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Synthesis and evaluation of tricyclic pyrrolopyrimidinones as dipeptide mimetics: Inhibition of interleukin-1β-converting enzyme

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Abstract—The application of a tricyclic pyrrolopyrimidinone scaffold for the synthesis of peptidomimetic inhibitors of interleukin-1β-converting enzyme (ICE) is reported. The synthesis of the tricyclic scaffold and conversion of it to a variety of target ICE inhibitors were accomplished in 4–5 steps. In vitro biological evaluation of the tricyclic pyrrolopyrimidinones revealed fair to good ICE inhibitors, with the most active compound exhibiting an IC_{50} of 14 nM in a caspase-1 enzyme binding assay.

Interleukin-1β (IL-1β) has been implicated in a number of disease states, including rheumatoid arthritis, osteoarthritis (OA), and sepsis.[1] In recent years, significant attention has been given to IL-1β, as it is widely believed to contribute significantly to the cartilage degradation, joint inflammation and pain associated with OA.[2,3] Interleukin-1β-converting enzyme (ICE) is known to be responsible for processing pro-IL-1β to its active form (IL-1β) and then releasing it extracellularly.[4,5] Therefore, inhibition of ICE should provide a means of controlling IL-1β levels therapeutically, providing disease modification and symptomatic relief for OA patients.

ICE, or caspase-1, is a member of the Caspase family of enzymes, a class of cysteine proteases sharing sequence homology and a preference for an aspartate residue at the P1 position of their substrates.[6] As a result of this preference, most ICE inhibitors reported to date possess an aspartic acid derived cysteine trap that is essential for inhibition. Additionally, it is well known that a hydrophobic P4 residue in the substrate is essential for selective inhibition of caspase-1 over other caspases.[6]

Since Merck's first report of a potent reversible tetrapeptide ICE inhibitor, **1**[7] (Fig. 1), there have been several potent inhibitors described.[8] While most of the focus for inhibitor design has been on constrained dipeptide mimics such as **2**[9], there have been reports of non-peptidic pyrimidinone and pyridone-based inhibitors of ICE, such as **3**–**5**.[10–13] These heteroaryl-based inhibitors are reported to exhibit modest potency for ICE inhibition, demonstrating that constraining the P3 region of the inhibitor in the form of a planar amino pyrimidinone/pyridone ring maintains activity. Tricyclic pyrimidinone **5** effectively constrains the P2 region as well as the P3, but sacrifices a key hydrogen bond donor in the P3 residue.

The key binding elements for ICE inhibition are well known based on the reported crystal structure of tetrapeptide **1** bound to the active site of ICE.[14] A successful scaffold for inhibition of ICE must properly orient the aspartate-derived trap and the P4 hydrophobic group, while maintaining certain hydrogen bonds with the peptide backbone in the S1 and S3 residues (Fig. 2). The P3 carbonyl and NH both participate in critical hydrogen bonds that help to orient the inhibitor in the active site. It was our belief that an appropriately substituted heteroaryl-fused pyrimidinone could effectively mimic

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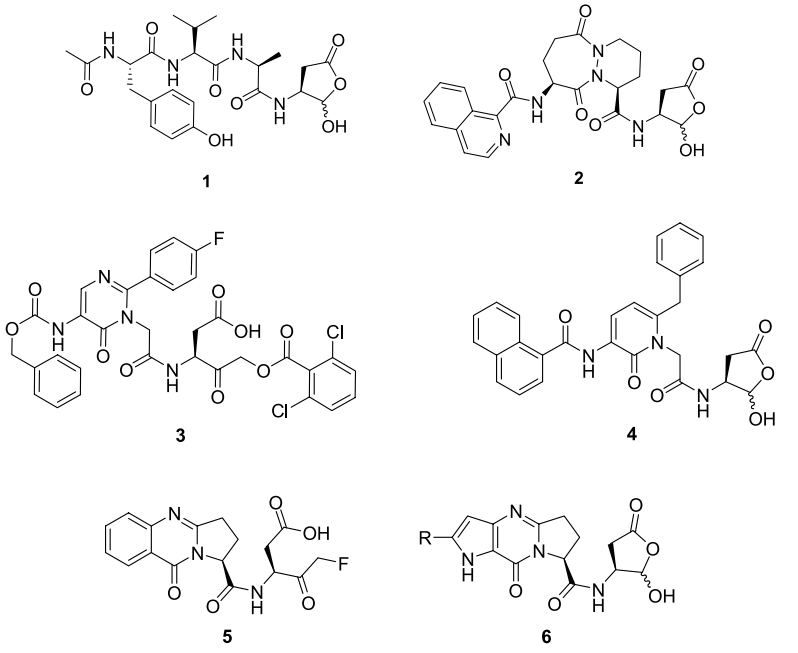


Figure 1. Selected inhibitors of ICE.

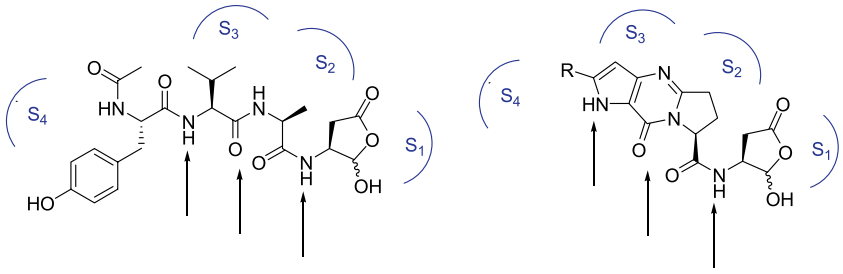
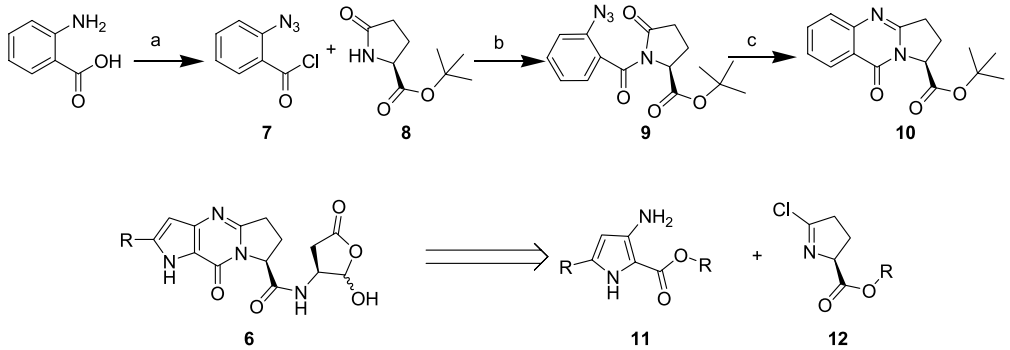


Figure 2. ICE inhibitor binding elements.

the preferred conformation for the P3–P4 region of the tetrapeptide while retaining the key hydrogen-bonding residues, thereby giving a potentially potent, non-peptide ICE inhibitor such as **6**. The R substituent of **6** may additionally provide the hydrophobic interactions essential for selectivity of caspase-1 over other caspases.

Though the proposed inhibitor **6** shares some structural similarity with compound **5**, we felt that incorporation of a pyrrole ring into our inhibitors would necessitate designing a new synthetic pathway. The reported synthesis of the related tricyclic core of **5** involved the diazotization of anthranilic acid, followed by acid chloride formation to yield **7**. Coupling of **7** with the lithium anion of *t*-butyl pyroglutamate gave compound **9**. The central ring was closed with an intramolecular aza-Wittig reaction to yield the tricyclic core **10** in four total steps.[13] (Scheme 1).

We believed that the tricyclic core of **6** could be prepared in a single step from the readily available amino esters **11** and chloroimine **12**. To test the validity of this



Scheme 1. Reagents and conditions: (a) 1: $NaNO_2$/HCl/NaN_3/NaOAc, 2: $SOCl_2$; (b) LDA; (c) PPh_3, xylene, reflux.

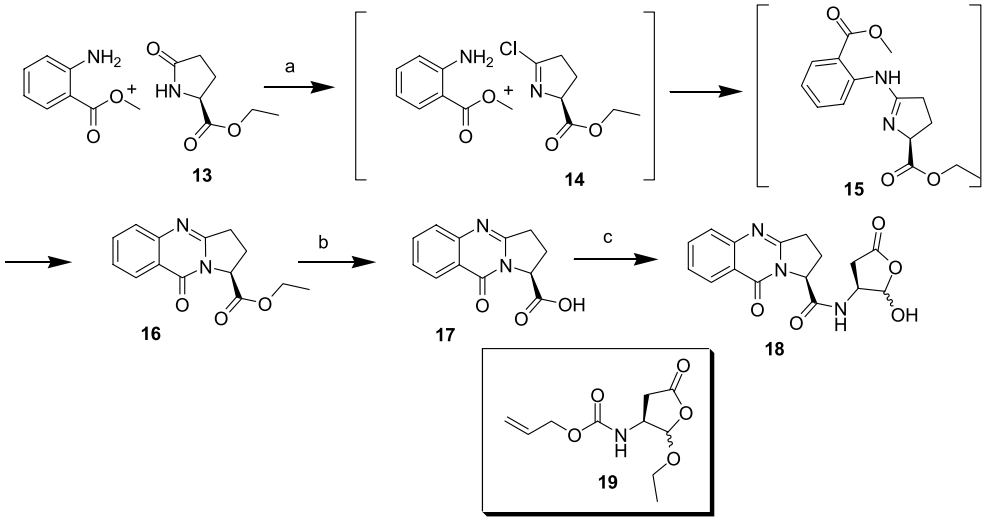
approach, compound **16**, possessing the same tricyclic core as **10**, was synthesized from methyl anthranilate and ethyl pyroglutamate, **13**. A mixture of methyl anthranilate and ethyl pyroglutamate in dichloroethane was treated with $POCl_3$ and heated at reflux for 3 h. It is believed that the pyroglutamate reacts with $POCl_3$ to form intermediate **14**, which rapidly reacts with the aniline nitrogen of methyl anthranilate to form amidine **15**. Intermediate **15** then undergoes spontaneous cyclization to form the desired tricyclic core, **16**, in a single step in 91% yield (Scheme 2).

The ethyl ester, **16**, was subsequently hydrolyzed with NaOH to give carboxylic acid **17**. After in situ deprotection of the Alloc protecting group, aspartate-derived cysteine trap **19**[15] was coupled with **17**. Acetal hydrolysis with TFA gave the final inhibitor **18**, a reversible ICE inhibitor analogous to **5**. These synthetic investigations provided us with good confidence that the new methodology could be applied to the readily available amino esters, **20a–e** to give our desired tricyclic core.

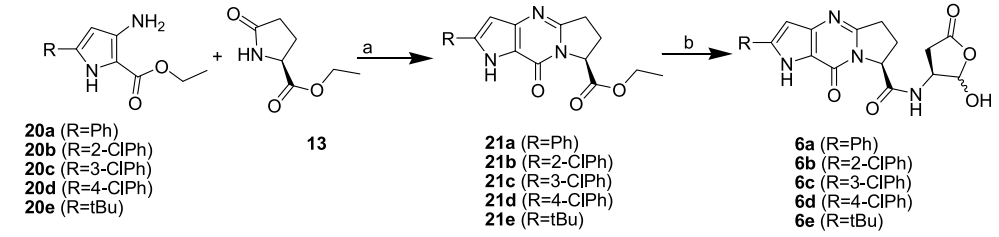
The synthesis of pyrroles **6a–e** (Scheme 3) from aryl substituted pyrrole amino esters **20a–e**[16] under the same conditions initially failed to provide the desired tricyclic core in appreciable amounts, presumably due to problems with the solubility of intermediates after the addition of $POCl_3$. This problem was accounted for with a solvent exchange. After initial reaction of pyrroles **20a–e** with ethyl pyroglutamate and $POCl_3$ in dichloroethane, the crude reaction mixture containing the intermediate amidine was concentrated in vacuo and redissolved in DMF. Heating the DMF solution at 110 °C affected the cyclization to provide tricyclic core, **21a–e**,[17] in 40–50% yield. Compounds **21a–e** were then carried on to the final inhibitors in a fashion analogous to that described for **18**.[18]

These novel ICE inhibitors were screened in a THP-1 whole cell assay[19] measuring IL-1β production to evaluate potency. Out of the five pyrrole scaffolds synthesized, four had whole cell $IC_{50} < 1000$ nM. These compounds were then evaluated in isolated enzyme assays[20] to evaluate selectivity for caspase-1 over caspase-3 and -8 (Table 1). The tricyclic pyrrolopyrimidinone-based inhibitors exhibited selectivity for caspase-1 of >100-fold over caspase-3 and >50-fold over caspase-8.

In summary, it has been demonstrated that hetero-aromatic rings can function as peptidomimetic backbones for ICE inhibition. The pyrrolopyrimidinone inhibitors described here were synthesized in 3–4 steps from readily available starting materials. The most potent inhibitor, **6b**, exhibited good activity (14 nM at caspase-1 and 252 nM in THP-1) while maintaining excellent selectivity over caspase-3 and -8.



Scheme 2. Reagents and conditions: (a) $POCl_3$, DCE, reflux, 3 h; (b) 1 N NaOH, MeOH; (c) 1: **19**, *N*,*N*-dimethylbarbituric acid, $Pd(PPh_3)_4$, HOBt, EDCI; 2: TFA, H_2O, AcCN.



Scheme 3. Reagents and conditions: (a) 1: $POCl_3$, DCE, RT, 10 min; 2: DMF, 110 °C, 3 h; (b) 1: 1 N NaOH, MeOH; 2: **19**, *N*,*N*-dimethylbarbituric acid, $Pd(PPh_3)_4$, HOBt, EDCI; 3: TFA, H_2O, AcCN.

Table 1. Caspase inhibition data

Compound	Ar	R	THP-1 IC_{50} (nM)	Casp-1 IC_{50} (nM)	Casp-3 IC_{50} (nM)	Casp-8 IC_{50} (nM)
18	Ph	H	10,000	4583	10,000	10,000
6a	Pyrrole	Ph	1064	264	10,000	3046
6b	Pyrrole	2-ClPh	252	14	1841	336
6c	Pyrrole	3-ClPh	529	55	4394	1823
6d	Pyrrole	4-ClPh	830	97	10,000	4811
6e	Pyrrole	tBu	975	421	2293	7540

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17. 2-(2-Chlorophenyl)-8-oxo-5,6,7,8-tetrahydro-1*H*-1,4,7a-triaza-*s*-indacene-7-carboxylic acid ethyl ester (**21b**): To a solution of **20b** (1.71 g, 6.46 mmol) and **13** (1.27 g, 8.08 mmol) in 100 mL 1,2-dichloroethane, $POCl_3$ (1.0 mL, 17.25 mmol) was added dropwise. The solution was heated to reflux for 10 min, and then concentrated in vacuo. The residue was re-dissolved in 50 mL of DMF and heated to 110 °C for 3 h. The solution was cooled, concentrated, and purified by flash chromatography (15% ethyl acetate in hexanes) to yield 1.03 g (45%) of **21b** as a yellow solid. ^1H (CD_3OD) δ 7.66 (m, 1H), 7.60 (m, 1H), 7.45 (m, 2H), 6.77 (s, 1H), 5.27 (dd, $J = 9.9$, 3.0 Hz, 1H), 4.31 (q, $J = 7.2$ Hz, 2H), 3.42–3.28 (br m, 3H), 2.78 (m, 1H), 2.47 (m, 1H), 1.34 (t, $J = 7.2$ Hz, 3H); MS 358 $(M + H)^+$.
18. 2-(2-Chlorophenyl)-8-oxo-5,6,7,8-tetrahydro-1*H*-1,4,7a-triaza-*s*-indacene-7-carboxylic acid (2-hydroxy-5-oxo-tetrahydro-furan-3-yl)-amide (**6b**): a solution of **21b** (500 mg, 1.40 mmol) in methanol (75 mL) was treated with excess 1 N NaOH (14.0 mL) and stirred for 15 h at rt. The solution was acidified with 1 N HCl and extracted with EtOAc. The crude acid of **21b** was isolated as a yellow solid, 446 mg (96%), MS 330 $(M + H)^+$. Catalytic $Pd(PPh_3)_4$ was added to a solution of **19** (670 mg, 2.92 mmol) and *N*,*N*-dimethylbarbituric acid (2.0 g, 12.81 mmol) in 75 mL CH_2Cl_2 at rt. The solution was stirred at rt for 15 min and then the crude carboxylic acid of **21b** prepared above was added as a solution in 15 mL DMF followed by HOBt (1.1 g, 8.14 mmol), and EDCI (1.4 g, 7.3 mmol). The solution was stirred for 3 h, diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated. After purification by preparative reverse phase HPLC the product was hydrolyzed with TFA in CH_3CN/H_2O. Further purification by preparative reverse phase HPLC yielded **6b** as a white solid, 346 mg (60%). ^1H NMR (CD_3OD) δ 7.66 (m, 1H), 7.60 (m, 1H), 7.46 (m, 2H), 6.76 (s, 1H), 5.30 (m, 1H), 4.71 (m, 1H), 4.35 (m, 1H), 3.35 (m, 2H), 2.70 (m, 4H); MS 429 $(M + H)^+$.
19. A suspension of human monocytic cells (THP-1, ATCC strain TIB202, 2×10^6/ml in RPMI 1640 medium from Gibco BRL) were 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.

20. The isolated Caspase enzyme (caspase-1, 3, and 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 was the initial velocity of substrate cleavage in the presence of inhibitor at concentration [I], and V_o was the initial velocity in the absence of inhibitor.