Bioorganic & Medicinal Chemistry Letters 19 (2009) 2162–2167

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and antiviral activities of novel acylhydrazone derivatives targeting HIV-1 capsid protein

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ARTICLE INFO

Article history:
Received 11 November 2008
Revised 14 January 2009
Accepted 27 February 2009
Available online 4 March 2009

Keywords:
Synthesis
Antiviral activity
Acylhydrazones
HIV-1 capsid

ABSTRACT

HIV-1 capsid protein (CA) plays important roles in the viral replication cycle. A number of acylhydrazone derivatives that act as inhibitors of HIV-1 CA assembly, were designed and synthesized. The synthesized compounds were tested for their antiviral activities and cytotoxicities using CEM cells. Some derivatives also were assayed for their ability to inhibit HIV-1 CA assembly in vitro. Among them, compounds **14f** and **14i** display the most promising potency with EC_{50} values of 0.21 and 0.17 μM, respectively.

Human immunodeficiency virus type-1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome (AIDS) which has become a major worldwide epidemic since 1981. Despite the therapeutic success of highly active anti-retroviral therapy (HAART), which includes the combined use of drugs targeting the viral enzymes reverse transcriptase (RT) and protease (PR), drug resistance and poor compliance caused by side effects hinder the achievement of sustainable antiviral effects.[1] With the advances in the understanding of HIV-1 life cycle, in addition to RT and PR many novel targets that may have potential antiviral intervention have been identified, including the other viral enzymes and viral proteins (e.g., integrase, envelope protein gp41 and regulatory protein tat). Drug discovery and development for anti-HIV has progressed significantly in the past decade, especially recently several novel antiviral drugs, such as enfuvirtide (fusion inhibitor), maraviroc (chemokine co-receptor antagonist) and raltegravir (integrase inhibitor), have been approved for the treatment of AIDS.[2] The successful design of such inhibitors could provide hints for the development of other new antiviral agents against HIV-1.

Recent studies reveal that HIV-1 capsid protein (CA) plays important roles in the life cycle of HIV-1 and has been an attractive target of high priority for anti-HIV.[3] During the viral replication cycle, HIV-1 CA can assemble into a conical core shell surrounding the viral nucleocapsid/RNA genome complex which is critical for viral replication.[4] Recently, several inhibitors that bind to CA and inhibit virus assembly have been reported.[5] CAP-1, one of the HIV CA assembly inhibitors, has been identified to bind to the CA N-terminal domain (NTD) and inhibit capsid assembly during viral maturation (Fig. 1).[6]

More recently, Prevelige and co-workers have reported a series of new small molecule inhibitors that target HIV-1 CA through the use of the high throughput assay (Fig. 2).[7] Among these compounds, we found that most of them have a core structure of acylhydrazone moiety, and two substituted phenyl ring in each end which seems to be the most important pharmacophores for their antiviral activities. In addition there are a few peptides which have been reported to bind to HIV-1 CA and interfere with the protein–protein interactions in HIV-1 CA.[8] Therefore, we designed novel acylhydrazone derivatives bearing hydrophobic or lipophobic groups in side chains by the introduction of different naturally occurring amino acids (such as glycine, L-phenylalanine and L-serine) into the backbone of these molecules so as to improve their binding affinities to the protein via hydrogen bonds, steric interactions, hydrophobic contacts, etc. To further explore the antiviral activities of these compounds, other molecular modifications were required in which various steric, electron-donating, electron-with-

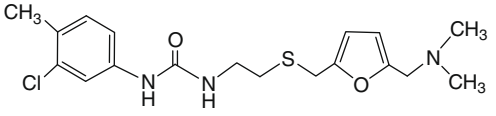

Figure 1. Structure of CAP-1.

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doi:10.1016/j.bmcl.2009.02.116

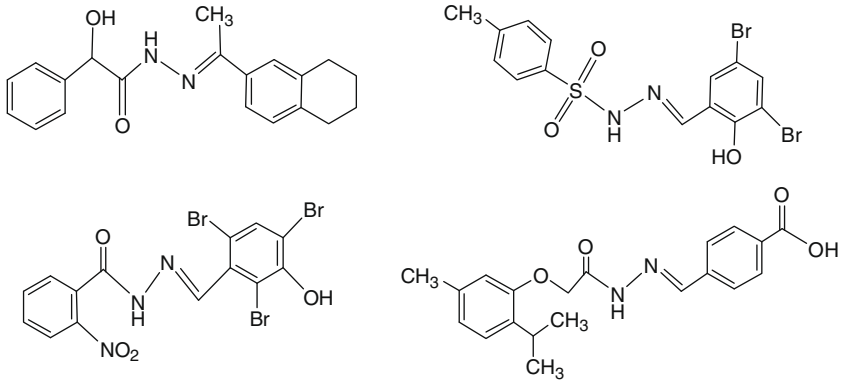
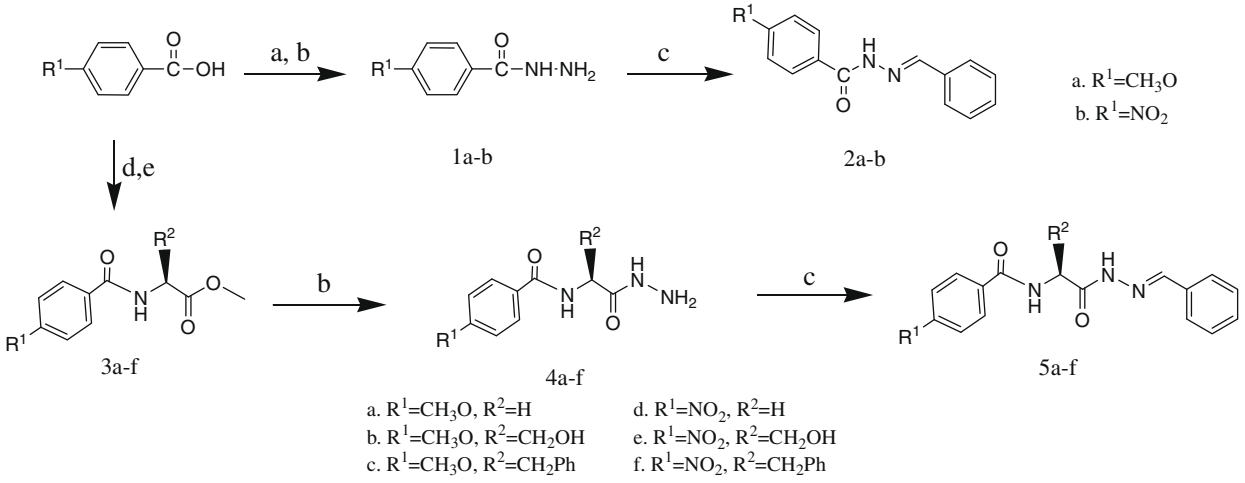


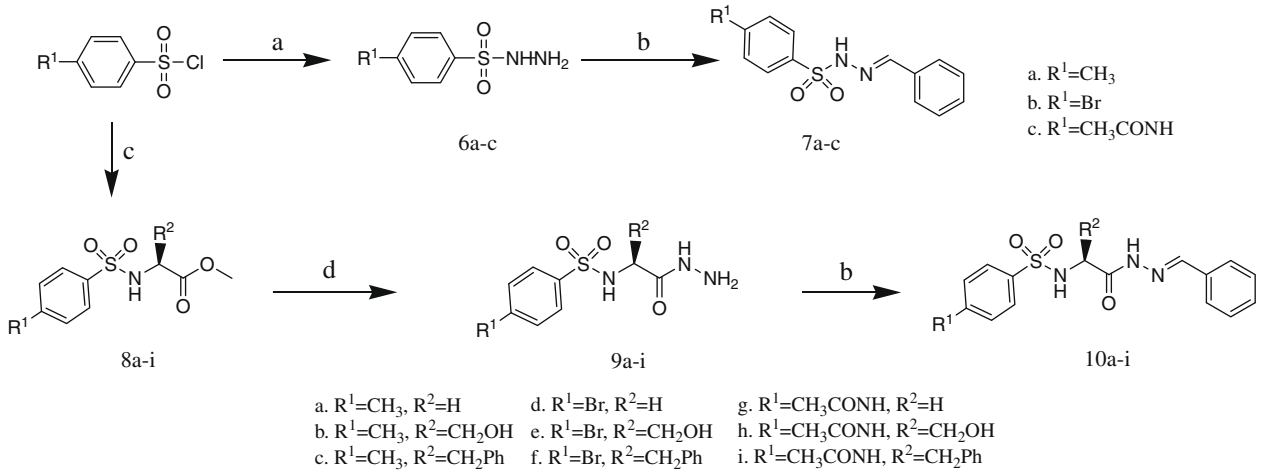
Figure 2. Small molecule inhibitors targeting HIV-1 CA.

drawing or hydrophobic groups were introduced into the aromatic ring in the end of the molecules.

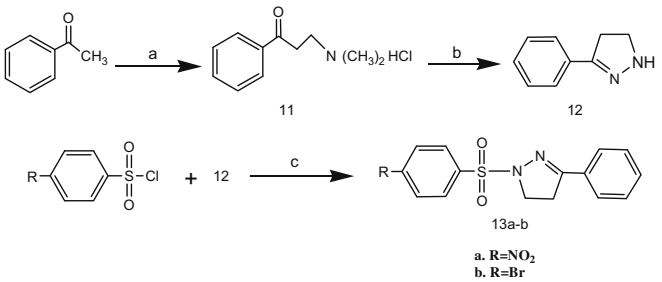
The synthetic route we applied for the synthesis of a variety of acylhydrazone analogues is summarized in Schemes 1–4. In Scheme 1, *N*-acylhydrazones **2(a–b)** were synthesized according to a general procedure described in the literature.[9] Starting from substituted benzoic acids, they were first converted to their corresponding acid chlorides by reaction with thionyl chloride, followed



Scheme 1. Synthesis of acylhydrazones and their derivatives. Reagents and conditions: (a) concentrated H_2SO_4, anhydrous CH_3CH_2OH, reflux, 20 h; (b) 85% $NH_2NH_2{\cdot}H_2O$, CH_3CH_2OH, reflux, 6–8 h; (c) PhCHO, anhydrous CH_3OH, rt, 1–2 h; (d) $SOCl_2$, $CHCl_3$, reflux, 6 h; (e) glycine/L-phenylalanine/L-serine methyl ester hydrochloride, Et_3N, CH_2Cl_2, 0 °C to rt, 5 h.



Scheme 2. Synthesis of sulfonylhydrazones and their derivatives. Reagents and conditions: (a) 85% $NH_2NH_2{\cdot}H_2O$, CH_3CH_2OH, H_2O, rt, 5 h; (b) PhCHO, anhydrous CH_3OH, rt, 1–2 h; (c) glycine/L-phenylalanine/L-serine methyl ester hydrochloride, Et_3N, CH_2Cl_2, 0 °C to rt, 20 h; (d) 85% $NH_2NH_2{\cdot}H_2O$, CH_3CH_2OH, reflux, 6–8 h.



Scheme 3. Synthesis of 1-(substituted benzenesulfonyl)-3-phenyl-2-pyrazolines. Reagents and conditions: (a) $(CH_2O)_n$, $HN(CH_3)_2{\cdot}HCl$, concentrated HCl, CH_3CH_2OH, reflux, 3 h; (b) 85% $NH_2NH_2{\cdot}H_2O$, 50% aqueous NaOH, CH_3OH, reflux, 45 min; (c) dry pyridine, on a boiling water bath, 20 min.

by addition of a variety of methyl ester hydrochloride of natural amino acids in the presence of triethylamine.[10] The yielded intermediates **3(a–f)** reacted with hydrazine hydrate in ethanol to obtain acid hydrazides **4(a–f)**, which underwent condensation with benzaldehyde affording the target compounds **5(a–f)**.[11]

The synthesis of sulfonylhydrazones and their derivatives is shown in Scheme 2. *N*-Arylsulfonyl hydrazones **7(a–c)** were prepared from substituted aromatic sulfonyl chlorides by the typical method.[12] Their derivatives **10(a–i)** were obtained by a route analogous to **5(a–f),** using the substituted aromatic sulfonyl chlorides as starting materials to react with natural amine acids methyl ester hydrochlorides,[13] subsequent hydrazinolysis with hydrazine hydrate and condensation with benzaldehyde.[14]

In Scheme 3, the hydrochloride of 3-dimethylaminopropiophenone **11** as Mannich Base was prepared by refluxing acetophenone, dimethylamine hydrochloride and paraformaldehyde in ethanol in the presence of concentrated hydrochloric acid according to the standard method.[15] The key intermediate **11** reacted with hydrazine hydrate providing 3-phenyl-2-pyrazoline **12** using a literature procedure, which was immediately treated with substituted aromatic sulfonyl chlorides to give the target compounds **13(a–b)**, respectively.[16]

To further investigate the antiviral activities of the acylhydrazone derivatives, further molecular modifications were carried out. The analogues possessing different substitutions in the right phenyl ring were synthesized through the route outlined in Scheme 4, using a variety of aromatic (or heteroaromatic) aldehydes and ketones as starting materials. The condensation of the acid hydrazides **9c** with appropriate aldehydes and ketones resulted in the formation of the corresponding acylhydrazone derivatives **14(a–l)**.[17]

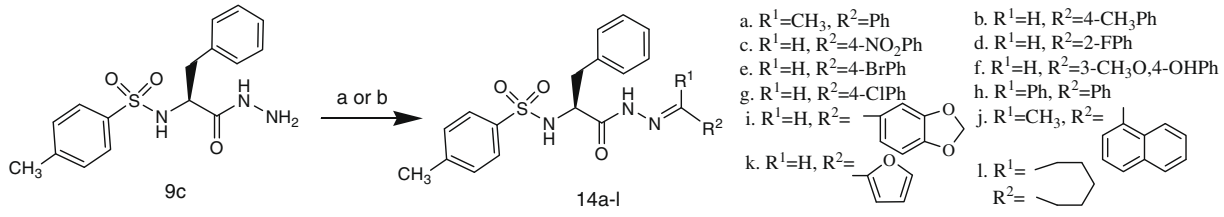
The purity of all the products was determined by TLC and melting points. The physical and spectral properties of previously reported compounds, such as melting points, were in accord with literature values, and the structures of the novel compounds were confirmed by ^{1}H NMR and EI-MS. According to the literature,[18] this class of acylhydrazones are in the form of *E* geometrical isomer for C=N double bond in DMSO-d_6, and the *E* geometrical isomer undergoes a rapid *cis*/*trans* amide equilibrium at room temperature, which is in agreement with the analytic results of the ^{1}H NMR spectra of our synthesized derivatives. For example, two sets of methylene, imine and amide protons signals of *cis*/*trans* amide conformers are observed in the ^{1}H NMR spectra of compounds **5a**, **5d**, **10a**, **10d** and **10g** in DMSO-d_6.

We also evaluated the biological activities of all our synthesized compounds using SIV-induced syncytium in CEM cells.[19] Their EC_{50} (antiviral activity), TC_{50} (cytotoxicity) and TI (therapy index) are listed in Table 1. Most of our synthesized compounds exhibited significant inhibitory activity against viral replication. Among the compounds (**2a**, **2b**, **5a–5f**, **7a–7c**, **10a–10i**), compounds **5c**, **10c**, **10d** and **10i** were the most potent with EC_{50} values ranging from 0.2 to 0.47 μM and TI values above 125. It is worth noting that each of these compounds except compound **10d** contains a L-phenylala-

Table 1
Antiviral activity and toxicity of acylhydrazone derivatives on SIV-induced syncytium

Compound[a]	EC_{50}[b] (μM)	TC_{50}[c] (μM)	TI[d]
2a	31.05	>100	>3.22
2b	5.59	29.6	5.3
5a	2	63.8	31.9
5b	0.6	45.6	76
5c	0.2	>100	>500
5d	1.03	>100	>97.1
5e	2	>100	>50
5f	3	18.71	6.2
7a	0.36	31.7	88.1
7b	0.95	>100	>105.3
7c	0.65	40	61.5
10a	1.5	83.5	55.7
10b	1.63	5.19	3.2
10c	0.4	50	125
10d	0.33	92.8	283.8
10e	35.98	80.2	2.2
10f	15.3	>100	>6.5
10g	15.1	>100	>6.6
10h	1.32	>100	>75.8
10i	0.47	>100	>212.8
13a	0.52	>100	>192.3
13b	0.1	38.2	382
14a	0.3	21.2	70.7
14b	9.93	>100	>10.1
14c	6.64	9.8	1.5
14d	4.45	>100	>22.5
14e	5.49	82.5	15
14f	0.21	>100	>476.2
14g	2.8	9.4	3.4
14h	0.27	>100	>365
14i	0.17	>100	>588.2
14j	0.4	49.1	122.8
14k	2.46	99.8	40.6
14l	0.89	78.3	87.6

[a] This assay used the antiviral drug Indinavir at 1×10^{-6} mol/L as positive control.
[b] Antiviral activity, concentration which inhibits viral replication by 50%.
[c] Cellular toxicity, concentration which is toxic to 50% of SIV-induced syncytium.
[d] Therapeutic index, TC_{50} value divided by EC_{50} value (TC_{50}/EC_{50}).



Scheme 4. Synthesis of acylhydrazone derivatives of various aldehydes and ketones. Reagents and conditions: (a) R^1COR^2, anhydrous CH_3OH, rt, 1–2 h; (b) R^1COR^2, anhydrous CH_3OH, glacial acetic acid, reflux, 3–8 h.

nine moiety which bears a benzyl side chain, and an electron-donating substituent (such as methyl, methoxy) in the left phenyl ring. One possible explanation is that the presence of these moieties enhance the binding affinity of the compounds to HIV-1 CA. In addition, we also synthesized the D-enantiomer of the acylhydrazone derivative **10c** as an example and evaluated for its antiviral activity. We found that it was nearly inactive (data not shown), which demonstrated that the configuration of these compounds greatly influenced their biological activities, and the L-enantiomers showed good antiviral activities in comparison with their D-enantiomers, so we just focused on the studies of these acylhydrazone derivatives with L-configuration in this paper. From this preliminary study, on the basis of compound **10c**, then we investigated the effect of structural modification of the right phenyl ring on their biological activities, which included incorporation of different electron-donating, electron-withdrawing, hydrophobic and steric substituents, such as methyl, halide, nitro groups, and replacement of the phenyl ring by a heterocyclic aromatic ring. Among the compounds **14a–14l**, compound **14f** and **14i** displayed most potent inhibitory activity compared with the parent **10c** with EC_{50} values from 0.4 μM to 0.21 μM and 0.17 μM, respectively, and the cytotoxicities were markedly reduced, resulting in increase of therapy index (TI) value from 125 to the above 476 and 588, respectively. Furthermore, the cyclization of NHN=CH moiety in sulfonylhydrazones produced 2-pyrazoline derivatives possessing a rigid conformation which led to a considerable increase in antiviral potency. For example, the compound **13b** showed significantly higher activity with EC_{50} value of 0.1 μM in comparison with the sulfonylhydrazone **7b** with EC_{50} value of 0.95 μM.

HIV-1 CA can assemble into tubes, which leads to increase in sample turbidity that can be measured spectrophotometrically.[6a] In our study this assay was used to probe for potential inhibitory effects of the acylhydrazone derivatives on in vitro capsid assembly.[20] As shown in Table 2, dissolution of native HIV-1 CA into assembly buffer led to an increase in absorbance at an initial rate of 59.16 mOD/min, while in the presence of CAP-1 the initial assembly rate decreased to 33.37 mOD/min. The assembly rates in the presence of the more potent compounds **5c**, **10c**, **10d**, **10i**, and **13b** decreased to 22.58 mOD/min, 18.90 mOD/min, 22.50 mOD/min, 20.78 mOD/min and 17.33 mOD/min, respectively, which are consistent with the antiviral activities of these compounds. Especially as shown in Table 2 and Figure 3, we found that in the presence of **14f** and **14i**, which showed high activities with EC_{50} values of 0.21 μM and 0.17 μM, respectively, the assembly rates decreased to 10.56 mOD/min and 7.89 mOD/min, but in the presence of **14b** and **14c**, which showed low activities with EC_{50} values of 9.93 μM and 6.64 μM, respectively, the assembly rates slightly decreased to 45.99 mOD/min and 31.92 mOD/min. These results indicated that these CA-binding compounds could inhibit capsid assembly in varying degrees and hence exhibit different level of antiviral activities, and the stronger ability to inhibit capsid assembly the compound had, the higher antiviral activity it showed. We next investigated the ability of acylhydrazone compounds **14f** and **14i** to inhibit HIV-1 capsid assembly under different concentrations, the result revealed the assembly rates were diminished in a dose-dependent manner, and increasing the doses of compounds could enhance the inhibition of HIV-1 CA assembly. The dose-dependence data for **14f** and **14i** are shown in Table 3.

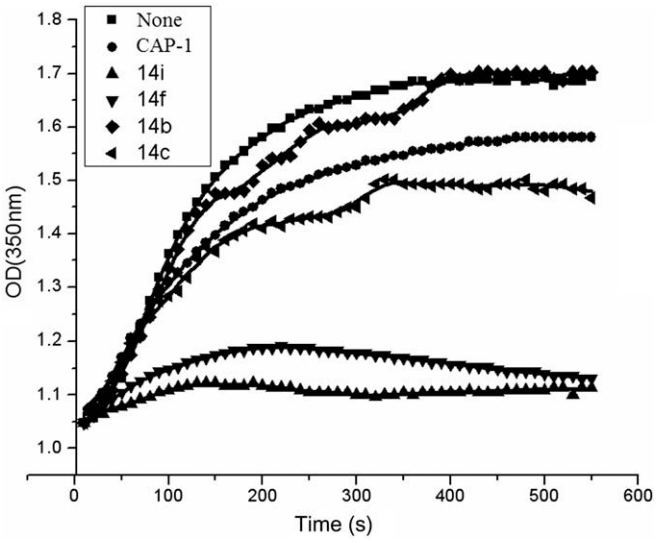


Figure 3. Turbidity assay results showing the effects of CA-binding compounds on in vitro capsid assembly.

In order to gain more insight into the interaction between this new series of acylhydrazone derivatives and HIV-1 capsid protein, molecular docking studies for the target compounds **5c**, **10c**, **10d**, **13b, 14f** and **14i** were performed using AUTODOCK 4 software based on the X-ray crystal structure of HIV-1 CA taken from the Protein Data Bank (PDB ID: 2JPR).[6b] We found that all the molecules in our series were docked into the binding pocket of the protein in the same manner as CAP-1. In addition they have also occupied two more other binding sites of the protein. The binding conformation with the lowest binding free energy (−13.49 kcal/mol) for the representative compound **14i**, one of the most active compounds, is shown in Figure 4, where the 3,4-methylenedioxyphenyl group of **14i** inserts into the deep hydrophobic pocket vacated by Phe32 just like the aromatic ring of CAP-1 (Fig. 4A). In addition, it seemed that the π–π stacking interaction between the phenyl

Table 2
Effects of the target compounds on HIV-1 CA assembly

Compound	Assembly rate[a] (mOD/min)
None	59.2 ± 0.3
5a	43.5 ± 1.4
5b	21.7 ± 1.3
5c	22.6 ± 0.6
10a	23.1 ± 0.6
10b	24.4 ± 0.5
10c	18.9 ± 0.8
10d	22.5 ± 1.9
CAP-1	33.4 ± 1.2
10i	20.8 ± 0.5
13b	17.3 ± 0.5
14b	46.0 ± 1.2
14c	31.9 ± 1.2
14f	10.6 ± 0.7
14i	7.9 ± 0.6

[a] Each value is reported as the mean ± SD (standard deviation) from two experiments in duplicate.

Table 3
Effects of different concentrations of the target compounds on HIV-1 CA assembly

Compound	Concentration (μM)	Ratio [a]	Assembly rate [b] (mOD/min)
14f	10	0.5:1	14.0 ± 0.1
	20	1:1	10.9 ± 0.5
	40	2:1	7.7 ± 0.1
14i	10	0.5:1	13.2 ± 1.2
	20	1:1	7.4 ± 0.6
	40	2:1	2.5 ± 0.1

[a] Ratio, compound/HIV-1 CA.
[b] Each value is reported as the mean ± SD (standard deviation) from two experiments in duplicate.

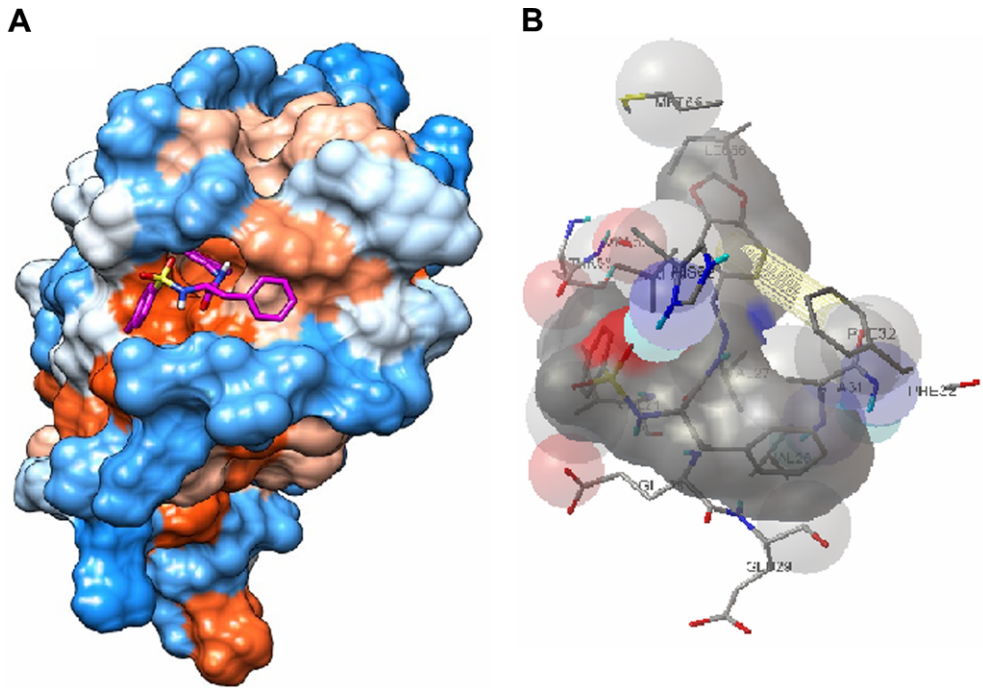


Figure 4. The docking result of compound **14i** in the complex with HIV-1 CA. (A) Stereo view of the complex of **14i** with HIV-1 CA. (B) The interactions between **14i** and CA in this model in which only a subset of residues closest to the ligand is displayed for sake of clarity. This figure highlights essential interactions: π–π interaction (indicated by light yellow column), close contacts (indicated by colored circular regions).

of 3,4-methylenedioxyphenyl group and that of Phe32 further stabilizes the binding. Moreover, the 4-methylphenyl group of **14i** can fully occupy the hydrophobic groove surrounded by Val24, Val27, Thr58 and Val59, and the benzyl side chain of **14i** with L-configuration just fits into the other groove formed by Val26, Glu29, Ala31 and Phe32. Figure 4B shows that besides the π–π interaction the binding between **14i** and HIV-1 CA was dominated by Van der Waals force, electrostatic and hydrophobic interactions. All of these reinforce the binding affinity, and could explain the high biological activity of compound **14i** theoretically.

In conclusion, a novel class of acylhydrazone derivatives was synthesized and assessed for their antiviral activities and cytotoxicities in vivo. The preliminary study shows that several compounds which contain a L-phenylalanine moiety and an electron-donating substituent in the left phenyl ring have good antiviral activities. Structural modification of compound **10c** produced a series of new analogues, of which compound **14f** and **14i** exhibited the most potent activities with IC_{50} values of 0.21 and 0.17 μM and TI values of above 476 and 588, respectively. The structure–activity relationships and molecular mechanisms of action of this novel class of antiviral agents will be further studied in our laboratory.

Acknowledgment

This project was supported by the National Natural Science Foundation of China (No. 30670415).

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11. *General procedure for the preparation of benzaldehyde N-(substituted benzoyl) amino acid hydrazones* **5(a–f)**. The appropriate N-(substituted benzoyl) amino acid methyl ester **3(a–f)** (20 mmol) was added in small portions to a stirred solution of 85% hydrazine hydrate (5 ml) in 10 ml ethanol. The mixture was heated under reflux for 6–8 h. while cooling to room temperature, the resulting precipitate was filtered in vacuo, washed with cold water and dried to give the corresponding hydrazide **4(a–f)**. To a magnetically suspension of the above hydrazide (0.5 mmol) in anhydrous methanol (2 ml) was added benzaldehyde (0.5 mmol) dropwise. Shortly the solution becomes homogeneous and within minutes the resulting hydrazone began to precipitate. After the mixture was stirred for further 1–2 h at room temperature, the precipitate was collected by filtration, washed with a small quantity of cold methanol and dried. Recrystallization of the reaction product from methanol gave the corresponding hydrazone **5(a–f)**. The analytical data of representative compounds **5a** and **5d** were as follows: *Compound* **5a:** mp: 195–196 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.82 (s, 3H), 3.96, 4.40 (2d, 2H, J = 5.7 Hz), 7.03 (d, 2H), 7.43–7.48 (m, 3H), 7.69–7.72 (m, 2H), 7.86–7.90 (m, 2H), 8.01, 8.24 (2s, 1H), 8.57, 8.75 (2t, 1H, J = 5.7 Hz), and 11.52 (s, 1H); EI-MS (m/z): 311 (M^+).*Compound* **5d:** mp: 234–236 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.03, 4.46 (2d, 2H, J = 6.0 Hz), 7.43–7.46 (m, 3H), 7.72 (t, 2H), 8.14 (d, 2H), 8.02, 8.24 (2s, 1H), 8.36 (d, 2H), 9.12, 9.28 (2t, 1H, J = 6.0 Hz), and 11.60 (s, 1H); EI-MS (m/z): 326 (M^+).
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14. Benzaldehyde N-(substituted benzenesulfonyl) amino acid hydrazones **10(a–i)** were prepared as described for benzaldehyde N-(substituted benzoyl) amino acid hydrazones **5(a–f)**, starting from N-(substituted benzenesulfonyl) amino acid methyl esters **8(a–i)**. The analytical data of representative compounds **10a**, **10d** and **10g** were as follows:*Compound* **10a**: mp: 158–159 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.37 (s, 1H), 3.54, 4.03 (2d, 2H, J = 5.7 Hz), 7.36–7.44 (m, 5H), 7.60–7.73 (m, 4H), 7.82, 8.02 (2t, 1H, J = 5.7 Hz), 7.93, 8.15 (2s, 1H), and 11.38, 11.46 (2s, 1H); EI-MS (m/z): 331 (M^+).*Compound* **10d**: mp: 195–

197 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.61, 4.10 (2d, 2H, J = 5.1 Hz), 7.42–7.44 (m, 3H), 7.61–7.83 (m, 6H), 7.93, 8.14 (2s, 1H, J = 5.1 Hz), 8.10, 8.26 (2t, 1H), and 11.42, 11.47 (2s, 1H); EI-MS (m/z): 397 (M+1$^+$), 395 (M−1$^+$).*Compound* **10g**: mp: 225–227 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.08 (s, 3H), 3.54, 4.03 (2d, 2H, J = 5.1 Hz), 7.42–7.44 (m, 4H), 7.61–7.76 (m, 6H), 7.93, 8.14 (2s, 1H, J = 5.1 Hz), 10.32 (s, 1H), and 11.39, 11.47 (2s, 1H); EI-MS (m/z): 374 (M$^+$).

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16. *General procedure for the preparation of 1-(substituted benzenesulfonyl)-3-phenyl-2-pyrazolines* **13(a–b)**. To a mixture of 14 ml of 85% hydrazine hydrate, 7.2 ml of 50% aqueous sodium hydroxide and 18 ml of methanol, a solution of of 3-dimethylaminopropiophenone hydrochloride (**11**) (20 mmol) in 70 ml of methanol was added over 10 min. After refluxing for 45 min, the methanol was distilled off at reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with water. Evaporation of the solvent gave the crude 3-phenyl-2-pyrazoline (**12**), which could be used without further purification. The crude 2-pyrazoline above obtained was dissolved in dry pyridine (20 ml). To the resulting solution was added in small portions the appropriate substituted benzenesulfonyl chloride (20 mmol). After the addition was complete, the reaction mixture was heated on a boiling water bath for 20 min, cooled and then poured onto crushed ice (30 g). The solid product separated was filtered, washed with water, dried and crystallized from ethanol. The analytical data of representative compound **13a** was as follows:*Compound* **13a**: mp: 178–180 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.15 (t, 2H, J = 9.6 Hz), 3.74 (t, 2H, J = 9.6 Hz), 7.26–7.45 (m, 3H), 7.66–7.69 (m, 2H), 8.13–8.16(m, 2H), and 8.35–8.38 (m, 2H); EI-MS (m/z): 331 (M$^+$).
17. The analytical data of representative compounds **14b** and **14k** were as follows:*Compound* **14b**: mp: 148–150 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2,23–2.36 (m, 6H), 2.71, 2.90 (2m, 2H), 3.96, 4.99 (2m, 1H), 7.15–7.34 (m, 9H), 7.41–7.55 (m, 4H), 7.82, 7.99 (2s, 1H), 8.15, 8.27 (2d, 1H, J = 8.7 Hz), and 11.26, 11.30 (2s, 1H); EI-MS (m/z): 435 (M$^+$).*Compound* **14k**: mp: 182–184 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.25, 2.31 (2s, 3H), 2.68, 2.90 (2m, 2H), 3.93, 4.87 (2m, 1H), 6.62–6.65 (m, 1H), 6.87 (t, 1H, J = 3.9 Hz), 7.14–7.21 (m, 7H), 7.37 (m, 1H), 7.48 (m, 1H), 7.78, 7.82 (2s, 1H), 7.90 (s, 1H), 8.11, 8.29 (2d, 1H, J = 9.0 Hz), and 11.28, 11.30 (2s, 1H); EI-MS (m/z): 411 (M$^+$).
18. (a) Palla, G.; Predieri, G.; Domiano, P. *Tetrahedron* **1986**, *42*(13), 3649; (b) Li, Y. J.; Wang, Y.; Jin, K.; Peng, Q. J.; Liu, S. N.; Zhang, Z. G. *Chin. J. Magn. Reson.* **2005**, *22*(1), 7.
19. (a) Chuang, A. J.; Killarn, K. F., Jr.; Chuang, R. Y.; Rice, W. G.; Schaeffer, C. A.; Mendeleyev, J.; Kun, E. *FEBS Lett.* **1993**, *326*, 140; (b) Antiviral inhibition assay: Inhibition of SIV-induced syncytium in CEM174 cell cultures was measured in a 96-well microplate containing 1×10^5 CEM cells/mL infected with 100 $TCID_{50}$ of SIV per well and containing appropriate dilutions of the tested compounds. After 5 days of incubation at 37 °C in 5% CO_2 containing humidified air, CEM giant (syncytium) cell formation was examined microscopically. The EC_{50} was defined as the compound concentration required to protect cells against the cytopathogenicity of SIV by 50%. The TC_{50} assay was performed in uninfected CEM174 cells under the same condition as above, and the value was determined as the concentration required to inhibit CEM cells proliferation by 50% in MTT reduction assay.
20. Ultraviolet spectrophotometry analysis: Ultraviolet spectrophotometry assay was performed at 350 nm on a Agilent 8453 spectrophotometer. A 1.0 μl of concentrated ligand in DMSO (1 mM) was added to a 75 μl aqueous solution (2 ml of 5 M NaCl mixed with 1 ml of 200 mM NaH_2PO_4, pH 8.0), and then added 25 μl capsid protein (40 μM) to initiate the reaction. Spectral measurements were made every 10 s, following a short initial delay to allow sample equilibration. Relative assembly rates were estimated from initial slopes of the plots of absorbance versus time.