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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1641–1645

Synthesis and SAR of diazepine and thiazepine TACE and MMP inhibitors

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Received 11 November 2004; revised 19 January 2005; accepted 21 January 2005

Abstract—Potent and selective TACE and MMP inhibitors utilizing the diazepine and thiazepine ring systems were synthesized and evaluated for biological activity in in vitro and in vivo models of TNF-α release. Oral activity in the mouse LPS model of TNF-α release was seen. Efficacy in the mouse collagen induced arthritis model was achieved with diazepine **20**.

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that is believed to play a role in the etiology of rheumatoid arthritis (RA), Crohn's disease and other disease states.[1] Small molecule mediators of TNF-α action are important targets for the treatment of these inflammatory diseases. Enbrel™, a soluble TNF receptor-Fc dimer, modulates TNF-α by interacting with both the 26 kDa membrane-bound form of TNF-α and 17 kDa soluble TNF-α and is a highly effective RA therapy.[2] An alternative paradigm for affecting TNF-α levels is via the inhibition of TNF-α converting enzyme (TACE/ADAM-17), the enzyme primarily responsible for the shedding of membrane-bound TNF-α to provide its soluble form.[3] A number of small molecule TACE inhibitors possessing excellent enzyme and cellular potency have recently been disclosed having diverse selectivity profiles versus TACE and the MMPs.[4] Since a variety of MMPs have been found to be overexpressed in RA synovial tissue and have been implicated in the destruction of cartilage in RA joints, the optimal TACE/MMP selectivity profile for a drug to treat rheumatoid arthritis is at present unresolved.[5] In addition, several MMP inhibitors have been reported to exhibit a musculoskeletal syndrome as a side effect, however the cause of this toxicity (e.g. inhibition of MMP-1, MMP-14 or sheddases) is unclear.[4b]

Molecular modeling studies of the TACE X-ray crystal structure[6] indicated that an acetylenic P1′ group could fit in the tunnel connecting the S1′ and S3′ pockets.[7] The butynyloxy P1′ group has been found to be effective for increasing potency versus the TACE enzyme in a cell-free assay, and in LPS-stimulated THP-1 cells for anthranilic acid-based and benzodiazepine-based sulfonamide hydroxamic acids (Fig. 1).[7,8] The butynyloxy group has also been found to enhance the MMP-1 selectivity of these compounds. More recent work on the incorporation of polar groups onto the benzodiazepine scaffold, to enhance aqueous solubility and in vivo activity, has also been described.[9] As in the benzodiazepines, the diazepine and thiazepine ring systems constrains the butynyloxy P1′ group relative to the hydroxamic acid. However, removal of the phenyl ring decreases the

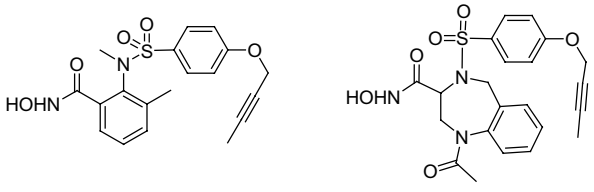

Figure 1. Butynyloxy TACE/MMP inhibitors.

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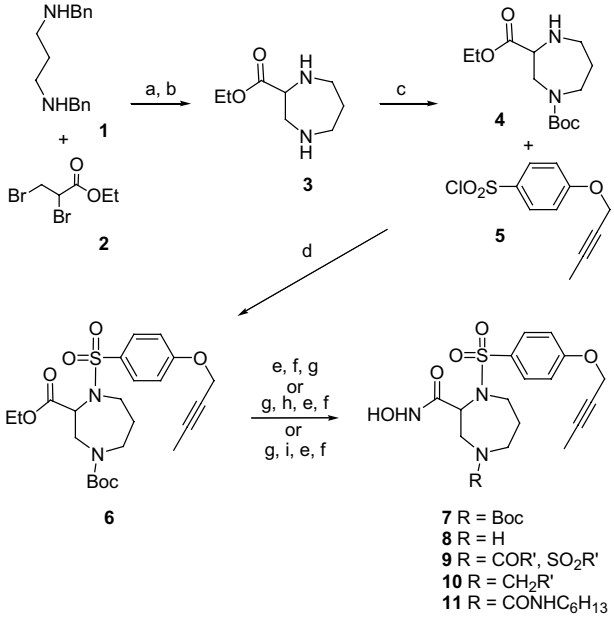
doi:10.1016/j.bmcl.2005.01.053

lipophilicity of the system, increases the basicity of the ring nitrogen in the diazepines and changes the conformation of the seven membered ring. Through the use of the two possible 1,4-diazepine isomers, the location (relative to the hydroxamic acid) of the basic nitrogens, as well as the substituents attached to them can be altered, providing an opportunity to explore the tolerances of the enzyme for interactions with these groups, as well as their effects on cellular and in vivo activity. Similarly, the two isomeric 1,4-thiazepines were synthesized and the effects of the sulfur and sulfone moieties on activity were evaluated.

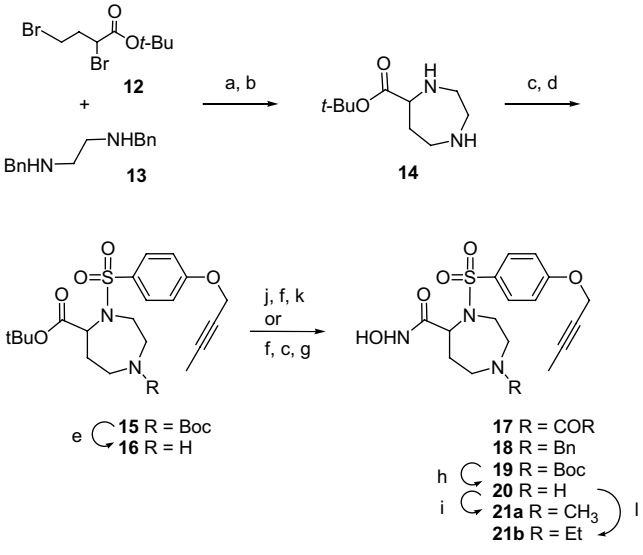
The 1,4-diazepine-2-carboxylate ring system was prepared by treating *N*,*N*′-dibenzyl-1,3-propanediamine (**1**) and ethyl 2,3-dibromopropionate (**2**) with triethylamine in benzene (Scheme 1).[10] The benzyl protecting groups were removed by catalytic hydrogenolysis using 10% Pd/C to give **3**. Selective protection of the more basic and sterically accessible amine using Boc_2O gave **4**. Sulfonylation of **4** with 4-(2-butynyloxy)benzenesulfonyl chloride (**5**) gave **6**. Saponification of the ethyl ester in **6** with NaOH gave the corresponding carboxylic acid, which was converted to the hydroxamic acid (**7**) by treatment with hydroxylamine in the presence of EDC and HOBT. Subsequent removal of the Boc protecting group with HCl gave **8**. Alternatively, removal of the Boc group in **6** with HCl released the basic amine, which could then be *N*-acylated, *N*-sulfonylated or *N*-alkylated by treatment with acyl chlorides, sulfonyl chlorides or alkyl halides, respectively. *N*-Acylation could also be effected by coupling the free nitrogen with a carboxylic acid in the presence of EDC and HOBT. Treatment of the basic amine with cyclohexyl isocyanate in the presence of Hunig's base gave the corresponding urea. Conversion of the ester to a hydroxamic acid as described above gave compounds **9**–**11**.

The isomeric 1,4-diazepine-5-carboxylate scaffold was synthesized in an analogous way by treating 2,4-dibromo-butyric acid *tert*-butyl ester (**12**) and *N*,*N*′-dibenzyl-1,2-ethanediamine (**13**) with Et_3N in CH_2Cl_2 (Scheme 2). The benzyl protecting groups were removed by catalytic hydrogenolysis using 10% Pd/C to give **14**. Selective protection of the more accessible nitrogen by treatment with Boc_2O followed by sulfonylation with **5** gave **15**. Selective removal of the *N*-Boc group by treatment of **15** with HCl in dioxane gave **16**, whose free amine group could be acylated or alkylated. Subsequent removal of the *t*-butyl group using TFA in CH_2Cl_2 gave the corresponding carboxylic acid, which was converted to the hydroxamic acid by treatment with oxalyl chloride and hydroxylamine to provide **17** and **18**. Alternatively, treatment of **15** with TFA in CH_2Cl_2 removed both protecting groups. The nitrogen was then Boc protected as above and the carboxylic acid was converted to the hydroxamic acid to give **19**. Removal of the Boc group with HCl in dioxane gave **20**, which could be selectively alkylated on the ring nitrogen with CH_3I and Et_3N to give **21a**. Alternatively, treatment of **20** with HOAc and $NaBH_4$ gave the *N*-ethyl analog **21b**.

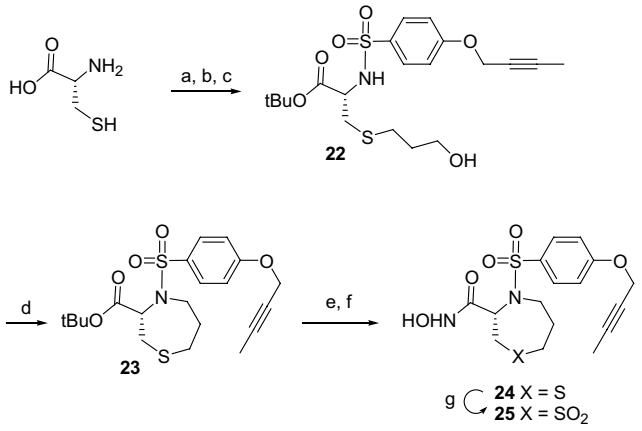
The isomeric 1,4-thiazepine-3-carboxylic acid scaffold was constructed beginning with D-cysteine (Scheme 3). Sequential treatment with 3-bromo-propan-1-ol and **5**, followed by conversion of the carboxylic acid to the *t*-butyl ester gave **22**. Cyclization by treatment with PPh_3 and diethyl azodicarboxylate gave **23**. Deprotection of the ester by treatment with TFA and conversion to the hydroxamic acid gave **24**. Oxidation of **24** with peracetic acid gave the sulfone **25**. In a similar fashion, the 1,4-thiazepine-5-carboxylic acid scaffold was



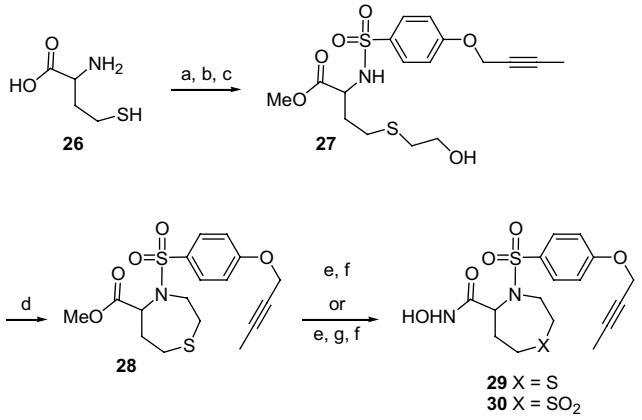
Scheme 1. Reagents and conditions (a) Et_3N, PhH; (b) H_2, 10% Pd/C; (c) Boc_2O, dioxane, NaOH (aq.); (d) pyridine; (e) NaOH; (f) EDC/HOBT/NH_2OH; (g) HCl; (h) RCOCl or RSO_2Cl or RX, *i*-Pr_2NEt or RCO_2H/EDC/HOBT; (i) $C_6H_{13}NCO$, *i*-Pr_2NEt.



Scheme 2. Reagents and conditions (a) Et_3N, CH_2Cl_2; (b) H_2, 10% Pd/C; (c) Boc_2O, dioxane, NaOH (aq.); (d) **5**, Et_3N, DMAP; (e) 4 N HCl/dioxane; (f) TFA, CH_2Cl_2; (g) EDC, HOBT, NH_2OH, DMF; (h) 2 N HCl/dioxane; (i) CH_3I, Et_3N, MeOH; (j) RCOCl or BnBr, Et_3N, CH_2Cl_2; (k) $(COCl)_2$, DMF, NH_2OH; (l) HOAc, $NaBH_4$.



Scheme 3. Reagents and conditions (a) $Br(CH_2)_3OH$, NaOH, EtOH; (b) **5**, Et_3N; (c) *t*-BuBr, DMA; (d) PPh_3, DEAD, THF; (e) TFA, CH_2Cl_2; (f) EDC, HOBT, NH_2OH, DMF; (g) CH_3CO_3H.



Scheme 4. Reagents and conditions (a) $Br(CH_2)_2OH$, NaOH, EtOH; (b) **5**, Et_3N; (c) $TMSCHN_2$; (d) PPh_3, DEAD, THF; (e) 5 N NaOH, MeOH; (f) EDC, HOBT, NH_2OH, DMF; (g) oxone.

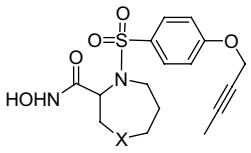
synthesized by *S*-alkylation of 2-amino-4-mercaptobutyric acid (**26**) with 2-bromoethanol (Scheme 4). Subsequent *N*-sulfonation with **5** and esterification using $TMSCHN_2$ gave **27**. Cyclization of **27** by treatment with PPh_3 and diethyl azodicarboxylate gave **28**, which was hydrolyzed and converted to the hydroxamic acid **29** as described above. Alternatively, saponification of **28** followed by oxidation to the sufone and conversion to the hydroxamic acid gave **30**.

Analogs were tested for their ability to inhibit TACE, MMP-1 and MMP-13.[11] The two collagenases, MMP-1 and MMP-13, are representative of enzymes with deep and shallow S1′ pockets, respectively, and thus may be predictive of selectivity towards other MMPs. Furthermore, MMP-13 is a key enzyme involved in type-II cartilage degradation and its inhibition may be particularly beneficial in arthritis. The 1,4-diazepine-2-hydroxamic acid scaffold provided potent inhibitors of cell-free TACE enzyme, with some analogs having IC_{50} values of less than 10 nM (**9c**, **d**, **g**) and greater than 100-fold selectivity for TACE over MMP-1 (**9c**–**e**, **g**) (Table 1). Inhibition of MMP-13 activity was seen as well. The most potent compounds in the TACE enzyme assay were the analogs with an *N*-$COCH_3$ (**9c**) group, an *N*-$COCH_2NHCO_2tBu$ (**9d**) group or a *N*-SO_2CH_3 (**9g**) group. Activity was also seen in the LPS stimulated THP-1 cellular assay.[12] Compounds **9c**, **9d** and **9g** with the most potent TACE enzyme activity were also among the best inhibitors in the cellular assay for this scaffold. Side chains with basic amine substituents (**9e**, **f**), or a basic ring nitrogen (**8**, **10a**, **10b**), were not effective in improving cellular activity in this series. Large groups on nitrogen were tolerated indicating interaction with a solvent exposed region in the enzyme. Several compounds in this series were orally active in the mouse LPS induced TNF-α release assay.[12,13] Compound **9d** showed the greatest inhibition, with 68% inhibition of TNF-α release 1 h after oral dosing at 25 mpk. The thiazepine analog **24** was a potent TACE inhibitor with 20-fold selectivity versus MMP-1. It performed somewhat better in the THP-1 cellular assay than the corresponding diazepine analogs, and gave 80% inhibition at 3 μM. The corresponding sulfone (**25**) was markedly better than **24** in the LPS assay, and gave 77% inhibition of TNF-α release after 1 h (50 mpk po) compared with 24% inhibition for **24**.

The isomeric 1,4-diazepine-5-hydroxamic acid scaffold provided TACE inhibitors that were comparable in potency to analogs utilizing the 1,4-diazepine-2-hydroxamic acid system (Table 2). Potent TACE activity was achieved independent of the nature of the substituent on nitrogen. Hydrogen (**20**), alkyl (**18**, **21**), carbonyl (**17a**–**d**) or Boc (**19**) substitution gave potent inhibitors. Moreover, this scaffold gave analogs that were more potent inhibitors in the cellular THP-1 assay relative to the isomeric diazepine scaffold. More potent MMP-13 inhibition was seen for this scaffold as well. Selectivity versus MMP-1 was approximately 10-fold in most cases. The potent cellular activity of this series led to potent activity in the mouse LPS induced TNF-α release assay. Several analogs had good activity 6 h after a single 50 mpk po dose. Although basicity had no effect on potency in the in vitro assays, in vivo activity was best for the basic NH compound, **20**, that displayed excellent activity both at 1 h (97% inhibition) and 6 h (62% inhibition) after dosing at 50 mpk po in the LPS assay. The Boc analog (**19**) similarly had good in vivo activity, although loss of the Boc group may occur in vivo. Due to its superior activity profile, compound **20** was tested in an efficacy model of arthritis, the mouse collagen induced arthritis (CIA) model of arthritis.[14] Upon dosing at 20 mpk bid po, **20** gave a 56% reduction of the mean clinical score relative to control (HulgG) after 35 days. The thiazepine analog **29**, and the corresponding sulfone **30**, showed potent TACE enzyme activity and excellent inhibition of TNF-α release in the THP-1 cellular assay. Both analogs showed potent oral activity in the mouse LPS assay of TNF-α release.

Due to the excellent in vivo activity of **20**, the pharmacokinetics were examined via three different modes of administration, intravenous (iv), intraperitoneal (ip) and oral (po), to female Balbc mice. Animals received a single iv bolus of 5 mpk, an ip dose of 10 mpk, and a po dose of 50 mpk. The compound exhibited moderate

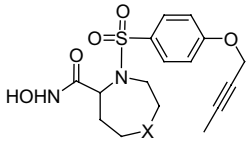
Table 1. 1,4-Diazepine-2-hydroxamic acids and 1,4-thiazepine-3-hydroxamic acids



Compound	X	MMP-1[a]	MMP-13[a]	TACE[a]	THP[b]	LPS[c]
7	$NCO_2{}^tBu$	26%	363	35	13	—
8	NH	576	225	11	43	4
9a	NCOPh	362	65	17	32	—
9b	NCO-2-thienyl	645	142	10	41	—
9c	$NCOCH_3$	2117	305	4	74	10
9d	$NCOCH_2NHCO_2{}^tBu$	4584	446	7	71	68
9e	$NCOCH_2NH_2$	3031	1063	12	62	—
9f	$NCO(CH_2)_3N(CH_3)_2$	19%	395	75	33	—
9g	NSO_2CH_3	40%	128	5	71	35
9h	$NSO_2(CH_2)_2CH_3$	1136	140	30	74	0
9i	NSO_2Ph	39%	88	68	62	46
10a	NCH_3	36%	180	24	42	—
10b	NCH_2Ph	212	52	61	41	—
11	$NCONHC_6H_3$	34%	307	14	36	—
24[d]	S	408	36	20	80	24[e]
25[d]	SO_2	334	311	28	83	77[e]

[a] IC_{50} (nM) or % inhibition at 1 μM.
[b] % inhibition at 3 μM.
[c] % inhibition after 1 h (25 mpk po).
[d] S configuration.
[e] % inhibition after 1 h (50 mpk po).

Table 2. 1,4-Diazepine-5-hydroxamic acids and 1,4-thiazepine-5-hydroxamic acids



Compound	X	MMP-1[a]	MMP-13[a]	TACE[a]	THP[b]	LPS (1 h)[c]	LPS (6 h)[c]
17a	NCOPh	113	4	10	93	35	0
17b	$NCOCH_2CH_3$	207	20	19	63[d]	—	—
17c	$NCOCH(CH_3)_2$	142	9	23	95	—	—
17d	$NCOCH_2OCH_3$	277	31	15	79	—	—
18	NCH_2Ph	287	7	83	45	17	0
19	$NCO_2{}^tBu$	92	5	74	75	88	43
20	NH	274	32	24	83	97	62
21a	NCH_3	238	21	23	82	32	39
21b	NEt	285	17	37	57[d]	—	—
29	S	171	11	13	87	70	41
30	SO_2	114	8	19	92	94	—

[a] IC_{50} (nM).
[b] % inhibition at 3 μM.
[c] % inhibition (50 mpk po).
[d] % inhibition at 1 μM.

clearance (3.86 L/h/kg) and volume of distribution (2.49 L/kg). The oral bioavailability was 18% and the half-life was similar and moderate in length by each mode of administration (iv = 1.43 h; ip = 2.64 h; po = 0.86 h). These results are consistent with the superior in vivo efficacy seen for this compound.

In summary, the 1,4-diazepine and 1,4-thiazepine ring systems are a useful scaffold for the synthesis of TACE and MMP inhibitors containing the butynyloxy P1′ group. Potent TACE and MMP-13 inhibitors that were selective versus MMP-1 were prepared. TACE enzyme activity translated into cellular activity in the LPS stimulated THP-1 assay. The 1,4-diazepine-5-hydroxamic acid and 1,4-thiazepine-5-hydroxamic acid scaffolds provided inhibitors with superior cellular and in vivo activity. Diazepine **20**, with a basic ring nitrogen, gave excellent in vivo activity in the mouse LPS induced

TNF-α release assay as well as oral efficacy in the CIA model of arthritis.

Acknowledgements

We thank R. Cowling and A. Sung and Wyeth DSM for technical support, and R. A. Black and K. M. Mohler for their insight and guidance during the course of this work.

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