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L-Serine and Glycine Based Ceramide Analogues as Transdermal Permeation Enhancers: Polar Head Size and Hydrogen Bonding

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Abstract—Novel transdermal permeation enhancers related to stratum corneum ceramides were investigated. The synthesis of a series of simple compounds based on two selected amino acids, L-serine and glycine, and their enhancement activities are reported. Glycine derivative **3** enhanced the permeation of theophylline through human skin in vitro 12.5±0.5 times. The relationships between properties of the polar head of the compounds and their activity are discussed.

The advantages of transdermal drug delivery are well documented and include the avoidance of first-pass metabolism by the liver, reduced fluctuations in plasma concentration, reduction of side effects, extended duration of activity, and convenient termination of drug administration.[1] The major limitation of the transdermal drug delivery is the outermost layer of the skin, the stratum corneum. The effective barrier function of the stratum corneum has been attributed to a specific lipid lamellae in the intercellular spaces.[2] The lamellae are composed mainly of ceramides, free fatty acids, cholesterol, cholesteryl esters, and cholesteryl sulphate. Ceramides (Fig. 1) are considered to be the key molecules in the lipid lamellar organization and the resistance to environmental changes, chemical and physical stress.[3] The ceramides comprise about 50% of the stratum corneum lipids and are structurally heterogenous.[4] The common feature is a relatively small polar head and two long, straight, saturated hydrophobic chains. The polar head contains two to four hydroxyl groups and the amide group. This enables ceramide to form strong hydrogen bonding network[5] necessary for the stability of a lamellar structure.[6]

The use of permeation enhancers is one of the approaches to facilitate drug diffusion through the skin. Enhancers can interact with the stratum corneum components and reversibly reduce skin barrier properties. Due to the structure of the stratum corneum—continuity of the lipid barrier—interaction with stratum corneum intercellular lipids is of crucial importance for the effectiveness of permeation enhancer action. Because of their structure, many permeation enhancers are capable of inserting themselves between the hydrophobic tails of the ceramide bilayer, thus disturbing their packing, increasing their fluidity and subsequently leading to easier diffusion of penetrants.[7,8]

There is a hypothesis that a certain similarity should exist between an enhancer molecule and ceramides.[9] However it is not clear where are the limits of the similarity. In other words what features of the ceramide molecule are necessary for maintaining the barrier function and, on the other hand, what change in the ceramide molecule will lead to a structure with permeation enhancing properties.

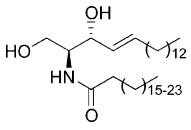

Figure 1. Ceramide **2**.

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The aim of this study was to synthesize a series of ceramide analogues with different polar head size and structure and to test their skin permeation enhancing properties in vitro. Furthermore, we wanted to assess the structure–activity relationships to obtain more information about the importance of the similarity of these enhancers to ceramides. Structures of the analogues were based upon the knowledge of ceramide biosynthesis (originating from L-serine).[10] In general, the substances consist of a polar head and two hydrophobic chains. The polar head contains amino acids or a hydroxy acid; and the hydrophobic chains are unbranched, both saturated and unsaturated, 11–17 carbons long.

The synthetic schemes are depicted in Figure. 2.[11] The first series of the analogues is the most similar to natural ceramides. The polar head is formed by one amino acid, serine or glycine. Primarily esters of L-serine **2** (yield 85%) and glycine **1** (yield 91%) were prepared via reaction with the pertinent alcohol and dry HCl under nitrogen.[12] Amino acid esters then reacted with *N*-hydroxysuccinimide ester (R_1COO-SI) to obtain compounds **3–6**. Lauric acid and oleic acid were converted into R_1COO-SI with *N*-hydroxysuccinimide and dicyclohexyl carbodiimide in ethyl acetate in yields of 73 and 76%.

N-Lauroyloxysuccinimide was also used for the preparation of *N*-acylated amino acids **7**,**8**[13] (90–93%), which then reacted with appropriate amino acid esters **1**,**2** to form ceramide analogues of the second series with a polar head formed by two amino acids **9–11**.

In the third series of the analogues, maleic acid monoester **12** (81%) was prepared first.[14] The monoester then reacted with amino acid esters **1**, **2** via the mixed anhydride method to yield **13**, **14**. The final products **15**, **16** were prepared by oxidizing the double bond with $KMnO_4$ in the presence of a crown-ether.[15]

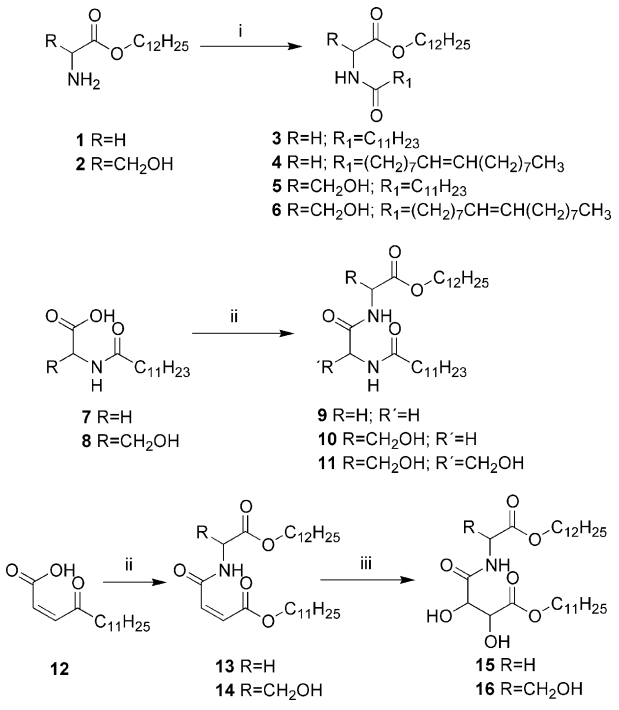

Figure 2. Synthesis of ceramide analogues: (i) R_1COO-SI, $CHCl_3$, rt, 24 h (55–82%); (ii) (1) TEA, THF, −20 °C, 15 min; (2) ClCOOEt, −20 °C, 15 min, (3) **1**(**2**), rt, 2 h (33–42% for **9–11**, 78 and 91% for **14** and **13**, resp); (iii) (1) $KMnO_4$, DH-18–C-6, CH_2Cl_2, −50 °C, 3 h; (2) Na_2SO_4; (3) H_2SO_4 (22–26%).

The compounds were tested in vitro using the modified Franz diffusion cell; theophylline was chosen as a model penetrant of medium polarity. Samples of excided human cadaver skin (300 μm thick, samples from identical areas on a thigh) were used. Donor samples (200 μL, occluded conditions) were prepared as 5% w/w suspensions of theophylline in a given vehicle (infinite dose) with 1% w/w of the suspended, partly dissolved enhancer (except control samples). Azone® (*N*-dodecylazepan-2-one)[16] was also tested under the same conditions to serve as a standard. Theophylline in the acceptor phase samples was determined by HPLC. For details of the permeation experiments and HPLC determination, see ref 17.

Cumulative amounts of theophylline (μg/cm^2) corrected for acceptor sample replacement were plotted against time (h) and fluxes (μg/cm^2/h) of theophylline through human skin were calculated. All the tested compounds are characterized by the enhancement ratio (ER, ratio of the means of the pertinent flux values and the control ones). Statistical analysis was performed using Student's *t*-test.

Results and Discussion

After the preliminary tests (data not shown) water and isopropyl myristate (IPM) were chosen as the representative hydrophilic and lipophilic vehicles. In the present study, we have chosen analogues with the same chain length (12C) to evaluate the characteristics of the polar head of the compounds. An oleic acid derivative was also tested, because the presence of *cis* double bond in the centre of the hydrophobic chain proved itself to disrupt ceramide lamellar organization more than a saturated chain.[18]

The ERs of the compounds from water and IPM suspensions are summarized in Table 1.

Surprisingly, the most potent enhancer was compound **3**, the simplest one, having glycine as the polar head.

Table 1. Enhancement ratios of the tested compounds in theophylline permeation through human skin from its aqueous and IPM suspensions

Compd	ER (water)±SD	ER (IPM)±SD
Control	1.00±0.15	1.00±0.08
3	12.50±0.50**,++	1.11±0.16
5	2.74±0.71	1.21±0.16*,+
6	1.26±0.25	1.49±0.27+
9	2.28±0.64	1.09±0.01
11	1.26±0.06	1.07±0.02
16	1.39±0.18*	1.45±0.34+
Azone®	2.50±0.93	0.60±0.36

$n = 3–4$.
*$p < 0.05$ versus control; **$p < 0.001$ versus control; +$p < 0.05$ versus Azone; ++$p < 0.001$ versus Azone.

The compound was five times more active than Azone® under the same conditions. The presence of the hydroxymethyl group in compound **5**, an L-serine derivative, decreased the permeation enhancing activity significantly. Compound **5** has stronger ability to form H-bonds compared to **3**. Since H-bonding is essential for the dense and rigid ceramide arrangement in the stratum corneum intercellular spaces, this finding can be related to the mechanism of action of this type of enhancers. The importance of H-bonding ability is also well documented in the process of permeation through the skin.[19]

Compound **6**, which has an oleyl residue instead of the lauryl one in compound **5**, shown substantially lower activity. The conformational kink in the oleyl chain due to the presence of the *cis* double bond, often reported as the cause of high enhancing activity of oleic acid and its derivatives, does not seem to contribute significantly to the activity of the present ceramide analogues with oleyl chain.

Compounds **9** and **11**, the dipeptide derivatives, exhibited lower activity compared to **3** and **5**. The possible explanation is that the bigger the polar head, the lower the ability of the compounds to incorporate themselves between the hydrophobic chains of ceramides in the stratum corneum. Similarly to the difference between **3** and **5**, compound **9** showed higher ER than **11**, which has two additional H-bonding groups.

A member of the third series, compound **16** has also low ER. This corresponds to the previously suggested relationships, as **16** has a large polar head and three hydroxyl groups.

The compounds were not active when in the IPM suspension (the reason was not estimated further).

From the previous studies it is only known that an enhancer molecule must have a polar head and a hydrophobic chain to ensure interaction with ceramides. The optimal chain length is 10–12 carbons. We hypothesize that a relatively small polar head of the ceramide analogues (similarly to ceramides) with a limited ability to act as a H-bond donor (in contrast to ceramides) is important for the enhancing activity. Further evaluation of this hypothesis is in progress.

Apart from these theoretical findings, ceramide analogue **3** derived from glycine seems to be a promising permeation enhancer,[20] because it has a high enhancement ratio (12.50) at low concentration together with expected low toxicity. As there is a variety of enzymes in the living epidermis, degradation of enhancer **3** into non-toxic metabolites after it reaches the stratum corneum/stratum granulosum interface could be expected.

Acknowledgements

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11. All compounds were fully characterized by spectral methods, and their purity checked by elemental analysis. Representative data of the most potent derivative; compound **3**: mp 81–82 °C; IR (KBr) ν_{max} 3333, 2918, 2849, 1740, 1641, 1548, 1244 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.97 (1H; bs; NH); 4.14 (2H; t; $J=6.9$ Hz; $COO–CH_2$); 4.03 (2H; d; $J=4.9$ Hz; CH_2NH); 2.23 (2H; t; $J=7.6$ Hz; $COCH_2$); 1.70–1.55 (4H; m; 2 CH_2); 1.40–1.15 (34H; m; 17 CH_2); 0.87 (6H; t; $J=6.6$ Hz; 2 CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2; 170.2; 65.7; 41.3; 36.4; 31.9; 29.6; 29.5; 29.5; 29.4; 29.3; 29.2; 28.5; 25.8; 25.6; 22.7; 14.1. Anal. calcd for $C_{26}H_{51}NO_3$: C, 73.36; H, 12,08; N, 3,29. Found: C, 72,98; H, 12,41; N, 3,49.
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