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Mechanochemistry applied to reformulation and scale-up production of Ethionamide: Salt selection and solubility enhancement

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ABSTRACT

Ethionamide (ETH), a Biopharmaceutics Classification System class II drug, is a second-line drug manufactured as an oral dosage form by Pfizer to treat tuberculosis. Since its discovery in 1956, only one reformulation was proposed in 2005 as part of the efforts to improve its solubility. Due to the limited scientific research on active pharmaceutical ingredients (APIs) for the treatment of neglected diseases, we focused on the development of an approachable and green supramolecular synthesis protocol for the production of novel solid forms of ETH. Initially, three salts were crystal engineered and supramolecular synthesized via slow evaporation of the solvent: a saccharinate, a maleate and an oxalate. The crystal structures of all salts were determined by single crystal X-ray diffraction. In sequence, mechanochemical protocols for them were developed, being the scale-up production of the maleate salt successfully reproducible and confirmed by powder X-ray diffraction. Finally, a more complete solid-state characterization was carried out for the ETH maleate salt, including thermal analysis, infrared spectroscopy, scanning electron microscopy and equilibrium solubility at different dissolution media. Although ETH maleate is thermodynamically less stable than ETH, the equilibrium solubility results revealed that this novel salt is much more soluble in purified water than ETH, thus being a suitable new candidate for future formulations.

1. Introduction

According to the World Health Organization (WHO), HIV, tuberculosis (TB), and malaria are the "big three" infectious diseases worldwide, being responsible for 4 million deaths annually (Dye et al., 2013). Unfortunately, these diseases are prevalent in poor countries, especially in Africa, where there is a lack of an effective, safe and affordable drug production for controlling them (Bourzac, 2014; Goldberg et al., 2012). In this context, this study focused on the development of an easy, large-scale, low-cost and ecological method for the production of novel solid forms of antituberculosis drugs — in particular, ethionamide (ETH). ETH, 2-ethyl-4-thiocarbamoylpyridine, is a second-line antituberculosis drug, analog to isoniazid (INH), which is considered the cornerstone of tuberculosis therapy. Although ETH and INH have the enoyl-[acyl-carrier-protein] reductase (InhA) as the primary molecular target, both are prodrugs activated by different mycobacterium enzymes and thus cross-resistance between them does not occur. ETH is marketed in oral form under the brand name of Trecator® (ethionamide tablets, USP) and it is in general administered in combination with others antimycobacterium drugs, such as pyrazinamide, rifampicin and ethambutol, in cases where it is observed intolerance or resistance by INH to the *Mycobacterium tuberculosis*. In addition, ETH can also be used in the treatment of infections caused by *Mycobacterium leprae* and *Mycobacterium avium* (Brossier et al., 2011; Anon., 2008; Wang et al., 2007; Picon et al., 2011; Vale et al., 2013). Even though it is so commonly used, ETH exhibits a low aqueous solubility. Since its discovery in 1956 only one reformulation was proposed in 2005 as part of the efforts to improve its solubility. At the time, the original ETH formulation, a sugar-coated tablet, was replaced by a film-coated tablet (Korth-Bradley et al., 2014). Also, since its discovery, besides the neutral structure reported in 1973, only three crystalline modifications of this API have been reported, viz., hexafluorosilicate, hydrochloride and hydrobromide salts, not reporting, however, possible improvements in ETH's physicochemical properties (Alléaume and Leroy, 1973; Gel'mbol'dt et al., 2010; Colleter and Gadret, 1968a,1968b).

It is well known that a common strategy employed to modify the solubility and stability of ionizable APIs is salt selection (Gould, 1986; Patel et al., 2009). Salts can be prepared using pharmaceutically acceptable bases or acids in order to protonate the API. For non-ionizable APIs, the co-crystallization is an option to improve the solid-state properties. A co-crystal can be defined as a molecular complex composed of a neutral API and one or more different co-formers in the same crystal lattice. Preferably, the co-former will belong to the generally regarded as safe (GRAS) list delivered by the FDA and will be solid at room temperature (Kawabata et al., 2011; Childs et al., 2004; Bond, 2012; Aateka et al., 2009; GRAS S. Food and Drug Administration). Over the last few years,

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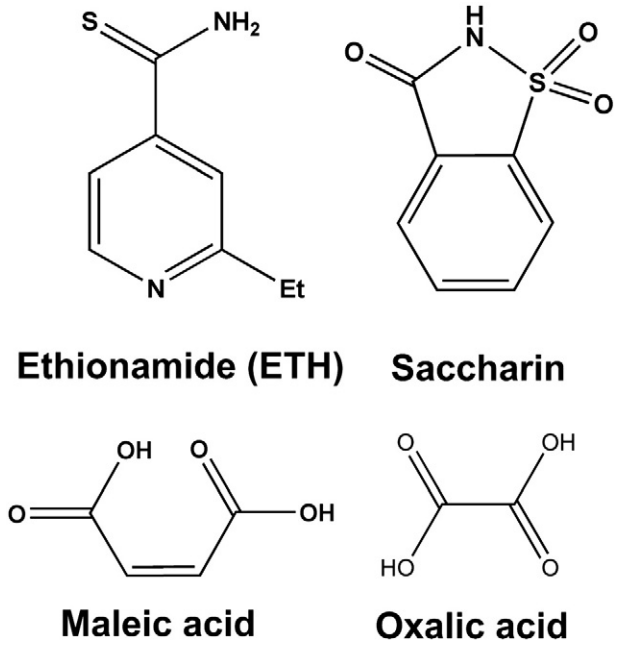
salts and co-crystals of several APIs have been designed and developed by our research group following a rational approach based on the crystal engineering principles (Da Silva et al., 2013; Martins et al., 2012; De Paula et al., 2013). Salts and co-crystals with enhanced physicochemical properties are recognized as a form of intellectual property and thus, subject to patent protection. In this sense, as part of an ongoing effort to develop new solid-state forms of ETH, in this work we report the rational synthesis and the crystal structures of three novel salts obtained from the reaction of ETH with saccharin and maleic and oxalic acids (Scheme 1). These molecules were chosen not only because of their pK_a values, but also because they are pharmaceutically accepted (Stahl and Wermuth, 2002; Haynes et al., 2005; Dighe et al., 2012). Furthermore, in the design of new solid-forms, it is important to consider the functional groups of the API and of the salt formers (or co-formers) in order to search for a specific synthon. In this case, we used the robust pyridine-carboxylic acid hetero-synthon for the designing of these salts. Initially, they were prepared by slow evaporation of the solvent, but in attempt to develop a scale-up and a green synthesis method for the production of ETH salts, we also worked on the development of production protocols involving mechanical grinding. Additionally, mechanical grinding has the advantage to use low or no solvent and to promote a reduction of the particle size, which could favor the solubility (Khadka et al., 2014; Vishweshwar et al., 2005). However, only the hydrogen maleate salt was successfully obtained by this method. Once the solubility of the counterion often drives the solubility of the resultant salt and considering that hydrogen maleate is much more soluble than oxalate and saccharinate counterions (Saal and Becker, 2013), herein we performed a detailed solid-state characterization of ETH maleate via infrared spectroscopy, thermogravimetric analysis, differential scanning calorimetry, scanning electron microscopy and equilibrium solubility at different dissolution media.

2. Experimental

2.1. Materials

ETH was purchased from Sigma-Aldrich Company, USA and used as received without any further purification. All chemicals used in this work were ACS grade reagents.



Scheme 1. Molecular structure of the ETH and the salt formers.

2.2. Preparation

ETH (10 mg, 0.06 mmol) was dissolved in 4 mL of ethanol and stirred at 40 °C for approximately 5 min. For the preparing of each salt, a stoichiometry amount of the corresponding acid (0.06 mmol of saccharin/maleic or oxalic acids) was added to the ETH solution and the resulting mixture was kept for 30 min at 40 °C, shaking constantly. Orange crystals were obtained after 2 days of slow solvent evaporation at room temperature. The ETH salts were also prepared via grinding using an oscillatory ball mill (model MM40; Retsch GmbH, Haan, Germany). An amount of 50.0 mg of ETH (0.301 mmol) and a stoichiometric quantity of the precursor acid was added to a 25 mL stainless steel jar with two 7 mm diameter stainless steel balls and milled at 25 Hz. The experiments were conducted by varying the stoichiometric ratio and the reaction time. For each experimental condition, the resulting sample was evaluated by X-ray powder diffraction technique and the results compared with the theoretical X-ray powder pattern obtained from the single-crystal structure of each salt. Only ETH maleate was successfully synthetized by this method at 1:1 M ratio and 30 min of milling time.

2.3. Single crystal x-ray diffraction

The single-crystal X-ray diffraction data of ETH maleate and ETH oxalate were collected at room temperature (293 K) on an Enraf-Nonius Kappa-CCD diffractometer using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). For ETH maleate, the crystallographic data was also obtained at low temperature (100 K). The software COLLECT (Anon., 1998) and the HKL package (Denzo, XDisplayF and Scalepack software) (Otwinowski and Minor, 1997) were applied for acquisition, indexing, integration and scaling of Bragg reflections. ETH saccharinate crystallographic data was collected at 120 K on a Bruker APEX II-DUO CCD diffractometer using Mo-Kα radiation. Unit cell determination, data collection and integration were performed with APEX II and SAINT software (Bruker, 2001). All structures were solved by direct methods and refined by full-matrix least squares on F^2 with SHELXL-2013 (Sheldrick, 2013). For refinement details, see Supplementary Information. The crystallographic software PARST95 (Nardelli, 1995), PLATON (Spek, 1990), WinGX (Farrugia, 2012), ORTEP-3 (Farrugia, 1997) and MERCURY (Macrae et al., 2008) were used to analyze and to prepare material for publication. All crystallographic information files were deposited in the Cambridge Structural Data Base under the codes CCDC 1017368 (ETH maleate at RT), CCDC 1017367 (ETH maleate at 100.0(2) K), CCDC 1408286 (ETH saccharinate) and CCDC 1407624 (ETH oxalate). Copies of these files may be solicited free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK; fax: (+44)1223-336033; e-mail: deposit@ccdc.cam.ac.uk.

2.4. Powder x-ray diffraction

PXRD analysis was performed on the samples obtained by ball milling. The sample was packed in a cavity-type sample holder and pressed to avoid preferred orientation. The X-ray powder diffraction data were collected at room temperature on a Rigaku Ultima IV diffractometer, in Bragg-Brentano reflective geometry, with CuKα radiation and Ni filter. The diffractograms were acquired in the 3–80° 2θ range with a step width of 0.02° and a constant counting time of 3 s/step.

2.5. Characterization of ETH maleate

2.5.1. Fourier transform infrared spectroscopy (FTIR)

FTIR measurements were performed on a Shimadzu IR Prestige-21 spectrophotometer (Kyoto, Japan) using KBr pellets. A blank KBr pellet was used for the background correction. The spectrum was recorded as an average of 128 scans, at a resolution of 2 cm^{-1} from 4000 to 400 cm^{-1}.