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Synthesis of Potential Impurities of Dabigatran Etexilate

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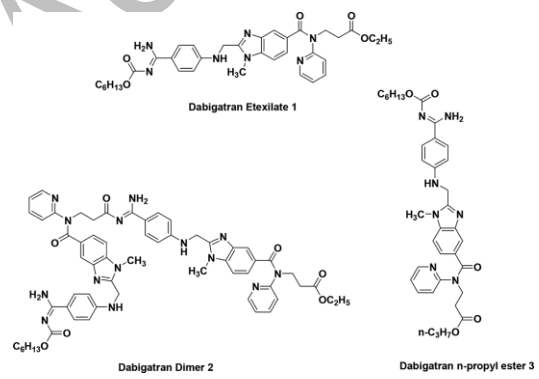
Abstract

The present work describes the origin, control and synthesis of two potent impurities of dabigatran etexilate **1**, dabigatran dimer **2** and dabigatran n-propyl ester **3** from the commercially available raw materials 2-[(4-Cyanophenyl)amino]acetic acid (**4**) and *N*-[3-Amino-4-(methylamino)benzoyl]-*N*-2-pyridinyl-β-alanine ethyl ester (**5**). These impurities are the process related impurities and may affect the quality of drug substance, during its manufacturing in large scale. These impurities are not only the crucial components in determining the quality and safety of the drug substance **1** but also provide a better understanding of impurity profiling.

Graphical Abstract



KEYWORDS: Dabigatran etexilate, dabigatran dimer, dabigatran n-propyl ester, related substance, impurities

INTRODUCTION

Dabigatran etexilate **1** (Fig. 1) is an oral pro-drug of thrombin inhibitor dabigatran.[1] Chemically it is known as *N*-{[2-({[4-({[(hexyloxy)carbonyl]amino}iminomethyl)phenyl] amino}methyl)-1-methyl-1*H*-benzimidazol-5-yl]carbonyl}-*N*-2-pyridinyl -β-alanine ethyl ester (**1)**. This drug is widely used to reduce the risk of stroke in patients with non-valvular atrial fibrillation (AF). [2] Many synthetic routes are available in the chemical literature to synthesis **1**, which involves the condensation of two commercially available key raw materials, 2-[(4-Cyanophenyl)amino]acetic acid (**4**) and *N*-[3-Amino-4-(methylamino)benzoyl]-*N*-2-pyridinyl-β-alanine ethyl ester (**5**) as shown in Figure 1. This condensed product after internal cyclization in acidic medium, followed by Pinner reaction and reaction with n-hexyl chloroformate yields **1**.[3]

During the process development of **1** in our laboratory, we came across two potent impurities of **1** namely Ethyl 3-(2-{[(4-{N-[3-(2-{[(4-{*N*-[(hexyloxy)carbonyl]carbamimidoyl}phenyl)amino] methyl}-1-methyl-*N*-(pyridin-2-yl)-1*H*-benzimidazol-5-carboxyamido)propanoyl]carbamimi doyl}phenyl)amino]methyl}-1-methyl-*N*-(pyridine-2-yl)-1*H*-benzimidazol-5-carboxamido) propanoate (**2**) and *N*-{[2-({[4-({[(hexyloxy)carbonyl]amino}iminomethyl)phenyl]amino} methyl)-1-methyl-1*H*-benzimidazol-5-yl]carbonyl}-*N*-2-pyridinyl -β-alanine - n-propyl ester (**3**), which are

present in a level of 0.1-0.5 %. It is recommended by ICH (International Council on Harmonization) that Impurities or related substances which are present in a level of < 0.1% w/w should be identified, based on the fact that unidentified impurities may cause health hazards by making the API (Active Pharmaceutical Ingredient) inferior in quality.[4] It is generally observed that during the multi-step chemical synthesis of API, trace level of unwanted chemical entity were also formed in the previous steps, which reacts in a similar way as do API and contaminates the drug substance.[5]

To have a better understanding of impurity profiling these impurities has to be identified, synthesized and controlled in the manufacturing process. Furthermore these impurities in a pure form are of great importance in determining the analytical parameters such as specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ) and relative retention factor.[6]

Based on the above facts and their importance among the drug regulatory authorities of United States, Europe and Japan, we have described here the origin, synthesis, characterization and control of this two impurities (**2** and **3**) which are not reported so far.[7–10]

RESULT AND DISCUSSION

Dabigatran dimer **2** is a process related impurity, which originates during the preparation of amidine **7.** Reaction of **4** with **5** leads to the formation of a condensed product **6** which on Pinner reaction yields amidine **7**. It is assumed that, during Pinner reaction while

neutralizing hydrochloric acid with ammonium carbonate leads to the dimerization of amidine **7** with a loss of one ethanol molecule. It was observed that during the amidination at basic medium resulted the dimerization of amidine **7** in a trace level. Amidine dimer which reacts in a similar way as **7** with n-hexyl chloroformate is considered as a route cause for the contamination of **2** in **1** at a level ~0.2-0.3%. Presence of such impurity was identified by Liquid Chromatography-Mass Spectrometry (LCMS), which has shown a higher mass with *m/z* 1080 amu, confirming the addition of amidine **7** and **1** with a loss one ethanol molecule.

Dabigatran dimer **2** was prepared as shown in Scheme 1, starting with **1**. Hydrolysis of **1** in THF using sodium hydroxide gave acid **8**.[11] Coupling of acid **8** with amidine **7** in presence of *N,N'*-dicyclohexylcarbodiimide and *N*,*N*-dimethyl-4-aminopyridine gave a crude product, which on column purification afforded **2** with a chromatographic purity of more than 95%. The chemical structure of **2** was confirmed by the spectral analysis and in comparison with **1**. In order to control this impurity, we have screened different conditions as well purification procedures to remove/ control **2**. Finally, promising results were obtained when we treated the solution of **1** with activated charcoal. It was observed that **2** have a tendency to adsorb on charcoal. Hence, to get rid of **2** we have introduced a charcoal treatment during the preparation of **1**.

The origin of impurity **3** is due to the contamination of ethyl ester **5** with n-propyl ester **9.** This n-propyl ester **9** reacts in a similar way as ethyl ester **5** and contaminates the drug substance **1** with **3**. Furthermore, it has been seen that during the isolation of **1** from

isopropyl alcohol could leads to the formation of **3**, based on the fact that the contamination of isopropyl alcohol with n-propyl alcohol in a level of ≤ 0.1%. The presence of dabigatran n-propyl ester **3** with *m/z* 641 amu was confirmed by LC-MS study with a level of 0.1-0.2% in **1.** Based on its presence, we have synthesized **3** and compared with the obtained LCMS peak. Dabigatran n-propyl ester **3** was prepared as shown in Scheme 2**,** starting with **1.** Transesterification of **1** in n-propyl alcohol leads to the formation of **3** as a major product**,** which on column purification produces **3** having a purity of more than 95% by HPLC. As **3** is believed to be arise from ethyl ester **5**, we have control the contamination of n-propyl ester **9** in **5** to not more than 0.3% based on the experimental and purge studies. Further, this impurity can be controlled to a specific level by adopting an effective purification procedure in **1**.

CONCLUSION

We have successfully demonstrated the synthesis and characterization of **2** and **3**, the potent impurities of dabigatran etexilate **1**. Preparation of these impurities not only helps in obtaining a good quality output for formulation but also helps in establishing the impurity profile of **1** by understanding the cause of its origin and control.

EXPERIMENTAL

Melting point was determined by a Reichert Thermopan apparatus. ^{1}H and ^{13}C NMR spectra were recorded by a Bruker Avance 300 MHz and Varian 500 MHz spectrometer using TMS as internal standard in DMSO-d_6. IR spectra were recorded using Perkin-Elmer Spectrum One Fourier Transform (FT) IR spectrophotometer. High-resolution

mass spectral (HRMS) analyses were performed using the electrospray ionization (ESI) method on Xevo G2 QTOF mass spectrometer. HPLC measurements were run on Inertsil ODS-4 (250 mm × 4.6 mm, 5 µm; make: GL Sciences) with a flow rate of 1.0 mL / min having a column oven temperature of 25 °C. UV detection occurred at λ □ 220 nm. Reagents and solvents were used from commercial sources.

Preparation Of Ethyl 3-(2-{[(4-{N-[3-(2-{[(4-{*N*-[(Hexyloxy)Carbonyl]Carbamimidoyl} Phenyl)Amino]Methyl}-1-Methyl-*N*-(Pyridin-2-Yl)-1*H*-Benzimidazol-5-Carboxyamido) Propanoyl]Carbamimidoyl}Phenyl)Amino]Methyl}-1-Methyl-*N*-(Pyridine-2-Yl)-1*H*-Benzimidazol-5-Carboxamido) Propanoate (Dabigatran Dimer, 2)

Amidine **7** (2.20 g, 4.10 mmol) along with 4-dimethylaminopyridine (0.45 g, 3.69 mmol) was dissolved in DMF (30 mL) at 25-30 °C. This solution was added over a mixture of acid **8** (2.22 g, 3.69 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (0.86 g, 4.17 mmol) in DMF (20 mL) under nitrogen atmosphere at 25-30 °C. The reaction mixture was stirred for 15 h at 25-30 °C, and further at 80 °C for another 2 h. The by-product (DCU) was filtered and the filtrate was diluted with a mixture of water (40 mL), dichloromethane (40 mL) and methanol (40 mL). The suspension was stirred for 25 min and the organic layer was separated. Organic layer was evaporated under vacuum and the residue was purified by column chromatography to obtain **2** as solid. Yield 1.70 g (42.5%); Mp 125-128 °C; HPLC Purity 96.33%; IR (KBr, cm^{-1}) 3367 (N-H), 1731 (C═O, ester), 1608 (C═O, amide); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, 3H, J = 6.5 Hz), 1.11 (t, 3H, J = 6.5 Hz), 1.28 (m, 6H), 1.57 (m, 2H), 2.68 (t, 4H, J = 6.0 Hz), 3.76 (s, 6H), 3.96 (m, 4H), 4.22

(m, 2H), 4.28 (m, 2H), 4.59 (m, 4H), 6.76-6.82 (m, 4H), 6.82-6.90 (m, 2H), 6.90-7.00 (m, 2H), 7.02-7.22 (m, 4H), 7.35-7.60 (m, 6H), 7.78-8.00 (m, 4H), 8.30-8.38 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 148.7, 140.8, 138.0, 129.2, 122.9, 122.2, 121.4, 119.5, 111.4, 109.5, 64.2, 60.1, 44.4, 39.4, 33.1, 31.0, 30.0, 28.6, 25.3, 22.1, 14.0; HRMS (ESI-QTOF) for $C_{59}H_{64}N_{14}O_7$ $[M+H]^+$: *m/z* calcd: 1081.5161; found: 1081.5179.

Preparation Of *N*-{[2-({[4-({[(Hexyloxy)Carbonyl]Amino}Iminomethyl)Phenyl]Amino} Methyl)-1-Methyl-1*H*-Benzimidazol-5-Yl]Carbonyl}-*N*-2-Pyridinyl-B-Alanine - N-Propyl Ester (Dabigatran N-Propyl Ester, 3)

Dabigatran etexilate **1** (5.0 g, 7.80 mmol) was suspended in n-propyl alcohol (50 mL) at 25 °C and stirred at reflux temperature for 18 h. The solvent was evaporated and the residue was purified by column chromatography to obtain **3. Y**ield 1.60 g (31.3%); Mp 72-75°C; HLPC Purity 96.78%; IR (KBr, cm^{-1}) 3387 (N-H), 1731 (C═O, ester), 1644 (C═O, amide); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82-0.88 (m, 6H), 1.28-1.32 (m, 6H), 1.51-1.58 (m, 4H), 2.69 (t, 2H, *J* = 6.9 Hz), 3.76 (s, 3H), 3.89 (t, 2H, *J* = 6.6 Hz), 3.97 (t, 2H, *J* = 6.6 Hz), 4.22 (t, 2H, *J* = 7.2 Hz), 4.59 (d, 2H, *J* = 5.4 Hz), 6.75 (d, 2H, *J* = 8.7 Hz), 6.88 (d, 1H, *J* = 7.8 Hz), 6.95 (t, 1H, *J* = 6.0 Hz), 7.14-7.17 (m, 2H), 7.39 (d, 1H, *J* = 8.4 Hz), 7.46 (d, 1H, *J* = 1.1 Hz), 7.54 (t, 1H), 7.79 (d, 2H, *J* = 8.7 Hz), 8.38 (dd,1H, J_1 = 1.2 Hz, J_2 = 4.8 Hz), 8.66 (br s, 1H), 9.11 (br s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 148.6, 137.8, 129.1, 122.7, 122.0, 121.2, 119.4, 111.3, 109.4, 29.8, 13.8, 10.2; HRMS (ESI-QTOF) for $C_{35}H_{43}N_7O_5[M+H]^+$: *m/z* calcd: 642.3326; found 642.3405.

SUPPORTING INFORMATION

IR, ^{1}H and ^{13}C NMR spectra, HPLC Traces, HRMS. This material can be found via the ‘‘Supplementary Content’’ section of this article’s webpage.’’

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REFERENCES

1. Van R, J.; Liesenfeld, K. H.; Stangier, J.; Haertter, S.; Wienen, W.; Clemens, A.; Feuring, M. *Thromb Haemost* **2010,** *103*, 1116-1127.

2. Sanford, M.; Plosker, G.L. *Drugs*. **2008**, *68*, 1699-1709.

3. Hauel, N.; Wienen, W.; Ries, U.; Priepke, H.; Stassen, J. U.S. Patent 6087380, 2000.

4. *ICH Q3A Impurities in New Drug Substances, R2*; International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use (ICH): Geneva, Switzerland, October 2006.

5. Miralles, S.; Rao, L.; Duran Lopez, E. PCT Patent WO2012152855 A1, 2012.

6. International Conference on Harmonization (ICH) Guidelines. Q_2B validation of analytical procedures: methodology; ICH Guidelines: Geneva, Switzerland, 1996.

7. Chen, Y.; Chen, H.; Yuan, L.; Liang, J. *Heterocycles*. **2013,** *87*, 1699-1710.

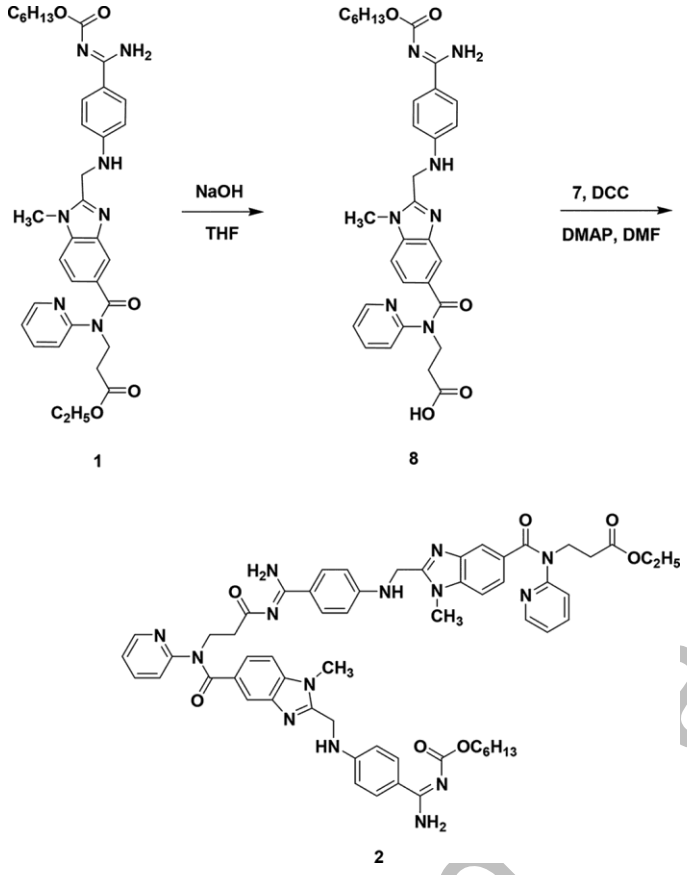
8. Zheng, Y. Y.; Shen, C.W.; Zhu, M.Y.; Zhou, Y. M.; Li, J. Q. *Org. Process Res. Dev*. **2014**, *18*, 744-750.

9. Sunil K. S.; Shekhar B. B.; Francis P, D.; Nagambar G, J.; Pravin, T.; Amit, S. PCT Patent WO2014041559 A2, 2014.

10. Libiao, L.; Jin, Z.; Xueyan, C.; Yun, C.; Ai, Z.; Yuan, L.; Junxuan, W. Chinese Patent CN105566296 A, 2016.

11. Yang, X. Z.; Yang, W. H.; Diao, X. J.; Xu, Y. G.; Gong, G. Q.; He, G.W. *European Journal of Medicinal Chemistry*. **2012**, *57*, 21-28.

Scheme 1. Synthesis of dabigatran dimer **2**



Scheme 2. Synthesis of dabigatran n-propyl ester **3**

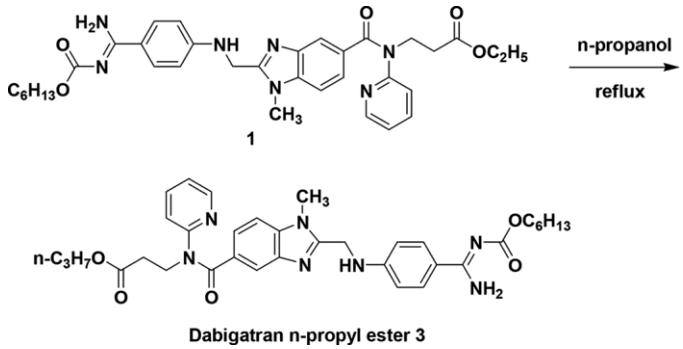


Figure 1. Synthesis of dabigatran etexilate **1** along with dabigatran dimer **2** and dabigatran n-propyl ester **3**