Discovery of γ-Lactam Hydroxamic Acids as Selective Inhibitors of Tumor Necrosis Factor α Converting Enzyme: Design, Synthesis, and Structure−Activity Relationships

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Figure 1. Representative MMP inhibitors (**1** and **2**) and targets considered during the discovery of γ-lactams (**3**−**5**).

Abstract: New γ-lactam TACE inhibitors were designed from known MMP inhibitors. A homology model of TACE was built and examined to identify the S1′ site as the key area for TACE selectivity over MMPs. Rational exploration of the P1′−S1′ interactions resulted in the discovery of the 3,5-disubstituted benzyl ether as a TACE-selective P1′ group. Further optimization led to the discovery of IK682 as a selective and orally bioavailable TACE inhibitor.

Introduction. Rheumatoid arthritis (RA) is an inflammatory autoimmune disease affecting more than 2 million people in the United States alone. It is traditionally treated with nonsteroidal antiinflammatory drugs (NSAID) or disease-modifying antirheumatoid drugs (DMARD). Owing to limited effectiveness and/or side effects of the two drug classes, there has been a continued search for improved therapeutics. One attractive target is the cytokine tumor necrosis factor α (TNFα),[1] which has been shown to be overproduced in the joint of RA patients. The clinical success of anti-TNFα biologics[2] has validated TNFα as a drug discovery target. While they are effective, these proteins are expensive to make and must be administered parenterally.

An alternative approach would be inhibiting formation of TNFα. TNFα is processed from its membrane-bound precursor by the metalloprotease TNFα converting enzyme (TACE).[3] TACE is a member of the reprolysin family of the metzincin superfamily that also includes MMPs (matrix metalloproteinases). Some inhibitors of MMPs have also been shown to block TACE, suggesting some similarities between the active sites of these enzymes. We[4] and others[5] have successfully drawn from the knowledge base of MMP inhibitor design to derive inhibitors of TACE. This paper will disclose the discovery of a new series of selective TACE inhibitors using a γ-lactam scaffold.[6]

Inhibitor Design. When our program was initiated, the tertiary structure of TACE was unknown and all known TACE inhibitors also inhibited MMPs. MMP inhibitors were therefore used to aid the initial design of TACE inhibitors. Succinate-derived hydroxamic acids
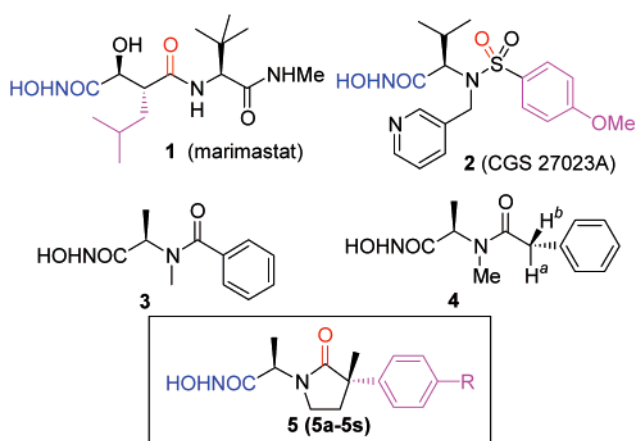
are a class of substrate-based inhibitors, represented by marimastat (**1**, Figure 1)[7] and have been investigated extensively in the MMP field. A new series of nonpeptidic inhibitors was subsequently reported by scientists at Novartis, typified by CGS 27023A (**2**).[8] The hydroxamate group of **2** binds to the catalytic zinc of MMP-3 in a fashion similar to that of the succinate-based inhibitors.[8b,c] Interestingly, only one oxygen (pro-*R*) of the SO₂ group is involved in H-bonding with MMP-3 (Leu164), which in turn projects the 4-methoxyphenyl group into the S1′ site. The picolyl group of **2** is projected toward solvent and does not appear to be critical for binding. Comparison of the binding mode of **1** and **2** revealed a zinc ligand (blue), a hydrogen-bond acceptor (red), and a P1′ group (pink) as three common features, which were used as key focal points in the design of a new scaffold.

Because only the pro-*R* oxygen of the sulfonyl group in **2** was involved in binding, we first attempted to dock benzamide **3** into MMP-3. However, it is impossible to maintain the three key interactions of **3** with the protein because the carbon is not only smaller than the sulfur but also has a planar rather than a tetrahedral geometry. To compensate for the planar and smaller carboxamide group, we inserted a methylene between the carbonyl and phenyl to create phenylacetamide **4**. It was gratifying to find that when modeled in MMP-3, **4** can be arranged in a conformation with the hydroxamate, acetamide oxygen, and phenyl group overlapping the corresponding groups of **2**, keeping the three key interactions we set out to achieve. In this model, the phenyl group is orientated perpendicular to the acetamide plane, placing the pro-*S* hydrogen of the benzylic methylene (H^a) and the methyl group of the tertiary amide in proximity. We envisioned that replacing H^a with a methylene and cyclization with the methyl group would enforce the conformation required for binding. γ-Lactam **5** was designed and shown to accomplish this goal. A methyl group was introduced at the α-position of the lactam to enhance the population of conformers with the phenyl group in a pseudoaxial orientation. A computer model of a γ-lactam analogue (**5a**, R = H) in MMP-3 revealed that the γ-lactam template could effectively project the hydroxamate, lactam carbonyl,

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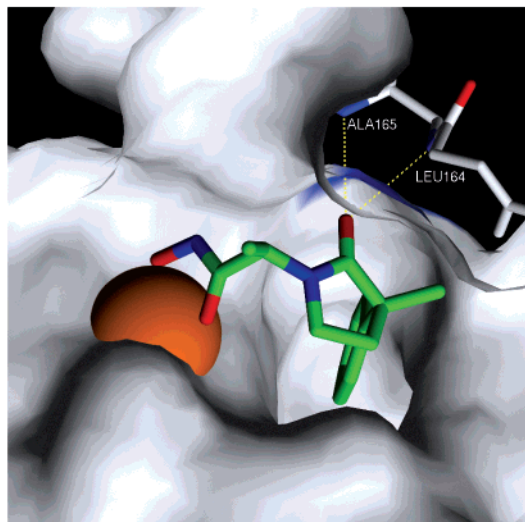§ Department of Metabolism and Pharmacokinetics.

Figure 2. Computer model of **5a** in MMP-3. The surface representation of MMP-3 (white except for nitrogen atoms of L164 and A165, blue) is truncated to expose backbone atoms within hydrogen bonding distance of the lactam's carbonyl. Oxygen atoms of the hydroxymate coordinate the zinc ion (orange), and the phenyl ring projects into the S1′ pocket.

and phenyl group to maintain the three previously mentioned binding interactions with active site of MMP-3 (Figure 2).

In clinical trials, broad-spectrum MMP inhibitors have been shown to cause tendonitis,[9] which may be attributed to nonspecific inhibition of MMPs required for normal physiological matrix turnover. Therefore, it would be desirable to discover TACE inhibitors having no effect on MMP activity. At the start of this work, all of the TACE inhibitors reported in the literature and from our in-house collection were found to be more potent against MMPs. As a starting point for TACE selectivity, a homology model of TACE was built on the basis of the structure of atrolysin, a related member of the reprolysin family. An overlay of the TACE model and MMP-3 crystal structure was examined to identify chemical opportunities for imparting TACE selectivity into lactam **5**. A striking difference was observed in S1′. MMP-3 is known to have a straight and deep S1′ pocket, while the TACE model predicts an L-shaped S1′ pocket. One basis for the difference is a tyrosine (Tyr223), which in MMP-3 forms one side of the S1′ pocket and is conserved across the MMP family. In our TACE model, this residue is replaced by the much smaller alanine. These observations suggested that selectivity could be achieved by suitable modification of P1′ such that the designated P1′ group should be able to adopt a bent conformation.

Synthesis. The synthesis of a γ-lactam analogue IK682 (**5s**) is depicted in Scheme 1. Phenylacetate **6** was reacted sequentially with iodomethane and allyl bromide under basic conditions. Ozonolysis of the crude material provided aldehyde **7** in 76% overall yield. Treatment of **7** with D-alanine methyl ester and Zn dust in HOAc at reflux facilitated reductive amination and cyclization in one pot. The two lactam diastereomers, epimeric at the quaternary center, were separated by chromatography, and the desired isomer **8** was debenzylated to give **9**. The structure of **9** was confirmed by single-crystal X-ray analysis. Phenol **9** was reacted with

Scheme 1. Synthesis of Lactam Analogue IK682[a]

IK682 (5s)
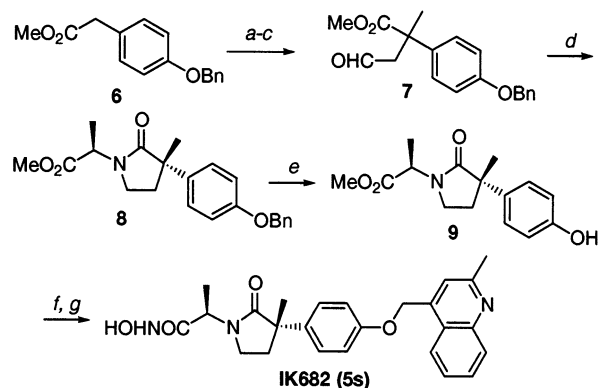
[a] Conditions: (a) NaHMDS, MeI, THF; (b) NaHMDS, allyl bromide, THF; (c) O₃, CH₂Cl₂, PPh₃ (76% for three steps); (d) D-alanine methyl ester, Zn, HOAc, at reflux (80% yield for both isomers, ratio 1:1); (e) H₂, Pd(OH)₂/C, MeOH (quantitative); (f) Cs₂CO₃, 4-chloromethyl-2-methylquinoline hydrochloride, NaI, DMSO (91%); (g) NH₂OH, KOH, MeOH (90%).

Table 1. In Vitro Potency of Lactams **5a**−**n**[a]

	R	pTACE, IC$_{50}$, nM	MMP (K_i, nM)		
			-1	-2	-9
5a	H	2200	221	108	242
5b	isobutyl	1000	4000	701	586
5c	methoxy	887	92	4.8	9
5d	*tert*-butoxy	>10000	b	b	b
5e	allyloxy	97	335	14	11
5f	benzyloxy	4	>5000	19	29
5g	phenyl	13000	2288	82	35
5h	phenoxy	185	143	<2.8	<2.1
5i	2-nitrobenzyloxy	4	>5000	35	66
5j	3-nitrobenzyloxy	6	>5000	38	39
5k	4-nitrobenzyloxy	6	>5000	11	40
5l	3,5-di-Me-benzyloxy	4	4747	5812	1634
5m	3,5-di-MeO-benzyloxy	7	>5000	>3000	>2000
5n	3,5-bis-CF₃-benzyloxy	2	>5000	>3000	>2000

[a] pTACE IC$_{50}$ and MMP K_i values are from a single determination. [b] Not tested.

4-chloromethyl-2-methylquinoline in the presence of Cs₂CO₃ and converted to IK682 using NH₂OH and KOH. Other lactam analogues were synthesized from either appropriately substituted phenylacetate in place of **6** or phenol intermediate **9**.

Results and Discussion. Lactam **5a** was found to have a submicromolar affinity for MMP-1, -2, and -9[10] (Table 1), validating the lactam model. In the porcine TACE (pTACE) assay,[11] **5a** is about 10-fold less potent. Docking and examination of **5a** in the TACE model suggested that the phenyl group of **5a** occupies the upper section of the S1′ pocket. From this projection, we anticipated that the TACE potency would be enhanced by substitution in the para position of the phenyl group. The isobutyl and methoxy analogues (**5b**,**c**) yielded only 2-fold improvement in affinity for pTACE. However, the methoxy compound **5c** increased MMP-2 and -9 potency by more than 20-fold over **5a**, indicating a divergent SAR for the two enzyme classes. A bulky *tert*-butoxy group was not tolerated, as evidenced by the loss of activity for pTACE (**5d**). The first encouraging result was obtained with an allyloxy group (**5e**), which boosted pTACE potency to 97 nM (>20-fold over **5a**). More dramatically, the benzyloxy substitution (**5f**) resulted in a 4 nM inhibitor, a 20-fold enhancement compared to **5e**, while MMP-2 and -9 activity remained
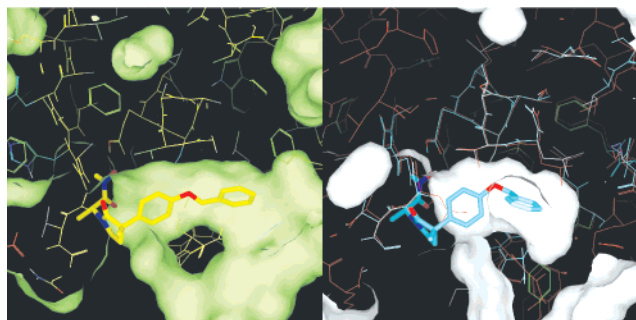
Figure 3. Model of **5f** in MMP-3 (left) and the TACE homology model (right). Nitrogen atoms are blue, oxygens red, and carbon atoms yellow and light-blue in the MMP-3 and TACE models, respectively.

essentially unchanged. The decreased affinity for MMP-1 was anticipated because the 4-(benzyloxy)phenyl group is too long to fit into the now well-established shallow S1′ pocket of MMP-1.[12] Docking **5f** in the TACE and MMP-3 active sites suggested that the flexible 4-(benzyloxy)phenyl group can adopt a bend predicted to be necessary for binding in the curved S1′ pocket of TACE, as well as a more linear staggered conformation when binding to MMPs (Figure 3). To further validate the models, phenyl and phenoxy analogues were synthesized. As shown in Table 1, as the linearity and rigidity of P1′ increased from phenoxyphenyl (**5h**) to biphenyl (**5g**), a more detrimental effect on pTACE potency was observed (40-fold and 3000-fold loss of potency, respectively, compared to **5f**). MMP-2 and -9 potency remained essentially unchanged for **5g** but increased considerably for **5h**, consistent with a deep but more linear S1′ pocket of these proteins.

Attempts to further improve selectivity for TACE by monosubstitution of the benzyl group of **5f** proved to be unsuccessful. For example, **5i**−**k** gave selectivity profiles almost identical to that of **5f**. The effect of a second substitution, specifically the 3,5-disubstitution, was dramatic. Dimethyl compound **5l** maintained pTACE potency and, more importantly, exhibited exquisite selectivity over MMP-1, -2, and -9 (1100-, 1400-, and 400-fold, respectively). The selectivity appears largely due to steric interactions because both electron-donating (OMe) and electron-withdrawing (CF$_3$) groups yielded inhibitors with comparable selectivity (**5m** and **5n**). The sum of these data suggested that the S1′ pocket of MMP-2 and -9 is narrower, while TACE appears to have a more spacious S1′.

Despite their potency for pTACE, **5f** and **5i**−**n** were determined to be weak inhibitors of TNFα production in the LPS-stimulated human whole blood assay (WBA; IC$_{50}$ > 10 μM).[11] It has been proposed that most of pro-TNFα processing occurs intracellularly,[13] indicating that compounds that lack cell membrane penetration are likely to have weak activity in the WBA. Protein binding could also adversely affect the potency in WBA. In our Caco-2 assay, **5f** had an excellent P_{app} value (49 × 10^{-6} cm/s), indicating that cell membrane penetration may not be an issue for the series. However, the series was found to be highly protein-bound (0.84% free for **5l**). To increase the free fraction, several polar bioisosteres of the benzyl group were introduced. The 4-picolyl ether **5o** increased the free fraction to 32%, and most significantly, the WBA potency improved to 5.9 μM (8-fold)

Table 2. In Vitro Potency of **5o**−**s** in pTACE and WBA[a]

	R	pTACE, IC$_{50}$, nM	WBA,[b] IC$_{50}$, nM
5o	(pyridin-4-yl)methoxy	21	5.9
5p	(2,6-di-Me-pyridin-4-yl)methoxy	16	8.1
5q	(2,6-di-Me-pyridin-4-yl)methoxy	3	6.6
5r	(quinolin-4-yl)methoxy	1.8	2.25
5s	(2-Me-quinolin-4-yl)methoxy	1[c]	0.35

[a] See footnotes a and b in Table 1. [b] Inhibition of TNFα release in WBA was determined with three donors. [c] Detection limit of the assay.

Table 3. Selectivity Profile of IK682

enzyme	K_i,[a] nM
pTACE[b]	0.56
MMP-1 (collagenase-1)	30000
MMP-2 (gelatinase A)	2050
MMP-3 (stremolysin-1)	141
MMP-7 (matrilysin)	259
MMP-8 (collagenase-2)	257
MMP-9 (gelatinase B)	10340
MMP-13 (collagenase-3)	1417
MMP-14 (membrane type-1 MMP)	15872
MMP-15 (membrane type-2 MMP)	3997
MMP-16 (membrane type-3 MMP)	1599

[a] $n = 3$. [b] Generated with a tight binding inhibition equation.

despite the 5-fold loss of affinity for pTACE compared to **5l** (Table 2). The 2,6-dimethyl- and 2,6-dichloro-4-picolyl analogues **5p** and **5q** maintained comparable potency in the WBA to **5o**. Analogous to the SAR in the benzyl ether series, **5p** and **5q** restored selectivity over MMP-2 and −9 (data not shown). The 4-quinolinyl-methoxy analogue **5r** further improved affinity to 2.25 μM in the WBA but suffered from MMP-2 and -9 activity (384 and 506 nM, respectively). 2-Methylquinoline analogue IK682 (**5s**) not only reestablished selectivity over MMP-2 and -9 but also resulted in a 6-fold increase in the WBA potency (0.35 μM). The WBA activity of IK682 can be partially contributed to the improved potency for pTACE (vide infra) and the relatively high free fraction in human serum (3.6%).

IK682 was found to be a potent inhibitor of pTACE with a K_i of 0.56 nM (Table 3). It exhibited excellent selectivity (>2000-fold) for pTACE relative to MMP-1, -2, -9, -13, -14, -15, and -16. Even though it has a submicromolar K_i for MMP-3, -7, and -8, IK682 is still more than 200-fold selective for pTACE.

The pharmacokinetics of IK682 administered intravenously and orally to Sprague Dawley rats and Beagle dogs was studied in a cassette-dose fashion, and the plasma samples were analyzed by a LC−MS−MS assay.[14] After iv administration, the systemic clearance, volume of distribution at steady state, and terminal half-life were 2.1 L h^{-1} kg^{-1}, 2 L kg^{-1}, and 11.8 h in rat and 0.9 L h^{-1} kg^{-1}, 0.9 L kg^{-1}, and 7.1 h in dog, respectively (Table 4). Oral absorption was rapid in both species with peak concentration occurring within 0.5 h. Oral bioavailability was good in both species, 41% (rat) and 32% (dog). The favorable oral bioavailability and long terminal half-life were likely due to the low to moderate clearance and good absorption consistent with the excellent Caco-2 P_{app} value (17.3 × 10^{-6} cm/s).

In conclusion, a novel lactam series of potent, selective, and orally bioavailable TACE inhibitors were designed using computer models, crystal structure of MMP-3, and known MMP inhibitors. To understand and

Table 4. Pharmacokinetic Parameters of IK682[a]

PK parameters	rat	dog
iv		
dose (mg/kg)	4	4
$t_{1/2}$ (h)	11.8	7.1
Cl (L h^{-1} kg^{-1})	2.1	0.94
V_{ss} (L kg^{-1})	2.0	0.93
AUC (nM h)	4171	10211
po		
dose (mg kg^{-1})	8	8
t_{max} (h)	0.38	0.38
F (%)	41	32

[a] Determination of two for each dosing group.

optimize TACE selectivity, a homology model of TACE was built, and the S1′ site was identified as a key area for selectivity. Rational exploration of the P1′–S1′ interactions using the lactam template resulted in potent and selective TACE inhibitors, exemplified by IK682. Discovery of these selective molecules should enable more precise studies delineating the biological roles of TACE and other MMP inhibitors in inflammatory, cardiovascular, and oncologic models of human disease and perhaps offer new therapeutic agents for the treatment of rheumatoid arthritis.

Supporting Information Available: Experimental details for IK682, spectroscopic data for **5a–r**, and X-ray data for **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Details of pTACE and WBA are described in ref 4a.

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