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7-Alkylcarbonyloxymethyl prodrugs of theophylline: topical delivery of theophylline

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Abstract

Five members of an homologous series of 7-*n*-alkylcarbonyloxymethyl (ACOM) prodrugs (C_1 to C_5, acetyl- to hexanoyloxymethyl) of a potential topical antiproliferative drug, theophylline (Th, **1**) have been synthesized, characterized and evaluated for their abilities to deliver Th into and through hairless mouse skin from suspensions in isopropyl myristate (IPM). The synthesis used a tertiary amine quaternary salt of the ACOM chlorides (**2**) as the alkylating agent to give about twice the percent yields as previously reported. Although all of the members of the 7-ACOM-Th prodrugs (C_1 to C_5, **3a** to **3e**) were more soluble in IPM than Th (10–200 times), the most water soluble members (C_1 and C_3, acetyl- and butyryloxymethyl) were only about 0.25 times as soluble as Th; C_2 (propionyloxymethyl) was only about 0.1 times as soluble. In addition to being almost as water soluble as C_1, C_3 was almost 10 times more lipid soluble than C_1 or C_2. Thus, the C_3 prodrug exhibited the best biphasic solubility properties in the series. In the diffusion cell experiments, the C_3 prodrug was the only member that was significantly (2 times) more effective than Th at delivering total Th species (J_i). In addition, all of the prodrugs of the series delivered significant amounts of intact prodrug through the skin (J_p): J_p/J_i for $C_1 = 47\%$, $C_2 = 29\%$, $C_3 = 32\%$, $C_4 = 7\%$ and $C_5 = 1\%$. None of the prodrugs delivered as much Th into the skin (C_s) as Th itself. Fluxes of Th from propylene glycol (J_j) subsequent to the initial application of prodrug or Th/IPM were all of a similar magnitude, so that the differences in J_i were not caused by differences in damage to the skins from the initial applications. 7-Hydroxymethyltheophylline (7-$HOCH_2$-Th, **4**) which was as lipid soluble as C_1 and C_2, but 10 times more water soluble than Th gave a J_i which was 2 times that of Th and a C_s value that was 2 times that of Th. 7-$HOCH_2$-Th was the best prodrug studied for increasing the delivery of Th.

Keywords: Theophylline; Alkylcarbonyloxymethyl prodrugs; Hydroxymethyl prodrug; Diffusion cell experiments; Lipid solubility; Water solubility

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1. Introduction

Alkylcarbonyloxymethyl (ACOM) type prodrugs are one of the most versatile approaches to transiently modifying the physicochemical properties of drugs containing a variety of functional groups. Drugs such as 5-fluorouracil (5-FU) containing acidic NH groups have been alkylated with ACOM halides, **2** (Mollgaard et al., 1982) to give highly lipophilic prodrugs that enhanced the topical delivery of the parent drug. Similarly, drugs such as benzylpenicillin containing an acidic CO_2H group (Jansen and Russell, 1965), or 6-mercaptopurine (6-MP) containing an acidic SH group (Sloan et al., 1983), have been alkylated to give prodrugs exhibiting enhanced oral or topical delivery, respectively. Even drugs such as cimetidine containing a basic NH group (Buur and Bundgaard, 1991) have been alkylated with ACOM halides to give prodrugs with improved lipid solubilities. On the other hand, drugs containing tertiary amine groups have also been alkylated to give 'soft' quaternary salts with improved water solubility (Davidson et al., 1994).

Although the 7-alkylcarbonyloxymethyltheophylline (7-ACOM-Th, **3**) series of prodrugs of theophylline (**1**) represents one of the first examples of the use of the ACOM promoiety to improve the topical delivery of a polar drug (Sloan and Bodor, 1982), there are a number of questions about the series that remain to be answered. First, only one intermediate length *n*-alkyl chain member (C_3) of the homologous series was evaluated in diffusion cell experiments, and its performance was not as good as the more polar 7-hydroxymethyltheophylline (7-$HOCH_2$-Th, **4**) —the intermediate in the hydrolysis of the 7-ACOM-Th (**3**) to Th. The 7-pivaloyloxymethyl prodrug was also evaluated but it is not a member of the homologous series because of the branched chain. Subsequently, results from the evaluation of 1-acyl prodrugs of 5-FU showed that the shorter length alkyl chain members of those series, the more water soluble members, were more effective at enhancing the topical delivery of 5-FU than more lipid soluble intermediate length alkyl chain members (Sloan et al., 1993; Beall et al., 1994; Beall and Sloan, 1996). Similarly, the shorter length alkyl chain, more water soluble, members of the ACOM prodrugs of 6-MP were the more effective members for delivering 6-MP through hairless mouse skin (Waranis and Sloan, 1987, 1988). And, more recently, the same trend has been observed for the 1-ACOM prodrugs of 5-FU (Taylor and Sloan, 1998). This suggests that the shorter length alkyl chain members of the 7-ACOM-Th prodrugs may be the more effective members of this series as well. Thus, the entire ACOM series needs to be evaluated in diffusion cell experiments. Second, lipid solubilities (in isopropyl myristate, S_{IPM}) and aqueous solubilities (in pH 4.0 acetate buffer, S_{H_2O}) were not determined for all of the shorter length alkyl chain members of the series. Thus, a more complete characterization of the physicochemical properties of all the members of the series needs to be done.

In this paper, an improved method of synthesizing the 7-ACOM-Th prodrugs is described. In addition, S_{H_2O}, S_{IPM} and partition coefficients between IPM and pH 4.0 acetate buffer (K) for the C_1 to C_5 (acetyl- to hexanoyloxymethyl) members of the series are reported. Finally, the abilities of the C_1 to C_5 7-ACOM-Th prodrugs to deliver Th and intact prodrug into and through hairless mouse skin are reported.

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. 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 37°C with a Fisher circulating water bath model 25. TLC analyses were run on Brinkman Polygram Sil G/UV 254 plates. Isopropyl myristate (IPM) was obtained from Givaudan (Clifton, NJ). Theophylline was purchased from Sigma Chemical Co., pivaloyloxymethyl chloride and all other reagent chemicals were from Aldrich Chemical Co. and all other solvents

were from Fisher. The female hairless mice (SKH-hr-1) were from Charles River. The alkylcarbonyloxymethyl chlorides (**2**) were synthesized according to a modification (Taylor, 1997) of the general procedure of Bigler et al. (1978).

2.1. General method for the synthesis of 7-alkylcarbonyloxymethyltheophyllines (3)

1-Methylpyrrolidine (3.5 g, 0.040 mol) was added to a well-stirred solution of alkylcarbonyloxymethyl chloride (0.012 mol) and 10 ml of dry acetonitrile in a round-bottom flask equipped with a reflux condenser and a $CaCl_2$ drying tube. The solution was stirred and heated at 70°C with an oil bath for 1 h. The solution was cooled to room temperature and allowed to react with theophylline (1.8 g, 0.01 mol) for 1 day with stirring. The solution was concentrated at reduced pressure and 40°C to a solid which was dissolved in 100 ml of CH_2Cl_2. The CH_2Cl_2 solution was extracted with 20 ml water, 20 ml of water containing 3 ml of concentrated HCl, and 20 ml of water, then dried over Na_2SO_4. The CH_2Cl_2 solution was concentrated at reduced pressure and 40°C to about 20 ml. Hot hexane (20–50 ml) was added and the suspension was allowed to sit at room temperature overnight. The precipitate was filtered to give the desired 7-alkylcarbonyloxymethyltheophylline prodrugs.

7-Acetyloxymethyltheophylline (**3a**,C_1) was synthesized from acetyloxymethyl chloride (89% pure) in 66% yield (recrystallized, 85% recovery): 1.67 g, mp 163.5–165°C (lit. mp (Sloan and Bodor, 1982) 163.5–166°C, 25% yield), R_f 0.27 (ethyl acetate), UV_{max} (CH_3CN) 276 nm ($\epsilon = 8.87 \times 10^3$ l/mol), UV_{max} (pH 4.0 buffer) 275 nm ($\epsilon = 9.28 \times 10^3$ l/mol).

7-Propionyloxymethyltheophylline (**3b**,C_2) was synthesized from propionyloxymethyl chloride (88% pure) in 95% yield: 2.54 g, mp 146–147°C (lit. mp (Sloan and Bodor, 1982) 142–144°C, 50% yield), R_f 0.34 (ethyl acetate), UV_{max} (CH_3CN) 276 nm ($\epsilon = 8.60 \times 10^3$ l/mol), UV_{max} (pH 4.0 buffer) 275 nm ($\epsilon = 9.12 \times 10^3$ l/mol).

7-Butyryloxymethyltheophylline (**3c**,C_3) was synthesized from butyryloxymethyl chloride (87% pure) in 87% yield: 2.44 g, mp 104–105°C (lit. mp (Sloan and Bodor, 1982) 102–105°C, 39% yield), R_f 0.38 (ethyl acetate), UV_{max} (CH_2CN) 276 nm ($\epsilon = 8.68 \times 10^3$ l/mol), UV_{max} (pH 4.0 buffer) 275 nm ($\epsilon = 9.13 \times 10^3$ l/mol).

7-Valeryloxymethyltheophylline (**3d**,C_4) was synthesized from valeryloxymethyl chloride (85% pure) in 81% yield: 2.37 g, mp 86–87°C, R_f 0.11 (diethyl ether), UV_{max} (CH_3CN) 276 nm ($\epsilon = 8.52 \times 10^3$ l/mol), UV_{max} (pH 4.0 buffer) 275 nm ($\epsilon = 9.04 \times 10^3$ l/mol).

Anal. Calcd for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04. Found: C, 53.07; H, 6.18; N, 19.08.

7-Hexanoyloxymethyltheophylline (**3e**,C_5) was synthesized from hexanoyloxymethyl chloride (93% pure) in 57% yield (recrystallized, 83% recovery): 1.95 g, mp 58–60°C (lit. mp (Sloan and Bodor, 1982) 65–68°C, 52% yield), R_f 0.19 (diethyl ether), UV_{max} (CH_3CN) 276 nm ($\epsilon = 8.21 \times 10^3$ l/mol).

7-Pivaloyloxymethyltheophylline (**3f**,C_{t4}) was synthesized from pivaloyloxymethyl chloride (99% pure) in 73% yield: 2.15 g, mp 109–110°C (lit. mp (Sloan and Bodor, 1982) 108–109.5°C, 38% yield), R_f 0.16 (diethyl ether), UV_{max} (CH_3CN) 275 nm ($\epsilon = 8.50 \times 10^3$ l/mol), UV_{max} (pH 4.0 buffer) 274 nm ($\epsilon = 8.98 \times 10^3$ l/mol).

7-Hydroxymethyltheophylline **(4)** was synthesized as previously described (Sloan and Bodor, 1982).

2.2. Analytical and physicochemical properties

Quantitation of all but one of the 7-ACOM-Th prodrugs was accomplished by HPLC using a Waters 501 HPLC pump at a flow rate of 1.0 ml/min, a Waters U6K 20 μl loop injector, Keystone Scientific Inc. 5 μm Nucleosil C8 150 × 4.6 mm column, a Waters 486 tunable detector ($\lambda_{anal} = 254$ nm) and a Hewlett-Packard 3396 Series III Integrator. The following mobile phase compositions (pH 5.0, 0.025 M acetate buffer: CH_3CN) gave the corresponding retention times for the prodrugs and internal standards: (80:20) $C_1 = 5.9$ min, $C_2 = 9.8$ min, phenacetin = 14.5 min; (70:30), $C_3 = 5.5$ min, $C_4 = 8.0$ min, ethyl paraben = 7.3 min. Quantitation of C_5 was accomplished by HPLC using a Beckman 110A pump at a flow rate of 1.0 ml/min, a Rheodyne

7125 20 μl loop injector, a Supelco 5 μm LC-8 150×4.6 mm column, an Upchurch Scientific guard column with Zorbax ODS packing, a Beckman model 153 fixed-wavelength detector ($\lambda_{anal} =$ 254 nm) and a Hewlett-Packard 3392A Integrator. Using a mobile phase of buffer: CH_3CN (56:44), C_5 had a retention time of 4.34 min.

Th was also quantitated by UV spectrophotometry from its absorbance at 270 nm ($\epsilon = 1.02 \times 10^4$ l/mol) in pH 7.1 phosphate buffer (0.11% formaldehyde) and at 276 nm ($\epsilon = 1.07 \times 10^4$ l/mol) in pH 9.0 phosphate buffer (0.11% formaldehyde).

The hydrolysis of $1.2–1.4 \times 10^{-4}$ M 7-ACOM-Th prodrugs in pH 9.02 buffer (0.01 M borate) and 46.7°C containing 1% CH_3CN was followed by UV spectrophotometry at 274–276 nm 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_o 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.

Lipid solubilities were determined in IPM according to a previously described procedure (Beall et al., 1994). Three suspensions of each prodrug 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 with dry acetonitrile and quantitated by UV spectrophotometry using molar absorptivities previously determined in triplicate at 275–276 nm.

Partition coefficients (K) were determined using the saturated IPM solutions ($n = 3$) from the lipid solubility determinations (Beall et al., 1994). The saturated IPM solutions were partitioned against 0.05 M acetate buffer (pH 4.0). The two phases were shaken vigorously for 10 s then allowed to separate for 60 s. The IPM layer was diluted with acetonitrile and the UV absorbance was determined. The K values were calculated as follows:

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

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 C_1 and C_2 the volume ratio was 2:1, for C_4 and C_{t4} it was 1:3 and for C_5 it was 1:5).

For aqueous solubilities, three suspensions of each prodrug in 0.05 M acetate buffer (pH 4.0) were vigorously stirred at 22 ± 1°C for 24 h. The suspensions were filtered through 0.45 μm nylon filters, then the saturated solutions were diluted with HPLC mobile phase and quantitated by HPLC as above.

2.3. Diffusion cell experiments

The diffusion cell experiments were run in essentially the same way as previously described (Beall et al., 1994). 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 7.1 phosphate buffer (0.05 M, $I = 0.11$ M, 32°C) containing 0.11% formaldehyde (2.7 ml of 36% aqueous formaldehyde/liter) to prevent microbial growth and to insure the integrity of the mouse skins during the course of the experiments: Th flux from PG applied 4 h after sacrifice was $6.1 \pm 0.6 \times 10^3$ μmol/cm^2 h; 24 h after sacrifice it was $8.3 \pm 1.9 \times 10^3$ μmol/cm^2 h; 48 h after sacrifice it was $9.4 \pm 1.2 \times 10^3$ μmol/cm^2 h; 120 h after sacrifice it was $10.0 \pm 1.2 \times 10^3$ μmol/cm^2 h (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.

Aliquots (0.5 ml, 0.25 M) of suspensions of each prodrug in IPM were applied to the donor side of each of three diffusion cells. The donor phase was removed and fresh donor phase (0.5 ml) was applied whenever the suspensions started to clear: for C_3 at 28 h; for C_4 at 44 h; for 7-$HOCH_2$-Th at 44 h. After the suspensions were applied, 6 ml samples of the receptor phases were

removed, generally at 8, 19, 22, 25, 28, 31, 45 and 48 h. The entire receptor phase was replaced with fresh receptor fluid each time a sample was removed. The amount of prodrug in each sample at 22, 25, 28 and 31 h was determined immediately by HPLC using the conditions described above. Aliquots (3 ml) of all samples were then adjusted to about pH 9 with aqueous NaOH and the prodrugs were allowed to hydrolyze until intact prodrug was not detected by HPLC. Theophylline (Th) in the samples was then quantitated from its UV absorption at 276 nm ($\epsilon = 1.07 \times 10^4$ l/mol, see above). At the same time, control experiments ($n = 3$), in which solutions of Th in receptor phase were also adjusted to about pH 9 with aqueous NaOH, were run to determine ϵ values for Th at that pH and if changes in the UV spectra of Th occurred with time which would indicate decomposition of Th: no changes occurred.

After the initial application period of 48 h, the donor phases were removed and the donor surfaces were quickly washed with 3×5 ml portions of methanol to remove any residual prodrug or Th. After the methanol wash, the skins were kept in contact with fresh receptor fluid for 23–24 h to allow any Th or Th prodrugs to leach out. Samples of the receptor phase were removed, the receptor phases were replaced with fresh receptor fluid, and 0.5 ml aliquots of a standard drug vehicle (theophylline/propylene glycol, Th/PG) were applied. Samples of the receptor phases (3 ml) from this second application were removed at 1, 2, 3 and 4 h, and the amounts of theophylline in the receptor phases were quantitated from its UV absorption ($\epsilon = 1.02 \times 10^4$ l/mol at pH 7.1). Each time a sample was removed the entire receptor phase was replaced with fresh receptor fluid. At the same time the samples from the 23–24 h leaching process were adjusted to about pH 9 with aqueous NaOH and any prodrug in the samples was allowed to hydrolyze to Th until no intact prodrug was detected by HPLC. Th in the sample was then quantitated from its UV absorption at 276 nm.

In all cases the rates of delivery of Th (J_i) and of intact Th prodrug (J_p) from the application of the prodrug, or of Th from the second application (J_j) through skin were determined by plotting the cumulative amount (μmol) of Th or intact Th prodrug measured in the receptor phase against time and dividing the steady-state portions of those plots by the surface area of the diffusion cells. Permeability coefficients (P_i) were determined by dividing the J_i values by the solubilities (S_{IPM}) of the prodrugs in IPM.

2.4. Solubility parameters

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

2.5. Statistical analyses

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

3. Results and discussions

3.1. Synthesis

The previous synthesis of most of the 7-ACOM-Th prodrugs studied here involved the acylation of 7-hydroxymethyltheophylline (7-$HOCH_2$-Th, **4**) with the corresponding acid chloride and base or with the carboxylic acid and dicyclohexylcarbodiimide (Sloan and Bodor, 1982). That synthesis gave considerable flexibility in the acyl portion of the promoiety that could be used, but was limited to the use of a simple oxamethylene spacer between the acyl portion of the promoiety and the 7-nitrogen of Th. However, the alternate approach, which had the potential for greater flexibility in the spacer, using the corresponding alkylcarbonyloxymethyl (ACOM) halide as an alkylating agent, and K_2CO_3 as the base, gave an even lower yield (26%) than the acylation reaction. Recently, improved yields and regiospecificity in the alkylation of 5-FU have been achieved using tertiary amine quaternary salts of ACOM halides in the presence of excess tertiary amine (Taylor and Sloan, 1998). When those conditions were applied to the synthesis of 7-ACOM-Th prodrugs, greatly improved yields

Table 1
Melting points (mp), lipid solubilities (S_{IPM}), pH 4.0 buffer solubilities (S_{H_2O}) and partition coefficients between IPM and pH 4.0 buffer (K) for 1-alkylcarbonyloxymethyl prodrugs of theophylline (7-ACOM-Th)

Alkyl =	mp (°C)	S_{IPM}(± S.D.) (μmol/ml)	S_{H_2O}(± S.D.) (μmol/ml)	K(± S.D.)
Th, **1**	270–274	0.34[a]	46.0[b]	—
CH_3, **3a**	163.5–165	2.75(0.099)	12.4(0.16)	0.141(0.006)[c]
C_2H_5, **3b**	146–147	2.93(0.11)	5.0(0.15)	0.634(0.05)[c]
C_3H_7, **3c**	104–105	25.4(0.32)	9.96(0.21)	2.41(0.27)[d]
C_4H_9, **3d**	86–87	44.0(1.5)	4.29(0.10)	8.42(0.18)[e]
C_5H_{11}, **3e**	58–60	77.8(0.45)	1.72(0.032)	28.0(2.5)[f]
$(CH_3)_3C$,**3f**	109–110	37.1(1.2)	—[g]	5.66(0.37)[e]
$ThCH_2OH$, **4**	260–262	3.52[b]	392(3.0)	—[g]

[a] From Sloan et al. (1986).
[b] From Sloan and Bodor (1982).
[c] IPM:buffer = 2:1.
[d] IPM:buffer = 1:1.
[e] IPM:buffer = 1:3.
[f] IPM:buffer = 1:5.
[g] Not determined.

were realized using a simple precipitation process to affect isolation. In only two examples (C_1 and C_5) was it necessary to recrystallize the initial product to give the desired prodrugs in acceptable purity, i.e. one component by TLC and HPLC and constant melting point.

The spectral properties and melting points of the 7-ACOM-Th prodrugs that were synthesized were consistent with those previously reported (Sloan and Bodor, 1982), and the new 7-ACOM-Th prodrug, C_4, was consistent with the other members of the series. The UV spectra all exhibited a maximum at about 276 nm in CH_3CN, which shifted to slightly higher molar absorptivities in pH 4.0 buffer. The ^1H NMR spectra ($CDCl_3$) exhibited C^8–**H** absorptions at about δ 7.9 and N–C**H**$_2$–O absorptions at about δ 6.25 which were consistent with the assigned structure.

3.2. Physicochemical properties

The solubilities in IPM (S_{IPM}), in pH 4.0 acetate buffer (S_{H_2O}) and the partition coefficients of the 7-ACOM-Th prodrugs between IPM and pH 4.0 buffer (K) are listed in Table 1. Although the first member of the series (C_1, acetyloxymethyl) was about 8 times more soluble in IPM than Th, the increase in IPM solubility for the first member of the series was not as great as observed with the first members of the series of ACOM prodrugs of 5-FU (Taylor and Sloan, 1998; 65 times) and of the bis- and mono-ACOM prodrugs of 6-MP (Waranis and Sloan, 1987, 1988; 250 and 50 times, respectively) or with the first few members of the series of 1-alkylcarbonyl (Beall and Sloan, 1996; 400 times) and 1-alkyloxycarbonyl (Beall et al., 1994; 45 times) prodrugs of 5-FU. In addition, the second member of the 7-ACOM-Th series is not significantly more soluble in IPM than the first member. In fact, even 7-$HOCH_2$-Th, which is a much more polar molecule based on its melting point, is marginally more soluble in IPM than the C_1 and C_2 prodrugs. Only the third member of the series, C_3 (butyryloxymethyl), exhibits increased solubility in IPM compared to Th (80 times) of the same order of magnitude as that observed for promoieties that are significantly effective (> 10-fold) at enhancing the topical delivery of the parent drug.

The water solubilities of the first two members of the 7-ACOM-Th series were also lower than expected based on the solubilities realized from the combination of this type of promoiety with other parent drugs, i.e. 1-ACOM of 5-FU (Taylor and Sloan, 1998; 2 times) and bis- and mono-ACOM of 6-MP (Waranis and Sloan, 1987, 1988;

Table 2
Values for log partition coefficients between IPM and pH 4.0 buffer (log K), ratios of S_{IPM}/S_{H_2O} (log SR), rate constants for hydrolyses at pH 9.02 and 46.7°C, and Charton's steric parameter (ν) for 7-ACOM-Th

Alkyl =	log K	π[a]	log SR[b]	π[a]	k_{obs} ($\pm$S.D.) ($min^{-1} \times 10^2$)	ν[c]
Th			−2.13			
CH_3	−0.85		−0.65		2.75(0.03)	0.52
C_2H_5	−0.20	0.65	−0.23	0.42	2.60(0.08)	0.56
C_3H_7	0.38	0.58	0.40	0.63	1.69(0.02)	0.68
C_4H_9	0.92	0.54	1.01	0.61	1.55(0.01)	0.68
C_5H_{11}	1.45	0.53	1.65	0.64	1.60(0.007)	0.68
$(CH_3)_3C$	5.66				0.296(0.013)	1.24
$ThCH_2OH$	—[d]		−2.04			

[a] $\pi = (\log K_{n+m} - \log K_n)/m$, 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 and where log SR can be substituted for log K.
[b] Solubility ratio, S_{IPM}/S_{H_2O}.
[c] Charton's steric parameters from Charton (1975).
[d] Not determined.

2.5 and 6 times, respectively). Generally, the first several members of the series are more water soluble than the parent drug, then the water solubility of the latter members decreases rapidly. In this series, C_1 exhibits only about 0.25 times and C_2 only about 0.1 times the water solubility of Th. On the other hand, C_3 is almost as water soluble and is almost 10 times as lipid soluble as C_1 (see above). It appears that the C_1 and C_2 prodrugs are less soluble in lipids and in water than expected, and C_3 exhibits the best biphasic solubility in the series. This result is achieved in spite of the fact that the water solubility of C_3 is limited by its two extra methylene groups compared to C_1.

The greatly enhanced water solubility of 7-$HOCH_2$-Th **(4)** should also be noted. Because of the predicted rapid hydrolysis (Bundgaard, 1985; $t_{1/2} = 0.019$ min at pH 7.4 and 37°C) of the hydroxymethyl derivative of molecules such as theophylline, which are relatively acidic (acidic $pK_a = 8.6$, Foye et al., 1995), the usual method of determining the water solubility by stirring an excess of **4** in water could not be used. Instead, excess **4** was stirred in 36% aqueous formaldehyde for 48 h (Bansal et al., 1981). Then the suspension was processed in the same way as the suspensions of the 7-ACOM-Th. The residue from the filtration of suspensions of **4** was **4** based on its ^1H NMR spectrum (C^8–**H** at δ 7.55 and N–CH_2O at δ 5.57 in $CDCl_3$). The 8.5-fold increase in water solubility achieved with **4** was consistent with the increase expected from literature precedents (Bundgaard, 1985).

The S_{H_2O} and S_{IPM} values determined here are less than those previously reported (Sloan and Bodor, 1982), except for C_{t4}. The higher solubilities previously reported were probably the result of sonication of samples to achieve dissolution. Previously (Sloan et al., 1988), solubilities obtained after sonication have been found to be higher than solubilities obtained by stirring at room temperature. Stirring at room temperature does not create the opportunity for supersaturated solutions to form from hot spots which are caused by sonication.

In spite of the fact that S_{H_2O} and S_{IPM} values for C_1 and C_2 were lower than expected, the trend in the K values for the whole series was well-behaved. Thus, the mean methylene π value (Table 2) was consistent ($\pi = 0.58 \pm 0.05$) with those from other series of homologous prodrugs (Beall and Sloan, 1996; Taylor and Sloan, 1998). In contradistinction to the results obtained from 1-ACOM-5-FU prodrugs, the mean methylene π value derived from the K values gave less variation than the π value derived from the SR values (Table 2, $\pi = 0.58 \pm 0.10$). In particular, the SR value for C_1 was almost twice that of the K value which resulted in a much smaller π value for the difference between log SR values for C_1 and C_2

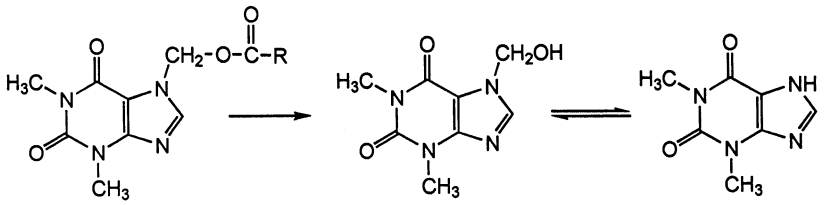

Fig. 1. The two-step hydrolysis of 7-ACOM-Th prodrugs to Th.

than for the differences between the other contiguous members of the series.

The hydrolyses of all the 7-ACOM-Th prodrugs were monitored at pH 9.02 and 46.7°C to facilitate the acquisition of data (Table 2). The hydrolysis of the pivaloyloxymethyl member of the series (C_{t4}, **4f**) had been studied previously, but only at pH 7.4 and 37°C (Johansen et al., 1983), so in the absence of a complete pH-rate profile, it is not possible to determine if the present data are consistent with their value of $k_{obs} = 5 \times 10^4$ min^{-1}. However, a plot of $\log k_{obs}$ for the hydrolyses of the 7-ACOM-Th prodrugs versus Charton's steric parameters (Charton, 1975) gives a good fit to: $\log k_{obs} = -1.35v - 0.86$ ($r = 0.998$). A similar plot of $\log k_{obs}$ for the hydrolyses of the 1-ACOM-5-FU prodrugs gave: $\log k_{obs} = -1.35v - 1.53$ ($r = 0.994$) (Taylor and Sloan, 1998). Thus, the identical relationship between steric effects and rates in the two series suggests that the mechanism (Fig. 1) for the hydrolyses is the same in the two series; only the intercept is different, i.e. the Th prodrugs hydrolyze faster than the 5-FU prodrugs. In addition, since the rates of hydrolyses are predicted by steric effects for an addition-elimination mechanism of hydrolysis, the mechanism for the hydrolyses of the ACOM prodrugs also appears to be an addition–elimination mechanism which is typical for the hydrolyses of esters. The 7-$HOCH_2$-Th intermediate that forms subsequently undergoes rapid loss of formaldehyde in a step that is dependent on the pK_a of the parent drug (see above). The fact that the 7-ACOM-Th prodrugs hydrolyze faster than the 1-ACOM-5-FU prodrugs is probably because the pK_a of N^3–**H** in the 5-FU prodrugs is 7.3 (Buur et al., 1985) so that, at pH values above 8.3, greater than 90% of the prodrug is ionized. This repulses the attack of hydroxide anion in the first step for ester hydrolyses. On the other hand, there are no acidic groups that would be ionized at pH 7–10 in the 7-ACOM-Th prodrugs so their hydrolyses would not be retarded by ionization of the substrate.

3.3. Diffusion cell experiment

Values for the delivery of total Th species (J_i) or intact prodrug (J_p) and total Th species retained in the skin (C_s) from the application of 7-ACOM-Th prodrugs are given in Table 3. Plots of cumulative total Th species and intact prodrug versus time for steady-state are given in Figs. 2 and 3, respectively. The values of J_i for the members of the 7-ACOM type prodrug do not follow the trends previously observed for combinations of this type of promoiety with 6-MP (Waranis and Sloan, 1987, 1988) or 5-FU (Taylor and Sloan, 1998). This is because the solubilities for the 7-ACOM-Th prodrugs did not follow the previous trends either. In both previously studied series several of the initial members of the series were more water soluble than the parent drug in addition to being more lipid soluble. The result was that several members in each series were more than a factor of 10 times more effective than the parent drug at delivering the parent drug through hairless mouse skin. Here, the most water-soluble member, C_1, is only 0.25 times as water soluble as Th and only about 9 times as lipid soluble. The most effective member of the series, albeit giving a modest 2-fold greater J_i than Th, is the C_3 prodrug which is almost as water soluble as C_1 and about 80 times more lipid soluble than Th.

A similar relatively low increase in lipid solubility and lower water solubility than the parent

Table 3
Flux and log permeability coefficient of total theophylline species (J_i and log P_i, respectively) and flux of intact 7-ACOM prodrug of theophylline (J_p) during steady state from application of 7-ACOM-Th/IPM, concentration of total Th species in skin (C_s) after application of 7-ACOM-Th/IPM, flux of Th from application of Th/PG (J_j) after 7-ACOM-Th/IPM removed and solubility parameter values for Th and 7-ACOM-Th (δ_i)

Alkyl =	J_i (± S.D.)[a] (μmol/cm^2 h)	log P_i (cm/h)	δ_i (cal/cm^3)$^{1/2}$	J_p (± S.D.)[b] (μmol/cm^2 h)	C_s (± S.D.)[c] (μmol)	J_j (± S.D.)[d] (μmol/cm^2 h)
Th	0.48 (0.16)	0.15	14.05	—	5.9 (0.35)	0.81 (0.094)
CH_3	0.58 (0.049)	−0.68	12.33	0.27 (0.034)	2.9 (0.90)	1.69 (0.19)
C_2H_5	0.31 (0.11)	−0.97	12.06	0.092 (0.032)	2.2 (1.4)	1.32 (0.21)
C_3H_7	1.06 (0.10)	−1.38	11.82	0.34 (0.076)	4.2 (0.61)	1.43 (0.096)
C_4H_9	0.59 (0.046)	−1.87	11.61	0.041 (0.0054)	3.8 (0.89)	1.22 (0.18)
C_5H_{11}	0.47 (0.042)	−2.22	11.43	0.0061 (0.0015)	1.6 (0.34)	1.20 (0.096)
$ThCH_2OH$	1.10 (0.18)	—	—	—	10.5 (2.0)	1.20 (0.17)
Control						0.74 (0.038)[e]

[a] J_i, flux of total Th species.
[b] J_p, flux of intact 7-ACOM-Th.
[c] C_s, concentration of total Th species in receptor phase after keeping the skin in contact for 24 h after donor phase from first application was removed to allow Th and intact 7-ACOM-Th to leach out.
[d] J_j, flux of Th from propylene glycol (PG) in a second application.
[e] Pretreatment with IPM (Sherertz et al., 1987).

drug has previously been observed for the 1-alkylaminocarbonyl prodrugs of 5-FU (Sloan et al., 1993). In that series only a 6-fold increase in lipid solubility and a 30-fold decrease in the water solubility was observed for the first member of the series, and no increase in delivery of 5-FU was achieved. In that series the greatest increase in delivery was achieved with the C_3 member, but it was only 3-fold. It also exhibited the best biphasic solubility. The C_3 member was the most water-soluble member of the series (only a 10-fold decrease in water solubility) and it was 240-fold more lipid soluble than 5-FU. The generally poor performance of the 1-alkylaminocarbonyl series was attributed to the fact that the promoiety masked one polar N–H group but introduced another in its place, so there was no significant decrease in crystal lattice energy. In the 7-ACOM-

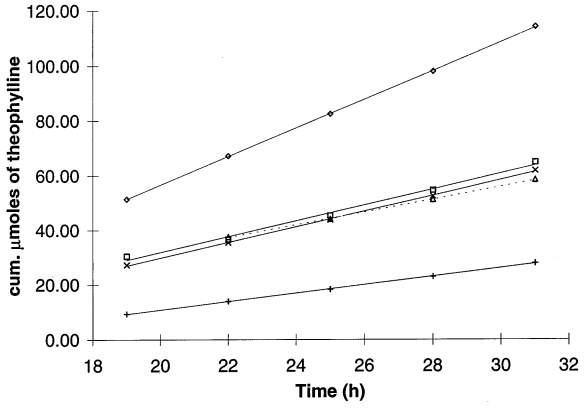

Fig. 2. Plots of cumulative concentrations of total Th in the receptor phases of diffusion cells after the application of the C_1 (×), C_2 (+), C_3 (◇), C_4 (□) or C_5 prodrugs (△).

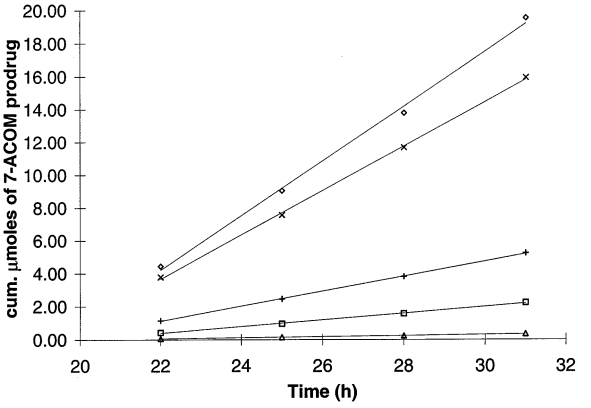

Fig. 3. Plots of cumulative concentrations of intact prodrug in the receptor phases of diffusion cells after the application of C_1 (×), C_2 (+), C_3 (◇), C_4 (□) or C_5 prodrugs (△).

Th series the poor performance may be due to the possibility that the promoiety does not sufficiently interfere with the stacking of theophylline (Sutor, 1958) in the crystal lattice.

The importance of water solubility in the optimization of the performance of prodrugs can be seen in the J_i value for 7-$HOCH_2$-Th. It was previously evaluated at the same time as the 7-ACOM-Th prodrugs (Sloan and Bodor, 1982) for its ability to deliver Th through hairless mouse skin. 7-$HOCH_2$-Th was the most effective prodrug for delivering Th in that study and in this study as well, yet its lipid solubility (S_{IPM}) is only about 10 times that of Th—about the same as the C_1 and C_2 prodrugs. The reason that 7-$HOCH_2$-Th performs so well is that it is almost 10 times as water soluble as Th. 7-$HOCH_2$-Th exhibits very good biphasic solubility, but with the greater emphasis on aqueous solubility instead of lipid solubility as in the example of the C_3 prodrug.

The J_i values previously reported (Sloan and Bodor, 1982) were from diffusion cell experiments that were run for only 12 h: steady-state had probably not been achieved. These experiments were run for 48 h and gave J_i values larger than those previously reported. The J_i values were all obtained from plots of cumulative μmol of Th versus time after 12 h and gave lag times of 9–14 h. However, the J_i values calculated from the first portion of these experiments gave J_i values that are consistent with those previously reported. The calculated J_i values from the first portion of the plots of cumulative Th versus time were: Th, 0.146 (0.097) μmol/cm^2 h; 7-$HOCH_2$-Th, 0.54 (0.62) μmol/cm^2 h; C_3, 0.44 (0.40) μmol/cm^2 h; with the previous values given in parentheses.

All of the 7-ACOM-Th prodrugs delivered intact prodrug through the skin. The percentage of intact prodrug (J_p/J_i) decreased as the length of the alkyl chain increased from 46% for C_1 to 1% for C_5, which is similar to the trend observed for the 1-ACOM-5-FU prodrugs (Taylor and Sloan, 1998). In the previous report on 7-ACOM-Th prodrugs (Sloan and Bodor, 1982), none of the C_3 prodrug permeated the hairless mouse skin intact, but here 32% has. The difference in delivery of intact prodrugs is due to the fact that in these experiments the skins were kept in contact with the receptor phases for 48 h before the suspensions were applied. This was done to keep the conditions for these experiments the same as those used in the study of other prodrugs (Beall et al., 1994; Beall and Sloan, 1996). On the other hand, the suspensions were applied immediately after the skins were placed in contact with the receptor phase in the previous study of 7-ACOM-Th prodrugs. During that 48 h difference, esterases responsible for hydrolyzing the prodrugs can leach out of the skin (Mollgaard et al., 1982; Waranis and Sloan, 1988) and decrease the ability of the skin to hydrolyze an ester substrate.

After the prodrug/IPM was removed, the skins were kept in contact with receptor phase for 24 h to allow any intact prodrug or Th in the skin to leach out. The amount of total Th species (C_s) found in the receptor phase after that 24 h has been used as an indication of the extent of delivery of drug/prodrug into the skin (Sloan et al., 1993). The values of C_s in Table 3 show that only 7-$HOCH_2$-Th is better (2 times) than Th itself at delivering Th into the skin. The best 7-ACOM-Th prodrug was only about 40% as effective as 7-$HOCH_2$-Th and 70% as effective as Th. The reason for the poor performance of the 7-ACOM-Th prodrugs at increasing C_s values is their poor performance at increasing J_i values.

Flux values from application of a standard solute/solvent after the initial application of the prodrug/IPM was removed has been used (Sloan et al., 1986) to determine if differences in J_i are due to differences in damage caused the initial prodrug/IPM combinations. In this case, the fluxes of Th from the application of Th/PG (J_j) showed that there were no significant differences between individual 7-ACOM-Th prodrugs except for the comparison between the C_1 and C_5 prodrugs. Thus, the differences in J_i values were not caused by differences in damage caused by the application of the individual prodrugs/IPM. On the other hand, the J_j value obtained after treatment with Th/IPM was significantly less than the other J_i values, although not significantly different

from a previously reported control value obtained under the same conditions (Sherertz et al., 1987; 0.74 ± 0.038 μmol/cm^2 h).

A plot of log permeability constants ($P_i = J_i/S_{IPM}$, Table 3) for the delivery of total Th species through hairless mouse skin against the calculated solubility parameters for the corresponding 7-ACOM-Th prodrug (δ_i, Table 3) gave a straight line with a slope which is similar to that observed for the ACOM prodrugs of 6-MP (Waranis and Sloan, 1987) and of 5-FU (Taylor and Sloan, 1998). The slopes and r values for all the prodrugs in the three series are: Th, 1.75, $r = 0.99$; 6-MP, 1.31, $r = 0.999$; 5-FU, 1.17, $r = 0.99$. It is apparent in all three series that as the members of the series become more lipophilic (decreasing absolute δ_i value), they become less efficient (decreasing $\log P_i$) at delivering the parent drug from a lipoidal vehicle, IPM. For the Th series, the decrease in efficiency is faster.

4. Conclusions

Although all of the 7-ACOM-Th prodrugs were more lipid soluble than Th, the first two members, which are usually more water soluble than the parent compound, were < 0.25 times as water soluble. The first several members of a series are also usually the members that give the greatest increase in delivery of the parent drug. The poor solubilities of the first several members of the 7-ACOM-Th series precludes significant increases in delivery of Th. The poor lipid and water solubilities may be attributed to the possibility that the short length alkyl chain 7-ACOM promoieties do not sufficiently interfere with the stacking of theophylline in the crystal lattice to give better solubilities (Sutor, 1958).

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