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Synthesis and evaluation of unsaturated caprolactams as interleukin-1β converting enzyme (ICE) inhibitors

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Abstract—Peptidomimetic compounds possessing a caprolactam ring constraint were prepared and evaluated as interleukin-1β converting enzyme (ICE) inhibitors. The caprolactam ring was used to constrain the P3 region of our inhibitors. This strategy proved to be effective for the synthesis of ICE inhibitors, maintaining key hydrogen bond interactions with the enzyme and invoking a preferred conformation for binding. Several compounds exhibited IC_{50} values less than 10 nM in a caspase-1 enzyme assay and less than 100 nM in a THP-1 whole cell assay measuring IL-1β production. Two compounds, **13c** and **13j**, were found to have good oral bioavailability (>50%) in rats when administered as prodrugs.

1. Introduction

In recent years, much attention has been given to interleukin-1β (IL-1β), a cytokine known to play an important role in a variety of inflammatory and autoimmune diseases, including rheumatoid arthritis (RA), osteoarthritis (OA), arthrosclerosis, septic shock, and inflammatory bowel syndrome.[1] Interleukin-1β converting enzyme (ICE) is responsible for cleaving an inactive 31 kDa precursor (pro-IL-1β) to release the active 17.5 kDa mature cytokine, IL-1β.[2] Therefore, inhibition of ICE offers an attractive therapeutic target for controlling IL-1β levels, potentially providing an effective means of treating a variety of diseases.

ICE (caspase-1) is a member of the caspase family of cysteine proteases and requires an aspartic acid residue at P1 for substrate recognition.[3] Numerous ICE inhibitors have been reported and most possess an aspartate recognition element that functions as a cysteine trap. Among them, the tetra-peptide Ac-YVAD-CHO (**1**) was reported to block the release of mature IL-1β from human whole blood stimulated with heat-killed *Staphylococcus aureus* with an IC_{50} of 4 μM.[4] From the X-ray crystallographic study of **1**, it is known that the tetrapeptide makes a reversible covalent bond with Cys285 of the enzyme while making several other key ionic and hydrogen bond interactions (Fig. 1).[5] Unfortunately, **1** was found to be poorly suited for therapeutic uses due to its peptidic nature.[6]

Pralnacasan® is one of the most studied ICE inhibitors to date.[7] It possesses a peptidomimetic bicyclic core which constrains the P2–P3 region to hold the inhibitor in a preferred conformation for binding. Pralnacasan® is a prodrug of the active species **2**, permitting the compound to be dosed orally.[8] We have previously reported that 8,5 and 8,6-fused bicyclic ring systems, **3** and **4**, were effective as P2–P3 constraints for ICE inhibition.[9,10] Unfortunately, the synthesis of these bicyclic inhibitors is rather lengthy, prompting us to explore monocyclic scaffolds such as **5** for ICE inhibition.[11]

2. Chemistry

Ring closing olefin metathesis (RCM) was selected as the most efficient means of synthesizing these monocyclic

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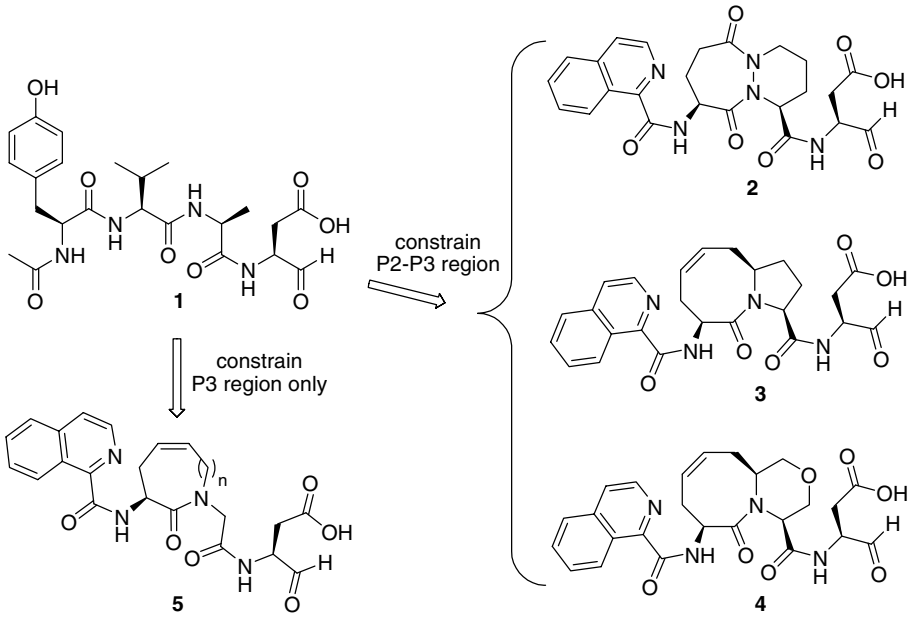


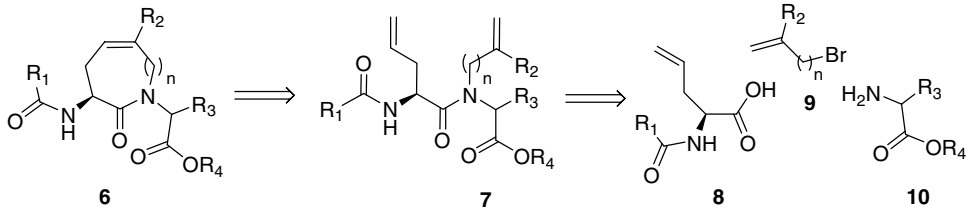
Figure 1.

peptidomimetic ring systems. The application of RCM to the synthesis of medium-sized rings and lactams in particular is well precedented.[12] In fact, the parent peptidomimetic scaffolds exploited here for ICE inhibition were first synthesized by RCM and we elected to follow an identical approach for the synthesis of our inhibitors.[13] The synthesis was carried out according to the general retrosynthesis depicted in Scheme 1. The metathesis precursor **7** can be rapidly assembled from readily attainable starting materials (**8**–**10**) by sequential and straightforward N-alkylation and acylation steps. Metathesis of **7** provides an efficient route to **6** which can be elaborated to a diverse set of inhibitors.

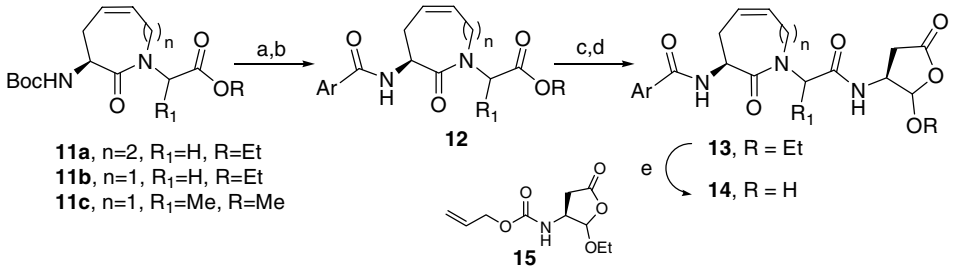
Inhibitors possessing no substitution on the olefin of the lactam ring (**14**) were synthesized from known scaffolds according to the sequence outlined in Scheme 2. Elaboration of **11** to desired inhibitors **14** began with the deprotection of the Boc-protected amine of **11**. This was achieved by treatment of **11** with TFA in CH_2Cl_2. The crude amines were converted to a variety of aryl amides through either carbodiimide-mediated couplings with the required carboxylic acid or by reaction of the crude amine with commercially available acid chlorides. Once the P4 aryl amide was introduced, the esters (**12**) were converted to the corresponding carboxylic acids upon hydrolysis with lithium hydroxide in THF/H_2O.

The aspartic acid aldehyde residue that serves as a cysteine trap in the enzyme was introduced utilizing intermediate **15**.[14] The free amine of **15** was generated in situ upon treatment with dimethylbarbituric acid and $Pd(Ph_3P)_4$ in CH_2Cl_2 at room temperature. Once deprotection of **15** was complete (typically <15 min), the carboxylic acid, EDAC, and HOBt were added. The coupling was generally complete within 2 h. The initially isolated crude product (**13**) could be purified by chromatography on silica gel, but was generally carried forward as crude. Compound **13** was treated with TFA in CH_3CN/H_2O for 3 h to form our final inhibitors **14** which were isolated and purified by preparative reverse phase HPLC.

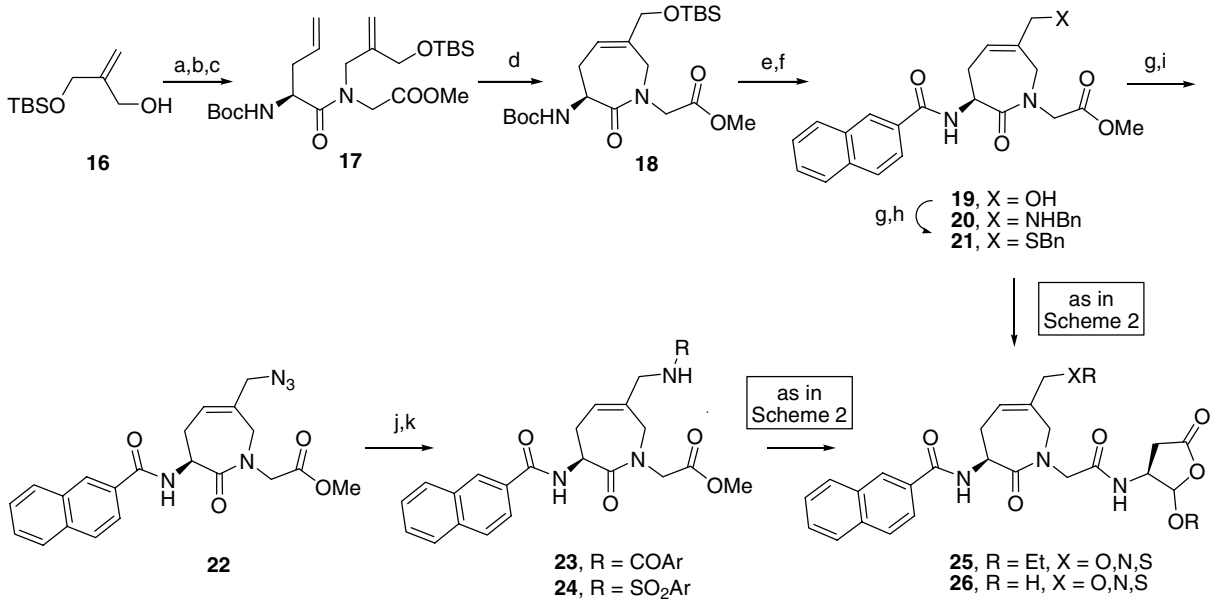
Compounds possessing substitution on the olefin of the lactam ring were prepared in an analogous manner, (Scheme 3) using a substituted allylbromide prepared from **16**.[15] The RCM precursor **17** was readily prepared on large scale and underwent metathesis cyclization smoothly in refluxing CH_2Cl_2 to yield the key intermediate **18** in 89% yield. Simultaneous deprotection of both the Boc and TBS groups of **18** was achieved by treatment of **17** with TFA in CH_2Cl_2. The resulting crude amine was acylated to obtain compound **19**. The allylic alcohol in **19** was converted to a mesylate and directly displaced with nucleophiles. Displacement of the mesy-



Scheme 1.



Scheme 2. Reagents and conditions: (a) TFA, CH_2Cl_2, rt; (b) ArCOCl, Et_3N, CH_2Cl_2, rt; or $ArCO_2H$, EDAC, HOBt, CH_2Cl_2; (c) LiOH, THF/H_2O, rt; (d) **15**, $Pd(Ph_3P)_4$, *N*,*N*-dimethylbarbituric acid, HOBt, EDAC, CH_2Cl_2/DMF; (e) TFA, CH_3CN/H_2O, rt.



Scheme 3. Reagents and conditions: (a) Ph_3P, NBS, CH_2Cl_2, −78 °C, 39%; (b) Glycine methyl ester hydrochloride, Et_3N, DMF, 0 °C to rt, 43%; (c) *N*-Boc-L-allylglycine, HOBt, EDAC, CH_2Cl_2, rt, 57%; (d) 2nd generation Grubb's catalyst,[16] CH_2Cl_2, 40 °C, 96%; (e) TFA/CH_2Cl_2, rt; (f) ArCOCl, Et_3N, CH_2Cl_2, rt, 89% (two steps); (g) $MeSO_2Cl$, Et_3N, CH_2Cl_2, −78 °C; (h) RNH_2, CH_2Cl_2, rt, 51% (two steps) or RSH, Et_3N, CH_2Cl_2, rt, 58% (two steps); (i) NaN_3, DMF, 50 °C, 83%; (j) Ph_3P, THF/H_2O; (k) PhCOCl or $PhSO_2Cl$, Et_3N, THF, 25%.

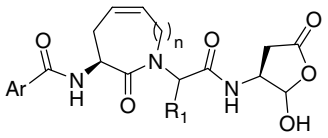
late with primary amines and thiols resulted in the formation of secondary amines (**20**) and thio ethers (**21**) which were converted to the desired inhibitors as described previously in Scheme 2. Alternatively, the mesylate of **19** was also converted to azide **22** upon treatment with NaN_3 in DMF. The azide was reduced to the corresponding amine and either acylated or sulfonylated to yield **23** and **24**, respectively. Intermediates **23** and **24** were also further elaborated to final inhibitors via the sequence outlined in Scheme 2.

3. Results and discussion

To properly evaluate the monocyclic scaffolds with respect to previously explored bicyclic scaffolds, we first synthesized and tested both the eight-membered (**14a**) and seven-membered (**14b**) lactams possessing a P4 isoquinolyl carboxamide identical to the bicyclic inhibitors, **2**–**4**. Compound **14b**, possessing a seven-membered lactam, proved to be about 3-fold more potent than **14a**, but was about 7-fold less potent than any of the bicyclic scaffolds that we had previously explored in the ICE enzyme assay (Table 1). Interestingly, when **14b** was tested in a THP-1 whole cell assay measuring IL-1β production, it was only 3-fold less active than either **3** or **4**. Though the unsaturated seven-membered monocyclic lactam resulted in some loss of activity with respect to the bicyclic inhibitors, it maintained enough potency to be a reasonable starting point for further optimization. We chose to explore SAR in three synthetically feasible regions of the molecule: (1) P4 variations, (2) P2 modifications, and (3) P3 modification to the lactam olefin.

We had previously established extensive P4 SAR for related bicyclic scaffolds indicating a preference for hydrophobic fused bicyclic aromatic amides and meta-substituted benzamides.[9] We synthesized several compounds possessing what we believed to be the better performing P4 substituents and observed modest improvements in potency with respect to **14b**, as measured by the ICE assay. Both of the fused bicyclic aromatic amides **14c** and **14d** proved to be quite potent

Table 1. Ring constraint, P2, and P4 SAR



Compound	Ar	n	R1	ICE (IC_{50}, nM)	Caspase-3 (IC_{50}, nM)	Caspase-8 (IC_{50}, nM)	THP-1 (IC_{50}, nM)
2	—	—	—	4	—	—	—
3	—	—	—	4	—	—	188
4	—	—	—	3	—	—	182
14a	1-Isoquinolinyl	2	H	77	—	—	—
14b	1-Isoquinolinyl	1	H	27	$>10^4$	2935	586
14c	2-Naphthyl	1	H	14	$>10^4$	2160	100
14d	2-Benzothiophene	1	H	20	$>10^4$	1451	712
14e	Ph	1	H	168	$>10^4$	4196	1078
14f	3-OMePh	1	H	53	$>10^4$	1231	1231
14g	3-CF_3Ph	1	H	11	$>10^4$	1957	554
14h	3-ClPh	1	H	29	$>10^4$	1184	383
14i	2,5-DiClPh	1	H	15	$>10^4$	554	71
14j	2-Naphthyl	1	Me(L)	1	7510	162	36
14k	2-Naphthyl	1	Me(D)	13	$>10^4$	537	871

with IC_{50}s = 14 nM and 20 nM, respectively. Additionally, two benzamide P4 substitutions, **14g** and **14i**, also exhibited IC_{50}s less than 20 nM. Although the enzyme potency for these compounds did not rival that of the bicyclic scaffolds, we were encouraged by compounds **14c** and **14i** which both proved to be more potent than either **3** or **4** in the THP-1 whole cell assay at 100 nM and 71 nM, respectively.

To examine how the P2 residue affects potency, compounds **14j** and **14k** were synthesized and directly compared with **14c**. Compound **14j**, possessing an L-alanine at P2, exhibited a significant increase in potency over **14c** in the enzyme assay (IC_{50} = 1 nM). The D-alanine isomer, **14k**, exhibited no increase in potency. When **14j** was tested in the THP-1 whole cell assay, we observed an IC_{50} = 36 nM. This level of whole cell potency was encouraging, because it represented a 5-fold improvement over the synthetically more complicated bicyclic scaffolds **2** and **3**.

We approximated the conformation of our lactam scaffold from a published crystal structure of a saturated caprolactam[17] and subsequently modeled the 2-naphthyl amide group at P4 and the P1 aspartic acid moiety to overlay on the X-ray structure of **1** in its active conformation (Fig. 2a).[18] This modeled compound was then docked into the enzyme active site (Fig. 2b). It was clear from the docked structure that the methyl group of an L-alanine at P2 possessed a minimal steric interaction with the enzyme in the S2 pocket, relative to the methyl group of the D-alanine analog, where significant interaction with the back of the pocket appeared plausible. This difference between D- and L-alanine in the modeled structure correlated well with our observed potencies for **14j** and **14k**, potentially providing a possible explanation as to why the L-alanine was better than D-alanine at the P2 position of our inhibitors.[19]

In an attempt to identify additional binding pockets in the enzyme, specifically in the P3 region, compounds **26a–i** were synthesized and compared directly with the unsubstituted lactam **14c** (Table 2). We installed a variety of functionalities on the olefin using the chemistry previously described in Scheme 3. Nearly all of the substitutions explored resulted in compounds equal to or

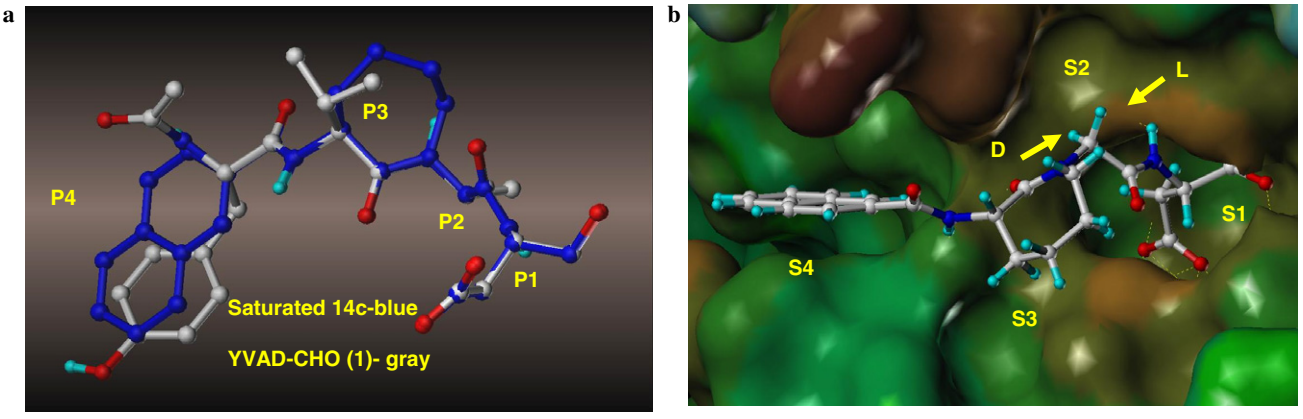
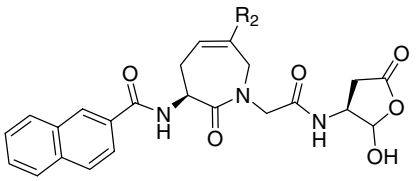


Figure 2. (a) Comparison of conformations of YVAD-CHO (**1**) with saturated caprolactam of **14c** scaffold, based on X-ray crystal data. (b) Model of caprolactam from (a) docked into ICE active site.

Table 2. P3 SAR



Compound	R_2	ICE (IC_{50}, nM)	Caspase-3 (IC_{50}, nM)	Caspase-8 (IC_{50}, nM)	THP-1 (IC_{50}, nM)
14c	H	14	$>10^4$	2160	100
26a[a]	Me	19	$>10^4$	2688	153
26b	CH_2OH	22	$>10^4$	2110	543
26c	CH_2SBn	8	$>10^4$	1606	1284
26d	CH_2NHBn	7	$>10^4$	10^4	232
26e	CH_2NHSO_2Ph	3	6810	109	326
26f	$CH_2NHCOPh$	2	1698	1085	466
26g	CH_2NHCO–2-OMePh	2	3372	887	1339
26h	CH_2NHCO–3-OMePh	1	1079	448	243
26i	CH_2NHCO–4-OMePh	1	1128	471	95

[a] Compound **26a** can be obtained through the same chemistry described in Scheme 2.

Table 3. PK results

Compound	Route	C_{max} (ng/mL)	$t_{1/2}$ (h)	$AUC_{0\rightarrow\infty}$[a] (h ng/mL)	% F[b]
14c	iv	6790	0.2	1970	—
14c	po	264	1.3	320	2
13c	po	9360	3.4	21000	>90
13j	po	720	5.1	4530	—
25i	po	9	7.1	30	—

[a] Values are based on the amount of free drug measured in the plasma.
[b] The oral bioavailability (% F) was calculated as the dose-normalized AUC_{PO}/AUC_{IV}.

more potent than **14c** in the caspase-1 enzyme assay, leading us to believe we may have identified a promising pocket for improving the potency of our inhibitors. A broad range of functional groups were well tolerated at this position, including hydroxyls (**26b**), thioethers (**26c**), amines (**26d**), sulfonamides (**26e**), and amides (**26f–i**). Unfortunately, these increases in enzyme potency were, in most cases, not accompanied by a corresponding increase in whole cell potency. In fact, all but one compound (**26i**) resulted in losses in whole cell potency. Compound **26i** was identified as a promising lead with an enzyme $IC_{50} = 1$ nM and a whole cell potency of 95 nM.

In addition to measuring ICE and whole cell potency, we also measured the selectivity of our inhibitors against caspase-3 and caspase-8 (Tables 1 and 2). We generally observed little or no activity against caspase-3 with only our most potent compounds (**14j** and **26g–i**) showing modest (>1000 nM) activity. We did however observe some activity against caspase-8, but generally found most of our inhibitors to be several hundred fold selective for caspase-1 over caspase-8.

Next, we decided to further evaluate a few of our better performing compounds (**14c**, **14j**, and **26i**) in a single dose pharmacokinetic study in male Sprague–Dawley rats. It is well documented that compound **2**, the active form of Pralnacasan®, is not orally bioavailable.[8] Instead, Pralnacasan® is administered as a prodrug to obtain acceptable oral bioavailability. We elected to test **14c** to see if the active form of our inhibitors suffered from the same problem. As we anticipated, compound **14c** was found to be only 2% orally bioavailable. With this result, we elected to evaluate the oral bioavailability of our compounds utilizing a prodrug strategy analogous to that reported for **2**. Prodrugs **13c** and **13j** exhibited very good PK parameters after oral administration (Table 3), demonstrating high plasma levels of free drug and a good half-life. However, with prodrug **25i** we observed very little of the active form (**26i**) in plasma. No additional studies were done to try to explain why **25i** was not orally bioavailable, but we hypothesized that this observation may be attributed to this compound's significantly larger molecular weight (629 vs 466 for **13c**).[20]

4. Conclusion

In summary, we have synthesized and evaluated both seven-membered and eight-membered monocyclic lactams as peptidomimetic constraints for ICE inhibition and found the seven-membered lactam to be more potent. We subsequently optimized the seven-membered lactam, exploring SAR in the P2, P3, and P4 regions of the inhibitors. These SAR investigations resulted in 14 compounds exhibiting enzyme IC_{50}s less than 20 nM. Additionally, three compounds (**14c**, **14j**, and **26i**) were identified with good whole cell potency ($IC_{50} < 100$ nM)

and were evaluated in pharmacokinetic studies. Two compounds (**14c** and **14j**) were identified as having good oral bioavailability (>50%) when administered as prodrugs (**13c** and **13j**, respectively). Further in vivo evaluation of these compounds will be disclosed elsewhere.

5. Experimental

5.1. General procedures

^{1}H NMR spectra were recorded on a Varian Unity Plus 300 MHz spectrometer and are referenced to either the $CDCl_3$ singlet at 7.27 ppm or the CD_3OD singlet at 4.87 ppm. ^{13}C spectra were recorded on a Varian Unity Plus 300 MHz spectrometer and are referenced to the residual protonated solvent ($CHCl_3$ or CH_3OH). Mass spectra were obtained on either a Fison Platform-II Quadrupole Mass Spectrometer or a Fison Trio2000 Quadrapole Mass Spectrometer. High-resolution mass spectra were obtained from the Procter and Gamble Pharmaceuticals Mass Spectrometry Lab (B. Regg). All solvents were purchased anhydrous (Aldrich Chemical) and used without further purification. All air-sensitive reactions were performed under an anhydrous nitrogen atmosphere. Flash chromatography was performed on silica gel (70–230 mesh; Aldrich). Thin-layer chromatography analysis was performed on glass-mounted silica gel plates (250 μm Analtech) and visualized using UV, iodine, or $KMnO_4$ in 5% NaOH.

5.2. Inhibition of ICE, caspase-3, and caspase-8 enzymes

The isolated enzyme (ICE, caspase-3, and caspase-8) assays were performed in a 96-well format using fluorogenic substrates, enzymes and control peptide inhibitors purchased from BioMol Research Laboratories (Plymouth Meeting, PA).[21] The assay was conducted according to the manufacturer's instructions. Enzyme inhibition was monitored over 30 min at 37 °C by measuring fluorescence using a BMG Fluostar plate reader (excitation filter 390 nm, emission filter 460 nm). IC_{50} values were calculated based on the equation $IC_{50} = [I]/(V_o/V_i) - 1$, where V_i is the initial velocity of substrate cleavage in the presence of inhibitor at concentration [I], and V_o is the initial velocity in the absence of inhibitor.

5.3. Inhibition of IL-1β production in a cell-based assay

A suspension of human monocytic cells (THP-1, ATCC strain TIB202, 2×10^6/mL in RPMI 1640 medium from Gibco BRL) was plated in 96-well plates, incubated with or without compounds (administered as solutions in DMSO, such that test concentrations ranged from 1 nM to 10 μM) for 15 min, and then stimulated with LPS (1 μg/mL) for a total of 4 h. Cells were centrifuged and the conditioned media was collected to quantify the release of IL-1β by an ELISA measurement according to the manufacturer's instructions (R&D Systems, catalog number DLB50) or stored at −20 °C for future use.

5.4. Pharmacokinetic analysis and oral bioavailability

Preliminary pharmacokinetic studies were conducted utilizing male Sprague–Dawley rats approximately 8–12 weeks old and weighing 225–300 g. A 40% hydroxypropyl-β-cyclodextrin solution containing test compound was administered at the dose level of 30 mg/kg and volume of 10 mL/kg to two animals via oral gavage. Blood samples were collected using the Culex automated blood collection system at time points: 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h. The blood samples were processed and the plasma frozen at −75 °C until analyzed. A pharmacokinetic analysis of the plasma concentration–time data was conducted using non-compartmental techniques.

5.5. General procedure for the synthesis of compounds 13 and 14

Compounds **11a**–**c** were prepared according to literature methods [13] and were treated with excess TFA in CH_2Cl_2. The reaction mixtures were stirred for 30 min to 1 h at room temperature and were subsequently concentrated under reduced pressure. The crude residue remaining was treated with saturated $NaHCO_3$ and extracted with EtOAc (3×). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. To the crude amine in CH_2Cl_2 were added acid chloride (2.5 equiv) and Et_3N (5 equiv). After the reaction mixture was stirred at room temperature for 10 min, MeOH was added to quench the reaction. Removal of the solvent under reduced pressure followed by flash chromatography on silica gel (EtOAc/hexane) yielded esters **12**. The esters were treated with excess LiOH in 3:1 THF/H_2O and stirred at room temperature until the reaction was complete. The reaction solution was neutralized with 1 N HCl and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield the crude carboxylic acids that were used without additional purification. Catalytic $Pd(Ph_3P)_4$ and *N*,*N*-dimethylbarbituric acid (6 equiv) were added to a solution of **15** (1 equiv) in CH_2Cl_2 at room temperature. The solution was stirred at room temperature for 15 min and then the carboxylic acid prepared above was added as a solution in CH_2Cl_2, followed by HOBt (2.3 equiv) and EDAC (2.3 equiv). The solution was stirred for 3 h at room temperature, diluted with EtOAc, washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by flash chromatography on silica gel to yield compounds **13a**–**k**, which were then further hydrolyzed by treatment with TFA in CH_3CN/H_2O. Purification of the final compounds was carried out by preparative reverse phase HPLC (CH_3CN/H_2O with 0.1% TFA) to give pure **14a**–**k** as a mixture of isomers at the acetal stereocenter.

5.5.1. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Ethoxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (13c). ^{1}H NMR (CD_3OD 300 MHz) δ 8.46 (s, 1H), 8.10–7.94 (m, 4H), 7.74–7.56 (m, 3H), 7.03 (d, J = 7.2 Hz, 0.5H, N–H proton), 6.87

(d, J = 7.5 Hz, 0.5H, N–H proton), 5.94–5.91 (m, 2H), 5.54 (d, J = 5.4 Hz, 0.5H), 5.49–5.42 (m, 1H), 5.42 (d, J = 1.5 Hz, 0.5H), 4.80–4.62 (m, 2H), 4.39–2.36 (series multiples, 9H), 1.26 (t, J = 6.9 Hz, 0.5 × 3H), 1.23 (t, J = 7.2 Hz, 0.5 × 3H); MS 465.7 $(M+H)^+$; HRMS Calcd for $C_{25}H_{28}N_3O_6$ $(M+H)^+$ 466.1978. Found: 466.1963.

5.5.2. *N*-((*S*,*Z*)-1-((*S*)-1-((3*S*)-2-Ethoxy-5-oxo-tetrahydrofuran-3-ylamino)-1-oxopropan-2-yl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (13j). ^{1}H NMR (CD_3OD 300 MHz) δ 8.46 (s, 1H), 8.09–7.94 (m, 4H), 7.79 (d, J = 6.6 Hz, 1H), 7.69–7.61 (m, 2H), 6.77 (d, J = 7.5 Hz, 1H, N–H proton), 5.92–5.88 (m, 2H), 5.54 (d, J = 5.7 Hz, 1H), 5.45–5.37 (m, 1H), 5.26 (q, J = 7.5 Hz, 1H), 4.76–4.65 (m, 1H), 4.42–4.36 (m, 1H), 3.90–3.72 (m, 1H), 3.71–3.61 (m, 2H), 2.86–2.73 (m, 2H), 2.50–2.39 (m, 2H), 1.36 (d, J = 6.9 Hz, 3H), 1.25 (t, J = 6.9 Hz, 3H); MS 480.1 $(M+H)^+$; HRMS Calcd for $C_{26}H_{30}N_3O_6$ $(M+H)^+$ 480.2135. Found: 480.2141.

5.5.3. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-1,2,3,4,7,8-hexahydroazocin-3-yl)isoquinoline-1-carboxamide (14a). ^{1}H NMR (CD_3OD) δ 9.10–9.08 (d, J = 6.9 Hz, 1H), 8.56 (br s, 1H), 8.07–8.04 (d, J = 7.8 Hz, 2H), 7.89–7.80 (m, 3H), 5.80–5.71 (m, 2H), 5.45 (br s, 1H), 4.62 (br s, 1H), 4.35 (m, 2H), 4.23 (m, 1H), 3.84–3.79 (m, 1H), 3.54–3.47 (m, 2H), 3.33 (br s, 1H), 3.14–1.96 (m, 6H); MS 452 $(M+H)^+$.

5.5.4. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)isoquinoline-1-carboxamide (14b). ^{1}H NMR (CD_3OD 300 MHz) δ 9.24 (d, J = 8.7 Hz, 1H), 8.57 (d, J = 5.7 Hz, 1H), 8.03 (dd, J = 6.9, 5.1 Hz, 2H), 7.85 (dd, J = 8.1, 6.9 Hz, 1H), 7.77 (dd, J = 8.1, 7.2 Hz, 1H), 5.99–5.92 (m, 2H), 5.55 (dd, J = 12.0, 3.9 Hz, 1H), 4.72 (m, 1H), 4.64–4.61 (m, 1H), 4.40–4.29 (m, 2H), 4.12 (dd, J = 16.5, 4.5 Hz, 1H), 3.62 (br d, J = 15.9 Hz, 1H), 2.88–2.65 (m, 2H), 2.59–2.46 (m, 2H); MS 439 $(M+H)^+$; HRMS Calcd for $C_{22}H_{22}N_4O_6$ $(M+H)^+$ 439.1618. Found: 439.1607.

5.5.5. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (14c). ^{1}H NMR (CD_3OD 300 MHz) δ 8.49 (s, 1H), 8.05–7.95 (m, 4H), 7.66–7.59 (m, 2H), 5.92 (br s, 2H), 5.55 (dd, J = 12.9, 4.2 Hz, 1H), 4.72 (br d, J = 17.4 Hz, 1H), 4.65 (d, J = 3.9 Hz, 0.5H), 4.64 (d, J = 4.8 Hz, 0.5H), 4.38–4.29 (m, 2H), 4.16 (dd, J = 16.5, 5.1 Hz, 1H), 3.62 (br d, J = 15.6 Hz, 1H), 2.79–2.66 (m, 2H), 2.61–2.47 (m, 1H); MS 438 $(M+H)^+$; HRMS Calcd for $C_{23}H_{24}N_3O_6$ $(M+H)^+$ 438.1665. Found: 438.1658.

5.5.6. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)benzo[*b*]thiophene-2-carboxamide (14d). ^{1}H NMR (CD_3OD 300 MHz) δ 8.07 (br s, 1H), 7.92 (d, J = 7.8 Hz, 2H), 7.49–7.41 (m, 2H), 5.94–5.84 (m, 2H), 5.46 (dd, J = 12.3, 3.3 Hz, 1H), 4.70–4.61 (m, 2H), 4.35–4.26 (m, 2H), 4.15 (dd, J = 16.5, 5.4 Hz, 1H), 3.59 (br d, J = 15.6 Hz), 2.74–2.65 (m, 2H), 2.58–2.47 (m, 2H); MS 444 $(M+H)^+$; HRMS Calcd for $C_{21}H_{22}N_3O_6S$ $(M+H)^+$ 444.1229. Found: 444.1244.

5.5.7. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)benzamide (14e). ^{1}H NMR (CD_3OD, 300 MHz) 7.90 (d, J = 7.2 Hz, 2H); 7.60–7.46 (m, 3H); 5.88 (m, 2H); 5.46 (dd, J = 8.4, 3.9 Hz, 1H); 4.70–4.58 (m, 4H); 4.36–4.30 (m, 2H); 4.10 (dd, J = 16.5, 4.8 Hz, 1H); 3.65–3.56 (m, 2H); 2.78–2.50 (m, 4H); MS (ESI+) 388.09.

5.5.8. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-3-methoxybenzamide (14f). ^{1}H NMR (CD_3OD 300 MHz) δ 7.48–7.38 (m, 3H), 7.16–7.12 (m, 1H), 5.91–5.89 (m, 2H), 5.46 (dd, J = 12.6, 4.2 Hz, 1H), 4.69 (br d, J = 18.3 Hz, 1H), 4.63 (d, J = 3.6, 0.5H), 4.62 (d, J = 4.8 Hz, 0.5H),4.38–4.27 (m, 2H), 4.17–4.09 (m, 1H), 3.87 (s, 3H), 3.60 (br d, J = 17.4Hz, 1H), 3.39–3.33 (m, 1H), 2.75–2.65 (m, 2H), 2.58–2.46 (m, 2H); MS 418 $(M+H)^+$.

5.5.9. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-3-(trifluoromethyl)benzamide (14g). ^{1}H NMR (CD_3OD 300 MHz) δ 8.24 (s, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.72 (dd, J = 7.8, 6.3 Hz, 1H), 5.92–5.86 (m, 2H), 5.52–5.46 (m, 1H), 4.71 (br d, J = 18.0 Hz, 1H), 4.64 (d, J = 3.9 Hz, 0.5H), 4.62 (d, J = 3.9 Hz, 0.5 H), 4.38–4.34 (m, 1H), 4.29 (dd, J = 13.2, 2.1 Hz, 1H), 4.15 (dd, J = 16.2, 4.5 Hz, 1H), 3.60 (br d, J = 18.7 Hz, 1H), 3.40–3.38 (m, 2H), 2.75–2.65 (m, 2H), 2.58–2.46 (m, 2H); MS 456 $(M+H)^+$. HRMS Calcd for $C_{20}H_{21}N_3O_6F_3$ $(M+H)^+$ 456.1382. Found: 456.1374.

5.5.10. 3-Chloro-*N*-((*S*,*Z*)-1-(2-((3*S*)-2-hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)benzamide (14h). ^{1}H NMR (CD_3OD 300 MHz) δ 7.93 (dd, J = 1.8, 1.5 Hz, 1H), 7.84 (br d, J = 7.5 Hz, 1H), 7.59 (ddd, J = 12.4, 8.1, 1.2 Hz, 1H), 7.50 (dd, J = 8.1, 7.5, Hz, 1H), 5.91–5.88 (m, 2), 5.46 (dd, J = 12.3, 4.5 Hz, 1H), 4.69 (br d, J = 18.3 Hz, 1H), 4.64 (d, J = 3.6 Hz, 0.5H), 4.62 (d, J = 3.9 Hz, 0.5H), 4.38–4.23(m, 2H), 4.14 (dd, J = 16.5, 5.1 Hz, 1H), 3.60 (br d, J = 18.7 Hz, 1H), 2.75–2.64 (m, 2H), 2.57–2.46 (m, 2H); MS 421 $(M+H)^+$; HRMS Calcd for $C_{19}H_{21}N_3O_6Cl$ $(M+H)^+$ 422.1119. Found: 422.1119.

5.5.11. 2,5-Dichloro-*N*-((*S*,*Z*)-1-(2-((3*S*)-2-hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)benzamide (14i). ^{1}H NMR (CD_3OD 300 MHz) δ 7.65 (dd, J = 0.9, 1.8 Hz, 1H), 7.51–7.50 (m, 2H), 5.96–5.84 (m, 2H), 5.46 (dd, J = 3.9, 12.6 Hz, 1H), 4.68 (br d, J = 18.3 Hz, 1H), 6.43 (d, J = 3.9 Hz, 0.5H), 4.62 (d, J = 4.2 Hz, 0.5H), 4.38–4.25 (m, 2H), 4.13 (dd, J = 16.5, 5.4 Hz, 1H), 3.60 (ddd, J = 17.1, 6.6, 2.4 Hz, 1H), 2.75–2.64 (m, 2H), 2.55–2.44 (m, 2H); MS 458 $(M+H)^+$; HRMS Calcd for $C_{19}H_{20}N_3O_6Cl_2$ $(M+H)^+$ 456.0729. Found: 456.0735.

5.5.12. *N*-((*S*,*Z*)-1-((*R*)-1-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-1-oxopropan-2-yl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (14j). ^{1}H NMR ($CDCl_3$ 300 MHz) δ 8.49 (s, 1H), 8.04–7.93 (m, 5H), 7.65–7.57 (m, 2H), 5.90–5.81 (m, 2H), 5.45 (dd, J = 12.6, 4.8 Hz, 1H), 5.24 (q, J = 7.5 Hz, 0.5H), 5.23 (q, J = 7.2 Hz, 0.5H), 4.65 (d, J = 4.2 Hz, 0.5H), 4.62 (d, J = 5.7 Hz, 0.5H), 4.45 (br d, J = 18.0 Hz, 1H), 4.32 (m, 1H), 3.76 (br d, J = 17.7 Hz, 1H), 2.70–2.43 (m, 4H), 1.40 (d, J = 7.2 Hz, 3H); MS 452 $(M+H)^+$; HRMS Calcd for $C_{24}H_{26}N_3O_6$ $(M+H)^+$ 452.1822. Found: 452.1843.

5.5.13. *N*-((*S*,*Z*)-1-((*S*)-1-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-1-oxopropan-2-yl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (14k). ^{1}H NMR ($CDCl_3$ 300 MHz) δ 8.47 (s, 1H), 8.00–7.92 (m, 5H), 7.63–7.55 (m, 2H), 5.95–5.80 (m, 2H), 5.55 (dd, J = 12.6, 4.2 Hz, 1H), 5.29 (q, J = 7.2 Hz, 0.5H), 5.26 (q, J = 6.7 Hz, 0.5H), 4.63 (d, J = 4.5 Hz, 0.5H), 4.61 (d, J = 4.5 Hz, 0.5H), 4.77 (br d, J = 17.7 Hz, 1H), 4.39–4.30 (m, 1H), 3.69–3.57 (m, 1H), 2.75–2.45 (m, 4H), 1.35 (d, J = 7.2Hz, 0.5 × 3H), 1.34 (d, J = 7.2 Hz, 0.5x3H); MS 452 $(M+H)^+$; HRMS Calcd for $C_{24}H_{26}N_3O_6$ $(M+H)^+$ 452.1822. Found: 452.1830.

5.6. (*S*)-Methyl 2-(2-(*tert*-butoxycarbonyl)-*N*-(2-((*tert*-butyldimethylsilyloxy)methyl)allyl)pent-4-enamido)acetate (17)

To a solution of 2-(*tert*-butyl-dimethyl-silanyloxymethyl)-prop-2-en-1-ol (**16**) (0.335 g, 1.65 mmol) in dry CH_2Cl_2 (5 mL), at −78 °C, were added triphenylphosphine (0.5 g, 1.90 mmol) and *N*-bromosuccinimide (0.3 g, 1.65 mmol), and stirring was maintained at −15 °C for 5 h. To this were added diethyl ether and water, and the mixture was portioned. The organic phase was washed with brine, dried (Na_2SO_4), and preabsorbed onto silica under reduced pressure. Chromatography on silica gel (EtOAc/hexane) afforded 0.17 g (39%) of (2-bromomethyl-allyloxy)-*tert*-butyl-dimethyl-silane. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 5.27–5.25 (m, 2H), 4.29 (s, 2H), 4.03 (s, 2H), 0.98 (s, 9H), 0.38–0.28 (m 6H); ^{13}C NMR ($CDCl_3$ 75 MHz) δ 145.0, 115.0, 109.2, 64.1, 63.7, 32.9, 31.8, 26.1, 22.9, 18.5, 14.3.

Glycine methyl ester hydrochloride (0.28 g, 2.26 mmol), Et_3N (0.63 mL, 4.54 mmol), and (2-bromomethyl-allyloxy)-*tert*-butyl-dimethyl-silane (0.24 g, 0.91 mmol) in DMF (5 mL) were stirred at room temperature for 24 h. To this were added diethyl ether and water, and the mixture was portioned. The organic phase was washed with brine, dried (Na_2SO_4) and concentrated. Flash chromatography on silica gel (EtOAc/hexane) yielded 0.107 g (43%) of [2-(*tert*-butyl-dimethyl-silanyloxymethyl)-allylamino]-acetic acid methyl ester. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 5.13 (br s, 1H), 4.99 (br s, 1H), 4.15 (br s, 2H), 3.71 (s, 3H), 3.38 (s, 2H), 3.25 (s, 2H), 1.71 (s, 1H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$ 75 MHz) δ 173.1, 146.3, 111.1, 65.0, 51.9, 51.6, 50.0, 26.1, 18.5, 5.1; MS 274 $(M+H)^+$.

A solution containing [2-(*tert*-butyl-dimethyl-silanyloxymethyl)-allylamino]-acetic acid methyl ester (6 g, 21.9 mmol), *N*-Boc-L-allylglycine (7.2 g, 32.9 mmol), HOBt (6 g, 43.9 mmol), and EDAC (8.5, 43.9 mmol) in 50 mL CH_2Cl_2 was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ and saturated NaCl, and dried (Na_2SO_4). Removal of the solvent under reduced pressure followed by flash chromatography on silica gel (EtOAc/hexane) yielded 5.9 g (57%) of **17**. (the NMR spectra were complicated by the presence of a mixture of amide rotamers) ^{1}H NMR ($CDCl_3$ 300 MHz) δ 5.77–5.61 (m, 1H), 5.15–4.85 (series of m, 4H), 4.64–3.76 (series of m, 7H), 3.63 (s, 3H), 2.47–2.42 (m, 1H), 2.34–2.23 (m, 1H), 1.34 (s, 9H), 0.82 (s, 9H), 0.04-0.02 (m, 6H); ^{13}C NMR ($CDCl_3$ 75 MHz, rotamers) δ 174.1, 174.0, 171.0, 170.7, 156.8, 156.5, 144.7, 134.7, 134.3, 120.8, 120.0, 1114.2, 113.7, 80.9, 80.7, 65.6, 65.3, 53.8, 53.4, 51.9, 51.4, 51.2, 50.1, 49.5, 48.5, 39.0, 38.6, 29.7, 27.3, 19.7, 3.9.; MS 471 $(M+H)^+$.

5.7. (*S*,*E*)-Methyl 2-(3-(*tert*-butoxycarbonyl)-6-((*tert*-butyldimethylsilyloxy)methyl)-2-oxo-3,4-dihydro-2*H*-azepin-1(7*H*)-yl)acetate (18)

Second-generation Grubbs catalyst (2 g) was added to a solution of **17** (4.1 g, 8.7 mmol) in 100 mL CH_2Cl_2. After refluxing the solution for 1 h, DMSO (10 mL) was added to the reaction mixture and stirring was continued at room temperature for another 12 h. Removal of the solvent under reduced pressure followed by flash chromatography on silica gel (EtOAc/hexane) yielded 3.7 g (96%) of **18**. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 5.79 (d, J = 6.3 Hz, 1H), 5.65 (br s, 1H), 5.03–4.96 (m, 1H), 4.56–4.48 (m, 2H), 3.98 (s, 2H), 3.95 (d, J = 17.4 Hz, 1H), 3.73 (s, 3H), 3.44–3.38 (d, J = 17.7, 1H), 2.71 (dd, J = 18, 3.3 Hz, 1H), 2.30–2.18 (m, 1H), 1.45 (s, 9H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$ 75 MHz) δ 172.9, 169.6, 155.2, 135.6, 124.6, 79.7, 66.9, 60.5, 52.4, 50.0, 47.6, 33.1, 28.5, 26.0, 18.4, 14.4, 5.0; MS 442 $(M+H)^+$.

5.8. (*S*,*E*)-Methyl 2-(3-(2-naphthamido)-6-(hydroxymethyl)-2-oxo-3,4-dihydro-2*H*-azepin-1(7*H*)-yl)acetate (19)

A solution containing **18** (5.2 g, 11.8 mmol) in 20 mL CH_2Cl_2 was treated with 5 mL of wet TFA and stirred at room temperature for 30 min. The solution was diluted with toluene and concentrated under reduced pressure. The crude residue obtained was dissolved in 50 mL of THF and treated with 2-naphthoyl chloride (2.69 g, 14.1 mmol) and Et_3N (3.3 mL, 23.7 mmol) at room temperature. The solution was stirred for 10 min and then 5 mL of MeOH was added to the reaction. Removal of the solvent under reduced pressure followed by flash chromatography on silica gel yielded 4.15 g (89% over two steps) of **19**. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 8.09 (s, 1 H), 7.67–7.53 (m, 5H), 7.32–7.23 (m, 2H), 5.48 (br s, 1H), 5.27–5.19 (m, 1H), 4.46–4.40 (d, J = 17.1 Hz, 1h), 4.04 (s, 2H), 3.79 (d, J = 12.5 Hz, 1H), 3.73 (d, J = 12.6 Hz, 1H), 3.46 (s, 3H), 3.35–3.19 (m, 2H), 2.68 (br d, J = 18.0 Hz, 1H), 2.08 (br dd, J = 15.5, 14.8 Hz, 1H); ^{13}C NMR ($CDCl_3$ 75 MHz) δ

170.8, 167.9, 164.9, 134.0, 133.0, 130.7, 129.1, 127.2, 126.3, 125.9, 124.9, 123.8, 121.7, 65.1, 50.9, 48.1, 47.5, 45.9, 30.2; MS 383 $(M+H)^+$.

5.9. (*S*,*Z*)-Methyl 2-(3-(2-naphthamido)-6-((benzylamino) methyl)-2-oxo-3,4-dihydro-2*H*-azepin-1(7*H*)-yl)acetate (20)

A solution containing **19** (0.11 g, 0.29 mmol) in 3 mL CH_2Cl_2 was treated with triethylamine (0.2 mL, 1.4 mmol) and methanesulfonyl chloride (0.1 mL, 1.3 mmol) at −78 °C. After stirring at −78 °C for 2 h, the reaction mixture was poured into a mixture of CH_2Cl_2 and saturated NaCl solution. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl, dried (Na_2SO_4) and concentrated. To this crude product in 3 mL of CH_2Cl_2, benzylamine (0.2 mL, 1.8 mmol) and Et_3N (0.5 mL, 3.6 mmol) were added at room temperature. The reaction mixture was stirred at room temperature for 30 min and then was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and saturated NaCl, and dried (Na_2SO_4). Removal of the solvent under reduced pressure followed by preparative reverse phase HPLC purification yielded 70 mg (51% over two steps) of **20**. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 8.36 (s, 1H), 7.96–7.87 (m, 4H), 7.75 (d, J = 6.3 Hz, 1H), 7.62–7.53 (m, 2H), 7.44–7.36 (m, 5H), 5.99 (br s, 1H), 5.43–5.36 (m, 1H), 4.67–4.61 (m, 2H), 4.25–4.12 (m, 2H), 3.91–3.81 (m, 2H), 3.74 (s, 3H), 3.45 (s, 2H), 3.02 (br d, J = 13.5 Hz, 1H), 2.43 (br dd, J = 15.3, 15.0 Hz, 1H); ^{13}C NMR ($CDCl_3$ 75 MHz) δ 172.7, 170.6, 166.8, 135.4, 135.2, 132.8, 131.1, 130.9, 130.2, 129.7, 128.8, 128.1, 127.9, 127.3, 127.1, 123.8, 53.0, 52.6, 50.3, 50.2, 49.2, 48.4, 32.6; MS 472 $(M+H)^+$.

5.10. (*S*,*E*)-Methyl 2-(3-(2-naphthamido)-6-(benzylthiomethyl)-2-oxo-3,4-dihydro-2*H*-azepin-1(7*H*)-yl)acetate (21)

A solution containing **19** (0.36 g, 1.09 mmol) in 5 mL CH_2Cl_2 was treated with triethyl amine (0.38 mL, 2.73 mmol) and methanesulfonyl chloride (0.19 mL, 2.45 mmol) at −78 °C. After stirring at −78 °C for 1.5 h, the reaction mixture was poured into a mixture of CH_2Cl_2 and brine. The organic layer was separated, washed with saturated $NaHCO_3$ and brine, dried ($NaSO_4$), and concentrated under reduced pressure. To this crude product in 1 mL CH_2Cl_2, benzyl mercaptan (0.126 mL, 2.2 mmol) was added at room temperature followed by triethyl amine (0.7 mL, 5.03 mmol). The reaction mixture was completed instantly. Removal of solvent under reduced pressure followed by flash chromatography (20% EtOAC/Hexane) on silica gel yielded 138 mg (58% over two steps) of **21**. ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1H), 7.96–7.87 (m, 4H), 7.77 (d, J = 5.7 Hz, 1H), 7.61–7.53 (m, 2H), 7.38–7.28 (m, 4H), 5.54–5.48 (m, 1H), 4.66 (dd, J = 17.1, 2.4 Hz, 1H), 4.50 (d, J = 17.1 Hz, 1H), 4.14 (d, J = 17.7 Hz, 1H), 3.77 (s, 3H), 3.64 (s, 2H), 3.46 (d, J = 17.4 Hz, 1H), 3.13 (d, J = 13.5 Hz, 1H), 3.02 (dd, J = 18.3, 3.9 Hz, 1H), 2.92 (d, J = 13.8 Hz, 1H), 2.43–2.34 (m, 2H); ^{13}C ($CDCl_3$) 172.9, 169.5, 166.6, 138.2, 135.1, 132.8, 131.8, 131.3, 129.2, 128.8, 128.7, 128.0, 127.9, 127.4, 127.2, 127.0, 123.8, 52.6, 49.7, 49.5, 39.2, 35.4, 32.6; MS 488 $(M+H)^+$.

5.11. (*S*,*E*)-Methyl 2-(3-(2-naphthamido)-6-(azidomethyl)-2-oxo-3,4-dihydro-2*H*-azepin-1(7*H*)- yl)acetate (22)

A solution of alcohol **19** (7.02 g, 17.7 mmol) in 50 mL CH_2Cl_2 was treated with triethyl amine (6.2 mL, 44.6 mmol) and methanesulfonyl chloride (3.1 mL, 40.1 mmol) at −78 °C. After stirring at −78 °C for 2 h, the reaction mixture was poured into a mixture of CH_2Cl_2 and saturated NaCl. The organic layer was separated, washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. To this crude product in 30 mL of DMF, sodium azide (5.8 g, 89.2 mmol) was added at room temperature. The reaction mixture was stirred at 47 °C for 12 h and then diluted with H_2O. The resulting mixture was extracted 3× with CH_2Cl_2 and the combined extracts were washed with 1 N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography to yield 6.2 g (83%) of azide **22**. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 8.38 (s, 1H), 7.96–7.87 (m, 4H), 7.74 (d, J = 6.3 Hz, 1H), 7.61–7.53 (m, 2H), 5.87–5.85 (m, 1H), 5.55–5.47 (m, 1H), 4.75 (br dd, J = 17.4, 2.7 Hz, 1H), 4.49 (d, J = 17.7 Hz, 1H), 4.19 (d, J = 17.4 Hz, 1H), 3.87 (d, J = 13.5 Hz, 1H), 3.78 (s, 3H), 3.70 (d, J = 13.5 Hz, 1H), 3.45 (d, J = 17.7 Hz, 1H), 3.05 (dd, J = 17.7, 3.6 Hz, 1H), 2.48–2.37 (m, 1H); ^{13}C NMR ($CDCl_3$ 75 MHz) δ 172.8, 169.4, 166.9, 135.1, 132.8, 131.3, 131.1, 129.3, 128.7, 128.0, 127.9, 127.0, 123.8, 57.8, 52.7, 50.3, 49.4, 48.3, 32.5; MS 408 (M+H).

5.12. (*S*,*Z*)-Methyl 2-(3-(2-naphthamido)-6-(benzamidomethyl)-2-oxo-3,4-dihydro-2*H*-azepin-1(7*H*)-yl)acetate (23f)

To the solution of the azide **22** (2.5 g, 5.9 mmol) in THF (30 mL) was added three drops of water. While stirring at room temperature, 3.12 g (11.9 mmol) triphenylphosphine was added. The reaction mixture was allowed to stir at room temperature under N_2 overnight. The solvent was then removed under vacuum and the residue chromatographed on C18 silica gel to obtain 2 g of the desired primary amine.

A solution of the amine (0.11 g, 0.29 mmol) in THF was cooled to 0 °C, and triethyl amine (0.19 mL, 1.4 mmol) and benzoyl chloride (0.2 mL, 1.7 mmol) were added while stirring. The reaction mixture was warmed to room temperature and stirred for 5 min. Methanol was added to the reaction mixture, and the solution was concentrated under reduced pressure. The crude residue was purified by preparative reverse phase HPLC to provide 58.6 mg (25%) of **23f**. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 8.36 (s, 1H), 7.94–7.85 (m, 5H), 7.72 (d, J = 6.3 Hz, 1H), 7.60–7.42 (m, 5H), 6.92–6.88 (m, 1H), 5.81–5.80 (m, 1H), 5.51–5.44 (m, 1H), 4.73 (dd, J = 17.1, 2.4 Hz, 1H), 4.55 (d, J = 17.4 Hz, 1H), 4.26 (dd, J = 14.4, 6.6 Hz, 1H), 3.97 (d, J = 17.4 Hz, 1H), 3.90 (dd, J = 14.4, 3.9 Hz, 1H), 3.59 (s, 3H), 3.51 (d, J = 17.7 Hz, 1H), 2.98 (br dd, J = 17.4, 3.3 Hz, 1H), 2.62 (br s, 1H), 2.39 (dd, J = 15.3, 14.7 Hz, 1H); ^{13}C NMR ($CDCl_3$ 75 MHz) 172.8, 170.2, 167.9, 166.7, 135.1, 134.2, 133.1, 132.8, 132.0, 131.3, 129.3, 128.8,

128.7, 128.0, 127.5, 127.4, 127.0, 123.8, 52.5, 50.5, 49.5, 49.1, 46.7, 32.5; *Intermediates **23g–i** and **24e** were synthesized by via an analogous procedure.

5.13. General procedure for the synthesis of compounds 25 and 26

Esters **19**, **20**, **21**, **23**, and **24** were hydrolyzed and coupled with the amine generated in situ from **15** according to the procedure outlined for the synthesis of **14**. The crude coupled product was purified by flash chromatography on silica gel to yield pure **25**. Further hydrolysis of **25** was accomplished by treatment with TFA in CH_3CN/H_2O. Purification of the final compounds was carried out by preparative reverse phase HPLC (CH_3CN/H_2O with 0.1% TFA) to give pure **26a–i** as a 1:1 mixture of isomers at the acetal stereocenter.

5.13.1. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Ethoxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-6-((4-methoxy-benzamido)-methyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (25i). ^{1}H NMR (CD_3OD 300 MHz) δ 8.47 (s, 1H), 8.11–7.63 (m, 6H), 7.30 (br s, 1H), 7.06–7.03 (m, 2H), 6.88 (d, J = 7.8 Hz, 1H), 5.85 (m, 1H), 5.51–5.47 (m, 1H), 5.39 (d, J = 5.4 Hz, 1H), 4.82–4.62 (m, 2H), 4.30–3.53 (series of multiplets, 8H), 3.02-2.71 (m, 2H), 2.54–2.43 (m, 2H), 2.23–2.15 (m, 5H), 1.27-1.16 (m, 3H); MS 629.3 $(M+H)^+$ HRMS Calcd for $C_{34}H_{37}N_4O_8$ $(M+H)^+$ 629.2611. Found: 629.2610.

5.13.2. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-6-methyl-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26a). ^{1}H NMR (CD_3OD 300 MHz) δ 8.45 (s, 1H), 8.00–7.91 (m, 4H), 7.62–7.54 (m, 2H), 5.57 (br s, 1H), 5.48 (dd, J = 12.3, 3.9 Hz, 1H), 4.77 (br d, J = 17.1 Hz, 1H), 4.64 (d, J = 3.9 Hz, 0.5H), 4.62 (d, J = 4.2 Hz, 0.5H), 4.39–4.29 (m, 1H), 4.23 (s, 2H), 3.44–3.35 (m, 2H), 2.75–2.62 (m, 2H), 2.55–2.45 (m, 2H), 1.82 (s, 3H); MS 452 $(M+H)^+$; HRMS Calcd for $C_{24}H_{26}N_3O_6$ $(M+H)^+$ 452.1822. Found: 452.1825.

5.13.3. *N*-((*S*,*E*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-6-(hydroxymethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26b). ^{1}H NMR (CD_3OD 300 MHz) δ 8.47 (s, 1H), 8.02–7.85 (m, 4H), 7.64–7.50 (m, 2H), 5.81 (br s, 1H), 5.56 (dd, J = 12.9, 3.9 Hz, 1H), 4.78 (br d, J = 18.3 Hz, 1H), 4.64 (d, J = 3.6 Hz, 0.5H), 4.63 (d, J = 4.2 Hz, 0.5H), 4.38–4.31 (m, 2H), 4.15 (dd, J = 15.9, 3.3 Hz, 1H), 4.03 (s, 2H), 3.66–3.56 (m, 1H), 3.40–3.39 (m, 1H), 2.79–2.65 (m, 2H), 2.59–2.48 (m, 2H); MS 468 $(M+H)^+$.

5.13.4. *N*-((*S*,*E*)-6-(Benzylthiomethyl)-1-(2-((3*S*)-2-hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26c). ^{1}H NMR (CD_3OD 300 MHz) δ 8.47 (s, 1H), 8.01–7.91 (m, 4H), 7.63–7.56 (m, 2H), 7.35–7.23 (m, 5H), 5.56–5.48 (m, 2H), 4.72 (br d, J = 17.1 Hz, 1H), 4.65 (d, J = 3.6 Hz, 0.5H), 4.63 (d, J = 3.6 Hz, 0.5H), 4.41–4.34 (m, 2H), 4.13 (dd, J = 16.5, 2.4 Hz, 1H), 3.68–3.51 (m, 3H), 3.39 (s, 2H), 3.16 (d, J = 14.1 Hz, 1H), 3.02 (d, J = 13.5 Hz, 1H), 2.79–2.67 (m, 2H), 2.58–2.49 (m, 2H); MS 574 $(M+H)^+$; HRMS Calcd for $C_{31}H_{32}N_3O_6S$ $(M+H)^+$ 574.2012. Found: 574.2012.

5.13.5. *N*-((*S*,*Z*)-6-((Benzylamino)methyl)-1-(2-((3*S*)-2-hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26d). ^{1}H NMR (CD_3OD 300 MHz) δ 8.48 (s, 1H), 8.03–7.93 (m, 4H), 7.66–7.57 (m, 4H), 7.51–7.47 (m, 3H), 6.12 (br s, 1H), 5.51 (dd, J = 12.6, 4.2 Hz, 1H), 4.82 (dd, J = 16.5, 2.7 Hz, 1H), 4.66 (d, J = 3.6 Hz, 0.5H), 4.62 (d, J = 3.9 Hz, 0.5H), 4.37–4.23 (m, 3H), 3.86–3.69 (m, 4H), 2.87–2.79 (m, 1H), 2.74–2.64 (m, 2H), 2.50 (m, 1H); MS 557 $(M+H)^+$.

5.13.6. *N*-((*S*,*E*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-6-(phenylsulfonamidomethyl)-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26e). ^{1}H NMR (CD_3OD 300 MHz) δ 8.45 (s, 1H), 8.01–7.89 (m, 6H), 7.67–7.55 (m, 5H), 5.67 (br s, 1H), 5.41 (dd, J = 12.3, 3.9 Hz, 1H), 4.69–4.59 (m, 2H), 4.40–4.32 (m, 1H), 4.19 (s, 2H), 3.51–3.46 (m, 3H), 2.77–2.64 (m, 2H), 2.60–2.41 (m, 2H); MS 607 $(M+H)^+$; HRMS Calcd for $C_{30}H_{31}N_4O_8S$ $(M+H)^+$ 607.1863. Found: 607.1855.

5.13.7. *N*-((*S*,*Z*)-6-(Benzamidomethyl)-1-(2-((3*S*)-2-hydroxy-5-oxo- tetrahydrofuran-3-ylamino)-2-oxoethyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26f). ^{1}H NMR (CD_3OD 300 MHz) δ 8.46 (s, 1H), 8.01–7.91 (m, 6H), 7.63–7.48 (m, 5H), 5.82 (br s, 1H), 5.55 (dd, J = 4.2, 12.6 Hz, 1H), 4.86 (br d, J = 17.7 Hz, 1H), 4.63 (d, J = 3.6 Hz, 0.5H), 4.61 (d, J = 3.9 Hz, 0.5H), 4.41–4.31 (m, 1H), 4.27–4.13 (m, 2H), 4.03 (s, 2H), 3.60 (dd, J = 17.7, 3.3 Hz, 1H), 2.79–2.47 (m, 4H); MS 571 $(M+H)^+$; HRMS Calcd for $C_{31}H_{31}N_4O_7$ $(M+H)^+$ 571.2172. Found: 571.2193.

5.13.8. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-6-((2-methoxybenz-amido)-methyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26g). ^{1}H NMR (CD_3OD 300 MHz) δ 8.47 (s, 1H), 8.02–7.88 (m, 5H), 7.64–7.50 (m, 3H), 7.18 (d, J = 8.1 Hz), 7.09 (ddd, J = 0.9, 7.5, 8.4 Hz, 1H), 5.82 (br s, 1H), 5.57 (dd, J = 12.9, 4.2 Hz, 1H), 4.86–4.83 (m, 1H), 4.63 (d, J = 3.9 Hz, 0.5H), 4.61 (d, J = 3.9 Hz, 0.5H), 4.35–4.13 (m, 3H), 4.08 (s, 2H), 4.01 (s, 3H), 3.60 (dd, J = 18.0, 3.0 Hz, 1H), 2.80–2.62 (m, 2H), 2.57 (m, 2H); MS 601 $(M+H)^+$; HRMS Calcd for $C_{32}H_{33}N_4O_8$ $(M+H)^+$ 601.2298. Found: 601.2320.

5.13.9. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-6-((3-methoxybenz-amido)-methyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26h). ^{1}H NMR (CD_3OD 300 MHz) δ 8.46 (s, 1H), 8.00–7.91 (m, 4H), 7.63–7.54 (m, 2H), 7.49–7.38 (m, 3H), 7.13–7.10 (m, 1H), 5.81 (br s, 1H), 5.54 (dd, J = 12.9, 4.5 Hz, 1H), 4.88–4.80 (m, 1H), 4.63–4.60 (d, J = 4.2 Hz, 0.5H), 4.61 (d, J = 4.2 Hz, 0.5H), 4.40–4.22 (m, 3H), 4.08 (s, 2H), 3.87 (s, 3H), 3.59 (dd, J = 17.7, 2.7 Hz, 1H), 2.78–2.65 (m, 2H), 2.60–2.47 (m, 2H); MS 601 $(M+H)^+$; HRMS Calcd for $C_{32}H_{33}N_4O_8$ $(M+H)^+$ 601.2298. Found: 601.2277.

5.13.10. *N*-((*S*,*Z*)-1-(2-((3*S*)-2-Hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2-oxoethyl)-6-((4-methoxybenz-amido)methyl)-2-oxo-2,3,4,7-tetrahydro-1*H*-azepin-3-yl)-2-naphthamide (26i). ^{1}H NMR (CD_3OD 300 MHz) δ 8.45 (s, 1H), 7.99–7.85 (m, 6H), 7.62–7.54 (m, 2H), 7.04-6.99 (m, 2H), 5.80 (br s, 1H), 5.53 (dd, J = 12.3, 3.9 Hz, 1H), 4.88–4.80 (m, 1H), 4.63 (d, J = 3.6 Hz, 0.5H), 4.61 (d, J = 3.0 Hz, 0.5H), 4.42–4.13 (m, 3H), 4.00 (s, 2H), 3.86 (s, 3H), 3.59 (dd, J = 17.1, 1.8 Hz, 1H), 2.77–2.65 (m, 2H), 2.55–2.47 (m, 2H); MS 601 $(M+H)^+$; HRMS Calcd for $C_{32}H_{33}N_4O_8$ $(M+H)^+$ 601.2298. Found: 601.2274.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.11.011.

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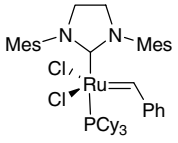
16. 

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20. The % *F* was calculated as a dose-normalized AUCPO-Parent/AUCPO-IV-Parent. For screening purposes, the intravenous studies with parent compound were conducted once, and data from that study were used for subsequent calculations with pro-drug analogs of that compound.

21. The kit used was the Caspase Fluorescent (AMC) Substrate/Inhibitor QuantiPak from BioMol Laboratories. The substrates were Ac-DEVD-AMC (Caspase-3), AC-YVAD-AMC (Caspase-1), and AC-IETD-AMC (Caspase-8).