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2-Substituted-2-amino-6-boronohexanoic acids as arginase inhibitors

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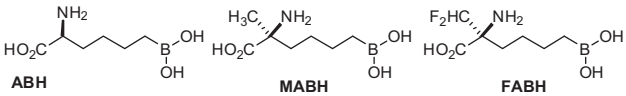
ABSTRACT

Substitution at the alpha center of the known human arginase inhibitor 2-amino-6-boronohexanoic acid (ABH) is acceptable in the active site pockets of both human arginase I and arginase II. In particular, substituents with a tertiary amine linked via a two carbon chain show improved inhibitory potency for both enzyme isoforms. This potency improvement can be rationalized by X-ray crystallography, which shows a water-mediated contact between the basic nitrogen and the carboxylic acid side chain of Asp200, which is situated at the mouth of the active site pocket of arginase II (Asp181 in arginase I). We believe that this is the first literature report of compounds with improved arginase inhibitory activity, relative to ABH, and represents a promising starting point for further optimization of in vitro potency and the identification of better tool molecules for in vivo investigations of the potential pathophysiological roles of arginases.

Arginase is a binuclear manganese metalloenzyme that catalyzes hydrolysis of L-arginine to urea and L-ornithine. Two isoforms are known, arginase I (Arg I) and arginase II (Arg II), which have different tissue distributions and biological functions. Arg I is expressed primarily in the liver, whereas Arg II is expressed in several extra-hepatic tissues, including the brain, spinal cord, kidney, small intestine, and mammary gland. The two isoforms are encoded by different genes, and have differing pI values, immunological reactivity, and subcellular locations, but share similar enzymatic reactivities.[1]

Human arginases are implicated in wide variety of diseases, including atherosclerosis, pulmonary hypertension, systemic hypertension, erectile dysfunction, asthma, wound healing, multiple sclerosis,[2] as well as several tropical diseases such as malaria and leishmaniasis.[3] Development of potent and selective arginase inhibitors would help to elucidate the pathophysio-logical roles that these enzymes play and potentially lead to novel therapies. Several compounds have already been reported, for example, 2(*S*)-amino-6-boronohexanoic acid (ABH),[4] which is the arginase inhibitor most widely used and reported in the literature.[5]

Recently, Ilies et al. have reported the synthesis and characterization of the quaternary amino acids 2-amino-6-borono-2-methyl-hexanoic acid (MABH) and 2-amino-6-borono-2-(difluoromethyl)-hexanoic acid (FABH), both of which showed decreased inhibitory potency, relative to ABH, against human Arg I.[6]



ABH racemic - our data: Arg1 1.4 uM (n=25) optically active ref 4: Arg1 0.8 uM;

MABH

FABH

Independently, we have developed a similar approach and present here the first steps in our research effort that, in contrast, resulted in significant improvements in arginase inhibitory potency compared to ABH.[7]

Our analysis of the binding mode of ABH to arginase I (PDB: 1D3V) revealed that while the whole molecule is tightly embedded in the active site pocket, its alpha-hydrogen projects out towards various surface residues of the enzyme and towards bulk solvent. Therefore, we reasoned that addition of appropriate functionality

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at this position might create new interactions and increase potency activity.

Most compounds were prepared by successive alkylations of the glycine equivalent **2**, using methodology developed by O'Donnell[8] (Scheme 1) or a variation thereof, that is, by alkylation of benzophenone protected amino acids (e.g., **1d** or **1j**).

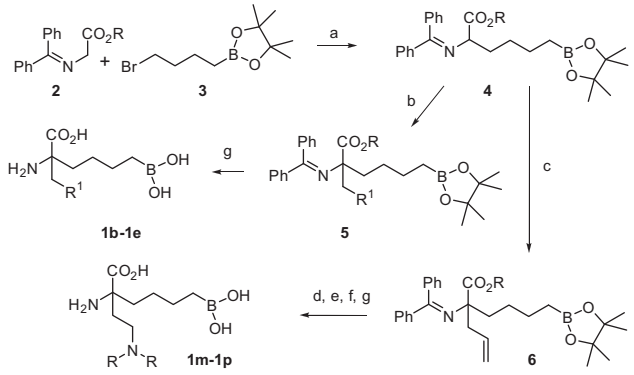
Alkylation with the commercially available bromide **3** allowed installation of the protected *n*-butyl boronic acid fragment in one easy step. The second alkylation (**4** → **5**) required more aggressive reaction conditions or the use of more active alkylating agents, which limited the choice of potential side chains. The alkylated products **5** were deprotected under acidic conditions to provide the desired final products **1b**–**1e** as racemates. Alternatively, alkylation with allyl bromide allowed us to prepare compound **6**. Ozonolysis of **6** followed by reductive amination and deprotection provided the desired products **1m**–**1p**. Final compounds obtained after acidic deprotection were very polar and were purified using reverse phase HPLC and lyophilized.

Compounds were tested for inhibition of human Arg I and Arg II using a colorimetric assay based upon the published method.[9,10] The inhibition of arginases by boronic acid inhibitors is strongly pH dependent.[11] Although the pH optimum for arginase is 9.0–9.5,[12] we decided to perform our assay at pH 7.4 which is more physiologically relevant. Our observed inhibitory potency for ABH for Arg I is very similar to the value reported in the literature (IC_{50} = 800 nM; optically active,[4] vs 1450 nM for racemic compound/our data).

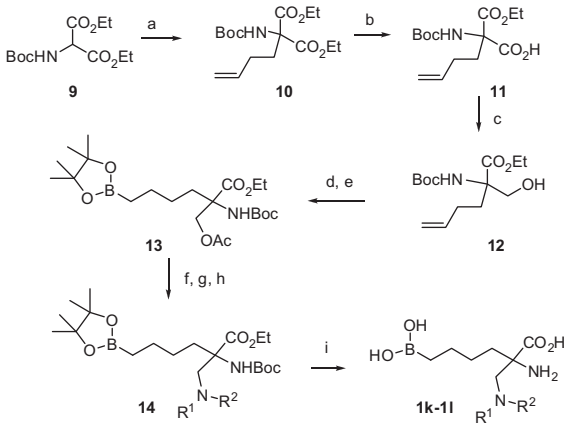
2,3-Diaminopropionic acid derivatives **1k** and **1l** were prepared starting from diethyl (Boc-amino)malonate (Scheme 2). Although the key step (the partial hydrolysis of the diester **10** to mono acid **11**) was achieved in non-selective way, it does provided a potential entry into enantioselective synthesis (e.g., via chemoenzymatic desymmetrization).[13] Acidic hydrolysis of intermediate **13** provided compound **1f**.

The initial compounds with alkyl and benzyl substituents at the alpha-position (**1b**–**1e**) had similar or lower potencies to the unsubstituted compound ABH (**1a**), but these findings confirmed that there is enough room to accommodate a larger alpha-substituent. Analogs with one to three carbon atom aliphatic chain-linked alcohols and ethers were synthesized (e.g., **1f**–**1i**) and tested in the hope of identifying new interactions at the opening of the active site pocket of Arg I, for example, Thr136, Ser137, Asp181 Asp183 (Ser155, Ser156, Asp200, Asp202, respectively in Arg II). Most of these compounds in this class showed little or no improvement in potency compared to ABH. An X-ray crystal structure of compound **1h** bound to Arg II measured at 2.2 Å resolution on a laboratory X-ray source,[14,15] revealed a contact distance of around 2.9 Å between the terminal hydroxyl group and a single oxygen atom of Asp202 (Fig. 1). The corresponding structure of **1h** bound to Arg I (2.0 Å resolution) revealed an almost identical binding mode, with the terminal hydroxyl group oriented toward Thr136 at distance 3.2 Å. In both Arg I and Arg II complexes, all other enzyme inhibitor interactions remained essentially unchanged compared to the respective ABH–enzyme complexes (data not shown).

Compounds **1f**–**1i** are representative examples of alpha-linked C1–C3 chains with non-basic hydrogen bond donor or acceptor groups. Several other aliphatic chain linked ureas, amides and sulfonamides were synthesized and tested with similar results (IC_{50} potencies in 1–5 μM range). Based on the improved potency seen for the histidine analog **1j**, compared to alpha-benzyl analog **1e**, efforts were focused on increasing the interaction of the



Scheme 1. Synthesis of inhibitors **1b**–**1e**, **1g**–**1j** and **1m**–**1p**. Reagents and conditions: (a) LiHMDS, THF, −78 °C to +50 °C (64%); (b) LiHMDS, R^1CH_2I, THF, −78 °C to rt; (c) LiHMDS, allyl bromide 3 equiv, THF, −78 °C to rt, 16 h (59%); (d) Et_2O–1 N HCl, rt then Boc_2O, EtOAc–$NaHCO_3$ sat (80%); (e) O_3, DCM, −78 °C then Ph_3P −78 °C to rt (93%); (f) R_2NH, 1,2-DCE, $NaBH(OAc)_3$, rt, 16 h (70–90% yield); (g) 6 N HCl, 95 °C, 16 h (50–90% yield).



Scheme 2. Synthesis of inhibitors **1k** and **1l**. Reagents and conditions: (a) 4-bromobut-1-ene, NaH, DMF, 90 °C (90%); (b) KOH, EtOH, 0 °C to rt, 19 h, (82%); (c) $ClCO_2Et$, Et_3N, then $NaBH_4$ −40 °C, 1 h, (78%); (d) Ac_2O, DMAP 1 equiv, DCM, rt, 16 h, (80%); (e) pinacol borane, chloro(1,5-cyclooctadiene)iridium(I) dimer, 1,2-bis(diphenyl-phosphino)ethane, DCM, rt, 10 h, (54%); (f) K_2CO_3, EtOH, rt, 2 h, (78%); (g) $(COCl)_2$, DMSO, Et_3N, DCM, −78 °C, 30 min, (57%); (h) R_2NH, 1,2-DCE, $NaBH(OAc)_3$, AcOH, 60 °C, 2 h (30–50% yield); (i) 6 N HCl, 95 °C, 16 h (50–80% yield).

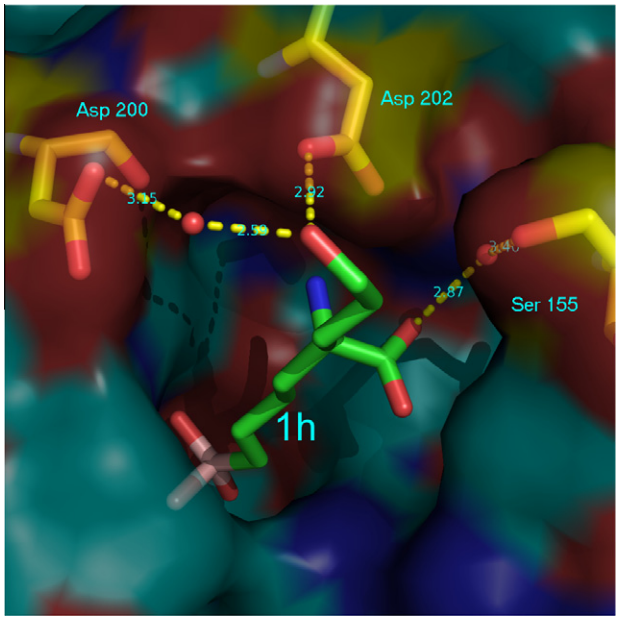


Figure 1. X-ray structure of compound **1h** in human Arg II.