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-iodo-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 prodrugs 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 (K_a) 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-iodo-2′-deoxyuridine; 5′-ester prodrugs; 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 prodrugs 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 prodrugs 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., abumin and α_1-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. ^{1}H-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 (C_8) All tech 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. Trifluorothymidine 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 (8000*g*) 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°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 (1.5×10^{-2} *M*) of each compound was added to 475 μl of plasma, previously equilibrated at 37°C in a shaker bath, and mixed thoroughly to generate an initial concentration of 7.5×10^{-4} *M*. At appropriate time points, 25 μl of the reaction mixture was withdrawn and the reaction was stopped by adding 150 μl of ice-cold methanol. Following thorough mixing, the solution was centrifuged at 8000*g* 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 α_1-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 1.5×10^{-4} to 1.5×10^{-3} *M*) was added to 0.25 ml of protein solution, previously equilibrated at 37°C in a shaker bath. Following an incubation for 3 hr at 37°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 ^{1}H-NMR (Me_2SO-d_6): δ1.07 (t,3,J = 7 Hz,CH_3), 2.18 (m,2,C_2H), 2.42 (q,2,J = 7 Hz,CH_2), 3.97 (m,1,C_4H), 4.22 (M,3,C_3H and C_5H), 6.09 (t,1,J = 7 Hz,C,H), and 7.97 (S,1,H_6). CI-MS (CH_4) m/e 411 (m + 1).

iii. 5′-Butyryl IDU ^{1}H-NMR ($CDCl_3$): δ0.99 (t,3,J = 7 Hz,CH_3), 1.73 (m,2,CH_2), 2.45 (m,4,C_2H and CH_2), 4.16–4.52 (m,4,C_3H,C_4H and C_5H), 6.24 (t,1,J = 7 Hz,C_1H), and 7.98 (S,1,H_6). CI-MS (CH_4) m/e 425 (m + 1).

iv. 5′-Isobutyryl IDU ^{1}H-NMR ($CDCl_3$): δ1.25 (d,6.J = 6.8 Hz,$2CH_3$), 2.53 (m,2,C_2H), 2.71 (septet,1,J = 6.8 Hz), 4.08 − 4.53 (m,4,C_3H,C_4H, and C_5H), 6.23 (t,1,J = 6.3 Hz,C,H), and 7.96 (S,1,H_6). CI-MS (CH_4) m/e 425 (m + 1).

v. 5′-Valeryl IDU ^{1}H-NMR ($CDCl_3$): δ0.97 (t,3,J = HZ,CH_3), 1.37 (M,2,CH_2), 1.65 (M,2,CH_2), 2.09–2.56 (M,4,CH_2 and C_2H), 4.13–4.49 (M,4,C_3H,C_4H and C_5H), 6.25 (t,1,J = 6 Hz,C,H), and 7.99 (S,1,H6). CI-MS (CH_4) m/e 439 (m + 1).

vi. 5′-Pivaloyl IDU ^{1}H-NMR ($CDCl_3$): δ1.27 (S,9,3,CH_3), 2.02–2.63 (m,2,C_2H), 4.24–4.47 (m,4,C_3H,C_4H, and C_5H), 6.24 (t,1,J = 7 Hz,C,H), and 7.9 (S,1,H6). CI-MS (CH_4) m/e 439 (m + 1).

vii. 5′-Benzoyl IDU ^{1}H-NMR (Me_2SO-d_6): δ2.19 (ddd,1,J = 3.4, 6.3, 13.6 Hz, C_2H), 2.29 (m,1,C_2H), 4.10 (M,1,C_4H), 4.35 (m,1,C_3H), 4.46 (dd,1,J = 5.6, 12 Hz, C_5H), 6.13 (t,1,J = 7.0 Hz,C_1H), 7.56 (t,3,J = 8.0 Hz, Ar-H), and 7.67 (m,1,ArH). CI-MS (isobutane) m/e 459 (m + 1).

viii. 5′-*p*-Nitrobenzoyl IDU ^{1}H-NMR (Me_2SO-d_6): δ2.20 (ddd,1,J = 3.7,6.4,13.6 H_Z C_2H), 2.33 (m,1, C_2H), 4.11 (dt,1,J = 3.7,5.5 H_Z, C_4H), 4.39 (m,1,C_3H), 4.49 (dd,1,J = 5.7, 12 Hz, C_5H), 4.58

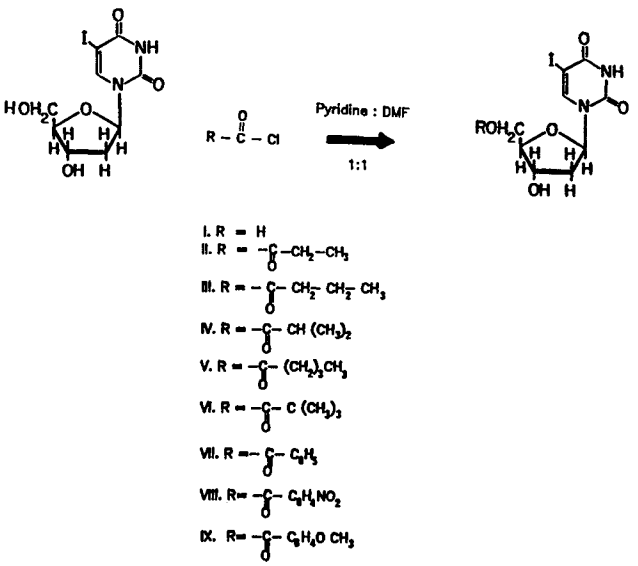

Fig. 1. Structures of derivatives of IDU included in the present study.

Table I. Reaction Conditions, Solubilities, Melting Points, and Elemental Analyses

Compound	Reaction condition	Solubility (mg/ml)	Melting point (°C)	Formula	Analysis (%) Calculated	Found
I	—	2.0	168–171	$C_9H_{11}IN_2O_5$		
II	Ice bath/2–3 days	1.42	167–168	$C_{12}H_{15}IN_2O_6$	C 35.12	35.31
					H 3.66	3.41
					N 6.83	7.13
III	Ice bath/2–3 days	0.614	145–146	$C_{13}H_{17}IN_2O_6$	C 36.79	36.93
					H 4.01	3.79
					N 6.61	6.32
IV	Ice bath/2–3 days	0.74	144–145	$C_{13}H_{17}IN_2O_6$	C 36.79	36.99
					H 4.01	4.14
					N 6.61	6.58
V	Ice bath/2–3 days	0.175	142–143	$C_{14}H_{19}IN_2O_6$	C 38.34	38.48
					H 4.34	4.46
					N 6.39	6.18
VI	Ice bath/2–3 days	0.2	106–107	$C_{14}H_{19}IN_2O_6$	C 36.83	36.94
					H 4.60	4.30
					N 6.14	6.46
VII	Room temp./5 hr	0.152	187–188	$C_{16}H_{15}IN_2O_6$	C 41.9	41.83
					H 3.2	3.27
					N 6.1	6.02
VIII	Ice bath/3.5 hr	0.25	197–198	$C_{16}H_{14}IN_3O_8$	C 38.18	38.3
					H 2.8	2.61
					N 8.3	7.85
IX	Room temp./5 hr	0.138	182–183	$C_{17}H_{17}IN_2O_7$	C 41.8	40.10
					H 3.5	3.34
					N 5.7	5.66

(dd,1,J = 3.6, 12 Hz, C_5H), 6.12 (t,1,J = 6.9 Hz,C,H,) 7.96 (S,1,H_6), 8.23 (d,2,J = 9.1 Hz,Ar-H), and 8.37 (d,2,J = 9.1 Hz, ar-H). CI-MS (isobutane) m/e 504 (m + 1).

ix. 5'-Anisoyl IDU ^{1}H-NMR (Me_2SO-d_6): δ2.16 (m,1,C_2H), 2.24 (m,1,C_2H), 3.81 (S,3,CH_3) 4.06 (m,1,C_3H), 4.31 (m,1,C_4H), 4.39 (m,1,C_5H) 4.44 (m,1,C_5H), 6.10 (t,1,J = 6.7 Hz,C_1H), 7.04 (m,1,Ar-H), 7.05 (m,1,Ar-H), 7.92 (S,1,H_6) 7.93 (d,1,J = 1.1 Hz,Ar-H), 7.95 (d,1,J = 1.1 Hz,Ar-H). CI-MS (isobutane) m/e 489 (m + 1).

RESULTS AND DISCUSSION

Physicochemical Properties

Chemical structures of IDU and its eight 5'-ester derivatives are depicted in Fig. 1. Melting points, aqueous solubilities, reaction conditions, and elemental analyses results are summarized in Table I. It is evident from the melting points that linear aliphatic substituents (C_2–C_5) cause a decrease in melting point. However, the aromatic substituents caused a significant increase in melting point, raising it over that of the parent compound.

The esters exhibited a lower aqueous solubility in pH 7.4 phosphate buffer than the parent compound. For the same number of carbon atoms, branched-chain esters exhibited a higher aqueous solubility and lower 1-octanol/pH 7.4 phosphate buffer partition coefficient as expected from the melting-point data. The lipophilicity of the derivatives were expressed on the basis of their 1-octanol/pH 7.4 phosphate

Table II. Partition Coefficients (P) and Chromatographic Capacity Factors (k') of IDU and Its Esters

Compound	log P	log k'
I	−0.95	—
II	0.678	0.16
III	0.87	0.23
IV	0.83	0.23
V	1.43	0.35
VI	1.34	0.28
VII	1.40	0.32
VIII	1.24	0.26
IX	1.38	0.3

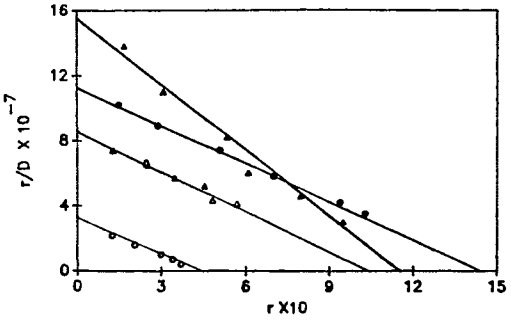

Fig. 2. Graphic determination of number of binding sites and association constants for interaction of IDU and its ester derivatives with albumin. r = moles drug bound per mole of albumin; D = molar concentration of unbound drug. (○——○) IDU; (△——△) isobutyryl; (●——●) propionyl; (▲——▲) pivaloyl IDU.

Table III. Association Constants (k_a) and the Number of Binding Sites (n) for IDU and Its Esters

Compound	Association constant (k_a) $\times 10^{-7}$	Number of binding sites (n)
I	6.8	0.43
II	7.8	1.46
III	11.9	1.13
IV	8.1	1.04
V	16.2	1.08
VI	13.72	1.14
VII	16.9	0.89
VIII	13.10	0.93
IX	14.62	1.08

buffer partition coefficients (P) and chromatographic capacity factors (k') on a reversed-phase column. As already mentioned the relative lipophilicities of the derivatives can be evaluated by reversed-phase HPLC techniques (5,6) where the capacity factor of a solute is considered as a measuring unit of its relative lipophilicity. The capacity factors of the ester derivatives were calculated on the basis of the retention times of solute (t_r) and solvent (t_o) using the relationship $k' = (t_r - t_o)/t_o$. With acetonitrile in water (40% v/v) as the mobile phase, the capacity factors for the ester derivatives III–IX exhibited a good linear correlation (0.989) with the 1-octanol/water partition coefficients. Rank order correlations of log P values (Table II) for the esters are mutually in good agreement with the log k' constants (7). A similar relationship has been reported for other ester derivatives (8,9). Hence, a proper selection of an ester substituent group may provide optimum lipophilicity for blood–brain barrier transport.

Plasma Protein Binding

Binding studies were performed with rat serum albumin and rat serum α_1-acid glycoprotein. No appreciable binding was observed with α_1-acid glycoprotein, whereas albumin produced measurable binding with IDU and 5′-esters. The association constants (K_a) and the number of binding sites (n) for each compound were calculated by means of a Scatchard plot. Figure 2 illustrates such plots where r/D versus r exhibited good linearity ($r^2 \approx 0.979$–0.985). The quantity r denotes the moles of drug bound per mole of protein and D is the amount of free drug present. The linearity of the Scatchard plots suggests that there is probably one type of binding site available for thymidine analogues. The n and K_a values calculated from these plots have been summarized in Table III. The parent compound IDU has a n value of 0.43, which tends to suggest that two moles of the parent drug bind to one mole of protein. However, masking of the 5′-hydroxyl group by esterification leads to 1:1 binding. Maximum K_a value was observed with 5′-valeryl ester, which was followed in order by benzoyl, anisoyl, pivaloyl, p-nitrobenzoyl, butyryl, isobutyryl, and proprionyl esters. Some recent reports, however, indicated that serum protein-bound ligands were taken up into the brain (10–12) and such a phenomenon was particularly observed with albumin and α_1-acid glycoprotein in the transport of imipramine and desipramine (13).

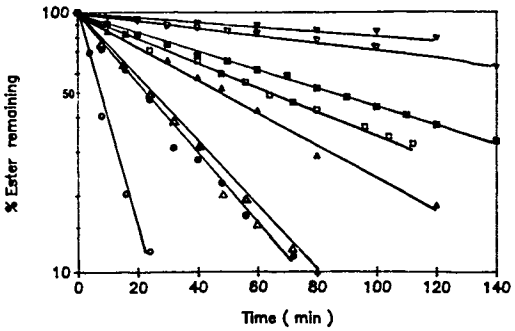

Fig. 3. Hydrolysis of various IDU esters by rat plasma as a function of time. (○——○) Valeryl IDU; (●——●) butyryl IDU (△——△) isobutyryl IDU, (▲——▲) p-nitrobenzoyl IDU; (□——□) pivaloyl IDU; (■——■) propionyl IDU; (▽——▽) benzoyl IDU; (▼——▼) anisoyl IDU.

Table IV. The Observed Hydrolytic Rate Constants (k_i) and Half-Lives ($t_{1/2}$) in Rat Plasma at 37°C

Compound	$k \times 10$ (min^{-1})	$t_{1/2}$ (min)
II	9.1	76.15
III	50.0	13.86
IV	26.2	26.45
V	94.6	7.32
VI	10.8	64.16
VII	3.4	203.00
VIII	14.3	48.46
IX	1.9	364.00

Hydrolysis of IDU Esters by Rat Plasma

The hydrolysis of IDU esters by rat plasma are illustrated graphically in Fig. 3. The decrease in IDU ester concentration was accompanied by an increase in the appearance of free IDU (data not shown), demonstrating the presence of esterase or esterase-like activity in rat plasma. No significant hydrolysis of the ester in the blank buffer solution was observed during the experiment. No other peak in the chromatograms other than that corresponding to IDU appeared during the hydrolysis of the esters. The esters were hydrolyzed at varying rates, depending on the nature of the substituent group. As demonstrated in Table IV maximum hydrolysis was obtained with valeryl ester, followed by butyryl, isobutyryl, p-nitrobenzoyl, pivaloyl, propionyl, benzoyl, and anisoyl derivatives. Enhanced hydrolysis with increasing chain length could be due to enhanced interaction between the substrate and the enzyme, a phenomenon described for many ester prodrugs (14,15). However, an increase in steric factor of 5′-acyl substituent tends to decrease the hydrolysis i.e., pivaloyl < isobutyryl < n-butyryl, demonstrating possible steric hindrance. Increased hydrolysis with p-nitrobenzoyl ester among aromatic esters might be due to a possible electrostatic interaction between the substrate and the enzyme since the p-nitro group has strong electron withdrawing properties. The hydrolytic stability of benzoyl and anisoyl derivatives also indicates that these

compounds may be useful in enhancing brain uptake of the parent nucleoside analogue because they will generate a steady or sustained level of the parent drug in the brain parenchyma.

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