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Preparation and characterization of a new harmine-based antiproliferative compound in complex with cyclodextrin: Increasing solubility while maintaining biological activity

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ABSTRACT

The trisubstituted harmine derivative, **2**, present a submicromolar antiproliferative activity on 5 cancer cell lines but a moderate kinetic solubility in pH 7.4 buffer. The aim of this work was to develop a **2**-cyclodextrin complex in order to increase the drug solubility while maintaining the biological activity. Firstly, the **2** increasing solubility in presence of several cyclodextrins (CDs) has been shown, with a maximum for 7 glucose subunit CD (βCD and 2HP-βCD). Phase solubility experiment in presence of 2HP-βCD has underline an A_L-type profile until 80 mM which suggest a 1:1 stoichiometry and a $K_{1:1}$ of 116 M^{-1} and a CE of 0.28 have been calculated. This 1:1 stoichiometry was confirmed by Job Plot experiment, following the CD H-3 proton by ^{1}H NMR. Secondly, ^{1}H NMR study of compound **2** in presence of βCD and geometry optimization of the complex has underline an inclusion of **2** into the CD, via the indole part of the drug. Finally, the efficiency of the **2** antiproliferative effect is not affected by the complexation, as shown by viability test.

1. Introduction

Harmine (**1**) (Fig. 1) is a natural β-carboline, which is a major alkaloid from *Peganum harmala* and *Banisteriopsis caapi*, presenting diverse pharmacological activities including anticancer activity (Cao et al., 2011; Dai et al., 2012; Hamsa and Kuttan, 2010). Based on this property, our group has synthesized three generations of harmine derivatives in order to obtain compounds combining a higher antiproliferative activity than harmine and a high solubility in pH 7.4 buffer (Frédérick et al., 2012; Meinguet et al., 2015). The best compound (**2**) (Fig. 1), belonging to the third generation, possesses a submicromolar growth inhibitory activity (IC_{50}) on 5 cancer cell lines associated with a moderate kinetic solubility of 189.2 ± 13.4 μg/mL (Meinguet et al., 2015).

Cyclodextrins (CDs) are natural cyclic oligomers composed of glucose subunits and presenting a truncated cone shape. Because of their conformation, their outer surface presents a polar character while their inner surface is described as an apolar cavity. This inner cavity has the characteristic to interact with apolar compounds, leading to partial or total inclusion of a large range of compounds. This inclusion can lead to a modification of the compound's solubility, bioavailability and stability, making CDs largely used in the pharmaceutical field (Hedges, 1998; Loftsson et al., 2005b).

β-carbolines, such as norharmane, harmane and harmine are described to interact with βCD apolar cavity (Mallick et al., 2005; Martín et al., 2003a,b). These complexes show weak interactions and models for a 1:1 stoichiometry suggest an inclusion of the indole part of the β-carboline in the β-CD apolar cavity.

Because of the benefit to increase drug solubility when using CDs and since some β-carbolines are described to interact with CD, we have investigated the ability of compound **2** to interact with the natural α, β, γCD and the modified 2HP-βCD. We studied compound **2** solubility in presence/absence of CDs, the stoichiometry and the geometry of the best complex, and we have determined the biological activity associated to each studied complex.

2. Experimental section

2.1. Materials

Compound **2** was synthesized following the method previously reported (Meinguet et al., 2015). The cyclodextrins [α-cyclodextrin (αCD); β-cyclodextrin (βCD); (2-hydroxypropyl)-β-cyclodextrin (2HP-βCD); γ-cyclodextrin (γCD)] were purchased from Sigma–

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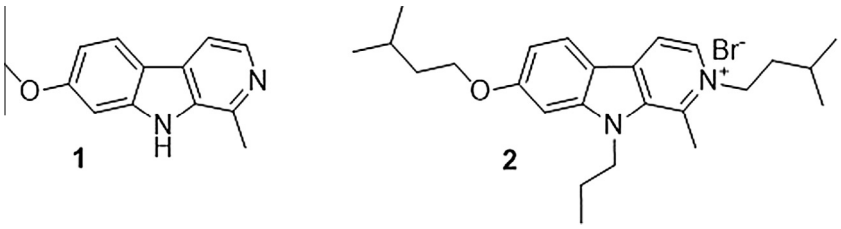

Fig. 1. Structure of harmine (**1**) and **2**.

Aldrich and were used as received. All other chemicals and solvents were of analytical grade and used as received.

2.2. Determination of **2** solubility in presence of αCD, βCD, 2HP-βCD and γCD

Screening was carried out by determining the concentration of soluble **2** in phosphate buffer (pH 7.4) in presence or absence of different CDs (α, β, γ, 2HP-βCD). On 11 μmol of **2**, 1 mL of the studied CD (5 mM) (presence) or 1 mL of buffer (absence) were added. Suspensions were sealed and stirred at 25 °C during 72 h. After filtration on 0.2 μm membrane filter (Life Sciences, 0.2 μm GHP Acrodisc), dilutions by a factor of 150 with phosphate buffer (pH 7.4) were carried out and concentration of **2** compound was determined by UV absorbance at 330 nm on an Agilent Cary 5000 spectrophotometer.

2.3. Phase solubility study of **2** with 2HP-βCD

Complexation of compound **2** with 2HP-βCD was studied following the method described by Higuchi and Connors (1965). An excess of **2** (15 mg) in presence of 1 mL of 2HP-βCD at the studied concentrations (from 0 mM to 200 mM in phosphate buffer pH 7.4) was sealed and stirred at 25 °C during 72 h. After filtration on 0.2 μm membrane filter, dilutions by a factor of 150 with phosphate buffer were carried out and **2** concentration was determined by UV absorbance at 330 nm. The global profile corresponds to an A_N-type phase solubility diagram. However, until 80 mM the complex is characterized by a linearity between soluble **2** and 2HP-βCD concentrations, corresponding to an A_L-type profile (Higuchi and Connors, 1965) until 80 mM. Consequently, the slope inferior to 1, indicates a 1:1 stoichiometry. The apparent stability constant ($K_{1:1}$) was calculated using the slope of the linear regression and the intrinsic solubility (S_0) following Eq. (1):

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

The complexation efficiency (CE) was then calculated using the intrinsic solubility and the apparent stability constant following Eq. (2) (Loftsson et al., 2005a):

$$\text{CE} = S_0 * K_{1:1} \quad (2)$$

2.4. Nuclear magnetic resonance studies

^{1}H NMR studies were carried out in D_2O. All NMR data were recorded on a Jeol spectrometer (JNM EX-400) at 25 °C. Chemical shifts are reported in parts per million (ppm) using the solvent residual peak as reference (δ_H = 4.79 ppm).

2.4.1. Continuous variation (Job's plot) method

Stoichiometry determination was realized following the continuous variation plot method (Job's plot). Solutions of **2** and βCD for several mole ratios r (r = [βCD]/([βCD] + [**2**])) varying from 0 to 1 and keeping the total concentration ([βCD] + [**2**]) equal to 2 mM for each experiment with a constant volume of 1 mL, were stirred at 25 °C during 72 h. The chemical shift of βCD H-3 proton was determined in absence (δ_{free}) and in presence ($\delta_{complex}$) of **2**. The difference $\Delta\delta = \delta_{complex} - \delta_{free}$ was then calculated and the product of $|\Delta\delta| \cdot$ [βCD] was then plotted against molar ratio of βCD. The maximal value obtained for r equal to 0.53 indicates a 1:1 stoichiometry complex.

2.4.2. Complex geometry study

^{1}H NMR of complex was performed by addition of **2** and βCD in D_2O in 1:1 stoichiometry, and was compared to ^{1}H NMR for **2** and βCD alone, in D_2O.

2.5. Complex geometry optimization

Water molecules and methyl 4-hydroxybenzoate ligand were removed from the crystallized structure of βCD complex that served as input geometry (CSD-AJUVEG) (Allen, 2002; Caira et al., 2003). Compound **2** was drawn in the "sketch molecules" tools from Discovery Studio 4.0. Complexes between **2** and βCD were then manually built avoiding interactions between Van der Waals surfaces of the two compounds constituting the complexes. Geometry optimization was performed on each complexes using GULP (Material Studio 6.1) with the Dreiding force field and default values.

2.6. Preparation of complexes

Complexes of **2**:βCD and **2**:2HP-βCD were prepared by stirring 40 mg (87 μmol) of **2** in 10 mL of the appropriate βCD or 2HP-βCD (87 μmol) in distilled water at 25 °C during 72 h. After 15 min centrifugation at 7800 rpm, supernatants were lyophilized using a Christ Alpha 2-4 LD freeze-dryer.

2.7. In vitro growth inhibitory activity experiment

The 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test was realized following the method described elsewhere (Dumont et al., 2007). Briefly, **2** and the two prepared complexes (**2**:βCD and **2**:2HP-βCD, in different concentrations (from 100 μM to 10 nM)), were added to A549 lung cancer cells during 72 h at 37 °C. The resulting metabolic activity was then determined by addition of MTT and detection of formazan at 570 nm. IC_{50} was then calculated as the concentration related to 50% of metabolic activity compared to a control (absence of compound).

3. Results and discussion

3.1. Determination of **2** solubility in presence of αCD, βCD, 2HP-βCD and γCD

Molecule **2** is a lipophilic compound (calculated log$P$ = 3.32 ± 1.51) (Meinguet et al., 2015) which presents an