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Design and SAR of new substituted purines bearing aryl groups at N9 position as HIV-1 Tat–TAR interaction inhibitors

Ruifang Pang, Chunlei Zhang, Dekai Yuan, Ming Yang *

The State Key Laboratory of Natural and Biomimetic Drugs, Peking University Health Science Center, PO Box 261, Beijing 100083, PR China

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ABSTRACT

Twenty-four purine derivatives bearing aryl groups at N9 position were designed and synthesized as HIV-1 Tat–TAR interaction inhibitors. All the compounds showed high antiviral activities in inhibiting the formation of SIV-induced syncytium in CEM174 cells. Ten of them with low cytotoxicities were evaluated by Tat dependent HIV-1 LTR-driven CAT gene expression colorimetric enzyme assay in human 293T cells at a concentration of 30 μM, indicating effective inhibitory activities of blocking the Tat–TAR interaction. The aryl groups at N9 position affected the binding affinities between compounds and TAR RNA, showing some specificities of aryl groups to TAR RNA.

1. Introduction

In spite of remarkable medical advances, acquired immune deficiency syndrome (AIDS) has reached worldwide epidemic proportions. Nowadays a variety of drugs are available for the clinical treatment of HIV-1 infection.[1–3] These drugs mainly inhibit two key enzymes in the HIV-1 life cycle, the reverse transcriptase and protease, and more recently viral entry.[4] However, viral rebound during therapy, the emergence of HIV drug resistance and the need for long-term treatment modalities often result in treatment failure with existing antiretrovirals.[5] These point to the needs of identifying and developing novel antiretroviral agents with different targets for inhibition of HIV-1 replication.[6,7] HIV replication relies on the transcription and translation machineries of its host cell. Transcription of HIV proviral DNA is regulated by the interaction of the viral transactivator protein Tat with the transactivation response element region (TAR), a 59-nucleotide stem-loop structure located at the 5′-end of all nascent transcribed HIV-1 mRNAs.[8,9] TAR contains a six-nucleotide loop and a three-pyrimidine nucleotide bulge, which connects two adjacent helical stem regions. The tri-nucleotide bulge (U23, C24, and U25) is essential for high-affinity and specific binding of the Tat protein. Tat is a protein with 86 amino acid residues. It includes a basic region (amino acid residues 48–59), termed arginine-rich motif (ARM), responsible for Tat–TAR specific interaction.[10] Tat binds to the three-base bulge of TAR and recognizes both the adjacent Watson–Crick base pairs and the surrounding phosphate groups.[11] In the absence of Tat, the transcription yields only short polyadenylated transcripts even though it does occur. Thus, the interaction between TAR and Tat is essential for virus replication and inhibiting Tat–TAR interaction is a potential approach for anti-HIV therapeutics.

Our previous work has described some β-carboline, isoquinoline, α,α-trehalose, and purine derivatives as HIV-1 Tat–TAR inhibitors.[12–17] We proposed a molecular model of new Tat–TAR inhibitors containing an 'activator', an 'anchor', and a 'linker'. 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. A 'linker' is the structure linking the 'activator' and the 'anchor' with optimal length.[17] The studies before show that the substituted purine derivatives possess more potent HIV-1 Tat–TAR inhibitory activities than β-carboline and isoquinoline derivatives due to the increased interaction between TAR RNA and the purine ring, which can be mutually complementary to the tri-based bulge. It was also indicated that the substitution at N9 position of the purine would affect the affinity of the compound toward TAR. Molecular modeling studies suggested that the compounds with side chains at N9 of the purine ring could bind to TAR in different modes from the compounds without side chains at N9. However, the structure–activity relationship (SAR) for N9 substituents was still not clear.[17] In order to make further study of their TAR RNA binding properties and to explore their SAR, we designed and synthesized 24 new purine derivatives bearing aryl groups at N9 position, as shown in Figure 1. According to our previous work,

* Corresponding author. Tel.: +86 10 82805264; fax: +86 10 82802724.
E-mail address: mingy@mail.bjmu.edu.cn (M. Yang).

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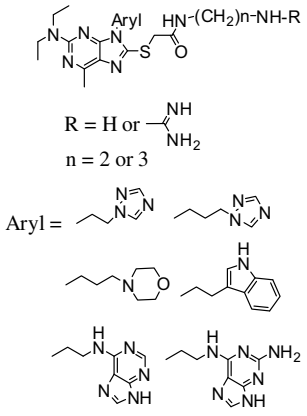
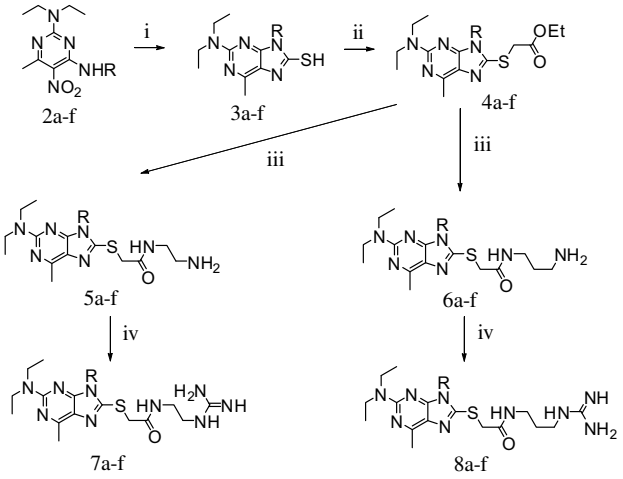


Figure 1. The structure of title compounds.



Scheme 2. Synthesis of compounds **5a–f**, **6a–f**, **7a–f**, and **8a–f**. Reagents and conditions: (i) H_2, 10% Pd/C, CH_3OH, rt, NaOH, CS_2, CH_3CH_2OH, reflux; (ii) K_2CO_3, $BrCH_2COOEt$, acetone, reflux; (iii) methanol, reflux; (iv) ethanol, aminoiminomethane sulfonic acid ($AIMSO_3H_2O$), K_2CO_3. R is the same as that shown in Scheme 1.

we assumed that the side chain terminated with substituted amino groups at N9 might contribute to the affinity with TAR by nonspecific electrostatic attraction. In this work we introduced aryl groups into the terminal of the side chain at N9 position, which might bind to TAR RNA in multiple ways, for example, forming hydrogen bond with unpaired base and aromatic π–π interaction in addition to nonspecific electrostatic attraction.

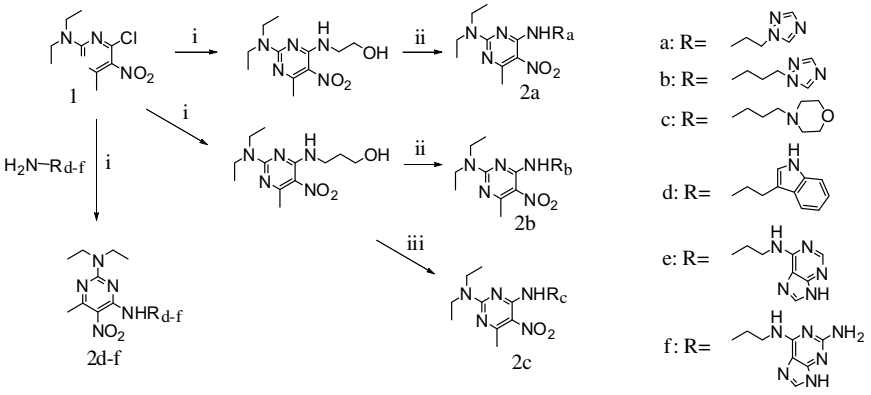
All the title compounds were synthesized for the first time and all of them were evaluated for their inhibitory ability to Tat–TAR interaction and antiviral activity

2. Results and discussion

The route used for the preparation of the title compounds (**5a–f**, **6a–f**, **7a–f**, and **8a–f**) was carried out as outlined in Scheme 1 and Scheme 2 and the synthetic procedures were explained in Section 4. Twenty-four compounds were obtained and their MS, ^1H NMR spectroscopy data were provided in Section 4.

The biological activities of all the title compounds were evaluated by SIV-induced syncytium in CEM cells. Their EC_{50}, TC_{50}, and SI values are listed in Table 1. As shown in Table 1, most of title compounds possessed a TC_{50} value more than 100 μM except **6b** (31.7 μM), **5c** (64.8 μM), **6c** (87.5 μM), **7e** (92.7 μM) and **5–8d** (6.5–29.5 μM). With regard to their antiviral activities, each of them possessed an EC_{50} value within the range from 1.6 μM to 20.0 μM, suggesting that all compounds possessed effective anti-SIV activity and some with low cytotoxicities. Furthermore, taking a detailed look at the data in Table 1, it was found as follows: (a) the length of the side chains at C8 position of the purine ring and terminal amino and guanidyl groups had some effect on the binding affinity with TAR but it was not decisive factor compared with the aryl group at N9 position; (b) considering aryl group at N9 position, the compounds bearing indol group (**5–8d**) possessed the most effective anti-SIV activities (EC_{50}: 1.6–7.9 μM) but the highest cytotoxicities among all these title compounds. It is likely that the aromaticity of the indol group contributed to enhance the binding affinity with TAR while its high hydrophobicity and strong intercalative binding interaction with host DNA might result in high cytotoxicity; (c) the compounds bearing purine group showed effective inhibitory activity without apparent cellular toxicity, and adenine (**5–8e**; their EC_{50}: 2.5–13.0 μM) group was better than guanine group (**5–8f**; their EC_{50}: 6.3–20.0 μM); (d) the anti-SIV activities of the compounds bearing trizole group (**5–8a**; their EC_{50}: 7.1–13.8 μM and **5–8b**; their EC_{50}: 5.4–8.9 μM) were lower than those of the compounds bearing purine group. And the length of the linker which connected the purine ring and the trizole group at N9 position also had effect on binding affinity, the longer linker with the stronger affinity; (e) the compounds bearing morpholine group possessed low anti-SIV activity compared with other title compounds. It might be due to the nonspecific binding of the protonated morpholine group with TAR RNA instead of specific binding.

To ascertain our assumption, we performed molecular modeling experiments of three title compounds with different substituents at N9 position (**5b**, 5c, and **5e**) using Autodock 3.0 to study the



Scheme 1. Synthesis of compounds **2a–f**. Reagents and conditions: (i) Et_3N, CH_2Cl_2, rt; (ii) Et_3N, CH_2Cl_2, methanesulfonyl chloride, 4–10 °C, sodium 1,2,4-triazol-1-ide, TBAB, DMF, 80 °C; (iii) Et_3N, CH_2Cl_2, methanesulfonyl chloride, 4–10 °C; morpholine, ethanol.

Table 1
Inhibiton 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})
5a	13.8	>100	>7.2
6a	11.5	>100	>8.7
7a	12.4	>100	>8.1
8a	7.1	>100	>14.1
5b	6.9	>100	>14.5
6b	8.9	31.7	3.6
7b	5.4	>100	>18.5
8b	5.8	>100	>17.2
5c	12.4	64.8	5.2
6c	11.8	87.5	7.4
7c	18.9	>100	>5.3
8c	11.2	>100	>8.9
5d	1.6	6.5	4.1
6d	4.2	10.2	2.4
7d	7.9	29.5	3.7
8d	5.6	29.1	5.2
5e	2.5	>100	>40
6e	5.5	>100	>18.2
7e	7.8	92.7	11.9
8e	13.0	>100	>7.7
5f	20.0	>100	>5
6f	6.5	>100	>15.4
7f	6.1	>100	>16.4
8f	8.3	>100	>12.0

[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.

interaction between these compounds and HIV-1 TAR RNA. As shown in Figure 2, the purine scaffold of compounds **5b**, 5c, and **5e** fitted into the major groove of TAR RNA. The adenine group at N9 position of compound **5e** formed a base-triple with the base pair G28:C37, so their π–π interaction and the H bond formed by the hydrogen at N9 of the adenine ring with the N7 of the base G28 as well as the cationic amino groups at C8 position interacted with the anionic phosphate groups surrounding the tri-base bulge of TAR might reinforce the binding affinity, which would explain the best biological activity of compound **5e**. Differently, the binding of compound **5b** and TAR RNA was stabilized by stacking the trizole group at N9 between A22 and U23. Considering physiologic conditions, the morpholine group of **5c** was assumed to be protonated in the process of computation. It seemed that the affinity obtained from computation was mainly caused by electrostatic attraction and more hydrogen bonds formed by two cationic side chains of **5c** with TAR RNA instead of interaction between the morpholine group at N9 position and TAR, which might be responsible for its lowest activity that exhibited in the biological tests. These results suggested that the suitable aryl group at N9 position, for example, purine might improve the binding specificity of the compound with the tri-base bulge region of TAR RNA.

In order to reveal whether the compounds we designed could inhibit HIV-1 Tat–TAR interaction, we evaluated ten title compounds with low cytotoxicities (**5b**, **7b**, **5c**, **6c**, **7c**, **8c**, **5e**, **6e**, 7e, and **8e**) by Tat dependent HIV-1 LTR-driven CAT gene expression colorimetric enzyme assay in human 293T cells 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 3, the range of inhibited CAT expression induced by the ten compounds from 48.2% to 83%. The compounds bearing adenine group (**5e**, 6e, and **8e**) showed the most depressed CAT expression (48.2%, 52.3%, and 58.6%), while those bearing morpholine group (**5c**, 6c, and **7c**) exhibited the weakest effection on CAT expression (80.4%, 83%, and 81.8%). These results complied with those of the

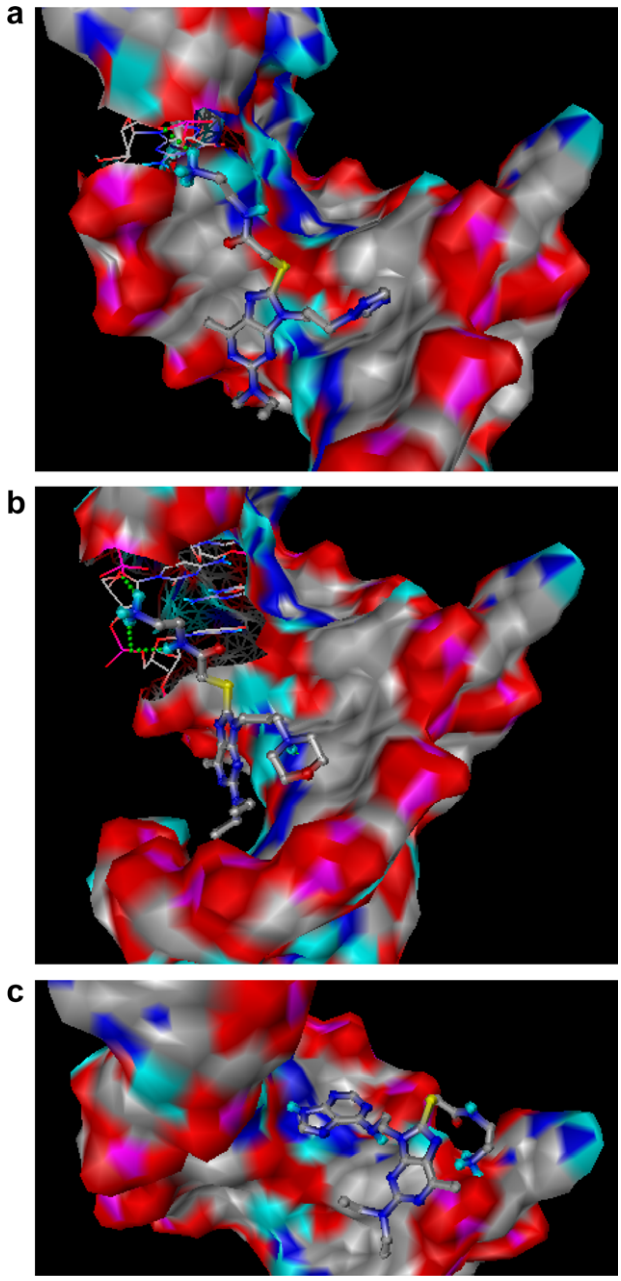


Figure 2. (a) Interaction of compound **5b** to TAR RNA; (b) Interaction of compound **5c** to TAR RNA; (c) Interaction of compound **5e** to TAR RNA.

SIV assay and were also agreed well with the molecular modeling studies. The decreased CAT activities in the presence of these ten title compounds suggested that all of them could effectively block the interaction of Tat–TAR in cell-based assay. And the aryl group at N9 position of purine ring did affect the binding specificity of compounds and TAR RNA.

However, as compared with all the title compounds, the substituted purines with a tri-substituted amino group at N9 position we reported before exhibited a little bit higher antiviral activities in inhibiting the formation of SIV-induced syncytium and blocked the TAR transactivation more effectively in CAT expression assay.[17] These results suggested that the size of the substituent at N9 position of purine ring was very important. Although the suitable substitution at N9 position would reinforce the binding affinity between the 'anchor'-substituted purine ring and TAR, the big aryl group, for example, guanine might reduce its binding affinity due

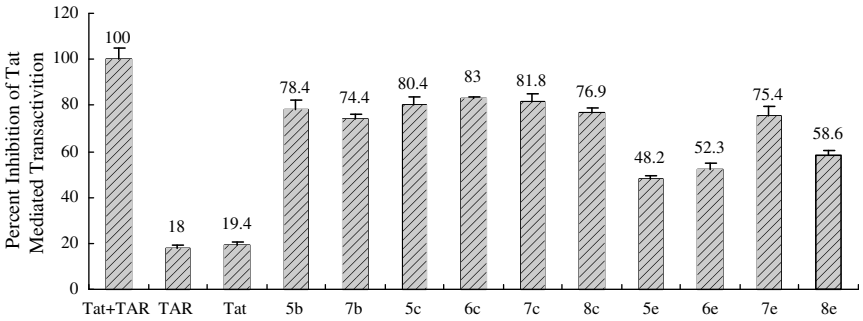


Figure 3. Effects of the ten title compounds on Tat-mediated Transactivation in 293T cells.

to the steric hindrance. We assumed that in the modification at N9 position of the purine ring, in addition to hydrogen bonds, electrostatic attraction and base-pair interaction, etc., the steric hindrance should be considered. The findings were in fact contrary to our assumption, but it provided us a new idea about the 'anchor' of our inhibitor model.

3. Conclusion

In this work, all the experiments reported here showed that the newly designed purine derivatives bearing aryl groups at N9 position could block the Tat–TAR interaction and had potent inhibition activity of SIV. The different aryl groups at N9 position could affect the binding affinity with TAR RNA in varying potency, thus bringing about different effects on the biological activity of the title compounds. However, due to the steric hindrance the aryl groups at N9 position did not significantly increase the biological activities of the title compounds compared with the substituted purines we reported before, which implied that the huge substituent groups of the purine ring were not helpful for improving the binding affinity between compounds and TAR RNA. These studies provide a new insight into the SAR of N9-substituted purines as HIV-1 Tat–TAR interaction inhibitors.

4. Experimental

4.1. Chemistry

All materials were commercially available and used without further purification. All the titled compounds were characterized by ^{1}H NMR spectra on a Varian 300 MHz spectrometer using the solvents described. Chemical shifts were reported in δ ppm (parts per million) relative to tetramethyl silane (TMS) except for deuteriorated water (D_2O) and the signals were quoted as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The mass spectra (EI or ESI) were recorded. Melting points were determined on a XA-4 instrument which was uncorrected.

4.2. General synthetic procedure

The synthetic work was started from 4-chloro-*N*,*N*-diethyl-6-methyl-5-nitro-pyrimidine-2-amine (compound **1**) as reported.[17,18] To a solution of compound **1** in chloroform, appropriate amines were added to afford compounds **2a**–**f**. The hydrogenation of compounds **2a**–**f** was performed in a GCD-500 hydrogen gas generator at 60 psi pressure using 10% Pt/C as catalyst. The products of reduction were used without purification for the cyclization reactions in alkaline ethanol with CS_2. Compounds **3a**–**f** obtained were heated in acetone under reflux with ethyl 2-bromoacetate to afford ethyl 2-(2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio) acetates (compounds **4a**–**f**). Ethane-1,2-diamine or 1,3-diaminopropane was heated in methanol under reflux with compounds **4a**–**f** to afford corresponding amides **5a**–**f** or **6a**–**f**, respectively. In the last step, aminoiminomethane sulfonic acid ($AIMSO_3H_2O$) was selected as guanidylation reagent in anhydrous ethanol considering its various advantages to afford compounds **7a**–**f** and **8a**–**f**.[19] All materials were commercially available and used without further purification.

4.2.1. N^4-(2-(1*H*-1,2,4-Triazol-1-yl)ethyl)-N^2,N^2-diethyl-6-methyl-5-nitro-pyrimidine-2,4-diamine (2a)

Compound **2a** was obtained through three steps of reactions. To a solution of 4-chloro-*N*,*N*-diethyl-6-methyl-5-nitro-pyrimidine-2-amine (compound **1**) (0.5 g, 2 mmol) and triethylamine (0.5 ml) in 15 ml chloroform, 0.23 ml of 2-aminoethanol (3.5 mmol) was added and the solution was stirred at room temperature for 6 h and then was concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel eluted with petroleum ether–ethyl acetate (10:1, v/v). 2-(2-(Diethylamino)-6-methyl-5-nitropyrimidin-4-ylamino)ethanol was obtained as yellow solid, 0.5 g, yield 93%, mp 75–77 °C. ^{1}H NMR ($CDCl_3$) δ 8.99 (s, 1H), 3.87–3.86 (d, 2H), 3.76–3.67 (m, 4H), 3.63–3.56 (q, 2H), 2.76 (s, 1H), 2.68 (s, 3H), 1.24–1.17 (m, 6H).

To a solution of 2-(2-(diethylamino)-6-methyl-5-nitropyrimidin-4-ylamino)ethanol (0.5 g, 1.86 mmol) and triethylamine (0.19 g, 1.86 mmol) in 15 ml CH_2Cl_2 under 10 °C, methanesulfonyl chloride (0.13 ml, 1.86 mmol) was added dropwise. The mixture was stirred at about 10 °C for 1.5 h. Then the solvent was evaporated under reduced pressure and the residue was dissolved in 15 ml dry DMF. Sodium 1,2,4-triazol-1-ide (0.45 g, 10 mmol) was added and the mixture was heated at 80–85 °C for 10 h. The resulting mixture was poured into 40 ml water and extracted with 30 ml × 3 ethyl acetate. The combined organic phase was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on a silica gel column eluted with petroleum ether–ethyl acetate–methanol (10:3:1, v/v) to give 0.5 g of **2a** as yellow solid, yield 84%, mp 148–150 °C. ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1H), 8.06 (s, 1H), 7.99 (s, 1H), 4.49–4.45 (t, 2H), 4.04–4.00 (t, 2H), 3.76–3.60 (m, 4H), 2.70 (s, 3H), 1.26–1.19 (q, 6H).

4.2.2. N^4-(3-(1*H*-1,2,4-Triazol-1-yl)propyl)-N^2,N^2-diethyl-6-methyl-5-nitro-pyrimidine-2,4-diamine (2b)

Compound **2b** was prepared from compound 1 (1.72 g, 7.0 mmol) and 3-aminopropan-1-ol (0.79 g, 10.5 mmol) according to the procedure for **2a**. The crude product was purified by flash chromatography on a silica gel column eluted with petroleum ether–ethyl acetate (5:1, v/v) to afford 1.64 g of 3-(2-(diethylamino)-6-methyl-5-nitropyrimidin-4-ylamino)propan-1-ol as yellow solid, yield 82%, mp 68–69 °C. ^{1}H NMR ($CDCl_3$) δ 8.89 (s, 1H), 3.76–3.67 (m, 6H), 3.65–3.58 (q, 2H), 2.69 (s, 3H), 2.19 (s, 1H), 1.93–1.87 (m, 2H), 1.24–1.18 (q, 6H).

This compound was activated by methanesulfonyl chloride and reacted with sodium 1,2,4-triazol-1-ide to give compound **2b**. The purification of compound **2b** was performed on silica gel column eluted with petroleum–CH_2Cl_2–methanol (20:5:1, v/v) and yellow solid was obtained, yield 87%, mp 103–105 °C. ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1H), 8.08 (s, 1H), 7.98 (s, 1H), 4.30–4.26 (t, 2H), 3.74–3.67 (q, 2H), 3.61–3.51 (m, 4H), 2.69 (s, 3H), 2.29–2.24 (m, 2H), 1.22–1.14 (q, 6H).

4.2.3. N^2,N^2-Diethyl-6-methyl-N^4-(3-morpholinopropyl)-5-nitro-pyrimidine-2,4-diamine (2c)

3-(2-(Diethylamino)-6-methyl-5-nitropyrimidin-4-ylamino) propan-1-ol (3.40 g, 12.0 mmol) was dissolved in 30 ml CH_2Cl_2 and then 3.3 ml triethylamine (24.0 mmol) was added. The solution was cooled in ice bath for 5 min and then the solution of methanesulfonyl chloride (1.40 ml, 18.0 mmol) in 20 ml CH_2Cl_2 was added dropwise. The resulting solution was stirred at about 15 °C overnight. The mixture was concentrated and subjected to column chromatography on silica gel eluted with petroleum ether–ethyl acetate (10:3, v/v). Yellow solid was obtained and dissolved in 30 ml ethanol. Fifteen milliliters of morpholine was added and the solution was heated at about 70 °C for 6 h. The resulting mixture was concentrated under reduced pressure and the residue was purified by chromatography on a silica gel column eluted with petroleum ether–ethyl acetate (3:1, v/v) to give 3.92 g of **2c** as yellow oil, yield 93%. ^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1H), 3.76–3.64 (m, 6H), 3.62–3.56 (m, 4H), 2.68 (s, 3H), 2.47–2.42 (t, 2H), 1.87–1.78 (m, 2H), 1.23–1.17 (m, 6H).

4.2.4. N^4-(2-(1*H*-Indol-3-yl)ethyl)-N^2,N^2-diethyl-6-methyl-5-nitro-pyrimidine-2,4-diamine (2d)

To a solution of compound **1** (2.45 g, 10.0 mmol) and triethylamine (2.0 ml, 14.3 mmol) in 50 ml chloroform, tryptamine (1.60 g, 10 mmol) was added and stirred at 15 °C overnight. The mixture was concentrated under reduced pressure and the residue was purified by chromatography on a silica gel column eluted petroleum ether–ethyl acetate (3:1, v/v) to give yellow solid which was recrystallized from CH_2Cl_2–methanol to give 3.17 g of **2d**, mp 158–160 °C. ^{1}H NMR ($CDCl_3$) δ 8.81 (s, 1H), 8.07 (s, 1H), 7.64–7.61 (d, 1H), 7.39–7.36 (d, 1H), 7.23–7.07 (m, 3H), 3.86–3.80 (q, 2H), 3.71–3.59 (m, 4H), 3.13–3.08 (t, 2H), 2.67 (s, 3H), 1.24–1.17 (q, 6H).

4.2.5. N^4-(2-(9*H*-Purin-6-ylamino)ethyl)-N^2,N^2-diethyl-6-methyl-5-nitro-pyrimidine-2,4-diamine (2e)

6-Chloro-9*H*-purine (1.55 g, 10.0 mmol) and 6.7 ml ethane-1,2-diamine (100 mmol) was dissolved in the mixture of 30 ml butan-1-ol and 5 ml water. The solution was heated at 80 °C for 24 h, cooled and concentrated under reduced pressure. The residue was suspended in 20 ml CH_2Cl_2 and filtered. The solid was washed with 10 ml acetone and 15 ml methanol. N^1-(9*H*-purin-6-yl)ethane-1,2-diamine was collected as pale yellow solid, 1.46 g, yield 82%. ^{1}H NMR (D_2O) δ 8.08 (s, 1H), 7.97 (s, 1H), 3.76–3.72 (t, 2H), 3.19–3.15 (t, 2H).

The obtained N^1-(9*H*-purin-6-yl)ethane-1,2-diamine (1.46 g, 8.2 mmol) and compound **1** (2.40 g, 9.8 mmol) was dissolved in the mixture of 50 ml ethanol and 10 ml water. 1.71 ml of triethylamine (12.3 mmol) was added. The mixture was heated at 50–60 °C for 40 h. The resulting mixture was cooled to room temperature, filtered and the solid was washed with 20 ml water. The filtrate was concentrated and filtered again. The solid was combined, washed with 10 ml methanol and 10 ml ethyl acetate to afford 2.8 g of **2e**, yield 88%, mp 246–248 °C. ^{1}H NMR (DMSO-d_6) δ 12.89 (s, 1H), 8.94 (s, 1H), 8.17 (s, 1H), 8.07 (s, 1H), 7.85 (s, 1H), 3.74 (s, 2H), 3.66–3.59 (q, 4H), 3.55–3.53 (d, 2H), 2.54 (s, 3H), 1.13–1.08 (t, 6H).

4.2.6. N^6-(2-(2-(Diethylamino)-6-methyl-5-nitropyrimidin-4-ylamino)ethyl)-9*H*-purine-2,6-diamine (2f)

6-Chloro-9*H*-purin-2-amine (1.70 g, 10.0 mmol) was dissolved in 10 ml ethane-1,2-diamine, and the solution was heated at 90–95 °C for 14 h. The ethane-1,2-diamine was removed under vacuum, and the residue was suspended in 20 ml methanol. To the mixture, 30 ml diethyl ether was added, mixed and filtered. The solid was collected. The filtrate was concentrated, and 30 ml diethyl ether was added and filtered again. The solid was combined and recrystallized from methanol to give 1.68 g of N^6-(2-aminoethyl)-9*H*-purine-2,6-diamine, yield 87%. ^{1}H NMR (D_2O) δ 7.61 (s, 1H), 3.66–3.63 (t, 2H), 3.15–3.11 (t, 2H).

1.93 g (10.0 mmol) of N^6-(2-aminoethyl)-9*H*-purine-2,6-diamine and 2.93 g of compound **1** (12.0 mmol) were dissolved in 50 ml methanol, and 2.1 ml of triethylamine (15.0 mmol) was added. The mixture was heated under reflux for 40 h, cooled, and filtered. The solid was washed with 30 ml diethyl ether. The filtrate was concentrated, and 60 ml of diethyl ether was added. The mixture was filtrated, and the solid was combined with the filter cake obtained previously to give 3.5 g of compound **2f**, yield 87%. A small amount of **2f** was suspended in 80 ml water and extracted with 50 ml $\times$2 of CH_2Cl_2. The organic phase was combined, dried (Na_2SO_4), and concentrated. The residue was recrystallized from methanol to afford yellow solid, mp 222–224 °C.^{1}H NMR (DMSO-*d*6) δ 12.06 (s, 1H), 8.87 (s, 1H), 7.64 (s, 1H), 7.35 (s, 1H), 5.64 (s, 2H), 3.73–3.72 (d, 2H), 3.67–3.60 (q, 4H), 3.55–3.53 (d, 2H), 2.51 (s, 3H), 1.14–1.09 (t, 6H).

4.2.7. 9-(2-(1*H*-1,2,4-Triazol-1-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purine-8-thiol (3a)

Compound **2a** (2.56 g, 8.0 mmol) was dissolved in 40 ml methanol and 10% Pt/C (0.3 g) was added. The mixture was hydrogenated in a GCD-500 hydrogen gas generator at 60 psi pressure for 3 h and then filtered to remove the catalyst. The solvent was concentrated to about 30 ml and 2.5 g of NaOH and 3.0 ml of CS_2 were added. The mixture was heated under reflux for 6.5 h, cooled and evaporated under reduced pressure to remove solvent and excess CS_2. The residue was acidified with conc. HCl to pH 2–3. White precipitates were generated and filtered. The crude product was recrystallized from methanol to give 1.78 g of compound **3a** as white needles, yield 67%, mp 240–242 °C. ^{1}H NMR (DMSO-d_6) δ 12.89 (s, 1H), 8.40 (s, 1H), 7.80 (s, 1H), 4.68–4.64 (t, 2H), 4.47–4.44 (t, 2H), 3.52–3.45 (q, 4H), 2.35 (s, 3H), 1.09–1.04 (t, 6H).

4.2.8. 9-(3-(1*H*-1,2,4-Triazol-1-yl)propyl)-2-(diethylamino)-6-methyl-9*H*-purine-8-thiol (3b)

Compound **3b** was prepared from compound **2b** (3.0 g), 10% Pt/C (0.3 g), NaOH (5.0 g), CS_2 (2.5 ml) according to the same procedure for **3a**, yield 74%. A small amount of the crude product was purified by chromatography on a silica gel column eluted with petroleum ether–ethyl acetate (3:1, v/v) to give yellow solid, mp 182–184 °C. ^{1}H NMR ($CDCl_3$) δ 11.24 (s, 1H), 8.26 (s, 1H), 7.94 (s, 1H), 4.34–4.28 (q, 4H), 3.65–3.58 (q, 4H), 2.55–2.51 (t, 2H), 2.46 (s, 1H), 1.19–1.15 (t, 6H).

4.2.9. 2-(Diethylamino)-6-methyl-9-(3-morpholinopropyl)-9*H*-purine-8-thiol (3c)

Compound **3c** was prepared from **2c** (3.92 g), 10% Pt/C (0.4 g), NaOH (3.0 g) and CS_2 (3.0 ml) according to the same procedure for **3a**. Purification of the crude product was performed on a silica gel column eluted petroleum ether–ethyl acetate–methanol (10:3:1, v/v) to give **3c** as white solid, yield 49%, mp 185–187 °C. ^{1}H NMR ($CDCl_3$) δ 11.39 (s, 1H), 4.32–4.27 (t, 2H), 3.68–3.59 (m, 8H), 2.51–2.44 (m, 9H), 2.08–2.03 (t, 2H), 1.20–1.15 (t, 6H).

4.2.10. 9-(2-(1*H*-Indol-3-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purine-8-thiol (3d)

Preparation of compound **3d** from **2d** was the same as that described for **3a**. The crude product was recrystallized from methanol to give white needles, yield 78%, mp 250 °C (dec). ^{1}H NMR (DMSO-d_6) δ 13.21 (s, 1H), 10.94 (s, 1H), 7.70–7.67 (d, 1H), 7.36–7.33 (d, 1H), 7.20 (s, 1H), 7.10–7.06 (t, 1H), 7.02–6.97 (t, 1H), 4.35–4.30 (t, 2H), 3.64–3.57 (q, 4H), 2.48 (s, 3H), 1.16–1.12 (t, 6H).

4.2.11. 9-(2-(9*H*-Purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purine-8-thiol (3e)

Compound **2e** (2.8 g, 7.3 mmol) was dissolve in about 40 ml 2 N aqueous H_2SO_4 and 10% Pd/C (0.3 g) was added. The mixture was hydrogenated in a GCD-500 hydrogen gas generator at 60 psi pressure for 7 h and then filtered to remove the catalyst. The filtrate was alkalized with solid NaOH in ice bath to pH 8–9 and additional 2.0 g of solid NaOH was added. To the mixture, 50 ml of ethanol and 2.5 ml of CS_2 were added and heated under reflux for 3 h. Another 1.0 ml of CS_2 was added and the solution was heated for another 4 h. The reaction mixture was cooled, concentrated under vacuum to remove ethanol. The residue was transferred into a 400 ml glass beaker and acidified with 6 N HCl to pH 3–4 in ice bath. White precipitates were generated, filtered, washed with water and dried in air to give 2.2 g of compound **3e** as pale yellow solid, yield 75%, mp 300 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.87 (s, 1H), 12.80 (s, 1H), 8.18 (s, 1H), 8.01 (s, 1H), 7.74 (s, 1H), 4.40 (s, 2H), 3.87 (s, 2H), 3.20 (s, 4H), 2.35 (s, 3H), 0.85 (s, 6H).

4.2.12. 9-(2-(2-Amino-9*H*-purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purine-8-thiol (3f)

Compound **2f** (3.5 g, 8.7 mmol) was suspended in 20 ml methanol and 40 ml 2 N aqueous H_2SO_4 was added to dissolve **2f**. 10% Pd/C (0.35 g) was added and the mixture was hydrogenated in a GCD-500 hydrogen gas generator at 60 psi pressure for 3 h and then filtered to remove the catalyst. The filtrate was alkalized with solid NaOH in ice bath to pH 3–4 and concentrated to about 30 ml. Solid NaOH (5.0 g) and 2.5 ml of CS_2 were added and heated under reflux for 4 h. Additional 1.0 ml of CS_2 was added and the solution was heated for another 3.5 h. The reaction mixture was cooled, evaporated under reduced pressure to remove ethanol. The residue was poured into about 100 ml crashed ice and acidified to pH 2–3 with 6 N HCl. White precipitates were generated and filtered. The filtrate was extracted with 75 ml ×3 ethyl acetate and the organic phase was combined and evaporated under vacuum to give green solid. The solids collected from filtration and extraction were combined and purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol (15:1, v/v) to give 3.5 g of **3f** as white solid, yield 90%, mp 280 °C (dec). ^{1}H NMR (DMSO-d_6) δ 12.06 (s, 1H), 8.87 (s, 1H), 7.64 (s, 1H), 7.35 (s, 1H), 5.64 (s, 2H), 3.73–3.72 (d, 2H), 3.67–3.60 (q, 4H), 3.55–3.53 (d, 2H), 2.51 (s, 3H), 1.14–1.09 (t, 6H).

4.2.13. Methyl 2-(9-(2-(1*H*-1,2,4-triazol-1-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)acetate (4a)

Compound **3a** (1.16 g, 3.5 mmol) was heated under reflux with anhydrous potassium carbonate (0.97 g, 7.0 mmol), ethyl 2-chloroacetate (0.86 g, 7.0 mmol) and potassium iodide (catalytic amounts) in 50 ml acetone for **9h**. The reaction mixture was cooled and filtered. The solid was washed with 30 ml acetone and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column eluted with petroleum–ethyl acetate (5:1, v/v) to give yellow oil. The oil was recrystallized from methanol to afford 1.2 g of **4a** as pale yellow solid, yield 88%, mp 88–90 °C. ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 1H), 7.87 (s, 1H), 4.65–4.62 (t, 2H), 4.42–4.38 (t, 2H), 4.11 (s, 2H), 3.65 (s, 3H), 3.64–3.54 (q, 4H), 2.43 (s, 3H), 1.14–1.09 (t, 6H).

4.2.14. Ethyl 2-(9-(3-(1H-1,2,4-triazol-1-yl)propyl)-2-(diethylamino)-6-methyl-9H-purin-8-ylthio)acetate (4b)

Compound **3b** (2.0 g, 5.8 mmol) was heated with anhydrous potassium carbonate (1.2 g, 8.7 mmol), ethyl 2-bromoacetate (1.2 g, 7.0 mmol) in 50 ml anhydrous ethanol at 55–60 °C for 12 h. The solvent was removed under reduced pressure and 80 ml ethyl acetate was added to the residue. The solution was filtered and the solid was washed with ethyl acetate. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on a silica gel column eluted with petroleum–ethyl acetate (4:1, v/v) to give 2.3 g of **4b** as white solid, yield 90%, mp 52–54 °C. ^{1}H NMR (DMSO-d_6) δ 8.53 (s, 1H), 7.99 (s, 1H), 4.28–4.24 (t, 2H), 4.19 (s, 2H), 4.17–4.10 (q, 2H), 4.06–4.01 (t, 2H), 3.62–3.55 (q, 4H), 2.45 (s, 3H), 2.32–2.27 (t, 2H), 1.21–1.16 (t, 3H), 1.13–1.09 (t, 6H).

4.2.15. Ethyl 2-(2-(diethylamino)-6-methyl-9-(3-morpholinopropyl)-9*H*-purin-8-ylthio)acetate (4c)

Compound **4c** was prepared from **3c** (1.90 g, 5.2 mmol), ethyl 2-bromoacetate (1.30 g, 7.8 mmol) and anhydrous potassium carbonate (1.08 g, 7.8 mmol) according to the procedure for **4b**. Purification was performed by flash chromatography on a silica gel column eluted with petroleum–ethyl acetate (3:1, v/v) to give 2.12 g of compound **4b** as pale yellow solid, yield 90%, mp 59–62 °C. ^{1}H NMR ($CDCl_3$) δ 4.25–4.18 (q, 2H), 4.15–4.11 (t, 4H), 3.72–3.62 (m, 8H), 2.56 (s, 3H), 2.45–2.41 (t, 6H), 2.05–2.00 (t, 2H), 1.30–1.26 (t, 3H), 1.20–1.15 (6H).

4.2.16. Methyl 2-(9-(2-(1*H*-indol-3-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)acetate (4d)

Compound **4d** was prepared from **3d** (1.42 g, 4.5 mmol), ethyl 2-chloroacetate (0.66 g, 5.4 mmol), anhydrous K_2CO_3 (0.62 g, 4.5 mmol) and KI (catalytic amounts) according to the procedure for **4a** while methanol was used as solvent. The crude product was recrystallized from methanol to afford 1.3 g of **4d** as white needles, yield 63%, mp 145–147 °C. ^{1}H NMR ($CDCl_3$) δ 8.01 (s, 1H), 7.81–7.79 (d, 1H), 7.40–7.37 (d, 1H), 7.26–7.13 (m, 2H), 7.00 (s, 1H), 4.36–4.30 (t, 2H), 4.06 (s, 2H), 3.76–3.66 (m, 7H), 2.58 (s, 3H), 1.24–1.20 (t, 6H).

4.2.17. Ethyl 2-(9-(2-(9*H*-purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)acetate (4e)

Compound **3e** (2.35 g, 5.9 mmol) was stirred with anhydrous K_2CO_3 (1.2 g, 8.7 mmol), ethyl 2-bromoacetate (1.1 g, 6.5 mmol) and TBAB (0.2 g) in 50 ml dry DMF for 14 h. Additional 0.9 g ethyl 2-bromoacetate was added and stirred for further 20 h. The mixture was filtered and the filtrate was concentrated under reduced pressure. To the residue, 60 ml of water was added and extracted with ethyl acetate (4 × 50 ml). The organic phase was combined, washed with brine (2 × 30 ml), dried (Na_2SO_4) and concentrated under vacuum. The residue was purified by chromatography on a silica gel column eluted with petroleum–ethyl acetate (3:1, v/v) to give 1.6 g of **4e** as white solid, yield 55%, mp 186–188 °C. ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H), 7.87 (s, 1H), 6.90 (s, 1H), 4.42–4.41 (d, 2H), 4.23–4.16 (q, 2H), 4.04 (s, 2H), 3.58–3.51 (q, 4H), 2.51 (s, 3H), 1.28–1.23 (t, 3H), 1.12–1.08 (t, 3H).

4.2.18. Ethyl 2-(9-(2-(2-amino-9*H*-purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)acetate (4f)

Compound **4f** was prepared from **3f** (3.3 g, 8.0 mmol), ethyl 2-bromoacetate (1.34 g, 8.0 mmol), anhydrous K_2CO_3 (1.66 g, 12.0 mmol) and TBAB (0.8 g) according to the procedure for com-

pound **3e** while the reaction time was 16 h and the purification of crude product was performed on a silica gel column eluted with petroleum–ethyl acetate–methanol (10:3:1, v/v) to give **4f** as white solid, yield 62%, mp 173–176 °C. ^{1}H NMR (DMSO-d_6) δ 12.09 (s, 1H), 7.63 (s, 1H), 7.37 (s, 1H), 5.64 (s, 2H), 4.28 (s, 2H), 4.14–4.02 (m, 4H), 3.75 (s, 2H), 3.52–3.47 (q, 4H), 2.43 (s, 3H), 1.19–1.15 (t, 3H), 1.06–1.01 (t, 6H).

4.2.19. 2-(9-(2-(1*H*-1,2,4-Triazol-1-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-aminoethyl)acetamide (5a)

Compound **3a** (0.478 g, 1.2 mmol) with 0.56 ml of ethane-1,2-diamine in 15 ml of methanol was heated under reflux in oil bath for 13 h. The solvent was removed under reduced pressure and the residue was purified by chromatography on a silica gel column eluted with CH_2Cl_2–CH_3OH–ammonia water (100:10:1, v/v) to give 0.366 g of compound **5a** as white solid, yield 70%. ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H), 7.94 (s, 1H), 7.72 (s, 1H), 4.70–4.66 (t, 2H), 4.47–4.43 (t, 2H), 3.76 (s, 2H), 3.69–3.62 (q, 4H), 3.34–3.28 (q, 2H), 2.82–2.78 (t, 2H), 2.56 (s, 3H), 1.22–1.17 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 432.22, found: 433.2 [(M+1)$^+$].

4.2.20. 2-(9-(3-(1*H*-1,2,4-Triazol-1-yl)propyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-aminoethyl)acetamide (5b)

Compound **5b** was prepared from **4b** (0.80 g, 1.85 mmol) and ethane-1,2-diamine (0.37 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (200:10:1, v/v) to give 0.666 g of compound **5b** as white solid, yield 79%.^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H), 8.14 (s, 1H), 7.97 (s, 1H), 4.25–4.20 (t, 2H), 4.13–4.08 (t, 2H), 3.87 (s, 2H), 3.69–3.62 (q, 4H), 3.36–3.30 (q, 2H), 2.83–2.80 (t, 2H), 2.58 (s, 3H), 2.48–2.44 (t, 2H), 1.21–1.16 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 446.23, found: 447.2 [(M+1)$^+$].

4.2.21. *N*-(2-Aminoethyl)-2-(2-(diethylamino)-6-methyl-9-(3-morpholinopropyl)-9*H*-purin-8-ylthio)acetamide (5c)

Compound **5c** was prepared from **4c** (0.80 g, 1.77 mmol) and ethane-1,2-diamine (1.2 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:10:1, v/v) to give 0.49 g (59%) of compound **5c** as white solid. ^{1}H NMR ($CDCl_3$) δ *8.61–8.55* (t, 1H), 4.12–4.05 (t, 2H), 3.86 (s, 2H), 3.69–3.60 (q, 8H), 3.40–3.31 (q, 2H), 2.88–2.83 (t, 2H), 2.57 (s, 3H), 2.42–2.32 (m, 6H), 2.04–1.90 (m, 2H), 1.22–1.15 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 464.27, found: 465.1 [(M+1)$^+$].

4.2.22. 2-(9-(2-(1*H*-Indol-3-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2- aminoethyl)acetamide (5d)

Compound **5d** was prepared from **4d** (0.56 g, 1.2 mmol) and ethane-1,2-diamine (0.56 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:10:1, v/v) to give 0.46 g of compound **5d** as white solid, yield 80%.^{1}H NMR ($CDCl_3$) δ 8.38 (s, 2H), 7.68–7.66 (d, 1H), 7.39–7.36 (d, 1H), 7.24–7.19 (t, 1H), 7.14–7.10 (t, 1H), 6.93 (s, 1H), 4.31–4.26 (t, 2H), 3.75–3.68 (q, 6H), 3.34–3.23 (m, 4H), 2.83–2.79 (t, 2H), 2.58 (s, 3H), 1.26–1.21 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 480.24, found: 481.2 [(M+1)$^+$].

4.2.23. 2-(9-(2-(9*H*-Purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-aminoethyl)acetamide (5e)

Compound **5e** was prepared from **4e** (0.70 g, 1.44 mmol) and ethane-1,2-diamine (1.2 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–triethylamine (200:15:2, v/v) to give 0.43 g of compound **5e** as white solid, yield 61%. ^{1}H NMR ($CDCl_3$) δ *8.50–8.45* (t, 1H), 8.37 (s, 1H), 4.37 (s, 2H), 4.02 (s, 2H), 3.76 (s, 2H), 3.57–3.46 (q, 4H), 3.39–3.31 (q, 2H), 2.86–2.80 (t, 2H), 2.67–2.56 (t, 2H), 2.50 (s, 3H), 1.11–1.04 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 498.24, found: 499.2 [(M+1)$^+$].

4.2.24. 2-(9-(2-(2-Amino-9*H*-purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-aminoethyl) acetamide (5f)

Compound **5f** was prepared from **4f** (0.80 g, 1.6 mmol) and ethane-1,2-diamine (0.56 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:20:1, v/v) to give 0.54 g of compound **5b** as white solid, yield 66%. ^{1}H NMR (DMSO-*d*6) δ 8.33–8.29 (t, 1H), 7.62 (s, 1H), 7.30 (s, 1H), 5.60 (S, 2H), 4.27 (s, 2H), 3.99 (S, 2H), 3.74 (s, 2H), 3.49–3.46 (d, 4H), 3.16–3.10 (q. 2H), 2.64–2.60 (t, 2H), 2.46 (s, 3H), 1.05–1.00 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 513.25, found: 514.3 [(M+1)$^+$].

4.2.25. 2-(9-(2-(1*H*-1,2,4-Triazol-1-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-aminopropyl)acetamide (6a)

Compound **6a** was prepared from **4a** (0.80 g, 2.0 mmol) and 1,3-diaminopropane (1.17 ml) according to the procedure for compound of **5a**. The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:10:1, v/v) to give 0.49 g of compound **6a** as white solid, yield 58%. ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 1H), 7.94 (s, 1H), 7.73 (s, 1H), 4.69–4.66 (t, 2H), 4.47–4.43 (t, 2H), 3.75 (s, 2H), 3.69–3.62 (q, 4H), 3.37–3.31 (q, 2H), 2.71–2.67 (t, 2H), 2.56 (s, 3H), 1.64–1.59 (t, 2H), 1.22–1.18 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 446.23, found: 447.2 [(M+1)$^+$].

4.2.26. 2-(9-(3-(1*H*-1,2,4-Triazol-1-yl)propyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-aminopropyl)acetamide (6b)

Compound **6b** was prepared from **4b** (0.75 g, 1.74 mmol) and 1,3-diaminopropane (0.72 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:12:1, v/v) to give 0.54 g of compound **6b** as waxy solid, yield 67%.^{1}H NMR ($CDCl_3$) δ 8.38 (s, 1H), 8.15 (s, 1H), 7.96 (s, 1H), 4.26–4.19 (t, 2H), 4.14–4.07 (s, 2H), 3.85 (s, 2H), 3.71–3.60 (q, 4H), 3.40–3.31 (q, 2H), 2.77–2.70 (t, 2H), 2.58 (s, 3H), 2.53–2.43 (q, 2H), 1.72–1.59 (m, 2H), 1.22–1.15 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 460.25, found: 461.3 [(M+1)$^+$].

4.2.27. *N*-(3-Aminopropyl)-2-(2-(diethylamino)-6-methyl-9-(3-morpholinopropyl)-9*H*-purin-8-ylthio)acetamide (6c)

Compound **6c** was prepared from **4c** (0.80 g, 1.77 mmol) and 1,3-diaminopropane (0.73 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:10:1, v/v) to give 0.75 g of compound **6b** as yellow oil, yield 88%.^{1}H NMR ($CDCl_3$) δ 8.54 (s, 1H), 4.11–4.06 (t, 2H), 3.83 (s, 2H), 3.67–3.62 (t, 8H), 3.37–3.31 (q, 2H), 2.72–2.68 (t, 2H), 2.57 (s, 3H), 2.40 (s,6H), 2.00–1.95 (t, 2H), 1.64–1.60 (t, 2H), 1.21–1.16 (t, 6H). MS (EI$^+$) *m*/*z* calcd: 478.28, found: 477 [(M−1)$^+$].

4.2.28. 2-(9-(2-(1*H*-Indol-3-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-aminopropyl)acetamide (6d)

Compound **6d** was prepared from **4d** (0.56 g, 1.2 mmol) and 1,3-diaminopropane (0.70 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:15:1, v/v) to give 0.47 g of compound **6d** as white solid, yield 80%. ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1H), 8.46 (s, 1H), 7.68–7.66 (d, 1H), 7.38–7.36 (d, 1H), 7.23–7.10 (m, 2H), 6.85 (s, 1H), 4.30–4.25 (t, 2H), 3.74–3.66 (m, 6H), 3.36–3.23 (m, 4H), 2.72–2.67 (t, 2H), 2.57 (s, 3H), 1.67–1.58 (m, 2H), 1.25–1.21 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 494.26, found: 495.3 [(M+1)$^+$].

4.2.29. 2-(9-(2-(9*H*-Purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-aminopropyl)acetamide (6e)

Compound **6e** was prepared from **4e** (0.70 g, 1.44 mmol) and 1,3-diaminopropane (0.60 ml). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:10:1, v/v) to give 0.40 g of compound **6e** as white solid, yield 55%. ^{1}H NMR ($CDCl_3$) δ 8.44 (s, 1H), 8.31 (s, 1H), 7.85 (s, 1H), 4.37 (s, 2H), 4.07 (s, 2H), 3.73 (s, 2H), 3.57–3.50 (q, 4H), 3.32–3.31 (d, 2H), 2.69–2.64 (t, 2H), 2.49 (s, 3H), 1.63–1.59 (t, 2H), 1.12–1.08 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 512.25, found: 513.3 [(M+1)$^+$].

4.2.30. 2-(9-(2-(2-Amino-9*H*-purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-aminopropyl)acetamide (6f)

Compound **6f** was prepared from **4f** (0.80 g, 1.6 mmol) and 1,3-diaminopropane (0.67 ml). The crude compound was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:10:1, v/v) to give 0.41 g of compound **6f** as white solid, yield 49%. ^{1}H NMR ($CDCl_3$) δ 8.52 (s, 1H), 7.53 (s, 1H), 6.54 (s, 1H), 4.84 (s, 2H), 4.30 (s, 2H), 3.96 (s, 2H), 3.69–3.59 (m, 6H), 3.30–3.28 (d, 2H), 2.71–2.66 (t, 2H), 2.52 (s, 3H), 1.64–1.60 (t, 2H), 1.18–1.13 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 527.27, found: 528.3 [(M+1)$^+$].

4.2.31. 2-(9-(2-(1*H*-1,2,4-Triazol-1-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-guanidinoethyl)acetamide (7a)

To a solution of compound **5a** (0.060 g, 0.14 mmol) in 10 ml of anhydrous ethanol, 0.030 g of aminoiminomethane sulfonic acid ($AIMSO_3H_2O$) (0.21 mmol) was added and the solution was stirred at 30–45 °C in water bath for 2 h and then at room temperature overnight. The solvent was evaporated under reduced pressure and purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (50:150:3, v/v) to give 49 mg of compound **7a** as white solid, yield 74%. ^{1}H NMR (DMSO-*d*6) δ 8.33 (s, 1H), 7.85 (s, 1H), 7.38 (s, 5H), 4.66–4.62 (t, 2H), 4.41–4.37 (t, 2H), 3.99 (s, 2H), 3.60–3.53 (q, 4H), 3.21–3.18 (t, 4H), 2.45 (s, 3H), 1.13–1.08 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 474.24, found: 475.2 [(M+1)$^+$].

4.2.32. 2-(9-(3-(1*H*-1,2,4-Triazol-1-yl)propyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-guanidinoethyl)acetamide (7b)

Compound **7b** was prepared from **5b** (0.096 g, 0.21 mmol) and $AIMSO_3H_2O$ (0.045 g, 0.32 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (100:300:3, v/v) to give 48 mg of compound **7b** as yellow solid, yield 47%.^{1}H NMR (DMSO-d_6) δ 8.73 (s, 1H), 8.52 (s, 1H), 8.21 (s, 1H), 7.95 (s, 1H), 7.46 (s, 3H), 7.32 (s, 1H), 4.26–4.21 (t, 2H), 4.08 (s, 2H), 4.02–3.97 (t, 2H), 3.58–3.51 (q, 4H), 3.19 (s, 4H), 2.43 (s, 3H), 2.28–2.23 (t, 2H), 1.10–1.05 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 488.25, found: 489.3 [(M+1)$^+$].

4.2.33. 2-(2-(Diethylamino)-6-methyl-9-(3-morpholinopropyl)-9*H*-purin-8-ylthio)-*N*-(2-guanidinoethyl)acetamide (7c)

Compound **7c** was prepared from **5c** (0.093 g, 0.20 mmol) and $AIMSO_3H_2O$ (0.043 g, 0.30 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (50:200:3, v/v) to give 79 mg of compound **7c** as white solid, yield 77%. ^{1}H NMR (DMSO-d_6) δ 7.60 (s, 5H), 4.07 (s, 2H), 4.01–3.96 (t, 2H), 3.61–3.54 (q, 4H), 3.49–3.48 (d, 4H), 3.21–3.19 (d, 2H), 3.14–3.13 (d, 2H), 2.43 (s, 3H), 2.27–2.25 (d, 6H), 1.86–1.82 (t, 2H), 1.13–1.08 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 506.29, found: 507.3 [(M+1)$^+$].

4.2.34. 2-(9-(2-(1*H*-Indol-3-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-guanidinoethyl)acetamide (7d)

Compound **7d** was prepared from **5d** (0.072 g, 0.15 mmol) and $AIMSO_3H_2O$ (0.043 g, 0.30 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (25:10:2, v/v) to give 28 mg of compound **7d** as white solid, yield 35%. ^{1}H NMR (DMSO-d_6) δ 10.93 (s, 1H), 8.57 (s, 1H), 7.78 (s, 1H), 7.67–7.64 (d, 1H), 7.37–7.06 (m, 8H), 7.02–6.97 (t, 1H), 4.25–4.20 (t, 2H), 4.08 (s, 2H), 3.66–3.62 (t, 4H), 3.21–3.12 (q, 6H), 2.48 (s, 3H), 1.17–1.13 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 522.26, found: 523.3 [(M+1)$^+$].

4.2.35. 2-(9-(2-(9*H*-Purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-guanidinoethyl)acetamide (7e)

Compound **7e** was prepared from **5e** (0.098 g, 0.20 mmol) and $AIMSO_3H_2O$ (0.085 g, 0.60 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (50:150:2, v/v) to give 97 mg of compound **7e** as white solid, yield 90%.^{1}H NMR (DMSO-d_6) δ 8.81 (s, 1H), 8.17 (s, 1H), 8.04 (s, 1H), 7.42–7.26 (d, 5H), 4.30 (s, 2H), 3.95 (s, 2H), 3.85 (s, 2H), 3.42–3.40 (d, 4H), 3.23 (s, 2H), 3.14 (s, 2H), 2.43 (s, 3H), 1.00–0.96 (t, 6H). MS (ESI$^+$) *m*/*z* calcd: 540.26, found: 541.3 [(M+1)$^+$].

4.2.36. 2-(9-(2-(2-Amino-9*H*-purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(2-guanidinoethyl)acetamide (7f)

Compound **7f** was prepared from **5f** (0.103 g, 0.20 mmol) and $AIMSO_3H_2O$ (0.043 g, 0.30 mmol). The crude product was purified by chromatography on a silica gel column eluted with methanol–ammonia water (20:1, v/v) to give 65 mg of compound **7f** as white solid, yield 58%. ^{1}H NMR (DMSO-d_6) δ 8.52 (s, 1H), 7.78 (s, 1H), 7.66 (s, 1H), 7.28–7.12 (d, 5H), 5.81 (s, 2H), 4.26 (s, 2H), 4.02 (s, 2H), 3.74 (s, 2H), 3.45–3.39 (m, 4H), 3.20 (s, 4H), 2.43 (s, 3H), 1.04–0.97 (m, 6H); MS (ESI$^+$) *m*/*z* calcd: 555.27, found: 556.3 [(M+1)$^+$].

4.2.37. 2-(9-(2-(1*H*-1,2,4-Triazol-1-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-guanidinopropyl)acetamide (8a)

Compound **8a** was prepared from **6a** (0.089 g, 0.20 mmol) and $AIMSO_3H_2O$ (0.085 g, 0.60 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (50:150:2, v/v) to give 55 mg of compound **8a** as white solid, yield 56%. ^{1}H NMR (DMSO-d_6) δ 8.41–8.40 (d, 1H), 8.32 (s, 1H), 7.91 (s, 1H), 7.83 (s, 1H), 7.40–7.26 (t, 4H), 6.90 (s, 1H), 4.63–4.62 (d, 2H), 4.37 (s, 2H), 3.96 (s, 2H), 3.56–3.50 (q, 4H), 3.11–3.10 (d, 4H), 2.42 (s, 3H), 1.61–1.56 (t, 2H), 1.08–1.06 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 488.25, found: 489.3 [(M+1)$^+$].

4.2.38. 2-(9-(3-(1*H*-1,2,4-Triazol-1-yl)propyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-guanidinopropyl)acetamide (8b)

Compound **8b** was prepared from **6b** (0.178 g, 0.39 mmol) and $AIMSO_3H_2O$ (0.082 g, 0.58 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (50:150:2, v/v) to give 157 mg of compound **8b** as white solid, yield 40%. ^{1}H NMR (DMSO-d_6) δ 8.53 (s, 2H), 8.03 (s, 1H), 7.97 (s, 1H), 7.35–7.23 (d, 4H), 4.27–4.23 (t, 2H), 4.06–4.00 (t, 4H), 3.61–3.52 (m, 4H), 3.14–3.08 (m, 4H), 2.45 (s, 3H), 1.62–1.58 (t, 2H), 1.12–1.08 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 502.27, found: 503.3 [(M+1)$^+$].

4.2.39. 2-(2-(Diethylamino)-6-methyl-9-(3-morpholinopropyl)-9*H*-purin-8-ylthio)-*N*-(3-guanidinopropyl)acetamide (8c)

Compound **8c** was prepared from **6a** (0.134 g, 0.28 mmol) and $AIMSO_3H_2O$ (0.060 g, 0.42 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–

methanol–ammonia water (50:150:2, v/v) to give 101 mg of compound **8c** as white solid, yield 69%. ^{1}H NMR (DMSO-d_6) δ 8.49 (s, 1H), 7.93 (s, 1H), 7.33 (s, 4H), 4.06–4.00 (d, 4H), 3.61–3.49 (m, 8H), 3.15–3.09 (t, 4H), 2.46 (s, 3H), 2.31 (s, 6H), 1.92–1.86 (t, 2H), 1.64–1.57 (t, 2H), 1.15–1.09 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 520.31, found: 521.3 [(M+1)$^+$].

4.2.40. 2-(9-(2-(1*H*-Indol-3-yl)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-guanidinopropyl)acetamide (8d)

Compound **8d** was prepared from **6a** (0.100 g, 0.20 mmol) and $AIMSO_3H_2O$ (0.085 g, 0.60 mmol). The crude compound was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (25:50:1, v/v) to give 113 mg of compound **8d** as white solid, yield 99%. ^{1}H NMR (DMSO-d_6) δ 10.94 (s, 1H), 8.42 (s, 1H), 7.78 (s, 1H), 7.67–7.65 (d, 1H), 7.38–6.98 (m, 8H), 4.26–4.21 (t, 2H), 4.04 (s, 2H), 3.66–3.60 (q, 4H), 3.15–3.12 (d, 4H), 2.47 (s, 3H), 1.62–1.58 (t, 2H), 1.17–1.13 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 536.28, found: 537.3 [(M+1)$^+$].

4.2.41. 2-(9-(2-(9*H*-Purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-guanidinopropyl)acetamide (8e)

Compound **8e** was prepared from **6a** (0.105 g, 0.20 mmol) and $AIMSO_3H_2O$ (0.085 g, 0.60 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (50:100:1, v/v) to give 91 mg of compound **8e** as white solid, yield 82%. ^{1}H NMR (DMSO-d_6) δ 8.39 (s, 1H), 8.12 (s, 1H), 7.93 (s, 1H), 7.71 (s, 1H), 4.31 (s, 2H), 3.96–3.88 (d, 4H), 3.46–3.40 (t, 4H), 3.17–3.11 (t, 4H), 2.44 (s, 3H), 1.64–1.58 (t, 2H), 1.02–0.95 (t, 6H); MS (ESI$^+$) *m*/*z* calcd: 554.28, found: 555.3 [(M+1)$^+$].

4.2.42. 2-(9-(2-(2-Amino-9*H*-purin-6-ylamino)ethyl)-2-(diethylamino)-6-methyl-9*H*-purin-8-ylthio)-*N*-(3-guanidinopropyl)acetamide (8f)

Compound **8a** was prepared from **6a** (0.120 g, 0.23 mmol) and $AIMSO_3H_2O$ (0.098 g, 0.69 mmol). The crude product was purified by chromatography on a silica gel column eluted with CH_2Cl_2–methanol–ammonia water (50:200:3, v/v) to give 56 mg of compound **8f** as white solid, yield 43%.^{1}H NMR (DMSO-d_6) δ 7.48 (s, 1H), 5.42 (s, 2H), 4.24 (s, 2H), 3.97 (s, 2H), 3.75 (s, 2H), 3.48–3.42 (q, 4H), 3.16–3.11 (d, 4H), 2.44 (s, 3H), 1.62 (s, 2H), 1.04–1.02 (d, 6H); MS (ESI$^+$) *m*/*z* calcd: 569.29, found: 570.3 [(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 TCID50 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 EC50 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 158 was recovered from the Protein Database (http://www.PDB.org) with the code as 1UUI. [20] 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 enerations, 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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Supplementary data

(a) Interaction of compound **5b** to TAR RNA; (b) Interaction of compound **5c** to TAR RNA; (c) Interaction of compound **5e** to TAR RNA.

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