Bioorganic & Medicinal Chemistry Letters 18 (2008) 5809–5814

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel hydroxamates as highly potent tumor necrosis factor-α converting enzyme inhibitors. Part II: Optimization of the S3′ pocket

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ARTICLE INFO

Article history:
Received 10 June 2008
Revised 8 September 2008
Accepted 10 September 2008
Available online 13 September 2008

Keywords:
TACE inhibitors
MMP inhibitors

ABSTRACT

A series of cyclopropyl hydroxamic acids were prepared. Many of the compounds displayed picomolar affinity for the TACE enzyme while maintaining good to excellent selectivity profiles versus MMP-1, -2, -3, -7, -14, and ADAM-10. X-ray analysis of an inhibitor in the TACE active site indicated that the molecules bound to the enzyme in the S1′–S3′ pocket.

TACE (TNF-α converting enzyme) is a member of the ADAM (a disintegrin and metalloprotease-containing enzyme) branch of the zinc metalloprotease family and TACE is responsible for cleaving membrane bound 26 kDa proTNF-α (tumor necrosis factor-α) to its soluble 17 kDa form.[1] TNF-α is a pro-inflammatory cytokine that has been reported to play a pivotal role in the disease progression of rheumatoid arthritisis (RA), psoriasis, and Crohn's disease through mediation of immuno-inflammatory responses of other pro-inflammatory cytokines.[2] In addition, elevated levels of TNF-α in the synovial fluid of RA patients has been reported.[3] With the commercial success of biologics, such as Remicade[4] and Enbrel,[5] that reduce soluble TNF-α levels, an intensive search for orally available small molecules that interrupt the TNF-α signaling pathway has garnered the interest of pharmaceutical community.[6] Of the many possible strategies to regulate TNF-α production, TACE inhibition has long been viewed as one of the most promising small molecule targets.[7]

TACE (ADAM-17) shares many active site similarities with many other matrix metalloproteinases (MMP) and ADAMs.[8] As a result, many of the early TACE inhibitors suffered from a lack of selectivity.[9] This broad spectrum MMP inhibition has been the suspected cause of musculoskeletal side effects observed with other hydroxamic acids.[9]

Recently, several TACE selective inhibitors have been reported in the literature for which Figure 1 shows three representative structures.[6] γ-Lactamhydroxamic acid **1**,[10] thiomorpholine hydroxamate **2**,[11] and cyclopropyl hydroxamate **3**[12] are all potent inhibitors of TACE, and were reported to have a good selectivity profile against many MMPs. Unfortunately, some level of activity was observed for several MMPs, in particular MMP-3, -7, for these representative compounds. Thus, a truly selective TACE inhibitor is still needed to determine the side effect profile of TACE inhibition.

As part of a structure based design approach, a series of cyclopropane based hydroxamic acid inhibitors was identified and two active site binding modes (termed modes A and B) to the TACE enzyme were described.[12] In this paper, we describe the further optimization of TACE potency and selectivity for inhibitors binding in the mode A binding conformation.

Examination of the X-ray crystal structure of **3** bound to TACE (PDB entry 3EDZ) reveals several structural features that appear to be important for efficient binding (Fig. 2): (1) a good zinc ligating group, (2) strategic placement of a hydrogen bond acceptor to participate in hydrogen bonding with Leu-348 and Gly-349, and (3) occupation of the largely hydrophobic S1′ and S3′ binding pockets.

With these design considerations in mind, further analysis suggested there was additional opportunity for optimization in the S3′ region of the active site. We, as well others, have noticed that the S1′ pocket is rather narrow and is connected to S3′ region through a distinctive bend that allows access to the large pocket.[12,13] It was believed that this unique shape and large binding pocket could provide added binding affinity and selectivity advantages.

Following our earlier work,[12] the construction of the cyclopropyl hydroxamate scaffold relied on the trans selective cyclopropanation of a substituted acrylate with 1-*tert*-butoxycarbonylmethyl-tetrahydrothiophenium bromide under basic conditions (Scheme 1).[14]

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doi:10.1016/j.bmcl.2008.09.045

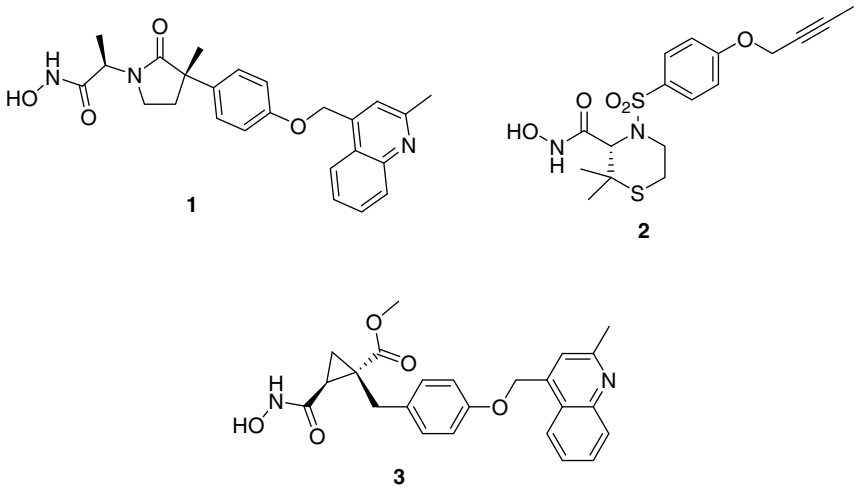


Figure 1. Recently published TACE inhibitors.

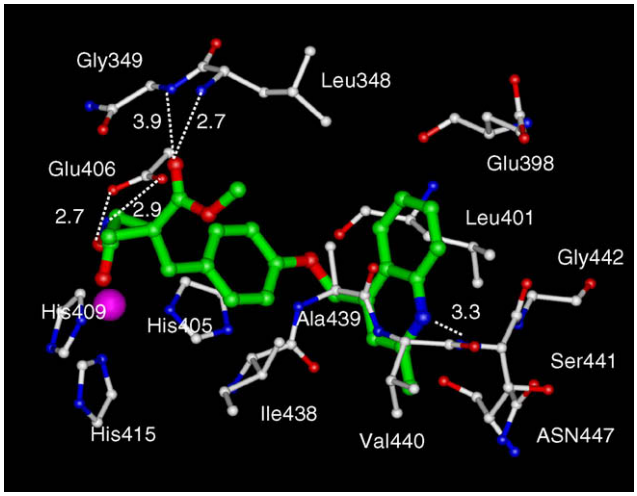
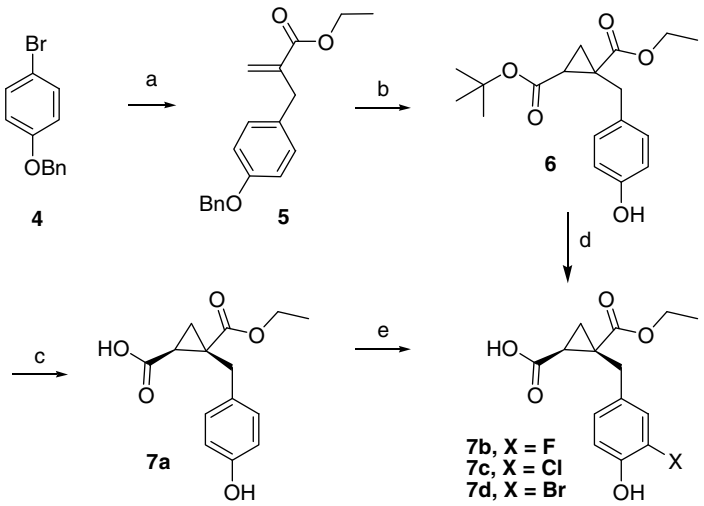


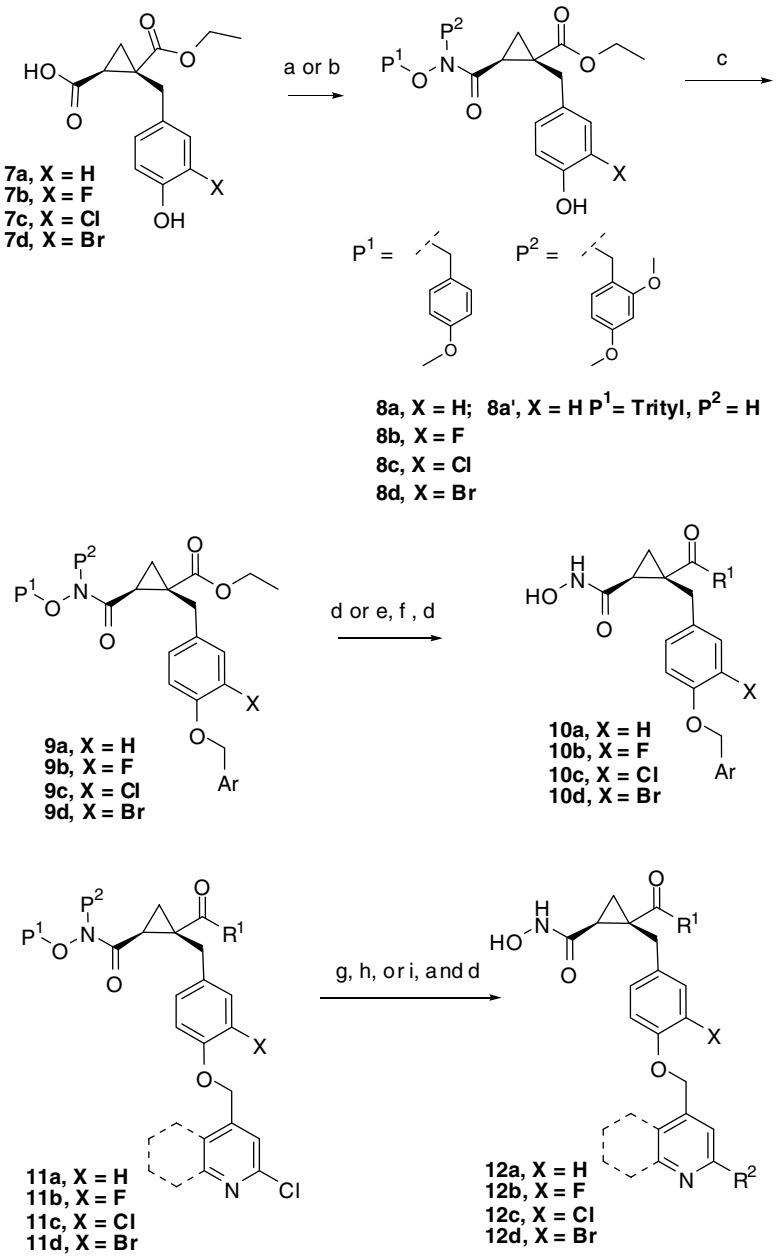
Figure 2. X-ray structure of inhibitor **3** (colored in green) bound to the active site of the TACE mutant enzyme (V353G).

Preparation of the desired acrylate was easily accomplished by treating the cuprate generated from commercially available 1-(benzyloxy)-4-bromobenze **4** with ethyl 2-(bromomethyl)acrylate to yield **5** in good yield.[15] Upon treatment with 1-*tert*-Butoxycarbonylmethyl-tetrahydrothiophenium bromide in the presence of DBU, compound **5** readily provided the desired cyclopropane with exclusive trans selectivity.[16] Subsequent hydrogenation yielded **6**. It was found very early on that the TACE binding affinity was exclusively associated with one enantiomer in this series. Thus, several separation conditions and techniques were surveyed. Although we were able to obtain enantiomerically pure material by several methods, the most convenient and reliable method was found to be chiral chromatography of the acid phenol intermediate from the treatment of **6** with 30% TFA/DCM to provide **7a** in >99% ee.

In an attempt to rapidly develop the SAR for the S3′ region from a late stage intermediate we prepared the *O*-trityl protected hydroxamic acid **8a**′ (Scheme 2). Unfortunately, in the next step, the unprotected NH was found to be significantly more reactive during the alkylation of the phenol. To overcome this problem, we realized a bis-protected hydroxamic acid was necessary to



Scheme 1. Reagents and conditions: (a) 1—*n*-BuLi, −78 °C, 1 h; 2—CuCN, 0 °C, 30 min; 3—ethyl 2-(bromomethyl)acrylate, THF, −78 °C, 30 min; 4—−78 °C to −10 °C; (b) 1—DBU, *tert*-butyloxymethyl-tetrahydro-thiophenium bromide, CH_3CN, 68%, 2—H_2, 10% Pd/C, CH_3OH; (c) 1—30% TFA in CH_2Cl_2, 2—Chiral Pak AS column, 40% IPA in hexane (0.1% AcOH); (d) 1—Selectflor, CH_3CN; 2—30% TFA in CH_2Cl_2, 3—Chiral Pak AS column, 40% IPA in hexane (0.1% AcOH); (e) NCS, CH_3CN or NBS, CH_3CN.



Scheme 2. Reagents and conditions: (a) *N*-(2,4-dimethoxybenzyl)-*O*-(4-methoxybenzyl) hydroxylamine, HOAt, EDCI, DIPEA, DMF; (b) 1—acetyl chloride, DMAP, DIPEA, CH_2Cl_2, 0 °C, 2—oxalyl chloride, cat. DMF, CH_2Cl_2, 3—*N*-(2,4-dimethoxybenzyl)-*O*-(4-methoxybenzyl)hydroxylamine, DIPEA, CH_2Cl_2; (4) 2 N NH_3 in CH_3OH; (c) $ArCH_2X$ (where X = Cl, Br, or I), K_2CO_3, TBAI, DMF; (d) $(CH_3CH_2)_3SiH$, 30% TFA, CH_2Cl_2; (e) LiOH, 1:1 THF/H_2O; (f) amine, HOBt, EDCI, DIPEA, DMF; (g) $ArB(OH)_2$, Pd(dppf)Cl_2·CH_2Cl_2, K_2CO_3, dioxane, water, μW; (h) R″MgX, Fe(acac)$_2$, THF, NMP; (i) amine, dioxane, μW.

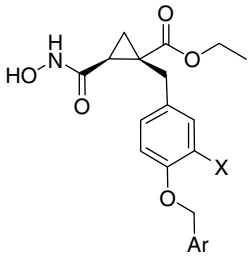
mask its reactivity, but the protecting groups should also be easily removed. Towards this end, *N*-(2,4-dimethoxybenzyl)-*O*-(4-methoxybenzyl) hydroxylamine was prepared in three steps from 4-methoxyl benzylchloride and *N*-hydroxyphthalimide.[17]

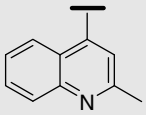
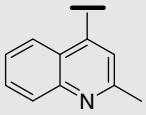
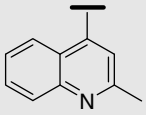
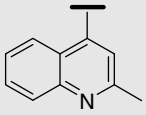
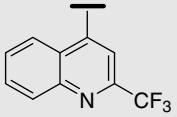
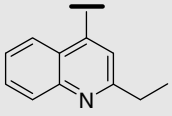
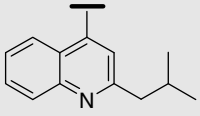
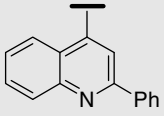
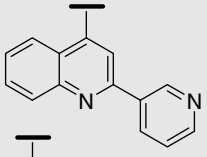
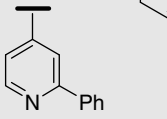
N-(2,4-Dimethoxybenzyl)-*O*-(4-methoxybenzyl) hydroxylamine was easily coupled to the cyclopropyl cores **7a–d** via a standard EDCI coupling procedure (Scheme 2). For large scale synthesis, it was more convenient to treat *N*-(2,4-dimethoxybenzyl)-*O*-(4-methoxybenzyl) hydroxylamine with the acid chloride generated from the cyclopropyl cores **7a–d** to furnish **8a–d**. Alkylation of **8a–d** proceeded smoothly with a variety of different benzylic halides to provide a diverse set of substituents designed to explore the S3′ region. To our delight, the bis-benzylated hydroxamate was easily deprotected with 30% TFA/DCM in the presence of triethyl silane to furnish the desired final targets **10a–d**. Additionally, the ethyl esters **9a–d** were saponified with lithium hydroxide and the subsequent carboxylic acid was coupled under standard peptide coupling conditions to provide a wide array of amides at this position (see Table 3 for examples). When the aryl group was a 2-chloro pyridine or quinoline, diversity could be incorporated via a number of metal mediated reactions or through direct displacement of the chloride with amines (**11**–**12**).

Affinity for TACE was measured by an internally quenched peptide substrate with its sequence derived from the pro-TNF-α cleavage site using a catalytic domain of recombinant human TACE enzyme. Selected compounds were initially counterscreened versus MMP-1, -2, -3, -7, -14, and ADAM-10.[18] Pharmacokinetic studies were conducted using cassette-dosing administered orally to Sprague–Dawley rats.[19]

As shown in Table 1, most of the compounds have K_i's in the low nanomolar range. Compounds **13** and **14** have excellent TACE enzyme activity with single digit nanomolar affinities. As the substituent becomes larger (i.e., where X = Cl (**15**) or Br (**16**)), a precipitous loss in potency was observed. Since docking studies suggested that the S1′ region of the TACE enzyme was quite narrow and consequently might not tolerate additional substitutions, this result was not surprising. Upon further analysis, there appeared to be unoccupied space in the S3′ binding pocket in particular the space where the methyl of the quinoline resided. To that end, several 2-substituted quinolines were prepared and analyzed. Remarkably, this region was found to be quite tolerant of a variety of substituents. Replacement of the 2-methyl group of the quinoline with a trifluoromethyl (**17**) or ethyl group (**18**) resulted in excellent TACE activity at 3 and 4 nM, respectively. As the size of this substituent became larger as indicated with **19** and **20**, a small drop in potency was observed although both compounds were still very active at 18 and 12 nM, respectively. Installment of a 3-pyridyl provided **21** which displayed a TACE K_i of 22 nM. Truncation of the quinoline aromatic ring thereby resulting in a 2-phenyl pyridine **22** displayed good affinity for the enzyme of 37 nM. Although **21** and **22** were somewhat less active, these modifications were viewed as important methods for improving the overall physical properties of these inhibitors by reducing the *c*Log*P* and/or adding polarity.

Table 1
Cyclopropyl ethyl ester SAR[a]



Compound	Ar	X	TACE K_i (nM)
13		H	6
14		F	5
15		Cl	158
16		Br	381
17		H	3
18		H	2
19		H	18
20		H	12
21		H	22
22		H	37

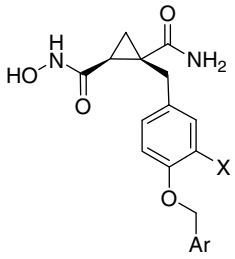
[a] Each K_i value is an average of three determinations, and the standard errors for all K_i determinations are less than 10%.

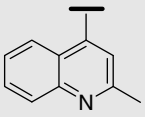
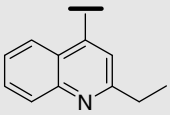
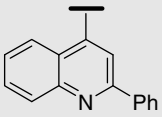
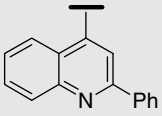
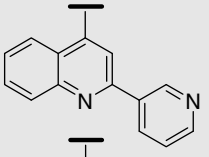
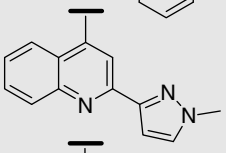
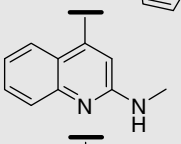
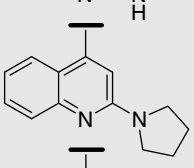
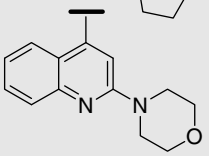
We next turned our attention to modifications of the ethyl ester group (Table 2). Because the carbonyl group in this region was designed to participate as a hydrogen bond acceptor it was anticipated that an amide should provide tighter TACE binding than an ester. Although **23** and **24** yielded comparable K_i's to their ester counterparts (3 and 1 nM versus 6 and 2 nM, respectively), a dramatic change was observed with larger substituents at the 2-position of the quinoline. When a 2-phenyl quinoline was combined with a primary amide, an approximately a 100-fold improvement in affinity was observed for **25** and **26** which now displayed low picomolar K_i's of 0.18 and 0.14 nM, respectively. Pyridyl and pyrazole replacement of the lipophilic phenyl (**27** and **28**, respectively) also yielded potent picomolar level compounds. Remarkably, N-linked heteroalkyls and heterocycles also displayed enhanced TACE inhibitory affinity to yield a series of subnanomolar inhibitors as represented by **29**–**31** despite concerns that increased basicity would diminish in vitro activity in this largely hydrophobic region.

Having identified the synergistic combination of S3′ 2-substituted quinoline and amide as a hydrogen bond acceptor, we next prepared a series of amides while holding the 2-phenyl quinoline constant to expand the SAR in this region (Table 3). In some early work, this area was found to be remarkably tolerant to a wide variety of modifications and substitution patterns. Gratifyingly, this trend remained intact with more elaborate S3′ derivatives. Preparation of the tertiary dimethyl amides **32** and **33** resulted in subnanomolar inhibition which was a similar level of activity to the primary amides (0.28 and 0.36 nM vs 0.18 and 0.14 nM, respectively). As we broadened our scope, we were delighted to see that more functionalized amides were also very potent. Prolinol amide **34** had a TACE K_i of 0.30 nM. The hydroxyl was transposed to the 3-position of the pyrolidine ring to provide **35** which was of equivalent potency. Interestingly, there was little dependence on the absolute chemistry of the substituent on the pyrolidine ring. This is exemplified with the comparison of 3-amino pyrolidine amides **36** and **37** where both are very potent compounds (0.38 and 0.54 nM). The basic nitrogen could be extended out, such as amino piperidine **38**, with little loss of activity (0.63 nM). When the hydrogen bond acceptor is a cyano, **39** a TACE K_i of 4 nM was observed, which was similar to ethyl ester **20**. One suggestion for this broad functional group acceptance is that the region around the amide is largely solvent exposed and has no defined interactions with the enzyme.

Evaluation of the selectivity data versus several other MMPs and ADAM-10 showed some interesting trends (Table 4). In the

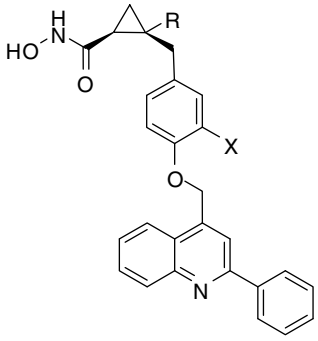
Table 2
SAR of primary amide and 2-substituted quinolines combination SAR[a]

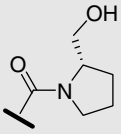
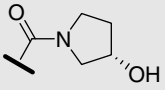
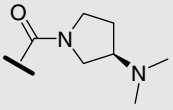
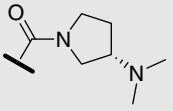
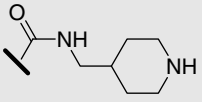


Compound	Ar	X	TACE K_i (nM)
23		H	3
24		H	1
25		H	0.18
26		F	0.14
27		F	0.14
28		F	0.15
29		F	0.76
30		F	0.15
31		F	0.21

[a] Each K_i value is an average of three determinations, and the standard errors for all K_i determinations are less than 10%.

Table 3
Amide SAR of 2-phenyl quinolines[a]



Compound	R	X	TACE K_i (nM)
32	–C(O)NMe$_2$	H	0.28
33	–C(O)NMe$_2$	F	0.36
34		H	0.30
35		H	0.25
36		H	0.38
37		H	0.54
38		H	0.63
39	–CN	H	4

[a] Each K_i value is an average of three determinations, and the standard errors for all K_i determinations are less than 10%.

Table 4
Selectivity of TACE inhibitors[a]

Compound	MMP-1	MMP-2	MMP-3	MMP-7	MMP-14	ADAM-10
13	>10,000	7000	952	1500	>10,000	>10,000
20	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
23	>10,000	1000	58	110	2400	>10,000
26	>10,000	2267	<14	128	4072	>10,000
33	>10,000	>10,000	>10,000	4617	>10,000	>10,000
34	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
39	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000

[a] Values are reported as K_i's. Each K_i value is an average of three determinations, and the standard errors for all K_i determinations are less than 10%.

ethyl ester series, 2-methyl quinoline **13** was shown to be quite selective versus MMP-1, MMP-2, MMP-14, and ADAM-10, but had low micromolar affinity for MMP-3 and MMP-7. Fortunately, simply switching to the 2-phenyl quinoline **20** provided a compound that was highly selective. Interestingly, when we evaluated the primary amide series similar behavior was not observed. In this case, both 2-methyl quinoline **23** and 2-phenyl quinoline **26** displayed low nanomolar inhibitory properties for MMP-3 and MMP-7 along with low micromolar affinities for MMP-2 and MMP-14. Despite selectivity ratios of 100-fold or greater (i.e., MMP K_i /TACE K_i), we were still concerned about the consequences of low nanomolar inhibition of MMP-3 and MMP-7. Gratifyingly, dimethyl amide **33** dramatically improved the selectivity profile

with a MMP-7 K_i of about 4 μM. Furthermore, as the amide became larger as in prolinol derivative **34** or when the amide was replaced with a cyano **39**, the selectivity was enhanced providing compounds with selectivity ratios of greater than 3000 versus all the MMPs and ADAMs we examined.

Despite the exceptional potency and selectivity profiles, many of these analogs displayed diminished activity in the human whole blood assay (HWB) and poor pharmacokinetic properties. For compounds **13**–**39**, HWB IC_{50}'s were found to be 2 μM or larger. Furthermore, compounds like **26** failed to display detectable plasma levels in a rat PK study,[19] which was characteristic for most of these amides. The low oral availability observed for **26** may, in part, be attributed to poor absorption as was suggested in a Caco-2 assay ((AP to BL) = 3 nm/s). In contrast, compound **39** displayed a reasonable AUC (>2500 nM h, 10 mpk, po) and stood out as having the best PK profile.

In summary, we have explored the effect of S3′ and hydrogen bond acceptor variations on potency and selectivity of a series of cyclopropyl hydroxamic acids. Many of these inhibitors displayed potent TACE K_i's in the picomolar range while maintaining good and sometimes excellent selectivity over MMP-1, MMP-2, MMP-3, MMP-7, MMP-14, and ADAM-10. More importantly, **39** displayed a good PK profile while maintaining a good in vitro profile.

Acknowledgments

We wish to thank Drs. Johnathan Piwinski, William Greenlee, and Michael Czarniecki for their continued support of the project. Also, we thank Emily Luk for the LC–MS analysis and Dr. T.M. Chan and Rebecca Osterman for help with NMR structural determinations.

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14. All new compounds gave satisfactory ^{1}H NMR and MS in accord with their assigned structure. All final targets showed LCMS purities >95%.
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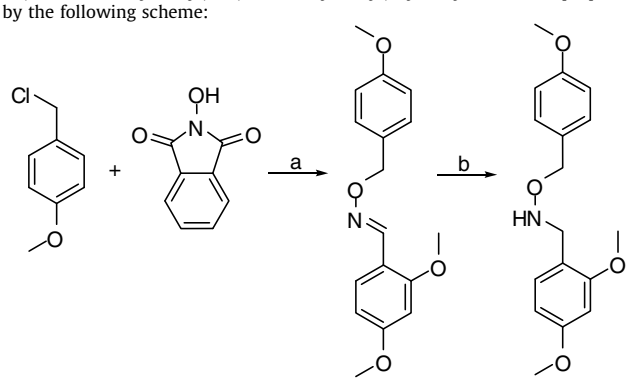
17. *N*-(2,4-Dimethoxybenzyl)-*O*-(4-methoxybenzyl) hydroxylamine was prepared by the following scheme:



Reagents and conditions: (a) 1—DIPEA, CH_3CN, reflux, 3 h; 2—20% $NH_2NH_2{\cdot}H_2O$, CH_3OH, reflux, 20 min; 3—2,4-dimethyoxybenzaldehyde, NaOAc, CH_3CO_2H/CH_3OH, reflux, 2 h, 50% (over 3 steps); (b) $Na(CN)BH_3$, CH_3CO_2H, 86%.

18. For details concerning all in vitro assay conditions used herein, please see Ref. 12.
19. For experimental details of the pharmacokinetic assay, see: Korfmacher, W. A.; Cox, K. A.; Ng, K. J.; Veals, J.; Hsieh, Y.; Weinhaus, S.; Broske, L.; Prelusky, D.; Nomeir, A.; White, R. E. *Rapid Commun. Mass Spectrum* **2001**, *15*, 335.