Journal of Molecular Structure 1105 (2016) 286–292

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Multinuclear NMR analysis of the antitubercular drug ethionamide

Nuno Vale [a, *], Alexandra Correia [b], Patrícia Figueiredo [a, b], Hélder A. Santos [b]

[a] UCIBIO/REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal

[b] Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Viikinkaari 5E (P.O. Box 56), FI-00014 Helsinki, Finland

ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form
30 September 2015
Accepted 19 October 2015
Available online 24 October 2015

Keywords:
Chemical reactivity
Ethionamide
^{15}N NMR
^{33}S NMR
Thioamide
Tuberculosis

ABSTRACT

Tuberculosis remains as the deadliest bacterial infection in developing countries, a situation that is particularly aggravated by the increasing spread of multidrug resistant mycobacteria (MDR-TB). In this view, not only new anti-tubercular drugs are urgently needed, but also a better understanding of the existing ones may aid in the future design of more efficient derivatives or surrogates. Ethionamide (ETA) is an anti-tubercular pro-drug used as second-line therapy against MDR-TB, being bio-activated by the mycobacterial monooxygenase EtA. ETA has been the focus of several research works, devoted either to the identification of ETA's metabolites or to the development of novel derivatives potentially useful to fight against tuberculosis. In either case, structural analysis of ETA and related structures is of undeniable relevance, while the presence of sulfur in ETA's structure brings about the possibility of including ^{33}S-NMR in the toolbox of structural analysis techniques. In this work, we have engaged into a multinuclear NMR characterization of ETA, through the study of the drug's solubility in seven deuterated solvents, and of the chemical shifts for different nuclei in ETA. Results showed which are the best conditions to study ETA by NMR and provided some important evidence on the low reactivity of the drug's thioamide group, which may be of relevance for future drug derivatization approaches.

1. Introduction

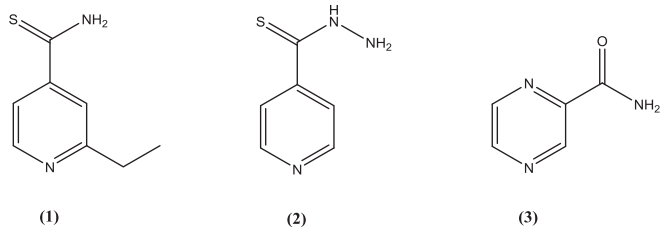
Ethionamide (ETA, 2-ethyl-4-thioamidopyridine, **1**; Scheme 1) is one of the most widely used drugs for the treatment of multidrug-resistant tuberculosis (MDR-TB). Like isoniazid (**2**; Scheme 1), and pyrazinamide (**3**; Scheme 1), ETA inhibits the mycobacterial enoyl reductase InhA, thus impairing the biosynthesis of mycolic acids, essential components of the bacterial wall [1]. ETA is actually a prodrug that needs to be activated by the mycobacterial enzyme EtaA, a FAD-containing enzyme that oxidizes ETA to the respective *S*-oxide (ETA-SO). ETA-SO is considered the main active metabolite of ETA [2–4].

The identification of active metabolites is an important aspect for the study of drugs and prodrugs, and Nuclear Magnetic Resonance (NMR) techniques have been employed towards this goal; for instance, three metabolites of ETA were identified using high-resolution magic angle spinning proton NMR (HRMAS ^{1}H NMR), whereby intra- and extracellular molecules could be distinguished [3]. HRMAS-NMR was also used to follow ETA activation directly within living mycobacterial cells or in mammalian liver [5,6]. In another example, ^{14}C-ETA (from 2-ethylpyridine and sodium ^{14}C-cyanide) has been used in a whole cell assay to study the metabolic conversions of ETA, using HPLC for identification of metabolites and ^{1}H/^{13}C NMR for characterization [1]. Given the relevance of multinuclear magnetic resonance spectroscopy (mNMR) in the study of novel compounds, and considering the chemical structure of ETA (**1**; Scheme 1), nitrogen- and sulfur-NMR may play an interesting role in the characterization of this drug, particularly of its thioamide group.

Nitrogen has two NMR-active nuclei, ^{15}N and ^{14}N, the former usually giving rise to sharp and intense peaks, whereas the latter is a medium sensitivity nucleus whose peaks are often significantly broadened by quadrupolar interactions, eventually leading to unobservable peaks even on high-resolution NMR spectrometers [7]. Still, ^{1}H and ^{13}C NMR are much more sensitive than ^{15}N NMR, which seldom is sensitive enough in a monodimensional analysis mode, making it necessary to use the projection of a heteronuclear correlation to improve sensitivity. In order to obtain a short range heteronuclear correlation, NH protons must be in slow exchange and not deuterated, which precludes the use of D_2O or deuterated alcohols as solvents [8,9].

* Corresponding author.
E-mail address: nuno.vale@fc.up.pt (N. Vale).

http://dx.doi.org/10.1016/j.molstruc.2015.10.047
0022-2860/



Scheme 1. Structures of ethionamide (**1**), isoniazid (**2**) and pyrazinamide (**3**).

Sulfur has only one NMR-active nucleus, ^{33}S, which is far from ideal for NMR observation, given the rather low natural abundance (0.76%), the nuclear spin of 3/2 and the substantial quadrupolar moment. Consequently, ^{33}S is best observed in solution only by employment of high-power NMR techniques and high-power NMR enables short 90° pulse widths, which in turn allow excitation of large spectral widths, ideal for broad lines. In addition, chemical shift differences vary linearly with magnetic field strength, but quadrupolar relation rates, and hence line widths, are independent of magnetic field. Thus, a high-power high-field (HP–HF) NMR spectrometer is ideal for the study of ^{33}S in solution [10]. In fact, HP–HF ^{33}S-NMR has been successfully employed for structural analysis of different sulfur-containing molecules, including an aromatic isothiocyanante, thiocyanate, thiophenol, alkyl aryl sulfide, thiazole, persulfate, sulfinic acid and its sulfinate salts, sulfonhydrazide, and sulfinyl halide [10–12].

The thioamide group is present in a number of biological active compounds besides ETA [1,13], which has been used as a peptide bond isostere [14], and is also a valuable building block for the synthesis of five and six-membered heterocycles [15,16]. Although thioamides are amide analogues, experimental and computational studies have shown that they differ from each other in many respects, such as the (i) longer C=S bond length, (ii) weaker hydrogen bond accepting character of thioamides, and (iii) steric influence of the large sulfur atom, which not only affects neighboring groups, but also leads to much higher rotational barriers about the N–C(S) bond, explaining why thioamides have stronger preference for planarity than amides do [17,18]. This means that the higher planarity of N–C(S) as compared to N–C(O) bonds stems from a greater contribution of the dipolar canonical structure (Fig. 1; **4a** and **4b**) in the resonance structures of the thioamide, because the C(2p)-S(3p) overlap in the thioamide bond is less effective than the C(2p)-O(2p) overlap in the amide bond [19–23].

In connection with the above, this work aimed at the application of multinuclear NMR analysis of ETA, with particular emphasis on its thioamide group, towards a better understanding of the drug's chemical reactivity.

2. Experimental

2.1. Materials

ETA was obtained from Sigma–Aldrich and used without further purification. All deuterated solvents were also purchased from Sigma–Aldrich. ETA was dissolved in 400 μL of each deuterated solvent, and the resulting solutions were flushed into a waiting 8 mm borosilicate NMR tube for analysis in a Bruker Avance III 400 spectrometer.

2.2. NMR spectra

^{13}C and ^{1}H measurements were made on a Bruker Avance III 400 spectrometer operating at 100 and 400 MHz, respectively. Carbon chemical shift data were obtained using standard WALTZ decoupling, and RINEPT + pulse sequences were used to collect coupling information. Both proton and carbon chemical shifts were referenced to internal standard tetramethylsilane (TMS). The natural abundance ^{15}N NMR measurements were made on the same spectrometer operating at 50.68 MHz. Nitromethane was used as a reference for the nitrogen chemical shift and spectral acquisition required about 20 min at a digital resolution of 0.0625 Hz; RINEPT + pulse sequences were used to obtain both chemical shift and coupling information. The resolution values for ^{13}C, ^{1}H and ^{15}N spectra were 0.898, 0.179 and 0.061 per point, respectively. ^{33}S-NMR spectrum was obtained at 38.4 MHz and the chemical shifts were measured relative to 2 M $CsSO_4$ solution in water as external reference standard. The temperature for all experiments was 298 K and the spectra were obtained using either a conventional quadrature or a quadrupole echo pulse sequence. The baseline artefacts were removed by subtracting data points from the FID as appropriate.

2.3. Fourier transmission infrared (FTIR)

ETA spectrum were studied by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The ATR-FTIR spectra of all samples were obtained using a Bruker VERTEX 70 series FTIR spectrometer (Bruker Optics, Germany) with a horizontal ATR sampling accessory (MIRacle, Pike Technology, Inc.). The ATR-FTIR spectra were recorded in the wavenumber region of 4000–650 cm^{-1} with a resolution of 4 cm^{-1} at room temperature using OPUS 5.5 software. The spectra were baseline corrected and normalized with OriginPro software (ver. 8.6).

3. Results and discussion

3.1. Influence of solvent used in the NMR characterization of ETA

ETA is very sparingly soluble in many common solvents, like water or low-molecular weight alcohols [24]. Still, we decided to include such solvents in the set of deuterated solvents used, namely, water, chloroform, methanol, acetone, acetonitrile, pyridine and dimethyl sulfoxide (DMSO), for future guidance of others on ETA's chemical shifts in different solvents, as long as concentrations reached were enough to have acceptable signal-to-noise ratios. The highest concentration of ETA reached in each of the

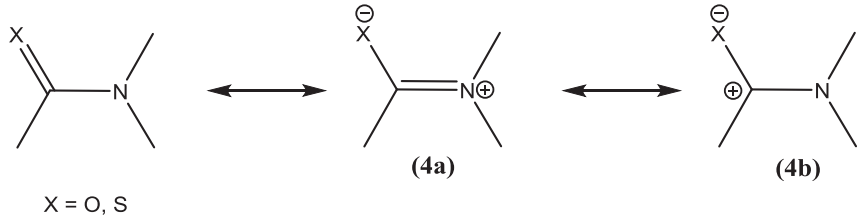


Fig. 1. Resonance structures in amide and thioamide bonds.