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PII: S0968-0896(16)31442-0
DOI: http://dx.doi.org/10.1016/j.bmc.2016.12.035
Reference: BMC 13462

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 13 August 2016
Revised Date: 26 November 2016
Accepted Date: 16 December 2016

Please cite this article as: Sun, Y., Xu, W., Fan, N., Sun, X., Ning, X., Ma, L., Liu, J., Wang, X., Design, Synthesis and Biological Evaluation of *(E)*-3,4-Dihydroxystyryl 4-Acylaminophenethyl Sulfone, Sulfoxide Derivatives as Dual Inhibitors of HIV-1 CCR5 and Integrase, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.12.035

Design, Synthesis and Biological Evaluation of *(E)*-3,4-Dihydroxystyryl 4-Acylaminophenethyl Sulfone, Sulfoxide Derivatives as Dual Inhibitors of HIV-1 CCR5 and Integrase

Yixing Sun[a,‡], Weisi Xu[b,‡], Ningning Fan[a], Xuefeng Sun[a], Xianling Ning[a], Liying Ma[b,*], Junyi Liu[a,c,*] and Xiaowei Wang[a,*]

[a] Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China.

[b] National Center for AIDS/STD Control and Prevention (NCAIDS), Chinese Center for Disease Control and Prevention, Beijing 102206, China

[c] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China

* Corresponding authors. Tel.: +86 10 82801706 (J.L.); tel.: +86 10 82805203 (X.W.). E-mail addresses: jyliu@bjmu.edu.cn (J. Liu), xiaoweiwang@bjmu.edu.cn (X. Wang), mal@chinaaids.cn (L. Ma).

‡ These Authors contribute equally.

ABSTRACT: Aiming at the limited effectiveness of current clinical therapeutic effect of AIDS, novel series of compounds bearing *(E)*-3,4-dihydroxystyryl sulfone (or sulfoxide) and anilide fragments were designed and synthesized as dual inhibitors of HIV-1 CCR5/IN. The biological results indicated that several target compounds showed inhibitory activity against HIV-1 Bal (R5) infection in TZM-bl cells. Besides targeting the chemokine receptor on the host cell surface, they also displayed binding affinities with HIV-1 integrase using the surface plasmon resonance (SPR) binding assays. Molecular docking studies have inferred the possible binding mode of target compounds against integrase. These data demonstrate that the structure of *(E)*-3,4-dihydroxystyryl sulfone and sulfoxide derivatives have the potential to derive potent dual inhibitors of HIV-1 Integrase and CCR5.

KEYWORDS: HIV dual inhibitors; CCR5 receptor; HIV integrase; Sulfones; Sulfoxides.

1. Introduction

In early stages of the development of highly active anti-retroviral therapy (HAART, a.k.a. “drug cocktails”), the treatments of HIV were limited to the combination therapies of reverse transcriptase (RT) inhibitors and protease inhibitors. With the application of integrase (IN) inhibitors, fusion inhibitors and co-receptor inhibitors, not only the selection scope of HAART has been widened, but the treatment outcome has also been improved.[1] Nevertheless, the poor patient compliance, the risk of drug interactions, and the emergence of resistant HIV strains brought by HAART have promoted to discovering new strategy against HIV, which include designing multi-targeted inhibitors.[2,3]

In order to block the HIV replication cycle at different stages, we planned to develop dual inhibitors of HIV-1 CCR5 and integrase. During the integration process in HIV life cycle, chemokine receptors CCR5, along with CXCR4, are required for virus’ membrane fusion with host cell.[4] In particular, the CCR5 has a main mediating role in the initial infection of HIV.[5] Also, with the action of integrase, cDNA strands assemble the host genomic DNA into a double-stranded DNA

to complete replication.[6] Additionally, the integrase serves as a feasible intracellular target since it hasn't been found in mammalian cells.[7] Accordingly, targeting both the integrase and CCR5 may provide low toxicity as well as great efficiency and timeliness.

Recent studies in the crystal structure of the CCR5-maraviroc complex have demonstrated the multiple forces between the receptor and the ligand.[8] These include a salt bridge interaction between the protonated tropane nitrogen and E283, several hydrogen bond forces including amide nitrogen to Y251, as well as the hydrophobic contacts between the phenyl group with the aromatic amino acid residues. In the meantime, previous crystallography studies have demonstrated the binding mode of raltegravir (RAL) with the Prototype Foamy Virus IN. The di-carbonyl moiety of diketo acids (DKAs) changed the conformation of IN by coordinating to the divalent ions in the active domain of the protein.[9,10]

Caffeic acid phenethyl ester (CAPE) has also been reported to inhibit the process of integration.[11,12] As shown in SAR studies, diphenol and carbonyl units are necessary to maintain the IN inhibitory activity.[13,14] Due to the susceptibility of carboxylic ester to hydrolase, bioisosteric replacement took place and sulfone (or sulfoxide) group was brought in to form the structure. Sulfone and sulfoxide groups are not only more stable than ester groups, but also more soluble in water. Moreover, Smithkline Beecham Corp. disclosed various benzanilides to acquire CCR5 inhibitory properties through generating corresponding hydrogen bonds and hydrophobic interactions.[15,16] With these evidences in mind, we highly integrated the structures of sulfone and sulfoxide derivatives of CAPE and aroyl aniline moiety which could acquire CCR5 inhibitory properties through generating corresponding hydrogen bonds and hydrophobic interactions, and formed new series of target compounds (Fig. 1).

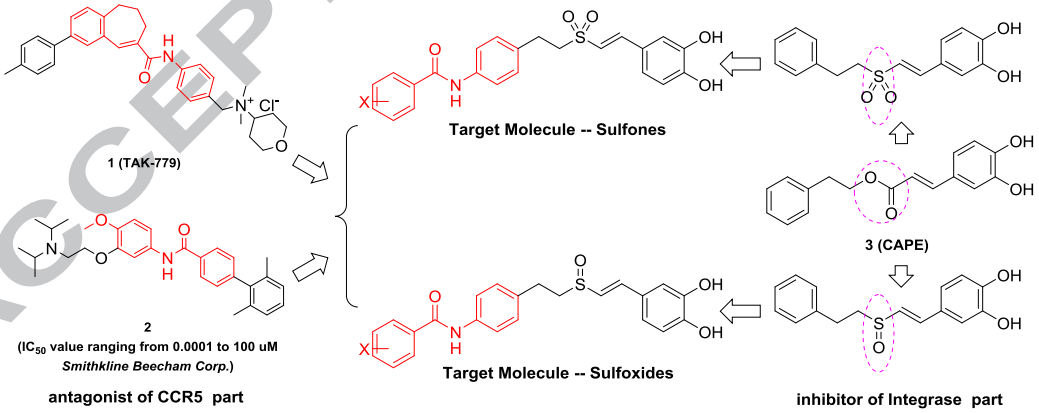


Figure 1. Design of dual inhibitors based on derivatives of CAPE and potent CCR5 antagonists.

Herein, we proposed to synthesize the target compounds to probe their antivirus activity against R5 HIV-1 strains and IN binding affinity. These results might provide useful information for developing novel dual inhibitors of HIV-1 IN and CCR5.

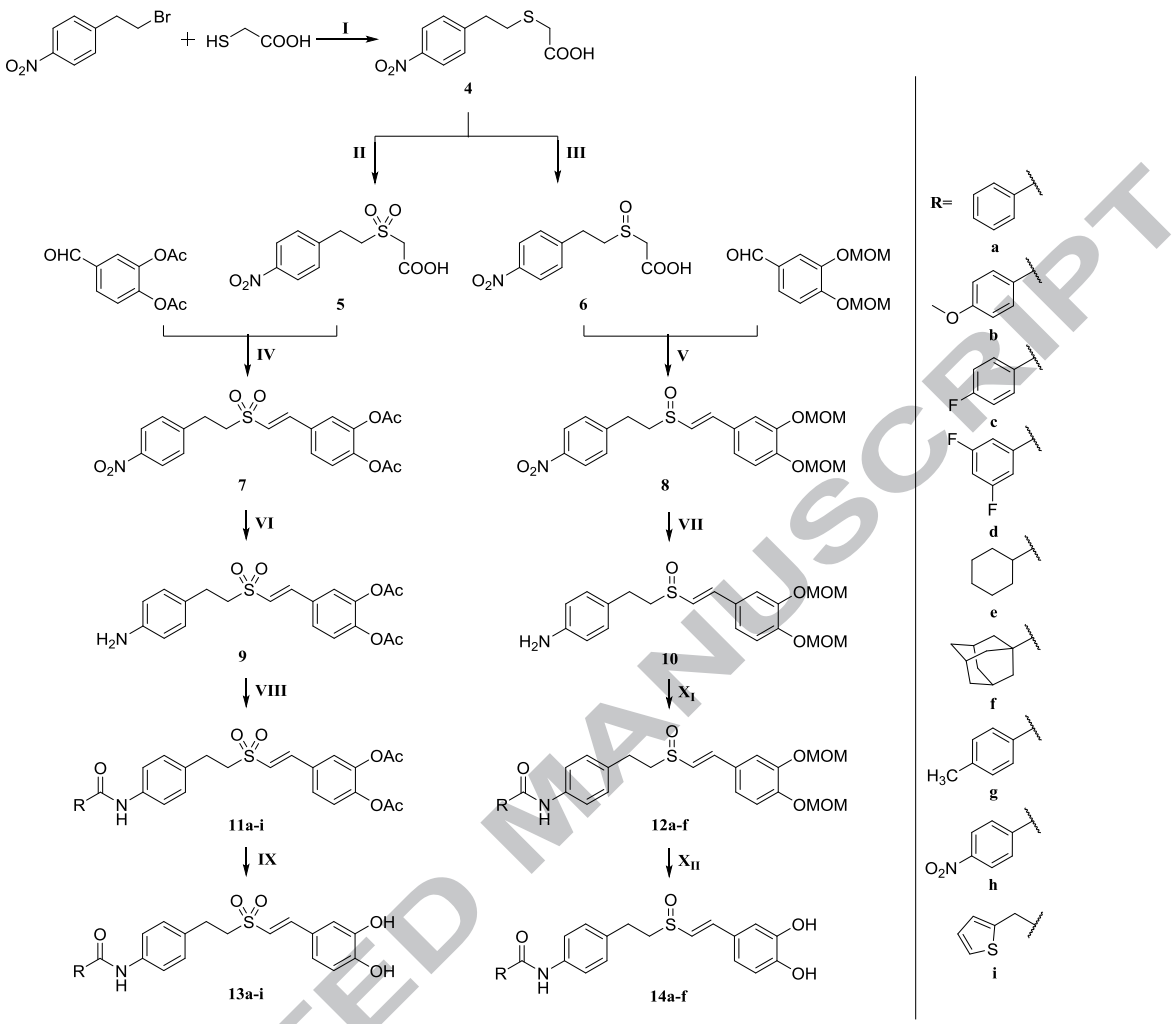
2. Results and discussion

2.1. Chemistry

For the preparation of the sulfone and sulfoxide target compounds, the synthetic routes (shown in Scheme 1) were explored respectively. The synthesis of **4**, **5**, and **6** were based on our previous report.[17] Commercially available 4-nitrophenethyl bromide was reacted with mercaptoacetic acid to obtain compound **4**, which was then oxidized with H_2O_2 in different solvents to afford **5** and **6** respectively.

In the presence of β-aminopropionic acid as catalyst, the sulfonyl intermediate **7** was obtained with high yield through Knoevenagel condensation of compound **5** with acetyl protected 3,4-dihydroxybenzaldehyde. However, pyrrolidine and HOAc was used as catalysts to give the sulfinyl intermediate **8**. The change of the Knoevenagel reaction condition is due to the decreased reactivity of the methylene structure.

Following the Knoevenagel condensation reaction, the nitro group of **7** was selectively reduced with palladium-catalyzed hydrogenation to afford the aromatic amine **9**. Due to the decrease of Pd/C catalytic activity that caused by the existence of the sulfoxide group, sodium hydrosulfite was used as reductant to obtain the intermediate **10**. Reaction of **9** and **10** with various acid chlorides gave the corresponding compounds **11a-i** and **12a-f.** Final deprotections of the acetyl and MOM groups gave the target compounds **13a-i** and **14a-f**, respectively.



Scheme 1. Synthetic routes of target compounds.

Reagents & conditions: (I) NaOH, MeOH, r.t., 2h, yields 80-93%; (II) 30%H_2O_2, HOAc, r.t., yields 74-97%; (III) 30%H_2O_2, MeOH, r.t., yields 83-95%; (IV) β-aminopropionic acid, THF, 4Å MS, reflux, 12h, yield 67-80%; (V) pyrrolidine, HOAc, THF, 4Å MS, reflux, 12h, yield 51-71%; (VI) H_2, Pd/C, CH_2Cl_2, r.t., 3h, yield 64-82%; (VII) $Na_2S_2O_4$, EtOH, H_2O, reflux, 12h, yield 25-45%; (VIII) RCOCl, Et_3N, CH_2Cl_2, ice bath, 2h, 16-75%; (IX) K_2CO_3, MeOH, r.t., 3h, yield 56-65%; (X_I,X_{II}) RCOCl, Et_3N, CH_2Cl_2, ice bath, 2h; HCl, MeOH, reflux, 3h, total yield 10-23%.

2.2. Biological results

2.2.1. Determination of the cytotoxicity and antivirus activity against R5 HIV-1 strains

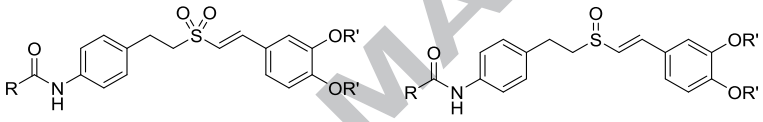
All the target compounds were sequentially tested for their cytotoxicity and their antivirus activity against R5 HIV-1 strains in TZM-bl cell line. For the *in vitro* entry inhibition assays, HIV-1 Bal (R5) virus strain was used to determine the type of chemokine receptors that mediate virus' entry.

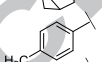
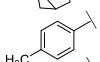
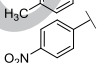
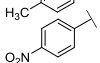
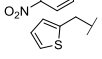
The TZM-bl cell line was selected to complete the cell based assays. Reporter genes such as firefly luciferase gene, which is promoted by HIV long terminal repeat sequence (LTR), and *E. coli*

β-galactosidase gene are carried and receptors including CCR5 were expressed simultaneously by this cell line. The intracellular viral load can therefore be tested by a fluorescence detector after the virus' entrance to the cell. Due to the specific mediation of CCR5 during the virus' entrance, HIV-1 Bal (R5) virus strain was used for measuring the CCR5 inhibitory activity of the target compounds.

All target compounds were tested for their cytotoxicity before testing for cell based anti-HIV activity. As shown in Table 1, the CC_{50} values of most active compounds are above 100μM. Meanwhile, the sulfone and sulfoxide derivatives at 50μM concentration displayed antiviral activity against R5 HIV-1 strains at different levels. Five compounds, including four sulfoxide derivative, showed at least 50% inhibition of HIV-1 replication in TZM-bl cells. The preliminary biological results suggested that sulfoxides showed better activity than sulfones. Besides, the difference of R substitution may affect the CCR5 inhibitory activity. Herein, 3,5-difluorophenyl group, cyclohexyl group, 4-nitrophenyl group, 1-adamantaneformyl group, and 4-fluorophenyl group are the more favored substituent groups.

Table 1. Inhibition of HIV-1 entry through CCR5 co-receptor as analyzed in TZM-bl cells using HIV-1 Bal (R5) virus



Compound	R	R'	Series	% Inhibition[a]	CC_{50}[b] (μM)	Compound	R	R'	Series	% Inhibition[a]	CC_{50}[b] (μM)
11a		Ac	Sulfone	<20	108	**13a**		H	Sulfone	<20	238
11b		Ac	Sulfone	<20	858	**13b**		H	Sulfone	<20	>1000
11c		Ac	Sulfone	26.2	162	**13c**		H	Sulfone	<20	>1000
11d		Ac	Sulfone	<20	225	**13d**		H	Sulfone	33.0	414
11e		Ac	Sulfone	<20	169	**13e**		H	Sulfone	45.9	638
11f		Ac	Sulfone	23.7	182	**13f**		H	Sulfone	21.6	786
11g		Ac	Sulfone	<20	177	**13g**		H	Sulfone	<20	168
11h		Ac	Sulfone	52.2	160	**13h**		H	Sulfone	23.4	483
11i		Ac	Sulfone	23.0	161	**13i**		H	Sulfone	<20	569
14a		H	Sulfoxide	68.4	157	**14d**		H	Sulfoxide	41.8	ND
14b		H	Sulfoxide	34.1	ND	**14e**		H	Sulfoxide	52.7	ND
14c		H	Sulfoxide	64.6	127	**14f**		H	Sulfoxide	64.7	ND
Maraviroc	--	--	--	IC_{50}=0.2nM	>1000						

ND = Not determined.

[a] % Decrease in fluorescent intensity in TZM-bl cells; data represent mean values for two wells.

[b] CC_{50} = concentration of compound required for 50% cytotoxicity against uninfected cells.

2.2.2. Assays for measuring the *in vitro* binding affinity of target compounds against HIV-1 IN

Having determined the antivirus activity against R5 HIV-1 strains, we carried out assays for the second target. In order to investigate the binding affinity of target compounds against HIV-1 IN, we used SPR based Biacore T200 biosensor for evaluation. The commercially available recombinant IN protein was immobilized on a CM5 chip and the binding response was recorded continuously in the response unit (RU) and graphically presented as a function of time. Preliminary screening of the 24 compounds showed that compound **11a, 13a, 13e,** and **14a** have better binding results (shown in Fig. 2). Then the dose-dependent relations was tested with various concentrations for the four compounds (200μM, 100μM, 50μM, 25μM, 12.5μM, etc.). To determine the equilibrium dissociation constant (K_D), the 1:1 binding fit model (kinetics) was used. According to the affinity estimates, the K_D value of compound **14a** was 141.8μM. As shown in Table 2, these SPR results determined that the *(E)*-3,4-dihydroxystyryl sulfone and sulfoxide derivatives have binding affinity against HIV-1 integrase.

Table 2. K_D values of the 4 representative compounds

Compound	**11a**	**13a**	**13e**	**14a**
K_D(μM)	207	160	115	142

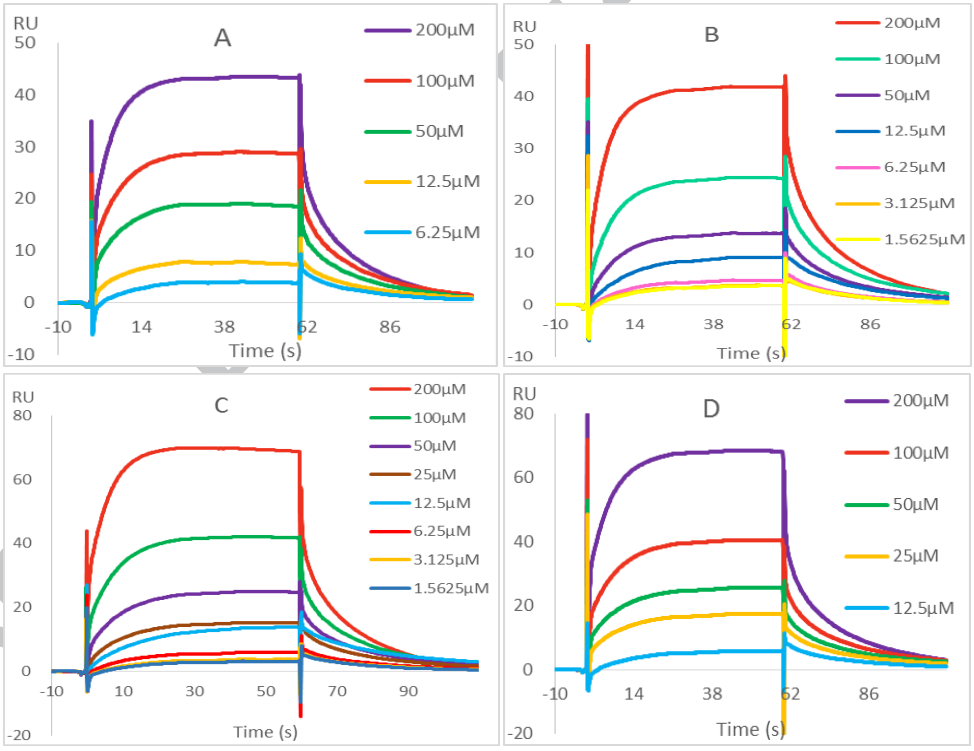


Figure 2. HIV-1 IN binding affinity using SPR biosensor technology. Representative sensorgram of **11a**(A), **13a**(B), **13e**(C), **14a**(D) were obtained.

It is noteworthy that compound **14a** demonstrate potency activity against R5 HIV-1 strains with relatively low toxicity to TZM-bl cells and moderate binding affinity with IN (K_D = 141.8μM). Therefore, the preliminary results suggested that target compound **14a** could be potent dual inhibitor of HIV-1 CCR5/IN.

2.3. Molecular docking

To assess the dual inhibitory properties of the target compounds, computational docking studies were carried out to explore the binding mode of the target compounds with active sites of CCR5 and prototype foamy virus (PFV) IN respectively.

Firstly, in docking studies with CCR5 (Fig. 3) (PBD: 4MBS), the results revealed that H bonds were observed between the phenolic oxygen and Tyr37, as well as amide nitrogen and Glu283. The bonding interactions have significant similarities with results from CCR5-maraviroc complex studies which are supposed to assist the molecule's positioning in the binding pocket,[7] and are recognized as the key points of exhibiting CCR5 inhibitory activity. The phenyl rings of inhibitors imparted π-π stacking with the CCR5 active site residues (Trp86, Tyr108, Phe109, Phe112, etc.). Additionally, hydrophobic interactions were developed by the positioning of aromatic rings into the hydrophobic pockets comprised by aromatic residues Tyr108, Phe109, Phe112, Trp248, and Tyr251.

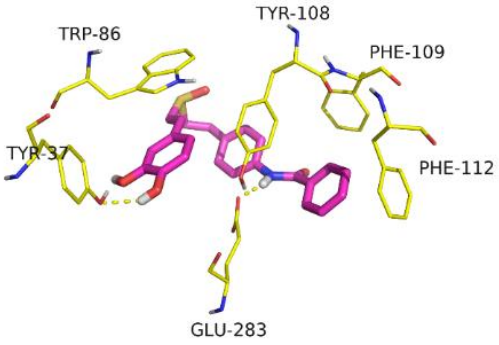


Figure 3. Molecular docking result of compound **14a** into HIV CCR5 (PBD: 4MBS). Hydrogen bonds were signed in yellow dotted line.

To further explore the potential of IN inhibitory activity of these compounds, docking works were performed using the PFV IN core domain (Fig. 4). Compound **13e** exhibited H bonding between one of the phenolic oxygen and the side chain of Glu221 residue, as well as coordination between the catechol oxygens and metal ion Mg^{2+} that results in the chelation. Meanwhile, in docking results of compound **14a**, Mg^{2+} metal ions coordinate with sulfinyl oxygens while H bonds are generated between acyl oxygen and Tyr212.

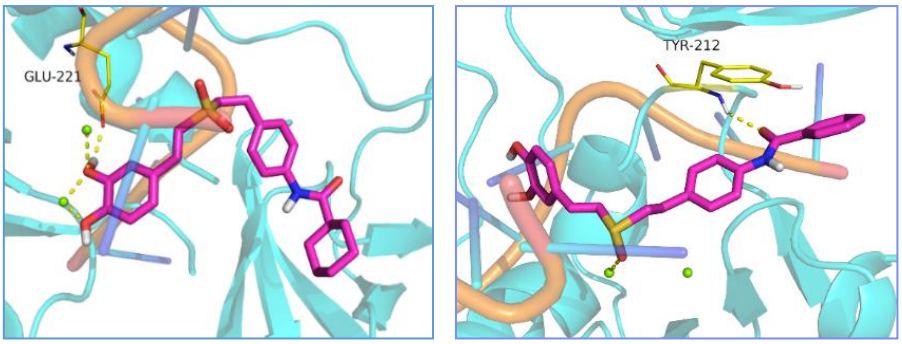


Figure 4. Molecular docking of compound **13e** (left), and **14a** (right) into PFV IN (PBD: 3OYA). The balls colored in green represents Mg^{2+} ions.

Therefore, the binding modes of the target compounds against HIV CCR5 and PFV IN were preliminarily revealed, laying a foundation for further development of these structures.

3. Conclusion

To sum up, series of *(E)*-3,4-dihydroxystyryl 4-acylaminophenethyl sulfones and sulfoxides were synthesized and tested for their *in vitro* HIV-1 cellular CCR5 inhibitory activity (close to 68.37% at 50μM) and binding affinity against HIV-1 integrase (K_D at around 10^{-4} mole) . The two independent mechanism enable the target compounds to block the entry and integration stages respectively, and it would possibly reduce the generation of drug resistant viruses. Taken the molecular docking results together, our study identified that the *(E)*-3,4-dihydroxystyryl sulfone and sulfoxide derivatives could derive novel compounds that display dual inhibition activities for HIV-1 virus.

4. Experimental procedures

4.1. Chemistry

4.1.1. General chemistry methods

The structural characterizations of compounds were performed by NMR, which were recorded on Bruker AVANCE III-400 spectrometer (400MHz) with TMS (tetramethylsilane, Me_4Si) as an internal standard. Chemical shifts were reported in δ (ppm) while coupling constants were measured in Hz. $CDCl_3$ and DMSO-d_6 were used as the solvents for NMR experiments. The purity of target compounds was determined by HPLC (Agilent 1260 infinity HPLC system). All the assayed compounds showed >95% purity by HPLC. All reactions were monitored by TLC, which was performed on precoated silica gel GF254 plates. The detection of TLC was done by UV light irradiation (UV lamp, model UV-IIB). Column chromatography was performed by silica gel H (200-300 or 500 mesh). Melting points were measured on an X-4 apparatus without correction. THF were purified and dried by standard methods. All other organic solutions were dried over anhydrous sodium sulfate. All other reagents were purchased as reagent grade from commercial sources and without further purification unless otherwise stated.

4.1.2. Synthesis of *(E)*-3,4-diacetoxystyryl 4-nitrophenethyl sulfone (7)

To a solution of 2-(4-nitrophenethylsulfonyl) acetic acid (**5**, 3mmol) in THF (15mL), 3,4-diacetoxybenzaldehyde (2mmol) and *β*-aminopropionic acid (1mmol) were added and the resulting solution was heated to reflux until absence of 3,4-diacetoxybenzaldehyde (checked by TLC). The solvent was evaporated under vacuum and the mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and concentrated in vacuo. Purification of the crude product by column chromatography (PE/EA) afforded the title compound **7**. Yield: 80%; Yellow solid; mp 86–87 ºC; ^{1}H

NMR (400MHz, $CDCl_3$) δ 8.17 (d, 2H, J = 8.5 Hz), 7.55 (d, 1H, J = 15.4 Hz), 7.27-7.41 (m, 5H), 6.72 (d, 1H, J = 15.4 Hz), 3.38 (m, 2H), 3.25 (m, 2H), 2.32 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 168.9, 168.7, 145.4, 145.1, 143.9, 143.2, 133.7, 132.5, 130.1, 130.1, 127.2, 124.3, 124.1, 123.9, 123.9, 122.8, 56.0, 28.3, 20.5, 20.5; MS (ESI) *m/z* 456.05 $[M+Na]^+$.

4.1.3. Synthesis of *(E)*-1,2-bis(methoxymethoxy)-4-(2-((4-nitrophenethyl)sulfinyl)vinyl) benzene (8)

To a solution of 2-(4-nitrophenethylsulfinyl) acetic acid (**6**, 3mmol) in THF (15mL), 3,4-methoxymethoxybenzaldehyde (2mmol), pyrrolidine (catalytic amount), and acetic acid (catalytic amount) were added and the resulting solution was heated to reflux until absence of 3,4-methoxymethoxybenzaldehyde (checked by TLC). The solvent was evaporated under vacuum and the mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and concentrated in vacuo. Purification of the crude product by column chromatography (PE/EA) afforded the title compound **8**. Yield: 71%; Yellow solid; mp 155–156 ºC; ^{1}H NMR (400MHz, $CDCl_3$) δ 8.16 (d, 2H, J = 8.7 Hz), 7.51 (d, 1H, J = 15.4 Hz), 7.19-7.40 (m, 5H), 6.62 (d, 1H, J = 15.4 Hz), 5.27 (d, 4H, J = 13.2 Hz), 3.53 (d, 6H, J = 6.6 Hz), 3.23-3.39 (m, 4H); ^{13}C NMR (100MHz, DMSO-d_6) δ 149.0, 146.8, 145.3, 145.0, 133.6, 132.4, 130.1, 130.1, 124.2, 123.9, 123.9, 123.3, 111.3, 108.1, 96.1, 96.1, 54.1, 53.9, 51.0, 34.3; MS (ESI) *m/z* 422.11 $[M+H]^+$ 444.11 $[M+Na]^+$.

4.1.4. Synthesis of *(E)*-3,4-diacetoxystyryl 4-aminophenethyl sulfone (9)

To a solution of *(E)*-3,4-diacetoxystyryl 4-nitrophenethyl sulfone (**7**, 0.5mmol) in dichloromethane (20mL), Palladium/C (20mg) were added and the resulting solution was allowed to stir under hydrogen gas for 3h at room temperature. The solvent was evaporated under vacuum and the mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and concentrated in vacuo. The crude product of **9** was washed with petroleum ether. Yield: 82%; Yellow oil; ^{1}H NMR (400MHz, $CDCl_3$) δ 7.46 (d, 1H, J = 15.4 Hz), 7.35 (d, 1H, J = 1.6 Hz), 7.22 (d, 1H, J = 15.4 Hz), 6.48-7.19 (m, 6H), 4.93 (s, 2H), 3.29 (m, 2H), 2.80 (m, 2H), 2.32 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 168.7, 168.7, 145.6, 143.7, 143.2, 133.7, 132.4, 129.3, 129.0, 129.0, 124.3, 124.1, 122.8, 115.3, 115.3, 55.7, 28.3, 20.6, 20.5; HRMS (ESI) *m/z* calcd for $C_{20}H_{21}NNaO_6S$ $[M+Na]^+$: 426.0987, found: 426.0988.

4.1.5. Synthesis of *(E)*-4-(2-((3,4-bis(methoxymethoxy)styryl)sulfinyl)ethyl)aniline (10)

To a solution of *(E)*-1,2-bis(methoxymethoxy)-4-(2-((4-nitrophenethyl)sulfinyl)vinyl)benzene (**8**, 0.5mmol) in ethanol (with 16.7% water, 20mL), sodium hydrosulfite (8mmol) were added several times and the resulting solution was heated to reflux until absence of compound **8** (checked by TLC). The solvent was evaporated under vacuum and the mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and concentrated in vacuo. The crude product of **10** was washed with petroleum ether. Yield: 45%;

Yellow oil; ^{1}H NMR (400MHz, $CDCl_3$) δ 7.48 (d, 1H, J = 15.4 Hz), 6.63-7.29 (m, 7H), 6.57 (d, 1H, J = 15.4 Hz), 5.27 (d, 4H, J = 4.4 Hz), 4.84 (s, 2H), 3.53 (s, 6H), 3.01-3.31 (m, 4H); ^{13}C NMR (100MHz, DMSO-d_6) δ 149.1, 146.9, 145.3, 134.0, 129.8, 128.9, 128.9, 128.8, 122.2, 121.3, 115.4, 115.4, 111.5, 108.2, 96.5, 96.3, 54.1, 54.0, 51.2, 34.1; MS (ESI) *m/z* 392.16 $[M+H]^+$.

4.1.6. General synthesis of *(E)*-3,4-diacetoxystyryl 4-acylaminophenethyl sulfones (11a – 11i)

To a solution of *(E)*-3,4-diacetoxystyryl 4-aminophenethyl sulfone (**9**, 0.1mmol) in dichloromethane (10mL), trimethylamine (0.2mmol), and various acid chlorides (0.22mmol) were added under the ice bath. The resulting solution was allowed to stir for 2h. The solvent was evaporated under vacuum and the mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and concentrated in vacuo. Purification of the crude product by column chromatography (PE/EA) afforded the target compounds **11a** to **11i**.

4.1.6.1. *(E)*-3,4-diacetoxystyryl 4-benzamidophenethyl sulfone (11a) 75%; Yellow solid; mp 92–93 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.21 (s, 1H), 6.79-7.95 (m, 14H), 3.42-3.46 (m, 2H), 2.94-2.98 (m, 2H), 2.33 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 168.6, 168.4, 165.2, 144.3, 142.7, 141.6, 138.2, 133.7, 133.3, 131.9, 131.7, 129.9, 129.9, 129.2, 129.2, 129.0, 129.0, 127.3, 124.7, 124.0, 120.8, 120.8, 114.9, 55.8, 28.1, 20.7, 20.6; MS (ESI) *m/z* 530.31 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{25}NNaO_7S$ $[M+Na]^+$: 530.1249, found: 530.1251.

4.1.6.2. *(E)*-3,4-diacetoxystyryl 4-4-methoxylbenzamidophenethyl sulfone (11b) 49%; Buff white solid; mp 106–107 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.04 (s, 1H), 7.06-7.95 (m, 13H), 3.84 (s, 3H), 3.49 (m, 2H), 2.98 (m, 2H), 2.30 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 168.6, 168.5, 165.3, 162.3, 144.4, 142.8, 141.7, 138.3, 133.4, 131.8, 130.0, 130.0, 129.1, 129.1, 128.0, 127.4, 124.8, 124.1, 120.9, 120.9, 115.0, 114.0, 114.0, 55.9, 55.3, 28.1, 20.8, 20.7; MS (ESI) *m/z* 560.21 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{28}H_{27}NNaO_8S$ $[M+Na]^+$: 560.1355, found: 560.1356.

4.1.6.3. *(E)*-3,4-diacetoxystyryl 4-4-fluorobenzamidophenethyl sulfone (11c) 39%; Buff white solid; mp 105–106 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.10 (s, 1H), 6.80-8.12 (m, 13H), 3.42 (m, 2H), 2.94 (m, 2H), 2.33 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 168.8, 168.6, 165.4, 144.7, 142.9, 141.8, 141.2, 138.2, 133.5, 131.9, 131.8, 130.1, 130.1, 129.8, 129.8, 129.2, 129.2, 127.2, 124.9, 124.2, 120.8, 120.8, 115.1, 56.0, 28.2, 21.0, 20.8; MS (ESI) *m/z* 548.30 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{24}FNNaO_7S$ $[M+Na]^+$: 548.1155, found: 548.1152.

4.1.6.4. *(E)*-3,4-diacetoxystyryl 4-3.5-difluorobenzamidophenethyl sulfone (11d) 36%; Yellow oil; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.14 (s, 1H), 6.83-7.79 (m, 12H), 3.44 (m, 2H), 2.96 (m, 2H), 2.33 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 164.7, 161.0, 161.0, 146.5, 145.9, 137.4, 135.1, 135.0, 134.5, 129.2, 127.9, 127.9, 124.2, 123.2, 121.5, 121.5, 117.2, 115.2, 110.1, 110.1, 108.1, 60.7,

27.5, 20.9, 20.8; MS (ESI) *m/z* 566.08 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{23}F_2NNaO_7S$ $[M+Na]^+$: 566.1061, found: 566.1058.

4.1.6.5. *(E)*-3,4-diacetoxystyryl 4-cyclohexylamidophenethyl sulfone (11e) 47%; Buff white solid; mp 98–99 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.12 (s, 1H), 6.74-7.85 (m, 9H), 3.44 (m, 2H), 2.95 (m, 2H), 2.29 (s, 6H), 1.20-1.76 (m, 11H); ^{13}C NMR (100MHz, DMSO-d_6) δ 174.6, 149.3, 146.1, 143.9, 138.4, 130.4, 130.0, 130.0, 124.3, 125.9, 125.9, 122.5, 119.6, 119.6, 116.2, 115.7, 55.5, 45.3, 29.6, 29.6, 28.2, 25.9, 25.8, 25.7; MS (ESI) *m/z* 536.22 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{31}NNaO_7S$ $[M+Na]^+$: 536.1719, found: 536.1718.

4.1.6.6. *(E)*-3,4-diacetoxystyryl 4-1-adamantaneformylamidophenethyl sulfone (11f) 18%; Yellow solid; mp 104–105 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.04 (s, 1H), 6.66-8.14 (m, 9H), 3.54 (m, 2H), 3.12 (m, 2H), 2.14 (s, 6H), 1.60-2.00 (m, 15H); ^{13}C NMR (100MHz, DMSO-d_6) δ 174.6, 169.3, 169.3, 149.3, 146.1, 143.9, 148.4, 132.9, 129.2, 129.1, 129.1, 124.3, 122.5, 122.4, 119.6, 119.6, 119.5, 116.2, 115.8, 55.6, 45.3, 29.6, 29.6, 29.6, 28.2, 25.9, 25.7, 25.7, 25.7, 24.7, 24.7, 24.7; MS (ESI) *m/z* 566.24 $[M+H]^+$ 588.17 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{31}H_{35}NNaO_7S$ $[M+Na]^+$: 588.2032, found: 588.2039.

4.1.6.7. *(E)*-3,4-diacetoxystyryl 4-4-methylbenzamidophenethyl sulfone (11g) 25%; White solid; mp 108–109 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.12 (s, 1H), 6.78-7.96 (m, 13H), 3.44 (m, 2H), 2.95 (m, 2H), 2.43 (s, 3H), 2.34 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 168.8, 168.6, 165.4, 144.5, 142.9, 141.8, 141.2, 138.4, 133.5, 131.9, 131.8, 130.1, 130.1, 129.4, 129.4, 129.2, 129.2, 127.5, 124.9, 124.2, 121.0, 121.0, 115.1, 56.0, 28.3, 23.5, 20.9, 20.8; MS (ESI) *m/z* 544.11 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{28}H_{27}NNaO_7S$ $[M+Na]^+$: 544.1406, found: 544.1399.

4.1.6.8. *(E)*-3,4-diacetoxystyryl 4-4-nitrobenzamidophenethyl sulfone (11h) 35%; Yellow solid; mp 126–127 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.21 (s, 1H), 6.80-7.95 (m, 13H), 3.43-3.46 (m, 2H), 2.94-2.98 (m, 2H), 2.33 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 168.6, 168.4, 165.7, 144.0, 142.7, 141.6, 138.2, 133.7, 133.3, 131.9, 131.6, 129.9, 129.9, 129.3, 129.3, 129.0, 129.0, 127.3, 124.7, 124.0, 120.8, 120.8, 114.9, 55.8, 28.1, 20.7, 20.6; MS (ESI) *m/z* 575.29 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{27}H_{24}N_2NaO_9S$ $[M+Na]^+$: 575.1100, found: 575.1101.

4.1.6.9. *(E)*-3,4-diacetoxystyryl 4-2-thiopheneacetylamidophenethyl sulfone (11i) 16%; Yellow brown solid; mp 92–93 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 7.00-8.03 (m, 12H), 6.80 (s, 1H), 3.48 (s, 2H), 3.43 (m, 2H), 2.95 (m, 2H), 2.30 (s, 6H); ^{13}C NMR (100MHz, DMSO-d_6) δ 169.5, 168.5, 168.5, 149.3, 146.1, 143.9, 140.2, 138.4, 135.9, 133.3, 130.1, 129.2, 129.2, 129.0, 128.4, 127.3, 124.7, 124.2, 120.8, 120.8, 113.5, 55.4, 45.5, 28.0, 20.7, 20.5; MS (ESI) *m/z* 550.18 $[M+Na]^+$; HRMS (ESI) *m/z* calcd for $C_{26}H_{25}NNaO_7S_2$ $[M+Na]^+$: 550.0970, found: 550.0968.

4.1.7. General synthesis of *(E)*-3,4-dihydroxystyryl 4-acylaminophenethyl sulfones (13a – 13i)

To a solution of *(E)*-3,4-diacetoxystyryl 4-acylaminophenethyl sulfones (**11a** – **11i**, 0.06mmol) in methanol (10mL), potassium carbonate (0.18mmol) was added and the resulting solution was allowed to stir for 3h at room temperature. The solvent was evaporated under vacuum and the mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and concentrated in vacuo. Purification of the crude product by column chromatography (PE/EA) afforded the target compounds **13a** to **13i**.

4.1.7.1. *(E)*-3,4-dihydroxystyryl 4-benzamidophenethyl sulfone (13a) 65%; White solid; mp 104–105 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.12 (s, 1H), 9.72 (s, 1H), 9.21 (s, 1H), 6.80-7.94 (m, 14H), 3.44 (m, 2H), 2.96 (m, 2H); ^{13}C NMR (100MHz, DMSO-d_6) δ 165.9, 149.4, 146.1, 144.0, 138.1, 135.4, 133.8, 132.0, 129.1, 129.1, 128.8, 128.8, 128.1, 128.1, 124.4, 122.5, 122.5, 120.9, 120.9, 116.2, 115.8, 55.5, 28.2; MS (ESI) *m/z* 422.25 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{20}NO_5S$ [M-H]$^-$: 422.1062, found: 422.1062.

4.1.7.2. *(E)*-3,4-dihydroxystyryl 4-4-methoxylbenzamidophenethyl sulfone (13b) 58%; Buff white solid; mp 108–109 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.20 (s, 1H), 9.71 (s, 1H), 9.22 (s, 1H), 6.81-7.96 (m, 13H), 3.81 (s, 3H), 3.43 (m, 2H), 2.95 (m, 2H); ^{13}C NMR (100MHz, DMSO-d_6) δ 165.3, 162.3, 149.3, 146.1, 144.0, 138.3, 133.6, 130.4, 130.0, 120.0, 129.1, 129.1, 127.4, 123.9, 122.5, 120.9, 120.9, 116.2, 115.7, 114.1, 114.1, 55.9, 55.5, 28.2; MS (ESI) *m/z* 452.10 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{24}H_{22}NO_6S$ [M+Na]$^+$: 452.1168, found: 452.1166.

4.1.7.3. *(E)*-3,4-dihydroxystyryl 4-4-fluorobenzamidophenethyl sulfone (13c) 61%; Buff white oil; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.21 (s, 1H), 9.71 (s, 1H), 9.20 (s, 1H), 6.79-8.12 (m, 13H), 3.43 (m, 2H), 2.97 (m, 2H); ^{13}C NMR (100MHz, DMSO-d_6) δ 164.7, 149.5, 146.5, 144.5, 138.0, 135.5, 134.2, 132.1, 129.2, 129.2, 128.8, 128.8, 124.8, 123.2, 121.5, 121.5, 115.5, 115.2, 55.7, 28.5; MS (ESI) *m/z* 440.26 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}FNO_5S$ [M-H]$^-$: 440.0968, found: 440.0972.

4.1.7.4. *(E)*-3,4-dihydroxystyryl 4-3.5-difluorobenzamidophenethyl sulfone (13d) 56%; Yellow oil; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.14 (s, 1H), 9.71 (s, 1H), 9.22 (s, 1H), 6.83-7.79 (m, 12H), 3.44 (m, 2H), 2.96 (m, 2H); ^{13}C NMR (100MHz, DMSO-d_6) δ 164.7, 161.0, 161.0, 146.5, 145.9, 137.4, 135.1, 135.0, 134.5, 129.2, 127.9, 127.9, 124.2, 123.2, 121.5, 121.5, 117.2, 115.2, 110.1, 110.1, 108.1, 60.7, 27.5; MS (ESI) *m/z* 458.21 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{18}F_2NO_5S$ [M-H]$^-$: 458.0874, found: 458.0878.

4.1.7.5. *(E)*-3,4-dihydroxystyryl 4-cyclohexylamidophenethyl sulfone (13e) 65%; White solid; mp 83–84 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.22 (s, 1H), 9.72 (s, 1H), 9.22 (s, 1H), 6.80-7.80 (m, 9H), 3.56 (m, 2H), 2.92 (m, 2H), 1.19-2.32 (m, 11H); ^{13}C NMR (100MHz, DMSO-d_6) δ 174.7, 149.3, 146.1, 143.9, 138.4, 130.4, 129.1, 129.1, 124.3, 123.9, 122.4, 119.6, 119.4, 116.2, 115.7, 55.5,

45.3, 29.6, 29.6, 28.2, 25.8, 25.7, 25.7; MS (ESI) *m/z* 428.32 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{26}NO_5S$ [M-H]$^-$: 428.1532, found: 428.1532.

4.1.7.6. *(E)*-3,4-dihydroxystyryl 4-1-adamantaneformylamidophenethyl sulfone (13f) 59%; Yellow solid; mp 122–123 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.65 (s, 1H), 9.05 (s, 1H), 9.05 (s, 1H), 6.76-8.13 (m, 9H), 3.54 (m, 2H), 3.12 (m, 2H), 1.55-2.07 (m, 15H); ^{13}C NMR (100MHz, DMSO-d_6) δ 174.6, 149.3, 146.0, 143.5, 138.4, 132.9, 129.2, 129.1, 124.3, 121.5, 121.4, 119.6, 119.6, 119.5, 116.0, 115.8, 55.6, 45.3, 29.6, 29.6, 29.6, 28.1, 25.9, 25.9, 25.9, 25.7, 25.7, 25.7; MS (ESI) *m/z* 480.17 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{27}H_{30}NO_5S$ [M-H]$^-$: 480.1845, found: 480.1843.

4.1.7.7. *(E)*-3,4-dihydroxystyryl 4-4-methylbenzamidophenethyl sulfone (13g) 58%; Light brown solid; mp 119–120 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.21 (s, 1H), 9.51 (s, 2H), 6.79-7.86 (m, 13H), 3.44 (m, 2H), 2.95 (m, 2H), 2.38 (s, 3H); ^{13}C NMR (100MHz, DMSO-d_6) δ 165.7, 149.4, 146.1, 144.0, 142.0, 138.2, 133.7, 132.5, 129.4, 129.4, 129.4, 129.1, 129.1, 128.1, 128.1, 124.3, 122.5, 120.9, 120.9, 116.2, 115.8, 55.5, 28.2, 21.4; MS (ESI) *m/z* 436.15 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{24}H_{22}NO_5S$ [M-H]$^-$: 436.1219, found: 436.1219.

4.1.7.8. *(E)*-3,4-dihydroxystyryl 4-4-nitrobenzamidophenethyl sulfone (13h) 60%; White solid; mp 138–139 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.21 (s, 1H), 9.72 (s, 1H), 9.21 (s, 1H), 6.78-8.39 (m, 13H), 3.44 (m, 2H), 2.95 (m, 2H); ^{13}C NMR (100MHz, DMSO-d_6) δ 164.7, 151.3, 146.5, 145.9, 136.8, 135.1, 135.0, 134.5, 129.6, 129.6, 127.9, 127.9, 124.2, 124.0, 123.2, 124.0, 121.5, 121.5, 117.2, 115.2, 60.7, 27.5; MS (ESI) *m/z* 467.08 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}N_2O_7S$ [M-H]$^-$: 467.0913, found: 467.0911.

4.1.7.9. *(E)*-3,4-dihydroxystyryl 4-2-thiopheneacetylamidophenethyl sulfone (13i) 59%; Yellow brown solid; mp 87–88 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 9.73 (s, 1H), 9.24 (s, 1H), 6.81-7.79 (m, 12H), 5.31 (s, 1H), 3.54 (m, 4H), 2.96 (m, 2H); ^{13}C NMR (100MHz, DMSO-d_6) δ 158.8, 146.5, 145.9, 137.4, 135.1, 135.0, 134.5, 129.2, 127.9, 127.9, 124.2, 123.2, 121.5, 121.5, 117.2, 115.2, 110.1, 110.1, 108.1, 60.7, 38.0, 27.6; MS (ESI) *m/z* 442.07 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{22}H_{20}NO_5S_2$ [M-H]$^-$: 442.0783, found: 442.0778.

4.1.8. General synthesis of *(E)*-*N*-(4-(2-((3,4-bis(methoxymethoxy)styryl)sulfinyl)ethyl)phenyl) amides (12a – 12f)

To a solution of *(E)*-4-(2-((3,4-bis(methoxymethoxy)styryl)sulfinyl)ethyl)aniline (**10**, 0.1mmol) in dichloromethane (10mL), trimethylamine (0.2mmol), and various acid chlorides (0.22mmol) were added under the ice bath. The resulting solution was allowed to stir for 2h. The solvent was evaporated under vacuum and the mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and

concentrated in vacuo. The crude products of **12a** to **12f** were washed with petroleum ether and were immediately added to the next step.

4.1.9. General synthesis of *(E)*-3,4-dihydroxystyryl 4-acylaminophenethyl sulfoxides (14a – 14f)

To a solution of *(E)*-*N*-(4-(2-((3,4-bis(methoxymethoxy)styryl)sulfinyl)ethyl)phenyl)amides (**12a – 12f**, 0.06mmol) in methanol (10mL), hydrochloric acid (catalytic amount) was added and the resulting solution was heated to reflux for 3h. The solvent was evaporated under vacuum and the mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried with sodium sulfate, and concentrated in vacuo. Purification of the crude product by column chromatography (PE/EA) afforded the target compounds **14a** to **14f**.

4.1.9.1. *(E)*-3,4-dihydroxystyryl 4-benzamidophenethyl sulfoxide (14a) 15%; White solid; mp 97–98 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.21 (s, 1H), 9.72 (s, 1H), 9.21 (s, 1H), 6.79-7.95 (m, 14H), 2.74-3.04 (m, 4H); ^{13}C NMR (100MHz, DMSO-d_6) δ 166.0, 149.4, 146.1, 143.9, 138.1, 135.5, 133.9, 132.0, 129.1, 129.1, 128.8, 128.8, 128.1, 128.1, 124.4, 122.5, 122.5, 120.9, 120.9, 116.2, 115.7, 50.3, 36.9; MS (ESI) *m/z* 406.10 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{20}NO_4S$ [M-H]$^-$: 406.1113, found: 406.1112.

4.1.9.2. *(E)*-3,4-dihydroxystyryl 4-4-methoxylbenzamidophenethyl sulfoxide (14b) 11%; Yellow oil; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.27 (s, 1H), 9.71 (s, 1H), 9.21 (s, 1H), 6.81-8.44 (m, 13H), 3.81 (s, 3H), 2.74-3.04 (m, 4H); ^{13}C NMR (100MHz, DMSO-d_6) δ 165.5, 162.5, 149.5, 146.3, 144.1, 138.5, 133.8, 130.6, 130.2, 130.2, 129.3, 129.3, 127.6, 124.1, 122.6, 121.1, 121.1, 116.4, 115.9, 114.3, 114.3, 56.1, 51.6, 33.1; MS (ESI) *m/z* 436.11 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{24}H_{22}NO_5S$ [M+Na]$^+$: 436.1219, found: 436.1216.

4.1.9.3. *(E)*-3,4-dihydroxystyryl 4-4-fluorobenzamidophenethyl sulfoxide (14c) 17%; Yellow oil; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.27 (s, 1H), 9.72 (s, 1H), 9.21 (s, 1H), 6.79-8.44 (m, 13H), 2.76-3.00 (m, 4H); ^{13}C NMR (100MHz, DMSO-d_6) δ 168.2, 165.9, 149.5, 146.3, 144.2, 142.1, 138.4, 133.9, 132.7, 129.5, 129.5, 129.2, 128.3, 128.3, 124.5, 122.6, 122.6, 121.1, 121.1, 126.4, 115.9, 52.8, 31.9; MS (ESI) *m/z* 424.09 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}FNO_4S$ [M-H]$^-$: 424.1019, found: 424.1022.

4.1.9.4. *(E)*-3,4-dihydroxystyryl 4-3.5-difluorobenzamidophenethyl sulfoxide (14d) 23%; Yellow oil; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.27 (s, 1H), 9.72 (s, 1H), 9.22 (s, 1H), 6.80-8.43 (m, 12H), 2.76-3.04 (m, 4H); ^{13}C NMR (100MHz, DMSO-d_6) δ 165.9, 161.0, 161.0, 149.4, 146.1, 144.0, 138.1, 135.5, 133.9, 132.0, 129.1, 128.9, 128.9, 122.5, 122.5, 121.0, 116.2, 115.7, 115.2, 110.6, 108.7, 108.7, 50.4, 36.9; MS (ESI) *m/z* 442.08 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{18}F_2NO_4S$ [M-H]$^-$: 442.0925, found: 442.0928.

4.1.9.5. *(E)*-3,4-dihydroxystyryl 4-cyclohexylamidophenethyl sulfoxide (14e) 10%; Yellow oil; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.27 (s, 1H), 9.72 (s, 1H), 9.22 (s, 1H), 6.80-7.94 (m, 9H), 2.74-3.04 (m, 4H), 1.19-1.76 (m, 11H); ^{13}C NMR (100MHz, DMSO-d_6) δ 174.8, 149.5, 146.3, 144.1, 138.6, 133.1, 129.4, 129.3, 124.5, 122.7, 122.6, 119.8, 119.7, 116.4, 116.0, 52.3, 45.5, 34.1, 29.8, 29.8, 26.1, 25.9, 25.9; MS (ESI) *m/z* 412.15 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{23}H_{26}NO_4S$ [M-H]$^-$: 412.1583, found: 412.1582.

4.1.9.6. *(E)*-3,4-dihydroxystyryl 4-1-adamantaneformylamidophenethyl sulfoxide (14f) 15%; Yellow solid; mp 111–112 ºC; ^{1}H NMR (400MHz, DMSO-d_6) δ 10.05 (s, 1H), 9.65 (s, 1H), 9.05 (s, 1H), 6.79-7.96 (m, 9H), 2.74-3.04 (m, 4H), 1.60-2.00 (m, 15H); ^{13}C NMR (100MHz, DMSO-d_6) δ 174.7, 149.3, 146.1, 143.9, 138.4, 130.4, 129.1, 129.1, 124.3, 123.9, 122.5, 122.5, 119.6, 119.6, 116.2, 115.7, 52.4, 35.7, 35.4, 35.4, 34.3, 34.3, 32.9, 32.5, 32.1, 32.1, 25.9; MS (ESI) *m/z* 464.18 [M-H]$^-$; HRMS (ESI) *m/z* calcd for $C_{27}H_{30}NO_4S$ [M-H]$^-$: 464.1896, found: 464.1893.

4.2. Biology

4.2.1. Cytotoxicity assays

The cytotoxicity of all target compounds were assessed *via* measurement of the amount of TZM-bl cells after exposure to the compounds for 2 to 3 days. In short, the target compounds were dissolved in DMSO cell freezing medium. TZM-bl cells were seeded in 96-well culture plates at 1×10^5 cells per mL (100μL per well). The cells were treated with various concentrations (1000μM, 200μM, 40μM, 8μM, and 1.6μM) of each compounds and were cultivated under 5% CO_2 at 37°C. Three wells were used in parallel for each concentration. Cells treated with culture medium were used as a negative control. Two days later, the culture medium was discarded and 10μL of coloring agent CCK-8 solution was added to each well before incubation was allowed to last for another three to four hours. The absorbance (*A*, at 450nm) was measured by a microplate reader. The (%) survival of TZM-bl cells was calculated using the following formula: (A_{450} of the experimental group/ A_{450} of the negative control group) × 100%.

4.2.2. Determination of antivirus activity against R5 HIV-1 strains

TZM-bl cells, the same type used in cytotoxicity assays, were seeded in 96-well plates. Complete medium were added to all sample wells, viral control wells and cell control wells. Twelve hours later, the culture medium was discarded before the solutions of target compounds were diluted to 50μM and added to sample wells. Two wells were used in parallel for each compound. HIV-1 Bal (R5) virus (200TCID50) were added into all sample wells and virus control wells and DEAE was added to a final concentration of 10μg/mL. The infected cells were cultivated for 3 days under 5% CO_2 at 37°C. The luciferase assay substrate were dissolved by luciferase lysis buffer and were added (100μL per well) into all experiment wells which had 100μL of the culture medium discarded beforehand. The luciferase assay system was shielded from light and was allowed to react for 2 minutes at room temperature. The mixtures in all wells were blown to well-distributed and

transferred to a black test plate for 150μL for each well. The results were measured by a fluorescence detector and the % inhibition of virus' infection was represented with the decrease in fluorescent intensity.

4.2.3. SPR binding assays

The SPR binding assays were performed as previously described by using SPR biosensor technology. The experiments were carried out on a Biacore T200 instrument that contains dual flow cells. Immobilization of IN proteins to sensor chip CM5 (Biacore) was carried out by primary amine coupling method resulting in an immobilization level of approximately 10000RU. By using automatic corrections of Biacore T200 Evaluation Software, all the raw sensorgrams were processed along with solvent corrected. The specific binding figures of compounds to immobilized IN were obtained after subtracting response signal from control flow cell. All Biacore data were collected at 25°C with PBST as running buffer at a constant flow of 30μL/min. The equilibrium dissociation constants (K_D) evaluating the binding affinity between IN and compounds were determined using 1:1 binding model and the curve fitting efficiency was checked by $\chi 2$ and residual plots.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grants 20972011, 21042009, and 21172014) and Ministry of Science and Technology of China (Grant 2009ZX09301-010).

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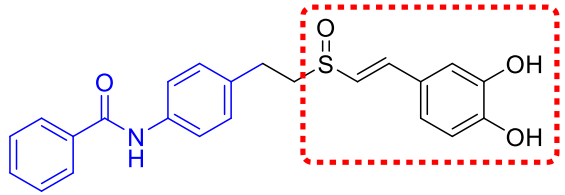
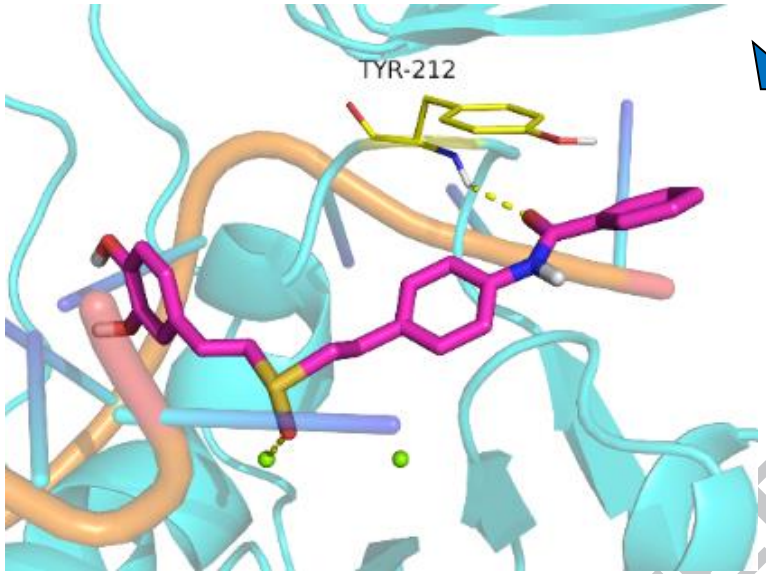
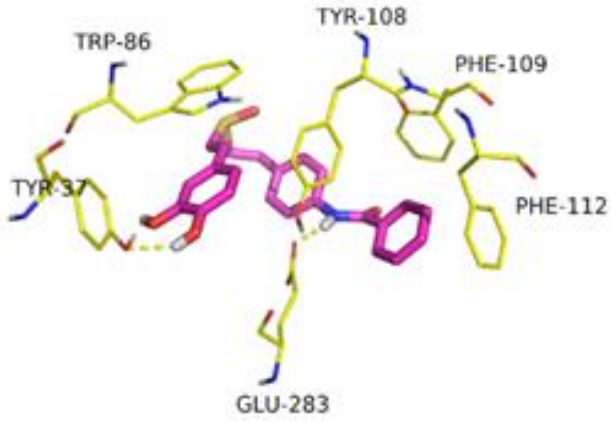


Novel Sulfoxide 14a as Dual Inhibitor of CCR5 / IN



Binding with IN.



Binding with CCR5.