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Synthesis and topical delivery of *N*-alkyl-*N*-alkyloxycarbonylaminomethyl prodrugs of a model phenolic drug: Acetaminophen

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Abstract

The synthesis, physicochemical characterization and flux of a homologous series of *N*-alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM) prodrugs of a model phenolic drug, acetaminophen (APAP), have been investigated. The most water soluble member of the series gave the highest transdermal delivery from isopropyl myristate (IPM) through hairless mouse skin. The flux of NANAOCAM prodrugs of APAP was accurately predicted by the Roberts–Sloan (RS) equation.

Keywords: APAP; NANAOCAM; Permeation; Lipid solubility; Water solubility; Roberts–Sloan equation

1. Introduction

Approaches to increasing topical delivery by transiently masking a phenolic functional group have been limited to morphine (Drustrup et al., 1991), naltrexone (Stinchcomb et al., 2002; Pillai et al., 2004; Valiveti et al., 2005a, 2005b; Hammell et al., 2005; Vaddi et al., 2005), buprenorphine (Stinchcomb et al., 1996), nalbuphine (Sung et al., 1998) and acetaminophen (Wasdo and Sloan, 2004). Only three promoieties, alkylcarbonyl (AC), alkyloxycarbonyl (AOC) and alkylaminocarbonyl (AAC), have been utilized but no soft alkylated derivatives of phenol have been used for topical delivery. Both AC and AOC derivatives of phenols rely on esterase catalysed hydrolysis or an addition–elimination type mechanism to revert to the parent drug. The soft alkyl analogs of the AC and AOC prodrugs, alkylcarbonyloxymethyl (ACOM) and alkyloxycarbonyloxymethyl (AOCOM) obtained by inserting an $–OCH_2–$ between the carbonyl carbon and the phenol oxygen, are presently being investigated in our lab as approaches to improving topical delivery of phenols. Insertion of a $–NR'CH_2–$ into the AOC (ROCO–) promoiety instead of $–OCH_2–$ gives the *N*-alkyl-*N*-alkyloxycarbonylaminomethyl, NANAOCAM ($ROCONR'CH_2–$), promoiety which should also act as a soft alkyl prodrug of phenols (Majumdar and Sloan, 2006a) by analogy to the NANAOCAM prodrugs of 6MP (Siver and Sloan, 1990) and ThH (Majumdar and Sloan, in press). Thus it is of interest to see if this insertion into the AOC promoiety improves the biphasic properties and flux of phenols through skin compared to the AOC prodrugs (Wasdo and Sloan, 2004). In order to test this hypothesis acetaminophen (APAP) was chosen as the model phenolic drug since the AOC prodrugs of APAP had been recently evaluated for their ability to increase the topical delivery of APAP.

The performance of the prodrug series in diffusion cell experiments will also be compared with that predicted by the Roberts–Sloan (RS) equation (Roberts and Sloan, 1999). The results will be added to the Sloan and coworkers database (Wasdo, 2005) to generate new coefficients for the parameters in the equation and give a more robust RS equation.

2. Materials and methods

Isopropyl myristate (IPM) was obtained from Givaudan Corp (Clifton, NJ). Theophylline (ThH) was purchased from Sigma Chemical Co. (St. Louis, MO, USA); all other reagent chemicals were from Aldrich Chemical Co. (Milwaukee, WI, USA). The water was obtained from a Millipore Milli-Q water ultra filtration system. Ultraviolet spectra were recorded on a Shimadzu UV-2501 PC spectrophotometer. A radiometer pH meter 26 was used to determine the pH of solutions. The vertical, Franz type

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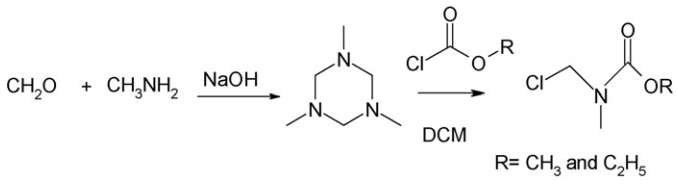


Fig. 1. Synthesis of NANAOCAM-Cl from 1,3,5-trimethylhexahydrotriazine.

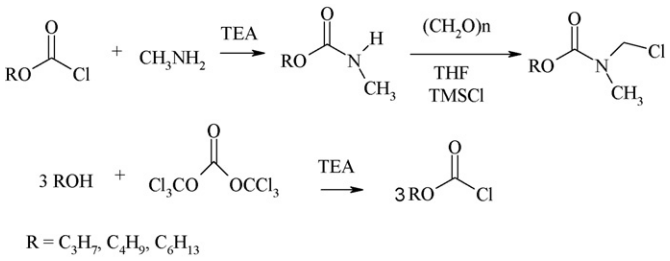


Fig. 2. Preparation of alkyl chloroformate in situ from alcohol and synthesis of NANAOCAM-Cl from alkyl amine.

diffusion cells were from Crown Glass (Somerville, NJ, USA) (surface area 4.9 cm^2, 20 mL receptor phase volume, 15 mL donor phase volume). The diffusion cells were maintained at 32 °C with a Fischer (Pittsburgh, PA, USA) circulating water bath model 25. The female hairless mice (SKH-hr-1) were from Charles River (Boston, MA, USA). Statistical analyses were carried out by using SAS 9.0. All animal sacrifices and preparation of membranes were carried out by Kenneth Sloan using IACUC approved protocols.

2.1. Synthesis of prodrug derivatives

Synthesis of the prodrugs (**1**–**5**) was accomplished by the alkylation of APAP with *N*-alkyl-*N*-alkyloxycarbonyl-aminomethyl chlorides (NANAOCAM-Cl) in the presence of a base like triethylamine and CH_2Cl_2 as the solvent. It was first necessary to synthesize the corresponding alkylating agent, NANAOCAM-Cl.

2.1.1. Synthesis of NANAOCAM-Cl

(a) 1,3,5-Trimethylhexahydrotriazine was synthesized from equimolar equivalents of aqueous formaldehyde, methyl amine and NaOH according to the protocol originally developed by Graymore (1932) and modified by Siver and Sloan (1990). Yield = 82%, ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 9H), δ 3.2 (s, 6H).

Next the 1,3,5-trimethylhexahydrotriazine (freshly prepared) in CH_2Cl_2 was added to an ice cold solution of 3 equiv. of alkyl chloroformate in CH_2Cl_2 over a period of 10 min as described previously to give NANAOCAM-Cl (Siver and Sloan, 1990) (Fig. 1).

N-Methyl-*N*-methyloxycarbonylaminomethyl chloride: yield = 90%, ^{1}H NMR ($CDCl_3$): δ 2.9 (s, 3H), δ 3.75 (s, 3H), δ 5.3 (s, 2H).

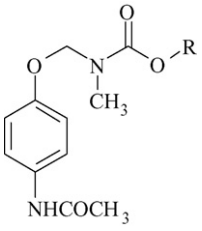
N-Methyl-*N*-ethyloxycarbonylaminomethyl chloride: yield = 89%, ^{1}H NMR ($CDCl_3$): δ 1.3 (t, 3H), δ 2.9 (s, 3H), δ 4.22 (q, 2H), δ 5.33 (s, 2H).

(b) *N*-Methyl-*N*-propyloxycarbonylaminomethyl chloride, *N*-methyl-*N*-butyloxycarbonyl-aminomethyl chloride and *N*-methyl-*N*-hexyloxycarbonylaminomethyl chloride were synthesized in an alternate way via a *N*-alkyl carbamic acid alkyl ester as previously described to give the desired alkylating agent (Majumdar and Sloan, 2006b) (Fig. 2).

N-Methyl-*N*-propyloxycarbonylaminomethyl chloride: yield = 85%, ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H), δ 1.7 (m, 2H), δ 3.0 (s, 3H), δ 4.12 (t, 2H), δ 5.35 (s, 2H).

N-Methyl-*N*-butyloxycarbonylaminomethyl chloride: yield = 82%, ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H), δ 1.41 (m, 2H), δ 1.65 (m, 2H), δ 3.0 (s, 3H), δ 4.17 (t, 2H), δ 5.33 (s, 2H).

Table 1
Molecular weights (M_w), melting points (mp), log solubilities in isopropyl myristate ($\log S_{IPM}$), log solubilities in water ($\log S_{AQ}$) and estimated log solubilities in pH 4.0 buffer ($\log_e S_{4.0}$)



Compound	*MW*	mp (°C)	$\log S_{IPM}$ (mM)	$\log S_{AQ}$ (mM)	$\log_e S_{4.0}$ (mM)	J_{MIPM} (μmol cm^{-2} h^{-1})
APAP	151	167–170	0.28	2	1.84	0.51 ± 0.18[a]
1, R = CH_3	252	86–88	1.15	1.66	1.61	1.11 ± 0.15[b]
2, R = C_2H_5	266	75–77	1.3	1.2	1.22	0.61 ± 0.097[c]
3, R = C_3H_7	280	59–60	1.61	0.95	0.91	0.51 ± 0.064[c]
4, R = C_4H_9	294	–	1.83	0.56	nd	0.43 ± 0.076[c]
5, R = C_6H_{13}	322	–	2.12	−0.16	nd	0.22 ± 0.006[d]

[a] Wasdo and Sloan (2004).
[b] **1** gave a statistically significant greater flux than APAP.
[c] **2**, **3** and **4** gave flux values that were not different from APAP.
[d] **5** gave a statistically significant lower flux than APAP.

N-Methyl-*N*-hexyloxycarbonylaminomethyl chloride: yield = 83%, ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H), δ 1.3 (m, 6H), δ 1.6 (m, 2H), δ 3.02 (s, 3H), δ 4.15 (t, 2H), δ 5.33 (s, 2H).

2.1.2. Synthesis of NANAOCAM-APAP prodrugs (Table 1)

Briefly a suspension of APAP (0.01 mol) and triethylamine (0.011 mol) in 25 mL dichloromethane was stirred under reflux conditions for an hour followed by the addition of the alkylating agent, NANAOCAM-Cl (0.011 mol). The contents were stirred overnight and subsequently diluted with 50 mL dichloromethane and washed with water (3× 10 mL). The dichloromethane layer was dried over Na_2SO_4 and concentrated at 40 °C using a rotary evaporator. The yellow oil obtained was then purified by column chromatography using ethyl acetate:hexane as eluent.

1 (R = CH_3) was obtained after recrystallization of an initial oil from CH_2Cl_2:hexane (1:3): yield = 70%, mp = 86–88 °C, R_f (0.36, ether), ^{1}H NMR ($CDCl_3$): δ 7.6 (s, 1H), δ 7.39 (d, 2H), δ 6.96–6.87 (2d, 2H), δ 5.28–5.21 (N–CH_2–O, 2s, 2H), δ 3.72–3.7 (O–CH_3, 2s, 3H), δ 3.0–2.97 (N–CH_3, 2s, 3H), δ 2.0 ($COCH_3$, s, 3H). Anal. calcd. for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.1. Found: C, 56.81; H, 6.34; N, 11.0.

2 (R = C_2H_5) was obtained after trituration of an initial oil with hexane overnight to give yellow crystals. These crystals were recrystallized from ethyl acetate:hexane (1:3) to give white crystals: yield = 65%, mp = 75–77 °C, R_f (0.6, ether), ^{1}H NMR ($CDCl_3$): δ 7.38 (d, 2H), δ 7.12 (s, 1H), δ 6.96 (dd, 2H), δ 5.3 (N–CH_2–O, s, 2H), δ 4.15 (O–CH_2, q, 2H), δ 3.0 (N–CH_3, s, 3H), δ 2.15 ($COCH_3$, s, 3H), δ 1.25 (CH_3, t, 3H). Anal. calcd. for $C_{13}H_{18}N_2O_4$: C, 58.64; H, 6.81; N, 10.52. Found: C, 58.53; H, 6.93; N, 10.47.

3 (R = C_3H_7) was obtained after silica gel column chromatography in ethyl acetate:hexane (3:2) followed by trituration in hexane overnight to give pale white crystals. These crystals were further recrystallized from ethyl acetate:hexane (3:2) to give white crystals: yield = 76%, mp = 58–59 °C, R_f (0.2, ethyl acetate:hexane, 3:2), ^{1}H NMR ($CDCl_3$): δ 7.38 (d, 2H), δ 7.14 (s, 1H), δ 6.96 (dd, 2H), δ 5.3 (N–CH_2–O, s, 2H), δ 4.05 (O–CH_2, t, 2H), δ 3.0 (N–CH_3, s, 3H), δ 2.15 ($COCH_3$, s, 3H), δ 1.65 (CH_2, m, 2H), δ 0.91 (CH_3, t, 3H). Anal. calcd. for $C_{14}H_{20}N_2O_4$: C, 60.01; H, 5.45; N, 12.73. Found: C, 60.1; H, 5.35; N, 12.58.

4 (R = C_4H_9) was obtained after silica gel column chromatography in ethyl acetate:hexane (3:2) followed by trituration in hexane overnight to give a colorless oil: yield = 66%, R_f (0.13, ethyl acetate:hexane, 3:7), ^{1}H NMR ($CDCl_3$): δ 7.38 (d, 2H), δ 7.14 (s, 1H), δ 6.96 (dd, 2H), δ 5.3 (N–CH_2–O, s, 2H), δ 4.05 (O–CH_2, t, 2H), δ 3.0 (N–CH_3, s, 3H), δ 2.15 ($COCH_3$, s, 3H), δ 1.65 (CH_2, m, 2H), δ 1.4 (CH_2, m, 2H), δ 0.91 (CH_3, t, 3H). Anal. calcd. for $C_{15}H_{23}N_2O_4$: C, 61.21; H, 7.53; N, 9.52. Found: C, 61.16; H, 7.65; N, 9.24.

5 (R = C_6H_{13}) was obtained after silica gel column chromatography in ethyl acetate:hexane (4:1) followed by trituration in hexane overnight to give a colorless oil: yield = 63%, R_f (0.36, ethyl acetate:hexane, 7:3), ^{1}H NMR ($CDCl_3$): δ 7.38 (d, 2H), δ 7.29 (s, 1H), δ 6.95–6.89 (2d, 2H), δ 5.29–5.23 (N–CH_2–O, 2s, 2H), δ 4.11–4.07 (O–CH_2, m, 2H), δ 3.02–2.98 (N–CH_3, 2s, 3H), δ 2.15 ($COCH_3$, s, 3H), δ 1.63 (CH_2, m, 2H), δ 1.3 (m, 6H), δ 0.89 (CH_3, m, 3H). Anal. calcd. for $C_{17}H_{27}N_2O_4$: C, 62.33; H, 8.13; N, 8.69. Found: C, 61.96; H, 8.14; N, 8.36.

2.2. Determination of solubilities and partition coefficients

Molar absorptivities were determined in triplicate for each member of the series in acetonitrile (CH_3CN) and pH 7.1 phosphate buffer at the maximum absorption wavelength. The molar absorptivities were calculated using Beer's law ($\varepsilon = A/c$). The solubilities of the prodrugs in isopropyl myristate (IPM) were determined in triplicate by stirring suspensions of each compound in 3 mL IPM with a magnetic stirrer for 24 h at room temperature (23 ± 1 °C) (Martin et al., 1985; Beall et al., 1994). The test tubes containing the suspensions were sealed and thermally insulated from the stirrer. After 24 h, the suspensions were filtered through a 0.45 μm nylon membrane filter. An aliquot (∼0.1–0.3 mL) was withdrawn from the clear filtrate and diluted to 10 mL in a volumetric flask with CH_3CN. The samples were then analyzed by UV spectroscopy using absorbances determined at 240 nm for APAP prodrugs. The solubility in IPM was calculated from the following relationship:

$$S_{IPM} = \left(\frac{A}{\varepsilon}\right)\left(\frac{V_{final}}{V_{aliquot}}\right) \quad (1)$$

where A is the absorbance of the sample at 240 nm, ε the molar absorptivity of the sample at 240 nm in CH_3CN, $V_{aliquot}$ the volume of the saturated filterate aliquot and V_{final} is the final diluted sample volume. In the case of prodrug derivatives which were oils, direct IPM solubility measurements were not possible. Thus partition coefficients between IPM and pH 4.0 buffers and S_{AQ} were used to estimate S_{IPM} (see below).

Solubilities in water (S_{AQ}) were determined by stirring suspensions in deionized water for 1 h to limit the extent of hydrolysis of the prodrugs. The samples were filtered through 0.45 μm nylon filters, diluted with CH_3CN and analyzed by UV spectroscopy. S_{AQ} was determined using the following relationship:

$$S_{AQ} = \left(\frac{A}{\varepsilon}\right)\left(\frac{V_{final}}{V_{aliquot}}\right)$$

Determination of S_{AQ} of compounds which were oils was carried out by stirring the compound in deionized water for 1 h and ensuring that a biphasic solution was present at all times. The test tubes containing the biphasic solution were centrifuged for 2 min; an aliquot (∼0.1–0.3 mL) was withdrawn from the water layer and diluted to 10 mL with CH_3CN in a volumetric flask. The samples were then analyzed by UV spectroscopy as above.

For determination of partition coefficients between IPM and pH 4.0 buffer ($K_{IPM:4.0}$) for compounds which were solids at room temperature, a measured volume (∼0.5–1 mL) of the filtered saturated IPM solutions from the lipid solubility experiments were mixed with a measured volume of pH 4.0 acetate buffer (∼1–5 mL) in a 10 mL test tube (Beall et al., 1993). The test tube was capped and vigorously shaken for 10 s and subsequently centrifuged for 2 min to allow the clear separation of

two phases. An aliquot (~0.3 mL) was withdrawn from the IPM layer and diluted to 10 mL with CH_3CN in a volumetric flask and analyzed by UV spectroscopy. The $K_{IPM:4.0}$ was calculated using the following relationship:

$$K_{IPM:4.0} = \left(\frac{V_{4.0}}{V_{IPM}}\right)\frac{A_F}{A_I - A_F}$$

where $V_{4.0}$ is the volume of pH 4.0 buffer used, V_{IPM} the volume of IPM used, A_I the initial absorbance of the saturated IPM solution before partitioning and A_F is the absorbance after partitioning. The pH 4.0 buffer solubility was estimated from $K_{IPM:4.0}$ using the following relationship:

$$S_{4.0} = \frac{S_{IPM}}{K_{IPM:4.0}}$$

Determination of partition coefficients between IPM and water ($K_{IPM:AQ}$) for compounds which were oils at room temperature was carried out by using a measured volume (~0.5–1.0 mL) of the saturated solutions used for the determination of S_{AQ} which was then placed in a 10 mL test tube with a measured volume (~1–10 mL) of IPM and shaken vigorously for 10 s as above. The test tubes were centrifuged; an aliquot was taken from the water layer and diluted with CH_3CN to 10 mL in a volumetric flask. Samples were subsequently analyzed by UV spectroscopy. $K_{IPM:AQ}$ and S_{IPM} were calculated using the following relationships:

$$K_{IPM:AQ} = \left(\frac{V_{AQ}}{V_{IPM}}\right)\frac{A_I - A_F}{A_F}, \qquad S_{IPM} = K_{IPM:AQ}S_{AQ}$$

Solubility ratios (SR) were calculated from the ratio of S_{IPM}/S_{AQ}. The methylene π values were calculated using the following equation:

$$\pi_{SR} = \frac{\log SR_{n+m} - \log SR_n}{m} \quad (2)$$

where n is the number of methylene units in the promoiety of one prodrug (the lowest member of the homologous series) and m is the number of additional units in the promoiety in the next member of the homologous series. Similarly the methylene π values (Leo et al., 1971; Hansch and Leo, 1979) using K values are also reported.

$$\pi_K = \frac{\log K_{n+m} - \log K_n}{m} \quad (3)$$

2.3. Determination of flux through hairless mice skins

The mice were rendered unconscious using CO_2 and sacrificed by cervical dislocation. Full thickness skins were removed by blunt dissection and placed dermal side down on the diffusion cells. The receptor phase was maintained at 32 °C with a circulating water bath. The receptor side was filled with 20 mL pH 7.1 buffer containing 0.1% (v/v) formaldehyde (2.7 mL of 37% aqueous formaldehyde/liter buffer) to prevent microbial growth (Sloan et al., 1991). No air bubbles were present in the receptor side. A magnetic stir bar was added through the side arm of the receptor compartment and suspended over a stir plate to mix the contents throughout the experiment. The dermal side of the mouse skins were kept in contact with the receptor phase buffer for 48 h prior to application of the donor phase to condition the membranes; the receptor phase was replaced with fresh buffer at least twice to leach out any water soluble UV absorbing material present in the skin which would interfere with the UV quantification of acetaminophen. Pre-application leach periods from 2 to 120 h were found to have no effect on the subsequent flux of theophylline from a standard theophylline/propylene glycol suspension (33 mg/0.5 mL) (Sloan et al., 1986a,b). In all cases, the prodrug was applied as a suspension in IPM. These suspensions were prepared by stirring the test compound for 24 h in 2 mL IPM at room temperature; the final suspension concentration exceeded the compounds solubility by at least 10-fold. Application of suspensions of test compound in the donor phase ensured that all compounds tested were at their maximum thermodynamic activity (Higuchi, 1960; Woodford and Barry, 1982) and allowed direct calculation of the maximum flux for a compound.

A 0.5 mL aliquot of a well stirred IPM suspension of each compound was evenly applied to each of three ($n = 3$) conditioned membrane surfaces. Samples, 5–6 mL of buffer, were removed and analyzed using UV spectroscopy. In order to maintain sink conditions, the entire receptor contents were changed each time a sample was removed. Samples were collected after 8, 19, 22, 25, 28, 31 and 48 h of the initial application of the donor phase. Receptor phases were analyzed by UV within 24 h of sample collection.

After the 48 h first application period, the remaining donor suspension was removed by thoroughly washing the skin with methanol. Methanol wash was found to have minimal effect on the barrier properties of the skin in control studies (Koch, 1986). In order to quantify the amount of dermal penetration of the prodrug, the skins were kept in contact with buffer for an additional period of 24 h. The length of the post-application leach period was sufficient to remove 85–90% of the residual compound in the skin (Siver, 1987).

To evaluate the integrity of membrane, a suspension of 33 mg/0.5 mL of ThH/propylene glycol was applied uniformly to each membrane surface as a second application. Samples were taken after 1, 2, 3, and 4 h and placed in test tubes for analysis by UV spectroscopy (theophylline, $\varepsilon = 1.02 \times 10^4$ L/mol). The entire receptor phase was changed with fresh buffer every time a sample from the receptor phase was removed. An increase in the flux of ThH compared to controls was an indication that the barrier function of the skin had been irreversibly affected by the drug/vehicle combination (Sloan et al., 1986b).

2.4. Determination of prodrug hydrolysis by UV spectroscopy

Absorbance at any wavelength was assumed to be a combination of the absorbances of drug and any intact prodrug. Using Beer's law, the mathematical expression is:

$$A\lambda = C_P\varepsilon_{P\lambda} + C_D\varepsilon_{D\lambda}$$

where $A\lambda$ was the absorbance at a particular wavelength, C_P the concentration of prodrug, C_D the concentration of drug, ε_P the molar absorptivity of prodrug and ε_D was the molar absorptiv-

ity of drug at wavelength λ. By measuring absorbances at two wavelengths it was possible to calculate C_D and C_P.

$$A\lambda_1 = C_P\varepsilon_{P\lambda_1} + C_D\varepsilon_{D\lambda_1}, \qquad A\lambda_2 = C_P\varepsilon_{P\lambda_2} + C_D\varepsilon_{D\lambda_2}$$

Simulataneously solving these equations we arrive at C_D and C_P.

$$C_P = \frac{A\lambda_1\varepsilon_{D\lambda_2} - A\lambda_2\varepsilon_{D\lambda_1}}{\varepsilon_{P\lambda_1}\varepsilon_{D\lambda_2} - \varepsilon_{P\lambda_2}\varepsilon_{D\lambda_1}}, \qquad C_D = \frac{A\lambda_1 - C_P\varepsilon_{P\lambda_1}}{\varepsilon_{D\lambda_1}}$$

C_P and C_D were then added to arrive at the total species of acetaminophen present.

For the APAP prodrugs, λ_1 was 240 nm and λ_2 was 280 nm.

2.5. Calculation of maximum flux

Maximum flux was calculated from the plot of the cumulative amounts of drug species in μmol delivered through the skin versus time in hours. The cumulative amount permeated was calculated by adding the amount that had permeated through the skin at each time interval to the previous total amount. The slope in μmol h^{-1} of the best fit line passing through the steady-state portion divided by the cross sectional area of the diffusion cell (4.9 cm^2) gave the maximum flux, J_M, in μmol cm^{-2} h^{-1}.

2.6. Statistical analyses and regression analyses

Statistical analyses was accomplished using Student's *t* test. Unless otherwise indicated, statistical significance is for $p < 0.05$. Linear regression analysis was accomplished using SAS 9.0.

3. Results and discussion

3.1. Physicochemical properties of NANAOCAM prodrugs of APAP

All prodrugs of APAP had lower melting points than the parent drug (Table 1). Only three of the five prodrugs were solids compared to the AOC-APAP series (Wasdo and Sloan, 2004) in which all the prodrugs were solids. The melting points decreased as carbon chain lengths increased. Thus insertion of –NR′CH_2– into the AOC (ROCO–) promoiety to give the NANAOCAM promoiety leads to a greater decrease in crystalline lattice energy, and hence melting points, compared to the AOC-APAP derivatives.

3.2. Solubilities

The S.D. of the solubilities in isopropyl myristate and water ($n > 3$) were all less than ±5%. The solubilities of prodrugs in IPM, water and estimated solubility in pH 4.0 buffer are shown in Table 1 while molar absorptivities, partition coefficients and π values are shown in Table 2. Lipid solubilities measured in IPM increased as carbon chain length increased. The most lipid soluble member of the series **5** was 69-fold more soluble than APAP while the first member of the series **1** was 7.4-fold more soluble than APAP. For the same alkyl chain length, the IPM solubilities of NANAOCAM-APAP were higher than AOC-APAP prodrugs (Wasdo and Sloan, 2004) probably because the melting points of these prodrugs were considerably lower. Also unlike the AOC-APAP series, a regular increase in solubilities in IPM with increasing chain length was seen in the NANAOCAM series.

The direct water solubilities or estimated pH 4.0 solubilities of all APAP prodrugs were lower than APAP. The most water soluble member of the series **1** was two-fold less water soluble than APAP. This is consistent with AOC-APAP prodrugs where the most soluble member of the series, C_1-AOC-APAP, was about four-fold less soluble in water than APAP. However for the same alkyl chain length, the NANAOCAM prodrugs were more soluble in water than the AOC prodrugs.

The ratios of the solubilities in IPM to AQ ($SR_{IPM:AQ}$) were reasonably well behaved. The average methylene π_{SR} was 0.57 ± 0.05. The π_{SR} value obtained for NANAOCAM-APAP prodrugs closely relate to AOC-APAP series which also had a π_{SR} value of 0.57. The S.D. for partition coefficients determined between IPM and pH 4.0 buffer ($K_{IPM:4.0}$) were all less than ±10%. The average methylene π_K was 0.56 ± 0.05 for the series. The π_K value obtained is in close agreement with that of other series of prodrugs (Beall and Sloan, 2001; Sloan and Wasdo, 2003; Wasdo and Sloan, 2004). Thus π values obtained using solubility ratios are in close agreement with π values

Table 2
Molar absorptivities in acetonitrile and buffer (ε), log solubility ratios between IPM and water (log $SR_{IPM:AQ}$), the differences between log $SR_{IPM:AQ}$ (π_{SR}), the log of partition coefficients between IPM and pH 4.0 buffer (log $K_{IPM:4.0}$), and the differences between log $K_{IPM:4.0}$(π_K)

Compound	CH_3CN[a]	Buffer[a,b]		log $SR_{IPM:AQ}$	π_{SR}	log $K_{IPM:4.0}$	π_K
	ε[c]	ε[c]	ε[d]				
APAP	1.36	0.8	0.12	−1.72			
1	1.43	1.11	0.1	−0.51		−0.45	
2	1.42	1.16	0.13	0.1	0.61	0.08	0.53
3	1.45	1.26	0.15	0.66	0.56	0.69	0.61
4	1.44	1.23	0.13	1.27	0.61	1.27	0.58
5	1.47	1.14	0.14	2.28	0.5	2.28	0.51

[a] Units of 1×10^4 L/mol.
[b] Buffer: pH 7.1 phosphate buffer with 0.11% formaldehyde.
[c] Molar absorptivities measured at 240 nm for compounds APAP, **1**–**5**.
[d] Molar absorptivities measured at 280 nm for compounds APAP, **1**–**5**.

obtained using partition coefficients between IPM and pH 4.0 buffer. This consistency makes π values a robust indicator of consistent homologous series behaviour.

3.2.1. Diffusion cell experiments

The maximum flux obtained for NANAOCAM prodrugs of APAP are presented in Table 1. All J_{MIPM} values for the prodrugs were within the ±30% variation in J values seen for in vitro hairless mouse skin diffusion cell experiments (Sloan et al., 2003; Wasdo and Sloan, 2004). Only two derivatives, **1** (2.18 times) and **2** (1.22 times) gave higher delivery of total species (APAP + prodrug) from IPM than APAP itself, while **3** gave a flux comparable to APAP. The C_6 derivative, **5**, delivered less APAP + prodrug species through the skin than APAP and performed the worst in the series inspite of being the most soluble in IPM. All prodrugs derivatives had higher lipid solubility than the parent drug however it was the most water soluble derivative that gave highest flux through the skin (Sloan et al., 1984; Sloan, 1989, 1992; Sloan and Wasdo, 2003). **1** gave 7.4 times the lipid solubility and 0.46 times the water solubility of APAP but **3** times the water solubility of other members of the series and consequently gave the greatest flux through skin. The most lipid soluble member of the series **5** (69 times higher than APAP) had the lowest flux (0.43 times that of APAP).

NANAOCAM prodrugs of APAP perform marginally better than the alkyloxycarbonyl (AOC) derivatives previously reported because they were more soluble in IPM and in water than the AOC derivatives. The best performing prodrug in the AOC series, C_1-AOC-APAP, was only 6.3 times more lipid soluble (compared to 7.4 times for **1**) and only 0.29 times as water soluble (compared to 0.46 times for **1**) as APAP and exhibited 1.7-fold higher flux than APAP through skin (compared to 2.2 times for **1**). The marginal increase in flux from C_1-NANAOCAM-APAP compared to C_1-AOC-APAP could be accounted for by its higher lipid and aqueous solubilities.

3.2.2. Prodrug bioconversion to parent drug

The percentage of intact prodrug found in the receptor phase was indicative of hydrolysis of prodrugs to the parent drug. For the APAP series, the percentages of intact prodrug C_1 to C_6 (**1**–**5**) in the receptor phase were 78, 77, 78, 76 and 80%, respectively. The poor bioconversion of these derivatives limits their application in topical delivery.

3.2.3. Permeability coefficients and solubility parameter values

When fluxes from IPM (J_{IPM}) were divided by their corresponding solubility in IPM (S_{IPM}), permeability coefficients (P_{MIPM}) were obtained. The $\log P_{MIPM}$ values of the prodrugs synthesized are given in Table 3. P_{MIPM} values decreased along with their respective calculated solubility parameter (Fedors, 1974; Sloan et al., 1986a; Sherertz et al., 1987). A plot of $\log P_{MIPM}$ versus δ_i values for APAP prodrugs (**1**–**5**) gave a positive slope (slope = 1.48, $r^2 = 0.99$, data not shown). Such dependence is consistent with previous results for lipophilic prodrugs of polar heterocyclic drugs like 5-FU (Beall and Sloan, 2001) and phenolic drugs like APAP (Wasdo and Sloan, 2004).

For these NANAOCAM-APAP prodrugs, $\log P_{MIPM}$ decreases linearly with increasing size of the promoiety. This decrease in P_{MIPM} can be explained by the increase in S_{IPM} and decrease of J_{MIPM} with increasing alkyl chain length.

3.2.4. Residual amounts in skin

The residual skin concentrations (C_{rs}) for NANAOCAM prodrugs of APAP are presented in Table 3. The most lipid soluble member of the series, **5**, gave the highest dermal delivery and also had the highest D/T ratio. D/T ratios increased with increased lipophilicity of the prodrugs except for **2**. Although compounds exhibiting higher J_{MIPM} (transdermal flux) were not substantially less effective in delivering total APAP species into the skin (dermal flux, C_{rs}), except for **2**, they had lower D/T ratios because they exhibited higher J_{MIPM}. However, the longer chain prodrugs could preferentially act as a depot form of APAP.

3.2.5. Second application fluxes

The second application theophylline flux (J_J) values are presented in Table 3. Skin penetration by theophylline from propylene glycol was approximately the same or lower than control for skins treated with NANAOCAM prodrugs of APAP. Normalization of the J_{MIPM} values by the respective J_J values

Table 3
log permeability values for prodrugs from IPM through hairless mouse skins ($\log P_{MIPM}$), solubility parameter values (δ_i), residual skin concentrations of total APAP containing species (C_{rs}), ratios of dermal vs. transdermal fluxes (D/T) and second application theophylline flux (J_J) data for flux of theophylline from propylene glycol

Compound	$\log P_{MIPM}$ (cm h^{-1})	δ_i (cal cm^{-3})$^{1/2}$	C_{rs} ± S.D. (μmol)	D/T	J_J ± S.D. (μmol cm^{-2} h^{-1})
1	−1.10	12.71	4.8 ± 1.8	4.3	0.49 ± 0.098[b]
2	−1.51	12.42	1.6 ± 0.1	2.5	0.87 ± 0.16[c]
3	−1.90	12.17	5.4 ± 0.7	10.7	0.52 ± 0.236[c]
4	−2.19	11.95	5.8 ± 0.9	13.5	1.23 ± 0.076[d]
5	−2.78	11.58	6.2 ± 0.9	28.3	0.49 ± 0.093[b]
Control[a]					0.74 ± 0.038

[a] Pretreatment with IPM (Sherertz et al., 1987).
[b] **1** and **5** gave significantly lower J_J values than control.
[c] J_J values for **2** and **3** were not significantly different from control.
[d] **4** gave significantly higher J_J values than control.

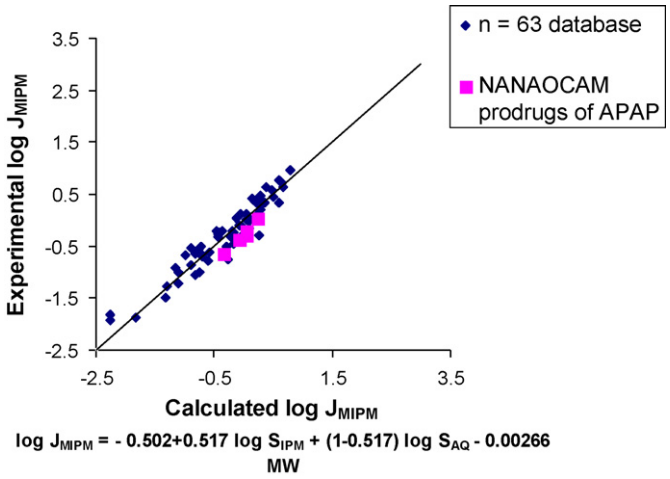


Fig. 3. Experimental vs. calculated log maximum flux values through hairless mouse skin from IPM using Eq. (4) ($n = 63$).

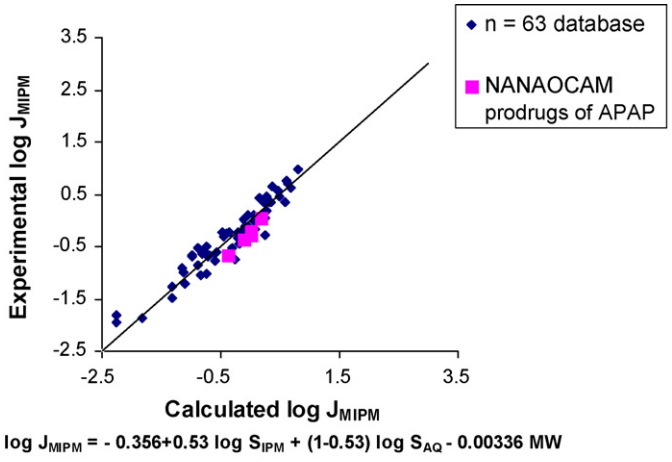


Fig. 4. Experimental vs. calculated log maximum flux values through hairless mouse skin from IPM using Eq. (5) ($n = 68$).

did not change the rank order of the performances of APAP, **1**–**5** (data not shown). Thus, the differences in J_{MIPM} must be due to differences in the abilities of prodrugs to deliver APAP and not damage to the skin barrier.

3.2.6. Modelling the flux of NANAOCAM prodrugs of APAP through hairless mouse skin from IPM using the RS equation

Analysis of flux obtained from NANAOCAM prodrugs data using the Roberts–Sloan (RS) equation allows quantification of the effect of IPM solubility, water solubility and molecular weight on flux through hairless mice skin. The first attempt to predict flux of the APAP prodrugs was carried out using the RS equation derived from the $n = 63$ (including APAP) compound database (Eq. (4); Wasdo, 2005).

$$\log J_{MIPM} = -0.502 + 0.517 \log S_{IPM} + (1 - 0.517)\log S_{AQ} - 0.00266\, MW (r^2 = 0.91) \quad (4)$$

The inclusion of five APAP prodrugs (**1**–**5**) revised the coefficients of the RS model as shown in the following equation:

$$\log J_{MIPM} = -0.356 + 0.53 \log S_{IPM} + (1 - 0.53)\log S_{AQ} - 0.00336\, MW (r^2 = 0.91, n = 68) \quad (5)$$

The NANAOCAM prodrugs of APAP underperformed regardless of the model used. The residual values (experimental $\log J_{MIPM}$ − calculated $\log J_{MIPM} = \Delta \log J_{MIPM}$) for compounds **1**–**5**, was 0.27 log units using Eq. (4) and 0.23 log units using Eq. (5). A plot experimental versus calculated $\log J_{MIPM}$ through hairless mouse skins is shown using Eqs. (4) and (5) in Figs. 3 and 4, respectively. The residual values for the entire database of $n = 68$ was ~0.16 using either Eqs. (4) or (5). Both the models correctly identified the best performing members of the APAP series and rank order of the performance of prodrugs was also correctly identified. The experimental flux, calculated flux and error in predicting flux ($\Delta \log J_{MIPM}$) using each model is given in Table 4. The result reinforces the importance of biphasic solubility to flux. The y coefficient in the RS equation (5) suggests the contribution of solubility in a lipid phase (S_{LIPID}) to be 0.53 and solubility in a water phase (S_{POLAR}) to be 0.47.

Table 4
Calculated flux (calc. $\log J_{MIPM}$) and error in predicting flux ($\Delta \log J_{MIPM}$) for compounds **1**–**5** through hairless mouse skins from IPM

Compound	Calc. $\log J_{MIPM}$ [a]	$\Delta \log J_{MIPM}$ [a]	Calc. $\log J_{MIPM}$ [b]	$\Delta \log J_{MIPM}$ [b]
APAP[c]	0.15	0.44	0.154	0.44
1	0.22	0.18	0.18	0.14
2	0.04	0.25	0.00	0.21
3	0.04	0.34	0.00	0.29
4	−0.07	0.29	−0.11	0.26
5	−0.34	0.32	−0.39	0.27

[a] Predicted from Eq. (4).
[b] Predicted from Eq. (5).
[c] Wasdo and Sloan (2004).

4. Conclusions

NANAOCAM derivatives of acetaminophen were poorly converted to APAP in diffusion cell experiments. This slow hydrolysis of NANAOCAM derivatives of phenol containing drugs may make them potentially useful depot forms of phenols or useful candidates to prevent the premature metabolism of phenols during oral drug delivery.

The delivery of total acetaminophen (APAP) containing species by one of its *N*-alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM) derivatives from IPM is enhanced compared to the parent drug molecule. The more water soluble member of this more lipid soluble series was the most effective at enhancing the delivery of total APAP species through mouse skin from IPM. Insertion of –NR′CH_2– into the AOC promoiety to give NANAOCAM derivatives increased the biphasic solubility and flux of APAP compared to AOC-APAP.

The amount of drug species delivered could be reasonably predicted by the RS equation. The addition of five new compounds to the database of existing 63 compounds for the RS equation gave new coefficients which were not substantially

different from the previous coefficients. The predicted dependence of flux on lipid and water solubility holds for this series of prodrugs.

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