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Modification and structure–activity relationship of a small molecule HIV-1 inhibitor targeting the viral envelope glycoprotein gp120

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This paper describes selected modification and structure–activity relationship of the small molecule HIV-1 inhibitor, 4-benzoyl-1-[(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methylpiperazine (BMS-378806). The results revealed: i) that both the presence and configuration (*R vs. S*) of the 3-methyl group on the piperazine moiety are important for the antiviral activity, with the 3-(*R*)-methyl derivatives showing the highest activity; ii) that the electronegativity of the C-4 substituent on the indole or azaindole ring seems to be important for the activity, with a small, electron-donating group such as a fluoro or a methoxy group showing enhanced activity, while a nitro group diminishes the activity; iii) that the N-1 position of the indole ring is not eligible for modification without losing activity; and iv) that bulky groups around the C-4 position of the indole or azaindole ring diminish the activity, probably due to steric hindrance in the binding. We found that a synthetic bivalent compound with two BMS-378806 moieties being tethered by a spacer demonstrated about 5-fold enhanced activity in an nM range against HIV-1 infection than the corresponding monomeric inhibitor. But the polyacrylamide-based polyvalent compounds did not show inhibitory activity at up to 200 nM.

Introduction

The disease AIDS is caused by the infection of the human immunodeficiency virus (HIV).[1] In the absence of a preventive vaccine, effective anti-HIV drugs are urged to combat the expanding global epidemic.[2–5] Almost all the FDA-approved anti-HIV drugs belong to two classes: the protease inhibitor and the reverse transcriptase inhibitor,[2,3] with the fusion peptide inhibitor T-20 (enfuvirtide) that blocks HIV entry as the only exception.[6,7] The highly active antiretroviral therapy (HAART) that combines three or more reverse transcriptase/protease inhibitors has prolonged the survival of AIDS patients and significantly reduced plasma viral loads in HIV infected patients. However, the emerging of drug-resistant strains as well as the toxicity associated with prolonged HAART therapy has limited the efficacy of the current combination regimen. New drugs with distinct mechanism of action and improved anti-HIV potency are highly desirable. In contrast to drugs targeting viral protease and reverse transcriptase, inhibitors that block HIV infection at the early stages of HIV entry have the advantage of preventing the establishment of the provirus.[8–10] A number of entry inhibitors have been discovered in recent years. Typical examples include: inhibition of the binding of HIV-1 gp120 to receptor CD4;[11] blockade of the interaction between gp120 and chemokine co-receptors;[12] and interference with the function of gp41 such as viral membrane fusion.[6,7] Recently, a novel class of small-molecule inhibitors, compounds **1–3** (Fig. 1), was discovered by a team at Bristol-Myers Squibb through high-throughput screening of a compound library.[13] Compound **3**, denoted BMS-378806, showed excellent potency against many HIV type 1 (HIV-1) laboratory and clinical isolates. Notably, BMS-378806 displays many attractive pharmacological properties such as minimal serum effect on anti-HIV-1 potency, excellent oral bioavailability, and a clean safety profile in animal toxicology studies.[14] Preliminary mechanistic studies suggested that BMS-378806 targets the HIV-1 envelope glycoprotein gp120 and blocks the binding of gp120 to CD4 receptor.[15,16] More recent studies indicated that the inhibitor exhibits its anti-HIV activity by interrupting the essential CD4 binding-induced conformational changes in gp120.[17,18]

As part of our project on developing multivalent HIV-1 entry inhibitors targeting the envelope glycoprotein oligomers,[19] we are particularly interested in modifying BMS-378806 and related inhibitors as lead compounds. It is hitherto not clear which part of the molecule can be modified while preserving its antiviral activity. We describe in this paper selected modifications and structure–activity relationship studies of this type of HIV-1 inhibitor to address the following questions: i) how do the (*R*)-methyl group on the piperazine moiety and its configuration affect the anti-HIV activity? ii) how does the electronegativity of the substituent at C-4 of the indole or azaindole ring influence the activity? and iii) whether can we improve its activity through multivalent assembly?

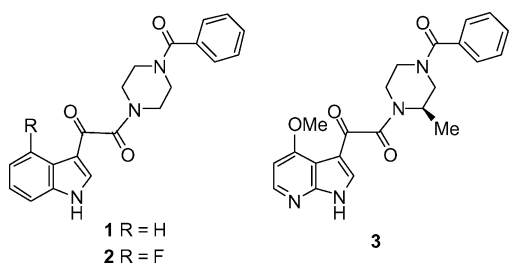

Fig. 1 Structures of small molecule HIV-1 attachment inhibitors.

Results and discussion

Synthesis

A facile synthesis of the BMS-378806 (compound **3**) was previously described by Wang and co-workers.[13] Therefore, the synthesis of the related inhibitors for the present studies was based on the reported procedure with corresponding modifications. The preparation of the indole derivatives is shown in Scheme 1. Starting with indole (**4**) or 4-fluoro-indole (**5**), the acyl chloride **6** or **7** was synthesized in one step by reacting with oxalyl chloride. Coupling of the acyl chloride **6** with the *N*-benzoylpiperazine (**8**), *N*-benzoyl-2-*R*-methyl-piperazine (**9**), and *N*-benzoyl-2-*S*-methyl-piperazine (**10**) gave compounds **1, 11, 12**, respectively. Similarly, coupling of the acyl chloride **7**

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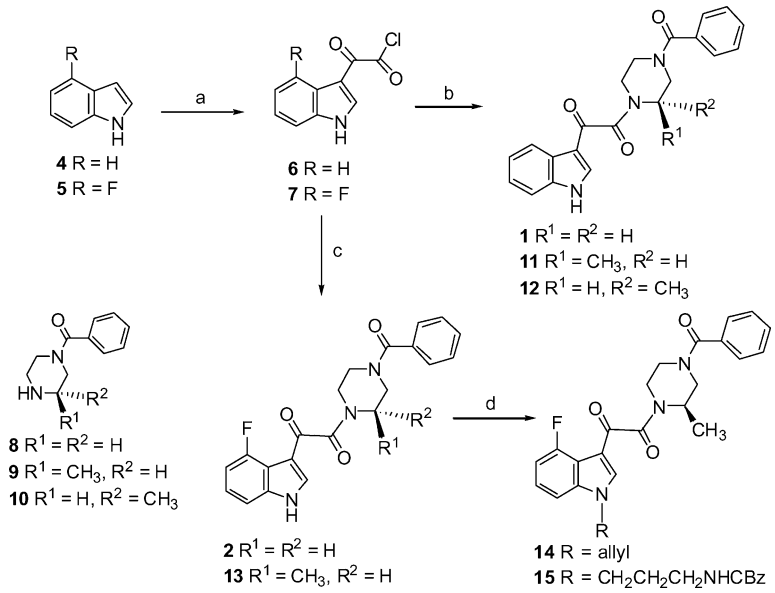
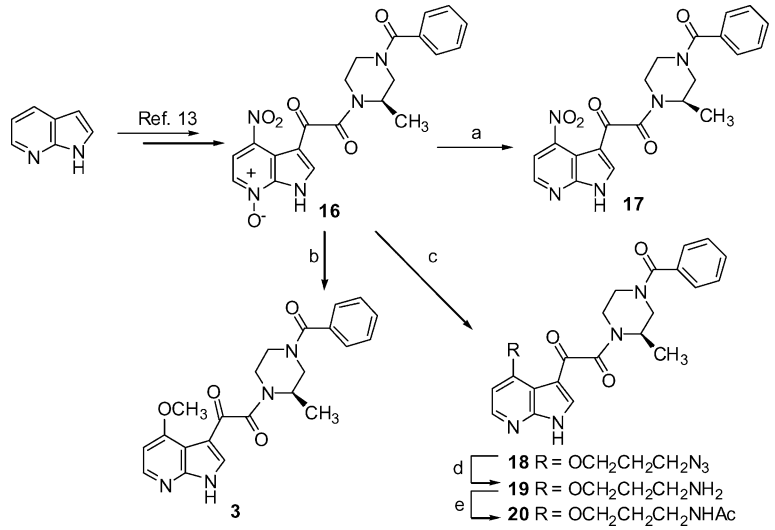


Scheme 1 a) Oxalyl chloride–Et_2O; b) *N*-benzoylpiperazine derivative (**8** or **9** or **10**), THF, DIPEA; c) *N*-benzoylpiperazine derivative (**8** or **9**), THF, DIPEA; d) NaH–THF–allyl bromide (for **14**) or NaH–THF–$BrCH_2CH_2CH_2NHCBz$ (for **15**).



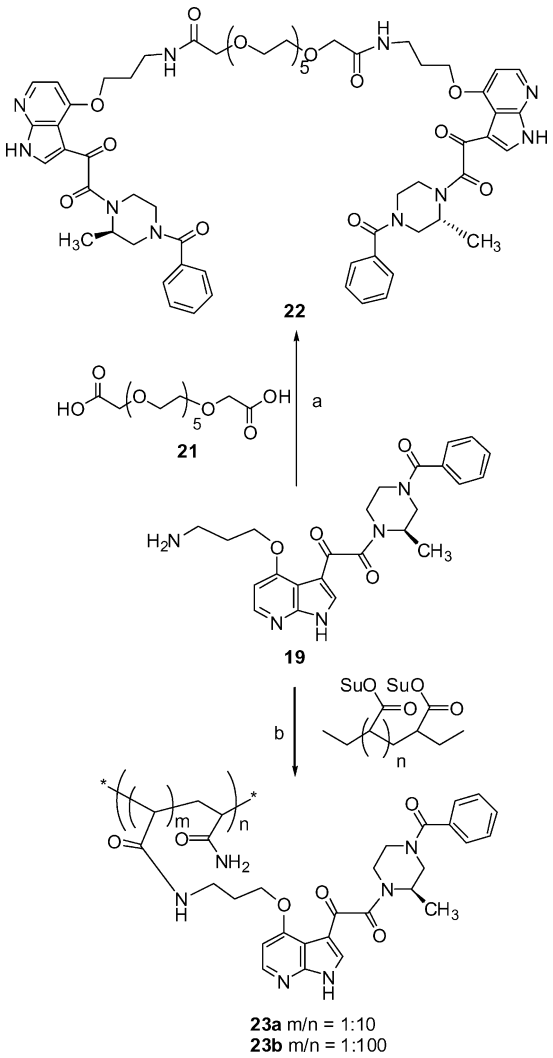
Scheme 2 a) PCl_3, EtOAc; b) (1) CH_3ONa, CH_3OH; (2) PCl_3, EtOAc; c) (1) $HOCH_2CH_2CH_2N_3$, NaH, ethylene glycol dimethyl ether; (2) PCl_3, EtOAc; d) Pd/C, H_2, MeOH; e) acetic anhydride, MeOH, aqueous $NaHCO_3$.

with the respective piperazine derivatives (**8** and **9**) afforded the fluoro-containing derivatives **2** and **13**, respectively. To functionalize the derivative, compound **13** was treated with allyl bromide in the presence of NaH to give the *N*-allyl derivative **14**. On the other hand, reaction of **13** with Br-$(CH_2)_3$-NHCBz gave the derivative **15**, which will provide a free amino group for further manipulation upon removal of the CBz protecting group.

To address the importance of the substituent at C-4 with concurrent functionalization of the molecule, we chose to modify the azaindole derivative (Scheme 2). The 4-nitro *N*-oxide intermediate **16** was prepared following the reported procedure.[13] Treatment of **16** with MeONa in MeOH at elevated temperature, followed by reduction of the *N*-oxide with PCl_3 in EtOAc, gave the known inhibitor **3**.[13] Removal of the *N*-oxide directly from **16** gave the 4-nitro compound **17**. On the other hand, a 3-azido-propoxyl group was introduced at the C-4 position by treatment of the 4-nitro-*N*-oxide derivative **16** with 3-azido-propanol and NaH in ethylene glycol dimethyl ether with subsequent removal of the *N*-oxide using PCl_3 to give the 4-(3-azido-propoxyl) derivative **18**. Reduction of the azido functionality by hydrogenation yielded the amino derivative **19**. Treatment of **19** with acetic anhydride gave the acetamido derivative **20** (Scheme 2). A bivalent compound **22** was synthesized by tethering two BMS-378806 moieties through a spacer. Thus, coupling of the amino compound **19** and 3,6,9,12,15,18-hexaoxaeicosane-1,20-dioic acid (**21**)[20] with EDCI gave the bivalent compound **22** (Scheme 3). The polyacrylamide-based polyvalent inhibitors were synthesized by reaction of the amino compound **19** with a pre-activated polymer poly[*N*-(acryloyloxy)succinimide].[21,22] The density of ligands in the polymer was controlled by altering the ratio of the amino compound **19** and the poly[*N*-(acryloyloxy)-succinimide] in the reaction, giving the polyvalent inhibitors **23a** (10% ligand substitution) and **23b** (1% ligand substitution), respectively.

Antiviral activity

The antiviral activities of the inhibitors were evaluated in the MT-2 cell line infected with HIV-1 IIIB. The results are summarized in Table 1. Comparison of the antiviral activities of compounds **1, 11**, and **12** revealed the importance of the (*R*)-methyl group on the piperazine ring. Compound **11**, with an (*R*)-methyl group, is 26-fold more active than compound **1** without the methyl group (see Table 1). Reversion of the configuration of the methyl substituent from (*R*) (compound **11**)



Scheme 3 a) EDCI, HOBt, DIPEA, DMF, rt; b) DMF, 60 °C, 24 h, then $NH_3 \cdot H_2O$, rt, 24 h.

Table 1 HIV-1 inhibitory activity and cytotoxicity of synthetic compounds[a]

Compound	IC_{50}/nM[b]	CC_{50}/nM[c]
1	990 ± 150	>1000
11	36 ± 8.0	>1000
12	400 ± 140[d]	>1000
2	38 ± 6.0	>1000
13	1.6 ± 0.40	>1000
14	>1000	>1000
15	>1000	>1000
3	2.3 ± 0.55	>1000
17	>1000	>1000
18	35 ± 3.0[d]	>1000
19	>1000	>1000
20	22 ± 2.5[d]	>1000
22	4.8 ± 0.7	>1000
23a	>200	>1000
23b	>200	>1000

[a] MT-2 cells were infected with HIV-1_{IIIB} in the presence or absence of the synthetic inhibitors at varied concentrations. Viral replication was assessed by measuring HIV-1 p24 antigen in the culture supernatants using a commercial p24 ELISA. [b] The IC_{50} was defined as the concentration of the inhibitor that reaches 50% inhibition of viral replication. [c] Cytotoxcity assays were performed in the presence of the inhibitors at varied concentrations for 7 days, and the cell viability was quantitated by using a MTT assay. CC_{50} was defined as the concentration of inhibitor causing 50% decrease in cell viability. [d] These compounds showed a normal dose response at 0–100 nM, but seemed to have reduced inhibitory activities at the high end of the concentrations (500–1000 nM). The reason is not clear.

to the (*S*)-configuration (compound **12**) led to a 10-fold decrease in antiviral activity. The positive effect of the (*R*)-methyl group was also demonstrated for the 4-fluoro derivatives. Thus, the compound with the (*R*)-methyl group (**13**, IC_{50} = 1.6 nM) is about 24-fold more potent than the one without the methyl group (**2**, IC_{50} = 38 nM). It was shown previously that introduction of a fluoro group at the C-4 of the indole ring (**1** *vs.* **2**) resulted in a significant enhancement of the antiviral activity against several types of HIV-1 strains including LAI, SF-2, and Bal.[13] Here, evaluation of the inhibitory activity of **1** and **2** against HIV-1 IIIB also revealed this tendency. Thus, compound **2** was found to be 26-fold more active than **1** against HIV-1 IIIB in the cell culture assay. Moreover, the fluoro type compound **13** (IC_{50} = 1.6 nM) showed 22-fold enhancement in antiviral activity against the proto type compound **11** (IC_{50} = 36 nM). On the other hand, the 1-*N*-alkylated compounds **14** and **15** showed 600-fold decrease in antiviral activity in comparison with the parent compound **13**. The results suggest that the N-7 position of the indole ring cannot be modified for functionalization.

The effects of electronegativity of the C-4 substituent on the azaindole ring were demonstrated by comparison of the 4-nitro derivative **17** (IC_{50} = 1000 nM) and the 4-methoxy derivative **3** (IC_{50} = 2.3 nM). Attachment of an electron-withdrawing group such as the nitro group at the C-4 position led to a dramatic decrease in the antiviral activity. Interestingly, changing the methoxy group to other alkoxy derivatives with a terminal functionality such as **18** (IC_{50} = 35 nM) and **20** (IC_{50} = 22 nM) resulted in a moderate decrease in activity, but the compounds are still potent in the nM range. However, the related alkoxy derivative **19** with a free amino group at the terminus did not show antiviral activity at up to 1000 nM. The results suggest that a positive charge around the C-4 position might cause unfavorable ionic interactions in its binding to the HIV-1 envelope glycoprotein gp120. As to the multivalent compounds, it was found that the bivalent compound **22**, which has two BMS-378806 moieties being tethered through a spacer at the C-4 position, demonstrated about 5-fold enhanced activity in an nM range against HIV-1 infection than the corresponding monomeric inhibitor **20**. The enhancement of the inhibitory activity of the bivalent compound over the monomeric compound might reflect the simultaneous binding of the bivalent compounds to the two interaction sites in the trimeric gp120 complex, as exemplified by the bivalent CD4-mimic miniproteins.[19] However, the loss of activity of the polyvalent compounds (**23a** and **23b**) suggest that steric hindrance around the C-4 position of the unit might result in the abortion of the binding for the polyvalent compounds. No compounds show any cytotoxicity at up to 1000 nM concentration (Table 1).

In summary, selected modification of the small molecule HIV-1 inhibitor, 4-benzoyl-1-[(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methylpiperazine (BMS-378806) was performed for evaluating its structure–activity relationship. The results indicate: i) that both the presence and configuration (*R vs. S*) of the 3-methyl group on the piperazine moiety are important for the antiviral activity, with the 3-(*R*)-methyl derivatives showing the highest activity; ii) that the electronegativity of the C-4 substituent on the indole or azaindole ring has great influence on the activity, with a small, electron-donating group such as a fluoro or a methoxy group showing enhanced activity, while the electron-withdrawing nitro group diminished the activity; iii) that the N-1 position is not eligible for modification (*e.g.*, *N*-alkylation); and iv) that bulky groups around the C-4 position of the indole or azaindole ring diminished the activity, probably due to steric hindrance in the binding. In order to develop an effective anti-HIV drug based on the lead compound, further structural modifications should be performed in connection with mechanistic studies and molecular modeling of the interactions between the inhibitor and HIV-1 gp120.

Experimental

General

All reagents were purchased from Aldrich Chemical Co. and used as received. Thin layer chromatography (TLC) was conducted on precoated plates (Merck 60F250). Flash column chromatography was performed with silica gel 60 (EM science, particle size 0.040–0.063 mm, 230–240 mesh). ^{1}H NMR spectra were measured on a QE-300/Tecmag AQuErius Spectrometer (300 MHz) or a Varian INOVA 500 spectrometer (500 MHz). ^{13}C NMR spectra were recorded on the QE-300/Tecmag AQuErius Spectrometer at 75 MHz. The ESI-MS spectra were measured on a micromass ZQ-4000 single quadruple mass spectrometer.

N-Benzoylpiperazine (8)

To a stirred solution of piperazine (1.72 g, 20 mmol) in dry CH_2Cl_2 (100 ml) under an argon atmosphere at room temperature were added methyl benzoate (2.72 g, 2.5 ml, 20 mmol) and Et_2AlCl_3 (1.0 M in hexanes, 20 ml, 20 mmol). The reaction mixture was stirred for 2 days and the reaction was then quenched by adding 2 M NaOH. The aqueous layer was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (EtOAc–MeOH, 10 : 1) to give **8** (2.50 g, 66%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37 (m, 5H), 3.73 (m, 2H), 3.37 (m, 2H), 2.92 (m, 2H), 2.82 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9, 135.4, 129.0, 127.9, 126.4, 45.6, 45.2.

(*R*)-*N*-(Benzoyl)-3-methylpiperazine (9)

The reaction of *R*-2-methyl-piperazine (1.0 g, 10 mmol), Et_2AlCl_3 (1.0 M in hexanes, 10 ml, 10 mmol) and methyl benzoate (1.36 g, 1.24 ml, 10 mmol) was performed as described for the preparation of **8** to give compound **9** (1.50 g, 74%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 5H), 4.50 (m, 1H, $J = 9.9$ Hz), 3.60 (m, 1H), 3.3–2.6 (m, 5H), 1.05 (broad peak, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 135.5, 129.0, 127.9, 126.4, 50.4, 48.6, 47.5, 45.7, 18.8.

(*S*)-*N*-(Benzoyl)-3-methylpiperazine (10)

The reaction of *S*-2-methyl-piperazine (1.0 g, 10 mmol), Et_2AlCl_3 (1.0 M in hexanes, 10 ml, 10 mmol) and methyl benzoate (1.35 g, 10 mmol) was performed as described for the preparation of **8** to give compound **10** (1.53 g, 75%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 5H), 4.50 (m, 1H, $J = 9.9$ Hz), 3.60 (m, 1H), 3.3–2.6 (m, 5H), 1.10 (m, 1.5H), 0.98 (m, 1.5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 135.5, 129.0, 127.9, 126.4, 50.4, 48.6, 47.5, 45.7, 18.8.

1-(Benzoyl)-3-*R*-methyl-4[(1*H*-indol-3-yl)oxoacetyl]piperazine (11)

To a solution of indole (200 mg, 1.71 mmol) in anhydrous diethyl ether (10 ml) was added oxalyl chloride (252 mg, 0.173 ml, 1.98 mmol) over 30 min. The reaction mixture was stirred at 0 °C for 3 h, then allowed to warm to room temperature over 1 h. The resulting yellow crystals were collected by filtration, washed with cold anhydrous ether (20 ml), and dried under vacuum to yield α-oxo-1*H*-indole-3-acetyl chloride (**6**) (320 mg, 91%), which was used immediately for the next step. A solution of (*R*)-*N*-benzoyl-3-methylpiperazine (**9**) (179 mg, 0.877 mmol) in dry THF (5 ml) was added to a stirred solution of **6** (182 mg, 0.877 mmol) in dry THF (5 ml). The mixture was cooled to 0 °C and diisopropylethylamine (135 mg, 0.104 mmol) was added dropwise. The mixture was warmed to room temperature and stirred for 3 h. The reaction mixture was evaporated and the residue was subject to flash chromatography to give compound **11** (293 mg, 90%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 10.88 (d, 1H, $J = 5.4$ Hz), 8.21 (s, 1H), 7.70 (dd, 1H, $J = 3.0$, 7.2 Hz), 7.40 (s, 5H), 7.20 (m, 4H), 5.02–2.80 (m, 7H), 1.30 (br s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 185.4, 171.5, 166.5, 136.7, 136.2, 134.4, 129.3, 127.8, 126.1, 124.5, 123.2, 122.1, 120.5, 113.0, 111.4, 49.7, 46.2, 14.4, 13.3. ESI-MS: 376.2 $(M + H)^+$.

1-(Benzoyl)-3-S-methyl-4[(1H-indole-3-yl)oxoacetyl]piperazine (12)

The reaction of (*S*)-*N*-benzoyl-3-methylpiperazine (**10**) (180 mg, 0.882 mmol) and α-oxo-1H-indole-3-acetyl chloride (**6**) (182 mg, 0.882 mmol) was carried out in the same way as described for the preparation of **11** to afford **12** (280 mg, 85%) as a white solid. The ^{1}H NMR, ^{13}C NMR, and mass spectra are identical to those of compound **11**.

1-(Benzoyl)-4[(1*H*-indol-3-yl)oxoacetyl]piperazine (1)

The reaction of *N*-benzoylpiperazine (**8**) (130 mg, 0.69 mmol) and α-oxo-1*H*-indole-3-acetyl chloride (**6**) (243 mg, 0.69 mmol) was performed in the same way as described for the preparation of **11** to give compound **1** (213 mg, 85%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.22 (d, 1H, $J = 2.2$ Hz), 8.11 (d, 1H, $J = 6.9$ Hz), 7.53 (d, 1H, $J = 8.7$ Hz), 7.45 (s, 5H), 7.27 (m, 2H), 4.8–3.3 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 186.4, 169.8, 166.6, 137.8, 137.4, 136.0, 130.2, 129.0, 127.5, 125.4, 124.2, 123.1, 121.4, 113.6, 113.2, 46.0, 41.1. ESI-MS: 362.1 $(M + H)^+$, 394.2 $(M + Na)^+$.

1-(Benzoyl)-4[(1*H*-4-fluoroindol-3-yl)oxoacetyl]piperazine (2)

The reaction of **7** (312 mg, 1.38 mmol), prepared from 4-fluoroindole in the same way as for the preparation of **6**, and **8** (263 mg, 1.38 mmol) was performed in the same way as described for the preparation of **11** to give compound **2** (390 mg, 75%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.23 (s, 1H), 7.41 (m, 5H), 7.33 (d, 1H, $J = 6.9$ Hz), 7.25 (m, 1H), 6.96 (dd, 1H, $J =$ 8.0, 10.6 Hz), 4.8–3.3 (m, 8H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 184.9, 169.9, 167.0, 140.5, 138.7, 136.0, 130.2, 129.0, 127.5, 125.1, 113.0, 109.6, 108.6, 108.5, 45.8, 42.0. ESI-MS: 380.2 $(M + H)^+$.

1-(Benzoyl)-3-*R*-methyl-4[(1*H*-4-fluoroindol-3-yl)oxoacetyl]piperazine (13)

The reaction of α-oxo-4-fluoro-1*H*-indole-3-acetyl chloride (**7**) (60 mg, 0.269 mmol) and (*R*)-*N*-(benzoyl)-3-methylpiperazine (**9**) (55 mg, 0.269 mmol) was carried out as described for the preparation of **11** to afford compound **13** (91 mg, 80%) as a white solid: ^{1}H NMR (300 MHz, CD_3OD): δ 11.2 (s, 1H), 7.73 (s, 1H), 7.40 (m, 5H), 7.07 (ddd, $J = 4.7$, 7.7, 8.4 Hz), 6.88 (dd, $J = 7.7$, 8.4 Hz), 4.9–2.9 (m, 7H), 1.30 (br s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 184.3, 171.5, 166.7, 154.0. 139.6, 136.8, 129.3, 127.8, 126.1, 124.0, 112.3, 107.9, 107.8, 107.6, 107.3, 59.5, 49.8, 14.3, 13.1. ESI-MS: 394.2 $(M + H)^+$.

1-(Benzoyl)-3-*R*-methyl-4[(1-allyl-4-fluoroindol-3-yl)oxoacetyl]piperazine (14)

To a stirred suspension of NaH (30 mg, 60% in mineral oil, 0.75 mmol) in dry THF (10 ml) was added compound **13** (147 mg, 0.374 mmol) in THF (5 ml), and the resulting mixture was stirred at room temperature for 30 min. Then allyl bromide (453 mg, 3.75 mmol) was added. The reaction mixture was refluxed for 30 min and the reaction was quenched by adding methanol (1 ml). The solvent was evaporated and the residue was partitioned between water and EtOAc. The organic layer was dried over $MgSO_4$, filtered, and the filtrate was concentrated under vacuum. The residue was purified by flash chromatography (EtOAc–hexanes, 1 : 1) to give compound **14** (136 mg, 84%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, 1H, $J = 2.5$ Hz), 7.41 (s, 5H), 7.28 (m, 2H), 7.15 (d, 1H, $J = 8.4$ Hz), 6.98 (t, 1H, $J = 8.4$ Hz), 5.98 (m, 1H), 5.34 (d, 1H,

J = 10.3 Hz), 5.20 (dd, 1H, J = 4.4, 6.8 Hz), 4.70 (d, 2H, J = 5.2 Hz), 4.7–2.95 (m, 7H), 1.3 (broad peak, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 183.8, 171.5, 166.6, 139.4, 134.4, 131.5, 129.3, 127.8, 126.1, 124.1, 124.0, 116.9, 116.8, 113.0, 111.5, 108.0, 107.8, 106.8, 106.7, 49.1, 48.8, 14.8, 13.0. ESI-MS: 434.3 $(M + H)^+$, 456.3 $(M + Na)^+$.

1-(Benzoyl)-3-*R*-methyl-4[1-(3-benzyloxycarbonyl-aminopropyl)-4-fluoroindol-3-yl-oxoacetyl]piperazine (15)

Compound **13** (90 mg, 0.228 mmol) was reacted with Br-$(CH_2)_3$-NHCBz (350 mg, 1.29 mmol) in the same way as described for the preparation of **14** to give compound **15** (115 mg, 88%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (d, 1H, J = 2.8 Hz), 7.47 (m, 5H), 7.38 (m, 5H), 7.27 (q, 1H, J = 6.5 Hz), 7.17 (d, 1H, J = 8.4 Hz), 7.02 (t, 1H, J = 8.4 Hz), 5.34 (br s, 1H), 5.15 (s, 2H), 4.9–3.0 (m, 11H), 2.08 (m, 2H), 1.35 (br s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 183.8, 139.4, 134.4, 127.8, 127.5, 127.0, 126.7, 126.1, 124.2, 124.1, 113.0, 111.2, 108.0, 107.6, 106.4, 65.5, 44.5, 43.9, 36.8, 28.9, 14.3, 13.1. ESI-MS: 585.2 $(M + H)^+$.

4-Benzoyl-1-[4-nitro-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine (17)

Benzoyl-2-(*R*)-methyl-1-[(4-nitro-7-oxido-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]piperazine **16** (85.1 mg, crude), prepared from azaindole according to the reported procedure,[13] was suspended in EtOAc (10 ml). To this suspension was added PCl_3 (0.8 ml) and the resulting mixture was stirred at room temperature for 5 h. The reaction was cooled down to 0 °C and neutralized to pH 6 with 2 M NaOH. The aqueous layer was extracted by EtOAc, and the combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was subject to flash chromatography (EtOAc–MeOH, 50 : 1) to give compound **17** (36 mg, 44%) as a yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.53 (d, 1H, J = 4.8 Hz), 8.48 (s, 0.5H), 8.44 (s, 0.5H) (these are from the rotamers), 7.59 (s, 1H), 7.41 (s, 5H), 4.7–3.3 (m, 7H), 1.3 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 184.0, 172.1, 166.2, 145.6, 139.9, 134.9, 130.0, 128.5, 126.8, 111.7, 111.4, 50.4, 45.5, 15.2, 13.9; ESI-MS: 422 $(M + H)^+$, 444 $(M + Na)^+$.

4-Benzoyl-1-[(4-methoxy-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methylpiperazine (3)

Compound **16** (230 mg, crude) was treated with MeONa–MeOH (0.5 M, 16 ml). Work-up of the reaction and purification of the product according to the reported procedure[13] gave **3** (85 mg, 40%) as white solid. The ^{1}H NMR, ^{13}C NMR, mass spectra were in agreement with the reported data.[13]

4-Benzoyl-1-[4-(3-azidopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine (18)

To a stirred suspension of NaH (240 mg, 60% in mineral oil, 6 mmol) in dry ethylene glycol dimethyl ether (15 ml) was added 3-azido-1-propanol (606 mg, 6 mmol, prepared by the reaction of sodium azide with 3-bromo-1-propanol) under an argon atmosphere. The mixture was stirred at room temperature for 2 h, then 4-benzoyl-2(*R*)-methyl-1-[(4-nitro-7-oxido-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]piperazine (**16**) (263 mg, crude) in ethylene glycol dimethyl ether (5 ml) was added. The resulting mixture was stirred at 70 °C overnight. The reaction was then quenched by adding MeOH (2 ml), concentrated and dried under vacuum to give a brown powder. The residue was suspended in EtOAc (8 ml), and PCl_3 (0.5 ml) was added. The mixture was stirred at room temperature for 5 h. The mixture was cooled down to 0 °C and neutralized to pH 6 by adding 2 M NaOH. The aqueous phase was extracted with ethyl acetate. The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under vacuum and the residue was subject to flash chromatography (EtOAc–MeOH, 50 : 1) to afford compound **18** (70 mg, 25%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.40 (d, 1H, J = 5.2 Hz), 8.30 (s, 0.5H), 8.21 (s, 0.5H), 7.40 (s, 5H), 7.28 (d, 1H, J = 5.2 Hz), 4.8–3.1 (m, 11H), 2.15 (m, 2H), 1.3 (broad peak, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 184.9, 176.4, 167.2, 145.5, 136.7, 134.9, 130.0, 128.5, 126.8, 112.5, 65.3, 50.4, 47.7, 28.1, 14.9, 14.3. ESI-MS: 476.23 $(M + H)^+$.

4-Benzoyl-1-[4-(3-aminopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine (19)

A solution of 4-benzoyl-1-[4-(3-azidopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridine-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine **18** (50 mg, 0.105 mmol) in MeOH (10 ml) containing Pd–C (20 mg) was hydrogenated under 40 psi for 2 h. The mixture was filtered through a pad of celite, and the pad was washed with MeOH (4 ml). The filtrate and washings were combined and evaporated at a reduced pressure to give compound **19** (42 mg, 90%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.18 (s, 0.5H), 8.10 (s, 0.5H), 7.81 (s, 2H), 7.40 (s, 5H), 4.6–3.3 (m, 9H), 2.65 (m, 2H), 2.21 (m, 2H), 1.3 (broad peak, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 185.0, 171.5, 160.0, 146.2, 137.9, 134.3, 129.4, 127.8, 126.1, 118.0, 66.9, 58.4, 50.2, 45.8, 37.5, 28.6, 14.3, 13.9. ESI-MS: 450 $(M + H)^+$.

4-Benzoyl-1-[4-(3-acetamidopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine (20)

To a solution of **19** (12 mg, 26.7 μmol) in aqueous MeOH containing $NaHCO_3$ (3 ml, pH = 8.2) was added Ac_2O (11 mg, 106 μmol). The mixture was stirred at room temperature until TLC analysis showed the disappearance of the starting material. MeOH (10 ml) was added to the mixture to quench the reaction. The mixture was concentrated and the residue was purified by flash chromatography to give compound **20** (10 mg, 76%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.18 (s, 2H), 8.11 (s, 1H), 7.50 (s, 5H), 4.50–3.05 (m, 11H), 2.18 (m, 2H), 1.97 (s, 3H), 1.32 (broad peak, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 182.1, 170.8, 170.2, 159.0, 145.9, 139.6, 135.2, 129.9, 128.4, 127.5, 126.7, 116.5, 65.6, 56.8, 45.7, 30.0, 21.2, 13.0, 11.9. ESI-MS: 492.2 $(M + H)^+$, 514.2 $(M + Na)^+$.

Preparation of the bivalent compound 22

A solution of compound **19** (8 mg, 17.8 μmol), the 3,6,9,12,15,18-hexaoxaeicosane-1,20-dioic acid (**21**)[20] (2.1 mg, 5. 94 μmol), EDCI (3.4 mg, 17.8 μmol), HOBt (17.8 μmol, 17.8 μl of 1 M NMP solution), and DIPEA (2 μl) in dry DMF (1 ml) was stirred at rt under an argon atmosphere for 24 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by preparative silica gel thin-layer chromatography (EtOAc–MeOH 10 : 1) to give the bivalent compound **22** (3.5 mg, 48%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.05 (s, 0.5H), 7.95 (s, 0.5H), 7.84 (s, 2H), 7.60–7.40 (br. s, 5H), 4.6–3.3 (br. s, 31H), 2.21 (m, 2H), 1.3 (br. s, 3H). ESI-MS: 1217.0 $(M + H)^+$.

Preparation of the polyacrylamide-based polymers (23a and 23b) of the 4-benzoyl-1-[4-(3-aminopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine

A solution of **19** (12 mg, 26.7 μmol) in DMF (1 ml) containing diisopropylethylamine (5 μl) was added to a stirred solution of poly[*N*-(acryloyloxy)succinimide] (45 mg), which was prepared according to the literature[21,22] (Mw = 216 kD; Mn = 157 kD; Mw/Mn = 1.4), in DMF (5 ml) under an argon atmosphere. The mixture was stirred at 60 °C for 24 h. Then concentrated $NH_3 \cdot H_2O$ (0.5 ml) was added dropwise and the resulting mixture was stirred at rt for 24 h. The reaction mixture was dialyzed exhaustively against distilled water and lyophilized to yield a polymer (**23a**) containing 4-benzoyl-1-[4-(3-aminopropoxy)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)oxoacetyl]-2-(*R*)-methyl-piperazine as a white powder (28 mg, 93%). The density (molar

ratio *m*/*n*) of the small molecule ligand in the polymer **23a** was determined to be about 1 : 10 (*cat.* 10% subsitution by the ligand) based on the integration of the aromatic signal (δ 7.40) for the small molecule moiety and the α-proton signal (δ 1.80) for the acrylamide moiety in the ^{1}H NMR (500 MHz, D_2O): δ 8.18 (br. s), 8.10 (br. s), 7.81 (br. s), 7.62 (br. s), 7.40 (br. s), 4.6–3.3 (m), 2.2 (m), 1.8 (m).

Changing the ratio of the amino compound **19** and the poly[*N*-(acryloyloxy)-succinimide] in the reaction yielded polymers with varied density of the ligands. Accordingly, the reaction of **19** (1.5 mg) with the poly[*N*-(acryloyloxy)-succinimide] (45 mg), followed by the work-up described above, gave the polymer **23b** with a *m*/*n* ratio of about 1 : 100 (*cat.* 1% substitution by the ligand).

Antiviral assays

The antiviral activities of the inhibitors were tested in the MT-2 cell line infected with HIV-1 IIIB at a multiplicity of infection (m.o.i) of 0.002. Virus inoculum was preincubated with serial dilutions of the inhibitors for 1 h at 37 °C prior to addition to the cells. Virus inoculum was then added to cells and the sample was incubated for 2 h at 37 °C. Infected cells were washed 3 times with PBS to remove non-adsorbed virus, and then plated in RPMI-10% FBS containing the inhibitors at the same concentration as in the preincubation step. Cells were plated in triplicate in a 96-well plate at 1×10^5 cells per well in a volume of 200 μl. On day 4 after infection, half of the medium was replenished with fresh medium and inhibitors. On day 7, virus replication was monitored by measuring p24 content in the culture supernatants using a commercial p24 ELISA (NCI, Frederick, MD). Cell viability in culture in the presence or absence of antiviral agents was measured by commercial MTT assay, as per the manufacturer's instructions (Boehringer Mannheim).

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