brief articles

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Inclusion Complex of a Bcl-2 Inhibitor with Cyclodextrin: Characterization, Cellular Accumulation, and in Vivo Antitumor Activity

Zhichao Zhang,*,† Guiye Wu,†,‡,§ Jin Gao,§,|| and Ting Song||

State Key Laboratory of Fine Chemicals, School of Chemistry, Dalian University of Technology, Dalian 116012, People's Republic of China, State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, People's Republic of China, and School of Life Science and Technology, Dalian University of Technology, Dalian 116024, People's Republic of China

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Abstract: Small molecule inhibitors always exhibit poor water solubility due to the inherent hydrophobic property. It is an important challenge when they are developed as a real drug. **S1**, a structure-specific Bcl-2 inhibitor encountered this issue when moved forward in preclinical development. Herein, we prepared a 1:1 type of **S1**−γ-cyclodextrin (**S1**−γ-CD) inclusion complex to enhance the solubility. Bioevaluation of this new formulation was carried out totally in water solution. The cell internalization and cellular accumulation of **S1**−γ-CD was illustrated by its fluorescence analogue **S2**. Disruption of Bcl-2/Bax heterodimerization in MCF-7 cells further revealed **S1**−γ-CD could reach the subcellular function site. Moreover, the even stronger disruption by **S1**−γ-CD than free **S1** was found due to the higher local concentrations. Furthermore, the in vivo antitumor activity of **S1**−γ-CD was evaluated in the H22 xenograft model. Results showed it exhibited significant antitumor activity with a decrease of tumor size (average tumor volume = 234 ± 76 mm^3 vs control group, 398 ± 121 mm^3, $P < 0.01$, and **S1** group, 296 ± 65 mm^3, $P < 0.05$), and a much longer survival time (the median time to the end point = 39.9 days vs control group, 29.2 days, $P < 0.01$). More importantly, the similar disruption of Bcl-2/Bax was found in **S1**−γ-CD treated mice and free **S1** treated ones. It demonstrated that **S1**−γ-CD not only obtains a pharmaceutical level in vivo but also maintains the mechanism-based antitumor ability of **S1** itself. It has been identified that cyclodextrin is appropriate to deliver a structure-specific molecule to its subcellular function site without any adverse effects on its mechanism-based potency, in either cultured cells or animals.

Keywords: Small molecule inhibitors; cyclodextrins; antitumor; in vivo

Introduction

Small-molecule inhibitors (SMIs) of protein−protein interactions (PPI) have huge therapeutic potential.[1,2] SMIs could sequester one protein in the subcellular location of its natural partner by occupying the hydrophobic groove located in their interface. This hydrophobic groove is termed as the hotspot. Proteins involved in PPI could be "promiscuous", binding to SMIs using the same hotspot region. As such, hydrophobicity and surface-shape complementarity are the two essential properties for SMIs. Unfortunately, the low aqueous solubility of hydrophobic SMIs frequently presents a number of problems when they are developed as a drug.

S1, for example, recently identified as a SMI of Bcl-2 family protein by our group[3] and placed on the list of 19 preclinical Bcl-2 inhibitor antitumor drugs,[4] encountered this

* Correspondence should be addressed to this author. Mailing address: Dalian University of Technology, State Key Laboratory of Fine Chemicals, No. 158-89, Zhongshan Road, Dalian 116012, People's Republic of China. E-mail: zczhang@dlut.edu.cn. Fax: 86-411-83673488. Tel: 86-411-39893872.

† State Key Laboratory of Fine Chemicals, School of Chemistry, Dalian University of Technology.

‡ Peking University.

§ These authors contributed equally to this work.

|| School of Biological Science and Technology, Dalian University of Technology.

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issue when it was moving forward in preclinical development. Bcl-2 family proteins are located either in cytoplasm or on mitochondrial membrane.[5] Bcl-2-like anti-apoptotic proteins contain a well-defined, hydrophobic surface binding groove, known as the BH3 binding groove, into which their pro-apoptotic relatives bind. The BH3-mediated interactions between pro-apoptotic and anti-apoptotic members of the Bcl-2 family determine cell fate. SMIs of the Bcl-2 family are thus thought to be the potent antitumor drugs.[6] **S1** is a molecule that mimics the BH3 domain to occupy the BH3 groove and then disrupt protein interactions between Bcl-2 members. As expected, this hydrophobic molecule has the disadvantage of being practically insoluble (<0.1 mg/mL) in water. All of the previous cell-based assays and in vivo evaluations were carried out by using its DMSO solution.

In order to overcome this limitation, we sought to prepare a water-soluble **S1** inclusion complex by cyclodextrins (CDs). CDs, annular water-soluble molecules of glucose units, have a bucket-shaped cavity with a relatively hydrophilic exterior and a relatively hydrophobic interior. They have been utilized in recent years as host molecules to produce inclusion complexes with a large variety of drugs and substances.[7] The resulting complexes generally exhibit enhanced solubility and other favorable changes of the characteristics of the guest molecule. Especially, CDs have been applied extensively to improve the solubility of antitumor agents. Paclitaxel, for example, was encapsulated into CDs and satisfactory solubility was obtained.[8] But there are few reports about the inclusion complex of a structure-specific molecule, especially for a molecule which could highly specifically recognize subcellular targets. It is of great interest to get the knowledge whether or not the inclusion complex can reach the subcellular function site and maintain structure-based potency.

In the present work, the water-soluble inclusion complex of **S1** with γ-CD was prepared. Rather than complex characterization, e.g. complexation ratios, complex confirmation, solubility diagrams, etc., the aims of this study were to illustrate the cell internalization, and to explore the structure-based antitumor ability of the complex inside cells and in an animal model in comparison with the free drug. To our knowledge, it is the first investigation of the molecular antitumor pharmacology of a CD-encapsulated structure-specific SMI.

Experimental Section

Chemicals. All solvents and reagents were purchased from commercial sources. The triple distilled water was used as solvent in all spectral and biological activity measurements. γ-Cyclodextrin (γ-CD) was purchased from Sigma-Aldrich (USA). **S1** was synthesized in our lab as previously described.[3]

Cell Lines, Antibodies and Probe. Human breast adenocarcinoma cell line (MCF-7) cells were purchased from China Center for Type Culture Collection (Wuhan, China) and cultured in RPMI 1640 medium with 10% (v/v) fetal calf serum. Cell culture was kept in a humidified incubator with 5% CO_2 at 37 °C. Exponentially growing cells were exposed to the correct concentrations of **S1** and **S1**−γ-CD inclusion, respectively for the indicated time periods. The antibodies used in the present research were rabbit antibodies against Bcl-2 (BS1220, Bioworld) and Bax (sc-526, Santa Cruz). Lyso-Tracker Red was from Invitrogen Co. Ltd. (C1046).

Animal. Mice bearing the ascitic-type hepatocarcinoma (H22 cells) were purchased from the Institute of Materia Medica, Chinese Academy of Medical Sciences (Beijing, China). Healthy BALB/c mice (CLA grade, 10 weeks, weight 34−36 g) were obtained from Dalian Laboratory Animal Center (Dalian, China) and were bred there. All experiments were performed according to institutional ethical guidelines on animal care approved by Dalian University of Medical Science.

Molecular Modeling. Gaussian 03[9] was used to calculate the energy-minimized geometries of **S1**.

Preparation and Characterization of S1−γ-CD Inclusion Complexes. γ-CD (1 mmol) was dissolved in 20 mL of water, while **S1** (1 mmol) was dissolved in 5 mL of acetone. **S1** solution was added dropwise into γ-CD solution under stirring within 30 min. A light purple solid was obtained after heating the aqueous solution at 60 °C for 24 h under continuous stirring. The solid was filtered and washed with distilled water and then with acetone. The obtained residue was dried under vacuum.

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A phase solubility study was carried out in distilled water according to Higuchi and Connors.[10] In brief, an excess amount of **S1** (1 μM) was added to 10 mL of aqueous solutions containing various concentrations of γ-CD (0−60 μM). Flasks were sealed and shaken at 25 °C for 7 days in the dark. After equilibrium was reached, the solution was filtered through a 0.45 μm hydrophilic membrane filter with the first one-third of the solution being discarded. The concentration of **S1** in the filtrate was determined by a UV−vis spectrophotometer at 592 nm (HP 8453 spectrophotometer, HP, USA).

The **S1**−γ-CD complexes were characterized by nuclear magnetic resonance (^{1}H NMR), circular dichroism (CD) studies, Fourier-transform infrared spectroscopy (FTIR), thermogravimetry−differential thermal analysis (TGA) and scanning electron microscopy (SEM). ^{1}H NMR spectra were measured on a Bruker AV-400 spectrometer (Bruker, Switzerland) using TMS as an internal standard. CD spectra were performed using a J-810 spectrophotometer (Jasco, Japan) at room temperature with scans from 200 to 600 nm. FTIR spectra were taken as KBr pellets at room temperature under nitrogen using a Nicolet-20DXB (Nicolet, USA). TGA was performed using a TGA/SDTA851e (Mettler-Toledo, Switzerland). Samples of about 5 mg were placed in a closed N_2 gas flow. The heating rate was 10 °C min^{-1} over the temperature range from 20 to 500 °C. SEM images were taken on KYKY2800B (KYKY, China) at an acceleration voltage of 15 kV and a working distance of 10 mm after gold coating.

Synthesis of S2 and Preparation of S2−γ-CD Complex. **S2** was synthesized in three steps:

(1) Synthesis of 3-Methoxyacenaphthenequinone. The 3-nitroacenaphthenequinone (2 mmol) was reacted with K_2CO_3 (2 mmol) in methanol (25 mL) at reflux for 8 h. After removal of methanol, the residue was purified by silica gel column chromatography using dichloromethane/petroleum ether (1:1, v/v) as eluent to afford 3-methoxyacenaphthenequinone. Yield: 0.34 g (80%).

(2) Synthesis of 3-Methoxy-dinitrilemethylene-oxy-acenaphthene. 3-Methoxyacenaphthenequinone (1 mmol) and propanedinitrile (2 mmol) were dissolved in dichloromethane (10 mL). After that, the solution was eluted by silica gel column chromatography using dichloromethane as eluent to afford 3-methoxy-dinitrilemethylene-oxy-acenaphthene. Silica gel was used as a catalyzer. Yield: 0.23 g (90%).

(3) Synthesis of **S2**. 3-Methoxy-dinitrilemethylene-oxy-acenaphthene (1 mmol) was reacted with K_2CO_3 (0.1 mmol) in acetonitrile (25 mL) at reflux for 1 h. After removal of acetonitrile, the residues were purified by silica gel column chromatography using dichloromethane/petroleum ether (2:1, v/v) as eluent to afford 3-methoxy-8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carbonitrile (**S2**). Yield: 0.09 g (35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (d, J = 8.4, 1H), 8.75 (d, J = 7.6, 1H), 8.40 (d, J = 7.6, 1H), 7.79 (t, J = 8.4, 1H), 7.25 (d, J = 8.0, 1H), 4.16 (s, 3H). IR (KBr) cm^{-1}: 2225,1629, 1581,1267, 1108. TOF MS (EI$^+$): $C_{16}H_8N_2O_2$, calcd for 260.0586, found 260.0590.

^{1}H NMR spectra were measured on a Bruker AV-400 spectrometer (Bruker, Switzerland) using TMS as an internal standard.

S2−γ-CD complex was prepared in the same way with **S1**−γ-CD and characterized by ^{1}H NMR, FTIR, TGA and phase solubility study.

Uptake Analysis by Confocal Laser Scanning Microscopy. S2 were dissolved in DMSO at the final concentration of 10 mM as a stock, while its γ-CD inclusion was dissolved in PBS (about 80 μM).

Exponentially growing MCF-7 cells were plated and cultured overnight in 24-well plates. The cell monolayers were then incubated with 10 μM **S2** and **S2**−γ-CD equivalent to 10 μM **S2**, respectively. Fluorescence microscopic imaging was taken by Nikon confocal microscope. (Nikon D-eclipse C1, Japan)

Coimmunoprecipitation from Cells. MCF-7 cells were incubated at 37 °C for 3 h with 10 μM **S1** DMSO solution and **S1**−γ-CD water solutions equivalent to 10 μM **S1**, respectively. Cells were harvested, and immunoprecipitated proteins were analyzed by Western blot using anti-Bcl-2 antibody as described.[11] Proteins were detected with an ECL kit.

Cytotoxicity Experiments. MCF-7 cells were cultured routinely in 24-well (1×10^4 cells per well) plates. After cells were cultured in the presence of various concentrations of **S1** (0.01−100 μM) from DMSO stock (1−10 mM) and **S1**−γ-CD water solution for 24 h, respectively, medium was aspirated and replenished with complete medium. IC_{50} was evaluated by metabolite 3-[4,5-dimethylthiazol- 2-yl]-2,5-diphentetrazolium bromide (MTT) metabolic labeling assay. Each experiment was performed three times. γ-CD was used as a control.

In Vivo Mechanism-Based Antitumor Ability Assay. The mouse bearing H22 tumor was sacrificed 6−8 days after tumor implant. The ascitic fluid was drawn through the abdominal cavity using a sterile syringe, and then diluted to a concentration of 1×10^7 cells per mL in PBS. 200 μL of diluted solution was injected into the shoulder of BALB/c mice by hypodermic needle. When tumors were apparent, animals were randomly divided into three groups of fifteen mice each and treatment was begun. **S1** was dissolved in DMSO while **S1**−γ-CD was in water. The control group was given DMSO (ip), while other 2 groups received ip injection every other day with 0.3 mg/kg body weight **S1** and **S1**−γ-CD equivalent to this dose, respectively for 10 days. The design of the dosage was based on our previous report.[3] Tumor sizes were determined before the treatment

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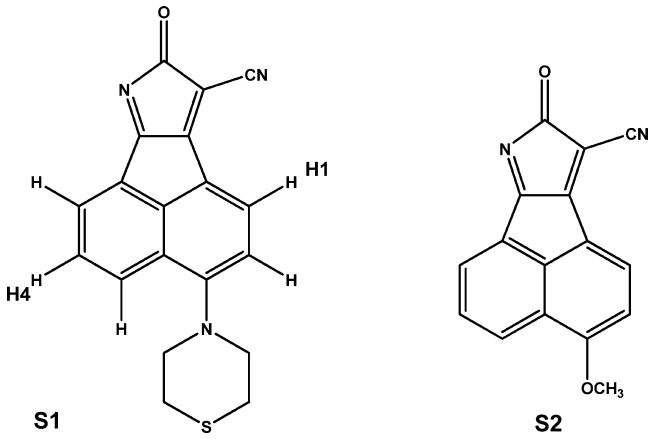

Figure 1. Structures of **S1** and **S2**. The distance of the two atoms about H1 and H4 is 0.718359 nm, which is labeled in **S1**.

and twice a week for the duration of the experiment by measuring the tumor in two dimensions with capilers and calculated using the formula tumor volume = (length × width^2)/2. On day 11, 5 mice were randomly selected from each group and killed. Tumors were stripped, excised and ground in 0.2% NP-40 buffer. Lysates equivalent to 500 μg of protein were precleared, and coimmunoprecipitation was performed. The other animals were monitored, and survival was recorded until death or the 45th day, when the mice were euthanized. The time to the end point (TTE) for each mouse was equal to the day of death, or the last day of the study (45 days). Tumor growth delay (TGD) is defined as the increase in the median time to TTE in a treatment group (T) compared to the control group (C) and expressed in days. % TGD = $(T - C)/C$.

Results and Discussion

First, the minimized energy conformation of **S1** is computed in Gaussian 03. As shown in Figure 1, the distance of H1 and H4 is 0.718359 nm, which is farmost in **S1**. Comparing with the inner diameter of β-CD which is 0.78 nm, γ-CD shows 0.95 nm.[12] So we chose γ-CD as the host molecule. The **S1**$-\gamma$-CD inclusion complex was synthesized in only one step.

The phase solubility profile of **S1**$-\gamma$-CD is presented in Figure 2, which could be classified as A_L-type according to Higuchi and Connors.[10] The solubility of **S1** increased linearly with the increasing concentrations of γ-CD, which is characterized as the formation of a 1:1 inclusion complex between **S1** and γ-CD.[10] The apparent stability constant K was calculated from the phase solubility diagram according to the following equation.[10]

$$K = \frac{\kappa}{S_0(1 - \kappa)}$$

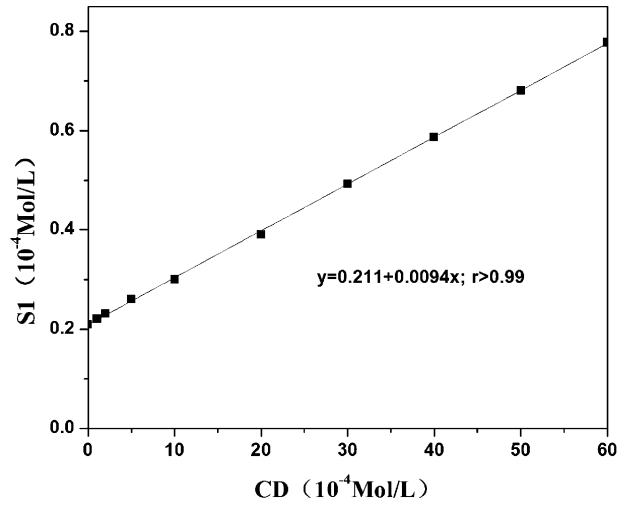

Figure 2. Phase solubility profile of **S1**$-\gamma$-CD.

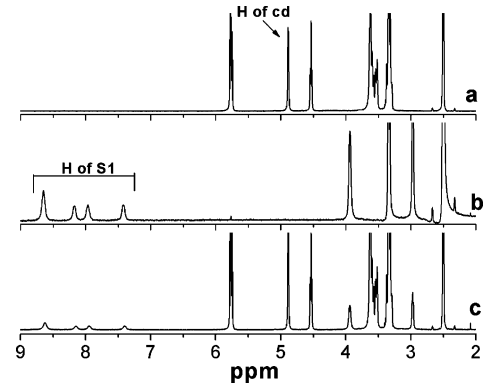

Figure 3. ^{1}H NMR spectra of (a) γ-CD, (b) **S1** and (c) **S1**$-\gamma$-CD (in DMSO).

where S_0 is the solubility of **S1** in the absence of γ-CD and κ is the slope of the straight line. The calculated apparent stability constant of the **S1**$-\gamma$-CD is 45.4 M^{-1}. As compared with the poor water solubility of **S1** (0.007 g/L), the complex of **S1** has a solubility value of 0.13 g/L. The maximum solubility of **S1**$-\gamma$-CD in pure water reached 80 μM, which could satisfy the cell-based antitumor assays and in vivo assays of **S1**$-\gamma$-CD in water solution.

^{1}H NMR spectra of **S1**$-\gamma$-CD (Figure 3) provided direct evidence for the formation of the 1:1 type inclusion complex. The peaks in the range 7.4−8.6 ppm of **S1** were assigned to phenyl protons and also noticed in the inclusion compound as well in addition to the characteristic peaks of CD. Notably, the phenyl protons of **S1** in Figure 3c were shifted slightly upfield about 0.09 ppm, which confirms the formation of an inclusion complex.[13] Additionally, the area ratio between phenyl protons of **S1** and C1−H protons (δ = 4.88 ppm) of the γ-CD in the complex is 1:9.1. Since a molecule of CD contains 7 C1−H protons,[14] we can calculate that the ratio of **S1** and γ-CD is 1:1.

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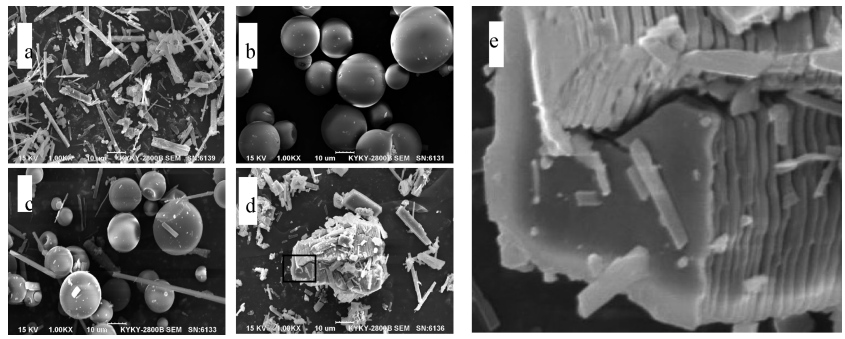

Figure 4. SEM images of (a) **S1**, (b) γ-CD, (c) **S1** and γ-CD physical mixture, (d) **S1**$-\gamma$-CD inclusion, and (e) magnified view of the boxed region in (d).

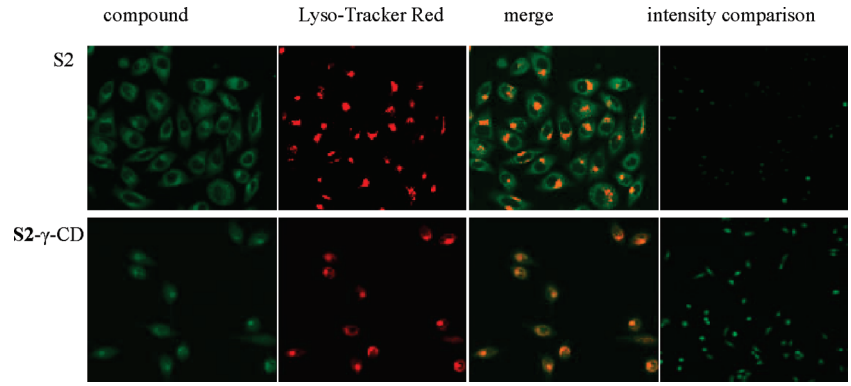

Figure 5. Fluorescence images of **S2** and **S2**$-\gamma$-CD uptake by cells. Images were taken by confocal microscope. Cells were coincubated with **S2** and **S2**$-\gamma$-CD at the same **S2** equivalent dose, respectively. After being washed 3 times, cells were dyed by Lyso-Tracker Red. The merge images demonstrate no colocalization of **S2** (green) with lysosomal probe (red), and colocalization of **S2**$-\gamma$-CD with Lyso-Tracker Red. A 60×/1.4 oil object lens was used. To compare the fluorescence intensity, 10 μM **S2** and **S2**$-\gamma$-CD equivalent to 10 μM **S2** was added into the medium, respectively. Fluorescence images were taken 10 min later (magnification 20×) under the same conditions.

FTIR data of **S1** and its inclusion complexes with γ-CD are listed in Supporting Information Figure S1, which corroborates the formation of an inclusion complex. The thermal stability of the inclusion complex is shown in Supporting Information Figure S2. It also indicated that the ratio of γ-CD and **S1** is of 1:1 type.[15] Additionally, the CD spectrum of the complex illustrated **S1** was embedded in the hydrophobic cavity almost parallel to the axis of γ-CD (Supporting Information Figure S3).[16−18]

Next, we applied SEM to characterize the surface morphology of powders derived from **S1**, γ-CD and their inclusion complexes. The 1:1 molar ratio **S1** and γ-CD physical mixtures were prepared by mixing the single components in an agate mortar until the mixture was homogeneous. As shown in Figure 4, a comparison of the images revealed that the complex is structurally distinct from free **S1**. **S1** displayed as an unregular morphology, while the γ-CD displayed as a ball. Specifically, the complex could be characterized as a regular platelike morphology (Figure 4e).

Importantly, we need to determine the cellular uptake of **S1**$-\gamma$-CD due to its subcellular function site. In addition, we need to determine whether **S1** and **S1**$-\gamma$-CD show different internalization. Because the binding constant of **S1**$-\gamma$-CD is relatively lower, we were wondering if **S1** can be released from the complex outside cell membrane or enter into cells included in CDs by endocytosis. Since **S1** is not a fluorescence molecule, we tested its fluorescence analogue **S2** (Figure 5). The structure of **S2** is shown in Figure 1. The ^{1}H NMR spectra, FTIR spectra, and the thermogravimetry−differential thermal analysis (TGA) of **S2**$-\gamma$-CD are in Supporting Information Figures S4, S5, and S6. The binding

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constant of **S2**−γ-CD (61.4 M^{-1}, Supporting Information Figure S7) is comparable with that of **S1**−γ-CD. The bright green fluorescence appeared inside MCF-7 cells after either **S2** or **S2**−γ-CD was added into the cell culture medium, indicating both **S2** and **S2**−γ-CD were internalized into cells. Considering the small and hydrophobic property, the passive diffusion should be the predominant way for **S2** to permeate cells. Interestingly, **S2**−γ-CD was mainly localized in lysosomes as shown by colocalization with lysosomal probe Lyso-Tracker Red. In contrast, no colocalization with lysosomes was found for free **S2**. Since CDs are hydrophilic, membrane impermeant molecules that can reach the inside of lysosomes via pinocytosis,[19] we concluded that the complex of **S2**−γ-CD was taken up by endocytosis. Because of the analogical structure, it is most likely that **S1**−γ-CD could internlize into cells in the same way with **S2**−γ-CD. More important, the fluorescence intensity of intracellular **S2**−γ-CD is much stranger than that of free **S2** under the same concentration, incubation time and exposure conditions. It suggests the inclusion complex may be easier to be taken up than free compound and accumulate inside cells.

Consequently, we investigated the disruption of Bcl-2/Bax complex by free **S1** and **S1**−γ-CD to compare their mechanism based antitumor ability in living cells. In general, Bax is constrained by overexpressed Bcl-2 in cancer cells so it cannot activate apoptosis.[1] The BH3 mimetics may disrupt this association to set free Bax,[6] which result in apoptosis. **S1**, for example, is a BH3 mimetic functioned to disrupt the Bcl-2/Bax complex.[20] Here, we determined at the same **S1** equivalent dose, which is 10 μM, **S1**−γ-CD can disrupt the Bcl-2/Bax even more strongly than the free drug (Figure 6). It could be explained that the local concentration of **S1**−γ-CD is higher than that of free **S1** due to the cellular accumulation as we observed in the fluorescence images of **S2**−γ-CD. Meanwhile, the IC_{50} values were measured to compare the cytotoxicity of **S1** and **S1**−γ-CD (Table 1). γ-CD was applied as a control. Consistent with the result of coimmunoprecipitation, **S1**−γ-CD exhibited stronger cytotoxicity (IC_{50} = 0.06 μM) against MCF-7 cells than free **S1** (IC_{50} = 0.09 μM). Meanwhile, the IC_{50} of γ-CD itself is more than 100 μM, consistent with a previous report.[21]

In order to investigate whether the inclusion could deliver the drug in vivo and whether the efficiency could reach and maintain pharmacologically active levels in tumor tissue, the H22 xenograft model was set up and administered with **S1**

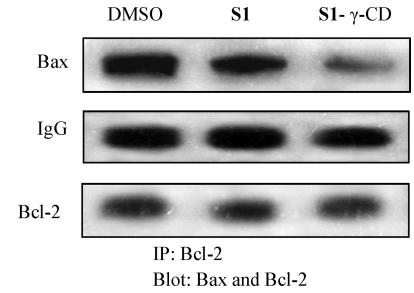

Figure 6. **S1** and **S1**−γ-CD disrupt Bcl-2/Bax heterodimerization in MCF-7 cells by coimmunoprecipitation. MCF-7 cells were exposed for 3 h to 10 μM **S1** and **S1**−γ-CD equivalent to 10 μM, respectively. Lysates equivalent to 100 μg of protein were precleared and then immunoprecipitated over 24 h with an antibody specific for Bax. Immunoprecipitates were analyzed by Western blot with these specific antibodies. The amount of Bcl-2/Bax in cell lysates prior to immunoprecipitation was determined with specific antibodies.

Table 1. Cytotoxicity Evaluation of the Compounds against MCF-7 Cell Line (IC_{50}, μM)

compound	IC_{50} (μM)
S1	0.09
S1−γ-CD	0.06
γ-CD	>100

and **S1**−γ-CD, respectively. Forty-five mice bearing palpable H22 tumors (average tumor volume = 101 ± 13 mm^3) were randomly placed into three separate groups (fifteen in each group) and were given intraperitoneal injections of **S1** DMSO solution (0.3 mg/kg body weight) and **S1**−γ-CD water solution equivalent to 0.3 mg/kg **S1**. As shown in Figure 7, by the 45th day, or the day of death, mice receiving **S1**−γ-CD had grown significantly smaller tumors (average tumor volume = 234 ± 76 mm^3) than the control (398 ± 121 mm^3, $P < 0.01$) and **S1** group (296 ± 65 mm^3, $P < 0.05$). In the meanwhile, the median TTE for **S1**−γ-CD was the maximum, 39.9 days (Table 2), which is comparable to the free **S1** group (37.9 days, $P > 0.05$). This TTE value corresponds to a significant 10.7 day $T - C$ and a 36.3% TGD, relative to the control mice. The survival of mice in the three groups

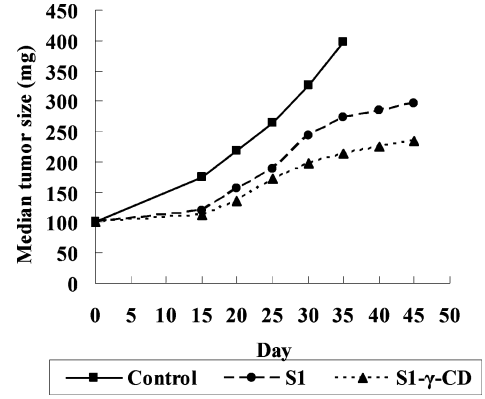

Figure 7. Antitumor efficiency using **S1** and **S1**−γ-CD in BALB/c mice bearing H22 tumor.

(19) Rosenbaum, A. I.; Zhang, G.; Warren, J. D.; Maxfield, F. R. Endocytosis of beta-cyclodextrins is responsible for cholesterol reduction in Niemann-Pick type C mutant cells. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 5477–5482.

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(21) Prior, M.; Lehmann, S.; Sy, M.; Molloy, B.; McMahon, H. Cyclodextrins Inhibit Replication of Scrapie Prion Protein in Cell Culture. *J. Virol.* **2007**, *81*, 11195–11207.

Table 2. Antitumor Efficiency Study in BALB/c Mice Bearing H22 Tumor

group	median TTE	$T - C$	% TGD
control	29.2		
S1	37.9[a]	8.7	29.8
S1$-\gamma$-CD	39.9[a]	10.7	36.6

[a] $P < 0.05$ vs control group.

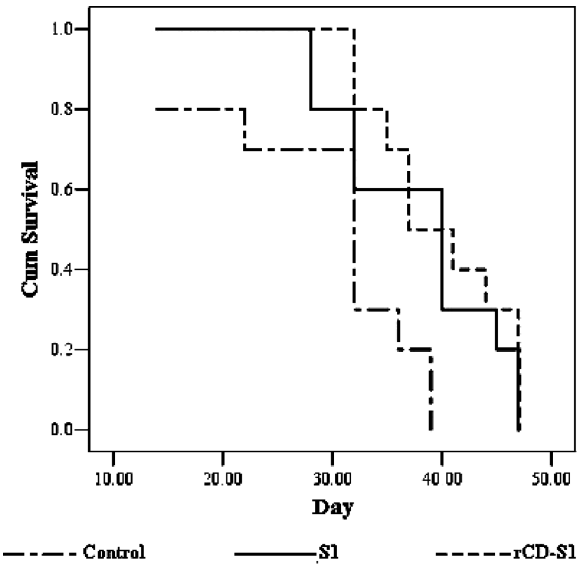

Figure 8. Antitumor efficiency using **S1** and **S1**$-\gamma$-CD in BALB/c mice bearing H22 tumor.

is illustrated in the Kaplan−Meier plot shown in Figure 8. All together, at the same **S1** equivalent dose, **S1**$-\gamma$-CD showed antitumor ability superior or equal to free **S1**. Additionally, the disruption of Bcl-2/Bax was also found in tumor tissue from the **S1**$-\gamma$-CD treated group, which is similar to that of free **S1** (Figure 9). It means the γ-CD inclusion could not only deliver the drug in animals and obtain a pharmaceutical level but also maintain the structure-specific antitumor ability.

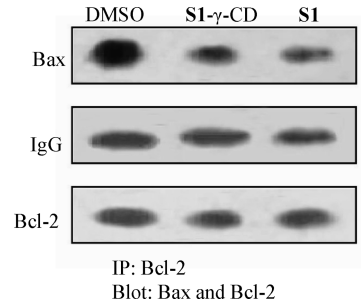

Figure 9. **S1** and **S1**$-\gamma$-CD disrupt Bcl-2/Bax heterodimerization by coimmunoprecipitation in BALB/c mice bearing H22 tumor. Solid tumors were stripped from tumor-bearing mice and ground in 0.2% NP-40 buffer. Lysates equivalent to 500 μg of protein were precleared, and then coimmunoprecipitation was performed.

Conclusions

A structure-specific antitumor lead **S1**, which was extremely insoluble in water, was encapsuled into γ-CD to form a water-soluble **S1**$-\gamma$-CD inclusion. It maintains or even improves the structure-specific antitumor potency both in MCF-7 cells and in a H22 xenograft model. These initial antitumor efficiency studies demonstrated that cyclodextrin can be used for structure-specific antitumor drug delivery to improve solubility and maintain mechanism-based antitumor ability.

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Supporting Information Available: IR, CD, and ^{1}H NMR spectra, thermal stability plot, and phase solubility profile. This material is available free of charge via the Internet at http://pubs.acs.org.

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