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Discovery and Pharmacokinetics of Sulfamides and Guanidines as Potent Human Arginase 1 Inhibitors

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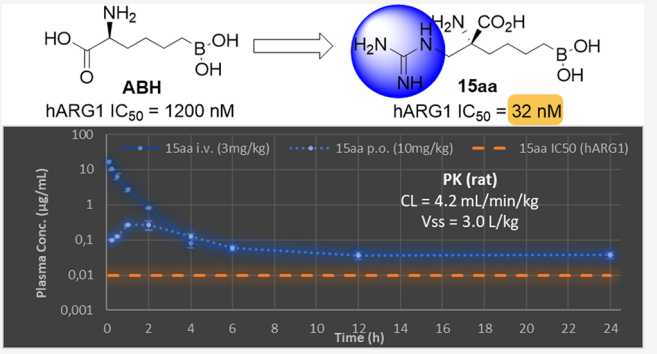
ABSTRACT: We designed and synthesized a series of arginase inhibitors as derivatives of the well-known 2-(*S*)-amino-6-boronohexanoic acid (ABH) with basic and neutral side chains in the α-position relative to the amino acid group. In an effort to improve the pharmacokinetic profile of literature examples and retain potent enzymatic activity, sulfamido moieties were introduced to generate hydrogen bond interaction with the aspartic acid residue in the arginase active site. The compounds with basic guanidine-containing side chains were even more potent arginase inhibitors. Both groups of compounds, as designed, demonstrated low clearance in their pharmacokinetic profile. The most active inhibitor **15aa** showed high nanomolar potency with IC_{50} = 32 nM toward human arginase 1 and demonstrated low clearance (4.2 mL/min/kg), long $t_{1/2}$, and moderate volume of distribution in rat pharmacokinetic studies.



KEYWORDS: *Arginase inhibitors, arginases, boronic acids, immuno-oncology, cancer immunotherapy, amino acids*

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Arginase (ARG) is a manganese-dependent enzyme that hydrolyzes arginine to ornithine and urea. Two isoforms of this protein are known (ARG1 and ARG2) and both catalyze the same reaction, but the occurrence of enzyme isoforms in cellular environments is different. ARG1 is a cytosolic protein, and ARG2 is localized in the mitochondrial matrix. Disorders related to arginases activity have been observed in patients with asthma, pulmonary hypertension, hypertension, T-cell dysfunction, erectile dysfunction, atherosclerosis, renal disease, ischemia-reperfusion injury, neurodegenerative disease, inflammatory disease, and fibrotic disease.[1−3] The role of ARG1 as a target in cancer immunotherapy is under investigation.[4] The myeloid-derived suppressor cells (MDSCs) secrete arginase 1 which depletes the local arginine concentration.[5] Depletion of arginine represents an important mechanism of immunosuppression, and high arginase activity in plasma and tumors demonstrated in patients with a wide spectrum of cancers correlates with a poor prognosis. Arginase promotes the immune escape of cancer cells by decreasing L-arginine concentration, which is required for proliferation and activation of cytotoxic T and NK cells.[4−12] Moreover, some cancer cells release ARG1-containing exosomes further suppressing antitumor immunity.[13] Restoration of arginine levels in the tumor microenvironment by ARG inhibitors induces T-cell activation and proliferation leading to T-cell-mediated antitumor responses.[13−17] The primary objective of our drug discovery program was to identify novel, highly potent and efficacious arginase inhibitors that could be used in cancer immunotherapy. The clinical relevance of arginase inhibitors in cancer immunotherapy is under investigation in ongoing phase 1/2 trials with the use of CB-1158 (compound **1**, Figure 1), as well as our molecule OATD-02 (phase 1 to be started mid-2020).[4,18]

Boronic acids are well-known pharmacophore fragments of arginase inhibitors.[19−33] Many simple α-substituted ABH (2-(*S*)-amino-6-boronohexanoic acid (**2**)) analogs exhibit potent inhibitory activity toward ARG1.[22,23,28] Conformationally restricted cyclic ABH analogs are even more active inhibitors (Figure 1).[24,27,29−33] Unfortunately, almost all of these compounds suffer from weak pharmacokinetics (PK), in particular, fast clearance and/or low oral bioavailability, due to their very polar zwitterionic nature (high PSA, negative logD).[23,24] On the one hand, the presence of a basic amino

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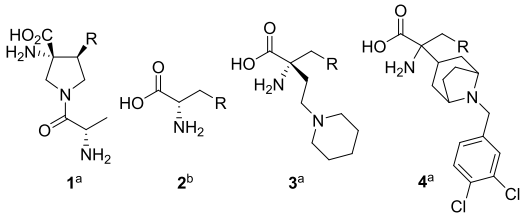


	1[a]	2[b]	3[a]	4[a]
hARG1 IC_{50} (nM):	98	1200	223	21
MW (g/mol):	287.1	175.0	286.2	443.2
CL (mL/min/kg; rat):	10	2.3	7.9	16.8
F (%; rat):	52	89	28	0.25

a) Literature data, b) *In house* data

Figure 1. Properties (inhibitory activity toward human ARG1, molecular weight, clearance and oral bioavailability in rats) of literature examples of ARG inhibitors.[23,24,34]

side chain increases the activity (**2** vs **3**; 1200 nM vs 223 nM; Figure 1) via interaction with aspartic acid Asp 183, but on the other hand, it has a negative influence on the PK profile (CL and F).[24]

As shown in Figure 1, compound **3** (MW = 286 g/mol) is relatively flexible and has a moderate oral bioavailability and clearance; however, it is probably not active enough for studies in humans. Compound **4** with a high molecular weight (443.2 g/mol) and rigid construction has a fast clearance and very low oral bioavailability.[24] It is noteworthy that cyclic compound **1** with low molecular weight and high potency shows unusually good oral bioavailability. Structurally, **1** differs from the others. Being a dipeptide-like compound it could be actively transported from the intestine via a peptide transporter system (e.g., PEPT1/2) rather than a simple amino acids transporter system (e.g., OATs, OCTs, CATs). However, it has a moderately fast clearance and because of that, this compound must be administered twice a day to humans. There is still a need to find compounds that are potent arginase inhibitors but with low clearance and high oral bioavailability.

As part of our arginase program, we designed and synthesized two groups of inhibitors: one with a nonbasic side chain and the second with a basic side chain, low molecular weight, and simple linear structure (Figure 2). We

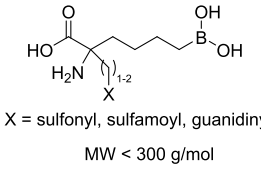


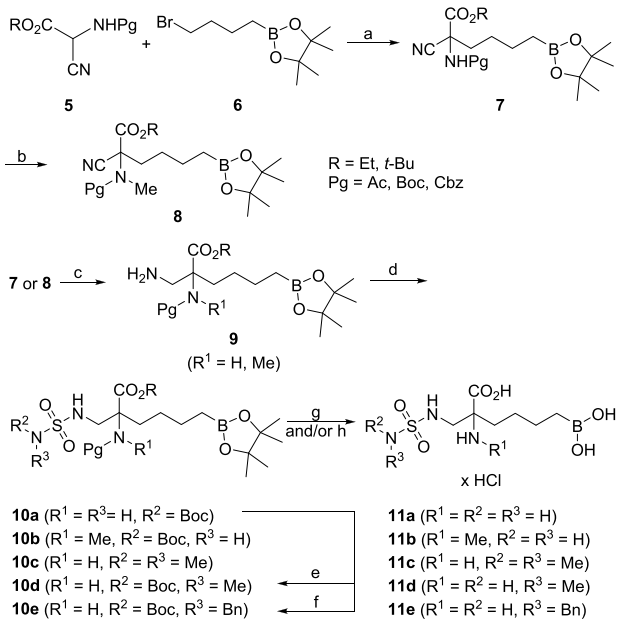
Figure 2. Compounds designed and synthesized in this work.

postulate that the only possible mechanisms of absorption in such polar, transition state analog inhibitors are active transport and/or paracellular transport and that these strongly hydrophilic compounds are not likely to undergo extensive first-pass metabolism. Moreover, reabsorption of the compounds, preventing renal clearance, must run via active transport predominantly. For these two mechanisms, the most important factors are molecular weight (<300 g/mol) and similarity to the naturally transported substances (amino acids) rather than polarity (log D < 0 is favorable).[35,36]

Sulfonamidyl and sulfamidyl are well-known as hydrogen bond-donor/acceptor functional groups with strong H-bond interaction and tetrahedral shape. Highly basic, planar guanidines also have very high affinity to H-bond formation. In addition, both groups possess drug-like properties, well-known for decades, which may explain the huge number of drugs incorporating these motifs.[37−41]

First, sulfamide derivatives were prepared. Alkylation of *N*-protected cyanoacetate **5** with pinacol-4-bromobutylboronate **6** afforded quaternary boronic cyanoaminoesters **7** that were further subjected to subsequent methylation, followed by reduction, or direct reduction to afford free primary amines **9**. Sulfamoylation of **9**, followed by hydrolysis of such formed sulfamides **10a−c**, gave the desired boronic acids **11a−c**. *N*-Terminally alkylated analogs **11d** and **11e** were obtained from **10a** using appropriate alcohol in Mitsunobu reaction and subsequent deprotection of such formed sulfamides **10d** and **10e** (Scheme 1).[42,43]

Scheme 1. Synthesis of Sulfamides 11[a]



[a]Reagents and conditions: (a) NaH, DMF; (b) NaH, DMF, then MeI; (c) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, MeOH; (d) $ClSO_2NCO$, *t*-BuOH, TEA, DCM, 0 °C (for **10a**); (*tert* butoxycarbonyl)((4-(methyliminio)pyridin-1(4*H*)-yl)sulfonyl)amide, TEA, DCM, RT, (for **10b**); $ClSO_2NMe_2$, TEA, DCM, 0 °C (for **10c**); (e) TPP, DIAD, THF, then MeOH; (f) TPP, DIAD, THF, then BnOH; (g) HCl/dioxane; (h) $HCl_{(aq)}$.

The racemic guanidine derivatives **15a−c** were obtained in a similar manner via guanidinylation of amines **9**, as depicted in Scheme 2.[44]

The asymmetric route was also developed. The synthesis of enantiomerically enriched guanidine **15aa** is illustrated in Scheme 3. Asymmetric, diastereoselective *aza*-Henry reaction led to the formation of nitro amino ester **19** that was subjected to hydroboration and subsequent reduction to obtain boronate **21**.[45] Amine **21** was next guanidylated and deprotected to give a desired amino acid **15aa**.[44] Enantiomeric purity of **15aa** was >98% ee based on the diastereomeric excess of the product after guanylation of **21**.

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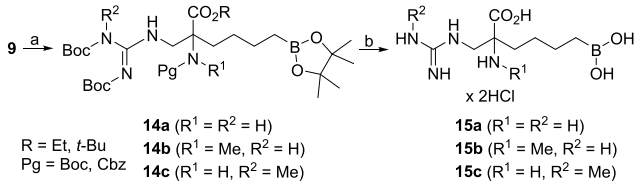
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of Guanidines 15[a]

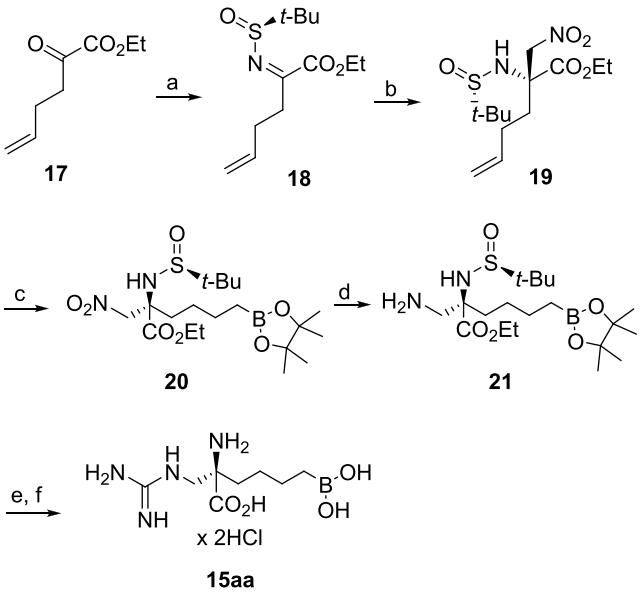


14a (R^1 = R^2 = H)
14b (R^1 = Me, R^2 = H)
14c (R^1 = H, R^2 = Me)

15a (R^1 = R^2 = H)
15b (R^1 = Me, R^2 = H)
15c (R^1 = H, R^2 = Me)

[a]Reagents and conditions: (a) *tert*-butyl (((*tert*-butoxycarbonyl)-amino)(1*H*-pyrazol-1-yl)methylene)carbamate, MeCN (for **14a** and **14b**); *tert*-butyl (((*tert*-butoxycarbonyl)-imino)-(1*H*-pyrazol-1-yl)-methyl)(methyl)carbamate, MeCN (for **14c**); (b) HCl/dioxane and/or 6 M HCl_{aq}.

Scheme 3. Asymmetric Synthesis of Guanidine 15aa



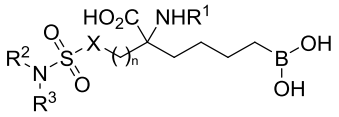
[a]Reagents and conditions: (a) (*S*)-(−)-2-methyl-2-propanesulfinamide, $Ti(OEt)_4$, DCM; (b) TBAF, $MeNO_2$; (c) pinacolborane, $[Ir(cod)Cl]_2$, DPPE, DCM; (d) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, MeOH; (e) *tert*-butyl (((*tert*-butoxycarbonyl)amino)(1*H*-pyrazol-1-yl)methylene) carbamate, Et_3N, MeCN; (f) 6M HCl_{aq}.

All final compounds described in this work were obtained and tested as hydrochloride salts. The novel sulfamide and guanidine analogs were evaluated in a human recombinant arginase 1 colorimetric enzymatic assay, measuring the inhibition of arginase-induced urea production.[46] The results from these experiments are listed in Tables 1 and 2, where the IC_{50} of synthesized compounds and reference inhibitors **1** and **2** are shown.

The introduction of the unsubstituted sulfamoylomethylene group in the α-position of ABH scaffold led to **11a** with more than 3.5-fold higher potency than ABH (0.33 vs 1.2 μM). It is worth noting that **11a** (MW = 283 g/mol) is much more active than unsubstituted basic primary aminomethylene analog (IC_{50} = 2 μM, lit. data)[22] and is the most active linear ABH analog with nonbasic side chain with the MW < 300 g/mol described so far.[22,25]

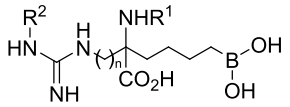
To improve the potency of **11a** simple methyl substituent was introduced in the R^1 position, but instead, a significant decrease in the inhibitory activity of **11b** was observed. Modification of the sulfamide moiety by the introduction of terminal *N*-alkyl substituents also generated less active

Table 1. *In Vitro* Activity of Sulfamoylo Derivatives



Cmpd	x	n	R^1	R^2	R^3	ARG1 IC_{50} (μM)
1		Reference compound				0.12
2		Reference compound				1.2
11a	NH	1	H	H	H	0.33
11b	NH	1	Me	H	H	2.3
11c	NH	1	H	Me	Me	0.73
11d	NH	1	H	H	Me	1.0
11e	NH	1	H	H	Bn	1.4
12	NH	2	H	H	H	2.8
13	CH_2	1	H	H	H	1.1

Table 2. *In Vitro* Activity of Guanidine Analogs



Cmpd	n	R^1	R^2	ARG1 IC_{50} (nM)
15a	1	H	H	67
15aa		R-enantiomer		32
15ab		S-enantiomer		6800
15b	1	Me	H	78
15c	1	H	Me	233
16	2	H	H	7000

compounds (**11c−e**). *N*,*N*-Dimethylsulfamoyl derivative (**11c**) was the most active of them (0.73 μM). In turn, bulkier, benzyl substituent as R^3 (in **11e**) was the least tolerated. We also found that potency decreases together with an extension of the spacer to two carbon atoms, and so simple ethylene-linked unsubstituted sulfamide homologue **12** exhibited substantially lower activity than **11a**. Interestingly, sulfonamide **13** demonstrated a clear impairment (3.3-fold) in potency compared to compounds being sulfamide **11a**. It clearly shows that NH in the methylene-bridge position is more favorable than the CH_2 group, likely due to its H-donor character. The most active compound in this series (**11a**) was nevertheless almost 3-fold weaker ARG1 inhibitor than **1**.

From the guanidine series presented in Table 2 the compounds with methylene-linker (**15a−15c**) showed a clear improvement over ABH and sulfamides **11** in the inhibitory potency toward arginase. In this context, the replacement of the sulfamide moiety in molecule **11a** into guanidine in inhibitor **15a** resulted in the formation of a 5-fold more active compound (**15a**) with IC_{50} = 67 nM. This presumably results from a more optimal and stronger binding interaction with Asp 181 and Asp 183 in the ARG1 active site by dual-ionic and hydrogen-bond interaction induced by guanidine. However, other interactions are also possible (especially with Ser137, Glu186, Gly142, Thr136, His126, and Asn130).[22−24]

As expected, the small highly polar enzyme active site accommodates only amino acids with the natural R-stereo configuration such as **15aa**. Unnatural amino acids with S-configuration, such as **15ab**, are much less active with IC_{50} = 6.8 μM. Enantiomerically enriched guanidine **15aa** is the most active hARG1 inhibitor with such a low molecular weight

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ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

(246.1 g/mol) as well as with the linear, noncyclic structure described to date. Because the α-amino group of the arginase inhibitors is involved in numerous important interactions with protein, only a limited choice of substituents at this group is possible. Interestingly, as seen with **15b**, incorporation of a simple methyl substituent at position R^1 has no high influence on the inhibitory potency. On the other hand, the methyl group on guanidine (in **15c**) resulted in decreased activity. The elongation of the carbonic linker in the guanidine series to two carbon atoms gave **16** with substantially lower activity. Thus, homologue **16** was the least active compound described in this work, with IC_{50} = 7 μM.

Next, the most potent racemic inhibitors from both series (**11a** and **15a**) were screened in mouse PK (Table 3).

Table 3. Pharmacokinetic Parameters of 11a, 15a, and 15aa

Compound	**11a** (mouse)	**15a** (mouse)	**15aa** (rat)
AUC,$n_{0\text{-}inf}$(i.v. kg·h/L)	4.13	1.66	3.92
C_0orC_{max}(i.v.mg/L)	34.59	18.83	21.22
CL (i.v.mL/min/kg)	4.0	10.0	4.2
$t_{1/2}$(i.v. h)	3.26	0.62	32.71
Vss (i.v.L/kg)	0.2	0.16	3.04
MRT (i.v. h)	0.81	0.26	11.91
F (p.o. %)	7	<1	4

Following intravenous administration in mice, compounds in this series generally exhibited low to moderate clearance, low volume of distribution, and low to moderate half-life. When compared to mouse PK of **1** the novel inhibitors **11a** and **15a** have over 5- and 2-fold slower clearance, respectively.[34] The bioavailability for oral dosing was low. Because of a low oral bioavailability in mice, antitumor efficacy studies in mouse tumor models have not been performed.

On the other hand, to compare PK profiles of the most active inhibitor **15aa** to other known inhibitors (e.g., **1**, **3**, and **4**, for which the rat PK parameters are known), the PK parameters of the compound in rats were measured.

The rat PK profile for optically active **15aa** was better than that for reference inhibitors **1**, **3**, and **4** after i.v. administration (slow clearance, a moderate volume of distribution, long $t_{1/2}$). Its oral bioavailability, however, was still very poor (4%). Nevertheless, the concentration of **15aa** is up to 4-fold higher than IC_{50} of human ARG1 for 24 h after compound administration i.v. as well as p.o. in low doses (Figure 3).

In summary, we have discovered a series of potent inhibitors of arginase 1, starting from ABH as a lead compound.

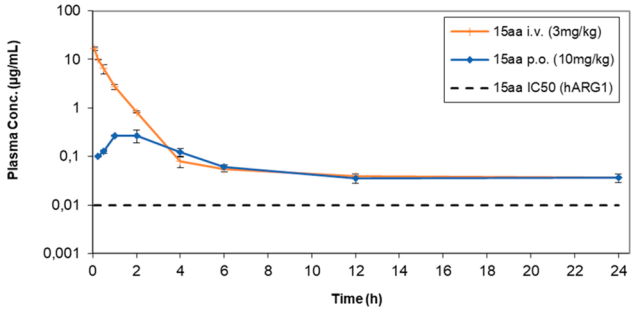

Figure 3. Pharmacokinetic profile of **15aa** in rats. Plasma concentrations following 3 mg/kg i.v. (orange line) and 10 mg/kg p.o. (blue line) administrations to male Sprague–Dawley rats.

Transforming an α position on the scaffold by the introduction of sulfamides and guanidines led to increasing activity providing compounds with low molecular weight and linear structure. Synthesized inhibitors demonstrated an improved pharmacokinetic profile after intravenous administration compared to the other known inhibitors; however, they suffered from low oral bioavailability.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.9b00508.

Chemistry and synthetic schemes, synthetic procedures and compound characterization, description of biochemical assays, PK (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

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Notes

The authors declare the following competing financial interest(s): Some of the authors are current employees of OncoArendi Therapeutics S.A. and own company stocks.

1. CB-1158 also known as INCB001158 was discovered by Calithera Biosciences and colicensed to Incyte Corporation. The compound is commercially available from several vendors. CAS: 2095732-06-0; UNII: IFD73D535A; DB15286.

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ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

2. Reference inhibitor **1** is compound no. **10** from patent US10065974(B2) (Calithera Biosciences). Both reference compounds, **1** and **2**, were tested in house as hydrochloride salts. ARG1/CB-1158 crystal structure has been resolved.[47]

■ ABBREVIATIONS

ABH, 2(*S*)-amino-6-boronohexanoic acid; AUC, area under curve; Boc, *tert*-butoxycarbonyl; CL, clearance; C_{max}, peak concentration; cmpd, compound; cod, 1,5-cyclooctadiene; DCM, dichloromethane; DIAD, diisopropyl azodicarboxylate; DPPE, ethylenebis(diphenylphosphine); F(%), oral bioavailability; H-bond, hydrogen bond; hARG1, human arginase 1; i.v., intravenous; LOQ, limit of quantification; MW, molecular weight; OAT, organic anion transporter; OCT, organic cation transporter; CAT, cationic amino acid transporter; Pg, protective group; PK, pharmacokinetics; p.o., per os; PSA, polar surface area; $t_{1/2}$, half-life; TBAF, tetra-*n*-butylammonium fluoride; TEA, triethylamine; TPP, triphenylphosphine; V_{ss}, volume of distribution

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