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ARTICLE

Design, synthesis and biological evaluation of caffeoyl - benzanilides as dual inhibitors of HIV integrase and CCR5†

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Novel series of caffeoyl-benzanilide compounds as dual inhibitors of HIV-1 CCR5/IN were designed and synthesized. The biological results indicated that the acetylated compounds with double bond reduced, especially compound **9a**, showed potential activity against HIV-1 CCR5 tropic viruses with EC_{50} value (4.85μM) as well as binding affinities with IN (K_D 2.4μM). Molecular modeling studies also suggested the possible binding mode of **9a** with CCR5 and IN respectively. These results indicated that **9a** has the possibility of being dual inhibitor of HIV-1.

Introduction

Human immunodeficiency virus-1 (HIV-1) is the causative agent of the acquired immunodeficiency syndrome (AIDS). Because of the variation and the persistence of viral, the clinical management of AIDS is very complicated. Currently, highly active antiretroviral therapy (HAART), constituted by inhibitors of reverse transcriptase (RT) and protease (PR) or integrase (IN) ones, successfully suppressed HIV-1 viral load to an undetectable level.[1] However, the efficiency of this therapy is compromised by complicated dosing and intolerable toxicities. Therefore, development of HIV-1 dual inhibitors, which two scaffolds merged into one entity and act on different sites of viral life cycle, would be an innovative approach to reduce drug toxicity as well as improving patient compliance.[2]

According to the virus' life cycle, the chemokine receptor CCR5 was identified as an essential coreceptor for the attachment of HIV-1 to the surface of $CD4^+$ macrophages and T-cells in entry level. Especially, CCR5 is an extracellular target on the host cell surface.[3] Therefore, inhibit the activity of CCR5 would be an effective strategy against HIV-1 virus.[4] In addition , the integrase, which integrated the proviral dsDNA into the host cell chromosome, could be an attractive target for the development of antiretroviral drugs.[3] Thus, we focused our attention on designing dual inhibitors of HIV-1 integrase and CCR5.

Caffeic acid phenethyl ester (CAPE) and flavonoid analogues have been reported to have potential activity against HIV-1[4,5] and considered safe with no mortality observed in experimental mice. The SAR study indicated that the dihydroxy styryl is a pharmacophore for anti-HIV activity.[6] And pharmaco-kinetic studies of CAPE indicated that the ester linkage is dramatically degraded by esterase and would also limit its oral bioavailability. Therefore, according to the principle of bioisostere, we decide to introduce the ketone group instead of the unstable ester group.

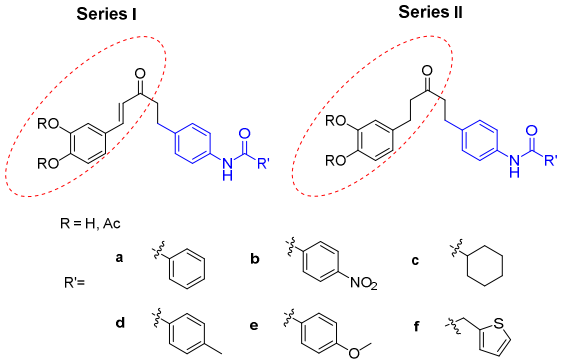

Figure1. Structures of target compounds

As we know, Kazmierski's research group has exploited and reported anilides as CCR5 antagonists comprehensively.[7]

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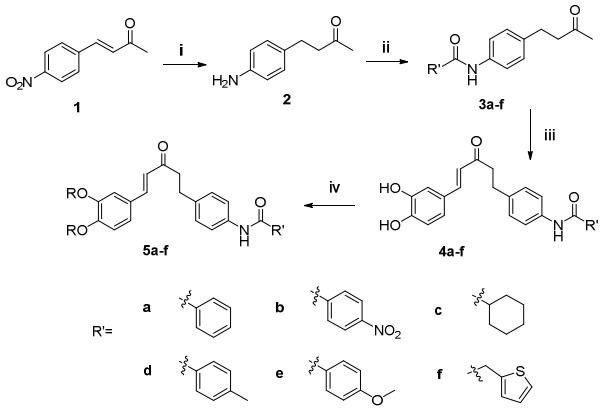
Especially Smithkline Beecham Corp. disclosed various benzanilides as antagonists of receptor. The docking study indicated that the benzanilides would provide more interactions with the binding pocket.[8] Herein, we have the caffeoyl moiety and benzanilides moiety merged into one entity and propose to design novel caffeoyl - benzanilides analogues as dual inhibitors of HIV-1 integrase and CCR5 (Figure 1).

In addition, to further explore the structure-activity relationships (SAR), the unsaturated double bond was reduced to single bond to enhance the flexibility and various substituted groups on the amide side chain were designed. To improve the permeability, the bisphenol acetylated compounds were also studied in our report.[9]

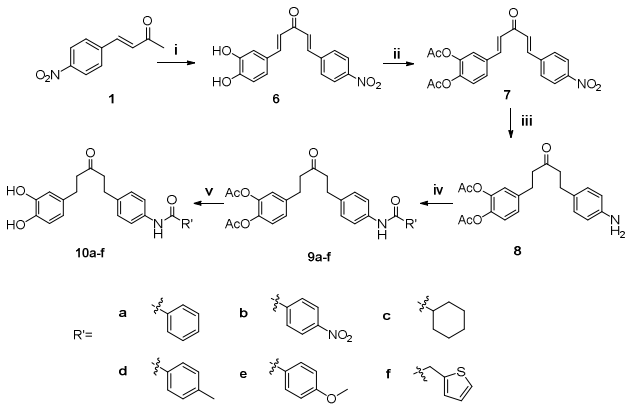
Results and Discussion

The synthesis of serious I target compounds is outlined in Schemes 1. (*E*)-4-(4-nitrophenyl)but-3-en-2-one (**1**), which was prepared by condensation of 4-nitrobenzaldehyde with acetone according to the reported procedure,[10] was hydrogenated using 10% Pd/C as the catalyst in CH_2Cl_2 to afford the saturated compound **2** in high yield.[11,12] Subsequently, reaction of **2** with various acid chlorides to furnish compounds **3a-f**, which condensed with 3,4-dihydroxy benzaldehyde in the presence of pyrrolidine and acetic acid to give the corresponding target compounds **4a-f**. To improve permeability, acetylation reaction was performed to obtain the corresponding compounds **5a-f** in high yields.



Scheme 1 Synthesis of serious **I** target compounds. Reagents and conditions:(□) 10% Pd/C, H_2, CH_2Cl_2, rt.; (□) R'COCl, CH_2Cl_2 rt. (□) 3,4-dihydroxy benzaldehyde, pyrrolidine, acetic acid, THF, reflux; (□) acetic anhydride, pyridine, rt, 5-15min.

Because of the low yield of preparing compounds **4a-f**, it would be difficult to get series II compounds (**10a-f**) by directly reduction. Therefore, another synthetic pathway was designed to obtain the corresponding saturated compounds **9a-f** and **10a-f**. (Scheme 2)



Scheme 2. Synthesis of serious II target compounds. Reagents and conditions: (□) 3,4-dihydroxy benzaldehyde, CH_3SO_3H, neat, rt, 5 min; (□) acetic anhydride, pyridine, rt, 15min; (□) 10% Pd/C, H_2, CH_2Cl_2, rt; (□) R'COCl, CH_2Cl_2 , rt; (□) HCl, H_2O, acetone, reflux.

Firstly, condensation reaction was carried out between **1** and 3,4-dihydroxy benzaldehyde with methane sulfonic acid as coupling agent to furnish the important intermediate compound **6**. Acetylation of **6** could afford compound **7** which was hydrogenated according to the methods mentioned above to yield the saturated compound **8**. Reaction of **8** with the corresponding acid chlorides to afford the series II compounds **9a-f**. Subsequently, deacetylation was performed to give the bisphenyl target compounds **10a-f** in high yields.

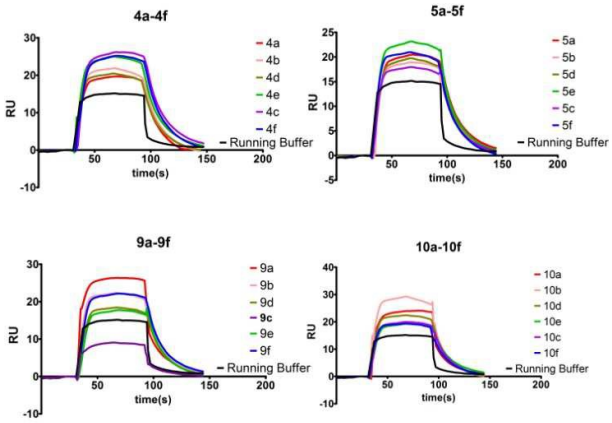


Figure 2 The RU value of target compounds at the concentration of 25μM.

Subsequently, the surface plasma resonance (SPR)-based competitive assay[12] was employed to evaluate the target compounds' binding affinity with IN by monitoring the alteration of response unit (RU) with the addition of compounds at different concentrations.[13] Firstly, IN was covalently immobilized to the sensor chip (CM5) surface by the standard primary amine coupling method. Then compounds were screened at the concentration of 25μM with running buffer as negative control. All the sensor grams

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were processed by automatic correction for non-specific bulk refractive index effects. The primary results were illustrated in Figure 2.

After the preliminary screen at one concentration, the seven promising ones (**4a, 4c, 4e, 4f, 9a, 10a, 10b**) were selected to perform in dose response assay with two models to determine the equilibrium dissociation constants (K_D) (Figure 3 and 4). The results were summarized in Table 1.

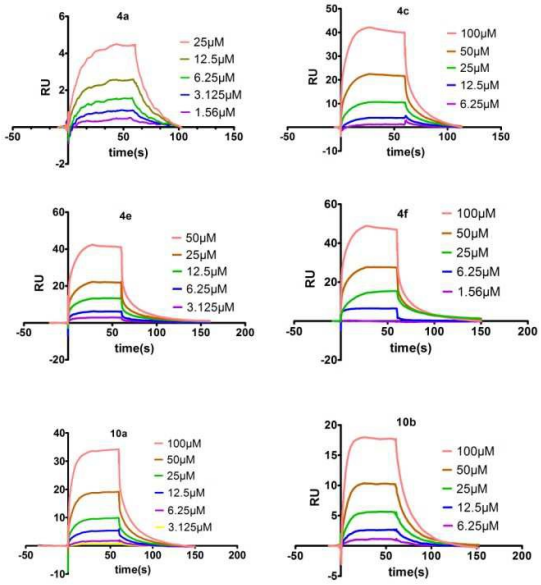


Figure 3 The curve fitting of six target compounds (**4a, 4c, 4e, 4f, 10a** and **10b**) with 1:1 binding model[13]

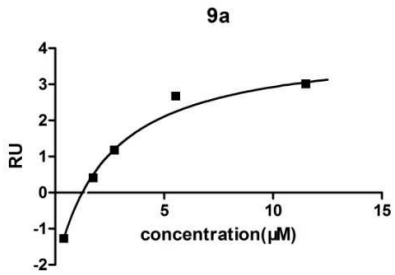
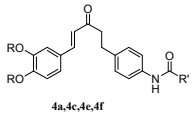
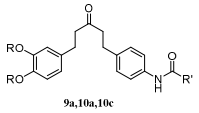


Figure 4 The curve fitting of compound **9a** with steady state model[14]

As shown in Table 1, the saturated compounds could be binding closely with IN than the corresponding ones with double bond. Especially, the bisphenyl acetylated compound **9a** exhibited excellently binding affinity with IN. The K_D value of **9a** (2.4μM), which was determined from the steady state model, was 46 folds and 68 folds lower than that of the unacetylated **10a** and **4a** respectively. Considering the different substitutions at amide moiety, thienyl (**4f** 61.3μM) and cyclohexyl (**6c** 89.8μM) substituents would be beneficial for binding.

Table 1 Equilibrium Dissociation Constants (K_D) of some compounds





compds	4a	4c	4e	4f	9a	10a	10c
R	H	H	H	H	Ac	H	H
R'							
K_D (μM)	163.6	89.8	43.2	61.3	2.4	110.5	80.9

The ability of these new analogues to inhibit HIV-1 CCR5 was evaluated using a cell based assay, which carried out in TZM-bl cells infected by HIV-1 Bal with Maraviroc as reference compound. TZM-bl cells are used for determination of anti-HIV activity due to easy and quick visualization of infected cells. Known as R5 isolate, Bal was used in screening studies.

Table 2 Inhibition of HIV-1 entry through CCR5 co-receptor as analyzed in TZM-bl cells using R5 tropic virus isolates (Bal)

Compd	EC_{50} (μM)[a]	SI[b]	Compd	EC_{50} (μM)[a]	SI[b]
4a	>50	< 1	**9a**	4.85±0.57	110
4b	>50	< 1	**9b**	16.83±2.34	15
4c	>50	< 3	**9c**	18.77±1.46	14
4d	>50	< 1	**9d**	23.14±4.23	9
4e	16.56±0.63	7	**9e**	30.63±4.75	21
4f	>50	< 2	**9f**	15.99±1.51	17
5a	>50	< 5	**10a**	22.84±1.03	26
5b	16.25±0.05	5	**10b**	42.46±0.31	6
5c	29.67±0.18	5	**10c**	18.34±0.82	10
5d	11.22±0.33	2	**10d**	36.30±1.15	5
5e	18.85±0.42	12	**10e**	>200	< 5
5f	>50	< 1	**10f**	55.37±1.06	8
Maraviroc	0.001	$>10^6$			

[a] Data represent the mean values of at least three experiments. [b] SI was calculated based on the CC_{50} and EC_{50}, respectively. Maraviroc was used as the reference compounds

All synthesized compounds were tested for their cytotoxicity before testing for cell based anti-HIV activity. The anti-HIV activity and cytotoxicity of the compounds were

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illustrated in Table 2. Most of the acetylated compounds exhibited better anti-HIV activity than that of the bisphenyl ones. One possible explanation for this result was the high permeability of acetylated compounds. Notably, compound **9a** once again exhibited significantly higher potency against R5 virus with low toxicity (EC_{50} = 4.85μM, SI = 110). These biological results indicate that **9a** would be the potent dual inhibitor of HIV-1 CCR5/IN.

To explore the binding molds of our compounds with CCR5 and IN, a representative inhibitor **9a** was docked into IN (PBD: 3OYA) and CCR5 (PBD: 4MBS) respectively using Glide of *Schrodinger suite 2015-1.*

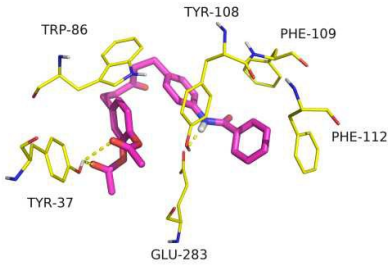

Figure 5. Binding mold of **9a** (magenta) into 4MBS of CCR5. Interactions are shown as yellow dashed line

Docking studies revealed that **9a** could be flexibly docked into the binding pocket of CCR5 and establish several ligand-receptor interactions, which were characterized as: (i) Two hydrogen bonds would be an important factor for anti-HIV-1 activity. One is donated by the NH function of the amide on the side chain to the carbonyl oxygen of Glu283. Another one is the acetyl ester with Tyr 37 residue. (ii) Favorable π-π stacking interactions could be observed between the phenyl ring of **9a** with the hydrophobic pocket defined by the side chains of Tyr108, Phe109, and Phe112. In addition, the aromatic ring of caffeoyl moiety interacted with TRP86 via π-π stacking. (Figure 5)

To further explore the binding mode of this analogue with IN, compound **9a** was docked into the catalytic core domain (CCD) of IN (Figure 6A), and several interactions could be observed. Firstly, the NH and the acyl of the amide formed two H-bonds with Gln186 and Tyr212 residues, respectively. Second, the carbonyl group of caffeoyl moiety coordinated with two Mg^{2+} metal ions (Figure 6A). Meanwhile, comparing the docking results of compound **10a**, we could find the binding mold of **10a** has changed. Only one Mg^{2+} ion coordinated with carbonyl oxygen, and the hydrogen bond of amide group with Gln186 residue disappeared (Figure 6B). In addition, the π-π stacking interactions between benzene ring of amide side chain and Tyr212 residue could be observed in both of **9a** and **10a**. Therefore, compound **9a** with more flexibility could fit well in the binding pocket of IN. This result coincidence quite well with our assumption.

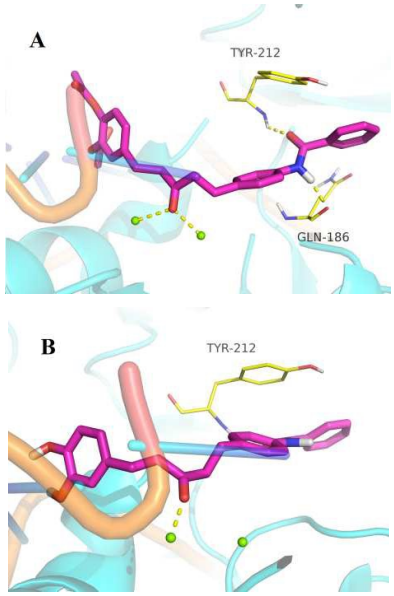

Figure 6. A: Binding mold of **9a** (magenta) into the 3OYA of HIV-1 IN; **B**: Binding mold of **10a** (magenta) into the 3OYA of HIV-1 IN. Hydrogen bonds are shown as yellow dashed line, green ball represent magnesium ion.

Conclusions

In conclusion, we have synthesized caffeoyl-benzanilide analogues as dual inhibitors of cellular CCR5 and HIV-1 IN. The biological evaluation data suggested that the acetylated compounds with double bond reduced could be active against R5 virus with low cytotoxicity. Interestingly, compound **9a** inhibited the entry of CCR5 tropic viruses with EC_{50} values of 4.85μM and also exhibited excellently binding affinity with IN (K_D 2.4μM). The biological and structural data provide more information on caffeoyl- benzanilides as a valid scaffold for dual inhibitors of HIV-1.

Acknowledgements

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References

1. P. Yeni, *Journal of hepatology*, 2006, **44**, S100-103.
2. W. C. Greene, Z. Debyser, Y. Ikeda, E. O. Freed, E. Stephens, W. Yonemoto, R. W. Buckheit, J. A. Este and T. Cihlar, *Antiviral research*, 2008, **80**, 251-265.
3. Vasu Nair, Guochen Chi, Roger Ptak and N. Neamati‡, *Journal of Medicinal*

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DOI: 10.1039/C6MD00311G

Chemistry, 2006, **49**.
4. S. U. Lee, C. G. Shin, C. K. Lee and Y. S. Lee, *European journal of medicinal chemistry*, 2007, **42**, 1309-1315.
5. C. C. Ho, S. S. Lin, M. Y. Chou, F. L. Chen, C. C. Hu, C. S. Chen, G. Y. Lu and C. C. Yang, *The Journal of antimicrobial chemotherapy*, 2005, **56**, 372-379.
6. T. R. Burke, Jr., M. Fesen, A. Mazumder, J. Yung, J. Wang, A. M. Carothers, D. Grunberger, J. Driscoll, Y. Pommier and K. Kohn, *Journal of Medicinal Chemistry*, 1995, **38**, 4171-4178.
7. W. Kazmierski, N. Bifulco, H. Yang, L. Boone, F. DeAnda, C. Watson and T. Kenakin, *Bioorganic & medicinal chemistry*, 2003, **11**, 2663-2676.
8. H. S. Bodiwala, S. Sabde, P. Gupta, R. Mukherjee, R. Kumar, P. Garg, K. K. Bhutani, D. Mitra and I. P. Singh, *Bioorganic & medicinal chemistry*, 2011, **19**, 1256-1263.
9. X. Ning, Y. Guo, X. Ma, R. Zhu, C. Tian, X. Wang, Z. Ma, Z. Zhang and J. Liu, *Bioorganic & medicinal chemistry letters*, 2013, **23**, 3700-3703.
10. N. Erathodiyil, S. Ooi, A. M. Seayad, Y. Han, S. S. Lee and J. Y. Ying, *Chemistry*, 2008, **14**, 3118-3125.
11. S. Dhuru, D. Bhedi, D. Gophane, K. Hirbhagat, V. Nadar, D. More, S. Parikh, R. Dalal, L. C. Fonseca, F. Kharas, P. Y. Vadnal, R. A. Vishwakarma and H. Sivaramakrishnan, *Bioorganic & medicinal chemistry letters*, 2011, **21**, 3784-3787.
12. M. Elinder, H. Nordstrom, M. Geitmann, M. Hamalainen, L. Vrang, B. Oberg and U. H. Danielson, *Journal of biomolecular screening*, 2009, **14**, 395-403.
13. N. J. de Mol, F. J. Dekker, I. Broutin, M. J. Fischer and R. M. Liskamp, *J Med Chem*, 2005, **48**, 753-763.
14. T. S. Peat, D. I. Rhodes, N. Vandegraaff, G. Le, J. A. Smith, L. J. Clark, E. D. Jones, J. A. Coates, N. Thienthong, J. Newman, O. Dolezal, R. Mulder, J. H. Ryan, G. P. Savage, C. L. Francis and J. J. Deadman, *PloS one*, 2012, **7**, e40147.

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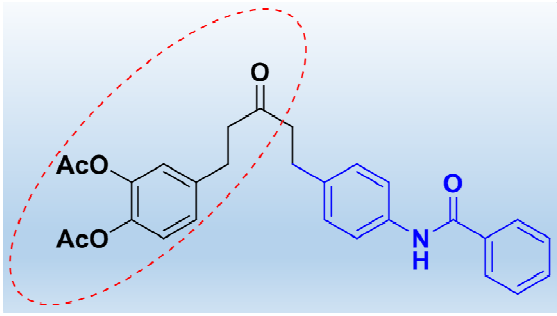
Graphical Abstract

Novel serious of caffeoyl-benzanilides have been synthesized and evaluated as dual inhibitors of HIV-1 CCR5/IN. Compound **9a** exhibited the possibility of being dual inhibitor of HIV-1.



9a EC50 = 4.8 µM
KD = 2.4 µM