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Influence of terminal branching on the transdermal permeation-enhancing activity in fatty alcohols and acids

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Abstract—In order to investigate the effect of terminal chain branching in the skin permeation enhancers, seven alcohols and seven acids with the chain length of 8–12 carbons and terminal methyl or ethyl branching were prepared. Their transdermal permeation-enhancing activities were evaluated in vitro using theophylline as a model permeant and porcine skin, and compared to those of the linear standards. Terminal methyl branching increased the enhancing activity only in 12C acid, no effect was seen in the shorter ones. Terminal ethyl however produced a significant increase in activity. In the alcohols, the branching was likely to change the mode of action, due to a different relationship between the activity and the chain length.

1. Introduction

The major limitation to transdermal drug delivery is the skin itself. The principal barrier to penetration of substances through the skin is provided by a superficial layer of the skin, the stratum corneum (SC), due to its compact structure. The use of penetration enhancers is one of the approaches to reversibly decrease the skin barrier resistance. Penetration enhancers are chemical compounds which are themselves pharmacologically inactive, but can partition into the SC and interact with its constituents when incorporated into a transdermal formulation, thereby reducing the resistance of skin to drug diffusion. A penetration enhancer may also increase the thermodynamic activity of a drug, thus resulting in augmented drug flux.[1]

Various fatty alcohols (FOL) and fatty acids (FA) have been studied previously as penetration enhancers for many types of permeants.[2,3] There are two general types of mechanisms, by which FOL and FA can increase the delivery of a drug through the skin. First, they can increase the solubility of the drug in a vehicle and consequently increase its partitioning into the skin. Second mechanism is the disruption of the densely packed lipids that fill the extra-cellular spaces of the SC.[4–6] In the group of FA these two effects are sometimes called the 'push-pull' mechanism.[7]

The influence of terminal branching in the group of FOL has not been studied yet. The only study dealing with branched alcohols has investigated the effect of chain branching situated next to hydroxyl.[8] In this study, the authors have reported that such alcohols are of a lower potency compared to the linear alcohols of the same chain length. Similar conclusion has been made in the study of Hrabálek et al.[9] for the group of 6-aminohexanoic acid esters with various branched and secondary alcohols.

Branched FA have been found widely in natural fats (particularly those of animal and bacterial origin). The methyl group close to the ω-end of the chain occurs in the *iso* or *anteiso* acids that are present in wool grease and in skin lipids.[10] Different branched FA have been found in the human skin surface lipids as a component of triglycerides.[11]

In the group of FA, the structure–activity relationships have been studied widely regarding the chain length, saturation versus unsaturation and the position of the double bond or branching.[3] The effect of unsaturated FA, especially of oleic acid, is the most investigated.[12] However, only a little progress has been made to find the

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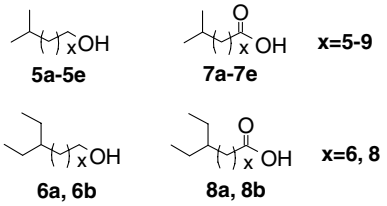

Figure 1. Fatty alcohols and acids investigated as transdermal permeation enhancers.

influence of branching. The work of Schneider et al.[13] studied the effect of 10-methylpalmitic acid (branching in the middle of the chain) and 10-methylhexadec-9-enoic acid (branching and unsaturation) on the dermal penetration of pyrene butyric acid and found out that their activity was comparable to that of oleic acid.

Aungst et al.[4] compared various linear FA with their branched isomers using naloxone as a model permeant and found out that the effects of the shorter-chain branched FA were not significantly different from those of the linear ones of the same carbon number. The only exception was represented by isostearic acid with the flux of naloxone ca. 3.5-fold higher compared to stearic acid. The authors suggested that the shorter-chain linear FA were already maximally disruptive of the lipid packing, so the branching did not increase the disruptive effect. On the other hand, stearic acid with the chain length similar to that of the SC lipids probably did not perturb their packed structure. That was why the branching in this case could disturb the skin barrier and the penetration of naloxone was increased.

Our aim was to evaluate the influence of terminal branching in the group of branched alcohols and acids (Fig. 1) with chain length of 8–12 carbons (which was reported previously to be the optimum chain length in unbranched saturated chains). Linear alcohols and acids of 8, 10 and 12 carbons have been chosen as linear standards for comparison. We aimed to investigate the hypothesis that the terminal branching causes greater disruption of the lipid chain packing than the linear chains.

2. Results and discussion

2.1. Synthesis

The alcohols **5a**–**5e** and **6a**–**6b** as well as the acids **7a**, **7c**, **8a**, and **8b** were commercially unavailable and the price of the rest was high. Therefore, they were prepared by linking ω-bromo acid sodium salts with the corresponding branched alkylmagnesium bromides.[14–16] The sodium salts of ω-bromo acids were prepared from their ω-lactones[17] or from the corresponding cycloalkanone (in the case of 8-bromooctanoic acid sodium salt). For the reaction schemes, see Figures 2 and 3, the list of the prepared alcohols and acids (including the precursors) is presented in Table 1.

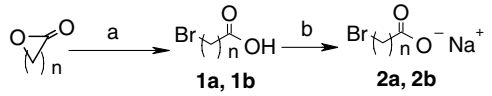

Figure 2. Preparation of the sodium salts of ω-bromo acids: (a) HBr, H_2SO_4, reflux, 4 h; (b) EtO^-Na^+, 0.5 h.

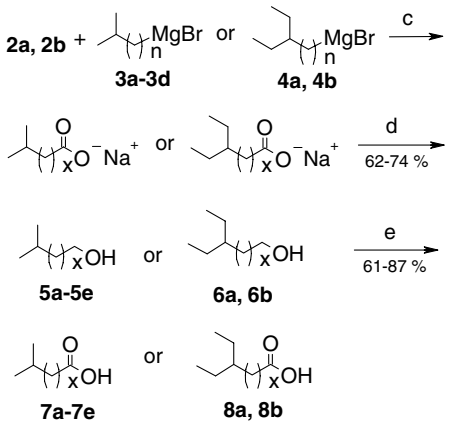

Figure 3. Preparation of the branched alcohols and acids: (c) Li_2CuCl_4, THF, −10 °C, 4 h; (d) $LiAlH_4$, THF, reflux, 1 h; (e) CrO_3, CH_3COOH, +5 °C, 2 h.

Table 1. List of the prepared compounds

Precursors	*n*	Alcohols	Acids	*x*	Reactants
1a, **2a**	5	**5a**	**7a**	5	**2a** + **3a**
1b, **2b**	7	**5b**	**7b**	6	**2a** + **3b**
3a	0	**5c**	**7c**	7	**2a** + **3c**
3b	1	**5d**	**7d**	8	**2a** + **3d**
3c	2	**5e**	**7e**	9	**2b** + **3c**
3d	3	**6a**	**8a**	6	**2a** + **4a**
4a	1	**6b**	**8b**	8	**2a** + **4b**
4b	3				

The branched alcohols and acids were prepared in good yields by the described method, which seemed to be suitable also for preparing them in higher amounts. The acids have been obtained by the oxidation of the prepared alcohols[18] although they occurred in the previous step as their sodium salts. However, recovering them from the reaction mixture appeared to be difficult and showed lower yields than carrying out the described method—reduction of the salts to alcohols followed by oxidation to acids.

2.2. Enhancement activity

The enhancement activity of each enhancer was evaluated using theophylline as a model drug, mixture of propylene glycol/water 3:2 as a donor medium and porcine skin as a model membrane. Theophylline has been selected as a model permeant of medium polarity that has been widely studied previously in various transdermal drug delivery systems.[7,19] The polar pathway[20] through the stratum corneum has previously been proposed for theophylline.[21] On the other hand, theophylline permeation can be successfully enhanced by

amphiphilic enhancers[22–24] that are likely to act in the lipid domains of the stratum corneum, therefore, its permeation through the intercellular pathway is highly probable. Thus, it is a suitable permeant for studying the activity of the prepared enhancers.

The enhancing activity was expressed as the enhancement ratio (ER) which is the ratio of flux of the model drug from the donor sample with the addition of the enhancer and without the enhancer (the control sample). The ER values are shown in Table 2.

The enhancing activities of the alcohols were significantly higher compared to those of the acids. In case of alcohols with the same chain length as dodecanol, the comparison showed that the terminal methyl branching did not increase the activity; the ER value of dodecanol was comparable to those of alcohols **5d** (with the same carbon number) and **5e** (with the same chain length). On the other hand, enlarging the terminal branching to ethyl increased the activity as seen in the alcohols **6a** and **6b**, which were both more active than dodecanol. Moreover, they were more potent than their methyl-branched analogues with the same chain length **5c** and **5e**, respectively. The ratio between the ER values of compounds **6a/5c**, **6b/5e** and **6b**/dodecanol varied around 1.5 (see Fig. 4).

Different result has been found for decanol, which exhibited an exceptional enhancing activity. Introduction of branching led to a significant decrease of its activity in both methyl- and ethyl-branched analogues. This might be due to a different pattern of activity. In the studied branched alcohols, the potency decreased with increasing chain length. On the other hand, the linear-chain alcohols exhibited a parabolic relationship with the maximum at 10C, which is consistent with previous studies; for a review on the structure-activity relationships see Kanikkannan et al.[25] and Vávrová et al.[26] The optimum chain length at approximately 10C was suggested to relate to the ability of the alcohols to disrupt the tight packing of the SC intercellular lipids. Apart from this mode of action, alcohols may also alter the solvent properties of SC thereby increasing the drug partitioning.[6]

Table 2. Enhancement ratios of the prepared compounds compared to the linear standards

Alcohols	ER ± SD	Acids	ER ± SD
5a	36.01 ± 9.84[a]	**7a**	2.49 ± 1.24[a]
5b	28.12 ± 3.94 [a]	**7b**	4.10 ± 1.20[a]
5c	19.93 ± 5.45[a,b]	**7c**	5.49 ± 2.97[a]
5d	14.32 ± 3.76[a]	**7d**	4.08 ± 1.41[a]
5e	11.92 ± 4.97[a]	**7e**	4.10 ± 2.34[a,b]
6a	32.55 ± 7.16[a,b]	**8a**	13.18 ± 3.93[a,b]
6b	18.13 ± 6.27[a,b]	**8b**	11.45 ± 3.92[a,b]
Octanol	34.77 ± 13.28[a]	Octanoic acid	2.15 ± 1.06[a]
Decanol	72.61 ± 9.75[a]	Decanoic acid	6.23 ± 1.35[a]
Dodecanol	12.16 ± 3.09[a]	Dodecanoic acid	2.43 ± 0.94[a]

n, 6–11 (skin fragments from 3 to 8 animals for each compound); SD, standard deviation.
[a] $p < 0.05$ versus control.
[b] $p < 0.05$ versus linear standard of the same chain length.

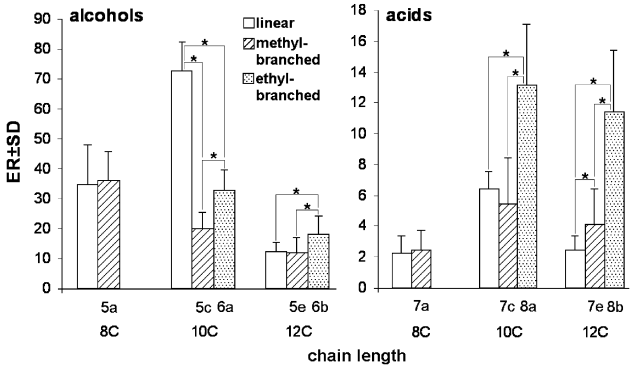

Figure 4. Comparison of the ER values of some of the unbranched and branched alcohols and acids of the same chain length. * significantly different ($p < 0.05$).

Since the chain length–activity relationship was different in linear and branched alcohols, we hypothesize that introduction of branching changed the mechanism of action of the alcohols. A possible explanation might be that branching restricted incorporation of the alcohols into the SC lipid lamellae. The observed enhancing effect was then caused by increased partitioning, which is consistent with the observed inverse linear relationship, that is, shorter alcohols were better enhancers. Another possibility is that the branched alcohols might form separated domains within the SC lipids providing permeability defects within the bilayer lipids, thus facilitating the permeation of the drug. Such behaviour was described for oleic acid, which is sterically hindered due to the 'kink' in the molecule compared to the linear chains.[12]

In the prepared acids, the activities reflected a parabolic relationship in both the linear and the branched groups. Comparing the activity of linear and methyl-branched acids of the same chain length, it was observed that the terminal methyl branching did not influence the enhancing activity. Only in the 12C acids, there was ca. 1.5-fold increase in the ER value of **7e** compared to dodecanoic acid. This result corresponded well with the results of Aungst et al.[4] The ethyl branching in the acids exhibited greater influence on their activities than in the alcohols. In the 10C acids, the ER values were increased approximately 2-fold compared to the linear and the methyl-branched. In the 12C acids, the increase was even higher, ca. 2.5- and 4.7-fold compared to the methyl analogue and to dodecanoic acid, respectively (see Fig. 4).

Hypothetically, the terminal chain branching could have a positive effect on permeation enhancement because the incorporation of such chains into the SC lipid lamellae would demand more space and thus cause more pronounced disturbance of the barrier. On the other hand, branching could decrease the incorporation of an enhancer into the SC lamellae although its lipophilicity would be higher than that of the linear one (the incre-

ment of terminal methyl and ethyl groups to the log *P* value of the studied linear compounds was 0.33 and 0.75, respectively[27]). These two effects might contribute markedly to the resulting activity of an enhancer, each one in an opposite direction, together with other effects such as altering the solubility in the vehicle and SC. However, the detailed interactions between the vehicle, theophylline and enhancers have not been investigated thus the relationships obtained are limited to the described or similar conditions.

The reports on effect of branching on the activity of the skin permeation enhancers are scarce. Chantasart et al.[8] and Hrabálek et al.[9] reported that branching near the polar head decreased the enhancing activity. Such branching, apart from the above-mentioned interactions, could sterically hinder the polar group and decrease, for example, its hydrogen bonding ability.

In the present study, the results differed for alcohols and acids. While branched acids reflected the same parabolic relationship between the chain length and enhancing activity as the linear ones, the alcohols behaved differently. Comparing the methyl- and ethyl-branched compounds, however, larger branching resulted in an increase of activity in all studied compounds. The influence of branching was relatively straightforward in the group of acids where the methyl branching led to almost the same activities and the ethyl one increased them significantly. On the other hand, in the alcohols, the branching probably changed the mode of action as suggested by the different relationship between the chain length and their activity.

3. Conclusion

A series of seven fatty alcohols and seven fatty acids with chain length of 8–12 carbons and terminal methyl and ethyl branching has been prepared. Their transdermal-permeation enhancing activities were compared to those of their linear analogues of the same chain length. Under the studied conditions, terminal methyl branching increased the enhancing activity only in 12C acid; no effect was seen in the shorter ones. Terminal ethyl however produced a significant increase in activity. In the alcohols, the branching was likely to change the mode of action, due to a different relationship between the activity and the chain length. In conclusion, the effect of branching was different in the two studied groups of compounds, thus the results of this preliminary work must be interpreted with caution. A more detailed study with branched enhancers with different polar head, for example, ester-linked to these branched alcohols or acids, is needed to elucidate the exact nature of the described interactions and relationships.

4. Experimental

All chemicals were purchased from Sigma–Aldrich (Schnelldorf, Germany); the commercially unavailable octanolide was prepared by the oxidation of cycloctanone by 30% hydrogen peroxide[17] and the resulting crude lactone was consumed without purification as described in Section 4.1.1. Silica gel 60 (230–400 mesh) for column chromatography, TLC plates (silica gel 60 F_{254}, aluminium back) and HPLC columns were obtained from Merck (Darmstadt, Germany).

The structure and purity of the prepared compounds were confirmed by FT-IR (Nicolet Impact 400 spectrophotometer) and ^{1}H and ^{13}C NMR spectra (Varian Mercury-Vx BB 300 instrument, operating at 300 MHz for ^{1}H, 75 MHz for ^{13}C, 5 mm tubes, standardized to internal tetramethylsilane). Melting points were measured using the Koffler apparatus and are uncorrected. HPLC analyses were performed using an LCP high-pressure pump (ECOM, Prague, Czech Republic), LCD 2083 UV detector (ECOM) and CSW 1.7 integrating software.

4.1. Synthesis

4.1.1. Preparation of ω-bromo acids 1a and 1b. ω-Lactone (0.561 mol) was dissolved in the mixture of 48% HBr (320 mL) and concd H_2SO_4 (77 mL). The mixture was left to stand at room temperature for 2 h and then it was heated to reflux for 4 h. After cooling to room temperature it was poured to 1.5 L of distilled water. The organic layer was separated and the water layer was saturated with NaCl and washed five times with diethyl ether. The extracts were combined with the organic layer, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The resulting light brown liquid was then purified by vacuum distillation.

4.1.1.1. Compound 1a (6-bromohexanoic acid). $C_6H_{11}BrO_2$, 195.06 $gmol^{-1}$, yield 75%, white waxy solid, bp 145–147 °C/12 torr (bp 134–136 °C/7 torr was reported[28]).

4.1.1.2. Compound 1b (8-bromooctanoic acid). $C_8H_{15}BrO_2$, 223.11 $gmol^{-1}$, yield 18% (related to the starting cyclooctanone), white waxy solid, bp 168–169 °C/12 torr (bp 147–150 °C/2 torr was reported[29]).

4.1.2. Preparation of the sodium salts of ω-bromo acids 2a and 2b. The sodium salts of the acids were prepared by adding the solution of sodium ethoxide prepared from Na (0.386 mol) and anhydrous ethanol (500 mL) to the solution of the ω-bromo acids **1a** and **1b** (0.386 mol) in anhydrous ethanol (320 mL). The mixture was stirred for 0.5 h, ethanol was evaporated in vacuum and the white precipitate was washed with diethyl ether, powdered in the mortar and dried at 70 °C in vacuum.

4.1.3. Preparation of the catalyst Li_2CuCl_4. $LiCl{\cdot}H_2O$ (0.002 mol) and $CuCl_2{\cdot}2H_2O$ (0.001 mol) were placed in a flask and retorted carefully to form brown (not black!) colour. Anhydrous THF (10 mL) was added to form orange solution and the flask was sealed. This solution was consumed immediately.

4.1.4. Preparation of the alcohols 5a–5e and 6a and 6b. First, the Grignard reagents **3a**–**3d** or **4a** and **4b** were prepared from the corresponding branched alkyl bro-

mide. The solution of alkyl bromide (0.250 mol) in anhydrous THF (50 mL) was slowly added to magnesium (0.252 mol) in anhydrous THF (250 mL). The reaction was initiated by adding a crystal of iodine. The mixture was then heated to reflux until all the magnesium dissolved.

The salt **2a** or **2b** (0.160 mol) was suspended in anhydrous THF (250 mL), cooled to −10 °C and the solution of the catalyst Li_2CuCl_4 (0.001 mol) in THF was added. Then the prepared solution of Grignard reagent **3a**–**3d** or **4a**–**4b** was added dropwise under vigorous stirring at the temperature not exceeding −5 °C. The colour of the mixture changed from orange through green and blue to white. After adding all the Grignard reagent the temperature was kept at −10 °C for another 4 h. After warming to room temperature $LiAlH_4$ (0.160 mol) was added and the mixture was heated to reflux for 1 h. Then the mixture was poured on crushed ice and the resulting white precipitate was dissolved by adding 30% H_2SO_4. The organic layer was separated and the water phase was extracted three times with diethyl ether. The organic layers were combined, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The resulting light brown oil was purified by fraction vacuum distillation.

4.1.4.1. Compound 5a (7-methyloctanol). $C_9H_{20}O$, 144.26 gmol^{-1}, yield 62%, colourless oil, bp 96–97 °C/10 torr (bp 100 °C/13 torr was reported[30]). FT-IR (neat): ν_{max} 3332, 2954, 2927, 2868, 2856, 1467, 1384, 1366, 1058, 724 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.61 (2H, t, J = 6.6, CH_2OH), 2.14 (1H, s, OH), 1.62–1.02 (11H, m, CH, 5CH_2), 0.84 (6H, d, J = 6.6, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 73.0, 38.9, 32.7, 29.6, 27.9, 27.3, 25.7, 22.6.

4.1.4.2. Compound 5b (8-methylnonanol). $C_{10}H_{22}O$, 158.29 gmol^{-1}, yield 62%, colourless oil, bp 103–104 °C/10 torr (bp 80 °C/6 torr was reported[31]). FT-IR (neat): ν_{max} 3331, 2953, 2927, 2866, 2854, 1467, 1384, 1366, 1058, 723 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.61 (2H, t, J = 6.6, CH_2OH), 1.94 (1H, s, OH), 1.60–1.02 (13H, m, CH, 6CH_2), 0.84 (6H, d, J = 6.6, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 63.0, 39.0, 32.7, 29.8, 29.4, 27.9, 27.3, 25.7, 22.6.

4.1.4.3. Compound 5c (9-methyldecanol). $C_{11}H_{24}O$, 172.31 gmol^{-1}, yield 63%, colourless oil, bp 119–120 °C/10 torr (bp 135–136 °C/12 torr was reported[32]). FT-IR (neat): ν_{max} 3328, 2953, 2926, 2854, 1467, 1384, 1366, 1056, 722 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.60 (2H, t, J = 6.7, CH_2OH), 1.93 (1H, s, OH), 1.60–1.02 (15H, m, CH, 7CH_2), 0.84 (6H, d, J = 7.2, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 62.9, 39.0, 32.7, 29.8, 29.6, 29.4, 27.9, 27.4, 25.7, 22.6.

4.1.4.4. Compound 5d (10-methylundecanol). $C_{12}H_{26}O$, 186.34 gmol^{-1}, yield 74%, colourless oil, bp 135–136 °C/10 torr (bp 147–149 °C/18 torr was reported[33]). FT-IR (neat): ν_{max} 3335, 2953, 2925, 2854, 1467, 1384, 1366, 1057, 721 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.62 (2H, t, J = 6.6, CH_2OH), 1.94 (1H, s, OH), 1.61–1.03 (17H, m, CH, 8CH_2), 0.84 (6H, d, J = 6.6, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 63.0, 39.0, 32.7, 29.9, 29.6, 29.4, 27.9, 27.4, 25.7, 22.6.

4.1.4.5. Compound 5e (11-methyldodecanol). $C_{13}H_{28}O$, 200.37 gmol^{-1}, yield 68%, colourless oil, bp 146–147 °C/10 torr (bp 139 °C/11 torr was reported[32]). FT-IR (neat): ν_{max} 3332, 2953, 2925, 2854, 1467, 1384, 1366, 1057, 721 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.62 (2H, t, J = 6.6, CH_2OH), 1.67 (1H, br s, OH), 1.61–1.03 (19H, m, CH, 9CH_2), 0.85 (6H, d, J = 6.6, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 63.0, 39.0, 32.8, 29.9, 29.7, 29.61, 29.59, 29.4, 27.9, 27.4, 25.7, 22.5.

4.1.4.6. Compound 6a (8-ethyldecanol). $C_{12}H_{26}O$, 186.34 gmol^{-1}, yield 66%, colourless oil, bp 131–132 °C/10 torr. FT-IR (neat): ν_{max} 3355, 2960, 2926, 2857, 1457, 1379, 1056, 721 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.62 (2H, t, J = 6.6, CH_2OH), 1.62–1.49 (1H, m, CH), 1.46 (1H, br s, OH), 1.38–1.06 (16H, m, 8CH_2), 0.82 (6H, t, J = 7.4, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 63.0, 40.3, 32.8, 30.1, 29.5, 26.7, 25.7, 25.4, 10.9.

4.1.4.7. Compound 6b (10-ethyldodecanol). $C_{14}H_{30}O$, 214.39 gmol^{-1}, yield 64%, colourless oil, bp 159–160 °C/10 torr (bp 155–160 °C/10 torr was reported[34]). FT-IR (neat): ν_{max} 3321, 2960, 2923, 2853, 1461, 1379, 1056, 721 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.67–3.55 (2H, m, CH_2OH), 2.01 (1H, br s, OH), 1.81–1.72 (1H, m, CH), 1.39–1.04 (20H, m, 10CH_2), 0.81 (6H, t, J = 7.4, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 62.9, 40.3, 32.74, 32.69, 30.1, 29.6, 29.4, 26.7, 25.7, 25.4, 10.9.

4.1.5. Preparation of the acids 7a–7e and 8a–8b. CrO_3 (0.056 mol) was dissolved in 90% w/w acetic acid (56 mL) and the solution was cooled to +5 °C. The alcohol **5a**–**5e** or **6a** and **6b** (0.014 mol) was added dropwise under vigorous stirring at the temperature not exceeding +5 °C. The temperature was kept for next 2 h and then the mixture was allowed to stand at room temperature overnight. Then it was diluted with water, extracted five times with diethyl ether and the organic extracts were washed by saturated solution of Na_2CO_3. The water layer was then acidified by HCl, washed five times by diethyl ether and the combined organic layers were washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The resulting yellow oil was purified by column chromatography on silica gel using hexane/ethyl acetate 9:1 as an eluent.

4.1.5.1. Compound 7a (7-methyloctanoic acid). $C_9H_{18}O_2$, 158.24 gmol^{-1}, yield 72%, pale yellow oil, mp 3–5 °C (mp 0 °C was reported[35]). FT-IR ($CHCl_3$): ν_{max} 3515, 3028, 2956, 2931, 2869, 1708, 1467, 1412, 1385, 1366, 1280 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 10.96 (1H, br s, COOH), 2.34 (2H, t, J = 7.4, CH_2COOH), 1.69–1.57 (2H, m, CH_2CH_2COOH), 1.57–1.10 (7H, m, CH, 3CH_2), 0.86 (6H, d, J = 6.6, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 180.5, 38.7, 34.1, 29.3, 27.9, 27.0, 25.7, 22.59, 22.55.

4.1.5.2. Compound 7b (8-methylnonanoic acid). $C_{10}H_{20}O_2$, 172.27 gmol^{-1}, yield 61%, pale yellow crystals, mp 15–19 °C (mp 23.7–24 °C was reported[36]). FT-IR ($CHCl_3$): ν_{max} 3515, 3026, 2955, 2928, 2868, 2857, 1708, 1467, 1412, 1384, 1366, 1291 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 10.16 (1H, br s, COOH), 2.33 (2H, t, $J = 7.4$, CH_2COOH), 1.69–1.56 (2H, m, CH_2CH_2COOH), 1.56–1.08 (9H, m, CH, 4CH_2), 0.86 (6H, d, $J = 6.6$, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 180.3, 38.9, 34.2, 29.5, 29.1, 27.9, 27.2, 24.7, 22.6.

4.1.5.3. Compound 7c (9-methyldecanoic acid). $C_{11}H_{22}O_2$, 186.30 gmol^{-1}, yield 70%, white crystals, mp 28–29.5 °C (mp 29–29.5 °C was reported[32]). FT-IR ($CHCl_3$): ν_{max} 3515, 3027, 2955, 2927, 2856, 1708, 1467, 1412, 1384, 1366, 1285 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 10.94 (1H, br s, COOH), 2.34 (2H, t, $J = 7.4$, CH_2COOH), 1.67–1.56 (2H, m, CH_2CH_2COOH), 1.56–1.08 (11H, m, CH, 5CH_2), 0.86 (6H, d, $J = 6.6$, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 180.4, 39.0, 34.1, 29.7, 29.3, 29.0, 27.9, 27.3, 24.7, 22.6.

4.1.5.4. Compound 7d (10-methylundecanoic acid). $C_{12}H_{24}O_2$, 200.32 gmol^{-1}, yield 70%, white crystals, mp 36.5–39 °C (mp 41.2 °C was reported[37]). FT-IR ($CHCl_3$): ν_{max} 3515, 3031, 2954, 2927, 2856, 1708, 1467, 1412, 1384, 1366, 1290 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 11.50 (1H, br s, COOH), 2.34 (2H, t, $J = 7.4$, CH_2COOH), 1.70–1.56 (2H, m, CH_2CH_2COOH), 1.56–1.08 (13H, m, CH, 6CH_2), 0.86 (6H, d, $J = 6.6$, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 180.4, 39.0, 34.1, 29.8, 29.5, 29.2, 29.1, 28.0, 27.4, 24.7, 22.7.

4.1.5.5. Compound 7e (11-methyldodecanoic acid). $C_{13}H_{26}O_2$, 214.35 gmol^{-1}, yield 87%, white crystals, mp 37–40 °C (mp 40.5–41 °C was reported[32]). FT-IR ($CHCl_3$): ν_{max} 3515, 3030, 2954, 2927, 2855, 1708, 1467, 1412, 1384, 1366, 1288 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 11.66 (1H, br s, COOH), 2.35 (2H, t, $J = 7.4$, CH_2COOH), 1.70–1.56 (2H, m, CH_2CH_2COOH), 1.56–1.08 (15H, m, CH, 7CH_2), 0.86 (6H, d, $J = 6.6$, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 180.5, 39.0, 34.1, 29.9, 29.6, 29.4, 29.2, 29.0, 27.9, 27.4, 24.6, 22.6.

4.1.5.6. Compound 8a (8-ethyldecanoic acid). $C_{12}H_{24}O_2$, 200.32 gmol^{-1}, yield 63%, colourless oil. FT-IR ($CHCl_3$): ν_{max} 3516, 3019, 2961, 2930, 2873, 2858, 2679, 1708, 1462, 1412, 1380, 1285 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.74 (1H, s, COOH), 2.34 (2H, t, $J = 7.7$, CH_2COOH), 1.69–1.55 (2H, m, CH_2CH_2COOH), 1.40–1.06 (13H, m, CH, 6CH_2), 0.82 (6H, t, $J = 7.4$, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 180.0, 63.6, 40.3, 34.1, 32.6, 29.7, 29.1, 26.5, 25.4, 24.7, 10.9.

4.1.5.7. Compound 8b (10-ethyldodecanoic acid). $C_{14}H_{28}O_2$, 228.38 gmol^{-1}, yield 68%, colourless oil. FT-IR ($CHCl_3$): ν_{max} 3516, 3019, 2961, 2927, 2873, 2857, 2674, 1708, 1462, 1412, 1380, 1287 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 3.73 (1H, s, COOH), 2.33 (2H, t, $J = 7.6$, CH_2COOH), 1.68–1.54 (2H, m, CH_2CH_2COOH), 1.38–1.06 (17H, m, CH, 8CH_2), 0.82 (6H, t, $J = 7.4$, 2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 179.7, 63.6, 40.3, 34.1, 32.7, 30.0, 29.5, 29.2, 29.1, 26.7, 25.4, 24.7, 10.9.

4.2. Permeation experiments

The transdermal enhancing activity was evaluated in vitro using Franz diffusion cell, theophylline as a model permeant and mixture of propylene glycol/water 3:2 as a donor vehicle. Porcine ear skin of full thickness was employed. Donor samples (200 μL/cm^2) were prepared by suspending 50 mg of theophylline in 1 mL of the vehicle with 10 mg of the enhancer. Control samples were prepared without the addition of enhancer. The samples were stirred at 50 °C for 5 min and then left to stand at 37 °C for 24 h to equilibrate. During the equilibration theophylline formed observable fine needles of its monohydrate,[6] which were resuspended mechanically before use. Theophylline was suspended with partial solution in the vehicle (i.e., at the maximum saturation level), the enhancers were all dissolved. The acceptor phase was phosphate-buffered saline (pH 7.4) with 0.03% of sodium azide as a preservative. Samples of the acceptor phase (0.6 mL) were taken at seven predetermined intervals during 48 h and replaced with fresh acceptor phase. Theophylline in the acceptor phase was determined by HPLC. The detailed preparation of the skin, donor samples and the theophylline determination have already been described in our previous study.[22]

Cumulative amounts of theophylline (μg/cm^2) were plotted against time and steady-state fluxes (μg/cm^2/h) were calculated from the linear region of the plot. The transdermal penetration-enhancing activity of each compound was then expressed as the enhancement ratio (ER)—ratio of the flux of theophylline with the addition of an enhancer and the flux of theophylline in the control sample. The data are presented as means ± standard deviation (SD) obtained from the skin fragments of 3–8 animals. The statistical significance of the differences was analysed with Student's t-test; value of $p < 0.05$ was considered significant.

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