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Studies of nonnucleoside HIV-1 reverse transcriptase inhibitors. Part 1: Design and synthesis of thiazolidenebenzenesulfonamides

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Abstract—A random high-throughput screening (HTS) program to discover novel nonnucleoside reverse transcriptase inhibitors (NNRTIs) has been carried out with MT-4 cells against a nevirapine-resistant virus, HIV-$1_{IIIB\text{-}R}$. The primary hit, a thiazolidenebenzenesulfonamide derivative, possessed good activity. A systematic modification program examining various substituents at the 3-, 4-, and 5-positions on the thiazole ring afforded compounds with enhanced anti-HIV-1 and reverse transcriptase (RT) inhibitory activities. These results confirm the important role of the substituents at these positions and the thiazolidenebenzenesulfonamide motif as a valuable lead series for the next generation NNRTIs.

1. Introduction

Acquired immunodeficiency syndrome (AIDS), which is caused by the human immunodeficiency virus type 1 (HIV-1) and results in life-threatening opportunistic infections and malignancies, has become a major worldwide pandemic.[1,2] Three million people had died from AIDS and 40 million people were living with HIV-1 or AIDS at the end of 2003.[3] From the beginning of anti-HIV-1 chemotherapy development, HIV-1 reverse transcriptase (RT) has been one of the main targets, and the majority of drugs used clinically are RT inhibitors. RT inhibitors can be classified into two groups: nucleoside reverse transcriptase inhibitors (NRTIs), which act as chain terminators to block the elongation of the HIV-1 viral DNA strand, and nonnucleoside reverse transcriptase inhibitors (NNRTIs), which directly inhibit RT enzyme by binding to the allosteric site near the polymerase active site.[4]

NRTIs inhibit RT selectively but are considerably toxic to cellular and mitochondrial DNA synthesis.[5] In this regard, NNRTIs are more specific and less toxic than NRTIs because they do not affect the activity of cellular polymerases. On the other hand, during NNRTI monotherapy for HIV-1-infected patients[6] and in vitro culture

Keywords: Thiazolidene; Benzenesulfonamide; Nonnucleoside HIV-1 reverse transcriptase inhibitor.
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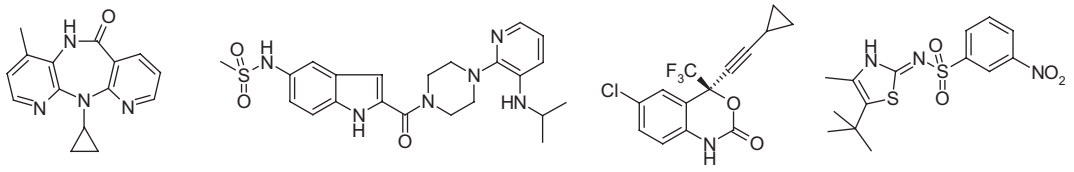


1 (nevirapine) **2** (delavirdine) **3** (efavirenz) **4**

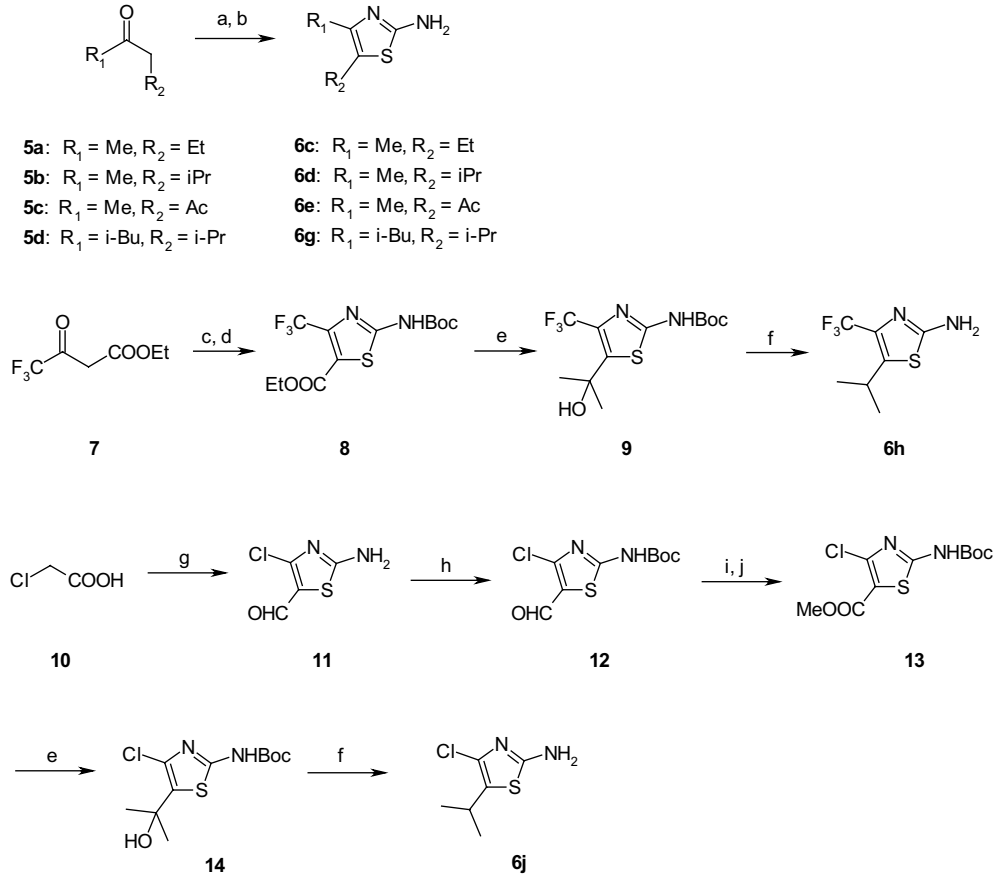
Figure 1. Structures of NNRTIs and lead compound **4**.

experiments,[7] rapid emergence of highly drug resistant viruses is often observed. In the case of nevirapine (**1**), the primary cause of drug resistance is the substitution of tyrosine by cysteine at the 181 position in HIV-1 RT (Y181C).[8] In fact, the Y181C mutant RT is less susceptible to nevirapine in vitro than the wild-type (WT) enzyme.[9] Furthermore, the RT mutation of the lysine at the 103 position to asparagine (K103N) is frequently observed in patients who have failed in the treatment with antiretroviral regimens containing currently available NNRTIs, such as nevirapine (**1**), delavirdine (**2**), and efavirenz (**3**).[10] Therefore, it is still important to find a new lead compound that may be able to overcome the resistance issue of NNRTIs (Fig. 1).

In this paper, we have examined the synthesis and structure–activity relationship (SAR) analysis of thiazolidenebenzenesulfonamide derivatives and found that a novel class of NNRTIs is active against the Y181C and K103N mutants.

2. Chemistry

A series of 4,5-dialkyl-2-aminothiazole derivatives, **6c–e** and **6g**, were synthesized (Scheme 1). The α-bromination of ketones **5a–d** under thermodynamic conditions[11] and subsequent cyclization using thiourea afforded 4,5-dialkyl-2-aminothiazoles **6c–e** and **6g**. 4-Trifluoromethyl-2-



5a: R_1 = Me, R_2 = Et
5b: R_1 = Me, R_2 = iPr
5c: R_1 = Me, R_2 = Ac
5d: R_1 = i-Bu, R_2 = i-Pr

6c: R_1 = Me, R_2 = Et
6d: R_1 = Me, R_2 = iPr
6e: R_1 = Me, R_2 = Ac
6g: R_1 = i-Bu, R_2 = i-Pr

7 **8** **9** **6h**

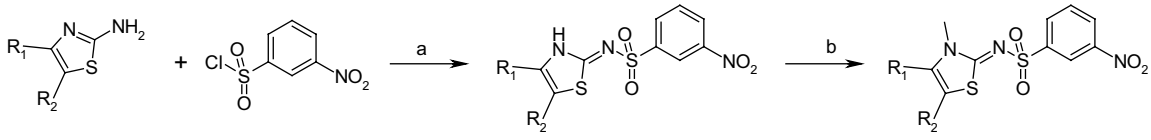
10 **11** **12** **13**

14 **6j**

Scheme 1. Reagents: (a) TMSBr, DMSO/CH_3CN; (b) thiourea/EtOH; (c) thiourea/I_2, *i*-PrOH; (d) Boc_2O, DMAP/THF; (e) MeLi/THF; (f) Et_3SiH/TFA; (g) thiourea, $POCl_3$, $Ca(OH)_2$, NaCl/DMF; (h) Boc_2O, DMAP/dioxane; (i) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene/*t*-BuOH; (j) MeOH, water soluble carbodiimide, HOBt, DMAP/DMF.

aminothiazole **6h** was synthesized from trifluoroacetoacetic acid ethyl ester **7** in five steps. The iodine-mediated cyclization of ester **7** with thiourea in isopropanol and the protection of the resultant amine with Boc anhydride provided compound **8**. The bis-alkylation of **8** using methyllithium provided 5-hydroxymethylethyl-2-aminothiazole **9**. The treatment of **9** with triethylsilane and trifluoroacetic acid effectively removed the tertiary hydroxy group and the Boc group, respectively, to afford the desired 4-trifluoromethyl-2-aminothiazole **6h**. 4-Chloro-2-aminothiazole **6j** was synthesized from chloroacetic acid **10** in six steps. The cyclization of chloroacetic acid **10** with thiourea, and subsequent formylation and chlorination, resulted in 5-formylthiazole **11**.[12] Treatment of **11** with Boc_2O, followed by oxidation and esterification, afforded the methyl ester **13**. Treatment of compound **13** with methyllithium, then triethylsilane and trifluoroacetic acid afforded the aminothiazole **6j**.

The 2-aminothiazoles **6a–j** were reacted with 3-nitrobenzenesulfonylchloride (**15**) to afford the thiazolidenebenzenesulfonamide derivatives **4** and **16a–j** (Scheme 2). Methylation of **4** and **16a–j** using methyl iodide in the presence of sodium hydride afforded the thiazolidenesulfonamides **17a–k**. Compound **4** was also converted into the corresponding *N*-ethyl derivative **17l** in a similar manner employing ethyl iodide. Alkylation of the nitrogen at the 3-position in **17a–l** was confirmed by NMR (Fig. 2). A nuclear Overhauser effect (NOE) was observed between the 3-methyl protons and the 4-methyl protons, but not between the 3-methyl protons

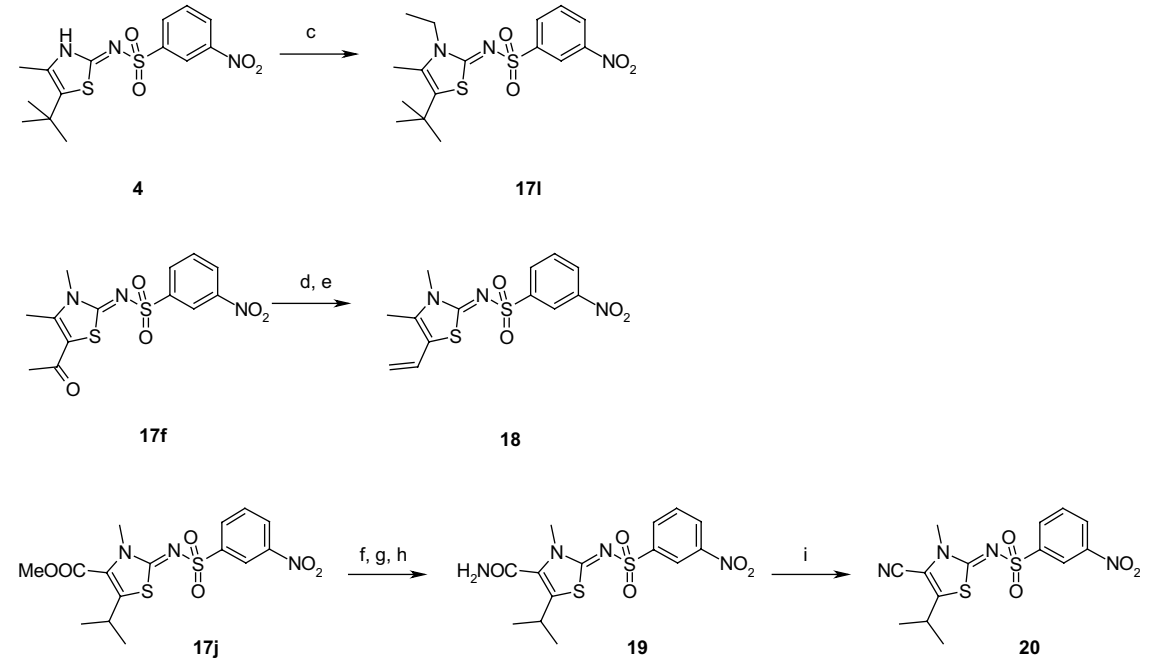


6a: R_1 = Me, R_2 = t-Bu
6b: R_1 = Me, R_2 = H
6c: R_1 = Me, R_2 = Et
6d: R_1 = Me, R_2 = i-Pr
6e: R_1 = Me, R_2 = Ac
6f: R_1 = H, R_2 = i-Pr
6g: R_1 = i-Bu, R_2 = i-Pr
6h: R_1 = CF_3, R_2 = i-Pr
6i: R_1 = COOMe, R_2 = i-Pr
6j: R_1 = Cl, R_2 = i-Pr

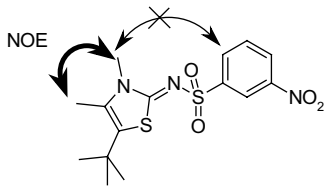
15

4: R_1 = Me, R_2 = t-Bu
16a: R_1 = Me, R_2 = H
16b: R_1 = Me, R_2 = Me
16c: R_1 = Me, R_2 = Et
16d: R_1 = Me, R_2 = i-Pr
16e: R_1 = Me, R_2 = Ac
16f: R_1 = H, R_2 = i-Pr
16g: R_1 = i-Bu, R_2 = i-Pr
16h: R_1 = CF_3, R_2 = i-Pr
16i: R_1 = COOMe, R_2 = i-Pr
16j: R_1 = Cl, R_2 = i-Pr

17a: R_1 = Me, R_2 = t-Bu
17b: R_1 = Me, R_2 = H
17c: R_1 = Me, R_2 = Me
17d: R_1 = Me, R_2 = Et
17e: R_1 = Me, R_2 = i-Pr
17f: R_1 = Me, R_2 = Ac
17g: R_1 = H, R_2 = i-Pr
17h: R_1 = i-Bu, R_2 = i-Pr
17i: R_1 = CF_3, R_2 = i-Pr
17j: R_1 = COOMe, R_2 = i-Pr
17k: R_1 = Cl, R_2 = i-Pr



Scheme 2. Reagents: (a) Py; (b) MeI, NaH/THF; (c) EtI, NaH/THF; (d) $NaBH_4$/EtOH; (e) MsCl, Et_3N/CH_2Cl_2; (f) NH_3aq/THF; (g) $SOCl_2$; (h) NH_3aq/$CHCl_3$; (i) $POCl_3$, DMF/1,2-dichloroethane.



17a

Figure 2. Confirmation of the structure of **17a**. An NOE was observed between the 3-methyl and 4-methyl protons on the thiazole ring, whereas no NOE signal was observed between the 3-methyl proton on the thiazole ring and the phenyl ring.

and aromatic protons, therefore the alkylated position was assigned to the ring nitrogen. Treatment of ketone **17f** with sodium borohydride and dehydration of the resulting alcohol generated vinyl derivative **18**. Hydrolysis of **17j** and subsequent amidation afforded carboxamide **19**, which on dehydration afforded nitrile **20**.

3. Results and discussion

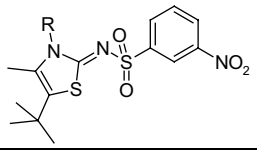
In order to identify the compounds that exhibit anti-HIV-1 activity against the Y181C mutant of HIV-1, we conducted a large-scale high-throughput screening (HTS) of in-house compound libraries in MT-4 cells acutely infected with HIV-$1_{IIIB\text{-}R}$. HIV-$1_{IIIB\text{-}R}$ is a nevirapine- and MKC-442-resistant HIV-1 strain, having the Y181C mutation. Our screening identified compound **4** as a primary hit, which possessed good anti-HIV-1 activity against both HIV-1_{IIIB} and HIV-$1_{IIIB\text{-}R}$ with EC_{50} values of 0.50 and 0.79 μM, respectively (data not shown). Compound **4** inhibited WT, Y181C, and K103N RTs with IC_{50} values of 0.37, 0.47, and 32 μM, respectively (Table 1).

In order to increase the inhibitory activity against WT, Y181C, and K103N RTs as well as HIV-1 replication, we synthesized a series of compound **4** analogues and evaluated for their inhibitory effects on these enzymes and HIV-1 replication in acutely infected MT-4 cells (Tables 1 and 2).

Previous studies have shown that two aromatic systems arranged in a 'butterfly-like orientation' are required for the inhibition of RT enzymes.[13,14] We therefore attempted to alkylate the ring nitrogen on the thiazole ring of compound **4** in order to confer a 'butterfly-like orientation' on the conformation of **4**. The introduction of the methyl group at the 3-position on the thiazole ring, compound **17a**, resulted in a 7- and 2.5-fold increase of the activity against Y181C and K103N RTs, respectively, when compared to unsubstituted compound **4**. In contrast, the introduction of an ethyl group (compound **17l**) at the 3-position significantly reduced the activity against the enzymes, confirming that the methyl group at the 3-position is crucial to the inhibition of these RTs. X-ray crystallography of **17a** revealed that the 3-methylated thiazolidenesulfonamide framework had a (*Z*)-thiazolidene conformation and provided a 'butterfly-like' structure in the two aromatic systems, as observed in other NNRTIs (Fig. 3).[13,14] In the case of **17a**, one oxygen atom of the sulfonyl group existed in the same plane of the phenyl ring, and the other existed in the plane on the thiazole ring. As a result, the RT inhibition was enhanced by the rigid conformation of the 'butterfly-like' (*Z*)-structure derived from methylation at the 3-position on the thiazole ring. Although the activity of **17a** against the WT RT was similar to that of **4**, the anti-HIV-1 activity of **17a** was 6-fold stronger than that of **4**. Consequently, the TI value of **17a** exceeded 290. Additionally, the introduction of the methyl group may be responsible for better cell permeability or compound stability under assay conditions.

We next investigated the effect of the substituents at the 5-position on the thiazole ring. As shown in Table 2, among the compounds possessing a methyl group at the 4-position, compounds **17a**, **17c–e**, and **18** retained the inhibitory activity against the WT RT. However, the introduction of an acetyl group at the 5-position (compound **17f**) led to a dramatic loss of the activity. Substitution with bulkier alkyl groups such as *tert*-butyl and isopropyl (compounds **17a** and **17e**; IC_{50} values of 0.066 and 0.071 μM, respectively) exhibited a superior activity against the Y181C RT but not against the WT or K103N RT: therefore the activity against the

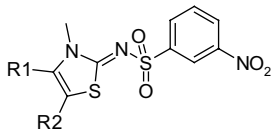
Table 1. In vitro activity of 3-substituted thiazolidene derivatives



Compounds	R	IC_{50} (μM)[a]			EC_{50} (μM)[b]	CC_{50} (μM)[c]	TI[d]
		WT	K103N	Y181C			
4	H	0.37	32	0.47	0.50	>25	>50
17a	Me	0.27	13	0.066	0.085	>25	>290
17l	Et	6.0	>50	0.12	>11.5	11.5	<1

[a] Compound concentration required to achieve 50% inhibition of recombinant HIV-1 RT activity.
[b] Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced CPE as determined by the MTT method.
[c] Compound concentration required to reduce the viability of mock-infected MT-4 cells as determined by the MTT method.
[d] Therapeutic index (CC_{50}/EC_{50}).

Table 2. In vitro activity of 4- and 5-substituted thiazolidene derivatives



Compounds	R_1	R_2	IC_{50} (μM)[a]			EC_{50} (μM)[b]	CC_{50} (μM)[c]	TI[d]
			WT	K103N	Y181C			
4			0.37	32	0.47	0.50	>25	>50
17a	Me	*t*-Bu	0.27	13	0.066	0.085	>25	>290
17b	Me	H	>50	>50	>50	>25	>25	—
17c	Me	Me	0.97	>50	33	1.1	>25	>23
17d	Me	Et	0.85	6.3	0.47	0.23	>25	>110
17e	Me	*i*-Pr	0.34	20	0.071	0.10	>25	>250
17f	Me	Ac	>50	>50	>50	>25	>25	—
17g	H	*i*-Pr	0.60	>50	0.70	3.1	>25	>8
17h	*i*-Bu	*i*-Pr	>50	>50	>25	>21.4	21.4	—
17i	CF_3	*i*-Pr	0.60	42	0.26	0.26	>25	>96
17j	COOMe	*i*-Pr	>50	>50	>25	>25	>25	—
17k	Cl	*i*-Pr	0.077	6.9	0.13	0.048	24	500
18	Me	CH=CH_2	0.15	6.2	9.6	0.097	4.4	45
19	$CONH_2$	*i*-Pr	39	>50	>25	>25	>25	—
20	CN	*i*-Pr	0.62	>50	0.64	0.71	>25	>35
Nevirapine (**1**)			0.0026	1.1	>1.9	0.0053	>25	>4700
Delavirdine (**2**)			0.042	4.8	7.5	0.0039	>25	>6400
Efavirenz (**3**)			0.0069	0.021	0.0040	0.0027	8.5	3200

[a–d]See footnotes in Table 1.

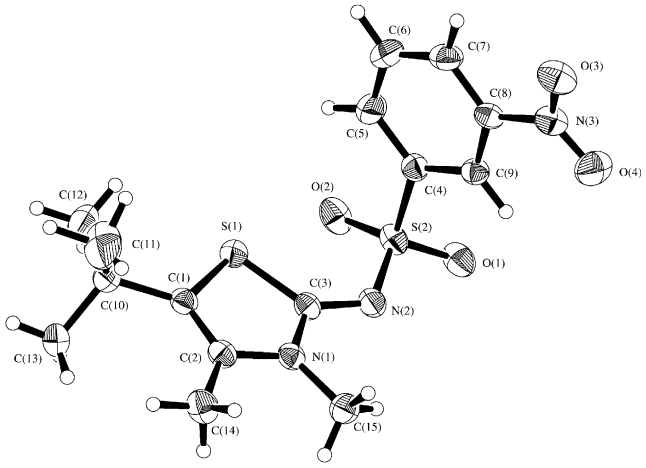


Figure 3. X-ray crystallography of compound **17a**.

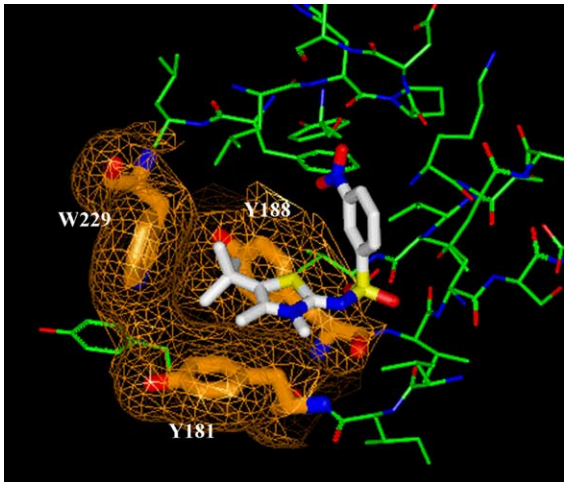


Figure 4. Docking study of **17a** within RT nonnucleoside binding site.

Y181C RT is affected by the nature of 5-position substituents. These results also suggest that the alkyl group at the 5-position exists near the Y181 of the HIV-1 RT. In the docking study of **17a** with a RT nonnucleoside binding site, it was predicted that the *tert*-butyl group would occupy the hydrophobic pocket constructed by Y181, Y188, and W229 and that it was positioned near the Y181 (Fig. 4). Therefore, the bulky alkyl groups at the 5-position on the thiazole ring seem to be crucial for the inhibition of the Y181C RT. With respect to the substituents at the 5-position on the thiazole ring, both ethyl (**17d**) and vinyl (**18**) groups improved the inhibitory activity against the K103N RT, and **17d** and **18** were significantly more potent than **4**. The K103N mutation reduced sensitivity to nevirapine and delavirdine 423- and 114-fold, respectively, but compounds **17a**, **17d**, and **18** were only 48-, 7-, and 41-fold less potent against the K103N mutant, respectively. Compounds **17a**, **17d**, **17e**, and **18** showed potent anti-HIV-1 activity with EC_{50} values of 0.085, 0.23, 0.10, and 0.097 μM, respectively. For each compound, its EC_{50} value was strongly associated with its IC_{50} value for WT RT.

In order to find more potent inhibitors, further modifications at the 4-position on the thiazole ring were performed. Among the 5-isopropyl derivatives prepared,

those with sterically small substituents at the 4-position exhibited the strong activity against both of the WT and the Y181C RTs: no apparent preference for either electron-withdrawing or electron-donating substituents was observed. Those compounds with methyl (**17e**), trifluoromethyl (**17i**), and cyano (**20**) substituents at the 4-position possessed the inhibitory activity similar to that of the unsubstituted compound (**17g**). However, the compounds with a bulkier *iso*-butyl group (**17h**) or hydrophilic groups, such as methoxycarbonyl (**17j**) and carbamoyl (**19**), showed a lack of RT inhibition. The 4-methyl derivative (**17e**) showed the strongest activity against the Y181C RT with an IC_{50} value of 0.071 μM, which was five times greater than its activity against the WT RT (IC_{50}: 0.34 μM). In contrast to **17e**, the 4-chloro derivative (**17k**) showed a stronger activity against the WT RT with an IC_{50} value of 0.077 μM, and this value was about twice stronger than against the Y181C RT (IC_{50}: 0.13 μM). The introduction of a chloro group to the 4-position (**17k**) also improved the activity against K103N RT (IC_{50}: 6.9 μM) when compared to **17e**. Among the 5-isopropyl derivatives, trifluoromethyl (**17i**), chloro (**17k**), and cyano derivatives (**20**) possessed potent anti-HIV-1 activity with EC_{50} values of 0.26, 0.048, and 0.71 μM, respectively. The SAR of their anti-HIV-1 activity also correlated well with the SAR deduced from the inhibitory activity against WT RT.

Taken together, three factors affect the activity against HIV-1 RT in this series. First, the 'butterfly-like' structure of the thiazolidenebenzenesulfonamide is crucial for RT inhibition. Second, the inclusion of bulky substituents, such as *tert*-butyl and isopropyl, at the 5-position on the thiazole ring enhances the inhibitory activity against the Y181C RT. Third, substituents with an appropriate size at the 4-position on the thiazole ring, such as methyl and chloro groups, are necessary for RT inhibition.

Since there was a good correlation between the EC_{50} values for HIV-1 replication and the IC_{50} values for the WT RT, we concluded that compound **4** and its related compounds were NNRTIs. Among them, the 5-*tert*-butyl-4-methyl (**17a**) and 4-chloro-5-isopropyl analogues (**17k**) showed the most potent anti-HIV-1 activity with EC_{50} values of 0.085 and 0.048 μM, respectively, which represent 6–10-fold improvements over the primary hit compound **4**, while still retaining good TI values.

These results present a significant springboard for the further improvement of the activity spectrum of thiazolidenebenzenesulfonamide analogues as novel NNRTIs.

4. Conclusion

The HTS of an in-house compound library in MT-4 cells acutely infected with HIV-$1_{IIIB\text{-}R}$ has been successfully applied to the discovery of a novel class of NNRTIs, which have potent inhibitory activity against both WT and Y181C RT. The SAR analysis of these thiazolidenebenzenesulfonamide derivatives indicates that the 'butterfly-like' conformation derived from the sulfonamide structure is important for the activity against the WT RT and that the methyl substituent at the 3-position on the thiazole ring is crucial for the fixed configuration to enhance the activity. The inclusion of hydrophobic bulky substituents at the 5-position on the thiazole ring is favorable for enhanced activity against the Y181C RT. Among the newly synthesized compounds, both **17a** and **17k** potently inhibit HIV-1 replication with EC_{50} values of 0.085 and 0.048 μM, respectively. They also have a potent activity against the WT and Y181C RT and to a lesser extent against the K103N RT. The thiazolidenebenzenesulfonamide analogues provide valuable leads for the discovery of the next generation NNRTIs.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yanaco micro-melting apparatus or Büchi B-545 melting point apparatus and are uncorrected. Proton magnetic resonance (^1H NMR) spectra were obtained in $CDCl_3$ or dimethylsulfoxide-d_6 (DMSO-d_6) using a JEOL JNM-EX90, JNM-EX400, JNM-GX500, or JNM-A500 spectrometer. Chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal standard (in NMR description, s: singlet, d: doublet, t: triplet, m: multiplet, br: broad peak). Mass spectra (MS) were recorded on a JEOL JMS-DX300 or a HITACHI M-80 mass spectrometer. Elemental analysis was carried out on Yanaco MT-3 or MT-5 CHN analyzer and a Yokogawa IC7000S Ion Chromatoanalyzer. Chromatographic separations were performed using a silica gel column (Merck Kieselgel 60). Analytical thin-layer chromatography (TLC) was carried out on precoated glass plates (Merck Kieselgel 60F254). The structure of target compounds were deduced from mass spectral and ^1H NMR data, and were verified by a single crystal X-ray structure determination for compound **17a** (Fig. 3).

The following known materials were prepared as described in the literature: **6a**,[15] **6f**,[16] **6i**,[17] or obtained from commercial suppliers: **6b**, **15**.

5.1.1. 5-Ethyl-4-methylthiazole-2-amine (6c). To a stirred solution of 2-butanone (**5a**, 5.00 g, 58.1 mmol) and bromotrimethylsilane (8.3 mL, 63.9 mmol) in acetonitrile (50 mL) was added slowly dropwise dimethylsulfoxide (4.5 mL, 63.9 mmol) under ice-bath cooling. After stirring for 15 min, the solution was warmed to room temperature and stirred for 2 h. The reaction mixture was poured into ice water and extracted with diethyl ether. The organic layer was washed with brine, then dried over anhydrous sodium sulfate, and evaporated under reduced pressure. Ethanol (50 mL) and thiourea (4.42 g, 58.1 mmol) was added to the residue and refluxed for 1 h. The mixture was cooled to room temperature, and the resulting precipitate was collected by filtration. The precipitate was washed with diethyl ether

and hexane to give **6c** hydrobromide (2.10 g, 15%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.10 (3H, t, J = 7.5 Hz, CH_3 of *t*-Bu), 2.11 (3H, s, CH_3 of Et), 2.58 (2H, q, J = 7.5 Hz, CH_2 of Et), 9.15 (2H, br s, NH_2); FAB-MS *m*/*z*: 143 (M^++1).

The following compounds were obtained in the same manner.

5.1.2. 4-Methyl-5-isopropylthiazole-2-amine (6d). 57% yield; ^{1}H NMR ($CDCl_3$) δ: 1.11 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 1.97 (3H, s, 4-Me), 3.02 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 6.49 (2H, br s, NH_2); FAB-MS *m*/*z*: 157 (M^++1).

5.1.3. 1-(2-Amino-4-methyl-1,3-thiazol-5-yl)ethanone (6e). 84% yield; ^{1}H NMR (DMSO-d_6) δ: 2.44 (3H, s, CH_3 of Ac), 2.52 (3H, s, 4-Me), 9.80 (2H, br s, NH_2); FAB-MS *m*/*z*: 157 (M^++1).

5.1.4. 4-Isobutyl-5-isopropylthiazole-2-amine (6g). 44% yield; ^{1}H NMR (DMSO-d_6) δ: 0.85 (6H, d, J = 6.8 Hz, CH_3 of *i*-Bu), 1.12 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 1.89 (1H, m, CH of *i*-Bu), 2.19 (2H, d, J = 6.8 Hz, CH_2 of *i*-Bu), 3.03 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 6.50 (2H, br s, NH_2); FAB-MS *m*/*z*: 199 (M^++1).

5.1.5. Ethyl 2-[(*tert*-butoxycarbonyl)amino]-4-trifluoromethyl-1,3-thiazole-5-carboxylate (8). To a stirred mixture of ethyl trifluoroacetoacetate (7.36 g, 60 mmol), thiourea (13.68 g, 180 mmol), and isopropanol (30 mL) was added iodine (11.42 g, 90.0 mmol) and the mixture was refluxed for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was recrystallized from methanol–water. To a tetrahydrofuran (70 mL) solution of the precipitate, di-*tert*-butyl dicarbonate (7.61 g, 34.9 mmol), and *N*,*N*-dimethylaminopyridine (178 mg, 1.46 mmol) was added. Then the mixture was stirred at 60 °C for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (ethyl acetate–toluene) to give **7** (10.43 g, 49%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.55 (9H, s, CH_3 of *t*-Bu), 1.57 (3H, t, J = 7.1 Hz, CH_3 of Et), 4.36 (2H, q, J = 7.1 Hz, CH_2 of Et), 8.48 (2H, br s, NH_2); FAB-MS *m*/*z*: 341 (M^++1).

5.1.6. *tert*-Butyl [5-(1-hydroxy-1-methylethyl)-4-trifluoromethyl-1,3-thiazol-2-yl]carbamate (9). Under argon atmosphere, to a tetrahydrofuran (200 mL) solution of **8** (4.25 g, 12.5 mmol) was added dropwise 1.14 M methyllithium diethyl ether solution (44 mL, 50 mmol) at −78 °C and the solution was stirred at the same temperature for 0.5 h. The reaction mixture was quenched with saturated aqueous ammonium chloride solution and extracted with ethyl acetate. The organic layer was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate–hexane) to give **9** (2.97 g, 73%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s, CH_3 of *t*-Bu), 1.71 (6H, s, Me), 2.43 (1H, s, OH), 8.36 (1H, br s, NH); FAB-MS *m*/*z*: 327 (M^++1).

5.1.7. 5-Isopropyl-4-trifluoromethyl-1,3-thiazol-2-amine (6h). To a stirred solution of **9** (2.97 g, 9.11 mmol) in trifluoroacetic acid (40 mL) was added triethylsilane (4.35 mL, 27.3 mmol) and stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate–hexane) to give **6h** (2.20 g, quantitative) as colorless syrup. ^{1}H NMR ($CDCl_3$) δ: 1.30 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 3.46 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 7.06 (2H, br s, NH_2); FAB-MS *m*/*z*: 211 (M^++1).

5.1.8. 2-Amino-4-chloro-1,3-thiazole-5-carbaldehyde (11). To a stirred solution of thiourea (100 g, 1.31 mol) in *N*,*N*-dimethylformamide (1.0 L) was added portionwise chloroacetic acid (124 g, 1.31 mol) and stirred at 40 °C for 2 h. To the stirred reaction mixture under ice-bath cooling was added dropwise phosphorous oxychloride (424 mL, 4.59 mol) for 1 h. The mixture was stirred at 60 °C for 30 min, and then stirred at 90 °C for 5 h. The reaction mixture was poured into ice-water and sodium chloride (456 g, 10.2 mol), calcium hydroxide (336 g, 5.93 mol) was added. The resulting precipitate was collected by filtration and washed with water until the filtrate was neutral. The precipitate was dried under reduced pressure to give **11** (165 g, 72%) as pink solid. Two tautomers were observed on ^{1}H NMR spectrum. ^{1}H NMR (DMSO-d_6) one tautomer: δ: 8.70 (1H, s, NH_2), 9.92 (1H, s, CHO), 13.20 (1H, br s, NH_2) and the other tautomer: 8.74 (2H, s, NH_2), 9.64 (1H, s, CHO); FAB-MS *m*/*z*: 237 (M^++1).

5.1.9. *tert*-Butyl (4-chloro-5-formyl-1,3-thiazol-2-yl)carbamate (12). To a stirred solution of **11** (34.4 g, 0.212 mol) in 1,4-dioxane (500 mL) was added di-*tert*-butyl dicarbonate (55.4 g, 0.254 mol) and *N*,*N*-dimethylaminopyridine (2.58 g, 0.021 mol). The reaction mixture was stirred at 60 °C for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The solution was washed with 5% aqueous potassium hydrogen sulfate solution and brine, and then dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give **12** (48.2 g, 87%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ: 1.50 (9H, s, CH_3 of *t*-Bu), 9.86 (1H, s, CHO), 12.53 (1H, s, NH); FAB-MS *m*/*z*: 263 (M^++1).

5.1.10. Methyl 2-[(*tert*-butoxycarbonyl)amino]-4-chloro-1,3-thiazole-5-carboxylate (13). A stirred mixture of **12** (30.0 g, 114 mmol), potassium dihydrogensulfate (46.5 g, 342 mmol) solution in water (200 mL) and 2-methyl-2-butene (157 mL, 1.48 mol) in *tert*-butanol (1.20 L) was added dropwise sodium chlorite (61.9 g, 684 mmol) in water (120 mL) under ice-bath cooling. The mixture was stirred at room temperature for 6 h. To the reaction mixture, ethyl acetate was added and washed with 5% aqueous potassium hydrogen sulfate solution. The organic layer was alkalinized with 1 M aqueous sodium hydroxide solution and washed with ethyl acetate. The aqueous layer was acidified to pH 3 with potassium hydrogen sulfate and extracted with chloroform. The organic layer was dried over sodium sulfate and evaporated. The residue was dissolved in

methanol (150 mL) and *N*,*N*-dimethylformamide (300 mL). To the solution, 1-hydroxybenzotriazole (23.1 g, 171 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (54.6 g, 285 mmol), and *N*,*N*-dimethylaminopyridine (134 mg, 1.1 mmol) was added and stirred at room temperature for 10 h. The solvent was removed under reduced pressure and 10% aqueous citric acid solution was added. The mixture was extracted with ethyl acetate–toluene and washed with water, saturated aqueous sodium hydrogen carbonate solution and brine. The organic layer was dried over sodium sulfate and evaporated to give **13** (19.0 g, 57%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.50 (9H, s, CH_3 of *t*-Bu), 3.79 (3H, s, CH_3 of COOMe), 12.30 (1H, s, NH); FAB-MS *m*/*z*: 293 (M^++1).

5.1.11. *tert*-Butyl [4-chloro-5-(1-hydroxy-1-methylethyl)-1,3-thiazol-2-yl]carbamate (14). Compound **14** was obtained from **13** in the same manner as described in the synthesis of **9**. 98% yield as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.47 (9H, s, CH_3 of *t*-Bu), 1.53 (6H, s, Me), 5.82 (1H, s, OH), 11.41 (1H, s, NH); FAB-MS *m*/*z*: 293 (M^++1).

5.1.12. 4-Chloro-5-isopropyl-1,3-thiazol-2-amine (6j). To a stirred solution of **14** (20 g, 6.82 mmol) in trifluoroacetic acid (20 mL) was added triethylsilane (10 mL) and stirred at room temperature for 1 h. The mixture was evaporated, saturated aqueous sodium hydrogen carbonate solution was added and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate–hexane) to give **6j** (1.17 g, 97%) as a red solid. ^{1}H NMR (DMSO-d_6) δ: 1.14 (6H, d, J = 6.9 Hz, CH_3 of *i*-Pr), 3.05 (1H, heptet, J = 6.9 Hz, CH of *i*-Pr), 7.20 (2H, br s, NH_2); FAB-MS *m*/*z*: 177 (M^++1).

5.1.13. *N*-(5-*tert*-Butyl-4-methyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (4). To a solution of **6a** (5.88 g, 34.6 mmol) in pyridine (200 mL) was added **15** (9.46 g, 42.7 mmol) and the solution was stirred at room temperature for 12 h. The reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution, 1 M hydrochloric acid, and brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (methanol–chloroform) and recrystallized from methanol to give **4** (8.49 g, 69%) as a yellow crystals. Mp 212–213 °C. ^{1}H NMR ($CDCl_3$) δ: 1.30 (9H, s, CH_3 of *t*-Bu), 2.16 (3H, s, 4-Me), 7.86 (1H, t, J = 8.3 Hz, benzene), 8.21 (1H, dd, J = 2.0, 8.3 Hz, benzene), 8.42 (1H, ddd, J = 1.8, 2.0, 8.3 Hz, benzene), 8.46 (1H, t, J = 1.8 Hz, benzene), 12.58 (1H, br s, NH); FAB-MS *m*/*z*: 356 (M^++1). Anal. Calcd for $C_{14}H_{17}N_3O_4S_2$: C, 47.31; H, 4.82; N, 11.82; S, 18.04. Found: C, 47.06; H, 4.84; N, 11.80; S, 17.84.

The following compounds were obtained in the same manner.

5.1.14. *N*-(4-Methyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16a). 60% yield; ^{1}H NMR (DMSO-d_6) δ: 2.10 (3H, s, Me), 6.48 (1H, s, thiazole), 7.85 (1H, t, J = 7.8 Hz, benzene), 8.22 (1H, ddd, J = 1.0, 2.0, 7.8 Hz, benzene), 8.43 (1H, ddd, J = 1.0, 1.5, 7.8 Hz, benzene), 8.51 (1H, dd, J = 1.5, 2.0 Hz, benzene), 12.88 (1H, br s, NH); FAB-MS *m*/*z*: 300 (M^++1).

5.1.15. *N*-(4,5-Dimethyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16b). 60% yield; ^{1}H NMR (DMSO-d_6) δ: 2.01 (3H, s, 4-Me), 2.10 (3H, s, 5-Me), 7.85 (1H, t, J = 7.8 Hz, benzene), 8.20 (1H, br d, J = 7.8 Hz, benzene), 8.42 (1H, ddd, J = 1.0, 2.0, 7.8 Hz, benzene), 8.46 (1H, t, J = 2.0 Hz, benzene), 12.63 (1H, br s, NH); FAB-MS *m*/*z*: 314 (M^++1).

5.1.16. *N*-(5-Ethyl-4-methyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16c). 11% yield; ^{1}H NMR (DMSO-d_6) δ: 1.09 (3H, t, J = 7.7 Hz, CH_3 of Et), 2.03 (3H, s, 4-Me), 2.50 (2H, q, J = 7.7 Hz, CH_2 of Et), 7.83 (1H, m, benzene), 8.22 (1H, dt, J = 1.5, 8.0 Hz, benzene), 8.42 (1H, m, benzene), 8.46 (1H, m, NH); FAB-MS *m*/*z*: 328 (M^++1).

5.1.17. *N*-(5-Isopropyl-4-methyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16d). 32% yield; ^{1}H NMR (DMSO-d_6) δ: 1.14 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 2.04 (3H, s, 4-Me), 3.10 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 7.85 (1H, dt, J = 0.9, 8.1 Hz, benzene), 8.22 (1H, dt, J = 1.4, 7.7 Hz, benzene), 8.43 (1H, m, benzene), 8.46 (1H, m, NH); FAB-MS *m*/*z*: 342 (M^++1).

5.1.18. *N*-(5-Acetyl-4-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (16e). 90% yield; ^{1}H NMR (DMSO-d_6) δ: 2.44 (3H, s, CH_3 of Ac), 2.46 (3H, s, 4-Me), 7.89 (1H, t, J = 8.1 Hz, benzene), 8.27 (1H, dt, J = 1.4, 8.1 Hz, benzene), 8.31 (1H, s, NH), 8.47 (1H, m, benzene); FAB-MS *m*/*z*: 342 (M^++1).

5.1.19. *N*-(5-Isopropyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16f). 51% yield; ^{1}H NMR (DMSO-d_6) δ: 1.19 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 2.94 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 7.05 (1H, s, thiazole), 7.86 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.23 (1H, br d, J = 7.8 Hz, benzene), 8.42 (1H, ddd, J = 1.5, 1.9, 8.3 Hz, benzene), 8.47 (1H, dd, J = 1.5, 1.9 Hz, benzene), 12.71 (1H, br s, NH); FAB-MS *m*/*z*: 328 (M^++1).

5.1.20. *N*-(4-Isobutyl-5-isopropyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16g). 85% yield; ^{1}H NMR (DMSO-d_6) δ: 0.84 (6H, d, J = 6.3 Hz, CH_3 of *i*-Bu), 1.16 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 1.84 (1H, m, CH of *i*-Bu), 2.30 (2H, d, J = 7.3 Hz, CH_2 of *i*-Bu), 3.12 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 7.86 (1H, t, J = 7.8 Hz, benzene), 8.22 (1H, br d, J = 7.8 Hz, benzene), 8.43 (1H, br d, J = 7.8 Hz, benzene), 8.48 (1H, t, J = 2.0 Hz, benzene), 12.60 (1H, br s, NH); FAB-MS *m*/*z*: 384 (M^++1).

5.1.21. *N*-(5-Isopropyl-4-trifluoromethyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16h). 78% yield; ^{1}H NMR ($CDCl_3$) δ: 1.14 (6H, d, J = 6.0 Hz, CH_3 of *i*-Pr), 3.24 (1H, m, CH of *i*-Pr), 7.71 (1H, dt, J = 1.1,

7.1 Hz, benzene), 8.13 (1H, dd, J = 1.1, 7.1 Hz, benzene), 8.26 (1H, dd, J = 1.1, 7.1 Hz, benzene), 8.49 (1H, d, J = 1.8 Hz, benzene); FAB-MS m/z: 393 (M^+-1).

5.1.22. Methyl 5-isopropyl-2-{[(3-nitrophenyl)sulfonyl]-amino}-1,3-thiazole-4-carboxylate (16i). 75% yield; ^{1}H NMR ($CDCl_3$) δ: 1.30 (6H, d, J = 7.0 Hz, CH_3 of i-Pr), 3.95 (1H, heptet, J = 7.0 Hz, CH of i-Pr), 3.93 (3H, s, CH_3 of COOMe), 7.69 (1H, dt, J = 7.7, 8.3 Hz, benzene), 8.27 (1H, ddd, J = 1.1, 1.8, 7.7 Hz, benzene), 8.38 (1H, ddd, J = 1.1, 2.4, 8.3 Hz, benzene), 8.77 (1H, t, J = 1.8 Hz, benzene); FAB-MS m/z: 386 (M^++1).

5.1.23. *N*-(4-Chloro-5-isopropyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16j). 35% yield; ^{1}H NMR (DMSO-d_6) δ: 1.21 (6H, d, J = 6.8 Hz, CH_3 of i-Pr), 3.12 (1H, heptet, J = 6.8 Hz, CH of i-Pr), 7.52 (1H, t, J = 8.1 Hz, benzene), 8.26 (2H, d, J = 8.1 Hz, benzene), 8.69 (2H, m, benzene and NH); FAB-MS m/z: 362 (M^++1).

5.1.24. *N*-(5-*tert*-Butyl-3,4-dimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17a). To a solution of **4** (8.49 g, 23.9 mmol) in tetrahydrofuran (150 mL) was added sodium hydride (60% dispersion in mineral oil: 1.44 g, 35.9 mmol) and iodomethane (2.98 mL, 47.8 mmol) under ice-bath cooling. The solution was warmed to room temperature and stirred for 12 h. The reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (chloroform) and recrystallized from methanol to give **17a** (6.56 g, 74%) as a yellow crystals. Mp 143–145 °C. ^{1}H NMR (DMSO-d_6) δ: 1.32 (9H, s, CH_3 of t-Bu), 2.12 (3H, s, 4-Me), 3.60 (3H, s, 3-Me), 7.84 (1H, dd, J = 7.9, 8.3 Hz, benzene), 8.26 (1H, br d, J = 7.9 Hz, benzene), 8.41 (1H, dd, J = 2.5, 8.3 Hz, benzene), 8.49 (1H, t, J = 2.5 Hz, benzene); FAB-MS m/z: 370 (M^++1). Anal. Calcd for $C_{15}H_{19}N_3O_4S_2$: C, 48.76; H, 5.18; N, 11.37; S, 17.36. Found: C, 48.69; H, 5.25; N, 11.31; S, 17.37.

The following compounds were obtained in the same manner.

5.1.25. *N*-(3,4-Dimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17b). 80% yield; mp 199–200 °C (H_2O–acetonitrile), ^{1}H NMR (DMSO-d_6) δ: 2.21 (3H, s, 4-Me), 3.42 (3H, s, 3-Me), 6.63 (1H, s, thiazole), 7.85 (1H, t, J = 7.8 Hz, benzene), 8.25 (1H, br d, J = 7.8 Hz, benzene), 8.42 (1H, ddd, J = 1.0, 2.0, 8.3 Hz, benzene), 8.49 (1H, dd, J = 1.5, 2.0 Hz, benzene), FAB-MS m/z: 313 (M^+). Anal. Calcd for $C_{11}H_{11}N_3O_4S_2 \cdot 0.3H_2O$: C, 41.45; H, 3.67; N, 13.18; S, 20.12. Found: C, 41.44; H, 3.40; N, 13.36; S, 20.24.

5.1.26. *N*-(3,4,5-Trimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17c). 88% yield; mp 156–158 °C (diethyl ether–acetonitrile), ^{1}H NMR (DMSO-d_6) δ: 2.13 (3H, s, 4-Me), 2.16 (3H, s, 5-Me), 3.43 (3H, s, 3-Me), 7.84 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.24 (1H, br d, J = 7.8 Hz, benzene), 8.42 (1H, ddd, J = 0.9, 2.0, 8.3 Hz, benzene), 8.48 (1H, t, J = 2.0 Hz, benzene); FAB-MS m/z: 328 (M^++1). Anal. Calcd for $C_{12}H_{13}N_3O_4S_2$: C, 44.03; H, 4.00; N, 12.84; S, 19.59. Found: C, 43.84; H, 3.90; N, 12.84; S, 19.49.

5.1.27. *N*-(5-Ethyl-3,4-dimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17d). 54% yield; mp 159–160 °C (ethanol). ^{1}H NMR (DMSO-d_6) δ: 1.09 (3H, t, J = 7.3 Hz, CH_3 of Et), 2.15 (3H, s, 4-Me), 2.61 (1H, q, J = 7.3 Hz, CH_2 of Et), 3.43 (3H, s, 3-Me), 7.85 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.25 (1H, ddd, J = 1.0, 1.9, 7.8 Hz, benzene), 8.44 (1H, ddd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.49 (1H, t, J = 1.9 Hz, benzene); FAB-MS m/z: 342 (M^++1). Anal. Calcd for $C_{13}H_{15}N_3O_4S_2$: C, 45.73; H, 4.43; N, 12.31; S, 18.78. Found: C, 45.78; H, 4.48; N, 12.45; S, 18.76.

5.1.28. *N*-(3,4-Dimethyl-5-isopropyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17e). 56% yield; mp 124–125 °C (diethyl ether–hexane). ^{1}H NMR (DMSO-d_6) δ: 1.15 (6H, d, J = 6.9 Hz, CH_3 of i-Pr), 2.17 (3H, s, 4-Me), 3.21 (1H, heptet, J = 6.9 Hz, CH of i-Pr), 3.42 (3H, s, 3-Me), 7.85 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.26 (1H, ddd, J = 1.0, 1.9, 7.8 Hz, benzene), 8.43 (1H, ddd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.49 (1H, t, J = 1.9 Hz, benzene); FAB-MS m/z: 356 (M^++1). Anal. Calcd for $C_{14}H_{17}N_3O_4S_2$: C, 47.31; H, 4.82; N, 11.82; S, 18.04. Found: C, 47.27; H, 4.70; N, 11.88; S, 18.09.

5.1.29. *N*-(5-Acetyl-3,4-dimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17f). 66% yield; mp 205–206 °C (acetonitrile). ^{1}H NMR (DMSO-d_6) δ: 2.08 (3H, s, 4-Me), 2.48 (3H, s, CH_3 of Ac), 2.58 (3H, s, 3-Me), 7.88 (1H, t, J = 8.0 Hz, benzene), 8.30 (1H, ddd, J = 0.9, 1.8, 8.0 Hz, benzene), 8.46 (1H, ddd, 0.9, 1.8, 8.0 Hz, benzene), 8.51 (1H, t, J = 1.8 Hz, benzene); FAB-MS m/z: 356 (M^++1). Anal. Calcd for $C_{13}H_{13}N_3O_5S_2$: C, 43.94; H, 3.69; N, 11.82; S, 18.05. Found: C, 44.11; H, 3.60; N, 11.99; S, 17.85.

5.1.30. *N*-(5-Isopropyl-3-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17g). 76% yield; mp 150–151 °C (isopropanol). ^{1}H NMR (DMSO-d_6) δ: 1.19 (6H, d, J = 6.9 Hz, CH_3 of i-Pr), 2.95 (1H, heptet, J = 6.9 Hz, CH of i-Pr), 3.45 (3H, s, 3-Me), 7.22 (1H, s, thiazole), 7.86 (1H, d, J = 7.8, 8.3 Hz, benzene), 8.26 (1H, br d, J = 7.8 Hz, benzene), 8.43 (1H, ddd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.49 (1H, t, J = 1.9 Hz, benzene); FAB-MS m/z: 342 (M^++1). Anal. Calcd for $C_{13}H_{15}N_3O_4S_2$: C, 45.73; H, 4.43; N, 12.31; S, 18.78. Found: C, 45.66; H, 4.31; N, 12.30; S, 18.89.

5.1.31. *N*-(4-Isobutyl-5-isopropyl-3-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17h). 41% yield; mp 125–126 °C (isopropanol). ^{1}H NMR (DMSO-d_6) δ: 0.90 (6H, d, J = 6.3 Hz, CH_3 of i-Bu), 1.16 (6H, d, J = 6.8 Hz, CH_3 of i-Pr), 1.78 (1H, m, CH of i-Bu), 2.48 (2H, d, J = 7.3 Hz, CH_2 of i-Bu), 3.12 (1H, heptet, J = 6.8 Hz, CH of i-Pr), 3.42 (3H, s, 3-Me), 7.86 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.23 (1H, br d, J = 7.8 Hz, benzene), 8.42 (1H, ddd, J = 1.0, 2.0, 7.8 Hz, benzene), 8.50 (1H, t, J = 2.0 Hz, benzene);

FAB-MS *m/z*: 398 (M^++1). Anal. Calcd for $C_{17}H_{23}N_3O_4S$: C, 51.36; H, 5.83; N, 10.57; S, 16.13. Found: C, 51.31; H, 5.82; N, 10.62; S, 15.96.

5.1.32. *N*-(5-Isopropyl-3-methyl-4-trifluoromethyl-1,3-thiazol-2(3*H*)-2-ylidene)-3-nitrobenzenesulfonamide (17i). 19% yield; mp 127–128 °C (ethyl acetate–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.31 (6H, d, J = 7.0 Hz, CH_3 of *i*-Pr), 3.53 (1H, m, CH of *i*-Pr), 3.58 (3H, br s, 3-Me), 7.70 (1H, t, J = 7.8 Hz, benzene), 8.30 (1H, dt, J = 2.0, 7.8 Hz, benzene), 8.39 (1H, m, benzene), 8.79 (1H, t, J = 2.0 Hz, benzene); FAB-MS *m/z*: 410 (M^++1). Anal. Calcd for $C_{14}H_{14}F_3N_3O_4S_2$: C, 41.07; H, 3.45; N, 10.26; S, 15.66; F, 13.92. Found: C, 41.02; H, 3.37; N, 10.23; S, 15.64; F, 13.83.

5.1.33. Methyl 5-isopropyl-3-methyl-2-{[(3-nitrophenyl)-sulfonyl]imino}-2,3-dihydro-1,3-thiazole-4-carboxylate (17j). 43% yield; mp 120–121 °C (ethyl acetate–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.29 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 3.67 (3H, s, 3-Me), 3.68 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 3.93 (3H, s, CH_3 of COOMe), 7.88 (1H, br t, J = 8.2 Hz, benzene), 8.29 (1H, br d, J = 7.7 Hz, benzene), 8.37 (1H, dd, J = 2.0, 8.2 Hz, benzene), 8.79 (1H, t, J = 2.0 Hz, benzene); FAB-MS *m/z*: 400 (M^++1). Anal. Calcd for $C_{15}H_{17}N_3O_6S_2$: C, 45.10; H, 4.29; N, 10.52; S, 16.06. Found: C, 44.93; H, 4.10; N, 10.62; S, 16.09.

5.1.34. *N*-(4-Chloro-5-isopropyl-3-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17k). 52% yield; mp 140–142 °C (diethyl ether). ^{1}H NMR (DMSO-d_6) δ: 1.20 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 3.18 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 3.47 (3H, s, 3-Me), 7.87 (1H, t, J = 7.8 Hz, benzene), 8.28 (1H, br d, J = 7.9 Hz, benzene), 8.45 (1H, br d, J = 7.9 Hz, benzene), 8.50 (1H, br s, benzene); FAB-MS *m/z*: 376 (M^++1). Anal. Calcd for $C_{13}H_{14}N_3O_4S_2Cl$: C, 41.54; H, 3.75; N, 11.18; S, 17.06; Cl, 9.43. Found: C, 41.89; H, 3.74; N, 10.95; S, 16.85; Cl, 9.81.

5.1.35. *N*-(5-*tert*-Butyl-3-ethyl-4-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17l). Compound **17l** was obtained in the same manner as described in the synthesis of **17a** with iodoethane instead of iodomethane. 50% yield; mp 139–141 °C (diethyl ether–hexane). ^{1}H NMR (DMSO-d_6) δ: 1.12 (3H, t, J = 6.8 Hz, CH_3 of Et), 1.32 (9H, s, CH_3 of *t*-Bu), 2.31 (3H, s, 4-Me), 3.96 (2H, q, J = 6.8 Hz, CH_2 of Et), 7.85 (1H, t, J = 8.3 Hz, benzene), 8.25 (1H, ddd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.42 (1H, dd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.48 (1H, t, J = 1.9 Hz, benzene); FAB-MS *m/z*: 384 (M^++1). Anal. Calcd for $C_{16}H_{21}N_3O_4S_2$: C, 50.11; H, 5.52; N, 10.96; S, 16.72. Found: C, 50.06; H, 5.49; N, 11.02; S, 16.81.

5.1.36. *N*-(3,4-Dimethyl-5-vinyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (18). To a solution of **17f** (1.78 g, 5.01 mmol) in methanol (50 mL) was added sodiumborohydride (0.38 g, 10.0 mmol) under ice-bath cooling. The mixture was warmed to room temperature and stirred for 2.5 h. Additional sodiumborohydride (0.19 g, 5.0 mmol) was added and stirred 12 h. The reaction mixture was quenched with aqueous acetone and evaporated. The residue was diluted with ethyl acetate and washed with water and brine, then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (chloroform–methanol) and recrystallized from isopropanol. To the solution of the product and triethylamine (1.39 mL, 10.0 mmol) in dichloromethane (20 mL) was added methanesulfonyl chloride (0.77 mL, 10.0 mmol) under ice-bath cooling. After the reaction mixture was stirred at the same temperature for 30 min, the solution was warmed to room temperature and stirred for 36 h. The reaction mixture was evaporated and diluted with chloroform. The solution was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate–hexane) and recrystallized from ethanol to give **18** (459 mg, 21%) as a yellow crystals. Mp 171–173 °C. ^{1}H NMR (DMSO-d_6) δ: 2.27 (3H, s, 4-Me), 3.44 (3H, s, 3-Me), 5.21 (1H, d, J = 10.8 Hz, –CH=C*H*$_2$), 5.33 (1H, d, J = 17.0 Hz, –CH=C*H*$_2$), 6.89 (1H, dd, J = 10.8, 17.0 Hz, –C*H*=CH$_2$), 7.86 (1H, t, J = 8.3 Hz, benzene), 8.28 (1H, br d, J = 7.8 Hz, benzene), 8.43 (1H, dd, J = 2.5, 7.8 Hz, benzene), 8.50 (1H, t, J = 2.5 Hz, benzene); FAB-MS *m/z*: 340 (M^++1). Anal. Calcd for $C_{13}H_{13}N_3O_4S_2$: C, 46.01; H, 3.86; N, 12.38; S, 18.90. Found: C, 45.93; H, 3.87; N, 12.10; S, 18.46.

5.1.37. 5-Isopropyl-3-methyl-2-{[(3-nitrophenyl)sulfonyl]imino}-2,3-dihydro-1,3-thiazole-4-carboxamide (19). To a solution of **17j** (877 mg, 2.19 mmol) in tetrahydrofuran (10 mL) was added saturated aqueous ammonia solution (10 mL) and heated to 120 °C in autoclave and stirred for 3 h. The mixture was evaporated in reduced pressure. To the residue was added thionyl chloride (1 mL), *N*,*N*-dimethylformamide (0.15 mL) and stirred at 70 °C for 1 h. Thionyl chloride was removed under reduced pressure and the residue was solved to chloroform (10 mL). To saturated aqueous ammonia (10 mL) was added dropwise the solution under ice-bath cooling and stirred at same temperature for 15 min. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was recrystallized from ethyl acetate–hexane to give **19** (547 mg, 65%) as an orange crystals. Mp 246–248 °C. ^{1}H NMR (DMSO-d_6) δ: 1.20 (6H, d, J = 7.0 Hz, CH_3 of *i*-Pr), 3.31 (1H, m, CH of *i*-Pr), 3.41 (3H, s, 3-Me), 7.87 (1H, t, J = 8.3 Hz, benzene), 8.15 (1H, s, NH_2), 8.19 (1H, s, NH_2), 8.28 (1H, br d, J = 8.3 Hz, benzene), 8.44 (1H, br d, J = 8.3 Hz, benzene), 8.50 (1H, m, benzene); FAB-MS *m/z*: 385 (M^++1). Anal. Calcd for $C_{14}H_{16}N_4O_5S_2$: C, 43.74; H, 4.20; N, 14.57; S, 16.68. Found: C, 43.68; H, 4.35; N, 14.34; S, 16.37.

5.1.38. *N*-(4-Cyano-5-isopropyl-3-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (20). To a solution of **19** (268 mg, 0.70 mmol) in 1,2-dichloroethane (5 mL) was added phosphorous oxychloride (0.33 mL, 3.50 mmol) and *N*,*N*-dimethylformamide (0.10 mL) un-

der ice-bath cooling. The mixture was heated to 70 °C and stirred for 1 h. The reaction mixture was pored into ice water and extracted with ethyl acetate. The organic layer was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was recrystallized from ethyl acetate–hexane to give **20** (180 mg, 70%) as an orange crystals. Mp 136–137 °C. ^{1}H NMR ($CDCl_3$) δ: 1.37 (6H, d, J = 7.0 Hz, CH_3 of *i*-Pr), 3.38 (1H, heptet, J = 7.0 Hz, CH of *i*-Pr), 3.59 (3H, s, 3-Me), 7.70 (1H, t, J = 7.9 Hz, benzene), 8.27 (1H, br d, J = 7.9 Hz, benzene), 8.40 (1H, ddd, J = 2.0, 2.2, 7.9 Hz, benzene), 8.76 (1H, t, J = 2.0 Hz, benzene); FAB-MS *m*/*z*: 367 (M^++1). Anal. Calcd for $C_{14}H_{14}N_4O_4S_2$: C, 45.89; H, 3.85; N, 15.29; S, 17.50. Found: C, 45.70; H, 3.65; N, 15.32; S, 17.24.

5.2. Pharmacology

5.2.1. Cells and viruses. MT-4 cells[18] and two strains of HIV-1, HIV-1_{IIIB} and HIV-$1_{IIIB\text{-}R}$, were used for the anti-HIV-1 assays. HIV-$1_{IIIB\text{-}R}$, which has a single amino acid change (Y181C) in its RT, is established by serial passages in cell cultures in the presence of escalating concentrations of MKC-442.[19] HIV-$1_{IIIB\text{-}R}$ is also resistant to nevirapine. MT-4 cells were grown and maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin G (100 units/mL), and gentamicin (20 mg/mL). MT-4 cells and HIV-1_{IIIB} were obtained from Rational Drug Design Laboratories (Fukushima, Japan).

5.2.2. Anti-HIV-1 assay. Determination of the antiviral activity of the test compounds against HIV-1_{IIIB} replication was based on the inhibition of virus-induced cytopathicity in MT-4 cells. Briefly, MT-4 cells were suspended in culture medium at 1×10^5 cells/mL and infected with virus at a multiplicity of infection (MOI) of 0.02. Immediately after virus infection, the cell suspension (100 μL) was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 5-day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[20] The HTS of our compound library was also performed using the MTT assay against HIV-$1_{IIIB\text{-}R}$.[19] The anti-HIV-1 activity and cytotoxicity of test compounds were expressed as EC_{50} and CC_{50}, respectively. EC_{50} is the concentration of a test compound that was able to suppress HIV-1 replication by 50%. CC_{50} is the concentration of a test sample that reduced viable cell number by 50% in mock-infected cells. The therapeutic index (TI) is the ratio of CC_{50} to EC_{50}.

5.2.3. In vitro RT inhibition assay. A expression plasmide, pG280, which encodes HIV-1 RT proteins as LacZ fusion proteins were used for the expression of WT RT and mutated RTs.[21] The single amino acid-substituted RTs (K103N RT and Y181C RT) were constructed using pG280 from a Quikchange™ Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). Recombinant RT enzymes were expressed in *E. coli*. UTX81 and purified by the scheme described by Saitoh et al.[21] In vitro RT assays were conducted according to the previously described method with the following modifications.[22] Test compounds and 0.01 unit of recombinant HIV-1 RT enzymes (either wild type or mutant) were incubated in a reaction mixture (50 μL) containing 50 mM Tris–HCl (pH 8.4), 100 mM KCl, 10 mM $MgCl_2$, 0.1% Triton X-100, 2 mM dithiothreitol, 0.01 OD_{260} of poly(rC)/oligo(dG)$_{12-18}$, and 1 μCi of [1′,2′-^{3}H]dGTP (33 Ci/mmol) at 37 °C for 1 h. The reaction was stopped with 200 μL of 5% cold trichloroacetic acid. The precipitated materials were analyzed for radio activity using a scintillation counter (Aloka Co., Ltd, Tokyo, Japan).

5.2.4. X-ray crystal structure analysis of 17a. A single crystal of **17a** was obtained by recrystallization from MeOH. Crystal data for **17a**: $C_{15}H_{19}N_3O_4S_2$; Mr = 369.45, colorless plate, crystal size 0.45 × 0.18 × 0.02 mm, triclinic, space group P-1, a = 10.1641(6) Å, b = 10.7000(6) Å, c = 9.6632(5) Å, α = 90.408(5)°, β = 106.252(5)°, γ = 63.298(4)°, V = 892.67(9) Å^3, Z = 2, D_{calc} = 1.374 g/cm^3, $F(000)$ = 388, μ(CuKα) = 29.21 cm^{-1}. Data were collected on a RIGAKU AFC5R diffractometer at 298 K. Lattice constants were obtained from a least-squares refinement using the setting angles of 25 reflections carefully centered in the range of 55.34° < 2θ < 62.31°. The structure of **17a** was solved by direct methods using the SIR92 program.[23] The data were corrected for Lorentz and polarization effects. An experimental absorption correction was also applied. All hydrogen atoms were located from difference Fourier synthesis. Full-matrix least-squares refinement was carried out and converged with a final calculated R-factor [$I > 3\sigma(I)$] of 0.043 (Rw = 0.062) and a goodness-of-fit of 1.189.

5.2.5. Docking study. Docking studies for compound **17a** with HIV reverse transcriptase[24] (RT) nonnucleoside binding sites were performed using the GOLD program.[25] Ten independent genetic algorithms (GA) in which a maximum number of 100,000 GA operations were performed on a single population of 100 individuals, were calculated. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively.

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