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Design and synthesis of novel β-diketo derivatives as HIV-1 integrase inhibitors

Liming Hu *, Sulei Zhang, Xianzhuo He, Zaigang Luo, Xiaoli Wang, Wei Liu, Xuemei Qin

College of Life Science and Bioengineering, Beijing University of Technology, Beijing 100124, China

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ABSTRACT

A series of novel β-diketo derivatives which combined the virtues of 1,3-diketo, 1,2,3-triazole and polyhydroxylated aromatics moieties, were designed and synthesized as potential HIV-1 integrase (IN) 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, but dihydroxylated aromatic derivatives and corresponding methoxy aromatic derivatives appear little inhibition to HIV-1 integrase.

1. Introduction

The viral enzyme integrase (IN), which mediates integration of the viral cDNA into the host genome by catalytic reactions—first catalyzes removal of the terminal dinucleotide from each 3′-end of the viral DNA (3′-processing) and subsequently mediates joining of the 3′-end of the viral DNA to the host DNA (strand transfer) during the viral replication cycle, is an attractive target for the development of new anti-HIV-1 inhibitors with high selectivity and low toxicity.[1] Recently, many structurally diverse compounds have been reported. Among these, compounds owning the β-diketoacids (DKA) represent the most convincing anti-integrase activity (Fig. 1, S-1360, L-731,988, 5-CITEP).[2] The diketo acid moiety was believed to be the most crucial pharmacophore for the inhibition of IN inhibitors,[3] and the structures of diketotriazole,[4] diketotetrazole,[5] and diketopyridine[6] were reported to be bioisosters of the diketo acid moiety. S-1360, a β-diketo bioisostere, which showed potent antiviral activity against HIV-1 integrase, is the first IN inhibitor for human clinical trials.[7] Unfortunately, S-1360 was withdrawn from clinical trials because of failed efficacy tests in infected patients due to its low concentrations attained in blood samples, ketone reduction and glucuronidation in vivo, and also suffered from a short half life.[8] It takes us great interests to study its structure–activity relationships (SAR). The structure of S-1360 consists of a hydrophobic subunit (4-fluoro-benzylfuran) and a pharmacophoric center (1,3-diketo and triazol moieties) which could binds to divalent metal ions (Mg^{2+} or Mn^{2+}) to block the 3′-end processing (3′-P) and the strand transfer (ST) in the HIV-1 integrase catalytic site.[9]

Amongst the other classes of HIV-1 integrase inhibitors, polyhydroxylated compounds represent of the family with the largest number of members (Fig. 1, CAPE, AG1717, Styrl-quinoline).[10,11a,b] Caffeic acid phenethyl ester, one of the typical polyhydroxylated compounds, displays potent anti-IN activities with weak cytotoxicity. The phenolic hydroxyl group, the pharma-cophoric center of this sort of compounds, plays an important role,[10,12] which can coordinate with metal ions (Mg^{2+} or Mn^{2+}) to block the 3′-P and ST reaction.

In previous studies, Catechol-DKA hybrids have been designed and tested as IN inhibitors following the goals of refining the pharmacophore, enhancing antiviral potency and improving cellular membrane permeability.[13,14] In order to study the possible interaction between the two main classes of inhibitors (polyphenol and DKAs), we synthesize new compounds that possess the two functions, that is, 3-hydroxy-1-oxo-triazol propoyl and polyhydroxyphenyl. The 1,2,3-triazole ring is not only a hydrogen-bond donor but also as a linking unit in the structure scaffold, its planar structure may facilitate the π stacking interaction with target enzymes similarly to phenyl ring. 1,2,3-Triazole moiety can be synthesized by application of synthetic strategy using organoazide and acetyl acetone.[15] Actually, many known 1,2,3-triazoles has been demonstrated to possess varied biochemical properties, including anti-HIV.[16a,b]

Based on the above consideration, we designed (*Z*)-1-(substitutedbenzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(polyhydroxy(or polymethoxy) phenyl) prop-2-en-1-one (Fig. 2) to explore the influence of chemical structure modification with the aim of gaining novel potential anti-IN agents. In this paper, we

* Corresponding author. Tel.: +86 10 6739 6211.
E-mail address: huliming@bjut.edu.cn (L. Hu).

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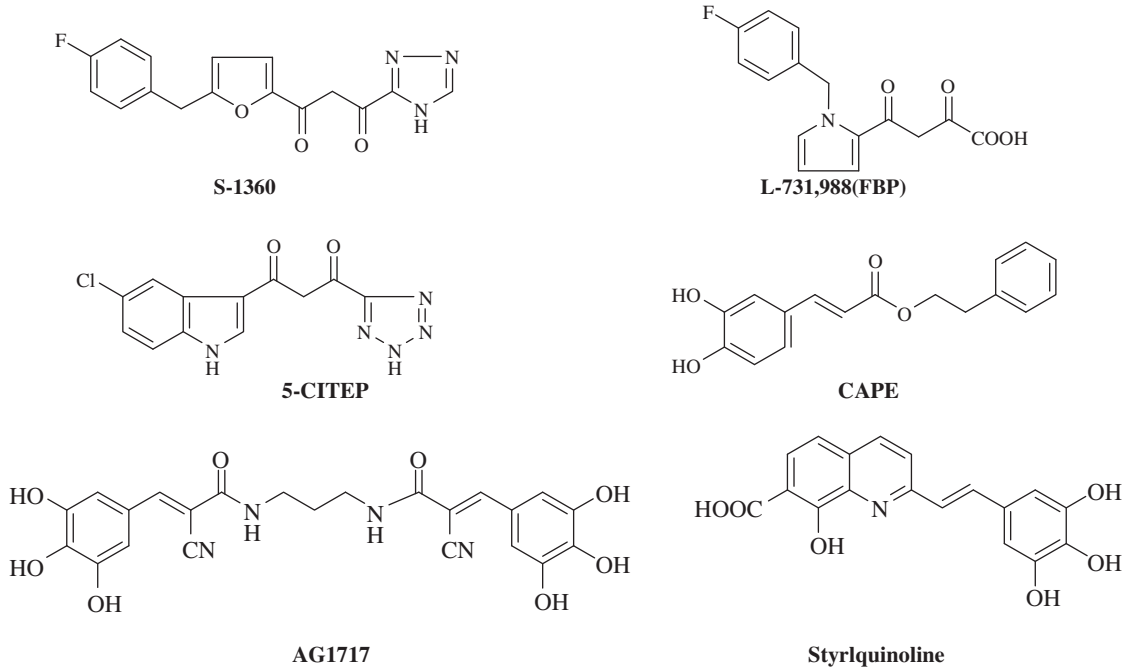


Figure 1. Structure of IN inhibitors.

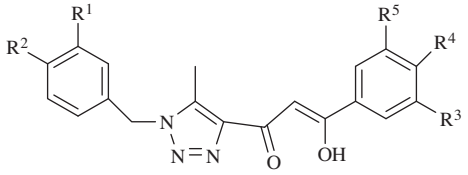


Figure 2. The structures of designed integrase inhibitors.

present the synthesis, the anti-integrase and antiviral activities of these new compounds.

2. Synthesis

(*Z*)-1-(substitutedbenzyl-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(polyhydroxy(or polymethoxy)phenyl)prop-2-en-1-one outlined in Scheme 1 is typical synthesis of 1,3-diketo derivatives. Starting from the commercially available benzyl bromide **1** and sodium azide, benzyl azide **2** can be facile prepared in excellent yields.[15] The key intermediate, 1-benzyl-4-acetyl-5-methyl-1,2,3-triazole **3**, was obtained by reaction of benzyl azide with acetyl acetone in the mixed solution of ethanol and acetonitrile under reflux in the presence of potassium carbonate with a moderate yield.[15] Compounds **3** underwent Claisen condensation with variable methyl polymethoxy benzoate **4** to afford the 1,3-diketo compounds **5** in the alkaline medium (NaH). The compounds **5** were isolated, purified, and also tested as IN inhibitors. Compounds **6** were obtained from compounds **5** by an exhaustive demethylation using boron tribromide in 43–78% yield.

3. Anti-integrase activity

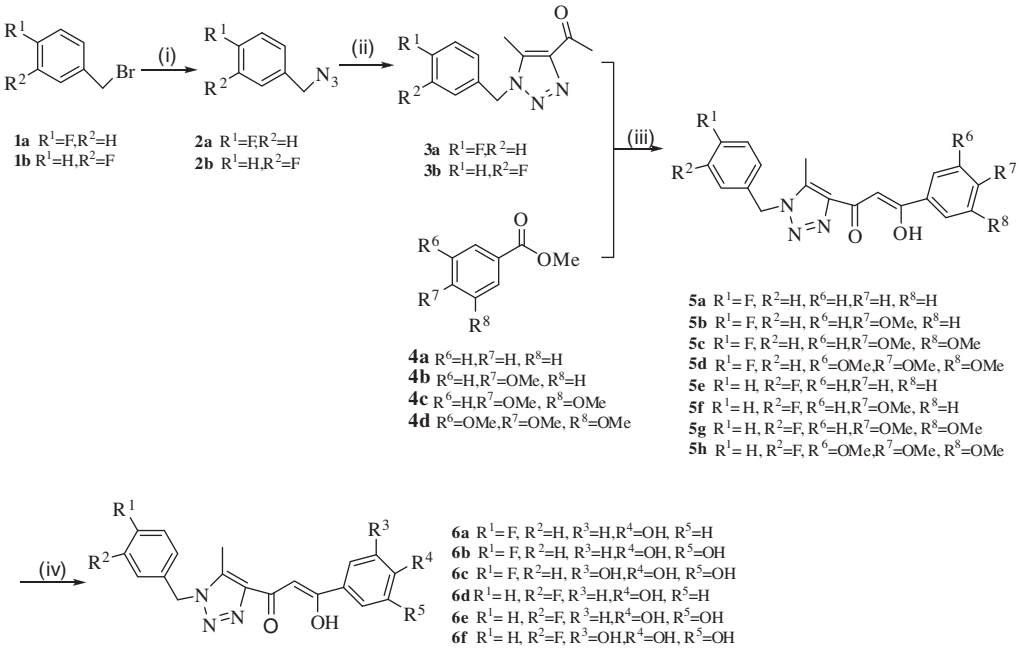
The inhibition effects of 1,3-diketo derivatives **5** and **6** were measured by HIV-1 integrase strand transfer (ST) activity assay, which was carried out as described previously.[17] Compounds **5a–h**, **6a–h** and positive control compound raltegravir were tested against purified IN and the data are summarized in Table 1.

4. Anti-HIV-1 activities

MAGI test, also called single life cycle, reflects only one round of infection. The test compound was added at different hours post the virus inoculation to observe which stage of the viral life cycle could be inhibited by the compound. The cells used were derived from HeLa cell line that both expresses high levels of CD4 and contains a single integrated copy of a β-galactosidase gene under the control of HIV-1 LTR. This cell line, called TZM-bl, can be used to determine quantitatively the titer of HIV wild type strains or HIV pseudoviruses. The inhibitive rate of the test compound could be calculated and the stage upon which the test compound acted could be determined. Compounds **6d** and **6h** were evaluated for their antiviral activity against HIV-1 replication in TZM-bl cells. TZM-bl cells were infected with HIV-1 and subsequently treated with increasing concentrations of drugs. The amount of virus was assayed by β-galactosidase assay, with HeLa-CD4-β-gal cells as reporting cells. Toxicity was estimated by MTT transformation assay. Antiviral properties are reported in Table 2.

5. Discussion

The selectivity of integrase inhibitors for the strand transfer reaction is of particular importance since it seem to be a prerequisite of an antiviral activity correlated with the integrase inhibition.[18] Catechol-DKA hybrids are the examples of the correlation between selectivity toward ST and antiviral activity.[13] The inhibition effects of 1,3-diketo derivatives **5** and **6** were measured by HIV-1 integrase strand transfer (ST) activity assay, which was carried out as described previously with some minor modifications. As shown in Table 1, compounds **5a–h** proved to be inactive in the ST assay (IC_{50} >47 μM), has no or variable methoxy substitutes on the phenyl ring. But replacing methoxy group by a hydroxyl affected the strand transfer reaction. 4-Hydroxylated aromatics derivatives **6a** and **6d** have little inhibition activity with IC_{50} >56 μM. While 3, 4-dihydroxylated aromatics derivatives **6b** and **6e** show anti-IN activity in the low micromolar range. The most potent derivatives are 3,4,5-trihydroxylated aromatics derivatives **6c** and **6f**, which



Scheme 1. Reagents and conditions: (i) NaN_3, CH_3COCH_3/H_2O, rt; (ii) $CH_3COCH_2COCH_3$, K_2CO_3, C_2H_5OH/CH_3CN, reflux; (iii) NaH, THF, reflux; (iv) 1 N BBr_3, CH_2Cl_2, rt, then H_2O.

Table 1
Inhibition of HIV-1integrase strand transfer catalytic activities[a]

Compounds	R^1	R^2	R^3	R^4	R^5	IC_{50}[b] (μM)
5a	F	H	H	H	H	>59
5b	F	H	H	OMe	H	>54
5c	F	H	H	OMe	OMe	>50
5d	F	H	OMe	OMe	OMe	>47
5e	H	F	H	H	H	>59
5f	H	F	H	OMe	H	>54
5g	H	F	H	OMe	OMe	>50
5h	H	F	OMe	OMe	OMe	>47
6a	F	H	H	OH	H	>56
6b	F	H	H	OH	OH	40
6c	F	H	OH	OH	OH	2.6
6d	H	F	H	OH	H	>56
6e	H	F	H	OH	OH	32
6f	H	F	OH	OH		0.78
Raltegravir						0.9

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

Table 2
Antiviral activities of compounds **6d** and **6h** compared to that of raltergravir

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	TI[c]
6c	8.92	33.4	3.7
6f	7.32	36.6	5.0
Raltegravir	0.025	>30	>1250

[a] Cytotoxic concentration 50%.
[b] Effective concentration 50%.
[c] Therapeutic index = CC_{50}/EC_{50}.

IC_{50} values for strand transfer are 2.6 and 0.78 μM, respectively. They are nearly 15-fold and 41-fold higher than **6b** and **6e**. The results indicate that the 1,3-diketo and triazol moieties are not the key active site for the compound. And the inhibitory activity correlates closely to the number of hydroxyl group on phenyl ring. The introduction of a hydroxyl at 3-position of the phenyl in **6b** and **6e** gave **6c** and **6f,** respectively, which exhibited high IC_{50} values. The result indicated the significance of the pyrogallol, and that the aryl substitution could assist the inhibitory activities. The fluorine on the benzyl group exhibit a little influence to inhibitory activity. The 3-fluoride substitute on benzyl group shows more active than 2-fluoride substitute do. Comparison of CC_{50} and EC_{50} of compounds **6c** and **6f** shows that their antiviral properties are quite similar. There are almost same CC_{50} of **6c**, 6f and raltegravir, compared to raltegravir, **6c** and **6f** are less active, and therefore the therapeutic index of **6c** and **6f** is low.

In summary, a series of 1,3-diketo derivatives were facile synthesized and have been identified as HIV-1 integrase inhibitors. The biological results showed that the poly-hydroxylated aromatic moiety plays an important role to inhibit HIV-1 integrase ST reaction. In particular, derivatives with 3, 4, 5-trihydroxylated aromatics subunit is the most potential compounds. And further work based on these structures is in progress.

6. Experimental

Unless otherwise noted, all materials were obtained from commercial suppliers and dried and purified by standard procedures. Melting points were determined on a Beijing keyi elec-opti instrument factory melting point apparatus. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Bruker Avance DRX400 spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent and tetramethyl-silane (TMS) as the internal standard. The chemical shifts were reported in δ (ppm). Mass spectra (MS) data were obtained using Esquire 6000 Mass Spectrometer. HRMS data were measured using Bruker APEX IV Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Petroleum ether used for column-chromatography had a boiling range of 60–90 °C. Purities of all target compounds **5** and **6** were determined by an Agilent 1200 HPLC system with a Agilent Zorbax Extend C-18 column, UV detector at 340 nm, mobile CH_3CN/H_2O (50–100%) and flow rate of 1 mL/min. Compounds **2** and **3** were prepared according to the corresponding literature procedures.[15,19]

6.1. General procedure for the synthesis of 1,3-diketo derivatives 5

To a suspension of sodium hydride (60% dispersion in oil) (20 mmol) in dry tetrahydrofuran (10 mL) was slowly added 1,2,3-triazoles **3** (10 mmol) in dry THF (10 mL) at 0 °Cand the mixture was stirred for 5 min. After that, variable methoxy substituted ethyl benzoate **4** (15 mmol) in dry THF (15 mL) was added to the above solution at 0 °Cand then the reaction mixture was slowly heated to refluxing for about 90 min with stirring till TLC confirmed that the reaction had finished. And then the cooled mixture was poured into a mixture of ice-water (20 mL) and concentrated HCl (5 mL), extracted with EtOAc and purified by flash chromatography on silica gel eluting with petroleum ether/ethyl acetate (4:1).

6.1.1. (*Z*)-1-(1-(4-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-phenylprop-2-en-1-one (5a)

White powder; yield 67%; mp 128–130 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.56 (s, 3H, triazole-CH_3), 5.51 (s, 2H, Ar-CH_2–), 7.07 (t, 2H, J = 8.4 Hz, Ar-*H*), 7.18–7.21 (m, 2H, Ar-*H*), 7.33 (s, 1H, –CO*CH*–), 7.45–7.55 (m, 3H, Ar-*H*), 8.01 (t, 2H, J = 6.4 Hz, Ar-*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 9.4, 51.0, 94.5, 116.3, 116.6, 127.0, 129.1, 129.2, 129.9, 132.3, 134.5, 136.6, 142.1, 181.0, 184.3; HRMS: *m/z* calcd for $C_{19}H_{17}FN_3O_2$: 338.1299; found: 338.1304; calcd for $C_{19}H_{16}FN_3NaO_2$: 360.1118; found: 360.1122; HPLC purity 99.26%.

6.1.2. (*Z*)-1-(1-(4-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(4-methoxyphenyl)prop-2-en-1-one (5b)

White powder; yield 56%; mp 145–146 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.55 (s, 3H, triazole-CH_3), 3.88 (s, 3H, Ar-OCH_3), 5.51 (s, 2H, Ar-CH_2–), 6.96 (d, 2H, J = 9.6 Hz, Ar-*H*), 7.06 (t, 2H, J = 6.4 Hz, Ar-*H*), 7.17–7.21 (m, 2H, Ar-*H*), 7.25 (s, 1H, –CO*CH*–), 7.99 (d, 2H, J = 6.4 Hz, Ar-*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 9.3, 51.0, 55.5, 93.3, 113.9, 116.3, 129.3, 129.9, 131.0, 136.3, 142.0, 163.2, 181.9, 182.6; HRMS: *m/z* calcd for $C_{20}H_{19}FN_3O_3$: 368.1410; found: 368.1408; HPLC purity 98.99%.

6.1.3. (*Z*)-3-(3,4-Dimethoxyphenyl)-1-(1-(4-fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxyprop-2-en-1-one (5c)

White powder; yield 60%; mp 141–142 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.56 (s, 3H, triazole-CH_3), 3.96 (s, 3H, Ar-OCH_3), 3.98 (s, 3H, Ar-OCH_3), 5.51 (s, 2H, Ar-CH_2–), 6.94 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.07 (t, 2H, J = 6.4 Hz, Ar-*H*), 7.17–7.21 (m, 2H, Ar-*H*), 7.27 (s, 1H, –CO*CH*–), 7.53 (d, 1H, J = 2.0 Hz, Ar-*H*), 7.68 (dd, 1H, J = 2.0 and 8.4 Hz, Ar-*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 9.4, 51.0, 56.0, 93.4, 109.5, 110.1, 110.6, 116.3, 121.4, 129.1, 129.3, 129.9, 136.3, 141.9, 149.0, 152.8, 161.5, 181.9, 182.5; HRMS: *m/z* calcd for $C_{21}H_{21}FN_3O_4$: 398.1516; found: 398.1513; HPLC purity 98.45%.

6.1.4. (*Z*)-1-(1-(4-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (5d)

White powder; yield 52%; mp 156–158 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.57 (s, 3H, triazole-CH_3), 3.94 (s, 3H, Ar-OCH_3), 3.96 (s, 6H, Ar-OCH_3), 5.52 (s, 2H, Ar-CH_2-), 7.08 (t, 2H, J = 8.4 Hz, Ar-*H*), 7.19–7.22 (m, 2H, Ar-*H*), 7.24 (s, 2H, Ar-*H*), 7.25 (s, 1H, –CO*CH*–); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 9.3, 51.1, 56.4, 60.9, 93.9, 104.6, 116.0, 116.3, 129.0, 129.2, 129.9, 136.4, 141.8, 153.1, 161.5, 181.7, 182.8; HRMS: *m/z* calcd for $C_{22}H_{23}FN_3O_5$: 428.1622; found: 428.1619; HPLC purity 98.89%.

6.1.5. (*Z*)-1-(1-(3-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-phenyl-prop-2-en-1-one (5e)

White powder; yield 75%; mp 105–107 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.56 (s, 3H, triazole-CH_3), 5.53 (s, 2H, Ar-CH_2–), 6.87–7.05 (m, 3H, Ar-*H*), 7.30–7.32 (m, 1H, Ar-*H*), 7.33 (s, 1H, –CO*CH*–), 7.45–7.55 (m, 3H, Ar-*H*), 7.99–8.01 (m, 2H, Ar-*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 9.4, 51.2, 94.4, 114.2, 114.5, 115.6, 115.9, 122.8, 127.1, 128.7, 130.9, 132.3, 134.5, 136.8, 142.2, 181.1, 184.2; HRMS: *m/z* calcd for $C_{19}H_{17}FN_3O_2$: 338.1299; found: 338.1304; calcd for $C_{19}H_{16}FN_3NaO_2$: 360.1118; found: 360.1123; HPLC purity 98.83%.

6.1.6. (*Z*)-1-(1-(3-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(4-methoxyphenyl)prop-2-en-1-one (5f)

White powder; yield 63%; mp 127–128 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.57 (s, 3H, triazole-CH_3), 3.89 (s, 3H, Ar-OCH_3), 5.55 (s, 2H, Ar-CH_2–), 6.91–6.96 (m, 2H, Ar-*H*), 6.98 (d, 2H, J = 8.8 Hz, Ar-*H*), 7.02–7.07 (m, 1H, Ar-*H*), 7.28 (s, 1H, –CO*CH*–), 7.32–7.38 (m, 2H, Ar-*H*), 8.02 (d, 2H, J = 9.2 Hz, Ar-*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 9.3, 51.1, 55.5, 93.3, 113.9, 114.2, 115.8, 122.7, 127.0, 129.2, 130.9, 136.5, 142.0, 163.2, 182.0, 182.5; HRMS: *m/z* calcd for $C_{20}H_{19}FN_3O_3$: 368.1410; found: 368.1405; HPLC purity 100%.

6.1.7. (*Z*)-3-(3,4-Dimethoxyphenyl)-1-(1-(3-fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxyprop-2-en-1-one (5g)

White powder; yield 61%; mp 122–124 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.57 (s, 3H, triazole-CH_3), 3.97 (s, 3H, Ar-OCH_3), 3.99 (s, 3H, Ar-OCH_3), 5.55 (s, 2H, Ar-CH_2–), 6.89–6.92 (m, 1H, Ar-*H*), 6.98 (t, 2H, J = 8.4 Hz, Ar-*H*), 7.02–7.07 (m, 1H, Ar-*H*), 7.28 (s, 1H, –CO*CH*–), 7.32–7.38 (m, 1H, Ar-*H*), 7.55 (d, 1H, J = 2.0 Hz, Ar-*H*), 7.70 (dd, 1H, J = 2.0 and 8.4 Hz, Ar-*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 9.3, 51.1, 56.1, 77.3, 93.5, 109.7, 110.1, 110.6, 114.2, 115.8, 121.4, 122.9, 127.4, 130.8, 136.5, 141.9, 149.1, 152.7, 181.9, 182.5; HRMS: *m/z* calcd for $C_{21}H_{21}FN_3O_4$: 398.1516; found: 398.1514; HPLC purity 99.54%.

6.1.8. (*Z*)-1-(1-(3-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (5h)

White powder; yield 72%; mp 112–113 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) 2.57 (s, 3H, triazole-CH_3), 3.94 (s, 3H, Ar-OCH_3), 3.96 (s, 6H, Ar-OCH_3), 5.55 (s, 2H, Ar-CH_2–), 6.89–7.07 (m, 3H, Ar-*H*), 7.25 (s, 2H, Ar-*H*), 7.28(s, 1H, –CO*CH*–), 7.31–7.38 (m, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) 9.3, 51.1, 56.3, 61.0, 93.9, 104.4, 114.2, 114.4, 115.6, 115.8, 122.7, 129.8, 130.9, 136.7, 141.9, 153.2, 164.3, 181.8, 182.9; HRMS: *m/z* calcd for $C_{22}H_{23}FN_3O_5$: 428.1622; found: 428.1619; HPLC purity 99.76%.

6.2. General procedure for the synthesis of 1,3-diketo derivatives 6

To a suspension of 1,3-diketo derivatives **6** with methyloxy groups (5 mmol) in dry CH_2Cl_2 (20 mL) was added 1 N tribromoborane solution (15 mL) at 0 °C and the mixture was stirred overnight at room tempreture. After that, water (15 mL) was slowly added to the above solution at 0 °C with stirring, followed by evaporation of the sovent under reduced pressure, extracted with EtOAc and purified by flash chromategraphy on silica gel eluting with petroleum ether/ethyl acetate (1:1).

6.2.1. (*Z*)-1-(1-(4-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(4-hydroxyphenyl)prop-2-en-1-one (6a)

Yellow powder; yield 78%; mp 208–210 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm) 2.58 (s, 3H, triazole-CH_3), 5.67 (s, 2H, Ar-CH_2–), 6.93 (d, 2H, J = 9.6 Hz, Ar-*H*), 7.13 (s, 1H, –CO*CH*–), 7.24 (t, 2H, J = 6.4 Hz, Ar-*H*), 7.29–7.33 (m, 2H, Ar-*H*), 7.91 (d, 2H, J = 6.4 Hz, Ar-*H*), 10.41 (s, 1H, Ar-*OH*); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) 9.4, 50.3, 92.6, 115.8, 116.1, 116.3, 124.8, 129.7, 130.2, 131.5, 137.4, 141.0, 161.0, 182.1, 182.7; HRMS: *m/z* calcd for

$C_{19}H_{17}FN_3O_3$: 354.1248; found: 354.1249; calcd for $C_{19}H_{16}FN_3NaO_3$: 376.1067; found: 376.1068; HPLC purity 98.95%.

6.2.2. (*Z*)-3-(3,4-Dihydroxyphenyl)-1-(1-(4-fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxyprop-2-en-1-one (6b)

Yellow powder; yield 43%; mp 192–194 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm) 2.56 (s, 3H, triazole-CH_3), 5.66 (s, 2H, Ar-CH_2–), 6.96 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.17 (s, 1H, –CO*CH*–), 7.32 (t, 2H, J = 8.8 Hz, Ar-*H*), 7.39–7.49 (m, 4H, Ar-*H*), 9.75 (br, 2H, Ar-*OH*); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) 9.4, 50.3, 92.6, 114.2, 116.1, 116.3, 120.3, 125.2, 130.1, 130.3, 131.8, 137.3, 141.0, 146.0, 151.1, 182.2, 182.4; HRMS: *m/z* calcd for $C_{19}H_{17}FN_3O_4$: 370.1197; found: 370.1203; calcd for $C_{19}H_{16}FN_3NaO_4$: 392.1017; found: 392.1021; HPLC purity 100%.

6.2.3. (*Z*)-1-(1-(4-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(3,4,5-trihydroxyphenyl)prop-2-en-1-one (6c)

Yellow powder; yield 57%; mp 187–189 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm) 2.56 (s, 3H, triazole-CH_3), 5.65 (s, 2H, Ar-CH_2–), 6.99 (s, 2H, Ar-*H*), 7.00 (s, 1H, –CO*CH*–), 7.23 (t, 2H, J = 8.8 Hz, Ar-*H*), 7.29–7.33 (m, 2H, Ar-*H*), 9.27 (br, 3H, Ar-*OH*); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) 9.4, 50.4, 92.6, 106.7, 108.5, 116.0, 116.3, 123.9, 130.2, 137.3, 139.2, 141.1, 146.5, 182.2, 182.3; HRMS: *m/z* calcd for $C_{19}H_{17}FN_3O_5$: 386.1146; found: 386.1151; calcd for $C_{19}H_{16}FN_3NaO_5$: 408.0966; found: 408.0972; HPLC purity 100%.

6.2.4. (*Z*)-1-(1-(3-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(4-hydroxyphenyl)prop-2-en-1-one (6d)

Yellow powder; yield 55%; mp 184–186 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm) 2.61 (s, 3H, triazole-CH_3), 5.78 (s, 2H, Ar-CH_2–), 6.98 (d, 2H, J = 6.4 Hz, Ar-*H*), 7.08–7.16 (m, 2H, Ar-*H*), 7.19 (s, 1H, –CO*CH*–), 7.21–7.26 (m, 1H, Ar-*H*), 7.46–7.51 (m, 1H, Ar-*H*), 7.91 (d, 2H, J = 6.4 Hz, Ar-*H*), 10.49 (s, 1H, Ar-*OH*); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) 9.4, 50.4, 92.6, 114.8, 115.4, 115.8, 116.2, 123.9, 124.8, 129.7, 131.5, 137.6, 138.3, 141.0, 162.9, 181.1, 182.4; HRMS: *m/z* calcd for $C_{19}H_{17}FN_3O_3$: 354.1248; found: 354.1251; calcd for $C_{19}H_{16}FN_3NaO_3$: 376.1067; found: 392.1021; HPLC purity 92.70%.

6.2.5. (*Z*)-3-(3,4-Dihydroxyphenyl)-1-(1-(3-fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxyprop-2-en-1-one (6e)

Yellow powder; yield 66%; mp 200–201 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm) 2.57 (s, 3H, triazole-CH_3), 5.70 (s, 2H, Ar-CH_2–), 6.90 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.05 (d, 1H, J = 7.6 Hz, Ar-*H*), 7.10 (s, 1H, –CO*CH*–), 7.20 (t, 2H, J = 8.4 Hz, Ar-*H*), 7.37–7.46 (m, 3H, Ar-*H*), 9.95 (br, 2H, Ar-*OH*); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) 9.4, 50.5, 92.6, 114.3, 114.9, 115.3, 115.6, 116.3, 120.3, 123.8, 125.2, 131.5, 137.5, 138.1, 141.0, 146.0, 151.1, 182.2, 182.4; HRMS: *m/z* calcd for $C_{19}H_{17}FN_3O_4$: 370.1197; found: 370.1201; calcd for $C_{19}H_{16}FN_3NaO_4$: 392.1017; found: 376.1020; HPLC purity 99.67%.

6.2.6. (*Z*)-1-(1-(3-Fluorobenzyl)-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxy-3-(3,4,5-trihydroxyphenyl)prop-2-en-1-one (6f)

Yellow powder; yield 59%; mp 213–215 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm) 2.57 (s, 3H, triazole-CH_3), 5.71 (s, 2H, Ar-CH_2–), 7.00 (s, 2H, Ar-*H*), 7.02 (s, 1H, –CO*CH*–), 7.04–7.21 (m, 3H, Ar-*H*), 7.41–7.47 (m, 1H, Ar-*H*), 9.14 (s, 1H, Ar-*OH*), 9.31 (s, 2H, Ar-*OH*); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm) 9.4, 50.4, 92.6, 106.7, 108.5, 114.7, 114.9, 123.9, 124.0, 131.5, 137.5, 139.2, 141.1, 146.1, 146.5, 182.2, 182.4; HRMS: *m/z* calcd for $C_{19}H_{17}FN_3O_5$: 386.1146; found: 386.1150; calcd for $C_{19}H_{16}FN_3NaO_5$: 408.0966; found: 408.0970; HPLC purity 97.33%.

6.3. HIV-1 integrase inhibitory assay: oligonucleotides

(1) Donor DNA: D01:5′biotin-CCCTTTTAGTCAGTGTG-GAAAATCTCT AGCA3′; D02: 3′GAAAATCAGTCACACCTTTTAGAGA TCGTCA5′.

(2)Target DNA: T01: 5′TGACCAAGGGCTAATTCACT-digoxin 3′; T02: 3′digoxin-ACTGGTTCCCGATTAAGTGA-5′.

D01 + D02 and T01 + T02 were mixed in a ratio of 1:1 at a concentration of 100 μm/L in purified water, heated to 94 °C for 5 min, slowly cooled down to room temperature, and stored at −20 °C until use.

6.4. HIV-1 integrase inhibitory assay

Compounds diluted in DMSO were pre-incubated with 800 ng integrase at 37.8 °C in the reaction buffer in the absence of Mn^{2+} for 10 min. Subsequently, 1.5 pmol donor DNA and 9 pmol target DNA were added and the reaction was initiated by the addition of 10 mmol/L Mn^{2+} into the final reaction volume. The reactions were carried out at 37.8 °C for 1 h and subsequent detection procedure was applied to detect the assay signals. Integrase inhibitor, raltergravir, was used as the control compound (positive control), whereas no compound but only DMSO in the reaction mixture was set as the drug-free control (negative control). The inhibition effects of 1,3-diketo derivatives **5** and **6** were calculated based on the positive and negative controls.

6.5. Antiviral assays

The HeLa-CD_4-LTR-β-gal indicator cells were plated in 96-well plates at 6000 cells per well. The highest concentration of the test compounds was 200 μg/mL, then diluted by four-fold serially. There were 5 dilutions and 4 duplicates for each dilution. The wells added only with virus-infected cells and without compounds were used as viral-control and the wells added only with mock-infected cells and without virus or compounds were used as cell-control. The supernatant in the test wells and the viral-control wells was discarded, and infected with 100 μL 2000 $TCID_{50}$ HIV-1 pseudoviruses. Then 100 μL diluted test compounds described above was added to the wells, making the final concentration of the test compounds decreased by half. The cell cultures were incubated at 37 °C in 5% CO_2 humidified atmosphere for 40–48 h, then fixed and stained. The blue cells were counted under an inverted microscope.

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References and notes

1. Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Disc.* **2005**, *4*, 236.
2. Ramkumar, K.; Serrao, E.; Odde, S.; Neamati, N. *Med. Res. Rev.* **2010**, *30*, 890.
3. Grobler, J. A.; Stillmock, K.; Hu, B.; Witmer, M.; Felock, P.; Espeseth, A. S.; Wolfe, A.; Egbertson, M.; Bourgeois, M.; Melamed, J.; Wai, J. S.; Young, S.; Vacca, J.; Hazuda, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6661.
4. Barreca, M. L.; Ferro, S.; Rao, A.; Luca, L. D.; Zappala, M.; Monforte, A. M.; Debyser, Z.; Witvrouw, M.; Chimirri, A. *J. Med. Chem.* **2005**, *48*, 7084.
5. Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinage, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13040.
6. Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M.; Guare, J. P., Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I. W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11233.
7. Billich, A. *Curr. Opin. Investig. Drugs* **2003**, *4*, 206.
8. Dayam, R.; Al-Mawsawi, L. Q.; Neamati, N. *Drugs R D* **2007**, *8*, 155.
9. Kawasuji, T.; Fuji, M.; Yoshinaga, T.; Sato, A.; Fujiwara, T.; Kiyama, R. *Bioorg. Med. Chem.* **2006**, *14*, 8420.
10. Wang, P.; Liu, C.; Sanches, T.; Zhong, Y.; Liu, B.; Xiong, J.; Neamati, N.; Zhao, G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4574.

11. (a) Mazumder, A.; Gazit, A.; Levitzki, A.; Nicklaus, M.; Yung, J.; Kohlhagen, G.; Pommiers, Y. *Biochemistry* **1995**, *34*, 15111; (b) Rabaaoui, S.; Zouhiri, F.; Lançon, A.; Leh, H.; Angelo, J.; Wattel, E. *Antimicrob. Agents Chemother.* **2008**, *52*, 3532.
12. Yang, L. F.; Xu, X. M.; Huang, Y. L.; Zhang, B.; Zeng, C. C.; He, H. Q.; Wang, C. X.; Hu, L. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5469.
13. Maurin, C.; Bailly, F.; Mbemba, G.; Mouscadet, J. F.; Cotelle, P. *Bioorg. Med. Chem.* **2006**, *14*, 2978.
14. Crosby, D. C.; Lei, X.; Gibbs, C. G.; McDougall, B. R.; Robinson, W. E.; Reinecke, M. G. *J. Med. Chem.* **2010**, *53*, 8161.
15. Kamalraj, V. R.; Senthil, S.; Kannan, P. *J. Mol. Struct.* **2008**, *892*, 210.
16. (a) Giffin, M. J.; Heaslet, H.; Brik, A.; Lin, Y. C.; Cauvi, G.; Wong, C. H.; McRee, D. E.; Elder, J. H.; Stout, C. D.; Torbett, B. E. *J. Med. Chem.* **2008**, *51*, 6263; (b) Alvarez, R.; Velazquez, S.; San-Felix, A.; Aquaro, S.; Clercq, E. D.; Perno, C. F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 4185.
17. He, H. Q.; Ma, X. H.; Liu, B.; Chen, W. Z.; Wang, C. X.; Cheng, S. H. *Acta Pharmacol. Sin.* **2008**, *29*, 397.
18. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. *Science* **2000**, *287*, 646.
19. Barral, K.; Moorhouse, A. D.; Moses, J. E. *Org. Lett.* **2007**, *9*, 1809.