Journal of Inclusion Phenomena and Macrocyclic Chemistry (2006) 56: 135–139
DOI 10.1007/s10847-006-9074-5
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Prominent Solubilizing Effect of 2-Hydroxypropyl-β-cyclodextrin on a New Thiazolidine Derivative (FPFS-410) with Antidiabetic and Lipid-lowering Activities through Inclusion Complex Formation

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Key words: 2-hydroxypropyl-β-cyclodextrin, FPFS-410, inclusion complex, solubility

Abstract

2-(*N*-Cyanoimino)-5-{(*E*)-4-styrylbenzylidene}-4-oxothiazolidine (FPFS-410) is a newly synthesized thiazolidine derivative having not only antidiabetic but also lipid-lowering activities. However, this compound has an extremely low aqueous solubility (2.8×10^{-8} M in phosphate buffer at 25 °C). In this study, we attempted to improve the solubility of FPFS-410 in water, by means of the complexation with 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD). Further, the interaction of FPFS-410 with HP-β-CyD in 50% v/v methanol/water mixed solution was investigated by ultraviolet and ^{1}H-nuclear magnetic resonance (NMR) spectroscopic methods, because the solubility of FPFS-410 in water was too low to study quantitatively the interaction. The results of the solubility method indicated that HP-β-CyD had a markedly high solubilizing ability to FPFS-410, e.g., the solubility of the compound was increased 200,000-fold by the addition of 40 mM HP-β-CyD. The continuous variation plot of the FPFS-410/HP-β-CyD system in 50% v/v methanol/water solution gave a maximum at a host/guest molar ratio of 1:1. ^{1}H-NMR spectroscopic studies suggested that the stilbene moiety of FPFS-410 is preferably included in the HP-β-CyD cavity to form the 1:1 complex in 50% v/v methanol/water solution. The present results suggest that HP-β-CyD is useful for improvement of the oral bioavailability of FPFS-410.

Introduction

Thiazolidinedione class of drugs (TZDs), such as rosiglitazone and pioglitazone, are new type of antidiabetic agents and widely used in clinical practice. However, in some cases they cause weight gain, plasma volume expansion, edema or hepatotoxicity [1, 2]. Because these unwelcome effects of TZDs are supposed to be due to activation of peroxisome proliferator-activated receptor γ (PPARγ), involved in the mode of action, various intensive attempts have been conducted to search drug candidates with potent antidiabetic and lipid-lowering activities, but with low affinity to PPARγ. 2-(*N*-cyanoimino)-5-{(*E*)-4-styrylbenzylidene}-4-oxothiazolidine (FPFS-410, Figure 1), a newly synthesized cyanoimino-oxothiazolidine with improving insulin sensitivity, has shown potent lowering effects on serum glucose and triglycerides at the preclinical stage. Furthermore, FPFS-410 has much less potency for PPARγ activation compared with pioglitazone and does not cause body weight gain in rodents [3]. Despite these valuable features, FPFS-410 exhibits an extremely low aqueous solubility (2.8×10^{-8} M in phosphate buffer at 25 °C), which is a drawback for its practical use. Cyclodextrins (CyDs) are known to form inclusion complexes with lipophilic drugs and improve their water solubility, dissolution rate and bioavailability [4–6]. In this study, we attempted to improve the solubility of FPFS-410 in water, by means of the complexation with 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD), because in a

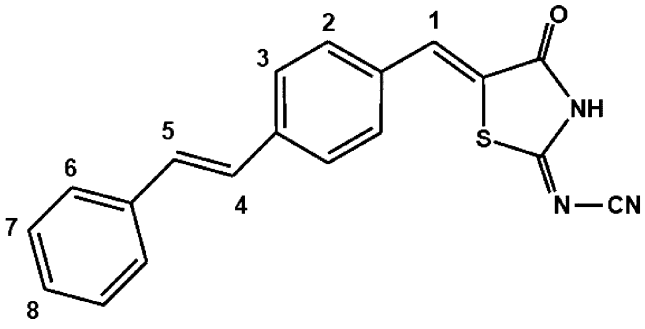

Figure 1. Chemical structure of 2-(*N*-cyanoimino)-5-{(*E*)-4-styrylbenzylidene}-4-oxothiazolidine (FPFS-410) and proton numbering.

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preliminary study HP-β-CyD showed the highest solubilizing ability among various CyDs such as parent α-, β- and γ-CyDs, their hydroxypropylated derivatives, and sulfobutyl ether β-CyD. Further, the interaction of FPFS-410 with HP-β-CyD in 50% v/v methanol/water mixed solution was investigated by ultraviolet and ^{1}H-nuclear magnetic resonance (NMR) spectroscopic methods, because the solubility of FPFS-410 in water was too low to study quantitatively the interaction.

Experimental

Materials

HP-β-CyD (average degree of substitution: 4.8) was obtained from Nihon Shokuhin Kako Co. (Tokyo, Japan). FPFS-410 was designed and synthesized by Fujimoto Pharmaceutical Co. (Osaka, Japan). Deuterium oxide (D_2O, 99%) and methanol-D_4 (CD_3OD, 99%) were purchased from Aldrich Chem. Co. (Milwaukee, WI). Other chemicals and solvents were of analytical reagent grade and double-distilled water was used throughout the study.

Solubility studies

The solubility studies were carried out according to the method of Higuchi and Connors [7]. An excess amount of FPFS-410 (about 1 mg) was added in the screw-capped vials (1.5 ml) containing HP-β-CyD solution at various concentrations in 1.0 M phosphate buffer (pH 7.0) or in 50% v/v methanol/1.0 M phosphate buffer (pH 7.0). The vials were shaken at 25 °C. After equilibrium was attained (about 3d), the solution was centrifuged (5500*g*, 15 min), filtered through a cotton plug, and analyzed for FPFS-410 by high-performance liquid chromatography (HPLC). The HPLC conditions were as follows: a Hitachi L-6000 pump (Tokyo, Japan) and a Hitachi L-4000 UV detector at 390 nm (Tokyo, Japan); a Hitachi D-2500 ChromatoIntegrator (Tokyo, Japan); a GL-Sciences Nucleosil C18 ODS column (4.6×250 mm, Tokyo, Japan); a mobile phase of acetonitrile/0.05 M ammonium acetate aqueous solution (11:9 v/v); a flow rate of 0.80 ml min^{-1}. The 1:1 stability constant ($K_{1:1}$) of the complex was calculated from an initial straight line portion of phase solubility diagram with Equation (1) [7]:

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

where S_0 and slope represent the intrinsic solubility of FPFS-410 and the slope of the phase-solubility diagram, respectively. On the other hand, the stability constants of higher order complexes ($K_{1:2}$) were analyzed according to the optimization technique as described previously [8].

UV and ^{1}H-NMR spectroscopic studies and molecular docking simulation

UV spectra were taken with a Hitachi U-2000A Spectrophotometer (Tokyo, Japan). The spectroscopic changes of FPFS-410 (0.025 mM) in the absence and presence of HP-β-CyD (0.25–2.0 mM) were analyzed in 50% v/v methanol/1.0 M phosphate buffer (pH 7.0) at 25 °C. The stability constant of 1:1 complexes ($K_{1:1}$) was calculated by the Scott equation (Equation 2) [9]:

$$\frac{ab}{d} = \frac{1}{K_{1:1}\varepsilon_c} + \frac{b}{\varepsilon_c} \quad (2)$$

where a and b are total concentrations of drug and CyD, respectively, ϵ_c is the difference in molar absorptivities of free and complexed drugs, and d is the change in absorbance of drug by the addition of CyD. The stoichiometry of the complex in 50% v/v methanol/1.0 M phosphate buffer (pH 7.0) at 25 °C was determined by the continuous variation method [10]. The total concentration of FPFS-410 and HP-β-CyD was 0.05 mM. ^{1}H-NMR spectra were taken at 25 °C on a JEOL JNM-ECP500 (Tokyo, Japan) operating at 500 MHz, using a 5-mm sample tube. 50% v/v Deuterated methanol/phosphate buffer (pH meter reading of 7.0) was used as a solvent and the water signal as an internal reference for ^{1}H-NMR. The assignment of ^{1}H-NMR signals of FPFS-410 and HP-β-CyD was performed by correlation spectroscopy (COSY) and according to the reference [11], respectively. Phase-sensitive ROESY spectra were acquired with F1 and F2 spectral widths of 400–3800 Hz with 36 scans. The relaxation delay was 4.0 s, the 90° pulse width was 19.5 μs, and the spin-lock mixing time was set to 250 ms.

Molecular docking simulations of the 1:1 FPFS-410/β-CyD complex were carried out using a MOE-AS Dock software (Molecular Operating Environment, Chemical Computing Group, Inc., Montreal, Canada; Ryoka System, Inc., Tokyo, Japan). The geometry parameters of parent β-CyD were taken from the previous paper [12], and those of FPFS-410 were constructed with Chem3D software (Cambridge Soft, Cambridge, MA). Various possible depositions (about 1000) and orientations of the guest molecule in the β-CyD cavity were generated using the program ASDock, and each structure was energy-minimized by the MMFF94x force filed calculation to obtain the total energy (U_{total}), the electrostatic and van der Waals interactions between the guest and β-CyD (U_{ele} and U_{vdw}, respectively), and the conformation energy of the ligand (U_{ligand}).

Results and discussion

Solubility studies

Figure 2 shows the phase solubility diagrams of FPFS-410 with HP-β-CyD in 1.0 M phosphate buffer (pH 7.0)

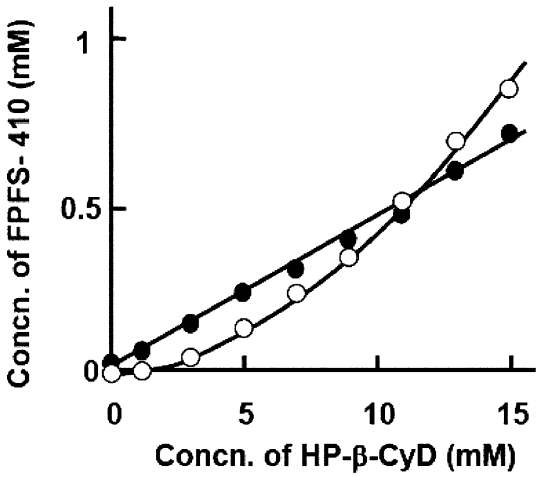

Figure 2. Phase solubility diagrams of FPFS-410/HP-β-CyD system in 1.0 M phosphate buffer (pH 7.0) (○) and in 50% v/v methanol/phosphate buffer (pH 7.0) (●). Each point represents the mean ± S.E. of 3 experiments.

and in 50% v/v methanol/phosphate buffer (pH 7.0). The latter solution was employed because UV spectroscopic studies indicated that FPFS-410 forms the 1:1 complex with HP-β-CyD in 50% v/v methanol/phosphate buffer (pH 7.0), as described later. HP-β-CyD showed a typical A_P type diagram in 1.0 M phosphate buffer (pH 7.0), with significant increase in the solubility of FPFS-410 at higher CyD concentrations. For example, the solubility of FPFS-410 increased about 200,000-fold at 40 mM HP-β-CyD (data not shown). On the other hand, a typical A_L type diagram was obtained when 50% v/v methanol/phosphate buffer (pH 7.0) was used as a solvent. These solubility curves were analyzed to obtain the stability constants of the complex, assuming that FPFS-410 forms the 1:1 and 1:2 complexes in 1.0 M phosphate buffer (pH 7.0) and the 1:1 complex in 50% v/v methanol/phosphate buffer (pH 7.0) under the present experimental conditions. The stability constants ($K_{1:1}$ and $K_{1:2}$) of the 1:1 and 1:2 (guest:host) complexes in 1.0 M phosphate buffer (pH 7.0) were 6.02×10^5 and 1.46×10^2 M^{-1}, respectively, and that of the 1:1 complex in 50% v/v methanol/phosphate buffer (pH 7.0) was 1.07×10^3 M^{-1}. The $K_{1:1}$ value was significantly larger than the $K_{1:2}$ value, indicating that the 1:1 FPFS-410/HP-β-CyD complex is a predominant species under the experimental conditions. The improved solubility of FPFS-410 through the complexation with HP-β-CyD was clearly reflected in the fast dissolution and the enhanced bioavailability of FPFS-410 in dogs, i.e., the oral bioavailability of FPFS-410 increased about 3-fold when it was administered as the HP-β-CyD complex, compared with that of the drug alone. These results will be reported elsewhere.

Interaction of FPFS-410 with HP-β-CyD in 50% v/v methanol/water solution

Figure 3A shows UV spectra of FPFS-410 in the absence and presence of HP-β-CyD in 50% v/v methanol/phosphate buffer (pH 7.0), in which the absorption intensity of the compound at 301 nm decreased with increasing CyD concentrations. Figure 3B shows the continuous variation plot of the FPFS-410/HP-β-CyD system monitored by the intensity change at 301 nm. The variation plot gave a maximum at a 1:1 molar ratio of the guest and host, indicating that FPFS-410 forms the 1:1 complex with HP-β-CyD in the mixed solvent. Therefore, the UV absorption change at 301 nm (Figure 3A) was analyzed to obtain the 1:1 stability constant of the complex, according to the Scott equation (Equation 2) [9], and the 1:1 stability constant of 990 M^{-1} was obtained. This value was almost identical to that obtained by the solubility method (1070 M^{-1}) in 50% v/v methanol/phosphate buffer (pH 7.0). The formation of the 1:2 complex may be difficult in 50% v/v methanol/phosphate buffer (pH 7.0), because of the competitive inclusion of methanol in the CyD cavity.

^{1}H-NMR spectroscopic studies were conducted to gain insight into the inclusion mode of the 1:1 FPFS-410/HP-β-CyD complex in 50% v/v methanol/phosphate buffer (pH 7.0). By the addition of FPFS-410, the

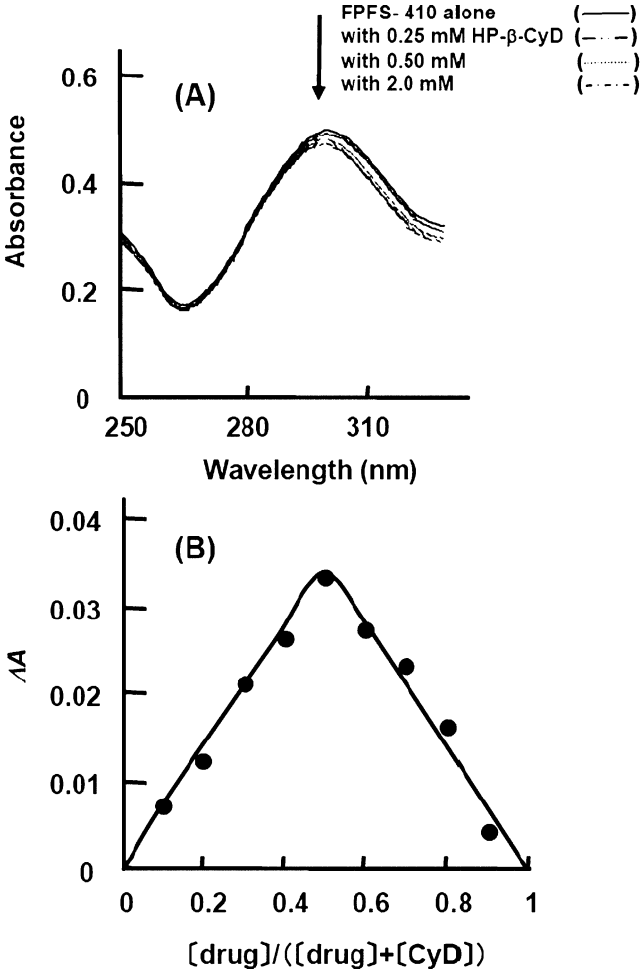

Figure 3. (A) UV spectra of FPFS-410 (0.025 mM) in the absence and presence of HP-β-CyD (0.25–2.0 mM) in 50% v/v methanol/phosphate buffer (pH 7.0) at 25 °C. (B) Continuous variation plot for FPFS-410/HP-β-CyD system in 50% v/v methanol/phosphate buffer (pH 7.0) at 25 °C. The total concentration of FPFS-410 and HP-β-CyD was 0.05 mM.

inner H3′ proton of HP-β-CyD significantly shifted upfield, whereas the outer H1′, H2′, H4′ and methyl protons of the 2-hydroxypropyl group shifted negligibly (data not shown). Unfortunately, the inner H5′ proton of HP-β-CyD could not be quantitatively monitored because of the overlapping with other protons. These results suggest that the guest is included in the HP-β-CyD cavity. On the other hand, by the addition of HP-β-CyD, large shift changes were observed in the central benzene moiety, the H2 and H3 protons, of FPFS-410 molecule (data not shown). The inclusion mode of the FPFS-410/HP-β-CyD complex was confirmed by ROESY spectroscopic studies. As shown in Figure 4, the correlation peaks were observed between the H2 and H3 protons of the guest and the inner H3′ proton of the host in the spectrum obtained in 50% v/v methanol/phosphate buffer (pH 7.0). These results suggest that the central benzene moiety is preferably included in the HP-β-CyD cavity to form the stable 1:1 complex. Figure 5 shows the MOE-optimized structure of the FPFS-410/β-CyD complex in a molar ratio of 1:1. The docking calculation was conducted using parent β-CyD instead of HP-β-CyD, because HP-β-CyD is a multi-component mixture of structurally related compounds. The top- and second-rank models of the 1:1 complex estimated by the docking calculation gave the almost identical inclusion structure with similar U_{total}, U_{ele}, U_{vdw}, and U_{lignad} energy levels, i.e., for the top-rank model 10.7, −5.87, −16.5 and 33.0 kcal mol^{-1}, respectively, and for the second-rank model 10.8, −4.33, −17.6 and 32.7 kcal mol^{-1}, respectively. As shown in Figure 5, the simulated structure of the 1:1 complex was coincided with that in 50% v/v methanol/phosphate buffer, i.e., the H2 and H3 protons of the benzene of FPFS-410 are in the vicinity of the inner H3′ proton at the secondary hydroxyl side of β-CyD, providing the correlation peaks between these protons in the ROESY spectrum. It is reasonable to assume that the 1:1 FPFS-410/HP-β-CyD complex has the similar structure to that of the β-CyD complex (Figure 5), because both β-CyDs gave the A_P type of phase solubility diagram in 1.0 M phosphate buffer, and the solubility curve of FPFS-410 tended to deviate slightly upward at higher HP-β-CyD concentrations (> 20 mM) even in the 50% v/v methanol/phosphate buffer. In 1.0 M phosphate buffer (pH 7.0), the second HP-β-CyD molecule may include the terminal benzene or oxothiazolidine moiety of FPFS-410 to form the 1:2 complex, although further studies should be done to demonstrate the 1:2 complexation.

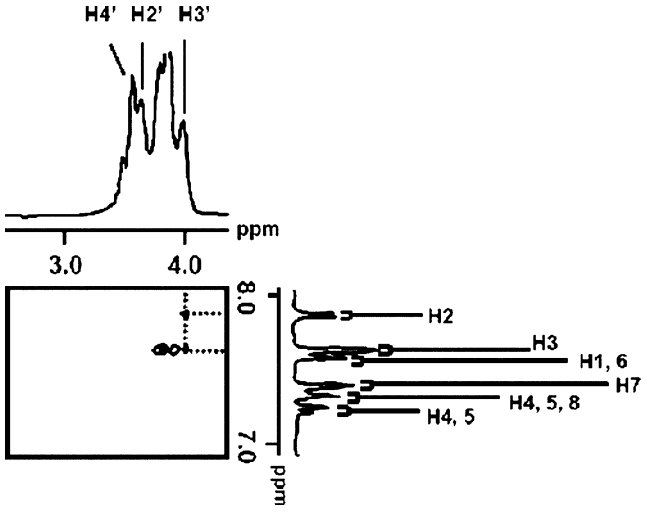

Figure 4. Partial contour plot of ROESY spectrum of FPFS-410/HP-β-CyD system in 50% v/v deuterated methanol/phosphate buffer (pH 7.0) at 25 °C. The concentration of the guest and the host was 15 and 150 mM, respectively.

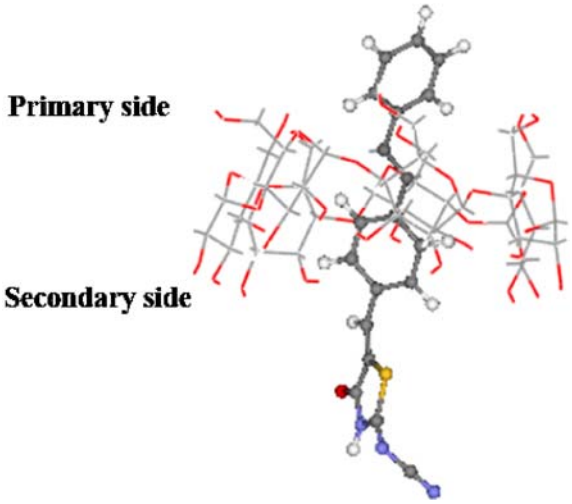

Figure 5. Inclusion structure of the 1:1 FPFS-410/β-CyD complex estimated by docking model calculation.

Conclusion

The newly synthesized FPFS-410 has both antidiabetic and lipid-lowing activities without unwelcome effects. However, the aqueous solubility of FPFS-410 is extremely low, preventing development of various dosage forms. HP-β-CyD markedly enhanced the solubility of FPFS-410 at higher CyD concentrations, e.g., the 200,000-fold increase of the solubility by the addition of 40 mM HP-β-CyD. HP-β-CyD predominantly included the central benzene moiety of FPFS-410 to form the 1:1 complex in 50% v/v methanol/water solution, while the terminal benzene or oxothiazolidine moiety may be additionally included to form the 1:2 complex in water. The markedly enhanced solubility of FPFS-410 is attributable to the A_P type solubility curve in water, probably resulted from the formation of the 1:2 inclusion complex or non-inclusion complexes of CyD aggregates [13].

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