

## INHIBITION OF MMP-1 AND MMP-13 WITH PHOSPHINIC ACIDS THAT EXPLOIT BINDING IN THE S<sub>2</sub> POCKET

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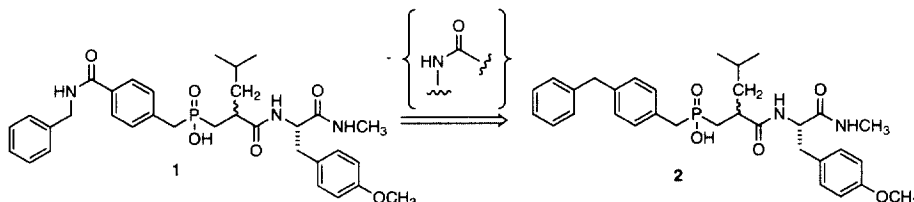
**Abstract:** Through the use of empirical and computational methods, phosphinate-based inhibitors of MMP-1 and MMP-13 that bind into the S<sub>2</sub> pocket of these enzymes were designed. The synthesis and testing of **2** suggested that binding was occurring as hypothesized. Structure determination of a co-crystal of **2** bound to the catalytic domain of MMP-1 confirmed the binding mode. Substituents binding into S<sub>2</sub>, S<sub>1</sub>', S<sub>2</sub>' and S<sub>3</sub>', were optimized yielding compounds with low double-digit nM IC<sub>50</sub>'s against these enzymes. © 1999 Elsevier Science Ltd. All rights reserved.

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.<sup>1</sup> As a result, the inhibition of MMP's has received an enormous amount of attention over the past few years.<sup>2</sup> 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.

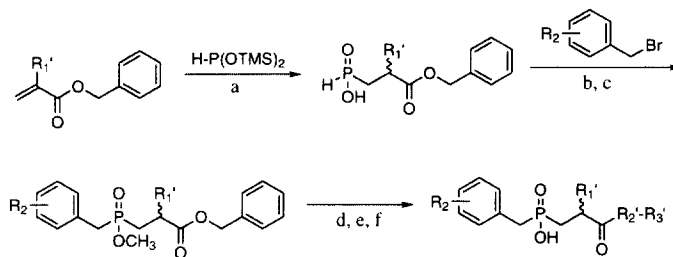
One of the first X-ray structures published was of MMP-8 (neutrophil collagenase) with a "left-hand side"-based hydroxamic acid bound in the active site.<sup>3</sup> In this structure a P<sub>2</sub> leucyl residue of the inhibitor occupies a pocket that normally accommodates the side chain of the P<sub>2</sub> 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<sub>1</sub>' 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<sub>1</sub> region of MMP-1 could be effectively spanned with a benzyl group as in phosphinate **1** which displays an IC<sub>50</sub> of 2100 ± 790 nM.<sup>4</sup> Computational studies with this compound and a homology model of MMP-1 (built from an X-ray crystal structure of

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<sup>5</sup> with an appropriate benzyl  $\alpha$ -substituted acrylate,<sup>6</sup> (2) Arbuzov reaction of the resulting mono-substituted phosphinic acid<sup>5b,7</sup> with an appropriately substituted benzyl bromide, (3) protection of the phosphinic acid as its methyl ester,<sup>8</sup> (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.<sup>9</sup> 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  $\alpha$ -methylbenzylamine to provide enantiomerically pure material,<sup>10</sup> which was then carried through the remaining steps of the synthesis to give a pure diastereomeric or enantiomeric product.

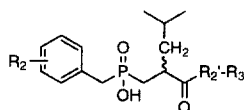


**Reagents and yields – range (mean):** (a)  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$  to room temp, 18 h, 83–100% (97%); (b) substituted benzyl bromide, N,O-bis-(trimethylsilyl)acetamide,  $\text{CH}_2\text{Cl}_2$ , 24 to 72 h; (c) excess  $\text{TMSCHN}_2$ , toluene:methanol – 7:3, 30 min, 22–100% (69%) yield for two steps; (d)  $\text{H}_2$ , Pd-BaSO<sub>4</sub>, EtOH, 1 h; (e)  $\text{R}_2\text{'-R}_3\text{'}$  amine, BOP, DIEA,  $\text{CH}_2\text{Cl}_2$ , 18 h, 4–98% (40%) yield for two steps; (f) TFA: $\text{H}_2\text{O}$  – 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  $\text{IC}_{50}$  against MMP-1<sup>11</sup> 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 cyclohexyl group (too "thick") to effectively occupy the  $S_2$  pocket.

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



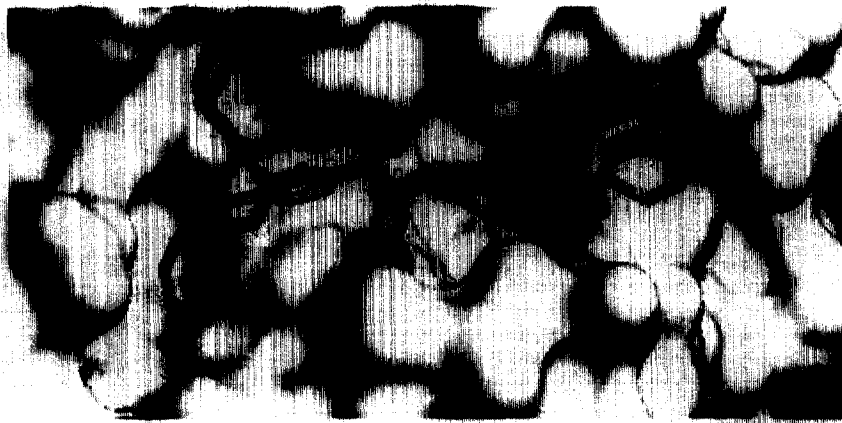
Compd <sup>a</sup>	$R_2$	$R_2'-R_3'^b$	MMP-1 IC <sub>50</sub> (nM) <sup>c</sup>	Ratio of <i>S/R</i> isomers at $P_1'$ side chain
<b>2</b>	4-PhCH <sub>2</sub> -	-TyrNHMe	270 ± 120	97:03
<b>3</b>	H-	"	15000 ± 610	49:51
<b>4</b>	2-Ph-	"	11000 ± 3200	52:48
<b>5</b>	3-Ph-	"	5100 ± 1400	50:50
<b>6</b>	4-Ph-	"	2200 ± 650	51:49
<b>7</b>	3-PhCH <sub>2</sub> CH <sub>2</sub> -	"	7400 ± 6600	51:49
<b>8</b>	4-(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> -	"	4500 ± 870	50:50
<b>9</b>	4-CyclohexylCH <sub>2</sub> -	"	1900 ± 420	32:68
<b>10</b>	4-PhCH <sub>2</sub> -	-TleNHMe	60 ± 0 (2)	100:0
<b>11</b>	"	-AlaNHMe	2300 ± 680	51:49
<b>12</b>	"	-NHCH(CH <sub>2</sub> OH)C(CH <sub>3</sub> ) <sub>3</sub>	18000 ± 4200	100:0
<b>13</b>	"	-NHCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>3</sub>	20000 (1)	100:0
<b>14</b>	4-PhSO <sub>2</sub> -	-TyrNHMe	1700 ± 170	50:50
<b>15</b>	4-PhO-	"	2800 ± 250	50:50
<b>16</b>	4-PhC(=O)-	-TleNHMe	750 ± 71 (2)	54:46

<sup>a</sup>All compounds were characterized by <sup>1</sup>H NMR, MS, and HPLC. The latter assured purity and allowed the diastereomeric ratio, if any, of the  $P_1'$  side chain to be determined. <sup>b</sup>TyrNHMe = (*S*) O-methyltyrosine N-methylamide; TleNHMe = (*S*) tert-leucine N-methylamide. <sup>c</sup>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.<sup>12</sup> 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.<sup>13</sup> 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<sup>2,13</sup> 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,<sup>13,14</sup> 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<sub>2</sub>'-P<sub>3</sub>' carboxamide would yield a positive energetic contribution is consistent with the hypothesis that the lipophilic P<sub>2</sub>' 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.



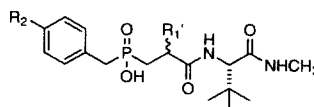
**Figure 1.** X-ray structure of phosphinate **2** (yellow) bound to the catalytic domain of MMP-1 superimposed with the X-ray structure of HProLeuGlyNHOH (green) as bound to MMP-8 (ref 3).

At the other end of the molecule we investigated the effect of replacing the methylene linker between the P<sub>2</sub> phenyl group and the P<sub>1</sub> 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<sub>1</sub>' pocket of MMP's has repeatedly been shown to be of primary importance for potency and selectivity of inhibitors.<sup>2,13</sup> In this regard we examined the effect of changing the P<sub>1</sub>' side chain on the activity against MMP-1, a shallow S<sub>1</sub>' pocket enzyme, vs activity against MMP-13, a deep S<sub>1</sub>' pocket enzyme. We maintained the P<sub>2</sub> 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<sub>2</sub> pocket similar to that of MMP-1. Therefore, the P<sub>2</sub> phenyl residue that bound effectively in the S<sub>2</sub> pocket of MMP-1 would likely be similarly accommodated in MMP-13. Consistent with SAR in other series,<sup>2,13</sup> 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<sub>1</sub>' pocket of MMP-1 somewhat more effectively than the "standard" isobutyl group. In contrast, the best MMP-13 inhibitors<sup>15</sup> had long lipophilic, but non-bulky, P<sub>1</sub>' 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<sub>1</sub>' pocket of either enzyme. Optimization for potency against these particular shallow and deep P<sub>1</sub>' 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<sup>16</sup> selective for MMP-13 over MMP-1.

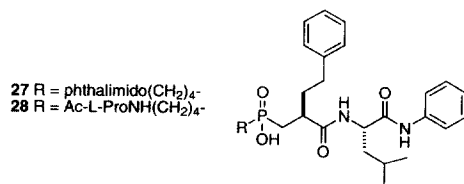
While selectivity between shallow and deep  $S_1'$  pocket enzymes can be obtained by selecting an appropriate  $P_1'$  side chain, obtaining selectivity between enzymes with similar  $S_1'$  pockets has not generally been achieved. That phosphinate-based inhibitors could provide such selectivity through 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<sub>2</sub>)<sub>4</sub>- residue, as in **28**, yields MMP-2 selectivity (Table 3).<sup>10</sup>

**Table 2.**  
Variation of the  
 $P_1'$  side chain



Compd <sup>a</sup>	R <sub>2</sub>	R <sub>1</sub> '	MMP-1 IC <sub>50</sub> (nM) <sup>b</sup>	MMP-13 IC <sub>50</sub> (nM) <sup>c</sup>	Ratio of <i>S/R</i> isomers at $P_1'$ side chain
<b>10</b>	PhCH <sub>2</sub> -	-CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	60 ± 0 (2)	180 ± 21 (2)	100:0
<b>17</b>	"	-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	340 ± 85 (2)	-	50:50
<b>18</b>	"	-CH <sub>2</sub> CH <sub>2</sub> CF <sub>3</sub>	45 ± 5	1600 ± 350	100:0
<b>19</b>	"	-CH <sub>2</sub> cyclopropyl	200 ± 170	1100 ± 1200	74:26
<b>20</b>	"	-CH <sub>2</sub> cyclobutyl	180 ± 190	110 ± 65	100:0
<b>21</b>	"	-CH <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	3500 ± 1500	>3000 (1)	53:47
<b>22</b>	"	-CH <sub>2</sub> CH <sub>2</sub> Ph	690 ± 370 (2)	14 ± 7 (2)	100:0
<b>23</b>	"	-CH <sub>2</sub> cyclohexyl	1800 ± 670	1600 ± 470	56:44
<b>24</b>	"	-CH <sub>2</sub> CH <sub>2</sub> cyclohexyl	19000 ± 4200	2000 ± 210	53:47
<b>25</b>	"	-(CH <sub>2</sub> ) <sub>4</sub> OPh	>30000 (1)	30 ± 1.4	100:0
<b>26</b>	H-	-CH <sub>2</sub> CH <sub>2</sub> Ph	22000 ± 5000 (2)	300 (1)	62:38

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



**Table 3.** Selective MMP inhibition (nM)

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

<sup>a</sup>MMP-13 IC<sub>50</sub>'s from Table 2. <sup>b</sup>MMP-3 IC<sub>50</sub>'s. <sup>c</sup>K<sub>i</sub>'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 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.<sup>17</sup>

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<sub>2</sub> and S<sub>1</sub>' 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<sub>1</sub>' isobutyl side chain. The majority of the activity in this and other mixtures of compounds reported herein resides in the *S* P<sub>1</sub>' isomer. For example, the isomer of **10** having a P<sub>1</sub>' side chain with the *R* configuration displayed an IC<sub>50</sub> of 6000 ± 1000 nM making it 100-fold less potent than the *S* isomer. Such a difference in activity between diastereomers at P<sub>1</sub>' is typical for compounds that bind with a peptide-like orientation in the S<sub>1</sub>' to S<sub>3</sub>' pockets (refs 2 and 13). Related benzyl amides containing phenyl or ethyl S<sub>1</sub> "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<sub>50</sub>'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<sub>1</sub>'.
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15. MMP-13 IC<sub>50</sub>'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<sub>1</sub>' 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<sub>1</sub>' 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<sub>a</sub> of **10** was measured to be 2.8.
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