SYNTHESIS AND CHARACTERIZATION OF IMPURITIES OF AN ANTICONVULSANT DRUG, LAMOTRIGINE

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ABSTRACT
Lamotrigine is a benzotriazine derivative, used to treat the disorders of central nervous system. During the process development of Lamotrigine (1), five unknown peaks [related compounds / impurities] were observed in HPLC analysis along with the Lamotrigine. All five impurities were identified, synthesized individually and characterized based on their spectral data (IR, Mass and NMR). The structures of these impurities were assigned as 2-(2,3-dichlorophenyl)-2-(guanidinylimino)acetonitrile (4), N-guanidinyl-2,3-dichlorobenzamide (6), 3-amino-6-(2,3-dichlorophenyl)-4H-1,2,4-triazin-5-one (8), N-[5-amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide (9) and 3,5-bis-(2,3-dichlorobenzamide)-6-(2,3-dichlorophenyl)-1,2,4-triazine (10) respectively. The present work describes the formation, synthesis and characterization of these impurities.

Keywords: Lamotigine, Impurities, Synthesis, Characterization, Spectroscopy

INTRODUCTION
Epilepsy is a neurological disorder characterized by unprovoked 50 million people worldwide. Lamotrigine 1, an anticonvulsant drug with a triazine structure is used for the treatment of disorders of central nervous system, in particular epilepsy. 2 There is a continuous demand for the development of new anticonvulsant agents and remarkable research is going on in this area. 3 Extensive research is going on for the preparation of different formulations of Lamotrigine. 4 Hence the study of the impurity profile of the drug is essential requirement. The International Conference on Harmonization (ICH) guidelines recommended identifying and characterizing all impurities that are present at a level of 0.10% or more. 5,6 In this context, a comprehensive study was undertaken to synthesize and characterize the impurities of Lamotrigine 1.

The synthesis of 2-(2,3-dichlorophenyl)-2-(guanidinylimino)acetonitrile 4 is reported in the literature. 7,8 The formation of impurities, 3-amino-6-(2,3-dichlorophenyl)-1H-1,2,4-triazin-5-one 8 and N-[5-amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide 9 is referred in the literature 9,10 but their synthesis is not reported. The identification and synthesis of N-guanidinyl-2,3-dichlorobenzamide 6 and 3,5-bis-(2,3-dichlorobenzamide)-6-(2,3-dichlorophenyl)-1,2,4-triazine 10 were reported here for the first time.

MATERIAL AND METHODS
The 1H-NMR was recorded in DMSO at 300 MHz on a Varian Gemini 200 MHz FT NMR spectrometer. The chemical shifts were reported in ppm relative to TMS. The FT-IR spectra were recorded in the solid state as KBr dispersion using a Perkin-Elmer 1650 FT -IR spectrophotometer. The mass spectrum (70 eV) was recorded on an Agilent-6310 LC-MS spectrometer. The solvents and reagents were used without any purification. The investigated samples of Lamotrigine bulk drug material were prepared in Sriniv Pharmaceuticals Limited, Hyderabad, India.

1) Synthesis of 2-(2,3-dichlorophenyl)-2-(guanidinylimino)acetonitrile (Schiff's base)
To a suspension of aminoguanidine bicarbonate (5.3 g, 0.023 mol) in water (40 mL) and subsequent drying at 65-70 °C under vacuum affords 2-(2,3-dichlorophenyl)-2-(guanidinylamino)acetonitrile (4, 9.6 g, 75%, HPLC purity 98%). IR (cm⁻¹): 3491.8, 3475.1, 2207.5, 1681.9, 1055.5; 1H NMR (DMSO-d₆, 8 ppm): 6.70 (s, 4H, NH₂), 7.41 (dd, Ar-H, 7.9 Hz), 7.66 (dd, 2H, Ar-H, J 7.7 Hz); 13C NMR (DMSO-d₆, 8 ppm): 131.03, 135.26, 132.44, 130.03, 129.47, 128.99, 128.17, 114.43, 113.82; MS-ESI m/z: 256.3 (M+1); Analysis calcd. for C₉H₇Cl₂N₄O: C, 42.10; H, 2.49; N, 27.69%.

2) Synthesis of N-Guanidinyl-2,3-dichlorobenzamide (Amide impurity)
To a solution of aminoguanidine bicarbonate (5.0, 0.036 mol) in dichlomethane (25 mL) was added triethyl amine (5.8 g, 0.057 mol) and stirred for 10-15 min to get a clear solution. A solution of 2,3-dichlorobenzyl chloride (5, 0.022 mol) in dichlomethane (5 mL) was added during 30-45 min at 25-30 °C. The reaction mixture was stirred at the same temperature for 1 h. Once the completion of reaction was confirmed by TLC (chloroform/methanol: 8:2), the separated solid was filtered and washed with dichlomethane (5 mL). The wet cake was suspended in water (50 mL) and adjusted the pH to 9.8-10.0 using aqueous sodium hydroxide (25%). The contents were stirred for 30-45 min at 20-25 °C. The filtration of the separated solid, washing with water (40.0 mL) and subsequent drying at 65-70 °C under vacuum affords 2-(2,3-dichlorophenyl)-1-(3-amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-5-yl)-2,3-dichlorobenzamide (9) and 3,5-bis-(2,3-dichlorobenzamide)-6-(2,3-dichlorophenyl)-1,2,4-triazine (10) respectively. The present work describes the formation, synthesis and characterization of these impurities.
reaction mass was extracted with dichloromethane (120 mL). The organic layer was separated and washed with water (40 mL) followed by 25% aqueous solution of sodium bicarbonate (50 mL). The solvent was distilled off completely under reduced pressure to get keto ester (7) as a residue. The residue was dissolved in isopropanol (100 mL) and added aminoguanidine bicarbonate (3.10 g, 0.073 mol) followed by p-toluene sulfonic acid (0.5 g). The reaction mixture was heated to reflux and maintained for 5 h. The reaction mass was cooled to 25-30 °C and stirred for 45-60 min. The filtration of the separated solid, washing with precooled isopropanol (25 mL) and subsequent drying at 50-55 °C under vacuum afforded 3-amino-6-(2,3-dichlorophenyl)-4H-1,2,4-triazin-3-one (8, 10.3 g, 40%, HPLC purity 99%). IR (cm⁻¹): 3355.1, 3149.7, 1697.9, 1675.3, 1605.7, 1598.5, 1481.1, 1460.6, 1385.5, 1352.4, 1343.0, 1325.6, 1323.1, 1279.7; MS - CI m/z: 258 (M+1); Analysis calcd. for C₁₅H₁₀Cl₂N₅O: C, 42.05; H, 2.35; N, 21.81% Found: C, 42.08; H, 2.15; N, 21.69%.

4) Synthesis of N-[5-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide (Mono benzoyl impurity)

To a stirred suspension of Lamotrigine (1, 5.0 g, 0.019 mol) in dichloromethane (25 mL) was added triethyl amine (5 mL) and the reaction mixture was cooled to 0-5°C. A solution of 2,3-dichlorobenzoyl chloride (5, 5.0 g, 0.023 mol) in dichloromethane (5 mL) was added during 30-45 min at 0-5°C. The reaction mixture was warmed to reach 25-30°C and stirred for 1-2 h at the same temperature. Once the completion of reaction was assessed by TLC (ethyl acetate/hexanes: 1:1, Rf 0.63), the separated solid was filtered and washed with dichloromethane (10 mL) to obtain crude compound. This was further purified column chromatography (silica gel) eluting with dichloromethane/methanol as eluent (0.2: 9.8) to afford N,N'-[3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide (10, 8.5 g, 72%), HPLC purity 97%). IR (cm⁻¹): 3211.1, 1660.5, 1364.8, 784.1. 1H NMR (DMSO-d₆, 8 ppm): 7.7-7.77 (t, 1H, Ar-H, J 8.0 Hz), 7.6-7.67 (d, 1H, Ar-H, J 8.0 Hz); 13C NMR (DMSO-d₆, 8 ppm): 166, 165.3, 163.0, 135.2, 134.8, 132.6, 132.1, 127.9; MS-Cl m/z: 603 (M+1); Analysis calcd. for C₂₃H₁₁Cl₆N₅O₂: C, 44.79; H, 2.15; N, 11.65% Found: C, 45.86; H, 1.84; N, 11.65%.

RESULTS AND DISCUSSIONS

Synthesis of Lamotrigine 5,6 involves the Schiff’s base formation of 2,3-dichlorobenzoyl cyanide 2 with aminoguanidine bicarbonate 3 in the presence of dilute nitric acid to yield compound 4, which on further cyclisation under basic conditions in methanol medium affords Lamotrigine 1 (Scheme 1).

![Scheme 1: Reagents and conditions a) dil. HNO₃, DMSO, b) KOH, methanol and isopropanol](image)
3499 cm\(^{-1}\) corresponding to amine \(-\text{NH}\). The NMR spectrum exhibited a broad peak at \(\delta\) 10.76 ppm corresponding to amide \(-\text{NH}\) and four protons at \(\delta\) 6.86 ppm corresponding to two amine groups.

The peaks at \(\delta\) 7.55, \(\delta\) 7.48 and at \(\delta\) 7.32 ppm correspond to aromatic protons. Based on the spectral data, the structure of this impurity was assigned as \(N\)-guanidinyl-2,3-dichlorobenzamide 6.

During the preparation of Lamotrigine 1 which involves Schiff's base formation of compound 2 with compound 3 followed by cyclization, as a side reaction the cyanide group gets hydrolyzed to keto acid and reacts with compound 3 followed by cyclization to form triazinone 8. As per the literature compound 8 is a base hydrolyzed product during the synthesis of Lamotrigine. This impurity was prepared quantitatively by \textit{in situ} esterification of compound 2 with ethanol under acidic conditions followed by imine formation with substance 3 and finally cyclization (Scheme 4). The mass spectrum of the substance 8 displayed a protonated molecular ion at \(m/z\) 258. The IR spectrum showed a band at 1675 cm\(^{-1}\) corresponding to cyclic amide carbonyl and a peak at 3355 cm\(^{-1}\) corresponding to amine.

The NMR spectrum exhibited a broad peak at \(\delta\) 6.35 ppm corresponding to aromatic amine and a triplet at \(\delta\) 7.34-7.38 ppm corresponding to aromatic proton meta position to the triazinone ring and peaks at \(\delta\) 7.3 and \(\delta\) 7.6 ppm corresponding to two aromatic protons. The amine protons were disappeared in \(D_2O\) exchange, which clearly indicates the structure of substance 8. Based on the spectral data, the structure was confirmed as 3-amino-6-(2,3-dichlorophenyl)-4\(H\)-1,2,4-triazin-5-one 8.

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In the synthetic consequence of 2,3-dichlorobenzoyl cyanide, a contaminant in the form of 2,3-dichlorobenzoic acid anhydride is produced, which reacts with Lamotrigine in subsequent reaction steps to form the related compounds 9 and 10. Mono benzoyl impurity 9 was synthesized by the reaction of Lamotrigine with 1.0 equivalent of 2,3-dichlorobenzoyl chloride and was purified by column chromatography (Scheme 5). The mass spectrum displayed a protonated molecular ion at m/z 430 whilst in the IR spectrum showed a band at 1704 cm⁻¹ corresponding to amide carbonyl and at 3325 cm⁻¹ corresponding to aromatic amine. The proton NMR spectrum displayed a multiplet at δ 7.39-7.52 ppm corresponding to four aromatic protons, a triplet at δ 7.66-7.73 ppm corresponding to two aromatic protons and a singlet at δ 11.01 ppm corresponding to amide –NH. Based on the spectral data, the structure was confirmed as N-[5-Amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide 9.

Substance 10 is a new compound and reported for the first time. Compound 10 was synthesized by treating Lamotrigine with excess equivalents of 2,3-dichlorobenzoyl chloride, which was isolated by column chromatography (Scheme 6). The mass spectrum displayed a protonated molecular ion at m/z 602. The IR spectrum showed a sharp peak at 1688 cm⁻¹ corresponding to amide carbonyl and a peak at 3211 cm⁻¹ corresponding to amide –NH. The proton NMR spectrum displayed a multiplet at δ 7.31-7.65 ppm corresponding to six aromatic protons, a triplet at δ 7.72-7.77 ppm corresponding to two aromatic protons and a singlet at δ 11.5 and δ 12.0 ppm corresponding to amide –NH. Based on this spectral data, the structure of this impurity was assigned as 3,5-bis-(2,3-dichlorobenzamido)-6-(2,3-dichlorophenyl)-1,2,4-triazine 10.

CONCLUSION

In conclusion, we have identified, synthesized and characterized five potential process-related impurities (4, 6, 8, 9 and 10) of Lamotrigine 1.

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