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Isolation and Identification of New Sildenafil Analogues from Dietary Supplements

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ABSTRACT

Three derivatized thio-sildenafil compounds were isolated from herbal health supplements. The structures of these three compounds were determined by NMR, high resolution MS, LC/MS/MS, UV and IR spectroscopy. Compound **1** and **2** have been identified as propoxyphenyl thioaildenafil and propoxyphenyl aildenafil, respectively. Compound **3**, named propoxyphenyl hydroxyethylthiosildenafil, is an analogue of thiosildenafil. The major difference between compound **3** and thiosildenafil is that propoxy and hydroxyethyl groups in compound **3** replaced the function groups of ethoxyl and methyl in thiosildenafil. Since their structures were similar to that of sildenafil or thiosildenafil, according to structure-activity relationship, the side effects of these analogues might be also associated with that of sildenafil or thio-sildenafil. Based on this study, these three analogues have been included in the inspection list of illegal adulterants in TFDA.

Key words: propoxyphenyl hydroxyethylthiosildenafil, propoxyphenyl aildenafil, propoxyphenyl thioaildenafil, NMR, TFDA

INTRODUCTION

In recent years, inhibitors of cyclic nucleotide phosphodiesterase type 5 (PDE-5) have been used as a therapeutic drug for the treatment of erectile dysfunction (ED)[(1)]. Nowadays, three PDE-5 inhibitor drugs have been approved by the Food and Drug Administration for the treatment of male ED: sildenafil, vardenafil, and tadalafil. Since then, more PDE-5 inhibitor drugs and their modified analogues have been found in the so-called "natural" herbal health supplements in the market[(2-11)]. Because the structurally modified analogues may pose some unknown adverse effects[(12,13)], it's important to find the presence of known or unknown analogues of PDE-5 inhibitor. Two novel adulterants, thiohomosildenafil and hydroxythiohomosildenafil, have been found in our laboratory[(14)]. These two sildenafil analogues were modified by replacing a carbonyl group in sildenafil with a thiocarbonyl group. Here we have further isolated three sildenafil analogues from herbal dietary supplements. These three compounds were all characterized by nuclear magnetic resonance (NMR), electrospray ionization-mass spectrometry (ESI-MS), infar-red (IR), ultraviolet (UV) spectroscopy. Compound **1** and **2** have been identified as propoxyphenyl thioaildenafil and propoxyphenyl aildenafil[(15)], which were found for the first time in Taiwan. Compounds **3**, named propoxyphenyl hydroxyethylthiosildenafil, has not been reported before. The structures of these analogues are illustrated in Figure 1.

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MATERIALS AND METHODS

I. *General*

Melting points were measured on a BŰCHI Melting Point B-540 unit; Optical rotations were taken on a JASCO-P-1030 polarimeter (cell length 10 mm); UV spectra were acquired on a JASCO CARY 300 Conc UV/Vis spectrophotometer; IR spectra were recorded on a JASCO FT-IR 480+ spectrophotometer; NMR spectra were recorded on a Bruker AVIII 800MHz NMR spectrometer. Chemical shift (δ) values are in ppm (part per million) with $CDCl_3$ as internal standard and coupling constants (J) are in Hz. High resolution ESI-MS (HRESI-MS) measurements were performed on Agilent 6530 Accurate-Mass Q-TOF LC/MS. Thin layer chromatography (TLC) was performed on Kieselgel 60, F_{254} (0.20 mm, Merck), and spots were observed under UV light at 254 and 366 nm, and stained by spraying with Dragendorff's reagent. For column chromatography, silica gel (Kieselgel 60, 70-230, and 230-400 mesh, Merck) and SUPELCO® Flash

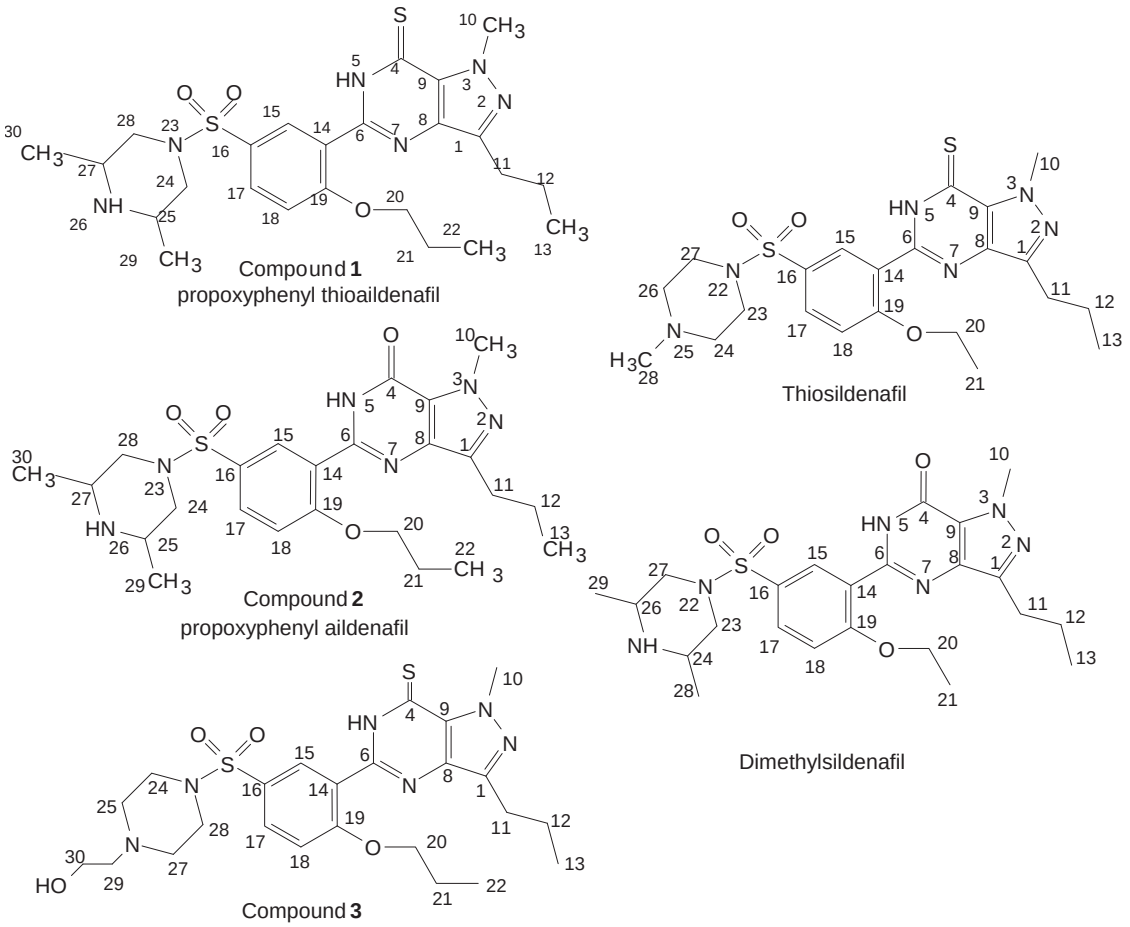

Figure 1. Structures of compounds **1-3** and sildenafil analogues.

Chromatography Starter Kit (Verspak silica gel column) were used.

II. *Materials*

Two herbal health supplements (samples 1 and 2) claimed for improving erectile function were randomly collected by the health officers from local markets in 2011.

III. *Extraction & Isolation*

(I) *Compound* **1**

Sample 1, a dried brown powder (22.1 g), was extracted with 95% ethanol (300 mL × 4). The crude extract (4.8 g) was dissolved with ethyl acetate (EtOAc). The EtOAc soluble layer (3.3 g) was chromatographed on an open silica gel column (packaged by Kieselgel 60, 70-230 mash, Merck) with EtOAc/methanol (MeOH) (10:1) to yield 3 fractions. Fraction 2 (344.2 mg) was recrystallized in the solvent system of CH_2Cl_2/MeOH (1:1) to give compound **1** (61.8 mg).

(II) *Compound* **2** *&* **3**

Sample 2, a dried brown powder (12.6 g), was extracted with 95% ethanol (300 mL × 4). The crude extract (3.9 g) was partitioned with CH_2Cl_2/MeOH/Water (20:10:10), and 2 fractions consisting of organic layer (2.0 g) and water layer were obtained. The organic layer was chromatographed over a Verspak silica gel column, using SUPELCO® Flash Chromatography Starter Kit, with CH_2Cl_2/MeOH (50:1) to yield 3 fractions.

Fraction 3 (124.8 mg) was further purified to afford compound **2** (67.3 mg) by recrystallization with CH_2Cl_2 and compound **3** (33.1 mg) was obtained from fraction 1 (44.2 mg) after washing with *n*-hexane.

IV. *Physical Data*

(I) *Compound* **1**

Common name: propoxyphenyl thio-aildenafil; IUPAC name: 5-[2-propoxy-5-((3R,5S)-3,5-dimethylpiperazin-1-ylsulfonyl)-phenyl]-1-methyl-3-*n*-propyl-1, 6-di-hydro-

7H-pyrazolo [4,3-d] pyrimidin-7-thione; formula: $C_{24}H_{34}N_6O_3S_2$; molecular mass (LC/MS/MS): 519.19 $(M+H)^+$; exacted mass (HRESI-MS): 519.2224 $(M+H)^+$; m.p.: 189~191°C; [α]25D : +46.3° (c1.1, CH_2Cl_2); IR (KBr) υmax: 3265 cm-1 (N-H), 1572 cm^{-1}(aromatic ring), 1257 cm^{-1} (thioketone group).

(II) *Compound* **2**

Common name: propoxyphenyl aildenafil; IUPAC name: 5-[2-propoxy-5-((3R,5S)-3,5-dimethylpiperazin-1-ylsulfonyl)-phenyl]-1-methyl-3-*n*-propyl-1,6-di-hydro-7H-pyrazolo[4,3-d] pyrimidin-7-one.; formula: $C_{24}H_{34}N_6O_4S$; molecular mass (LC/MS/MS): 503.25 $(M+H)^+$; exacted mass (HRESI-MS): 503.2440 $(M+H)^+$; m.p.: 181~183°C; $[\alpha]^{25}_D$: +50.2° (*c*0.3, CH_2Cl_2); IR (KBr) υ_{max}: 3312 cm^{-1} (N-H), 1690 cm^{-1} (carbonyl group).

(III) *Compound* **3**

Common name: propoxyphenyl hydroxyethylthiosildenafil;

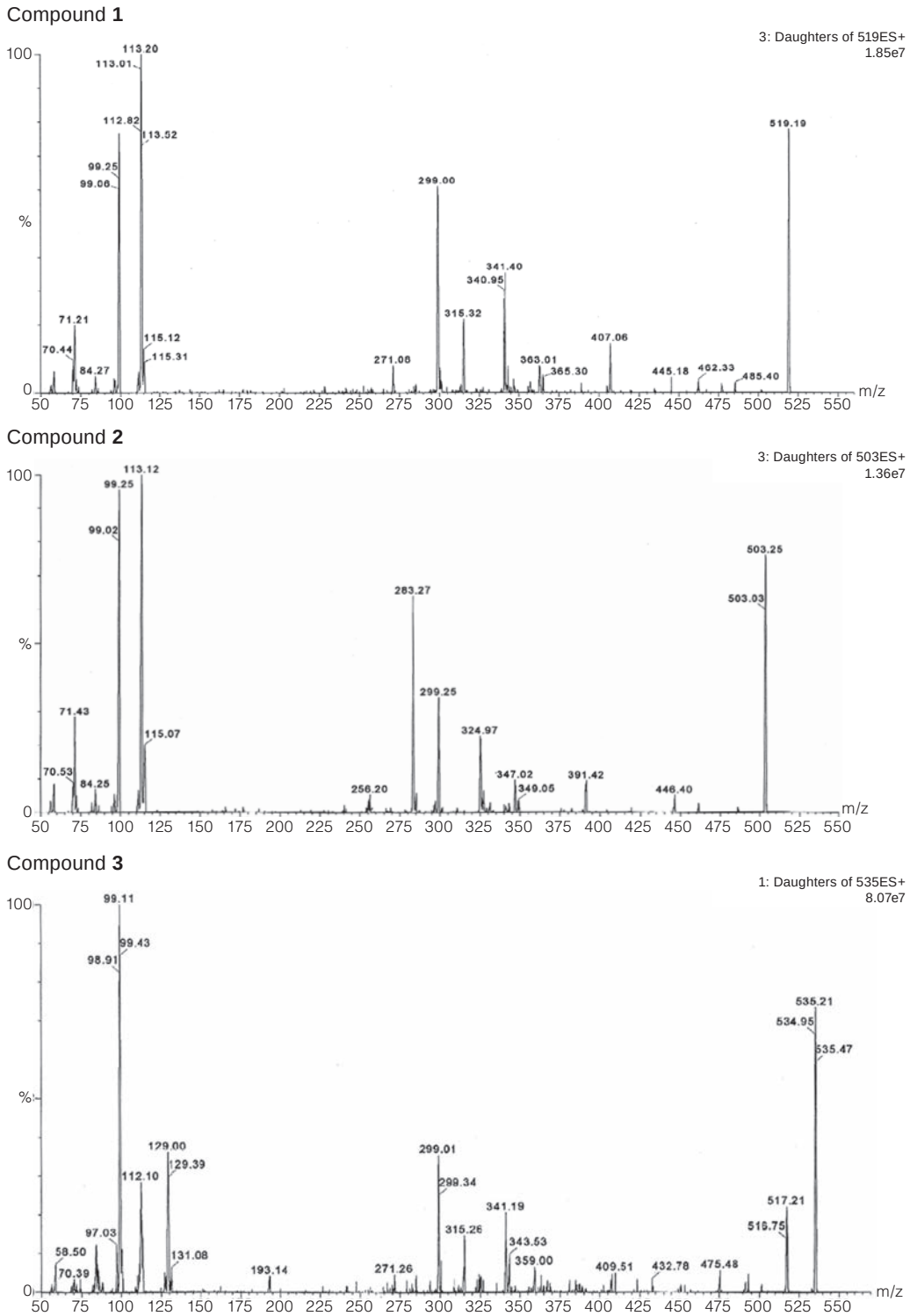

Figure 2. LC/MS/MS spectra of compounds **1, 2** and **3**.

IUPAC name: 5-[2-propoxy-5-(4-hydroxyethylpiperazin-1-ylsulfonyl)-phenyl]-1-methyl-3-*n*-propyl-1,6-di-hydro-7H-pyrazolo[4,3-d] pyrimidin-7-thione.; formula: $C_{24}H_{34}N_6O_4S_2$; molecular mass (LC/MS/MS): 535.28 $(M+H)^+$; exacted mass (HRESI-MS): 535.2166 $(M+H)^+$; m.p.: 158~160°C; $[\alpha]^{25}_D$: +33.8° (*c*0.2, CH_2Cl_2); IR (KBr) υ_{max}: 3448 cm^{-1} (N-H), 1571 cm^{-1} (aromatic ring), 1245 cm^{-1} (thioketone group).

RESULTS AND DISCUSSION

I. *Compound* **1**

The compound **1** was purified and recrystalized as yellow powder from CH_2Cl_2. The melting point was between 189 and 191°C. Compound **1** showed $[M+H]^+$ ion at m/z 519.19, corresponding to the molecular formula $C_{24}H_{34}N_6O_3S_2$ (Figure 2) and was reconfirmed by HRESIMS with $[M+H]^+$ ion 519.2224. The UV spectrum of compound **1** showed λ max at 226 and 295 nm, with a similar spectral pattern to that of thio-sildenafil (Figure 3).

The IR spectrum (Figure 4) of compound **1** showed absorption peaks with the characteristics of an amine at 3265 cm^{-1}, an aromatic ring at 1572, 1497 and 3092 cm^{-1} , and a thioketone group at 1257 cm^{-1}, suggesting that compound **1** was a thio-sildenafil analogue.

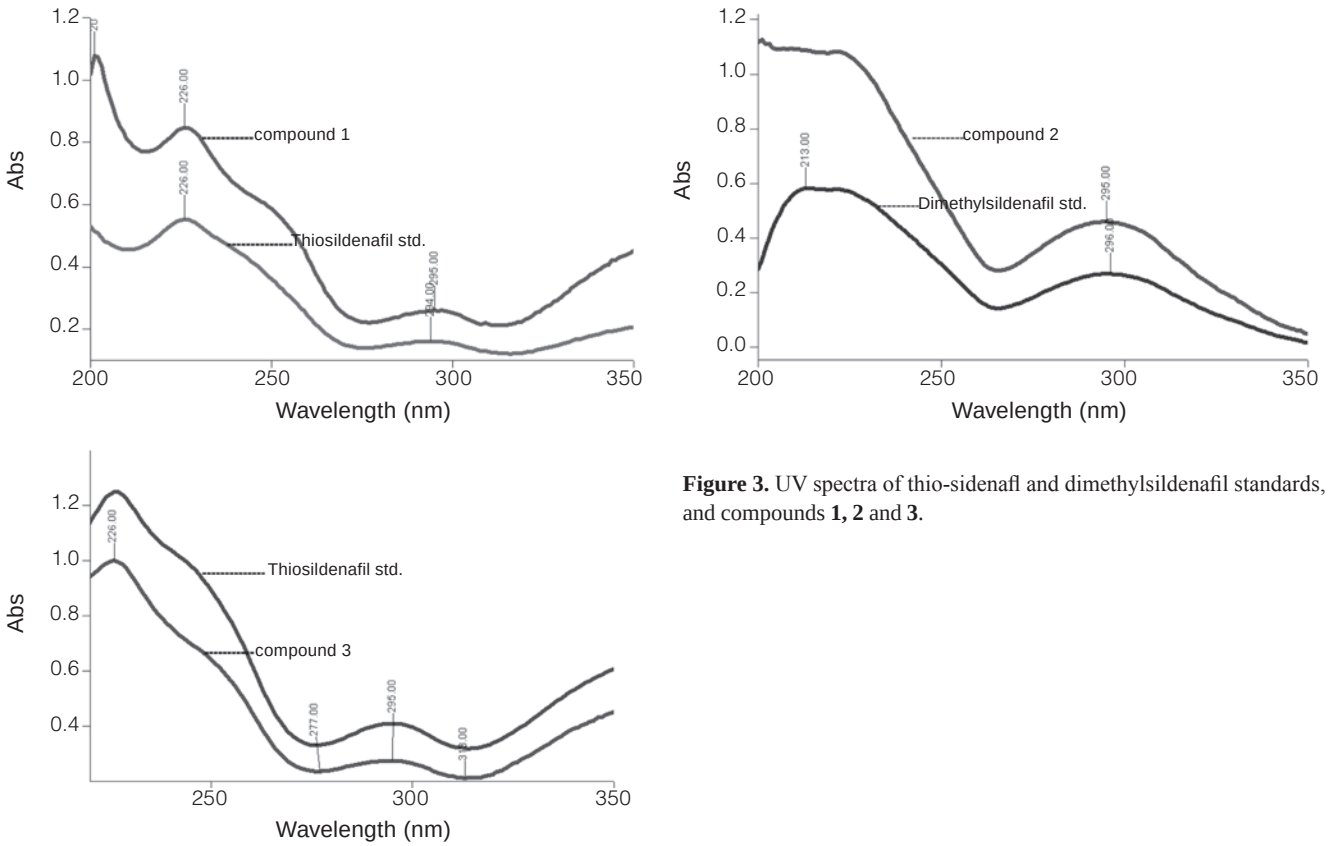

Figure 3. UV spectra of thio-sidenafl and dimethylsildenafil standards, and compounds **1, 2** and **3**.

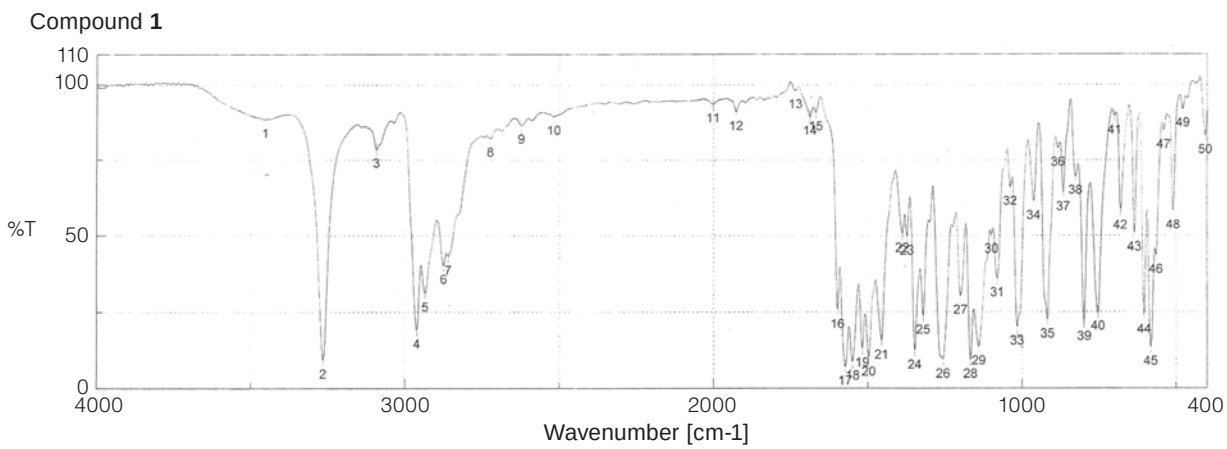

Figure 4. IR spectra of compounds **1, 2** and **3**.

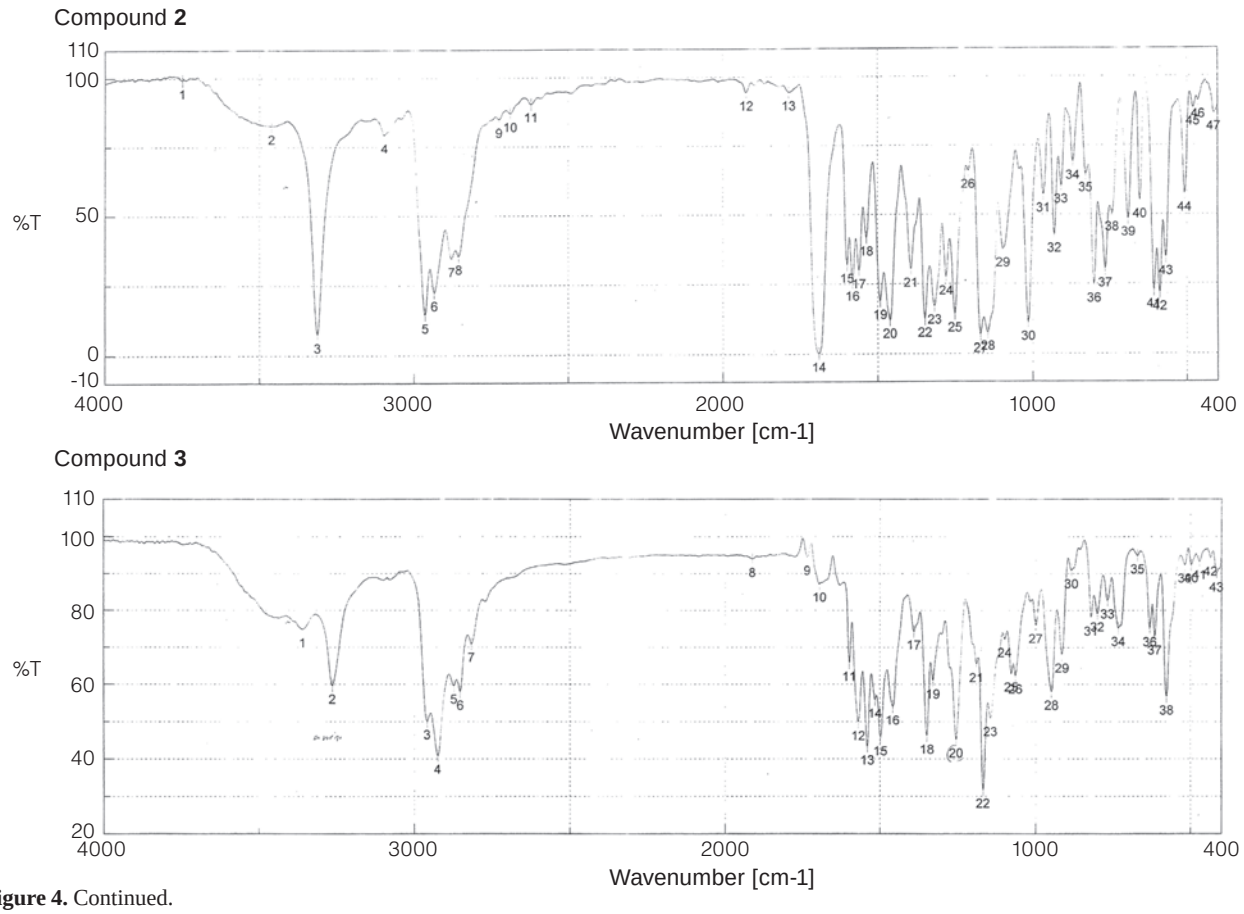

Figure 4. Continued.

Table 1. 1D, 2D NMR data of compound **1**

No.	$^{1}H(\delta_H)$	$^{13}C(\delta_C)$	COSY	HMBC
1	-	146.3(s)	-	-
4	-	171.7(s)	-	-
6	-	146.0(s)	-	-
8	-	133.7(s)	-	-
9	-	132.2(s)	-	-
10	4.46, 3H, s	39.2(q)	-	C-9
11	2.89, 2H, *t*, *J* = 7.4 Hz	27.5(t)	H-12	C-13, C-12, C-8, C-1
12	1.78, 2H, *q*, *J* = 7.3 Hz	22.0(t)	H-11, H-13	C-13, C-11, C-1
13	0.94, 3H, *t*, *J* = 7.3 Hz	13.9(q)	H-12	C-13, C-12
14	-	119.8(s)	-	
15	8.74, 1H, *d*, *J* = 2.0 Hz	130.4(d)	-	C-16, C-17, C-14, C-6
16	-	129.2(s)	-	
17	7.78, 1H, *dd*, *J* = 2.0,8.7 Hz	131.8(d)	H-18	C-16, C-19
18	7.14, 1H, *d*, *J* = 8.7 Hz	113.8(d)	H-17	C-19, C-16, C-19
19	-	159.4(s)	-	
20#	4.23, 2H, *t*, *J* = 9.3 Hz	72.1(t)	H-21	C-19, C-21, C-22
21#	2.05, 2H, *m*	22.2(t)	H-20, H-22	C-22, C-20
22	1.15, 3H, *t*, *J* = 10.6 Hz	10.6(q)	H-21	C-21, C-20
24(28)ax	1.85,2H, *t*, *J* = 10.7 Hz	52.0(t)	H-25&H-27	C-25&27, C-29&30
25&27	2.94, 2H, *m*	50.1(d)	H-28, H-24	C-24&28
28(24)eq	3.62, 2H, *d*, *J* = 10.3 Hz	52.0(t)	H-25&H-27	C-25&27, C-29&30
29&30	0.97, 6H, *d*, *J* = 6.2 Hz	19.1(q)	H-25&H-27	C-25&27, C-24&28

δ ppm in $CDCl_3$, *J* in H_Z, 200 MH_Z for ^{13}C, 800 MH_Z for ^{1}H.

The ^{1}H-NMR spectrum of compound **1** showed characteristics of three ABX-type (1,3,4-trisubstituted) aromatic protons at δ_H 7.14 (1H, *d*, *J* = 8.7 Hz), δ_H 7.78 (1H, *dd*, *J* = 2.0, 8.7 Hz), δ_H 8.74 (1H, *d*, *J* = 2.0 Hz) and a singlet methyl attached to an aromatic amine at δ_H 4.46 (3H, *s*). Six methylenes at δ_H 1.78 (2H, *q*, *J* = 7.3 Hz), δ_H 1.85 (2H, *t*, *J* = 10.7 Hz), δ_H 2.05(2H, *m*), δ_H 2.89 (2H, *t*, *J* = 7.4 Hz), δ_H 3.62 (2H, *d*, *J* = 10.3 Hz), δ_H 4.23(2H, *t*, *J* = 9.3 Hz) , two methines at δ_H 2.94 (2H, *m*) and four methyls at δ_H 0.94 (3H, *t*, *J* = 7.3 Hz), δ_H 1.15 (3H, *t*, *J* = 10.6 Hz), δ_H 0.97 (6H, *d*, *J* = 6.2 Hz). With the correlation spectroscopy (COSY) (δ_H2.94/ δ_H 1.85/ δ_H 0.97) (Figure 5) and heteronuclear multiple bond coherence **(**HMBC) spectrum, two methylenes at δ_H 3.62 and 1.85 were assigned as the geminal methylene protons of a dimethylpiperazine for H-24 and 28, the methine at δ_H 2.94 was assigned for H-25 and 27, and the methyl at δ_H 0.97 was the dimethyl groups for H-29 and 30.

The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra indicated five primary carbons, six secondary carbons, five tertiary carbons and eight quaternary carbons. One quaternary carbon at δ_C 171.7 was assigned to thio-carbonyl group for C-4, which was supported by IR spectrum (Figure 4).

The major difference between compound **1** and thiosildenafil[14] was related to piperazine group. Except the piperazinyl group, compound **1** had another difference with thio-sildenafil at position 19. The key-point COSY correlation (δ_H 4.23/ δ_H 2.05/δ_H 1.15) (Figure 5) and HMBC correlation (δ_H 4.23/ δ_C 22.0/δ_C 159.4/δ_C 10.6) (Figure 7) indicated that the exthoxyl group at position 19 in thio-sildenafil[14] has been replaced as a propoxyl group in compound **1**.

By integrating these spectral data, the structure of compound **1** was determined as propoxyphenyl thioaildenafil by comparing with the literature data[15]. It was determined a thiosildenafil analogue, except that the ethoxy group bonded to phenyl ring is replaced by a propoxy group at position 19.

II. *Compound 2*

Compound **2**, obtained as white powder, melting point 181 - 183°C, the molecular formula $C_{24}H_{34}N_6O_4S$ (m/z 503.25) was determined by LC/MS/MS (Figure 2) and reconfirmed by HRESIMS($[M+H]^+$ ion at m/z 503.2440). The UV spectrum of compound **2** showed absorption at λmax 296 as similar to sildenafil (Figure 3). The IR spectrum (Figure 4) of compound **2** showed absorption peaks with the characteristics of an amine at 3312 cm^{-1}, an aromatic ring at 1598 and 1491 cm^{-1}, and a carbonyl group at 1690 cm^{-1}. Compared with the UV and IR spectra of sildenafil in reference, compound **2** was speculated as a sildenafil analogue.

The spectral data of ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY and HMBC of compound **2** were similar to those of compound **1**, as shown in Table 2. The ^{1}H-NMR spectrum of compound **2** also exhibited signals for one set of ABX-type (1,3,4-trisubstituted) aromatic protons at δ_H 7.12 (1H, *d*, *J* = 8.4 Hz), δ_H 7.78 (1H, *dd*, *J* = 2.4, 8.4 Hz) and δ_H 8.74 (1H, *d*, *J* = 2.4 Hz), as well as a singlet methyl attached to an aromatic

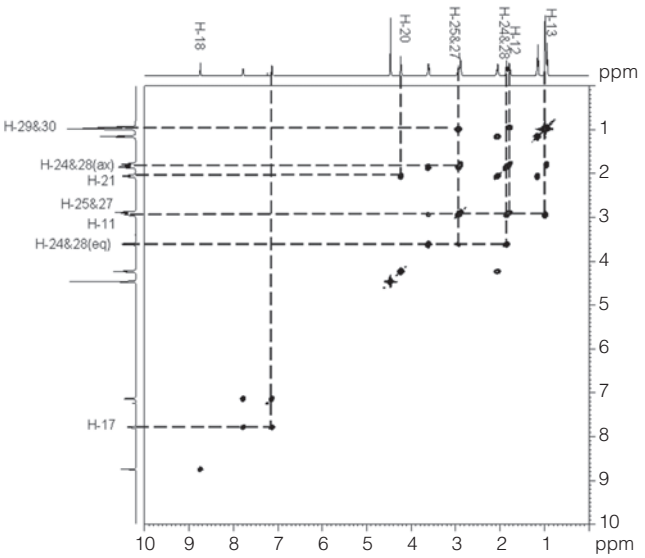

Figure 5. COSY spectrum of compound **1**.

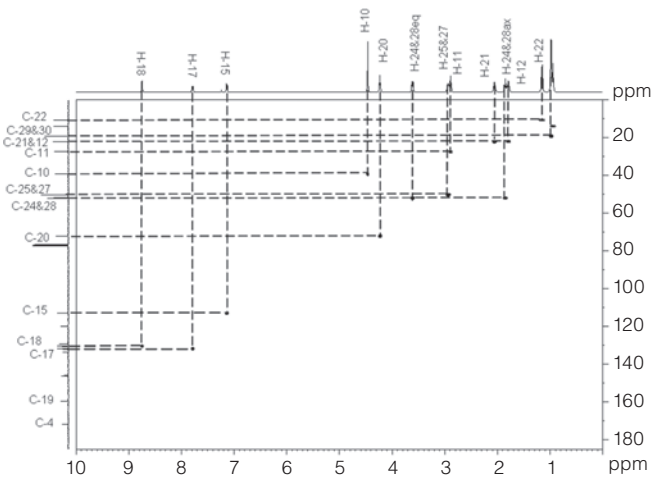

Figure 6. HMQC spectrum of compound **1**.

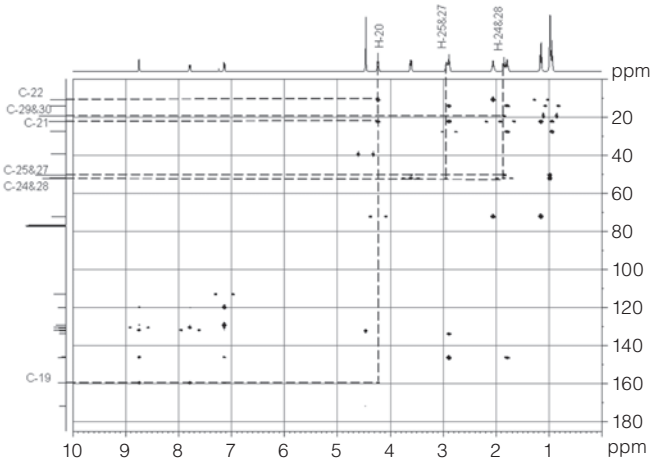

Figure 7. HMBC spectrum of compound **1**.

Table 2. 1D, 2D NMR data of compound **2**

No.	$^{1}H(\delta_H)$	$^{13}C(\delta_C)$	COSY	HMBC
1	-	146.8(s)	-	-
4#	-	153.5**(s)**	-	-
6	-	146.4(s)	-	-
8	-	138.3(s)	-	-
9	-	124.4(s)	-	-
10	4.22, 3H, *s*	38.1(q)	-	C-9
11	2.88, 2H, *t*, *J* = 7.7 Hz	27.7(t)	H-12	C-13, C-12, C-8, C-1
12	1.81, 2H, *m*	22.2(t)	H-11,H-13	C-13, C-11, C-1
13	0.97, 3H, *t*, *J* = 7.3 Hz	13.9(q)	H-12	C-13, C-12
14	-	120.1(s)	-	
15	8.74, 1H, *d*, *J* = 2.4 Hz	130.8(q)	-	C-16, C-17, C-14, C-6
16	-	129.1(s)	-	
17	7.78, 1H, *dd*, *J* = 2.4,8.4 Hz	131.5(d)	H-18	C-16, C-19
18	7.12, 1H, *d*, *J* = 8.4 Hz	112.9(d)	H-17	C-16, C-19
19	-	159.3(s)	-	
20	4.22, 2H, *t*, *J* = 9.3 Hz	71.7(t)	H-21	C-19, C-21, C-22
21	2.00, 2H, *m*	22.2(t)	H-20,H-22	C-22, C-20
22	1.14, 3H, *t*, *J* = 7.4 Hz	10.5(q)	H-21	C-21, C-20
$24(28)_{ax}$	1.86, 2H, *t*, *J* = 10.7 Hz	52.0(t)	H-25&H-27	C-25&27, C-29&30
25&27	2.95, 2H, *m*	50.2(d)	H-28,H-24	C-24&28
$28(24)_{eq}$	3.63, 2H, *d*, *J* = 10.3 Hz	52.0(t)	H-25&H-27	C-25&27, C-29&30
29&30	1.00, 3H, *d*, *J* = 6.3 Hz	19.2(q)	H-25&H-27	C-25&27, C-24&28

δppm in $CDCl_3$, *J* in H_Z, 200 MH_Z for ^{13}C, 800 MH_Z for ^{1}H.

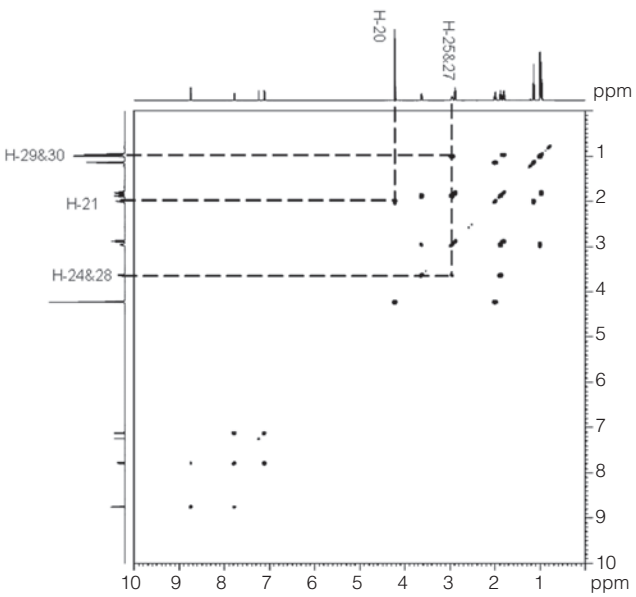

Figure 8. COSY spectrum of compound **2**.

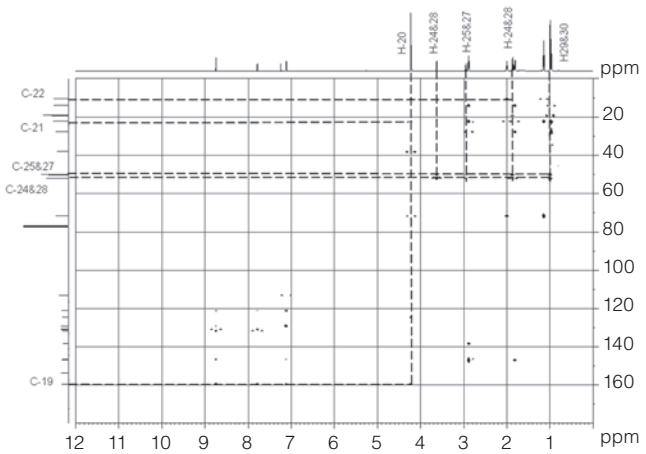

Figure 9. HMBC spectrum of compound **2**.

amine at δ_H 4.46 (3H, *s*), six methylenes, two methines and four methyls. In the ^{1}H-^{1}H COSY (Figure 8) and HMBC (Figure 9) spectra, the same structure of dimethylpiperazine with two methylenes at δ_H 3.63 (H-24&28 eq) and 1.86 (H-24&28 ax), methines at δ_H 2.95 (H-25&27) and a dimethyl groups at δ_H 0.97 (H-29&30) was revealed. Furthermore, as compound **1**, the propoxyl group on position 19 of compound **2** was observed by COSY correlation (δ_H 4.22/ δ_H 2.00) and HMBC correlation (δ_H 4.22/ δ_C 22.0/ δ_C 10.5/δ_C 159.3). However, the ^{13}C-NMR and DEPT indicated that the signal of thio-carbonyl (δ_C 171.7) in compound **1** was replaced to carbonyl group (δ_C 153.5) in compound **2**, which was supported by 1690 cm^{-1} of IR spectra (Figure 4).

Based on these spectra data, compound **2** was determined as propoxyphenyl aildenafil[15] (Figure 1).

Table 3. 1D, 2D NMR data of compound **3**

No.	$^{1}H(\delta_H)$	$^{13}C(\delta_C)$	DEPT	COSY	HMBC
1	-	146.4(s)	0	-	
4	-	171.8(s)	0	-	
6	-	146.0(s)	0	-	
8	-	133.8(s)	0	-	
9	-	132.3(s)	0	-	
10	4.49, 3H, *s*	39.3(q)	1	-	C-9
11	2.92, 2H, *t*, *J* = 7.6 Hz	27.5(t)	2	H-12	C-13, C-12, C-8, C-1
12	1.83, 2H, *t*, *J* = 7.4 Hz	22.0(t)	2	H-11, H-13	C-13, C-11, C-1
13	0.99, 3H, *t*, *J* = 7.4 Hz	13.9(q)	1	H-12	C-13, C-12
14	-	120.0(s)	0	-	
15	8.81, 1H, *d*, *J* = 2.0 Hz	130.8(d)	3	-	C-16, C-17,C-14, C-6, C-19
16	-	128.9(s)	0	-	
17	7.83, 1H, *dd*, *J* = 2.4, 8.8 Hz	131.9(d)	3	H-18	C-16, C-19
18	7.17, 1H, *d*, *J* = 8.7 Hz	113.1(d)	3	H-17	C-16, C-19
19	-	159.6(s)	0	-	
20	4.26, 2H, *t*, *J* = 6.4 Hz	72.2(t)	2	H-21	C-19, C-21, C-22
21	2.08, 2H, *m*	22.3(t)	2	H-20, H-22	C-22, C-20
22	1.18, 3H, *t*, *J* = 7.4 Hz	10.7(q)	1	H-21	C-21, C-20
24&28#	3.08, 4H, *brs*, *J* = 10.7 Hz	46.0(t)	2	H-25&H-27	-
25&27#	2.59, 4H, *t*, *J* = 4.4 Hz	51.9(t)	2	H-28&H-24	C-24&28, C-29
29#	2.52, 2H, *t*, *J* = 5.3 Hz	58.9(t)	2	H-30	C-25&27, C-30
30#	3.55, 2H, *t*, *J* = 5.3 Hz	57.7(t)	2	H-29	C-29

δppm in $CDCl_3$, *J* in H_Z, 200 MH_Z for ^{13}C, 800 MH_Z for ^{1}H.

III. *Compound **3***

Compound **3**, obtained as yellow powder as compound **1**, melting point 158 - 160°C. The molecular formula $C_{24}H_{34}N_6O_4S_2$ (m/z 535.28) has been determined by LC/MS/MS (Figure 2) and reconfirmed by HRESI MS ($[M+H]^+$ ion at m/z 535.2166). The UV spectrum showed λmax at 226 and 295, with similar spectral pattern as thio-sildenafil and compound **1** (Figure 3). The IR spectrum (Figure 4) of compound **3** showed the same characteristic peaks with an amine at 3448 cm^{-1}, an aromatic ring at 1571 cm^{-1} and a thio-carbonyl group at 1245 cm^{-1} as compound **1**. These spectra indicated that compound **3** was a thiosildenafil analogue, too.

The spectral data of ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY and HMBC for compound **3** were shown in Table 3. Same as compound **1**, the ^{1}H-NMR spectrum of compound **3** exhibited signals for one set of ABX-type (1,3,4-trisubstituted) aromatic protons at δ7.17 (1H, *d*, *J* = 8.7 Hz), δ_H 7.83 (1H, *dd*, *J* = 2.4, 8.8 Hz), δ_H 8.81 (1H, *d*, *J* = 2.4 Hz), and a singlet methyl attached to an aromatic amine at δ_H 4.49 (3H, *s*). The ^{13}C-NMR and DEPT spectra indicated three primary carbons, ten secondary carbons, three tertiary carbons and eight quaternary carbons. One quaternary carbon at δ_C 171.8 was assigned to thio-carbonyl group for C-4 as compound **1**, and it was also supported by IR spectra. In ^{1}H-^{1}H COSY (Figure 10) and HMBC (Figure 11) spectra, the same propoxyl group on position 19 was determined by the COSY

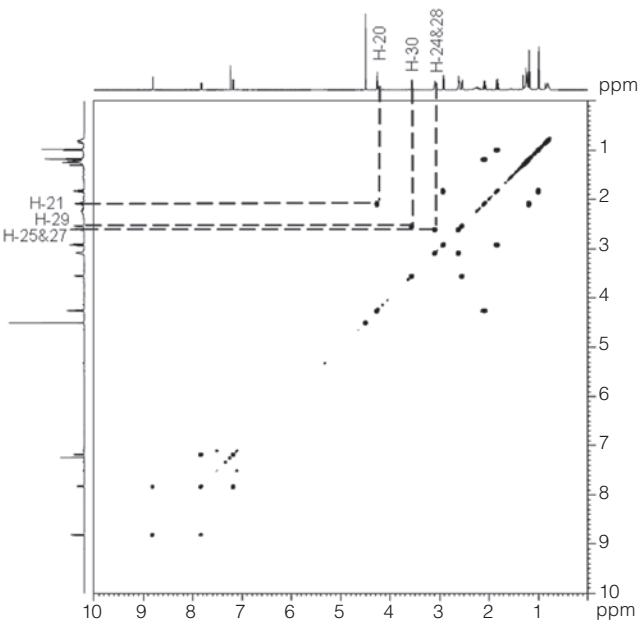

Figure 10. COSY spectrum of compound **3**.

corrlelation (δ_H 4.26 /δ_H 2.08) and HMBC correlation (δ_H 4.26/ δ_C 22.3/ δ_C 10.7).

However, the main difference between compound **3** and compound **1** was observed in addition to the above-mentioned

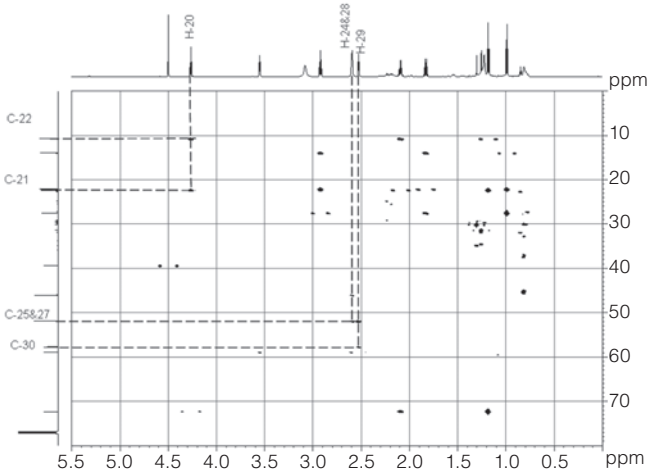

Figure 11. HMBC spectrum of compound **3**.

similarities. First, with the COSY (δ_H 3.08/ δ_H 2.59) (Figure 10) and HMBC correlation (δ_H 3.08/ δ_C 51.9) (Figure 11), two multiple peaks at δ_H 3.08 (4H, *brs*) and 2.59 (4H, *t*) were assigned as methylenes of a piperazinyl for H-24&28 and H-25&27, respectively. Both the low field chemical shifts at δ_H 3.55 and δ_C 57.7 of C-30 methylene, and δ_H 2.52 and δ_C 58.9 of C-29 methylene, indicated the linkage of hetero-atom. The COSY correlation (δ_H 2.52 /δ_H 3.55*)* and HMBC correlation *(*δ_H 2.52/ δ_C 51.9 /δ_C 57.7) indicated that these two methylenes were assigned to hydroxyl-propoyl which was attached to piperazinyl nitrogen. By comparing those data with compound **1**, the 3, 5-dimethylpiperazine in compound **1** has been changed to a hydroxyl-propyl piperazine.

Based on these spectra, the structure of compound **3** was confirmed as thio-sildenafil analogue 5-[2-propoxy-5-(4-hydroxyethylpiperazin-1-ylsulfonyl)-Phenyl]-1-methyl-3-*n*-propyl-1,6-di-hydro-7H-pyrazolo[*4,3-d*]-pyrimidin-7-thione (Figure 1**)** and named as propoxyphenyl hydroxyethylthiosildenafil.

CONCLUSIONS

In this study, compound **1**, **2** and **3** were isolated from the routine inspection samples, and their structures were elucidated by IR, NMR, LC/MS/MS and HRESI MS to be propoxyphenyl thioaildenafil (**1**), propoxyphenyl aildenafil (**2**) and propoxyphenyl hydroxyethylthiosildenafil (**3**). Compound **3** is a new compound whose structure has not been reported previously. The results will offer as the reference to formulate the regulation of natural supplements.

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REFERENCES

1. Terrett, N. K., Bell, A. K., Brown, D. and Ellis, P. 1996. Sildenafil (Viagra) a potent and selective inhibitor of type-5 cyclic GMP phosphodiesterase with utility for the treatment of male erectile dysfunction. Bioorg. Med. Chem. Lett. 6: 1819-1824.
2. Shin, M. H., Hong, M. K., Kim, W. S., Lee, Y. J. and Jeoung, Y. C. 2003. Identification of a new analogue of sildenafil added illegally to a functional food marketed for penile erectile dysfunction. Food Addit. Contam. 20: 793-796.
3. Zou, P., Oh, S. S. Y., Hou, P., Low, M. Y. and Koh, H. L. 2006. Simultaneous determination of synthetic phosphodiesterase-5 inhibitors found in a dietary supplement and pre-mixed bulk powders for dietary supplements using high-performance liquid chromatography with diode array detection and liquid chromatography–electrospray ionization tandem mass spectrometry. J. Chromatogr. A 1104: 113-122.
4. Blok-Tip, L., Zomer, B., Bakker, F., Hartog, K. D., Hamzink, M., Ten Hove, J., Vredenbregt, M. and De Kaste, D. 2004. Structure elucidation of sildenafil analogues in herbal products. Food Addit. Contam. 21: 737-748.
5. Poon, W. T., Lam, Y. H., Lai, C. K., Chan, A. Y. and Mak, T. W. 2007. Analogues of erectile dysfunction drugs: an under recognized threat. Hong Kong Med. J. 13: 359-363.
6. Shin, C., Hong, M., Kim, D. and Lim, Y. 2004. Structure determination of a sildenafil analogue contained in commercial herb drinks. Magn. Reson. Chem. 42: 1060-1062.
7. Hou, P., Zou, P., Low, M. Y., Chan, E. and Koh, H. L. 2006. Structural identification of a new acetildenafil analogue from pre-mixed bulk powder intended as a dietary supplement, Food Addit. Contam. 23: 870-875.
8. Park, H. J., Jeong, H. K., Chang, M. I., Im, M. H., Jeong, J. Y., Choi, D. M., Park, K., Hong, M. K., Youm, J., Han, S. B., Kim, D. J., Park, J. H. and Kwon, S. W. 2007. Structure determination of new analogues of vardenafil and sildenafil in dietary supplements. Food Addit. Contam. 24: 122-129.
9. Zou, P., Hou, P., Low, M. Y. and Koh, H. L. 2006. Structural elucidation of a tadalafil analogue found as an adulterant of a herbal product. Food Addit. Contam. 23: 446-451.
10. Zou, P., Hou, P., Oh, S. S. Y., Chong, Y. M., Bloodworth, B. C., Low, M. Y. and Koh, H. L. 2008. Isolation and identification of thiohomosildenafil and thiosildenafil in health supplements. J. Pharm. Biomed. Anal. 47: 279-284.
11. Kumasaka, K., Kawahara, N., Doi, K., Kojima, T. and

Goda, Y. 2008. Determination of (R)-xanthoanthrafil, a phosphodiesterase-5 inhibitor, in a dietary supplement promoted for sexual enhancement. Chem. Pharm. Bull. (Tokyo) 56: 227-230.

12. The USP convention Inc., 2005, Drug Information for the Health Care Professional, 25th ed., The United States Pharmacopeial Convention Inc. MD, USA.
13. Mosby. 2005. Mosby's Drug Consult, 15 th ed., Elsevier Inc., FL, USA.
14. Lai, K. C., Liu, Y. C., Liao, Y. C., Lin, Y. L., Tsai, L. Y., Lin, J. H. and Lo, C. F., 2010. Isolation and identification of three thio-sildenafil analogues in dietary supplements. J. Food Drug Anal.18: 269-278
15. Kee, C. L., Ge, X.W., Koh, K. L., Low, M. Y., 2012. Isolation and characterization of propoxyphenyl linked sildenafil and thiosildenafil analogues in health supplements. J. of Pharm. and Biomed. Anal., Article in press.