DISCOVERY OF SELECTIVE, ORALLY BIOAVAILABLE INHIBITOR OF HUMAN ACIDIC MAMMALIAN CHITINASE

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Introduction

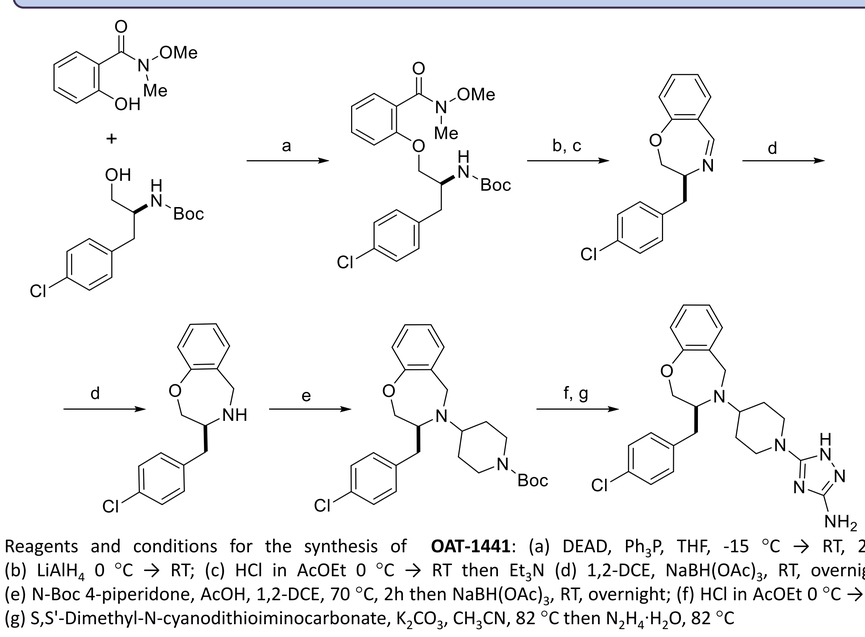
Human acidic mammalian chitinase (hAMCase) is a 52kDa enzyme expressed in the stomach, salivary gland and lungs (the other chitinolytically-active protein found in mammals is chitotriosidase CHIT1). Elevated levels of AMCase have been detected during type 2 inflammation in both murine asthma models[1,2] and allergic asthma patients[1].

Preliminary studies have shown that inhibition of the enzymatic activity of AMCase with the allosamidin or demethylallosamidin resulted in suppression of IL-13 mediated allergic inflammation and ovalbumin (OVA) induced allergy in murine lungs.[3]

Our ongoing studies are focused on finding selective and potent compounds towards each of chitinases mentioned above. Recently we have reported a highly selective mouse CHIT1 inhibitor **OAT-2068**[4] and selective mouse AMCase inhibitor **OAT-177**.[5] The latter was effective in house dust mite (HDM)-induced allergic airway inflammation model in mice.

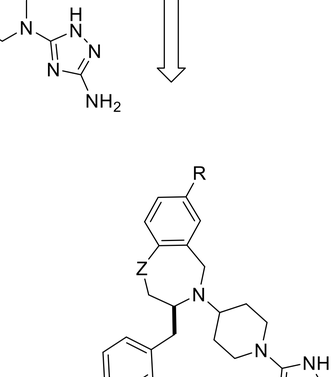
Herein, we report the synthesis and structure-based optimization of a new series of compounds that led us to the discovery of **OAT-1441**. The introduction of the benzoxazepine moiety resulted in high activity and selectivity along with significant decrease in inhibition of hERG. These characteristics together with improved in vitro and pharmacokinetic profile make OAT-1441 a suitable candidate for further preclinical development.

Synthesis of OAT-1441

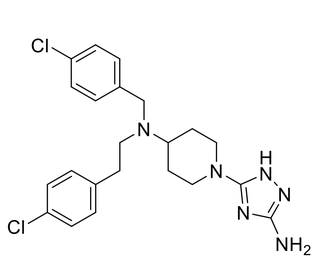


Reagents and conditions for the synthesis of **OAT-1441**: (a) DEAD, Ph_3P, THF, -15 °C → RT, 24h; (b) $LiAlH_4$ 0 °C → RT; (c) HCl in AcOEt 0 °C → RT then Et_3N (d) 1,2-DCE, $NaBH(OAc)_3$, RT, overnight; (e) N-Boc 4-piperidone, AcOH, 1,2-DCE, 70 °C, 2h then $NaBH(OAc)_3$, RT, overnight; (f) HCl in AcOEt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

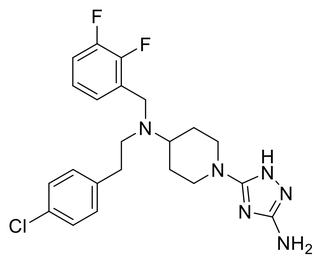
Structure Activity Relationship Leading to OAT-1441



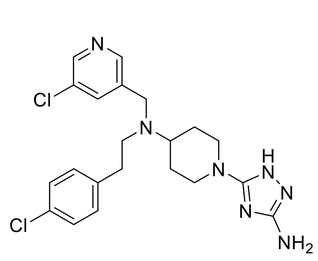
OAT-177
hAMCase IC_{50} = 14 nM
hCHIT1 IC_{50} = 232 nM
16x
hERG IC_{50} = 4000 nM



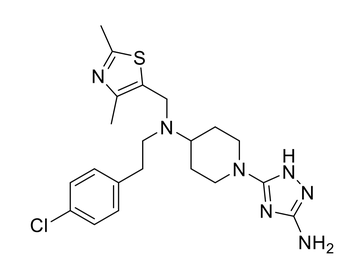
1
hAMCase IC_{50} = 6 nM
hCHIT1 IC_{50} = 149 nM
25x
hERG IC_{50} = 400 nM



2
hAMCase IC_{50} = 4 nM
hCHIT1 IC_{50} = 312 nM
78x
hERG IC_{50} = 5300 nM

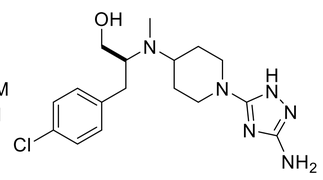


3
hAMCase IC_{50} = 21 nM
hCHIT1 IC_{50} = 742 nM
35x
hERG IC_{50} = 3800 nM



4
hAMCase IC_{50} = 9 nM
hCHIT1 IC_{50} = 205 nM
23x
hERG IC_{50} = 16000 nM
poor PK

5
10x
hAMCase IC_{50} = 223 nM
hCHIT1 IC_{50} = 2200 nM

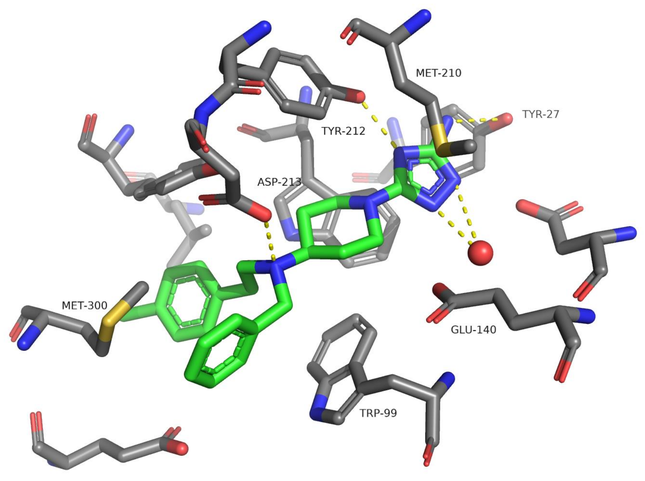


6
Z = NMe; R = Cl
600x
hAMCase IC_{50} = 0.7 nM
hCHIT1 IC_{50} = 420 nM
hERG IC_{50} = 800 nM

7
Z = NMe; R = H
196x
hAMCase IC_{50} = 1.4 nM
hCHIT1 IC_{50} = 275 nM
hERG IC_{50} = 2600 nM

8
Z = O; R = Cl
hAMCase IC_{50} = 7.6 nM
303x
hCHIT1 IC_{50} = 2300 nM
hERG IC_{50} = 6700 nM

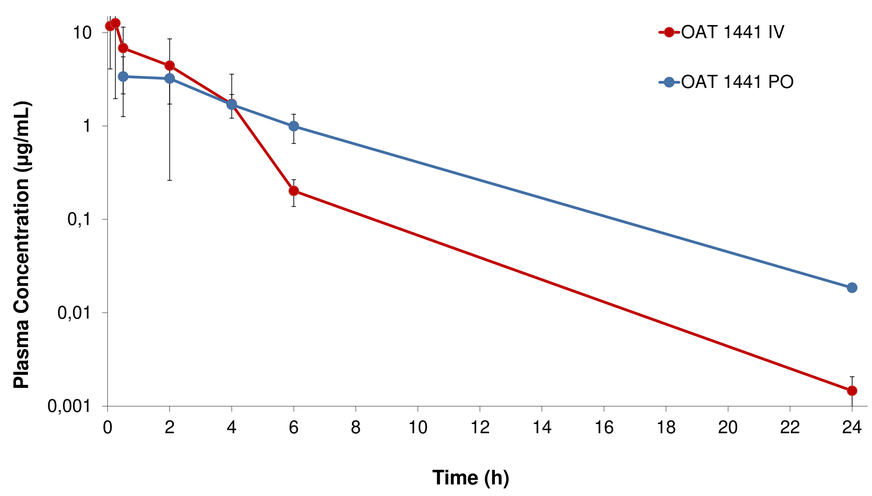
OAT-1441
Z = O; R = H
hAMCase IC_{50} = 4.7 nM
hCHIT1 IC_{50} = 830 nM
177x
hERG IC_{50} = 22000nM



Structure of the binding site of AMCase with **1** determined by X-ray crystallography.

Pharmacokinetic Parameters 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-inf}(mg*h/L)	23.7	22.5
AUC_{0-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%

Conclusions

In summary, the SAR for *N*-benzylic analogues of OAT-177 was established. Changing electronic and steric factors in aromatic ring as exemplified for structures 1-4 caused significant improvement of hAMCase/hChit1 selectivity however hERG parameter remained major concern. Further optimization by decreasing structural lability resulted in improvement of aforementioned *in vitro* parameters as well as pharmacokinetic properties of OAT-1441 .

Literature

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