Anti-Inflammatory Activity of Geldanamycin and Its Derivatives in LPS-Induced RAW 264.7 Cells

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Abstract
Geldanamycin (1) had been isolated as a major compound from Streptomyces zerumbet W14; an endophyte of Zingiber zerumbet (L.) Smith. Two new geldanamycin derivatives; 17-(tryptamine)-17-demethoxygeldanamycin (2) and 17-(5’-methoxytryptamine)-17-demethoxygeldanamycin (3) were synthesized and their anti-inflammatory activity was evaluated in LPS-induced RAW 264.7 cells by investigating their effects on the inhibition of production of NO, PGE2, TNF-α, IL-1β, IL-6 and IL-10. The data obtained were consistent with the modulation of TNF-α, IL-1β, IL-6 and IL-10 production by these derivatives at concentration of 1 to 5 µg/ml. A similar effect was also observed when LPS-induced NO release and PGE2 production were tested. The inhibitory effects were shown in concentration-dependent manners. From the obtained results, it was concluded that two new geldanamycin derivatives possess anti-inflammatory activity on LPS-induced RAW 264.7 cells. They could be useful for the management of inflammatory diseases.

Keywords
Anti-Inflammatory Activity, Geldanamycin Derivatives, Streptomyces zerumbet, RAW 264.7 Cells

1. Introduction
Geldanamycin molecule composes of a benzoquinone structure and a planar
macrocyclic ansamycin bridge. The target of geldanamycin is heat shock protein 90 (Hsp90), it deactivates Hsp90 specifically to inhibit tumor growth or virus replication and also interfere normal function of Hsp90 in normal cells [1]. The synthesized a series of new 17- and 19-modified derivatives of geldanamycin to obtain new types of Hsp90 inhibitor with weaker toxicities and higher efficiencies have been attempting [2]-[9]. In our previous study, geldanamycin had been isolated as a major compound from Streptomyces zerumbet W14; an endophyte of Zingiber zerumbet (L.) Smith. It had in vitro anti-inflammatory activity on LPS-induced RAW 264.7 cells by inhibition of mRNA expression and production of inducible NO synthase (iNOS), tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) [10].

Tryptamine, a monoamine alkaloid containing an indole ring structure was derived by the decarboxylation of amino acid tryptophan. Tryptamine played important biomedical role as neurotransmitter and neuromodulator, vasoconstrictor and vasodilator, antibacterial, antifungal and antioxidant agents [11]. Its modification at different position led to many compounds of pharmacological importance. Recently, tryptamine-gallic acid hybrid molecule have been synthesized as SEGA (3a), which prevented non-steroidal anti-inflammatory drug-induced mitochondrial pathology, apoptosis, and gastropathy by blocking mitochondrial oxidative stress, chelating intramitochondrial free iron, and correcting mitochondrial pathology entering into mitochondria [12]. According to this effect, the invention of tryptamine-geldanamycin hybrids has been designed and evaluated the in vitro anti-inflammatory activity. The C17 methoxyl of geldanamycin molecule could allow for the introduction of various nucleophiles, hence geldanamycin from the beginning had been a popular template for semi-synthetic analogs [2]-[9].

In this study, a novel 17-substituted geldanamycin derivatives with tryptamine and 5-methoxytryptamine were synthesized and their anti-inflammatory activity was evaluated on LPS-activated macrophage RAW 264.7 cells by investigating their effects on the inhibition of production of NO, PGE₂, TNF-α, IL-1β, IL-6 and IL-10.

2. Materials and Methods

2.1. Extraction and Isolation

Streptomyces zerumbet W14 was isolated from the rhizome of Zingiber zerumbet (L.) Smith by surface-sterilization technique and identified by morphological, cultural, physiological and biochemical characteristics and also 16S rDNA gene sequencing as described by Taechowisan et al. [10]. This strain was grown on ISP-2 agar at 30°C for 14 days and then the culture medium was cut into small pieces that were extracted with ethyl acetate (3 x 500 ml). This organic solvent was pooled and then taken to dryness under rotary evaporation to give a dark brown solid. The solid was separated by column chromatography using silica gel
60 (Merck, 0.040 - 0.063 mm) and 30%, 50%, 75% and 100% of ethyl acetate in hexane as the eluent to give 17 main fractions (F1-F17). Fraction F13 (30.3 mg) gave a very prominent single spot of pure compound on TLC and was undertaken to investigate on NMR spectroscopy. The spectral data revealed this compound to be geldanamycin (1).

2.2. Synthesis of Geldanamycin Derivatives

The following experimental information was representative and describes the complete details of geldanamycin derivative synthesis.

Geldanamycin derivatives; 17-(tryptamine)-17-demethoxygeldanamycin (2) and 17-(5’-methoxytryptamine)-17-demethoxygeldanamycin (3) were synthesized from geldanamycin (Figure 1); To a solution of geldanamycin (0.84 g, 100 mmol) in dichloromethane (15 ml) at 25˚C was added tryptamine (0.29 g, 150 mmol) (Sigma-Aldrich) or 5’-methoxytryptamine (0.36 g, 150 mmol) (Sigma-Aldrich). The reaction mixture was stirred at 25˚C for 30 minutes before addition of saturated aqueous CaCl₂ (5 ml). The organic layer was removed and washed with saturated CaCl₂ solution (3 × 5 ml) and dried (Na₂SO₄). The mixture was filtered over Celite, rinsed with ethyl acetate, and concentrated under reduced pressure to give a dark purple solid. Purification by flash chromatography (silica, 60% ethyl acetate/hexanes) affords 17-(tryptamine)-17-demethoxygeldanamycin (1.01 g, 97 mmol, 96.8%) or 17-(5’-methoxytryptamine)-17-demethoxygeldanamycin (1.03 g, 95 mmol, 94.7%) (Figure 2).

2.3. Cell Culture and Sample Treatment

RAW 264.7 murine macrophage cell line was obtained from the Korean Cell Line Bank (Seoul, Korea). These cells were grown at 37˚C in DMEM medium supplement with 10% FBS, penicillin (100 units/ml), and streptomycin sulfate (100 µg/ml) in a humidified atmosphere of 5% CO₂. Cells were incubated with the compound at increasing concentrations and stimulated with LPS 1 µg/ml for 24 h.

2.4. MTT Assay for Cell Viability

Cytotoxicity studies were performed on a 96-well plate. RAW 264.7 cells were mechanically scraped and plated 2 × 10⁵ per well on 96-well plate containing 100 µl of DMEM medium with 10% FBS and incubated overnight. The compounds were dissolved in dimethylsulfoxide (DMSO) for stock solution. The DMSO concentrations in all assays did not exceed 0.1%. Twenty-four h after seeding, 100 µl new media or test compound was added, and the plates were incubated for 24 h. Cells were washed once before adding 50 µl FBS-free medium containing 5 mg/ml MTT. After 4 h of inoculation at 37˚C, the medium was discarded and the formazan blue, which formed in the cells, was dissolved in 50 µl DMSO. The optical density was measured at 450 nm. The concentration required for reducing the absorbance by 50% (IC₅₀) compared to the control cells was determined.
Figure 1. Geldanamycin chemical structure (1) and structures and synthesis of two derivatives; 17-(tryptamine)-17-demethoxygeldanamycin (2) and 17-(5'-methoxytryptamine)-17-demethoxygeldanamycin (3).

Figure 2. Silica gel TLC (GF<sub>254</sub>) of geldanamycin derivative synthesis; 17-(5'-methoxytryptamine)-17-demethoxygeldanamycin on 60% of ethyl acetate in hexane after color reaction with vanillin-sulfuric acid. (G: Geldanamycin, M: 5'-methoxytryptamine, N: New compound synthesis, N + G: New compound synthesis plus geldanamycin.

2.5. Nitrite Assay

Nitrite accumulation, an indicator of NO synthesis, was measured in the culture medium by Griess reaction [13]. Briefly, 100 µl of cell culture medium was
mixed with 100 µl of Griess reagent [equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthylethylenediamine-HCl] and incubated at room temperature for 100 min, and then the absorbance at 550 nm was measured in a microplate reader. Fresh culture medium was used as the blank in all experiments. The amount of nitrite in the samples was calculated from a sodium nitrite standard curve freshly prepared in culture medium.

2.6. PGE2, TNF-α, IL-1β, IL-6 and IL-10 Assay

PGE2, TNF-α, IL-1β, IL-6 and IL-10 level in macrophage culture medium were quantified by ELISA kits (PeproTech, NJ, USA) according to the manufacturer’s instructions.

2.7. Data Analysis

All calculations were done using the SPSS version 15 statistical software package for analysis of the data. The data were presented as means ± SEM values of three independent determinations and statistical analysis carried out using one-way ANOVA. Differences were considered to be of statistical significance at an error probability of less than 0.05 (p < 0.05).

3. Results

3.1. Synthesis of Geldanamycin Derivatives

The mass spectral data of geldanamycin and geldanamycin derivatives were carried out by 1H-NMR, 13C-NMR as following.

Compound (1): The IR spectrum displayed characteristic absorption bands of NH and OH stretches at n 3478, 3440, 3336 and 3297 cm⁻¹, CH stretch in CH₃ and CH₂ at n 2927 cm⁻¹, CH stretch in methyl ether at n 2853 cm⁻¹, C=O stretch in OCONH₂ at n 1729 cm⁻¹, C=O stretch in a,b-unsaturated amide at n 1701 cm⁻¹ and C=O stretches in quinone at n 1675 and 1652 cm⁻¹. The MS gave a [M+Na]⁺ ion at m/z 583.2571 which corresponded to the molecular formula C₂₉H₄₈N₂O₉ for the compound, indicating eleven double bond equivalents in the molecule. The structure was fully elucidated by ¹H NMR, ¹³C NMR spectroscopy, DEPT-135, and 2D NMR spectral studies. The ¹H-NMR spectral data (DMSO-d₆) of compound 1 showed four methyl groups at δH 0.75 (3H, brs), 0.97 (3H, brs), 1.62 (3H, s) and 1.93 (3H, s) three methoxy protons at δH 3.23 (3H, s), 3.24 (3H, s) and 3.96 (3H, s), five olefin protons at δH 5.50 (1H, d, J = 8.5 Hz), 5.81 (1H, br), 6.58 (1H, t), 6.95 (1H, d) and 7.04 (1H, s), four oxygenated methines at δH 3.09 (2H, br), 4.36 (1H, d, J = 7.6 Hz) and 4.88 (1H, br), amine hydrogen at δH 9.18 (1H, br), two methanefriyl group at δH 1.93 (1H, s) and 2.56 (1H) and methanediyl groups at δH 1.45 (2H, br), 2.18 (1H, dd, J = 4.8 and 12.5 Hz) and 2.43 (1H, dd, J = 9.9 and 12.5 Hz) ppm. The ¹³C-NMR spectrum exhibited 39 signals which were classified by the DEPT-135 and HMQC spectra as four methyl carbons at δC 12.8 (2-Me), 13.0 (8-Me), 13.4 (8-Me) and 23.9 (14-Me), three methoxy carbons at δC 56.5 (12-OMe), 57.1 (6-OMe), and 61.6
(17-OMe), five olefin carbons at δC 111.3 (C-19), 126.3 (C-4), 128.7 (C-3), 132.4 (C-9) and 138.7 (C-5), five quarternary olefin carbons at δC 128.7 (C-16), 129.1 (C-8), 133.2 (C-2), 140.1 (C-20) and 156.9 (C-17), two methanetriyl carbons at δC 27.1 (C-14) and 32.6 (C-10), two methanediyl carbons at δC 31.3 (C-13) and 32.2 (C-15), four oxygenated methines at δC 72.4 (C-11), 80.7 (C-12), 81.1 (C-7) and 82.3 (C-6), four carbonyl carbons at δC 156.6 (7-OCONH2), 169.7 (C-1), 183.6 (C-21) and 184.3 (C-18). The 1H-1H COSY spectrum revealed the connectivity, in DMSO-d6 from H-3 through H-4; H-4 through H-3 and H-5; H-5 through H-4 and H-6; H-6 through H-5 and H-7; H-7 through H-6; H-9 through H-10; H-12 through H-9 and 10-Me; 10-Me through H-10; H-12 through H-13; H-13 through H-12 and H-14; H-14 through H-13, 14-Me and H-15; 14-Me through H-14 and H-15 through H-14. The HMBC spectrum showed the following long-range correlations: 2-Me (δH 1.93) to C-1 (δC 169.7), C-2 (δC 133.2) to C-3 (δC 128.7) and C-4 (δC 126.3); H-4 (δH 6.58) to C-2 (δC 133.2) and C-6 (δC 82.3); H-6 (δH 4.36) to C-4 (δC 126.3), 6-OMe (δC 57.1); 6-OMe (δH 3.24) to C-6 (δC 82.3); H-7 (δH 4.88) to C-5 (δC 138.7), 7-OCONH2 (δC 156.6), C-9 (δC 132.4) and 8-Me (δC 13.0); 8-Me (δH 1.62) to C-7 (δC 81.1) and C-9 (δC 132.4); H-9 (δH 5.50) to C-7 (δC 81.1), and 8-Me (δC 13.0); 10-Me (δH 0.75) to C-9 (δC 132.4), C-10 (δC 32.6), and C-11 (δC 72.4); H-11 (δH 3.09) to 10-Me (δC 13.4); H-12 (δH 3.09) to 12-OMe (δC 56.5); 12-OMe (δH 3.23) to C-12 (δC 80.7); H-13 (δH 1.45) to 14-Me (δC 23.9); H-14 (δH 1.93) to C-12 (δC 80.7) and C-16 (δC 128.7); 14-Me (δH 0.97) to C-14 (δC 27.1) and C-15 (δC 32.2); H-15 (δH 2.18, 2.43) to C-13 (δC 31.3), C-14 (δC 27.1), C-16 (δC 128.7), C-17 (δC 156.9) and C-21 (δC 183.6); 17-OMe (δH 3.96) to C-17 (δC 156.9) and NH (δH 9.18) to C-1 (δC 169.7), C-19 (δC 111.3) and C-21 (δC 183.6). The spectral data revealed the compound 1 to be geldanamycin. Its 1H- and 13C-NMR spectral data of which were in good agreement with those of geldanamycin (Table 1) previously reported by Ōmura et al. [14] and Qin and Panek [15].

Compound (2): The MS gave a [M + Na]+ ion at m/z 711.3384 which corresponded to the molecular formula C38H48N4O8. The structure was fully elucidated by 1H NMR, 13C NMR spectroscopy, DEPT-135, and 2D NMR spectral studies. The 1H-NMR spectral data (CDCl3) of compound 2 showed four methyl groups at δH 0.93 (3H, d, J = 6.3 Hz), 0.99 (3H, d, J = 6.9 Hz), 1.80 (3H, s) and 2.02 (3H, s), two methoxy proton at δH 3.26 (3H, s) and 3.36 (3H, s), five olifin protons at δH 5.86 (1H, m), 5.89 (1H, m), 6.58 (1H, t, J = 12 Hz), 6.95 (1H, d, J = 12 Hz) and 7.24 (1H, s), four oxygenated methines at δH 3.45 (1H, m), 3.57 (1H, d, J = 9.0 Hz), 4.30 (1H, d, J = 9.9 Hz) and 5.18 (1H, s), nitrogenated methines at δH 3.77 (1H, m) and 3.91 (1H, m), five aromatic protons at δH 7.13 (1H, d, m), 7.14 (1H, m), 7.15 (1H, m), 7.40 (1H, d, J = 7.8 Hz) and 7.60 (1H, d, J = 7.8 Hz), amine hydrogen at δH 8.25 (1H, s), two methanetriyl groups at δH 1.77 (1H, m) and 2.74 (1H, m) and three methanediyl groups at δH 1.77 (2H, m), 2.40 (1H, m), 2.70 (1H, m) and 3.15 (1H, t, J = 6.6 Hz) ppm. The 13C-NMR spectrum exhibited 38 signals which were classified by the DEPT-135 and HMQC spectra as
Table 1. Comparison of the spectral data of the compound 1 and GDA<sup>a</sup>.

| No. | δ<sup>C</sup> compound 1 | δ<sup>C</sup> GDA | δ<sup>H</sup> compound 1 | δ<sup>H</sup> GDA | HMBC (H→C) | COSY | NOESY |
|-----|------------------------|---------------|---------------------|---------------|------------|------|-------|
| 1   | 169.7 C                | 169.1         | -                   | -             | -          | -    | -     |
| 2   | 133.2 C                | 133.2         | -                   | -             | -          | -    | -     |
| 2-Me | 12.8 CH<sub>3</sub> | 12.2          | 1.93 s              | 1.91, s       | 1, 2, 3, 4 | -    | -     |
| 3   | 128.7 CH               | 128.4         | 6.95 d              | 6.95, d       | -          | 4    | NH, 4, 6, 7 |
| 4   | 126.3 CH               | 125.7         | 6.58 t              | 6.56, t       | 2, 6       | 3, 5 | 3, 5  |
| 5   | 138.7 CH               | 137.8         | 5.81 br             | 5.80, t       | -          | 4, 6 | 4, 6  |
| 6   | 82.3 CH                | 81.6          | 4.36 d (7.6)        | 4.34, d       | 4, 6-OMe   | 5, 7 | 3, 5, 7 |
| 6-OMe | 57.1 CH<sub>3</sub> | 56.0          | 3.24 s              | 3.22, s       | 6          | -    | -     |
| 7   | 81.1 CH                | 80.6          | 4.88 br             | 4.86, br      | 5, 7-OCNH<sub>2</sub>, 9, 8-Me | 6 | 3, 6, 9 |
| 7-OCNH<sub>2</sub> | 156.6 C  | 156.0  | -                | 6.45, br      | -          | -    | -     |
| 8   | 129.1 C                | 132.6         | -                   | -             | -          | -    | -     |
| 8-Me | 13.0 CH<sub>3</sub> | 12.5          | 1.62 s              | 1.61, s       | 7, 9       | -    | 10    |
| 9   | 132.4 CH               | 131.9         | 5.50 d (8.5)        | 5.51, d       | 7, 8-Me    | 10   | 7     |
| 10  | 32.6 CH                | 32.1          | 2.56               | 3.61, m       | -          | 9, 10-Me | 8-Me, 10-Me, 11, 12 |
| 10-Me | 23.4 CH<sub>3</sub> | 23.3          | 0.75 d (6.8)       | 0.97, d       | 9, 10, 11  | 10   | 10, 11, 12 |
| 11  | 72.4 CH                | 71.9          | 3.09 br             | 3.29, s       | 10-Me      | -    | 10, 10-Me, 13, 14 |
| 11-OH | -                    | -             | -                   | -             | -          | -    | -     |
| 12  | 80.7 CH                | 80.2          | 3.09 br             | 3.07, m       | 12-OMe     | 13   | 10, 10-Me, 13, 14 |
| 12-OMe | 56.5 CH<sub>3</sub> | 56.6          | 3.23 s              | 3.23, s       | 12         | -    | -     |
| 13  | 31.3 CH<sub>2</sub>   | 31.0          | 1.45 br             | 1.45, m       | 14-Me      | 12, 14| 11, 12, 14 |
| 14  | 27.1 CH                | 26.6          | 1.93 s              | 1.91, br      | 12, 16     | 13, 14-Me, 15a, 15b | 13 |
| 14-Me | 23.9 CH<sub>3</sub> | 23.0          | 0.97 br             | 0.76, d       | 14, 15     | 14   | -     |
| 15a | 32.2 CH<sub>2</sub>  | 31.7          | 2.43 dd (12.5, 9.9) | 2.42, m       | 13, 14, 16, 17, 21 | 14, 15b | - |
| 15b | 32.2 CH<sub>2</sub>  | 31.7          | 2.18 dd (12.5, 4.8) | 13, 14, 16, 17, 21 | 14, 15a   | -    | -     |
| 16  | 128.7 C                | 128.1         | -                   | -             | -          | -    | -     |
| 17  | 156.9 C                | 156.4         | -                   | -             | -          | -    | -     |
| 17-OMe | 61.6 CH<sub>3</sub> | 61.0          | 3.96 s              | 3.93, s       | 17         | -    | -     |
| 18  | 184.3 C                | 183.6         | -                   | -             | -          | -    | -     |
| 19  | 111.3 CH               | 110.9         | 7.04 s              | 7.02, s       | -          | -    | -     |
| 20  | 140.1 C                | 139.6         | -                   | -             | -          | -    | -     |
| 21  | 183.6 C                | 183.1         | -                   | -             | -          | -    | -     |
| NH  | -                      | -             | 9.18, NH, br        | 9.14, NH, br  | 1, 19, 21 | -    | 3     |

<sup>a</sup>GDA, geldanamycin (data from Ōmura et al. [14]). <sup>1</sup>H and <sup>13</sup>C-NMR assignments for compound 1 [<sup>1</sup>H (400 MHz), <sup>13</sup>C-NMR (100 MHz), DMSO-<sup>d6</sup>, J = Hz]; Geldanamycin [<sup>1</sup>H (400 MHz),<sup>13</sup>C-NMR (100 MHz), DMSO-<sup>d6</sup>, J = Hz].
four methyl carbons at $\delta_C$ 12.3 (10-Me), 12.5 (2-Me), 12.7 (8-Me) and 22.8 (14-Me), two methoxy carbons at $\delta_C$ 56.7 (12-OMe) and 57.1 (6-OMe), five olefin carbons at $\delta_C$ 108.0 (C-19), $\delta_C$ 126.5 (C-4), $\delta_C$ 126.9 (C-3), $\delta_C$ 133.8 (C-9) and $\delta_C$ 135.8 (C-5), five quaternary olefin carbons at $\delta_C$ 108.6 (C-16), 132.7 (C-8), 135.0 (C-2), $\delta_C$ 141.4 (C-20) and 144.9 (C-17), four oxygenated methines at $\delta_C$ 72.6 (C-11), 81.2 (C-6), 81.5 (C-12) and 81.7 (C-7), nitrogenated methines at 45.7 (C-22), five aromatic carbons at $\delta_C$ 111.5 (C-28), 118.5 (C-31), 119.8 (C-29), 122.5 (C-25) and 125.6 (C-30), three quaternary aromatic carbons at $\delta_C$ 111.3 (C-24), 126.8 (C-27) and 136.6 (C-26), two methanetriyl carbons at $\delta_C$ 28.5 (C-14) and 32.3 (C-10), three methanediyl carbons at $\delta_C$ 25.75 (C-23), 34.4 (C-15) and 35.0 (C-13) and four carbonyl quaternary carbons at $\delta_C$ 156.1 (7-OCONH$_2$), 168.4 (C-1), 180.5 (C-21) and 183.8 (C-18) ppm.

Compound (3): The MS gave a [M + Na]$^+$ ion at m/z 741.3482 which corresponded to the molecular formula C$_{39}$H$_{50}$N$_4$O$_9$. The structure was fully elucidated by $^1$H-NMR, $^{13}$C-NMR spectroscopy, DEPT-135, and 2D NMR spectral studies.

Table 2. The $^1$H-NMR spectral data (CDCl$_3$) of compound 3 showed four methyl groups at $\delta_H$ 0.94 (3H, d, $J = 6.3$ Hz), 1.00 (3H, d, $J = 6.9$ Hz), 1.80 (3H, s) and 2.02 (3H, s), three methoxy protons at $\delta_H$ 5.86 (1H, m), 5.89 (1H, m), 6.57 (1H, t, $J = 11.4$ Hz), 6.95 (1H, d, $J = 11.4$ Hz) and 7.24 (1H, s), four oxygenated methines at $\delta_H$ 3.45 (1H, m), 3.57 (1H, m), 4.31 (1H, d, $J = 9.9$ Hz) and 5.18 (1H, s), nitrogenated methines at $\delta_H$ 3.92 (1H, m) and 3.76 (1H, m), four aromatic protons at $\delta_H$ 6.90 (1H, d, $J = 9.0$ Hz) and 7.00 (1H, s) and 7.09, amine hydrogen at $\delta_H$ 6.47 (1H, t, $J = 6.0$ Hz), 8.14 (1H, s) and 9.71 (1H, s), two methanetriyl groups at $\delta_H$ 1.77 (1H, m), 2.48 (1H, m) and 2.68 (1H, m) and three methanediyl groups at $\delta_H$ 1.77 (2H, m), 2.44 (1H, m), 2.68 (1H, m) and 2.74 (1H, m) ppm. The $^{13}$C-NMR spectrum exhibited 39 signals which were classified by the DEPT-135 and HMQC spectra as four methyl carbons at $\delta_C$ 12.4 (10-Me), 12.6 (2-Me), 12.8 (8-Me) and 23.0 (14-Me), three methoxy carbons at $\delta_C$ 56.0 (29-OMe), 56.7 (12-OMe) and 57.1 (6-OMe), five olefin carbons at $\delta_C$ 108.7 (C-19), $\delta_C$ 126.6 (C-4), $\delta_C$ 107.0 (C-3), $\delta_C$ 133.8 (C-9) and $\delta_C$ 135.8 (C-5), five quaternary olefin carbons at $\delta_C$ 108.5 (C-16), 132.8 (C-8), 135.0 (C-2), $\delta_C$ 141.4 (C-20) and 145.0 (C-17), four oxygenated methines at $\delta_C$ 72.7 (C-11), 81.3 (C-6), 81.6 (C-12) and 81.8 (C-7), nitrogenated methines at 45.6 (C-22), four aromatic carbons at $\delta_C$ 100.4 (C-28), 112.3 (C-30), 112.7 (C-31) and 123.4 (C-25), four quaternary aromatic carbons at $\delta_C$ 111.0 (C-24), 127.3 (C-27), 131.8 (C-26) and 154.3 (C-29), two methanetriyl carbons at $\delta_C$ 28.6 (C-14) and 32.4 (C-10), three methanediyl carbons at $\delta_C$ 25.8 (C-23), 34.5 (C-15) and 35.2 (C-13) and four carbonyl carbons at $\delta_C$ 156.1 (7-OCONH$_2$), 168.4 (C-1), 180.6 (C-21) and 183.9 (C-18) ppm. The $^1$H-$^1$H COSY spectrum revealed the connectivity in CDCl$_3$ from H-3 through H-4; H-4 through H-3 and H-5; H-5 through H-4 and H-6; H-6 through H-5 and H-7; H-7 through H-6; H-9 through H-10; H-10 through H-9, 10-Me and H-11; 10-Me through H-10; H-11 through H-10 and H-12; H-12 through H-11 and H-13; H-13 through H-12; H-14 through 14-Me and 15; 14-Me through
H-14; H-15 through H-14; H-22 through 22-NH and H-23; H-23 through H-22; H-30 through H-31 and H-31 through H-30. The HMBC spectrum showed the following long-range correlations; 14-Me (δH 0.94) to C-13 (δC 35.2), C-14 (δC 28.6) and C-15 (δC 34.5); 10-Me (δH 1.00) to C-9 (δC 133.8), C-10 (δC 32.4) and C-11 (δC 72.7); H-13 (δH 1.77) to C-11 (δC 72.7), C-12 (δC 81.6) and C-14 (δC 28.6); H-14 (δH 1.77) to C-12 (δC 81.6) and C-13 (δC 35.2); 8-Me (δH 1.80) to C-7 (δC 81.8), C-8 (δC 132.8) and C-9 (δC 133.8); 2-Me (δH 2.02) to C-1 (δC 168.4), C-2 (δC 135.0) and C-3 (δC 127.0); H-15 (δH 2.44, 2.68) to C-13 (δC 35.2), C-14 (δC 28.6), 14-Me (δC 23.0), C-16 (δC 108.5), C-17 (δC 145.0) and C-21 (δC 180.6); H-10 (δH [2.74] 2.73 - 2.78) to C-8 (δC 132.8), C-9 (δC 133.8) and 10-Me (δC 12.4); H-23 (δH 3.11) to C-22 (δC 45.6), C-24 (δC 111.0) and C-25 (δC 123.4); 6-OMe (δH 3.27) to C-6 (δC 81.3); 12-OMe (δH 3.36) to C-12 (δC 81.6); H-12 (δH 3.45) to C-10 (δC 32.4), C-11 (δC 72.7), 12-OMe (δC 56.7) and C-14 (δC 28.6); H-11 (δH [3.57] 3.57 - 3.60) to C-9 (δC 133.8), C-10 (δC 32.4), 10-Me (δC 12.4) and C-12 (δC 81.6); H-22 (δH 3.76, 3.92) to C-23 (δC 25.8) and C-24 (δC 111.0); 29-OMe (δH 3.87) to C-29 (δC 154.3); H-6 (δH 4.31) to C-4 (δC 126.6) and 6-OMe (δC 57.1); H-7 (δH 5.18) to C-5 (δC 135.8), 7-OCONH₂ (δC 156.1), C-8 (δC 132.8), 8-Me (δC 12.8) and C-9 (δC 133.8); H-5 (δH [5.86] 5.85 - 5.87) to C-3 (δC 127.0), C-4 (δC 126.6), C-6 (δC 81.3) and C-7 (δC 81.8); H-9 (δH 5.89*) to C-7 (δC 81.8), 8-Me (δC 12.8), 10-Me (δC 12.4) and C-11 (δC 72.7); 22-NH (δH 6.47) to C-16 (δC 108.5), C-18 (δC 183.9), C-22 (δC 45.6) and C-23 (δC 25.8); H-4 (δH 6.57) to C-2 (δC 135.0), C-3 (δC 127.0) and C-5 (δC 135.8); H-31 (δH 6.90) to C-26 (δC 131.8), C-27 (δC 127.3) and C-29 (δC 154.3); H-3 (δH 6.95) to C-1 (δC 168.4), C-2 (δC 135.0), 2-Me (δC 12.6), C-4 (δC 126.6) and C-5 (δC 135.8); H-28 (δH 7.00) to C-24 (δC 111.0), C-26 (δC 131.8), C-29 (δC 154.3) and C-30 (δC 112.3); H-25 (δH 7.09*) to C-23 (δC 25.8), C-24 (δC 111.0), C-26 (δC 131.8) and C-27 (δC 127.3); H-19 (δH 7.24) to C-17 (δC 145.0) and C-21 (δC 180.6); H-30 (δH 7.29) to C-29 (δC 154.3); 25-NH (δH 8.14) to C-24 (δC 111.0), C-25 (δC 123.4), C-26 (δC 131.8) and C-27 (δC 127.3); 1-NH (δH 9.17) to C-1 (δC 168.4), C-19 (δC 108.7) and C-21 (δC 180.6)

Table 2. Comparison of the spectral data of the compound 2 and compound 3.

| No. | δC compound 2 | δC compound 3 | δH compound 2 | δH compound 3 |
|-----|----------------|----------------|----------------|----------------|
| 1   | 168.4 C        | 168.4 C        | -              | -              |
| 2   | 135.0 C        | 135.0 C        | -              | -              |
| 2-Me| 12.5 CH₃       | 12.6 CH₃       | 2.02 s         | 2.02 s         |
| 3   | 126.9 CH       | 127.0 CH       | 6.95 d (12)    | 6.95 d (11.4)  |
| 4   | 126.5 CH       | 126.6 CH       | 6.58 t (12)    | 6.57 t (11.4)  |
| 5   | 135.8 CH       | 138.7 CH       | 5.86 m         | 5.86 m         |
| 6   | 81.2 CH        | 81.3 CH        | 4.30 d (9.9)   | 4.31 d (9.9)   |
| 6-OMe| 57.1 CH₃      | 57.1 CH₃      | 3.26 s         | 3.27 s         |
| 7   | 81.7 CH        | 81.1 CH        | 5.18 s         | 5.18 s         |
|     | 1H (300 MHz) | 13C-NMR (75 MHz) | CDCl3, \( J = \text{Hz} \) |
|-----|--------------|------------------|--------------------------|
| 7-OCO \( \text{NH}_2 \) | 156.1 C | 156.1 C | - |
| 8 | 132.7 C | 132.8 C | - |
| 8-Me | 12.7 CH\(_3\) | 12.8 CH\(_3\) | 1.80 s | 1.80 s |
| 9 | 133.8 CH | 133.8 CH | 5.89 m | 5.89 |
| 10 | 32.3 CH | 32.4 CH | 2.74 m | 2.74 m |
| 10-Me | 12.3 CH\(_3\) | 12.4 CH\(_3\) | 0.99 d (6.9) | 1.00 d (6.9) |
| 11 | 72.6 CH | 72.7 CH | 3.57 d (9) | 3.57 m |
| 11-OH | - | - | - |
| 12 | 81.5 CH | 81.6 CH | 3.45 m | 3.45 m |
| 12-OMe | 56.7 OCH\(_3\) | 56.7 OCH\(_3\) | 3.36 s | 3.36 s |
| 13 | 35.0 CH\(_2\) | 35.2 CH\(_2\) | 1.77 m | 1.77 m |
| 14 | 28.5 CH | 28.6 CH | 1.77 m | 1.77 m |
| 14-Me | 22.8 CH\(_3\) | 23.0 CH\(_3\) | 0.93 d (6.3) | 0.94 d (6.3) |
| 15a | 34.4 CH\(_2\) | 34.5 CH\(_2\) | 2.70 | 2.68 m (12.5, 9.9) |
| 15b | - | - | - |
| 16 | 108.6 C | 108.5 C | - |
| 17 | 144.9 C | 145.0 C | - |
| 17-OMe | - | - | - |
| 18 | 183.8 C | 183.9 C | - |
| 19 | 108.7 CH | 108.7 CH | 7.24 s | 7.24 s |
| 20 | 141.4 C | 141.4 C | - |
| 21 | 180.5 C | 180.6 C | - |
| 22a | 45.7 CH\(_2\) | 45.6 CH\(_2\) | 3.91 m | 3.92 m |
| 22b | - | - | - |
| 23 | 25.75 CH\(_2\) | 25.8 CH\(_2\) | 3.15 t (6.6) | 3.11 t (6.6) |
| 24 | 111.3 C | 111.0 C | - |
| 25 | 122.5 CH | 123.4 CH | 7.13 m | 7.09 |
| 26 | 136.6 C | 131.8 C | - |
| 27 | 126.8 C | 127.3 C | - |
| 28 | 111.5 CH | 100.4 CH | 7.40 d (7.8) | 7.00 s |
| 29 | 119.8 CH | 154.3 C | 7.13 m | - |
| 29-OMe | - | 56.0 OCH\(_3\) | - | 3.87 s |
| 30 | 125.6 CH | 112.3 CH | 7.15 m | 7.29 d (9) |
| 31 | 118.5 CH | 112.7 CH | 7.60 d (7.8) | 6.90 d (9) |
| 1-NH | - | - | 9.17 s | 9.17 s |
| 22-NH | - | - | 6.47 brs (6.0) | 6.47 t (6.0) |
| 25-NH | - | - | 8.25 s | 8.14 s |

\(^{1}\)H and \(^{13}\)C-NMR assignments for the compound 2 and compound 3; \(^{1}\)H (300 MHz), \(^{13}\)C-NMR (75 MHz), CDCl\(_3\), \( J = \text{Hz} \).
Table 3. Comparison of the spectral data of the compound 3 and compound 1.

| No. | δ_C compound 3 | δ_C compound 1 | δ_H compound 3 | δ_H compound 1 | HMBC (H→C) | COSY |
|-----|----------------|---------------|----------------|----------------|-------------|------|
| 1   | 168.4 C        | 169.7 C       | -              | -              | -           | -    |
| 2   | 135.0 C        | 133.2 C       | -              | -              | -           | -    |
| 2-Me| 12.6 CH_3      | 12.8 CH_3     | 2.02 s         | 1.93 s         | 1, 2, 3     | -    |
| 3   | 127.0 CH       | 128.7 CH      | 6.95 d (11.4)  | 6.95 d         | 1, 2, 2-Me, 4, 5 | 4    |
| 4   | 126.6 CH       | 126.3 CH      | 6.57 t (11.4)  | 6.58 t         | 2, 3, 5     | 3, 5 |
| 5   | 135.8 CH       | 138.7 CH      | 5.86 m         | 5.81 brs       | 3, 4, 6, 7  | 4, 6 |
| 6   | 81.3 CH        | 82.3 CH       | 4.31 d (9.9)   | 4.36 d (7.6)   | 4, 6-OMe    | 5, 7 |
| 6-OMe| 57.1 CH_3      | 57.1 CH_3     | 3.27 s         | 3.24 s         | 6           | -    |
| 7   | 81.8 CH        | 81.1 CH       | 5.18 s         | 4.88 brs       | 5, 7-OCONH_2, 8, 8-Me, 9 | 6    |
| 7-OCONH_2| 156.1 C      | 156.6 C       | -              | -              | -           | -    |
| 8   | 132.8 C        | 129.1 C       | -              | -              | -           | -    |
| 8-Me| 12.8 CH_3      | 13.0 CH_3     | 1.80 s         | 1.62 s         | 7, 8, 9     | -    |
| 9   | 133.8 CH       | 132.4 CH      | 5.89*          | 5.50 d (8.5)   | 7, 8-Me, 10-Me, 11 | 10  |
| 10  | 32.4 CH        | 32.6 CH       | 2.74 m         | 2.56           | 8, 9, 10-Me | 9, 10-Me, 11 |
| 10-Me| 12.4 CH_3      | 13.4 CH_3     | 1.00 d (6.9)   | 0.75 d (6.8)   | 9, 10, 11   | 10   |
| 11  | 72.7 CH        | 72.4 CH       | 3.57 m         | 3.09 brs       | 9, 10, 10-Me, 12 | 10, 12 |
| 11-OH| -             | -             | -              | -              | -           | -    |
| 12  | 81.6 CH        | 80.7 CH       | 3.45 m         | 3.09 brs       | 10, 11, 12-OMe, 14 | 11, 13 |
| 12-OMe| 56.7 OCH_3     | 56.5 CH_3     | 3.36 s         | 3.23 s         | 12          | -    |
| 13  | 35.2 CH_3      | 31.3 CH_2     | 1.77 m         | 1.45 brs       | 11, 12, 14  | 12   |
| 14  | 28.6 CH        | 27.1 CH       | 1.77 m         | 1.93 s         | 12, 13      | 14-Me, 15b |
| 14-Me| 23.0 CH_3      | 23.9 CH_3     | 0.94 d (6.3)   | 0.97 brs       | 13, 14, 15  | 14   |
| 15a | 34.5 CH_3      | 32.2 CH_2     | 2.68 m         | 2.43 dd (12.5, 9.9) | 13, 14, 14-Me, 16, 17, 21 | 15b |
| 15b | 34.5 CH_3      | 32.2 CH_2     | 2.44 m         | 2.18 dd (12.5, 4.8) | 14, 16, 17, 21 | 14, 15a |
| 16  | 108.5 C        | 128.7 C       | -              | -              | -           | -    |
| 17  | 145.0 C        | 156.9 C       | -              | -              | -           | -    |
| 17-OMe| -             | 61.6 CH_3     | -              | 3.96 s         | -           | -    |
| 18  | 183.9 C        | 184.3 C       | -              | -              | -           | -    |
| 19  | 108.7 CH       | 111.3 CH      | 7.24 s         | 7.04 s         | 17, 21      | -    |
| 20  | 141.4 C        | 140.1 C       | -              | -              | -           | -    |
| 21  | 180.6 C        | 183.6 C       | -              | -              | -           | -    |
| 22a | 45.6 CH_3      | -             | 3.92 m         | -              | 23, 24      | 22b, 22-NH, 23 |
| 22b | 45.6 CH_3      | -             | 3.76 m         | -              | 23, 24      | 22a, 22-NH, 23 |
| 23  | 25.8 CH_2      | -             | 3.11 t (6.6)   | -              | 22, 24, 25  | 22a, 22b |
| 24  | 111.0 C        | -             | -              | -              | -           | -    |
| 25  | 123.4 CH       | -             | 7.09*          | -              | 23, 24, 26, 27 | -    |
3.2. Effects of Geldanamycin Derivatives on Cell Viability in RAW 264.7 Cells

In this study, geldanamycin and its derivatives at concentrations of 10 and 40 µg/ml caused a significant reduction in cell viability ($p < 0.05$). However, these compounds at concentrations of 1 to 5 µg/ml did not show any cytotoxic activity in MTT assays. In detail, the cell viability in RAW 264.7 cells which were incubated with compound 1 at concentrations of 1, 2.5, 5, 10, 20 and 40 µg/ml for 24 h were 97.42% ± 6.68%, 94.71% ± 5.33%, 91.06% ± 4.25%, 73.58% ± 6.91%, 56.44% ± 4.56%, and 45.92% ± 4.37%, respectively, while the cell viability of compound 2 at different concentrations were 102.18% ± 4.63%, 101.73% ± 4.49%, 98.84% ± 5.21%, 83.13% ± 4.67%, 68.39% ± 5.87%, and 58.74% ± 5.06%, respectively, and the cell viability of compound 3 at different concentrations were 101.94% ± 4.38%, 100.57% ± 3.67%, 97.26% ± 5.31%, 84.51% ± 5.06%, 66.25% ± 6.76%, and 60.65% ± 6.72% of the control group treated with LPS only, respectively, while the cell viability of the blank group treated with media only was 102.73% ± 3.66% (Figure 3). Therefore, the non-toxic concentrations of up to 5 µg/ml were chosen for subsequent experiments.

3.3. Effects of Geldanamycin Derivatives on NO and PGE$_2$ Production in LPS-Induced RAW 264.7 Cells

LPS caused a significant increase in NO and PGE$_2$ production when compared with the blank control, geldanamycin and its derivatives at concentrations of 1, 2.5 and 5 µg/ml caused a significant reduction in NO and PGE$_2$ production when compared with LPS-induced control group ($p < 0.05$). In detail, the production of NO in LPS-induced RAW 264.7 incubated with compound 1 at concentrations of 1, 2.5 and 5 µg/ml for 24 h were 48.72 ± 7.43, 36.51 ± 5.84 and 20.28 ± 4.66 µM, respectively, the production of NO in LPS-induced RAW 264.7 incubated with compound 2 at different concentrations were 34.59 ± 6.83, 26.56 ± 6.15 and 17.45 ± 4.86 µM, respectively, and with compound 3 at different
concentrations were 31.76 ± 5.87, 22.52 ± 5.63 and 15.47 ± 4.55 µM, respectively, while the production of NO in the group treated with LPS only was 52.64 ± 6.11 µM. The production of PGE₂ in LPS-induced RAW 264.7 incubated with compound 1 at concentrations of 1, 2.5 and 5 µg/ml for 24 h were 36.75 ± 6.05, 26.67 ± 6.81 and 20.87 ± 3.70 ng/ml, respectively, the production of PGE₂ in LPS-induced RAW 264.7 incubated with compound 2 at different concentrations were 30.74 ± 6.05, 20.62 ± 6.23 and 15.77 ± 3.48 ng/ml, respectively, and with compound 3 at different concentrations were 27.06 ± 5.53, 17.61 ± 4.39 and 13.27 ± 3.10 ng/ml, respectively, while the production of PGE₂ in the group treated with LPS only was 51.75 ± 6.56 ng/ml. Therefore, the inhibitory levels of geldanamycin and its derivatives on NO and PGE₂ production also showed a dose-dependent manner (Figure 4 (NO) and Figure 5 (PGE₂)).

3.4. Effects of Geldanamycin Derivatives on Pro-Inflammatory Cytokine Production in LPS-Induced RAW 264.7 Cells

In this study, data showed that geldanamycin and its derivatives decreased production of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-10 in LPS-induced RAW 264.7 cells (p < 0.05) (Figure 6-9). Treatment with LPS alone in RAW 264.7 cells resulted in a significant increase of pro-inflammatory cytokine productions compared with the blank control group. The detailed results of this assay are as follows: TNF-α productions in LPS-induced RAW 264.7 cells incubated with compound 1 at concentrations of 1, 2.5 and 5 µg/ml for 24 h were 36.56 ± 5.51, 22.79 ± 4.80 and 14.73 ± 4.52 ng/ml, respectively,
Figure 4. Evaluation of nitrite production by RAW 264.7 cells stimulated for 24 h with LPS alone or in combination with increasing concentrations (1 - 5 µg/ml) of geldanamycin and its derivatives. The values are the means of at least three determinations ± SEM. Probability level (Student’s t-test): *p < 0.05 vs LPS-treated group.

Figure 5. Effect of geldanamycin and its derivatives on PGE$_2$ production in LPS-induced RAW 264.7 macrophage for 24 h. The values are the means of at least three determinations ± SEM. Probability level (Student’s t-test): *p < 0.05 vs LPS-treated group.

Figure 6. Effect of geldanamycin and its derivatives on LPS-induced TNF-α production by RAW 264.7 cells. The values are the means of at least three determinations ± SEM. Probability level (Student’s t-test): *p < 0.05 vs LPS-treated group.
Figure 7. Effect of geldanamycin and its derivatives on LPS-induced IL-1β production by RAW 264.7 cells. The values are the means of at least three determinations ± SEM. Probability level (Student’s t-test): *p < 0.05 vs. LPS-treated group.

Figure 8. Effect of geldanamycin and its derivatives on LPS-induced IL-6 production by RAW 264.7 cells. The values are the means of at least three determinations ± SEM. Probability level (Student’s t-test): *p < 0.05 vs. LPS-treated group.

Figure 9. Effect of geldanamycin and its derivatives on LPS-induced IL-10 production by RAW 264.7 cells. The values are the means of at least three determinations ± SEM. Probability level (Student’s t-test): *p < 0.05 vs. LPS-treated group.
TNF-α productions in LPS-induced RAW 264.7 incubated with compound 2 at different concentrations were 29.28 ± 4.61, 18.47 ± 3.69 and 12.82 ± 3.17 ng/ml, respectively, and TNF-α productions in LPS-induced RAW 264.7 incubated with compound 3 at different concentrations were 27.78 ± 3.95, 16.88 ± 3.47 and 11.21 ± 2.87 ng/ml, respectively while the production of TNF-α in the group treated with LPS only was 42.73 ± 4.78 ng/ml, IL-1β productions in LPS-induced RAW 264.7 cells incubated with compound 1 at different concentrations were 29.57 ± 6.28, 15.65 ± 7.26 and 9.62 ± 4.58 ng/ml, respectively, IL-1β productions in LPS-induced RAW 264.7 cells incubated with compound 2 at different concentrations were 22.16 ± 5.43, 12.77 ± 4.35 and 9.08 ± 3.57 ng/ml, respectively, and IL-1β productions in LPS-induced RAW 264.7 cells incubated with compound 3 at different concentrations were 20.67 ± 4.96, 17.45 ± 3.67 and 9.46 ± 3.34 ng/ml, respectively, IL-6 productions in LPS-induced RAW 264.7 cells incubated with compound 1 at different concentrations were 20.04 ± 3.20, 12.45 ± 2.84 and 6.63 ± 2.37 ng/ml, respectively, and IL-6 productions in LPS-induced RAW 264.7 cells incubated with compound 2 at different concentrations were 17.92 ± 2.81, 10.53 ± 2.62 and 5.27 ± 2.01 ng/ml, respectively, while the production of IL-1β in the group treated with LPS only was 42.39 ± 5.48 ng/ml, IL-10 productions in LPS-induced RAW 264.7 cells incubated with compound 1 at different concentrations were 22.13 ± 3.64, 17.61 ± 3.56 and 13.32 ± 2.34 ng/ml, respectively, IL-10 productions in LPS-induced RAW 264.7 cells incubated with compound 2 at different concentrations were 18.11 ± 3.07, 14.06 ± 2.96 and 10.72 ± 2.67 ng/ml, respectively, and IL-10 productions in LPS-induced RAW 264.7 cells incubated with compound 3 at different concentrations were 16.55 ± 2.22, 12.39 ± 2.07 and 8.97 ± 2.05 ng/ml, respectively, while the production of IL-10 in the group treated with LPS only was 28.31 ± 5.69 ng/ml. Therefore, treatment with geldanamycin derivatives (1, 2.5 and 5 µg/ml) remarkably inhibited in a LPS-induced TNF-α, IL-1β, IL-6 and IL-10 production in a dose-dependent manner.

4. Discussion

LPS was a typical inflammatory stimulus, which could trigger inflammation and activate the cellular signal transduction, and caused the intracellular cascades [16]. LPS activates macrophages and detects the characteristics of the cells, which is a common means to evaluate the anti-inflammatory activity of many drugs. Therefore, in this study, LPS was utilized to induce macrophage RAW 264.7 cells inflammatory response, and the anti-inflammatory effect of geldanamycin and its derivatives was investigated. The experimental results showed that LPS could activate RAW 264.7 cells effectively, and induced expression of pro-inflammatory mediators (NO and PGE₂) and cytokines (TNF-α, IL-1β, IL-6 and
Furthermore, it also could promote the gene transcription of iNOS, TNF-α, IL-1β and IL-6 mRNA [10]. In addition, the experimental results also showed that the doses of geldanamycin and its derivatives (1, 2.5 and 5 µg/ml) had no effect on the viability of RAW 264.7 cells experimentally. These results further demonstrated the pro-inflammatory effect of LPS on macrophages, and provided a reliable inflammatory model as well as a proper concentration range of geldanamycin and its derivatives for the follow-up experiments.

Inflammatory is a very complex process, including the accumulation of inflammatory cells, proliferation and production of inflammatory mediators and cytokines [17]. Macrophages function by releasing pro-inflammatory mediators and cytokines, such as COX-2, iNOS, MMP-2, MMP-9, NO, PGE2, ROS, TNF-α, IL-1β, IL-6, IL-10 and chemokines (chemokine C-C motif ligand 2; CCL2) [18] [19] [20] [21] [22]. These mediators and cytokines play a principal role in inflammatory diseases and processes [23] [24] [25]. Hence, it is an effective means to prevent the occurrence and development of inflammatory response by blocking the excessive production of these inflammatory mediators and cytokines [26] [27] [28]. TNF-α, which can cause pro-inflammatory effects to many types of cells, is produced by macrophages, mononuclear cells and T cells. IL-1β, IL-6 and IL-10 are regarded as an endogenous mediator of LPS-induced fever [29] [30]. This study found that, under certain conditions, geldanamycin and its derivatives can significantly reduce the level of these cytokine productions in LPS-induced RAW 264.7 cells. These results suggested that geldanamycin and its derivatives could play an anti-inflammatory role by inhibiting TNF-α, IL-1β, IL-6 and IL-10 productions. NO and PGE2 are also produced by inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) respectively [31] [32] [33]. These mediators drive the recruitment and initiation of macrophages and other immune cells to complete a full cycle of inflammation and interfere with metabolic functions [34]. In this study found that LPS can increase the expression of NO and PGE2 obviously, but if co-incubated with geldanamycin and its derivatives simultaneously, the expression of NO and PGE2 was significantly decreased and the decrease is dose-dependent. The data demonstrated that geldanamycin and its derivatives can suppress inflammatory response by inhibiting the production of NO and PGE2 in LPS-stimulated RAW 264.7 cells. In the literatures, geldanamycin has been reported as a potent anti-inflammatory compound that target heat-shock protein 90 and glucose-related protein 96 [35] [36] [37], which act as intracellular chaperones that maintain the structural integrity of cytoplasmic and endoplasmic reticulum-associated proteins, respectively. It binds to, and inactivates the function of, an Hsp90-Hsp70 multichaperone machine [38]. This multisubunit complex maintains the conformation and activity of regulatory kinases (e.g. c-src, cyclin-dependent protein kinase 4 [CDK4], Raf-1, and p38) [39] [40] [41], and nitric oxide synthase (NOS) [42] in eukaryotic cells. Because several Hsp90 substrates promote cellular activation and cell growth by maintaining the structural integrity of kinases such as c-Src, Raf-1, CDK4, extracellular signal-regulated kinase 1, JNK, p38 MAPK, lymphocyte-specific...
protein tyrosine kinase [43], protein kinase R [44] [45] [46] [47], and transcription factors (e.g. NF-kB) [48] [49], steroid hormone receptors [50] [51] [52], these proteins have potent antiproliferative effects both in vitro and in vivo [53]. Other Hsp90 substrates (e.g. NOS) promote inflammation. Because of this, geldanamycin has potent anti-inflammatory effects [37] [54] by binding to the amino-terminal ATPase domain of Hsp90 and inactivates its function [35] [55]. Inhibition of the function of Hsp90 substrates with the use of geldanamycin has antiproliferative and anti-inflammatory effects [36] [56] [57] [58] [59]. However, geldanamycin was toxic to normal cells, it exhibited hepatotoxicity at its effective concentrations [60], thereby limiting dose regimens should be considered for patient safety with medication administration [61] [62]. As previous studies have verified that derivatization of geldanamycin by substituting the C17-position of geldanamycin with an amino group has greatly decreased the toxicity while retaining the anti-tumor activity. [63] [64] [65] [66]. According to this effect, numerous geldanamycin derivatives with reduced hepatotoxicity have been designed, and several of them have entered clinical trials to treat patients [67] [68] [69]. In this study, synthetic geldanamycin derivatives devoid of C17 methoxy group by tryptamine and 5’methoxytryptamine substitution could potentially provide new agents with improved toxicity profiles over geldanamycin by increasing the percentage of cell viability with retaining the inhibitory effect of pro-inflammatory cytokines and mediators in LPS-induced RAW 264.7 cells. However, it should be noted that geldanamycin and its derivatives showed to be toxic at the concentration of 10 to 40 µg/ml in LPS-induced RAW 264.7 cells. The reason for this phenomenon is due the benzoquinon moiety which has an affinity for Hsp90 [70].

In this study, the inhibitory effects of geldanamycin derivatives on the productions of proinflammatory cytokines and mediators in LPS-stimulated RAW 264.7 cells were more potent than that of geldanamycin. A literature review revealed only one study of the anti-inflammatory effects of tryptamine hybrid compounds [71]. The reason for this phenomenon is due to tryptamine group could synergize with geldanamycin in the inhibitory effects on production of inflammatory mediators and proinflammatory cytokines in LPS-stimulated RAW 264.7 cells. In addition, cytotoxic effect of geldanamycin and its derivatives was evaluated in the absence or presence of LPS. When treated alone, these compounds did not affect the cell viability at all concentrations used (1 to 5 µg/ml). The potencies of the inhibitory activities of geldanamycin and its derivatives on RAW 264.7 macrophage cells are expected that these compounds would be useful for the treatment of inflammatory diseases that show the increased expression of proinflammatory cytokines and mediators.

The results obtained here demonstrate that the effects of geldanamycin derivatives (17-(tryptamine)-17-demethoxygeldanamycin and (17-(5’-methoxytryptamine)-17-demethoxygeldanamycin) on the productions of proinflammatory cytokines and mediators in LPS-stimulated RAW 264.7 cells were more potent than that of geldanamycin in a dose-dependant manner with less toxicity. These
results suggest that tryptamine hybrid geldanamycins can inhibit inflammatory response and may be a potential therapeutic candidate for the treatment of chronic inflammation and autoimmune inflammatory diseases. They could be useful for future drug development.

**Acknowledgements**

The authors are grateful to Mr Suttinun Vicharn in the Department of Chemistry, Faculty of Science, Silpakorn University, Thailand, for measuring NMR and MS data, respectively. This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, Thailand.

**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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