Effects of 5'-Ester Modification on the Physicochemical Properties and Plasma Protein Binding of 5-Iodo-2'-deoxyuridine

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A series of 5'-((O-acyl and O-benzoyl) derivatives of 5-ido-2'-deoxyuridine (IDU) was synthesized by direct acylation of the parent nucleoside in a pyridine-N,N'-dimethylformamide mixture (1:1). Aqueous solubilities in phosphate buffer (pH 7.4), partition coefficients in 1-octanol/phosphate buffer (pH 7.4), plasma protein binding properties, and plasma reversion kinetics of these potential produgs were evaluated. The esters showed an expected increase in lipophilicity with a corresponding decrease in aqueous solubility relative to the parent compound. The association constants (Kₐ) with albumin also exhibited a good linear correlation with the lipophilicity of the compounds. However, the reversion rate constants in plasma varied with the steric and polar nature of the acyl or benzoyl substituent.

KEY WORDS: 5-ido-2'-deoxyuridine; 5'-ester produgs; aqueous solubility; lipophilicity; plasma protein binding; plasma reversion kinetics.

INTRODUCTION

In an attempt to overcome the problems associated with effective IDU delivery to the brain, a series of 5'-mono esters of IDU has been synthesized. The choice of using esters as produgs stems primarily from the fact that the distribution of esterases is ubiquitous and the molecule containing hydroxyl or carboxyl groups can be converted to desired lipophilicity by the selection of an appropriate ester side chain (1). It is assumed that antiviral activity will result from initial hydrolysis of the ester in vivo, followed by the intracellular formation of the triphosphate derivative and eventual incorporation into viral DNA. We have recently reported the synthesis of a few ester produgs of IDU (2) which exhibited enhanced lipophilicity and reduced cytotoxicity and were capable of regenerating parent compound on hydrolysis by esterases present in the rabbit ocular tissues (3). In this article synthesis of some additional novel 5'-esters of IDU, their physicochemical properties, interaction with serum proteins, and reversion kinetics in plasma are described.

MATERIALS AND METHODS

Chemicals

IDU and trimethyl acetyl chloride were obtained from Sigma Chemical Co., St. Louis, MO. All other acid chlorides were procured from Aldrich Chemical Co., Milwaukee, WI. The chemicals and solvents used were of reagent grade and were used as received. The rat serum proteins, i.e., albumin and α₁-acid glycoprotein were also obtained from Sigma Chemical Co. Distilled, deionized water was used for the preparation of buffer solutions as well as mobile phases.

Methods

Melting points were determined on a Thomas Hoover Unimelt capillary device and are uncorrected. ¹H-NMR spectra were run on a Chemagnetics A-200 spectrometer at 200 MHz. Chemical ionization mass spectra were obtained from a Finnegan 4000 mass spectrometer. pH measurements were taken at the temperature of the study using a Corning Model 125 pH meter equipped with a combination electrode (Corning Science Products, Medfield, MA).

Analytical Method

A high-pressure liquid chromatographic (HPLC) method was developed for the analyses of IDU and its derivatives. The system was comprised of a Model 510 solvent delivery module, U6K injector, and 480 UV-Vis variable wavelength detector, all from Waters Associates (Milford, MA). A 25 cm × 4.6-mm-ID reversed phase (C₈) Alltech column, operated at ambient temperature, was used for all separations. The mobile phase consisted of 8% (v/v) (for IDU) and 40% (v/v) (for IDU esters) acetonitrile in water. For propionyl ester, the proportion of acetonitrile was reduced to 30% (v/v). The detection was carried out at 261 nm and the flow rate was maintained at 1.0 ml/min in all cases. Trifluoroacetic acid and ethyl paraben were used as internal standards for IDU and esters analyses respectively. The retention time for IDU was found to be 8 min and for esters it ranged from 6 to 12 min. The presence of a particular compound in the incubation medium was verified by comparing the analyte retention time with that of the freshly prepared standard solutions of authentic samples.

Determination of Aqueous Solubility

A suspension of each compound was prepared by adding excess solid in 0.05 M phosphate buffer (pH 7.4) and was stirred for 72 hr at 25°C. The suspension was centrifuged (8000g) and the supernatant was filtered through 0.45-µm nylon-66 filter (Rainin) and the filtrate was analyzed by HPLC as described earlier.

Determination of Partition Coefficient

Apparent partition coefficients were determined by shake flask method (4) using mutually presaturated aqueous and organic phases at 34°C between 1-octanol and 0.05 M phosphate buffer (pH 7.4). The mixture containing equal volumes of drug solution (in 0.05 M phosphate buffer, pH 7.4) and 1-octanol was stirred at 34°C for 24 hr. The aqueous phase was sampled and analyzed by HPLC. The apparent partition coefficient was determined according to the following equation:

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\[ K = \frac{C_{aq} - C_{eq}}{C_{eq}} \]

\( C_{aq} \) is the total IDU or prodrug concentration at the start and \( C_{eq} \) is the concentration at equilibrium. Hydrolysis of the prodrugs during the time course of the experiment was found to be insignificant.

**Determination of the Enzymatic Hydrolysis Rates in Rat Plasma**

Plasma was obtained from freshly collected heparinized rat blood. The plasma samples were stored in aliquots at \(-20^\circ C\) to avoid repeated freezing and thawing. The ester hydrolyzing activity was determined by monitoring the concentration of IDU ester at different postincubation times. Twenty-five microliters of a freshly prepared solution (\(1.5 \times 10^{-2} \, M\)) of each compound was added to 475 \(\mu l\) of plasma, previously equilibrated at 37\(^\circ\)C in a shaker bath, and mixed thoroughly to generate an initial concentration of 7.5 \(\times 10^{-4} \, M\). At appropriate time points, 25 \(\mu l\) of the reaction mixture was withdrawn and the reaction was stopped by adding 150 \(\mu l\) of ice-cold methanol. Following thorough mixing, the solution was centrifuged at 8000g for 10 min and the supernatant was analyzed by HPLC. No measurable chemical hydrolysis of the ester occurred during the time course of the experiment as shown by the presence of same amount of ester in a control experiment where the plasma was replaced with the same volume of buffer.

**Protein-Binding Studies**

The binding of IDU and its derivatives with two major serum components i.e., albumin and \(\alpha\)-acid glycoprotein, was carried out in a total volume of 0.275 ml. Twenty-five microliters of a freshly prepared solution of the compound of varying concentrations (ranging from \(1.5 \times 10^{-4}\) to \(1.5 \times 10^{-3}\) \(M\)) was added to 0.25 ml of protein solution, previously equilibrated at 37\(^\circ\)C in a shaker bath. Following an incubation for 3 hr at 37\(^\circ\)C and overnight at room temperature, the mixture was filtered through Centricon-10 (Amicon) and the filtrate was analyzed by HPLC. The values for the association constants and the number of binding sites were obtained from Scatchard plot.

**General Procedure for the Synthesis of 5'-Esters of IDU**

The synthesis of 5'-ester derivatives of IDU was carried out according to Narurkar and Mitra with slight modification (2). In short a 10% molar excess of an appropriate acid chloride was added to a chilled solution of IDU in a 1:1 mixture of \(N,N'\)-dimethylformamide and pyridine. The reaction was allowed to continue with constant stirring in an ice bath and the progress of the reaction was periodically monitored by thin-layer chromatography (TLC) using a chloroform-methanol mixture as a developing solvent. At the completion of reaction, i.e., when most of the IDU had been converted, the reaction mixture was dried in vacuo. The residue was dissolved in ethyl acetate and the organic layer was washed twice with water to remove any unreacted reagents. The ethyl acetate layer was evaporated and the residue was purified by silica gel chromatography using a chloroform-methanol mixture as eluent. Purity of the compound was ascertained by HPLC, elemental analysis, TLC, and melting-point determinations. Structural confirmation was made by NMR and CI-MS.

ii. 5'-Propionyl IDU 1H-NMR (Me2SO-d6): 81.07 (t, 3J = 7 Hz, CH3), 2.18 (m, 2, C2H3), 2.42 (q, 2J = 7 Hz, CH2), 3.97 (m, 1, CH3), 4.22 (M, 3, C1H and C2H), 6.09 (t, 1J = 7 Hz, C1H), and 7.97 (S, 1, H2). CI-MS (CH3) m/e 411 (m + 1).

iii. 5'-Butyryl IDU 1H-NMR (CDCl3): 80.99 (t, 3J = 7 Hz, CH3), 1.73 (m, 2, CH2), 2.45 (m, 4, C2H and CH2), 4.16-4.52 (m, 4, C2H, CH, and C2H), 6.24 (t, 1J = 7 Hz, C1H), and 7.98 (S, 1, H2). CI-MS (CH3) m/e 425 (m + 1).

iv. 5'-Isobutyryl IDU 1H-NMR (CDCl3): 81.25 (d, 6J = 6.8 Hz, CH3), 2.53 (m, 2, CH2), 2.71 (septet, 1J = 6.8 Hz), 4.08 - 4.53 (m, 4, C2H, CH, and C2H), 6.23 (t, 1J = 6.3 Hz, C1H), and 7.96 (S, 1, H2). CI-MS (CH3) m/e 425 (m + 1).

v. 5'-Valeryl IDU 1H-NMR (CDCl3): 80.97 (t, 3J = 6.8 Hz, CH3), 1.37 (M, 2, CH2), 1.65 (M, 2, CH2), 2.09 - 2.56 (M, 4, CH, and C2H), 4.13 - 4.49 (M, 4, C2H, C1H, and C2H), 6.25 (t, 1J = 6 Hz, C1H), and 7.99 (S, 1, H6). CI-MS (CH3) m/e 439 (m + 1).

vi. 5'-Pivaloyl IDU 1H-NMR (CDCl3): 81.27 (S, 3, CH3), 2.02 - 2.63 (m, 2, CH2), 4.24 - 4.47 (m, 4, C2H, C1H, and C2H), 6.24 (t, 1J = 7 Hz, C1H), and 7.9 (S, 1, H6). CI-MS (CH3) m/e 439 (m + 1).

vii. 5'-Benzoyl IDU 1H-NMR (Me2SO-d6): 82.19 (ddd, 1J = 3.4, 6.3, 13.6 Hz, C1H), 2.29 (m, 1, C2H), 4.10 (M, 1, C2H), 4.35 (m, 1, C2H), 4.46 (dd, 1J = 5.6, 12 Hz, C3H), 6.13 (t, 1J = 7 Hz, C2H), 7.56 (l, 3J = 8.0 Hz, Ar-H), and 7.67 (m, 1, ArH). CI-MS (isobutane) m/e 459 (m + 1).

viii. 5'-p-Nitrobenzoyl IDU 1H-NMR (Me2SO-d6): 82.20 (ddd, 1J = 3.7, 6.4, 13.6 Hz, C1H), 2.33 (m, 1, C2H), 4.11 (dt, 1J = 3.7, 5.5 Hz, C3H), 4.39 (m, 1, C3H), 4.49 (dd, 1J = 5.7, 12 Hz, C2H), 4.58

Fig. 1. Structures of derivatives of IDU included in the present study.

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\( I = H \)
\( II = \text{CH}_2 \text{CH}_2 \text{CH}_3 \)
\( III = \text{CH}_2 = \text{CH}_2 \text{CH}_3 \)
\( IV = \text{CH}_2 \text{C} (\text{CH}_3)_2 \)
\( V = \text{CH}_2 \text{C} (\text{CH}_3)_2 \)
\( VI = \text{CH}_2 \text{C} (\text{CH}_3) \)
\( VII = \text{CH}_2 \text{C} (\text{CH}_3) \)
\( VIII = \text{CH}_2 \text{C} (\text{CH}_3) \)
\( IX = \text{CH}_2 \text{C} (\text{CH}_3) \)