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Development of a salt drug with improved solubility: Ethionamide nitrate

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ABSTRACT

To avoid drug resistance, an adequate tuberculosis treatment should include not only a first-line drug but also at least one second-line drug such as, for example, Ethionamide (ETH). However, the dissolution rate and oral absorption of ETH is highly limited by its low aqueous solubility. Considering that a salt is in general more soluble than its parent compound, herein we depicted a new supramolecular modification of ETH, an Ethionamide nitrate salt ($ETHNO_3$). This salt is the first ETH structure that has been crystallized with four independent ionic pairs ($ETH^{+}NO_3^{-}$) in the asymmetric unit. In addition to the structural study, the salt formation was also identified on the FT-IR and FT-Raman spectra. The thermal behavior of $ETHNO_3$ was also investigated here together with its solubility profile in three dissolution media (purified water, pH 4.0 and 7.0).

1. Introduction

Salt formation is well recognized and accepted as the most effective and simple method for modifying the solubility of ionizable drugs [1–4]. For weakly basic APIs, salt formation experiments are usually conducted using inorganic acids, mainly HCl (*c.a* ~54% of pharmaceutical salts) [5–7]. However, the study of different salt formers is mandatory since the influence of a given counterion on the API's physicochemical properties is still difficult to predict. Consequently, the screening and selection of an optimal salt form with the desire physico-chemical proprieties as well as pharmacodynamics behavior has become a crucial stage on the drug discovery pathway [5–7].

Tuberculosis (TB) is one of the major causes of mortality in developing countries [8]. Its high incidence and prevalence turns TB a global health problem. The success of first-line drugs in TB treatment is low and often requires the application of second-line drugs [9]. In addition, the high incidence of TB in HIV patients has highly contributed to the emergence and spread of multidrug-resistant TB (MDR-TB). Although the second-line drugs are less effective and tolerated than the first-line ones, they are essential to avoid the growth of resistance strains and to reduce the treatment time [9]. Ethionamide (ETH, Scheme 1), 2-ethylpyridine-4-carbothioamide, is a Biopharmaceutical Classification System (BCS) class II anti-TB drug. It is the most frequently used second-line drug in the treatment of MDR-TB [10,11]. Its limited solubility [12] and unfavored bioavailability has stimulated the design of new solid forms, such as salts and cocrystals, in order to enhance it pharmaceutical performance. In our recent study [13], we synthetized and study three ETH salts: a maleate, a saccharinate and an oxalate. Following this approach, Mannava et al. [14] has been reported a series of ETH co-crystals with glutaric, adipic, suberic, sebacic and fumaric acids. Within this framework, herein we describe a novel ETH salt form with an inorganic acid, HNO_3. Besides the two halides of ETH (hydrochloride and hydrobromide salts [15,16]), Ethionamide nitrate ($ETHNO_3$) represents the third inorganic salt reported to date. In addition to the solubility studies at different dissolution media (purified water, pH 4.0 and 7.0), a solid state characterization of $ETHNO_3$ was perform using Single-Crystal X-ray Diffraction (SCXRD), Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Hot-stage Microscopy (HSM), Fourier Transform Infrared (FT-IR) and Raman (FT-Raman) spectroscopy.

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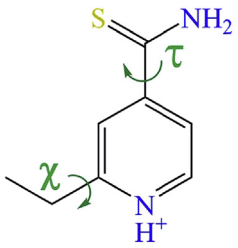

Scheme 1. Structure of Ethionamide (ETH) showing the conformational flexible substituents (thionamide and ethyl groups) and the τ and χ torsion angles.

2. Experimental

2.1. Preparation

Ethionamide was purchased from Sigma-Aldrich and used without further purification. All other chemicals, such as nitric acid and ethanol, were from analytical grade. $ETHNO_3$ was obtained by the reaction of ETH and HNO_3 acid. 20 mg of ETH was mechanically reacted with excess of HNO_3 (14 mol L^{-1}). After that the system suffer a change in its color and the resultant solid was dissolved in ethanol 95% solution and left for slow evaporation at room temperature. Red plate crystals were obtained after a few days upon solvent evaporation. X-ray powder diffraction was used to verify the purity of synthesized sample. The experimental powder XRD pattern of $ETHNO_3$ is in good agreement with the calculated indicating that the sample presents high purity (Fig. S2).

2.2. Single crystal structure determination (SCSD)

The X-ray diffraction data for the $ETHNO_3$ was collected at room temperature on an Agilent Super Nova diffractometer with CCD detector system equipped with a Mo source (λ = 0.71073 Å). The data integration, the Lorentz and polarization effect and absorption corrections were applied with CrysAlis(Pro) [17]. Using Olex2 [18], the structure was solved by direct methods and the model obtained was refined by full−matrix least squares on F^2 (SHELXTL−97 [19]). All the hydrogen atoms were placed in calculated positions and refined with fixed individual displacement parameters [$U_{iso}(H) = 1.2U_{eq}$ or $1.5U_{eq}$] according to the riding model (C−H bond lengths of 0.97 Å and 0.96 Å, for methylene and methyl groups, respectively). An analysis of the diffraction data revealed a twinned structure and disordered ethyl groups (for more details see ESI†). Molecular representations were generated by Olex2 [18] and MERCURY 3.2 [20]. The CIF file of the $ETHNO_3$ was deposit in the Cambridge Structural Data Base [21] under the code CCDC 1504368. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk.

2.3. Thermal analyses

DSC measurements were performed on a DSC-60 Schimatzu instrument with constant sample weight (2.5 ± 0.5 mg), scanning rate of 10 °C min^{-1}, under dry N_2 as the purge gas. TGA was carried out on a Shimadzu TGA-60 thermobalance. Approximately 5.0 mg of sample were placed on an alumina pan and heated under N_2 flow from 25 to 300 °C at a heating rate of 10 °C/min. All thermal analyses were performed on the temperature range of 25−250 °C.

2.4. Hot-stage polarized optical microscopy

HSM was performed on a Leica DM2500P microscope connected to a Linkam T95-PE hot-stage equipment under air atmosphere. Data were visualized with a Linksys 32 software for hot-stage control. Crystal $ETHNO_3$ was placed on a 13 mm glass coverslip within a 22 mm diameter pure silver heating block inside of the stage. The sample was heated at a ramp rate of 10 °C.min^{-1} up to a final temperature of 200 °C but discontinued on melting of all material.

2.5. Vibrational spectroscopy analysis

The infrared spectra were recorded on Alpha Bruker FT-IR spectrophotometer using KBr pellets in the range of 4000−400 cm^{-1} with an average of 64 scans and 2 cm^{-1} of spectral resolution. FT-Raman spectroscopy was performed using a Bruker RFS 100 instrument with Nd^{3+}/YAG laser operating at 1064 nm in the near-infrared and a CCD detector cooled with liquid nitrogen using a spectral resolution of 4 cm^{-1}.

2.6. Solubility measurements

Aqueous solubility of ETH and $ETHNO_3$ was determined by the flask saturation method at 25 °C in three dissolution media (purified water, pH 4.0 and 7.0) [22]. Saturated solutions of $ETHNO_3$ were prepared stirring an excess amount of this salt (100 mg) into 500 μL of each dissolution media for a period of 48 h. After 48 h of sedimentation, the solutions were filtered through a 0.45 mm filter (Millipore). The identity of the solid sediments was checked by PXRD analysis. UV−Vis spectroscopy was employed to analyze the $ETHNO_3$ supernatant concentration. Samples were diluted in their respective dissolution media before we start measuring. ETH and $ETHNO_3$ showed absorbance maxima at 282 nm in pH 4.0 and 286 nm in purified water and pH 7.0 (Figs. S5 and S6, Supplementary Information Section). ETH and $ETHNO_3$ solubility were measured by interpolating their maximum absorbance readings to a calibration curve. The calibration curve was constructed for each dissolution media studied at 25 °C using the maximum absorbance of ETH in concentrations ranging from 0.005 to 0.2 mg mL^{-1} (Table S1).

3. Result and discussion

3.1. Structure determination

Ethionamide nitrate, $ETHNO_3$, has been prepared considering the protonable pyridine nitrogen atom of ETH (pK_a: 4.49 [13]) and the possible resultant pyridinium anion synthon [23–26]. Red-plate crystals only grow up at an extremely acid crystallization medium. $ETHNO_3$ crystallizes in the $P\bar{1}$ Triclinic centrosymmetric space group (Z = 2) with four ETH^+ NO_3^- pairs per asymmetric unit (Z' = 4) (Fig. 1) [27–29]. Details of the structure determination and refinement procedures are presented in Table 1. As expected, each independent ETH^+ cation is H-bonded to a NO_3^- anion through the pyridinium ring. Pyridine protonation was confirmed by the proton location in the Fourier map and the analysis of the C4−N2−C5 angle in the pyridine ring (Table 2). This angle, which assumes the value of 118° in the neutral ETH, appears in the four independent molecules widened in the 122−124° range.

Structurally, ETH is a pyridine derivative with flexible substituents (Scheme 1), which explains the high number of ETH^+ conformers found in the ASU (Z'>1). Fig. 2 shows the overlay of the four conformers, obtained by superimposing the pyridinium rings. Notoriously, conformer A differs significantly from the other ones (see τ and χ torsion values in Table 2). Conformers B and C are practically equivalent, showing the same τ and χ values (Table 2). Conformer D in turn differs from those by the value of χ.

In the crystal packing of $ETHNO_3$, each NO_3^- anion is H-bonded