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Oxal Hydroxamic Acid Derivatives with Inhibitory Activity against Matrix Metalloproteinases

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Abstract—Several amines, amino acid derivatives and low molecular weight peptides containing an amide-bound oxal hydroxamic acid moiety have been synthesized and tested for their inhibitory effects towards native human gelatinase B (MMP-9) and the catalytic domains of the membrane type MT1-MMP (MMP-14) and of neutrophil collagenase (MMP-8). A number of these compounds exhibited considerable inhibitory activity against the tested metalloproteinases.

Recently, the development of non-substrate-based inhibitors of matrix metalloproteinases[1,2] (MMPs) or matrixins has gained attention in pharmaceutical research.[3,4] Since the zinc-dependent proteinases are involved in various physiological and pathological processes, potent and selective synthetic inhibitors could counteract excessive activity of these enzymes. MMPs play an important role in most processes involved with remodeling or destruction of peptide connective tissue components. Examples are inflammatory events, degenerative joint diseases, multiple sclerosis or cancer.[5,6] Today several MMP inhibitors are in clinical trials with varying degrees of success and the research in potent and selective compounds remains of immediate interest.[7]

In this article, a new class of hydroxamic acid based MMP inhibitors is described. Potential inhibitors of metalloproteinases should be provided with a core, mediate attachment to the subsites of the enzyme and have a zinc chelating group such as a hydroxamic acid function. Carboxy-terminal compounds, covering the primed subsites of the MMPs' active center, are much more effective than those binding at the unprimed sites. Therefore a method for easy extension of the amino terminus of small peptides was desired to equip the structure with a carboxylic functional moiety. This element was required to be built in and converted easily to the hydroxamic acid. Since a simple possibility for turning the amino terminus of a peptide into a carboxylic one is an amide-bound dicarbonic acid and considerable success has been had with malonic acid derivatives,[8] here the most compact one was chosen. Several compounds containing an oxal hydroxamic acid moiety peptide-bound to small peptides or pseudo peptides and also to aliphatic or aromatic amines were synthesized. The hydroxamic acids were screened for effects on the activity of native human gelatinase B (MMP-9), which was our main target, the catalytic domains (cd) of the membrane type MT1-MMP (MMP-14) and of neutrophil collagenase (MMP-8). Several of these compounds were found to be potent inhibitors of the tested enzymes.

Figure 1 shows oxal hydroxamates with aliphatic amino acid derivatives as the core element. The examples clarify the importance of the presence of at least one aromatic function with respect to the activity of this inhibitor species (Table 1). Compound **4**, containing only aliphatic residues, has no significant influence on the tested MMPs. Better results were obtained by introduction of chiral aromatic amines bound at the carboxylic terminus. The most suitable was L(−)-phenyl ethyl amine (**1**, **3**) whereas the D-derivative (**2**) generally was not well tolerated in this position. Activity enhancement was observed when additional aromatic moieties were present in the amino acid side chains. Compounds **5** to **9** contain aspartic acid, serine or threonine carrying benzyl ester or the more stable benzyl ether group, respectively. In general, the L(−)-phenyl ethyl amine in the second position is more active than the small aliphatic dimethyl amide.

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As is shown in compound **10**, the influence of the aliphatic character of the first residue could not be fully 'compensated for' by more complex aromatic residues in the second position. The best choices in position one were aromatic amino acids followed by the well-studied L(−)-phenyl ethyl amine such as the phenylalanine derivative **11** (Fig. 2).

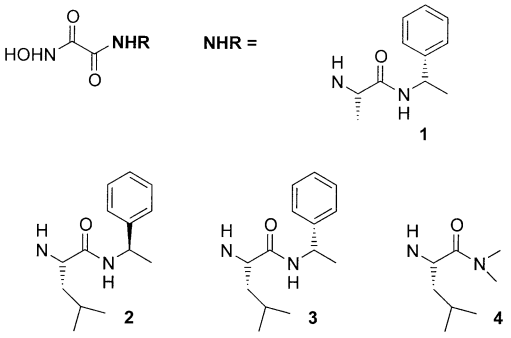

Figure 1. Oxal hydroxamic acid inhibitors with aliphatic amino acid derivatives.

Substitution of the terminal amine by a 1-(1-naphthyl)-ethyl amine residue (**13**) was also well tolerated but the most remarkable of the phenylalanine derivatives is compound **14**. With its tyrosine benzyl ether in the second position, the maximum number of unsubstituted single aromatic moieties useful for this inhibitor species was realized. Thus both a high aromatic and hydrophobic character is favorable for inhibitory activity, while the compounds must be sufficiently soluble in aqueous buffer to provide physiological availability. The activity of **14** against the tested metalloproteinases is almost constant in all cases at about 1 μM. Generally MMP-14 is much less affected by the reported oxal hydroxamates.

The next logical step in inhibitor design is to take the best one and improve it. A rational target for modification was the amino acid's aromatic moiety. Substitution of the phenylalanine in **11** by tryptophan led to the most potent compound **15**. This submicromolar gelatinase and collagenase inhibitor also showed high selectivity in discriminating for MMP-14 by about 100-fold. Replacement of the trypthophan's indole by a 1-naphthyl system, resulting in the more hydrophobic derivative **17**, was well tolerated. The presence of a more flexible 2-naphthylalanine (**19**) caused a decrease in the activity. As described above for the hydroxamates, the phenyl ethyl amine in the second position was favored. The dimethyl amides **16**, **18** and **20** exhibited less activity towards MMP-9 and MMP-8 only. MMP-14 was slightly better inhibited, a behavior more evident in the properties of **11** and **12**.

Another possible modification of the phenyl group in **11** was the introduction of single aromatic substituents. In

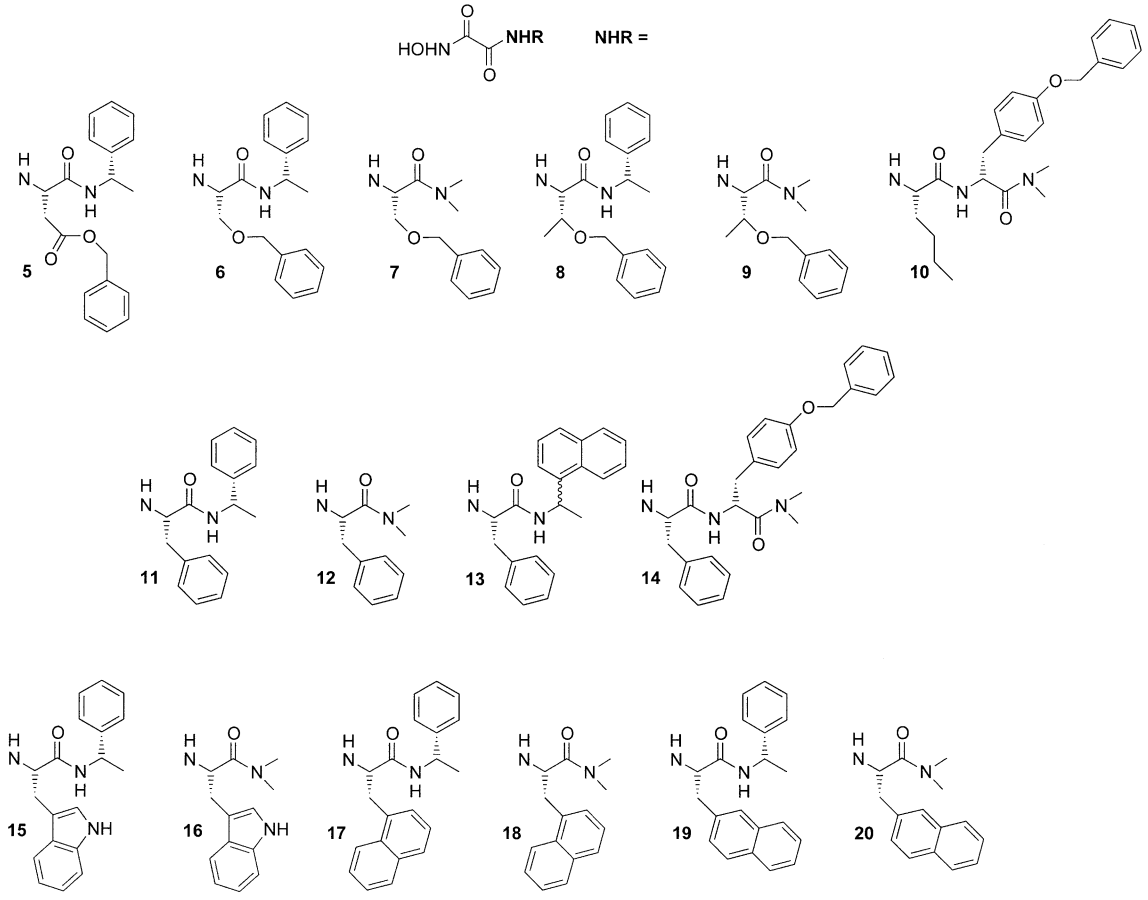

Figure 2. Oxal hydroxamic acid inhibitors with amino acid inhibitors containing aromatic side chain residues.

Table 1. Inhibition of MMPs with compounds **1**–**34**

Compd	MMP-9		cdMMP-14		cdMMP-8	
	K_i (μM)	Ra (%)[a]	K_i (μM)	Ra (%)[a]	K_i (μM)	Ra (%)[a]
1	9.0	35	180	80	6.0	9
2		60		79		74
3	5.5	9	20	54	1.7	3
4		55		61		72
5	0.6	3		53		1
6	3.3	6	11	19		1
7		40		41		56
8	3.2	11		71	3.0	8
9	4.0	18		66	75	67
10	7.5	13	33	29		34
11	0.9	1	14	36	0.43	1
12	14	25	9.5	15	38	48
13		2		42		5
14	1.0	3	1.4	5		10
15	0.1	1		36	0.4	2
16	4.4	25	15	33	28	44
17	0.15	1		29	0.3	0
18	8.2	15		26		47
19	0.5	5		37	1.0	4
20	44	19		30	6.0	15
21	0.6	0		54	0.25	0
22		11		69		5
23	3.0	7		37	22	43
24	2.0	1		53	1.5	4
25		2		23		5
26		22		76		32
27	0.8	5		74	1.0	6
28	3.3	7		54		43
29		67		80		89
30	0.35	2	35	35	14	15
31	0.25	3	9	14	7.0	12
32		5		48		39
33	0.1	0		20		14
34	0.8	3		48		18

[a]Remaining enzyme activity in percent according to the standard procedure described below. The Ra values are uncorrected and are considered to be a half-quantitative guideline of the inhibitor's potential.

our studies of hydroxamic acid MMP inhibitors, the benzyl ether moiety proved to be favorable and peptide compounds with the tyrosine benzyl ether residue at the C-terminal side of the reactive site showed high affinity towards MMPs. The oxal hydroxamic acid compounds **22** to **25** with this voluminous moiety exhibited moderate activity. Here, an H-donor at the carboxyl terminus seemed to be required for binding to MMP-8. The dimethyl amide **23**, lacking this feature for H-bonding, showed lower affinity. The highest activity is achieved by leaving out the benzyl function realized with the tyrosine derivative **21** (Fig. 3).

The influence of H-bond affinity enhancement by side chain functions was also demonstrated with compounds **26** and **27**. The slightly more compact **27** with a more hydrophilic urea group was preferred by MMP-9 and MMP-8. Clearly the influence of the size of the compound on the binding mode of these inhibitors must be considered. Even if neither a substrate analogue nor a carboxyl terminal association with the enzyme is assumed, it is remarkable that only natural L-amino acids and their derivatives were tolerated. On the other hand the large diphenylurea moiety of the side chain of **27** would be difficult to fit in the P_1'-site of the proteinases even with those possessing large binding sites such as MMP-9 and MMP-8. Hence, a combined binding mode of these large compounds needs to be considered in which contacts of the aminophenylalanine derivative are made with one side of the enzyme's active site and of the phenyl ethyl amine with the other. This is an interesting hypothesis as this allows realization of more possibilities for ligand–enzyme interactions by optimal placement of the zinc binding function and steric hindrance of the active center.

In contrast to these larger inhibitors with their flexible pseudo peptide cores, oxal hydroxamic acids with compact aromatic amines were also synthesized. Three examples are shown in Figure 4. The (2-naphthyl)ethyl amine derivatives **28** and **29**, with different chiralities, also exhibited different activity, especially towards MMP-9. Significantly, **28**, which is the better gelatinase inhibitor, is the one with an absolute configuration similar to that of a reduced L-amino acid.

The chloro tryptamine derivative **30** is a further example where a small modification results in a large effect. The activity of the analogous non-halogenated compound (**34**) towards MMP-9 was less than half that of **30**. The other MMPs were also less affected. The high MMP-9-affinity of the non-chiral compound **30** with a mass of less than 300 Da was quite remarkable. Its flexible ethylene linker connecting the zinc ligating group and the indole system, whose aromatic interactions with enzyme functions probably afford the

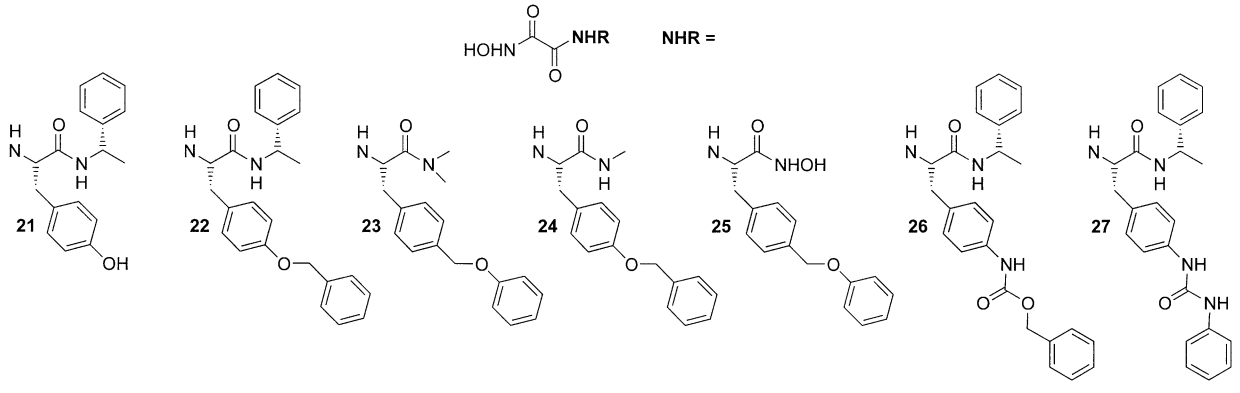

Figure 3. Oxal hydroxamic acid inhibitors with *p*-substituted aromatic amino acid derivatives.

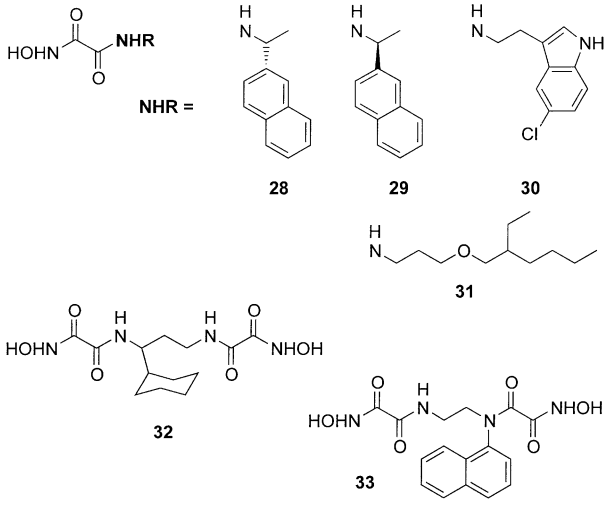

Figure 4. Oxal hydroxamic acid derivatives containing amines or diamines.

main part of the binding enthalpy, could be unfavorable for affinity because of entropic reasons or essential for correct orientation of the two moieties as well.

Oxal hydroxamic acids containing aliphatic amines or diamines were synthesized and tested as well. Interesting examples are the very flexible compound **31**, whose high activity is almost certainly due to hydrophobic interactions, and the difunctional compounds **32** and **33**. The *N*-naphthyl ethylenediamine derivative **33** is one of the most potent inhibitors of MMP-9 found here.

The novel oxal hydroxamic acid based MMP inhibitors presented in this work will certainly give encouragement for the investigation of further modifications and improvements. Their selectivity and low molecular weight raise hopes for the design of new potent and pharmacologically relevant inhibitors of MMPs based on these interesting compounds as highly developed lead structures.

Experimental

K_i values were determined by the established method of Dixon with MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 acetate as fluorogenic substrate. The buffer was aqueous 20 mM Tris–HCl, 100 mM NaCl, 5 mM $CaCl_2$ and 0.05% Brij35^{TM}. A standard screening procedure was used for determining the inhibitory activity. Measurements were carried out with a fluorescence spectrometer LS 50B from Perkin-Elmer in 10 mm cuvettes containing 2 mL buffer plus 50 μL DMSO. For reproducible determination of the inhibited enzyme's remaining activity, a definite amount of unblocked enzyme was needed. The concentration of enzyme was adjusted to produce a fixed linear increase (2.7 system specific units) of fluorescence after addition of 10 μL of 0.1 mg/mL substrate solution in DMSO. By addition of 0.1 μmol inhibitor the remaining, relative enzyme activity was determined. The concentrations were optimized to screen for inhibitors with activity in the lower micromolar range.

The peptides or pseudo peptides of **1**–**27** were synthesized by coupling of protected amino acid derivatives and amines. The amino-terminal deprotected peptide or amine was treated with oxalic acid monomethyl or ethyl ester chloride in organic solvents with surplus tertiary amine under dry conditions at 0 °C. The compounds **1**–**34** were produced by subsequent aminolysis of the corresponding oxalic acid ester with hydroxyl amine in methanol[9] and were purified by extraction or chromatographic procedures. The compounds were characterized by NMR spectroscopy and mass spectrometry.

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