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Benzodiazepine Inhibitors of the MMPs and TACE

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Abstract—A series of benzodiazepine inhibitors of the MMPs and TACE has been developed. These compounds display an interesting selectivity profile and should be useful tools for exploring the biological relevance of such selectivity.

The matrix metalloproteinases (MMPs) are a family of over 20 different enzymes that are involved in a variety of biological processes including degradation of the extracellular matrix, embryonic development, angiogenesis, wound healing, apoptosis and normal tissue remodeling. Under normal circumstances, the activity of the MMPs is tightly controlled, as the disregulation of these enzymes can lead to various disease states, including osteoarthritis (OA),[1] rheumatoid arthritis (RA)[1] and cancer.[2] The ADAM class of enzymes is structurally related to the MMPs and includes TNF-α converting enzyme (TACE), the enzyme responsible for cleaving membrane-bound TNF, which is believed to have a major role in the pathology of rheumatoid arthritis, inflammation and other disease states.[3] A variety of inhibitors of MMPs have been shown to be potent inhibitors of TACE.[4]

Given their role in the above diseases, it is expected that small molecule inhibitors of MMPs and TACE would have utility for treating many disorders. Thus, several MMP inhibitors have already entered clinical trials for the treatment of cancer and OA. Furthermore, there are two clinically effective RA therapies available that block the action of TNF-α, Enbrel® and Remicade®, giving credence to the idea that small molecule inhibitors of TACE may be effective in treating RA.[5]

One problem that has been broached frequently during the course of developing MMP inhibitors for clinical trials is how to achieve selective inhibition of individual members of the MMP and ADAM family of enzymes, and whether such selectivity would be advantageous. The current goal is to exploit known differences in the binding domains of the various enzymes in order to produce small molecule inhibitors with different selectivity profiles and evaluate their efficacy and toxicological profiles.[6] It is hoped that by doing so, we can begin to address what type of selectivity is necessary in order to minimize detrimental side effects of MMP and TACE inhibitors while retaining their therapeutic potential.

Numerous examples of both peptide and nonpeptide small molecule MMP inhibitors are known.[6] In the area of nonpeptide based inhibitors, CGS-27023A is a classic example (Fig. 1A).[7] We now wish to disclose our work in this area using a benzodiazepine template as a framework for designing potent MMP inhibitors (Fig. 1B).[8]

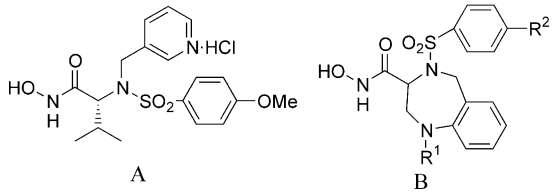

Figure 1. (A) CGS-27023A; (B) general structure of benzodiazepine inhibitors.

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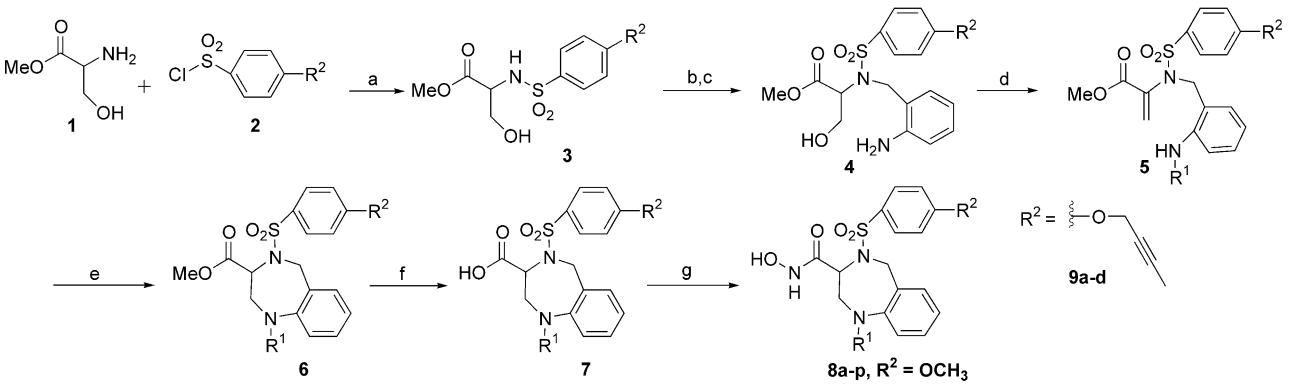


Scheme 1. Reagents and conditions: (a) Et_3N, CH_2Cl_2; (b) 2-nitrobenzyl bromide, NaH, DMF; (c) ammonium formate, 10% Pd/C; (d) Et_3N, CH_2Cl_2, R^1Cl; (e) sodium bicarbonate, CH_3OH; (f) LiOH, THF, CH_3OH, H_2O; (g) (i) oxalyl chloride, DMF, CH_2Cl_2, 0 °C; (ii) hydroxylamine hydrochloride, Et_3N, DMF.

These compounds exhibit potent inhibitory activity as well as interesting selectivity profiles against MMPs and TACE and as such may be useful in treating many of the conditions stated above.

Chemistry

Synthesis of the benzodiazepines commences with the sulfonylation of D,L-serine methyl ester with *p*-methoxybenzene sulfonyl chloride to give a good yield (79–84%) of sulfonamide **3** (Scheme 1).[9] Alternative sulfonyl chlorides could be used to provide analogues with different R^2 substituents. The sulfonamide is then alkylated with 2-nitrobenzyl bromide to give the nitro intermediate in 54–69% yield. The moderate yield of this reaction is due to alkylation of the primary alcohol followed by elimination to give the α,β-unsaturated ester as a by-product. The nitro group is then reduced with ammonium formate and 10% palladium on carbon in ethanol to give 65–74% of amine **4**, after recrystallization from ethyl acetate. The benzodiazepine ring is constructed via acylation of the aniline with concomitant elimination of the hydroxyl moiety to provide **5**, which undergoes a Michael addition into the resulting α,β-unsaturated ester to give **6** in 80–96% yield (two steps). A variety of acid chlorides were used in this acylation/elimination/cyclization sequence. Hydrolysis of methyl ester **6**, followed by subsequent transformation to the hydroxamic acid gives the final products, **8** and **9**. Compounds **9a**–**d**, where R^2 is a butynyl ether were prepared via the cleavage of the methyl ether in compound **6** with BBr_3, followed by alkylation of the resulting phenol using the Mitsunobu protocol (DEAD, PPh_3, alcohol, THF, toluene). The butynyl analogues were then converted to the hydroxamic acids as before.

Discussion

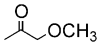
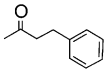
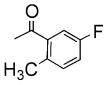
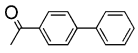
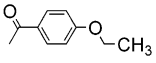
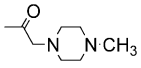
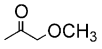
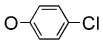
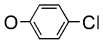
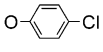
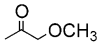
The compounds synthesized as shown above were evaluated as inhibitors of MMP-1, MMP-9, MMP-13, and TNF-α converting enzyme (TACE).[10] All of the compounds in Table 1 show selectivity for MMP-9 and MMP-13 versus MMP-1 and TACE. As the size of R^1 increases, the potency against TACE decreases, while the activity for MMP-9 and MMP-13 remains largely unaffected. Two notable compounds are **8e** and **8l**. Compound **8e** displays high potency against all four enzymes while **8l** shows a dramatic decrease in potency for all four enzymes. This reduction in potency must have an electronic component and is clearly not due just to sterics. The biphenyl analogue **8i** and the phenyl derivative **8c** are the most potent in the series with subnanomolar IC_{50}'s for both MMP-9 and MMP-13; **8i** is also the most selective member of the series with MMP-1/MMP-13 = 132 and TACE/MMP-13 = 498. MMP-1 is also sensitive to the size of the R^1 substituent, however the tolerance is somewhat different than TACE. Compounds with an sp^3 center α to the carbonyl (**8j**, MMP-1 = 465 nM) are typically less active than those with an sp^2 center in the same position (**8c**, MMP-1 = 16 nM).

In order to provide compounds with improved selectivity for MMP-13, compounds **8m**–**8p** were prepared. In other series, it is known that P1′ substituents of this nature have conferred increased selectivity for MMP-13.[6] Interestingly, this effect is not seen to any great extent for **8m**–**8o**. However, **8n** does exhibit a decrease in potency for TACE, although this is not accompanied by a significant improvement in selectivity relative to **8c**. Example **8p**, with the 4-pyridyl ether, displays the most pronounced effects in this series with 381-fold selectivity for MMP-13 over MMP-1 and 78-fold selectivity for MMP-13 over TACE.

Select compounds were tested in two advanced models (Table 2). In both models, CGS-27023A (Fig. 1A) was used as a standard. This compound was the first of the sulfonamide based MMP inhibitors and has been used in clinical trials for the treatment of cancer.[7] Initially, compounds were screened in a dialysis tubing implant assay to measure in vivo bioactivity versus MMP-13.[11] In this assay a section of dialysis tubing containing enzyme is implanted subcutaneously in the back of a rat and inhibitor is administered ip or orally. After a period of time the tubing is recovered and enzyme activity is analyzed.

Many of the compounds performed quite well and were equipotent or superior to CGS-27023A. Two compounds, **8a** and **8d**, were tested by two routes of

Table 1. In vitro data for benzodiazepines **8a**–**8p**

Compd	R^1	R^2	MMP-1[a]	MMP-9[a]	MMP-13[a]	TACE[a]
8a		OCH_3	18	1.4	1	103
8b		OCH_3	157	8	3	104
8c		OCH_3	16	0.6	0.4	95
8d		OCH_3	171	4	3	69
8e		OCH_3	20	1	1	13
8f		OCH_3	34	2	2	95
8g		OCH_3	523	18	26	207
8h		OCH_3	55	4	2	271
8i		OCH_3	53	0.7	0.4	199
8j		OCH_3	465	13	7	318
8k		OCH_3	24	2	2	157
8l		OCH_3	4982	187	317	808
8m			61	2	2	157
8n			59	2	2	528
8o			35	2	4	254
8p			763	2	2	157

[a]IC_{50} (nM).

administration. Both showed a significant decrease in activity when going from ip to po administration. The poorest performer in this assay was **8g**, which displayed only 22% of the activity of the control at the 2 h time point when dosed ip. Thus, while CGS-27023A gave 35.3% inhibition of MMP-13, **8g** displayed a modest 7.8% inhibition when dosed in the same assay. Compounds **8d**, **8j** and **8p** had less than 50% of the activity of the control when dosed orally. A small subset of the compounds tested in dialysis went on to the bovine

Table 2. Dialysis and cartilage explant data for select compounds

Compd	Dialysis activity[a] (route, time)	Cartilage explant, activity[c] (at 1 μM)
CGS-27023A	1	1
8a	1.7 (2 h, ip)	NT
8a	0.90 (1 h, po)	
8c	1.2 (2 h, ip)	0.73
8d	0.80 (1 h, ip)	NT
8d	0.49 (1 h, po)	
8e	1.1 (2 h, ip)	1.2
8f	1.0 (1 h, po)	2.5
8g	0.22 (2 h, ip)	NT
8j	0.47 (1 h, po)	NT
8m	IA	NT
8p	0.32 (1 h, po)[b]	NT

[a]Compounds were dosed at 50 mpk and the data are expressed relative to activity of CGS-27023A (such that control = 1) versus MMP-13 in the same experiment.
[b]Compound was dosed at 25 mpk.
[c]Control was CGS-27023A. Data are expressed as in the dialysis assay.
IA = inactive; NT = not tested.

Table 3. In vitro data for compounds **9a**–**9d**

Compd	R_1	MMP-1[a]	MMP-9[a]	MMP-13[a]	TACE[a]	THP-1[b] (%)
9a		835	228	77	16	60
9b		260	95.5	34.6	20	44
9c		165	35.8	9.5	59	25
9d		841	33	29	10	59

[a]IC_{50}, nM.
[b]% Inhibition @ 3 μM.

cartilage explant model.[12] All four compounds that were tested provided a level of inhibition of cartilage degradation that was comparable or superior to CGS-27023A at a dose of 1 μM. Compound **8c**, for example, displayed 46.3% inhibition of cartilage degradation while CGS-27023A gave 63% inhibition, again in the same assay.

Replacement of the methoxy group with but-2-ynyloxy (Table 3) changes the selectivity dramatically for TACE. Compounds **9a**–**9d** all display a 1.6- to 6.9-fold increase in potency for TACE relative to their methoxy substituted counterparts (**8a**–**8d**). Interestingly, the potency for the other enzymes decreased with the addition of this but-2-ynyloxy substituent. Notably, the selectivity for TACE over MMP-1 is drastically improved, with compound **9d** showing an 84-fold preference for TACE. These compounds were also tested for their ability to inhibit TNF-α in a cellular assay using THP-1 cells.[13] All compounds displayed a reasonable level of inhibition at 3 μM. Upon dosing down, only **9a** maintained a good level of inhibition (44%) at 1 μM. Within the butynyl series, we have therefore succeeded in providing compounds that are selective for TACE over MMP-1 and have demonstrated the ability to inhibit TNF-α in a cellular system.

Conclusion

In conclusion, we have succeeded in synthesizing a novel series of benzodiazepine inhibitors of the MMPs and TACE. These compounds demonstrate an interesting selectivity profile and several have also shown high levels of activity in two advanced models. By appropriate manipulation of the R^2 position, we have successfully transformed the methoxy-substituted compounds into a series that now displays a vastly improved selectivity profile for TACE. Given this data, these compounds might be useful in the treatment of the various disease states mediated by these enzymes and serve as probes for the importance of selectivity in the disease process.

References and Notes

1. (a) Elliott, S.; Cawston, T. *Drugs Aging* **2001**, *18*, 87. (b) Shaw, T. S.; Nixon, J. S.; Bottomley, K. M. *Exp. Opin. Invest. Drugs* **2000**, *9*, 1469.
2. (a) Sternlicht, M. D.; Bergers, G. *Emerg. Ther. Targets* **2000**, *4*, 609. (b) Lauer-Fields, J. L.; Fields, G. B. *Exp. Opin. Ther. Pat.* **2000**, *10*, 1873.
3. Moss, M. L.; White, J. M.; Lambert, M. H.; Andrews, R. C. *Drug Discov. Today* **2001**, *6*, 417.
4. Nelson, F. C.; Zask, A. *Exp. Opin. Ther. Pat.* **1999**, *8*, 383.
5. Lorenz, H.-M. *Curr. Opin. Invest. Drugs* **2000**, *1*, 188.
6. (a) Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. *Curr. Med. Chem.* **2001**, *8*, 425. (b) Skotnicki, J. S.; Levin, J. I.; Killar, L. M.; Zask, A. In *Metalloproteinases as Targets for Anti-Inflammatory Drugs*; Bottomley, K. M. K., Bradshaw, D., Nixon, J. S., Eds.; Birkhauser: Basel, 1999: p 17. (c) Montana, J.; Baxter, A. *Curr. Opin. Drug Disc. Devel.* **2000**, *3*, 353.
7. MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, S.; Melton, R. A.; Fryer, L.; Goldberg, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V.; Parker, D. T. *J. Med. Chem.* **1997**, *40*, 2525.
8. (a) Albright, J. D; Delos Santos, E. G.; Du, X. U.S. Patent 6,071,903, 2000; *Chem. Abstr.* **2000**, *133*, 17487. (b) Delos Santos, E. G.; Albright, J. D.; Nelson, F. C.; Du, X.; Levin, J. I.; Chen, J.; Skotnicki, J. S.; Killar, L. M.; DiJoseph, J. F.; Sung, A.; Sharr, M. A.; Roth, C. E.; Walter, T.; Cowling, R.; Jin, G. *Abstracts of Papers*, 222nd National Meeting of the American Chemical Society, Chicago, IL, Aug 26–30, 2001; American Chemical Society: Washington DC, 2001; Medi 246.
9. All new compounds gave satisfactory ^{1}H NMR, IR and MS in accord with their assigned structure.
10. (a) Weingarten, H.; Feder, J. *Anal. Biochem.* **1985**, *147*, 437. (b) Jin, G.; Huang, X.; Black, R.; Wolfson, M.; Rauch, C.; McGregor, H.; Ellestad, G.; Cowling, R. *Anal. Biochem.* **2002**, *302*, 269.
11. DiJoseph, J. F.; Sharr, M. A. *Drug Dev. Res.* **1998**, *43*, 200.
12. Lewis, E. J.; Bishop, J.; Bottomley, K. M.; Bradshaw, D.; Brewster, M.; Broadhurst, N. J.; Brown, P. A.; Budd, J. M.; Elliott, L.; Greenham, A. K.; Johnson, W. H.; Nixon, J. S.; Rose, F.; Sutton, B.; Wilson, K. *Br. J. Pharm.* **1997**, *121*, 540.
13. Levin, J. I.; Chen, J. M.; Cole, D. C. WO 0044709, 2000. *Chem. Abstr.* **2000**, *133*, 150908.