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Synthesis and activity of quinolinylmethyl P1′ α-sulfone piperidine hydroxamate inhibitors of TACE

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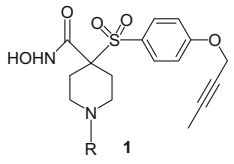
ABSTRACT

A series of α-sulfone piperidine hydroxamate TACE inhibitors **11a–n** bearing a quinolinyl methyl P1′ group was prepared, and their activity was compared to analogous α- and β-sulfone piperidine hydroxamates with a butynyloxy P1′ group. The quinolinyl methyl P1′ group affords increased inhibitory enzyme activity relative to the corresponding butynyloxy P1′ analogs in the α-sulfone piperidine hydroxamate series, and greater selectivity than the corresponding butynyloxy P1′ analogs in the β-sulfone piperidine hydroxamate series.

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that has been demonstrated to play a pivotal role in a variety of inflammatory diseases including rheumatoid arthritis (RA).[1] The clinical success of biologic anti-TNF therapies including Enbrel®,[2] Humira®, and Remicade®,[3] administered via injection or infusion to treat RA via modulation of TNF-α levels, has validated TNF-α as a target for pharmaceutical intervention. TNF-α converting enzyme (TACE) is a membrane bound zinc proteinase and is the primary enzyme responsible for the cleavage of membrane bound TNF-α to afford soluble TNF-α.[4] The potential for the development of orally active, small molecule inhibitors of TACE that modulate levels of soluble TNF-α to provide an alternative treatment for RA and other inflammatory diseases has made the design of drugs for this target the focus of intense interest.[5]

We have previously discovered that both α- and β-sulfone piperidine hydroxamate scaffolds, **1** and **2**, can provide potent TACE inhibitors, particularly when a butynyloxyphenyl P1′ moiety is attached.[6,7] Inhibitors bearing the butynyloxyl group can provide excellent cellular activity and selectivity for TACE, depending on the scaffold used.[8] The β-sulfone piperdine hydroxamates, **2**, are generally more potent than α-sulfone piperdine hydroxamates, **1**, in both the TACE enzyme and cellular assays. For example, **2** (R = Ac) is a 2 nM inhibitor of TACE enzyme, with greater than 75-fold selectivity over MMP-1, -2, -9, -13, and -14.[6a] Compound **2** also possesses reasonable cellular activity, as measured by its ability to inhibit LPS-induced TNF production in Raw cells with an IC_{50} of 490 nM. In comparison, α-sulfone hydroxamates **1** are less selective and generally have inferior cellular activity relative to the corresponding β-sulfone analogs.[6b] Thus, compound **1** (R = Ac) shows less than 5-fold selectivity over MMP-13 and has moderate TACE enzyme activity with an IC_{50} of 47 nM.[6c]



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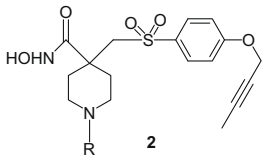

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Despite their reasonable enzyme and cellular activity, even the best α- and β-sulfone hydroxamates **1** and **2** show only moderate activity in human whole blood (HWB). In addition, this class of compounds demonstrates generally poor pharmacokinetic properties, making it difficult to achieve and sustain plasma levels above the HWB IC_{50}. Optimization of the piperidine α-sulfone hydroxamates has so far focused on varying the substituent on the piperidine nitrogen in an effort to further increase activity and selectivity, while keeping the P1′ butynyloxyl group constant.[6b] In light of the fact that the quinolinylmethyl ether P1′ moiety has been shown to provide very active and extremely selective TACE inhibitors,[9] we have explored the impact of this group on the α-sulfone hydroxamate scaffold.

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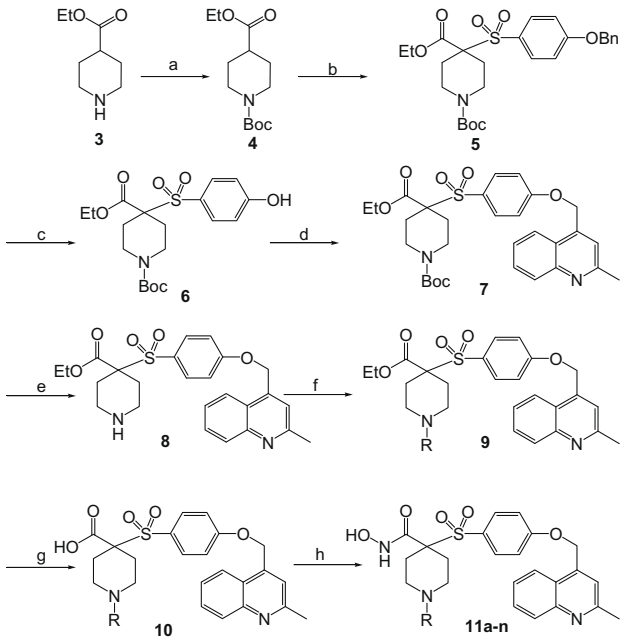


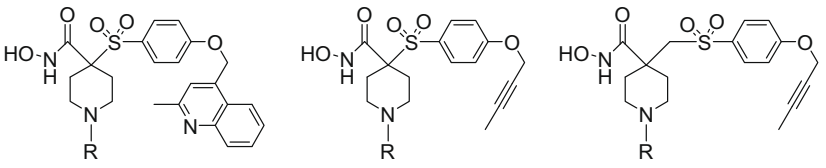
Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, THF, rt, 95%; (b)LDA/−78 °C; 4-(benzyloxy)benzene-1-sulfonylfluoride; (c) 10% Pd/C, ammonium formate, MeOH; (d) Cs_2CO_3/DMF, 4-(chloromethyl)-2-methylquinoline; (e) TFA, 93%; (f) RCOCl or RSO_2Cl or RNCO, TEA, DMAP, CH_2Cl_2, rt, 12 h, 85–95%; (g) LiOH, THF/MeOH/H_2O (3:2:2); (h) NH_2OH, HOBT, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide, DMF, 81%.

The synthetic route to the desired targets is illustrated in Scheme 1. Thus, protection of ethyl piperidine-4-carboxylate, **3**, with di-*tert*-butyl-dicarbonate affords the *N*-Boc derivative **4**. Deprotonation of **4** with lithium diisopropylamide, followed by quenching with 4-(benzyloxy)benzene-1-sulfonyl fluoride, gives the desired α-sulfonyl ester **5** in good yield (71%).[10] Cleavage of the benzyl ether of **5** under transfer hydrogenation conditions to provide phenol **6**, and subsequent alkylation with 4-(chloromethyl)-2-methylquinoline then affords quinolinylmethyl ether **7**. Removal of the *N*-Boc protecting group from the piperidine with trifluoroacetic acid then gives the secondary amine **8** and allows the introduction of a variety of functionality on the piperidine nitrogen via alkylation, acylation, and sulfonylation, affording **9**. Hydrolysis of the ester of **9** with lithium hydroxide to provide carboxylic acid **10**, followed by hydroxamate formation under peptide coupling conditions gives final products **11a–n**.

The 14 analogs bearing the quinolinyl methyl ether P1′ group, **11a–n**, were all evaluated for in vitro enzyme activity in a FRET assay using the catalytic domain of TACE, and next profiled for selectivity against MMP-2 and MMP-13 (Table 1).[11] These MMPs were chosen for profiling because in our MMP/TACE programs, historically, they have been the most challenging for achieving substantial selectivity.[6a,12]

Of all of these analogs, the least active inhibitors of TACE enzyme are the NH-piperidine **11a** and the *N*-dichlorobenzyl derivative **11l** with IC_{50}s of 33 nM and 65 nM, respectively. The amides **11b–11f**, carbamate **11g**, and ureas **11h–i** are all extremely active enzyme inhibitors with IC_{50}s of 3 nM or less, despite the wide size range of the substituents on the piperidine nitrogen of these compounds. The sulfonamides **11m** and **11n** show a greater dependence on steric bulk, with the smaller methyl sulfonamide **11m** being 10-fold more active than the bulkier isopropyl sulfonamide **11n**. Methylation of the piperidine nitrogen of **11a** produces a 3-fold improvement in enzyme activity for **11j** (IC_{50} = 11 nM). The 4-picolyl derivative **11k** is essentially equipotent to **11j**, indicating a tolerance for a basic moiety at this position.

Table 1
IC_{50}s of quinolinylmethyl α-sulfone piperidine hydroxamic acid



11a-n **1a-e** **2a-d**

Compound	R	TACE (nM)	MMP-2 (nM)	MMP-13 (nM)	Raw Cells (nM)	HWB (μM)
11a	H	33	>15,000	8624	334	2.4
1a	H	201		4019		
2a	H	1	1590	1900		
11b	CHO	2	1401	440	68	1.3
11c	Ac	1	6958	1336	385	1.5
1b	Ac	47		136		
2b	Ac	2	341	155	490	8.7
11d	COiPr	2	1241	415	>1000	14
11e	COPh	1	516	204	>1000	28
1c	COPh	73		105		
2c	COPh	1	62	59	400	2.5
11f	CO-4-Py	2	1093	349	230	1.9
11g	Boc	<1	2674	1110	>1000	>50
1d	Boc	134		284		
2d	Boc	2		135	620	14
11h	CONHEt	2	3614	835	240	2.5
11i	$CONEt_2$	3	96	57	>1000	>50
11j	Me	11	15,099	1451	125	2.4
11k	CH_2-4-Py	9	855	266	378	11
11l	CH_2Ph-3,4-Cl	65	145	80	>1000	>50
1e	CH_2Ph-3,4-Cl	149		72		
11m	SO_2Me	1.2	1743	393	345	2.8
11n	SO_2iPr	10	2140	926	>1000	14

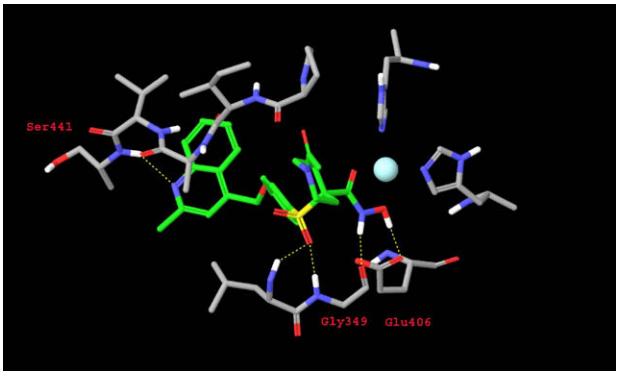


Figure 1. Compound **11c** docked to 2FVS. The zinc atom is colored in light blue.

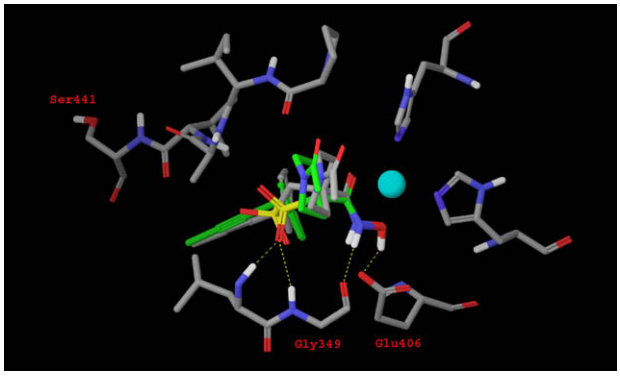


Figure 2. Compounds **1b** (green) and **2b** (gray) docked to 2i47. The zinc atom is colored in light blue.

The selectivity of the quinolinyl methyl P1′ analogs **11a–h**, **11j**, **11m**, and **11n** for TACE is greater than 90-fold over MMP-2 and MMP-13, and can exceed 1000-fold. Compounds **11a** and **11b** were also screened against MMP-9, and were found to be very selective against this enzyme with IC_{50}s of >12,000 nM. Diethyl urea **11i** is the most active of the quinoline derivatives against MMP-2 and MMP-13 with IC_{50}s of less than 100 nM against both, more than 10-fold more active against these two MMPs than the analogous ethyl urea analog **11h**. The *N*-picolyl analog **11k** is only moderately active against MMP-2 and MMP-13, but loses selectivity due to its modest TACE activity (IC_{50} = 9 nM), while the 3,4-dichlorobenzyl compound **11l** is essentially equipotent against all three enzymes. The structural basis for these differing selectivity profiles is unclear.

For five of the α-sulfone piperidine hydroxamates bearing the quinolinyl methyl P1′ group, **11a**, **11c**, **11e**, **11g**, and **11l**, a direct comparator with a butynyl P1′ group was prepared.[6c] In addition, three direct comparators, again with butynyl P1′ groups, in the β-sulfone piperidine hydroxamate series have previously been disclosed.[6] The butynyloxy P1′ derivatives in the α-sulfone piperidine hydroxamate series, **1a–e**, are each substantially less active against TACE enzyme and more active against MMP-13 than their quinolinyl methyl P1′ counterparts, with none of these analogs affording even 10-fold TACE selectivity. In contrast, all of the β-sulfone piperidine hydroxamate compounds, **2a–2d**, are equipotent to the corresponding quinolinyl analogs **11c** and **11e**, respectively. However, although the unsubstituted piperidine analog **2a** is greater than 1000-fold selective for TACE over MMP-2 and MMP-13, none of **2b–2d** has a level of selectivity approaching that provided by the quinolinyl methyl P1′ moiety.

In an effort to elucidate the binding modes of these compounds, **11c, 1b**, and **2b** were docked to TACE.[13] As there are conformational changes upon introduction of the quinolinomethyl tail[14] two different PDBs were used for developing models for the docking evaluations. The PDB **2FV5** contains a pyrrolidinone hydroxamate with the quinolinomethyl tail and was used for docking **11c** (Fig. 1). The PDB **2i47** has a β-sulfone hydroxamate with a butynyloxy P1′ group as a ligand and was therefore used for docking **1b** and **2b** (Fig. 2). While compound **2b** is more potent than **1b** in the TACE FRET assay, the reason for this increased activity is not clear from the docking studies. Replacement of the butynyloxy P1′ moiety of **1b** with a quinolinylmethyl tail to give compound **11c** provides increased van der Waals contacts, as well as a hydrogen bond to Ser141. The increased potency of **11c** is likely due to these additional interactions.

The analogs **11a–n** were also evaluated for their ability to inhibit LPS-stimulated TNF production in Raw cells and in human whole blood. The most potent compound in Raw cells is the formamide derivative **11b** with an IC_{50} of 68 nM, the only analog with a cell IC_{50} below 100 nM. Several other analogs, **11a**, **11c**, **11f**, **11h**, **11j**, **11k**, and **11m**, have sub-micromolar cell IC_{50}s. There is no correlation between enzyme IC_{50} and cell activity, as several compounds with very potent activity in the TACE enzyme assay have IC_{50}s greater than one micromolar in Raw cells. Activity in human whole blood tracks reasonably well with Raw cell activity for the quinolinyl methyl analogs, but none of the analogs tested has sub-micromolar HWB IC_{50}s. Only in the case of the *N*-acetyl analog **11c** does HWB activity improve for the quinolinyl methyl P1′ compound, relative to the corresponding β-sulfone hydroxamate, **2b**, bearing a butynyloxy P1′ group.

Finally, in vivo activity was demonstrated for compound **11f**, with a HWB IC_{50} of 1.9 μM. This α-sulfone piperidine hydroxamate provided 68% inhibition of LPS-stimulated TNF production after an oral dose of 25 mg/kg dose in a mouse model.[11]

In summary, we have prepared a series of α-sulfone piperidine hydroxamate TACE inhibitors bearing a quinolinyl methyl P1′ group. These compounds have been shown to be extremely potent inhibitors of TACE enzyme and, depending on the substituent on the piperidine nitrogen, can provide excellent selectivity over MMP-2 and MMP-13. The quinolinyl methyl P1′ group affords increased inhibitory enzyme activity relative to the corresponding butynyloxy P1′ analogs in the α-sulfone piperidine hydroxamate series, and greater selectivity than the corresponding butynyloxy P1′ analogs of the β-sulfone piperidine hydroxamate series. Although all of the compounds disclosed suffer from moderate activity in human whole blood, oral activity has been shown in a mouse model of TNF production for compound **11f**, indicating that this series may provide useful leads for potent, selective and in vivo active inhibitors of TACE.

Acknowledgments

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