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IDENTIFICATION, CHARACTERIZATION AND SYNTHESIS OF POTENTIAL RELATED SUBSTANCES OF ETHIONAMIDE

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ABSTRACT

During process optimization of Ethionamide **1**, it was observed that an unknown impurity **7**, at levels 0.06 -0.25% in HPLC analysis along with known potential impurities. This new unknown impurity was isolated using preparative high performance liquid chromatography, based on the complete spectral analysis (^{1}H-NMR,^{13}C-NMR, Mass and IR) this new impurity was designated as 2-(1-(2-Ethylpyridin-4-yl)prop-1-en-2-yl)pyridine-4-carbothioamide **7**. The present work describes the formation, synthesis and characterization of the impurity.

Keywords: Ethionamide, tuberculosis, impurities, synthesis, characterization.

INTRODUCTION

Tuberculosis Bacillus (TB) is a growing global health problem because lack of proper therapeutic agents for its remedy.[1] There is another serious and alarming problem due to the resurgence of TB especially for the synergy with global human immunodeficiency virus (HIV) and the emergence of multi-drug-resistant (MDR) strains.[2] Ethionamide (2-Ethylthioisonicotinamide) is a prodrug[3] used for the treatment of Tuberculosis. Ethionamide is indicated in combination with other antituberculosis agents for the treatment of all forms of Tuberculosis caused by mycobacterium tuberculosis and also reported to cause hypothyroidism for treatment of multidrug resistant tuberculosis.[4, 5] Few of the antituberculosis drugs (such as Prothionamide) synthesis involves the basic chemistry of this Ethionamide drug synthesis (Scheme-1).

EXPERIMENTAL

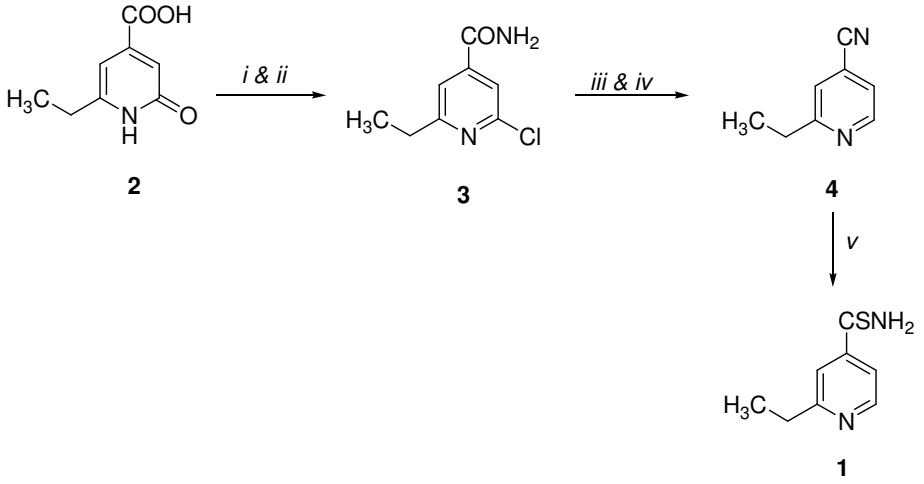
General Procedures

All melting points were determined with Polmon melting point apparatus. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 300 MHz spectrometer. Chemical shifts are reported in ppm downfield from TMS as an internal standard. Mass spectra were measured on Perkin Elmer PE SCIEX-API 2000 mass spectrometer. Elemental analyses were performed using a Heraeus CHN-O-Rapid instrument. Analytical HPLC were run with Inertsil ODS-3 (150 X 4.6) mm, 3µm, Inertsil sustain (150 X 4.6) mm, 3µm, YMC ODS.AQ (150 X 4.6) mm, and 3µm at 290 nm "RT" denotes room temperature.

2-Chloro-6-(1-(2-chloro-6-ethylpyridin-4-yl)-1-oxopropan-2-yl) isonicotinamide (5)

Compound 2 (100 g, 0.5988 mmole) was treated with phosphorous oxychloride (328 g, 2.139 mmole) and subsequent quenching of resulting acid chloride in aqueous ammonia (25%, 250 g, 7.126 mmole) resulted mixture of products Compound 3 (purity 90% by HPLC) and dimer impurity 5 (7.0-9.0% purity by HPLC) and is crystallized three times with acetone (1.0 L) to get pure Compound 3. The acetone mother liquor, enriched with dimmer impurity is subjected to purification on silica gel [Eluent: ethyl acetate/

hexane (0.2 :9.8)] affords 5 (5.0 g, 0.023 %) with purity 95% (by HPLC) mp:- 142°C; IR (KBr, cm^{-1}): 3368, 3313, 1782,1701,1053, 844, 810; ^{1}H-NMR (DMSO-d_6, δ ppm) :1.14-1.19 (t, 3H, *J*=7.5 Hz),1.45-1.47 (d, 3H, *J*= 6.9 Hz),2.73-2.80 (m, 2H, *J*=7.8 Hz), 5.14-5.21 (m, 1H, *J*=6.9 Hz),7.531- 7.535 (d, 2H, *J*=1.2 Hz), 7.582 – 7.586 (d, 1H, *J*=1.2 Hz),7.745 -7.749 (t, 1H,*J*=1.2 Hz), 7.8 & 8.3(s, 2H, NH_2 D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, ppm):13.2, 16.5, 30.1, 49.2, 119.4, 119.6, 120.4, 120.6, 145.9, 146.2, 150.1, 150.4, 161.5, 164.7, 165.5, 197.7. MS (ESI, m/z): 353.0.



Scheme-1:
Where, (i.) $POCl_3$, 95°C; (ii.) Aq. Ammonia, -5°C; (iii.)Pd/C, Methanol, 30°C; (iv.)$POCl_3$, 80°C; (v.)H_2S, Isopropanol, 25°C.

2-(-(1-(2-Ethylpyridin-4-yl)prop-1-en-2-yl)isonicotinonitrile (6)

2-Chloro-6-(1-(2-chloro-6-ethylpyridin-4-yl)-1-oxopropan-2-yl)isonicotinamide 5 (4.0 g, 0.0569 mmole) was dehalogenated by hydrogenation over palladium on charcoal (10%) (1.2 g). After removal of carbon, is concentrated and treated with phosphorous oxychloride (9.0 g, 0.0586 mmole) at reflux for 2 hrs. The resulting mass is further quenched in ice, neutralized with aqueous NaOH solution, extraction with toluene. Then, toluene extract is concentrated to get the residue, was crystallized from cyclohexane (100 mL) to get compound **6**, dried to yield (2.12 g, 60%) off white solid purity 98.0% (by HPLC)) mp:- 82°C; IR (KBr, cm^{-1}): 2939, 2912, 2237, 1685, 1543, 1053; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.22-1.27(t, 3H, *J*= 7.5 Hz), 2.32 (s, 3H), 2.7-2.8 (dd, 2H, *J*=7.5 Hz),7.23-7.26 (m, 1H, *J*=1.2 Hz),7.29 (s,1H),7.56 (s,1H),7.78-7.8 (dd,1H,),8.2 (s,1H), 8.49-8.51(d,1H, *J*=5.1 Hz),8.84-8.86 (d,1H, *J*=5.1Hz), ^{13}C-NMR (DMSO-d_6, ppm):13.9, 15.5, 30.7, 117.1, 120.3, 121.2, 122.1, 122.5, 124.2, 129.4, 138.1, 144.7, 149.2, 150.1, 159.3, 163.1 , MS (ESI, m/z) 250.

2-(1-(2-Ethylpyridin-4-yl)prop-1`-en-2-yl)pyridine-4-carbothioamide (7)

Added stoichiometric amount of hydrogen sulphide to a solution of Compound **6** in isoprapanol (1.5 g 0.006 mmole), triethanolamine (0.2 g, 0.0012 mmole) gives product and is further refluxed in hexane, cooled to 25-30°C filtered and dried at 55-60°C Yield (1.65 g, 90%) as yellow solid. Purity 99.2% (by HPLC mp-170°C; IR (KBr, cm^{-1}): 3661, 3190, 1600, 1546, 1080; ^{1}H-NMR (DMSO-d_6, δ ppm): 1.22-1.27(t, 3H, *J*= 7.8 Hz) 2.34(s, 3H), 2.74-2.81(m,2H, *J*=7.5 Hz), 7.25-7.26 (d,1H, *J*= 5.1 Hz), 7.3 (s,1H), 7.48 (s, 1H),7.67-7.68 (d,1H, *J*=5.1 Hz), 8.0(s, 1H), 8.49-8.50(d, 1H, *J*=5.1 Hz),8.67-8.68(d,1H, *J*= 4.8Hz), 9.85 & 10.25 (s, 2H –NH_2 proton D_2O exchangeable);^{13}C-NMR (DMSO-d_6, ppm): 13.7, 15.6, 30.6, 117.04, 120.2, 121.0, 121.9, 127.8, 139.1, 144.9,147.1, 148.9,149.0, 158.4, 162.9, 198.3; MS (ESI m/z) 284 $[M+H]^+$ Analysis Calcd. for $C_{16}H_{17}N_3S$ (283.0); C, 67.81; H, 6.05; N, 14.83; S, 11.31. Found: C, 67.80; H, 6.08; N, 14.85; S, 11.26.

RESULTS AND DISCUSSION

During process optimization of Ethionamide **1**, observed an unknown impurity in the range from 0.06% to 0.25 %. Further, International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) guideline indicates that unknown impurities at or above 0.05% in the drug substance require identification depending on the maximum daily dosage.[6, 7] (**Figure 1**). In order to meet the stringent regulatory requirements the impurity needs to be identified and characterized.[8] Hence **1** was initially analyzed by LCMS (**Figure 2**) to provide parent ion at m/z 284 of unknown compound along with m/z 167 of Ethionamide **1** and thus provide a basis for initial identification. Then, a comprehensive study had been carried out to identify the origin of this contaminant.

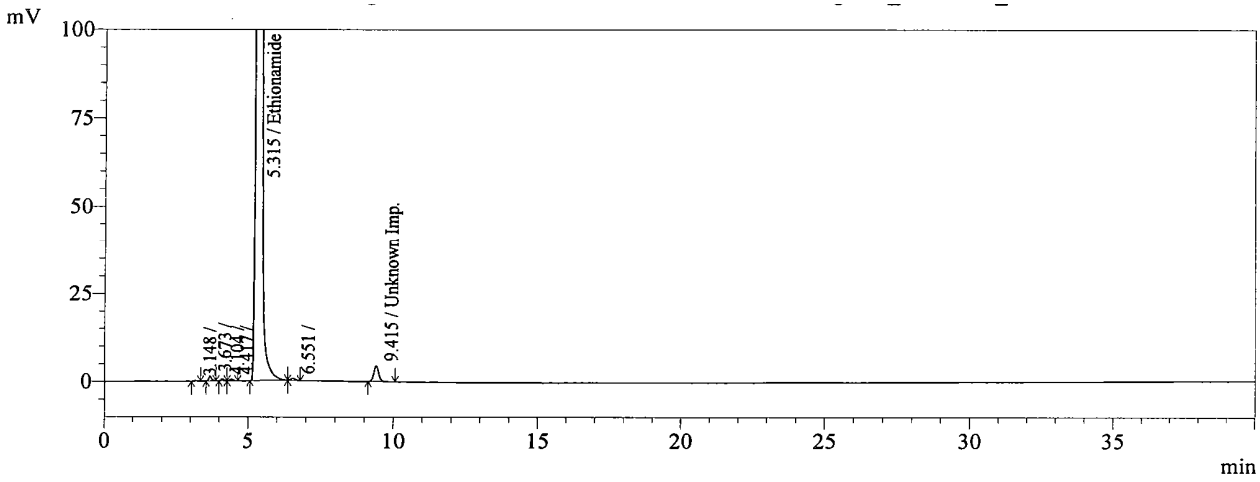


Fig.-1: HPLC Chromatogram of Ethionamide

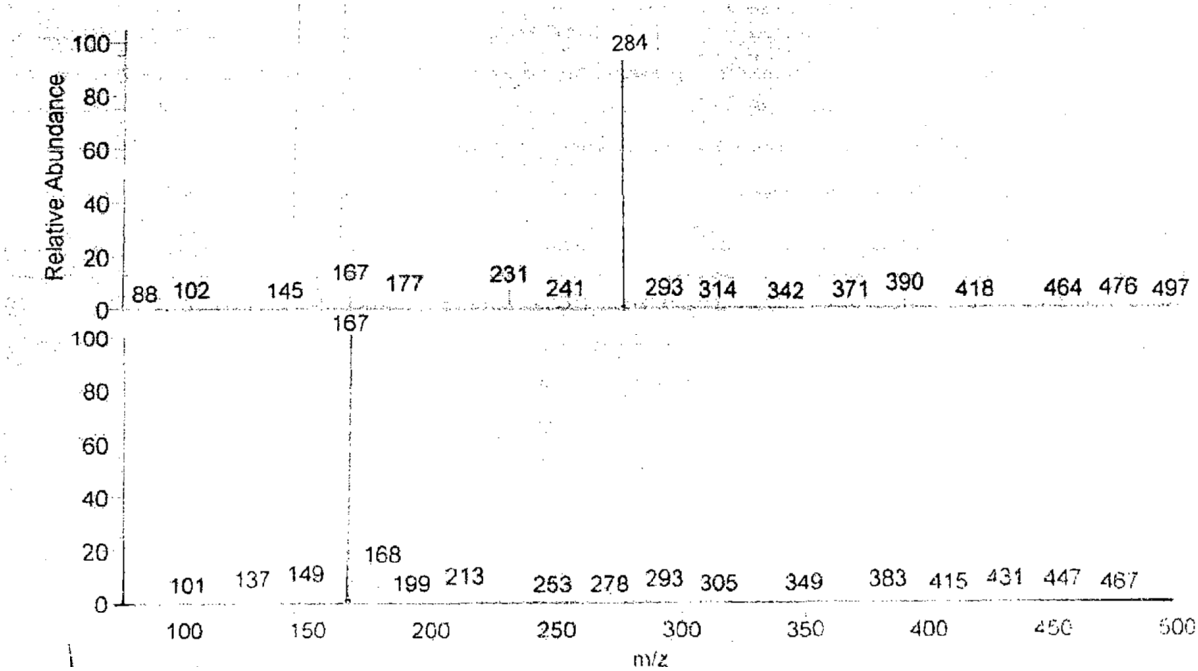
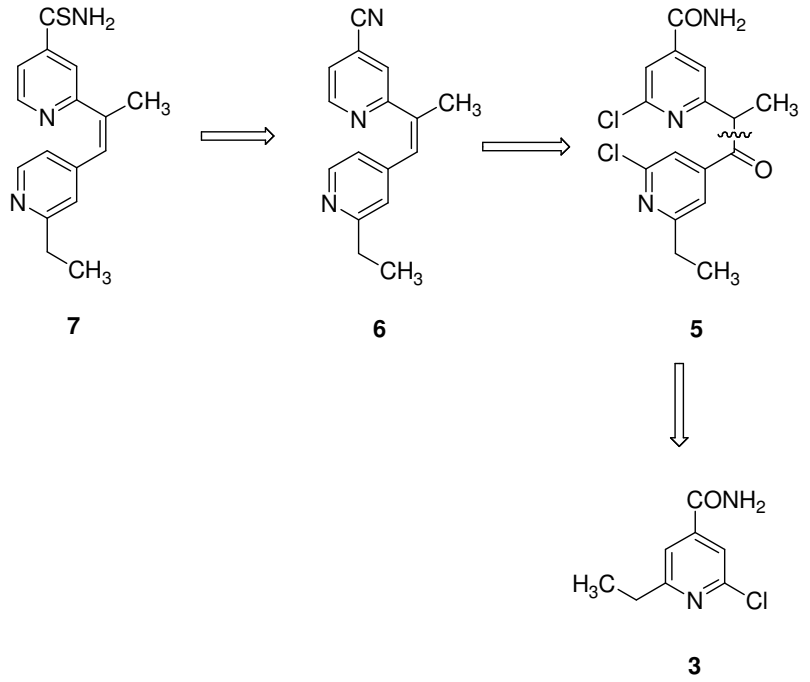
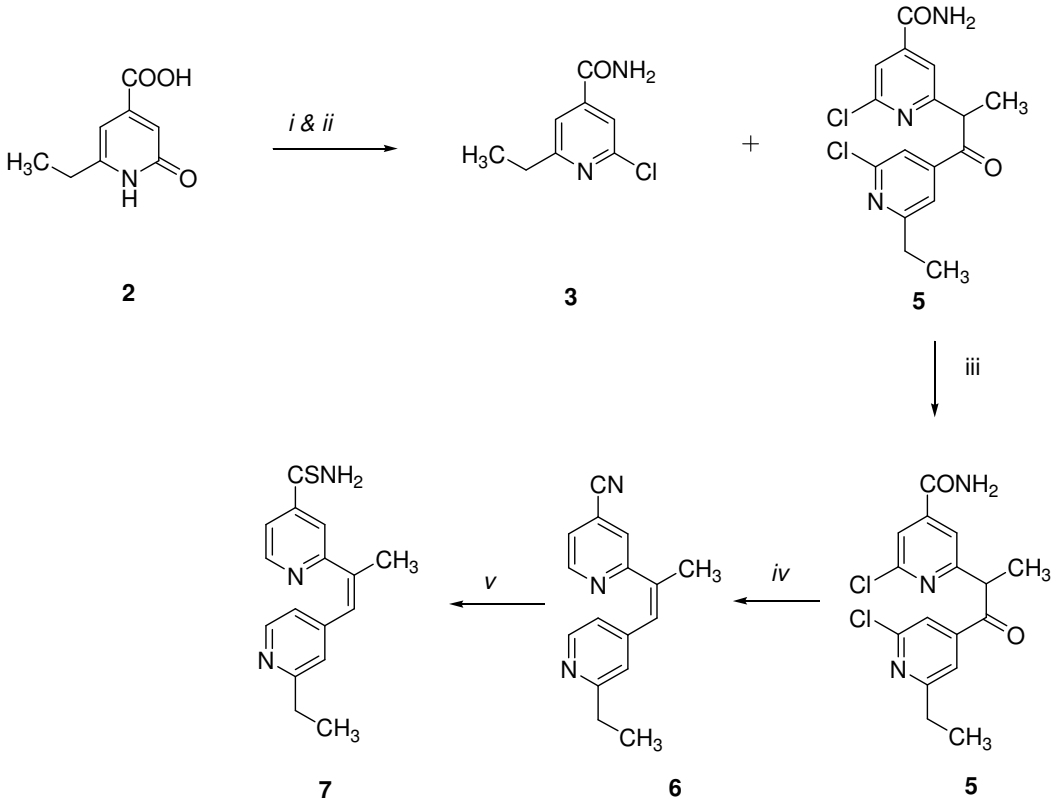


Fig.-2: LC-MS Spectrum of Ethionamide and its unknown impurity.



Scheme-2: Retro synthesis analysis of compound **7**



Scheme-3: (i.) $POCl_3$,95°C; (ii.) Aq. Ammonia, -5°C; (iii.) Acetone; (iv.) Pd/C, Methanol 30°C, $POCl_3$, 80°C; (v.) H_2S, Isoprapanol, 25°C

Along with the other listed impurities of Ethionamide (USP 37, EP 8.1), an unknown impurity formed in the process was identified as dimer impurity**7**. Dimer impurity formation is very common in drug substances and most of the times elimination of this impurity was very difficult from the drug substances because of no solubility difference. Therefore it was needed to prepare this compound in pure form by identifying the origin.

Our retro synthetic analysis for compound **7**, is shown in Scheme-2. 2-(1-(2-Ethylpyridin-4-yl) prop-1-en-2-yl) isonicotino-nitrile **6** is the precursor of **7** Isonicotinonitrile **6** might be obtained from compound **5**. 2-Chloro-6-ethylisonicotinamide **3** which could be prepared from 4-carboxy-6-ethyl-2-pyridone **2**. It observed that dimer impurity formation during preparation of 2-Chloro-6-ethylisonicotinamide **3**.

Compound **2** is treated with phosphorous oxychloride and subsequent quenching of the resulting acid chloride in ammonia solution yields 2-Chloro-6-ethylisonicotinamide **3**, along with formation of 2-Chloro-6-(1-(2-chloro-6-ethylpyridin-4-yl)-1-oxopropan-2-yl)isonicotinamide (dimer compound) **5** which is separated from acetone mother liquor followed by column chromatography. Further, dehydration and dehalogenation by hydrogenating over palladium on charcoal followed by phosphorous oxychloride reaction to give Compound **6**. Addition of stoichiometric amount of hydrogen sulphide to a solution of Isonicotinonitrile **6** in isopropanol give Compound **7** (Scheme-3).

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REFERENCES

1. (a) I. Bastian, R. Colebunders, *Drugs.* **58 (4)**, 633(1999**)** (b) D. Butler, *Nature.*, **406**, 670 (2000).
2. A. Gazdic, *Med. Arch.,* 52, 207(1998).
3. T. A. Vannelli, A. Dykman, P.R. Ortiz de Montellano, J. *Biol. Chem,* 277 **(15)**, 12824(2002).
4. D. Drucker, M. C. Eggo, I. E. Salit, G. N. Burrow, *Ann. Intern. Med.***, 100 (6)**, 837(1984).
5. Centre for Disease Control and Prevention (CDC). Treatment of tuberculosis. *Morb. Mortal. Wkly Rep.,* **52**, 26(2003).
6. International Conference on Harmonization guidelines Q3A (R) Impurities in New Drug Substances February 2002
7. International Conference on Harmonization guidelines Q3B (R) Impurities in New Drug Substances February 2002
8. P.O Kane, *Nature.* 195, 495(1962).
9. M. H. Cynamon, M. Sklaney, *Antimicrob Agents Chemother.,* **47 (8)**, 2442(2003).

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