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IMIDAZO[1,2-a]PIPERAZINES AS MECHANISTIC INHIBITORS OF SERINE PROTEINASES

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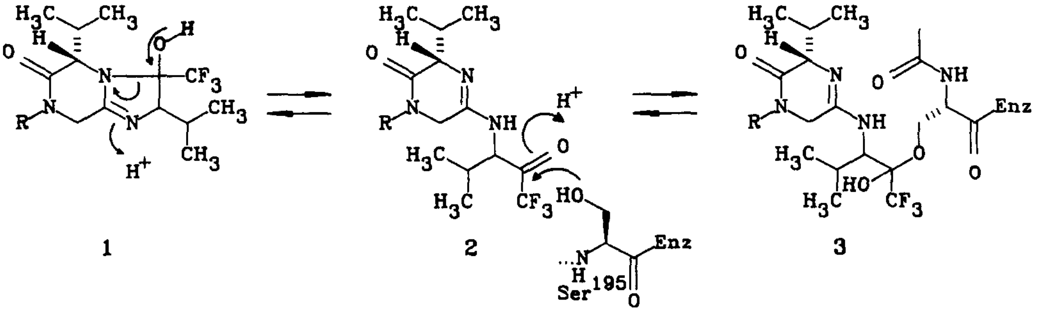
Summary: The synthesis of imidazo[1,2-a]piperazines bearing a trifluoromethyl substituent, potential inhibitors of serine proteinases, is illustrated.

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 or inflammation, the normal lung is protected from proteolytic digestion by the endogenous protease inhibitor α_1-antitrypsin (α_1-PI). The protective mechanism appears to be nonoperative in individuals with an α_1-PI deficiency due to genetic or other causes, such as cigarette smoking which oxidizes Met^{365} 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 expected to be potentially useful in the treatment of pulmonary emphysema and other related diseases.

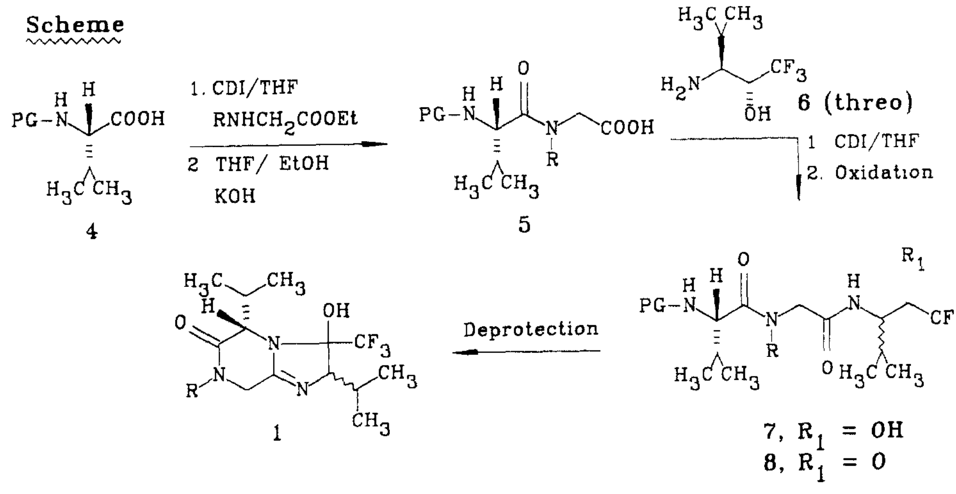
In an ongoing search for new and therapeutically useful low molecular weight inhibitors of serine proteinases, and in particular HLE, we were interested in preparing novel peptidyl trifluoromethyl ketone derivatives of the type which have been shown to be effective *in vitro* inhibitors[2] of HLE. During this synthetic program, we elucidated the structure of an unexpected class of heterocyclic compounds (imidazo[1,2-a]piperazines) **1**[3] which might be capable of functioning as inhibitors of HLE *via* the intermediacy of the corresponding piperazine open form system **2** to ultimately give the hemiketal **3**. These may be effective mechanism based inhibitors, since trifluoromethyl ketones presumably act *via* hemiketal formation with the catalytic site Ser^{195} of HLE[2].



1 **2** **3**

In order to test our hypothesis a series of imidazo[1,2-a]piperazines was prepared in high yield as is illustrated in the Table. The required tripeptide trifluoromethyl ketones **8** were readily prepared from N-protected-L-valine **4** as is illustrated in the Scheme. Condensation of **4** with the appropriately N-substituted glycine ethyl ester utilizing CDI afforded, after hydrolysis, the *N*-protected acids **5**. The trifluoromethyl alcohol derivative **6** of (*dl*)-valine was prepared by means analogous to those previously described[4] to give a mixture of *threo* and *erythro* isomers. The *threo*-isomer of **6**, [(*2R,3S*) + (*2S,3R*)], could easily be separated from the corresponding *erythro*-set of enantiomers by crystallization. The *threo*-isomer **6** was obtained as a colorless solid, mp 80 - 82 °C, whereas the *erythro*-isomer **6** remained as an oil.The amine **6** was condensed with the dipeptides **5** employing CDI to give the tripeptide alcohols **7**. The CBZ or *tert*-BOC tripeptide alcohols **7** could cleanly be oxidized to the corresponding protected trifluoromethyl ketones **8** by the Dess-Martin periodinane reagent.[5] The ketones **8** were deprotected catalytically (Pd/C, H_2, 45 psi, EtOH or Pd/C H_2NNH_2, EtOH) in the case in which PG = CBZ or treated with *p*-dioxane saturated with dry hydrogen chloride in the case in which PG = *tert*-BOC to give directly the imidazo[1,2-a]piperazines **1**[3,6] as mixtures of diastereomers in > 85 % yields after silica-gel purification. The tricyclic inhibitor **1g** was prepared in 85 % yield in an analogous manner to that described above by utilizing *tert*-Boc-L-proline instead of *N*-substituted glycines. The surprising stability of the tertiary alcohols **1** towards elimination may be due to the destabilization of the incipient carbonium cation by the electron-withdrawing CF_3 group.

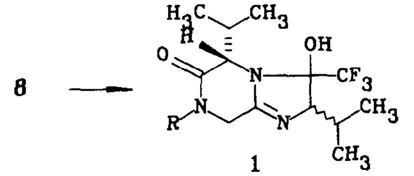
Scheme

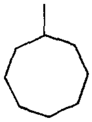
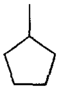
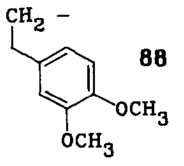
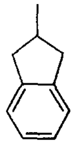
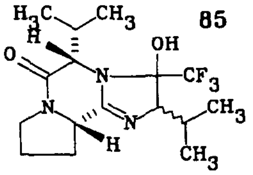


PG = t-BOC or CBZ

The catalytic triad of HLE is composed of Ser^{195}, Asp^{102}, and His^{57}. The hydroxy group of the side chain of active site Ser^{195} acts as a nucleophile and is activated by the close proximity of the imidazole of His^{57}. HLE cleaves amide bonds of substrates by the nucleophilic addition of Ser^{195} to carbonyls to form a tetrahedral intermediate. The inhibition of HLE by such heterocyclic molecules as **1** may be explained by several different mechanisms. The intact inhibitors **1** may inhibit HLE by their resemblance to the transition-state of cleavage. Alternatively through acid-base catalysis, facilitated by Asp^{102} and His^{57}, the piperazines **1** may be transformed into the species **2**. The trifluoromethyl ketone **2** generated may then react with the enzyme active site Ser^{195} or with water to generate transition-state analogs *in situ* (i.e., the former is illustrated). The exact manner of inhibition by the heterocycles has not yet been determined.

Table



Entry[a]	R	Yield (%)	Entry[a]	R	Yield (%)
1a	H	86	1e		85
1b	CH_3	80			
1c		84	1f		88
1d		86			
1g		85			

[a] Mixture of diastereomers.

Our preliminary finding is that the heterocyclic compound **1d** is a moderate inhibitor of HLE (IC$_{50}$ = 3.7 µg/mL).[7] Inhibition is presumed to occur *via* covalent, but reversible, bond formation with catalytic site Ser^{195} of HLE *via* the intermediacy of **2** to generate a hemiketal **3** or by transition state inhibition.

In conclusion this communication describes the facile synthesis of a series of imidazo[1,2-a]piperazines **1** as mechanistic inhibitors of HLE from amino-keto tripeptides **8**.

Acknowledgement: We wish to thank Ms. G. Chow and Dr. M. Skoog for conducting all biological evaluations.

References and notes

1. a) Eriksson, S. *Acta. Med. Scand.* **1965**, 177 (suppl. 432).

   b) Stockley, R.A. *Clin. Sci.* **1983**, *64*, 119.

   c) Stockley, R.A. *Lungs* **1987**, *165*, 61

   d) Stine, P.J. *Clinics In Chest Med.* **1983**, *4*, 405

2. a) Impenali, B., Abeles, R.H. *Biochemistry* **1986**, *25*, 3760.

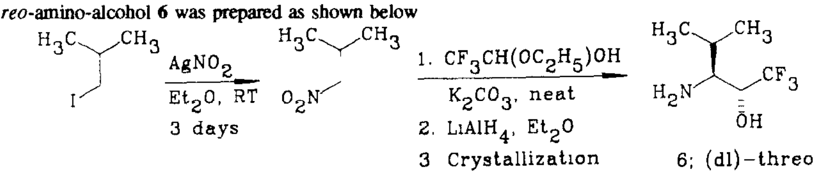
   b) Abeles, R.H. *Drug Develop. Res.* **1987**, *10*, 221

   c) Skiles, J.W., Fuchs, V., Chow, G.; Skoog, M. *Res. Commun. Chem. Pathol. Pharmacol* **1990**, *68*, 365.

3. The structure of the inhibitor **1d** has recently been verified by X-ray crystallography, the details of which will be reported elsewhere.

4. a) Impenali, B.; Abeles, R.H *Tetrahedron Lett.* **1986**, *27*, 135.

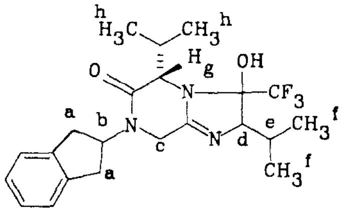
   b) The *threo*-amino-alcohol **6** was prepared as shown below



6; (dl)-threo

5. 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.

6. Compound **1d**: ^{1}H and ^{13}C NMR and MS data are listed below:



250.13 MHz ^{1}H NMR (CDCl$_3$, TMS) δ 0.99 (d, 3 H$_h$, CH$_3$), 1.04 (d, 3 H$_f$, CH$_3$), 1.11 (d, 6 H$_{f+h}$, 2 X CH$_3$), 2.07 (m, 1 H$_e$, CH), 2.15 (m, 1 H$_i$, CH), 2.90 (dd, 1 H$_a$, CH, J$_{a\text{-}a'}$ = - 16.6 Hz, J$_{a\text{-}b}$ = 5.9 Hz), 2.91 (dd, 1 H$_a$, CH, J$_{a\text{-}a'}$ = -16.6 Hz, J$_{a\text{-}b}$ = 5.9 Hz), 3.24 (dd, 1 H$_a$, CH, J$_{a\text{-}a'}$ = -16.6 Hz, J$_{a\text{-}b}$ = 8.5 Hz), 3.26 (dd, 1 H, CH, H$_{a\text{-}a'}$ = -16.6 Hz, J$_{a\text{-}b}$ = 8.5 Hz), 3.77 (dd, 1 H$_e$, CH, J$_{d\text{-}e}$ = 5.4 Hz, J$_{d\text{-}e}$ = 3 0 Hz), 3.86 (d, 1 H$_c$, CH, J$_{c\text{-}c'}$ = - 15.3 Hz), 3.92 (dd, 1 H$_c$, CH, J$_{c\text{-}c'}$ = -15.3 Hz, J$_{c\text{-}d}$ = 3.0 Hz), 4.03 (d, 1 H$_g$, CH, J$_{g\text{-}i}$ = 4.8 Hz), 5.38 (m, 1 H$_b$, CH), 5.82 (bs, 1 H, OH), 7 20 (bs, 4 H, aromatic CH); 67.93 MHz ^{13}C NMR (CDCl$_3$, TMS) δ 17.36 (CH$_3$), 19.18 (CH$_3$), 20.26 (CH$_3$), 22.00 (CH$_3$), 28.21 (^{e}CH), 33.54 (^{i}CH), 36.25 (indanyl CH$_2$), 36.38 (indanyl CH$_2$), 40.97 (^{c}CH$_2$), 53.57 (indanyl CH), 60.35 (^{g}CH), 75.77 (^{d}CH), 94.04 (q, J = + 30.5 Hz, C(OH)CF$_3$), 123.51 (q, J = - 283.4 Hz, CF$_3$), 124.43 (aromatic CH), 124.42 (aromatic CH), 127.17 (aromatic 2 X CH), 139.95 (quaternary C), 140.11 (quaternary C), 155.04 (C = N), 168.08 (C = O); MS (CI/CH$_4$) [(M + H)$^+$/(%)] 464 (M + C$_3$H$_5$)$^+$ (13.74), 452 (M + C$_2$H$_5$)$^+$ (36.91), 424 (100), 422 (M-H)$^+$ (17.63), 406 (M-H-H$_2$O)$^+$ (36.65), 404 (M-H-H$_2$O)$^+$, (16.75), 308 (15.95); FAB accurate mass calcd. for C$_{22}$H$_{29}$F$_3$N$_3$O$_2$ 424 2212 (MH$^+$), 424.2207 (MH$^+$, found).

7. For comparison the elastase inhibitory activity of the heterocycle **1d** (IC$_{50}$ = 3.7 μM) should be compared with the tripeptide derivative **8** (PG=CBZ; R = 2-indanyl)(IC$_{50}$=0.365 μM), Skiles, J.W. et al. *J. Med. Chem.*, in press. The ten fold decrease in potency of the heterocycles **1** relative to the tripeptide trifluoromethyl ketones **8** which span the S$_3$-S$_1$ subsites of HLE may be due to decreased interaction of **1** with HLE. It is assumed that deactivation of HLE by **1** proceeds through the intermediacy of the piperazine trifluoromethyl ketone **2** which then forms a hemiketal **3** with active site serine,^{195}Ser, as is known to occur with other trifluoromethyl ketones. Thus the isprpopyl group adjacent to the trifluoromethyl ketone substituent of **2** should thus occupy the S$_1$ subsite of HLE. However this would displace the second isopropyl group of the piperazine **2** away from the S$_3$ subsite thus leading to decreased binding and potency.