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THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NOVEL SERIES OF DIAZEPINE MMP INHIBITORS

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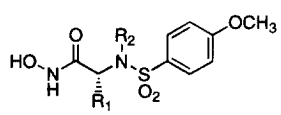
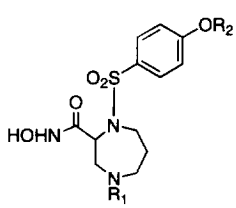
Abstract: A novel series of diazepine-based hydroxamic acid inhibitors of MMP-1, MMP-9, and MMP-13 were prepared and evaluated both in vitro and in vivo.

The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that have been implicated in the etiology of a wide variety of disease states including atherosclerosis,[1] rheumatoid arthritis,[2] osteoarthritis,[3] and cancer.[4] The therapeutic potential for potent, orally bioavailable small molecule inhibitors of MMPs to treat pathologies that are presently intractable has made them prime targets for drug development.[5] In that regard the discovery of sulfonamide-based hydroxamic acid inhibitors of stromelysin (MMP-3), exemplified by CGS 27023A (**1**; R_1 = *i*Pr, R_2 = 3-Picolyl), has proven to be the seminal work in the rapidly expanding area of nonpeptide MMP inhibitors.[6] The recent disclosure of conformationally constrained analogs related to CGS 27023A, encompassing the piperazine,[7] morpholine,[7] thiomorpholine,[7] and thiazepine[8] ring systems, now prompts us to report on the synthesis and biological evaluation of a novel series of diazepine MMP inhibitors.

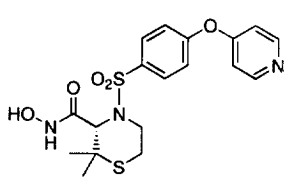
The diazepine-sulfonamides, **2**, arise conceptually from the connection, via a nitrogen linker, of the P1 substituent of CGS 27023A (**1**, R_1), attached to the carbon adjacent to the hydroxamic acid, with the $P2^1$ substituent (**1**, R_2) borne by the sulfonamide nitrogen. That such a strategy results in compounds which can retain or improve upon the activity of the acyclic sulfonamides has been demonstrated by AG3340 (**3**), now in clinical trials for the treatment of cancer.[4e,5a]



1
CGS 27023A (R_1 = *i*Pr; R_2 = 3-Picolyl)



2



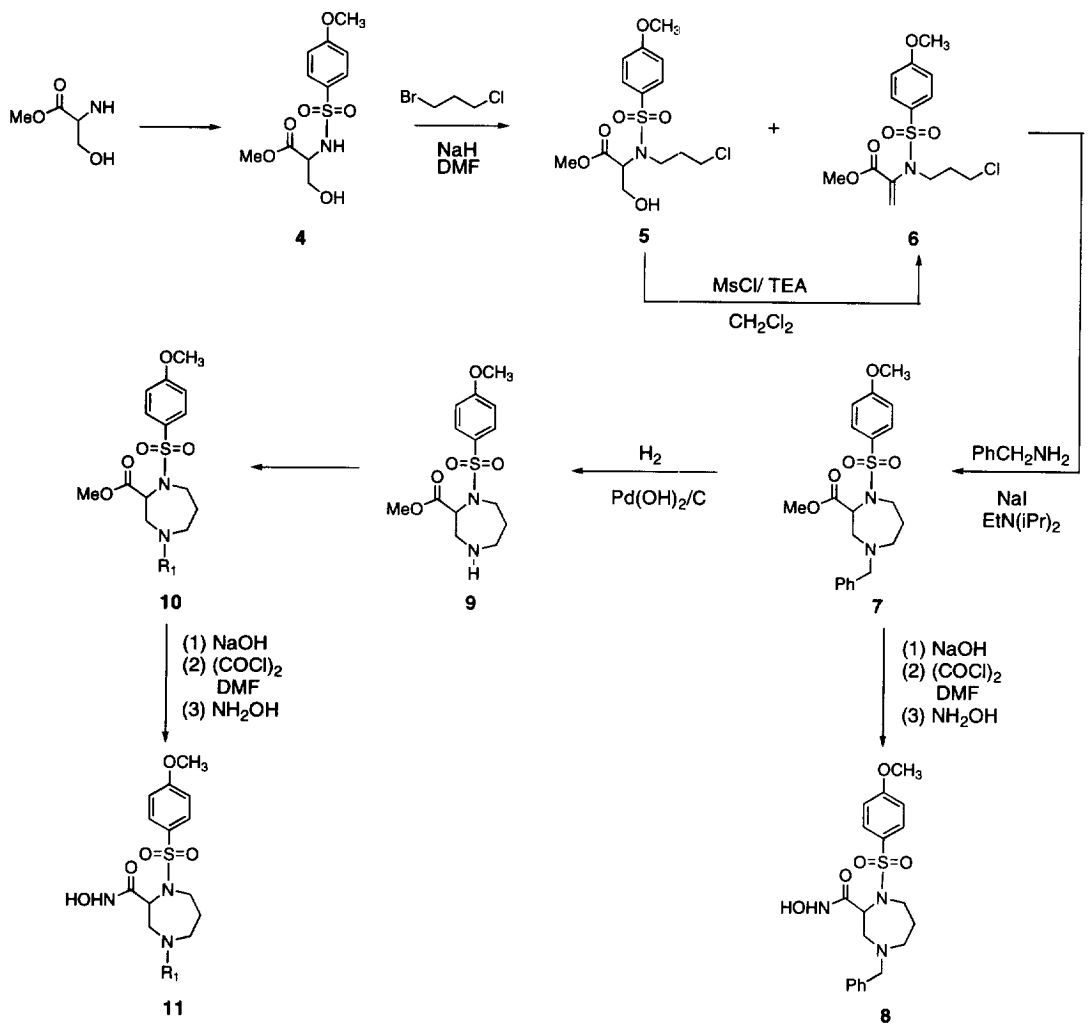
3 (AG3340)

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00473-9

Chemistry:

The target diazepines were synthesized in racemic form starting from D,L-serine methyl ester (Scheme 1).[9] Sulfonylation of the amino-ester with 4-methoxybenzenesulfonyl chloride in the presence of triethylamine provides a 54% yield of the sulfonamide **4**. The sulfonamide is then N-alkylated with 1-bromo-3-chloropropane (3 equiv) in the presence of 1.2 equiv of sodium hydride in DMF to provide a mixture of the chloro-alcohol (47%) **5**, and the chloro-olefin (18%) **6**. Chloro-alcohol **5** is next converted into olefin **6** in 86% yield through treatment with methanesulfonyl chloride and triethylamine. The diazepine **7** is then formed

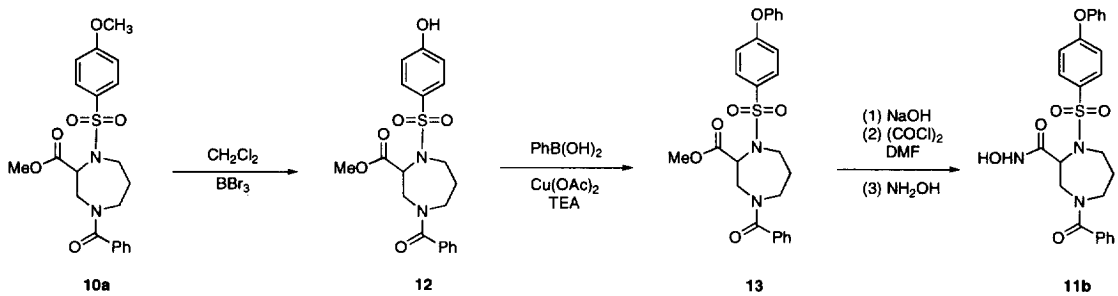
Scheme 1. Preparation of Diazepine-Hydroxamic Acids.



in 76% yield by the reaction of olefin **6** with benzylamine, diisopropylethylamine and sodium iodide for 3 h at 80 °C in DMF. The N-benzyl protected diazepine-ester is converted into the corresponding hydroxamic acid **8** via hydrolysis with aqueous sodium hydroxide (93%) followed by acid chloride formation and subsequent reaction with excess hydroxylamine (78%). Acylated diazepine analogs **11** were accessed by hydrogenation of **7** with Pearlman's catalyst to provide the secondary amine **9** in 89% yield, which is then coupled with carboxylic acids, acid chlorides, anhydrides or isocyanates to give **10**. Ester hydrolysis and conversion of the resulting carboxylic acid into the desired hydroxamic acid provides the final products. Compounds **11f** and **11i** are obtained from trifluoroacetic acid mediated BOC-deprotection of **11e** and **11h**, respectively.

Diphenyl ether **11b** is obtained from benzamide **10a** (Scheme 2) via cleavage of the methyl ether with boron tribromide (35%) to give phenol **12**, followed by aryl ether formation with phenyl boronic acid according to the method of Chan[10] to give **13**, which is then transformed into the corresponding hydroxamate as before.

Scheme 2. Preparation of Diphenyl Ether 11b.



10a **12** **13** **11b**

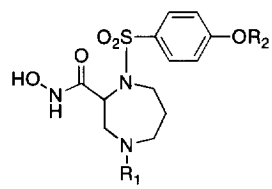
Biology:

All diazepine-hydroxamic acids were tested in vitro[11] for their ability to inhibit MMP-1, MMP-9 and MMP-13 (Table 1). Inhibitors of MMP-9 are potentially valuable as inhibitors of tumor metastasis,[4] while MMP-13 inhibitors may offer protection from cartilage degradation associated with arthritis.[3] Potency against MMP-1 was not deemed to be desirable due to the possibility that the inhibition of this MMP isoform has been associated with deleterious side effects in clinical settings.[12]

All of the hydroxamic acids shown in Table 1 are potent in vitro inhibitors of both MMP-9 and MMP-13, although none of these compounds show any significant selectivity between the two enzymes. The most potent members of the series are the benzamide derivatives, **11a**,**b**,**c** and **d** which are from two- to eight-fold more active, in the same assay, than CGS 27023A (**1**) versus MMP-9 and MMP-13. Interestingly, compound **11b** is no more selective against MMP-1 than analog **11a**, despite its lengthy biaryl ether moiety. If it is assumed that these diazepine-hydroxamates bind to MMP-1 and MMP-13 in the same manner as described for the binding of CGS 27023A to stromelysin,[13] then we postulated that the biaryl substituent of **11b** would lie in

the $S1^1$ pocket of these enzymes and preclude tight binding in the shallow $S1^1$ pocket of MMP-1. It is possible that the arginine residue that forms the bottom of the MMP-1 $S1^1$ pocket has sufficient conformational mobility to swing away from the distal phenyl ring without the loss of binding energy. Amide-carbamate **11e**, though less potent than the benzamides versus MMP-9 and MMP-13, is the most selective (43-fold) of the diazepine-hydroxamates for MMP-13 over MMP-1. Benzylic amine **8** and glycinamide derivative **11f** are the least potent azepine derivatives. However, the unsubstituted diazepine **11i**, also containing a basic amine, is comparable in potency to the amides. The bulky *t*-butyl carboxamide **11g**, ureas **11j** and **11k**, and carbamate **11h** are at least 5-fold less active than **11a** versus MMP-13.

Table 1. In Vitro and In Vivo Activities of Diazepine-Hydroxamates.



Compound	R_1	R_2	IC_{50} (nM) MMP-1	MMP-9	MMP-13	Dialysis Implant Inhibition[a,b] (MMP-13)
CGS 27023A			15	8.8	8.2	1.0
8	-CH_2Ph	Me	445	31	65	0.89
11a	-C(O)Ph	Me	45	2.0	2.2	0.92
11b	-C(O)Ph	Ph	22	1.2	1.3	1.0
11c	-C(O)Ph-4-OCF_3	Me	128	4.0	6.2	IA
11d	-C(O)Ph-2-Ph	Me	116	3.3	4.6	0.51
11e	-C(O)CH_2NHBOC	Me	690	23	16	0.41
11f	-C(O)CH_2NH_2-HCl	Me	703	157	46	0.16
11g	-C(O)tBu	Me	251	4.8	11	NT
11h	-C(O)OtBu	Me	618	9.1	26	IA
11i	-H-HCl	Me	91	60	5.1	0.79
11j	-C(O)NHPh	Me	309	18	17	IA
11k	-C(O)NH(*S*)-CH_3-Bn	Me	312	9.2	10.4	0.77

[a] %Inhibition/%Inhibition for CGS27023A @ 50 mg/kg po (n = 6)

[b] %Inhibition for CGS27023A @ 50 mg/kg po ranged from 75-86%.

NT = Not Tested.

IA = Inactive.

Several of the diazepine-hydroxamates were also tested in a bovine articular cartilage explant assay.[14] The aryl ether **11b** and unsubstituted diazepine **11i** provide levels of inhibition of collagen degradation comparable to CGS 27023A (58%) at a dose of 1 μM. At the same dose diazepine-hydroxamates **11c**, **11d**, **11g**, and **11k** were significantly less active than CGS 27023A.

The in vivo bioactivity of several of the diazepine-hydroxamates was assessed through the use of a dialysis tubing implant assay.[15] In this model a solution containing MMP-13 is placed in a dialysis bag that is then implanted subcutaneously in the back of a mouse 30 min after oral dosing of the inhibitor. Forty-five minutes after implanting the dialysis tubing, 75 min after dosing the inhibitor, the tubing is recovered and the enzyme activity of the contents is measured. All of the compounds tested were compared to CGS 27023A in the same experiment. The most active of the compounds tested in this assay are benzamides **11a** and **11b**, which are essentially equipotent to CGS 27023A. This is interesting in light of the reported in vivo SAR for CGS 27023A and its congeners, in which a basic nitrogen is required for good oral potency.[6] The ortho-substituted biphenyl carboxamide **11d** is less potent and the trifluoromethoxy-substituted benzamide is inactive at 50 mg/kg, though there is little difference in their in vitro potencies. Glycinamide **11f** and urea **11j** are also inactive at this dose. The unsubstituted diazepine **11i**, a potent in vitro inhibitor containing a basic nitrogen for increased water solubility, is slightly less potent than benzamide **11a**. Benzylic amine **8**, also containing a basic nitrogen, is slightly more potent than **11i** in the dialysis implant model even though it is ten-fold less active versus MMP-13 in vitro.

In conclusion, we have synthesized a novel series of diazepine-hydroxamic acid MMP inhibitors. All of these compounds are potent inhibitors of MMP-9 and MMP-13 in vitro. Diazepines **11b** and **11i** are also active in an in vitro cartilage degradation model. Furthermore, evaluation of several of these compounds in an in vivo mouse bioactivity model indicates that some of them are potent inhibitors of MMP-13 after oral dosing.

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