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Discovery of novel hydantoins as selective non-hydroxamate inhibitors of tumor necrosis factor-α converting enzyme (TACE)☆

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Abstract—A series of novel hydantoins was designed and synthesized as structural alternatives to hydroxamate inhibitors of TACE. 5-Mono- and di-substituted hydantoins exhibited activity with IC_{50} values of 11–60 nM against porcine TACE in vitro and excellent selectivity against other MMPs.

Tumor necrosis factor-α (TNF-α) is a well-established pro-inflammatory cytokine, primarily produced by activated macrophages and T-cells, that is implicated in a variety of autoimmune disorders such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis.[1] Anti-TNF-α drugs such as Remicade®, Enbrel®, and Humira® have clinically validated the approach of sequestering TNF-α with antibodies and binding proteins to block its action at the TNF-α receptor (TNF-R) and are a significant advance in treating these diseases.[2] However, the high cost of manufacturing these agents and the need to administer them parenterally have led to a strong interest to develop orally active molecules that suppress TNF-α activity.

An alternative approach to modulate the action of TNF-α is to use selective small molecule inhibitors of TNF-α converting enzyme (TACE, ADAM-17). TACE is a membrane-bound zinc metalloprotease that is primarily responsible for proteolytically processing the 26-kDa membrane-bound form of pro-TNF-α to its 17 kDa soluble form.[3] In principle, TACE inhibitors would halt the processing of TNF-α thereby precluding it from activating TNF-R and short-circuiting the inflammatory signal transduction cascade.[4] The development of TACE inhibitors has grown out of the much larger effort directed at inhibiting the dozens of related matrix metalloproteases (MMPs). Synonymous with Zn-metalloprotease inhibition is the hydroxamic acid structural element found in the vast majority of inhibitors (Fig. 1).[5] X-ray co-crystal structures of hydroxamate inhibitors and MMPs show five strong non-covalent interactions in the active site alone which largely account for the typical nM K_i's observed for this class.

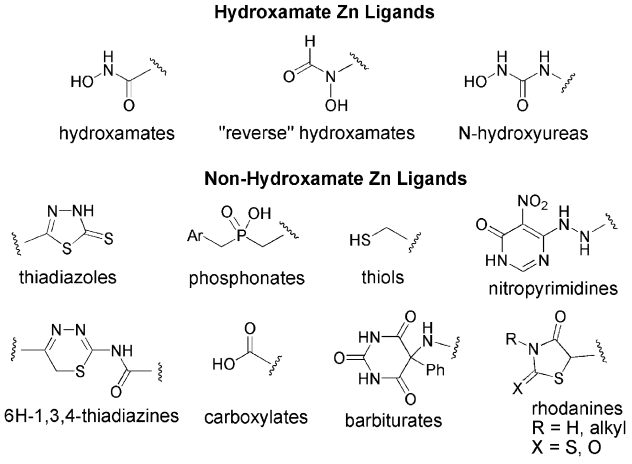

Figure 1. Precedented zinc ligands found in MMP inhibitors.[7]

Keywords: TACE; TACE inhibitor; Non-hydroxamate; MMP; Matrix metalloprotease; Matrix metalloproteinase; TNF.

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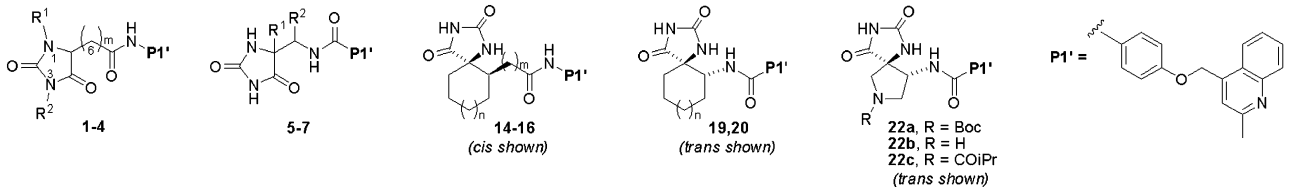
Although we[6] and others have exploited the intrinsic potency of the hydroxamic acid for TACE and MMPs, there remains considerable interest to discover a non-hydroxamate inhibitor. The advantages of a non-hydroxamate metalloprotease inhibitor include the potential for improved pharmacokinetics and avoiding the possibility of high clearance due to hydroxamate hydrolysis or proteolysis in vivo to the corresponding acid and toxic hydroxylamine.[8] A variety of non-hydroxamate MMP inhibitors have been developed that bear zinc-ligating functional groups (Fig. 1) but many suffer from inherent liabilities such as metabolic instability, poor bioavailability or cellular penetration, and cytotoxic functionality.[7] Of these hydroxamate replacements, we were intrigued by the potency of the rhodanines (thiazolidinediones) which showed IC_{50} values of 1–5 nM against MMP-13 despite their simple structures.[7e] All efforts to transpose the rhodanines into TACE inhibitors failed. Attempts to add chemical stability to the rhodanines led to the synthesis of hydantoin isosteres which when substituted with a TACE-selective 4-[(2-methyl-4-quinolinyl)methoxy]-phenyl P1′ group,[9] provided the first leads (Table 1). The synthesis of hydantoins is most efficiently achieved from ketones using the Bucherer–Bergs reaction. The simple acyclic hydantoins **1**–**7** (Table 1) were prepared using literature procedures.[10]

Spirocyclic hydantoins possessing ester-containing side chains were synthesized from α-substituted cyclopentanone or cyclohexanone again applying the Bucherer–Bergs conditions[11] (Scheme 1). Diastereo-selectivity varied as shown. Acid hydrolysis of the ester followed by standard peptide coupling with the TACE-selective 4-[(2-methyl-4-quinolinyl)-methoxy]aniline P1′ group gave the final substituted spirocyclopentyl and spirocyclohexyl products **14**–**16**.

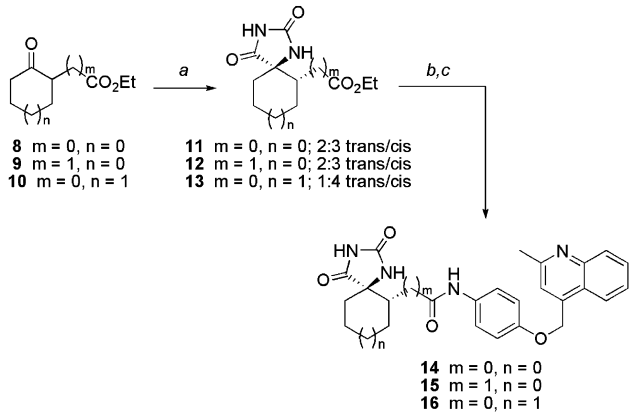
Spirocyclic hydantoins that contain a 'reversed' amide side chain were prepared using the complimentary approach outlined in Scheme 2. Commercially available racemic 1,2-aminocyclopentanol and 1,2-aminocyclo-

Table 1. In vitro potency of hydantoins for porcine TACE, MMP-1, -2, -9, and -13

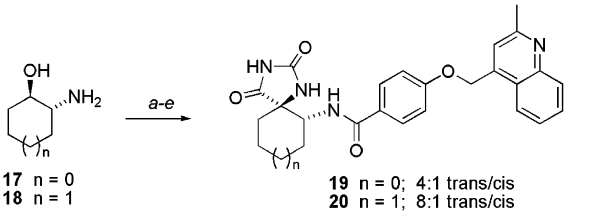


Compound	R^1	R^2	*m*	*n*	pTACE IC_{50}[a] (nM)	K_i[a] (nM)			
						MMP-1	MMP-2	MMP-9	MMP-13
(rac)-**1**[b]	H	H	1	—	210	>4946	>3333	>2128	>5025
(5*S*)-**1**[b]	H	H	1	—	3700	>4946	>3333	>2128	>5025
(5*R*)-**1**[b]	H	H	1	—	170	2472	567	1000	4120
(5*R*)-**2**[b]	H	H	2	—	150	>4946	>3333	>2128	>5025
(rac)-**3**[b]	H	Me	1	—	>100000	>4946	>3333	>2128	>5025
(rac)-**4**[b]	Me	H	1	—	>100000	>4946	>3333	>2128	>5025
(5*R*)-**5**[b]	H	H	—	—	98	>4946	>3333	>2128	>5025
(rac)-**6**[b]	Me	H	—	—	29	>4946	>3333	>2128	>5025
(rac)-**7**[b,c]	H	Me	—	—	28	>4946	>3333	>2128	>5025
(rac)-*trans*-**14**[d]	—	—	0	0	230	988	307	179	392
(rac)-*cis*-**14**[d]	—	—	0	0	64	>4946	>3333	>2128	>5025
(rac)-*cis*-**15**[d]	—	—	1	0	7400	>4946	>3333	>2128	>5025
(rac)-*trans*-**15**[d]	—	—	1	0	17	>4946	>3333	>2128	>5025
(5*R*,6*S*)-*trans*-**15**[d,e,f]	—	—	1	0	11	>4946	>3333	>2128	>5025
(5*S*,6*R*)-*trans*-**15**[d,f]	—	—	1	0	900	>4946	>3333	>2128	>5025
(rac)-*cis*-**16**[d]	—	—	0	1	270	>4946	>3333	>2128	>5025
(rac)-*trans*-**19**[d]	—	—	—	0	54	>4946	>3333	>2128	>5025
(rac)-*cis*-**19**[d]	—	—	—	0	5500	>4946	>3333	>2128	>5025
(rac)-*trans*-**20**[d]	—	—	—	1	24	>4946	>3333	>2128	>5025
(5*R*,6*R*)-*trans*-**22a**[d,f]	—	—	—	—	13	>4946	>3333	>2128	>5025
(5*R*,6*R*)-*trans*-**22b**[d,f]	—	—	—	—	25	>4946	>3333	>2128	>5025
(5*R*,6*R*)-*trans*-**22c**[d,f]	—	—	—	—	24	—	>3333	—	>5025

[a] pTACE IC50 and MMP K_i data are from a single determination.
[b] Synthesized as described in Ref. 10.
[c] Compound **7** is diastereomerically pure but its relative stereochemistry was not determined.
[d] All relative stereochemistry was determined using NOESY ^{1}H NMR.
[e] Compound *trans*-**15** was resolved by chiral HPLC and the absolute stereochemistry was tentatively assigned (5*R*,6*S*) based on analogy to the TACE activity of (5*R*)-**1**, (5*R*)-**2**, (5*R*)-**5**, and (5*R*,6*R*)-**22a**–**c**.
[f] Absolute and relative stereochemistry was determined via X-ray crystal structure of a CSA-salt of a **22** synthetic intermediate that had been resolved by chiral HPLC and was used to make (5*R*,6*R*)-*trans*-**22a**–**c**.



Scheme 1. Reagents and conditions: (a) $(NH_4)_2CO_3$, KCN, EtOH/H_2O, sealed tube 80 °C; (b) 4 M HCl (37%, 34%, 32% for two steps); (c) 4-[(2-methyl-4-quinolinyl)methoxy]aniline·HCl, HATU or PyBOP, DIEA, DMSO (8%, 35%, 32%).

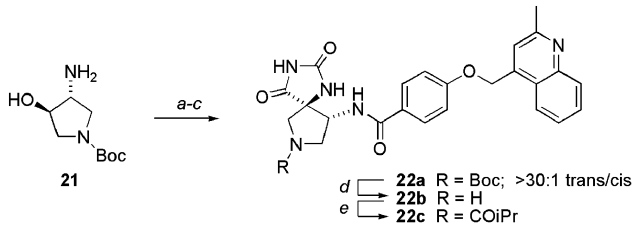


Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, MeOH; (b) TPAP, NMO, DCM (90%, 88% for two steps); (c) $(NH_4)_2CO_3$, KCN, EtOH/H_2O, sealed tube 80 °C (25%, 81%); (d) 2:1 TFA/DCM (100%, 100%); (e) 4-[(2-methyl-4-quinolinyl)-methoxy]benzoic acid, BOP, iPr$_2$NEt, DMSO (18%, 12%).

hexanol were Boc-protected and the alcohol oxidized to the ketone using Ley's catalyst or Dess–Martin periodinane.[12] Bucherer–Bergs reaction gave a 4:1 *trans*/*cis* mixture of hydantoins for cyclopentane and 8:1 *trans*/*cis* for cyclohexane. Acid deprotection of the Boc group followed by BOP coupling to 4-[(2-methyl-4-quinolinyl)methoxy]benzoic acid—a TACE-selective P1′ group that we had developed in our hydroxamate work[6] gave the final 'reversed' amide hydantoins **19** and **20**.

The synthesis of spiropyrrolidinyl hydantoins was performed starting from racemic compound **21**[13] as shown in Scheme 3. The primary amine was coupled to the TACE-selective P1′ group previously mentioned followed by oxidation of the hydroxyl group to a ketone with Dess–Martin periodinane and hydantoin formation to furnish **22a**. Boc-deprotection of **22a** with TFA/DCM provided **22b** which was then coupled to isovaleric anhydride to form the pyrrolidinyl amide **22c**.

The chemistry used to synthesize compounds **14**–**22** gave variable diastereoselectivity, which allowed each diastereomer to be isolated and assayed independently. All relative stereochemistry was determined by NOESY ^{1}H NMR and the absolute stereochemistry of compounds **1**, **2**, **5**, and **22** was uniformly (5*R*), originating from the chiral pool or purified by chiral HPLC and elucidated by small molecule crystallography. The in vitro activity



Scheme 3. Reagents and conditions: (a) 4-[(2-methyl-4-quinolinyl)methoxy]benzoyl chloride, 10% $NaHCO_3$, DCM; (b) Dess–Martin periodinane, DCM (40%, two steps); (c) $(NH_4)_2CO_3$, KCN, EtOH/H_2O, sealed tube 80 °C, (48%); (d) 2:1 TFA/DCM (100%); (e) $(i$PrCO$)_2$O, DIEA, DCM (70%).

of the synthesized hydantoins was evaluated using porcine TACE (pTACE) because of its availability and high homology to human TACE.[14] MMP-1, -2, -9, and -13 were used as a representative selectivity panel that possess both shallow and deep S1′ pockets.[6]

The hydantoins shown in Table 1 were the first leads discovered to have pTACE activity. Compound **1**, prepared from racemic aspartic acid, had an IC_{50} of 210 nM against pTACE and was selective against MMP-1, -2, -9, and -13. When the same compound was made discretely from (*S*) and (*R*) aspartic acid, it was the 5-(*R*) stereochemistry that accounted for the pTACE activity. The one methylene longer homolog (*R*)-**2** (prepared from R–Glu) was marginally more active at 150 nM. Independent N-methylation of the hydantoin nitrogens (compounds **3** and **4**) established that they must be unsubstituted to retain activity. Reversal of the pendant amide side chain led to additional improvement in pTACE activity such as compound **5** (Table 1) which had an IC_{50} of 98 nM against pTACE. Addition of a methyl substituent in the 5- or 6-position to bias the side-chain conformation gave compounds **6** and **7** with improved IC_{50}s of 29 and 28 nM, respectively.

In an effort to elucidate the bound conformation of these acyclic inhibitors, the 5- and 6-positions were annulated to make the conformationally constrained 5,5-spirocyclic hydantoins. These analogs have the added benefit of circumventing racemization that 5-monosubstituted hydantoins (compounds **1**–**5** and **7**) are prone to. The observation that methyl substitution in the 5 and 6 positions (compounds **6** and **7**) enhanced pTACE activity suggested that such an annulation strategy might further increase potency.

Compound **14** (the spirocyclopentyl version of compound **1**) indeed showed an improvement in pTACE activity from 210 to 64 nM (comparing racemates, Table 1). Although *trans*-**14** had some activity with diminished selectivity, *cis*-**14** proved to be the more active diastereomer. When the side chain of **14** was homologated by one carbon to give *cis*/*trans*-**15** (the spirocyclopentyl version of compound **2**), the active stereoisomer reversed with *trans*-**15** having pTACE activity of 17 nM, whereas *cis*-**15** had an IC_{50} of 7400 nM. This apparent paradox is easily rationalized

by comparing the 3-dimensional structures of *cis*-**14** and *trans*-**15** which both project the side-chain amide carbonyl in the same orientation with different cyclopentane puckering (see accompanying paper). Racemic *trans*-**15** was separated into its enantiomers using chiral HPLC and the putative (5*R*,6*S*)-*trans*-**15** enantiomer accounted for the pTACE activity at 11 nM. Reversing the amide side chain in the spirocyclic hydantoins (akin to compound (5*R*)–**5**) produced an even more conformationally constrained analog, compound **19**, as a pair of diastereomers. Like its isosteric analog *trans*-**15**, *trans*-**19** proved to be the more active diastereomer albeit 3-fold less active for pTACE at 54 nM. The conformational effects of spirocyclohexane analogs were also investigated. Compound *cis*-**16** was 4-fold less active than the cyclopentyl analog **14**, presumably due to a less than optimal orientation of the equatorial P1′ side chain. Nevertheless, reversing the amide in the cyclohexyl series gave *trans*-**20** which had good pTACE activity at 24 nM. In an effort to elaborate on the good pTACE activity of compound **19**, the spirocyclopentane was replaced with a pyrrolidine ring to give compounds **22a–c**. The pTACE activity of these analogs was good with IC_{50}s of 13, 25, and 28 nM, respectively, with excellent selectivity.

In summary, hydantoins have been known for over 100 years and 5,5-diphenylhydantoin (Dilantin®, phenytoin) has been used for decades to treat epilepsy. The use of hydantoins as a privileged scaffold for MMP inhibition has not been reported in the literature[15]—remarkable considering this century old heterocycle, mass produced in the early 1990s as the first combinatorial libraries,[16] has no doubt passed through extensive high-throughput screening against all varieties of metalloproteases. When incorporated into a TACE inhibitor, the hydantoin is a good albeit intrinsically less potent Zn ligand than an analogous hydroxamate. Our evidence suggests that the stringent requirement for unsubstituted hydantoin nitrogens, the unnatural (5*R*) stereochemistry, and an H bond acceptor appended to an appropriately functionalized hydrophobic P1′ side chain that complements the MMP S1′ pocket are what confer activity to this new class of molecules.[17]

Acknowledgment

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