Development of EP547: A Potent, Highly Selective MRGPRX4 Inverse Agonist for the Treatment of Cholestatic and Uremic Pruritus

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INTRODUCTION

EP547 is a novel MRGPRX4 inverse agonist in development for the treatment of cholestatic and uremic pruritus:

- Over 50% of patients with cholestatic liver disease or chronic kidney disease suffer from pruritus that is often debilitating and poorly responsive to current pharmacological treatments.[1,2] Bile acids and/or heme metabolites, which may accumulate in blood and skin, have been implicated as pruritogens in these conditions.
- MRGPRX4 is expressed in a subset of pruriceptive sensory neurons in the skin and has been shown to be the only receptor to be activated by bile acids in human dorsal root ganglia (DRG). Recent publications provide compelling preclinical and clinical rationale for the development of an MRGPRX4 antagonist for the treatment of cholestatic (CP) and uremic (UP) pruritus.[3-5]
- Here, we describe the discovery and pre-clinical development of EP547, a novel orally available, mechanistically targeted MRGPRX4 inverse agonist for the treatment of CP and UP.

AIM

To identify a potent, selective, MRGPRX4 inverse agonist capable of blocking a diverse set of known agonists as a clinical development candidate for the treatment of CP and UP.

METHODS AND MATERIALS

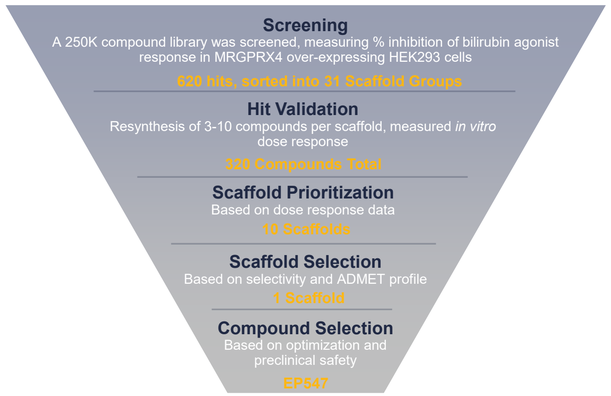
In Vitro studies: HEK293 cells were stably transfected to express human MRGPRX4 with an N-terminal HA tag (Beacon Discovery, San Diego, CA). IP-1 was measured using the IP-One – G_q Kit purchased from Cisbio.

Pharmacokinetic and safety studies were conducted at Eurofins, Pharmaron and Wuxi Apptec utilizing standard procedures.

RESULTS

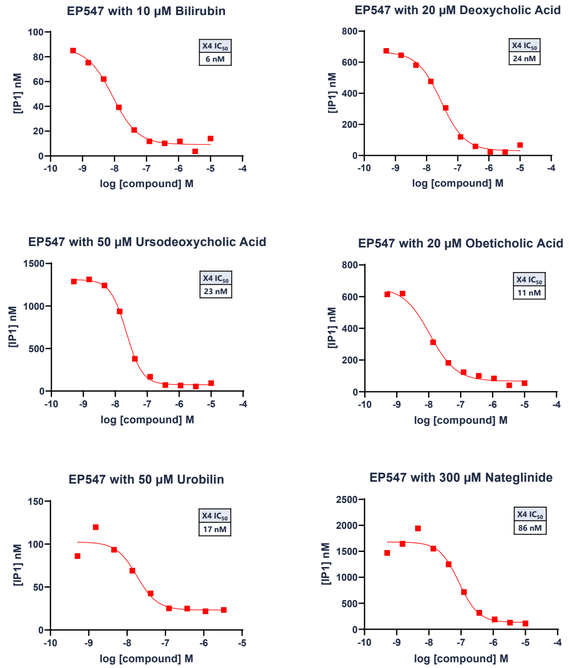
High throughput screening and structure activity relationship optimization enabled the discovery of EP547, a potent small molecule MRGPRX4 inverse agonist

Figure 1. EP547 was identified in 18 months using a rapid hit-to-lead paradigm



EP547 is a potent, selective inverse agonist of MRGPRX4, with the ability to block the activity of all agonists tested (at maximally effective concentrations)

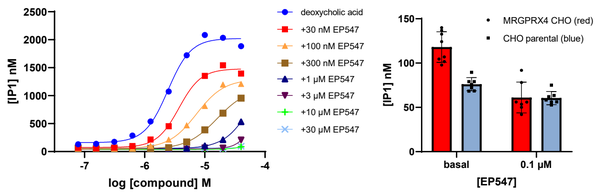
Figure 2. EP547 blocks a wide variety of structurally diverse [endogenous (left panels) and pharmacological (right panels)] MRGPRX4 agonists



Pharmacological mechanism: EP547 blocks MRGPRX4 activity in Schild assays and agonist-independent constitutive activity as an inverse allosteric agonist

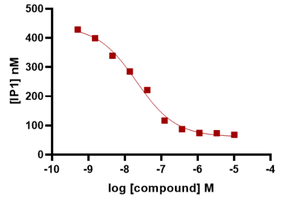
Figure 3. Schild plot analysis shows that EP547 dose-dependently flattens deoxycholic acid induced agonism of MRGPRX4, indicating that EP547 binds non-competitively (allosteric) to the human MRGPRX4 receptor (left)

EP547 blocks the constitutive activity of MRGPRX4 (no ligand present) in over-expressed CHO cells, consistent with inverse agonistic modulation (right)



The potency of EP547 was further validated in SH4 melanoma cells that endogenously express MRGPRX4; EP547 showed similar potency against MRGPRX4 agonists

Figure 4. EP547 inhibits the activity of 60 μM deoxycholic acid in SH4 cells with an IC_{50} of 20 nM, similar to MRGPRX4 over-expressing HEK293 cells (IC_{50} of 24 nM)



EP547 demonstrates acceptable MRGPRX4 selectivity which, along with its *in vitro* and *in vivo* safety profile, makes it a suitable candidate for clinical development

- EP547 was found to be highly selective for MRGPRX4 with no significant agonist or antagonist activity at any of the other 7 human MRGPRs. In addition, EP547 is selective against a large panel of additional receptor targets, including GPCRs, ion-channels, nuclear receptors, and kinases (results not shown).
- EP547 has demonstrated ADME, toxicology, and safety profiles that support its selection as a clinical candidate.

EP547 demonstrates excellent pharmacokinetic properties including high oral bioavailability, low total clearance, and a long terminal half-life (Table 1)

Table 1. Oral single dose pharmacokinetics of EP457 in various species

Species	Dose (mg/kg)	C_{max} (μM)	T_{max} (h)	AUC (μMxh)	Terminal Half-Life (h)	Total Clearance (mL/min/kg)	Oral Bioavailability (%)
Mouse	50	230	0.7	2150	11.2	0.83	ND
Rat	10	40	6.0	670	12.3	0.74	100
Rabbit	5	99	4.0	1968	13.0	0.12	100
Monkey	5	47	0.6	202	9.4	0.68	67

EP547 demonstrated a favorable *in vivo* safety profile when evaluated in a comprehensive non-clinical safety program, comprising general toxicology and safety pharmacology studies (Table 2).

- In chronic general toxicology studies in rat and monkey, the no observed adverse effect levels (NOAELs) were obtained at high EP547 exposure levels (C_{max} ≥ 190 μM).
- EP547 also showed no adverse findings in a battery of respiratory, CNS, and cardiovascular safety studies at high exposures (C_{max} ≥ 419 μM). These NOAEL average plasma concentrations exceed the *in vitro* potency by > 3000-fold.

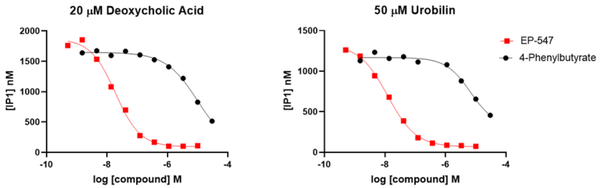
Table 2. Summary of in vivo safety evaluations conducted with EP547

Study Type	Species	Longest Duration	NOAEL (mg/kg/day)	Exposures at NOAEL	
				C_{max} (μM)	AUC (μMxh)
General Toxicology	Rat	6 months	30	357	5693
	Monkey	9 months	20	190	1904
Respiratory Safety	Rat	Single Dose	150	419	6539
CNS Safety	Rat	28 days	150	559	8647
CV Safety	Monkey	Single Dose	100	468	5822

Identification of 4-phenylbutyrate (4-PB) as a low potency MRGPRX4 antagonist

- 4-PB has been reported to markedly decrease intractable CP in 3 pediatric patients with progressive familial intrahepatic cholestasis type 1 (PFIC-1), in the face of persistent and marked elevations of bile acid levels. The mechanism was unknown.[6]
- We have discovered that 4-PB is an antagonist of MRGPRX4, capable of inhibiting the response of distinct agonists, albeit with a potency >100-fold less than that of EP547.
- At the high doses used in patients with PFIC-1, we postulate that the reported marked alleviation of CP with 4-PB treatment may be explained by its activity on MRGPRX4, providing clinical support for the therapeutic potential of MRGPRX4 antagonism in CP.

Figure 5. EP547 is >100-fold more potent than 4-PB on MRGPRX4 when evaluating deoxycholic acid and urobilin as representative agonists



CONCLUSION

- **MRGPRX4 antagonism, which blocks activation of the receptor by bile acids and heme metabolites, has the potential to be a highly targeted, mechanism-based therapeutic intervention for both cholestatic and uremic pruritus.**
- **We have discovered EP547, a novel, highly selective MRGPRX4 inverse agonist with optimized pharmaceutical characteristics for clinical use**.
  - EP547 is a potent inverse agonist that antagonizes a large variety of known MRGPRX4 agonists and blocks constitutive activity of MRGPRX4.
  - EP547 shows favorable *in vivo* pharmacokinetic properties including high oral bioavailability, linear dose-proportional pharmacokinetics, low total clearance, and a long terminal half-life, supporting once daily dosing in humans.
  - EP547 shows a suitable pharmaceutical and safety profile for advancement into clinical development.

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DISCLOSURES

All authors are employees of and hold stock in Escient Pharmaceuticals, Inc. (Escient).

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