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SULFONAMIDE-BASED HYDROXAMIC ACIDS AS POTENT INHIBITORS OF MOUSE MACROPHAGE METALLOELASTASE

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Abstract: The structural requirements of sulfonamide-based hydroxamic acid **1** for inhibition of macrophage metalloelastase (MME) were investigated. A short aliphatic group at the R_2 position together with an aromatic group at the R_3 position significantly improved the inhibitory activity. Compounds **32**, **34**, and **40** were the most potent inhibitors of MME with IC_{50} values between 5 and 6 nM. © 1998 Elsevier Science Ltd. All rights reserved.

Chronic inflammation is often maintained by continuous recruitment and activation of monocytes and macrophages in response to persistent inflammatory stimuli at the site of injury.[1] These macrophages release proteolytic enzymes that degrade extracellular matrix, and chronic secretion of these enzymes can result in tissue damage.[2] For years, it has been hypothesized that neutrophil elastase is primarily involved in the pathogenesis of emphysema, a disease that is characterized by destruction of elastin in the lung alveolar wall.[3,4] However, increasing evidence has suggested a prominent role of macrophage metalloelastase (MME) in causing this disease.[5,6] Therefore, inhibitors of this enzyme may have therapeutic value.

Both the murine and human MME's have been cloned.[7,8] In this study, mouse MME was expressed in *Escherichia coli*, and the enzyme was purified according to a published protocol.[9] Since compounds containing a hydroxamic acid group have been shown to be effective inhibitors of metalloproteases such as collagenase,[10] neutral endopeptidase 24.11,[11] and endothelin converting enzyme,[12,13] they were therefore tested in the MME enzyme assay. The syntheses of the sulfonamide-based hydroxamic acid **1** and its analogs were reported previously.[14] [^{3}H]Elastin was used as a substrate for purified MME.[15] Table 1 shows the effects of R_4 modifications of compound **1** on MME inhibition. Compound **1** is a modest inhibitor of MME; a 27% inhibition of the enzyme activity was obtained at 100 nM. No significant change in the inhibitory activity was observed with fluorine substitution **2**, but the potency of the chlorine-containing analog **3** was greatly improved. Interestingly, a methyl substitution **4** resulted in 56% inhibition of MME activity, while an amino or dimethylamino (**5**, **6**) was not as effective. A methoxy group (**7**) in the R_4 position was found to be the most

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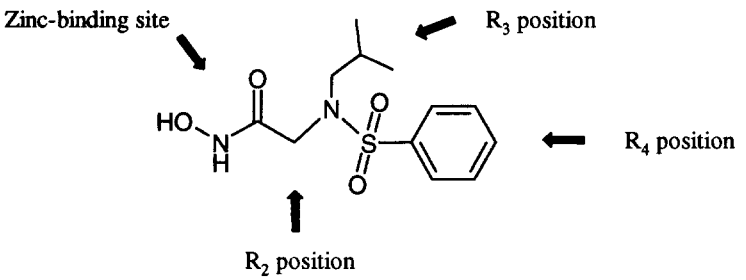
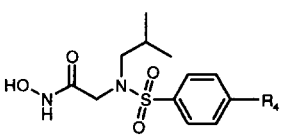
potent, with 70% inhibition of MME activity at 100 nM. No further improvement was obtained with elongated alkoxy chains (**8**). Therefore, a methoxy group was fixed at this position for additional modifications.



Compound **1**

Apparently, both the length and branching of an aliphatic chain at the R_3 position are critical for MME inhibition, since any slight structural variation resulted in a significant decrease in potency (Table 2). For example, a total loss of inhibitory activity was seen in compounds with shorter chains at the R_3 position (**9**, **10**), and a drastic decrease in the potency was observed in compound **12** with an additional methylene when compared with compound **7**. The effects of an aromatic group at the R_3 position were also investigated. The cyclohexyl **13** was a weak inhibitor, while the cyclohexylmethyl **14** was as potent as compound **7**. Surprisingly, an additional methylene (**15**) did not affect the inhibitory activity as was shown in compound **12**. This observation was also confirmed in the alkyl phenyl substituents **16-18**. No substantial changes in the inhibitory activity in compounds with a *p*-methyl (**19**), *p*-chloro (**20**), *p*-methoxy (**21**), or *m*-methoxy (**22**) substituent were noted when compared with compound **16**. Likewise, replacing the phenyl ring with a biphenyl (**23**) or pyridyl

Table 1. Effects of R_4 modifications on MME inhibition

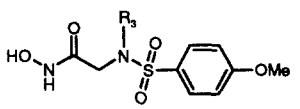


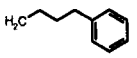
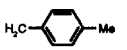
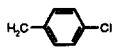
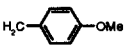
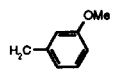
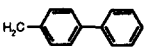
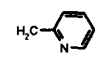
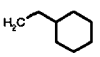
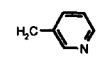
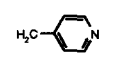
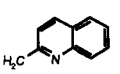
Compd	R_4	% Inhibition @ 100 nM	Compd	R_4	% Inhibition @ 100 nM
1	H	27	**5**	NH_2	22
2	F	22	**6**	$N(CH_3)_2$	34
3	Cl	68	**7**	OCH_3	70
4	CH_3	56	**8**	$O(CH_2)_2OCH_2CH_3$	69

The values for % inhibition of MME activity represent the means of 3 determinations with less than 7% in SEM.

group (**24-26**) only resulted in a marginal improvement of the potency. On the other hand, quinolyl **27** was less potent compared with compound **16**. These results strongly suggest that the enzyme may contain a narrow but deep hydrophobic pocket. Further improvement was attempted with a benzyl group fixed at the R_3 position.

Table 2. Effects of R_3 modifications on MME inhibition



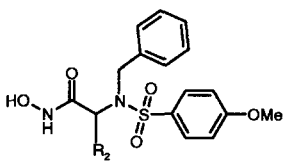
Compd	R_3	% Inhibition @ 100 nM	Compd	R_3	% Inhibition @ 100 nM
9	H	0	**18**		83
10	$CH(CH_3)_2$	0	**19**		82
11	$CH(CH_3)CH_2CH_3$	35	**20**		76
7	$CH_2CH(CH_3)_2$	70	**21**		89
12	$(CH_2)_2CH(CH_3)_2$	11	**22**		75
13	C_6H_{11}	13	**23**		86
14		77	**24**		88
15		79	**25**		80
16		74	**26**		92
17		88	**27**		50

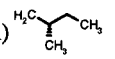
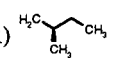
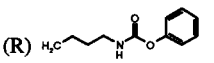
The values for % inhibition of MME activity represent the means of 3 determinations with less than 7% in SEM.

The effects of R_2 modification are shown in Table 3. A significant increase in potency was achieved with the inclusion of a methyl group (compound **28**). Separation of the isomers showed that the R-enantiomer **29** was about 3-fold more potent than its S-enantiomer **30**, with respective IC_{50} values of 9.6 and 35 nM for inhibition of MME. The same preference of the R-enantiomer was also confirmed with the isopropyl (**31**, **32**) and isobutyl (**34**, **35**) substituents. Compounds **32** and **34** were found to have the optimal chain lengths as the most potent

inhibitors of MME with IC_{50} values of about 5 nM. Decreasing (compound **29**) or increasing (compound **33**) the length of the alkyl chain by one methylene group resulted in slightly weaker inhibitors. Substitution with a cyclopentyl, piperidyl, or phenyl (compounds **36-39**) group at the R_2 position decreased the inhibitory potency in general.

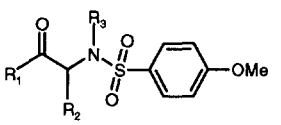
Table 3. Effects of R_2 modifications on MME inhibition

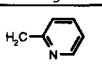
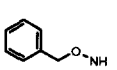


Compd	R_2	IC_{50} (nM)	Compd	R_2	IC_{50} (nM)
28	(R, S) CH_3	13	**34**	(R) 	4.8
29	(R) CH_3	9.6	**35**	(R) 	16
30	(S) CH_3	35	**36**	(R) C_5H_9	15
31	(R, S) $CH(CH_3)_2$	16	**37**	(R) 4-piperidine	> 100
32	(R) $CH(CH_3)_2$	4.9	**38**	(R) Ph	31
33	(R) $CH_2CH(CH_3)_2$	13	**39**	(R) 	67

The IC_{50} values represent the means of 3-6 determinations with less than 11% in SEM.

Table 4. Effects of modifications of the zinc binding, R_2, and R_3 sites on MME inhibition



Compd	R_1	R_2	R_3	IC_{50} (nM)
40	HOHN	(R) $CH(CH_3)_2$		6.0
41	HOHN	(S) $CH(CH_3)_2$		37
42	HOHN	(R) $CH_2CH(CH_3)_2$		6.9
43	HOHN	(R) $CH(CH_3)_2$		7.7
44		(R) $CH(CH_3)_2$		No inhibition @ 100 nM
45	HO	(R) $CH(CH_3)_2$		No inhibition @ 100 nM

The IC_{50} values represent the means of 3 determinations with less than 11% in SEM

Since compounds with a pyridyl group at the R_3 position (compounds **24-26**, Table 2) might be more potent than that with a phenyl group (**16**) as MME inhibitors, compound **32** was therefore further optimized. However, the resulting compounds **40** and **43** did not show improvement in MME inhibition when compared with compound **32**. On the other hand, a slightly more potent inhibition was seen with a pyridyl group at the R_3 position in compounds containing a longer alkyl R_2 side-chain (compare compounds **33** and **42**). As expected, the S-enantiomer **41** was found to be a weaker MME inhibitor than its corresponding R-enantiomer **40**. The hydroxamic acid moiety of this series of compounds is critical for MME inhibition; any modification of this zinc-binding group resulted in a total loss of the inhibitory activity (compounds **44** and **45**).

In conclusion, optimization of sulfonamide-based hydroxamic acid series of compounds has led to the discovery of potent inhibitors of MME. Recently, it has been shown that MME-deficient ($MME^{-/-}$) mice, in contrast to wild-type mice, do not develop emphysema upon long-term exposure to cigarette smoke.[16] These results strongly suggest that MME inhibitors may be useful for the treatment of chronic respiratory diseases such as emphysema.

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15. To test the inhibitory activity, a compound at the desired concentration was pre-incubated with 0.5 μg of purified, recombinant mouse MME for 20 min at room temperature in a buffer containing 5 mM $CaCl_2$ and 400 mM NaCl in 20 mM Tris, pH 8.0, prior to addition of [^{3}H]elastin (90,000 cpm). The mixture was incubated at 37 °C for 4 h in a total volume of 200 μL, and the reaction was terminated by centrifugation. The radioactivity in 140 μL of supernatant was counted in a scintillation counter to quantitate the amount of degraded elastin. In order to determine the IC_{50} values for MME inhibition, at least 7 concentrations of each inhibitor in triplicate samples were used, and the full concentration-response curve of each inhibitor was repeated 2-3 times. The IC_{50} values were determined by a nonlinear least squares curve-fitting program.

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