

Synthesis and characterization of impurities of an anti-psychotic drug substance, Olanzapine

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Abstract

HPLC analysis of olanzapine **1**, a known anti-psychotic drug, showed impurity peaks ranging from 0.05 to 0.15 % during process development. These samples were analyzed by LCMS and the peaks were identified at *m/z* 230, 341, 511, 326, 361 and 329. All six impurities were synthesized individually and characterized based on their spectral data (IR, NMR and Mass). The structure of these impurities were assigned as 2-methyl-4,9-dihydro-3-thia-4,9-diazabenzof[*f*]azulen-10-one **4**, 1-[4-(2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulen-10-yl)piperazin-1-yl]ethanone **5**, bis-[10-(2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulene)]-1,4-piperazine **6**, 2,4-dimethyl-10-(4-methylpiperazin-1-yl)-4*H*-3-thia-4,9-diazabenzof[*f*]azulene **7**, 10-(4-chloromethyl-4-methylpiperazin-1-ylum)-2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulene chloride **8** and 2-methyl-10-(4-methyl-*N*-oxopiperazin-1-yl)-4*H*-3-thia-4,9-diazabenzof[*f*]azulene **9** respectively. The formation, synthesis and characterisation of the olanzapine impurities are discussed.

Keywords: Olanzapine, impurity profile, related substances

Introduction

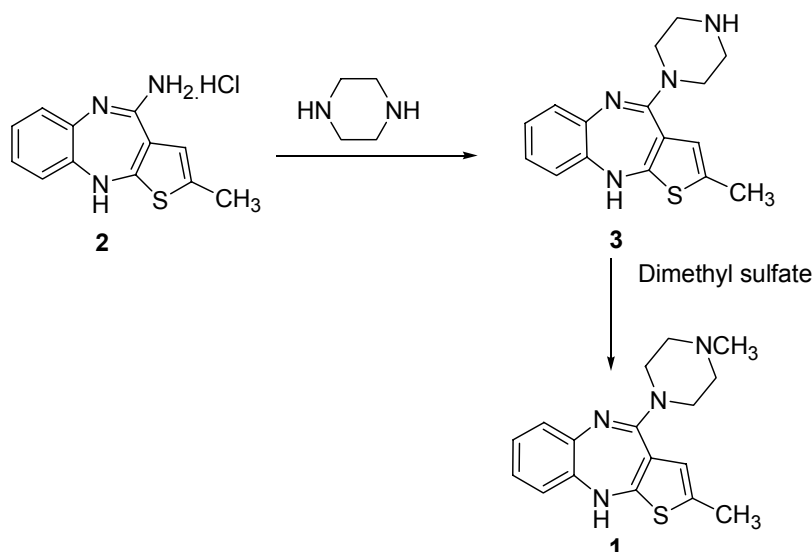
Olanzapine **1**, an atypical anti-psychotic drug^{1,2} with a thienobenzodiazepinyl structure, is indicated for the treatment of schizophrenia. It displays a broad pharmacological profile and is a selective monoaminergic antagonist with high affinity binding to serotonin 5HT_{2A/2C}, dopamine D₁₄, muscarinic M₁₋₅ and adrenergic α_1 receptors^{3,4,5}.

The HPLC analysis of olanzapine displayed six impurity peaks in the range of 0.05 to 0.15% levels along with the olanzapine peak. As per the guidelines recommended by ICH, the acceptable level for a known or unknown related compound (impurity) is less than 0.15 and 0.10

% respectively in a drug substance. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified and characterized. Our present work deals with the identification, synthesis and characterization of impurities/related substances of olanzapine **1**.

Results and Discussion

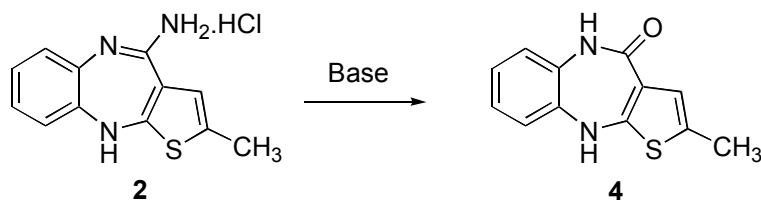
Olanzapine was synthesized in the laboratory by a known pathway (Scheme 1) as per the literature method⁶ which involved the reaction of 2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulen-10-ylamine hydrochloride **2** with piperazine to give **3**, which on further methylation with dimethyl sulfate yielded olanzapine **1**.



Scheme 1

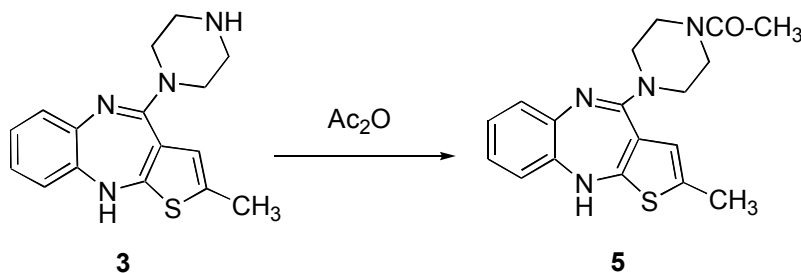
A typical analytical LC chromatogram of a laboratory sample of olanzapine displayed impurities/related compounds over a range of 0.05-0.15%. These impurities were identified, synthesized and characterized by spectral analysis. The assigned structures of these impurities are namely 2-methyl-4,9-dihydro-3-thia-4,9-diaza-benzo[*f*]azulen-10-one **4**, 1-[4-(2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulen-10-yl)-piperazin-1-yl]ethanone **5**, bis-[10-(2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulene)]-1,4-piperazine **6**, 2,4-dimethyl-10-(4-methylpiperazin-1-yl)-4*H*-3-thia-4,9-diazabenzof[*f*]azulene **7**, 10-(4-chloromethyl-4-methylpiperazin-1-ylum)-2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulene chloride **8** and 2-methyl-10-(4-methyl-*N*-oxopiperazin-1-yl)-4*H*-3-thia-4,9-diazabenzof[*f*]azulene **9**.

Impurity **4**, a potential impurity formed during the synthesis of olanzapine due to basic reaction conditions, was prepared by reacting compound **2** with sodium hydroxide (Scheme 2).



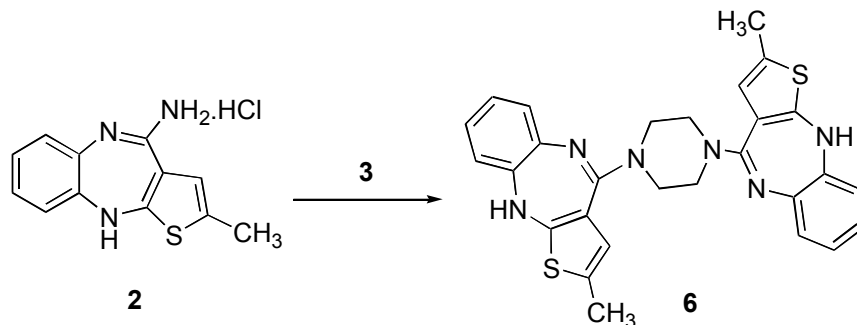
Scheme 2

Acetic acid is used in the work-up of the olanzapine synthesis. Thus compound **3** was *N*-acetylated resulting in the formation of impurity **5**. This impurity was synthesized quantitatively by the reaction of compound **3** with acetic anhydride (Scheme 3).



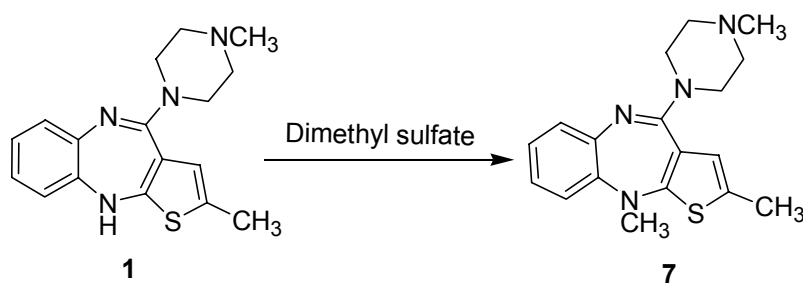
Scheme 3

Any residual **2** present in the reaction reacts with compound **3** to give impurity **6**. This impurity is formed only in trace quantities (Scheme 4).



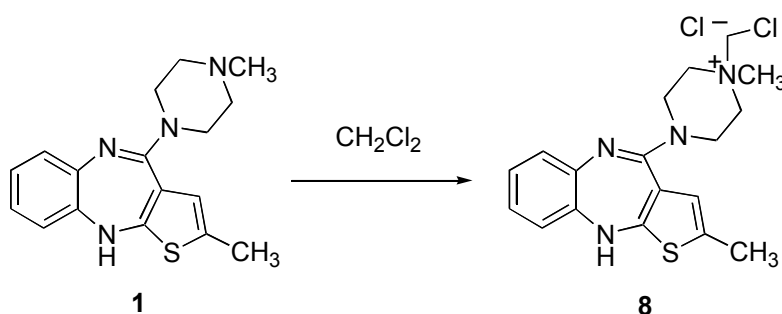
Scheme 4

Methylation of compound **3** with dimethyl sulfate forms olanzapine **1** (Scheme 1) however, as a side reaction, the free NH group present in the diazabenzothiazole moiety of **1** is methylated, resulting in impurity **7**. This impurity was synthesized by the reaction of olanzapine with excess dimethyl sulfate (Scheme 5).



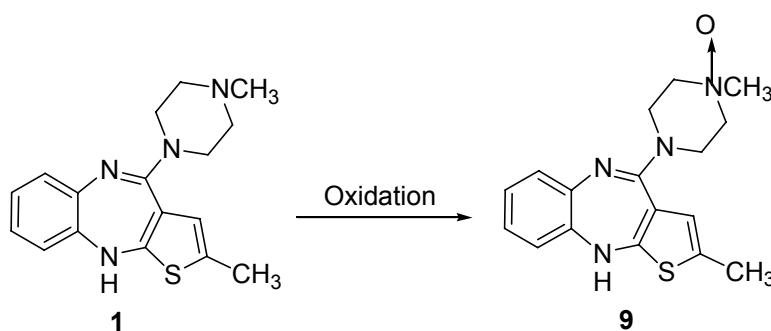
Scheme 5

Compound **8**, is formed by the reaction of olanzapine **1** with dichloromethane, a solvent that is used for the final purification of olanzapine. This impurity was synthesized by prolonged contact of olanzapine with dichloromethane at elevated temperatures and its structure was further confirmed by its spectral data (Scheme 6).



Scheme 6

Impurity **9** is formed in traces due to aerial oxidation of olanzapine **1**. This impurity was synthesized by the oxidation of olanzapine **1** with *m*-chloroperbenzoic acid and was purified by column chromatography (Scheme 7).



Scheme 7

Experimental Section

General Procedures. ^1H NMR spectra were recorded on a Gemini 200 MHz FT NMR spectrometer; the chemical shifts are reported in δ ppm relative to TMS. The IR spectra were recorded in the solid state as KBr dispersion using Perkin Elmer FT-IR Spectrophotometer. The mass spectra were recorded on Shimadzu LCMS-QP8000, LC-MS and AB-4000 Q-trap LC-MS/MS.

2-Methyl-4,9-dihydro-3-thia-4,9-diazabenzof[*f*]azulen-10-one (4)

A mixture of 2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulen-10-ylamine hydrochloride (**2**, 50.0 g, 0.19 mol) and 30% aqueous sodium hydroxide (200 mL) were heated to reflux till reaction was complete. The reaction mixture was cooled to room temperature and the resulting solid was filtered and washed with water (100 mL). The resultant wet cake was dissolved in methanol (1000 mL) at reflux, treated with carbon (5.0 g) and finally water (500 mL) was added precipitating a solid. The isolated solid was filtered, washed with water (100 mL) and dried at 70 °C to yield compound **4** (Yield: 35 g; HPLC Purity: >99 %). IR (cm^{-1}): 3281, 3191 (NH), 1637 (CO); ^1H NMR (DMSO- d_6 , δ ppm): 2.2 (s, 3H, CH₃), 6.6 (s, 1H, CH), 6.9 (m, 4H, Ar-H), 7.6 (s, 1H, NH); Mass: 230 (M^+); C H N Analysis Calcd. for C₁₂H₁₀N₂OS: C, 62.59; H, 4.38; N, 12.16% Found: C, 62.36; H, 4.57; N, 12.20%

1-[4-(2-Methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulen-10-yl)piperazin-1-yl]ethanone (5)

To a stirred mixture of 2-methyl-10-piperazin-1-yl-4*H*-3-thia-4,9-diazabenzof[*f*]azulene (**3**, 2.5 g, 0.008 mol) and dichloromethane (50 mL), triethylamine (1.3 mL) was added slowly. The reaction mixture was cooled to 5 °C and to it, acetic anhydride (1.0 mL) was added and the mixture stirred until the reaction was complete. Water (10 mL) was added and the organic and aqueous layers were separated. The organic layer was washed with water (4x10 mL) and concentrated under reduced pressure to a minimum volume for solid separation. The separated solid was filtered, washed with dichloromethane (5 mL) and dried to a constant weight to yield compound **5** (Yield: 2.5 g; HPLC purity: 97 %); IR (cm^{-1}): 1642 (C=O), 3248 (NH); ^1H NMR (CDCl₃, δ ppm): 2.0-2.1 (s, 3H, CH₃), 2.3 (s, 3H, CH₃), 3.0-3.6 (m, 8H, CH₂), 6.4 (s, 1H, Ar-H), 6.7-6.9 (m, 4H, Ar-H), 7.6 (s, 1H, NH); Mass: 341 (M^+); C H N Analysis Calcd. for C₁₈H₂₀N₄OS: C, 63.50; H, 5.92; N, 16.46% Found: C, 63.72; H, 5.99; N, 16.35%

Bis-[10-(2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulene)]-1,4-piperazine (6)

A mixture of 2-methyl-10-piperazin-1-yl-4*H*-3-thia-4,9-diazabenzof[*f*]azulene (**3**, 10.0 g, 0.034 mol), dimethyl sulfoxide (10 mL), toluene (40 mL) and 2-methyl-4*H*-3-thia-4,9-diazabenzof[*f*]azulen-10-ylamine hydrochloride (**2**, 9.0 g, 0.034 mol) was heated to reflux. Triethylamine (20 mL) was added in three equal portions to the reaction mixture at reflux temperature and the reaction mass was stirred for reaction completion. The reaction mixture was cooled to 35 °C and the undissolved material was filtered off. The filtrate was concentrated under

reduced pressure. To the residue, water (50 mL) was added and the mixture was stirred for solid separation. The isolated solid was filtered, washed with aqueous methanol and dried at 60 °C to a constant weight to yield compound **6** (Yield: 10.1 g, HPLC purity: 93.0 %); IR (cm⁻¹): 3375 (NH); ¹H NMR (CDCl₃, δ ppm): 2.2-2.3 (s, 6H, CH₃), 3.2-3.5 (m, 8H, CH₂), 6.4 (s, 2H, Ar-H), 6.7-6.9 (m, 8H, Ar-H), 7.7 (s, 2H, NH); Mass: 511 (M⁺); C H N Analysis Calcd. for C₂₈H₂₆N₆S₂: C, 65.85; H, 5.13; N, 16.46% Found: C, 65.82; H, 5.21; N, 16.35%

2, 4-Dimethyl-10-(4-methylpiperazin-1-yl)-4H-3-thia-4,9-diazabenzof]azulene (7)

To a stirred mixture of olanzapine (**1**, 2.5 g) and dichloromethane (15 mL), dimethyl sulfate (3.5 mL) was added slowly at 35 °C and the mixture stirred for reaction completion. The resultant solid was filtered off, washed successively with dichloromethane (10 mL), *n*-hexane (10 mL), dried at 65 °C to a constant weight and finally recrystallized from toluene (15 mL) to yield compound **7** (Yield: 2.4 g, HPLC Purity: 98%); ¹H NMR (CDCl₃, δ ppm): 2.2 (s, 3H, CH₃), 2.4 (s, 3H, CH₃), 2.8 (s, 3H, CH₃), 3.3-3.5 (m, 4H, CH₂), 4.0 (m, 4H, CH₂), 6.3 (s, 1H, Ar-H), 6.5 (d, 1H, Ar-H), 6.8-7.0 (m, 3H, Ar-H); Mass: 326 (M⁺); C H N Analysis Calcd. for C₁₈H₂₂N₄S: C, 66.22; H, 6.79; N, 17.16% Found: C, 66.32; H, 6.53; N, 17.22%

10-(4-Chloromethyl-4-methylpiperazin-1-ylum)-2-methyl-4H-3-thia-4,9-diazabenzof]-azulene chloride (8)

Olanzapine (**1**, 12.5 g, 0.034 mol) in dichloromethane (125 mL) was stirred at reflux for reaction completion. The reaction mass was concentrated to a minimum volume at 45 °C. The isolated solid was filtered off, washed with dichloromethane (65.0 mL) and dried at 35 °C to a constant weight. The solid was stirred in hot methanol (10.0 mL) at 65 °C, filtered, washed with methanol (3.0 mL) and dried at 35 °C to yield **8** (Yield: 10.5 g, HPLC purity: 97%); IR (cm⁻¹): 3212 (NH); ¹H NMR (CDCl₃, δ ppm): 2.3 (s, 3H, CH₃), 3.3 (s, 3H, CH₃), 3.5-3.9 (m, 8H, CH₂), 5.7 (s, 2H, CH₂), 6.4 (s, 1H, Ar-H), 6.7-6.9 (m, 4H, Ar-H), 7.7 (s, 1H, NH); Mass: 361 (M-35.5); C H N Analysis Calcd. for C₁₈H₂₂Cl₂N₄S : C, 54.35; H, 5.54; N, 14.09% Found: C, 54.38; H, 5.65; N, 14.05%

2-Methyl-10-(4-methyl-N-oxopiperazin-1-yl)-4H-3-thia-4,9-diazabenzof]azulene (9)

To a mixture of olanzapine (**1**, 12.5 g, 0.034 mol) in acetic acid (62.5 mL) *m*-chloroperbenzoic acid (7.0 g, 0.04 mol) was added and the mixture stirred at 50 °C for reaction completion. The reaction mass was concentrated under reduced pressure and the residual mass was dissolved in dichloromethane (50 mL). The resultant solution was washed with water, concentrated under reduced pressure and finally purified by column chromatography to yield compound **9** (Yield: 8 g, HPLC purity: 94%); IR (cm⁻¹): 3218 (NH); ¹H NMR (CDCl₃, δ ppm): 2.3 (s, 3H, CH₃), 3.2 (s, 3H, CH₃), 3.3-3.5 (m, 4H, CH₂), 4.0 (m, 4H, CH₂), 6.3 (s, 1H, Ar-H), 6.5 (d, 1H, Ar-H), 6.8-7.0 (m, 3H, Ar-H), 7.6 (s, 1H, NH); Mass: 329 (M⁺); C H N Analysis Calcd. for C₁₇H₂₀N₄OS: C, 62.17; H, 6.14; N, 17.06% Found: C, 62.15; H, 6.18; N, 17.12%

Acknowledgements

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