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Synthesis, physico-chemical properties and penetration activity of alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoates as potential transdermal penetration enhancers

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ABSTRACT

Skin penetration enhancers are used to allow formulation of transdermal delivery systems for drugs that are otherwise insufficiently skin-permeable. The series of seven esters of substituted 6-aminohexanoic acid as potential transdermal penetration enhancers was formed by multistep 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 method for the lipophilicity measurement and their lipophilicity (log *k*) was determined. Hydrophobicities (log *P*/*C* log *P*) of the studied compounds were also calculated using two commercially available programs and 3D structures of the selected compounds were investigated by means of ab initio/DFT calculations of geometry. All the synthesized esters were tested for their in vitro transdermal penetration enhancer activity. The relationships between the lipophilicity and the chemical structure (SLR) of the studied compounds as well as the relationships between their chemical structure and transdermal penetration activity are mentioned.

1. Introduction

A transdermal route offers several advantages compared with other routes for delivery of drugs with systemic activity. The advantages include elimination of hepatic first-pass metabolism, easy use and withdrawal in case of side effects, and continuous non-invasive infusion of drugs having short biological half-times. However, the outermost layer of skin, *stratum corneum* (SC), forms a strong barrier for most of exogenous substances including drugs. The barrier function of the SC is attributed to its multilayered 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 means using penetration enhancers, and biochemical means such as liposomal vesicles and enzyme inhibition.[1,2]

Transdermal penetration enhancers (also called sorption promoters or accelerants) are special pharmaceutical excipients that interact with skin components to increase penetration of drugs from the topical dosage forms to blood circulation. Numerous compounds (different chemical structures) were evaluated as penetration enhancers and a number of potential sites and modes of action were identified for them.[3–5] In spite of the extensive research in this field, chemical penetration enhancers have not reached their full potential in transdermal or topical systems so far.

The penetration enhancers are generally classified as membrane-acting types or co-solvent types. The membrane-acting types achieve effects using small amounts by altering the condition of the lipid membrane constituting the *stratum corneum*. The co-solvent types, such as propylene glycol (PG), polyethylene glycol and ethanol, achieve effects by improving the solubility of drugs or enhancing permeation of drugs through the skin.[6] It has been suggested that the mechanisms by which these enhancers affect skin permeability are (a) disruption of the ordered lipid bilayer structures and eventual fluidization of the lipid environment of the *stratum corneum*, (b) interaction with intracellular proteins of the *stratum corneum*, and (c) improvement of partitioning and solubility of the drug in the *stratum corneum*.[3,7,8]

Besides the use of chemical penetration enhancers also skin occlusion often, but not always, increases percutaneous absorption of drugs. The occlusion of skin substantially changes many properties of the skin, including hydration, permeability of the skin barrier to some but not all exogenous chemicals, epidermal lipid composition, DNA synthesis, microbial flora, and other molecular and cellular processes.[9] The occlusion also increases penetration of other chemicals and antigens, and hence may exacerbate irritant

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and allergic reactions.[10] Also drug delivery from many transdermal patches benefits from occlusion.

Some of the important penetration enhancers are terpenes and terpenoids, pyrrolidinones, fatty acids and esters, sulfoxides, alcohols and glycerides and miscellaneous enhancers including phospholipids, cyclodextrin complexes, amino acid derivatives, lipid synthesis inhibitors, clofibric acid, dodecyl-*N*,*N*-dimethylamino acetate and enzymes.[11] 1-Dodecylazacycloheptan-2-one (Azone®) is one of the most studied penetration enhancers possessing a large polar head group and a lipid alkyl chain which are thought to bee necessary for its activity, see Figure 1. As it could be expected from its chemical structure, Azone® is a highly lipophilic material with log *P* around 6.2. It enhances the skin transport of a wide variety of drugs with different lipophilicity. Its possible mechanism of action has been previously reported.[3,12,13] The chemical structure of Azone® was considered to be a combination of pyrrolidone and decylmethylsulfoxide, both of which are potential penetration enhancers.[14] To date, many Azone related compounds (including modifications of heterocyclic moiety or/and lipophilic chain) have been made and studied as penetration enhancers. 6-Aminohexanoic acid esters were also designed as acyclic Azone analogs[15] and fragments of pyrrolidin-2,5-dione and pyrrolidin-2-one are parts of described potent penetration enhancers as well.[16]

The traditional lipophilicity parameter log *P* is a well-known physico-chemical descriptor largely used in QSAR analyses. In some experimental studies of penetration enhancement the lipophilicity (non-polarity) of enhancers was measured and the corresponding relationship between enhancer non-polarity and penetration enhancement potency was investigated.[17–21]

The lipophilicity is one of the most important physical properties both for biologically active compounds and for excipients. Classical methods for determination of the partition (log *P*) coefficient are time consuming and not always sufficiently reliable. Therefore, reversed phase high performance liquid chromatography (RP-HPLC) methods have become popular and widely used for lipophilicity measurement. The general procedure is measurement of directly accessible retention time under isocratic conditions with various amounts of an organic modifier (methanol) in the mobile phase using end-capped non-polar C_{18} stationary RP columns and calculating the capacity factor *k*. Log *k*, calculated from the capacity factor *k*, is used as a lipophilicity index converted to the log *P* scale.[22]

The multistep synthesis of seven alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoates with C_6–C_{12} linear alkyl ester chains is described here. The lipophilicity (log *k*) of the compounds was determined using RP-HPLC. Primary in vitro screening of transdermal penetration activity of all synthesized esters was evaluated using a Franz cell.[23] The geometry of two selected compounds was investigated by means of ab initio/DFT calculations, and 3D structures are presented. The structure–activity relationships of the studied compounds are discussed in this study.

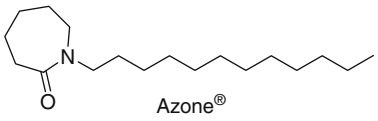


Figure 1. 1-Dodecylazacycloheptan-2-one or *N*-dodecylcaprolactam (Azone®).

2. Results and discussion

2.1. Chemistry

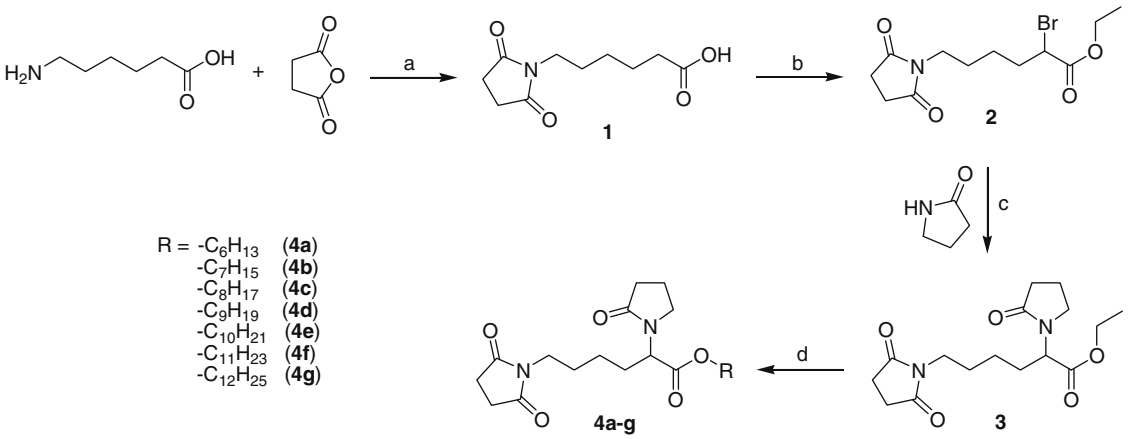
6-Aminohexanoic acid and succinic anhydride were used as the starting materials for multistep synthesis, and by their reaction 6-(2,5-dioxopyrrolidin-1-yl)hexanoic acid (**1**) was obtained. Under the optimized Schwenk and Papa procedure[24,25] acid **1** in the one-pot synthesis gave ethyl-2-bromo-6-(2,5-dioxopyrrolidin-1-yl)hexanoate (**2**).

The critical step of the synthesis was the C–N nucleophilic coupling of pyrrolidin-2-one and ethyl-2-bromo-6-(2,5-dioxopyrrolidin-1-yl)hexanoate (**2**). This C–N bond-forming reaction of α-bromocarboxylic compound **2** and a 5-membered ω-lactam ring was carried out under catalysis by powdered copper(I) oxide, and ethyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (**3**) was obtained. Various conditions and yields of the C–N coupling of non-aromatic *N*-heterocycle starting materials were reported recently.[26]

The series of seven target alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoates (**4a–4g**) was formed by conventional base-catalyzed transesterification[27] of the key intermediate **3** in excess of corresponding primary unbranched alcohol. The discussed compounds were synthesized according to Scheme 1.

2.2. Lipophilicity properties of the prepared compounds

Hydrophobicities (log *P*/*C* log *P* values) of the studied compounds **3**, **4a–4g** were calculated using two commercially available programmes (ChemDraw Ultra 10.0 and ACD/LogP DB) and measured by means of RP-HPLC determination of capacity factors *k*



Scheme 1. Synthesis of target ω-lactams **4a**–**4g**. Reagents and conditions: (a) acetone, 25 °C, 24 h; (b) one-pot synthesis: $SOCl_2$, Br_2, EtOH; (c) NaH, DMF, Cu_2O; (d) Na, R–OH.

with subsequent calculation of log *k*. The results are shown in Table 1.

As expected, ethyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (**3**) showed the lowest lipophilicity, whereas dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (**4g**) possessed the highest lipophilicity. It can be assumed, that the calculated log *P*/*C* log *P* data and the determined log *k* values correspond to the expected lipophilicity increasing within the series of the evaluated compounds (ethyl ⋘ hexyl < heptyl < nonyl < decyl < undecyl < dodecyl derivatives). This dependence is approximately linear. Log *k* data specify lipophilicity within this series of the discussed compounds.

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 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.[28] For this reason, structures of compounds **4a** and **4g** as the shortest and the longest potential enhancers within the series were investigated. Unfortunately, the determination of 3D structures of both compounds was a complicated task due to their large conformational flexibility and complex media. If only three orientations were taken into account for each torsion in the molecule, the number of initial conformations would be 1,594,323 and 1,162,261,467, respectively. Of course, many of them would be prohibited due to close contacts of some atoms. Anyway, the number of possible conformations precludes the use of systematic mapping of conformational space. Calculations with an explicit solvent or even a solvent mixture are generally not feasible at ab initio level and the dielectric continuum solvation model[29] must be used. It is not so sensitive to the dielectric constant and thus water as a dielectric medium is a good approximation for geometry optimizations. The initial scan of geometries was performed using the Conformational Search utility in HyperChem and corresponds to vacuo. 39,682 and 30,214 initial conformations were explored for compounds **4a** and **4g**, respectively. Structures within 3 kcal/mol were then reoptimized in water at HF/4-31G ab initio level in GAUSSIAN 03W.[30,31] For both compounds this resulted in more than 100 possible conformations. Global optima for *S*-enantiomers of compounds **4a** and **4g** are illustrated in Figures 2 and 3, respectively. Used *S*-configuration does not influence the predicted properties. However, their populations would be no more than 1%. According to Figures 2 and 3 it can be assumed, that all the presented compounds show folded conformation, that is, all the compounds represent coned (coiled) molecules.

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/LogP DB
3	−1.0629	−0.39/0.1972	−0.16 ± 0.56
4a	−0.3005	1.35/2.3132	1.88 ± 0.56
4b	−0.1314	1.77/2.8422	2.39 ± 0.56
4c	0.0349	2.19/3.3371	2.90 ± 0.56
4d	0.2013	2.60/3.9002	3.41 ± 0.56
4e	0.3640	3.02/4.4292	3.92 ± 0.56
4f	0.5285	3.44/4.9582	4.43 ± 0.56
4g	0.6906	3.86/5.4872	4.94 ± 0.56
Oleic acid	—[a]	6.29/7.7860	7.42 ± 0.20
Azone®	—	7.07/6.5400	7.82 ± 0.35

[a] Log *k* value was not determined by the described method.

2.4. In vitro screening of penetration enhancing activity

The enhancement 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 was used as a model drug of medium polarity (log *P* −0.06; log D_8 −0.05)[32,33] as it has been extensively studied in transdermal penetration experiments.[34–36] Most of the studies involved the use of propylene glycol (PG) or its mixture with water or ethanol as a donor vehicle. Previous works has 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.[37–39] 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.[40,41] The porcine skin has shown to be histologically and biochemically similar to the human skin. The same as full-thickness pig ear skin it was used in numerous percutaneous absorption studies.[42] Nevertheless for testing of hydrophobic penetrants dermatomated skin is highly recommended.[43] The skin permeation experiments were performed

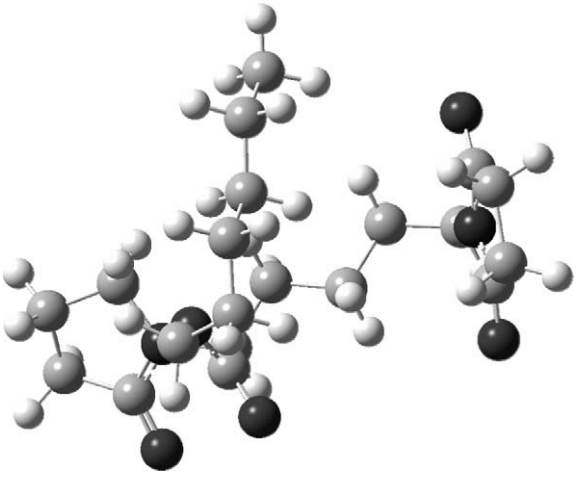


Figure 2. 3D structure of *S*-enantiomer of hexyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (**4a**).

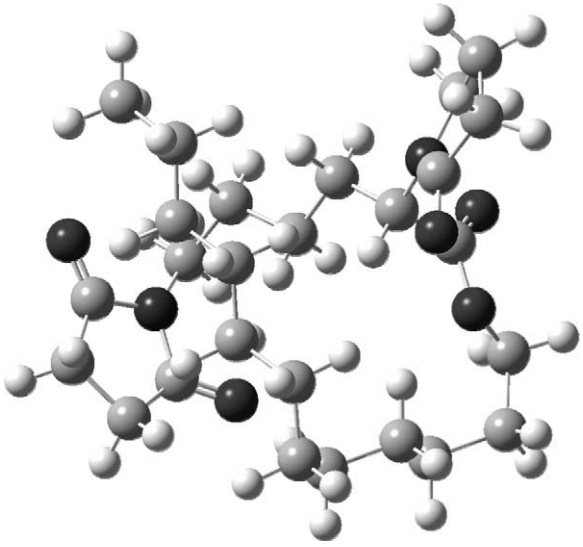


Figure 3. 3D structure of *S*-enantiomer of dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (**4g**).

using static Franz diffusion cells.[23] Generally, the use of static cells would yield the same results as the use of flow-through systems when it is not necessary to maintain skin viability. The effect of the perfusion rate in the receptor medium has shown to influence results particularly if a permeant is very lipophilic in nature.[44–46]

The effect of alkyl esters **4a**–**4g** on the penetration of theophylline through the porcine skin is presented in Figure 4. All prepared compounds **4a**–**4g** enhanced the penetration of theophylline through the porcine skin and showed better enhancement ratios (ERs) than oleic acid, which was used for comparison and belongs to the most studied enhancers of the group of fatty acids.[47] Control experiments were run with only theophylline in donor vehicle and without any enhancer. The highest enhancement ratios (ERs) were obtained with compounds **4d** (nonyl) with ER 2.45 and **4g** (dodecyl) with ER 2.38. The ERs are presented in Table 2.

The penetration of the enhancers through the skin was further investigated. Only compounds **4a** and **4b** were detected in the receptor compartment after 24 h since the start of the experiment. All other alkyl esters **4c–4g** were not detected, and it was assumed that due to their higher lipophilicity they did not get through the skin barrier or remained incorporated in the skin structures.

3. Conclusion

In this work a series of seven alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoates with C_6–C_{12} linear alkyl ester chain as potential transdermal penetration enhancers was prepared. The ability of these compounds to enhance the penetration of theophylline through porcine skin was tested. All prepared compounds showed higher ERs than oleic acid. The highest enhancement ratios (ERs) in this study were exhibited by compound **4d** (C_9 ester chain) and **4g** (C_{12} ester chain). The prepared compounds did not get through the skin, except for compounds **4a** and **4b**, which were detected in the receptor phase in 24 h. The action mechanism seems to be in the interaction of enhancers with intercellular lipids of the *stratum corneum*.

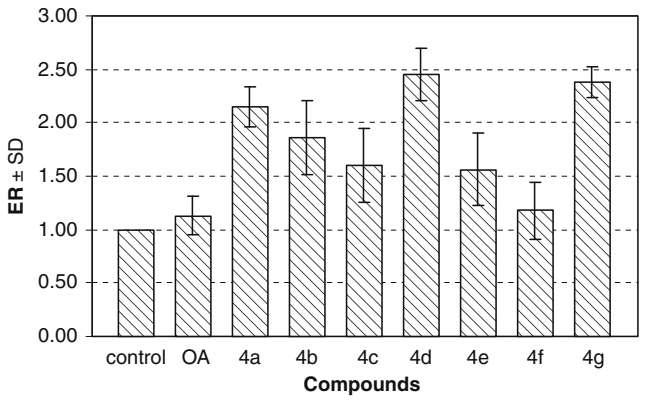


Figure 4. Enhancement ratios (ERs) of the prepared compounds **4a–4g** and oleic acid (**OA**), control is with theophylline only without any enhancer. *Error bars* correspond to the standard deviation of three experiments.

Table 2
Enhancement ratios (ERs) of the prepared compounds **4a–4g** and oleic acid (OA), data are expressed as means ± SD ($n = 3$)

Compd	ER ± SD
4a	2.15 ± 0.19
4b	1.86 ± 0.35
4c	1.60 ± 0.35
4d	2.45 ± 0.24
4e	1.56 ± 0.34
4f	1.86 ± 0.27
4g	2.38 ± 0.15
OA	1.13 ± 0.18

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. 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 vapor. The melting points were determined on a Boetius PHMK apparatus (Nagema, 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 region of 4000–600 cm^{-1}. 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 (δ) to internal $Si(CH_3)_4$, when diffused easily exchangeable signals are omitted. Mass spectra were measured using a LTQ Orbitrap Hybrid Mass spectrometer (Thermo Electron Corporation, USA) with direct injection into APCI source (400 °C) in the positive mode.

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

The solution of succinic anhydride (45.0 g, 450.0 mmol) in acetone (230 mL) was added dropwise to the suspension of 6-aminohexanoic acid (34.4 g, 262.0 mmol) in acetone (140 mL). The reaction mixture was stirred at room temperature for 24 h, after which it was filtered and a pure white powder product was washed with acetone. Yield: 82%. Mp 100–102 °C. IR (cm^{-1}): 3315, 2929, 1688, 1560, 1414, 1250, 1183. ^1H NMR (500 MHz, DMSO-d_6), δ: 12.05 (s, 1H, OH), 3.02 (t, 2H, $J = 7.0$ Hz, NCH_2), 2.90 (s, 4H, $OCCH_2CH_2CO$), 2.41 (t, 2H, $J = 6.0$ Hz, $OOCCH_2$), 2.32–2.15 (m, 4H, CH_2), 1.52–1.24 (m, 2H, CH_2). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 174.25, 173.66, 38.27, 33.52, 28.72, 28.62, 25.83, 24.11. HR-MS: for $C_{10}H_{16}O_4N$ $[M+H]^+$ calcd 214.2378 *m/z*, found 214.2377 *m/z*.

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

To organic acid **1** (45.8 g, 214.8 mmol), held at 30 °C, thionyl chloride (29.4 g, 247.0 mmol, 17.9 mL) was added slowly dropwise and the mixture was stirred at 60–80 °C until the gas evolution essentially stopped. At 80 °C Br_2 (36.1 g, 225.5 mmol, 11.6 mL) was added dropwise at approximately the rate, at which Br_2 was consumed. Stirring continued for several hours until the evolution of HBr nearly stopped. Absolute ethanol (27 mL) was added slowly to the crude acid chloride at 20–30 °C. After stirring overnight, the mixture was evaporated until dry in a vacuum and the residue was dissolved in diethyl ether (50 mL). The solution was washed with dilute $NaHSO_3$ and water, the organic layer was dried over anhydrous $MgSO_4$, filtered and the organic solvent was removed under rotary evaporation. The crude product (yield: 94%) was purified by vacuum distillation using a Vigreux column (bp 160–165 °C/0.35 mbar) to yield 81% of a colourless oil. R_F 0.44 (propan-2-ol 100%). IR (cm^{-1}): 2939, 1730, 1692, 1436, 1399, 1143, 818. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.16 (q, 2H, $J = 7.0$ Hz, OCH_2), 4.11 (t, 1H, $J = 7.3$ Hz, BrCH), 3.44 (t, 2H, $J = 7.2$ Hz, NCH_2), 2.64 (s, 4H, $OCCH_2CH_2CO$), 1.99 (q, 2H, $J = 7.3$ Hz, $CHCH_2$), 1.54 (qi, 2H, $J = 7.0$ Hz, CH_2), 1.43–1.15(m, 2H, CH_2), 1.23 (t, 3H, $J = 7.1$ Hz, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 176.94, 169.43, 61.84, 45.63, 38.23, 34.17, 28.08, 26.77, 24.43, 13.82. HR-MS: for $C_{12}H_{19}O_4NBr$ $[M+H]^+$ calcd 320.0492 *m/z*, found 320.0491 *m/z*.

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

Pyrrolidin-2-one (4.0 g, 46.9 mmol) was added slowly to the 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 stopped. Compound **3** (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 anhydrous $MgSO_4$, filtered and the organic solvent was removed under rotary evaporation. The crude product was purified by flash chromatography on silica gel (ethyl acetate/petroleum ether 3:1) provided a light yellow oil. Yield 66%. R_F 0.45 (propan-2-ol 100%). IR (cm^{-1}): 2927, 1767, 1687, 1401, 1284, 1187, 1153, 1027. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.66 (dd, 1H, J^1 = 5.0 Hz, J^2 = 10.6 Hz, CH), 4.16 (q, 2H, J = 7.1 Hz, OCH_2), 3.50 (t, 2H, J = 7.2 Hz, NCH_2), 3.54–3.29 (m, 2H, CH_2pyrr.), 2.70 (s, 4H, $OCCH_2CH_2CO$), 2.42 (t, 2H, J = 8.0 Hz, CH_2pyrr.), 2.17–1.95 (m, 2H, CH_2pyrr. and 1H from CH_2–CH), 1.78–1.56 (m, 2H, CH_2 and 1H from CH_2–CH), 1.34–1.28 (m, 2H, CH_2), 1.26 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 177.13, 175.78, 170.76, 61.12, 53.51, 43.53, 38.26, 30.73, 28.08, 27.03, 23.35, 18.21, 14.07. HR-MS: for $C_{16}H_{25}O_5N_2$ $[M+H]^+$ calcd 325.1758 *m/z*, found 325.1757 *m/z*.

4.1.4. General procedure for preparation alkyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoates (4a–g)

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

4.1.4.1. Hexyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (4a). Light yellow oil. Yield 62%. R_F 0.27 (ethyl acetate/petroleum ether 5:1). IR (cm^{-1}): 3300, 2980, 1848, 1736, 1688, 1592, 1448, 1336, 1272, 1192, 1096, 920, 712, 632. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.70 (dd, J^1 = 10.61 Hz, J^2 = 4.79 Hz, 1H, CH), 4.06 (t, J = 6.61 Hz, 2H, $COOCH_2$), 3.52–3.24 (m, 2H, CH_2pyrr.), 3.46 (t, J = 6.98 Hz, 2H, NCH_2), 2.68 (s, 4H, $COCH_2$), 2.40 (t, J = 7.95 Hz, 2H, CH_2pyrr.), 2.15–1.85 (m, 4H, CH_2pyrr., $CHCH_2$), 1.77–1.51 (m, 4H, CH_2), 1.35–1.18 (m, 8H, CH_2), 0.86 (t, J = 6.15 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 177.23, 175.76, 170.82, 65.33, 53.55, 43.56, 38.28, 31.34, 30.75, 28.50, 28.39, 28.10, 27.05, 25.43, 22.42, 20.63, 18.21, 13.89. HR-MS: for $C_{20}H_{33}O_5N_2$ $[M+H]^+$ calcd 381.2384 *m/z*, found 381.2385 *m/z*.

4.1.4.2. Heptyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (4b). Light yellow oil. Yield 59%. R_F 0.28 (ethyl acetate/petroleum ether 5:1). IR (cm^{-1}): 3380, 3020, 1768, 1736, 1672, 1640, 1432, 1352, 1192, 1160, 1032, 808, 632. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.67 (dd, J^1 = 10.70 Hz, J^2 = 4.97 Hz, 1H, CH), 4.03 (t, J = 6.59 Hz, 2H, $COOCH_2$), 3.44 (t, J = 7.22 Hz, 2H, NCH_2), 3.36 (dq, J^1 = 15.40 Hz, J^2 = 9.27 Hz, 2H, CH_2pyrr.), 2.65 (s, 4H, $COCH_2$), 2.37 (t, J = 7.84 Hz, 2H, CH_2pyrr.), 2.08–1.82 (m, 4H, CH_2pyrr., $CHCH_2$), 1.75–1.45 (m, 4H, CH_2), 1.30–1.19 (m, 10H, CH_2), 0.83 (t, J = 6.60 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 177.13, 175.66, 170.78, 65.25, 53.46, 43.47, 38.22, 31.53, 30.69, 28.69, 28.38, 28.05, 27.00, 25.68, 23.31, 22.41, 18.16, 13.90. HR-MS: for $C_{21}H_{35}O_5N_2$ $[M+H]^+$ calcd 395.2540 *m/z*, found 395.2542 *m/z*.

4.1.4.3. Octyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (4c). Light yellow oil. Yield 62%. R_F 0.25 (ethyl acetate/petroleum ether 5:1). IR (cm^{-1}): 3380, 1688, 1592, 1512, 1432, 1320, 1160, 1096, 984, 824, 632. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.71 (dd, J^1 = 10.74 Hz, J^2 = 4.92 Hz, 1H, CH), 4.06 (t, J = 6.67 Hz, 2H, $COOCH_2$), 3.47 (t, J = 7.23 Hz, 2H, NCH_2), 3.39 (dq, J^1 = 15.34 Hz, J^2 = 7.7 Hz, 2H, CH_2pyrr.), 2.69 (s, 4H, $COCH_2$), 2.41 (t, J = 7.90 Hz, 2H, CH_2pyrr.), 2.11–1.86 (m, 4H, CH_2pyrr., $CHCH_2$), 1.76–1.48 (m, 4H, CH_2), 1.33–1.20 (m, 12H, CH_2), 0.86 (t, J = 6.40 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 177.13, 175.68, 170.87, 65.33, 54.45, 53.59, 43.57, 38.33, 31.70, 30.77, 29.08, 28.50, 28.19, 28.13, 27.10, 25.82, 23.42, 22.56, 18.27, 13.99. HR-MS: for $C_{22}H_{37}O_5N_2$ $[M+H]^+$ calcd 409.2697 *m/z*, found 409.2696 *m/z*.

4.1.4.4. Nonyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (4d). Light yellow oil. Yield 55%. R_F 0.25 (ethyl acetate/petroleum ether 5:1). IR (cm^{-1}): 3380, 3020, 1816, 1736, 1672, 1592, 1528, 1432, 1352, 1288, 1192, 1160, 1032, 856, 824, 728, 632. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.71 (dd, J^1 = 10.78 Hz, J^2 = 4.92 Hz, 1H, CH), 4.07 (t, J = 6.72 Hz, 2H, $COOCH_2$), 3.48 (t, J = 7.24 Hz, 2H, NCH_2), 3.39 (dq, J^1 = 15.34 Hz, J^2 = 7.7 Hz, 2H, CH_2pyrr.), 2.69 (s, 4H, $COCH_2$), 2.41 (t, J = 8.11 Hz, 2H, CH_2pyrr.), 2.12–1.86 (m, 4H, CH_2pyrr., $CHCH_2$), 1.79–1.52 (m, 4H, CH_2), 1.33–1.21 (m, 14H, CH_2), 0.86 (t, J = 6.40 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 177.21, 175.75, 170.89, 65.36, 53.54, 43.54, 38.33, 31.79, 30.79, 29.41, 29.17, 28.48, 28.14, 27.11, 25.82, 23.42, 22.61, 18.26, 14.06. HR-MS: for $C_{23}H_{39}O_5N_2$ $[M+H]^+$ calcd 423.2853 *m/z*, found 423.2854 *m/z*.

4.1.4.5. Decyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (4e). Light yellow oil. Yield 58%. R_F 0.23 (ethyl acetate/petroleum ether 5:1). IR (cm^{-1}): 3380, 2980, 1752, 1688, 1592, 1432, 1272, 1160, 1080, 840, 728. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.69 (dd, J^1 = 10.71 Hz, J^2 = 4.94 Hz, 1H, CH), 4.05 (t, J = 6.59 Hz, 2H, $COOCH_2$), 3.45 (t, J = 7.22 Hz, 2H, NCH_2), 3.37 (dq, J^1 = 15.39 Hz, J^2 = 9.27 Hz, 2H, CH_2pyrr.), 2.67 (s, 4H, $COCH_2$), 2.38 (t, J = 7.98 Hz, 2H, CH_2pyrr.), 2.09–1.84 (m, 4H, CH_2pyrr., $CHCH_2$), 1.77–1.50 (m, 4H, CH_2), 1.31–1.15 (m, 16H, CH_2), 0.84 (t, J = 6.40 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 177.09, 175.65, 170.85, 65.31, 53.58, 43.56, 38.31, 31.80, 30.75, 29.43, 29.20, 29.11, 28.49, 28.12, 27.08, 25.80, 23.40, 22.58, 18.26, 13.99. HR-MS: for $C_{24}H_{41}O_5N_2$ $[M+H]^+$ calcd 437.3010 *m/z*, found 437.3010 *m/z*.

4.1.4.6. Undecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (4f). Light yellow oil. Yield 54%. R_F 0.24 (ethyl acetate/petroleum ether 7:1). IR (cm^{-1}): 3379, 3020, 1736, 1672, 1592, 1528, 1432, 1352, 1288, 1192, 1160, 1032, 824, 632. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.70 (dd, J^1 = 10.70 Hz, J^2 = 4.96 Hz, 1H, CH), 4.06 (t, J = 6.57 Hz, 2H, $COOCH_2$), 3.53–3.24 (m, 2H, CH_2pyrr.), 3.47 (t, J = 7.22 Hz, 2H, NCH_2), 2.68 (s, 4H, $COCH_2$), 2.40 (t, J = 7.90 Hz, 2H, CH_2pyrr.), 2.11–1.78 (m, 4H, CH_2pyrr., $CHCH_2$), 1.74–1.52(m, 4H, CH_2),1.33–1.17 (m, 18H), 0.86 (t, J = 6.45 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 177.05, 175.60, 170.89, 65.33, 53.67, 43.62, 38.36, 31.86, 30.78, 29.53, 29.45, 29.25, 29.15, 28.56, 28.25, 28.15, 27.13, 25.85, 23.45, 22.61, 18.31, 13.99. HR-MS: for $C_{25}H_{43}O_5N_2$ $[M+H]^+$ calcd 451.3166 *m/z*, found 451.3168 *m/z*.

4.1.4.7. Dodecyl-6-(2,5-dioxopyrrolidin-1-yl)-2-(2-oxopyrrolidin-1-yl)hexanoate (4g). Light yellow oil. Yield 56%. R_F 0.24 (ethyl acetate/petroleum ether 7:1). IR (cm^{-1}): 3380, 2940, 1752, 1672, 1624, 1592, 1432, 1272, 1192, 1160, 1016, 952, 904, 824, 744, 648. ^1H NMR (500 MHz, $CDCl_3$), δ: 4.71 (dd, J^1 = 10.77 Hz, J^2 = 4.84 Hz, 1H, CH), 4.06 (t, J = 6.72 Hz, 2H, $COOCH_2$), 3.53–3.24

(m, 2H, CH_2pyrr.), 3.47 (t, J = 7.25 Hz, 2H, NCH_2), 2.69 (s, 4H, $COCH_2$), 2.40 (t, J = 7.97 Hz, 2H, CH_2pyrr.), 2.11–1.86 (m, 4H, CH_2pyrr., $CHCH_2$), 1.78–1.50 (m, 4H, CH_2), 1.40–1.15 (m, 20H), 0.86 (t, J = 6.35 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 177.08, 175.64, 170.87, 65.34, 53.65, 43.62, 38.35, 35.35, 31.87, 30.78, 29.58, 29.53, 29.46, 29.28, 29.15, 28.55, 28.23, 28.15, 27.12, 25.85, 23.44, 22.62, 18.31, 14.01. HR-MS: for $C_{26}H_{45}O_5N_2$ $[M+H]^+$ calcd 465.3323 *m/z*, found 465.3323 *m/z*.

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

The HPLC separation system Agilent 1200 series instrument was used, equipped with a diode array detection (DAD) system, a quarternary model pump, and an automatic injector (Agilent Technologies, Germany). Data acquisition was performed using the ChemStation chromatography software. The chromatographic column Zorbax Eclipse XDB (Agilent Technologies, Germany), C_{18} 5 μm, 4.6 × 150 mm, was used. The 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, injection 10 μL, column temperature 25 °C. The detection wavelength of 204 nm and the bandwidth of 8 nm were chosen. The 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 the 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.[22] The log k values of the individual compounds are shown in Table 1.

4.3. Lipophilicity and geometry calculations

Log P values, that is, the logarithm of the partition coefficient for n-octanol/water, were predicted using the CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) and the ACD/LogP 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 by means of the 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 the HyperChem (HyperChem for Windows, ver. 8.0.3, Hypercube, Inc., Gainesville, 2007). Structures within 3 kcal/mol were then reoptimized in water at HF/4-31G ab initio level in GAUSSIAN 03W.[30] Water was simulated using the CPCM polarizable conductor calculation solvation model.[31] The results are illustrated in Figures 2 and 3.

4.4. In vitro screening of penetration enhancing activity

Skin samples were obtained from porcine ear. Full thickness dorsal skin was cut in fragments and stored at −18 °C until utilized. Skin samples were than slowly thawed (at 4 °C overnight and then at ambient temperature) before each experiment. The penetration enhancing activities of newly synthesized compounds **4a–4g** were evaluated in vitro using vertical Franz diffusion cell (PermeGear Inc., USA), donor surface area 0.635 cm^2, receptor volume 5.2 mL. The skin was mounted between the donor and receptor compartments of a Franz diffusion cell with the epidermal side up. The receptor compartment was filled with the phosphate buffer saline (pH 7.4) and was maintained at 37 ± 0.5 °C through the use of circulating water bath. The receptor compartment was continuously stirred using magnetic stirring bar. The skin was kept in the contact with receptor phase for 0.5 h prior to experiment. The donor samples were prepared by dissolving of tested enhancer (20 mg) in PG (0.5 mL) and the solution of theophylline (5 mg) in water (0.5 mL) was added. This mixture was shaken vigorously and then sonicated for 10 minutes at 40 °C, than this stable system was applicated on the skin surface, and the donor compartment of the cell was covered by Parafilm®. The control samples were prepared likewise without enhancers. Samples (0.5 mL) of the receptor phase were withdrawn at six predetermined 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. Minimal three determinations for each compound were performed using skin fragments from minimal two animals for each compound. The samples were stored at −18 °C until HPLC analysis.

4.5. Sample analysis

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

The cumulative amounts of theophylline penetrated across the skin into receptor compartment (μg/cm^2) corrected for sample removal were plotted against time (hours). An approximately linear dependence was found ($R^2 \geqslant 0.98$) and steady state fluxes (μg/cm^2/h) were calculated from 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 2.

Determination of the penetrated enhancer was performed using HPLC method described in Section 4.2, injection 10 μL, and the samples of the receptor phase withdrawn at the mentioned intervals were analyzed.

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