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Short Note

N'^1,N'^4-bis(2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethylidene)succinohydrazide

Taishu Kawada [1], Katsuhito Kino [2,*], Yoshihiko Matsuzawa [2], Masayuki Morikawa [3], Yasuko Okamoto [3], Takanobu Kobayashi [1] and Yoshiyuki Tanaka [3]

[1] Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, 1314-1 Shido, Sanuki-shi, Kagawa 769-2193, Japan
[2] Faculty of Science and Engineering, Tokushima Bunri University, 1314-1 Shido, Sanuki-shi, Kagawa 769-2193, Japan
[3] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 180 Nishihama-Boji, Yamashiro-cho, Tokushima 770-8514, Japan
* Correspondence: kkino@kph.bunri-u.ac.jp; Tel.: +81-87-899-7290

Abstract: The title compound, N'^1,N'^4-bis(2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethylidene)succinohydrazide (**1**), was obtained by the reaction of formylmethylflavin and succinic acid dihydrazide. The product **1** was characterized by ^{1}H-NMR, ^{13}C-NMR, HRMS and UV.

Keywords: flavin; bisflavin; hydrazide; Schiff base

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1. Introduction

Flavins, chemical compounds containing an isoalloxazine ring in their molecule, are part of the complex redox centers of proton pumps and act as photoreceptors in phototropism [1]. Flavins are a large group of compounds involved in biological processes such as photosynthesis or phototropism [1]. They are found in food products, and they are present in enzymes and photoreceptors [1]. They are active chromophores in the processes of oxidation and reduction that take place in cells. It is well known that flavin acts as a cofactor for oxidoreductases, and this property is exploited in several biological and chemical oxidation reactions [1]. For example, it was previously reported that DNA in the presence of riboflavin is damaged under UV-A irradiation [2–6], with the ability to cause mutations associated with this [7–9]. To apply the oxidizing capacity of flavin for various purposes, formylmethylflavin (FMF) is used as a raw material for further derivatization [10–19].

Previous studies have reported bisflavin compounds having two flavins [20], and these molecules have a new catalytic activity or molecular recognition [21–24]. We report here the facile synthesis of a novel bisflavin. The title compound, N'^1,N'^4-bis(2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethylidene)succinohydrazide (**1**), was synthesized and characterized as follows.

2. Results and Discussion

Formylmethylflavin (FMF) was dissolved in acetic acid, a 50% (*v*/*v*) aqueous solution. Succinic acid dihydrazide was added to this solution, and the mixture was stirred for 30 min at room temperature (Figure 1), and the precipitation was formed. This precipitation, product **1**, was characterized as the desired product by NMR (^{1}H-NMR and ^{13}C-NMR), HRMS, and UV analysis (see Supplementary Files). The structure of **1** is shown in Figure 1.

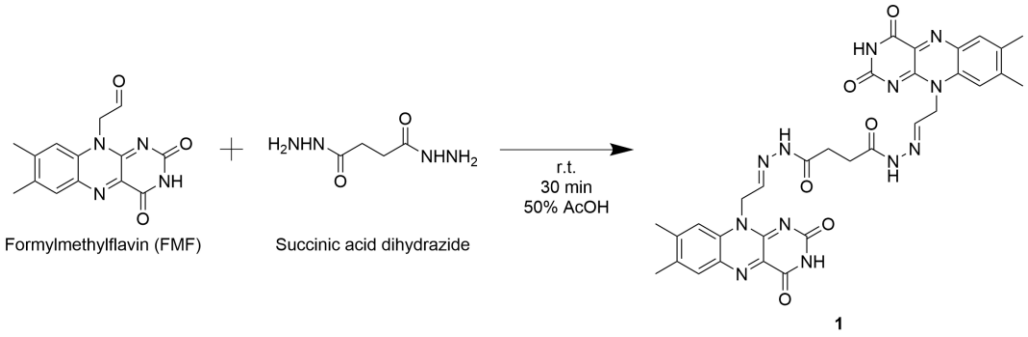

Figure 1. Synthesis of N'^1,N'^4-bis(2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethylidene)succinohydrazide (**1**).

The precipitate was dissolved in AcOH, and silica gel TLC showed a large spot (R_f = 0.40) and a small one (R_f = 0.80). Only the small spot was extracted from the silica gel with solvent, and the TLC analysis of the extracted sample showed both spots having R_f = 0.40 and 0.80, respectively. When the solvent was changed from AcOH to TFA, the spot with an R_f = 0.80 was larger than that with an R_f = 0.40. Therefore, the sample with the R_f = 0.80 spot may have contained a protonated bisflavin.

Bisflavin **1** was poorly soluble in many solvents and barely soluble in DMSO. The ^{1}H-NMR measurement of this compound was determined by supersaturation concentration. However, because the supersaturation state could not be maintained for the ^{13}C-NMR measurements over a long time, a mixture of AcOH-d_4 and TFA-*d* was used. The peak assignments were made based on a previous report [16] and predictions using ChemOffice. In addition, the ^{1}H-NMR measurements were confirmed by determining the exchangeable protons via the addition of D_2O and by COSY analysis. We also believe that the complexity of the spectrum is due to structural polymorphism. For the ^{13}C-NMR measurements, DEPT was also performed. Peak assignments are shown in the Materials and Methods section.

It has been previously reported that bisflavin makes π-π interactions, which change its redox state [24,25]. Therefore, bisflavin may have a different photoreactivity than riboflavin, which will be examined in future works. In addition, bisflavin has been investigated as a potential inhibitor of amyloid-β [26], and the synthesized product **1** might act as some kind of inhibitor. Finally, the simple synthetic method reported here will lead to the rapid synthesis of other bisflavins.

3. Materials and Methods

FMF was synthesized via known methods [13]. FMF (142 mg, 0.5 mmol) was dissolved in 9 M acetic acid (20 mL, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Succinic acid dihydrazide (36 mg, 0.25 mmol, Kanto Chemical Co., Inc., Tokyo, Japan) was added to this solution and the mixture was stirred for 30 min at room temperature. The resulting precipitate was filtered under a vacuum and washed with water and MeOH (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The precipitate yielded 101 mg (60%, based on succinic acid dihydrazide) of a light-yellow solid. The reactions were monitored by chromatography on TLC Silica Gel 60 glass plates (Merck KGaA, Darmstadt, Germany) using BuOH:AcOH:H_2O = 4:1:1 (BuOH; Kishida chemical Co., Ltd., Osaka, Japan) as an eluent. FMF had an R_f value of 0.82. The precipitate contained a product showing two R_f values of 0.40 and 0.80.

The melting/decomposition point was determined with "OptiMelt Automated Melting Point System" (MPA100; Stanford Research Systems, Sunnyvale, CA, USA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on an Avance 400 (Bruker BioSpin, Rheinstetten, Germany) using DMSO-d_6 (Acros Organics, Geel, Belgium) and AcOH-d_4:TFA-*d* = 10:1 (Cambridge isotope laboratories, Inc., Tewksbury, Massachusetts, USA) as the solvent. Mass spectra were recorded on a mass spectrometer "SYNAPT G2-Si HDMS" (Waters, Milford, MA, USA). UV absorption spectra (220 to 900 nm) were recorded on an Ultrospec

3100 pro (GE Healthcare Japan Corporation, Tokyo, Japan) in DMSO (Wako Pure Chemical Industries, Ltd.).

Melting point: >233 °C (decomposition)

UV (DMSO): λ_{max} 440 nm (log ε 4.29), 345 nm (4.10), 270 nm (4.70)

HRESIQ-TOFMS *m*/*z*: 677.2333 $[M-H]^-$ (calculated for $C_{32}H_{29}N_{12}O_6$, 677.2333)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.38 (s, 1H), 11.36 (s, 1H), 11.35 (s, 1H), 11.20 (s, 1H), 11.18 (s, 1H), 11.17 (s, 1H), 11.15 (s, 1H) (11.38–11.15; succinohydrazide 1,4-CONH and flavin 3-NH), 7.95 (s, 1H), 7.92 (s, 1H), 7.91 (s, 1H), 7.90 (s, 1H), 7.86 (s, 1H), 7.81 (s, 1H), 7.77 (s, 1H), 7.74 (s, 1H), 7.72 (s, 1H) (7.95–7.72; flavin H-6,9), 7.73 (t, 1H, *J* = 2.8 Hz), 7.37 (t, 1H, *J* = 3.6 Hz), 7.35 (t, 1H, *J* = 1.5 Hz) (7.73–7.35; flavin N^{10}-CH_2-CHN), 5.44 (d, 2H, *J* = 1.5 Hz), 5.41 (d, 2H, *J* = 3.6 Hz), 5.37 (d, 2H, *J* = 2.8 Hz) (5.44–5.37; flavin N^{10}-CH_2-CHN), 2.48–2.18 ppm (16H, flavin 7,8-CH_3 and CO-CH_2-CH_2-CO, overlap with the signal of DMSO)

^{13}C-NMR (100 MHz, AcOH-d_4:TFA-*d* = 10:1): δ 172.5 (succinohydrazide 1-CONH), 156.7 (flavin 2, 4-CO), 156.6 (flavin 2, 4-CO), 145.5 (flavin $C^{4a, 5a, 7, 8, 9a, 10a}$), 144.8 (flavin $C^{4a, 5a, 7, 8, 9a, 10a}$), 141.0 (flavin $C^{4a, 5a, 7, 8, 9a, 10a}$), 140.8 (flavin $C^{4a, 5a, 7, 8, 9a, 10a}$), 132.0 (flavin 6,9-CH), 129.2 (flavin $C^{4a, 5a, 7, 8, 9a, 10a}$), 128.0 (flavin $C^{4a, 5a, 7, 8, 9a, 10a}$), 82.1 (flavin N^{10}-CH_2-CH), 57.9 (flavin N^{10}-CH_2-CH), 53.6 (flavin 7,8-CH_3), 27.0 ppm (CO-CH_2-CH_2-CO)

Silica gel TLC (BuOH:AcOH:H_2O = 4:1:1) showed spots (UV, 254 nm) with an R_f = 0.40 and 0.80.

Supplementary Materials: The following supporting information can be downloaded online, Supplementary File S1: mol file; Supplementary File S2: ^{1}H-NMR; Supplementary File S3: ^{13}C-NMR; Supplementary File S4: HRMS; Supplementary File S5: UV-VIS.

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