Identification, Synthesis, Isolation And Spectral Characterization Of Direct Factor Xa Inhibitor Related-Substances

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ABSTRACT: Most potential related-substances of Betrixaban maleate drug substance were synthesized and characterized. Among these, two related-substances were found to be intermediates. Proposed structures were further confirmed by characterization using NMR, FT-IR, and HRMS techniques. Based on the spectroscopic, spectrometric and elemental analysis data, unknown related-substances were characterized as O-Desmethyl Betrixaban (OR) N-(5-Chloropyridin-2-yl)-2-(4-(N,N-dimethylcarbamimidoyl)benzamido)-5-hydroxybenzamide hydrobromide, Betrixaban amide (OR) N1-(2-((5-chloropyridin-2-yl)carbamoyl)-4-methoxyphenyl)terephthalamide, N-DesmethylBetrixaban (OR) N-(5-chloropyridin-2-yl)-5-methoxy-2-{[4-(N-methylcarbamimidoyl)benzoyl]amino}benzamide hydrochloride, Deschloro Betrixaban (OR) 2-{[4-(N,N-dimethylcarbamimidoyl)benzoyl]amino}-5-methoxy-N-(pyridin-2-yl)benzamide hydrochloride, N-(5-hromopyridin-2-yl)-2-(4-(N,N-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide hydrochloride and 2-amino-N-(5-chloropyridin-2-yl)-5-methoxybenzamide and N-(5-chloropyridin-2-yl)-2-(4-cyanobenzamido)-5-methoxybenzamide. A plausible mechanism for the formation of these related-substances was also proposed.

KEY WORDS: Direct factor Xa inhibitor; related-substances; anticoagulant agent; pulmonary; embolism (PE); deep vein thrombosis; venous thromboembolism; Betrixaban

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1. Introduction

Coagulation factor Xa (FXa) is a particularly attractive target for the development of effective and safe anticoagulants1. Betrixaban (Bevyxxa) is the fourth orally administered anticoagulant that acts by inhibiting Factor Xa (FXa) activity2,3, joining the group of Rivaroxaban (Xarelto), Apixaban (Eliquis), and Edoxaban (Savaysa). Rivaroxaban was the first of these agents and was initially approved in mid-2011 for the prophylaxis of deep vein thrombosis (DVT), which can lead to pulmonary embolism (PE) in patients undergoing knee or hip replacement surgery. Apixaban (Eliquis) was approved in late 2012 to reduce the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation. In early 2015, Edoxaban (Savaysa) was approved to reduce the risk of stroke and systemic embolism. Betrixaban selectively blocks the active site of FXa and inhibits free FXa and prothrombinase activity4. It decreases thrombin generation but has no direct effect on platelet aggregation. The new anticoagulant is specifically indicated for the prophylaxis of venous thromboembolism (VTE) in adult patients who are hospitalized for an acute medical illness and are at risk for thromboembolic complications because of moderate or severe restricted mobility and other risk factors for VTE5,6.

The presence of its related compounds in a drug substance can have a significant impact on the quality and safety of the drug product. Therefore, it is necessary to study the impurity profile of any active pharmaceutical ingredient (API) and control it during the manufacturing of a drug product. As per the general guidelines recommended by ICH [4] to qualify the drug substance, the amount of acceptable level for a known and unknown related compound (impurity) should be less than 0.15 and 0.10%, respectively. In order to meet the stringent regulatory requirements, the impurities present in the drug substance need be identified and characterized completely.

Very recently, we have described an efficient, industrial scale synthesis of Betrixaban maleate. During the synthesis of Betrixaban maleate, we came across many process-related impurities and some of them were captured in our prior art also. To obtain a better understanding of the complete impurity profile of Betrixaban maleate and to compare the extent of contamination of the impurities in Betrixaban maleate, we decided to synthesize all the possible impurities. During the process development of Betrixaban, six unknown related-substances were found. Hence, a comprehensive study was undertaken to identify, synthesize and characterize these six unknown related-substances of Betrixaban [Table-
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In this article, we have reported the synthesis, isolation and spectral characterization of related-substances obtained during our process development of Betrixaban.

SYNTHETIC SCHEME OF BETRIXABAN MALEATE

Experimental Section:

Unless stated, all reagents and solvents used in this study were commercially available. All reactions were monitored by TLC using commercial silica gel plates. IR spectra were obtained using KBr disks on the FT-IR Perkin Elmer. UV spectrum was scanned from 200 – 400 nm by using Perkin Elmer Lambda 35 UV–Visible spectrophotometer. NMR spectra were recorded on Bruker 300MHz Avance NMR spectrometer with tetramethylsilane as an internal reference. Melting points were observed on a Buchi M-565 Melting Point Tester and are uncorrected. Mass spectra were recorded on Waters Xevo G2-XS Q-TOF LC/MS/MS system.

Preparation of Betrixaban maleate or N-(5-Chloro-2-pyridinyl)-2-[(4-(N,N-dimethylcarbamimidoyl)benzoyl)amino]-5-methoxybenzamide (2Z)-2-butenedioate (OR) N-(5-chloropyridin-2-yl)-2-[(4-(N,N-dimethylcarbamimidoyl)benzoyl)amino]-5-methoxybenzamide (2Z)-but-2-enedioate (1:1) [9]:

Under nitrogen atmosphere, 29% w/w dimethylamine solution in tetrahydrofuran (57.2g, 0.368 moles) was added to a suspension of the cyano Intermediate (25g, 0.061 moles) in tetrahydrofuran (250ml) at 0-5°C. Subsequently, 20% w/v isopropyl magnesium chloride (221ml, 0.43 moles) was added at the same temperature. Thereafter, reaction mass temperature was raised to 15-20°C and stirred for 1-2hrs, at that temperature. Further, the reaction mass was quenched into ~ 25% aq. HCl and concentrated under reduced pressure to remove tetrahydrofuran and filtered. Thus, obtained product was dried and purified from N,N-dimethylacetamide / dichloromethane to yield Betrixaban monohydrochloride. Further, Betrixaban monohydrochloride compound (5g, 0.01 moles) and maleic acid (2.4g, 0.02 moles) were sequentially charged into a mixture of water and methanol (1:1, 50mL). After stirring the reaction mass for 3-4 hrs. at ambient temperature, aqueous sodium carbonate solution was added (1.09g, 0.01 moles) and stirring was
continued for another hour. After concentrating the reaction mass under reduced pressure to remove methanol, the product was filtered and washed with water (5mL). Finally, the obtained product was dried under reduced pressure to yield Betrixaban maleate as pale yellow colored crystalline powder; Yield 50%; M.P. 195-198°C; UV λ max 204 nm; 1H NMR (300 MHz, DMSO-d6): δ 11.16 (br s, 1H), 11.04 (br s, 1H), 9.39 (br s, 1H), 9.03 (br s, 1H), 8.45 (d, J = 2.4 Hz, 1H), 8.14-7.95 (m, 5H), 7.77 (d, J = 8.1 Hz, 2H), 7.44 (d, J = 2.7 Hz, 1H), 7.22 (dd, J = 9.0 Hz, 1H), 6.03 (s, 2H), 3.87 (s, 3H), 3.24 (br s, 3H), 2.99 (br s, 3H); 13C NMR (75 MHz, DMSO-d6): δ 167.2, 164.0, 154.2, 150.5, 146.2, 138.0, 137.5, 132.1, 128.6, 128.3, 128.2, 127.8, 125.7, 116.0, 115.8, 118.6, 41.9, 38.9, 155.6, 150.6, 146.4, 137.8, 136.2, 130.4, 128.6, 127.8, 125.9, 118.2, 116.2, 113.9, 55.6, 41.9, 38.9; m/z: 452 (M+H). The percentage of Carbon, Hydrogen, Chlorine and Oxygen in Betrixaban maleate were determined by using Waters Xevo G2-XS Q-TOF LC/MS/MS system. The protonated molecular ion at m/z 452 (M+1) confirms the Betrixaban maleate base monoisotopic mass as 451 corresponding to molecular formula of C23H23ClN4O3.

Preparation of O-Desmethyl Betrixaban (OR) N-(5-Chloropyridin-2-yl)-2-[(N,N-dimethylcarbamimidoyl)benzamido]-5-hydroxybenzamide hydrobromide (OR) N-(5-Chloropyridin-2-yl)-2-[(N,N-dimethylcarbamimidoyl)benzoyl]amino)-5-hydroxybenzamide hydrobromide [10]: Related-substance 10, which forms during work-up operations of Betrixaban synthesis, due to acid hydrolysis and was prepared by reacting compound 7 with Boron tribromide.

Table 1

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the Element</th>
<th>Theoretical value (%)</th>
<th>Experimental value (% Calculated based on Q-TOF MS data)</th>
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<tr>
<td>1</td>
<td>Carbon</td>
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<td>60.99</td>
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<td>2</td>
<td>Hydrogen</td>
<td>4.91</td>
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<tr>
<td>3</td>
<td>Chlorine</td>
<td>7.85</td>
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<tr>
<td>4</td>
<td>Nitrogen</td>
<td>15.50</td>
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<tr>
<td>5</td>
<td>Oxygen</td>
<td>10.62</td>
<td>10.60</td>
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</table>

Preparation of Betrixaban Amide (OR) N-2-((5-chloropyridin-2-yl)carbamoyl)-4-methoxyphenyl)terephthalalimde (OR) N-2-[(5-chloropyridin-2-yl)carbamoyl]-4-methoxyphenyl]benzene-1,4-dicarboxamide [11]: Related-substance 11, formulaeas in basic reaction conditions during the synthesis of Betrixaban, was prepared by reacting compound 7 with Di-potassium hydrogen phosphate in the presence of peroxide.
A mixture of N-(5-Chloropyridin-2-yl)-2-(4-(N,N-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide hydrochloride or Betrixaban hydrochloride (7) (5g, 10.24 mmol) and Di-potassium hydrogen phosphate (2.6g, 14.92 mmol) in N,N-Dimethyl formamide (50 mL) was stirred at ambient temperature for 20 mins and hydrogen peroxide (0.55g, 16.17 mmol) was added at the same temperature. Stirring was continued at room temperature for about 24 hrs. (reaction monitored by TLC). After completion of reaction, reaction mixture was quenched into pre-cooled dil. hydrochloric acid (25 mL) and suspended product was filtered to yield the desired Amide related-substance as a grey colored powder; yield 80% (3.5g); m.p. 265-270°C; UV λ max 292 nm; 1H NMR (300 MHz, DMSO-d6): δ 11.13 (br, 1H), 10.98 (br, 1H), 8.44 (d, J = 2.4 Hz, 1H), 8.17-7.93 (m, 8H), 7.55 (br, 1H), 7.43 (d, J = 2.7 Hz, 1H), 7.21 (dd, J = 9.0 Hz, 1H), 3.86 (s, 3H); 13C NMR (75 MHz, DMSO-d6): 167.2, 167.1, 164.3, 155.4, 150.6, 146.3, 146.3, 137.8, 137.0, 136.9, 130.6, 127.8, 127.1, 126.2, 125.8, 124.6, 118.2, 116.2, 113.8, 55.5; m/z: 425 (M+H).

Preparation of Desmethyl Betrixaban (OR) N-(5-chloropyridin-2-yl)-5-methoxy-2-[(4-(N-methylcarbamimidoyl)benzoyl]amino]benzamide hydrochloride [12]: Related-substance 12, shapes during Grignard reaction, carried out to synthesise Betrixaban, was prepared by reacting compound 6 with methylamine.

Under inert atmosphere, methylamine, 2M solution in tetrahydrofuran (67.5 mL, 135.2 mmol) was added dropwise to a suspension of (N-(5-Chloropyridin-2-yl)-2-(4-cyanobenzamido)-5-methoxybenzamide (OR) N-(5-Chloropyridin-2-yl)-2-[(4-cyanobenzoyl]amino]5-methoxy benzamide (OR) cyano intermediate (5g, 12.29 mmol) in tetrahydrofuran (50 mL) at 0-5°C. Thereafter, isopropyl magnesiumchloride, 2M solution in tetrahydrofuran (80.5 mL, 159.8 mmol) was slowly transferred to the reaction mixture and maintained for an hour at the same temperature. Thereafter, reaction mass was quenched into pre-chilled aq. hydrochloric acid (150 mL) and solvent was evaporated under reduced pressure. Finally, obtained solids were filtered and dried under vacuum to yield N-Desmethyl Betrixaban as a yellow colored crystalline powder, yield 75% (3.6g); m.p. 197-201°C; UV λ max 232 nm; 1H NMR (300 MHz, DMSO-d6): δ 11.14 (s, 1H), 11.06 (s, 1H), 10.18 (s, 1H), 9.69 (s, 1H), 9.23 (s, 1H), 8.44 (d, J = 2.4 Hz, 1H), 8.14-8.06 (m, 3H), 8.00-7.93 (m, 4H), 7.42 (d, J = 2.7 Hz, 1H), 7.21 (dd, J = 8.9 Hz, 1H), 3.86 (s, 3H), 3.04 (s, 3H); 13C NMR (75 MHz, DMSO-d6): δ 167.1, 163.8, 162.6, 155.6, 150.6, 146.4, 138.6, 137.9, 131.3, 130.3, 128.6, 127.6, 126.8, 125.9, 125.0, 118.1, 116.3, 113.9, 55.6, 29.8; m/z: 438 (M+H).

Preparation of Deschloro Betrixaban (OR) 2-[(4-(N,N-dimethyl-carbamimido]yl)benzoyl]amino]5-methoxy-N-(pyridin-2-yl)benzamide hydrochloride [13]: Related-substance 13, forms under Grignard reaction conditions during the synthesis of Betrixaban, was prepared by subjecting compound 7 under palladium catalysis.
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N-(5-Chloropyridin-2-yl)-2-(4-(N,N-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide hydrochloride or Betrixaban hydrochloride (7) (5g, 10.24 mmol) was dissolved in methanol (350 mL) and obtained solution was transferred into a autoclave vessel. Solution was degassed with nitrogen and subsequently flushed with hydrogen gas. Thereafter, 10% Pd/C (2g) was charged and 2-3Kg/m² hydrogen pressure was applied. Stirring was continued for 5-6 hrs. and reaction completion was monitored by TLC. Afterwards, reaction completion catalyst was removed by filtration and obtained filtrate was concentrated to yield crude impurity, which was further purified by column chromatography from methanol and dichloromethane as an eluent to get pure Deschloro Betrixaban as light-yellow colored crystalline solid. yield 28% (1.3g); m.p. 198-200°C; UV λ max 203 nm; 1H NMR (300 MHz, CD3OD-d4): δ 8.37-8.30 (m, 2H), 8.18-8.14 (m, 2H), 7.86-7.80 (m, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 2.7 Hz, 1H), 7.23-7.16 (m, 3H), 3.91 (s, 3H), 3.35-3.32 (m, 3H), 3.10 (s, 3H); 13C NMR (75 MHz, DMSO-d6): δ 169.3, 166.6, 166.0, 157.8, 152.9, 149.2, 139.6, 139.4, 133.7, 132.4, 129.7, 129.3, 126.3, 125.4, 121.6, 119.2, 116.8, 114.8, 56.3, 42.7, 39.5; m/z: 418 (M+H).

Preparation of Bromo analogue of Betrixaban (OR) N1-(2-((5-bromopyridin-2-yl)carbamoyl)-4-methoxyphenyl)-N4,N4-dimethylterephthalamide [15]: Related-substance 15, a carryover of corresponding contaminat of key starting material i.e. 2-Amino-5-chloropyridine, was prepared by subjecting compound 14, by following the reaction conditions of Betrixaban.

Under inert atmosphere, dimethylamine, 2M solution in tetrahydrofuran (27.7 mL, 55.4 mmol) was added dropwise to a suspension of N-(5-Bromopyridin-2-yl)-2-(4-cyanobenzamido)-5-methoxybenzamide (OR) bromo analogue of cyano intermediate (5g, 11.08 mmol) in tetrahydrofuran (50 mL) at 0-5°C. Isopropyl magnesium chloride, 2M solution in tetrahydrofuran (33.2 mL, 66.5 mmol) was slowly transferred to the reaction mixture and maintained for an hour at the same temperature. Thereafter, reaction mass was quenched into pre-chilled aq. hydrochloric acid (70 mL) and the solvent was evaporated under reduced pressure. Obtained product was filtered and dried under vacuum to yield Bromo analogue of Betrixaban as a yellow colored solid. Yield 75% (4.1g); m.p. 217-220°C; UV λ max 232 nm; 1H NMR (300 MHz, DMSO-d6): δ 11.15 (br s, 1H), 11.06 (br s, 1H), 9.47 (br s, 1H), 9.28 (br s, 1H), 8.52 (t, J = 1.5 Hz, 1H), 8.10-8.07 (m, 4H), 8.00 (d, J = 8.7 Hz, 1H), 7.77 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 3.0 Hz, 1H), 7.21 (dd, J = 9.0, 1H), 3.86 (s, 3H), 3.27 (s, 3H), 2.98 (s, 3H); 13C NMR (75 MHz, DMSO-d6): δ 167.1, 163.9, 155.6, 150.9, 148.5, 140.5, 137.4, 132.3, 130.3, 128.6, 127.8, 126.9, 125.0, 118.1, 116.8, 114.3, 113.8, 55.6; m/z: 452 (M+H).
### Table-2

<table>
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<th>Related-Substance identification number</th>
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<td>O-Desmethyl Betrixaban</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>Betrixaban Amide</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>N-Desmethyl Betrixaban</td>
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<table>
<thead>
<tr>
<th>13</th>
<th>Dechloro Betrixaban</th>
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<tbody>
<tr>
<td>14</td>
<td>Bromo analogue of Cyano Intermediate</td>
</tr>
<tr>
<td>15</td>
<td>Bromo analogue of Betrixaban</td>
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</tbody>
</table>

Acknowledgement

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References

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[3].

[4].

[5].
