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Pre-formulation of GSK2251052, A Novel Boron-Containing Small Molecule Designed with Ideal CMC Properties and In Development for the Treatment of Serious Gram Negative Bacterial Infections

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Abstract

Purpose: To characterize the physicochemical properties and conduct pre-formulation on GSK2251052 (formerly AN3365), a novel, boron-containing, small molecule in development for the treatment of serious Gram-negative bacterial infections. GSK2251052 was rationally designed to target leucyl-tRNA synthetase and has *in vitro* and *in vivo* activity against Gram-negative bacteria.

Methods: Pre-formulation activities in support of a sterile intravenous formulation for use in Phase I clinical study included salt screening, solid state stability, solution stability, determination of pKa, logD, pH solubility profile, PXRD characterization, thermal analysis, GVS, excipient compatibility, and autoclave studies.

Results: GSK2251052 is a small molecule with a crystalline and stable HCl salt (MW 273.52) of high aqueous solubility (>200 mg/mL at pH 5), a stable polymorph form, and excellent solid state stability both as a powder and as a lyophilized drug product. Due to its crystalline behavior, solid state stability, high water solubility, and fast dissolution kinetics, the HCl salt of GSK2251052 was successfully formulated as a sterile lyophilized cake without needs for additional excipients. The GSK2251052.HCl lyophilized prototype formulation was shown to be chemically and physically stable at 5°C, 25°C/60%RH, and 40°C/75%RH for at least 9 months.

Conclusion: GSK2251052.HCl is a novel boron-containing small molecule with a very attractive CMC profile, high aqueous solubility and excellent solid state stability. Pre-formulation activities were successful in identifying a simple intravenous formulation for use in the first-in-human clinical trial.

Introduction

GSK2251052 was rationally designed to target leucyl-tRNA synthetase and has *in vitro* and *in vivo* activity against Gram-negative bacteria. Pre-formulation activities in support of a sterile intravenous formulation for use in Phase I clinical study included salt screening, solid state stability, solution stability, determination of pKa, LogP, logD, pH solubility profile, PXRD characterization, thermal analysis, GVS, excipient compatibility, autoclave, and lyophilization studies.

Results

Physico-Chemical Characterization of GSK2251052.HCl

GSK2251052.HCl is a chiral (S enantiomer), small molecular weight (FW 273.52) compound (Figure 1). The lipophilicity profile was measured experimentally and shows the high hydrophilicity of GSK2251052.HCl (Figure 2). This high hydrophilicity translates into uncommonly high water solubility (260 mg/mL at pH 5.0, and 235 mg/mL at pH 3.3). Salt screening studies identified the hydrochloride salt as the most stable. Polymorph screening studies identified Form 4 of the hydrochloride salt as the most stable polymorph. Physico-chemical stability at stressed conditions of 40°C/75%RH and 50°C for up to 12 weeks showed minimal change in the HPLC purity and PXRD profile of GSK2251052.HCl. On that basis, the Form 4 of the hydrochloride salt was selected for early development. The PXRD pattern of the HCl Form 4 is shown in Figure 3.

Figure 1. Chemical Structure of GSK2251052.HCl

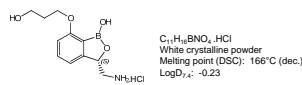


Figure 2. GSK2251052.HCl Lipophilicity LogP/LogD Profile

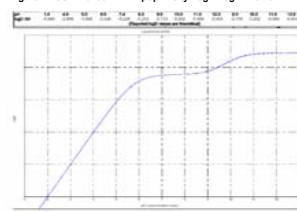
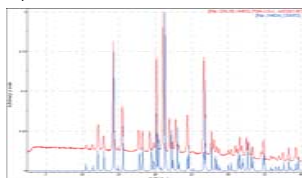


Figure 3. Comparison of the Calculated PXRD Pattern to the Experimental PXRD Pattern of GSK2251052.HCl



Stability of GSK2251052.HCl in Solution Formulations

GSK2251052.HCl is a well behaved small molecule with high water solubility, which makes it suitable for an IV formulation. Solution formulation screening included the following studies:

- Stability of solutions in water and saline at room temperature and after autoclave conditions.
- Stability of solutions in commonly acceptable pharmaceutical buffers at different pHs (Figures 4 and 5).
- Excipient compatibility studies (Figures 6 and 7):
 - pH Modification, adjustment with inorganic and organic acids and bases (HCl, NaOH, diethanolamine)
 - Anti-oxidants (ascorbic acid, sodium metabisulfite, citric acid, tartaric acid, monothylglycerol, malic acid)
 - Chelating agents (EDTA)
 - Other stabilizing agents (sucrose, sorbitol, serine, fructose, cyclodextrine)

These studies demonstrated:

- Stability of GSK2251052.HCl in solution is optimal at acidic pH (pH 2)
- Stability is improved by the presence of edetate disodium, malic acid, and sucrose
- However, long-term aqueous chemical stability of GSK2251052.HCl solutions was unlikely to be improved sufficiently by pH control and/or addition of excipients to yield a shelf life long enough to support the duration of a Phase I clinical study.

Thus, formulation development efforts were focused on the development of lyophilized formulations.

Figure 4. GSK2251052.HCl in Pharmaceutical Buffers at Room Temperature

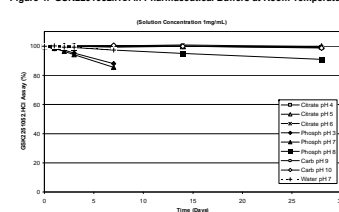


Figure 5. GSK2251052.HCl in Pharm. Buffers, Autoclave 121°C 30 minutes

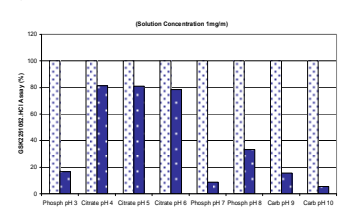


Figure 6. GSK2251052.HCl Effect of Anti-Oxidants and Chelating Agents

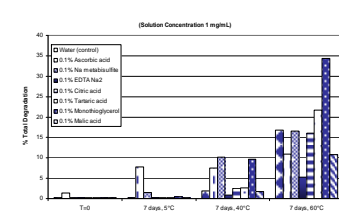
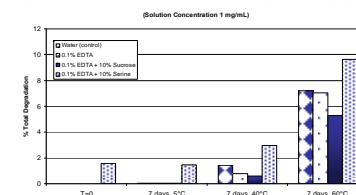


Figure 7. GSK2251052.HCl Effect of Combined Chelating and Stabilizing Agents



Stability and Suitability of GSK2251052.HCl as a Lyophilized Powder for Reconstitution

Lyophilized prototype formulations were prepared by dissolving GSK2251052.HCl at a concentration of 100 mg/g or 120mg/g in aqueous media containing various combinations of excipients. Solutions were filtered through 25mm, 0.2um nylon syringe-tip filters before being delivered in 1 g aliquots to 2 mL, clear glass lyophilization vials and freeze-dried.

Tables 1 and 2 describe the R&D screening lyophilization cycle and setup parameters used to freeze-dry all lyophilized prototype formulations for stability screening. Eleven lyophilized prototype formulations (Table 3) were prepared and followed up on stability for 2 weeks at the conditions of 5°C/ambient, 25°C/60%RH, and 40°C/75%RH.

Table 1. R&D Freeze-Dry Screening Cycle

Phase	Temperature (°C)	Rate (m/s)	End criteria	Pressure (mTorr)
Freezing	-20	None	100	NA
Primary Drying	-20	None	100	100
Secondary	-20	None	100	100
Termination	-20	None	None	None

Table 2. R&D Freeze-Dry Parameters

Parameter	Condition
Start up temperature	-20.5°C
End temperature	-20.5°C
Start up pressure	100 mTorr
End pressure	100 mTorr

Table 3. Screening of GSK2251052.HCl Lyophilized Prototype Formulations

Lyophilized Prototype Formulation	Formulation Description	Appearance	Reconstitution Time	% Total Protein Residual after 2 Weeks (25°C/60%RH)
LP-1	GSK2251052.HCl	White cake	30 minutes	0.08
LP-2	GSK2251052.HCl + 1% EDTA	White cake	30 minutes	0.43
LP-3	GSK2251052.HCl + 1% EDTA + 10% Sucrose	White precipitate	120 minutes	0.02
LP-4	GSK2251052.HCl + 1% EDTA + 10% Sucrose + 10% Fructose	White precipitate	120 minutes	0.02
LP-5	GSK2251052.HCl + 1% EDTA + 10% Sucrose + 10% Fructose + 10% Sorbitol	White precipitate	120 minutes	0.02
LP-6	GSK2251052.HCl + 1% EDTA + 10% Sucrose + 10% Fructose + 10% Sorbitol + 10% Serine	White precipitate	120 minutes	0.02
LP-7	GSK2251052.HCl + 1% EDTA + 10% Sucrose + 10% Fructose + 10% Sorbitol + 10% Serine + 10% Malic acid	White precipitate	120 minutes	0.02
LP-8	GSK2251052.HCl + 1% EDTA + 10% Sucrose + 10% Fructose + 10% Sorbitol + 10% Serine + 10% Malic acid + 10% Monothylglycerol	White cake	30 minutes	1.76
LP-9	GSK2251052.HCl + 1% EDTA + 10% Sucrose + 10% Fructose + 10% Sorbitol + 10% Serine + 10% Malic acid + 10% Monothylglycerol + 10% Cyclodextrin	White cake	30 minutes	0.31
LP-10	GSK2251052.HCl + 1% EDTA + 10% Sucrose + 10% Fructose + 10% Sorbitol + 10% Serine + 10% Malic acid + 10% Monothylglycerol + 10% Cyclodextrin + 10% Diethanolamine	White cake	30 minutes	0.77
LP-11	GSK2251052.HCl + 1% EDTA + 10% Sucrose + 10% Fructose + 10% Sorbitol + 10% Serine + 10% Malic acid + 10% Monothylglycerol + 10% Cyclodextrin + 10% Diethanolamine + 10% Sodium Hydroxide	White cake	30 minutes	0.36

Pre-formulation studies for a lyophilized formulation of GSK2251052.HCl demonstrated:

- Acidification of the formulation adversely affected the reconstitution of the lyophilized drug product.
- Incorporation of sucrose in the formulation produced dual-layered stratification of the final lyophilized drug product.
- Inclusion of excipients such as EDTA disodium, sucrose, citrate and lactate buffering systems, and diethanolamine and sodium hydroxide adversely affected the chemical stability of the lyophilized drug product.

Based on 2 weeks stability at 40°C/75%RH, the LP1 formulation was selected as the lead GSK2251052.HCl drug product formulation for clinical use. The stability of LP1 was continued for 9 months (Table 4).

Key stability test attributes monitored in the selected lyophilized lead Prototype 1 formulation included: pre- and post-reconstitution appearance, reconstitution time, pH of the solution, osmolality, assay and related substances, water content, weight gain, and particulates per container.

Table 4. GSK2251052.HCl Lyophilized Prototype 1 Formulation Stability

Condition	Pre-Reconstitution Appearance	Reconstitution Time (seconds)	Post-Reconstitution Appearance	pH	Conductivity (mS/cm)	Assay (%)	Total Res. Subst. (%)	Water content (wt %)	Weight gain (wt %)	Particulates per container (≤ 10µm, ≥ 25µm µm)	
5°C	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0
	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0
	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0
25°C	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0
	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0
	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0
40°C	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0
	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0
	White cake	30	White cake	5.5	100	100	0.05	100	0.02	100	0

Result Summary

- GSK2251052.HCl is a small molecule (MW 273.52) with a crystalline and stable HCl salt of high aqueous solubility (>200 mg/mL at pH 5), a stable polymorph form, and excellent solid state stability both as a powder and as a lyophilized drug product.
- Due to its crystalline behavior, solid state stability, high water solubility and fast dissolution kinetics, the HCl salt of GSK2251052 was successfully formulated as a sterile lyophilized cake without need for additional excipients.
- GSK2251052.HCl formulated as a lyophilized powder for reconstitution was shown to be chemically and physically stable at 5°C, 25°C/60%RH, and 40°C/75%RH for at least 9 months.

Conclusions

- GSK2251052.HCl is a novel boron-containing small molecule with a very attractive CMC profile, high aqueous solubility and excellent solid state stability.

- Pre-formulation activities were successful in identifying a simple intravenous formulation for use in the first-in-human clinical trial.
- Lyophilized GSK2251052.HCl powder for reconstitution was successfully manufactured and released for use in a Phase I clinical study. (See AAPS 2011, Poster No. T3424).

Acknowledgments

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