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Synthetic, structural mimetics of the β-hairpin flap of HIV-1 protease inhibit enzyme function

Jay Chauhan [a], Shen-En Chen [b], Katherine J. Fenstermacher [c], Aurash Naser-Tavakolian [b], Tali Reingewertz [d], Rosene Salmo [a], Christian Lee [e], Emori Williams [f], Mithun Raje [a], Eric Sundberg [d], Jeffrey J. DeStefano [c], Ernesto Freire [b], Steven Fletcher [a,g,*]

[a] Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, 20 N. Pine St., Baltimore, MD 21201, USA
[b] Department of Biology, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, USA
[c] Department of Cell Biology & Molecular Genetics, University of Maryland, College Park, MD 20742, USA
[d] Department of Microbiology and Immunology, University of Maryland School of Medicine, 725 West Lombard St., Baltimore, MD 21201, USA
[e] PharmD Program, University of Maryland School of Pharmacy, 20 N. Pine St., Baltimore, MD 21201, USA
[f] Vivien T Thomas Medical Arts Academy, 100 N Calhoun St., Baltimore, MD 21223, USA
[g] University of Maryland Greenebaum Cancer Center, 22 S. Greene St., Baltimore, MD 21201, USA

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ABSTRACT

Small-molecule mimetics of the β-hairpin flap of HIV-1 protease (HIV-1 PR) were designed based on a 1,4-benzodiazepine scaffold as a strategy to interfere with the flap–flap protein–protein interaction, which functions as a gated mechanism to control access to the active site. Michaelis–Menten kinetics suggested our small-molecules are competitive inhibitors, which indicates the mode of inhibition is through binding the active site or sterically blocking access to the active site and preventing flap closure, as designed. More generally, a new bioactive scaffold for HIV-1PR inhibition has been discovered, with the most potent compound inhibiting the protease with a modest K_i of 11 μM.

1. Introduction

HIV-1 protease (HIV-1 PR) is a retroviral enzyme that is essential for the life-cycle of HIV, and is, therefore, an important target for AIDS therapy.[1–3] Indeed, there are now eleven FDA-approved HIV-1PR inhibitors.[3] HIV-1 PR cleaves newly synthesized gag and gag-pol polyproteins at the appropriate places to create mature protein components of an infectious virion that proceeds to further infect normal host cells.[1–3] Owing to the high mutation rate within the active site, however, the enzyme becomes resistant to current drugs that target this region, which encompasses all of the FDA-approved drugs.[3] Presently, effective management of the disease is afforded through a cocktail of drugs, or "HAART" (Highly Active AntiRetroviral Therapy), which simultaneously inhibit several viral proteins.[3] Thus, novel modes of HIV-1 PR inhibition are urgently required.[4,5] A complementary strategy to targeting the active site is to disrupt the protein–protein interaction (PPI) in the 'whiskers' region of the dimeric complex that is comprised of four interdigitating β-strands, two from each monomer (the N-terminal residues 1–4 and the C-terminal residues 96–99). This is a particularly appealing target since this PPI accounts for 75% of the dimerization stabilization energy,[6] and the mutation rate here is lower than in the active site, suggesting inhibitors targeting this region may be more refractory to resistance.[7] However, there is a considerable entropic challenge here since the isolated monomers are not thermodynamically stable[6] and so an inhibitor designed to recognize the folded β-strand conformations as found in the active dimer would need to organize its target peptide sequences into said β-strands. Nevertheless, Schramm, Chimelewksi and others have made some progress in this area with 'interfacial peptides',[8–17] although new drugs with this mechanism of action are yet to be identified.

Extensive X-ray crystal structures have revealed that HIV-1 PR is a C2-symmetric homodimer where the enzyme's active site is 'gated' by two glycine-rich, anti-parallel β-hairpin flaps (residues 43–58) that recognize each other predominantly through hydrophobic interactions.[18] These flaps 'open' and 'close' to allow peptide substrates to enter, and products to exit, the active site of

* Corresponding author. Tel.: +1 410 706 6361; fax: +1 410 706 5017.
E-mail address: sfletcher@rx.umaryland.edu (S. Fletcher).

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0968-0896/

HIV-1 PR; moreover, they are essential for proper placement of the substrate within the active site. Accordingly, interfering with this PPI in some manner might be expected to compromise enzymatic activity. In fact, Král and Konvalinka have demonstrated that metallacarboranes are capable of competitively inhibiting HIV-1 PR through an unconventional binding mode wherein the metallacarboranes bind the flap-proximal region of the active site, approximately the S3 and S3′ substrate binding sites, trapping the enzyme in the open conformation.[19] It is noteworthy that this binding mode was unprecedented as all reported crystal structures of ligands bound to HIV-1 PR had shown the enzyme in the closed conformation. Konvalinka and colleagues have speculated that the metallacarboranes inhibit HIV-1 PR through preventing flap closure, and hence improper formation of the active site, as this would still deliver competitive inhibition kinetics.[19] Klebe and coworkers have also targeted the open-flap conformation of HIV-1 PR with pyrrolidine-based inhibitors.[20] Thus, there exists precedent for trapping the enzyme in the (semi-)open, inactive conformation. We herein describe our efforts to disrupt the enzymatic activity of HIV-1 PR through abrogating the PPI between the β-hairpin flaps with rationally designed synthetic flap mimetics.

2. Results and discussion

As a novel strategy to inhibit HIV-1 PR, we reasoned that the equilibrium between the 'semi-open' and 'closed' states of the enzyme could be intercepted by small-molecules that mimic one of the β-hairpin flaps found at the flap–flap PPI. Interaction of one β-hairpin flap with a flap surrogate would prevent flap closure and result in the incomplete formation of the enzyme's activity site, providing a novel mechanism for the inhibition of HIV-1 PR. More simply, binding the flap region directly above the active site may be considered to confer steric hindrance to incoming substrates. As mimetics of β-turns, β-strands and α-helices, 2-amido-2′-carboxamido-diphenylacetylene derivatives exhibit a privileged role in biomimicry, but their syntheses can be lengthy.[21–23] In addition, 1,4-benzodiazepines (BZD) have also been utilized to imitate β-turns.[24] Given their simpler synthesis, we selected the BZD scaffold to reproduce the epitope of one of the β-hairpin turns located at the dynamic PPI between the two HIV-1PR monomers.

Residues 47–54 of the β-hairpin flap are shown in Figure 2A and B. A corresponding BZD proteomimetic of the flap turn is given in Figure 2C, whose MM2 energy-minimized conformation is overlaid with the turn of the β-hairpin flap (Figure 2D). In order to simplify the synthetic chemistry as well as attempt the mimicry of I47 and I54, the BZD was inverted and then elaborated as shown in Figure 2E. [I50] of the mimetic was intended to interact with I47/I54 of the target β-hairpin flap, whilst [I47] and [I54] were designed to recognize I50 of the flap (residues in square brackets indicate those residues being mimicked). R^1 represents the side chain of the I50 mimetic and may be readily varied by invoking different amino acids in the construction of the BZD ring. To capture additional hydrophobic interactions with the flap, the side-chain of G51 was emulated by a methyl group, instead of a hydrogen atom. We hypothesized that bulky, hydrophobic R^2 groups would putatively mimic I47, and *tert*-butyl esters at the R^3 position were designed to mimic I54. We proposed to also include carboxylic acids here to assist in compound solubility. Accordingly, β-hairpin flap mimetics based on the generic structure in Figure 1D were prepared as in Scheme 1.

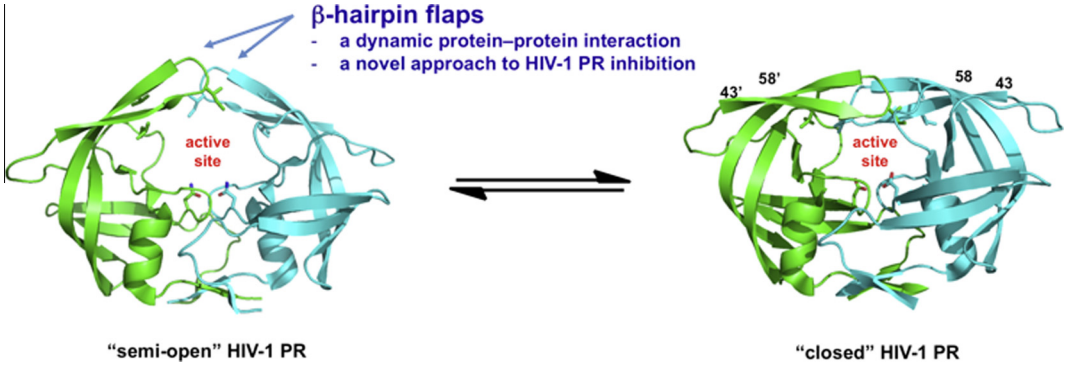
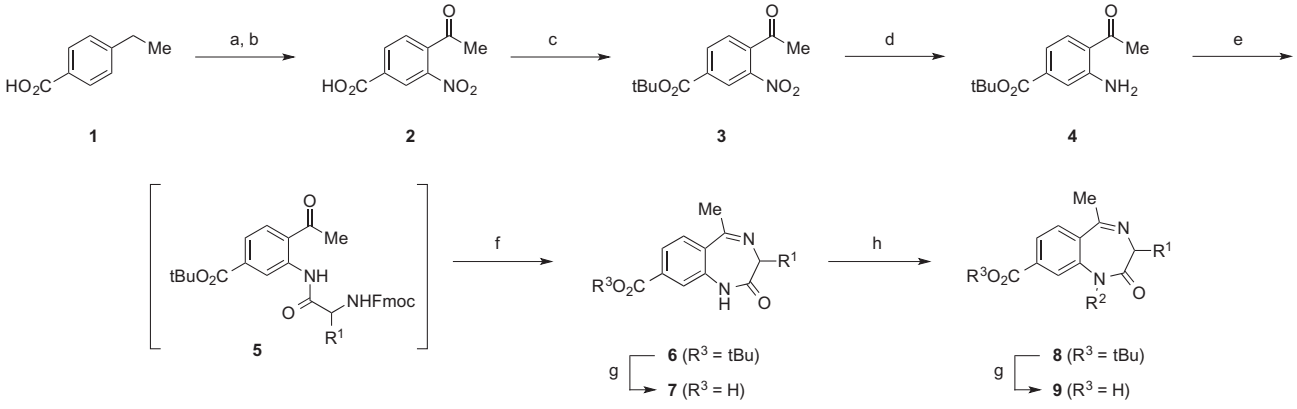


Figure 1. The semi-open and closed forms of HIV-1 PR, with the active site indicated, including the catalytic aspartate residues Asp25 and Asp25′), and the β-hairpin flap regions highlighted (residues 43–58 and 43′–58′). Green and cyan represent the two HIV-1 PR monomers.



Scheme 1. Reagents and conditions: (a) HNO_3, H_2SO_4, −10 °C; room temperature (RT), 1 h; (b) H_5IO_6, CrO_3, CH_3CN, RT, 16 h; (c) *t*BuOH, DCC, DMAP, CH_2Cl_2, RT, 3 h; (d) 10% Pd/C, H_2, MeOH, RT, 1 h; (e) (1) FmocNH(R^1)CO_2H, isobutyl chloroformate, NMM, THF or CH_2Cl_2, 0 °C, 30 min; (2) **4**, reflux, 20 h; (f) $HNEt_2$, CH_3CN, RT, 3 h; (g) TFA, CH_2Cl_2, RT, 4 h; (h) R^2CH_2Br or R^2CH_2Cl, K_2CO_3, DMF, RT, 3 h.

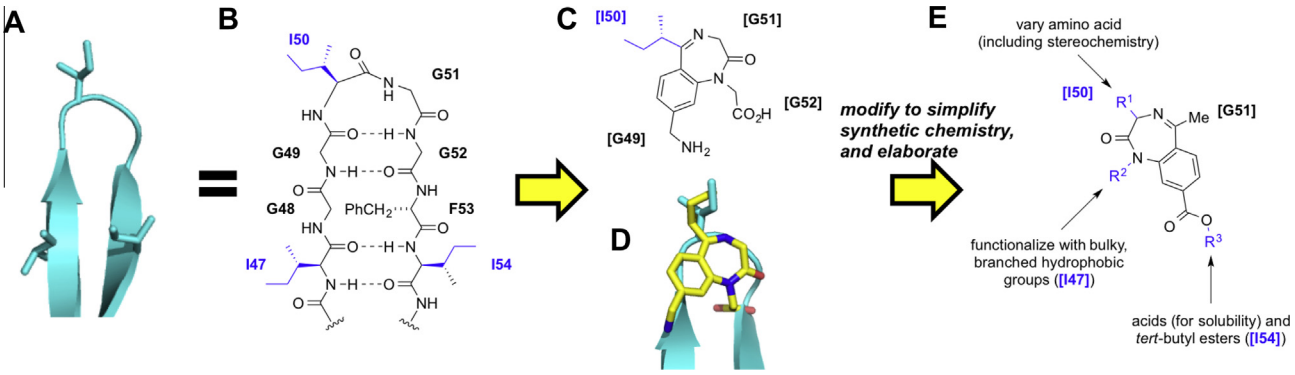
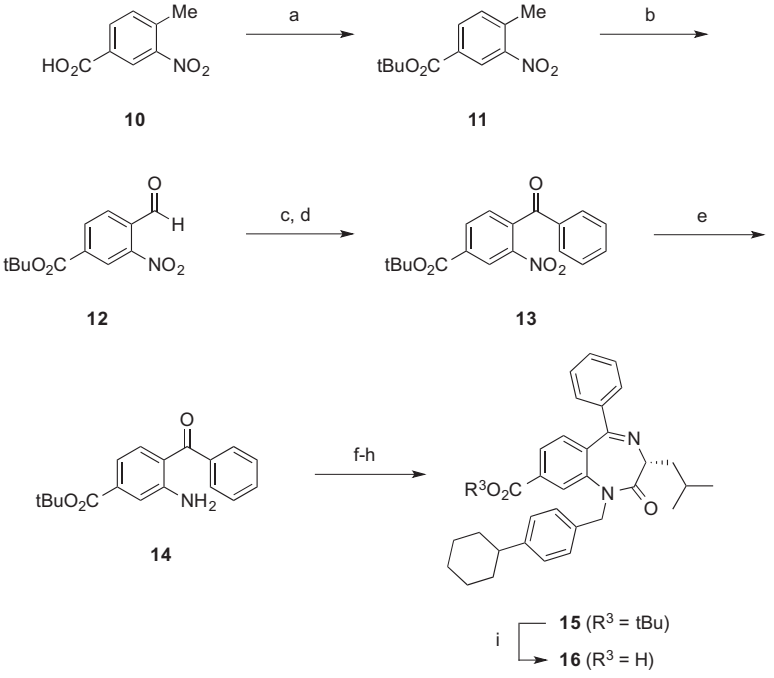


Figure 2. (A) Cartoon form of the β-hairpin flap (residues 47–54) from a monomer of HIV-1PR; dashed lines = hydrogen bonds; (B) the peptide sequence of HIV-1PR$^{47-54}$; (C) a 1,4-benzodiazepine (BZD)-based mimetic of the four residues at the turn of the β-hairpin flap; (D) MM2 energy minimized conformation of C (yellow, colored by atom type) overlaid on β-turn; (E) flipped BZD from C to simplify the synthetic chemistry, and elaboration towards the additional mimicry of I47 and I54. Residues in square brackets indicate those residues being mimicked.

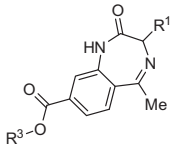
Regioselective nitration of **1** followed by benzylic oxidation with Jones reagent afforded ketone **2**. Esterification with *tert*-butanol and *N*,*N*-dicyclohexylcarbodiimide (DCC) then delivered *tert*-butyl ester **3**. Attempted reduction of the nitro group in **3** with stannous chloride ($SnCl_2{\cdot}2H_2O$) yielded a mixture of the desired product **4** along with the corresponding 3-methyl-2,1-benzisoxazole (anthranil) that was presumably generated through heterocyclization of the intermediate hydroxylamine with the ketone. We investigated this observation more fully, and have published our findings elsewhere.[25] Complete transformation of **3** into **4** was instead accomplished via catalytic hydrogenation, which may have occurred through direct reduction of the aryl nitro group to the respective aniline and/or reduction of the weak N–O bond within the 2,1-benzisoxazole generated in situ. Fmoc-protected amino acids (R^1 group) were pre-activated with isobutyl chloroformate then coupled to aniline **4** to deliver amides **5**, which were not isolated; subsequent base-mediated cyclization delivered the key BZD nucleus, as in compounds **6**. Deprotection of the *tert*-butyl esters in **6** with TFA furnished acids **7**. Alternatively, the anilide nitrogen of **6** was alkylated under standard conditions with R^2 bromides or chlorides to yield target compounds **8**, which, upon treatment with TFA, provided the additional target compounds **9**. We also prepared two congeners of **8** and **9** in which the methyl imine was replaced by a phenyl imine, as depicted in Scheme 2, using similar chemistry to that described previously. Briefly, **10** was esterified to give **11** whose benzylic methyl group was oxidized to deliver aldehyde **12**. A Grignard reaction with phenyl magnesium bromide followed by re-oxidation of the resulting secondary alcohol furnished ketone **13**. Finally, reduction, heterocyclization and deprotection then afforded products **15** and **16**.

As shown in Table 1, a small batch of simpler BZDs lacking the R^2 group was initially prepared to gauge if this novel scaffold could elicit inhibition of HIV-1 PR. Protocols measuring HIV-1 PR activities using chromogenic and fluorogenic substrates have been well-established.[26–29] The HIV-1 PR activities were measured spectroscopically allowing inhibition constants, K_i, of the newly synthesized compounds to be determined. Although most of the compounds in Table 1 did not inhibit the enzyme, two of the most hydrophobic compounds, *tert*-butyl esters **6f** and **6g**, exhibited appreciable inhibitory activity (K_i = 64 μM and 88 μM, respectively). Thus, a second



Scheme 2. Reagents and conditions: (a) *t*BuOH, DCC, DMAP, CH_2Cl_2, RT, 3 h; (b) (1) DMF-dimethyl acetal, DMF, 140 °C, 5 h; (2) $NaIO_4$, THF-H_2O, 1:1, RT, 2 h; (c) PhMgBr, THF, 0 °C, 3 h; (d) CrO_3, H_2SO_4, $(CH_3)_2CO$, 0 °C, 2 h; (e) 10% Pd/C, H_2, MeOH, RT, 1 h; (f) (1) FmocNH-D-Leu-CO_2H, isobutyl chloroformate, NMM, CH_2Cl_2,0 °C, 30 min; (2) **14**, reflux, 20 h; (g) $HNEt_2$, CH_3CN, RT, 3 h; (h) 1-(bromomethyl)-4-cyclohexylbenzene, K_2CO_3, DMF, RT, 3 h; (i) TFA, CH_2Cl_2, RT, 4 h.

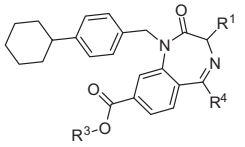
Table 1
Inhibition of HIV-1 PR by BZDs unfunctionalized at the anilide nitrogen



Compound	R^1	R^3	K_i (μM)	Compound	R^1	R^3	K_i (μM)
6a	—H		>200	**6e**			>200
7a	—H	—H	199 ± 16	**7e**		—H	158 ± 5
6b	Me		>200	**6f**			64 ± 9
7b	Me	—H	>200				
6c			193 ± 32	**7f**		—H	>200
7c		—H	>200	**6g**			88 ± 14
6d			>200	**7g**		—H	>200
7d		—H	>200				

K_i is the inhibition constant, the concentration of inhibitor that produces half-maximal inhibition.

Table 2
Inhibition of HIV-1 PR by BZDs alkylated at the anilide nitrogen with a 4-cyclohexylbenzyl moiety

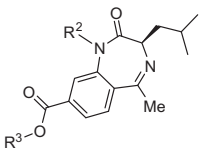


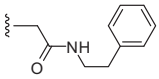
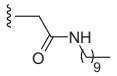
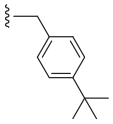
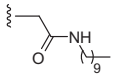
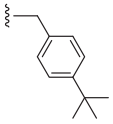
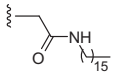
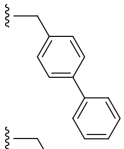
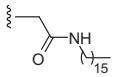
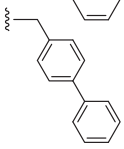
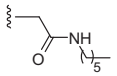
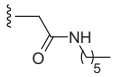
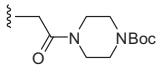
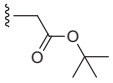
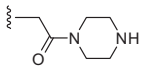
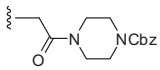
Compound	R^1	R^3	R^4	K_i (μM)	Compound	R^1	R^3	R^4	K_i (μM)
8a	—H		—Me	169 ± 27	**16**		—H	—Ph	68 ± 13
9a	—H	—H	—Me	85 ± 24	**8e**			—Me	108 ± 12
8b	Me		—Me	88 ± 6	**9e**		—H	—Me	53 ± 7
9b	Me	—H	—Me	56 ± 9					
8c			—Me	76 ± 7	**8f**			—Me	54 ± 7
9c		—H	—Me	27 ± 4	**9f**		—H	—Me	47 ± 12
8d			—Me	65 ± 12	**8g**			—Me	131 ± 8
9d		—H	—Me	19 ± 4					
15			—Ph	88 ± 5	**9g**		—H	—Me	72 ± 11

K_i is the inhibition constant, the concentration of inhibitor that produces half-maximal inhibition.

set of compounds was prepared in which the anilide nitrogen atom was alkylated with 4-cyclohexylbenzyl (R^2) bromide to afford the series of compounds illustrated in Table 2 whose R^2 groups were proposed to mimic I47. In general, the bulkier and more hydrophobic the I50-mimicking R^1 group, the more potent was the inhibitor. Interestingly, the L- and D-leucine derivatives **9c** (K_i = 27 μM) and **9d** (K_i = 19 μM) were more potent than the L-isoleucine derivative **9e** (K_i = 53 μM) wherein the R^1 group was a perfect match for I50. Whilst there appeared little impact of the stereogenic centre on the activity of the leucine derivatives **8c**–**9d**, the L-phenylalanine

Table 3
SAR of the anilide R^2 group of the BZD compounds



Compound	R^2	R^3	K_i (μM)	Compound	R^2	R^3	K_i (μM)
8h			>200	**9n**			75 ± 7
9h			>200	**8o**			>200
8i			147 ± 19	**9o**			11 ± 3
9i			46 ± 7	**8p**			165 ± 11
8j			62 ± 5	**9p**			11 ± 3
9j			21 ± 2	**8q**			>200
8k			89 ± 7	**9q**			75 ± 7
9k			>200	**8r**			143 ± 9
8l			119 ± 40	**9r**			>200
9l			>200	**8s**			>200
8m			>200	**9s**			81 ± 28
9m			>200	**8t**			>200
8n			95 ± 9	**9t**			49 ± 15

K_i is the inhibition constant, the concentration of inhibitor that produces half-maximal inhibition.

derivatives **8f** and **9f** were more potent than their D-counterparts. In contrast to most instances in Table 1, the free acids (R^3 = H) were more potent than their corresponding *tert*-butyl esters, the latter of which were designed to approximate to I54. We surmise this finding may be related to compound solubility. In order to gauge if enhancing the hydrophobicity at the tip of the flap mimetic would confer improved inhibition, the 5-methyl R^4 group was replaced with a phenyl ring, although this resulted in reduced inhibition (compare **9d** with **16**), which is consistent with this group required to imitate only a small glycine side chain. To expand our library further, we selected the most potent compound from Table 2, **9d**, and replaced the 4-cyclohexylbenzyl group with a variety of alternative hydrophobic groups as possible mimetics of I47; these compounds are shown in Table 3. Once more, acids were more potent than their corresponding *tert*-butyl esters. Compounds bearing larger, more hydrophobic R^2 groups translated into increasingly improved inhibition of HIV-1 PR. For example, compare **9h** (R^2 = Bn) with **9i** (R^2 = 4-*tert*-butylbenzyl) and **9j** (R^2 = 4-phenylbenzyl) where the inhibitory activities increase in that order. For the series of alkyl amides, the larger derivatives **9o** and **9p**, both with K_is of 11 μM, were more potent than the smaller derivative **9q** (K_i = 75 μM), which may be due to mimicry of I54 as well as I47. Others have also observed improved inhibition of HIV-1 PR through the addition of long chain alkyl groups to moderately active inhibitors.[16] An MM2 energy-minimized conformation of **9d** overlaid with one of the β-hairpin flaps of HIV-1PR is shown in Figure 3, which reveals good mimicry of I47 and I50.

To investigate the mode of inhibition of our BZD-based compounds, **9o** was studied further. According to Figure 4, **9o** is a competitive inhibitor of HIV-1 PR. While these findings indicate that **9o** possibly binds the active site of HIV-1 PR, it does not rule out a potential mechanism by which the small-molecule sterically blocks access to the active site, as originally designed, and as reported elsewhere.[19,20] Attempts to acquire a co-crystal structure of **9o** with HIV-1 PR failed, possibly due to the modest binding affinity of the compound coupled with its poor solubility. We are presently developing some second-generation compounds that might address these drawbacks.

To examine if our compounds could inhibit HIV virus replication within cells, we first studied the general cytotoxicity of four of our most potent compounds: **9d**, **9j**, **9o** and **9p**. For this purpose, MT-4 cells were utilized, which are a lymphocyte-derived cell line used for HIV tissue culture, particularly for assessing anti-retroviral

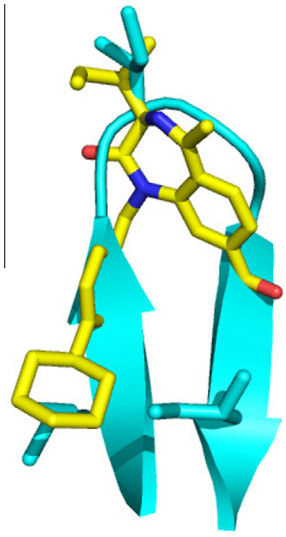


Figure 3. An MM2 energy minimized conformation of **9d** (yellow, colored by atom type) overlaid with one of the β-hairpin flaps from HIV-1PR (cyan).

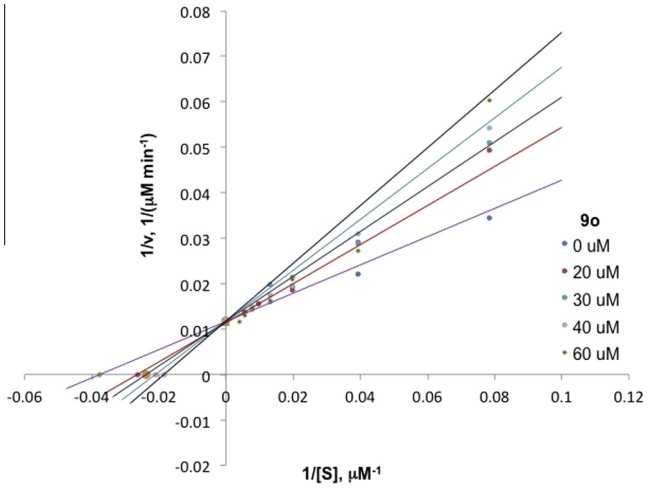


Figure 4. Compound **9o** is a competitive inhibitor of HIV-1 PR.

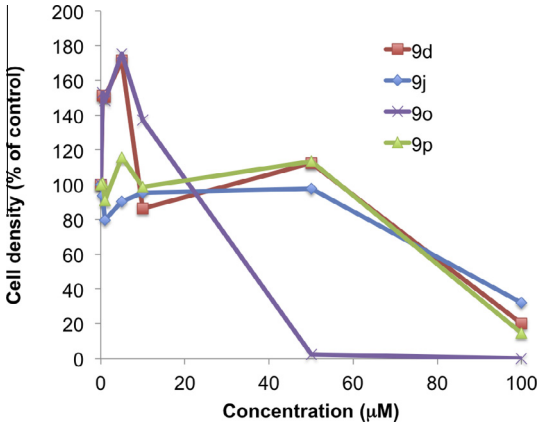


Figure 5. Cytotoxicities of select HIV-1PR inhibitors to MT-4 cells.

cytotoxicity. The data is shown in Figure 5. All but **9p** were tolerated up to 50 μM, but all proved particularly cytotoxic at 100 μM. We then tested the ability of the compounds to inhibit HIV replication in TZM-bl cells, a HeLa-derived cell line expressing CCR5, CXCR4, and CD4 that contains a luciferase gene under the control of a HIV tat-driven promoter. As shown in Figure 6, which is presented as relative luminescence units (RLUs) of a control (HIV infection without any inhibitor), all compounds appeared to inhibit HIV replication within cells at doses of 50 μM or higher. However,

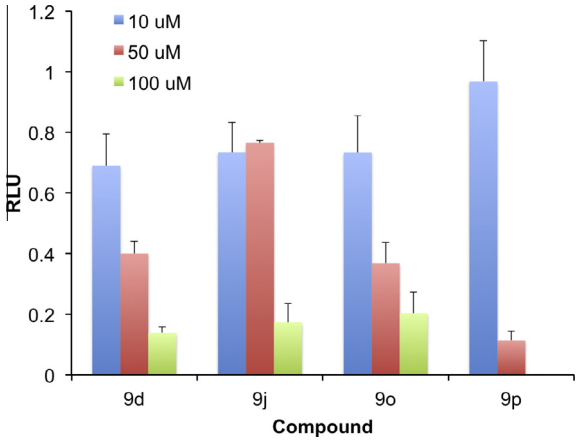


Figure 6. HIV-1 virus replication inhibition by select HIV-1PR inhibitors in HeLa CD4+ TZM-bl cells. RLU = relative luminescence units, relative to untreated control.

according to Figure 5, the HIV infectivity data for **9j** and **9p** may be attributed in part, or completely, to the general cytotoxicities exhibited by these compounds. Two of our more promising compounds, **9d** and **9o**, will be subjected to further structure–activity refinement to enhance the inhibition of HIV-1 PR in vitro towards the development of more potent, and less cytotoxic, inhibitors of HIV replication within cells.

In conclusion, a new series of HIV-1 PR inhibitors was developed based on BZD-inspired small-molecule mimicry of one of the β-hairpin turns found at the flap–flap PPI, which is the gated access to the enzyme's active site. Our molecules possibly mimic I47, I50 and G51. Although the exact binding mode remains unknown at this time, Michaelis–Menten analysis indicates that our compounds function as competitive inhibitors either through (a) binding in the active site, or (b) sterically blocking access to the active site. Efforts towards the determination of the specific binding mode of these new HIV-1 PR inhibitors is underway, and second-generation inhibitors based on lead compounds **9d** and **9o** are presently a focus of our laboratory (see Figure 6).

3. Experimental

3.1. Chemistry

3.1.1. General

Unless otherwise stated, all reaction were performed under an inert atmosphere (N_2). Reagents and solvents were ACS grade, and purchased from Sigma–Aldrich, Alfa Aesar, Oakwood and TCI America. Anhydrous solvents were used as provided from Sigma–Aldrich. Reactions were monitored by thin-layer chromatography (TLC), visualizing with a UV lamp and/or $KMnO_4$ stain. Flash column chromatography was performed with silica gel 60 Å (70–230 mesh, Merck). ^1H and ^{13}C NMR spectra were recorded on a Varian INOVA 400 MHz NMR spectrometer at 25 °C. Chemical shifts are reported in parts per million (ppm). Data for ^1H NMR are reported thus: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration), where multiplicities are: s = singlet, d = doublet, t = triplet, m = multiplet. The residual solvent peak was used as an internal reference: $CDCl_3$ (δ_H 7.26; δ_C 77.21) and d_6-DMSO (δ_H 2.50; δ_C 39.51). Mass spectra were obtained on an Electrospray TOF (ESI-TOF) mass spectrometer (Bruker AmaZon X). All final molecules were deemed to be >90% pure by reversed-phased HPLC using a Waters 1525 analytical/preparative HPLC fitted with a C18 reversed-phase column (Atlantis T3 (T3) or Symmetry (S): 4.6 mm × 150 mm) according to the following conditions with solvents (A) H_2O/0.1% TFA, (B) CH_3CN–H_2O, 9:1 with 0.1% TFA at 1 ml min^{-1}: (I) a gradient of 100% A to 100% B over 22 min (T3); (II) a gradient of 100% A to 100% B over 22 min, then maintained for 13 min at 100% B (T3); (III) a gradient of 100% A to 100% B over 10 min (S); (IV) a gradient of 50% A to 100% B over 22 min (S); (V) an isocratic gradient of 100% B over 22 min (S). HPLC data are presented as retention time (t_R (min)), purity (%), condition (I, II, II, IV or V).

3.1.1.1. 4-Acetyl-3-nitrobenzoic acid (2)[25]. A round bottom flask equipped with a magnetic stirrer bar was charged with HNO_3 (25 mL) and 4-ethylbenzoic acid (5 g, 33.3 mmol). The reaction was cooled to −10 °C and H_2SO_4 (20 mL) was added, and then the reaction was allowed to stir at room temperature for 1 h. The reaction was poured over ice and the precipitate filtered to give 4-ethyl-3-nitrobenzoic acid as a cream solid (5.9 g, 92%): δ_H (DMSO-d_6, 500 MHz) 8.36 (s, 1H, Ar), 8.15 (d, J = 7.5, 1H, Ar), 7.67 (d, J = 7.5, 1H, Ar), 2.89 (q, 2 H, CH_2), 1.23 (t, 3 H, CH_3). To a round bottom flask equipped with a magnetic stirrer bar was charged with H_5IO_6 (4.54 g, 20.00 mmol) in MeCN (50 mL). 0.1 M CrO_3 in MeCN (5 mL, 0.50 mmol) and 4-ethyl-3-nitrobenzoic acid (1.95 g, 10.0 mmol) were added to the reaction mixture, which was allowed to stir at room temperature overnight. The reaction mixture was decanted, washed with EtOAc and reduced in vacuo. The residue was partitioned between EtOAc (100 mL) and H_2O (50 mL), washed with 5% $Na_2S_2O_3$ (50 mL), saturated aqueous NaCl (50 mL), dried over $MgSO_4$, filtered and reduced in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to provide the title compound as a cream solid (1.56 g): δ_H (DMSO-d_6, 500 MHz) 13.90 (br s, 1H, CO_2H), 8.50 (s, 1H, Ar), 8.34 (d, J = 8.0, 1H, Ar), 7.88 (d, J = 8.0, 1H, Ar), 2.60 (s, 3 H, CH_3).

3.1.1.2. *tert*-Butyl 4-acetyl-3-nitrobenzoate (3). A round bottom flask equipped with a magnetic stirrer bar was charged with **2** (209 mg, 1.00 mmol), *t*BuOH (115 μL, 1.20 mmol) and DMAP (12 mg, 0.10 mmol) in CH_2Cl_2 (6 mL). *N*,*N*-Dicyclohexylcarbodiimide (DCC; 218 mg, 1.20 mmol) was added, and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was reduced in vacuo, and the residue was purified by column chromatography (hexane/EtOAc) to provide the title compound as a yellow oil (217 mg, 88%): δ_H (DMSO-d_6, 500 MHz) 8.46 (s, 1H, Ar), 8.31 (d, J = 8.0, 1H, Ar), 7.89 (d, J = 8.0, 1H, Ar), 2.60 (s, 3 H, CH_3), 1.59 (s, 9 H, 3 × CH_3).

3.1.1.3. *tert*-Butyl 4-acetyl-3-aminobenzoate (4). A round bottom flask equipped with a magnetic stirrer bar was charged with **3** (300 mg, 1.44 mmol) in MeOH (10 mL). Pd/C (60 mg, 20 mol%) was added and the reaction put under H_2 gas (balloon for 1 h). The reaction mixture was filtered through Celite, reduced in vacuo and the residue was purified by silica gel column chromatography (hexane/EtOAc) to provide the title compound as a yellow solid (150 mg): δ_H (DMSO-d_6, 500 MHz) 7.82 (d, J = 8.0, 1H, Ar), 7.35 (br s, 3 H, Ar, NH_2), 6.99 (d, J = 8.0, 1H, Ar), 2.53 (s, 3 H, CH_3), 1.54 (s, 9 H, 3 × CH_3).

3.1.2. General procedure A: preparation of 1,4-benzodiazepines 6

A round bottom flask equipped with a magnetic stirrer bar was charged with *N*-Fmoc amino acid **4** (1.50 mmol), *N*-methylmorpholine (1.60 mmol) and CH_2Cl_2 or THF (10 mL), depending on amino acid solubility. The reaction was cooled to 0 °C and isobutyl chloroformate (1.60 mmol) was added dropwise. The reaction was stirred at 0 °C for 30 min, aniline **4** (1.00 mmol) was added in one portion and the reaction refluxed for 20 h. The reaction was cooled, quenched with saturated aqueous NH_4Cl (1 mL), then partitioned between EtOAc (60 mL) and H_2O (30 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL), then the organic portions combined, washed with H_2O (10 mL), brine (10 mL), dried over $MgSO_4$, filtered and reduced in vacuo. The residue (crude **5**) was dissolved in MeCN (10 mL), Et_2NH (2 mL) was added at room temperature, and then the reaction was stirred for 3 h. The reaction was reduced in vacuo, then purified by silica gel column chromatography (hexane/EtOAc) to provide the title compound.

3.1.3. General procedure B: preparation of *N*-alkylated-1,4-benzodiazepines 8

A round bottom flask equipped with a magnetic stirrer bar was charged with 1,4-benzodiazepine (0.15 mmol), K_2CO_3 (0.45 mmol) and DMF (3 mL). The reaction was cooled to 0 °C and alkyl halide (0.30 mmol) in DMF (1 mL) was added. The reaction was stirred at room temperature for 3 h. The reaction was partitioned between EtOAc (30 mL) and H_2O (10 mL). The organic phase was washed with H_2O (3 × 10 mL), brine (10 mL), dried over $MgSO_4$, filtered and reduced in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc) to provide the title compound.

3.1.4. General procedure C: deprotection of 1,4-benzodiazepines 6 and 8

To a round bottom flask equipped with a magnetic stirrer bar was charged with 1,4-benzodiazepines **6** or **8** (0.10 mmol) and

CH_2Cl_2 (1 mL). TFA (1 mL) was added at room temperature and stirred for 4 h. The reaction was reduced in vacuo, co-evaporated with chloroform and lyophilized from MeCN/H_2O (1:1) to provide the title compound **7** or **9** as its TFA salt.

3.1.4.1. *tert*-Butyl 5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (6a). Prepared according to General Procedure A with Fmoc-Gly-OH, to give the title compound as a white solid (246 mg, 90%): mp 186–188 °C; δ_H (DMSO-d_6, 400 MHz) 10.53 (s, 1H, NH), 7.80 (d, J = 8.4, 1H, Ar), 7.70 (s, 1H, Ar), 7.64 (d, J = 8.4, 1H, Ar), 3.90 (s, 2 H, CH_2CO), 2.40 (s, 3H, CH_3-CN), 1.55 (s, 9 H, 3 × CH_3); δ_C (DMSO-d_6, 100 MHz) 170.1, 168.5, 163.9, 137.9, 133.3, 131.3, 129.1, 123.1, 121.7, 81.4, 56.2, 27.7, 25.6; LRMS: m/z $C_{15}H_{18}N_2O_3$ requires: 274.1, found: 275.3 [M + H]; t_R = 1.59 (98.7%, III).

3.1.4.2. 5-Methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (7a). Prepared according to General Procedure C with **6a**, to give the title compound as a cream solid (33 mg, 99%): mp > 250 °C; δ_H (DMSO-d_6, 400 MHz) 11.11 (s, 1H, NH), 8.00 (d, J = 8.0, 1H, Ar), 7.82 (d, J = 1.6, 1H, Ar), 7.78 (dd, J = 8.0, 1.6, 1H, Ar), 4.13 (s, 2H, CH_2CO), 2.69 (s, 3H, CH_3CN); δ_C (DMSO-d_6, 100 MHz) 168.3, 166.0, 138.9, 135.5, 131.1 127.6, 123.8, 122.6, 52.6, 24.4; LRMS: m/z $C_{11}H_{10}N_2O_3$ requires: 218.1, found: 219.3 [M+H]; t_R = 1.27 (98.1%, III).

3.1.4.3. (*S*)-*tert*-Butyl 3,5-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (6b). Prepared according to General Procedure A with Fmoc-Ala-OH, to give the title compound as a white solid (267 mg, 93%): mp 80–82 °C; δ_H (DMSO-d_6, 400 MHz) 10.53 (s, 1H, NH), 7.80 (d, J = 8.4, 1H, Ar), 7.69 (d, J = 1.2, 1H, Ar), 7.65 (dd, J = 8.4, 1.2, 1H, Ar), 3.44 (q, J = 6.4, 1H, CHCO), 2.38 (s, 3H, CH_3CN), 1.55 (s, 9H, 3 × CH_3), 1.54 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 171.0, 166.4, 163.9, 137.5, 133.3, 131.6, 128.8, 123.0, 121.7, 81.4, 57.7, 27.7, 25.4, 16.9; LRMS: m/z $C_{16}H_{20}N_2O_3$ requires: 288.1, found: 289.2 [M+H]; t_R = 1.57 (90.8%, III).

3.1.4.4. (*S*)-3,5-Dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]-diazepine-8-carboxylic acid (7b). Prepared according to General Procedure C with **6b**, to give the title compound as a cream solid (34 mg, 98%): mp 58–60 °C; δ_H (DMSO-d_6, 400 MHz) 11.25 (s, 1H, NH), 8.05 (d, J = 8.0, 1H, Ar), 7.84 (d, J = 1.2, 1H, Ar), 7.81 (dd, J = 8.0, 1.2, 1H, Ar), 3.96 (q, J = 6.4, 1H, CHCO), 2.74 (s, 3H, CH_3CN), 1.50 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.4, 166.3, 139.3, 136.4, 131.6, 124.2, 123.1, 56.0, 24.3, 14.1; LRMS: m/z $C_{12}H_{12}N_2O_3$ requires: 232.1, found: 233.2 [M+H]; t_R = 1.25 (97.6%, III).

3.1.4.5. (*S*)-*tert*-Butyl 3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (6c). Prepared according to General Procedure A with Fmoc-Leu-OH, to give the title compound as a cream solid (280 mg, 85%): mp 84–86 °C; δ_H (DMSO-d_6, 400 MHz) 10.55 (s, 1H, NH), 7.83 (d, J = 8.4, 1H, Ar), 7.70 (s, 1H, Ar), 7.65 (d, J = 8.4, 1H, Ar), 3.32–3.25 (m, 1H, CHCO), 2.83 (s, 3H, CH_3CN), 1.88–1.81 (m, 1H, C*H*Me_2), 1.78–1.68 (m, 2H, CH_2iPr), 1.55 (s, 9H, 3 × CH_3), 0.85 (d, J = 5.6, 3H, CH_3), 0.71 (d, J = 5.6, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 170.2, 166.7, 163.9, 137.5, 133.3, 131.5, 128.8, 123.1, 121.7, 81.4, 60.5, 27.7, 25.4, 23.9, 23.2, 21.8; LRMS: m/z $C_{19}H_{26}N_2O_3$ requires: 330.2, found: 331.3 [M+H]; t_R = 5.61 (97.0%, I).

3.1.4.6. (*S*)-3-Isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (7c). Prepared according to General Procedure C with **6c**, to give the title compound as a cream solid (38 mg, 99%): mp 113–115 °C; δ_H (DMSO-d_6, 400 MHz) 11.09 (s, 1H, NH), 7.99 (d, J = 8.4, 1H, Ar), 7.81 (d, J = 1.2, 1H, Ar), 7.78 (dd, J = 8.4, 1.2, 1H, Ar), 3.66 (t, J = 6.4, 1H, CHCO), 2.66 (s, 3H, CH_3CN), 1.92–1.71 (m, 3H, C*H*Me_2, CH_2iPr), 0.89 (d, J = 6.0, 3H, CH_3), 0.77 (d, J = 6.0, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.2, 166.5, 138.9, 135.6, 131.1, 128.7, 124.1, 122.9, 114.8, 59.2, 37.6, 24.9, 24.3, 23.4, 22.1; LRMS: m/z $C_{15}H_{18}N_2O_3$ requires: 274.1, found: 275.2 [M+H]; t_R = 3.63 (92.6%, I).

3.1.4.7. (*R*)-*tert*-Butyl 3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (6d). Prepared according to General Procedure A with Fmoc-D-Leu-OH, to give the title compound as a yellow solid (273 mg, 83%): mp 85–87 °C; δ_H (DMSO-d_6, 400 MHz) 10.55 (s, 1H, NH), 7.83 (d, J = 8.4, 1H, Ar), 7.70 (s, 1H, Ar), 7.65 (d, J = 8.4, 1H, Ar), 3.32–3.25 (m, 1H, CHCO), 2.83 (s, 3H, CH_3CN), 1.88–1.81 (m, 1H, C*H*Me_2), 1.78–1.68 (m, 2H, CH_2iPr), 1.55 (s, 9H, 3 × CH_3), 0.85 (d, J = 5.6, 3H, CH_3), 0.71 (d, J = 5.6, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 170.2, 166.7, 163.9, 137.5, 133.3, 131.5, 128.8, 123.1, 121.7, 81.4, 60.5, 27.7, 25.4, 23.9, 23.2, 21.8; LRMS: m/z $C_{19}H_{26}N_2O_3$ requires: 330.2, found: 331.2 [M+H]; t_R = 4.48 (99.1%, I).

3.1.4.8. (*R*)-3-Isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (7d). Prepared according to General Procedure C with **6d**, to give the title compound as a yellow solid (39 mg, 99%): mp 121–123 °C; δ_H (DMSO-d_6, 400 MHz) 11.09 (s, 1H, NH), 7.99 (d, J = 8.4, 1H, Ar), 7.81 (d, J = 1.2, 1H, Ar), 7.78 (dd, J = 8.4, 1.2, 1H, Ar), 3.66 (t, J = 6.4, 1H, CHCO), 2.66 (s, 3H, CH_3CN), 1.92–1.71 (m, 3H, C*H*Me_2, CH_2iPr), 0.89 (d, J = 6.0, 3H, CH_3), 0.77 (d, J = 6.0, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.2, 166.5, 138.9, 135.6, 131.1, 128.7, 124.1, 122.9, 114.8, 59.2, 37.6, 24.9, 24.3, 23.4, 22.1; LRMS: m/z $C_{15}H_{18}N_2O_3$ requires: 274.1, found: 275.2 [M+H]; t_R = 3.68 (92.1%, I).

3.1.4.9. (*S*)-*tert*-Butyl 3-((*R*)-sec-butyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (6e). Prepared according to General Procedure A with Fmoc-Ile-OH, to give the title compound as a white solid (277 mg, 84%): mp 81–83 °C; δ_H (DMSO-d_6, 400 MHz) 10.51 (s, 1H, NH), 7.82 (d, J = 8.4, 1H, Ar), 7.69 (s, 1H, Ar), 7.65 (d, J = 8.0, 1H, Ar), 2.89 (d, J = 9.2, 1H, CHCO), 2.39 (s, 3H, CH_3CN), 2.18–2.12 (m, 1H, CH), 1.79–1.73 (m, 1H, CH), 1.55 (s, 9H, 3 × CH_3), 1.09–1.02 (m, 1H, CH), 0.88 (d, J = 6.5, 3H, CH*C*H_3), 0.82 (t, J = 7.6, 3H, CH_2CH_3); δ_C (DMSO-d_6, 100 MHz) 168.9, 166.3, 163.9, 137.4, 133.3, 131.5, 128.8, 123.1, 121.7, 81.4, 67.2, 34.4, 27.7, 25.4, 24.4, 15.8, 10.8; LRMS: m/z $C_{19}H_{26}N_2O_3$ requires: 330.2, found: 331.2 [M+H]; t_R = 3.98 (91.2%, III).

3.1.4.10. (*S*)-3-((*R*)-sec-Butyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (7e). Prepared according to General Procedure C with **6e**, to give the title compound as a cream solid (38 mg, 99%): mp 73–75 °C; δ_H (DMSO-d_6, 400 MHz) 11.0 (s, 1H, NH), 7.96 (d, J = 8.0, 1H, Ar), 7.78–7.75 (m, 2H, Ar), 3.40–3.23 (m, 1H, CHCO), 2.61 (s, 3H, CH_3CN), 2.22–2.12 (m, 1H, CH), 1.80–1.66 (m, 1H, CH), 1.14–1.06 (m, 1H, CH), 0.94 (d, J = 6.8, 3H, CH*C*H_3), 0.84 (t, J = 7.6, 3H, CH_2CH_3); δ_C (DMSO-d_6, 100 MHz) 168.0, 166.5, 138.6, 130.6, 124.1, 122.7, 114.5, 66.0, 33.7, 25.1, 24.8, 16.0, 11.1; LRMS: m/z $C_{15}H_{18}N_2O_3$ requires: 274.1, found: 275.2 [M+H]; t_R = 3.70 (92.2%, I).

3.1.4.11. (*S*)-*tert*-Butyl 3-benzyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (6f). Prepared according to General Procedure A with Fmoc-Phe-OH, to give the title compound as a white solid (316 mg, 87%): mp 85–87 °C; δ_H (DMSO-d_6, 400 MHz) 10.59 (s, 1H, NH), 7.78 (d, J = 8.0, 1H, Ar), 7.68 (s, 1H, Ar), 7.63 (d, J = 8.0, 1H, Ar), 7.24–7.18 (m, 4H, Ar), 7.14–7.10 (m, 1H, Ar), 3.54 (t, J = 6.0, 1H, CHCO), 3.36 (dd, J = 13.6, 6.0, 1H, C$H_a$$H_b$Ar), 3.16 (dd, J = 13.6, 6.0, 1H, CH$_a$$H_b$Ar),

2.37 (s, 3H, CH_3CN), 1.53 (s, 9H, 3 × CH_3); δ_C (DMSO-d_6, 100 MHz) 169.6, 166.8, 163.9, 139.2, 137.3, 133.4, 131.6, 129.4, 128.9, 128.0, 125.9, 123.1, 121.7, 81.4, 64.3, 36.9, 27.7, 25.4; LRMS: *m/z* $C_{22}H_{24}N_2O_3$ requires: 364.2, found: 365.2 [M+H]; t_R = 5.38 (100%, III).

3.1.4.12. (*S*)-3-Benzyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (7f). Prepared according to General Procedure C with **6f**, to give the title compound as a cream solid (41 mg, 99%): mp 78–80 °C; δ_H (DMSO-d_6, 400 MHz) 11.00 (s, 1H, NH), 7.91 (d, *J* = 8.4, 1H, Ar), 7.76 (d, *J* = 1.6, 1H, Ar), 7.74 (dd, *J* = 8.0, 1.6, 1H, Ar), 7.29–7.21 (m, 4H, Ar), 7.18–7.13 (m, 1H, Ar), 3.85 (t, *J* = 6.4, 1H, CHCO), 3.40 (dd, *J* = 14.0, 6.4, 1H, C*H*$_a$H$_b$Ar), 3.22 (dd, *J* = 14.0, 6.4, 1H, CH$_a$*H*$_b$Ar), 2.56 (s, 3H, CH_3CN); δ_C (DMSO-d_6, 100 MHz) 169.0, 166.5, 138.5, 138.4, 135.0, 130.6, 129.9, 128.6, 126.7,124.1, 122.7, 63.1, 35.6, 25.2; LRMS: *m/z* $C_{18}H_{16}N_2O_3$ requires: 308.1, found: 309.2 [M+H]; t_R = 4.61 (95.5%, I).

3.1.4.13. (*R*)-*tert*-Butyl 3-benzyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (6g). Prepared according to General Procedure A with Fmoc-D-Phe-OH, to give the title compound as a white solid (291 mg, 80%): mp 90–92 °C; δ_H (DMSO-d_6, 400 MHz) 10.59 (s, 1H, NH), 7.78 (d, *J* = 8.0, 1H, Ar), 7.68 (s, 1H, Ar), 7.63 (d, *J* = 8.0, 1H, Ar), 7.24–7.18 (m, 4H, Ar), 7.14–7.10 (m, 1H, Ar), 3.54 (t, *J* = 6.0, 1H, CHCO), 3.36 (dd, *J* = 13.6, 6.0, 1H, C*H*$_a$H$_b$Ar), 3.16 (dd, *J* = 13.6, 6.0, 1H, CH$_a$*H*$_b$Ar), 2.37 (s, 3H, CH_3CN), 1.53 (s, 9H, 3 × CH_3); δ_C (DMSO-d_6, 100 MHz) 169.6, 166.8, 163.9, 139.2, 137.3, 133.4, 131.6, 129.4, 128.9, 128.0, 125.9, 123.1, 121.7, 81.4, 64.3, 36.9, 27.7, 25.4; LRMS: *m/z* $C_{22}H_{24}N_2O_3$ requires: 364.2, found: 365.2 [M+H]; t_R = 3.53 (96.1%, III).

3.1.4.14. (*R*)-3-Benzyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (7g). Prepared according to General Procedure C with **6g**, to give the title compound as a cream solid (41 mg, 88%): mp 83–85 °C; δ_H (DMSO-d_6, 400 MHz) 11.00 (s, 1H, NH), 7.91 (d, *J* = 8.4, 1H, Ar), 7.76 (d, *J* = 1.6, 1H, Ar), 7.74 (dd, *J* = 8.0, 1.6, 1H, Ar), 7.29–7.21 (m, 4H, Ar), 7.18–7.13 (m, 1H, Ar), 3.85 (t, *J* = 6.4, 1H, CHCO), 3.40 (dd, *J* = 14.0, 6.4, 1H, C*H*$_a$H$_b$Ar), 3.22 (dd, *J* = 14.0, 6.4, 1H, CH$_a$*H*$_b$Ar), 2.56 (s, 3H, CH_3CN); δ_C (DMSO-d_6, 100 MHz) 169.0, 166.5, 138.5, 138.4, 135.0, 130.6, 129.9, 128.6, 126.7,124.1, 122.7, 63.1, 35.6, 25.2; LRMS: *m/z* $C_{18}H_{16}N_2O_3$ requires: 308.1, found: 309.2 [M+H]; t_R = 3.91 (90.0%, I).

3.1.4.15. *tert*-Butyl 1-(4-cyclohexylbenzyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8a). Prepared according to General Procedure B with **6a** and 1-(bromomethyl)-4-cyclohexylbenzene, to give the title compound as a white solid (52 mg, 78%): mp 171–173 °C; δ_H (DMSO-d_6, 400 MHz) 7.85 (s, 1H, Ar), 7.75 (d, *J* = 8.4, 1H, Ar), 7.68 (d, *J* = 8.4, 1H, Ar), 7.12 (d, *J* = 8.0, 2H, Ar), 7.00 (d, *J* = 8.0, 2H, Ar), 5.05 (d, *J* = 16.0, 1H, C*H*$_a$H$_b$Ar), 4.98 (d, *J* = 16.0, 1H, CH$_a$*H*$_b$Ar), 4.40 (d, *J* = 11.2, 1H, C*H*$_a$H$_b$CO), 3.69 (d, *J* = 11.2, 1H, CH$_a$*H*$_b$CO), 2.46–2.40 (m, 1H, Cy), 2.38 (s, 3H, CH_3CN), 1.77–1.66 (m, 5H, Cy), 1.46 (s, 9H, 3 × CH_3), 1.39–1.17 (m, 5H, Cy); δ_C (DMSO-d_6, 100 MHz) 168.6, 168.2, 163.6, 146.3, 140.8, 134.5, 133.8, 133.4, 128.1, 126.7, 126.4, 124.8, 122.6, 81.3, 79.2, 78.9, 78.6, 55.9, 49.6, 43.3, 33.9, 33.8, 27.6, 26.3, 25.5, 24.9; LRMS: *m/z* $C_{28}H_{34}N_2O_3$ requires: 446.3, found: 447.2 [M+H]; t_R = 27.74 (100%, II).

3.1.4.16. 1-(4-Cyclohexylbenzyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9a). Prepared according to General Procedure C with **8a**, to gie the title compound as a cream solid (49 mg, 98%): mp 103–105 °C; δ_H (DMSO-d_6, 400 MHz) 7.97 (s, 1H, Ar), 7.88 (d, *J* = 8.0, 1H, Ar), 7.80 (d, *J* = 8.0, 1H, Ar), 7.12 (d, *J* = 8.0, 2H, Ar), 6.99 (d, *J* = 8.0, 2H, Ar), 5.14 (d, *J* = 16.0, 1H, C*H*$_a$H$_b$Ar), 4.98 (d, *J* = 16.0, 1H, CH$_a$*H*$_b$Ar), 4.42 (d, *J* = 11.2, 1H, C*H*$_a$H$_b$CO), 3.90 (d, *J* = 11.2, 1H, CH$_a$*H*$_b$CO), 2.52 (s, 3H, CH_3CN), 2.46–2.38 (m, 1H, Cy), 1.76–1.65 (m, 5H, Cy), 1.38–1.16 (m, 5H, Cy); δ_C (DMSO-d_6, 100 MHz) 167.3, 165.9, 146.4, 141.1, 134.2, 134.1, 129.1, 126.7, 126.4, 125.3, 123.3, 54.1, 49.8, 33.8, 26.3, 25.5, 24.4; LRMS: *m/z* $C_{24}H_{26}N_2O_3$ requires: 390.2, found: 391.2 [M+H]; t_R = 4.66 (100%, I).

3.1.4.17. (*S*)-*tert*-Butyl 1-(4-cyclohexylbenzyl)-3,5-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8b). Prepared according to General Procedure B with **6b** and 1-(bromomethyl)-4-cyclohexylbenzene, to give the title compound as a white solid (55 mg, 81%): mp 66–68 °C; δ_H (DMSO-d_6, 400 MHz) 7.88 (s, 1H, Ar), 7.78 (d, *J* = 8.0, 1H, Ar), 7.70 (d, *J* = 8.0, 1H, Ar), 7.12 (d, *J* = 7.6, 2H, Ar), 6.97 (d, *J* = 7.6, 2H, Ar), 5.04 (s, 2H, C*H*$_2$Ar), 3.65 (q, *J* = 6.4, 1H, CHCO), 2.46–2.37 (m, 1H, Cy), 2.35 (s, 3H, CH_3CN), 1.77–1.65 (m, 5H, Cy), 1.48–1.40 (m, 12H, 4 × CH_3), 1.39–1.17 (m, 5H, Cy); δ_C (DMSO-d_6, 100 MHz) 169.7, 166.4, 163.7, 146.3, 140.5, 134.7, 134.3, 133.4, 127.9, 126.7, 126.5, 124.7, 122.8; LRMS: *m/z* $C_{29}H_{36}N_2O_3$ requires: 460.3, found: 461.3 [M+H]; t_R = 5.97 (96.3%, VI).

3.1.4.18. (*S*)-1-(4-Cyclohexylbenzyl)-3,5-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9b). Prepared according to General Procedure C with **8b**, to give the title compound as a cream solid (51 mg, 99%): mp 70–72 °C; δ_H (DMSO-d_6, 400 MHz) 8.00 (s, 1H, Ar), 7.89 (d, *J* = 8.0, 1H, Ar), 7.81 (d, *J* = 8.0, 1H, Ar), 7.11 (d, *J* = 8.0, 2H, Ar), 6.95 (d, *J* = 8.0, 2H, Ar), 5.20 (d, *J* = 16.0, 1H, C*H*$_a$H$_b$Ar), 4.99 (d, *J* = 16.0, 1H, CH$_a$*H*$_b$Ar), 3.89 (q, *J* = 6.8, 1H, CHCO), 2.49 (s, 3H, CH_3CN), 2.46–2.38 (m, 1H, Cy), 1.75–1.65 (m, 5H, Cy), 1.48 (d, *J* = 6.8, 3H, CH3), 1.35–1.16 (m, 5H, Cy); δ_C (DMSO-d_6, 100 MHz) 168.7, 166.0, 146.4, 140.8, 134.4, 134.2, 129.0, 126.7, 126.5, 125.3, 123.6, 56.3, 50.1, 33.9, 33.8, 26.2, 25.5, 24.0, 15.7; LRMS: *m/z* $C_{25}H_{28}N_2O_3$ requires: 404.2, found: 405.2 [M+H]; t_R = 4.59 (90.2%, V).

3.1.4.19. (*S*)-*tert*-Butyl 1-(4-cyclohexylbenzyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8c). Prepared according to General Procedure B with **6c** and 1-(bromomethyl)-4-cyclohexylbenzene, to give the title compound as a white solid (63 mg, 84%): mp 77–79 °C; δ_H (DMSO-d_6, 400 MHz) 7.90 (s, 1H, Ar), 7.79 (d, *J* = 8.4, 1H, Ar), 7.71 (d, *J* = 8.4, 1H, Ar), 7.12 (d, *J* = 8.0, 2H, Ar), 6.96 (d, *J* = 8.0, 2H, Ar), 5.04 (s, 2H, C*H*$_2$Ar), 3.49 (t, *J* = 6.8, 1H, CHCO), 2.47–2.38 (m, 1H, Cy), 2.35 (s, 3H, CH_3CN), 2.00–1.62 (m, 8H, C*H*Me$_2$, C*H*$_2$iPr, Cy), 1.47 (s, 9H, 3 × CH_3), 1.41–1.10 (m, 5H, Cy), 0.86 (d, *J* = 6.4, 3H, CH_3), 0.72 (d, *J* = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.9, 166.8, 163.7, 146.4, 140.5, 134.7, 134.2, 133.5, 127.9, 126.8, 126.5, 124.9, 122.8, 81.4, 60.4, 49.8, 43.3, 33.9, 33.8, 27.6, 26.3, 25.5, 24.8, 24.0, 23.2, 21.9; LRMS: *m/z* $C_{32}H_{42}N_2O_3$ requires: 502.3, found: 503.3 [M+H]; t_R = 29.28 (90.5%, II).

3.1.4.20. (*S*)-1-(4-cyclohexylbenzyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9c). Prepared according to the General Procedure C with **8c**, to give the title compound as a cream solid (55 mg, 99%): mp 63–65 °C; δ_H (DMSO-d_6, 400 MHz) 8.01 (d, *J* = 1.2, 1H, Ar), 7.86 (d, *J* = 8.0, 1H, Ar), 7.80 (dd, *J* = 8.4, 1.2, 1H, Ar), 7.10 (d, *J* = 8.4, 2H, Ar), 6.93 (d, *J* = 8.4, 2H, Ar), 5.21 (d, *J* = 16.0, 1H, C*H*$_a$H$_b$Ar), 4.98 (d, *J* = 16.0, 1H, CH$_a$*H*$_b$Ar), 3.65 (t, *J* = 7.2, 1H, CHCO), 2.45 (s, 3H, CH_3CN), 2.44–2.37 (m, 1H, Cy), 2.01–1.62 (m, 8H, C*H*Me$_2$, C*H*$_2$iPr, Cy), 1.38–1.11 (m, 5H, Cy), 0.87 (d, *J* = 6.4, 3H, CH_3), 0.74 (d, *J* = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.2, 166.1, 146.4, 140.7, 134.3, 134.1, 133.1, 128.6, 126.7, 126.5, 125.7, 123.7, 59.6, 50.0, 43.3, 33.9, 33.8, 26.3, 25.5, 24.4, 23.9, 23.0, 21.8; LRMS: *m/z* $C_{28}H_{34}N_2O_3$ requires: 446.3, found: 447.3 [M+H]; t_R = 4.72 (90.2%, V).

3.1.4.21. (*R*)-*tert*-Butyl 1-(4-cyclohexylbenzyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8d). Prepared according to General Procedure B with **6d** and 1-(bromomethyl)-4-cyclohexylbenzene, to give the title compound as a white solid (64 mg, 85%): mp 75–77 °C; δ_H (DMSO-d_6, 400 MHz) 7.90 (s, 1H, Ar), 7.79 (d, J = 8.4, 1H, Ar), 7.71 (d, J = 8.4, 1H, Ar), 7.12 (d, J = 8.0, 2H, Ar), 6.96 (d, J = 8.0, 2H, Ar), 5.04 (s, 2H, CH_2Ar), 3.49 (t, J = 6.8, 1H, CHCO), 2.47–2.38 (m, 1H, Cy), 2.35 (s, 3H, CH_3CN), 2.00–1.62 (m, 8H, C*H*Me_2, C*H*$_2$iPr, Cy), 1.47 (s, 9H, 3 × CH_3), 1.41–1.10 (m, 5H, Cy), 0.86 (d, J = 6.4, 3H, CH_3), 0.72 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.9, 166.8, 163.7, 146.4, 140.5, 134.7, 134.2, 133.5, 127.9, 126.8, 126.5, 124.9, 122.8, 81.4, 60.4, 49.8, 43.3, 33.9, 33.8, 27.6, 26.3, 25.5, 24.8, 24.0, 23.2, 21.9; LRMS: m/z $C_{32}H_{42}N_2O_3$ requires: 502.3, found: 503.3 [M+H]; t_R = 28.86 (96.5%, II).

3.1.4.22. (*R*)-1-(4-Cyclohexylbenzyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9d). Prepared according to General Procedure C with **8d**, to give the title compound as a yellow solid (55 mg, 99%): mp 87–89 °C; δ_H (DMSO-d_6, 400 MHz) 8.01 (d, J = 1.2, 1H, Ar), 7.86 (d, J = 8.0, 1H, Ar), 7.80 (dd, J = 8.4, 1.2, 1H, Ar), 7.10 (d, J = 8.4, 2H, Ar), 6.93 (d, J = 8.4, 2H, Ar), 5.21 (d, J = 16.0, 1H, C*H*$_a$H$_b$Ar), 4.98 (d, J = 16.0, 1H, CH$_a$*H*$_b$Ar), 3.65 (t, J = 7.2, 1H, CHCO), 2.45 (s, 3H, CH_3CN), 2.44–2.37 (m, 1H, Cy), 2.01–1.62 (m, 8H, C*H*Me_2, C*H*$_2$iPr, Cy), 1.38–1.11 (m, 5H, Cy), 0.87 (d, J = 6.4, 3H, CH_3), 0.74 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.2, 166.1, 146.4, 140.7, 134.3, 134.1, 133.1, 128.6, 126.7, 126.5, 125.7, 123.7, 59.6, 50.0, 43.3, 33.9, 33.8, 26.3, 25.5, 24.4, 23.9, 23.0, 21.8; LRMS: m/z $C_{28}H_{34}N_2O_3$ requires: 446.3, found: 447.3 [M+H]; t_R = 6.57 (100%, I).

3.1.4.23. (*S*)-*tert*-Butyl 3-((*R*)-sec-butyl)-1-(4-cyclohexylbenzyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8e). Prepared according to General Procedure B with **6e** and 1-(bromomethyl)-4-cyclohexylbenzene, to give the title compound as a white solid (70 mg, 93%): mp 75–77 °C; δ_H (DMSO-d_6, 400 MHz) 7.89 (s, 1H, Ar), 7.79 (d, J = 8.0, 1H, Ar), 7.70 (d, J = 8.0, 1H, Ar), 7.12 (d, J = 7.6, 2H, Ar), 6.95 (d, J = 7.6, 2H, Ar), 5.07 (d, J = 16.0, 1H, C*H*$_a$H$_b$Ar), 5.00 (d, J = 16.0, 1H, CH$_a$*H*$_b$-Ar), 3.07 (d, J = 9.6, 1H, CHCO), 2.46–2.37 (m, 1H, Cy), 2.34 (s, 3H, CH_3CN), 2.31–2.65 (m, 1H, CH), 1.81–1.62 (m, 6H, CH, Cy), 1.46 (s, 9H, 3 × CH_3), 1.38–1.13 (m, 5H, Cy), 1.04–0.96 (m, 1H, CH), 0.89 (d, J = 6.4, 3H, CHC*H*$_3$), 0.81 (t, J = 7.6, 3H, CH_2C*H*$_3$); δ_C (DMSO-d_6, 100 MHz) 167.8, 166.4, 163.6, 146.4, 140.5, 134.7, 134.0, 133.4, 127.8, 126.8, 126.4, 124.8, 122.7, 81.4, 67.3, 49.7, 43.3, 34.6, 33.9, 33.8, 27.6, 26.3, 25.5, 24.8, 24.3, 15.6, 10.7; LRMS: m/z $C_{32}H_{42}N_2O_3$ requires: 502.3, found: 503.3 [M+H]; t_R = 25.48 (95.6%, II).

3.1.4.24. (*S*)-3-((*R*)-sec-Butyl)-1-(4-cyclohexylbenzyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9e). Prepared according to General Procedure C with **8e**, to give the title compound as a yellow solid (55 mg, 98%): mp 94–96 °C; δ_H (DMSO-d_6, 400 MHz) 7.99 (s, 1H, Ar), 7.83 (d, J = 8.4, 1H, Ar), 7.78 (d, J = 8.4, 1H, Ar), 7.10 (d, J = 8.4, 2H, Ar), 6.90 (d, J = 8.4, 2H, Ar), 5.20 (d, J = 16.0, 1H, C*H*$_a$H$_b$Ar), 4.97 (d, J = 16.0, 1H, CH$_a$*H*$_b$Ar), 3.21 (d, J = 10.0, 1H, CHCO), 2.59–2.54 (m, 1H, Cy), 2.40 (s, 3H, CH_3CN), 2.29–2.25 (m, 1H, CH), 1.82–1.62 (m, 6H, CH, Cy), 1.43–1.15 (m, 5H, Cy), 1.07–0.95 (m, 1H, CH), 0.90 (d, J = 6.4, 3H, CHC*H*$_3$), 0.82 (t, J = 7.2, 3H, CH_2C*H*$_3$); δ_C (DMSO-d_6, 100 MHz) 167.2,166.1, 146.4, 140.5, 134.3, 133.7, 133.5, 128.3, 126.7, 126.4, 125.3, 123.4, 66.6, 49.8, 34.2, 33.9, 26.3, 25.5, 24.4, 24.3, 15.5, 10.6; LRMS: m/z $C_{28}H_{34}N_2O_3$ requires: 446.3, found: 447.2 [M+H]; t_R = 20.77 (100%, I).

3.1.4.25. (*S*)-*tert*-Butyl 3-benzyl-1-(4-cyclohexylbenzyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8f). Prepared according to General Procedure B with **6f** and 1-(bromomethyl)-4-cyclohexylbenzene, to give the title compound as a white solid (62 mg, 78%): mp 81–83 °C; δ_H (DMSO-d_6, 400 MHz) 7.85 (s, 1H, Ar), 7.75 (d, J = 8.4, 1H, Ar), 7.67 (d, J = 8.4, 1H, Ar), 7.25–7.19 (m, 4H, Ar), 7.16–7.13 (m, 1H, Ar), 7.08 (d, J = 7.6, 2H, Ar), 6.90 (d, J = 7.6, 2H, Ar), 5.03 (s, 2H, C*H*$_2$Ar), 3.74 (t, J = 6.8, 1H, CHCO), 3.43 (dd, J = 13.2, 6.8, 1H, C*H*$_a$H$_b$Ar), 3.22 (dd, J = 13.2, 6.8, 1H, CH$_a$*H*$_b$Ar), 2.47–2.36 (m, 1H, Cy), 2.33 (s, 3H, CH_3CN), 1.81–1.61 (m, 5H, Cy), 1.45 (s, 9H, 3 × CH_3), 1.34–1.16 (m, 5H, Cy); δ_C (DMSO-d_6, 100 MHz) 168.3, 166.9, 163.6, 146.4, 140.3, 138.9, 134.5, 134.1, 133.5, 129.5, 128.0, 127.9, 126.7, 126.5, 126.0, 124.8, 122.7, 81.4, 64.2, 49.8, 43.3, 37.2, 33.9, 33.8, 27.6, 26.2, 25.5, 24.8. LRMS: m/z $C_{35}H_{40}N_2O_3$ requires: 536.3, found: 537.3 [M+H]; t_R = 27.86 (95.8%, II).

3.1.4.26. (*S*)-3-Benzyl-1-(4-cyclohexylbenzyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9f). Prepared according to General Procedure C with **8f**, to give the title compound as a cream solid (58 mg, 98%): mp 94–96 °C; δ_H (DMSO-d_6, 400 MHz) 7.97 (s, 1H, Ar), 7.78 (d, J = 8.4, 1H, Ar), 7.74 (d, J = 8.4, 1H, Ar), 7.25–7.19 (m, 4H, Ar), 7.17–7.13 (m, 1H, Ar), 7.07 (d, J = 8.0, 2H, Ar), 6.87 (d, J = 8.0, 2H, Ar), 5.24 (d, J = 16.0, 1H, C*H*$_a$H$_b$Ar), 4.94 (d, J = 16.0, 1H, CH$_a$*H*$_b$Ar), 3.83 (t, J = 6.4, 1H, CHCO), 3.42 (dd, J = 14.0, 6.4, 1H, C*H*$_a$H$_b$Ar), 3.24 (dd, J = 14.0, 6.4, 1H, CH$_a$*H*$_b$Ar), 2.44–2.36 (m, 1H, Cy), 2.37 (s, 3H, CH_3CN), 1.76–1.63 (m, 5H, Cy), 1.37–1.15 (m, 5H, Cy); δ_C (DMSO-d_6, 100 MHz) 168.0, 166.0, 146.4, 140.2, 138.5, 134.2, 133.8, 133.6, 129.4, 128.2, 128.0, 126.7, 126.5, 126.0,125.3, 123.4, 63.6, 49.7, 43.2, 36.6, 33.9, 33.8, 26.2, 25.5, 24.6; LRMS: m/z $C_{31}H_{32}N_2O_3$ requires: 480.2, found: 481.2 [M+H]; t_R = 23.88 (99.0%, II).

3.1.4.27. (*R*)-*tert*-Butyl 3-benzyl-1-(4-cyclohexylbenzyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8g). Prepared according to General Procedure B with **6g** and 1-(bromomethyl)-4-cyclohexylbenzene, to give the title compound as a white solid (73 mg, 91%): mp 78–80 °C; δ_H (DMSO-d_6, 400 MHz) 7.85 (s, 1H, Ar), 7.75 (d, J = 8.4, 1H, Ar), 7.67 (d, J = 8.4, 1H, Ar), 7.25–7.19 (m, 4H, Ar), 7.16–7.13 (m, 1H, Ar), 7.08 (d, J = 7.6, 2H, Ar), 6.90 (d, J = 7.6, 2H, Ar), 5.03 (s, 2H, C*H*$_2$Ar), 3.74 (t, J = 6.8, 1H, CHCO), 3.43 (dd, J = 13.2, 6.8, 1H, C*H*$_a$H$_b$Ar), 3.22 (dd, J = 13.2, 6.8, 1H, CH$_a$*H*$_b$Ar), 2.47–2.36 (m, 1H, Cy), 2.33 (s, 3H, CH_3CN), 1.81–1.61 (m, 5H, Cy), 1.45 (s, 9H, 3 × CH_3), 1.34–1.16 (m, 5H, Cy); δ_C (DMSO-d_6, 100 MHz) 168.3, 166.9, 163.6, 146.4, 140.3, 138.9, 134.5, 134.1, 133.5, 129.5, 128.0, 127.9, 126.7, 126.5, 126.0, 124.8, 122.7, 81.4, 64.2, 49.8, 43.3, 37.2, 33.9, 33.8, 27.6, 26.2, 25.5, 24.8; LRMS: m/z $C_{35}H_{40}N_2O_3$ requires: 536.3, found: 537.3 [M+H]; t_R = 27.61 (95.6%, II).

3.1.4.28. (*R*)-3-Benzyl-1-(4-cyclohexylbenzyl)-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9g). Prepared according to General Procedure C with **8g**, to give the title compound as a cream solid (58 mg, 98%): mp 97–99 °C; δ_H (DMSO-d_6, 400 MHz) 7.97 (s, 1H, Ar), 7.78 (d, J = 8.4, 1H, Ar), 7.74 (d, J = 8.4, 1H, Ar), 7.25–7.19 (m, 4H, Ar), 7.17–7.13 (m, 1H, Ar), 7.07 (d, J = 8.0, 2H, Ar), 6.87 (d, J = 8.0, 2H, Ar), 5.24 (d, J = 16.0, 1H, C*H*$_a$H$_b$Ar), 4.94 (d, J = 16.0, 1H, CH$_a$*H*$_b$Ar), 3.83 (t, J = 6.4, 1H, CHCO), 3.42 (dd, J = 14.0, 6.4, 1H, C*H*$_a$H$_b$Ar), 3.24 (dd, J = 14.0, 6.4, 1H, CH$_a$*H*$_b$Ar), 2.44–2.36 (m, 1H, Cy), 2.37 (s, 3H, CH_3CN), 1.76–1.63 (m, 5H, Cy), 1.37–1.15 (m, 5H, Cy); δ_C (DMSO-d_6, 100 MHz) 168.0, 166.0, 146.4, 140.2, 138.5, 134.2, 133.8, 133.6, 129.4, 128.2, 128.0, 126.7, 126.5, 126.0,125.3, 123.4, 63.6, 49.7, 43.2, 36.6, 33.9, 33.8, 26.2, 25.5, 24.6; LRMS: m/z $C_{31}H_{32}N_2O_3$ requires: 480.2, found: 481.2 [M+H]; t_R = 23.84 (100%, II).

3.1.4.29. (*R*)-*tert*-Butyl 1-benzyl-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8h). Prepared according to General Procedure B with **6d** and benzyl bromide, to give the title compound as a yellow solid (55 mg, 88%): mp 94–96 °C; δ_H (DMSO-d_6, 400 MHz) 7.90 (d, J = 1.6, 1H, Ar), 7.79 (d, J = 8.4, 1H, Ar), 7.70 (dd, J = 8.4, 1.6, 1H, Ar), 7.30 (t, J = 7.2, 2H, Ar), 7.20 (t, J = 7.2, 1H, Ar), 7.04 (d, J = 7.2, 2H, Ar), 5.15 (d, J = 16.0, 1H, CH_aH$_b$Ar), 5.06 (d, J = 16.0, 1H, CH$_a$$H_b$Ar), 3.66 (t, J = 5.6, 1H, CHCO), 2.36 (s, 3H, CH_3CN), 1.99–1.91 (m, 1H, CHMe$_2$), 1.86–1.68 (m, 2H, CH_2iPr), 1.49 (s, 9H, 3 × CH_3), 0.87 (d, J = 6.4, 3H, CH_3), 0.72 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.1, 166.8, 163.7, 140.3, 137.3, 134.3, 133.5, 128.5, 127.9, 127.1, 126.6, 124.9, 122.8, 81.5, 60.4, 49.8, 27.6, 24.8, 24.0, 23.2, 21.9; LRMS: m/z $C_{26}H_{32}N_2O_3$ requires: 420.2, found: 421.2 [M+H]; t_R = 23.20 (97.6%, II).

3.1.4.30. (*R*)-1-Benzyl-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9h). Prepared according to General Procedure C with **8h**, to give the title compound as a yellow solid (47 mg, 99%): mp 81–83 °C; δ_H (DMSO-d_6, 400 MHz) 7.99 (d, J = 1.2, 1H, Ar), 7.83 (d, J = 8.4, 1H, Ar), 7.77 (dd, J = 8.4, 1.2, 1H, Ar), 7.26 (t, J = 7.6, 2H, Ar), 7.19 (t, J = 7.6, 1H, Ar), 7.02 (d, J = 7.6, 2H, Ar), 5.26 (d, J = 16.0, 1H, CH_aH$_b$Ar), 5.02 (d, J = 16.0, 1H, CH$_a$$H_b$Ar), 3.61 (t, J = 6.8, 1H, CHCO), 2.43 (s, 3H, CH_3CN), 1.99–1.91 (m, 1H, CHMe$_2$), 1.88–1.68 (m, 2H, CH_2iPr), 0.88 (d, J = 6.8, 3H, CH_3), 0.73 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.5, 166.1, 140.4, 137.0, 133.7, 128.5, 128.4, 127.1, 126.6, 125.3, 123.4, 59.9, 49.9, 24.6, 24.0, 23.1, 22.2, 21.9; LRMS: m/z $C_{22}H_{24}N_2O_3$ requires: 364.2, found: 365.2 [M+H]; t_R = 4.63 (98.1%, I).

3.1.4.31. (*R*)-*tert*-Butyl 1-(4-(*tert*-butyl)benzyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8i). Prepared according to General Procedure B with 6d and 4-*tert*-butylbenzyl bromide, to give the title compound as a white solid (65 mg, 92%): mp 82–83 °C; δ_H (DMSO-d_6, 400 MHz) 7.88 (s, 1H, Ar), 7.80 (d, J = 8.0, 1H, Ar), 7.71 (d, J = 8.0, 1H, Ar), 7.30 (d, J = 8.0, 2H, Ar), 7.00 (d, J = 8.0, 2H, Ar), 5.08 (d, J = 16.0, 1H, CH_aH$_b$Ar), 4.99 (d, J = 16.0, 1H, CH$_a$$H_b$Ar), 3.50 (t, J = 6.8, 1H, CHCO), 2.36 (s, 3H, CH3CN), 1.98–1.89 (m, 1H, CHMe$_2$), 1.86–1.68 (m, 2H, CH_2iPr), 1.46 (s, 9H, 3 × CH_3), 1.23 (s, 9H, 3 × CH_3), 0.86 (d, J = 6.4, 3H, CH_3), 0.72 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.0, 166.8, 163.7, 149.4, 140.6, 134.9, 134.1, 133.5, 127.9, 126.2, 125.3, 124.9, 122.7, 81.5, 60.4, 49.8, 34.1, 27.6, 24.9, 24.0, 23.2, 21.9; LRMS: m/z $C_{30}H_{40}N_2O_3$ requires: 476.3, found: 477.3 [M+H]; t_R = 6.45 (95.6%, V).

3.1.4.32. (*R*)-1-(4-(*tert*-Butyl)benzyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9i). Prepared according to General Procedure C with **8i**, to give the title compound as a cream solid (51 mg, 98%): mp 95–97 °C; δ_H (DMSO-d_6, 400 MHz) 8.00 (s, 1H, Ar), 7.84 (d, J = 8.4, 1H, Ar), 7.78 (d, J = 8.4, 1H, Ar), 7.27 (d, J = 8.4, 2H, Ar), 6.93 (d, J = 7.6, 2H, Ar), 5.23 (d, J = 16.0, 1H, CH_aH$_b$Ar), 4.96 (d, J = 16.0, 1H, CH$_a$$H_b$Ar), 3.59 (t, J = 6.0, 1H, CHCO), 2.41 (s, 3H, CH_3CN), 1.98–1.90 (m, 1H, CHMe$_2$), 1.86–1.67 (m, 2H, CH_2iPr), 1.21 (s, 9H, 3 × CH_3), 0.87 (d, J = 6.0, 3H, CH_3), 0.73 (d, J = 6.0, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.4, 166.1, 149.4, 140.5, 134.0, 133.7, 128.4, 126.2, 125.3, 125.2, 123.4, 60.0, 49.8, 34.1, 31.2, 24.6, 24.0, 23.1, 21.9; LRMS: m/z $C_{26}H_{32}N_2O_3$ requires: 420.2, found: 421.2 [M+H]; t_R = 5.37 (100%, I).

3.1.4.33. (*R*)-*tert*-Butyl 1-([1,1′-biphenyl]-4-ylmethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8j). Prepared according to General Procedure B with **6d** and 4-phenylbenzyl bromide, to give the title compound as a white solid (61 mg, 82%): mp 76–78 °C; δ_H (DMSO-d_6, 400 MHz) 7.93 (s, 1H, Ar), 7.82 (d, J = 8.0, 1H, Ar), 7.72 (d, J = 8.0, 1H, Ar), 7.63–7.57 (m, 4H, Ar), 7.44 (t, J = 7.6, 2H, Ar), 7.34 (t, J = 7.6, 1H, Ar), 7.15 (d, J = 8.4, 2H, Ar), 5.18 (d, J = 16.8, 1H, CH_aH$_b$Ar), 5.13 (d, J = 16.8, 1H, CH$_a$$H_b$Ar), 3.53 (t, J = 6.8, 1H, CHCO), 2.39 (s, 3H, CH_3CN), 2.01–1.93 (m, 1H, CHMe$_2$), 1.88–1.68 (m, 2H, CH_2iPr), 1.47 (s, 9H, 3 × CH_3), 0.88 (d, J = 6.8, 3H, CH_3), 0.73 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.1, 166.9, 163.7, 140.4, 139.6, 138.9, 136.6, 134.3, 133.5, 128.9, 128.0, 127.4, 127.2, 126.8, 126.5, 124.9, 122.8, 81.5, 60.4, 49.6, 27.6, 24.9, 24.0, 23.2, 21.9; LRMS: m/z $C_{32}H_{36}N_2O_3$ requires: 496.3, found: 497.3 [M+H]; t_R = 6.03 (91.8%, V).

3.1.4.34. (*R*)-1-([1,1′-Biphenyl]-4-ylmethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9j). Prepared according to General Procedure C with **8j**, to give the title compound as a cream solid (53 mg, 97%): mp 108–110 °C; δ_H (DMSO-d_6, 400 MHz) 8.04 (d, J = 1.6. 1H, Ar), 7.89 (d, J = 8.4, 1H, Ar), 7.81 (dd, J = 8.4, 1.6, 1H, Ar), 7.64–7.56 (m, 4H, Ar), 7.43 (t, J = 8.0, 2H, Ar), 7.33 (t, J = 7.6, 1H, Ar), 7.14 (d, J = 8.0, 2H, Ar), 5.30 (d, J = 16.4, 1H, CH_aH$_b$Ar), 5.09 (d, J = 16.4, 1H, CH_aH$_b$-Ar), 3.70 (t, J = 7.2, 1H, CHCO), 2.49 (s, 3H, CH_3CN), 2.02–1.93 (m, 1H, CHMe$_2$), 1.89–1.70 (m, 2H, CH_2iPr), 0.89 (d, J = 6.8, 3H, CH_3), 0.75 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.4, 166.1, 140.6, 139.5, 138.9, 136.3, 134.1, 133.1, 128.9, 128.7, 127.4, 127.2, 126.8, 126.5, 125.4, 123.5, 59.7, 49.9, 24.5, 24.0, 23.1, 21.9; LRMS: m/z $C_{28}H_{28}N_2O_3$ requires: 440.2, found: 441.2 [M+H]; t_R = 5.08 (99.3%, I).

3.1.4.35. (*R*)-*tert*-Butyl 1,3-diisobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8k). Prepared according to General Procedure B with **6d** and 1-bromo-2-methylpropane, to give the title compound as a white solid (44 mg, 76%): mp 116–118 °C; δ_H (DMSO-d_6, 400 MHz) 7.94 (s, 1H, Ar), 7.83 (d, J = 8.4, 1H, Ar), 7.75 (d, J = 8.4, 1H, Ar), 4.14 (dd, J = 14.0, 9.2, 1H, CH_aH$_b$Ar), 3.46 (dd, J = 14.0, 9.2, 1H, CH$_a$$H_b$Ar), 3.34–3.32 (m, 1H, CHCO), 2.40 (s, 3H, CH_3CN), 1.94–1.85 (m, 1H, CHMe$_2$), 1.76–1.62 (m, 3H, CHMe$_2$, CH_2iPr), 1.56 (s, 9H, 3 × CH_3), 0.83 (d, J = 6.4, 3H, CH_3), 0.71–0.66 (m, 6H, 2 × CH_3) 0.59 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.9, 166.9, 164.3, 140.7, 135.2, 134.0, 128.2, 125.2, 123.6, 82.0, 79.7, 79.4, 79.1, 60.9, 52.9, 28.1, 27.0, 25.2, 24.3, 23.6, 22.2, 20.3, 19.5; LRMS: m/z $C_{23}H_{34}N_2O_3$ requires: 386.3, found: 387.2 [M+H]; t_R = 4.34 (91.3%, I).

3.1.4.36. (*R*)-1,3-Diisobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9k). Prepared according to General Procedure C with **8k**, to give the title compound as a cream solid (43 mg, 99%): mp 97–99 °C; δ_H (DMSO-d_6, 400 MHz) 8.02 (d, J = 1.2, 1H, Ar), 7.91 (d, J = 8.0, 1H, Ar), 7.84 (dd, J = 8.0, 1.2, 1H, Ar), 4.14 (dd, J = 14.0, 9.2, 1H, CH_aH$_b$iPr), 3.52 (dd, J = 14.0, 9.2, 1H, CH$_a$$H_b$iPr), 3.44 (t, J = 5.6, 1H, CHCO), 2.49 (s, 3H, CH_3CN), 1.95–1.88 (m, 1H, CHMe$_2$), 1.79–1.52 (m, 3H, CHMe$_2$, CH_2iPr), 0.84 (d, J = 6.8, 3H, CH_3), 0.71–0.68 (m, 6H, 2 × CH_3), 0.57 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.2, 166.3, 140.4, 133.8, 128.5, 125.2, 123.6, 60.0, 52.5, 26.5, 24.5, 23.9, 23.1, 21.8, 19.8, 19.1; LRMS: m/z $C_{19}H_{26}N_2O_3$ requires: 330.2, found: 331.2 [M+H]; t_R = 1.28 (96.8%, III).

3.1.4.37. (*R*)-*tert*-Butyl 1-(2-(*tert*-butoxy)-2-oxoethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8l). Prepared according to General Procedure B with **6d** and *tert*-butyl bromoacetate, to give the title compound as a white solid (61 mg, 92%): mp 62–64 °C; δ_H (DMSO-d_6, 400 MHz) 7.86 (d, J = 8.4, 1H, Ar), 7.79–7.77 (m, 2H, Ar), 4.48 (d, J = 17.2, 1H, CH_aH$_b$CO$_2$), 4.39 (d, J = 17.2, 1H, CH$_a$$H_bCO_2$), 3.37 (t, J = 6.4, 1H, CHCO), 2.42 (s, 3H, CH_3CN), 1.91–1.62 (m, 3H, CHMe$_2$, CH_2iPr), 1.55 (s, 9H, 3 × CH_3), 1.40 (s, 9H, 3 × CH_3), 0.84

(d, J = 6.8, 3H, CH_3), 0.71 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.4, 167.8, 167.0, 163.8, 140.5, 133.6, 133.5, 127.9, 124.9, 122.1, 81.6, 81.4, 60.1, 50.3, 27.7, 27.6, 25.1, 24.0, 23.0, 21.9; LRMS: m/z $C_{25}H_{36}N_2O_5$ requires: 444.3, found: 445.2 [M+H]; t_R = 21.17 (99.5%, II).

3.1.4.38. (*R*)-1-(Carboxymethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9l). Prepared according to General Procedure C with **8l**, to give the title compound as a yellow solid (43 mg, 98%): mp 83–85 °C; δ_H (DMSO-d_6, 400 MHz) 7.93 (d, J = 8.4, 1H, Ar), 7.90 (s, 1H, Ar), 7.86 (d, J = 8.4, 1H, Ar), 4.51 (s, 2H, CH_2CO), 3.56 (t, J = 6.8, 1H, CHCO), 2.52 (s, 3H, CH_3CN), 1.94–1.64 (m, 3H, C*H*Me_2, CH_2iPr), 0.85 (d, J = 6.8, 3H, CH_3), 0.72 (d, J = 6.8, 3H, CH3); δ_C (DMSO-d_6, 100 MHz) 170.2, 168.6, 166.1, 140.9, 134.1, 132.7, 128.6, 125.4, 123.1, 59.4, 49.7, 24.6, 24.0, 23.0, 21.8; LRMS: m/z $C_{17}H_{20}N_2O_5$ requires: 332.2, found: 333.3 [M+H]; t_R = 1.29 (100%, III).

3.1.4.39. (*R*)-*tert*-Butyl 3-isobutyl-5-methyl-2-oxo-1-(pyridin-4-ylmethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8m). Prepared according to General Procedure B with **6d** and 4-(chloromethyl)pyridine, to give the title compound as a white solid (56 mg, 89%): mp 64–66 °C; δ_H (DMSO-d_6, 400 MHz) 8.49 (d, J = 5.2, 2H, Py), 7.85 (d, J = 8.0, 1H, Ar), 7.78 (s, 1H, Ar), 7.73 (d, J = 8.0, 1H, Ar), 7.10 (d, J = 5.2, 2H, Py), 5.16 (d, J = 17.2, 1H, C$H_a$$H_b$Py), 5.06 (d, J = 17.2, 1H, C$H_a$$H_b$Py), 3.56 (t, J = 6.4, 1H, CHCO), 2.42 (s, 3H, CH_3CN), 1.99–1.91 (m, 1H, C*H*Me_2), 1.82–1.68 (m, 2H, CH_2iPr), 1.47 (s, 9H, 3 × CH_3), 0.88 (d, J = 6.8, 3H, CH_3), 0.72 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.4, 167.0, 163.6, 149.7, 146.6, 140.3, 134.0, 133.5, 128.1, 125.0, 122.5, 121.6, 81.5, 60.3, 49.4, 27.6, 25.0, 23.9, 23.2, 21.8; LRMS: m/z $C_{25}H_{31}N_3O_3$ requires: 421.2, found: 422.2 [M+H]; t_R = 3.94 (96.3%, I).

3.1.4.40. (*R*)-3-Isobutyl-5-methyl-2-oxo-1-(pyridin-4-ylmethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9m). Prepared according to General Procedure C with **8m** to give the title compound as a yellow solid (47 mg, 99%): mp 98–100 °C; δ_H (DMSO-d_6, 400 MHz) 8.79 (d, J = 6.4, 2H, Py), 7.93 (d, J = 8.8, 1H, Ar), 7.86–7.83 (m, 2H, Ar), 7.62 (d, J = 6.4, 2H, Py), 5.32 (s, 2H, CH_2Py), 3.69–3.65 (m, 1H, CHCO), 2.52 (s, 3H, CH_3CN), 1.98–1.90 (m, 1H, C*H*Me_2), 1.78–1.69 (m, 2H, CH_2iPr), 0.88 (d, J = 6.8, 3H, CH_3), 0.72 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.3, 168.8, 166.1, 155.2, 144.0, 140.3, 133.8, 133.5, 128.5, 125.6, 123.6, 122.9, 59.9, 50.3, 24.9, 23.9, 23.3, 21.7; LRMS: m/z $C_{21}H_{23}N_3O_3$ requires: 365.2, found: 366.2 [M+H]; t_R = 3.48 (94.6%, I).

3.1.4.41. (*R*)-*tert*-Butyl 3-isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(phenethylamino)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8n). Prepared according to General Procedure B with **6d** and 2-bromo-*N*-phenethylacetamide, to give the title compound as a white solid (70 mg, 95%): mp 80–82 °C; δ_H (DMSO-d_6, 400 MHz) 8.27 (t, J = 5.6, 1H, NH), 7.88 (s, 1H, Ar), 7.84 (d, J = 8.0, 1H, Ar), 7.76 (d, J = 8.0, 1H, Ar), 7.28 (t, J = 7.2, 2H, Ar), 7.22–7.17 (m, 3H, Ar), 4.48 (d, J = 17.6, 1H, C$H_a$$H_b$CONH), 4.21 (d, J = 17.6, 1H, C$H_a$$H_b$CONH), 3.40 (t, J = 6.4, 1H, CHCO), 3.33–3.26 (m, 2H, CH_2NH), 2.72 (t, J = 6.4, 2H, CH_2Ar), 2.43 (s, 3H, CH_3CN), 1.95–1.88 (m, 1H, C*H*Me_2), 1.77–1.65 (m, 2H, CH_2iPr), 1.54 (s, 9H, 3 × CH_3), 0.85 (d, J = 6.4, 3H, CH_3), 0.70 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.1, 167.4, 163.3, 141.2, 139.3, 133.6, 133.5, 128.6, 128.3, 127.8, 126.1, 124.7, 122.8, 81.5, 60.1, 50.5, 40.5, 40.1, 38.9, 35.1, 27.6, 25.0, 23.9, 23.2, 21.7; LRMS: m/z $C_{29}H_{37}N_3O_4$ requires: 491.3, found: 492.2 [M+H]; t_R = 5.54 (100%, I).

3.1.4.42. (*R*)-3-Isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(phenethylamino)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9n). Prepared according to General Procedure C with **8n**, to give the title compound as a cream solid (54 mg, 99%): mp 69–71 °C; δ_H (DMSO-d_6, 400 MHz) 8.25 (t, J = 5.6, 1H, NH), 7.95 (d, J = 1.6, 1H, Ar), 7.92 (d, J = 8.0, 1H, Ar), 7.85 (dd, J = 8.0, 1.6, 1H, Ar), 7.24 (t, J = 7.2, 2H, Ar), 7.17–7.13 (m, 3H, Ar), 4.46 (d, J = 16.4, 1H, C$H_a$$H_b$CONH), 4.32 (d, J = 16.4, 1H, C$H_a$$H_b$CONH), 3.67 (t, J = 6.8, 1H, CHCO), 3.31–3.26 (m, 2H, CH_2NH), 2.70 (t, J = 7.2, 2H, CH_2Ar), 2.60 (s, 3H, CH_3CN), 1.97–1.89 (m, 1H, C*H*Me_2), 1.84–1.68 (m, 2H, CH_2iPr), 0.87 (d, J = 6.8, 3H, CH_3), 0.74 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.0, 167.3, 166.1, 141.6, 139.3, 134.6, 131.8, 129.0, 128.6, 128.3, 126.1, 125.3, 123.7, 58.9, 50.9, 40.4, 38.1, 35.1, 24.4, 23.9, 23.0, 21.8; LRMS: m/z $C_{25}H_{29}N_3O_4$ requires: 435.2, found: 436.2 [M+H]; t_R = 4.16 (97.3%, I).

3.1.4.43. (*R*)-*tert*-Butyl 3-isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(decylamino)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8o). Prepared according to General Procedure B with **6d** and 2-bromo-*N*-decylacetamide, to give the title compound as a white solid (68 mg, 86%): mp 64–66 °C; δ_H (DMSO-d_6, 400 MHz) 8.10 (t, J = 5.2, 1H, NH), 7.87–7.83 (m, 2H, Ar), 7.76 (d, J = 8.4, 1H, Ar), 4.46 (d, J = 16.4, 1H, C$H_a$$H_b$CONH), 4.21 (d, J = 16.4, 1H, C$H_a$$H_b$CONH), 3.45 (t, J = 6.8, 1H, CHCO), 3.08–3.03 (m, 2H, CH_2NH), 2.44 (s, 3H, CH_3CN), 1.93–1.86 (m, 1H, C*H*Me_2), 1.77–1.64 (m, 2H, CH_2iPr), 1.54 (s, 9H, 3 × CH_3), 1.42–1.36 (m, 2H, Alk), 1.30–1.18 (m, 15H, Alk), 0.86–0.82 (m, 5H, CH_3, Alk), 0.70 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.9, 167.1, 163.8, 141.3, 133.7, 133.2, 128.0, 124.6, 122.8, 81.5, 59.9, 50.5, 38.6, 31.3, 29.1, 29.0, 28.8, 28.7, 27.6, 26.3, 24.9, 23.9, 23.2, 22.1, 21.7, 13.9; LRMS: m/z $C_{31}H_{49}N_3O_4$ requires: 527.4, found: 528.4 [M+H]; t_R = 27.36 (99.2%, II).

3.1.4.44. (*R*)-3-Isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(decylamino)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9o). Prepared according to General Procedure C with **8o**, to give the title compound as a cream solid (58 mg, 99%): mp 139–141 °C; δ_H (DMSO-d_6, 400 MHz) 8.11 (t, J = 5.6, 1H, NH), 7.96 (d, J = 1.6, 1H, Ar), 7.92 (d, J = 8.4, 1H, Ar), 7.85 (dd, J = 8.4, 1.6, 1H, Ar), 4.44 (d, J = 16.8, 1H, C$H_a$$H_b$CONH), 4.28 (d, J = 16.8, 1H, C$H_a$$H_b$CONH), 3.60 (t, J = 6.8, 1H, CHCO), 3.08–3.03 (m, 2H, CH_2NH), 2.55 (s, 3H, CH_3CN), 1.95–1.88 (m, 1H, C*H*Me_2), 1.81–1.66 (m, 2H, CH_2iPr), 1.42–1.35 (m, 2H, Alk), 1.29–1.16 (m, 15H, Alk), 0.87 (d, J = 6.4, 3H, CH_3), 0.85 (t, J = 7.2, 3H, Alk), 0.73 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.3, 167.1, 166.1, 141.5, 134.2, 132.2, 128.7, 125.2, 123.5, 59.3, 50.9, 38.6,38.5, 31.3, 29.1, 29.0, 28.9, 28.8,28.7, 26.3, 24.6, 23.9, 23.1, 22.1, 21.8, 14.0; LRMS: m/z $C_{27}H_{41}N_3O_4$ requires: 471.3, found: 472.3 [M+H]; t_R = 5.93 (100%, I).

3.1.4.45. (*R*)-*tert*-Butyl 3-isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(hexadecylamino)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]-diazepine-8-carboxylate (8p). Prepared according to General Procedure B with **6d** and 2-bromo-*N*-hexadecylacetamide, to give the title compound as a yellow oil (82 mg, 90%): δ_H (DMSO-d_6, 400 MHz) 8.06 (t, J = 5.6, 1H, NH), 7.85 (s, 1H, Ar), 7.78 (d, J = 8.0, 1H, Ar), 7.74 (d, J = 8.0, 1H, Ar), 4.47 (d, J = 16.4, 1H, C$H_a$$H_b$CONH), 4.17 (d, J = 16.4, 1H, C$H_a$$H_b$CONH), 3.42–3.37 (m, 1H, CHCO), 3.09–3.03 (m, 2H, CH_2NH), 2.41 (s, 3H, CH_3CN), 1.93–1.86 (m, 1H, C*H*Me_2), 1.77–1.64 (m, 2H, CH_2iPr), 1.54 (s, 9H, 3 × CH_3), 1.42–1.36 (m, 2H, Alk), 1.30–1.15 (m, 27H, Alk), 0.86–0.82 (m, 5H, CH_3, Alk), 0.70 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.1, 167.1, 163.7, 141.1, 133.4, 127.6, 124.5, 122.7, 81.2, 79.2, 78.9, 78.6, 60.1, 50.5, 38.7, 31.3, 29.2, 29.1, 29.0, 28.8, 28.7, 27.6, 26.3, 25.0, 23.9, 23.2, 22.1, 21.7, 13.9;

LRMS: m/z $C_{37}H_{61}N_3O_4$ requires: 611.5, found: 612.4 [M+H]; t_R = 34.07 (100%, II).

3.1.4.46. (*R*)-3-isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(hexadecylamino)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9p). Prepared according to General Procedure C with **8p**, to give the title compound as a cream solid (66 mg, 99%): mp 96–98 °C; δ_H (DMSO-d_6, 400 MHz) 8.11 (t, J = 5.6, 1H, NH), 7.95 (d, J = 1.2, 1H, Ar), 7.91 (d, J = 8.0, 1H, Ar), 7.84 (dd, J = 8.0, 1.2, 1H, Ar), 4.44 (d, J = 16.4, 1H, CH_aH$_b$CONH), 4.27 (d, J = 16.4, 1H, CH$_a$$H_b$ CONH), 3.58 (t, J = 6.8, 1H, CHCO), 3.08–3.02 (m, 2H, CH_2NH), 2.54 (s, 3H, CH_3CN), 1.95–1.87 (m, 1H, CHMe$_2$), 1.81–1.66 (m, 2H, CH_2iPr), 1.38–1.35 (m, 2H, Alk), 1.27–1.17 (m, 27H, Alk), 0.86 (d, J = 6.4, 3H, CH_3), 0.84 (t, J = 7.2, 3H, Alk), 0.72 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 173.5, 172.3, 171.3, 146.7, 133.7, 130.3, 128.6, 122.3, 64.5, 56.1, 43.7, 36.5, 34.2, 34.1, 34.0, 33.9, 33.8, 31.4, 29.8, 29.1, 28.2, 27.3, 26.9, 19.1; LRMS: m/z $C_{33}H_{53}N_3O_4$ requires: 555.4, found: 556.4 [M+H]; t_R = 29.83 (95.4%, II).

3.1.4.47. (*R*)-*tert*-Butyl 3-isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(hexylamino)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8q). Prepared according to General Procedure B with **6d** and 2-bromo-*N*-hexylacetamide, to give the title compound as a white solid (61 mg, 87%): mp 66–68 °C; δ_H (DMSO-d_6, 400 MHz) 8.11 (t, J = 5.6, 1H, NH), 7.87–7.83 (m, 2H, Ar), 7.76 (d, J = 8.4, 1H, Ar), 4.47 (d, J = 17.2, 1H, CH_aH$_b$CONH), 4.22 (d, J = 17.2, 1H, CH$_a$$H_b$CONH), 3.45 (t, J = 6.8, 1H, CHCO), 3.08–3.04 (m, 2H, CH_2NH), 2.44 (s, 3H, CH_3CN), 1.93–1.86 (m, 1H, CHMe$_2$), 1.78–1.66 (m, 2H, CH_2iPr), 1.54 (s, 9H, 3 × CH_3), 1.40–1.36 (m, 2H, Alk), 1.27–1.19 (m, 7H, Alk), 0.86–0.84 (m, 5H, CH_3, Alk), 0.70 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.9, 167.1, 163.8, 141.3, 133.7, 133.2, 128.0, 124.6, 122.8, 81.5, 59.9, 50.5, 38.6, 30.9, 29.1, 27.6, 26.0, 24.9, 23.9, 23.2, 22.0, 21.8, 13.9; LRMS: m/z $C_{27}H_{41}N_3O_4$ requires: 471.3, found: 472.3 [M+H]; t_R = 6.05 (91.8%, II).

3.1.4.48. (*R*)-3-Isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(hexylamino)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9q). Prepared according to General Procedure C with **8q**, to give the title compound as a cream solid (52 mg, 99%): mp 130–132 °C; δ_H (DMSO-d_6, 400 MHz) 8.10 (t, J = 5.6, 1H, NH), 7.93 (d, J = 1.2, 1H, Ar), 7.91 (d, J = 8.4, 1H, Ar), 7.82 (dd, J = 8.4, 1.2, 1H, Ar), 4.42 (d, J = 16.4, 1H, CH_aH$_b$CONH), 4.26 (d, J = 16.4, 1H, CH$_a$$H_b$CONH), 3.61 (t, J = 6.4, 1H, CHCO), 3.04–3.00 (m, 2H, CH_2NH), 2.55 (s, 3H, CH_3CN), 1.92–1.84 (m, 1H, CHMe$_2$), 1.79–1.62 (m, 2H, CH_2iPr), 1.35–1.30 (m, 2H, Alk), 1.25–1.16 (m, 7H, Alk), 0.83 (d, J = 6.4, 3H, CH_3), 0.82 (t, J = 7.2, 3H, Alk), 0.71 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.1, 167.1, 166.1, 141.2, 134.5, 131.9, 128.9, 125.2, 123.6, 117.0, 114.1, 59.1, 50.9, 38.2, 31.0, 29.0, 25.9, 24.5, 23.9, 23.0, 22.0, 21.8, 13.9; LRMS: m/z $C_{23}H_{33}N_3O_4$ requires: 415.2, found: 416.3 [M+H]; t_R = 4.23 (98.3%, I).

3.1.4.49. (*R*)-*tert*-Butyl 1-(2-(4-(*tert*-butoxycarbonyl)piperazin-1-yl)-2-oxoethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8r). Prepared according to General Procedure B with **6d** and *tert*-butyl 4-(2-bromoacetyl) piperazine-1-carboxylate, to give the title compound as a white solid (55 mg, 81%): mp 128–130; δ_H (DMSO-d_6, 400 MHz) 7.84 (s, 1H, Ar), 7.78–7.71 (m, 2H, Ar), 4.82 (d, J = 16.4, 1H, CH_aH$_b$CO), 4.63 (d, J = 16.4, 1H, CH$_a$$H_b$CO), 3.55–3.20 (m, 9H, piperazine, CHCO), 2.41 (s, 3H, CH_3-CN), 1.92–1.83 (m, 1H, CHMe$_2$), 1.82–1.61 (m, 2H, CH_2iPr), 1.54 (s, 9H, 3 × CH_3), 1.41 (s, 9H, 3 × CH_3), 0.84 (d, J = 6.4, 3H, CH_3), 0.71 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.1, 165.8, 163.8, 153.8, 140.9, 133.8, 133.4, 127.8, 124.6, 122.5, 81.5, 79.2, 60.1, 49.0, 44.0, 41.3, 28.0, 27.7, 25.0, 24.0, 23.1, 21.9; LRMS: m/z $C_{30}H_{44}N_4O_6$ requires: 556.3, found: 557.3 [M+H]; t_R = 4.65 (97.7%, IV).

3.1.4.50. (*R*)-3-Isobutyl-5-methyl-2-oxo-1-(2-oxo-2-(piperazin-1-yl)ethyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9r). Prepared according to General Procedure C with **8r**, to give the title compound as a cream solid (52 mg, 99%): mp 69–71; δ_H (DMSO-d_6, 400 MHz) 8.88 (br s, 1H, NH), 7.93–7.81 (m, 3H, Ar), 4.86–4.72 (m, 2H, CH_2CO), 3.78–3.50 (m, 5H, piperazine, CHCO), 3.22–3.00 (m, 4H, piperazine), 2.50 (s, 3H, CH_3CN), 1.96–1.85 (m, 1H, CHMe$_2$), 1.80–1.62 (m, 2H, CH_2iPr), 0.85 (d, J = 6.4, 3H, CH_3), 0.71 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.7, 166.3, 166.2, 141.0, 133.6, 128.1, 125.3, 123.5, 117.3, 114.4, 59.6, 48.9, 42.7, 24.7, 24.0, 23.1, 21.9; LRMS: m/z $C_{21}H_{28}N_4O_4$ requires: 400.2, found: 401.2 [M+H]; t_R = 3.36 (94.0%, I).

3.1.4.51. (*R*)-*tert*-Butyl 1-(2-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-oxoethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8s). Prepared according to General Procedure B with **6d** and benzyl 4-(2-bromoacetyl)piperazine-1-carboxylate, to give the title compound as a white solid (82 mg, 77%): mp 109–111; δ_H (DMSO-d_6, 400 MHz) 7.81 (d, J = 8.8, 1H, Ar), 7.75 (d, J = 8.8, 1H, Ar), 7.74 (s, 1H, Ar), 7.42–7.29 (m, 5H, Cbz), 5.11 (s, 2H, Cbz), 4.83 (d, J = 17.2, 1H, CH_aH$_b$CO), 4.64 (d, J = 17.2, 1H, CH$_a$$H_b$CO), 3.57–3.34 (m, 9H, piperazine, CHCO), 2.41 (s, 3H, CH_3CN), 1.90–1.83 (m, 1H, CHMe$_2$), 1.80–1.64 (m, 2H, CH_2iPr), 1.53 (s, 9H, 3 × CH_3), 0.84 (d, J = 6.4, 3H, CH_3), 0.71 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.5, 166.3, 164.3, 154.9, 141.4, 137.2, 134.3, 133.8, 128.9, 128.3, 128.2, 128.1, 125.1, 123.0, 81.9, 66.9, 60.6,49.5, 44.3, 41.7, 28.1, 25.4, 24.4, 23.5, 22.3; LRMS: m/z $C_{33}H_{42}N_4O_6$ requires: 590.3, found: 591.3 [M+H]; t_R = 4.74 (98.4%, IV).

3.1.4.52. (*R*)-1-(2-(4-((Benzyloxy)carbonyl)piperazin-1-yl)-2-oxoethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9s). Prepared according to General Procedure C with **8s**, to give the title compound as a white solid (62 mg, 98%): mp 88–90; δ_H (DMSO-d_6, 400 MHz) 7.95–7.82 (m, 3H, Ar), 7.42–7.29 (m, 5H, Ar), 5.10 (s, 2H, CH_2Ph), 4.87–4.68 (m, 2H, CH_2CO), 3.64–3.26 (m, 9H, piperazine, CHCO), 2.54 (s, 3H, CH_3CN), 1.96–1.85 (m, 1H, CHMe$_2$), 1.80–1.62 (m, 2H, CH_2iPr), 0.85 (d, J = 6.4, 3H, CH_3), 0.73 (d, J = 6.4, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 166.2, 165.8, 154.4, 141.2, 136.7, 128.5, 127.9, 127.6, 125.3, 117.5, 66.4, 59.3, 49.2, 43.9, 41.3, 24.5, 23.9, 23.0, 21.9; LRMS: m/z $C_{29}H_{34}N_4O_6$ requires: 534.2, found: 535.1 [M+H]; t_R = 5.25 (90.1%, I).

3.1.4.53. (*R*)-*tert*-Butyl 1-(2-(4-(((9H-fluoren-9-yl)methoxy)carbonyl) piperazin-1-yl)-2-oxoethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (8t). Prepared according to General Procedure B with **6d** and (9*H*-fluoren-9-yl) methyl 4-(2-bromoacetylpiperazine-1-carboxylate, to give the title compound as a white solid (82 mg, 77%): mp 124–126; δ_H (DMSO-d_6, 400 MHz) 7.90 (d, J = 7.4, 2H, Fmoc), 7.84 (d, J = 8.0, 1H, Ar), 7.77–7.72 (m, 2H, Ar), 7.64 (d, J = 7.4, 2H, Fmoc), 7.42 (t, J = 7.4, 2H, Fmoc), 7.34 (t, J = 7.4, 2H, Fmoc), 4.82 (d, J = 17.2, 1H, CH_aH$_b$CO), 4.63 (d, J = 17.2, 1H, CH$_a$$H_b$CO), 4.42 (d, J = 6.0, 2H, Fmoc), 4.29 (t, J = 6.0, 1H, Fmoc), 3.55–3.20 (m, 9H, piperazine, CHCO), 2.41 (d, J = 0.8, 3H, CH_3CN), 1.92–1.83 (m, 1H, CHMe2), 1.82–1.61 (m, 2H, CH_2iPr), 1.54 (s, 9H, 3 × CH_3), 0.84 (d, J = 6.8, 3H, CH_3), 0.71 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.1, 167.1, 165.9, 163.9, 154.3, 143.8, 140.9, 140.8, 133.9, 133.4, 128.9, 127.8, 127.7, 127.2, 125.0, 124.6, 122.6, 120.2, 81.5, 66.6, 60.2, 49.0, 46.8, 27.7, 25.0, 24.0, 23.1, 21.9; LRMS: m/z $C_{40}H_{46}N_4O_6$ requires: 678.3, found: 679.2 [M+H]; t_R = 4.67 (98.5%, IV).

3.1.4.54. (*R*)-1-(2-(4-(((9H-Fluoren-9-yl)methoxy)carbonyl)piperazin-1-yl)-2-oxoethyl)-3-isobutyl-5-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (9t). Prepared according to General Procedure C with **8t**, to give the title

compound as a white solid (37 mg, 97%): mp 80–82; δ_H (DMSO-d_6, 400 MHz) 7.98–7.77 (m, 5H, Ar, Fmoc), 7.64 (d, J = 7.2, 2H, Fmoc), 7.42 (t, J = 7.2, 2H, Fmoc), 7.34 (t, J = 7.2, 2H, Fmoc), 4.82 (d, J = 17.2, 1H, CH_aH_bCO), 4.73 (d, J = 17.2, 1H, CH_aH_bCO), 4.41 (s, 2H, Fmoc), 4.29 (s, 1H, Fmoc), 3.64–3.58 (m, 1H, CHCO), 3.51–3.20 (m, 8H, piperazine), 2.55 (s, 3H, CH_3CN), 1.97–1.87 (m, 1H, $CHMe_2$), 1.82–1.64 (m, 2H, CH_2iPr), 0.86 (d, J = 6.0, 3H, CH_3), 0.74 (d, J = 6.8, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.3, 166.2, 165.8, 162.3, 154.3, 143.8, 141.2, 140.8, 128.5, 127.7, 127.2, 125.3, 125.0, 123.7, 120.1, 66.6, 59.3, 49.2, 46.8, 41.2, 35.8, 24.5, 23.9, 23.0, 21.9; LRMS: m/z $C_{36}H_{38}N_4O_6$ requires: 622.3, found: 401.2 [(M+H)−Fmoc]; t_R = 5.04 (91.0%, I).

3.1.4.55. *tert*-Butyl 4-methyl-3-nitrobenzoate (11). A round bottom flask equipped with a magnetic stirrer bar was charged with 4-methyl-3-nitobenzoic acid (2.30 mg, 12.70 mmol), *t*BuOH (1.45 mL, 15.24 mmol) and DMAP (50 mg, 0.03 mmol) in CH_2Cl_2 (60 mL). DCC (3.14 g, 15.24 mmol) was added and then the reaction was stirred at room temperature for 3 h. The reaction mixture was reduced in vacuo and then the residue was purified by column chromatography (hexane/EtOAc) to provide the title compound as a white solid (1.21 g, 40%): δ_H (DMSO-d_6, 500 MHz) 8.50 (d, J = 1.6, 1H, Ar), 8.07 (dd, J = 8.0, 1.6, 1H, Ar), 7.39 (d, J = 8.0, 1H, Ar), 2.63 (s, 3H, CH_3), 1.59 (s, 9H, 3 × CH_3).

3.1.4.56. *tert*-Butyl 4-formyl-3-nitrobenzoate (12)[24]. A round bottom flask equipped with a magnetic stirrer bar was charged with *tert*-butyl 4-methyl-3-nitrobenzoate (**11**; 237 mg, 1.00 mmol) in DMF (2 mL). *N*,*N*-Dimethylformamide dimethylacetal (172 μL, 1.30 mmol) was added and the reaction mixture was heated to 140 °C for 5 h, then cooled and reduced in vacuo. The residue was dissolved in 50% THF in H_2O (6 mL), $NaIO_4$ (642 mg, 3.00 mmol) was added and allowed to stir at room temperature for 2 h. The reaction mixture was decanted with CH_2Cl_2, washed with H_2O, brine (10 mL), dried over $MgSO_4$, filtered and reduced in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc) to provide the title compound as a cream solid (100 mg, 40%): δ_H ($CDCl_3$, 400 MHz) 10.46 (s, 1H, CHO), 8.67 (s, 1H, Ar), 8.36 (d, J = 8.0, 1H, Ar), 7.99 (d, J = 8.0, 1H, Ar), 1.64 (s, 9H, 3 × CH_3).

3.1.4.57. *tert*-Butyl 4-benzoyl-3-nitrobenzoate (13). A round bottom flask equipped with a magnetic stirrer bar was charged with *tert*-butyl 4-formyl-3-nitrobenzoate (**12**; 277 mg, 1.10 mmol) in THF (20 mL). The reaction was cooled to 0 °C and 1 M phenylmagnesium bromide (1.32 mL, 1.32 mmol) was added and stirred at 0 °C for 3 h. The reaction was quenched with saturated aqueous NH_4Cl (1 mL), partitioned between EtOAc (60 mL) and H_2O (30 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL) and the organic portions combined, washed with H_2O (10 mL), brine (10 mL), dried over $MgSO_4$, filtered and reduced in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc) to provide a yellow oil that was subsequently dissolved in acetone (20 mL), 2.6 M Jones Reagent (634 μL, 1.65 mmol) was added at 0 °C and the mixture was stirred for 2 h. The reaction was filtered through Celite, reduced in vacuo, and then purified by column chromatography (hexane/EtOAc) to provide the title compound as a yellow solid (292 mg, 81% (two steps)): δ_H ($CDCl_3$, 400 MHz) 8.79 (d, J = 1.2, 1H, Ar), 8.37 (dd, J = 8.0, 1.2, 1H, Ar), 7.23 (d, J = 7.6, 2H, Ar), 7.61 (t, J = 7.6, 1H, Ar), 7.55 (d, J = 8.0, 1H, Ar), 7.46 (t, J = 7.6, 2H, Ar), 1.65 (s, 9H, 3 × CH_3).

3.1.4.58. *tert*-Butyl 3-amino-4-benzoylbenzoate (14). A round bottom flask equipped with a magnetic stirrer bar was charged with **13** (47 mg, 0.14 mmol) in MeOH (3 mL). Pd/C (10 mg) was added and the reaction placed under 1 atm of H_2 gas (balloon) for 1 h. The reaction mixture was filtered through Celite, reduced in vacuo and the residue was purified by silica gel column chromatography (hexane/EtOAc) to provide the title compound as a yellow oil (40 mg, 95%): δ_H ($CDCl_3$, 400 MHz) 7.63 (d, J = 7.2, 2H, Ar), 7.53 (t, J = 7.2, 1H, Ar), 7.49–7.41 (m, 3H, Ar), 7.35 (s, 1H, Ar), 7.16 (d, J = 8.0, 1H, Ar), 6.04 (br s, 2H, NH_2), 1.58 (s, 9H, 3 × CH_3).

3.1.4.59. (*R*)-*tert*-Butyl 1-(4-cyclohexylbenzyl)-3-isobutyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylate (15). Prepared according to General Procedure A and B, to give the title compound as a yellow solid (43 mg, 99%): mp 93–95 °C; δ_H (DMSO-d_6, 400 MHz) 8.07 (s, 1H, Ar), 7.69 (d, J = 7.6, 1H, Ar), 7.51 (t, J = 7.6, 1H, Ar), 7.41 (t, J = 7.6, 2H, Ar), 7.33–7.26 (m, 3H, Ar), 6.99 (d, J = 7.6, 2H, Ar), 6.91 (d, J = 7.6, 2H, Ar), 5.30 (d, J = 15.6, 1H, CH_aH_bAr), 4.99 (d, J = 15.6, 1H, CH_aH_bAr), 3.69–3.65 (m, 1H, CHCO), 2.44–2.34 (m, 1H, Cy), 2.17–2.11 (m, 1H, $CHMe_2$), 1.95–1.60 (m, 7H, CH_2iPr, Cy), 1.52 (s, 9H, 3 × CH_3), 1.38–1.12 (m, 5H, Cy), 0.95 (d, J = 5.6, 3H, CH_3), 0.78 (d, J = 5.6, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 169.4, 167.6, 164.2, 146.9, 142.4, 138.3, 135.0, 134.3, 133.5, 131.0, 130.3, 129.5, 128.7, 127.3, 127.2, 125.1,123.8, 82.1, 61.7, 49.8, 43.7, 34.4, 34.2, 28.1, 26.8, 26.7, 26.0,24.7, 23.8, 22.4; LRMS: m/z $C_{37}H_{44}N_2O_3$ requires: 564.3, found: 565.3 [M+H]; t_R = 19.01 (100%, V).

3.1.4.60. (*R*)-1-(4-Cyclohexylbenzyl)-3-isobutyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepine-8-carboxylic acid (16). Prepared according to General Procedure C to give the title compound as a yellow solid (43 mg, 99%): mp 104–105 °C; δ_H (DMSO-d_6, 400 MHz) 8.17 (s, 1H, Ar), 7.73 (d, J = 7.6, 1H, Ar), 7.51 (t, J = 7.2, 1H, Ar), 7.40 (t, J = 7.2, 2H, Ar), 7.30–7.21 (m, 3H, Ar), 6.94 (d, J = 7.6, 2H, Ar), 6.84 (d, J = 7.6, 2H, Ar), 5.47 (d, J = 15.6, 1H, CH_aH_bAr), 4.90 (d, J = 15.6, 1H, CH_aH_bAr), 3.67–3.64 (m, 1H, CHCO), 2.40–2.32 (m, 1H, Cy), 2.19–2.05 (m, 1H, $CHMe_2$), 1.95–1.60 (m, 7H, CH_2iPr, Cy), 1.38–1.09 (m, 5H, Cy), 0.94 (d, J = 5.6, 3H, CH_3), 0.77 (d, J = 5.6, 3H, CH_3); δ_C (DMSO-d_6, 100 MHz) 168.8, 167.3, 166.3, 146.4, 141.7, 137.9, 134.3, 133.5, 133.3, 130.5, 129.8, 129.0, 128.2, 127.0, 126.7, 125.1, 123.9, 61.2, 48.9, 43.2, 34.0, 33.7, 26.3, 25.5, 24.3, 23.3, 22.0; LRMS: m/z $C_{33}H_{36}N_2O_3$ requires: 508.3, found: 509.3 [M+H]; t_R = 27.29 (100%, II).

3.2. HIV-1PR assay

3.2.1. Materials

Recombinant HIV-1PR was prepared from *Escherichia coli* inclusion bodies and purified according to previously published procedures.[26–29] Stock solutions (20 mM) of inhibitors were prepared in 100% DMSO. The catalytic activities of the HIV-1PR were monitored by the hydrolysis of the chromogenic substrate Lys-Ala-Arg-Val-Nle-nPhe-Glu-Ala-Nle-NH_2, where Nle stands for norleucine and nPhe stands for 4-nitrophenylalanine (California Peptide Research, Napa, CA), and the fluorogenic substrate Arg-Glu (EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DABCYL)-Arg (Molecular Probes).

3.2.2. Determination of kinetic parameters

Inhibition constants, K_i, for the inhibitors were obtained at 25 °C by measuring the rate of fluorogenic substrate hydrolysis using final concentrations of 30–40 nM HIV-1 protease in 10 mM sodium acetate, 1 M sodium chloride, 2% DMSO, pH 5.0, and 16.7 μM substrate at different inhibitor concentrations in the range of 0–200 μM. Inhibition constants were obtained by fitting the data to standard equations for tight-binding competitive inhibitors.

Kinetic parameters, K_m, k_{cat}, and V_{max} were determined by initial rate measurements at 25 °C. HIV-1 protease was added to

a 120-μl microcuvette containing substrate at 25 °C. Final concentrations in the assay were: 30–40 nM active HIV-1 protease, 0–255 μM substrate, 10 mM sodium acetate, 1 M sodium chloride, and 2% DMSO, pH 5.0. In the spectrophotometric assay, the absorbance was monitored at 300 nm by using a Cary 100 spectrophotometer (Varian Instruments, Palo Alto, CA, USA). An extinction coefficient for the difference in absorbance upon hydrolysis ($1800\ M^{-1}\ cm^{-1}$ at 300 nm) was used to convert absorbance change to reaction rates.[27] Hydrolysis rates were obtained from the initial portion of the data, where at least 80% of the substrate remains unhydrolyzed. In the spectrofluorometric assay, the fluorescence was monitored by using a Cary Eclipse fluorescence spectrophotometer (Instruments, Palo Alto, CA, USA) with excitation and emission wavelengths of 340 nm and 490 nm, respectively. The concentration of active protease was determined by performing active site titrations with KNI-727, a very potent inhibitor (at pH 5.0, $K_i \approx 1.4$ nM), using protease concentrations much higher (≈2 μM) than the corresponding K_i.

3.3. Cell assays

3.3.1. Cells and viruses

TZM-bl cells were a gift from Dr. Eric O. Freed (National Cancer Institute, Frederick, MD) and MT-4 cells were obtained from the NIH AIDS Reagent Program (Germantown, MD). Infected and uninfected cells were maintained in DMEM or RPMI, respectively, containing 10% heat-inactivated fetal bovine serum and 1× penicillin–streptomycin, at 37 °C with 5% CO_2. Replication-competent HIV was generated by transfection of 293T cells using the pNL4-3 proviral sequence. The inhibitor compounds were initially resuspended in DMSO then diluted in the appropriate media, resulting in a final carrier DMSO concentration of 0.25%.

3.3.2. Endpoint cytotoxicity assays

Cytotoxicity assays were performed in triplicate on MT-4 cells, which were seeded at 2×10^5 cells/ml into 24 well plates in media containing the indicated concentration of inhibitor (carrier only, 10 μM, 50 μM, or 100 μM). After three days, the number of viable cells in each well was determined by trypan-blue dye exclusion.

3.3.3. Viral replication inhibition assays

TZM-bl cells were seeded onto 96 well plates at 1×10^4 cells/mL in 200 μL and incubated overnight. After 24 h, the media was removed and diluted virus (MOI = 0.01) or mock was added to the cells in media containing either the carrier or inhibitor (final concentrations of 25 μM, 50 μM, or 100 μM). All conditions were done in triplicate.

At the indicated days post infection, the cells were washed once with 1x phosphate-buffered saline and then luciferase production was measured with the Britelite™plus Reporter Gene Assay System (Perkin-Elmer), according to manufacturer's instructions. Plates were read on the Centro XS3 LB 960 Microplate Luminometer from Berthold Technologies and recorded with the MikroWin 2000 software.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.09.002.

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