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Acetylenic TACE Inhibitors. Part 1. SAR of the Acyclic Sulfonamide Hydroxamates

J. I. Levin,[a,*] J. M. Chen,[a] K. Cheung,[a] D. Cole,[a] C. Crago,[a] E. Delos Santos,[a] X. Du,[a] G. Khafizova,[a] G. MacEwan,[a] C. Niu,[a] E. J. Salaski,[a] A. Zask,[a] T. Cummons,[b] A. Sung,[b] J. Xu,[a] Y. Zhang,[c] W. Xu,[c] S. Ayral-Kaloustian,[a] G. Jin,[a] R. Cowling,[a] D. Barone,[d] K. M. Mohler,[d] R. A. Black[d] and J. S. Skotnicki[a]

[a]*Wyeth Research, 401 N. Middletown Rd., Pearl River, NY 10965, USA*
[b]*Wyeth Research, PO Box CN-8000, Princeton, NJ 08543, USA*
[c]*Wyeth Research, 200 Cambridge Park Drive, Cambridge, MA 02140, USA*
[d]*Immunex Corporation, Seattle, WA 98101, USA*

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Abstract—The SAR of a series of potent sulfonamide hydroxamate TACE inhibitors, all bearing a butynyloxy P1′ group, was explored. In particular, compound **5j** has excellent in vitro potency against isolated TACE enzyme and in cells, good selectivity over MMP-1 and MMP-9, and oral activity in an in vivo model of TNF-α production and a collagen-induced arthritis model.

Small molecule mediators of tumor necrosis factor-α (TNF-α) levels are sought after agents for the treatment of several inflammatory diseases including rheumatoid arthritis (RA) and Crohn's disease.[1] 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] However, Enbrel™ must be parenterally dosed and an orally bioavailable agent would therefore be highly desirable.

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 (Fig. 1).[4–7] Some of these are selective for TACE over most MMPs (e.g., **1**, IK-682[4]), others are broad-spectrum inhibitors of TACE and MMPs (e.g., **2**, SDZ 242–484[5]) and some display selectivity over MMP-1 (e.g., **3**[6] and **4**[7]). Since a variety of MMPs have been found to be over-expressed in RA synovial tissue and have been implicated in the destruction of cartilage in RA joints the optimal MMP/TACE selectivity profile for a drug to treat rheumatoid arthritis is at present unresolved.[8]

We have previously disclosed a series of anthranilic acid based sulfonamide hydroxamic acid TACE inhibitors bearing novel propargylic P1′ groups, exemplified by compound **4**.[7] A number of these propargyl ethers are excellent inhibitors of TACE in a cell-free enzyme assay and also potently inhibit the LPS-induced release of

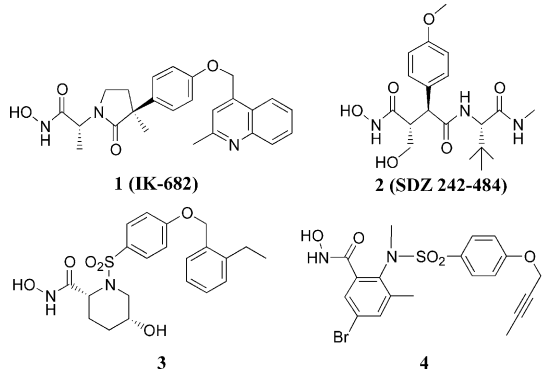

Figure 1. Literature TACE inhibitors.

*Corresponding author. Tel.: +1-845-602-3053; fax: +1-845-602-5561; e-mail: levinji@wyeth.com

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soluble TNF in THP-1 cells. Selectivity for TACE over MMP-1 and potent inhibitory activity in an in vivo model of TNF-α production has also been demonstrated. We now disclose the extension of the use of the butynyloxy P1′ group to a series of sulfonamide hydroxamates, **5**, derived from acyclic amino acids (Fig. 2).

Sulfonamide hydroxamic acids **5a–i** (Table 1) were prepared as shown in Scheme 1. Thus, 4-butynyloxybenzene sulfonyl chloride, **6**, was readily available from the reaction of 1-bromo-2-butyne with 4-hydroxybenzenesulfonic acid, followed by treatment with oxalyl chloride. Sulfonylation of various amino-esters with **6** gave sulfonamides **7** in good yield. These N-H sulfonamides could then be converted into the corresponding hydroxamates, or first *N*-alkylated to afford **8** followed by hydroxamate formation. The cysteine and penicillamine derivatives **5k–n** resulted from initial S-alkylation of the amino acids, and subsequent sulfonylation and hydroxamate formation according to Scheme 1. Derivative **5o** was available by appending a Boc protected *N*-methyl ethanolamine sidechain on Fmoc protected 4-hydroxyphenylglycine methyl ester via Mitsunobu reaction prior to Fmoc removal and sulfonylation with **6**. Compound **5p** required sulfonylation of TBS-protected 4-hydroxyphenylglycine methyl ester, followed by *N*-methylation of the sulfonamide, desilylation and functionalization of the phenol. Alternatively, N-H sulfonamides, including **5j**, could be obtained via solid phase synthesis. Coupling of Fmoc protected amino acids with a hydroxylamine-linked resin[9] followed by removal of the Fmoc, sulfonylation with **6** and cleavage of the linker with TFA gave the desired hydroxamates **5**.

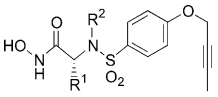

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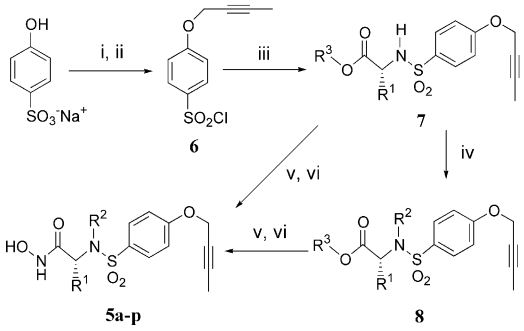
Figure 2. Acyclic α-sulfonamide hydroxamates.

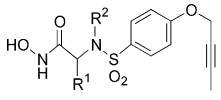
Variants of the butynyloxy P1′ group were available from glycine according to the routes described in Scheme 2. Initial formation of 4-fluorophenylsulfonamide **9a** was followed by formation of the *t*-butyl ester and alkylation of the sulfonamide to give **10a**. Displacement of the fluorine with propargylamine led to **11a**, while reaction with potassium ethyl xanthate and reduction of the resulting disulfide, followed immediately by *S*-alkylation with 1-bromo-2-butyne, led to **11b**. Conversion of **11a–b** into hydroxamates **12a–b** proceeded via the acids, with HOBT/EDC and hydroxylamine. Nitrile analogue **12c** resulted from a Heck coupling of 4-bromophenylsulfonamide **10b** with acrylonitrile and subsequent hydrogenation to give intermediate **11c**, followed by hydroxamate formation.

β-Amino acid derivatives **13a–b** were prepared by sulfonylation of β-alanine *t*-butyl ester and subsequent conversion into the desired NH- or N-methyl sulfonamide hydroxamates similarly to Scheme 1.



Scheme 1. (i) 1-Bromo-2-butyne, NaOH; (ii) $(COCl)_2$, DMF; (iii) amino-ester; (iv) R^2X, K_2CO_3 or NaH; (v) NaOH; (vi) (a) $(COCl)_2$, DMF or EDC/HOBT; (b) NH_2OH.

Table 1. In vitro potency of substituted α-sulfonamide hydroxamic acids



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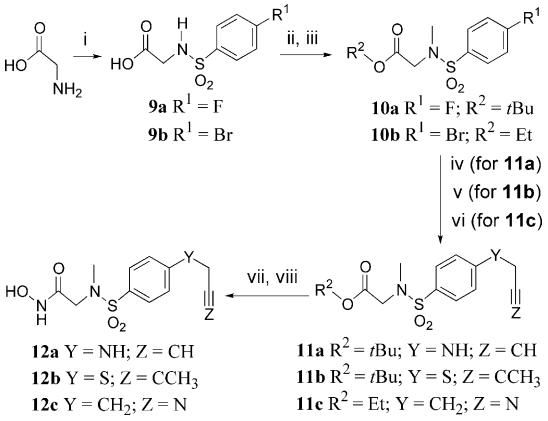
Compd	D/L	R^1	R^2	TACE[a]	THP[b]	MMP-1[a]	MMP-9[a]	MMP-13[a]
5a	D	H	H	5	15	47% (10)	753	185
5b	D	H	CH_3	7	22	1895	309	99
5c	D	H	CH_2-3-Py	7	45	1156	40	20
5d	D	CH_3	H	4	40	4031	796	195
5e	D	CH_3	CH_3	5	60	330	68	28
5f	D	$CH(CH_3)_2$	H	10	58	2965	42	18
5g	D	$CH(CH_3)_2$	CH_3	15	79	259	11	38
5h	D	$C(CH_3)_3$	H	17	78	880	224	41
5i	D	$(CH_3)_2$	H	13	9	10,000	1377	396
5j	D	$CH(CH_3)OH$	H	10	50	2471	777	86
5k	D,L	CH_2SCH_2-3-Py	H	3	56	1908	160	28
5l	D,L	CH_2SCH_2-3-Py	CH_3	6	80	61	14	5
5m	D,L	$C(CH_3)_2SCH_2$-3-Py	H	6	49	476	130	8
5n	D,L	$C(CH_3)_2SCH_2$-3-Py	CH_3	54	52	9	4	2
5o	D	Ph-4-$O(CH_2)_2NHCH_3$	H	18	48	10,000	349	146
5p	D	Ph-4-$O(CH_2)_2NHCH_3$	CH_3	26	87	927	11	27

[a]IC_{50}, nM or % inhibition (μM).
[b]% Inhibition at 1 μM.



Scheme 2. (i) 4-Br or 4-F-$PhSO_2Cl$; (ii) DMF-*t*-butyl acetal; (iii) MeI, K_2CO_3; (iv) H_2NCH_2CCH, DMSO, 80 °C; (v) (a) $C_2H_5OCS_2K$, DMSO, 100 °C; (b) PPh_3, HCl, H_2O; (c) 1-Bromo-2-butyne, NaH; (vi) (a) $CH_2=CHCN$, $(PPh_3)_2PdCl_2$, TEA; (b) Pd/C, NH_4CO_2H (vii) TFA, TES (for **11a–b**); LiOH (for **11c**) (viii) HOBT/EDC/NH_2OH.

All of the sulfonamide hydroxamic acids were tested in vitro[10] for their ability to inhibit MMP-1, MMP-9, MMP-13 and TACE.[11] The inhibition of these enzymes may help prevent cartilage degradation in RA, and therefore be therapeutically desirable.[8] However, inhibitors of TACE with various MMP inhibition profiles were also sought in order to gain insight into the possible source of musculoskeletal side effects seen in clinical trials of some broad-spectrum MMP inhibitors.[12]

The in vitro potencies for a series of acyclic sulfonamide hydroxamic acid analogues bearing butynyl P1′ groups are shown in Table 1.

All of the α-amino acids used to prepare the sulfonamide hydroxamates in Table 1 afforded potent inhibitors of TACE enzyme in a cell-free assay. The D-α-amino acids were generally greater than 50-fold more potent than the analogous L-α-amino acid derivatives (not shown). For each series the NH-sulfonamides (**5a**, **5d**, **5f**, **5h–k**, **5m**, and **5o**) are far more selective for TACE over MMP-1 than the analogous *N*-methyl sulfonamides (**5b**, **5e**, **5g**, **5l**, **5n**, and **5p**), with selectivity ratios ranging from 80- to 2000-fold. That the butynyl group of these analogues does in fact reside in the S1′-S3′ channel of TACE is confirmed by the X-ray structure of **5d** (Fig. 3). A potential polar interaction between the sulfonamide NH of **5d**, and its analogues, and the backbone carbonyl of an active-site proline residue in both MMP-1 and TACE may serve to reduce the conformational entropy of these ligands. This would adversely affect the ability of the butynyl moiety of the NH-sulfonamides to fit in the S1′ pocket of MMP-1, and effectively increase selectivity for TACE over MMP-1. The difference in selectivity between the NH and *N*-methyl sulfonamide series might then be rationalized by the observation, through NMR analysis, of multiple binding conformers of compound **5g** bound to MMP-1.[13]

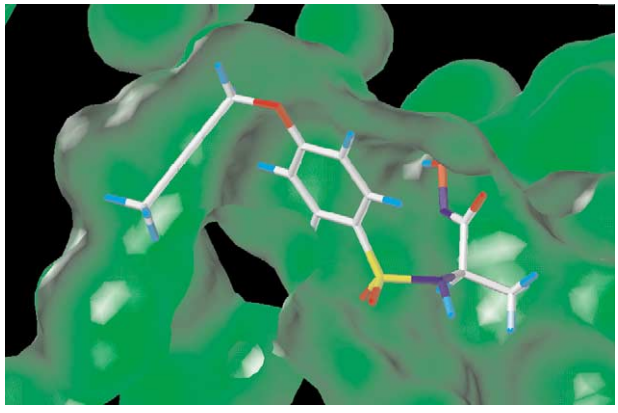


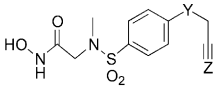
Figure 3. X-ray structure of compound **5d** bound to TACE.

In contrast to selectivity, activity in the THP-1 cellular assay[14] is improved for the *N*-methyl sulfonamides relative to the NH-derivatives, with the exception of the glycine (**5a**, **5b**) and penicillamine (**5m**, **5n**) series. Cellular potency also improves with increasing steric bulk of the alkyl amino acids in both the NH and *N*-methyl sulfonamide series, but selectivity over MMP-1 decreases as substitution on the amino acid β-carbon increases (compare **5d**, **5f**, and **5h**), presumably due in part to beneficial interactions with the S2′ pocket of the MMPs. An exception is the geminal dimethyl analogue **5i**, which has poor cell activity, although it has >100-fold selctivity for TACE over both MMP-1 and MMP-9.

In search of a boost in cellular potency several analogues were prepared with polar functionality incorporated in the inhibitor P1, or P2′ groups. Appending a picolyl group to the sulfonamide nitrogen in the glycine series (**5c**) does not affect enzyme potency, but increases cell activity relative to the corresponding NH and *N*-methyl analogues (**5a–b**). A threonine residue (**5j**) provides good selectivity over each of the MMPs tested along with moderate cellular potency. Compounds **5k–p**, with basic amines in the P1 substituent, also offered no distinct advantage over the alanine and valine analogues in terms of their potency in the THP assay.

To investigate whether the butynyloxy P1′ group, optimal for TACE enzyme and cell activity in the anthranilate sulfonamide series,[7] was preferred for the α-amino hydroxamate series the analogues in Table 2 were prepared. The propargyl amine **12a** and thioether **12b** are both less potent than butynyl ether **5b** in the enzyme and cellular assays. Surprisingly, nitrile **12c** is poorly

Table 2. In vitro potency of P1′ variants



Compd	Y	Z	TACE[a]	THP[b]	MMP-1[a]	MMP-9[a]	MMP-13[a]
5b	O	CCH_3	7	33	1895	309	99
12a	NH	CH	120	8	~10,000	1511	751
12b	S	CCH_3	38	14	4948	111	84
12c	CH_2	N	6% (1)	7	>10,000	74	207

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

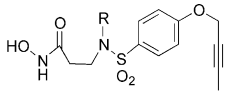
active against TACE, although whether it is the carbon linker or the nitrile that is not tolerated has not been determined.

The acyclic β-amino acid derivatives, **13a**–**b**, are not as potent against TACE enzyme as **5a**–**b**, but appear to be greater than 100-fold selective over MMP-1, -9, and-13 (Table 3). Unfortunately, both compounds are also less active in the cellular assay at 3 μM. Additional work in this series aimed at enhanced cellular potency will be reported in due course.

The in vivo activity of compounds **5d**–**5p** was initially measured by their ability to inhibit the LPS-stimulated production of TNF-α in a mouse.[14] Several of these compounds (**5d**, **5h**, and **5p**), dosed at 50 mg/kg po, provided greater than 75% inhibition of TNF-α levels one h after administration of LPS, and two (**5f** and **5j**) gave greater than 50% inhibition at 25 mg/kg po. The most potent analogue in this model was threonine derivative **5j** with an ED_{50} of 3 mg/kg po. The potential of these compounds to be effective treatments for RA was then assessed by activity in a mouse prophylactic collagen-induced arthritis (CIA) efficacy model, a standard model of human RA.[15] Compound **5j** is reproducibly active at reducing clinical severity scores in this efficacy model at 20 mg/kg po bid ($n=4$) and has shown activity at 10 mg/kg po bid ($n=2$). Furthermore, **5j** has excellent bioavailability at 10 mg/kg in the mouse (100%), rat (46%), dog (75%) and monkey (17%), and inhibits LPS-stimulated TNF-α production in human whole blood with an IC_{50} of 1 μM. Compound **5j** is stable in liver microsomes of several species, including humans. The glucuronide, observed in CD-1 mouse and cynomologous monkey, is the only metabolite seen.

In summary, we have synthesized a series of butynyloxy-based α-sulfonamide hydroxamate inhibitors of MMPs and TACE. Many of these compounds are potent inhibitors of TACE in an isolated enzyme assay and in THP-1 cells, with several possessing greater than 100-fold selectivity for TACE over MMP-1 and MMP-9. Oral activity has been demonstrated for several of these compounds in a mouse model of LPS-stimulated TNF-α production and one (**5j**) shows good oral potency in a prophylactic CIA efficacy model. The extension of this work to additional inhibitor scaffolds will be reported in due course.

Table 3. In vitro potency of β-sulfonamide hydroxamates



Compd	R	TACE[a]	THP[b]	MMP-1[a] (%)	MMP-9[a] (%)	MMP-13[a] (%)
13a	H	145	0	9 (10)	18 (10)	37 (10)
13b	CH_3	73	9	33 (10)	53 (10)	57 (10)

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

Acknowledgements

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15. Arthritis was induced in female mice, aged 6–8 weeks, by intradermal injection at the base of the tail with 100 μL (100 μg) of type II collagen emulsified in complete Freund's adjuvant (CFA) supplemented with additional 2 mg/mL of Mycobacterium tuberculosis H37 Ra. Mice were dosed with drug twice a day, while control mice received vehicle only. Dosing began on day 18 prior to the onset of arthritis and continued for 17 days. On day 21, disease was induced by injecting 40 μg of LPS intraperitoneally.