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Synthesis and evaluation of novel 1-(2-acylhydrazinocarbonyl)-cycloalkyl carboxamides as interleukin-1β converting enzyme (ICE) inhibitors

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Abstract—Novel 1-(2-acylhydrazinocarbonyl)cycloalkyl carboxamides were designed as peptidomimetic inhibitors of interleukin-1β converting enzyme (ICE). A short synthesis was developed and moderately potent ICE inhibitors were identified (IC_{50} values <100 nM). Most of the synthesized examples were selective for ICE versus the related cysteine proteases caspase-3 and caspase-8, although several dual-acting inhibitors of ICE and caspase-8 were identified. Several of the more potent ICE inhibitors were also shown to inhibit IL-1β production in a whole cell assay (IC_{50} < 500 nM).

Interleukin-1 (IL-1) produces both inflammatory and immunoregulatory responses in the cell and can contribute to a variety of disease states. IL-1 is comprised of two proteins IL-1α and IL-1β. Both have roles in the progression of inflammation, but IL-1β is produced in larger quantities by the cell.[1] IL-1β is synthesized as a biologically inactive precursor (pro-IL-1β) that requires processing by interleukin-1β converting enzyme (ICE or caspase-1) at the Asp116-Ala117 site for activation.[2] Increased cellular levels of ICE were found in explant cartilage under osteoarthritic conditions, and concurrently increased levels of IL-1β were found in the surrounding synovial fluid.[3] The inhibition of ICE may become a treatment for IL-1β mediated diseases, which includes the potential treatment of rheumatoid and osteoarthritis.

The tetrapeptide Ac-YVAD-CHO (**1**, Fig. 1) is a potent competitive inhibitor of ICE and showed that inhibition of ICE in whole blood prevented the secretion of IL-1β.[4] Since the discovery of peptide **1**, several other ICE inhibitors have been reported.[5] A number of these inhibitors possessed a geometrically constrained bicyclic backbone which preserved key hydrogen bonding interactions and allowed the proper positioning of the P1 aspartic aldehyde moiety and the P4 hydrophobic moiety. One of the most advanced inhibitors is based on a pyridazinodiazepine bicyclic scaffold of the P2 and P3 areas of peptide **1** (**2**, Pralnacasan free drug).[6] However,

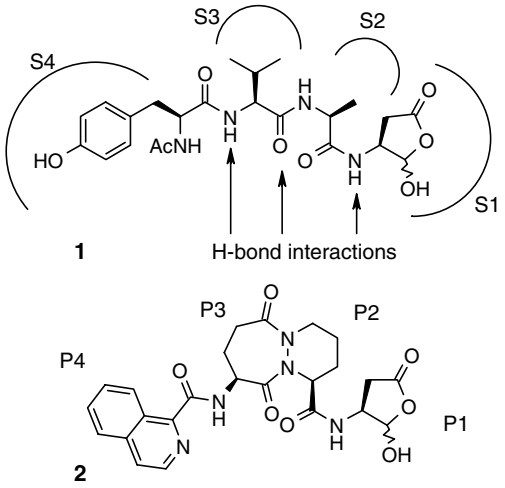

Figure 1. Known ICE inhibitors and proposed binding interactions.

Keywords: Interleukin-1β converting enzyme; ICE; Inhibitor; Peptidomimetic scaffold.
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the synthesis of **2** and related bicyclic inhibitors is lengthy, requiring >10 steps.

We now report the synthesis and evaluation of 1-(2-acylhydrazinocarbonyl)cycloalkyl carboxamides (**3**, Fig. 2) as reversible ICE inhibitors. The target compounds were designed to hold the positioning of the aspartic aldehyde moiety in the S1 pocket, and to present an aryl group into the S4 hydrophobic region. We hypothesized that the azapeptide scaffold would also maintain key hydrogen bonding interactions in the ICE active site.[7] The 1-(2-acylhydrazinocarbonyl)cycloalkyl carboxamide scaffold allowed for the removal of a several points of chirality, and greatly simplified the synthesis the target inhibitors.

A series of 1,1-dicarboxycycloalkanes was used as the core building blocks in order to mimic the P2 area of the inhibitor **1**. 1,1-Dicarboxycyclopropane and cyclobutane were obtained commercially. The cyclopentyl, cyclohexyl, and indanyl dicarboxylates were synthesized in two steps using a literature procedure.[8] The valine (P3) moiety of compound **1** was replaced by an acyl hydrazide linkage allowing for diversity to be conveniently introduced at the P4 region using an acylation reaction. Finally, the remaining carboxy group provided the attachment point for the aspartic aldehyde moiety as P1.

The synthesis of compound **16** is representative (Scheme 1).[9] 1,1-Dicarboxycyclopentane was coupled to 1-naphthoyl hydrazide using 1-ethyl-3-dimethylaminopropyl-carbodiimide (EDAC) and hydroxybenzotriazole (HOBt) to give compound **4**. For other examples, the synthesis was altered to produce higher yields in this coupling step through the use of the mono-ethyl ester of the malonate precursor. The mono-esters were then saponified to give analogs of compound **4**.

Compound **4** was then coupled (EDAC and HOBt) with the protected aspartic aldehyde moiety **5**.[10] The reaction proceeded via an in situ deprotection of the amine of **5** with dimethylbarbituric acid and triphenylphosphine in dichloromethane at room temperature. Final conversion to provide compound **16** occurred by treating the semi-purified coupling product with TFA in acetonitrile and water.

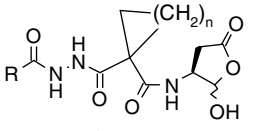

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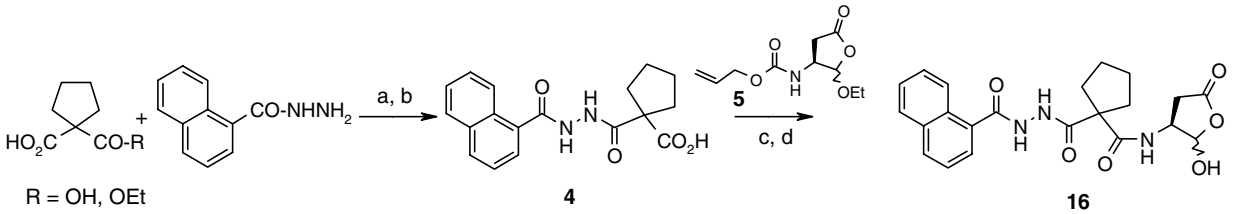
Figure 2. 1-(2-Acylhydrazinocarbonyl)cycloalkyl carboxamides.

The synthesized 1-(2-acylhydrazinocarbonyl)cycloalkyl carboxamides were evaluated for their ability to inhibit ICE and the related cysteine proteases caspase-3 and caspase-8.[11] In addition, the compounds were tested in a whole cell THP-1 assay to measure their ability to inhibit the production and secretion of IL-1β.[12] The results are presented in Table 1. Several of the analogs possessed strong activity as ICE inhibitors with IC_{50} values <100 nM. Particularly noteworthy were compounds **12**, **16**, **20**, **25**, and **26**.

All of the described compounds were inactive as inhibitors of caspase-3 (IC_{50} values >10 μM), but they were found to be variously active against caspase-8. Although a majority of the analogs tested were selective for ICE over caspase-8, a number of compounds exhibited comparable potency. In particular, it was observed that most of the 1-naphthyl substituted compounds (e.g., compounds **12**, **16**, and **20**) were comparably potent ICE and caspase-8 inhibitors (IC_{50} values <150 nM). It was also observed that compound **9** (an analog possessing an *ortho*-substituted aryl group in the P4 position) was equipotent at inhibiting ICE and caspase-8. This led to the investigation of the 2,6-disubstituted compounds **22** and **23**, which displayed a reversed selectivity, being more potent inhibitors of caspase-8 over ICE.

The structure–activity trends observed for variations at the P4 position of these 1-(2-acylhydrazinocarbonyl)cycloalkyl carboxamides revealed that *ortho*- and *meta*-substituted aromatic moieties were preferred over *para*-substitution. For example, in the cyclobutyl series, the methyl-containing compounds **9**–**11** showed a preference for *ortho*- and *meta*-substitution on the phenyl ring, with the *para*-substituted analog being 2- to 4-fold less potent. The 1-naphthyl derivatives (**12**, **16**, **20**, and **25**) were among the most potent analogs.

For the P2 site, the various cycloalkane moieties showed minor changes in potency with the exception of the cyclopropane analogs. The activity of derivatives based on cyclopropane (**6** and **7**) was dramatically decreased relative to the larger ring analogs. In contrast, the compounds in the indanyl series (**24**–**26**), which were designed as larger and more hydrophobic analogs, were



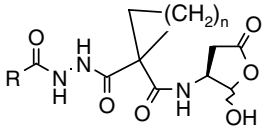
R = OH, OEt

4

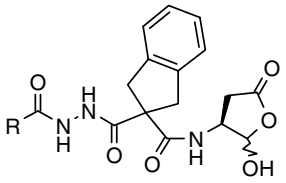
16

Scheme 1. Reagents and conditions: (a) EDAC, HOBt, 18 h, 30–95%; (b) LiOH, THF–H_2O (3:1), 95%; (c) compound **4**, *N*,*N*-dimethylbarbituric acid, $(Ph_3P)_4Pd$, CH_2Cl_2, HOBt, EDAC, 40–80%; (d) TFA, CH_3CN, H_2O, 60–95%.

Table 1. In vitro enzyme inhibition and whole cell THP-1 IC_{50} data for compounds **8**–**26**



Compounds **6-23**



Compounds **24-26**

Compound	*n*	R	Enzyme IC_{50}[a] (nM)		Whole Cell IC_{50}[b] (nM) THP-1
			ICE	Caspase-8	
2		Pralnacasan (free drug)	3.6	40	190
6	1	*m*-Methoxyphenyl	⩾10,000	⩾10,000	⩾10,000
7	1	2-Naphthyl	2900	⩾10,000	6400
8	2	*m*-Methoxyphenyl	260	2200	400
9	2	*o*-Methylphenyl	310	310	610
10	2	*m*-Methylphenyl	150	2700	620
11	2	*p*-Methylphenyl	580	4300	6900
12	2	1-Naphthyl	50	130	470
13	2	2-Naphthyl	240	6300	Not tested
14	3	*m*-Methoxyphenyl	200	1170	550
15	3	*m*-Methylphenyl	490	1200	2000
16	3	1-Naphthyl	40	90	480
17	3	2-Naphthyl	150	3200	570
18	4	*m*-Methoxyphenyl	240	1800	1300
19	4	*m*-Methylphenyl	430	3900	470
20	4	1-Naphthyl	50	70	450
21	4	2-Naphthyl	90	2600	790
22	4	2,6-Dimethylphenyl	⩾10,000	230	⩾2500[c]
23	4	2,6-Dimethoxyphenyl	700	300	⩾2500[c]
24	2-Indanyl	*m*-Methylphenyl	430	3120	⩾2500[c]
25	2-Indanyl	1-Naphthyl	40	420	690
26	2-Indanyl	2-Naphthyl	30	2100	500

[a] Enzyme IC_{50} results are expressed as ±30% or less.
[b] Whole cell THP-1 IC_{50} results varied approximately ±2-fold.
[c] 2500 nM was the maximum drug concentration used for this compound.

found to possess activity as ICE inhibitors that was comparable to the simpler cycloalkyl analogs.

The more potent ICE inhibitors were found to exhibit good activity in the whole cell THP-1 assay with IC_{50} values <1 μM. With the current series, a 2- to 10-fold reduction in potency for the THP-1 assay relative to the ICE enzyme activity was observed. We interpreted the whole cell results as a combined measure of the compounds' ability to penetrate the cell membrane and their potencies as enzyme inhibitors.

In conclusion, we developed an expedient synthesis of 1-(2-acylhydrazinocarbonyl)cycloalkyl carboxamides, a unique class of monocyclic ICE inhibitors. Of the various substitutions evaluated, the indanyl analog **26** showed the best overall activity profile: good enzyme potency for ICE (albeit, approximately 10-fold less active than the bicyclic inhibitor **2**), good selectivity for ICE over caspase-8, and moderate activity in the THP-1 assay. Finally, the potency against caspase-8 was shown to be tunable based on the selection of an appropriate P4 substitution. For example, the 1-naphthyl P4 substituted analogs generally provided potent dual-acting inhibitors of ICE and caspase-8, as exemplified by compounds **12**, **16**, and **20**. These compounds may serve as prototypes for identifying selective caspase-8 inhibitors with potential therapeutic utility in modulating apoptosis, NF-κB signaling, and T-cell activation.[13]

Due to the modest overall potency of these compounds, further work on this class was terminated. However, this activity in combination with the comparative ease of synthesis did prompt us to further evaluate other classes of monocyclic ICE inhibitors. The results of these studies are reported separately.[14]

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9. Preparation of *N*-[(3*S*)-2-hydroxy-5-oxotetrahydrofuran-3-yl]-1-{[2-(1-naphthoyl)hydrazino]carbonyl}cyclopentane carboxamide (**16**). To naphthalene-1-carboxylic acid hydrazide hydrochloride (0.445 g, 2.0 mmol) in dichloromethane (50 mL) were sequentially added triethylamine (1.5 mL, 10 mmol), cyclopentane-1,1-dicarboxylic acid (1.58 g, 10 mmol), and EDAC hydrochloride (0.383 g, 2.0 mmol). This solution was stirred for one week. The reaction solution was extracted with a 1:1 solution of saturated sodium bicarbonate and water (4× 100 mL). The basic aqueous layers were acidified with concentrated HCl to pH 2. The acidic aqueous layers were extracted with ethyl acetate (3× 300 mL). The organic extractions were reduced under vacuum to give a white solid that was purified by HPLC. The combined fractions from chromatography were reduced under vacuum to give compound **4** as a white solid (0.129 g, 20%). ^{1}H NMR (300 MHz, CD_3OD): δ 1.77–1.84 (br m, 4H), 2.33–2.42 (br m, 4H), 7.54–7.63 (m, 3H), 7.78–7.81 (m, 1H), 7.94–7.97 (m, 1H), 8.03–8.06 (d, 1H, J = 8.4 Hz), 8.41–8.44 (m, 1H). MS (ESI): *m/e* = 327.17 (M+H).To a solution of compound **5** (0.218 g, 0.95 mmol) in dichloromethane (20 mL) was added tetrakis(triphenylphosphine)palladium(0) (0.102 g, 0.088 mmol). After a few seconds of stirring, bubbles evolved. After stirring for an additional 10 min, compound **4** (0.115 g, 0.35 mmol), EDAC hydrochloride (0.162 g, 0.84 mmol), 1-HOBt (0.114 g, 0.84 mmol), and barbituric acid (0.357 g, 2.3 mmol) were added. Extra dichloromethane (10 mL) and *N*,*N*-dimethylformamide (1 mL) were added to aid the solubility of all reagents. After 1 h, the reaction solution was diluted with ethyl acetate (50 mL), washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL). The organic layer was reduced under vacuum to furnish an orange residue that was carefully chromatographed using flash column chromatography (long hold with 100% CH_2Cl_2 and then slow gradient to MeOH–CH_2Cl_2 2:98). A pale orange residue was obtained, which contained *N*-[(3*S*)-2-ethoxy-5-oxotetrahydrofuran-3-yl]-1-{[2-(1-naphthoyl)hydrazino]carbonyl}cyclopentane carboxamide as a diastereomeric mixture, plus a less polar impurity (observed by thin-layer chromatography). This material was used without further purification. MS (ESI): *m/e* = 454.19 (M+H).This partially purified material was dissolved in acetonitrile (10 mL) and water (5 mL), and trifluoroacetic acid (10 mL) was added. After 1 h of stirring, the solvent was removed under reduced pressure to furnish a faintly orange residue that was purified by HPLC. The combined fractions from chromatography were concentrated under reduced pressure to furnish compound **16** as a clear residue (0.054 g, 35% over two steps). ^{1}H NMR (300 MHz, CD_3OD): δ 1.76–1.81 (br m, 4H), 2.27–2.39 (br m, 4H), 2.60–2.80 (m, 2H),4.37–4.46 (m, 1H), 4.65–4.71 (dd, 1H, J = 13.2, 4.8 Hz), 7.54–7.64 (m, 3H), 7.79–7.81 (m, 1H), 7.93–7.98 (m, 1H), 8.04–8.07 (d, 1H, J = 8.4 Hz), 8.43–8.46 (m, 1H); ^{13}C NMR (75.5 MHz, CD_3OD): δ 24.91, 33.67, 34.82, 61.54, 105.05, 124.66, 125.37, 125.83, 126.45, 126.98, 128.19, 130.53, 131.04, 131.85, 133.93, 173.19. MS (ESI): *m/e* = 426.14 (M+H).
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12. 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 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.
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