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Design, synthesis, and biological evaluation of novel quinoline derivatives as HIV-1 Tat–TAR interaction inhibitors

Shuguang Chen, Ran Chen, Meizi He, Ruifang Pang, Zhiwu Tan, Ming Yang *

State Key Laboratory of Natural and Biomimetic Drugs, Peking University, PO Box 261, Beijing 100191, China

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ABSTRACT

Thirty-two quinoline derivatives 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. Nine of them with low cytotoxicities were evaluated by Tat dependent HIV-1 LTR-driven CAT gene expression colorimetric enzyme assay in human 293T cells, indicating effective inhibitory activities of blocking the Tat–TAR interaction. Molecular modeling experiments indicated that these compounds may inhibit Tat–TAR interaction by binding to Tat protein instead of TAR RNA.

1. Introduction

Since the 1980s, Acquired Immure Deficiency Syndrome (AIDS) has become a disastrous epidemic which greatly threatens the health of humankind and brings us crisis and challenges in the health care system, with more than 38 million people infected worldwide.[1] Today, there are more than 20 drugs available in clinic for the treatment of HIV-1 infection. However, emergence of drug-resistant viruses caused by mutations of the enzymes often results in treatment failure with existing antiretrovirals.[2] The cross-resistance to the same class of compounds is also a problem for the treatment process.[3] Thus, efforts were made to develop new antiretroviral agents with different targets for inhibition of HIV-1 replication. One of the most promising targets is the process of Tat/TAR RNA interaction. This Tat-mediated trans-activation process requires the structural integrity of TAR RNA and the cooperative interaction of human host cell proteins, such as positive transcription elongation factor b (P-TEFb), a complex composed of cyclin T1 (CycT1) and cyclin-dependent kinase 9 (CDK9).[4–7] CDK9 hyperphosphorylates the carboxy-terminal domain (CTD) of RNA polymerase II and induces efficient promoter clearance and transcriptional elongation. Furthermore, CycT1 remodels the structure of Tat to enhance its affinity for the TAR RNA, and the TAR RNA further enhances the interaction between Tat and CycT1.[8] Due to the crucial role that Tat/TAR complex plays, lots of compounds which could efficiently inhibit the Tat/TAR RNA interaction have been developed, such as acridine derivative CGP 40336A[9] and diphenylfuran derivative DB60.[10] Our laboratory also proposed a molecular model of new Tat–TAR interaction inhibitors containing an 'anchor', a 'linker' and a positively charged 'activator'.[11] The positively charged 'activator', just like the role that the arginine-rich region of Tat plays, reinforces the binding affinity between the compound and the negatively charged TAR RNA, thus disrupting the formation of Tat/TAR complex. According to this model we have successfully designed series of inhibitors like α,α-trehalose derivatives,[12] isoquinoline derivatives,[13] β-carboline derivatives,[14] and purine derivatives.[11,15]

When screening the compound library in our lab for inhibitory activity of Tat–TAR interaction, we found that one compound named **CS3** revealed mild inhibitory activity and antiviral potency. However, it was very interesting that **CS3** bore no positively charged group as in the molecular model mentioned above, suggesting that it may inhibit Tat–TAR interaction in a different way. We scrutinized the structure of **CS3**, and its features were found as follows (as shown in Fig. 1): (a) it contained a planar aromatic

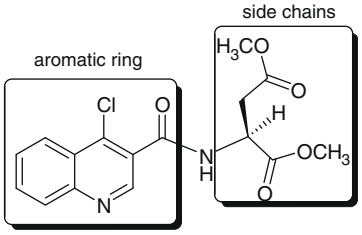

Figure 1. The structure of **CS3**.

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

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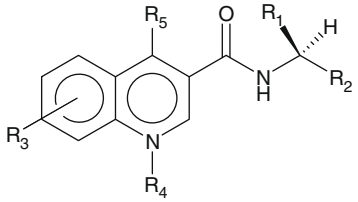
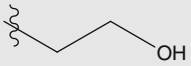
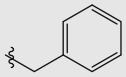
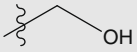
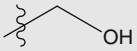
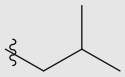
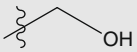
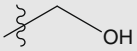
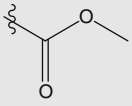
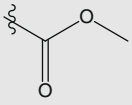
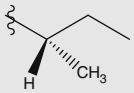
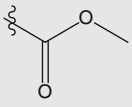
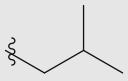
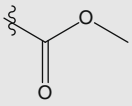
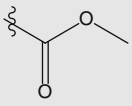
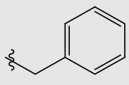
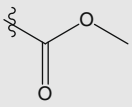
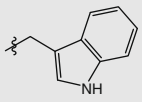
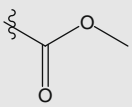
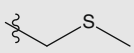
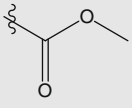
Table 1
Inhibition effect and cytotoxicity of the title compounds on SIV induced syncytium



Compound[a]	R_1	R_2	R_3	R_4	R_5	EC_{50}[b] (μM)	TC_{50}[c] (μM)	SI[d] (TC_{50}/EC_{50})
5a	H		H		Cl	6.6	>100	>15
5b			H		Cl	4.0	>100	>25
5c	CH_3		H		Cl	7.0	>100	>14
5d			H		Cl	7.9	>100	>12
5e	H		H		Cl	2.7	>100	>37
5f	H		H		Cl	5.4	>100	>18
5g	CH_3		H		Cl	5.7	81.3	.14
5h			H		Cl	1.3	23.3	17
5i			H		Cl	9.4	49.1	5.2
5j			H		Cl	5.6	17.6	3.1
5k			H		Cl	7.9	>100	>13
5l			H		Cl	10.0	>100	>19
5m			H		Cl	2.9	68.4	22

(*continued on next page*)

Table 1 (*continued*)

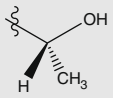
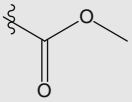
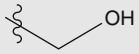
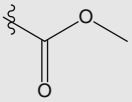
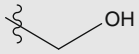
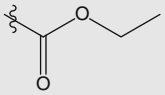
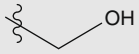
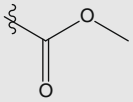
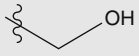
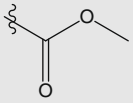
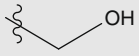
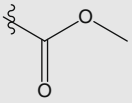
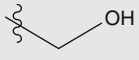
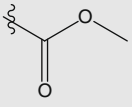
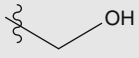
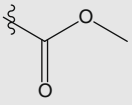
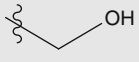
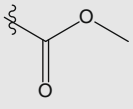
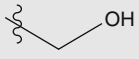
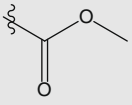
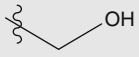
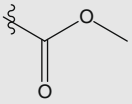
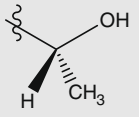
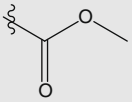
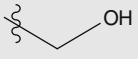
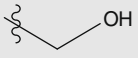
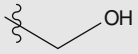
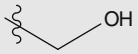
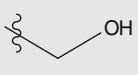
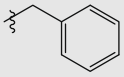
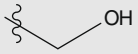
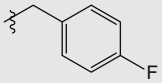
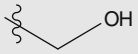
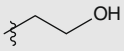
Compound[a]	R_1	R_2	R_3	R_4	R_5	EC_{50}[b] (μM)	TC_{50}[c] (μM)	SI[d] (TC_{50}/EC_{50})
5n			H		Cl	7.0	>100	>14
5p			H		Cl	1.1	>100	>90
5q			H		Cl	4.7	>100	>21
5r			8-CH_3		Cl	2.8	>100	>36
5s			7-CH_3		Cl	4.7	59.6	13
5t			6-CH_3		Cl	11.6	>100	>8.6
5u			8-Cl		Cl	6.7	>100	>15
5v			7-Cl		Cl	8.2	>100	>12
5w			6-Cl		Cl	12.0	>100	>8.3
5x			6-F		Cl	5.8	>100	>17
5y			6,8-F		Cl	8.8	>100	>11
5z			7-CH_3		Cl	13.0	>100	>14
6a	H		6-Phenoxy	H	Carbonyl	4.9	>100	>20
6b	H		H	H	Carbonyl	3.7	>100	>27

Table 1 (*continued*)

Compound[a]	R_1	R_2	R_3	R_4	R_5	EC_{50}[b] (μM)	TC_{50}[c] (μM)	SI[d] (TC_{50}/EC_{50})
6c	H		6-Cl	H	Carbonyl	5.1	>100	>19
8a	H		H	CH_3	Carbonyl	8.7	>100	>11
8b	H		H		Carbonyl	6.0	>100	>16
8c	H		H		Carbonyl	6.9	>100	>14
8d	H		H		Carbonyl	7.3	>100	>13

[a] Indinavir was used as the positive control at a concentration of 10 μM here. Its EC_{50} was 0.0108 μM and TC_{50} was above 100 μM in this system.
[b] EC_{50}, concentration was required to protect cells against the cytopathogenicity of SIV by 50%.
[c] TC_{50}, concentration was required to inhibit uninfected cells proliferation by 50%.
[d] SI, selective index.

ring, namely 4-chloroquinoline; (b) it contained two side chains which were connected with the aromatic ring by an amide linker. So we proposed these two features as our structure template for further modification to improve the antiviral activity. In order to perform the modification more efficiently and reasonably, the research was divided into three steps: (1) With the aromatic ring remaining the same as 4-chloroquinoline, the two side chains moiety was modified. (2) Then substitution of the 4-chloroquinoline ring was explored. (3) Finally, the nitrogen atom of the quinoline ring was substituted, while the chloride atom of the quinoline ring was replaced by hydroxyl or carbonyl at the same time. Ultimately, we designed and synthesized thirty-two compounds possessing the features above, as shown in Table 1.

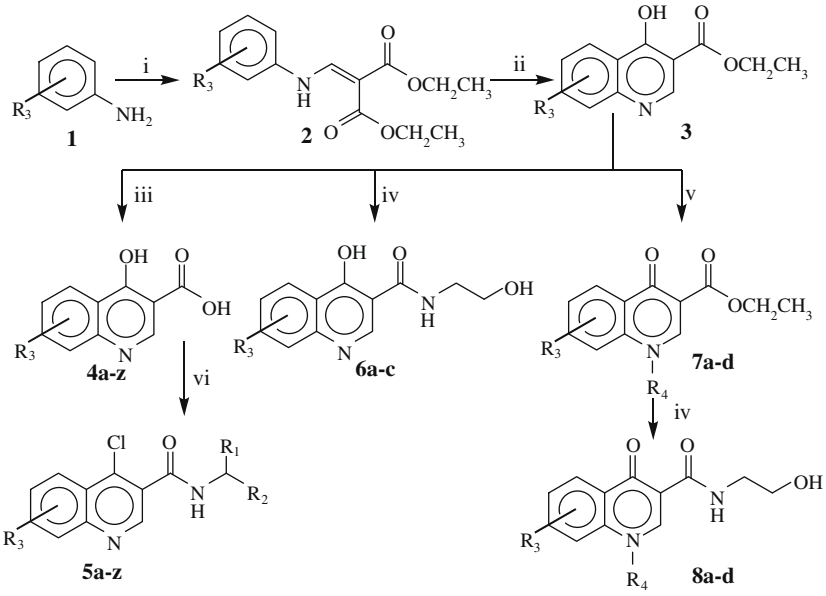
Thirty of all thirty-two title compounds (except **6b** and **6c**) were synthesized for the first time and all of them were evaluated for their antiviral activity, while nine compounds were evaluated for their inhibitory ability to Tat–TAR interaction.

2. Result and discussion

The route used for preparation of the title compounds (**5a–z**, **6a–c** and **8a–e**) is illustrated in Scheme 1 and the synthetic procedure is explained in Section 4. Thirty-two compounds were obtained and their MS, ^1H NMR spectroscopy data are 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 the title compounds possessed a TC_{50} value more than 100 μM except **5g**, **5h**, **6i**, **5j**, **5m**, and **5s**. With regard to their antiviral activities, each of the title compounds exhibited an EC_{50} value within the range from 1.1 μM to 12.0 μM, suggesting that all compounds possessed effective anti-SIV activities while most of them possessed low cytotoxicity.

Compounds **5a–m** were synthesized to investigate the structure-activity relationship (SAR) of the side chains moiety men-



Scheme 1. Synthesis of quinoline derivatives. Reagents and conditions: (i) EMME, 3 h, rt, 130 °C; (ii) diphenyl ether, 2 h, rt, 250 °C; (iii) 1 M NaOH, 2 h, reflux; acetic acid was added until pH 2–3; (iv) ethanolamine, 2 h, rt, 170 °C; (v) R_4I, DMF, 18 h, 60 °C; (vi) $SOCl_2$, $CHCl_3$, 4 h, rt, reflux; evaporate; NHR_1R_2 was added.

tioned in Section 1. By taking a detailed look at the data in Table 1, we found that: (1) compounds bearing a hydroxyl alkane side chain (**5a**–**e**) all exhibited good anti-SIV activities and low cytotoxicities. (2) Compounds bearing a methyl ester side chain (**5f**–**m**) also possessed effective anti-SIV activities, however, it exhibited fairly high cytotoxicity if the other chain was an alkane (**5g**–**j**) or a structure containing sulfur atom (**5m**). So based on the conclusions above, we designed and synthesized two compounds bearing both a hydroxyl alkane side chain and a methyl ester side chain (**5n** and **5p**), which turned out to possess good anti-SIV activities and low cytotoxicities, especially **5p** (EC_{50}: 1.1 μM). Maybe it was the Hydrogen bond interaction being hindered by methyl that made **5n** have higher EC_{50} value than **5p**. To investigate SAR of the aromatic ring moiety, we synthesized a series of compounds (**5q**–**y**) bearing the two side chains of **5p** with various substituents of quinoline ring and listed their data in Table 1. It was found that substituent changes of quinoline ring had little influence on anti-SIV activity and cytotoxicity, whether it was electron-donating or electron-withdrawing substituent at any position. In addition, we synthesized **5z** to testify the steric hindrance effect of methyl mentioned above. Actually **5z** (EC_{50}: 13.0 μM) did possess higher EC_{50} value than **5s** (EC_{50}: 4.7 μM). Finally, we synthesized compounds bearing different substituent groups on 1-position (the nitrogen atom) of quinoline ring while 4-position turned into carbonyl (**6a**–**c** and **8a**–**d**). And again, this kind of modification did not show significant changes in anti-SIV activity and cytotoxicity.

To ascertain that the compounds we designed could inhibit HIV-1 Tat–TAR interaction, we evaluated nine title compounds with low cytotoxicities (**CS3**, **5d**, **5f**, **5n**, **5p**, **5s**, **5z**, **8a**, **8b**) by Tat dependent HIV-1 LTR-driven CAT gene expression colorimetric enzyme assay (see Section 4). As a report gene, the depressed CAT expression indicated the high inhibitory activity of the compound. As shown in Figure 2, the range of inhibited CAT expression induced by the nine compounds was from 44.2% to 92.7%. The compounds bearing hydroxymethyl chain (**5p** and **5s**) showed lower depressed CAT expression (59.6% versus 82.4% and 53.3% versus 92.7%) than their 2-OH-ethyl counterparts (**5n** and **5z**). This result complied with that of the SIV assay. The decreased CAT activities in the presence of these nine title compounds suggested that most of them could effectively block the interaction of Tat–TAR in

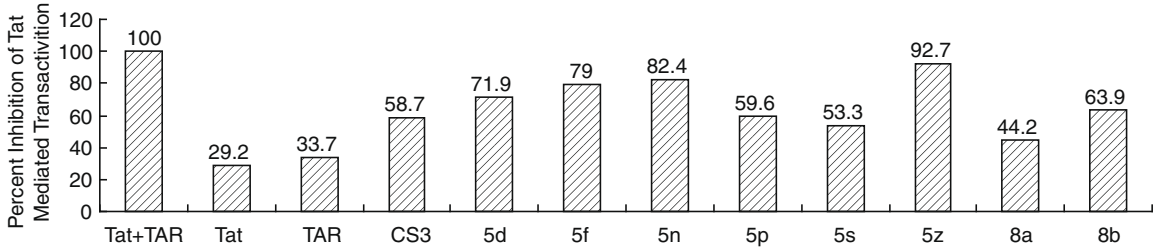


Figure 2. Effects of the nine title compounds on Tat-mediated transactivation in 293T cells.

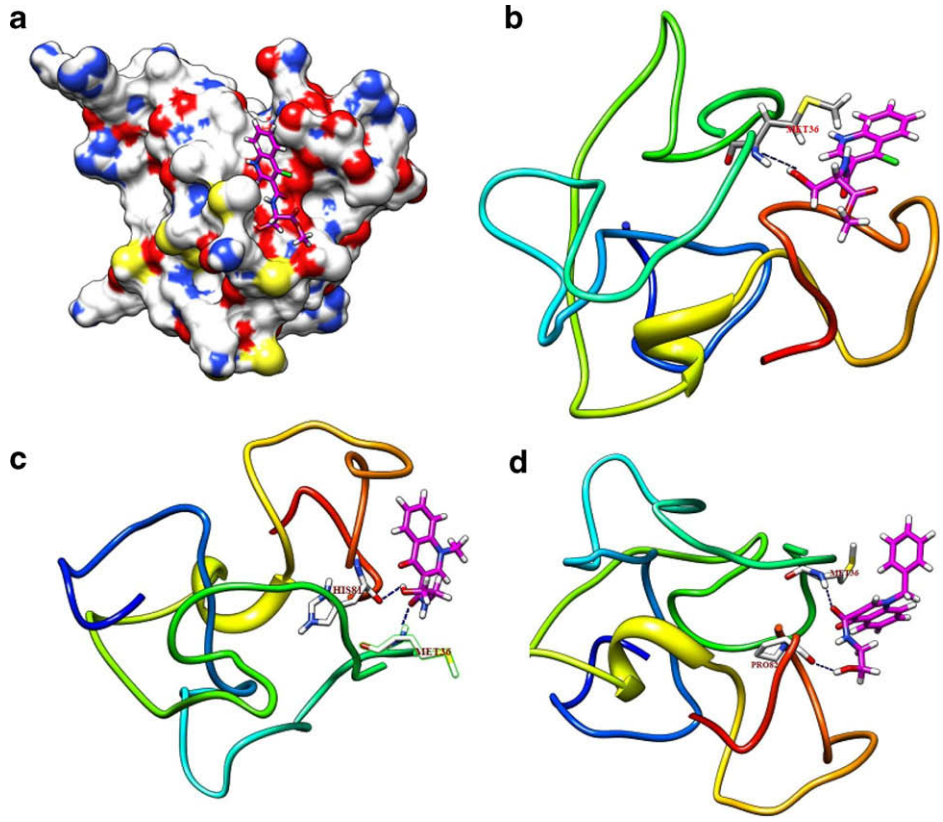


Figure 3. (a, b) Interaction of compound **5p** to Tat protein; (c) interaction of compound **8a** to Tat protein; (d) interaction of compound **8b** to Tat protein.

cell-based assay. Among all the nine compounds, compound **8a** showed the lowest depressed CAT expression (44.2%).

To ascertain our assumption, we performed molecular modeling experiments of three title compounds with different substituents (**5p**, **8a**, and **8b**) using Autodock 4.0 to study the interaction between these compounds and HIV-1 TAR RNA. However, the free energies which reflected the binding affinity of the compounds and the TAR RNA element all turned out to be above-5 kcal/mol, which means these compounds may not inhibit Tat–TAR interaction in the way of binding to TAR RNA. Then we studied the interaction between these compounds and Tat protein. As shown in Figure 3, the bound conformations of all these compounds occupied a pocket surrounded by amino acid residues M36, H81, P82, Q35, C34 with fairly low free energies below −10 kcal/mol (Fig. 3a). H-bond interactions were observed between the side chains of **5p**, **8a**, **8b** and the amide backbone of M36, H81, and P82, indicating that the H-bond interaction may play an important role in enhancing the binding preferences of the compounds (Fig. 3b–d), thus confirming our assumption that it was the H-bond interaction being hindered by methyl that made **5n** have higher EC_{50} value than **5p**. Besides, the aromatic ring moiety of all the three compounds were out of the pocket, which could explain why the modifications on the quinoline ring did not significantly influence the biological activity. We supposed that these compounds could change the conformation of Tat protein by binding to it, which may cause a failure in forming Tat–TAR RNA complex. That may be the reason why compounds like **CS3** could inhibit Tat–TAR interaction although they bore no positively charged groups.

3. Conclusion

In this work, all experiments reported here showed that the newly designed quinoline derivatives could block the Tat–TAR interaction and had potent inhibition activity of SIV. Molecular modeling experiments indicated that these compounds may inhibit Tat–TAR interaction by binding to Tat protein instead of TAR RNA. Different side chains of the title compounds could affect the binding affinity with Tat protein in varying potency, thus bringing about different effects on the biological activities. However, modifications on the quinoline ring did not significantly influence the biological activities of the title compounds, which implied that the substituent groups of the quinoline ring were not helpful for improving the binding affinity between compounds and Tat protein. These studies provide an insight into the SAR of quinoline derivate 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 or Bruke AM-300 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 an XA-4 instrument which was uncorrected.

4.2. General synthetic procedure

The synthesis of the target compounds required access to different substituted 4-hydroyl-3-quinolinecarboxylic ester derivatives (compound **3**). In detail, substituted aniline (compound **1**) was heated with diethyl ethoxymethylenemalonate (EMME) at 130 °C to give malonates (compound **2**), which generally without purification, was cyclized to 4-hydroxyquinoline-3-carboxylic acid ethyl esters by heating at 250 °C in diphenyl ether, as shown in Scheme 1.[16,17] Compound **3** was hydrolyzed to give compound **4**. Then compound **4** was treated with an excess of $SOCl_2$ in the presence of several drops of chloroform to afford 4-chloroquinoline-3-carbonyl chloride, which was then reacted with the amine to afford **5a**–**z**. Compounds **6a**–**c** were prepared by heating compound **3** with ethanolamine at 130 °C.[18] Alkylation of compound **3** by treatment with alkyl halides and anhydrous potassium carbonate gave l-alkyl-1, 4-dihydro-4-oxoquinoline esters (compounds **7a**–**d**).[19] Compounds **8a**–**d** were obtained by heating compounds **7a**–**d** with ethanolamine.

4.2.1. 4-Chloro-*N*-(3-hydroxypropyl) quinoline-3-carboxamide (5a)

A mixture of compound **4a** (0.19 g; 1 mmol), $SOCl_2$ (3 ml) and several drops of chloroform was stirred under reflux for 3 h and then concentrated under reduced pressure to give 4-chloroquinoline-3-carbonyl chloride. The chloride was then mixed with anhydrous THF (5 ml) and added dropwise to the solution of propanolamine (1.2 mmol) and triethylamine (2 mmol) in anhydrous THF in ice bath. After stirring overnight, the mixture was filtered. The filtrate was concentrated under reduced pressure then purified by column chromatography (silica gel, dichloromethane–methanol (100:1, v/v)), Compound **5a** was obtained as yellow solid, mp 143–144 °C, yield 84.9%. ^{1}H NMR ($CDCl_3$) δ 9.09 (s, 1H), 8.34–8.31 (d, 1H), 8.19–8.16 (d, 1H), 7.87–7.82 (t, 1H), 7.77–7.70 (t, 1H), 6.72–6.70 (d, 1H), 3.96–3.92 (m, 2H), 3.79–3.74 (t, 2H), 3.70–3.66 (t, 2H); MS (ESI^+) *m*/*z* calcd: 264.07, found: 265.1 [$(M+1)^+$].

4.2.2. (*S*)-4-Chloro-*N*-(1-hydroxy-3-phenylpropan-2-yl) quinoline-3-carboxamide (5b)

Compound **5b** was prepared in the same way as (**5a**), pale-white solid, mp 149–151 °C yield 73.6%. ^{1}H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.63 (s, 1H), 8.28–8.25 (d, 1H), 8.14–8.11 (d, 1H), 7.95–7.89 (t, 1H), 7.85–7.80 (t, 1H), 7.36–7.29 (m, 5H), 4.97 (s, 1H), 4.24–4.20 (m, 1H), 3.58–3.54 (m, 1H), 3.50–3.45 (m, 1H), 3.05–2.99 (m, 1H), 2.77–2.69 (m, 1H); MS (ESI^+) *m*/*z* calcd: 340.10, found: 341.0 [$(M+1)^+$].

4.2.3. (*S*)-4-Chloro-*N*-(1-hydroxypropan-2-yl) quinoline-3-carboxamide (5c)

Compound **5c** was prepared in the same way as (**5a**), yellow wax, yield 85.4%. ^{1}H NMR (DMSO-d_6) δ 8.86 (s, 1H), 8.58–8.56 (d, 1H), 8.31–8.28 (d, 1H), 8.16–8.13 (d, 1H), 7.96–7.91 (t, 1H), 7.86–7.81 (t, 1H), 4.85–4.81 (t, 1H), 4.06–4.00 (m, 1H), 3.56–3.48 (m, 1H), 3.40–3.32 (m, 1H), 1.18–1.16 (m, 1H); MS (ESI^+) *m*/*z* calcd: 264.07, found: 264.9 [$(M+1)^+$].

4.2.4. (*S*)-4-Chloro-*N*-(1-hydroxy-4-methylpentan-2-yl) quinoline-3-carboxamide (5d)

Compound **5d** was prepared in the same way as (**5a**), white needles, mp 124–126 °C yield 90.3%. ^{1}H NMR ($CDCl_3$) δ 8.97 (s, 1H), 8.23–8.20 (d, 1H), 8.10–8.07 (d, 1H), 7.83–7.77 (t, 1H), 7.69–7.64 (t, 1H), 6.61–6.58 (d, 1H), 4.39–4.32 (m, 1H), 3.90–3.85 (m, 1H), 3.75–3.70 (m, 1H), 2.78 (br s, 1H), 1.84–1.73 (m, 1H) 1.65–1.44 (m, 2H), 1.09–0.94 (m, 6H); MS (ESI^+) *m*/*z* calcd: 306.11, found: 307.2 [$(M+1)^+$].

4.2.5. 4-Chloro-*N*-(2-hydroxyethyl) quinoline-3-carboxamide (5e)

Compound **5e** was prepared in the same way as (**5a**), pale-yellow solid, mp 151–152 °C, yield 88.4%. ^{1}H NMR ($CDCl_3$) δ 9.09 (s,

1H), 8.36–8.31 (d, 1H), 8.19–8.15 (d, 1H), 7.89–7.83 (t, 1H), 7.79–7.69 (t, 1H), 3.96–3.92 (m, 2H), 3.79–3.74 (m, 2H); MS (ESI^+) *m*/*z* calcd: 250.05, found: 251.2 [$(M+1)^+$].

4.2.6. Methyl 2-(4-chloroquinoline-3-carboxamido) acetate (5f)

Compound **5f** was prepared in the same way as (**5a**), pale-yellow solid, mp 128–130 °C, yield 79.0%. ^1H NMR ($CDCl_3$) δ 9.11 (s, 1H), 8.36–8.32 (d, 1H), 8.19–8.14 (d, 1H), 7.90–7.82 (t, 1H), 7.80–7.70 (t, 1H), 7.05 (s, 1H), 4.38–4.36 (d, 2H), 3.85 (s, 3H); MS (EI^+) *m*/*z* calcd: 278.05, found: 278 [$(M)^+$].

4.2.7. (*S*)-Methyl 2-(4-chloroquinoline-3-carboxamido) propanoate (5g)

Compound **5g** was prepared in the same way as (**5a**), pale-yellow solid, mp 129–131 °C, yield 85.9%. ^1H NMR ($CDCl_3$) δ 9.07 (s, 1H), 8.36–8.31 (d, 1H), 8.18–8.14 (d, 1H), 7.90–7.81 (t, 1H), 7.77–7.68 (t, 1H), 7.08–7.06 (d, 1H), 4.94–4.87 (m, 1H), 3.87 (s, 3H), 1.66–1.60 (d, 3H); MS (ESI^+) *m*/*z* calcd: 292.06, found: 293.1 [$(M+1)^+$].

4.2.8. (2*S*,3*S*)-Methyl 2-(4-chloroquinoline-3-carboxamido)-3-methylpentanoate (5h)

Compound **5h** was prepared in the same way as (**5a**), pale-yellow sticky jelly, yield 93.6%. ^1H NMR ($CDCl_3$) δ 9.05 (s, 1H), 8.32–8.29 (dd, 1H), 8.16–8.13 (d, 1H), 7.87–7.82 (dt, 1H), 7.74–7.70 (dt, 1H), 7.05–7.04 (d, 1H), 4.95–4.91 (m, 1H), 3.82 (s, 3H), 2.15–2.10 (m, 1H), 1.60–1.53 (m, 1H), 1.37–1.26 (m, 3H), 1.08–0.95 (m, 6H); MS (EI^+) *m*/*z* calcd: 334.11, found: 334 [$(M)^+$].

4.2.9. (*S*)-Methyl 2-(4-chloroquinoline-3-carboxamido)-4-methylpentanoate (5i)

Compound **5i** was prepared in the same way as (**5a**), pale-yellow solid, mp 81–83 °C, yield 77.9%. ^1H NMR ($CDCl_3$) δ 10.54 (s, 1H), 8.32–8.34 (d, 1H), 7.85–7.80 (m, 1H), 7.69–7.63 (m, 1H), 7.56–7.53 (d, 1H), 7.38–7.33 (d, 1H), 4.74–4.71 (m, 1H), 3.77 (s, 3H), 3.44–3.38 (m, 2H); MS (ESI^+) *m*/*z* calcd: 334.11, found: 335.1 [$(M+1)^+$].

4.2.10. (*S*)-Methyl 2-(4-chloroquinoline-3-carboxamido)-3-methylbutanoate (5j)

Compound **5j** was prepared in the same way as (**5a**), pale-yellow sticky jelly, yield 83.2%. ^1H NMR ($CDCl_3$) δ 9.03 (s, 1H), 8.29–8.25 (d, 1H), 8.15–8.11 (d, 1H), 7.87–7.80 (m, 2H), 7.55–7.54 (m, 1H), 4.80–4.73 (m, 1H), 3.75 (s, 3H), 2.12–2.00 (m, 1H), 1.92–1.77 (m, 2H), 1.05–0.97 (m, 6H); MS (ESI^+) *m*/*z* calcd: 320.09, found: 321.1 [$(M+1)^+$].

4.2.11. (*S*)-Methyl 2-(4-chloroquinoline-3-carboxamido)-3-phenylpropanoate (5k)

Compound **5k** was prepared in the same way as (**5a**), pale-yellow solid, mp 116–118 °C, yield 81.0%. ^1H NMR ($CDCl_3$) δ 8.99 (s, 1H), 8.33–8.30 (d, 1H), 8.16–8.13 (d, 1H), 7.88–7.82 (t, 1H), 7.75–7.70 (t, 1H), 7.35–7.25 (m, 3H), 7.22–7.19 (m, 2H), 6.86–6.83 (d, 1H), 5.22–5.15 (m 1H), 3.84 (s, 3H), 3.44–3.23 (dd,2H); MS (EI^+) *m*/*z* calcd: 368.09, found: 368 [$(M)^+$].

4.2.12. (*S*)-Methyl 2-(4-chloroquinoline-3-carboxamido)-3-(1H-inden-3-yl) propanoate (5l)

Compound **5l** was prepared in the same way as (**5a**), yellow solid, mp 84–86 °C, yield 91.0%. ^1H NMR ($CDCl_3$) δ 8.98 (s, 1H), 8.31–8.29 (d, 1H), 8.13 (s, 2H), 7.85–7.83 (t, 1H), 7.73–7.70 (t, 2H), 7.63–7.60 (d, 1H), 7.40–7.36 (d, 1H), 7.22–7.10 (m, 3H), 6.93–6.90 (d, 1H), 5.24–5.21 (m, 1H), 3.79 (s, 3H), 3.68–3.50 (dd, 2H); MS (EI^+) *m*/*z* calcd: 407.10, found: 407 [$(M)^+$].

4.2.13. (*R*)-Methyl 2-(4-chloroquinoline-3-carboxamido)-3-(methylthio) propanoate (5m)

Compound **5m** was prepared in the same way as (**5a**), yellow sticky jelly, yield 83.2%. ^1H NMR ($CDCl_3$) δ 9.09 (s, 1H), 8.37–8.34 (d, 1H), 8.19–8.16 (d, 1H), 7.90–7.85 (t, 1H), 7.77–7.72 (t, 2H), 7.16–7.14 (d, 1H), 5.05–5.01 (m, 1H), 3.86–3.85 (d, 3H), 2.70–2.62 (m, 2H), 2.41–2.36 (m, 2H); MS (ESI^+) *m*/*z* calcd: 352.06, found: 353.0 [$(M+1)^+$].

4.2.14. (2*S*,3*R*)-Methyl 2-(4-chloroquinoline-3-carboxamido)-3-hydroxybutanoate (5n)

Compound **5n** was prepared in the same way as (**5a**), yellow solid, mp 220–222 °C, yield 92.4%. ^1H NMR ($CDCl_3$) δ 9.11 (s, 1H), 8.38–8.35 (d, 1H), 8.20–8.17 (d, 1H), 7.90–7.85 (t, 1H), 7.77–7.73(d, 1H), 7.14–7.11 (d, 1H), 4.94–4.91 (dd, 1H), 4.56 (br s, 2H), 3.86 (s, 3H), 1.42–1.40 (d, 3H); MS (ESI^+) *m*/*z* calcd: 322.07, found: 323.0 [$(M+1)^+$].

4.2.15. (*S*)-Methyl 2-(4-chloroquinoline-3-carboxamido)-3-hydroxypropanoate (5p)

Compound **5p** was prepared in the same way as (**5a**), pale-yellow solid, mp 191–192 °C, yield 79.5%. ^1H NMR ($CDCl_3$) δ 9.12 (s, 1H), 8.37–8.35 (d, 1H), 8.20–8.17 (d, 1H), 7.91–7.85 (t, 1H), 7.78–7.73 (t, 1H), 5.02–4.98 (m, 1H), 4.19 (s, 2H), 3.88 (s, 3H); MS (ESI^+) *m*/*z* calcd: 308.06, found: 309.0 [$(M+1)^+$].

4.2.16. (*S*)-Ethyl 2-(4-chloroquinoline-3-carboxamido)-3-hydroxypropanoate (5q)

Compound **5q** was prepared in the same way as (**5a**), yellow wax, yield 77.7%. ^1H NMR ($CDCl_3$) δ 9.15–9.12 (s, 1H), 8.86 (s, 1H), 8.33–8.30 (d, 1H), 8.17–8.14 (d, 1H), 7.98–7.92 (t, 1H), 7.87–7.84 (t, 1H), 5.14–5.10 (t, 1H), 4.61–4.59 (q, 1H), 4.21–4.14 (q, 2H), 3.83–3.78 (m, 2H), 1,27–1.22(t, 3H); MS (ESI^+) *m*/*z* calcd: 322.07, found: 322.9 [$(M+1)^+$].

4.2.17. (*S*)-Methyl 2-(4-chloro-8-methylquinoline-3-carboxamido)-3-hydroxypropanoate (5r)

Compound **5r** was prepared in the same way as (**5a**), pale-yellow solid, mp 186–188 °C, yield 89.3%. ^1H NMR ($CDCl_3$) δ 12.09 (br s, 1H), 9.07 (s, 1H), 8.22–8.18 (d, 1H), 7.93 (s, 1H), 7.57–7.53 (d, 2H), 4.98–4.95 (m, 1H), 4.17 (s, 2H), 3.87 (s, 3H), 2.61 (s, 3H); MS (ESI^+) *m*/*z* calcd: 322.07, found: 323.1 [$(M+1)^+$].

4.2.18. (*S*)-Methyl 2-(4-chloro-7-methylquinoline-3-carboxamido)-3-hydroxypropanoate (5s)

Compound **5s** was prepared in the same way as (**5a**), pale-yellow solid, mp 167–169 °C, yield 79.3%. ^1H NMR ($CDCl_3$) δ 11.98 (br s, 1H), 9.06 (s, 1H), 8.19–8.17 (d, 1H), 7.93 (s, 1H), 7.67–7.64 (d, 1H), 7.55–7.53 (d, 1H), 4.97–4.94 (m, 1H), 4.19–4.17 (m, 2H), 3.91–3.89 (d, 3H), 2.66–2.61 (m, 3H); MS (ESI^+) *m*/*z* calcd: 322.07, found: 323.1 [$(M+1)^+$].

4.2.19. (*S*)-Methyl 2-(4-chloro-6-methylquinoline-3-carboxamido)-3-hydroxypropanoate (5t)

Compound **5t** was prepared in the same way as (**5a**), pale-yellow solid, mp 193–195 °C, yield 88.6%. ^1H NMR ($CDCl_3$) δ 9.11 (s, 1H), 8.20–8.18 (d, 1H), 7.71–7.69 (d, 1H), 7.63–7.58 (t, 1H), 7.39–7.37 (d, 1H), 5.00–4.97 (t, 1H), 4.18 (s, 2H), 3.87 (s, 3H), 2.83 (s, 3H); MS (ESI^+) *m*/*z* calcd: 322.07, found: 323.1 [$(M+1)^+$].

4.2.20. (*S*)-Methyl 2-(4,8-dichloroquinoline-3-carboxamido)-3-hydroxypropanoate (5u)

Compound **5u** was prepared in the same way as (**5a**), yellow solid, mp 290–291 °C, yield 90.6%. ^1H NMR ($CDCl_3$) δ 9.08 (s, 1H), 8.21–8.18 (d, 1H), 7.93 (s, 1H), 7.57 (s, 1H), 7.53 (s, 1H), 5.00–4.94 (q, 1H), 4.17 (s, 2H), 3.87 (s, 3H); MS (ESI^+) *m*/*z* calcd: 344.01, found: 345.1 [$(M+1)^+$].

4.2.21. (*S*)-Methyl 2-(4,7-dichloroquinoline-3-carboxamido)-3-hydroxypropanoate (5v)

Compound **5v** was prepared in the same way as (**5a**), yellow solid, mp 277–279 °C, yield 84.5%. ^{1}H NMR ($CDCl_3$) δ 9.11 (s, 1H), 8.21–8.19 (d, 1H), 7.92 (s, 1H), 7.66 (s, 1H), 7.55–7.53 (d, 1H), 4.99–4.93 (q, 1H), 4.18 (s, 2H), 3.89 (s, 3H); MS (ESI$^+$) *m*/*z* calcd: 344.01, found: 345.1 [(M+1)$^+$].

4.2.22. (*S*)-Methyl 2-(4,6-dichloroquinoline-3-carboxamido)-3-hydroxypropanoate (5w)

Compound **5w** was prepared in the same way as (**5a**), pale-yellow solid, mp 303–305 °C, yield 92.3%. ^{1}H NMR ($CDCl_3$) δ 9.09 (s, 1H), 8.21–8.18 (d, 1H), 7.73–7.70 (d, 1H), 7.63–7.59 (t, 1H), 7.38–7.34 (d, 1H), 5.00–4.96 (q, 1H), 4.18 (s, 2H), 3.89 (s, 3H); MS (ESI$^+$) *m*/*z* calcd: 344.01, found: 345.1 [(M+1)$^+$].

4.2.23. (*S*)-Methyl 2-(4-chloro-6-fluoroquinoline-3-carboxamido)-3-hydroxypropanoate (5x)

Compound **5x** was prepared in the same way as (**5a**), pale-yellow solid, mp 303–305 °C, yield 74.1%. ^{1}H NMR ($CDCl_3$) δ 9.09 (s, 1H), 8.21–8.18 (d, 1H), 7.72–7.70 (d, 1H), 7.63–7.60 (t, 1H), 7.38–7.34 (d, 1H), 4.99–4.96 (q, 1H), 4.18 (s, 2H), 3.88 (s, 3H); MS (ESI$^+$) *m*/*z* calcd: 326.05, found: 327.1 [(M+1)$^+$].

4.2.24. (*S*)-Methyl 2-(4-chloro-6, 8-difluoroquinoline-3-carboxamido)-3-hydroxypropanoate (5y)

Compound **5y** was prepared in the same way as (**5a**), pale-yellow solid, mp 219–221 °C, yield 81.8%. ^{1}H NMR ($CDCl_3$) δ 9.09 (s, 1H), 7.73–7.69 (d, 1H), 7.63–7.60 (d, 1H), 7.38–7.34 (d, 1H), 4.99–4.96 (q, 1H), 4.18 (s, 2H), 3.87 (s, 3H); MS (ESI$^+$) *m*/*z* calcd: 344.04, found: 345.1 [(M+1)$^+$].

4.2.25. (2*S*,3*R*)-Methyl 2-(4-chloro-7-methylquinoline-3-carboxamido)-3-hydroxybutanoate (5z)

Compound **5z** was prepared in the same way as (**5a**), pale-yellow solid, mp 195–196 °C, yield 81.4%. ^{1}H NMR ($CDCl_3$) δ 9.11 (s, 1H), 8.18–8.16 (d, 1H), 7.93 (s, 1H), 7.66 (s, 1H), 7.57–7.52 (d, 1H), 7.15–7.10 (d, 1H), 4.95–4.89 (q, 1H), 4.60–4.62 (m, 1H), 3.86 (s, 3H), 2.64 (s, 3H), 1.39–1.43 (d, 3H); MS (ESI$^+$) *m*/*z* calcd: 336.09, found: 337.2 [(M+1)$^+$].

4.2.26. *N*-(2-Hydroxyethyl)-4-oxo-6-phenoxy-1,4-dihydroquinoline-3-carboxamide (6a)

A mixture of compound **3** (2 mmol) and an excess of ethanolamine was stirred under reflux for 2 h and then the ethanolamine was evaporated under reduced pressure, then the residue was recrystallized from ethanol to give **6a** as white needles, mp > 250 °C, yield 57.9%. ^{1}H NMR (DMSO-d_6) δ 12.75 (s, 1H), 10.07–10.03 (t, 1H), 8.72 (s, 1H), 7.79–7.76 (d, 1H), 7.61–7.53 (m, 2H), 7.48–7.43 (t, 2H), 7.26–7.21 (t, 1H), 7.13–7.10 (d, 2H), 4.80–4.77 (t, 1H), 3.51–3.47 (m, 2H), 3.40–3.37 (m, 2H); MS (ESI$^+$) *m*/*z* calcd: 324.11, found: 325.0 [(M+1)$^+$].

4.2.27. *N*-(2-Hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (6b)

Compound **6b** was prepared in the same way as (**6a**), dilute-brow needles, mp > 250 °C, yield 66.6%. ^{1}H NMR (DMSO-d_6) δ 12.63 (br s, 1H), 10.15–10.11 (t, 1H), 8.75 (s, 1H), 8.27–8.25 (m, 1H), 7.80–7.68 (m, 2H), 7.51–7.46 (m, 1H), 4.83 (br s, 1H), 3.55–3.52 (m, 2H), 3.44–3.38 (m, 2H); MS (ESI$^+$) *m*/*z* calcd: 232.08, found: 232.9 [(M+1)$^+$].

4.2.28. 6-Chloro-*N*-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (6c)

Compound **6c** was prepared in the same way as (**6a**), dilute-brow needles, mp > 250 °C, yield 75.1%. ^{1}H NMR (DMSO-d_6) δ 12.79 (br s, 1H), 10.01–9.97 (t, 1H), 8.77 (s, 1H), 8.16–8.17 (m, 1H), 7.82–8.72 (m, 2H), 4.85 (br s, 1H), 3.53–3.57 (m, 2H), 3.39–3.45 (m, 2H); MS (ESI$^+$) *m*/*z* calcd: 266.05, found: 266.9 [(M+1)$^+$].

4.2.29. *N*-(2-Hydroxyethyl)-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (8a)

A mixture of compound **3** (2 mmol), anhydrous potassium carbonate (3 mmol) and an excess of iodomethane was stirred in DMF (30 ml) at 60 °C for 24 h then extracted with 20 ml × 3 dichloromethane, the extraction was concentrated under reduced pressure. Then the residue was recrystallized from ethanol to give **7a** as white needles, which was heated with an excess of ethanolamine under reflux for 2 h and then the ethanolamine was evaporated under reduced pressure. The residue was recrystallized from ethanol to give **6a** as white needles, mp > 250 °C, yield 74.7%. ^{1}H NMR (DMSO-d_6) δ 10.13–10.09 (t, 1H), 8.88 (s, 1H), 8.37–8.33 (d, 1H), 7.89–7.85 (m, 2H), 7.61–7.56 (t, 1H), 4.89–4.84 (t, 1H), 4.02 (s, 3H), 3.58–3.50 (m, 2H), 3.45–3.37 (m, 2H); MS (ESI$^+$) *m*/*z* calcd: 246.10, found: 246.9 [(M+1)$^+$].

4.2.30. 1-Benzyl-*N*-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (8b)

Compound **8c** was prepared in the same way as (**8a**), white needles, mp > 250 °C, yield 43.2%.. ^{1}H NMR (DMSO-d_6) δ 10.44 (s, 1H), 8.94 (s, 1H), 8.55–8.52 (d, 1H), 7.65–7.60 (t, 1H), 7.50–7.42 (m, 2H), 7.38–7.32 (m, 3H), 7.18–7.15 (d, 2H), 5.48 (s, 1H), 3.89–3.84 (m, 2H), 3.70–3.65 (m, 2H), 3.59–3.56 (t, 1H); MS (ESI$^+$) *m*/*z* calcd: 322.13, found: 323.0 [(M+1)$^+$].

4.2.31. 1-(4-Fluorobenzyl)-*N*-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (8c)

Compound **8d** was prepared in the same way as (**8a**), white needles, mp > 250 °C, yield 39.8%. ^{1}H NMR (DMSO-d_6) δ 10.40 (s, 1H), 8.92 (s, 1H), 8.54–8.52 (d, 1H), 7.64–7.61 (t, 1H), 7.50–7.45 (t, 1H), 7.41–7.38 (d, 1H), 7.18–7.13 (m, 2H), 7.08–7.02 (m, 2H), 5.45 (s, 2H), 3.88–3.84 (m, 2H), 3.74–3.66 (m, 2H), 1.63 (br s, 1H); MS (ESI$^+$) *m*/*z* calcd: 340.12, found: 341.0 [(M+1)$^+$].

4.2.32. *N*,1-Bis(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (8d)

Compound **8e** was prepared in the same way as (**8a**), white needles, mp > 250 °C, yield 61.7%. ^{1}H NMR (DMSO-d_6) δ 10.12–10.09 (t, 1H), 8.77 (s, 1H), 8.38–8.35 (d, 1H), 7.94–7.91 (d, 1H), 7.82–7.80 (t, 1H), 7.57–7.52 (t, 1H), 5.04–5.01 (t, 1H), 4.86–4.82 (t, 1H), 4.54–4.51 (m, 2H), 3.75–3.74 (m, 2H), 3.55–3.50 (m, 2H), 3.43–3.35 (m, 2H); MS (ESI$^+$) *m*/*z* calcd: 276.11, found: 277.0 [(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 2×105 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 EC_{50} was defined as the compound concentration required to protect cells against the cytopathogenicity of SIV by 50%. Indinavir 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 Tat Mal protein was recovered from the Protein Database (http://www.PDB.org) with the code as 1k5k. The advanced docking program Auto-dock 4.0 was used to perform the automatic molecular docking with our compounds.[20] 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 Tat and Gasteiger–Hückel for the compounds. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco.[21]

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