Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4291–4294
Bioorganic & Medicinal Chemistry Letters

Discovery of novel conformationally restricted diazocan peptidomimetics as inhibitors of interleukin-1β synthesis

Kofi A. Oppong,* Christopher D. Ellis, Michael C. Laufersweiler, Steven V. O'Neil, Yili Wang, David L. Soper, Mark W. Baize, John A. Wos, Biswanath De, Gregory K. Bosch, Amy N. Fancher, Wei Lu, Maureen K. Suchanek, Richard L. Wang and Thomas P. Demuth, Jr.

Procter & Gamble Pharmaceuticals, Inc., 8700 Mason-Montgomery Road, Mason, OH 45040, USA

Received 3 May 2005; revised 16 June 2005; accepted 20 June 2005
Available online 19 July 2005

Abstract—A novel diazocan containing dipeptide mimetic was synthesized via reductive N–N bond cleavage of a pyrazolidino-pyrazolidine using Raney-Ni and evaluated as an ICE inhibitor. This versatile 8-membered ring containing scaffold possesses an N-5 ring nitrogen that was used to explore structure-activity relationships in a cell-based assay measuring inhibition of interleukin-1β.

In recent years, there has been a substantial effort toward the development of therapeutic agents in the area of arthritis.[1] The cytokine (interleukin-1β), a critical mediator in the inflammatory pathway, has been implicated in the progression of this disease, and one method for modulating the levels of the IL-1β is through inhibition of IL-1β converting enzyme (ICE).[2,3] ICE, also known as caspase-1, is a member of the caspase (cysteine aspartate proteases) family of enzymes which require an aspartic acid residue for recognition and is responsible for the processing of IL-1β from an inactive pro-form into the mature cytokine.[3] It has been recently reported that a selective peptide ICE inhibitor blocks IL-1β production in a human cartilage explant culture.[4] Furthermore, ICE inhibitors show both anti-inflammatory and analgesic effects in animal models.[5] The validity of IL-1 modulation as a therapeutically relevant approach gained additional credibility with the FDA approval of Kineret®, an injectable IL-1 receptor antagonist for rheumatoid arthritis (RA) therapy.[6]

At present, most small molecule inhibitors of ICE have been based on the tetrapeptide (Ac-YVAD-CHO) **1** (Fig. 1), which is reported to block the release of IL-1β from human whole blood cells with an IC_{50} of 4 μM.[7] A widely utilized approach for the development of ICE inhibitors has been to constrain the Val-Ala portion (Fig. 1, structure **2**) of the tetrapeptide, thereby maintaining the important hydrogen bond interactions of the peptide backbone and properly orienting the important P1 and P4 recognition regions of the molecule in the enzyme pocket.[8] One notable example of the application of this strategy is Pralnacasan® **3**, a reversible ICE inhibitor, which constricts the P2–P3 region of the molecule as a pyridazinodiazepine. [5] Pralnacasan® progressed into clinical trials,[9] but the phase II program was discontinued due to liver abnormalities in dogs after a nine-month exposure to a high dose of Pralnacasan®.

In this article, we describe the development of a novel diazocan peptidomimetic **4**, and the evaluation of it as an ICE inhibitor. Our approach to this design was guided by four main criteria: (a) preservation of key binding interactions with ICE, (b) structural novelty of scaffold, (c) ease of synthesis, and (d) the ability to explore new binding interactions in the S3 region of the enzyme pocket. In addition, we believed that the secondary nitrogen at N-5 may also serve as a handle for optimization of the physical/chemical properties of the molecule.[10]

Monocyclic 8-membered lactams have generally been elusive targets due to the inability to overcome entropic barriers associated with traditional lactam formation.[11] Our approach hinged on a reductive N–N bond scission

Keywords: ICE; Caspase-1; Diazocan; Peptidomimetic; Interleukin-1β.
* Corresponding author: Tel.:+1 513 622 0481; fax:+1 513 622 5338; e-mail: Oppong.ka@pg.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.06.050

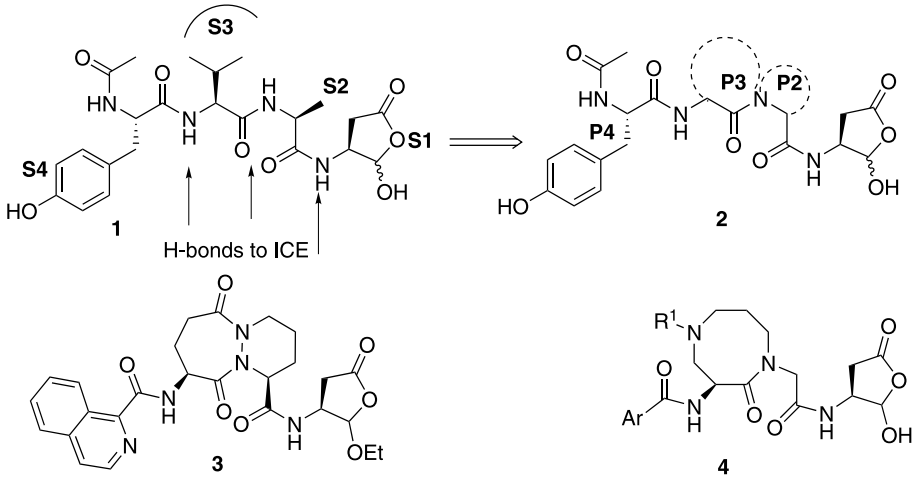


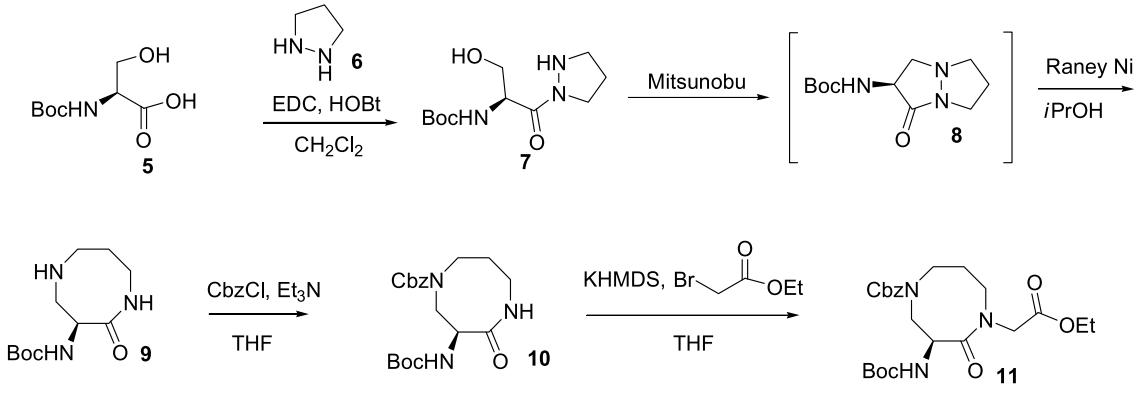
Figure 1.

of the intermediate pyrazolidino-pyrazolidine (**8**) with Raney-Ni.[12]

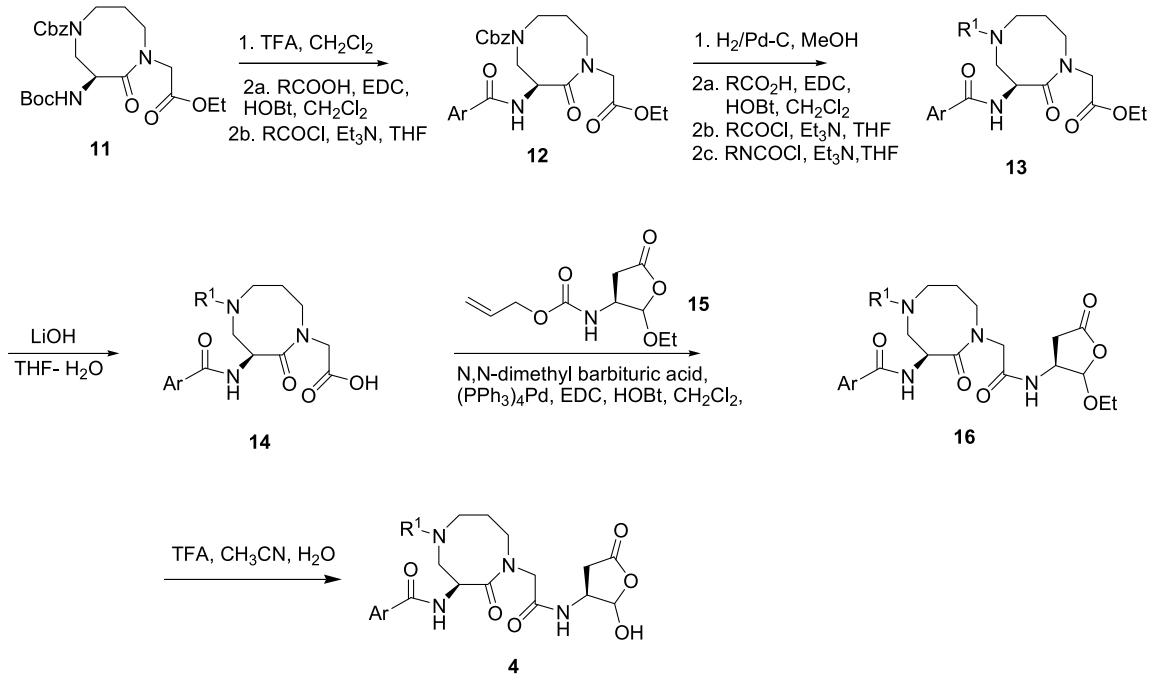
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) coupling of pyrazolidine **6** with Boc-L-serine led to **7** in 75% yield (Scheme 1). Standard Mitsunobu[13] reaction conditions were employed to convert alcohol **7** into the intermediate pyrazolidino-pyrazolidine (**8**), which is carried forward without isolation into the next sequence of reactions. Reductive cleavage of the N–N bond in **8**[14] with Raney-Ni in isopropanol at 50 °C for 2 h led to isolation of target diazocan intermediate **9** in 75–84% yield over two steps. Dipeptide **11** was obtained by a sequence that involved initial protection of the secondary amine as the benzyl carbamate, followed by alkylation of the amide nitrogen with ethyl bromoacetate.

Conversion of dipeptide **11** into an ICE inhibitor (Scheme 2) involved an initial deprotection of the Boc-carbamate with TFA in CH_2Cl_2, followed by acylation of the amine with a variety of aryl acids or aryl acid chlorides. Standard deprotection of the benzyl carbamate with H_2/Pd-C was followed by conversion of the resultant secondary amine into a variety of amides and ureas. The final sequence involved initial base hydrolysis of the ester of **13** using LiOH, followed by EDC coupling to the cysteine trap precursor **15**.[15] Final acid hydrolysis of the acetal of **16** affords the final compound **4**.

Using SAR developed for another series of ICE inhibitors,[16] we synthesized compounds bearing a small number of P4 substituent groups (**18**–**23**, Table 1), keeping the N-5 substituent as a benzamide. For screening purposes, we selected a THP-1[17] whole-cell assay measuring IL-1β production as our primary tool to evaluate potency. Since ICE is an intracellular target, we believed that a whole cell assay, taking into account properties such as cell permeability, would be more predictive of the future success in animal models. Among the six analogs screened, compound **21** distinguished itself as the most active with an IC_{50} of 205 nM.[18] With these results in hand, we retained the naphthyl amide as the preferred P4 substituent and made variations at N-5 (**24**–**36**, Table 1). We observed a slight preference at this position for small amide substituents, as exemplified by the formamide (**33**) and the acetamide (**34**). The morpholino-urea substituent (**36**) also proved to be a potent inhibitor in the THP whole-cell assay at 224 nM. Overall, none of the substituents screened at N-5 showed a remarkable improvement compared to the parent benzamide (**21**). A selection of diazocan ICE inhibitors was screened in caspase-1, -3, and -8[19] enzyme inhibitor assays to confirm their ability to inhibit ICE and to assess the

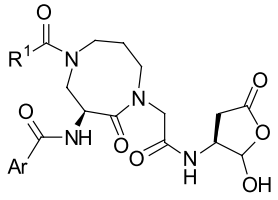


Scheme 1.



Scheme 2.

Table 1. Diazocan SAR



Compound	Ar	R1	THP-1 IC_{50} (nM)	Casp-1 IC_{50} (nM)	Casp-3 IC_{50} (nM)	Casp-8 IC_{50} (nM)
18	Ph	Ph	1015			
19	3-CF_3Ph	Ph	1019			
20	5-Cl-2-MePh	Ph	298			
21	2-Naphthyl	Ph	205	19	>10,000	>10,000
22	1-Isoquinoline	Ph	280	30	>10,000	>10,000
23	2-Benzothiophene	Ph	560			
24	2-Napthyl	Ph(2-OMe)	246	38	>10,000	>10,000
25	2-Napthyl	Ph(3-OMe)	229	15	>10,000	>10,000
27	2-Napthyl	Ph(3-F)	397	22	>10,000	>10,000
28	2-Napthyl	Ph(2-CF_3)	1525			
29	2-Napthyl	Ph(3-CF_3)	1736			
30	2-Napthyl	Ph(5-Cl-2-Me)	>2500			
31	2-Napthyl	Piperonyl	285	24	>10,000	>10,000
32	2-Napthyl	CH_2Ph	808			
33	2-Napthyl	H	219			
34	2-Napthyl	Me	216			
35	2-Napthyl	Cyclohexyl	577			
36	2-Napthyl	N-morpholine	224	44	>10,000	>10,000
3	Pralnacasan®		120	2		

selectivity for caspase-1 over other caspases. Overall, the diazocan inhibitors showed an excellent selectivity profile against caspase-3 and -8. The most active inhibitor **21** was less potent than Pralnacasan® in the caspase-1 enzyme assay, but showed comparable activity in the THP-1 whole-cell assay.

In summary, we have developed an efficient synthesis of a potent novel diazocan ICE inhibitor starting from Boc-serine and pyrazolidine. Though the diazocan scaffold possessed a versatile secondary nitrogen at N-5, variations at this position had little effect on the observed activity. In the THP whole cell assay, we

identified the naphthalene-2-carboxylic acid diazocan amide (**21**)[20] as the most potent inhibitor of ICE in this series, comparable to Pralnacasan®. We plan to discuss data from ongoing in-vivo studies in the near future.

References and notes

1. For a review on IL-1 biology, see: (a) Dinarello, C. A.; Wolff, S. M. *N. Engl. J. Med.* **1993**, *328*, 106; (b) Pelletier, J. P.; DiBattistal, J. A.; Roughley, P.; McCollum, R.; Martel-Pelletier, J. *Rheum. Dis. Clin. North Am.* **1993**, *19*, 545; (c) Fernandez-Madrid, F.; Karvonen, R. L.; Teitge, R. A.; Miller, P. R.; Negendank, W. G. *J. Magn. Reson. Imaging* **1994**, *12*, 703.
2. (a) Dinarello, C. A.; Wolff, S. M. *N. Engl. J. Med.* **1993**, *328*, 106; (b) Ator, M. A.; Dolle, R. E. *Curr. Pharm. Des.* **1995**, *1*, 191.
3. (a) Wilson, K. P.; Black, J.; Thompson, J. A.; Kim, E. E.; Griffith, J. P.; Navia, M. A.; Murcko, M. A.; Chambers, S. P.; Aldape, J. P.; Raybuck, S. A.; Livingston, D. J. *Nature* **1994**, *370*, 270; (b) Sleath, P. R.; Hendrickson, R. C.; Kronheim, S. R.; March, C. J.; Black, R. A. *J. Biol. Chem.* **1990**, *265*, 14526.
4. Saha, N.; Moldovan, F.; Tardif, G.; Pelletier, J. P.; Cloutier, J. M.; Martel-Pelletier, J. *Arthritis Rheum.* **1999**, *42*, 1577.
5. Rudolphi, K.; Gerwin, N.; Verzijl, N.; Van der Kraan, P.; Van den Berg, W. *Osteoarthritis and Cartilage* **2003**, *11*, 738.
6. Bresnihan, B.; Alvaro-Gracia, J. M.; Cobby, M.; Doherty, M.; Domljan, Z.; Emery, P.; Nuki, G.; Pavelka, K.; Rau, R.; Rozman, B.; Watt, I.; Williams, B.; Aitchison, R.; McCabe, D.; Musikic, P. *Arthritis Rheum.* **1999**, *42*, 1781.
7. Thornberry, N. A.; Peterson, E. P.; Zhao, J. J.; Howard, A. D.; Griffin, P. R.; Chapman, K. T. *Biochemistry* **1992**, *33*, 3934.
8. Dolle, R. E.; Prasad, C. V. C.; Prouty, C. P.; Salvino, J. M.; Awad, M. M. A.; Schmidt, S. J.; Hoyer, D.; Ross, T. M.; Graybill, T. L.; Speier, G. J.; Uhl, J.; Miller, B. E.; Helasek, C. T.; Ator, M. A. *J. Med. Chem.* **1997**, *40*, 1941.
9. Randle, J. C. Abstracts of Papers, 228th National Meeting of the American Chemical Society, Philadelphia, PA; American Chemical Society: Washington, DC, 2004; Abstract U944.
10. Hanessian, S.; McNaughtonSmith, G.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789.
11. (a) Hocquemiller, R.; Cavè, A.; Husson, H. P. *Tetrahedron* **1977**, *33*, 653; (b) Evans, P. A.; Holmes, B. *Tetrahedron* **1991**, *47*, 9131; (c) Nubbemeyer, U. *Top. Curr. Chem.* **2001**, *216*, 125; (d) Maier, M. E. *Angew. Chem. Int. Ed.* **2000**, *39*, 2073.
12. Martinez-Perez, J. A.; Pickel, M. A.; Caroff, E.; Woggon, W. *Syn. Lett* **1999**, *12*, 1875.
13. Mitsunobu, O. *Synthesis* **1981**, *1*, 1.
14. The intermediate **8** is stable to column chromatography but decomposes over a period of one week when kept neat at room temperature. However it can be stored for extended periods as a cold solution in isopropanol.
15. Batchelor, M. J.; Bebbington, D.; Bemis, G. W.; Fridman, W. H.; Gillespie, R. J.; Golec, J. M. C.; Gu, Y.; Lauffer, D. J.; Livingston, D. J.; Matharu, S. S.; Mullican, M. D.; Murcko, M. A.; Murdoch, R.; Nyce, P. L.; Robidoux, A. L. C.; Su, M.; Wannamaker, M.; Wilson, K. P.; Zelle, R. E. PCT Intl. Appl. WO9722619, 1997.
16. Soper, D. L.; O'Neil, S. V.; Wang, Y.; Oppong, K. A.; Ellis, C. D.; Laufersweiler, M. C.; Demuth, T. P.; Fancher, A. N.; Lu, W.; Wang, R. L.; Schwecke, W. P.; Cruze, C. A.; Buchalova, M.; Belkin, M.; Wos, J. A. Abstracts of Papers, 228th National Meeting of the American Chemical Society, Philadelphia, PA; American Chemical Society: Washington DC 2004; Abstract U956.
17. Inhibition of IL-1β production in a cell-based assay: A suspension of human monocytic cells (THP-1, ATCC strain TIB202, 2×106/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.
18. The values obtained for the free drug form of Pralnacasan® 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 reference 5.
19. Inhibition of caspase-1, -3, and -8 enzymes: 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, USA). 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.
20. ^{1}H NMR (CD_3OD): δ 8.38 (s, 1H), 7.97–7.85 (m, 4H), 7.62–7.48 (m, 8H), 5.60 (dd, $J = 11.1$, 3.9 Hz, 1H), 4.66 (dd, $J = 6.0$, 3.9 Hz, 1H), 4.48–4.25 (m, 2H), 3.99–3.68 (m, 4H), 3.50 (m, 1H), 2.73 (ddd, $J = 16.2$, 6.3, 5.4 Hz, 1H), 2.56 (ddd, $J = 16.2$, 8.1, 2.7 Hz, 1H), 2.38 (bs, 1H), 2.01 (bs, 1H); FAB HRMS 559.217560 $(M + H)^+$.