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Identification of second-generation P2X3 antagonists for treatment of pain

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ABSTRACT

A second-generation small molecule P2X3 receptor antagonist has been developed. The lead optimization strategy to address shortcomings of the first-generation preclinical lead compound is described herein. These studies were directed towards the identification and amelioration of preclinical hepatobiliary findings, reducing potential for drug-drug interactions, and decreasing the projected human dose of the first-generation lead.

Despite the presence of numerous treatment options, pain due to osteoarthritis is still a primary complaint of older populations; furthermore, only a small percentage of pain sufferers receive a prescription treatment. New drugs with minimal side effects, improved efficacy, and ease of administration are sought for the treatment of pain. The P2X3 receptor is an ATP-gated ion channel expressed on nociceptors (primary afferent neurons that sense painful stimuli) with limited receptor expression in other tissues.[1] Its role in the transmission of nociceptive stimuli along with its selective expression pattern makes the P2X3 receptor an attractive target with the potential to provide treatment of inflammatory, visceral, and neuropathic pain states.[2] Indeed, P2X3 receptor antagonists identified in our laboratories have already demonstrated dose-dependent reversal of pain in the rat Complete Freund's Adjuvant (CFA) assay.[3]

MK-3901, disclosed previously, was the first P2X3 receptor antagonist preclinical candidate identified by our laboratories. MK-3901 is a potent antagonist of the P2X3 receptor as measured by our Ca^{++} mobilization FLIPR and patch clamp electrophysiology (EP) assays (FLIPR IP = 21 nM, EP IC_{50} = 24 nM).[4] Importantly, when evaluated in the rat CFA model of inflammatory pain, MK-3901 demonstrated efficacy similar to that of our positive comparator, naproxen, after 60 mg/kg (p.o.) administration with a measured EC_{90} of approximately 3 μM.[3] However, in non-human preclinical safety studies MK-3901 was shown to induce hyperbilirubinemia across several species, most notably a greater than 15-fold increase in total bilirubin levels in rhesus at exposures approximately 6-fold over the projected clinical AUC. The elevated bilirubin levels associated with MK-3901 could potentially be attributed to off-target inhibition of UGT1A1 (IC_{50} = 1 μM), a glucuronosyltransferase involved in bilirubin metabolism.[5] MK-3901 exhibited excellent bioavailability (%F) in rat, dog, and rhesus monkey pharmacokinetic (PK) studies (F = 60%, 68%, and 47%, respectively), however the compound was found to be extensively metabolized in human liver microsomes. Analysis of these metabolites identified both the northern pyridine methyl group and the isoxazoline ring as metabolic soft-spots. These factors lead to unacceptably high predicted dose and dose frequency (420 mg TID). In addition to hepatobiliary findings and a high projected human dose, MK-3901 also pre-

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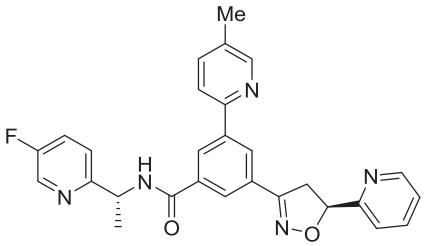


MK-3901

FLIPR IP = 21 nM
EP IC_{50} = 24 nM
Rat CFA: EC_{90} ~ 3 μM

CYP2C9 IC_{50} = 5.7 μM
PXR EC_{50} = 47% @ 10 μM
UGT1A1 IC_{50} = 1 μM

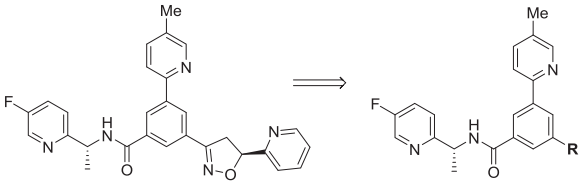
Rat Pharmacokinetics
%F = 60
PPB = 98.4%
$Cl_{int,\ in\ vivo}$ = 133 mL/min/kg
$t_{1/2}$ = 1.0 h

Predicted human dose:
420 mg TID

Fig. 1. Profile of MK-3901.

sented the potential for drug-drug interactions (DDI) due to cytochrome P450 (CYP2C9) inhibition and pregnane X receptor (PXR) activation as shown in Fig. 1.

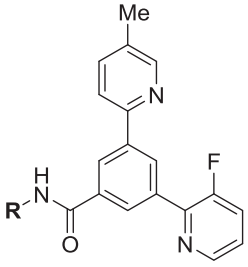
Table 1
SAR of eastern substituents.

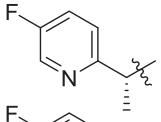
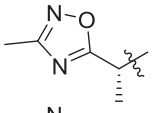
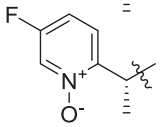
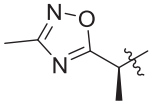
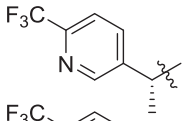
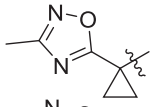
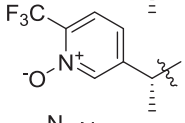
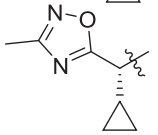
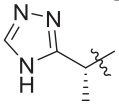
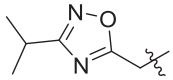
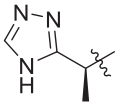
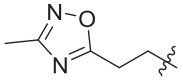


Compound	R	P2X3 FLIPR IP (nM)	$Cl_{int,\ in\ vivo}$ (mL/min/kg)[a]	PPB[a]	PXR EC_{50} (μM)
1		37	3911	97.4%	NA
2		8	24780	99.2%	6
3		47	533	85.1%	>30
4		67	NA	82.0%	2

[a] values in rat.

Table 2
SAR of western amides.



Compound	R	FLIPR IP (nM)	PPB (r)	Compound	R	FLIPR IP (nM)	PPB (r)
2		8	99.2%	10		3	99.6%
5		2	90.4%	11		17	NA
6		8	98.6%	12		170	NA
7		5	95.2%	13		30	NA
8		284	NA	14		155	NA
9		10	98.8%	15		45	NA