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Hydantoins, triazolones, and imidazolones as selective non-hydroxamate inhibitors of tumor necrosis factor-α converting enzyme (TACE)

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Abstract—We have discovered selective and potent inhibitors of TACE that replace the common hydroxamate zinc binding group with a hydantoin, triazolone, and imidazolone heterocycle. These novel heterocyclic inhibitors of a zinc metalloprotease were designed using a pharmacophore model that we previously described while developing hydantoin and pyrimidinetrione (barbiturate) inhibitors of TACE. The potency and binding orientation of these inhibitors is discussed and they are modeled into the X-ray crystal structure of TACE and compared to hydroxamate and earlier hydantoin TACE inhibitors which share the same 4-[(2-methyl-4-quinolinyl)methoxy]benzoyl P1′ group.
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Tumor necrosis factor-α (TNF-α) is a cytokine that is produced by activated monocytes and macrophages, and plays a central role in immunity and inflammation.[1] Binding proteins or antibodies that intercept TNF-α (infliximab, adalimumab, etanercept) block its ability to activate the TNF-α receptor and have become highly effective treatments for rheumatoid arthritis, Crohn's disease, and psoriasis.[2] One of the many strategies to downregulate the activity of TNF-α is the use of small molecules to inhibit its convertase, 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 that is secreted from cells.[3]

Because TACE inhibitors logically started from the better studied matrix metalloprotease (MMP) inhibitors, virtually all of them contain the ubiquitous hydroxamate and more recently, the reverse hydroxamate zinc binding groups (ZBGs; Fig. 1).[4] Inhibitors containing these bidentate ligands generally have excellent potency but often suffer from intrinsically poor pharmacokinetics or metabolic liabilities.[5] Over the past several decades, numerous zinc-ligating, non-hydroxamate chemotypes have been incorporated into small molecules with the goal of finding potent and druglike inhibitors of zinc metalloproteases (Fig. 1).[6]

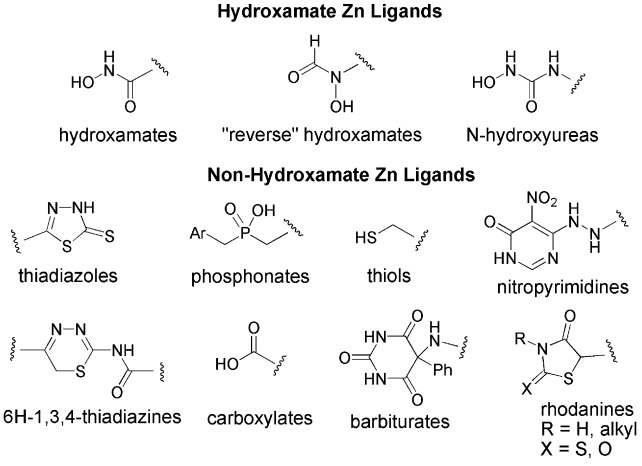

Figure 1. Precedented ZBGs found in zinc metalloprotease inhibitors.

Keywords: TACE; TACE inhibitor; TNF-α; MMP; Matrix metalloprotease; Non-hydroxamate.
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In our quest to find more 'druglike' inhibitors of TACE and other zinc metalloproteases, we discovered that hydantoins and barbiturates make for excellent replacements of hydroxamates.[7] Extensive SAR and modeling of the hydantoins elucidated a pharmacophore that consists of:

(1) A cyclic urea that coordinates the active site zinc, putatively in monodentate fashion via its carbonyl. One of the urea N–H groups must have a low pK_a for hydrogen bonding to (or deprotonation by) a conserved active site glutamate general base and the other N–H is required for a H-bond to a proximal Gly amide carbonyl.
(2) A linker that conformationally induces a U-turn between the ZBG and the P1′ group.
(3) An H-bond acceptor in the linker capable of interacting with mainchain Gly and Leu amide N–H.
(4) An appropriately functionalized P1′ group that complements the metalloprotease S1′ subsite.

Having validated this model with highly constrained hydantoins and barbiturates, we sought to prospectively identify new non-hydroxamate ZBGs using the above criteria as structural prerequisites. With these design constraints in place, both triazolones and imidazolones (Fig. 2) can achieve the same hydrogen bonding complimentarity as do the hydantoins and barbiturates in the active site of TACE as their enol tautomers.

The immediate problem of transposing a hydantoin TACE inhibitor into a triazolone or imidazolone was the trajectory of the P1′ side chain. Since one of the prerequisites for activity in the hydantoins is a U-shaped turn to engage main chain Leu348 and Gly349 in H-bonds and angle the P1′ appropriately, an even greater turn would be required for the sp^2 triazolones/imidazolones to compensate for the absent hydantoin 5-position chiral center. Since we had reliable models of both hydroxamates in TACE (later confirmed by an X-ray co-crystal structure[8]) and hydantoins in TACE,[7b] all that remained to do was search for optimal bridging groups between the triazolone/imidazolone ZBG and the 4-[(2-methyl-4-quinolinyl)methoxy]phenyl P1′ side chain. Molecular modeling was used to prioritize the extensive number of potential linkers based on conformational analysis and their adherence to the hydantoin model.

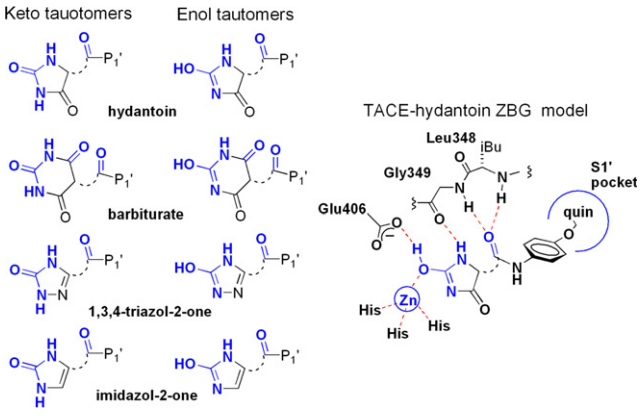
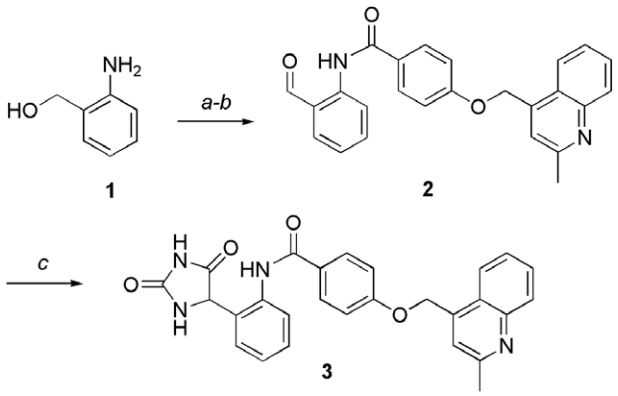
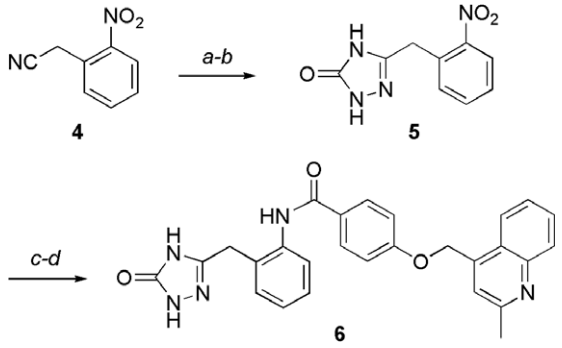


Figure 2. Pharmacophore elements of heterocyclic ZBGs.

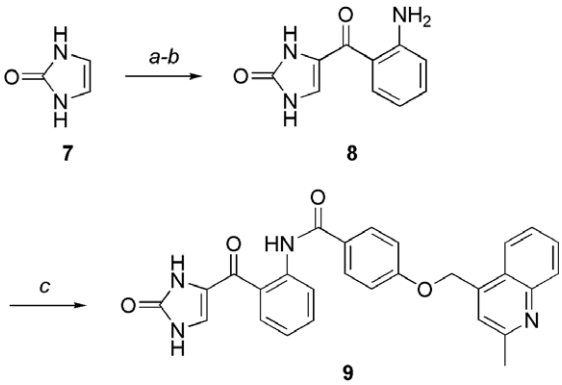
Hydantoin **3** (Scheme 1) was synthesized starting from 2-hydroxymethylaniline by coupling it to our preferred P1′ group to form an amide. Dess–Martin periodinane oxidation of the alcohol to the aldehyde followed by



Scheme 1. Reagents and conditions: (a) 4-[(2-methyl-4-quinolinyl)methoxy]benzoyl chloride, DCM; 10% $NaHCO_3$ (88%); (b) Dess–Martin periodinane, 1:1 DCM/DMF (100%); (c) $(NH_4)_2CO_3$, KCN, EtOH/H_2O, 80 °C sealed tube (10%).



Scheme 2. Reagents and conditions: (a) HCl, EtOH (84%); (b) semicarbazide·HCl, *N*-methylmorpholine, DMF, 120 °C, 12 h (54%); (c) 5% Pd/C, H_2, MeOH, THF, water (100%); (d) 4-[(2-methyl-4-quinolinyl)methoxy]-benzoic acid, $POCl_3$, pyridine (76%).

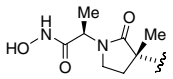
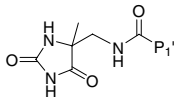
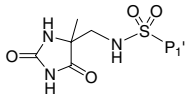
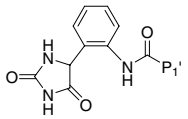
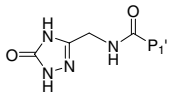
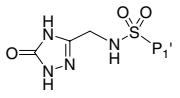
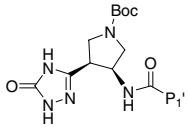
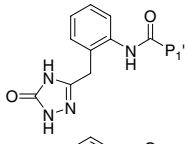
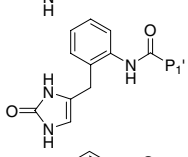
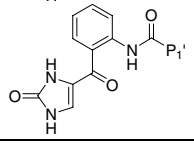


Scheme 3. Reagents and conditions: (a) 2-nitrobenzoyl chloride, $AlCl_3$, nitrobenzene (45%); (b) 5% Pd/C, H_2, MeOH (98%); (c) 4-[(2-methyl-4-quinolinyl)methoxy]-benzoic acid, $POCl_3$, pyridine (6%).

the Bucherer-Bergs reaction provided compound **3** in low yield due to significant competing benzoin condensation. Scheme 2 illustrates how triazolone **6** was prepared. Pinner reaction with nitrile **4** gave an imidate that condensed with semicarbazide to form triazolone **5**. Palladium-catalyzed hydrogenation of the nitro substituent followed by coupling to the P1′ acid using the method of Alves et al.[9] gave product **6**. Imidazolone **9** was prepared by treatment of commercially available imidazol-2-one hydrogen sulfate with 2-nitrobenzoyl chloride under Friedel–Crafts acylation conditions (Scheme 3). Reduction of the nitro group by catalytic hydrogenation and coupling to the P1′ as before provided compound **9** albeit in very low yield due to the unreactivity of the aniline. The synthesis of the remaining compounds in Table 1 is straightforward.[10]

The pharmacophore model described in Figure 2 above was built upon an extensive hydantoin SAR[7a] which provided a logical starting point for triazolone and imidazolone design. Hydantoins **10** and **11**, containing amido or sulfonamide side chains, showed good TACE activity at 29 and 37 nM, respectively. Another active compound was hydantoin **3** that contains a phenyl linker which makes a longer turn to the P1′ group to give a potent inhibitor at 14 nM.

Table 1. In vitro potency of selected hydantoins, triazolones, and imidazolones in pTACE, MMP-2, −3, −7, −12, and −13[a]

Compound	Structure[b]	pTACE IC_{50}, nM	MMP-2 Ki, nM	MMP-3 Ki, nM	MMP-7 Ki, nM	MMP-12 Ki, nM	MMP-13 Ki, nM
IK682		1	2050	141	259	—	1417
10[c]		29	>3333	—	—	>5025	—
11[c]		37	>3333	—	—	>5025	—
3		14	>3333	>4501	>6368	782	>5025
13[c]		>1000	>3333	—	—	>5025	—
14		439	2472	567	1000	4120	>5025
(3R,4S)-7[c]		162	>3333	—	—	>5025	—
6		34	>3333	>4501	>6368	3611	4314
15		1600	>3333	>4501	>6368	>6023	>5025
9		9	>3333	>4501	>6368	>6023	4314

[a] pTACE IC_{50}s are an average of two determinations; MMP Ki data are from a single determination. All compounds are racemic with the exception of **7**.

[b] The P1′ group in all cases is 4-[(2-methyl-4-quinolinyl)methoxy]phenyl.

[c] These compounds were also >4946 nM for MMP-1 and >2128 nM for MMP-9.

Simply substituting a hydantoin ZBG such as **10** for a triazolone **13** proved ineffective for the amide side chain and models of the latter suggest that **13** is unable to make an acute enough angle to access the S1′ site. However, the same change in ZBG when the side chain incorporates a sulfonamide (**14**) can better achieve the requisite turn and is 439 nM in pTACE albeit not as active as the hydantoin analog **11** at 37 nM. A more acute turn is achieved by the pyrrolidinyl linker **rac-cis-7** which is 3-fold more potent at 162 nM against TACE. Lastly, a benzyl linker was appended to a triazolone ZBG to make an even sharper albeit longer turn to provide compound **6** with an IC_{50} of 34 nM. As previously observed with hydantoin and barbiturate ZBGs, the 4-[(2-methyl-4-quinolinyl)methoxy]phenyl P1′ group confers excellent selectivity against other MMPs. It is interesting to compare triazolone **6** with hydantoin **3** because the added methylene in **6** appears to compensate for the chiral center in **3** to achieve a similar presentation of the ZBG and P1′ group (see Fig. 3b). Based on the pharmacophore model in Figure 2, we also wanted to explore an imidazolone. Transposition of the triazolone **6** into the corresponding imidazolone **15** surprisingly yielded an inactive compound.

Closer analysis reveals that the pK_a of an imidazolone is approximately 12, far less acidic than any of the other ZBGs and unlikely to be deprotonated, even with the assistance of the active site Lewis acidic zinc ion. We reasoned that replacement of the imidazolone benzyl linker with a benzoyl linker should have the effect of lowering the imidazolone pK_a by several log units and further preorganizing the P1′ side chain into the optimal U-shape conformation to access S1′. Indeed, compound **9** proved to be the most potent TACE compound in the series at 9 nM and retained its excellent selectivity.[11]

Figure 3a shows a model of compound **9** superimposed with the IK682-TACE crystal structure.[12] The carbonyl of the imidazolone is in close contact with zinc via a monodentate interaction and strongly hydrogen bonded to E406 while its N–H forms another hydrogen bond to mainchain Gly349. The ketone-containing linker may increase the potency of compound **9** by making an additional interaction to Leu348 in addition to the pK_a enhancement it confers. The amide side chain forms a hydrogen bond with Leu348 analogous to the side chains of the hydantoins such as **10**, **11**, and **3**. Shown in Figure 3b is an overlay of hydantoin **3**, triazolone **6**, and imidazolone **9**, and their shared interactions to zinc and residues in the vicinity of the active site. Clearly visible is how each unique linking group bridges the ZBG to the P1′ in a low energy conformation. Each molecule is able to engage Glu406 in a strong H-bond (or be deprotonated by it) which serves to enhance its interaction to the Zn, especially as an anion. Density functional computational methods of TACE by Cross et al.[13] calculate the theoretical pK_a of the active site Glu406 at 5.9. Furthermore, the pK_a of a hydroxamic acid (8.9) upon coordination to the active site Lewis acidic Zn is reduced to 5.3.

Table 2 shows the aqueous pK_a's of ZBGs that we have successfully incorporated into TACE inhibitors. If the magnitude of the pK_a reduction from the above analysis holds for the non-hydroxamate ZBGs in Table 2, their pK_a would likewise decrease to approximately the predicted value of Glu406 and in some cases, below. Generally, our data suggest that the potency of the heterocyclic ZBGs roughly correlates with their pK_a.

The quest for inhibitors of zinc-dependent metallo-proteases has been a fertile area of research for over 20

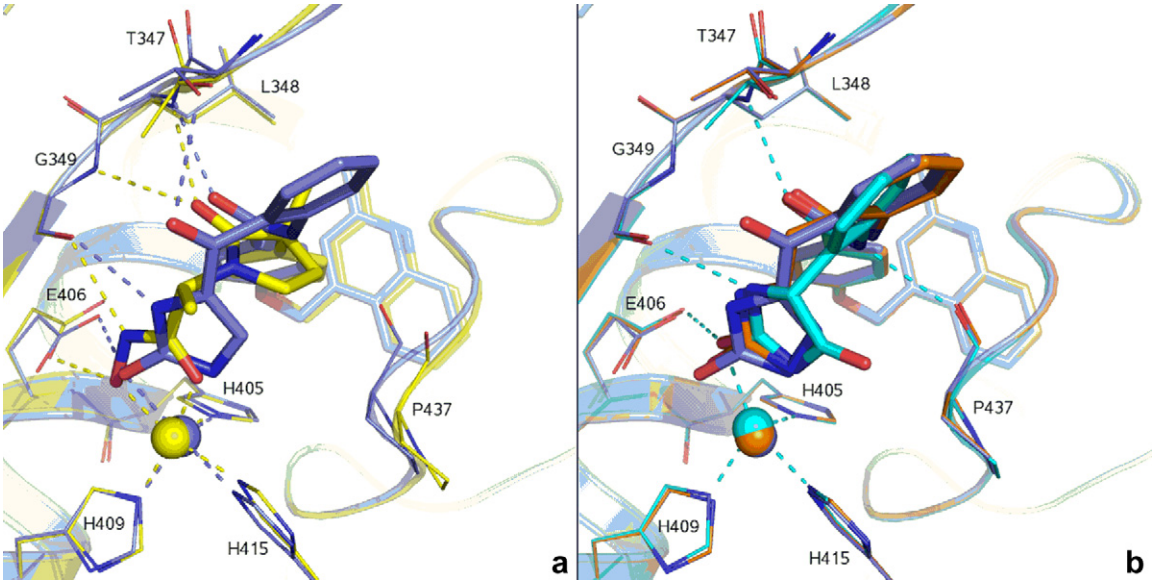


Figure 3. (a) Overlay of the modeled binding mode of compound **9**, blue, and the crystal structure of IK682, yellow. Hydrogen bonds and metal interactions between **9** and TACE are denoted by the dashed blue lines. (b) Modeled binding modes of compounds **3**, cyan, **6**, orange, and **9**, blue. Representative hydrogen bonds between **3** and TACE are indicated with dashed lines.

Table 2. Aqueous pK_a's of ZBGs[a]

ZBG	pK_a (aq)
Hydroxamic acid	8.9
Barbiturate	7.0
Hydantoin	9.0
1,3,4-Triazol-2-one	8
Imidazol-2-one	~12
4-Benzoyl-imidazol-2-one	—
1,3,4-Triazole-2-thione[b]	7.0

[a] pK_a's were experimentally measured with the exception of triazolone and imidazolone which were taken from comparably substituted examples reported in Beilstein.

[b] An account of our triazolethione-based TACE inhibitors is forthcoming in this journal.

years. Despite this massive effort however, there are no marketed inhibitors of zinc-dependent metalloproteases due to poor PK, lack of efficacy, and toxicity. Many have attempted to develop druglike, non-hydroxamate zinc metalloprotease inhibitors using ZBGs that have a strong intrinsic Kd for zinc.[14] Such an approach may be handicapped by promiscuous chelation to metals other than zinc (e.g., hydroxamates bind Fe(III) 10^6- to 10^{11}-fold stronger than Zn(II)).[15] The heterocycles described herein have compensated for the reduced, intrinsic Kd's for Zn exhibited by the bidentate hydroxamates using a functional group with putatively weaker monodentate metal interactions supplemented by additional hydrogen bonds in the vicinity of the active site. The 4-[(2-methyl-4-quinolinyl)methoxy]phenyl P1′ group maintains good TACE selectivity across series. Use of these more druglike ZBGs[16] with other P1′ groups should find broad applicability to the inhibition of zinc metalloproteases in the future.

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10. (a) For the synthesis of **10**, **11**, **12**, see Ref. 7a; (b) Compounds **13** and **14** were prepared by coupling the P1′ sulfonyl chloride or P1′ acid chloride to glycine under Schotten-Baumann conditions. The acid was coupled to semicarbazide·HCl (HATU, Et_3N, DMF) followed by treatment with 2 M NaOH overnight to give the triazolone; (c) (3R,4S)-**7** was prepared by coupling the β-amidopyrrolidinyl carboxylate (prepared in Duan, J.; Ott, G.; Chen, L.; Lu, Z.; Maduskuie, T.P.; Voss, M.E.; Xue, C.-B. WO2001/070673, 2001) with semicarbazide (HATU, Et_3N, DMF) followed by treating with 2 M NaOH overnight followed by Boc deprotection with TFA/DCM; (d) Synthesis of **15** started from 2-chloro-1-(2-nitrophenyl)ethanone (Terrier, C.; Mary, A.; Thal, C. *Tetrahedron* **1994**, *50*, 6287) which was then treated with KOCN in water at rt for 12 h to form the imidazolone (29% yield). Reduction of the nitro group (Pd/C, MeOH, H_2; 100% yield) followed by coupling to the P1′ acid using the same method as used for compounds **6** and **9** provided **15**.
11. While it would be intriguing to see the activity of the benzoyl linked triazolone version of **6**, 5-ketotriazolones are virtually unknown in the literature and we were not successful in making it. Also, it is unlikely that the potency enhancement would mirror compound **9** (a vinylogous acylurea) in magnitude because its pK_a would not benefit as much from resonance stabilization due to cross-conjugation.
12. Compounds were modeled in chain A of the TACE crystal structure 2FV5. Waters were removed from the structure except for those within the active site that formed >1 direct interactions with the protein. The complex was minimized with the OPLSAA-2005 force field using the GB/SA water model as implemented in Macromodel. IK682 and residues within 5 Å were allowed to move freely, residues 5–10 Å were constrained with a force constant of 200, and residues >10 Å were frozen. The structure was first subjected to 500 steps of SD minimization followed by TNCG minimization to a gradient

change of <0.05. The resulting minimized structure was used as the starting template for ligand modeling. Modeled ligands were constructed manually by removing the hydroxamate moiety from IK682 and replacing it with the appropriate functional group. The ZBGs were assumed to be in the enol tautomeric form. A conformational search was then carried out using the conformational search module of Macromodel allowing only the newly constructed functional group to move while freezing the remaining coordinates. The resulting conformations were aligned onto IK682 in the TACE/IK682 minimized complex and the best fitting conformation (as judged by sterics and hydrogen bonding) selected manually. The complex of the new ligand in TACE was then refined by the minimization protocol described above.

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16. The hydantoin group is found in phenytoin, the barbiturate is found in pentobarbital, and the triazolone is found in aprepitant.