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Phosphinic Acid-Based MMP-13 Inhibitors That Spare MMP-1 and MMP-3

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Abstract—Phosphinic acid-based inhibitors of MMP-13 have been investigated with the aim of identifying potent inhibitors with high selectivity versus MMP-1. Independent variation of the substituents on a P_1' phenethyl group and a P_2 benzyl group improved potencies in both cases around 3-fold over the unsubstituted parent. Combining improved P_1' and P_2 groups into a single molecule gave an inhibitor with a 4.5 nM IC_{50} against MMP-13 and which is 270-fold selective over MMP-1.

The quest to identify therapeutically useful inhibitors of MMP's has entered its third decade[1], and this quest remains to be fulfilled. The early studies, which were initiated in the pre-genomics era, aimed at inhibiting MMP-1 and focused on identifying molecules which were potent and which had favorable pharmacokinetic (PK) properties. These early endeavors have been replaced with more fundamental work aimed at identifying the many MMP's present in humans, assessing their relative abundance and distribution, and most critically, determining their relevance to different disease states.[2] The search for therapeutically useful molecules has likewise evolved. Early studies focused on peptidic structures some of which displayed potent MMP-1 inhibition but which had unknown (and un-appreciated lack of) specificity and poor PK properties. While progress has been made in identifying compounds with improved PK in both peptidic and non-peptidic motifs, clinical experience with broad spectrum inhibitors has revealed a common, intolerable side effect, musculoskeletal syndrome (MSS).[3] Avoiding MSS is likely to require identification of the MMP(s) responsible for the side effect and the discovery and development of appropriately selective inhibitors which in an ideal case, would inhibit only the target MMP(s).

That MMP-1 plays a role in the development of MSS has been postulated[4] and efforts to identify MMP inhibitors that spare MMP-1 have been reported.[4,5] The target MMPs for these studies, though, have varied depending on which disease is being studied. In the case of osteoarthritis, the presence of MMP-13 in OA cartilage tissue in humans,[6,7] its ability to turn over type II collagen,[6,8] its co-localization with cleaved type II collagen in OA cartilage tissue,[9] and it degradative properties in cartilage,[10] all point to its likely role in the pathology of the disease.[11] Additional evidence that MMP-13 is a key driver of cartilage destruction (vs MMP-1) was provided by the study of a relatively broad spectrum inhibitor, CGS 27023A,[12] versus **1**, a MMP-1-sparing MMP-13 inhibitor, in the bovine nasal cartilage (BNC) model of cartilage degradation (Fig. 1).[13] In this model CGS 27023A displayed an IC_{50} for inhibition of hydroxyproline release of $\sim$50 nM which is about 10-fold higher than its IC_{50} against recombinant MMP-13 (3 nM) and 3-fold higher than its MMP-1 IC_{50} (18 nM). The phosphinate **1** displayed an IC_{50} in the BNC model of $\sim$500 nM, about 10-fold higher than its MMP-13 IC_{50} (70 nM), consistent with the result with CGS 27023A. Furthermore, the IC_{50} of **1** in the BNC model is about 40-fold less than its MMP-1 IC_{50} (22,000 nM) clearly demonstrating that MMP-1 does not play significant role in this model.

We recently reported a series of phosphinate-based inhibitors, including **2**, the *t*-butylglycine-containing analogue of **1** (MMP-13 IC_{50} 30 nM, MMP-1 IC_{50}

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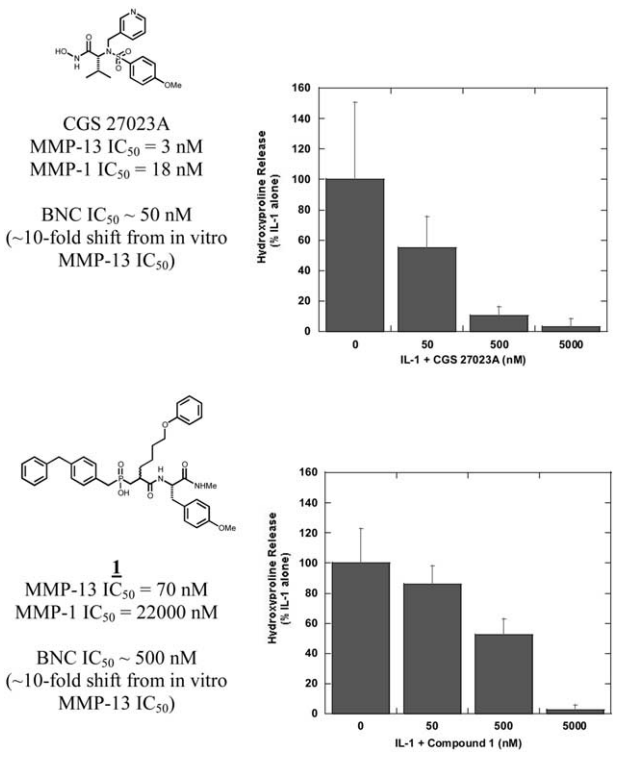
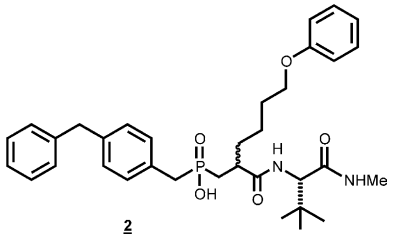


Figure 1. Inhibition of IL-1 induced degradation of bovine nasal cartilage (BNC) explants.

>30,000 nM), that take advantage of binding into pockets on both sides of the scissile bond.[14] In particular, key interactions occur in the S_2 and S_1' pockets and with the catalytic zinc atom. The premise of this work was that, through the interaction with more subsites of the enzyme, the identification of selective compounds may be possible. Since our group is focused on potential treatments for osteoarthritis, we wished to continue to explore this premise in the context of MMP-13 inhibition. We especially sought compounds with >100-fold selectivity versus MMP-1. The most potent MMP-13 inhibitor identified in our previous work was **3**, having an MMP-13 IC_{50} of 21 nM and 37-fold selectivity over MMP-1 (Table 1). Although **2** was more selective versus MMP-1, the somewhat greater potency of **3** and the synthetic accessibility and lower molecular weight of P_1' phenethyl derivatives led us to explore the latter sub-series. Herein we report the results of our studies in which we have varied the P_2 and P_1' substituents seeking potent and selective MMP-13 inhibitors.



2

The synthesis of P_1' analogues was accomplished in 4 synthetic steps (Scheme 1). Appropriately substituted ethyl α-phenethylacrylates were prepared through reaction of various benzyl cuprates with ethyl α-bromomethylacrylate.[15] The use of an excess of cuprate drove the reaction to completion leading to a crude product containing the desired acrylate and the substituted toluene derived from the quenched cuprate. This latter, neutral impurity was easily removed after the next synthetic step in which the mono-substituted phosphinic acid **4**, prepared through an Arbuzov reaction of the corresponding benzyl bromide with bis(trimethylsilyl)phosphonite,[16] was reacted with each α-substituted acrylate.[17] The crude di-substituted phosphinic acid was purified by absorption onto MP-carbonate (Argonaut Technologies) resin, washing to remove non-acidic components and release of product with TFA in CH_2Cl_2. The phosphinates thus prepared were pure by NMR and LC-MS. Subsequent saponification and selective coupling of the carboxylic acid to *S tert*-leucine *N*-methylamide using PyBOP[18] gave the crude desired compounds as a mixture of diastereomers. Preparative RP (reverse phase) chromatography yielded the pure compounds **5**–**13**.

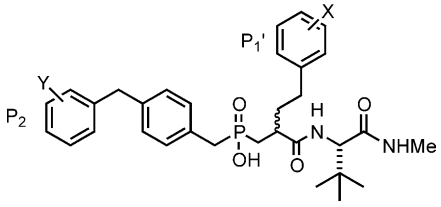
The synthesis of the P_2 analogues made use of intermediate **14** in order to facilitate the rapid preparation of the target compounds (Scheme 2). This was prepared from benzyl α-phenethylacrylate and 4-iodobenzylbromide as previously described.[14] The intermediate mono-substituted phosphinic acid adduct of bis(trimethylsilyl)phosphonite and benzyl α-phenethylacrylate was partially resolved (~3/1 *S*/*R*) by one crystallization of its *S* α-methylbenzylamine salt.[19] Negishi coupling of **14** with benzyl zinc reagents[20] gave the intermediates containing the various P_2 substituents. Conversion into the target phosphinic acids **15**–**27** was achieved as previously described.[14] Owing to the partial resolution of the earlier intermediate, the diastereomeric mixtures were all biased toward the more potent isomer. Nevertheless, if the diastereomeric mixture was sufficiently potent, the pure isomer was isolated by preparative RP chromatography (e.g., **15**, **18**, **21**, and **26**).

The analogue combining the better P_1' and P_2 substituents, **28**, was prepared from 4-(2-methoxy)benzylbenzyl bromide and ethyl α-(4-chlorophenyl)ethylacrylate as described above for the other P_1' analogues.

Substitution on the terminus of the phenethyl P_1' group modulated MMP-13 potency and/or selectivity versus MMP-1. In all cases but one, substitution either had a slight effect (e.g., **6**) or modestly increased MMP-13 potency from 2- to 4-fold. The exception, the 2-methyl derivative **8**, was substantially less active. Although increases in potency were only modest at best, these, coupled with modest reductions in MMP-1 potency, led to selectivities of greater than 100-fold for some of the 4-substituted derivatives. The loss of MMP-1 potency for compounds with additional substitution at C-4 of the phenylethyl group is consistent with the MMP-1 S_1' pocket being shallow.

In contrast to the considerable published SAR for P_1' substitution of MMP-13 inhibitors,[2] little is known about the S_2 pocket of MMP's.[21] As a result, we chose to initially survey the effect of fluoro, methyl and

Table 1. Variation of P_1' and P_2 residues. MMP-13 and MMP-1 activity



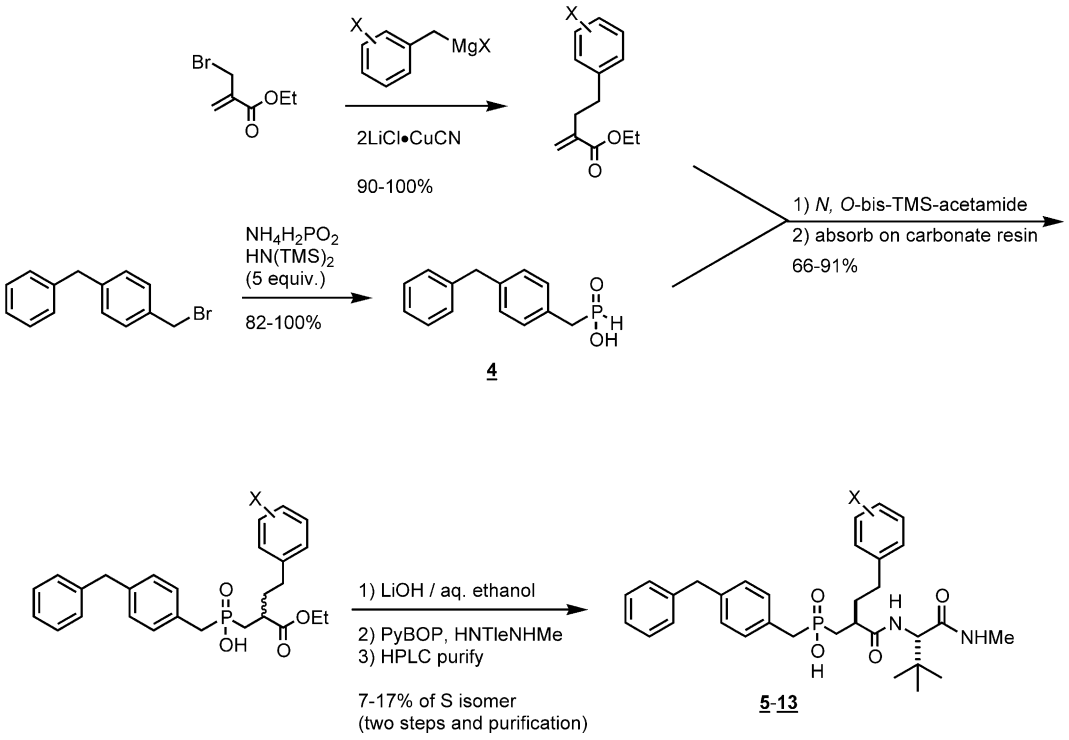
CPD[a]	Y	X	MMP-1[b] IC_{50} (nM)[d]	MMP-13[c] IC_{50} (nM)[d]	MMP-1/ MMP-13 IC_{50} ratio	Ratio of *S*/*R* isomers at P_1' side chain
3	H–	H–	770±300	21±9	37	100/0
5	H–	2-F–	280±40	10±3	28	99/1
6	H–	3-F–	860±190	19±6	45	100/0
7	H–	4-F–	530±110	13±3	41	100/0
8	H–	2-Me–	12,000±900	>300 (3)	<40	100/0
9	H–	3-Me–	120±10	4.3±0.8	28	100/0
10	H–	4-Me–	630±30	7.7±2.4	82	100/0
11	H–	4-Cl–	1100±200	8.3±2.8	130	100/0
12	H–	4-Br–	1400±100	9.4±1.2	150	100/0
13	H–	4-MeO–	1600±200	7.7±2.0	210	100/0
15	2-F–	H–	690±320	18±7.8	38	96/04
16	3-F–	H–	1600±300	58±41	28	75/25
17	4-F–	H–	970±50	44±31	22	74/26
18	2-Me–	H–	1100±500	24±21	46	96/04
19	3-Me–	H–	2300±1000	37±13	62	80/20
20	4-Me–	H–	1600±300	29±9	55	79/21
21	2-MeO–	H–	770±290	7.0±3.8	110	99/01
22	3-MeO–	H–	3000±800	43±18	70	78/22
23	4-MeO–	H–	5200±500	54±23	96	79/21
24	2-Cl–	H–	430±190	17±7	25	86/14
25	2-Et–	H–	350±40	13±7	27	86/14
26	2-EtO–	H–	540±190	11±4	49	98/02
27	2-CF_3–	H–	650±370	31±23	21	73/23
28	2-MeO–	4-Cl–	1200±10 (2)	4.5±2.2	270	100/0

[a]All compounds were characterized by ^{1}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.
[b]Determined using full-length MMP-1 by the method of Bickett (ref 22).
[c]Determined using full-length MMP-13 by the method of Bickett (ref 22).
[d]Values are the mean±S.D. of 3 determination unless otherwise noted (*n*).

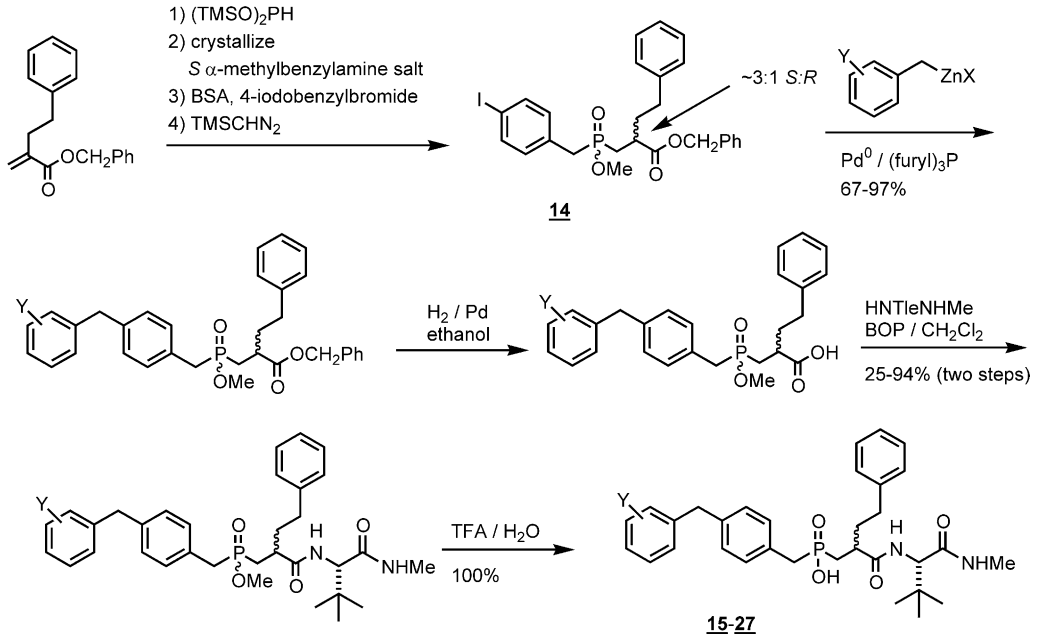
methoxy groups at the 2-, 3- and 4-positions of the terminal P_2 benzyl group. In contrast to the findings with P_1' substituents, most such substitutions led to decreases in activity. However, within each substituent set, the 2-substituted derivatives were the most potent with the 2-methoxy analogue **21** being both more potent and more selective than **3**. The observed increase in selectivity of **21** derives from its increased MMP-13 potency (3-fold) as MMP-1 potency remained the same. This suggested that a 2-substituent may be interacting in a favorable way with the S_2 pocket of MMP-13 while enjoying little additional positive interaction with the S_2 pocket of MMP-1. In order to possibly capitalize on this, we prepared additional 2-substituted derivatives, **24**–**27**. However, none of these were more potent or selective than **21**.

Having defined improved substituents for both the P_2 benzyl group and the P_1' phenethyl group, we then prepared **28** which combines these substituents—a 2-methoxybenzyl group at P_2 and a 4-chlorophenethyl group at P_1'—in a single molecule. As we had hoped, the substituents in this compound did display modest synergism; excellent potency (MMP-13 IC_{50} 4.5 nM) and high selectivity versus MMP-1 (270-fold) was observed.

Having achieved our key goal of identifying compounds with >100-fold selectivity over MMP-1, we next examined the selectivity of this series of compounds versus other MMP's. All of the compounds in Table 1 were tested against MMP-2 and MMP-3; however, only data for selected compounds, **3**, **11**, **21** and **28**, are presented in Table 2; these data are representative of what was found for the entire series. The compounds were in general very selective for MMP-13 versus MMP-3 with virtually all analogues being 100-fold or more selective. On the other hand, selectivity versus MMP-2 was absent. In fact, most of these compounds were more potent against MMP-2 than against MMP-13. In our earlier report,[14] we argued that the difference in potencies of **3** and **29**, a P_2 'debenzyl' analogue, indicated that the P_2 benzyl group is interacting with the S_2 pocket of MMP-13. Similarly, the MMP-2 potencies of **3** and **29** vary 11-fold suggesting that, as with MMP-13, the P_2 benzyl group is interacting with the S_2 pocket (or another adjacent region) of MMP-2. To broaden the profile of the series, selected compounds were also screened against MMP-8 and MMP-12, two other deep pocket MMPs. As with MMP-2, selectivity for MMP-13 inhibition over either MMP-8 or MMP-12 was not observed (Table 2). Also, like MMP-2, the ~10-fold



Scheme 1.

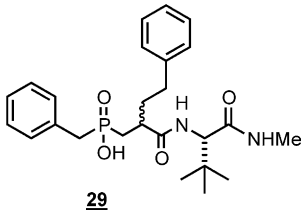


Scheme 2.

shift in potency between the 'de-benzyl' analogue **29** and **3** suggests that the inhibitors are interacting in the S_2 pocket as well as the S_1' pocket.

Through systematic variation of substituents we have improved the potency of phosphinate-based MMP-13 inhibitors over those reported earlier. Furthermore, high selectivity between MMP-13 and MMP-1 has been demonstrated. With the exception of MMP-3, selectivity against other MMPs was not observed. However, comparisons of MMP-2, -8, -12 and -13 potencies for the 'de-benzyl' analogue **29** versus **3** ($\sim$10-fold difference in all cases) suggests that key interactions are occurring with both the P_2 and P_1' groups. Therefore, further manipulation of these groups may lead to compounds selective towards these other enzymes. Selectivity against these particular MMP's may or may not ultimately be sought depending on whether selectivity

Table 2. MMP-13, MMP-3, MMP-2, MMP-8 and MMP-12 activity of selected phosphinic acids



29

	MMP-13[a] (IC_{50} nM)[f]	MMP-3[b] (IC_{50} nM)[f]	MMP-2[c] (IC_{50} nM)[f]	MMP-8[d] (K_i nM)[f]	MMP-12[e] (IC_{50} nM)[f]
3	21±9	1600±500	10±4	0.77±0.44	8.2±2.5
11	8.3±2.8	900±100	2.6±0.6	0.56±0.10	1.7±0.7
21	7.0±3.8	800±200	6.6±2.4	2.1±0.1	4.0±0.8
28	4.5±2.2	1600±400	6.6±0.5	2.4±0.4	5.0±4.2
29[g]	210±80	2700 (1)	110 (1)	12±2	93±12
Ratio of IC_{50}'s **29** versus **3**	*10*	—	*11*	*16*	*11*

[a]Determined using full-length MMP-13 by the method of Bickett (ref 22).
[b]Determined using full-length MMP-3 by the method of Nagase (ref 23).
[c]Determined using full-length MMP-2 by the method of Knight (ref 24).
[d]Determined using the catalytic domain of MMP-8 by the method of Bickett (ref 22).
[e]Determined using the catalytic domain of MMP-12 by the method of Bickett (ref 22).
[f]Values are the mean±SD of 3 determinations unless otherwise noted (*n*).
[g]Compound **29**, reported in our earlier work (ref 14), is a 62/38 mixture of *S*/*R* P_1' isomers.

versus MMP-1 is sufficient to avoid MSS or on what other MMPs are eventually identified as being key contributors to this undesired side effect.

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