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Research Paper

Dimethylamino Acid Esters as Biodegradable and Reversible Transdermal Permeation Enhancers: Effects of Linking Chain Length, Chirality and Polyfluorination

Jakub Novotný,[1] Petra Kovaříková,[2] Michal Novotný,[1] Barbora Janůšová,[1] Alexandr Hrabálek,[1] and Kateřina Vávrová[1,3]

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Purpose. Series of *N,N*-dimethylamino acid esters was synthesized to study their transdermal permeation-enhancing potency, biodegradability and reversibility of action. Effects of chirality, linking chain length and polyfluorination were investigated.
Materials and Methods. *In vitro* activities were evaluated using porcine skin and four model drugs—theophylline, hydrocortisone, adefovir and indomethacin. Biodegradability was determined using porcine esterase, reversibility was measured using electrical resistance.
Results. No differences in activity were found between (*R*), (*S*) and racemic dodecyl 2-(dimethylamino) propanoate (DDAIP). Substitution of hydrocarbon tail by fluorocarbon one resulted in loss of activity. Replacement of branched linking chain between nitrogen and ester of DDAIP by linear one markedly improved penetration-enhancing activity with optimum in 4–6C acid derivatives. Dodecyl 6-(dimethylamino) hexanoate (DDAK) was more potent than clinically used skin absorption enhancer DDAIP for theophylline (enhancement ratio of DDAK and DDAIP was 17.3 and 5.9, respectively), hydrocortisone (43.2 and 11.5) and adefovir (13.6 and 2.8), while DDAIP was better enhancer for indomethacin (8.7 and 22.8). DDAK was rapidly metabolized by porcine esterase, and displayed low acute toxicity. Electrical resistance of DDAK-treated skin barrier promptly recovered to control values.
Conclusion. DDAK, highly effective, broad-spectrum, biodegradable and reversible transdermal permeation enhancer, is promising candidate for future research.

KEY WORDS: biodegradability; permeation enhancers; reversibility; structure–activity relationships; transdermal drug delivery.

INTRODUCTION

Transdermal drug delivery offers many advantages compared to the conventional routes of application including avoidance of the first pass effect, stable blood levels, easy application and higher compliance of the patient (1,2). However, physicochemical properties of the majority of clinically used drugs do not allow them to overcome the skin barrier, which is represented mainly by the uppermost epidermal layer, the stratum corneum (SC). One of the possibilities to temporarily decrease the skin barrier resistance is the use of permeation enhancers (3–5). These compounds promote the permeation of topically applied drugs through SC to achieve the therapeutic concentrations necessary for local or systemic effect. Although hundreds of permeation enhancers have been identified to date, no ideal compound possessing high activity and low toxicity has been found, and the structure-activity relationships are still poorly understood.

Amino acid derivatives belong to the most promising groups of permeation enhancers. Dodecyl 2-(dimethylamino) propanoate (DDAIP, NexACT®, Fig. 1), based on the amino acid alanine, is a clinically used biodegradable transdermal permeation enhancer (6). It is effective in promoting the transdermal permeation of several types of drugs by mechanisms including disordering the lipid organization (7–9), keratin interaction (10) and drug complexation (11). Moreover, DDAIP and its hydrochloride salt have low toxicity, are rapidly metabolized by esterases, and are well tolerated on skin (12). For a review on dimethylamino acid-based enhancers, see Ref. (10).

Being an alanine derivative, DDAIP bears a chiral centre within its polar head. Since the SC lipids, in particular the polar head groups of ceramides, represent a chiral environment, the interaction between DDAIP and skin lipids (7) may be of a stereoselective nature. For a review on chirality in skin permeation, see (5) and (13). Previously, no difference in enhancing effect of (*R*), (*S*) and racemic 6-aminohexanoic acid

[1] Centre for New Antivirals and Antineoplastics, Department of Inorganic and Organic Chemistry, Faculty of Pharmacy Hradec Králové, Charles University in Prague, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic.
[2] Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Hradec Králové, Czech Republic.
[3] To whom correspondence should be addressed. (e-mail: katerina.vavrova@faf.cuni.cz)

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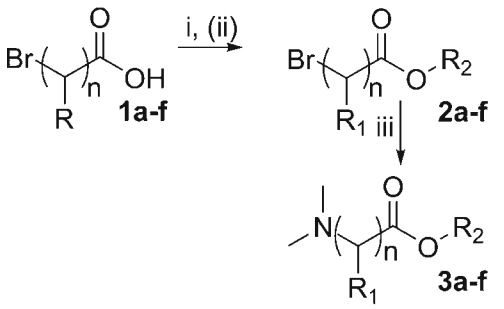

	n	R_1	R_2	synth.
a	1	$-CH_3$	$-C_{12}H_{25}$	i, iii
b	2	-H	$-C_{12}H_{25}$	ii, iii
c	3	-H	$-C_{12}H_{25}$	i, iii
d	4	-H	$-C_{12}H_{25}$	i, iii
e	5	-H	$-C_{12}H_{25}$	i, iii
f	7	-H	$-C_{12}H_{25}$	i, iii
g	1	$-CH_3$	$-C_{10}H_4F_{17}$	i, iii

Fig. 1. Synthesis of *N,N*-dimethylamino acid esters. Reagents and conditions: *i* dodecanol or 1H,1H,2H,2H-perfluoro-1-decanol/DCC/DMAP/ethyl acetate /0°C; *ii* (1) $SOCl_2$, reflux; (2) dodecanol/$CHCl_3$, reflux; *iii* $(CH_3)_2NH$/THF.

2-octyl ester was found (14). However, those compounds are relatively weak enhancers with the chiral centre in the hydrophobic chain, not in the polar head, which is expected to interact with the chiral polar heads of ceramides. Therefore, we focused on DDAIP as a model chiral enhancer to evaluate the hypothesis that the enhancer action may be dependent on its configuration.

DDAIP is an amphiphilic substance possessing a bulky polar head and a 12C alkyl chain. The structure-activity relationships of enhancer hydrophobic chain(s) are well documented with the optimum usually at around 10–12 carbons in saturated chains (15,16). Nevertheless, there is no study concerned with the effect of polyfluorination of an enhancer. Fluorocarbon chains in general have exceptional chemical and biological inertness, unique hydro and lipophobicity, have greater cross-sectional area, are stiffer, and fluorocarbon surfactants are more surface-active than their hydrocarbon analogues (17,18). Highly fluorinated materials have potential as pulmonary, topical and ophthalmological drug delivery systems (19). All of these properties may influence the enhancer behavior in the skin barrier.

Another structural feature of DDAIP is that the dimethylamino group is positioned on the α-carbon resulting in a sterically demanding polar head group. We have previously described dodecyl 6-(dimethylamino)hexanoate (DDAK, Fig. 1) being even more active enhancer than DDAIP for theophylline (20) and adefovir (21,22). DDAK was designed by combining the 5-carbon linking group between the ionizable nitrogen and the enzymatically labile ester group of Transkarbam 12, a highly potent non-toxic permeation enhancer (21,23), and the *N,N*-dimethylamino polar head from DDAIP. Based on these findings, we aimed to compare DDAIP and DDAK in a greater detail, particularly the linking chain structure.

The purpose of this study was to synthesize a series of DDAIP analogues to study the effects of chirality, polyfluorination and linking chain length on their transdermal permeation-enhancing potency. We aimed to evaluate their activity using four model drugs with distinct physicochemical properties, determine the biodegradability of the most potent compound by porcine esterase, and confirm the reversibility of its action by transdermal electrical resistance measurement.

MATERIALS AND METHODS

Chemicals and Instrumentation

All chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany). Silica gel 60 (230–400 mesh) for column chromatography and TLC plates (silica gel 60 F_{254}, aluminum back) were obtained from Merck (Darmstadt, Germany). The structure and purity of the synthesized compounds were confirmed by FTIR (Nicolet Impact 400 spectrophotometer) and ^1H and ^{13}C NMR spectra (Varian Mercury-Vx BB 300 instrument, operating at 300 MHz for ^1H, 75 MHz for ^{13}C) and optically active compounds were characterized by their optical rotation (ADP Bellingham and Stanley Polarimeter; 1.0 dm cell). The melting points were measured with a Kofler apparatus, and are uncorrected.

Synthesis

*General procedure for the preparation of the bromocarboxylic acid esters (**2a, 2c-2g**).* Bromo acid (9.8 mmol), 9.8 mmol of an alcohol and 0.12 g (0.98 mmol) of 4-dimethylaminopyridine (DMAP) in 20 ml of ethyl acetate was cooled to 0°C and 2.22 g (10.8 mmol) of dicyclohexylcarbodiimide (DCC) in 15 ml of ethyl acetate was added. The reaction was allowed to reach room temperature, and then was stirred overnight. The unreacted DCC was removed by addition of a droplet of acetic acid. The resulting dicyclohexylurea was filtered off and washed with small amount of ethyl acetate. After a water/diethyl ether extraction work up, the pure product was obtained as a colorless liquid by column chromatography using ethyl acetate/hexane elution system.

Dodecyl 2-bromopropanoate (**2a**). Yield=87%. ^1H NMR (300 MHz, $CHCl_3$): δ 4.36 (q; J=6.9 Hz; 1H), 4.09–4.22 (m; 2H), 1.82 (d; J=6.9; 3H), 1.61–1.71 (m; 2H), 1.26–1.38 (m; 18H), 0.88 (t; J=6.6 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 170.3, 66.1, 40.3, 31.9, 29.6, 29.5, 29.3, 29.2, 28.4, 25.7, 22.7, 21.7, 14.1; IR (ATR): ν_{max} 2,922, 2,853, 1,739, 1,219, 1,157 cm^{-1}.

Dodecyl 4-bromobutanoate (**2c**). Yield=72%. ^1H NMR (300 MHz, $CHCl_3$): δ 4.07 (t, J=6.7 Hz, 2H), 3.46 (t, J=6.5 Hz, 2H), 2.49 (t, J=7.2 Hz, 2H), 2.12–2.21 (m, 2H), 1.57–1.67 (m, 2H), 1.25–1.41 (m; 18H), 0.87 (t, J=6.7 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 170.3, 65.2, 37.8, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 28.5, 25.8, 22.7, 21.7, 14.1; IR (ATR): ν_{max} 2,922, 2,853, 1,734, 1,198, 1,170 cm^{-1}.

Dodecyl 5-bromopentanoate (**2d**). Yield=84%. ^1H NMR (300 MHz, $CHCl_3$): δ 4.06 (t, J=6.7 Hz, 2H), 3.41 (t, J=6.5 Hz, 2H), 2.33 (t, J=7.5 Hz, 2H), 1.74–1.94 (m, 4H), 1.56–1.69 (m, 2H), 1.25–1.41 (m; 18H), 0.87 (t, J=6.7 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.2, 64.6, 33.3, 33.0, 31.0, 29.6, 29.5, 29.3, 29.2, 28.6, 25.9, 23.5, 22.7, 14.1; IR (ATR): ν_{max} 2,922, 2,853, 1,731, 1,458, 1,253, 1,170 cm^{-1}.

Dodecyl 6-bromohexanoate (**2e**). Yield=83%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.05 (t; J=6.7 Hz; 2H), 3.40 (t; J=6.7 Hz; 2H), 2.31 (t; J=7.4 Hz; 2H), 1.83–1.92 (m; 2H), 1.56–1.70 (m; 4H), 1.42–1.52 (m; 2H), 1.25–1.30 (m; 18H), 0.87 (t; J=6.7 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.5, 64.5, 34.1, 33.4, 32.4, 31.9, 29.6, 29.5, 29.3, 29.2, 28.6, 27.6, 25.9, 24.1, 22.7, 14.1; IR (ATR): ν_{max} 2,923, 2,853, 1,734, 1,463, 1,253, 1,173 cm^{-1}.

Dodecyl 8-bromooctanoate (**2f**). Yield=56%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.05 (t; J=6.9 Hz; 2H), 3.40 (t; J=6.9 Hz; 2H), 2.29 (t; J=7.5 Hz; 2H), 1.80–1.90 (m; 2H), 1.56–1.67 (m; 4H), 1.26–1.48 (m; 24H), 0.88 (t; J=6.6 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.9, 64.5, 34.3, 33.9, 32.7, 31.9, 29.6, 29.3, 29.2, 28.9, 28.6, 28.4, 27.9, 25.9, 24.9, 22.7, 14.1; IR (ATR): ν_{max} 2,923, 2,853, 1,735, 1,465, 1,458, 1,235, 1,173 cm^{-1}.

1H,1H,2H,2H-Perfluorodecyl 2-bromopropanoate (**2g**). Yield=88%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.48 (t; J=6.4 Hz; 2H), 4.37 (q; J=6.9 Hz; 1H), 2.60–2.44 (m; 2H), 1.83 (d; J=7.0 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 169.8, 57.7, 39.3, 30.6, 30.3, 30.1, 21.4; IR (ATR): ν_{max} 2,982, 2,930, 1,748, 1,449, 1,206, 1,152 cm^{-1}.

*Dodecyl 3-bromopropanoate (**2b**).* A mass of 1.50 g (9.8 mmol) of 3-bromopropanoic acid was refluxed with 5 ml of thionyl chloride for 2 h. Thionyl chloride was evaporated under vacuum, the residue dissolved in 5 ml of chloroform and added to the solution of 1.83 g (9.8 mmol) of dodecanol in 8 ml of chloroform. The mixture was kept under reflux for 6 h. The product was purified on silica with hexane/ethyl acetate. Yield=66%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.12 (t; J=6.7 Hz; 2H), 3.58 (t; J=6.9 Hz; 2H), 2.91 (t; J=6.9 Hz; 2H), 1.58–1.68 (m; 2H), 1.26–1.44 (m; 16H), 0.88 (t; J=6.7 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 172.6, 64.8, 32.7, 32.5, 31.9, 29.6, 29.5, 29.3, 29.2, 28.6, 27.8, 25.9, 22.7, 14.1; IR (ATR): ν_{max} 2,922, 2,853, 1,737, 1,466, 1,234, 1,172 cm^{-1}.

*General procedure for the preparation of N,N-dimethylaminoalkanoates (**3a–3g**).* Bromo ester **2a–2g** (4.67 mmol) was dissolved in 10 ml of tetrahydrofurane (THF). Eleven milliliter of 2.0 M dimethylamine solution in THF was added by syringe and the mixture was stirred for 24 h at room temperature. The suspension was filtered and the filtrate was concentrated under vacuum. Compounds **3a, 3e, 3f** and **3g** were purified on silica column using hexane/ethyl acetate–ethyl acetate. The other compounds (**3b–d**) were dissolved in 50 ml of dry diethyl ether and gently bubbled with hydrogen chloride. White crystals of an ammonium salt appeared immediately and the mixture was bubbled with nitrogen to remove the unreacted hydrogen chloride. The solid was filtered off and recrystallized from chloroform/diethyl ether. The crystals were suspended in diethyl ether, corresponding amount of 5% solution of hydrogen carbonate was added and the free base was extracted. The organic phase was separated, treated with saturated solution of KBr, dried over sodium sulfate and concentrated in vacuum yielding colorless oily liquid.

Dodecyl 2-(dimethylamino)propanoate (**3a**, DDAIP). Yield=96%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.08–4.13 (m; 2H), 3.18–3.25 (q; J=7.0 Hz; 1H), 2.34 (s; 6H), 1.59–1.69 (m; 2H), 1.25–1.29 (m; 21H), 0.87 (t; J=6.9 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.3, 64.5, 62.9, 41.8, 31.9, 29.6, 29.5, 29.3, 29.2, 28.7, 25.9, 22.7, 15.1, 14.1; IR (ATR): ν_{max} 2,923, 2,853, 1,731, 1,454, 1,167 cm^{-1}.

Dodecyl 3-(dimethylamino)propanoate (**3b**). Yield=86%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.06 (t; J=6.9 Hz; 2H), 2.61 (t; J=7.2 Hz; 2H), 2.47 (t; J=6.9 Hz; 2H), 2.24 (s; 6H), 1.56–1.65 (m; 2H), 1.25–1.35 (m; 18H), 0.87 (t; J=6.9 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 172.6, 64.6, 54.7, 45.2, 32.9, 31.9, 29.6, 29.5, 29.3, 29.2, 28.6, 25.9, 22.7, 14.1; IR (ATR): ν_{max} 2,923, 2,853, 1,736, 1,461, 1,168 cm^{-1}.

Dodecyl 4-(dimethylamino)butanoate (**3c**). Yield=71%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.05 (t; J=6.9 Hz; 2H), 2.26–2.36 (m; 4H), 2.22 (s; 6H), 1.74–1.84 (m; 2H), 1.56–1.65 (m; 2H), 1.25–1.35 (m; 18H), 0.88 (t; J=6.9 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.6, 64.5, 58.5, 45.3, 32.1, 31.9, 29.6, 29.5, 29.3, 29.2, 28.6, 25.9, 22.9, 22.6, 14.1; IR (ATR): ν_{max} 2,923, 2,853, 1,736, 1,461, 1,181 cm^{-1}.

Dodecyl 5-(dimethylamino)pentanoate (**3d**). Yield=60%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.04 (t; J=6.9 Hz; 2H), 2.31(t; J=7.2 Hz; 2H), 2.25 (t; J=7.2 Hz; 2H), 2.20 (s; 6H), 1.55–1.56 (m; 4H), 1.43–1.53 (m; 2H), 1.25–1.36 (m; 18H), 0.87 (t; J=6.9 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.7, 64.5, 59.3, 45.5, 34.2, 31.9, 29.6, 29.5, 29.3, 29.2, 28.6, 27.2, 25.9, 22.8, 22.7, 14.1; IR (ATR): ν_{max} 2,923, 2,853, 1,736, 1,459, 1,174 cm^{-1}.

Dodecyl 6-(dimethylamino)hexanoate (**3e**, DDAK). Yield=80%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.04 (t; J=6.9 Hz; 2H), 2.29 (t; J=7.5 Hz; 2H), 2.23 (t; J=7.5 Hz; 2H), 2.20 (s; 6H), 1.55–1.68 (m; 4H), 1.42–1.52 (m; 2H), 1.25–1.37 (m; 20H), 0.87 (t; J=6.9 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.8, 64.4, 59.6, 45.5, 34.3, 31.9, 29.6, 29.5, 29.3, 29.2, 28.6, 27.4, 27.0, 25.9, 24.9, 22.7, 14.1; IR (ATR): ν_{max} 2,923, 2,853, 1,736, 1,459, 1,170 cm^{-1}.

Dodecyl 8-(dimethylamino)octanoate (**3f**). Yield=87%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.05 (t; J=6.9 Hz; 2H), 2.28 (t; J=7.5 Hz; 2H), 2.22 (t; J=7.2 Hz; 2H), 2.20 (s; 6H), 1.56–1.66 (m; 4H), 1.39–1.49 (m; 2H), 1.25–1.31 (m; 24H), 0.87 (t; J=6.9 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.9, 64.4, 59.9, 45.5, 34.4, 31.9, 29.6, 29.5, 29.3, 29.2, 29.1, 28.6, 27.7, 27.3, 25.9, 25.0, 22.7; 14.1; IR (ATR): ν_{max} 2,924, 2,853, 1,736, 1,459, 1,168 cm^{-1}.

1H,1H,2H,2H-Perfluorodecyl 2-(dimethylamino)propanoate (**3g**). Yield=86%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 4.42 (t; J=6.4 Hz; 2H), 3.26 (q; J=7.0 Hz; 1H), 2.41–2.58 (m; 2H), 2.34 (s; 6H), 1.29 (d; J=7.0 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 172.8, 62.7, 56.2, 41.6, 30.9, 30.6, 30.3, 14.7; IR (ATR): ν_{max} 2,985, 2,945, 2,873, 2,834, 2,787, 1,735, 1,458 cm^{-1}.

Preparation of DDAIP enantiomers. 2-(*tert*-butoxycarbonylamino)propanoic acid (**4h**, **4i**). A mass of 1.47 g (6.74 mmol) of di-*tert*-butyl dicarbonate in 6 ml of dioxane was added to an ice cold solution of 0.5 g (5.61 mmol) of L- or D-alanine in 10 ml of 1M sodium hydroxide. The reaction was stirred for 0.5 h at 5°C and subsequently for 3.5 h at room temperature. The mixture was concentrated to half of its volume on rotary evaporator, cooled to 0°C, and acidified to pH 2–3 by slow addition of 1 N $KHSO_4$. Product was extracted with ethyl acetate, dried over Na_2SO_4 and concentrated under vacuum providing white crystalline solid with melting point of 81–82°C. (*R*)-isomer (**4h**). Yield=90%. (*S*)-isomer (**4i**). Yield=93%. ^{1}H NMR (300 MHz, $CHCl_3$): δ 5.05 (d; J=5.7 Hz; 1H), 4.34 (m; 1H), 1.45 (s; 9H), 1.43 (s; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 177.9, 155.4, 80.3, 49.1, 28.3, 18.3.

Dodecyl 2-(tert-butoxycarbonylamino)propanoate (**5h**, **5i**). A mass of 1.00 g (5.29 mmol) of the protected acid **4h**

or **4i**, 0.99 g (5.29 mmol) of dodecanol and 0.065 g (0.53 mmol) of DMAP was dissolved in 10 ml of ethyl acetate. The mixture was cooled to 0°C and 1.20 g (5.82 mmol) of DCC in 10 ml of ethyl acetate was added. The mixture was allowed to warm to room temperature and stirred for additional 24 h. Subsequently, the reaction was quenched by addition of a droplet of acetic acid and filtered. The filtrate was concentrated under vacuum, and purified on silica column using hexane/ethyl acetate 19:1. (*R*)-isomer (**5h**). Yield=65%. $[\alpha]_D^{22.7°C}$=2.77° (1.0, $CHCl_3$), (*S*)-isomer (**5i**). Yield=68%. $[\alpha]_D^{22.9°C}$=−2.84° (1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CHCl_3$): δ 5.05 (d; *J*=5.7 Hz; 1H), 4.29 (m; 1H), 4.9–4.15 (m; 2H), 1.63 (m; 2H), 1.44 (s; 9H), 1.37 (d; *J*=7.2 Hz; 3H), 1.25–1.32 (m; 18H), 0.87 (t; *J*=6.7 Hz; 3H); ^{13}C NMR (75 MHz, $CHCl_3$): δ 173.4, 155.1, 79.7, 65.5, 49.2, 31.9, 29.6, 29.5, 29.3, 29.2, 28.5, 28.3, 25.8, 22.7, 18.8, 14.1.

Dodecyl 2-aminopropanoate. 5 ml of trifluoroacetic acid (TFA)/dichlomethane (1:1 *v*/*v*) mixture was added to 0.5 g (1.40 mmol) of the *N*-protected ester (**5h**, **5i**). TLC (butanol/water/acetic acid 4:1:1, Rf=0.79) indicated a full deprotection of the amino group in 30 min. The solvent was evaporated in vacuum, oily residue dissolved in 20 ml of dichloromethane, cooled on ice and neutralized with 25 ml of ice cold 2% solution of sodium bicarbonate. The aqueous phase was extracted with additional 2×20 ml of dichloromethane. The organic phase was dried over sodium sulfate, concentrated and used without further purification.

Dodecyl 2-(dimethylamino)propanoate (**6h**, **6i**). A mass of 0.35 g (1.35 mmol) of dodecyl 2-aminopropanoate was dissolved in 20 ml of dry dichloromethane and 214 µl of 35% formaldehyde solution was added. Then, 1.14 g (5.38 mmol) of sodium triacetoxyborohydride (24) was added and the mixture was stirred for 2 h at room temperature. The reaction was cooled to 0°C and extracted with 0.25 M sodium bicarbonate. The products were obtained after separation on silica using hexane/ethyl acetate 4:1 as a colorless liquid. (*R*)-isomer (**6h**). Yield=82%. $[\alpha]_D^{22°C}$=12.5° (1.0, $CHCl_3$), (*S*)-isomer (**6i**). Yield=82%. $[\alpha]_D^{22°C}$=-12.8° (1.0, $CHCl_3$). The spectra were in accordance with racemic DDAIP (**3a**).

Donor Samples

The composition of the donor samples is listed in Table I. The enhancers were added in 1% concentration (*w*/*v*). The suspension was stirred for 5 min at 50°C and then allowed to equilibrate at 37°C for 24 h before the application on the skin. All the donor samples were saturated with the pertinent model drug at these concentrations.

For the determination of the effect of the studied enhancers on the solubility of the model drugs in the donor vehicle, the samples, either with or without the enhancer, were prepared in triplicate as described above. The samples were centrifuged at 10,000×*g* for 5 min, the supernatant was withdrawn, diluted with the pertinent mobile phase and the concentration of the drug was determined by HPLC.

Skin

For the *in vitro* experiments, porcine skin was selected due to it is availability and permeability similar to the human skin (25–27). Porcine ears were purchased from a local slaughterhouse. To ensure integrity of the skin barrier, ears were removed post-sacrifice before the carcass was exposed to the high-temperature cleaning procedure. Full-thickness dorsal skin was excised by blunt dissection, and hairs were carefully trimmed. The skin was then immersed in 0.03% sodium azide solution in saline for 5 min for preservation. The skin fragments were stored at −20°C up to two months.

Permeation Experiments

The skin permeability was evaluated using modified Franz diffusion cells with an available diffusion area of 1 cm^2 and acceptor volume of approximately 17 ml. The porcine skin was slowly thawed, cut into pieces of 2×2 cm, mounted into the diffusion cells dermal side down and sealed with silicone grease. The acceptor compartment was filled with PBS at pH 7.4 with 0.03% of sodium azide as a preservative and the volume of the acceptor phase was measured and included into the calculation. The Franz diffusion cells with mounted skin samples were placed in a thermostated water bath with a constant temperature of 32°C equipped with a multi-place magnetic stirrer. After equilibration period of 1 h, 200 µl (i.e. an infinite dose) of the donor sample was applied to the SC side of the skin and covered with a glass slide. The acceptor phase was stirred at 32°C throughout the experiment. Sink conditions were maintained for all the drugs. Samples of the acceptor phase (0.6 ml) were withdrawn at predetermined time intervals during 48 h (52 h in the case of hydrocortisone) and replaced

Table I. The Properties of the Model Drugs and the Composition of the Donor Samples Used for the Permeation Experiments

	Physicochemical properties				Donor sample		
Model drug	MW (g/mol)	mp (°C)	logP	p*K*a	Drug amount (%)	Vehicle	Solubility (mg/ml)
Theophylline	180	273[a]	-0.02[a]	1.5, 8.6[b]	5	60% PG	28±3
Hydrocortisone	362	220[a]	1.61[a]	–	2	60% PG	8.3±0.6
Adefovir	273	301[c]	−2.06[b]	1.2, 4.2, 6.8[d]	2	PB pH 4.8	70±7
Indomethacin	358	158[a]	4.27[a]	4.5[a]	2	60% PG	0.9±0.1

PG propylene glycol, *PB* phosphate buffer
[a] Data retrieved from SRC PhysProp database (www.syrres.com)
[b] Calculated using ACD/Labs Software V8.14 for Solaris
[c] Taken from (39)
[d] Taken from (40)

with fresh buffer solution. The permeation experiment with hydrocortisone had to be prolonged to reach the pseudo steady-state. The cumulative amount of the drug permeated across the skin, corrected for the acceptor phase replacement was plotted against time, and the steady state flux was calculated from the linear region of the plot. Enhancement ratio (ER) was calculated as a ratio of the flux with and without the enhancer.

At the end of the permeation experiment, the diffusion cells were dismounted; the skin surface washed with 0.5 ml of ethanol and 0.5 ml of water and blotted dry. The exposed area of 1 cm^2 was punched out and weighted. The skin sample was then extracted with 5 ml of the appropriate mobile phase (or PBS at pH 7.4 for adefovir) for 48 h. The recovery was 98±2% for theophylline, 101±7% for indomethacin, 93±1% for hydrocortisone and 97±2% for adefovir (28). The concentration of the drug in the extract was determined by HPLC.

Enzymatic Hydrolysis of DDAK

DDAK (10 mg, 0.03 mmol) was dissolved in 10 ml of acetonitrile. 100 μl of this stock solution was added to 0.2 IU porcine esterase in 9.9 ml of PBS at pH 7.4 and the solution was incubated at 32°C. The samples of 0.1 ml were withdrawn in predetermined intervals during 120 min and 0.1 ml of acetonitrile was added to deactivate the enzyme. The sample was diluted with 1.8 ml of acetonitrile/ water (4:1) and assayed on HPLC/MS. Since this analytical method describes only the decomposition of DDAK, the presence of the expected hydrolysis product dodecanol was confirmed by TLC on silica gel using chloroform/methanol 9:1. The Rf values for dodecanol and DDAK were 0.82 and 0.25, respectively. The negative control containing DDAK without the esterase was prepared likewise.

HPLC Conditions

The model drugs were determined by isocratic reversed-phase HPLC using LC-20AD pump, SIL-20AC autosampler and SPD-20A UV/VIS detector (Shimadzu, Kyoto, Japan). The data were analyzed using CSW v. 1.7 for Windows integrating software (Data Apex, Prague, Czech Republic).

Separation of theophylline was achieved on LiChroCART 250–4 column (LiChrospher 100 RP-18, 5 μm, Merck) at 35°C using methanol/0.1M NaH_2PO_4 4:6 (*v*/*v*) as a mobile phase. A flow rate of 1.2 ml/min was employed and the effluent was measured at 272 nm. The retention time of theophylline was 2.9±0.1 min.

Indomethacin samples were analyzed on LiChroCART 250–4 column (LiChrospher 100 RP-18, 5 μm, Merck) using a mobile phase containing acetonitrile/water/acetic acid 90:60:5 (*v*/*v*/*v*) at a flow rate of 1.5 ml/min at 40°C. UV absorption was monitored at 270 nm and the retention time was 3.9±0.1 min.

Hydrocortisone was determined on LiChroCART 250–4 column (LiChrospher 100 RP-18, 5 μm, Merck) at 40°C using methanol/water/THF 60:40:1 (*v*/*v*/*v*). The flow rate was adjusted at 1.2 ml/min, absorption was measured at 252 nm. The retention time of hydrocortisone was 4.2±0.1 min.

Adefovir samples were analyzed on LiChroCART 250-4 column (Purospher STAR, RP-18e, 5 μm, Merck) with LiChroCART 4-4 guard column containing the same sorbent at 40°C. The mobile phase consisted of 10 mM KH_2PO_4 and 2 mM Bu_4NHSO_4 at pH 6.0 with 7% of acetonitrile at a flow rate of 1.5 ml/min. The detector wavelength was set at 260 nm (28).

HPLC-MS analysis of DDAK was performed using a chromatographic system LC 20A Prominence (Shimadzu, Kyoto, Japan) coupled with LCQ Max advantage mass spectrometer (Thermo Finnigan, San Jose, USA) with ESI source and an ion trap analyzer. The data were processed using Xcalibur software (Thermo Finnigan, San Jose, USA). DDAK was determined on Luna, phenyl-hexyl column (150× 30 mm, 5 μm, Phenomenex, Aschaffenburg, Germany) at 40°C using a mixture of 0.01% HCOOH and acetonitrile (30:70; *v*/*v*) as a mobile phase. A flow rate of 0.3 ml/min and an injection volume of 2 μl were used. The determination was made in selected ion monitoring mode on $[M+H]^+$ at m/z 328 for DDAK and m/z 356 for internal standard (dodecyl 8-dimethylaminooctanoate (**3f**)). The retention times of DDAK and the internal standard were 2.4±0.1 min and 2.7± 0.1 min, respectively.

Reversibility of DDAK Action

The reversibility of the skin barrier function after DDAK treatment was studied by measuring the transdermal electrical resistance using an LCR meter 4080 (Conrad electronic, Hirschau, Germany, measuring range 20 Ω–10 MΩ, error at kΩ values <0.5%, measuring frequency 120 kHz). The skin samples were mounted into the Franz diffusion cells, the acceptor compartments were filled with PBS at pH 7.4 and the cells were equilibrated at 32°C for 0.5 h as described above. 0.5 ml of PBS was introduced into a donor compartment and the baseline skin resistance ($k\Omega/cm^2$) was measured by stainless steel electrodes inserted into the donor and acceptor compartment. The buffer solution was removed from the donor compartment by a cotton swab, and 150 μl of the donor sample containing 1% (*w*/*v*) of DDAK in 60% propylene glycol (PG) was applied. The control cells received 150 μl of 60% PG without the enhancer. The donor samples were removed after two hours and the skin surface was washed with 0.5 ml of distilled water and gently blotted dry. The resistance was measured at predetermined time intervals during 8 h.

Data Analysis

Kruskal–Wallis one way analysis of variance on Ranks with Dunn's or Student–Newman–Keuls post test method was used for the statistical analysis. The skin electrical resistances were compared using *t*-test or Mann–Whitney Rank Sum Test (SigmaStat for Windows version 3.0.1). Data are presented as means ± SEM and the number of replicates is given in the pertinent figure.

RESULTS

Synthesis

The *N,N*-dimethylamino acid esters were prepared via carbodiimide coupling of a bromo acid and dodecanol or 1H,1H,2H,2H-perfluoro-1-decanol, except for dodecyl 3-bromopropanoate **2b**, which was synthesized from an acyl

chloride, followed by a nucleophilic substitution of bromine with dimethylamine (Fig. 1). This synthetic pathway via the bromo esters was more convenient and provided better yields with easy purification of the products in comparison with our previous work starting from dimethylamino acid (20). Solution of dimethylamine in THF provided similar yields but was easier to handle than liquid dimethylamine (6).

The above procedure could not be applied to the synthesis of (*R*)- and (*S*)-DDAIP because the substitution occurs at the chiral centre. Hence, reductive dimethylation of alanine dodecyl ester using sodium triacetoxyborohydride and formaldehyde was applied (Fig. 2) (24).

Permeation Experiments

The effect of the linking chain length on the enhancing activity of the prepared compounds was evaluated using theophylline as the model drug. The basic physicochemical characteristics of this drug are listed in Table I. The theophylline flux through the porcine skin without an enhancer was 2.4±2.5 μg cm^{-2} h^{-1}, and the skin concentration was 436 μg/g. All of the synthesized dimethylamino acid dodecyl esters significantly increased the theophylline permeation (Fig. 3). DDAIP enhanced the theophylline flux 5.9 times to 13.9 μg cm^{-2} h^{-1} with no significant difference between the (*R*) and (*S*)-enantiomers and the racemate. Its straight-chain isomer **3b**, i.e. 3-(dimethylamino)propionate, displayed significantly higher activity than DDAIP with enhancement ratio (ER) of 15.0. The highest activity was observed in aminobutanoic (**3c**), aminopentanoic (**3d**) and aminohexanoic acid (DDAK) derivatives, i.e. those with 3 to 5-carbon linking chain between the tertiary nitrogen and ester carbonyl. These enhancers allowed for reaching theophylline flux values up to 42.2±14.3μg cm^{-2} h^{-1}, with the corresponding ER being 17.8, i.e. approximately three times higher activity than DDAIP. Further prolongation of the linking chain to 7 carbons in **3f** resulted in diminished theophylline permeation. Interestingly, replacement of a hydrocarbon chain in DDAIP by a polyfluorinated one in **3g** led to a complete loss of its permeation-enhancing activity.

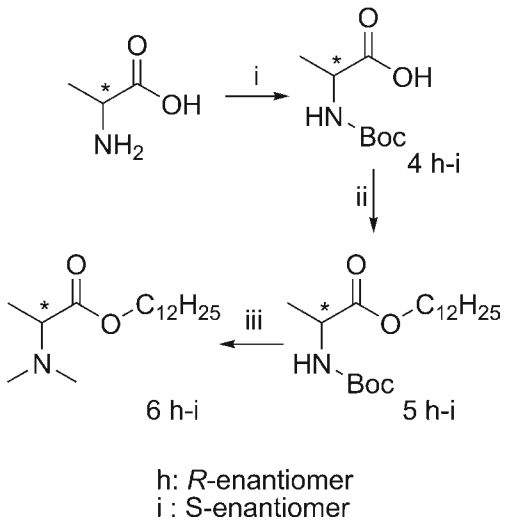

Fig. 2. Synthesis of DDAIP enantiomers. Reagents and conditions: *i* $(Boc)_2O$/1M NaOH/dioxane; *ii* $C_{12}H_{25}OH$/DCC/DMAP/ethyl acetate/0°C; *iii* (1) TFA/CH_2Cl_2 (1:1); (2) Na[$(CH_3CO_2)_3BH$]/35% HCHO/CH_2Cl_2.

When considering the effects of the enhancers on the theophylline skin concentration, neither racemic DDAIP nor its enantiomers showed any effect. On the other hand, DDAK significantly increased theophylline skin concentration to 1,149±278 μg/g (Fig. 3). The solubility of the theophylline in the donor medium was 27.9±3.3 mg/ml, and was not significantly affected by any of the tested compounds.

DDAIP—both the racemate and the enantiomers—and DDAK were further compared using hydrocortisone, indomethacin and adefovir as the model drugs covering a wide range of physicochemical properties (Table I).

The flux of hydrocortisone without an enhancer was 0.11±0.07 μg cm^{-2} h^{-1}, and DDAIP increased it 11.5 times to 1.27 μg cm^{-2} h^{-1}. The corresponding skin concentration was 168±39 μg/g. Again, no significant difference was found between the (*R*) and (*S*)-enantiomers and the racemic DDAIP. DDAK proved to be a more potent enhancer than DDAIP for hydrocortisone with the flux of 4.78 μg cm^{-2} h^{-1} (ER=43.2) and the skin concentration of 484±96 μg/g (Fig. 4). The solubility of hydrocortisone in the donor vehicle was 8.3±0.6 mg/ml, and it was not significantly affected by any of the enhancers.

The flux of adefovir through the porcine skin was 1.4±0.6 μg cm^{-2} h^{-1}, and the skin concentration was 219 μg/g. DDAIP increased the adefovir flux approximately three times, but had no effect on the amount retained in the skin. No stereoselectivity in DDAIP action was observed. The addition of DDAK into the donor sample resulted in significantly higher adefovir flux (ER=13.6) and skin concentration (714 μg/g) in comparison with DDAIP. The permeability characteristics of the racemic DDAIP and DDAK measured under the same conditions were taken from our previous work (21,22), and were included in Fig. 5 for comparison. The solubility of adefovir in the donor vehicle was 70±7 mg/ml, and was not significantly changed by any of the enhancers.

On the other hand, DDAIP (ER=22.8) was more effective than DDAK (ER=8.7) in enhancing the permeation of indomethacin through the porcine skin. Similar results were obtained for the indomethacin skin concentration. The donor sample with DDAIP and DDAK produced indomethacin skin concentration of 1012±173 and 482±166 μg/g, respectively (Fig. 6). The solubility of indomethacin in the donor media significantly increased from 0.9±0.1 mg/ml in the control sample to 3.4±0.2 and 11.6±1.2 mg/ml in the samples with DDAIP and DDAK, respectively.

Enzymatic Hydrolysis of DDAK

DDAK was hydrolyzed in the presence of porcine esterase - the degradation followed a first order kinetics with the estimated half time $T_{1/2}$ of approximately 17.2 min (Fig. 7). The negative control without the enzyme showed that the compound was stable toward chemical hydrolysis.

Reversibility of DDAK Action

The baseline transdermal electrical resistance was 9.1–14.6 kΩ/cm^2. The resistance decreased to 35% (4.8 kΩ/cm^2) and 81% (8.9 kΩ/cm^2, significant difference at $p<0.05$) of the initial value after 2-h application of DDAK and control sample, respectively (Fig. 8). The resistance of the control

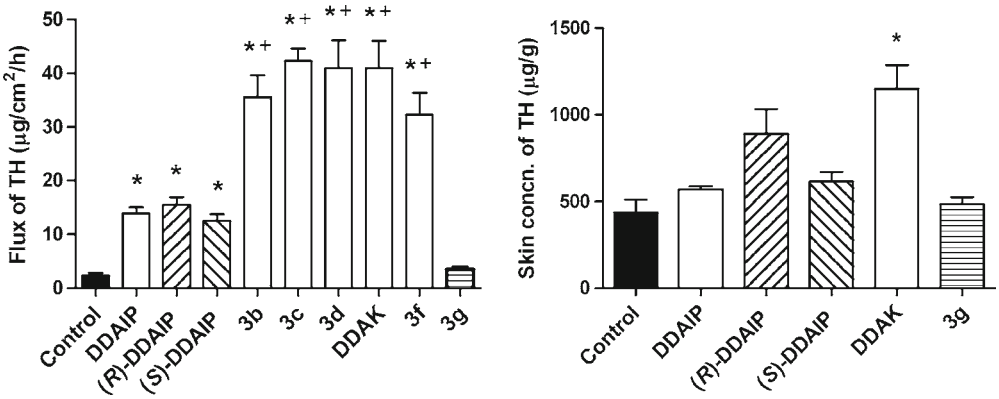

Fig. 3. Effects of the *N,N*-dimethylamino acid esters on theophylline (TH) flux through (left) and its concentration in the porcine skin (right). Means±SEM, n=8–11 (three donors). *Asterisk* indicates significant difference against control (p<0.05), *plus symbol* indicates significant difference against DDAIP (p<0.05).

(PG-treated sample) continued to slowly decrease until it reached approximately 5.5 kΩ/cm^2. The DDAK-treated skin resistance further dropped to 20% (2.7 kΩ/cm^2) at 3 h, i.e. 1 h after the sample removal. Then, the resistance started to increase continually up to values of the control samples. At 6 h, i.e. 4 h after the removal of the donor samples, no significant difference between DDAK and control was observed.

DISCUSSION

Chirality of DDAIP

The purpose of this study was to investigate the structure-activity relationships in a group of dimethylamino acid transdermal permeation enhancers. The parent compound of this series, DDAIP, is a chiral compound. Since DDAIP increases the skin permeability by interacting with the skin barrier lipids (7), which represent a chiral environment, its enhancing activity may be of a stereoselective nature. The results from this study, however, did not show any significant difference between (*R*), (*S*) and racemic DDAIP in increasing either the flux or skin concentration of any of the four model drugs. This is in accordance with our previous study with 2-octyl 6-aminohexanoate as an enhancer with the chiral centre located in the hydrophobic chain (14). Thus, the interaction of DDAIP with the skin lipids and/or the consequent barrier permeabilization is not dependent on the exact spatial orientation of its polar head.

Polyfluorination of DDAIP Tail

In order to explore the impact of polyfluorination on the behavior of permeation enhancers, fluorocarbon DDAIP analogue **3g** was synthesized, and its effect on the skin transport of theophylline was evaluated. However, this compound was completely inactive. It should be noted that the chain length in **3g** was 2C-shorter than that in DDAIP—1H,1H,2H,2H-perfluoro-1-decanol was used for the synthesis because it was commercially available. However, such shortening of the chain could not be responsible for the observed change as the decyl derivatives are usually equally or more active than their dodecyl counterparts (5,15). Thus, the loss of enhancing activity can be attributed to the presence of a fluorocarbon instead of a comparable hydrocarbon chain.

This effect of chain fluorination may be explained either by insufficient penetration of **3g** into the SC lipid bilayers, or its inability to disrupt the barrier lipid packing. The former explanation is probably not feasible since polyfluorinated compounds have been found to cross the skin barrier. For example, *in vitro* dermal absorption of 8–2 fluorotelomer alcohol, i.e. 1H,1H,2H,2H-perfluoro-1-decanol (the same

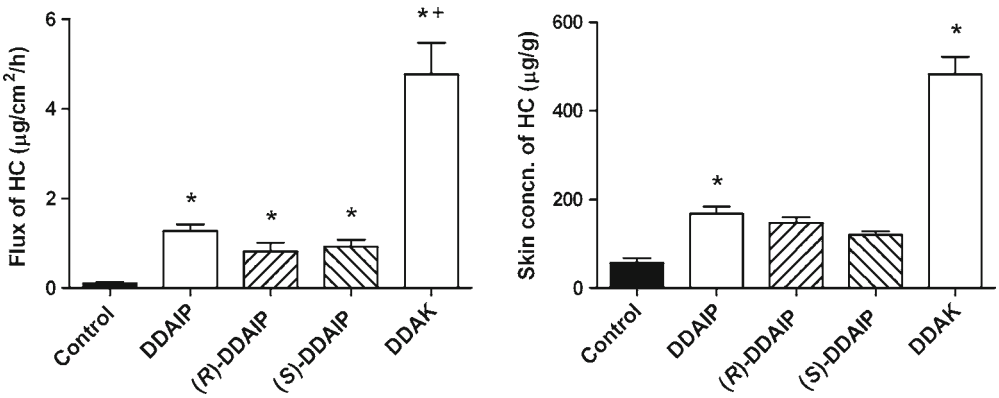

Fig. 4. Effects of the *N,N*-dimethylamino acid esters on hydrocortisone (HC) flux through (*left*) and its concentration in the porcine skin (*right*). Means±SEM, n=6 (two donors). *Asterisk* indicates significant difference against control (p<0.05), *plus symbol* indicates significant difference against DDAIP (p<0.05).

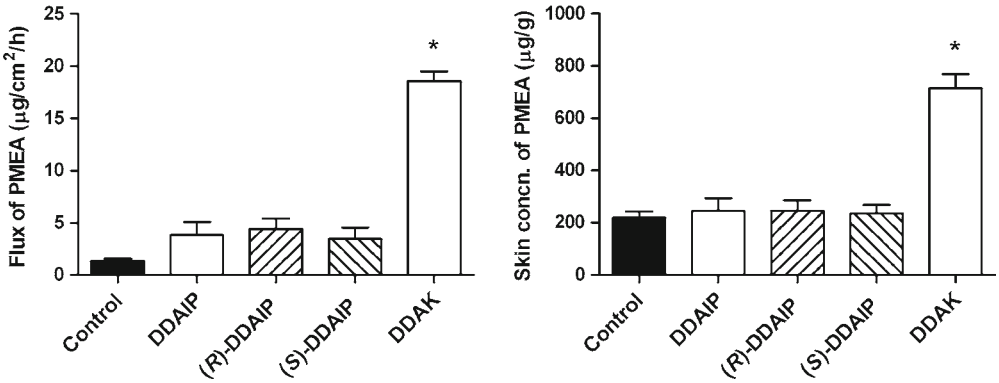

Fig. 5. Effects of the *N*,*N*-dimethylamino acid esters on adefovir (PMEA) flux through (*left*) and its concentration in the porcine skin (*right*). Means±SEM, n=4–11 (two to four donors). *Asterisk* indicates significant difference against control ($p<0.05$).

alcohol as incorporated in **3g** molecule in this study), from a 0.5% methylcellulose vehicle and ethanol was approximately 7 and 30%, respectively, for human skin (DuPont-6997 unpublished, in (29)). Moreover, perfluorinated poison ivy allergens were elicitors of allergic contact dermatitis in pentadecylcatechol-sensitized mice (30).

The latter possible explanation, i.e. that polyfluorinated DDAIP did not disrupt the skin barrier lipid lamellae, may be connected with different behavior of a fluorocarbon chain. It was suggested that phospholipid membrane permeability after replacing a part of the hydrocarbon tail for a fluorinated one resulted from a compromise between (a) increased packing disorder due to weaker lateral interaction and augmented steric repulsions and (b) increased order related to increased hydrophobicity of the fluorocarbon chains. In short C_4F_9 fluorinated tails, both effects appeared to be balanced while with longer C_8F_{17} chains the hydrophobic interactions predominated, resulting in enhanced membrane ordering and rigidity (17). This may provide a reasonable explanation of the inability of **3g** to perturb the tight SC lipid packing.

Linking Chain Structure

DDAK is a DDAIP analogue with longer linking chain between the tertiary nitrogen and ester bond. Its exceptional transdermal permeation-enhancing activity was identified using theophylline (20) and adefovir (21,22), where it was even more active than DDAIP. To compare these two structurally similar enhancers in a greater detail, their effects on the permeation of four model permeants having different physicochemical properties were studied.

First, the effect of the linking chain structure between the dimethylamino group and ester carbonyl was investigated using theophylline as a model drug. DDAIP is an alanine derivative bearing the basic nitrogen on the α-carbon together with a methyl group, i.e. its linking chain could be regarded as "branched". Its isomer **3b** having linear ethylene linking chain, i.e. a β-alanine derivative, showed significantly higher activity, suggesting a negative effect of the linking chain branching on the activity of DDAIP.

The effect of branching on the enhancing properties of various compounds was discussed in several articles. Aungst described similar enhancement produced by 5C-14C branched and unbranched fatty acids (31). Likewise, no significant difference in permeation-enhancing ability of fatty alcohols and acids (32) and carbamates (33) with terminal methyl branching with the only exception of 12C acid was observed by Klimentova. However, similar ethyl-branched alcohols and acids showed significantly higher activity (32). On the other hand, Chantasart described a decrease in the intrinsic enhancing potency with a higher degree of branching (34). Hrabálek suggested that the relatively small degree of branching in the hydrophobic chain of 6-aminohexanoic acid esters did not prevent them from interacting with the lipid

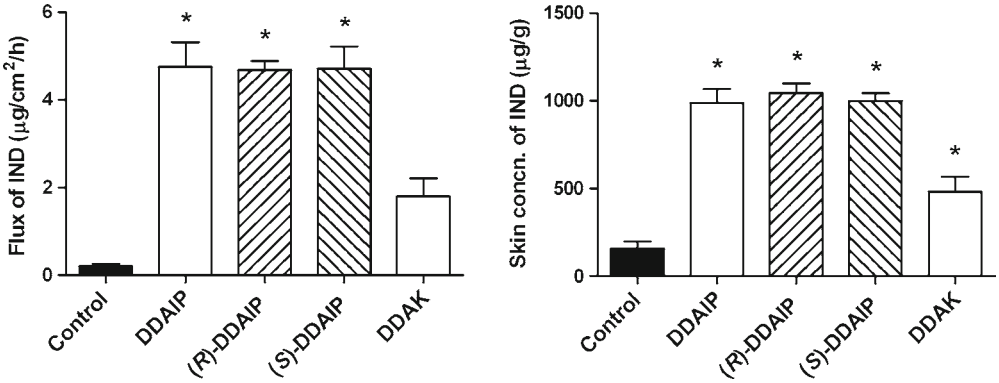

Fig. 6. Effects of the *N*,*N*-dimethylamino acid esters on indomethacin (IND) flux through (*left*) and its concentration in the porcine skin (*right*). Means±SEM, n=7 (two donors). *Asterisk* indicates significant difference against control ($p<0.05$).

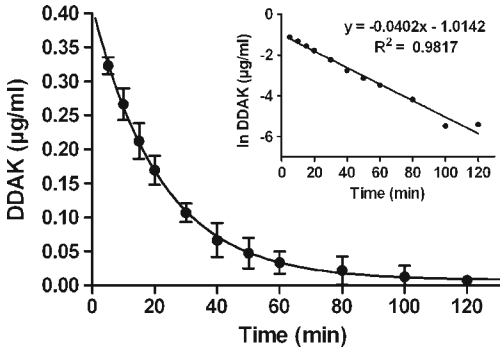

Fig. 7. *In vitro* hydrolysis of DDAK in the presence of porcine esterase (first order reaction, $T_{1/2}$=17.2 min). Means±SEM, n=5. *Insert* Plot of ln of DDAK concentration against time.

components of SC. However, a higher degree of branching, cyclization of the chain, and presence of an aromatic ring resulted in a loss of activity (35). The results from this study with DDAIP and **3b** seem to support the latter studies that branching close to the polar head has negative effect on the enhancing activity.

With prolonging the linking chain between the nitrogen and ester carbonyl, the potency of the enhancers increased first, and then remained comparable in **3c**, **3d** and DDAK, i.e. in derivatives of butanoic, pentanoic and hexanoic acid, and decreased in octanoic acid-based enhancer **3f**. Neither of these enhancers changed the solubility, i.e. the thermodynamic activity of the model drug in the vehicle. The difference between DDAK and DDAIP can be explained by diverse interaction within the SC lipid bilayers. DDAK is a substance with the same hydrogen bonding activity as DDAIP, but more lipophilic and with a flexible linking chain, which could adopt an optimal conformation and probably interact more readily with SC components.

DDAK *Versus* DDAIP

The activity of DDAK and DDAIP was further studied using hydrocortisone, adefovir and indomethacin (Table I) to find whether DDAK was able to facilitate absorption of a broader spectrum of drugs. This enhancer was more potent than DDAIP in increasing both the flux and skin concentration of hydrocortisone and adefovir confirming the results with theophylline. Neither DDAIP nor DDAK changed the solubility of these drugs in the donor vehicle, *i.e.* their thermodynamic activity. Thus, the reported enhancement ratios reflect a direct increase in the skin permeability.

On the other hand, the enhancing effect of DDAIP on the permeation of indomethacin was 2.6 times higher than that of DDAK. The enhancing potency of DDAIP towards indomethacin permeation was previously explained by a complex formation via hydrogen bonding between the carboxyl group of indomethacin and the tertiary amine of the enhancer (11). This is consistent with 3.8 times increased solubility of the drug in the donor vehicle observed in this work. On the other hand, DDAK possesses similar dimethylamino group capable of interacting with indomethacin. Indeed, the solubility of indomethacin in 60% PG increased ten times after the addition of DDAK. However, its ability to enhance indomethacin permeation through the skin was lower than that of DDAIP. In order to study the nature of the interaction, NMR and IR spectra of indomethacin, DDAIP, DDAK and their equimolar mixtures were recorded. ^{13}C-NMR spectra showed larger upfield shifts of the carbons next to nitrogen in DDAK-indomethacin complex than in the DDAIP one, consistent with the shifts of protonated DDAK. Similar changes were observed in the ^{1}H-NMR and IR spectra. Thus, DDAK was likely protonated by indomethacin. This may be explained by different basicity of the two enhancers: DDAK is a stronger base that DDAIP due to better steric availability and higher electron density of the nitrogen lone pair and is therefore capable of (partly) dissociating indomethacin, i.e. a carboxylic acid. However, the formation of an ion pair in the donor vehicle may be decreased due to its high dielectric constant and the two ionic species are likely solvated. This may explain the lower activity of DDAK towards indomethacin.

Biodegradability of DDAK

DDAK was designed as a biodegradable permeation enhancer. Similar to DDAIP, it contains ester bond and may thus be hydrolyzed by skin esterases. To determine DDAK biodegradability, we used the same porcine esterase method as was applied previously to DDAIP (6), tranexamic acid-based enhancers (16) and Transkarbam 12 (23). However, the conditions for HPLC determination of DDAIP described by Büyütimkin *et al.* (6) were not applicable to DDAK analysis due to its low UV absorption, which limited the sensitivity of the assay. The enhancer concentration was thus determined using HPLC/MS. The results demonstrated that DDAK was rapidly hydrolyzed by porcine esterase with $T_{1/2}$=17.2 min. This is in a good agreement with DDAIP showing half life of 18.5 min (6). The longer half life of DDAIP may be explained by steric hindrance of the ester group by the adjacent methyl. As esterases are present in the human epidermis (36,37), the hydrolysis is likely to occur *in vivo* as well, preventing any possible harmful action of this compound on living cells. Moreover, pilot oral toxicity tests in mice and rats showed

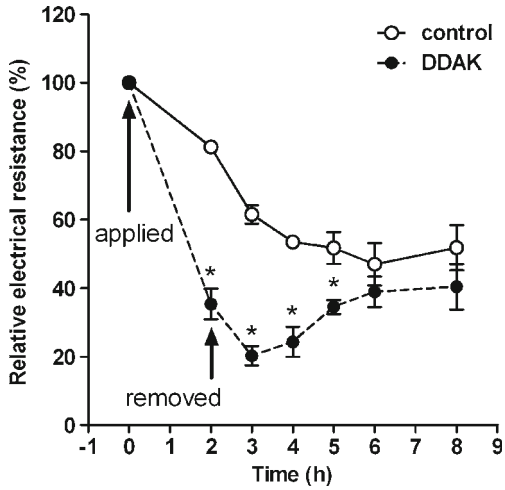

Fig. 8. *In vitro* transdermal electrical resistance of DDAK-treated and control skin as a function of time. The *arrows* indicate the application and removal of the donor samples. *Asterisk* indicates significant difference against control. Means±SEM, n=4 (two donors).

that all animals survived a DDAK dose of 2 g/kg without any signs of toxicity (BioTest Ltd, Konárovice, Czech Republic).

Reversibility of DDAK Action

Electrical resistance is a simple, quick and robust method for measuring the skin barrier integrity (38). In this study, we used this method for evaluation of the reversibility of DDAK action. The results confirmed that (a) DDAK acts by direct decrease of the skin barrier resistance as suggested previously (22), (b) the action of this enhancer on the skin is temporary and c) the barrier changes induced by DDAK are reversible. The reversibility is probably connected with rapid clearance of DDAK from the SC with subsequent hydrolysis of this compound by epidermal esterases. The rapid reversibility may also explain the previously found difference in activity of this enhancer in the co-application and pretreatment protocol (22).

CONCLUSION

In this study, the transdermal permeation-enhancing potency of a series of *N,N*-dimethylamino acid esters was studied. No stereoselectivity in action of DDAIP was found. Polyfluorination of a hydrophobic chain of DDAIP resulted in a complete loss of activity. Replacement of a "branched" linking chain between nitrogen and ester of DDAIP by a linear one markedly improved the enhancing activity with optimum linking chain length found in 4–6C acid derivatives. DDAK was more potent enhancer than DDAIP for theophylline, hydrocortisone and adefovir, while DDAIP was better enhancer for indomethacin. DDAK was rapidly metabolized by porcine esterase, displayed low acute toxicity and reversible action. These results suggest that DDAK, a highly effective biodegradable transdermal permeation enhancer for a broad spectrum of drugs, is a promising candidate for future research.

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