JOURNAL OF
MEDICINAL
CHEMISTRY

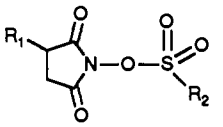
Volume 33, Number 4 April 1990

Communications to the Editor

Inhibitors of Human Leukocyte Proteinase-3

Sir:

Human leukocyte proteinase-3 (PR-3) is a serine endopeptidase that has been isolated recently from the granules of polymorphonuclear leukocytes.[1] The degradative action of PR-3 on elastin in vitro is comparable to that of human leukocyte elastase at a pH of 6.5. Furthermore, PR-3 induces emphysema in hamsters when administered intratracheally that is commensurate with that of HLE. PR-3 is the *second* neutrophil enzyme, besides HLE, that has the capacity to induce emphysema and may play a critical role in the pathogenesis of the disease. Like elastase, PR-3 is inhibited by α-1-proteinase inhibitor.[2,3] It is, however, unaffected by low molecular weight inhibitors such as, for example, methoxysuccinyl-Ala-Ala-Pro-Val chloromethyl ketone, a known inhibitor of elastase.[1] Although a large number of inhibitors of HLE have been reported in the literature,[4] no inhibitors have been reported for PR-3. This paper describes the utilization of a series of compounds represented by structure I as active site probes and as modulators of the activity of PR-3.



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Chemistry. Compounds 1–16 were synthesized by using procedures similar to those described previously.[5,6] The infrared and NMR spectra of the synthesized compounds were recorded on a Perkin-Elmer 1330 spectrophotometer and a Varian XL-300 NMR spectrometer, respectively.

Biochemistry. Proteinase-3 was isolated as described previously[1] and assayed by mixing 100 μL of a 375 nM enzyme solution, 475 μL of deionized water, 400 μL of 0.1 M phosphate buffer, pH 6.5, in a thermostated cuvette, followed by the addition of 25 μL of 20 mM BOC-Ala-*p*-nitrophenol in acetonitrile. The change in absorbance was monitored at 347.5 nm for 5 min.

In a representative inhibition run, 10 μL of a 0.22 μM solution of the inhibitor in dimethyl sulfoxide was mixed with 10 μL of 22.0 μM enzyme solution and 980 μL of 0.1 M phosphate buffer, pH 6.5, and placed in a constant-temperature bath. Aliquots (100 μL) were withdrawn at different time intervals and transferred to a cuvette containing 25 μL of 20 mM BOC-Ala-*p*-nitrophenol in acetonitrile and 875 μL of phosphate buffer. After incubating for 30 s, the absorbance was monitored at 347.5 nm for 1 min. The pseudo-first-order inactivation rate constants (k_{obs}) were obtained from plots of ln (v_t/v_0) vs time and expressed in terms of the apparent second-order inactivation rate constants, $k_{obs}/[I]$ (M^{-1} s^{-1}).[7] These are the

Table I. Inhibition of Human Leukocyte Proteinase-3 by Compound I

compd	R_1	R_2	$k_{obs}/[I]$,[a] M^{-1} s^{-1}
1	H	methyl	inactive
2	H	*trans*-styryl	*b*
3	methyl	methyl	2300
4	methyl	*trans*-styryl	4500
5	ethyl	methyl	13400
6	ethyl	*trans*-styryl	*c*
7	*n*-propyl	methyl	8600 (8600)
8	*n*-propyl	*trans*-styryl	*c* (*c*)
9	isopropyl	methyl	2200 (3800)[d]
10	isopropyl	*trans*-styryl	8100 (100000)[d]
11	*n*-butyl	methyl	800 (4100)
12	*n*-butyl	*trans*-styryl	3800 (*c*)
13	isobutyl	methyl	1200 (50000)[d]
14	isobutyl	*trans*-styryl	10400 (*c*)[d]
15	benzyl	methyl	inactive (1100)[d]
16	benzyl	*trans*-styryl	*b* (9400)[d]

[a] Values in parentheses are for HLE. [b] Less than 35% inhibition following a 10-min incubation of PR-3 with a 50-fold excess of inhibitor. [c] Inactivation was too fast to measure by sampling techniques. [d] Corresponding values for HLE taken from Groutas et al.

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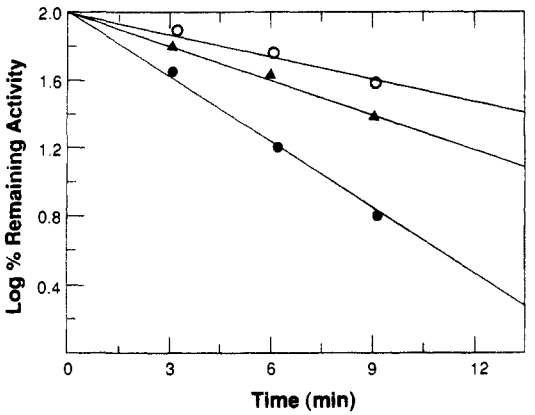

Figure 1. Kinetics of inactivation of proteinase-3 by compound 11. Proteinase-3 (284 nM) was incubated with 10-fold, 20-fold, and 30-fold excess of compound 11 in 0.1 M phosphate buffer, pH 6.5, and 2% organic solvent. Aliquots were removed periodically and assayed for enzymatic activity with use of BOC-Ala-*p*-nitrophenol.

average of two or three determinations. The k_2/K_i value for inhibitor 11 was computed by determining the k_{obs} values at various inhibitor concentrations and replotting the data according to the equation shown below:[7]

$$1/k_{obs} = (K_i/k_2)(1/[I]) + 1/k_2$$

Results and Discussion

A series of compounds represented by structure I have been investigated as inhibitors of PR-3. The results, expressed in terms of the bimolecular rate constant $k_{obs}/[I]$ (M^{-1} s^{-1}), are summarized in Table I.

The synthesized compounds inactivate PR-3 in a time-dependent and highly efficient manner (Figure 1). Furthermore, the interaction of PR-3 with these compounds involves the active site, since the presence of substrate in the incubation mix partially protects the enzyme from inactivation. With use of compound 7, for example, the $k_{obs}/[I]$ values in the absence and presence (0.4 mM) of substrate were found to be 8600 and 6350 M^{-1} s^{-1}, respectively.

The preference of PR-3 for compounds with small hydrophobic alkyl chains is clearly evident by the results in Table I. This is in agreement with experimental observations involving the specificity of action of PR-3 on the oxidized insulin A and B chains, shown to occur at locations involving amino acids with small hydrophobic side chains.[2,8,9] This predilection of PR-3 for hydrophobic substrates is also apparent by the avidity of PR-3 for elastin, the hydrophobic component of lung connective tissue.

Recent observations[10] strongly suggested that structure I may represent a general class of mechanism-based inhibitors of serine proteinases and that specificity for a target proteinase can be tailored by appropriate manipulation of R_1. Indeed, by varying the side chain (R_1), it has been possible to obtain fairly potent inhibitors of PR-3. Figure 2 shows the effect that the R_1 group, believed to be occupying the S_1 subsite,[11] has on the $k_{obs}/[I]$ ratio. It is clear that the presence of an alkyl group at the C-3 position is a prerequisite for inhibitory activity (compound

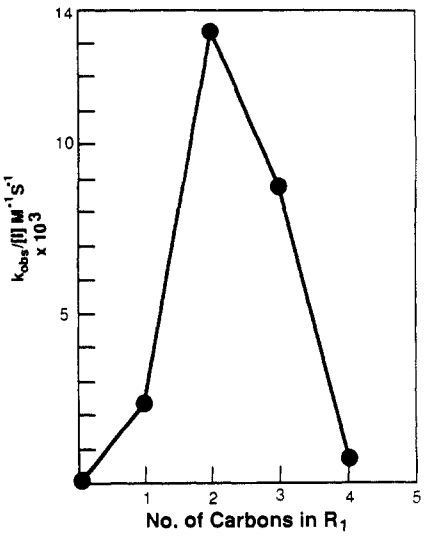

Figure 2. Effect of the size of R_1 on inhibitory activity.

1) and that inhibitory activity is abolished when the R_1 is bulky (15 and 16). Optimum inhibitory activity is reached with an ethyl group as the P_1 residue and further enhancement in inhibitory activity can be realized by introducing a *trans*-styryl group. The beneficial influence of the *trans*-styryl group appears to be invariant to the nature of the R_1 group (4, 6, 8, 10, 12, 14), with the exception of the inactive compounds. A similar effect was noted earlier with HLE, and on the basis of some preliminary modeling studies, this was tentatively ascribed to a favorable interaction between the phenyl group of the *trans*-styryl group and Phe-41, a residue that is known to span the S_1' and S_3' subsites of HLE.[12,13]

The P_1 specificity of HLE has been shown to be dependent on substrate chain length, becoming broader with decreasing chain length.[14] Thus, reactive monomeric substrates and inhibitors with bulky side chains can be accommodated at the active site of the enzyme. This may not be the case with PR-3 (compound 15). Interestingly, the HLE substrates Suc-Ala_3-pNA and Suc-Ala_2-Val-pNA do *not* serve as substrates of PR-3.[1] Steady-steady kinetic studies with peptidyl *p*-nitroanilides and benzylthio esters aimed at probing further the active site of PR-3 and unraveling the substrate-specificity requirements of this enzyme are currently in progress.

Although the precise nature of the interaction of PR-3 with these inhibitors is uncertain at the moment, it very likely involves formation of an acyl enzyme followed by either slow deacylation and/or formation of a reactive electrophilic species capable of reacting with an active-site nucleophilic residue (His-57).[10] We have observed that the incubation of PR-3 with a 10-fold excess of compound 5 results in an initial rapid and total inactivation of the enzyme, followed by a slow and partial regain in enzymatic activity (60% and 65% after 24 and 48 h, respectively).

In conclusion, we have reported the first class of time-dependent inhibitors of the recently discovered neutrophil serine endopeptidase proteinase-3. Further biochemical studies with this elastolytic enzyme are currently in progress and will be reported in due course.

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Acknowledgment. This work was supported by the National Institutes of Health (HL 38048 to W.C.G. and HL 37616 to J.R.H.).

† Wichita State University.
‡ University of Utah Health Sciences Center.
§ University of Minnesota.

W. C. Groutas,*,† J. R. Hoidal,*,‡ M. J. Brubaker† M. A. Stanga,† R. Venkataraman† B. H. Gray,§ N. V. Rao‡

Department of Chemistry
Wichita State University
Wichita, Kansas 67208
School of Medicine
Division of Respiratory, Critical Care and Occupational Medicine
University of Utah Health Sciences Center
Salt Lake City, Utah 84132
Department of Microbiology and Medicine
University of Minnesota
Minneapolis, Minnesota 55455

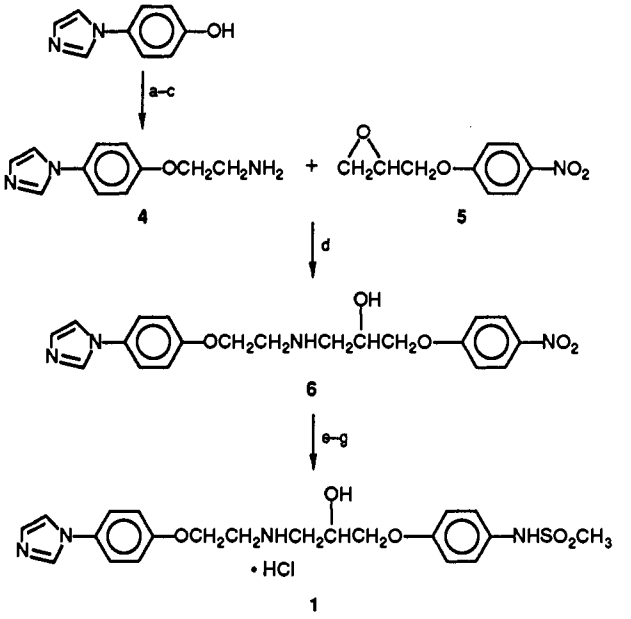
Received November 29, 1989

Synthesis and Pharmacological Studies of *N*-[4-[2-Hydroxy-3-[[2-[4-(1*H*-imidazol-1-yl)phenoxy]ethyl]amino]propoxy]phenyl]methanesulfonamide, a Novel Antiarrhythmic Agent with Class II and Class III Activities

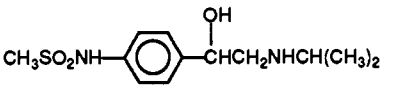
Sir:

Sudden cardiac death (SCD) claims approximately 400 000 lives annually in the United States.[1] Most of these deaths are due to reentrant ventricular arrhythmias.[2] No single antiarrhythmic agent is effective in a majority of arrhythmia patients due to the variety of etiologies contributing to SCD. Our approach to the treatment of SCD was to design compounds with multiple focussed activities in order to obtain agents with a broader therapeutic application. We chose to combine Class III electrophysiological activity (prolonging cardiac refractoriness)[3] with Class II (β-blocking) activity. Class III antiarrhythmic agents have been shown to be effective in models of reentrant arrhythmias.[4-8] These arrhythmias are thought to be major contributors to SCD.[8-10] Class II agents reduce enhanced sympathetic activity which has been implicated as a potential trigger for reentrant arrhythmias.[11,12] In fact, β-blockers are the only agents approved for the purpose of reducing mortality after a myocardial infarction. Thus, a combination Class II/III agent should not only decrease the opportunity for a triggering event, but should also prevent the establishment of a reentrant rhythm by increasing cardiac refractoriness. (±)-Sotalol can be considered as a prototype of these agents; however, this compound lacks potency as a Class III agent relative to its Class II activity and is a nonselective β-blocker.

Scheme I. Synthesis of Compound 1[a]



[a] (a) 2-Ethyl-2-oxazoline; (b) 6 N HCl; (c) NaOH; (d) $(CH_3)_3Al/CH_2Cl_2$; (e) H_2, Pd–C/EtOH; (f) CH_3SO_3H, CH_3SO_2Cl/H_2O (pH = 5–6); (g) HCl/MeOH.



(±)-sotalol

Our goal was to design potent Class III agents with a *balanced* amount of cardioselective β-blockade which would be effective against reentrant arrhythmias and catecholamine dependent arrhythmias without adverse conduction slowing or hemodynamic activity. The approach to these agents was to combine a Class III pharmacophore with a β-blocking pharmacophore using a common nitrogen moiety. We describe the synthesis and pharmacology of the most interesting compound from this work, **1**, its enantiomers and a limited series of related analogues. A full report of the compounds which led to **1** will be given in a subsequent publication.

We had observed that *N*,*N*-diethyl-2-[4-(1*H*-imidazol-1-yl)phenoxy]ethanamine (**2**) and *N*-[4-[3-(diethylamino)-2-hydroxypropoxy]phenyl]methanesulfonamide (**3a**) were potent Class III electrophysiological agents in canine Purkinje fibers (see Table II). Further, Smith[13] has

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