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Pharmacophore and structure–activity relationships of integrase inhibition within a dual inhibitor scaffold of HIV reverse transcriptase and integrase

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ABSTRACT

Rational design of dually active inhibitors against human immunodeficiency virus (HIV) reverse transcriptase (RT) and integrase (IN) has proved viable with 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) type of non-nucleoside RT inhibitors (NNRTIs). To establish the pharmacophore and study the structure–activity relationships (SAR) of integrase inhibition within a previously disclosed RT/IN dual inhibitor scaffold, new analogues featuring substitution at different sites of the HEPT ring were designed and synthesized. These studies have revealed an IN inhibition pharmacophore that is merged with the known RT pharmacophore through a shared C-6 benzyl group. Further SAR also demonstrated that optimal IN inhibition within our dual inhibitor scaffold requires a regiospecific (N-1) diketoacid (DKA)-carrying pendant with a certain length.

1. Introduction

The clinical management of acquired immunodeficiency syndrome (AIDS) caused by HIV[1] is complicated by the lack of vaccines[2] and the persistence of viral reservoirs.[3] Treatment of HIV/AIDS is essentially limited to highly active antiretroviral therapy (HAART),[4] a multi-target regimen that combines antivirals with orthogonal mechanisms of action, thus creating a larger barrier to resistance selection than monotherapy. HAART is centered around inhibitors of RT and protease (PR), and is enhanced by recently approved antivirals targeting new molecular mechanisms of HIV replication, such as CCR5 antagonist maraviroc[5] and IN inhibitor raltegravir.[6,7] Nevertheless, the adherence required by multi-target therapy to effectively curb resistance hinges largely on its tolerance as well as regimen simplicity, which could be difficult to achieve with HAART cocktails.[8,9] Instead, a maximally simplified multi-target therapy in which a single chemical scaffold confers inhibitory activities against multiple viral enzymes offers an appealing alternative with less problems of dosing complexity, drug–drug interactions and toxicities.[10,11] Such multi-functional single-structure compounds are referred to as designed multiple ligands (DMLs) and represent a subject of tremendous challenge and growing interest in medicinal chemistry.[12–14] We have previously disclosed the first rationally designed dually active inhibitors against HIV RT and IN based on a few NNRTI scaffolds.[15–17] Amongst these the best ones were designed based on TNK-651 (**1**, Fig. 1), an RT inhibitor of the HEPT family[18] that has an N-1 pendant extending from the NNRTI binding pocket to the protein/solvent interface. This orientation allowed the introduction of a relatively hydrophilic chelating group, namely a DKA functionality, to induce inhibitory activity against IN.[15] Compounds thus designed (e.g., **2**, Fig. 1) were found active against both enzymes, and showed exceptional anti-HIV activity.[15] This design, however, was largely based on a well-known RT pharmacophore with only a minimal DKA functionality designed in for IN inhibition. It remains unknown as for what other structural moieties within this dual inhibitor scaffold may have contributed to the observed anti-IN activity and whether this activity is optimized. Given the lack of critical structural information on HIV IN catalysis, studies aimed at unraveling the pharmacophore and SAR of IN inhibition within a dual inhibitor scaffold would provide knowledge and insights necessary for future design of novel RT/IN dual inhibitors with improved IN inhibition.

Notably major chemotypes of IN inhibitors all feature a DKA functionality or its heterocyclic bioisostere, and selectively inhibit strand transfer (ST).[19–21] Despite success in clinical development of ST inhibitors as manifested by the recently approved raltegravir, structure based design of IN inhibitors is prohibitively hindered by the insufficient characterization of detailed binding mode, which most likely involves a DNA–IN interface.[19] The DNA bound IN crystal structure has eluded the field until very recently.[22] As

Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; RT, reverse transcriptase; IN, integrase; HEPT, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine; NNRTI, non-nucleoside reverse transcriptase inhibitors; SAR, structure–activity relationship; DKA, diketoacid; HAART, highly active antiretroviral therapy; DML, designed multiple ligand; CPE, cytopathic effect.

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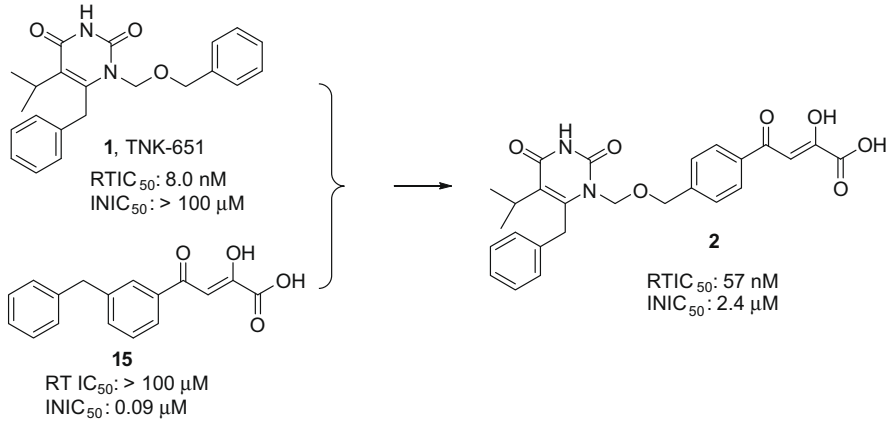


Figure 1. Rationally designed RT/IN dual inhibitor **2** based on HEPT NNRTI **1** and DKA IN inhibitor **15**.[15]

a result, docking models[23,24] constructed based on partial IN structure or homologous enzymes generally lack this critical DNA–IN interface, hence the biological relevance and predictability.[25] Nevertheless, a vastly simplified pharmacophore model implicated in major SAR studies has revealed two minimal structural components required for IN binding: a chelating triad which binds with two Mg^{2+} ions in a relatively hydrophilic region, anchoring the inhibitor onto the protein surface; and a hydrophobic benzyl moiety that fits in a highly hydrophobic cavity near the active site (Fig. 2).[26–29] As observed in compound **15**, a stereotypical DKA IN inhibitor has a benzyl group immediately connected to an aromatic ring on which the DKA functionality is hanging (Fig. 1). Intriguingly, our dual inhibitor **2** exhibits potent inhibitory activity against IN without having such a typical pharmacophore. We hypothesize that the C-6 benzyl group (circled in blue, Fig. 2) of the HEPT RT pharmacophore may have also contributed to IN inhibition by binding to the hydrophobic cavity. We intend to verify this pharmacophore hypothesis and explore its SAR by studying the spatial relationship between the C-6 benzyl group and the DKA chelator. Such a relationship has proved central to the SAR of typical DKA IN inhibitors.

Detailed SAR design is delineated in Figure 3. We first designed compounds **3**–**6** to probe if an additional benzyl group is required for IN binding (Fig. 3). Introducing a benzyl in N-1 side chain would render the right half of these compounds identical to stereotypical DKA IN inhibitor **15** (Fig. 1). Compounds **7**–**8** were designed to evaluate how the length of the N-1 linker affects IN binding, whereas compounds **9**–**10** (substituted at C-2) and compounds **11**–**14** (substituted at N-3 position) were included to study the impact of the angle between the chelator and benzyl on IN inhibition. As the focus of this study is primarily on understanding IN inhibition, potential loss of RT binding affinity incurred from structural modifications is anticipated. Balancing inhibitory activities against different enzyme targets will be the concern of future studies and can be best achieved with well defined pharmacophores, which is the main goal of this particular study.

2. Results and discussion

2.1. Chemical synthesis

The chemistry involved in this study primarily concerns the derivatization at N-1, C-2 and N-3 sites of pyrimidine compounds to prepare analogues proposed in Figure 3. Particularly the regioselective functionalization at N-1/N-3 is not easily accessible. Exclusive N-1 alkylation can be achieved via bissilylated intermediates **24**–**27** (Scheme 1), though this reaction works with highly reactive electrophiles only, such as chloromethyl ether (e.g., **19**), benzylic/allylic halide or methyl iodide. This strategy allowed the preparation of target compounds **3**–**6** (Scheme 1). The synthesis starts with a Suzuki coupling reaction to assemble the benzyl alcohol intermediate **18**, which was converted into chloromethyl ether **19** using previously reported method.[15] The subsequent alkylation provided the access to key methyl ketone intermediates **28**–**31**. These methyl ketones were further elaborated into desired DKA compounds **3**–**6** via a Claisen type condensation and a saponification reaction (Scheme 1).

A similar synthetic route was employed for the synthesis of target compounds **7**–**8** (Scheme 2), wherein the requisite acetophenones **34**–**35** were prepared from alcohols **32**–**33** through a Friedel–Crafts acylation.[30]

C-2 derivatization was easily achieved through an S-alkylation of thiopyrimidine compound **43** (Scheme 3). The alkylating agents, bromides **41**–**42**, were prepared by brominating alcohols **40** and **18**. Conversion of alkylated intermediates, acetophenones **44**–**45**, into DKA target compounds **9**–**10** was effected through an aforementioned two-step process consisting of a condensation and a saponification.

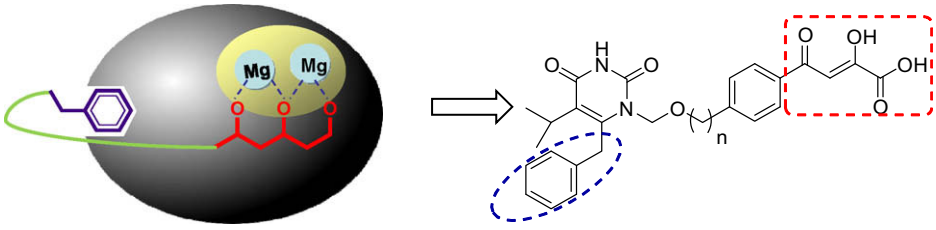


Figure 2. Schematic representation of IN binding mode: the DKA chelator (red) anchors onto the hydrophilic surface of IN and binds to two Mg^{2+} ions, while a terminal benzyl group (blue) fits in a hydrophobic cavity nearby. These two structural moieties are connected through a linker (green).

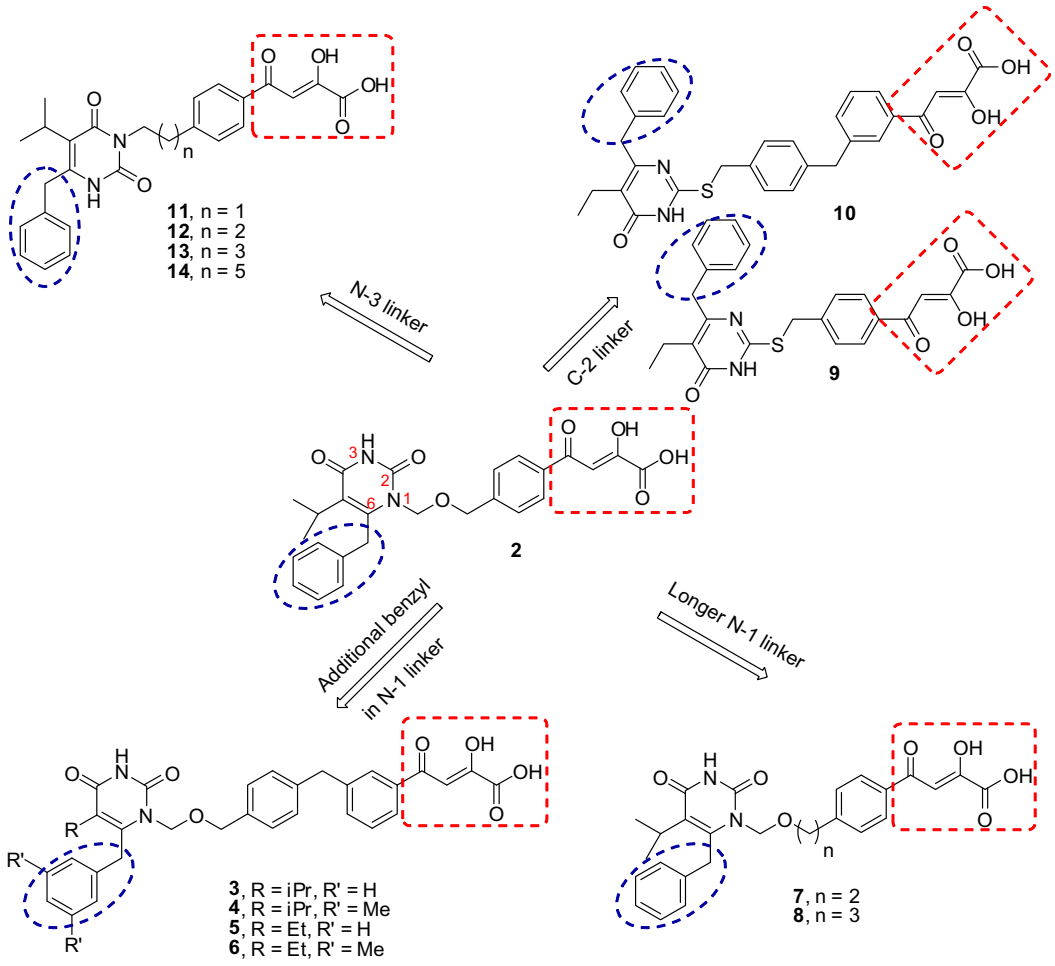
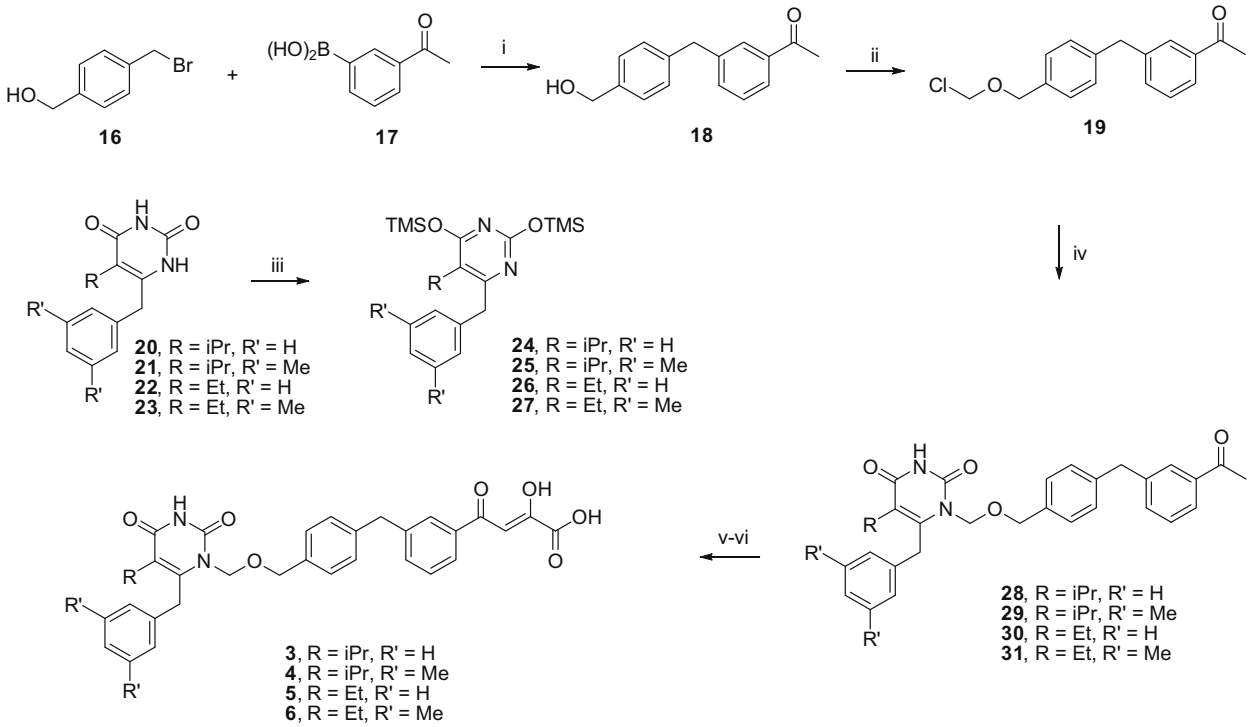
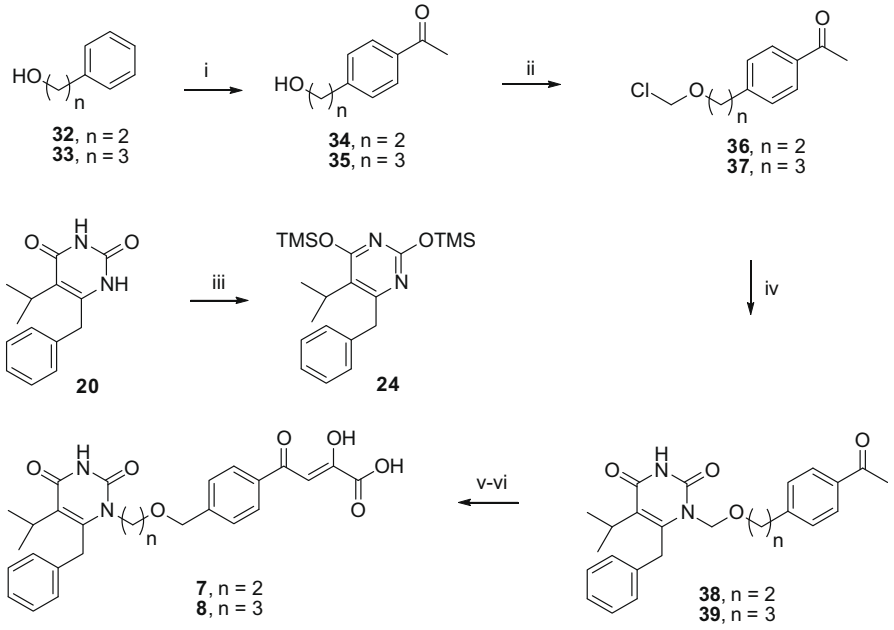


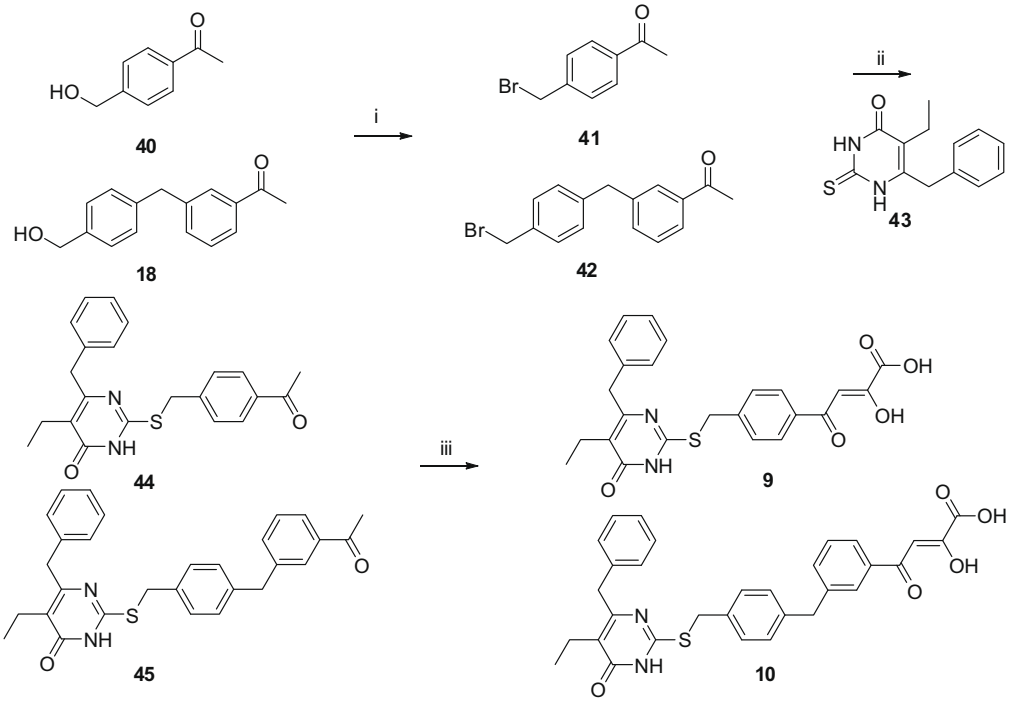
Figure 3. Design of SAR: minimal pharmacophores are circled (red for the DKA chelating triad and blue for the terminal benzyl group).



Scheme 1. Synthesis of compounds **3**–**6**. Reagents and conditions: (i) $Pd(OAc)_2$, PPh_3, K_3PO_4, toluene, rt, 81%; (ii) $(HCHO)_n$, TMSCl, rt; (iii) *N*,*O*-bistrimethylsilylacetamide (BSA, 2.2 equiv), CH_2Cl_2, rt; (iv) **24**/**25**/**26**/**27**, TBAI, rt, 77–88%; (v) Na/EtOH, diethyloxalate, rt; (vi) NaOH, rt, 30 min, 61–72%.



Scheme 2. Synthesis of compounds **7**–**8**. Reagents and conditions: (i) AcCl/$AlCl_3$, hexanes, −10 °C to rt, 80–81%; (ii) $(HCHO)_n$, TMSCl, rt; (iii) *N*,*O*-bistrimethylsilylacetamide (BSA, 2.2 equiv), CH_2Cl_2, rt; (iv) **24**, TBAI, rt, 70–99%; (v) Na/EtOH, diethyloxalate, rt; (vi) NaOH, rt, 30 min, 49–60%.

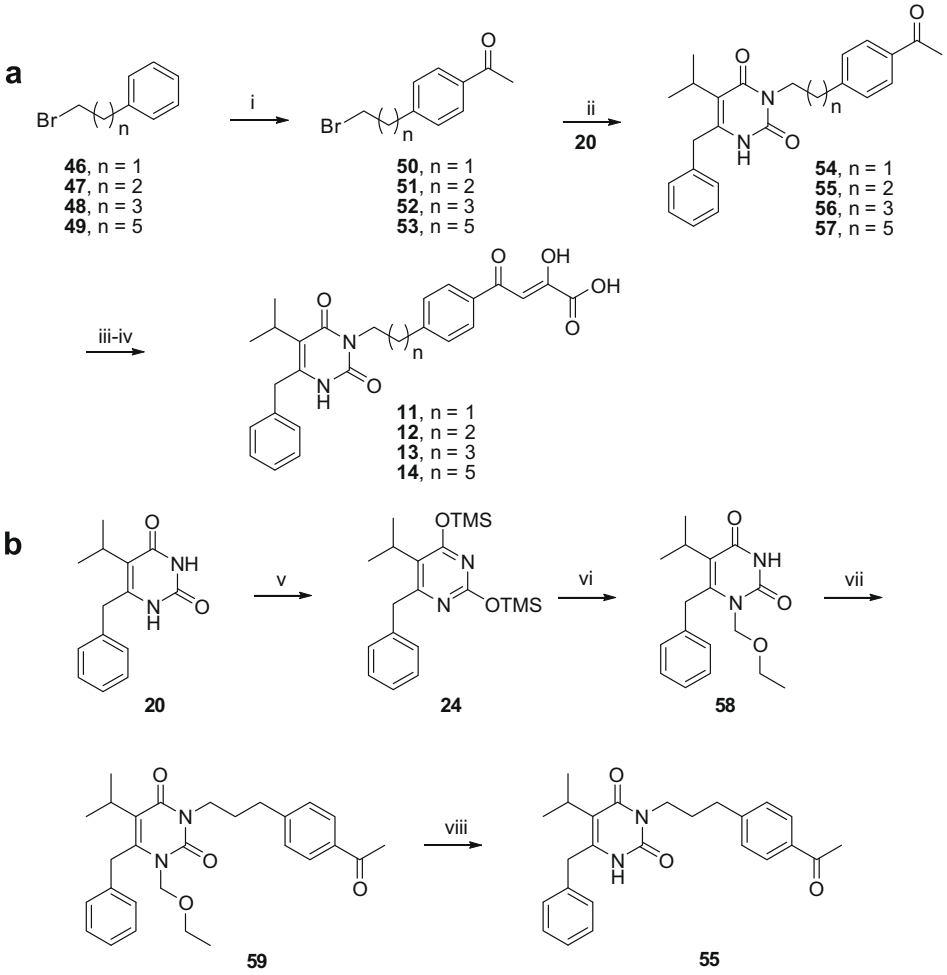


Scheme 3. Synthesis of compounds **9**–**10**. Reagents and conditions: (i) NBS/PPh_3, THF, 0 °C, 1 h, 80%; (ii) **43**, K_2CO_3, DMF, rt, 6 h, 50–80%; (iii) (a) Na/EtOH, diethyloxalate, rt; (b) NaOH, rt, 30 min, 62–67%.

Functionalization at N-3 position was accomplished by direct alkylation (Scheme 4a) in modest yields. Notably direct alkylation with uracil or thymine generally occurs at N-1 site.[31] The unusual regioselectivity observed herein may be attributed to steric hindrance. Nevertheless, the efficiency of this reaction is considerably compromised by the undesired N-1 and N-3 dialkylation. An alternative route[32] involving selective N-1 protection and deprotection was found to produce N-3 regioisomers in 65% yield over three steps for compound **55** (Scheme 4b). Comparison on NMR spectra showed that compounds prepared via both routes are identical, confirming the N-3 regioselectivity of the direct alkylation.

2.2. Biological evaluation

All compounds were tested biochemically against both RT and IN using recombinant enzymes. Their ability to inhibit HIV replica-



Scheme 4. Synthesis of compounds **11**–**14**. Reagents and conditions: (i) AcCl/$AlCl_3$, CS_2, 0 °C, 1 h, 75–83%; (ii) Cs_2CO_3, DMF, 50 °C, 2 h, 20–40%; (iii) Na/EtOH, diethyloxalate, rt; (iv) NaOH, rt, 30 min, 46–52%; (v) *N*,*O*-bistrimethylsilylacetamide (BSA, 2.2 equiv), CH_2Cl_2, rt; (vi) Ethoxymethylchloride, TBAI, rt, 83%; (vii) Cs_2CO_3, DMF, 80 °C, 2 h, 93%; (viii) 90% TFA (aqueous), reflux, 2 h, 84%.

Table 1
Effects of an additional benzyl group in N-1 side chain on IN binding

Compound	RT IC_{50}[a] (μM)	IN IC_{50}[a] (μM)	HIV EC_{50}[a] (μM)	TC_{50}[b] (μM)	TI[c]
3	0.16	11	0.46	>100	>220
4	0.10	22	0.17	96	560
5	0.71	16	0.56	32	56
6	0.028	14	0.014	>100	>710
2[d]	0.057	2.4	0.033	>10	>310
1[d]	0.016	>100	0.016	>10	>610
15[d]	>100	0.093	0.16	>10	>61

[a] Concentration of 50% inhibition.
[b] Concentration of 50% cytotoxicity.
[c] Therapeutic index, defined as the ratio of TC_{50}/EC_{50}.
[d] Data taken from previous publication.[15]

tion was evaluated using a cytoprotection (reduction of CPE) assay with CEM-SS cells infected with the IIIB strain of HIV-1. Table 1 summarizes assay results for compounds **3**–**6**. Significantly, compound **3** which has an additional benzyl group confers an anti-HIV activity 14-fold lower than compound **2**, along with a reduced anti-RT activity (3-fold) and anti-IN activity (5-fold). The latter is particularly intriguing as the right half of molecule **3** is identical to compound **15** which is a potent IN inhibitor (Fig. 1). Apparently the additional benzyl group in the N-1 side chain severely hinders IN binding, which strongly suggests that only the terminal C-6 benzyl group can fit into the hydrophobic cavity (Fig. 2), and that this terminal benzyl group is removed too far from the chelating DKA by the additional benzyl group in N-1 linker. These results lend support to our hypothesis that within our dual inhibitor scaffold the C-6 benzyl group on the HEPT ring is an important part of the pharmacophore for IN inhibition. This is significant as the same benzyl group is also essential for RT binding, indicating that the two pharmacophores responsible for dual inhibitory activities are merged, which is a highly desirable yet not readily achievable feature for dual inhibitors (or DMLs).[13,14]

To further study the impact of the spatial arrangement between the DKA and the C-6 benzyl group on IN inhibition, more analogues with variable distances and angles were designed and synthesized. Since our focus is primarily on IN binding, dose response studies were carried out against IN only. Inhibition against HIV and RT was evaluated at single and double concentrations, respectively (Table 2). Interestingly, a 3–4-fold drop in anti-IN activity was observed with compounds **7**–**8** compared to compound **2**, confirming that elongating the N-1 linker adversely affects IN binding affinity. The most drastic loss of IN inhibition was observed with compounds **9** and **10** (inactive at 100 μM), in which the side chain carrying the DKA chelator is placed on the C-2 of the pyrimidine ring. Such an orientation dramatically alters the angle between the terminal benzyl group and the DKA, preventing the former from binding to the hydrophobic cavity. By contrast, the placement of the DKA-bearing pendant at the N-3 position does not seem to prohibit

Table 2
Effects of distance and angle between DKA and C-6 benzyl on IN binding

Compound	% Inhibition against RT		IN IC_{50} (μM)	% HIV CPE reduction at 10 μM
	10 μM	100 μM		
7	100	100	7.4	100
8	100	100	8.2	91
9	0	0	>100	NA[a]
10	0	0	>100	NA[a]
11	0	0	8.0	28
12	0	0	12	0.6
13	0	0	37	0
14	0	0	>100	9.0

[a] Compounds are cytotoxic.

IN binding, though a considerable decrease in inhibition was observed (3–15-fold for compounds **11**–**13**), with a 2–3 atom linker being most active. When a linker as long as six carbons was used (compound **14**), the compound was found devoid of inhibition against IN. Not surprisingly, the derivatization at N-3 of the pyrimidine ring (compounds **11**–**14**) causes a complete loss of activity against RT (Table 2). The proton at N-3 has proved crucial for RT binding as it forms a key hydrogen bond with K101 in the NNRTI binding pocket.[33] However, the loss of anti-RT activity observed with C-2 derivatives (compounds **9**, **10**) is somewhat unexpected as similar scaffolds have been found in dihydro(alkylthio)benzyloxopyrimidines (s-DABO) compounds which are generally active against RT.[34,35]

3. Conclusions

We have designed and synthesized various derivatives of a previously reported HIV RT/IN dual inhibitor (**2**) to establish the pharmacophore of IN inhibition and explore the impact on IN binding of spatial arrangements between the DKA chelating triad and the C-6 terminal benzyl group. Through these studies we have demonstrated that for RT/IN dual inhibitors based on HEPT scaffold, the C-6 benzyl group which constitutes part of the RT pharmacophore also fulfils the hydrophobic binding requirement for IN, and that to achieve optimal inhibition against IN the DKA-carrying pendant should be at N-1 or N-3 site of the HEPT ring and the linker between the two minimal pharmacophores should be 2–3 atoms long (compounds **2**, **11**, **12**). It was also observed that the N-3 substitution causes a complete loss of anti-RT activity, leaving N-1 as the sole site for the incorporation of the DKA chelator. Due to the dearth of structural information on IN binding, these findings provide an important source of knowledge and inspiration for our ongoing efforts in the design and synthesis of improved HIV RT/IN dual inhibitors.

4. Experimental section

4.1. Chemistry

4.1.1. 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 on a Teledyne Combiflash RF-200 with RediSep columns (silica) and 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.

4.1.1.1. 1-(4-(2-Hydroxyethyl)phenyl)ethanone (34). $AlCl_3$ (19.56 g, 0.147 mol) was added to 30 mL of pre-cooled (−10 °C) hexanes followed by the slow addition of alcohol **32** (6.0 g, 0.049 mol). To the resulting slurry was added acetyl chloride dropwise. Upon completion of addition, the mixture was allowed to stir at −10 °C for an additional 2 h. The reaction was quenched by pouring the mixture into 50 mL of ice water. After separation, the aqueous phase was extracted with CH_2Cl_2 (40 mL × 3). The combined organics were concentrated and the residue was re-dissolved into 50 mL of THF. To the solution was then added 50 mL of 2 N NaOH and the resulting mixture was stirred at room temperature for 2 h. After separation the aqueous was extracted with CH_2Cl_2 (50 mL × 3). The combined organic extracts were washed with saturated aqueous NH_4Cl, water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, hexanes/EtOAc, 4:1) to afford compound **34** (6.5 g, 81%) as a pale yellow oil: ^1H NMR (600 MHz, $CDCl_3$) δ 7.76 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 3.75 (q, J = 6.0 Hz, 2H), 3.41 (t, J = 5.4 Hz, 1H), 2.80 (t, J = 6.6 Hz, 2H), 2.44 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.6, 145.3, 135.3, 129.4, 128.7, 63.1, 39.3, 26.7.

4.1.1.2. 1-(4-(3-Hydroxypropyl)phenyl)ethanone (35). This compound was prepared from alcohol **33** following the procedure described for the synthesis of **34**. ^1H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 3.63 (q, J = 5.4 Hz, 2H), 2.73 (t, J = 6.0 Hz, 2H), 2.53 (s, 3H), 2.27 (s, 1H), 1.86 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.3, 148.2, 135.2, 128.9, 128.8, 62.0, 34.0, 32.3, 26.7.

4.1.1.3. 1-(3-(4-(Hydroxymethyl)benzyl)phenyl)ethanone (18). A mixture of alcohol **16** (2.01 g, 10 mmol), boronic acid **17** (2.46 g, 15 mmol), K_3PO_4 (20 mmol), PPh_3 (104 mg, 0.4 mmol) and $Pd(OAc)_2$ (45 mg, 0.2 mmol) was vacuumed and purged with argon. To this was then added 30 mL of toluene. The resulting yellow suspension was stirred at room temperature for 2 days. The reaction mixture was then taken up in 100 mL of ether and washed with 1 N NaOH and brine. The organic was dried over Na_2SO_4 and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, hexanes/EtOAc, 2:1) to afford compound **18** (1.9 g, 81%) as a light yellow solid: ^1H NMR (600 MHz, $CDCl_3$) δ 7.80–7.77 (m, 2H), 7.37–7.36 (m, 2H), 7.28 (d, J = 7.8 Hz, 2H), 7.16 (d, J = 7.8 Hz, 2H), 4.63 (d, J = 5.4 Hz, 2H), 4.01 (s, 2H), 2.55 (s, 3H), 2.07 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.6, 141.9, 140.0, 139.3, 137.6, 133.9, 129.3, 129.0, 128.8, 127.6, 126.6, 65.2, 41.7, 26.9.

4.1.1.4. 1-(4-(Bromomethyl)phenyl)ethanone (41). To a solution of alcohol **40** (0.45 g, 0.003 mol) and PPh_3 (0.786 g, 0.003 mol) in THF was added NBS (0.534 g, 0.003 mol) in one portion at 0 °C. The resulting yellow solution was stirred at 0 °C for 1 h. The reaction was then quenched by adding 20 mL of H_2O and was extracted with CH_2Cl_2 (20 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, hexanes/EtOAc, 2:1) to afford compound **41** (0.48 g, 80%) as a pale yellow oil: ^1H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, J = 7.8 Hz, 2H), 7.43 (d, J = 7.8 Hz, 2H), 4.46 (s, 2H), 2.55 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.5, 143.0, 137.1, 129.5, 129.0, 32.4, 26.9.

4.1.1.5. 1-(3-(4-(Bromomethyl)benzyl)phenyl)ethanone (42). This compound was prepared from alcohol **18** as an orange oil following the procedure described for the preparation of **41**. Yield: 80%, ^1H NMR (600 MHz, $CDCl_3$) δ 7.81–7.79 (m, 2H), 7.37 (m, 2H), 7.31 (d, J = 7.8 Hz, 2H), 7.15 (d, J = 7.8 Hz, 2H), 4.45 (s, 2H), 4.01 (s, 2H), 2.56 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.3, 141.5, 141.1, 137.7, 136.1, 134.0, 129.6, 129.5, 129.1, 128.9, 126.7, 41.7, 33.7, 27.0.

4.1.1.6. 1-(4-(2-Bromoethyl)phenyl)ethanone (50). A mixture of $AlCl_3$ (8.3 g, 0.063 mol), acetyl chloride (4.7 mL) and CS_2 (30 mL) was stirred at 0 °C while a solution of bromide **46** (12.4 g, 0.067 mol) in 9.5 mL of acetyl chloride was added. The resulting mixture was stirred at 0 °C for 1 h and then poured into a mixture of concentrated HCl (20 mL) and ice (200 g). This mixture was extracted with CH_2Cl_2 (30 mL × 4). The combined organic extracts were washed with 1 N NaOH, water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, hexanes/EtOAc, 9:1) to afford compound **50** (11.4 g, 75%) as a pale yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 3.52 (t, J = 7.8 Hz, 2H), 3.15 (t, J = 7.2 Hz, 2H), 2.51 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.8, 144.5, 136.1, 129.1, 128.9, 39.2, 32.5, .26.8.

4.1.1.7. 1-(4-(3-Bromopropyl)phenyl)ethanone (51). This compound was prepared from bromide **47** as a pale yellow oil following the procedure described for the preparation of **50**. Yield: 81%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, J = 7.8 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 3.38 (t, J = 6.6 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 2.58 (s, 3H), 2.18 (quintet, J = 7.2 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.9, 146.5, 135.6, 129.0, 128.9, 34.2, 33.9, 33.0, 26.8.

4.1.1.8. 1-(4-(4-Bromobutyl)phenyl)ethanone (52). This compound was prepared from bromide **48** as a pale yellow oil following the procedure described for the preparation of **50**. Yield: 83%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, J = 7.2 Hz, 2H), 7.23 (d, J = 7.8 Hz, 2H), 3.38 (t, J = 6.6 Hz, 2H), 2.66 (t, J = 7.2 Hz, 2H), 2.54 (s, 3H), 1.85 (quintet, J = 6.6 Hz, 2H), 1.76 (quintet, J = 7.2 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.9, 147.8, 135.4, 128.8 (2), 35.2, 33.6, 32.3, 29.6, 26.8.

4.1.1.9. 1-((4-Acetylphenethoxy)methyl)-6-benzyl-5-isopropylpyrimidine-2,4(1*H*,3*H*)-dione (38). To a suspension of pyrimidine **20** (0.23 g, 1.0 mmol) in CH_2Cl_2 (2.0 mL) was added BSA (0.538 mL, 2.2 mmol) at room temperature under argon. The resulting mixture was stirred until a clear solution was achieved (ca. 30 min, **24** obtained). A separate flask was charged with alcohol **34** (246 mg, 1.5 mmol) and 1.0 mL of TMSCl. To this was added paraformaldehyde (54 mg, 1.8 mmol). The mixture was stirred until a homogeneous solution was observed (ca. 30 min). The solution was concentrated and the residue was dissolved in 2.0 mL and added to the freshly prepared intermediate **24**, followed by the addition of a catalytic amount of TBAI. The reaction mixture was kept overnight and then quenched by adding a saturated aqueous solution of $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (10 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, hexanes/EtOAc, 2:1) to afford compound **38** (0.414 g, 99%) as a pale yellow solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.24 (s, 1H), 7.83 (d, J = 7.8 Hz, 2H), 7.28–7.20 (m, 5H), 7.02 (d, J = 7.8 Hz, 2H), 5.09 (br, 2H), 3.98 (s, 2H), 3.81 (t, J = 6.0 Hz, 2H), 2.84 (t, J = 5.4 Hz, 2H), 2.77 (m, 1H), 2.51 (s, 3H), 2.54 (s, 3H), 1.22 (d, J = 6.0 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.9, 163.0, 152.6, 148.6, 144.7, 135.6 (2), 129.4, 129.3, 128.7, 127.4 (2), 120.0, 73.2, 69.8, 36.2, 33.5, 26.7, 20.6.

4.1.1.10. 1-((4-(3-Acetylbenzyl)benzyloxy)methyl)-6-benzyl-5-isopropylpyrimidine-2,4(1*H*, 3*H*)-dione (28). This compound was prepared from pyrimidine **20** as a pale yellow oil following the procedure described for the preparation of **38**. Yield: 87%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.77 (s, 1H), 7.81–7.78 (m, 2H), 7.37–7.24 (m, 7H), 7.15 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 7.2 Hz, 2H), 5.21 (br, 2H), 4.63 (s, 2H), 4.17 (s, 2H), 4.01 (s, 2H), 2.85 (m, 1H), 2.56 (s, 3H), 1.27 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.5, 162.8, 152.4, 148.6, 141.7, 140.5, 137.6, 135.7, 135.6, 134.0, 129.4, 129.2, 129.0, 128.9, 128.4, 127.5 (2), 126.6, 120.1, 73.2, 71.8, 41.7, 33.7, 28.5, 26.9, 20.7.

4.1.1.11. 1-((4-(3-Acetylbenzyl)benzyloxy)methyl)-6-(3,5-dimethylbenzyl)-5-isopropyl pyrimidine-2,4(1*H*,3*H*)-dione (29). This compound was prepared from pyrimidine **21** as a pale yellow oil following the procedure described for the preparation of **38**. Yield: 77%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.58 (s, 1H), 7.80–7.78 (m, 2H), 7.37 (m, 2H), 7.26 (d, J = 7.2 Hz, 2H), 7.15 (d, J = 7.8 Hz, 2H), 6.88 (s, 1H), 6.67 (s, 2H), 5.22 (br, 2H), 4.63 (s, 2H), 4.10 (s, 2H), 4.02 (s, 2H), 2.85 (m, 1H), 2.57 (s, 3H), 2.26 (s, 6H), 1.28 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.5, 162.7, 152.3, 148.8, 141.7, 140.4, 139.0, 137.6, 135.7, 135.3, 134.0, 129.2, 129.1, 129.0, 128.9, 128.4, 126.6, 125.3, 119.9, 73.2, 71.8, 41.7, 33.5, 28.6, 26.9, 21.5, 20.7.

4.1.1.12. 1-((4-(3-Acetylbenzyl)benzyloxy)methyl)-6-benzyl-5-ethylpyrimidine-2,4(1*H*,3*H*)-dione (30). This compound was prepared from pyrimidine **22** as a pale yellow oil following the procedure described for the preparation of **38**. Yield: 81%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.73 (s, 1H), 7.81–7.78 (m, 2H), 7.37 (m, 2H), 7.30–7.23 (m, 5H), 7.15 (d, J = 7.8 Hz, 2H), 7.06 (d, J = 7.2 Hz, 2H), 5.18 (br, 2H), 4.61 (s, 2H), 4.14 (s, 2H), 4.02 (s, 2H), 2.57 (s, 3H), 2.45 (q, J = 7.2 Hz, 2H), 1.04 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.5, 171.4, 163.6, 152.2, 149.1, 141.7, 140.5, 137.6, 135.6, 135.4, 133.9, 129.5, 129.2, 129.0, 128.8, 128.4, 127.6, 126.6, 117.2, 73.0, 71.8, 41.7, 33.6, 26.9, 19.4, 14.0.

4.1.1.13. 1-((4-(3-Acetylbenzyl)benzyloxy)methyl)-6-(3,5-dimethylbenzyl)-5-ethylpyrimidine-2,4(1*H*,3*H*)-dione (31). This compound was prepared from pyrimidine **23** as a pale yellow oil following the procedure described for the preparation of **38**. Yield: 88%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.80–7.78 (m, 2H), 7.37 (m, 2H), 7.26 (m, 2H), 7.15 (d, J = 7.8 Hz, 2H), 6.87 (s, 1H), 6.66 (s, 2H), 5.21 (br, 2H), 4.62 (s, 2H), 4.07 (s, 2H), 4.02 (s, 2H), 2.57 (s, 3H), 2.45 (q, J = 7.2 Hz, 2H), 2.26 (s, 6H), 1.05 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.5, 163.7, 152.3, 149.5, 141.7, 140.5, 139.1, 137.6, 135.7, 135.1, 134.0, 129.2 (2), 129.0, 128.9, 128.4, 126.6, 125.3, 117.1, 73.1, 71.8, 41.7, 33.5, 26.9, 21.5, 19.4, 14.0.

4.1.1.14. 1-((3-(4-Acetylphenyl)propoxy)methyl)-6-benzyl-5-isopropylpyrimidine-2,4(1*H*,3*H*)-dione (39). This compound was prepared from bromide **35** as a pale yellow oil following the procedure described for the preparation of **38**. Yield: 70%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.34 (t, J = 7.8 Hz, 2H), 7.27 (t, J = 7.2 Hz, 1H), 7.24 (t, J = 7.8 Hz, 2H), 7.10 (d, J = 7.2 Hz, 2H), 5.11 (br, 2H), 4.17 (s, 2H), 3.56 (t, J = 6.6 Hz, 2H), 2.86 (quintet, J = 7.2 Hz, 2H), 2.70 (t, J = 7.8 Hz, 2H), 2.56 (s, 3H), 1.87 (m, 1H), 1.28 (d, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.0, 163.0, 152.5, 148.6, 147.6, 135.6, 135.4, 129.4, 128.8 (2), 127.5 (2), 120.1, 73.3, 68.6, 33.7, 32.4, 30.8, 28.6, 26.7, 20.6.

4.1.1.15. 3-(4-Acetylphenethyl)-6-benzyl-5-isopropylpyridine-2,4(1*H*,3*H*)-dione (54). A solution of pyrimidine **20** (0.244 g, 1.0 mmol) in DMF (3 mL) was treated with Cs_2CO_3 (0.326 g, 1.0 mmol) and stirred for 30 min. To this was added a solution of bromide **50** (0.241 g, 1.0 mmol) in 2 mL of DMF. The resulting yellow mix was heated at 50 °C for 2 h. The reaction was quenched by adding 10 mL of H_2O and was extracted with ethyl acetate (10 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, toluene/EtOAc, 4:1) to afford compound **54** (0.08 g, 20%) as a pale yel-

low oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.81 (s, 1H), 7.86–7.79 (m, 2H), 7.46–7.24 (m, 6H), 4.11 (m, 2H), 3.83 (s, 2H), 3.03–2.95 (m, 3H), 2.56 (s, 3H), 1.25 (d, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.4, 162.7, 152.5, 145.8, 144.5, 139.4, 137.5, 135.5, 134.0, 129.4, 129.3, 129.0, 128.8, 128.7, 127.8, 126.7, 116.3, 41.5, 36.5, 33.9, 27.7, 26.9, 20.5.

4.1.1.16. 3-(3-(4-Acetylphenyl)propyl)-6-benzyl-1-(ethoxymethyl)-5-isopropylpyrimidine-2,4(1*H*,3*H*)-dione (59). A solution of **58** (40 mg, 1.0 mmol) in DMF (1.0 mL) was treated with Cs_2CO_3 (65 mg, 1.5 mmol) and stirred for 30 min. To this was added bromide **50** (38 mg, 1.2 mmol). The resulting yellow mix was heated at 80 °C for 2 h. The reaction was quenched by adding 10 mL of H_2O and was extracted with ethyl acetate (10 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The resultant residue was subjected to flash chromatography (silica gel, hexane/EtOAc, 3:1) to afford compound **59** (57 mg, 93%) as a pale yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, J = 7.8 Hz, 2H), 7.30–7.20 (m, 3H), 7.25 (m, 2H), 7.11 (d, J = 7.8 Hz, 2H), 5.13 (s, 2H), 4.16 (s, 2H), 4.04 (t, J = 7.8 Hz, 2H), 3.61 (q, J = 7.2 Hz, 2H), 2.88 (septet, J = 7.2 Hz, 1H), 2.78 (t, J = 7.2 Hz, 2H), 2.57 (s, 3H), 2.04 (quintet, J = 7.8 Hz, 2H), 1.28 (d, J = 6.6 Hz, 6H), 1.19 (t, J = 7.2 Hz, 2H), ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.1, 161.9, 152.5, 147.6, 146.7, 135.8, 135.4, 129.4, 128.8, 128.7, 127.5, 127.4, 119.1, 73.9, 65.2, 41.5, 33.7, 33.6, 28.8, 28.7, 26.8, 20.6, 15.2.

4.1.1.17. 3-(3-(4-Acetylphenyl)propyl)-6-benzyl-5-isopropylpyrimidine-2,4(1*H*,3*H*)-dione (55). *Method* (a): This compound was prepared from bromide **51** as a pale yellow oil following the procedure described for the preparation of **54**. Yield: 40%.

Method (b): Compound **59** (50 mg) was dissolved in 90% aqueous CF_3COOH (3 mL). The resulting mixture was refluxed for 2 h and then allowed to cool to room temperature and evaporated to dryness, the residue was subjected to flash chromatography (silica gel, Hexane/EtOAc, 1.5:1) to afford compound **55** (37 mg, 84%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.97 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.30–7.20 (m, 7H), 3.92 (t, J = 7.2 Hz, 2H), 3.77 (s, 2 H), 3.03 (septet, J = 7.2 Hz, 1H), 2.72 (t, J = 7.8 Hz, 2H), 2.53 (s, 3H), 1.98 (quintet, J = 7.8 Hz, 2H), 1.28 (d, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.1, 162.9, 152.7, 147.5, 145.9, 135.6, 135.3, 129.2, 128.8, 128.7, 127.7, 116.1, 40.4, 36.5, 33.6, 28.7, 27.7, 26.7, 20.5.

4.1.1.18. 3-(4-(4-Acetylphenyl)butyl)-6-benzyl-5-isopropylpyrimidine-2,4(1*H*,3*H*)-dione (56). This compound was prepared from bromide **52** as a pale yellow oil following the procedure described for the preparation of **54**. Yield: 33%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.07 (s, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.29–7.23 (m, 7H), 3.89 (br, 2H), 3.78 (s, 2 H), 3.02 (septet, J = 7.2 Hz, 1H), 2.70 (br, 2H), 2.56 (s, 3H), 1.66 (br, 4H), 1.28 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.1, 162.9, 152.8, 148.4, 145.9, 138.1, 135.8, 135.2, 129.3, 129.2, 128.9, 128.8, 128.4, 127.6, 125.5, 116.2, 40.3, 36.5, 35.7, 28.7, 27.8, 27.5, 26.8, 20.5.

4.1.1.19. 3-(6-(4-Acetylphenyl)hexyl)-6-benzyl-5-isopropylpyrimidine-2,4(1*H*,3*H*)-dione (57). This compound was prepared from bromide **53** as a pale yellow oil following the procedure described for the preparation of **54**. Yield: 34%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.76 (s, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.31–7.23 (m, 7H), 3.84 (t, J = 7.8 Hz, 2H), 3.79 (s, 2 H), 3.02 (septet, J = 7.2 Hz, 1H), 2.63 (t, J = 7.8 Hz, 2H), 2.57 (s, 3H), 1.61 (m, 4H), 1.36 (br, 4H), 1.28 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.1, 162.9, 152.6, 148.8, 145.6, 135.6, 135.2, 129.2, 128.8, 128.7, 127.7, 116.1, 40.7, 36.5, 36.1, 31.2, 29.1, 27.8, 27.7, 27.1, 26.8, 20.6.

4.1.1.20. 2-(4-Acetylbenzylthio)-6-benzyl-5-ethylpyrimidin-4(3*H*)-one (44). To a solution of thiopyrimidine **43** (0.246 g, 1.0 mmol) in DMF (1.0 mL) was added bromide **41** (0.235 g, 1.1 mmol) and K_2CO_3 (0.138 g, 1.0 mmol). The resulting mixture was stirred at room temperature for 6 h and was quenched by adding 10 mL of H_2O. The precipitate was filtered, washed with H_2O and ether, then dried under vacuum to afford compound **44** (0.19 g, 50%) as a white solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.76 (br, 2H), 7.27–7.20 (m, 7H), 4.33 (br, 2H), 3.93 (br, 2H), 2.56 (m, 5H), 1.07 (br, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 197.8, 165.4, 161.8, 155.8, 143.1, 138.4, 136.3, 129.4, 129.2, 128.7 (2), 126.7, 123.0, 40.6, 34.1, 26.8, 19.0, 13.4.

4.1.1.21. 2-(4-(3-Acetylbenzyl)benzylthio)-6-benzyl-5-ethylpyrimidin-4(3*H*)-one (45). This compound was prepared from bromide **42** as a white solid following the procedure described for the preparation of **44**. Yield: 80%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.78 (br, 1H), 7.79 (m, 2H), 7.35 (t, J = 7.8 Hz, 2H), 7.22 (m, 4H), 7.18–7.13 (m, 3H), 7.01 (d, J = 8.4 Hz, 2H), 4.28 (s, 2 H), 3.97 (s, 2H), 3.91 (s, 2H), 2.58–2.54 (m, 5H), 1.05 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.5, 165.5, 161.9, 156.4, 141.8, 139.8, 138.6, 137.6, 135.4, 134.0, 129.6, 129.3, 129.2, 129.0, 128.9, 128.7, 126.7, 122.7, 41.7, 40.7, 34.3, 27.0, 19.0, 13.5.

4.1.1.22. (*Z*)-4-(4-(2-((6-Benzyl-5-isopropyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methoxy)ethyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (7). Sodium (92 mg, 4.0 mmol) was added to 2 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 (0.219 mL, 1.6 mmol) and a suspension of compound **38** (347 mg, 0.8 mmol) in 2 mL of anhydrous EtOH. The solution turned yellow immediately and precipitate formed. This mixture was stirred for 2 h and then quenched with a saturated aqueous solution of NH_4Cl. The resulting white precipitate was collected by filtration, washed with water and then dried under vacuum to give an ester intermediate as off-white solid. This intermediate was then dissolved into EtOH/CH_2Cl_2 (1:1, 2 mL) and treated with 1 N NaOH (2 mL). The resulting mixture was stirred for 30 min 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 compound **7** (195 mg, 49%) as a pale yellow solid: ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.05 (s, 1H), 7.85 (d, J = 7.8 Hz, 2H), 7.25 (m, 3H), 7.19 (m, 2H), 7.07 (s, 1H), 6.98 (d, J = 7.8 Hz, 2H), 5.05 (s, 2H), 3.97 (s, 2H), 3.83 (t, J = 6.6 Hz, 2H), 2.83 (t, J = 6.6 Hz, 2H), 2.73 (m, 1H), 1.17 (d, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 189.8, 170.6, 164.8, 163.8, 152.0, 149.6, 145.8, 135.2, 132.9, 129.8, 129.5, 128.4, 127.6, 127.4, 119.8, 97.7, 73.5, 70.3, 36.4, 33.7, 28.5, 20.5; HRMS (ESI-) calcd for $C_{27}H_{27}N_2O_7$ $[M-H]^-$ 491.1823, found 491.1828 (E = 0.9 ppm).

4.1.1.23. (*Z*)-4-(3-(4-(((6-Benzyl-5-isopropyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)yl)methoxy)methyl)benzyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (3). This compound was prepared from intermediate **28** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 65%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.73 (s, 1H), 7.84 (s, 2H), 7.42 (d, J = 3.6 Hz, 2H), 7.31 (t, J = 7.8 Hz, 2H), 7.25 (d, J = 7.2 Hz, 4H), 7.15 (d, J = 8.4 Hz, 3H), 7.05 (d, J = 7.2 Hz, 2H), 5.21 (br, 2H), 4.63 (s, 2H), 4.18 (s, 2H), 4.03 (s, 2H), 2.85 (m, 1H), 1.25 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 189.7, 171.5, 164.3, 163.5, 152.1, 149.5, 142.1, 140.3, 135.6, 135.3, 134.9, 134.7, 129.5, 129.4, 129.2, 128.6, 128.4, 127.6, 127.5, 126.2, 119.9, 97.5, 73.3, 71.9, 41.6, 33.8, 28.5, 20.5; HRMS (ESI-) calcd for $C_{33}H_{31}N_2O_7$ $[M-H]^-$ 567.2131, found 567.2204 (E = 13 ppm).

4.1.1.24. ((*Z*)-4-(3-(4-(((6-(3,5-Dimethylbenzyl)-5-isopropyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methoxy)methyl)benzyl)-phenyl)-2-hydroxy-4-oxobut-2-enoic acid (4). This compound was prepared from intermediate **29** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 61%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.64 (s, 1H), 7.83 (s, 2H), 7.40 (s, 2H), 7.25 (d, J = 6.6 Hz, 2H), 7.15 (d, J = 6.6 Hz, 3H), 6.87 (s, 1H), 6.65 (s, 2H), 5.22 (br, 2H), 4.63 (s, 2H), 4.10 (s, 2H), 4.02 (s, 2H), 2.84 (m, 1H), 2.26 (s, 6H), 1.26 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 189.4, 172.1, 164.3, 163.2, 151.9, 149.4, 141.9, 140.0, 138.9, 135.5, 134.8, 134.6, 134.5, 129.2, 128.9, 128.3, 128.2, 126.0, 125.0, 119.5, 97.3, 73.1, 71.6, 41.4, 33.4, 28.3, 21.3, 20.3; HRMS (ESI-) calcd for $C_{35}H_{35}N_2O_7$ $[M-H]^-$ 595.2444, found 595.2451 (E = 1.0 ppm).

4.1.1.25. (*Z*)-4-(3-(4-(((6-Benzyl-5-ethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methoxy)methyl)benzyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (5). This compound was prepared from intermediate **30** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 68%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.86 (s, 1H), 7.84 (s, 2H), 7.41 (d, J = 3.6 Hz, 2H), 7.30 (t, J = 7.8 Hz, 2H), 7.25 (d, J = 7.8 Hz, 3H), 7.16–7.14 (m, 3H), 7.05 (d, J = 7.2 Hz, 2H), 5.19 (br, 2H), 4.62 (s, 2H), 4.15 (s, 2H), 4.03 (s, 2H), 2.44 (q, J = 7.2 Hz, 2H), 1.03 (t, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 189.8, 171.4, 164.3, 164.2, 152.1, 150.0, 142.1, 140.3, 135.6, 135.1, 134.9, 134.8, 129.5, 129.4, 129.2, 128.5, 128.4, 127.6, 127.5, 126.2, 117.1, 97.6, 73.1, 71.9, 41.6, 33.7, 19.3, 13.9; HRMS (ESI-) calcd for $C_{32}H_{29}N_2O_7$ $[M-H]^-$ 553.1974, found 553.2042 (E = 12 ppm).

4.1.1.26. (*Z*)-4-(3-(4-(((6-(3,5-Dimethylbenzyl)-5-ethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methoxy)methyl)benzyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (6). This compound was prepared from intermediate **31** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 72%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.87 (s, 1H), 7.83 (s, 2H), 7.40 (s, 2H), 7.25 (d, J = 6.6 Hz, 2H), 7.15 (d, J = 7.8 Hz, 3H), 6.87 (s, 1H), 6.64 (s, 2H), 5.21 (br, 2H), 4.62 (s, 2H), 4.07 (s, 2H), 4.02 (s, 2H), 2.44 (q, J = 7.2 Hz, 2H), 2.25 (s, 6H), 1.04 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 189.8, 171.6, 164.3, 164.3, 152.2, 150.3, 142.1, 140.3, 139.2, 135.7, 134.9, 134.8, 129.4, 129.3, 129.2, 128.5, 128.4, 126.2, 125.2, 117.0, 97.6, 73.2, 71.9, 41.6, 33.5, 21.5, 19.3, 13.9; HRMS (ESI-) calcd for $C_{34}H_{23}N_2O_7$ $[M-H]^-$ 581.2247, found 581.2354 (E = 11 ppm).

4.1.1.27. (*Z*)-4-(4-(3-((6-benzyl-5-isopropyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methox)propyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (8). This compound was prepared from intermediate **39** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 60%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.34 (t, J = 7.8 Hz, 2H), 7.26 (m, 2H), 7.14 (s, 1H), 7.10 (d, J = 7.2 Hz, 2H), 5.13 (br s, 2H), 4.18 (s, 2H), 3.58 (t, J = 6.0 Hz, 2H), 2.87 (m, 1H), 2.72 (t, J = 7.8 Hz, 2H), 1.89 (q, J = 7.2 Hz, 2H), 1.29 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 189.6, 171.1, 164.5, 163.6, 152.1, 149.5, 148.7, 135.3, 132.4, 129.5, 129.3, 128.4, 127.6, 127.5, 120.0, 97.4, 73.4, 68.7, 33.8, 32.5, 30.6, 28.6, 20.6; HRMS (ESI-) calcd for $C_{28}H_{29}N_2O_7$ $[M-H]^-$ 505.1980, found 505.1995 (E = 2.9 ppm).

4.1.1.28. (*Z*)-4-(4-((4-Benzyl-5-ethyl-6-oxo-1,6-dihydropyrimidin-2-ylthio)methyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (9). This compound was prepared from intermediate **44** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 67%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.76 (br, 2H), 7.31 (br, 2H), 7.22–7.15 (m, 5H), 4.30 (s, 2H), 3.83 (s, 2H), 2.39 (q, J = 7.8 Hz, 2H), 0.87 (t, J = 7.2 Hz, 3H); HRMS (ESI-) calcd for $C_{24}H_{21}N_2O_5S$ $[M-H]^-$ 449.1176, found 449.1167 (E = 2.0 ppm).

4.1.1.29. (*Z*)-4-(3-(4-((4-Benzyl-5-ethyl-6-oxo-1,6-dihydropyrimidin-2-ylthio)methyl)benzyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (10). This compound was prepared from intermediate **45** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 62%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.78 (br, 2H), 7.37 (br, 2H), 7.16–7.02 (m, 9H), 4.19 (s, 2H), 3.91 (s, 2H), 3.81 (s, 2H), 2.37 (q, J = 7.2 Hz, 2H), 0.86 (t, J = 6.6 Hz, 3H); HRMS (ESI-) calcd for $C_{31}H_{27}N_2O_5S$ $[M-H]^-$ 539.1646, found 539.1689 (E = 8.0 ppm).

4.1.1.30. (*Z*)-4-(4-(2-(4-Benzyl-5-isopropyl-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)yl)ethyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (11). This compound was prepared from intermediate **54** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 48%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.03 (br, 1H), 7.96 (d, J = 7.8 Hz, 2H), 7.41 (d, J = 7.8 Hz, 2H), 7.27–7.15 (m, 5H), 7.04 (s, 1H), 4.46 (t, J = 6.0 Hz, 2H), 3.80 (s, 2H), 3.03 (m, 3H), 1.10 (d, J = 6.6 Hz, 6H); HRMS (ESI-) calcd for $C_{26}H_{25}N_2O_6$ $[M-H]^-$ 461.1718, found 461.1722 (E = 0.9 ppm).

4.1.1.31. (*Z*)-4-(4-(3-(4-Benzyl-5-isopropyl-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)-yl)propyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (12). This compound was prepared from intermediate **55** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 52%, ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.50 (s, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.34 (t, J = 7.8 Hz, 2H), 7.28 (d, J = 7.8 Hz, 1H), 7.23 (d, J = 7.2 Hz, 2H), 7.15 (s, 1H), 3.93 (t, J = 7.8 Hz, 2H), 3.80 (s, 3H), 3.03 (septet, J = 7.2 Hz, 1H), 2.80 (t, J = 7.2 Hz, 2H), 2.07 (quintet, J = 7.2 Hz, 2H), 1.28 (d, J = 6.6 Hz, 6H); HRMS (ESI-) calcd for $C_{27}H_{27}N_2O_6$ $[M-H]^-$ 475.1869, found 475.1874 (E = 1.0 ppm).

4.1.1.32. (*Z*)-4-(4-(4-(4-Benzyl-5-isopropyl-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)yl)butyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (13). This compound was prepared from intermediate **56** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 46%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.95 (s, 1H), 7.92 (d, J = 6.6 Hz, 2H), 7.76 (d, J = 7.8 Hz, 1H), 7.36–7.19 (m, 7H), 3.75 (m, 4H), 2.82 (septet, J = 7.2 Hz, 1H), 2.67 (t, J = 7.2 Hz, 1H), 2.63 (t, J = 7.2 Hz, 1H), 1.52 (m, 4H), 1.03 (d, J = 6.6 Hz, 6H); HRMS (ESI-) calcd for $C_{28}H_{29}N_2O_6$ $[M-H]^-$ 489.2026, found 489.2053 (E = 5.5 ppm).

4.1.1.33. (*Z*)-4-(4-(6-(4-Benzyl-5-isopropyl-2,6-dioxo-2,3-dihydropyrimidin-1(6*H*)-yl)hexyl)phenyl)-2-hydroxy-4-oxobut-2-enoic acid (14). This compound was prepared from intermediate **57** as a pale yellow solid following the procedure described for the preparation of **7**. Yield: 48%, ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.94 (s, 1H), 7.94 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.29 (t, J = 7.2 Hz, 2H), 7.21 (d, J = 7.8 Hz, 3H), 7.01 (br, 1H), 3.75 (s, 2H), 3.69 (t, J = 7.2 Hz, 2H), 2.81 (septet, J = 7.2 Hz, 1H), 2.63 (t, J = 7.2 Hz, 2H), 1.56 (quintet, J = 7.2 Hz, 2H), 1.46 (quintet, J = 7.2 Hz, 2H), 1.28 (m, 4H), 1.03 (d, J = 6.6 Hz, 6H); HRMS (ESI-) calcd for $C_{30}H_{33}N_2O_6$ $[M-H]^-$ 517.2339, found 517.2391 (E = 10.0 ppm).

4.2. Biology

4.2.1. 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 has 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.

4.2.2. IN assay

IN was expressed using *Escherichia coli* BL21(DE3) and standard IPTG induction.[36] In a typical assay, DNA substrate 5′biotin ATG TGGAAAATCTCTAGCAGT and 3′cy5 ACTGCTAGA GATTTTCCACAT (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 temp with mild agitation. Nonspecific 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. 90 μL 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.

4.2.3. HIV-1 assay

The HIV Cytoprotection assay used CEM-SS cells and the IIIB 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 70–95% 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%), TC_{50} (concentration resulting in 50% cell death) and a calculated TI (therapeutic index TC_{50}/EC_{50}) were provided. Each assay included the HIV RT inhibitor AZT as a positive control.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.004.

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