(R)-2-(3-Mercapto-2(S)-methyl-1-oxopropoxy)-3-(methylthio)propanoic Acid, the First Ultra-Short-Acting Angiotensin Converting Enzyme Inhibitor[1]

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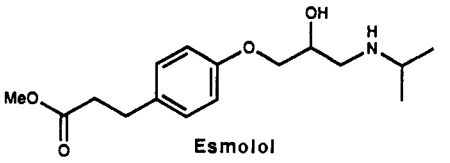
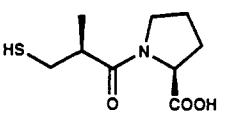
The rational design of ultra-short-acting (USA) agents for use in critical care has been described for β-blockers,[2] antiarrythmics,[3] and analgesics.[4] Their short duration of action is based on the inactive metabolite approach[5] whereby the active species is enzymatically degraded in vivo to less or inactive products. The advantages of these drugs over their longer acting counterparts is that the short biological half-life—normally 10–15 min—provides, on intravenous infusion, a rapid achievement of a steady-state plasma concentration and ready adjustment of the desired therapeutic effect. On cessation of infusion the action of the drug rapidly dissipates. This profile is of particular importance in critical care as it should allow ready titration and alteration of dose to balance changes in the state of the patient. An example of this concept is the ultra-short-acting β-blocker esmolol which is marketed for use as a continuous intravenous infusion.[6]

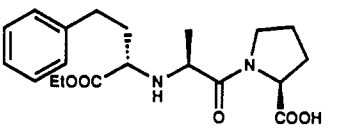
Sodium nitroprusside and glycerol trinitrate are commonly used vasodilators in the treatment of acute hypertension[7] and acute heart failure.[8] The use of these agents, however, is limited by tolerance,[9] stimulation of adverse neuroendocrine reflexes,[10] risk of hypotension,[7] and, in the case of sodium nitroprusside, thiocyanate toxicity.[7] Angiotensin converting enzyme (ACE) inhibitors, the best known examples of which are captopril and enalapril, are widely accepted vasodilators in chronic heart failure,[11] although their use in acute conditions has been restricted due to their prolonged duration of effect.[12] We therefore initiated a program to discover an ultra-short-acting ACE inhibitor (USACEI) whose very short duration of action should allow it to be suitable for critical care use. A further advantage for USACEI use is that once the desired therapeutic effect is observed and the patient has stabilized, then continued ACE inhibition can be provided via oral therapy using more conventional inhibitors.



Esmolol



Captopril



Enalapril

Three factors were considered particularly important in deciding on the required profile for a clinically useful USACEI. Firstly, high potency was seen as being vital in an agent that would be continuously infused and degraded. Captopril, the prototype ACE inhibitor, is active in the low nanomolar range in our screens; potency in this range (around 6 nM or less) was considered adequate. Secondly, the degradation product(s) should have little or no ACE inhibitory activity and be able to be rapidly cleared from the body. Small hydrophilic products were seen to be ideal. Thirdly, blood enzymes should be capable of breaking down the compound. This would have two main advantages. One is that, as human blood is readily available from volunteers, we would be able to screen compounds for rate of degradation in blood. By using the target species, i.e. man, for this measurement we would be able to avoid problems associated with extrapolation from laboratory animals to man. This was seen as being particularly important as variation of enzyme types, levels, structure, and substrate requirements between different species is well-known.[13] A second advantage of blood-borne degradation is that there would be no need to rely on metabolism or clearance by other organs, e.g., liver or kidneys. It is known that in critically ill patients blood flow to organs and enzyme activity can be seriously impaired.[10] A half-life for degradation of 10–15 min in human blood was seen to be rapid enough to give ready titration of dose. In summary, we required a potent ACE inhibitor that was degraded by human blood with a half life of 10–15 min to give small, inactive, hydrophilic products.

Captopril (1), being the smallest of the well known potent ACE inhibitors, was chosen as the starting point. The

(1) This work was presented in part at the 23rd National Medicinal Chemistry Symposium, Buffalo, NY, June 1992; Abstract Number 26, and at the 14th Scientific Meeting of the International Society of Hypertension, Madrid, Spain, June 1992; Poster Number D.P113 and B.P25.

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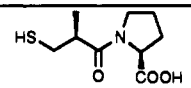
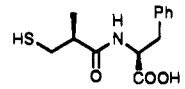
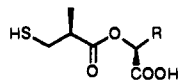
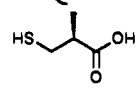
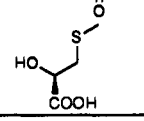
Table I. ACE Inhibition and Human Blood Hydrolysis Rates

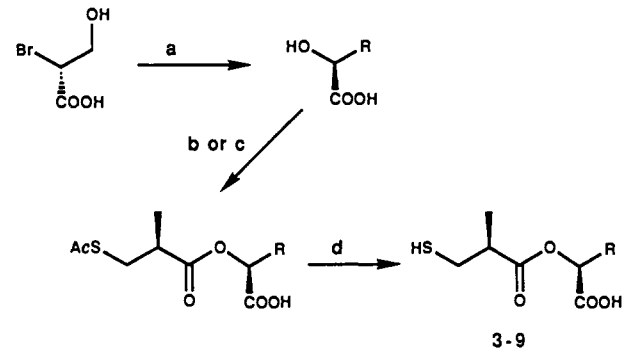
compound			ACE inhib, nM	blood $T_{1/2}$,[a] min
captopril		1	2.7	>60
		2	84.7	>60
 3–9	R = H	3	123.0	53
	CH_2Ph	4	10.6	23
	CH_2SCH_3	5	5.7	14
	$CH_2SCH(CH_3)_2$	6	2.8	20
	CH_2S-cyclopentyl	7	1.8	27
	$CH_2SCH(CH_2CH_3)_2$	8	2.0	37
	CH_2SCH_2Ph	9	3.2	>60
		10	30% inhib at 10^{-4} M	
		11	no inhib at 10^{-5} M	

[a] $T_{1/2}$ for mercapto acid 10 formation; see text and supplementary data for explanation and full details.

proline amide bond was chosen as the site of degradation, molecular alterations focusing on promoting its hydrolytic cleavage. The assay of ACE inhibitory activity was based on a published method using purified rabbit lung ACE.[14] This was modified to include the addition of Zn^{2+} (20 μM) to purified enzyme and the use of radiolabeled substrate. Hydrolysis rate in human blood was analyzed by HPLC of the reaction products after derivatization of the thiols with *N*-pyrenemaleimide.[15] These measurements were complicated by the fact that both parent compound and mercapto acid product were lost from the incubation by disulfide formation. However the rate of product loss by this route was relatively slow, $T_{1/2} > 40$ min, so measurements were made over the first 15 min and extrapolated to give $T_{1/2}$ values for mercapto acid formation. (Full details of the procedure is included as supplementary material.)

Tertiary amides and proline amides in particular, i.e. that present in captopril (1), are known to be resistant to cleavage by peptidases.[16] Indeed no captopril hydrolysis was observed in human blood (Table I). Replacement of proline with phenylalanine (compound 2)[17] should give a compound with an amide bond more susceptible to peptidases. Again no evidence for hydrolysis in human blood was observed. Depsipeptide bonds, i.e. replacement of NH by O, are known on occasions to lead to increased hydrolysis rates.[16] We therefore prepared the simplest of these compounds based on glycolic acid (compound 3).[18] We were pleased to observe slow hydrolysis of this compound in human blood as evidenced by the appearance of the mercapto acid 10.

Scheme I [a]



3-9

[a] Reagents: (a) $NaOCH_3$, CH_3OH then sodium thiolate; (b) (*S*)-$AcSCH_2CH(CH_3)COOH$, CDI, THF then add hydroxy acid, $(CH_3CH_2)_3N$, THF; (c) (*R*)-$AcSCH_2CH(CH_3)COCl$; (d) NH_3, H_2O.

Structural variation of the hydroxy acid portion of this compound was undertaken to give increased potency and hydrolysis rate. The synthetic route to these compounds is shown in Scheme I. The chiral hydroxy acids either were commercially available or were prepared by thiolate ring opening of the epoxy acid generated in situ from (*R*)-2-bromo-3-hydroxypropanoic acid, which in turn was prepared by nitrosation of D-serine in the presence of bromide ion.[19] Coupling to (*S*)-3-(acetylthio)-2-methylpropanoic acid was effected via the imidazolide[18] or acid chloride.[20] Deprotection using aqueous ammonia[18] gave the final compounds which were isolated as solid dicyclohexylamine or 1-adamantanamine salts.

Full details of the structure–activity relationships will be given in a full paper, but some general points are illustrated by compounds 3–9 (Table I). The presence of a substituent on the carbon atom α to the carboxy group gave both increased potency and altered hydrolysis rate. While potency could be further increased with larger substituents, hydrolysis rates of these more potent com-

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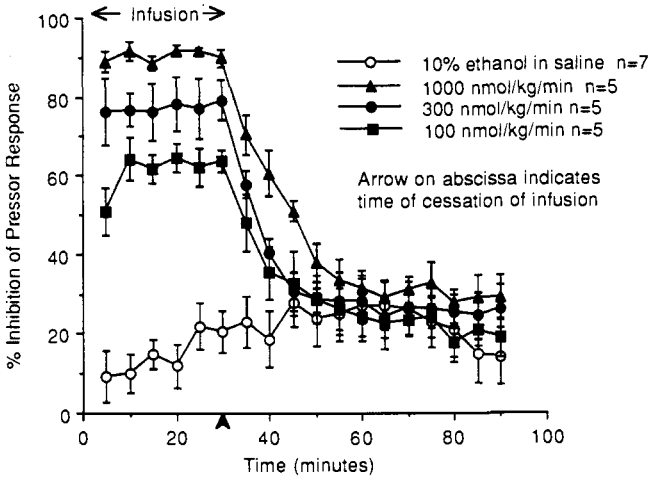

Figure 1. Effect of infusion of FPL 66564 on angiotensin I pressor responses in the urethane anesthetized rat.

pounds (6–9) became unacceptably long. The required balance of potency (5.7 nM) and degradation rate (14 min) was only observed in the (methylthio)methyl substituted compound 5 (FPL 66564). The hydrolysis products of this compound, the mercapto acid 10 and the hydroxy acid 11, are both without ACE inhibitory activity and, being small hydrophilic molecules, should not present any clearance problems.

As an illustration of the action of this USACE inhibitor in vivo, Figure 1 shows the effect on angiotensin I pressor responses in the anesthetized rat of initiating and terminating an intravenous infusion of compound 5. Rapid equilibrium is reached followed by a sustained plateau. On cessation of the infusion the effect rapidly dissipates to base-line levels. Further details of the pharmacodynamics and pharmacokinetics in this and other species will be published separately.

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Supplementary Material Available: Full experimental details and analytical data and human blood and anesthetized rat protocols (8 pages). Ordering information is given on any current masthead page.