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Design and synthesis of novel nitrogen-containing polyhydroxylated aromatics as HIV-1 integrase inhibitors from caffeic acid phenethyl ester

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ABSTRACT

A series of nitrogen-containing polyhydroxylated aromatics from caffeic acid phenethyl ester were designed and synthesized as HIV-1 integrase inhibitors. Most of these compounds exhibited potent inhibitory activities at micromolar concentrations against HIV-1 integrase in the 3′-end processing and the strand transfer. Their key structure–activity relationship was also discussed.

HIV-1 integrase catalyzes the integration of HIV-1 DNA into the host cell DNA, enabling HIV-1 viruses to infect CD4 cells.[1] Thus, HIV-1 integrase is essential for HIV-1 replication and sustained viral infection.[1] Inhibition of HIV-1 integrase therefore provides an attractive strategy for antiretroviral drug design. There has been yet no human integrase homolog in the literature, and the reaction catalyzed by HIV-1 integrase is rather unique.[2,3] Therefore, in principle, selective inhibitors with few or no side effects can be designed, and the characteristics of HIV-1 integrase make it an attractive target for therapeutic interventions.

Over the past several years, extensive studies focusing on HIV-1 integrase have resulted in a large number of inhibitors with diverse structural features, such as oligonucleotides, peptides, polyhydroxylated compounds, quinoline derivatives, hydrazide and quinolones.[4–11] Previous studies have shown that polyhydroxylated compounds, especially caffeic acid phenethyl ester (**1**) display potent activities against HIV-1 integrase with weak cytotoxicity (Fig. 1).[12,13] Being a pharmacophore of caffeic acid phenethyl ester, the phenolic hydroxyl group is coordinated with metal ions, like Mg^{2+} or Mn^{2+}. Such coordination in the HIV-1 integrase catalytic core blocks the 3′-end processing (3′-P) and the strand transfer (ST).[14,15] However, the linker and the phenyl ring are supposed to interact with the hydrophobic pocket and residues around the

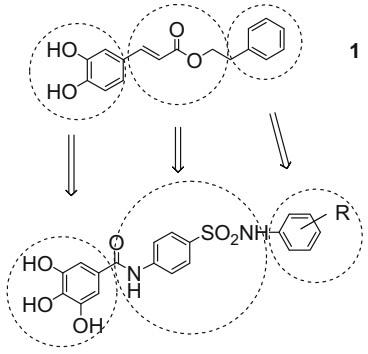

Figure 1. Caffeic acid phenethyl ester and designed integrase inhibitors.

domain to reinforce their affinity and selectivity to HIV-1 integrase.[16] Based on these studies and theories, several polyhydroxylated compounds have been identified as effective HIV-1 integrase inhibitors in clinical trials, and their further clinical research is currently in process.[17]

In this Letter, we described our lead-optimization work starting from caffeic acid phenethyl ester. The phenolic hydroxyl moiety was reserved as a necessary pharmacophore, while the catechol was replaced by a galloyl group, which was reported having higher affinity to metal ions.[18] A sulfanilamide replaced the ester as the linker, because it was suggested that compounds containing sulfanilamide groups show higher antiretroviral activities.[19,20] In order

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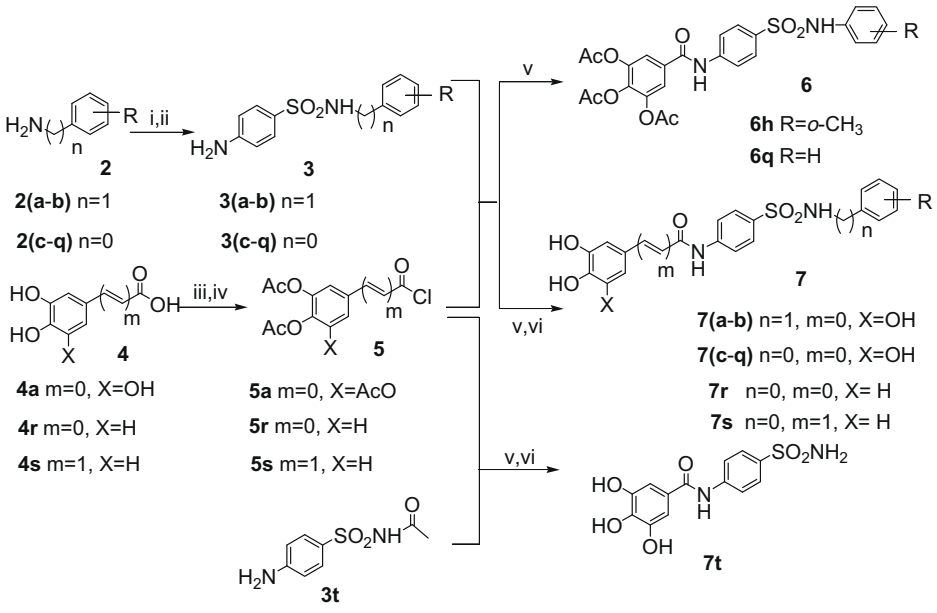


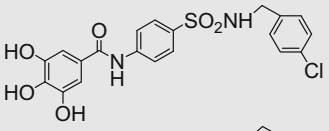
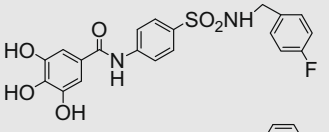
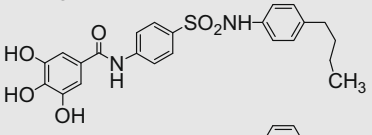
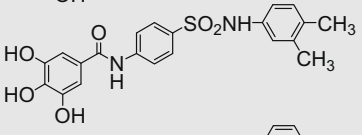
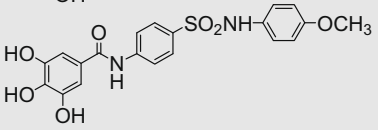
Scheme 1. Reagents and conditions: (i) 4-acetamidobenzene-1-sulfonyl chloride, pyridine, 0 °C–rt, 4 h; (ii) 5 M NaOH, MeOH, 70 °C, 3 h; (iii) Ac_2O, pyridine, rt, 24 h; (iv) $SOCl_2$, 80 °C, 5 h; (v) pyridine, acetone, 0 °C–rt, 20 h; and (vi) MeOH, THF, HCl, 60 °C, 1 h.

to investigate the interactions between the phenyl ring and the hydrophobic pocket and residues around the HIV-1 integrase domain, compounds containing a series of substituted phenyl were synthesized.

Outlined in Scheme 1 is a typical synthesis of the nitrogen-containing polyhydroxylated aromatics-based HIV-1 integrase inhibitors. Direct mono-sulfonylation of **2** with 4-acetamidobenzene-1-sulfonyl chloride gave sulfonamide. Following the acetyl removal under alkaline conditions in methanol yielded an intermediate **3**. Another intermediate **5** was obtained from **4** with the treatment of acetic anhydride first to protect hydroxyl groups, and then with thionyl chloride. Amidation between **3** and **5** was carried out in acetone to have sufficient solubility for **3** to convert to compounds **6**. Using tetrahydrofuran–methanol–concentrated hydrochloric acid (2:2:1, v/v/v) as a solvent avoided the hydrolysis of the amide linkage, and finally, the acetyl removal led to our target compounds **7**.[21]

Above compounds were tested for inhibitory activities against HIV-1 integrase using ^{32}P-labeled assays.[12] As shown in Table 1, these inhibitors demonstrate activities against HIV-1 integrase in

Table 1
Enzyme assay results for compounds **7a–t** and **6h**, **6q** in vitro

Compounds	Structures	Inhibition of HIV-1 IN (IC_{50})[a]	
		3′-P (μM)	ST (μM)
7a		5 ± 2	2 ± 1
7b		7 ± 3	2 ± 1
7c		3 ± 1	2.5 ± 0.5
7d		8 ± 3	8 ± 1
7e		5	4.5

(*continued on next page*)

Table 1 (*continued*)

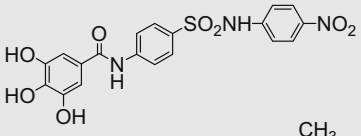
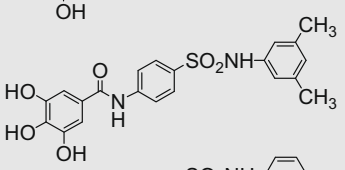
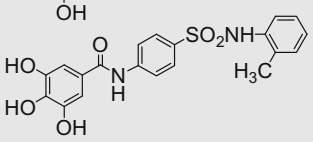
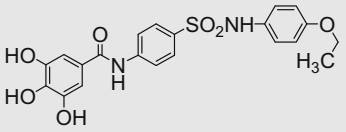
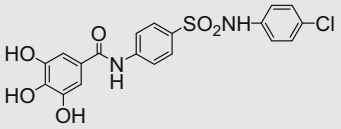
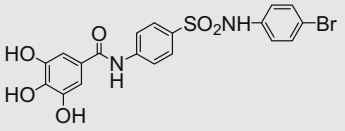
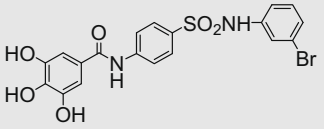
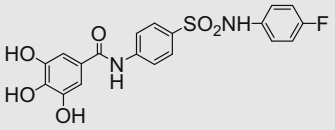
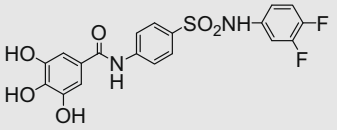
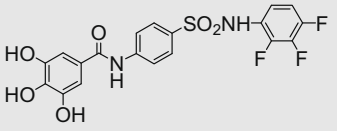
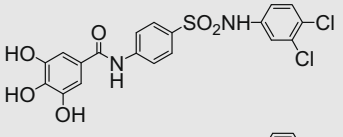
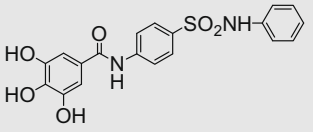
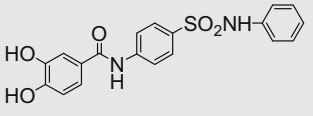
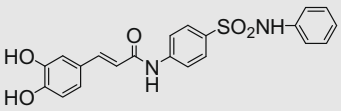
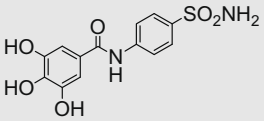
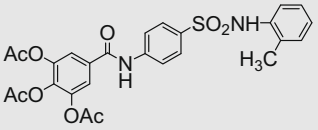
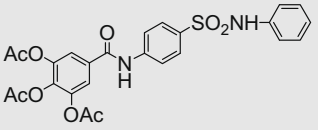
Compounds	Structures	Inhibition of HIV-1 IN (IC_{50})[a]	
		3′-P (μM)	ST (μM)
7f		4 ± 2	3 ± 0.2
7g		8 ± 2	2.5 ± 0.5
7h		7 ± 3	2 ± 1
7i		6	1.3
7j		6 ± 2	2 ± 1
7k		3 ± 2	0.7 ± 0.3
7l		2 ± 1	0.7 ± 0.3
7m		7	0.9 ± 0.1
7n		4 ± 2	1.5
7o		7 ± 2	3 ± 2
7p		5 ± 1	1.7
7q		7 ± 3	1.5 ± 0.1
7r		100	50 ± 10

Table 1 (*continued*)

Compounds	Structures	Inhibition of HIV-1 IN (IC_{50})[a]	
		3′-P (μM)	ST (μM)
7s		16 ± 5	12 ± 4
7t		22 ± 11	2 ± 1
6h		>10	1.6
6q		8	1.6

[a] HIV-1 IN inhibitory activities were measured according to the procedure described in Ref. 12.

the 3′-end processing and strand transfer at micromolar concentrations. Compound **7r** shows a weak activity with an IC_{50} value of 100 μM towards 3′-end processing compared to **7s** (16 ± 5 μM), with the only difference of a double bond between a caffeoyl and an amide structurally. Interestingly, the introduction of a hydroxyl at 5-position of the caffeoyl in **7r** gave **7q**, which exhibited high IC_{50} values of both 3′-end processing and strand transfer at 7 ± 3 and 1.5 ± 0.1 μM, respectively. These results indicate the significance of the galloyl as a core pharmacophore, and that the aryl substitution could assist and improve the inhibitory activities. Compound **7t**, without a phenyl in the sulfonamide group, shows a slight activity drop against 3′-end processing (22 ± 11 μM). However, an unexpected selectivity was observed although the selectivity index is small (3′-end processing/strand transfer = 11). A methylene linker was introduced into **7j** and **7m**, leading to compounds **7a** and **7b**. However, the presence of longer spans caused a slight impact on their activities (Table 1).

In order to investigate the substitution effect on the phenyl ring, electron-donating and electron-withdrawing groups were preferred and utilized (**7c**–**p**). All these compounds display significant potent inhibitory activities (Table 1) without any marked fluctuation. This observation somewhat indicates that such substitution has no notable impact on their activities. However, compounds **7k** and **7l** exhibit the most potent inhibitory activities at 0.7 ± 0.3 μM to strand transfer and 3 ± 2/2 ± 1 μM to 3′-end processing, respectively. Similarly, compounds with halogenic substitution (**7a**, **7b**, **7j**–**p**) show IC_{50}'s in the range of 0.7–7 μM without any significant dependence on the number or position of the substitutions.

Intrigued by the blockage from the phenolic hydroxyl group, more substitution was studies. Surprisingly, the introduction of an acetyl to the oxygen atom has caused no substantial loss of the HIV-1 integrase inhibitory activities in **6h** or **6q**. We believe that the oxygen atoms in phenolic hydroxyl groups are exposed sufficiently to interact with Mg^{2+} or Mn^{2+}. Meanwhile, another possible explanation is that the carbonyl, instead of the phenolic hydroxyl group, binds to Mg^{2+} or Mn^{2+} (Date not shown).

In conclusion, a series of novel nitrogen-containing polyhydroxylated aromatics with sulfanilamide linkers have been identified as HIV-1 integrase inhibitors. The current work represents the first synthetic example of such structures for the development of HIV-1 integrase inhibitors. They exhibit potent inhibitory activities at micromolar concentrations, and further research based on these structures will be continued.

Acknowledgments

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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.2009.06.100.

References and notes

1. Vodicka, M. A. *Somat. Cell Mol. Genet.* **2001**, *26*, 35.
2. Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Disc.* **2005**, *4*, 236.
3. Zhao, G. S.; Wang, C.; Liu, C.; Lou, H. X. *Mini-Rev. Med. Chem.* **2007**, *7*, 707.
4. Jing, N. J.; Marchand, C.; Liu, J.; Mitra, R.; Hogan, M. E.; Pommier, Y. *J. Biol. Chem.* **2000**, *275*, 21460.
5. Krajewski, K.; Long, Y. Q.; Marchand, C.; Pommier, Y.; Roller, P. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3203.
6. De Soultrait, V. R.; Desjobert, C.; Tarrago-Litvak, L. *Curr. Med. Chem.* **2003**, *10*, 1765.
7. Xu, Y. W.; Zhao, G. S.; Shin, C. G.; Zang, H. C.; Lee, C. K.; Lee, Y. S. *Bioorg. Med. Chem.* **2003**, *11*, 3589.
8. Costi, R.; Santo, R. D.; Artico, M.; Massa, S.; Ragno, R.; Loddo, R.; La Colla, M.; Tramontano, E.; La Colla, P.; Pani, A. *Bioorg. Med. Chem.* **2004**, *12*, 199.
9. Bénard, C.; Zouhiri, F.; Normand-Bayle, M.; Danet, M.; Desmaële, D.; Leh, H.; Mouscadet, J. F.; Mbemba, G.; Thomas, C. M.; Bonnenfant, S.; Le Bret, M.; d'Angelo, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2473.
10. Zhao, H.; Neamati, N.; Sunder, S.; Hong, H. X.; Wang, S. M.; Milne, G. W. A.; Pommier, Y.; Burke, T. R., Jr. *J. Med. Chem.* **1997**, *40*, 937.
11. Sato, M.; Motomura, T.; Aramaki, H.; Matsuda, T.; Yamashita, M.; Ito, Y.; Kawakami, H.; Matsuzaki, Y.; Watanabe, W.; Yamataka, K.; Ikeda, S.; Kodama, E.; Kodama, E.; Matsuoka, M.; Shinkai, H. *J. Med. Chem.* **2006**, *49*, 1506.
12. Zhang, X. C.; Neamati, N.; Lee, Y. K.; Orr, A.; Brown, R. D.; Whitaker, N.; Pommier, Y.; Burke, T. R., Jr. *Bioorg. Med. Chem.* **2001**, *9*, 1649.
13. Charvat, T. T.; Lee, D. J.; Robinson, W. E.; Chamberlin, A. R. *Bioorg. Med. Chem.* **2006**, *14*, 4552.

14. Neamati, N.; Lin, Z. W.; Karki, R. G.; Orr, A.; Cowansage, K.; Strumberg, D.; Pais, G. C.; Voigt, J. H.; Nicklaus, M. C.; Winslow, H. E.; Zhao, H.; Turpin, J. A.; Yi, J.; Skalka, A. M.; Burke, T. R.; Pommier, Y., Jr. *J. Med. Chem.* **2002**, *45*, 5661.
15. Tchertanov, L.; Mouscadet, J. F. *J. Med. Chem.* **2007**, *50*, 1133.
16. Kawasuji, T.; Fuji, M.; Yoshinaga, T.; Sato, A.; Fujiwara, T.; Kiyama, R. *Bioorg. Med. Chem.* **2006**, *14*, 8420.
17. Dayam, R.; Al-Mawsawi, L. Q.; Neamati, N. *Drugs R. D.* **2007**, *8*, 155.
18. King, P. J.; Ma, G.; Miao, W. F.; Jia, Q.; McDougall, B. R.; Reinecke, M. G.; Cornell, C.; Kuan, J.; Kim, T. R.; Robinson, W. E., Jr. *J. Med. Chem.* **1999**, *42*, 497.
19. Supuran, C. T.; Innocenti, A.; Mastrolorenzo, A.; Scozzafava, A. *Mini-Rev. Med. Chem.* **2004**, *4*, 189.
20. Barbaro, G.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Pharm. Des.* **2005**, *11*, 1805.
21. See the Supplementary data.