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PRELIMINARY COMMUNICATIONS

INHIBITION OF ANGIOTENSIN-CONVERTING ENZYME BY DERIVATIVES OF 3-MERCAPTO-2-METHYLPROPANOYL GLYCINE

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In recent years potent and specific inhibitors of ACE have been discovered (1,2) and have been shown to be effective as antihypertensive agents* (3,4). An imino acid residue (e.g. proline) is a common structural feature of most potent ACE inhibitors reported in the literature (1,2). However, mercaptoacylamino acids which contain an acyclic tertiary amide group have not been studied extensively as inhibitors of ACE activity. In this communication we present data indicating that such mercaptoacylamino acids are potent inhibitors of ACE activity *in vitro*.

Methods: A crude preparation of ACE was obtained by extracting rabbit lung acetone powder (Pel-Freez) with phosphate buffer, pH 8.3, using the procedure described by Cushman and Cheung (5). The activity of the crude ACE was determined in 0.1M KH_2PO_4-0.3M NaCl-2% DMSO at pH 8.3 and 37° using HHL, 2mM, as substrate and the method of Cushman and Cheung (6). The quantity of enzyme used was sufficient to catalyze the hydrolysis of 10 to 15% of the substrate in 10 min. To determine I_{50} values, assays were initiated by adding enzyme to a buffered solution of substrate ± inhibitor.

Partially purified rabbit lung ACE was prepared according to the procedure described by Cheung *et al.*(7). It had a specific activity of 24 units/mg using HHL (5mM) as the substrate. Kinetic studies of captopril and the calcium salt of RHC 3454 were done with this partially purified ACE preparation. For these experiments ACE activity was determined in 0.1M KH_2PO_4-0.17M NaCl-2% DMSO, pH 8.0, at 37° using CBZ-Phe-His-Leu as substrate and the method described by Piquillod *et al.*(8); the ACE concentration was estimated to be 2.1×10^{-10}M. The rates of product formation were linear for 10 minutes under these conditions at all substrate and inhibitor concentrations. Solutions of inhibitors were prepared fresh daily. Fluorescence measurements were carried out as described by Cheung *et al.*(7) using excitation and emission wavelengths of 360 nm and 480 nm, respectively.

The syntheses of compounds listed in Table I have been described earlier (9).

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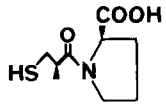
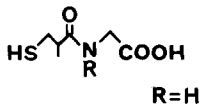
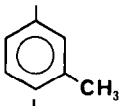
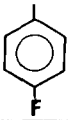
*Abbreviations used: ACE, angiotensin-converting enzyme; DMSO, dimethylsulfoxide; HHL, hippuryl-histidyl-leucine; CBZ, carbobenzyloxy; MK-421, N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]-L-Ala-L-Pro.

TABLE I.

Derivatives of 3-Mercapto-2-methylpropanoyl Glycine and their Inhibitory Effects on Rabbit Lung ACE Activity

Compound	RHCG ID NO.	Structure	I_{50} (μM)
captopril			0.018
1	2973	 R=H	0.21
2	3147	R=CH_3	0.13
3	3171-W*	R=*i*-Pr	0.072
4	3206	R= 	0.033
5	3471	R= 	0.018
6	3454	R= 	0.018
7	3402-W*	R= 	0.035
8	3319	R= 	0.30
9	3582-V**	R= 	0.019
10	3588	R= 	0.023

*Dicyclohexylammonium salt
**Benzathine salt

Results and Discussion: The data presented in Table I indicate that compound 1, 3-mercapto-2-methylpropanoyl glycine, is about 10 times less potent than captopril as an inhibitor of rabbit lung ACE. Substitution of the amide hydrogen atom of compound 1 by methyl or isopropyl groups resulted in two-fold and three-fold increases, respectively, in the inhibitory effect. An additional two-fold increase in inhibitory potency was achieved by cyclizing the isopropyl substituent on the amide nitrogen atom (compound 4).

The maximum inhibitory effect on ACE activity was obtained when a cyclobutyl (compound 5) or cyclopentyl (compound 6) group was the substituent on the amide nitrogen atom. Changing the substituent to a phenyl group (compound 8) resulted in a ten-fold decrease in potency, but substitution of the aromatic ring (compounds 9 and 10) resulted in ACE inhibitors that were essentially equipotent with captopril.

RHC 3454 (compound 6), one of the most potent ACE inhibitors listed in Table I, was characterized further by determining the type of ACE inhibition and the inhibitor constant, K_i, using CBZ-Phe-His-Leu as substrate. The double-reciprocal plots shown in Fig. 1 indicate that RHC 3454 is a mixed inhibitor of ACE activity. A K_m value of 0.10 mM was obtained from the double-reciprocal plot for the uninhibited reaction and a K_i value of 0.044 μM was obtained from a secondary plot of the slopes versus inhibitor concentrations. An identical experiment using captopril as the ACE inhibitor indicated that captopril was also a mixed inhibitor of ACE activity (Fig. 2) with a K_i of 0.030 μM. Captopril has been reported to be both a mixed (10) and a competitive inhibitor (1) of ACE activity with HHL as substrate.

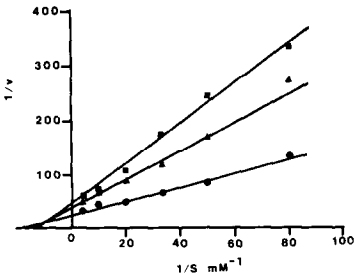


Fig. 1. Double reciprocal plots for the inhibition of rabbit lung ACE by RHC 3454. (●) control, (▲) 0.04 μM (■) 0.08 μM.

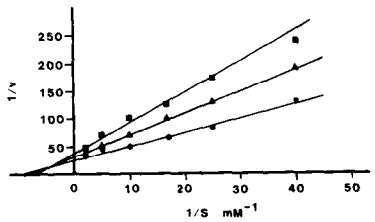


Fig. 2. Double reciprocal plots for the inhibition of rabbit lung ACE by captopril. (●) control, (▲) 0.02 μM (■) 0.04 μM.

The kinetic studies, together with the data presented in Table I, demonstrate that mercaptoacylamino acids containing an acyclic tertiary amide group are effective ACE inhibitors <u>in vitro</u> with a potency comparable to that of compounds containing the cyclic tertiary amide group. Recently, RHC 3659 (pivalopril), the pivaloyl thioester of RHC 3454, was shown to inhibit ACE in normal volunteers with an efficacy comparable to that of captopril and MK-421 (11).

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