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Isolation, identification and characterization of two novel process-related impurities in olanzapine

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ABSTRACT

Olanzapine is a marketed antipsychotic agent for treatment of both positive and negative symptoms of schizophrenia. The chemical synthesis of olanzapine drug substance involved the reaction of thienobenzodiazepine hydrochloride with *N*-methylpiperazine. During the manufacture of olanzapine, two unknown impurities were present in pilot batches in the range of 0.08–0.22% based upon HPLC analysis. These unknown impurities were enriched from the mother liquor of reaction by preparative HPLC, and characterized by UV, FT-IR, LC–MS/TOF, 1D-NMR (^{1}H, ^{13}C, DEPT), 2D-NMR (^{1}H-^{1}H COSY, HSQC, HMBC, ROESY) and single-crystal X-ray diffraction analysis. Based on the complete spectral analysis and knowledge of the synthetic route of olanzapine, these two new impurities were identified as 2-methyl-4-(4-methyl piperazin-1-yl)-10-((methylthio)methyl)-thieno[2,3-*b*][1,5] benzodiazepine (impurity-I) and 10-(3-(1H-benzo[d]imidazol-2-yl)-5-methylthiophen-2-yl)-2-methyl-4-(4-methyl piperazin-1-yl)-thieno[2,3-*b*][1,5]benzodiazepine (impurity-II). Finally, prospects to the formation and controlling of impurity-I and II were discussed in detail.

1. Introduction

Olanzapine (Fig. 1A), an efficacious and well-tolerated atypical anti-psychotic drug with a thienobenzodiazepinyl structure, is widely used for both treatment of schizophrenia and acute manic or mixed episodes, along with maintenance therapy in bipolar disorder and related psychiatric disorders [1]. It has a multireceptorial binding profile including a greater affinity for serotonin 5-HT_{2A} than for dopamine D_2 receptors [2]. In view of its wide use, the stability indicating methods for the determination of process-related and degradation impurities in olanzapine [3–9] have received much attention. And a series of impurities related to oxidative degradation and side reactions of olanzapine have been reported in literature and pharmacopoeia [6–14], especially degradation products resulting from oxidation of the thiophene ring. However, only limited literature is available regarding N-10 substituted byproducts of olanzapine [14,15].

The synthetic route of olanzapine was widely reported and applied in laboratory sample preparation and commercial production for its advantages of low-cost materials, simple preparation process, and in good yield. The key synthetic step (Fig. 1B) involved the reaction of 4-amino-2-methyl-l0*H*-thieno[2,3-*b*][1,5]benzodiazepine hydrochloride (compound 1) with an excess amount of *N*-methylpiperazine under nitrogen atmosphere [16–18]. During the manufacture of olanzapine, two unknown process-related impurities were observed in different batches in the range of 0.08–0.22% based upon HPLC analysis.

It is well known that the safety of a drug product is dependent not only on the toxicological properties of the active drug substance itself, but also on the impurities that it may contain. As per International Conference on Harmonization (ICH) guidelines for impurities in new drug substances, reporting threshold is 0.05% and identification threshold is 0.1% or 1.0 mg per day intake (whichever is lower) for maximum daily dose ≤2 g/day [19]. Considering that the daily dose of olanzapine should not exceed 20 mg [1], these two unknown impurities in olanzapine need to be identified and characterized.

In the continuing research efforts on the impurity profiling [20], herein, a comprehensive study has been undertaken to identify and characterize two unknown process-related impurities present in

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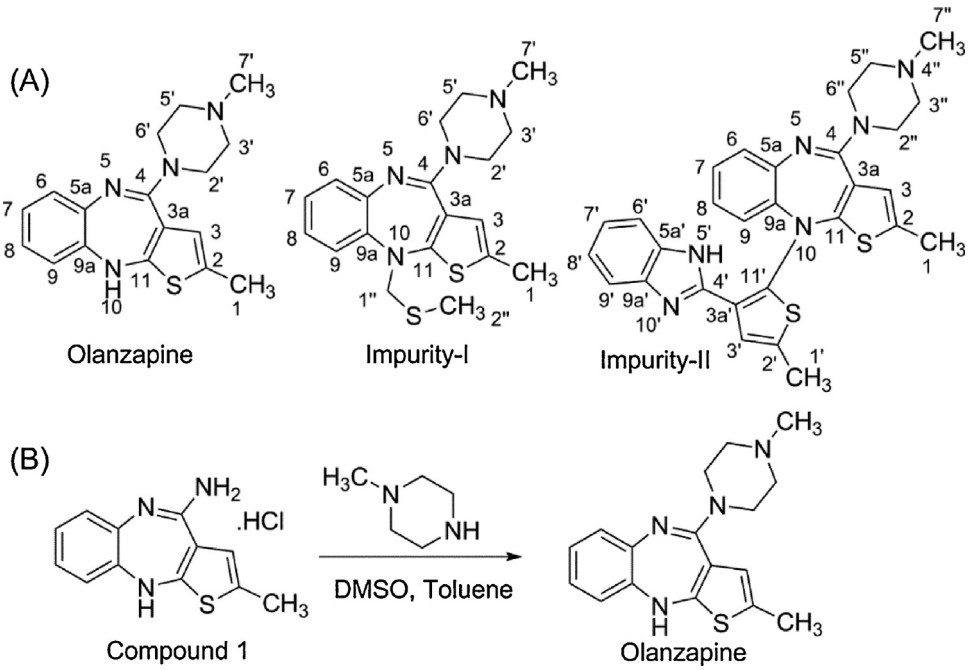

Fig. 1. (A) Chemical structures of olanzapine, impurity-I and impurity-II with numbering; (B) The key synthetic step of olanzapine.

olanzapine. To the best of our knowledge, the identification, separation and structural elucidation of two N-10 substituted byproducts of olanzapine (Impurity-I and II, Fig. 1A) by UV, FT-IR, LC–MS/TOF, 1D-NMR, 2D-NMR and single-crystal X-ray diffraction analysis, and the plausible prospects to the formation and controlling of impurities are first detailed in this paper.

2. Experimental

2.1. Chemicals and reagents

The various pilot batches samples and reference standard (No. 20170215-B, 99.99%) of olanzapine were obtained in our laboratory. Impurity-I and II were purified from the enriched mother liquor of olanzapine by preparative HPLC. Acetonitrile (HPLC grade) and sodium dodecyl sulfate (SDS, AR grade) were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Phosphoric acid (85%, w/w, AR grade) and sodium hydroxide (AR grade) were purchased from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). Edetate disodium (EDTA, AR grade) and dichloromethane (AR grade) were obtained from Shanghai Lingfeng Chemical Regent Co., Ltd. (Shanghai, China). Water used for the preparation of mobile phase was purified using Millipore Milli-Q plus (Milford, MA, USA) purification system. Dimethyl sulphoxide-d_6 (for NMR) was purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China).

2.2. High performance liquid chromatography (analytical)

The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of a Shimadzu LC-20AD high pressure pump with a Gradient Valve Kit (LPGE UNIT 20A) attached to it, a DGU-20A_5 on-line degasser, a CTO-20A column oven set at 35 °C, and a SPD-20A UV–vis variable wavelength detector set at 220 nm. Data acquisition was performed with LabSolutions software. The separation of olanzapine was performed with an Agilent ZORBAX Eclipse XDB-C_8 column (250 mm × 4.6 mm, 5 μm). The mobile phase A and B were acetonitrile-buffer (48:52, v/v) and acetonitrile-buffer (70:30, v/v), respectively. The HPLC buffer was prepared with a process which started from dissolving 13 g of sodium dodecyl sulfate to 1450 mL of water, then added 5 mL of phosphoric acid (85%, w/w), adjusted with sodium hydroxide (50% w/w) to pH 2.5, and finally diluted with water to 1500 mL. The linear gradient programme was set as follows: T_{min}/B(%); T_0/0; T_{10}/0; T_{20}/100; T_{25}/100; T_{27}/0; T_{35}/0. Flow rate was set at 1.5 mL/min and injection volume was 20 μL. The sample diluent was a mixture of SDS-phosphoric acid-EDTA (30 mM: 50 mM: 0.1 mM) of pH 2.5 adjusted with orthophosphoric acid and acetonitrile in the ratio of 60:40 (v/v).

2.3. High performance liquid chromatography (preparative)

Preparative isolation work was performed on SHIMADZU LC-20A preparative HPLC system which was equipped with an automated fraction collector and UV detector. Data was collected and processed using LabSolutions software. Approximately 50 mg/mL of olanzapine raw product was prepared to load on to the column. A Welch Xtimate C_{18} column (250 × 30 mm, 5 μm) was employed. Separation of olanzapine and its process-related impurities was achieved under gradient mode (T_{min}/B(%):T_0/25, T_4/25, T_9/50, T_{15}/60, $T_{15.5}$/25, T_{16}/stop) using a mobile phase of 0.1% formic acid in water (A)-acetonitrile (B) and detection wavelength at 220 nm. Flow rate was set at 20 mL/min and injection volume was 200 μL.

The fractions corresponding to impurity-I and II were co1lected into dry ice cooled flasks and concentrated by evaporating acetonitrile at room temperature under high vacuum on a BC-R203 Rotary Evaporator (Shanghai Biochemical Equipment CO., LTD.). The aqueous layer was adjusted to pH 8.0 with 0.6 mol/L NaOH and extracted with methylene chloride. Methylene chloride layer was evaporated at room temperature under vacuum by means of BC-R203 Rotary Evaporator. And impurity-I (HPLC purity: 99.1%) and II (HPLC purity: 97.1%) were obtained.

2.4. Liquid chromatography-mass spectrophotometry (LC–MS/MS)

Mass spectrometry was performed using an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system equipped with an Agilent Jet Stream electrospray source and con-