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EXAMINATION OF PEPTIDIC α',β-DIAMINO-α,α-DIFLUOROKETONES AS INHIBITORS OF HUMAN LEUKOCYTE ELASTASE

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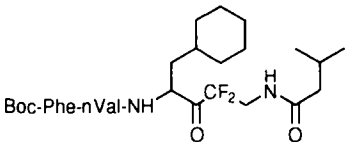
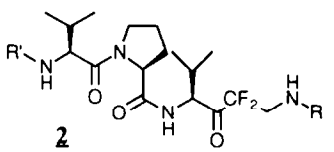
Abstract: The preparation and in vitro evaluation of a series of peptidic α',β-diamino-α,α-difluoroketones are described. Comparison of the efficacy of these compounds to both their corresponding trifluoromethylketone and α-carboxamido-α,α-difluoroketone inhibitors reveals a divergent set of structure-activity relationships. This divergence indicates that the inhibitor's P_1'-amino group is strongly interacting with the enzyme's S_1'-subsite.

The possible clinical utility of potent, specific inhibitors of human leukocyte elastase (EC 3.4.21.37, HLE) for the treatment of a variety of pathological states has resulted in such compounds being an important target for chemists.[1] At ZENECA Pharmaceuticals (formerly ICI Pharmaceuticals) several series of reversible, low molecular-weight, peptidic inhibitors of HLE have been explored. Those efforts examined a variety of electrophilic carbonyl derivatives which were capable of forming reversible tetrahedral adducts with the active-site serine of HLE, e.g. trifluoromethylketone (TFMK),[2] α-carboxamido-α,α-difluoroketone (CDFK),[3] α-carbonyl [e.g. keto, amido, carboxy] ketone,[3] and α-ketoheterocycle[4] based inhibitors, and ultimately led to the selection of a trifluoromethylketone (ICI 200,880),[5] for clinical development.

Recently Schirlin et al.[6] have developed peptidic α',β-diamino-α,α-difluoroketones as electrophilic carbonyl based inhibitors of several proteases, with emphasis on inhibitors, e.g. **1**,[6b,c] of the aspartic acid protease human renin. Since interest in new inhibitors of HLE remains high[1] we chose to prepare a series of analogues **2** designed to examine how this pharmacophore functions for inhibition of HLE when coupled to a backbone (R-Val-Pro-Val-) known to afford potent HLE inhibitors.[2,3,4]



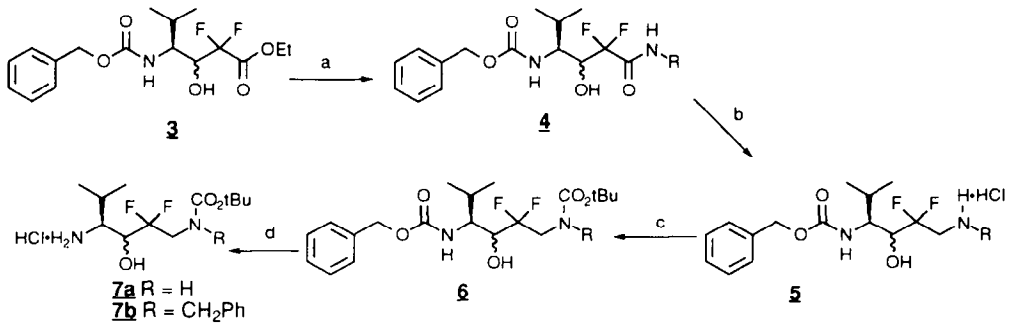
1, IC_{50} = 3.5 nM on human renin[6b]



2

Synthesis of the intermediate difluoroamines **7a,b** was accomplished using the general methodology described by Schirlin et al. for **7a**[6a] and exemplified here by the synthesis of **7b** (Scheme 1).[7,8]

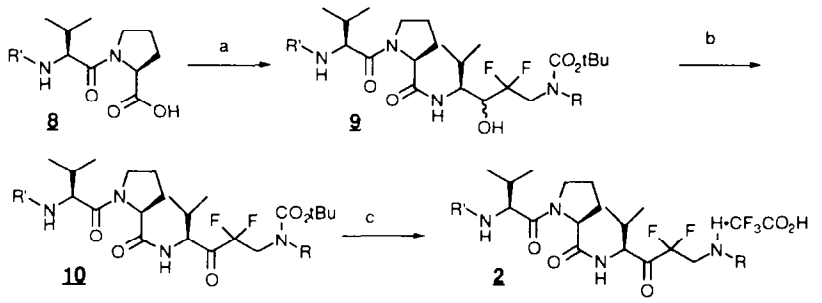
Scheme 1[a,b]



[a] (a) NH_3, EtOH or $PhCH_2NH_2$, EtOH (60%); (b) i. $Me_2S{\cdot}BH_3$, THF, ii HCl, Et_2O (78%); (c) $(BOC)_2O$, K_2CO_3, THF/H_2O (75%); (d) H_2, 10% Pd/C, EtOH/HCl (>95%)
[b] The yields reported (%) are for the R = CH_2Ph series

Conversion of these intermediate amines into the test compounds was achieved as outlined in Scheme 2. The appropriately *N*-protected dipeptide acids **8** were coupled with difluoroamines **7a,b** using 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, a water soluble carbodiimide (WSCDI), to afford alcohols **9**. Modified Pfitzner-Moffatt oxidation[9] of these alcohols yielded the α',β-diamino-α,α-difluoroketones **10**. Deprotection of the P_1'-amino nitrogen atom and direct conversion to the trifluoroacetic acid salt of the product amines, **2**, was effected by treatment of **10** with neat trifluoroacetic acid.

Scheme 2[a,b]



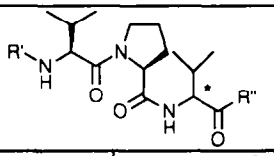
[a] (a) WSCDI, triethylamine, **7**, THF (87%). (b) WSCDI, Cl_2CHCO_2H, DMSO, Toluene (87%); (c) CF_3CO_2H (61%)
[b] R = H or CH_2Ph. R' = 4-(MeO)C_6H_4CO-, $PhCH_2O_2C$- or PhO_2C-. Representative yields (%) are for the R = CH_2Ph, R' = $PhCH_2O_2C$- series

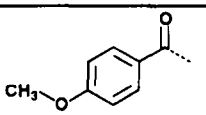
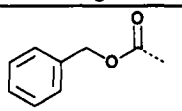
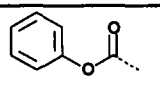
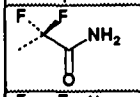
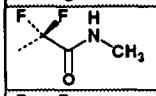
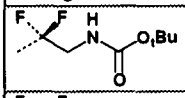
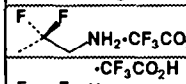
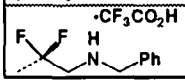
We chose to prepare a set of analogues, **19**-**27**, in which the P_3-amine was substituted with either the 4-methoxybenzoyl, CBZ or phenoxycarbonyl groups (see Table 1). For comparison purposes we also examined several similarly substituted TFMK's (**11**-**13**)[2] and CDFK's (**14**-**18**).[10] The β-amino-α,α-difluoroketone analogues (ADFK) **19**-**27** displayed relatively large differences in potency, in subsets that contained either a constant P_3-amino substituent (e.g. compare **22** to **25** [43-fold] or **23** to **20** [118-fold]) or a constant P_1'-amino group (e.g. compare **24** to **23** [143-fold]. In contrast, the greatest potency differences seen in the earlier sets of

inhibitors were much smaller in similar subsets with either a constant P_3-amino substituent (e.g. compare **17** to **15** [8-fold] or P_1'-region (e.g. compare **16** to **18** [4-fold]]. Furthermore the differences between the TFMK's and CDFK's which had a constant P_3-amino substituent were also relatively small (e.g. compare **15** to **12** [7-fold]).

The reasons for the greater degree of potency variation are not obvious and indicate that the β-amino regions of these α',β-diamino-α,α-difluoroketones have significant interactions with HLE. Since these amines are only weakly basic (pKa > 6.7)[6a] and should be only partially protonated under the conditions of the assay (pH 7.6) it is probable that these interactions are not of the charge-charge variety. Previously, X-ray studies in the TFMK[11] and CDFK[12] inhibitor series had shown similar conformations for both bound inhibitors. However, the greater effect of the P_3-amino substituent on K_i values seen in the ADFK's as compared to those found in either of the other two series of inhibitors, indicates that the β-amino-region interactions must result in significant changes in the conformation of the bound inhibitors from that previously observed in these other two series.

Table 1. Test compounds and in-vitro inhibition of HLE.[a,b]



R' / R"			
	comp. / K_i (nM)[b]	**comp.** / K_i (nM)	**comp.** / K_i (nM)
	**11** / 3.1 ± 0.8	**12** / 2.5 ± 0.3	**13** / 2.3 ± 0.5
	**14** / 0.65 ± 0.27	**15** / 0.38 ± 0.13	**Not prepared**
	**16** / 3.4 ± 0.4	**17** / 3.0 ± 0.9	**18** / 0.85± 0.2
	**19** / 3.4 ± 0.9	**20** / 1.1 ± 0.4	**21** / 0.39± 0.04
	**22** / 15.0 ± 2.5	**23** / 130.0 ± 7.0	**24** / 0.81±
	**25** / 0.35 ± 0.09	**26** / 1.6 ± 0.3	**27** / 4.1± 0.4

[a] The trifluoromethylketones (**11**-**13**) were mixtures of diastereomers at the indicated carbon (*) whereas the α-carboxamido-α,α-difluoroketones (**14**-**18**) and the α',β-diamino-α,α-difluoroketones (**19**-**27**) were single *S*-enantiomers.

[b] The inhibition constrant (K_i) versus HLE was determined using a synthetic substrate as described in detail in reference 5.

These results are especially interesting since Powers et al.[13] had also examined the effect of varying the P'-region, on substrate recognition, in a series of P'-extended substrates, and found only relatively limited

differences in enzyme affinity. Preliminary structural analysis of these molecules docked into a molecular model of the active-site of HLE did not provide a clear explanation. Nonetheless these results exemplify that the P'-groups in reversible peptidic HLE inhibitors can play an important role in enzyme recognition.

References and Notes

1 For two recent, complementary reviews on inhibitors of human leukocyte elastase, see:
a) Edwards, P.D.; Bernstein, P.R. *Med. Res. Rev.* **1994**, *14*, 127.
b) Bernstein, P.R.; Edwards, P.D. and Williams, J.C. *Prog. Med. Chem.*; Ellis, G.W. and Luscombe, D.K., Eds.; Elsevier, Amsterdam, 1994; Volume 31, pp. 59-120.

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5 Williams, J.C.; Falcone, R.C.; Knee, C.; Stein, R.L.; Strimpler, A.M.; Reaves, B.; Giles, R.E.; Krell, R.D. *Am. Rev. Respir. Dis.* **1991**, *144*, 875-883.

6 a) Schirlin, D.; Baltzer, S.; Altenburger, J.M. *Tet. Lett.* **1988**, *29*, 3687.
b) Tarnus, C.; Jung, M.J.; Rémy, J.-M.; Baltzer, S. and Schirlin, D.G. *FEBS Lett.* **1989**, *249*, 47.
c) Schirlin, D.; Tarnus, C.; Baltzer, S. and Rémy, J.-M. *BioMed. Chem. Lett.* **1992**, *2*, 651.
d) Altenburger, A.M.; Schirlin, D. *Tet. Lett.* **1991**, *32*, 7255.

7 All new compounds were characterized by ^{1}H NMR, MS and combustion analysis. Like many peptidic trifluoromethyl ketones, several of these compounds held on to water tightly and analyzed as partial or full hydrates.

8 As an example, 9.7g of compound **6b** was dissolved in 80 mL EtOH to which was added 0.95g 10% Pd/C and 1 mL concentrated HCl. Treatment with H_2 (50 PSI, at room temperature, overnight) followed by filtration and evacuation in vacuo afforded a quantitative yield of **7b** as an off-white foam.

9 Pfitzner, K.E.; Moffatt, J.G. *J. Am. Chem. Soc.* **1965**, *87*, 5661.

10 These compounds were prepared using the procedures and intermediates described in reference 3: unpublished results of Bernstein, P.R. and Veale, C.A., these laboratories.

11 Takahashi, L.H.; Radhakrishnan, R.; Rosenfield, R.E.; Meyer, E.F.; Trainor, D.A. and Stein, M. *J. Mol. Biol.* **1988**, *201*, 423.

12 Takahashi, L.H.; Radhakrishnan, R.; Rosenfield, R.E.; Meyer, E.F. and Trainor, D.A. *J. Am. Chem. Soc.* **1989**, *111*, 3368.

13 McRae, B.; Nakajima, K.; Travis, J. and Powers, J.C. *Biochemistry* **1980**, *19*, 3973.

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