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Cytotoxicity activity of geldanamycin derivatives against various cancer cell lines

Thongchai Taechowisan[1*], Tipparat Samsawat[1], Winyou Puckdee[1], Waya S. Phutdhawong[2]

[1]Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom 7300, Thailand.
[2]Department of Chemistry, Faculty of Science Silpakorn University, Nakhon Pathom 7300, Thailand.

ABSTRACT

Geldanamycin (**1**) was isolated as a major compound from *Streptomyces zerumbet* W14. It was then used as a precursor to synthesize two new geldanamycins: 17-(tryptamine)-17-demethoxygeldanamycin (**2**) and 17-(5′-methoxytryptamine)-17-demethoxygeldanamycin (**3**). The cytotoxicity activity of these two new compounds was evaluated and compared with the cytotoxicity of compound **1**. Cytotoxicity activity was evaluated against a normal cell line, and three cancer cell lines using an 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay. The solubility of these compounds was also determined. The solubility of compounds **2** and **3** in water was 290.69 and 348.18 μM, higher than that of compound **1** by about 1.91 and 2.29 times, respectively. Compounds **2** and **3** showed moderate cytotoxic activity on Vero and human cervical carcinoma cells with IC_{50} values of >200.00 μg/ml. The strongest cytotoxicity of compound **3** was observed in human breast carcinoma cells (MCF-7) and human hepatocellular carcinoma cell line (HepG2) cells with IC_{50} values of 82.50 and 114.35 μg/ml, respectively, while the IC_{50} values of compound **2** against MCF-7 and HepG2 cells were 105.62 and 124.57 μg/ml, respectively. The findings showed that these new geldanamycin derivatives exhibited selective cytotoxicity toward some cancer cells at a lower concentration. Therefore, future studies on these compounds could be useful for the management of some cancers.

INTRODUCTION

Geldanamycin was the first benzoquinone ansamycin antibiotic that exhibited anticancer activity by inhibiting kinase folding by the Hsp90 chaperone complex in a wide range of cancers (Whitesell *et al*., 1994). The blockage of Hsp90 function induced the proteasome-dependent degradation of cancer relevant target proteins, known as client proteins (Mimnaugh *et al*., 1996). Despite its anticancer potential *in vitro*, the clinical use of geldanamycin has not been considered due to several limitations (Supko *et al*., 1995). First, it exhibited severe hepatotoxicity and nephrotoxicity at therapeutic recommended doses in animal models, limiting effective doses, and thus was

unacceptable as a therapeutic profile. This toxicity seemed to be caused by metabolisms of the benzoquinone moiety. Second, geldanamycin is metabolically unstable and poorly soluble in water. Accordingly, variants of geldanamycin have been developed, most notably by altering the quinone ring structure, which have led to improvements in tolerance, potency, metabolic stability, and water solubility (Le Brazidec *et al*., 2004). Although, the synthesized series of geldanamycin derivatives to make new types of Hsp90 inhibitor with weak toxicity and high efficiency have been attempting (Jurczyszyn *et al*., 2014; Lee, 2018; Lin *et al*., 2015; Vasilevskaya *et al*., 2003). However, there was a limit number of water solubility of these geldanamycin derivatives.

Tryptamine was derived by the decarboxylation of tryptophan. Tryptamine has been used in the past as a neurotransmitter and neuromodulator, vasoconstrictor, and vasodilator, and as antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory agent (Kousara *et al*., 2017).

*Corresponding Author
Thongchai Taechowisan, Department of Microbiology, Faculty of Science,
Silpakorn University, Nakhon Pathom 7300, Thailand.
E-mail: tewson84 @ hotmail.com*

Its modification has led to many compounds of pharmacological importance. Recently, tryptamine-based compounds have been synthesized as anticancer agents (Guo *et al.*, 2019; Malik *et al.*, 2019; Wang *et al.*, 2016). It has been an effective tool for improving water solubility, biological potency, and pharmacokinetic properties of many natural products (Kousara *et al.*, 2017). According to these effects, the invention of tryptamine-geldanamycin hybrids has been designed. The C17 methoxyl of the geldanamycin molecule can allow for various nucleophiles to be introduced. Thus, geldanamycin from the beginning has been a popular template for semi-synthetic analogs (Lin *et al.*, 2015; Modi *et al.*, 2011; Supko *et al.*, 1995; Tian *et al.*, 2004; Wrona *et al.*, 2010). In this study, two new geldanamycin derivatives were synthesized in form tryptamine-geldanamycin hybrids by substituting with tryptamine and 5-methoxytryptamine that consist of heterocyclic and strong polar groups in the 17-position to improve the pharmacokinetic properties. To our knowledge, this is the first report to describe the screening of tryptamine-geldanamycin hybrids as anticancer agents. The aim of the present study was to evaluate the anticancer activity of the synthesized tryptamine-geldanamycin hybrids against some cancer cell lines: [human breast carcinoma cells (MCF-7); human cervical carcinoma cells (HeLa); and human hepatocellular carcinoma cells (HepG2)] and their water solubility was also determined.

MATERIALS AND METHODS

Isolation and cultivation of endophytic actinomycete

Actinomycete strain W14 was isolated from the rhizome tissue of *Zingiber zerumbet* (L.) Smith, collected from Chanthaburi province, Thailand. The samples were used to isolate the endophytic actinomycetes by surface-sterilization technique and validation of surface sterilization was performed as described in the previous studies (Coombs and Franco, 2003; Taechowisan *et al.*, 2017). Strain W14 was selected and identified using morphological, cultural, physiological, and biochemical characteristics, chemotaxonomy and 16S rDNA sequencing (Taechowisan and Lumyong, 2003; Taechowisan *et al.*, 2019). 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 × 500 ml). This organic solvent was taken to dryness under rotary evaporation. 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 thin-layer chromatography (TLC) and was used to investigate on nuclear magnetic resonance (NMR) spectroscopy. The spectral data revealed this compound to be geldanamycin ($C_{29}H_{40}N_2O_9$) (**1**).
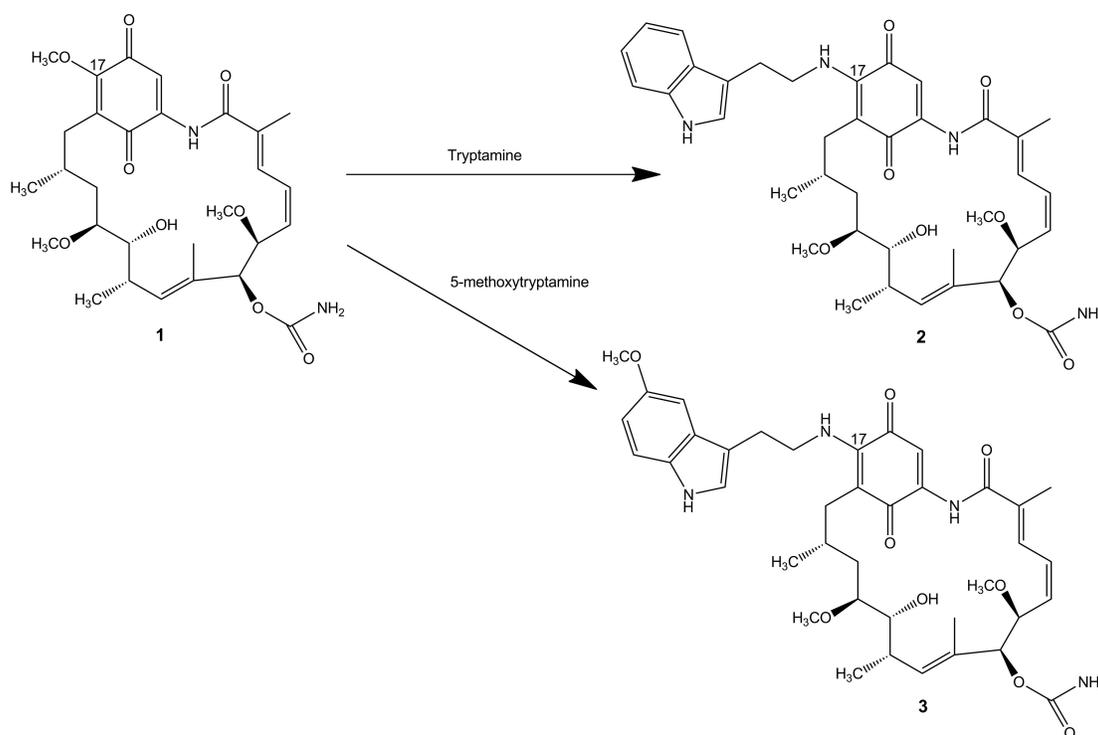
Figure 1. Geldanamycin chemical structure (**1**) and structures and synthesis of two derivatives; 17-(tryptamine)-17-demethoxygeldanamycin (**2**) and 17-(5′-methoxytryptamine)-17-demethoxygeldanamycin (**3**).

Synthesis of geldanamycin derivatives

All procedures and experiments described were geladanamycin derivative synthesis.

Geladanamycin derivatives; 17-(tryptamine)-17-demethoxygeldanamycin ($C_{38}H_{48}N_4O_8$) (**2**) and 17-(5′-methoxytryptamine)-17-demethoxygeldanamycin ($C_{39}H_{50}N_4O_9$) (**3**) were synthesized from geldanamycin (Fig. 1). To a solution of geldanamycin (0.84 g, 100 mmol) in dichloromethane (15 ml) at 25°C, tryptamine (0.29 g, 150 mmol) (Sigma-Aldrich) or 5′-methoxytryptamine (0.36 g, 150 mmol) (Sigma-Aldrich) was added. The reaction mixture was stirred at 25°C for 30 minutes before the addition of saturated aqueous $CaCl_2$ (5 ml). The organic layer was removed and washed with saturated $CaCl_2$ solution (3 × 5 ml) and dried (Na_2SO_4). 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 compound **2** (1.01 g, 97 mmol, 96.8%) or compound **3** (1.03 g, 95 mmol, 94.7%).

Water solubility test

The water solubility of geldanamycin and its derivatives was carried out by UV-spectroscopic assay in 96-well plates and measured using a SpectraMax Plus plate reader (Molecular Devices Sunnyvale, CA) as described by Hoelke *et al.* (2009). Briefly, the compounds were solubilized in water at various concentrations and incubated on a shaker deck at 30°C for 30 minutes. The samples were filtered and 200 µl of the samples were transferred to 96-well plates. The absorbance was measured at 450 nm for compound **1** and at 570 nm for compounds **2** and **3**. The calibration of UV-spectroscopic measurements for solubility of each compound was plotted against the concentrations. The stable part of the optical density change was ascribed to water solubility of the compounds. A check of the photometric linearity of the UV plate reader using solutions of $K_2Cr_2O_7$ at variable concentrations led to a linear range of the instrument from 0.003 to 3.5 absorption units for wavelengths 350 nm.

MTT assay for cytotoxicity activity

A normal cell line [African green monkey kidney cells (Vero)] and three cancer cell lines (MCF-7; HeLa; HepG2) were obtained from the Korean Cell Line Bank (Seoul, Korea). These cells were grown at 37°C in Dulbecco's modified eagle's medium (DMEM) supplement with 10% fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycein sulfate (100 µg/ml) in a humidified atmosphere of 5% CO_2. Cytotoxicity studies were performed on a 96-well plate. The cells were mechanically scraped and plated 2×10^5 per well containing 100 µl of DMEM medium with 10% FBS and incubated overnight. The purified compound was dissolved in dimethylsulfoxide for stock solution. Cells were incubated with purified compound at increasing concentrations (1.5625, 3.125, 6.25, 12.5, 25, 50, 100, and 200 µl/ml) in FBS-free medium for 24 hours. The

dimethyl sulfoxide (DMSO) concentrations in all assays did not exceed 0.1%. Cells were washed once before adding 50 µl FBS-free medium containing 5 mg/ml 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). After 4 hours 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 570 nm. Consecutively, measurements for concentration required for 50% (IC_{50}) inhibition were noted. Cell viability percent was calculated using the formula given below:

$$\text{Percentage (\%) of cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100$$

The graph was plotted with the *Y*-axis showing the percentage of viability of cells and *X*-axis showing the compound concentration.

The therapeutic index was calculated as the ratio of IC_{50} of normal cells to IC_{50} of cancer cells.

Statistical analysis

Values are expressed as means ± standard deviation (SD) of three experiments. The SPSS v.16.0 software (SPSS Inc., Chicago, IL) was used for data analysis. Comparisons between two groups were analyzed using the two-tailed Dunnett *t*-tests treated compound **1** as a control group. A *p*-value <0.05 is considered as statistically significant.

RESULTS

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

Compound (**1**): The infrared (IR) spectrum displayed characteristic absorption bands of NH and OH stretches at n 3,478, 3,440, 3,336, and 3,297cm^{-1}, CH stretch in CH_3 and CH_2 at n 2,927 cm^{-1}, CH stretch in methyl ether at n 2,853 cm^{-1}, C=O stretch in $OCONH_2$ at n 1,729 cm^{-1}, C=O stretch in a,b-unsaturated amide at n 1,701 cm^{-1} and C=O stretches in quinone at n 1,675 and 1,652 cm^{-1} (Fig. 2). The MS gave a [M+Na]$^+$ ion at *m/z* 583.2571 (Fig. 3a) which corresponded to the molecular formula $C_{29}H_{40}N_2O_9$ for the compound indicating eleven double bond equivalents in the molecule. The structure was fully elucidated by ^{1}H-NMR, ^{13}C-NMR spectroscopy, DEPT-135, and 2D-NMR spectral studies. The spectral data revealed the compound **1** to be geldanamycin. Its ^{1}H-NMR and ^{13}C-NMR spectral data of which were in good agreement with those of geldanamycin (Table 1) previously reported by Ōmura *et al.* (1979) and Qin and Panek (2008).

Compound (**2**): The MS gave a [M + Na]$^+$ ion at *m/z* 711.3384 (Fig. 3b) which corresponded to the molecular formula $C_{38}H_{48}N_4O_8$. The structure was fully elucidated by ^{1}H-NMR, ^{13}C-NMR spectroscopy, DEPT-135, and 2D NMR spectral studies as shown in Table 2.
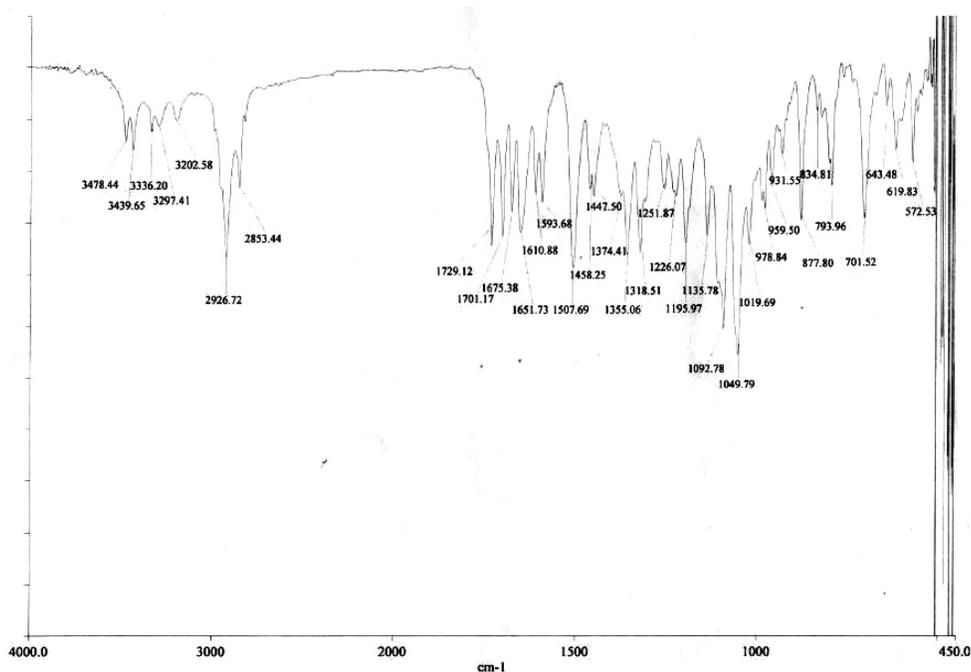
Figure 2. IR spectrum of compound **1**.
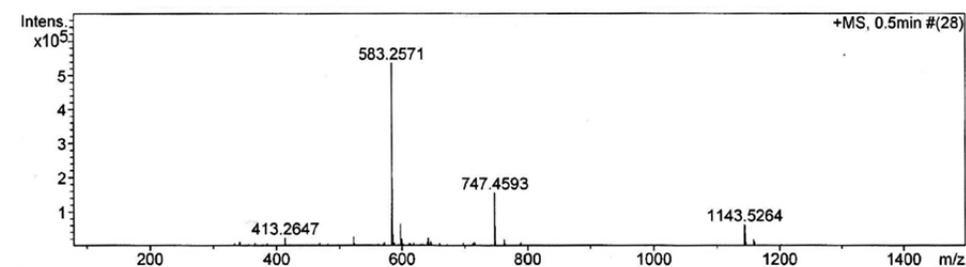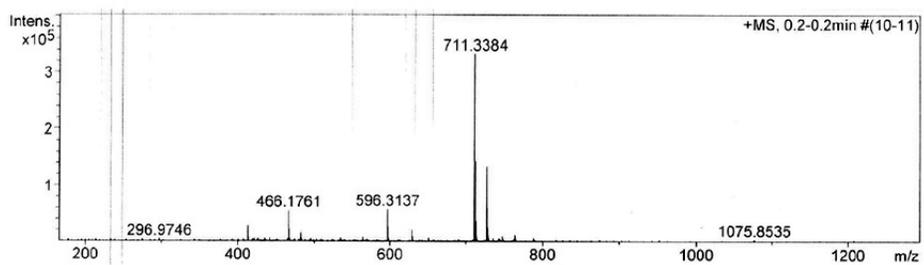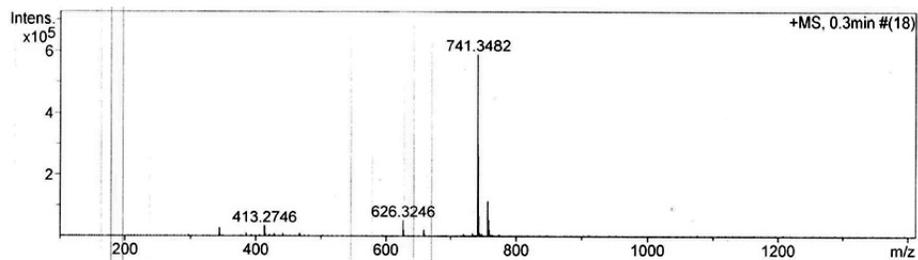
Figure 3. Mass spectrum of compounds **1** (**a**), **2** (**b**) and **3** (**c**).

Table 1. Comparison of the spectral data of the compound **1** and GDA[a].

No.	δ_C compound 1	δ_C GDA	δ_H compound 1	δ_H GDA	HMBC (H→C)	COSY	NOESY
1	169.7 C	169.1	–	–	–	–	–
2	133.2 C	133.2	–	–	–	–	–
2-Me	12.8 CH_3	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_3	56.0	3.24 s	3.22, s	6	–	–
7	81.1 CH	80.6	4.88 br	4.86, br	5, 7-OCONH$_2$, 9, 8-Me	6	3, 6, 9
7-OCONH$_2$	156.6 C	156.0	–	6.45, br	–	–	–
8	129.1 C	132.6	–	–	–	–	–
8-Me	13.0 CH_3	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_3	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_3	56.6	3.23 s	3.23, s	12	–	–
13	31.3 CH_2	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_3	23.0	0.97 br	0.76, d	14, 15	14	–
15a	32.2 CH_2	31.7	2.43 dd (12.5, 9.9)	2.42, m	13, 14, 16, 17, 21	14, 15b	–
15b		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_3	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

[a]GDA, geldanamycin (Ōmura *et al.*, 1979).

Compound (**3**): The MS gave a [M + Na]+ ion at *m/z* 741.3482 (Fig. 3c) which corresponded to the molecular formula $C_{39}H_{50}N_4O_9$. The structure was fully elucidated by ^{1}H-NMR, ^{13}C-NMR spectroscopy, DEPT-135, and 2D NMR spectral studies as shown in Table 2. The detailed comparison of spectral data of compounds **3** and **1** is shown in Table 3.

The water solubility of geldanamycin (**1**) was found to be 151.78 µM (Table 4). In contrast, the solubility of its derivatives (**2** and **3**) in water was 290.69 and 348.18 µM, respectively, about 1.91 and 2.29 times, respectively, higher than that of geldanamycin. These data suggest that the attachment of a tryptamine moiety to geldanamycin at the C17 position greatly enhanced its water solubility.

Geldanamycin and its derivatives were evaluated for cytotoxicity activity against Vero, MCF-7, HeLa, and HepG2 cell lines using the MTT assay. Compounds **2** and **3** exhibited weak cytotoxicity activity toward Vero and HeLa cells with IC_{50} values of >200.00 µg/ml (Table 5 and Fig. 4). However, these compounds were able to exhibit stronger cytotoxicity activity to MCF-7 and HepG2 than geldanamycin. These data were of interest, as it suggested that two novel geldanamycin derivatives were more toxic to some cancer cells than normal cells. Accordingly, they could be considered potential anticancer drugs in appropriate dosages.

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

No.	δ_C compound 2	δ_C compound 3	δ_H compound 2	δ_H compound 3
1	168.4 C	168.4 C	–	–
2	135.0 C	135.0 C	–	–
2-Me	12.5 CH$_3$	12.6 CH$_3$	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 CH$_3$	57.1 CH$_3$	3.26 s	3.27 s
7	81.7 CH	81.1 CH	5.18 s	5.18 s
7-OCONH$_2$	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			2.40	2.44 m (12.5, 4.8)
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			3.77 m	3.76 m
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

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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$_2$	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$_2$	32.2 CH$_2$	2.68 m	2.43 dd (12.5, 9.9)	13, 14, 14-Me, 16, 17, 21	15b
15b			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$_2$	–	3.92 m	–	23, 24	22b, 22-NH, 23
22b			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	–
26	131.8 C	–	–	–	–	–
27	127.3 C	–	–	–	–	–
28	100.4 CH	–	7.00 s	–	24, 26, 29, 30	–
29	154.3 C	–	–	–	–	–
29-OMe	56.0 OCH$_3$	–	3.87 s	–	29	–
30	112.3 CH	–	7.29 d (9)	–	29	31
31	112.7 CH	–	6.90 d (9)	–	26, 27, 29	30
1-NH	–	–	9.17 s	9.18, NH, brs	1, 19, 21	–
22-NH	–	–	6.47 t (6.0)	–	16, 18, 22, 23	22a, 22b
25-NH	–	–	8.14 s	–	24, 25, 26, 27	25
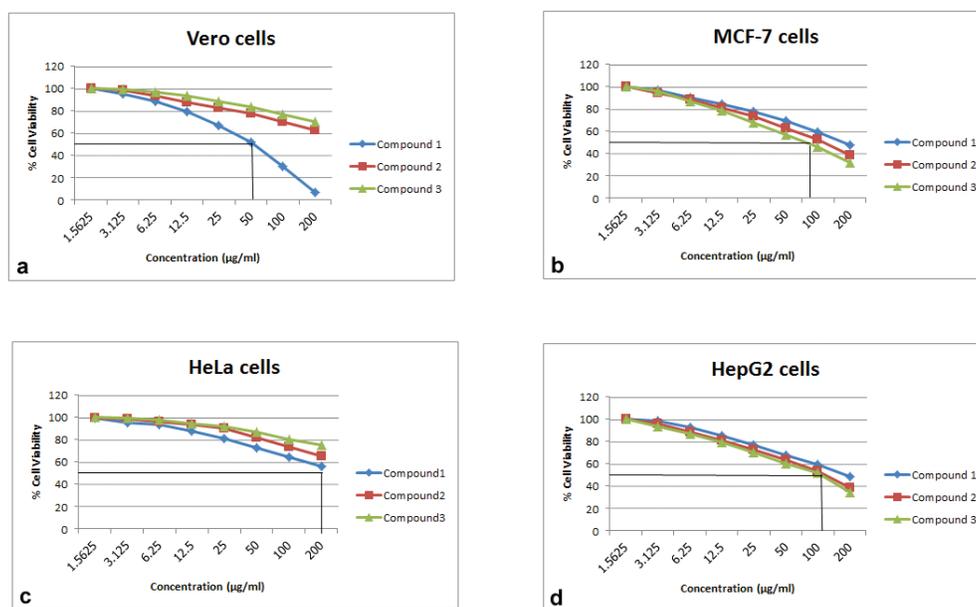
Figure 4. Cytotoxicity effects of compounds 1(♦), 2(■) and 3(▲) on Vero cells (**a**), MCF-7 cells (**b**), HeLa cells (**c**) and HepG2 cells (**d**) using an MTT colorimetric assay.

Table 4. Water solubility of geldanamycin (**1**) and its derivatives (**2** and **3**).

Compounds	MW	Solubility in water (mg/ml)[b]	Solubility in water (μM)[a]	Relative solubility
1	560	0.085 ± 0.004	151.78 ± 7.14[b]	1.00
2	688	0.200 ± 0.003	290.69 ± 4.36[c]	1.91
3	718	0.250 ± 0.003	348.18 ± 5.02[d]	2.29

[a]The results presented represent the average of three separate experiments (mean ± SD).

[b,c,d]Significant differences ($p < 0.05$).

Table 5. Cytotoxicity activity (IC$_{50}$) of geldanamycin (**1**) and its derivatives (**2** and **3**).

Compounds	IC$_{50a}$ (μg/ml)				Therapeutic index[b]		
	Vero[c]	MCF-7	HeLa	HepG2	MCF-7	HeLa	HepG2
1	54.25	178.43	>200.00	184.92	0.30	ND[i]	0.11
2	>200.00[d]	105.62[e]	>200.00	124.57[f]	>1.90[g]	ND	>1.60[h]
3	>200.00[d]	82.50[e]	>200.00	114.35[f]	>2.42[g]	ND	>1.75[h]

[a]IC$_{50}$ values represent the concentration causing 50% growth inhibition. They were determined by linear regression analysis of the average of three separate experiments.

[b]Therapeutic index is defined as the ratio of the median toxic dose on normal cells to the median effective dose on cancer cells.

[c]Vero = African green monkey kidney cell line, MCF-7 = human breast carcinoma cell line, HeLa = human cervical carcinoma cell line, HepG2 = human hepatocellular carcinoma cell line.

[d,e,f,g,h]Significant differences from the compound **1** ($p < 0.05$).

[i]ND = not determined.

DISCUSSION

The benzoquinone ansamycins are an important class of Hsp90 inhibitors that possess potent anticancer activity in preclinical models and may emerge as efficacious therapeutic agents for the treatment of cancer (Isaacs, 2005). More generally, Hsp90 is an attractive therapeutic anticancer target because inhibition of its chaperone activity results in lower cellular levels of multiple client proteins that are critical for cancer-cell survival (Neckers, 2002). Geldanamycin, an ansamycin antibiotic, along with its analogues, had significant anticancer properties (Schnur *et al.*, 1995; Whitesell *et al.*, 1992). These compounds bind specifically to the adinosine triphosphate (ATP) binding site of Hsp90 agents, these disrupted Hsp90 association with client protein (Grenert *et al.*, 1997; Prodromou *et al.*, 1997; Stebbins *et al.*, 1997). Therefore, the

prevention of binding of Hsp90 with ATP affected extremely the composition of Hsp90-containing chaperone complexes (An *et al.*, 1997; Obermann *et al.*, 1998; Workman *et al.*, 2002). However, the use of geldanamycin and its derivatives as a chemotherapeutic agent did not proceed owing to its severe hepatotoxicity, metabolic instability, and poor water solubility (Fukuyo *et al.*, 2009; Supko *et al.*, 1995). The improvement of water solubility leads to drug potency and advantageous pharmacological profiles. Therefore, water-soluble geldanamycin derivatives with improved pharmacokinetic and pharmacological properties are necessary (Cheng *et al.*, 2005; Wu *et al.*, 2012). Tryptamine has been an effective tool for improving the water solubility, biological potency, and pharmacokinetic properties of many natural products (Kousara *et al.*, 2017). Recently, tryptamine derivatives have showed moderate to good anticancer

activity against HepG2, HeLa, CNE1, and A549 human cancer cell lines with IC$_{50}$ values of 16.5–18.7 μM (Guo *et al.*, 2019). In this study, geldanamycin exhibited lower solubility in water than its derivatives. It also exhibited more cytotoxicity against normal cells than cancer cells, but no cytotoxicity against HeLa cells. This differs from those reports that geldanamycin was extremely active against KB cells, an ubiquitous keratin-forming HeLa cell line (<0.001 μg/ml) (Deboer *et al.*, 1970), but showed moderate cytotoxicity against MCF-7 and HepG2. According to other reports, geldanamycin was active against various human breast cancer cell lines including: SKBr3 (IC$_{50}$ value of 37 nM) (Hu *et al.*, 2004); BC (IC$_{50}$ value of 0.01 μg/ml) (Jongrungruangchok *et al.*, 2006); MDA-MB-231, MCF-7 and T-47D (IC$_{50}$ value of 100 μM) and human hepatocellular carcinoma cell lines, HepG2, and SMMC7721 (IC$_{50}$ value of 200 μM) (Zhang *et al.*, 2016). In contrast, cytotoxicity activity of tryptaminated geldanamycins against cancer cells (MCF-7 and HepG2) exhibited greater activity than geldanamycin. This was due to the increased water solubility of geldanamycin derivatives and enhanced anticancer activity of tryptaminated geldanamycins.

CONCLUSION

In summary, the two new tryptaminated-geldanamycins have been synthesized; they showed the water solubility improvement. They also have potent cytotoxic effects on MCF-7; human breast cancer cells, and HepG2 with low cytotoxicity against Vero cells. These results suggest that the tryptaminated-geldanamycin derivatives may be a potential therapeutic candidate for the treatment of some cancers. They could be useful for future drug development.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflicts of interest.

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