Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 773-778, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00
Pergamon Press Ltd

ELASTASE INHIBITORS CONTAINING CONFORMATIONALLY RESTRICTED LACTAMS AS P_3-P_2 DIPEPTIDE REPLACEMENTS

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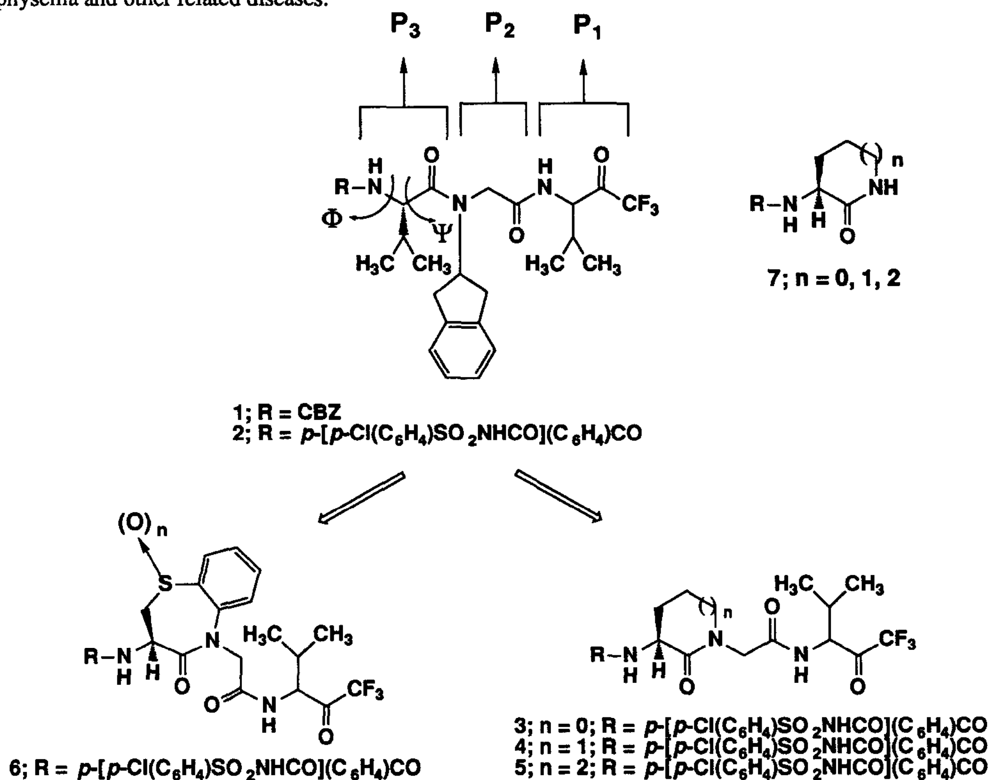
(*Received in USA* 18 *January* 1993)

Abstract: The synthesis of potential human leukocyte elastase (HLE) inhibitors containing conformationally restricted lactams as peptidomimetic replacements at P_3- P_2 and which contain a trifluoromethyl ketone of valine at P_1 are described.

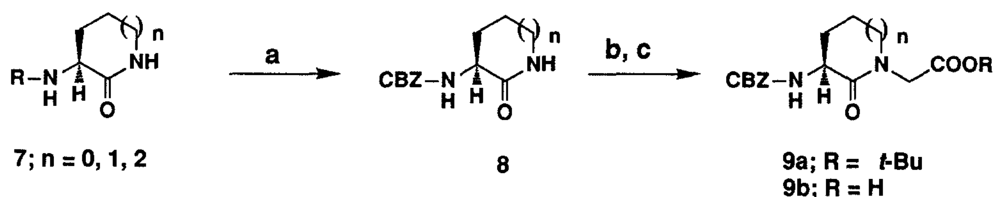
Proteinases from polymorphonuclear leukocytes (PMN) and macrophages such as elastase and cathepsin G have been implicated in the chronic tissue destruction associated with inflammation, arthritis and, in particular, emphysema.[1] During infection in inflammation, the normal lung is protected from proteolytic digestion by the endogenbus protease inhibitor α_1-antitrypsin (α_1-PI). The protective mechanism appears to be inoperative in individuals with a α_1-PI deficiency due to genetic or other causes such as cigarette smoking which oxidizes Met^{358} of α_1-PI to the corresponding sulfoxide causing the inhibitor to be nonfunctional. Synthetic small molecule inhibitors of human leukocyte elastase (HLE) may therefore be potentially useful in the treatment of pulmonary emphysema and other related diseases.



In our ongoing search for new and therapeutically useful low molecular weight inhibitors of serine proteinases,[2] and in particular HLE, we were interested in preparing novel peptidyl trifluoromethyl ketone inhibitors of HLE which contain conformationally restricted lactams at P3-P2 as peptide bond surrogates.[3] The incorporation of conformationally restricted lactams into peptidyl inhibitors of HLE has not previously been reported upon. We have previously reported[2] that the primary specificity site of HLE (i.e., S_1) is very discriminating in accommodating only small lipophilic residues such as valine or isoleucine residues. Large groups such as leucine and phenylalanine or smaller residues such as glycine or alanine are all inactive. On the other hand, it also has been shown that the P_2-position is very promiscuous in allowing a wide variety of substituents from very small residues such as glycine to large residues such as *N*-(octanyl)glycine.[2]

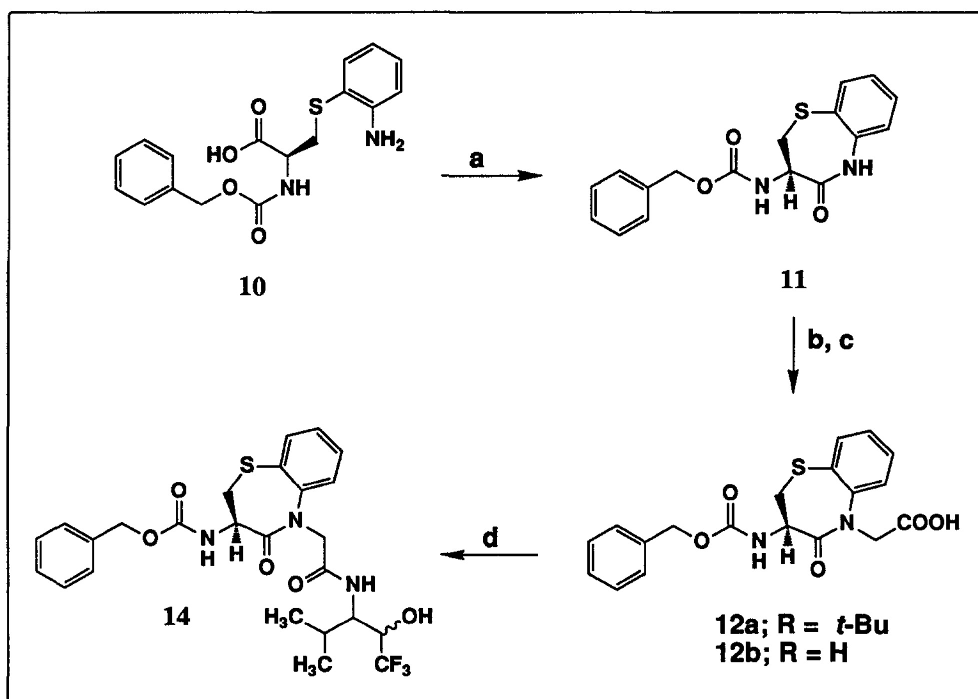
The incorporation of conformationally restricted lactams is a common strategy for improving the potency, selectivity, and metabolic stability of peptide inhibitors. Lactams which maintain a given dipeptidyl unit in the *trans*-amide conformer and bias neighboring ψ and ϕ angles, have proven particularly useful in a number of applications of the renin-angiotensin system.[4, 5] Tripeptides which are composed of non-proteinogenic achiral *N*-substituted glycine residues at P_2 and which possess a trifluoromethyl ketone of valine at P_1 are effective *in vitro* inhibitors of HLE with IC_{50} values in the submicromolar range. This type of inhibitor is exemplified by **1** and **2**, IC_{50} = 0.365 and 0.084 μM respectively.[2] In pursuing the design of conformationally restricted analogues of **1** and **2** we have assumed that the amide bond remains in the *trans* conformation in the HLE:inhibitor complex. This is supported by prior evidence[2] that in molecular docking studies of the low energy conformation of **1** with the X-ray structure of HLE it is seen that the *trans* form is clearly maintained and of lower energy than the *cis* form. The P_3-carbonyl group of **1** is hydrogen bonded to the NH of Val^{216} of HLE (Val^{216} NH to O of P_3-Val; 166.85; 1.90 Å). The calculated ψ and ϕ values for the P_3-Val of the lowest energy conformation of **1** are 142.81° and -63.93° respectively.[6]

Conformationally restricted analogues of **1** and **2** were created by bridging a carbon chain between the *beta*-carbon of the P_3 L-Val with the nitrogen of the P_2 Gly. For synthetic simplicity the *gamma*-carbon of L-Val was omitted. The angle ψ is now clearly a function of the lactam size. The type of inhibitors that we were interested in exploring contain a 5, 6, or 7 membered monocyclic lactam as P_3-P_2 dipeptide surrogates (i.e., **3**, **4**, and **5**). In addition we were also interested in constructing the bicyclic lactam **6**. The conformational result of incorporating lactams into potential inhibitors is to restrict the available low energy ψ-values. The required five, six, and seven membered lactams **7** (R = H) were prepared as previously reported.[7] The lactams **7** were treated with CBZ-Cl by standard means to afford the urethanes **8**. Treatment of **8** in THF with NaH and *tert*- butyl bromoacetate gave the *tert*-butyl esters **9a** which were then deprotected with dry hydrogen chloride in *p*-dioxane to give the acids **9b**.



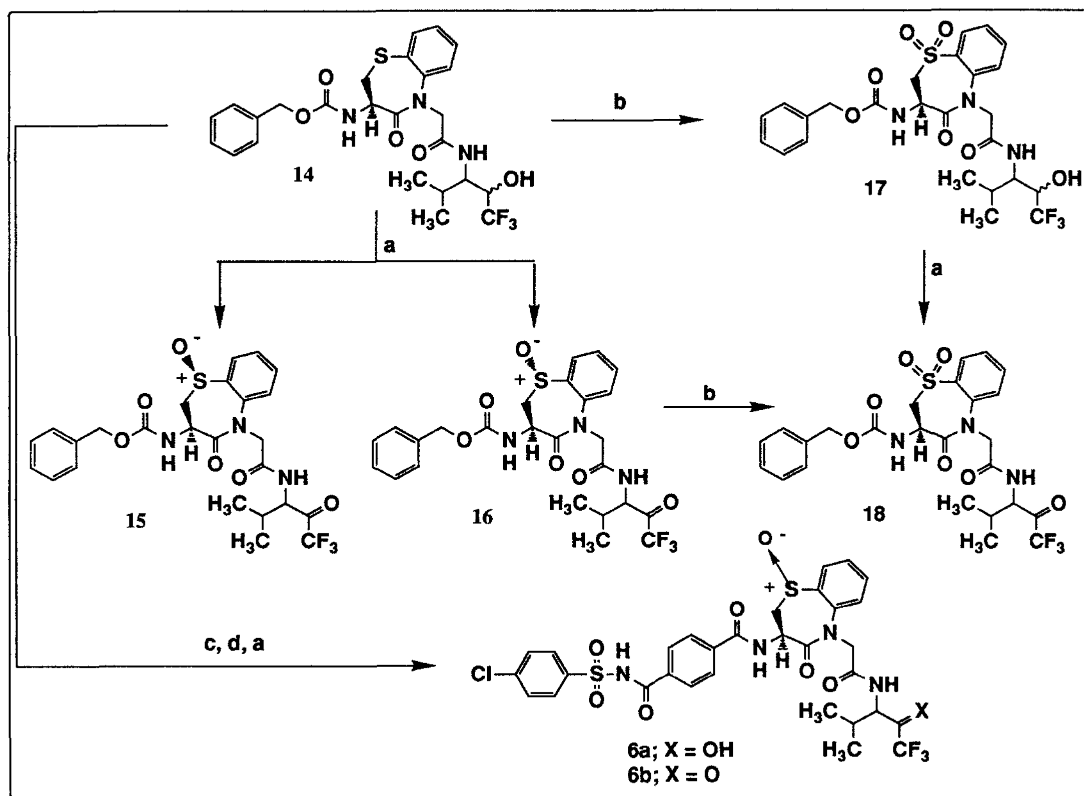
Reagents: (a) CBZ-Cl; (b) NaH, THF, *tert*-butyl bromoacetate; (c) HCl/ *p*-dioxane.

The known bicyclic lactam **11** was prepared in a similar manner to that previously reported.[5b] The ring closure of the acid **10** was effected using a water soluble carbodiimide (WSCDI), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, in DMF to afford the bicyclic lactam **11**. Alkylation of the lactam **11** with *tert*-butyl bromoacetate gave, after deprotection with dry HCl in *p*-dioxane, the acid **12b**.



Reagents: (a) WSCDI; (b) NaH, THF, *tert*-butyl bromoacetate; (c) HCl/ *p*-dioxane; (d) CDI, THF, **13**.

The synthesis of the potential inhibitors **3**, **4**, and **5** is illustrated by the synthesis of **6b**. The trifluoromethyl alcohol of the type exemplified by **14** was prepared by condensing the acid **12b** with the (*dl*)-*threo* amine $H_2NCH[CH(CH_3)_2]CH(OH)CF_3$ (**13**)[8a] through the employment of CDI as the amide generating reagent. The alcohol **14** was oxidized with Dess-Martin periodinane[9] to afford the two isomeric sulfoxide trifluoromethyl ketones **15** and **16**. The isomeric sulfoxides **15** and **16** could be separated by column chromatography. When the sulfide **14** was oxidized with MCPBA the sulfone alcohol **17** was obtained which was then oxidized *via* Dess-Martin periodinane to the sulfone trifluoromethyl ketone **18**. The sulfoxide ketones **15** and **16** could also be oxidized to the sulfone trifluoromethyl ketone **18** *via* Dess-Martin periodinane. The bicyclic lactam **14** was deprotected catalyticaly over 10 % Pd/C and then condensed with *p*-[*p*-$Cl(C_6H_4)SO_2NHCO](C_6H_4)COOH$ *via* WSCDI to give the alcohol **6a**.[2] The diastereomeric alcohols **6a** were oxidized with Dess-Martin periodinane to give the trifluoromethyl ketone **6b**.



Reagents: (a) Dess-Martin periodinane, TFA, CH_2Cl_2; (b) MCPBA, CH_2Cl_2; (c) H_2, Pd/C; (d) WSCDI, CH_2Cl_2, *p*-[*p*-Cl(C_6H_4)SO_2NHCO](C_6H_4)COOH

As seen from the table, the 5, 6, and 7-membered monocyclic lactams are all totally devoid of HLE inhibitory activity *in vitro*. The calculated[6] torsional angle ψ of the low energy conformation of the previously reported inhibitor **1** is 142.81°. The ψ angles in the lowest energy conformers of the 5, 6, and 7-membered lactams were calculated to be -110°, -124.41°, and 154.59° respectively. The ψ torsional angle for the bicyclic system **6** is 143.07°. The similarity in ψ torsional angles between the 7-membered monocyclic lactam **5** and the peptide **2** suggest that factors other than this particular torsional may be involved in tight binding to HLE.

The bicyclic lactam **6b**; however, showed good HLE inhibitory activity both *in vitro* (IC_{50} = 6.2 μM)[10] and *in vivo*. The *in vitro* inhibitory activity of **6b** should be compared with the tripetides **1** (IC_{50} = 0.365 μM) and **2** (IC_{50} = 0.084 μM).[11] There is a seventy fold decrease in the potency of the lactam **6b** relative to the tripeptide trifluoromethyl ketone **2**. Both **2** and **6b** span the S_5 - S_1 subsites of HLE. The inhibitor **6b** has been tested against representative examples of all four classes of proteinases (e.g., serine, cysteine, aspartic, and metallo) and has been found to inhibit only HLE. Enzymes such as cathepsins D, B, and G, urokinase, TPA, thrombin, C_1-esterase, renin, plasmin, HIV-protease, thrombin, and trypsin are not inhibited when assayed at 100 μM.

The inhibitor **6b** has been studied in an elastase induced pulmonary hemorrhage (EPH) model in the hamster.[12] HLE induces acute hemorrhage in the hamster lung when administered intratracheally (it.).[12] Hemorrhage can be quantitated 18 h later by measuring red blood cell concentration in bronchial alveolar lavage

fluid. In this model administration of **6b** (20 μg, it. per animal) five minutes prior to HLE challenge, effectively inhibited hemorrhage in a dose dependent manner by 76.4 %. In a similar manner the tripeptide **2** exhibited an ED_{50} of 4.8 μg it. per animal in this model. In accord with our previous report on peptidyl inhibitors of the type exemplified by **2**, the *p*-[*p*-Cl(C_6H_4)SO_2NHCO](C_6H_4)CO functionality at the amino terminal end of tripeptides is required for *in vivo* activity.[2]

TABLE

Cmpd. No.		IC_{50} (μM)
	P_3 P_2 P_1	
3	R-NH–(5-membered lactam)–N–CH₂–C(O)–NH–CH(CH(CH₃)₂)–C(O)CF₃	>> 5
4	R-NH–(6-membered lactam)–N–CH₂–C(O)–NH–CH(CH(CH₃)₂)–C(O)CF₃	>> 5
5	R-NH–(7-membered lactam)–N–CH₂–C(O)–NH–CH(CH(CH₃)₂)–C(O)CF₃	>> 5
6b	R-NH–(benzothiazepinone S-oxide)–N–CH₂–C(O)–NH–CH(CH(CH₃)₂)–C(O)CF₃	6.8

It is tempting to speculate as to why **6b** should exhibit a greater potency than the corresponding 5, 6, and 7 membered monocyclic lactams which are totally devoid of HLE inhibitory activity. We have earlier established that benzo fusion into inhibitors such as **1** and **2** at the P_2-position results in potent and selective inhibitors of HLE.[2] As described above in a likewise fashion benzolactams such as **6b** are significantly more potent than their monocyclic lactam counterparts (i.e. **5**) although the ψ torsional angles are similar in both series. This same trend has been observed in the design of ACE inhibitors, e.g. benzolactams are more potent than monocyclic lactams.[4] The above observations may be due to the fact that the S_3 and S_2 subsites of HLE are highly hydrophobic in nature and thus

the highly lipophilic phenyl of **6b** may contribute to overall tight binding. Alternatively, the sulfoxide of **6b** is very polar and may thus effectively hydrogen bond with remote residues of HLE.

In conclusion this communication describes the syntheses of a series of tripeptides which contain conformationally restricted lactams at the P_3-P_2 positions and which contain a trifluoromethyl ketone of valine at P_1. One of the designed molecules **6b** exhibited good *in vitro* (IC_{50} = 6.8 μM) and *in vivo* (76.4 % reduction in hemorrhage at 20 μg, it. per animal) inhibition of HLE.

Acknowledgment: We wish to thank Ms. G. Chow and Dr. M. Skoog for conducting all *in vitro* evaluations, and Mr. S. Weldon and Dr. G. Possanza for conducting *in vivo* evaluation of **6b**.

References and Notes

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1. (a) Erikson, S. *Acta. Med. Scand.* **1965**, 177 (suppl. 432). (b) Janoff, A. *Am. Rev Respir. Dis.* **1985**, *132*, 417. (c) Ekerot, L.; Ohlsson, K. *Adv. Exp. Med. Biol.* **1984**, *167*, 335.

2. (a) Skiles, J.W.; Fuchs, V.; Miao, C.; Sorcek, R.; Grozinger, K.G.; Mauldin, S.C.; Vitous, J.; Mui, P.W.; Jacober, S.; Chow, G.; Matteo, M.; Skoog, M.;Weldon, S.M.; Possanza, G.; Keims, J.; Letts, G.; Rosenthal, A.S. *J. Med. Chem.* **1992**, *35*, 641. (b). Skiles, J.W.; Miao, C.; Sorcek, R.; Jacober, S.; Mui, P.W.; Chow, G.; Weldon, S.; Possanza, G.; Skoog, M.; Rosenthal, A.S. *J. Med. Chem.* **1992**, *35*, 4795. (c) Skiles, J.W.; Fuchs, V.; Chow, G.; Skoog, M. *Res. Commun. Chem. Pathol. Pharmacol.* **1990**, *68*, 365.

3. The nomenclature used for describing the individual amino acid residues (P_2, P_1, P_1', P_2', etc.) of a peptide substrate and the corresponding subsites (S_2, S_1, S_1', S_2', etc.) of a protease is that of Schecter and Berger: Schecter, I; Berger, A. *Biochem Biophys Res. Commun.* 1967, *27*, 157-162.

4. (a) Thorsett, E.D.; Harris, E.E.; Aster, S.; Peterson, D.; Taub, D.; Patchett, A.A.; Ulm, E.H.; Vassil, T.C. *Biochem. Biophys. Res. Commun* **1983**, *111*, 166. (b) Hassal, C.H.; Krohn, A.; Moody, C.J.; Thomas, W.A. *J. Chem. Soc Perkin Trans. I* **1984**, 155.

5. (a) Watthey, J. W. H.; Gavin, T.; Desai, M. *J Med. Chem.* **1984**, *27*, 816. (b) Slade, J.; Stanton, J.L.; Ben-David, D.; Mazzenga, G.C. *J. Med. Chem.* **1985**, *28*, 517.

6. Minimizations of the inhibitors were done using the program CHARMm (Molecular Simulations Inc., Waltham, MA) and utilizing a Silicon Graphics 4D/50 (Mountain View, CA) computer. Molecular graphics interpretation of the results was done *via* QUANTA (Molecular Simulations Inc.).

7. Pellegata, M.; Pifferi, G. *Synthesis* **1978**, 614.

8. (a) The *threo*-amino-alcohol **13** was prepared according to reference 2a. (b) For the preparation of similar trifluoromethyl nitro alcohols see: Imperiali, B.; Abeles, R.H. *Tetrahedron Lett.* **1986**, 27, 135.

H_3C CH_3 / I —($AgNO_2$)→ H_3C CH_3 / O_2N —(1. $CF_3CH(OC_2H_5)OH$, K_2CO_3; 2. Crystallization; 3. $LiAlH_4$)→ H_3C CH_3 / H_2N CF_3 / OH

13; (*dl*)-*threo*

9. Dess, D.B.; Martin, J.C. *J. Org. Chem.* **1983**, *48*, 4155. **Caution**, care should be taken in the handling of this reagent. The explosive nature of periodinane and its precursor, 2-iodoxybenzoic acid, has recently been described; Plumb, J.B.; Harper, D. *J. Chem. Eng. News* **1990**, July 16, p 3.

10. Concentration inhibiting 50 % of the activity of HLE at pH 7.5 in 0.1 M Tris buffer containing 0.5 M NaCl with the substrate MeO-Suc-Ala-Ala-Pro-Val-pNA at a concentration of 0.5 mM. Compounds were incubated for 20 min prior to starting the reaction by addition of substrate. Biological *in vitro* evaluations were conducted according to references 2a and 2b.

11. Peptidyl trifluoromethyl ketone inhibitors such as **1**, **2**, and **6b** are presumed to deactivate HLE *via* transition state inhibition. The enhanced electrophilicity of fluorinated ketones over non fluorinated ketones may facilitate an enzyme-catalyzed addition of the active site Ser195 of HLE to the ketone carbonyl to form a metastable hemiketal, which resembles the tetrahedral intermediate in the reaction pathway for enzyme-substrate hydrolysis. For further details see references 2a and 2b and references within.

12. For details of the elastase induced pulmonary hemorrhage (EPH) model in hamsters see references 2a and 2b.