Synthesis and pharmacological characterization of the dipeptide piperidine derivatives as a novel orally available arginase inhibitors

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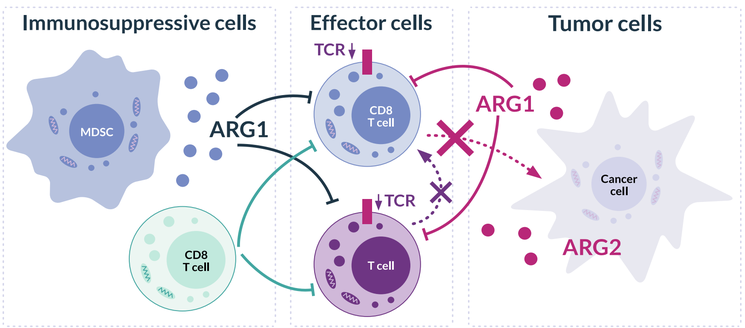
Introduction

The role of arginine and arginases in immune function

Arginases (ARG1 and ARG2) are the enzymes that hydrolyze arginine to ornithine and urea. The appropriate concentration of L-arginine plays an important role in the full immune response in mammals. L-Arginine deficiency down-regulates the expression of the CD3 ζ chain, a critical signaling element of the TCR, thereby impairing T cell function. Moreover, depletion of L-arginine leads to an arrest in T cell cycle progression, inhibition of IFN-γ production, and blocking of signaling through the T cell receptor. ARG1 is mainly produced by myeloid-derived suppressor cells (MDSC) that are highly enriched in the tumor-bearing state but also by TAMs, neutrophils, and some tumor cells themselves. Arginase was shown to participate in the suppression of tumor-infiltrating lymphocytes in patients with prostate carcinoma, non-small cell lung carcinoma and multiple myeloma. Moreover, some cancer cells release ARG1-containing exosomes, further suppressing antitumor immunity. Therefore, inhibition of arginase has great therapeutic potential.[1]



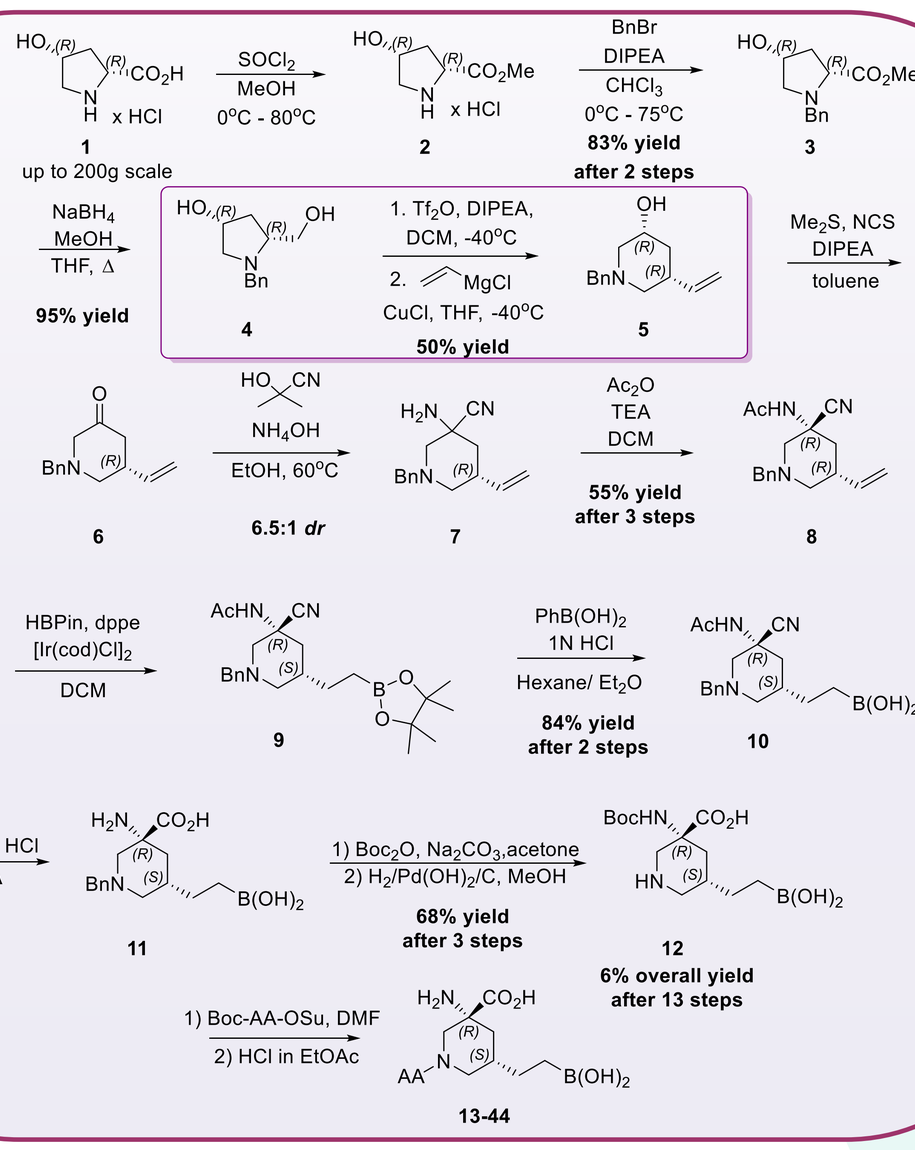
First-time disclosure of novel orally available Arg1 inhibitors

Herein, we present a novel class of arginase inhibitors[2] which are piperidine dipeptides exhibiting a different pharmacological profile compared to our clinical candidate – **OATD-02**[3] *a first-in-class*, low nanomolar dual ARG1/2 inhibitor with high intracellular activity. Compounds from this series poorly penetrate cellular membranes, hence these inhibitors are able to inhibit mainly the extracellular ARG1. We focused on developing arginase inhibitors that can be transported across cell membranes and into the body by specific transporters (PEPT1/2), which could potentially result in improved bioavailability.

Results

Innovative stereospecific synthetic pathway

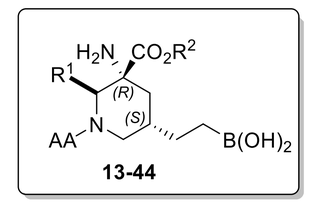
We developed an innovative stereospecific synthetic pathway starting from the commercially available *cis*-4-hydroxy-D-proline hydrochloride **1**. In two steps *via* ester **2**, methyl (2*R*,4*R*)-1-benzyl-4-hydroxypyrrolidine-2-carboxylate **3** was obtained. Reduction of the ester **3** to (3*R*,5*R*)-1-benzyl-5-(hydroxymethyl)-pyrrolidin-3-ol **4** and subsequent stereospecific expansion of the five-membered to the six-membered ring by reaction of derivative **4** (*via* aziridinium intermediate) with vinyl magnesium chloride in the presence of DIPEA, gave hydroxypiperidine **5**, as a single diastereoisomer with good yield (50%). It is worth mentioning that the ring expansion of unprotected hydroxypyrrolidines, using the vinyl Gringard reagent has not been described in the literature so far. The ketone **6** obtained *via* Corey-Kim oxidation was subjected to the Strecker reaction, and the obtained aminonitrile **7** (6.5: 1 *dr*), was protected with an acetyl group. After the chromatographic separation of the diastereoisomers, acetamide **8** was hydroborated under iridium-catalyzed conditions. The formed boronic ester **9** was subjected to transesterification with phenylboronic acid and hydrolyzed with 12N hydrochloric acid to form amino acid **11**. After protection of the primary amino group with Boc, followed by deprotection of the benzyl group, the resulting amino acid **12** was then used for coupling with Boc-protected *N*-hydroxysuccinimide esters of several aminoacids (Boc-AA-OSu).



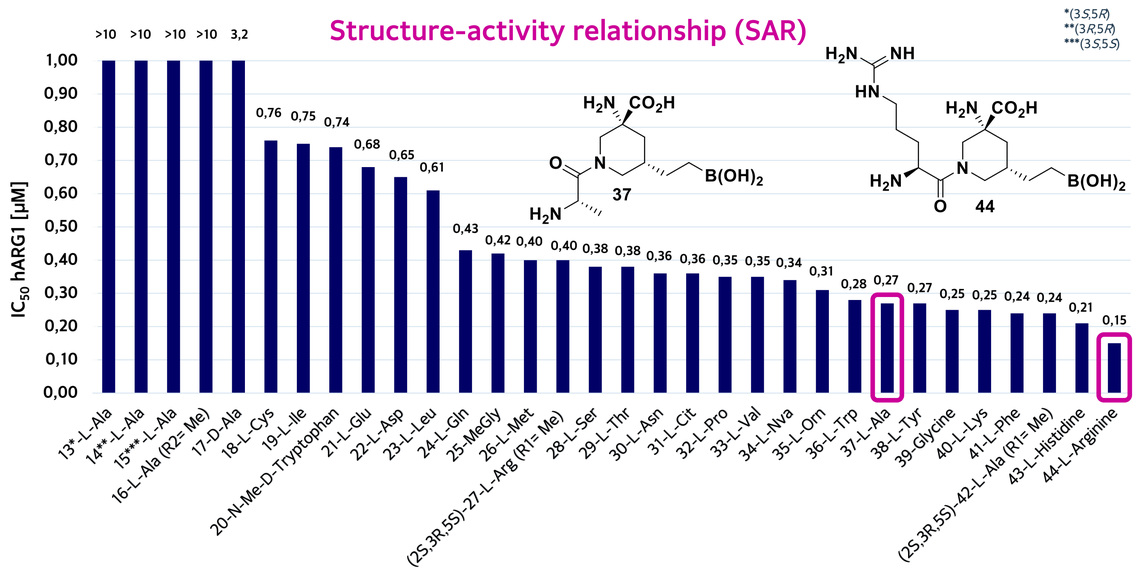
In vitro studies

All possible diastereoisomers (**13**, **14**, **15**, **37**) with attached L-alanine, the effect of the presence of methyl ester on the activity (**16**), the opposite enantiomer of L-alanine (**17**) and a series of derivatives with attached natural and unnatural amino acids (**18**-**44**) were tested.

The selected compounds were tested toward intracellular inhibition of mouse arginase using bone marrow-derived M2-polarized macrophages, which surrogate immunosuppressive TME. Poor intracellular activity (>100μM) was observed for the tested compounds, probably due to their low penetration through cell membranes.

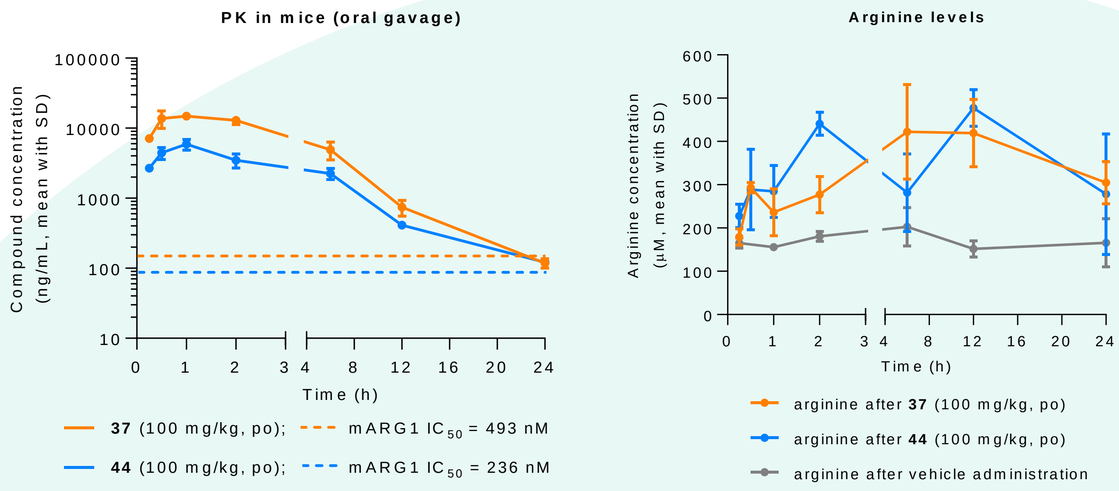


Structure-activity relationship (SAR)



PK/PD profile

For the selected compounds we performed PK/PD studies in mice and/or rats (bioavailability up to 67%). Compounds **37** and **44** showed sufficient plasma exposure after PO administration in mice. Concentrations of these compounds remained (up to 24h) above the IC_{50} (mouse arginase (mARG1)). After oral administration of **44**, a similar increase in arginine (PD effect) was observed, in comparison to **37,** despite higher exposure of **37**.



Conclusions

The novel class of arginase inhibitors, unnatural boronic acid-based dipeptides exhibiting a different pharmacological profile compared to our clinical candidate – **OATD-02,** has been discovered:

- An innovative, multi-step, stereospecific synthetic pathway was developed
- The disclosed series showed good *in vitro* activity against hARG1 (IC_{50} up to 150 nM)
- Favorable pharmacokinetics in animal models (oral bioavailability up to 67%)
- Clean *in vitro* (eg.: cytotoxicity, hERG, Diversity Panel) and *in vivo* safety profiles for **44**
- After oral administration, a significant arginine level increase was observed in plasma

The developed arginase inhibitor **44** was tested *in vivo* in several animal tumor models and showed weak antitumor activity. This is probably due to poor penetration of cellular membranes and thus inhibits only extracellular ARG1. Based on the results obtained for **44**, we believe that a better application of this type of compound may be potential therapies where extracellular ARG1 plays a major role e.g. cardiovascular indications.[4]

References

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