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DESIGN AND SYNTHESIS OF THIOL CONTAINING INHIBITORS OF MATRIX METALLOPROTEINASES

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Abstract: A series of thiol containing derivatives was prepared. Several of these compounds were found to inhibit matrix metalloproteinases 1, 3, and 9 with selectivity towards 3 and 9. Compounds **15**, **20**, and **22** were administered to rats orally at 75 μmol/kg. Drug levels of compounds **20** and **22** in the plasma were found to exceed the IC_{50} values for MMP 3 and 9 four hours after administration.

Matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that are involved in regulating breakdown and remodeling of extracellular matrix.[1] The overexpression and activation of these enzymes has been implicated in the destruction of tissue in diseases such as arthritis, cancer, and multiple sclerosis.[2-4]

Even though there is little homology between the MMPs and either angiotensin converting enzyme (ACE) or neutral endopeptidase (NEP), both of which are also zinc-containing metalloproteinases, we were interested by the results detailed by Chiroscience, where a series of thiol containing MMP inhibitors were

ACE/NEP TEMPLATE **MMP TEMPLATE**

identified to be structurally similar to thiol containing dual ACE/NEP inhibitors.[5,6] One of the major changes in the molecules was the modification of the C-terminal acid in the ACE/NEP template to an amide. We had previously reported on a series of thiol containing dual ACE/NEP inhibitors.[7] We planned to use this ACE/NEP template (**1**) as our starting point and determine if we could also impart MMP inhibitory activity by varying the design of these molecules.[8] Many of the compounds from template **1** were already proven to be long acting inhibitors of ACE and NEP in vivo after oral admistration, further making this template an attractive starting point.

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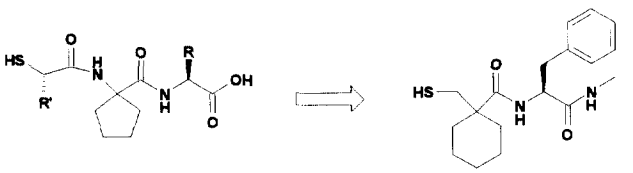
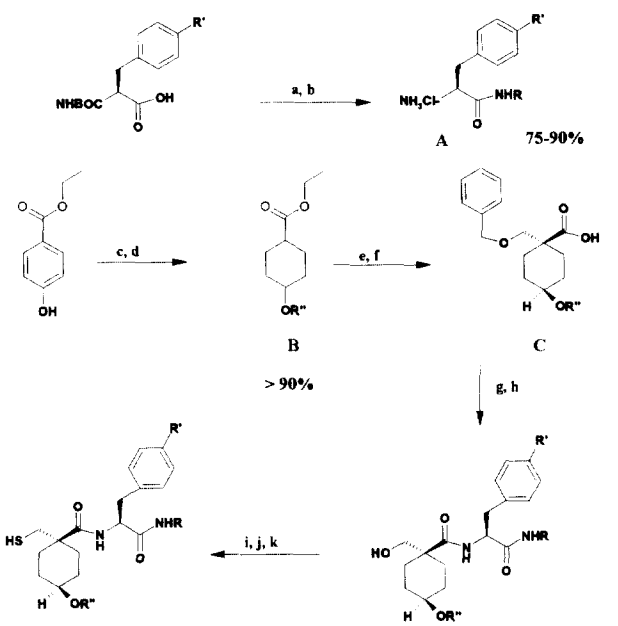
By simply converting the C-terminal carboxylic acid in our lead thiol ACE/NEP template (**1**) to an N-methyl amide, we obtained compounds with very little activity against the MMPs (MMP-1, 3, 9; IC_{50} > 2,000 nM). However, by removing the amide bond next to the thiol we obtained our early lead MMP inhibitor (**2**) which possessed micromolar activity against MMPs 1, 3, and 9.[9] Further optimization of this molecule, in particular by the addition of substituents to the 4- position of the cyclohexyl unit, led to nanomolar inhibitors of MMPs 1, 3, and 9. The synthetic pathway used to contruct the thiol containing MMP inhibitors is shown



1 **2**

in Scheme 1. This route is convergent and can be used to easily prepare a variety of different derivatives. The key 4-alkoxy substituted cyclohexylcarboxylic acid was prepared starting from ethyl-4-hydroxybenzoate. Alkylation of the phenol with an alkyl bromide in the presence of potassium carbonate afforded an ether

Scheme 1



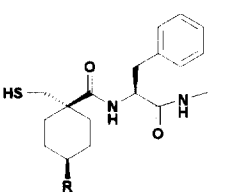
a. RNH_2, EDCI, HOBt, CH_2Cl_2; b. HCl(g), CH_2Cl_2; c. R"Br, K_2CO_3; d. 5% Rh/C, EtOH, H_2 50 psi.; e. LDA, BOMCl, 0°C; f. KOH, EtOH, reflux; g. A, DCC, HOAt, Et_3N, CH_2Cl_2; h. 10% Pd/C, EtOH, HCl, H_2 60 psi; i. MsCl, Et_3N, CH_2Cl_2; j. AcS^-K^+, CH_3CN; k. 1N NaOH, MeOH.

which was hydrogenated to the saturated cyclohexane ring with 5% rhodium on carbon in good overall yield. Treatment of the ester with lithium diisopropyl amide and benzyl chloromethyl ether gave primarily (80 to 95%) the equatorially alkylated product. The stereochemistry of the alkylated product was initially assigned through a series of NOE NMR experiments and further confirmed by obtaining an X-Ray crystal structure of intermediate **D** (R = Me, R' = OMe, R" = Me) .[10] The 4-substituted alkyl and CF_3 cyclohexyl acids which were used in the preparation of analogs 7, **8**, and **9** are commercially available and can be converted to the needed benzyloxy intermediates (**C**) as shown in Scheme 1.

The ester was then hydrolyzed with potassium hydroxide and coupled to an amino acid amide (**A**) in the presence of 1-hydroxy-7-azabenzotriazole (HOAt) and 1,3-dicyclohexylcarbodiimide (DCC).[11] The benzyl group was removed by catalytic hydrogenation and the resulting alcohol (**D**) was converted to a mesylate. The mesylate was displaced with potassium thioacetate and then treated with 1 N sodium hydroxide to provide the free thiol analog.

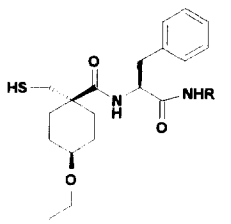
Many of the compounds prepared by this synthetic route are shown in Tables 1, 2, and 3. In our initial SAR investigations, we also prepared the cyclopentyl analog of **2** which was found to have no in vitro activity.[12] As a result, we focused our efforts on 6-membered ring analogs.

Table 1. Cyclohexyl Modifications



		MMP IC_{50} nM		
Entry	R	1	3	9
2	H	5,030 ± 890	9,200 ± 1,130	350 ± 130
3	OMe	110 ± 6	926 ± 57	35 ± 7
4	3-OMe[13]	--	> 10,000	> 10,000
5	OEt	700 ± 250	640 ± 50	18 ± 3
6	OPr	6,032 ± 917	187 ± 21	630 ± 29
7	Pr	--	> 10,000	> 10,000
8	tBu	--	> 10,000	> 10,000
9	CF_3	669 ± 33	4,474 ± 285	108 ± 11

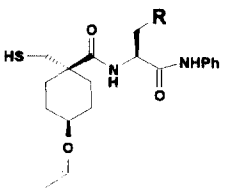
Table 2. C-Terminal Amide Modifications



		MMP IC_{50} nM		
Entry	R	1	3	9
10	isopropyl	680 ± 105	568 ± 137	40 ± 11
11	cyclopentyl	561 ± 133	666 ± 220	24 ± 5
12	cyclohexyl	2,933 ± 702	617 ± 76	79 ±23
13	phenyl	1,223 ± 119	76 ± 4	49 ± 13
14	phenyl[14]	--	> 10,000	> 10,000
15	*p*-(MeO)phenyl	1,273 ± 261	39 ± 6	46 ± 11
16	*p*-(F)phenyl	2,153 ± 301	87 ± 18	52 ± 14
17	*p*-(Cl)phenyl	5,909 ± 765	113 ± 13	79 ± 30
18	*p*-(NMe_2)phenyl	1,330 ± 83	77 ± 14	63+/-9
19	*p*-(OCF_3)phenyl	> 10,000	175 ± 27	81 ± 20
20	benzyl	823 ± 139	207 ± 4	26 ±5
21	2-pyridyl	3,473 ± 614	449 ± 84	121 ± 30
22	3-pyridyl	135 ± 64	73 ± 31	17 ± 4

Substitution of the cyclohexane ring was probed primarily at the 4-position to simplify the stereochemical complexity of the series. We did however synthesize the 3-methoxy analog, compound **4**.[13] It was found to be inactive. As can be seen from Table 1, having a small alkoxy group in the 4-position such as methoxy or ethoxy enhances the compound's activity against MMPs 1, 3, and 9. Interestingly, the propyl analog (**7**) has no in vitro activity. Perhaps the ether oxygen is acting as a hydrogen bond acceptor and thus this additional interaction with the enzymes leads to the observed increase in potency. Since the ethoxy derivative (**5**) had the best overall MMP inhibitory activity, additional analogs of this compound were prepared. Further modification of the C-terminal amide as shown in Table 2 can lead to increased potency against MMP-1 as seen in particular for analogs **11** and **22**. Interestingly the *p*-(OCF_3) phenyl analog (**19**) lost all activity against MMP-1 whereas the para- methoxy (**15**) and fluoro (**16**) analogs still had low micromolar activity. To investigate the importance of the orientation of the substituents on the cyclohexyl ring we also prepared the other diastereomer of compound **13**.[14] This analog, compound **14** was inactive in vitro. In order to probe the effect of varying the amino acid side chain, a series of N-phenyl amino acid derivatives of **13** were prepared as shown in Table 3. The best analog prepared with respect to overall MMP activity was the 3-pyridyl analog (**28**). No large increase in in vitro potency or selectivity was seen in this series.

Table 3. C-Terminal Amino Acid Modifications



		MMP IC_{50} nM		
Entry	R	1	3	9
13	**phenyl**	**1,223 ± 119**	**76 ± 4**	**49 ±13**
23	*p*-(OH)phenyl	739 ± 218	52 ± 8	14 ± 3
24	*p*-(MeO)phenyl	1,059 ± 136	62 ± 3	20 ± 2
25	*p*-(OEt)phenyl	3,271 ± 754	139 ± 29	34 ± 9
26	*p*-(Cl)-phenyl	1,938 ± 252	223 ± 14	66 ± 12
27	*p*-(F)phenyl	6,259 ± 3,058	134 ± 15	83 ± 39
28	3-pyridyl	564 ± 81	49 ± 2	21 ± 1
29	2-thienyl	953 ± 110	47 ± 9	42 ± 8

From preliminary modeling studies and SAR information we believe the compounds are filling the S_1', S_2' and S_3' subsites of the enzymes, with the cyclohexyl group in S_1'.

Compounds **15**, **20**, and **22** were administered to rats, orally at 75 μmol/kg. As seen in Table 4, LC/ESI/MS analysis of the plasma samples taken from these rats, showed that drug levels exceed the IC_{50} values for MMP 3 and 9 for at least 4 h after administration for compounds **20** and **22**.[15]

Although we did make a number of modifications to the starting ACE/NEP template we were encouraged with the fact that a few of these compounds could be detected in blood samples after oral administration. Overall it seemed that these compounds had better in vitro activity for MMP 3 and 9 than for MMP 1. In addition, by selecting the appropriate substituents it is possible to make these compounds more selective for MMP 9. Further testing of these compounds in a variety of disease models will determine if they have therapeutic utility.

Table 4. Pharmacokinetic Parameters of MMP Compounds Obtained Following Oral Administration in Rats

	Plasma Concentration		
Entry	2 h (nM)	4 h (nM)	number of rats
15	not detected	not detected	2
20	837 ± 60	491 ± 68	3
22	253 ± 71	289 ± 10	2

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8. For a related series of thiol containing MMP-1 inhibitors see Beszant, B.; Bird, J.; Gaster, L. M.; Harper, G. P.; Hughes, I.; Karran, E. H.; Markwell, R. E.; Miles-Williams, J.; Smith S. A. *J. Med. Chem.***1993**, *36*, 4030.
9. Assay contitions for MMP 1, 3, and 9 determinations are as described in MacPherson et al. *J. Med. Chem.* **1997**, *40*, 2525. All measurements are at least n = 3 except for compound **2** where there is an n =2 .
10. Details of the X-Ray crystal analysis are available upon request.
11. EDCI could also be used in place of DCC. For these amide bond forming reactions HOAt generally gave higher yields, see Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
12. Cyclopentyl derivative IC_{50}(MMP-1) = > 10,000 nM; IC_{50}(MMP-3) = > 10,000 nM ; IC_{50}(MMP-9) = > 10,000 nM.
13. Prepared in a similar manner as shown in Scheme 1, from the commercially available acid. Submitted as a mixture of diastereomers.
14. Collected the opposite diastereomer of **C** as shown in Scheme 1 by chromatography and converted to analog **14**.
15. A liquid chromatography/electrospray/tandem mass spectrometry assay was developed to determine plasma concentration of drugs levels in male Sprague Dawley rats administered a single oral dose of 75 μmol/kg (oral gavage in 3% corn starch). Plasma samples were collected at 0.5, 1, 2, and 4 h. An internal standard was added along with dithiothreitol. The samples underwent protein precipitation with acetonitrile, filtered, and the filtrate was injected onto an HPLC system.