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Novel thiol-based TACE inhibitors: Rational design, synthesis, and SAR of thiol-containing aryl sulfonamides

B. Govinda Rao,* Upul K. Bandarage,* Tiansheng Wang, Jon H. Come, Emanuele Perola, Yunyi Wei, Shi-Kai Tian and Jeffrey O. Saunders

Vertex Pharmaceuticals Inc., 130 Waverly Street, Cambridge, MA 02139, USA

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Abstract—A series of potent thiol-containing aryl sulfonamide TACE inhibitors was designed and synthesized. The SAR and MMP selectivity of the series were investigated. In particular, compound **4b** has shown excellent in vitro potency against the isolated TACE enzyme and good selectivity over MMP-2, -7, -8, -9, and -13. The X-ray structure of **4b** bound to TACE was obtained.

Inhibition of TACE (TNF-α converting enzyme) is considered an attractive mechanism to control the release of TNF-α (tumor necrosis factor-α) and a viable therapy for the treatment of rheumatoid arthritis and Crohn's disease, as these illnesses are caused by overexpression of TNF-α, a pro-inflammatory cytokine.[1] The active site domain of TACE is homologous to matrix metalloproteinases (MMPs).[2] A large number of MMP inhibitors have been reported over the years and some were tested in clinical trials.[3] Not surprisingly, many of these MMP inhibitors are also found to be good inhibitors of TACE. The structural similarity between the active sites of various MMPs and TACE offered a big challenge for the design of specific inhibitors. One major approach used to overcome this challenge was to exploit the differences in the primary and secondary structures of the active site loops of MMPs and TACE that form the S1′ pocket. In particular, the narrow channel between the S1′ and S3′ pockets offers an avenue to build selectivity over other MMPs.[4,5] Another approach used successfully for designing selective MMP inhibitors,[3] but seldom applied to design specific TACE inhibitors, is to use non-hydroxamate zinc-binding groups (ZBGs) such as a thiol.[6] In this investigation, we have applied a combination of these two approaches to design specific TACE inhibitors with novel scaffolds designed using the TACE crystal structure.

Compound **1** (K_i = 10 nM) is a potent and modestly specific inhibitor of TACE.[4] It has a hydroxamate as the zinc-binding group and a sulfonamide scaffold that directs the phenyl ring into the S1′ pocket. The specificity of the inhibitor is imparted by the butynyloxy tail of the inhibitor that reaches into the S3′ pocket from the S1′ pocket via a narrow channel observed only in the crystal structure of TACE (PDB code: 1BKC[2]) (Fig. 1). We were interested in exploring non-hydroxamates as ZBGs for our initial studies because hydroxamic acids are often poorly absorbed in vivo and hence carry metabolic liabilities.[7] It is also known that inhibitors with thiol as the ZBG group (e.g., compound **2**) offer an advantage in imparting specificity in inhibition of MMPs.[6,8] Hybrids of compounds **1** and **2** led to the synthesis of compound **3**. Modeling of these compounds in the active site of TACE suggested that the two methyl substituents of compound **3** might be connected to form a ring to produce compound **4b** without losing the potency. A crystal structure of compound **4b** bound to the active site of the TACE enzyme was obtained to guide structure-based optimization of these novel thiol-based inhibitors.

The general synthesis of sulfonamide-thiols is shown in Scheme 1. Sulfonylation of various cyclic 3-hydroxy secondary amines **5** (n = 1, 2, and 3) with 4-butynyloxybenzenesulfonyl chloride[9] **6** in THF gave sulfonamides **7** in good yield. These sulfonamides **7** were then subjected to standard Mitsunobu conditions to

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* Corresponding authors. Tel.: +1 617 444 6334 (B.G.R); tel.: +1 617 444 6882; fax: +1 617 444 7827 (U.K.B); e-mail addresses: govinda_rao@vrtx.com; upul_bandarage@vrtx.com

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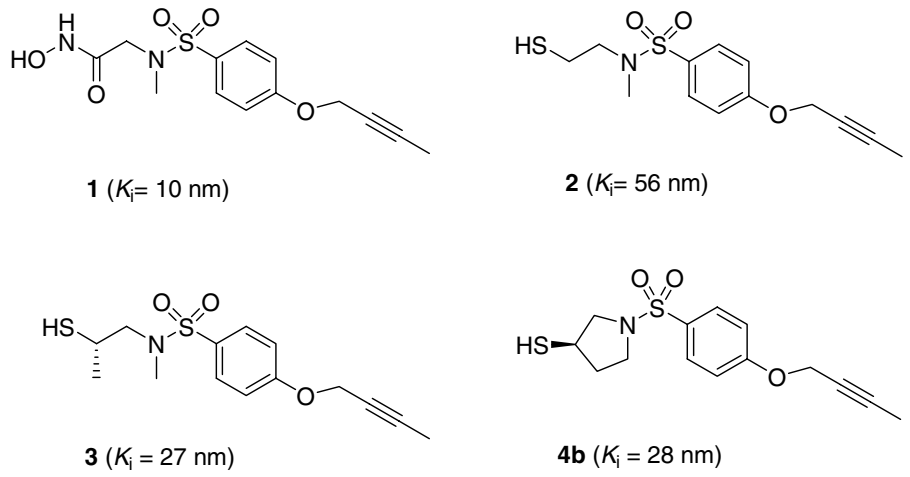
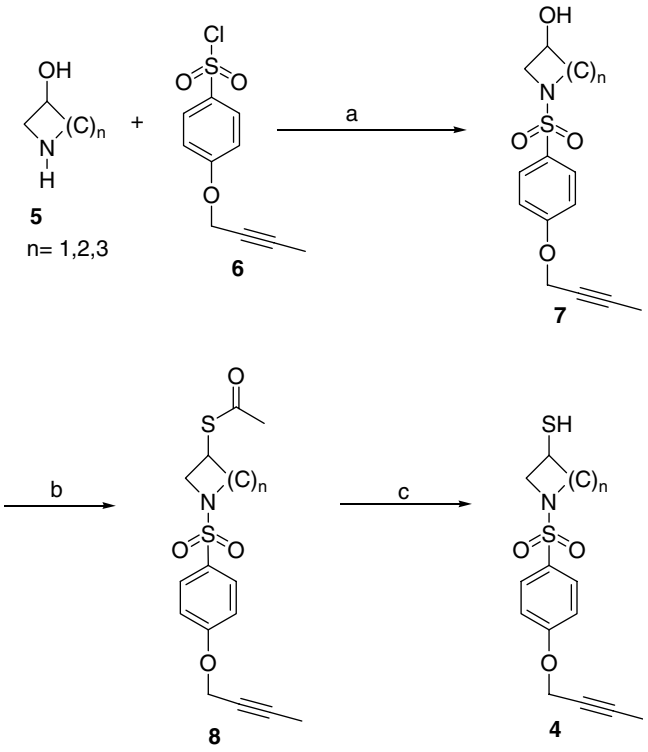


Figure 1. TACE inhibitors bearing butynyloxy group.



Scheme 1. Reagents: (a) Et_3N, THF/H_2O, 88–92%; (b) Ph_3P, DEAD, CH_3COSH, THF 76–82%; (c) i—10% $NaOCH_3$, MeOH, ii—10% HCl 95–98%.

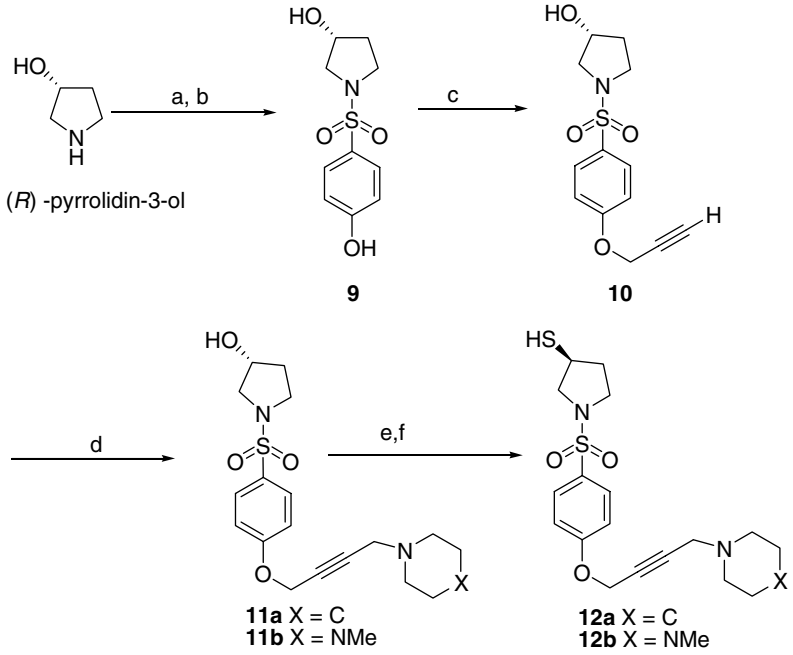
obtain thioacetates **8** in good yield. Hydrolysis of thioacetates **8** with aqueous sodium methoxide followed by acidification afforded the desired thiols **4** in high yields.

Synthesis of thiols containing solubilizing groups is shown in Scheme 2. The reaction of 4-benzyloxybenzene-1-sulfonyl chloride[10] with (*R*)-pyrrolidin-3-ol followed by debenzylation of the resulting benzyloxy intermediate gave compound **9** in high yield. Compound **9** was alkylated with propargyl bromide in the presence of K_2CO_3 in DMF at 60 °C to give **10** also in high yield. Mannich condensation of **10** with a mixture of paraformaldehyde and piperidine or *N*-methyl piperazine in the presence of $CuSO_4$ afforded **11a** or **11b** in good to moderate yield. Compound **11a** or **11b** was then converted to target thiol **12a** or **12b** as described in Scheme 1.

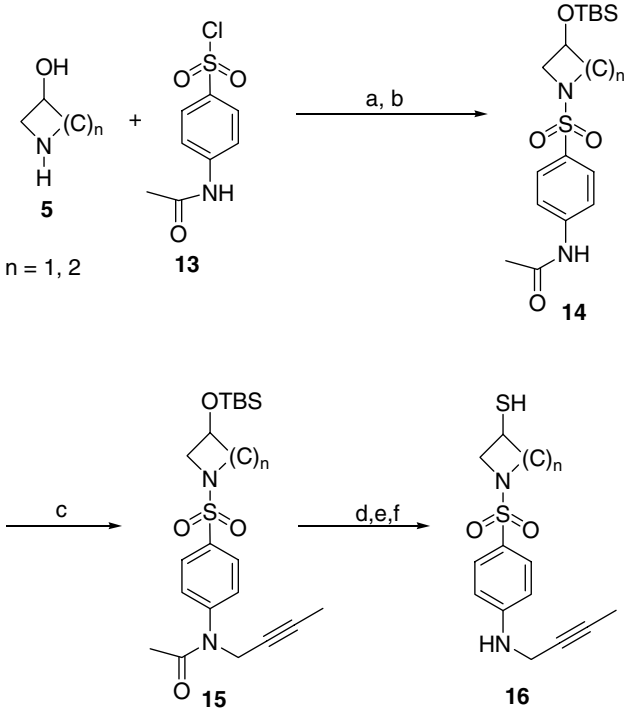
Syntheses of the butynylamine tail containing sulfonamide-thiols **16** are shown in Scheme 3. Sulfonylation of **5** with commercially available sulfonyl chloride **13** followed by protection of hydroxyl group with TBSCl afforded **14** in good yield. Alkylation of **14** with 1-bromo-2-butyne gave **15** in moderate yield. Removal of the TBS group of **15** followed by Mitsunobu reaction and subsequent hydrolysis afforded desired thiols **16**.

As shown in Table 1, the azetidine thiols **4a**, **16a** turned out to be very potent TACE inhibitors (entries 1 and 7), while solubilizing group containing thiols **12a** and **12b** were the least potent compounds in this set (entries 5 and 6). Changing the stereochemistry from *R* to *S* did not improve TACE potency in the five-membered-series (entries 2 and 3). Replacement of the ether oxygen of the butynyloxy tail with NH did not produce a significant change in TACE potency in the four-membered-series (azetidine series) (entries 1 and 7). On the other hand, similar changes in the five-membered-series (pyrrolidine series) increased the TACE potency by 5-fold (entries 2 and 8). Increasing the ring size from five to six did not improve the TACE affinity (entry 4). Introduction of basic amine-containing tails diminished the TACE potency. The compound with a piperidine-containing tail **12a** was approximately 20-fold less potent than **4b**, while the *N*-methylpiperazine-containing compound **12b** was approximately 120-fold less potent than **4b** (entries 2, 5, and 6). This observation suggests that the large cyclic basic amine moieties are not well tolerated in the S3′ pocket despite the fact these analogs were designed based on the X-ray crystal structure of **4b**.

An X-ray crystal structure has been obtained for **4b** bound to the active site of TACE (Fig. 2).[11] In this structure, the thiol group of the inhibitor makes a strong interaction with the active site Zn^{2+} ion providing the



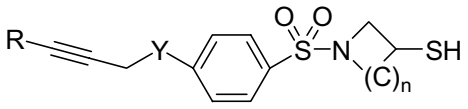
Scheme 2. Reagents and conditions: (a) 4-benzyloxybenzene-1-sulfonyl chloride, Et_3N, THF/H_2O, 91%; (b) 10% Pd/C, H_2, MeOH /EtOAC, 99%; (c) K_2CO_3, 3-bromoprop-1-yne, DMF, 60 °C, 82%; (d) HCHO, $CuSO_4$, piperidine, 85 °C, **11a** 78%, **11b** 62%; (e) Ph_3P, DEAD, CH_3COSH, THF 88% and 36%; (f) i—10% $NaOCH_3$, MeOH, ii—10% HCl, **12a** 94%, **12b** 92%.

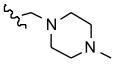


Scheme 3. Reagents: (a) Et_3N, THF/H_2O, 88–92%; (b) TBSCI, imidazole, CH_2Cl_2, 93%; (c) NAH, $CH_3CH{\equiv}CHCH_2Br$, DMF, 76–83%; (d) TBAF, THF, 100%; (e) Ph_3P, DEAD, CH_3COSH, THF, 76–88%; (f) 4 N HCl in dioxane, 90–96%.

fourth ligand of the tetrahedral coordination in addition to the three histidine side chains (His-405, His-409, and His-415). The only other observed specific interaction is the hydrogen bond between the sulfonamide oxygen of the inhibitor and the NH of Gly-349. The phenyl ring

Table 1. In vitro potency of thiol–arylsulfonamide series



Entry	Compound	*n*	Y	R	TACE K_i (nM)
1	**4a**	1	O	CH_3	13
2	**4b** (3*R*)	2	O	CH_3	28
3	**4c** (3*S*)	2	O	CH_3	33
4	**4d** (racemic)	3	O	CH_3	55
5	**12a** (3*R*)	2	O		530
6	**12b** (3*R*)	2	O		3400
7	**16a**	1	NH	CH_3	11
8	**16b** (3*R*)	2	NH	CH_3	5

stacks against the His-405 side chain and is surrounded by other hydrophobic side chains of the S1′ pocket. The butynyloxy tail bends into a narrow channel connecting the S1′ and S3′ pockets. A similar binding mode was observed recently for a different class of TACE inhibitors.[12] The crystal structure suggests that the tail can be extended with bigger groups to fill the S3′ pocket, and gain potency.

We tested a few of the potent TACE inhibitors against a set of closely related MMPs (MMP-2, -7, -8, -9, and -13) and found them to be selective (Table 2). Compounds **2**, **4b**, and **4d** are more selective than compound **1** (with hydroxamate as ZBG). To understand this selectivity, we overlaid the crystal structure of TACE:**4b** complex with the crystal structures of the five MMPs. As stated

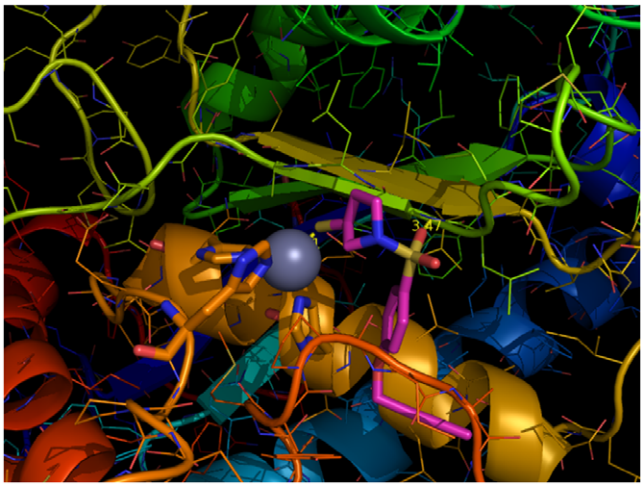


Figure 2. Crystal structure of compound **4b** bound to TACE. The Zn atom is shown as a light blue sphere. The three His side chains coordinating the Zn shown as sticks along with the inhibitor (color coded: C, purple; O, red; N, blue; and S, yellow). The thiol sulfur is at a distance of 2.2 Å from the Zn^{2+} ion.

Table 2. Selectivity profile of selected compounds

Compound	K_i (nM)					
	TACE	MMP-2	MMP-7	MMP-8	MMP-9	MMP-13
1	10	27	3400	43	800	17
2	52	5700	33000	1300	200	2000
4b	28	5000	>6000	1900	200	1200
4d	55	>3000	>3000	800	90	700

earlier, the butynyloxy group of **4b** occupies a channel-like space between the S1′ and S3′ subsites in TACE. This channel is blocked in these MMPs by a conserved Tyr (Tyr-423 in MMP-9) present in all MMPs at the beginning of the S1′ specificity loop.[3] The same residue in TACE is Ala-439. Therefore, it is not surprising that compound **4b** is less potent against these MMPs. It is very likely that the butynyloxy group swings away to occupy a different part of the S1′ subsite in MMPs as observed by NMR for a similar inhibitor with butynyloxy tail.[13] The differences in the shape and size of this part of the pocket may be responsible for reduced selectivity of **4b** against MMP-9.

In summary, we have designed and synthesized a novel series of thiol-containing aryl sulfonamides as inhibitors of TACE. Most of these compounds show very potent inhibition in an enzyme assay using the isolated TACE enzyme. One of the potent TACE compounds, **4b** possesses 200-fold selectivity over MMP-2 and MMP-7 as well as 40-fold selectivity over MMP-8 and MMP-13. Discovery of this novel class of TACE inhibitors offers a therapeutic potential for the treatment of rheumatoid arthritis and Crohn's disease.

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