International Journal of Pharmaceutics 371 (2009) 25–32

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Evaluation of alkyloxycarbonyloxymethyl (AOCOM) ethers as novel prodrugs of phenols for topical delivery: AOCOM prodrugs of acetaminophen

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ARTICLE INFO

Article history:
Received 25 September 2008
Received in revised form 10 December 2008
Accepted 11 December 2008
Available online 24 December 2008

Keywords:
Prodrugs
Diffusion cell experiments
Roberts–Sloan equation
Lipid solubility
Water solubility
Topical delivery

ABSTRACT

The maximum fluxes of a series of alkyloxycarbonyloxymethyl (AOCOM) ethers of acetaminophen (APAP) through hairless mouse skin from isopropyl myristate, IPM (J_{MMIPM}) were measured. The J_{MMIPM}, solubilities in IPM (S_{IPM}), water (S_{AQ}) and pH 4.0 buffer ($S_{4.0}$) and molecular weights MW were then fitted to the Roberts–Sloan (RS) equation: $\log J_M = x + y \log S_{LIPID} + (1-y) \log S_{AQ} - z\text{MW}$. Only one of the prodrugs gave an improvement in the flux obtained by APAP itself. The general lack of improvement in flux seems to be due to the fact that there was no improvement in the S_{AQ} values of the AOCOM derivatives compared to APAP. When the $n = 5$ members of the AOCOM series were added to the $n = 66$ database of J_{MMIPM} to give $n = 71$ and fitted to the RS equation where S_{LIPID} was S_{IPM}, the following coefficients were obtained: $x = -0.562$, $y = 0.501$, $z = 0.00248$, $r^2 = 0.923$. These results demonstrate the importance of improving S_{AQ} for prodrugs to improve their solubilities in the skin and hence the flux of the parent drug. The RS equation, which is derived directly from Fick's law, explains this dependence of flux on S_{AQ}.

1. Introduction

Alkylcarbonyloxymethyl (ACOM) or α-(alkylcarbonyloxy)alkyl (ACOA) derivatives have been frequently used as soft alkyl prodrugs of a variety of functional groups in drug molecules. For instance, ACOM derivatives of amides (Bundgaard and Nielsen, 1987), imides (Buur et al., 1985; Taylor and Sloan, 1998), thioamides (Sloan et al., 1983; Waranis and Sloan, 1987, 1988), carboxylic acids (Jansen and Russell, 1965; Bundgaard et al., 1989), amines (Sloan and Koch, 1983a) and phenols (Sloan and Koch, 1983b; Thomas and Sloan, 2008b) have been reported. However there have been many fewer reports of the use of the close relative of ACOM: alkyloxycarbonyloxymethyl (AOCOM) derivatives. There is a report of the synthesis of one AOCOM derivative of theophylline among several ACOM derivatives designed to enhance the topical delivery of theophylline, but it was never evaluated in a diffusion cell experiment (Sloan and Bodor, 1982). There is another report of the synthesis of several AOCOM derivatives of 5-fluorouracil (Buur et al., 1986) together with their solubilities and rates of hydrolysis but no evaluation in a biological application. On the other hand, there is at least one α-(alkyloxylcarbonyloxy)alkyl (AOCOA) derivative of a carboxylic acid that is widely marketed—Cefpodoxine Proxetil (Beale, 2004). And there has been a recent report of the synthesis of AOCOM derivatives of a phenol (Thomas and Sloan, 2007).

Drugs containing a phenolic group represent quite important classes of drugs such as narcotic agonists and antagonists, estrogens and neurotransmitters, to name a few (Dhareshwar and Stella, 2007). Because they are rapidly conjugated and inactivated through the phenolic groups when given orally, they have been the subject of numerous attempts to protect the phenolic group with prodrugs. They are also ideal candidates for topical delivery since their therapeutic doses can be quite low. However, since their physicochemical properties are often not conducive to effective topical delivery, prodrugs have been often used to try to improve those physicochemical properties. For instance, a large number of types of prodrugs of naltrexone have been synthesized and evaluated in diffusion cell experiments to determine which type was the most effective: alkylcarbonyl (AC, Stinchcomb et al., 2002), alkyloxycarbonyl (AOC, Pillai et al., 2004) and alkylaminocarbonyl (AAC, Valiveti et al., 2005). As part of a systematic investigation of the effectiveness of different types of soft alkyl derivatives of a model phenolic drug, acetaminophen (APAP), at enhancing topical delivery, we have previously reported the results from diffusion cell experiments where AOC (Wasdo and Sloan, 2004) derivatives were compared with the results from soft alkyl ACOM (Thomas and Sloan, 2008a) derivatives. Here we report the results from the evaluation of AOCOM soft alkyl derivatives of APAP (Fig. 1) and compare those with previous results. We also report the results of incorporating this dataset into the Roberts–Sloan database ($n = 61$, Wasdo and Sloan, 2004 plus the ACOM dataset $n = 5$, Thomas and Sloan, 2008a,b) $n = 66$ to give an $n = 71$ database.

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doi:10.1016/j.ijpharm.2008.12.020

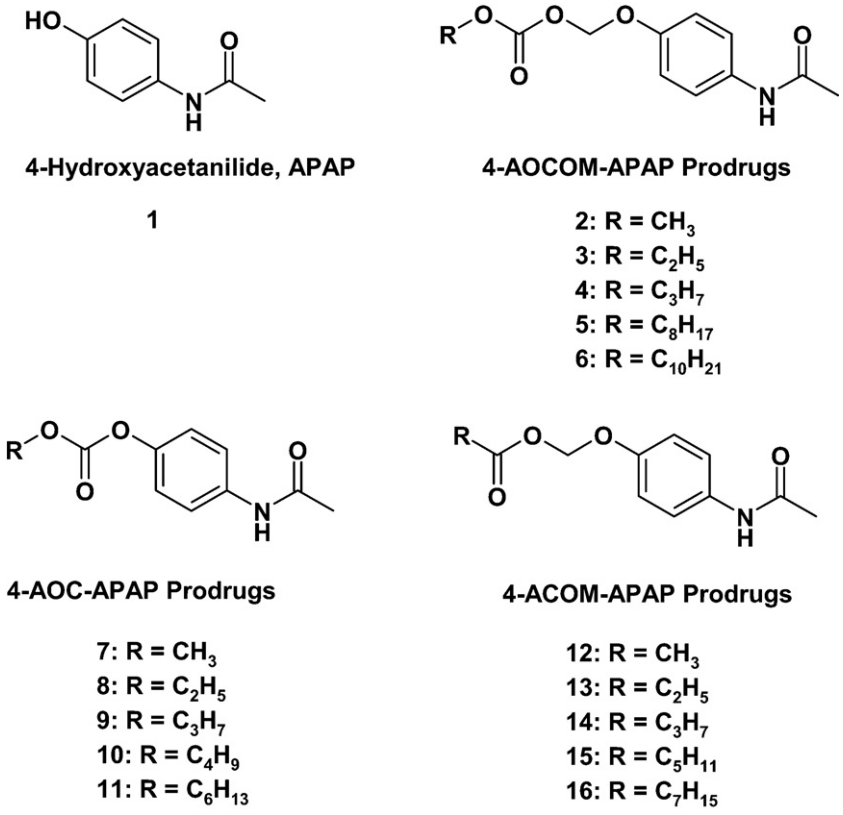

Fig. 1. Structures of APAP and its prodrugs.

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–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 synthesis and characterization of AOCOM APAP prodrugs is described elsewhere (Thomas and Sloan, 2007). 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."

2.2. Physicochemical properties and analysis

The molar absorptivity of each prodrug at 240 nm (ε_{240}) in acetonitrile (Table 1) was determined in triplicate by dissolving a known amount of prodrug in acetonitrile, and analyzing the dilute solution by UV spectrophotometry (Thomas, 2006). For each prodrug, the solubility in isopropyl myristate, S_{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 analyzed by UV spectrophotometry. The absorbance at 240 nm (A_{240}) was used to calculate the prodrug concentration in the IPM solution.

Solubilities in water, S_{AQ}, were also determined in triplicate using a protocol identical 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 (Wasdo and Sloan, 2004; Thomas and Sloan, 2008a). In each case, a sample of the filtrate was diluted with acetonitrile and analyzed 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 pH 4.0 buffer ($S_{4.0}$) as well as S_{AQ} is a parameter in the Roberts–Sloan, RS, database (Roberts

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

Compound[a]	ε_{240} in ACN[b,c]	ε_{240} in buffer[b,d]	ε_{280} in buffer[b,e]
1	1.36[f]	1.01 ± 0.053	0.174 ± 0.020
2, C1	1.44 ± 0.023		
3, C2	1.53 ± 0.041	1.11 ± 0.036	0.101 ± 0.014
4, C3	1.46 ± 0.056		
5, C8	1.52 ± 0.048		
6, C10	1.54 ± 0.0027		

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

and Sloan, 1999), acetate buffer (0.01 M, pH 4.0) was used as the aqueous phase in the partition coefficient experiments: $K_{IPM:4.0}$. 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. 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** and **4**: 0.7, 2.5, and 10, respectively. The two phases were vigorously shaken for 10 s (Beall et al., 1993), and 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. Using the previously measured absorbance at 240 nm for the saturated solution, the partition coefficient was calculated as follows:

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

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. It was not possible to experimentally determine partition coefficients for compounds **5** and **6** since their respective solubility ratios (S_{IPM}/S_{AQ}) were much too high. Therefore, in these cases $K_{IPM:4.0}$ was estimated from the average methylene π_K obtained for compounds **2**–**4** according to the following relationship:

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

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 with which it is compared.

Similarly the solubility ratio between IPM and AQ ($SR_{IPM:AQ}$) can be calculated for **2**–**5** and the average methylene π_{SR} calculated from $\log SR_{n+m} = (\pi_{SR})(m) + \log SR_n$ can be used to estimate $\log SR_{IPM:AQ}$ for **6**. In this way S_{AQ} for **6** can be estimated from $S_{IPM}/SR_{IPM:AQ}$ values for **6**.

UV spectrophotometry was also used to determine the amount of **1** (APAP) 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 **3** and **1** were determined in pH 7.1 phosphate buffer (0.05 M, $I = 0.11$ M) containing 0.11% formaldehyde by first dissolving a known amount of either compound in acetonitrile ($n = 5$). An aliquot (1 mL) of the acetonitrile solution was removed, diluted with buffer, and analyzed by UV spectrophotometry to obtain the molar absorptivities shown in Table 1. Because there is considerable overlap between the UV spectra of APAP and its AOCOM prodrugs **2**–**6**, the relative concentrations of each were determined using the following approach. The differences in absorption were found to be greatest at 240 nm and at 280 nm. Therefore, considering the additive nature of absorption, the absorbance at each wavelength (assuming constant cell length) is:

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

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

where A is the absorbance at the respective wavelengths, ε is 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:

$$C_P = \frac{\varepsilon_{A_{280}} A_{240} - \varepsilon_{A_{240}} A_{280}}{\varepsilon_{A_{280}} \varepsilon_{P_{240}} - \varepsilon_{A_{240}} \varepsilon_{P_{280}}} \quad (5)$$

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

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

2.3. Diffusion cell experiments

The maximum flux of each prodrug was measured according to a previously described procedure (Sloan et al., 1986) 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 allow any UV absorbing material to leach out. During this time, the receptor phase was removed and replaced with buffer 3 times in order to facilitate the leaching process. Twenty-four hours before application of the prodrug, a suspension (0.095–0.664 M, i.e. generally $10 \times S_{IPM}$) 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) as the first application. 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 as above to determine the amounts of permeated APAP and prodrug. 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 species containing APAP (i.e. APAP and prodrug) to leach from the skin. Following this second leaching period, the receptor phase was replaced with fresh buffer and an aliquot (0.5 mL) of a standard drug/vehicle (theophylline/propylene glycol, Th/PG, 200 mg/3 mL) was applied to the skin surface as the second application. Samples of the receptor phase were taken at 1–4 h and analyzed by UV spectrophotometry. No absorption at 240 nm due to APAP or its prodrugs was observed which suggested that 24 h was sufficient to leach all APAP and its prodrugs from the skins before the second application. The concentration of theophylline in the receptor phase was determined by measuring its absorbance at 270 nm ($\varepsilon = 10{,}200\ L\,mol^{-1}$). At each sampling time, the entire receptor phase was removed and replaced with fresh buffer.

In each experiment, the flux was determined by plotting the cumulative amount of species containing APAP (APAP plus prodrug) or 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 in IPM (S_{IPM}) and in water (S_{AQ}) for prodrugs **2**–**6** are listed in Table 2. The relative standard deviations of the S_{IPM} and S_{AQ} values were all $\leq \pm 5\%$ except for the S_{AQ} value for **5** which

Table 2
Physicochemical properties of AOCOM, AOC (Wasdo and Sloan, 2004), and ACOM (Thomas and Sloan, 2008a) prodrugs of acetaminophen (APAP).

Compound[a]	MW	Mp (°C)[b]	S_{IPM}[c]	S_{AQ}[c]	$S_{4.0}$[c,d]	$K_{IPM:4.0}$
APAP, **1**	151	167–170	1.9	100	–	–
AOCOM **2**, C1	239	104–106	7.93 ± 0.14	7.20 ± 0.14	8.39	0.946 ± 0.022
AOCOM **3**, C2	253	83–85	20.7 ± 1.0	7.76 ± 0.41	7.51	2.76 ± 0.22
AOCOM **4**, C3	267	68–69	45.8 ± 1.5	2.00 ± 0.09	4.97	9.21 ± 0.51
AOCOM **5**, C8	337	64–65	66.4 ± 1.9	0.004 ± 0.0005	0.029	2720[e]
AOCOM **6**, C10	365	54–56	130 ± 2.4	0.00056[f]	0.0062	26500[e]
AOC **7**, C1	209	112–115	12.0	20.4	17.0	0.692
AOC **8**, C2	223	120–122	9.33	3.80	4.47	2.09
AOC **9**, C3	237	104–106	23.4	2.70	3.02	7.94
AOC **10**, C4	251	118–120	13.8	0.43	0.447	31.6
AOC **11**, C6	279	108–110	16.7	0.048	0.032	513
ACOM **12**, C1	223	95–96	8.41	15.2	16.2	0.519
ACOM **13**, C2	237	56–59	62.0	24.7	26.6	2.33
ACOM **14**, C3	251	56–58	73.5	7.12	8.26	8.90
ACOM **15**, C5	279	50–52	109	0.597	0.90	121
ACOM **16**, C7	307	53–54	98.7	0.0637	0.048	2077[e]

[a] C1, C2, etc. refer to the length of the alkyl chain in the promoiety.
[b] Uncorrected.
[c] Units of mM.
[d] Estimated from $S_{IPM}/K_{IPM:4.0}$.
[e] Extrapolated from previous $K_{IPM:AQ}$ in the series as described in the text.
[f] Estimated from $S_{IPM}/SR_{IPM:AQ}$ (SR = solubility ratio).

was ±11%. As expected, all of the AOCOM prodrugs exhibited lower melting points than APAP and were more soluble in IPM than APAP. There was a steady increase in S_{IPM} on going from the first to the last member of the series, with the last member of the series (**6**, C10) exhibiting the greatest increase (68-fold) in S_{IPM} over APAP. As seen in the alkyloxycarbonyl (AOC, Wasdo and Sloan, 2004) and ACOM (Thomas and Sloan, 2008a) prodrugs of APAP, all of the AOCOM derivatives were much less soluble in water than APAP. In fact, the most water-soluble member of this series, **3**, exhibited only 0.08-times the S_{AQ} of APAP. In general, the S_{AQ} values decreased along the series except for a slight increase in S_{AQ} on going from C1 to C2. The present S_{AQ} value for **3** (C2) is twice as high as the value previously reported by Seki et al. (1988). Although the reason for this discrepancy is unclear, it must be noted that the S_{AQ} value of 4-ethyloxycarbonyloxyacetanilide, **8**, measured by Seki et al. (1988) (S_{AQ} = 2.15 mM, 25 °C, 0.01 M phosphate buffer, pH 7.0) is also about one-half the S_{AQ} value measured by Wasdo and Sloan (2004) under these same conditions.

In order to incorporate the physicochemical property data for **2**–**6** into the Roberts–Sloan, RS, database (Wasdo and Sloan, 2004), pH 4.0 buffer was used as the aqueous phase in partition coefficient determinations ($K_{IPM:4.0}$). 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) from $S_{IPM}/K_{IPM:4.0}$. Partition coefficients between IPM and buffer could only be determined for the first three members of the series. The last two homologs, **5** and **6**, exhibited such low solubilities in water that the present method for measuring partition coefficients was not useful.

However, the relative standard deviations for the $K_{IPM:4.0}$ values were all ≤±8%. Also, although the two methylene π_K for this series (0.46 and 0.52) calculated from a transposition of the terms in Eq. (2) were much lower than the average π_K for the ACOM APAP series (π_K = 0.60 ± 0.05), the average (0.49) was within the standard deviation of the average π_K for the AOC APAP series (π_K = 0.55 ± 0.06, Wasdo and Sloan, 2004). Since the $K_{IPM:4.0}$ values obtained for the first three homologs were reasonable, the average π_K value was used to calculate $K_{IPM:4.0}$ for the last two members of the series (**5** and **6**) which was then used to estimate their $S_{4.0}$ values. The estimated solubility in pH 4.0 buffer was somewhat higher than the experimentally determined S_{AQ} in the case of **2** and somewhat lower in the case of **3**. For **4** and **5**, the estimated values for $S_{4.0}$ were all much higher (2.5 and 5.5-times higher, respectively) than the corresponding experimental values for S_{AQ}. The use of these higher estimated $S_{4.0}$ values could ultimately lead to an overprediction of J_{MMIPM} from the RS equation (see below).

Since experimental log S_{AQ} values for **4** and **5** were available, a log S_{AQ} value for **6** was estimated from the log of the solubility ratios (log SR = log S_{IPM} − log S_{AQ}) of **2**–**5**. The average of the differences in log SR for **2**–**5** was 0.59. The log SR for **5** was 4.18 and the log SR for **6** was then estimated to be 5.36, and the value for **6** log S_{AQ} was calculated from **6** log S_{IPM}(2.11) − **6** log SR(5.36) = −3.25. Thus the log S_{AQ} value estimated from log SR and log S_{IPM} was much less than the log $S_{4.0}$ value of −2.21 estimated from log $K_{IPM:4.0}$ and log S_{IPM}. Again, the use of the estimated $S_{4.0}$ value instead of the estimated S_{AQ} for **6** could lead to an overprediction of J_{MMIPM} from the RS equation.

In order to facilitate comparisons between the AOCOM APAP series and other APAP derivatives, the relevant physicochemical property data of the 4-AOC APAP (Wasdo and Sloan, 2004) and 4-ACOM APAP series (Thomas and Sloan, 2008a) have also been included in Table 2. If comparisons are made between members of the same alkyl chain length (C1 to C3), the AOCOM series is generally more soluble in IPM and less soluble in water than the AOC series. For instance, C2 and C3 AOCOM are 2.2 and 2.0-times, respectively, more soluble in IPM than the corresponding members of the AOC series, while C1 and C3 AOC are 2.8 and 1.4-times, respectively, more soluble in water than the corresponding members of the AOCOM series. If similar comparisons are made between the AOCOM and ACOM series, the C1 to C3 ACOM derivatives exhibit higher solubilities in both water and IPM than the corresponding members of the AOCOM series.

If the structural differences between the promoieties are taken into account (Sloan and Wasdo, 2006), slightly different conclusions are reached. Since the AOCOM derivatives contain a CH_2O linker between the phenoxy group of APAP and the carbonyl of the prodrug, the alkyl chain in this series is extended two atoms farther from APAP than members of the same alkyl chain length in the AOC series. Therefore, rather than simply counting the number of methylene units in the alkyl chain, it may be more appropriate to include this two-atom unit in the total chain length when making comparisons between homologs of approximately equal size. Using this rationale, C1 and C2 AOCOM are 2.7 and 18-times, respectively,

more soluble in water than the corresponding members of the AOC series (C3 and C4). The differences in S_{IPM} are not as one-sided. In one case the AOC member (C3) is more soluble in IPM (compared to C1 AOCOM), while in the other case the AOCOM member (C2) is more soluble (compared to C4 AOC).

This approach may also be used to compare the AOCOM derivatives with the corresponding ACOM derivatives. In this case, C2 and C3 ACOM are 7.8 and 3.6-times more soluble in IPM than the corresponding members of the AOCOM series (C1 and C2). In addition, C2 ACOM is 3.4-times more soluble in water than C1 AOCOM while C3 ACOM exhibits 92% of the solubility of C2 AOCOM in water. Based on these results, it appears that substitution of oxygen for a methylene unit in the carbonyl group of the prodrug (ACOM → AOCOM) results in a decrease in lipid solubility with little improvement in or a decrease in water solubility.

3.2. Diffusion cell experiments

Hairless mice have been used as the skin source because it has been shown to be a reasonable model membrane for diffusion cell experiments in previous reports from our laboratory (Sloan et al., 1986; Thomas and Sloan, 2007) and others (Kai et al., 1990). In addition to giving more reproducible results than human skin, hairless mouse skin is more sensitive to vehicle and potential solute effects on permeability than human skin (Kai et al., 1990) or pig skin by inference so that subtle differences in permeation are more easily quantitated.

Results from the diffusion cell experiments for the delivery of total APAP containing species through hairless mouse skin from IPM by AOCOM APAP prodrugs, EXP J_{MMIPM}, are listed in Table 3. For most of the prodrugs, samples of the receptor phase were taken every 3 h once steady-state flux was established. The exception was compound **6** in which samples only were taken every 12 h to allow measurable amounts of permeant to accumulate in the receptor phases. As a consequence, the flux value for **6** listed in Table 3 is an estimate of J_{MMIPM} based on the samples taken at 31 and 43 h.

Compounds **5** and **6** gave the lowest flux values. The limit of detection (LOD) is the blank reading plus 3 times the S.D. obtained for the blank (0 ± 0.002 absorbance units, Abs, for the Shimadzu UV-2501 PC) or 0.006 Abs. Since $\varepsilon_{240} = 1.01 \times 10^4$ L mol^{-1} for APAP and the volume of each sample is 0.020 L, the LOD is 0.0119 μmol. For compound **5** the amount in a typical 3 h, sample that gave a flux of 0.021 μmol cm^{-2} h^{-1} is 0.309 μmol while for compound **6** the amount in a typical 12 h sample that gave a flux of 0.0074 μmol cm^{-2} h^{-1} is 0.435 μmol. Thus, both **5** and **6** gave sample amounts well above the LOD. The limit of quantification (LOQ) is the blank plus 10 times the S.D. so LOQ is 0.020 Abs or 0.0396 μmol which is well below the sample amounts as well.

Also included in Table 3 are the diffusion cell results from the AOC APAP (Wasdo and Sloan, 2004) and ACOM APAP (Thomas and Sloan, 2008a) series. With the exception of **3** (±47%) and **4** (±32%), the fluxes of **2**–**6** were all within the ±30% variation typically observed (Wasdo and Sloan, 2004) in diffusion cell experiments with hairless mice. As a whole, the AOCOM derivatives were not very effective at increasing the transdermal delivery of APAP. In the one case **3** where the flux of the prodrug was greater than that of APAP, the improvement was only marginal (1.3-fold). If the fluxes of members of the same alkyl chain length are compared, the first three homologs of the AOCOM series (C1 to C3) performed worse on average than the corresponding members of the ACOM series: the corresponding ACOM gave 1.65, 2.81 and 2.75 times the flux of the AOCOM. Compared to the AOC series the AOCOM gave mixed results: The corresponding AOC gave 2.25, 0.26 and 1.27 times the flux of the AOCOM. If structural differences between the promoieties are taken into account (as above for the solubility comparisons), the AOCOM series is more effective at delivering APAP than the AOC series. The C1 and C2 members of the AOCOM series gave 1.23 and 6.73 times the flux of the C3 and C4 members of the AOC series. On the other hand the AOCOM series remained less effective at delivering APAP than the ACOM series. The C2 and C3 members of the ACOM series gave 4.20 and 1.18 times, respectively, the flux of the C1 and C2 members of the AOCOM series.

Table 3
Experimental maximum flux of total species containing APAP through hairless mouse skin from IPM (EXP J_{MMIPM}) as the first application, maximum flux of theophylline through hairless mouse skin from propylene glycol (J_J) as the second application, percent intact prodrug in receptor phase at 31 h (%), the absolute value of the individual difference between EXP log J_{MMIPM} and log J_{MMIPM} calculated by the $n = 71$ database coefficients to RS (ΔCALC log J_{MMIPM}), concentration of total species containing APAP leached from the skin after first application (C_S) and the dermal to transdermal delivery ratio (D/T).

Compound[a]	EXP J_{MMIPM}[b]	EXP log J_{MMIPM}[b]	%	J_J[b]	ΔCALC log J_{MMIPM}[b,c]	C_S[d]	D/T[e]
APAP, **1**	0.51	−0.29		0.74	0.492	2.74	0.046
AOCOM **2**, C1	0.443	−0.35 ± −1.29	32	0.88	0.077	2.83	0.054
AOCOM **3**, C2	0.660	−0.18 ± −0.51	46	1.12	0.094	3.03	0.039
AOCOM **4**, C3	0.283	−0.55 ± −1.04	25	1.12	0.305	4.53	0.14
AOCOM **5**, C8	0.021	−1.67 ± −2.74	0	1.03	0.014	1.57	0.63
AOCOM **6**, C10	0.0074	−2.13 ± −3.74	0	0.71	0.100	0.83	0.95
AOC **7**, C1	1.00	0.00	64	1.12	0.074	5.45	0.046
AOC **8**, C2	0.174	−0.76	14	0.64	0.455	1.08	0.053
AOC **9**, C3	0.355	−0.45	25	1.14	0.226	2.84	0.068
AOC **10**, C4	0.098	−1.01	0	0.85	0.221	1.91	0.17
AOC **11**, C6	0.032	−1.49	0	0.76	0.103	1.79	0.47
ACOM **12**, C1	0.730	−0.136	0	0.93	0.088	2.67	0.031
ACOM **13**, C2	1.86	0.270	9	0.94	0.188	13.1	0.060
ACOM **14**, C3	0.777	−0.109	0	0.78	0.317	5.56	0.061
ACOM **15**, C5	0.344	−0.464	0	0.86	0.207	3.55	0.088
ACOM **16**, C7	0.110	−0.957	0	0.69	0.028	2.72	0.21
Control[f]				1.02			

[a] C1, C2, etc. refer to the length of the alkyl chain in the promoiety.
[b] Units of μmol cm^{-2} h^{-1}.
[c] Calculated using Eq. (14) for $n = 71$ compounds.
[d] Units of μmol.
[e] Calculated from $[C_S/(4.9\ \text{cm}^2\ 24\ \text{h})]/J_{MMIPM} = D/T$ ratio.
[f] From Sloan et al. (1986).

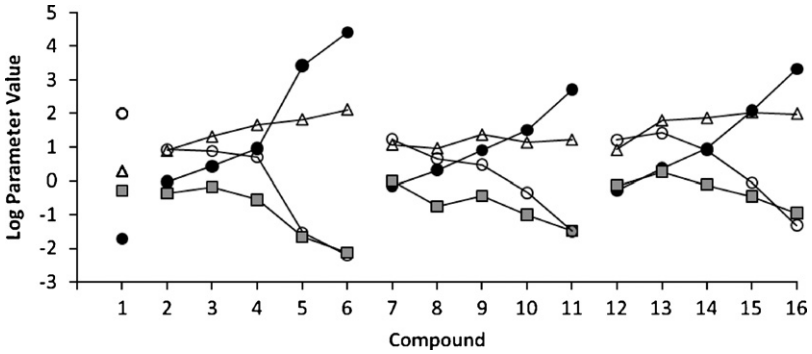

Fig. 2. Log S_{IPM} (Δ), log $S_{4.0}$ (○), log $K_{IPM:4.0}$ (●), log EXP J_{MMIPM} (■). Values for APAP **1**, AOCOM **2**–**6**, AOC **7**–**11**, ACOM **12**–**16**.

When the receptor phases from the application of **2**–**6** were analyzed during steady-state flux conditions, various percentages of intact prodrug and APAP were found (Table 3). The entries are from samples taken at 31 h and are representative of percentages of intact prodrug observed at other times during steady-state. Although no effort was made to determine the half-lives of **2**–**6** in the receptor phase buffer, aqueous stability may be estimated based on the work of others. For example, Seki et al. (1988) found that **3** exhibited a half-life of 200 h in pH 7.0 phosphate buffer (0.01 M) at 25 °C. Thus, under the present experimental conditions it is reasonable to assume that presence of APAP in the receptor phase is due to enzymatic hydrolysis of the prodrugs in the skin and is not the result of chemical hydrolysis in the receptor phase since there was only 3 h between samples and each sample was analyzed within 1 h. In general, the percent of intact prodrug decreased as the alkyl chain length increased. A similar trend was previously observed (Wasdo and Sloan, 2004) in the APAP series (Table 3) and in the literature which suggests that shorter chain length homologs are more stable enzymatically but less stable chemically than the longer chain homologs (Waranis and Sloan, 1987; Buur et al., 1985; Taylor and Sloan, 1998).

Apparently, the fluxes of **2**–**6** are not artificially high due to damage sustained by the skin over the course of the first application or the leaching periods (Sloan et al., 1986). The 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 (Table 3). However, it is important to recognize that IPM is a well-known penetration enhancer which can increase flux 50-fold compared to experiments where water was the vehicle (Sloan et al., 2003). Although the apparent flux values of **2**–**6** are likely inflated due to using IPM as a vehicle compared to water, this should not change the rank order of flux within or between series nor does it affect the balanced dependence of J_{MMIPM} on S_{IPM} and S_{AQ} (Sloan et al., 2003).

In order to ascertain the relative impact of solubility in a lipid, solubility in water, and partition coefficient on flux, the trends in log S_{IPM}, log $S_{4.0}$, log $K_{IPM:4.0}$, and log J_{MMIPM} for APAP **1** and its prodrugs **2**–**6**, **7**–**11** and **12**–**16** are graphically represented in Fig. 2. The most consistent trend between the series is the steady increase in log $K_{IPM:4.0}$ with increasing alkyl chain length. This is in spite of the fact that the log J_{MMIPM} generally decreased along each series. Thus it is clear from the present results that log $K_{IPM:4.0}$ is of little positive predictive value in determining the rank order of flux. Similarly, there is no obvious relationship between log S_{IPM} and flux as log S_{IPM} grows larger along the AOCOM series **2**–**6**, but remains relatively constant along the ACOM **12**–**16** and AOC **7**–**11** series. On the other hand, the trends in log $S_{4.0}$ generally mirror the trends in flux as previously shown (Sloan et al., 2003; Sloan, 1989). Although this dependence of flux on log S_{AQ} (or log $S_{4.0}$) is most apparent in homologous series of compounds, such dependence has recently been demonstrated for a large number of unrelated compounds through human skin from water *in vitro* (J_{MHAQ}, Juntunen et al., 2008) and for a small set of nonsteroidal anti-inflammatory drugs through human skin from mineral oil (MO) *in vivo* (J_{MHMO}, Roberts and Sloan, 2001) using the Roberts–Sloan, RS, model (Roberts and Sloan, 1999).

The RS equation (Eq. (7)) is the only model for transdermal permeation to incorporate a dependence of maximum flux, J_M, on S_{AQ}. RS is directly derived from Fick's law (Eq. (8)). It is assumed that the concentration of the permeant in the last layer of the membrane, C_{Mn}, is negligible and the concentration of the permeant in the first few layers of the membrane, C_{M1}, can be calculated from the product of the partition coefficient between the membrane, MEM, and the vehicle, VEH ($K_{MEM:VEH}$) and the concentration of the permeant in the vehicle, C_{VEH} (Eq. (9)). Since J_M obtains when C_{VEH} is the solubility in the vehicle, S_{VEH} (saturated donor phase), C_{M1} becomes the solubility in the first few layers of the membrane, S_{M1}, and Eq. (9) gives Eq. (10). Transformation of $K_{MEM:VEH}$ (Surber et al., 1990) when the vehicle is a lipid, LIPID, gives Eq. (11) where $(K_{LIPID:AQ})^Y c$ has been substituted for $K_{MEM:AQ}$. Substitution of solubilities for K gives Eq. (12). Substitution of $\log D_0 - \beta MW$ for log D and Eq. (12) for C_{M1} into Eq. (8), then collection of constants ($\log D_0/L$ and log c) gives Eq. (7) (Roberts and Sloan, 1999).

$$\log J_M = x + y \log S_{LIPID} + (1-y)\log S_{AQ} - z\,MW \tag{7}$$

$$J = \frac{D(C_{M1} - C_{Mn})}{L} \tag{8}$$

$$C_{M1} = (K_{MEM:VEH})C_{VEH} \tag{9}$$

$$S_{M1} = (K_{MEM:VEH})S_{VEH} \tag{10}$$

$$K_{MEM:VEH} = K_{MEM:LIPID} = \frac{(K_{LIPID:AQ})^Y c}{K_{LIPID:AQ}} \tag{11}$$

$$\begin{aligned} \log S_{M1} &= y \log K_{LIPID:AQ} + \log c - \log K_{LIPID:AQ} + \log S_{LIPID} \\ &= y \log S_{LIPID} - y \log S_{AQ} + \log c - \log S_{LIPID} \\ &\quad + \log S_{AQ} + \log S_{LIPID} \\ &= y \log S_{LIPID} + (1-y)\log S_{AQ} + \log c \end{aligned} \tag{12}$$

Previously, the ACOM APAP prodrugs (**12**–**16**) had been added to the $n = 61$ database (Wasdo and Sloan, 2004) to give $n = 66$ and the following fit of log J_{MMIPM}, log S_{IPM}, log $S_{4.0}$ and MW to RS (Thomas and Sloan, 2008a).

$$\log J_{MMIPM} = -0.545 + 0.511 \log S_{IPM} + 0.489 \log S_{AQ} - 0.00253MW \tag{13}$$

When the coefficients from Eq. (13) were used to predict log J_{MMIPM} for the AOCOM APAP prodrugs, the average of the absolute differences between the experimental, EXP log J_{MMIPM}, and

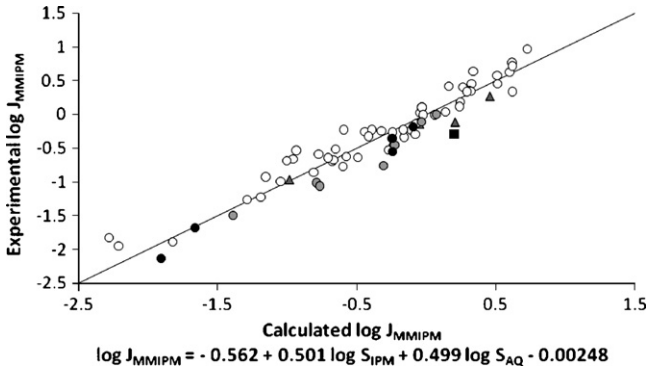

Fig. 3. Plot of experimental versus calculated flux for prodrugs of various heterocycles (○), APAP (■), 4-AOCOM-APAP (●), 4-AOC-APAP (●), 4-ACOM-APAP (▲).

predicted $\log J_{MMIPM}$, PRE $\log J_{MMIPM}$ (which is ΔPRE $\log J_{MMIPM}$), was 0.143 ± 0.106 log units when the $\log S_{AQ}$ values were used and 0.368 ± 0.252 log units when $\log S_{4.0}$ values were used (data not shown). In both cases the ΔPRE $\log J_{MMIPM}$ were greater than predicted by Eq. (13) but by not nearly as great if $\log S_{AQ}$ values were used: 1.39 versus 2.33 times. The main contributors to the overprediction were by **4**–**6** which gave much higher $\log S_{4.0}$ values than $\log S_{AQ}$ values (see above). The average of the absolute differences between EXP $\log J_{MMIPM}$ and calculated $\log J_{MMIPM}$, CALC $\log J_{MMIPM}$ (which is ΔCALC $\log J_{MMIPM}$) for the $n = 66$ database was 0.155 ± 0.118 log units, so ΔPRE $\log J_{MMIPM}$ for the AOCOM APAP prodrugs is better than the average for the entire database when $\log S_{AQ}$ values are used.

When the AOCOM APAP prodrugs were then added to the $n = 66$ database and a new fit of the new $n = 71$ database to RS was obtained, the following new coefficients were obtained ($r^2 = 0.923$):

$$\log J_{MMIPM} = -0.562 + 0.501 \log S_{IPM} + 0.499 \log S_{AQ} - 0.00248MW \quad (14)$$

Eq. (14) was then used to calculate $\log J_{MMIPM}$ and ΔCALC $\log J_{MMIPM}$ were obtained (Table 3). A plot of EXP $\log J_{MMIPM}$ versus CALC $\log J_{MMIPM}$ is given in Fig. 3. Using the fit to Eq. (14), the ΔCALC $\log J_{MMIPM}$ values for the AOCOM (using the S_{AQ} values) improved to 0.118 ± 0.110 log units which is even lower than the average for the $n = 71$ database: 0.156 ± 0.117 log units.

In order to determine whether AOCOM prodrugs of phenols would be more effective at delivering the parent compound to the skin (dermal delivery) or through the skin and into the systemic circulation (transdermal delivery), the skins were left in contact with buffer for 24 h after removing the donor phase to allow APAP and prodrug to leach out. The amount of total species containing APAP leached from the skin (C_S) is shown in Table 3 and has been used as a measure of dermal (D) compared to transdermal (T) delivery based on EXP J_{MMIPM}. Comparing the first three members of the three series (AOCOM, AOC and ACOM prodrugs) in Table 3, the AOCOM are comparable to both the AOC and ACOM (except for the C_S of ACOM C2, **14**) in both C_S and D/T values. When the first two members of the AOCOM series were compared with the members of the AOC, **9** and **10**, and ACOM, **13** and **14**, series of comparable molecular weight, the AOCOM prodrugs were again comparable to the AOC and ACOM series in C_S values (except for C2 ACOM) and D/T values (except for C4 AOC).

4. Conclusions

The AOCOM derivatives of APAP did not improve the delivery of total species containing APAP compared to the previously reported AOC and ACOM derivatives. When comparing members of each series of about the same molecular weight, the ACOM (C2 and C3) gave higher fluxes than the AOCOM (C1 and C2), while the AOCOM gave higher fluxes than the AOC (C3 and C4). The trend in fluxes followed almost exactly the trend in water solubility. The more water-soluble derivative usually gave the higher flux value in this comparison of AOC, ACOM and AOCOM prodrugs. Thus, the paradigm that the more water-soluble members of a series of more lipid soluble derivatives will give the higher flux (Sloan, 1989) because maximum flux, J_M, depends on S_{M1} and S_{M1} depends on S_{LIPID} and S_{AQ} (Sloan et al., 2006) remains true; and the reason the AOCOM did not perform better than the AOC and ACOM derivatives is that there was no improvement in the water solubilities of the AOCOM.

The calculation of the delivery of total species containing APAP by the AOCOM prodrugs from the Roberts–Sloan model (Eq. (14)) gave the best fit of any of these three series of prodrugs of APAP with a ΔCALC $\log J_{MMIPM}$ of only 0.118 log units compared to 0.156 for the entire $n = 71$ database. The CALC $\log J_{MMIPM}$ also correctly predicted the rank order of performance of the members of the series except for C1 and C3. Eq. (14) calculated a much higher $\log J_{MMIPM}$ than the experimental value for C3: ΔCALC $\log J_{MMIPM} = 0.31$ log units. In fact, that one discrepancy between EXP and CALC values is primarily responsible for ΔCALC $\log J_{MMIPM}$ being as high as it is for the AOCOM APAP series.

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