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# Antiviral activity of geldanamycin and its derivatives against influenza virus

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## ARTICLE INFO

## ABSTRACT

Two new geldanamycin derivatives such as 17-(tryptamine)-17-demethoxygeldanamycin (**2**) and 17-(5'-methoxytryptamine)-17-demethoxygeldanamycin (3) were synthesized by nucleophilic substitution of the C17 methoxyl of geldanamycin (**1**). Their antiviral activity was evaluated based on influenza virus propagation in embryonated chicken eggs and viral absorption by hemagglutination (HA) inhibition test. The findings indicated that these compounds inhibited viral propagation at a concentration of 12.5 µg/ml. For the viral absorption, only compounds **2** and **3** inhibited HA at a concentration of 50 µg/ml. The solubility of compounds **2** and **3** in water was 290 and 306 µM, higher than that of compound **1** about 1.91 and 2.01 times, respectively. The compounds **2** and **3** showed a moderate cytotoxic activity on LLC-MK2 and Vero cells with $IC_{50}$ values of >200.00 µg/ml. These results demonstrated the invention of tryptamine-geldanamycin hybrids to prevent influenza virus infection in viral absorption and viral propagation steps.

## INTRODUCTION

Geldanamycin is a benzoquinone ansamycin, and it binds specifically to heat shock protein 90 (Hsp90) (Prodromou *et al.*, 1997; Sullivan *et al.*, 1997), resulting in dysfunction and rapid degradation of Hsp90-associated client proteins (Blagosklonny, 2002; Richter and Buckner, 2001). As a specific inhibitor of Hsp90 function, its derivatives showed antitumor activity (Miyata, 2005; Ochel *et al.*, 2001) and has been used for the treatment of various cancers (Biamonte *et al.*, 2010; Jhaveri *et al.*, 2012; Pacey *et al.*, 2006). Intervention with the Hsp90 function is the mode of action of geldanamycin (Roe *et al.*, 1999).

For viral replications, Hsp90 is necessary for the viral protein synthesis (Burch and Weller, 2005; Basha *et al.*, 2005; Connor *et al.*, 2007; Geller *et al.*, 2007; Hu and Seeger, 1996; Hu *et al.*, 1997; Hung *et al.*, 2002; Li *et al.*, 2004; Smith *et al.*, 2010; Shan *et al.*, 2011; Waxman *et al.*, 2001). It has been shown that geldanamycin blocks the replication of the viruses both *in vitro* and *in vivo* via inhibition of Hsp90 (Amraiz *et al.*, 2017; Li *et al.*, 2004; 2010; 2012; Luo *et al.*, 2003; Schang *et al.*, 2002; Smith *et al.*, 2010; Wang *et al.*, 2017). However, the therapeutic utilization of this compound has been restricted by low water solubility, metabolic instability, and severe hepatotoxicity (Fukuyo *et al.*, 2009; Supko *et al.*, 1995). Therefore, its derivatives with improved pharmacokinetic profiles have been developed. The synthesized series of geldanamycin derivatives to make new types of Hsp90 inhibitor with weak toxicity and high efficiency have been seeking (Kitson *et al.*, 2013; Li *et al.*, 2010; Lin *et al.*, 2015; Modi *et al.*, 2011; Shan *et al.*, 2011; Supko *et al.*, 1995; Tian *et al.*, 2004; Wrona *et al.*, 2010).

Tryptamine was the product of the decarboxylation of tryptophan. It has been used in the past as a vasodilator, neurotransmitter, antiviral, antibacterial, antifungal, anti-inflammatory, and antioxidant agent (Kousara *et al.*, 2017). Its modification has been conducted to be pharmacologically active compounds. Recently, tryptamine has been synthesized as a novel non nucleosidic compound against hepatitis B virus (Qu *et al.*, 2011). It has been a useful tool for improving the solubility, biological activities, and pharmacological properties of numerous natural

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products (Kousara *et al.*, 2017). According to these effects, the invention of tryptamine-geldanamycin hybrids has been designed. The C17 methoxyl group of geldanamycin molecule can permit for various nucleophiles to be introduced. Thus, geldanamycin has been a popular template for producing various types of bioactive compounds (Lin *et al.*, 2015; Modi *et al.*, 2011; Supko *et al.*, 1995; Tian *et al.*, 2004; Wrona *et al.*, 2010). Furthermore, the other report showed that some of the 17-substituted geldanamycin derivatives contained stronger activity against hepatitis B virus than geldanamycin with higher LD$_{50}$ values than that of geldanamycin (Li *et al.*, 2010). It has been reported that influenza virus replication could be inhibited by interfering the Hsp90 function (Chase *et al.*, 2008). However, the activity of geldanamycin and its derivatives against influenza virus has been not reported. Therefore, geldanamycin could inhibit the functions of viral proteins by interfering with the complex formation of Hsp90 and viral proteins. Inhibition of Hsp90 activity also causes the inhibition of viral protein synthesis. Furthermore, it has been reported that geldanamycin could inhibit viral replication by preventing the chaperone-mediated process in viral protein folding and functions (Li *et al.*, 2004).

In this study, novel tryptamine-geldanamycin hybrids were synthesized, their antiviral activity against influenza virus was evaluated based on virus propagation in embryonated chicken eggs and viral absorption by hemagglutination (HA) inhibition test, and their water solubility and cytotoxicity were also determined.

## MATERIALS AND METHODS

### Isolation and cultivation of the actinomycete

Eighteen actinomycetes were isolated from the various tissues (leaf, pseudostem, rhizome, and root) of *Zingiber zerumbet* (L.) Smith (Taechowisan *et al.*, 2017). Among the 18 isolates, the isolate W14 was found to be the best producer of antibacterial

substances (Taechowisan *et al.*, 2019). This isolate was selected and identified using a polyphasic approach (Taechowisan and Lumyong, 2003; Taechowisan *et al.*, 2019). The strain was cultured on ISP-2 agar plates at 30°C for 14 days and then was extracted with ethyl acetate. The crude extract was fractionated using low-to-high polar solvents (ethyl acetate in hexane) and purified on Thin Layer Chromatography (TLC) (Taechowisan *et al.*, 2019). The purified compound was undertaken to investigate on Nuclear Magnetic Resonance (NMR) spectroscopy. The spectral data of this compound corresponded to be geldanamycin ($C_{29}H_{40}N_2O_9$) (**1**).

### Synthesis and solubility of geldanamycin derivatives

Geldanamycin derivatives were synthesized from geldanamycin (Fig. 1) as described earlier (Taechowisan *et al.* 2019). The solubility of the novel geldanamycins in water was determined by comparing with geldanamycin.

### Viral strain propagation

Influenza viruses A/free-grazing duck/Nakhon Pathom/1/2017 (H5N2) (Taechowisan *et al.*, 2018) were cultivated in embryonated eggs. Viral titer was determined using the HA assay as previously described (Brauer and Chen, 2015).

#### *Virus cultivation inhibition assay*

Virus cultivation inhibition assay was carried out by embryonated chicken egg inoculation. About 100 µl of tested compounds (12.5, 25, and 50 µg/ml) was incubated with 100 µl of seed virus ($2.86 \times 10^8$ virus particles/ml) at 37°C for 30 minutes, and then, 100 µl of the mixture was inoculated into each embryonated chicken egg and incubated at 37°C for 4 days. The allantoic fluid was harvested and then was tested by HA assay (Brauer and Chen, 2015). About 20 µg/ml of heparin (AppliChem, Germany) was used as a positive control.
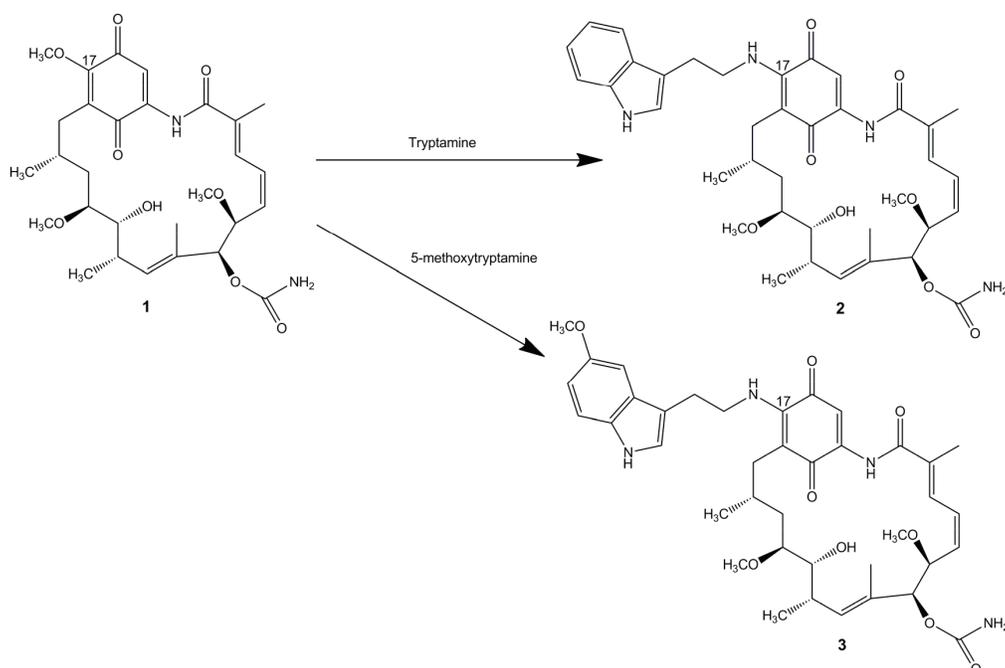


**Figure 1.** The semisynthetic route of 17-(tryptamine)-17-demethoxygeldanamycin (**2**) and 17-(5′-methoxytryptamine) -17-demethoxygeldanamycin (**3**) from geldanamycin (**1**).

*HA inhibition (HAI) assay*

HAI assay was used to evaluate the effect of the compounds in virus adsorption to target cells. The compounds (25 µl) with two-fold serial dilution with Phosphate Buffered Saline (PBS) were mixed with an equal volume of seed influenza virus (400 HAU per 50 µl). After incubation at room temperature for 30 minutes, 50 µl of the solution was mixed with an equal volume of 1% chicken erythrocyte suspension and then was incubated at 4°C for 30 minutes. The highest dilution of the compounds that prevented HA is called the HAI titer. About 20 µg/ml of heparin (AppliChem, Germany) was used as a positive control.

## MTT assay for cell viability

The normal cells (LLC-MK2: rhesus monkey kidney epithelial cell line and Vero: African green monkey kidney cell line) were grown at 37°C in Dubecco's Modified Eagle Medium (DMEM) medium supplement with 10% FBS, penicillin (100 units/ml), and streptomycin sulfate (100 µg/ml) in a humidified atmosphere of 5% $CO_2$. Cytotoxicity studies were performed on a 96-well plate. The cells were trypsinized and plated on a 96-well plate ($2 \times 10^5$ per well) containing DMEM medium with 10% FBS and incubated overnight. Cells were incubated with the compounds at increasing concentrations in FBS-free medium for 24 hours. Cells were washed once, and 50 µl of FBS-free medium containing 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and incubated in 5% $CO_2$ at 37°C for 4 hours. The medium was replaced with 50 µl of DMSO to dissolve the formazan product. The optical density was measured at 450 nm. The half inhibitory concentration ($IC_{50}$) was defined as a 50% reduction of the absorbance compared with the control assay.

## RESULTS

The characterization of geldanamycins was carried out by $^1$H-NMR, $^{13}$C-NMR, and mass spectral methods as follows.

Compound (**1**): The mass spectrum showed a [M+Na]$^+$ ion at *m/z* 583.2571 (molecular formula : $C_{29}H_{40}N_2O_9$). The spectral data revealed this compound to be a geldanamycin, which was

**Table 1.** $^1$H-NMR and $^{13}$C-NMR spectral data of compound **1** and GDA[a].

| No. | $\delta_C$ compound 1 | $\delta_C$ GDA | $\delta_H$ compound 1 | $\delta_H$ 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 (data from Ōmura *et al.*, 1979).

in agreement with those of geldanamycin (Table 1), previously described by Omura *et al.* (1979) and Qin and Panek (2008).

Compound (**2**): The mass spectrum showed a [M+Na]$^+$ ion at *m/z* 711.3384 (molecular formula : $C_{38}H_{48}N_4O_8$). The structure

**Table 2.** $^1$H-NMR and $^{13}$C-NMR spectral data of compound **2** and compound **3**.

| No. | $\delta_C$ compound 2 | $\delta_C$ compound 3 | $\delta_H$ compound 2 | $\delta_H$ 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 |

was fully elucidated by $^1$H-NMR, $^{13}$C-NMR spectroscopy, DEPT-135, and 2D-NMR spectral data (Table 2).

Compound (**3**): The mass spectrum showed a [M+Na]$^+$ ion at *m/z* 741.3482 (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 data (Table 2), and the spectral data of this compound were compared with the spectral data of compound **1** (Table 3).

**Table 3.** $^1$H-NMR and $^{13}$C-NMR spectral data of compound **3** and compound **1**.

| No. | $\delta_C$ compound 3 | $\delta_C$ compound 1 | $\delta_H$ compound 3 | $\delta_H$ 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 |

The water solubility of geldanamycin (**1**) was found to be 151.60 µM (Table 4). In contrast, the solubility of its derivatives (**2** and **3**) in water was 290 and 306 µM, respectively, about 1.91 and 2.01 times higher than that of geldanamycin, respectively. These data suggest that the conjugation of a tryptamine moiety to geldanamycin at the C17 position greatly enhanced their water solubility.

The effect of geldanamycin and its derivatives on influenza virus propagation was evaluated at various concentrations in embryonated chicken eggs. The virus yields were determined by HA test. The virus propagation was obtained only in control, whereas no virus was detected in the compound treatments. In addition, the effect of geldanamycin and its derivatives on viral adsorption to chicken erythrocytes was carried out. Interestingly, as expected, the compounds **2** and **3** inhibited viral binding to the cells with HAI titer of 1:50, whereas geldanamycin could not inhibit viral binding to the cells (Table 4). These data suggested that geldanamycin and its derivatives inhibited influenza virus propagation, but tryptamine-geldanamycin hybrids could inhibit the viral adsorption (early step) of influenza virus infection. Heparin at the concentration of 20 µg/ml could completely inhibit both viral propagation and viral absorption (data not shown).

Geldanamycin and its derivatives were evaluated for cytotoxicity activity against LLC-MK2 and Vero cell lines using the MTT assay. The compounds **2** and **3** exhibited weak cytotoxicity activity toward LLC-MK2 and Vero cells with $IC_{50}$ values of >200.00 µg/ml (Table 5). The results show that two novel geldanamycin possesses low toxicity to normal cells and can display potential application in antiviral chemoprevention and chemotherapy.

## DISCUSSION

Influenza virus causes seasonal outbreaks in temperate regions, with an increase in disease and mortality rate which is a serious problem. With the expectation of exploiting the potency of Hsp90 inhibitor against influenza virus, we investigated an *in vitro* inhibitory activity of geldanamycin and its derivatives against influenza virus as they were promising candidates *in vitro*.

**Table 4.** Water solubility and HAI titers of geldanamycins.

| Compounds | MW | Solubility in water (mg/ml) | Solubility in water (µM) | Relative solubility | Hemagglutination inhibition titers |
|---|---|---|---|---|---|
| 1 | 560 | 0.085 | 151.60 | 1.00 | ND[a] |
| 2 | 688 | 0.200 | 290 | 1.90 | 1:50 |
| 3 | 718 | 0.250 | 306 | 2.00 | 1:50 |

[a]ND; not determined.

**Table 5.** Cytotoxicity activity ($IC_{50}$) of geldanamycins.

| Compounds | $IC_{50}$[a] (µg/ml) | |
|---|---|---|
| | LLC-MK2[b] | Vero |
| 1 | 73.67 | 54.25 |
| 2 | >200.00 | >200.00 |
| 3 | >200.00 | >200.00 |

[a]$IC_{50}$ values represent the concentration causing 50% growth inhibition.
[b]LLC-MK2; rhesus monkey kidney epithelial cell lines, Vero; African green monkey kidney cell lines.

The toxicity and water solubility of geldanamycin have been a marked hindrance for its development for chemotherapy use. These have incentive scientists to pay attention to develop less toxic geldanamycin derivatives. In this study, compounds **2** and **3** also exhibited less cytotoxicity than geldanamycin in the normal cell lines. We also found that these compounds showed a greater increase in water solubility. It should be mentioned that the antiviral activity of geldanamycin and its derivatives appeared in influenza virus propagation, which suggests that geldanamycin and its derivatives are an option choice in terms of antiviral agents in the viral propagation step. The introduction of a tryptamine or 5′-methoxytryptamine group at the C17-position of geldanamycin could not interfere with the binding of geldanamycin derivatives to Hsp90, but it had a largely decreased toxicity and increased water solubility. As stated by the crystal structure of the geldanamycin-Hsp90 complex (Stebbins *et al.*, 1997), the substitution in the C17 methoxyl of geldanamycin is revealed to the external cavity of the Hsp90 protein, while the difference of substituents of geldanamycin is crucial for the interaction with the Hsp90 protein. According to the report by Li *et al.* (2010), 17-amino-17-demethoxygeldanamycin derivatives have a great potential for antiviral activity, whereas the 19-substituted geldanamycin modification was not a possibility in terms of antiviral agents. Due to the introduction of a group, the C19-position of geldanamycin could interfere with the binding of geldanamycin derivatives to Hsp90 by the steric effects (Li *et al.*, 2010). These results led us to contemplate that Hsp90 could be a target for antiviral infection and that geldanamycin and its derivatives have a great potential for antiviral propagation by interfering with Hsp90 in the protein folding and stabilizing of virus-infected cells.

The invention of tryptamine-geldanamycin hybrids has been designed at the C17-position of geldanamycin by nucleophilic substitution reactions. These compounds inhibited not only on viral propagation but also on viral absorption. It suggested that tryptamine-geldanamycin hybrids could protect viral infection in both the steps. According to this effect, Sun *et al.* (2019) reported that tryptophan dendrimers could block receptor binding of enterovirus A71, and these compounds could prevent binding and internalization of the virus. Furthermore, the Chinese Academy of Science, Shanghai, has shown that tryptamine derivatives had an antiviral activity against hepatitis B virus (Qu *et al.*, 2017). This study discovered the effects of the tryptamine-geldanamycins on inhibition of influenza virus propagation and adsorption. This study will help the researcher to uncover the structural modifications of the compounds to improve biological activities.

## CONCLUSION

In summary, the antiviral activity, low toxicity, and enhanced water solubility of two new tryptamine-geldanamycins, compounds **2** and **3**, were presented in this work, in comparison with geldanamycin. In particular, all of these compounds showed antiviral activity in viral propagation, whereas the tryptamine-geldanamycins not only inhibit viral propagation but also inhibit viral absorption which has low toxicity and good water solubility better than geldanamycin. These results show that the functions of Hsp90 can be inhibited by these compounds and the virus cannot be propagated. This suggests a new antiviral approach. Therefore, Hsp90 could be an excellent antiviral target, and the tryptamine-geldanamycins could be considered as a new choice for antiviral agents.

**REFERENCES**

Amraiz D, Zaidi NSS, Fatima M. Antiviral evaluation of an Hsp90 inhibitor, gedunin, against dengue virus. Trop J Pharm Res, 2017; 16:997–1004.

Basha W, Kitagawa R, Uhara M, Imazu H, Uechi K, Tanaka J. Geldanamycin, a potent and specific inhibitor of Hsp90, inhibit gene expression and replication of human cytomegalovirus. Antivir Chem Chemother, 2005; 16:135–46.

Biamonte MA, Van de Water R, Arndt JW, Scannevin RH, Perret D, Lee WC. Heat shock protein 90: inhibitors in clinical trials. J Med Chem, 2010; 53:3–17.

Blagosklonny MV. Hsp-90-associated oncoproteins: multiple targets for geldanamycin and its analogs. Leukemia, 2002; 16**:**455–62.

Brauer R, Chen P. Influenza virus propagation in embryonated chicken eggs. J Visualized Exp, 2015; 97:e52421; doi:10.3791/52421

Burch AD, Weller SK. Herpes simplex virus type 1 DNA polymerase requires the mammalian chaperone hsp90 for proper localization to the nucleus. J Virol, 2005; 79:10740–49.

Chase G, Deng T, Fodor E. HSP90 inhibitors reduce influenza virus replication in cell culture. Virology, 2008; 377:431–9.

Connor JH, McKenzie MO, Parks GD, Lyles DS. Antiviral activity and RNA polymerase degradation following Hsp90 inhibition in a range of negative strand viruses. Virology, 2007; 362:109–19.

Fukuyo Y, Hunt CR, Horikoshi N. Geldanamycin and its anti-cancer activities. Cancer Lett, 2009; 290:24–35.

Geller R, Vignuzzi M, Andino R, Frydman J. Evolutionary constraints on chaperone-mediated folding provide an antiviral approach refractory to development of drug resistance. Gen Dev, 2007; 21:195–205.

Hu J, Seeger C. Hsp90 is required for the activity of a hepatitis B virus reverse transcriptase. Proc Natl Acad Sci USA, 1996; 93:1060–4.

Hu J, Toft DO, Seeger C. Hepadnavirus assembly and reverse transcription require a multi-component chaperone complex which is incorporated into nucleocapsids. EMBO J, 1997; 16:59–68.

Hung JJ, Chung CS, Chang W. Molecular chaperone Hsp90 is important for vaccinia virus growth in cells. J Virol, 2002; 76**:**1379–90.

Jhaveri K, Taldone T, Modi S, Chiosis G. Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers. Biochim Biophys Acta, 2012; 1823:742–55.

Kitson RR, Chang CH, Xiong R, Williams HE, Davis AL, Lewis W, Dehn DL, Siegel D, Roe SM, Prodromou C, Ross D. Synthesis of 19-substituted geldanamycins with altered conformations and their binding to heat shock protein hsp90. Nat Chem, 2013; 5:307–14.

Kousara S, Anjuma SN, Jaleela F, Khana J, Naseema S. Biomedical significance of tryptamine: a review. J Pharmacovigil, 2017; 5:239.

Li YH, Lu QN, Wang HQ, Tao PZ, Jiang JD. Geldanamycin, a ligand of heat shock protein 90, inhibits herpes simplex virus type 2 replication both *in vitro* and *in vivo*. J Antibiot, 2012; 65:509–12.

Li YH, Tao PZ, Liu YZ, Jiang JD. Geldanamycin, a ligand of heat shock protein 90, inhibits the replication herpes simplex virus type 1 *in vitro*. Antimicrob Agents Chemother, 2004; 48:867–72.

Li YP, Shan GZ, Peng ZG, Zhu JH, Meng S, Zhang T, Gao LY, Tao PZ, Gao RM, Li YH, Jiang JD, Li ZR. Synthesis and biological evaluation of heat-shock protein 90 inhibitors: geldanamycin derivatives with broad antiviral activities. Antivir Chem Chemother, 2010; 20:259–68.

Lin Z, Peng R, Li Z, Wang Y, Lu C, Shen Y, Wang J, Shi G. 17-ABAG, a Novel geldanamycin derivative, inhibits LNCaP-cell proliferation through heat shock protein 90 inhibition. Int J Mol Med, 2015; 36:424–32.

Luo H, Zhang J, Dastvan F, Yanagawa B, Reidy MA, Zhang HM, Yang D, Wilson JE, McManus BM. Ubiquitin-dependent proteolysis of cyclin D1 is associated with coxsackievirus-induced cell growth arrest. J Virol, 2003; 77**:**1–9.

Miyata Y. Hsp90 inhibitor geldanamycin and its derivatives as novel cancer chemotherapeutic agents. Curr Pharm Des, 2005; 11: 1131–8.

Modi S, Stopeck A, Linden H, Solit D, Chandarlapaty S, Rosen N, D'Andrea G, Dickler M, Moynahan ME, Sugarman S, Ma W. HSP90 inhibition is effective in breast cancer: a phase II trial of tanespimycin (17-AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. Clin Cancer Res, 2011; 17:5132–9.

Ochel HJ, Eichhorn K, Gademann G. Geldanamycin: the prototype of a class of antitumor drugs targeting the heat shock protein 90 family of molecular chaperones. Cell Stress Chaperones, 2001; 6**:**105–12.

Ōmura S, Nakagawa A, Sadakane N. Structure of herbimycin a new ansamycin antibiotic. Tetrahedron Lett, 1979; 20:4323–6.

Pacey S, Banerji U, Judson I, Workman P. Hsp90 inhibitors in the clinic. Handb Exp Pharmacol, 2006; 172:331–58.

Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell, 1997; 90**:**65–75.

Qin HL, Panek JS. Total synthesis of the Hsp90 inhibitor geldanamycin. Organic Lett, 2008; 10:2477–9.

Qu SJ, Wang GF, Duan WH, Yao SY, Zuo JP, Tan CH, Zhu DY. Tryptamine derivatives as novel non-nucleosidic inhibitors against hepatitis B virus. Bioorg Med Chem, 2011; 19:3120–7.

Richter K, Buckner J. Hsp90: chaperoning signal transduction. J Cell Physiol, 2001; 188**:**281–90.

Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. J Med Chem, 1999; 42**:**260–6.

Schang LM, Bantly A, Knockaert M, Shaheen F, Meijer L, Malim MH, Gray NS, Schaffer PA. Pharmacological cyclin-dependent kinase inhibitors inhibit replication of wild-type and drug-resistant strains of herpes simplex virus and HIV-1 by targeting cellular, not viral, proteins. J Virol, 2002; 76:7874–82.

Shan GZ, Peng ZG, Li YH, Li D, Li YP, Meng S, Gao LY, Jiang JD, Li ZR. A novel class of geldanamycin derivatives as HCV replication inhibitors targeting on Hsp90: synthesis, structure-activity relationships and anti-HCV activity in GS4.3 replicon cells. J Antibiot, 2011; 64:177–82.

Smith DR, McCarthy S, Chrovian A, Olinger G, Stossel A, Geisbert TW, Hensley LE, Connor JH. Inhibition of heatshock protein 90 reduces Ebola virus replication. Antiviral Res, 2010; 87:187–94.

Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FL, Pavletich NP. Crystal structure of an HSP90–geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell, 1997; 89: 239–50.

Sullivan W, Stensgard B, Caucutt G, Bartha B, McMahon N, Alnemri ES, Litwack G, Toft D. Nucleotides and two functional states of hsp90. J Biol Chem, 1997; 272**:**8007–12.

Sun L, Lee H, Thibaut HJ, Lanko K, Rivero-Buceta E, Bator C, Martinez-Gualda B, Dallmeier K, Delang L, Leyssen P, Gago F. Viral engagement with host receptors blocked by a novel class of tryptophan dendrimers that targets the 5-fold-axis of the enterovirus-A71 capsid. PLoS Pathog, 2019; 15:e1007760.

Supko JG, Hickman RL, Grever MR, Malspeis L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. Cancer Chemother Pharmacol, 1995; 36:305–15.

Taechowisan T, Chaisaeng S, Phutdhawong WS. Antibacterial, antioxidant and anticancer activities of biphenyls from Streptomyces sp. BO-07; an endophyte in *Boesenbergia rotunda* (L.) Mansf A. Food Agri Immunol, 2017; 28:1330–46.

Taechowisan T, Dumpin K, Phutdhawong WS. Isolation of avian influenza a (H5N2) from free-grazing ducks in Thailand and antiviral effects of tea extracts on viral propagation. Asian J Poult Sci, 2018; 12:7–13.

Taechowisan T, Puckdee W, Phutdhawong WS. *Streptomyces zerumbet*, a Novel species from *Zingiber zerumbet* (L.) Smith and isolation of its bioactive compounds. Adv Microbiol, 2019; 9:194–219.

Taechowisan T, Lumyong S. Activity of endophytic actinomycetes from roots of Zingiber officinale and Alpinia galanga against phytopathogenic fungi. Ann Microbiol, 2003; 53: 291-8.

Tian ZQ, Liu Y, Zhang D, Wang Z, Dong SD, Carreras CW, Zhou Y, Rastelli G, Santi DV, Myles DC. Synthesis and biological activities of novel 17-aminogeldanamycin derivatives. Bioorg Med Chem, 2004; 12:5317–29.

Wang C, Liu P, Luo J, Ding H, Gao Y, Sun L, Luo F, Liu X, He H. Geldanamycin reduces acute respiratory distress syndrome and promotes the survival of mice infected with the highly virulent H5N1 influenza virus. Front Cell Infect Microbiol, 2017; 15:267.

Waxman L, Whitney M, Pollok BA, Kuo LC, Darke PL. Host cell factor requirement for hepatitis C virus enzyme maturation. Proc Natl Acad Sci USA, 2001; 98:13931–5.

Wrona IE, Gozman A, Taldone T, Chiosis G, Panek JS. Synthesis of reblastatin, autolytimycin, non-benzoquinone analogues: potent inhibitors of heat shock protein 90. J Org Chem, 2010; 75:2820–35.