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Synthesis and Characterization of Potential Impurities of Eltrombopag Olamine

KATUROJU SRINIVASACHARY[1,*], CHENNURI RAJESH [1], SHASHIKANT TIWARI[1], Y.S. SOMANNAVAR[1], SAILENDRA KUMAR NADAKUDITY[1], KRISHNA MURTHY V.R. MOTURU[1], SAMALA BHANUCHADAR[1] and BHOOMIREDDY RAMADEVI[2]

[1]Chemical Research and Development, APL Research Centre-II, Aurobindo Pharma Ltd., Survey No.71 & 72, Indrakaran Village, Kandi Mandal, Sangareddy District-502329, India
[2]Department of Chemistry, Jawaharlal Nehru Technological University Hyderabad, Hyderabad-500072, India

*Corresponding author: E-mail: kathrojusrinivasachary@gmail.com

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This work reports the feasibility of recently developed industrial viable process for eltrombopag olamine starting from 2-bromo-6-nitro phenol and reports the identification of four potential impurities related to eltrombopag olamine, namely eltrombopag olamine ester (**1**), 2-aminophenol analogue of eltrombopag (**2**), 3,3′-(2-amino-3-oxo-3*H*-phenoxazine-4,6-diyl dibenzoic acid (**3**), 2′-hydroxy[1,1-biphenyl]-3-carboxylic acid (**4**). These impurities are the crucial components in determining the quality of the drug substance, eltrombopag olamine during its manufacturing. These impurities have impact on the quality of eltrombopag olamine and controlled these impurities results excellent yields of active pharma ingredient of eltrombopag olamine

Keywords: Eltrombopag olamine, 2-Amino phenol, Impurities.

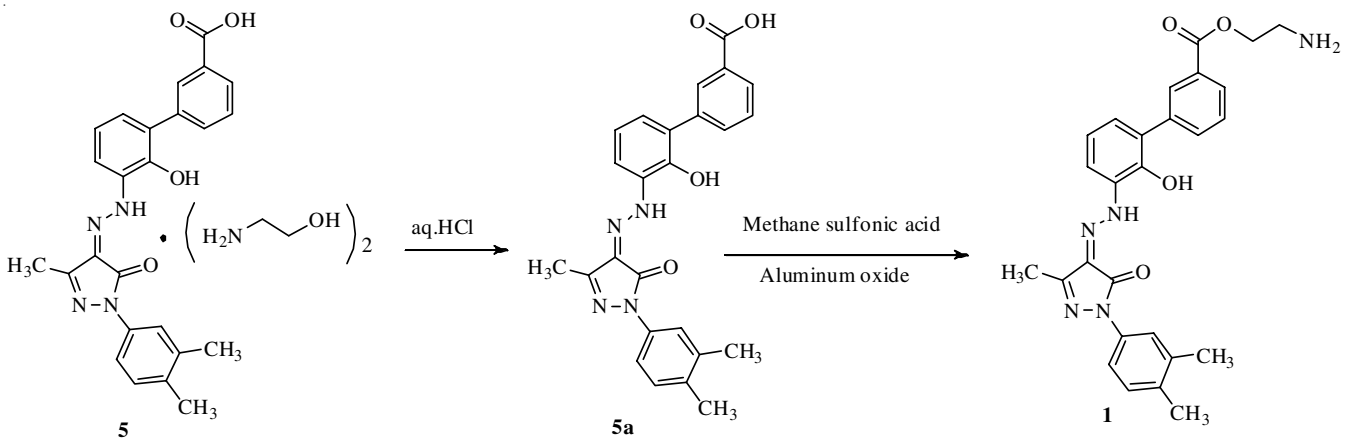
INTRODUCTION

Eltrombopag olamine is an anti-thrombocytopenic drug and chemically known as 3′-{(2*Z*)-2-[1(3,4-dimethyl phenyl)-3-methyl-5-oxo-1,5-dihydro-4*H*-pyrazol-4-ylidene]hydrazino}-2′-hydroxy-3-biphenyl carboxylic acid, bisethanol amine [1,2]. This drug is a small thromboprotein receptor agonist for oral administration [3-5] and interacts with the transmembrane domain of the thromboprotein receptor leading to increase the platelet production.

The major challenge for the organic chemist is to produce a quality, safe, reproducible synthetic schemes within the developmental space. Furthermore, it is extremely challenging to identify the impurities or related substances which are important components arise in trace level during the synthesis of drug substance. Besides, the presence of impurities in an active pharmaceutical ingredient (API) has a significant impact on the quality and safety of the drug. Therefore , it is always recommended by ICH guidelines to study the origin and control of such impurities which are present in the API at a level ≤ 0.05% w/w [6,7] and also important to identify, synthesize and characterize such impurities in a pure form to check the analytical parameters such as specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ) and relative retention [7]. Due to the stringent impurity profiling of API from the drug regulatory authorities and their importance we herein report the synthesis and characterization of four potential impurities related to eltrombopag olamine, an anti-thrombocytopenic drug *viz*. eltrombopag olamine ester (**1**), 2-amino-phenol analogue of eltrombopag (**2**), 3,3′-(2-amino-3-oxo-3*H*-phenoxazine-4,6-diyl dibenzoic acid (**3**), 2′-hydroxy[1,1-biphenyl]-3-carboxylic acid (**4**) are reported. In the above mentioned impurities **2** and **3** are the process related impurities from the key intermediate 3′-amino-2′-hydroxy biphenyl-3-carboxylic acid, while impurities **1** and **4** are the process degradant products from eltrombopag olamine.

EXPERIMENTAL

Melting points were determined using Reichert Thermopan apparatus. ^{1}H & ^{13}C NMR spectra were recorded with a Bruker Avance 300MHz and Varian 500 MHz spectrometers, respectively using TMS as internal standard in DMSO-d_6. IR spectra were recorded using Perkin-Elmer Spectrum One Fourier

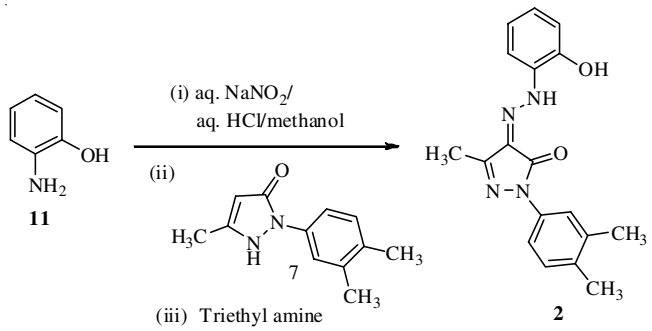


Scheme-I: Synthesis of eltrombopag olamine ester (**1**)

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 ºC. UV detection occurred at λ = 240 nm. Reagents and solvents were procured from the commercial sources.

Preparation of eltrombopag olamine ester (1): Suspended aluminium oxide (14 g, 135.7 mmol) in methane sulfonic acid (65.15 g, 678 mmol) was mixed with eltrombopag free base (20 g, 45 mmol), monoethanol amine (3 g, 41.5 mmol) at 25-30 ºC. Heated the reaction mass to 75-80 ºC and stirred the reaction mass for 24 h and poured the reaction mass in to ice cold demineralized water resulted in a solid crude, which was precipitated out. Purified the solid crude by column chromatography with tetrahydrofuran:methylene chloride to yield eltrombopag olamine ester (**1**) as a orange colour solid [8] (**Scheme-I**). Yield: 15 g (68%); Purity: 96.28%, m.p.: 182 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.085-2.188 (t, 1H), 2.225-2.513 (m, 9H), 2.730-2.732 (s, 1H), 2.890 (s,1H), 3.168-3.546 (t, 2H), 4.467 (s, 2H), 7.057-8.338 (m, 10H), 14.923 (brs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 11.562, 18.865, 19.673, 38.053, 41.199, 57.443, 61.644, 114.058, 115.368, 118.929, 121.990, 127.210, 128.416, 128.701, 128.923, 129.535, 129.807, 130.184, 130.656, 132.249, 132.857, 134.150, 135.756, 136.811, 137.810, 142.540, 147.838, 150.826, 156.895, 165.599; HRMS (ESI, QTOF) for $C_{27}H_{27}N_5O_4$[M+H]$^+$: *m/z* calcd. (found): 486.2141 (486.2158).

Synthesis of 2-amino phenol analogue of eltrombopag olamine (2): Added aqueous 4 M HCl (24 mL) to suspension of 2-amino phenol (5 g, 46 mmol) in methanol (150 mL) followed by the addition of 35% w/w aqueous sodium nitrite solution (10 g, 50 mmol) at 0-5 ºC and stirred the reaction mixture at the same temperature for 2 h. Now added compound **7** (50 mmol), triethylamine (13.95 g, 137.85 mmol) at 0-5 ºC and stirred the reaction mixture for 30 min, then slowly raised the reaction mixture to 20-25 ºC, again stirred the reaction mixture at the same temperature for 2 h, filtered, washed with methanol (10 mL) and dried under reduced pressure at 55 ºC to obtained compound **2** as orange colour solid (**Scheme-II**).

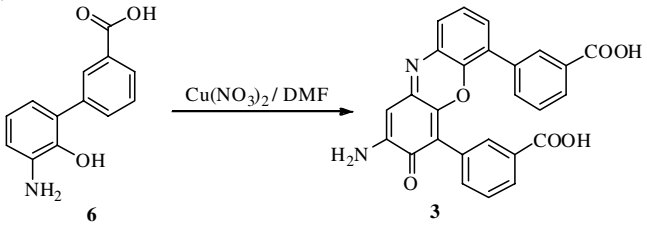


Scheme-II: Synthesis of 2-aminophenol analogue of eltrombopag (**2**)

Yield: 10 g (68%); Purity: 99.61%; m.p.: 195 ºC. IR (KBr, ν_{max}, cm^{-1}): 3402 (phenolic-OH), 3048 (mononuclear arom. 1,3,4-substituted), 1704 (C=O, amide); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.226-2.488 (s, 6H), 2.494-2.512 (s, 3H), 6.918-7.211 (m, 4H), 7.608-7.712 (m, 3H), 10.623 (brs, 1H), 13.625 (brs, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 11.508, 18.845, 19.626, 41.163, 57.429, 114.320, 115.312, 115.816, 118.856, 120.170, 126.295, 127.931, 128.904, 129.744, 132.724, 135.789, 136.752, 146.289, 147.670, 156.946; HRMS (ESI, QTOF) for $C_{18}H_{18}N_4O_2$[M+H]$^+$: *m/z* calcd. (found): 323.1508 (323.1478).

Synthesis of 3,3′-(2-amino-3-oxo-3*H*-phenoxazine-4,6-diyl)dibenzoic acid (3): Cupric nitrate (2.65 g, 100 mmoL) was added to a solution of compound **6** (25 g, 109 mmol) in DMF (150 mL) at 25-30 ºC. Stirred the reaction mass at 60-65 ºC for 24 h and thereafter it was concentrated under vacuum at 55-60 ºC. Purified the crude product by column chromatography [9] (**Scheme-III**). Yield: 22 g (45%); Purity: 95.47%; m.p.: 175 ºC; IR (KBr, ν_{max}, cm^{-1}): 3329 (arom. NH_2), 2955 (OH), 1266 (C-O). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.2-7.9 (m, 12H), 1.15-1.20 (brs, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 38.661, 38.939, 39.218, 39.774, 40.330, 98.155, 114.797, 125.120, 127.398, 127.786, 128.094, 128.310, 129.396, 129.945, 130.315, 130.845, 131.048, 131.667, 133.511, 133.951, 134.851, 135.198, 139.028, 144.531, 147.061, 147.815, 166.889, 167.100, 178.660; HRMS (ESI, QTOF) for $C_{26}H_{16}N_2O_6$[M+H]$^+$: *m/z* calcd. (found): 453.1087 (453.1091).

Synthesis of 2′-hydroxy[1,1-biphenyl]-3-carboxylic acid (4): Compound **6** (5 g, 21.8 mmol) was added to mixture



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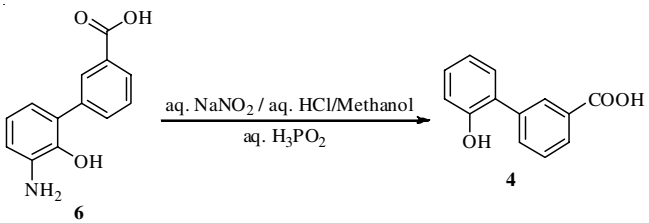
Scheme-III: Synthesis of 3,3′-(2-amino-3-oxo-3*H*-phenoxazine-4,6-diyl)-dibenzoic acid (**3**)

of methanol (100 mL) and 4 M aqueous HCl (8 mL) at 0-5 ºC and followed by the addition of sodium nitrite (1.65 g, 7 mmol). Now added demineralized water (5 mL) and stirred the reaction for 1 h at 0-5 ºC. Added 50% w/w aqueous hypophosphorous acid solution (4.3 g, 32 mmol) and raised the reaction mixture to 25-30 ºC. Thereafter, slowly raised the reaction mass temperature to 65-70 ºC and reaction was completed in 6 h, by checking TLC and concentrated the reaction mass under reduced pressure. Dissolved the residue in methylene chloride (100 mL) and then added demineralized water (50 mL). Now separated the organic layer and evaporated under vacuum and purified the crude compound by colum chromatography with using ethyl acetate and hexane (**Scheme-IV**). Yield: 3.74 g (80%); Purity: 92.24%; m.p.: 143 ºC. IR (KBr, ν_{max}, cm^{-1}): 3329 (arom. NH_2), 2955 (OH), 1266 (C-O);^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 6.873-8.137 (m, 8H), 9.620 (s, 1H), 12.896 (brs, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 116.146, 119.606, 126.759, 127.441, 128.301, 128.988, 129.991, 130.284, 130.510, 133.416, 138.836, 154.317, 167.456; HRMS (ESI, QTOF) for $C_{26}H_{16}N_2O_6$[M-H]$^-$: *m/z* calcd. (found): 213.0552 (213.0557).



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Scheme-IV: Synthesis of 2′-hydroxy[1,1-biphenyl]-3-carboxylic acid (**4**)

RESULTS AND DISCUSSION

Various synthetic routes are available in literature for the preparation of eltrombopag olamine [10,11]. Duffy *et al*. [4] reported the synthesis of eltrombopag, which is poorly soluble in water. However, this compound has adverse effects and further developed as pharmaceutically acceptable salt namely eltrombopag olamine [12,13]. The process involves usage of expensive reagents and very low isolation yield. Currently, a developed method [14-16] involving inexpensive reagents is shown in **Scheme-V**. According to **Scheme-V**, the synthesis of eltrombopag olamine (**5**) involves the protection of 2-bromo-6-nitro phenol (**12**) with benzyl chloride in the presence of K_2CO_3 and catalytic amount of tetrabutylammonium iodide in acetonitrile to afford benzyl protected-2-bromo-6-nitro phenol (**13**). Compound **13** was condensed with 3-carboxy phenyl boranic acid with using 10% PdC (50% wet), Na_2CO_3 in methanol to afford 2′-benzyloxy-3′-nitro biphenyl-3-carboxylic acid (**10**).

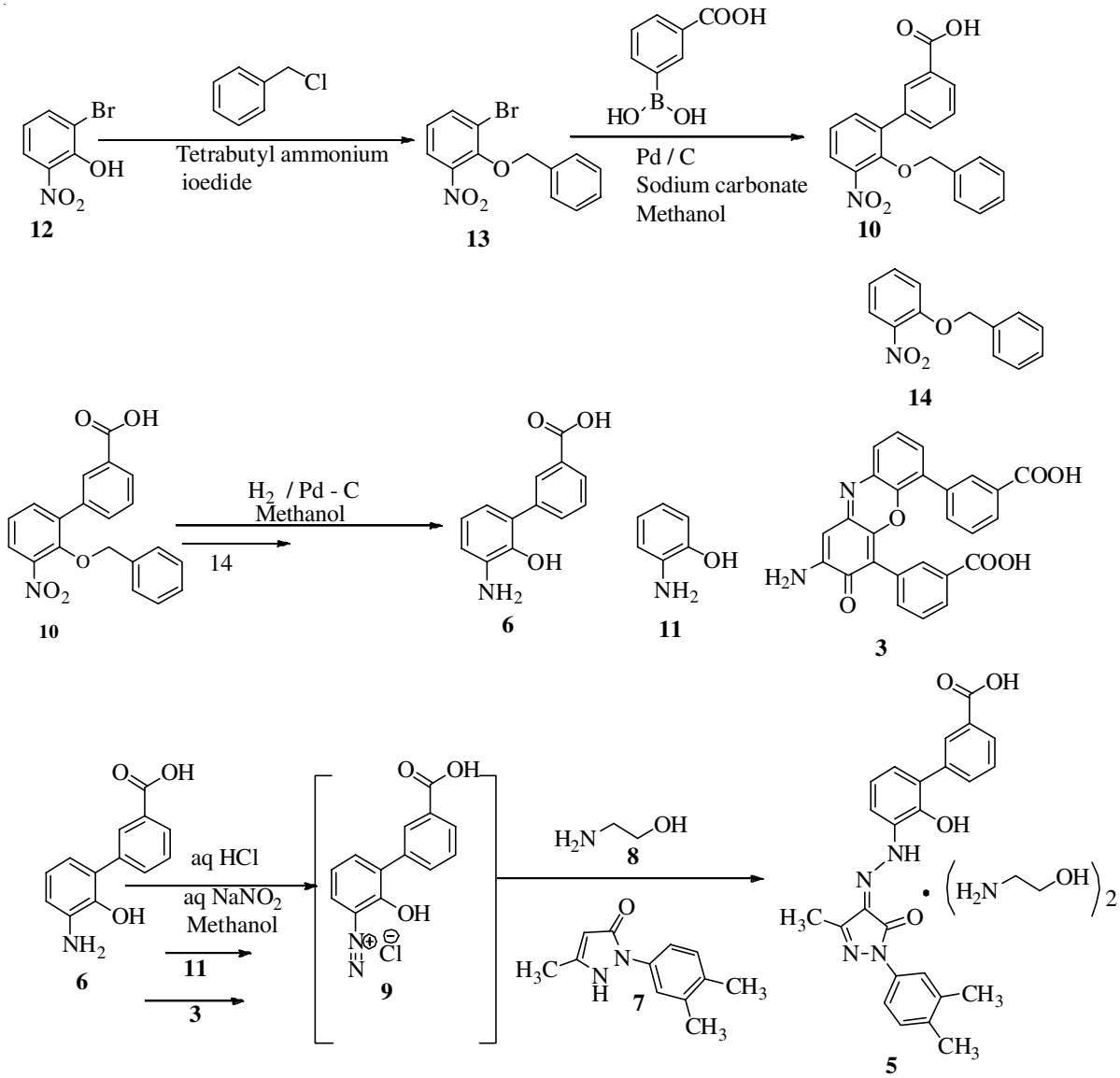
While searching the literature [17], it is observed that 2′-benzyloxy-3′-nitro biphenyl-3-carboxylic acid (**10**) undergoes a hydrogenation with PdC in methanol to afford [18] 3′-amino-2′-hydroxy biphenyl-3-carboxylic acid (**6**) during these process leads to a formation of impurities namely 3,3′-(2-amino-3-oxo-3*H*-phenoxazine-4,6-diyl)-dibenzoic acid (**3**) and surprisingly another identified the process degradant impurity 2-amino phenol (**11**). Impurity **3** did not participate in the reaction of **5** but it appeared as a contaminant in crude eltrombopag olamine (**5**) and 2-amino phenol (**11**) was identified by HPLC. It is further participated in eltrombopag olamine (**5**) reaction, which involves in the diazotization with aqueous HCl and $NaNO_2$ followed by condensation with 2′-(3,4-dimethyl phenyl)-5-methyl-(1*H*)-pyrazole-3-(2*H*)-one (**7**) in the presence of base mono ethanolamine (**8**) to afforded 2-amino phenol analogue of eltrombopag (**2**) with crude eltrombopag olamine (**5**) [19].

Impurity **1** was formed in trace level during the condensation of biphenyl-3-carboxy-2′-hydroxy-3′-diazonium chloride (**9**) with 2′-(3,4-dimethyl phenyl)-5-methyl-(1*H*)-pyrazole-3-(2*H*)-one (**7**) in the presence of base mono ethanol amine (**8**) at 60-65 ºC for longer time (48 h). Impurity **4** was formed in trace during the condensation of compound **9** with compound **7**, where a loss of azo group of compound **9** leads to afford impurity **4** [14,15].

The formation of impurity **1** is due to the thermal degradation of eltrombopag olamine, results in the esterification of eltrombopag with monoethanol amine. It is difficult to synthesize impurity **1** in pure form because every time amide impurity was obtained since the carboxylic group of eltrombopag having high vicinity to react with amine group of mono ethanol amine. However, we succeeded to prepare impurity **1** using an alternative route, acidified compound **5** with aqueous HCl results in the formation of a free acid compound **5a**. Coupling of free acid compound **5a** with mono ethanolamine (**8**) in the presence of methane sulfonic acid, aluminium oxide to afford a crude compound [8], which was purified by column chromatography. For controlling of this impurity, attempted several experiments and finally achieved the suitable procedure for the synthesis of compound **5** and performed the reaction at 5-30 ºC.

It is very interesting to study of the formation of 2-amino phenol analogue of eltrombopag olamine (**2**). A Suzuki reaction of compound **13** was condensed with 3-carboxy phenyl boranic acid with Pd/C and Na_2CO_3 in methanol medium leads to the formation of compound **14** along with compound **10**, which undergoes hydrogenation with Pd/C with H_2 gas results in the formation of compounds **6** and **11**. Compound **11** also participates in the final reaction of compound **5** to form trace level of impurity **2** (**Scheme-III**). Alternatively, synthesis of impurity **2** can also be obtained by treating 2-aminophenol with aqueous sodium nitrite and aqueous HCl and followed by condensation with 2′-(3,4-dimethyl phenyl)-5-methyl-(1*H*)-pyrazole-3-(2*H*)-one (**7**) in the presence of triethylamine leads to the formation of impurity **2**

To control this impurity in compound **5**, 2-aminophenol content is limited to not more than 0.1% (w/w) in the specification of 3′-amino-2′-hydroxy biphenyl-3-carboxylic acid (**6**)

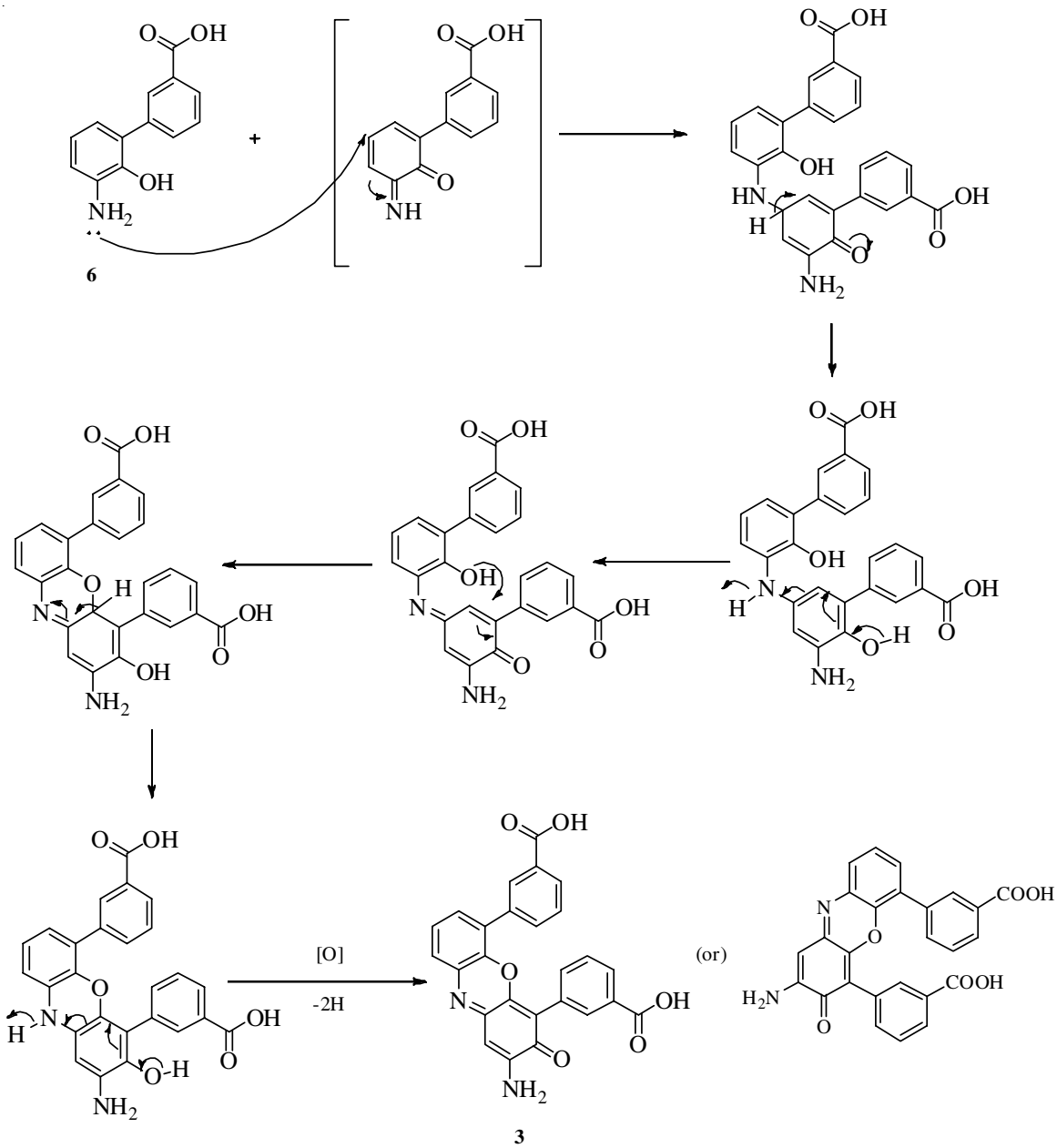


Scheme-V: Synthetic scheme of eltrombopag olamine (**5**)

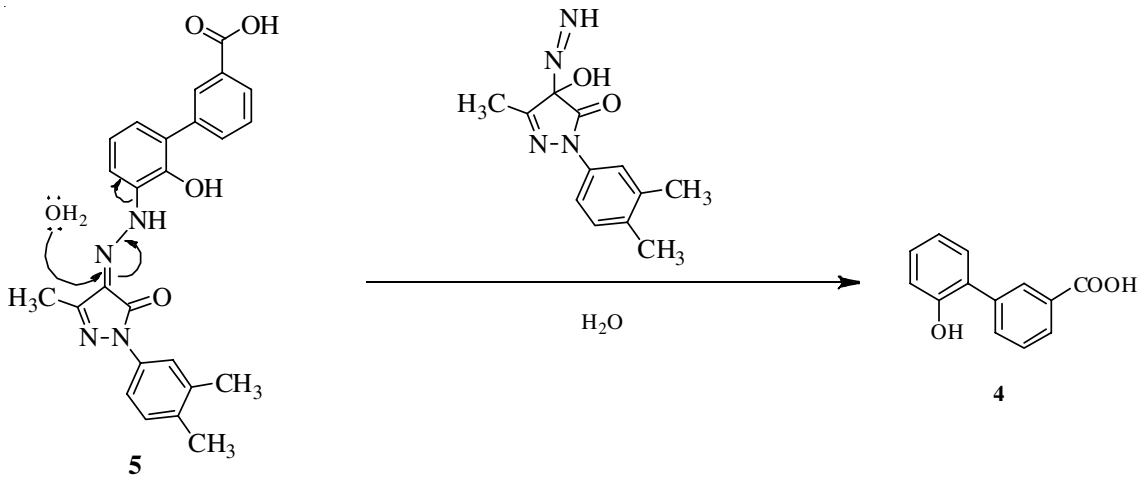
controls 2-aminophenol analogue of eltrombopag. Further, the above specification limit of 2-aminophenol in compound **6** also controls 2-aminophenol less than 31.3 µg (30% of acceptable limit) in eltrombopag olamine drug substance as per ICH M7.

It is quite interesting to know the formation of impurity **3**, during the preparation of compound **6**. This can be understood from the plausible mechanism of the formation of compound **3**. Preparation of impurity **3** involves (**Scheme-VI**) by treating of compound **6** with cupric nitrate in DMF medium leads to the formation of compound **3** and further purified by column chromatography. This impurity was carry forward from compound **6** to compound **5** but it not participated in the final reaction of compound **5**. The plausible mechanism of impurity **3** reveals the route that is during the synthesis of compound **6** from compound **10**, the oxidative degradation leads to to form impurity **3**. In order to control this impurity, this impurity was washed out during purification of compound **5** with DMSO and ethanol. This impurity is controlled to the limit less than 0.1% in eltrombopag olamine drug substance as per ICH Q3A.

2′-Hydroxy[1,1′-biphenyl]-3-carboxylic acid (**4**) is a degradation impurity. It was believed that this impurity originates due to elimination of diazonium group from biphenyl-3-carboxy-2-hydroxy-3′-diazonium chloride (**9**), during the synthesis of eltrombopag olamine crude and can carry over to eltrombopag olamine drug substance, and also further the hydrolytic degradation of Eltrombopag results in impurity **4**. To control the impurity **4** during the isolation of eltrombopag olamine crude implemented aqueous methanol followed by purification from a mixture of DMSO and ethanol this impurity is controlled to the limit (< 0.15%) in eltrombopag olamine drug substance as per ICH Q3A. Synthesis of this impurity (**Scheme-VII**) involves by treating of compound **6** with aqueous sodium nitrite, aqueous HCl, aqueous hypo phosphorous acid solution leads to form impurity **4** and isolated in form by using column chromatography.



Scheme-VI: Plausible mechanism of 3,3′-(2-amino-3-oxo-3*H*-phenoxazine-4,6-diyl)dibenzoic acid (**3**)



Scheme-VII: Plausible mechanism of 2′-hydroxy[1,1-biphenyl]-3-carboxylic acid (**4**)

Conclusion

In this work, a successful effort was made to synthesize the four potential impurities related to eltrombopag olamine *viz*. eltrombopag olamine ester (**1**), 2-amino phenol analogue of eltrombopag (**2**), 3,3′-(2-amino-3-oxo-3*H*-phenoxazine-4,6-diyl dibenzoic acid (**3**), 2′-hydroxy[1,1-biphenyl]-3-carboxylic acid (**4**). These potential impurities will allow to understand the actual cause for its generation and control in future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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