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Synthesis and evaluation of thiazepines as interleukin-1β converting enzyme (ICE) inhibitors☆

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Abstract—A series of monocyclic thiazepine inhibitors of interleukin-1β converting enzyme (ICE) were synthesized in eight steps from commercially available intermediates. In vitro biological evaluation showed the thiazepines to be moderately potent ICE inhibitors, with the most active compound exhibiting an IC_{50} value of 30 nM in an enzyme inhibition assay. Compounds of this class possessed good selectivity against the related enzymes caspase-3 and caspase-8.

The cytokine interleukin-1β (IL-1β) has been shown to play an important role in a variety of disease processes, including inflammation, septic shock, wound healing, and arthritis.[2] The IL-1 receptor antagonist Kineret™ has been approved for use in the treatment of rheumatoid arthritis (RA), indicating that modulation of IL-1β activity provides a clinical benefit. IL-1β is produced as an inactive pro-form 31 kDa precursor (pro-IL-1β), which is cleaved to the biologically active 17.5 kDa cytokine by the enzyme interleukin-1β converting enzyme (ICE, caspase-1).[3,4] The enzyme is a member of the caspase (*cys*teine *asp*artate specific prote*ase*) family of cysteine proteases, and as such requires Asp116–Ala117 recognition from the substrate for activation and cleavage to the mature cytokine. As a result, ICE potentially offers an attractive target for inhibition of IL-1β production for a variety of disease indications.

To date, 15 mammalian caspases with varying biological functions and substrate specificities have been reported.[5] Using positional scanning libraries, the tetrapeptide fragment Ac-YVADCHO (**1**, Fig. 1) was identified as a potent and selective inhibitor ($Ki \sim 5$ nM) of ICE.[6] Most small molecule inhibitors of ICE are peptidomimetic compounds[7] based on Ac-YVADCHO, which is reported to block the release of IL-1β from human whole blood cells with an IC_{50} of 4 μM. The major focus in ICE peptidomimetics has been to restrict the Val–Ala portion in the tetrapeptide, maintaining the important interaction of the peptide backbone which results in properly orienting the important P1 and P4 recognition regions of the molecule in the enzyme pocket.[3] One

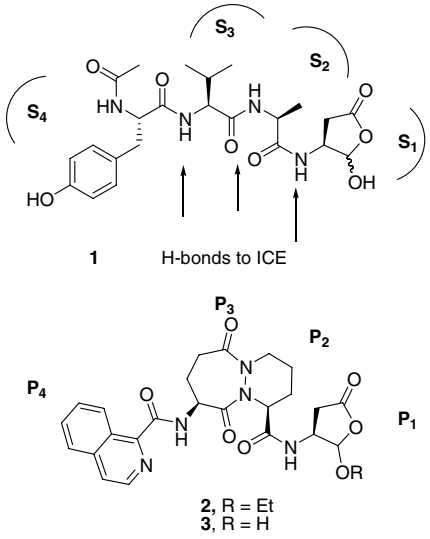

Figure 1. Selected ICE Inhibitors.

Keywords: ICE; Caspase-1; Thiazepine; Peptidomimetic; Interleukin-1β.
☆ Ref. 1.
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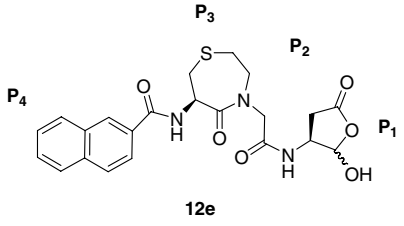
notable example is Pralnacasan® (**2**), the prodrug form of a reversible ICE inhibitor which constricts the P2–P3 region of the molecule as a pyridazinodiazepine and was progressed into late-stage clinical trials.[8] The free drug form of Pralnacasan® is **3**.

In this paper, we report the synthesis and evaluation of thiazepines as monocyclic peptidomimetic ICE inhibitors. We theorized that we could achieve the required conformation to maintain the interaction at P1 using an aspartate group and at P4 with aromatic groups to mimic Tyr. The sulfur of the thiazepine could point into the S3 pocket to occupy space similar to the Val of Ac-YVADCHO (see Fig. 2). In addition, the monocyclic thiazepines should be more readily synthesized than bicyclic scaffolds such as Pralnacasan®. The thiazepine scaffold was studied in conjunction with a number of other easily prepared scaffolds, including diazocans, pyrrolopyrimidinones, cycloalkyl carboxamides, and caprolactams.[9]

Figures 3 and 4 compare the published crystal structure of Ac-YVADCHO bound to ICE[3] and a model of our thiazepine based on the crystal structure of a previously reported ACE inhibitor.[10] Based on the single crystal X-ray structure of the reported thiazolidinone ring, a model of inhibitor **12e** was constructed. The rotatable bonds of the P1 and P4 moieties of the model were oriented in low-energy conformations that overlapped with the corresponding P1 and P4 substituents in the AcYVAD-CHO structure from the crystal structure. Finally, this model was docked into the active site of the ICE protein crystal structure in place of the



12e

Figure 2. Thiazepine ICE Inhibitor.

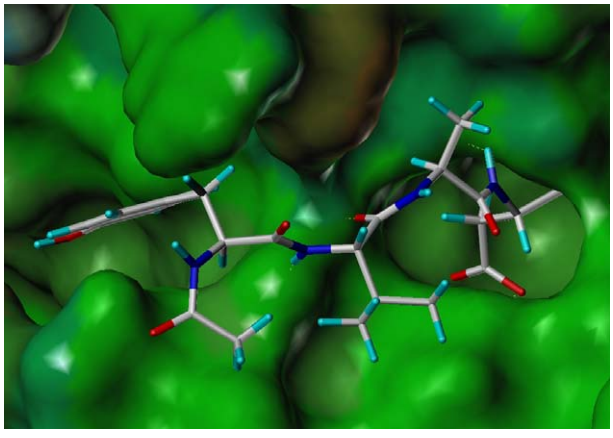


Figure 3. X-ray structure of AcYVAD-CHO complexed to ICE.

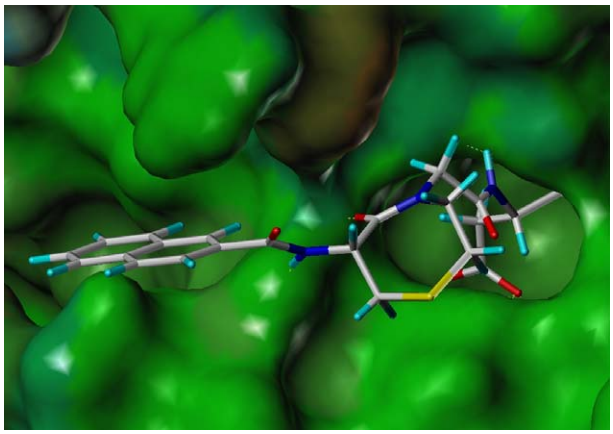
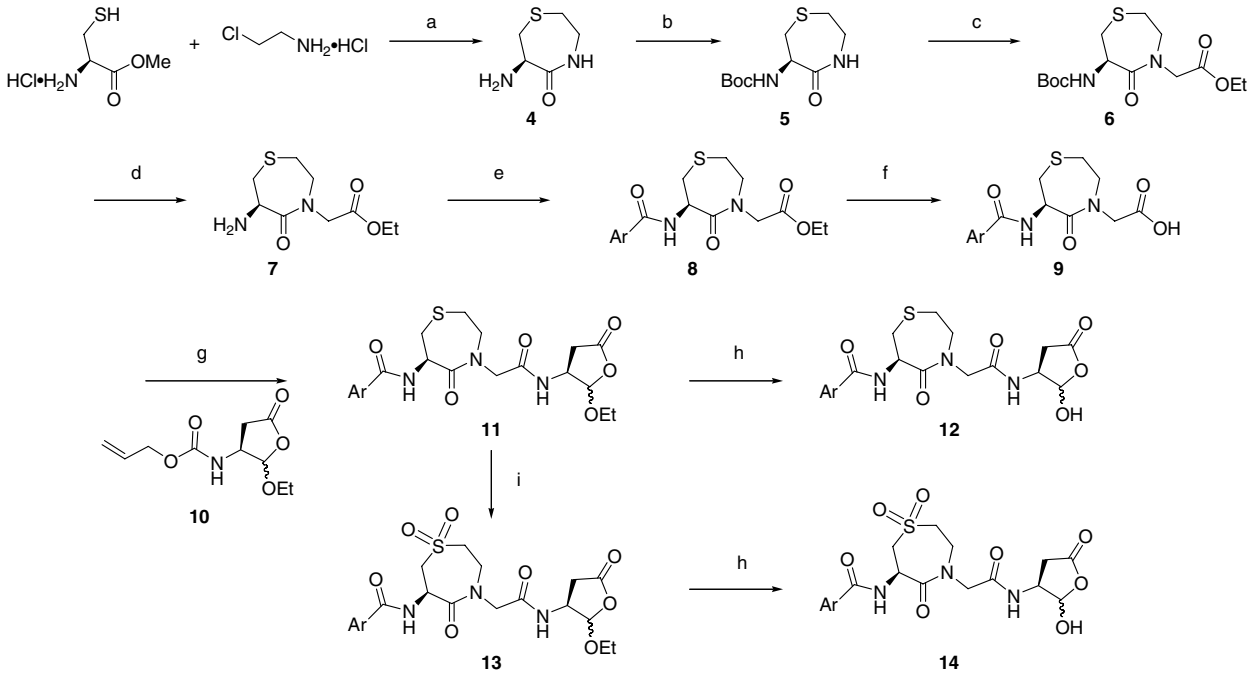


Figure 4. Molecular model of thiazepine **12e**.

AcYVAD-CHO structure. The monocyclic thiazepine scaffold can potentially orient the P1 and P4 substituents well for interaction with the enzyme similar to that of Ac-YVADCHO while maintaining the desired H-bond interactions of the peptidomimetic backbone.

In order to test our hypothesis, the thiazepine scaffold was synthesized. L-Cysteine methyl ester hydrochloride and 2-chloroethylamine hydrochloride (Scheme 1) were reacted using literature procedures to afford the cyclized thiazepine scaffold **4**,[11] which was then protected using $(Boc)_2O$ and Et_3N in methanol yielding **5** in 70–80% yield. Alkylation with ethyl bromoacetate in THF using KHMDS as the base produced **6** in yields of 50–70%. Boc-deprotection to **7**[11] followed by acylation with an aryl acid chloride or aryl acid afforded **8**,[12] with 35–70% yields over two steps. Following saponification of **8**, the resulting acid **9** was coupled with the aspartate trap **10**[13] using EDCI and HOBT, to provide compound **11** (40–70%, two steps). At this point in the synthesis, proton NMR showed the presence of only two compounds (due to the racemic acetal). This provided evidence that little or no racemization took place in the earlier KHMDS deprotonation. Hydrolysis of the ethyl acetal gave the final sulfide compounds **12**[14] in 50–80% yield. Oxidation of the ring sulfur of **11** using *m*-CPBA afforded **13** (50–65%), which was hydrolyzed yielding the final sulfone compounds **14**[15] in 35–50% yield.

The thiazepine compounds showed generally good potency in the ICE inhibition assay,[16] ranging from 30 to 350 nM (see Table 1). They were generally $\geqslant$100-fold selective for ICE over caspase-8 and were all inactive against caspase-3. The analogs with fused bicyclic P4 substituents (**12c–e**) were more active than the monocyclic aromatics (**12a–b**). In the enzyme assay, we observed a 4- to 5-fold decrease in the activity when moving from the sulfides to the sulfones (for example, compare compounds **12e** and **14e**). The compounds were also tested in the THP-1 whole cell assay[17] measuring the inhibition of IL-1β production caused by exposure to the ICE inhibitor. In the THP-1 assay, most of the compounds had IC_{50} values which were 5- to 15- fold higher than the corresponding ICE enzyme IC_{50}.



Scheme 1. Reagents and conditions: (a) Et_3N, MeOH, reflux; (b) $(Boc)_2O$, Et_3N, MeOH; (c) ethyl bromoacetate, KHMDS, THF; (d) TFA CH_2Cl_2; (e) ArC(O)Cl, Et_3N, THF or $ArCO_2H$, DIC, DMAP, DMF; (f) $LiOH \cdot H_2O$, THF/H_2O; (g) **10**, $(Ph_3P)_4Pd$, 1,3-dimethylbarbituric acid, HOBT, EDCI, CH_2Cl_2; (h) TFA, CH_3CN/H_2O; (i) mCPBA, $NaHCO_3$, CH_2Cl_2.

Table 1. In vitro data for the thiazepine series

Comp	Ar	IC_{50} (nM)[a]			IC_{50} (nM)[b]
		ICE	Casp-3	Casp-8	THP-1
12a	Ph	350	>10,000	5200	1700
12b	3-CF_3Ph	150	>10,000	>10,000	1000
12c	2-Benzo[*b*]thiophene	80	>10,000	9500	640
12d	1-Isoquinolyl	70	>10,000	7000	730
12e	2-Naphthyl	30	>10,000	9400	480
14a	Ph	2300	>10,000	>10,000	3200
14b	3-CF_3Ph	Not tested	Not tested	Not tested	3400
14c	2-Benzo[*b*]thiophene	320	>10,000	8500	1700
14d	1-Isoquinolyl	130	>10,000	5000	1400
14e	2-Naphthyl	150	>10,000	>10,000	1100
3	Pralnacasan®[18]	3.6	1300	40	190

[a] Enzyme IC_{50} results are expressed as ±30% or less.
[b] Whole cell THP IC_{50} results vary approximately ±2-fold.

In summary, a series of monocyclic thiazepines was prepared as a new class of ICE inhibitors. These compounds offer the advantage of a short (eight step) synthesis while also reducing the number of chiral centers in the molecule. The thiazepines were moderately potent ICE inhibitors possessing good selectivity against caspase-3 and -8. This approach demonstrated that the monocyclic scaffold maintained the key hydrogen-bonding interactions of previously reported inhibitors and presented the P1 and P4 substituents in the necessary conformation. Although the better performing thiazepines, such as compound **12e**, were less active than the clinical candidate Pralnacasan®, we have shown the potential utility of monocycles as scaffolds for effective ICE inhibition. Alternative monocyclic scaffolds may provide opportunities for the design of more competitive ICE inhibitors.

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14. Preparation of *N*-((*R*)-4-(2-((*S*)-2-hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2- oxoethyl)-5-oxo-1,4-thiazepan-6-yl)-2-naphthamide (**12e**). A solution of **8e** (0.20 g, 0.51 mmol) in THF (6 mL) and water (2 mL) was treated with $LiOH \cdot H_2O$ (43 mg, 1.0 mmol) and stirred for 1 h at rt. The solution was acidified (pH3) with 1 N HCl and extracted with Et_2O, washed with saturated brine, dried ($MgSO_4$), and concentrated. The crude acid (**9e**) was isolated as a white solid, 0.17 g (91%), MS 359.1 $(M + H)^+$. Catalytic $Pd(PPh_3)_4$ was added to a solution of **10** (0.32 g, 1.4 mmol) and 1,3-dimethylbarbituric acid (0.41 g, 2.6 mmol) in 5 mL CH_2Cl_2 at rt. The solution was stirred at rt for 15 min and then the crude carboxylic acid (**9e**) prepared above was added as a solution in 5 mL CH_2Cl_2 and 0.5 mL DMF followed by HOBt (0.19 g, 1.4 mmol) and EDCI (0.26 g, 1.3 mmol). The solution was stirred for 2 h, washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. After purification by preparative reverse-phase HPLC, **11e** was recovered as a pale yellow solid (0.13 g, 60% yield), MS 486.2 $(M + H)^+$. **11e** (45 mg, 0.093 mmol) was hydrolyzed with TFA in CH_3CN/H_2O. Purification by preparative reverse phase HPLC yielded **12e** as a white solid, 19 mg (45%). ^{1}H NMR (CD_3OD): δ 8.47 (s, 1H), 8.01 (q, 1H), 7.97 (m, 3H), 7.61 (m, 2H), 5.42 (d, J = 8.4 Hz, 1H), 4.64 (m, 1H), 4.36 (m, 1H), 4.20 (m, 3H), 3.67 (d, J = 15.3 Hz, 1H), 2.99 (m, 3H), 2.70 (m, 2H), 2.53 (m, 1H). ESI-MS 458.1 $(M + H)^+$.
15. Preparation of *N*-((*R*)-4-(2-((*S*)-2-hydroxy-5-oxo-tetrahydrofuran-3-ylamino)-2- oxoethyl)-1,1-dioxido-5-oxo-1,4-thiazepan-6-yl)-2-naphthamide (**14e**). To a solution of **11e** (83 mg, 0.17 mmol) in 5 mL CH_2Cl_2, $NaHCO_3$ (40 mg, 0.48 mmol), and mCPBA (86 mg, 0.35 mmol) were added. After stirring at rt for 2 h, the solids were filtered off and the solution concentrated. After purification by preparative reverse-phase HPLC, **13e** was recovered as an off-white solid (85 mg, 96% yield), MS 518.2 $(M + H)^+$. **13e** was hydrolyzed with TFA in CH_3CN/H_2O. Purification by preparative reverse phase HPLC yielded **14e** as a white solid, 33 mg (41%). ^{1}H NMR (CD_3OD): δ 8.45 (s, 1H), 7.95 (m, 1H), 7.93 (s, 3H), 7.59 (m, 2H), 5.55 (d, J = 10.2 Hz, 1H), 4.65 (dd, J = 3.9, 9.0 Hz, 1H), 4.52 (dd, J = 5.1, 16.2 Hz, 1H), 4.39 (m, 2H), 4.08 (dd, J = 3.0, 16.5 Hz, 1H), 3.79 (m, 3H), 3.50 (dd, J = 2.4, 13.8 Hz, 1H), 3.28 (m, 1H), 2.71 (m, 1H), 2.53 (m, 1H). ESI-MS 490.1 $(M + H)^+$.
16. 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). 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.
17. 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 4 h. Cells were centrifuged and the conditioned media were 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.

18. The values for the free drug form of Pralnacasan® (compound **3b**) in our assays were comparable to those reported in similar bioassays; see Rudolphi, K.; Gerwin, N.; Verzijl, N.; van der Kraan, P.; van den Berg, W. *OsteoArthritis Cartilage* **2003**, *11*, 738.