29**, respectively.

Synthesis of benzo[*e*][1,3]thiazine derivatives with fused cyclic amidines

Benzo[*e*][1,3]thiazine derivatives with various ring-sized and/or modified cyclic amidine moieties **35** were also synthesized by the consecutive heterocumulene addition and S_NAr reactions (Scheme 6). Oxidative amidination using several diamines **32** proceeded efficiently to form five- or six-membered rings (**33a–d**). The same reaction for the seven-membered amidine (**33e**) was incomplete, but purification of the Boc-protected amidine **36** followed by subsequent deprotection of the Boc group gave the pure seven-membered amidine **33e**. The resulting amidines were converted to cyclic-amidine-fused benzo[*e*][1,3]-thiazin-2-imines **34** *via tert*-butyl isothiocyanate addition and an S_NAr reaction. TFA-mediated deprotection gave the expected derivatives **35**.

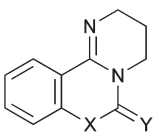


Scheme 6 Synthesis of benzo[*e*][1,3]thiazine derivatives with fused cyclic amidines. Reagents and conditions: (a) 2-fluorobenzaldehyde or 2-bromobenzaldehyde, I_2, K_2CO_3, *t*-BuOH, 70 °C, 68–79%; (b) NaH, *t*-BuNCS, DMF, rt −80 °C, 18–50%; (c) TFA, MS4Å, $CHCl_3$, reflux, 16–86%; (d) Boc_2O, Et_3N, DMAP, CH_2Cl_2, rt, 37% [2 steps (a,d)]; (e) TFA, CH_2Cl_2, reflux, 80%.

Structure–activity relationships of the central heterocyclic core in pyrimido[1,2-*c*][1,3]benzothiazines

Initially, the structural requirements of the 1,3-thiazin-2-imine core substructure in **4** (PD 404182) for anti-HIV activity were investigated (Table 1). The antiviral activities against the HIV-1_{IIIB} strain were evaluated using the MAGI assay. Substitution of the imino group in **4** with a carbonyl group (**14**) resulted in a significant decrease in anti-HIV activity (EC_{50} = 8.94 μM). Pyrimido[1,2-*c*][1,3]benzoxazines (**7–9**), pyrimido [1,2-*c*]quinazolines (**10–12**), and pyrimido[1,2-*c*][1,3]benzothiazine-6-thione (**13**), in which the 1-sulfur and/or 2-imino groups in **4** were modified, showed no activity. These results suggested that both the 1-sulfur atom and the 2-imino group are indispensable functional groups for the inhibitory activity against HIV infection, and may be involved in potential interactions with the target molecules.

Table 1 SARs for 1,3-thiazin-2-imine core



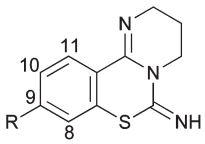
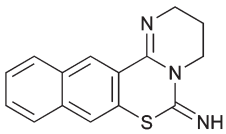
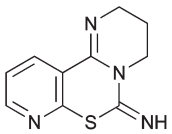
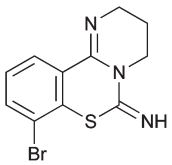
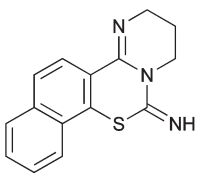
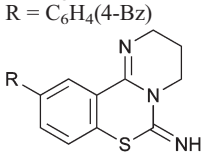
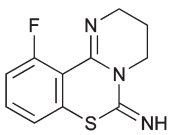
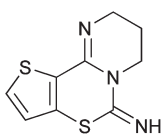
Compound	X	Y	EC_{50} (μM)[a]
4	S	NH	0.44 ± 0.08
7	O	O	>10
8	O	S	>10
9	O	NH	>10
10	NH	O	>10
11	NH	S	>10
12	NH	NH	>10
13	S	S	>10
14	S	O	8.94 ± 1.07

[a] EC_{50} values were the concentration that blocks HIV-1 infection by 50% and derived from three independent experiments.

Structure–activity relationships of the benzene substructure in pyrimido[1,2-*c*][1,3]benzothiazine

A series of derivatives with modification of the benzene substructure in the pyrimido[1,2-*c*][1,3]benzothiazine were evaluated for anti-HIV activity (Table 2). The addition of positively charged *N*,*N*-dimethylamino (**28a**) and *N*-methylamino groups (**28b**) at the 9-position significantly decreased the anti-HIV activity. The 9-acetamide group (**28c**), which has hydrogen-bond donor/acceptor abilities, also attenuated the bioactivity. The acetyl (**28d**) and nitro (**28e**) groups, with hydrogen acceptor properties, induced slight decreases in the anti-HIV activity. In contrast, derivatives with less-polarized substituents (**28f–o** and **28q**) at this position generally reproduced the potent anti-HIV activity of **4**. In terms of the electron-donating or -withdrawing properties of the substituent groups on the benzene substructure, good correlations were not observed. For example, the electron-donating methoxy (**28f**), methyl (**28g**), and *n*-butyl groups (**28h**), and the electron-withdrawing fluoro (**28i**) and trifluoromethyl groups (**28j**) exhibited similar anti-HIV activities (EC_{50} =

Table 2 SARs for benzene part

Compound		EC_{50} (μM)[a]	Compound		EC_{50} (μM)[a]
			**30r**		0.56 ± 0.13
4	R = H	0.44 ± 0.08			
28a	R = NMe_2	4.74 ± 1.07			
28b	R = NHMe	>10			
28c	R = NHAc	>10	**30s**		2.55 ± 0.26
28d	R = COMe	1.44 ± 0.33			
28e	R = NO_2	1.13 ± 0.18			
28f	R = OMe	0.57 ± 0.09			
28g	R = Me	0.49 ± 0.10			
28h	R = *n*-butyl	0.44 ± 0.09			
28i	R = F	0.50 ± 0.07	**30k**		>10
28j	R = CF_3	0.53 ± 0.12			
28k	R = Br	0.25 ± 0.09			
28l	R = Ph	0.24 ± 0.04			
28m	R = vinyl	0.18 ± 0.05			
28n	R = styryl	0.25 ± 0.05			
28o	R = pentenyl	0.24 ± 0.11	**30t**		>10
28p	R = N_3	0.43 ± 0.06			
28q	R = C_6H_4(4-Bz)	0.53 ± 0.12			
			**30i**		1.68 ± 0.19
29a	R = NMe_2	2.12 ± 0.26			
29e	R = NO_2	3.00 ± 0.59			
29f	R = OMe	0.53 ± 0.04			
29g	R = Me	0.38 ± 0.04			
29k	R = Br	0.24 ± 0.05			
29l	R = Ph	0.24 ± 0.05	**30u**		>10
29m	R = vinyl	0.40 ± 0.09			
29q	R = C_6H_4(4-Bz)	0.67 ± 0.16			

[a] EC_{50} values were the concentration that blocks HIV-1 infection by 50% and derived from three independent experiments.

0.44–0.57 μM), indicating that the antiviral activity is independent of the electronic state of the 1,3-benzothiazin-2-imine core in forming potential π-stacking interaction(s) with the target molecules. Among the hydrophobic substituents at this position, bromo (**28k**), phenyl (**28l**), vinyl (**28m**), styryl (**28n**), and pentenyl groups (**28o**) induced inhibitory activity two or three times greater than that of **4** (EC_{50} = 0.18–0.25 μM). Modification with photoreactive azido (**28p**) and benzoylphenyl groups (**28q**) maintained the inhibitory activity; these could be used as probe molecules to identify the target molecule(s) of **4**.[26]

Similar SARs were observed for modification at the 10-position of pyrimido[1,2-*c*][1,3]benzothiazine. Addition of positively charged *N*,*N*-dimethylamino (**29a**) and polarized nitro groups (**29e**) reduced the anti-HIV activity (EC_{50} = 2.12 and 3.00 μM, respectively). Hydrophobic groups including methoxy (**29f**), methyl (**29g**), bromo (**29k**), phenyl (**29l**), vinyl (**29m**), and 4-benzoylphenyl (**29q**) (EC_{50} = 0.24–0.67 μM) had favorable effects on the bioactivity, suggesting potential hydrophobic interactions of these additional functional groups with the target molecule(s).

Further miscellaneous modifications of benzothiazine substructure were also investigated (Table 2). The naphtho[2,3-*e*][1,3]thiazine derivative **30r**, with a 9,10-fused benzene, exhibited anti-HIV activity equipotent to that of the parent **4** (EC_{50} = 0.56 μM). A 6-fold decrease in the anti-HIV activity of the pyridine-fused pyrido[3,2-*e*][1,3]thiazine derivative (**30s**) was observed (EC_{50} = 2.55 μM). In addition, introduction of 8-bromo (**30k**) and 8,9-fused benzene (**30t**, naphtho[2,1-*e*][1,3]thiazine) substituents on benzothiazine resulted in a loss of activity, suggesting that modification at the 8-position was inappropriate for favorable interactions with the target molecule(s). The 11-fluoro derivative **30i** and thiophene-fused **30u**, which has a 5-6-6 framework (thieno[2,3-*e*][1,3]thiazine), exhibited four times lower and no inhibitory potencies, respectively.

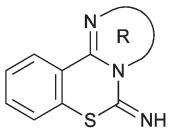
Structure–activity relationships of cyclic amidine part of pyrimido[1,2-*c*][1,3]benzothiazine

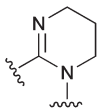
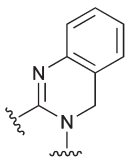
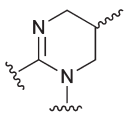
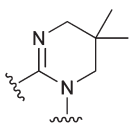
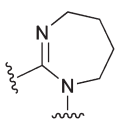
A SAR study of the top-right cyclic amidine substructure was carried out. The five-membered dihydroimidazole derivative **35a** had no anti-HIV activity (Table 3), suggesting that the five-membered ring may impair the critical interactions with the target molecule(s) *via* its small-sized ring strain or indirect effects on the thiazinimine core with a possibly altered conformation. Similarly, compound **35b** with the phenyl-fused dihydropyrimidine substructure showed lower inhibitory activity (EC_{50} = 3.78 μM). Appending one or two methyl groups on the six-membered pyrimidine (**35c** and **35d**) induced 1.5- to 2-fold higher inhibitory potencies (EC_{50} = 0.35 and 0.24 μM, respectively) compared with that of the parent compound **4**. In addition, compound **35e** with a seven-membered tetrahydro-1,3-diazepine substructure exhibited similar anti-HIV activity to that of **4** (EC_{50} = 0.31 μM).

Mechanistic studies of anti-HIV pyrimido[1,2-*c*][1,3]-benzothiazin-6-imines and related tricyclic heterocycles

To investigate the mechanism of action of PD 404182 derivatives, a time of drug addition study was carried out (Fig. 3). In this experiment, the anti-HIV activity profiles of **4**[27] and its derivatives **29k**[27] were compared with those of well-known anti-HIV agents such as an adsorption inhibitor (DS 5000),[28] fusion inhibitor [enfuvirtide (T-20)],[5] NRTI (AZT),[29] NNRTI (nevirapine),[30] and integrase inhibitor (raltegravir).[6] After inoculation of HeLa-CD4/CCR5-LTR/β-gal cells with HIV-1_{IIIB}, each anti-HIV-1 drug was added at a 90% inhibitory effect concentration at the indicated time points. The inhibitory effects on the infection were determined by counting the blue cells 48 h later. This investigation revealed that compound **4** (PD 404182) had an inhibitory profile in the early stage of viral infection similar to those of DS 5000 and enfuvirtide (Fig. 3). Identical profiles were observed for derivative **29k**.

Table 3 SARs for cyclic amidine



Compound		EC_{50} (μM)[a]
4		0.44 ± 0.08
35a		>10
35b		3.78 ± 1.39
35c		0.35 ± 0.09
35d		0.24 ± 0.04
35e		0.31 ± 0.06

[a] EC_{50} values were the concentration that blocks HIV-1 infection by 50% and derived from three independent experiments.

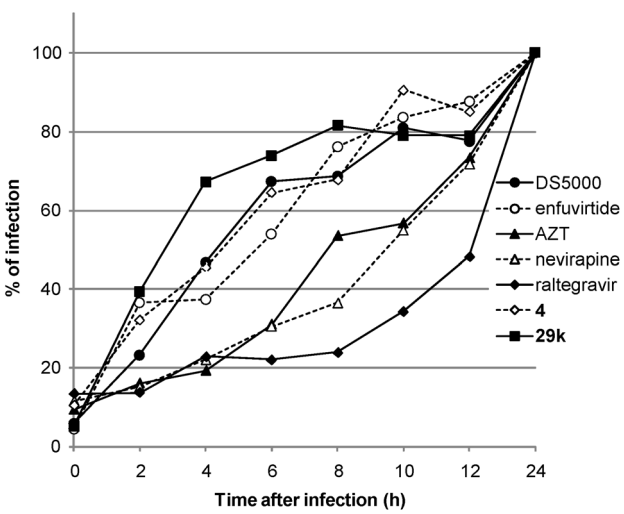


Fig. 3 Time of drug addition profiles for infection by HIV-1_{IIIB} strain of HeLa-CD4/CCR5-LTR/β-gal cells.

Table 4 Anti-HIV activity of **4** and **35d** against other HIV strains

Strain	EC_{50} (μM)[a]	
	4	**35d**
$HIV\text{-}1_{NL4\text{-}3}$	0.38 ± 0.06	0.25 ± 0.03
$HIV\text{-}1_{BaL}$	0.37 ± 0.06	0.16 ± 0.02
$HIV\text{-}2_{EHO}$	0.31 ±0.06	0.17 ± 0.03
$HIV\text{-}2_{ROD}$	0.30 ± 0.06	0.11 ± 0.03

[a] EC_{50} values were the concentration that blocks HIV infection by 50% and derived from three independent experiments.

To gain additional insights into the mechanism of action of PD 404182 derivatives, the antiviral activities against other HIV subtypes were evaluated (Table 4). Compound **4** was effective against not only $HIV\text{-}1_{IIIB}$ but also other two HIV-1 strains ($HIV\text{-}1_{NL4\text{-}3}$ and $HIV\text{-}1_{BaL}$) with similar potency. Both $HIV\text{-}1_{IIIB}$ and $HIV\text{-}1_{NL4\text{-}3}$ strains utilize CXCR4 as a coreceptor for entry, while $HIV\text{-}1_{BaL}$ strain does CCR5, indicating that chemokine receptors CXCR4 and CCR5 are not the molecular targets of PD 404182 derivatives. The similar level of antiviral activity of **4** against HIV-2 ($HIV\text{-}2_{EHO}$ and $HIV\text{-}2_{ROD}$), which is mainly distributed in West Africa, was observed. Highly potent inhibitory activities of a derivative **35d**[27] against these HIV strains were observed, as in the case of the SAR study of the $HIV\text{-}1_{IIIB}$ strain discussed above. It has been well known that NNRTIs are not effective against HIV-2, highlighting that PD 404182 derivatives do not act as NNRTIs. Although PD 404182 derivatives and enfuvirtide showed similar anti-HIV-1 profile in the time of drug addition assay, $HIV\text{-}2_{EHO}$ and $HIV\text{-}2_{ROD}$ infection were affected by PD 404182 derivatives, in contrast with the less effective enfuvirtide,[31] suggesting that PD 404182 derivatives may not be directed at the HIV gp41 envelope protein. Recent reports have suggested that the antiviral activities of compound **4** against HIV, HCV, and pseudotype lentiviruses were derived from disruption of the structural integrities of virions.[17] Although the mechanism of action of PD 404182 derivatives is not fully understood at this stage, the unidentified biomolecule(s) in viruses or host cells, including envelope protein(s), lipid membranes and/or sugar chain(s), could be promising molecular targets for this new class of anti-HIV agents.

Conclusion

In conclusion, we have designed and synthesized PD 404182 derivatives for a novel series of anti-HIV agents. Comprehensive SAR studies demonstrated that the 6-6-6 fused pyrimido[1,2-*c*]-[1,3]benzothiazine scaffold and the heteroatom arrangement in the thiazinimine moiety are indispensable for the inhibitory activity of **4** (PD 404182) against HIV infection. Optimization studies of the benzene and cyclic amidine rings indicate that the introduction of a hydrophobic group on the benzene ring and the amidine group is more effective in improving the antiviral activity, giving potential favorable interaction(s) with the target molecule(s). In addition, PD 404182 derivatives could be promising agents for treatment of HIV-2 infection. We also revealed, using time of drug addition experiments, that PD 404182 derivatives prevent the HIV infection process at an early stage. For iterative molecular design of more effective derivatives based on binding modes, the identification of the target molecule(s) of PD 404182 derivatives is being investigated using derivatives such as **28p** and **28q**.

Experimental section

General

^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 Me_4Si as an internal standard. ^{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), TLC Aluminium oxide 60 F_{254} basic (Merck), or NH_2 Silica Gel 60 F_{254} Plate (Wako) 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 cm^3 min^{-1} 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% unless otherwise stated.

General procedure of oxidative amidination: synthesis of 2-(3-bromo-2-fluorophenyl)-1,4,5,6-tetrahydropyrimidine (23k). To a solution of 3-bromo-2-fluorobenzaldehyde **20k** (0.71 g, 3.5 mmol) in *t*-BuOH (33 cm^3) was added propylenediamine (285.4 mg, 3.9 mmol). The mixture was stirred at 70 °C for 30 min, and then K_2CO_3 (1.45 g, 10.5 mmol) and I_2 (1.11 g, 4.4 mmol) were added. After being stirred at the same temperature for 3 h, the mixture was quenched with sat. Na_2SO_3. The organic layer was separated and concentrated. The resulting solid was dissolved in H_2O, and then pH was adjusted to 12–14 with 2N NaOH. The whole was extracted with $CHCl_3$. The extract was dried over $MgSO_4$. After concentration, the resulting solid was recrystallized from $CHCl_3$–*n*-hexane to give compound **23k** as colorless crystals (0.62 g, 69%): mp 99 °C; IR (neat) ν_{max}/cm^{-1}: 1624 (C═N); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.84–1.89 (2H, m, CH_2), 3.50 (4H, t, J = 5.7 Hz, 2 × CH_2), 5.13 (1H, br s, NH), 7.03 (1H, td, J = 8.0, 0.9 Hz, Ar), 7.54 (1H, ddd, J = 8.0, 6.4, 1.3 Hz, Ar) and 7.69 (1H, ddd, J = 8.0, 6.5, 1.3 Hz, Ar). δ_C (100 MHz; $CDCl_3$) 20.6, 42.1 (2C), 109.6 (d, J = 22.3 Hz), 125.1 (d, J = 4.1 Hz), 126.3 (d, J = 13.2 Hz), 129.8 (d, J = 3.3 Hz), 134.3, 150.8 and 156.3 (d, J = 248.3 Hz); δ_F (500 MHz; $CDCl_3$) −110.7; *Anal.* Calc. for $C_{10}H_{10}BrFN_2$: C, 46.72; H, 3.92; N, 10.90. Found: C, 46.64; H, 4.10; N, 10.93%.

General procedure of CS_2-mediated cyclization for pyrimido-[1,2-*c*][1,3]benzothiazine-6-thiones 24: synthesis of 3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*]thieno[2,3-*e*][1,3]thiazin-6-thione (24u). To a mixture of 2-(3-bromothiophen-2-yl)-1,4,5,6-tetrahydropyrimidine **23u** (122.6 mg, 0.50 mmol) and NaH (40.0 mg,

1.0 mmol; 60% oil suspension) in DMF (1.7 cm^3) was added CS_2 (0.060 cm^3, 1.0 mmol) under an Ar atmosphere. After being stirred at 80 °C for 12 h, the mixture was concentrated. The residue was purified by flash chromatography over silica gel with *n*-hexane–EtOAc (8 : 2) to give the compound **24u** as a pale yellow solid (80.5 mg, 67%): mp 167 °C (from $CHCl_3$–*n*-hexane); IR (neat) ν_{max}/cm^{-1}: 1624 (C═N); δ_H (400 MHz; $CDCl_3$; Me_4Si) 2.04–2.10 (2H, m, CH_2), 3.68 (2H, t, J = 5.5 Hz, CH_2), 4.42 (2H, t, J = 6.1 Hz, CH_2), 6.76 (1H, d, J = 5.4 Hz, Ar) and 7.49 (1H, d, J = 5.4 Hz, Ar). δ_C (100 MHz; $CDCl_3$) 21.5, 45.0, 48.5, 122.3, 128.4, 130.8, 131.0, 141.7 and 189.7; HRMS (FAB): *m*/*z* Calc. for $C_9H_9N_2S_3$ $[M + H]^+$ 240.9928; found: 240.9936.

General procedure of *t*-BuNCS-mediated cyclization for *t*-Bu protected pyrimido[1,2-*c*][1,3]thiazin-6-imines 25–27, and 34: synthesis of *N*-(*tert*-butyl)-3,4-dihydro-9-nitro-2*H*,6*H*-pyrimido[1,2-*c*]-[1,3]benzothiazin-6-imine (25e). To a mixture of 2-(2-fluoro-4-nitrophenyl)-1,4,5,6-tetrahydropyrimidine **21e** (2.0 g, 8.96 mmol) and NaH (716.8 mg, 17.92 mmol; 60% oil suspension) in DMF (29.8 cm^3) was added *t*-BuNCS (2.28 cm^3, 17.92 mmol) under an Ar atmosphere. After being stirred at −20 °C to rt for 2 days, 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 aluminium oxide with *n*-hexane–EtOAc (10 : 0 to 9 : 1) to give compound **25e** as a pale yellow solid (1.77 g, 62%): mp 152–153 °C (from $CHCl_3$–*n*-hexane); IR (neat) ν_{max}/cm^{-1}: 1604 (C═N), 1591 (NO_2), 1581 (C═N), 1523 (NO_2); δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.39 (9H, s, 3 × CH_3), 1.91–1.96 (2H, m, CH_2), 3.66 (2H, t, J = 5.2 Hz, CH_2), 3.88 (2H, t, J = 5.7 Hz, CH_2), 7.97 (2H, dd, J = 9.7, 2.3 Hz, Ar), 8.01 (2H, d, J = 2.3 Hz, Ar) and 8.39 (1H, d, J = 9.2 Hz, Ar). δ_C (125 MHz; $CDCl_3$) 21.7, 30.0, 45.3, 45.5, 54.5, 119.9, 120.3, 130.0, 131.1, 132.8, 136.1, 146.5 and 148.5; HRMS (FAB): *m*/*z* Calc. for $C_{15}H_{19}N_4O_2S$ $[M + H]^+$ 319.1229; found: 319.1229.

General procedure of BrCN-mediated cyclization for pyrimido-[1,2-*c*][1,3]thiazin-6-imines 28 and 30: synthesis of 3,4-dihydro-9-methyl-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (28g). 3,4-Dihydro-9-methyl-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazine-6-thione **24g** (62.1 mg, 0.25 mmol) was suspended in a 0.1 M solution of NaOH in MeOH–H_2O (9 : 1) (5 cm^3). After being stirred for 12 h under reflux, the mixture was concentrated. The residue was suspended in anhydrous EtOH (1 cm^3) and BrCN (53.0 mg, 0.50 mmol) was added under Ar atmosphere. After stirring for 2 h under reflux, the reaction mixture was quenched 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 *n*-hexane–EtOAc (9 : 1) to give the compound **28g** as colorless solid (39.2 mg, 68%): mp 121 °C (from $CHCl_3$–*n*-hexane); IR (neat) ν_{max}/cm^{-1}: 1620 (C═N), 1569 (C═N); δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.94–1.99 (2H, m, CH_2), 2.32 (3H, s, CH_3), 3.67 (2H, t, J = 5.7 Hz, CH_2), 4.01 (2H, t, J = 6.3 Hz, CH_2), 6.84 (1H, s, Ar), 7.02 (1H, d, J = 8.6 Hz, Ar), 7.16 (1H, br s, NH) and 8.10 (1H, d, J = 8.6 Hz, Ar). δ_C (125 MHz; $CDCl_3$) 21.1, 21.1, 43.8, 44.9, 123.6, 124.1, 127.4, 128.6, 128.8, 141.1, 146.6 and 153.6; HRMS (FAB): *m*/*z* Calc. for $C_{12}H_{14}N_3S$ $[M + H]^+$ 232.0908; found: 232.0912.

General procedure of *tert*-butyl deprotection for pyrimido[1,2-*c*][1,3]benzothiazin-6-imines 28–30: synthesis of 3,4-dihydro-9-nitro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine (28e). TFA (1.5 cm^3) was added to a mixture of *N*-(*tert*-butyl)-3,4-dihydro-9-nitro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine **25e** (47.8 mg, 0.15 mmol) in $CHCl_3$ and molecular sieves 4 Å (225 mg, powder, activated by heating with Bunsen burner). After being stirred under reflux for 1.5 h, the mixture was concentrated. To a mixture of this residue in $CHCl_3$ was added dropwise Et_3N at 0 °C to adjust the pH to 8–9. The whole was extracted with EtOAc, and the extract was washed with sat. $NaHCO_3$, brine, and dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over aluminium oxide with *n*-hexane–EtOAc (19 : 1 to 1 : 1) to give compound **28e** as a pale yellow solid (24.9 mg, 63%): mp 170–172 °C (from $CHCl_3$–*n*-hexane); IR (neat) ν_{max}/cm^{-1}: 1620 (C═N), 1587 (NO_2), 1568 (C═N), 1523 (NO_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.97–2.03 (2H, m, CH_2), 3.74 (2H, t, J = 5.6 Hz, CH_2), 4.04 (2H, t, J = 6.2 Hz, CH_2), 7.41 (1H, br s, NH), 7.93 (1H, d, J = 2.2 Hz, Ar), 8.00 (1H, dd, J = 9.0, 2.2 Hz, Ar) and 8.42 (1H, d, J = 9.0 Hz, Ar). δ_C (100 MHz; $CDCl_3$) 20.8, 43.8, 45.2, 118.9, 120.5, 130.4, 130.8, 131.7, 145.1, 148.7 and 151.3; *Anal.* Calc. for $C_{11}H_{10}N_4O_2S$: C, 50.37; H, 3.84; N, 21.36. Found: C, 50.29; H, 4.03; N, 21.08%.

General procedure of Suzuki–Miyaura cross coupling for 9-aryl pyrimido[1,2-*c*][1,3]thiazine derivatives: synthesis of *N*-(*tert*-butyl)-3,4-dihydro-9-phenyl-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine 25l. To a solution of 9-bromo-*N*-(*tert*-butyl)-3,4-dihydro-2*H*,6*H*-pyrimido[1,2-*c*][1,3]benzothiazin-6-imine **25k** (52.8 mg, 0.15 mmol) and phenylboronic acid (21.9 mg, 0.18 mmol) in a mixture of toluene (1.5 cm^3), EtOH (0.9 cm^3) and 1 M aq. K_2CO_3 (1.5 cm^3) was added $Pd(PPh_3)_4$ (6.9 mg, 4 mol%) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (3.67 mg, 3 mol%). After being stirred at 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 aluminium oxide with *n*-hexane–EtOAc (10 : 0 to 9 : 1) to give the compound **25l** as colorless solid (44.8 mg, 85%): mp 122.5–124 °C (from $CHCl_3$–*n*-hexane); IR (neat) ν_{max}/cm^{-1}: 1592 (C═N); δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.40 (9H, s, 3 × CH_3), 1.90–1.95 (2H, m, CH_2), 3.64 (2H, t, J = 5.4 Hz, CH_2), 3.89 (2H, t, J = 6.0 Hz, CH_2), 7.33–7.37 (2H, m, Ar), 7.41–7.44 (3H, m, Ar), 7.58 (2H, d, J = 6.9 Hz, Ar) and 8.25 (1H, d, J = 8.6 Hz, Ar). δ_C (125 MHz; $CDCl_3$) 21.9, 30.0 (3C), 45.1, 45.4, 54.2, 122.7, 124.8, 126.5, 127.0 (2C), 128.0, 128.8 (2C), 128.9, 129.5, 138.3, 139.4, 142.9 and 147.7; HRMS (FAB): *m*/*z* Calc. for $C_{21}H_{24}N_3S$ $[M + H]^+$ 350.1691; found: 350.1683.

Determination of anti-HIV activity

The sensitivity of three HIV-1 strains and two HIV-2 strains was determined by the MAGI assay.[32] The target cells (HeLa-CD4/CCR5-LTR/β-gal; 104 cells per 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 per well, giving 60 blue

cells after 48 h of incubation) and cultured in the presence of various concentrations of the drugs in fresh medium. Forty-eight hours after viral exposure, all the blue cells strained 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:

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

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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