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The design, synthesis, and biological evaluation of novel substituted purines as HIV-1 Tat–TAR inhibitors

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Abstract—A series of novel substituted purines containing a side chain with a terminal amino or guanidyl group were designed and synthesized as HIV-1 Tat–TAR inhibitors. All the compounds could effectively block the TAR transactivation in human 293T cells with the CAT expression percentage ranging from 34.4% to 65.7% and showed high antiviral effects with low cytotoxicities in inhibiting the formation of SIV-induced syncytium in CEM174 cells. Molecular modeling studies by Auto-dock process suggest that the compounds bind to TAR RNA in two different modes.

1. Introduction

Trans-activator of transcription (Tat) protein plays a determinant role in HIV replication by specific interaction with the trans-activation response element region (TAR), a 59-nucleotide stem-loop structure containing a six-nucleotide loop, a three-nucleotide bulge, and two single-nucleotide bulges at the 5'-end of all nascent transcribed HIV-1 mRNAs.[1,2] The tri-nucleotide bulge (U23, C24, U25) of HIV TAR is essential for high-affinity and specific binding to the basic domain (RKKRRQRRR, 49–57) of Tat protein. The arginine 52 residue of Tat directly binds the tri-nucleotide bulge and its guanidyl group is largely responsible for the Tat–TAR interaction.[3–6] The arginine residue (or arginineamide) can bind to TAR and induce a change in RNA conformation largely mimicking a portion of Tat–TAR complex.[7] It has also been proved that the high-affinity binding of some small molecules with amino, guanidyl group or arginine residue to TAR RNA is governed by electrostatic interaction between these groups and the region near the UCU bulge.[8,9] Due to the specificity of Tat–TAR interaction and the high conservation of TAR sequence, the interaction of Tat–TAR is an attractive target for designing novel anti-HIV drugs.[10,11]

Our previous work has demonstrated that some substituted β-carboline, isoquinoline, α,α-trehalose derivatives with a flexible side -chain with a terminal amino or guanidyl group could interact with TAR and inhibit the replication of HIV-1.[12] These studies above provide us an idea for designing one type of new Tat–TAR inhibitors containing an 'activator,' an 'anchor', and a 'linker' with suitable length. An 'activator' is defined as a group that could recognize and bind to the tri-based bulge of TAR, usually an amino or guanidyl group. An 'anchor' is a functional group that could interact with TAR in different ways from the 'activator', such as stacking into tri-based bulge, forming hydrogen bond with unpaired base, intercalating into the upper or lower stem, or falling into the major groove near the bulge. Its structure may vary in a wide range, such as single, linked or fused aromatic cycles, glucosides, etc. A suitable 'anchor' can not only reinforce the affinity but also improve the solubility and lyphohydrophilic character of the compound which would affect the final activity. A 'linker' is the structure

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linking the 'activator' and the 'anchor' with optimal length. If the linker could form additional interactions such as hydrogen bond or electrostatic interaction with TAR, the interaction between the small molecule and TAR will be reinforced. The β-carboline, isoquinoline, and α,α-trehalose derivatives as well as several other kinds of small molecular inhibitors of Tat–TAR fall into the model we proposed.[7,8,11–17]

In order to obtain more potential Tat–TAR inhibitors and make further study of the TAR RNA binding property of them, we have designed and synthesized a novel series of substituted purines as HIV-1 Tat–TAR inhibitors, as shown in Figure 1, on the basis of the molecular model we proposed above. Multi-substituted purine is selected as an 'anchor' here for the following reasons: (1) Purine and its analogues attract much attention for serving as key recognition and anchoring elements in a variety of cofactors and signaling molecules in bio-systems and are usually studied as anti-cancer and anti-virus agents.[18–21] (2) The purine would bind to TAR RNA with higher affinity than β-carboline or isoquinoline for it could mimic Watson–Crick interactions with pyrimidine bases of the tri-based bulge, or form hydrogen bonds between its N-atom and the nucleotides of TAR. (3) The amphiphlic 'anchor' would give the compounds higher solubility than β-carbolines and isoquinolines. The 'linker' here is located at C8 position of the purine and is somewhat longer but more flexible than our former compounds. The 'activator' is an amino or guanidyl group for its specific binding with TAR at the tri-nucleotide bulge.

All the title compounds are synthesized for the first time and biological evaluation proves that all of them possess inhibitory activity to Tat–TAR interaction with low cytotoxicity as compared with the substituted β-carboline, isoquinoline, α,α-trehalose derivatives we reported before.

2. Results and discussion

The synthetic work is illustrated in Scheme 1 and the synthetic procedure is explained in Section 4. Twelve title compounds were obtained and their MS, ^{1}H and ^{13}C NMR spectroscopy data are provided in Section 4.

All the 12 title compounds (**6a**–**6f** and **7a**–**7f**) were evaluated for inhibiting HIV-1 Tat–TAR interaction in human 293T cells using Tat dependent HIV-1 LTR-driven CAT gene expression colorimetric enzyme assays at a concentration of 30 μM. As a report gene, the depressed CAT expression indicated the high inhibitory activity of the compound. As shown in Figure 2, the decreased CAT activities in the presence of the title compounds suggested that all of them could effectively block the interaction of Tat–TAR RNA in vivo.

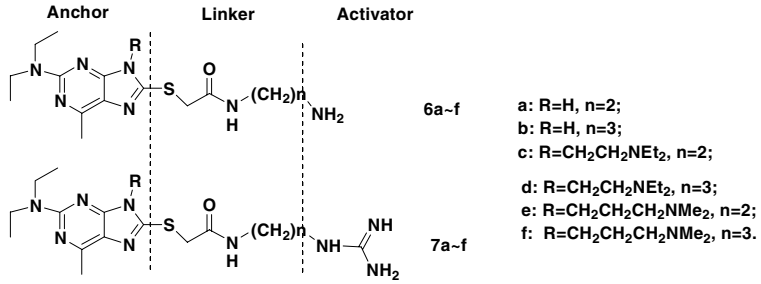
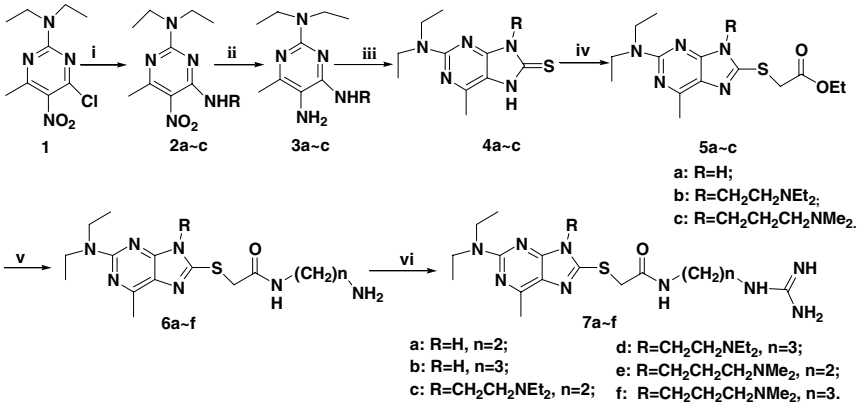


Figure 1. The structure of title compounds.



Scheme 1. Synthesis for substituted purines. Reagents and conditions: (i) amine, ethanol, 24 h, rt; (ii) H_2 (1 atm), Pd/C, methanol, 36 h, rt; (iii) CS_2, NaOH, ethanol, 5 h, reflux; (iv) $ClCH_2CO_2Et$, KI, K_2CO_3, THF, 12 h, rt; (v) ω,ω-diamino alkane (5 equiv), methanol, 8 h, reflux; (vi) $AIMSO_3H{\cdot}H_2O$ (1.1 equiv), anhydrous ethanol, 4 h, 35–45 °C.

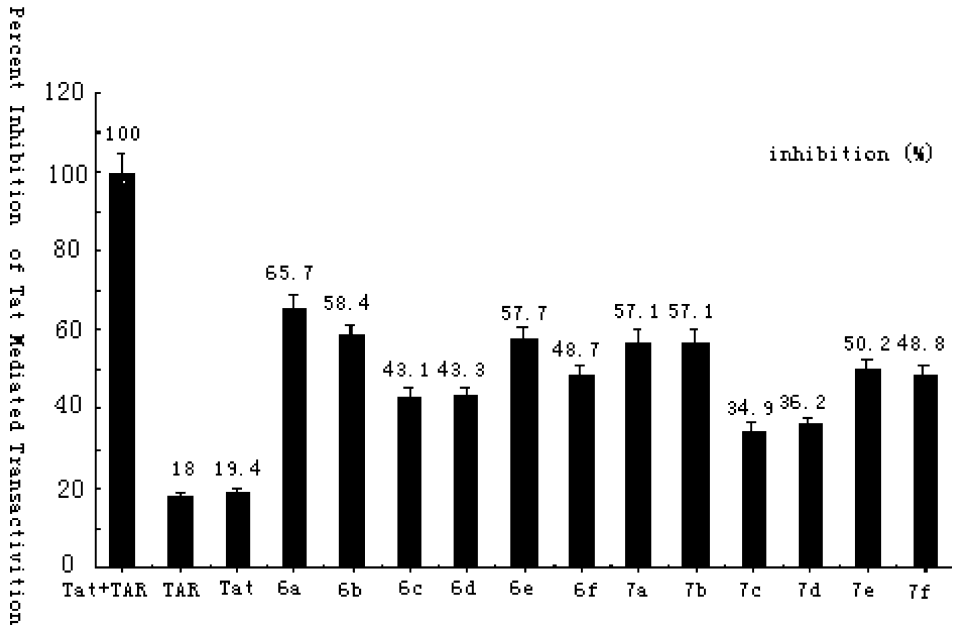


Figure 2. Effects of title compounds on Tat Trans-activation in 293T cells.

The range of inhibited CAT expression induced by the title compounds is from 34.9% to 65.7%, and the lowest data, 34.9% and 36.2%, appeared in the presence of **7c** and **7d**, respectively, while **6a** showed the highest (65.7%). As we reported before, at the same concentration, only two of the twelve β-carbolines depressed CAT expression below 60% (Refs. 12a,d), and for isoquinoline derivatives and α,α-trehalose–arginine conjugates, CAT expressions were all above 40% (Refs. 12e,b,c). It is very clear that the purine derivatives contain the best activity in the CAT assay. This might come from the ability of purine to mimic Watson–Crick interactions with pyrimidine bases.

Taking a detailed look at the data in Figure 2, it is found as follows: (a) when the amino group at the end of the side chain was converted to the guanidyl group, the inhibitory activities of the compounds would be reinforced, such as **6a** to **7a**, **6c** to **7c**, **6e** to **7e**, etc.; (b) with a side chain terminated with a trisubstituted amino group at N9 of the purine ring, the inhibition of CAT expression in 293T cells is increased by **6c**, **6d**, **6e**, and **6f** compared to **6a** and **6b** as well as **7c**, **7d**, **7e**, and **7f** compared to **7a** and **7b**. Among them, **6c**, **6d**, **7c**, and **7d** appeared to be the most effective compounds in restraining the CAT expression; (c) the length of the side chain at C8 had little effect on the activities of the title compounds for there was no significant difference in activity between compounds **6c** and **6d**, **7a** and **7b**, **7c** and **7d**, **7e** and **7f**, respectively (Fig. 3).

We also evaluated the biological activities of the title compounds using SIV-induced syncytium in CEM cells. Their EC_{50}, TC_{50} and SI values are listed in Table 1. As shown in Table 1, each of them possessed an $\mathbf{EC}_{50}$ value within the range from 0.2 μM to 4.7 μM, and a $\mathbf{TC}_{50}$ value more than 100 μM except **6c** (52.5 μM). And this demonstrated that nearly all compounds we mentioned in this article possessed effective anti-SIV activity with low cytotoxicity, especially **6b** and **6f** with a SI value more than 200 and 500, respectively.

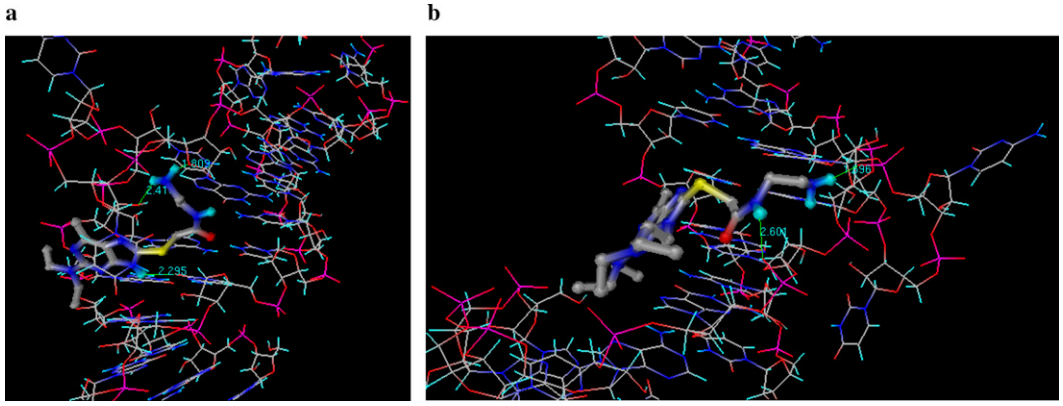


Figure 3. (a) Interaction of compound **6a** to TAR RNA; (b) interaction of compound **6c** to TAR RNA.

Table 1. Inhibition effect and cytotoxicity of the title compounds on SIV induced syncytium

Compound[a]	EC_{50}[b] (μM)	TC_{50}[c](μM)	SI[d] (TC_{50}/EC_{50})
6a	2.0	>100	>50
6b	0.5	>100	>200
6c	1.3	52.2	40.2
6d	1.4	>100	>71.4
6e	3.4	>100	>29.4
6f	0.2	>100	>500
7a	4.2	>100	>23.8
7b	0.7	>100	>142.9
7c	0.7	>100	>142.9
7d	4.7	>100	>21.3
7e	7.2	>100	>13.9
7f	3.2	>100	>31.25

[a] AZT was used as the positive control at a concentration of 10 μM here. Its EC_{50} is 0.0122 μM and TC_{50} is above 100 μM in this system.
[b] EC_{50}, concentration required to protect cells against the cytopathogenicity of SIV by 50%.
[c] TC_{50}, concentration required to inhibit uninfected cells proliferation by 50%.
[d] SI, selective index.

Compounds with an amino group as the 'activator' possessed smaller EC_{50} than those with an guanidyl group which might be due to the lyphohydrophilic characters of the compounds. Under the physiological condition, compounds with an 'activator' of amino group would possess higher lyphophilicity and could be easily absorbed by the cells. But with a guanidyl group as a cation, it was difficult for the compounds to be absorbed by the cells. We used 293T cell line in CAT assay while CEM174 cell line in anti-SIV assay, which might account for some differences of activities inside the cells. These two different biological systems in which the compounds might present different solubilities, ionic forms and especially different absorptions by cells would affect the final activities.

Here using Auto-dock 3.0 process,[22] we studied the interaction between title compounds and HIV-1 TAR RNA and the energetic data of the interaction and the figures of the compounds binding to TAR were obtained.

In Table 2, the free energy and K_i values of the title compounds reflect the binding affinity of the compounds and the TAR RNA element. The negative free energy and low K_i values suggest that the title compounds could bind to TAR RNA element in theory. A terminal guanidyl group on the side chain could raise the K_i value and these results are consistent with those of the SIV assay. However, the substitution at N9 seems to depress the binding affinity, which is different from the CAT assay. Considering that the model we used here came from the binding complex of rbt203 and TAR, we assume that **6a** and **6b** with similar conformation to rbt203 could fit the model better than those with a side -chain at N9 of the ring.

Compounds **6a**, **6b**, **7a**, and **7b** inserted into the enlarged minor groove with the rings underneath U23 of the tri-based bugle and the amino or guanidyl groups binding to the backbone of the bulge or the lower stem region near A22:U40 and G21:C41. Hydrogen bonds were formed by the amino group with the backbone of the bulge and by the N9 or N7 of the ring with the amino group of the base. While compounds **6c**, **6d**, **6e**, **6f**, **7c**, **7e**, and **7f**, each with an additional side chain at N9, presented a different binding mode: they bound to the major groove of TAR RNA with their rings and to the bulge with their amino or guanidyl groups inserted between the base A22 and U23. In this pattern, the binding was stabilized by hydrogen bonds formed by the N7 or N9 atom with the backbone or the base in the groove. Although the side chain at N9 did not form hydrogen bond, it might contribute to the affinity of the anchor by electrostatic attraction. The number of hydrogen bond, formed by compounds **7a**–**7f** with TAR was fewer than compounds **6a**–**6f** and it was due to the fact that under physiologic conditions guanidyl group was fully protonated.

The fact that more potent HIV-1 Tat–TAR inhibitory activities of the 12 title compounds than those of our previous β-carboline and isoquinoline derivatives might suggest the increased interaction with TAR RNA of the purine ring. The substitution at N9 of the purine ring would affect the interaction between the compound and TAR. Unlike the previous work, there was no significant relationship between the biological activity of the title compounds and the length of the 'linker.' It was indicated that our purine derivatives might interact with TAR in a mode somewhat different from β-carboline and isoquinoline derivatives.

Table 2. Energy data of the title compounds in Autodock process

Compound	Docked energy (kcal/mol)	Free energy (kcal/mol)	K_i	Intermolecular energy (kcal/mol)	Internal energy (kcal/mol)
6a	−14.38	−11.35	9.78e−09	−14.15	−0.23
6b	−15.01	−11.7	2.67e−09	−14.81	−0.2
6c	−15.29	−10.23	3.7e−08	−14.59	−0.7
6d	−15.83	−10.34	2.63e−08	−15.01	−0.82
6e	−15.58	−11.14	6.84e−09	−15.19	−0.4
6f	−15.47	−10.57	1.78e−08	−14.93	−0.54
7a	−14.44	−11.04	8.08e−09	−14.15	−0.29
7b	−13.82	−10.03	4.49e−08	−13.45	−0.37
7c	−14.26	−8.96	2.71e−07	−13.63	−0.63
7d	−15.2	−9.52	1.06e−07	−14.5	−0.7
7e	−13.53	−8.72	4.04e−07	−13.08	−0.45
7f	−15.71	−10.48	2.09e−08	−15.15	−0.56

From the molecular modeling results, we found that the interaction pattern to TAR RNA of the title compounds is related to the structure of 'anchor.' Without a side chain at N9, the ring of compounds (**6a**, **6b**, **7a**, and **7b**) inserted into the minor groove near the tri-based bulge. In the other case, with a side chain at N9 of the ring, compounds (**6c**, **6d**, **6e**, **6f**, **7c**, **7e**, and **7f**) presented a different binding mode: the rings stayed in the major groove of TAR RNA and the amino or guanidly groups bound to the bulge by inserting between the bases A22 and U23. This is some different from the newly reported TAR RNA binding reagents such as rbt 203 and rbt 550.[14,15] Both of the binding models would efficiently block the Tat–TAR interaction. as compared with the TAR RNA binding reagents we reported previously.

3. Conclusion

In this work, we proposed a general molecular design for Tat–TAR inhibitors and synthesized a novel series of substituted purines as HIV-1 Tat–TAR inhibitors. Compared with our previous β-carboline and isoquinoline derivatives, the title compounds were more active in inhibiting HIV-1 Tat–TAR interaction in 239T cells and depressed SIV-induced syncytium in CEM174 cells, which might be due to the increased interaction with TAR RNA of the purine ring. Molecular modeling studies suggested the compounds could bind to TAR in two different modes according to the purine structures. All the findings provide us with new ideas in designing novel Tat–TAR inhibitors.

4. Experimental

4.1. Chemistry

All reactions were performed with commercially available reagents and they were used without further purification. Solvents were dried by standard methods and stored over molecular sieves. All reactions were monitored by thin-layer chromatography (TLC) and viewed with UV light. Melting points were determined on a XA-4 instrument and are uncorrected. All the title compounds were characterized by ^{1}H NMR spectra on a Brucker 300 MHz photometer using the solvents described. Chemical shifts were reported in δ ppm (parts per million) relative to Tetramethyl Silane (TMS) for deuteriorated water (D_2O). Signals were quoted as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The mass spectra (EI and FAB+) were recorded on JEOL-JMS-SX-PNBA, (*p*-nitrobenzyl alcohol) was used as matrix (M^+) which showed M+1 peak at 154, 2M+1 peak at 307.

4.2. General synthetic procedure

The synthetic work was carried out starting from 4-chloro-*N*,*N*-diethyl-6-methyl-5-nitro-pyrimidine-2-amine **1** and 5-nitro-pyrimidine-2,4-diamine **2** was easily prepared from it by amination.[23] Hydrogen reduction was preferred to prepare pyrimidine-2,4,5-triamine **3**. Without further purification, cyclization of **3** with CS_2 was performed in alkali ethanol and substituted purine-8-thiol **4** was obtained in a high yield after acidization of the reaction system. Under the catalysis of K_2CO_3/KI in THF, (purin-8-ylsulfanyl)-acetic acid ethyl ester **5** was obtained from **4** in a moderate yield at reflux temperature. By reactions with 5 equiv or more 1,2-ethylenediamine or 1,3-propylenediamine in methanol, **5** was conveniently converted to **6** in good yields. As a result the side chain with full length was introduced into the purine ring at C8. In the last step, amino iminomethane sulfonic acid ($AIMSO_3H{\cdot}H_2O$) was selected as guanidylation reagent in anhydrous ethanol considering the amphiphilicity or hydrophilicity of target compounds. $AIMSO_3H{\cdot}H_2O$ was chosen to construct the guanidyl group at the end of the side chain for its high reactivity, mild condition, simple procedure, and easy final treatment,[24] and which could be efficiently used in preparing other amphiphilic or hydrophilic compounds with guanidyl groups as well.

4.2.1. Aminoiminomethane sulfonic acid ($AIMSO_3H{\cdot}H_2O$). $AIMSO_3H{\cdot}H_2O$ was prepared from thiourea by the method reported in reference and obtained as a colorless crystal, mp 132–134 °C (dec).[24]

4.2.2. 4-Chloro-*N*,*N*-diethyl-6-methyl-5-nitro-pyrimidine-2-amine (1). This intermediate was obtained as yellow crystal, mp 69–71 °C.[23]

4.2.3. N^2,N^2-Diethyl-6-methyl-5-nitro-pyrimidine-2,4-diamine (2a). Compound **1** (1.5 g; 0.06 mol) was dissolved in anhydrous ethanol (20 mL) and $NH_3{\cdot}H_2O$ (26%, 10 mL) was added dropwise under stirring. After being reacted for 24 h at room temperature, the mixture was poured into water (100 mL) and (**2a**) was precipitated as yellow solid and then purified by column chromatography (silica gel, petroleum ether/ethyl acetate = 5:1, v/v), 1.35 g, mp 89–92 °C, yield = 98%.

4.2.4. N^4-(2-Diethylaminoethyl)-N^2,N^2-diethyl-6-methyl-5-nitro-pyrimidine-2,4-diamine (2b). Intermediate compounds (**2b**) were obtained from(**1**) and N^1,N^1-diethyl-ethane-1,2-diamine, After purified by column chromatography (silica gel, organic layer of $CHCl_3/CH_3OH/NH_3{\cdot}H_2O$ = 40:2:1, v/v/v), **2b** was obtained as yellow jelly in nearly stoichiometric yield, respectively. Without further purification, it was used in the next step directly.

4.2.5. N^4-(3-Dimethylaminopropyl)-N^2,N^2-diethyl-6-methyl-5-nitro-pyrimidine-2,4-diamine (2c). Compound **2c** was also obtained from N^1,N^1-dimethyl-propane-1,3-diamine and (**1**) by the same procedure and used in the next step directly.

4.2.6. N^2,N^2-Dimethyl-6-methyl-pyrimidine-2,4,5-triamine (3a). Compound **2a** (2.0 g; 0.0089 mol) was dissolved in anhydrous methanol (30 mL) containing Pd/C (10%, 0.1 g) and H_2 gas was bubbled into the solution under stirring for 36 h. Compound **3a** was obtained as colorless needle-like solid in stoichiometric yield when methanol was removed under reduced pressure. Without further purification, it was used in the next step directly.

4.2.7. N^4-(2-Diethylaminoethyl)-N^2,N^2-diethyl-6-methyl-pyrimidine-2,4,5-triamine (3b). Compound **3b** was obtained in the same procedure for **3a** after reaction for 8 h as pale-green jelly in stoichiometric yield, Without further purification, **3b** was directly used in the next step.

4.2.8. N^4-(3-Dimethylaminopropyl)-N^2,N^2-diethyl-6-methyl-pyrimidine-2,4,5-triamine (3c). Compound **3c** was obtained in the same procedure for **3a** after reaction for 8 h as pale-green jelly in stoichiometric yield. Without further purification, **3c** was directly used in the next step.

4.2.9. 2-Diethylamino-6-methyl-7,9-dihydro-purine-8-thione (4a). Compound **3a** (2.0 g; 0.0102 mol) was dissolved in ethanol (20 mL) containing KOH (0.58 g; 0.0102 mol) and CS_2 (0.5 mL) was added dropwise under stirring. After being reacted at room temperature for 1.5 h, the mixture was then heated to 78 °C and refluxed for 5 h. After ethanol was removed, the residue was dissolved in water (100 mL) and neutralized with dilute HCl (10%) to pH 5–6, then (**4a**) precipitated as pale-pink solid. After being purified by column chromatography (silica gel, petroleum ether/ethyl acetate = 3:1, v/v), **4a** was obtained as white flocculent solid, 2.16 g, mp 285 °C (dec), yield = 88.5%.

4.2.10. 2-Diethylamino-9-(2-diethylaminoethyl)-6-methyl-9*H*-purine-8-thiol (4b). The compound was obtained from **3b** in the same procedure for (**4a**). After being purified by column chromatography (silica gel, petroleum ether/ethyl acetate = 1:2, v/v), **4b** was obtained as white flocculent solid, mp 211–214 °C, yield = 87.1%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.12–1.21 (m, 12H), 2.43 (s, 3H), 2.70 (q, 4H), 2.90 (m, 2H), 3.64 (q, 4H), 4.25 (m, 2H). MS (EI) *m*/*z* calcd: 336.21, found: 336 ($M^{+\cdot}$).

4.2.11. 2-Diethylamino-9-(3-dimethylaminopropyl)-6-methyl-9*H*-purine-8-thiol (4c). Compound **4c** was also prepared from **3c** and purified by column chromatography (silica gel, ethyl acetate), white solid, mp 190–193 °C, yield = 83.3%. ^{1}H NMR (300 MHz, CD_3OD) δ1.17 (t, 6H), 2.01–2.12 (m, 2H), 2.28 (s, 6H), 2.44 (s, 3H), 2.42–2.50 (m, 2H) 3.62 (q, 4H), 4.23–4.30 (m, 2H). MS (EI) *m*/*z* calcd: 322.19, found: 322 ($M^{+\cdot}$).

4.2.12. (2-Diethylamino-6-methyl-9*H*-purin-8-ylsulfanyl)-acetic acid ethyl ester (5a). Compound **4a** (1.5 g; 0.0063 mol) was dissolved in HTF (50 mL) containing ethyl chloroacetate (1.16 g; 0.010 mol), K_2CO_3 (1.3 g; 0.0095 mol) KI (0.05 g) and reacted at room temperature for 12 h. After the residue was purified by column chromatography (silica gel, petroleum ether/ethyl acetate = 3:1,v/v), **5a** was obtained as white solid, 1.3 g, mp 84–88 °C, yield = 78.6%.

4.2.13. Ethyl [2-diethylamino-9-(2-diethylaminoethyl)-6-methyl-9*H*-purin-8-ylsulfanyl]acetate (5b). The compound was prepared from **4b** in the same way as (**5a**). After column chromatography (silica gel, petroleum ether/ethyl acetate = 1:1, v/v), **5b** was obtained as yellow oil, yield = 73.5%. ^{1}H NMR (300 MHz, CD_3Cl_3) δ 1.00–1.26 (t t, 15H), 2.52 (s, 3H), 2.57–2.61 (q, 4H), 2.73–2.76 (m, 2H), 3.59–3.63 (q, 4H), 4.06 (s, 2H), 4.06–4.10 (m, 2H), 4.17–4.19 (q, 2H). MS (EI) *m*/*z* calcd: 422.25, found: 422 ($M^{+\cdot}$).

4.2.14. Ethyl [2-diethylamino-9-(3-dimethylaminopropyl)-6-methyl-9*H*-purin-8-ylsulfanyl]-acetate (5c). Compound **5c** was prepared from (**4c**) and column chromatography (silica gel, methanol/ethyl acetate = 1:1, v/v), pale-pink solid, yield = 78.9%. ^{1}H NMR (300 MHz, CD_3Cl_3) δ 1.16–1.20 (m, 9H), 2.09–2.11 (m, 2H), 2.35 (s, 6H), 2.51–2.56 (m, 2H), 2.56 (s, 3H), 3.25–3.67 (m, 6H), 3.76 (s, 2H), 4.12–4.14 (m, 2H). MS (EI) *m*/*z* calcd: 408.23. found: 408 ($M^{+\cdot}$).

4.2.15. *N*-(2-Aminoethyl)-2-(2-diethylamino-6-methyl-9*H*-purin-8-ylsulfanyl)acetamide (6a). Compound **5a** (0.45 g; 0.0014 mol) was dissolved in anhydrous methanol (10 mL) containing 1,2-ethylenediamine (0.42 g; 0.0070 mol) and refluxed for 8 h. After methanol was removed under reduced pressure, the residue was purified by column chromatography (silica gel, the organic layer of MeOH/$CHCl_3$/$NH_3{\cdot}H_2O$ (26%) = 5:3:1, v/v/v). Compound **6a** was obtained as pale-yellow sticky jelly, yield = 85.2%. ^{1}H NMR (300 MHz, CD_3OD) δ1.01 (t, 6H, *J* = 6.54 Hz), 2.43 (s, 3H), 2.67 (m, 2H), 3.23 (m, 2H), 3.44 (dq, 4H, *J* = 6.54 Hz), 3.49 (s, 2H); MS (EI) *m*/*z* calcd: 337.17, found: 337 ($M^{+\cdot}$).

4.2.16. *N*-(3-Aminopropyl)-2-(2-diethylamino-6-methyl-9*H*-purin-8-ylsulfanyl)acetamide (6b). Compound **6b** was prepared in the same way as(**6a**), pale-yellow sticky jelly, yield = 73.6%. ^{1}H NMR (300 MHz, CD_3OD) δ 0.98 (m, 6H), 1.20, 1.50 (d t, 2H, *J* = 6.5 Hz), 2.20, 2.50 (d t, 2H, *J* = 6.51 Hz), 2.41 (s, 3H), 3.00, 3.15 (dt, 2H, *J* = 6.5 Hz), 3.21 (s, 2H), 3.49 (m, 4H); MS (EI) *m*/*z* calcd: 351.18, found: 351 ($M^{+\cdot}$).

4.2.17. *N*-(2-Aminoethyl)-2-[2-diethylamino-9-(2-diethylaminoethyl)-6-methyl-9*H*-purin-8-ylsulfanyl]acetamide (6c). Compound **6c** was obtained from **5b**. After purified by column chromatography (silica gel, the organic layer of $CHCl_3$/MeOH/$NH_3{\cdot}H_2O$ (26%) = 20:3:1, v/v/v). Compound **6c** was obtained as yellow oil, yield = 94.9%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.10–1.27 (m, 12H), 2.56–2.63 (m, 4H), 2.63 (s, 3H), 2.75–2.82 (dm, 4H), 3.29–3.35 (m, 2H), 3.62–3.69 (q, 4H), 3.83 (s, 2H), 4.06 (t, 2H), 8.59 (s, 1H); MS (FAB+) *m*/*z* calcd: 436.27, found: 437.2 [$(M+1)^+$].

4.2.18. *N*-(3-Aminopropyl)-2-[2-diethylamino-9-(2-diethylaminoethyl)-6-methyl-9*H*-purin-8-ylsulfanyl]acetamide (6d). Compound **6d** was obtained from **5b** and purified by column chromatography as (**6c**). Compound **6d**: yellow oil, yield = 95.7%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.00–1.20 (d t, 12H), 1.56–1.65 (m, 2H), 2.55 (s, 3H), 2.60–2.79 (m, 4H), 2.52–2.58 (m, 4H), 3.30–3.37 (m, 2H), 3.66 (q, 4H), 3.82 (s, 2H), 4.03–4.08 (t, 2H), 8.57 (s, 1H). MS (FAB+) *m*/*z* calcd: 450.29, 450.7 ($M^{+\cdot}$).

4.2.19. *N*-(2-Aminoethyl)-2-[2-diethylamino-9-(3-dimethylaminopropyl)-6-methyl-9*H*- -purin-8-ylsulfanyl]acetamide (6e). Compound **6e** was prepared from **5c** and purified by column chromatography (silica gel, the

organic layer of $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (26%) = 40:5:1, v/v/v). Compound **6e**: yellow oil, yield = 96.7%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.20 (t, 6H), 1.93–1.98 (m, 2H), 2.24 (s, 6H), 2.32 (t, 2H), 2.57 (s, 3H), 2.78–2.82 (m, 2H), 3.29–3.35 (m, 2H), 3.66 (q, 4H), 3.84 (s, 2H), 4.04–4.09 (m, 2H), 8.54 (s, 1H). MS (FAB+) *m*/*z* calcd: 426.26, found: 426.0 (M^+).

4.2.20. *N*-(3-Aminopropyl)-2-[2-diethylamino-9-(3-dimethylaminopropyl)-6-methyl-9*H*-purin-8-ylsulfanyl]acetamide (6f). Compound **6f** was prepared from **5c** and purified by column chromatography as (**6e**). Compound **6f**: yellow oil, yield = 93.6%. ^{1}H NMR (300 MHz, CD_3OD) δ1.19 (t, 6H), 1.56–1.63 (m, 2H), 1.92–1.97 (m, 2H), 2.24 (s, 6H), 2.30–2.35 (m, 2H), 2.55 (s, 3H), 2.68–2.71 (m, 2H), 3.30–3.34 (m, 2H) 3.66 (q, 4H), 3.83 (s, 2H), 4.03–4.08 (m, 2H). MS (FAB+) *m*/*z* calcd: 436.27, found: 436.8 ($M^{+\cdot}$).

4.2.21. *N*-(2-Guanidinoethyl)-2-(2-diethylamino-6-methyl-9*H*-purin-8-ylsulfanyl)acetamide hydrosulfite (7a). $AIMSO_3H \cdot H_2O$ (0.15 g, 0.001 mol) was added by portion to the solution of **6a** (0.4 g: 0.00091 mol) in 15 ml anhydrous ethanol in water bath at 35–45 °C in 1 h. After being stirred for 2 h, the solvent was moved under reduced pressure and a pale-yellow jelly was obtained. After recrystallized in isopropanol, **7a** was obtained as moisture regained jelly, 0.42 g, yield = 87.7%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.02 (m, 6H), 2.62 (s, 3H), 3.20 (m, 2H), 3.32 (dq, 4H, J = 6.54 Hz), 3.90 (m, 2H), 4.10 (s, 2H); MS (FAB+) *m*/*z* calcd: 379.19, found: 379.8 [$(M+1)^+$].

4.2.22. *N*-(3-Guanidinopropyl)-2-(2-diethylamino-6-methyl-9*H*-purin-8-ylsulfanyl) acetamide hydrosulfite (7b). Compound **7b** was prepared from **6b** moisture regained jelly, 0.45 g, yield = 83.1%. ^{1}H NMR (300 MHz, CD_3OD) δ 1.01 (t, 6H, J = 6.18 Hz), 1.20, 1.50 (dt, 2H, J = 6.9 Hz), 2.43 (s, 3H), 2.63, 3.20 (dt, 2H, J = 6.8 Hz), 3.08 (m, 4H, J = 5.8 Hz), 3.48 (m, 2H, J = 6.8 Hz); MS (FAB+) *m*/*z* calcd: 393.21, found: 393.4 ($M^{+\cdot}$).

4.2.23. 2-[2-Diethylamino-9-(2-diethylaminoethyl)-6-methyl-9*H*-purin-8-ylsulfanyl]-*N*-(2-guanidinoethyl)acetamide (7c). Compound **7c** was prepared from **6c** in the same way as (**7a**) and (**7b**). After being purified by column chromatography (silica gel, the organic layer of $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ (26%) = 40:60:7, v/v/v), **7c** was obtained as pale-yellow jelly, yield = 91.2%. ^{1}H NMR (300 MHz, D_2O) δ 0.97, 1.13 (d t, 12H), 2.34 (s, 3H), 2.99–3.06 (m, 2H), 3.11–3.23 (m, 4H), 3.21–3.24 (m, 4H), 3.43 (q, 4H), 3.81 (s, 1H), 4.28, 4.63 (d t, 2H). MS (FAB+) *m*/*z* calcd: 478.30, found: 479.1 [$(M+1)^+$].

4.2.24. 2-[2-Diethylamino-9-(2-diethylaminoethyl)-6-methyl-9*H*-purin-8-ylsulfanyl]-*N*-(3-guanidino-propyl)acetamide (7d). Compound **7d** was prepared from (**6d**) and purified by column chromatography as **7c**: pale-yellow jelly, yield = 91.5%. ^{1}H NMR (300 MHz, D_2O) δ1.00, 1.13 (dt, 12H), 1.40–1.70 (m, 2H), 2.41 (s, 3H), 3.00–3.10 (m, 2H), 3.12–3.17 (m, 4H), 3.28–3.33 (m, 2H), 3.76 (s, 2H), 4.59 (q, 2H); MS (FAB+) *m*/*z* calcd: 492.31, found: 493.4 [$(M+1)^+$].

4.2.25. 2-[2-Diethylamino-9-(3-dimethylaminopropyl)-6-methyl-9*H*-purin-8-ylsulfanyl]-*N*-(2-guanidino-ethyl)acetamide (7e). Compound **7e**: pale-yellow jelly, yield = 90.9%. ^{1}H NMR (300 MHz, D_2O) δ 0.99 (t, 6H), 2.16–2.21 (m, 2H), 2.40 (s, 3H), 2.68 (s, 6H), 3.01–3.07 (m, 2H), 3.18–3.29 (d m, 4H), 3.46 (q, 4H), 3.61 (s, 2H), 4.26–4.31 (m, 2H). MS (FAB+) *m*/*z* calcd: 464.28, found: 464.9 ($M^{+\cdot}$).

4.2.26. 2-[2-Diethylamino-9-(3-dimethylaminopropyl)-6-methyl-9*H*-purin-8-ylsulfanyl]-*N*-(3-guanidino-propyl)acetamide (7f). Compound **7f**: pale-yellow jelly, yield = 91.2%. ^{1}H NMR (300 MHz, D_2O) δ 0.99 (t, 6H), 1.62 (m, 2H), 2.19 (m, 2H), 2.41 (s, 3H), 2.68 (s, 6H), 3.04–3.17 (d m, 4H), 3.37 (t, 2H), 3.48 (q, 4H), 3.61 (s, 2H), 4.30 (t, 2H). MS (FAB+) *m*/*z* calcd: 478.30, found: 477.8 [$(M-1)^+$].

4.3. Biological evaluation

Transient transfection and CAT assays: 293T cells were grown as monolayer in Dulbecco's modified Eagle's medium (DMEM) (Gibco–BRL) supplemented with 10% (v/v) fetal calf serum, penicillin (100 U mL^{-1}), and streptomycin (100 U mL^{-1}) at 37 °C in 5% CO_2 containing humidified air. The cells were seeded at a six-well plate 24 h prior to transfection which was performed by standard calcium phosphate coprecipitation techniques with optimum amounts of the plasmids pLTRCAT and pSVCMVTAT. Twenty four hours later, the culture medium was removed and the cells were washed twice with phosphate-buffered saline (PBS). Then the transfected cells were added to fresh medium together with diluted compounds at final concentration of 30 μM, respectively, and incubated for another 24 h. After 48-h post-transfection, the cells were harvested and analyzed for CAT activity using a commercial CAT ELISA kit (Roche Molecular Biochemicals) in accordance with the manufacturer's protocol. All data were reported as a percentage of CAT activity (±SD). Results shown were representative of three independent experiments.

Inhibition of SIV-induced syncytium in CEM174 cell cultures was measured in a 96-well microplate containing 2×10^5 CEM cells/mL infected with 100 $TCID_{50}$ of SIV per well and containing appropriate dilutions of the tested compounds. After 5 days of incubation at 37 °C in 5% CO_2 containing humidified air, CEM giant (syncytium) cell formation was examined microscopically. The EC_{50} was defined as the compound concentration required to protect cells against the cytopathogenicity of SIV by 50%. AZT was used as the positive control at a concentration of 10 μM here.

4.4. Molecular modeling

The initial structures of our compounds were subjected to minimization using MOPAC in Chemoffice 2002 and the 3D structure of HIV-1 TAR RNA in complex

with its inhibitor rbt 203 was recovered from the Protein Database (http://www.PDB.org) with the code as 1UUI. The advanced docking program Auto-dock 3.0 was used to remove the small molecule and perform the automatic molecular docking with our compounds. The number of generations, energy evaluation, and docking runs were set to 370,000, 1,500,000, and 30, respectively, and the kinds of atomic charges were taken as Kollman-all-atom for HIV-1 TAR RNA and Gasteiger-Hücel for the compounds.

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