

*Short Note*

***N'*-[2-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethylidene]-4-nitrobenzohydrazide**

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**Abstract:** The title compound, *N'*-[2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethylidene]-4-nitrobenzohydrazide (**1**), was obtained by the reaction of formylmethylflavin and *p*-nitrobenzohydrazide. The product **1** inhibited the DNA binding of nuclear factor- $\kappa$ B, and was characterized by  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR, electrospray ionization mass spectrometry, elemental analysis, IR and UV.

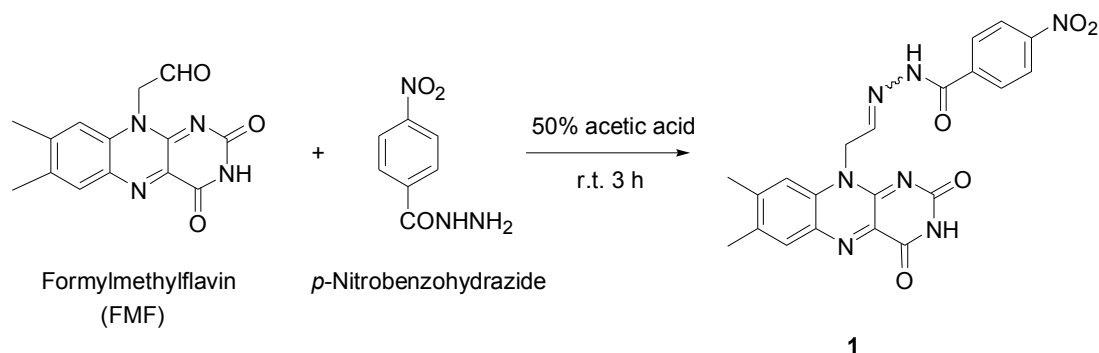
**Keywords:** flavin; Nuclear factor- $\kappa$ B; inhibitor; DNA binding

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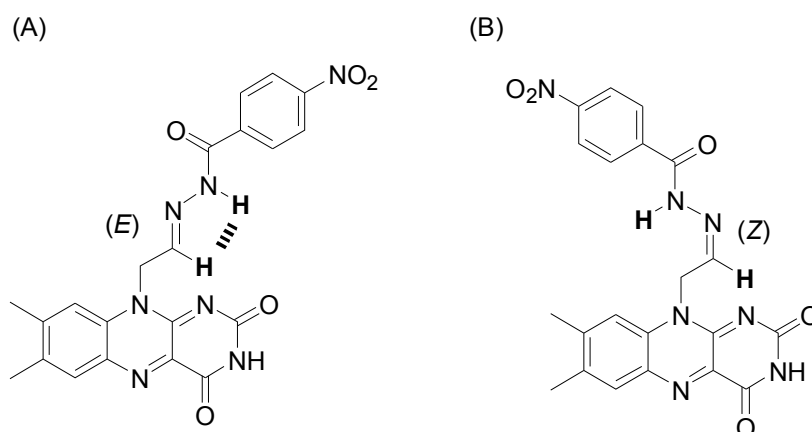
Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is a transcription factor which regulates the expression of proteins that play key roles in immunity and inflammation [1–4]. Consequently, NF- $\kappa$ B is considered a good target for the treatment of many diseases such as cancers and chronic inflammatory diseases [5–10]. To find NF- $\kappa$ B inhibitors, we screened our chemical library of small molecule compounds, and found triazine and flavin. Triazine derivatives directly inhibit the DNA binding of NF- $\kappa$ B [11,12]. Moreover, we synthesized several flavin derivatives [13,14] and tested their inhibitory effects on the DNA binding of NF- $\kappa$ B by performing electrophoretic mobility shift assays (EMSA) as previously reported [11,12]. The title compound, *N'*-[2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethylidene]-4-nitrobenzohydrazide (**1**; 100  $\mu\text{M}$ ), inhibited the DNA binding of NF- $\kappa$ B p50 to 23% of the amount of DNA-bound NF- $\kappa$ B p50 in the control (data not shown). Product **1** was synthesized and characterized as follows.

Formylmethylflavin (FMF) was dissolved in 50% acetic acid. *p*-Nitrobenzohydrazide was added to this solution and the mixture was stirred for 3 h at room temperature (Scheme 1). This compound was characterized as the product **1** by NMR ( $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR and NOESY), electrospray ionization mass spectrometry (ESI-MS), IR and UV (see Supporting Information). The structure of **1** is shown in Scheme 1. Two diastereomers of **1** were observed by NMR. Although the two diastereomers could not be separated by HPLC, they were confirmed by their NOESY spectrum. The  $^1\text{H}$ -NMR spectrum revealed that the ratio of the major diastereomer to the minor diastereomer is 1.84. A correlation between the flavin  $^{10}\text{N-CH}_2\text{CH}$  (8.05 ppm) and Ar 1-CONH (12.04 ppm) of the major diastereomer was detected in the NOESY spectrum. Therefore, the geometry of the major diastereomer was the *E* form (Figure 1A). In contrast, the geometry of the minor diastereomer was the *Z* form (Figure 1B) because no correlation was observed between the flavin  $^{10}\text{N-CH}_2\text{CH}$  (7.63 ppm) and Ar 1-CONH (11.95 ppm).

**Scheme 1.** Synthesis of *N*-[2-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)ethylidene]-4-nitrobenzohydrazide (**1**).



**Figure 1.** The geometric structures of the product **1**. (A) *E* configuration. (B) *Z* configuration.



## Experimental

FMF was synthesized by a known method [13]. FMF (28.4 mg, 0.1 mmol) was dissolved in 9 M acetic acid (2.8 mL, Wako Pure Chemical Industries, Ltd., Osaka, Japan). *p*-Nitrobenzohydrazide (18.1 mg, 0.1 mmol, Wako Pure Chemical Industries, Ltd.) was added to this solution and the mixture was stirred for 3 h at room temperature. The reactions were monitored by chromatography on TLC Silica Gel 60

glass plates (Merck KGaA, Darmstadt, Germany) using 2-propanol (Nacalai Tesque Inc., Kyoto, Japan) as eluent. FMF and *p*-Nitrobenzohydrazide had  $R_f$  values of 0.7 and 0.6, respectively. Instead, the solution contained a product showing an  $R_f$  of 0.4. The resulting precipitate was filtered under vacuum and washed with water. The precipitate yielded 40 mg (90%, based on FMF) of a light yellow solid.

The melting/decomposition point was determined with a cartridge heater (ATM-01; AS ONE Corporation, Osaka, Japan); the sample was placed in an aluminum pan, then heated. ATR/FT-IR absorption spectra were recorded on an FT/IR-6300 type A (JASCO Corporation, Tokyo, Japan). Elemental analyses were performed using PE2400 Series II CHNS/O Analyzer (PerkinElmer, Inc., Waltham, MA, USA).  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra were recorded on an Avance 700 (Bruker BioSpin, Rheinstetten, Germany) using  $\text{DMSO}-d_6$  (Kanto Chemical Co., Inc., Tokyo, Japan) as solvent. NOESY spectra were recorded on an Avance 400 (Bruker BioSpin) using  $\text{DMSO}-d_6$  (Kanto Chemical Co., Inc.) as solvent. Mass spectra were recorded on an APEX-Qe 9.4T AS (Bruker Daltonics, Billerica, 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:  $>273\text{ }^\circ\text{C}$  (decomposition).

UV (DMSO):  $\lambda_{\text{max}}$  445 nm ( $\log \epsilon$  4.10), 340 nm (3.97), 272 nm (4.63).

FT-IR (ATR):  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3173 (NH), 3076 (NH), 1719 (C=O), 1695 (C=O), 1651 (C=O).

HRESIFTMS  $m/z$ : 448.13839  $[\text{M}+\text{H}]^+$  (calculated for  $\text{C}_{21}\text{H}_{18}\text{N}_7\text{O}_5$ , 448.13639).

$^1\text{H}$ -NMR (700 MHz,  $\text{DMSO}-d_6$ ,  $E$ ):  $\delta$  12.04 (s, 1H, Ar 1-CONH), 11.40 (s, 1H, flavin 3-NH), 8.33 (d,  $J = 8.8$  Hz, 2H, ArH-3, 5), 8.05 (t,  $J = 3.2$  Hz, 1H, flavin  $N^{10}$ -CH $\underline{2}$ CH), 8.04 (d,  $J = 8.8$  Hz, 2H, ArH-2, 6), 7.96 (s, 1H, flavin H-6), 7.81 (s, 1H, flavin H-9), 5.54 (d,  $J = 3.2$  Hz, 2H, flavin  $N^{10}$ -CH $\underline{2}$ CH), 2.51 (flavin 8-CH $_3$ , overlap with the signal of DMSO), 2.41 (s, 3H, flavin 7-CH $_3$ ) ppm.

$^1\text{H}$ -NMR (700 MHz,  $\text{DMSO}-d_6$ ,  $Z$ ):  $\delta$  11.95 (s, 1H, Ar 1-CONH), 11.34 (s, 1H, flavin 3-NH), 7.78 (d,  $J = 8.4$  Hz, 2H, ArH-3, 5), 7.75 (s, 1H, flavin H-6), 7.63 (br t,  $J = 1.9$  Hz, 1H, flavin  $N^{10}$ -CH $\underline{2}$ CH), 7.55 (s, 1H, flavin H-9), 7.35 (d,  $J = 8.4$  Hz, 2H, ArH-2, 6), 5.40 (br d,  $J = 1.9$  Hz, 2H, flavin  $N^{10}$ -CH $\underline{2}$ CH), 2.37 (s, 3H, flavin 8-CH $_3$ ), 2.35 (s, 3H, flavin 7-CH $_3$ ) ppm.

$^{13}\text{C}$ -NMR (175 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  167.94, 161.32, 159.78, 159.71, 151.45, 150.18, 149.71, 149.25, 147.43, 146.73, 146.30, 145.82, 140.18, 138.70, 137.12, 136.82, 136.00, 135.60, 133.72, 133.48, 131.06, 131.00, 130.47, 129.35, 129.04, 123.59, 122.00, 116.62, 116.38, 45.33, 44.57, 20.70, 20.42, 18.75, 18.62 ppm.

Elemental analysis: Calculated for  $(\text{C}_{21}\text{H}_{17}\text{N}_7\text{O}_5) \cdot 1.2\text{H}_2\text{O}$ , C, 53.78%; H, 4.17%; N, 20.90%. Found: C, 53.77%; H, 4.11%; N, 21.06%.

Silica gel TLC (2-propanol) showed a spot (UV, 254 nm) with  $R_f = 0.4$ .

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## Author Contributions

K. Kino designed research; M. Morikawa, K. Kino and E. Asada analyzed the data; M. Morikawa performed identification using ESI-MS and UV; M. Morikawa and E. Asada performed synthesis and purification; E. Asada determined melting/decomposition point; K. Katagiri performed identification using FT-IR and elemental analysis; K. Mori-Yasumoto performed identification using NMR; T. Kobayashi performed EMSA; M. Morikawa, K. Kino, E. Asada, K. Katagiri, K. Mori-Yasumoto, M. Suzuki, T. Kobayashi and H. Miyazawa contributed in the preparing of the manuscript; M. Morikawa, K. Kino, K. Katagiri, K. Mori-Yasumoto, M. Suzuki, T. Kobayashi and H. Miyazawa contributed with valuable discussions and scientific input. All authors read and approved the final manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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