Identification of Mutaprodenafil in a Dietary Supplement and Its Subsequent Synthesis

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Received June 16, 2011; accepted July 12, 2011; published online July 14, 2011

We isolated a new illegal sildenafil analogue named mutaprodenafil from a dietary supplement for erectile dysfunction (ED) and proposed that it is an aildenafil derivative containing an imidazole moiety. We subsequently synthesized mutaprodenafil from a thioaildenafil and authenticated its structure.

Key words aildenafil prodrug; sildenafil analogue; mutaprodenafil; phosphodiesterase-5 inhibitor; erectile dysfunction

In recent years, we have seen a dramatic increase in the consumption of dietary supplements along with the rise in the public's awareness of health matters. However, some of these products are illegally advertised as being effective for male sexual enhancement. Among these products, most are adulterated with synthetic compounds, such as sildenafil, vardenafil, and tadalafil, which are active ingredients used for the treatment of penile erectile dysfunction (ED), and their analogs or other unapproved phosphodiesterase-5 (PDE-5) inhibitors.[1—14)] In the last year, a new illegal sildenafil analogue was isolated from a dietary supplement for ED. It was reported that the compound is converted to aildenafil, a structural analogue of sildenafil,[15)] under physiological acidic conditions.[16)] Recently, Venhuis's group reported its structural determination, mainly based on a detailed discussion of the results of MS analyses, and concluded that it was a nitrosated prodrug (**A**) of aildenafil (Fig. 1A).[16)] We have also independently isolated the same compound from a dietary supplement and analyzed its structure. Most of our analytical data (1D-, ^{1}H-, ^{13}C-NMR, and LC-MS spectra) matched Venhuis's data, and we predicted the structure to be an aildenafil derivative (Table 1). However, our proposed structure (Fig. 1B) was different from that of **A**.

The critical discussion points regarding the compound's structure are as follows: 1) The three carbons outside of the aildenafil moiety must be located in positions appropriate to their ^{13}C chemical shifts (δ_C 150.1, 139.2, 115.2) because the chemical shifts assigned to the positions in the structure of **A** are abnormal. 2) The observed heteronuclear multiple bond connectivity (HMBC) correlations between the imino proton at δ_H 3.78 and the two carbons at δ_C 115.2 and δ_C 139.2 must be explained by the proposed structure. 3) The observed two paired N-HMBC correlations, namely, those between the methine proton at δ_H 7.86 or the imino proton at δ_H 3.78 and the nitrogens at δ_N 177.0 and δ_N 252.0, must be explained. 4) The structure must also satisfy the 2D INADEQUATE (the Incredible Natural-Abundance Double-Quantum Transfer Experiment) findings for the compound, which showed correlations between the carbons at δ_C 150.1 and δ_C 115.2. As shown in Fig. 2, our proposed structure completely satisfies all of these critical points.

Considering these results, we firmly believe that the structure of the isolated aildenafil derivative is as illustrated in Fig. 1B, and hence, contains an imidazole moiety. Therefore, we synthesized compound **1** to confirm its structure.

We were able to synthesize **1** as follows (Chart 1): A mixture of thioaildenafil (25 mg, 0.05 mmol),[17)] 5-chloro-1-methyl-4-nitroimidazole (8.0 mg, 0.05 mmol), and anhydrous sodium acetate (4.1 mg, 0.05 mmol) in 0.5 ml dimethylsulfoxide was stirred at 100 °C for 2 h.[18)] The reaction mixture was then poured into water, extracted with $CHCl_3$, and dried

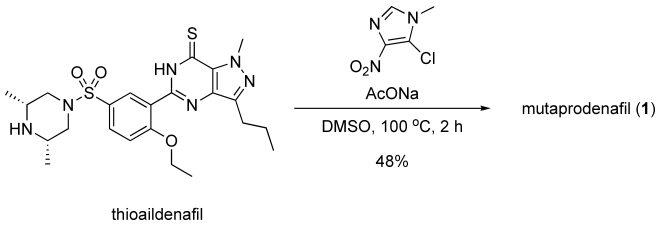

Chart 1. Synthesis of Compound **1**

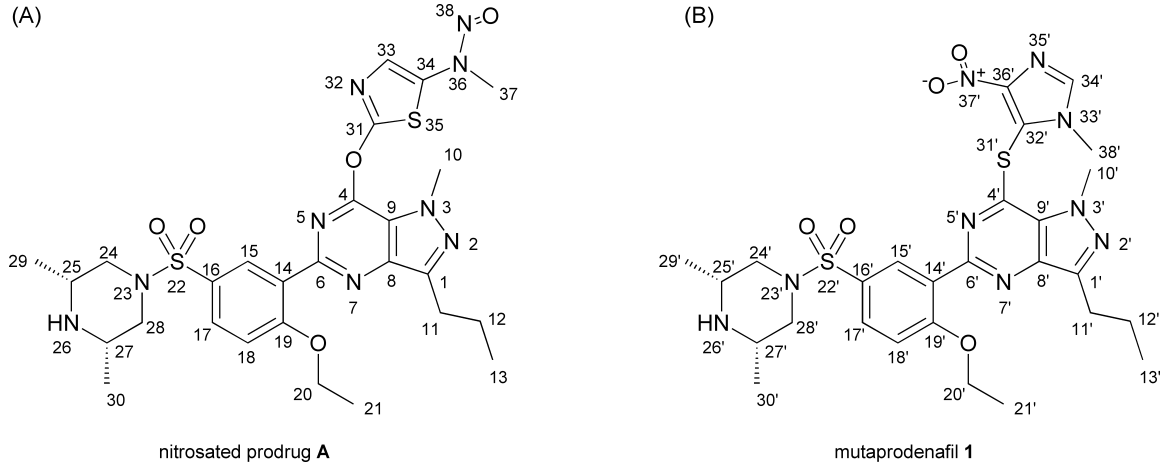

Fig. 1. Proposed Chemical Structures of the New Aildenafil Derivative

(A) Compound **A** proposed by Venhuis's group. (B) Compound **1** proposed by our group.

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Table 1. ^{1}H- and ^{13}C-NMR Data of Isolated Aildenafil Derivative in Chloroform-*d*

Atom	Mutaprodenafil (**1**)	
	δ_C	δ_H (J in Hz)
1′	146.7	—
4′	149.0	—
6′	154.6	—
8′	143.5	—
9′	128.2	—
10′	39.1	4.37 s
11′	27.7	2.94 t (7.6)
12′	21.9	1.84 m
13′	13.8	0.95 t (7.3)
14′	127.3	—
15′	131.5	7.79 d (2.7)
16′	126.6	—
17′	130.0	7.60 dd (2.7, 9.2)
18′	113.1	6.99 d (9.2)
19′	160.7	—
20′	64.7	4.06 q (6.9)
21′	14.3	1.32 t (6.9)
24′/28′	51.7	1.71 t (11.0), 3.47 dd (2.8, 11.0)
25′/27′	50.1	2.88 m
29′/30′	19.2	0.96 d (6.3)
32′	115.2	—
34′	139.2	7.86 s
36′	150.1	—
38′	33.0	3.78 s

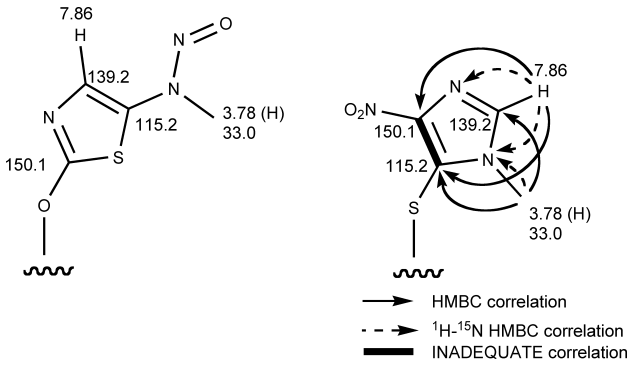

Fig. 2. HMBC, ^{1}H–^{15}N HMBC, and INADEQUATE Correlations for Compound **1**

over anhydrous sodium sulfate. Evaporation of the solvent gave an oily residue, which was purified by gel permeation chromatography (GPC) to give **1** (15 mg, 48%) as a white solid.[19]

Then, we measured the ^{1}H- and ^{13}C-NMR spectra of the synthesized **1** and found that the data were almost identical to those of the isolated molecule. Furthermore, the retention time of the LC-MS spectrum of the synthesized **1** was the same as that of the isolated molecule (Fig. 3).[20] These results indicate that the structure of the isolated aildenafil derivative can not be a nitrosated prodrug **A**, but rather that compound **1** contains an imidazole moiety. Since compound **1** can be converted to aildenafil by acidic treatment and we revised the structure proposed by Venhuis's group (nitroso−prodenafil), we named it mutaprodenafil (mutatis+prodenafil). In this communication, we have synthesized mutaprodenafil **1** and authenticated its veridical structure.

In conclusion, we isolated a new illegal sildenafil analogue

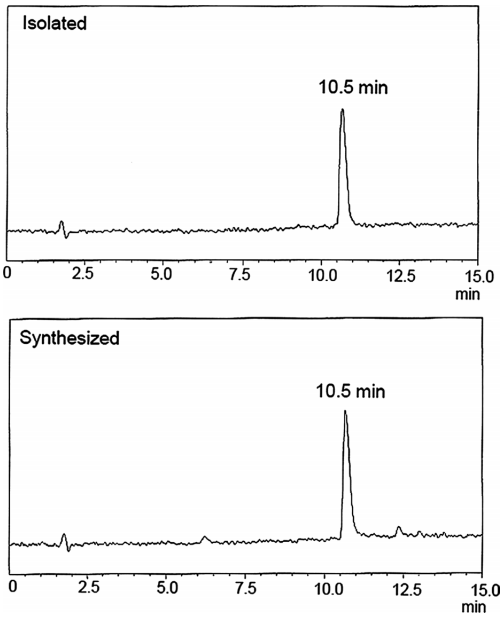

Fig. 3. LC-MS Spectra of the Isolated and Synthesized Compounds

named mutaprodenafil from a dietary supplement for ED and proposed that it is an aildenafil derivative with an imidazole moiety. Furthermore, we synthesized mutaprodenafil (**1**) from thioaildenafil to confirm its structure. We found that mutaprodenafil could be synthesized from thioaildenafil and commercially available 5-chloro-1-methyl-4-nitroimidazole. Mutaprodenafil is the first prodrug type analogue of the legal drugs used for the treatment of penile ED. In order to avoid illegal compounds being detected by routine inspections, counterfeit drug manufacturers synthesize new types of illegal compounds whenever possible. Normally, their detailed pharmacological activities are not examined. Thus, patients who unknowingly take dietary supplements adulterated with such compounds are at risk of suffering harmful side effects. Therefore, we must continuously monitor and prevent illegal drug manufacturing.

Acknowledgements This work was partly supported by a Health and Labour Science Research Grant from the Ministry of Health, Labour, and Welfare of Japan.

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19) ESI-MS *m*/*z*: 630.2272 ($[M+H]^+$, Calcd for $C_{27}H_{36}N_9O_5S_2$: 630.2275). IR (ATR) cm^{-1}: 1700, 1600, 1580, 1520, 1500, 1460. UV λ_{max} (MeOH) nm ($\log \varepsilon$): 218 (4.44), 240 (4.60), 283 (4.10), 297 (4.11), 335 (3.98, sh).
20) HPLC analysis was carried out on a Hypersil GOLD column (2.1 mm×100 mm, Thermo Fischer Scientific Inc., U.S.A.) with isocratic elution with 40% acetonitrile and 60% water solution added 0.1% formic acid. The flow rate was 0.2 ml/min. LC-MS-2010EV liquid chromatography-mass spectrometer (Shimadzu, Japan), equipped with an LC-20AB pumps, DGU-$20A_5$ degasser, SIL-20AC auto sampler, were employed.