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Short communication

Rapid screening and structural elucidation of a novel sibutramine analogue in a weight loss supplement: 11-Desisobutyl-11-benzylsibutramine☆

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ABSTRACT

A novel analogue of sibutramine, 11-desisobutyl-11-benzylsibutramine, has been discovered. During routine ion mobility spectrometry (IMS) screening of a weight loss supplement collected at an US FDA import operation facility an unknown peak was observed. Further analysis of the supplement by liquid chromatography-mass spectrometry (LC–MS) and high resolution mass spectrometry revealed an unknown peak with a relative retention time of 1.04 with respect to sibutramine and a predicted formula of $C_{20}H_{24}NCl$. In order to elucidate the analogue's structure, it was isolated from the supplement and characterized by tandem mass spectrometry and nuclear magnetic resonance (NMR), which revealed the analogue possessed a benzyl moiety at the 11 position in place of the isobutyl group associated with sibutramine.

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1. Introduction

According to the Centers for Disease Control and Prevention, more than one-third of adults and approximately 17% of children and adolescents aged 2–19 years in the United States are obese [1]. Given the epidemic of obesity in the United States and worldwide, treatment of obesity has emerged as one of the greatest present unmet medical needs and a target for pharmacological interventions [2]. Over the past 80 years, pharmaceutical attempts to address the problem have been severely limited by adverse events. Currently only two FDA approved treatments exist on the market: Orlistat® and the recently approved Qsymia® [3].

Sibutramine (*N*-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl-*N*,*N*-dimethylamine **1**, Fig. 1) was first reported in 1987 by Jeffery and Whybrow as a pharmaceutical treatment for depression [4], but the serotonin and noradrenaline re-uptake inhibitor soon emerged as a novel compound for the treatment of obesity [5–7]. It was approved by the FDA in 1997 for obesity treatment and marketed by Abbott labs as Meridia®. However, a number of cardiovascular events were reported and subsequent studies on the drug determined the risks for heart attack and stroke outweighed the weight loss benefits of sibutramine [8]. In October 2010 the FDA requested voluntary withdrawal of sibutramine from the market, to which Abbott acquiesced [9]. Nonetheless, sibutramine continues to be found in the US dietary supplement market along with its major *N*-desmethyl and *N*-didesmethyl analogues by our laboratory and others [10–13]. These supplements are advertised to be a natural way for promoting weight loss, yet the true efficacy of the supplement is often exhibited through the action of illegally added sibutramine, its analogues, or other pharmaceutical components such as phenolphthalein. The active pharmaceutical ingredient (API) is frequently left off the package label which misleads consumers and puts them at risk for adverse events.

In an attempt to survey many products and ingredients on the US market, the FDA has developed a rapid-screening program employing IMS, Raman, X-ray fluorescence and infrared techniques [10,11,14–20] which serve as general "pass/fail" screening methods. IMS (benchtop and portable) analysis of weight-loss supplements has been employed previously by our laboratory [10,11], and this technique was applied to the current work. Herein we report the detection and identification of a new sibutramine analogue found in a supplement advertised for weight loss: 11-desisobutyl-11-benzylsibutramine (**2**, Fig. 1).

2. Experimental

2.1. Materials

A dietary supplement (which consisted of a blister pack of capsules containing a brown powder) that was advertised for

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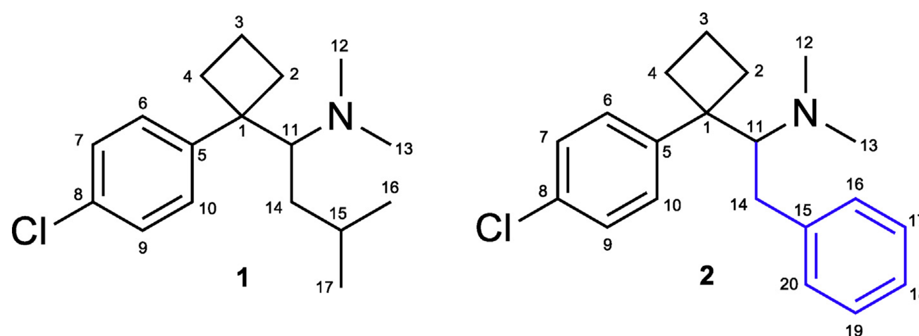

Fig. 1. Sibutramine **1** and analogue **2**.

weight-loss was collected at a US FDA import operation facility. Water was purified to 18 MΩ cm using a Milli-Q water system from Millipore (Bedford, MA, USA). HPLC grade acetonitrile, isopropyl alcohol, dichloromethane, sodium hydroxide and formic acid (99+%) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). $CDCl_3$ (99.8 atom % D), D_2O (100.0% atom D) and magnesium sulfate (97%, anhydrous) were purchased from Acros (Morris Plains, NJ, USA). Sibutramine HCl monohydrate standard was purchased from USP (Rockville, MA, USA). Meridia® 5 mg capsules were obtained from Abbott Laboratories (Chicago, IL, USA). Polytetrafluoroethylene (PTFE) syringe filters (0.45 μm pore size, 25 mm membrane diameter) were purchased from Millipore (Marlborough, MA). The sampling swabs used with the portable ion mobility spectrometer (SABRE 4000) were purchased from Smiths Detection (Warren, NJ). Substrates (PTFE filter, 2 μm pore size, 46.2 mm membrane diameter) used with the benchtop ion mobility spectrometer (IONSCAN-LS) were obtained from Whatman (Florham Park, NJ).

2.2. Instrumentation and methods

2.2.1. Ion mobility spectrometry

An IONSCAN®-LS (Smiths Detection, Danbury, CT, USA) benchtop ion mobility spectrometer operated in the positive ion mode was used to screen a weight loss supplement for the presence of illegal adulterants including sibutramine, desmethylsibutramine, didesmethylsibutramine, phenolphthalein, fluoxetine, rimonabant, fenfluramine, and sertraline [11]. The system was equipped with an internal isobutyramide calibrant. Ion mobility spectra were recorded using the IONSCAN®-LS IM-Station Software version 5.389. Prior to analysis 1 μl of sample was deposited onto a PTFE substrate and the solvents were allowed to evaporate. The substrate was then introduced into the IMS system and placed on the desorber heater. Parameters included an analysis time of 13.75 s with a scan period of 50 ms, a shutter grid width of 0.2 ms, 28 segments with 10 co-added scans per segment and a drift flow of 300 cc/min. The desorber, inlet and drift tube temperatures were set to 291, 289 and 232 °C, respectively. A sibutramine reference standard solution was prepared by adding 15 ml of isopropyl alcohol to the contents of one 5 mg capsule of Meridia® (Abbott Laboratories). After shaking by inversion for 30 s, the standard solution was filtered using a 0.45 μm PTFE syringe filter (Millipore) and diluted 1:100 in isopropyl alcohol for IMS analysis. The weight loss supplement sample was prepared in the same manner. The isolated analogue in D_2O (see Sections 2.2.3 and 2.2.4) was analyzed using the benchtop IMS device.

A SABRE® 4000 (Smiths Detection, Danbury, CT) portable ion mobility spectrometer (dimensions of 14.5 in × 4 in × 4.5 in and weight of 7 lbs) with Instrument Manager software version 5.057 (Smiths Detection, Danbury, CT) was used in this study. The instrument contained nicotinamide as an internal calibrant. The preset narcotics control parameters (positive ion) and particle mode were used for the detection of sibutramine and the analogue [10]. The analysis time was 15 s with a scan period of 27 ms, a shutter grid width of 0.3 ms, 12 segments with 50 co-added scans per segment and a drift flow of 200 cc/min. Using a type K thermocouple (Control Company model 4131CC, Friendswood, Texas), the desorber temperature of the portable IMS instrument was measured and set to 200 °C. The drift tube temperature was preset to approximately 130 °C. The instrument was baked-out for 2 h at 180 °C and cooled for 2 h prior to the analysis. After each analysis, the instrument was purged with clean air as needed. The sample that was previously analyzed using the benchtop spectrometer was also analyzed using the portable IMS instrument. In order to obtain a reduced mobility of the sibutramine analogue using the portable IMS device, the isolated analogue in D_2O (see Sections 2.2.3 and 2.2.4) was analyzed directly. In order to obtain a peak area that was similar to that found from the analysis of the sample, the isolated analogue D_2O solution was diluted with isopropyl alcohol (approximately 1:3 dilution) and analyzed.

2.2.2. Liquid chromatography–mass spectrometry and high resolution mass spectrometry

For direct infusion mass spectrometric studies, the sample was analyzed using a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a Triversa Nanomate nanospray source (Advion, Ithaca, NY). High resolution mass spectra were acquired in the positive mode over the mass range from m/z 100 to 1000 at an operating resolution of 30,000 at m/z 400. MS/MS spectra were obtained by using collision-induced dissociation at relative collision energy of 45%. LC–MS analysis was performed using a modified in-house method. The system consisted of an Agilent 1200 HPLC coupled to a Thermo LTQ Orbitrap XL mass spectrometer with a Luna Phenyl Hexyl Column, 2.0 mm × 150 mm, 3 μm particle size (Phenomenex, Torrance, CA). Mobile phase A consisted of 0.1% formic acid in water while mobile phase B consisted of 0.1% formic acid in acetonitrile. The gradient started at 30% B and was held there for 4 min before ramping to 95% B over 4 min. The gradient was then held at 95% B for 8 min, after which the system returned to 30% B for column re-equilibration for a total run time of 25 min. The sample was prepared by taking the filtered extract prepared above for benchtop IMS and diluting 1:1000 in 49:49:2 acetonitrile:water:formic acid for mass spectrometric analysis. Data were processed using Xcalibur QualBrowser software (version 2.0.7).

2.2.3. Isolation of the analogue from the dietary supplement

Semi-prep chromatography was conducted on an Agilent 1100 system with fraction collection performed using an Agilent 1200 series G1364C analytical fraction collector. A Phenomenex Luna 5 μm PFP(2) 100 Å 100 × 10.0 mm column was used for isolation of the analogue. Mobile phase A consisted of Milli-Q water with 0.1% formic acid and B was acetonitrile with 0.1% formic acid.

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