DISCOVERY OF OAT-1441 – HIGHLY POTENT, SELECTIVE AND ORALLY BIOAVAILABLE INHIBITOR OF HUMAN ACIDIC MAMMALIAN CHITINASE

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BACKGROUND

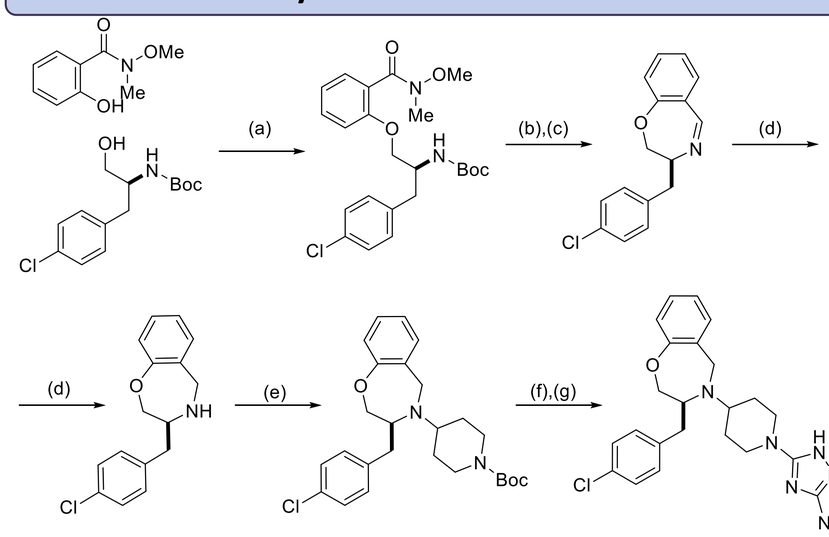
Acidic mammalian chitinase (AMCase) belongs to a family of evolutionary conserved GH18 glycoside hydrolases and is one of two catalytically active proteins in mammals (chitotriosidase CHIT1 is the other one).[1]

Elevated levels of AMCase are detected during Th2 airway inflammation associated with allergies and asthma. It was observed in mouse model of asthma, that administration of bisdionin F – a small molecule AMCase inhibitor strongly suppressed those inflammatory effects.[2]

As a part of our program targeting chitinases inhibition as a potential therapy for pulmonary diseases, our research focuses on finding specific and potent compounds towards each of enzymes mentioned above. Recently we have reported a highly selective mouse CHIT1 inhibitor **OAT-2068**[3] and selective mouse AMCase inhibitor **OAT-177**. Both compounds displayed excellent PK profiles and *in vitro* activities. Additionally **OAT-177** was effective in clinically relevant aeroallergen-induced asthma model in mice (HDM-induced allergic airway inflammation).[4]

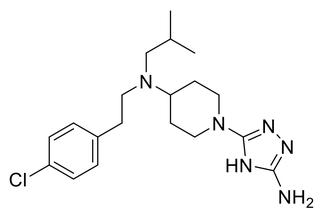
Herein we describe the continuation of these studies focused on improvement of characteristics of inhibitor **OAT-177** in terms of its *in vitro* activity against human AMCase, high selectivity versus human CHIT1, lower hERG inhibition, and adequate pharmacokinetic properties in rats.

Synthesis of OAT-1441



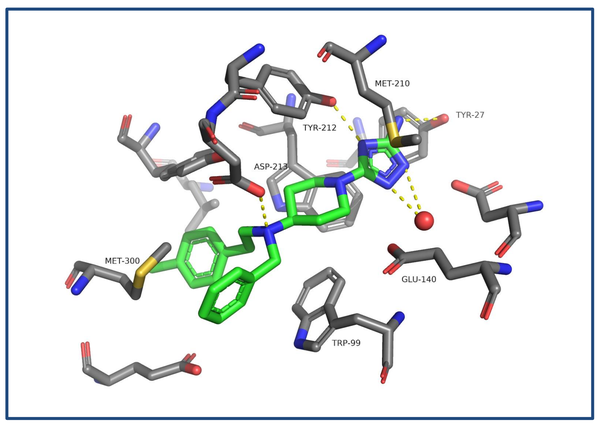
Reagents and conditions for the synthesis of **OAT-1441**: (a) DEAD, Ph_3P, THF, -15 °C → RT, 24h; (b) $LiAlH_4$ 0 °C → RT; (c) HCl in AcOEt 0 °C → RT then Et_3N (d) 1,2-DCE, $NaBH(OAc)_3$, RT, overnight; (e) N-Boc 4-piperidone, AcOH, 1,2-DCE, 70 °C, 2h then $NaBH(OAc)_3$, RT, overnight; (f) HCl in AcOEt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



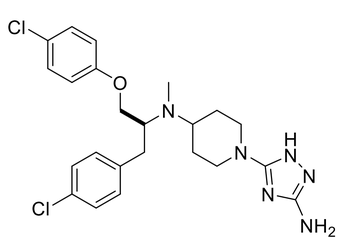
OAT-177
hAMCase IC_{50} = 14 nM
hCHIT1 IC_{50} = 232 nM
hERG IC_{50} = 4000 nM

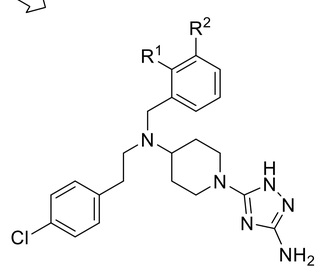
16x



31x

hAMCase IC_{50} = 580 nM
hCHIT1 IC_{50} = 16000 nM





R^1 = H; R^2 = OH
hAMCase IC_{50} = 2 nM
hCHIT1 IC_{50} = 98 nM
hERG IC_{50} = 150 nM

49x

R^1 = H; R^2 = H
hAMCase IC_{50} = 5 nM
hCHIT1 IC_{50} = 163 nM

33x

R^1 = OH; R^2 = H
hAMCase IC_{50} = 2 nM
hCHIT1 IC_{50} = 49 nM

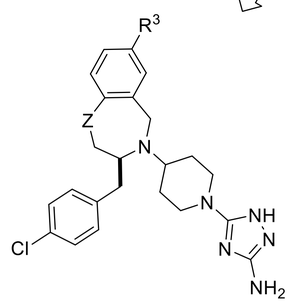
25x

196x

Z = CH_3N; R^3 = H
hAMCase IC_{50} = 1.4 nM
hCHIT1 IC_{50} = 275 nM
hERG IC_{50} = 2600 nM

600x

Z = CH_3N; R^3 = Cl
hAMCase IC_{50} = 0.7 nM
hCHIT1 IC_{50} = 420 nM
hERG IC_{50} = 800 nM



Z = O; R^3 = F
hAMCase IC_{50} = 7 nM
hCHIT1 IC_{50} = 930 nM
hERG IC_{50} = 6700 nM

133x

Z = O; R^3 = Cl
hAMCase IC_{50} = 7.6 nM
hCHIT1 IC_{50} = 2300 nM
hERG IC_{50} = 6700 nM

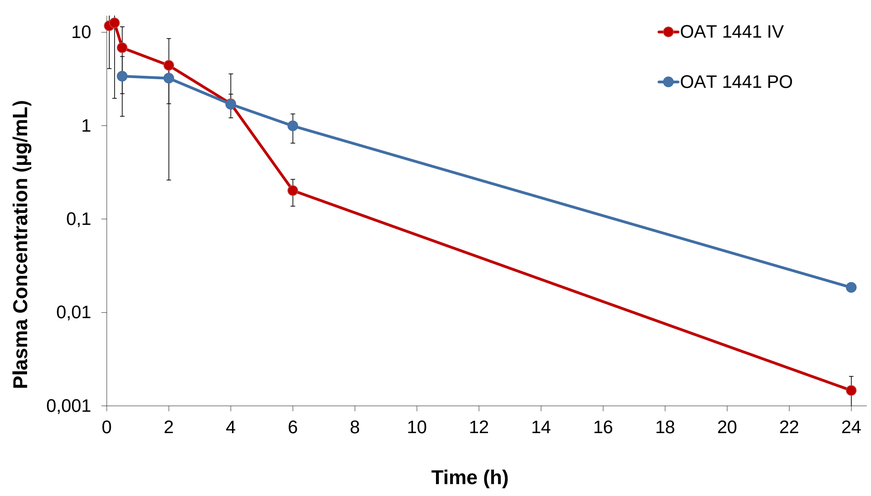
303x

OAT-1441
Z = O; R^3 = H
hAMCase IC_{50} = 4.7 nM
hCHIT1 IC_{50} = 830 nM
hERG IC_{50} = 22000nM

177x

Pharmacokinetic Profile of OAT-1441 in Rats

Plasma Concentrations of OAT-1441 Following 3 mg/kg IV and 10 mg/kg PO Administrations to Male Sprague-Dawley Rats



Pharmacokinetic parameters

Route	IV	PO
Dose (mg/kg)	3	10
$AUC_{0\text{-}inf}$(mg*h/L)	23.7	22.5
$AUC_{0\text{-}t}$(mg*h/L)	23.7	22.5
C_0 or C_{max}(mg/L)	11.3	3.4
T_{max}(h)	n/a	0.5
CL (L/h/kg)	0.13	n/a
Vss (L/kg)	0.2	n/a
$T_{½}$ (h)	5.4	4.2
MRT (h)	1.9	4.2
Bioavailability (F%)	n/a	28%

Materials and 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. The proteins were purified by nickel-affinity chromatography. Chitinolytic activity of the enzymes was measured using standard assay as previously described.

For determination of enzymatic activity 103 µM 4-methylumbelliferyl-β-D-N,N'-diacetylchitobioside hydrate and 5.2 ng per well of hAMCase, 46 µM 4-methylumbelliferyl-β-D-N,N'-diacetylchitobioside hydrate and 3 ng per well of mAMCase, 5 µM 4-methylumbelliferyl-β-D-N,N',N''-triacetylchitotrioside and 0.2 ng per well of hCHIT1 or 20 µM 4-methylumbelliferyl-β-D-N,N',N''-triacetylchitotrioside and 2 ng per well of mCHIT1 were used. Appropriate substrate, enzyme, and varying concentrations of compounds in assay buffer (0.1 M citrate, 0.2 M dibasic phosphate, 1 mg/mL BSA) were incubated in a 96-well black microtiter plate with shaking in the dark at 37 °C for 60 min followed by addition of stop solution (0.3 M glycine/NaOH buffer, pH 10.5). Substrate hydrolysis product 4-methlyumbelliferone was measured fluorometrically using Spark M10 (Tecan) microplate reader (excitation 355 nm/emission 460 nm). IC_{50} values of all inhibitors against AMCase and CHIT1 were determined from dose-response sigmoidal curves of the % of inhibition vs log(inhibitor concentration) using GraphPad Prism version 6.0. 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.

CONCLUSIONS

In summary, the new highly potent and selective AMCase inhibitors have been discovered and preliminary SAR was established. An advanced lead OAT-1441 has been fully characterized and was shown to have highly improved *in vitro* and pharmacokinetic profiles. Additionally, significantly reduced hERG activity, compared to OAT-177, was observed.

LITERATURE

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