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Research Article
Antiviral Activity of Dopamine Geldanamycin Hybrids Against Influenza Virus and Association with Molecular Docking Analysis

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Abstract

Background and Objective: Geldanamycin (GDM) is an antibiotic isolated from *Streptomyces zerumbet* W14 that specifically targets and deactivates heat shock protein 90 (Hsp90) to inhibit virus replication. The therapeutic utilization of GDM has been restricted by its low water solubility and severe hepatotoxicity. The aim of the present study was to synthesis the novel geldanamycin derivatives and evaluate their biological properties. **Materials and Methods:** Five new Dopamine Geldanamycin Hybrids (DGH); compounds **2-6** were synthesized by nucleophilic substitution of GDM (**1**). Solubility, cytotoxicity, antiviral activity and molecular docking analyses were carried out. **Results:** The solubility of DGH in water was 0.386-5.464 mM, higher than that of compound **1**. These compounds exhibited weak cytotoxic activity against LLC-MK2 and Vero cells, with IC_{50} values in the range of 104.52-496.31 μg mL^{-1}. These compounds (except compound **5**) inhibited influenza virus propagation in embryonated chicken eggs at the minimum inhibitory concentration of 6.25 μg mL^{-1}. They interacted positively with Hsp90, showing binding free energy (ΔG) of -100.50 to -114.28 kcal mol^{-1}, which indicated lower Hsp90 affinity compared with that of geldanamycin (-141.296 kcal mol^{-1}) and 17-dimethylamino ethylamino-17-demethoxygeldanamycin (-145.307 kcal mol^{-1}), despite being partly bound in the active site (compounds **2**, **3** and **6**) or outside the active site (compound **4**). **Conclusion:** The study findings revealed, through molecular docking analysis, that the development of DGH improved the pharmacokinetic profiles of solubility, cytotoxicity and antiviral activities. It is, therefore, recommended DGH that is a potential alternative treatment agent for influenza virus infection.

Key words: Antiviral activity, cytotoxicity activity, dopamine-geldanamycin hybrids, heat shock protein 90, influenza virus, molecular docking, water solubility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Geldanamycin (GDM) is a benzoquinone ansamycin antibiotic. Its molecular structure consists of a benzoquinone unit and a planet macrocyclic ansamycin bridge. Recently, GDM was isolated from *Streptomyces zerumbet* W14, an endophyte of *Zingiber zerumbet* (L.) Smith[1]. The target of GDM is a heat shock protein 90 (Hsp90), which it specifically deactivates to inhibit tumor growth or virus replication[2-6]. It has been shown that GDM blocks viral replication both *in vitro* and *in vivo* via inhibition of Hsp90[7-9]. However, the therapeutic utilization of this compound has been restricted by its low water solubility, metabolic instability and severe hepatotoxicity[10,11]. Therefore, GDM derivatives with improved pharmacokinetic profiles, have been developed. A series of synthetic GDM derivatives that will generate new types of Hsp90 inhibitors with weak toxicity and high efficiency have been sought[12-16]. Several GDM analogs have been synthesized, including 17-allylamino-17-demethoxy geldanamycin (17-AAG) and 17-dimethylamino ethylamino-17-demethoxy geldanamycin (17-DMAG); however, their water solubility was limited[17]. Recently, tryptamine geldanamycin hybrids have been synthesized. These compounds inhibited influenza virus propagation in embryonated chicken eggs. Their water solubility was increased above that of GDM[6].

Dopamine is a neurotransmitter responsible for transmitting signals between nerve cells. It is used in the treatment of severe hypotension, bradycardia, circulatory shock and cardiac arrest[18]. Dopamine is polar a covalent compound that is soluble in polar molecules such as water. In this study, it is a useful tool for the development of Dopamine Geldanamycin Hybrids (DGH) with improved solubility and biological activities. The C17 methoxy group of the GDM molecule can permit the introduction of various nucleophiles. Thus, GDM has been a popular template for producing various types of bioactive compounds[12-14]. Furthermore, another report showed that, compared with GDM, some of the 17-substituted GDM derivatives exhibited stronger activity against hepatitis B virus and had higher LD_{50} values[7]. It has been reported that influenza virus replication could be inhibited by interfering with Hsp90 function[19]. Inhibition of Hsp90 activity also causes inhibition of viral protein synthesis. Furthermore, it has been reported that GDM could inhibit viral replication by preventing chaperone-mediated processes in viral protein folding and functions[2]. Therefore, molecular docking studies were performed with Hsp90 and the new DGH.

In this study, novel DGH were synthesized and their antiviral activity against the influenza virus was evaluated based on virus propagation in embryonated chicken eggs. Their water solubility, cytotoxicity and molecular docking on Hsp90 were also determined.

MATERIALS AND METHODS

Study area: The study was carried out in the Department of Microbiology and Department of Chemistry, Silpakorn University, Nakhon Pathom, Thailand from November, 2019-December, 2020.

Cultivation of actinomycetes and product isolation: *Streptomyces zerumbet* W14 was obtained as an endophyte from *Zingiber zerumbet* (L.) Smith using the surface-sterilization technique[1]. The bacterium was grown on ISP-2 agar at 30°C for 14 days. The initial steps of antibiotic isolation and purification were as previously described[6]. The purified compound was subjected to investigation by NMR spectroscopy. The spectral data for this compound identified it as geldanamycin ($C_{29}H_{40}N_2O_9$) (1).

Chemical reagents and materials: All chemicals were purchased from Tokyo Chemical Industry (Tokyo, Japan), Sigma-Aldrich (Darmstadt, Germany) and Fluka Chemical (Buchs, Switzerland) Companies. All solvents were dried by using standard methods. The ^1H and ^{13}C NMR spectra were recorded with a Bruker Avance 300 spectrometer (Bruker, Massachusetts, USA), (300 MHz for ^1H, 75 MHz for ^{13}C). Mass spectra were determined with a micrOTOF (Bruker, Massachusetts, USA). Melting points were measured with a Stuart Scientific SMP 2 melting point apparatus (Cole-Parmer Ltd, Staffordshire, UK) and are uncorrected. The reaction was monitored by TLC, performed on aluminum sheets pre-coated with silica gel 60 F254 (Darmstadt, Germany). Column chromatography was performed using a Merck Kieselgel 60 column chromatography (Darmstadt, Germany).

Synthesis of dopamine-geldanamycin hybrids

Synthesis of *N*-*tert*-butyl 3,4-dihydroxy phenethyl carbamate (8): To a solution of dopamine hydrochloride (7) (0.48 g, 2.54 mmol) in a mixture of dioxane (5 mL) and H_2O (2.5 mL) was added 2N NaOH (1.27 mL, 2.54 mmol) and stirred for 10 min at room temperature. Then, a solution of *tert*-butyl dicarbonate (0.6 mL, 2.54 mmol) in dioxane (4 mL) was added to the mixture. The reaction was stirred at room temperature

overnight under an argon (Ar) atmosphere. The resulting mixture was cooled. EtOAc was added and then the mixture was acidified with 1N HCl to adjust the pH approximately 3. The aqueous layer was washed with EtOAc. The organic layers were combined, washed with water, then and brine and dried over with anhydrous (anh.) Na_2SO_4. The organic layer was then concentrated under reduced pressure. The crude product was purified by column chromatography using hexane: EtOAc 3:1 as the eluent to provide product **8** (0.47 g, 73% yield) as a brown solid.

Synthesis of *N-tert*-butyl 3,4-dimethoxy phenethyl carbamate (9a): *N-tert*-butyl 3,4-dihydroxy phenethyl carbamate (**8**) (0.39 g, 1.56 mmol) was dissolved in anh. MeOH (10 mL). K_2CO_3 (0.95 g, 6.87 mmol) and dimethyl sulphate (0.33 mL, 3.44 mmol) were added. The mixture was refluxed for 5 hrs under an Ar atmosphere. The resulting mixture was filtered using sintered glass and washed with MeOH. The filtrate was concentrated to provide a residue, which was then purified by flash column chromatography using hexane: EtOAc 2:1 as the eluent to provide product **9a** (0.31 g, 76% yield) as a pale yellow solid.

Synthesis of *N-tert*-butyl 3,4-bis(benzyloxy) phenethyl carbamate (9b): *N-tert*-butyl 3,4-dihydroxy phenethyl carbamate (**8**) (0.04 g, 0.17 mmol) was dissolved in anh. DMF (2 mL), then K_2CO_3 (0.14 g, 1.02 mmol) and benzyl chloride (0.04 mL, 0.37 mmol) were added. The mixture was stirred at room temperature for 24 hrs under an Ar atmosphere, filtered through celite and then washed with EtOAc. The filtrate was washed with water and brine. The organic layer was dried over with anh. Na_2SO_4 and concentrated. The crude product was purified by column chromatography using hexane: EtOAc 2:1 as the eluent to provide product **9b** (0.065 g, 88% yield) as a white solid.

Synthesis of *N-tert*-butyl 3,4-bis((2-bromobenzyl)oxy) phenethyl carbamate (9c): *N-tert*-butyl 3,4-dihydroxy phenethyl carbamate (**8**) (0.06 g, 0.24 mmol) was dissolved in anh. DMF (2 mL), then K_2CO_3 (0.27 g, 1.42 mmol) and 2-bromobenzyl bromide (0.06 mL, 0.46 mmol) were added. The mixture was stirred at room temperature for 24 hrs under an Ar atmosphere, filtered through celite and then washed with EtOAc. The filtrate was washed with water and brine. The organic layer was dried over with anh. Na_2SO_4 and concentrated. The crude product was purified by column chromatography using hexane: EtOAc (4:1) as the eluent to provide **9c** (0.10 g, 72% yield) as a white solid.

Synthesis of *N-tert*-butyl 3,4-dibutoxy phenethyl carbamate (9d): *N-tert*-butyl 3,4-dihydroxy phenethyl carbamate (8) (0.12 g, 0.47 mmol) was dissolved in anh. DMF (4 mL), then K_2CO_3 (0.39 g, 2.84 mmol) and *n*-butyl bromide (0.10 mL, 0.95 mmol) were added. The mixture was stirred at room temperature for 24 hrs under an Ar atmosphere, filtered through celite and then washed with EtOAc. The filtrate was washed with water and brine. The organic layer was dried over with anh. Na_2SO_4 and concentrated. The crude product was purified by preparative chromatography using hexane: EtOAc (4:1) as a mobile phase to provide **9d** (0.02 g, 12% yield) as a colorless oil.

Synthesis of dopamine derivatives (10a-10c): Compound **9a**-**9c** (1 equiv.) was reacted with 5% TFA in CH_2Cl_2 (20 equiv.) and stirred at room temperature for 2 hrs. The solvent was removed under vacuum and purified by preparative chromatography using 15% MeOH in CH_2Cl_2 as a mobile phase to provide the products **10a-c**: 2-(3,4-dimethoxy phenyl) ethanamine (**10a**), 2-(3,4-bis (benzyloxy)phenyl) ethanamine (**10b**) and 2-(3,4-bis((2-bromobenzyl)oxy)phenyl) ethanamine (**10c**).

Synthesis of 17-demethoxy geldanamycin derivatives (2-6): To a solution of geldanamycin (**1**) (1 equiv.) in CH_2Cl_2 was added with dopamine derivative (**7**, **10a**-**10c**) (2 equiv.) in MeOH and Et_3N (2.3 equiv.). The reaction was shielded away from light and stirred at room temperature for 48 hrs. The solvent was removed, diluted with EtOAc and then washed with 1N HCl, H_2O and brine. The organic phases were combined and dried over with anh. Na_2SO_4. The organic phase was concentrated under reduced pressure. The crude product was purified by preparative chromatography to yield a purple solid product.

(4E, 6Z, 8S, 9S, 10E, 12S, 13R, 14S, 16R)-19-((3,4-dihydroxyphenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo [16.3.1]docosa-1(21),4,6,10,18-pentaen-9-yl carbamate (**2**): 0.0167 g. HMS calculated for $C_{36}H_{47}N_3O_{10}$ $(M+Na)^+$ 704.3154, found 704.3157.

(4E, 6Z, 8S, 9S, 10E, 12S, 13R, 14S, 16R)-19-((3,4-dimethoxyphenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo [16.3.1]docosa-1(21),4,6,10,18-pentaen-9-yl carbamate (**3**): 0.0508 g. HMS calculated for $C_{38}H_{51}N_3O_{10}$ $(M+Na)^+$ 732.3467, found 732.3469.

(4E, 6Z, 8S, 9S, 10E, 12S, 13R, 14S, 16R)-19-((3,4-bis(benzyloxy)phenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo [16.3.1]docosa-1(21),4,6,10,18-pentaen-9-yl carbamate (**4**): 0.0501 g. HMS calculated for $C_{50}H_{59}N_3O_{10}$ $(M+Na)^+$ 884.4093, found 884.4094.

(4E, 6Z, 8S, 9S, 10E, 12S, 13R, 14S, 16R)-19-((3,4-bis((2-bromobenzyl)oxy)phenethyl) amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo [16.3.1]do-cosa-1(21),4,6,10,18-pentaen-9-yl carbamate (**5**): 0.0757 g. HMS calculated for $C_{50}H_{57}Br_2N_3O_{10}$ $(M+Na)^+$ 1040.2303, found 1040.2323.

(4E, 6Z, 8S, 9S, 10E, 12S, 13R, 14S,16R)-19-((3,4-dibutoxy phenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo [16.3.1]docosa-1(21),4,6,10,18-pentaen-9-yl carbamate (**6**): Compound **9d** (1 equiv.) was reacted with 5% TFA in CH_2Cl_2 (20 equiv.) and stirred at room temperature for 2 hrs. The solvent was removed under vacuum to provide the crude product **10d**, which was used without further purification. To a solution of geldanamycin (**1**) (1 equiv.) in CH_2Cl_2 has added the crude product of the dopamine derivative **10d** (2 equiv.) in MeOH and Et_3N (2.3 equiv.). The reaction was shielded from light and stirred at room temperature for 48 hrs. The solvent was removed, diluted with EtOAc and then washed with 1N HCl, H_2O and brine. The organic phases were combined and dried over with anh. Na_2SO_4. The organic phase was concentrated under reduced pressure. The crude product was purified by preparative chromatography to yield product **6** as a purple solid 0.0097 g. HMS calculated for $C_{44}H_{63}N_3O_{10}$ $(M+Na)^+$ 816.4406, found 816.4402.

The solubility of the novel DGH in water was determined by comparison with GDM as previously described[6].

Viral strain propagation: Influenza viruses A/free-grazing duck/Nakhon Pathom/1/2017 (H5N2)[20] were cultivated in embryonated eggs. Viral titers were determined using the hemagglutination (HA) assay as previously described[21].

Virus cultivation inhibition assay: Virus cultivation inhibition assays were carried out by embryonated chicken egg inoculation. One hundred microlitres of test compounds at various concentrations (12.5, 25, 50 and 100 μg mL^{-1}) was incubated with 100 μL of seed virus (2.86×10^8 virus particles mL^{-1}) at 37°C for 30 min, 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 tested by HA assay[21]. About 20 mg mL^{-1} of hepapin (Applichem, Germany) was used as a positive control.

MTT assay for cell viability: The normal cell lines (LLC-MK2: Rhesus monkey kidney cells and Vero cells: African green monkey kidney cells) were obtained from the Korean Cell Line Bank (Seoul, Korea). These cells were grown in DMEM medium supplemented with 10% FBS, penicillin (100 U mL^{-1}) and streptomycin sulphate (100 μg mL^{-1}) at 37°C in a humidified atmosphere of 5% CO_2. Cytotoxicity studies were performed in a 96-well plate. Details of the procedures have been described in a previous publication[6].

Sequence and domain analysis: The protein sequences of vertebrate Hsp90 were available in the NCBI database. Multiple Sequence Alignment (MSA) was performed using Clustal Omega (version 1.2.4). The domain analysis for the same protein was performed using Prosite.

Molecular docking studies: The two-dimensional structures of geldanamycin and DGH were drawn and converted to SMILES strings with ChemDraw software (http://cambridgesoft.com) and the Online SMILES Translator and Structure File Generator (https://cactus.nci.nih.gov/translate/), respectively. The energies of these compounds were optimized and converted to #D format, saved as Protein Data Bank (PDB) files using UCSF Chimera v.1.14 (University of California, CA) and further used for docking studies.

The three-dimensional structure of Hsp90 with the co-crystallized geldanamycin (PDB ID: 1OSF) was retrieved from the Research Collaboratory for Structural Bioinformatics PDB and chosen for molecular docking studies. The crystal structure of 1OSF is employed for docking geldanamycin and DGH to obtain reliable predictions of ligand binding. The water molecules were removed from the crystal structure using Discovery Studio software, followed by the addition of Gasteiger charges to targets.

Docking simulations were undertaken with Hsp90 as the target (1OSF) and geldanamycin, 17-DMAG and DGH as the ligands using Auto Dock Vina to predict the ligand-binding sites on Hsp90. The target confirmation was set as a rigid unit while the ligands were considered to be flexible and adaptable to the target. Vina explored the lowest binding affinity conformations and provided five different conformations for the Hsp90 target. The lowest binding energy docking conformations of each compound were selected. Auto Dock Vina was processed using an exhaustiveness of four and a grid box with dimensions for the centre of x = 30.2535, y = 45.3258 and z = 52.7852 with a size of X = 41.3526, Y = 43.2578 and Z = 50.8467 for 1OSF. The UCSF Chimera v.1.14 was chosen for visual inspection and preparations. The protein-ligand interactions were analyzed with the aid of BIOVIA Discovery Studio and Lig Plot v.4.5.3.

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

RESULTS

In the present work, a series of DGH were synthesized via nucleophilic substitution of the C17 methoxyl of GDM (1) as described in the methods section (Fig. 1-2).

The water solubility of compound **1** was found to be 0.152 mM (Table 1). In contrast, the solubility of DGH in water

Table 1: Water solubility of geldanamycin (**1**) and dopamine-geldanamycin hybrids (**2-6**)

Compounds	MW	Solubility in water (mg mL^{-1})[a]	Solubility in water (mM)[a]	Relative solubility
1	560	0.085±0.004	0.152±0.002[b]	1.00
2	681	3.333±1.154	4.894±1.695[f]	32.19
3	709	0.666±0.577	0.940±0.814[d]	6.18
4	862	0.333±0.577	0.386±0.669[c]	2.53
5	1019	1.333±0.577	1.308±0.566[e]	8.60
6	793	4.333±0.577	5.464±0.728[f]	35.94

[a]Results presented represent the average of three separate experiments (Mean±SD). [b-f]Significant differences ($p<0.05$)

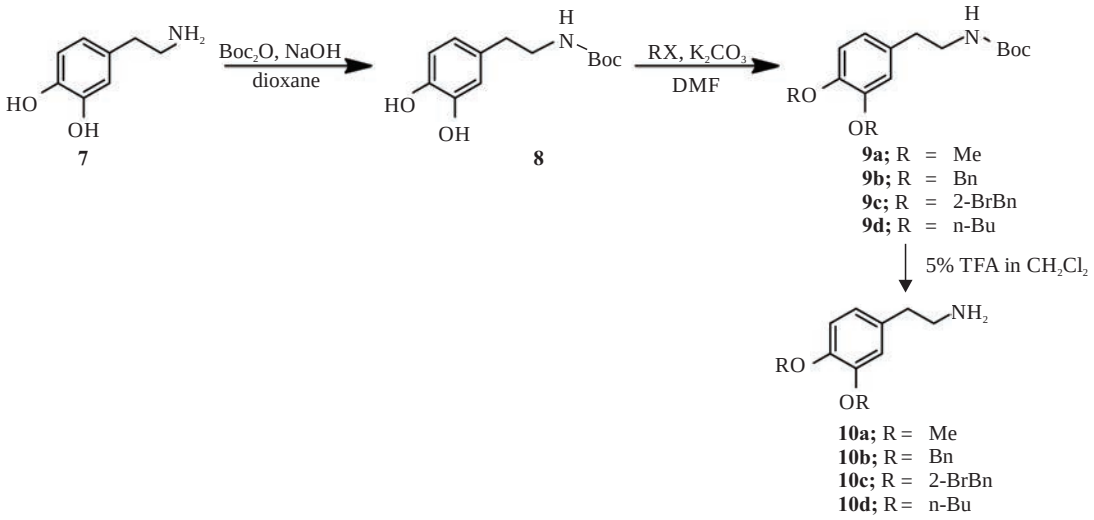

Fig. 1: Syntheses of dopamine derivatives **10a-10d**

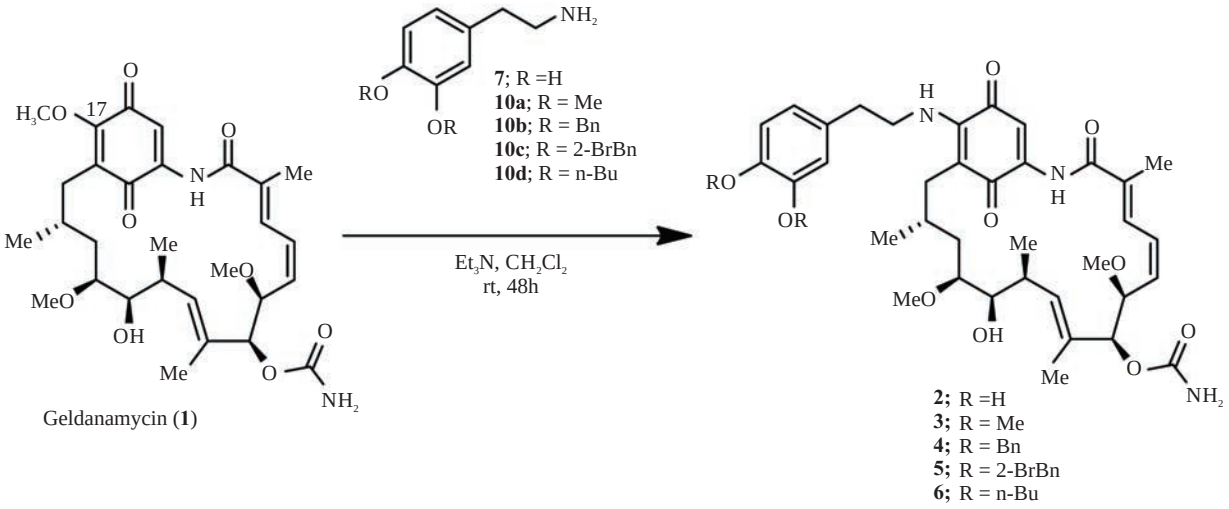

Fig. 2: Syntheses of 17-demethoxy geldanamycin derivatives **2-6**

Table 2: Cytotoxicity activity (IC_{50}) of geldanamycin (**1**) and dopamine-geldanamycin hybrids (**2-6**)

	IC_{50}[a] (μg mL^{-1})	
Compounds	LLC-MK2[b]	Vero
1	45.61	54.25
2	397.84	104.52
3	429.17	181.00
4	496.31	376.70
5	458.95	364.31
6	221.19	369.44

[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

was between 0.386 and 5.464 mM, approximately 2.53-35.94 times higher than that of GDM. These data suggest that the conjugation of a dopamine moiety to GDM at the C17 position greatly enhanced their water solubility.

GDM and DGH were evaluated for cytotoxicity activity against LLC-MK2 and Vero cell lines using the MTT assay. All DGH exhibited weak cytotoxicity activity toward LLC-MK2 and Vero cells with IC_{50} values of >100.00 μg mL^{-1} (Table 2). The results show that the novel DGH possesses low toxicity to normal cells compared with GDM.

The effect of GDM and its derivatives on influenza virus propagation was evaluated at various concentrations in embryonated chicken eggs. Virus yields were determined by the hemagglutination test. Virus propagation was obtained in the control and compound 5 treatments at the highest final concentration (50 μg mL^{-1}), while no virus was detected in compounds **1, 2, 3, 4** and **6** treatments at the lowest final concentration (6.25 μg mL^{-1}). Besides, the effect of GDM and DGH on viral adsorption to chicken erythrocytes was carried out. Interestingly, as expected compounds **2** and **3** inhibited viral binding to the cells with an HAI titre of 1:50, while GDM and compounds **1, 4, 5** and **6** could not inhibit viral binding to chicken red blood cells. These data suggested that GDM and DGH (except compound **5**) inhibited influenza virus propagation, but some of them (compounds **2** and **3**) could inhibit the viral adsorption (early step) of influenza virus infection. Heparin at a concentration of 20 μg mL^{-1} could completely inhibit both viral propagation and viral absorption (data not shown). The results show that the novel DGH can display potential application in antiviral chemoprevention and chemotherapy.

MSA indicated that all of the vertebrate Hsp90 sequences, exhibited 99.53% similarity (Fig. 3). According to domain analysis, all of the Hsp90 sequences contained the domain PS00298 (Prosite entry), which was conserved throughout the Hsp90 family and all such domains possessed a similar ATP binding site (Fig. 4). Subsequently, Hsp90; PBD ID: 1OSF was selected for molecular docking studies. Comparative docking of 1OSF with 17-DMAG, GDM and DGH was carried out to provide evidence in support of their *in vivo* antiviral activity.

The results of docking studies (as shown in Table 3) revealed that 17-DMAG participated in interactions through six hydrogen bonds with Asp54, Lys58, Asp93, Asn106, Lys112 and Phe138 to the N-terminal domain pocket of Hsp90 (Fig. 5a), with a binding energy of -145.307 kcal mol^{-1}. Geldanamycin formed five hydrogen bonds with Lys58, Asp93, Asn106, Lys112 and Phe138 to the N-terminal domain pocket of Hsp90 (Fig. 5b), with a binding energy of -141.296 kcal mol^{-1}. Compound **2** formed two hydrogen bonds with Asn106 and Gly137 to the N-terminal part of the domain pocket (Fig. 5c), with a binding energy of -101.67 kcal mol^{-1}. Compound **3** formed four hydrogen bonds with Lys58 (two positions), Gly132 and Gly137 to the N-terminal part of the domain pocket (Fig. 5d), with a binding energy of -102.36 kcal mol^{-1}. Compound **4** formed two hydrogen bonds with Met12 and Glu14 to the N-terminal part of Hsp90 (outside active site) (Fig. 5e), with a binding energy of -114.28 kcal mol^{-1}. Compound **5** did not form a hydrogen bond to any part of the Hsp90 molecule; however, its binding energy was -100.71 kcal mol^{-1}, nearly equal to that of compound **6**. This interaction may occur through several mechanisms, including van der Waals forces, hydrophobic interactions, etc. Compound **6** formed two hydrogen bonds with Glu47 and Gly137 to the N-terminal part of Hsp90, with a binding energy of -100.50 kcal mol^{-1}. Figure 5 displays the 2D interactions of the compounds and Hsp90 obtained from molecular docking using Ligplot. As can be seen, that 17-DMAG formed a higher number of hydrogen bonds than the other compounds (Fig. 5a). As expected, we observed that 17-DMAG (Fig. 6a) and geldanamycin (Fig. 6b), the binding site of Hsp90 was identical, they participated in interactions to the active site of Hsp90 N-terminal domain, while compounds **2** (Fig. 6c), **3** (Fig. 6d) partly bound in the active site of Hsp90 N-terminal domain. To display the docking results, that compound **4** participated in interactions outside the active site of the N-terminal part of Hsp90 and compound **5** did not form a hydrogen bond to any part of the Hsp90 molecule, they were carried out using the BIOVIA Discovery Studio as shown in Fig. 6e-f, respectively. Compound **6** is also partly bound in the active site of the Hsp90 N-terminal domain (Fig. 6g). The

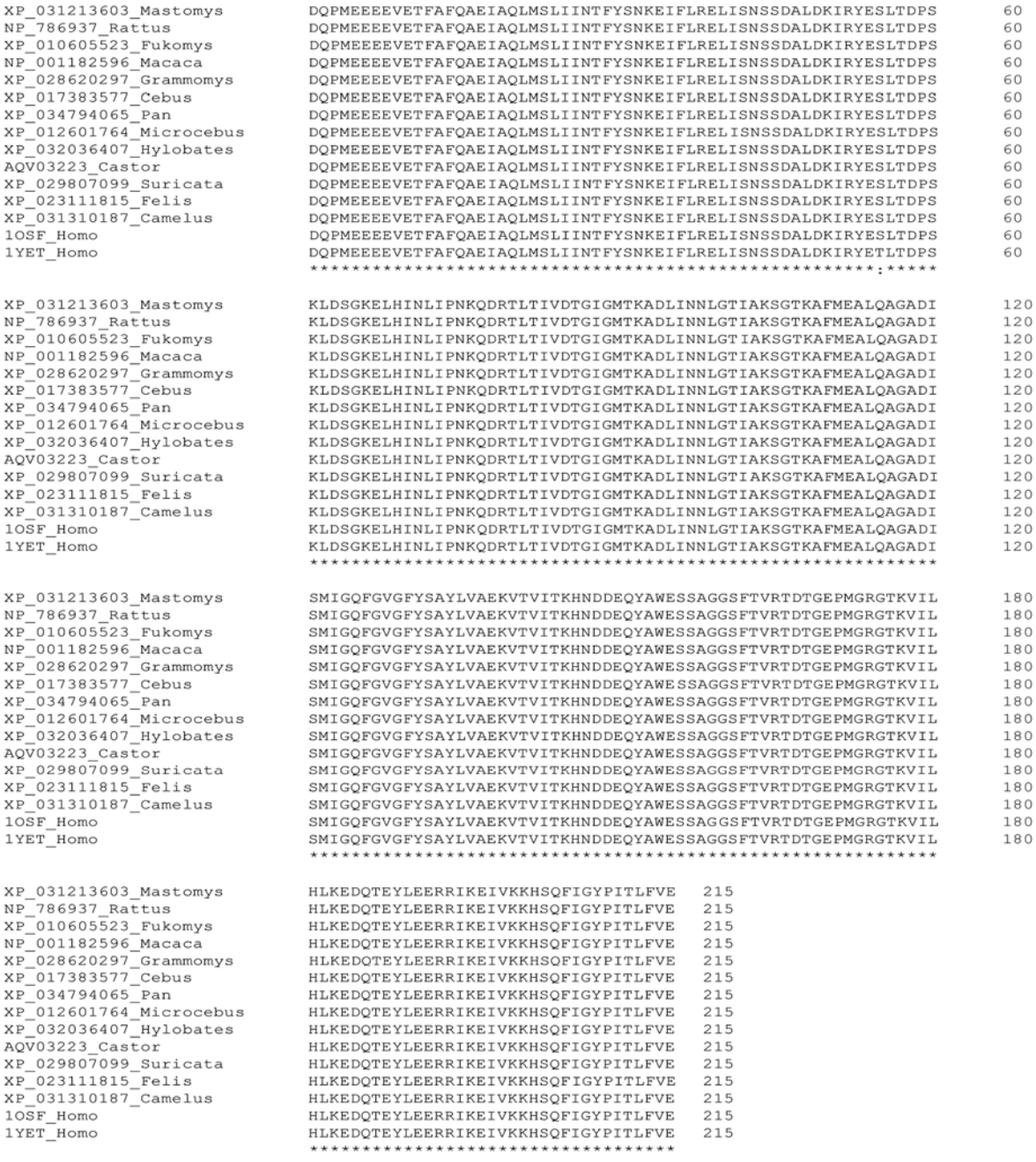

Fig. 3: MSA generated for Hsp90 sequences of fourteen vertebrates showing the conserved regions

result of Fig. 7 shows the hydrogen bond acceptors and hydrogen bond donors and how the ligand is set inside the cavity. The green patches present denote the hydrogen bond acceptor and the pink patches represent the hydrogen bond donor and it shows how the ligand is present inside the cavity. As can be seen, that 17-DMAG (Fig. 7a) and geldanamycin (Fig. 7b) participated in interactions to the active site, compounds **2** (Fig. 7c), **3** (Fig. 7d) partly bound in the active site and compound **4** (Fig.7e) participated in interactions outside active site, these compounds exhibit the hydrogen bond acceptors and hydrogen-bond donors by docking results of Hsp90, while compound **5** did not participate in the hydrogen bond acceptor or a donor to any part of the Hsp90 molecule (Fig. 7f). Compound **6** partly bound in the active site and also exhibit the hydrogen bond acceptors and hydrogen-bond donors by docking results of Hsp90 (Fig. 7g).

Fig. 4: Domain analysis of Hsp90 sequence revealed that the domain PS00298 (30th-39th amino acids) is conserved in all Hsp90 family, has a similar ATP binding site in all the sequences

Table 3: Geldanamycin and dopamine-geldanamycin hybrids to inhibit Hsp90 protein based on the molecular docking simulation

		Conventional hydrogen bond		
Compounds	$\Delta G_{binding}$ (kcal mol^{-1})	H-donors	H-acceptors	Docking site
17-DMAG[a]	-145.307	17-DMAG: H38	1OSF: ASP93:OD2	In active site
		1OSF:LYS58:HZ2	17-DMAG:O5	
		17-DMAG:H11	1OSF: ASP54:OD2	
		1OSF:ASN106:HD21	17-DMAG:O6	
		1OSF:LYS112:HZ1	17-DMAG:O9	
		1OSF:PHE138:HN	17-DMAG:O1	
1	-141.296	Compound 1:H38	1OSF: ASP93:OD2	In active site
		1OSF:LYS58:HZ2	Compound 1:O5	
		1OSF:ASN106:HD21	Compound 1:O6	
		1OSF:LYS112:HZ1	Compound 1:O9	
		1OSF:PHE138:HN	Compound 1:O1	
2	-101.67	1OSF:ASN106:HD21	Compound 2:O2	Partly bound in active site
		1OSF:GLY137:HN	Compound 2:O4	
3	-102.36	1OSF:LYS58:HZ1	Compound 3:O3	Partly bound in active site
		1OSF:LYS58:HZ1	Compound 3:O9	
		Compound 3: HN2	1OSF: GLY132:O	
		1OSF:GLY137:HN	Compound 3:O4	
4	-114.28	Compound 4:HN3	1OSF:MET12:O	Outside active site
		1OSF:GLU14:HN	Compound 4:O7	
5	-100.71	-	-	Did not bind to the active site
6	-100.50	Compound 5:H40	1OSF: GLU47:OE1	Partly bound in active site
		1OSF:GLY137:HN	Compound 5:O4	

[a]17-DMAG: 17-dimethylamino ethylamino-17-demethoxy geldanamycin

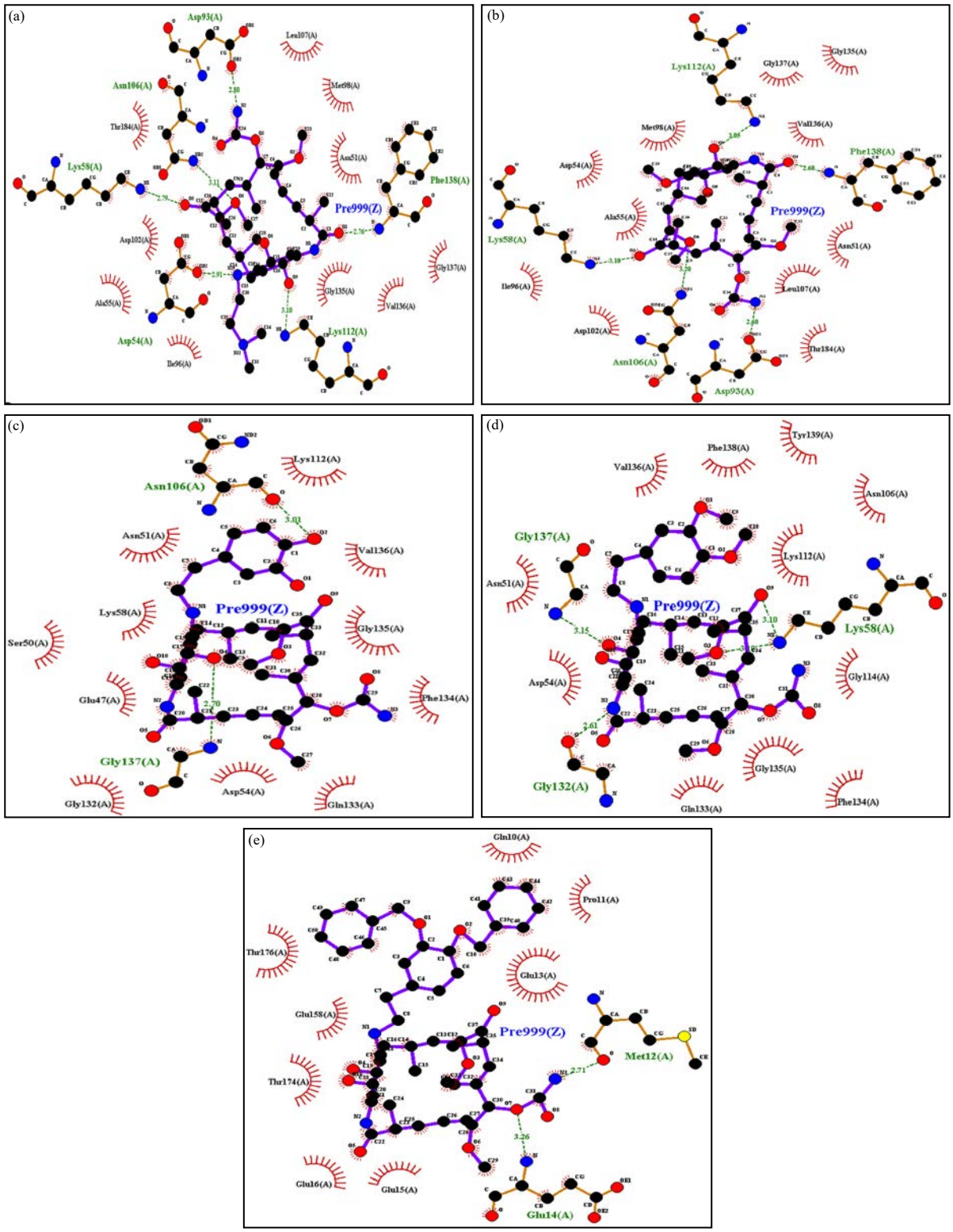

Fig. 5(a-e): Ligplot showing hydrogen bonding interactions (with green dashed lines) and hydrophobic contacts (red arcs with radiating lines) for the ligand

(a) 17-DMAG and (b-e) Compounds **1-4** with Hsp90 molecule, respectively

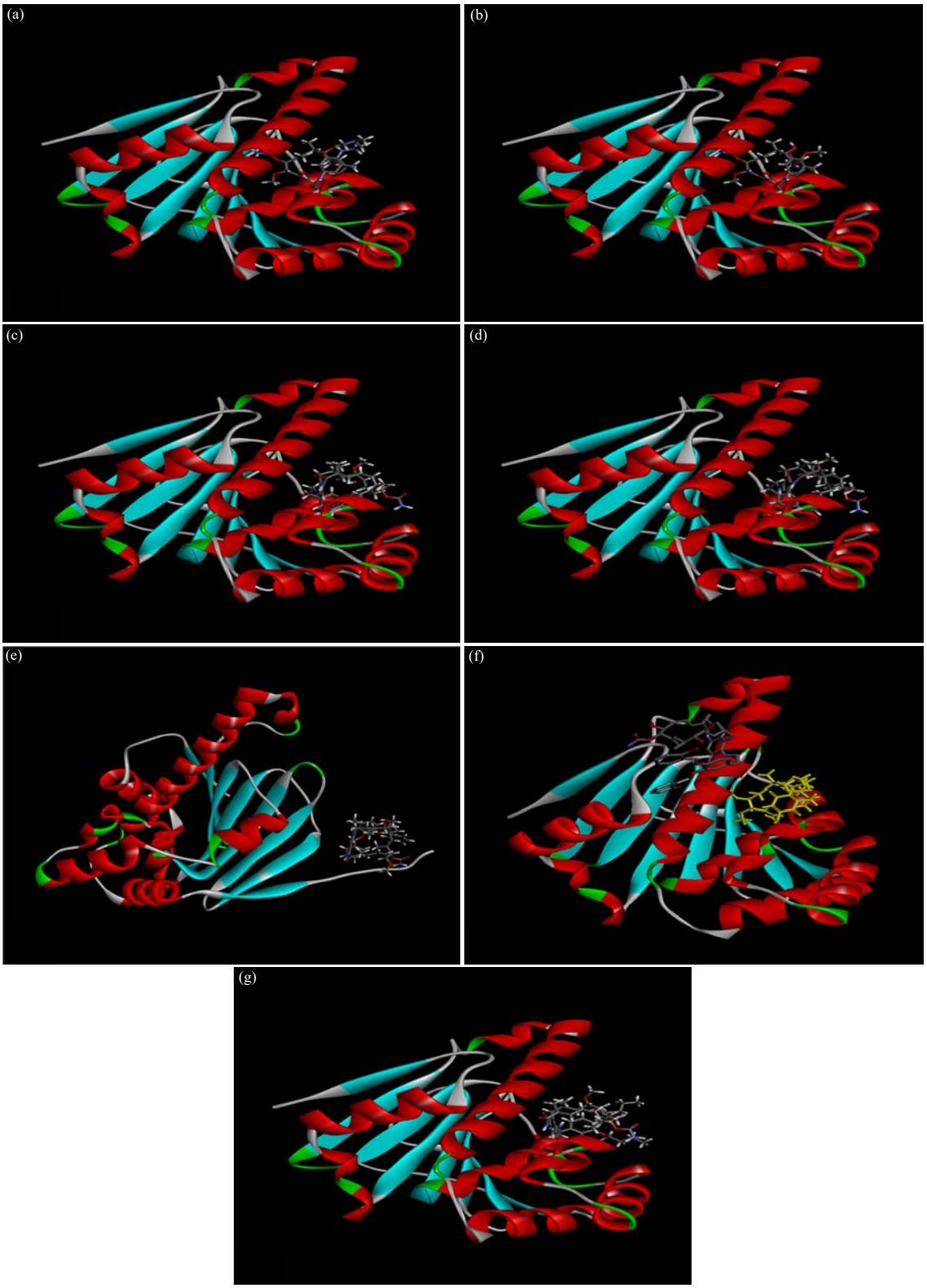

Fig. 6(a-g): Docked ligands with Hsp90 (1OSF)

(a) 17-DMAG, (b) Compound **1**, (c) Compound **2**, (d) Compound **3**, (e) Compound **4**, (f) Compound **5** and (g) Compound **6**. In the case of compound **5**, the brown molecule was represented by geldanamycin (f)

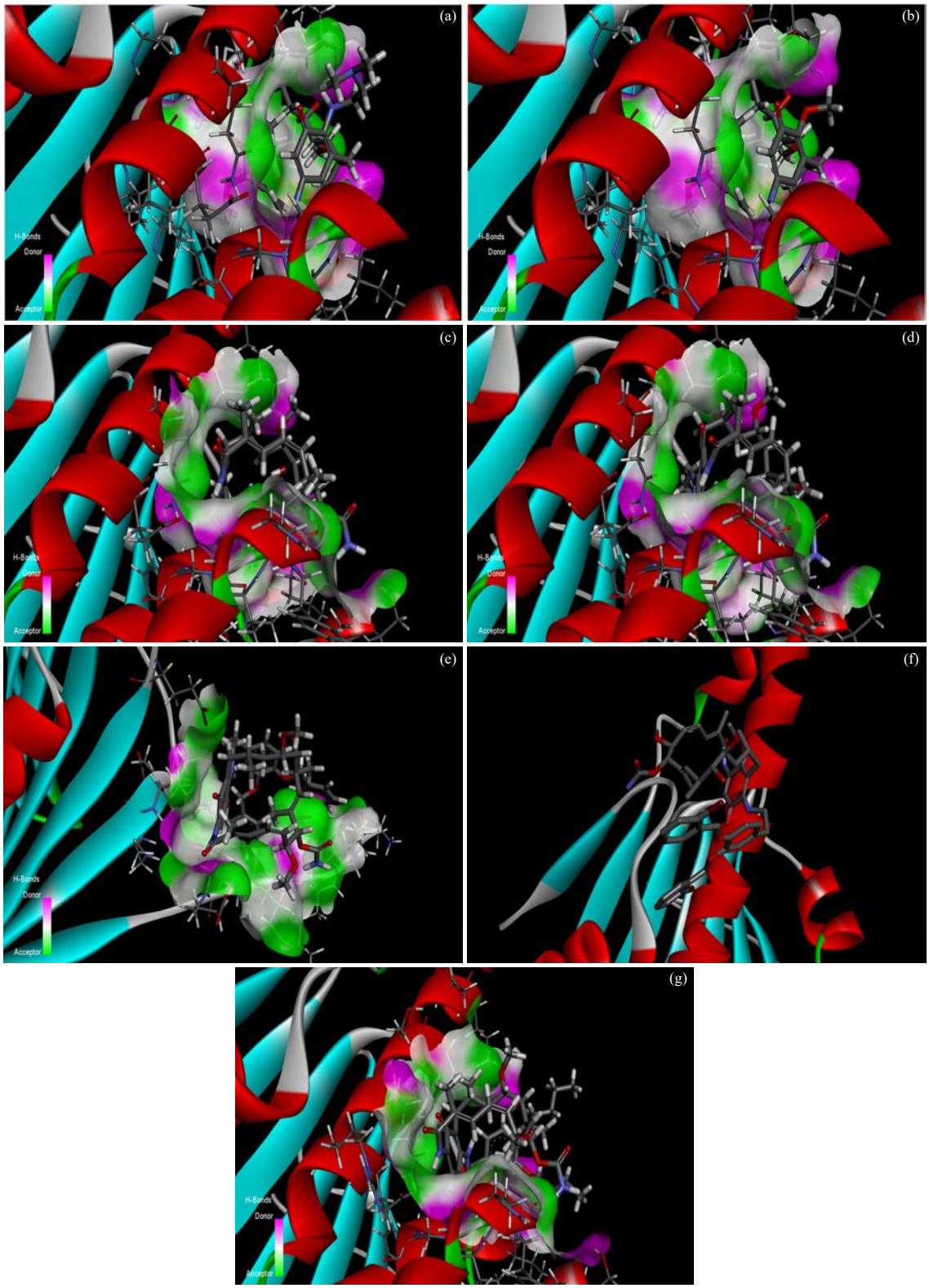

Fig. 7(a-g): Hydrogen bond acceptor and hydrogen bond donor interactions of docked ligands with Hsp90 (1OSF)
(a) 17-DMAG, (b) Compound **1**, (c) Compound **2**, (d) Compound **3**, which were docked in the part of the active pocket site, (e) Compound **4** molecule was docked at the outside of the active pocket site, (f) Compound **5** molecule did not bind to any part of Hsp90 and (g) Compound **6** was docked in the part of the active pocket site. The green patches present denote the hydrogen bond acceptor and the pink patches represent the hydrogen bond donor

DISCUSSION

Influenza virus causes seasonal outbreaks in temperate regions, along with an increase in disease and mortality rates, which poses a serious health problem. With the expectation of exploiting the potency of the Hsp90 inhibitor against the influenza virus, we investigated *in vitro* inhibitory activity of GDM and its derivatives against the influenza virus, as they were promising candidates *in vitro*. The toxicity and water solubility of GDM has been a marked hindrance to its development for chemotherapy use. These have incentivized scientists to turn their attention to developing less toxic GDM derivatives. One such compound, 17-allylamino-17-demethoxy geldanamycin has been reported to be a less toxic Hsp90 inhibitor for the treatment of tumors and has been enrolled in multiple clinic trials[22]. In our study, the synthesized DGH exhibited less cytotoxicity than GDM in the normal cell lines and showed a greater increase in water solubility. We also found that some of these compounds inhibited influenza virus propagation in embryonated chicken eggs. It should be mentioned that GDM and some of its derivatives have antiviral activity, which suggests that GDM and some of its derivatives are optional choices in terms of antiviral agents in the viral propagation step.

The introduction of dopamine or dopamine-derivative group (except in compounds **4** and **5**) at the C17-position of GDM did not interfere with the binding of GDM derivatives to Hsp90 but greatly decreased their toxicity and increased their water solubility. As indicated by the crystal structure of the geldanamycin-Hsp90 complex[23], substitution of the C17 methoxyl of GDM is revealed to the external cavity of the Hsp90 protein, while the difference of substituents of GDM are crucial for the interaction with the Hsp90 protein which according to the report by Li *et al.*[7], that 17-amino-17-demethoxy geldanamycin derivatives have a great potential for antiviral activity, while the 19-substituted geldanamycin modification was not a possibility in terms of antiviral agents. The introduction of a group at the C19-position of GDM could interfere with the binding of GDM derivatives to Hsp90 due to steric effects[7]. These results led us to contemplate that Hsp90 could be a target for antiviral infection and that geldanamycin and some of its derivatives have great potential for antiviral propagation by interfering with Hsp90 in the protein folding and stabilization of virus-infected cells.

The development of DGH was carried out through nucleophilic substitution reactions at the C17-position of GDM. Some of these compounds inhibited not only viral propagation but also viral absorption, which suggests that DGH could protect against viral infection at both steps. To this end, Sarkar *et al.*[24] reported that dopamine not only affects behavior, movement, endocrine, cardiovascular, renal and gastrointestinal functions but also regulates the immune system. Dopamine receptors are expressed on almost all immune cells. Activation of such receptors by dopamine or its agonists has been reported to modulate activation, proliferation and cytokine production in immune cells[25,26]. This study discovered the effects of GDM and some DGH (compounds **2, 3, 4** and **6**) on inhibition of influenza virus propagation and viral adsorption (compounds **2** and **3**). These properties are due to structural relationships between dopamine moieties in the GDM molecule. For the synthesized derivatives, our results are in good agreement with those of previous reports[27,28], that the dopamine moiety increases water solubility. Moreover, the length of the conjugation plays a crucial role in the activity of the synthesized derivatives[29,30]. This study will help the researcher to uncover structural modifications of compounds to improve their biological activities.

The DGH contained both stronger and weaker antiviral activity than GDM, but all had greatly decreased toxicity and increased water solubility. According to the crystal structure of the geldanamycin-Hsp90 comples[23], substituents at the C17-position of GDM are not crucial for the interaction of the test compounds with the Hsp90 protein. However, if an introduced molecule is too large (compounds **4** and **5**), it may not fit the active site pocket. In the case of compound **4**, it has antiviral activity, but it binds outside the active site of the Hsp90 molecule, while compound **5** cannot bind to any part of the Hsp90 molecule, so it has no antiviral activity. This observation also helps us to understand why there was obvious regularity observed between antiviral activity and structural differences in the C17-position, which was in good agreement with the molecular docking study. Noticeably, compounds **2, 3, 4** and **6** demonstrated potent antiviral activity against the influenza virus. This led us to speculate that Hsp90 could be an antiviral target and that some DGH have antiviral activity because they interfere with chaperone assistance in the protein folding and stabilization processes of viral proteins. Moreover, compounds **2, 3** and **6**, possessed Hsp90-binding ability that was almost similar to that of GDM and 17-DMAG. However, the hydrogen bonding interactions of these molecules with Hsp90 were less than those of GDM and 17-DMAG.

CONCLUSION

In summary, novel DGH with antiviral activity, low toxicity and enhanced water-solubility were presented in this work, in comparison with GDM. In particular, compounds **2, 3, 4** and

6 showed antiviral activity in terms of viral propagation; moreover, compounds **2** and **3** inhibited not only viral propagation but also viral absorption and had low toxicity and good water solubility in comparison with GDM. These results show these compounds can inhibit the functions of Hsp90 and hence, virus propagation. This suggests a new antiviral approach. Therefore, Hsp90 could be an excellent antiviral target and some DGH could be considered a new choice for antiviral agents.

SIGNIFICANCE STATEMENT

In this study, novel DGH were synthesized with improved solubility and biological activities. Some DGH affected antiviral activity against influenza virus propagation in embryonated chicken eggs and the results were supported by molecular docking studies. This study will help researchers to uncover novel GDM derivative compounds as potential alternative agents for treatments of influenza virus infection.

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