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Synthesis and Characterisation of Fingolimod Impurities: A Drug for Multiple Sclerosis

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ABSTRACT

Fingolimod (1) is an immunomodulating drug, frequently used for treating Multiple Sclerosis (MS) approved by the US-FDA. During the process development of Fingolimod, some process related impurities were observed along with the final API. These impurities were identified as N,N-dimethyl (2), N-methyl (3), nitromono methyl (4), monomethyl (5), nitrohydroxy (6) and hydroxy (7) impurities. The present work describes the synthesis and characterization of these impurities.

Keywords: Fingolimod, N N-dimethyl, N-methyl, Mono methyl, Impurities

INTRODUCTION

In the year 1992, fingolimod (1, trade name as Gilenya) was first synthesized [1,2]. It is an immunomodulating drug, and was approved by US-FDA for treating multiple sclerosis (MS) in 2010. *In-vivo* this drug forms sphingosine 1-phosphate receptor modulator by phosphorylation with sphingosine kinase 2, later this phosphate moiety binds to extracellular G-protein-coupled receptors and prevents the lease of lymphocytes from lymphoid tissue thus preventing them from contributing to an autoimmune reaction. This process results a neural restoration and protection process, which can reduce MS recurrence rate, drops the progression of damage, decrease intracranial Magnetic Resonance Imaging (MRI), the number of lesions and decrease the severity of the lesions [3,4]. As Gilenya is the present FDA approved first-line treatment for Multiple Sclerosis (MS) that is orally available, fingolimod (1) has the potential to recast the therapy of this debilitating disease [5-7].

Now a day's medicines or drugs have become an essential element of our human life to combat with various existing and new coming deceases. Unlike earlier days, most of the medicine in current years is entirely synthetically made and the synthetic medicine certainly contain different impurities either chemical or microbial majorly the impurities are chemical impurities only. The process-related impurities in a drug (API) can have a significant impact on the quality and safety of the drug products/drug substance. The impurity levels in any drug substance are determined as per its biological or toxicological data. It is very important for "regulatory" point of view for drug approval and to select the limitations of "related impurities." Therefore, it is very essential to study the impurity profile of any drugs (API) and minimize it during the development and manufacturing process of a drug product or drug substance. As per the ICH Q3 guidelines, any impurities which are forming at a level of ≥ 0.10% w/w with respect to the API should be synthesized, identified, and characterized thoroughly throughout the development [8].

Fingolimod (1), whose chemical name is 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride, is commonly called Fingolimod hydrochloride, The chemical structural is shown in Figure 1.

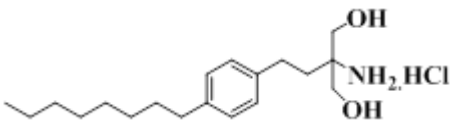


Figure 1: Fingolimod chemical structure

To date, several synthetic routes for Fingolimod have been reported [9-19]. In these synthetic routes some impurities like N,N-dimethyl impurity (2), N-methyl impurity (3), nitromonomethyl impurity (4), monomethyl impurity (5), nitro hydroxy impurity (6) and hydroxy impurity (7) were observed and synthesized, characterized by NMR and mass spectroscopy. These impurities shown in Figure 2.

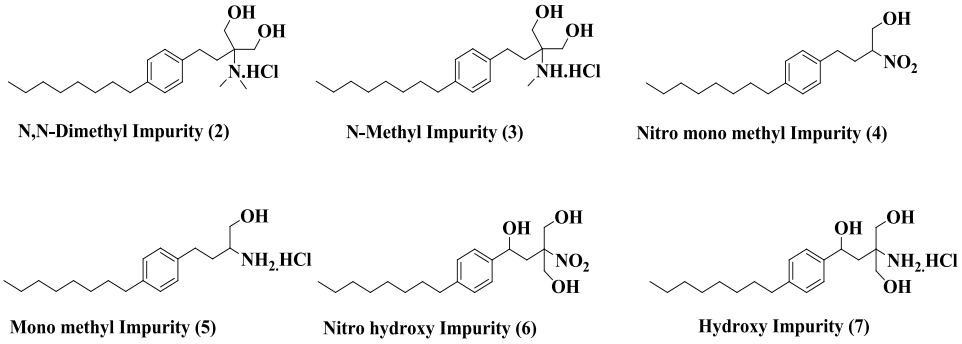


Figure 2: Fingolimod impurities

MATERIALS AND METHODS

All commercial reagents and solvents were used without additional purification. Reactions were monitored by Thin Layer Chromatography (TLC) on silica gel plates (60 F254), visualizing with iodine spray or ultraviolet light (254 nm). Column chromatography was performed on silica gel (60-120 mesh) using a proper eluent. ^{1}H-NMR spectra were determined in $CDCl_3$ and DMSO solutions using Varian MR 400 (400 MHz) spectrometers respectively. Proton chemical shifts (δ) are relative to Tetramethylsilane (TMS, δ=0.0) as internal standard and expressed in parts per million. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as br (broad). Coupling constants (J) are given in Hertz. MS spectra were recorded using an Agilent 6430 triple quadruple mass spectrometer.

Preparation of N, N-dimethyl impurity (2)

The compound-16 (15 g, 44.4 mmol) and dichloromethane (150 ml) are charged into a RBF at 25-30°C and stirred. p-Toluenesulfonic acid (75.68 mg, 0.44 mmol) is charged followed by 2, 2-dimethoxy propane (7.38 g, 71 mmol) into the flask. Then the reaction mixture was stirred for 6 h at 25-30°C. Solvents evaporated under reduced pressure. To a reactor, was added crude compound- 17, 150 ml of methanol and 1.5 g of 10% Pd/C. The reaction mixture was allowed to stir at room temperature for 16 h under 1 kg/cm^3 hydrogen pressure. The reaction was filtered through a pad of celite, and washed with methanol. The combine filtrate was concentrate under reducing pressure to afford the compound-18. The compound-18 and 150 ml of tetrahydrofuran are charged into a RBF at 25-30°C and stirred. Potassium carbonate (12.27 g, 88.8 mmol) is charged followed by methyl iodide (31.5 g, 222 mmol) into the flask. Stir the reaction mixture for 16 h at 25-30°C. Solvents evaporated under reducing pressure. Crude purified by column chromatography on silica gel in 40% ethylacetate/hexanes to get the compound-19 as brown solid (7 g, yield=42%). To a RBF, was added compound-19 (7 g, 18.63 mmol), 70 ml of dichloromethane and HCl (0.7 ml). Stir the reaction for 3 h at 25-30°C to get N, N-Dimethyl impurity (2) (6.0 g, yield=86.5%) as brown color solid R_f=0.2 (10% MeOH/DCM). ^{1}H-NMR (400 MHz, DMSO-d^6) δ (ppm): 7.20-7.18 (d, 2H), 7.12-7.10 (d, 2H), 5.70-5.68 (t, 2H), 3.92-3.87 (dd, 2H), 3.80-3.75 (dd, 2H), 3.33 (s, 3H), 3.16 (s, 6H), 2.62-2.58 (m, 2H), 2.54-2.50 (m, 2H), 2.08-2.03 (m, 2H), 1.55-1.51 (t, 2H), 1.27-1.24 (m, 10H), 0.83-0.86 (t, 3H); Mass: (m/z) 336.4 (M^{+1}-HCl); Anal. Calcd. for $C_{21}H_{38}ClNO_2$ (371.26): C, 67.81; H, 10.30; Cl, 9.53; N, 3.77; O, 8.60. Found: C, 67.87; H, 10.35; Cl, 9.50; N, 3.74; O, 8.61.

Preparation of N-methyl impurity (3)

Compound-18 (10 g, 28.7 mmol), tetrahydrofuran (100 ml) and potassium carbonate (7.9 g, 57.4 mmol) are charged in RBF, stirred and cooled to 0-5°C. Boc anhydride (9.4 g, 43.0 mmol) is added to the reaction mixture at 0-5°C and the reaction mixture is stirred at a temperature of 25-30°C for about 3 h. Solids filtered and distilled the solvents under reduced pressure at below 40°C. Water (50 ml) and DCM (50 ml) were charged and stirred for 30 min, separated the organic layer. Evaporated the solvents under reduced pressure to get the compound-20. Compound-20 and 100 ml of tetrahydrofuran are charged into a RBF and stirred at 25-30°C. The reaction mixture is cooled to 0-5°C. 60% sodium hydride (1.66 g, 43.0 mmol) is charged at 0-5°C and stirred for 30 min. Methyl iodide (6.1 g, 43.0 mmol) is charged drop wise at 0-5°C. The reaction mixture stirred for about 3 h at a temperature 25-30°C. Water (100 ml) precooled to 0-10°C is slowly added at the same temperature of 0-5°C. 100 ml of dichloromethane is charged. The organic layer is separated and solvents evaporated under reducing pressure to get the crude compound-21, which was purified by flash chromatography (EA: Hex=4:6) to afford a brown solid (6.0 g, yield: 45%). Compound-21 (6 g, 13.0 mmol), 60 ml of dichloromethane and 0.6 ml of Conc. HCl are charged in RBF at a temperature 25-30°C and stirred the reaction mixture at the same temperature for 3 h. The solvent was removed under reducing pressure to afford the N- methyl impurity (3) a brown solid (4.0 g, yield=78%). R_f=0.2 (10% MeOH/DCM). ^{1}H-NMR (400 MHz, DMSO-d^6) δ (ppm): 7.21-7.19 (d, 2H), 7.11-7.13 (d, 2H), 3.92-3.89 (dd, 2H), 3.80-3.75 (dd, 2H), 3.16 (s, 6H), 2.63-2.58 (m, 2H), 2.08-2.04 (m, 2H), 1.54-1.61 (t, 2H), 1.26-1.23 (m, 10H), 0.86-0.83 (t, 3H); Mass (m/z): 322.4 (M^{+1}-HCl); Anal. Calcd. for $C_{20}H_{36}ClNO_2$ (357.24): C, 67.11; H, 10.14; Cl, 9.90; N, 3.91; O, 8.94. Found: C, 67.10; H, 10.11; Cl, 9.91; N, 3.89; O, 8.90.

Preparation of nitro monomethyl impurity (4)

Compound-11 (15 g, 51.47 mmol) and dichloromethane (75 ml) are charged into a RBF at 25-30°C and cooled to -5°C-10°C. Triethylsilane (TES) (14.96 g, 128.67 mmol) is added. A solution of titanium tetrachloride (14.64 g, 77.2 mmol) in dichloromethane (75 ml) is added over a period of 15 min and the obtained reaction mixture is maintained at the same temperature -5°C-10 °C for 4 h. Water (150 ml) precooled to 0-10°C is slowly added to reaction mixture and stirred for 1 at 25-30°C. Separated the organic layer and evaporated the solvent under reducing pressure at below 45°C to afford compound-12. The compund-12 and 150 ml of toluene are charged in RBF at 25-30°C. Para formaldehyde (463 mg, 15.44 mmol) is charged followed by sodium carbonate (5.45 g, 51.47 mmol) into the flask and stirred the reaction mixture for 4 h at a temperature 100-110°C. The reaction mixture is filtered and water (100 ml) charged. Separated the organic layer and evaporated the solvent under reducing pressure at below 45°C to get the crude-4, which was purified by flash chromatography (EA: Hex=4:6) to afford nitromono hydroxy methyl impurity (4) a white solid (5.6 g, yield: 35%). ^{1}H-NMR (400 MHz, DMSO-d^6) δ (ppm): 7.09-7.06 (d, 4H), 4.63-4.56 (m, 1H), 3.81-3.72 (m, 2H), 2.56-2.47 (m, 4H), 2.11-1.94 (m, 2H), 1.54-1.50 (t, 2H), 1.25-1.22 (m, 10H), 0.86-0.82 (t, 3H); Mass (*m/z*): 306.6 (M^{-1}-HCl); Anal. Calcd. for $C_{18}H_{29}NO_3$ (307.21): C, 70.32; H, 9.51; N, 4.56; O, 15.61. Found: C, 70.30; H, 9.55; N, 4.57; O, 15.63.

Preparation of monomethyl impurity (5)

To a 500 ml autoclave, was added compound-4 (5.6 g, 18.21 mmol), 56 ml of methanol and 10% Pd/C. The reaction mixture was allowed to stir at 25-30°C for 15 h under 1 kg/cm^3 hydrogen pressure. The reaction was filtered through pad of celite and washed with methanol. The combined filtrate was concentrate under reduced pressure to affords crude compound-14, which was purified by flash chromatography (methanol: dichloromethane=0.1: 9.9) to afford compound-14 (4.8 g, yield=95%), a white solid. The compound-14 (4.8 g, 17.3 mmol) and 48 ml of methanol are charged into a RBF at 25-30°C. Ammonium chloride (0.92 g, 17.3 mmol) is charged at 25-30°C and stirred the reaction mixture at the same temperature for 4 h. The solid filter and washed with methanol to get the title compound (5) as white solid (5 g, yield: 92%), R_f=0.1(10% MeOH/DCM). ^{1}H-NMR (400 MHz, DMSO-d^6) δ (ppm): 7.14-7.09 (dd, 4H), 3.73-3.62 (m, 1H), 3.53-3.48 (m, 1H), 3.09-3.03 (m, 1H), 2.64-2.60 (m, 2H), 2.54-2.50 (m, 1H), 1.86-1.77 (m, 2H), 1.54-1.51 (t, 2H), 1.26-1.23 (m, 10H), 0.83-0.86 (m, 3H); Mass (*m/z*): 278.3 (M^{+1}-HCl); Anal. Calcd. for $C_{18}H_{32}ClNO$ (313.22): C, 68.87; H, 10.28; Cl, 11.29; N, 4.46; O, 5.10. Found: C, 68.83; H, 10.25; Cl, 11.24; N, 4.42; O, 5.12.

Preparation of nitrohydroxyl impurity (6)

The compound-11 (15 g, 51.4 mmol) and 150 ml of methanol are taken into a RBF at a temperature of 25-30°C. Sodium borohydride (1.16 g, 30.8 mmol) is charged slowly at -5-0°C. The reaction mixture stirred for 3 h at 25-30°C. Evaporated the solvent under reduced pressure to get the compound-13. The compound-13 and toluene (150 ml) are charged into a RBF. Para formaldehyde (1.93 g, 64.3 mmol) is charged fallowed by potassium carbonate (24.89 g, 180.1 mmol) into the flask. The reaction mixture stirred for 4 h at a temperature of 100-110°C. Filter the solids and evaporated the solvent under reduced pressure to get the crude compound-6, which was purified by flash chromatography (methanol: dichloromethane=0.1: 9.9) to afford compound-6 (6.6 g, yield: 36.28%), a brown syrup. R_f=0.1(10% MeOH/DCM). ^{1}H-NMR (400 MHz, DMSO-D6) δ (ppm): 7.22-7.21 (dd, 2H), 7.11-7.09 (dd, 2H), 4.84-4.81 (t, 1H), 3.51-3.40 (m, 2H), 3.33-3.23 (m, 2H), 2.54-2.50 (t, 2H), 1.53-1.52 (d, 4H), 1.26-1.23 (m, 10 H), 0.84-0.83 (t, 3H); Mass (*m/z*): 354.5 (M+1); Anal. Calcd. for $C_{19}H_{31}NO_5$ (353.22): C, 64.56; H, 8.84; N, 3.96; O, 22.63 Found: C, 64.50; H, 8.81; N, 3.93; O, 22.60.

Preparation of hydroxyl impurity (7)

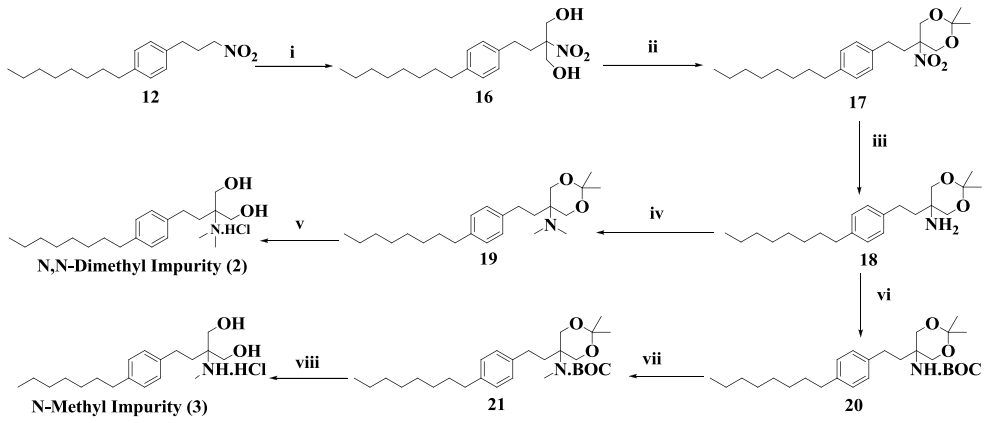
To a 500 ml reactor, was added compound-6 (6 g, 16.97 mmol), 60 ml methanol and 0.6 g of 10% Pd/C. The reaction mixture was allowed to stir at 25-30°C for 15 h under a 1 kg/cm^3 hydrogen pressure. The reaction mixture filtered through a pad of celite. Evaporated the solvent under reduced pressure to get the compound-15, which was purified by flash chromatography (methanol: dichloromethane=0.2: 9.8) to afford compound-15 (5.1 g, yield: 92.89%), a white solid. The compound-15 (5 g, 15.45 mmol) and 50 ml of methanol are charged into a RBF 25-30^{0}C. Ammonium chloride (826 mg, 15.45 mmol) is charged at 25-30°C. The reaction mixture stirred at a temperature of 25-30°C for 4 h. Filter the solid and washed with methanol to get the titled compound-7 a white solid (4.5 g, yield=81%). R_f=0.1(10% MeOH/DCM). ^{1}H-NMR (400 MHz, DMSO-D6) δ (ppm): 7.22-7.21 (dd, 2H), 7.11-7.09 (dd, 2H), 4.84-4.81 (t, 1H), 3.51-3.40 (m, 2H), 3.33-3.23 (m, 2H), 2.54-2.50 (t, 2H), 1.53-1.52 (d, 4H), 1.26-1.23 (m, 10 H), 0.84-0.83 (t, 3H); Mass (*m/z*): 324.5 (M^{+1}-HCl); Anal. Calcd. for $C_{19}H_{34}ClNO_3$ (359.22): C, 63.40; H, 9.52; Cl, 9.85; N, 3.89; O, 13.34. Found: C, 63.42; H, 9.50; Cl, 9.80; N, 3.86; O, 13.30.

RESULTS AND DISCUSSION

During the process development of fingolimod, various process-related impurities have been identified. Synthesis of N, N-dimethyl impurity (2) and N-methyl impurity (3) are shown in the Scheme 1, nitro monomethyl impurity (4), monomethyl impurity (5), nitro hydroxy impurity (6) and hydroxy impurity (7) are shown in the Scheme 2.

Treatment of the commercially available octanophenone 8 with TES under Titanium Tetrachloride ($TiCl_4$) gave compound 9, which undergo friedel craft acylation with 3-chloro propyl chloride to get the compound 10, then SN^2 displacement with sodium nitrite to yields intermediate 11 [20]. The treatment of 11 with TES and titanium tetrachloride gave compound 12. The hydroxymethyl moiety was introduced to compound 12 on the alpha carbon to form nitromonomethyl impurity 4, and then nitro group reduced with Pd/C followed by hydrochloric salt formation yields Mono methyl impurity 5.

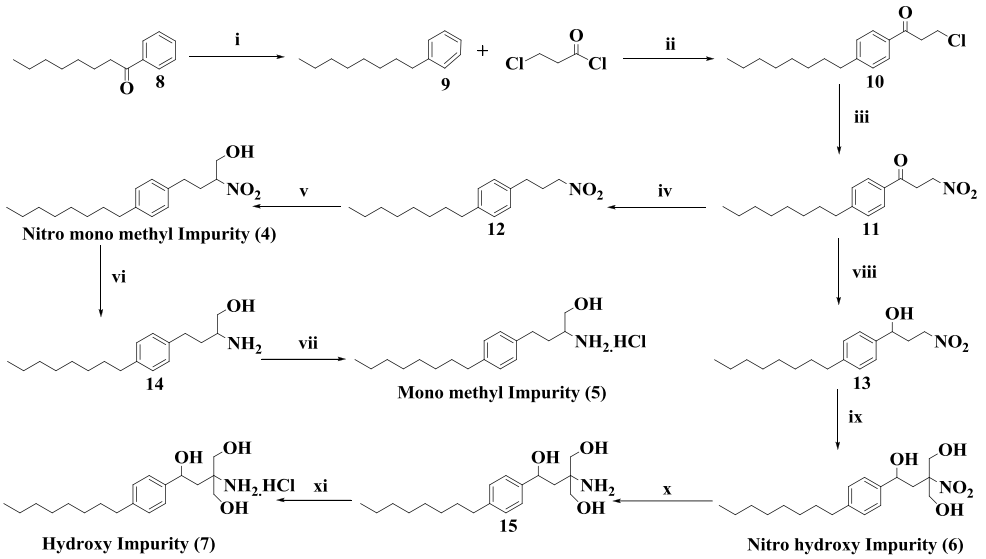
Compound 11 reduced with sodium borohydride to get compound 13. The hydroxymethyl moiety was introduced to compound 13 on the alpha carbon to form nitro hydroxy impurity 6, and then nitro group reduced with Pd/C followed by hydrochloric salt formation yields hydroxy impurity 7 (Scheme 2).



Scheme 1: Synthesis of N,N-dimethyl impurity (2) and N-Methyl impurity (3)

Reagents, Conditions**:** (i) $(HCHO)_n$, K_2CO_3, Toluene, Reflux, 5 h; (ii) 2,2-Dimethoxy propane, PTSA, DCM, RT, 6 h; (iii) H_2, 10% Pd/C, MeOH, RT, 16 h; (iv) CH_3I, K_2CO_3, THF, RT, 16 h; (v) HCl, DCM, RT, 3 h; (vi) $(Boc)_2O$, K_2CO_3, THF, 0-5°C, 3 h; (vii) CH_3I, NaH, THF, 0-5°C, 1 h; (viii) HCl, DCM, RT, 3 h.

The hydroxy methyl moiety was introduced to compound 12 on the alpha carbon to form 16, which was protected with 2,2-dimethoxy propane in the presence of PTSA to results compound 17. Nitro group reduced with Pd/C to yields compound 18. Thus, treatment of compound 18 with methyl iodide gave compound 19, followed by deprotection and hydrochloric acid salt (2) formation with hydrochloric acid. The compound 18 was protected with Boc anhydride followed by methylation with methyl iodide yields to compound 21. This was undergo deprotection and hydrochloric acid salt formation with hydrochloric acid gave N- methyl impurity (3) (Scheme 1).



Scheme 2: Synthesis of nitromono methyl impurity (4), monomethyl impurity (5), nitrohydroxy impurity (6) and hydroxy impurity (7)

Reagents, Conditions: (i) TES, $TiCl_4$,-5°C to RT, 5 h; (ii) $AlCl_3$, 0°C to RT, 2 h; (iii) $NaNO_2$, DMF, RT, 5 h; (iv) TES, $TiCl_4$, DCM, -5°C to RT, 5 h; (v) $(HCHO)_n$, K_2CO_3, Toluene, Reflux, 5 h; (vi) 10% Pd/C, MeOH, RT, 16 h; (vii) NH_4Cl, MeOH, RT, 4 h; (viii) $NaBH_4$, MeOH, -5°C to RT, 3 h; (ix) $(HCHO)_n$, K_2CO_3, Toluene, Reflux, 5 h; (x) 10% Pd/C, MeOH, RT, 16 h; (xi) NH_4Cl, MeOH, RT, 4 h

CONCLUSION

The six processes related impurities in manufacturing of Fingolimod were synthesized and characterized. Innovatively prepared of N, N-dimethyl impurity (2) by protecting dihydroxy groups (16) and N-methyl impurity (3) by Boc protection followed by methylation.

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