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Identification of novel sildenafil-analogues in an adulterated herbal food supplement

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ABSTRACT

A new herbal product advertised as potency pill was sent for analysis by the local authority. The product was tested for the presence of potential derivatives of PDE-5 inhibitors, such as sildenafil, vardenafil, and tadalafil. Sildenafil analogues were identified, in which the piperazine ring and the sulfonyl group were replaced by a piperazinone and a hydroxyethyl structure, respectively. The chemical structures were established by LC–MS in ESI negative mode, UV and NMR spectroscopy (including DEPT, HSQC, HMBC, H,H-COSY, H,H-TOCSY and H,H-NOESY experiments). This is the first report of piperazinonafil and isopiperazinonafil as adulterant in an herbal food supplement.

1. Introduction

In Germany, sildenafil (Viagra®), tadalafil (Cialis®) and vardenafil (Levitra®) are the only phosphodiesterase-5 inhibitors approved by authority for the treatment of erectile dysfunction. These drugs are only available on prescription and must be used under medical advise. Adverse effects of PDE-5 inhibitors are well documented. Precaution should be taken when patients suffer from cardiovascular incidence or heart failure. There could be higher risk of strong side effects when patients take food supplements declared as herbal potency pills. These products, mainly marketed as capsules, do not only contain dried extracts from plants as indicated and are frequently adulterated with synthetic drugs for the treatment of erectile dysfunction, corresponding analogues [1] or entirely different synthetic drugs [2,3]. These related compounds do not carry the status of legal drug substances and are only described in scientific literature or patents protecting the three legal ED-drugs. For example Shin et al. did the first close scrutiny on functional food in 2003 and discovered the analogue homosildenafil [4]. Later on acetildenafil was first reported by Blok-Tip et al. in 2004 [5]. Until today, more than 30 structurally modified analogues are described in the scientific literature. They were detected in herbal products and characterized by several research groups [6–12]. Although their structures and chemical properties are known, information about their pharmacological effects is often poor. To asses some risk, Venhuis et al. reported a IC_{50} value of 7.6 nM for acetildenafil which was comparable to sildenafil, when tested on an in vitro assay on human PDE-5 [1]. In contrast, hydroxyhomosildenafil showed a 2.1 fold activity while bearing a hydroxyethylpiperazine moiety instead of the methylpiperazine ring of sildenafil. There is less knowledge about the activity and potential side effects of many other illegal added derivatives, so it is necessary for official medicinal control laboratories to make strong efforts to discover such products and remove them from the market.

The analogues identified in the current study resulted from a product that was tested for adulteration with sildenafil, tadalafil, and vardenafil. It was also tested for acetildenafil and other structurally modified analogues using LC–MS/MS following Ng's methodology [13]. The first data achieved by LC–DAD and LC–MS showed similarity to sildenafil, but the UV-spectra did not meet the absorption lines produced by the reference substances like sildenafil or acetildenafil. Even the mass spectra disallowed conclusions to already known structures. Thus, NMR was used to identify the structure of these compounds.

2. Experimental

2.1. Chemicals

Reference standards of sildenafil citrate and hydroxyacetildenafil were obtained from Pfizer (Berlin, Germany), tadalafil

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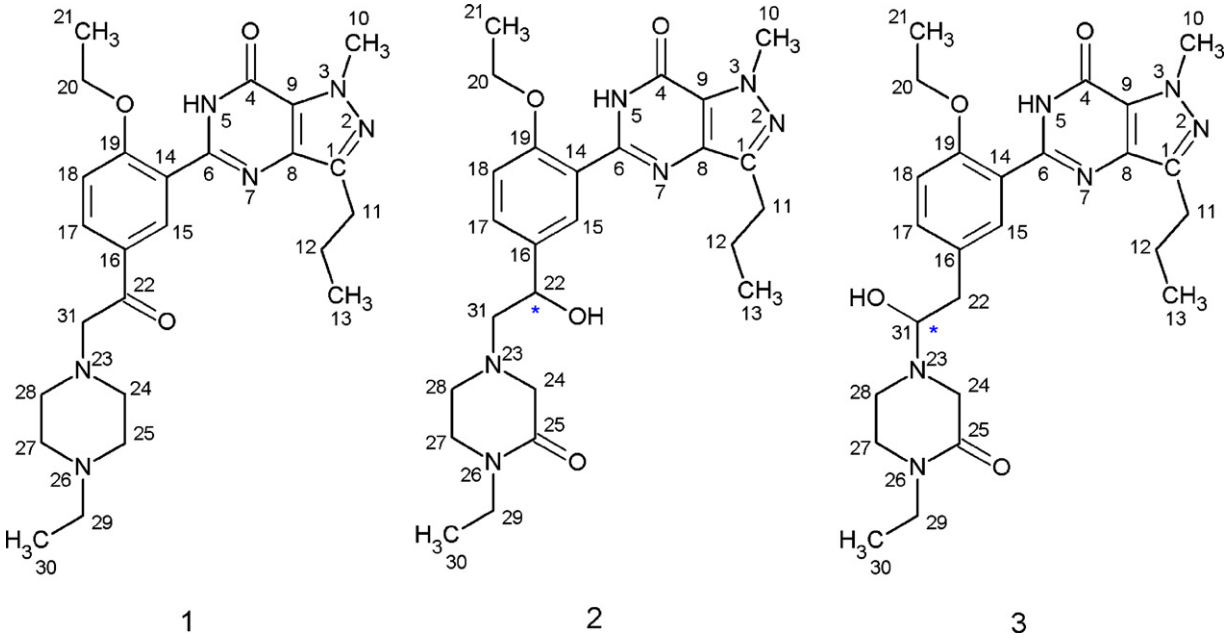

Fig. 1. Chemical structures of acetildenafil **1**, piperazinonafil **2** and isopiperazinonafil **3**.

reference standard from Eli Lilly (Bad Homburg, Germany), and vardenafil hydrochloride trihydrate from Bayer (Leverkusen, Germany). Acetildenafil was kindly provided by the National Institute for Public Health and the Environment (Bilthoven, The Netherlands). Ammonium formate, methylenechloride, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). All LC solvents were obtained in LC–MS grade (Chromasolv®) from Fluka (Buchs, Switzerland).

2.2. Sample preparation

The content of one capsule was mixed with 10 mL of methylenechloride and 2 mL of 2 M sodium hydroxide solution. The organic layer was collected using a separation funnel. The aqueous layer was extracted again with 5 mL of methylenechloride. The combined organic solvents were dried under a stream of nitrogen. The residue was reconstituted in 1 mL of acetonitrile. This solution was suitably diluted with the same solvent for LC–DAD analysis and for LC–MS analysis. All samples and solutions were filtered before use through a 0.45 μm Spartan filter from Whatman (Dassel, Germany).

2.3. LC–DAD

LC–DAD analysis was performed on an Elite La Chrom system, equipped with a L-2455 diode array detector (VWR, Darmstadt) operated in a detection range from 200 to 320 nm. For chromatographic separation, a Zorbax-SB C_{18} column (250 mm × 4.6 mm × 5.0 μm; Agilent Deutschland GmbH, Böblingen, Germany) was used. The elution conditions were as follows: gradient elution with solvent A – 10 mM ammonium formate and solvent B – acetonitrile. Chromatography was started with solvent B 20% which was raised to 80% within 20 min. Solvent B was subsequently decreased within 5 min to 20% again to allow reconditioning the analytical column for an additional of 5 min. The column temperature was kept at 25 °C, the flow rate was set to 1.0 mL/min with an injection volume being 10 μL of each sample. Isolation of the compounds was carried out using the chromatographic system described above, but with an injection volume of 80 μL. The eluate was collected in a sample tube after the detection of the peak in the chromatographic run. The solvent was removed under reduced pressure and the residue was subjected to NMR-analysis.

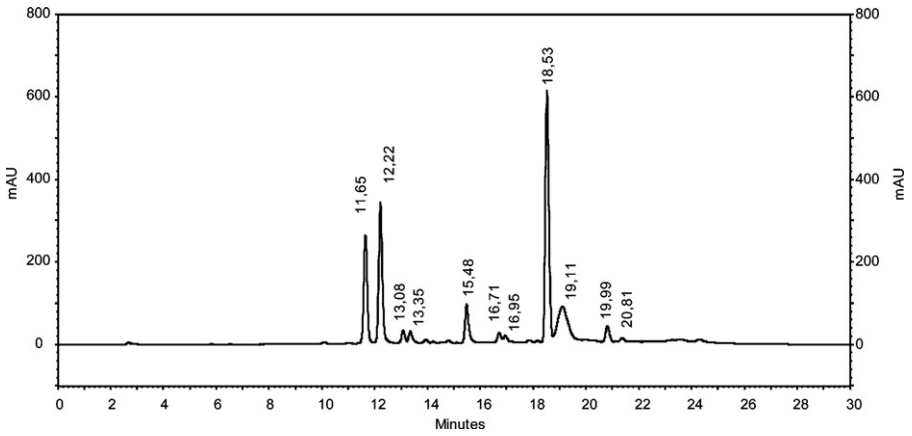

Fig. 2. LC–DAD run (wavelength 254 nm) of prepared sample.

Table 1
Identified compounds **2** and **3** and corresponding retention times.

Compound number	Name	Retention time (LC–DAD)	Retention time (LC–MS)
2	Piperazinonafil	12.22 min	12.69 min
3	Isopiperazinonafil	11.65 min	12.36 min

2.4. LC–MS

LC–MS studies were carried out by using a Shimadzu prominence LC-20 (Shimadzu, Kyoto, Japan) system connected to AB Sciex 5500 Qtrap™ triple quadrupole linear ion trap mass spectrometer (AB Sciex, Foster City, USA) operated in electrospray negative mode by Analyst software version 1.5.1. Separation conditions were comparable to the parameters mentioned under LC–DAD but using a Luna C_{18} column (150 mm × 2.0 mm × 3.0 μm; Phenomenex, Torrance, USA) and a flow rate of 0.2 mL/min. The MS parameters were: entrance potential: −10 V; declustering potential: −10 V; collision energy: −10 V; collision cell exit potential: −11 V; curtain gas: 30 psi; ion spray voltage: −4.5 kV; source temperature: 600 °C; ion source gas 1: 40 psi; ion source gas 2: 60 psi. The MS/MS method was set up by using an enhanced MS scan (EMS) with information dependent acquisition (IDA) which would initiate an additional experiment, namely enhanced product ion scan (EPI). The IDA was set for ions greater than *m*/*z* 400 and

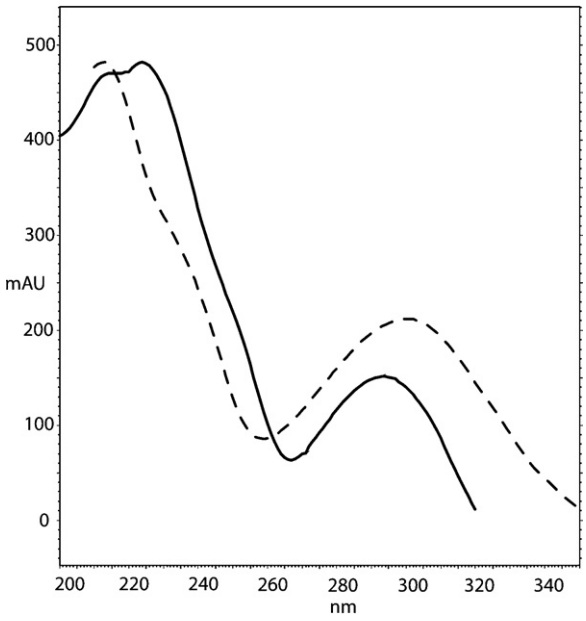

Fig. 3. UV-spectra of compounds **2** and **3** (—), showing maximum absorbance at 221 nm and 290 nm. The UV-spectrum of sildenafil reference substance (—-) is given for comparison.

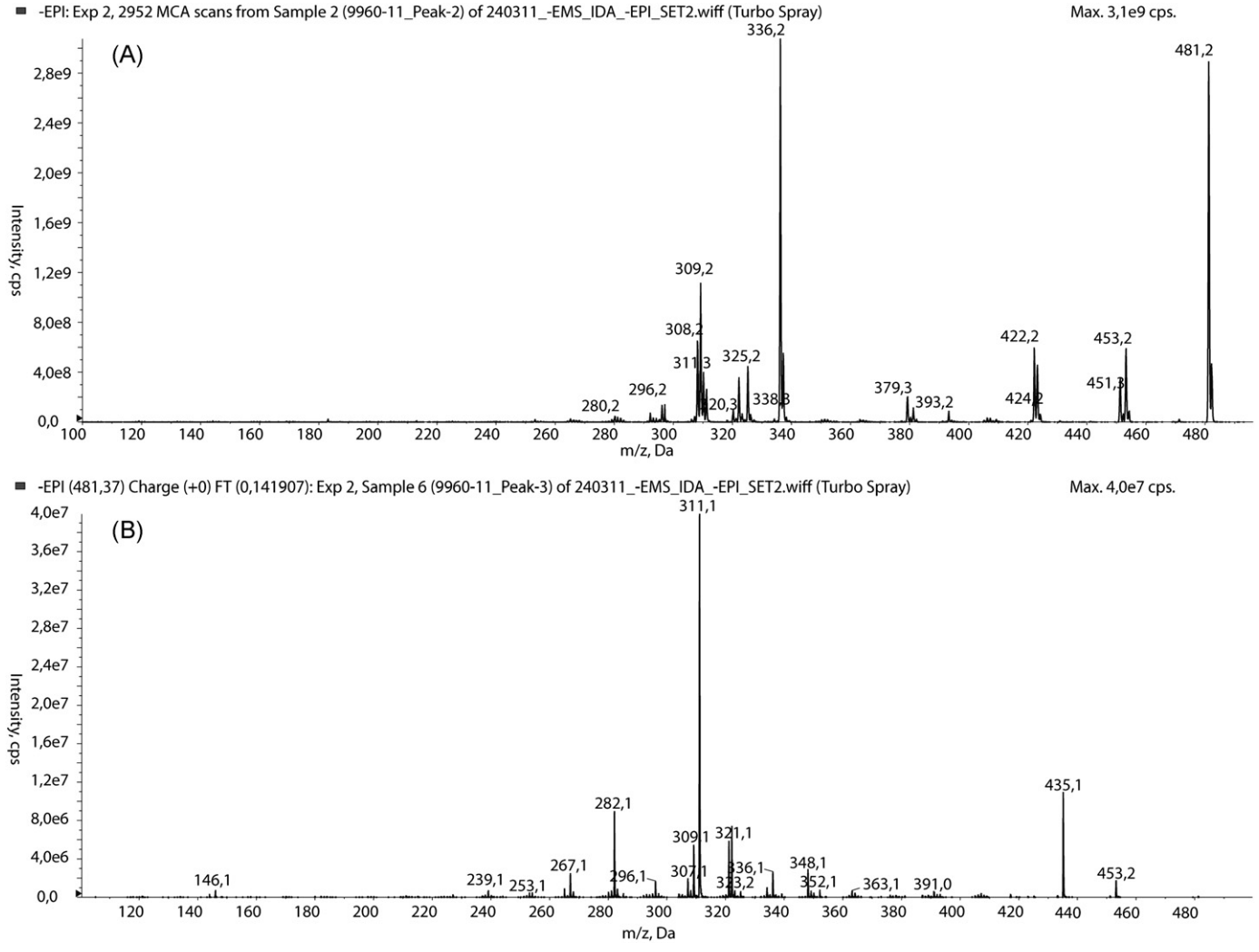

Fig. 4. Mass spectra from enhanced product ion scan of compound **3** (A) and compound **2** (B).

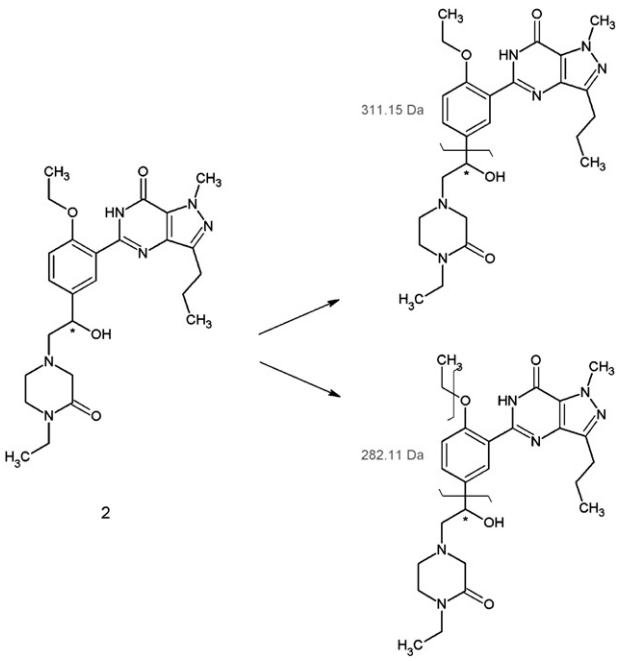

Fig. 5. Proposed fragmentation of compound **2**.

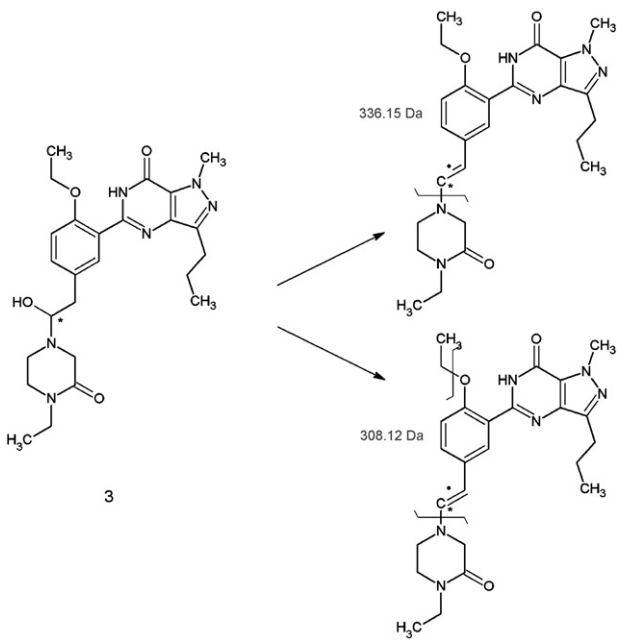

Fig. 6. Proposed fragmentation of compound **3**.

smaller than *m/z* 600 in the survey experiment of EMS. When exceeding an intensity of 100,000 cps, the EPI was initiated with Q1 fixed and Q3 scanning from 100 Da to 600 Da with collision energy of −45 V and spread of 15.0 V.

2.5. NMR

Each of the purified compounds **2** and **3** was dissolved in 0.6 mL of CD_2Cl_2. NMR experiments with detection of ^1H (one-dimensional ^1H NMR, two-dimensional H,H-COSY, H,H-TOCSY, H,H-NOESY, HSQC and HMBC) were performed with a Bruker AVANCE-I 500 MHz spectrometer, equipped with an inverse 5 mm $^1H/^{13}C$ probehead. NMR experiments with detection of ^{13}C (one-dimensional ^{13}C NMR with ^1H decoupling, DEPT-135, DEPT-90) were carried out with a Bruker AVANCE-III spectrometer, equipped with a 5 mm CP-QNP X-detect cryoprobehead at 125.6 MHz ^{13}C NMR frequency. Chemical shifts are reported in ppm. Data were processed with TOPSPIN 3.0 or MestreNova software.

3. Results and discussion

3.1. LC–DAD

The unknown compounds were detected during analysis of an herbal food supplement. Using HPLC–DAD, compound **2** (Fig. 1) eluted at an RT of 12.22 min, shortly after compound **3** (Fig. 1) which resolved at an RT of 11.65 min (Fig. 2 and Table 1). Both peaks

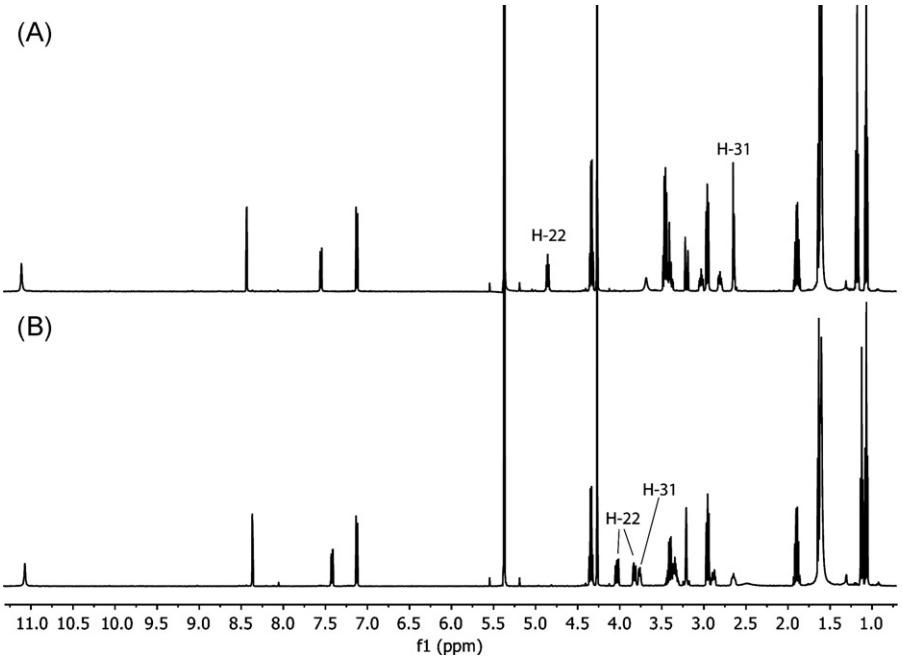

Fig. 7. ^1H NMR spectrum of (A) compound **2** and (B) compound **3**.

Table 2
NMR data of piperazinonafil **2**, isopiperazinonafil **3** and acetildenafil **1** [5]; Positions 1 to 31 indicate either hydrogen (H,H-COSY/H,H-TOCSY) or carbon (HMBC) signal.

Pos.	Compound **2** (piperazinonafil)						Compound **3** (isopiperazinonafil)						Acetildenafil	
	δ ^{1}H	Multiplicity	δ ^{13}C	DEPT[a]	H,H-COSY/ TOCSY	HMBC	δ ^{1}H	Multiplicity	δ ^{13}C	DEPT[a]	H,H-COSY	HMBC	δ ^{1}H	δ ^{13}C
1			146.2	0		H-11/H-12			146.3	0		H-11/H-12		146.8
2														
3														
4			153.6	0					153.6	0				153.7
5	11.10	1H, br s					11.06	1H, br s					10.8	
6			148.3	0		H-15			148.1	0		H-15		147.4
7														
8			138.4	0		H-11			138.4	0		H-11		138.5
9			124.4	0		H-10			124.4	0		H-10		124.5
10	4.25	3H, s	38.0	3			4.25	3H, s	38.0	3			4.27	38.2
11	2.94	2H, t, J = 7.7 Hz	27.6	2	H-12	H-12/H-13	2.94	2H, t, J = 7.4 Hz	27.7	2	H-12/H-13	H-12/H-13	2.94	27.8
12	1.88	2H, m, J = 7.4 Hz	22.3	2	H-11/H-13	H-11/H-13	1.88	2H, m, J = 7.4 Hz	22.3	2	H-11/H-13	H-11/H-13	1.87	22.4
13	1.05	3H, t, J = 7.4 Hz	13.8	3	H-12	H-11/H-12	1.05	3H, t, J = 7.4 Hz	13.8	3	H12	H-11/H-12	1.08	14.1
14			120.0	0		H-18			120.1	0				129.9
15	8.42	1H, d, J = 2.3 Hz	128.4	1	H-17	H-17/H-22	8.35	1H, d, J = 2.3 Hz	131.1	1	H-17	H-17	9.14	132.3
16			135.0	0		H-18/H-22/H-31			129.0	0		H-18/H-22/H-31		120.0
17	7.54	1H, dd, J = 8.6, 2.3 Hz	129.9	1	H-18	H-15/H-22	7.40	1H, dd, J = 8.6, 2.3 Hz	132.4	1	H-15/H-18	H-15	8.15	132.5
18	7.11	1H, d, J = 8.6 Hz	113.2	1	H-17		7.11	1H, dd, J = 8.6 Hz	113.1	1	H-17		7.09	112.7
19			156.1	0		H-15/H-17/H-18/H-20			156.4	0		H-15/H-17/H-18/H-20		159.8
20	4.32	2H, q, J = 7.0 Hz	65.5	2	H-21	H-21	4.33	2H, q, J = 7.0 Hz	65.6	2	H-21	H-21	4.36	65.8
21	1.61	3H, t, J = 7.0 Hz	14.5	3	H-20	H-20	1.62	3H, t, J = 7.0 Hz	14.5	3	H-20	H-20	1.63	14.6
22[b]	4.84	1H, m, J = 6.6 Hz	68.4	1	H-31	H-15/H-31	4.02	1H, m	61.4	2	H-31	H-31		194.9
22[b]							3.82	1H, m	61.4		H-31			
23														
24[b]	3.40	1H, m	57.1	2	H-24	H-28/H-31	3.20	2H, s	54.0	2		H-31	2.68	52.2
24[b]	3.19	1H, d, J = 16.1 Hz	57.1		H-24	H-28/H-31								
25			165.5	0		H-24/H-27/H29			165.9	0		H-24/H-29	2.54	52.5
26														
27[b]	3.43	2H, m	45.8	2	H-28	H-29	2.87	1H, m	45.9	2	H-28	H-28/H-29	2.54	52.5
27[b]			45.8				3.33	1H, m	45.9		H-28			
28[b]	3.02	1H, m	49.9	2	H-27	H-24/H-31/H-27	2.63	1H, m	46.5	2	H-27	H-24/H-31	2.68	52.2
28[b]	2.79	1H, m	49.9		H-27	H-24/H-31/H-27	3.31	1H, m	46.5		H-27			
29	3.45	2H, q, J = 7.2 Hz	41.0	2	H-30	H-30	3.39	2H, m, J = 7.1 Hz	40.9	2	H-30	H-30	2.41	53.3
30	1.16	3H, t, J = 7.2 Hz	11.9	3	H-29	H-29	1.10	3H, t, J = 7.1 Hz	11.8	3	H-29	H-29	1.05	11.8
31	2.62	2H, m, J = 6.6 Hz	65.0	2	H-22	H-22/H-24	3.75	1H, m	68.6	1	H-22	H-22/H-24/H-28	3.82	64.6

δppm in CD_2Cl_2 (compounds **2** and **3**) and $CDCl_3$ (acetildenafil), J in Hz.

[a] Number in DEPT is the number of attached protons.

[b] Proton signals are double-specified due to the influence of chiral carbon.

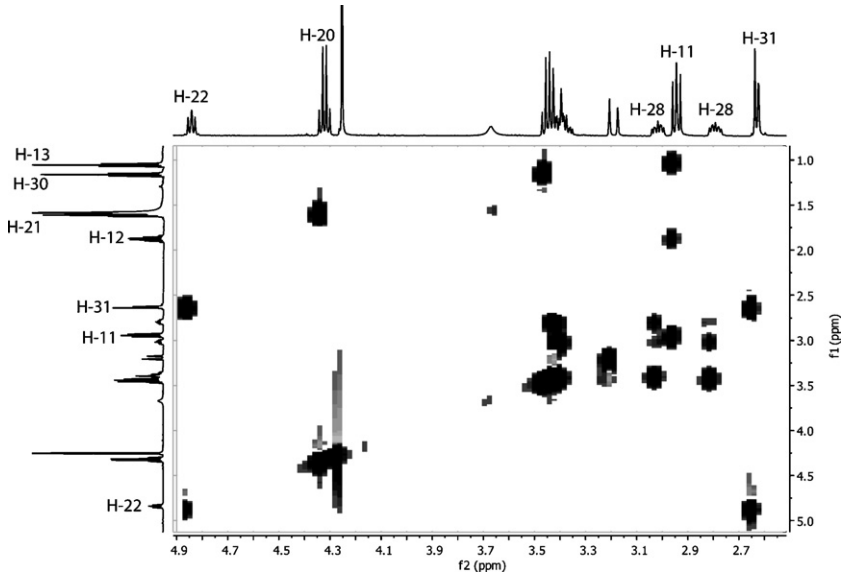

Fig. 8. H,H-TOCSY spectrum of compound **2**.

showed identical UV absorption between 200 nm and 320 nm. The UV-traces shown in Fig. 3 clearly demonstrate the differences between compounds **2** and **3**, and sildenafil.

3.2. LC–MS

The extract of the herbal food supplement was subjected to LC–MS analysis. Different to the method reported by Ng et al. [13] using a precursor ion scan as survey scan, an EMS was set up for the initial MS experiment. The scan of the extract produced two major peaks at RT 12.36 min and RT 12.69 min with a parent ion mass of *m*/*z* 481. This indicates that the unknown compounds **2** and **3** had same molecular weights of 482 Da. The corresponding mass spectrum of the $[M-H]^-$ ion at RT 12.69 min is shown in Fig. 4B. The two fragments (*m*/*z* 311 and *m*/*z* 282) were characteristic for PDE-5 inhibitors, but there is no established structure with a molecular mass of 482 Da. A hydroxyethyl moiety could be assigned by the following fragments: *m*/*z* 453 formed by neutral loss of ethylene (−28) from the molecular ion, followed by loss of water from the alcohol function in position 22 of the molecule to give ion of *m*/*z* 435. The resulting fragments (*m*/*z* 311 and *m*/*z* 282) seem to obey the same cleavage mechanism reported for acetildenafil [13]. The *m*/*z* 311 fragment results from the cleavage of the carbon bond between position 16 and 22 of the molecule (Fig. 5). Fragment *m*/*z* 282 is generated in similar manner but with prior neutral loss of the ethylene part.

The first peak at RT 12.36 min also had a parent ion of *m*/*z* 481 but generated different daughter ions. Just ion *m*/*z* 453 resulted from neutral loss of ethylene as described before. All other fragments could be explained by shifting of the alcohol function from position

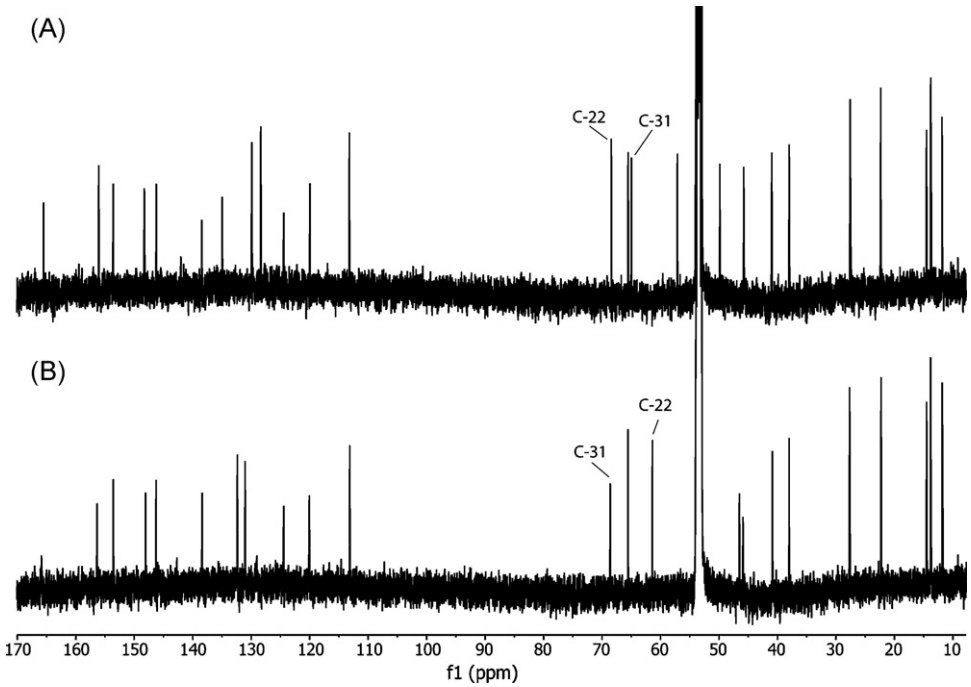

Fig. 9. ^{13}C NMR spectrum of (A) compound **2** and (B) compound **3**.

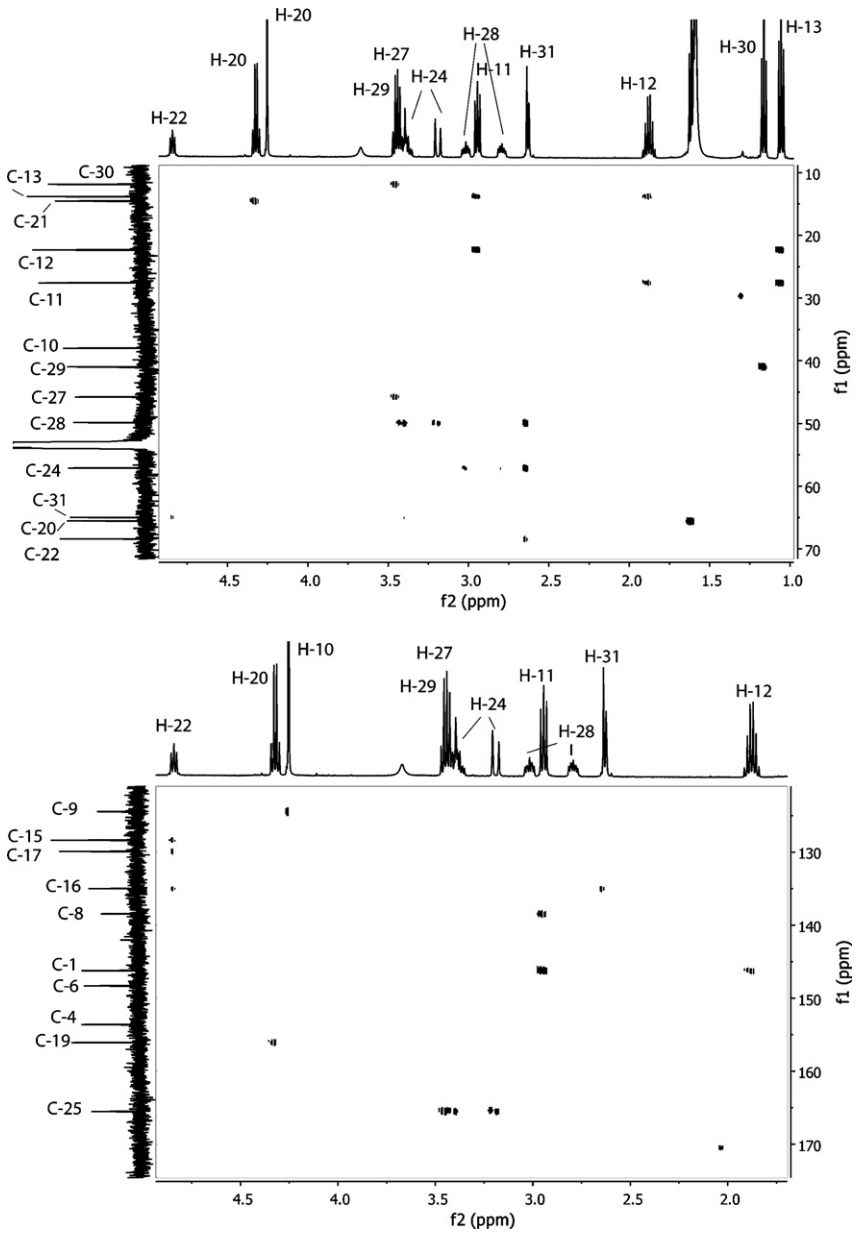

Fig. 10. HMBC spectrum of compound **2**.

22 to 31 which gives compound **3** an acetal function (mixed *N*,*O*-acetal). The first fragmentation occurs at position 31 with loss of the alcohol function (−18), the first acetal half, which resulted in *m*/*z* 336. Afterwards, the ESI negative conditions induce breakage of the second acetal half between position 31 and nitrogen 24 of the piperazinone together with neutral loss of ethylene (Fig. 6) to give *m*/*z* 308. All ions observed for compound **3** (Fig. 4A) are the evidence for the isomer hydroxyethyl moiety in comparison to structure **2**.

3.3. NMR

^{1}H and ^{13}C NMR data of acetildenafil (**1**) [5] and compounds **2** and **3** are summarized in Table 2. The ^{1}H NMR spectrum of **2** (Fig. 7A) shows a signal at 11.1 ppm which can be tentatively assigned as an amide proton. The three signals at 8.42 ppm (d, J = 2.3 Hz), 7.54 ppm (dd, J = 8.6, 2.3 Hz), and 7.11 ppm (d, J = 8.6 Hz) resemble the coupling pattern of a 1,2,4-substituted phenyl ring. Signals were detected at the same frequencies as for the N-methyl, O-ethyl and C-propyl moieties in acetildenafil.

The proton signal for H-31 is shifted to higher field which indicates a structural modification (in comparison with acetildenafil) nearby. Moreover, a proton signal at 4.84 ppm (m, J = 6.6 Hz) is observed, which is not present in acetildenafil. The H,H-TOCSY data (Fig. 8) shows a correlation between the signals at 2.62 ppm and 4.84 ppm. These observations suggest the presence of a hydroxyethyl moiety.

The proton signals representing the piperazinonyl methylene resonances also differ significantly in comparison to the respective ones for acetildenafil. Instead of two complex signals for H-24/28 and H-25/27 in acetildenafil, discrete signals for H-24 (3.19, 3.40 ppm), H-28 (2.79, 3.02 ppm) and H-27 (3.43 ppm) are observed. No proton signal for H-25 is observed. The signal pattern is consistent with a piperazinone moiety. Interestingly, the signals for the geminal protons at C-24 and C-28 are found at different frequencies indicating the neighbourhood of a chiral atom.

The ^{13}C NMR spectrum (Fig. 9A) shows most of the characteristic resonances for acetildenafil [5]. On the other hand, the characteristic signal of the carbonyl group at position 22 is missing. Instead

the signal for C-22 is observed at 68.4 ppm. Moreover, a signal at 165.5 ppm consistent with an amid carbon can be assigned to C-25 leading to the proposed piperazinone structure.

All conclusion mentioned above are in line with all signals detected in HSQC-NMR spectra, such as the correlations between δ_C 68.4/δ_H 4.84 (C-22/H-22), δ_C 49.9/δ_H 2.79, 3.02 (C-28/H-28), δ_C 45.8/δ_H 3.43 (C-27/H-27), and δ_C 57.1/δ_H 3.19, 3.40 (C-24/H-24). The HMBC spectroscopic data (Fig. 10) also provided strong confirmation of the structure with indicative correlations of δ_H 4.84/δ_C 128.4, 135.0, 129.9, 65.0 (H-22/C-15, C-16, C-17, C-31), δ_H 2.62/δ_C 135.0, 68.4, 57.1, 49.9 (H-31/C-16, C-22, C-24, C-28), and δ_H 3.19, 3.40/δ_C 165.5, 49.9, 65.0 (H-24/C-25, C-28, C-31).

A 2D-NOESY experiment shows a correlation between H-22 and the aromatic protons H-15 and H-17. Moreover, a NOE occurs between H-31 and the aromatic protons H-15 and H-17. A weak NOE is observed between the piperazinone protons H-24/H-28 and H-22. Moreover, a very weak NOE occurs between H-28 (2.79 ppm) and H-15 (8.42 ppm). These correlations also support the signal assignments and indicate an orientation of the piperazinone ring towards the phenyl ring [14].

In summary, the NMR spectroscopic data provide firm evidence that the unknown compound **2** is 5-{2-ethoxy-5-[1-hydroxy-2-(1-ethylpiperazin-2-on-4-yl)ethyl]phenyl}-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (Fig. 1).

The chemical structure of compound **3** was definitely identified by comparison of its NMR data with those of compound **2**. Roughly, the ^{1}H NMR (Fig. 7B) and ^{13}C NMR (Fig. 9B) spectra of compound **3** were similar to those of compound **2**. However, the proton signals for H-22 at δ_H 4.84 and H-31 at δ_H 2.62 of **2** disappear and new signals at δ_H 4.02, 3.82 and 3.75 appear for **3**.

The comparison of the ^{13}C NMR spectra of compounds **2** (Fig. 9A) and **3** (Fig. 9B) showed that the secondary carbon signals of C-31 at δ_C 65.0 and C-24 at δ_C 57.1 disappear and a new secondary carbon signal at δ_C 61.4 appears.

The H,H-COSY correlation between H-31 (3.75 ppm) and H-22 (4.02, 3.82 ppm) again indicates the presence of a hydroxyethyl moiety. In conjunction with many correlations observed in HSQC and HMBC experiments (Table 2), the structure of compound **3** is assigned as 5-{2-ethoxy-5-[2-hydroxy-2-(1-ethylpiperazin-2-on-4-yl)ethyl]phenyl}-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (Fig. 1). Specifically, the HSQC correlations between δ_C 68.6/δ_H 3.75 (C-31/H-31), δ_C 61.4/δ_H 4.02, 3.82 (C-22/H-22), and δ_C 54.0/δ_H 3.20 (C-24/H-24), and the HMBC correlations of δ_H 3.75/δ_C 129.1, 61.4, 54.0, 46.5 (H-31/C-16, C-22, C-24, C-28) are strongly indicative for the signal assignments.

Compared with the chemical structure of compound **2**, the *N*,*O*-acetal structure of compound **3** explains the significant shift of the proton and carbon signals for H/C-31 to lower field as well as the shift of the proton and carbon signals for H/C-22 to higher field. The *N*,*O*-acetal structure also leads to a slightly higher electron density at C-24 and C-28 which explains the shift of both signals to higher field.

4. Conclusion

In the present study, two analogues of sildenafil were isolated from a herbal product. The chemical structures of both compounds were elucidated using LC–MS, UV and NMR spectroscopic data. Based on one- and two-dimensional NMR data, the chemical structures were determined as 5-{2-ethoxy-5-[1-hydroxy-2-(1-ethylpiperazin-2-on-4-yl)ethyl]phenyl}-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (compound **2**) and 5-{2-ethoxy-5-[2-hydroxy-2-(1-ethylpiperazin-2-on-4-yl)ethyl]phenyl}-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (compound **3**). Based on the piperazinone structure, the compounds were named piperazinonafil **2** and isopiperazinonafil **3**. Both were quantitated in the product in varying but approximately equal amounts per capsule. Notably, similar structures bearing an hydroxyethyl moiety without piperazinone functionality were reported by Piazza et al. [15], but these purinone derivatives were intended to treat patients with precancerous lesions. Taking into account the structure similarity of both compounds to acetildenafil, which shows activity similar to sildenafil, **2** and **3** might exhibit PDE-5 inhibition [14]. However, the efficacy and safety/toxicity profile of **2** and **3** is not yet known. Quite obviously, this puts consumers' health at substantial risk.

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References

[1] B.J. Venhuis, D.M. Barends, M.E. Zwaagstra, D. de Kaste, Recent Developments in Counterfeits and Imitations of Viagra, Cialis and Levitra: A 2005–2006 Update, RIVM Report 370030001/2007, National Institute for Public Health and the Environment, The Netherlands, 2007, 49 pp.
[2] M.J. Bogusz, H. Hassan, E. Al-Enazi, Z. Ibrahim, M. Al-Tufail, Application of LC–ESI-MS–MS for detection of synthetic adulterants in herbal remedies, J. Pharm. Biomed. Anal. 41 (2006) 554–564.
[3] Y. Chen, L. Zhao, F. Lu, Y. Yu, Y. Chai, Y. Wu, Determination of synthetic drugs used to adulterate botanical dietary supplements using QTRAP LC–MS/MS, Food Addit. Contam. 26 (2009) 595–603.
[4] M.H. Shin, M.K. Hong, W.S. Kim, Y.J. Lee, Y.C. Jeoung, Identification of a new analogue of sildenafil added illegally to a functional food marketed for penile erectile dysfunction, Food Addit. Contam. 20 (2003) 793–796.
[5] L. Blok-Tip, B. Zomer, F. Bakker, K.D. Hartog, M. Hamzink, J. ten Hove, M. Vredenbregt, D. de Kaste, Structure elucidation of sildenafil analogues in herbal products, Food Addit. Contam. 21 (2004) 737–748.
[6] J.C. Reepmeyer, J.T. Woodruff, Use of liquid chromatography–mass spectrometry and a chemical cleavage reaction for the structure elucidation of a new sildenafil analogue detected as an adulterant in an herbal dietary supplement, J. Pharm. Biomed. Anal. 44 (2007) 887–893.
[7] H. Göker, M. Coskun, M. Alp, Isolation and identification of a new acetildenafil analogue used to adulterate a dietary supplement: dimethylacetildenafil, Turk. J. Chem. 34 (2010) 157–163.
[8] B.J. Venhuis, G. Zomer, M. Hamzink, H.D. Meiring, Y. Aubin, D. de Kaste, The identification of a nitrosated prodrug of the PDE-5 inhibitor aildenafil in a dietary supplement: a Viagra with a pop, J. Pharm. Biomed. Anal. 54 (2011) 735–741.
[9] P. Zou, P. Hou, S.S.-Y. Oh, X. Ge, B.C. Bloodworth, M.-Y. Low, H.-L. Koh, Identification of benzamidenafil, a new class of phosphodiesterase-5 inhibitor, as an adulterant in a dietary supplement, J. Pharm. Biomed. Anal. 47 (2008) 255–259.
[10] K.-C. Lai, Y.-C. Liu, M.-C. Tseng, J.-H. Lin, Isolation and identification of a sildenafil analogue illegally added in dietary supplements, J. Food Drug Anal. 14 (2006) 19–23.
[11] M.-C. Lin, Y.-C. Liu, Y.-L. Lin, J.-H. Lin, Identification of a tadalafil analogue adulterated in a dietary supplement, J. Food Drug Anal. 17 (2009) 451–458.
[12] P. Zou, P. Hou, S.S.-Y. Oh, Y.M. Chong, B.C. Bloodworth, M.-Y. Low, H.-L. Koh, Isolation and identification of thiohomosildenafil and thiosildenafil in health supplements, J. Pharm. Biomed. Anal. 47 (2008) 279–284.
[13] C.S. Ng, T.Y. Law, Y.K. Cheung, P.C. Ng, K.K. Choi, Development of a screening method for the detection of analogues of sildenafil and vardenafil by the use of liquid chromatograph coupled with triple quadrupole linear ion trap mass spectrometer, Anal. Meth. 2 (2010) 890–896.
[14] J.C. Reepmeyer, D.A. d'Avignon, Structure elucidation of thioketone analogues of sildenafil detected as adulterants in herbal aphrodisiacs, J. Pharm. Biomed. Anal. 49 (2009) 145–150.
[15] G.A. Piazza, R. Pamukcu, US 6,200,980, Cell Pathways Inc., 2001.