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Inclusion complex of GA-13316 with β-cyclodextrin: Preparation, characterization, molecular modeling, and *in vitro* evaluation

Rui Yang[a,b,1], Jing-Bo Chen[a,1], Chuan-Fan Xiao[a], Zhi-Cheng Liu[b], Zhan-Yong Gao[a], Sheng-Jiao Yan[a], Ji-Hong Zhang[b,*], Hong-Bin Zhang[a,*], Jun Lin[a,*]

[a] *Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, PR China*
[b] *Faculty of Science, Kunming University of Science and Technology, Kunming 650093, PR China*

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ABSTRACT

The inclusion complex of GA-13316 with β-cyclodextrin (β-CD) is one of a unique series of gibberellin derivatives possessed of potential anticancer activities. The complex with β-CD was characterized by means of UV, XRD, DSC, TG, ^{1}H, and 2D NMR spectroscopy. In addition, we investigated the main aspects of the interaction between GA-13316 and β-CD using both experimental and molecular modeling approaches. The complex still maintained its anticancer activity, as shown by *in vitro* cell survival assay on the human colon carcinoma cell line (HCT116) and the human lung cancer cell line (H460). The results showed that the use of β-CD could be obviously improved the water solubility and stability of GA-13316, implying that the inclusion complex could be a promising future therapeutic agent.

1. Introduction

Cancer is currently a challenging disease for medical professionals and, therefore, developing anticancer drugs has always been a central task in Drug Research and Development. It was reported that a series of gibberellins-based molecules were designed and synthesized which started from gibberellic acid (GA_3), and they showed strong anticancer activities in a number of human cancer cell lines by MTT assay (Chen et al., 2009). The results showed that there were high levels of activities for compounds with the α,β-unsaturated ketone unit present in both the A-ring and the D-ring. Despite these high activities, the poor aqueous solubility and stabilization limited the usage of the gibberellin derivative as anticancer drugs.

Cyclodextrins (CDs) are cyclic oligosaccharides that consist of (α-1,4)-linked α-D-glucopyranose units with a lipophilic central cavity and a hydrophilic outer surface (Connors, 1997; Szejtli, 1998, 2004). The inclusion complexation of drug molecules with CDs usually results in favorable changes in the physicochemical properties, such as solubility, dissolution rate, stability, and bioavailability, of the drug (Liu et al., 2013; Qiu et al., 2014; Zhang et al., 2013), thus making them more suitable for delivery. Recently, we reported one of the gibberellin derivatives (GA-13315) was complexed with cyclodextrins, which resulted in improvement of the physiochemical properties of GA-13315, especially in solubility and stability (Yang et al., 2012). Another gibberellin (13-chlorine-3,15-dioxygibberllic acid *p*-methoxybenzyl ester, GA-13316) was also found possess high activities, the only structure difference is formed at C-7, that the methyl ester is GA-13315, and *p*-methoxybenzyl ester is GA-13316 (Fig. 1). Because of the good results we obtained from GA-13315/CDs, and also curiously to find whether the slight difference in structure will or will not result to the difference in molecular recognition, we attempted to prepare the complexes and study the physiochemical properties of GA-13316/CDs.

In this experiment, a new inclusion complex was formed by β-cyclodextrin and GA-13316. We were particularly interested in exploring the solubilization effect of β-CD on GA-13316 and the binding ability of the resulting inclusion complex, which would provide a useful approach for obtaining novel GA-13316-based pharmaceutical drugs with high water solubility, high bioavailability, and low toxicity. Additionally, we performed a molecular

* Corresponding authors. Tel.: +86 871 503 3215; fax: +86 871 503 3215.
E-mail addresses: zhjihong2000@yahoo.com.cn (J.-H. Zhang), zhanghb@ynu.edu.cn (H.-B. Zhang), linjun@ynu.edu.cn, linjunyn@yahoo.com.cn (J. Lin).
[1] These authors contributed equally to this paper.

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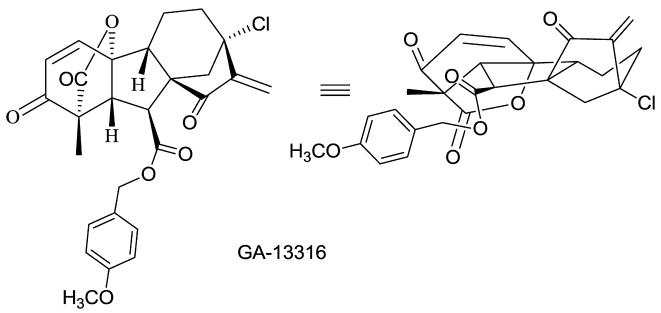


Fig. 1. The structure of GA-13316.

modeling study of the interaction between GA-13316 and β-CD to elucidate the supramolecular structure of the complex and as complementary evidence to support the experimental studies.

2. Experimental

2.1. Materials

Pure GA-13316 (FW = 496), obtained from GA_3 by a semi-synthetic route (Chen et al., 2009), was supplied by Prof. Zhang's group. β-CD (average substitution degree = 1135) was purchased from ABCR GmbH & Co. KG and used without further purification. Other reagents and chemicals were of analytical reagent grade. All experiments were performed using ultrapure water.

2.2. Apparatuses

NMR spectra were conducted on a Bruker Avance DRX spectrometer at 500 MHz and 298 K in D_2O. The one-dimensional spectra of both solutions were run with an FID resolution of 0.18 Hz/point. The residual HDO line had a line width at a half-height of 2.59 Hz. Two-dimensional (2D) ROESY spectra were acquired at 298 K with presaturation of the residual water resonance and a mixing (spin-lock) time of 350 ms at a field of ~2 kHz using the TPPI method, with a 1024 K time domain in F2 (FID resolution 5.87 Hz) and 460 experiments in F1. Processing was carried out with zero-filling to 2 K in both dimensions using sine (F2) and qsine (F1) window functions, respectively.

The UV–vis spectrum was recorded on a Shimadzu UV 2401 (Japan) equipped with a conventional 1 cm path (1 cm × 1 cm × 4 cm) quartz cell in a thermostated compartment, which was kept at 25 °C by a Shimadzu TB-85 Thermo bath unit.

A powder X-ray diffraction spectrum was taken using a Rigaku TTRIII Rotating Target diffractometer with Cu Kα radiation (40 kV, 100 mA) at a scanning rate of 5° min^{-1}. Powder samples were mounted on a vitreous sample holder and scanned with a step size of $2\theta = 0.02°$ between $2\theta = 3°$ and 50°, except the physical mixture $2\theta = 10°$ and 50°.

Differential scanning calorimetry (DSC) and thermogravimetric (TG) measurements were conducted on a 2960 SDT V3.0F instrument and NETZSCH STA 449F3, respectively, and 3–3.5 mg of each sample was heated at a rate of 10 °C/min from room temperature to 400 °C under dynamic nitrogen atmosphere and at a flow rate of 70 mL/min.

2.3. Preparation of GA-13316/β-CD

CD was used in excess to improve the yield of the complex. GA-13316 (0.02 mM, 9.9 mg) and CD (0.01 mM) were completely dissolved in a mixture of ethanol and water (ca. 7 mL, V:V = 1:5), and the mixture was stirred for 5 days at room temperature. Ethanol was used due to the poor water solubility of GA-13316. After evaporating the ethanol from the reaction mixture, the uncomplexed GA-13316 was removed by filtration. The filtrate was evaporated under reduced pressure at 37 °C to remove the solvent and then dried in a vacuum to produce the GA-13316/β-CD complex. GA-13316/β-CD complex (yield 92%): ^1H NMR (500 MHz, D_2O, TMS): δ 7.24–7.30 (d, 3H, aromatic and H-1′, 2′ of A ring protons for GA-13316), 6.91–6.92 (d, 2H, aromatic of GA-13316), 6.12 (s, H-17 of GA-13316), 5.71 (s, H-17 of GA-13316), 5.01–5.02 (s, 7H, H-1 of β-CD and part protons for GA-13316), 3.40–3.90 (m, 47H, H-2–6 of β-CD and part protons for GA-13316), and 1.28–3.43 (m, 12H, H-6′, 9′, 11′, 12′, 14′ of B, C and D ring protons for GA-13316).

2.4. Preparation of GA-13316/β-CD physical mixture

The physical mixture, to test for possible inclusion, was performed by mixing the powders in a 1:1 molar ratio of GA-13316 and β-CD in an agate mortar.

2.5. Phase-solubility diagram

Phase-solubility studies were performed as described by Higuchi and Connors (Higuchi & Connors, 1965). An excess amount of GA-13316 relative to the most concentrated β-CD solution was added to β-CD solutions of increasing concentrations (0.1058–0.6526 mM). The mixtures were stirred for 48 h at room temperature protected from light. After equilibrium was achieved, the solution was filtered through a 0.45 μm membrane filter and the concentration of GA-13316 in solution was determined using a UV spectrophotometer at 265 nm and by comparing the absorbance with a standard curve of pure GA-13316. The β-CD did not interfere with UV measurements. This experiment was carried out in triplicate. The apparent stability constant (K_c) of the GA-13316/β-CD complex was calculated from the phase-solubility diagram according to the following equation:

$$K_c = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the solubility of GA-13316 in the absence of β-CD and "slope" is the corresponding slope of the phase solubility diagram.

2.6. Aqueous solubility of GA-13316/β-CD

An adequate amount of the complex was added to 2 mL water (ca. pH 6.0) to ensure the solution reached saturation under nitrogen, and the mixture sheltered from light and was stirred for 1 h at 20 ± 2 °C. Then, the remaining solid in the solution was removed by filtration through a 0.45 μm cellulose acetate membrane. The filtrate was evaporated under reduced pressure to dryness and the residue was dosed by the weighing method.

2.7. Molecular modeling

In order to investigate and confirm the inclusion behavior of guest (GA-13316) into host (β-CD), a CHARMm-based MD Docking algorithm (Wu, Robertson, Brooks, & Vieth, 2003) was performed using the Discovery Studio 3.5 Client and the originally placed DS CDOCKER module.

Cartesian coordinates of the host (β-CD) and the guest (GA-13316) were extracted and built from the ChemBioOffice Ultra 12.0. Subsequently, the preliminary DFT calculation for optimizing all species was carried out using B3LYP functional (Becke, 1993; Lee, Yang, & Parr, 1988; Miehlich, Savin, Stoll, & Preuss, 1989) and the 6-311G (d,p) basis set (Hariharan & Pople, 1973) with the Gaussian 03 program (Frisch et al., 2004). To mimic the inclusion mode, β-CD and GA-13316 were separately defined as receptor or ligand, and

then the binding site of β-CD was specified by a site sphere at the centroid of the narrow rim with a radius of 6 angstrom. Simultaneously, the CHARMm force field was applied to the GA-13316. After performing the docking experiments, the docked complex of the host-guest was selected according to the CDOCKER energy and CDOCKER interaction energy, and combined with geometrical matching quality.

2.8. Measurement of cytotoxicity (Chen et al., 2008)

The cytotoxicity of the samples on tumor cells was measured by (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. HCT116 (a human colon carcinoma cell line) and H460 (a human lung cancer cell line) were seeded into 96-well microculture plates. For adherent cells (HCT116), they were allowed to culture 24 h to allow for adhesion before the addition of the samples, while suspended cells (H460) were seeded just before the addition of the samples. The cell densities were selected based on the results of preliminary tests to ensure that the control cells were in an exponential phase of growth during the experiment and to obtain a linear relationship between the optical density and the number of viable cells. The tumor cells were exposed to 1.0, 10 and 100 μM of the samples for either 72 h for the adherent cells or 48 h for the suspended cells. Each concentration was tested in triplicate. At the end of exposure, 20 μl of 5 mg/ml MTT (Sigma chemical Co) was added to each well and the plates were incubated for 4 h at 37 °C. Then, triplex solution (10% SDS, 5% isobutanol, 0.012 M HCl) was added to the wells and the plates were incubated for 12–20 h at 37 °C. The optical density (OD) was read using a plate reader at 570 nm. DMSO was used as the solvent for GA-13316, injection water was used as the solvent for the other samples (GA-13316/β-CD) and cis-dichlorodiamineplatimum (II) (DDP, positive control). DMSO and injection water control wells, in which there was no sample, were included in all of the experiments in order to eliminate the influence of solvents. The inhibitory rate of cell proliferation was calculated using the following formula:

$$\text{growth inhibition}\,(\%) = \left(\frac{\text{OD}_{\text{control}} - \text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}}\right) \times 100\%.$$

The cytotoxicity of the samples on the tumor cells was expressed as IC_{50} values, which is the sample concentrations that reduced the absorbance of treated cells by 50% with respect to untreated cells, and were calculated by the LOGIT method.

2.9. Stability test

Comparative tests involving the stability of aqueous solutions of free and GA-13316 complexed with β-CD was tracked using the absorbance changes at 265 nm by means of the UV spectra obtained at room temperature (during 20 d, all experiments were carried out in triplicate at predetermined time intervals). The concentration of free GA-13316 is 2.4 μM, and GA-13315/β-CD complex is 6.8 μM. The results were expressed as percentages of the remaining GA-13316, i.e. the $A/A_0 \times 100$ ratio, where A_0 is the initial concentration of GA-13316 alone or the GA-13316/β-CD complex and A is the concentration at the specified time interval.

3. Results and discussion

3.1. Phase-solubility diagram

As shown in Fig. 2, there was a linear increase in GA-13316 solubility with increasing concentrations of β-CD, indicating the formation of a soluble complex between GA-13316 and β-CD. The phase solubility curve obtained can be classified as a typical A_L type (Higuchi & Connors, 1965). Since such a profile was characterized by a slope of less than one, it was assumed that the solubility increase was due to the formation of 1:1 complex (Higuchi & Connors, 1965). The K_c was calculated from the slope of the phase-solubility diagram according to Eq. (1), where the S_0 is the intrinsic water solubility of GA-13316 (S_0 = 1.2 mg/L, 2.4 μM). K_c was calculated to be 21,100 M^{-1}. This high K_c value indicates that the inclusion complex formed between GA-13316 and β-CD is quite stable.

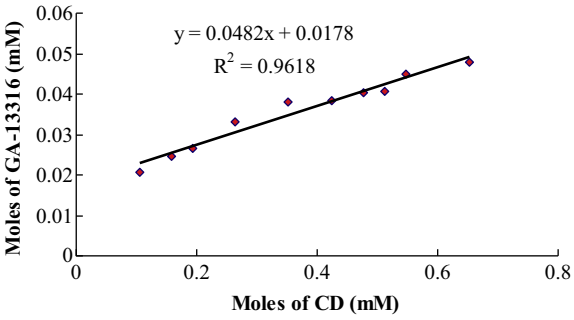


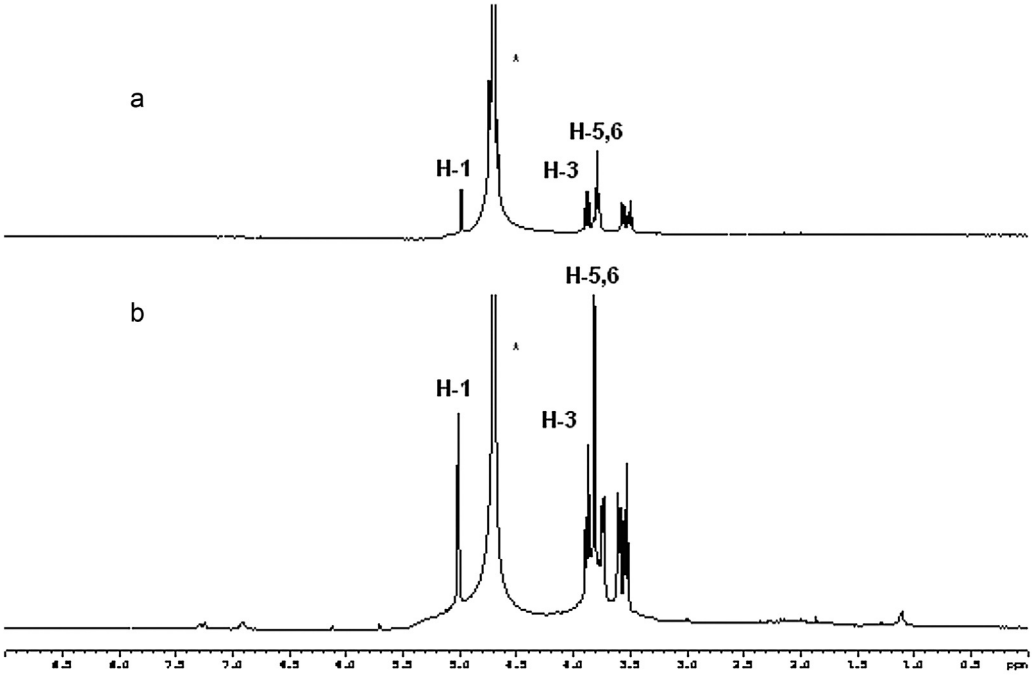
Fig. 2. Phase solubility of β-CD with GA-13316.

3.2. ^1H and 2D NMR analysis

In order to compare GA-13316 and GA-13315 in molecular recognition, NMR was used to obtain the possible inclusion modes of GA-13316 like GA-13315. The ^1H NMR spectra of β-CD and GA-13316/β-CD in D_2O are shown in Fig. 3a and b. In the spectrum for GA-13316/β-CD inclusion complex, appreciable chemical shift changes were observed with respect to the spectra for the free β-CD. The ^1H chemical shifts of β-CD protons in the absence and presence of GA-13316 are listed in Fig. 3e. Inclusion complexation with GA-13316 had effects on the δ values of the H-1, H-2, H-3, H-4, H-5, and H-6 protons of β-CD exhibited relatively weak, but significant changes (0.01–0.03 ppm), which could have been caused by the hydrogen bond between the hydroxyl arms of β-CD and the oxygen atoms of GA-13316. It is worth noting that the H-5 protons shifted ca. 0.02 ppm, but that the H-3 protons shifted ca. 0.01 ppm after inclusion complexation. Both H-3 and H-5 are situated in the inner part of β-CD, wherein H-3 is on the wide side and H-5 is on the narrow side of the cavity, we can propose from the ^1H NMR data that GA-13316 was entrapped in the β-CD cavity, and should insert into the β-CD cavity from the narrower rim. By comparing the integration area of these protons with that of the H-1 protons of β-CD, we determined that the inclusion stoichiometry of the GA-13316/β-CD complex was 1:1, which is in good correlation with the result obtained through phase-solubility diagram.

Meanwhile we compared the ^1H chemical shifts of GA-13316 protons in the absence and presence of β-CD. The ^1H NMR spectra of GA-13316 in $CDCl_3$ and GA-13316/β-CD in D_2O were shown in the supporting information. Some of the resonances of GA-13316 also shifted upon complex formation, from δ 7.29–7.30 (H-1 and H-Ph of GA-13316), 7.24–7.26 (H-2 of GA-13316), 6.91–6.92 (H-Ph of GA-13316), 6.12 (H-17 of GA-13316), 5.71 (H-17 of GA-13316), 1.28–1.29 (H-18 of GA-13316) in the free compound to δ 7.19–7.21 (H-1 and H-Ph of GA-13316), 6.84–6.87 (H-Ph of GA-13316), 6.20 (H-17 of GA-13316), 6.09–6.11 (H-2 of GA-13316) 5.87 (H-17 of GA-13316) and 1.35 (H-18 of GA-13316) ppm. Although the displacement of the signals could be attributed to the inclusion of the phenyl moiety of GA-13316 into the hydrophobic cavity of β-CD, it is important to note that the use of different solvent systems ($CDCl_3$ versus D_2O) must also influence the observed chemical shifts.

The information on the spatial proximity of the molecules in the inclusion complex was confirmed by two-dimensional



c		δ (ppm)	
		β-CD	GA-13316/β-CD complex
H-1	d	4.99	5.02
H-2	dd	3.57	3.58
H-3	dd	3.88	3.87
H-4	dd	3.50	3.48
H-5	m	3.80	3.82
H-6	dd	3.77	3.76

Fig. 3. ^{1}H NMR spectra of GA-13316 in the absence and presence of β-CD in D_2O at 25 °C, respectively: (a) β-CD, (b) GA-13316/β-CD complex (asterisk highlights the water peak); and the chemical shifts (δ) of the β-CD and GA-13316/β-CD (c).

(2D) NMR spectroscopy, since cross-peaks in the ROESY spectra are expected for protons that are closer than 0.4 nm in space (Correia et al., 2002). For this purpose, we obtained 2D ROESY of the inclusion complex of GA-13316 with β-CD. The ROESY spectrum of the GA-13316/β-CD complex (Fig. 4A) showed an appreciable correlation of aromatic protons of GA-13316 with the H-3 and H-5 protons of β-CD. These results indicate that the aromatic ring in GA-13316 was included in the β-CD cavity.

Based on these observations, and together with the 1:1 stoichiometry, we deduced the possible inclusion modes of GA-13316 with β-CD that are illustrated in Fig. 4B. For GA-13315, NMR indicated that the A, B and C ring of GA-13315 was included in the β-CD cavity (Fig. 4C) (Yang et al., 2012). Although it is slight difference of configuration in C-7 of GA-13316 and GA-13315, it is obviously difference in the possible inclusion mode when they are complexed with β-CD.

3.3. Molecular modeling studies

The supramolecular complex of GA-13316 with β-CD was investigated by molecular docking to confirm and rationalize the observed inclusion geometry. To achieve this, head inclusion orientations from the β-CD narrow rim was considered. The 3D structure with the lowest energy and the measured distance of H···H correlation are presented in Fig. 5a and 5b. Since the distance between the aromatic hydrogen (H_a, H_b, H_c, and H_d) of the GA-13316 and the interior wall hydrogen (H_5 and H_3) of β-CD ranged from 2.545 to 3.032 Å, this implied that the weak H···H correlation, which has been confirmed by 2D NMR spectroscopy above, is rational. On the other hand, the intermolecular hydrogen bond, i.e. O—H···O, was generated by the complexion, but the inherent intramolecular hydrogen bond that was located on the wide rim of the β-CD maintained the same pattern *versus* the uncomplexed mode (Fig. 5c). Thus, the newly formed hydrogen bond may favor the contribution

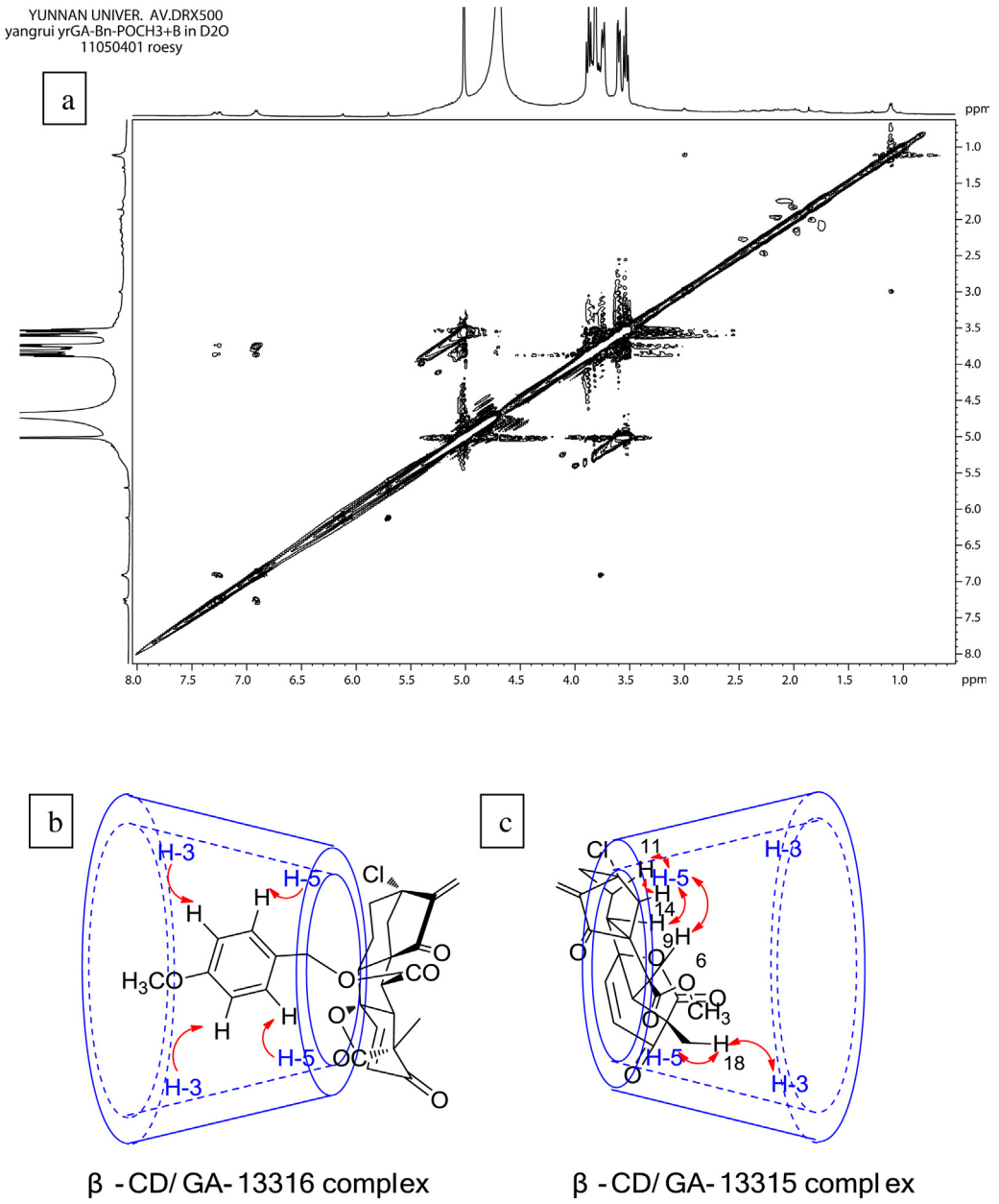


Fig. 4. (a) ROESY spectrum of GA-13316/β-CD complex in D_2O; and (b) possible inclusion mode and significant NOESY (↔) correlations of the GA-13316/β-CD inclusion complex; (c) possible inclusion mode and significant NOESY (↔) correlations of the GA-13315/β-CD inclusion complexes.

of the stability of the host–guest complex. Moreover, the binding energy ($\Delta_b G^\circ$) and the theoretical binding constant (K_b) of the complex were also calculated at room temperature in a gaseous phase (Fig. 5d) using Eqs. (2) and (3), respectively. Finally, the calculated theoretical binding constant ($K_b = 7.66 \times 10^{10}$) hinted at the high stability of the complex, which was in agreement with the K_c of 2.11×10^5. Once again, the docking results agreed well with the experimental data, highlighting the structural outcome of the supramolecular aggregates.

$$\Delta_b G^\circ = \Delta G^\circ(\text{complex}) - \Delta G^\circ(\beta\text{-CD}) - \Delta G^\circ(\text{GA-13316}) \quad (2)$$

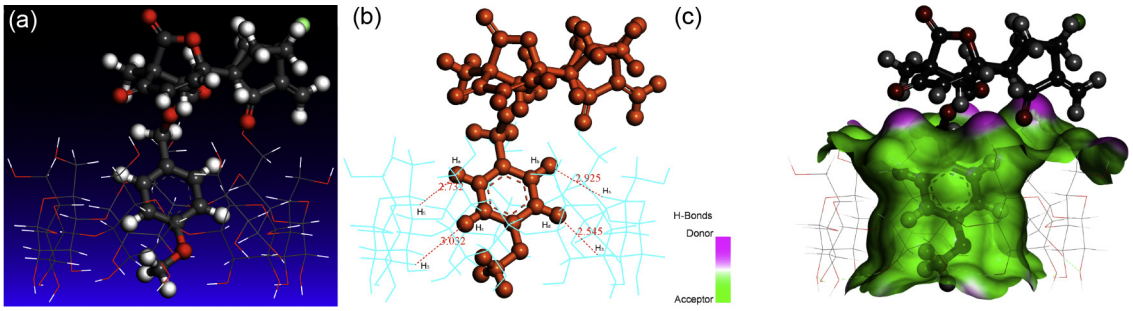
$$K_b = e^{-\frac{\Delta_b G^\circ}{RT}} \quad (3)$$

3.4. XRD analysis

The lack of crystallinity is an added evidence for the formation of inclusion complex. Fig. 6A shows the powder X-ray diffraction (XRD) patterns for GA-13316 (guest), β-CD (host), their solid inclusion complex, and their physical mixture. As indicated in Fig. 6A, β-CD (Fig. 6A(b)) shows several sharp intensity peaks suggesting that β-CD existed as crystalline nature. On the other hand, the GA-13316 was amorphous (Fig. 6A(a)), while a diffuse halo-pattern was recorded for GA-13315/β-CD complex (Fig. 6A(c)) demonstrating its amorphous nature, and the diffraction pattern of the physical mixture (Fig. 6A(d)) is different from the complex (Fig. 6A(c)), indicating that there was an interaction between β-CD and GA-13316.

3.5. DSC analysis

The thermal properties of the GA-13316/β-CD complex were investigated by differential scanning calorimetry (DSC). DSC revealed some information about the solid state interactions between the drug and cyclodextrin. As shown in Fig. 6B, the DSC curve of GA-13316 contained an endothermic peak at 68 °C



(d)

ΔG° or K_b	ΔG°(Complex)	ΔG°(β-CD)	ΔG°(GA-13316)	$\Delta_b G^\circ$	K_b
kcal·mol^{-1}	-42.3967	-17.3529	-10.2049	-14.8389	7.66×10^{10}

Fig. 5. The lowest energy conformation of the inclusion complex (a); a schematic for H···H correlation (b); inter- and intramolecular hydrogen bonds (O—H···O) of the inclusion complex (c); Binding energy ($\Delta_b G^\circ$) and the theoretical binding constant (K_b) of the inclusion complex (d).

(Fig. 6B(a)). In contrast, the DSC curves of pristine β-CD had an endothermic peak at 113 °C (Fig. 6B(b)). However, in the GA-13316/β-CD complex DSC curves, the endothermic peaks at ca. 68 °C, which corresponded to free GA-13316, disappeared. This coincided with the appearance of a new endothermic peak at 73 and 100 °C in the case of GA-13316/β-CD (Fig. 6B(c)). These results further confirm the formation of the GA-13316/β-CD complex.

3.6. TG analysis

The thermal properties of the GA-13316/β-CD complex were investigated by TG methods. A systematic analysis of the TG curves revealed that GA-13316 decomposed at ca. 172 °C and β-CD at ca. 260 °C (Fig. 7A). However, the thermal stability of their inclusion complexes differed, with the decomposition temperature for the GA-13316/β-CD inclusion complex being ca. 230 °C. The results indicate that GA-13316's usual thermal properties were altered after inclusion complexation, and that the GA-13316/β-CD complex possessed a high decomposition temperature, which means that the complex is fairly stable from a thermal viewpoint.

3.7. Solubilization

The water solubility of the GA-13316/β-CD complex was assessed through the preparation of its saturated solution (Montassier, Duchêne, & Poelman, 1997). An excess amount of the complex was placed in 2 mL of water (pH ca. 7.0) and the mixture was stirred for 1 h. After removing the insoluble substance by filtration, the filtrate was evaporated under reduced pressure to dryness and the residue was dosed by the weighing method. The results showed that the water solubility of this GA-13316, when compared to that of free GA-13316 (ca. 1.2 μg/mL), was remarkably increased to approximately 1.1 mg/mL by the solubilizing effect of β-CD. In the control experiment, a clear solution was obtained after dissolving the GA-13316/β-CD complex (3.6 mg), which was equivalent to 1.1 mg of GA-13316, in 1 mL of water at room temperature. This confirmed the reliability of the obtained satisfactory water solubility of the GA-13316/β-CD complex, which will be beneficial for the medical utilization of this compound.

3.8. In vitro cytotoxicity

The cytotoxicity of free GA-13316 and GA-13316 complexed with β-CD on HCT116 and H460 cells was individually evaluated using the MTT assay (Carmichael, DeGraff, Gazdar, Minna, & Mitchell, 1987; Mossman, 1983), with adriamycin (DDP) as the positive control. The IC_{50} values are presented in Table 1. Free GA-13316 displayed superior cytotoxicity when compared to DDP against HCT116 cells. However, cytotoxicity against HCT116 cells displayed that complex still maintain in anticancer activity, while against H460 cells the complex displayed superior cytotoxicity compared to free GA-13316.

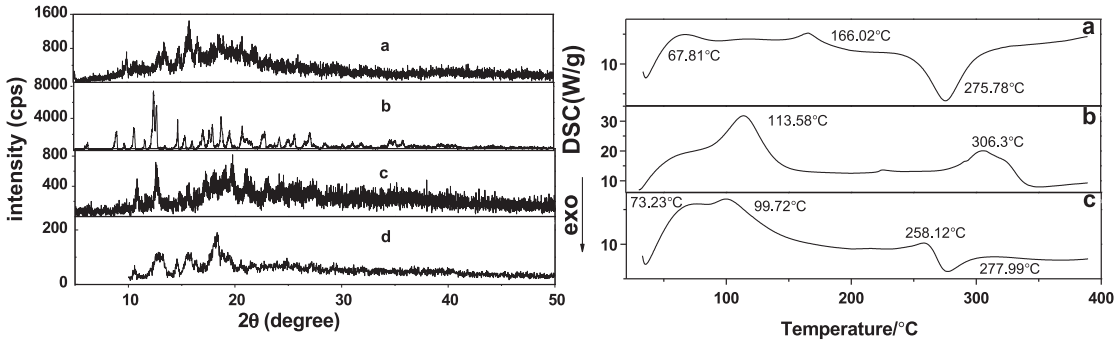


Fig. 6. (A) XRD patterns: (a) GA-13316, (b) β-CD, (c) GA-13316/β-CD inclusion complex, (d) GA-13316/β-CD physical mixture (1:1); (B) DSC thermograms: (a) GA-13316, (b) β-CD, (c) GA-13316/β-CD inclusion complex.

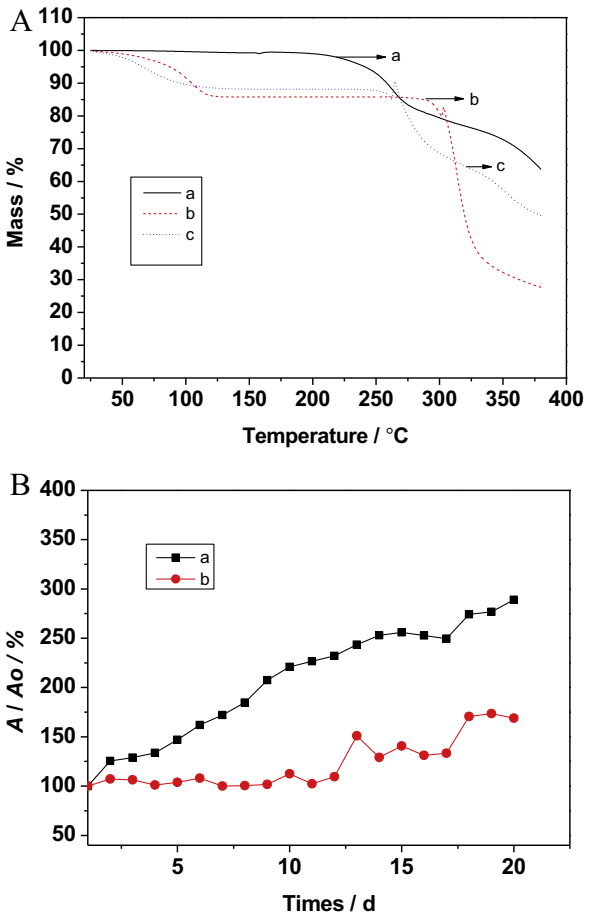


Fig. 7. (A) TG thermograms: (a) GA-13316, (b) β-CD, (c) GA-13316/β-CD inclusion complex. (B) Accretion profiles of GA-13316 decomposition in an aqueous solution containing: (a) free GA-13316, (b) GA-13316/β-CD complex, with an interval of 1 d.

3.9. Stability

In order to evaluate the stability of GA-13316/β-CD, we tracked the absorbance changes of GA-13316 and GA-13316/β-CD (Yang, Chen, Lin, & Liu, 2008). The solid GA-13316 or GA-13316/β-CD quickly dissolved thoroughly and was kept at room temperature, and the absorbance was analyzed at 265 nm by UV–vis spectra each day. Fig. 7B shows the trend of the relative absorbance A/A_0 change for GA-13316 and GA-13316/β-CD on each day. The change in free GA-13316 was very marked upon dissolving in water. The relative absorbance of GA-13316 quickly rose 2.5 times in the first 13 days when compared to the starting absorbance, then it slowed down from the 14 to 17 day, and it increased again. The accumulation of GA-13316 was close to 289% on the twentieth day. However, the accretion of GA-13316 was slowed when GA-13316 was included with β-CD, as shown in Fig. 7B. Until day 12, the percentage of remaining GA-13316 in the GA-13316/β-CD complex solution kept slowly increasing, close to 110%, and then the percentage tended to increase at late eight days, when the highest percentage was close to 174%. This indicated that GA-13316/β-CD was much more stable than free GA-13316.

Table 1
In vitro cytotoxic activities of GA-13316 and GA-13316/β-CD.

	Anti-cancer activity IC_{50} (μM)	
	HCT116	H460
GA-13316	0.93	5.28
GA-13316/βCD	1.13	2.17
ADM	2.37	0.9

4. Conclusions

In conclusion, GA-13316 was capable of forming an inclusion complex with β-CD in the stoichiometric ratio of 1:1, resulting in an A_L-type phase-diagram. The results of the UV–vis, NMR, DSC, TG, XRD and molecular modeling analyses demonstrated that GA-13316/β-CD has different physicochemical characteristics than GA-13316. Upon complexation with β-CD, the water solubility and stability of GA-13316 were significantly enhanced. Given the shortage of applications for GA-13316 and the easy and environmentally-friendly preparation of the GA-13316/β-CD complex, this inclusion complexation should be regarded as a promising strategy in the design of a novel formulation of GA-13316 for anticancer medicine.

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