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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 log₁₀ CFU reduction in the spleen of animals co-treated with SB-PT091 and RIF and an additional 1.7 log₁₀ reduction in the spleen with animals treated with both SB-PT070 and RIF.

Keywords

Formulation; Drug Discovery; tuberculosis; diphenyl ethers; Rifampin; combinatorial treatment

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Ethical Approval: Procedures were under protocols approved by the Institutional Animal Care and Use Committee at the Colorado State University facilities, which are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

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.³⁴⁵

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).⁶⁷⁻⁹¹⁰ 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.⁸⁹ 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.⁶¹⁰⁷¹¹ 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

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.

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 0-30%. 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 (Nicom 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 (C_{max}), 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 formulation performance.^{18, 19} In this rapid model animals are infected using the low dose aerosol infection followed by treatment for 10 consecutive days starting 5 days post infection. Compounds were solubilized in specified formulations and delivered at the given route and dose. For combinatorial studies, RIF was suspended in Phosphate Buffered Saline. Spleens were harvested and processed for colony forming units. CFU counts from infected treated animals were compared to infected untreated animals using a one-way ANOVA test or a one-way t-test (Prism).

2.6. Synergy Studies

A checkerboard plate technique was used to examine the potential interaction between SB-PT091 and RIF *in vitro*.¹⁹ The fractional inhibitory concentrations (FICs) were calculated to determine synergy in the drug combinations as described.²⁰²¹ FIC is defined as the MIC of a drug in combination divided by the MIC of that drug alone. The fractional inhibitory index (FICI) is the sum of the FIC (Σ FIC) for the drugs tested in the combination. Drugs are considered synergistic when the Σ FIC is less than 0.5, indifferent when the Σ FIC is between 0.5 and 4, and antagonistic when the Σ FIC is greater than 4.

2.7. Ethics Statement

Procedures were under protocols approved by the Institutional Animal Care and Use Committee at the Colorado State University facilities, which are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

3. Results and Discussion

In drug discovery, early lead candidates often have poor physiochemical properties that present problems for efficacy studies. The first generation diphenyl ether lead compound SB-PT004, and the second generation diphenyl ether lead compounds SB-PT070 and SB-PT091 have high logP values, 5.76, 6.24 and 6.31 respectively, and the physical characteristic of oil.⁶⁹⁷ The solubility of a drug under aqueous conditions is a key parameter that governs its bioavailability because only the fraction of the drug in solution can be absorbed. Since keeping a formulation as simple as possible is a primary goal in evaluation of drug activity and efficacy, one of the first approaches is to solubilize in a solvent such as 5% EtOH or 1% DMSO, followed by dilution with water. Initially, both SB-PT004⁸ and SB-PT070 (data not published) were delivered to animals in an Mtb infection model at 300 mg/kg in 5% EtOH with no efficacy detected. We hypothesized that we could improve the deliverability and bioavailability of the compounds by re-formulation and achieve efficacy. Accordingly, SB-PT004 was reformulated in co-solvents and SMEDDS and the pharmacokinetics of SB-PT004 in these formulations and rapid efficacy study in the murine model of Mtb infection with the second-generation leads follow.

3.1. Co-solvent Formulations and Efficacy

A strategy to improve the solubility of lipophilic compounds is to formulate in a combination of water and water miscible solvents resulting in a co-solvent formulation. Examples of solvents used in oral commercial formulations include ethanol, polyethylene glycol, and propylene glycol.²² These co-solvent formulations can be delivered PO and, with a dilution step, IP. The advantages of co-solvent formulations are they are easy and fast to formulate. The solubility of SB-PT004 in the individual co-solvents at approved concentrations encountered in commercially available oral formulations was well below the targeted dose of 40 mg/mL routinely used for delivery of humanized doses of candidates in murine models of infection (Table 1). Since combining co-solvents can improve solubility, we chose a combination of excipients, 15% EtOH, 20% PG and 40% PEG, for further pharmacokinetic study. In pharmacokinetic studies using the same dose and PO route, the $T_{1/2}$ for SB-PT004 in the co-solvent formula was 2.7X the $T_{1/2}$ when compared to SB-PT004 delivered in the 5% EtOH and the T_{max} for the compound delivered in the co-solvent was 2X that of the compound delivered in 5% EtOH. However, the C_{max} and AUC were lower in the co-solvent formulation (Table 2). To see the effect of this change on bioavailability due to formulation, both SB-PT070 and SB-PT091 were delivered PO in the co-solvent formulation at 200 mg/kg once daily to animals in the Mtb infection model. While no significant CFU reduction was observed in the lungs of the treated animals, a significant 0.99 log₁₀ CFU reduction was detected in the spleens from infected animals treated with SB-PT070 and a significant 1.47 log₁₀ CFU reduction was detected in the spleens of infected animals treated with SB-PT091 (Figure 3). In contrast, no efficacy in either the lungs or spleens was observed when the compounds were delivered in 5% EtOH (data not shown).

3.2. SDLFs

Self-Emulsifying Drug Delivery Systems (SEDDS) and Self-Micro Emulsifying Drug Delivery Systems (SMEDDS)

SDLFs are mixtures of oils and surfactants that have been used to improve the bioavailability of difficult to solubilize and deliver compounds due to high logP.²³ By delivering to and maintaining the drug in a homogenized solubilized form in the Gastrointestinal tract (GI), lipid based formulations eliminate the dissolution step necessary for absorption and may increase drug absorption through various mechanics including the stimulation of intestinal lymphatic uptake and increasing gastric transit time.^{24,25} Since poorly water-soluble drugs are becoming more prevalent in drug discovery, a lipid based classification system (LFCS) was introduced to identify important characteristics and to recognize the advantages and disadvantages of lipid-based formulations.^{13,14}

The development of SDLFs begins with assessing the effect of excipients on solubility and dispersion. Oils are some of the most important excipients and are chosen first because solubility within the oil is critical for drug loading and prevention of drug precipitation after dilution in the gut lumen.¹³ Representative oils were evaluated as possible solvents for oral delivery (Table 1). Captex 200P was found to be the best oil because it solubilized nearly 500 mg of SB-PT004 per mL.

Oral lipid-based formulations often contain emulsifiers or surfactants to facilitate dispersion of the drug and formulation components in the GI tract following ingestion. The most widely recommended being surfactants with a high hydrophilic-lipophilic balance (HLB).³ The solubility of SB-PT004 was determined in 10%(w/v) solutions (Table 1) of four surfactants, Tween 20, Tween 80, Solutol HS 15, and Cremophor EL, with HLB values ranging from 16.4-21.5. Surfactant selection was based on miscibility with the oil and its emulsification efficiency when combined with the oil dispersed in water.^{12, 26} Large transmittance percentages correlate to the production of smaller sized oil droplets in the dispersion and smaller droplet size has been reported to increase bioavailability.²⁷ Cremophor EL, Solutol HS 15, Tween 80, and Tween 20 generated dispersions with transmission values of 84%, 70.5 %, 67.9% and 11.2% respectively. The addition of a co-surfactant has been reported to improve lipid-based formulations by aiding in dispersability and drug absorption from the intestinal tract.^{25, 28, 29} Co-surfactants and solvents (Capmul MCM NF, Capmul PG-12, EtOH, PG, and PEG-400) were added and their transmittance values evaluated (Table 3). The degradation of the emulsions by the addition of hydrophilic co-solvents (EtOH, PEG-400, and PG) maybe attributed to the fact that co-solvents possess greater aqueous solubility than the principal surfactants in lipid-based formulations. When the formulation is dispersed, the co-surfactant preferentially partitions into the aqueous phase. This reduces the co-solvent concentration at the oil/water interface, thus destabilizing the emulsion droplet.³⁰ The addition of Capmul MCM NF increased the transmittance values of Solutol HS 15 and Cremophor EL, however, Cremophor EL has been reported to reduce absorption when delivered orally due to micellar entrapment of drug compound in the intestine.^{31, 32} Since an objective was to enhance drug exposure through formulation modification, we chose to proceed with Solutol HS 15-Captex 200P -Capmul MCM. Additional studies were carried out to optimize this formulation for oral delivery.

The self-emulsification process is related to the oil/surfactant combination, the ratio of the combination, the concentration of the surfactant and the temperature. The addition of a drug to SDLFs may interfere with the self-emulsification process. Therefore, to identify the self-emulsifying existence fields for the lipid-based formulas pseudo-ternary phase diagrams were constructed (Figure 2). The systems identified contained between 40- 60% (w/w) oils and surfactants with HLB >12. Under the LFCS these solutions were Type IIIA lipid formulations and can be referred to as SMEDDS formulations. Emulsion droplet size is an important factor in the performance of a SMEDDS. The size of the droplet affects the surface area, which affects the rate and extent of drug release as well as absorption. The SBPT004/SMEDDS formulation was further characterized to determine effects of formula composition and dispersing solution on the globule size of the resulting emulsions (Table 4). Since the droplet size distribution can influence the *in vivo* fate of emulsions,²⁷ the SMEDDS formula A (40% Captex 200P, 40% Solutol HS15, 20% Capmul MCM NF) was chosen for pharmacokinetic analysis.

3.3. Pharmacokinetic Evaluation

A murine *in vivo* absorption study determined that SMEDDS was successful in improving the pharmacokinetic profile of lead centroid molecule SB-PT004 by a 1.7-fold increase in AUC₀₋₂₄ compared to SB-PT004 formulated in 5% EtOH (Table 2). Most significantly,

formulation of SB-PT004 in SMEDDS extended the length of time to reach maximum plasma concentrations ($T_{\max}$) compared to delivery in the 5% EtOH and co-solvent formulation. The $T_{1/2}$ for the SMEDDS, co-solvent and 5% EtOH formulation were 8.85, 5.51 and 2.08 h respectively, suggesting the improvement in the plasma drug concentration is not a result of the vehicle affecting the clearance rate of the compound. The increase in AUC in this study is related to the increase in $T_{1/2}$ and $T_{\max}$ which indicates improved bioavailability and drug exposure, thus utilizing the SMEDDS improved several factors that impact absorption of lipophilic drugs: solution homogeneity, minimization of particle/globule size, and aqueous solubility.

3.4. Synergy Studies

Our drug discovery program focusing on the diphenyl ether pharmacophore has the goal to develop a potent and specific enoyl reductase, InhA inhibitor that is not susceptible to mechanisms associated with isoniazid resistance. Further, since any new TB chemotherapeutics would be given in combination with a current frontline compound we assessed potential antagonism between the diphenyl ether drug class and RIF. RIF was chosen because its target is DNA dependent RNA synthesis whereas the diphenyl ethers target the enoyl-ACP reductase, InhA. SB-PT091 and RIF when tested in combination *in vitro* were found to have an Σ FIC index of 1 (SB-PT091 FIC=0.5; RIF FIC=0.5) indicating that diphenyl ether drug class was not antagonistic to the activity of RIF.

3.5. Murine Mtb Infection Model

To assess the performance of the SMEDDS formulation on the delivery and efficacy of diphenyl ether lead candidates, and co-treatment, SB-PT070 and SB-PT091 were tested in combination with RIF using SMEDDS in our rapid murine Mtb infection model of dissemination described previously^{18, 19}. In this rapid model to assess drug efficacy, bacteria are delivered to the lungs and treatment is initiated day 5 post infection before the bacteria disseminate to the spleen. Since we have found that dissemination is important to the pathogenesis of tuberculosis as well as other respiratory pathogens, drugs and drug candidates that control dissemination from the infection site of the lungs to secondary locations such as the spleen are more efficacious at controlling prolonged infection and relapse³³⁻³⁷. The SMEDDS formulation for diphenyl ethers was diluted immediately prior to delivery, and dosed at 50 mg/kg BID (SB-PT070) and 25 mg/kg BID (SB-PT091), in the acute murine Mtb infection model. The doses were given at sub-therapeutic levels to ensure detection of improved efficacy in the combinatorial therapy. When treated alone at this level, SB-PT091 and SB-PT070 demonstrated marginal efficacy as intended as indicated by a 0.78 $\log_{10}$ and a $\log_{10}$ 0.2 reduction in spleen bacterial burden, respectively (data not shown). RIF given by itself at a therapeutic dose of 10 mg/kg reduced the bacterial load in the lung ~ 0.5 $\log_{10}$ and reduced the bacterial load by almost 2 $\log_{10}$ in the spleen. Significantly, the animal group that were given both SB-PT091 and RIF had an additional 1.4 $\log_{10}$ reduction of bacterial burden in the spleen and those given SB-PT070 and RIF had an additional 1.7 $\log_{10}$ reduction of bacterial burden in the spleen compared to RIF alone (Figure 4). Notably, an animal in each combination group had no detectable CFU's in the spleen. While there was a reduction in the bacterial load in the lungs for each treatment group compared to untreated controls, the reduction was not statistically significant. Importantly, co-treatment of the

diphenyl ether class of InhA inhibitors improved treatment efficacy over RIF alone in our rapid model of Mtb. This observation indicates that lead compounds of the diphenyl class have potential as members in combinatorial regimens where total CFU reduction in the spleen was not observed, and may afford treatment options against clinical strains with various drug susceptibility profiles and prevent bacterial resistance to RIF from emerging.

The discovery of potent small molecules with poor physiochemical properties that limits evaluation of efficacy in animal models of infection is increasingly common in early drug discovery. Indeed, our ongoing drug discovery programs often face this hurdle in evaluating SAR relationships with efficacy. In response to this problem, we have developed a series of SDLFs formulations appropriate for delivery of compounds with high logP values. As a proof of concept for improvement of delivery, C_{max} and T_{max} , and overall drug exposure we utilized a SMEDDS from this series that was optimized for hydrophobic substituted diphenyl ethers. In addition, to the study described here, we have employed SMEDDS formulations from this series in our other drug discovery programs with success. Specifically, SB-PT052 and SB-PT068 are lipophilic diphenyl ethers that target the enoyl-ACP reductase FabI1 in *Burkholderia pseudomallei*, and were both delivered IP in SMEDDS in an acute *Burkholderia pseudomallei* murine infection model.³⁸ SB-PT052 significantly reduced the bacterial load in the lung ($-0.6 \log_{10}$ CFU/ml) and in the spleen ($-1.1 \log_{10}$ CFU/ml) whereas; SB-PT068 only significantly reduced the bacteria load in the spleen ($-1.3 \log_{10}$ CFU/ml). Further, we have utilized SMEDDS formula to deliver substituted benzimidazoles that target Mtb FtsZ. In this study, the lead compounds were delivered PO in SMEDDS in our rapid murine Mtb infection model and demonstrated significant efficacy, equivalent to isoniazid demonstrated by a $\sim 6 \log_{10}$ CFU/mL reduction in the lung and spleen in the Mtb murine infection model.³⁹ This is the first report of a series of SDLFs formulations that improve drug candidate deliverability and drug exposure for compounds with poor physiochemical properties for evaluation in efficacy models of infection.

4. Conclusion

We developed formulations that improved the solubility and the efficacy of diphenyl ethers in murine infection models. The co-solvent formulation, 15% EtOH, 20% PG and 40% PEG, improved the bioavailability and drug exposure of SB-PT070 and SB-PT091 to achieve efficacy in our rapid murine Mtb infection model. A SMEDDS formula, 40% captex, 40% Solutol and 20% capmul MCN, was derived that had the most potential in improving bioavailability by pharmacokinetics and was used effectively in the combinatorial therapy experiment with RIF and diphenyl ethers. This study demonstrated that formulation can improve drug exposure of compounds and that efficacy of a series of compounds is related to SAR, formulation, T_{max} , $T_{1/2}$, potency, route, toxicity, and dose. Although the SMEDDS was optimized for the delivery of the parent compound SB-PT004 and related derivative compounds it also proved useful in our drug discovery with the benzimidazoles without further changes. These results support the use of SMEDDS to improve systemic availability of difficult to solubilize hydrophobic compounds.

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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.

Figure 2.

Phase diagrams. Ternary phase diagrams of oil, surfactant, and co-surfactant of the selected self-emulsifying systems. (A) Cremaphor EL, Capmul MCM and Captex 200, and (B) Solutol HS, Capmul MCM and Captex 200. The concentration of oil and surfactants varied from 30% to 70% and the concentration of co-surfactant was evaluated from 0-30%. 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.

Figure 3. Bacterial burden in mouse lungs and spleen treated with SB-PT070 or SB-PT091. The diphenyl ethers were delivered PO in the co-solvent formulation, at 200 mg/kg once daily. The solid symbols represent the lung data and the open symbols represent the spleen data. Significance was determined using a one-way t test.

Figure 4.

Bacterial burden in mouse lungs and spleen co-treated with rifampin and SB-PT070 or SB-PT091. The diphenyl ethers were formulated in the SMEDDS formulation, and the animals were dosed with SB-PT070 (50 mg/kg), SB-PT091 (25mg/kg) or Rifampin (10mg/kg) alone or in combination. The solid symbols represent the lung data and the open symbols represent the spleen data. Significance was determined using a one-way ANOVA with Tukeys multiple comparison test.

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 Glycol (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

Table 2

Pharmacokinetic parameters of SB-PT004

	5%EtOH	5% EtOH	5% EtOH	Co-Solvent *	SMEDDS **
Dose	50mg/kg	200mg/kg	200mg/kg	200mg/kg	200mg/kg
Route	PO	PO	IP	PO	PO
T_{1/2} (h)	0.539	2.08	3.04	5.54	8.85
T_{max} (h)	0.5	0.5	1.0	1.0	5
C_{max} ng/ml	54.89	146.33	381.22	108.6	167.65
AUC₍₀₋₁₎ mg*h/L		345.50		332.74	N/A
AUC₍₀₋₂₄₎ mg*h/L		0.61		N/A	1.05

* **Co-solvent:** 15% EtOH, 20% PG and 40%PEG

** **SMEDDS:** 40% Captyec 200P, 40% Solutol HS15, 20% Capmul MCM NF

Table 3

Surfactant/Co-surfactant Emulsification Efficiency

Co-Surfactant	Percent Transmittance		
	Solutol HS 15	Cremophore EL	Tween 80
Capmul MCM	99.4	97.7	47.2
Capmul PG 12	3.9	79.6	19
Ethanol	3.7	4.9	11.2
Propylene Glycol	15.4	5.1	2.9
PEG-400	7.6	67.4	28

Table 4

Composition and assessment of SMEDDS

	A	B	C
Composition Formulation			
SB-PT004(g)	0.04	0.04	0.04
Captex 200 (g)	0.4	0.5	0.6
Solutol HS 15 (g)	0.4	0.3	0.2
Capmul MCM NF (g)	0.2	0.2	0.2
Globule size following dilution (1:200) Media (nm)			
Water	37±6.2	45±19.8	78±28.6
Phosphate Buffer	34±13	46±20.6	75±12.3
HCl	38±7.9	67±11.8	87±23.3