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Short Note

2-[(2,6-Dimethylmorpholin-4-yl)methyl]-4-[(*E*)-2-{3-[(*E*)-2-{3-[(2,6-dimethylmorpholin-4-yl)methyl]-4-hydroxy-5-methoxyphenyl}ethenyl]-1*H*-pyrazol-5-yl}ethenyl]-6-methoxyphenol

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Abstract: A novel di-Mannich derivative of curcumin pyrazole, 2-[(2,6-dimethyl morpholin-4-yl)methyl]-4-[(*E*)-2-{3-[(*E*)-2-{3-[(2,6-dimethylmorpholin-4-yl)methyl]-4-hydroxy-5-methoxyphenyl}ethenyl]-1*H*-pyrazol-5-yl}ethenyl]-6-methoxyphenol (**2**), has been synthesized through a Mannich reaction of curcumin pyrazole (**1**), formaldehyde, and 2,6-dimethylmorpholine. The structure of the synthesized compound was confirmed on the basis of FTIR, ^{1}H-NMR, ^{13}C-NMR, 2D Heteronuclear Single-Quantum Correlation (HSQC) and 2D Heteronuclear Multiple Bond Correlation (HMBC), and mass spectral data. The water solubility was evaluated and the result showed that compound **2** was three times more soluble than that of curcumin pyrazole (**1**) and curcumin.

Keywords: di-Mannich derivative; curcumin pyrazole; 2,6-dimethylmorpholine; water solubility

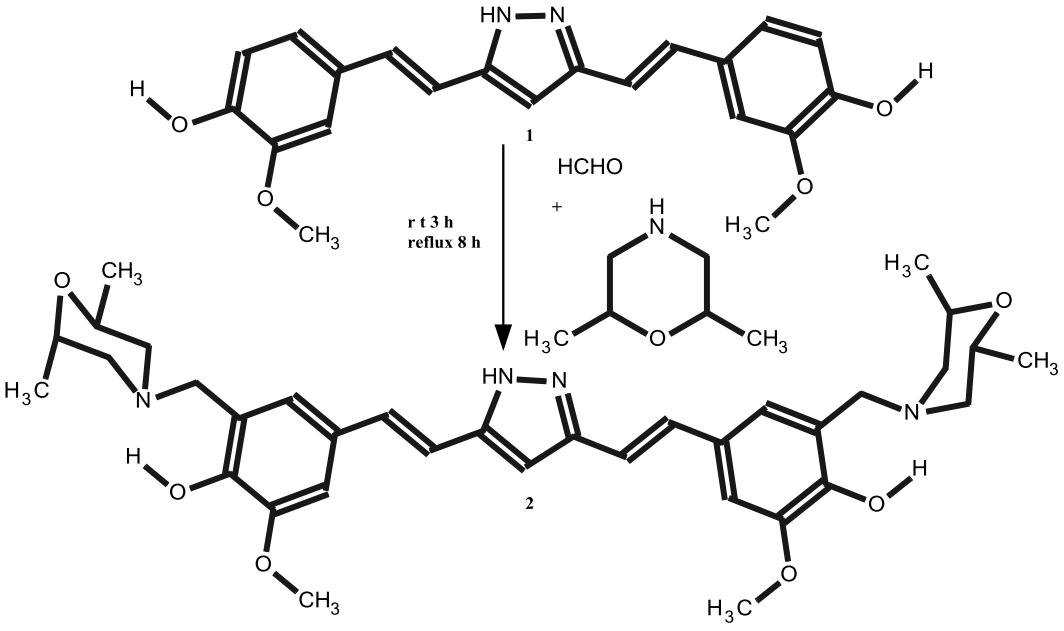
1. Introduction

Several curcuminoids bearing a pyrazole ring showed enhancement in the potency of their biological activity as anti-malarials, anti-proliferates, antiplatelet inhibitors, anti-inflammatory agents, antiviral agents, and NOS inhibitors, in comparison with curcumin [1–7]. Contrary to curcumin, which is rapidly degraded at physiological pH, they are stable and exhibit minimal metal chelating properties [8,9]. However, the water solubility prediction (log S) of curcumin pyrazole is not different enough from curcumin [10]. A Mannich reaction provides a suitable method to introduce an aminoalkyl substituent into a molecule. In several instances, the Mannich derivatives exhibit better activity than the corresponding parent analogs. Moreover, the presence of a Mannich side chain increases the solubility and hence the bioavailability of the drug molecule [11–13]. The substitution of aminomethyl into curcumin and into 1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one by means of the Mannich reaction was reported [14,15]. Herein, we report the aminomethylation of curcumin pyrazole (**1**) with 2,6-dimethylmorpholine as an amine reagent.

2. Results and Discussion

2.1. Chemistry

The starting material, curcumin pyrazole (**1**), was prepared according to the reported method [2]. The aminoalkylation of compound **1** was achieved through a Mannich reaction using formaldehyde solution and 2,6-dimethylmorpholine at a reflux temperature for 8 h, to give the title compound, 2-[(2,6-dimethylmorpholin-4-yl)methyl]-4-[(*E*)-2-{3-[(*E*)-2-{3-[(2,6-dimethylmorpholin-4-yl)methyl]-4-hydroxy-5-methoxyphenyl}ethenyl]-1*H*-pyrazol-5-yl}ethenyl]-6-methoxyphenol (**2**) (Scheme 1).



Scheme 1. Synthesis pathway of the title compound.

The high-resolution electrospray ionisation mass spectrum (HRESIMS) of the title compound **2** showed the molecular ion peak at m/z 619.3474 ([M + H]$^+$). The IR spectrum of the compound showed the disappearance of OH phenolic and NH pyrazole, and revealed CH aliphatic bands at 2823, 2937, and 2972 cm^{-1}. In the ^{1}H-NMR spectra of the compound, protons of OH phenolic and NH pyrazole appeared at δ 4.08 and 2.25 ppm as broad peaks, respectively. The appearance of the protons of the two phenyl rings as two singlet peaks at δ 6.72 ppm (2H) and 6.97 ppm (2H) indicate that the Mannich bases substituted H of the phenyl ring at the ortho position relative to OH groups. Protons of –CH_2– connecting N of the morpholine skeleton to the to the phenyl ring (N-CH_2-Ar) were observed at δ 3.67 ppm (4H, s). The of protons of CH_3, CH_2, and CH in the 2,6-dimethylmorpholine moiety and the methoxyphenyl appeared at more than one chemical shifts. These appearances indicate that the compound consists of a mixture of two isomers [16]. The Fourier-Transform (FT) NMR spectrum of material 2,6-dimethylmorpholine used in the reaction showed that the material consisted of a mixture of cis-form and trans-form [17], hence the obtained target compound consisted of two isomers [18]. In the 2D Heteronuclear Single-Quantum Correlation (HSQC) of the compound (Figure 1), the protons of methyl groups ($\underline{CH_3}$-CH-) appearing as two peaks at δ 1.15 and 1.24 ppm (12H, d, J = 6.0 Hz) correlated to two carbon peaks at δ 19.1 and 18.0 ppm. Protons of CH_2 connected to N (CH-$\underline{CH_2}$-N-) appearing as a doublet peak at δ 2.83 ppm (4H, d, J = 11 Hz) and as a triplet peak at δ 1.86 ppm (4H, t, J = 11 Hz) correlated to a carbon peak at δ 58.5 ppm, and protons of CH-O appearing as three multiplet peaks at δ 3.77, 3.71 (overlapped with the peak of N-CH_2-Ar), and 3.52 ppm (4H, m) correlated to two carbon peaks at δ 61,9 and 71,8 ppm. Meanwhile, protons of the methoxyphenyl group (CH_3O-Ar) appearing as two singlet peaks at δ 3.89 and 3.90 ppm (6H, s) correlated to a carbon peak δ 56.0 ppm. The ^{1}H-NMR, ^{13}C-NMR, 2D HSQC, and 2D Heteronuclear Multiple Bond Correlation (HMBC) spectra provided the expected number and types of protons and carbons of the aromatic, ethylenic, and pyrazole moieties of compound **2**. These values are in complete agreement with the structure assigned.

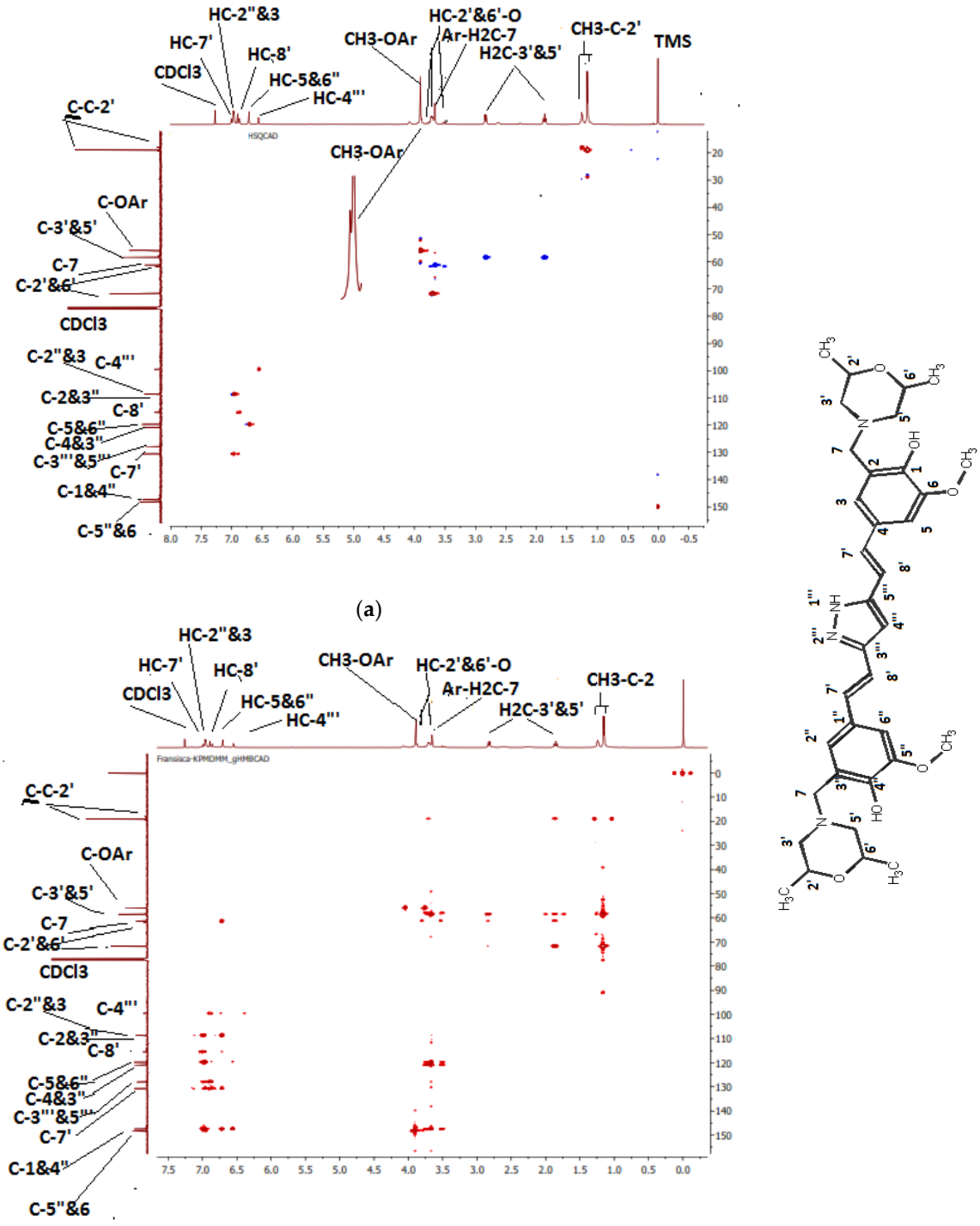


Figure 1. 2D Heteronuclear Single-Quantum Correlation (HSQC) (**a**) and 2D Heteronuclear Multiple Bond Correlation (HMBC) (**b**) of the title compound.

2.2. Solubility Evaluation

The solubility of compound **2** in water (pH 7.4) was three times more soluble than that of curcumin pyrazole **1** and curcumin (Table 1). The introduction of the aminoalkyl and alkoxy group to curcumin pyrazole increases the number of hydrogen bond formations and the potential to undergo protonation

of the compound in water. However, it is followed by the increase of many carbon atoms, so that the increase in solubility of compound 2 is not high [19].

Table 1. Water solubility of curcumin, curcumin pyrazole (**1**) and the title compound (**2**).

Compound	Molecular Formula	Molecular Weight	λ Max	Solubility [1] (mg/L)
Curcumin	$C_{21}H_{20}O_6$	368.12	428.0	3.2
1	$C_{21}H_{20}N_2O_4$	364.14	333.5	3.1
2	$C_{35}H_{46}N_4O_6$	618.35	333.0	9.2

[1] Values are the mean (n = 3).

3. Materials and Methods

3.1. General

All solvents, chemicals, and reagents were obtained commercially and used without purification. Purity test of the product was performed by the thin layer chromatographic (TLC) method on silica gel 60 F254 plates (Merck, Darmstadt, Germany). Melting points were determined in the capillary tube using a melting point apparatus (Stuart Scientific, Bibby Sterilin, Staffordshire, UK) and are uncorrected. Infrared (IR) spectra were recorded on an FTIR spectrophotometer (8400S, Shimadzu, Kyoto, Japan); NMR spectra were recorded on an NMR spectrometer (JEOL JNM 500, Peabody, MA, USA) at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard; coupling constant (J) are expressed in Hz; $CDCl_3$ were used as solvents. High-resolution mass spectrum was recorded on an ESI-TOP Water LCT Premier XE mass spectrometer (Waters Corp., Milford, MA, USA) in positive mode.

3.2. Synthesis of 2-[(2,6-Dimethylmorpholin-4-yl)methyl]-4-[(E)-2-{3-[(E)-2-{3-[(2,6-dimethylmorpholin-4-yl) methyl]-4-hydroxy-5-methoxyphenyl}ethenyl]-1H-pyrazol-5-yl}ethenyl]-6-methoxyphenol (**2**)

Curcumin pyrazole (728 mg, 2 mmol) was dissolved in 5 mL ethanol. The solution was then cooled in an ice bath and 2,6-dimethylmorpholine (862 µL, 7 mmol) was added slowly to the cold solution, after which 600 µL (7 mmol) of a solution of 37% aqueous formaldehyde were added dropwise. The mixture was allowed to remain at room temperature for 3 h, then was refluxed for about 8 h. The progress of the reaction was monitored by TLC. After the reaction was completed, solvent and other volatile components of the reaction mixture were evaporated until a dark gummy residue remained. This was taken up with 5 mL of methanol and evaporated again to a residue. The residue was then dissolved in 10 mL of warm methanol and poured slowly with constant stirring into about 400 mL of cold distilled water. The precipitate was allowed to settle overnight and the water with impurities was decanted from the product. The latter was filtered and washed with distilled water, and then dried at room temperature. The desired product was obtained as a brownish-yellow powder in a 73% yield, mp: 193–194 °C and R_f = 0.65 (EtOH:$CHCl_3$ = 1:12).

HRESIMS (m/z): found 619.3474 ($[M + H]^+$), calculated masses of $C_{35}H_{47}N_4O_6$: 619.3496 (error −3.6 ppm). FTIR (KBr) υ_{max} cm^{-1}: 2823, 2937 and 2972 (CH aliphatic), 1568 (C=N), 1490, 1468 (C=C), 1375 (C–N), 1252 (C-O phenolic), 1147 and 1084 (C-O-C). ^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.15 and 1.24 (12H, d, $\underline{CH_3}$-C-2′), 1.86 and 2.83 (8H, H_2C-3′ and H_2C-5′(N-C$\underline{H_2}$-CH-)), 3.67 (4H, s, Ar-H_2C-7 (N-CH_2-Ar)), 3.77, 3.71 and 3,52 (4H, m, HC-2′ and HC-6′ (CH_3-$\underline{CH}$(CH_2)-O)), 3.89 and 3.90 (6H, s, Ar-O-$\underline{CH_3}$), 6.55 (1H, s, HC-4‴ (CH-$_{Pyrazole}$)), 6.72 (2H, s, H_{Ar}), 6.87 (2H, d, J = 16 Hz, HC-8′ ($\underline{HC}$=$C_{ethenyl}$)), 6.97 (2H, s, H_{Ar}), 6.98 (2H, d, J = 16 Hz, HC-7′ ($\underline{HC}$=$C_{ethenyl}$)). ^{13}C-NMR (500 MHz, $CDCl_3$), δ/ppm: 18.0 and 19.1 (4C, C-2′-$\underline{C}H_3$), 56.1 (2C, $\underline{C}H_3$-O-Ar), 58.5 (4C, C-3′ and C-5′ (N-$\underline{C}H_2$-CH-)), 61.3 (2C, C-7 (N-C-Ar)), 61,9 and 71.8 (4C, C-2′ and C-6′ (C-O-C)), 99.7, (1C, C-4‴$_{pyrazole}$), 108.8 (2C, C-2″ and C-3$_{\underline{Ar}H}$), 108.9, (2C, C-2 and C-3″$_{ArH}$), 115.5 (2C, C-8′$_{ethenyl}$), 119.9 (2C, C-5 and C-6″$_{ArH}$), 121.0 (2C, C-1″ and C-4$_{Ar}$), 128.1 (2C, C-3‴ and C‴$_{pyrazole}$), 130.7

(2C, C-7′$_{ethenyl}$), 147.5 (2C, C-1 and C-4″$_{Ar\text{-}OH}$), and 148.3 (2C, C-5″ and C-6$_{Ar\text{-}O\text{-}C}$) [16]. The supporting FTIR, HRESIMS, ^{1}H-NMR, and ^{13}C-NMR, spectra (Figures S1–4) are presented in the supplementary material file.

3.3. Solubility Evaluation

The water solubility of the title compound (**2**), curcumin pyrazole (**1**), and curcumin were evaluated according to the method reported previously [20]. Five milligrams of the test compounds were separately dissolved in 5 mL solution of phosphate buffer pH 7.4 and vortexed. When the compound was completely dissolved, the solution was added to the compound until it was visibly saturated, then centrifuged at 3000 rpm for 10 min. The absorbance (A) of the supernatant was measured spectrophotometrically at λ maximum of the test compounds, respectively.

Supplementary Materials: The following are available online at www.mdpi.com/1422-8599/2017/3/M949. Figure S1: FTIR spectrum, Figure S2: HRESI Mass spectrum, Figure S3: ^{1}H-NMR and Figure S4: ^{13}C-NMR of the title compound.

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Author Contributions: H.H., I.I., and J.U. conceived and designed the experiments; J.U. performed the experiments; H.H., I.I., and J.U. analyzed the data; H.H. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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