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Arylsulphonyl Hydroxamic Acids: Potent and Selective Matrix Metalloproteinase Inhibitors

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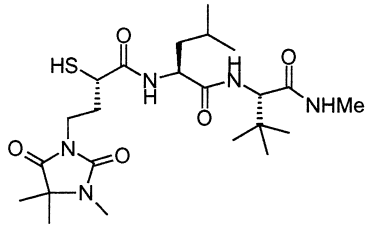
Abstract—A series of novel matrix metalloproteinase inhibitors is described in which selectivity between MMP and 'sheddase' activity has been achieved and which demonstrate potent in vivo activity in models of arthritis and cancer. © 2001 Elsevier Science Ltd. All rights reserved.

The association between overexpression of matrix metalloproteinases and diseases such as metastatic tumours has long been established.[1] Within our laboratories, development of inhibitors which mimic the natural enzyme subtrate led to the discovery of BMS275291 **1**, a thiol which was found to be a potent and broad spectrum inhibitor of matrix metalloproteinases with no activity against shedding of ectodomain proteins.[2]

The development of MMP inhibitors has been particularly hampered by side effects of early clinical candidates such as Marimastat **2**,[1b] which causes musculoskeletal pain and stiffness, limiting the maximum tolerable dose and necessitating treatment 'holidays' in patients. Given that there are both dose limitations and a lack of efficacy of early MMP inhibitors, there is an urgent need to provide compounds that can be dosed at clinically useful levels without the requirement to cut short the treatment. There is considerable debate as to whether side effects arise from inhibition of specific MMPs, or from the broad spectrum of activity of early compounds against so-called sheddase enzymes.[1b] With the observation that **1** was incapable of generating tendinitic effects in animal models, in contrast to compounds such as **2**, we felt that sheddase inhibition may play a part in MMP-inhibitor-related tendinitis.[2–5]

Other research groups have postulated that inhibition of MMP-1 may contribute to side effects[6] as the enzyme plays a crucial role in wound healing and tissue remodelling. In the current programme of work, we therefore elected to design and prepare MMP inhibitors, which spared MMP1 and ectodomain shedding.



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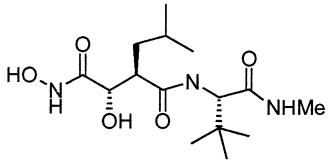

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To assist in the identification of non-peptidic MMP inhibitors, we employed computer-aided molecular design (CAMD) techniques to search databases for likely lead compounds. The UNITY database searching package within Sybyl (Tripos, St Louis, USA) was used for this purpose. Using the crystal structure of

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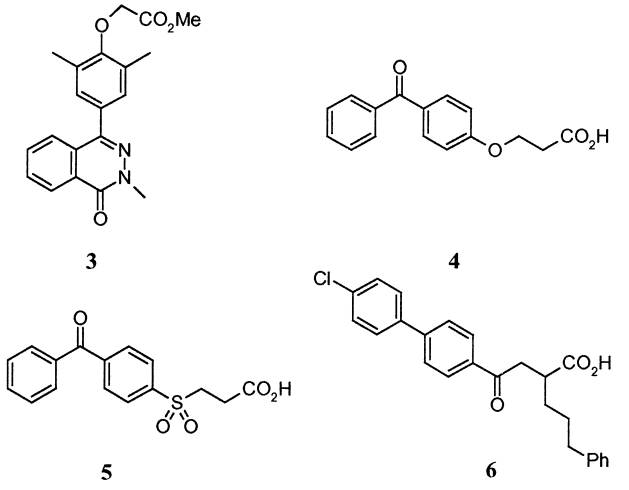
neutrophil collagenase (MMP-8, PDB code 1mnc) the location of a carboxylate group as a potential zinc binder was defined. This simply employed the atomic coordinates of three of the atoms of the hydroxamate group (carbonyl carbon and oxygen atoms, and the nitrogen atom) onto which the carboxylate moiety was superimposed. Another atomic position was also defined corresponding to the 'first' carbonyl oxygen position on the inhibitor that interacts with the main-chain amide NH group of Leu 181. Distance constraints were applied between the two molecular fragments and the crystal structure of MMP-8 was used to define regions of steric occlusion. The search results were examined 'on screen' to refine the lists of compounds to be screened against MMPs 1, 8 and 9 at a concentration of 20 μM. Hits were then followed up in order to obtain IC_{50} values against these enzymes.

The SAR by NMR work of Fesik et al. at Abbott[7] addressed a similar issue, finding small molecules which bound into ^{15}N-labelled stromelysis and optimising the linkage between them.

After completion of our screening exercise, a number of modestly potent inhibitors were identified, of which we selected the ester **3** as a starting point for a medicinal chemistry lead optimisation exercise.



It was quickly found that simplification of the aromatic hydrophobe to a benzophenone did not delete inhibitory activity and that a carboxylic acid was equipotent to the ester. A three-atom linker to the carboxylate gave a modest increase in potency, however, **4** remained only weakly active at around 5 μM.

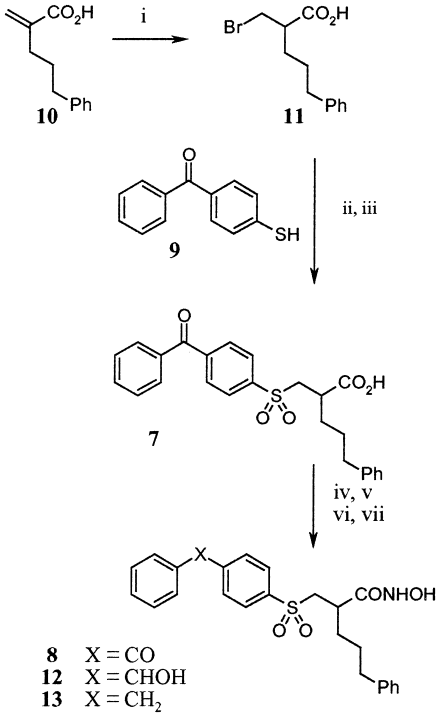
Replacement of the ether with a thioether linker reduced activity, however the sulphone **5** was accommodated.

Seeking additional binding interactions at the alpha position, we incorporated the phenpropyl α chain used to good effect in the Bayer compound **6** to give us the sulphone **7**. We then sought to increase binding affinity to the enzyme active site zinc atom, by preparation of the hydroxamic acid **8** as shown in Scheme 1.

Michael addition of 4-mercaptobenzophenone **9** with the acrylic acid **10** proved capricious under a variety of conditions, however the derived bromide **11** was found to couple rapidly and in high yield under very mild conditions. Oxone oxidation of the crude product allowed us to isolate the acid **7** in high yield by crystallisation. Conversion of this to the desired hydroxamic acid was carried out by coupling with TBDMS protected hydroxylamine, followed by deprotection using dry HCl to give **8** in good overall yield.

Reduction of **8** using borohydride gave efficient conversion to the alcohol **12**, while the use of triethyl silane gave the diaryl methane **13**.

The hydroxamic acid **8** was found to possess extremely poor aqueous solubility (< 0.001 mg/mL) and we therefore prepared the analogous pyridophenone **14** by a similar route to that shown in Scheme 1, achieving a significant improvement in solubility. The *N*-oxide **15** was also isolated as some oxidation with oxone was observed during the synthesis.



Scheme 1. Reagents: (i) HBr, AcOH; (ii) Et_3N, DMF; (iii) Oxone, MeOH, water; (iv) EDC, H_2NOTBS, DCM; (v) HCl, ether, DCM; (vi) $NaBH_4$, EtOH; (vii) TFA, Et_3SiH.

Table 1. Assay results for compounds **1**–**5** and **9**–**19** (IC_{50}, nanomolar)

Compounds	MMP1	MMP2	MMP3	MMP8	MMP9
3	7000	> 10,000	> 10,000	3000	6000
7	20,000	800	1200	300	400
8	2,000	4	100	15	5
12	25,000	1700	2500	3000	2500
13	> 30,000	200	> 2000	300	150
14	10,000	3	100	3	15
15	20,000	50	350	80	150

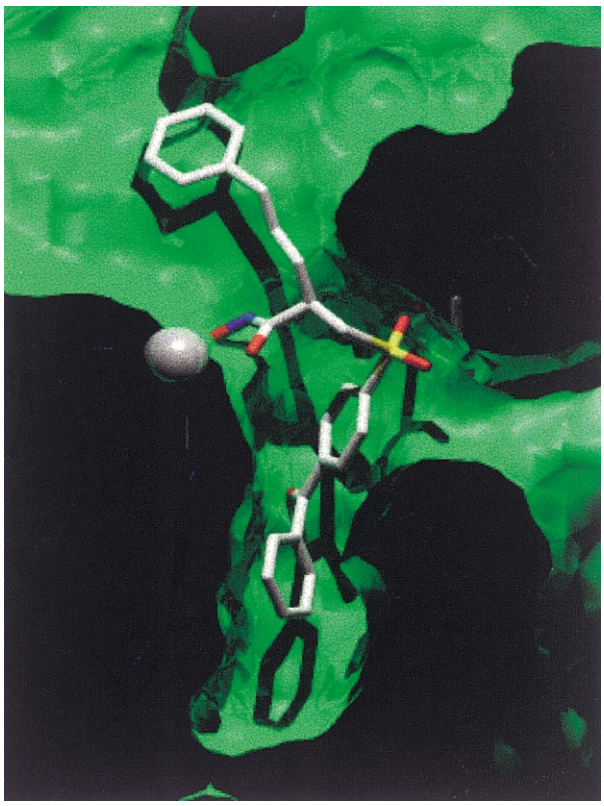
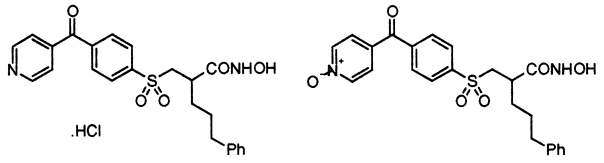


Figure 1. Predicted binding mode of **8** in MMP-8.



14 **15**

Compounds were initially screened against MMPs 1, 2, 3, 8 and 9 as shown in Table 1, using previously reported fluorimetric assays.[5] The acid **5** was modestly potent, with selectivity over MMP1, while the additional substituent in **7** conferred a significant increase in potency versus MMPs 2, 8 and 9.

Low nanomolar potency was achieved with the hydroxamic acid **8** with a selectivity ratio of 500 between MMPs 1 and 2. Reduction of the ketone had a significant detrimental effect, with the alcohol **12** being 500-fold less active against MMP9 than the ketone **8**. The diarylmethane **13** had intermediate potency.

We were encouraged to find that the pyridophenone **14** retained excellent potency against MMPs 2, 3, 8 and 9, and a superior selectivity profile with respect to MMP1 when compared to compound **8**. The derived *N*-oxide was significantly less potent.

It is well known that access to the S1′ pocket can lead to efficient and selective MMP inhibitors and that this pocket in MMP-1 is truncated by arginine 214.[8] MMPs 2, 3, 8 and 9 all have much longer S1′ pockets than MMP-1. To test whether the lower efficacy of **8** in inhibiting MMP-1 relative to these other MMPs can be explained by the shorter S1′ of the former enzyme, a docking study was carried out. To this end, **8**(*R*) was docked into a crystal structure of MMP-8 (PDB code 1mnc) and of MMP-1 (PDB code 1hfc) using the program GOLD (CCDC, Cambridge, UK). In order for this program to be able to reproduce the correct binding modes of the similar MMP inhibitors as found in crystal structures, two distance constraints of 2.2 Å are required to anchor the two hydroxamate oxygens to the catalytic zinc ion and a hydrogen bond constraint is needed between a sulphone oxygen and a protein backbone NH (leucine 181 in both MMP-1 and 8).

The predicted binding mode in MMP-8 (shown in Fig. 1) puts the arylketone moiety of **8** in the S1′ pocket. In MMP-1, this substituent is predicted to bind along the prime side substrate binding groove and not in the S1′ pocket. These results are consistent with the hypothesis that **8** has little activity at MMP-1 due to its inability to bind in the S1′ pocket.

Having achieved good potency against the enzymes of interest and good selectivity versus MMP1 with compounds **8** and **14**, we compared their activity in a range of shedding events against Marimastat **2** (Table 2).[5] Both of our compounds were inactive in these assays, while Marimastat **2** gave significant activity at micromolar concentrations. We were thus satisfied that our compounds possessed the desired in vitro inhibitory profile, sparing MMP1 and sheddases and potent against the enzymes of interest.

As anticipated from its poor aqueous solubility, **8** was found to have very poor oral bioavailability in the rat, while **14** gave significant plasma levels, up to 300 ng/mL following oral dosing at 10 mg/kg.[9]

Whittaker and co-workers have reported that only broad spectrum MMP inhibitors showed efficacy in their B16 melanoma model and postulated that competitors' compounds may act as broad spectrum agents at the doses used in animal models.[1b] With this in mind, we were intrigued to investigate whether we could

Table 2. IC_{50} values (μM) against shedding of ectodomain proteins from stimulated PBMCs

Compound	TNFa	L-Selectin	TNF RII	IL1 RII	IL6-R
2	2	5	1	10	10
8	>100	>100	>100	>100	>100
14	>100	>100	>100	37	>100

Table 3. In vivo evaluation of test compounds

Compound	Adjuvant arthritis[10]	B16-F10 mouse melanoma[11]
Marimastat	50%@10 mg/kg	40%@30 mg/kg
8	40%@10 mg/kg	40%@30 mg/kg
14	50%@3 mg/kg	80%@30 mg/kg

achieve in vivo activity with our highly selective compounds.

We were pleased to find that, in in vivo models of arthritis and cancer, **8** was equipotent to Marimastat, while **14** gave significantly better inhibition in both models (Table 3).

In summary, we have identified potent and selective MMP inhibitors, which have provided us with excellent leads for optimisation. Further work in this area will be reported at a later date.

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9. Plasma concentration of compound **13** was determined by addition of a standard solution of **8** (20 mL of a 2.5 μg/mL solution) to 50 μL of plasma, which was then diluted to 1100 μL. This was loaded onto a C_{18} SPE cartridge, washed with water and 30% methanol/water, then eluted with 400 μL acetonitrile. 100 mL of the eluent was injected onto a 5 mM C_{18} Hypersil BDS 100×4.6 mm column, eluted with acetonitrile/0.1% formic acid into a Turbo Ionspray interface on a Sciex API300 mass specrometer.
10. Adjuvant arthritis was induced in female Sprague–Dawley rats by intradermal injection of *Mycobacterium butyricum* in mineral oil into the left hind paw. Foot volume was monitored over days 1–22 and compounds were dosed orally from days 12–22. Percent inhibition was determined at day 21 relative to untreated control.
11. The compounds were evaluated in a B16 lung melanoma model of metastasis. Briefly, B16-F10 cells were maintained in culture until required. These were administered intravenously to C57BL6 mice (day 0) and 14 days later these animals were killed and the lungs removed. These tissues were fixed and the number of colonies present on the surface of the lungs was counted. Test compounds were administered 4 and 2 h prior to inoculation of the mice with B16-F10 cells and at 1, 6, 24, 48 and 72 h thereafter.