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Short communication

Structure elucidation of a process-related impurity of dapoxetine

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ABSTRACT

Unknown by-product associated with the synthesis of dapoxetine was isolated. The structure elucidation of this new compound using accurate mass data and NMR spectroscopy is presented herein. The unambiguous resonance assignment concluded to the formation of a tricyclic compound 4-phenyl-2H,3H,4H-naphtho[1,2-b]pyran, a new impurity of dapoxetine which has never been reported previously. A proposed mechanism for the formation of the new carbon–carbon bond is discussed. For the separation of dapoxetine and the process-related impurities, a gradient HPLC method was developed.

1. Introduction

Dapoxetine (Dpx), (*S*)-*N*,*N*-dimethyl[3-(naphthalen-1-yloxy)-1-phenylpropyl]amine hydrochloride, Priligy®, is a novel short acting selective serotonin reuptake inhibitor (SSRI) that is being developed specifically as an on-demand oral treatment of premature ejaculation with a unique pharmacokinetic profile [1]. Dpx attains its peak plasma concentration in about 1.5 h after dosing which is much faster than conventional SSRIs and by 24 h the plasma concentration decreases to approximately 5% of the peak concentration. These pharmacokinetic properties make Dpx an excellent candidate for on-demand treatment of premature ejaculation. The eutomer (*S*)-Dpx is 3.5 times more potent SSRI than (*R*)-Dpx, that is why Dpx is marketed as a single enantiomer drug [2]. A wide range of synthetic procedures were developed to synthesize racemic and enantiopure Dpx [3–7].

Our previous study reported a robust, sensitive and validated method for the chiral separation of Dpx enantiomers *via* cyclodextrin-modified capillary electrophoresis [7] along with a synthetic procedure for racemic Dpx following the main literature methods. In this communication, the identification of a by-product is discussed, which was formed in the last step of a common synthetic pathway using *in situ* mesylation for dapoxetine synthesis [6,8–15] (Fig. 1).

This well-characterized and widely accepted scheme for Dpx synthesis utilizes mesylate as an excellent leaving group in nucleophilic substitution reactions. The mesylate intermediate (**5**) is formed *in situ* and usually converted to Dpx without isolation. There is a single reference reporting the characteristics of this intermediate [16], however the isolation of this compound is missing. Several attempts failed to isolate compound **5**, but provided an unknown by-product.

The goal of this communication is the structure elucidation of this new by-product using NMR spectroscopy and mass spectrometry. Following the structural characterization of this impurity, a mechanism for the formation was also proposed. For the separation of Dpx and the process-related impurities, a gradient HPLC method was developed.

2. Materials and methods

2.1. Instrumentation

All NMR experiments were carried out on a 600 MHz Varian DDR NMR spectrometer equipped with a 5 mm inverse-detection gradient (IDPFG) probehead. Standard pulse sequences and processing routines available in VnmrJ 3.2 C/Chempack 5.1 were used for structure identifications. The complete resonance assignments were established from direct ^{1}H–^{13}C, long-range ^{1}H–^{13}C, and scalar spin–spin connectivities using 1D ^{1}H, ^{13}C, ^{1}H–^{1}H gCOSY, ^{1}H–^{13}C gHSQCAD (J = 140 Hz), ^{1}H–^{13}C gHMBCAD (J = 8 Hz) experiments, respectively. The probe temperature was maintained at 298 K and standard 5 mm NMR tubes were used. The ^{1}H chemical shifts were

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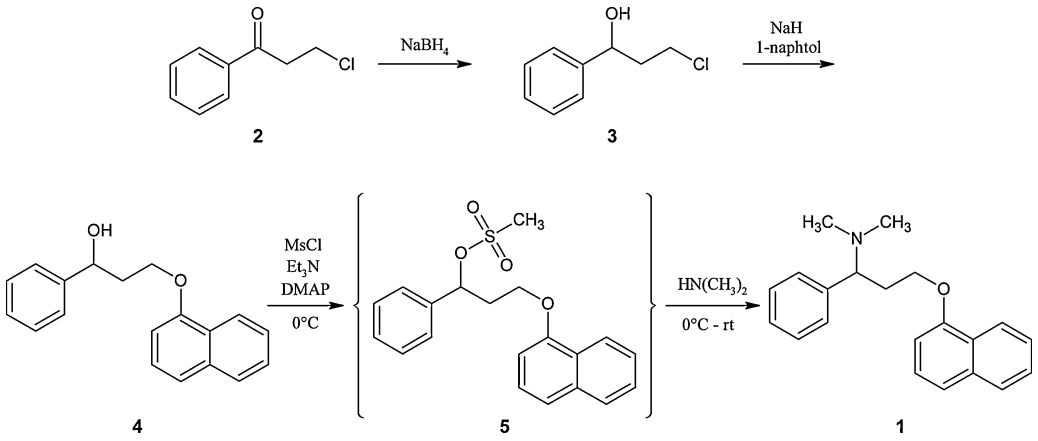

Fig. 1. Synthetic scheme of dapoxetine involving *in situ* mesylation in the last step.

referenced to TMS (0.00 ppm) while ^{13}C chemical shifts were referenced to the applied NMR solvent $CDCl_3$ (77.16 ppm).

The accurate mass of the products were determined with an Agilent 6230 time-of-flight mass spectrometer. Samples were introduced by the Agilent 1260 Infinity LC system, the mass spectrometer was operated in conjunction with a JetStream (ESI) ion source in positive ion mode. Reference masses of *m/z* 121.050873 and 922.009798 were used to calibrate the mass axis during analysis. Mass spectra were processed using Agilent MassHunter B.02.00 software.

HPLC–DAD analyses were performed using an Agilent 1260 Infinity LC apparatus. An Agilent Zorbax Eclipse Plus C18, 100 mm × 4.6 mm i.d. (3.5 μm particle size) column was applied. The mobile phase consisted of methanol and water with 0.1 (v/v%) formic acid using the following gradient program: 0 min 50% MeOH, 4 min 50% MeOH, 6 min 90% MeOH, 10.0 min 90% MeOH, 11 min 50% MeOH, 15 min 50% MeOH. The flow rate was set to 1 ml/min, while the column temperature was kept at 50 °C. The UV spectra were recorded ranging from 200 to 400 nm.

2.2. Chemicals

All reagents for synthesis, HPLC grade solvents used for LC–MS analyses and $CDCl_3$ (99.8 atom% D) for NMR were purchased from Sigma–Aldrich. Water was produced by a Millipore Milli-Q Direct 8 water purifying system. TLC was performed using precoated Silica gel 60 F_{254} TLC plates and visualized with ultraviolet light at 254 nm. For column chromatography, the 40–63 μm silica was used.

2.3. Synthesis of 3-chloro-1-phenyl-1-propanol (**3**)

To the solution of 3-chloropropriophenone (1 g; 6 mmol) in THF (14 ml) and water (1 ml), $NaBH_4$ (0.27 g; 7 mmol) was added at 0 °C. The mixture was stirred for 24 h at room temperature.

After completion (checked by TLC using CH_2Cl_2, starting material R_f = 0.77, product R_f = 0.48), dilute acetic acid (12%) was added slowly to the reaction mixture with stirring at the same temperature to set the pH to 4.5. The crude mixture was treated with water (10 ml), extracted with ethyl acetate (2 × 150 ml). The separated organic layers were combined and washed with 5% $NaHCO_3$ solution. The organic layer was washed with water and dried over sodium sulfate and filtered, followed by solvent evaporation.

The crude product was the racemic 3-chloro-1-phenyl-1-propanol (**3**, 1.02 g, 99%) as yellow oil and subsequently crystallized overnight.

^1H NMR ($CDCl_3$, 600 MHz): δ (ppm): 7.39–7.34 (m, 4H), 7.33–7.29 (m, 1H), 4.94 (dd, *J* = 8.5, 4.7 Hz, 1H), 3.78–3.70 (m, 1H), 3.59–3.53 (m, 1H), 2.28–2.20 (m, 1H), 2.13–2.06 (m, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm): 143.83, 128.80, 128.05, 125.90, 71.47, 41.85, 41.58. HRMS: calc. $[M+Na]^+$ 193.0391, found $[M+Na]^+$ 193.0396.

2.4. Synthesis of 3-(1-naphthalenyloxy)-1-pheny-l-propanol (**4**)

Operating under inert atmosphere of nitrogen gas, to a mixture of DMF (2.3 ml) and 60% sodium hydride in mineral oil (0.12 g, 2.9 mmol) at 0 °C in an ice bath a solution of 1-naphthol (0.43 g, 2.9 mmol) of DMF (2.3 ml) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h and a solution of **3** (0.53 g, 3.1 mmol) in DMF (2.1 ml) was added. After stirring overnight at room temperature and completion (checked by TLC using *n*-hexane and EtOAc (9:1, v/v), starting material R_f = 0.21, product R_f = 0.14), the reaction mixture was poured into water (10 ml) and extracted with ethyl acetate (3 × 25 ml). The extracts were combined and washed with water (30 ml) and sodium hydroxide solution (2 × 20 ml, 1 N), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The 0.84 g crude product was purified by column chromatography using *n*-hexane and EtOAc (9:1, v/v) as an eluent, to afford purified 3-(1-naphthalenyloxy)-1-pheny-l-propanol (**4**, 0.58 g, 70.2%).

^1H NMR ($CDCl_3$, 600 MHz): δ (ppm): 8.29–8.24 (m, 1H), 7.85–7.80 (m, 1H), 7.54–7.48 (m, 2H), 7.47–7.42 (m, 3H), 7.41–7.35 (m, 3H), 7.31 (t, *J* = 7.3 Hz, 1H), 6.81 (d, *J* = 7.6 Hz, 1H), 5.14 (dd, *J* = 8.2, 4.9 Hz, 1H), 4.38–4.33 (m, 1H), 4.23–4.18 (m, 1H), 2.46–2.39 (m, 1H), 2.37–2.30 (m, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm): 154.57, 144.30, 134.63, 128.74, 127.84, 127.67, 126.54, 126.00, 125.96, 125.71, 125.40, 121.96, 120.55, 104.93, 72.28, 65.51, 38.65. HRMS: calc. $[M+Na]^+$ 301.1199, found $[M+Na]^+$ 301.1204.

2.5. Synthesis of 4-phenyl-2H,3H,4H-naphtho[1,2-b]pyran (**6**)

To a solution of **4** (0.54 g, 1.9 mmol) in CH_2Cl_2 (8 ml) triethylamine (0.29 g, 2.9 mmol) and 4-dimethylaminopyridine (DMAP) (1.02 mg, 8.2 μmol) were added under nitrogen atmosphere. The reaction mixture was cooled to 0 °C. Methanesulfonyl chloride (0.29 g, 2.5 mmol) was added dropwise to the reaction mixture and stirred at 0 °C for 2 h. The reaction mixture was stirred at room temperature for additional 21 h. After completion (checked by TLC using *n*-hexane and EtOAc (9:1, v/v), starting material R_f = 0.12, product R_f = 0.64), the reaction mixture was added to CH_2Cl_2 (30 ml) and extracted with 0.5 M HCl (2 × 20 ml), saturated sodium bicarbonate solution (2 × 20 ml), washed with water (2 × 20 ml), dried