Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

SYNTHESIS AND CHARECTERIZATION OF IMPURITIES OF AN ANTICONVULSANT DRUG, LAMOTRIGINE

SOMISETTI NARENDER RAO [1], SRIPATHI SOMAIAH [1], T. RAVISANKAR [1] AND K. SUDHAKAR BABU [2*]

[1.] Department of Research and Development, Srini Pharmaceuticals Ltd., Plot No.10, Type-C, Road No.8, Film nagar, Jubilee Hills, Hyderabad 500033, Andhra Pradesh, India,[2.]Department of Chemistry, Sri Krishna Devaraya University, Anantapur, A.P, India 515002, *Email: ksbabusku@rediffmail.com, ss_naren@rediffmail.com

Received: 12 Feb 2011, Revised and Accepted: 18 May 2011

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-(guanidiny limino) 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-dichloro phenyl)-1,2,4-triazin-3-yl]-2,3-dichloro benzamide (**9)** and 3,5-bis-(2,3-dichloro benzamido)-6-(2,3-dichloro phenyl)-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[1] 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)-4H-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] and these are listed impurities of pharmacopeias, [11,12,13] but their synthesis is not reported. The identification and synthesis of *N*-guanidinyl-2,3-dichlorobenzamide **6** and 3,5-bis-(2,3-dichlorobenzamido)-6-(2,3-dichlorophenyl)-1,2,4-triazine **10** were reported here for the first time.

MATERIAL AND METHODS

The ^{1}H-NMR was recorded in DMSO at 300 MHz on a Varian Gemini 200 MHz FT NMR spectrometer. The chemical shifts were reported in δ 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 Srini Pharmaceuticals Limited., Hyderabad, India.

1) Synthesis of 2-(2,3-dichlorophenyl)-2-(guanidinylimino) acetonitrile (Schiff's base)

To a solution of sulfuric acid (55.0 g, 0.561 mol) in water (30.0 mL) was added aminoguanidine bicarbonate (**3**, 10.0 g, 0.073 mol) in portion wise for 1-2 h at 20-25 °C. Added 2,3-dichlorobenzoyl cyanide (**2**, 10.0 g, 0.05 mol), heated the reaction mixture to 50-55 °C and maintained for 5-6 h. The completion of the reaction can be monitored by TLC (chloroform/methanol: 8.5:1.5, R_f: 0.29). The reaction mass was cooled to 20-25°C and stirred for 20-30 min. The separated solid was filtered and washed with water (10 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)-2-(guanidinylimino)acetonitrile (**4**, 9.6 g, 75%, HPLC purity 98%). IR (cm^{-1}): 3491.8, 3457.1, 2207.5, 1681.9, 1055.5; ^{1}H NMR (DMSO-d_6, δ ppm): 6.70 (s, 4H, NH_2), 7.41 (dd, Ar-H, *J* 7.9 Hz), 7.66 (ad, 2H, Ar-H, *J* 7.9 Hz); ^{13}C NMR (DMSO-d_6, δ ppm): 163.55, 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 $C_9H_7Cl_2N_5$: C, 42.21; H, 2.76; N, 27.35%; found: C, 42.10; H, 2.49; N, 27.69%.

2) Synthesis of N-Guanidinyl-2, 3-dichlorobenzamide (Amide impurity)

To a suspension of aminoguanidine bicarbonate (**3**, 5.0 g, 0.036 mol) in dichloromethane (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-dichlorobenzoyl chloride (**5**, 5.0 g, 0.023 mol) in dichloromethane (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: 8.5:1.5, R_f: 0.22), the separated solid was filtered and washed with dichloromethane (5 mL). The wet cake was dried at 40-45 °C under vacuum to afford *N*-guanidinyl-2,3-dichlorobenzamide (**6**, 4.7 g, 80%, HPLC purity 97%). IR (cm^{-1}): 3489.5, 3375.4, 1656.0, 1045.6; ^{1}H NMR (DMSO-d_6, δ ppm): 6.86 (s, 4H, NH_2), 7.32 (dd, 1H, Ar-H, *J* 7.7Hz), 7.48 (dd, 1H, Ar-H, *J* 7.7 Hz), 7.55 (dd, 1H, Ar-H, *J* 8.0 Hz), 10.76 (s, 1H, Amide-NH); ^{13}C NMR (DMSO-d_6, δ ppm): 161.50, 153.07, 142.27, 131.64, 129.17, 129.02, 128.61, 127.56; MS-ESI *m/z*: 247.3 (M+1); Analysis calcd. for $C_8H_8Cl_2N_4O$: C, 38.89; H, 23.26; N, 22.68%; found: C, 39.04; H, 3.10; N, 22.48%.

3) Synthesis of 3-Amino-6-(2,3-dichlorophenyl)-4H-1,2,4-triazin-5-one (Triazinone impurity)

To a solution of ammonium chloride (5.3 g, 0.01 mol) in water (5.5 mL) was added aq. HCl (16.5 mL) followed by sulfuric acid (10.2 g, 0.104 mol) and stirred for 10-15 min. The reaction mixture was slowly heated 40 °C and added 2,3-dichlorobenzoyl cyanide (**2**, 20.0 g, 0.10 mol) in portion wise during 20-30 min. The contents were stirred for 3 h at 40 °C. Added ethanol (14 mL) to the reaction mixture and the temperature was raised to 75 °C. The reaction mixture was maintained for 4 h at the same temperature. Once the completion of the reaction was assessed by TLC (chloroform/methanol: 9:1, R_f: 0.22), it was cooled to 25-30 °C. The

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 (2.5 mL) and subsequent drying at 50-55 °C under vacuum affords 3-amino-6-(2,3-dichlorophenyl)-4*H*-1,2,4-triazin-5-one (**8**, 10.3 g, 40%, HPLC purity 99%). IR (cm^{-1}): 3355.1, 3149.7, 1697.9, 1675.3, 737.4; ^1H NMR (DMSO-d_6, δ ppm): 4.65 (s, 1H, NH), 6.35 (s, 2H, NH_2), 7.30 (d, 1H, Ar-H, *J* 6.0 Hz), 7.34-7.38 (t, 1H, Ar-H, *J* 8.0 Hz), 7.60-7.62 (d, 1H, Ar-H, *J* 8.0 Hz); ^{13}C NMR (DMSO-d_6, δ ppm): 166, 163.5, 163.0, 135.2, 134, 132.6, 132.1, 127.9; MS-CI *m/z*: 258 (M+1); Analysis calcd. for $C_9H_6Cl_2N_4O$: C, 42.05; H, 2.35; N, 21.81% Found: C, 42.08; H, 2.30; N, 21.79%.

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

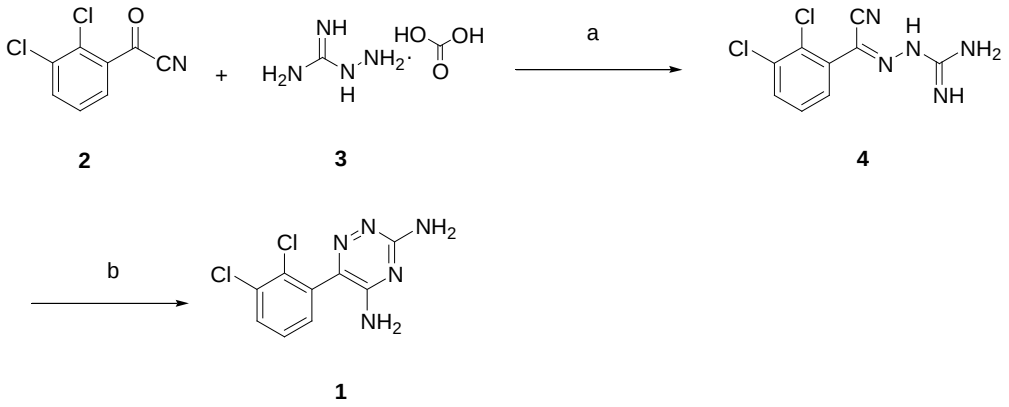
To a stirred suspension of Lamotrigine (**1,** 5.0 g, 0.019 mol) in dichloromethane (25 mL) was added triethyl amine (5 mL) and the reaction mixture was cooled to 0-5°C. A solution of 2,3-dichlorobenzoyl chloride (**5,** 5.0 g, 0.023 mol) in dichloromethane (5 mL) was added during 30-45 min at 0-5°C. The reaction mixture was warmed to reach 25-30°C and stirred for 1 h. The completion of the reaction can be monitored by TLC (chloroform/ethyl acetate/methanol: 6:3.5:0.5, R_f: 0.54). The separated solid was filtered and washed with dichloromethane (10 mL) to obtain crude compound which was further purified column chromatography (silica gel) eluting with dichloromethane/methanol (0.5: 9.5) to afford *N*-[5-amino-6-(2,3-dichlorophenyl)-1,2,4-triazin-3-yl]-2,3-dichlorobenzamide (**9,** 4.7 g, 56%, HPLC purity 97%). IR (cm^{-1}): 3325.3, 1704.2, 1623.9, 1387.9, 798.0; ^1H NMR (DMSO-d_6, δ ppm): 7.39-7.52 (m, 4H, Ar-H), 7.66-7.73 (t, 2H, Ar-H, *J* 9.0), 11.01 (s, 1H, Amide-NH); ^{13}C NMR (DMSO-d_6, δ ppm): 164.2, 138.1, 136, 135.3, 133.7, 134.1, 132.7, 130.3, 128.5, 127.56, 126.7; MS-CI *m/z*: 430 (M+1); Analysis calcd. for $C_{16}H_9Cl_4N_5O_2$: C, 44.79; H, 2.15; N, 16.29% Found: C, 44.81; H, 2.19; N, 16.26%.

5) Synthesis of 3, 5-Bis-(2,3-dichlorobenzamido)-6-(2,3-dichlorophenyl)-1,2,4-triazine (Dibenzoyl 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 cooled the contents to 0-5°C. A solution of 2,3-dichlorobenzoyl chloride (**5,** 8.4 g, 0.040 mol) in dichloromethane (5 mL) was added during 30-45 min at 0-5 °C. The reaction mixture was allowed 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, R_f: 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, 1688.5, 1364.8, 784.1; ^1H NMR (DMSO-d_6, δ ppm): 7.31-7.65 (m, 6H, Ar-H), 7.72-7.77 (t, 3H, Ar-H,*J* 9.0), 11.5 (s, 1H, Amide-NH), 12.0 (s, 1H, Amide-NH); ^{13}C NMR (DMSO-d_6, δ ppm): 165.2, 138.3, 136.3, 134.8, 134.3, 133.7, 133.0, 130.5, 128.7, 128.0, 126.5; MS-CI *m/z*: 603 (M+1); Analysis calcd. for $C_{23}H_{11}Cl_6N_5O_2$: C, 45.88; H, 1.84; N, 11.65% Found: C, 45.86; H, 1.83; N, 11.62%.

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** (Scheme1).



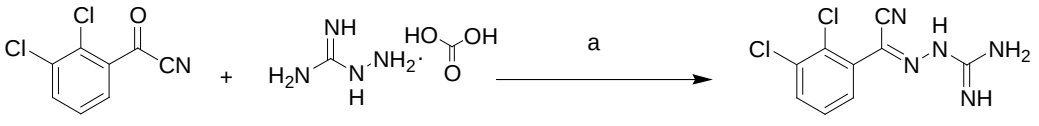
Scheme 1: Reagents and conditions a) dil. HNO_3, DMSO, b) KOH, methanol and isopropanol

A typical HPLC chromatogram (Figure.1) of laboratory sample of Lamotrigine displayed impurities/related compounds over a range of 0.05-0.15%. These impurities were identified by LC-MS, synthesized and characterized by spectral analysis. The assigned structures of these impurities are namely 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-dichlorobenzamido)-6-(2,3-dichlorophenyl)-1,2,4-triazine **10.**

Compound **4** is a potential impurity, which is an unreacted product during cyclisation step in the synthesis of Lamotrigine (Scheme 1). It was prepared by reacting 2,3-dichlorobenzoyl cyanide **2** with aminoguanidine bicarbonate **3** in aq. sulfuric acid (Scheme 2). The mass spectrum of the substance **4** showed a protonated molecular ion at *m/z* 256.3. The IR spectrum showed a band at 3491 cm^{-1} corresponding to amine –NH, at 2207 cm^{-1} corresponding to nitrile and at 1681 cm^{-1} corresponding to imine C=N. The NMR spectrum displayed broad a peak at δ 6.70 ppm corresponding to four amine protons and peaks at δ 7.41 and at δ 7.66 ppm corresponding to aromatic protons. Based on the spectral data, the structure was confirmed as 2-(2,3-dichlorophenyl)-2-(guanidinylimino)acetonitrile **4**.

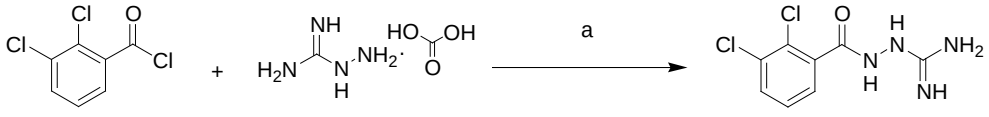
Any traces of 2,3-dichlorobenzoyl chloride that is present in 2,3-dichlorobenzoyl cyanide **2** reacts with aminoguanidine bicarbonate **3** which leads to the formation of impurity **6.** Substance **6** was synthesized by the condensation of 2,3-dichlorobenzoylchloride **5** with aminoguanidine bicarbonate **3** in the presence of triethyl amine in dichloromethane (Scheme 3). The mass spectrum of the substance **8** displayed a protonated molecular ion at *m/z* 247.3. The IR spectrum showed a sharp band at 1656 cm^{-1} corresponding to amide carbonyl, at 3375 cm^{-1} corresponding to amide -NH and a peak at

3489 cm^{-1} corresponding to amine –NH. The NMR spectrum exhibited a broad peak at δ 10.76 ppm corresponding to amide –NH and four protons at δ 6.86 ppm corresponding to two amine groups. The peaks at δ 7.55, δ 7.48 and at δ 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**.



2 **3** **4**

Scheme 2: Reagents and conditions a) aqueous H_2SO_4



5 **3** **6**

Scheme 3: Reagents and conditions a) TEA, dichloromethane

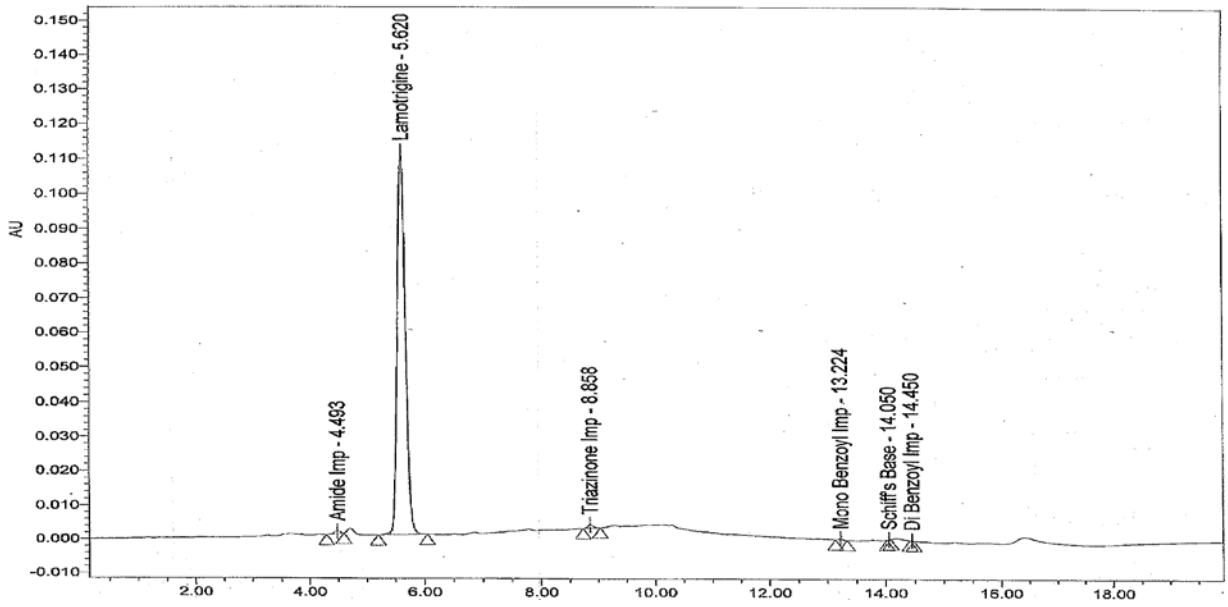
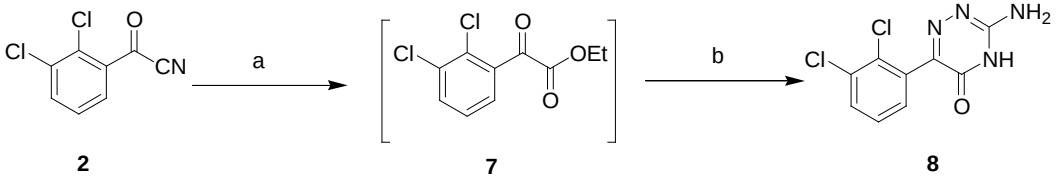


Fig. 1: HPLC Chromatogram of Lamotrigine

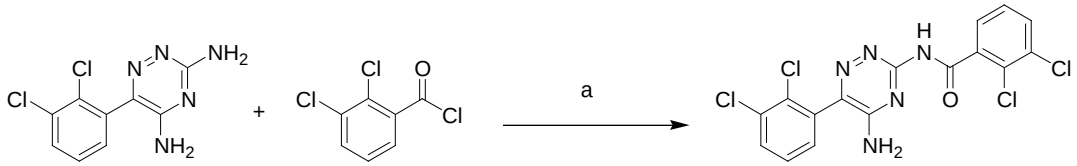
During the preparation of Lamotrigine **1** which involves Schiff's base formation of compound **2** with compound **3** followed by cyclization, as a side reaction the cyanide group gets hydrolyzed to keto acid and reacts with compound **3** followed by cyclization to form triazinone **8**. As per the literature compound **8** is a base hydrolyzed product during the synthesis of Lamotrigine. This impurity was prepared quantitatively by *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 δ 6.35 ppm corresponding to aromatic amine and a triplet at δ 7.34-7.38 ppm corresponding to aromatic proton meta position to the triazinone ring and peaks at δ 7.3 and δ 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-on**e 8**.



2 **7** **8**

Scheme 4: Reagents and conditions a) Aqueous HCl, H_2SO_4, ethanol b) aminoguanidine bicarbonate, isopropanol

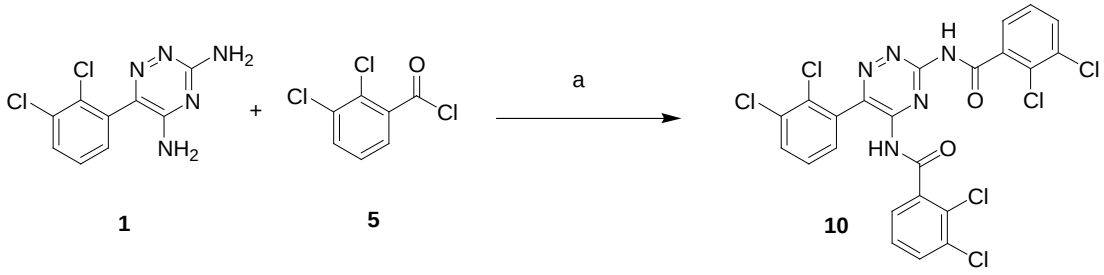
In the synthetic consequence of 2,3-dichlorobenzoyl cyanide **2**, a contaminant in the form of 2,3-dichlorobenzoic acid anhydride is produced, which reacts with Lamotrigine in the subsequent reaction steps to form the related compounds **9** and **10**. Mono benzoyl impurity **9** was synthesized by the reaction of Lamotrigine **1** with 1.0 equivalent of 2,3-dichlorobenzoyl chloride **5** 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 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**.



1 **5** **9**

Scheme 5: Reagents and conditions a) TEA, dichloromethane

Substance **10** is a new compound and reported for the first time. Compound **10** was synthesized by treating Lamotrigine **1** with excess equivalents of 2,3-dichlorobenzoyl chloride **5**, 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**.



1 **5** **10**

Scheme 6: Reagents and conditions a) TEA, dichloromethane

CONCLUSION

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

REFERENCES

1. Fitton A, Goa K L. Drugs. 1995; 50: 691.
2. McNamara J O. Nature. 1999; A15-A22.
3. Neha Garg, Trilok Chandra, Ashok Kumar. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 2(2): 88.
4. Prakash Goudanavar, Shivam H Shah, Doddayya Hiremath. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(3): 208.
5. International Conference on Harmonization guidelines Q3A (R) Impurities in New Drug Substances February 2002 (this guideline provides guidance for registration application on the content and qualification of impurities in new drug substances produced by the chemical synthesis).
6. International Conference on Harmonization guidelines Q3B (R) Impurities in New Drug Substances February 2002 (Guidance for registration or marketing application on the content and qualification of impurities in new drug product. Sawyer D A, Baxter M G, Miller A A. US Patent 4602017, 1986.
7. Rees R W A, Russell P B, Foell T J, Bright R E. J. Med. Chem. 1972; 15: 859.
8. Schneider G, Gego C L, Ondi L, Mate A G, Lukacs F, Nyerges M, Garaczi S. US Patent 6683182 B2, 2004.
9. Edmeades L M, Griffith-Skinner N A, Hill D A, Hill G T, Packham T W. Eur. Pat. Appl. 295980 A2, 1999.
10. European Pharmacopeia 6.3: 4195.
11. United States Pharmacopeia 32: 4069.
12. Pharmeuropa. 2004; 16(3): 398.