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Benzodiazepine inhibitors of the MMPs and TACE. Part 2

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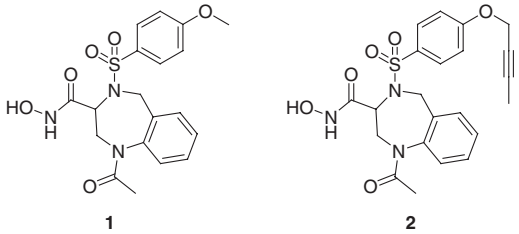
Abstract—A series of benzodiazepine MMP/TACE inhibitors bearing polar moieties has been synthesized in an effort to optimize inhibitory activity against LPS-stimulated TNF production in human monocytes and oral activity in a murine LPS model.

TNF-α converting enzyme (TACE) is the enzyme responsible for cleaving 26 kDa membrane-bound TNF to provide 17 kDa soluble TNF, a pro-inflammatory cytokine.[1] The modulation of levels of both membrane-bound and soluble TNF through the use of biologics such as TNF antibodies or soluble TNF receptors has been shown to be an effective treatment for rheumatoid arthritis (RA),[2] and TACE expression has been found to be up-regulated in the synovium of RA patients.[3] Small molecule inhibitors of TACE therefore have the potential to provide an orally active treatment for TNF-mediated diseases including RA, and this has led to a number of reports on these agents.[4] Of the reported inhibitors of TACE,[4] most have also been potent inhibitors of one or more matrix metalloproteinases (MMPs), a related family of enzymes that have been implicated in arthritis, tumor metastasis and other pathologies.[5] Recently more selective TACE inhibitors have been disclosed that take advantage of the difference in shape between the TACE S1′ pocket and that of the MMPs.[6] Since levels of several MMPs involved in cartilage degradation are over-expressed in the synovial tissue of RA patients,[7] dual MMP/TACE inhibitors may have clinical advantages over inhibitors of TACE alone, if they can avoid the dose limiting toxicities that have plagued this class of compounds.

We have previously disclosed our work on benzodiazepine-sulfonamide hydroxamic acids with potent activity against MMPs (**1**, Fig. 1).[8] In addition, the use of a butynyl ether P1′ moiety on the benzodiazepine scaffold enhanced the MMP-1 selectivity of these compounds as well as their ability to inhibit both cell-free TACE and TNF production in human monocytes (**2**, Fig. 1). We now present our efforts to optimize the cellular potency and oral activity of our initial set of benzodiazepine MMP/TACE inhibitors. Since the incorporation of basic amines into sulfonamide hydroxamate MMP/TACE inhibitors had previously been shown to be beneficial for cell and in vivo activity,[9] we have investigated the effect of appending basic amines and other polar functionality onto solvent exposed portions of the benzodiazepine scaffold.

The synthesis of the benzodiazepine analogs **3** varied at R^1 (Scheme 1) commenced with aniline-alcohol **4**,



1 **2**

Figure 1. Benzodiazepine MMP/TACE inhibitors.

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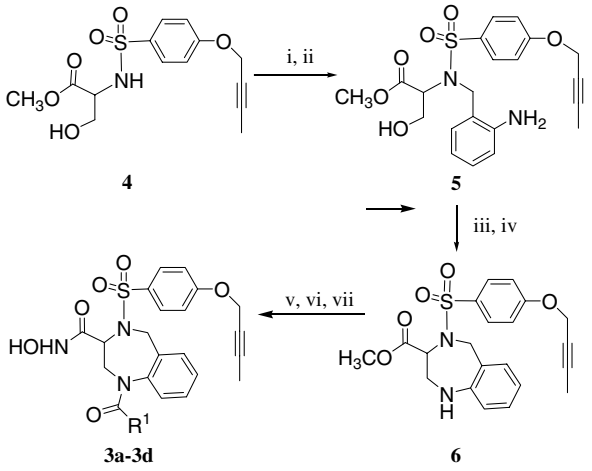
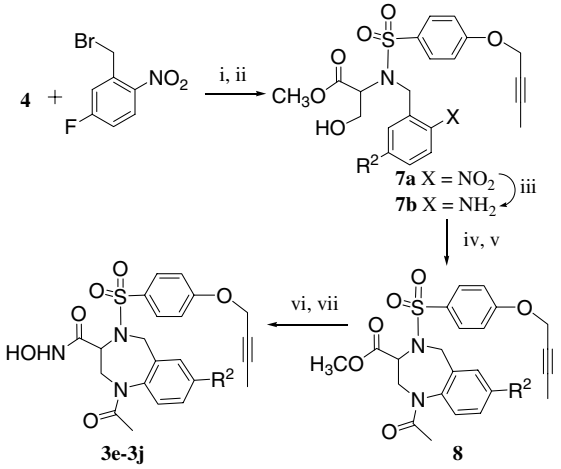


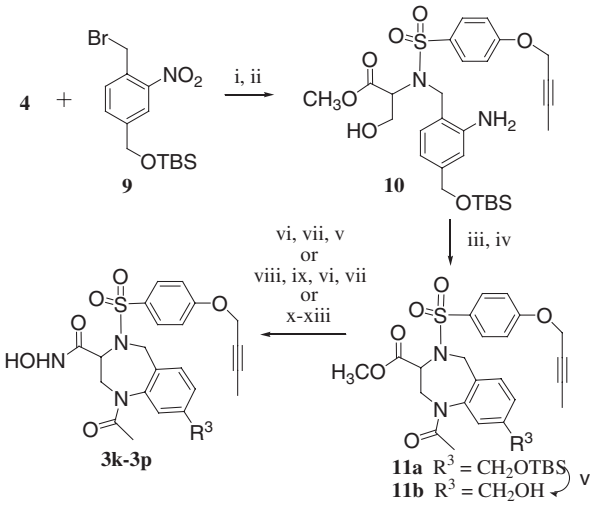
Scheme 1. Reagents and conditions: (i) 2-NO_2PhCH_2Br, Bu_4NI, NaH, DMF; (ii) $SnCl_2$, EtOH; (iii) BnOCOCl, TEA; (iv) $NaHCO_3$, MeOH, 45°; (v) R^1COCl, NaH, THF; (vi) LiOH, THF, MeOH; (vii) a. $(COCl)_2$, DMF, b. NH_2OH–HCl, TEA, DMF.

prepared in three steps from D,L-serine methyl ester.[8,10] Thus, serine methyl ester was sulfonylated with butynyloxyphenylsulfonyl chloride to give sulfonamide **4**. This sulfonamide was then alkylated with 2-nitrobenzyl bromide followed by reduction of the nitro aryl to the requisite aniline, **5**. Reaction of **5** with benzyl chloroformate in the presence of triethylamine followed by sodium bicarbonate-mediated ring closure provided NH-benzodiazepine **6** directly in 35% yield, rather than the expected benzyl carbamate. Acylation of **6**, followed by ester hydrolysis and subsequent hydroxamate formation via the acid chloride afforded analogs **3a**–**d**.

Analogs varied at R^2 were prepared according to Scheme 2. Alkylation of **4** with 2-nitro-4-fluorobenzyl bromide followed by SN_{Ar} displacement of the fluorine with the desired amine or alkoxide provided **7a**. Reduction of the nitro group with tin(II) chloride gave



Scheme 2. Reagents and conditions: (i) Bu_4NI, NaH, DMF; (ii) R^2H, $(iPr)_2NEt$, DMF or R^2H, NaH, DMF; (iii) $SnCl_2$, EtOH; (iv) AcCl/TEA; (v) $NaHCO_3$, MeOH, 45°; (vi) LiOH, THF, MeOH; (vii) a. $(COCl)_2$, DMF, b. NH_2OH–HCl, TEA, DMF.



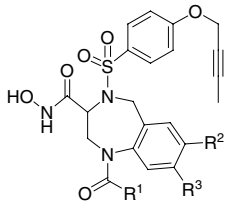
Scheme 3. Reagents and conditions: (i) NaH, DMF; (ii) Fe, NH_4Cl, EtOH, H_2O; (iii) AcCl/TEA; (iv) $NaHCO_3$, MeOH, rt; (v) HF, ACN; (vi) LiI, EtOAc, reflux; (vii) a. EDC, HOBt, DMF, b. NH_2OH, H_2O; (viii) Ms_2O, TEA; (ix) amine, Bu_4NI, THF, rt; (x) a. PCC, DMF; b. NaH_2PO_4, $NaClO_2$, *t*-BuOH, $(CH_3)_2CCHCH_3$; (xi) a. $(COCl)_2$, DMF, b. amine; (xii) LiOH, THF, MeOH; (xiii) a. $(COCl)_2$, DMF, b. NH_2OH–HCl, TEA, DMF.

aniline **7b**. Acylation of the aniline with concomitant elimination of the serine hydroxyl moiety followed by formation of the benzodiazepine ring via intramolecular Michael addition into the α,β-unsaturated ester gave ester **8**. Compound **8** was then converted into the desired hydroxamates, **3e**–**j**.

Derivatives functionalized at R^3 were synthesized as shown in Scheme 3. Thus, sulfonamide **4** was first alkylated with benzylic bromide **9**, which had been synthesized from commercial 2-nitro-4-carboxybenzyl bromide by reduction with borane and silylation of the resulting alcohol, and the aromatic nitro group was then reduced to give aniline **10**. As before, acetylation of **10** and intramolecular Michael reaction yielded benzodiazepine **11a**. Conversion of ester **11a** into the corresponding carboxylic acid was followed by hydroxamate formation and desilylation to give **3k**. Desilylation of **11a** gave alcohol **11b**, which was mesylated followed by displacement with amines to give **3l**–**n**. Secondary amines resulting from this displacement were protected as the corresponding *t*-butyl carbamates prior to installation of the hydroxamic acid moiety. Displacement of the mesylate of **11b** with sodium azide and subsequent reduction gave the primary amine that was next acylated to ultimately afford amide **3o**. Alcohol **11b** could also be oxidized to the corresponding carboxylic acid and then converted into the *N*-methylamide, followed ultimately by hydroxamate formation to provide **3p**.

The inhibitors synthesized as shown in Schemes 1–3 were evaluated in vitro against MMP-1, MMP-13, and TNF-α converting enzyme (TACE) and the data are shown in Table 1.[11] Additionally, all compounds were tested for their ability to inhibit LPS-stimulated TNF

Table 1. In vitro activity of substituted benzodiazepine hydroxamates



2, 3a-p

Compd	R^1	R^2	R^3	TACE[a]	THP[b]	MMP-1[a]	MMP-13[a]
2	CH_3	H	H	16	60	835	77
3a	CH_2OCH_3	H	H	81	73	853	97
3b	2-Furyl	H	H	33	74	125	7
3c	3-Pyridyl	H	H	89	33	238	19
3d	Pyrazine	H	H	69	41	356	20
3e	CH_3	$N(CH_3)_2$	H	197	33	682	17
3f	CH_3	$N[(CH_2)_2]_2O$	H	130	73	1255	33
3g	CH_3	$N[(CH_2)_2]_2NCH_3$	H	92	75	852	21
3h	CH_3	$N(CH_3)(CH_2)_2$-2-pyridyl	H	121	82	19% (1)	53
3i	CH_3	$NH(CH_2)_2$-2-pyridyl	H	49	44	30% (1)	47
3j	CH_3	$O(CH_2)_2Ph$	H	36	83	45% (1)	28
3k	CH_3	H	CH_2OH	12	63	500	20
3l	CH_3	H	CH_2NHCH_3	14	81	456	32
3m	CH_3	H	$CH_2N(CH_3)_2$	18	70	904	67
3n	CH_3	H	$CH_2N[(CH_2)_2]_2NH$	14	72	483	17
3o	CH_3	H	$CH_2NHCOCH_3$	31	46	578	12
3p	CH_3	H	$CONHCH_3$	41	64	813	42

[a] IC_{50}, nM or % inhibition (μM).
[b] % Inhibition at 3 μM.

production in THP-1 cells.[12] Activity in human whole blood was not assessed.

The replacement of the acetyl R^1 moiety of **2** with more polar functionality does not result in increased potency against cell-free TACE. Thus, the methoxyacetyl derivative **3a** is fivefold less potent than the acetyl analog against TACE and fivefold less selective over MMP-1. Interestingly, despite its reduced enzyme activity, **3a** gives slightly better inhibition at 3 and 1 μM (73% and 47%, respectively) than **2** in the THP-1 cellular assay. Furan derivative **3b** is similar in activity to the acetyl derivative against cell-free TACE and in cells, but it has no significant selectivity over MMP-1 and is a more potent inhibitor of MMP-13 than TACE. Pyridyl and diazine analogs, **3c** and **3d**, show the same selectivity profile as furyl derivative **3b**, but are much less potent inhibitors of TACE, particularly in LPS-stimulated THP-1 cells.

Compounds **3e**–**h** with a tertiary amine substituent at R^2 are significantly less potent in the TACE FRET assay compared to the parent analog **2**. However, these inhibitors have enhanced potency against MMP-13 relative to **2**. Removal of the N-methyl group of **3h** results in a more than twofold increase in TACE potency for secondary amine **3i**, although it is still somewhat less potent than **2**. Phenethyl ether **3j** is slightly more potent than the isosteric secondary amine **3i** versus both TACE and MMP-13, but still less potent than **2**, lacking substitution at R^2. Again, the cellular activity of **3e**–**i** did not parallel the activity of these derivatives against cell-free TACE. Thus, **3f**–**h**, which range from approximately 6-fold to 12-fold less potent than **2** against isolated TACE enzyme, are all superior to **2**, as well as secondary amine **3i**, in terms of cellular activity. Ether **3j** is also relatively potent in cells, equivalent to the tertiary amines. Since TACE is located intracellularly as well as on the cell surface,[13] correlations have been suggested between the cell permeability of a compound, as measured in Caco-2 cells, and its potency in inhibiting TNF production in cells.[14] However, measurement of Caco-2 permeability for aniline **3e** and morpholine derivative **3f**, which have comparable activity against cell-free TACE, showed that **3e** was highly permeable and had relatively poor activity in THP-1 cells, while **3f** had poor permeability but was among the most potent of the benzodiazepine series in THP-1 cells. For comparison, compound **2** is also highly permeable in the Caco-2 assay, and 10-fold more potent than **3e** and **3f** in the TACE FRET assay, but less potent than **3f** at 3 μM in the THP assay. Also, the small amount of protein present in the THP-1 cellular assay is not sufficient for protein binding to be a significant factor in these differences.

Benzodiazepines **3k**–**n**, substituted at R^3, are essentially equipotent to the parent compound **2** against TACE enzyme and significantly more potent than analogs **3e**–**j** substituted at R^2. These analogs are also highly active against MMP-13 and 30–40-fold selective for TACE over MMP-1. In contrast to the R^2 substituted analogs,

little difference is seen in TACE potency between alcohol **3k**, secondary and tertiary amines **3l** and **3m**, and the dibasic piperazine **3n**. Amide derivatives **3o** and **3p** are less potent TACE inhibitors than **2** and have a reduced level of selectivity over MMP-1 relative to **2** and the R^2 substituted analogs. In LPS-stimulated THP-1 cells compounds **3k**–**n** were all equal to or better than **2**, with N-methyl amine **3l** the most active, providing 81% inhibition at 3 μM. The amides **3o** and **3p** are also both less potent in cells than the compounds bearing a basic amine moiety at R^3.

The in vivo activity of the benzodiazepine analogs was assessed by measuring their ability to inhibit LPS-stimulated TNF production in mice.[15] In this assay compound **2** provided 71% inhibition at an oral dose of 50 mg/kg, but was not dosed down further. None of compounds **3a**–**d** afforded greater than 35% inhibition at 25 mg/kg po. Of the analogs bearing a substituent at R^2 (**3e**–**j**) the most potent on oral dosing were dimethylaniline **3e** and piperazine **3g** with 88% ($n = 1$) and 67% ($n = 2$) inhibition, respectively, at 50 mg/kg, and ether **3j**, which gave 42% inhibition at 25 mg/kg.

The pharmacokinetics of **2** and **3g** were examined after a 5 mg/kg iv dose in Balb/c mice. Piperazine **3g** had a longer half-life than **2**, 1.3 and 0.2 h, respectively, but both were rapidly cleared (6.0 vs 8.3 L/h kg). The metabolic stability of **3g** after incubation with CD-1 mouse microsomes was also evaluated and compared to **2**. Piperazine **3g** was far more stable than **2** and had only one significant metabolite, resulting from conversion of the hydroxamate into the corresponding carboxylic acid. Products resulting from cleavage of the butynyl ether were not detected for **3g**. In contrast, **2** had three major metabolites, including oxidation of the phenyl sulfonamide ring, cleavage of the butynyl ether P1′ group and, primarily, reduction of the hydroxamate to the corresponding amide.

Compounds **3l**, **3n**, and **3p**, all substituted at R^3, were each tested at 25 mg/kg po and unfortunately none gave better than 25% inhibition of LPS-stimulated TNF production.

In conclusion, we have developed efficient synthetic routes to a variety of functionalized racemic benzodiazepine-sulfonamide hydroxamic acids. Many of these analogs have been shown to be potent inhibitors of TACE and MMP-13 and some demonstrate selectivity over MMP-1. The incorporation of polar functionality into the benzodiazepine scaffold at any of three different positions was also found to provide greatly increased aqueous solubility (>60 μg/mL) for all of the compounds that were assessed (**3c**–**e**, **3i**, **3n**, and **3p**) relative to compound **2** (5 μg/mL). Furthermore, three members of this series, **2**, **3e**, and **3g**, were shown to be effective at inhibiting LPS-stimulated TNF production on oral dosing in mice at 50 mg/kg. The isolation of the active enantiomer of each of these racemates should further enhance activity and the potential utility of these compounds for the treatment of TNF-mediated inflammatory diseases.

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