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Rational Design, Synthesis and Structure–Activity Relationships of a Cyclic Succinate Series of TNF-α Converting Enzyme Inhibitors. Part 1: Lead Identification

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Abstract—Rational design based on the broad spectrum MMP inhibitor CGS 27023A led to the identification of a novel series of cyclic succinate TACE inhibitors. As a mixture of two enantiomers, the lead compound **17b** exhibited potent enzyme activity ($IC_{50} = 8$ nM) in the inhibition of porcine TNF-α converting enzyme (pTACE) and excellent selectivity over aggrecanase and MMP-1, -2 and -9.

Tumor necrosis factor-α (TNF-α)[1] is a key proinflammatory cytokine that plays a pivotal role in a number of autoimmune diseases such as rheumatoid arthritis (RA).[2] The therapeutic application of anti-TNF-α has been relentlessly pursued in the past through several approaches. One of them is the neutralization of TNF-α activity by forming biologically inactive complex with the cytokine using macromolecules. Efforts in this approach have culminated in the discovery of the monoclonal TNF-α antibody infliximab[3] and the soluble TNF p75 receptor fusion protein entanercept.[4] The clinical success of these biologics in RA patients clearly demonstrates that anti-TNF-α is a valid target for the treatment of autoimmune pathologies.

TNF-α exists in two forms, the membrane-bound proform comprising 233 amino acids with a molecular mass of 26 kDa and the soluble form of 17 kDa comprising 157 nonglycosylated amino acids. It has been shown recently that the shedding of the biologically active TNF-α from its membrane-anchored proform is mediated by a metalloproteinase called TNF-α converting enzyme (TACE)[5] and inhibition of TACE blocks TNF-α release.[6] These experimental evidences imply that inhibition of TNF-α through inhibition of TACE may represent an alternative approach in anti-TNF-α therapy. As a result, TACE has emerged as a new potential therapeutic target for small molecule drug design.[7]

TACE is a member of the ADAM (a disintegrin and a metalloprotease domian) subfamily of the metzincin superfamily that also includes the astacins, serralysins, and matrix metalloproteinases (MMPs). X-ray crystal structure of human TACE revealed that TACE shows relatively low overall sequence homology to MMPs but has significant similarity in the active site.[8] This probably explains why broad-spectrum MMP inhibitors are capable of inhibiting TNF-α processing and in turn, most TACE inhibitors derived from MMP leads inhibit MMPs broadly.[7] Since broad-spectrum MMP inhibitors have been found to cause side-effects in oncology clinical trials,[9] it is desirable to develop selective TACE inhibitors for the long-term treatment of TNF-α mediated disorders.

Efforts directed towards the search for TACE inhibitors during the past have been focused on rational design based on two classes of MMP leads, the succinate-based pseudopeptides and non-peptidic sulfonamides as represented by marimastat (**1**)[10] and CGS 27023A (**2**),[11] respectively. Intensive studies have led to the identification of a number of new templates which are selective

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TACE inhibitors with moderate to potent cellular activity.[12] The discovery of a series of succinate-based macrocyclic hydroxamic acids containing biphenylmethyl residues at P1′ (e.g., **3**) in our laboratory has provided important information that TACE has an S1′ pocket different to MMPs and accordingly, selective TACE inhibitors can be achieved by elaboration at P1′.[12a] Unfortunately, those macrocycles have low oral bioavailability, probably due to high molecular weight (Fig. 1).

To identify smaller molecule TACE inhibitors, we elected to explore new templates using CGS 27023A (**2**) as our starting point for structure-based rational design. The crystal structure of CGS 27023A bound to MMP-3 revealed that the hydroxamic acid which chelates to the catalytic zinc, the pro-*R* oxygen of the sulfonyl group which is hydrogen-bonded to the NH group of Leu-164, and the 4-methoxyphenyl which binds in the S1′ pocket are three key structure features for binding.[11b,c] Modeling studies suggested that since only one of the sulfonyl oxygens in CGS 27023A is involved in hydrogen bonding, the sulfonyl could be replaced with a carbonyl. To compensate for the alteration in geometry from SP^3 hybridization for sulfonyl to SP^2 hybridization for carbonyl, the amino α to the hydroxamate in **2** needs to be replaced with an SP^3 methylene. Thus we selected a succinate as the key structural component in our design. One of the carboxylate of the succinate would be derived to a hydroxamic acid as the chelator and the other would form an amide bond with a P1′ residue

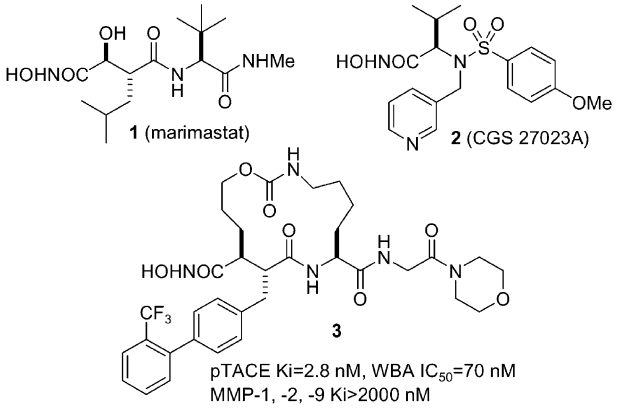


Figure 1.

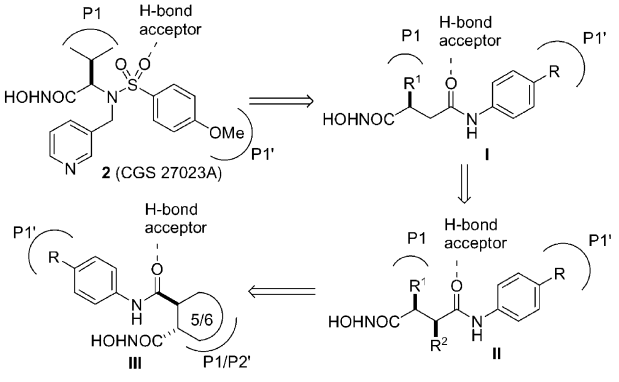
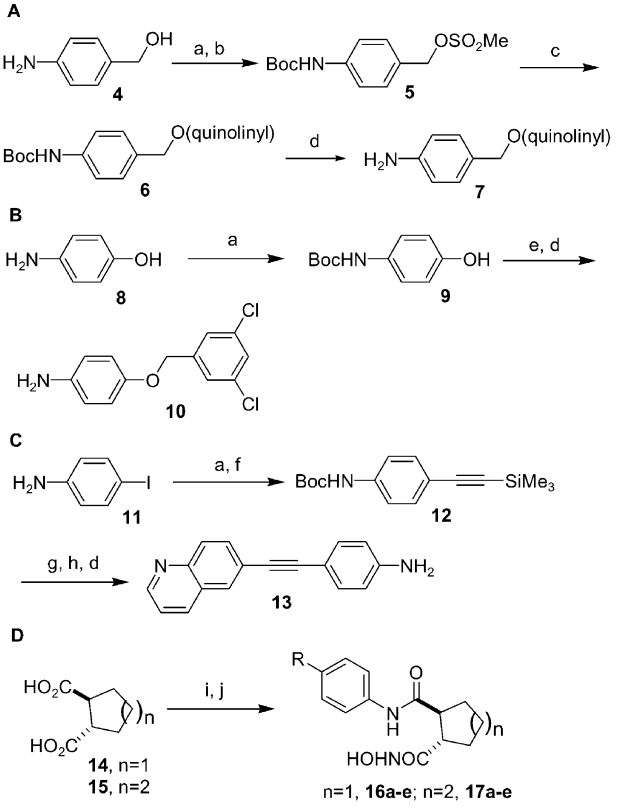


Figure 2.

such as an aniline derivative (**I** in Fig. 2). To constrain the orientation of the aniline P1′ residue into the S1′ pocket, the 3-position of the succinate could be substituted with an R^2 group (**II** in Fig. 2) and the resulting R^2 group could be cyclized with the R^1 group at the 2-position to form a five- or six-membered carbocycle or heterocycle. To maintain the three key binding interactions observed in CGS 27023A, the two carboxylates of the five- or six-membered cycle should assume a *trans* configuration (**III** in Fig. 2).

To test our design hypothesis, a series of *trans*-cyclopentanedicarboxylate and *trans*-cyclohexanedicarboxylate derivatives were synthesized as depicted in Scheme 1. The 4-(quinolinyloxymethyl)aniline residues were prepared starting from 4-aminobenzyl alcohol **4** (Scheme 1A). Boc protection followed by mesylation provided the mesylate **5**. Alkylation of **5** with 4-, 5- or 6-hydroxyquinoline using K_2CO_3 at an elevated temperature provided the ether derivative **6** which was treated with an acid to give the amine **7**. 4-(3,5-Dichlorobenzyloxy)-aniline **10** was prepared by Boc protection of 4-aminophenol **8** followed by alkylation of the resulting phenol with 3,5-dichlorobenzyl bromide and subsequent removal of the Boc group (Scheme 1B).



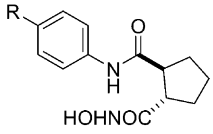
Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, THF, DIEA; (b) $MeSO_2Cl$, DIEA, CH_2Cl_2, 85%; (c) 4-, 5- or 6-hydroxyquinoline, K_2CO_3, DMF, 60–80 °C; (d) 4 N HCl in dioxane; (e) 3,5-dichlorobenzyl bromide, K_2CO_3, DMF, 60 °C, 80%; (f) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, DIEA, THF, 75%; (g) K_2CO_3, MeOH, 90%; (h) 6-trifluoromethanesulfonyloxyquinoline, $Pd(PPh_3)_4$, CuI, DIEA, THF, 65%; (i) an aniline derivative (0.5×), BOP (0.55×), DIEA, DMF; (j) hydroxylamine hydrochloride, BOP, DIEA, DMF.

The synthesis of 4-[2-(6-quinolinyl)ethynyl]aniline **13** involved two sequential palladium mediated couplings (Scheme 1C). Boc protection of **11** followed by coupling with trimethylsilylacetylene gave rise to intermediate **12**. After desilylation, coupling of the resulting acetylene with the triflate of 6-hydroxyquinoline followed by acid removal of the Boc group yielded **13**. The final hydroxamic acids **16a–e** and **17a–e** were obtained by coupling of an aniline derivative with an excess amount (2 equivalents) of the commercially available *trans*-1,2-cyclopentanedicarboxylic acid **14** or *trans*-1,2-cyclohexanedicarboxylic acid **15** using BOP (1.1 equivalent) followed by condensation of the resulting carboxylic acid with hydroxylamine hydrochloride using BOP (Scheme 1D).

Porcine TACE (pTACE) assay was used to assess the affinity for TACE and LPS-stimulated human whole blood assay (WBA) was employed to determine the inhibition of TNF-α production. MMP-1, -2, and -9 and aggrecanase were selected as the representative enzymes for counterscreen to understand the selectivity profile.[13] As shown in Table 1, with a benzyloxy at the 4-position of the aniline, the five-membered cyclic succinate derivative **16a** (racemate) displayed a submicromolar affinity for pTACE with a decent selectivity over MMP-1, -2, -9 and aggrecanase. While the selectivity of **16a** over MMP-1 was anticipated as MMP-1 possesses a shallow S1′ pocket,[12a] the selectivity over MMP-2 and-9 which have a narrow and deep S1′ pocket[12a] was a surprise to us since it is in striking contrast to what we observed with the γ-lactam series discovered in the same group, in which the small and deep benzyloxy residue at the P1′ position resulted in potent MMP-2 and -9 activity and selectivity for TACE over MMP-2 and -9 requires 3,5-disubstitution on the phenyl ring.[12g] The selectivity of **16a** over MMP-2, -9 and aggrecanase probably stems from the combination of the ring rigidity of the cyclic succinate and the optimal projection of the benzyloxy residue in the TACE S1′ pocket but not in the MMP and aggrecanase S1′ pockets. Despite the decent affinity for pTACE, **16a** showed $<50\%$ inhibition at 50 μM concentration in WBA, which is probably caused by high protein binding. To improve the polarity, the benzyl residue in **16a** was replaced with (4-pyridinyl)methyl to provide the more polar analogue **16b**, which led to a six-fold loss in pTACE affinity. Removal of the oxygen between the (4-pyridinyl)methyl and the aniline in **16b** enhanced the pTACE affinity by 3-fold but slightly reduced the selectivity over MMP-2 (**16c**). 3,5-Dichloro-substitution on the pheny ring of the benzyloxy in **16a** or replacement of the benzyloxy in **16a** with (4-quinolinyl)oxymethyl improved the pTACE potency by 2–3-fold but did not affect the cellular activity (**16d** and **16e**, $IC_{50}>50$ μM).

We next examined the six-membered cyclic succinate template. Compound **17a** exhibited a profile in activity and selectivity similar to its five-membered counterpart **16a** (Table 2). With (4-quinolinyl)oxymethyl at the 4-position of the aniline residue, compound **17b** displayed an IC_{50} value of 8 nM in the pTACE assay as a mixture of two enantiomers, which is about 5-fold better than its five-membered counterpart **16b**. In addition, **17b** exhibited an excellent TACE selectivity profile versus MMP-1, -2, and -9 and aggrecanse but remained inactive in WBA ($IC_{50}>50$ μM). Replacement of 4-quinolinyl in **17b** with 5-quinolinyl (**17c**) or 6-quinolinyl (**17d**) reduced the pTACE potency by 5- and 7-fold, respectively, and suffered from a loss in selectivity over MMP-2 and -9. With a linear

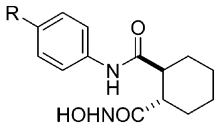
Table 1. In vitro potency of the five-membered cyclic succinates **16a–e**[a–c]



Compd	R	pTACE	WBA	MMP-1, MMP-2, MMP-9 K_i, nM
		IC_{50}, μM		
16a	Benzyloxy	0.11	>50	>4949 >3333 >2128
16b	(4-Pyridinyl)methoxy	0.62	>50	>4949 >3333 >2128
16c	(4-Pyridinyl)methyl	0.21	>50	>4949 675 >2128
16d	3,5-Dichlorobenzyloxy	0.053	>50	>4949 >3333 >2128
16e	(4-Quinolinyl)oxymethyl	0.039	>50	>4949 >3333 >2128

[a]All compounds are racemic.
[b]All compounds showed IC_{50} values of >500 nM in the inhibition of aggrecanase.
[c]pTACE, WBA, aggrecanase IC_{50} and MMP K_i values are from single determination.

Table 2. In vitro potency of the six-membered cyclic succinates **17a–e**[a–c]



Compd	R	pTACE	WBA	MMP-1, MMP-2, MMP-9 K_i, nM
		IC_{50}, μM		
17a	Benzyloxy	0.089	> 50	> 4949 > 3333 > 2128
17b	(4-Quinolinyl)oxymethyl	0.008	> 50	> 4949 > 3333 > 2128
17c	(5-Quinolinyl)oxymethyl	0.045	> 50	> 4949 585 1359
17d	(6-Quinolinyl)oxymethyl	0.056	> 50	> 4949 1079 876
17e	(6-Quinolinyl)ethynyl	7.24	> 50	> 4949 > 3333 > 2128

[a]All compounds are racemic.
[b]All compounds showed IC_{50} values of > 500 nM in the inhibition of aggrecanse.
[c]pTACE, WBA, aggrecanase IC_{50} and MMP K_i values are from single determination.

and rigid (6-quinolinyl)ethynyl at the 4-position of the aniline, compound **17e** displayed a weak affinity for pTACE (IC_{50} = 7.24 μM), in agreement with our previous observation from the homology model that TACE has a curved or boomerang-like shape S1′ pocket.[12a,14]

In summary, rational design based on the broad spectrum MMP inhibitor CGS 27023A led to the discovery of a novel series of cyclic succinate TACE inhibitors. With 4-[(4-quinolinyl)oxymethyl]anilide as a P1′ residue, the six-membered analogue **17b** displayed an IC_{50} of 8 nM for pTACE as a mixture of two enantiomers. Excellent selectivity was observed for pTACE relative to MMP-1, -2, -9 and aggrecanase, which are members of the related metalloproteinase family. Unfortunately, compound **17b** is ineffective in the inhibition of TNF-α release, with an IC_{50} value of > 50 μM in WBA. To optimize the cellular potency, modifications at the cyclohexyl ring and the quinoline moiety of this lead compound have been carried out in our laboratory, leading to the identification of a potent TACE inhibitor in the cellular assay, which will be presented in the following communication.

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