Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 387–389

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Evaluation of δ-Lactams (Piperazones) as Elastase Inhibitors

Jürgen Seibel,[a] David Brown,[c] Augustin Amour,[c] Simon J. Macdonald,[b] Neil J. Oldham[a] and Christopher J. Schofield[a,*]

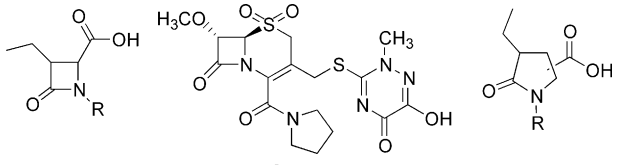
[a]*The Oxford Centre for Molecular Sciences and The Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, UK*
[b]*Medicinal Chemistry 1, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage SG1 2NY, UK*
[c]*Systems Research, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage SG1 2NY, UK*

Received 1 August 2002; revised 28 October 2002; accepted 6 November 2002

Abstract—A series of monocyclic δ-lactams (piperazones) was prepared and analysed as inhibitors of porcine pancreatic elastase and human neutrophil elastase.

Serine proteases and related enzymes are a diverse and ancient family of hydrolytic enzymes that share common active site characteristics including a nucleophilic serine residue and an 'oxy-anion' hole.[1] Included in the best characterized members of the family are the pancreatic serine proteases, trypsin, and chymotrypsin. Elastases are relatively non-selective enzymes belonging to the chymoptrypsin sub-family. They have been implicated in the development of various human diseases including emphysema, cystic fibrosis, and rheumatoid arthritis.[2] Elastases and other serine proteases, including those involved in blood clotting and infection, are current targets for medicinal chemistry.

Many different types of serine protease inhibitor have been developed. Amongst those forming a covalent bond with the target enzyme, extensive studies have been carried out with acylating agents, including studies on the inhibition of elastases by both monocyclic β-lactams (**1**) and bicyclic β-lactams (**2**).[3] Both a sufficient rate of formation and a threshold stability of the complex are required for useful inhibition via an acyl–enzyme complex.



1 **2** **3**

*Corresponding author. Fax: +44-1865-275674; e-mail:

β-Lactam inhibitors of serine proteases react to form relatively stable acyl–enzyme complexes (cf. that involved in catalysis) via ring-opening of the lactam by the nucleophilic side chain of Ser-195.[4–7] In one case the stability of the acyl–enzyme complex is related to rotation of the carbonyl group of the ester link out of the oxy-anion hole.[6]

5,5-*trans*-Fused γ-lactams are potent inhibitors of human neutrophil elastase (HNE); IC_{50} values of $<0.05\,\mu M$ being observed in some cases.[8,9] Certain monocyclic γ-lactams (**3**) have also been shown to inhibit elastase, but are relatively weak inhibitors compared to their β-lactam analogues. In one γ-lactam series, this is due, in part at least, to reversible formation of the γ-lactam from the acyl–enzyme and in another series also due to the hydrolytic lability of the acyl–enzyme in which the ester carbonyl was located in the oxyanion hole. Hydrolytically stable acyl–enzyme complexes are thus only formed when the hydrolytic water molecule itself is displaced or another aspect of the catalytic machinery is perturbed.[10,11]

Imming et al. have reported an interesting NMR study on the rates of hydrolysis of a series of lactams.[12] γ-Butyrolactam was found to be hydrolyzed considerably slower than β-propiolactam, but it was noted that 'δ-valerolactam and β-propiolactam had the same reactivity'. Penicillin antibiotics were more reactive than both by approximately a factor of 10^3. Medium-sized lactams were the least susceptible lactams to hydrolysis.

0960-894X/03/$ - see front matter
PII: S0960-894X(02)00995-2

This study suggested that δ-lactams have potential for use as antibacterials and serine protease inhibitors.

Here, we report the synthesis of a series of monocyclic δ-lactams/piperaz-2-ones and its evaluation for elastase inhibition. The choice of target took into account previous structure–activity work on β- and γ-lactams with respect to the group α- to the lactam carbonyl and the *N*-substituent. The choice of piperaz-2-ones as templates reflected their potential ease of synthesis, including a desire to minimize the number of chiral centres, and the possibility of modification in a 'combinatorial' manner.

The first explored route involved lactam formation as the final or penultimate step. Alkylation of a *tert*-butyl ester derivative of isoleucine **5** with (2-chloro-ethyl)carbamic acid *tert*-butyl ester **4** followed by double-deprotection of amine **6** with CF_3CO_2H yielded the cyclization precursor **7**. Lactamization could be achieved with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and pyridine in moderate yields to give the δ-lactam **8**. Sulfamoylation of both amide and amine nitrogens was achieved by reaction 2.1 equiv BuLi in THF at 0 °C followed by TsCl (2.2 equiv) to provide **9**. Although this approach could be improved by differential *N*-protection, it was limited since lactam formation could not be achieved (in sufficiently high yield if at all) when electron withdrawing groups (e.g., *para*-toluenesulphonyl) were present on the primary amine of the cyclisation precursor (Scheme 1).

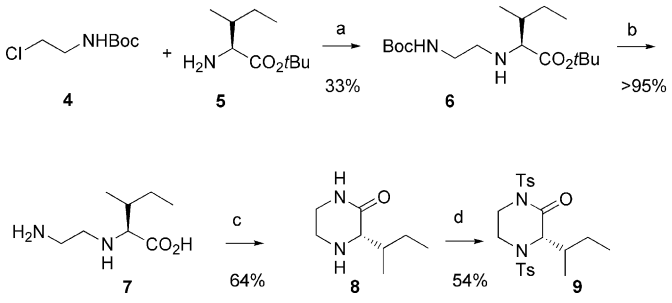
A second approach was based on the Mitsunobu cyclization reaction and is exemplified in Scheme 2. This route proved to be straightforward and flexible. Coupling of *N*-*t*-Boc amino acids **11** with *N*-benzylethanolamine **10** mediated by 1,1′-carbonyldiimidazole (CDI) gave **12**. Subsequent removal of the Boc group followed by cyclization via the Mitsunobu protocol gave the piperazin-2-ones **14** in 66–68% overall yield from **11**. Different *N*-substituents could be introduced via Boc protection and removal of the benzyl group by Birch reduction (Schemes 3 and 4). The amides were reacted with LHMDS (lithium hexamethyldisilazide) or *n*BuLi, followed by reaction with TsCl or MsCl to form targets **19**, **20**, **21**.

Both synthetic strategies, but particularly the latter, are amenable to the production of large numbers of piperaz-2-ones via variation of the starting materials and/or the *N*-substituents.

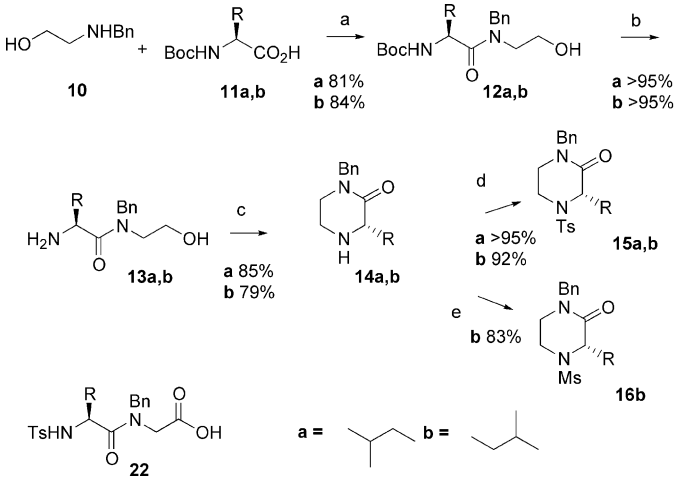
Positive ion electrospray mass spectrometry (ESI-MS) studies on the interaction of **15b**, **19**, **20** and **21** with PPE gave rise to mass shifts corresponding to the formation of both 1:1 and 1:2 complexes (Table 1). In each case the E^*I_2 complexes were observed at lower levels (ca. 10% relative intensity of $E + EI + EI_2$). For **15b**, further MS analyses were carried out. No peak was observed for the anticipated hydrolysis product **22**. Further incubation of **22** with PPE in H_2O^{18} followed by ESI-MS analysis did not result in incorporation of ^{18}O label into **22**. This result contrasts with that obtained with certain monocyclic-γ-lactam inhibitors **3** under analogous conditions, where clear evidence for acylation of Ser-195 was obtained.[6]

The lactam derivatives (**15**), (**19**), (**20**) and (**21**) were tested for inhibition of both HNE and PPE by standard kinetic analyses.[6,13] No inhibition of either was observed for either enzyme for **8ab**, **9**, **14ab**, **15a**, or **18a**,**b**. In some cases, for example, **9**, lack of solubility was problematic and may explain the lack of observed inhibition for these and other compounds. Weak inhibition of PPE, but not HNE, was observed in the case of **16b** (IC_{50} ca. 2000 μM), **19** (IC_{50} ca. 293 μM), and **20** (IC_{50} 923 μM).[14] Each of these three compounds displayed apparent slow binding kinetics. Compound **15b** was a more potent inhibitor, but was still slow binding with IC_{50} values of 28 and 62 μM versus HNE and PPE, respectively. In the latter case a K_i value of 154 μM was obtained. The apparent difference in activity between **15a** (inactive) and **15b** (active) which differ only in the substituent α- to the lactam carbonyl, being 1-methylpropyl and isobutyl in the latter is interesting and supports the argument that in order to achieve inhibition correct fit is as important as reactivity of the amide moiety. In this regard it is also notable that the most active compound (**15b**) has an *N*-benzyl substituent on its lactam nitrogen and is thus chemically less reactive than the targets with *N*-sulphonyl groups most of which were inactive.

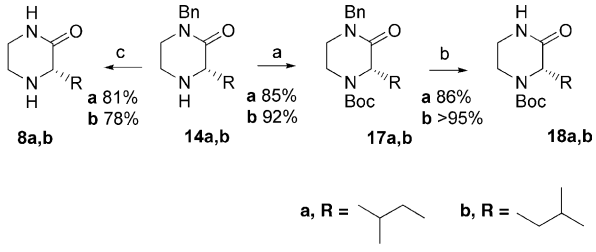
In conclusion, we have prepared a series of piperaz-2-ones/δ-lactams, containing a single chiral centre, via synthetic routes that are amenable to optimization by



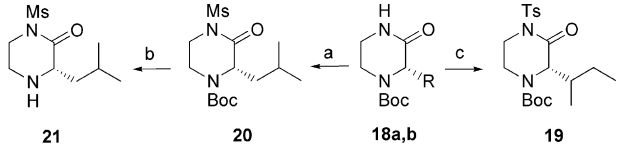
Scheme 1. Synthesis of δ-lactams. Reagents and conditions: (a) (^{i}Pr$_2$)EtN (2 equiv), (NBu_4)I, MeCN, reflux, 12 h; (b) CF_3CO_2H/CH_2Cl_2 (1:1), rt, 3 h; (c) DCC, pyridine (2 equiv), MeCN, rt, 6 h; (d) BuLi (2.1 equiv), THF, TsCl (2.2 equiv), 0 °C, 3 h.



Scheme 2. Synthesis of δ-lactams using a Mitsunobu strategy. Reagents and conditions: (a) **11**, CDI, THF, rt, 1 h, then **10**, THF, 12 h; (b) TFA/CH_2Cl_2 (1:2), 0 °C, 90 min; (c) PPh_3 (1.3 equiv), DEAD (1.3 equiv), THF, rt, 5 h; (d) NEt_3 (1.5 equiv), TsCl (1.3 equiv), CH_2Cl_2, rt, 12 h; (e) NEt_3 (1.1 equiv), MsCl (1.1 equiv), CH_2Cl_2, rt, 12 h.



Scheme 3. Reagents and conditions: (a) Boc_2O (1.2 equiv), CH_2Cl_2, rt, 12 h; (b) Na, NH_3, THF, −78 °C, 20 min.



Scheme 4. Reagents and conditions: (a) LHMDS (1 equiv), MsCl (1.2 equiv), THF, −78 °C, 2.5 h; (b) TFA/CH_2Cl_2 (1:1), 0 °C, 90 min; (c) BuLi (1.3 equiv), THF, TsCl (1.5 equiv), 0 °C, 3 h.

Table 1. ESIMS studies on the Interaction of δ-Lactams with PPE[a]

Compd	Mass inhibitor	PPE	E/I	Relative int. (%)[b]	Diff.[c]
15b	400.5	25,904	26,303	42	399
19	410.5	25,908	26,317	74	409
20	334.4	25,901	26,236	64	335
21	234.3	25,903	26,137	28	234

[a]Incubations contained PPE/inhibitor in a 1:5 ratio (except for compound **15b** which was a 1:2 ratio). The incubation times for **15b**, **19**, **20** and **21** were 30, 12, 5 and 10 min.
[b]Relative intensity refers to the intensity of the adduct peaks normalized to E + EI + EI_2.
[c]Mass shift relative to unmodified PPE observed in the same analysis.

solid phase synthesis. Some of the compounds were shown to be inhibitors of PPE. Whilst inhibition has not been shown to occur via acylation, and other modes of inhibition are possible, the results support the proposal of Imming[12] that δ-lactams be evaluated as inhibitors of serine-enzymes.

References and Notes

1. Krem, M.; Rose, T.; Di Cera, E. *Trends Cardiovasc. Med.* **2000**, *10*, 171 and references therein.
2. Vender, R. L. *J. Invest. Med.* **1996**, *44*, 531.
3. Babine, R. E.; Vender, R. L. *Chem. Rev.* **1997**, *97*, 1359.
4. Knight, W. B.; Chabin, R.; Green, B. G. *Arch. Biochem. Biophys.* **1992**, *296*, 704.
5. Knight, W. B.; Swiderek, K. M.; Sakuma, T.; Calaycay, J.; Shively, J. E.; Lee, T. D.; Covey, T. R.; Shushan, B.; Green, B. G.; Chabin, R.; Shah, S.; Mumford, R.; Dickinson, T. A.; Griffin, P. R. *Biochemistry* **1993**, *22*, 2031.
6. Wilmouth, R. C.; Westwood, N. J.; Anderson, K.; Brownlee, W.; Claridge, T. D. W.; Clifton, I. J.; Pritchard, G. J.; Aplin, R. T.; Schofield, C. J. *Biochemistry* **1998**, *37*, 17506.
7. Taylor, P.; Anderson, V.; Dowden, J.; Flitsch, S. L.; Turner, N. J.; Loughran, K.; Walkinshaw, M. D. *J. Biol. Chem.* **1999**, *274*, 24901.
8. Macdonald, S. J. F.; Belton, D. J.; Buckley, D. M.; Spooner, J. E.; Anson, M. S.; Harrison, L. E.; Mills, K.; Upton, R. J.; Dowle, M. D.; Smith, R. A.; Molloy, C. R.; Risley, C. *J. Med. Chem.* **1998**, *41*, 3919.
9. Macdonald, S. J. F.; Dowle, M. D.; Harrison, L. A.; Shah, P.; Johnson, M. R.; Inglis, G. G. A.; Clarke, G. D. E.; Smith, R. A.; Humphreys, D.; Molloy, C. R.; Amour, A.; Dixon, M.; Murkitt, G.; Godward, R. E.; Padfield, T.; Skarzynski, T.; Singh, O. M. P.; Kumar, K. A.; Fleetwood, G.; Hodgson, S. T.; Hardy, G. W.; Finch, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 895 and references therein.
10. Westwood, N. J.; Claridge, T. D. W.; Edwards, P. N.; Schofield, C. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2973.
11. Wilmouth, R. C.; Kassamally, S.; Westwood, N. J.; Sheppard, R. J.; Claridge, T. D. W.; Aplin, R. T.; Wright, P. A.; Pritchard, G. J.; Schofield, C. J. *Biochemistry* **1999**, *38*, 7989.
12. Imming, P.; Klar, B.; Dix, D. *J. Med. Chem.* **2000**, *43*, 4328.
13. Macdonald, S. J. F.; Dowle, M. D.; Harrison, L. A.; Spooner, J. E.; Shah, P.; Johnson, M. R.; Inglis, G. G. A.; Clarke, G. D. E.; Belton, D. J.; Smith, R. A.; Molloy, C. R.; Dixon, M.; Murkitt, G.; Godward, R. E.; Skarzynski, T.; Singh, O. M. P.; Kumar, K. A.; Hodgson, S. T.; McDonald, E.; Hardy, G. W.; Finch, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 243.
14. Values are means of at least three experiments.