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Research Article

Comprehensive Review on Therapeutic Relevance of Arginase 1 and Arginase 2

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Abstract
Arginase, an important enzyme in the urea cycle, has two isoforms. These two isoforms are cytoplasmic arginase 1 (ARG1) found mostly in the liver and mitochondrial arginase 2 (ARG2) which is mainly found in the kidneys and extra hepatic tissues. ARG1 is necessary for the clearance of nitrogen while ARG2 has a role in the regulation of arginine metabolism and the functionality of mitochondria. Additionally, both arginase isoforms also modulate immune response, metabolic and signalling pathways. Arginase mediated tissue repair and tolerance to immune system by arginine depletion resulting in limited nitric oxide (NO) and macrophage activity is sustained by ARG1. Mitochondrial bioenergetics, secretion of insulin from the cells and the inflammatory response are all controlled by ARG2. Alterations of these isoforms are linked to a variety of conditions. Hyperargininaemia, asthma, fibrosis and cancer have been linked with the action of arginase 1, on the other hand chronic kidney disease, atherosclerosis, neurodegeneration and metabolic syndrome are associated with arginase2. T-cell activities have both isoforms acting on them in the tumour microenvironment and thus assist tumour development. In atherosclerosis and metabolic disorders, they enhance the vascular system, insulin action and adipose turbulence. This review focuses on the molecular ecological processes, physiological roles, and pathological activities of arginase 1 and arginase 2 as more and more studies have illustrated their roles in health as well as the disease context. It further reviews the discovered inhibitors of arginases implicated to various pathological states.

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Introduction to Arginase Isoforms

L-arginine, in mammals, is metabolized by the enzyme arginase to L-ornithine and urea, which is necessary for nitrogen balance in the body (1,2,3). Two distinct isoforms of arginase are known in mammals: arginase 1 (ARG1) and arginase 2 (ARG2) which are genetically different and perform uniquely specialized functions (4,5). The existence of these isoforms in evolution signifies that they are functional in regulating a plethora of physiological processes including metabolic detoxification and immune responses (6,7,8). This allows the clear localization of ARG1 to the liver, as opposed to ARG2 which is widely distributed in tissues (9). The presence of two isoforms allows compartmentalization of metabolic processes, ensuring that arginine metabolism adapts to varying physiological conditions of the system (9). Initially considered in relation to the urea cycle, ARG1 has more recently also been recognized as an important player in the immune system and tissue healing (10,11,12). The subsequent

discovery of ARG2 is associated with cell energetics including its mitochondrial localization (13,14). Functional relations of ARG1 and ARG2 situate metabolism and cell signalling pathways in a hierarchical relation system (15). Some isoforms have also been identified to have changing expression levels in other tissues but remain prominent in the tissues where they are physiologically required. High concentrations of ARG2 in hepatocytes indicate a role in the detoxification of ammonia (16).

Conversely, ARG2 is highly expressed in the kidney implying that it has a possible function in renal arginine metabolism and polyamines synthesis (17,18). The two isoforms are present in immune cells, which take part in the inflammatory and reparative responses (18). Recent studies have revealed that their expression is modified by nutrients, stress or diseases, illustrating the plasticity of arginase activity. Alterations in the activity of any of the isoforms are associated with diverse pathological states including metabolic and immune diseases. Interestingly, there is an overlap between the two isoforms since ARG2 is said to affect the activity of ARG1 indirectly by means of shared intermediates of metabolism (9). This complex interaction emphasizes their common effect in cellular and systemic physiology. Although they are distinct genes, ARG1 and ARG2 can be distinguished from one another by their cofactor and substrate binding, which means that they have specific functional roles.

Structural Insights

Overall Structure

Both ARG1 (Fig-1) and ARG2 (Fig-2), possess a similar folded structure known as the ureohydrolase fold (5), which has a catalytic site that is enveloped by regulatory and stabilizing sites. Since the active site of the enzyme is deeply embedded within the structure, it is very well conserved and contains a binuclear manganese cluster that is catalytically essential (19). The coordination of manganese ions is secured through the binding of amino acid residues such as His101, Asp124 and Glu277, which help in catalysis of the substrate by maintaining the geometry that is required. It is noted that ARG1 expressed in the cytosol does not possess specific mitochondrial targeting sequences; in contrast, ARG2 does contain such a sequence allowing for mitochondrial import (20). Using the X-ray crystallography technique, some structural variations in the loops surrounding the active sites of ARG1 and ARG2 are characterized to account for the differential substrate preferences of the two enzymes (5). Because of the architecture of both isoforms as trimers, structural stability and cooperative activity between subunits to enhance enzymatic efficiency are mandated. ARG2 contains an active site with higher degrees of responsiveness which allows it to process larger sized substrates and intermediates under some conditions.

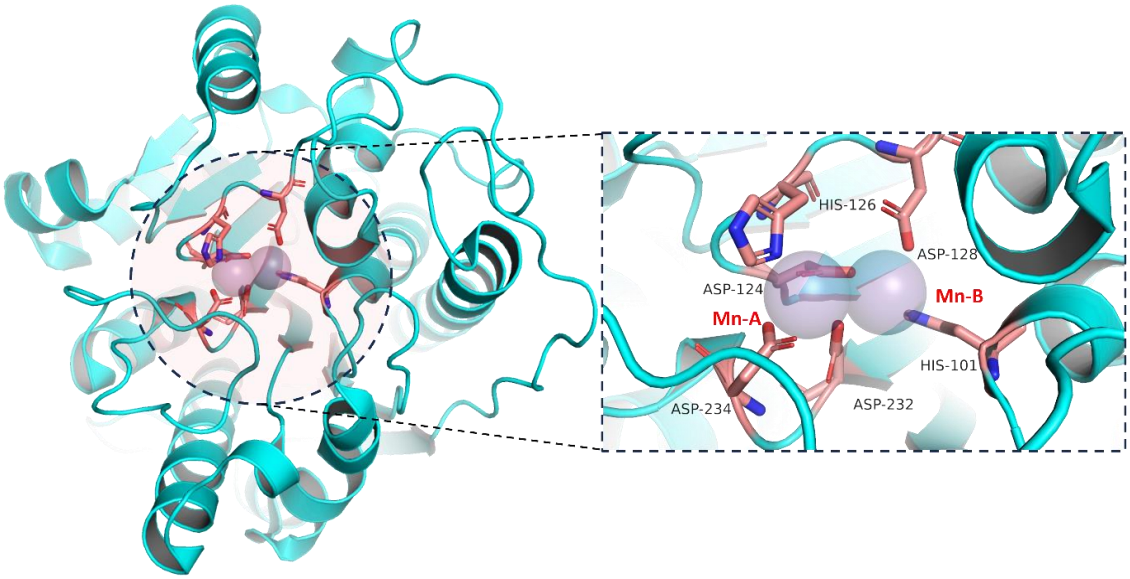

Fig-1: Structure of Arginase-1 (PDB: 2ZAV)

With the variability of enzyme activities accompanying changes in the metabolic state of mitochondria, the state of the ARG2 structure is distinct from that of ARG1 which is permanently fixed in the cytosol and is ideal for a steady state. The alteration of the catalytic residues occurs through substrate binding that is required for successful hydrolysis of the enzyme. Solvent interactions with ARG1 are prevented because of its catalytic active site being buried and this in turn decreases any undesired side reactions. There is a broad range of metabolic functions expected from ARG2 which is also exhibited by it more exposed active site.

The trimers of arginase are able to withstand hydrolysis conditions due not just to the catalytic activity of the ARG1, but also due to salt bridges and hydrogen bonds between the subunits. Being situated within the mitochondria, however, constrains the structure of ARG2 as it needs to maintain structural stability under the thermal as well as oxidative conditions. Recent studies have determined crystal structures of both ARG1 (21,22) and ARG2 (23,24), meaning that further studies can design inhibitors and allosteric regulators to understand the binding interactions further.

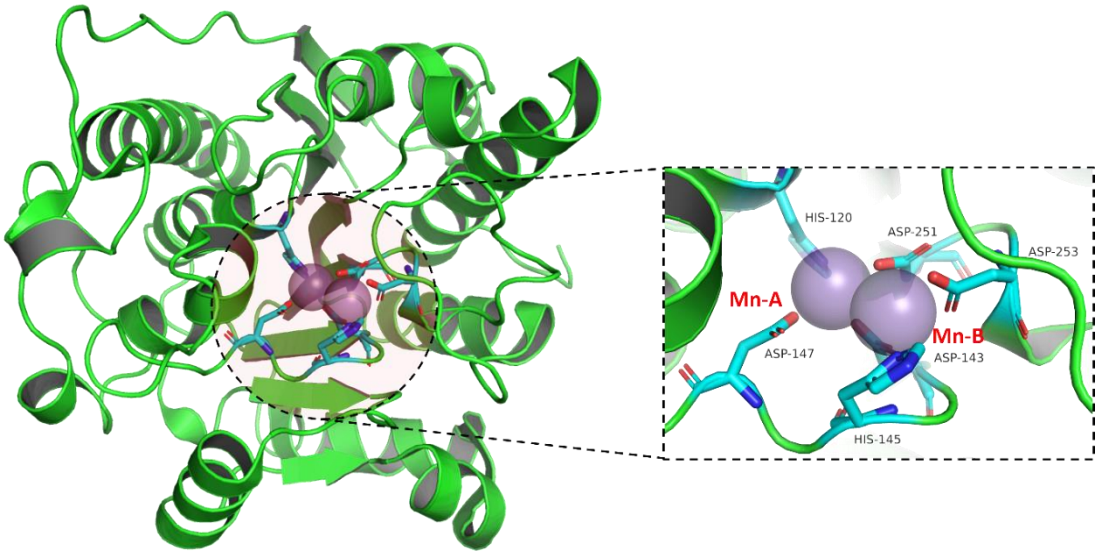

Fig-2: Structure of Arginase-2 (PDB: 1PQ3)

Substrate Binding and Catalysis

There are evidences to suggest that the binding pocket of both isoforms has a preference for binding L-arginine but slight differences in the composition of residues determine their kinetic parameters which are different. ARG1's catalytic efficiency would seem to fit high arginine flux, for example, during detoxification of ammonia in the liver. This information also indicates that because ARG2 has a slower turnover rate, it can maintain arginine levels in tissue where its availability is paramount to the issue such as the kidney and brain. ARG1 also gets substrate specificity by incorporating hydrogen bonds and van der Waals forces in several conservative residues that surround the active site. Although ARG2's active site is primarily designed for L-arginine as its substrate, it is sometimes capable of alternative substrates which associates it with pathways such also nitric oxide biosynthesis and polyamines metabolism. Both isoforms hydrolyse the L-arginine-containing bonds with water through a nucleophilic mechanism assisted by manganese cluster. There are many factors that affect the transition-state stabilization due to enzymatic activity, and recent sequencing of met mutations have confirmed which residues are necessary for the processes in both isoforms. Kinetic analysis has shown that ARG1 works efficiently at alkaline pH close to 8 as found in the liver, whereas ARG2 has shown a higher tolerance to pH changes (25). The fact that ARG2 can process other substrates like citrulline indicate that ARg2 can participate in arginine recycling (26,27).

Catalytic Mechanism of Arginase

ARG1 and ARG2 share an identical arrangement and chemical nature of residues at the binding site level as well as the catalytic mechanism. The catalytic triad consists of Asp128, His141 and Glu277 in ARG1, corresponding to Asp147, His160 and Glu296 in ARG2 (28). Manganese ions also participate in the catalytic mechanism and show different coordination patterns. Specifically, the $Mn2^+$ ion deeply located in the binding site (MnA) is coordinated by His1011 (ARG2: His1202), Asp1241 (ARG2: Asp1432), Asp1281 (ARG2: Asp1472), Asp2321 (ARG2: Asp2512), and the catalytic water molecule. The second $Mn2^+$ ion (MnB) is coordinated by His1261 (ARG2: His1452), Asp1241 (ARG2: Asp1432), Asp2321 (ARG2:Asp2512), Asp2341 (ARG2: Asp2532) (in a bidentate way), along with the catalytic water (29). The catalytic water molecule is converted into a hydroxyl ion due to Mn2+-mediated coordination and, likely, to the presence of an amino acid acting as a base. The guanidinium group of L-arginine is stabilized and maintained in the correct orientation through H-bonding by Glu2771 (ARG2: Glu2962), which in turn is stabilized by His1411 (ARG2: His1602) (30). The hydroxyl ion performs a nucleophilic attack on the carbon atom of the guanidinium group, which acts as an electrophilic site. This nucleophilic attack generates a tetrahedral intermediate.

The proton from the hydroxyl group is transferred first to Asp1281 and subsequently to the nitrogen of the departing L-ornithine. Subsequently, the tetrahedral intermediate collapses, resulting in the production of urea and L-ornithine. The regeneration of the catalytic site remains somewhat unclear. It has been suggested that a water molecule displaces the urea in the coordination spheres of MnA and MnB. This is followed by proton transfer to the bulk solvent by His1411 and the reformation of the nucleophilic hydroxide anion (31). Alternative mechanisms have been proposed, like the formation of a neutral guanidine group in L-Arg assisted by His1411. The neutral guanidine ligand could then coordinate one of the Mn2+ ions via the imino group, displacing the water molecule from the coordination sphere. A proton transfer from the water molecule to the amino group of L-Arg could follow, leading to a nucleophilic attack on the carbon atom of the guanidinium group by the newly generated hydroxyl anion. According to Di Costanzo et al., the proton transfer mentioned earlier is facilitated by proton shuttling from the bulk solvent before product dissociation, adding another layer of complexity to the understanding of ARG's catalytic function (22). The

similarities in the catalytic sites and mechanisms of action of ARG1 and ARG2 account for the lack of selective inhibitors toward one of the isoforms and highlight the need to adopt new strategies for designing more selective molecules able to achieve targeted therapeutic effects.

It is indicated from structural modeling that the inhibitors which bind closer to the manganese cluster have the capacity to block the catalysis and, therefore, making it possible to develop isoform specific drugs. Also, the effect brought by co-factors like NADPH, especially in relation to the mitochondrial localization of ARG2, on arginase activity has been noted. Such differences in cofactor availability in different bodies may alternatively act as an extra layer of control on the activity of arginase isoforms. Frozen-out states in advanced techniques, like molecular dynamics simulations, began illustrating the conformational changes taking place during substrate binding and product releasing. It has been noted in studies that there exist differences in the kinetics of ARG1 and ARG2 when each is expressed under bio–pathological conditions due to the diverse functions ARG1 and ARG2 have in systemic arginine metabolism.

Tissue Distribution and Expression

It is important to note that ARG1 and ARG2 display unique as well as overlapping tissue expression which suggests that these enzymes have different yet cooperative physiological roles (32,33,34). Eukaryotic cells modify the expression of ARG1 in response to the nutritional status of an organism, which is commonly found in hepatocytes where the enzyme plays a primary role in the urea cycle for ammonia detoxification and nitrogen homeostasis. ARG1 has a liver predominant expression that is tightly controlled by the absolute levels of nutrition and hormonal signals, providing flexibility to adapt to different amounts of protein in an organism (35). During prolonged fasting, glucagon and glucocorticoids induce ARG1 expression to increase gluconeogenesis, thereby optimizing nitrogen disposal alongside energy metabolism (36). In contrast, when the organism is in an anabolic state, ARG1 transcription is prevented via insulin. In the kidney, ARG2 is also expressed where it helps in renal arginine metabolism and ammonium buffering in acidic pH levels (37,38). Furthermore, renal ARG2 is implicated in the regulation of the systemic acid-base balance during metabolic acidosis where bicarbonate precursors are generated. ARG2 is localized to the mitochondria of pancreatic beta cells where it is suggested to modulate insulin secretion via intracellular arginine and ornithine levels. It is evident that both isoforms are expressed in immune cells namely macrophages and myeloid-derived suppressor cells (MDSCs); however, their functions are not the same where M2 macrophages have a higher level of ARG1 that blocks the inflammatory response while ARG2 facilitates inflammation by assisting in the metabolic shifting of M1 macrophages.

Functional Roles of Arginase 1 and 2

Arginase 1

One of the main important activities of human ARG1 or Arginase 1 is the urea cycle where it helps to bring about the chemical transformation of ammonia from protein metabolism into urea that can be eliminated from the body by kidneys. This action maintains the balance of nitrogen in the body especially during times of greater than normal protein intake. Apart from its liver role, however, ARG1 also plays a role in the immune system mainly through the regulation of L-arginine metabolism in macrophages and its effect over nitric oxide (NO) production (39). It was demonstrated that in macrophages, ARG1 promotes tissue regeneration by channelling arginine metabolism towards proline creation, which is needed for collagen formation (40). There is also some evidence on the potential inhibition of inflammation from ARG1 activity, since it would cause a switch in macrophage type from a pro-inflammatory state M1 to an M2 anti-inflammatory state. In an asthma context, on the other hand, ARG1 induced airway hyperreactivity and airway epithelium cell fibrosis which suggests that it's a possible target site for therapeutic interventions (41). Furthermore, the role of ARG1 remains crucial in neuroprotection since, in microglia, the expression of the gene is shown to reduce neuroinflammation through the inhibition of NO production (42).

In constraining vascular biology, ARG1 controls endothelial actions by controlling the supply of arginine which is required to synthesize nitric oxide by endothelial cells (eNOS) (43). It has been found out recently that there is a link between ARG1 activity and metabolic syndrome as its dysregulation can lead to the inability to metabolize glucose and lipids (44). Malignant tumors associated with macrophages express ARG1 and in doing so depletes L-arginine within the tumor microenvironment leading to the downregulation of T cell activation that is needed for immune sensing. Cancer therapy has utilized arginase inhibitors to target ARG1 activity which is the main switch for immune suppression in cancer. Upon overexpressing in macrophages, ARG1 also regulates the intracellular catalysis of womb bacteria by balancing the availability of arginine.

Arginase 2

Involved in the metabolism of arginine and downstream processes such as polyamine and proline synthesis, ARG2 which is associated with mitochondria is considered essential for cellular bioenergetics (45). In the kidney, ARG2 contributes to ammonia buffering and recycling of arginines, crucial activities that serve to restore acid-base balance in the body (46). Beta cells of the pancreas are regulated by the activity of ARG2 which regulates Insulin levels by controlling levels of arginine and ornithine (47). In the brain, ARG2 is involved in the processes that control the supply of messengers in the synthesis of glutamate and GABA. The activities of ARG2 in endothelial cells also play a role in vascular stability as it interferes with nitric oxide (NO) production and oxidative stress level (48). In cancer, through supporting assimilation metabolism and inhibiting immune responses by arginine-depletion,

ARG2 promotes the growth of the tumour (49). In inflammatory disorders, especially, inflammation related to the tumor microenvironment, however, activates ARG2 expression which further assists in the metabolic rewiring of the immune cells enabling their activation and survival in nutrient limiting conditions. Due to its the ability to cause oxidative stress and mitochondrial damage, dysregulation of ARG2 has been associated with neurodegenerative diseases (50). Collagen synthesis in fibroblasts is linked to the activity of ARG2 which plays a role in wound healing and fibrosis.

ARG2, it has been suggested in recent studies, has autophagy modulating capabilities through its interaction with intracellular arginine levels in relation to cellular stress response (51). Some metabolic disorders such as obesity and type 2 diabetes are associated with ARG2 expression which can modulate insulin sensitivity as well as lipid metabolism. In cardiac tissues where ARG2 is expressed, it participates in arginine metabolism as well as aids stressed myocardial cells. ARG2 has been linked to atrophy and growth of muscle by virtue of its function in modulation of both protein synthesis and degradation in skeletal muscle tissues. The role of ARG2 in T-cell metabolism has also been described whereby T-cell activity and differentiation are affected by ARG2 activity. ARG2 inhibitors are currently being vetted as therapeutic agents for various diseases such as metabolic syndrome and cancer.

Arginase inhibitors

Arginase inhibitors are a subclass of compounds developed purposely to thwart the activity of arginase which is the enzyme responsible for the hydrolysis of L-arginine to urea and ornithine. These inhibitors (Fig-3) have generated interest and noticeable research efforts in medicine, particularly in the areas of oncology and other fields like cardiovascular diseases and immunotherapy where there is considerable potential therapeutic efficacy. In relation to cancer, it has been observed that high levels of arginase activity led to depletion of L-arginine, which adversely affects T-cell activity, which is an essential component for tumour defeat. This has the effect of enhancing anti-tumour immunity through the differentiation of these compounds that inhibit arginase activity and lower L-arginine depletion levels. These compounds however have wider application as biologics, for instance in cardiovascular diseases they have been shown to facilitate better endothelial function while offering vascular preservation of nitric oxide which is an important enzyme. Clinical research on such inhibitors (28,52,53,54) has advanced to the point where the library of these compounds contains as natural origin, as well as synthesized ones and current work is focused on increasing their specificity and activity in order to achieve effective clinical use.

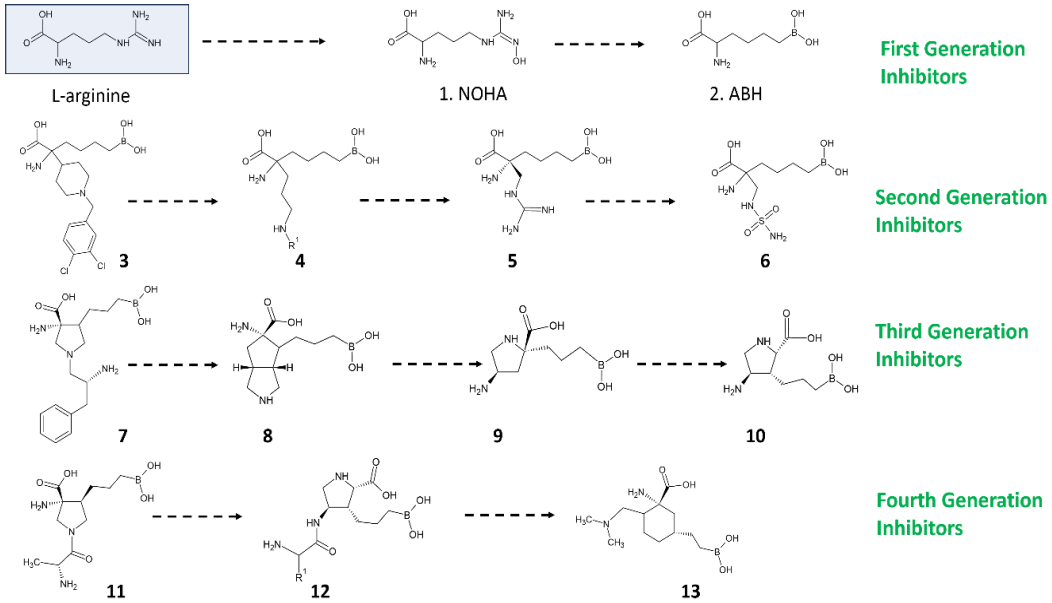

Fig-3: Discovery and Evolution of Arginase Inhibitors

First Generation: Simple Structural Mimics

1. **Key Inhibitors**:
   - **1 (NOHA)**: Nω-Hydroxy-L-arginine, a direct mimic of L-arginine's guanidinium group with the addition of a hydroxyl group.
   - **2 (ABH)**: 2(S)-Amino-6-boronohexanoic acid, introducing a boronic acid moiety to improve binding affinity.
2. **Mechanistic Insights**:
   - These inhibitors mimic the natural substrate, L-arginine, targeting the arginase active site.

Second Generation: Modified Functional Groups

1. **Key Inhibitors**:
   - **3 (ABH(pip))**: A derivative of ABH with a piperidine group for enhanced hydrophobic interactions.
   - **4**: ABH analogue with a basic side chain, designed to improve binding to the enzyme's acidic residues.
   - **5**: A guanidine derivative, mimicking the guanidinium group of L-arginine with enhanced electronic properties.

- **6**: Sulfamoyl derivative, introducing a sulfonamide group for additional hydrogen-bonding capabilities.

2. **Mechanistic Enhancements**:
- Modifications in this generation aim to exploit additional enzyme-substrate interactions while improving selectivity.
- Increased chemical diversity provides better pharmacokinetic properties compared to the first generation.

Third Generation: Complex Cyclic Structures

1. **Key Inhibitors**:
- **7**: Pyrrolidine derivative (NED-3228), featuring a pyrrolidine ring to stabilize binding through hydrophobic and electrostatic interactions.
- **8**: A bicyclic derivative designed to rigidify the structure for improved binding affinity.
- **9 and 10**: Proline derivatives with varying substitutions, enhancing flexibility and enzyme specificity.

2. **Mechanistic Refinements**:
- Cyclic scaffolds stabilize inhibitor conformation, reducing entropy loss upon binding.
- Enhanced boronic acid positioning allows for stronger interactions with the catalytic Mn2+ ions and active site residues.

Fourth Generation: Advanced Peptide and Cyclic Derivatives

1. **Key Inhibitors**:
- **11**: Peptide derivative with a pyrrolidine scaffold (Numidargistat), combining structural mimicry with peptide backbone flexibility.
- **12**: Peptide derivative with a proline scaffold, incorporating features to target both Mn2+ coordination and the surrounding residues.
- **13**: Cyclohexane derivative (OATD-02), focusing on non-peptide-based scaffolds for improved stability and bioavailability.

2. **Mechanistic Innovations**:
- Peptide-based scaffolds (12) are designed to optimize interactions with specific residues, enhancing selectivity between ARG1 and ARG2 isoforms.
- Non-peptide derivatives like OATD-02 (13) offer better metabolic stability and therapeutic potential.

Overall Observations

1. **Progressive Design Strategy**:
- Each generation reflects advancements in structural complexity, moving from simple substrate mimics to cyclic and peptide-based scaffolds.
- Functional group modifications have been tailored to improve binding affinity, selectivity, and stability.

2. **Challenges**:
- Selectivity between ARG1 and ARG2 remains a critical issue due to structural similarities in the active sites.
- Pharmacokinetics, including bioavailability and metabolic stability, need continuous optimization in later generations.

3. **Therapeutic Potential**:
- These inhibitors hold promise for various therapeutic applications, including cancer, cardiovascular diseases, and immune modulation.

This stepwise evolution of arginase inhibitors showcases the iterative approach of rational drug design, where structural modifications are guided by mechanistic insights to achieve optimized therapeutic agents.

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