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Synthesis and Characterization of Related Substances Observed in Macitentan, an Endothelien Receptor Antagonist

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ABSTRACT

During the process development of Macitentan (1), four related substance (impurities) were detected by high performance liquid chromatography (HPLC) in the final crude material ranging from 0.20 to 0.50% and these are identified by Liquid chromatography-mass spectrometry (LC–MS). All impurities were subsequently synthesized, characterized by spectroscopic techniques and further analyzed and confirmed by chromatographic techniques by spiking and purging study and this impurities are namely 2, 2'-{[5-(4-bromophenyl) pyrimidine-4,6-diyl]bis(oxy)}diethanol (8), 5-(4-bromo-phenyl)-4,6-bis-[2-(5-bromo-pyrimidin-2-yloxy)-ethoxy]- pyrimidine (9), N-[5-(4-bromophenyl)-6-(2-{[5-(4-bromophenyl)-6-chloropyrimidin-4-yl]oxy}ethoxy)-4-pyrimidinyl] -N'-propylsulfamide (10) and 2-{[5-(4-bromophenyl)-6-{[(propylamino)sulfonyl]amino} pyrimidin-4-yl]oxy}ethylacetate (11).

Keywords: Macitentan, Antagonist, Impurities, Synthesis.

I. INTRODUCTION

The Macitentan (OPSUMIT®), an orally active endothelin receptor antagonist (ERA), is approved for treatment of pulmonary arterial hypertension (PAH), and is chemically also known as *N*-[5-(4-bromophenyl)-6-[2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy]-4-pyrimidinyl]-*N*'-propylsulfamide [1]. Macitentan is a dual ERA, i.e. acts as an antagonist of two endothelin (ET) receptor subtype, ETA and ETB [1]. However, Macitentan has 50-fold increased selectivity for the ET_A subtype compared to the ETB subtype [2]. Macitentan blocks the ET1-dependent rise in intracellular calcium by inhibiting the binding of ET-1 to ET receptors. Blocking of the ET_A receptor subtype seems to be of more importance in the treatment of PAH than blocking of ET_B, likely because there are higher numbers of ET_A receptors than ET_B receptors in pulmonary arterial smooth muscle cells [3-7].

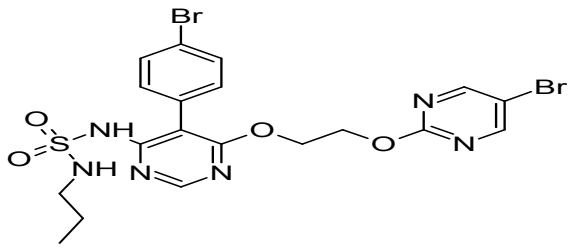
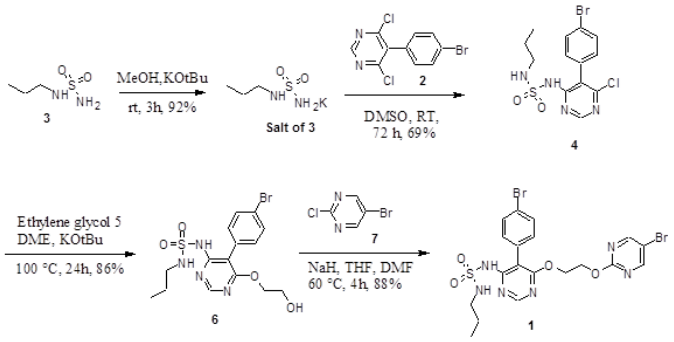


Figure 1. Structure of Macitentan **(1)**

The presence of impurities in drug substance can have a significant impact on the quality and safety of the drug product. The acceptance criteria of these impurities are stringent i.e. acceptable level for all impurities present in the drug should be less than 0.10% based on the guideline laid down by the international conference on harmonization (ICH) and pharmacopeia [8]. In order to meet these impurities present in the drug substance greater than above mentioned value must be identified and characterized. It is equally important to have impurity in pure form required for analytical development such as specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), and relative retention factor. Thus; it is essential and important to establish the facile and robust synthesis for the related substances and their characterization during drug development activity. During the laboratory development of Macitentan various related substances were observed in crude samples, based on LC-MS study and its fragmentation patterns, probable structures were predicted, synthesized the predicted compounds and further confirmed by spiking & purging study by HPLC. Present work describes identification, synthesis, and characterization of four related impurities (**8, 9, 10,** and **11**) during development of Macitentan (**1**).

II. RESULT AND DISCUSSION

Macitentan (**1**) was synthesized by known literature synthetic methods [9, 10] (Scheme-1). It involves the reaction of *N*-propylsulfamide (**3**) with potassium tertiary butoxide (KOtBu) in presence methanol to provide potassium salt of **3**, which is then condensed with 5-(4-bromophenyl)-4,6-dichloropyrimidine (**2**) in dimethylsulfoxide (DMSO) to provide 6-chloro compound (**4**). Compound **4** was then reacted with ethylene glycol (**5**) in presence of potassium tertiary butoxide in dimethoxy ethane (DME) to provide 2-hydroxyethoxy compound (**6**). Finally, nucleophilic substitution of **6** with 5-bromo-2-chloropyrimidine (**7**) in presence of sodium hydride (NaH) in tetrahydrofuran (THF) and dimethylformamide (DMF) resulted in desired Macitentan (**1**).



Scheme 1. Reported synthetic scheme for Macitentan **(1)**

During the development we observed that crude sample of Macitentan containing 4 unknown peaks, further our purification process for **1** is capable to remove these impurities but resulted in less yield of **1**. Hence, for identification of unknown peaks appeared at RRT 0.10, 1.50 and 1.60 and 0.50 and further avoiding their formation in reaction the crude sample of Macitentan (**1**) was subjected for LC-MS analysis. The relative molecular mass of these of unknown peaks appeared at RRT 0.10, 1.50 and 1.60 and 0.50 are 357 (**8**), 475 (**9**), 671 (**10**) and 699 (**11**) respectively by LC-MS based on the molecular mass of compound predicted the probable structures for these impurities and they are namely 2,2'-{[5-(4-bromophenyl)pyrimidine-4,6-diyl]bis(oxy)}diethanol (**8**), 5-(4-bromo-phenyl)-4,6-bis-[2-(5-bromo-pyrimidin-2-yloxy)-ethoxy]-pyrimidine **(9),** *N*-[5-(4-bromophenyl)-6-(2-{[5-(4-bromophenyl)-6-chloropyrimidin-4-yl]oxy}ethoxy)-4-pyrimidinyl] -*N*'-propylsulfamide (**10),** 2-{[5-(4-bromophenyl)-6-{[(propylamino)sulfonyl] amino} pyrimidin-4-yl] oxy}ethylacetate (**11**) and further theire chemical structures are captured in Figure 2.

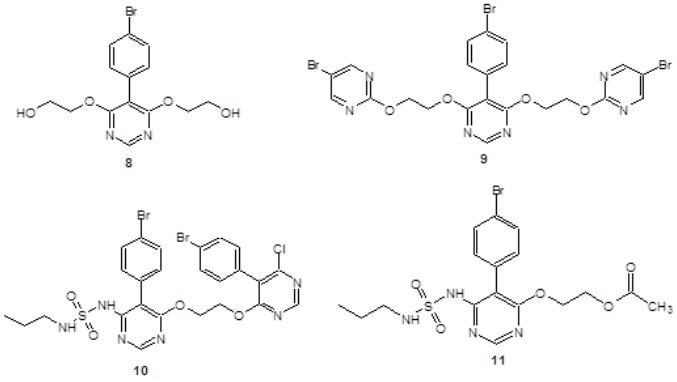
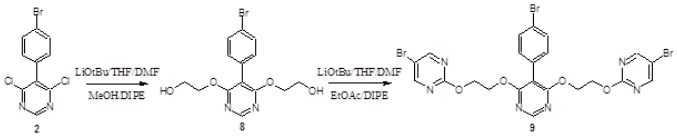


Figure 2. Structure of Macitentan related substances.

Further to confirm these probable impurities **8, 9, 10** and **11** we synthesized these impurities by possible synthetic path and were further confirmed by spectroscopic and chromatographic data followed by spiking and purging study by HPLC. The detailed synthesis of impurities **8, 9, 10** & **11** and subsequently their formation in reaction mass of **1** are as follows,

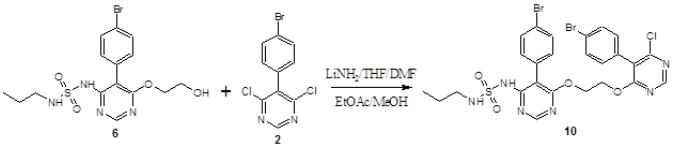
Synthesis of impurity 8: The nucleophilic substitution of unreacted 5-(4-bromophenyl)-4,6-dichloropyrimidine (**2**) with two molecules of ethylene glycol (**5**) in presence of potassium tert. butoxide in DMSO leads to formation of impurity **8**. It is further synthesized by condensing **2** with **5** in presence of lithium *tert*. butoxide in DMSO at elevated temperature to provide white solid powder of **8** (Scheme 2)**.**

Synthesis of impurity 9: The impurity **9** was formed due to traces amount of **8** was present in **6**, which was condensed with two molecules of **7** in the presence of KOtBu in DMF and THF. The impurity **9** was synthesized by condensing **8** with **7** in presence of lithium tert. butoxide in THF and DMF at elevated temperature (Scheme 2).



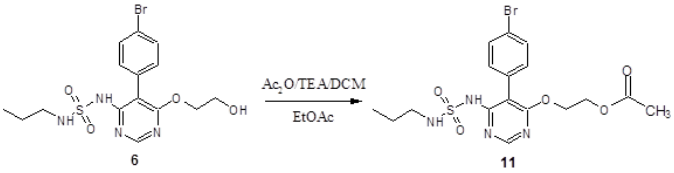
Scheme 2. Synthesis of impurity **8**, and **9**

Synthesis of impurity 10: The impurity **10** was formed during the synthesis of **6;** compound **6** was condensed with readily available **2** in presence of KOtBu in DMSO. Impurity **10** was synthesized by condensation of **6** with **2** in the presence of lithium tert. butoxide in THF and DMF at elevated temperature to give compound **10** (scheme 3).



Scheme 3. Synthesis of impurity **10**

Synthesis of impurity 11: Impurity **11** was formed due to side reaction of **6** with ethyl acetate which is used as a solvent for the extraction, leads to the formation of acetate compound **11**. This impurity was synthesized by the reaction of **9** with acetic anhydride in the presence of lithium tert. Butoxide to provide impurity **11** (Scheme 4).



Scheme 4. Synthesis of impurity **11**

III. CONCLUSIONS

In conclusion, we have successfully identified, four process related impurities of Macitentan namely, **8**, **9**, **10,** and **11** by LC-MS data and which is further confirmed by spiking and purging study after synthesizing and characterization of these impurities.

IV. EXPERIMENTAL SECTION

Melting points were determined on Analab melting point apparatus, in open capillary tubes and are uncorrected. The 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded on a Varian Gemini 400 MHz FT NMR spectrometer. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane as internal standard and are given in δ units. The solvents used for NMR spectra were deuterated chloroform and deuterated dimethylsulfoxide unless otherwise stated. Infrared spectra were taken on Perkin Elmer Spectrum 100 in potassium bromide pallets unless otherwise stated. Elemental analyses were performed on a Hosli CH-Analyzer and the results were within ±0.3 % of the calculated values. High-resolution mass spectra were obtained with a Shimadzu GC–MS QP mass spectrometer with an ionization potential of 70 eV. All the reaction were monitored by thin layer chromatography (TLC), carried out on 0.2 mm silica gel 60F254 (Merck) plates using UV light (254 and 366 nm) or High performance liquid chromatography (HPLC) on Agilent Technologies 1200 series for detection. Gas chromatography on Agilent Technologies 7683B with head space was used for analyzing the residual solvents. Common reagent–grade chemicals are either commercially available and were used without further purification or were prepared by standard literature procedures.

Synthesis of 2,2'-{[5-(4-bromophenyl)pyrimidine-4,6-diyl]bis(oxy)}diethanol (8)

To a solution of lithium tert. butoxide (17.0 g, 0.21 mol) in DMSO (100 ml) and ethylene glycol **5** (250 ml), added compound **2** (25.0 g, 0.08 mol) at 25-30°C. The reaction mass was heated to 55-60 °C and maintained for 2-6 hrs. After completion of reaction monitored by HPLC, reaction mass was cooled to 25-30 °C. Quenched the reaction mass by 5% citric acid solution in water (250 ml), and product were extracted with ethyl acetate (250 x 2 ml), organic layer was washed with 5% brine solution (250 ml). The organic layer was concentrated under reduced pressure at below 55 °C to obtain syrup. The resulting syrup was dissolved in methanol (75 ml) and heated to 60-65 °C, added DIPE (200 ml) at 60-65 °C. The precipitated solid was cooled to 25-30 °C and maintained for 45-60 min. The obtained solid was filtered, washed, and dried under vacuum at 45-50°C for 2-4 h to provide title compound **8**. Yield: 16.5 g (72.0 %). IR (cm^{-1}): 3383.75, 2950, 1577, 1438, 1314, 1100, 827. MS *m/z* (%): 357.0 [M^+ + 1]. ^{1}HNMR (CDCl3, 300 MHz): δ 8.72 (s, 1H), 7.48-7.51 (d, 2H), 7.40-7.42 (d, 2H), 4.25-4.28 (t, 4H), 3.91-3.95 (t, 4H), 2.93-2.95 (t, 2H). ^{13}C NMR (DMSO, 300 MHz): δ 172.21, 152.34, 142.23, 134.62, 129.2, 122.21, 114.43, 78.34, 63.32. Chemical purity by HPLC: 97.15%

Synthesis of 5-(4-bromo-phenyl)-4,6-bis-[2-(5-bromo-pyrimidin-2-yloxy)-ethoxy]-pyrimidine (9)

To a stirred solution of **8** (10 g, 0.028 mol) in a mixture of DMF (100 ml) and THF (100 ml) at 25-30°C, were added lithium tert. butoxide (7.0 g, 2.5 mol) followed by **7** (12.0 g, 0.0 mol). The reaction mass was heated to 55-60 °C and maintained for 4-6 h. After completion of

reaction monitored by HPLC, cooled the reaction mass to 25-30°C. The reaction mass was quenched with water (500.0 ml), and adjusted pH 5-6 using acetic acid. The product was extracted with ethyl acetate, and organic layer was washed with 5 % brine solution in water (250 ml). The organic layer were concentrated under vacuum at below 50 °C to obtain syrup. The syrup was diluted in DIPE (100 ml) and heated to 55-60 °C. Precipitated solid was cooled to 25-30 °C and maintained for 60 min. The obtained solid was filtered, washed, and dried under vacuum at 45-50°C for 3 h to offer title compound **9**. Yield: 7.6 g, (40 %). Purity by HPLC: 96.21%. IR (cm^{-1}): 3445, 2957, 1569, 1430, 1308, 1120, 1064, 822. MS *m/z* (%): 670.9 [M^+ + 1]. ^{1}HNMR (DMSO, 300 MHz): δ 8.71 (s, 4H), 8.46 (s, 1H), 7.40-7.42 (d, 2H), 7.25-7.28 (d, 2H), 4.60-4.69 (t, 8H). ^{13}C NMR (DMSO, 300 MHz): δ 166.16, 163.11, 159.72, 155.70, 132.33, 130.49, 129.12, 120.65, 111.88, 103.87, 65.50, 64.91.

Synthesis of *N*-[5-(4-bromophenyl)-6-(2-{[5-(4-bromophenyl)-6-chloropyrimidin-4-yl]oxy}ethoxy)-4-pyrimidinyl]-*N*'-propylsulfamide (10)

To a stirred solution of compound (**6**) (25.0 g, 0.057 mol) in a mixture of THF (100 ml) and DMF (100 ml) at 25-30 °C, added lithium amide (), followed by **2** (18.0 g, 0.059 mol). Heated the reaction mass to 55-60 °C and maintained for 2-4 h. After completion of reaction monitered by HPLC, cooled the reaction mass to 25-30°C and quenched with 5 % citric acid solution in water (500 ml). The product was extracted with ethyl acetate (500 X 2 ml), washed with water (500 x 2 ml), and concentrated organic layer under vacuum to obtain syrup. The syrup was diluted in methanol (100 ml) and heated to 60-65 °C. Cooled the reaction mass to 25-30 °C and maintained for 60 min. The obtained solid was filtered, washed, and dried under vacuum at 45-50 °C for 2h to provide title compound 10. Yield: 35.0 g, (86.40%). IR (cm^{-1}): 3314, 2953, 1571, 1434, 1305, 1173, 1045, 826. MS *m/z* (%): 699.0 [M^+ + 1]. ^{1}HNMR (CDCl3, 300 MHz): δ 9.23 (s, 1H), 8.12 (s, 1H), 7.52-7.49 (dd, 4H), 7.24-7.29 (dd, 4H), 4.46-4.48 (t, 4H), 3.2 (t, 2H), 2.1 (m, 3H), 0.98-1.01 (t, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 172.09, 170.01, 169.36, 163.24, 154.21, 152,21, 140.83, 139.84, 132.21, 128.06, 128.26, 127.66, 127.76, 127.41, 127.48, 114.96, 41.1, 23.54, 11.10. Chemical purity by HPLC: 98.57%.

Synthesis of 2-{[5-(4-bromophenyl)-6-{[(propylamino)sulfonyl]amino}pyrimidin-4-yl]oxy}ethyl acetate (11)

To a stirred solution of compound (**6**) (10.0 g, 0.23 mol) in DCM (50 ml) at 25-30 °C, added triethyl amine (25 ml, 0.24 mol) followed by acetic anhydride (3.6 g, 0.035 mol), and maintained for 25-30 °C for 2-4 h. After completion reaction monitored by HPLC, quenched reaction mass with water (100 ml) and product was extracted in DCM (100 ml). The organic layer was washed with water (50 ml), and concentrated under vacuum below 45 °C to provide title compound **11** as an oil. Yield: 8.0 g, (73.0 %). IR (cm^{-1}): 3282, 2968, 1738, 1658, 1574, 1436, 1390, 1171, 1087, 829. MS *m/z* (%): 475.0 [M^+ + 1]. ^{1}HNMR (CDCl3, 300 MHz): δ 8.47 (s, 1H), 7.58-7.64 (dd, 2H), 7.17-7.27 (dd, 2H), 4.54-4.57 (t, 2H), 4.29-4.32 (t, 2H), 2.96 (t, 2H), 2.00 (s, 3H), 1.55-1.62 (m, 2H), 0.91-1.05 (m, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 170.54, 165.95, 157.68-156.18, 151.68, 132.38-136.46, 131.57-131.77, 127.89-12859, 122.78-123.09, 104.48, 65.29, 64.42-64.59, 61.83-61.92, 53.51, 46.39, 22.29, 20.60-20.90, 10.87-11.18. Chemical purity by HPLC: 98.65%.

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