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In vitro evaluation of alkylcarbonyloxymethyl (ACOM) ethers as novel prodrugs of phenols for topical delivery: ACOM prodrugs of acetaminophen

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Abstract

The fluxes (J_{IPM}) of a series of alkylcarbonyloxymethyl (ACOM) ethers of acetaminophen (APAP) were measured through hairless mouse skin from suspensions of each prodrug in isopropyl myristate (IPM). Solubilities in IPM, estimated solubilities in pH 4.0 buffer ($S_{4.0}$) and flux data for the 4-ACOM-APAP prodrugs were incorporated into the Roberts–Sloan (RS) database to give new estimates for the independent variables of the RS equation: $\log J_{IPM} = x + y \log S_{IPM} + (1 - y) \log S_{4.0} - z M_W$. All but one of the 4-ACOM-APAP derivatives hydrolyzed completely on permeation through mouse skin. Three out of the five prodrugs permeated the skin better than APAP, with a maximum fourfold increase in flux. Biphasic solubility – not solubility in a single solvent – was shown to have the greatest impact on flux. A fit of the new $n = 66$ database to the RS equation gave the following values for x, y, z, and r^2: $x = -0.545$, $y = 0.511$, $z = 0.00253$, $r^2 = 0.915$. These results demonstrate that the topical delivery of a model phenol, acetaminophen, can be improved by transiently masking the phenolic hydroxyl group as an ACOM ether.

Keywords: Prodrugs; Diffusion cell experiments; Topical delivery; Roberts–Sloan equation; Transformed Potts–Guy equation; Water solubility

1. Introduction

Drugs that contain phenolic functional groups are attractive candidates for topical delivery via prodrug derivatization for several reasons. From a pharmacokinetic point of view, orally administered phenols typically exhibit poor bioavailability due to first-pass metabolism in the gastrointestinal tract and liver—a problem which is avoided in transdermal delivery. From a synthetic organic point of view, the phenolic OH is a convenient handle upon which to attach a wide range of promoieties. Most of the previous work on the topical delivery of phenols via a prodrug approach has focused on the corresponding ester (alkylcarbonyl, AC), carbonate (alkyloxycarbonyl, AOC) and carbamate derivatives (alkylaminocarbonyl, AAC) (Drustrup et al., 1991; Hansen et al., 1992; Sloan, 1992; Stinchcomb et al., 1996, 2002; Sung et al., 2000; Pillai et al., 2004; Wasdo and Sloan, 2004; Valiveti et al., 2005). One of the most studied classes of drug in that respect is the narcotic analgesics (Drustrup et al., 1991; Hansen et al., 1992; Stinchcomb et al., 1996, 2002; Sung et al., 2000; Pillai et al., 2004; Valiveti et al., 2005). Surprisingly, there are currently no examples of the topical delivery of phenols by alkylcarbonyloxymethyl (ACOM) derivatives. This is in spite of their well-documented effectiveness at improving the oral bioavailability (Beaumont et al., 2003) of phosphates and carboxylic acids, and the topical delivery (Sloan, 1992) of amides, imides, thioamide, and carboxylic acids.

Although there does not appear to be great differences in permeation enhancement when an ACOM promoiety is used in place of an AC in the same parent drug (1-AC (Beall and Sloan, 1996) versus 1-ACOM-5-fluorouracil (Taylor and Sloan, 1998) and 3-AC (Beall and Sloan, 2001) versus 3-ACOM-5-fluorouracil (Roberts and Sloan, 2003)), the ACOM promoiety offers certain advantages not provided by the corresponding AC or AOC prodrugs. Since the carbonyl moiety of the ACOM prodrug is separated from the parent compound by a methylene spacer, the physicochemical properties of ACOM derivatives are governed less by the parent drug and more by the promoiety. Another consequence of the methylene spacer is that ACOM

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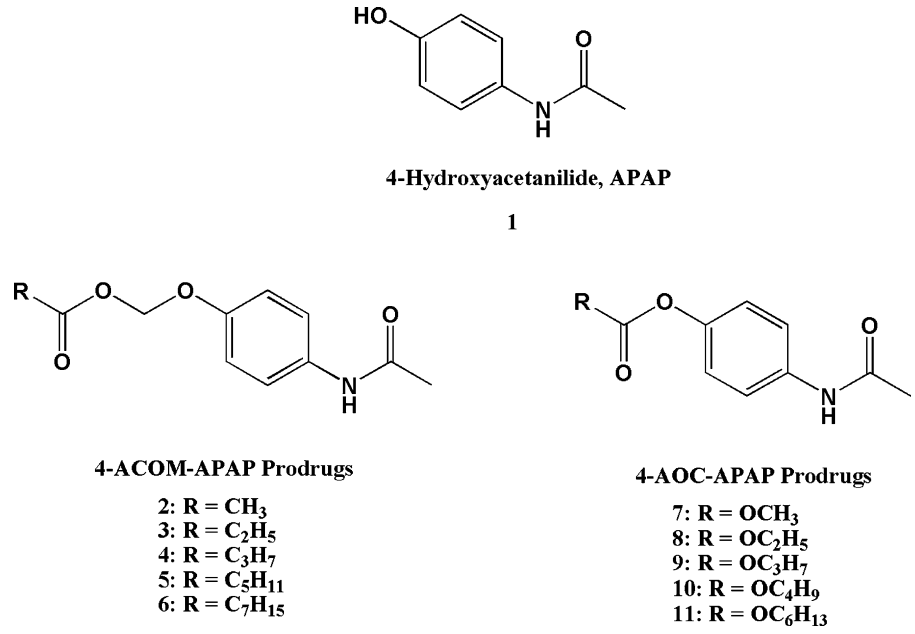

Fig. 1. Structures of APAP and its prodrugs.

derivatives are more hydrolytically stable than AC derivatives since the leaving group during hydrolysis is an aryl hemiacetal (approximate pK_a 11) (McClelland et al., 1991) rather than a phenol (approximate pK_a 8–10) (Jencks and Regenstein, 1976). The result is that soft alkyl prodrugs such as ACOMs are more easily customized to meet the particular objectives (drug solubility, stability, etc.) of the investigator (Sloan, 1992; Sloan and Wasdo, 2003, 2006).

As a first step in determining whether the ACOM promoiety is capable of improving the topical delivery of phenols, we have evaluated a series of simple ACOM ethers (Fig. 1) of 4-hydroxyacetanilide (acetaminophen, APAP) in diffusion cell experiments. In addition, the results have been compared to a previously reported series of AOC prodrugs of APAP (Fig. 1) (Wasdo and Sloan, 2004). Finally, the Roberts–Sloan equation (Roberts and Sloan, 1999) – a flux model based mainly on a database of heterocyclic prodrugs – has been evaluated for its ability to predict the flux of these nonheterocyclic ACOM prodrugs.

2. Materials and methods

2.1. Materials

Melting points were determined on a Meltemp capillary melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were obtained on a Shimadzu UV-265 or UV-2501 PC spectrophotometer. The vertical Franz diffusion cells (surface area 4.9 cm^2, 20 mL receptor phase volume, 15 mL donor phase volume) were purchased from Crown Glass (Somerville, NJ, USA). A Fisher (Pittsburgh, PA, USA) circulating water bath was used to maintain a constant temperature of 32 °C in the receptor phase. Isopropyl myristate (IPM) was purchased from Givaudan (Clifton, NJ, USA). Theophylline (Th) was purchased from Sigma Chemical Co. (St. Louis, MO, USA); all other chemicals were purchased from Fisher. The female hairless mice (SKH-hr-1) were obtained from Charles River (Boston, MA, USA). All procedures involving the care and experimental treatment of animals were in agreement with the NIH "Principles of Laboratory Animal Care." The synthesis and characterization of the 4-ACOM-APAP prodrugs is described elsewhere (Thomas, 2006).

2.2. Physicochemical properties and analysis

For each prodrug, the solubility in isopropyl myristate (IPM) was determined in triplicate as previously described (Beall et al., 1993) by crushing a sample of the prodrug into a fine powder. Excess powder was added to a test tube containing 3 mL IPM. The test tube was then insulated and the suspension was allowed to stir at room temperature (23 ± 1 °C) for 24 h on a magnetic stir plate. The suspension was filtered through a 0.25 μm nylon syringe filter. A sample of the filtrate was diluted with acetonitrile and quantitated by UV spectrophotometry using the previously determined molar absorptivities (Thomas, 2006) of each prodrug at 240 nm (ε_{240}) in acetonitrile (Table 1).

Solubilities in water were also determined in triplicate using an identical protocol to the one described above, except that the suspensions were only stirred for 1 h before filtering. This was done in order to make direct comparisons between the present investigation and previous studies (Beall et al., 1994; Wasdo and Sloan, 2004). In each case, a sample of the filtrate was diluted with acetonitrile and quantitated by UV spectrophotometry using ε_{240} in acetonitrile (Table 1).

Partition coefficients were also determined in triplicate for each prodrug by using the saturated IPM solutions obtained from the solubility determinations. Since solubility in water, and particularly in pH 4.0 buffer ($S_{4.0}$), is a parameter in the

Table 1
Molar absorptivities (ε) of APAP **1** and prodrugs **2–6**

Compound	ε_{240} in ACN[a,b]	ε_{240} in buffer[a,c]	ε_{280} in buffer[a,d]
1, APAP	1.36[e]	1.01 ± 0.053[f]	0.174 ± 0.020[f]
2, C1[g]	1.48 ± 0.011		
3, C2[g]	1.64 ± 0.067		
4, C3[g]	1.56 ± 0.057	1.20 ± 0.025[h]	0.119 ± 0.0025[h]
5, C5[g]	1.58 ±0.050		
6, C7[g]	1.46 ±0.044		

[a] Units of 1×10^4 L mol^{-1}.
[b] Molar absorptivities at 240 nm acetonitrile (±S.D., $n = 3$).
[c] Molar absorptivities at 240 nm in pH 7.1 phosphate buffer with 0.11% formaldehyde.
[d] Molar absorptivities at 280 nm in pH 7.1 phosphate buffer with 0.11% formaldehyde.
[e] Taken from Wasdo and Sloan (2004).
[f] $n = 5$ (±S.D.).
[g] C1, C2, . . ., refer to the length of the alkyl chain.
[h] $n = 6$ (±S.D.)

Roberts–Sloan (RS) database (Roberts and Sloan, 1999; Sloan and Wasdo, 2003), acetate buffer (0.01 M, pH 4.0) was used as the aqueous phase in the partition coefficient experiments. In this way, $S_{4.0}$ could be estimated from $(S_{IPM})/(K_{IPM:4.0})$ as described previously (Beall et al., 1993) and the values included in the database (pH 4.0 buffer was previously used to decrease ionization for other members of the RS database such as 5-fluorouracil (5-FU) and 6-mercaptopurine (6-MP) which still contain ionizable N–H groups after derivatization). Thus, an aliquot of the saturated IPM solution was partitioned against pH 4.0 buffer using the following volume ratios ($V_{4.0}/V_{IPM}$) for compounds **2**, **3**, **4**, and **5**: 0.5, 2, 10, and 20, respectively. The two phases were vigorously shaken for 10 s (Beall et al., 1993), then allowed to separate via centrifugation. An aliquot of the IPM layer was removed, diluted with acetonitrile, and analyzed by UV spectrophotometry as described above. Thus:

$$K_{IPM:4.0} = \left[\frac{A_a}{(A_b - A_a)}\right]\frac{V_{4.0}}{V_{IPM}} \quad (1)$$

where A_b and A_a are the respective absorbances before and after partitioning, and $V_{4.0}$ and V_{IPM} are the respective volumes of buffer and IPM in each phase. Due to the high solubility ratio exhibited by compound **6**, it was not possible to accurately determine its partition coefficient using this procedure. Therefore, in this case $K_{IPM:4.0}$ was estimated from the average methylene π_K obtained for compounds **2–5** according to the following relationship:

$$\log K_{n+m} = (\pi_K)(m) + \log K_n \quad (2)$$

where π_K is determined in Table 3, 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 with which it is compared.

UV spectrophotometry was also used to determine the amount of APAP (**1**) and prodrug present in the receptor phase of the diffusion cell. Since all the prodrugs in this study were part of a homologous series, it was assumed that satisfactory results would obtain for the entire series from the use of the molar absorptivity of one homolog. Thus, the molar absorptivities of compounds **4** and APAP (**1**) were determined in pH 7.1 phosphate buffer (0.05 M, $I = 0.11$ M) containing 0.11% formaldehyde ($n = 5$ and 6) by first dissolving a known amount of either compound in acetonitrile. An aliquot (0.500 mL) of the acetonitrile solution was removed, diluted with buffer, and analyzed by UV spectrophotometry to give the molar absorptivities shown in Table 1. Because there is considerable overlap between the UV spectra of APAP, **1**, and its ACOM prodrugs, **2–6**, the relative concentrations of each were determined using a previously described method (Wasdo and Sloan, 2004). The differences in absorption were found to be greatest at 240 and at 280 nm. Therefore, considering the additive nature of absorption, the absorbance at each wavelength (assuming constant cell length) is

$$A_{240} = \varepsilon_{P240}C_P + \varepsilon_{A240}C_A \quad (3)$$

$$A_{280} = \varepsilon_{P280}C_P + \varepsilon_{A280}C_A \quad (4)$$

where A is the absorbance at the respective wavelengths, ε the molar absorptivity of either the prodrug (P) or APAP (A) at the respective wavelengths, and C is the concentration of the respective compounds in the mixture. Solving the two simultaneous equations gives the following solution for the prodrug concentration C_P:

$$C_P = \frac{\varepsilon_{A280}A_{240} - \varepsilon_{A240}A_{280}}{\varepsilon_{A280}\varepsilon_{P240} - \varepsilon_{A240}\varepsilon_{P280}} \quad (5)$$

Once C_P is known, it may be inserted into Eq. (3) to give the following solution for the concentration of APAP (C_A):

$$C_A = \frac{A_{240} - \varepsilon_{P240}C_P}{\varepsilon_{A240}} \quad (6)$$

2.3. Solubility parameters

Solubility parameters were calculated by the method of Fedors (1974) as demonstrated by Martin et al. (1985) and Sloan et al. (1986).

2.4. Diffusion cell experiments

The diffusion cell experiments were conducted according to a previously described procedure (Sloan et al., 1986). Briefly, the flux of each prodrug was measured using skin samples from three different mice. Prior to skin removal, the mice were rendered unconscious by CO_2 then sacrificed via cervical dislocation. Skins were removed by blunt dissection and placed dermal side down 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/L) to inhibit microbial growth and maintain the integrity of the skins (Sloan et al., 1991) throughout the experiment. A rubber O-ring was placed on top of the skin to ensure a tight seal, and the donor and receiver compartments were fastened together with a metal clamp.

Prior to the application of the prodrug, the skins were kept in contact with buffer for 48 h to condition the skins and to allow any UV absorbing material to leach out. During this time, the

receptor phase was removed and replaced with buffer three times in order to facilitate the leaching process. Twenty-four hours before application of the prodrug, a suspension (0.09–0.80 M, i.e. roughly 10× S_{IPM}) of the prodrug in IPM was prepared and allowed to mix until it was needed in the diffusion cell experiments. After the 48 h leaching period, an aliquot (0.5 mL) of the prodrug suspension was added to the surface of the skin (donor phase). Samples of the receptor phase were usually taken at 8, 19, 22, 25, 28, 31, 34, and 48 h and analyzed within 1 h by UV spectrophotometry using the molar absorptivities in Table 1 to determine the amounts of permeated APAP and prodrug. Flux was determined by plotting the cumulative amounts of APAP plus APAP prodrug against time. Flux could then be calculated by dividing the slope of the steady-state portion of the graph by the surface area of the skin (4.9 cm^2). At each sampling time, the entire receptor phase was replaced with fresh buffer in order to maintain sink conditions.

After the 48 h of the first application period, the donor suspension was removed and the skins were washed three times with methanol (3–5 mL) to remove any residual prodrug from the surface of the skin. The skins were kept in contact with buffer for an additional 24 h to allow all APAP species (i.e. APAP and prodrug) to leach from the skin. That buffer was analyzed by UV spectroscopy for APAP and prodrug that had leached from the skin to give values for C_S (Table 5).

Following this second leaching period, the receptor phase was replaced with fresh buffer and an aliquot (0.5 mL) of a standard saturated drug/vehicle (theophylline/propylene glycol, 200 mg/3 mL) was applied to the skin surface: the second application period. Samples of the receptor phase were taken at 1, 2, 3, and 4 h and analyzed by UV spectrophotometry. The concentration of theophylline in the receptor phase was determined by measuring its absorbance at 270 nm (ε = 10,200 L mol^{-1}). No absorption at 240 nm due to APAP or ACOM-APAP was observed which suggested that 24 h was sufficient to leach all APAP and ACOM–APAP out of the skin before the second application. At each sampling time, the entire receptor phase was removed and replaced with fresh buffer. Flux was determined by first plotting the cumulative amounts of Th against time. Flux could then be calculated by dividing the slope of the steady-state portion of the graph by the surface area of the skin (4.9 cm^2).

3. Results and discussion

3.1. Physicochemical properties

The solubilities of compounds **2–6** in IPM (S_{IPM}) and in water (S_{AQ}) are shown in Table 2. The relative standard deviations were all ≤ ±5% except for the S_{AQ} value measured for compound **6** (±9%). As expected, all the prodrugs were more soluble in IPM than APAP (Table 2). The biggest increase in S_{IPM} (sevenfold) occurred on going from the first (C1) to the second member (C2) of the series. Beyond C2, S_{IPM} gradually increased until the fourth member of the series (C5), but began to decrease thereafter. In general, the trends in S_{IPM} for **2–6** appear to follow the trends in melting point, though there was less variation in melting point among **3–6** than there was in S_{IPM}. It is important to note that the trends in S_{IPM} shown here were observed previously in other prodrug series including 1-ACOM-5-fluorouracil (1-ACOM-5U) (Taylor and Sloan, 1998), 3-ACOM-5-FU (Roberts and Sloan, 2003), 1-AOC-5-FU (Beall et al., 1994), and bis-6,9-ACOM-6-mercaptopurine (6,9-ACOM-6-MP) (Waranis and Sloan, 1987).

In addition to the 4-ACOM-APAP series, physicochemical data from a recently described (Wasdo and Sloan, 2004) series of 4-alkyloxycarbonyl (AOC) derivatives of APAP is also listed in Table 2. If homologs of the same alkyl chain length are compared (**2–4** versus **7–9**), the ACOM derivatives all exhibit lower

Table 2
Physicochemical properties of APAP **1**, 4-ACOM-APAP prodrugs **2–6** and 4-AOC-APAP[a] prodrugs **7–11**

Compound	M_W[b]	mp °C[c]	S_{IPM}[d,e,f]	S_{AQ}[d,f,g]	$S_{4.0}$[d,h]	$K_{IPM:4.0}$[i]
1, APAP	151	167–170	1.9[a]	100[a]		
2, C1	223	92–95	8.41 ± 0.44	15.2 ± 0.34	16.2	0.52 ± 0.016
3, C2	237	56–59	62.0 ± 1.91	24.7 ± 0.33	26.6	2.33 ±0.039
4, C3	251	56–58	73.5 ± 1.45	7.12 ± 0.0073	8.26	8.90 ± 1.00
5, C5	279	50–52	109 ± 1.48	0.597 ± 0.018	0.90	121 ± 19.1
6, C7	307	53–54	98.7 ± 3.77	0.0637 ±0.0060	0.048	2077[j]
7, C1[a]	209	112–115	12.0	20.4	17.0	0.692
8, C2[a]	223	120–122	9.33	3.80	4.47	2.09
9, C3[a]	237	104–106	23.4	2.70	3.02	7.94
10, C4[a]	251	118–120	13.8	0.427	0.447	31.6
11, C6[a]	279	108–110	16.7	0.0479	0.0324	513

[a] Data from Wasdo and Sloan (2004).
[b] Molecular weight.
[c] Melting point (uncorrected).
[d] Units of mM.
[e] Solubility in isopropyl myristate (IPM).
[f] Measured at 23 ± 1 °C.
[g] Solubility in water.
[h] Solubility in pH 4.0 buffer estimated from $S_{IPM}/K_{IPM:4.0}$.
[i] Partition coefficient between IPM and pH 4.0 acetate buffer.
[j] Extrapolated from previous $K_{IPM:4.0}$ in the series as described in the text.

melting points and, with the exception of **2** (C1), are more soluble in IPM and water than the corresponding members of the AOC series. However, comparisons such as this do not take into account the structural differences between the promoieties in question. The members of the ACOM series contain a CH_2O spacer between the phenoxy group of APAP and the carbonyl of the promoiety which extends the alkyl chain further from the phenyl ring of the parent, while the members of the AOC series contain an extra oxygen atom in the alkyl chain. The difference is one methylene unit, so it may be more appropriate to compare the C1 member of the ACOM series to the C2 member of the AOC series. If similar comparisons are made for the remainder of the two series, the ACOM prodrugs are 4–17-times more soluble in water and, with the exception of **2**, are three to five times more soluble in IPM than the corresponding members of the AOC series.

Although **2**–**6** were 4–60 times more lipid soluble than APAP, they were all much less soluble in water than APAP. In fact, the most water soluble member of the series, C2, exhibited only one-fourth the aqueous solubility of APAP (Table 2). S_{AQ} increased on going from C1 (**2**) to C2 (**3**), but dropped off quickly as the alkyl chain length increased. The C2 member was also the most water soluble member of the 1-ACOM-5-FU (Taylor and Sloan, 1998) and 3-ACOM-5-FU (Roberts and Sloan, 2003) prodrug series, so it is not unusual for the second member of such a series to be the most soluble in water. Although masking a hydrogen bond donor in the parent compound will invariably lead to higher S_{IPM}, it may also lead to higher S_{AQ} relative to the parent. This was not the case in the present study or in some previous prodrug series including 7-ACOM-theophylline (7-ACOM-Th) (Kerr et al., 1998), 1-alkylaminocarbonyl-5-FU (1-AAC-5-FU) (Sloan et al., 1993), and 4-AOC-APAP (Wasdo and Sloan, 2004).

In order to incorporate the physicochemical property data for **2**–**6** into the Roberts–Sloan database (Sloan and Wasdo, 2003), pH 4.0 buffer was used as the aqueous phase in determinations of partition coefficients. Partition coefficients obtained in this manner were then used to estimate the solubilities of **2**–**6** in pH 4.0 buffer ($S_{4.0}$, Table 2). Partition coefficients between IPM and pH 4.0 buffer ($K_{IPM:4.0}$) were experimentally determined for all compounds except for **6** (Table 2). The relative standard deviations in $K_{IPM:AQ}$ were all less than ±10% except for **4** (±11%) and **5** (±16%). Although the average methylene π_K for the 4-ACOM-APAP series (0.60 ± 0.05) is somewhat higher than the 4-AOC-APAP series (0.55 ± 0.06)(Wasdo and Sloan, 2004), it is consistent with average methylene π_K values seen in other ACOM prodrug series: 1-ACOM-5-FU (Taylor and Sloan, 1998), $\pi_K = 0.60 \pm 0.14$; 3-ACOM-5-FU (Roberts and Sloan, 2003), $\pi_K = 0.59 \pm 0.01$; 7-ACOM-Th (Kerr et al., 1998), $\pi_K = 0.58 \pm 0.05$. Since the partition coefficients and π_K values for **2**–**5** (Table 3) were reasonably well-behaved, the average π_K value was used to estimate the partition coefficient for **6** (Table 3). Use of the solubility ratios $SR_{IPM:AQ}$ (Table 3) as a surrogate for $K_{IPM:4.0}$, resulted in an average methylene π_{SR} value that was slightly higher than π_K, but exhibited a smaller standard deviation (0.62 ± 0.03). The estimated solubilities in pH 4.0 buffer $S_{4.0}$ were somewhat higher than S_{AQ} for **2**–**4** ($10 \pm 5\%$), while the calculated $S_{4.0}$ for **6** was only 0.75 times the experimentally measured S_{AQ} for **6**. On the other hand, $S_{4.0}$ for **5** was 1.5 times higher than its S_{AQ}, which is somewhat greater than the largest variation observed previously in the 4-AOC-APAP series (Wasdo and Sloan, 2004). It was difficult to accurately measure $K_{IPM:4.0}$ for **5** and **6** because of the big differences in their SR, so the $S_{4.0}$ values are also less accurate than for **2**–**4**.

Table 3
Log solubility ratios ($\log SR_{IPM:AQ}$), differences between $\log SR_{IPM:AQ}$ (π_{SR}), log partition coefficients ($\log K_{IPM:4.0}$), differences between $\log K_{IPM:4.0}$ (π_K), and solubility parameters (δ_i) for prodrugs **2**–**6**

Prodrug	$\log SR_{IPM:AQ}$[a]	π_{SR}[b]	$\log K_{IPM:4.0}$[c]	π_K[d]	δ_i[e]
2	−0.257		−0.285		12.04
3	0.400	0.66	0.368	0.65	11.77
4	1.01	0.61	0.949	0.58	11.54
5	2.26	0.62	2.09	0.57	11.18
6	3.19	0.57	3.32[f]		10.89

[a] log of the ratio of the solubilities in IPM (S_{IPM}) and water (S_{AQ}).
[b] $\pi_{SR} = (\log SR_{n+m} - \log SR_n)/m$; 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 with which it is compared.
[c] log of the partition coefficient between IPM and pH 4.0 buffer.
[d] Same definition as in b with the exception that $\log K_{IPM:4.0}$ is used in place of $\log SR_{IPM:AQ}$.
[e] Calculated as described in Sloan et al. (1986) units = $(\text{cal cm}^{-3})^{1/2}$.
[f] Extrapolated from previous $K_{IPM:4.0}$ in the series as described in the text.

3.2. Diffusion cell experiments

To date, there has been only one report of the topical delivery of total species containing 4-hydroxyacetanilide (APAP) (hereinafter referred to as delivery, flux or permeation of APAP) by a homologous series of prodrugs (Wasdo and Sloan, 2004). In order to facilitate comparisons between the results of the present investigation to those of the prior study of 4-AOC-APAP derivatives, data from both prodrug series are listed in Table 4. As shown in Table 4, the fluxes, J_M (±S.D.) of the ACOM prodrugs with the exception of **6** (±32%) were within the typical (Wasdo and Sloan, 2004) ±30% variation of *in vitro* experiments with hairless mice. Three of the five members of the ACOM series were more effective at delivering APAP through the skin than APAP itself. This is in contrast to the AOC series in which only one member (C1) delivered more APAP. If comparisons are made between members of the same alkyl chain length (**2**–**4** versus **7**–**9**), the ACOM derivatives are, with the exception of **2** versus **7**, 2–11-times more effective at delivery of APAP than the corresponding members of the AOC series. The flux of the most permeable derivative **3** was 3.6 times greater than that of APAP. An improvement of this magnitude is modest when compared to the results of other prodrug series. For instance, 6-ACOM derivatives of 6-mercaptopurine (6-MP) (Waranis and Sloan, 1988) and 1-ACOM derivatives of 5-fluorouracil (Taylor and Sloan, 1998) improve the flux of the parent by as much as 69 and 16 times, respectively. The apparent ineffectiveness of the ACOM promoiety in the present case may be explained by considering the differences in the physicochemical properties of the parent compounds. Compared to APAP, 5-FU and 6-MP are much less soluble in IPM and water (Sloan and Wasdo, 2003).

Table 4
Flux of total APAP species through hairless mouse skin from suspensions of 4-ACOM-APAP and 4-AOC-APAP[a] prodrugs in IPM (J_M), second application flux of theophylline through hairless mouse skin from a suspension in propylene glycol (J_J), error in predicting log J_M using the Roberts–Sloan equation ($\Delta \log J_{predicted}$), error in calculating log J_M using the Roberts–Sloan equation ($\Delta \log J_{calculated}$), and ratio of the flux of the prodrug to the flux of APAP ($J_{prodrug}/J_{APAP}$)

Compound	J_M[b]	J_J[b]	log J_M[b]	$\Delta \log J_{predicted}$[c]	$\Delta \log J_{calculated}$[d]	$J_{prodrug}/J_{APAP}$
1, APAP	0.51[a]	0.74[a]	−0.29[a]	−0.496[e]	−0.484	
2, C1	0.730 ± 0.23	0.934 ± 0.136	−0.136	−0.104	−0.0911	1.4
3, C2	1.86 ± 0.24	0.935 ± 0.0764	0.270	−0.213	−0.197	3.6
4, C3	0.777 ±0.20	0.780 ± 0.224	−0.109	−0.350	−0.331	1.5
5, C5	0.344 ± 0.062	0.857 ±0.148	−0.464	−0.254	−0.231	0.67
6, C7	0.110 ± 0.028	0.687 ± 0.147	−0.957	−0.0366	−0.00703	0.22
7, C1	1.00	1.12	0.00	−0.0953[e]	−0.0794	2.0
8, C2	0.174	0.64	−0.76	−0.482[e]	−0.464	0.51
9, C3	0.355	1.14	−0.45	−0.260[e]	−0.240	0.69
10, C4	0.0977	0.85	−1.01	−0.264[e]	−0.241	0.20
11, C6	0.0324	0.76	−1.49	−0.162[e]	−0.133	0.063
Control[f]		1.02 ± 0.13[g]				

[a] From Wasdo and Sloan (2004).
[b] Units of μmol cm^{-2} h^{-1}.
[c] Predicted from $\log J_M = -0.497 + 0.519 \log S_{IPM} + 0.481 S_{4.0} - 0.00268 M_W$ (coefficients from $n = 61$ database (Wasdo and Sloan, 2004), were recalculated using SAS 8.1). Error in prediction = log experimental J_M − log predicted J_M.
[d] Calculated from $\log J_M = -0.545 + 0.511 \log S_{IPM} + 0.489 S_{4.0} - 0.00253 M_W$ ($n = 61$ + current data gives a new database of $n = 66$ compounds). Error in calculation was from log experimental J_M − log calculated J_M.
[e] Already included in the $n = 61$ database, so the value listed here is actually the difference between log experimental J_M and a calculated value for flux, log $J_{calculated}$.
[f] Skins were sequentially subjected to 48 h conditioning, 48 h contact with IPM, methanol wash, 24 h leaching.
[g] From Sloan et al. (1986).

Thus it is not surprising to find that the flux of APAP is twofold higher than the flux of 5-FU and 134 times greater than that of 6-MP. As a consequence of its relatively high S_{IPM} and S_{AQ} values, it is more difficult to improve the flux of APAP than it is to improve the flux of polar heterocycles such as 5-FU and 6-MP with simple *n*-alkyl ACOM derivatives. It is also worth mentioning that the 7-ACOM derivatives of theophylline (Th) (Kerr et al., 1998), a polar heterocycle, exhibited only modest (twofold) improvements in flux. Though Th is less soluble in lipid and aqueous solvents than APAP, it is seven times more soluble in IPM than 5-FU while still exhibiting 54% of the water solubility of 5-FU. Again, the better the biphasic solubility of the parent compound, the more difficult it is to improve the flux via a prodrug approach using a simple *n*-alkyl promoiety.

When the receptor phases from the application of **2**–**6** were analyzed during steady-state flux conditions, only APAP was found with the exception of compound **3**. Complete or substantial enzymatic hydrolysis of ACOM derivatives during permeation of hairless mouse skin is not unusual and has been reported previously many times (Waranis and Sloan, 1987, 1988; Taylor and Sloan, 1998; Roberts and Sloan, 2003). For **3**, the intact prodrug accounted for 9% of the total APAP species in the receptor phase (Table 5). Since this particular derivative was also the most permeable member of the series, the system of cutaneous esterases in this case may have been overwhelmed and unable to completely hydrolyze the prodrug on its way through the skin. A similar phenomenon was observed in the 4-AOC-APAP series (Wasdo and Sloan, 2004) in which the derivative that exhibited the highest flux also delivered the highest percentage of intact prodrug through the skin (Table 5). Although no effort was made to determine the half-lives of **2**–**6** in the receptor phase buffer, the compounds were observed to be stable for at least 3 h (the time between receptor phase samples when chemical hydrolysis could occur) in the receptor phase buffer while molar absorptivities and solubilities were determined. In addition, the aqueous stability may be estimated based on similar studies by others (Bauguess et al., 1975; Bundgaard et al., 1986). For example, Bundgaard et al. (1986) found that the 2-acetyloxymethyl and 2-butyryloxymethyl derivatives of salicylamide exhibit half-lives of 46 and 98 h, respectively at 37 °C in pH 7.4 buffer. On the other hand, 4-hexanoyloxyacetanilide

Table 5
Percent intact prodrug detected in receptor phase during steady-state (% intact), log permeability coefficients (log P_M), concentrations of APAP species in skin (C_S), and dermal/transdermal delivery ratios for APAP **1**, 4-ACOM-APAP **2**–**6**, and 4-AOC-APAP prodrugs[a] **7**–**11**

Compound	% Intact[b]	log P_M[c]	C_S[d]	D/T[e]
1, APAP		−0.571	2.74 ± 0.70[f]	0.046
2, C1	0	−1.06	2.67 ± 0.572	0.031
3, C2	9	−1.52	13.1 ± 2.10	0.060
4, C3	0	−1.98	5.56 ± 0.535	0.061
5, C5	0	−2.50	3.55 ± 1.05	0.088
6, C7	0	−2.95	2.72 ± 1.55	0.21
7, C1	64	−1.08	5.45 ± 1.57[f]	0.046
8, C2	14	−1.73	1.08 ± 0.13[f]	0.053
9, C3	25	−1.82	2.84 ± 1.44[f]	0.068
10, C4	0	−2.15	1.91 ± 0.08[f]	0.17
11, C6	0	−2.71	1.79 ± 0.43[f]	0.47

[a] From Wasdo and Sloan (2004).
[b] Percent intact prodrug detected in the 31 h receptor phase sample.
[c] Calculated from log experimental J_M − log S_{IPM}, units of cm h^{-1}.
[d] Amount of total APAP species (in units of μmol) in receptor phase after 24 h following donor phase removal to allow APAP and prodrug to leach out of skin.
[e] Calculated from $D/T = [(C_S/4.9\ cm^2\ 24\ h)]/J_M$.
[f] From Wasdo (2005).

displays an approximate half-life of 19 h at 37 °C in pH 7.8 buffer (Bauguess et al., 1975). Based on the later value and given the generally higher pK_a of an aryl hemiacetal (McClelland et al., 1991) compared to its corresponding phenol, the ACOM derivatives **2–6** should exhibit half-lives greater than 19 h under the present experimental conditions. It is also unlikely that the prodrugs were hydrolyzed in the donor phases since compound **6** (used as a representative of the series) was stable (as indicated by ^{1}H NMR) for at least 1 week in IPM. Therefore, it is reasonable to assume that the absence of intact prodrug in the receptor phase is due to extensive enzymatic hydrolysis in the skin and is not the result of substantial chemical hydrolysis in the receptor or donor phases.

Although formaldehyde is generated during the hydrolysis of ACOM derivatives (Bundgaard, 1989), such prodrugs have been used for decades to treat a variety of conditions (Beaumont et al., 2003) without any overt formaldehyde-related toxicity. Besides, formaldehyde is generated metabolically under normal *in vivo* conditions. Thus, low levels of the hydrolysis byproducts generated in the present *in vitro* case are not expected to cause more toxicity than formaldehyde generated *in vivo*.

Apparently, the fluxes of **2–6** are not artificially high due to damage sustained by the skin over the course of the experiment. This assessment is based on control experiments in which a suspension of theophylline in propylene glycol (Th/PG) was applied to the skin following the removal of the prodrug donor phase. This second application of Th/PG resulted in Th flux values, J_J, that were not significantly different from those through skins treated with IPM alone (control, Table 4). IPM is known to increase flux by up to 50-fold compared to experiments where water is the vehicle because of its supposed ability to decrease the resistance of skin to permeation (Sloan et al., 2003). Thus, the apparent flux values of **2–6** are likely inflated due to IPM, but this is not expected to change the rank order of flux within or between series based on literature precedent (Sloan et al., 2003).

If the fluxes of **2–6** are normalized by their respective solubilities in IPM, the corresponding permeability coefficients P_M are obtained (Table 5). P_M has units of distance per time (usually cm h^{-1}). Thus, P_M gives no indication of the amount, or dose, of the permeant that is entering the body, so it is not clinically useful. Nevertheless, P_M is frequently used in the literature to quantify the permeation efficiency of a compound through skin (Hadgraft and Guy, 2003; Williams, 2003). One of the most popular expressions of P_M, the Potts–Guy Eq. (7) (Potts and Guy, 1992), shows that P_M is positively dependent on the octanol–water partition coefficient ($K_{OCT:AQ}$) and negatively dependent on molecular weight (M_W):

$$\log P_M = -6.3 + 0.71 \log K_{OCT:AQ} - 0.0061 M_W \quad (7)$$

Such a relationship of log P_M to log $K_{OCT:AQ}$ suggests that percutaneous absorption is positively dependant on lipid solubility and negatively dependant on the water solubility of a permeant. However, a plot of the log P_M values for **2–6** versus their respective log $K_{IPM:4.0}$ values gave a negative slope (−0.519, $r^2 = 0.975$, plot not shown).

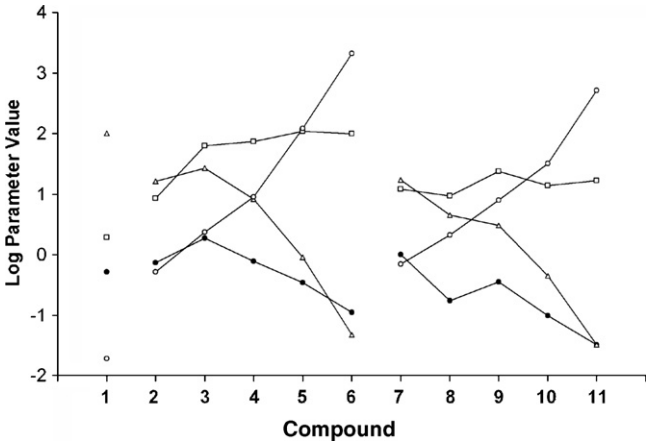

Fig. 2. log S_{IPM} (□), log $S_{4.0}$ (△), log $K_{IPM:4.0}$ (◯), and log J_M (●). Values for APAP **1**, 4-ACOM-APAP prodrugs **2–6**, and 4-AOC-APAP prodrugs **7–11**.

On the other hand, a plot of log P_M versus the calculated solubility parameters of **2–6** gave a positive slope (1.642, $r^2 = 0.997$, plot not shown), demonstrating an inverse relationship between log P_M and alkyl chain length (i.e. higher S_{IPM}, lower δ_i). These results are consistent with the findings of others (Roberts and Sloan, 2003; Sloan et al., 2003; Wasdo and Sloan, 2004), and support the idea (Sloan and Wasdo, 2003) that $K_{OCT:AQ}$ alone is a poor positive predictor of flux.

In order to ascertain the relative impact of solubility in a lipid, solubility in water, and partition coefficient on flux, the trends in S_{IPM}, $S_{4.0}$, $K_{IPM:4.0}$, and J_M for APAP **1** and its prodrugs **2–6** and **7–11** are graphically represented (Sloan et al., 2003; Wasdo, 2005) in Fig. 2. What is clear from such a representation is that $K_{IPM:4.0}$ is of little positive predictive value in determining the rank order of flux. For each increase in alkyl chain length, there is a corresponding increase in $K_{IPM:4.0}$ regardless of the trends in J_M. In addition, within the AOC series and to a lesser extent in the ACOM series, the trends in S_{IPM} are relatively flat across the series despite the fact that J_M grows progressively smaller. In contrast, the trends in $S_{4.0}$ generally mirror the trends in J_M across a series. Although such trends imply that water solubility is a better predictor of flux than lipid solubility, the reality is that flux is best predicted when both properties are considered (Roberts and Sloan, 1999; Sloan et al., 2006). This is demonstrated in the present case by the fact that the most permeable members of both series (**3** and **7**) exhibit the best mixture of high S_{IPM} and high $S_{4.0}$. Such behavior is no doubt related to the biphasic nature of the absorption barrier presented by the stratum corneum (Madison et al., 1987; Hou et al., 1991; Roberts and Sloan, 2000).

There is currently only one mathematical model available for quantifying the positive dependence of flux on lipid and aqueous solubility:

$$\log J_M = x + y \log S_{IPM} + (1 - y) \log S_{4.0} - z M_W \quad (8)$$

$$\log J_M = -0.491 + 0.520 \log S_{IPM} + 0.480 \log S_{4.0} - 0.00271 M_W \quad (9)$$

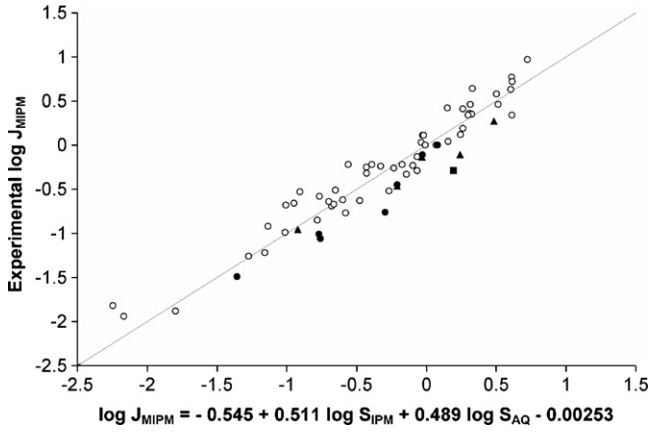

Fig. 3. Plot of experimental vs. calculated flux for 5-FU, 6-MP, and Th prodrugs (◯, $n = 53$), APAP (■), 4-AOC-APAP prodrugs (●), and 4-ACOM-APAP prodrugs (▲) to give $n = 66$.

Eq. (8), or the Roberts–Sloan (RS) model (Roberts and Sloan, 1999; Sloan et al., 2006), was originally based on a database ($n = 42$) of seven different series of prodrugs of polar heterocycles. This database was recently updated (Wasdo and Sloan, 2004) to include two new series of heterocyclic prodrugs and one new series of phenolic prodrugs (4-AOC-APAP) resulting in a more structurally diverse database of 61 compounds. A fit of that $n = 61$ data to Eq. (8) gave the form of RS expressed by Eq. (9) (Wasdo and Sloan, 2004). In its present state, the model is heavily dependent on data from heterocyclic compounds: 59% 5-FU related entries, 18% 6-MP related entries, and 10% Th related entries in the database. Only 8 of the 61 entries (13%) are of a phenol (i.e. APAP). Therefore, it was of interest to determine whether Eq. (9) could accurately predict the flux of the 4-ACOM prodrugs **2**–**6** of APAP. Application of Eq. (9) to prodrugs **2**–**6** resulted in predicted flux values ($J_{predicted}$, data not shown) that were consistently higher than the experimentally determined fluxes (J_M). The differences between $\log J_M$ and $\log J_{predicted}$ ($\Delta \log J_{predicted}$) for **2**–**6** are listed in Table 4. On average, the error in predicting $\log J_M$ ($\Delta \log J_{predicted}$) was 0.192 ± 0.124 log units.

In order to increase the diversity of the database and improve the predictive power of RS, prodrugs **2**–**6** were incorporated into the database. A fit of the independent variables $\log S_{IPM}$, $\log S_{4.0}$, and M_W for the resulting $n = 66$ entries to Eq. (8) using $\log J_M$ as the dependent variable gave the following estimates for x, y, and z: $x = -0.545$, $y = 0.511$, $z = 0.00253$, $r^2 = 0.915$. These parameter estimates were then used to calculate J_M for all 66 compounds (data not shown). A plot of $\log J_M$ versus the calculated $\log J_M$ values is shown in Fig. 3. The differences between the experimental and calculated fluxes ($\Delta \log J_{calculated}$) for APAP **1** and its prodrugs **2**–**6** and **7**–**11** are listed in Table 4. As shown in Table 4, the $\Delta \log J_{calculated}$ for **1**, **2**–**6**, and **7**–**11** decreased with the incorporation of the 4-ACOM-APAP data into the database. On average, the $\Delta \log J_{calculated}$ for **2**–**6** (0.171 ± 0.126 log units) was somewhat higher than the average $\Delta \log J_{calculated}$ for the entire $n = 66$ database (0.155 ± 0.118 log units), but was much lower than the average $\Delta \log J_{calculated}$ for **7**–**11** (0.231 ± 0.148 log units). Interestingly, APAP and its prodrugs all exhibit lower than expected fluxes based on the present form of RS. In addition, the average $\Delta \log J_{calculated}$ for APAP and its prodrugs (**1** plus **2**–**6**, plus **7**–**11**; 0.227 ± 0.133 log units) is quite a bit higher than the average $\Delta \log J_{calculated}$ for the database as a whole and may represent a bias in the database for prodrugs of heterocycles.

In order to determine whether 4-ACOM-APAP prodrugs function better as dermal (delivery into the skin itself) or transdermal (delivery through the skin and into the systemic circulation) delivery agents, the skins were kept in contact with buffer for 24 h after removing the donor phase to allow APAP and prodrugs to leach out. The amount of total APAP species leached from the skin (C_S) is shown in Table 5. As shown in Table 5, the rank order of C_S generally follows the rank order of flux. In other words, the most permeable members of the series were also the most effective at increasing the concentration of APAP in the skin. Three out of the five ACOM derivatives were able to deliver more APAP into the skin than suspensions of topically applied APAP alone, with derivative **3** delivering up to five-times more total species containing APAP. Using the C_S values as an estimate for the amount of total APAP species delivered into the skin, dermal/transdermal delivery ratios (D/T, Table 5) were calculated from Eq. (10):

$$\frac{D}{T} = \frac{(C_S/4.9\,\text{cm}^2 24\,\text{h})}{J_M} \tag{10}$$

Most of the prodrugs exhibited D/T ratios that were higher than APAP. Thus, compared with topically applied APAP alone, all but one of the ACOM prodrugs (**2**) were more effective than APAP itself at delivering total APAP species into the skin than through it. Among **2**–**6**, the prodrugs that preferentially delivered more total species containing APAP into the skin itself were also the most lipophilic and least permeable members of the series. Thus, compounds such as **5** and **6** may be best suited for a therapeutic regimen involving sustained delivery of low levels of a drug, while the shorter chain derivatives would allow for maximum exposure of the drug to the systemic circulation.

4. Conclusions

Despite the success of ACOM prodrugs in improving the transdermal delivery of heterocyclic drugs, there are currently no examples of this approach being applied to a phenol. The results presented here demonstrate for the first time that ACOM derivatives are capable of improving the topical delivery of a phenol. In general, the ACOM derivatives of acetaminophen (APAP) exhibited better biphasic solubility and lower melting points than the previously studied (Wasdo and Sloan, 2004) AOC derivatives. As a result, the 4-ACOM-APAP prodrugs were capable of improving the delivery of acetaminophen by approximately fourfold. The trends in flux were found to depend on a balance between lipid and aqueous solubility. Addition of the 4-ACOM-APAP prodrugs to the Roberts–Sloan database increased the structural diversity of the current database and resulted in a more robust RS model. Given that all of the 4-ACOM-APAP derivatives contained simple aliphatic groups in the acyl chain, it is likely that even greater improvements in flux will be realized by

incorporating more hydrophilic functional groups into the acyl chain (Sloan and Wasdo, 2003).

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