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Topical delivery of *N*-alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM) prodrugs of theophylline (ThH)

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Abstract

N^7-(*N*-Alkyl-*N*-alkyloxycarbonyl) aminomethyl (NANAOCAM) prodrugs of theophylline (ThH) have been synthesized and characterized by their solubilities in isopropyl myristate (S_{IPM}), solubilities in water (S_{AQ}), partition coefficients between IPM and pH 4.0 buffer ($K_{IPM:4.0}$) and by their ability to penetrate hairless mouse skin from IPM (J_{MIPM}). The most lipid soluble and water soluble member, *N*-methyl-*N*-ethyloxycarbonylaminomethyltheophylline, gave the highest flux through hairless mouse skin from IPM compared to ThH. The flux of NANAOCAM prodrugs of ThH can be accurately predicted by the Roberts–Sloan (RS) equation.

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

1. Introduction

Psoriasis is a chronic skin disease characterized by scaling and inflammation. When cells in the outer layer of skin reproduce faster than normal and pile up on the skin's surface, scaling occurs. It presently affects 2.6% of the United States population, or almost 5.8–7 million people. When psoriasis develops, patches of skin thicken, redden, and become covered with silvery scales. Psoriasis most often occurs on the elbows, knees, scalp, lower back, face, palms, and soles of the feet.

Theophylline is commonly used to treat asthma and is partially effective when given orally or topically to treat psoriasis (Berenbein et al., 1979; Iancu et al., 1979). Its mechanism of action is believed to be the inhibition of phosphodiesterases which leads to an increase in cAMP levels whose levels in psoriatic skin are severely compromised (Voorhees and Duell, 1971; Bourne et al., 1974). Since theophylline exhibits a narrow therapeutic range, increasing the oral dose to treat psoriasis is not a viable option. On the other hand, topical delivery of theophylline is limited by its poor solubility in the skin. Prodrugs of theophylline could potentially solve this problem, enabling this old drug to be topically effective in treating psoriasis. Sloan and coworkers have reported acyloxymethyl ($RCOOCH_2$, ACOM), alkyloxycarbonyl (ROCO, AOC), alkylcarbonyl (RCO, AC) and Mannich bases ($–CH_2NRR'$) of ThH as useful prodrugs of ThH (Sloan and Bodor, 1982; Sloan et al., 1984, 2000; Kerr et al., 1998). AOC, AC and Mannich bases of ThH rely on chemical hydrolysis to give ThH. While AOC and AC hydrolyse by an addition-elimination type of mechanism (Fig. 1a), Mannich bases hydrolyse by a S_N1 type of mechanism (Fig. 2). Since the leaving group in both cases is Th^- where ThH has a pK_a of 8.6, the rates of hydrolysis are very fast; e.g. 7-AOC-Th has a $t_{1/2}$ = 1–3 min, 7-AC-Th has a $t_{1/2} < 1$ min and Mannich bases of ThH have $t_{1/2} < 1$ min. Prodrugs with such short half lives make pharmaceutical formulation very difficult, the shelf life of compounds with such short $t_{1/2}$ in water means they require anhydrous storage conditions. This makes it difficult to keep costs of producing the prodrugs down. Thus, a more stable prodrug derivative of ThH needs to be designed which offers the same advantages that AOC, AC or Mannich bases offer in increasing flux through the skin.

7-ACOM-Th derivatives are more stable than the AOC, AC and Mannich bases since they revert to ThH by esterase mediated hydrolysis to give hydroxymethylTh. HydroxymethylTh then falls apart owing to its chemical instability to give formaldehyde and ThH (Bundgaard, 1991). Unfortunately, the increase in flux of ThH generated by 7-ACOM-Th derivatives was only four times.

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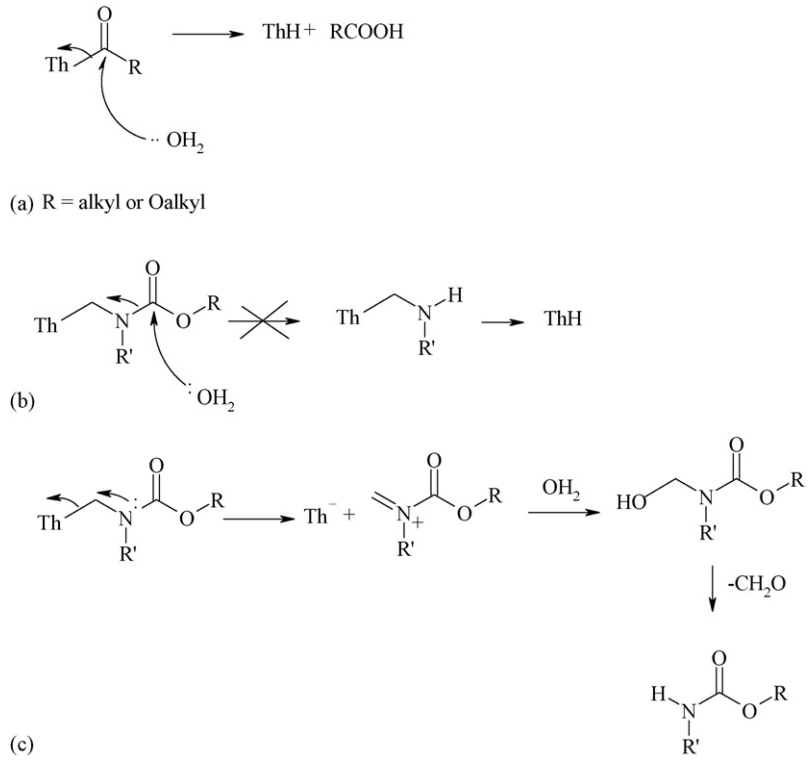


Fig. 1. Hydrolysis of esters and carbonate esters of ThH by addition–elimination (a and b) and hydrolysis of NANAOCAM-Th by a S_N1 type of pathway instead of addition–elimination (c).

N-Alkyl-*N*-alkyloxycarbonylaminomethyl ($ROCONR'CH_2$, NANAOCAM) promoieties, where a $NR'–CH_2$ group is inserted into the AOC promoiety, were designed as chemically more stable, enzymatically labile derivatives of ThH which would exhibit enhanced biphasic solubility. Since the carbonyl functionality in the resulting carbamate is less electrophilic than carbonyls in AC and AOC derivatives, direct nucleophillic attack of water by an addition-elimination type of pathway on the carbonyl of a carbamate does not occur as easily as for an AOC or AC group, thereby imparting even greater chemical stablility over AC and AOC derivatives (Fig. 1b). However, it was subsequently found that NANAOCAM derivatives of ThH hydrolyse by a facile S_N1 type of pathway where the NR'-CH_2 nitrogen donates its pair of electrons to stabilize an incipient carbocation with Th^- leaving (Fig. 1c) (Majumdar and Sloan, 2006a).

The ability of the nitrogen to donate electrons and stabilize the carbocation formed in the transition state is diminished in NANAOCAM derivatives compared to Mannich bases. This is because of the presence of an electron withdrawing alkyloxycarbonyl (ROCO) attached directly to the $NR'–CH_2$ nitrogen in NANAOCAM compared to Mannich bases where the nitrogen is attached to electron donating alkyl groups, edg. The edg aids in stabilizing the carbocation in the Mannich base and increasing its rate of hydrolysis over the NANAOCAM promoiety (Fig. 2).

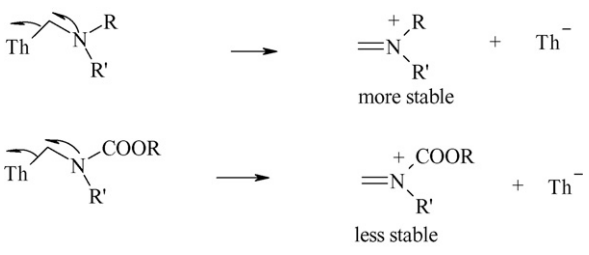


Fig. 2. Carbocations formed by hydrolysis of Mannich bases of theophylline and NANAOCAM-Th.

NANAOCAM derivatives of ThH should improve biphasic solubility in comparison to the ACOM promoiety because a more basic nitrogen atom replaces an oxygen atom. The NANAOCAM promoiety, thus gives medicinal chemists additional flexibility to improve stability as well as solubility (lipid versus water) of prodrugs derived from ThH. For example, NANAOCAM prodrugs of 6-mercaptopurine (6MP) have been reported and the best performing prodrug improved permeation across the skin by four-fold (Siver and Sloan, 1990).

We thereby decided to extend the use of the NANAOCAM promoiety to imides (acidic –NH containing compounds) like ThH and evaluate the diffusion of NANAOCAM-Th derivatives through hairless mouse skins *in vitro* from IPM. The physicochemical characterization of the homologous series of NANAOCAM prodrugs of ThH and their ability to increase skin permeation were evaluated. The performance of the prodrug series in diffusion cell experiments were compared with that predicted by the Roberts–Sloan (RS) equation (Eq. (1)) (Roberts and Sloan, 1999). The data were then added to the Sloan and coworkers database to generate new coefficients for the parameters in the equation and give a more robust RS equation.

$$\log J_{MIPM} = x + y \log S_{IPM} + (1 - y) \log S_{AQ} + z\,MW \quad (1)$$

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. ^{1}H NMR spectra were obtained at 400 MHz on a Varian Mercury-400BB spectrometer. TLC analyses were run on Brinkman Polygram Sil G/uv 254 plates. Ultraviolet spectra were recorded on a Shimadzu UV-2501 PC spectrophotometer. A radiometer pH meter 26 was used to determine pH of solutions. The vertical, Franz type 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). The animal research adhered to the NIH "Principles of Laboratory Animal Care." Statistical analyses were carried out using SAS 9.0.

2.1. Synthesis of prodrug derivatives (Table 1)

Synthesis of the prodrugs (**2**–**7**) was accomplished by the alkylation of ThH with *N*-alkyl-*N*-alkyloxycarbonylaminomethyl 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, as previously reported (Majumdar and Sloan, 2006b).

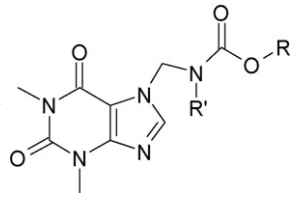
Then theophylline (5.5 mol, 1 equiv.), triethylamine (1.1 equiv.) and 20 mL CH_2Cl_2 were stirred for 20 min. A white suspension formed. The alkylating agent (*N*-alkyl-*N*-alkyloxycarbonylaminomethyl chloride, 1.1 equiv.) was then added to the suspension. An exothermic reaction occurred and the suspension cleared to give a solution which was stirred overnight at room temperature. The reaction mixture was diluted with 50 mL CH_2Cl_2 and extracted with 10 mL 1N HCl, 20 mL $NaHCO_3$ solution and 3× 50 mL water. The CH_2Cl_2 layer was then dried over Na_2SO_4 and concentrated to give a white solid which was crystallized from CH_2Cl_2: ether (1:4) to give pure white crystals of N^7-(*N*-alkyl-*N*-alkyloxycarbonyl)aminomethyl theophyllines (Table 1) as previously reported (Majumdar and Sloan, 2006b).

2.2. Determination of solubilities and partition coefficients

Molar absorbtivities were determined in triplicate for each member of the series in acetonitrile (ACN) and pH 7.1 phosphate buffer (0.05 M, I = 0.11 M, 32 °C) containing 0.1% formaldehyde. The molar absorbtivities were calculated using Beer's law ($\varepsilon = A/c$). The solubilities of the prodrugs in isopropyl myristate (IPM) (Beall et al., 1994) were determined in triplicate by stirring suspensions of the compound in 2 mL IPM with a magnetic stirrer for 24 h at room temperature (23 ± 1 °C). The test tubes containing the suspensions were sealed and thermally insulated from the stirrer. After stirring, 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 of saturated solutions and diluted to 10 mL in a volumetric flask with ACN. The samples were then analyzed by UV spectroscopy and absorbances determined at 273 nm for the ThH prodrugs. The solubility in IPM was calculated using molar absortivities previously determined in triplicate at 273 nm (Table 2).

Table 1

NANAOCAM prodrugs of ThH



Compound	N–R′	O–R
2	CH_3	CH_3
3	CH_3	C_2H_5
4	CH_3	C_3H_7
5	CH_3	C_4H_9
6	CH_3	C_6H_{13}
7	C_2H_5	CH_3

Solubilities in water (S_{AQ}) (Beall et al., 1994) were determined by stirring suspensions in deionized water for 1 h to limit the extent of hydrolysis (Majumdar and Sloan, 2006a) of the prodrugs. The samples were filtered through nylon filters and analyzed by UV spectroscopy. S_{AQ} values were determined using molar absortivities measured at 273 nm (Table 2).

For determination of partition coefficients ($K_{IPM:4.0}$), (Beall et al., 1993) between IPM and pH 4.0 acetate buffer (0.05 M, 32 °C), 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. 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 ACN in a volumetric flask and analyzed by UV spectroscopy as above. The $K_{IPM:4.0}$ was calculated using the following relationship:

$$K_{IPM:4.0} = \frac{V_{4.0}}{V_{IPM}} \times \frac{A_F}{A_I - A_F} \quad (2)$$

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 of the compound remaining after partitioning. The solubility in pH 4.0 buffer can be estimated from $K_{IPM:4.0}$ using the following equation:

$$S_{4.0} = \frac{S_{IPM}}{K_{IPM:4.0}} \quad (3)$$

Solubility ratios (SR) were calculated from the ratio of S_{IPM}/S_{AQ}. The methylene π values were calculated using Eq. (4):

$$\pi = \frac{\log\ SRn + m - \log\ SRn}{m} \quad (4)$$

Table 2
Molar absortivities in acetonitrile and pH 7.1 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] ε[b]	Buffer [a,c]		log $SR_{IPM:AQ}$	π_{SR}	log $K_{IPM:4.0}$	π_K
		ε[d]	ε[e]				
1	0.8	0.62	0.51	−2.12		−2.14	
2	0.79	0.45	0.63	−0.55	0.44	−0.51	
3	0.78	0.47	0.63	−0.11	0.55	−0.04	0.47
4	0.83	0.49	0.65	0.44	0.68	0.41	0.45
5	0.82	0.44	0.6	1.12	0.52	1.07	0.61
6	0.79	0.47	0.55	2.16	0.52	1.97	0.45
7	0.84	0.53	0.64	−0.04	0.51	−0.13	0.38

[a] Units of 1×10^4 L/mol.
[b] Molar absortivities measured at 273 nm for compounds **1**–**7**.
[c] Buffer: pH 7.1 phosphate buffer with 0.11% formaldehyde.
[d] Molar absortivities measured at 260 nm for compounds **1**–**7**.
[e] Molar absortivities measured at 284 nm for compounds **1**–**7**.

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 higher member of the homologous series. Similarly the methylene π values (Leo et al., 1971; Hansch and Leo, 1979) using $K_{IPM:4.0}$ values were also reported.

$$\pi = \frac{\log K_{n+m} - \log K_n}{m} \quad (5)$$

2.3. Determination of flux through hairless mice skins

The diffusion cell experiments were run in essentially the same way as described before (Beall et al., 1994). The mice were rendered unconscious using CO_2 and sacrificed by cervical dislocation. Full thickness skins were removed by dissection along the length of the abdomen; the pieces were scraped to remove excess fat, cut into proper sizes and placed dermal side down on the diffusion cells with the dorsal side in contact with the applied phase. The receptor side of the Franz diffusion cell was filled with about 20 mL of pH 7.1 buffer at 32 °C containing 0.1% (v/v) formaldehyde (2.7 mL of 37% aq. formaldehyde per liter) to prevent microbial growth (Sloan et al., 1991) ensuring that 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 stir the contents throughout the experiment. The mouse skins were kept in contact with 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 quantification of theophylline.

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. A 0.5-mL aliquot of a well-stirred IPM suspension was evenly applied to the conditioned membrane surface. To obtain a sample from the receptor compartment, 5–6 mL of buffer was removed using a Pasteur pipette from the side arm of the receptor and placed in a test tube for quantification using UV spectroscopy. In order to maintain sink conditions, the entire receptor contents were emptied and filled with fresh buffer. Samples were collected 8, 19, 22, 25, 28, 31 and 48 h after the initial application of the donor phase.

After the 48 h initial 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 35 mg/0.5 mL of ThH/propylene glycol was applied uniformly to the membrane surface as a second application. Samples were taken after 1, 2, 3 and 4 h and placed in test tubes for further analysis. The receptor phase was refilled with fresh buffer every time a sample from the receptor phase was taken. An increase in the flux of theophylline 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., 1986).

2.4. Determination of prodrug hydrolysis by UV spectroscopy

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

$$A\lambda = C_P\varepsilon_{P\lambda} + C_D\varepsilon_{D\lambda} \quad (6)$$

where $A\lambda$ was the absorbance at a particular wavelength, C_P concentration of prodrug, C_D concentration of drug, ε_P, molar absortivity of prodrug and ε_D molar absortivity of drug at wavelength λ. By measuring absorbances at two wavelengths it was possible to calculate C_D and C_P.

$$A\lambda_1 = C_P\varepsilon_{P\lambda_1} + C_D\varepsilon_{D\lambda_1} \quad (7)$$

$$A\lambda_2 = C_P\varepsilon_{P\lambda_2} + C_D\varepsilon_{D\lambda_2} \quad (8)$$

Simultaneously, solving these equations gave C_D and C_P.

$$C_P = \frac{A_{\lambda_1}\varepsilon_{D\lambda_2} - A_{\lambda_2}\varepsilon_{D\lambda_1}}{\varepsilon_{P\lambda_1}\varepsilon_{D\lambda_2} - \varepsilon_{P\lambda_2}\varepsilon_{D\lambda_1}} \quad (9)$$

$$C_D = \frac{A\lambda_1 - C_P\varepsilon_{P\lambda_1}}{\varepsilon_{D\lambda_1}} \quad (10)$$

C_P and C_D were then added to give the total species of theophylline present. For ThH prodrugs, λ_1 was 260 nm and λ_2 was 284 nm (Table 2).

2.5. Calculation of maximum flux

Maximum flux was calculated from the plot of the cumulative amounts of drug species in μmol that permeated the skin versus time. The slope 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_{MIPM} in μmol cm^{-2} h^{-1}.

3. Results and discussions

3.1. Melting point behaviour of NANAOCAM prodrugs of ThH

All prodrugs of ThH had lower melting points than the parent drug (Table 3). The melting points decrease as carbon chain lengths increase except for **4**. The even carbon chain member of the series had higher melting points than the odd carbon chain length member. This alternate rise and fall of melting points on addition of a methylene unit to the alkyl chain of a promoiety along a series has previously been observed for several series of homologous series (Chikos and Nichols, 2001; Wasdo and Sloan, 2004; Yalkowsky, 1977).

3.2. Solubilities

The solubilities of prodrugs in IPM and water are shown in Table 3. The S.D. of the solubilities in isopropyl myristate and water were all less than ±5%. All of the prodrugs were more lipid soluble than ThH. IPM solubility generally increased as melting point decreased along the series. The most lipid member of the series **3** was 66-fold more soluble than ThH. The increase in solubility occurs because the promoiety masks a polar N–H group and the short alkyl chains decrease crystal packing efficiency. All NANAOCAM prodrugs of ThH were less water soluble than the parent drug. Water solubility increased on going from **2** to **3** and decreased thereafter from **4** to **6** The most lipid soluble member of the series, **3**, was also the most soluble in water, albeit 2.5-fold less soluble than ThH in water.

Regardless of the irregular behaviour of the absolute solubilities, the ratios of the solubilities in IPM and AQ ($SR_{IPM:AQ}$) (Table 2) were reasonably well behaved. The average methylene π_{SR} was 0.55 ± 0.09 for the series. The S.D. for partition coefficients determined between IPM and pH 4.0 buffer ($K_{IPM:4.0}$) (Table 2) were all less than ±10%. The average methylene π_K was 0.50 ± 0.08 for the series. The π value obtained is in close agreement with other series of prodrugs (Wasdo and Sloan, 2004; Beall and Sloan, 2001; Sloan et al., 2003). Thus, π values obtained using solubility ratios are in close agreement with π values obtained using partition coefficients between IPM and pH 4.0 buffer. This consistency makes π values a robust indicator of homologous series behaviour.

3.3. Diffusion cell experiments

The maximum flux values obtained for NANAOCAM prodrugs of ThH are presented in Table 3. All J_{MIPM} values were within the ±30% variation in J values seen in *in vitro* hairless mouse skin diffusion cell experiments. Only **3** (1.58 times) in the ThH series performed better than ThH at delivering total species from IPM. All other prodrugs delivered less total ThH drug species (ThH + prodrug) through the skin than by ThH itself. The C_6 derivative, **6**, performed the worst of all derivatives even though it was the second most soluble molecule in IPM because it was the least soluble in water. **3** was the most lipid soluble (64 times) and water soluble member (0.39 times) of the series and hence, gave the best maximum flux through skin. In the 7-AOC-Th series, (Sloan et al., 2000) the best performing prodrug was 133-fold more lipid soluble and 0.77 as water soluble as ThH and gave a three-fold increase of flux across the skin. Unfortunately, insertion of the 'N(R′)CH_2' moiety into the AOC moiety does not increase lipid solubility by as much as, and is less water soluble than the best member in the 7-AOC-Th series. As a result, increased delivery of total ThH containing species by the AOC moiety was twice that of the NANAOCAM moiety. Similarly, in the 7-ACOM-Th series (Kerr et al., 1998), the best performing prodrug C_3 was about 0.65 times as water soluble as ThH and 41 times more lipid soluble than ThH and improved flux by

Table 3
Molecular weights (MW), melting points (mp), log solubilities in isopropyl myristate (log S_{IPM}), log solubilities in water (log S_{AQ}), log estimated solubilities in pH 4.0 Buffer ($\log_e S_{4.0}$) and log experimental fluxes of total ThH species from suspensions of **1** to **7** in IPM through hairless mouse skin (log J_{MIPM})

Compound	MW	mp (°C)	log S_{IPM} (mM)	log S_{AQ} (mM)	$\log_e S_{4.0}$ (mM)	log J_{MIPM} (μ mol cm^2 h^{-1})
1, ThH	179	270–274	−0.47	1.66	1.67	−0.32
2, R = CH_3, R′ = CH_3	280	165–166	0.43	0.97	0.93	−0.79
3, R = C_2H_5, R′ = CH_3	294	115–117	1.34	1.25	1.38	−0.12
4, R = C_3H_7, R′ = CH_3	308	128–129	0.88	0.44	0.47	−1.05
5, R = C_4H_9, R′ = CH_3	322	103	1.04	−0.08	−0.02	−1.15
6, R = C_6H_{13}, R′ = CH_3	350	75–77	1.27	−0.88	−0.7	−1.52
7, R = CH_3, R′ = C_2H_5	294	145–147	0.95	0.99	1.17	−0.47

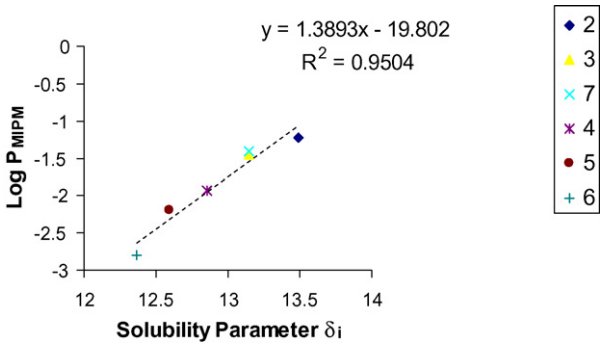


Fig. 3. Plot of solubility parameter vs. log P_{MIPM} for **2–7**.

about four-fold. Thus, the decreased aqueous solubility of the NANAOCAM-Th prodrugs also leads to decreased flux across hairless mouse skins compared to the 7-AOC and 7-ACOM-Th prodrugs.

3.4. Prodrug bioconversion to parent drug

The percentages of intact prodrug **2–7** in the receptor phase were 37, 26, 34, 28, 29 and 32%, respectively. The percentage of intact prodrug remained constant irrespective of chain length.

3.5. Permeability coefficients and solubility parameter values

When flux from IPM (J_{IPM}) was divided by their corresponding solubility in IPM (S_{IPM}), the permeability coefficient (P_{MIPM}) was obtained. The log P_{MIPM} values of the prodrugs synthesized here are given in Table 4. P_{MIPM} values decreased along with their respective calculated solubility parameter. A plot of log P_{MIPM} versus δ_i values for the ThH series (**2–7**) gave a positive slope (Fig. 3). Such dependence has been seen before for lipophilic drugs of polar heterocyclic drugs like 5-FU and phenolic drugs like APAP (Sloan et al., 2003; Wasdo and Sloan, 2004).

For NANAOCAM-Th prodrugs, log P_{MIPM} decreases linearly with increasing chain length 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. However, **3** had a higher J_{MIPM} and S_{IPM} than **2** but a lower P_{MIPM} because the increase in J_{MIPM} on going from **2** to **3** is lower than the increase in S_{IPM} from **2** to **3**. Hence, the ratio J_{MIPM}/S_{IPM} (P_{MIPM}) is lower.

3.6. Residual amounts in skin

The residual skin concentrations (C_{rs}) for NANAOCAM-Th prodrugs are presented in Table 4. Of the homologous series, **3** gave the highest C_{rs} values. This derivative also gave the highest flux through the skin. Thus, **3** gave the highest combination of transdermal and dermal flux in the homologous series. The other prodrugs in the ThH series except for **7** deliver similar amounts of ThH into the skin and no regular trend was seen. Compound **7** actually gives a higher C_{rs} value and gives the second highest flux of all NANAOCAM prodrugs.

3.7. Second application fluxes

The second application theophylline flux (J_{JIPM}) are presented in Table 4. Skin penetration by theophylline from propylene glycol was approximately the same or lower for skins treated with NANAOCAM prodrugs of ThH compared to the control (IPM). Normalization of the J_{MIPM} values by the respective J_{JIPM} values did not change the rank order of the performances of **2–7** (data not shown). Thus, the differences in J_{JIPM} must be due to differences in the abilities of prodrugs to deliver ThH and not damage to the skin barrier.

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

Application of the Roberts–Sloan equation (RS) to analysis of flux obtained from NANAOCAM prodrugs data allows

Table 4
Log permeability coefficients for the ThH prodrugs from IPM through hairless mouse skins (log P_{MIPM}), solubility parameter values (δ_i), residual skin concentration of total ThH species (C_{rs}), second application ThH flux from propylene glycol (J_{JIPM}), error in predicting log J_{MIPM} from RS equation ($n = 63$) and error in predicting log J_{MIPM} from RS equation ($n = 69$)

Compound	log P_{MIPM}[a]	δ_i (cal cm^{-3})$^{1/2}$	C_{rs} (μmol)	J_{JIPM}[b]	Δ log J_{MIPM}[c]	Δ log J_{MIPM}[d]
ThH	0.15	14.05		0.81	0.1	0.12
2	−1.23	13.49	1.358	0.713	0.239	0.148
3	−1.46	13.15	2.064	0.596	0.131	0.0427
4	−1.93	12.85	1.19	0.827	0.391	0.297
5	−2.19	12.59	1.204	0.6	0.295	0.205
6	−2.79	12.36	1.3	0.52	0.321	0.230
7	−1.42	13.15	2.31	0.69	0.154	0.0606
Control				0.74[e]		

[a] Units of cm h^{-1}, calculated from log J_{MIPM} − log S_{IPM} = log P_{MIPM}.
[b] Units of μmol cm^{-2} h^{-1}.
[c] Predicted log $J_{MIPM} = -0.502 + 0.517 \log S_{IPM} + (1–0.517) \log S_{AQ} - 0.00266$ MW from Wasdo, 2005 ($n = 63$). Error in prediction was from experimental log J_{MIPM} − predicted log J_{MIPM}.
[d] Predicted log $J_{MIPM} = -0.232 + 0.549 \log S_{IPM} + (1–0.549) \log S_{AQ} - 0.00389$ MW. Error in prediction was from experimental log J_{MIPM} − predicted log J_{MIPM}.
[e] Pretreatment with IPM (Sheretz et al., 1987).

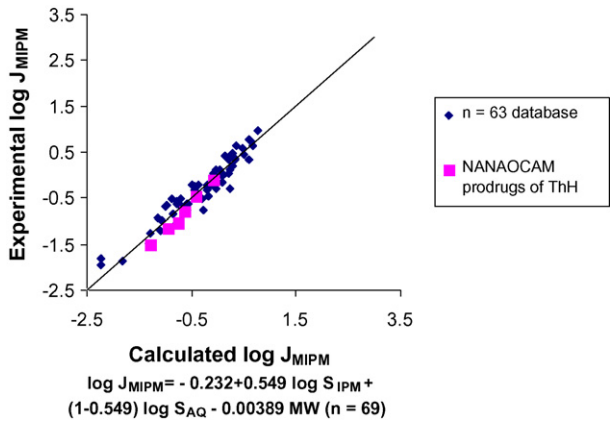


Fig. 4. Experimental vs. calculated log maximum flux values through hairless mouse skin from IPM using Eq. (4).

quantification of the effect of IPM solubility, water solubility and molecular weight on flux through hairless mice skin. First, flux of the ThH prodrugs was predicted using the RS equation derived from the $n = 63$ compound database (Wasdo, 2005) as shown in Eq. (11).

$$\log J_{\mathrm{MIPM}} = -0.502 + 0.517 \log S_{\mathrm{IPM}} + (1 - 0.517) \log S_{\mathrm{AQ}} - 0.00266\ \mathrm{MW}\ \ (r^2 = 0.91) \quad (11)$$

Inclusion of ThH prodrugs (**2–7**) revised the coefficients of the RS model as shown in Eq. (12).

$$\log J_{\mathrm{MIPM}} = -0.232 + 0.549 \log S_{\mathrm{IPM}} + (1 - 0.549) \log S_{\mathrm{AQ}} - 0.00389\,\mathrm{MW}\ \ (r^2 = 0.91,\ \ n = 69) \quad (12)$$

NANAOCAM prodrugs of ThH underperformed regardless of the RS equation used. The residual values (experimental $\log J_{\mathrm{MIPM}}$ − predicted $\log J_{\mathrm{MIPM}} = \Delta \log J_{\mathrm{MIPM}}$) for compounds **2–7** was 0.28 log units according to Eq. (11) and 0.16 log units using Eq. (12). The average error in calculating $\log J_{\mathrm{MIPM}}$ was 0.17 log units for the entire $n = 69$ database which is comparable to the $\log J_{\mathrm{MIPM}}$ of 0.16 log units obtained for the $n = 63$ database. A plot of experimental versus calculated $\log J_{\mathrm{MIPM}}$ through hairless mouse skins is shown using Eq. (12) in Fig. 4. The behaviour of these prodrugs is similar to AOC–APAP prodrugs (Wasdo and Sloan, 2004) which also underperformed. Both equations correctly identified the best performing members of the NANAOCAM-Th series and their rank order of performance was also correctly identified. The errors in predicting flux ($\Delta \log J_{\mathrm{MIPM}}$) using both equations is given in Table 4.

4. Conclusion

For the 7-NANAOCAM-Th series of prodrugs, the most lipid soluble and the most water soluble member of the series was the most effective in delivering total ThH species from IPM through hairless mouse skins. The 7-NANAOCAM-Th prodrugs were generally less effective than both 7-AOC and 7-ACOM prodrugs of ThH because they were generally less soluble in lipid (IPM) and water. However, they were more stable ($t_{1/2} = 420$ min at pH 8.8 buffer and 46 °C; Majumdar and Sloan, 2006a) than their AOC counterparts which should make formulation more feasible. The addition of the 7-NANAOCAM-Th series to the RS database and subsequent fitting of $n = 69$ to the RS equation gave new coefficients that were not substantially different from the previous parameter estimates. The flux of NANAOCAM prodrugs is accurately predicted by the RS equation which reflects the fact that solubility in lipid and water are important criteria for optimizing flux through the skin.

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