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The discovery of novel tartrate-based TNF-α converting enzyme (TACE) inhibitors

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ABSTRACT

A novel series of TNF-α convertase (TACE) inhibitors which are non-hydroxamate have been discovered. These compounds are bis-amides of L-tartaric acid (tartrate) and coordinate to the active site zinc in a tridentate manner. They are selective for TACE over other MMP's. We report the first X-ray crystal structure for a tartrate-based TACE inhibitor.

Tumor necrosis factor-α (TNF-α) is one of the major immunomodulatory and proinflammatory cytokines. The overproduction of TNF-α has been implicated in many autoimmune disorders such as rheumatoid arthritis, Crohn's disease and psoriasis.[1] The successful treatment of inflammatory diseases, by the reduction of TNF-α levels, has been validated by the biologics such as Remicade®, Enbrel® and Humira®.[2] The discovery of a cost-effective, orally active small molecule drug which could modulate TNF-α levels is of high interest.

One potential approach to reduce the levels of soluble TNF-α is to block the release of TNF-α from the cell surface by the inhibition of TNF-α converting enzyme (TACE/ADAM17).[3] TACE is a membrane-bound zinc-metalloprotease which converts the 26-kD transmembrane pro-form of TNF-α to the mature 17-kD soluble form.[4] Crystal structures of inhibitors bound to TACE show that the active site of TACE shares many common features with the matrix metalloproteinases (MMP's).[5] However, one distinguishing feature of TACE is a tunnel interconnecting the S1′ and S3′ pockets into a single large cavity. It has been demonstrated[6] that TACE selectivity may be achieved by appropriately incorporating groups that bind in the narrow S1′ tunnel and large S3′ pocket. As musculoskeletal side effects have been associated with broad spectrum MMP inhibitors, there is interest in selective TACE inhibitors.[7]

While many TACE inhibitors are hydroxamate based,[8] our interests focused on the discovery of a selective non-hydroxamate TACE inhibitor. To achieve this goal we screened our proprietary mixture-based combinatorial library with the Automated Ligand Identification System (ALIS).[9] The screen identified four compounds (**1**–**4**) with moderate TACE affinities (Table 1). Compounds **1** and **2** were equipotent and showed sub-micromolar TACE inhibition. Interestingly the structures were found to be bis-amides of L-tartaric acid (tartrates).[10] To the best of our knowledge, this is the first report of a tartrate-based inhibitor of TACE. The activity is specific to the L-tartrates as the corresponding D-tartrates were found to be inactive.[11]

Herein we report the first crystal structure of a tartrate-based inhibitor **2** bound to the TACE enzyme (Fig. 1a).[12] A unique *tridentate* zinc binding mode was revealed with the tartrate scaffold and is defined by the two hydroxyl groups and the non-prime amide carbonyl interacting with the catalytic zinc atom (O-Zn distances = 2.0 Å, 2.4 Å and 2.5 Å, respectively) (Fig. 1b). The zinc atom maintains its coordination with the three imidazole nitrogens of His405, His409 and His415. Therefore, the Zn ion takes a

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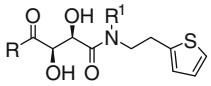
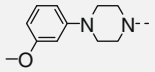
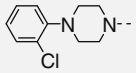
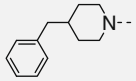
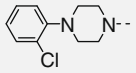
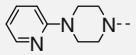
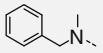
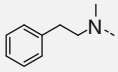
Table 1
ALIS screening hits and early SAR



Compound	R	R^1	TACE K_i[a] (nM)
1		H	400
2		H	400
3		H	1100
4		H	1400
9		H	na[b]
10		Me	na[b]
11		H	2500
12		H	1300
13		H	764
14		H	1280

[a] The compounds were tested in a FRET assay using the catalytic domain of TACE.
[b] na = not active.

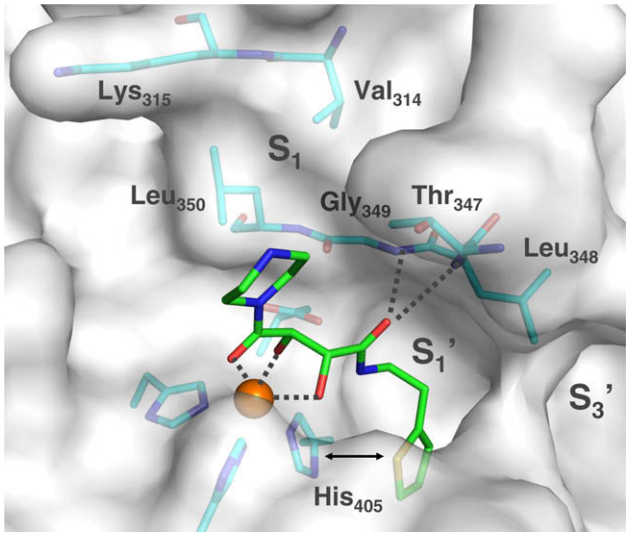


Figure 1a. X-ray structure of **2** (green carbon, stick) bound to the TACE catalytic domain (surface). The 2-chlorophenyl group was missing from the crystal structure. The S1′ and S3′ subsites are indicated. The zinc catalytic site residues and residues that form the hydrophobic region of the S1 subsite are shown (cyan carbon, stick). His405 which makes pi-stacking interactions with the thiophene is also labeled

pseudo-octahedral coordination configuration in this binding mode. The prime amide carbonyl oxygen makes hydrogen bonds with the backbone –NH of both Leu348 and Gly349 (O–N distance = 3.1 Å and 3.4 Å). The OH near the non-prime side also forms hydrogen bonds with the carboxylate oxygens of Glu406 (O–O distance = 2.8 Å and 3.2 Å). It is interesting to note that all the four oxygen atoms on the tartrate core collectively make

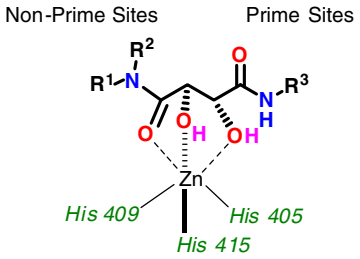


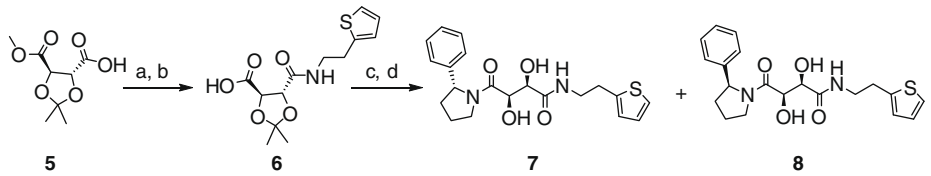
Figure 1b. Tridentate chelation of the zinc atom with the tartrate core.

important interactions with the TACE protein. The ethylthiophene group binds in the S1′ pocket which is formed by Leu401, Val434 and Val440 at the bottom and Leu348, Val402, Ala439 and His405 on the side. Besides hydrophobic interactions with the S1′ residues, the thiophene group also makes a pi-stacking interaction with His405. The 2-chlorophenylpiperazine group binds to the S1 subsite defined by Val314, Lys315, Thr347 and Leu350 and is a rather flat hydrophobic patch that is solvent exposed. Note that the chlorophenyl group was excluded from the model as it appears disordered and lacks *2fofc* electron density.

Due to its binding mode, the tartrate series provides a scaffold to explore both the non-prime and prime binding interactions. In order to explore the effect of modifications to the P1 group on the activity of the TACE inhibitors, a general procedure was used to prepare analogs (Scheme 1). Compound **5**[13] was converted to **6** in 2 steps via amide bond formation and saponification of the ester. Coupling of the racemic 2-phenylpyrrolidine to **6** followed by reverse phase HPLC separation of the diastereomers and acetonide deprotection afforded **7** and **8**.

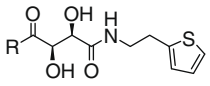
As shown in Table 1, the preference for a tertiary amide at the non-prime site was demonstrated by the comparison of compounds **9** and **13**.[14] The crystal structure does not provide an obvious explanation for this preference. The *N*-aryl piperazine was tolerant of substitution as shown with compounds **1** and **2.** Replacement of the *N*-aryl ring with 2-pyridyl of **11** resulted in a moderate loss in activity. Acyclic tertiary amides **12**–**14** were also well tolerated and equipotent to the screening hit **4**. The loss of activity observed when a tertiary amide was at the prime site demonstrated the preference for secondary amides (compound **2** vs **10**).

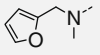
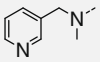
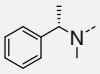
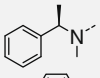
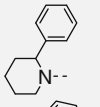
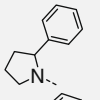
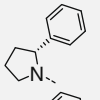
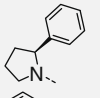
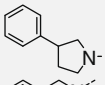
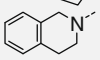
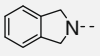
In order to further explore the role of the non-prime amide, we focused our efforts on the structure activity relationships (SAR) around compound **13**. Heteroaryl analogs **15** and **16** were equipotent to the corresponding phenyl analog **13** (Table 2). A benzylic methyl groups (**17** and **18**) moderately improved the potency relative to **13**. We speculated that we could gain additional potency by designing a compound that would project functionality back towards the surface of the protein. Our first attempt was to constrain the benzyl amide **13** into the ring system of (±)-2-phenylpiperidine amide **19** (K_i = 325 nM) which had comparable potency to compounds **17** and **18**. The (±)-2-phenylpyrrolidine amide **20** proved to be more potent (K_i = 96 nM) analog. The crystal structure of **20** (Fig. 2)[15] confirmed our hypothesis; the compound maintained similar interactions with the zinc and prime site observed in the crystal structure of **2,** and added a new face-edge interaction of the 2-phenylpyrrolidine with His415. In the crystal structure only the 2*R*-phenylpyrrolidine diastereomer of **20** was observed, suggesting that the resolved compound would be more potent. Compound **20** was resolved into diastereomers **7** and **8** as described in Scheme 1. The stereochemical assignment was confirmed by synthesis of **8** from 2*S*-phenylpyrrolidine.[16] The 2*R*-phenylpyrrolidine **7** (K_i = 20 nM), as predicted by the X-ray structure of **20**, was the more active isomer. Moving the aryl substitution to the



Scheme 1. Reagents and conditions: (a) 2-thiopheneethylamine, DIEA, HATU, DMF (64%); (b) 1.0 M LiOH, THF (53%); (c) racemic 2-phenyl-pyrrolidine, DIEA, HATU (70%); 90:10 TFA/H_2O (87%).

Table 2
Tertiary P1 amide optimization



Compound	R	TACE K_i (nM)
15		1020
16		943
17		487
18		238
19		325
20		96
7		20
8		725
21		456
22		91
23		66

3-position of pyrrolidine was not as preferred (compound **20** vs **21**). Modeling suggested that compound **21** would lose the face-edge interaction which was important in the potency of compound **20**. Exploration of fused bicyclic systems led to compounds **22** (K_i = 91 nM) and **23** (K_i = 66 nM). Similar to compound **2**, compounds **22** and **23** would bind to the S1 region and make favorable hydrophobic contacts with Val314, Lys315, Thr347 and Leu350 sidechains.

An exploration of 2-aryl and 2-heteroarylpyrrolidines to further probe the face-edge interaction with His415 was carried out. (Table 3) Replacement of the phenyl ring with a heterocycle afforded compounds **24** and **25** which were less active than the phenyl analog **20**. Compounds **26**–**28** demonstrate that meta-substitution on the phenyl ring was preferred. Modeling indicated that this may be due to the additional hydrophobic contacts of the meta-substituent with the protein surface residues such as Pro437. The electronic effect of the substituent had little effect on the potency (compounds **27**, **29**, **30** and **31**).

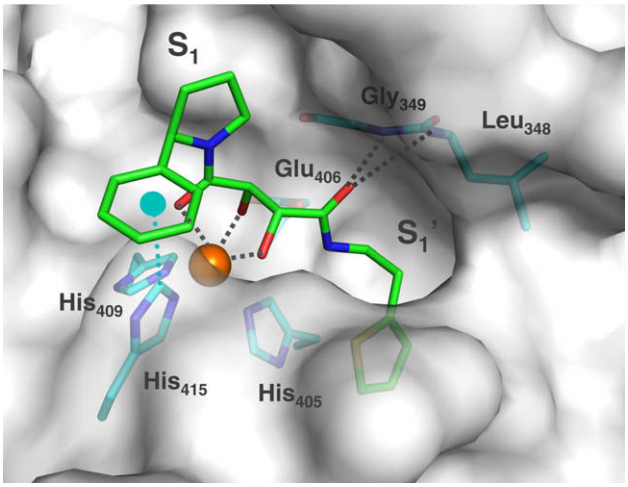
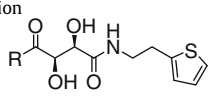
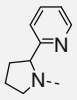
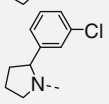
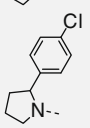
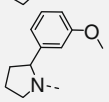
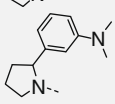


Figure 2. X-ray structure of **20** (green carbon, stick) bound to the TACE catalytic domain (surface). Residues near the zinc catalytic site are shown (cyan carbon, stick). The face-edge interactions between the phenyl ring and His415 are indicated.

Table 3
2-Aryl pyrrolidine optimization



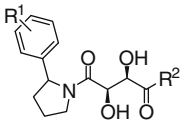
Compound	R	TACE K_i (nM)
24		188
25		164
26		323
27		11
28		755
29		13
30		33
31		41

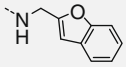
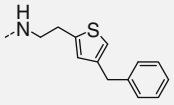
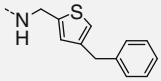
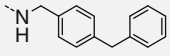
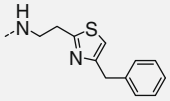
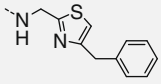
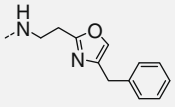
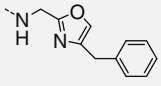
Our early efforts to explore the SAR of the secondary prime amides are shown in Table 4. Shortening of the ethylene linker of compound **27** to the methylene linker of compound **32** resulted in a loss of activity. The benzyl amide **33** was significantly less active than compound **27**. However, compound **34** (K_i = 160 nM) is of comparable activity to compound **20** (K_i = 96 nM). Extension into the S3′ pocket via the addition of a benzyl group modestly improved the potency for the ethylene linked thiophene (compound **35** vs **20**). A greater increase in potency was observed for compounds **36** (K_i = 93 nM) and **37** (K_i = 70 nM) by the addition of a P3′ substituent to the corresponding parent compounds **32** and **33**. Replacement of the thiophene with either thiazole or oxazole resulted in active compounds (compounds **35** and **36** vs **38**–**41**) and a preference for the methylene linked systems was observed.

Having identified TACE inhibitors with nanomolar affinities, we focused our investigation on the key binding elements of the tartrate series. As shown in Scheme 2, mono acid/mono ester **42** was used to prepare the mono-hydroxy analogs in 3 steps. Deletion of either hydroxyl group from the tartrate core resulted in the complete loss of TACE inhibition for compounds **43** and **44**. (Table 5) Comparison of the diamide **45** with the monothioamides **46** and **47** demonstrates the necessity of both amide carbonyls. For thioamide **46**, the lesser activity highlights the importance of zinc coordination of the non-prime amide carbonyl. We speculated that the weaker affinity of compound **46** may be attributed to the intrinsic weaker zinc binding ability of C=S versus C=O and also potential steric conflict of the sulfur atom with the imidazole ring of His415. The decrease in activity of thioamide **47** may be explained by sulfur being a poorer hydrogen-bond acceptor than oxygen. Therefore, the hydrogen bonds between the prime amide carbonyl and Leu348 and Gly349 are also key binding interactions. Replacement of the non-prime amide with amide isosteres, such as a thiazole or imidazole, afforded compounds with weaker TACE inhibition (compounds **48** and **49**) and was not advantageous. The loss of activity was attributed mainly to the lack of interactions with the S1 subsite or the face-edge interactions with His415.

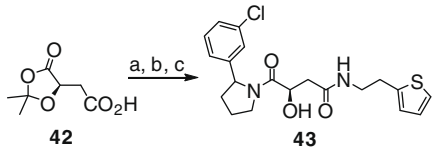
The binding mode of the tartrate series provided the opportunity to explore the effect of the prime and non-prime substituents on selectivity. Screening hit **2** had good selectivity relative to the MMP panel and was equipotent against ADAM10. (Table 6) Compound **27** showed that optimization of the non-prime substituent maintained the good MMP selectivity. In contrast to compound **27**, compound **34** is a TACE and pan-MMP inhibitor; thus suggesting the prime interactions may influence the selectivity profile more greatly than the non-prime interactions. Extension into the

Table 4
Secondary P1′ amide optimization



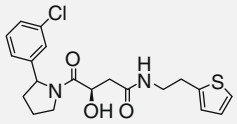
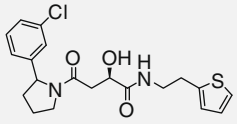
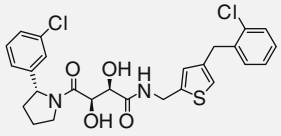
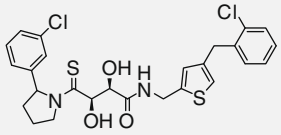
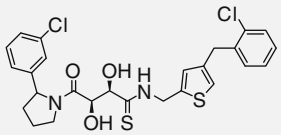
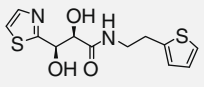
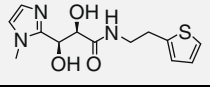
Compound	R^1	R2[a]	TACE K_i (nM)
32	3-Cl		44% @ 500 nM[b]
33	3-Cl		1440
34	H		160
35	H		66
36	4-F		93
37	3-Cl		70
38	H		48
39	H		8
40	H		461
41	H		22

[a] Synthetic procedures for these amines may be found in Ref. 10.
[b] The data is reported as % inhibition.



Scheme 2. Reagents and conditions: (a) 2-thiopheneethylamine, DIEA, HATU, DMF (100%); (b) 1.0 M HCl, THF (73%); (c) (±)-2-(3-chlorophenyl)-pyrrolidine, DIEA, HATU, DMF (49%).

Table 5
Tartrate core modifications

Compound	Structure[a]	TACE K_i (nM)
43		na[b]
44		na[b]
45		13
46		1520
47		1110
48		5530
49		10,600

[a] Synthetic procedures for these compounds may be found in Ref. 10.
[b] na = not active.

Table 6
Selectivity data

K_i (μM)	**2**	**27**	**34**	**35**
TACE	0.40	0.011	0.160	0.066
ADAM10	0.40	0.029	0.734	>25
MMP1	>90	>50	18.7	>50
MMP2	>30	33	0.3	>50
MMP3	>50	17	7.7	—
MMP7	>60	>50	>50	6
MMP12	9	>50	—	0.7
MMP13	7	20	0.51	3.6
MMP14	>40	11	0.14	>30

S3′ pocket improved the ADAM10 selectivity while maintaining the favorable MMP selectivity, as shown by compound **35**. Encouraged by the selectivity profile of the tartrate series we tested compounds **2** and **20** in the human whole blood assay (HWBA);[17] unfortunately they were inactive. Our efforts to optimize for HWBA activity will be discussed in future publications.

In summary, we have reported the discovery of the first tartrate scaffold that utilizes tridentate coordination to the active site zinc of a TACE from a diverse mixture-based library. The tartrate series is of particular interest because it has been found to be a suitable replacement for the hydroxamate based TACE inhibitors and their unique binding mode allows for access to both the prime and non-prime binding pockets. Using ligand and structure-based design our initial optimization efforts were successful in improving potency and highlighting new interactions for further refinement. This work enables the possibility of designing potent and selective small molecule tartrate TACE inhibitors and our additional efforts towards improving potency and selectivity will be discussed in future publications.

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17. Human whole blood is diluted 1:1 with serum free medium (RPMI, L-glutamine, Pen-Strep, HEPES) and incubated with a compound in a final volume of 360 μL for 1 h at 37 °C. Forty microliters of LPS (10 μg/mL) is then added. Supernatant is collected after 3.5 h incubation and the concentration of TNF-α is determined by ELISA (R&D Systems).