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Synthesis and characterization of process related impurities of an anti-tuberculosis drug-Prothionamide

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ABSTRACT

*The synthesis of **7** contaminants of prothionamide **4a** formed during the preparation of prothionamide bulk drug, is described. The products are 2,6–dipropylpyridine-4-carbothioamide **4b**, 3-propylpyridine-4-carbothioamide **4c**, 2-ethylpyridine-4-carbothioamide **4d**, 2-propylisonicotinamide **6,** pyridine-4-carbothioamide **4e,** 2-propylisonicotinicacid **5,** prothionamide sulfoxide **7.** Unknown Compounds of **4c** and **5-7** were isolated and characterized for the first time. The side products formed during the synthesis of prothionamide intermediate 2-propyl-4-cyanopyridine **3a** were also synthesized and characterized as 2,6-dipropyl-4-cyanopyridine **3b**, 3-propyl-4-cyanopyridine **3c**, 2-ethyl-4-cyanopyridine **3d**. The structures of all these compounds were established on the basis of spectral data (IR, 1H-NMR and MS).*

Keywords: Prothionamide, impurities, bulk drug, synthesis, contaminants.

INTRODUCTION

Tuberculosis [1,2] infections were treated initially with a cocktail of drugs to prevent the development of drug resistance. The first line drugs normally employed in this cocktail for drug-sensitive tuberculosis are isoniazid (1952), ethambutol (1961), pyrazinamide (1952) and rifamcin (1966). Second line drugs ethionamide, prothionamide [3,4], thiacetazone and isoxyl come into play when the infection is unresponsive to treatment with first line drugs, usually as a result of the development of resistance to one or more of the agents.

Prothionamide **4a**, chemically known as 2-propyl-4-pyridinecarbothioamide, is a second line drug, for the treatment of tuberculosis. Several methods are reported in the literature for the preparation of prothionamide [5-8], but the most preferable and convenient route for synthesis of prothionamide is preparation of 2-propyl-4-cyanpyridine **3a** intermediate starting from 4-cyano pyridine by alkylation (Scheme 1) followed by thionation of cyano group (Scheme 2). However, synthesis of related compounds was not yet discussed. International Conference on Harmonization (ICH) has published guidelines on impurities in new drug substances [9], product [10] and residual solvents [11]. The preparation and characterization data of these related substances has been necessary for the preparation of reference compounds for the quality assurance of bulk drugs and drug formulations.

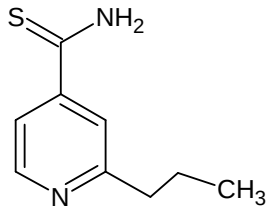


Fig. 1. Structure of Prothionamide 4a

MATERIALS AND METHODS

Experimental. All reagents and solvents employed were of commercial grade and were used as such, unless otherwise specified. All melting points were determined with Polmon melting point apparatus. 1H-NMR and 13C-NMR spectra were recorded on a Bruker 300 spectrometer. Chemical shifts are reported in ppm downfield from TMS as internal standard. Mass spectra were measured on Thermo Finningan LCQ mass spectrometer. Infrared spectra were recorded on a Shimadzu spectrometer. Elemental analyses were performed using a Heraeus CHN-O-Rapid instrument.

General procedure for alkylation of 4-cyanopyridine (1)

2-propyl-4-cyanopyridine (3a). Aq. ammonium persulphate solution (10 % w/w, 228 g, 990 mmol) was added drop wise to a mixture of 4-cyanopyridine 1 (100 g, 960 mmol), $AgNO_3$ (4 g, 23 mmol), n-butyric acid (90 g, 1020 mmol) and nitric acid (70% w/w, 43 g, 470 mmol) in water (300 mL) at 90-100°C over a period of 30 min. The reaction mass after 30 min, was neutralized with aqueous ammonia at 25-30°C and extracted with cyclohexane, concentrated. Oxalic acid dihydrate (145 g, 1150 mmol) was added to the above residue in acetone (600 mL). The resulting 4-cyanopyridine oxalate salt was removed by filtration. The obtained acetone filtrate was concentrated, diluted with water (400 mL), neutralized with aq. ammonia. The product was extracted with cyclohexane (400 mL) and concentrated. This crude material was further purified by fractional distillation (140-160°C under reduced pressure) to get **3a** (45 g, 32%) as a colourless liquid; purity 97% (by HPLC); IR (KBr, cm^{-1}) 2962, 2874, 2230, 1674, 1550, 1419; ^{1}H-NMR (300 MHz, DMSO) δ 0.90 (t, 3H, *J* 7.2 Hz), 1.70 (m, 2H), 2.76 (t, 2H, *J* 7.5 Hz), 7.65 (d, 1H, *J* 4.8 Hz), 7.73 (s, 1H), 8.73 (d, 1H, *J* 5.1 Hz); ^{13}C-NMR (75 MHz, DMSO) δ 13.6, 22.1, 39.3, 117.1, 119.6, 122.9, 124.6, 150.3, 163.3; MS (ESI, *m/z*): 147.0 $[M+H]^+$. Anal. Calcd. For $C_9H_{10}N_2$ (146.19): C, 73.94; H, 6.89; N, 19.16. Found: C, 73.92; H, 6.90; N, 19.17.

2,6-Dipropyl-4-cyanopyridine (3b)

This compound was prepared in a similar way to **3a**, using 4-cyanopyridine **1** (100 g, 960 mmol) and excess of n-butyric acid (127 g, 1440 mmol). But, purified by silica gel column chromatography (hexane: ethyl acetate) to get compound **3b** (12 g, 6.6 %) as a colourless liquid; purity 96% (by HPLC); IR (KBr, cm^{-1}) 2931, 2237, 1597, 1558, 1411; ^{1}H-NMR (300 MHz, DMSO) δ 0.90 (t, 6H, *J* 7.5 Hz), 1.69 (m, 4H), 2.72 (t, 4H, *J* 7.5 Hz), 7.52 (s, 2H); ^{13}C-NMR (75 MHz, DMSO) δ 13.6, 22.2, 38.8, 117.4, 119.7, 121.7, 162.8; MS (ESI, *m/z*): 189.0 $[M+H]^+$. Anal. Calcd. For $C_{12}H_{16}N_2$ (188.13): C, 76.55; H, 8.57; N, 14.88. Found: C, 76.54; H, 8.59; N, 14.88.

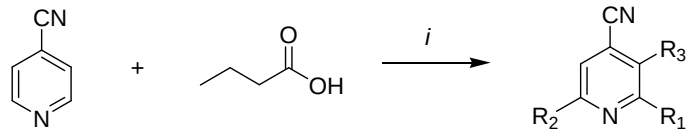
3-Propyl-4-cyanopyridine (3c)

This compound was prepared in a similar way to **3a**, using 4-cyanopyridine **1** (100 g, 960 mmol) and n-butyric acid (90 g, 1020 mmol). However, the crude material, before fractional distillation is again treated with oxalic acid (20 g, 160 mmol) in water, filtered the solid. The oxalate salt was neutralized with aqueous ammonia at room temperature, extract with cyclohexane (100 mL), concentrated to get **3c** (6 g, 4.3%) as a colourless liquid; purity 94% (by HPLC); IR (KBr, cm^{-1}) 2962, 2229, 1589, 1550, 1411; ^{1}H-NMR (300 MHz, DMSO) δ 0.92 (t, 1H, *J* 7.5 Hz), 1.64 (m, 2H), 2.77 (t, 2H, *J* 7.5 Hz), 7.8 (d, 1H, *J* 5.1 Hz), 8.65 (d, 1H, *J* 4.2 Hz), 8.76 (s, 1H); ^{13}C-NMR (75 MHz, DMSO) δ 13.3, 23.3, 32.8, 115.8, 119.1, 125.5, 139.0, 148.0, 151.0; MS (ESI, *m/z*): 147.0 $[M+H]^+$. Anal. Calcd. For $C_9H_{10}N_2$ (146.19): C, 73.94; H, 6.89; N, 19.16. Found: C, 73.91; H, 6.91; N, 19.17.

2-Ethyl-4-cyanopyridine (3d)

This compound was prepared in a similar way to **3a**, using 4-cyanopyridine **1** (100 g, 960 mmol) and n-propionic acid (85 g, 1150 mmol). Obtained crude was fractionally distilled at 105-115°C under reduced pressure to get **3d** (48 g, 38%) as a colourless liquid; purity 97% (by HPLC); IR (KBr, cm^{-1}) 2928, 2237, 1593, 1550, 1462; ^{1}H-NMR (300

MHz, DMSO) δ 1.23 (t, 3H, *J* 7.5 Hz), 2.8 (m, 2H), 7.65 (d, 1H, *J* 5.1 Hz), 7.75 (s, 1H), 8.73 (d, 1H, *J* 5.1 Hz); ^{13}C-NMR (75 MHz, DMSO) δ 13.4, 30.5, 117.1, 119.7, 122.9, 124.1, 150.3, 164.5;MS (ESI, *m/z*): 133 $[M+H]^+$. Anal. Calcd. For $C_8H_8N_2$ (132.16): C, 72.70; H, 6.10; N, 21.20. Found: C, 72.72; H, 6.08; N, 21.20.



1 **2**

3a *R_1=$CH_2CH_2CH_3$; R_2=H; R_3=H*

3b *R_1 & R_2=$CH_2CH_2CH_3$; R_3=H*

3c *R_1 & R_2=H; R_3=$CH_2CH_2CH_3$*

3d *R_1=CH_2CH_3; R_2=H; R_3=H*

1 *R1& R2 & R3 = H*

Scheme 1. *i.* $(NH_4)_2S_2O_8$, $AgNO_3$ and HNO_3, 90-100°C

General procedure for thionation of alkyl-4-cyanopyridine (3a-d)

Add 2-alkyl-4-cyanopyridine to a solution of Na_2S and S in water at 25-30°C. After 2 hrs adjusted the aqueous mass pH to 8.5-9.0 with con. HCl. The resulting solid was filtered and dried.

2-Propylpyridine-4-carbothioamide (4a)

This compound was prepared in a similar way of above general procedure for thionation, using 2-propyl-4-cyanopyridine **3a** (100 g, 684 mmol) , Na_2S (181 g, 950 mmol) and S (7.2 g, 225 mmol) in water (300 mL). The obtained wet solid was purified with acetonitrile (400 mL) followed by crystallisation from methanol (800 mL) to get pure **4a** (60 g, 49%)) as a yellow crystals; purity 99.6% (by HPLC); mp 140-142°C, *lit.*[5] 142°C, *lit.*[12] 142-145°C; IR (KBr, cm^{-1}) 3267, 2962, 2873, 1674, 1593, 1550, 1458, 1419, 1392; ^{1}H-NMR (300 MHz, DMSO) δ 0.90 (t, 3H, *J* 7.5 Hz), 1.71 (m, 2H), 2.74 (t, 2H, *J* 7.5 Hz), 7.52 (d, 1H, *J* 5.4 Hz), 7.55 (s, 1H), 8.54 (d, 1H, *J* 5.1 Hz), 9.72 (s, 1H), 10.15 (s, 1H); ^{13}C-NMR (75 MHz, DMSO) δ 13.8, 22.4, 39.6, 118.5, 119.8, 146.8, 149.3, 162.1, 198.8; MS (ESI, *m/z*): 181 $[M+H]^+$. Anal. Calcd. For $C_9H_{12}N_2S$ (180.27): C, 59.96; H, 6.71; N, 15.54; S, 17.79. Found: C, 59.95; H, 6.72; N, 15.52; S, 17.78.

2,6-Dipropylpyridine-4-carbothioamide (4b)

This compound was prepared in a similar way of general procedure for thionation, using **3b** (10 g, 52 mmol), Na_2S (14 g, 58 mmol) and sulphur (0.72 g, 22 mmol) and water , to get **4b** (6 g, 27 mmol) as a yellow powder; purity 99.6% (by HPLC); mp 115-117 °C; IR (KBr, cm^{-1}) 3267, 2962, 2873, 1674, 1593, 1550, 1458, 1419, 1392; ^{1}H-NMR (300 MHz, DMSO) δ 0.90 (t, 6H, *J* 7.2 Hz), 1.69 (m, 4H), 2.69 (t, 4H, *J* 7.2 Hz), 7.35 (s, 2H), 9.66 (s, 1H), 10.08 (s, 1H); ^{13}C-NMR (75 MHz, DMSO) δ 13.6, 22.3, 38.6, 117.0, 147.0, 161.7, 199.0; MS (ESI, m/z): 223.0 $[M+H]^+$. Anal. Calcd. For $C_{12}H_{18}N_2S$ (222.35): C, 64.82; H, 8.16; N, 12.16; S, 14.42. Found: C, 64.80; H, 8.15; N, 12.18; S, 14.43.

3-Propylpyridine-4-carbothioamide (4c)

This compound was prepared in a similar way of general procedure for thionation, using **3c** (10 g, 68 mmol), Na_2S (18 g, 75 mmol) and S (0.66 g, 20 mmol), to get **4c** (6 g, 48.7%) as a yellow powder; purity 99.6% (by HPLC); mp 142-144 °C; IR (KBr, cm^{-1}) 3267, 2962, 2873, 1674, 1593, 1550, 1458, 1419, 1392; ^{1}H-NMR (300 MHz, DMSO) δ 0.89 (t, 3H, *J* 7.2 Hz), 1.63 (m, 2H), 2.66 (t, 2H, *J* 7.5 Hz), 7.12 (d, 1H, *J* 4.8 Hz), 8.39 (d, 1H, *J* 5.1 Hz), 8.43 (s, 1H), 9.72 (s, 1H), 10.18 (s, 1H); ^{13}C-NMR (75 MHz, DMSO) δ 14.1, 23.6, 31.7, 120.2, 131.4, 147.1, 150.1, 150.7, 200.7; MS (ESI, *m/z*): 181 $[M+H]^+$. Anal. Calcd. For $C_9H_{12}N_2S$ (180.27): C, 59.96; H, 6.71; N, 15.54; S, 17.79. Found: C, 59.94; H, 6.72; N, 15.53; S, 17.78.

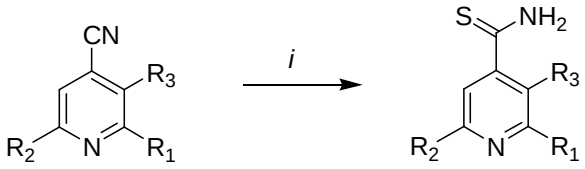
2-Ethylpyridine-4-carbothioamide (4d)

This compound was prepared in a similar way of general procedure for thionation, using **3d** (100 g, 757 mmol), Na_2S (200 g, 830 mmol) and S (7.3 g, 230 mmol). The obtained wet solid was purified with acetonitrile (400 mL)

followed by crystallisation from methanol (800 mL) to get **4d** (60 g, 48%) as a yellow crystals; purity 99.6% (by HPLC); mp 160-162°C, *lit.*[5] 166°C, *lit.*[13] 158-164°C; IR (KBr, cm^{-1} 3267, 2357, 1662, 1593, 1419; ^{1}H-NMR (300 MHz, DMSO) δ 1.23 (t, 3H, *J* 7.5 Hz), 2.8 (m, 2H), 7.46 (dd, 1H, *J* 1.5, 5.4 Hz), 7.56 (s, 1H), 8.54 (d, 1H, *J* 5.1 Hz), 9.72 (s, 1H), 10.15 (s, 1H); ^{13}C-NMR (75 MHz, DMSO) δ 13.8, 30.77, 118.5, 119.1, 146.8, 149.3, 162.1, 198.8; MS (ESI, *m/z*): 167 $[M+H]^+$. Anal. Calcd. For $C_8H_{10}N_2S$ (166.24): C, 57.80; H, 6.06; N, 16.85; S, 19.29. Found: C, 57.82; H, 6.04; N, 16.84, S, 19.27.

Pyridine-4-carbothioamide (4e)

This compound was prepared in a similar way of general procedure for thionation, using **1** (10 g, 960 mmol), Na_2S (25.4 g, 105 mmol) and S (0.98 g, 31 mmol), to get pyridine-4-carbothioamide **4e** (10 g, 76%) as a yellow crystals; purity 99.8% (by HPLC); mp 194-196°C; IR (KBr, cm^{-1}) 2337, 1674, 1427, 1149; ^{1}H-NMR (300 MHz, DMSO) δ 7.71 (d, 2H, *J* 6.3 Hz), 8.65 (d, 2H, *J* 6.0 Hz), 9.78-10.2 (s, 2H); ^{13}C-NMR (75 MHz, DMSO) δ 121.0, 146.4, 149.9, 198.5; MS (ESI, *m/z*): 139.0 $[M+H]^+$. Anal. Calcd. For $C_6H_6N_2S$ (138.19): C, 52.15; H, 4.38; N, 20.27; S, 23.20. Found: C, 52.14; H, 4.37; N, 20.28; S, 23.21.



3a *R_1=$CH_2CH_2CH_3$; R_2=H; R_3=H* ***4a*** *R_1=$CH_2CH_2CH_3$; R_2=H; R_3=H*

3b *R_1 & R_2=$CH_2CH_2CH_3$; R_3=H* ***4b*** *R_1 & R_2=$CH_2CH_2CH_3$; R_3=H*

3c *R_1 & R_2=H; R_3=$CH_2CH_2CH_3$* ***4c*** *R_1 & R_2=H; R_3=$CH_2CH_2CH_3$*

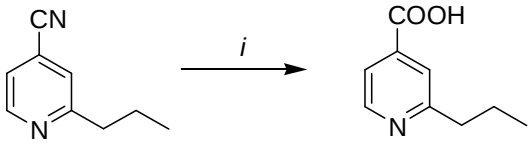
3d *R_1=CH_2CH_3; R_2=H; R_3=H* ***4d*** *R_1=CH_2CH_3; R_2=H; R_3=H*

1 *R1& R2 & R3 = H* ***4e*** *R1& R2 & R3 = H*

Scheme 2. *i*. Na_2S, S, H_2O, RT

2-Propylisonicotinicacid (5)

Mixture of 2-propyl-4-cyanopyridine **3a** (10 g, 68 mmol), NaOH (13.7 g, 340 mmol) in water (100 mL) was heated for 4 hr at 95-100°C. After completion of reaction, the reaction mass was adjusted to pH 3.0-3.5 with conc. HCl, filtered the solid, to get **5** (5 g, 44%) as a off white solid; purity 99.2% (by HPLC); mp 194-197°C; IR (KBr, cm^{-1}) 2964, 2387, 1728, 1568, 1464; ^{1}H-NMR (300 MHz, DMSO) δ 0.89 (t, 1H, *J* 7.2 Hz), 1.71 (m, 2H), 2.78 (t, 2H, *J* 7.8 Hz), 7.6 (dd, 1H, *J* 1.5, 5.1 Hz), 7.6 (s, 1H), 8.6 (d, 1H, *J* 5.1 Hz), 13.5 (s, 1H); ^{13}C-NMR (75 MHz, DMSO) δ 13.8, 22.6, 38.8, 120.4, 121.9, 138.6, 150.3, 163.1, 166.6; MS (ESI, *m/z*): 166 $[M+H]^+$. Anal. Calcd. For $C_9H_{11}NO_2$ (165.19): C, 65.44; H, 6.71; N, 8.48; O, 19.37. Found: C, 65.42; H, 6.69; N, 8.47; O, 19.38.

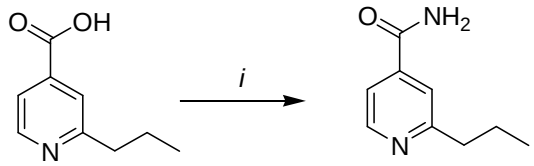


3a **5**

Scheme 3. *i*. aq. NaOH, 80-85°C

2-Propylisonicotinamide (6)

Mixture of 2-propylisonicotinicacid **5** (10 g, 60 mmol) and phosphoryl chloride (18.6 g, 120 mmol) heated for 2 hr at 95-100°C. After completion of reaction, quenched the mass carefully in precooled ammonia (45 mL) at 5-10°C, filtered and washed with water to yield **6** (6 g, 60%) as a off white solid; purity 99.2% (by HPLC); mp 125-130°C; IR (KBr, cm^{-1}) 3346, 2359, 1672, 1550, 1419; ^{1}H-NMR (300 MHz, DMSO) δ 0.92 (t, 3H, *J* 7.2 Hz), 1.71 (m, 2H), 2.75 (t, 2H, *J* 7.2 Hz), 7.3 (s, 1H), 7.57 (d, 1H, *J* 5.1 Hz), 7.63 (s, 1H), 8.19 (s, 1H), 8.57(d, 1H, *J* 5.1 Hz); ^{13}C-NMR (75 MHz, DMSO) δ 13.8, 22.4, 39.7, 119.0, 120.5, 141.7, 149.7, 162.5, 166.8; MS (ESI, *m/z*): 165 $[M+H]^+$. Anal. Calcd. For $C_9H_{11}NO_2$ (164.20): C, 65.44; H, 6.71; N, 8.48; O, 19.37. Found: C, 65.42; H, 6.70; N, 8.49; O,19.39.

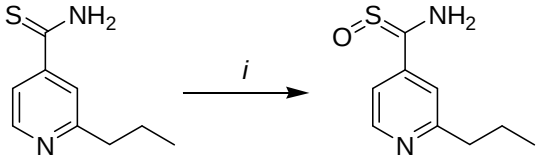


5 **6**

Scheme 4. *i*. $POCl_3$, aq.NH_3, 95-100°C

Prothionamide sulfoxide (7)

Compound **4a** (10 g, 55 mmol) and $KMnO_4$ (17.6 g, 110 mmol) dissolved in water (80 mL) mixture was stirred for 4 hr at 25-30°C, extract the product with dichloromethane (100 mL), concentrated, isolated with hexane(50 mL) to yield **7** (7 g, 74%) as a yellow solid; mp 112-115°C; IR (KBr, cm^{-1}) 3078, 1647, 1600, 1558, 1419; ^{1}H-NMR (300 MHz, DMSO) δ 0.89 (t, 3H, *J* 7.5 Hz), 1.70 (m, 2H), 2.72 (t, 2H, *J* 7.5 Hz), 7.30 (d, 1H, *J* 4.8 Hz), 8.55 (d, 1H, *J* 5.1 Hz), 7.37 (s, 1H), 8.49 (s, 1H), 9.39 (s, 1H); ^{13}C-NMR (75 MHz, DMSO) δ 13.8, 22.3, 38.8, 116.2, 117.5, 136.4, 150.1, 163.0, 189.6; MS (ESI, *m/z*): 197 $[M+H]^+$. Anal. Calcd. For $C_9H_{12}N_2OS$ (196.27): C, 55.08; H, 6.16; N, 14.27; O, 18.15; S, 16.34. Found: C, 55.06; H, 6.15; N, 15.30; O, 18.16; S, 16.33.



4a **7**

Scheme 5. *i*. $KMnO_4$, H_2O, RT

RESULTS AND DISCUSSION

It was observed during the process development of **4a** that some of the batches were contaminated with the unknown impurities **4c & 5-7** along with the known impurities of **4b, 4d, 4e** in the range from 0.1% to 0.5%. These impurities were confirmed and synthesized after identification by HPLC and detection of mass by LC-MS. It was also observed during the alkylation of 4-cyanopyridine **1**, undesired alkylated compounds **3b-3d** were formed due to free radical reactions. In the subsequent reaction of thionation of cyano group, these alkylated compounds results the formation of **4b-4e** along with the degradation products **5-7**. Therefore it is necessary to synthesize these impurities **4b-4e** and **5-7** in pure form for the analytical method validation of prothionamide bulk drug. Then, a comprehensive study has been carried out to prepare these contaminants.

2,6-Dipropyl-4-cyanopyridine **3b** was prepared using excess of alkylating reagent butyric acid (Scheme 1), and it was further purified with silica gel column chromatography to get pure **3b**. This, on further treatment with sodium sulphide and sulphur in water result in the formation of 2,6-dipropylpyridine-4-carbothioamide **4b.** 3-Propyl -4-cyanopyridine **3c** also formed (~10%) during free radical reaction of **1**. The resulting crude (mixture of compounds) is treated with oxalic acid salt in water. The product is extracted with solvent after neutralizing the oxalic acid. This compound on further treatment with Na_2S and S in water resulted the compound **4c.** 2-Ethyl-4-cyanopyridine **3d** was observed during the preparation of **3a** due to propyl radical formed from butyric acid. It was prepared by the reaction of 4-cyanopyridine **1** with n-propionic acid. This, on further treatment with Na_2S and S in water results the formation of **4d**.

Pyridine-4-carbothioamide **4e** is formed due to the thionation of unreacted 4-cyanopyridine present in the 2-propyl-4-cyanopyridine **3a**. It was prepared in the pure form by treating **1** with Na_2S and S in water.

It was observed that during the thionation of cyano group with aqueous Na_2S, compound **5** was formed in the range from 0.2% to 0.5% due to hydrolysis. Compound **5** was prepared by treating **3a** with aqueous NaOH (Scheme 3). The compound **6** (~ 1.0% to 2.0%) is observed during the thioamide formation of cyano group with Na_2S in aqueous media due to partial hydrolysis. Compound **6** was prepared by converting compound **5** with acid chloride then to

amide (Scheme 4). Prothionamide gets oxidized with oxygen to form prothionamide sulfoxide **7**. It was prepared by oxidation of prothionamide **4a** with aqueous potassium permanganate (Scheme 5).

CONCLUSION

In conclusion, the process related impurities formed during the synthetic study of the manufacturing process of prothionamide, were identified and characterized, and their structures were confirmed by chemical synthesis. Also, the spectral data (IR, 1H-NMR and MS) of these compounds was described.

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REFERENCES

[1] T. T. Fajardo, R. S. Guinto, R. V. Cellona, R. M. Abalos, E. C. Dela Cruz, R. H. Gelber, *Am. J. Trop. Med. Hyg.*, **2006,** 74 (3), 457.
[2] A. Koul, E. Arnoult, N. Lounis, J. Guillemont, K. Andries, *Nature* **2011,** 469, 483.
[3] P. R. Donald, H. I. Seifart, *J. Pediatr.*, **1989,** 115(3), 483.
[4] I. E. Hughes, H. Smith, P. O. Kane, Lancet **1962,** 279, 616.
[5] D. Liberman, U.S. Patent 2,901,488, (**1957**).
[6] W. Cheng-Hsia, H. Fang-Yu, H. Jhy-Ming, *Heterocycles* **1979**, 12(9), 1191.
[7] F. Minisci, F. Bertini, R. Galli, A. Quilico, C. Del Pizzo, Ger. Offen. 2,153,234 (**1972**), *Chem. Abstr*. **1972**, 77, 48278.
[8] F. Minisci, R. Bernardi, F. Bertini, R. Galli, M. Perchinummo, *Tetrahedron* **1971**, 27, 3575.
[9] International Conference on Harmonization guidelines, Q3A(R). *Federal Register*. **2000**, 65(140), 45085.
[10] International Conference on Harmonization guidelines, Q3B(R). *Federal Register.* **2000**, 65(139), 44791.
[11] International Conference on Harmonization guidelines, Q3C. *Federal Register.* **1997**, 62(247), 67377.
[12] Japanese Pharmacopoeia, 16th ed. **2011**.
[13] European Pharmacopoeia, 5th ed. **2005**.