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Short communication

# Structural characterization of sulfoaildenafil, an analog of sildenafil

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ABSTRACT

Phosphodiesterase type 5 (PDE-5) inhibitors represent a class of drugs used primarily in the treatment of erectile dysfunction. Currently, three PDE-5 inhibitors have been approved by the U.S. Food and Drug Administration (FDA) for use in the United States: sildenafil citrate, tadalafil, and vardenafil hydrochloride trihydrate. A bulk material, labeled as an ingredient for a dietary supplement, was analyzed for the presence of PDE-5 inhibitors. The compound that was detected displayed structural similarities to sildenafil, and was characterized further using LC–$MS^n$, FTICRMS, X-ray crystallography and NMR. The compound was given the name sulfoaildenafil. When compared to sildenafil, sulfoaildenafil contains a sulfur atom substitution for the oxygen atom in the pyrazolopyrimidine portion of the molecule, and a 3,5-dimethyl substitution on the piperazine ring, rather than the 4-methyl moiety. The X-ray crystallographic data indicate that the material in this sample is comprised of two polymorphs, which may affect the chemical and/or biological properties of any product formulated with this compound.

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## 1. Introduction

Phosphodiesterase type 5 (PDE-5) inhibitors represent a class of drugs used primarily in the treatment of erectile dysfunction. For the past several years, synthetic PDE-5 inhibitors such as sildenafil, tadalafil and vardenafil have been identified routinely in "all natural" herbal supplements [1–6]. More recently, the trend has shifted toward the introduction of analogs of the approved PDE-5 inhibitors. These compounds exhibit minor structural alterations compared to their approved or commercial counterparts. Based on these structural similarities, it is reasonable to expect these analogs to exhibit similar biological activities [7–9]. The presence of analogs in herbal supplements or in unapproved drug products could pose a significant risk to public health, because these analogs are not declared on the labeling. In many cases, there is no information available to the public with regard to toxicological or pharmacological effects. However, because herbal products are often considered to be "all natural", there exists a widespread belief that they are inherently safer and healthier than products that contain synthetic ingredients. This belief may be especially true for individuals for whom synthetic PDE-5 inhibitors are contraindicated, such as patients who take nitrate medications for treatment of diabetes, hypertension, hyperlipidemia and ischemic heart disease. The possibility of an individual taking nitrates in combination with supplements that have been adulterated with synthetic PDE-5 inhibitors or their analogs may have serious health consequences [10].

Numerous analogs of sildenafil, tadalafil and vardenafil have been reported in the literature [2,11–21]. In general, analogs of sildenafil, such as homosildenafil [2,11], hydroxyhomosildenafil [11,12] and aildenafil (dimethylsildenafil, methisosildenafil) [18,19], incorporate structural changes in the piperazine portion of the molecule. Recently, sildenafil thione derivatives have been reported, in which the oxygen atom in the pyrazolopyrimidine portion of sildenafil has been replaced by a sulfur atom [22–25].

This article describes the structural characterization of sulfoaildenafil, a thione derivative of aildenafil. The compound was detected during an analysis of a suspect bulk material using liquid chromatography–electrospray ionization mass spectrometry (LC–$MS^n$). Determination of the compound's accurate mass was accomplished using Fourier-transform ion cyclotron resonance mass spectrometry (FTICRMS).

Because the placement of the two methyl groups on the piperazine ring cannot be determined solely using mass spectrometry, the bulk material was also subjected to X-ray crystallographic and nuclear magnetic resonance (NMR) spectroscopic characterization. These experiments provide unequivocal structural information for sulfoaildenafil. This compound, also called thiomethisosildenafil, has been reported recently as an adulterant in a herbal supplement

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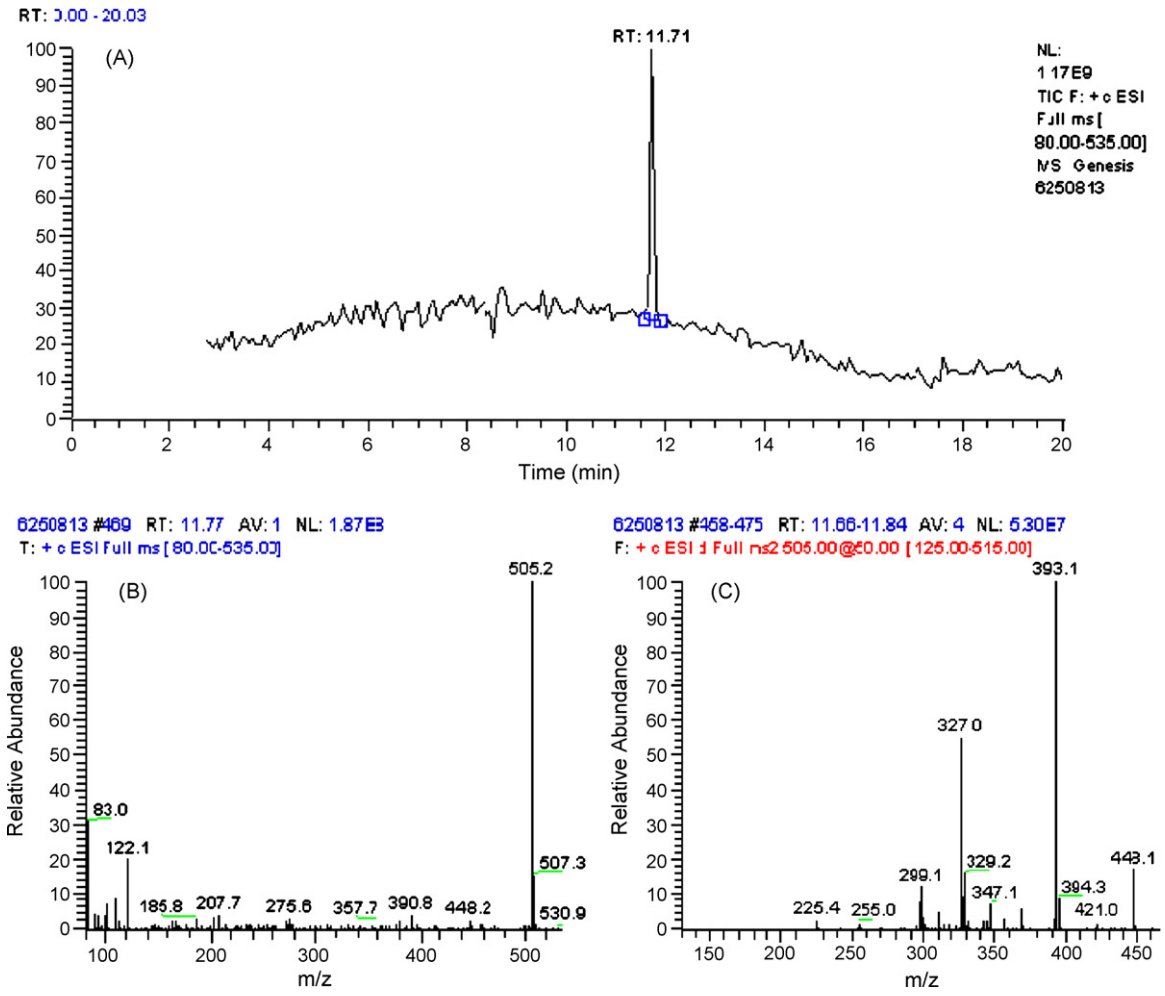


**Fig. 1.** Mass spectrometric characterization of sulfoaildenafil. (A) Total ion chromatogram. (B) Full scan $ESI^+$ mass spectrum for sulfoaildenafil. (C) Product ion spectrum ($MS^2$ of $m/z$ 505).

[25]. The accurate mass and X-ray crystallographic data presented in this article supplement the data reported previously [25].

## 2. Experimental

### 2.1. Chemicals and sample

The suspect bulk material was submitted to the laboratory for analysis. HPLC grade $CH_3CN$, HPLC grade $CH_3OH$ and acetic acid were purchased from Fisher Scientific (St. Louis, MO, USA). Formic acid (88%), diethyl ether, tetramethylsilane, dimethylformamide and dimethyl sulfoxide-$d_6$ were purchased from Sigma–Aldrich (St. Louis, MO, USA). The 18 MΩ cm deionized $H_2O$ was generated using a Milli-Q system (Millipore, Bedford, MA, USA).

### 2.2. LC–$MS^n$ analysis

The column used was a Zorbax SB-C18, 2.1 mm × 150 mm, 3.5 μm particle size (Agilent Technologies, Wilmington, DE, USA). A gradient mobile phase was used at a flow rate of 0.2 mL/min, and consisted of 0.1% formic acid in $H_2O$ and $CH_3CN$ (0–15 min linear from 5% $CH_3CN$ to 95% $CH_3CN$; 15–20 min 95% $CH_3CN$; post-run equilibration of 7 min).

Samples were dissolved in 5–10 mL of $CH_3CN$:$H_2O$ (50:50, v/v). A portion of the solution was filtered through a 0.2 μm PTFE syringe filter (Fisher) and further diluted with $CH_3CN$ prior to analysis.

Initial LC–$MS^n$ screening analyses were performed using a Thermo-Finnigan LCQ Deca XP Plus ion trap mass spectrometer (ThermoElectron Corp., San Jose, CA, USA) coupled to an Agilent 1100 Series capillary HPLC system. Data acquisition and analysis were accomplished using Xcalibur version 1.3. Source parameters (capillary voltage, lens voltage, etc.) were optimized for maximum ion transmission, using the manufacturer's calibrant at $m/z$ 524, with the ESI source in positive ionization mode. The following parameters were constant through the analyses: sheath gas flow = 60 arbitrary units; sweep gas flow = 20 arbitrary units; spray voltage = 3.5 kV; capillary temperature = 325 °C. The analysis employed two scan events for the $MS^n$ analysis. The first was to collect MS data over the range $m/z$ 80–535. The second event was to collect data-dependent MS/MS spectra on the most intense ion from scan event 1, $m/z$ 505, with normalized collision energy of 50% using helium as the collision gas.

### 2.3. FTICRMS analysis

Accurate mass measurements were acquired using a Thermo-Finnigan LTQ FT mass spectrometer. For accurate mass measurement, the analyte was passed through a SEP-PAK C18 SPE cartridge (Millipore), preconditioned with 2 mL of $CH_3CN$ and 2 mL of water. Elution from the cartridge was done with 1 mL of $CH_3OH$. The eluate was diluted with $CH_3OH$:$H_2O$:acetic acid (50:50:1, v/v) for infusion.

The FTICR mass spectrometer was calibrated according to manufacturer's specifications using a solution of caffeine ($m/z$ 195), MRFA

($m/z$ 524) and Ultramark mix ($m/z$ 1122, 1222, 1322, 1422, 1522, 1622, 1722 and 1822). Data acquisition and analysis were accomplished using Xcalibur version 2.2. Mass spectra were acquired by syringe infusion of the extract at a flow rate of 5 μL/min. The ESI spray voltage was set to 3.5 kV. The resolving power was 100,000 at $m/z$ 400. The automatic gain control ion population target for full MS was $5e^{+5}$. The maximum ion injection time was 2000 ms for full MS.

### 2.4. X-ray crystallography

Diffraction data were collected on a Bruker AXS SMART APEX CCD diffractometer (Bruker AXS Inc., Madison, WI, USA) with software running the program ApexII (Apex2 v2008.2-4). The structures were solved by direct methods and refined by full matrix least squares against $F^2$ with all reflections using SHELXTL (SAINT (Version 6.02), SMART for WNT/2000 (Version 5.625) and SHELXTL (Version 6.14)) (Bruker). A portion of the bulk material was dissolved in dimethylformamide; single crystals were grown by slow diffusion of diethyl ether into this solution.

### 2.5. NMR analysis

$^1H$, $^{13}C$ and $^1H$ COSY nuclear magnetic resonance data were collected on a 400 MHz Bruker Avance 400 Ultrashield NMR equipped with a triple resonance probe and pulsed-field gradient accessories. Data were collected at 300 K with dimethyl sulfoxide-$d_6$ as the solvent. All shifts were reported against tetramethylsilane as internal standard.

## 3. Results and discussion

### 3.1. Mass spectrometry

The total ion chromatogram and product ion spectrum for the unknown compound are shown in Fig. 1, indicating an $[M+H]^+$ ion at $m/z$ 505, and product ions at $m/z$ 448, 393, 327 and 299. Hydroxyhomosildenafil [11], thiohomosildenafil [22–24] and thiomethisosildenafil [25] produce an $[M+H]^+$ ion at $m/z$ 505. Hydroxyhomosildenafil yields product ions at $m/z$ 489 and 377 [11], thiohomosildenafil at $m/z$ 477, 421, 393, 357, 355, 327, 315, 299 and/or 271 [22–24], and thiomethisosildenafil at $m/z$ 448, 393, 329, 327, 315 and 299 [25]. Aildenafil has a 3,5-dimethyl substitution on the piperazine ring, versus the 4-methyl group of sildenafil, and yields an ion at $m/z$ 489, and product ions at $m/z$ 377, 311, 283 and 113 [18,19]. Homosildenafil has a 4-ethyl substitution versus the 4-methyl group, has an ion at $m/z$ 489, and product ions at $m/z$ 377, 313, 311 and 283 [11]. Fig. 2 compares the structures of sildenafil, aildenafil and sulfoaildenafil.

The ions observed at $m/z$ 327 and 299 in the compound of interest, coupled with the lack of ions at $m/z$ 313 and 283, indicate the substitution of a sulfur atom for the oxygen atom in the pyrazolopyrimidine portion of sildenafil, as is observed in thiohomosildenafil and thiomethisosildenafil. The compound exhibits the loss of the piperazine portion of the molecule, which is deduced from the transition $m/z$ 505 → 393, and the loss of the piperazine and sulfonyl portion of the molecule, represented by the transition $m/z$ 505 → 327. The product ion at $m/z$ 299 represents the loss of $C_2H_4$ from the fragment represented by $m/z$ 327.

The accurate mass for this compound, $m/z$ 505.20496, is equivalent to a molecular formula for the $[M+H]^+$ species of $C_{23}H_{33}O_3N_6S_2$ [error −0.08906 ppm]. This supports the conclusion that the unknown compound is a thione derivative of sildenafil, and also indicates that it contains an additional modification of the piperazine portion of the molecule. However, it is also clear, based on the product ions observed with both homosildenafil and aildenafil, that the differentiation between a 3,5-dimethyl or a 4-ethyl substitution is not possible using mass spectrometry alone. The bulk material was then subjected to both X-ray crystallographic and NMR spectroscopic analyses.

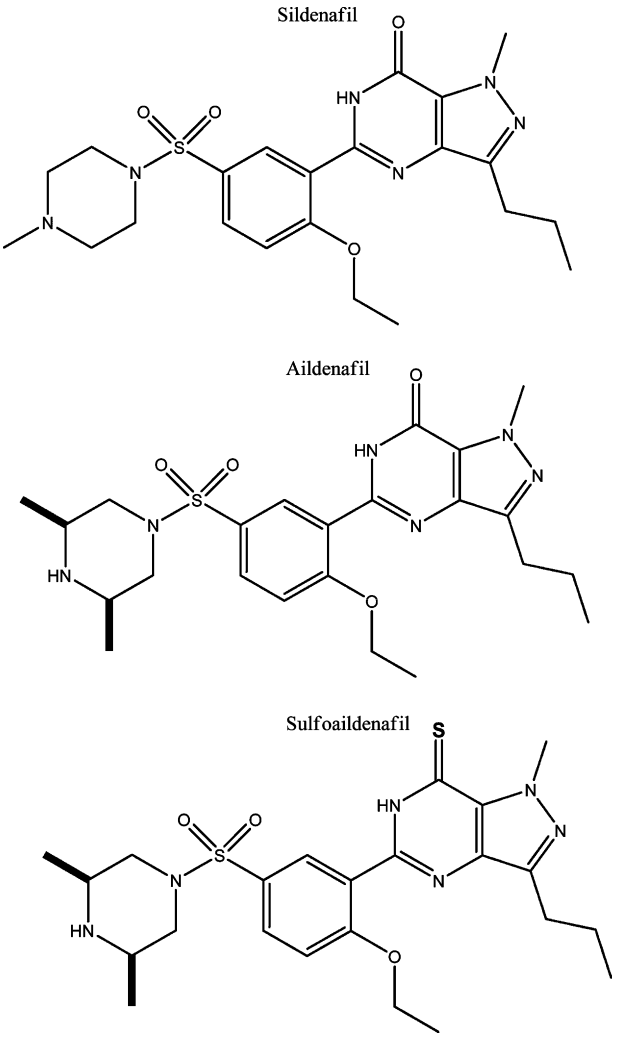


**Fig. 2.** Structures of sildenafil, aildenafil and sulfoaildenafil. Structural differences are emphasized in bold.

### 3.2. X-ray crystallography

Two polymorphs were identified by single crystal diffraction. Both polymorphs indicated methanesulfonate as the counterion, and differed only in the orientation of flexible side chains and packing of the molecules. It is possible that the two forms could possess different chemical properties and/or biological activities, which could lead to significant effects on *in vivo* activity [26]. No additional experiments were conducted in an attempt to further characterize either the chemical or biological properties of the polymorphs.

Fig. 3 shows the structure for Polymorph A of the compound, as well as the methanesulfonate counterion. Experimental details and crystallographic data for both polymorphs, including the structure for Polymorph B, are given in the supplementary material provided. The data for CCDC 715089 (Polymorph A) and CCDC 715090 (Polymorph B) can also be obtained via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 01223 336033.

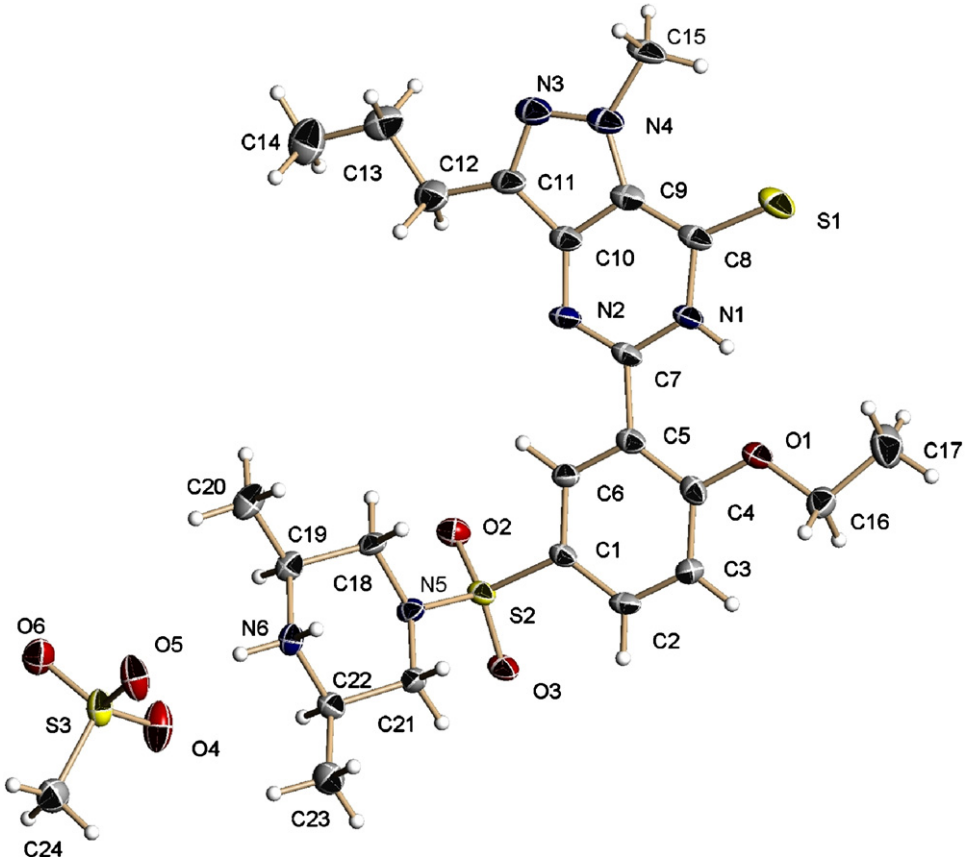


**Fig. 3.** X-ray crystal structure of Polymorph A of sulfoaildenafil methanesulfonate.

### 3.3. NMR spectroscopy

The identity of the compound was verified using $^{1}H$, $^{13}C$ and $^{1}H$ COSY NMR spectroscopy. The data obtained were comparable to those obtained by Reepmeyer and d'Avignon [25], using $CDCl_3$ as the solvent. The bulk material was pure by NMR standards, at >99%.

## 4. Conclusion

In the present study, the structure of sulfoaildenafil (or thiomethisosildenafil), a derivative of sildenafil, was determined using LC–ESI–MS/MS, FTICRMS, X-ray crystallography and NMR. The bulk material, declared as an ingredient for a dietary supplement, was, in fact, sulfoaildenafil methanesulfonate having a purity of greater than 99%. Sulfoaildenafil differs from sildenafil in two areas of the molecule: first, the oxygen atom in the pyrazolopyrimidine portion of sildenafil has been replaced with a sulfur atom; secondly, the methyl substitution in the 4-position of the piperazine ring has been replaced by a 3,5-dimethyl substitution.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2009.04.003.

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