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# A molecular modeling analysis of novel non-hydroxamate inhibitors of TACE

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**Abstract**—Recently, an X-ray co-crystal structure of our hydroxamate inhibitor IK682 and TACE [Niu, X.; Umland, S.; Ingram, R.; Beyer, B. M.; Liu, Y.-H.; Sun, J.; Lundell, D.; Orth, P. *Arch. Biochem. Biophys.* **2006**, *451*, 43–50] was published that explicitly shows the orientation of the hydroxamate and the TACE-selective 4-[(2-methyl-4-quinolinyl)methoxy]phenyl P1′ group in the S1′ and S3′ sites. The preceding paper described a novel series of potent and TACE-selective hydantoins and we previously described pyrimidinetrione (barbiturate) inhibitors of TACE, both of which contain the same P1′ group as IK682. Using this TACE-selective P1′ group as an anchor, stereochemical and conformational constraints in the inhibitors, and restrictions to the active site Zn coordination geometry, we developed a highly plausible and predictive pharmacophore model that rationalizes the observed TACE activity of all three inhibitors.


Matrix metalloproteases are a family of zinc endopeptidases that are responsible for the proteolytic breakdown of extracellular matrix during normal tissue homeostasis. Of the close to 30 MMPs discovered to date, the aberrant activity of several members of this class has been linked to numerous disease states such as rheumatoid arthritis, osteoarthritis, metastasis, angiogenesis, and autoimmune disorders.[1] Beyond MMPs, there has been considerable effort directed at finding selective small molecule inhibitors for the zinc-dependent metalloprotease TACE, which is responsible for processing pro-TNF-α into its soluble, inflammatory form.[2] The clinical success of Remicade®, Enbrel®, and Humira® (biologics that sequester TNF-α) in treating RA, IBD, and psoriasis proves that attenuating the effects of TNF-α can mitigate the severity of numerous autoimmune diseases.[3]

The catalytic domain of zinc metalloproteases possesses a conserved HEXXHXXGXXH zinc ligating sequence that forms an active site zinc coordinatively saturated by a water molecule that catalyzes the hydrolysis of amide bonds in protein substrates. Inhibitors of MMPs typically consist of a zinc binding group (ZBG, e.g., hydroxamates) appended to a large P1′ substituent that binds in the large hydrophobic S1′ pocket. Much of the MMP selectivity of these inhibitors comes from taking advantage of the structural differences found in the S1′ subsite.[1]

To date, several crystal structures of TACE in complex with inhibitors containing the hydroxamic acid Zn ligating group have been reported.[4] The preceding manuscript[5a] described the chemistry and structure–activity relationships of a new class of potent hydantoin TACE inhibitors. In addition, the 4-[(2-methyl-4-quinolinyl)methoxy]phenyl group was found to broadly confer excellent TACE-selectivity to our hydantoin, barbiturate and hydroxamate inhibitors.[5] The goal of this modeling study was to compare the interactions that hydroxamate and non-hydroxamate ZBGs (that all bear the same P1′ group) make with the active site of TACE and rationalize their observed activity and orientation in TACE. Such models might also be used to improve or expand upon the design of these and other non-hydroxamate zinc metalloprotease inhibitors.

*Keywords*: TACE; TACE inhibitor; Modeling; Hydantoin; Barbiturate; Hydroxamate; MMP; Metalloprotease; Metalloproteinase; TNF; Non-hydroxamate.
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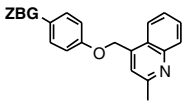
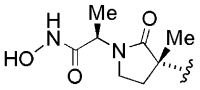
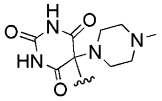
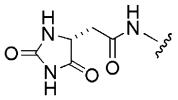
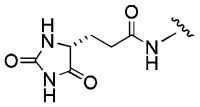
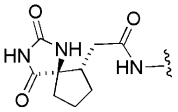
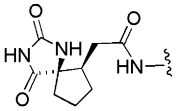
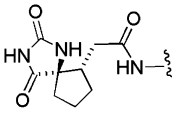
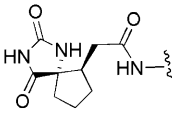
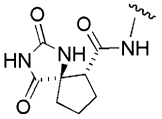
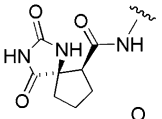
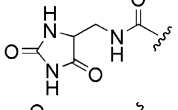
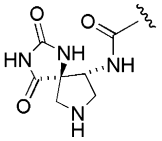
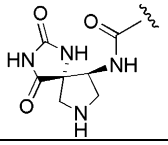
Several of the compounds in Table 1 were modeled in chain A of the TACE crystal structure 2fv5.[3c] The TACE/IK682 complex was minimized and the resulting coordinates used as the starting structure for model building. Each non-hydroxamate ZBG was then built manually onto the shared 4-[(2-methyl-4-quinolinyl)methoxy]phenyl P1′ group and the resulting complex was then fully minimized.[6] Figure 1 shows the modeling results of conformationally constrained hydantoins and barbiturates in TACE compared to the IK682–TACE and barbiturate–MMP-8 crystal structures.

**Table 1.** In vitro potency of various inhibitors of pTACE




| Compound | Zinc binding group (ZBG) | Stereochem | pTACE $IC_{50}$ (nM) |
|---|---|---|---|
| **IK682** |  | (2*R*, 3′*S*) | <1 |
| **2** |  | (rac) | 91 |
| **3** |  | (5*R*) | 170 |
| **4** |  | (5*R*) | 150 |
| **5a** |  | (rac)-trans | 17 |
| **5b** |  | (rac)-cis | 7400 |
| **5c** |  | (5*R*,6*S*)-trans | 11 |
| **5d** |  | (5*S*,6*R*)-trans | 900 |
| **6a** |  | (rac)-trans | 230 |
| **6b** |  | (rac)-cis | 64 |
| **7** |  | (rac) | 98 |
| **8a** |  | (5*R*,6*S*)-trans | 25 |
| **8b** |  | (5*S*,6*R*)-trans | 900 |

The position of the metal ligating group is largely determined by a set of hydrogen bonds observed crystallographically and in the models of the hydantoin containing inhibitors. A subset of these interactions are observed between the protein and the hydroxamic acid moiety in the TACE crystal structure 2fv5 (Fig. 1B). The hydroxamic acid is anchored in the active site by four interactions: two hydrogen bonds via the hydroxyl in contact with E406 and the hydroxamate NH interacting with the backbone carbonyl of G349, and a bidentate ligation of the hydroxyl and carbonyl oxygens to the zinc atom.

The interactions of the non-hydroxamate ZBGs with the TACE active site differ from those of the hydroxamate because they ligate Zn in monodentate fashion and hydrogen bond the same residues but in a fundamentally different way. In the pyrimidinetriones (Fig. 1A), one such interaction is the hydrogen bond between the backbone NH of L348 and the carbonyl at the 4-position of the pyrimidinetrione present in both the MMP-8 (human neutrophil elastase) crystal structure[7] and our model of compound **2**.[5e] Another hydrogen bond is observed between the E406 acid and the C2 carbonyl of the pyrimidinetrione (which in turn coordinates the Zn in monodentate fashion) via the enol tautomer of the heterocycle. The MMP-8 X-ray structure shows a bidentate pyrimidinetrione Zn interaction while our model of compound **2** was consistently monodentate. The difference between the two appears to arise from the highly optimized P1′ group in **2** that will not allow the pyrimidinetrione to shift into the bidentate orientation observed in MMP-8. An additional hydrogen bond between the amide NH and the backbone carbonyl of P437 (observed in several MMP/hydroxamate crystal structures) is sometimes observed, but not strictly conserved.

Models of the hydantoins show a similar hydrogen bonding pattern to the pyrimidinetriones with some important differences (Figs. 1B–D). On the surface, the modeling suggests that the key metal interaction may be bidentate as both the oxygen and nitrogen atom are consistently observed within ~2.0 Å of the zinc. However, while the hydantoin nitrogen is in close proximity to the zinc, its lone pair is not properly oriented to form a direct interaction because it is directed away from the zinc. This suggests that, unlike the hydroxamates, the hydantoins form only a monodentate interaction with the zinc through the C2 carbonyl. Based on the models, we predict that the hydantoin has been ionized by the active site E406 general base in what resembles an enol tautomeric form stabilized by hydrogen bonding to

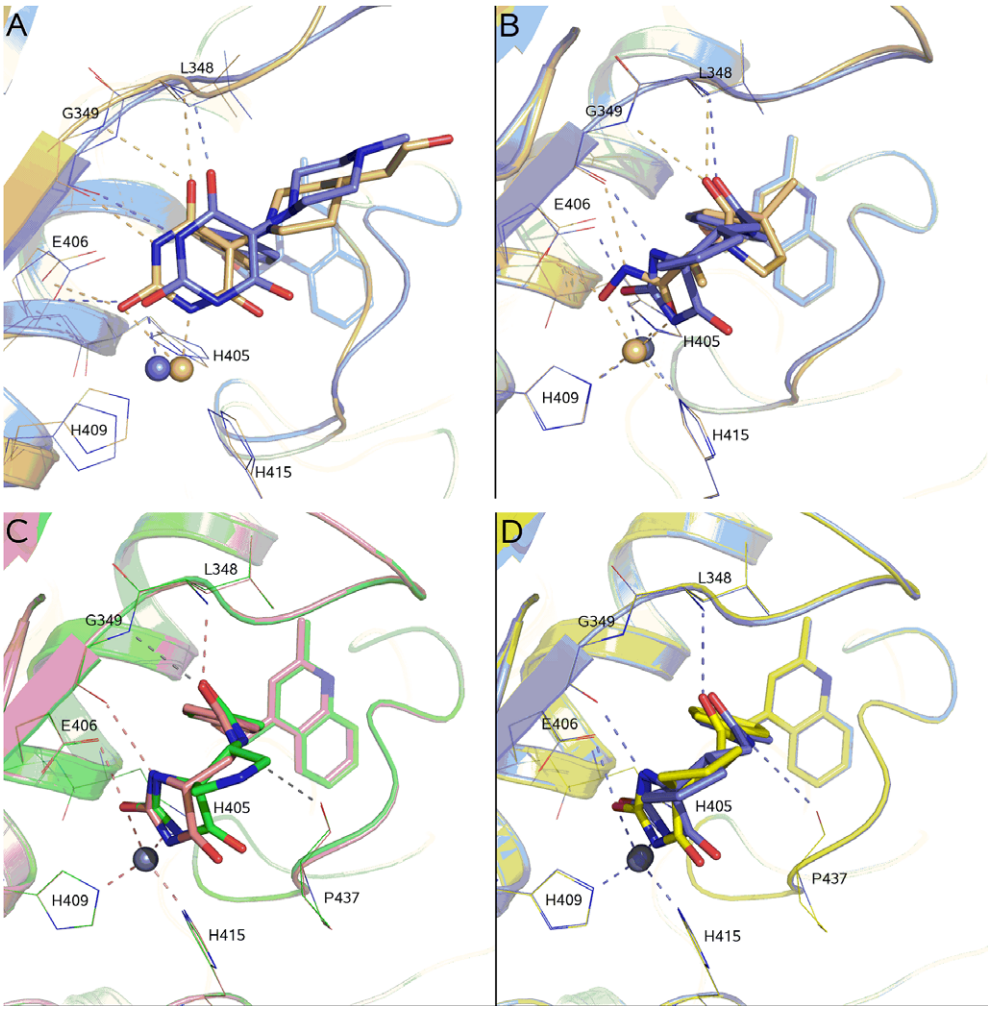



**Figure 1.** (A) Hydrogen bonding of 5-(4-(2-hydroxyethyl)piperidin-1-yl)-5-phenylpyrimidine-2,4,6-trione in the MMP-8 crystal complex (orange) as compared with the 2,4,6-pyrimidinetrione **2** modeled in TACE (blue). (B) Hydrogen bonding interactions of the spirohydantoin **5c** modeled in TACE (blue) and compared to the crystallographic binding mode of IK682, shown in yellow. (C) Conserved interactions in the zinc binding region for acyclic hydantoin (5*R*)-**7** (pink) and spirohydantoin **8a** (green) in TACE. (D) Conformational comparison of **5c** (blue) and **6b** (yellow) modeled in TACE. Representative hydrogen bonds and metal interactions (2.0–3.5 Å) are indicated with dashed lines. The dashed blue line is an optional hydrogen bond to G349 and the P437 interaction is also intermittently observed. Figure generated with Pymol.

E406, but the precise location of the proton that forms this hydrogen bond is not clear. Because the hydantoins lack the extra heterocyclic carbonyl found in the pyrimidinetriones, they can compensate by using the exocyclic amide carbonyl in both the forward amide (**3**, **4**, **5**, and **6**) and reverse amide (**7** and **8**) to hydrogen bond to the backbone NH of L348 and G349 analogous to hydroxamate **IK682**.

Figure 1C illustrates how spirohydantoin **8a** has been constrained by annulation into a single conformation of its acyclic (and less active) congener (5*R*)-**7**. The TACE-selective 4-[(2-methyl-4-methyl-4-quinolinyl)-methoxy]phenyl P1′ group resides snugly in the S1′ and S3′ sites of TACE. An apparent paradox of the data in Table 1 is how **5c** (trans; 11 nM) and its 1 methylene shorter homolog **6b** (cis; 64 nM) are both active and yet have opposite relative stereochemistry. Inspection of the models in Figure 1D shows that in fact, the two achieve superimposable low energy conformation.

From the ligand perspective, the measured p$K_a$ of hydantoin **5d** (Table 1) in water is 8.9, almost exactly the same as the p$K_a$ of hydroxamate **IK682** which is 9.0, but less acidic than pyrimidinetrione **2** with a p$K_a$ of 7.2–7.8. However, initial coordination of the carbonyl of these inhibitors to the Lewis acidic active site Zn ion decreases their p$K_a$ by several orders of magnitude for deprotonation by (or H-bonding to) the active site E406 general base. Density functional computational methods have been used to calculate a decrease in 3.3 p$K_a$ units for a hydroxamic acid upon coordinating the TACE active site.[8] A comparable decrease in acidity for the hydantoins (p$K_a$ of 5.6) and pyrimidinetriones (p$K_a$ of ~4.2) upon coordination to the Zn ion likewise puts them in the range of 'deprotonated' by the active site Glu406 (calcd p$K_a$ of 5.9)—a prototropic tautomer that for all intensive purposes is the enol form. It is interesting to note that both pyrimidinetriones and hydantoins are known to be in equilibrium with their enol tautomers in solution.[9] As enol tautomers, the hydantoins

and pyrimidinetriones may act more like a transition state mimics than substrates and it is conceivable that it is the enol rather than keto form that initially binds TACE. Analysis of the inhibitor SAR and stereochemical selectivity provides further validation of the models (Table 1). The hydantoin group of **3** is intolerant of N-substitution as methylation of either results in a complete loss of potency.[5a] The model predicts a hydrogen bond between the N1 amide NH of the hydantoin and the backbone carbonyl of G349, which would be destroyed by the methyl substituent. Methylation of the N3 nitrogen disrupts the metal interaction both directly via a steric clash with the Zn ion and indirectly by precluding deprotonation of the hydantoin by, or hydrogen bonding to, E406.

Conformationally constraining the 5 and 6 positions via cyclization to spirocyclopentyl **5c** provides a 13-fold increase in TACE potency to 11 nM. Since the spiro linkage is predicted to be solvent exposed, this region should be tolerant to substitution. Indeed, **8** as well as a variety of pyrrolidine substituents (see preceding paper) do not affect TACE potency nor does adding an additional carbon to form the spirocyclohexyl hydantoin.

The stereochemical preference of the enzyme is also consistent with the models. The trans relative stereochemistry of **5a** was found to be 435-fold more active than its cis diastereomer (**5b**). Upon resolution and determination of absolute stereochemistry, it was found that the trans-(5*R*,6*S*) enantiomer is 81-fold more active than its antipode trans-(5*S*,6*R*) (**5c** and **5d**, respectively). Inspection of the model reveals that the cis compound cannot achieve a conformation that simultaneously makes hydrogen bonds to L348 or G349 and the hydantoin hydrogen bonding/metal interactions. The trans-(5*S*,6*R*) stereochemistry pushes the spiro linkage into the protein backbone, completely disrupting the hydrogen bonding and metal interactions. The same stereochemical prerequisite holds true for **8a** and its much less active enantiomer **8b**. Another interesting observation is that diastereomers **6a** and **6b** differ by only 3.5-fold in TACE activity. Modeling of both stereochemistries shows that the hydantoin interactions are not substantially disrupted in the trans configuration due to compensatory cyclopentane puckering (not shown).

In conclusion, we favor a mechanism of inhibition whereby the carbonyl of the ZBG displaces the active site zinc-bound water (like amide substrate) which decreases its p$K_a$ by several log units. The general base E406 that typically deprotonates this water molecule for nucleophilic attack on an amide substrate instead deprotonates the hydroxamate/barbiturate/hydantoin, further enhancing its interaction with the Zn metal—a reasonable possibility considering the Lewis acid-induced acidity of ZBGs with a p$K_a$ of 7–9 should be less than or equal to the E406 conjugate base. In addition, all three ZBGs have an NH that hydrogen bonds to G349. While the pyrimidinetriones hydrogen bond to L348 and G349 directly via the heterocycle. The hydantoins and hydroxamates use a pendant amide carbonyl to hydrogen bond to L348 and optionally, G349.

Many have attempted to develop drug-like, non-hydroxamate zinc metalloprotease inhibitors based on the assumption that a strong $K_d$ for zinc will translate into a potent $K_i$ for the inhibitor.[10] While logical from a bioinorganic perspective, this approach does not address the 'drugability' of the ligand which may exhibit general toxicity or complex PK/PD due to adventitious chelation to metals other than zinc (e.g., hydroxamates bind Fe(III) $10^6$- to $10^{11}$-fold stronger than Zn(II)).[11] The success of hydantoins and pyrimidinetriones as TACE inhibitors does not rely upon their strong association with the active site metal.[12] Rather, these heterocycles have compensated for the reduced, intrinsic $K_d$'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. When substituted with the same 4-[(2-methyl-4-quinolinyl)methoxy]phenyl P1′ group, the hydantoins display good $IC_{50}$s (11- to 91-fold less potent than hydroxamates—a difference of 1–3 kcal/mol)[13] while retaining high selectivity against several MMPs. The use of hydantoins and other non-hydroxamate heterocycles as obligatory Zn metal ligands for the inhibition of zinc metalloproteases will be reported in due course.

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6. 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 were allowed to move freely, residues 5–10 were constrained with a force constant of 200, and residues >10 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 barbiturate and hydantoins 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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12. Zn(II)-barbiturate complexes are rare in the inorganic literature and Zn(II)-hydantoinoate complexes are virtually unknown.
13. Naturally, this comparison discounts important differences in ligand desolvation, H-bonding strength, and entropy. Nevertheless, it is impressive that a TACE-optimized hydantoin bearing the same P1′ group as a TACE-optimized hydroxamate is just 10-fold less potent, especially considering hydroxamates get superior binding via a bidentate $Zn(His)_3$ interaction in place of what is quite likely a less optimal monodentate $Zn(His)_3$ interaction for the hydantoins.