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Short communication

Isolation and identification of a new sildenafil analogue adulterated in energy drink: Propoxyphenyl sildenafil

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ABSTRACT

A new sildenafil analogue was found to be added illegally to a energy drink marketed for the enhancement of sexual function. The structure was determined as 1-[4-propoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulfonyl]-4-methylpiperazine. Owing to the inclusion of one more methyl group to sildenafil (on *C*-21), the detected compound was called "propoxyphenyl sildenafil". The sample was purified with column chromatography. The UV, IR, LC/MS (ESI) and completely assigned NMR data of propoxyphenyl sildenafil is reported. Having compared the structure with sildenafil, the results showed that the 2-ethoxy group (at the position on C-19) has been replaced by propoxy group.

1. Introduction

Synthetic phosphodiesterase-5 (PDE-5) inhibitors, such as Sildenafil citrate (Viagra®, Pfizer), Vardenafil hydrochloride (Levitra®, Bayer), and Tadalafil (Cialis®, Lilly) are widely used for the treatment of erectile dysfunction (ED) [1,2]. However, a number of important adverse effects of these approved drugs have been reported [1], such as headache, flushing, cardiovascular disorders [3], visual disturbances [4,5], sudden hearing loss [6]. It is well known that, food supplement are suitable vehicles for adulteration with some drug substance or undeclared synthetic chemical compounds [1,7]. Some herbal products advertised as "all natural", in contrast had been found to contain synthetic PDE-5 inhibitors [7,8]. Legal PDE-5 inhibitors or their unapproved new analogues as adulterants are frequently detected in dietary herbal supplements, for enhancing sexual men performance. Recently, the use of new modified derivatives is increasing steadily and the current number of analogues is estimated to be at least 50 [1,7]. At this point, the question is: why do the manufacturers use these structure-modified new compounds for their production? The answer is: it is difficult to detect these modified adulterants by means of ordinary laboratory inspection system using reference standards, without advanced instruments like nuclear magnetic resonance spectroscopy (NMR) [1,9–11]. NMR is the only analytical technique that provides full structural information of novel compounds [10]. Briefly, these manufacturers have only one intention: escaping from detection. On the other hand, consumers are at risk if dietary supplements are adulterated with these new analogues, because of their unknown safety and toxicity profile. Hence, it is very important to detect the presence of known or unknown synthetic PDE-5 inhibitors, in health supplements. In our central research laboratory, we are analysing these commercially available dietary supplements which have been sent by the Ministry of Food, Agriculture and Livestock, to check whether they possess these kind of analogues [2,12]. In the present study, a new sildenafil analogue (**1**) was isolated from an energy drink and its structure was determined using NMR, MS and IR (Fig. 1).

2. Materials and methods

2.1. Equipments

Uncorrected melting points were measured on a Büchi B-540 capillary melting point apparatus. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded employing a VARIAN MERCURY 400 MHz FT spectrometer, with $CDCl_3$ as solvent. Chemical shifts (δ) are in ppm relative to TMS. The LC/MS spectra were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC, using ESI(+) method, with C-18 column. Elemental analyses were performed by Leco CHNS-932. The infrared spectrum was recorded in the 600–3600 cm^{-1} range using a Perkin-Elmer Spectrum 100 FT-IR spectrometer and KBr pellets. The UV spectrum was recorded with Shimadzu UV-1601 UV/Vis spectrophotometer.

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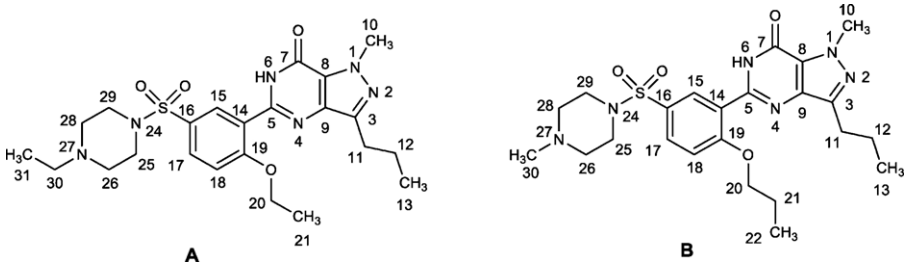

Fig. 1. Structures of homosildenafil (A) and propoxyphenyl sildenafil (Compound **1**) (B).

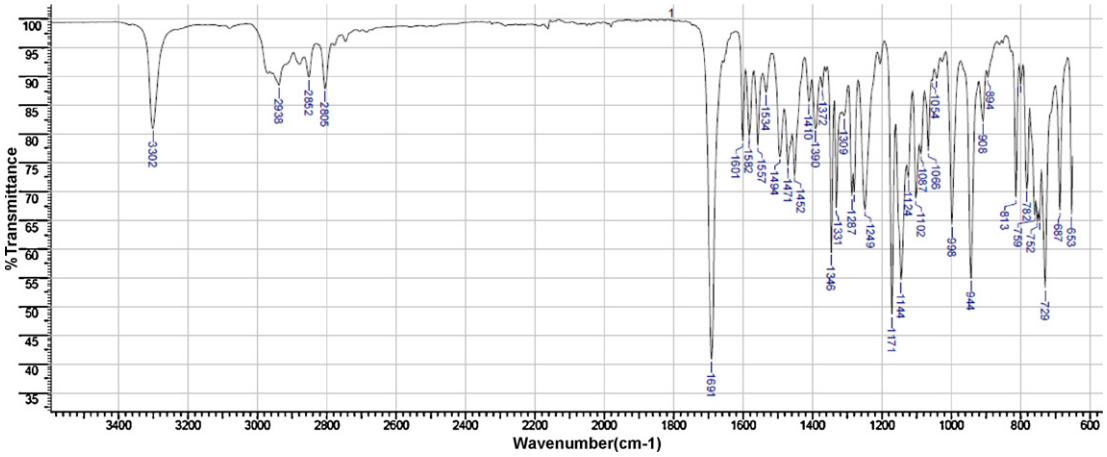

Fig. 2. IR spectrum of propoxyphenyl sildenafil.

2.2. Extraction and isolation

The contents of two aluminium cans (each containing 150 ml) of energy drink samples were extracted with the mixture of CH_2Cl_2—MeOH (95:5), evaporated and directly transferred to an open column with silica gel 60 (0.04–0.063 mm) and eluted with CH_2Cl_2—MeOH (100:1.5). Fractions were collected and analysed by TLC. All fractions containing the target compound were collected and the solvent was evaporated and the residue crystallised from MeOH. 0.033 g of white powder was obtained, m.p. 160–161 °C. Analysis calculated for $C_{23}H_{32}N_6O_4S$: C 56.54, H 6.60, N 17.2, S 6.56; found: C 56.56, H 6.33, N 16.9, S 6.45.

2.3. NMR analysis

Unknown compound (**1**) was dissolved in $CDCl_3$ and subjected to 1D and 2D NMR spectroscopic analysis (^1H, ^{13}C, DEPT, Homo-COSY, HSQC and HMBC) at room temperature.

2.4. LC/MS analysis

LC-MS coupled with positive (ESI+) Electro Spray method was used to determine its molecular weight. The HPLC of LC/MS was carried out on a column XTerra® MS C-18 (4.6 mm × 250 mm, 5 μm) with acetonitrile:methanol: 0.01 M ammonium acetate in water (65:20:15) as the mobile phase. The flow rate was 1.0 ml/min, the injection volume was 10 μl and the running time 20 min. The eluate was monitored by a photo-diode array detector at 290 nm. The analytical condition of mass was as follows: capillary voltage: 3.71 kV, cone voltage: 75 V, source temperature: 100 °C, desolvation temperature: 350 °C. Compound (**1**) showed its $[M+H]^+$ ion at *m/z* 489.2 which is corresponding to the molecular formula $C_{23}H_{32}N_6O_4S$ (488).

3. Results and discussion

The molecular weight of homosildenafil is 488, that of the unknown compound is the same, however its HPLC retention time was different: it was between those of sildenafil and homosildenafil. Therefore, the unknown compound isolated from commercially available energy drink is considered to be one of the new sildenafil analogues. Compound (**1**) was purified using column chromatographic techniques as well as crystallisation from energy drink.

Compound **(1)** exhibited two UV maxima at 215 and 292 nm in MeOH. The IR spectrum (KBr) showed absorption bands with the characteristics of an amine at 3302 cm^{-1}, C—H stretching at 2938 and 2852 cm^{-1}, an α,β-unsaturated lactam at 1691 cm^{-1}, an aromatic ring at 1558 cm^{-1}, asymmetric sulfonamide at 1346 cm^{-1} and symmetric sulfonamide at 1171 cm^{-1} (Fig. 2).

The mass spectra and fragmentation product ions of sildenafil and propoxy sildenafil are shown in Fig. 3 and Scheme 1, respectively. Both compounds exhibit similar fragmentation with the initial loss of the piperazine containing portion of the molecule. Evidence of this loss can be deduced from the product ions *m/z* 311 for sildenafil and *m/z* 325 (due to inclusion of one methylene) for propoxy sildenafil. The product ions, with *m/z* 299, 283 and 99 are common, because of their similar structure.

Table 1 shows the ^1H NMR, ^{13}C NMR, DEPT, ^1H–^1H–COSY, HSQC and HMBC spectral data of compound (1). The spectroscopic numbering and the structure of the unknown compound (1) are shown in Fig. 1. The ^1H NMR spectrum showed characteristics of amide proton at δ_H 10.86 (1H, *br.s*). While the splitting pattern of the protons of C-20 is a quartet in homosildenafil [11–14] due to the neighbourhood to CH_3 group (*C*-21), corresponding protons of this unknown compound (**1**) is observed as a triplet at δ_H 4.25 ppm (2H, t, J = 7.6 Hz). This means, that there is one more carbon atom