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New bedaquiline salt with improved bioavailability and reduced food effect

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Bedaquiline (BDQ) is an orally administered diarylquinoline class anti-tuberculosis drug used in the combination therapy of MDR-TB. Currently, it is marketed as Bedaquiline Fumarate (BDQ-FA). However, due to its poor water solubility, BDQ-FA must be taken with food to enhance bioavailability. Concurrent administration reduces patient compliance and may increases the risk of inconsistent serum levels due to differences in dietary habits. This study designed and synthesized the saccharin salt of Bedaquiline (BDQ-SA). Compared to the marketed fumarate salt, BDQ-SA exhibits better solubility and absorption. The food effect of commercially available BDQ-FA in Beagle dogs was 7.2 times, while BDQ-SA reduced to 3.2 times. This study provides a good example of using a new salt form to improve drug solubility and mitigate the food effect of the drug.

Introduction

Bedaquiline (BDQ) is a diarylquinoline compound, first discovered by Andries et al.1 in 2005. BDQ represents the first drug approved by the United States Food and Drug Administration (US FDA) since 1971 with a novel mechanism of action and activity against Mycobacterium tuberculosis.2-4 It functions by binding to the c subunit of the ATP synthase transmembrane complex F$_{0}$, inhibiting proton flow and thus hindering ATP synthesis, thereby suppressing the growth and proliferation of tuberculosis bacteria.5-10

BDQ belongs to BCS class II drug with poor aqueous solubility resulting in poor bioavailability.11, 12 The currently marketed salt form is BDQ-FA, with an absolute bioavailability of 36%.9 Co-administration with food can double its bioavailability.9 However, taking BDQ-FA with food reduces patient compliance and increases the risk of inconsistent serum levels due to varying dietary habits. Therefore, reducing the food effect of BDQ is advantageous for both clinicians and patients.

A drug cocrystal refers to a multi-component crystal formed by the combination of an active pharmaceutical ingredient (API) and a cocrystal former (CCF) through non-covalent interactions in a specific stoichiometric ratio within the same lattice.13 As a solid form of a drug, a cocrystal can alter the physicochemical properties (solubility, dissolution rate, permeability, and stability, etc.) of the API without changing its chemical structure, thereby improving bioavailability, enhancing the inherent pharmacological activity of the drug, and achieving better therapeutic effects.14 When proton transfer occurs between the API and the CCF, the resulting multi-component crystal is considered as a salt; when there is no proton transfer between the API and the CCF, the resulting multi-component crystal is referred to as a cocrystal.15

This study introduces the preparation process of BDQ-SA and comprehensively characterizes its physicochemical properties using single-crystal X-ray diffraction (SCXRD), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and so on. The solubility and stability of the salt were investigated using HPLC. Additionally, pharmacokinetic parameters of the commercially available salt form and the new salt form were studied in beagle dogs. This research opens up new avenues for increasing the solubility of certain poorly water-soluble drugs and mitigating their food effect.

Experimental

Chemicals and Reagents

Samples of BDQ-FA (purity 99.09%) and BDQ (purity 99.87%) were synthesized by the Novel Technology Center of Pharmaceutical Chemistry, Shanghai Institute of Pharmaceutical Industry Co., Ltd. (Shanghai, China). Saccharin (purity 98%) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). HPLC-grade methanol and acetonitrile were purchased from CINC High Purity Solvents Co., Ltd. (Shanghai, China). Other analytical grade reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Water was purified using a Millipore Milli Q-Plus system (Millipore Corp., Billerica, Massachusetts, United States).

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Screening of Bedaquiline Salt Forms

BDQ, as an API, contains functional groups such as -N(CH₂)₃-, -OH, and -Br, which can act as donors or acceptors in hydrogen bonding or halogen bonding interactions. The structure of BDQ also includes benzene rings, naphthalene rings, and quinoline rings, which readily engage in π-π stacking interactions with the aromatic rings present in coformers. Based on the structural characteristics of BDQ, GRAS additives with relatively simple structures that are prone to forming hydrogen bonds were selected for screening. BDQ and various coformers were placed in 2 mL centrifuge tubes at a molar ratio of 1:1. The screening of BDQ coformers was conducted using evaporation crystallization, cooling crystallization, suspension crystallization, and grinding methods. The obtained powders and crystals were characterized by PXRD after vacuum drying, and the diffraction patterns were compared with those of BDQ and the corresponding CCFs to observe the appearance of any new diffraction peaks (Table S1). The SA was ultimately chosen as the ligand to prepare a new salt form of BDQ. The structural formulas of BDQ and SA are shown in Figure 1.

Preparation of BDQ-SA

The synthesis of the new salt form BDQ-SA was carried out using a reaction crystallization method. BDQ (555.5 mg, 1 mmol) was accurately weighed and placed in a 50 mL round-bottom flask, followed by the addition of 20 mL isopropanol. The mixture was refluxed at 80-90 °C in an oil bath with stirring until dissolved. SA (183.2 mg, 1 mmol) was then slowly added, and the stirring continued for 30 minutes to obtain a clear solution. The reaction mixture was filtered hot, and the filtrate was transferred to another round-bottom flask and slowly cooled to 0-10 °C with stirring for 1 hour. The resulting precipitate was filtered, washed twice with 5 mL isopropanol, and then vacuum dried (vacuum pressure -0.08 MPa, temperature 50-60 °C) for 12 hours. Obtain 614.53 mg of BDQ-SA powder with a purity of 99.74% and a yield of 83.19%.

Powder X-Ray Diffraction

PXRD characterization was performed using a D8 Advance powder X-ray diffractometer from Bruker (Germany) with a voltage of 40 kV and a current of 40 mA. The test sample powder (2-5 mg) was spread evenly on a glass plate, and data were collected in the 2θ range of 3-40° at a scanning rate of 5°/min under room temperature conditions. The collected data were integrated and imaged using RINT Rapid, and the diffraction peaks were analyzed using Rigaku’s Jade 6.0.

Preparation of Single Crystals

BDQ-SA single crystals were grown using a solvent evaporation method. BDQ and SA were accurately weighed and placed in a 5 mL glass vial, and then 4 mL of a 50% acetonitrile aqueous solution was added. The mixture was stirred at 200 rpm at 50 °C until fully dissolved, and the reaction solution was filtered hot through a needle filter membrane and then slowly crystallized at room temperature.

Single Crystal X-Ray Diffraction

SCXRD characterization was performed using an APEX II CCD single X-ray diffractometer from Bruker (Germany) with Mo Kα radiation (λ = 0.71073 Å) as the light source at a test temperature of 100 K. The collected data were integrated and scaled using the SAINT program, and the absorption effects were corrected using SADABS. The crystal structure was refined and reduced using the full-matrix least squares method with SHELXL-2017 software. The crystal structure was plotted using Diamond software.

Thermal Analyses

DSC characterization was conducted using a TA Q2000 differential scanning calorimeter from Waters (USA) with a nitrogen flow rate of 50 mL/min and a heating rate of 10 °C/min. The test sample powder (3-5 mg) was placed in an alumina crucible, and the temperature range was set from 40 to 250 °C. TGA characterization was performed using a TG 209 F3 Tarsus thermal gravimetric analyzer from NETZSCH (Germany) with a nitrogen flow rate of 50 mL/min and a heating rate of 10 °C/min. The test sample powder (3-5 mg) was placed in an aluminum tray, and the temperature range was set from 10 to 400 °C.

Fourier Transformation Infrared

FT-IR characterization was conducted using a NICOLET MAGNA-IR 750 infrared spectrometer from Thermo Scientific (USA) with a scanning range of 350-4000 cm⁻¹ and a resolution of 4 cm⁻¹. The sample was thoroughly ground with an appropriate amount of KBr powder in an agate mortar, then pressed into a transparent thin film using a tablet press for detection.

1H Nuclear Magnetic Resonance Spectra

1H-NMR characterization was performed using an AC AVANCE nuclear magnetic resonance spectrometer from Bruker (Germany) with deuterated chloroform (CDCl₃) as the solvent and tetramethylsilane (TMS) as the internal standard at a detection frequency of 400 MHz.

Physicochemical Stability Experiment

Spread the BDQ-SA powder evenly in an open, flat-bottomed weighing bottle, ensuring that the thickness does not exceed 3 mm. Place the mouth of the weighing bottle in a constant temperature drying oven at 60 °C, as well as in constant temperature and humidity chambers set at 25 °C/ RH 60% and 45 °C/ RH 75%, respectively. Samples are to be collected at 0, 5, 10, and 30 days for content determination. Additionally, PXRD characterization on samples collected at different time points to observe any changes in characteristic diffraction peaks of BDQ-SA.

Powder Dissolution

The test samples were prepared by grinding and sieving the powders through a 150 μm (100 mesh) sieve to ensure uniform
particle size. The sieved BDQ-SA and BDQ-FA powders were separately placed in 20 mL of pH 1.0, 2.0, 4.5, 6.8 buffer solutions, and water. The dissolution test was conducted at 37 °C with a rotation speed of 200 rpm. Samples were collected at predetermined time points, and the concentration of BDQ was determined using HPLC.

PK in Beagle Dogs

Conducting pharmacokinetic (PK) studies in accordance with the "Guidelines for the Care and Use of Laboratory Animals" and approved by the Laboratory Animal Welfare and Ethics Committee of the Shanghai Institute of Pharmaceutical Industry. Sixteen healthy male beagle dogs were divided into four groups, with four dogs in each group. Male beagle dogs (9-11 kg) are divided into four groups, with four dogs in each group. The fasting group fasted for 12 hours before administration and had a uniform meal 4 hours after administration. The fed group fasted for 12 hours before administration and received a standard meal containing approximately 22 g of fat (558 kcal) at the time of administration. Table S2 outlines the dosing regimen.

Blood plasma samples were collected from all four groups of administration. Table S2 outlines the dosing regimen. For the powder dissolution, hygroscopicity, and stability experiments, the content of BDQ was determined using HPLC. The ratio of BDQ to saccharin in BDQ-SA is 1:1, dissociating into free base and SA in aqueous solution. We determined the content of the free base BDQ in the final product using HPLC with an external standard method, and subsequently calculated the purity of BDQ-SA. The chromatographic column used was the Waters SunFire C18 column (4.6 × 250 mm, 5 μm). The mobile phase consisted of a mixture of 10 mmol/L potassium dihydrogen phosphate and 10 mmol/L dipotassium hydrogen phosphate in water (pH 3.0) and acetonitrile at a ratio of 35:65. The column temperature was maintained at 30 °C, and the flow rate was set at 1 mL/min. The injection volume was 5 μL, and detection was performed at a wavelength of 224 nm. The total run time was 10 minutes.

For the analysis of BDQ in biological samples, LC-MS/MS was employed, with 20(S)-protopanaxatriol chosen as the internal standard. The chromatographic column used was the Agilent ZORBAX SB-C18 column (2.1×50 mm, 1.7 μm). The mobile phase consisted of acetonitrile (Phase A) and 0.1% formic acid aqueous solution (Phase B). The gradient elution program is as follows: time (min)/Phase A (%): 0/50, 0.6/50, 1/90, 5/95, 5.1/50, 6/50. The column temperature was maintained at 50 °C, and the flow rate was set at 0.3 mL/min. A sample injection volume of 3 μL was utilized, with the sample tray temperature set at 4 °C. An ESI source was employed, operating in positive ion mode. MRM mode was utilized for detection. The monitored ion pairs were m/z 555.23→58.21 (BDQ), m/z 541.25→58.01 (N-BDQ), and m/z 441.45→123.2 (internal standard).

RESULTS AND DISCUSSION

SCXRD and Crystal Structure Analysis.

The SCXRD results indicate that BDQ-SA incorporates water molecules and acetonitrile solvent during single crystal growth. Crystallographic data analysis was conducted on this single crystal to elucidate the binding mode between BDQ and SA. Crystallographic data are presented in Table S3, and the lengths and angles of the main hydrogen bonds are listed in Table S4, the CCDC number is 2356517.

Based on the collected crystallographic data, the crystal structure of BDQ-SA solvate belongs to the orthorhombic crystal system with the P2₁_2₁_2₁ space group. Protons from the sulfonfyl amine group in SA molecules are transferred to the tertiary amine N atom of BDQ molecules, forming a quaternary ammonium salt. The formyl group of SA forms N2-H1...O1 (d=2.71Å) hydrogen bonds with the tertiary amine of BDQ, as shown in Figure 2 (b). BDQ-SA forms one-dimensional chain structures along the a-axis through three types of O-H...O hydrogen bonds, as shown in Figure 2 (c). One-dimensional chains form two-dimensional and three-dimensional structures through van der Waals forces, short contact interactions, etc., as shown in Figure 2 (d). According to the pKa rule, pKa = 13.05 for API and pKa = 1.31 for SA, ΔpKa (pKa (conjugate acid of base) - pKa (acid)) > 3, it is classified as a salt18.
The single crystal of BDQ-SA solvent compound was subjected to vacuum drying (vacuum pressure \( \leq -0.08\) MPa, temperature 110 °C) for 12 hours for desolvation experiments. PXRD analysis was performed, and the results showed that the characteristic diffraction peaks of the solvent-removed BDQ-SA after treatment were essentially consistent with those of BDQ-SA prepared by the reaction crystallization method. However, the crystallinity of the solvent-removed BDQ-SA powder decreased. Prediction of the PXRD spectrum of the BDQ-SA solvent compound using Mercury software revealed significant differences between the characteristic diffraction peaks of the BDQ-SA solvent compound and those of the solvent-removed BDQ-SA (Figure S1). Therefore, the removal of solvent molecules from the lattice leads to structural rearrangement and a simultaneous loss of some crystallinity.

**Thermal Analysis**

The TGA and DSC profiles of BDQ, SA, and BDQ-SA are shown in Figure S2 and S3. Thermal analysis results show that BDQ-SA exhibits an endothermic peak at 133.7 °C, corresponding to its melting point, followed by a thermal weight loss at 145 °C. The single sharp endothermic peak of the solid-state form of BDQ-SA indicates its high purity and crystallinity. For BDQ-SA, no mass loss before the melting event was observed in the TG plot, indicating its non-solvating nature.

**FT-IR and \(^1\)H-NMR Analysis**

Due to BDQ being a derivative of biphenylquinoline, its molecular structure is complex with numerous functional groups, resulting in a complex infrared spectrum (Figure S4). SA contains benzene rings, carbonyl groups, sulfonyl groups, and amino groups, with characteristic absorption peaks in the range of 1800 to 1100 cm\(^{-1}\).

For BDQ-SA, no mass loss before the melting event was determined to be 1:1, thereby confirming its molecular structure. After the -N(CH\(_3\))\(_2\) in the BDQ molecule was converted to a quaternary ammonium group, the chemical shift of the hydrogens on the two methyl groups increased and towards the lower field. The assignments of the H-NMR spectrum of BDQ-SA are presented in Table S6.

**Stability**

The results of stability are presented in Table S7. BDQ-SA exhibits negligible or virtually no hygroscopicity. When BDQ-SA samples were stored at 60 °C, 25 °C/ RH 60%, and 45 °C/ RH 75% conditions for 30 days, there was no significant change in sample purity, and characteristic diffraction peaks remained unchanged (Figure S5), indicating good chemical stability of BDQ-SA.

**Powder Dissolution and PK Studies in Beagles**

During the oral administration of solid dosage forms, the dissolution of drugs plays a pivotal role in their absorption within the body. To compare the dissolution profiles of desolvated BDQ-SA and BDQ-FA in simulated gastric and intestinal fluids, we selected pH 1.0, 2.0, 4.5, 6.8 buffer solutions, and water as dissolution media for conducting in vitro dissolution studies. Due to the very low solubility of BDQ in pH 4.5, 6.8, and water, rendering them indistinguishable (Table S8), 0.1% Tween 80 was added to pH 4.5, 6.8, and water to enhance solubility.

After vacuum drying for 12 hours, the BDQ-SA and BDQ-FA powders were ground in an agate mortar and pestle, then sieved through a 150μm (100 mesh) sieve to ensure uniform particle size. Appropriate amounts of the sieved BDQ-SA and BDQ-FA powders were taken and placed separately in 20 mL of pH 1.0, 2.0, 4.5, 6.8 buffer solutions, and water, with a stirring speed of 200 rpm and a heating temperature of 37°C. Four samples were prepared in parallel for each batch under different pH conditions. Sampling time points were 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 min. Immediately after withdrawing the dissolution solution each time, an equal volume of buffer...
solution was replenished to avoid errors. The content of BDQ was determined using HPLC. The dissolution-time curves of BDQ-SA and BDQ-FA under different pH conditions were shown in Figure 3. The experimental results demonstrate that the dissolution rate of BDQ-SA in buffer solutions with pH values of 1.0, 2.0, 4.5, 6.8, and water was higher than that of the commercially available BDQ-FA salt. Furthermore, the solubility of BDQ-SA at 180 minutes is 1.5 to 5 times as that of BDQ-FA. Among these, BDQ-SA demonstrates the most significant solubility advantage in pH 4.5 buffer solution, with its solubility being 5 times that of BDQ-FA. Both exhibit a notable “umbrella effect” trend, characterized by an initial increase followed by a decrease in solubility, particularly evident in pH 4.5, 6.8 buffer solutions, and water19-21. However, this trend is less pronounced in pH 1.0 and 2.0 buffer solutions. Under pH 1.0 and 2.0 conditions, a portion of BDQ-SA may convert to an amorphous form, increasing its solubility in the buffer solution and maintaining the solution at a higher concentration level. After the experiment, the pH of the buffer solutions was measured, and no significant changes in pH values were observed. PXRD analysis was performed on the residual solids, and the results showed that their crystal form remained unchanged. The specific data have been added to Table S9 and Figure S6 in the ESI.

Desolvated BDQ-SA and BDQ-FA were administered by gavage to Beagle dogs at a dose of 10 mg/kg (as free base) under both fasting and fed conditions. Plasma BDQ concentrations were determined using LC-MS/MS. The results of BDQ plasma concentrations in Beagle dogs are depicted in Figure 4, with PK parameters summarized in Table 1. The results indicate that under fasting conditions in Beagle dogs, BDQ-SA and BDQ-FA exhibit similar time to peak concentration (T \(_{\text{max}}\)), with BDQ-SA demonstrating a peak concentration (C \(_{\text{max}}\)) 2.2 times as that of BDQ-FA and an area under the concentration-time curve (AUC) 2.4 times as that of BDQ-FA. Compared to BDQ-FA, BDQ-SA shows a significantly higher total drug exposure in Beagle dogs. Under fed conditions in Beagle dogs, BDQ-SA and BDQ-FA exhibit similar T \(_{\text{max}}\), with BDQ-SA showing a C \(_{\text{max}}\) 1.3 times that of BDQ-FA and an AUC 1.1 times that of BDQ-FA. BDQ-SA and BDQ-FA demonstrate similar total drug exposure in Beagle dogs under fed conditions. BDQ-SA mitigates the food effect observed with the commercially available BDQ-FA salt form.

<table>
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<td>SD</td>
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Figure 3 Dissolution profile of BDQ-SA and BDQ-FA (a) pH 1.0, (b) pH 2.0, (c) pH 4.5 with 0.1% Tween 80, (d) pH 6.8 with 0.1% Tween 80, (e) water with 0.1% Tween 80.
Conclusions
In summary, based on the principles of multi-component crystal engineering and supramolecular chemistry, this study screened and prepared a new salt form of BDQ and conducted comprehensive structural characterization and property investigations. BDQ-SA exhibits significant advantages in vitro dissolution compared to the commercially available salt forms. Pharmacokinetic studies in Beagle dogs revealed that under fasting conditions, the AUC of BDQ-SA is 2.4 times as that of BDQ-FA, while under fed conditions, the AUC of BDQ-SA is 1.1 times as that of BDQ-FA. The food effect of commercially available BDQ-FA in Beagle dogs was 7.2 times, while BDQ-SA reduced to 3.2 times. BDQ-SA mitigates the food effect observed with the commercially available BDQ-FA salt form. These experimental findings provide a new salt form for improving the physicochemical properties of BDQ and offer reference for the systematic development and in vivo sample detection of BDQ new salt forms.

Conflicts of interest
The authors show no conflict of interest.

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References


New bedaquiline salt with improved bioavailability and reduced food effect

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1. The authors confirm that the data supporting the findings of this study are available within the article.
2. The data supporting this article have been included as part of the Supplementary Information.
3. Crystallographic data for BDQ-SA solvate has been deposited at the CCDC under 2356517 and can be obtained from https://www.ccdc.cam.ac.uk.