Novel Applications of Tetrazoles Derived from the TMSN3-Ugi Reaction
Zhao, Ting

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Chapter 6

Review: effective arginase inhibitors as promising therapeutics for the treatment of arginase induced NO deficiency related diseases

Ting Zhao, Alexander Dömling, In preparation.
6.1 Introduction

Arginase is a binuclear manganese containing enzyme that catalyzes the hydrolysis of L-arginine with release of urea and ornithine (Figure 6.1). In mammals two types of arginase are identified: arginase I and II. They are encoded by different genes\(^1\) and show varied relative levels in different tissues,\(^2\)-\(^5\) but share \(\sim 60\%\) sequence identity. Human arginase I is a cytosolic enzyme containing 322 amino acid residues, which is expressed mainly in liver and also found at a low level in nonhepatic tissues. It mainly functions in the urea cycle for ammonia detoxification. Human arginase II is a mitochondrial enzyme dominantly expressed in nonhepatic tissues such as kidney and blood vessels.\(^6\) It primarily functions in biosynthesis of polyamines and amino acids including ornithine, proline, and glutamate.\(^6\) Arginase competes for the common substrate L-arginine with nitric oxide synthase (NOS) which produces nitric oxide (NO). In human living system, NO plays crucial roles in vascular and neuronal signal transduction, smooth muscle contractility, bioenergetics, platelet adhesion and aggregation, immunity, and cell death regulation.\(^7\)-\(^11\) The decreased supply of NO induces many pathologies, including inflammatory disorders, and immune system imbalance, which are associated with various symptoms like hypertension, cardiovascular dysfunctions, neurodegeneration, arthritis, asthma and septic shock (Table 6.1).\(^12\)-\(^16\) However, it has been proven that increasing the administration of L-arginine did not lead to a higher level of NO, but resulted in a higher arginase activity. This is due to the fact that the \(V_{\text{max}}\) of arginase is approximately 1000-fold greater than that of NOS, although NOS possess a much higher affinity (\(K_m\)) for L-arginine than arginase.\(^17\) In fact, the upregulation of arginase is not the only approach to decrease the intracellular L-arginine levels, which are regulated by at least three distinct mechanisms including cellular uptake of cation amino acid (CAT) transporters, metabolism of NO synthase (NOS) and arginase, and recycling of L-citrulline and reutilizing it in other tissues. In this chapter, we focus on research about effective arginase inhibitors as promising therapeutics for the treatment of arginase induced NO deficiency related diseases without interfering with the bioavailability of L-arginine for NOS. In the following sections, the developments in this field are summarized, and the structure-activity relationship (SAR) is discussed to reveal how these inhibitors function for the arginase inhibition.
Figure 6.1. The metabolism of L-arginine uptake and the proceeding process in NO-producing cells. Firstly cationic amino acid transporters (CAT) facilitate L-arginine to enter into the cell. Subsequently, L-arginine is metabolized by nitric oxide synthase (NOS) or arginase with the products L-citrulline and NO. In the citrulline-NO cycle, L-citrulline is resynthesized to L-arginine catalyzed by the enzymes argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL)) with argininosuccinate as intermediate. Besides, L-arginine is converted by arginase into L-ornithine and urea. Ornithine decarboxylase (ODC) converted L-ornithine to putrescine, which might be further metabolized to aminotransferase (OAT). L-proline might be derived from pyrrolidine-5-carboxylate metabolism. Noteworthy is that these pathways are not required to be coexpressed within the same cell type.

Table 6.1. The role of NO in various biological systems and the related NO deficiency induced diseases

<table>
<thead>
<tr>
<th>Biological system</th>
<th>The role of NO</th>
<th>Typical disease</th>
</tr>
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<tbody>
<tr>
<td>Cardiovascular system</td>
<td>Vasodilatate vessels</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td></td>
<td>Inhibit of vasoconstrictor influence and platelet adhesion to the vascular</td>
<td>Hypertension</td>
</tr>
<tr>
<td></td>
<td>endothelium, also leukocyte adhesion to vascular endothelium</td>
<td>Hypercholesterolaemia</td>
</tr>
<tr>
<td></td>
<td>Antiproliferation</td>
<td>Diabetes</td>
</tr>
<tr>
<td>System</td>
<td>Function</td>
<td>Diseases/Diseases</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Reproductive system</td>
<td>Modulate vaginal smooth muscle contractility and blood flow in the animal model</td>
<td>Abnormal male and female genital sexual arousal response</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genital arousal disorders, Penile erectile dysfunction in men, Vaginal and clitoral engorgement insufficiency in women</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>Induce the relaxation of pulmonary vessel</td>
<td>Asthma</td>
</tr>
<tr>
<td></td>
<td>Mediating inflammatory reaction in airway</td>
<td>Chronic obstructive pulmonary disease (COPD)</td>
</tr>
<tr>
<td>Immune system</td>
<td>Induce apoptosis in chondrocytes, as observed in cartilage destruction typical of osteoarthritis</td>
<td>Allergic asthma, Pulmonary hypertension, Arthritis, Cancer, Autoimmunity</td>
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<td></td>
<td>Promote tumor growth via polyamine synthesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Down-regulate NO-mediated tumor cytotoxicity</td>
<td></td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Diffuse back to the presynaptic terminal and increase cGMP levels through the activation of soluble guanylate cyclase (sGC)</td>
<td>Cerebral ischemia, Neurodegeneration, Parkinson’s disease, Alzheimer’s disease, Multiple sclerosis, Amyotrophic lateral sclerosis and dementia</td>
</tr>
<tr>
<td></td>
<td>Signal through ion channels including sodium, voltage-gated calcium, calcium-activated and ATP-sensitive potassium, and cyclic nucleotide-gated channels, as well as AMPA receptors (AMPAR) to modulate synaptic strength and intrinsic postsynaptic neuronal excitability</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal system</td>
<td>Initiate and sustain the ulcerative colitis</td>
<td>The ulcerative colitis: a nasty human inflammatory bowel disease (IBD)</td>
</tr>
<tr>
<td>6.2 Structures of human arginase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 6.2.1 Human arginase I

Human arginase I exists mainly as a 105 kDa trimer. Each monomer contains a binuclear Mn (II) center that is critical for its catalytic activity. The overall fold of this monomer belongs to the α/β family, consisting of a parallel, 8 stranded β-sheet flanked on both sides by several α-helices. For the wild-type arginase I, the binuclear Mn (II) center (MnA and MnB) is located at the bottom of a ~15 Å-deep active site cleft in each monomer with a distance of 3.3 Å.\(^{19,20}\) MnA, the more deeply situated metal ion, has a square pyramidal coordination formed by His101 (Nδ), Asp124 (Oδ1, bidentate), Asp128 (Oδ1), His141 (Nδ), Asp232 (Oδ1, monodentate) and a solvent molecule. The solvent molecule bridges both MnA and MnB, as well as, donating a
hydrogen bond to Asp128. MnB is coordinated with terminal ligands His126 (N\(\delta\)), Asp124 (O\(\delta_2\)), Asp232 (O\(\delta_1\)), Asp234 (bidentate O\(\delta_1\) and O\(\delta_2\)), Thr246 (O\(\gamma\)) and the bridging solvent molecule in distorted octahedral fashion (Figure 6.2).

**Figure 6.2.** (a) The overview of human arginase I with 8 stranded \(\beta\)-sheet flanked on both sides by several \(\alpha\)-helices; and (b) Contacts of amino acid residues of human arginase I (water mediated hydrogen bonds: green line) and contacts of the Mn atoms with human arginase I (purple line) (PDB: 2PHA).

### 6.2.2 Human arginase II

Human arginase II exists primarily as a 129 kDa trimer, which is a bit larger than human arginase I.\(^{21}\) Higher order oligomers (hexamer, nonamer, and 18-mer) of human arginase II were also observed.\(^{21}\) At present, the biological function of human arginase II is still poorly understood, however, it is generally acknowledged that human arginase II mainly contributes to polyamines and collagen synthesis due to the incomplete urea cycle in nonheptic tissues. Noteworthy to mention is that the apo structure of human arginase II has not been disclosed, yet.

### 6.2.3 Catalytic mechanism of human arginase

The catalytic mechanism of human arginase proposed by Kanyo et al. is most consistent with available biochemical, enzymological, and structural data (Scheme 6.1).\(^{20}\) Several important features are included in this mechanism: (1) a precatalytic binding side chain for arginine in which the side chain of Glu277 plays a key role in L-arginine recognition and formation of a salt bridge between Glu277 and L-arginine to orient its scissile guanidinium carbon directly in
line for nucleophilic attack by the metal-bridging hydroxide ion, (2) attack of a nucleophilic metal-bridging hydroxide ion, (3) formation of a neutral tetrahedral intermediate that is stabilized by the binuclear Mn$^{2+}$ center, and (4) a possible role for His141 as a proton shuttle in mediating proton transfer between the active site and bulk solvent.

The role of the Mn$^{2+}$ center was studied by Carvajal et al. They showed that the presence of Mn$^{2+}$ on the quaternary structure of human arginase I is apparently required to maintain the active conformation of the enzyme. They found that withdrawal of the metal ions results in the disassociation of arginase to subunits. These subunits without manganese are detected to be inactive in an enzyme assay. However, the activity can be completely restored by the addition of Mn ions. Moreover, the reactivation treatment normally causes subunits to form an active tetrazyme. Later they confirmed that human arginase I can also be activated by Ni$^{2+}$ and Co$^{2+}$. In addition, the replacement of Mn$^{2+}$ with other metal ions in human arginase I resulted in a significant change in $V_{\text{max}}$ but showed no effect on the $K_m$ or $K_i$. This suggested that the metal ion in human arginase I is not responsible for binding L-arginine to arginase. The metal ion probably serve as a catalytic entity to facilitate the activation and hydrolytic breakdown of the enzyme-bound substrate. Furthermore, the true catalytic groups may be moved into reactive positions as a consequence of a conformational change induced by the metal ion.

![Scheme 6.1. Proposed mechanism for the hydrolysis of L-arginine catalyzed by arginase.](image)

Additionally, Glu 277 and His 141 are important amino acid residues for the catalytic activity of human arginase. Glu 277 is located deeply in the active-site cleft, 4.5 Å away from Mn$^{2+}$.

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An ideal salt linkage with the substrate guanidinium group would come from a roughly 20° rotation about $\chi_1$ of this side chain. This interaction would introduce the electrophilic guanidinium carbon of L-arginine directly over the solvent molecule that bridges two metals. This solvent molecule probably functions as a nucleophilic hydroxide ion in the active catalyst. Since the guanidium group of L-arginine has a high $pK_a$ value of 13.5, it is impossible to directly bind to the metal(s). However, the $K_m$ of L-arginine is not affected by the metal variations, even by metal depletion. This indicates that the Michaelis complex is not derived from a substrate-metal interaction.

On the other hand, His141 possibly acts as a shuttle which transfers protons to and from bulk solvent. It was found that arginase remains about 10% activity compared to the wild-type enzyme when His141 is substituted with asparagine (H141N). Given the fact that the side chain of His141 is located about half-way out of the active site cleft and 4.2 Å away from the metal-bridging solvent molecule, the remained catalytic activity of H141N arginase could be explained by the fact that the direct proton transfer with bulk solvent still occurs even in the absence of His141.

6.3 The inhibitors for human arginase I and II

Arginase activity is an effective therapeutic target for NO-deficiency induced diseases. Many moderate and potent inhibitors have been synthesized and their inhibitory potency assays in vitro are tested.

6.3.1 ABH and BEC

In designing protease inhibitors, it is thought that the electron-deficient boron atom can facilitate the addition of a suitable nucleophile, yielding a stable, anionic tetrahedral intermediate. This could be attributed to the reason that the conversion of boronic acids from neutral sp² to anionic sp³ form facilitates the formation of transition state analogues. Through attack of hydroxyl nucleophile of the solvent, the tetracoordinate boronate complex mimics the tetrahedral intermediate produced in the hydrolysis of L-arginine. Moreover, boronic acids are effective serine protease and aminopeptidase inhibitors. Based on the ternary arginase-ornithine-borate complex, Christianson et al. synthesized a boronic acid analogue of arginine, 2(S)-amino-6-boronohexanoic acid (ABH, Scheme 6.2). This is the first boronic acid-based arginine analogue which functions as human arginase inhibitor, with an IC₅₀ and $K_d$ value of 0.8 µM, and ≤ 0.1 µM (pH 7.5 and 9.0), respectively. The measured high affinity between human arginase I and ABH indicated that the tetrahedral borate mimics binding interactions.
postulated for the tetrahedral transition state(s) in the catalytic reaction. Moreover, they analyzed the cocrystal structure of human arginase I-ABH complex determined at 1.7 Å resolution from a crystal perfectly twinned by hemihedry. The result showed that ABH binding changes the geometry of the Mn$^{2+}$A coordination polyhedron from square pyramidal in the wild-type enzyme to distorted octahedral in the enzyme–inhibitor complex. There is no net change in the coordination geometry of Mn$^{2+}$B, which still remains distorted octahedral. The Mn$^{2+}$A–Mn$^{2+}$B separation increases slightly, from 3.3 Å in the native enzyme to 3.4 Å in the enzyme–inhibitor complex. However, this slight difference may be within the experimental error (Figure 6.3).

**Scheme 6.2.** The boronic acid analogues of L-arginine, (S)-amino-2-borono-6-hexanoic acid (ABH) and (S)-(2-boronoethyl)-L-cysteine (BEC), undergo a nucleophilic attack by the metal-bridging hydroxide ion in the arginase active site. The resulting tetrahedral boronate anions mimic the tetrahedral intermediate (and its flanking transition states) in the hydrolysis of L-arginine.

**Figure 6.3.** (a) The three direct and four water-mediated hydrogen bonds with enzyme residues at the mouth site of human arginase I (direct hydrogen bonds: green line, water...
mediated hydrogen bonds: purple line); and (b) the overview of hydrogen bonding between ABH and human arginase I (green line) and contacts of the Mn atoms with human arginase I (purple line) (PDB: 2AEB).

Interestingly, the lack of a corresponding hydrogen bond between the negatively charged carboxylate group of Glu 277 and both boronate hydroxyl groups O2 and O3 which exists in L-arginine binding state of human arginase I may result from electrostatic repulsion with the negatively charged boronate anion. However, an extensive network of hydrogen bonds at the mouth site of α-NH2 and α-COOH of human arginase I significantly compensated to this electron static repulsion. This well explain the corresponding high affinity and potent inhibitory activity (Figure 6.3).

In addition, a sulfur atom was introduced into the ABH backbone, which resulted in another boronic acid-based arginine analogue (S)-(2-boronoethyl)-L-cysteine (BEC). BEC is a slow-binding competitive inhibitor for human arginase I and II without inferencing with NOS bioactivity.26, 27 The $K_d$ of BEC was 270 nM at pH 8.5 to human arginase I,26 and the $K_i$ was 30 nM at pH 9.5 to human arginase II.27 The X-ray crystal structures of human arginase I and II-BEC complex (Figures 6.4 and 6.5) are similar to that of human arginase I and II–ABH complexes. Besides, the boronic acid moiety undergoes a same nucleophilic attack in a similar fashion to that of ABH, forming a tetrahedral boronate anion that bridges the binuclear manganese cluster, thereby mimicking the tetrahedral intermediate (and its flanking transition states) in human arginine hydrolysis reaction.

The overview of human arginase I and II-BEC complex are shown in Figures 6.4 and 6.5. Several differences are observed in the binding mode of BEC to the mouth site of human arginase I and II, and also found in the tetrahedral boronate binding fashion.26 On the one hand, α-COOH and α-NH2 groups of BEC are bonded to the mouth site of the catalytic pocket of human arginase I through three direct and four water-mediated hydrogen bonds, whereas in the human arginase II-BEC complex, two direct and five water-mediated hydrogen bonds were observed. The more water-mediated interactions between human arginase II and BEC are probably derived from the larger volume of the human arginase II active site cleft (554 Å3) than that of human arginase I (440 Å3).27 On the other hand, the arginase I-BEC complex presents a slightly weaker hydrogen bond (3.3 Å) donated by boronate hydroxyl O2 to E277 than that in the arginase II-BEC complex (3.0 Å).
According to enzyme assays, the affinity of human arginase I-BEC complex is about 50-fold lower than that of human arginase I-ABH (ABH, $K_d = 5$ nM, BEC, $K_d = 270$ nM). It was found that the tetrahedral boronate anions of BEC and ABH form the nearly identical coordination interactions with the binuclear manganese cluster, and hydrogen bond interactions with Asp128, as well as the backbone carbonyl of His141. However, the hydrogen bond interactions of the $\alpha$-COOH and $\alpha$-NH$_2$ groups of BEC and ABH have slightly different
geometries and bond lengths. These subtle differences probably are caused by the geometric differences in the thioester moiety of BEC. One is that the C-S bond length (1.8 Å) is a little bit longer than that of the C-C bond (1.5 Å). Another is that the C-S-C bond angle is 114°, larger than that of C-C-C bond (97°). As a result, human arginase-BEC possesses a slightly lower affinity than that of human arginase-ABH.28

6.3.2 NOHA and nor-NOHA

\(N\)-hydroxy-L-arginine (NOHA), an intermediate of NO biosynthesis, is a moderate inhibitor for arginase (Figure 6.6). A Crystal structure of an arginase-NOHA complex reveals that NOHA displaces the metal-bridging hydroxide ion and bridges the binuclear manganese cluster (Figure 6.7). Moreover, early studies showed that amino acid L-lysine is an arginase inhibitor.29-31 To improve the inhibitory potency of NOHA, Chrisianson et al. synthesized a novel human arginase inhibitor \(N^{\omega}\)-hydroxy-nor-L-arginine (nor-NOHA).32 Among nor-NOHA, NOHA and L-lysine, nor-NOHA showed the highest affinity, with a \(K_d\) of 517 nM. The crystal structure of human arginase-nor-NOHA suggests that inhibitor binding does not cause any significant conformational changes in the active site. A reasonable higher potency of nor-NOHA could be explained by the shorter coordination distances of \(\text{Mn}^{2+}_A \cdots \cdot \text{O}\) and \(\text{Mn}^{2+}_B \cdots \cdot \text{O}\). This is because the \(N^\zeta\)-OH group of nor-NOHA displaces the metal-bridging hydroxide ion and nearly symmetrically bridges the binuclear manganese cluster. The metal ion coordination geometry is improved, which is tighter (\(\text{Mn}^{2+}_A \cdots \cdot \text{O} = 2.1 \text{ Å}\) and \(\text{Mn}^{2+}_B \cdots \cdot \text{O} = 2.2 \text{ Å}\)) (Figure 6.8).

\[\text{Figure 6.6. The structures of human arginase inhibitors L-lysine, NOHA and nor-NOHA.}\]

In addition, the \(\alpha\)-COOH and \(\alpha\)-NH\(_2\) substituents of the human arginase I-nor NOHA complex form three direct and four water-mediated hydrogen bonds with amino acid residues of the enzyme, similar to that of ABH (Figure 6.3 and Figure 6.8). However, the hydroxyl guanidinium group is sandwiched between the imidazole side chains of His141 and His126 and appears to form \(\pi\text{-}\pi\) stacking interactions, which is different from that of ABH.
Figure 6.7. (a) The overview of hydrogen bonding of human arginase I-NOHA complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line); (b) the overview of hydrogen bonding between NOHA and human arginase I (green line) and contacts of the Mn atoms with human arginase I (purple line) (PDB: 3LP7).

Figure 6.8. The overview of hydrogen bonding of human arginase I-nor-NOHA complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line); (b) the overview of hydrogen bonding between nor-NOHA and human arginase I (green line) and contacts of the Mn atoms with human arginase I (purple line) (PDB: 3KV2).

6.3.3 α-Substituted ABH derivatives

6.3.3.1 Simple alkyl substituted ABH derivatives at α center

The crystal structures of human arginase-ABH and -BEC complexes indicates that a conserved hydrogen bond network is very important for the molecular recognition of α-NH₂ and α-COOH groups of inhibitors. This recognition significantly contributes to enzyme-
inhibitor affinity. Inspired by the fact that α-difluoromethylornithine (DFMO) is an analogue of ornithine which is a hydrolysis product of L-arginine, Christianson et al. synthesized racemic 2-amino-6-borono-2-methylhexanoic acid (MABH) and racemic 2-amino-6-borono-2-(difluoromethyl)hexanoic acid (FABH) as human arginase I inhibitors (Figure 6.9), and studied their inhibitory activities. Both MABH and FABH showed a lower affinity compared with ABH: human arginase I-MABH and -FABH were 49-fold and 1889-fold less potent, respectively. The weak affinity could not be explained by the crystal structures of the human arginase I-MABH and -FABH, which reveal that the α-NH₂ and α-COOH groups of MABH and FABH bind similarly within the active sites of human arginase I; and no significant conformational changes are induced in the active site or elsewhere in the protein structure (PDB: 3GN0, 3SJT and 3SKK) (Figures 6.10 - 6.12). However, it might be possible to recapture and enhance the affinity with a longer α-substituent which could introduce more interactions directly towards the Thr136 region. Furthermore, the replacement of hydrogen by fluorine increases the lipophilicity and metabolic resistance of organic molecule, as fluorine can confer greater pharmacokinetic and pharmacodynamics stability. 

![Figure 6.9](image)

**Figure 6.9.** The structures of human arginase inhibitors DMFO, MABH and FABH.

![Figure 6.10](image)

**Figure 6.10.** (a) The overview of hydrogen bonding of human arginase I-DMFO complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line); (b) the overview of hydrogen bonding between DMFO and human arginase I (water mediated...
hydrogen bonds: purple line) and contacts of the Mn atoms with human arginase I (purple line) (PDB: 3GN0).

**Figure 6.11.** (a) The overview of hydrogen bonding of human arginase I-MABH complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line); (b) contacts of human arginase I with MABH and the Mn atoms (purple line) (PDB: 3SJT).

![Diagram](image1)

**Figure 6.12.** (a) The overview of hydrogen bonding of human arginase I-FABH complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line); (b) contacts of human arginase I with FABH and the Mn atoms (purple line) (PDB: 3SKK).

![Diagram](image2)

6.3.3.2 ABH derivatives with long chain substitution at α center

Recently, a series of novel arginase inhibitors were reported, which are substituted at the α center with a tertiary amine linked via a two carbon chain (Figure 6.13). Among the
synthesized inhibitors, ABDEH, ABHPEH and ABPEH showed remarkable improved inhibitory potency for both human arginase I and II, which is significantly higher (5- 10- fold) than that of ABH. The improved potency could be explained by an additional water-mediated interaction between the tertiary amine and the carboxylic acid side chain of Asp200. Besides, these two functional groups might function as ammonium and carboxylate ions, respectively, due to the close proximity of the water molecule to the amine nitrogen (2.47 Å) and the nearly symmetrical interactions of the water with both carboxylic acid oxygens (3.19 and 3.72 Å) (Figure 6.14). However, the chiral properties of these inhibitors was not mentioned.

**Figure 6.13.** A selected list of human arginase inhibitors with a tertiary amine linked via a two carbon chain at α center.

**Figure 6.14.** The additional hydrogen bonding between the tertiary amine of ABHPEH and Asp200 (purple line), and the overview of hydrogen bonding of α-COOH and α-NH₂ with human arginase I (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB: 4IE3).

Later, the same research group reported another potent human arginase inhibitor (R)-ABPEH, which possessed a much lower IC₅₀ (223 nM for human arginase I and 559 nM for human arginase II) than that of ABH. This could be rationalized by a formation of new water mediated hydrogen bonds with Asp181 and Asp183 (Figure 6.15).³⁶
Moreover, methyl substitution at \( \alpha \)-N position, the resulting inhibitor \((R)\)-BMPEH was found to be a more potent human arginase inhibitor. The tested IC\(_{50}\) of the inhibitor \((R)\)-BMPEH for human arginase I and II reduced to 60 and 67 nM, respectively. Compared with ABH, the inhibitory potency increased 24-fold for human arginase I and 28-fold for human arginase II. This improved potency could be attributed to a gain in entropy due to two reasons. The first is the liberation of water molecule, while the second is the enhancement of existing hydrogen bonds probably due to the minor conformational changes of the inhibitor and enzyme. On the other hand, the more thermodynamically stable chair conformation for inhibitor \((R)\)-BMPEH could explain its higher inhibitory potency than \((R)\)-ABPEH (Figures 6.15 and 6.16).

The inhibitor \((R)\)-BMPEH showed nearly the same inhibitory potency for human arginase I and II, indicating it has no selectivity for both the enzymes. These could reasonably explain by two reasons: (1) the crystal structures of human arginase I- and II-(\(R)\)-BMPEH are quite similar; (2) the distances between the water molecule and the aspartic acids are all within the hydrogen bonding range (Figure 6.16).

**Figure 6.15.** (a) The overview of hydrogen bonding of human arginase I-(\(R)\)-ABPEH complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB: 4HWW); (b) the overview of hydrogen bonding of human arginase II-(\(R)\)-ABPEH complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB 4HZE).
6.3.3.3 ABH derivatives containing direct cyclic substitution at α center

By combining multicomponent reaction and classical synthesis, series of inhibitors containing direct cyclic substitution at α center were obtained. The substituted groups included a piperidine ring linked directly to a quaternary amino acid center, and tropane in which two carbons bridge in the piperidine ring (Figure 6.17).37

![Diagram of inhibitors]

**Figure 6.16.** (a) The overview of hydrogen bonding of human arginase I-(R)-BMPEH complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB: 4HXQ); (b) the overview of hydrogen bonding of human arginase II-(R)-BMPEH complex at the mouth site (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB: 4I06).

**Figure 6.17.** The structures of human arginase inhibitors with a piperidine ring linked directly to a quaternary amino acid center and with two carbon bridged in the piperidine ring.
For inhibitors containing a piperidine ring linked directly to a quaternary amino acid center, a water-mediated interaction was identified between the basic nitrogen and the carboxylic acid side chain of Asp181 and Asp183 in human arginase I, and Asp200 and Asp202 in human arginase II. This interaction reasonably explain their higher potency than that of ABH in most cases.

Surprisingly, with an additional two carbon bridge, the obtained inhibitors containing tropane moiety possess significantly much better inhibition for human arginase I and II. The tropane moiety of inhibitors benefits to lock the piperidine ring in a boat conformation to further enhance the interaction between the nitrogen atom of tropane and residues of enzyme, which explain the improvement of inhibitory potency.

![Chemical structures of inhibitors](image)

**Figure 6.18.** The most two potent inhibitors for both human arginase I and II, as reported in literature up till now.

Inhibitors 2-amino-6-borono-2-(6-(4-chlorobenzyl)-6-azabicyclo[3.1.1]heptan-3-yl)hexanoic acid (ABCAHH) and 2-amino-6-borono-2-(6-(3,4-dichlorobenzyl)-6-azabicyclo[3.1.1]heptan-3-yl)hexanoic acid (ABDCAHH), are the two most potent inhibitors for human arginase I and II, as reported in literature up till now (Figure 6.18). However, the X-ray crystal structure of the human arginase-ABCAHH complex is not disclosed yet. In fact, ABCAHH and ABDCAHH possess almost identical structures, with a tiny difference at meta position (with/without Cl substituent). According to the X-ray crystal structure of human arginase-ABDCAHH complex, it was claimed that the excellent inhibitory potency can be
explained by two facts. One is that the fixation of the geometry of the ring forces side chain nitrogen atom towards the right position, with gaining additional entropy. The other is due to the direct interaction between ABDCAHH and Asp202 instead of water mediated contact (Figure 6.19).

Figure 6.19. (a) The overview of hydrogen bonding of compound 2-amino-6-borono-2-(6-(3,4-dichlorobenzyl)-6-azabicyclo[3.1.1]heptan-3-yl)hexanoic acid (ABDCAHH) containing tropane moiety with human arginase II (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB: 4IXU); and (b) the overview of hydrogen bonding of compound 2-amino-6-borono-2-(1-(4-chlorobenzyl)piperidin-4-yl)hexanoic acid (ABCPHA) containing a piperidine ring linked directly to a quaternary amino acid center with human arginase II (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB: 4IXV).

6.3.4 Other human arginase inhibitors

Other types of human arginase inhibitors are also reported, with however low inhibitory potency in most cases. The chemical structures, inhibitory potency and PDB codes of the crystal of inhibitor-human arginase complex are summarized in Table 6.2. Moreover, the detailed interactions between the inhibitor and human arginase are depicted in Figures 6.20 – 6.24.

In general, the low inhibitory potency could be explained by the short side chain of the inhibitors which could not coordinate with the binuclear Mn (II) center in active site of human arginase, resulting in the lack of the tetrahedral intermediate formation. As for (S)-2-amino-6-nitrohexanoic acid (ANH), this is attributed to the fact that the nitro group forms the trigonal planar structure rather than the tetrahedron.
<table>
<thead>
<tr>
<th>Inhibitor[a]</th>
<th>Structure</th>
<th>Potency</th>
<th>Enzyme</th>
<th>PDB code</th>
<th>The reason for the weak potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGPA 38</td>
<td><img src="image" alt="structure" /></td>
<td>55 mM ((K_i))</td>
<td>human arginase I</td>
<td>4FCI</td>
<td>no favorable interactions between the short guanidinium side chain and the enzyme active site.</td>
</tr>
<tr>
<td>AECA 39</td>
<td><img src="image" alt="structure" /></td>
<td>([b])</td>
<td>([b])</td>
<td>3DJ8</td>
<td>no covalent bond between the enzyme and inhibitor; H141 and E277 are neither too far away nor too poorly oriented for nucleophilic attack</td>
</tr>
<tr>
<td>TSC 40</td>
<td><img src="image" alt="structure" /></td>
<td>([b])</td>
<td>([b])</td>
<td>2PHO</td>
<td></td>
</tr>
<tr>
<td>2AI 41</td>
<td><img src="image" alt="structure" /></td>
<td>3600 ± 20 (\mu M) ((K_i))</td>
<td>human arginase I</td>
<td>3MJL</td>
<td>without a direct interact with the binuclear manganese cluster</td>
</tr>
<tr>
<td>2AH 41</td>
<td><img src="image" alt="structure" /></td>
<td>300 ± 9 (\mu M) ((K_i))</td>
<td>human arginase I</td>
<td>3MFV</td>
<td>the short side chain could not interact directly with the binuclear manganese cluster</td>
</tr>
<tr>
<td>AHH 41</td>
<td><img src="image" alt="structure" /></td>
<td>3000 ± 10 ((K_i))</td>
<td>human arginase I</td>
<td>3MFV</td>
<td>the short side chain could not interact directly with the binuclear manganese cluster</td>
</tr>
<tr>
<td>A1P 41</td>
<td><img src="image" alt="structure" /></td>
<td>4.0 ± 0.2 ((K_i))</td>
<td>human arginase I</td>
<td>([b])</td>
<td></td>
</tr>
<tr>
<td>A4P 41</td>
<td><img src="image" alt="structure" /></td>
<td>800000 ((K_i))</td>
<td>human arginase I</td>
<td>([b])</td>
<td></td>
</tr>
<tr>
<td>APP 41</td>
<td><img src="image" alt="structure" /></td>
<td>500 ± 8 ((K_i))</td>
<td>human arginase I</td>
<td>([b])</td>
<td>probably due to the longer amino acid side chain</td>
</tr>
<tr>
<td>ANH 42</td>
<td><img src="image" alt="structure" /></td>
<td>60 (\mu M) ((K_i))</td>
<td>human arginase I</td>
<td>3F80</td>
<td>no typical tetrahedral intermediate binding to arginase instead of a trigonal planar nitro group</td>
</tr>
<tr>
<td>AHD 42</td>
<td><img src="image" alt="structure" /></td>
<td>30 mM ((K_i))</td>
<td>human arginase I</td>
<td>([b])</td>
<td></td>
</tr>
</tbody>
</table>

[a] AGPA = L-2-amino-3-guanidinopropionic acid; AECA = (2-S)-2-amino-7,8-epoxyoctanoic acid; TSC = thiosemicarbazide; 2AI = 2-aminimidazole; 2AH = (S)-2-amino-3-(2-amino-1H-imidazol-4-yl)propanoic acid; AHH = 2-amino-5-(2-amino-1H-imidazol-4-yl)pentanoic acid; A1P = (S)-2-amino-5-(2-amino-1H-imidazol-1-yl)pentanoic acid; A4P = 2-amino-5-(2-amino-1H-imidazol-1-yl)pentanoic acid; APP = (S)-5-((1H-imidazol-2-
yl)amino)-2-aminopentanoic acid; ANH = (S)-2-amino-6-nitrohexanoic acid; AHD = (S)-aminoheptanedioic acid; [b] Not mentioned in the literature.

**Figure 6.20.** (a) The hydrogen bonds recognition of α-NH₂ and α-COOH of L-2-amino-3-guanidinopropionic acid (AGPA) at the mouth site of active pocket of human arginase I; and (b) contacts of human arginase I with AGPA (green line) and the Mn atoms (purple line) (PDB: 4FCI).

**Figure 6.21.** (a) The hydrogen bonds recognition of α-NH₂ and α-COOH of (2S)-2-amino-7,8-epoxyoctanoic acid (AECA) at the mouth site of active pocket of human arginase I; (b) the interactions of the oxirane oxygen with human arginase I (green line) and contacts of the Mn atoms with human arginase I (purple line) (PDB: 3DJ8).
Figure 6.22. The cocrystal structure of human arginase I-thiosemicarbazide (TSC) complex (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB: 2PHO).

Figure 6.23. (a) The cocrystal structure of human arginase I-2-aminoimidazole (2AI) complex and contacts of the Mn atoms with human arginase I (purple line) (PDB: 3MJL); and (b) the cocrystal structure of human arginase I-(S)-2-amino-3-(2-amino-1H-imidazol-4-yl)propanoic acid (2AH) complex (direct hydrogen bonds: green line, water mediated hydrogen bonds: purple line) (PDB: 3MFW).
6.4 Conclusions

The upregulation of arginase imbalanced the bioavailability of L-arginine for NOS. It can further induce several NO dependent diseases in living system, such as an abnormal immune reaction, the disorder of epithelial cells in many tissues and the proliferation of tumor cells. To inhibit arginase activity will be a broad therapeutic potential for the treatment of a range of pathological conditions. This review focus on discussing the interactions between inhibitors and human arginase, to reveal their relevant structure and activity relationship. With this, we could draw the conclusion that a potent human arginase inhibitor should contain proper substituted groups which can recognize the hydrogen bond network composed of amino acid residues at the mouth site of human arginase. Meanwhile, to mimic the tetrahedral intermediate as the substrate attacked by the nucleophilic hydroxide ion at the guanidinium carbon in hydrolysis process, a tight coordination with binuclear Mn (II) center in active site of human arginases is requisite. To date, none of the reported inhibitors showed a selectivity for inhibiting two isozymes of arginase. Furthermore, to the best of our knowledge, up until now, the developed human arginase inhibitors possess poor pharmacokinetic profiles, and they are not applied in clinic treatment. Therefore, it is challenging and demanding to develop more potent inhibitors with more reliable properties.
6.5 References


