Discovery of OAT-1441 – highly active, selective and orally bioavailable inhibitor of human AMCase

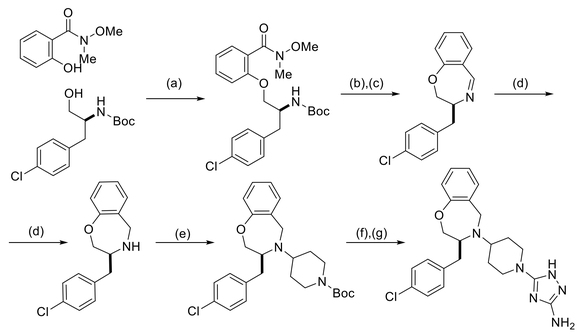
Gleb Andryianau, Michał Kowalski, Michał Piotrowicz, Barbara Dymek, Piotr Sklepkiewicz, Adam Rajkiewicz, Magdalena Salamon, Agnieszka Zagożdżon, Aleksandra Rymaszewska, Marcin Mazurkiewicz, Szymon Klossowski, Marzena Mazur, Sylwia Olejniczak, Robert Koralewski, Krzysztof Matyszewski, Bartłomiej Borek, Wojciech Czestkowski, Piotr Niedziejko, Agnieszka Bartoszewicz, Elżbieta Pluta, Mariusz Gruza, Karolina Dzwonek, Filip Stefaniak, Jacek Olczak, Adam Gołębiowski

OncoArendi Therapeutics S.A., Żwirki i Wigury 101, 02-089 Warsaw, Poland

Background

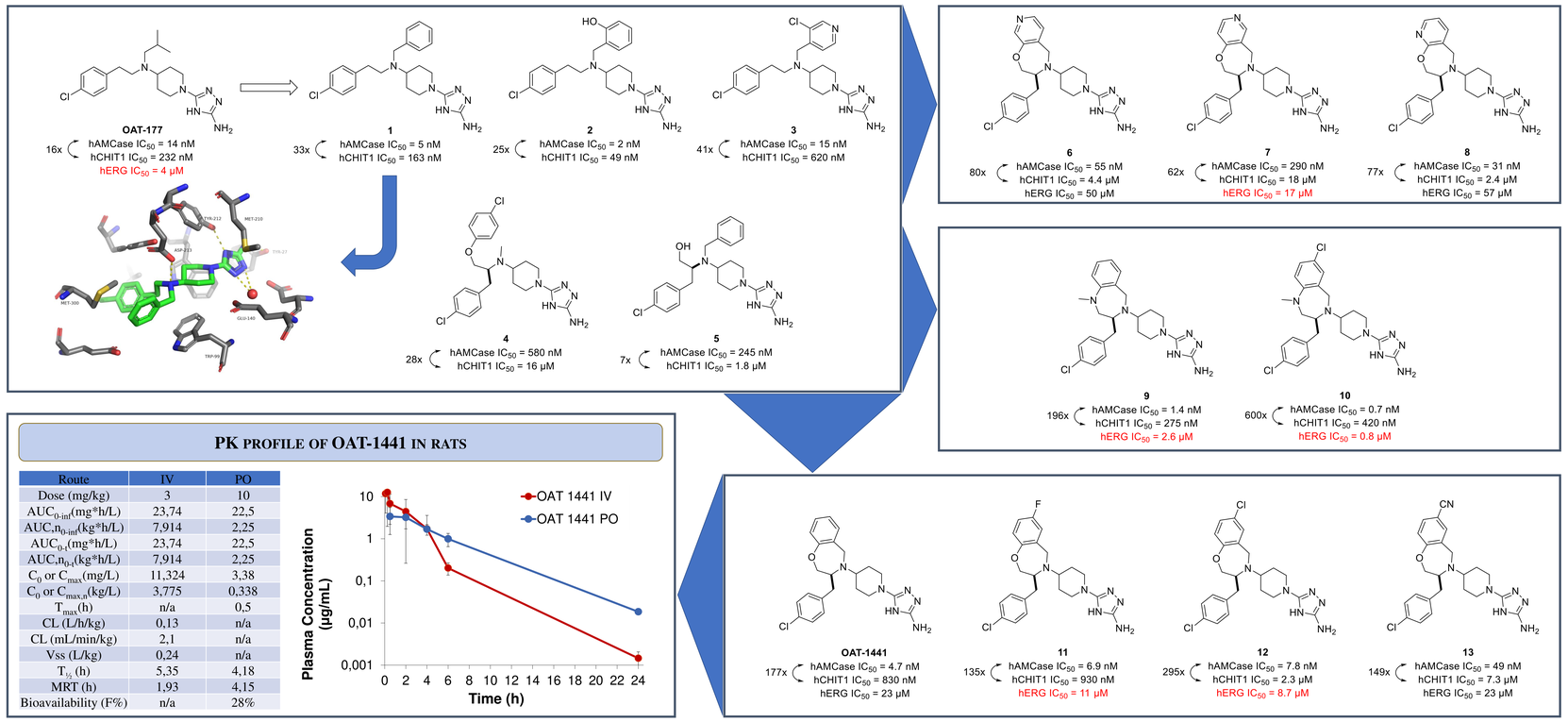
Acidic mammalian chitinase (AMCase) is one of two catalytically active proteins in mammals (chitotriosidase CHIT1 is the other one) which is a representative of evolutionary conserved family of GH18 glycoside hydrolases. [1] Elevated levels of AMCase suggest Th2 airway inflammation process which is associated with allergies and asthma. It has been shown, that administration of bisdionin F – a small molecule AMCase inhibitor strongly suppressed those inflammatory effects in mouse model of asthma. [2] In related studies, as a part of our in-house Chitinase Inhibitors Platform we confirmed that mAMCase selective molecule **OAT-177** is efficacious in the clinically relevant asthma mouse HDM model. [3] Additionally we had developed and reported **OAT-2068** – selective mCHIT1 inhibitor. [4] Both compounds have been shown to have excellent PK parameters which make them suitable for *in vivo* studies of the role of these biological targets in mouse models. The discovery of **OAT-1441** (along with our in-house hCHIT1 selective inhibitors program) completes the set of selective chitinases inhibitors and provide the right tools to dissect chitinases roles in the biological processes.

Synthesis of OAT-1441



Reagents and conditions for the synthesis of OAT-1441: (a) DEAD, Ph_3P, THF, -15 °C → RT, 24h; (b) $LiAlH_4$ 0 °C → RT; (c) HCl in AcOEt 0 °C → RT then Et_3N (d) 1,2-DCE, $NaBH(OAc)_3$, RT, overnight; (e) N-Boc-4-piperidone, AcOH, 1,2-DCE, 70 °C, 2h then $NaBH(OAc)_3$, RT, overnight; (f) HCl in AcOEt 0 °C → RT; (g) S,S'-Dimethyl-N-cyanodithioiminocarbonate, K_2CO_3, CH_3CN, 82 °C then $N_2H_4 \cdot H_2O$, 82 °C

Structure Activity Relationship for AMCase selective inhibitors



PK profile of OAT-1441 in rats

Route	IV	PO
Dose (mg/kg)	3	10
$AUC_{0\text{-}inf}$(mg*h/L)	23,74	22,5
$AUC,n_{0\text{-}inf}$(kg*h/L)	7,914	2,25
$AUC_{0\text{-}t}$(mg*h/L)	23,74	22,5
$AUC,n_{0\text{-}t}$(kg*h/L)	7,914	2,25
C_0 or C_{max}(mg/L)	11,324	3,38
C_0 or $C_{max,n}$(kg/L)	3,775	0,338
T_{max}(h)	n/a	0,5
CL (L/h/kg)	0,13	n/a
CL (mL/min/kg)	2,1	n/a
Vss (L/kg)	0,24	n/a
$T_{½}$ (h)	5,35	4,18
MRT (h)	1,93	4,15
Bioavailability (F%)	n/a	28%

Methods

IN VITRO ACTIVITY MEASUREMENTS

Enzymatic Assays: IC_{50} Determination toward Human and Mouse AMCase and Human and Mouse CHIT1. Human and mouse AMCase and human and mouse CHIT1 recombinant proteins were produced in CHO-K1 cells after transient transfection with plasmid coding full-length protein with C-terminal His-tag. IC_{50} values of all inhibitors against hAMCase and hCHIT1 were determined from dose-response sigmoidal curves of the % of inhibition vs log(inhibitor concentration) using GraphPad Prism version 6.0 according to previously published protocols. [3, 4] Experiments were performed in duplicate or triplicate.

For evaluation of binding of selected compounds to hERG channel, Predictor™ hERG Fluorescence Polarization Assay Kit (Themofisher Scientific) was used according to manufacturer's protocol.

IN VIVO PHARMACOKINETIC PROFILE STUDY

The pharmacokinetic properties of **OAT-1441** were evaluated in male Sprague-Dawley rats following single intravenous bolus or oral administration. The solution was prepared in a 10% EtOH/10 % solutol/80% water vehicle for intravenous bolus and oral administrations at 3 mg/kg or 10 mg/kg doses, respectively. Blood collection was performed according to state-of-the-art procedure under anesthesia with sampling of blood into K_2EDTA anti-coagulant tubes, followed by centrifugation to obtain plasma. Samples were stored frozen at − 20 °C or lower prior to compound extraction and LC/MS/MS analysis. Pharmacokinetic parameters were calculated by standard modeling from the systemic plasma concentration

Literature

[1] R. Hamid *et al*, *J. Pharm. Bioallied Sci.*, **2013**, *5*, pp 21−29
[2] T. E. Sutherland *et al*, *Chem. Biol.*, **2011**, *18*, pp 569−579
[3] M. Mazur *et al*, *Biorg. Med. Chem. Lett.*, **2018**, *28*, pp 310−314
[4] M. Mazur *et al*, *J. Med. Chem.*, **2018**, *61*, pp 695−710

Summary

In summary, we have developed **OAT-1441**, novel inhibitor of human AMCase with extraordinary potency, selectivity and PK profile. Starting from **OAT-177**, we added aromatic ring system to the central nitrogen atom and subsequently locked the conformation into 7-membered ring system, which helps to decrease N-metabolism and significantly improves the final pharmacokinetic profile. Compared to our starting point (**OAT-177**), **OAT-1441** exhibits significantly decreased hERG activity (both binding & functional patch-clamp assays).

Financial support:

„Preclinical research and clinical trials of first-in-class development candidate in therapy of asthma and inflammatory bowel disease”

European Funds Smart Growth

European Union
European Regional Development Fund