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Synthesis and characterization of related substances of Paliperidone, an antipsychotic agent

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ABSTRACT

Paliperidone, is a known orally active antipsychotic agent. Five related substances (Impurities) were observed during laboratory process development and pilot scale preparation of Paliperidone. They are Didehydro paliperidone, 9-Alkyl analogue, Dehydro-9-alkyl analogue, Desfluoro paliperidone and Hydroxy keto paliperidone. The present work describes the detection, origin, synthesis, characterization and control of these related substances, thereby providing a commercial method to synthesize substantially pure paliperidone.

Keywords: Antipsychotic agent, paliperidone, risperidone, related substances, metabolite.

INTRODUCTION

Paliperidone is a typical antipsychotic agent belonging to benzisoxazole class of compounds. Paliperidone (9-hydroxy risperidone) is the major active metabolite of risperidone.[1,7] It is a second generation antipsychotic drug, used for the treatment of schizophrenia and related disorders. Paliperidone is a racemic mixture and chemically known as (±)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2a] pyrimidin-4-one. It is marketed by Johnson & Johnson Pharmaceuticals under the trade name Invega ®.

Paliperidone is a centrally active dopamine D_2 and steotinergic 5-HT_{2A} antagonist, as demonstrated in both *in-vitro* and *in-vivo* animal and human studies. It is also active as an antagonist at α-1 and α-2 adrenergic receptors and H_1 histaminergic receptors.[2]

The presence of impurities in an Active Pharmaceutical Ingredient (API) can impact on the quality and safety of the drug product. International Conference on Harmonization (ICH)

guidelines recommend identifying and characterizing all impurities present in API at a level of ≥ 0.10%. [11] Impurities are required in pure form to check the analytical performance characteristics such as specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, system suitability testing and relative retention factor. [12]

During the laboratory process development and pilot scale preparation of Paliperidone various related substances were observed, based on LC-MS studies and its fragmentation patterns, structures are assigned tentatively, synthesized and confirmed by spiking studies by HPLC. In our current work, we identified five related substances during laboratory process development and pilot scale preparation of paliperidone **(1)**. Their detection, origin, synthesis, characterization and control of these related substances **(2)** to **(6)** are described in this article.

*Following are the related substances identified in paliperidone **(1)** synthetic process. 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (Didehydro paliperidone) **(2)**, (±)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (9-Alkyl analogue) **(3)**, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7-dihydro-9-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (Dehydro-9-alkyl analogue) **(4)**, (±)-3-[2-[4-(1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (Desfluoro paliperidone) **(5)** and (±)-3-[2-[4-(4-fluoro-2-hydroxybenzoyl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (Hydroxy keto paliperidone) **(6)**.*

MATERIALS AND METHODS

Solvents and reagents were obtained from commercial sources and used without purification. The IR spectra (*v* max cm^{-1}) were recorded in solid state KBr dispersion using perkin Elmer FT-IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker-Avance 300 MHz spectrometer. The chemical shifts were reported in δ ppm relative to TMS. The mass spectra were recorded on API 2000 Perkin Elmer PE-SCIEX mass spectrometer. Melting points were determined by Polman melting point apparatus (Model No. MP-96) and are uncorrected. . The compounds **3, 4, 5** are novel and **2, 6** are known in literature. [2,7,3(i)]. We synthesized all these compounds as described in this section.

Preparation of 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (2)

3-[2-Chloroethyl]-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one monohydrochloride **12** (5.00 g, 18.18 mmol) and 6-fluoro-3-(4-piperidinyl)1,2-benzisoxazole hydrochloride **14** (4.57 g, 17.82 mmol) were added to methanol (60 mL) at 25-30 °C. Diisopropylethylamine (9.38 g, 72.71 mmol) was added to above suspension at 25-30 °C. Temperature raised to reflux and maintained for 12 h. The reaction mass was cooled to 10-15 °C and the resulting solid product was filtered and washed with chilled methanol (2 x 8 mL, 2-8 °C) to give compound **2** as pale yellow crystals. Further, recrystallization from methanol gave pure compound **2** (6.2 g, 80.2 %); mp 157-159 °C; IR (KBr pellet), cm^{-1}: 3064.2 (Aromatic C-H, str.), 2937.8 (Aliphatic C-H, str.), 1655.2 (C=O, str.), 1576.5, 1547.5 (C=C & C=N, str.), 1480.6, 1448.4, 1346.6 (C-H, bending in CH_2,CH_3), 1270.6 (C-N, str.), 1129.8 (C-F, str.), 787.4 & 756.3 (Aromatic CH Out-of-plane bend); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.80-2.08 (m, 4H, CH_2), 2.25 (t, 2H, CH_2, *J* = 11.00 Hz), 2.50 (s, 3H, CH_3), 2.83 (m, 2H, CH_2), 3.12-3.16 (m, 5H, 2xCH_2, CH), 7.14 (d, 2H, Ar-H, *J* = 4.10 Hz), 7.28 (dd, 1H, Ar-H, *J* = 9.06Hz, 1.65 Hz), 7.70 (dd, 1H, Ar-H, *J* = 9.06Hz, 1.65 Hz), 8.01 (dd, 1H, Ar-H, *J* = 9.06Hz, 5.20Hz), 8.41 (t,1H, Ar-H, *J* = 4.12 Hz); ^{13}C NMR (75 MHz,

DMSO-d_6): δ 23.0, 24.8, 30.9, 34.2, 53.7, 57.2, 98.0, 98.4, 113.2, 113.5, 113.6, 114.5, 116.5, 117.9, 118.1, 124.6, 124.7, 143.1, 150.4, 158.1, 160.3, 162.2; MS *m/z:* 423.1[$(M+H)^+$]; Anal. Calcd. for $C_{23}H_{23}FN_4O_3$ (422.4), C, 65.39; H, 5.49; N, 13.26%; found: C, 65.35; H, 5.41; N, 13.22%; HPLC retention time ~41.8 min (RRT ~1.22).

Preparation of (±)-3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-[4-(6-fluro-1,2-benzisoxazol-3-yl)-1-piperidinyl]-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (3)

(±)-3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one **1** (5.00 g, 11.74 mmol) was suspended in dichloromethane (50 mL) containing few drops of *N*,*N*-dimethylformamide. Slowly added thionylchloride (1.46 g, 12.32 mmol) over 10 min at 5-8 °C and stirred for 1 h, filtered off and washed with precooled dichloromethane (2 x 5 mL, 2-8 °C) and dried under reduced pressure to give compound **15** (4.5 g, 86.3 %). Further, compound **15** (3.50 g, 12.32 mmol) was mixed with **14** (2.08 g, 8.11 mmol) and taken in methyl isobutyl ketone (40 mL). Added sodium bicarbonate (3.05 g, 36.33 mmol), potassium iodide (25 mg, catalytic amount) successively to the above suspension at 25-30 °C. Reaction mass was maintained at 80-85 °C for 15 h and was concentrated under reduced pressure upto ~50% reaction volume. Cooled to 25-30 °C and filtered off precipitated salts. The filtrate was distilled under reduced pressure to obtain glassy wool solid mixture of compound **3** and **4** (Chromatographic purity ratio ~85:15). Further, compound **3** was purified by repeated crystallizations from toluene to yield **3** in pure form (2.3 g, 46.5 %); mp 223-225 °C; IR (KBr pellet), cm^{-1}: 3066.5 (Aromatic C-H, str.), 2943.7, 2853.9 (Aliphatic C-H, str.), 1650.9 (C=O, str.), 1615.6, 1532.8, 1494.3 (C=C& C=N, str.), 1467.9, 1446.9, 1360.2 (C-H, bending in CH_2,CH_3), 1272.1 (C-N, str.), 1122.8 (C-F, str.), 788.9, 759.6 (Aromatic CH out-of-plane bend); ^{1}H NMR (300 MHz,$CDCl_3$): δ 1.91-2.12 (m, 12H, CH_2), 2.31 (m, 2 H, CH_2), 2.38 (S, 3H, CH_3), 2.44& 2.69 (2m, 2H, CH_2), 2.58, 2.82 & 3.19 (3m, 8H, CH_2), 3.10 (m, 2H, 2 x CH), 3.64 (t, 1H, CH, *J* = 5.20 Hz), 3.80 & 4.44 (2m, 2H, CH_2), 7.05 (2dd, 2H, Ar-H, *J* = 9.06 Hz, 1.92 Hz), 7.24 (d, 2H, Ar-H, *J* = 9.06 Hz), 7.70 (m, 2H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.8, 21.6, 22.8, 23.8, 29.7, 30.3, 30.8, 31.0, 34.5, 40.3, 49.8, 51.1, 53.2, 56.4, 64.4, 97.2, 97.3, 97.5, 97.6, 112.2, 112.5, 117.3, 120.2, 122.4, 122.5, 122.6, 122.7, 156.1, 158.2, 160.9, 162.4, 163.1, 165.1; MS *m/z:* 629.3 [$(M+H)^+$]; Anal. Calcd. for $C_{35}H_{38}F_2N_6O_3$ (628.8); C, 66.85; H, 6.09; N,13.36%; found: C, 66.81; H, 6.07; N, 13.37%;HPLC retention time ~53.1 min (RRT ~1.56).

Preparation of 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7-dihydro-9-[4-(6-fluro-1,2-benzisoxazol-3-yl)-1-piperidinyl]-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (4)

Diketo paliperidone **16** (2.50 g, 5.90 mmol) was added to methanol (20 mL) followed by successive addition of isoxazole intermediate **14** (1.52 g, 5.92 mmol), sodium bicarbonate (2.47 g, 29.40 mmol) and potassium iodide (25 mg, catalytic amount) at 25-30 °C. The reaction mass was maintained at 60-65 °C for 14 h. Completion of reaction was monitored by HPLC. Reaction mass was cooled to 25-30 °C and filtered off precipitated salts. The filtrate was distilled under reduced pressure upto a mass volume of ~10 mL. Cooled to 0-5 °C and filtered off product and washed with precooled methanol (2 x 5 mL, 2-8 °C) to give compound **4** as yellow crystals (2.0 g, 53.6 %); mp 218-222 °C; IR (KBr pellet), cm^1: 3069.6, 3000.1 (Aromatic C-H, str.), 2944.1,2925.2 (Aliphatic C-H, str.), 1650.6 (C=O, str.), 1616.8, 1585.4, 1527.5 (C=C & C=N, str.), 1466.2, 1447.3, 1344.0 (C-H, bending in CH_2,CH_3), 1272.3 (C-N, str), 1127.5 (C-F, str.), 780.2, 759.9 (Aromatic CH out-of-plane bend); ^{1}H NMR (300 MHz,$CDCl_3$): δ 2.04-2.33 (m, 10H, CH_2), 2.39 (s, 3H, CH_3), 2.46 (m, 2H, CH_2), 2.58, 2.80, 3.19 & 3.61 (4m, 8H, CH_2), 2.64 (t, 2H, CH_2, *J* = 12.00 Hz), 3.09 (m, 2H, 2 x CH), 4.09 (t, 2H, CH_2, *J* = 6.80 Hz), 5.77 (t, 1H, CH, *J* = 4.60 Hz), 7.07 (2dd, 2H, Ar-H, *J* = 9.06 Hz & 1.92Hz), 7.25 (2dd, 2H, Ar-H, *J* =7.96 Hz & 1.92Hz), 7.73 (m,2H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.5, 21.9, 23.8, 29.7, 30.0, 30.1,

34.4, 38.5, 50.8, 52.9, 53.4, 56.2, 97.3, 97.4, 97.5, 97.6, 112.2, 112.3, 112.4, 112.5, 114.1, 117.2, 122.4, 122.5, 122.6, 122.7, 143.6, 147.8, 158.7, 160.8, 161.7, 163.1, 163.8, 163.9, 165.1; MS *m/z:* 627.4[$(M+H)^+$]; Anal. Calcd. for $C_{35}H_{36}F_2N_6O_3$ (626.7); C, 67.08; H, 5.79; N, 13.41%; found: C, 67.01; H, 5.73; N, 13.40%; HPLC retention time ~56.0 min (RRT ~1.65).

Preparation of (±)-3-[2-[4-(1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (5)

(±)-3-(2-Chloroethyl)-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one **8** (9.00 g, 37.19 mmol) and 3-(4-piperidinyl)-1,2-benzisoxazole monohydrochloride **17** (9.31 g, 39.04 mmol) were added to methanol (90 mL) at 25-30°C. Triethylamine (14.27 g, 141.28 mmol) was added to above suspension at 25-30 °C. Suspension was heated to 62-65 °C and maintained for 14 h. Reaction mass was cooled to 25-30 °C. Product was filtered and washed with methanol (2 x 10 mL). Further, dried to obtain compound **5** (10.9 g, 72.2%); mp 184-186 °C; IR (KBr pellet), cm^{-1}: 3053.3, 3016.1 (Aromatic C-H, str.), 2960.8, 2938.0 (Aliphatic C-H, str.), 1629.9 (C=O, str.), 1587.9, 1536.9 (C=C & C=N, str.), 1468.1, 1438.2, 1339.9 (C-H, bending in CH_2,CH_3), 1273.3 (C-N, str.), 795.6, 769.1 (Aromatic CH out-of-plane bend); ^{1}H NMR (300 MHz,$CDCl_3$): δ 1.70-2.25 (m, 8H, CH_2), 2.30 & 3.18 (2m, 4H, CH_2), 2.35 (s, 3H, CH_3), 2.54 (m, 2H, CH_2), 2.78 (m, 2H, CH_2), 3.11 (m, 1H, CH), 3.94 (m, 2H, CH_2), 4.26 (brs, OH), 4.50 (m, 1H, CH), 7.28 (m, 1H, Ar-H), 7.55 (m, 2H, Ar-H), 7.77 (d, 1H, Ar-H, *J* = 8.23 Hz); ^{13}C NMR (75 MHz,$CDCl_3$): δ 18.9, 21.6, 24.3, 27.3, 31.0, 35.2, 42.7, 53.9 57.0, 67.5, 110.5, 121.0, 121.2, 122.1, 123.4, 130.0, 157.8, 158.2, 161.6, 162.4, 163.6; MS *m/z:* 409.1 [$(M+H)^+$]; Anal. Calcd. for $C_{23}H_{28}N_4O_3$ (408.5); C, 67.63; H, 6.91; N, 13.72%; found: C, 67.59; H, 6.87; N, 13.71%; HPLC retention time ~27.4 min (RRT ~0.80).

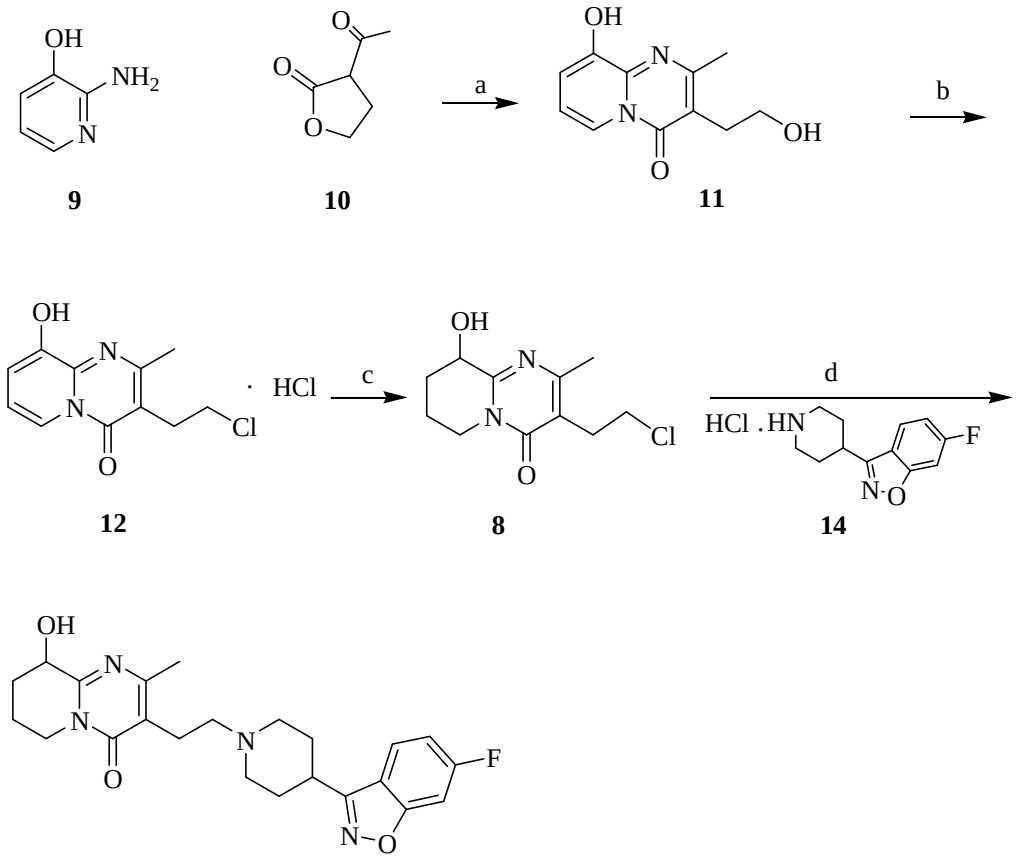
Preparation of (±)-3-[2-[4-(4-fluoro-2-hydroxybenzoyl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (6)

(±)-3-(2-Chloroethyl)-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one **8** (5.00 g, 20.62 mmol) and (4-fluoro-2-hydroxyphenyl) (piperidin-4-yl)methanone hydrochloride **18** (5.51 g, 24.71 mmol) were added to methanol (70 mL). Triethylamine (9.37 g, 92.77 mmol) was added at 25-30 °C and raised the reaction mass temperature to reflux (60-63 °C) and continued the reflux for 14 h. Completion of reaction was monitored by HPLC. Reaction mixture cooled to 10-15 °C. The product was isolated by filtration to yield compound **6** (crude, 4.97g). Further, repeated crystallizations from methanol gave substantially pure compound **6** (3.9 g, 44.3 %); mp 301-304 °C; IR (KBr pellet), cm^{-1}: 3045.5 (Aromatic C-H, str.), 2960.3, 2919.6 (Aliphatic C-H, str.), 1655.1 (C=O, str.), 1535.2, 1511.0 (C=C & C=N, str.), 1471.2, 1439.6, 1360.9 (C-H, bending in CH_2,CH_3), 1273.4 (C-N, str.), 1121.1 (C-F, str.), 800.6, 752.4 (Aromatic CH out-of-plane bend); ^{1}H NMR (300 MHz, DMSO-d_6): δ1.70-2.03 (m, 8H, CH_2), 2.19 (m, 2H, CH_2), 2.33 (s, 3H, CH_3), 2.53 (m, 2H, CH_2), 2.76 (t, 2H, CH_2, *J* = 6.30 Hz), 3.12-3.25 (m, 3H, CH_2, CH), 3.94 (m, 2H, CH_2), 4.18 (brs, OH), 4.49 (dd, 1H, CH, *J* = 6.30 Hz & 10.10Hz), 6.58-6.70 (m, 2H, Ar-H), 7.77 (dd, 1H, Ar-H, *J* = 6.59 Hz & 9.06 Hz), 12.83 (brs, Ar-OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 17.0, 17.1, 20.8, 26.0, 26.5, 41.9, 43.4, 46.0, 51.3, 53.4, 65.1, 104.5, 104.8, 107.3, 107.6, 121.2, 133.7, 133.8, 151.0, 162.7, 163.1, 163.3, 204.9; MS *m/z:* 430.1[$(M+H)^+$]; Anal. Calcd. for $C_{23}H_{28}FN_3O_4$ (429.4), C, 64.32; H, 6.57; N, 9.78%; found: C, 64.29; H, 6.56; N,9.75%; HPLC retention time ~36.0 min (RRT ~1.06).

RESULTS AND DISCUSSION

Paliperidone **1** was synthesized by known literature synthetic methods. [3,4] (Scheme-1). A key intermediate, (±)-3-[2-chloroethyl]-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4H-pyrido[1,2-a]-pyrimidin-4-one **(8)**, was synthesized by reacting 2-amino-3-hydroxy pyridine **(9)** with 2-

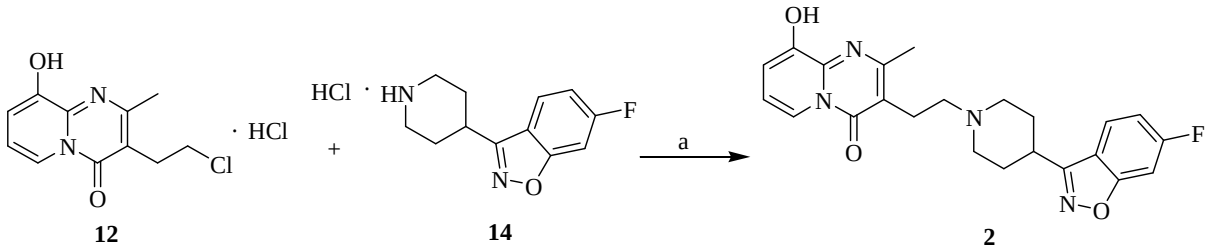
acetylbutyrolactone **(10)** in presence of o-xylene and catalyzed by *p*-toluenesulfonic acid, which was further chlorinated with thionyl chloride in N,N-dimethylformamide to give 3-[2-chloroethyl]-9-hydroxy-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one mono hydrochloride **(12)**, compound **12** thus prepared was hydrogenated with 10% Pd/C (2-4 kg hydrogen pressure) in methanol to yield compound **8**. Further, condensation of compound **8** with 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole hydrochloride **(14)** [4] in methanol using triethylamine as base resulted in desired paliperidone **1**.



Scheme-1: Synthetic scheme of paliperidone

1. Reagents and conditions: (a) o-xylene, PTSA, 17 h, 70.6%; (b) DMF,$SOCl_2$, 2h, Ethyl acetate, acetone, methanol, 72.6%; (c) Methanol, Pd/C (10% w/w), H_2 (↑), 10h, 60.6%; (d) Methanol, triethylamine, 16h, 60.1%.

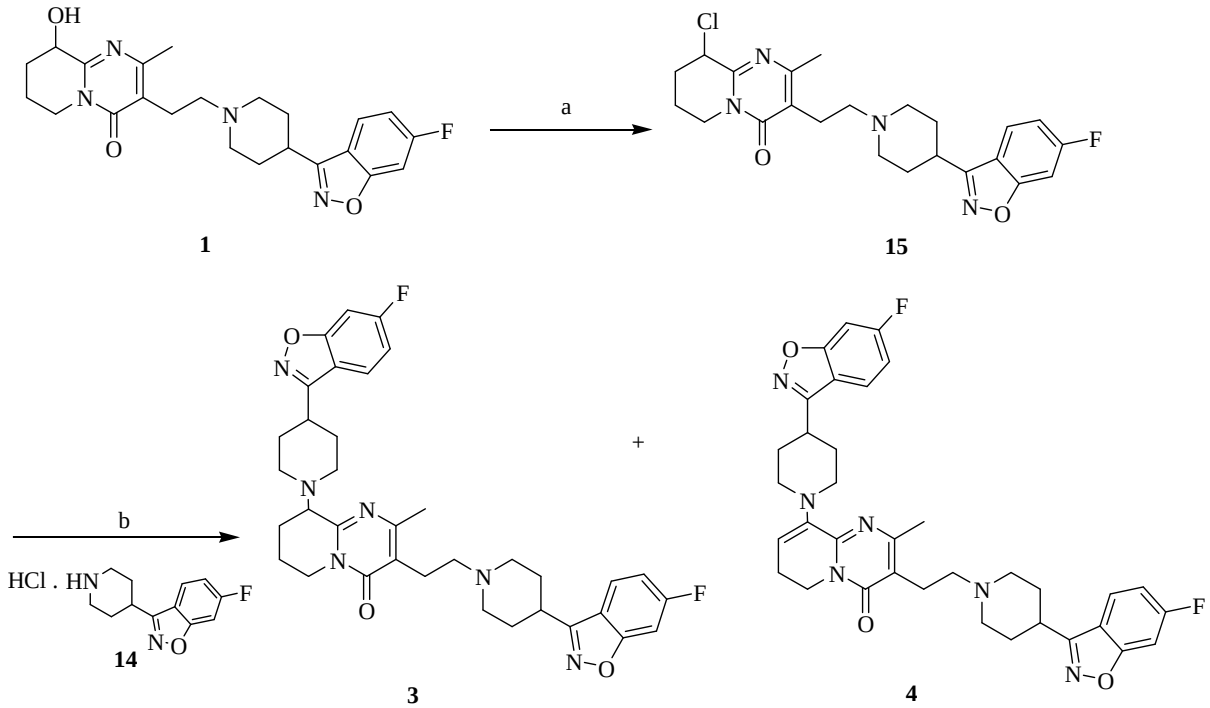
Didehydro paliperidone **2** is observed as an impurity in synthesis of paliperidone **1**. This impurity originated from 3-[2-chloroethyl]-9-hydroxy-2-methyl-4H-pyrido [1,2-a]pyrimidin-4-one **12[5]**. Compound **12** present as a contaminant in compound **8**. Further, reacted with compound **14** to produce compound **2**. Related substance **2** was independently prepared from compound **12** and **14** in methanol and diisopropylethylamine (Scheme-2). The mass spectrum showed a molecular ion at *m/z* 423.1amu [$(M+H)^+$] and its sodium adduct at *m/z* 445.3amu [$(M+H+Na)^+$]. In comparison with Paliperidone ^{1}H NMR, observed three additional aromatic protons at δ 7.14 (2H), 8.41(1H) ppm and also some of the specific protons not observed at δ 1.22-1.84 ppm, (4H) & 3.97 ppm, (2H). ^{13}C NMR showed additional characteristic aromatic carbon signals at δ 113.5, 113.6, 117.9 ppm corresponding to double bonds in pyridopyrimidine ring and also observed absence of $3CH_2$, 1CH characteristic carbon signals at δ 18.7, 27.5, 42.8, 67.3 ppm. This related substance was spiked with paliperidone sample containing didehydro paliperidone and confirmed related substance.



12 **14** **2**

Scheme-2: Synthetic scheme of didehydro paliperidone

2. Reagents and conditions: (a) Methanol, diisopropylethyl amine, reflux, 12h, 80.2%.



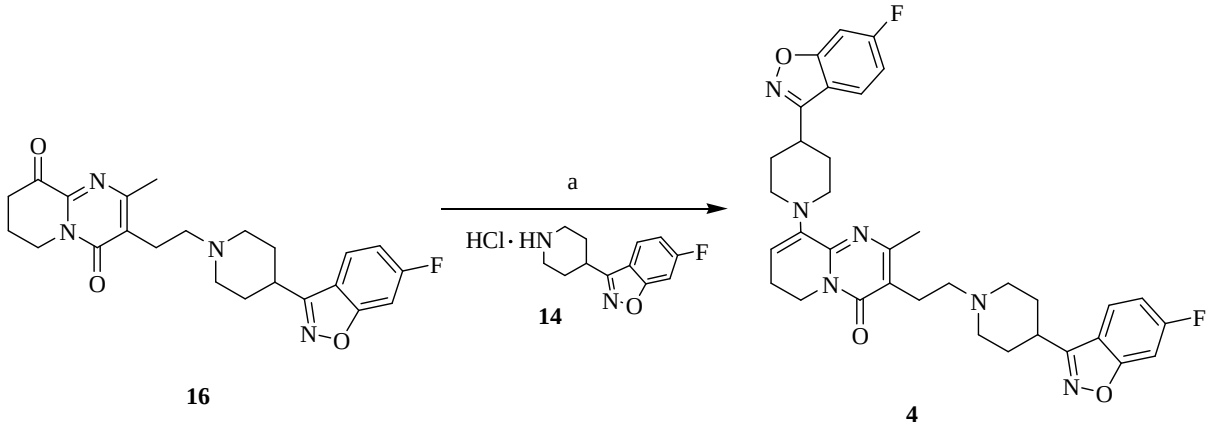
1 **15** **14** **3** **4**

Scheme-3: Synthetic scheme of 9-alkyl analogue

3. Reagents and conditions: (a) dichloromethane, thionyl chloride, DMF, 60 min, 86.3%; (b) Methyl isobutyl ketone, $NaHCO_3$, KI, 80-85^{0}C, 16h, 46.5%.

9-Alkyl analogue **3** is observed as an impurity in paliperidone. This impurity may originate from dichloro compound present in compound **8**, which leads to form 9-chloro risperidone **(15)**. Further, **15** was condensed with **14** to produce **3** and **4**, (ratio 85:15%). This related substance **3** was independently prepared by compound **1** reaction with thionyl chloride, in methylene dichloride and few drops of N,N-dimethylformamide to give 9-chloro risperidone **15**, which was further reacted with isoxazole intermediate **14** in methyl isobutyl ketone medium, sodium bicarbonate used as base to produces **3** and **4** (ratio ~85:15) (Scheme-3). Further, compound **3** was purified by repeated crystallizations in toluene to obtain pure compound **3**. This related substance also can originate due to dilution factor of organic reaction solvents. The mass spectrum of **3** showed the molecular ion peak at *m/z* 629.3 amu [$(M+H)^+$] and its sodium adduct at *m/z* 651.4 amu [$(M+H+Na)^+$]. Also observed 9-alkyl cleaved fragmentation to produce molecular ion peak at *m/z* 409.4 amu and *m/z* 219.7 amu, it indicated another isoxazole moiety is attached at 9-position. In comparison with paliperidone, we observed double the isoxazole moiety protons in ^{1}H NMR and carbon signals in ^{13}C NMR. ^{1}H NMR, ^{13}C NMR and Mass Spectra (MS) data of prepared compound **3** were identical with that of sample isolated from paliperidone.

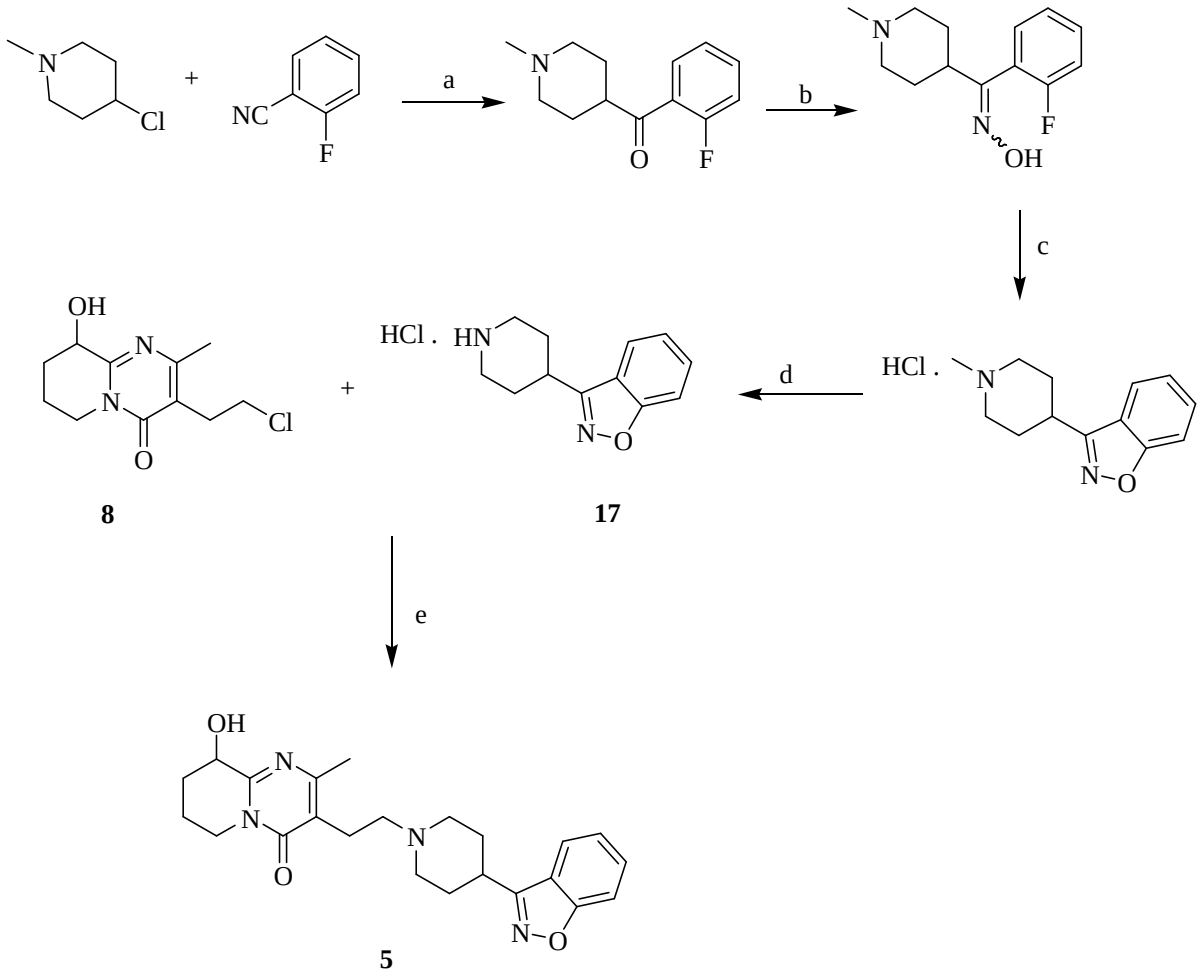
Dehydro 9-alkyl analogue **4** is also observed as an impurity in synthesis of paliperidone **1**. This related substance originated from diketo paliperidone **16,** [10] which was a potential impurity in paliperidone preparation. Compound **16** was formed due to presence of peroxides in reaction medium. This related substance **4** was independently prepared from compound **16**, which was prepared from known literature methods [9,10] and further, compound **16** condensed with **14** in presence of sodium bicarbonate, potassium iodide in methanol medium to yield compound **4** (Scheme-4). The mass spectrum of **4** showed the molecular ion peak at *m/z* 627.4 amu $[(M+H)^+]$ and its sodium adduct at *m/z* 649.4 amu $[(M + H + Na)^+]$, observed 9-alkyl cleaved fragmentation to produce molecular ion peak at *m/z* 407.1 amu and *m/z* 219.6 amu, it indicated one more isoxazole moiety is attached at 9-position. In comparison with compound **3**, compound **4** having 2 amu less, which indicates possibility of one double bond formation in pyridopyrimidine ring. In view of 9-alkyl paliperidone **3**, we observed one additional triplet at δ 5.77 ppm in ^{1}H NMR spectrum and characteristic signal at δ 114.1 ppm in ^{13}C NMR corresponding to one aromatic carbon at 8-position of pyridopyrimidine ring. In view of didehydro paliperidone **2** ^{1}H NMR**,** we observed additional two methylene protons at δ 2.46 & 4.09 ppm, indicates one double bond present in pyridopyrimidine ring. Related substance **4** was spiked with paliperidone sample containing dehydro 9-alkyl analogue and confirmed the related substance.



Scheme-4: Synthetic scheme of Dehydro 9-alkyl analogue

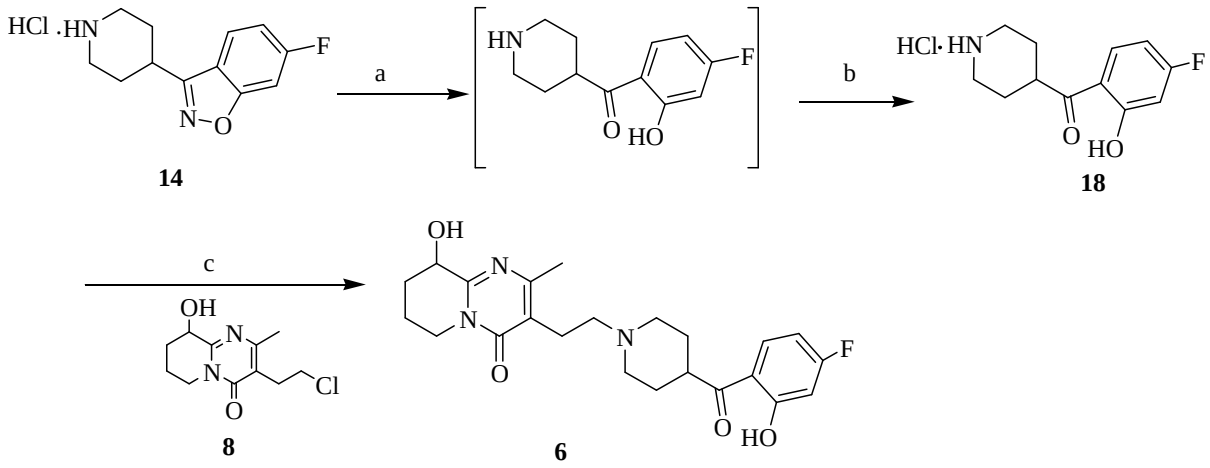
***4**. Reagents and conditions: (a) methanol, $NaHCO_3$, KI, 60-65^{o}C, 14h, 53.7%.*

Desfluoro paliperidone **5** is observed as an impurity in paliperidone **1**. Contamination of isoxazole intermediate **14** with desfluoro isoxazole intermediate (**17**) leads to this related substances **5**. Related substance **5** was independently prepared from compound **17**, which was prepared from known literature methods[6] and further, compound **17** condensed with **8** in presence of triethylamine as base in methanol to provide **5** (Scheme-5). The protonated molecular ion of compound **5** appeared at *m/z* 409.1 $[(M+H)^+]$ and its sodium adduct at *m/z* 431.2 amu $[(M+H+Na)^+]$. In view of paliperidone, IR spectrum not displayed C-F stretching frequency at 1131.2 cm^{-1}. ^{1}H NMR indicated one additional aromatic proton at δ 7.55 ppm and ^{13}C NMR also indicated its corresponding carbon signal at δ 130.0 ppm. In addition to this, we have not observed quaternary carbon signals at δ 164.1 & 164.3 ppm. This desfluoro paliperidone **5** was spiked with paliperidone sample containing desfluoro paliperidone and confirmed related substance.



Scheme-5: Synthetic scheme of Desfluoro paliperidone

5. Reagents and conditions: (a) Mg turnings, dry tetrahydrofuran, 1,2-dibromoethane, 13h, NH_4Cl, H_20, Et_2O; (b) $NH_2OH.HCl$, TEA, methanol; (c) KOH, toluene, H_2O, IPA.HCl; (d) 1-Chloroethylchloroformate, MDC, TEA, methanol; (e) Methanol, TEA, 14h, 72.2%.



Scheme-6: Synthetic scheme of hydroxy keto paliperidone

6. Reagents and conditions: (a) Methanol, TEA, 10%Pd/C, 12 h; (b) 5%Aq.HCl; (c) Methanol, TEA, 12 h, 44.3%.

Hydroxy keto paliperidone **6**[2,7] is known as metabolite of paliperidone **1**. Compound **6** is also identified as an impurity in paliperidone synthesis. This related substance **6** was independently prepared from compound **18**. Further, compound **18** was condensed with **8** in presence of triethylamine as base in methanol medium to provide related substance **6** (Scheme-6).

Compound **18** is prepared from known literature process [8] through catalytic reductive cleavage of 3-substituted 1,2-benzisoxazole **14** to provide compound **18**. The protonated molecular ion of compound **6** appeared at *m/z* 430.1 [$(M+H)^+$]. In view of paliperidone **1**, ^{1}H NMR spectrum showed *brs at* δ 12.83 ppm corresponding to one phenolic OH and it was confirmed by D_2O exchange analysis. ^{13}C NMR showed additional characteristic carbonyl carbon signal of at δ 204.9 ppm. This compound **6** was spiked with paliperidone sample containing compound **6** and confirmed the related substance.

Paliperidone related substances **2, 3** and **5** originate from corresponding raw material impurities. These are controlled by raw material specification. Further, any traces of these impurities can be removed during isolation of paliperidone. Paliperidone related substance **6** is removed by the purification of paliperidone during recrystallization from methanol. Paliperidone related substances **3** and **4** are process related impurities. Paliperidone related substance **3** is a raw material impurity as well as a process related impurity. Paliperidone related substances **3** and **4** can be controlled by carrying out reaction using lower volume of organic reaction solvent. Further, these impurities can be removed from paliperidone by additional purification in toluene.

CONCLUSION

To have a thorough understanding of impurity formation for origin of antipsychotic agent paliperidone, it is essential to have detailed information about various potential impurities and their mechanism of formation. In view of regulatory importance of the impurities in the API, a detailed study on various impurities in paliperidone was conducted. Different process related substances and metabolite of paliperidone were identified. They were synthesized and characterized by using various spectroscopic techniques like ^{1}H NMR, ^{13}C NMR and infrared (IR) spectrum. These characterizations are well supported by liquid chromatography-mass spectrometry (LC-MS) and Mass spectral data.

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