Salt Screen of Bedaquiline using a Combination of Techniques to Identify Promising New Salts for Development

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PURPOSE

Bedaquiline fumarate is a currently marketed drug product approved for use as part of combination therapy for the treatment of multi-drug resistant Tuberculosis (MDR-TB) in adults and children twelve years and older[1]. The Bill and Melinda Gates Foundation is interested in finding additional Bedaquiline salts with acceptable development properties to be used as a treatment in developing countries where TB is a highly prevalent threat[2]. This poster describes the results of salt screen activities using powerful, orthogonal analytical techniques to expedite the process, as well as a potential development path forward for select salts and/or cocrystals of Bedaquiline.

OBJECTIVE(S)

1. Prepare salts and/or cocrystals of Bedaquiline (Figure 1) using pharmaceutically acceptable counterions.
2. Characterize the salts and/or cocrystals using appropriate orthogonal physico-chemical techniques.
3. Identify select salts for further development based upon all results.

METHOD(S)

- Selected various pharmaceutically accepted counterions based upon their inherent properties to form suitable salts with Bedaquiline API: acetic acid, benzoic acid, benzenesulfonic acid, hydrobromic acid, hydrochloric acid, lactic acid, malic acid, maleic acid, methanesulfonic acid, and succinic acid (1:1 and 1:2, counterion:API).
- Prepared salts by: slow (SE), fast evaporation (FE), heating, and solvent/anti-solvent addition; chose solvents based upon solubility of Bedaquiline and all counterions, polarity and dielectric constant; conducted experiments in two separate laboratories to increase the likelihood of finding additional salts.
- Screened solids obtained by polarized light microscopy (PLM) to identify those solids that were crystalline, Raman microscopy and/or infrared microspectroscopy (R/A and/or ATR) and compared to a spectrum of Bedaquiline (free form) to determine if a salt had been made.
- Analyzed potential Bedaquiline salts by proton nuclear magnetic resonance spectrometry (^{1}H NMR) and laboratory single crystal X-ray diffraction to confirm the counterion stoichiometry and possible solvent inclusion in the crystal lattice. Laboratory (Purdue) or synchrotron (Argonne National Labs) X-ray powder diffraction (XRPD) were used for further characterization and to identify possible polymorphs of formed salts.
- Analyzed selected Bedaquiline salts by hot stage optical microscopy (HSOM), melting point, differential scanning calorimetry (DSC), thermogravimetry (TG), weight gain to evaluate hygroscopicity, and solubility in simulated gastric fluid (SGF) using HPLC with UV /VIS detection to identify appropriate salts for development.

RESULT(S)

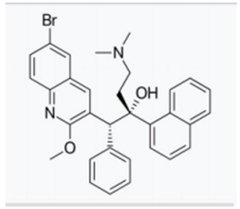

Figure 1. Structure of Bedaquiline free base

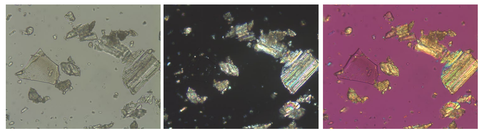

Figure 2. PLM images of Bedaquiline benzoate. Left to right plane polarized light, cross-polarized light, cross-polarized light with red compensator

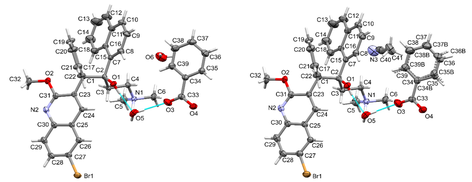

Figure 4. Single crystal structures for Bedaquiline benzoic acid salt: left) hydrate, right) ACN solvate

Five crystalline salt candidates of Bedaquiline were obtained (1:1): benzoic, maleic, besylate, mesylate and hydrochloric. The salts displayed low solubility in SGF (<15 μg/mL) which is not uncommon for Bedaquiline. All physicochemical characterization data is displayed in Table 1 for these salts. An example of one of the Bedaquiline salts (benzoate) is presented in this poster.

The crystalline benzoate salt has been confirmed by PLM (Figure 2), IR microspectroscopy using both R/A and ATR (Figure 3), XRPD, ^{1}H NMR and single crystal x-ray. The structure exists as a hydrate with 1.17 mols of water or an ACN solvate with 0.75 mols ACN occupancy and 1 mol water (Figure 4). High resolution synchrotron XRPD data revealed subtle differences between hydrate and solvated salts (Figure 5). The material is moderately hygroscopic (<5% weight gain) upon exposure to high relative humidity (75% RH). Thermal properties were evaluated further by TG (3.2% volatiles supporting single crystal data), DSC (desolvation/melting endotherms of 109.0/124.7 °C) and HSOM (supporting melt behavior) as shown in Figures 6 – 7. A small-scale polymorph screen revealed no change in crystalline structure.

For Bedaquiline, Raman microscopy could not be used as a pre-screening tool for determination of small scale crystallization experiments due to its lack of specificity as shown in Figure 8.

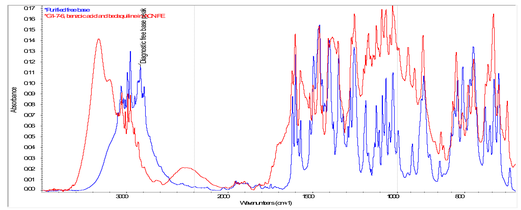

Figure 3. Infrared R/A spectra of Bedaquiline benzoate (red) and Bedaquiline free base (blue). Diagnostic peaks are labeled

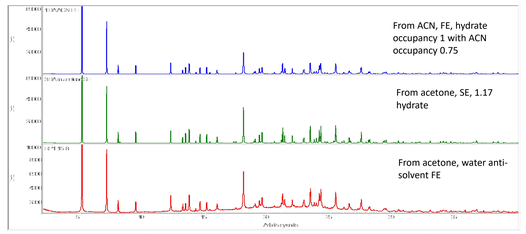

Figure 5. Synchotron XRPD of Bedaquiline benzoic acid salts, top to bottom: crystallized from ACN, acetone, and acetone/water

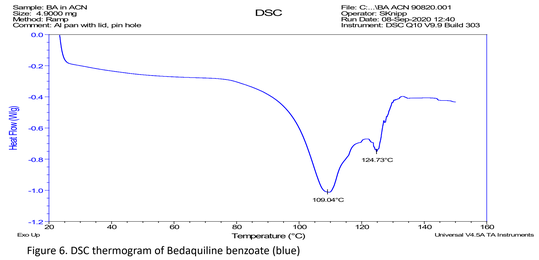

Figure 6. DSC thermogram of Bedaquiline benzoate (blue)

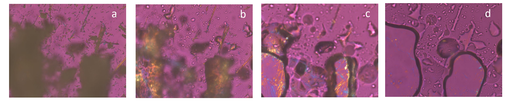

Figure 7. HSOM images of Bedaquiline benzoate using cross-polarized light with red compensator. Left to right: a) 92.2 °C, start of melt; b) 100.5 °C, melting; c) 105.0 °C, melting; d) 109.5 °C, melt nearly complete

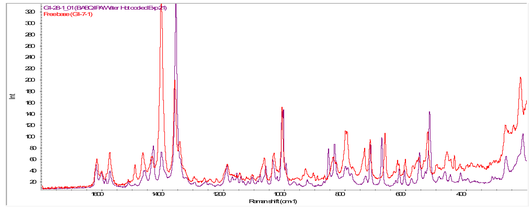

Figure 8. Raman spectra of Bedaquiline benzoate (purple) and Bedaquiline free base (red). Note lack of specificity between the spectra

Table 1. Physicochemical Characterization Data for Bedaquiline Salts

	Benzoate	Maleate	Besylate	Mesylate	HCl
^{1}H NMR	(1:1) Salt confirmed	(1:1) Salt confirmed	(1:1) Salt confirmed	(1:1) Salt confirmed	(1:1) Salt confirmed
Synchotron XRPD	Crystalline	Crystalline	Crystalline	Crystalline	Crystalline
Single Crystal	1.17 mols water or 0.75 mol ACN with 1 mol water	2 mols THF	1 mol THF and 1 mol water	–	2 mols acetone, 1 mol water
IR (R/A)	Salt confirmed	Salt confirmed	Salt confirmed	Salt confirmed	Salt confirmed
IR (ATR)	Salt confirmed	Salt confirmed	Salt confirmed	Salt confirmed	Salt confirmed
HSOM	Desolvation?/melt	Desolvation?/melt	Desolvation/melt/ recrystallization/melt	Desolvation/melt	Desolvation/melt
DSC	Endos @ 109.0, 124.7 °C	Endos @ 143.0 °C	Endos @ 81.8, 106.7, 192.4°C; exo @ 198.8°C	Endos @ 89.9, 113.3, 186.9 °C	–
TG	~3.2% volatiles	–	–	–	–
PLM	Blades, Anhedral-platy	Anhedral-platy, polycrystalline, agglomerates	Anhedral-platy, polycrystalline, agglomerates	Anhedral-platy, polycrystalline, agglomerates	Blades, Anhedral-platy
HPLC (solubility)	~12.7 μg/mL (SGF)	~2.9 μg/mL (SGF)	~7.6 μg/mL (SGF)	~2.3 μg/mL (SGF)	~7.0 μg/mL (SGF)

CONCLUSION(S)

Five different Bedaquiline salts (1:1) were made and successfully scaled-up using various pharmaceutically acceptable counterions that are suitable for further development: benzoic acid, maleic acid, benzenesulfonic acid, methansulfonic acid and hydrochloric acid. PLM and IR micro-spectroscopy (R/A and/or ATR) were shown to be successful pre-screening tools for determining salt formation; however, Raman microscopy, due to lack of specificity, was not a useful pre-screening tool for Bedaquiline salt formation. These newly identified salts were characterized using physico-chemical techniques including ^{1}H NMR, XRPD, HSOM, DSC, and/or TG. The increased sensitivity and resolution of Argonne National Labs synchrotron XRPD data allowed for detection of possible polymorphs, and/or contaminants. Upon successful growth of single crystals from select salts, the single crystal x-ray structure was used to confirm stoichiometry and solvent inclusion for the benzoic acid, maleic acid, benzenesulfonic acid, and hydrochloride salts. Finally, the solubility of the five select salts was determined in simulated gastric fluid to be < 15 μg/mL.

FUNDING

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REFERENCES

1. Janssen Therapeutics. Sirturo ™ website http://www.sirturo.com/.
2. World Health Organization. Global Tuberculosis Report 2019.

ACKNOWLEDGEMENTS

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