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DRUG DISCOVERY AND RESISTANCE

Formulation studies of InhA inhibitors and combination therapy to improve efficacy against *Mycobacterium tuberculosis*

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SUMMARY

Previously, structure-based drug design was used to develop substituted diphenyl ethers with potency against the *Mycobacterium tuberculosis* (Mtb) enoyl-ACP reductase (InhA), however, the highly lipophilic centroid compound, SB-PT004, lacked sufficient efficacy in the acute murine Mtb infection model. A next generation series of compounds were designed with improved specificity, potency against InhA, and reduced cytotoxicity *in vitro*, but these compounds also had limited solubility. Accordingly, solubility and pharmacokinetics studies were performed to develop formulations for this class and other experimental drug candidates with high logP values often encountered in drug discovery. Lead diphenyl ethers were formulated in co-solvent and Self-Dispersing Lipid Formulations (SDLFs) and evaluated in a rapid murine Mtb infection model that assesses dissemination to and bacterial burden in the spleen. *In vitro* synergy studies were performed with the lead diphenyl ether compounds, SB-PT070 and SB-PT091, and rifampin (RIF), which demonstrated an additive effect, and that guided the *in vivo* studies. Combinatorial therapy *in vivo* studies with these compounds delivered in our Self-Micro Emulsifying Drug Delivery System (SMEDDS) resulted in an additional 1.4 $\log_{10}$ CFU reduction in the spleen of animals co-treated with SB-PT091 and RIF and an additional 1.7 $\log_{10}$ reduction in the spleen with animals treated with both SB-PT070 and RIF.

1. Introduction

Academic drug discovery programs have a significant value in the development of novel chemotherapeutics through performing lengthy basic drug discovery and structure-activity relationship (SAR) analyses, that sets the foundation for advanced drug discovery and Pre-investigational new drug (IND) filings. The limited progression of lead candidates suitable for IND studies is often attributed to poor physiochemical properties of drug candidates that impact the feasibility of evaluation. In fact, rational drug discovery and high throughput screening approaches have been linked to the increase of compounds with lower bioavailability and poor solubility [1]. There are numerous examples of compounds that display excellent *in vitro* potency but fail in development due to poor biopharmaceutical performance [2]. In recent years appropriate delivery systems for these highly lipophilic drugs have been under investigation [3,4,5].

Our drug discovery program has developed substituted diphenyl ethers that target the Mtb protein InhA, and the substituted diphenyl ether SB-PT004, with a hexyl substituent at position 5, was selected as an early lead compound (Figure 1) [6,7–9,10]. Although, SB-PT004 had potency against Mtb *in vitro*, limited efficacy in the murine Mtb infection model was demonstrated when SB-PT004 was delivered in 5% EtOH [8]. The lack of efficacy was most likely due to insufficient *in vivo* drug exposure [8,9]. Further SAR studies that resulted in the modification of the B-ring changed the drug–protein interaction kinetics but not the physiochemical properties that govern bioavailability [6,7,10,11]. Two second-generation lead compounds; SB-PT070, with an ortho methyl group, and SB-PT091, with an ortho substituted chlorine were identified and prioritized based on drug–protein interaction kinetics. Both SB-PT070 and SB-PT091 are slow-onset, tight-binding nM inhibitors of InhA with MIC values of 3.13 and 1.57 μg/mL respectively against Mtb H37Rv (Figure 1) [7].

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Our focus for this study was to improve the pharmacokinetics and bioavailability of the substituted diphenyl ethers, SB-PT070 and SB-PT091 *via* formulation by improving solubility, absorption, and prolonging the time to reach the maximum drug concentration (T_{max}). To achieve this we employed co-solvent and Self-Micro Emulsifying Drug Delivery Systems (SMEDDS). The pharmacokinetic profile of SB-PT004 in these formulations was compared to the previously used 5% EtOH formulation. Efficacy of both SB-PT070 and SB-PT091 alone in co-solvents and in combination with rifampin (RIF) in SMEDDS were tested in a rapid murine Mtb infection model designed to assess dissemination and bacterial burden in the spleen. The formulations are presented as a resource for drug discovery programs to optimize deliverability and bioavailability of compounds and the efficacy results are provided to demonstrate performance of the formulation systems.

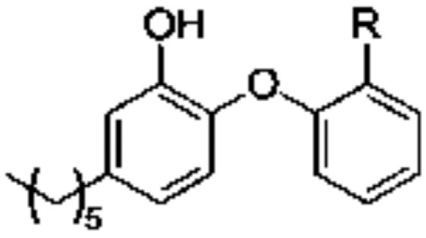
2. Materials and methods

2.1. Evaluation of co-solvents

The solubility of SB-PT004 was determined in various co-solvents, surfactants and oils (Table 1). All samples were prepared in duplicate by adding an excess amount of SB-PT004 to a vial containing a known amount of each vehicle followed by vortex mixing for 30 s. Mixtures were then shaken for 48 h in an environmental orbital shaker at 30 °C. Upon equilibration, solutions were visually inspected for presence of drug not in solution; samples were then centrifuged at 14,000 rpm for 10 min. The supernatant was collected and diluted as necessary with methanol for analysis with LC/MS/MS.

2.2. Screening of surfactants and co-surfactants

The emulsification ability of various surfactants was screened with a method described in the literature [12]. The mixtures were gently heated at 55 °C to homogenize the components. Emulsions were formed and allowed to stand for 2 h and % transmittance was evaluated using a spectrophotometer (Beckman Coulter DU640 UV/VIS Scanning Spectrophotometer) at 650 nm using distilled water as a blank. The turbidometric method was also used to assess the efficiency of the co-surfactants to improve the emulsification ability of selected oil and surfactants [12]. A surfactant/co-surfactant was added to an oil and homogenized while gently heating (55 °C). The isotropic mixture was diluted with distilled water at 37 °C, mixed gently via flask inversion to form a fine dispersion, allowed to stand for 2 h, and then evaluated.



SB-PT004: R1=H
SB-PT070: R1=Me
SB-PT091: R1=H,R2=Cl

Figure 1. Structure of the three lead diphenyl ethers used in this study.

2.3. Construction of ternary phase diagrams

Ternary phase diagrams of oil, surfactant, and co-surfactant of the selected self-emulsifying systems were constructed (Figure 2). The percentages of each component were decided based on the published requirements for spontaneously emulsifying systems [13,14]. The concentration of oil and surfactants varied from 30% to 70% and the concentration of co-surfactant was evaluated from 0 to 30%. For each diagram, 23 mixtures were prepared. Each component of the mixture was accurately weighed out and homogenized via gentle heating (55 °C) and vortex mixing. An aliquot of the self-emulsifying pre-concentrate was diluted in glass vials containing distilled water at 37 °C. After equilibration, dispersibility and appearance were visually evaluated and scored [15–17]. The globule size of selected formulations was measured following dilutions in various media including distilled water, 0.1 N HCl, and phosphate buffer pH 7.4. Mean globule size was measured using dynamic light scattering (Nicomp 380 ZLS particle size).

2.4. Pharmacokinetic evaluation

C57/BL6 mice (6–8 week old) were used to evaluate SB-PT004 formulations. Animals were provided free choice water and fasted overnight then fed 4 h after dose administration. Animals were anesthetized via inhalational anesthetic and blood was collected via terminal cardiac puncture at 15, 30, 60, 180, 300, 480, 720, and 1440 min. Whole blood samples were immediately transferred to lithium heparin tubes to prevent clotting.

Plasma was separated from whole blood via centrifugation at 14,000 rpm at 4 °C for 10 min and stored at minus 80 °C until analysis. Samples were prepared for analysis by mixing with 3 parts of ice-cold methanol: acetonitrile (1:1), shaken for 10 min and then centrifuged at 14,000 rpm at 4 °C for 15 min. Supernatants were collected and placed into sample vials for direct analysis via LC/MS/MS methodology. The pharmacokinetic parameters of maximum plasma concentration (Cmax), time to maximum plasma concentration (T_{max}), half-life ($T_{1/2}$), and area under the curve (AUC_{0-24}) calculated using the PK2 add-in for Microsoft Excel (Joel Usansky and Atul Desai, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA, USA).

2.5. Murine Mtb infection model

A modified rapid murine Mtb infection model designed to assess dissemination to the secondary site the spleen was used to assess

Table 1
Solubility of SB-PT004 in various excipients.

Vehicle		Percent solution, solubility mg/ml	HLB
Co-solvents	Ethanol (EtOH, Aaper)	20% 0.0034 ± 0.00033	
	Propylene Gycol (PG, Sigma Aldrich)	40% 0.0268 ± 0.0073	
	Polyethylene Glycol 400 (PEG-400, Sigma Aldrich)	40% 0.125 ± 0.0248	
Surfactants	Tween 20	10% 21.5 ± 0.1	16.7
	Tween 80	10% 16.4 ± 0.6	15
	Solutol HS15(BASF)	10% 19 ± 1.8	16.3
	Cremohor EL (BASF)	10% 19 ± 2.3	13
Lipids	Captex 200 P (Abitec Corp)	496.5 ± 47.4	3.8
	Captex 300 (Abitec Corp)	216 ± 13.5	
	Capmul MCM NF (Abitec Corp)	N/A	5–6