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7-alkylcarbonyl and 7-alkyloxycarbonyl prodrugs of theophylline

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Abstract

Five members (**6**–**10**) of an homologous series of 7-alkyloxycarbonyltheophylline (7-AOC-Th) and four members (**2**–**5**) of a homologous series of 7-alkylcarbonyltheophylline (7-AC-Th) prodrugs have been synthesized by known methods and characterized. All of the members in both series were much more soluble in isopropyl myristate (S_{IMP}) (10–200 times) and in each series, at least one member was more soluble in pH 4.0 buffer (S_{AQ}) than Th. However, in the 7-AC-Th series, only the acetyl member, **2**, which exhibited about 90% of the S_{AQ} of Th, was sufficiently stable to be evaluated — it gave four times the flux of Th/IPM (isopropyl myristate). In the 7-AOC-Th series, all the members were sufficiently stable to be evaluated but the member which exhibited the greatest S_{AQ}, **6** (methyloxycarbonyl), did not exhibit the greatest flux. Instead, **8** (propyloxycarbonyl), which exhibited the second greatest S_{AQ} (about 80% of the S_{AQ} of Th), but exhibited over ten times the S_{IPM} of **6** gave the greatest flux — two times the flux of Th/IPM. Thus, good biphasic solubility was the best predictor of increased flux. All of the prodrugs delivered only Th through the mouse skin. Only **2**/IPM actually delivered more Th into the skin than Th/IPM which correlated with its ability to increase the flux of Th through the skin.

Keywords: Theophylline; Alkylcarbonyl prodrugs; Alkyloxycarbonyl prodrugs; Lipid solubility; Water solubility; Diffusion cell experiments; Stability

1. Introduction

Psoriasis is a chronic, hyperproliferative skin disease characterized by itchy, scaly plaque formation especially on the elbows, knees and scalp but which in extreme cases can involve most areas of the skin. This disease is difficult to treat, with no known cure so that palliative treatment is the mainstay of therapy. Theophylline, a drug more commonly used to manage asthma, has been shown to be partially effective in the treatment of psoriasis when given orally or topically (Iancu et al., 1979; Berenbein et al., 1979, respectively). Its mechanism of action in treating psoriasis is believed to be its ability to inhibit phosphodiesterase activity and hence to increase cyclic adenosine

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3′,5′-monophosphate (cAMP) concentrations (Bourne et al., 1974), which are low in psoriatic skin (Voorhees and Duell, 1971). However, theophylline exhibits a narrow therapeutic range (the difference between efficacy and toxicity is small) so that increasing the oral dose to increase the local concentration of theophylline is not a viable option. On the other hand, increasing the local concentration by increasing the topical delivery of theophylline is potentially attractive.

There are two primary methods of increasing the topical delivery of a drug. The first method is the modification of the formulation to incorporate penetration enhancers. The second method is the synthesis of transient derivatives of the drug (prodrugs) that exhibit increased lipid and aqueous solubilities; properties that have been shown to result in the enhanced ability of the derivatives to permeate skin (Sloan, 1992; Roberts and Sloan, 1999). An example of the first method is the use of alcohols other than propylene glycol (PG) in simple, one component vehicles to deliver theophylline. Increases in theophylline flux of 500-fold were obtained but, except for propanol, damage to the skin increased proportionately (Sloan et al., 1998). Thus, an estimate of effective enhancement normalized for damage was only 10-fold. An example of the second method is the use of alkylcarbonyloxymethyl (ACOM) prodrugs to enhance the topical delivery of theophylline (Kerr et al., 1998). Although the prodrugs were all more lipid soluble than theophylline, none of them were more water soluble so that the best ACOM prodrugs only gave a 2-fold increase in delivery. For other heterocyclic drugs such as 5-fluorouracil (5-FU) (Taylor and Sloan, 1998) or 6-mercaptopurine (6-MP) (Waranis and Sloan, 1986) the ACOM prodrugs were more water soluble as well as more lipid soluble so that 16- to 70-fold increases, respectively, in topical delivery were achieved. Thus, in order to significantly improve the topical delivery of theophylline, it was a logical progression to identify a type of prodrug that is more water soluble as well as more lipid soluble than the parent drug.

Some members of different types of *N*-acyl prodrugs of 5-FU (alkyloxycarbonyl- and alkylcarbonyl-5-FU) have been shown to exhibit increased water as well as increased lipid solubility and to increase the delivery of 5-FU by 25–40-fold, respectively, from suspensions in isopropyl myristate (IMP) (Beall and Sloan, 1996; Beall et al., 1994). In this paper, we report the synthesis of 7-alkyloxycarbonyl (AOC) and 7-alkylcarbonyl (AC) prodrugs of theophylline, their physicochemical properties and their abilities to delivery theophylline through hairless mouse skin from suspensions in IMP. The hypothesis is, if the prodrugs are more water soluble as well as more lipid soluble than theophylline, they will give much better than 2-fold increase in its topical delivery.

2. Methods and materials

Melting points were determined with a Meltemp capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Ultraviolet (UV) spectra were obtained on Shimadzu UV-265 or 2501PC spectrophotometers, while IR spectra were obtained on a Perkin–Elmer 1420 ratio recording spectrophotometer. 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. TLC analyses were run on Brinkman Polygram Sil G/UV 254 plates. IPM was obtained from Givaudan, Clifton, NJ. Theophylline was purchased from Sigma Chemical Co.; acid chlorides, acid anhydrides, alkyl chloroformates and all other reagent chemicals were from Aldrich Chemical Co.; all other solvents were from Fisher. The female hairless mice (25–30 g, 12–16 weeks old SKH-hr-1) were from Charles River. Microanalyses were obtained from Atlantic Microlab Inc., Norcross, GA.

2.1. Synthesis

2.1.1. General procedures for 7-alkylcarbonyltheophyllines

To the corresponding acid anhydride (15 ml) was added 3.0 g (0.017 mol) of theophylline (**1**) in

a flask equipped with a reflux condenser and a drying tube. The suspension was stirred and heated at 130°C for 3 h; the suspension cleared. When the solution cooled, a precipitate formed which was filtered. The residue was washed with ether and dried in a vacuum desiccator. This procedure gave 3.08 g 7-acetyltheophylline (**2**) in 83% yield. The initial product from the reaction of **1** with propionic anhydride was resuspended in 10 ml more of anhydride and the reaction repeated to give 1.92 g of 7-propionyltheophylline (**3**) in 49% yield.

To the corresponding acid chloride (0.013 mol) in 20 ml of ice-cooled dichloromethane was added 1.8 g (0.01 mol) of **1** followed by 1.18 g (0.012 mol) of triethylamine in 20 ml of dichloromethane over 20 min. The suspension was stirred at room temperature for 3 h, then diluted with 150 ml of ether, and filtered. The filtrate was concentrated at reduced pressure to about 10 ml, then slowly diluted with hexane with stirring. The suspension that resulted was filtered. The residue was dried in a vacuum desiccator. This procedure gave 1.99 g of 7-butyryltheophylline (**4**) in 80% yield, and 1.90 g of 7-valeryltheophylline (**5**) in 72% yield.

2.1.2. General procedure for 7-alkyloxycarbonyltheophyllines

To 1.80 g (0.01 mol) of theophylline and 0.012 mol of the corresponding alkyl chloroformate in 35 ml of well-stirred dichloromethane at room temperature was added 1.24 g (0.012 mol) of triethylamine in 15 ml of dichloromethane. The solution that resulted was stirred for 30 min, then extracted with 20 ml H_2O. The dichloromethane layer was separated, dried over anhydrous Na_2SO_4 for 30 min, and concentrated at reduced pressure to dryness. The residue was then crystallized from mixtures of dichloromethane and ethyl ether, pet ether or ethyl ether alone. This procedure gave 1.72 g of 7-methyloxycarbonyltheophylline (**6**) in 72% yield from dichloromethane:ethyl ether; 2.05 g of 7-ethyloxycarbonyltheophylline (**7**) in 81% yield from dichloromethane:ethyl ether; 1.85 g of 7-propyloxycarbonyltheophylline (**8**) in 75% yield from dichloromethane:pet ether; 2.06 g of 7-butyloxycarbonyltheophylline (**9**) in 74% yield from dichloromethane:pet ether; 1.55 g of 7-hexyloxycarbonyltheophylline (**10**) in 71% yield (from 0.007 mol of theophylline) from ethyl ether.

2.2. Physicochemical properties and hydrolyses

Lipid solubilities were determined in IPM according to a previously described procedure (Beall et al., 1993). Three suspensions of each prodrug were stirred at 22 ± 1°C for 48 h. The suspensions were filtered through 0.45 μm nylon filters. For **6**–**10**, aliquots from the saturated solutions were diluted with dry acetonitrile and quantitated by UV spectrophotometry using molar absorptivities

Table 1
Melting point (mp) and elemental analysis data

Compound	mp, °C (lit mp, °C)	Formula	Calculated (found)		
			Carbon	Hydrogen	Nitrogen
2	155–159 (158)[a]				
3	128–132 (129–130)[b]				
4	93–94 (88–90)[b]				
5	96–98				
6	174–175	$C_9H_{10}N_4O_4$	45.38 (45.41)	4.23 (4.30)	23.52 (23.34)
7	139–141 (139–141)[c]	$C_{10}H_{12}N_4O_4$	47.62 (47.66)	4.80 (4.83)	22.21 (22.11)
8	85.5–87.5	$C_{11}H_{14}N_4O_4$	49.62 (49.67)	5.30 (5.37)	21.04 (20.91)
9	80.5–82	$C_{12}H_{16}N_4O_4$	51.42 (51.47)	5.75 (5.83)	19.99 (19.86)
10	77–78.5	$C_{14}H_{20}N_4C_4$	54.53(54.59)	6.54 (6.59)	18.17 (18.30)

[a] Ref. Biltz and Strufe (1914).
[b] Ref. Ishido et al. (1964).
[c] Ref. Giani and Molteni (1957).

for **6**–**10** previously determined in triplicate at 284 nm in acetonitrile (Table 1). For **2**–**5**, aliquots of the saturated solutions were immediately diluted with methanol and after 3 h, quantitated for theophylline by UV spectroscopy using the molar absorptivity of theophylline previously determined in triplicate at 270 nm in methanol ($\varepsilon = 9.81 \times 10^3$ l mol^{-1}).

Partition coefficients (K) were determined using the saturated IPM solutions ($n = 3$) from the lipid solubility determinations (Beall et al., 1993). The saturated IPM solutions were partitioned against 0.05 M acetate buffer (pH 4.0). The two phases were shaken vigorously for 10 s and then allowed to separate for 60 s. Aliquots from the IPM layers for **6**–**10** were diluted with dry acetonitrile and the UV absorbances was determined. The K values were calculated as follows:

$$K = \left[\frac{A_a}{(A_b - A_a)}\right]\frac{V_{H_2O}}{V_{IPM}}$$

where A_b and A_a were the absorbances from the IPM layer before and after partitioning, respectively, and V_{H_2O} and V_{IPM} were the volumes of the buffer and IPM phases, respectively. For those compounds exhibiting large solubility differences in the two phases, volume ratios (IPM:buffer) other than 1:1 were necessary to achieve accurate results but the ratio never exceeded 10:1 or 1:10. For **6**, the ratio was 5:1 while for **10** it was 1:4. Aliquots from the IPM layers for **2**–**5** were immediately diluted with methanol and after 3 h the UV absorbance for theophylline was determined as in the determination of the lipid solubilities. The K values for **2**–**5** were then determined as above using the UV absorbances due to theophylline instead of the intact prodrug. For **2**–**5**, the IPM:buffer volume ratios different from 1:1 were 4:1 for **2** and **3** and 1:2 for **5**.

The hydrolyses of $1.2–1.4 \times 10^{-4}$ M 7-AOC-Th prodrugs (**6**–**10**) in pH 9.02 buffer (0.01 M borate) containing 1% CH_3CN at 32°C were followed by UV spectrophotometry at 273 nm ($\varepsilon = 1.07 \times 10^4$ l/mol for theophylline) to 12 half-lives. Multiple non-linear regression (SPSS program, version 6.1 for Windows) analysis of absorbance data from the first five half-lives was used to solve for A_∞, A_0 and $t_{1/2}$. There was less than 1% difference between observed A_∞ and that calculated from multiple non-linear regression analysis of the data, and r^2 values were > 0.999.

2.3. Diffusion cell experiments

The diffusion cell experiments were run according to previously described procedures (Sloan et al., 1986). Briefly, female hairless mice were sacrificed by cervical dislocation. Their skins were removed by blunt dissection and placed epidermal side up in glass Franz diffusion cells with the dermal side in contact with pH 7.4 phosphate buffer (0.005 M; $I = 0.11$ M; 32°C) containing 0.11% formaldehyde (2.7 ml of 36% aqueous formaldehyde per l) to prevent microbial growth and to insure the integrity of the mouse skins during the course of the experiment. (In control experiments to determine the effect of time on the integrity of the mouse skin, Th flux from PG applied 4 h after sacrifice was $6.1 \pm 0.6 \times 10^{-3}$ μmol cm^{-2} h^{-1}; 24 h after sacrifice was $8.3 \pm 1.9 \times 10^{-3}$ μmol cm^{-2} h^{-1}; 48 h after sacrifice was $9.4 \pm 1.2 \times 10^{-3}$ μmol cm^{-2} h^{-1}; 120 h after sacrifice was $10.0 \pm 1.2 \times 10^{-3}$ μmol cm^{-2} h^{-1} (Sloan et al., 1991)). The skins were kept in contact with the buffer for at least 48 h to condition the skins and to allow UV absorbing materials to leach from the skins; the receptor phases were changed at least three times during this time to facilitate the leaching process. The epidermal sides of the skins were exposed to ambient conditions and were untreated during the pre-application period.

After the pre-application period, 0.5-ml aliquots from suspensions of **6**–**10** in IPM were applied to the epidermal sides of the skins (the IPM suspensions were stirred at 22 ± 1°C for 48 h prior to application to ensure that saturation was attained). Total concentrations of the IPM suspensions ranged from 0.2 M for **6**, **7** and **10** to 0.3 M for **8** and **9**, which ensured that enough excess solid was present to maintain saturation for the duration of the application period (see below). Each drug–vehicle combination was run in triplicate. For **8** and **9**, the donor phases were replaced at 23 and 20 h, respectively, and saved for analysis by ^{1}H NMR spectroscopy, along with donor

phases at 48 h from the application of **6**–**10**. Samples were taken from the receptor phases at 8, 20, 23, 26, 29, 32, 35, 44 and 48 h after donor phase application. The receptor phases were changed following removal of each sample so that sink conditions were maintained. Samples were analyzed by UV spectroscopy ($\varepsilon = 1.02 \times 10^4$ l mol^{-1} at 271 nm) for Th that had diffused. Cumulative amounts of Th in the receptor phase (μmol) were plotted against time (h), and the slopes of the linear, steady-state regions were calculated using linear regression. The slopes divided by 4.9 cm^2 (the area of the donor surface) gave the steady-state fluxes (J_i in μmol cm^{-2} h^{-1}). Permeability coefficients (P_i) were determined by dividing the values of J_i by the solubility of the corresponding prodrugs in IPM (S_{IPM}).

Only **2** and **3** of the 7-AC-Th prodrugs were evaluated in diffusion cells. The IPM suspensions (0.3 M) of **2** and **3** were only stirred for 12 h at 22 ± 1°C before application and the 0.5 ml donor phases were replaced every 12 h so that fresh donor phases were constantly being prepared for application. Each donor phase that was removed was immediately analyzed by ^{1}H NMR spectroscopy. Samples of the receptor phases were taken at 7, 12, 24, 27, 30, 33, 36 and 48 h and samples were analyzed for Th as above for **6**–**10**.

Following removal of the donor phases after the 48-h application period, the epidermal sides of the skins were washed three times with 5 ml portions of methanol to remove all remnants of prodrug and vehicle from the skin surfaces. This was accomplished quickly (< 3 min) to minimize contact time between the skins and methanol. The receptor phases were changed again, and the dermal sides were kept in contact with the fresh buffer for 24 h while the epidermal sides were again left exposed to the air. After this leaching period, another sample was taken from each cell to measure the skin accumulation of Th (C_s).

Second applications to the epidermal sides of the skins were made after the leaching period with a standard drug–vehicle suspension. Theophylline in propylene glycol (0.4 M) was applied to assess the damage to the skins from application of the initial drug–vehicle combinations. Samples were taken at 1, 2, 3 and 4 h after application. The samples were analyzed for theophylline by UV spectroscopy ($\varepsilon = 1.02 \times 10^4$ l mol^{-1} at 271 nm) and second application fluxes (J_j) were determined as described above.

The ^{1}H NMR spectra of the donor phases from the applications of **2**, **3**, **6**–**10** were run in $CDCl_3$. The C^8–H for Th appears at δ7.73 and the C^8–H for the 7-AC and 7-AOC prodrugs appears at δ8.23–8.33 in $CDCl_3$. Since this area of the spectra is free from interference by IPM absorbances, the two signals were easily quantified by integration.

2.4. Solubility parameters

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

2.5. Statistical analysis

Statistical analyses were accomplished using Student's *t*-test. Unless otherwise indicated, statistical significance is for $P < 0.05$.

3. Results and discussion

3.1. Syntheses

The 7-AC-Th prodrugs were synthesized either by reaction of Th with the corresponding acid anhydrides (Biltz and Strufe, 1914) or with the corresponding acid chloride in the presence of triethylamine (Bodor et al., 1978). The reaction with the acid anhydrides only worked well for the first member of the series (**2**), but a second reaction of the initial product with fresh anhydride was necessary to give complete conversion of Th to **3**. The reaction of Th with the acid chlorides worked well for the preparation of **4** and **5**. The mp of **2**, **3** and **4** were consistent with literature values (Table 1). The ^{1}H NMR spectra of **2**–**5** were consistent with those previously reported (Bodor et al., 1978) for the longer alkyl chain members of the 7-AC-Th series (Table 2); the C^8–H absorption was previously reported to be at

Table 2
Spectroscopic data (UV and ^{1}HNMR), rates of hydrolysis ($t_{1/2}$) and calculated solubility parameters (δ_i) for 7-RCO-Th

Compound R=	UV[a], ($\varepsilon \times 10^{-3}$ ± S.D., 1 mol^{-1})	^{1}H NMR[b]		$t_{1/2}$[c] (min)	δ_i[d] (cal cm^{-3})$^{1/2}$
		C^8–H	CH$_2$OC=O		
2, CH_3	6.46 (0.34)[e]	8.33[f]	s 2.97[f,g]	–	–
3, C_2H_5	–	8.33	–	–	–
4, C_2H_7		8.30[f]	–	–	–
5, C_4H_9		8.30	–	–	–
6, OCH_3	6.74 (0.126)	8.27	s 4.15	1.8	13.61
7, OC_2H_5	6.83 (0.0)	8.23	q 4.55	3.4	13.19
8, OC_3H_7	6.83 (0.095)	8.25	t 4.45	3.6	12.84
9, OC_4H_9	6.85 (0.12)	8.23	t 4.49	3.4	12.54
10, OC_6H_{13}	6.82 (0.25)	8.23	t 4.45	–	12.05

[a] UV_{max} at 284 nm in CH_3CN for **6**–**10**.
[b] ^{1}H NMR run in $CDCl_3$ with TMS as internal standard; s, singlet; q, quartet; t, triplet; units of δ.
[c] Hydrolyses run at 32°C in pH 9.02 borate buffer.
[d] Calculated according to method of Martin et al. (1985).
[e] UV_{max} at 296 nm in CH_2Cl_2.
[f] ^{1}H NMR also run in $(CD_3)_2S$=O where C^8–H was at δ8.60 for **2** and δ8.63 for **4** and CH$_3$C=O was at δ2.80 for **2**.
[g] ^{1}H NMR absorption due to CH$_3$C=O.

δ8.37, here it is at δ8.33. In addition, the position of the CH$_3$C=O absorption in **2** at δ2.97 was consistent with that previously reported for **2** at δ3.00 (Higuchi et al., 1971) and with that previously reported for 1-acetyl-5-FU — an analogous *N*-acetyl heterocycle — at δ2.73 (Beall et al., 1996). The UV spectra of **2** was also consistent with the UV spectrum previously reported (Bodor et al., 1978) for 7,7′-succinylditheophylline; the λ_{max} (CH_2Cl_2) was previously reported at 300 nm ($\varepsilon = 1.31 \times 10^4$ l mol^{-1}), here at 296 nm ($\varepsilon = 6.46 \times 10^3$ l mol^{-1}) for **2**. Since each molecule of 7,7′-succinylditheophylline contains two molecules of acylated theophylline, its ϵ should be twice as large as **2**. Finally, the IR spectra of **2**–**5** were consistent with those previously reported (Bodor et al., 1978); the broad intense NH absorptions between 2400 and 2900 cm^{-1} in Th were absent in **2**–**5** and a strong new carbonyl absorption was present at 1730–1750 cm^{-1}.

The 7-AOC-Th prodrugs were synthesized by reaction of Th with the corresponding alkyl chloroformates by analogy to the reaction of Th with the acid chlorides above. The products exhibited ^{1}H NMR spectra (Table 2) similar to those of **2**–**5** (C^8–H at δ8.23–8.27 for **6**–**10** compared with δ8.30–8.33 for **2**–**5**) and that of 1-ethyloxycarbonyl-5-FU, **11** (CH$_2$–O at δ4.45–4.55 for **6**–**10** compared with δ4.40 for **11**; Roberts, unpublished work). The UV spectra of **6**–**10** (Table 2) were consistent with those of **2**, exhibiting a shift of the UV_{max} to a longer wavelength than that exhibited by Th and a slightly higher molar absorptivity than **2**. Although the synthesis of 7-AOC-Th prodrugs **6** and **7** had been previously reported (Giani and Molteni, 1957) and the mp of **7** isolated here was consistent with the literature melting point, **6** was not, so elemental analyses were obtained for **6**–**10**, all of which were consistent with their elemental formulae (Table 1).

3.2. Physicochemical properties and hydrolyses

All the members of both series of prodrugs were more lipid soluble (S_{IPM}) than Th (Table 3), with the initial members of the 7-AC series being somewhat more lipid soluble than the corresponding members of the 7-AOC series, which may be due to the lower mp of the 7-AC prodrugs. In each series, S_{IPM} increased with increasing alkyl chain length and then decreased with further increases beyond C_3 in the 7-AC series and C_4 in the 7-AOC series. Similar trends in S_{IPM} have previously been reported not only for the analogous

1-AC-5-FU (Beall and Sloan, 1996) and 1-AOC-5-FU series (Beall et al., 1994), but also, for example, for the 1-alkylcarbonyloxymethyl-5-FU (1-ACOM-5-FU) series (Taylor and Sloan, 1998). The causes of this behavior have been discussed by Yalkowsky (1977), Taylor and Sloan (1998) among many others.

In each series, at least one member exhibited increased solubility in pH 4.0 buffer (S_{AQ}) compared with Th, and at least one other member had an S_{AQ} value comparable to the S_{AQ} value of Th. The decreased S_{AQ} for **7** (the C_2 member) compared with **6** or **8** in the 7-AOC series is a result that has also been observed for the C_2 member of the 7-ACOM-Th series (Kerr et al., 1998). In the present series, S_{AQ} values were all estimated from the measured partition coefficient values (K) between pH 4.0 buffer and IPM whereas in the 7-ACOM-Th series, they were measured directly. Good correlations between estimated S_{AQ} and directly measured S_{AQ} are usually obtained for prodrugs that are hydrolytically stable, so the decreased S_{AQ} for **7** is probably not an artifact of the experimental techniques. The S_{AQ} values for **6** and **7** are significantly different from the values previously reported (1 part in 100 parts (42 mM) and 1 part in 35 parts cold water (113 mM), respectively; Vieth and Leube, 1925). However, the mp reported by Vieth and Leube are also quite different (mp, 255–260°C for **6**) or quite broad (mp, 135–140°C for **7**) compared with those obtained here; and no spectroscopic evidence was available to support the structures or purities of the previously reported **6** and **7**.

The K values for the members of the 7-AOC series were consistent with those of other homologous series of prodrugs of Th and 5-FU based on the mean of the methylene π values calculated from the log K values (Table 3). The K values for the 7-AC series, and hence the S_{AQ} values, were more difficult to measure than those for the 7-AOC series because of the very rapid hydrolysis of this type of prodrug. In fact, in order to obtain reproducible values, not only for K but also for S_{IPM}, it was necessary to immediately dilute the saturated IPM solutions with methanol allow the 7-AC prodrug to undergo rapid solvolysis, and then measure Th concentrations in the methanol solutions that resulted.

Although the values for K and S_{IPM} in the 7-AC-Th series were reproducible, the values for K were not consistent with those of other homologous series of prodrugs of Th and 5-FU. The value for the mean methylene π value is low and

Table 3
Lipid solubilities (S_{IPM}), pH 4.0 buffer solubilities (S_{AQ}), partition coefficients between IPM and pH 4.0 buffer (K_i), log K_i and calculated π values

Compound	S_{IPM}[a] ($\pm$S.D.)	S_{AQ}[a]	K_i ($\pm$S.D.)	log K_i	π[b]
Th, **1**	0.34	46.0	–	–	
2	6.40 (0.05)	40.3	0.159 (0.0064)[c]	−0.80	
3	25.5 (0.35)	79.9	0.319 (0.014)[c]	−0.50	0.30
4	71.0 (0.75)	29.8	2.38 (0.37)	0.38	0.88
5	35.7 (3.5)	7.6	4.71 (0.14)[d]	0.67	0.29
6	3.56 (0.05)	57.3	0.062 (0.0006)[e]	−1.21	
7	6.70 (0.22)	22.6	0.30 (0.02)	−0.52	0.69
8	45.5 (1.9)	35.5	1.29 (0.04)	0.11	0.63
9	64.8 (2.8)	11.8	5.48 (0.68)	0.74	0.63
10	56.5 (3.2)	1.0	56.9 (8.1)[f]	1.76	0.51

[a] Units of mM or μmol ml^{-1}.

[b] $\pi = (\log K_{n+m} - \log K_n)/m$, where n is the number of methylene units in the promoiety of one prodrug and m is the number of additional methylene units in the promoiety of the prodrug with which it is compared.

[c] Ratio of IPM/pH 4.0 buffer 4:1.

[d] Ratio of IPM/pH 4.0 buffer 1:2.

[e] Ratio of IPM/pH 4.0 buffer 5:1.

[f] Ratio of IPM/pH 4.0 buffer 1:4.

Table 4
Flux of Th (J_i) and log permeability constants (log P_i) from the application of the 7-AC- and 7-AOC-Th prodrugs in IPM, flux of Th (J_j) from the application of Th/PG after removal of the prodrugs in IPM, and concentration Th in skin (C_s) after application of prodrug in IPM

Compound	J_i ($\pm$S.D.)[a]	log P_i[b]	J_j ($\pm$S.D.)[a]	C_s ($\pm$S.D.)[c]
Th, **1**[d]	0.48 (0.16)	0.15	0.81 (0.084)	5.9 (0.4)
2	1.84 (0.17)	−0.54	1.24 (0.18)	15.5 (2.1)
6	0.286 (0.11)	−1.10	0.71 (0.19)	2.2 (0.6)
7	0.207 (0.058)	−1.51	0.85 (032)	1.6 (0.3)
8	1.06 (0.28)	−1.63	0.96 (0.15)	3.0 (0.9)
9	0.637 (0.016)	−2.01	0.81 (0.054)	3.0 (0.3)
10	0.146 (0.044)	−2.59	0.73 (0.17)	1.5 (0.6)
Control			0.74 (0.038)[e]	

[a] Units of μmol cm^{-2} h^{-1}.
[b] Units of cm h^{-1}.
[c] Units of μmol.
[d] Standard values from Kerr et al. (1998).
[e] Pretreatment with IPM (Sherertz et al., 1987).

the S.D. is unacceptable; $\pi = 0.49 \pm 0.33$. The only S_{AQ} value previously reported for any member of this series was 12.7 mM at 25°C in water for **2** by Higuchi et al. (1971). However, this estimated value was based on the dissolution rate of **2** and required that the authors' estimated diffusion constants for both Th and **2** and their assumptions that all the particles were of the same size and densities were correct.

Although the rates of hydrolysis of **2** and similar N-AC prodrugs are very fast ($t_{1/2} = 40$ s for **2** in water at room temperature; Lee et al., 1979), the rates of hydrolysis of N-AOC prodrugs **6**–**10** were considerably slower by comparison (Table 2). This is expected for prodrugs containing an O=C–O-alkyl instead of an O=C-alkyl group, where the oxyalkyl groups can decrease the electrophilicity of the carbonyl carbon by resonance compared with a simple alkyl group. The longer alkyl chain members, **7**–**9**, of the series were more stable than **6** because of steric hindrance, which impedes an addition–elimination type mechanism of hydrolysis. The 7-AOC-Th prodrugs all hydrolyzed completely during their diffusion through the skin. UV spectra of receptor phase samples obtained immediately after the samples had been taken showed no signs of any absorption peaks at longer than 271 nm.

3.3. Diffusion cell experiments

The results from the diffusion cell experiments are given in Table 4. In the 7-AC-Th series, only the application of **2** in IPM gave a donor phase that contained any intact prodrug (60%) after 12 h of contact with hydrated skin and exposure to ambient moisture. Prodrug **3** was also evaluated in the diffusion cell experiment, but no intact prodrug was observed in the donor phases after any of the 12-h application periods. Thus the value for J_i (0.72 ± 0.26 μmol cm^{-2} h^{-1}) for **3** is much lower than expected based on the performance of **2**. Compound **2** was also the only member of the 7-AC-Th series for which a UV spectrum exhibiting a single absorption in dichloromethane could be obtained; **3**, **4** and **5** all showed multiple absorptions at 272, 284 and 296 nm. Thus neither of the other two members of the series were evaluated in diffusion cell experiments since it was assumed that they would decompose too quickly like **3**. Although the J_i value for **2**/IPM is almost four times that of Th/IPM (Table 3), the fact that the donor phase was also probably saturated with Th means that the J_i value for Th/IPM has to be subtracted from the J_i for **2**/IPM to give a more accurate J_i value for **2**/IPM. However, even that normalized value, 1.36 μmol cm^{-2} h^{-1}, gives the largest increase in flux (3-

fold) for any of the acyl type prodrugs of Th evaluated here or in Kerr et al. (1998). It is unfortunate that the 7-AC-Th series of prodrugs were not sufficiently stable to remain intact during their topical application so they could be evaluated. Based on the flux of **2**/IPM and the greater S_{IPM} (4-fold) and S_{AQ} values (2-fold) for **3** compared with **2**, **3**/IPM should theoretically have been significantly more effective at delivering Th through skin than Th/IPM.

In the 7-AOC-Th series all of the prodrugs were stable when suspended in IPM in contact with hydrated skin and exposure to ambient moisture for at least 24 h for two prodrugs (**8** and **9**) and 48 h for the other three. However, although the prodrugs were stable in IPM, their J_i values (Table 4) were no better than those obtained from the 7-ACOM-Th prodrugs (Kerr et al., 1998) which were slightly less soluble in IPM and three to four times less soluble in pH 4.0 buffer for the first four members of the two series. In fact, the J_i values for **7**, **8** and **9** were quite similar to those for the corresponding alkyl chain length members of the 7-ACOM-Th series, and the rank order of all the members of each series was the same. In each series, the member of the more lipophilic series of homologous prodrugs that exhibited the greatest S_{AQ} value was not the member that gave the greatest J_i value. In each series, it was the member (C_3) that was ten times more soluble in IPM than the most water soluble member of the five (C_1), yet retained a significant percentage (60–80%) of the water solubility of C_1, that gave the greatest increase in delivery of Th through skin. In each series, the relatively poor performance of the second member of the series (C_2) can be directly attributed to its poor lipid and water solubility compared with adjacent members in the series.

In order to determine if the observed flux values for **6**–**10** were consistent with their S_{AQ} and S_{IPM} values, predicted log J_M were calculated from the molecular weights (MW), S_{AQ} and S_{IPM} using the transformed Potts–Guy equation (Roberts and Sloan, 1999) where $J_M = -0.211 + 0.534 \log S_{IPM} + 0.466 \log S_{AQ} - 0.00364$ MW. The average error in predicting log J_M (experimental log J_M-predicted log $J_M = \Delta \log J_M$) for the 7-AOC-Th series (0.50 ± 0.10 log units) was over four-times larger than the average $\Delta \log J_M$ for the members of the $n = 42$ database of prodrugs from which the parameter estimates for the transformed Potts–Guy equation had been obtained, and 2.5 times larger than the previously most poorly predicted series of prodrugs. The rank order of ability of the members of the series to deliver Th through the mouse skin was correctly predicted by the transformed Potts–Guy equation; however, the magnitude of the predicted flux was consistently almost three-times larger than that observed. On the other hand, the transformed Potts–Guy equation predicted log J_M for **2** reasonably accurately compared with the average of $\Delta \log J_M$ for the $n = 42$ database (average $\Delta \log J_M = 0.128$ log units for $n = 42$ compared with 0.10 log units for **2**.

After the prodrug/IPM combinations were removed, the skins were kept in contact with receptor phase for 24 h to allow any Th in the skin to leach out. The amount of prodrug or drug found in the receptor phase after 24 h (C_s) has been used as an indicator of the extent of delivery of drug into the skin or dermal delivery (Sloan et al., 1993). The values of C_s in Table 4 show that only the 7-AC prodrug, **2**, is better (three times) than Th itself at delivering Th into the skin. The best 7-AOC-Th prodrug, **8**, is only about 50% as effective as Th. This can be attributed to the poor performances of the 7-AOC-Th prodrugs at increasing values for J_i. On the other hand, the only 7-AC-Th derivative that could be evaluated, **2**, gave the greatest value for C_s which was commensurate with the fact that it gave the greatest value for J_i of any prodrug of Th evaluated here or by Kerr et al. (1998). None of the normalized ratio of C_s/J_i (referred to as the D/T ratio; Beall et al., 1994) for any of the prodrugs were as large as that for Th itself (calculations not shown), so none of the prodrugs exhibited preferential dermal delivery of Th.

Flux values J_j from application of a standard solute/solvent (Th/propylene glycol (PG)) after the initial application of prodrug/IPM was removed (Sloan et al., 1986) were used to determine if differences in J_j were due to differences in damage caused by components of the initial appli-

cation. For the 7-AOC-Th series, the J_j values after the application of prodrug/IPM were not significantly different from each other or from J_j after the application of Th/IPM itself (Table 4). Thus, the differences in J_i are not due to differences in damage caused by the components of the initial application. On the other hand, the value for J_j after the application of **2** is significantly higher than J_j after Th/IPM. However, J_j for **2** is not significantly different from J_j for the members of the 7-AOCM-Th series after their application in IPM.

A plot of log permeability constants ($P_i = J_i/S_{IPM}$, Table 4) for the delivery of Th through hairless mouse skin against the calculated solubility parameters of the 7-AOC-Th prodrugs (δ_i, Table 2) gave a straight line (plot not shown) with a slope (0.93, $r = 0.98$) which is similar to that observed for AOC (Beall et al., 1994) and AC (Beall and Sloan, 1996) prodrugs of 5-FU (0.86, $r = 0.99$; 0.89, $r = 0.99$, respectively). Thus, as the members of a series of homologous prodrugs became more lipophilic (decreasing absolute δ_i value) they became less efficient at delivering the parent drug from a lipoidal vehicle (decreasing log P_i value) as previously observed.

4. Conclusion

The physicochemical properties of the 7-AC-Th and 7-AOC-Th prodrugs were as expected based on the previous results obtained for the similar 1-AC-5-FU and 1-AOC-5-FU series: they were much more soluble in a lipid (S_{IPM}) and at least one member in each series was also more soluble in pH 4.0 buffer (S_{AQ}) than Th. However, in spite of their generally much greater water solubility than the corresponding members of the 7-ACOM-Th series, the 7-AOC-Th series did not perform any better than the 7-ACOM-Th series at increasing the delivery of Th into or through hairless mouse skin. On the other hand, the one member of the 7-AC-Th series that was sufficiently stable to be evaluated, performed as expected based on its S_{AQ} and S_{IPM} values. It is not clear why the 7-AOC-Th series performed as badly as it did.

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