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N-Alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM) prodrugs of carboxylic acid containing drugs

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Abstract—Synthesis and hydrolysis in aqueous buffers of novel *N*-alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM) and *N*-aryl-*N*-alkyloxycarbonylaminomethyl (NArNAOCAM) derivatives of carboxylic acid containing drugs were carried out. The hydrolysis follows a S_N1 type mechanism and is dependent on the nucleofugacity of the leaving group. Topical delivery of the NANAOCAM derivative of naproxen from IPM across hairless mice skin was examined in in vitro diffusion cell experiments. The prodrug was 4.5-fold less lipid soluble, 2.4-fold less water soluble and 3.6-fold less permeable than the parent drug.

Carboxylic acid containing drugs are ionized at physiological pH and find it difficult to breach biological membrane barriers. The prodrug approach which transiently masks polar functional groups has proven useful in a number of cases.[1,2] Most carboxylic acid containing drugs are derivatized as alkyl esters, acyloxymethyl (ACOM) or alkyloxycarbonyloxymethyl (AOCOM) esters. These carboxylic acid esters are hydrolysed in vivo by ubiquitous esterases to yield the parent drug.[3] Since these types of prodrugs rely on enzymes to generate the active principle, they are prone to biological variability in tissues and cells and may not generate sufficient amounts of the active principle at the target site to be therapeutically useful.[3] Replacing the oxygen atom in AOCOM by nitrogen gives the *N*-alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM) promoiety. NANAOCAM conjugates of phenols, imides and thiols hydrolysed by a S_N1 type of pathway in aqueous buffers[4] and exhibited improved biphasic solubility and flux from isopropyl myristate (IPM) across hairless mouse skins.[4,5] Extension of the application of the NANAOCAM promoiety to carboxylic acid containing drugs, which exhibit lower pK_a values (~3–5) compared to phenols and imides (~8.6–10), should give prodrugs that hydrolyse chemically rather than relying on enzymes, and may improve permeation through the skin as previously observed.

In the present paper, we report the synthesis of NANAOCAM derivatives of carboxylic acid containing drugs, investigate its mechanism of hydrolysis in aqueous buffers and probe the effect of the NANAOCAM promoiety on penetration of a model carboxylic acid containing drug, naproxen, across hairless mouse skin in vitro from IPM.

Synthesis and hydrolysis studies. NANAOCAM derivatives of five different carboxylic acids were made by alkylating the parent drug with *N*-alkyl-*N*-alkyloxycarbonylaminomethyl chloride (NANAOCAM-Cl)[6] (Table 1). The alkylating agent was synthesized in two steps. Methylamine was reacted with formaldehyde in the presence of an equivalent of NaOH to generate 1, 3, 5-trimethylhexahydrotriazine. Reaction of 1, 3, 5-trimethylhexahydrotriazine with methylchloroformate overnight in dichloromethane gave NANAOCAM-Cl in quantitative amounts.[4,7]

N-Aryl-*N*-alkyloxycarbonylaminomethyl (NArNAOCAM) derivatives of naproxen were also made to illustrate the mechanism of hydrolysis. An aromatic amine was reacted with equimolar amounts of pyridine and methyl chloroformate to yield *N*-arylcarbamic acid methylester. Chloromethylation of *N*-arylcarbamic acid methylester with paraformaldehyde and trimethylsilyl chloride (TMSCl) gave the corresponding

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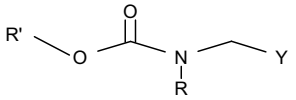
Table 1. Hydrolysis of NANAOCAM derivatives of carboxylic acid containing agents and NArNAOCAM derivatives of naproxen at pH 7.1 and 39 °C and the influence of pK_a of leaving groups on rates of hydrolysis



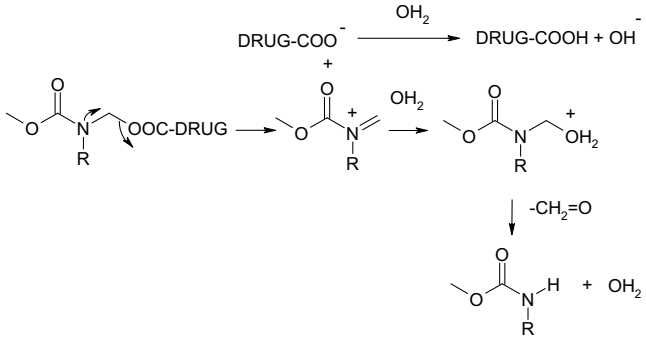
Y = -OOC-Drug
R'= CH_3; R = Alkyl or Aryl.

Compound	R	Y	log$k$ (s^{-1})	$t_{1/2}$ (min)	pK_a
1	CH_3	Naproxen	−2.24	2	4.2
2	CH_3	Indomethacin	−2.5	3.65	4.5
3	CH_3	Ibuprofen	−2.55	4.11	4.41
4	CH_3	Dimethylaminobenzoic acid	−2.72	6	5.2
5	CH_3	Nalidixic acid	−3.55	40	6.0
6	C_6H_5	Naproxen	−4.02	119.62	4.2
7	*p*-MeO–C_6H_4–	Naproxen	−3.63	49.83	4.2
8	*p*-EtOOC–C_6H_4–	Naproxen	−4.13	156.46	4.2

NArNAOCAM-Cl. Alkylation of naproxen in the following step with NArNAOCAM-Cl gave the desired NArNAOCAM prodrugs of naproxen. All compounds synthesized were fully characterized by UV,[8] NMR[8] and elemental analysis.[8]

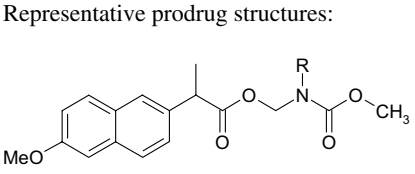
The hydrolysis of NANAOCAM and NArNAOCAM conjugates was investigated in pH 7.1 buffer at 39 °C.[9] All the NANAOCAM-carboxylic acid prodrugs hydrolysed in aqueous buffers to release the parent molecule exhibiting pseudo-unimolecular first order kinetics. The lower pK_a of carboxylic acids makes the rates of hydrolysis faster than phenols, imides and thiols. A plot of log$k$ versus pK_a for **1**–**5** was linear and indicated that rates of hydrolysis were dependent on the nucleofugacity of the leaving group (slope = $\rho = 0.65$, $r^2 = 0.92$, plot not shown). Previously the mechanism of hydrolysis of NANAOCAM derivatives of phenols, imides and thiols was established to be S_N1. To investigate if a S_N1 type of hydrolysis also holds for NANAOCAM derivatives of carboxylic acids, NArNAOCAM derivatives of a model carboxylic acid drug, naproxen, were synthesized (**6**–**8**) and their rates of hydrolysis measured (Table 1).

If the mechanism is S_N1, the rate of hydrolysis should be decreased by the *N*-aryl group where a positive charge on CH_2 in N–CH_2–O is destabilized. Furthermore, as in the case of phenols, the order of rates of hydrolyses should be –COOEt > –H > –OMe which would be consistent with a S_N1 type of hydrolysis (Scheme 1). An electron-donating substituent on the *N*-aryl ring like –OCH_3 stabilizes the S_N1 transition state more than an

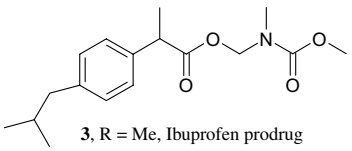


Scheme 1.

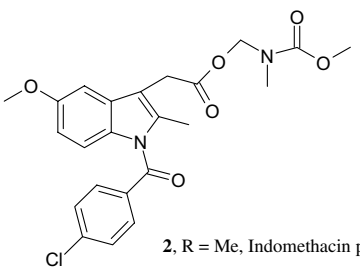
Representative prodrug structures:



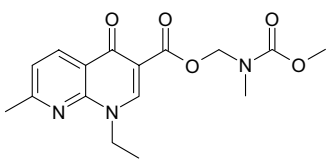
1, R = Me, Naproxen prodrug
6-8, R = Aryl, Naproxen prodrug



3, R = Me, Ibuprofen prodrug



2, R = Me, Indomethacin prodrug



5, R = Me, Nalidixic acid prodrug

electron-withdrawing group like –COOEt. Indeed, the substituted *N*-aryl derivatives increased the half-lives of hydrolyses from 2 min to between 50 and 150 min in the order –COOEt > –H > $–OCH_3$. *N*-Alkylamidomethyl esters of carboxylic acids also hydrolyse by a S_N1 mechanism[2] so there is some precedence for this result.

The kinetics of decomposition of *N*-(4′-ethyloxycarbonylphenyl)-*N*-methyloxycarbonylaminomethyl ester of naproxen, **8**, was also studied in aqueous buffers at 39 °C over a wide pH range (Fig. 1). The rates of hydrolysis were independent of the pH of buffers from pH 4.0 to 8.25 so the mechanism of hydrolyses along this pH range is believed to be S_N1. At pH 9.2 a sharp increase in rates of hydrolysis was observed. This is probably due to a change in the mechanism of hydrolysis from a S_N1 to an addition-elimination where nucleophilic addition

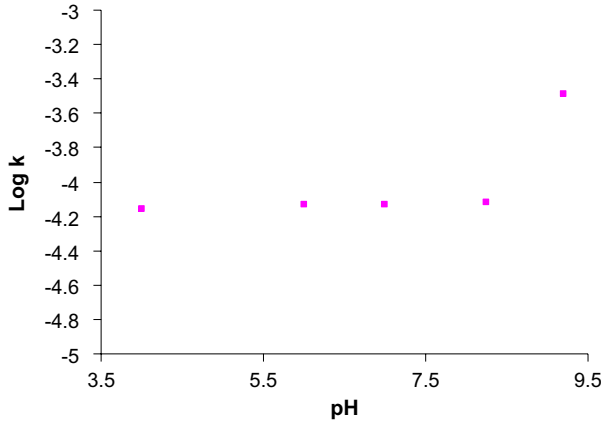
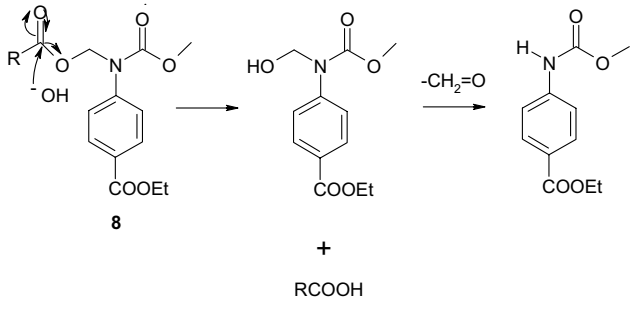


Figure 1. pH rate profile of **8**.



Scheme 2.

occurs on the carbonyl functional group of the ester followed by elimination of carboxylate ion to form *N*-hydroxymethyl-*N*-arylcarbamic acid methyl ester (Scheme 2).[10]

All three NArNAOCAM naproxen derivatives represent potentially useful prodrugs which are reasonably stable but should be sufficiently labile to release the drug at an appropriate rate to express its pharmacological activity. Moreover, since **8** would eventually release *p*-aminobenzoic acid, a component of sunscreens, it may be the most attractive candidate.

The physicochemical characterizations of NANAOCAM prodrugs of naproxen and dimethylaminobenzoic acid and the parent drugs were also carried out (Table 2).[11] Solubility in IPM (S_{IPM}) and partition coefficient between IPM and pH 4.0 buffer ($K_{IPM:4.0}$) were determined. Solubility in pH 4.0 buffer ($S_{e4.0}$) was estimated from the product of S_{IPM} and $K_{IPM:4.0}$ since direct measurement of $S_{4.0}$ was not possible owing to the instability of **1** and **4** in buffer. **1** was 4.5-fold less lipid-soluble and 2.5-fold less water-soluble than naproxen, while **4** was 11.7-fold more lipid soluble and 5.5-fold more water soluble than dimethylaminobenzoic acid. Since naproxen is therapeutically more interesting, we investigated the delivery of **1** across hairless mice skin in vitro from IPM. However, the prodrug, being less water and lipid soluble than the parent drug, performed poorly in comparison to naproxen since it is necessary to improve both the water and lipid solubility of the prodrug compared to the parent drug[12] to improve topical delivery.

Here we have reported NANAOCAM and NArNAOCAM promoieties as novel prodrugs of carboxylic acid containing drugs which hydrolyse by a S_N1 type of pathway. NANAOCAM-carboxylic acids are chemically too unstable to be therapeutically useful, however NArNAOCAM derivatives, being more stable, may be more useful for pharmaceutical formulation. NArNAOCAM-naproxen conjugates are thus prodrugs of a carboxylic acid which unlike ACOM or AOCOM esters are not dependent on enzymatic hydrolysis and have sufficient chemical stability to be formulated. The NArNAOCAM promoiety can also act as spacers or linkers for conjugation of water solubilizing groups like amines and peptides to a carboxylic acid functional group of a drug molecule and thereby increase biphasic solubility and flux through skin. Attempts to make NArNAOCAM-naproxen derivatives with enhanced water solubility are currently under investigation.

Table 2. Estimated solubility in pH 4.0 buffer ($S_{e4.0}$), solubility in IPM (S_{IPM}), log partition coefficients between IPM and pH 4.0 buffer ($\log K_{IPM:4.0}$), flux from IPM through hairless mice skins (J_{MIPM}), residual skin concentration of total drug species (C_{rs}) and permeability coefficients from IPM through hairless mouse skins (P_{MIPM}) of NANAOCAM prodrugs and parent drugs

Compound	$S_{e4.0}$ (mM)	S_{IPM} (mM)	$\log K_{IPM:4.0}$	J_{MIPM} (μmol cm^{-2} h^{-1})	C_{rs} (μmol)	P_{MIPM}[a] (cm h^{-1})	% of parent drug obtained after diffusion
Naproxen	0.27	23.49	1.95	0.36	2.71	0.015	—
1	0.11	5.16	1.67	0.1	4.21	0.019	100
Dimethylaminobenzoic acid	0.38	4.21	1.05				
4	2.09	46.83	1.35				

[a] Calculated from J_{MIPM}/S_{IPM}.

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6. General procedure for alkylation reactions: The alkylation of carboxylic acids with NANAOCAM-Cl and NArNAOCAM-Cl was carried out by reacting equimolar equivalents of carboxylic acid and TEA in CH_2Cl_2 for 1 h followed by addition of the alkylating agent to the reaction mixture. The contents were stirred overnight and worked up by washing with water. The CH_2Cl_2 solution was dried over Na_2SO_4 and then concentrated to an oil which was purified by crystallization or column chromatography.
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8. ^{1}H NMR(400 MHz; $CDCl_3$; Me_4Si) of **1**: δ 7.69 (t, 3H), 7.4 (d, 1H), 7.11–7.15 (m, 2H), 5.41–5.29 (dd, 2H), 3.91 (s, 3H), 3.85 (q, 1H), 3.7–3.65 (2s, 3H), 2.89–2.85 (2s, 3H), 1.57 (d, 3H). UV: λ_{max} (pH 7.1 buffer) 271.5 and 245 nm (ε) 0.52×10^4, 0.77×10^4 L mol^{-1}. Elemental analysis (Found: C, 65.05; H, 6.45; N, 4.2 Calcd for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23%), yield = 97%, mp = 100–101 °C, R_f (0.49, ethyl acetate/hexane, 1:4). ^{1}H NMR(400 MHz; $CDCl_3$; Me_4Si) of **6**: δ 7.69 (t, 3H), 7.36–7.39 (dd, 1H), 7.13–7.17 (m, 5H), 6.9 (d, 2H), 5.5–5.68 (dd, 2H), 3.93 (s, 3H), 3.88 (q, 1H), 3.65 (s, 3H), 1.57 (d, 3H). UV: λ_{max} (pH 7.1 buffer) 272.8 and 245 nm (ε 0.35×10^4, 0.83×10^4 L mol^{-1}), yield = 60%, R_f (0.17, ethyl acetate/hexane, 1:5).
9. The rates of hydrolysis in aqueous buffers were determined by UV spectroscopy. An aliquot (100 μl) of a stock solution of compound dissolved in acetonitrile was added to 2.9 ml of buffer in a cuvette such that the final concentration was about 10^{-5} M. Half-lives were calculated from the plot of $\log(A_\infty - A_t)$ or $\log(-(A_\infty - A_t))$ versus time.
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