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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 3587–3595
Bioorganic & Medicinal Chemistry

Design and synthesis of dual inhibitors of HIV reverse transcriptase and integrase: Introducing a diketoacid functionality into delavirdine

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Received 8 January 2008; revised 1 February 2008; accepted 5 February 2008
Available online 8 February 2008

Abstract—Cost and toxicity problems associated with highly active antiretroviral therapy (HAART) in HIV/AIDS treatment could be alleviated by using designed multiple ligands (DMLs). Dual inhibitors of HIV reverse transcriptase (RT) and integrase (IN) were rationally designed by introducing a diketoacid (DKA) functionality into the tolerant C-5 site of RT inhibitor delavirdine. The resulting compounds all demonstrate good activity against both RT and IN in enzymatic assays and HIV in cell-based assay, whereas their C-7 regioisomers are all inactive in these assays. Balanced activities were observed with C-3 halogenated inhibitors.

1. Introduction

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS).[1,2] Despite FDA approval of over 30 antiviral regimens for the treatment of AIDS,[3] HIV continues to be a devastating pandemic that claimed 2.1 million lives in 2007 alone.[4] The increasing mortality of HIV/AIDS is largely due to the lack of vaccines[5] and the persistence of viral reservoirs,[6] which render this disease unpreventable and incurable. These complications underscore the imperativeness of chemotherapeutic agents as they provide the only relief for HIV/AIDS patients. Unfortunately, clinical usage of singly dosed antivirals is limited by the rapid selection of resistant viral strains. Instead, cocktails combining mechanistically distinct drugs are administered to form highly active antiretroviral therapy (HAART),[7] which can effectively suppress viral replication to undetectable level. Inhibitors of reverse transcriptase (RT) and protease (PR) constitute the cornerstone of HAART, which is enhanced by recent FDA-approved anti-HIV drugs that target new molecular mechanisms of HIV replication, such as entry inhibitors enfuvirtide[8] and maraviroc,[9] and integrase (IN) inhibitor raltegravir (MK-0518).[10] Nevertheless, the benefits of HAART as the standard AIDS chemotherapy are still compromised by major issues that originate from using multiple drugs, such as high cost and toxicity.[11] Side effects can be particularly problematic as they normally lead to poor patient adherence, and as a result, patients experience viral rebound, and even worse, multi-drug resistance (MDR).[12] We envision that these issues could be alleviated by using designed multiple ligands (DMLs)[13–15] which are single structures engaging multiple biological targets. These multifunctional compounds should work to contain much of the resistance as HAART does, albeit at a lower cost and possibly with less side effects. Identifying multiple ligands through rational design has been a subject of growing interest in medicinal chemistry.[14]

2. Design of RT/IN dual inhibitors

HIV RT and IN are two virally encoded enzymes that function sequentially in two closely related steps of HIV replication. Interestingly, RT has two distinct active sites for polymerase and RNase H activities, respectively, whereas IN uses the same binding site for two catalytic activities: 3′ processing and strand transfer. It could be more amenable to target RNase H and IN as these two share a common binding mechanism. However, targeting dissimilar binding sites could be essential to avoiding quick emergence of drug resistance. Therefore, our design is focused on the polymerase active site of RT along with

Keywords: HIV; Dual inhibitors; Reverse transcriptase; Integrase.
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doi:10.1016/j.bmc.2008.02.007

IN. Strategically, our design generally involves the incorporation of an IN pharmacophore to a known potent RT inhibitor as illustrated by the design of the first rationally designed RT/ IN inhibitor **1** (Fig. 1).[16] Apparently the key to this strategy would be to identify a tolerant region in the RT structure. Crystal structure of TNK-651, a RT inhibitor of the 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) family,[17] reveals that the N-1 pendant of the pyrimidine core extends from the non-nucleoside RT inhibitor (NNRTI) binding pocket to the protein/solvent interface, which presents an attractive site for incorporating a moiety to generate anti-IN activity (Fig. 1).

Similarly, crystallographic study has shown that the methylsulfonamide group at the C-5 position of delavirdine (**2**),[18] an RT inhibitor of the bis(heteroaryl)piperazine (BHAP) family, is situated in an open area where structural modification could be tolerated. Therefore, replacing this sulfonamide group with a DKA could generate inhibitory activity against IN without significantly affecting the RT binding (Fig. 2). Notably, known pharmacophore model[19,20] indicates that, besides a DKA type of metal-chelating functionality, IN binding also requires a hydrophobic aromatic ring directly connected to the chelator, which can be satisfied by the indole ring of **3a** in our design.

Other FDA-approved RT inhibitors nevirapine[21] and efavirenz[22] are almost entirely enclosed by the NNRTI binding pocket, which is largely hydrophobic and therefore an inappropriate environment for the polar metal-chelating pharmacophores of IN inhibitors.

3. Chemistry

Our synthetic strategy for inhibitor **3a** is outlined in Scheme 1. We envision that inhibitor **3a** can be synthetically accessed from acyl intermediate **4**, which can be prepared from piperazine **5** and indole ester **6**. Direct acylation of commercial ester **7** could give **6**, which might be complicated by the regioselectivity issue. Alternatively, indole ester **6** can be synthesized via Fisher indole synthesis[23] from hydrazone **11**, which can be prepared via a classic Japp–Klingeman reaction[24] between aniline **9** and malonate **10**.

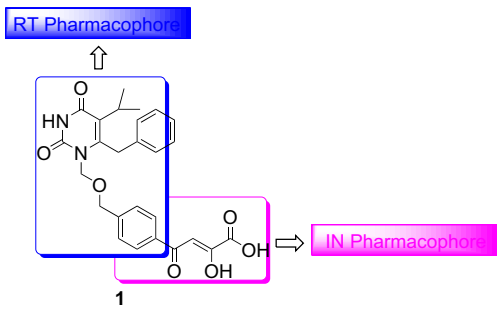


Figure 1. A rationally designed RT/IN dual inhibitor featuring a HEPT pharmacophore for anti-RT activity and a DKA pharmacophore for IN activity.

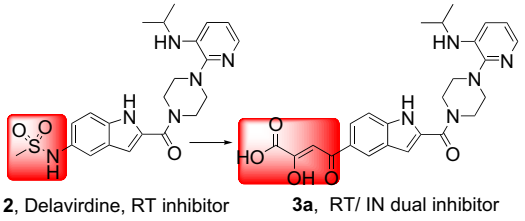


Figure 2. The design of RT/IN dual inhibitor **3a**: replacing the methylsulfonamide group of RT inhibitor **2** with a DKA functionality.

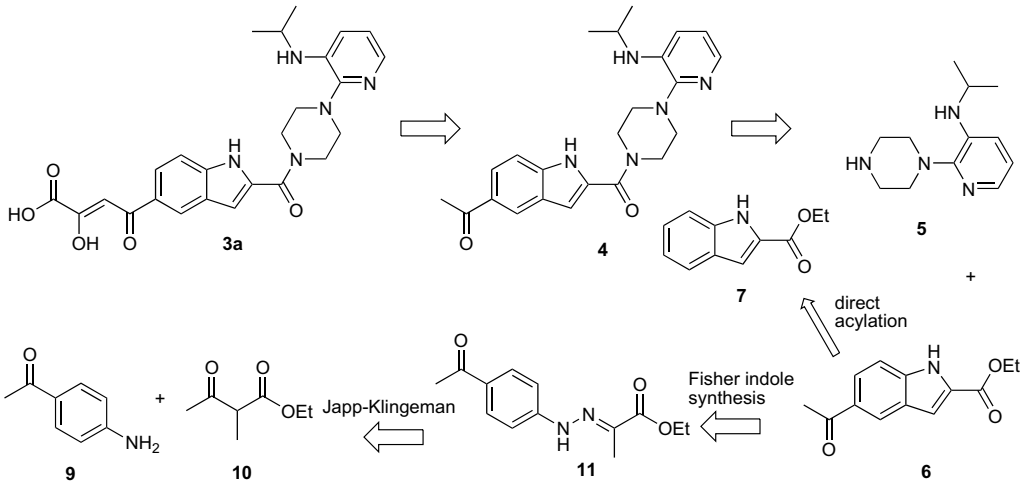
The synthesis starts with the preparation of **6a** (Scheme 2). Unfortunately, the direct Friedel–Crafts acylation of ethyl indole-2-carboxylate (**7**) yielded a mixture of three regioisomers (Scheme 2a).[25] Clearly acylation at C-3 position of the indole ring is largely favored to give **8** (62%), and the desired 5-acyl compound **6a** was produced as a minor product (15%) only, which was found inseparable from **8**. Remarkably, 7-acyl product **6b** is well separated from the inseparable mixture of **6a** and **8** and has a significantly different solubility in organic solvents, presumably due to an internal H-bonding in **6b** (Scheme 2a). Nevertheless, the direct Friedel–Crafts acylation provides a viable synthesis for isomeric 7-acyl indole-2-carboxylate **6b** instead of the desired compound **6a**.

The alternative Fisher indole synthesis was then pursued for the synthesis of **6a** (Scheme 2b). In this event, aniline **9** was diazotized and reacted with enolized malonate **10** to produce the requisite hydrazone **11** in excellent yield. The subsequent Fisher indole synthesis[26] was effected by microwaving the hydrazone in trifluoroacetic acid (TFA) at 140 °C for 15 min. Gratifyingly, the microwave reaction gave a much higher yield (45%) than the same reaction with conventional heating (4%).[25] Another strategy to get around the regioselectivity issue in the acylation reaction is to block the most reactive C-3 site. We chose to halogenate this site because (a) the dehalogenation could be viable; (b) halogens are bioisosteric to hydrogen; and (c) these halogens could be used as handles for further functionalization to study the SAR at this site. As expected, the bromination (**12a**) and chlorination (**12b**) occurred exclusively at C-3 position (Scheme 2c), and the subsequent acylation at C-5 and C-7 positions yielded products (**6c**, **6d** and **6e**, **6f**) that are easily separated.

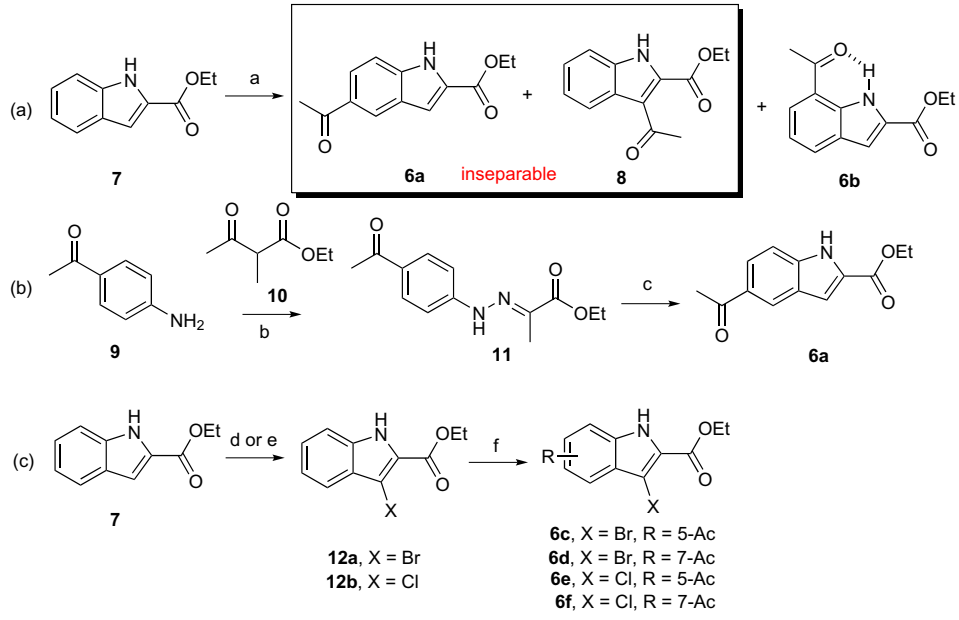
To complete the synthesis (Scheme 3), ethyl 5-acyl-indole-2-carboxylates **6a–f** were saponified and coupled with piperazine compound **5** [27] to produce intermediates **4a–f**, which were further elaborated to produce inhibitors **3a–f** through a condensation with ethyl oxalate and a subsequent saponification.

4. Results and discussion

Results of anti-RT, anti-IN, and anti-HIV assays of synthesized RT/IN inhibitors are summarized in Table 1. Remarkably, the substitution pattern appears to affect both RT binding and IN binding dramatically. As



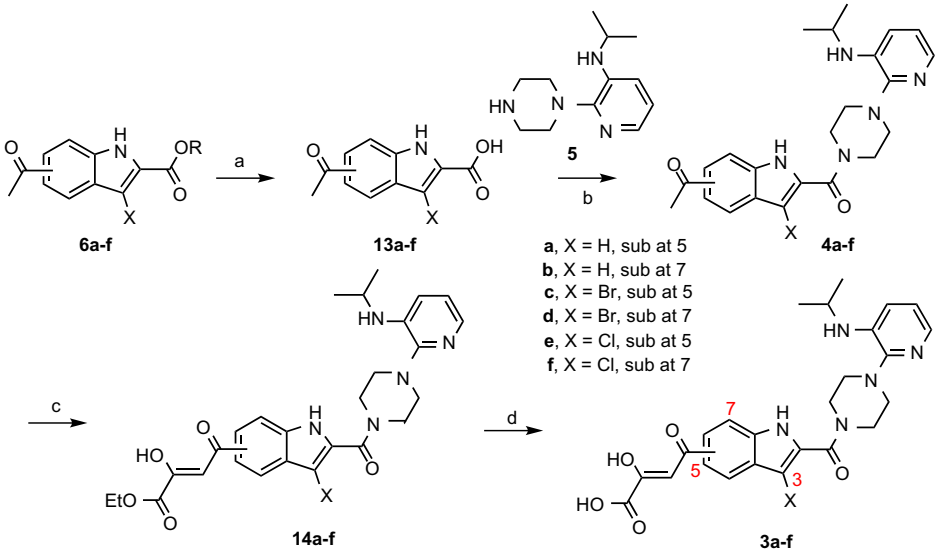
Scheme 1. Synthetic plan for dual inhibitor **3a**.



Scheme 2. Preparation of acyl indole-2-carboxylates **6a–f**. Reagents and conditions: (a) AcCl, $AlCl_3$, CH_2ClCH_2Cl, rt, 1 h, 100%, **6a/8/6b** = 15:62:23; (b) $NaNO_2$, HCl; **10**, KOH, 91%; (c) microwave, 140 °C, 15 min, 45%; (d) NBS, DMF, rt, 96% (**12a**); (e) NCS, DMF, rt, 95% (**12b**); (f) AcCl, $AlCl_3$, CH_2ClCH_2Cl, rt, 1 h; for **12a**: 73%, **6c/6d** = 1.5:1; for **12b**: 75%, **6e/6f** = 1.6:1.

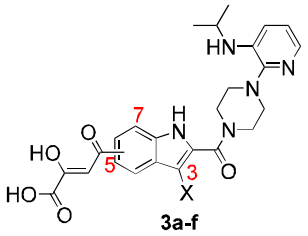
clearly shown in Table 1, compounds with DKA substitution at C-7 position (**3b**, **3d**, **3f**) are virtually inactive against both enzymes, whereas compounds with C-5 DKA functionality (**3a**, **3c**, **3e**) show sub- or low micromolar activity against RT and IN in enzymatic assays as well as HIV in cell-based assay. Apparently C-7 substitution results in angular compounds with nearly closed conformations which might not favor binding to IN, while RT modeling[28] shows that the DKA functionality of compounds with C-7 substitution has unfavorable electrostatic interactions with the backbone carbonyls of K103 and K104. By contrast, inhibitors with C-5 substitution have a pseudo-linear conformation resembling delavirdine, which apparently benefits both RT and IN binding. This observed overwhelming preference of C-5 substitution over C-7 proves the importance of identifying tolerant regions in the design of dual inhibitors with two distinct pharmacophores.

Significantly, it was also observed that introducing a halogen (Cl or Br) atom at C-3 position of the indole ring reduces the anti-RT potency while slightly enhancing the anti-IN activity, reflecting the dissimilarity of these two binding sites. As a result, balanced binding affinity against both RT and IN is achieved with inhibitors **3c** and **3e** (Table 1), particularly the latter (IC_{50}: 1.1 μM against RT, 4.7 μM against IN and 0.98 μM against HIV-1). By contrast, inhibitor **3a** lacks the halogen substituent at C-3 and its activities against RT and IN are separated by 3 orders of magnitude, which is similar to inhibitor **1**. Importantly, the major thrust for developing dual inhibitors active against two dissimilar targets is to



Scheme 3. Synthesis of inhibitors **3a**–**f**. Reagents and conditions: (a) NaOH (aq), EtOH, reflux, 3 h, 44%; (b) **5**, CDI, DMF, rt, 62%; (c) Na/EtOH, diethyl oxalate, rt, 3 h; (d) NaOH (aq), EtOH/$CHCl_3$, rt, 1 h, 41% over 2 steps.

Table 1. Anti-RT, anti-IN and anti-HIV assay results for inhibitors **3a**–**f**[a]



Inhibitor	X	Sub	RT IC_{50} (μM)	IN IC_{50} (μM)[b]	HIV EC_{50} (μM)
1[c]	—	—	0.057	2.4	0.033
2	—	—	0.036	>100	0.021
3a	H	5	0.0059	11	0.52
3b	H	7	>100	>100	>10
3c	Br	5	0.12	3.9	0.79
3d	Br	7	>100	75	>10
3e	Cl	5	1.1	4.7	0.98
3f	Cl	7	>100	>100	>10

[a] IC_{50}: 50% inhibitory concentration; EC_{50}: 50% effective concentration.
[b] Average activity against 3′ processing and strand transfer.
[c] Data taken from Ref. 16.

achieve the potential advantage of avoiding selection of resistance mutations. If the activities are largely unbalanced, as in the case of inhibitor **3a**, the selection of resistant mutation at the more potent site (RT) would almost certainly lead to treatment failure, as the activity against the less potent site (IN) would not be sufficient to suppress viral replication. In this scenario, an even worse consequence of resistance mutations at both RT and IN sites is highly possible. Therefore, achieving balanced binding affinity against dissimilar targets is essential, which, unfortunately, remains the biggest challenge in designing multiple ligands.[14] The examples of balanced activities with **3c** and **3e** are not ideal as they reflect a loss of anti-RT activity compared to **3a**. However, these results do demonstrate that activities can be balanced via functionalizing the initial lead compound.

In conclusion, we have designed a scaffold for dual inhibitors of HIV reverse transcriptase (RT) and integrase (IN) by introducing a diketoacid (DKA) functionality into the tolerant C-5 site of RT inhibitor delavirdine. Assay results show that C-5 substituted inhibitors **3a**, **3c**, and **3e** all demonstrate good activity against both RT and IN in enzymatic assays and HIV in cell-based assay, whereas their C-7 regioisomers are all inactive. We have also demonstrated that balanced activities can be achieved via functionalizing the initial lead compound. Inhibitor **3e** demonstrates the best balance against RT (IC_{50}: 1.1 μM), IN (IC_{50}: 4.7 μM), and HIV (EC_{50}: 0.98 μM).

5. Experimental

5.1. Chemistry

5.1.1. General procedures. All commercial chemicals were used as supplied unless otherwise indicated. Dry solvents (THF, Et_2O, CH_2Cl_2, and DMF) were dispensed under argon from an anhydrous solvent system with two packed columns of neutral alumina or molecular sieves. Anhydrous ethanol was purchased from Sigma–Aldrich. Flash chromatography was performed with Silia-P flash silica gel (silicycle, 230–400 mesh) with indicated mobile phase. All reactions were performed under inert atmosphere of ultra-pure argon with oven-dried glassware. ^1H and ^{13}C NMR spectra were recorded on a Varian 600 MHz spectrometer. High resolution mass data were acquired on an Agilent TOF II TOS/MS spectrometer capable of ESI and APCI ion sources. Microwave reactions were conducted with a Personal Chemistry Emrys Optimizer Microwave Reactor.

5.1.1.1. (*E*)-Ethyl 2-(2-(4-acetylphenyl)hydrazono)propanoate (11). This compound was prepared according to

a known procedure.[25] The crude hydrazone was obtained in 91% yield, which was recrystallized from toluene to give pure hydrazone **11** (65%) as a purple solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 4.31 (q, J = 7.2 Hz, 2H), 2.53(s, 3H), 2.12 (s, 3H), 1.36 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.1, 165.2, 147.5, 135.4, 131.2, 130.6, 113.7, 61.7, 26.5, 14.5, 10.9.

5.1.1.2. Ethyl 5-acyl-1H-indole-2-carboxylate (6a). Hydrazone **11** (1.00 g × 5, 4.00 mmol × 5) was placed into 5 reaction tubes. To each tube was added 6.0 mL of TFA. The resulting dark purple suspension was microwaved (140 °C) for 15 min. TFA was then removed and the residue was subjected to flash chromatography (silica gel, hexanes/EtOAc = 4:1) to afford compounds **6a** (2.05 g, 45%) as a light purple solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.34 (s, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.31 (s, 1H), 4.43 (q, J = 7.8 Hz, 2H), 2.65 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.3, 162.0, 139.5, 131.0, 129.4, 127.1, 125.3, 125.2, 112.3, 110.3, 61.6, 26.8, 14.6.

5.1.1.3. Ethyl 3-bromo-1*H*-indole-2-carboxylate (12a). A solution of *N*-bromosuccinimide (NBS, 3.92 g, 22.0 mmol) in DMF (15.0 mL) was added dropwise to an ice-cooled solution of ethyl 1*H*-indole-2-carboxylate (3.78 g, 20.0 mmol) in DMF (10.0 mL). Upon completion of addition (ca. 20 min), the resulting mixture was allowed to warm to room temperature and stirred for an additional 2 h. This mixture was then poured into 300 mL of ice water and the resulting precipitate was collected by filtration, washed with water and dried under vacuum to afford compound **12a** (5.25 g, 98%) as a yellow solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.30 (br s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.40–7.34 (m, 2H), 7.21(t, J = 7.8 Hz, 1H), 4.45 (q, J = 6.6 Hz, 2H), 1.44 (t, J = 6.6 Hz, 3H).

5.1.1.4. Ethyl 3-chloro-1H-indole-2-carboxylate (12b). This compound was prepared using NCS following a similar procedure described for the preparation of **12a**: yield (95%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.25 (br s, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.39-7.35 (m, 2H), 7.21(t, J = 7.2 Hz, 1H), 4.47 (q, J = 7.2 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.5, 135.1, 126.8, 126.4, 122.6, 121.5, 120.4, 112.6, 112.3, 61.7, 14.6.

5.1.2. General procedure for the Friedel–Crafts acylation[25]. A suspension of $AlCl_3$ (30.0 mmol) in dichloroethane (DCE, 100 mL) was cooled to 0 °C. To this was added acetyl chloride (30.0 mmol) and the mixture was stirred for 5 min. A solution of an indole-2-carboxylate in 20.0 mL of DCE was then added dropwise. The resulting mixture was stirred at room temperature for 2 h and then poured into 100 mL of ice water. The aqueous was extracted with EtOAc (100 mL × 2) and the combined organics were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to flash chromatography (silica gel, hexanes/EtOAc, 6:1) to afford desired acyl compounds.

5.1.2.1. Ethyl 7-acyl-3-1*H*-indole-2-carboxylate (6b). Yield (23%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.75 (s, 1H), 7.87 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 7.2 Hz, 1H), 7.21 (d, J = 1.8 Hz, 1H), 7.16 (t, J = 7.2 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 2.65 (s, 3H), 1.40 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 199.8, 161.5, 135.4, 129.5, 129.1, 129.0, 128.2, 121.2, 120.1, 108.5, 61.3, 26.6, 14.6.

5.1.2.2. Ethyl 5-acyl-3-bromo-1*H*-indole-2-carboxylate (6c). Yield (50%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.51(br s, 1H), 8.28 (s, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 4.47 (q, J = 7.2 Hz, 2H), 2.69 (s, 3H), 1.44 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.8, 160.9, 137.9, 131.6, 127.7, 126.4, 126.0, 123.9, 112.5, 100.1, 62.1, 26.8, 14.5.

5.1.2.3. Ethyl 7-acyl-3-bromo-1*H*-indole-2-carboxylate (6d). Yield (33%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.79 (br s, 1H), 7.89 (d, J = 7.2 Hz, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.24 (d, J = 7.2 Hz, 1H), 4.44 (q, J = 7.2 Hz, 2H), 2.68 (s, 3H), 1.44 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 199.8, 160.3, 134.0, 129.4, 129.3, 127.8, 125.8, 121.1, 120.7, 98.6, 61.7, 26.8, 14.6.

5.1.2.4. Ethyl 5-acyl-3-chloro-1*H*-indole-2-carboxylate (6e). Yield (45%); ^{1}H NMR (600 MHz, CD_3OD) δ 8.24 (s, 1H), 7.89 (d, J = 9.0 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 2.63 (s, 3H), 1.40 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 199.1, 160.8, 137.9, 130.4, 125.5, 125.4, 124.3, 122.5, 113.5, 112.8, 61.4, 26.2, 14.0.

5.1.2.5. Ethyl 7-acyl-3-chloro-1*H*-indole-2-carboxylate (6f). Yield (45%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.60 (br s, 1H), 7.85 (d, J = 7.2 Hz, 2H), 7.19 (t, J = 7.8 Hz, 1H), 4.43 (q, J = 7.2 Hz, 2H), 2.65 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 199.8, 160.2, 133.2, 129.3, 127.5, 126.5, 124.0, 121.0, 120.5, 112.7, 61.6, 26.7, 14.6.

5.1.3. General procedure for the hydrolysis of indole ester 6a–f. A solution of ester (3.20 mmol) in EtOH/CH_2Cl_2 (1:1, 10.0 mL) was treated with 1 N NaOH (13.0 mL, 13.0 mmol). The resulting mixture was stirred for 5 h. After separation, the aqueous was extracted with 20% MeOH in CH_2Cl_2 (15.0 mL × 2) and then acidified with 1 N HCl to pH 4. The precipitate was collected by filtration, washed thoroughly with water, and dried under vacuum to give acids **13a**–**f**.

5.1.3.1. 5-Acyl-1*H*-indole-2-carboxylic acid (13a). Yield (44%,); ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.06 (s, 1H), 10.09 (s, 1H), 8.38 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.23 (s, 1H), 2.57 (s, 3H), 1.42 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 198.0, 163.1, 140.2, 131.0, 130.5, 127.0, 125.5, 124.4, 113.1, 109.7, 27.2.

5.1.3.2. 7-Acyl-1*H*-indole-2-carboxylic acid (13b). Yield (37%,); ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.37 (s, 1H), 10.65 (s, 1H), 8.03 (d, J = 7.2 Hz, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.22 (d,

J = 2.4 Hz, 1H), 2.66 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 200.7, 162.8, 135.1, 130.3, 129.6, 129.2, 129.1, 121.3, 120.9, 108.4, 27.2.

5.1.3.3. 5-Acyl-3-bromo-1*H*-indole-2-carboxylic acid (13c). Yield (66%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.45 (br s, 1H), 12.51 (s, 1H), 8.14 (s, 1H), 7.89 (d, J = 9.0 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 2.61 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 197.8, 161.9, 138.7, 131.1, 127.5, 127.2, 125.6, 122.9, 113.9, 97.8, 27.4.

5.1.3.4. 7-Acyl-3-bromo-1*H*-indole-2-carboxylic acid (13d). Yield (66%,); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.78 (s, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 2.66 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 200.4, 162.1, 134.0, 129.6, 129.4, 127.8, 126.2, 120.7, 112.5, 98.5, 26.4.

5.1.3.5. 5-Acyl-3-chloro-1*H*-indole-2-carboxylic acid (13e). Yield (90%,); ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.64 (br s, 1H), 12.38 (s, 1H), 8.23 (s, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 2.61 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 197.8, 161.9, 137.8, 130.9, 125.7, 125.6, 125.3, 122.0, 113.8, 111.5, 27.3.

5.1.3.6. 7-Acyl-3-chloro-1*H*-indole-2-carboxylic acid (13f). Yield (52%,); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.59 (br s, 1H), 7.81 (d, J = 7.2 Hz, 2H), 7.16 (t, J = 7.8 Hz, 1H), 2.63 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.3, 162.5, 133.1, 129.3, 127.4, 126.3, 123.9, 121.0, 120.3, 112.5, 26.3.

5.1.4. General procedure for the coupling of acids 13a–f with amine 5. To a solution of acid (2.13 mmol) in 8.00 mL of DMF was added carbonyl diimidazole (CDI, 0.42 g, 2.56 mmol) at 0 °C. After the mixture was stirred for 30 min, a solution of amine **5** (0.56 g, 2.56 mmol) in 2.00 mL of DMF was added and the resulting mixture was stirred for 18 h. The precipitate was filtered via a pad of celite. The filtrate was diluted with CH_2Cl_2, washed with a saturated $NaHCO_3$ aqueous solution and brine, and dried over Na_2SO_4. After the solvent was removed, the residue was subjected to flash chromatography (silica gel, hexanes/EtOAc, 1:1) to afford compounds **4a**–**f**.

5.1.4.1. 1-(2-(4-(3-(Isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-5-yl)ethanone (4a). Yield (62%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.02 (s, 1H), 8.28(s, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 4.2 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 6.90–6.89 (m, 2H), 4.44 (d, J = 7.8 Hz, 1H), 3.90 (br s, 4H), 3.56 (m, 1H), 3.02 (d, J = 4.8 Hz, 4H), 2.86 (s, 3H), 1.16 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.3, 163.0, 150.4, 136.6, 134.7, 133.8, 132.3, 129.3, 128.9, 128.0, 121.2, 120.7, 120.5, 117.3, 104.9, 71.0, 43.6, 36.4, 31.4, 27.3, 23.0.

5.1.4.2. 1-(2-(4-(3-(Isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-7-yl)ethanone (4b). Yield (58%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.97 (s, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.55–7.54 (m, 1H), 7.23 (t, J = 7.8 Hz, 1H), 6.98 (d, J = 1.8 Hz, 1H), 6.92–6.89 (m, 2H), 6.83 (d, J = 7.8 Hz, 1H), 4.18 (s, 1H), 4.09 (br s, 4H), 3.54 (m, 1H), 3.18 (br s, 4H), 2.61 (s, 3H), 1.23 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 198.2, 162.8, 150.0, 138.9, 136.7, 135.1, 131.1, 130.6, 127.0, 124.5, 124.3, 120.8, 117.0, 112.2, 107.0, 49.4, 44.0, 36.7, 31.7, 26.8, 23.1.

5.1.4.3. 1-(3-Bromo-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-5-yl)ethanone (4c). Yield (92%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.21 (s, 1H), 8.13 (s, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 4.8 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 6.89 (dd, J = 7.8 Hz, 4.8 Hz, 1H), 6.80 (d, J = 7.8 Hz, 1H), 4.15 (d, J = 6.6 Hz, 1H), 3.85 (br s, 4H), 3.51 (m, 1H), 3.14 (t, J = 4.8 Hz, 4H), 2.62 (s, 3H), 1.19 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.9, 162.8, 149.9, 138.4, 136.7, 135.0, 131.0, 130.3, 126.6, 124.5, 122.3, 120.9, 117.0, 112.5, 93.4, 43.9, 36.7, 31.6, 26.8, 23.1; HRMS (ESI+) calcd for $C_{23}H_{27}N_5O_2Br$ $[M+H]^+$ 484.1353, found 484.1338 (E = −3.2 ppm).

5.1.4.4. 1-(3-Bromo-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-7-yl)ethanone (4d). Yield (44%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.80 (s, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.63 (m, 1H), 7.25–7.20 (m, 1H), 6.91–6.88 (m, 1H), 6.81 (d, J = 7.8 Hz, 1H), 3.84 (br s, 4H), 3.51 (m, 1H), 3.15 (br s, 4H), 3.11 (m, 1H), 2.66 (s, 3H), 1.20 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 200.0, 162.3, 150.0, 136.7, 135.0, 134.1, 130.2, 128.4, 127.8, 126.6, 124.7, 121.0, 120.8, 117.1, 112.3, 92.5, 50.7, 44.0, 26.7, 23.1, 23.0.

5.1.4.5. 1-(3-Chloro-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-5-yl)ethanone (4e). Yield (55%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.37 (s, 1H), 8.20(s, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 4.2 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 6.91–6.87 (m, 2H), 4.50 (d, J = 8.4 Hz, 1H), 3.85 (br s, 2H), 3.65 (br s, 2H), 3.56 (m, 1H), 3.00 (br s, 4H), 2.62 (s, 3H), 1.14 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 197.8, 161.0, 150.2, 137.6, 136.6, 134.6, 130.8, 129.7, 124.5, 124.3, 120.9, 117.3, 113.3, 104.8, 79.9, 43.6, 36.5, 31.4, 27.4, 22.9.

5.1.4.6. 1-(3-Chloro-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-7-yl)ethanone (4f). Yield (67%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.64 (s, 1H), 7.82 (t, J = 7.8 Hz, 2H), 7.65 (d, J = 4.8 Hz, 1H), 7.20 (t, J = 7.8 Hz, 1H), 6.87 (m, 1H), 6.79 (d, J = 7.8 Hz, 1H), 4.12 (d, J = 4.8 Hz, 1H), 3.84 (br s, 4H), 3.50 (m, 1H), 3.15 (m, 4H), 2.64 (s, 3H), 1.20 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 199.9, 161.7, 150.1, 136.6, 135.1, 133.5, 128.1, 127.8, 126.8, 125.6, 121.0, 120.6, 120.5, 116.9, 106.2, 49.8, 44.0, 26.7, 23.1.

5.1.5. General procedure for the synthesis of final acid compounds 3a–f from 4a–f. Sodium (4.38 mmol) was added to 3.00 mL of anhydrous EtOH at room temperature under argon. The mixture was stirred until a clear solution was obtained. To this was added diethyl oxalate

(0.237 mL, 1.75 mmol) and a suspension of compound **4a–f** (0.88 mmol) in 2 mL of anhydrous EtOH. The solution turned yellow immediately and precipitate formed. This mixture was stirred for 4 h then quenched with a saturated aqueous solution of NH_4Cl (5.0 mL), extracted with 20% MeOH in CH_2Cl_2 (10.0 mL × 3). The combined organics were washed with brine (15.0 mL) and dried over Na_2SO_4. After the solvent was removed, the residue was directly taken to next step, except for the intermediate from **4c**, which was purified by flash chromatography (silica gel, 5% MeOH in CH_2Cl_2) to afford compound **14c**.

The intermediate (**14c** and the crude residue for those from **4a**, **4b**, **4d–f**, 0.45 mmol) was dissolved into EtOH/CH_2Cl_2 (1:1, 2 mL) and treated with 1N NaOH (2 mL). The resulting mixture was stirred for 2 h and then thoroughly extracted with 20% MeOH in CH_2Cl_2. The aqueous phase was then acidified with 1 N HCl to pH 4. The precipitate was collected by filtration, washed with water, and dried under vacuum to give acids **3a–f**.

5.1.5.1. (*Z*)-Ethyl-4-(3-bromo-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-5-yl)-2-hydroxy-4-oxobut-2-enoate (14c). Yield (45%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.43 (s, 1H), 7.96 (s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 3.6 Hz, 1H), 7.35 (d, J = 7.2 Hz, 1H), 7.10 (s, 1H), 6.90 (dd, J = 7.8 Hz, 4.8 Hz, 1H), 6.81 (d, J = 7.8 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 3.65 (q, J = 7.2 Hz, 1H), 3.50 (m, 1H), 3.85 (br s, 4H), 3.13 (s, 4H), 1.37 (t, J = 7.2 Hz, 3H), 1.18 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.6, 168.3, 163.0, 162.8, 149.8, 139.0, 136.8, 134.8, 130.6, 128.4, 126.9, 124.0, 121.9, 121.0, 117.2, 113.0, 98.3, 93.5, 62.7, 50.6, 44.0, 36.8, 31.7, 18.5, 14.3.

5.1.5.2. (*Z*)-4-(2-(4-(3-(Isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-5-yl)-2-hydroxy-4-oxobut-2-enoic acid (3a). Yield (41% over two steps); ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 8.28 (s, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.56 (s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 7.8 Hz, 1H), 7.21–7.18 (m, 1H), 6.93 (s, 1H), 4.04 (br s, 4H), 3.63 (m, 1H), 3.29 (s, 4H), 1.24 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 191.4, 169.1, 164.7, 162.9, 146.5, 139.5, 138.8, 131.4, 127.7, 127.6, 127.2, 123.9, 123.1, 121.2, 121.1, 112.3, 106.8, 97.5, 48.7, 44.1, 21.2; HRMS (ESI−) calcd for $C_{25}H_{25}N_5O_5$ $[M-H]^-$ 476.1934. Found: 476.1938.

5.1.5.3. (*Z*)-4-(2-(4-(3-(Isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-7-yl)-2-hydroxy-4-oxobut-2-enoic acid (3b). Yield (24% over two steps); ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 8.15 (d, J = 7.8 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.58–7.53 (m, 3H), 6.97–6.86 (m, 2H), 6.68 (s, 1H), 3.95 (br s, 4H), 3.56 (m, 1H), 3.14 (s, 4H), 1.22 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 197.8, 167.0, 163.1, 150.0, 138.8, 137.3, 135.9, 134.3, 133.9, 128.9, 128.0, 125.5, 123.8, 122.2, 121.0, 117.5, 114.2, 111.6, 43.8, 42.5, 38.5, 22.5; HRMS (ESI−) calcd for $C_{25}H_{25}N_5O_5$ $[M-H]^-$ 476.1934. Found: 476.1925.

5.1.5.4. (*Z*)-4-(3-Bromo-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-5-yl)-2-hydroxy-4-oxobut-2-enoic acid (3c). Yield (49% from **14c**); ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 8.23 (s, 1H), 7.91 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 3.6 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.45 (s, 1H), 7.07–7.01 (m 2H), 3.84 (br s, 4H), 3.57 (m, 1H), 3.19 (s, 4H), 1.22 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.5, 168.4, 164.4, 163.5, 162.6, 148.2, 139.0, 137.9, 131.9, 130.5, 128.5, 126.8, 123.9, 121.7, 121.4, 119.2, 113.0, 98.3, 93.0, 44.1, 36.7, 29.7, 22.4; HRMS (ESI−) calcd for $C_{25}H_{25}N_5O_5Br$ $[M-H]^-$ 554.1039. Found: 554.1076.

5.1.5.5. (*Z*)-4-(3-Bromo-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-7-yl)-2-hydroxy-4-oxobut-2-enoic acid (3d). Yield (47% over two steps); ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 7.83 (d, J = 7.8 Hz, 1H), 7.77 (d, J = 8.4 Hz, 2H), 7.30 (t, J = 7.8 Hz, 1H), 7.25 (s, 1H), 7.14–7.11 (m, 1H), 7.06 (d, J = 7.8 Hz, 1H), 3.84 (br s, 4H), 3.47 (m, 1H), 3.16 (br s, 4H), 3.13 (m, 1H), 1.24 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.7, 166.3, 163.0, 162.8, 148.7, 136.9, 135.2, 134.1, 130.4, 128.6, 127.9, 126.6, 124.4, 121.3, 120.9, 117.5, 112.7, 92.8, 44.2, 26.7, 23.4, 23.1; HRMS (ESI−) calcd for $C_{25}H_{25}N_5O_5Br$ $[M-H]^-$ 554.1039. Found: 554.1028.

5.1.5.6. (*Z*)-4-(3-Chloro-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-5-yl)-2-hydroxy-4-oxobut-2-enoic acid (3e). Yield (34% over two steps); ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 8.28 (s, 1H), 7.90 (d, J = 9.0 Hz, 1H), 7.57 (d, J = 3.6 Hz, 1H), 7.48 (d, J = 9.0 Hz, 1H), 7.07–7.02 (m 2H), 3.86 (br s, 4H), 3.58 (m, 1H), 3.19 (s, 4H), 1.23 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 191.2, 169.2, 164.7, 162.1 (2), 148.4, 138.4, 137.9, 132.0, 128.3, 125.1, 123.9, 121.3, 120.7, 119.1, 113.0, 106.9, 44.0, 22.2; HRMS (ESI+) calcd. for $C_{25}H_{27}N_5O_5Cl$ $[M+H]^+$ 512.1701. Found: 512.1708.

5.1.5.7. (*Z*)-4-(3-Chloro-2-(4-(3-(isopropylamino)pyridin-2-yl)piperazine-1-carbonyl)-1*H*-indol-7-yl)-2-hydroxy-4-oxobut-2-enoic acid (3f). Yield (35% over two steps); ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$) δ 7.93 (d, J = 7.2 Hz, 1H), 7.82 (t, J = 7.2 Hz, 2H), 7.78 (d, J = 4.8 Hz, 1H), 7.74 (d, J = 4.8 Hz, 1H), 7.16 (d, J = 6.6 Hz, 1H), 7.03–7.00 (m, 1H), 4.15 (d, J = 7.2 Hz, 1H), 3.89 (br s, 4H), 3.22 (br s, 4H), 3.17 (m, 1H), 1.24 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 191.9, 166.4, 163.1, 162.8, 148.9, 137.1, 135.3 134.2, 130.3, 128.6, 127.8, 126.7, 124.5, 121.1, 120.8, 117.8, 112.8, 93.0, 44.4, 27.1, 23.6, 23.2; HRMS (ESI+) calcd for $C_{25}H_{27}N_5O_5Cl$ $[M+H]^+$ 512.1701. Found: 512.1702.

5.2. Biology

5.2.1. RT assay. This assay was performed using the *Quan-T-RT Assay System* kit from Amersham Biosciences (TRK 1022). Experiments were done using 0.65 mL snap cap tubes with a SPA (scintillation proximity assay) bead, where a short poly (A) tail and a oligo (T) primer have been coupled via a biotin linkage to a resin bead containing scintillation cocktail. This resin

was incubated with a drug compound, the [^{3}H]-TTP tracer and the reaction was initiated by the addition of the RT (RT) enzyme from Ambion: the RNA Company (AM2045). The [^{3}H]TTP was incorporated into the poly (A) tail by RT enzyme but in the presence of the drug candidate this incorporation should be inhibited. This reaction was incubated for 3 h at 37 °C and quenched by the addition of the stop buffer. These tubes were then transferred to 20 mL scintillation tubes (glass) and counted for 1 min per sample using a beta counter.

5.2.2. IN assay. IN was expressed using *Escherichia coli* BL21(DE3) and standard IPTG induction.[29] In a typical assay, DNA substrate 5′-biotin ATGTGGAAAA TCTCTAGCAGT and 3′-cy5 ACTGCTAGAGATTTT CCACAT (IDT) were annealed in DNA annealing buffer (50 mM Tris, pH 7.5, 10 mM $MgCl_2$) by heating to 95 °C and allowed to cool over 10 min in a thermocycler. For each inhibitor concentration 12.5 μL of 600 nM IN in reaction buffer (10 mM HEPES, pH 7.5, 10 mM $MnCl_2$, 1 mM DTT, 10% glycerol, 0.1 mg/ml BSA 0.05% Brij-35) was added to each well in a 96-well plate. 0.5 μL of inhibitor or DMSO was added to each sample and allowed to equilibrate with the enzyme for 10 min at room temperature. After the incubation, 12.5 μL of 50 nM DNA substrate in reaction buffer was added to each well to initiate the reaction. The reaction was incubated at 37 °C for 2 h, after which 75 μL of 4× binding buffer (20 mM Tris, pH 8.0, 1.6 M NaCl, 40 mM EDTA, 0.4 mg/ml salmon sperm DNA) was added and the entire mixture was transferred to a StreptaWell High bind plate (Roche). The DNA was allowed to bind to the plate for 1 h at room temperature with mild agitation. Non-specific DNA was removed from the plate by washing 3 × 5 min with 200 μL denaturing buffer (30 mM NaOH, 0.2 M NaCl, 1 mM EDTA). The plate was washed briefly with 200 μL of TE. To remove the DNA for fluorescence detection 100 μL of formamide was added to each well and incubated for 15 min at room temp with mild agitation. Ninety microliters of the formamide solution was transferred into a black 384-well plate and read on a molecular devices analyst using the continuous lamp excitation 620 nm, emission 665 nm.

5.2.3. HIV-1 assay. The HIV Cytoprotection assay used CEM-SS cells or human PBMCs and the IIIB or RF strain of HIV-1. Briefly virus and cells were mixed in the presence of test compound and incubated for 6 days. The virus was pre-titered such that control wells exhibit 70% to 95% loss of cell viability due to virus replication. Therefore, antiviral effect or cytoprotection was observed when compounds prevent virus replication. Each assay plate contained cell control wells (cells only), virus control wells (cells plus virus), compound toxicity control wells (cells plus compound only), compound colorimetric control wells (compound only) as well as experimental wells (compound plus cells plus virus). Cytoprotection and compound cytotoxicity were assessed by MTS (CellTiter® 96 Reagent, Promega, Madison WI) or XTT dye reduction and the EC_{50} (concentration inhibiting virus replication by 50%) values were provided. Each assay included the HIV RT inhibitor AZT as a positive control.

Acknowledgments

This research was supported by the Center for Drug Design at the University of Minnesota. We thank Dr. Eric Bennett for discussion, Dr. Christine Salomon for IN assay and Christine Dreis for RT assay. We also thank Roger Ptak at Southern Research Institute for cell-based assay, Dr. Robert Craigie of NIH for the integrase plasmid and Dr. William Shannon for consultation and discussion.

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