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Identification of potent and selective TACE inhibitors via the S1 pocket

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Abstract—By focusing on the P1 portion of the piperidine β-sulfone ligands we identified a motif that induces selectivity and resulted in a series of TACE inhibitors that demonstrated excellent in vitro potency against isolated TACE enzyme and excellent selectivity over MMPs 1, 2, 9, 13, and 14.

TNF-α converting enzyme (TACE) is the protease responsible for the release of soluble tumor necrosis factor-α (TNF-α) from its parent membrane bound pro-TNF-α.[1–4] TNF-α is an important cytokine that mediates the immuno-inflammatory responses of other pro-inflammatory cytokines. These cytokines have been linked to the pathogenesis of rheumatoid arthritis (RA) and are involved in promoting inflammation as well as bone and cartilage destruction. That TNF-α is an ideal target for pharmaceutical intervention in the treatment of RA has been demonstrated by the success of TNF-α monoclonal antibodies (Remicade) and TNF-soluble receptors (Enbrel).[5,6] Herein we report on our progress toward potent and selective small-molecule inhibitors of TACE with the goal of inhibiting the production of soluble TNF-α.

Studies from our labs[7–10] (**C**, Fig. 1) and others[11] illustrate the difficulty of gaining selectivity for TACE over various MMPs. Historically the design of selective TACE inhibitors has focused on the S1′ pocket for increasing selectivity over the MMPs.[11,12] As a result of these efforts highly TACE-specific inhibitors have been disclosed that take advantage of protein–inhibitor interactions in this region (**A** and **B**, Fig. 1).[13–17] However, reports of selectivity-inducing inhibitor modifications in areas other than the P1′ group have been less frequent.

We have recently disclosed a 4,4′-piperidine β-sulfone hydroxamate inhibitor (**E**) that shows good selectivity for TACE over MMPs 1, 2, 9, 13, and 14 due to its sterically bulky isopropyl sulfonamide P1 group.[18] We now report the extension of that work to the design and synthesis of several additional highly selective TACE inhibitors through the systematic variation of P1 substituents at the piperidine nitrogen. It is noteworthy that neither the β-piperidine sulfone hydroxamate scaffold, nor the P1′ butyne is responsible for this selectivity as illustrated by the broad spectrum inhibitory activity of compounds **C** and **D**.[8,19] As past efforts from our labs[9,18] indicate, gaining selectivity against MMP-2 and MMP-13 is more challenging than MMPs 1, 9, and 14, and we have focused our screening paradigm on those more challenging MMPs.[20]

Initial efforts focused on the exploration of alkyl sulfonamide variants of **E** that were prepared as outlined in Scheme 1.[21] While all of the compounds in Table 1 are active against TACE, only a few followed the pattern of selectivity established by compound **E**. Of the alkyl sulfonamides, **1**–**9**, only cyclopropyl sulfonamide (**7**) proved to be >100-fold selective for TACE over MMP-2 and -13. We were surprised to discover that compound **6**, a morpholine-substituted analog of **E**,

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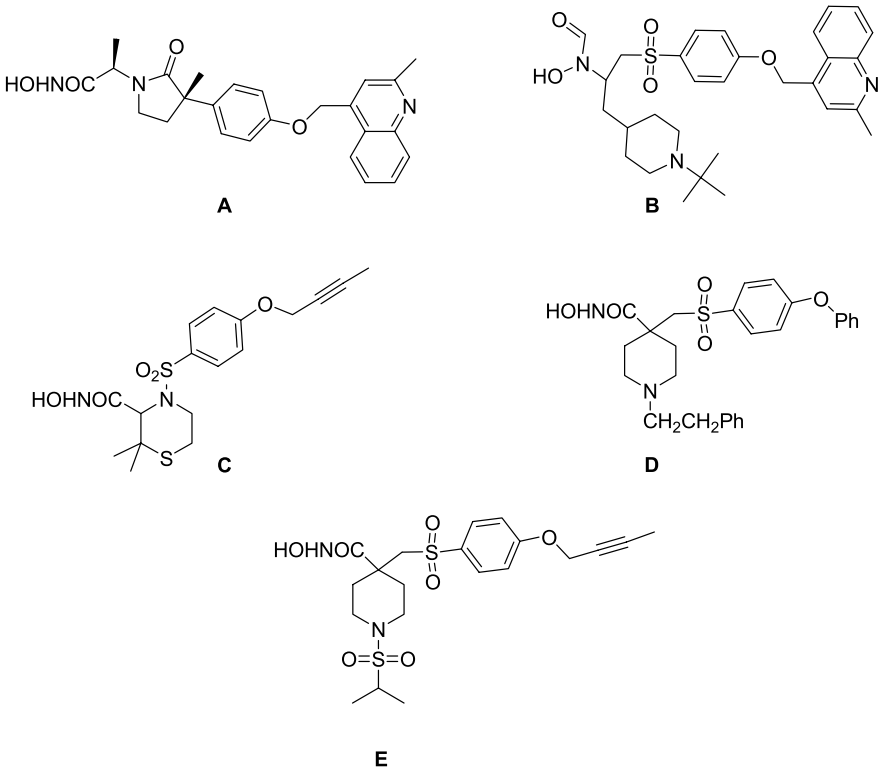
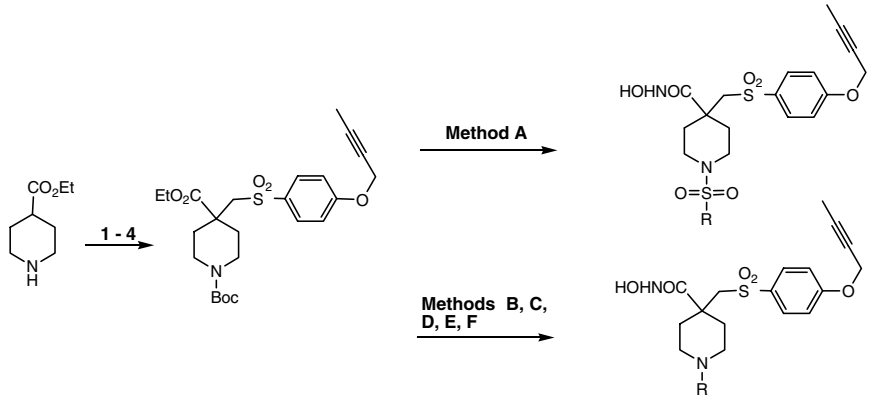


Compound	TACE[a]	MMP-1[a]	MMP-2[a]	MMP-3[a]	MMP-9[a]	MMP-13[a]
A	0.56	30000	2050	141	10340	1417
B	12	-	5000	1500	90000	4000
C	9.0	201	-	-	71	9.0
D	-	700	0.3	42.5	9.0	1.1
E	1.5	8780	355	-	1670	230

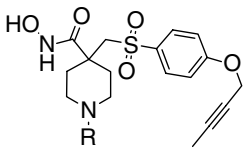
[a]IC_{50}(nM)

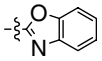
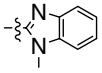
Figure 1. Recently published MMP/TACE inhibitors.



Scheme 1. Reagents and conditions: (1) di-*tert*-butyl dicarbonate, THF; (2) a—LDA, THF; b—CH_2I_2; (3) a—4-mercaptophenol, K_2CO_3, DMF; b—*m*-CPBA, CH_2Cl_2; (4) 1-bromo-2-butyne, Cs_2CO_3, DMF. Method A: (i) NaOH, THF, MeOH; (ii) BOP, *t*-Bu–ONH_2, DMF, Cs_2CO_3; (iii) TFA, CH_2Cl_2; (iv) RSO_2Cl, CH_2Cl_2, $NaHCO_3$ (satd aq); (v) TFA, 45 °C. Method B: (i) TFA, CH_2Cl_2; (ii) *tert*-butylsulfinyl chloride, $NaHCO_3$ (satd aq), CH_2Cl_2; (iii) *m*-CPBA, CH_2Cl_2; (iv) NaOH, THF, MeOH; (v) BOP, H_2NOH, DMF, Et_3N. Method C: (i) TFA, CH_2Cl_2; (ii) 2-chloro-1-ethanesulfonyl chloride, Et_3N, THF; (iii) morpholine, *n*-BuOH, 90 °C; (iv) NaOH, THF, MeOH; (v) BOP, H_2NOH, DMF, Et_3N. Method D: (i) TFA, CH_2Cl_2; (ii) 1-chloropropane-2-sulfonyl chloride, Et_3N, THF; (iii) morpholine, *n*-BuOH, 100 °C; (iv) NaOH, THF, MeOH; (v) BOP, H_2NOH, DMF, Et_3N. Method E: (i) NaOH, THF, MeOH; (ii) BOP, *t*-Bu–ONH_2, DMF, Et_3N; (iii) TFA, CH_2Cl_2; (iv) ArBr or ArCl, *i*-Pr_2EtN, DMA, microwave, 140 °C; (v) TFA, 45 °C. Method F: (i) NaOH, THF, MeOH; (ii) BOP, *t*-Bu–ONH_2, DMF, Et_3N; (iii) TFA, CH_2Cl_2; (iv) SO_2Cl_2, CH_2Cl_2, −90 °C, amine, *i*-Pr_2EtN; (v) TFA, 45 °C.

Table 1.



Compound	Method	R	TACE[a]	MMP-2[a]	MMP-13[a]
E	—	SO_2CHMe_2	1.5	355	230
1	B	SO_2–*tert*-butyl	<1	73	238
2	A	SO_2CH_2–4-pyridine	2.0	82	295
3	A	SO_2CHEt_2	1.2	58	136
4	A	SO_2CH_2–*cyc*-C_6H_{11}	<1	11	72
5	C	$SO_2CH_2CH_2$–*N*-morpholine	2.2	37	43
6	D	$SO_2CH(Me)CH_2$–*N*-morpholine	1.4	37	130
7	A	SO_2–*cyc*-C_3H_5	1.6	161	1159
8	A	SO_2–*cyc*-C_5H_9	1.3	54	155
9	A	SO_2–*cyc*-C_6H_{11}	2.8	4	24.4
10	E	2-Pyridine	1.33	62.6	205
11	E	4-Pyridine	5.6	2058	2040
12	E		1.1	40	56
13	E		<1	176	166

[a] IC_{50} (nM).

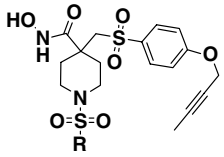
showed poor selectivity. Contrary to expectations, the MMP selectivity of the alicyclic sulfonamides **7**–**9** diminishes as the cycloalkyl ring size expands.

Selective inhibitors were also accessed through heteroaryl piperidine N-arylation (compounds **10**–**13**, Table 1). Although the 4-pyridyl derivative **11** is somewhat less active against TACE than the corresponding 2-pyridyl analog **10**, it achieves >300-fold selectivity over both MMP-2 and MMP-13. The benzoxazole **12** is not exceptionally selective over the MMPs, but the addition of a flanking methyl group in benzimidazole **13** boosts selectivity to >100-fold while retaining potency against TACE.

Heterocyclic sulfonamides were also investigated (compounds **14**–**19**, Table 2). The best of these proved to be the 3,5-dimethyl isoxazole (**14**) that demonstrated >300-fold selectivity against MMPs 2 and 13. A similarly sterically encumbered analog, the dimethyl pyrazole **15**, is at least 100-fold selective for TACE. Contrast this to compounds **18** and **19**, each having a single substituent β to the sulfonamide. Both have greater potency against MMP-2 and MMP-13 than **14**, but their exceptional potency against TACE precludes accurate assessment of their selectivity. When there are no substituents β to the sulfonamide (compounds **16** and **17**), selectivity is greatly reduced.

The X-ray structure of compound **14** bound to TACE (PDB entry 2I47[22]) illustrates how the dimethyl isoxazole ring is accommodated by TACE. Modeling[23] of **14** to an X-ray structure of MMP-13 (1ZTQ[22,24]) (Fig. 2), we observe the isoxazole ring popping out of the S1 pocket, leaving it largely solvent exposed. This is due to the somewhat larger and more hydrophobic MMP-13 Tyr 151 residue replacing Lys 315 of TACE. As such, Tyr 151 cannot readily adopt a conformation that

Table 2.



Compound[a]	R	TACE[b]	MMP-2[b]	MMP-13[b]
14		2.2	664	2277
15		<1	99.5	385
16		3.0	19	147
17		7	33	—
18		<1	22	23
19		<1	32	38

[a] Compounds prepared via Method A.
[b] IC_{50} (nM).

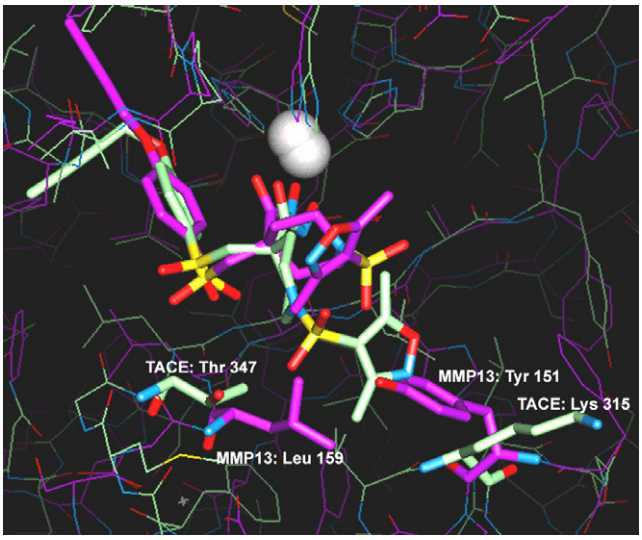


Figure 2. Superposition of the X-ray structure of compound **14** bound to TACE (carbons shown in green) and a model of compound **14** bound to MMP-13 (carbons in purple). The ligands and residue differences between TACE and MMP-13 in the S1 pocket are shown in thick stick representation; zinc is shown in a space-filling model in white.

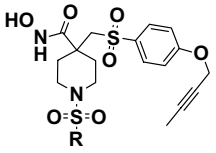
allows the isoxazole ring to effectively bind in the MMP-13 S1 pocket. Further steric clashes of compound **14** bound to MMP-13 can also be seen between an isoxazole methyl and Leu 159 (Thr 347 in TACE); this also destabilizes the **14**–MMP-13 interactions.

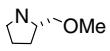
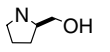
Having identified β,β′-disubstitution of the substituent borne by the P1 sulfonamide of the piperidine ring as a motif that supplied selectivity, we next prepared a series of sulfamide derivatives **20**–**26** (Table 3) according to Method F of Scheme 1. Thus, compound **20** was conceived as a direct mimetic of isopropyl sulfonamide **E** and had selectivity comparable to **E** over MMP-13, but was only 30-fold selective over MMP-2. Sulfamides **21**–**24** derived from cyclic amines were also approximately 100-fold selective over MMP-13, but were less selective over MMP-2. Compounds **25** and **26**, with the additional functionality flanking the sulfamide nitrogen, both attained the desired level of selectivity over MMP-13, and the pyrrolidinol derivative **26** was also more than 100-fold selective over MMP-2. Unfortunately, analogs of **26** prepared from 2,5-disubstituted pyrrolidines proved to be inaccessible.

Despite the fact that there was now ready access to TACE-selective piperidine sulfone hydroxamates through variations of the P1 moiety, preparing inhibitors with substantial potency in human whole blood (HWB) was a complicating issue. Of compounds **1**–**26**, only sulfamides **21** and **26** had IC_{50}s of better than 2 μM in human whole blood. Previously, we reported that the 4-picolyl derivative **F** and 4-pyridyl amide **G** had promising activity in the HWB assay,[18] with IC_{50}s of 2.7 and 1.3 μM, respectively, though they lacked selectivity over MMP-2 and -13. We therefore sought to synthesize 3,5-disubstituted pyridyl analogs of **F** and **G** in an effort to enhance their selectivity profiles while retaining HWB activity. Compounds **27** and **28** were prepared according to Methods G and H (Scheme 2), respectively (see Table 4). We were gratified to find that each of these now possessed >100-fold selectivity against MMP-2 and -13, demonstrating that the effect of the P1 group on selectivity applies to alkyl and amide linkages as well. Furthermore, we were gratified to see that compound **28** did retain its 1.3 μM IC_{50} in the HWB assay, validating this approach.

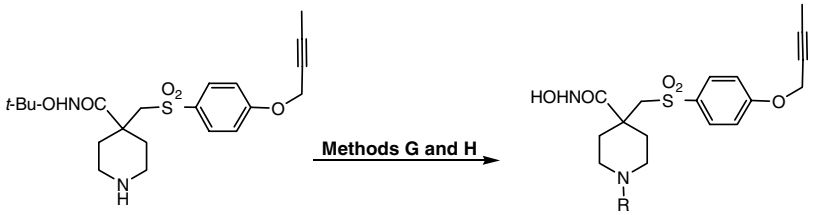
In summary, we have disclosed an approach for increasing the selectivity of 4,4′-piperidine β-sulfone hydroxamate inhibitors for TACE over MMP-2 and MMP-13 by manipulating the P1 substituent. The incorporation of steric bulk adjacent to the attachment point of the P1 group to the piperidine nitrogen, as exemplified best

Table 3.



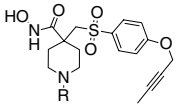
Compound[a]	R	TACE[b]	MMP-2[b]	MMP-13[b]
20	NMe_2	1.8	61.3	229
21	*N*-Morpholine	1.0	51	195
22	*N*-Piperidine	1.2	51	144
23	*N*-Pyrrolidine	1.0	31	117
24	1-(4-*N*-Acetyl)piperazine	1.5	20	104
25		1.5	58	190
26		1.4	172	354

[a] Compounds **21**–**26** prepared via Method F.
[b] IC_{50} (nM).



Scheme 2. Reagents and conditions: Method G: (i) 2,6-dichlorobenzaldehyde, $NaBH(OAc)_3$, CH_2Cl_2; (ii) TFA, 45 °C. Method H: (i) 2,6-dichlorobenzoic acid, BOP, Et_3N, DMF; (ii) TFA, 45 °C.

Table 4. Hybridization of selectivity-inducing motifs



Compound	R	TACE[a]	MMP-1[a]	MMP-2[a]	MMP-9[a]	MMP-13[a]	MMP-14[a]
F	CH_2–4-Pyridine	2.1	7,250	77	—	114	784
G	C(O)–4-Pyridine	2.6	3,010	46	191	21.5	583
14		2.2	37,300	664	5500	2277	24,000
27[b]		1.6	2,680	555	920	259	14,800
28[c]		1.7		183	—	449	

[a] IC_{50} (nM).
[b] Method G.
[c] Method H.

by analogs **14**, **15**, and **27**, provides TACE-specific inhibitors while in some cases retaining significant levels of activity in human whole blood. These inhibitors are therefore excellent leads for the development of highly potent and selective inhibitors of TACE for the treatment of RA.

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