Structure Based Discovery and Anti-fibrotic Activity of Novel Antagonists of Lysophosphatidic Acid Receptor 1 (LPAR1)

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ABSTRACT

BACKGROUND
Lysophosphatidic acid (LPA) has been identified as an important mediator of fibrosis by stimulating its G-protein coupled receptor, lysophosphatidic acid receptor 1 (LPAR1).[1-3] The 1st generation LPAR1 antagonist, BMS-986020, demonstrated a significant reduction in forced vital capacity (FVC) decline in a 6-month phase II trial in IPF patients but was terminated due to compound-specific hepatobiliary toxicity.[4] Therefore, next generation LPAR1 antagonists with improved properties and an improved safety profile may represent important new anti-fibrotic agents. Here, we describe the discovery and characteristics of our lead compounds, potential best-in-class oral small molecule LPAR1 antagonists.

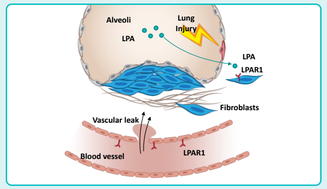
METHOD
Novel LPAR1 antagonists were designed through structure-based drug design leveraging LPAR1 receptor structures in complex with antagonist lead molecules integrated with cutting-edge free energy perturbation (FEP) technology. The compounds were initially screened in the Ca^{2+} flux assay using LPAR1 overexpression cells, followed by testing in a human lung fibroblast migration inhibition assay. Selected series were optimized for in vitro and in vivo ADME, and safety properties paying close attention to hepatic bile acid transporter inhibition. Lead compounds were evaluated for LPAR1 antagonist activity in a mouse model of LPA-mediated histamine release, anti-fibrotic efficacy in a mouse model of bleomycin induced lung fibrosis, and toxicity in preclinical species.

RESULTS
Structure-based drug design and Schrödinger FEP modeling dramatically shortened compound synthesis and testing cycles resulting in the identification of multiple novel LPAR1 antagonist chemical series with nanomolar potency in LPAR1 Ca^{2+} flux assay. During lead optimization, multiple lead compounds from different series displayed balanced profiles. Two lead compounds LTSE A and B showed potent in vitro activity with nanomolar IC_{50} in Ca^{2+} flux assay and fibroblast migration inhibition. In vivo, LTSE A and B inhibited LPA-mediated histamine release in mice with IC_{80} of 1.1 and 2.1 nM, respectively. Both compounds showed strong anti-fibrotic activities in therapeutic treatment of bleomycin-induced lung fibrosis mice by decreasing Ashcroft scores. In addition, both LTSE A and B demonstrated excellent ADME properties in multiple preclinical species, and minimal inhibition for multiple hepatic bile acid transporters (BSEP IC_{50} = 51 and 40 μM, respectively; MRP3 and MRP4 IC_{50} > 50 μM). Both compounds demonstrated large safety margins in preclinical toxicity evaluation and were free of hepatobiliary toxicity.

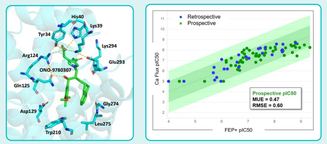
CONCLUSION
Novel oral LPAR1 antagonists were discovered through structure-based drug design. The advanced leads with superior potency and balanced ADME and safety properties exhibited potential best-in-class profiles.

INTRODUCTION

LPA/LPAR1 Axis in PulmonaryFibrosis[5]



STRUCTURE-BASED DRUG DESIGN

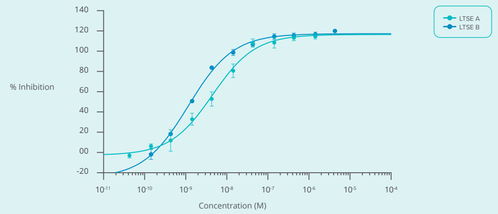


- The crystal structure[6] of small molecule antagonist bound hLPAR1 inspired SBDD and detailed interactions revealed facilitated FEP+ application

IN VITRO POTENCY

	IC_{50}, nM	
LPAR1 Antagonist	LPAR1 Ca^{2+} Flux	LPA Induced Fibroblast Migration
LTSE A	< 10	< 10
LTSE B	< 10	< 10

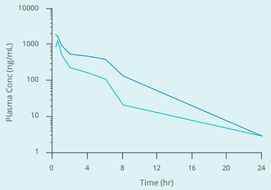
LPA Chemotaxis Assay



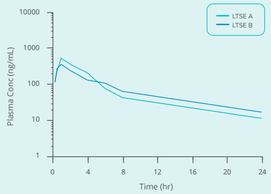
- LTSE A and LTSE B showed potent *in vitro* activity with nanomolar IC_{50} in Ca^{2+} flux assay and LPA induced fibroblast migration assay

PHARMACOKINETICS

Rat PO, 5 mg/kg



Monkey PO, 2 mg/kg



Rat
IV 1 mg/kg,
PO 5 mg/kg

	Route	$T_{1/2}$ (h)	CL_p (mL/min/kg)	V_{ss} (L/kg)	C_{max} (ng/mL)	AUC_{0-inf} (ng*h/mL)	F (%)
LTSE A	IV	4	20	1.0	—	838	—
LTSE A	PO	2	—	—	1238	2497	50
LTSE B	IV	3	11	0.8	—	1676	—
LTSE B	PO	3	—	—	2043	5421	65

Monkey
IV 1 mg/kg,
PO 5 mg/kg

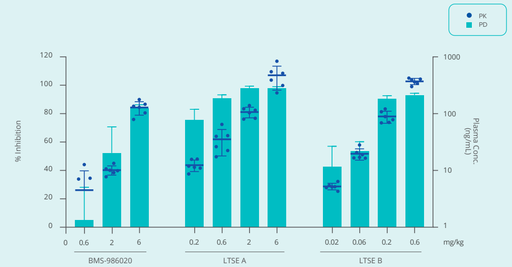
	Route	$T_{1/2}$ (h)	CL_p (mL/min/kg)	V_{ss} (L/kg)	C_{max} (ng/mL)	AUC_{0-inf} (ng*h/mL)	F (%)
LTSE A	IV	8	6	1.1	—	2758	—
LTSE A	PO	8	-	-	577	2328	42
LTSE B	IV	6	6	1.0	—	2868	—
LTSE B	PO	8	—	—	396	2255	39

- Low to moderate clearance, moderate volume of distribution and good oral bioavailability

PK PD STUDY

LPA INDUCED HISTAMINE RELEASE PD MODEL

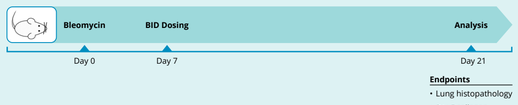
LPA Induced Histamine Release in Mice



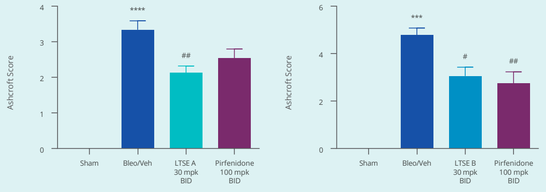
- Oral treatment of LTSE A and LTSE B demonstrated dose dependent inhibition in LPA mediated histamine release and achieved activity at low dose

EFFICACY STUDY

BLEOMYCIN INDUCED LUNG FIBROSIS MODEL



Ashcroft Score in the Lung



- Therapeutic treatment of LTSE A and LTSE B demonstrated significant reductions in Ashcroft score

n = 6 for Sham and n = 11 for other groups; Data represent mean ± SEM
*** $P < 0.001$, **** $P < 0.0001$ verse Sham, Mann-Whitney U test
# $P < 0.05$, ## $P < 0.01$, verse Bleo group, Kruskal-Wallis test with a Dunn's analysis

TOXICOLOGY

LIVER TRANSPORTER INHIBITION

	IC_{50}, μM		
LPAR1 Antagonist	BSEP	MRP3	MRP4
BMS-986020	3.4	21	11
LTSE A	51	72	> 100
LTSE B	51	54	58

- The inhibition of BSEP and other transporters by LTSE A and LTSE B are markedly improved compared to BMS-986020

PRECLINICAL TOXICOLOGY
In preclinical dose range finding studies, there was no toxicity signal in rats treated with LTSE A and B up to 1000 mg/kg/day for 7 days and cynomolgus monkeys treated with LTSE A up to 1000 mg/kg/day for 7 days.

CONCLUSIONS

- Novel LPAR1 antagonists, LTSE A and LTSE B, were designed through structure-based drug design leveraging LPAR1 receptor structures integrated with FEP technology
- LTSE A and LTSE B demonstrated excellent potency in Ca^{2+} flux assay, LPA induced fibroblast migration assay and histamine release PD model
- LTSE A and LTSE B showed excellent PK properties in preclinical species
- LTSE A and LTSE B demonstrated significant improvement in lung fibrosis in a therapeutic bleomycin induced lung fibrosis model
- LTSE A and LTSE B showed minimal inhibition of liver transporters and large safety margins in preclinical toxicity evaluation
- LTSE development candidate is currently in IND enabling studies

REFERENCES:

1. Tager AM, et al. The lysophosphatidic acid receptor LPA 1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. *Nat Med.* 2008;14(1):45. **2.** Yung YC, et al. LPA receptor signaling: pharmacology, physiology, and pathophysiology. *J Lipid Res.* 2014;55(7):1192. **3.** Geraldo LHM, et al. Role of lysophosphatidic acid and its receptors in health and disease: novel therapeutic strategies. *Signal Transduct Target Ther.* 2021;6(1):45. **4.** Palmer SM, et al. Randomized, double-blind, placebo-controlled, phase 2 trial of BMS-986020, a lysophosphatidic acid receptor antagonist for the treatment of idiopathic pulmonary fibrosis. *Chest.* 2018;154(5):1061. **5.** Aikawa S, et al. Lysophosphatidic acid as a lipid mediator with multiple biological actions. *J Biochem.* 2015;157(2):81. **6.** Chrencik JE, et al. Crystal Structure of Antagonist Bound Human Lysophosphatidic Acid Receptor 1. *Cell.* 2015;161(7):1633.