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Synthesis and biological properties of novel arginase inhibitors

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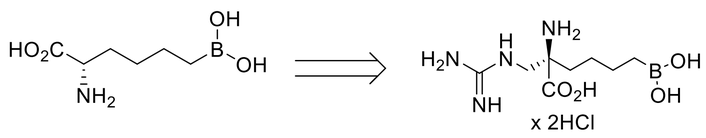
Introduction

Arginase is a manganese-dependent enzyme that hydrolyzes arginine to ornithine and urea. Two isoforms of this enzyme are known (ARG-1 and ARG-2) and both catalyze the same reaction, but the occurrence of enzyme isoforms in cellular environment is different (ARG-1 is cytosolic protein and ARG-2 is localized in mitochondrial matrix) [1]. Disorders related to arginases activity have been observed in patients with various diseases such as: asthma, pulmonary hypertension, hypertension, T-cell dysfunction, erectile dysfunction, atherosclerosis, renal disease, ischemia reperfusion injury, neurodegenerative disease, wound healing, inflammatory disease and fibrotic disease [2]. Arginase also promotes the immune escape of cancer cells by decreasing arginine concentrations that is required for proliferation and activation of cytotoxic T and NK cells. High plasma and tumor arginase (ARG) activity has been demonstrated in patients with a wide spectrum of cancers and correlated with a poor prognosis [3,4].

Herein we present results of our early studies. Based on the well-known 2-(*S*)-amino-6-boronohexanoic acid (ABH) **1** arginase inhibitor [5] we designed and synthesized a linear compound, enantiomerically pure guanidine derivative **12aa** with basic side chain in the α-position related to amino acid functional group [6].



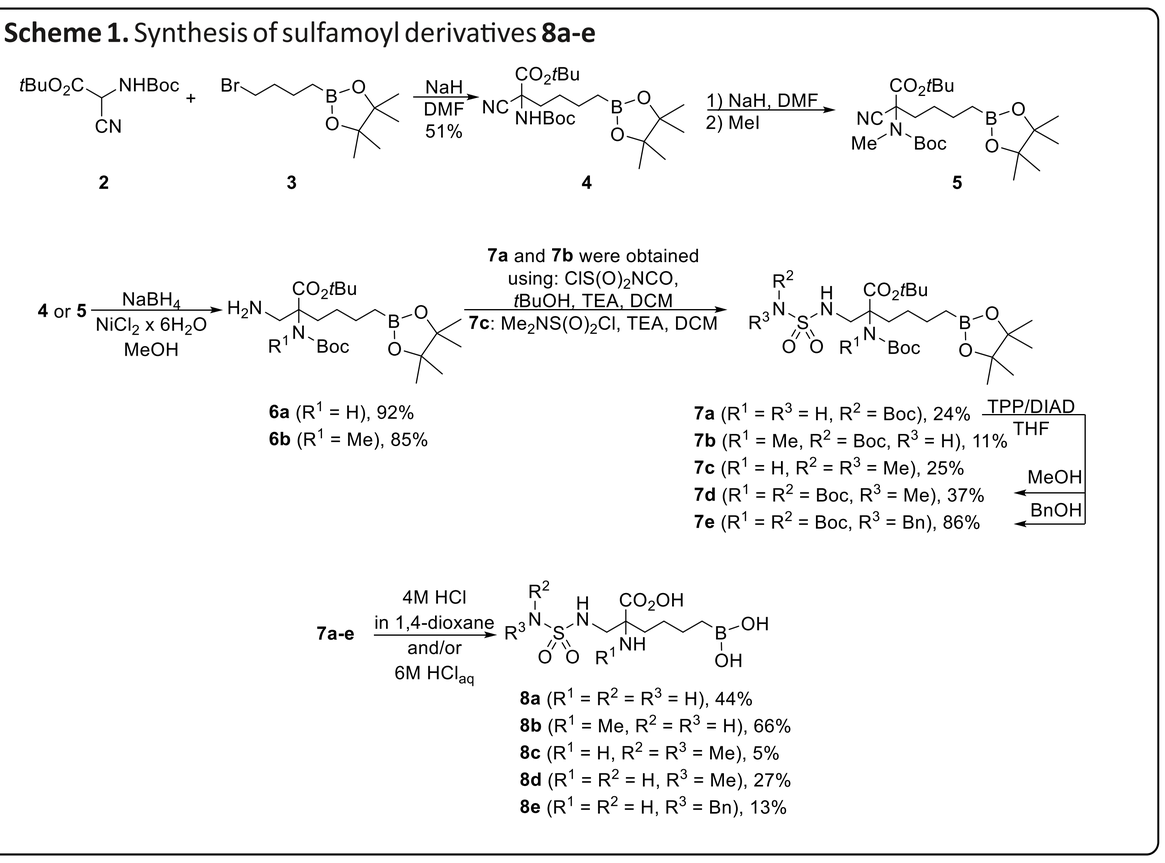
1 hARG1 IC_{50}= 800nM — **12aa** hARG1 IC_{50}= 32nM

Results

Chemistry and in vitro activity

First, sulfamide derivatives were prepared. Alkylation of *N*-Boc-protected cyanoacetate **2** with pinacol-4-bromobutylboronate **3** afforded quaternary boronic cyanoaminoesters **4** which were subjected to subsequent methylation or direct reduction to free primary amines **6b** and **6a** respectively. Sulfamoylation of **6**, followed by hydrolysis of the formed sulfamides **7a-e** gave the desired boronic acids **8a-c**. *N*-terminally alkylated analogs **8d** and **8e** were obtained from **7a** by the use of appropriate alcohol in Mitsunobu conditions and subsequent deprotection of sulfamides **7d** and **7e** (Scheme 1).

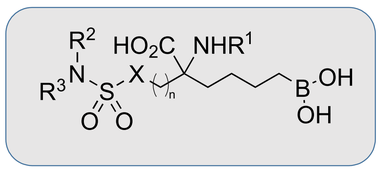
Scheme 1. Synthesis of sulfamoyl derivatives **8a-e**



In vitro activity of sulfamoyl derivatives **8a-e** are listed in Table 1. We also synthesized analogues **9** and **10** and we found that their potency decreases together with extension of the spacer to two carbon atoms (**9**) or changing NH in methylene-bridge position to CH_2 group (**10**).

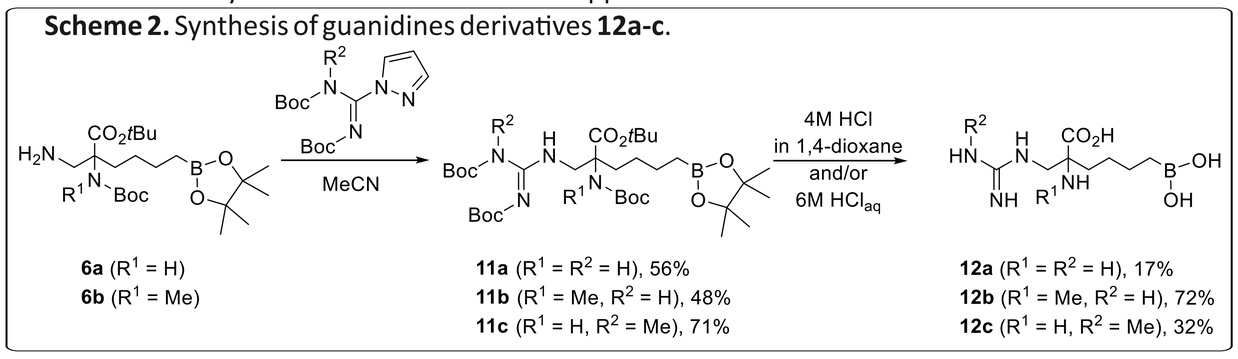
Table 1. In vitro activity of sulfamoyl derivatives.

Comp No.	X	n	R^1	R^2	R^3	hARG1 IC_{50} (μM)
8a	NH	1	H	H	H	0.30
8b	NH	1	Me	H	H	2.3
8c	NH	1	H	Me	Me	0.73
8d	NH	1	H	H	Me	1.0
8e	NH	1	H	H	Bn	1.4
9	NH	2	H	H	H	2.8
10	CH_2	1	H	H	H	1.1



The racemic guanidine derivatives **12a-c** were obtain in the similar manner but guanidinylations instead of sulfamoylations of amines **6a-b** were applied.

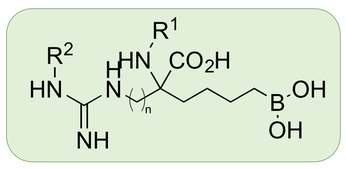
Scheme 2. Synthesis of guanidines derivatives **12a-c**.



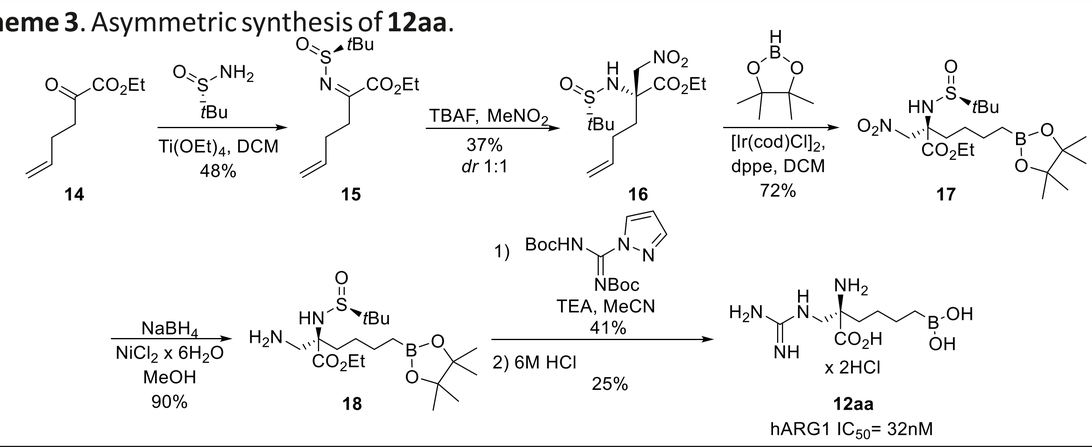
From the guanidine series presented in Table 2 compounds with methylene-linker (**12a-c**) showed a clear improvement in the inhibitory potency toward arginase over ABH (IC_{50} = 800 nM). Independent synthetic pathway was developed to obtain enantiomerically pure gunidines **12aa** (Scheme 3) and **12ab**.

Table 2. In vitro activity of guanidines derivatives.

Comp No.	n	R^1	R^2	hARG1 IC_{50} (nM)
12a	1	H	H	67
12aa	*R*-enantiomer			32
12ab	*S*-enantiomer			6800
12b	1	Me	H	78
12c	1	H	Me	233
13	2	H	H	7000



Scheme 3. Asymmetric synthesis of **12aa**.



Piperidines analogs **21a-c** were also synthesized (Scheme 4), but their in vitro activity was poor (Table 3).

Scheme 4. Synthesis of piperidines **21a-c**.

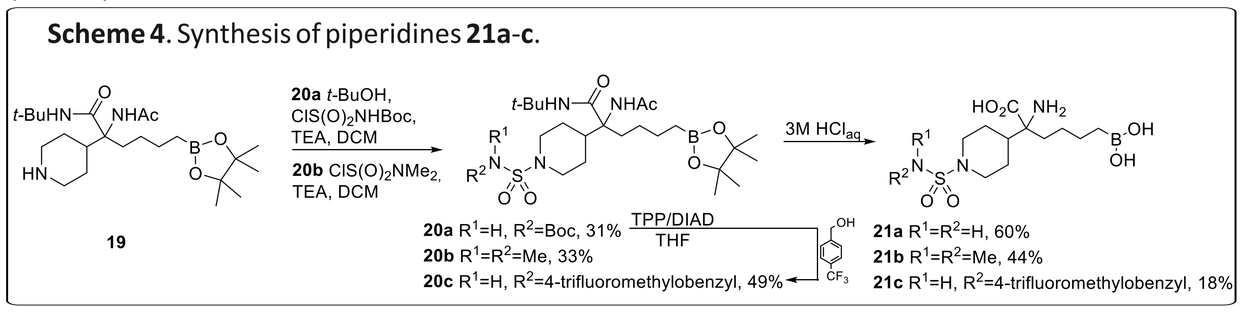
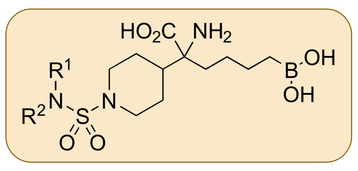
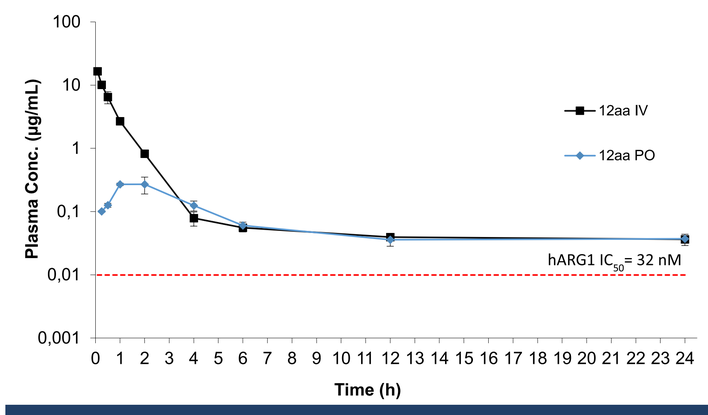


Table 3. In vitro activity of piperidines derivatives **21a-c**.

Comp No.	R^1	R^2	hARG1 IC_{50} (μM)
21a	H	H	2.8
21b	Me	Me	12.3
21c	H	4-(trifluoromethyl)benzyl	6.8



Pharmacokinetic parameters of 12aa in rats



Route	IV	PO
Dose (mg/kg)	3	10
AUC_{0-inf}(mg*h/L)	11.77	1.4
C_0 or C_{max}(mg/L)	21.23	0.27
T_{max}(h)	n/a	2.0
CL (mL/min/kg)	**4.2**	**n/a**
Vss (L/kg)	3.04	n/a
$T_{½}$ (h)	**32.71**	**30.82**
Bioavailability (F%)	n/a	4%

Conclusions

We present the results of our early studies on the novel class of small-molecule inhibitors of arginase. We have discovered three series of potent inhibitors of arginase 1. The sulfamoyl and guanidine analogues with low molecular weight displayed high enzymatic activity. Rats pharmacokinetics for synthesised compounds demonstrated low clearance and poor oral bioavailability. The most active, linear compound is enantiomerically pure guanidine derivative **12aa** with the natural *R*-stereo configuration (IC_{50} = 32 nM).

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