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Transdermal delivery of 5-fluorouracil (5-FU) through hairless mouse skin by 1-alkyloxycarbonyl-5-FU prodrugs: Physicochemical characterization of prodrugs and correlations with transdermal delivery

Howard Beall [a,1], Richard Prankerd [b,2], Kenneth Sloan [a,*]

[a] *Department of Medicinal Chemistry, P.O. Box 100485, University of Florida, Gainesville, FL 32610, USA*
[b] *Department of Pharmaceutics, University of Florida, Gainesville, FL 32610, USA*

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Abstract

The members of a series of 1-alkyloxycarbonyl-5-fluorouracil (5-FU) derivatives have been synthesized, characterized, and evaluated for their abilities to deliver total 5-FU species into and through skin. The most effective member of the series at delivering 5-FU through skin (flux) was the ethyl derivative, **3** (25 times as effective as 5-FU), which was also the most water soluble member of the series. There was a good correlation between flux and water solubility for the entire series but none between flux and lipid solubility. **3** was also the most effective (4.9 times as effective as 5-FU) member of the series at delivering 5-FU into the skin (C_s), and there was a good correlation between C_s and flux except for the hexyl derivative, **6**. Although the partition coefficients of the first four members of the 1-alkyloxycarbonyl series were less than those of the corresponding members of the 1-alkylaminocarbonyl series, which were previously studied, their water solubilities were 5–30 times greater and they were 3–10 times more effective at delivering total 5-FU species through hairless mouse skin. However, the 1-alkyloxycarbonyl derivatives delivered mostly intact prodrug (42–90%) through skin while the 1-alkylaminocarbonyl derivatives delivered mostly (> 90%) 5-FU. In spite of this difference, there was a good correlation between permeability coefficients for total 5-FU species delivered and calculated solubility parameters for both series.

Keywords: Transdermal delivery; Prodrug; Water solubility; 1-Alkyloxycarbonyl-5-fluorouracil; Partition coefficient; Isopropyl myristate solubility; Solubility parameters; Thermal stability

1. Introduction

A recent report (Sloan et al., 1993) on the abilities of 1-alkylaminocarbonyl derivatives of 5-FU (**1**, 5-FU) to deliver 5-FU through hairless mouse skin has given support to previous observations that the most water soluble member of a homologous series of more lipid soluble prodrugs

* Corresponding author.
[1] Present address: University of Colorado, School of Pharmacy, Denver, Colorado 80262.
[2] Present address: Department of Pharmacy, University of Queensland, 4072, Australia.

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will be the member which is the most efficient at enhancing transdermal delivery (Waranis and Sloan, 1987, 1988; Sloan, 1992a). However, although all the 1-alkylaminocarbonyl prodrugs were much more lipid soluble than 5-FU, even the most water soluble member of that series of 1-acyl type prodrugs was only about one-tenth as soluble in water as 5-FU. Thus, the rather small (3-fold, compared to 5-FU) increase in the transdermal delivery of 5-FU realized by the best prodrug in the series was attributed to the low water solubility exhibited by that type of 1-acyl prodrug. In addition, the lack of water solubility exhibited by the 1-alkylaminocarbonyl type of 1-acyl prodrug was attributed to the fact that, although derivatization had masked one N-H group in 5-FU which was capable of forming intermolecular hydrogen bonds, the 1-alkylaminocarbonyl promoiety introduced another N-H group. The net result was that there was no decrease in the number of N-H groups that were capable of hydrogen bond formation.

In order to determine if the low water solubility and an amide-like N-H group in the promoiety were responsible for the lack of performance by the 1-alkylaminocarbonyl prodrugs of 5-FU, members of other series of 1-acyl type derivatives which lack an amide-like N-H group in the promoiety have been investigated for their abilities to deliver 5-FU through hairless mouse skin. In this case, the 1-alkyloxycarbonyl derivatives were chosen for investigation. All the members of the series of 1-alkyloxycarbonyl derivatives of 5-FU had been reported to be more lipophilic than 5-FU based on their octanol/water partition coefficients (Buur and Bundgaard, 1986, 1987), and at least one of the members of the series had been reported to be more water soluble than 5-FU (Buur and Bundgaard, 1986). In addition, although the 1-alkyloxycarbonyl-5-FU derivatives had been reported to be reasonably stable at pH 7.4 and 37°C, it had also been reported that they hydrolyzed very rapidly in human plasma: $t_{1/2}$ = 1–3 min (Buur and Bundgaard, 1986). Thus, it was anticipated that the 1-alkyloxycarbonyl-5-FU derivatives would function effectively as prodrugs of 5-FU.

The synthesis and physicochemical characterization of the members of a homologous series of 1-alkyloxycarbonyl-5-FU derivatives are reported here, together with an evaluation of their ability to deliver 5-FU into and through hairless mouse skin.

2. Experimental

Melting points (m.p.) were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental microanalyses were obtained for all novel compounds through Atlantic Microlab, Incorporated (Norcross, GA). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Infrared (IR) spectra were recorded with a Perkin-Elmer 1420 spectrophotometer. Ultraviolet (UV) spectra were obtained with a Cary 210 or Shimadzu UV-265 spectrophotometer. The HPLC system consisted of a Beckman model 110A pump with a model 153 UV detector, a Rheodyne 7125 injector with a 20 μl loop, and a Hewlett-Packard 3392A integrator. The column was a Lichrosorb RP-8 10 μm reverse-phase column, 250 mm × 4.6 mm. The diffusion cells were from Crown Glass, Somerville, NJ (surface area 4.9 cm^2, 20 ml receptor phase volume). The diffusion cells were maintained at 32°C with a Fisher circulating water bath model 25. DSC analyses in hermetically sealed pans were carried out using a Perkin Elmer DSC-7 scanning calorimeter, and thermogravimetric analyses (TGA) were performed using a Perkin Elmer TGA-7 thermogravimetric analyzer, both controlled by a Perkin-Elmer TAC-7 interface and IBM PS/2 Model 50Z microcomputer. Isopropyl myristate was obtained from Givaudan, Clifton, NJ. 5-FU was purchased from Sigma Chemical Co. All other reagents were obtained from Aldrich Chemical Co. The female hairless mice (SKH-hr-1) were from Temple University Skin and Cancer Hospital.

2.1. Synthesis (Beall, 1991)

2.1.1. Preparation of 1-alkyloxycarbonyl-5-FU (general procedure)

To 0.66 g (0.01 mol) of 85% potassium hydroxide dissolved in methanol (20–50 ml) was added

1.33 g of 5-FU (**1**, 0.0102 mol). Slightly more than an equivalent of 5-FU was used to prevent excess base from being present during the reaction. The methanol suspension was stirred for 30 min, and the methanol was evaporated under reduced pressure. The potassium salt was suspended in acetone (25–50 ml) which was evaporated under reduced pressure to remove residual methanol. The salt was resuspended in acetone (25–50 ml), and the suspension was added dropwise over a 3 min period to a well stirred acetone (20 ml) solution containing 1.0–1.2 equivalents of the appropriate alkyl chloroformate. The mixture was stirred at room temperature for 60 min. The mixture was filtered, and the residue was washed with acetone (20 ml). The combined acetone solutions were evaporated under reduced pressure, and the solid residue was recrystallized from an appropriate solvent or solvent combination to give the following compounds.

2.1.2. 1-Methyloxycarbonyl-5-fluorouracil (2)

Recrystallization from acetone gave 1.36 g of **2** (72%): m.p. 158–60°C (lit. (Buur and Bundgaard, 1986) m.p. 159–60°C); IR (KBr) 1695, 1710, 1740, and 1760 cm^{-1} (C = O); ^{1}H-NMR [$(CD_3)_2SO$] δ 3.86 (s, 3H, $C\underline{H}_3$) and 8.16 (d, $J = 7$ Hz, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon = 9.63 \times 10^3$ l/mol).

2.1.3. 1-Ethyloxycarbonyl-5-fluorouracil (3)

Recrystallization from acetone/ether gave 1.31 g of **3** (65%): m.p. 127–8°C (lit. (Buur and Bundgaard, 1986) m.p. 126–8°C); IR (KBr) 1690, 1730 and 1750 cm^{-1} (C = O); ^{1}H-NMR [$(CD_3)_2SO$] δ 1.31 (t, $J = 7$ Hz, 3H, $C\underline{H}_3$), 4.31 (q, $J = 7$ Hz, 2H, $OC\underline{H}_2$), and 8.16 (d, $J = 7$ Hz, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon = 9.86 \times 10^3$ l/mol).

2.1.4. 1-Propyloxycarbonyl-5-fluorouracil (4)

Recrystallization from acetone/ether gave 1.37 g of **4** (64%): m.p. 124–6°C; IR (KBr) 1690, 1730, and 1755 cm^{-1} (C = O); ^{1}H-NMR [$(CD_3)_2SO$] δ 0.95 (t, $J = 7$ Hz, 3H, $C\underline{H}_3$), 1.70 (m, 2H, $OCH_2C\underline{H}_2$), 4.23 (t, $J = 7$ Hz, 2H, $OC\underline{H}_2$), and 8.15 (d, $J = 7$ Hz, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon = 1.001 \times 10^4$ l/mol).

Anal. Calc. for $C_8H_9FN_2O_4$: C, 44.45; H, 4.20; N, 12.96; Found: C, 44.53; H, 4.23; N, 12.89.

2.1.5. 1-Butyloxycarbonyl-5-fuorouracil (5)

Recrystallization from dichloromethane/hexane gave 1.33 g of **5** (58%): m.p. 97–8°C (lit. (Buur and Bundgaard, 1986) m.p. 102–4°C); IR (KBr) 1695, 1735, and 1765 cm^{-1} (C = O); ^{1}H-NMR [$(CD_3)_2SO$] δ 0.91 (t, $J = 7$ Hz, 3H, $C\underline{H}_3$), 1.3–1.8 (m, 4H, $OCH_2C\underline{H}_2C\underline{H}_2$), 4.27 (t, $J = 6$ Hz, 2H, $OC\underline{H}_2$), and 8.13 (d, $J = 7$ Hz, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon = 9.93 \times 10^3$ l/mol).

2.1.6. 1-Hexyloxycarbonyl-5-fluorouracil (6)

Recrystallization from dichloromethane/hexane gave 1.27 g of **6** (49%): m.p. 66–7°C (lit. (Buur and Bundgaard, 1987) m.p. 68–9°C); IR (KBr) 1690, 1730, and 1750 cm^{-1} (C = O); ^{1}H-NMR [$(CD_3)_2SO$] δ 0.87 (t,3H, $C\underline{H}_3$), 1.1–1.8 (m, 8H, $OCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 4.26 (t, $J = 6$ Hz, 2H, $OC\underline{H}_2$), and 8.13 (d, $J = 7$ Hz, 1H, C^6-$\underline{H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon = 1.004 \times 10^4$ l/mol).

2.1.7. 1-Octyloxycarbonyl-5-fluorouracil (7)

Recrystallization from dichloromethane/hexane gave 1.80 g of **7** (61%): m.p. 97–8°C; IR (KBr) 1690, 1730, and 1750 cm^{-1} (C = O); ^{1}H-NMR [$(CD_3)_2SO$] δ 0.87 (t, 3H, $C\underline{H}_3$), 1.2–1.9 (m, 12H, $OCH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 4.27 (t, $J = 6$ Hz, 2H, $OC\underline{H}_2$), and 8.15 (d, $J = 7$ Hz, 1H C^6-$\underline{H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon = 1.009 \times 10^4$ l/mol).

Anal. Calc. for $C_{13}H_{19}FN_2O_4$: C, 54.53; H, 6.69; N, 9.79; Found: C, 54.46; H, 6.73; N, 9.77.

2.2. Solubilities and partition coefficients

Lipid solubilities were determined in isopropyl myristate (IPM) according to a previously described procedure (Beall, 1991; Beall et al., 1993a). Three suspensions of each derivative were stirred at 22 ± 1°C for 48 h. The suspensions were filtered through 0.45 μm nylon filters, then the saturated solutions were diluted in dry acetonitrile and quantitated by UV spectroscopy. Molar absorptivities were predetermined in triplicate in acetonitrile at 254 nm.

For aqueous solubilities, three suspensions of each derivative were vigorously stirred in 0.05 M acetate buffer (pH 4.0) at 22 ± 1°C for 60 min (Beall, 1991; Beall et al., 1993a). The suspensions were filtered through 0.45 μm nylon filters, then the saturated solutions were diluted in acetonitrile and quantitated using UV spectroscopy as above. In addition, the samples of derivatives **2–4** were quantitated using HPLC (see below for conditions).

The partition coefficients (K) were determined using the saturated IPM solutions from the lipid solubility study (Beall, 1991; Beall et al., 1993a). For most compounds, equal volumes (1 ml) of saturated IPM solution and 0.05 M acetate buffer (pH 4.0) were used. The two phases were shaken vigorously for 10 s then allowed to separate for 60 s. The IPM layers were diluted with acetonitrile and analyzed by UV spectroscopy. The IPM-buffer partition coefficients were calculated as follows:

$$K = [A_a/(A_b - A_a)]V_{H_2O}/V_{IPM}$$

where A_a is the absorbance from the IPM layer after partitioning, A_b denotes that from the IPM layer before partitioning, V_{H_2O} is the volume of the aqueous phase, and V_{IPM} represents that of the IPM phase. Partitioning was carried out in triplicate for a fixed volume ratio for each derivative. For those compounds with large differences in solubility in one phase relative to the other, volume ratios (IPM/buffer) other than 1:1 were necessary, but the ratio never exceeded 10:1 or 1:10.

2.3. Hydrolysis kinetics

Hydrolysis rates were determined at 32°C for 1-methyloxycarbonyl-5-FU (**2**) in 0.05 M phosphate buffer (pH 7.1, $\mu = 0.12$ M) and in the same buffer with 0.11% formaldehyde (3.6×10^{-2} M). The rate in the presence of formaldehyde was determined for comparison with the rate in plain buffer since formaldehyde was used as a preservative in the diffusion cell experiments described in the following section. The hydrolyses were followed by HPLC. The mobile phase contained 10% methanol and 90% 0.025 M acetate buffer (pH 5.0) v/v with a flow rate of 1.0 ml/min. The column effluent was monitored at 254 nm. Hydrolysis was initiated by adding 0.4 ml of a stock solution of compound **2** in acetonitrile to 25 ml of buffer prewarmed to 32°C in a constant temperature water bath to give final concentrations of 1.8×10^{-4} M. Each hydrolysis reaction was run in triplicate and was followed for a minimum of three half-lives. The correlation coefficients for the pseudo-first order plots were ≥ 0.999.

2.4. Diffusion cell experiments

The diffusion cell experiments were run in essentially the same way as previously described (Sloan et al., 1993). Briefly, the hairless mice were killed and the dorsal portion of each mouse skin was removed and placed in contact with the receptor phase (pH 7.1 phosphate buffer, 0.05 M, $\mu = 0.11$ M, containing 2.7 ml of 36% formaldehyde/l of buffer) at 32°C for 48 h. The use of 0.1% formaldehyde as a preservative has been shown to be essential to maintain the integrity of hairless mouse skin during the diffusion cell experiments (Sloan et al., 1991). The receptor phases were changed three times during this preapplication leaching-conditioning period. Then, 0.5 ml aliquots of 0.3–0.8 M suspensions of each prodrug in IPM were applied to the donor side of each of three diffusion cells. The suspensions were prepared in the same way that samples were prepared for the solubility experiments: excess prodrug in IPM was stirred for 48 h at room temperature. These donor phases were removed every 12 h without methanol wash, and fresh 0.5 ml aliquots of the suspensions were applied. These donor phases were saved for later analyses. After the suspensions were applied, 3 ml aliquots of the receptor phases were removed, generally at 4, 8, 12, 21, 24, 27, 30, 33, 36, 45 and 48 h. The amount of 5-FU in each aliquot was determined from the UV absorption at 265 nm ($\epsilon = 7.13 \times 10^3$ l/mol) after the aliquot had been allowed to sit at room temperature for 72 h. The aliquot were kept for 72 h to ensure that all the prodrug had hydrolyzed to 5-FU before analysis. Each time an

aliquot was removed, the entire receptor phase was replaced with fresh receptor fluid.

When the initial application period of 48 h was completed, the donor phases were carefully removed and the solid portions of all the donor phases were analyzed for intact prodrug by ^{1}H-NMR (C<u>H</u> = absorption at δ 8.13–8.16). The donor surfaces were quickly washed with three 5 ml portions of methanol to remove any residual prodrug or 5-FU. After the methanol wash, the skins were kept in contact with fresh receptor fluid for 23–24 h to allow any 5-FU or 5-FU prodrug in the skins to leach out. Subsequently, these receptor phases were analyzed for 5-FU by UV spectrophotometry as above to give the amount of 5-FU in the skins (C_s). The receptor phases were replaced with fresh fluid and 0.5 ml aliquots of a standard drug/vehicle (theophylline/propylene glycol) were applied. This second application period was only for up to 12 h. Aliquots (3 ml) of receptor phases were generally taken at 1, 2, 3, 4, 6 and 12 h, and the amounts of theophylline in the receptor phases were quantitated from the UV absorptions at 270 nm ($\epsilon = 1.02 \times 10^4$ l/mol). Each time an aliquot was removed the entire receptor phase was replaced with fresh receptor fluid.

In all cases the rates of delivery of 5-FU (J_i) or theophylline (J_j) through skin were determined by plotting the cumulative amount (mg) of 5-FU or theophylline measured in the receptor phase against time, and dividing the slopes of the steady-state portions of those plots by the surface area of the diffusion cells. Permeability coefficients were determined by dividing the J values by the equivalent mg of 5-FU solubility of the corresponding prodrugs in IPM.

In separate diffusion cell experiments, a suspension of each prodrug in IPM was applied to one diffusion cell. The same procedure as above was used except that intact prodrug content in the receptor phases was determined at each sampling time by HPLC and the donor phases were only changed once. Mobile phase containing 18 or 50% v/v methanol in 0.025 M acetate buffer (pH 5.0) with a flow rate of 1.0 ml/min was used with the system described earlier. The 18% methanol mobile phase was used to quantitate the methyl- (**2**), ethyl- (**3**) and propyloxycarbonyl-5-FU (**4**) derivatives: retention times were 6.0, 11.5 and 28.8 min, respectively. The 50% methanol mobile phase was used to quantitate the butyl- (**5**) and hexyloxycarbonyl-5-FU (**6**) derivatives: retention times were 8.2 and 27.7 min, respectively. Aliquots were removed and chromatographed immediately after they were taken. Prodrug fluxes were calculated in the same manner as fluxes for total 5-FU.

2.5. Solubility parameters

The calculated solubility parameters were obtained using the method of Fedors (1974) as illustrated by Martin et al. (1985) and Sloan et al. (1986).

2.6. Statistical analysis

Statistical analysis was accomplished using Student's t-test. Unless otherwise indicated, statistical significance is for $p < 0.05$.

3. Results and discussion

3.1. Syntheses and characterization

Four of the six 1-alkyloxycarbonyl-5-FU prodrugs that were synthesized for this work had been described before (Buur and Bundgaard, 1986, 1987). However, the method described in this paper (the inverse addition of a suspension of the potassium salt of 5-FU to a solution of the chloroformate) was found to give consistently better yields of derivatives, probably because the presence of excess base during the reaction was avoided. The ^{1}H-NMR, UV and IR spectra of the two derivatives that had not been reported before were consistent with the spectra of those that had. In particular, the position of the C<u>H</u> = absorption in all these derivatives was at about δ 8.13–8.16 in DMSO-d_6. The position of the C<u>H</u> = absorption is diagnostic of a 1-acyl type derivative where the C<u>H</u> = absorption is shifted downfield, compared to the same absorption in 5-FU or in 3-acyl derivatives of 5-FU, because of the

anisotropic effect of the 1-acyl carbonyl group which is oriented toward it (Sloan et al., 1993).

The melting points of the derivatives were consistent with those reported in the literature (Buur and Bundgaard, 1986, 1987). However, the methyloxycarbonyl derivative, **2**, did not melt but started to foam at about 158°C and had completely changed from a solid to a foam by about 160°C. A clear liquid was never observed even after the temperature was raised to 170°C; the sample continued to foam. All of the derivatives exhibited endotherms at approximately the same temperatures as the observed melting points upon differential scanning calorimetric (DSC) analyses. However, DSC analysis of **2** showed that if **2** was heated at 5°C/min, an endotherm at 168°C was observed; but, if a sample of **2** was first heated at 167°C for 10 min then cooled to 40°C before being analyzed, an endotherm at 137°C was observed. Similar behavior was observed for **3** where an endotherm was observed at 128°C if **3** was heated at 5°C/min, but where an endotherm was observed at 119°C if the sample was first heated at 129°C for 15 min and cooled to 40°C. Derivatives **2** and **3** were also the only ones that exhibited broad endotherms before the peak endotherm that corresponded to the observed melting point. After the preliminary heat treatment, only a single endotherm was observed for **2** and **3** upon DSC analyses.

The thermal gravimetric analyses (TGA) of **2** showed that onset of mass loss occurred at about 195°C but that by about 230°C there was an abrupt stop in mass loss with about 15% mass remaining in the sample. The remaining mass was lost completely by about 270°C as a second phase. A similar TGA profile was observed for **3**, but the mass loss profiles of **4** and **5** were somewhat less pronounced with only 4 and 6%, respectively, mass remaining before the second phase of mass loss occurred and mass loss was complete at about 260 and 250°C, respectively. On the other hand, smooth loss of sample upon TGA was complete at about 255 and 260°C for **6** and **7**, respectively; no second phase was observed.

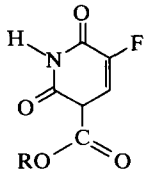
The DSC and TGA data suggest that thermal decomposition, rearrangement or both may be taking place with some of the 1-alkyloxycarbonyl derivatives. Although there is a considerable amount of literature for the similar DSC and TGA behavior of the 1-alkylaminocarbonyl derivatives being due to thermal loss of alkylisocyanate to regenerate 5-FU (Ozaki et al., 1977; Sloan et al., 1993), no literature precedent exists describing the thermal behavior of 1-alkyloxycarbonyl derivatives. Preliminary data suggest that *N*-alkyl derivatives are formed from the thermal decomposition/rearrangement as well as 5-FU. Work is ongoing on this result to determine the mechanism.

The hydrolyses of the methyloxycarbonyl derivative, **2**, in buffer and in buffer containing 0.11% formaldehyde were run to determine the effect of formaldehyde. Pseudo-first-order rate constants for the hydrolysis of **2** were obtained in both buffer ($3.33 \pm 0.04 \times 10^{-3}$ min^{-1}, $t_{1/2} = 208$ min, pH 7.1 and 32°C; lit. (Buur and Bundgaard, 1986) $t_{1/2} = 190$ min at pH 7.4 and 37°C), and buffer containing formaldehyde ($3.78 \pm 0.05 \times 10^{-3}$ min^{-1}, $t_{1/2} = 183$ min). Only 5-FU was observed as the product in both cases. The hydrolysis was significantly faster in the presence of formaldehyde, suggesting that general base catalysis by formaldehyde hydrate may be involved (Beall et al., 1993b).

3.2. Solubilities and partition coefficients

The solubilities of the 1-alkyloxycarbonyl prodrugs in IPM (S_{IPM}), values for partition coefficients between IPM and pH 4.0 buffer (K), direct pH 4.0 buffer solubilities (S_{H_2O}), and literature values for S_{H_2O} have been discussed in a previous paper (Beall et al., 1993a). Those values are reproduced here (Table 1) for convenience. The water solubilities for **2**, **5** and **6** agree reasonably well with those in the literature. However, the water solubility reported here and in the previous paper for **3** is almost 8 times greater than that reported in the literature. There is no question that the structure of the compound designated as **3** is correct. The ^{1}H-NMR, UV and IR spectra, DSC analysis and TGA behavior (see above) of **3** are all consistent with those of the other 1-alkyloxycarbonyl derivatives that have been synthesized. The melting point was also consistent with

Table 1
Solubilities in isopropyl myristate (S_{IPM}) and in water (S_{H_2O}), partition coefficients (K), solubility ratio (SR) and methylene π values



R =	S_{IPM} [a]	K [b]	S_{H_2O} [a]	S_{H_2O} [a,c]	SR [d]	π [e]
5-FU	0.0064	0.00058	12.5	11.0	0.00051	
2, CH_3	0.39 (0.27)	0.019 (6:1)	22.6 (15.6)	23.3	0.017	
3, C_2H_5	2.6 (1.7)	0.075	53.1 (34.2)	6.9	0.049	0.59
4, C_3H_7	3.2 (1.9)	0.36	11.9 (7.2)	–	0.27	0.67
5, C_4H_9	7.8 (4.4)	1.4	6.7 (3.8)	6.0	1.2	0.61
6, C_6H_{13}	39.5 (19.9)	31 (1:6)	1.4 (0.70)	1.5	29	0.66
7, C_8H_{17}	10.3 (4.7)	285 (1:10)	0.040 (0.018)	–	257	0.52

[a] Solubility in mg/ml (equivalent mg of 5-FU).
[b] Partition coefficient between IPM and pH 4.0 buffer (IPM: buffer phase volume ratio different from 1:1).
[c] Buur and Bundgaard (1987).
[d] Solubility ratio from S_{IPM}/S_{H_2O}.
[e] $\pi = (\log K_{n+m} - \log K_n)/m$, where n is the number of methylene units in the promoiety of one prodrug and m denotes the number of additional methylene units in the promoiety of the prodrug with which it is compared.

the literature value for **3** (Buur and Bundgaard, 1986).

Analysis of the solubility properties of **3** suggests that the higher S_{H_2O} value reported here is correct. If the literature value for S_{H_2O} is used to calculate methylene π values for the 1-alkyloxycarbonyl series based on log SR (solubility ratio) values rather than on log K values, the π value derived from the comparison of **2** with **3** is a very large number (1.34), while the value derived from the comparison of **3** with **4** is a negative number (−0.15). The mean ± standard deviation for the methylene π values for the 1-alkyloxycarbonyl series calculated from log SR and using the literature S_{H_2O} value for **3** is 0.60 ± 0.53. On the other hand, the mean ± standard deviation using the S_{H_2O} value reported here is 0.60 ± 0.13. For comparison purposes, the mean methylene π value ± standard deviation for the 1-alkyloxycarbonyl series calculated from their log K values is 0.61 ± 0.06. This mean π value for the CH_2 group is consistent with those for CH_2 groups from other series of *N*-acyl derivatives (Beall et al., 1993a), and the standard deviation, using the S_{H_2O} value reported here, is acceptable. Thus, the S_{H_2O} value reported here is much more consistent with the S_{H_2O} values observed for the other members of the series. The reason for the previously reported S_{H_2O} value for **3** is not clear, although polymorphic differences cannot be completely ruled out.

The S_{IPM} values for the members of the 1-alkyloxycarbonyl series are all higher than those for the corresponding members of the 1-alkylaminocarbonyl series except for the R = C_8H_{17} member, which is about 20% less soluble. The biggest difference is for the first two members of the series where the R = CH_3 and R = C_2H_5 members are almost 8 and 5 times more soluble in IPM, respectively. Since solubility depends in part on crystal lattice energies, this trend suggests that the crystal structures for both types of 1-acyl derivatives are dominated by the functional groups in 5-FU and the N-(C = O)-NHR or N-(C = O)-OR enabling functional groups (Sloan, 1992a) for lower homologs in the two series but by the aliphatic chain in R for the higher homologs (Sloan et al., 1993).

3.3. Diffusion cell experiments

The results from the diffusion cell experiments are given in Table 2. Almost all of the 1-alkyloxycarbonyl derivatives are significantly more

Table 2
Rates of delivery of total 5-FU by 1-alkyloxycarbonyl-5-FU prodrugs (J_i) and rates of delivery of theophylline (J_j) through hairless mouse skin, log permeability coefficients (log P_i), solubility parameters of prodrugs (δ_i), amounts of 5-FU retained in skin (C_s), and dermal/transdermal (D/T) delivery ratios ($n = 3$)

Compound (δ_i) [a]	J_i ($\pm$SD) [b]	C_s ($\pm$SD) [c]	J_j ($\pm$SD) [b,d]	Log P_i [e]	D/T [f]
5-FU (15.0)	0.031 (0.012)	0.48 (0.12)	0.16 (0.026)	0.68	0.13
2 (14.10)	0.34 (0.078)	1.08 (0.013)	0.25 (0.013)	0.10	0.027
3 (13.50)	0.77 (0.17)	2.34 (0.52)	0.23 (0.052)	−0.34	0.026
4 (13.02)	0.30 (0.026)	0.65 (0.18)	0.22 (0.039)	−0.80	0.019
5 (12.62)	0.29 (0.013)	0.55 (0.065)	0.23 (0.026)	−1.18	0.016
6 (12.02)	0.20 (0.013)	1.43 (0.0)	0.23 (0.013)	−1.99	0.062
7 (11.57)	0.038 (0.0026)	0.42 (0.065)	0.23 (0.026)	−2.09	0.094

[a] Units of $(cal/cm^3)^{1/2}$.
[b] Units of mg of 5-FU/cm^2 per h.
[c] Units of mg of 5-FU.
[d] The application of theophylline/propylene glycol to hairless mouse skins in diffusion cell experiments without pretreatment with 5-FU/IPM or prodrug/IPM, but after brief washing with methanol, gave $J_j = 0.0024$ mg/cm^2 per h.
[e] Units of cm/h from J_i/S_{IPM} (in equivalent mg of 5-FU).
[f] Calculated from $[C_s/(4.9\ cm^2\ 24\ h)]/J_i$ to give a dimensionless ratio.

effective (6–25 times) than 5-FU in IPM at delivering total 5-FU species through hairless mouse skin. The only member that is not significantly more effective is one of the more lipid soluble members of the series – the octyloxycarbonyl derivative, **7**. Also, although all of the derivatives are more soluble in IPM than 5-FU, the two derivatives that are the least soluble in IPM are the methyl- (**2**) and ethyloxycarbonyl (**3**) derivatives, and they are the two derivatives that are the most effective at delivering total 5-FU species. Finally, octanol solubilities for **2**, **3**, **5** and **6** can be estimated from octanol/pH 4.0 buffer partition coefficient values determined by Buur and Bundgaard (1986, 1987) and the pH 4.0 buffer solubility values determined here ($S_{OCT} = S_{H_2O}K$). Those S_{OCT} values are 4.52, 35.6, 52.0 and 152.6 mg/ml, respectively. The S_{OCT} values follow the same trend as the S_{IPM} values. Thus, there is no correlation between lipid solubilities (S_{IPM} or S_{OCT}) and relative abilities to deliver total 5-FU species.

On the other hand, there is a correlation between water solubilities (S_{H_2O}) and relative abilities of the members of this series of 1-alkyloxycarbonyl derivatives to deliver total 5-FU species through hairless mouse skin. The more water soluble (Table 1) the derivative is, the more effective it is at delivering total 5-FU (Table 2). The same correlation was observed with the 1-alkylaminocarbonyl derivatives. However, the first four members of this series are 5–30 times more soluble in water and they deliver 3–10 times more total 5-FU through skin than the corresponding first four members of the 1-alkylaminocarbonyl series. The first four members of the series of 1-alkyloxycarbonyl derivatives are also 1.2–6 times more lipid soluble than the corresponding members of the series of 1-alkylaminocarbonyl derivatives. However, the fact that in each series there is a correlation between rank order of flux of 5-FU and rank order of water solubility, but not lipid solubility, suggests that water solubility is the more dominant feature. Thus, the more water soluble the type of 1-acyl derivative of 5-FU is, the more effective it is at delivering total 5-FU through skin. This assumes that the two types of derivatives being compared are each more soluble in lipids than 5-FU. It also assumes that only the first four or five members of each series are being compared where the solubilities are determined by the effect of the functional groups in 5-FU and the enabling functional group in the promoiety and not by the aliphatic chain in the promoiety (see above).

In two separate experiments, the first five members of this series were evaluated for their abilities to deliver intact prodrug through skin, each in one diffusion cell. The rates of delivery of

Table 3
Rates of delivery of intact prodrug (J_{PD}) and total 5-FU (J_i) at steady state, and percent intact prodrug delivered at steady state (SS) and at 11 h ($n = 1$)

Compound	J_{PD} [a]	J_i [a]	Percent prodrug	
			SS	11 h
2	0.057	0.14	42	16
3	0.48	0.53	90	75
4	0.13	0.16	78	43
5	0.13	0.17	73	32
6	0.14	0.17	79	14

[a] Units of mg equivalents of 5-FU/cm^2 per h.

total 5-FU species were lower (Table 3) than those observed in the other diffusion cell experiments (Table 2), and the order of effectiveness was not reproduced except for the fact that **3** was still much more effective than the other members of the series. This may have been the result of the fact that fresh donor phases were applied only twice in the experiments reported in Table 3 compared to four times in those reported in Table 2. Regardless, values for the percent of intact prodrug delivered are indicative of the effect of the hydrolytic stability of this type of 1-acyl prodrug on which species were delivered. The results in Table 3 show that a large portion of 1-alkyloxycarbonyl prodrugs diffuse through hairless mouse skin intact under conditions where 10% or less of the 1-alkylaminocarbonyl-5-FU derivatives diffused through intact. This is especially true during the steady-state portion of the experiments where up to 90% (for **3**) of intact prodrug diffused into the receptor phase. On the other hand, quite a bit less intact prodrug diffused through during the initial portions of the experiments (11 h, Table 3). This observation and the observation that higher rates of delivery of total 5-FU correlate with higher percentages of intact prodrug delivered, suggest, at least in part, that the large amounts of prodrug delivered into the skin are simply too much for the skin enzymes to hydrolyze. Other possibilities are (a) that continuous changing of the receptor phase during the conditioning and sampling parts of the diffusion cell experiments depleted the enzymatic activity, or (b) that the formaldehyde that has been used as a preservative has reacted with the enzymes responsible for the hydrolysis and deactivated them. However, it has previously been shown that S^6-acyloxymethyl derivatives of 6-mercaptopurine (6-MP), which also require enzymatic hydrolysis, delivered only 6-MP in diffusion cell experiments using this same protocol (Waranis and Sloan, 1988).

The trend in the skin accumulation (C_s) data is similar to the trend in the flux data, except for the 1-hexyloxycarbonyl derivative, **6**, where the value for C_s is much higher than expected based on correlations with either S_{H_2O} or J_i. However, generally higher fluxes of total 5-FU species correlate with higher amounts of total 5-FU species accumulated in the skin. This is quite different from the results for skin accumulation observed for the 1-alkylaminocarbonyl series (Sloan et al., 1993). In that case, there was no correlation between flux and C_s. However, it should be noted that the trends in that flux data were weakened by the fact that differences in flux among the three more effective members of the series were not significant; and there was no trend at all in the C_s values.

The trend in the ratios between C_s (normalized for skin area and time during which the sample was collected) and J_i, which has been designated as the dermal/transdermal (D/T) delivery ratio, is similar to the trend in the D/T delivery ratios observed for the 1-alkylaminocarbonyl series (Sloan et al., 1993). The value for the ratio becomes progressively smaller as the group becomes larger until R = C_4H_9 is reached, then the value for the ratio becomes progressively larger. Assuming that the skin accumulation values are reasonable estimates of relative dermal delivery (delivery into the skin) as opposed to transdermal delivery (delivery through the skin), this trend in D/T ratios suggests that the most effective derivatives with which to enhance dermal delivery without unduly enhancing transdermal delivery are among the first and the later members of a series of homologous derivatives. In this series, none of the derivatives is as effective as 5-FU at selectively delivering total 5-FU species into the skin even though some are much more effective at delivering total 5-FU species through the skin. However, these in vitro C_s

values may not give estimates of relative dermal delivery that correlate with relative biological activity in vivo. This needs to be determined.

There are no apparent trends in the rates of delivery of theophylline from propylene glycol (J_j) vs J_i; all of the J_j values were essentially the same. Since J_j values represent one measure of how much damage was done to the mouse skins by the initial application of the prodrugs in IPM (Sloan et al., 1986), the large differences in J_i values cannot be due to differences in damage to the skins caused by application of the prodrugs in IPM.

The solubility parameters calculated for the prodrugs (δ_i) are also given in Table 2 along with their respective log permeability coefficients (log P_i) calculated from J_i/S_{IPM}. Results for previous homologous series of prodrugs that deliver mostly parent drug have shown that as the members of the series become increasingly more lipophilic (decreasing δ value) they become less effective at delivering the parent drug from lipoidal vehicles (Sloan, 1992b). In this series, which delivers mainly intact prodrug, it was of interest to see if a similar relationship between δ_i and log P_i would be obtained. The results are shown in Fig. 1 along with the results from the 1-alkylaminocarbonyl-5-FU series. Clearly, whether the prodrug delivers the parent drug or diffuses through intact has no effect on the relationship between δ_i and log P_i. The 1-alkyloxycarbonyl derivatives of 5-FU behave like all the other prodrugs by this criteria.

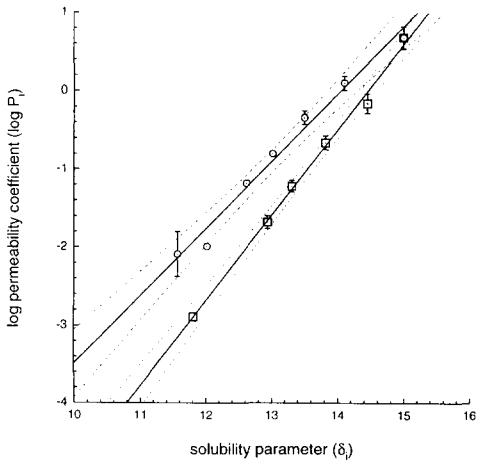


Fig. 1. Plots of experimental log permeability coefficients (P_i) vs the solubility parameters for the 1-alkyloxycarbonyl-5-FU prodrugs (δ_i) for the delivery of total 5-FU through hairless mouse skin (○) and plots of the same data for the 1-alkylaminocarbonyl-5-FU prodrugs (□).

4. Conclusions

The solubilities of the 1-alkyloxycarbonyl series of 5-FU prodrugs show that removing the amide-like NH group from the promoiety and replacing it with an oxygen group significantly increases the water solubilities of at least the first four members of the series compared to the 1-alkylaminocarbonyl series. The change improves their comparative lipid solubilities as well but not as much as the water solubilities so that the partition coefficient values exhibited by the 1-alkyloxycarbonyl series are actually less than those exhibited by the members of the corresponding 1-alkylaminocarbonyl series. This result is an example of increased lipid solubilities not necessarily leading to an increase in partition coefficients in a comparison of two types of prodrugs.

The results from the diffusion cell experiments show that changes in the promoiety that result in increased water solubilities of more lipid soluble derivatives lead to increased rates of delivery of total 5-FU species through skin. Thus, the first four members of the 1-alkyloxycarbonyl series are much more water soluble than the corresponding members of the 1-alkylaminocarbonyl series and they are also much more effective at delivering total 5-FU through the skin.

There is also a good correlation between water solubility and flux within the series of more lipid soluble prodrugs. The most water soluble member of the series, **3**, is the most effective member of the series at enhancing flux. However, the first four members of the 1-alkyloxycarbonyl series are all much more lipid soluble than the corresponding members of the 1-alkylaminocarbonyl series as well. Thus, the effect of water solubility seems to be a major factor in optimizing transdermal delivery within a series or between two different series, but the importance of the effect of enhanced lipid solubility cannot be ignored.

Finally, the in vitro experiments suggest that the 1-alkyloxycarbonyl derivatives may be too stable to function effectively as dermal delivery agents as opposed to transdermal delivery agents. Because these prodrugs are so stable, they retain the solubility properties that allow them to partition into the skin more effectively than 5-FU long enough that they partition through the skin more effectively as well. Thus, no improvement in the dermal/transdermal delivery ratio relative to 5-FU is achieved in vitro. This result should not preclude members of this series from being tested in vivo where enzymatic activity should be much higher and the conversion of the prodrug to 5-FU would be more complete. However, it does suggest that, in order to achieve improvement in the dermal/transdermal delivery ratio, it may be necessary to identify other types of prodrugs with similar solubility properties but that hydrolyze more rapidly than the 1-alkyloxycarbonyl type prodrugs of 5-FU.

Similarly, it is not clear what effect the highly hydrated skin will have on the potential of in vitro diffusion cell experiments, in general, to predict trends in vivo. Because the in vitro mouse skin is highly hydrated, it may be more permeable to the more water soluble members of a series of more lipid soluble derivatives than normally hydrated skin in vivo. However, it should be noted that the same trend observed with hairless mouse skin has also been observed with human skin in vitro (Bonina et al., 1991), so that, if hydration of the membrane in diffusion cell experiments gives misleading predictions of in vivo trends, changing to human skin will not correct the problem.

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