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N,N′-Dialkylaminoalkylcarbonyl (DAAC) prodrugs and aminoalkylcarbonyl (AAC) prodrugs of 4-hydroxyacetanilide and naltrexone with improved skin permeation properties

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ABSTRACT

N,N′-Dialkylaminoalkylcarbonyl (DAAC) and aminoalkylcarbonyl (AAC) prodrugs of phenolic drugs acetaminophen (APAP) and naltrexone (NTX) are reported. The effects of incorporation of a basic amine group into the promoiety of an acyl prodrug of a phenolic drug on its skin permeation properties are also presented. DAAC-APAP prodrugs were synthesized via a three-step procedure starting with haloalkylcarbonyl esters which were reacted with five different amines: dimethylamine, diethylamine, dipropylamine, morpholine, and piperidine. The spacing between the amino group and the carbonyl group of the acyl group was 1–3 CH_2. After the hydrolysis of the ester, the carboxylic acid product was subsequently coupled with the parent drug via a dicyclohexyl carbodiimide (DCC) mediated coupling to yield the DAAC-APAP-HCl prodrugs in excellent yields. The AAC prodrugs were synthesized using commercially available Boc-protected amino acids using DCC or EDCI as coupling agents. The yields of the prodrugs synthesized using these two different methods have been compared. Half-lives ($t_{1/2}$) of a few members of the DAAC and AAC series were measured in buffer (pH 6.0, 20 mM). The members evaluated in hydrolysis experiments exhibit a $t_{1/2}$ range of 15–113 min. Among AAC-APAP prodrugs, the isopropyl group in valinate-APAP-HCl exerted a steric effect that increased the $t_{1/2}$ value for this prodrug compared to alaninate-APAP-HCl or prolinate-APAP-HCl. The 2-morpholinylacetate-APAP prodrug was able to achieve twice the flux of APAP in in vitro diffusion cell experiments through hairless mouse skin.

Optimization of the topical delivery of phenols is an attractive goal for research in the areas of pain management (narcotic analgetics) and hormone replacement therapy (estradiol). The delivery of any topically applied drug is defined as the amount of drug diffusing into and through the skin per unit area and time (J, i.e., $\mu mol\ cm^{-2}\ h^{-1}$). Therefore, to enhance the delivery of a topically applied drug, improvement in its flux (J) needs to be achieved.[1] The physicochemical properties of a drug that predict its maximum flux (J_M) are its aqueous and lipid solubilities (S_{AQ}, S_{LIPID}) and its molecular weight (MW): the Roberts–Sloan equation:[2]

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

Since $y = 0.5$–0.6 (above), delivery into (dermal) and through (transdermal) the skin of phenolic drugs is a challenging goal due to a lack of balance in S_{LIPID} and S_{AQ} of these drugs. Obviously, one approach to achieving that balance required by the Roberts–Sloan equation to optimize J_M of the drug is to improve the S_{LIPID} and S_{AQ} of the drug without greatly increasing MW, or changing the ability of the drug to interact with its target receptor or enzyme. Prodrugs, which transiently modify the S_{LIPID} and S_{AQ} of the drug to improve those properties yet revert to the parent drugs in a controlled and predictable manner, is one strategy to accomplish this goal.

In the case of the phenolic drugs, masking the polar OH group may lead to an increase in S_{LIPID} but not S_{AQ} which will not lead to optimization of J_M. In fact, in a homologous series of prodrugs, the member of the series exhibiting the best balance of S_{LIPID} and S_{AQ}, but not necessarily the most lipid soluble member, shows the best J_M. Alkyloxycarbonyl (AOC)[3] acyl type and alkylcarbonyloxymethyl (ACOM),[4] alkyloxycarbonyloxymethyl (AOCOM)[5] and *N*-alkyl-*N*-alkyloxycarbonylaminomethyl (NANAOCAM)[6] soft alkyl type prodrugs of a model phenolic drug, acetaminophen (APAP), have been synthesized, characterized and evaluated in diffusion cell experiments in our lab. Similarly, simple acyl type prodrugs of naltrexone (NTX), a narcotic analgetic, have been synthesized by others.[7,8]

However, optimization of the prodrugs was not sufficient to give clinically effective delivery of NTX. The enhancement in J was primarily due to the increase S_{LIPID} exhibited by the prodrugs

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while only one exhibited increased S_{AQ} and it gave one of the higher J_M values.

More recently, studies investigating the effect of incorporation of ethyleneoxy groups into the promoieties of prodrugs of APAP[9] and NTX[10] on J_M have been reported. Significant increases in S_{LIPID} and S_{AQ} obtained for promoieties containing three ethyleneoxy groups was apparently offset by increased MW caused by ethyleneoxy-associated water molecules which compromised their effectiveness. Thus, in terms of prodrug design, an ideal promoiety is one that increases S_{LIPID} and S_{AQ} without dramatically increasing MW.

The incorporation of basic amine groups into the promoiety should also lead to increased S_{LIPID} (as the free base), and especially S_{AQ}, of prodrugs of phenols. The increased S_{AQ} is the consequence of the ionization of the amine group in water which in turn depends on the pK_a of the amine. Only a few reports describing the synthesis of acyl type aminoalkylcarbonyl (AAC) prodrugs of phenolic drugs containing a basic amine group in the promoiety have been published,[11–13] and in none of those reports was transdermal delivery observed. On the other hand, Milosovich et al. have shown a *N*,*N*-dialkylaminoalkylcarbonyl ester of the aliphatic hydroxy group in testosterone did increase the transdermal delivery of testosterone.[14] However, no synthesis of *N*,*N*-dialkylaminoalkylcarbonyl (DAAC) prodrugs of phenols with different alkyl chain lengths between the amine and the carbonyl groups have been reported. Increasing the alkyl chain lengths should result in increasing pK_a values of the amine groups as they become insulated from the electron withdrawing effect of the carbonyl group and should result in increased S_{AQ} values. Thus, it was of interest to synthesize DAAC and more AAC prodrugs of phenolic drugs and to determine what effect changes in pK_a may have on S_{AQ} and hence on J_M.

Here, we report on efficient synthesis of DAAC and AAC prodrugs of APAP and an AAC prodrug of NTX (Fig. 1). The half lives ($t_{1/2}$) of some of the DAAC-APAP, AAC-APAP and AAC-NTX prodrugs are reported. The pK_a values of the amine group in each prodrug are estimated using the pK_a prediction software pK_a DB 12.0 (ACD Labs, Inc.) (Table 3). The results from an in vitro diffusion cell experiment on one member of the series and its hydrochloride salt using hairless mouse skin and an isopropyl myristate (IPM) vehicle are also reported.

The DAAC prodrugs of APAP (**5a**–**5k**) were synthesized in three steps (Scheme 1). The choice of the amine incorporated into the promoiety was based on the pK_a and the steric bulk of the amine. Five secondary amines were chosen for the synthesis of **3b**, **3c**, **3e**–**3k** which exhibited pK_a values between 10.2 and 8.3: dimethyl-, diethyl-, and dipropylamine, piperidine and morpholine. **3a** and **3d** were commercially available. An alpha (α), beta (β) or gamma (γ) halo ester was then reacted with the secondary amines to give the corresponding *N*,*N*-dialkylaminoalkyl esters **2c**, **2e**–**2k**: **2b** was commercially available. In the cases of the synthesis of **2c** and **2e** (more sterically hindered amines), the ethyl chloroacetate was converted to ethyl iodoacetate by treatment with 1 equivalent of NaI in 10 mL acetone prior to reaction with the amine. The ethyl iodoacetate was not isolated but immediately used. For the synthesis of **2g**, **2h**, **2g**, **2j** and **2k** the corresponding bromo esters were commercially available. The esters were hydrolyzed with HCl to give their corresponding amino acid salts (**3b**, **3c**, **3e**–**3k**) which, along with **3a** and **3d**, were coupled to APAP using dicyclohexylcarbodiimide (DCC) in pyridine to give the hydrochloride salts of the prodrugs (**4a**–**4k**). The free bases of the prodrugs were isolated by rapid (<30 s) partitioning of the hydrochlorides between saturated aqueous $NaHCO_3$ and dichloromethane at 0–4 °C as the starting temperature. If this procedure was not used significant (20%–60%) hydrolysis was observed.

The AAC-APAP prodrugs were synthesized by coupling the corresponding N-Boc protected amino acid with the phenol (APAP or

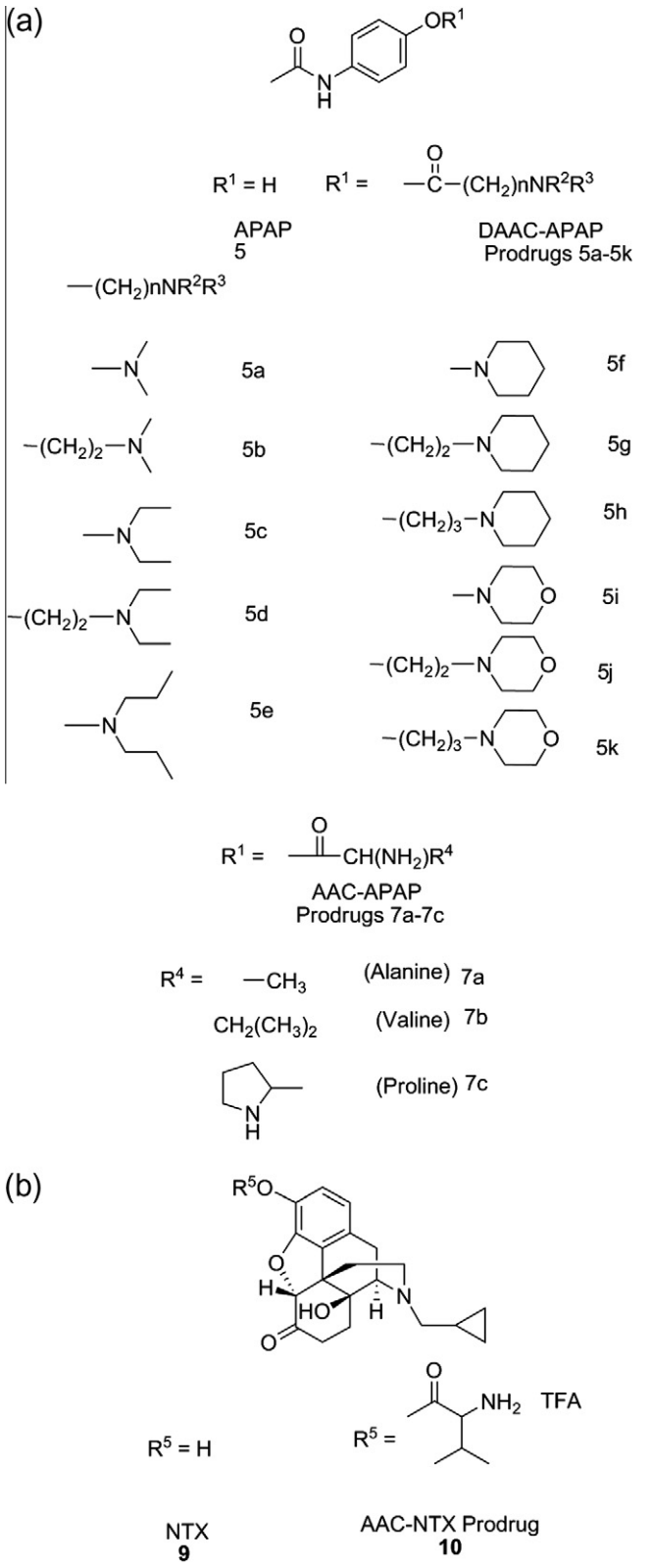
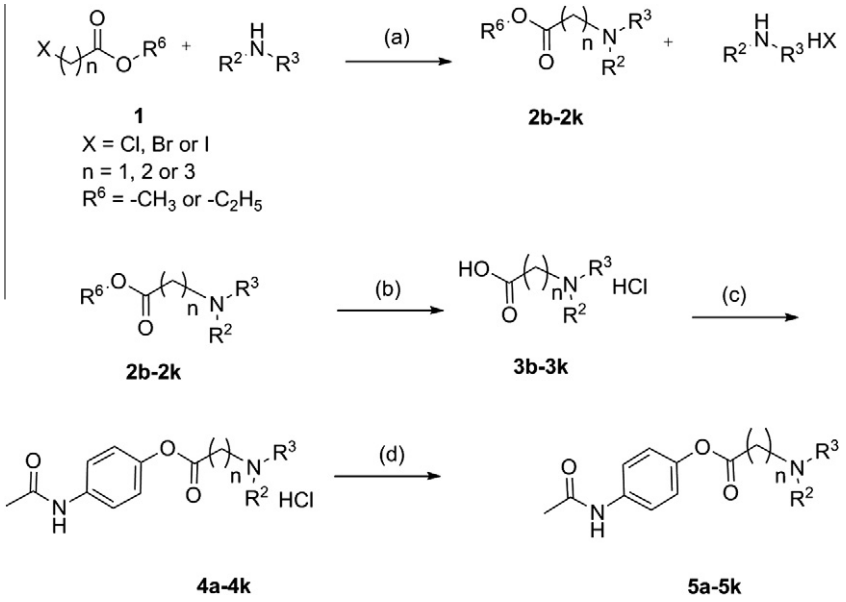


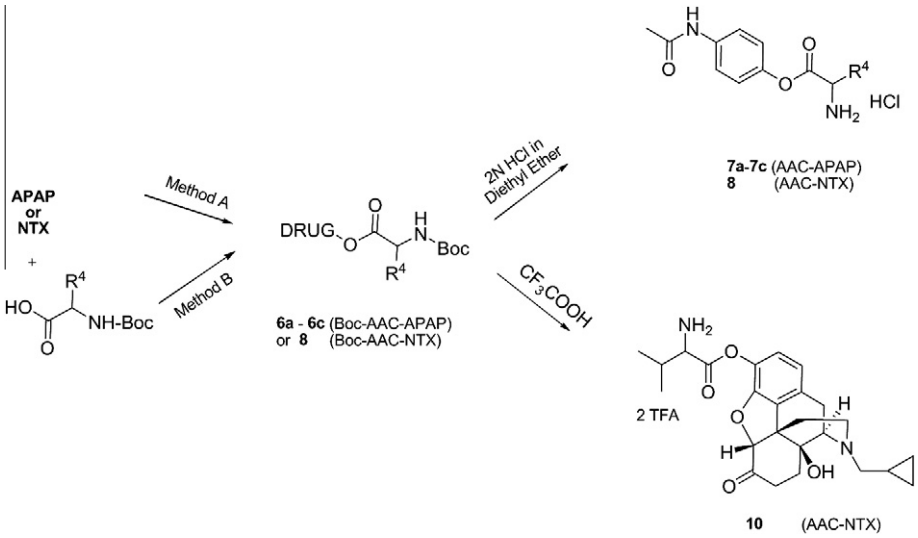
Figure 1. Chemical structures of (a) APAP; (b) NTX and the synthesized DAAC and AAC prodrugs.

NTX) in dichloromethane to give the Boc protected AAC prodrug (**6a**–**6c**). Deprotection with HCl/ether gave the prodrugs as HCl salts (**7a**–**7c**) in good yield (Scheme 2).

When DCC/DMAP was used as the coupling reagent (Method A) to give the Boc protected compounds, purification and further



Scheme 1. Synthesis of DAAC prodrugs of APAP. Reagents and conditions: (a) ACN, 60 °C, 8–12 h; (b) concd HCl, excess, 100 °C, 12 h; (c) DCC/Py, **5**, rt, 24–48 h; (d) aq satd $NaHCO_3$, 0–4 °C, 30 s.



Scheme 2. Synthesis of AAC prodrugs of APAP and NTX. Reagents and conditions: Method A: DCC/DMAP, rt, 8–12 h; Method B: EDCI/CH_2Cl_2, rt, 6 h; (a) 2 N HCl in diethylether, rt, 8 h.

characterization of these compounds was a problem because of the presence of the DCC coupling by-product: Dicyclohexyl urea (DCU). So the Boc-AAC-APAP prodrugs (**6a**–**6c**) were deprotected without purification to give **7a**–**7c** in good yields. In order to obtain pure Boc protected AAC prodrugs, *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide (EDCI) was used as the coupling reagent. However, the yields of **7a**–**7c** were lower. For the coupling of APAP with Boc-amino acid, the presence of DMAP as a catalyst was essential. In the absence of the catalyst the reaction did not go to completion even after 48 h of reaction time. An attempt to synthesize the Boc-AAC-APAP compounds using DCC coupling in pyridine also led to an incomplete (40%–50%) reaction.

The overall yields of AAC prodrugs synthesized by DCC mediated coupling were relatively higher than that obtained with EDCI mediated coupling. Since the Boc-AAC-APAP prodrugs exhibit some water solubility, the lower yield using the EDCI mediated coupling may be due to the loss of the Boc-AAC prodrugs during the aqueous work-up of the reaction mixture.

The relative yields of the compounds synthesized using Method A or Method B have been shown in Table 2. Generally, the yields of DAAC-APAP prodrugs were much higher (Table 1) than the AAC series of prodrugs (Table 2). The results from the elemental analysis of the synthesized prodrugs have been shown in Table S1 (Supplementary data).

The trend in the pK_a values of DAAC-APAP prodrugs (Table 3) can be explained in terms of inductive effect of the carbonyl group. The presence of an electron withdrawing carbonyl group in the beta position causes the electron pair on the N atom to be less 'available'. The increase in the distance between the carbonyl carbon and the amine group makes the amine increasingly more ba-

Table 1
Percentage yields of the DAAC prodrugs (DAAC-APAP-HCl) and the corresponding free base forms (DAAC-APAP) from the hydrochlorides

	% Yield
DAAC-APAP HCl	
4a	75
4b	85
4c	70
4d	80
4e	50
4f	71
4g	65
4h	78
4i	70
4j	50
4k	61
DAAC-APAP	
5a	95
5b	95
5c	78
5d	61
5e	—
5f	71
5g	75
5h	85
5i	70
5j	80
5k	61

Table 2
Percentage yields of the Boc-AAC-APAP compounds and the corresponding AAC prodrugs synthesized from Method A or Method B

% Yield Method A		% Yield Method B
Boc-AAC-APAP		
6a	—	60
6b	—	46[a]
6c	—	31[a]
AAC-APAP-HCl		
7a	74	44
7b	69	57
7c	84	27
AAC-NTX		
8	—	52
10	—	90

[a] Average of two experiments.

Table 3
Half lives ($t_{1/2}$, min) and predicted pK_a values of DAAC-APAP-HCl, AAC-APAP-HCl and VAL-NTX-TFA prodrugs in buffer (pH 6.0, Phosphate, 50 mM, I = 0.5) at 37 ± 2 °C

	pK_a	$t_{1/2}$[a] (min)
4a	6.94	76.92 ± 0.24
4b	8.66	113.03 ± 2.85
4c	8.08	105.71 ± 4.45
4d	9.59	79.32 ± 11.92
4f	6.98	—
4g	8.65	79.67 ± 1.25
7a	8.62	32.33 ± 0.28
7b	7.46	91.37 ± 3.45
7c	8.65	14.90 ± 0.81
10	—	31.78 ± 1.52

[a] All experiments were run in triplicate.

sic. The pK_a of ethyl *N*,*N*-dimethylaminobutyrate is very close to dimethyl amine indicating a weak inductive effect over three methylene groups.[15]

To investigate the transient nature of DAAC and the AAC prodrugs of APAP and NTX, kinetics of hydrolysis of some members of the series was studied. The $t_{1/2}$ values of the prodrugs have been shown in Table 3. The presence of an amine group activates the acyl group towards hydroxide ion attack because of the ($-I$) effect of the amine group and promotes its intramolecular general base catalyzed hydrolysis.[15,16] The $t_{1/2}$ of these activated esters is much smaller than unactivated ester groups which may make these compounds attractive prodrug candidates. Kinetic behavior of esters containing such an activated acyl group (metranidazole *N*,*N*-dimethyl glycinate ester,[17] hydrocortisone lysine ester[18] and acetaminophen glycine, α-aspartic acid and β-aspartic acid esters[12]) has been studied in detail. Bungaard et al. and other investigators have showed that the protonated and unprotonated form of the prodrug undergoes hydrolysis at different rates.[17–19] The half-lives of the amino acid esters show a dependence on the pK_a of the amine in the molecule which governs the ratio of the protonated to the unprotonated form at a particular pH.

The shortest $t_{1/2}$ amongst all the prodrugs evaluated was 14.9 min for the PRO-APAP-HCl prodrug, **7c**. This is an unexpected result based on the steric factors alone. However, it has been previously observed that piperazine-2, 5-diones are formed readily from dipeptide esters particularly those containing N-methylamino acids or proline. In the case of peptides containing proline, geometrical factors take control.[20] For the cyclization of dipeptide esters to piperazines to occur, the peptide bond must be in the cis conformation so that the terminal amino group and the ester carbonyl carbon can interact to form the six-membered ring. For example, glycylproline ethyl ester cyclizes more readily than the prolylglycine ester because of the ease in assumption of the cis conformation. The case of the **7c** can be explained by a similar explanation. Presence of the proline ring system 'fixes' the –NH group in an orientation that favors more effective general base catalysis by the –NH group. In this regard it is worth while to mention that Wu et al.[13] recently reported a decrease in the $t_{1/2}$ of the proline ester prodrug of APAP from 65 min at pH 5.0 to 3.5 min at pH 7.4, which correlates well with our $t_{1/2}$ value of 14.9 min at pH 6.0.

The procedure for the diffusion cell experiments has been described earlier.[3] The measurement of the steady state flux of the prodrug or the parent drug was followed by the measurement of the steady state flux of a standard drug/vehicle combination (theophylline/propylene glycol) as a control experiment to validate the absence of any damage caused to the skin sample during the course of the experiment: control value of 1.02 μmol cm^{-2} h^{-1}.[21] The results from diffusion cell experiments through hairless mouse skin with MORn1-APAP (**5i**) and its hydrochloride (MORn1-APAP.HCL, **4i**) are listed in Table 4. The experimental flux value (EXP log J_M) for **5i** was two times higher compared to APAP whereas the EXP log J_M value for the corresponding hydrochloride,

Table 4
Maximum flux of the prodrugs through hairless mouse skin from a saturated isopropyl myristate (IPM) donor phase (J_{MIPM}), maximum flux of theophylline through hairless mouse skin from a saturated propylene glycol donor phase (J_S), log experimental (EXP) J_{MIPM} calculated using Roberts–Sloan equation (CALC log J_{MIPM}), absolute difference between EXP and CALC log J_{MIPM} values (Δ log J_{MIPM})

	J_{MIPM}[a]	J_S[a]	EXP log J_{MIPM}	CALC log J_{MIPM}[c]	Δlog J_{MIPM}
5i	1.05 ± 0.17	1.35 ± 0.65	0.021	−0.203	0.224
4i	0.54 ± 0.02	0.96 ± 0.25	−0.26[b]	—	—
APAP	0.51[d]	0.74[d]	−0.19	0.262	0.472

[a] Units of μmol cm^{-2} h^{-1}.
[b] Experiment run in duplicate.
[c] Calculated from log CALC $J_{MIPM} = -0.599 + 0.502 \times \log S_{IPM} + 0.498 \times \log S_{4.0} - 0.00235 \times MW$, where S_{IPM} = 2.52 mM and $S_{4.0}$ = 50.51 mM (values from Ref. 18).
[d] Values from Wasdo et al. (2000).

4i was only as high as APAP. The second application flux values J_S were not greater than the control value indicating that the flux enhancement was not a result of damage to the skin sample by the vehicle. The Roberts–Sloan equation[21] was used to calculate the flux of **5i** (CALC $\log J_M$). The absolute difference between EXP $\log J_M$ and CALC $\log J_M$ for **5i** was 0.22 log units. A detailed evaluation of the solubility and permeation properties of the prodrug candidates will be published elsewhere.

In conclusion, a highly efficient synthesis of DAAC and AAC prodrugs of phenol containing drugs, APAP and NTX has been reported. The DAAC-APAP compounds can be scaled up without the requirement of tedious column purification process by a simple recrystallization step. In terms of prodrug design, an important advantage of incorporating a basic amine functional group into an acyl prodrug is that the physicochemical properties, for example, basicity, aqueous or lipid solubilities (unpublished results) and chemical stability of the corresponding prodrugs, can be tailored by varying the amine group, adjusting the alkyl chain length in the promoiety of the DAAC or an appropriate choice or R group in the AAC prodrugs.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.118.

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