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# Investigating the activity of 2-substituted alkyl-6-(2,5-dioxopyrrolidin-1-yl)hexanoates as skin penetration enhancers

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ABSTRACT

Skin penetration enhancers are used in the formulation of transdermal delivery systems for drugs that are otherwise not sufficiently skin-permeable. We generated two series of esters by multi-step synthesis with substituted 6-aminohexanoic acid as potential transdermal penetration enhancers by multi-step synthesis. The synthesis of all newly prepared compounds is presented here. Structure confirmation of all generated compounds was accomplished by $^{1}$H NMR, $^{13}$C NMR, IR and MS spectroscopy. All the prepared compounds were analyzed using RP-HPLC and their lipophilicity (log *k*) was determined. The hydrophobicity (log *P*/*C* log *P*) of the studied compounds was also calculated using two commercially available programs and 3D structures of the selected compounds were investigated by means of ab initio calculations of geometry and molecular dynamic simulations. All the synthesized esters were tested for their in vitro transdermal penetration-enhancing activity and showed higher enhancement ratios than oleic acid. The highest enhancement ratios were exhibited by compound **5f** ($C_{(2)}$ substituted with piperidine-2-one, $C_{11}$ ester chain) and **5a** ($C_{(2)}$ substituted with piperidine-2-one, $C_6$ ester chain). The series with a ω-lactam ring (piperidin-2-one; **5a**–**g**), showed slightly higher activities than those with morpholine (**6a**–**6g**). All of the agents showed minimal anti-proliferative activity ($IC_{50}$ >6.25 μM), indicating they would have low cytotoxicity when administered as chemical penetration enhancers. The relationships between the lipophilicity and the chemical structure of the studied compounds, as well as the correlation between their chemical structure and transdermal penetration-enhancing activity, are discussed.



## 1. Introduction

Developments in the field of pharmaceutical administration have resulted in the discovery of highly sophisticated drug delivery systems that allow for the maintenance of a constant drug level in an organism. Transdermal therapeutic systems are an excellent alternative to conventional pharmaceutical administration forms. However, the application of transdermal drug delivery faces the problem of insufficient or no penetration of active pharmaceutical substances through the skin. The outermost layer of skin, namely the stratum corneum (SC), forms a barrier for most exogenous substances, including drugs. The barrier function of the SC is attributed to its multi-layered wall-like structure, in which terminally differentiated keratin-rich epidermal cells (corneocytes) are embedded in an intercellular lipid-rich matrix. Several technological advances have been made in the recent decades to overcome skin barrier properties. Examples include physical means such as iontophoresis, sonophoresis, microneedles, chemical techniques using penetration enhancers, and biochemical methods such as liposomal vesicles.[1–3]

Transdermal penetration enhancers (also called sorption promoters or accelerants) are special pharmaceutical excipients that interact with skin components to increase penetration of drugs from topical application to entrance into the circulation. Numerous compounds of different chemical varieties were evaluated as penetration enhancers and a number of potential sites and modes of action have been identified.[3–6] In spite of the extensive research in this field, chemical penetration enhancers have not reached their full potential in transdermal or topical systems thus far.

Chemical penetration enhancers (CPEs) are compounds which can partition into and interact with the SC constituents when incorporated into a transdermal formulation, thereby reducing the resistance of the skin to drug diffusion. Several possible mechanisms of action of enhancers have been hypothesized, but the exact

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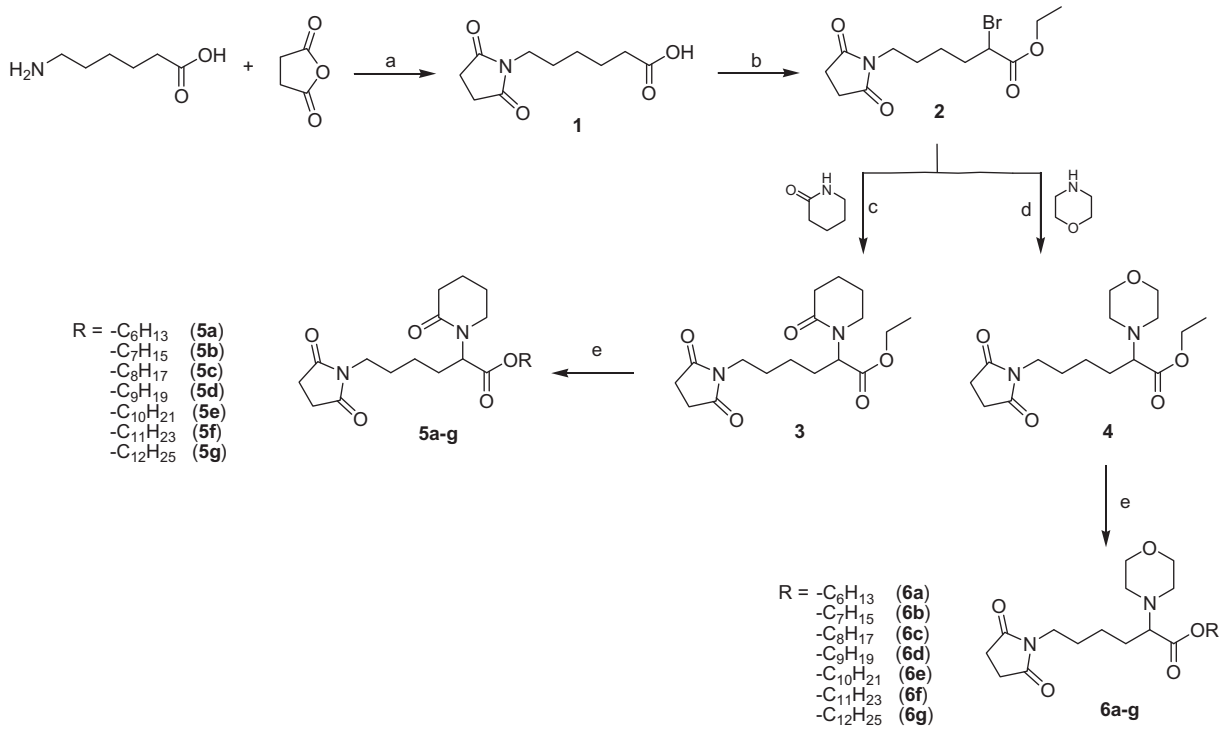


**Scheme 1.** Synthesis of target compounds **5a–g** and **6a–g**. Reagents: (a) acetone; (b) one pot synthesis: $SOCl_2$, $Br_2$, EtOH; (c) NaH, DMF, $Cu_2O$; (d) toluene; (e) Na, R-OH.

**Table 1**
Comparison of calculated lipophilicities (log *P*/*C* log *P*) with determined log *k* values

| Compd | Log *k* | Log *P*/*C* log *P* (ChemOffice) | Log *P* (ACD/Log *P* DB) |
|---|---|---|---|
| **3** | −0.9565 | 0.03/0.7562 | 0.37 ± 0.56 |
| **4** | −0.7951 | −0.20/0.55 | −0.33 ± 0.48 |
| **5a** | −0.2356 | 1.77/2.8722 | 2.41 ± 0.56 |
| **5b** | −0.0685 | 2.19/3.4012 | 2.92 ± 0.56 |
| **5c** | 0.0973 | 2.60/3.9302 | 3.43 ± 0.56 |
| **5d** | 0.2638 | 3.02/4.4592 | 3.94 ± 0.56 |
| **5e** | 0.4285 | 3.44/4.9882 | 4.45 ± 0.56 |
| **5f** | 0.5894 | 3.86/5.5172 | 4.96 ± 0.56 |
| **5g** | 0.7413 | 4.27/6.0462 | 5.47 ± 0.56 |
| **6a** | −0.0736 | 1.54/2.606 | 1.71 ± 0.48 |
| **6b** | 0.0934 | 1.95/3.195 | 2.22 ± 0.45 |
| **6c** | 0.2613 | 2.37/3.724 | 2.73 ± 0.48 |
| **6d** | 0.4177 | 2.79/4.253 | 3.23 ± 0.48 |
| **6e** | 0.5834 | 3.21/4.782 | 3.74 ± 0.48 |
| **6f** | 0.7588 | 3.62/5.311 | 4.25 ± 0.48 |
| **6g** | 0.9221 | 4.02/5.84 | 4.76 ± 0.48 |
| Oleic acid | —[a] | 6.29/7.7860 | 7.42 ± 0.20 |

[a] Log *k* value was not determined.

mechanisms have not been elucidated. It is almost certain that they have multiple effects. Generally, it can be stated that CPEs can (i) interact with the intercellular lipid matrix (especially ceramides), (ii) interact with protein structures (influencing the conformation of keratin in the corneocytes or proteins in desmosomes), or (iii) promote partitioning (influencing the SC nature leading to an increase in the penetrant concentration gradient and thus increasing the flux, i.e., increasing the concentration of the drug in the skin).[3]

Dodecylazacycloheptan-2-one (Azone®) is one of the most studied penetration enhancers and possesses a large polar head group and a lipid alkyl chain which are thought to be necessary for its activity.[3,4] As it could be expected from its chemical structure, Azone® is a highly lipophilic chemical with a log *P* of 6.2.[3,4] It enhances the skin transport of a wide variety of drugs with different lipophilicities. Its possible mechanism of action has been reported.[3,4,7,8] The chemical structure of Azone® is a combination of pyrrolidone and decylmethylsulfoxide, both of which are potential penetration enhancers.[9] To date, many Azone®-related compounds (including modifications of heterocyclic moiety and/or lipophilic chain) have been made and studied as penetration enhancers.[3] The 6-aminohexanoic acid esters were also designed as acyclic Azone® analogs[3] and pyrrolidin-2,5-dione, piperidin-2-one and morpholine are also fragments of described potent penetration enhancers. The novelty of the new compounds described herein is that they represent a combination of drug fragments which showed high efficiency as transdermal penetration enhancers.[3,10,11]

The multi-step synthesis of a series of seven alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoates and seven alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoates with $C_6$–$C_{12}$ linear alkyl ester chains as novel CPEs is described herein. The traditional lipophilicity parameter, log *P*, is a well-known physico-chemical descriptor largely used for quantitative structure–activity relationship analysis. In some experimental studies of penetration enhancement, the lipophilicity (non-polarity) of enhancers was measured and the corresponding relationship between enhancer lipophilicity and penetration enhancement potency was investigated.[12–16] Therefore, we have examined both the experimental lipophilicity log *k* data[17] and calculated lipophilicity log *P*/*C* log *P* of all compounds. The geometry of four selected compounds was investigated by means of ab initio calculations and molecular dynamic simulations and their 3D structures are presented. This is significant, as physico-chemical properties such as planarity can influence transdermal penetration. Primary in vitro screening of transdermal penetration-enhancing activity of all synthesized esters was evaluated using a Franz cell.[18] Compounds possessing carbonyl moieties are potential iron chelating agents with possible anti-proliferative activity.[19] Therefore, all the discussed compounds were evaluated for their anti-proliferative activity against the human SK-N-MC neuroepithelioma cell line. The structure–activity relationships of the studied compounds are discussed.

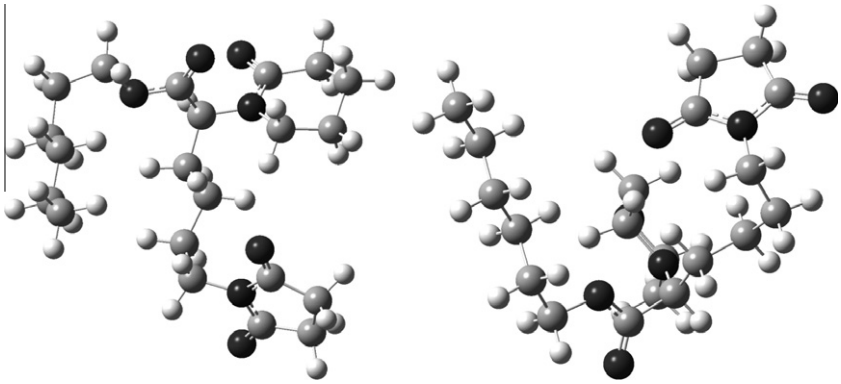

**Figure 1.** 3D structure of the *S*-enantiomer of hexyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (**5a**) and hexyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoate (**6a**).

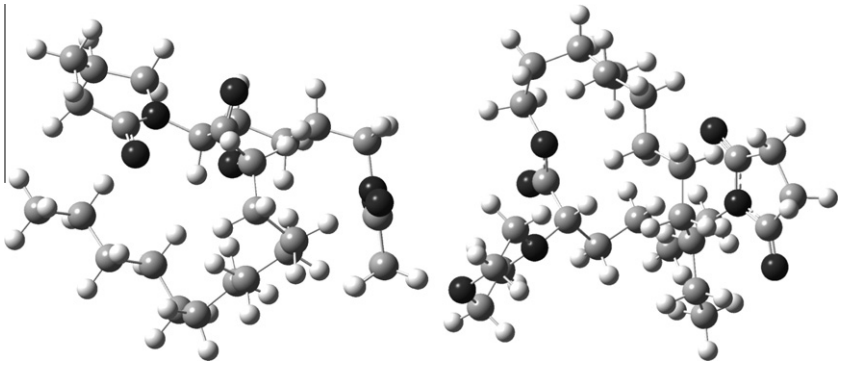

**Figure 2.** 3D structure of the *S*-enantiomer of dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (**5g**) and dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoate (**6g**).

**Table 2**
Exocyclic torsion angles (ExTors) of compounds **5a** and **5g** from molecular dynamics (MD) and ab initio (AI) calculations

| ExTor [°] | **5a** (MD) | **5a** (AI) | **5g** (MD) | **5g** (AI) |
|---|---|---|---|---|
| 1 | 180 | 182 | 180 | 177 |
| 2 | 56; 179 | 311 | 172; 133; 7 | 241 |
| 3 | 175; 69 | 177 | 175; 63 | 61 |
| 4 | 182; 292 | 195 | 181; 77; 289 | 174 |
| 5 | 182; 292 | 293 | 181; 67 | 181 |
| 6 | 183; 296; 63 | 294 | 178; 64; 296 | 57 |
| 7 | 109 | 105 | 271; 72 | 254 |
| 8 | 244 | 264 | 64 | 290 |
| 9 | 180; 73; 288 | 183 | 178; 72; 286 | 182 |
| 10 | 299; 64; 180 | 64 | 301; 179 | 67 |
| 11 | 180; 69 | 66 | 181; 290 | 289 |
| 12 | 181; 67; 290 | 176 | 180; 295; 65 | 60 |
| 13 | 180; 292 | 67 | 180; 290; 67 | 188 |
| 14 | 300; 60; 180 | 180 | 180; 64;295 | 290 |
| 15 | — | — | 179; 297; 3 | 187 |
| 16 | — | — | 118; 58;298 | 171 |
| 17 | — | — | 182; 357 | 69 |
| 18 | — | — | 182; 357 | 179 |
| 19 | — | — | 180; 64; 295 | 182 |
| 20 | — | — | 59; 298; 180 | 178 |

## 2. Result and discussion

### 2.1. Chemistry

6-Aminohexanoic acid and succinic anhydride were used as the starting materials for the multi-step synthesis and by their reaction, 6-(2,5-dioxopyrrolidin-1-yl)hexanoic acid (**1**) was obtained (Scheme 1). Using an optimised Schwenk and Papa procedure,[20,21] **1** gave ethyl-2-bromo-6-(2,5-dioxopyrrolidin-1-yl)hexanoate (**2**) through a one-pot synthesis, which we have reported previously.[22]

The key intermediate, ethyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (**3**), was prepared from the α-bromocarboxylic compound, **2**, and piperidin-2-one using the heterogeneous catalyst, powdered copper(I) oxide. The preparation of a morpholine adduct, **4**, was carried out under conventional conditions.[23]

The series of desired alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoates (**5a**–**g**) and alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoates (**6a**–**g**) were formed by conventional base-catalyzed transesterification[24] of the key intermediates, **3** or **4**, in excess of the corresponding primary unbranched alcohol, according to Scheme 1.

### 2.2. Lipophilicity of the prepared compounds

The hydrophobicity (log *P*/*C* log *P* values) of the studied compounds **3**, **4**, **5a**–**5g**, and **6a**–**6g** were calculated using two commercially available programs (ChemDraw Ultra 10.0 and ACD/Log P DB). Their hydrophobicities were measured experimentally by RP-HPLC determination of their capacity factors (*k*) with subsequent calculation of log *k*. This procedure measures the retention times of the compounds under isocratic conditions with various amounts of an organic modifier (methanol) in the mobile phase. Using an end-capped non-polar $C_{18}$ stationary RP column, the capacity factor *k* can be calculated. log *k* is used as a lipophilicity index converted to the log *P* scale.[17] The results are shown in Table 1.

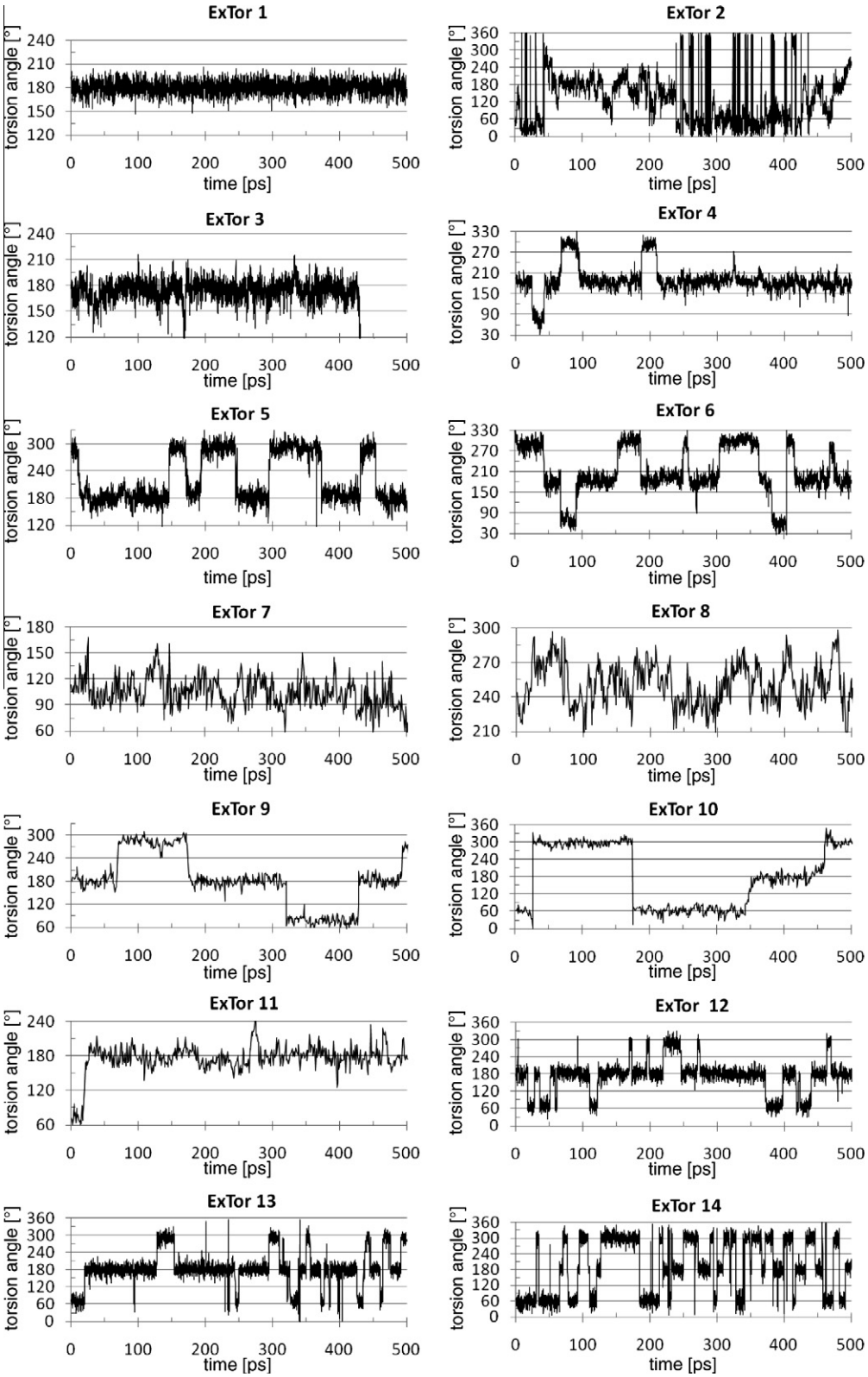


**Figure 3.** Exocyclic torsion angles (ExTors) from molecular dynamic calculations.

The data obtained with all the compounds show that the experimentally-determined lipophilicities (log *k*) of compounds **3**–**6g** are lower than those indicated by the calculated log *P*/*C* log *P*. Contrary to our experimental results, the calculated data showed that the morpholine series, **6a**–**6g**, possess lower lipophilicity than the piperidin-2-one series, **5a**–**5g**. This is probably caused by intramolecular interactions between the lactam carbonyl moiety and other sterically close carbonyl groups.

As expected, **3** and **4** showed the lowest lipophilicity, whereas dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)

hexanoate (**5g**) and dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoate (**6g**) were the most lipophilic within each series. The calculated log *P*/*C* log *P* data and the determined log *k* values correspond to the expected trend in lipophilicity, increasing within each series of evaluated compounds (ethyl ⋘ hexyl < heptyl < nonyl < decyl < undecyl < dodecyl derivatives). This dependence is approximately linear. The log *k* data corresponds to the lipophilicity within the series of compounds discussed.

### 2.3. Geometries—3D structures

The chemical structure as well as exact 3D geometries of the studied penetration enhancers under study influence their physico-chemical properties and, thus, moderate their penetration-enhancing activity. Furthermore, they are also used for the derivation of a series of structural descriptors like molecular dimensions, volume and surface, dipole moment etc., which are often used for in silico screening and design of new penetration enhancers.[3,25] One of several possible mechanisms of action of Azone®-like enhancers is that they intercalate between ceramides in the SC.[3,7] By doing this, they disrupt ceramide-ceramide *H*-bonds and bind firmly to the ceramide molecule, forming a "channel" in the SC.[3,7] As intercalation potency is dependent on a planar conformation, understanding the 3D structures of the studied compounds is of importance. Therefore, this section describes the ab initio calculations of geometry and molecular dynamic (MD) simulations. To correlate penetration-enhancing activity and other physico-chemical properties with structure, the exact 3D geometries of the (*S*)-enantiomers of compounds **5a** and **5g**, as well as **6a** and **6g** were studied by computational methods. The reason why these compounds were chosen is that they represent the shortest and the longest potential enhancers within each series under investigation. Due to the size of the molecules, which was discussed previously,[11] as well as for a better representation of the solvent media, a combination of molecular mechanics and ab initio quantum chemical methods were used to examine the geometries of our compounds.

The initial scan of geometries for all compounds was performed in vacuo using the conformational search utility in the program, HyperChem. For structures **5a**, **5g**, **6a** and **6g**, 28671, 49564, 39286 and 19091 initial conformations were explored, respectively. For subsequent ab initio calculations, this number was reduced and only conformations with relative energies within 2 kcal/mole were considered. These conformations were then re-optimized in water at HF/4-31G level in GAUSSIAN 09 W.[26,27] Global minima for all compounds are illustrated in Figures 1 and 2.

While ab initio calculations are appropriate for prediction of exact geometries of a limited number of conformers and their spectral (NMR, IR) or chiroptical properties (optical rotation, VCD) in a gas phase, they are not suitable for the scanning of a large number of conformers, as it is a time-consuming process. As noted previously,[11] the solvent media are usually quite poorly represented by ab initio calculations. For the above calculations at the ab initio level, the actual solvent used for experiments (propylene glycol/water 1:1 v/v) was simplified so that propylene glycol molecules were omitted and water molecules were replaced by a dielectric continuum using the CPCM (conductor-like polarizable continuum model) solvation model.

As the first step for a better representation of the solvent at the ab initio level, as well as a better scan of possible conformations and their dynamics, we performed a series of molecular MD runs at the molecular mechanics level with explicit solvent molecules under periodic boundary conditions. For this purpose, a periodic box was created and filled with a mixture of (*R*)-propylene glycol/(*S*)-propylene glycol/water 0.5:0.5:1 v/v. After pre-equilibration, a solute molecule was soaked and selected solvent molecules were deleted so as to preserve the composition and macroscopic density. The whole molecular system was optimized, heated to room temperature and equilibrated. A series of MD runs for compounds **5a** and **5g** were started from several best conformations after the conformational search.

Mean values of all exocyclic torsions from MD simulations are summarized in Table 2 (in decreasing order of frequency) and their graphical representations for compound **5g** are given in Figure 3. For comparison, global minima from ab initio calculations are also given in Table 2. Designation of exocyclic torsions are illustrated in Figure 4.

MD simulations starting from different conformers led usually to the same torsions with more or less different frequencies and consecutive MD runs produced only slightly different frequencies. This means that longer MD runs should be used for more realistic frequencies, but otherwise the qualitative picture is satisfactory. The MD torsion values agree well with ab initio values in Table 2. However, conformational flexibility is best revealed from the graphs in Figure 3 which show independent changes of almost all torsions. It is clearly evident that the conformer lifetimes are of the order of only few tens of picoseconds (Fig. 3) and for derivatives with longer alkyl chains it would be even less.

It can be concluded in this respect that MD simulations with explicit solvent molecules provide appropriate sampling of available conformations of the studied compounds which is far beyond the possibilities of ab initio calculations at present. A detailed analysis of all torsions and their changes as well as of hydrogen bonds which were observed during simulations would be interesting, but beyond the scope of this paper.

Considering the above discussed data, the compounds examined in this study show a non-planar conformation, the consequence of which can be lower penetration activity. Indeed, a planar configuration is advantageous due to preferable interactions with skin components, especially ceramides.[3,7]

### 2.4. In vitro screening of transdermal penetration-enhancing activity

The penetration-enhancing activity of the prepared compounds was evaluated using theophylline as a model penetrant and propylene glycol/water 1:1 v/v as a donor vehicle. Theophylline has been used as a model drug of medium polarity (log *P* −0.06; log $D_8$ −0.05),[28,29] as it has been extensively studied in transdermal penetration experiments.[30,31] Most of the studies involved the use of propylene glycol (PG) or its mixture with water or ethanol as a donor vehicle. Previous studies have indicated that PG by itself (or a PG/water co-solvent system) does not interfere with membranes, but rather exhibits a synergistic effect in combination with other penetration enhancers.[32–34] Porcine ear skin was selected for initial evaluation of enhancement activity of prepared compounds as this tissue is a suitable in vitro model for human skin.[35,36] Porcine skin has been shown to be histologically and biochemically similar to human skin and full-thickness pig ear skin has been used in numerous percutaneous absorption studies.[37] Nevertheless, for testing of hydrophobic penetrants, dermatomed skin has been recommended.[38] The skin permeation experiments were performed using static Franz diffusion cells.[18]

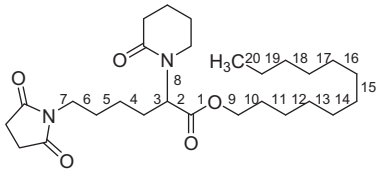


**Figure 4.** Designation of exocyclic torsion angles (ExTors).

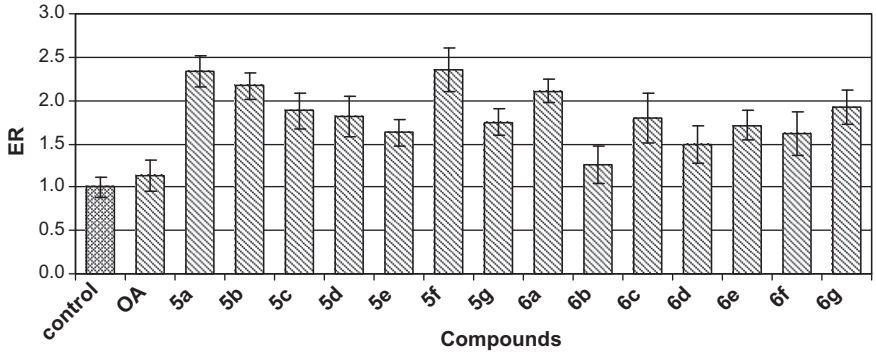


**Figure 5.** Enhancement ratios (ERs) of the prepared compounds **5a**–**g**, **6a**–**g** and oleic acid (**OA**). Control experiments used theophylline in the donor vehicle without any enhancer. Results are expressed as mean ± SD ($n$ = 3 experiments).

The effect of alkyl esters, **5a**–**g** and **6a**–**g**, on the penetration of theophylline through porcine skin is presented in Figure 5. All prepared compounds, **5a**–**g** and **6a**–**g**, enhanced the penetration of theophylline through the porcine skin and showed better enhancement ratios (ERs) than oleic acid (OA), which was used for comparison and belongs to the most studied enhancers in the fatty acid group of compounds.[39] Control experiments were run with only theophylline in the donor vehicle in the absence of any enhancer. The highest enhancement ratios (ERs) were obtained with compounds **5f** (undecyl, piperidin-2-one) and **5a** (hexyl, piperidin-2-one), with ERs of 2.36 and 2.33, respectively. The ERs are presented in Table 3.

According to the above presented data (Table 3, Fig. 5), it can be concluded that all the discussed compounds showed only moderate penetration activity. This moderate activity may be predicted from the 3D structures of the selected compounds (Figs. 1 and 2) in the medium propylene glycol/water 1:1 v/v, where non-planar (coned/coiled) conformations of compounds are illustrated. Due to the non-planar conformations of all the discussed compounds, their intercalation potency is decreased, contrary to planar (open) conformations, which can cause their moderate penetration activity.[3,7]

The penetration of the enhancers through the skin was further investigated. Only compounds **5a**, **5b**, **6a** and **6b** were detected in the receptor compartment after 24 h. All other alkyl esters **5c**–**5g** and **6c**–**g** were not detected and it was assumed that they did not get through the skin barrier or remained incorporated in skin structures due to their high lipophilicity.

**Table 3**
Enhancement ratios (ERs) of the prepared compounds, **5a**–**5g** and **6a**–**6g**, and oleic acid (OA) and in vitro anti-proliferative activity of the studied compounds using SK-N-MC neuroepithelioma cells

| Compd | ER | SK-N-MC $IC_{50}$ (μM) |
|---|---|---|
| **5a** | 2.33 ± 0.18 | >6.25 |
| **5b** | 2.17 ± 0.15 | >6.25 |
| **5c** | 1.88 ± 0.21 | >6.25 |
| **5d** | 1.81 ± 0.23 | >6.25 |
| **5e** | 1.63 ± 0.15 | >6.25 |
| **5f** | 2.36 ± 0.25 | >6.25 |
| **5g** | 1.75 ± 0.16 | >6.25 |
| **6a** | 2.11 ± 0.13 | >6.25 |
| **6b** | 1.26 ± 0.22 | >6.25 |
| **6c** | 1.80 ± 0.29 | >6.25 |
| **6d** | 1.49 ± 0.21 | >6.25 |
| **6e** | 1.71 ± 0.17 | >6.25 |
| **6f** | 1.61 ± 0.25 | >6.25 |
| **6g** | 1.92 ± 0.20 | >6.25 |
| OA | 1.13 ± 0.18 | — |
| Dp44mT | — | 0.01 ± 0.01 |
| DFO | — | 17.07 ± 3.77 |

ERs data are expressed as mean ± SD ($n$ = 3 experiments) and anti-proliferative activity of the compounds are expressed as mean ± SD ($n$ = 3 experiments).

## 2.5. In vitro anti-proliferative activity

Carbonyl-containing moieties are potential iron chelating agents, with many demonstrating anti-proliferative activity due to their ability to bind cellular iron which is essential for proliferation.[40] Therefore, we examined the anti-proliferative effects of our novel CPEs against the human SK-N-MC neuroepithelioma cell line to examine if there may be any undesired cytotoxic side-effects associated with CPE treatment (Table 3). The SK-N-MC cell line was chosen as the effect of iron chelators on their proliferation has been extensively examined.[41,42] The anti-proliferative activity of these novel compounds was assessed in comparison to the well-known clinically used iron chelator, desferrioxamine (DFO), and the highly cytotoxic chelator, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT).[40]

All alkyl esters, **5a**–**g** and **6a**–**g**, demonstrated poor anti-proliferative effects against the SK-N-MC cell line, with $IC_{50}$ values greater than 6.25 μM (Table 3). As expected from our previous studies,[41,42] the iron chelator, DFO, demonstrated poor anti-cancer activity, with an $IC_{50}$ value of 17.07 μM, while the cytotoxic chelator, Dp44mT ($IC_{50}$: 0.01 μM), showed potent anti-proliferative effects (Table 3). These results suggest that the poor anti-proliferative activity of our alkyl ester, **5a**–**g** and **6a**–**g**, will lead to limited cytotoxicity when used in vivo as CPEs.

## 3. Conclusions

In the current study, a series of seven alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoates and seven alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoates with $C_6$–$C_{12}$ linear alkyl ester chains were prepared as potential transdermal penetration enhancers. The ability of these compounds to enhance the penetration of theophylline through porcine skin was examined. All prepared compounds showed higher enhancement ratios (ERs) than oleic acid. The highest ERs in this study were exhibited by compound **5f** ($C_{(2)}$ substituted with piperidine-2-one, $C_{11}$ ester chain) and **5a** ($C_{(2)}$ substituted with piperidine-2-one, $C_6$ ester chain). We can conclude that the series with a ω-lactam ring (piperidin-2-one), **5a**–**g**, showed slightly higher activities than those with morpholine **6a**–**6g**. Most of the prepared compounds did not penetrate through the skin, except for compounds **5a**, **5b**, **6a** and **6b** which were detected in the receptor phase in 24 h. All the compounds were additionally evaluated for their anti-proliferative activity against the human SK-N-MC neuroepithelioma cell line. None of the studied compounds showed any significant anti-proliferative activity, suggesting that these compounds would have low cytotoxic side-effects when administered as CPEs. Thus, our novel alkyl esters, **5a** and **5f**, show promise as CPEs with minimal cytotoxicity.

## 4. Experimental

### 4.1. Chemistry

All reagents were purchased from Sigma-Aldrich (Schnelldorf, Germany) and Merck (Darmstadt, Germany). Kieselgel 60, 0.040–0.063 mm (Merck, Darmstadt, Germany) was used for column chromatography. Thin layer chromatography (TLC) experiments were performed on alumina-backed Silica Gel 40 F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and evaluated in iodine vapour. The melting points were determined on a Mikro-Heiztisch System PolyTherm A apparatus (Wagner & Munz, Munich and Hund, Wetzlar, Germany) and are uncorrected. Infrared (IR) spectra were recorded on a Smart MIRacle™ ATR ZnSe for Nicolet™ 6700 FT-IR Spectrometer (Thermo Scientific, USA). The spectra were obtained by accumulation of 256 scans with 2 $cm^{-1}$ resolution in the 4000–600 $cm^{-1}$ region. All $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker Avance-500 FT-NMR spectrometer (500 MHz for $^1H$ and 125 MHz for $^{13}C$, Bruker Comp., Karlsruhe, Germany). Chemicals shifts are reported in ppm ($\delta$) using internal $Si(CH_3)_4$ as the reference, with diffuse, easily exchangeable signals being omitted. Mass spectra were measured using a LTQ Orbitrap Hybrid Mass Spectrometer (Thermo Electron Corporation, USA) with direct injection into an APCI source (400 °C) in the positive mode.

#### 4.1.1. 6-(2,5-Dioxopyrrolidin-1-yl)hexanoic acid (1)

This was synthesised as described previously by Brychtova et al.[11,22]

#### 4.1.2. Ethyl-2-bromo-6-(2,5-dioxopyrrolidin-1-yl)hexanoate (2)

This was synthesised as described by Brychtova et al.[11,22]

#### 4.1.3. Ethyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl) hexanoate (3)

Piperidin-2-one (46.9 mmol) was added slowly to a suspension of NaH (51.5 mmol, 60% dispersion in mineral oil) in dry DMF (100 mL). The mixture was stirred for a few minutes until the evolution of hydrogen gas ceased. Compound **2** (10.0 g, 31.2 mmol) and $Cu_2O$ (1.1 g, 7.8 mmol) were then added and the mixture was refluxed under argon for 9 h. The cooled mixture was poured onto ice, filtered and extracted with chloroform. The combined organic extracts were washed with water, dried over $MgSO_4$, filtered and the organic solvent was removed under vacuum. The crude product was purified by flash chromatography on silica gel (ethyl acetate/petroleum ether/TEA 10:1:0.1). This provided a light yellow oil. Yield 7.3 g (70%). $^1H$ NMR (500 MHz, $CDCl_3$), $\delta$: 5.14 (dd, $J^1$ = 5.0 Hz, $J^2$ = 10.3 Hz, 1H, CH), 4.22–4.06 (m, 2H, $COOCH_2$), 3.50 (t, $J$ = 7.1 Hz, 2H, $NCH_2$), 3.29–3.17 (m, 2H, $NCH_2$pip.), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.48–2.42 (m, 2H, $CH_2$pip.), 2.01–1.53 (m, 8H, $CH_2$), 1.37–1.23 (m, 2H, $CH_2$), 1.26 (t, $J$ = 7.2 Hz, 3H, $CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$), $\delta$: 177.13, 171.16, 170.47, 61.00, 55.72, 44.13, 38.42, 32.21, 28.13, 27.48, 27.22, 23.46, 23.12, 20.98, 14.15. IR ($cm^{-1}$): 2941, 2868, 1731, 1698, 1637, 1401, 1178. HR-MS: for $C_{17}H_{27}O_5N_2$ $[M+H]^+$ calcd 339.1914 $m/z$, found 339.1914 $m/z$.

#### 4.1.4. Ethyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoate (4)

Morpholine (5.4 g, 62.4 mmol) was dissolved in toluene (50 mL) and compound **2** (10.0 g, 31.2 mmol) was added. The mixture was refluxed under argon for 5 h. The solvent was evaporated and the residue was suspended in $Et_2O$. The solid was filtered, washed with $Et_2O$ and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel ($CH_2Cl_2$/MeOH 95:5). This provided a yellow oil. Yield: 8.1 g (80%). $^1H$ NMR (500 MHz, $CDCl_3$), $\delta$: 4.18 (q, $J$ = 7.1 Hz, 2H, $COOCH_2$), 3.74–3.63 (m, 4H, $OCH_2$ morph.), 3.50 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.10 (t, $J$ = 7.4 Hz, 1H, CH), 2.71 (s, 4H, $OCCH_2CH_2CO$), 2.66–2.48 (m, 4H, $NCH_2$ morph.), 1.80–1.52 (m, 4H, $CH_2$), 1.45–1.27 (m, 2H, $CH_2$), 1.29 (t, $J$ = 7.1 Hz, 3H, $CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$), $\delta$: 177.07, 171.72, 67.54, 67.38, 60.19, 49.95, 38.57, 28.29, 28.13, 27.38, 23.32, 14.48. IR ($cm^{-1}$): 2948, 2855, 1697, 1400, 1150, 1114. HR-MS: for $C_{16}H_{27}O_5N_2$ $[M+H]^+$ calcd 327.1914 $m/z$, found 327.1915 $m/z$.

#### 4.1.5. General procedure for preparation alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoates (5a–g) and alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl) hexanoates (6a–g)

The mixture of ethyl ester **3** or **4** (7.7 mmol), appropriate primary alcohol (38.5 mmol) and metallic sodium (3.85 mmol) was stirred at 90 °C in an oil bath until the sodium was completely dissolved. The mixture was then heated at 130 °C for 5–7 h and ethanol was distilled off during the reaction as it formed. The excess of longer-chain alkyl alcohol was distilled off under reduced pressure and the rest was extracted with AcOH (0.5 M) and $Et_2O$. The organic layer was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography on silica gel using ethyl acetate/petroleum ether (5:1) as the eluent.

**4.1.5.1. Hexyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (5a).** Colorless oil, yield 39%. $^1H$ NMR (500 MHz, $CDCl_3$), $\delta$: 5.16 (dd, $J^1$ = 10.4 Hz, $J^2$ = 5.3 Hz, 1H, CH), 4.18–4.03 (m, 2H, $COOCH_2$), 3.50 (t, $J$ = 7.3 Hz, 2H, $NCH_2$), 3.27–3.17 (m, 2H, $NCH_2$ pip.), 2.71 (s, 4H, $OCCH_2CH_2CO$), 2.48–2.41 (m, 2H, $CH_2$pip.), 2.05–1.53 (m, 10H, $CH_2$), 1.36–1.26 (m, 8H, $CH_2$), 0.89 (t, $J$ = 6.5 Hz, 3H, $CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$), $\delta$: 177.16, 171.26, 170.44, 65.22, 55.79, 44.19, 38.45, 32.24, 31.33, 28.49, 28.14, 27.49, 27.25, 25.54, 23.49, 23.14, 22.48, 21.01, 13.92. IR ($cm^{-1}$): 2932, 2868, 1731, 1697, 1640, 1401, 1166. HR-MS: for $C_{21}H_{35}O_5N_2$ $[M+H]^+$ calcd 395.2540 $m/z$, found 395.2541 $m/z$.

**4.1.5.2. Heptyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (5b).** Colorless oil, yield 45%. $^1H$ NMR (500 MHz, $CDCl_3$), $\delta$: 5.16 (dd, $J^1$ = 10.4 Hz, $J^2$ = 5.2 Hz, 1H, CH), 4.15–4.02 (m, 2H, $COOCH_2$), 3.50 (t, $J$ = 7.3 Hz, 2H, $NCH_2$), 3.28–3.17 (m, 2H, $NCH_2$pip.), 2.71 (s, 4H, $OCCH_2CH_2CO$), 2.48–2.41 (m, 2H, $CH_2$pip.), 2.05–1.52 (m, 10H, $CH_2$), 1.41–1.21 (m, 10H, $CH_2$), 0.88 (t, $J$ = 6.5 Hz, 3H, $CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$), $\delta$: 177.16, 171.25, 170.44, 65.22, 55.78, 44.18, 38.45, 32.23, 31.67, 28.81, 28.53, 28.14, 27.49, 27.26, 25.83, 23.49, 23.14, 22.52, 21.01, 13.99. IR ($cm^{-1}$): 2931, 2859, 1731, 1697, 1640, 1401, 1166. HR-MS: for $C_{22}H_{37}O_5N_2$ $[M+H]^+$ calcd 409.2697 $m/z$, found 409.2697 $m/z$.

**4.1.5.3. Octyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (5c).** Colorless oil, yield 53%. $^1H$ NMR (500 MHz, $CDCl_3$), $\delta$: 5.15 (dd, $J^1$ = 10.4 Hz, $J^2$ = 5.3 Hz, 1H, CH), 4.15–4.02 (m, 2H, $COOCH_2$), 3.50 (t, $J$ = 7.3 Hz, 2H, $NCH_2$), 3.27–3.17 (m, 2H, $NCH_2$pip.), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.48–2.41 (m, 2H, $CH_2$pip.), 2.03–1.53 (m, 10H, $CH_2$), 1.37–1.21 (m, 12H, $CH_2$), 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$), $\delta$: 177.09, 171.21, 170.38, 65.18, 55.79, 44.17, 38.42, 32.21, 31.70, 29.09, 28.52, 28.12, 27.48, 27.23, 25.85, 23.46, 23.13, 22.55, 20.99, 13.98. IR ($cm^{-1}$): 2928, 2857, 1732, 1697, 1641, 1401, 1166. HR-MS: for $C_{23}H_{39}O_5N_2$ $[M+H]^+$ calcd 423.2853 $m/z$, found 423.2854 $m/z$.

**4.1.5.4. Nonyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (5d).** Colorless oil, yield 45%. $^1H$ NMR (500 MHz, $CDCl_3$), $\delta$: 5.16 (dd, $J^1$ = 10.4 Hz, $J^2$ = 5.3 Hz, 1H, CH), 4.15–4.02 (m, 2H, $COOCH_2$), 3.50 (t, $J$ = 7.3 Hz, 2H, $NCH_2$), 3.27–3.17 (m, 2H,

$NCH_2$pip.), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.48–2.41 (m, 2H, $CH_2$pip.), 2.03–1.53 (m, 10H, $CH_2$), 1.37–1.23 (m, 14H, $CH_2$), 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.12, 171.25, 170.43, 65.22, 55.81, 44.20, 38.46, 32.25, 31.82, 29.44, 29.18, 28.55, 28.15, 27.51, 27.26, 25.88, 23.50, 23.16, 22.62, 21.03, 14.03. IR ($cm^{-1}$): 2926, 2855, 1732, 1698, 1641, 1401, 1166. HR-MS: for $C_{24}H_{41}O_5N_2$ $[M+H]^+$ calcd 437.3010 $m/z$, found 437.3011 $m/z$.

**4.1.5.5. Decyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (5e).** Colorless oil, yield 47%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 5.16 (dd, $J^1$ = 10.5 Hz, $J^2$ = 5.4 Hz, 1H, CH), 4.14–4.02 (m, 2H, $COOCH_2$), 3.50 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.26–3.17 (m, 2H, $NCH_2$pip.), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.48–2.40 (m, 2H, $CH_2$pip.), 2.02–1.52 (m, 10H, $CH_2$), 1.41–1.21 (m, 16H, $CH_2$), 0.88 (t, $J$ = 6.5 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.14, 171.27, 170.45, 65.24, 55.81, 44.20, 38.47, 32.26, 31.86, 29.50, 29.26, 29.19, 28.56, 28.16, 27.51, 27.27, 25.90, 23.51, 23.17, 22.65, 21.04, 14.06. IR ($cm^{-1}$): 2925, 2854, 1732, 1698, 1642, 1401, 1166. HR-MS: for $C_{25}H_{43}O_5N_2$ $[M+H]^+$ calcd 451.3166 $m/z$, found 451.3167 $m/z$.

**4.1.5.6. Undecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (5f).** Colorless oil, yield 37%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 5.16 (dd, $J^1$ = 10.5 Hz, $J^2$ = 5.3 Hz, 1H, CH), 4.15–4.02 (m, 2H, $COOCH_2$), 3.50 (t, $J$ = 7.3 Hz, 2H, $NCH_2$), 3.25–3.16 (m, 2H, $NCH_2$pip.), 2.71 (s, 4H, $OCCH_2CH_2CO$), 2.48–2.41 (m, 2H, $CH_2$pip.), 2.05–1.50 (m, 10H, $CH_2$), 1.40–1.18 (m, 18H, $CH_2$), 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.13, 171.25, 170.42, 65.22, 55.79, 44.19, 38.45, 32.24, 31.87, 29.55, 29.48, 29.28, 29.18, 28.55, 28.15, 27.49, 27.26, 25.88, 23.49, 23.15, 22.64, 21.02, 14.05. IR ($cm^{-1}$): 2924, 2854, 1732, 1698, 1642, 1401, 1166. HR-MS: for $C_{25}H_{43}O_5N_2$ $[M+H]^+$ calcd 465.3323 $m/z$, found 465.3323 $m/z$.

**4.1.5.7. Dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopiperidin-1-yl)hexanoate (5g).** Colorless oil, yield 42%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 5.16 (dd, $J^1$ = 10.5 Hz, $J^2$ = 5.3 Hz, 1H, CH), 4.15–4.02 (m, 2H, $COOCH_2$), 3.50 (t, $J$ = 7.3 Hz, 2H, $NCH_2$), 3.25–3.17 (m, 2H, $NCH_2$pip.), 2.71 (s, 4H, $OCCH_2CH_2CO$), 2.48–2.41 (m, 2H, $CH_2$pip.), 2.05–1.50 (m, 10H, $CH_2$), 1.41–1.12 (m, 20H, $CH_2$), 0.88 (t, $J$ = 6.5 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.12, 171.24, 170.41, 65.22, 55.79, 44.19, 38.45, 32.23, 31.87, 29.58, 29.53, 29.48, 29.29, 29.17, 28.54, 28.14, 27.49, 27.25, 25.88, 23.48, 23.15, 22.64, 21.02, 14.05. IR ($cm^{-1}$): 2923, 2853, 1732, 1698, 1642, 1401, 1166. HR-MS: for $C_{27}H_{47}O_5N_2$ $[M+H]^+$ calcd 479.3479 $m/z$, found 479.3480 $m/z$.

**4.1.5.8. Hexyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl) hexanoate (6a).** Light yellow oil, yield 53%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 4.03 (t, $J$ = 6.6 Hz, 2H, $COOCH_2$), 3.63–3.57 (m, 4H, $OCH_2$ morph.), 3.43 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.03 (t, $J$ = 7.4 Hz, 1H, CH), 2.63 (s, 4H, $OCCH_2CH_2CO$), 2.57–2.47 (m, 4H, $NCH_2$ morph.), 1.69–1.45 (m, 6H, $CH_2$), 1.38–1.15 (m, 8H, $CH_2$), 0.82 (t, $J$ = 6.5 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 176.98, 171.70, 67.43, 67.25, 64.27, 49.80, 38.40, 31.20, 28.56, 28.17, 28.01, 27.25, 25.49, 23.20, 22.35, 13.81. IR ($cm^{-1}$): 2932, 2856, 1722, 1697, 1401, 1149, 1116. HR-MS: for $C_{20}H_{34}O_5N_2$ $[M+H]^+$ calcd 383.2540 $m/z$, found 383.2541 $m/z$.

**4.1.5.9. Heptyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl) hexanoate (6b).** Light yellow oil, yield 55%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 4.10 (t, $J$ = 6.6 Hz, 2H, $COOCH_2$), 3.71–3.65 (m, 4H, $OCH_2$ morph.), 3.50 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.10 (t, $J$ = 7.4 Hz, 1H, CH), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.64–2.55 (m, 4H, $NCH_2$ morph.), 1.76–1.52 (m, 6H, $CH_2$), 1.42–1.20 (m, 10H, $CH_2$), 0.89 (t, $J$ = 6.5 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.06, 171.84, 67.58, 67.39, 64.42, 49.94, 38.56, 31.66, 28.81, 28.74, 28.31, 28.13, 27.39, 25.92, 23.34, 22.52, 13.99. IR ($cm^{-1}$): 2930, 2855, 1722, 1698, 1400, 1152, 1116. HR-MS: for $C_{21}H_{36}O_5N_2$ $[M+H]^+$ calcd 397.2697 $m/z$, found 397.2696 $m/z$.

**4.1.5.10. Octyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl) hexanoate (6c).** Light yellow crystalline compound, mp. 40.4-41.6 °C, yield 58%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 4.11 (t, $J$ = 6.7 Hz, 2H, $COOCH_2$), 3.71–3.62 (m, 4H, $OCH_2$ morph.), 3.50 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.10 (t, $J$ = 7.4 Hz, 1H, CH), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.64–2.55 (m, 4H, $NCH_2$ morph.), 1.76–1.52 (m, 6H, $CH_2$), 1.46–1.23 (m, 12H, $CH_2$), 0.88 (t, $J$ = 6.5 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.10, 171.87, 67.59, 67.41, 64.45, 49.95, 38.58, 31.76, 29.14, 28.75, 28.33, 28.15, 27.41, 25.98, 23.36, 22.61, 14.05. IR ($cm^{-1}$): 2930, 2854, 1718, 1692, 1402, 1149, 1117. HR-MS: for $C_{22}H_{38}O_5N_2$ $[M+H]^+$ calcd 411.2853 $m/z$, found 411.2855 $m/z$.

**4.1.5.11. Nonyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl) hexanoate (6d).** Light yellow crystalline compound, mp 42.3-43.1 °C, yield 51%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 4.10 (t, $J$ = 6.6 Hz, 2H, $COOCH_2$), 3.71–3.65 (m, 4H, $OCH_2$ morph.), 3.50 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.10 (t, $J$ = 7.4 Hz, 1H, CH), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.64–2.55 (m, 4H, $NCH_2$ morph.), 1.75–1.52 (m, 6H, $CH_2$), 1.42–1.20 (m, 14H, $CH_2$), 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.10, 171.86, 67.58, 67.40, 64.45, 49.94, 38.58, 31.82, 29.44, 29.19, 28.75, 28.32, 28.14, 27.40, 25.98, 23.35, 22.63, 14.06. IR ($cm^{-1}$): 2916, 2850, 1728, 1699, 1400, 1158, 1117. HR-MS: for $C_{23}H_{40}O_5N_2$ $[M+H]^+$ calcd 425.3010 $m/z$, found 425.3011 $m/z$.

**4.1.5.12. Decyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl) hexanoate (6e).** Light yellow crystalline compound, mp 45.5-46.1 °C, yield 53%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 4.10 (t, $J$ = 6.7 Hz, 2H, $COOCH_2$), 3.71–3.65 (m, 4H, $OCH_2$ morph.), 3.50 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.10 (t, $J$ = 7.4 Hz, 1H, CH), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.64–2.55 (m, 4H, $NCH_2$ morph.), 1.76–1.52 (m, 6H, $CH_2$), 1.42–1.20 (m, 16H, $CH_2$), 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.06, 171.82, 67.54, 67.36, 64.41, 49.91, 38.53, 31.83, 29.46, 29.23, 29.15, 28.71, 28.28, 28.11, 27.37, 25.95, 23.31, 22.61, 14.04. IR ($cm^{-1}$): 2924, 2854, 1699, 1400, 1155, 1117. HR-MS: for $C_{24}H_{42}O_5N_2$ $[M+H]^+$ calcd 439.3166 $m/z$, found 439.3166 $m/z$.

**4.1.5.13. Undecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoate (6f).** Light yellow crystalline compound, mp 48.9-49.7 °C, yield 52%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 4.10 (t, $J$ = 6.7 Hz, 2H, $COOCH_2$), 3.71–3.65 (m, 4H, $OCH_2$ morph.), 3.50 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.10 (t, $J$ = 7.4 Hz, 1H, CH), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.64–2.55 (m, 4H, $NCH_2$ morph.), 1.76–1.52 (m, 6H, $CH_2$), 1.42–1.17 (m, 18H, $CH_2$), 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.08, 171.84, 67.57, 67.39, 64.43, 49.93, 38.56, 31.87, 29.55, 29.48, 29.29, 29.17, 28.74, 28.31, 28.13, 27.39, 25.97, 23.34, 22.64, 14.06. IR ($cm^{-1}$): 2924, 2853, 1699, 1401, 1156, 1117. HR-MS: for $C_{25}H_{44}O_5N_2$ $[M+H]^+$ calcd 453.3323 $m/z$, found 453.3323 $m/z$.

**4.1.5.14. Dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(morpholin-4-yl)hexanoate (6g).** Light yellow crystalline compound, mp 52.6-53.4 °C, yield 55%. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 4.10 (t, $J$ = 6.7 Hz, 2H, $COOCH_2$), 3.71–3.65 (m, 4H, $OCH_2$ morph.), 3.50 (t, $J$ = 7.4 Hz, 2H, $NCH_2$), 3.10 (t, $J$ = 7.4 Hz, 1H, CH), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.64–2,55 (m, 4H, $NCH_2$ morph.), 1.76–1.52 (m, 6H, $CH_2$), 1.42–1.17 (m, 20H, $CH_2$), 0.88 (t, $J$ = 6.4 Hz, 3H, $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$: 177.09, 171.86, 67.58, 67.39, 64.45,

49.94, 38.57, 31.89, 29.60, 29.55, 29.49, 29.31, 29.19, 28.75, 28.31, 28.14, 27.40, 25.98, 23.35, 22.65, 14.07. IR ($cm^{-1}$): 2919, 2852, 1717, 1694, 1401, 1155, 1117. HR-MS: for $C_{26}H_{46}O_5N_2$ $[M+H]^+$ calcd 467.3479 *m*/*z*, found 467.3480 *m*/*z*.

### 4.2. Lipophilicity HPLC determination (capacity factor *k*/calculated log *k*)

An Agilent 1200 series HPLC system was used equipped with a diode array detection (DAD) system, a quaternary model pump and an automatic injector (Agilent Technologies, Germany). Data acquisition was performed using ChemStation chromatography software. The $C_{18}$ 5 μm, 4.6 × 150 mm Zorbax Eclipse XDB chromatographic column (Agilent Technologies, Germany) was used for these studies. A mixture of MeOH (HPLC grade, 85.0%) and $H_2O$ (HPLC grade, 15.0%) was used as a mobile phase. The total flow of the column was 0.4 mL/min. An injection volume of 10 μL and column temperature of 25 °C were used. A detection wavelength of 204 nm and the bandwidth of 8 nm were chosen. A KI methanolic solution was used for dead time ($t_D$) determination. Retention times ($t_R$) were measured in minutes.

The capacity factors *k* were calculated using ChemStation chromatography software according to the formula $k = (t_R - t_D)/t_D$, where $t_R$ is the retention time of the solute, whereas $t_D$ denotes the dead time obtained via an unretained analyte. Log *k*, calculated from the capacity factor *k*, is used as a lipophilicity index converted to the log *P* scale.[17] The log *k* values of the individual compounds are shown in Table 1.

### 4.3. Lipophilicity and geometry calculations

Log *P* values (i.e., the logarithm of the partition coefficient for *n*-octanol/water) were predicted using CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) and ACD/Log *P* DB software (ACD/Labs, ver. 11.01, Advanced Chemistry Development, Inc., Canada, Toronto, 2007). *C* log *P* values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were generated using CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) software. The results are shown in Table 1.

The initial scan of geometries was performed using the conformational search utility in HyperChem (HyperChem for Windows, ver. 8.0.3, Hypercube, Inc., Gainesville, 2007). Structures within 2 kcal/mole were then re-optimized in water at HF/4-31G ab initio level in GAUSSIAN 09 W.[26] Water was simulated using the CPCM polarizable conductor calculation solvation model.[27] The results are illustrated in Figures 1 and 2.

MD simulations were performed in HyperChem using the MM+ force field. A periodic box with dimensions *a* = *b* = *c* = 20 Å was filled with solvent molecules using a published method[43] and adjusted using our own code. The resulting system consisted of 15 molecules of (*R*)-propylene glycol, 15 molecules of the (*S*)-enantiomer, 120 molecules of water and a solute molecule. The resulting macroscopic density was 1.02 g/$cm^3$. MD data were accumulated from a series of 500 ps runs with a 1 fs time step at 298 K within a thermostatically controlled water bath. Data were collected every 10 steps.

### 4.4. In vitro screening of transdermal penetration-enhancing activity

Skin samples were obtained from porcine ear. Full thickness dorsal skin was cut in fragments and stored at −20 °C until utilized. Skin samples were then slowly thawed (at 4 °C overnight and then at ambient temperature) before each experiment. The penetration-enhancing activity of newly synthesized compounds **5a**–**5g** and **6a**–**6g** were evaluated in vitro using a vertical Franz diffusion cell (PermeGear Inc., USA), with a donor surface area of 0.635 $cm^2$ and receptor volume of 5.2 mL. The skin was mounted between the donor and receptor compartments of the diffusion cell with the epidermal side up. The receptor compartment was filled with phosphate buffered saline (pH 7.4) and was maintained at 37 ± 0.5 °C through the use of a circulating water bath. The receptor compartment was continuously stirred using a magnetic stirring bar. The skin was kept in contact with the receptor phase for 0.5 h prior to the experiment. The donor samples were prepared by dissolving the tested enhancer (20 mg) in PG (0.5 mL) and a solution of theophylline (5 mg) in water (0.5 mL) was added. This mixture was shaken vigorously and then sonicated for 10 min at 40 °C. This stable system was applied to the skin surface and the donor compartment of the cell was covered by Parafilm®. The control samples were prepared in the same manner without enhancers. Samples (0.5 mL) of the receptor phase were withdrawn at six pre-determined time intervals over 24 h (1, 2, 4, 8, 12 and 24 h) and the cell was refilled with an equivalent amount of fresh buffer solution. A minimum of three determinations were performed using skin fragments from a minimum of two animals for each compound. The samples were stored at −18 °C until analyzed by HPLC.

### 4.5. Sample analysis

Analysis of samples for theophylline content from the transdermal penetration-enhancing activity studies above was performed using an Agilent 1200 series HPLC system. This was equipped with a diode array detection (DAD) system, quaternary model pump and automatic injector (Agilent Technologies, Germany). Data acquisition was performed using ChemStation chromatography software. A Waters Symmetry, $C_8$ 5 μm, 4.6 × 250 mm chromatographic column was used. A mixture of acetonitrile (HPLC grade, 50.0%) and $H_2O$ (HPLC grade, 50.0%) was used as the mobile phase. The total flow of the column was 0.5 mL/min, with an injection volume of 10 μL and a column temperature of 25 °C. A detection wavelength of 280 nm with a bandwidth of 8 nm was chosen. The retention time ($t_R$) of theophylline was 5.07 ± 0.05 min.

The cumulative amounts of theophylline that penetrated across the skin into the receptor compartment (μg/$cm^2$), which were corrected for sample removal, were plotted against time (h). A linear dependence was found ($R^2 \geqslant 0.98$) and steady state fluxes (μg/$cm^2$/h) were calculated using the linear region of the plots. Enhancement ratios (ERs) were calculated as the ratio of the flux of theophylline with and without the enhancer. The results are summarized in Table 3.

Determination of the penetrated enhancer was performed using the HPLC method described in Section 4.2. An injection volume of 10 μL was utilized and the samples of the receptor phase withdrawn at the mentioned intervals were then analysed.

### 4.6. Cell culture and in vitro anti-proliferative activity

The human SK-N-MC neuroepithelioma cell type was obtained from the American Type Culture Collection (Manassas, VA, USA) and was cultured in minimum essential medium (MEM; Gibco, Melbourne Australia) containing 10% (v/v) FBS, 1.0 mM sodium pyruvate (Gibco), 1% (v/v) non-essential amino acids (Gibco), 2 mM L-glutamine (Gibco), 100 U/mL penicillin (Gibco), streptomycin (Gibco) and 0.28 μg/mL fungizone (Squibb Pharmaceuticals, Montreal, Canada). Cells were cultured under standard conditions at 37 °C, in a humidified atmosphere at 5% $CO_2$.

The effect of the compounds on cellular proliferation were determined by the MTT [1-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium] assay using standard techniques.[41,42] Previous studies have demonstrated that MTT reduction is proportional to

viable cell counts using SK-N-MC cells.[44] The SK-N-MC cells were seeded in 96-well microtiter plates at $1.5 \times 10^4$ cells/well in medium containing human diferric transferrin (Tf) at 1.25 μM ([Fe] = 2.5 μM) and compounds at a range of concentrations (0–25 μM). Control samples contained medium with Tf (1.25 μM) without the compounds. The chelators, DFO and Dp44mT, were also included as internal controls, as their effects are well characterized in this cell line.[41,42] The cells were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$ and 95% air for 72 h. After 72 h, 10 μL of MTT solution (stock solution: 5 mg/mL) was added to each well and incubated for 2 h at 37 °C. After solubilization of the cells with 100 μL of 10% SDS-50% isobutanol in 0.01 M HCl, the plates were read at 570 nm using a scanning multi-well spectrophotometer. The inhibitory concentration ($IC_{50}$) was defined as the compound concentration necessary to reduce the absorbance to 50% of the untreated control.

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## References and notes

1. Smith, E. W.; Maibach, H. I. *Percutaneous Penetration Enhancers*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2006.
2. Thong, H.-Y.; Zhai, H.; Maibach, H. I. *Skin Pharmacol. Physiol.* **2007**, *20*, 272.
3. Jampilek, J.; Brychtova, K. *Med. Res. Rev. 30*, in press.
4. Williams, A. C.; Barry, B. W. *Adv. Drug Deliv. Rev.* **2004**, *56*, 603.
5. Benson, H. A. E. *Curr. Drug Deliv.* **2005**, *2*, 23.
6. Sinha, V. R.; Kaur, M. P. *Drug Dev. Ind. Pharm.* **2000**, *26*, 1131.
7. Hadgraft, J.; Peck, J.; Williams, D. G.; Pugh, W. J.; Allan, G. *Int. J. Pharm.* **1996**, *141*, 1725.
8. Lopez-Cervantes, M.; Marquez-Mejia, E.; Cazares-Delgadillo, J.; Quintanar-Guerrero, D.; Ganem-Quintanar, A.; Angeles-Anguiano, E. *Drug Dev. Ind. Pharm.* **2006**, *32*, 267.
9. Williams, A. C.; Barry, B. W. *Crit. Rev. Ther. Drug Carr. Syst.* **1992**, *9*, 305.
10. Pugh, W. J.; Wong, R.; Falson, F.; Michniak, B. B.; Moss, G. P. *J. Pharm. Pharmacol.* **2005**, *57*, 1389.
11. Brychtova, K.; Jampilek, J.; Opatrilova, R.; Raich, I.; Farsa, O.; Csollei, J. *Bioorg. Med. Chem.* **2010**, *18*, 73.
12. Zheng, T.; Hopfinger, A. J.; Esposito, E. X.; Liu, J.; Tseng, Y. J. *J. Chem. Inf. Model.* **2008**, *48*, 1238.
13. Ghafourian, T.; Zandasrar, P.; Hamishekar, H.; Nokhodchi, A. *J. Controlled Release* **2004**, *99*, 113.
14. Barry, B. W. *J. Controlled Release* **1987**, *6*, 85.
15. Kim, N.; El-Kattan, A. F.; Asbill, C. S.; Kennette, R. J.; Sowell, J. W.; Latour, R.; Michniak, B. B. *J. Controlled Release* **2001**, *73*, 183.
16. Baert, B.; Deconinck, E.; Van Gele, M.; Slodicka, M.; Stoppie, P.; Bode, S.; Slegers, G.; Vander Heyden, Y.; Lambert, J.; Beetens, J.; De Spiegeleer, B. *Bioorg. Med. Chem.* **2007**, *15*, 6943.
17. Pliska, V. In *Lipophilicity in Drug Action and Toxicology*; Pliska, V., Testa, B., van der Waterbeemd, H., Eds., 1st ed.; Methods and Principles in Medicinal Chemistry; Wiley-VCH: Weinheim, 1996; Vol. 4, pp 1–6.
18. Franz, T. J. *J. Invest. Dermatol.* **1975**, *64*, 190.
19. Lau, J.; Dorwald, F. Z.; Stephensen, H.; Bloch, P.; Hansen, T. K.; Madsen, K. PCT Int. Appl. 2006; WO/2006/097537.
20. Schwenk, E.; Papa, D. *J. Am. Chem. Soc.* **1948**, *70*, 3626.
21. Berry, J. P.; Isbell, A. F.; Hunt, G. E. *J. Org. Chem.* **1972**, *37*, 4396.
22. Brychtova, K.; Farsa, O.; Csollei, J. *Lett. Org. Chem.* **2009**, *6*, 25.
23. Brychtova, K.; Slaba, B.; Placek, L.; Jampilek, J.; Raich, I.; Csollei, J. *Molecules* **2009**, *14*, 3019.
24. Otera, J. *Chem. Rev.* **1993**, *93*, 1449. and references cited therein.
25. Karande, P.; Jain, A.; Ergun, K.; Kispersky, V.; Mitragotri, S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4688.
26. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. GAUSSIAN 09, Revision A.2, Gaussian, Inc., Wallingford CT, USA, 2009.
27. Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995.
28. Ni, N.; El-Sayed, M. M.; Sanghvi, T.; Yalkowsky, S. H. *J. Pharm. Sci.* **2000**, *89*, 1620.
29. Katz, M.; Ben-Shlush, I.; Kolusheva, S.; Jelinek, R. *Pharm. Res.* **2006**, *23*, 580.
30. Fang, J. Y.; Tsai, T. H.; Hung, C. F.; Wong, W. W. *J. Pharm. Pharmacol.* **2004**, *56*, 1493.
31. Sloan, K. B.; Beall, H. D.; Taylor, H. E.; Getz, J. J.; Villaneuva, R.; Nipper, R.; Smith, K. *Int. J. Pharm.* **1998**, *171*, 185.
32. Evrard, D.; Touitou, E.; Kolusheva, S.; Fishov, Y.; Jelinek, R. *Pharm. Res.* **2001**, *18*, 943.
33. Williams, A. C.; Barry, B. W. *Int. J. Pharm.* **1989**, *56*, 43.
34. Yamane, M. A.; Williams, A. C.; Barry, B. W. *J. Pharm. Pharmacol.* **1995**, *47*, 978.
35. Jacobi, U.; Kaiser, M.; Toll, R.; Mangelsdorf, S.; Audring, H.; Otberg, N.; Sterry, W.; Lademann, J. *Skin Res. Technol.* **2007**, *13*, 19.
36. Herkenne, C.; Naik, A.; Kalia, Y. N.; Hadgraft, J.; Guy, R. H. *Pharm. Res.* **2006**, *23*, 1850.
37. Meyer, W.; Schwarz, K.; Neurand, K. T. *Curr. Probl. Dermatol.* **1978**, *7*, 39.
38. Wilkinson, S. C.; Maas, W. J. M.; Nielsen, J. B.; Greaves, L. C.; Sandt, J. J. M.; Williams, F. M. *Int. Arch. Occup. Environ. Health* **2006**, *79*, 405.
39. Rowat, A. C.; Kitson, N.; Thewalt, J. L. *Int. J. Pharm.* **2006**, *307*, 225.
40. Kalinowski, D. S.; Richardson, D. R. *Pharmacol. Rev.* **2005**, *57*, 547.
41. Richardson, D. R.; Sharpe, P. C.; Lovejoy, D. B.; Senaratne, D.; Kalinowski, D. S.; Islam, M.; Bernhardt, P. V. *J. Med. Chem.* **2006**, *49*, 6510.
42. Kalinowski, D. S.; Yu, Y.; Sharpe, P. C.; Islam, M.; Liao, Y.-T.; Lovejoy, D. B.; Kumar, N.; Bernhardt, P. V.; Richardson, D. R. *J. Med. Chem.* **2007**, *50*, 3716.
43. Jin, Y.; Lee, S.K.; Lee, D.W. Yonsei University, Seoul, South Korea.
44. Richardson, D. R.; Tran, E. H.; Ponka, P. *Blood* **1995**, *86*, 4295.