Solubilization of Thiazolobenzimidazole Using a Combination of pH Adjustment and Complexation with 2-Hydroxypropyl-β-Cyclodextrin

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The thiazolobenzimidazole 1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-d]benzimidazole, TBI, is an experimental drug for the treatment of AIDS which exhibits a low water solubility (11 μg/mL) and is therefore difficult to administer in an injectable solution dosage form at a target solution concentration of 10 mg/mL. The compound has a single ionizable functional group and exhibits an increase in solubility with decreasing pH consistent with a pK_a of 3.55, but the maximum solubility attainable by pH adjustment has been shown to be only 0.4 mg/mL (at pH 2). TBI has been found to form inclusion complexes in either its neutral or its protonated form with 2-hydroxypropyl-β-cyclodextrin (HPCD). The equilibrium constants for 1:1 complex formation were found to be 81 and 1033 M⁻¹ for the protonated and neutral species, respectively. Although the formation of protonated complex is less favored in comparison to the neutral complex, the contribution of this species to the overall solubility of TBI predominates at low pH. Thus, using a combined approach of pH adjustment and complexation with HPCD, a solubility enhancement of 3 orders of magnitude is possible. NMR proton spectroscopy and molecular modeling studies, conducted to understand the orientation of TBI in the complex and the effect of protonation, are described.

KEY WORDS: solubilization; complexation; 2-hydroxypropyl-β-cyclodextrin; cyclodextrins; NMR proton spectroscopy; computer simulation; molecular dynamics; AIDS chemotherapy.

INTRODUCTION

A frequently encountered difficulty in the formulation of new drug candidates in injectable dosage forms is the limited solubility of many such agents in aqueous solutions. Although a variety of methods has been employed for solubilization of such compounds (1,2), there continues to be a need for novel approaches for solubilizing water-insoluble drugs for their development as parenteral solutions.

The thiazolobenzimidazole (TBI) depicted in Fig. 1 is an experimental agent under consideration for clinical testing by the National Cancer Institute for the treatment of AIDS. The compound has a very low water solubility (11 μg/mL), a factor of 10⁴ below the target solution concentration of 10 mg/mL. Although TBI possesses an ionizable functionality, its pK_a (=3.55) is too low to provide adequate solubilization within a physiologically acceptable range (pH >2) by simple pH adjustment. Attempts to solubilize TBI using other classical approaches such as cosolvent solubilization, incorporation into the oil phase of lipid emulsions, and complexation using chemically modified cyclodextrins were unsuccessful. An examination of the structure of TBI suggested, however, that it might be possible for this drug to form inclusion complexes with cyclodextrins such as 2-hydroxypropyl-β-cyclodextrin (HPCD) in either its neutral or its protonated form, since it appeared possible for the aromatic portions of the molecular to be included in the cyclodextrin cavity while allowing the protonated imidazolyl portion to reside outside the cavity in a largely aqueous environment. This paper describes both experimental studies and molecular dynamics simulations that were conducted to explore a combined approach of pH adjustment coupled with complexation using HPCD to achieve the desired solubility enhancement of ≥3 orders of magnitude.

MATERIALS AND METHODS

TBI [1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-d]benzimidazole; NSC No. 625487] was supplied by the National Cancer Institute (Bethesda, MD). 2-Hydroxypropyl-β-cyclodextrin (Molecusol), having an average molecular weight of 1540 and therefore an average degree of substitution of seven 2-hydroxypropyl residues per molecule, was a gift from Pharmatec, Inc. (Alachua, FL). All other compounds were reagent grade from commercial sources and used without further purification.

Solubility Determinations

Solubility studies were carried out in 0.01 ionic strength buffers (3) varying in pH from 2 to 8 and in aqueous solutions of HPCD ranging from 7 to 40% at various pH values and ionic strengths. An amount of sample well in excess of its estimated solubility was suspended in 1–2 mL of the solvent in a 4-mL glass vial sealed with a Teflon-lined cap. The samples were rotated in a water bath (Haake A81, Berlin, Germany) or oven (Shel Oven Lab., Sheldon Manufacturing Inc., Cornelius, OR) maintained at 25 ± 0.5°C for periods of time ranging from 1 to 3 days to ensure equilibrium. All determinations were done in duplicate or triplicate. The equilibrium pH of each solution was measured (pH M82, Radiometer America, Cleveland, OH) and the samples were filtered using 0.45-μm filters (Acro LC 35 or Acro LC 3A, Gelman Sciences, Ann Arbor, MI), suitably diluted, and analyzed by HPLC.

HPLC Analyses

TBI solutions were determined by high-performance liquid chromatography (HPLC) using a reverse-phase C₁₈ column (Brownlee OD224, Applied Biosystems Inc., San Jose, CA, or Supelcosil LC-18-S, Supelco Inc., Bellefonte, PA) with a mobile phase of 70% methanol and 30% ammonium acetate buffer (0.01 M).

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NMR Proton Spectroscopy

The 1D and 2D proton spectra were obtained on a Varian Unity-500 NMR spectrometer at 25°C. The spectra were referenced to DSS in D₄O (99.9 atom% D, Cambridge Isotope Laboratories) and TMS in 1,4-dioxane-d₆ (99.5 atom% D, Aldrich Chemical Co., Milwaukee, WI). Both α-D-glucose (Sigma Chemical Co., St. Louis, MO) and 2-hydroxypropyl-β-cyclodextrin were exchanged for deuterium before use. Samples for NMR analysis were prepared by dissolving 600 mg of exchanged α-D-glucose or HPCD and 5 mg of TBI into 3 mL of D₂O. NMR analyses of the neutral complexes were carried out without further treatment. NMR spectra of the protonated complexes were generated in samples at pD values ≤2.0, adjusted by adding a small amount of concentrated DCl. Based on the equilibrium binding constants determined in this study (cf. Table III), the above conditions should have resulted in >90% of the drug being in complexed form.

Computer Simulations of Binding of TBI with HPCD

Constant-volume, constant-temperature molecular dynamics (MD) simulations of the inclusion complexes were performed on the DISCOVER (Version 2.7) molecular dynamics simulation program (Biosym. Technologies, San Diego, CA). Energy minimizations were conducted for three systems, all of which assumed 1 molecule of protonated TBI, 1 molecule of β-cyclodextrin (CD), and 749 water molecules. β-Cyclodextrin was used in the simulations rather than 2-hydroxypropyl-β-cyclodextrin because the incorporation of seven 2-hydroxypropyl residues into the β-cyclodextrin molecules resulted in too many additional conformational possibilities. The systems simulated were (i) TBI and CD separated from each other by a distance greater than the non-bonded cutoff distance (>8.5 Å); (ii) TBI complexed with CD with its imidazole ring inserted into the CD cavity; and (iii) TBI complexed with CD with its phenyl ring inserted into the CD cavity. The structure of β-cyclodextrin was constructed from single glucopyranose units. Adjusting the torsion angles about the oxygen atom connecting the monomers and repeated energy minimization resulted in a stable seven-membered torus-shaped ring similar to the crystal structure reported by Lindner and Saenger (4). Each molecular system was contained in a box size of 25.5 x 25.5 x 37.0 Å with periodic boundary conditions (density = 1.03 g/cm³). The step size was 1 fs. To start the simulations, different seed numbers were used for initial Maxwellian velocity distribution for each system. TBI was inserted into the wide end of the CD torus using molecular graphics. In order to remove this initial bias, the complex was subjected to simulated annealing; the temperature of the complex was increased at a rate of 50 K/100 steps from 50 to 1000 K. After an additional 2 psec of simulation at 1000 K, the temperature was decreased back to 300 K. Simulations were continued and the coordinates were saved every 0.1 psec for analysis.

RESULTS AND DISCUSSION

TBI Solubility in Various Solvent Systems

Preliminary solubility studies employed a variety of classical cosolvent systems and a lipid emulsion in an attempt to identify systems providing a solubility of ≥10 mg/mL. These solubility data are listed in Table I. TBI is relatively lipophilic, with a soybean oil/water partition coefficient of approximately 10³ (from the solubility ratios) and a soybean oil solubility of 15 mg/mL. However, the highest concentration achievable in a lipid emulsion (Liposyn II, 20%) was found to be only 3 mg/mL. Relatively high percentages of the cosolvents dimethyl sulfoxide (DMSO), propylene glycol, and N-methyl-2-pyrrolidinone were required to reach solubilities in excess of 10 mg/mL, and therefore such systems would be physiologically unacceptable for intravenous administration. Of the cosolvents, N-methyl-2-pyrrolidinone is the most lipophilic and therefore provided higher solubilization at a lower percentage of organic component, but a percentage greater than 50% was necessary to achieve the target concentration.

Table 1. Solubility of TBI in Various Solvent Systems at 25°C

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (mg/mL)</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.011</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Dimethylsulfoxide (DMSO)</td>
<td>&gt;50*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% DMSO/30% water</td>
<td>7.6</td>
<td>2.4</td>
<td>2</td>
</tr>
<tr>
<td>50% DMSO/50% water</td>
<td>1.11</td>
<td>0.0</td>
<td>2</td>
</tr>
<tr>
<td>30% DMSO/70% water</td>
<td>0.21</td>
<td>5.5</td>
<td>3</td>
</tr>
<tr>
<td>70% DMSO/30% 0.01 M HCl</td>
<td>7.6</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>50% DMSO/50% 0.01 M HCl</td>
<td>1.28</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>30% DMSO/70% 0.01 M HCl</td>
<td>0.87</td>
<td>0.0</td>
<td>2</td>
</tr>
<tr>
<td>Propylene glycol (PG)</td>
<td>&gt;12*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% PG/50% water</td>
<td>0.75</td>
<td>4.8</td>
<td>4</td>
</tr>
<tr>
<td>40% PG/60% water</td>
<td>0.22</td>
<td>5.6</td>
<td>2</td>
</tr>
<tr>
<td>33% PG/67% water</td>
<td>0.15</td>
<td>1.1</td>
<td>2</td>
</tr>
<tr>
<td>10% PG/90% water</td>
<td>0.021</td>
<td>4.4</td>
<td>2</td>
</tr>
<tr>
<td>50% PG/50% 0.01 M HCl</td>
<td>1.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Methylpyrrolidinone (NMP)</td>
<td>&gt;40*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70% NMP/30% water</td>
<td>7.9</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td>50% NMP/50% water</td>
<td>3.1</td>
<td>0.6</td>
<td>2</td>
</tr>
<tr>
<td>40% NMP/60% water</td>
<td>1.2</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>15</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Liposyn II 20%</td>
<td>3.1</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Liposyn II 10%</td>
<td>1.8</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

* Visual estimate.