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Report

Synthesis, Physicochemical Properties, and Cytotoxicity of a Series of 5′-Ester Prodrugs of 5-Iodo-2′-deoxyuridine

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Five aliphatic 5′-esters of 5-iodo-2′deoxyuridine (IDU) were synthesized via an acid chloride alcoholysis reaction. The solubility in pH 7.4 phosphate buffer, lipophilicity as determined by partition experiments in octanol/pH 7.4 buffer, and cytotoxicity of these potential prodrugs were evaluated. The esters showed a 43- to 250-fold increase in lipophilicity and a 1.6- to 14-fold decrease in aqueous solubility relative to IDU. At a concentration of 50 μM, all esters showed reduced cytotoxicity toward uninfected Vero cells relative to IDU.

KEY WORDS: 5-iodo-2′-deoxyuridine; 5′-ester prodrugs; solubility; partition coefficient; cytotoxicity.

INTRODUCTION

Herpes simplex virus type I infection (HSV-1) is the leading cause of corneal scarring and visual impairment in the United States (1,2). 5-Iodo-2′-deoxyuridine (IDU) is used in the treatment of ocular HSV-1 infections such as keratitis. Its therapeutic usefulness is limited, however, by frequent administration (3), a high incidence of treatment failure unassociated with resistant viral strains, and the inability to eradicate virus particles from deep ocular tissues (4). These problems can be attributed largely to the polar nature of IDU resulting in poor permeability of the drug across the lipoidal epithelial layer of the corneal membrane.

Incomplete ocular absorption of drugs is due primarily to low corneal permeability and/or rapid drainage from the application site (5,6). The prodrug approach has been successfully applied to improve the ocular bioavailability of epinephrine (7–9), nadolol (10), various prostaglandins (11), pilocarpine (12), and timolol (13).

Five aliphatic 5′-ester derivatives of IDU (II–VI) (Fig. 1) were synthesized as potential prodrugs and tested for cytotoxicity. It was felt that transient blocking of the 5′-hydroxyl group will enhance the lipophilicity of IDU and also help reduce phosphorylation at the 5′- postion by thymidine kinase. Prodrug-based delivery, while resulting in appreciable IDU concentrations at the infected ocular sites, may also help in minimizing its toxicity, due to reduction in drug entrapment by uninfected normal cells.

MATERIALS AND METHODS

Chemicals

IDU and trimethyl acetyl chloride were obtained from Sigma Chemical Co., Mo. All other acid chlorides were obtained from Aldrich Chemical Co., Wis. Chemicals and solvents used were of reagent grade and were used as received. 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 made at the temperature of the study using a Corning Model 125 pH meter equipped with a combination electrode (Corning Science Products, Medfield, Mass.).

The high-performance liquid chromatographic (HPLC) setup consisted of a Waters model 510 pump equipped with a Waters U6K injector, a Waters Lambda Max Model 481 variable wavelength LC spectrophotometer, and a Fisher Recordall series 5000 strip chart recorder. The mobile phase for prodrug analyses was 25–40% (v/v) acetonitrite in water

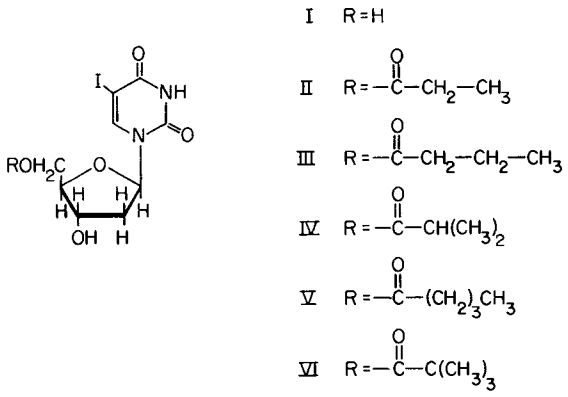

Fig. 1. IDU and five aliphatic 5′-ester derivatives.

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at a flow rate of 1.0 ml/min. Alltech C_{18} econosphere octadecyl silane column was used. IDU was analyzed with a mobile phase of 25% (v/v) of methanol in water at a flow rate of 1.0 ml/min on a Waters μBondapak phenyl column. Ultraviolet detection was performed at 261 nm. Temperature was ambient. Parabens and benzamide were used as internal standards for prodrugs and IDU analyses, respectively.

General Procedure for Synthesis of 5'-Esters

Direct acylation of IDU was effected by adding slowly a 10% molar excess of the appropriate acyl chloride to a chilled solution of IDU in a 1:1 mixture of pyridine:*N,N*-dimethyl formamide. The solvent–base combination was found to facilitate greatly the selectivity of acylation of the primary hydroxyl group over the secondary hydroxyl group (14). This could be a consequence of having the acylating agent as a charged species (*N*-acyl pydridinium chloride) in an aprotic, polar solvent such as DMF (15,16). The reaction was allowed to continue in an ice bath for 2–3 days. At completion, the mixture was evaporated to dryness with a rotary evaporator, under reduced pressure. The residue was dissolved in chloroform and the organic phase was washed with water, saturated sodium bicarbonate solution, and water. Evaporation of chloroform under reduced pressure gave the corresponding 5'-ester derivatives.

Compounds II–VI were recrystallized from benzene–methanol in over a 50% yield. Purity was determined by HPLC, elemental analysis, 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_1H), and 7.97 (s, 1, H_6). CI-MS (CH_4) *m/e* 411 (m + 1).

Anal. Calcd. for $C_{12}H_{15}IN_2O_6$ (410.1): C, 35.12; H, 3.66; N, 6.83. Found: C, 35.31; H, 3.41; N, 7.13.

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).

Anal. Calcd. for $C_{13}H_{17}IN_2O_6$ (424.1): C, 36.79; H, 4.01; N, 6.61. Found: C, 36.93; H, 3.79; N, 6.32.

IV. 5'-Isobutyryl IDU. ^{1}H-NMR ($CDCl_3$): δ1.25 (d, 6, J = 6.8 Hz, 2 CH_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_1H), and 7.96 (s, 1, H_6) CI-MS (CH_4) *m/e* 425 (m + 1).

Anal. Calcd. for $C_{13}H_{17}IN_2O_6$ (424.1): C, 36.79; H, 4.01; N, 6.61. Found: C, 36.99; H, 4.14; N, 6.58.

V. 5'-Valeryl IDU. ^{1}H-NMR ($CDCl_3$,: δ0.97 (t, 3, J = 7 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_1H), and 7.99 (s, 1, H_6). CI-MS (CH_4) *m/e* 439 (m + 1).

Anal. Calcd. for $C_{14}H_{19}IN_2O_6$ (438.1): C, 38.34; H, 4.34; N, 6.39. Found: C, 38.48; M, 4.46; N, 6.18.

VI. 5'-Pivaloyl IDU. ^{1}H-NMR ($CDCl_3$): δ1.27 (s, 9, $3CH_3$), 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_1H), and 7.9 (s, 1, H_6). CI-MS (CH_4) *m/e* 439 (m + 1).

Anal. Calcd. for $C_{14}H_{19}IN_2O_6 \cdot H_20$ (456.1): C, 36.83; H, 4.60; N, 6.14. Found: C, 36.94; H, 4.30; N, 6.46.

Determination of Aqueous Solubility

Excess solid in 0.05 *M* phosphate buffer (pH 7.4) was allowed to equilibrate at 25°C with continous stirring for 72 hr. The suspensions were then filtered through 0.45-μM nylon-66 filters (Rainin) and the filtrate was analyzed by HPLC. Triplicate samples were run and the mean aqueous solubility was calculated.

Determination of Partition Coefficients

Apparent partition coefficients were determined at 34°C between 1-octanol and 0.05 *M* phosphate buffer at pH 7.4. Mutually presaturated aqueous and organic phases were used. The compounds were dissolved in the aqueous buffer phase and then mixed with an equal volume of 1-octanol and stirred at 34°C till HPLC analysis ensured equilibrium. The aqueous phase was sampled and analyzed by HPLC. Partition coefficients were calculated from Eq. (1).

$$K = \frac{C_{aq} - C_{eq}}{C_{aq}} \quad (1)$$

where C_{aq} is the total IDU or prodrug concentration and C_{eq} is the aqueous IDU or prodrug concentration at equilibrium. Hydrolysis of prodrugs during the experiment was insignificant.

Protocol for Cytotoxicity Study

Vero cells in MEM–Earl's salts with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin were plated in fifty-four 25-cm^2 flasks at a density of 220,000 cells/2.5 ml/flask. The cells were al-

Table I. Physicochemical Properties of IDU and Its 5'-Ester Prodrugs

Compound	m.p. (°C)	Solubility[a] in pH 7.4 phosphate buffer, 25°C [M/L ± SD (×10^3)]	K^a ± SD (octanol/water)
I	168–171 (dec)	5.65 (0.5)	0.11 (0.02)
II	167–168	3.48 (0.3)	4.77 (0.1)
III	145–146	1.45 (0.1)	7.50 (0.3)
IV	144–145	1.75 (0.3)	6.92 (0.8)
V	142–143	0.40 (0.2)	27.54 (2.0)
VI	106–107	0.44 (0.1)	22.10 (1.5)

[a] $N = 3$.

Table II. Effect of IDU and Its 5′-Ester Prodrugs on the Replication of Uninfected Vero Cells *In Vitro*[a]

Compound	Percentage inhibition of Vero cells	
	50 μ*M*	400 μ*M*
I	25.9	58
II	6.0	36
III	21.1	50
IV	18.8	33
V	12.9	41
VI	9.3	72

[a] Assays were carried out in triplicate with appropriate controls.

lowed to attach for 24 hr. The cytotoxicity of compounds (I–VI) on uninfected Vero cells was determined at 50 and 400 μ*M*.

On day 1, test compounds were added to the medium to yield a concentration of 50 and 400 μ*M*. Immediately after adding test samples to flasks, two flasks from each group were counted. The flasks were washed with phosphate-deficient buffered saline and then trypsinized with 1 ml of trypsin–Versene, 0.05% trypsin, and 0.02% EDTA, until the cells were dislodged. The number of cells was counted using a hemocytometer after adding 1 ml of trypan blue solution.

Each day for the next 2 days, two of the remaining flasks were harvested in the aforementioned manner for determination of cell number. Assays were carried out in triplicate with appropriate controls.

RESULTS AND DISCUSSION

Melting points, aqueous solubilities, and octanol/water partition coefficients of compounds are shown in Table I. All prodrugs have lower melting points than that of IDU, which may be due to the loss of tightly bound crystal structure of IDU resulting from the weakening of intramolecular hydrogen bonding. The aqueous solubility measurements showed the expected decrease, whereas octanol/water partition coefficients exhibited an increase with an increase in the number of carbon atoms in the promoiety. For the same number of carbons, branched-chain esters were more soluble and had a lower partition coefficient than their normal-chain analogues.

The effect of IDU and 5′-ester prodrugs on the replication of uninfected host Vero cells is shown in Table II. All 5′-ester derivatives (II–VI) appear to be less cytotoxic than IDU to Vero cells at a concentration of 50 μ*M*. Similar results are obtained at a concentration of 400 μ*M* for compounds II–V. Compound VI, however, appears to be more toxic to Vero cells than the parent drug at a concentration of 400 μ*M*. As postulated in Scheme I, IDU is sequentially phosphorylated at the 5′-position to the triphosphate by both viral and cellular thymidine kinase. Viral thymidine kinase, however, possesses a higher affinity for IDU and is able to trap greater amounts of IDU than the cellular enzyme. IDU triphosphate is then incorporated into viral DNA instead of deoxythymidine triphosphate by viral DNA polymerase, which is the basis for antiviral activity of IDU.

Esterification of the 5′-hydroxyl group probably results in reduction of the molecule's ability to serve as a substrate for thymidine kinase. Consequently, the ester is not phosphorylated and does not get incorporated into viral and cellular DNA. This may explain the reduced cytotoxicity to Vero cells observed with almost all esters. Studies on adenosine and related compounds have shown that a free hydroxyl group at the 5′ position was required to serve as a good substrate for adenosine deaminase (17,18).

The purpose of this study is to identify IDU prodrugs for improved ocular delivery. Enzymatic hydrolysis in ocular tissue homogenates and *in vitro* corneal transport of prodrugs will be discussed in a future communication.

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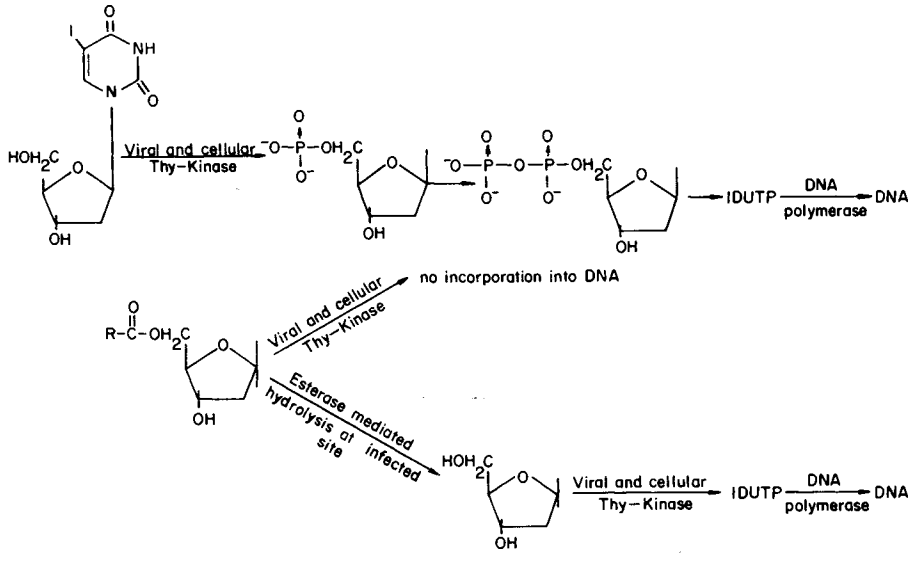

Scheme I

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