Supporting information

Design, synthesis and biological evaluation of caffeoyl – benzanilidesas dual inhibitors of HIV integrase and CCR5

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Supporting information

Chemistry

NMR spectra were recorded on a Bruker AVANCE III-400 spectrometer with TMS as an internal standard, and $CDCl_3$ and DMSO-d6 as the solvents. The chemical shifts were expressed in parts per million (δ ppm). Coupling constants (J) were measured in Hz. MS were taken using a Bruker Apex IV FTMS with methanol as the solvent. Melting points were measured on an X-4 apparatus without correction. Silica gel H was used for column chromatography and silica gel GF_{254} was used for TLC plates, which were used to monitor the reactions. All reagents were purchased from commercial sources. When necessary, they were purified and dried by standard methods.

General procedure for the synthesis of compounds 4a-f.

To the solution of **3a-f** (3 mmol) in THF (20 mL), 3, 4-dihydroxy benzaldehyde (3.2 mmol), the pyrrolidine (catalytic amount) and acetic acid (catalytic amount) were added. The resulting mixture was heated to reflux. After the reaction completed, the mixture was concentrated in vacuo, diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried by Na_2SO_4, and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether: ethyl acetate) afforded the corresponding compounds **4a-f**.

***(E)*-N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopent-4-en-1-yl)phenyl)benzamide (4a)** White solid(347.1mg, 30%), mp: 198-200°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.84-2.87(m, 2H, CH_2), 2.96-3.00(m, 2H, CH_2), 6.58-7.97 (m, 14H, ArH,CH=CH), 10.20(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 29.7, 41.7, 115.2, 116.3, 120.9, 128.1, 128.8, 128.9, 131.9, 135.5, 137.2, 137.5, 143.6, 165.8, 199.3; HRMS (ESI): m/z, calcd. for $C_{24}H_{21}NO_4$ $[M-H]^-$: 386.1471, found 386.1459.

***(E)*-N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopent-4-en-1-yl)phenyl)-4-nitrobenzamide(4b)**White solid (323.2mg, 25%). mp: 193-196°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.84-2.87(m, 2H, CH_2),

2.97-3.00(m, 2H, CH_2), 6.59-8.19 (m, 13H, ArH,CH=CH), 9.643(s, 1H, OH), 9.651(s, 1H, OH), 10.517(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 29.6, 41.6, 115.3, 116.2, 120.9, 123.5, 124.0, 126.3, 128.8, 129.0, 129.6, 137.0, 137.7, 141.1, 143.5, 146.1, 149.5, 166.8, 199.2; HRMS (ESI): m/z, calcd. for $C_{24}H_{20}N_2O_6$ $[M-H]^-$: 431.1321, found 431.1303.

***(E)*-N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopent-4-en-1-yl)phenyl)cyclohexanecarboxamide(4c)**
White solid(258.9mg, 22%). mp: 86-89°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 1.15-1.23(m, 11H, C_5H_{11}), 2.92-2.95 (m, 2H, CH_2), 3.40-3.50(m, 2H, CH_2), 6.57-7.726 (m, 9H, ArH,CH=CH), 9.172(s, 1H, NH).**^{13}C-NMR** (100MHz, DMSO-d_6) δ: 21.2, 25.7, 25.9, 27.7, 29.6, 45.3, 115.2, 118.0, 119.5, 122.1, 126.3, 128.8, 129.1, 131.4, 132.0, 137.9, 143.4, 145.7, 146.1, 170.8, 199.2;HRMS (ESI): m/z, calcd. for $C_{24}H_{27}NO_4$ $[M-H]^-$: 392.1940, found 392.1904.

***(E)*-N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopent-4-en-1-yl)phenyl)-4-methylbenzamide (4d)** White solid(228.1mg, 19%). mp: 209-212°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.38(s, 3H, CH_3), 2.82-2.86(m,2H, CH_2), 2.95-2.99(m, 2H, CH_2), 6.58-7.88 (m, 13H, ArH,CH=CH), 10.095(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 28.2, 29.6, 41.7, 115.2, 116.3, 120.8, 122.2, 123.3, 126.0, 128.6, 136.5, 137.7, 143.5, 146.3, 176.3, 199.3;HRMS (ESI): m/z, calcd. for $C_{25}H_{23}NO_4$ $[M-H]^-$: 400.1627, found 400.1602.

***(E)*-N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopent-4-en-1-yl)phenyl)-4-methoxybenzamide (4e)**
White solid(149.8mg, 12%). mp: 215-218°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.84-2.88(m, 2H, CH_2), 2.96-3.00 (m, 2H, CH_2), 3.84(s, 3H, OCH_3), 6.59-7.99(m, 13H, ArH,CH=CH), 10.017(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 29.6, 41.7, 55.7, 113.9, 115.2, 116.2, 120.8, 122.0, 123.4, 126.3, 127.5, 128.8, 130.0, 136.8, 137.7, 143.4, 146.1, 148.9, 162.3, 165.1, 170.7, 172.4, 199.1;HRMS (ESI): m/z, calcd. for $C_{25}H_{23}NO_5$ $[M-H]^-$: 416.1576, found 416.1561.

***(E)*-N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopent-4-en-1-yl)phenyl)-2-(thiophen-2-yl)acetamide (4f)**
White solid(353.22mg, 29%). mp: 234-238°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.80-2.83(m, 2H, CH_2), 2.93-2.97(m, 2H, CH_2), 3.85(s, 2H, CH_2), 6.58-7.499 (m, 12H, ArH, CH=CH), 10.118(s, 1H, NH).**^{13}C-NMR** (100MHz, DMSO-d_6) δ: 29.6, 38.0, 41.7, 115.3, 116.3, 119.7, 122.1, 123.5, 125.5, 126.3, 126.7, 127.1, 129.0, 136.8, 137.4, 137.7, 143.4, 146.1, 148.9, 168.2, 199.2;HRMS (ESI): m/z, calcd. for $C_{23}H_{21}NO_4S$ $[M-H]^-$:406.1113, found 406.1103

General procedure for the synthesis of compounds 5a-f
The E-3,4-dihydroxy styryl aralkyl ketones **4a-f** (1 mmol) was added to a solution of the pyridine (2.2 mmol) solution in acetic anhydride (3 mL) and stirred at r.t . After the reaction was completed (TLC), the reaction mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried by Na_2SO_4, and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether: ethyl acetate) to give the compound **5a-f**.

***(E)*-N-(4-(5-(3,4-diacetylphenyl)-3-oxopent-4-en-1-yl)phenyl) benzamide(5a)** white solid(378.5mg, 80%). mp: 66-69°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.30(s, 6H, $2CH_3$), 2.52-4.19(m, 4H, $2CH_2$), 6.10-7.87(m, 14H, ArH,CH=CH),. **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 20.6,

29.7, 42.7, 120.7, 123.4, 124.4, 127.1, 128.8, 129.0, 130.8, 136.4, 141.1, 142.1, 168.4, 171.3;HRMS (ESI): m/z, calcd. for $C_{28}H_{25}NO_6$ $[M+H]^+$: 472.1682, found 472.1672.

***(E)*-N-(4-(5-(3,4-diacetylphenyl)-3-oxopent-4-en-1-yl)phenyl) -4-nitrobenzamide** (**5b**)white solid (408.9mg, 79%). mp: 189-191°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.29-2.30(d, 6H, $2CH_3$), 2.88-3.04(m, 4H, $2CH_2$,), 6.92-7.69(m, 13H, ArH,CH=CH), 10.51(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 20.7, 29.8, 42.5, 120.8, 122.9, 124.0, 126.7, 127.0, 128.3, 129.2, 133.3, 135.4, 138.2, 140.5, 140.8, 142.5, 143.6, 149.7, 168.0, 168.2, 198.955; HRMS (ESI): m/z, calcd. for $C_{28}H_{24}N_2O_8$ $[M+H]^+$: 517.1533, found 517.1514.

***(E)*-N-(4-(5-(3,4-diacetylphenyl)-3-oxopent-4-en-1-yl)phenyl) cyclohexanecarboxamide**(**5c**)white solid (396.7mg, 83%). mp: 130-132°C. **^{1}H-NMR**(400 MHz, DMSO-d_6) δ: 1.28-2.25(m, 11H, C_6H_{11}), 2.32 (s, 6H, $2OCH_3$), 2.94-2.96(m, 4H, $2CH_2$), 6.64-7.47(m, 9H, ArH, CH=CH); **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 20.7, 25.7, 29.6, 29.7, 42.8, 46.5, 120.0, 122.9, 124.0, 126.7, 127.0, 128.9, 133.4, 136.3, 136.8, 140.7, 142.5, 143.6, 168.0, 168.1, 174.3, 199.0; HRMS (ESI): m/z, calcd. for $C_{28}H_{31}NO_6$ $[M+H]^+$: 478.2151, found 478.2139.

(E)-N-(4-(5-(3,4-diacetylphenyl)-3-oxopent-4-en-1-yl)phenyl)-4-methylbenzamide (**5d**) white solid (393.8mg, 81%). mp: 152-155°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.32 (d, 6H, $2CH_3$), 2.44-2.99(m, 4H, $2CH_2$), 3.00(s, 3H, CH_3), 6.67-7.80 (m, 13H, ArH;CH=CH), 7.82(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 20.7, 21.5, 29.6, 42.8, 120.4, 122.9, 124.0, 126.7, 127.0, 129.0, 129.4, 133.4, 136.2, 137.2, 140.7, 142.3, 142.5, 143.6, 168.1, 199.0; HRMS (ESI): m/z, calcd. for $C_{29}H_{27}NO_6$ $[M+H]^+$: 486.1838, found 486.1817.

(E)-N-(4-(5-(3,4-diacetylphenyl)-3-oxopent-4-en-1-yl)phenyl)-4-methoxybenzamide (**5e**) white solid (391.7mg, 78%). mp: 142-145°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 1.72(s, 6H, $2CH_3$), 2.84(s, 3H, OCH_3), 3.64-3.84(m, 4H, $2CH_2$), 6.26-7.97(m, 13H, ArH,CH=CH), **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 19.2, 20.6, 29.6, 42.7, 55.5, 114.0, 120.5, 122.9, 124.0, 126.7, 127.0, 127.1, 128.9, 129.0, 130.9, 133.4, 136.3, 137.1, 140.7, 142.5, 143.6, 165.2, 168.0, 168.1, 199.0; HRMS (ESI): m/z, calcd. for $C_{29}H_{27}NO_7$ $[M+H]^+$: 502.1788, found 502.1763..

***(E)*-N-(4-(5-(3,4-diacetylphenyl)-3-oxopent-4-en-1-yl)phenyl)-2-(thiophen-2-yl)acetamide**(**5f**) white solid (393.7mg, 80%). mp: 192-194°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.29-2.30(d, 6H, $2OCH_3$), 2.82-2.85(m, 2H, CH_2), 2.96-3.02(m, 2H, CH_2), 3.86(s, 2H, CH_2), 6.83-7.68(m, 12H, ArH, CH=CH); **^{13}C-NMR** (100MHz, DMSO-d6) δ: 19.2, 20.7, 29.6, 38.4, 42.6, 120.3, 122.9, 124.0, 126.0, 126.7, 126.9, 127.5, 127.7, 128.9, 131.0, 133.3, 135.8, 137.4, 140.8, 142.5, 143.6, 168.0, 168.1, 199.0;HRMS (ESI): m/z, calcd. for $C_{24}H_{21}NO_4SI$ $[M-H]^+$:492.1481, found 492.1465.

General procedure for the synthesis of compounds 9a-f.

To the solution of **8** in CH_2Cl_2, various acid chlorides (1.0 equiv) were added. Reaction mixture was allowed to stir for 2h at room temperature. After the reaction was completed (TLC), the combined organic fractions were washed with brine, dried by Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by column chromatography (petroleum ether: ethyl acetate) to give the compound **9a-f**.

N-(4-(5-(3,4-diacetylphenyl)-3-oxopentyl)phenyl)benzamide (**9a**) White solid (384.1mg, 81%). mp: 123- 124°C. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ: 2.24(s, 3H, CH_3), 2.26(s, 3H, CH_3), 2.63-2.70(m, 4H, $2CH_2$), 2.82-2.88(m, 4H, $2CH_2$), 6.97-7.90(m, 12H, ArH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 20.6, 28.6, 29.5, 44.3, 44.4, 120.5, 123,2, 123.4, 126.7, 127.2, 128.4, 128.6, 128.8, 130.1, 131.7, 133.5, 135.1, 136.3, 136.8, 140.1, 141.8, 165.8, 168.6, 208.9; HRMS (ESI): m/z, calcd. for $C_{28}H_{27}NO_6$ $[M+H]^+$: 474.1838, found 474.1827.

N-(4-(5-(3,4- diacetylphenyl)-3-oxopentyl)phenyl) -4-nitrobenzamide(**9b**) White solid(414.5mg, 80%). mp: 127-129°C. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ: 2.21-2.29(d, 6H, $2CH_3$), 2.61-2.71(m, 4H, $COCH_2$; CH_2), 2.82-2.91(m, 4H, $COCH_2$; CH_2), 6.99-7.08(m, 5H, ArH), 7.47-7.49(d, 2H, m-ArH, J = 8 Hz), 8.05-8.08(d, 2H, o-ArH, J = 8.4 Hz), 8.29-8.31(d, 2H, m-ArH, J = 8.4 Hz). **^{13}C-NMR** (100MHz, $CDCl_3$) δ: 20.6, 20.8, 28.5, 29.8, 44.0, 44.7, 120.6, 123.1, 123.5, 123.8, 126.9, 128.4, 128.9, 135.6, 137.5, 140.2, 140.6, 141.7, 149.6, 163.6, 168.9, 209.1; HRMS (ESI): m/z, calcd. for $C_{28}H_{26}N_2O_8$ $[M+H]^+$: 518.1689, found 518.1676.

N-(4-(5-(3,4-diacetylphenyl)-3-oxopentyl)phenyl)cyclohexanecarboxamide(**9c**)Yellow solid(369.8mg, 77%). mp: 97-100°C. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ: 1.25-1.58(m, 11H, C_6H_{11}), 2.13(s,6H, $2CH_3$), 2.64-2.69(m, 4H, $2CH_2$), 2.83-2.86(m, 4H, $2CH_2$), 6.99-7.41(m, 7H, ArH). **^{13}C-NMR** (100MHz, $CDCl_3$) δ: 20.7, 25.7, 28.7, 29.6, 44.3, 44.4, 46.4, 120.0, 123.0, 123.4, 126.7, 128.7, 136.8, 140.1, 141.8, 168.4, 168.6, 174.5, 208.9; HRMS (ESI): m/z, calcd. for $C_{28}H_{33}NO_6$ $[M+H]^+$: 480.2308, found 480.2289.

N-(4-(5-(3,4-diacetylphenyl)-3-oxopentyl)phenyl) -4-methylbenzamide(**9d**) White solid(366.1mg, 75%). mp: 89-92°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.26(s, 3H, CH_3), 2.28(s, 3H, CH_3), 2.43(s, 3H, CH_3), 2.64-2.71(m, 4H, $2CH_2$), 2.84-2.90(m, 4H, $2CH_2$), 6.98-7.80(m, 11H, ArH), 8.03(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 20.6, 20.7, 21.5, 28.7, 29.5, 44.3, 44.4, 120.4, 123.2, 123.4, 126.7, 127.1, 128.8, 129.3, 132.2, 136.3, 136.7, 140.2, 141.8, 142.2, 168.4, 168.6, 208.9; HRMS (ESI): m/z, calcd. for $C_{29}H_{29}NO_6$ $[M-H]^+$: 488.1995, found 488.1982.

N-(4-(5-(3,4-diacetylphenyl)-3-oxopentyl)phenyl)-4-methoxybenzamide(**9e**)Gray solid (403.2mg, 80%). mp: 111-113°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.27(s, 3H, CH_3), 2.28(s, 3H, CH_3), 2.66-2.70(m, 4H, $2CH_2$), 2.86-2.88(m, 4H, $2CH_2$), 3.88(s, 3H, CH_3), 6.95-7.10(m, 11H, ArH), 7.97(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 20.6, 20.7, 28.7, 29.5, 44.3, 44.4, 55.5, 113.9, 120.4, 123.2, 123.4, 126.7, 127.2, 128.8, 129.0, 136.4, 136.6, 140.1, 140.2, 141.8, 162.4, 165.2, 168.4, 168.6, 208.9; HRMS (ESI): m/z, calcd. for $C_{29}H_{29}NO_7$ $[M-H]^+$: 504.1944, found 504.1932.

N-(4-(5-(3,4-diacetylphenyl)-3-oxopentyl)phenyl)-2-(thiophen-2-yl)acetamide(**9f**) Brown solid (360.1mg, 75%). mp: 58-61°C. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ: 2.30(s, 6H, $2CH_3$), 2.64-2.68(m, 4H, $2CH_2$), 2.83-2.86(m, 4H, $2CH_2$), 7.00-7.07(m, 10H, ArH). **^{13}C-NMR** (100MHz, $CDCl_3$) δ: 20.6, 21.0, 28.7, 29.3, 44.2, 44.3, 120.1, 123.2, 123.3, 125.7, 126.6, 127.4, 128.7, 135.8, 135.9, 137.0, 140.0, 141.8, 167.9, 168.3, 168.5, 208.7; HRMS (ESI): m/z, calcd. for $C_{26}H_{25}NO_6S$ $[M-H]^+$:

480.1403, found 480.1392.

General procedure for the synthesis of compounds 10a-f.

Hydrochloric acid were added to a solution of the **9a-f** (1 mmol) solution in a mix solution of cetone and methanol (10 mL) and the resulting mixture was heated to reflux. After the reaction was completed (TLC), the combined organic fractions were washed with brine, dried by Na_2SO_4, and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether: ethyl acetate) to give the title compound.

N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopentyl)phenyl)benzamide(**10a**)Whitesolid(232.8mg, 60%). mp: 107-111°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.61-2.67(m, 4H, $2CH_2$), 2.75(m, 4H, $2CH_2$), 6.42-7.96(m, 12H, ArH), 8.61(s, 1H, OH), 8.70(s, 1H, OH), 10.17(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 29.0, 43.9, 44.3, 115.9, 119.2, 120.9, 128.1, 128.8, 131.9, 132.4, 135.5, 137.0, 137.5, 143.8, 145.5, 165.8, 209.7; HRMS (ESI): m/z, calcd. for $C_{24}H_{23}NO_4$ $[M-H]^-$: 388.1627, found 388.1614.

N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopentyl)phenyl) -4-nitrobenzamide(**10b**) White solid (259.6mg, 66%). mp: 60-62°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.60-2.66(m, 4H, $2CH_2$), 2.75(m, 4H, $2CH_2$), 6.40-8.38(m, 11H, ArH), 8.61(s, 1H, OH), 8.70(s, 1H, OH), 10.20(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 29.0, 43.9, 44.3, 55.4, 60.2, 115.9, 116.1, 119.2, 121.0, 124.0, 128.9, 129.6, 132.3, 137.0, 137.5, 141.1, 143.7, 145.5, 149.6, 209.6; HRMS (ESI): m/z, calcd. for $C_{24}H_{22}N_2O_6$ $[M-H]^-$: 433.1478, found 433.1465.

N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopentyl)phenyl)cyclohexanecarboxamide(**10c**) Yellow solid (323.08mg, 82%). mp: 122-125°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 1.17-2.33(t, 11H, C_6H_{11}), 2.59-2.65(m, 4H, $2CH_2$), 2.70(m, 4H, $2CH_2$), 6.40-7.09(m, 7H, ArH), 8.60(s, 1H, OH), 8.69(s, 1H, OH), 9.69(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 25.7, 29.0, 29.6, 44.0, 44.3, 45.3, 115.9, 116.1, 119.2, 128.7, 132.4, 136.0, 137.9, 143.7, 145.5, 174.5, 209.7; HRMS (ESI): m/z, calcd. for $C_{24}H_{29}NO_4$ $[M-H]^-$: 394.2097, found 394.2088.

N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopentyl)phenyl)-4-methylbenzamide (**10d**) White solid (273.36mg, 68%). mp: 118-121°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.39(s, 3H, CH_3), 2.60-2.66(m, 4H, $2CH_2$), 2.74(m, 4H,$2CH_2$), 6.40-7.88(m, 11H, ArH), 8.60(s, 1H, OH), 8.69 (s, 1H, OH), 10.07(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 21.2, 29.0, 43.9, 44.3, 115.9, 116.1, 119.2, 120.9, 128.1, 128.7, 129.3, 132.4, 132.6, 136.8, 137.6, 141.9, 143.8, 145.5, 165.6, 209.7; HRMS (ESI): m/z, calcd. for C25H25NO4 $[M-H]^-$: 402.1784, found 402.1773.

N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopentyl)phenyl)-4-methoxybenzamide (**10e**) Brown solid (271.2mg, 65%). mp: 122-125°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.60-2.66(m, 4H, $2CH_2$), 2.73(m, 4H, $2CH_2$), 3.84(s, 3H, OCH_3), 6.40-7.97(m, 11H, ArH), 10.03(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 29.0, 43.9, 44.3, 55.9, 114.0, 116.1, 116.3, 119.1, 120.9, 127.5, 128.7, 130.0, 136.7, 143.9, 145.6, 162.3, 165.2, 209.7; HRMS (ESI): m/z, calcd. for $C_{25}H_{25}NO_5$ $[M-H]^-$:

418.1733, found 418.1714.

N-(4-(5-(3,4-dihydroxyphenyl)-3-oxopentyl)phenyl) -2-(thiophen-2-yl)acetamide（**10f**）Brown solid (330.6mg, 81%). mp: 58-61°C. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 2.51 (m, 4H, $2CH_2$), 2.70(m, 4H, $2CH_2$), 3.86(s, 2H, $COCH_2$), 6.39-7.50(m, 10H, ArH), 10.20(s, 1H, NH). **^{13}C-NMR** (100MHz, DMSO-d_6) δ: 29.0, 37.9, 44.3, 115.9, 116.1, 119.2, 119.8, 125.5, 126.7, 127.1, 128.9, 132.3, 136.6, 137.4, 137.7, 143.8, 145.5, 168.3, 209.7; HRMS (ESI): m/z, calcd. for $C_{23}H_{23}NO_4S$ $[M-H]^-$: 408.1348, found 408.1339.

Biological assays

SPR

Immobilization of proteins. IN proteins were immobilized onto a CM5 sensor chip using standard amine-coupling chemistry at 25 °C. PBS-P was used as the running buffer. The carboxymethyl dextran surface was activated with a 7-min injection of a 1:1 ratio of 400 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)/100 mM N-hydroxysuccinimide (NHS). IN were diluted in 10 mM sodium acetate (pH 5.0) to 50 μg/mL and coupled in separate flow cells with a 7-min injection. Remaining activated groups were blocked with a 7-min injection of 1M ethanolamine (pH 4.5). Protein immobilization levels typically achieved were 10000RU.

Compound screening. The 10mM compound stocks were diluted a further 20-fold in PBS-P screening buffer to obtain 500μM concentrations in PBS-P buffer containing 5% (v/v) DMSO. Screening experiments were run at 20 °C with PBS-P running buffer supplemented with 5% (v/v) DMSO. Compounds were screened using a 96-well format with an association and dissociation time of 60s each. A concentration series in 2-fold dilutions (1.56–100 μM) in PBS-P/5% DMSO under the same conditions as described above was used to confirm hits.

Data processing. Raw sensorgram data were reduced, solvent corrected, and double referenced using the Scrubber 2 software package (BioLogic Software, Campbell, Australia). The binding affinity of the compounds was fit to a 1:1 binding model or steady state affinity.

Cell based assay about CCR5

Cytotoxicity

The 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay was used to assess the cytotoxicity of the compounds tested for anti-HIV-1 activity. Briefly, graded concentrations of inhibitors were added to TZM-bl cells at 5×10^4/well and incubated at 37°C for 3 days. Add 10μL CCK-8 reagent and incubation at 37°C for 4h to allow color development of the XTT formazan product, the absorbance at 450 nm of each well was read in a Victor2 1420 Multilabel Counter (Wallace-PerkinElmer Life and Analytical Sci ences Inc., Boston, MA). Then calculated the percent cytotoxicity and CC_{50} (50% cytotoxic concentration).

Assay of the inhibitory activity of compounds on Bal.

TZM-bl was obtained from the NIH AIDS Research and Reference Reagent Program (NIH, Bethesda, MD, USA). The inhibitory activities of the compounds on laboratory adapted HIV-1 strain Bal, was tested in TZM-bl cells. Briefly, cells (4×10^4/well) were infected by the addition of 200 $TCID_{50}$s of HIV-1strain Bal, then incubate for 2 h at 37°C before the addition of compounds at serial dilutions. After further incubation at 37°C for 3 days, add 100μL Luciferase Assay System

incubate 2min. Luciferase activity was measured with luciferase assay reagent (Promega) and a LuminescenceCounter (Perkin-Elmer) according to the manufacturers' instructions.