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Research Article

Identification and Characterisation of New Vardenafil Analogue Illegally Added to Pressed Candies

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Abstract: The illegal addition of a vardenafil analogue to pressed candies was identified using high-performance liquid chromatography (HPLC). Subsequently, the compound was extracted with acetonitrile under sonication, followed by separation and purification by semi-preparative HPLC. The structure of the compound was determined using high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy. The results indicated that the compound was a vardenafil analogue with a molecular formula of $C_{24}H_{34}N_6O_4S$. In this compound, the ethoxy group attached to the benzene ring of vardenafil was replaced with a propoxy group. Therefore, the chemical name of this analogue is propoxyphenyl vardenafil. The illegal addition of this compound to functional foods has not been previously reported; therefore, this should be brought to the attention of regulatory authorities, and the compound should be considered in tests for illegal additives in functional and health foods.

***Keywords*:** pressed candies, vardenafil analogue, illegal addition, food safety, phosphodiesterase 5 (PDE-5) inhibitor analogues, high-resolution mass spectrometry, nuclear magnetic resonance, regulatory implications

1. Introduction

Sildenafil, tadalafil, and vardenafil, famously known as the "Viagra Trio", are phosphodiesterase 5 (PDE-5) inhibitors that have revolutionized the treatment of erectile dysfunction (ED) [1-2]. Their groundbreaking discovery has not only saved countless relationships, but has also brought a sense of normalcy to millions of men worldwide. However, despite their clinical success, these drugs, as well as their novel analogues, have found their way into an unlikely setting-our food.

Their use in functional and health foods is strictly prohibited, but they have not stopped unscrupulous manufacturers from adding these compounds [3-4] and their innovative analogues [5-13] to food products to cater to high market demand. This trend aligns with the historical patterns of adulteration, in which structural modifications are intentionally introduced to evade regulatory detection [14]. In recent years, the addition of novel PDE-5 inhibitor analogues has become increasingly prevalent [15] to avoid detection by regulatory authorities.

Among the various PDE-5 inhibitor analogues, sildenafil analogues are the most frequently reported [16-17], followed by tadalafil analogues. In contrast, vardenafil analogues have seldom been reported [16]. Recent studies have emphasized that even minor structural changes, such as alkyl chain elongation or heteroatom substitution, can

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significantly alter pharmacological profiles and toxicity, necessitating advanced analytical methods for identification [15]. The toxicity of these novel PDE-5 inhibitor analogues is yet to be established, and their dosage is not controlled when they are used as additives [14, 18], which can be harmful to the health of consumers and may even lead to death [19]. For instance, prolonged exposure has been linked to severe cardiovascular events and irreversible retinal damage [19]. Regulatory frameworks such as the EU's Rapid Alert System for Food and Feed (RASFF) and the FDA's Dietary Supplement Ingredient Advisory List have been pivotal in mitigating risks, yet gaps persist in detecting structurally evolved analogues [14]. Therefore, the illegal addition of these new analogues requires significant attention.

Pressed candies, also known as tablet candies, are new food products made from sugar and extracts of foods or medicinal substances. They are prepared by granulating and pressing ingredients into tablets, giving them a compact and portable form. Their high demand in China can be attributed to their small size, highly concentrated extract components, and high absorption efficiency. However, their popularity has made them a prime target for adulteration, as evidenced by multiple reports of PDE-5 inhibitor analogues in similar matrices [4, 20]. In a disturbing development, there have been reports in Chinese literature on the illegal addition of PDE-5 type analogues in pressed candy [20].

In this study, high-performance liquid chromatography (HPLC) was used for preliminary screening and semi-preparative HPLC for purification, and a novel vardenafil analogue was identified in a functional pressed candy product, and its structure was confirmed by high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance spectroscopy (NMR). This discovery not only highlights the prevalence of such illegal practices, but also underlines the need for stringent regulations and enforcement to protect public health.

2. Materials and methods

2.1 *Sample and chemicals*

Standard vardenafil (purity: 99.7%), homosildenafil (purity: 99.6%), aminotadalafil (purity: 99.8%), tadalafil (purity: 99.8%), thioaildenafil (purity: 99.5%), and pseudovardenafil (purity: 99.0%) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Standard norneosildenafil (purity: 99.5%) was purchased from Beijing Manhage Bio-Technology Company (Beijing, China).

Acetonitrile and methanol (HPLC grade) were supplied by Merck (Darmstadt, Germany). Formic acid (HPLC grade) was supplied by TGI (Tokyo, Japan). Analytical Research (AR) grade phosphoric acid and triethylamine (AR grade) were obtained from Shanghai Clinical Research Center (SCRC) (Shanghai, China). Deuterated dimethyl sulfoxide (d-DMSO, 99.9%) was obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA).

A pressed candy named Younaili ginseng extract oyster peptide was randomly selected by regulatory authorities from the commercial markets. The main ingredients listed on the label included maltodextrin, glucose, ginseng (cultivated for five years or less) 10%, polygonatum sibiricum (8%), oyster peptide (1%), mulberry, magnesium stearate, and ascorbic acid.

2.2 *HPLC screening*

To prepare a 1 mg/mL PDE-5 standard stock solution, 10 mg of vardenafil, homosildenafil, aminotadalafil, tadalafil, thioaildenafil, pseudovardenafil, and norneosildenafil standards were weighed, transferred to a 10 mL volumetric flask, dissolved in acetonitrile, and diluted to the mark. Then, 1 mL of the stock solution was transferred to a 10 mL volumetric flask and diluted to the mark with acetonitrile to obtain a 100 μg/mL solution.

Ten pressed candies were finely ground and thoroughly mixed. The powder (0.5 g) was precisely weighed and transferred to a 50 mL volumetric flask, to which 40 mL of acetonitrile was added. The mixture was sonicated for 15 min and cooled to room temperature(10-30 ℃). The solution was diluted with acetonitrile to 50 mL. The resultant solution was shaken well, passed through a 0.45 μm nylon membrane filter, and stored in a refrigerator at 4 ℃ until analysis.

Separation was performed as previously described [7]. The sample solutions were separated on a Thermo Syncronil column (4.6 × 250 mm, 4.6 μm) using an UltiMate 3000 DGLC HPLC system (Thermo Fisher, USA) with a diode array detector (DAD) scanning in the wavelength range of 190-400 nm. Mobile phase A consisted of a triethylamine

phosphate solution (7 mL of triethylamine was diluted to 1,000 mL with water, and the pH was adjusted to 2.8 with phosphoric acid), methanol, and acetonitrile (60 : 20 : 20, v/v/v). Mobile phase B consisted of a triethylamine phosphate solution, methanol, and acetonitrile (8 : 46 : 46, v/v/v). The gradient elution conditions were as follows: 0-12 min, 0% B; 12-25 min, 0%-100% B; 25-30 min, 100% B; 30-31 min, 100%-0% B; and 31-41 min, 0% B, with a flow rate of 1 mL/min; column temperature, 35 ℃; and injection volume, 10 μL. Detection was performed at 230 nm wavelength. The resultant chromatograms and ultraviolet (UV) spectra of the vardenafil standard and sample solutions are shown in Figure 1.

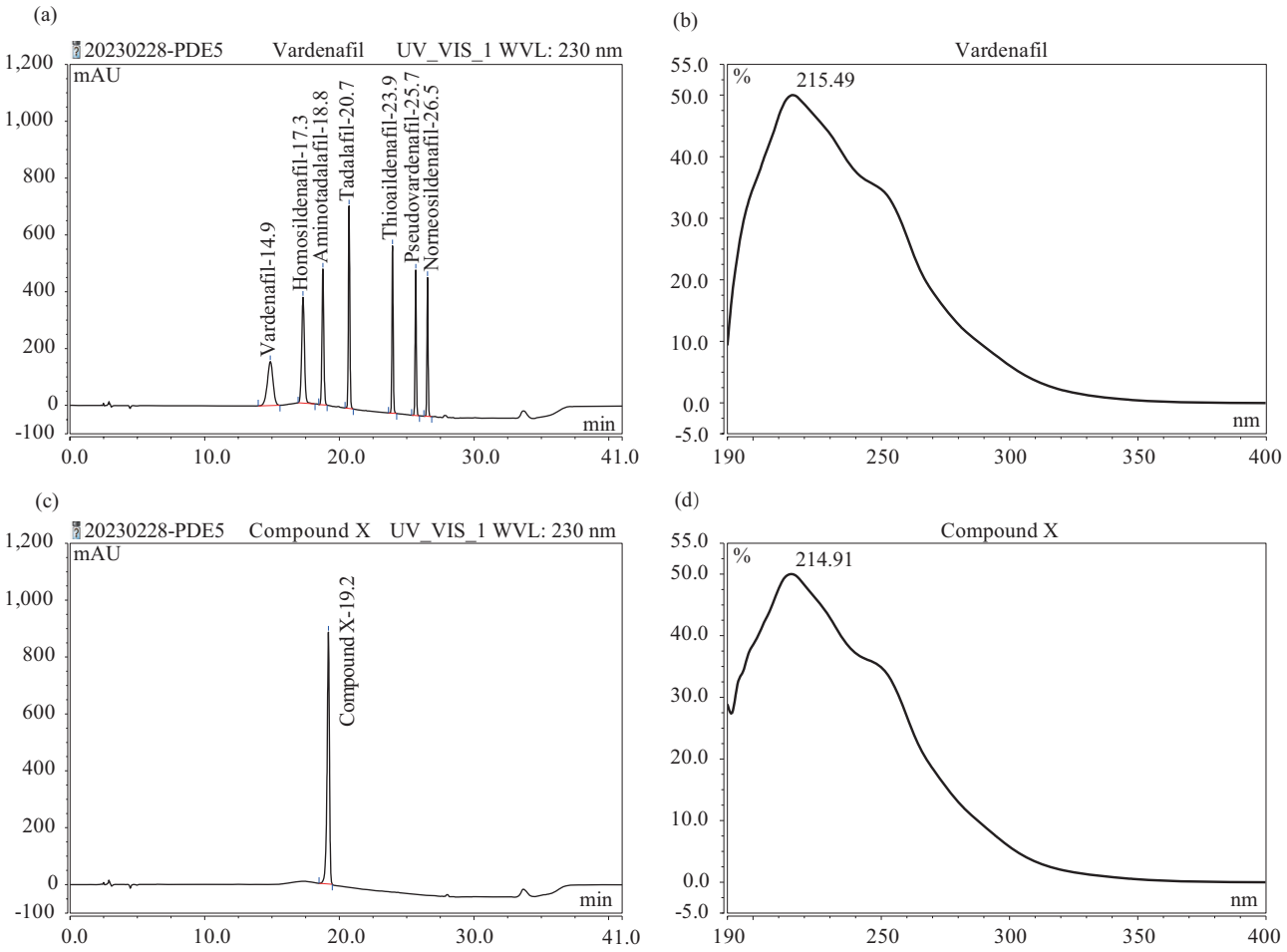

Figure 1. HPLC chromatograms recorded at 230 nm and UV spectra of vardenafil and Compound X. (a) Chromatogram of Vardenafil (RT: 14.9 min) and six PDE-5 inhibitor standards, (b) UV spectra of Vardenafil showing characteristic absorbance at ~215 nm, (c) Chromatogram of Compound X with a distinct peak at RT 19.2 min, indicating a longer retention time compared to vardenafil, (d) UV spectra of Compound X, displaying a similar absorbance profile to vardenafil

2.3 *HRMS analysis*

The vardenafil standard solution was diluted to 100 ng/mL, and the sample solution was diluted with an acetonitrile/water mixture (50/50 v/v) to obtain a solution containing approximately 100 ng/mL vardenafil. All solutions were passed through a 0.22 μm nylon membrane filter.

The analysis was conducted on the Thermo Fisher Scientific Orbitrap Q-Exactive LC-tandem HRMS system (Bremen, Germany).

Chromatographic separation was performed using a Thermo Syncronis C18 column (2.1 × 100 mm, 2.7 μm) with mobile phases of 0.1% formic acid in water (A) and acetonitrile (B). The gradient elution was programmed as follows: 0-6

min (25-50% B), 6-12 min (50-90% B), 12-13 min (90-25% B), and 13-20 min (25% B), at a flow rate of 0.2 mL/min and column temperature of 25°C, with an injection volume of 2 μL.

The MS parameters included positive electrospray ionization (ESI+), spray voltage of 3.5 kV; inlet capillary temperature of 320 ℃, sheath gas flow rate of 8 arb, auxiliary gas flow rate of 30 arb, and collision energy of 45 eV. Full-scan MS and MS/MS spectra were acquired at *m/z* 70-1,050. The corresponding primary and secondary mass spectra of the vardenafil standard and the sample are shown in Figures 2 and 3, respectively.

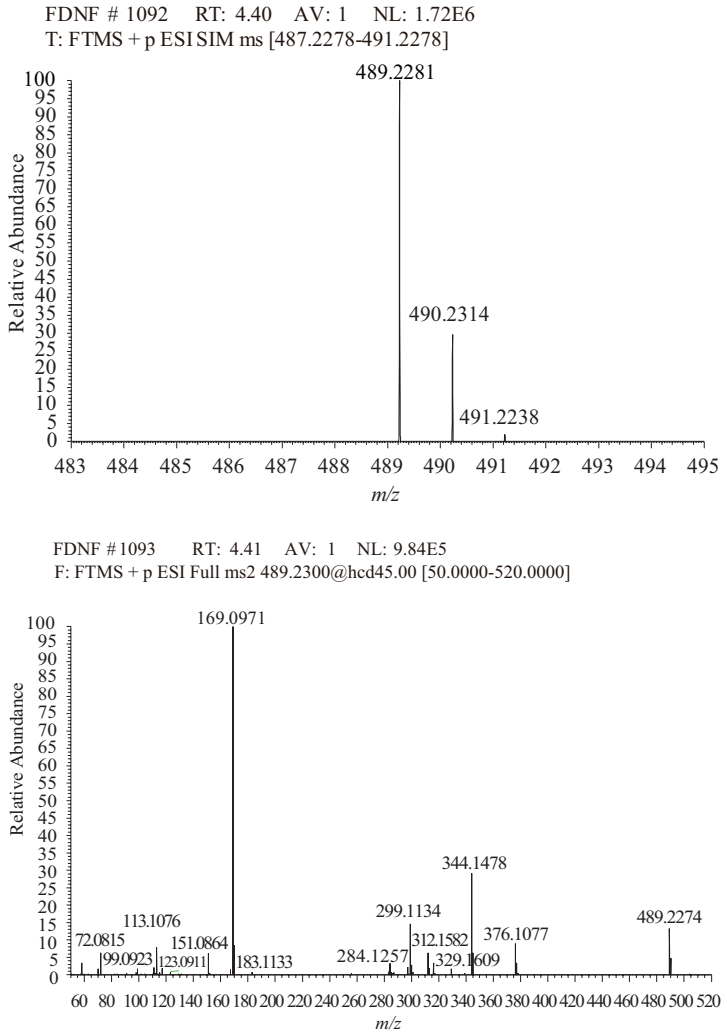

Figure 2. MS and MS^2 spectra of Vardenafil ($[M+H]^+$ *m/z* 489.2281). Key fragment ions at *m/z* 377, 329, and 312 corresponding to the cleavage of the ethoxy group (C_2H_5O-)

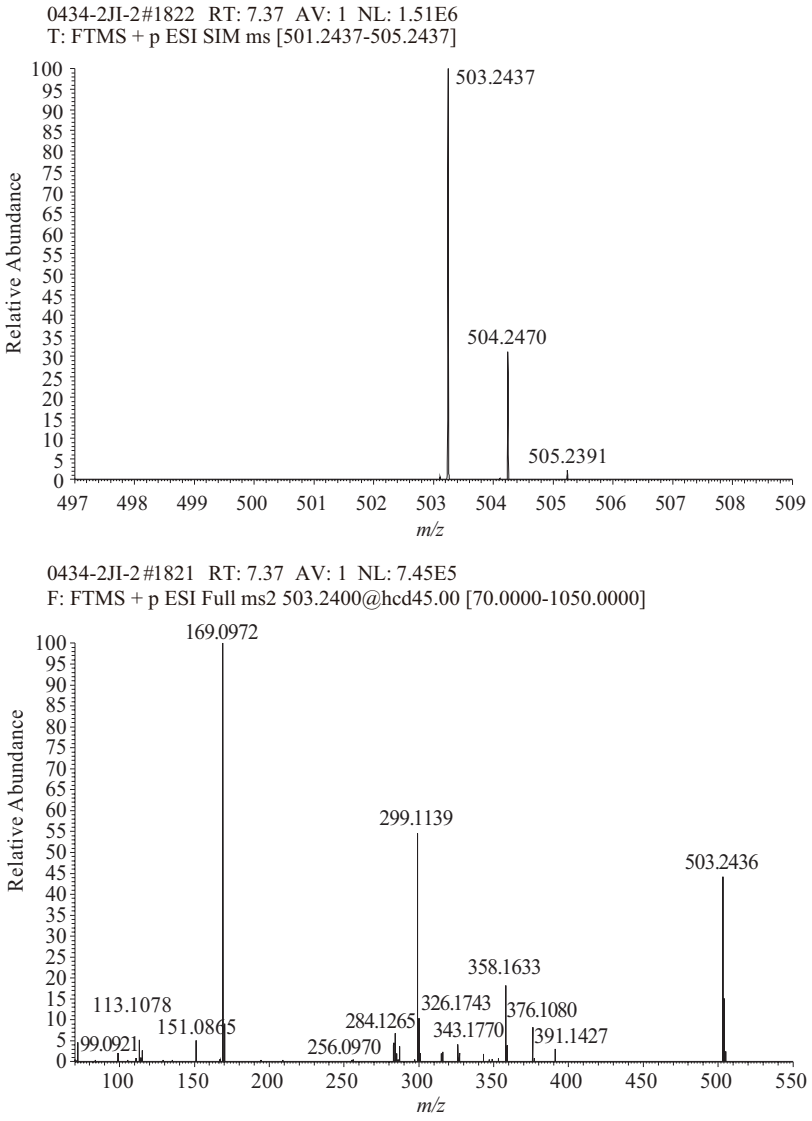

Figure 3. MS and MS^2 spectra of Compound X ($[M+H]^+$ *m/z* 503.2437)

2.4 *Semi-preparative separation and purification of samples*

For semi-preparative purification, 2.5 g of the sample was weighed and transferred to a 50 mL polypropylene centrifuge tube. The sample was extracted by adding 30 mL acetonitrile to the tube, ultrasonicating for 15 min, and centrifuging at 8,000 rpm for 3 min. The extraction was repeated twice and the extracts were combined and concentrated to approximately 10 mL under reduced pressure at room temperature (10-30 ℃). The resultant solution was passed through a 0.45 μm nylon membrane filter and stored until analysis.

Separation was performed using an Ultimate 3,000 semi-preparative HPLC system (Thermo Fisher, USA) on a Thermo Hypersil Gold C18 column (10 × 150 mm, 5 μm). Mobile phases were water (A) and acetonitrile (B) with the following gradient: 0-2 min (5% B), 2-6 min (5-90% B), 6-9 min (90% B), 9-9.5 min (90-5% B), and 9.5-17 min (5% B), at 3.5 mL/min and 35 °C, with injection volume of 0.5 mL, detection wavelength of 230 nm. The target fraction (7.2-8.2 min) was collected, concentrated under vacuum (60 °C), and yielded 30 mg of white powder.

2.5 *NMR analysis*

The purified compounds were dissolved in DMSO-d_6 and assessed by ^{1}H, ^{13}C, ^{1}H-^{1}H correlation spectroscopy (COSY), and heteronuclear single quantum coherence spectroscopy (HSQC) using a Bruker Ultrashield 400 Plus spectrometer system (Fällanden, Switzerland). Data processing and structural assignments were performed using the Mestrenova software package (version 12.0.0), and the relevant spectra and results are shown in Figures 4 and 5 and Table 1, respectively.

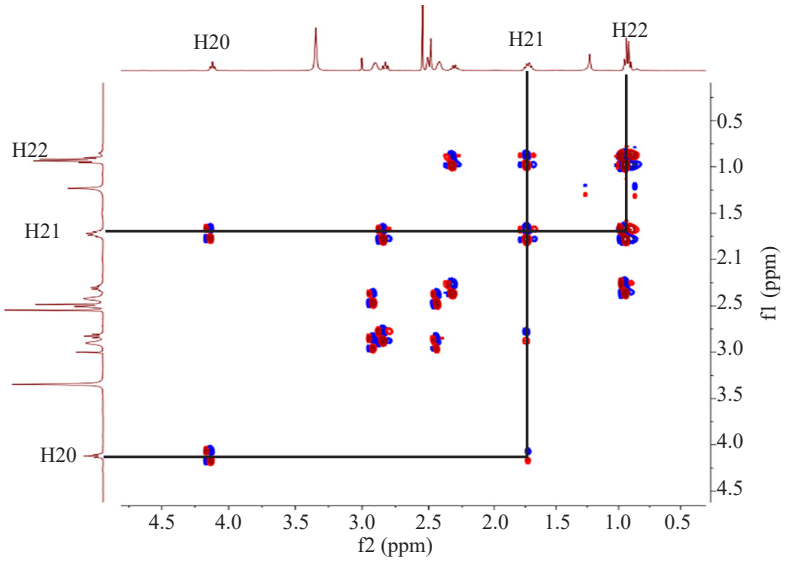

Figure 4. ^{1}H-^{1}H COSY spectrum of Compound X in DMSO-d6

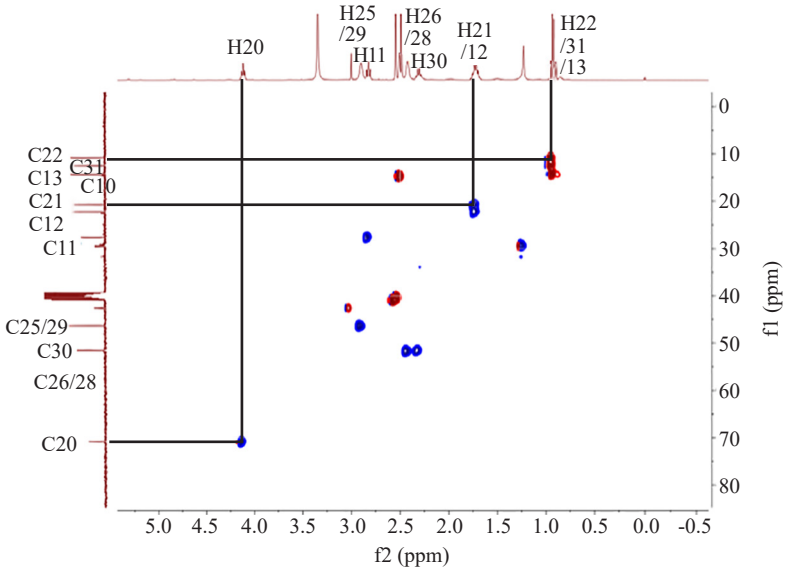

Figure 5. ^{1}H-^{13}C HSQC spectrum of Compound X in DMSO-d6

Table 1. NMR data of vardenafil and Compound X in DMSO-d6 (δ in ppm)

Pos	Vardenafil (DMSO-d6) [12]		Compound X		
	δ_H	δ_C	δ_H	δ_C	COSY
1	-	-	-	-	-
2	-	146.1	-	146.6	-
3	11.82 (1H, s)	-	11.68 (1H, s)	-	-
4	-	155.0	-	155.5	-
5	-	137.6	-	138.1	-
6	-	-	-	-	-
7	-	144.4	-	144.8	-
8	-	-	-	-	-
9	-	113.3	-	114.2	-
10	2.50 (3H, s)	14.1	2.48 (3H, s)	14.7	-
11	2.86 (2H, t)	27.1	2.83 (2H, t)	27.6	H-12
12	1.76 (2H, m)	20.2	1.73 (2H, m)	20.7	H-11/H-13
13	0.94 (3H, t)	13.7	0.93 (3H, m)	14.2	H-12
14	-	126.4	-	126.4	-
15	-	160.7	-	160.8	-
16	7.45 (1H, d)	113.6	7.40 (1H, d)	113.6	H-17
17	7.95 (1H, dd)	132.2	7.87 (1H, d)	132.6	H-16
18		120.9	-	121.5	-
19	7.97 (1H, d)	130.3	7.85 (1H, s)	130.5	-
20	4.24 (2H, q)	65.1	4.12 (2H, t)	70.8	H-21
21	1.35 (3H, t)	14.3	1.73 (2H, m)	22.2	H-20/H-22
22	-	-	0.93 (3H, m)	10.8	H-21
23	-	-	-	-	-
24	3.50, 3.81 (2H, br)	43.0	-	-	-
25	3.81(2H, br)	49.4	2.90 (2H, br)	46.4	H-26
26	-	-	2.42 (2H, br)	51.7	H-25
27	3.81(2H, br)	49.4	-	-	-
28	3.50, 3.81 (2H, br)	43.0	2.42 (2H, br)	51.7	H-29
29	2.90 (2H, q)	50.6	2.90 (2H, br)	46.4	H-28
30	1.23 (3H, t)	8.7	2.31 (2H, q)	51.5	H-31
31	-	-	0.93 (3H, m)	12.3	H-30

3. Results

3.1 *HPLC-DAD results*

The HPLC-DAD chromatogram revealed two notable peaks, corresponding to the vardenafil standard at a retention time of 14.9 min and an unknown compound (Compound X) at a retention time of 19.2 min (Figure 1). The UV spectra of Compound X were similar to those of vardenafil, with both compounds exhibiting maximum absorbance at approximately 215 nm. These results suggested that Compound X was a vardenafil analogue.

3.2 *HRMS results*

The primary mass spectra (Figures 2 and 3) revealed that the isotopic abundance ratio of Compound X ([M + H]$^+$; 503.2437 *m/z*) was 503.2437 : 504.2470 : 505.2391 = 100 : 32 : 2 and that of the vardenafil standard ([M + H]$^+$; 489.2281 *m/z*) was 489.2281 : 490.2314 : 491.2238 = 100 : 30 : 2. The difference between their *m/z* values was 14.0156, and their isotopic abundance ratios were similar, indicating that the elemental compositions of these two compounds were approximately the same. Vardenafil has a molecular formula of $C_{23}H_{32}N_6O_4S$; its five compositional elements (C, H, O, N, and S) were input into the Q-Exactive HRMS TraceFinder 4.1 software and the molecular weight of Compound X (502.2357) was considered to calculate that the closest matching compound with an *m/z* of 503.2357 had a molecular formula of $C_{24}H_{34}N_6O_4S$, which contains an additional-CH_2 compared with vardenafil.

The secondary mass spectra (Figures 2 and 3) showed that the fragment ions at *m/z* 376, 299, 169, and 151 were common between the two compounds, indicating that their main molecular structures were similar. The *m/z* difference between Compound X fragments at *m/z* 391, 343, and 326 and the vardenafil fragments at *m/z* 377, 329, and 312 was 14 *m/z*. Based on the structures of vardenafil fragments previously described in the literature [17], the structural modifications were tentatively determined to have occurred at the 15th carbon of the benzene ring, where the ethoxy (-O-CH_2-CH_3) group was replaced by a propoxy (-O-CH_2-CH_2-CH_3) group. The postulated chemical structure of Compound X is shown in Figure 6, and the fragmentation pathways are shown in Figure 7.

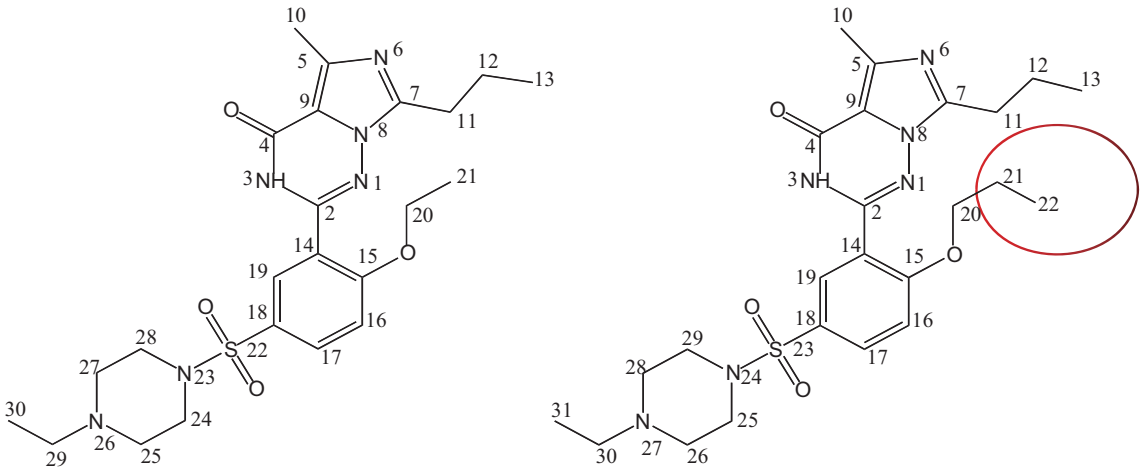

Figure 6. Chemical structures of Vardenafil and Compound X

Note: Fragment ions at *m/z* 391, 343, and 326 show a consistent +14 Da shift compared to vardenafil, confirming the substitution of ethoxy (-OC_2H_5) with propoxy (-OC_3H_7). The *m/z* difference of 14.0156 between vardenafil and Compound X aligns with the molecular formula change ($C_{23}H_{32}N_6O_4S \rightarrow C_{24}H_{34}N_6O_4S$), supporting the propoxy modification.

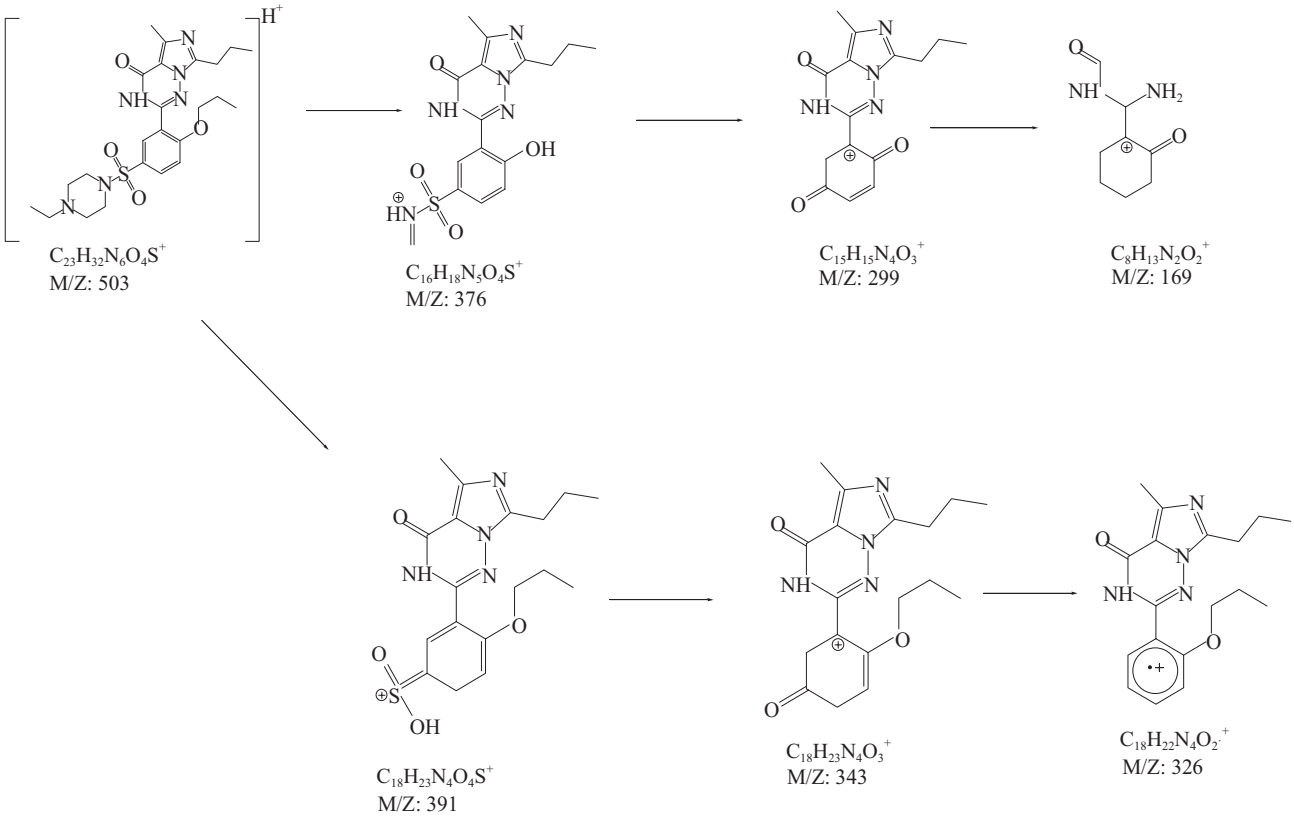

Figure 7. Proposed fragmentation pathways of Compound X

3.3 *NMR results*

The ^{1}H-^{13}C HSQC and ^{1}H-^{1}H COSY spectra of Compound X were analyzed; the spectral data are presented in Table 1. The complete assignments of the hydrogen and carbon spectra indicated that Compound X contained 24 carbon and 34 hydrogen atoms, which is consistent with the HRMS results.

The δ_H and δ_C chemical shifts of Compound X were slightly different from those of vardenafil [12]; however, the two compounds exhibited a high level of overall similarity (Table 1). Compound X contained two additional Hs near δ_H1.73, and the ^{1}H-^{1}H COSY data (Figure 4) indicated that δ_H1.73 was coupled with δ_H0.93 and δ_H4.12, indicating their proximity to the -CH_2-CH_2-CH_3 structure. The ^{1}H-^{13}C HSQC data (Figure 5) clearly indicated that this structure corresponded to the propoxy group at sites 20, 21, and 22, as depicted in Figure 6. Therefore, Compound X was positively identified as propoxyphenyl vardenafil, and key differences in the δ_H values (e.g., H-20 and H-21) directly reflect the elongation of the alkoxy chain from ethoxy to propoxy.

4. Discussion

HPLC-DAD screening revealed the illegal addition of an unknown compound to a pressed candy product. This unknown compound was purified using semi-preparative HPLC and dried in a rotary evaporator under reduced pressure, yielding approximately 30 mg of a white powder. Based on HRMS and NMR data, the structure of the compound was determined to be 2-[5-(4-ethyl-piperazine-1-sulfonyl)-2-propoxyphenyl]-5-methyl-7-propyl-3H-imidazo[1, 5-f][1, 2, 4]triazin-4-one. In this compound, the ethoxy group attached to the benzene ring of vardenafil was replaced with a propoxy group. Hence, the chemical name of this compound was propoxyphenyl vardenafil.

We also tested the ginseng extract using the same method and found no ingredients similar to propoxyphenyl vardenafil. Literature studies [21] have shown that ginseng primarily contains saponins (dammarane-type, ocotillol-

type, and oleanane-type), polysaccharides (starch-like glucans and pectin), amino acids, and volatile oils (aldehydes, heterocycles, sesquiterpenoids, fatty acids, fatty acid ester compounds, and alkane hydrocarbons). In addition to the above-mentioned substances, salicylic acid amine, maltol and its glucoside, 10 kinds of organic acids, and non-saponin water-soluble glycosides have been isolated and identified from ginseng. Ginseng also contains more than 12 alkaloids, including adenosine, spermine, and choline. Furthermore, ginseng contains various trace elements, vitamins, and enzymes, and there is no literature suggesting that ginseng contains vardenafil or its analogues.

The discovery of propoxyphenyl vardenafil in pressed candies raises critical concerns about its potential health impact on consumers. As a PDE-5 inhibitor analogue, this compound may exhibit pharmacological activity similar to vardenafil, potentially leading to unregulated vasodilation and hypotension, particularly in individuals with pre-existing cardiovascular conditions [4, 19]. Recent studies have shown that unapproved PDE-5 analogues often lack rigorous toxicity assessments, and their unpredictable pharmacokinetic profiles may result in overdose risks or drug-drug interactions [5-6]. For instance, co-administration with nitrates, commonly prescribed for angina, could synergistically lower blood pressure to dangerous levels, as reported in cases involving other PDE-5 analogues [14, 22].

Recent studies on PDE-5 inhibitor adulteration have predominantly focused on sildenafil and tadalafil analogues, such as desmethyl carbodenafil [19] and N-cyclohexyl nortadalafil [5]. These analogues often evade detection owing to subtle structural modifications, similar to the propoxy substitution observed in Compound X. However, unlike previously reported cases, the elongation of the alkoxy chain in propoxyphenyl vardenafil introduces distinct chromatographic (e.g., RT shift) and spectroscopic (e.g., δ_H changes in NMR) signatures, which can be used for targeted screening [23].

Furthermore, the substitution of an ethoxy group with a propoxy moiety in propoxyphenyl vardenafil may alter its metabolic pathway. Propoxy chains are known to increase lipophilicity, potentially enhancing tissue accumulation and prolonging half-life [15-16]. Such modifications can exacerbate long-term toxicity, including hepatotoxicity or nephrotoxicity, as observed in structurally related designer drugs [11, 18].

The absence of controlled dosing in adulterated foods increases the risks. Consumers unknowingly ingesting propoxyphenyl vardenafil may experience adverse effects ranging from headache and dyspepsia to severe cardiovascular events [3]. Vulnerable populations, such as the elderly or those with undiagnosed heart conditions, are particularly at risk.

In comparison to previously reported cases, the illegal addition of PDE-5 inhibitors such as sildenafil and tadalafil analogues in food products has been well documented. For example, sildenafil analogues like desmethyl carbodenafil have been linked to fatal toxicity due to their potent vasodilatory effects [19]. Similarly, tadalafil analogues have been found in dietary supplements, posing significant health risks due to their unregulated use [5]. The discovery of propoxyphenyl vardenafil adds to this growing list of PDE-5 inhibitor analogues, highlighting the need for more stringent regulatory oversight and advanced detection methods to safeguard public health.

It is the first report of the illegal addition of this compound to functional foods. This finding of a novel vardenafil analogue in a pressed candy product is alarming and requires immediate attention from regulatory authorities and health organizations. The illegal addition of such drugs and their analogues to food products not only violates existing laws and regulations but also puts consumers at risk of potential health hazards.

The pressing need for a stricter regulatory framework to govern the food industry is evident in this case. Stringent guidelines and enforcement mechanisms need to be established to prevent the illegal addition of PDE-5 inhibitor analogues and other drugs to food products. Regular monitoring and surveillance programs should be implemented to ensure the safety and quality of the food supply chain.

5. Conclusion

The discovery of propoxyphenyl vardenafil in pressed candies underscores the urgent need for enhanced regulatory frameworks and consumer education. Regulatory authorities should prioritize the following actions: (1) establish a comprehensive database of known and emerging PDE-5 inhibitor analogues to facilitate rapid identification; (2) mandate pre-market testing for functional foods and dietary supplements to ensure compliance with safety standards; (3) launch public awareness campaigns to educate consumers about the risks of unregulated products. In addition, food

manufacturers should adopt rigorous quality control measures and transparent labeling practices to build consumer trust and ensure product safety.

This study also highlights the urgent need for toxicological evaluation of propoxyphenyl vardenafil. Future research should prioritize in vivo studies to assess its acute and chronic effects, establish safe exposure thresholds, and elucidate its mechanisms of action. Additionally, public health campaigns must educate consumers about the dangers of unregulated functional foods, while regulatory agencies should implement advanced screening protocols targeting emerging PDE-5 analogues.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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