New Oligoethylene Ester Derivatives of 5-Iodo-2′-deoxyuridine as Dermal Prodrugs: Synthesis, Physicochemical Properties, and Skin Permeation Studies

FRANCESCO PAOLO BONINA,[1] MARIA GRAZIA RIMOLI,[2] LUCIA AVALLONE,[2] FRANCESCO BARBATO,[2] MARZIA AMATO,[2] CARMELO PUGLIA,[1] MAURIZIO RICCI,[3] PAOLO DE CAPRARIIS[4]

[1]Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università degli Studi di Catania, Viale A. Doria 6, 95125 Catania, Italy

[2]Dipartimento di Chimica Farmaceutica e Tossicologica, Facoltà di Farmacia, Università degli Studi di Napoli Federico II, Via Domenico Montesano 49, 80131 Napoli., Italy

[3]Dipartimento di Chimica e Tecnologia del Farmaco, Facoltà di Farmacia, Università degli Studi di Perugia, Via del Liceo 1, 06100 Perugia, Italy

[4]Dipartimento Farmaco Chimico Tossicologico, Facoltà di Farmacia, Università di Sassari, Via Moroni 23, 07100 Sassari, Italy

Received 29 February 2000; revised 7 May 2001; accepted 25 May 2001

ABSTRACT: Five new oligoethylene ester derivatives (**9–13**) of 5-iodo-2′-deoxyuridine (IDU) were synthesized and assayed to determine their lipophilicity by both experimental lipophilicity indices ($\log K'$) and calculated partition coefficients (CLOGP). *In vitro* experiments were carried out to evaluate the chemical and enzymatic stability and fluxes through excised human skin of these new IDU derivatives. Esters **9–13** showed increased lipophilicity compared with the parent drug (IDU), had good stability in phosphate buffer (pH 7.4), and were readily hydrolyzed by porcine esterase. No correlation between lipophilicity and skin permeation fluxes of synthesized esters **9–13** was observed. Results from *in vitro* percutaneous absorption studies showed that, among all esters synthesized, only esters **9** and **10** significantly increased the cumulative amount of IDU that penetrated through excised human skin compared with the parent drug (IDU). © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 91:171–179, 2002

Keywords: IDU; dermal prodrugs; SCE membranes; in vitro

INTRODUCTION

Although 5-iodo-2′-deoxyuridine (IDU) is useful in the treatment of skin herpes simplex keratitis,[1] its therapeutic usefulness is limited by its poor skin permeability, which is due to the polar nature of this drug. To overcome this problem, various strategies have been used to enhance IDU skin permeation. Approaches have included formulation additives, such as chemical penetration enhancers, and vehicles that maximize partitioning of IDU into the skin.[2–4] The penetration enhancers are chemical compounds that are themselves pharmacologically inactive but can partition into the skin and interact with the constituents of the superficial skin layer and reduce the resistance of the skin to drug diffusion.[5] The use of penetration enhancers shows some potential disadvantages:[6] the inclusion of these compounds within a formulation may increase the absorption of components other than the drug, which can provoke skin damage and

Correspondence to: Francesco Paolo Bonina (Telephone: 0039-95-222258, Fax: 0039-95-222258; E-mail: boninafi@mbox.unict.it)

Journal of Pharmaceutical Sciences, Vol. 91, 171–179 (2002)

toxicity. The prodrug approach represents an alternative and very promising method of enhancing the skin permeability of a topically applied drug.

The prodrug concept involves the chemical modification of a drug into a bioreversible form to change its pharmacological and pharmacokinetic properties and thus enhance its skin delivery. At the same time, the prodrug should be able to readily regenerate enzymatically to the parent drug in the target tissues (viable epidermis and/or dermis). The prodrug strategy has been used to increase IDU permeability through various biological membranes such as blood–brain barrier[7,8] and cornea.[9]

In the recent past, we have applied a new promising strategy in the dermal prodrug design that consists of conjugating the parent drug with promoieties that have an inherent ability to enhance skin permeability. We have obtained several dermal prodrugs of nonsteroidal antiinflammatory drugs, such as indomethacin and naproxen, conjugating them by ester linkage to different penetration enhancers such as *N*-alkyllactames,[10,11] *N*-acyllactames[12] polyoxyethylene glycols,[13] and terpenoids.[14]

Some of these prodrugs with good water stability, rapid enzymatic hydrolysis, and increased fluxes through excised human skin can be regarded as promising indomethacin and naproxen dermal prodrugs. To increase IDU dermal delivery, we describe the synthesis of new ester derivatives of IDU (**9–13**) by coupling this drug with polyoxyethylene glycols via a succinic acid spacer (see Scheme 1). IDU derivatives **9–13** were tested to determine their hydrolytic stability in water and susceptibility to undergoing *in vitro* enzymatic cleavage and fluxes through excised human skin.

EXPERIMENTAL SECTION

Chemicals and Apparatus

IDU and the diethylene glycol (PEG2), triethylene glycol (PEG3), tetraethylene glycol (PEG4), pentaethylene glycol (PEG5), and hexaethylene glycol (PEG6) were obtained from Fluka. All the other chemicals were of analytical or reagent grade and were obtained from commercial sources.

The course of reactions and the purity of products were monitored by thin layer chromatography (TLC) using precoated silica gel aluminium plates (Merck 60 $_{F254s}$). Detection of spots was made by ultraviolet (UV) radiation, exposure to iodine vapor, and spraying with $Ce(SO_4)_2$ aqueous solution. Preparative separations were performed in columns containing Merck 60 silica gel (70–230 mesh). Melting points were determined with a Kofler hot stage microscope and are uncorrected.

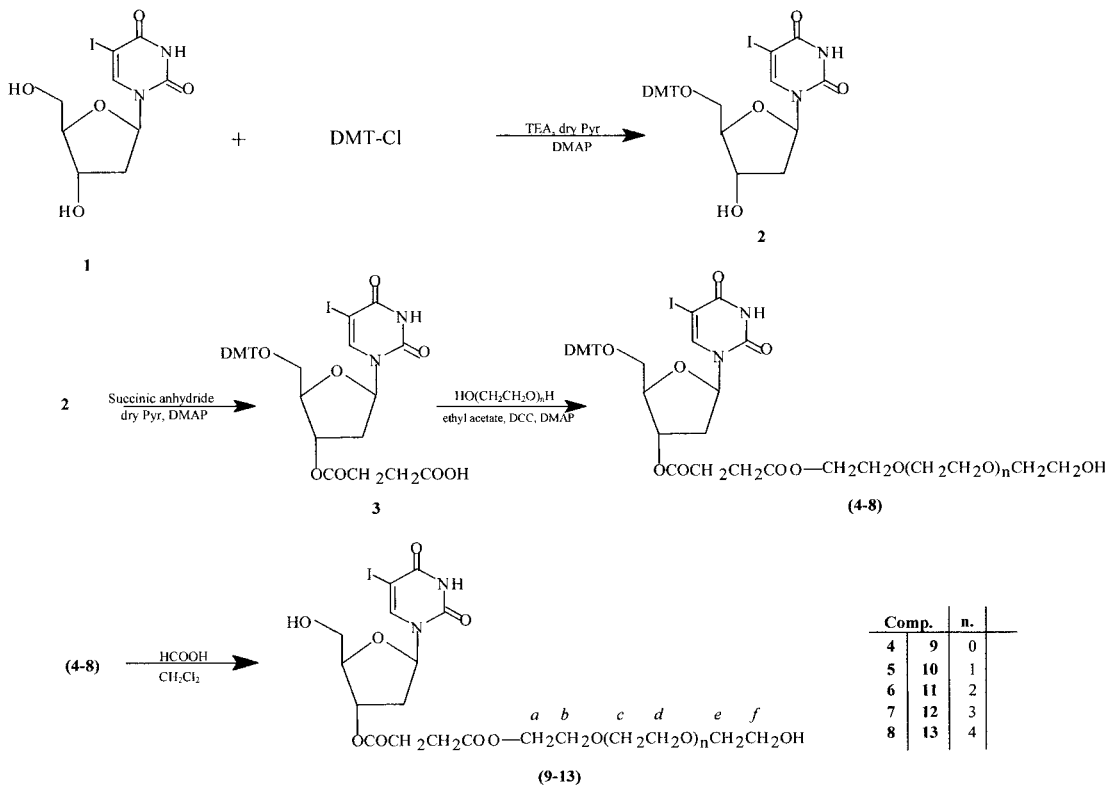
Elemental analyses were carried out with a Carlo Erba model 1106 analyzer. The product characterization by proton (^{1}H) and carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopy was carried out with a Bruker AMX-500 spectrometer ($CDCl_3$ and CD_3OD as solvents). Chemical shift values are reported in δ units (ppm).

General Methods of Prodrug Synthesis

For the preparation of the IDU esters **9–13** (Scheme 1), initially the 5′-OH of IDU (1) was blocked by reaction with 4,4′-dimethoxytrityl chloride (DMT-Cl) to give 5′-*O*-dimethoxytrityl-5-iodo-2′-deoxyuridine (2); then, the 3′-OH of IDU was esterified by succinic anhydride yielding 5′-*O*-dimethoxytrityl-5-iodo-2′-deoxyuridine-3′-*O*-succinate (3). The syntheses of **2** and **3** were reported in the previous paper.[15]

The product **3** was coupled respectively with the different glycols to give 3′-*O*-(polyoxyethyleneglycol-succinyl)-5′-dimethoxytrityl-5-iodo-2′-deoxyuridine derivatives (**4–8**). Briefly, dicyclohexylcarbodiimide (DCC; 270 mg; 1.30 mmol) was added to a solution consisting of **3** (1 g; 1.30 mmol), 4-*N*,*N*-dimethylaminopyridine (DMAP), and the appropriate oligoethylene glycol (5.28 mmol) in ethyl acetate. The reaction mixture was stirred at room temperature for ~6 h under anhydrous conditions. The progress of the reaction was periodically monitored by TLC using a chloroform–methanol mixture (99:1) as a developing solvent. At the completion of reaction, the separated dicyclohexylurea (DCU) was filtered off. The filtrate was washed twice with water to remove any unreacted reagents and dried over anhydrous sodium sulfate. The organic phase was evaporated *in vacuo*, and the residue was purified by silica gel chromatography, using as eluent chloroform containing 0.5% pyridine and then with a step gradient up to ~5% methanol in chloroform.

Successively, the dimethoxytriphenyl group was removed by HCOOH, yielding 3′-*O*-(polyoxyethyleneglycol-succinyl)-5-iodo-2′-deoxyuridine conjugates **9–13** by a method reported in



Scheme 1. Synthesis of esters **9–13**.

literature.[16] In particular, a solution of derivatives **4–8** in CH_2Cl_2–HCOOH (6 mL, 5:1, v/v) was stirred for 45 min at room temperature. The reaction mixture was chromatographed, eluting first with chloroform until the dimethoxytriphenyl–methanol was removed and then with chloroform–methanol (95:5 v/v) to obtain the required products (**9–13**).

The esters **9–13** were obtained in oil forms and they failed to crystallize. Elemental analysis (C, H, N) of all synthesized products were within ±0.3% of the theoretical values and the infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectral data were consistent with their structures.

PEG2-3′-O-succinyl-5-iodo-2′-deoxyuridine Ester (9)

^{1}H NMR ($CDCl_3$): δ 2.30–2.40 (m, 2H, 2′-H); 2.65 (m, 4H, $COCH_2CH_2CO$); 3.55 (t, 2H, *e*); 3.65 (AB system, 2H, 5′-H); 3.75 (m, (4H, *b*, *f*); 4.05 (m, 1H, 4′-H); 4.25 (t, 2H, *a*); 5.20 (m, 1H, 3′-H); 6.15 (m, 1H, 1′-H); 8.20 (s, 1H, C-6-H). ^{13}C NMR ($CDCl_3$): δ 173.78–173.23 (two succinic spacer CO); 162.47 (4-CO); 151.69 (2-CO); 146.75 (6-CH); 86.65 (4′-CH); 86.47 (1′-CH); 76.56 (3′-CH); 73.31 (*e*); 69.73 (*b*); 68.55 (5-C); 64.74 (5′-CH_2); 62.54 (*a*); 61.88 (*f*); 38.67 (2′-CH_2); 29.70, 29.52 (two succinic spacer CH_2).

PEG3-3′-O-succinyl-5-iodo-2′-deoxyuridine Ester (10)

^{1}H NMR ($CDCl_3$): δ 2.35–2.45 (m, 2H, 2′-H); 2.60 (m, 4H, $COCH_2CH_2CO$); 3.30–3.40 (AB system, 2H, 5′-H); 3.50–3.70 (10H, *b*, *c*, *d*, *e*, *f*); 4.10 (m, 1H, 4′-H); 4.20 (t, 2H, *a*); 5.40 (m, 1H, 3′-H); 6.25 (m, 1H, 1′-H); 8.10 (s, 1H, C-6-H). ^{13}C NMR ($CDCl_3$): δ 172.06–171.61 (two succinic spacer CO); 159.72 (4-CO, 2-CO); 143.96 (6-CH); 85.09 (4′-CH); 84.34 (1′-CH); 75.47 (3′-CH); 72.45 (*e*); 70.53 (*c*); 70.29 (*d*); 68.97 (*b*, 5-C); 63.81 (*a*); 63.53 (5′-CH_2); 61.69 (*f*); 38.49 (2′-CH_2); 29.08, 28.86 (two succinic spacer CH_2).

PEG4-3′-O-succinyl-5-iodo-2′-deoxyuridine Ester (11)

^{1}H NMR ($CDCl_3$): δ 2.35–2.45 (m, 2H, 2′-H); 2.65 (m, 4H, $COCH_2CH_2CO$); 3.30–3.40 (AB system, 2H, 5′-H); 3.60–3.70 (m, 14H, *b, c, d, e, f*); 4.10 (m, 1H, 4′-H); 4.20 (t, 2H, *a*); 5.40 (m, 1H, 3′-H); 6.22 (m, 1H, 1′-H); 8.09 (s, 1H, C-6-H). ^{13}C NMR

($CDCl_3$): δ 171.61–172.06 (two succinic spacer CO); 160.00 (4-CO); 150.00 (2-CO); 145.45 (6-CH); 85.96 (4′-CH); 85.45 (1′-CH); 75.15 (3′-CH); 72.49 (*e*); 70.47, 70.38, 70.34, 70.15 (4C, *c, d*); 68.98 (*b*); 68.95 (5-C); 63.90 (*a*); 62.14 (5′-CH_2); 61.51 (*f*); 38.06 (2′-CH_2); 29.14, 28.97 (two succinic spacer CH_2).

PEG5-3′-O-succinyl-5-iodo-2′-deoxyuridine Ester (12)

^{1}H NMR ($CDCl_3$): δ 2.35–2.45 (m, 2H, 2′-H); 2.50 (m, 4H, $COCH_2CH_2CO$); 3.30–3.40 (AB system, 2H, 5′-H); 3.40–3.60 (m, 18H, *b, c, d, e, f*); 4.00 (m, 1H, 4′-H); 4.10 (t, 2H, *a*); 5.30 (m, 1H, 3′-H); 6.20 (m, 1H, 1′-H); 8.09 (s, 1H, C-6-H). ^{13}C NMR ($CDCl_3$): δ 174.87–174.11 (two succinic spacer CO); 163.09 (4-CO); 151.39 (2-CO); 146.40 (6-CH); 85.85 (4′-CH); 84.98 (1′-CH); 75.04 (3′-CH); 71.91 (*e*); 69.84, 69.78, 69.72, 69.65 (6C, *c*, *d*); 68.60 (*b*); 68.28 (5-C); 64.36 (*a*); 61.30 (5′-CH_2); 60.57 (*f*); 36.92 (2′-CH_2); 29.17, 29.00 (two succinic spacer CH_2).

PEG6-3′-O-succinyl-5-iodo-2′-deoxyuridine Ester (13)

^{1}H NMR ($CDCl_3$): δ 2.35–2.45 (m, 2H, 2′-H); 2.55 (m, 4H, $COCH_2CH_2CO$); 3.25–3.35 (AB system, 2H, 5′-H); 3.45-3.65 (m, 22H, *b, c, d, e, f*); 4.00 (m, 1H, 4′-H); 4.12 (t, 2H, *a*); 5.35 (m, 1H, 3′-H); 6.19 (m, 1H, 1′-H); 8.00 (s, 1H, C-6-H). ^{13}C NMR ($CDCl_3$): δ 171.61–172.06 (two succinic spacer CO); 160.00 (4-CO); 150.00 (2-CO); 145.45 (6-CH); 85.00 (4′-CH); 84.05 (1′-CH); 76.71 (3′-CH); 72.39 (*e*); 70.28, 70.23, 69.96 (8C, *c*, *d*); 68.80 (5-C); 68.73 (*b*); 63.75 (*a*); 63.29 (5′-CH_2); 61.32 (*f*); 38.00 (2′-CH_2); 28.80, 28.60 (two succinic spacer CH_2).

Chemical and Enzymatic Hydrolysis

Initially, solutions were prepared by dissolving an aliquot of each ester **9–13** in methanol (20 mg/mL). The hydrolysis rate for all esters **9–13** was determined by dissolving 200 μL of methanolic solution in 10 mL of phosphate buffer saline (PBS; 0.05 M, pH 7.4), which was thermostatically controlled at 37°C, and following the disappearance of the chromatographic peak of the esters (**9–13**) by the HPLC method reported later.

Enzymatic hydrolysis of esters was determined as previously reported.[10] Porcine esterase was diluted 1000-fold with phosphate buffer, pH 7.4, prior to use. A total of 200 μL of each ester (**9–13**) methanolic solution was added to 10 mL of phosphate buffer (0.05 M, pH 7.4), which was thermostatically controlled at 37°C, and then 200 μL of the esterase solution was added. Both the disappearance of the derivatives (**9–13**) and the formation of IDU in the solution were monitored by high-performance liquid chromatography (HPLC) at different times.

Pseudo-first-order half-times ($t_{1/2}$) for the chemical and enzymatic hydrolysis of esters (**9–13**) were calculated from the linear slopes of plots of the logarithm of remaining ester against time. In enzymatic hydrolysis studies, the pseudo-first-order times at which 50% of the total parent compound had been formed ($f_{50\%}$) were determined from the linear slopes of the logarithm of unformed parent drug (**1**) [log(parent $drug_{max}$ − parent $drug_t$)] over time as reported in literature.[17,18]

Lipophilicity Indices (Log *k′*) and Calculated Partition Coefficients (CLOGP) of 1 and Esters 9–13

Lipophilicity indices of PEG derivatives esters **9–13** were obtained by the isocratic HPLC method described later, measuring compounds retention times expressed as log *k′*.[19] It is well known that the ranking of elution in reversed-phase HPLC represents a relative scale of lipophilicity of analytes. Estimates of lipophilicity of derivatives **9–13** were also obtained considering the theoretically calculated values of log *P* (CLOGP; CLOGP for Windows 2.0 version).

HPLC Analysis

Compound **1** and esters **3** and **9–13** were determined by HPLC. In particular, we used a Model 600E (Waters-Millipore, Milford, MA) liquid chromatograph equipped with a Model 7125 Rheodyne injection valve (fitted with a 20-μL loop) and a Water Associates Model 486 ultraviolet (UV) detector set at 254 nm was used. The chromatograms were recorded by a Data Module Model 746 (Millipore). The stainless steel column was a Spherisorb S-5-ODS2 (4 × 250 mm; Phase Separations, Clwid, U.K.). The eluent was a mixture of acetonitrile–PBS (0.01 M, pH 7.0, 20/80, v/v) at a flow rate of 1.5 mL/min. The aqueous portion of eluent was filtered by membrane filters (type HA, Millipore).

The eluent mixture was obtained directly from the chromatographic apparatus by mixing, at low pressure, the organic modifier and the aqueous

phase that had been deaerated previously by bubbling helium. The chromatography was carried out at room temperature. Chromatographic retention data are expressed by the logarithm of capacity factor (lipophylic index, log k'), defined as log $k' = \log [(t_r - t_0)/t_0]$, where t_r and t_0 are the retention time of the analyte and a non-retained compound (methanol), respectively.

In Vitro Skin Permeability

Samples of adult human skin (mean age 43±8 years) were obtained from breast reduction operations. Subcutaneous fat was removed, and the skin was immersed in distilled water at 60±1°C for 2 min,[20] then the stratum corneum and epidermis (SCE) were peeled off. SCE membranes were dried in a desiccator at ~25% relative humidity (RH) and then wrapped in aluminium foil and stored at 4±1°C until use.[21] Dried SCE samples were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in Franz-type diffusion cells (LGA, Berkeley, CA). The skin surface available for absorption was 0.75 cm^2 and the receptor volume was 4.5 mL. The receiving compartment contained ethanol–water (50:50) to ensure sink conditions.[22,23] The receiving solution was stirred and maintained at 35±1°C throughout the experiments. IDU and its esters (**9–13**) were dissolved in ethanol (5 mg/mL) and 200 μL was placed on the skin surface. The solvent was allowed to evaporate, and the experiment was run for 24 h. Samples of the receiving solution were withdrawn at different time intervals and replaced with fresh solutions. The samples were analyzed for IDU and esters **9–13** content by HPLC as already described. In the case of esters **9–13**, intact ester was found in the receptor phase together with a variable amount of IDU. Therefore, the fluxes through the skin of these esters were obtained by plotting the total cumulative amount of IDU equivalents permeated (IDU free permeated + IDU equivalent permeated as ester) versus time and dividing the slopes of the steady-state portions of the graphs by the area of the diffusion cells.

RESULTS AND DISCUSSION

Chemical and Enzymatic Hydrolysis

The degradations of IDU prodrugs (**9–13**) were studied in buffer solution (pH 7.4) at 37°C. The degradation of each ester followed first-order kinetics, and the corresponding $t_{1/2}$ values ranged from 103 to 143 h (see Table 1). As shown in Table 1, ester **12** presents higher chemical stability compared with the other esters whose values of $t_{1/2}$ appear to be similar.

In the chemical hydrolysis studies we observed the formation of only a small amount of intermediate **3** (log $k' = 0.60$), scarcely detectable by HPLC, and time-increased IDU concentrations. These findings suggest that hydrolytic cleavage of esters **9–13** occurs preferentially at the 3′-*O*-ester linkage.

Table 1. Chemical and Enzymatic Hydrolysis, Lipophilic Index, Calculated Partition Coefficient, and Flux Through Excised Human Skin for IDU and Esters 9–13

	$t_{1/2}$ (h)					
Compound	Buffer pH 7.4[c]	Porcine Esterase[c]	$f_{50\%}$ (h)[a]	Log K'	CLOGP	Flux±SD[b] ($\mu g \cdot cm^{-2} \cdot h^{-1}$)
IDU	—	—	—	−0.52	−0.98	0.76±0.24
9	113.4±7.2	1.92±0.34	2.41	0.35	−0.42	2.88±0.65[e]
10	103.3±8.2	2.32±0.20	2.68	0.46	−0.36	2.17±0.53[e]
11	104.8±7.5	1.97±0.31	2.22	0.54	−0.29	1.24±0.22
12	143.7±8.5[d]	4.53±0.49[d]	4.93	0.64	−0.23	0.68±0.24
13	107.3±8.2	2.04±0.30	2.88	0.75	−0.17	0.57±0.18

[a] $f_{50\%}$ is the time by which 50% of total IDU has been formed.
[b] The *in vitro* skin permeation experiments were carried out in duplicate using SCE membrane samples obtained from three different subjects.
[c] The hydrolytic (chemical and enzymatic) experiments were carried out in duplicate.
[d] $p < 0.01$ ester **12** versus other esters (one-way ANOVA, Newman–Keuls Multiple Comparison Test).
[e] $p < 0.01$ esters **9** and **10** versus esters **12** and **13** and IDU; $p < 0.01$ ester **9** versus ester **11**; $p < 0.05$ ester **10** versus ester **11**; $p < 0.05$ ester **9** versus ester **10** (one-way ANOVA, Newman–Keuls Multiple Comparison Test).

Because an essential prerequisite for the successful use of dermal prodrugs is their reconversion into the parent drug within the skin, we assessed the enzymatic cleavage of esters **9–13** with porcine liver esterases, which are regarded as good models for skin esterase enzymatic activity.[10,24,25]

As may be noted in Table 1, all the esters were susceptible to undergoing enzymatic hydrolysis by porcine liver esterase with $t_{1/2}$ ranging from ~2 to 4.5 h. The ester **12** appears more stable against enzymatic hydrolysis with respect to the other esters that present similar values of $t_{1/2}$.

Table 1 includes $f_{50\%}$ values for IDU, which represent the time at which 50% of total parent drug (**1**) was formed in a porcine esterase solution. The $f_{50\%}$ values have been reported in literature[17,18,26] as useful parameters in enzymatic hydrolysis evaluation of prodrugs that could require more than one step to generate the parent drug. Therefore, $f_{50\%}$ can be considered as a more relevant indicator of the formation of the parent drug than the prodrug degradation rate. In Figure 1, a typical time course of ester **9** degradation and IDU formation in enzymatic hydrolysis experiments is reported: both these processes exhibit a pseudo-first-order kinetics for a limited time interval.

As reported in Table 1, the values of the ester **9–13** degradation ($t_{1/2}$) are similar to determined $f_{50\%}$ values for IDU appearance: this evidence suggests that the formation of the parent drug (**1**) takes place at the same rate as the loss of these ester prodrugs **9–13**. These findings, together with the small amount of ester **3** detected in enzymatic hydrolysis medium, suggest to us that the enzymatic hydrolysis occurs too preferentially in 3′-*O*-ester linkage without formation of compound **3**.

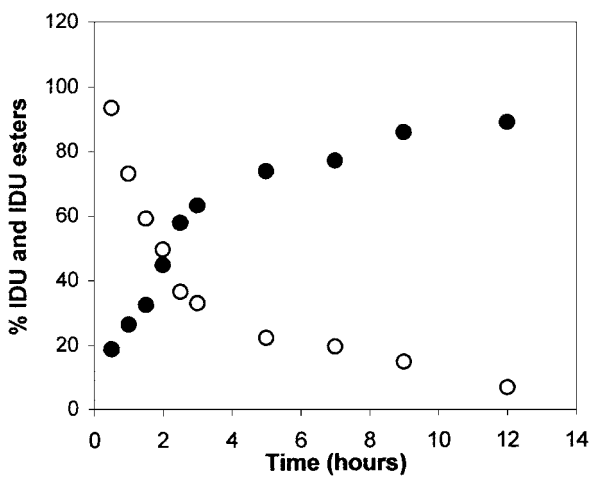


Figure 1. Time courses for ester **9** (○) and IDU (●) during hydrolysis of the prodrug in porcine esterase aqueous solution (pH 7.4) at 37°C.

Lipophilicity

Stratum corneum is generally believed to be the main barrier to drug skin permeation process. Because the horny layer is basically a lipophilic barrier, drug lipophilicity is regarded as one of the key parameters that controls drug skin permeation. One of the main objectives in dermal prodrug design is, therefore, to obtain prodrugs with increased lipophilicity compared with the parent drug. Many authors[27,28] have outlined that more lipophilic drug derivatives could show better partitioning and solubility into the stratum corneum, which could result in enhanced skin permeation.

To assess the lipophilicity of esters **9–13**, two different parameters were considered : CLOG data from theoretical calculation and log K' chromatographic indices. The esterification of 3′-OH group of IDU gave esters **9–13** with increased lipophilicity compared to the parent drug (see Table 1).

As showed in Figure 2, both lipophilicity parameters (CLOGP and log K') of esters **9–13** increased as the polyoxyethylene chain lengthened. In addition, as noted in Figure 2, the good correlation observed between log K' and CLOGP suggests that both these parameters can be suitably used to evaluate the lipophilicity of synthesized esters **9–13**.

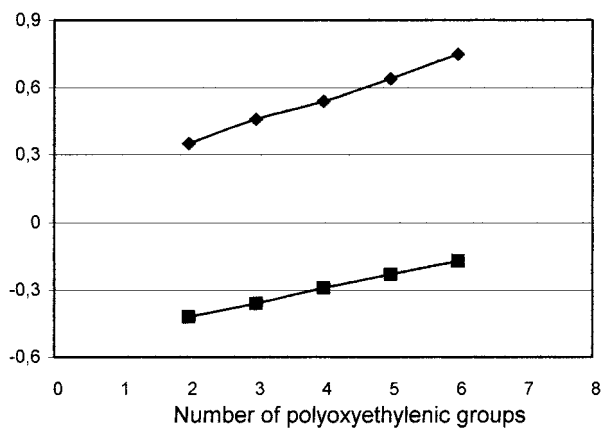


Figure 2. CLOGP (■) and log K' (◆) values versus number of polyoxyethylene groups of esters (**9–13**).

In Vitro Skin Permeation Study

Skin permeation of IDU and esters (**9–13**) was evaluated through excised human skin (SCE membranes) because other authors reported that the dermis *in vitro* can act as a significant additional barrier to the permeation of some compounds.[29] In the case of esters (**9–13**) skin permeation experiments, intact ester was found in the receptor phase together with a variable amount of IDU ranging between 4 and 8%, compared with the amount of ester permeated. The observed hydrolysis should be ascribed mainly to the chemical hydrolysis given that in previous *in vitro* skin permeation studies of dermal prodrugs[10,14] we observed a very poor enzymatic activity of SCE membranes. Furthermore, the large amounts of ethanol used in the receptor compartment for ensuring the sink conditions could notably decrease the chemical hydrolysis in this medium with respect to that obtained in pH 7.4 buffer aqueous solution used for *in vitro* chemical stability studies.

Plotting the total cumulative amount of IDU (IDU free + IDU equivalent permeated as ester prodrug) against time we obtained typical plots similar to that reported in Figure 3. steady-state fluxes of cumulative IDU, obtained after IDU or esters **9–13** skin application, are reported in Table 1. As may be noted, esters 11–13 did not significantly increase IDU cumulative amount penetrated through the skin, whereas applying esters 9 and 10 onto the skin resulted in the cumulative amount of IDU penetrating through the SCE being 3.8- and 2.8-fold higher, respectively, compared with that obtained after topical application of 1.

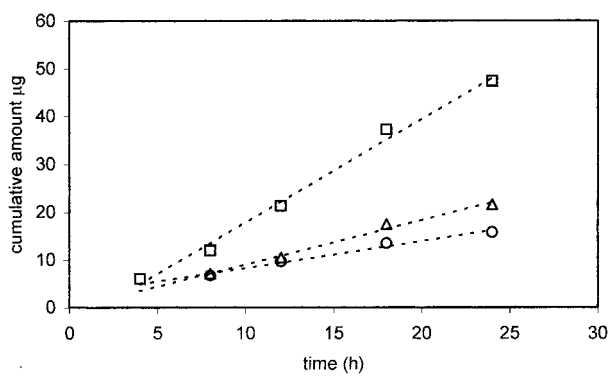


Figure 3. Plot of the cumulative amount (µg) of IDU (○) and ester **11** (△) penetrated through excised human skin versus time.

Lipophilicity and water solubility are regarded as the key parameters for a successful dermal prodrug design.[27,28,30] Accordingly, an effective dermal prodrug must possess an increased lipophilicity, compared with the parent drug, together with adequate aqueous solubility.

Because the skin is regarded as a lipophilic rate-limiting barrier, enhancement of prodrug lipophilicity seems to be important to improve both diffusional characteristics and solubility of the parent drug in the stratum corneum. The enhancement of water solubility is equally important for at least two reasons:[27] (a) at the molecular level, because the lipid domain of the stratum corneum consist of multilamellar bilayers, the transporting species must be able to repetitively cross lipid–aqueous phase interfaces; (b) lipophilic derivatization to obtain prodrugs increases partitioning into the stratum corneum, forming a reservoir, but the subsequent transport into the aqueous milieu beneath may be limited by both prodrug aqueous solubility and the ability of epidermal enzymes to convert the prodrug into a more polar metabolite. In this way, viable epidermis, rather than the stratum corneum, would be the rate-limiting membrane[31] and, therefore, prodrugs should possess suitable water solubility. With regard to our synthesised esters (**9–13**), we evaluated their lipophilicity by both measured lipophilic indices (log k') and calculated CLOGP but we haven't been able to determine the experimental water solubility of these compounds because their oil form creates difficulties indetermining this parameter. Furthermore, in attempting to calculate the theoretical water solubility, we unsuccessfully applied the theoretical methods reported by Yalkowsky et al.[32,33] and previously used to estimate the water solubility of lipophilic dermal prodrugs.[12,13]

The application of these theoretical methods to estimate the water solubility of our esters **9–13** produced unrealistic data: probably these theoretical models can be useful to evaluate only the water solubility of very lipophilic compounds. So, we are unable to evaluate the effects of water solubility of esters **9–13** on their skin permeation fluxes. On the basis of the *in vitro* percutaneous data, we obtained no correlation between lipophilicity and skin permeation fluxes of synthesized esters **9–13**. In our previous papers[10–13] on dermal prodrugs, we obtained an increase in the skin permeation of drugs only for derivatives with both increased lipophilicity and water solubility (compared with the parent drug).

With regard to our results, the IDU skin permeation enhancement, showed by esters 9 and 10, could be explained on the basis of their increased lipophilicity and, probably, their moderate water solubility with respect to parent drug (**1**).

Despite the more lipophilic character of esters **11–13**, these derivatives showed lower fluxes and the lack of IDU skin enhancement. These findings could be attributed to both higher molecular size and higher ability to form H-bonds (due to more polyoxyethylene groups), which, as reported in literature,[34,35] could decrease the diffusional properties of these esters through the skin. Furthermore, the increased lipophilicity of esters **11–13** could result in a reduction of water solubility, resulting, therefore, in a reduction of the skin permeation of the esters.

In conclusion, synthesised IDU esters **9–13** proved moderately stable in aqueous media and were readily hydrolyzed by porcine esterase. Among all esters **9–13** synthesized, only **9** and **10** seem to penetrate through excised human skin better than the parent drug (1). Further *in vivo* pharmacological studies are planned to investigate the potential use of esters **9** and **10** as dermal prodrugs of IDU.

REFERENCES

1. Spadari S, Ciarrocchi G, Focher F, Verri A, Maga G, Arcamone F, Iafrate E, Manzini S, Garbesi A, Tondelli L. 1995. 5-Iodo-2′-deoxy-L-uridine and (*E*)-5-(2-bromovinyl)-2′-deoxy-L-uridine: Selective phosphorylation by herpes simplex virus type 1 thymidine kinase, antiherpetic activity, and cytotoxicity studies. Mol Pharmacol 47(6):1231–1238.
2. Touitou E, Segal R, Pisanty S, Milogoldzweig I. 1988. Glycirrhyzin gel as vehicle for idoxuridine topical preparation: Skin permeation behaviour. Drug Des Deliv 3(3):267–272.
3. Sheth NV, McKeough MB, Spruance SL. 1987. Measurement of the stratum corneum drug reservoir to predict the therapeutic efficacy of topical iododeoxyuridine for herpes virus infection. J Invest Dermatol 89(6):598–602.
4. Bravo ML, Auladell C, Navarro C. Evaluation absorption and safety of idoxuridine after topical administration. Methods Find Exp Clin Pharmacol 10(11):705–709.
5. Barry BW. 1987. Mode of action of penetration enhancers in human skin. J Controlled Release 6: 85–97.
6. Hadgraft J. 1989. Formulation of anti-inflammatory agents. In: Lowe NJ, Hensby CN, editors. Nonsteroidal Anti-inflammatory Drugs. Basel (Switzerland): Karger, pp. 21–43.
7. Ghosh MK, Mitra AK. 1992. Enhanced delivery of 5-iodo-2′-deoxyuridine to the brain parenchyma. Pharm Res 9(9):1173–1176.
8. Ghosh MK, Mitra AK. 1992. Brain parenchymal metabolism of 5-iodo-2′-deoxyuridine and 5′-ester prodrugs. Pharm Res 9(8):1048–1052.
9. Narurkar MM, Mitra AK. 1989. Prodrugs of 5-iodo-2′-deoxyuridine for enhanced ocular transport. Pharm Res 6(10):887–891.
10. Bonina FP, Montenegro L, de Caprariis P, Bousquet E, Tirendi S. 1991. 1-Alkylazacycloalkan-2-one esters as prodrugs of indomethacin for improved delivery through human skin. Int J Pharm 77:21–29.
11. Bonina FP, Montenegro L, Guerrera F. 1993. Naproxen 1-alkylazacycloalkan-2-one esters as dermal prodrugs: *In vitro* evaluation. Int J Pharm 100:99–105.
12. Bonina FP, Montenegro L, Trapani G, Franco M, Liso G. 1995. *In vitro* evaluation of *N*-acyllactam esters of indomethacin as dermal prodrugs. Int J Pharm 124:45–51.
13. Bonina FP, Montenegro L, de Caprariis P, Palagiano F, Trapani G, Liso G. 1995. *In vitro* and *in vivo* evaluation of polyoxyethilene indomethacin esters as dermal prodrugs. J Controlled Release 34:223–232.
14. Palagiano F, Arenare L, Barbato F, La Rotonda MI, Quaglia F, Bonina FP, Montenegro L, de Caprariis P. 1997. *In vitro* and *in vivo* evaluation of terpenoid esters of indomethacin as dermal prodrugs. Int J Pharm 149:171–182.
15. Rimoli MG, Avallone L, de Caprariis P, Galeone A, Forni F, Vandelli MA. 1999. Synthesis and characterisation of poly(D,L-lactic acid)-idoxuridine conjugate. J Controlled Release 58:61–68.
16. Bessodes M, Komiotis D, Antonakis K. 1986. Rapid and selective detritylation of primary alcohols using formic acid. Tetrahedron Lett 27: 579.
17. Rautio J, Nevalainen T, Taipale H, Vepsäläinen J, Gynther J, Pedersen T, Järvinen T. 1999. Synthesis and *in vitro* evaluation of aminoacyloxyalkyl esters of 2-(6-methoxy-2-naphthyl) propionic acid as novel naproxen prodrugs for dermal drug delivery. Pharm Res 16(8):1172–1178.
18. Järvinen T, Poikolainen M, Suhonen P, Vepsäläinen J, Alaranta S, Urtti A. 1995. Comparison of enzymatic hydrolysis of pilocarpine prodrugs in human plasma, rabbit cornea and butyrylcholinesterase solutions. J Pharm Sci 84:656–660.
19. Lambert WJ. 1993. Modelling oil–water partioning and membrane permeation using reversed-phase chromatography. J Chromatogr A 656:469–484.
20. Kligman AM, Christophers E. 1963. Preparation of isolated sheets of human skin. Arch Dermatol 88:702–705.

21. Swarbrick J, Lee G, Brom J, Gensmantel NP. 1982. Drug permeation through human skin I : Effect of storage conditions of skin. J Invest Dermatol 78: 63–66.
22. Mueller LG. Novel anti-inflammatory esters, pharmaceutical composition and methods for reducing inflammation. U.K. Patent GB 2 204 869 A, 1988.
23. Toitou E, Fabin B. 1988. Altered skin permeation of a highly lipophilic molecule: Tetrahydrocannabinol. Int J Pharm 43:17–22.
24. Cheung YW, Li Wan Po A, Irwin WJ. 1985. Cutaneous biotransformation as a parameter in the modulation of the activity of topical corticosteroids. Int J Pharm 26:175–189.
25. Wong O, Tsuzuki N, Nghiem B, Kuehnhoff J, Itoh T, Masaki K, Huntington J, Konishi R, Rytting JH, Higuchi T. 1989. Unsaturated cyclic ureas as a new non-toxic biodegradable transdermal penetration enhancers: II. Evaluation study. Int J Pharm 52: 191–202.
26. Rautio J, Taipale H, Gynther J, Versäläinen J, Nevalainen T, Jarvinen T. *In vitro* evaluation of acyloxyalkyl esters as dermal prodrugs of ketoprofen and naproxen. J Pharm Sci 87:1622–1628.
27. Guy RH, Hadgraft J. 1992. Percutaneous penetration enhancement: Physicochemical consideration and implications for prodrug design. In: Sloan KB, editor. Prodrugs: Topical and ocular drug delivery. New York: Marcel Dekker. pp. 1–16.
28. Sloan KB. 1989. Prodrugs for dermal delivery. Adv Drug Del Rev 3:912–922.
29. Bronaugh RL, Stewart RF. 1984. Methods for *in vitro* percutaneous absorption studies. III. Hydrophobic compounds. J Pharm Sci 73:1255–1258.
30. Waranis RP, Sloan KB. 1987. Effect of vehicles and prodrug properties and their interactions on the delivery of 6-mercaptopurine through skin: Bisacyloxymethyl-6-mercaptopurine prodrugs. J Pharm Sci 76:587–595.
31. Friend D, Catz P, Heller J, Reid J, Baker R. 1988. Transdermal delivery of levonorgestrel: II. Effect of prodrug structure on skin permeability *in vitro*. J Controlled Release 7:251–261.
32. Yalkowsky SH, Valvani SC. 1980. Solubility and partitioning I: Solubility of nonelectrolytes in water. J Pharm Sci 69:912–922.
33. Yalkowsky SH, Valvani SC, Roseman TJ. 1983. Solubility and partitioning VI: Octanol solubility and octanol–water partition coefficients. J Pharm Sci 72:866–870.
34. Lien EJ, Gao H. 1995. QSAR analysis of skin permeability of various drugs in man as compared to *in vivo* and *in vitro* studies in rodents. Pharm Res 12(4):583–587.
35. El Tayar N, Tsai RS, Testa B, Carrupt PA, Hansch C, Leo A. 1991. Percutaneous penetration of drugs: A quantitative structure–permeability relationship study. J Pharm Sci 80(8):744–749.