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Synthesis of polyhydroxylated aromatics having amidation of piperazine nitrogen as HIV-1 integrase inhibitor

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ABSTRACT

(*E*)-*N*-[3-(4-Cinnamoylpiperazin-1-yl)propyl]-3,4-dihydroxybenzamide and (*E*)-*N*-[3-(4-cinnamoylpiperazin-1-yl)propyl]-3,4,5-trihydroxybenzamide were designed and synthesized as potential HIV-1 integrase inhibitors and evaluated their inhibition to the strand transfer process of HIV-1 integrase. The result indicates that 3,4,5-trihydroxylated aromatic derivatives exhibit good inhibition to HIV-1 integrase, however, corresponding 3,4-dihydroxylated aromatic derivatives appear little inhibition of HIV-1 integrase.

The human immunodeficiency virus type 1 (HIV-1) is the causative agent of the acquired immunodeficiency syndrome (AIDS).[1] Highly active antiretroviral therapy (HAART) is now the common treatment for HIV-1 patients, but the infection has been difficult to cure because of drug resistance[2,3] and immune response. In the essential steps for viral replication, the viral enzyme integrase mediated viral integration catalyzes the insertion of the proviral DNA into the genome of host cells,[4] therefore integrase is an attractive strategy for antiretroviral drug design.

Polyhydroxylated compound caffeic acid phenethyl ester display potent activities against HIV-1 integrase with weak cytotoxicity.[5] Being a pharmacophore of caffeic acid phenethyl ester, the phenolic hydroxyl group is coordinated with metal ions, such as Mg^{2+} or Mn^{2+}, which block the 3′-end processing (3′-P) and the strand transfer (ST) in the HIV-1 integrase catalytic core. Previous studies have made great strides in the design and discovery of integrase inhibitors, such as phenalenones isolated from fungal extracts,[6] chalcone derivatives (**1**),[7] caffeic acid phenethyl ester (**2**), caffeoylamino acid derivatives,[8] sulfonamide (**3**),[9,10] L-chicoric acid[11] and catechol-diketoacid hybrid,[12] which show inhibitory activity against HIV-1 integrase. The structure of inhibitors consists of a hydrophobic subunit, a hydrophilic polyhydroxylated aromatic domain and a linker segment (Fig. 1).[13]

The linker and phenyl ring are responsible for the interaction with the hydrophobic pocket and residues around the domain to reinforce their affinity and selectivity to HIV-1 integrase.[14] The hydrophilic domain, being polyhydroxylated aromatic moiety, is essential for the enzyme inhibitory activity as a pharmacophore, through the sequestration of the divalent cofactor in the IN active core and then blocking the access of a host DNA to the integrase enzyme. Full coplanarity of polyhydroxylated aromatics inhibitor structure is not an absolute requirement for high activity, computational studies performed that certain IN inhibitors, such as AG593, which linker segment is piperazine, cannot assume an entirely coplanar conformation.[7] Based on the nature and the length of the linker between piperazine amide nitrogens, we have

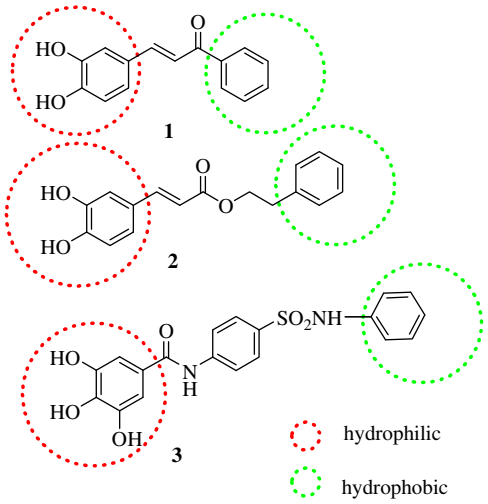

Figure 1. Structural features of polyhydroxylated aromatics integrase inhibitors.

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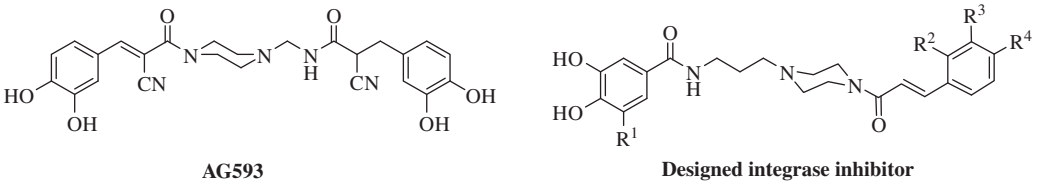
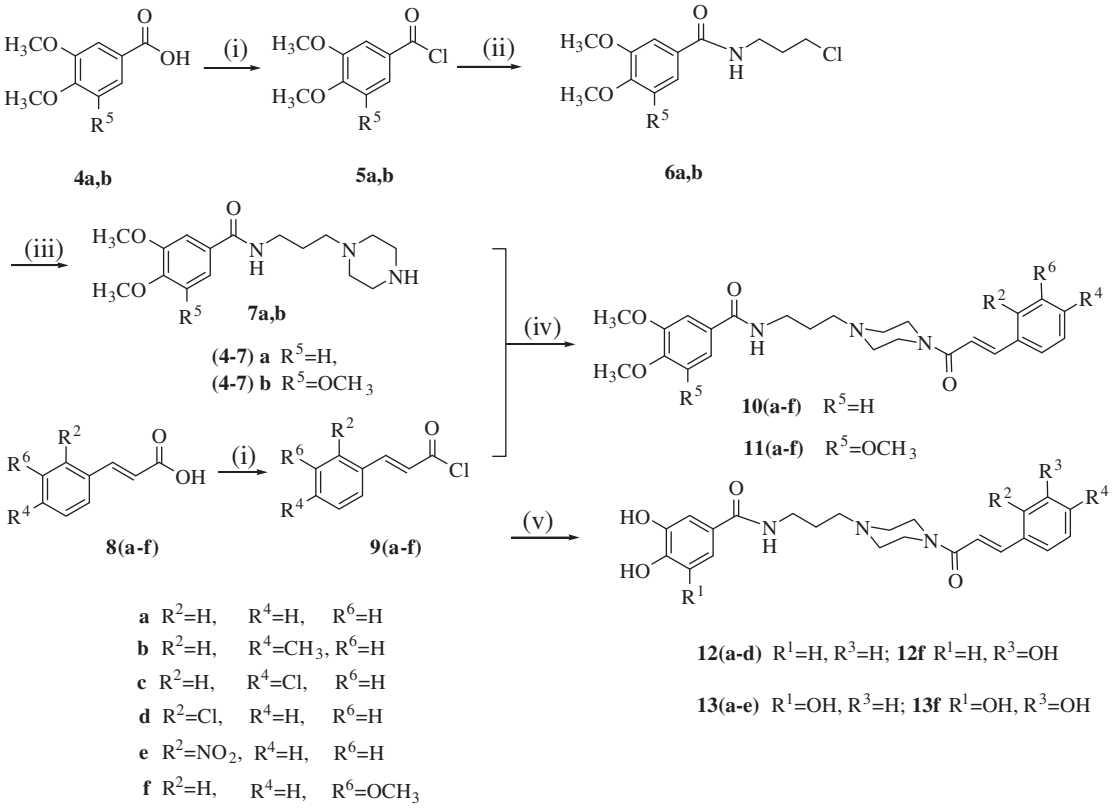


AG593 **Designed integrase inhibitor**

Figure 2. The structures of AG593 and designed integrase inhibitor.



4a,b **5a,b** **6a,b**

7a,b

(4-7) a R^5=H,
(4-7) b R^5=OCH_3

10(a-f) R^5=H

11(a-f) R^5=OCH_3

8(a-f) **9(a-f)**

a R^2=H, R^4=H, R^6=H
b R^2=H, R^4=CH_3, R^6=H
c R^2=H, R^4=Cl, R^6=H
d R^2=Cl, R^4=H, R^6=H
e R^2=NO_2, R^4=H, R^6=H
f R^2=H, R^4=H, R^6=OCH_3

12(a-d) R^1=H, R^3=H; **12f** R^1=H, R^3=OH

13(a-e) R^1=OH, R^3=H; **13f** R^1=OH, R^3=OH

Scheme 1. Reagents and conditions: (i) $SOCl_2$, reflux; (ii) $NH_2CH_2CH_2CH_2Cl$, CH_2Cl_2, 0 °C–rt, overnight; (iii) piperazine, K_2CO_3/KI, $CHCl_3$, reflux; (iv) CH_2Cl_2, 0 °C–rt; (v) (1) BBr_3,CH_2Cl_2, rt, then H_2O.

designed and synthesized polyhydroxylated aromatics compounds as integrase inhibitors (Fig. 2).

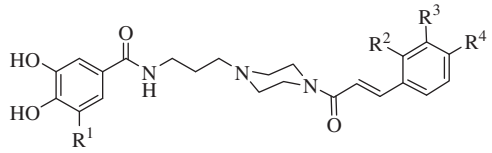
Depicted in Scheme 1 is our synthetic route developed to allow for efficient introduction of aromatic substituent on the piperazine nitrogen toward the end of the synthesis.

The synthesis of inhibitors **12** and **13** starts with treatment of 3,4,5-trimethoxy benzoic acid or 3,4-dimethoxy benzoic acid **4** with thionyl chloride, to give benzoyl chloride **5** in excellent yield, which was treated with 3-chloropropen-1-amine to provide the 3-chloropropyl benzamide **6**. Direct mono-amination of **6** with piperazine in chloroform obtained intermediate **7** in a good yield. Another intermediate **9** was obtained from cinnamic acid **8** reacting with thionyl chloride. Amidation between **7** and **9** was carried out in dichloromethane to convert to *N*-[3-(4-cinnamoylpiperazin-1-yl)propyl]benzamide **10** and **11**. Finally, the methyl removal led to our target compounds **12** and **13** using tribromoborane.

Compounds **12** and **13** were tested in vitro for strand transfer in the enzyme inhibition assay according to a previous method.[15] As shown in Table 1, 3,4-dihydroxylated aromatics derivatives **12** show less activity towards strand transfer, but 3,4,5-trihydroxylated aromatics derivatives **13** exhibit activities at micromolar concentrations. In comparing the potencies of **12** and **13** in the strand transfer assay, it was seen that introduction of a hydroxyl at 5-position of the caffeoyl in **12** gave **13** to improve IN inhibition activities. The results indicate the significance of galloyl as a core pharmacophore. In order to investigate the substituent effect on the phenyl ring in cinnamoyl moiety, electron-donating and electron-withdrawing groups were utilized (**13a–f**). All these compounds display significant potent inhibitory activities without any marked fluctuation. This observation somewhat indicates that such substitution has no notable impact on their activities. The HIV-IN inhibitory effect of compound **13** is nearly as much as that of the control compound L-708906.

In conclusion, a series of nitrogen-containing polyhydroxylated aromatics with piperazine linker have been identified as HIV-1 integrase inhibitors. Based on the nature and the length of the linker piperazine between amide nitrogens, it cannot assume that conformation of polyhydroxylated aromatics structure is entirely coplanar. The current work appears that (*E*)-*N*-[3-(4-cinnamoylpiperazin-1-yl)propyl]-3,4-dihydroxybenzamide and (*E*)-*N*-[3-(4-cinnamoylpiperazin-1-yl)propyl]-3,4,5-trihydroxybenzamide were designed and synthesized for HIV-1 integrase inhibitors and (*E*)-*N*-[3-(4-cinnamoylpiperazin-1-yl)propyl]-3,4,5-trihydroxybenzamide can inhibit replication of HIV-1 in vitro against integrase in strand transfer at micromolar concentrations. We believe that the oxygen atoms in phenolic hydroxyl groups are coordinated with

Table 1
Inhibition of HIV-1 integrase catalytic activities of compounds **12** and **13**[a]



Compds	R^1	R^2	R^3	R^4	IC_{50}[b] (μM)
12a	H	H	H	H	>100
12b	H	H	H	CH_3	>100
12c	H	H	H	Cl	>100
12d	H	Cl	H	H	>100
12f	H	H	OH	H	>100
13a	OH	H	H	H	12.83
13b	OH	H	H	CH_3	17.47
13c	OH	H	H	Cl	24.94
13d	OH	Cl	H	H	19.27
13e	OH	NO_2	H	H	31.31
13f	OH	H	OH	H	15.41
L-708906 (control)					0.77

[a] HIV-1 IN inhibitory activities were measured according to the procedure described in Ref. 15.
[b] Inhibition of strand transfer.

metal ions in enzyme. And further research based on these structures will be continued.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.087.

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