Bioorganic & Medicinal Chemistry Letters, Vol.2, No.7, pp. 651-654, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

MDL 74147, A NOVEL SELECTIVE AND SOLUBLE INHIBITOR OF HUMAN RENIN. SYNTHESIS, STRUCTURE-ACTIVITY RELATIONSHIP, SPECIES AND PROTEASE SELECTIVITIES.

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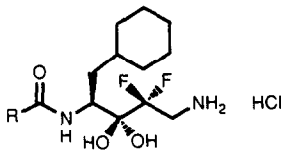
(*Received* 12 *March* 1992)

Abstract. *The synthesis of a novel, potent and soluble inhibitor of human renin bearing a β-amino-α,α-difluoroketone functionality is described. Its structure-activity relationship, species and protease selectivities are discussed.*

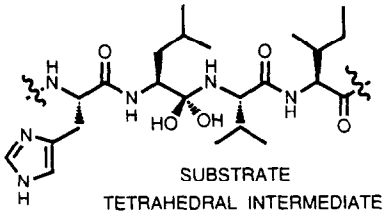
The search for potent and specific inhibitors of human renin [EC 3.4.23.15] has been a challenge to medicinal chemists for over twenty years[1]. Bioavailability problems have limited their use as antihypertensive agents[1]. Several concepts such as transition state analogues have been developed to optimally interact with the active site residues of aspartyl proteases, in particular renin.To overcome the solubilization problem, introduction of polar groups at either the carboxy or amino terminus of the known renin inhibitors has proven to be the most effective approach[1]. A few attempts to improve solubilization dealt with the formation of ion pairs between the active site aspartic acid residues and a basic site on the inhibitors, such as primary amine[2], secondary amine[3] or an imidazole[4].

Except for the latter example, inhibitory activities of the derived structures were relatively disappointing with decreasing size of the inhibitory structures. The difficulty of counterbalancing the energy required for desolvation of the ammonium group is one of the reasons invoked by the authors for that phenomenon[1,2].

Recently, the combination of a β,β-difluoroamine and a difluoromethyleneketone functionalities led us to a new class of highly selective,potent and soluble inhibitors of human renin (formula A, hydrate form).



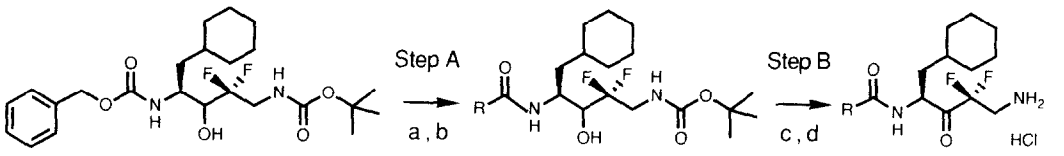
INHIBITOR
FORMULA A



SUBSTRATE
TETRAHEDRAL INTERMEDIATE

The general synthesis of this class of inhibitors is described in Scheme 1. The fully protected key intermediate 1 is easily accessible through a three step sequence described in reference 5. As illustrated for the aminoketone 3a, removal of the N4-protecting group by hydrogenolysis and subsequent derivatization (Iva (p.OCH_3)Phe nValOH, iBuOCOCl, NMM[11], CH_3CN) affords the expected alcohol 2a in 75 % overall yield. Oxidation (PDC[11], AcOH, 3 Å mol. sieves, CH_2Cl_2) and cleavage of the N_1 protecting group (saturated HCl/Et_2O; 0°C) yields the desired aminoketone 3a in 75 % yield. Additional examples are presented in Table 1.

SCHEME 1

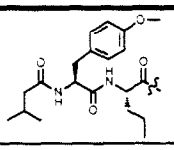
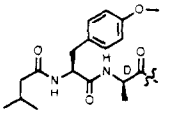
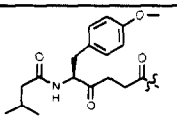
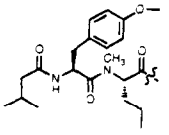


1 2a 3a

RCO : Iva(p.CH_3O)PhenVal

a) H_2, 10% Pd/C, C_2H_5OH, 95%, b) Iva(p CH_3O) PhenValOH , iC_4H_9OCOCl , NMM , CH_3CN ,79% , c) PDC 3Å molecular sieves , AcOH , CH_2Cl_2 , RT , 86% , d) HCl / $(C_2H_5)_2O$, 0° C, 87%

TABLE 1

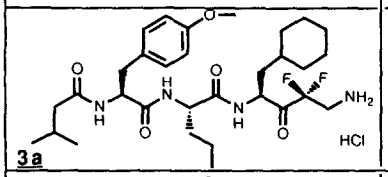
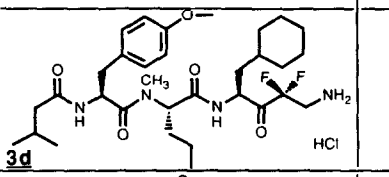
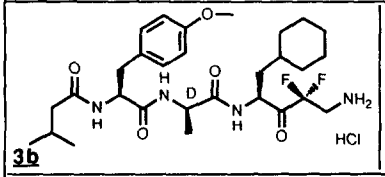
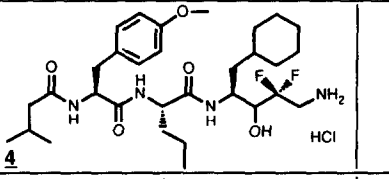
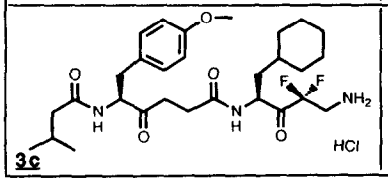
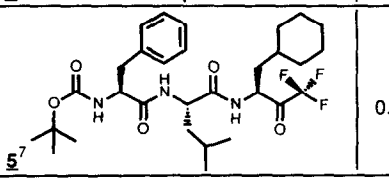
RCO	YIELDS OF CONVERSION[9] STEP A 1 → 2	STEP B 2 → 3
	75%	75%
	49%	58%
	60%	56%
	51%	35%

As shown in Table 2, MDL 74147 (3a) is species selective and effectively inhibits human and monkey plasma renins whereas dog or rat plasma renins are much less affected. 3a is also protease selective, inhibiting Pepsin, Cathepsin D and α Chymotrypsin at concentrations 2 to 3 orders of magnitude higher than for human or primate renins (Table 2).

TABLE 2

ENZYMES	PLASMA RENINS IC_{50}[10] (μM)				K_i[10] (μM)		
	HUMAN	MONKEY	DOG	RAT	PEPSIN	CATHEPSIN D	CHYMO-TRYPSIN
3a	0.016	0.022	3	100	40	4	2.5

TABLE 3

STRUCTURES	IC_{50} (μM)	STRUCTURES	IC_{50} (μM)
 3a	0.016	 3d	40
 3b	27	 4	>1
 3c	25	 5[7]	0.250[7]

The mode of binding of 3a to human renin is probably very similar to other renin inhibitors in terms of P2[6] or P3[6] selectivities. Replacement of the P2 L-n-Valine residue by D-Alanine (→3b), propionic acid (→3c) or N-CH3 L-n-Valine (→ 3d) moieties resulted in a virtual loss of activity (Table 3) showing the importance of both the amide NH and the α-S-configuration of the P2 residue. Results shown in Table 3 also clearly demonstrate that both the ketone and the amine contribute to efficient binding. Reduction of the ketone to the alcohol (→4) or replacement of the amino alkyl side chain by a fluorine atom (→5)[7] considerably reduces the potency of the inhibitors.

The fluoromethylene building block presumably plays the dual role of simultaneously inducing the hydration of the ketone[8] and weakening the basicity of the terminal aminefunction (measured pKa of 6.7, formula A).

These combined electronic effects help overcome the problem of the energy requirement for the desolvation of the ammonium group as the inhibitor binds to the enzyme. Interestingly, the weakly basic amino alkyl side chain also enhances the solubility of the inhibitor in aqueous media (Table 4).

TABLE 4

SOLUBILITIES IN AQUEOUS MEDIA

	H2O	Phosphate buffer 0.1M pH 7.4	NaCl 0.15M 0.9‰
3a	4.20	0.65	2.87
3b	2.02	0.068	ND
3c	99.6	0.41	ND

X-ray analysis should allow an accurate assignment of the hydrogen bond network and the putative ion pair that the difluoroaminoketone present in 3a might establish with the aspartyl residues of the active site of renin.
In vivo results will be reported in a following paper. The extension of this new concept of inhibition of renin to other aspartyl protease of therapeutic interest is under current investigation.

Acknowledgement. We acknowledge the excellent work of B. Heintzelmann, C. Colin and C. Kugel for measuring pKa value and providing solubilities data.

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8) MDL 74147: elemental analysis calculated for $C_{31}H_{48}N_4O_5F_2$ HCl, 1.5 H_2O: C, 56.57, H, 7.96; N, 8.51. Found C, 56.61, H, 7.66, N, 8.52. ^{19}F NMR (DMSO-d_6, 338 MHz, C_6F_6 ext. ref) ketone/hydrate ~ 55/45, υ_A 53.69 ppm, υ_B: 51.47 ppm (J_{FAFB} = 265.2 Hz, ketone); υ_A: 48.32 ppm, υ_B = 46.52 ppm (J_{FAFB} = 245.8 Hz, hydrate).

9) All new compounds were characterized by ^1H NMR and ^{19}F NMR, MS and/or combustion analysis.

10) Values were determined under the following conditions: **Enzyme**/substrate/buffer/temperature/analysis. **Renin**/endogeneous angiotensinogen/phosphate buffer pH 6.0/37°C/radioimmuno-assay; **Pepsin**/N-acetyl-L-Phe-3,5-diiodoTyr/0.05 M Formate pH 2.0/37°C/HPLC; **Cathepsin D**/porcine tetradecapeptide/0.25 M Citrate pH 3.5/37°C/HPLC; **α-chymotrypsin**/Benzoyl-Tyr ethylester/0.1 M phosphate pH 8/37°C/HPLC.

11) Abbreviations used: NMM, N-methyl morpholine; PDC, pyridinium dichromate.