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INHIBITION OF MMP-1 AND MMP-13 WITH PHOSPHINIC ACIDS THAT EXPLOIT BINDING IN THE S_2 POCKET

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Abstract: Through the use of empirical and computational methods, phosphinate-based inhibitors of MMP-1 and MMP-13 that bind into the S_2 pocket of these enzymes were designed. The synthesis and testing of **2** suggested that binding was occurring as hypothesized. Structure determination of a co-crystal of **2** bound to the catalytic domain of MMP-1 confirmed the binding mode. Substituents binding into S_2, S_1', S_2' and S_3', were optimized yielding compounds with low double-digit nM IC_{50}'s against these enzymes.

Matrix metalloproteases (MMP's) are responsible for the turnover of extracellular protein matrices and their over expression and activation appears to play a role in the pathology of various diseases such as cancer and arthritis.[1] As a result, the inhibition of MMP's has received an enormous amount of attention over the past few years.[2] Of the many published studies the large majority have dealt with hydroxamic acid-based inhibitors. The lesser effort expended on inhibitors of other classes presumably derives from the typical potency advantage that hydroxamic acids have over related thiol-, carboxylate-, or phosphinate-based inhibitors. Despite the potency disadvantage, other classes of inhibitors may offer advantages of their own. For example, phosphinate-based inhibitors may possess useful properties deriving from their capacity to accommodate side chains that bind in pockets on both sides of an enzyme's catalytic residues. Given that the binding pockets of related enzymes will be similar but not identical, binding of an inhibitor into more pockets should allow for the identification of compounds more selective for a specific enzyme. In addition, the binding energy gained through the interaction with additional pockets may allow the apparent inherent potency disadvantage of phosphinate-based inhibitors to be overcome.

One of the first X-ray structures published was of MMP-8 (neutrophil collagenase) with a "left-hand side"-based hydroxamic acid bound in the active site.[3] In this structure a P_2 leucyl residue of the inhibitor occupies a pocket that normally accommodates the side chain of the P_2 leucine or glutamine present in the enzyme's substrate, collagen. We speculated that a phosphinate-based inhibitor that took advantage of binding in this pocket, in addition to binding in the critical S_1' pocket, would have the affinity required for therapeutically useful levels of inhibition as well as selectivity over other MMP's.

Earlier work in our laboratories had demonstrated that the S_1 region of MMP-1 could be effectively spanned with a benzyl group as in phosphinate **1** which displays an IC_{50} of 2100 ± 790 nM.[4] Computational studies with this compound and a homology model of MMP-1 (built from an X-ray crystal structure of

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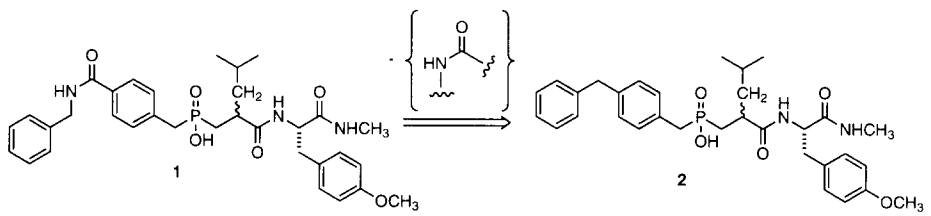
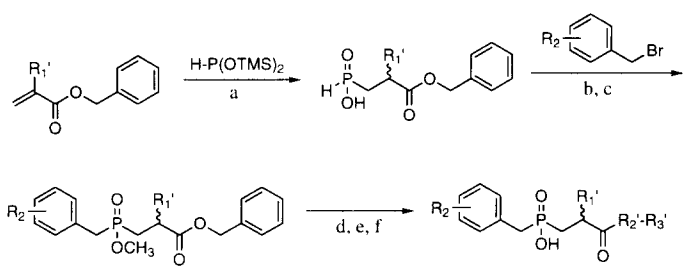
MMP-8) revealed that in a phosphinate with a benzyl group spanning the S_1 region, a carboxamido group was not likely to be a suitable linker for directing a substituent into the S_2 pocket. The amide functionality of **1** extends beyond the S_2 pocket and is torsionally too rigid to allow a substituent thereon to reach the S_2 pocket. Modeling indicated that excision of the carboxamide linker of **1** may allow the terminal phenyl residue to occupy the S_2 pocket. We therefore sought to synthesize **2** as a probe of this hypothesis.



Chemistry: Compound **2** and other compounds discussed below were prepared in six steps by: (1) Michael addition of bis(trimethylsilyl)phosphonite[5] with an appropriate benzyl α-substituted acrylate,[6] (2) Arbuzov reaction of the resulting mono-substituted phosphinic acid[5b,7] with an appropriately substituted benzyl bromide, (3) protection of the phosphinic acid as its methyl ester,[8] (4) hydrogenolysis of the terminal benzyl ester, (5) coupling of the resulting acid with the requisite amine, and (6) hydrolysis of the phosphinic acid ester.[9] The final compounds were generally tested as a mixture of diastereomers since the P_1' side chain stereochemistry was not controlled. Pure diastereomers were isolated in a few cases by direct crystallization from the crude product while in other cases, generally when the activity of the mixture warranted, we separated the isomers by reverse phase (C-18) flash chromatography. The more active (*S*) isomer was in all cases the diastereomer with the shorter retention time. In a few cases the intermediate mono-substituted phosphinic acid was recrystallized with α-methylbenzylamine to provide enantiomerically pure material,[10] which was then carried through the remaining steps of the synthesis to give a pure disastereomeric or enantiomeric product.

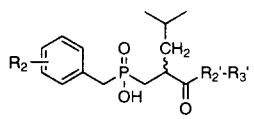


Reagents and yields – range (mean): (a) CH_2Cl_2, 0°C to room temp, 18 h, 83–100% (97%); (b) substituted benzyl bromide, N,O-bis-(trimethylsilyl)acetamide, CH_2Cl_2, 24 to 72 h; (c) excess $TMSCHN_2$, toluene:methanol – 7:3, 30 min, 22–100% (69%) yield for two steps; (d) H_2, Pd-$BaSO_4$, EtOH, 1 h; (e) R_2'-R_3' amine, BOP, DIEA, CH_2Cl_2, 18 h, 4–98% (40%) yield for two steps; (f) TFA:H_2O – 9:1, 1 to 2 h, 36–100% (88%).

Results: Compound **2** was isolated as a nearly pure isomer (97/3, *S/R* at the P_1' isobutyl side chain) by direct crystallization of the crude product and displayed an IC_{50} against MMP-1[11] of 270 nM (Table 1). The increase in potency over **1** indicated that **2** did possess additional favorable binding interactions with the enzyme; a result consistent with our modeling studies. The relatively weak MMP-1 activity of the P_2

unsubstituted compound **3** and the biphenyl analogs **4–6**, which contain lipophilic groups but which cannot reach the S_2 pocket, further supported our hypothesis regarding the binding of **2**. Modeling indicates that the terminal phenyl group of **7** can reach the S_2 pocket; however, it was weakly active which may relate to the additional degrees of freedom inherent in the compound's ethyl linker and the attendant entropic penalty paid on removing this potential motion. The relatively weak activity of **8** and **9** presumably reflects the inability of an isopropyl group (too small) or cylclohexyl group (too "thick") to effectively occupy the S_2 pocket.

Table 1.
Variation of the
P_2 and P_1'-P_3' side chains



Compd[a]	R_2	R_2'-R_3'[b]	MMP-1 IC_{50} (nM)[c]		Ratio of *S/R* isomers at P_1' side chain
2	4-$PhCH_2$-	-TyrNHMe	270	± 120	97:03
3	H-	"	15000	± 610	49:51
4	2-Ph-	"	11000	± 3200	52:48
5	3-Ph-	"	5100	± 1400	50:50
6	4-Ph-	"	2200	± 650	51:49
7	3-$PhCH_2CH_2$-	"	7400	± 6600	51:49
8	4-$(CH_3)_2CHCH_2$-	"	4500	± 870	50:50
9	4-$CyclohexylCH_2$-	"	1900	± 420	32:68
10	4-$PhCH_2$-	-TleNHMe	60	± 0 (2)	100:0
11	"	-AlaNHMe	2300	± 680	51:49
12	"	-$NHCH(CH_2OH)C(CH_3)_3$	18000	± 4200	100:0
13	"	-$NHCH_2C(CH_3)_3$	20000	(1)	100:0
14	4-$PhSO_2$-	-TyrNHMe	1700	± 170	50:50
15	4-PhO-	"	2800	± 250	50:50
16	4-PhC(=O)-	-TleNHMe	750	± 71 (2)	54:46

[a]All compounds were characterized by ^1H NMR, MS, and HPLC. The latter assured purity and allowed the diastereomeric ratio, if any, of the P_1' side chain to be determined. [b]TyrNHMe = (*S*) O-methyltyrosine N-methylamide; TleNHMe = (*S*) tert-leucine N-methylamide. [c]Values are the mean ± SD of 3 determinations unless otherwise noted (n).

The binding mode of **2** was ultimately confirmed through the structure determination of a co-crystal of **2** with the catalytic domain of MMP-1 which revealed that the P_2 phenyl group was indeed occupying the S_2 pocket (Figure 1). The phosphinic acid was observed to be bound to the active site zinc and the isobutyl side chain occupied the S_1' pocket.[12] In addition, the P_2' and P_3' carboxamides were hydrogen bonded to enzyme residues much as has been observed in other inhibitors that have a substrate-like structure in this region.[13] Having confirmed that binding in the S_2 pocket was possible and productive, we continued our investigation of the series.

Modification of the P_2' and P_3' substituents in other series of MMP inhibitors has been reported to affect activity to some degree[2,13] and this experience was reflected in the present series. Thus, replacing the *O*-methyltyrosine P_2' residue of **2** with a (*S*) *tert*-leucine residue as in **10** increased activity 4–fold while decreasing the size of the side chain by incorporating a P_2' alanine residue as in **11** reduced activity substantially. As has been postulated elsewhere,[13,14] the trend towards greater potency with bulkier, lipophilic P_2' side chains probably represents a combination of differences in hydration of the adjacent carboxamide groups and torsional constraints imparted by the side chain. Truncation of the P_2'-P_3' *N*-methylcarboxamide

to a hydroxymethyl residue as in **12** or its complete removal as in **13** reduced activity greatly indicating the importance of hydrogen bonds to the terminal carboxamide. That hydrogen bonding to the P_2'-P_3' carboxamide would yield a positive energetic contribution is consistent with the hypothesis that the lipophilic P_2' side chain prevents the complete hydration of the adjacent carboxamido groups in bulk solution but does not affect their participation in hydrogen bonding networks when the inhibitor is bound to the enzyme.

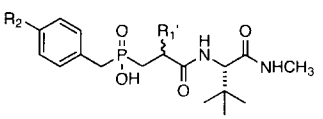
Figure 1. X-ray structure of phosphinate **2** (yellow) bound to the catalytic domain of MMP-1 superimposed with the X-ray structure of HProLeuGlyNHOH (green) as bound to MMP-8 (ref 3).

At the other end of the molecule we investigated the effect of replacing the methylene linker between the P_2 phenyl group and the P_1 benzyl spacer with sulfonyl, ether or carbonyl linkages. Since all of these compounds **14–16** were less active than the corresponding methylene linked compounds (**14** and **15** vs **2** and **16** vs **10**), we utilized the methylene linker in the remainder of our studies.

Binding in the S_1' pocket of MMP's has repeatedly been shown to be of primary importance for potency and selectivity of inhibitors.[2,13] In this regard we examined the effect of changing the P_1' side chain on the activity against MMP-1, a shallow S_1' pocket enzyme, vs activity against MMP-13, a deep S_1' pocket enzyme. We maintained the P_2 phenyl group in all of these compounds since a homology model of MMP-13 (built from an X-ray crystal structure of MMP-8) led us to believe that MMP-13 would have an S_2 pocket similar to that of MMP-1. Therefore, the P_2 phenyl residue that bound effectively in the S_2 pocket of MMP-1 would likely be similarly accommodated in MMP-13. Consistent with SAR in other series,[2,13] the most potent compounds against MMP-1 were those with short, lipophilic side chains (Table 2). Given the potency advantage of **18** over **10**, we conclude that the 3,3,3-trifluoropropyl side chain of **18** occupies the shallow S_1' pocket of MMP-1 somewhat more effectively than the "standard" isobutyl group. In contrast, the best MMP-13 inhibitors[15] had long lipophilic, but non-bulky, P_1' side chains. The most potent MMP-13 inhibitors, **22** and **25**, contain phenethyl and phenoxybutyl side chains, respectively. The similarly lipophilic cyclohexylmethyl- and cyclohexylethyl-containing compounds, **23** and **24**, were less active with their respective side chains being too bulky to enter the S_1' pocket of either enzyme. Optimization for potency against these particular shallow and deep P_1' pocket enzymes also produced the most selective inhibitors. Thus, **18** was 35–fold selective for MMP-1 over MMP-13 and **22** and **25** were 49– and >1000–fold[16] selective for MMP-13 over MMP-1.

While selectivity between shallow and deep S_1' pocket enzymes can be obtained by selecting an appropriate P_1' side chain, obtaining selectivity between enzymes with similar S_1' pockets has not generally been achieved. That phosphinate-based inhibitors could provide such selectivity through binding to pockets on both sides of an enzyme's catalytic residues was one of our initial postulates. This potential advantage of phosphinate-based inhibitors is exemplified by **22** which is a potent and 85–fold selective inhibitor of MMP-13 over MMP-3, another deep S_1' pocket enzyme (Table 3). The increase in MMP-13 potency of **22** over **26** and their similar, weak MMP-3 inhibition indicates that the P_2 phenyl residue of **22** makes productive contacts in the S_2 pocket of MMP-13 but not in that of MMP-3. Additional, although not as dramatic, examples of the potential of phosphinates to display selectivity between enzymes with similar S_1' pockets is provided by the work of Caldwell et al. wherein they report that a S_3-S_1 phthalimidobutyl group, as in **27**, leads to MMP-3 selectivity while a S_3-S_1 Ac-*L*-ProNH$(CH_2)_4$- residue, as in **28**, yields MMP-2 selectivity (Table 3).[10]

Table 2.
Variation of the P_1' side chain



Compd[a]	R_2	R_1'	MMP-1 IC_{50} (nM)[b]		MMP-13 IC_{50} (nM)[c]		Ratio of *S/R* isomers at P_1' side chain
10	$PhCH_2$-	$-CH_2CH(CH_3)_2$	60	± 0 (2)	180	± 21 (2)	100:0
17	"	$-CH_2CH_2CH_3$	340	± 85 (2)	-		50:50
18	"	$-CH_2CH_2CF_3$	45	± 5	1600	± 350	100:0
19	"	-CH_2cyclopropyl	200	± 170	1100	± 1200	74:26
20	"	-CH_2cyclobutyl	180	± 190	110	± 65	100:0
21	"	$-CH_2CH_2CH(CH_3)_2$	3500	± 1500	>3000	(1)	53:47
22	"	$-CH_2CH_2Ph$	690	± 370 (2)	14	± 7 (2)	100:0
23	"	-CH_2cyclohexyl	1800	± 670	1600	± 470	56:44
24	"	-CH_2CH_2cyclohexyl	19000	± 4200	2000	± 210	53:47
25	"	$-(CH_2)_4OPh$	>30000	(1)	30	± 1.4	100:0
26	H-	$-CH_2CH_2Ph$	22000	± 5000 (2)	300	(1)	62:38

[a]See footnote a, Table 1. [b]See footnote c, Table 1. [c]Values are the mean ± SD of 3 determinations unless otherwise noted (n).

27 R = phthalimido$(CH_2)_4$-
28 R = Ac-L-ProNH$(CH_2)_4$-

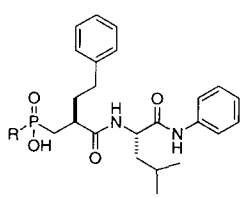


Table 3. Selective MMP inhibition (nM)

Compd	MMP-13	MMP-3	MMP-2	MMP selectivity
22	14[a]	1200[b]	-	85-fold
26	300[a]	2700[b]	-	-
27	-	1.4[c]	20[c]	14-fold
28	-	62[c]	6.1[c]	10-fold

[a]MMP-13 IC_{50}'s from Table 2. [b]MMP-3 IC_{50}'s. [c]K_i's from Caldwell et al. (ref 10).

Having identified compounds with reasonably potent in vitro activity, we examined the pharmacokinetics of one of these in rats in order to assess the therapeutic prospects of the series. Phosphinate **22** when dosed iv in rats displayed good pharmacokinetics with low clearance (12 mL/min/kg) and a small steady state volume of distribution (0.2 L/kg) which translate to a terminal half-life of 2.3 h. However, when dosed orally the compound was not detected in the plasma. Considering the low clearance, we presume the absence of

compound in plasma after oral dosing is due to poor absorption and this likely derives from the charged nature of the molecule at physiological pH.[17]

In conclusion, we have demonstrated that the design and synthesis of phosphinate-based inhibitors of MMP's that are potent and selective by virtue of their interactions with the S_2 and S_1' sites is possible and that an X-ray crystal structure and computer modeling can contribute to the design process. The iv pharmacokinetics of a compound from the series are promising and we continue to explore the SAR of related phosphinates seeking compounds with greater potency and selectivity as well as with oral activity.

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4. Phosphinate **1** is a 57:43 mixture of *S* and *R* diastereomers at the P_1' isobutyl side chain. The majority of the activity in this and other mixtures of compounds reported herein resides in the *S* P_1' isomer. For example, the isomer of **10** having a P_1' side chain with the *R* configuration displayed an IC_{50} of 6000 ± 1000 nM making it 100–fold less potent than the *S* isomer. Such a difference in activity between diastereomers at P_1' is typical for compounds that bind with a peptide-like orientation in the S_1' to S_3' pockets (refs 2 and 13). Related benzyl amides containing phenyl or ethyl S_1 "spacers" showed only very weak inhibition of MMP-1 activity at 30000 nM.
5. Bis(trimethylsilyl)phosphonite was prepared as described in: (a) Boyd, E. A.; Regan, A. C.; James, K. *Tetrahedron Lett.* **1992**, *33*, 813. (b) ibid. **1994**, *35*, 4223. (c) The Michael reaction was performed as described in: Boyd, E. A.; Corless, M.; James, K.; Regan, A. C. *Tetrahedron Lett.* **1990**, *31*, 2933.
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11. MMP-1 IC_{50}'s were determined by the method of Bickett et al. (ref 18) using recombinant MMP-1 (expressed in baculovirus).
12. The phosphinate oxygens of **2** are not symmetrically oriented around the zinc atom. The solvent accessible phosphinate oxygen is 2.15 Å from the zinc atom while the "buried" oxygen is 2.90 Å away indicating monodentate ligation. A similar result has been reported by Babine and Bender (ref 13) for a phosphinate bound to MMP-7. The "buried" phosphinate oxygen in our structure is hydrogen bonded to the catalytic glutamic acid as indicated by an O-O distance of 2.71 Å. The crystal structure also confirmed that **2** has the *S* configuration at P_1'.
13. For a discussion of the binding of inhibitors to various MMP's see: Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359.
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15. MMP-13 IC_{50}'s were determined by the method of Bickett et al. (ref 18) using recombinant MMP-13 (expressed in baculovirus).
16. The high selectivity of **25**, which derives from the compound's very weak MMP-1 inhibition, stands in contrast to the work of Gowravaram et al. (ref 19) who reported hydroxamate-based inhibitors which contain a phenoxybutyl P_1' side chain and which are potent MMP-1 inhibitors. The difference between their hydroxamates and our phosphinate presumably derives from the different angles at which the phenoxybutyl side chains enter the S_1' pocket of MMP-1. These angles are greatly influenced by the geometry that the hydroxamate and phosphinate ligands assume upon binding to the active site zinc.
17. For example, the pK_a of **10** was measured to be 2.8.
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