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## Review

# Novel chemical permeation enhancers for transdermal drug delivery

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### ARTICLE INFO

### ABSTRACT

Transdermal drug delivery has been accepted as a potential non-invasive route of drug administration, with advantages of prolonged therapeutic action, decreased side effect, easy use and better patient compliance. However, development of transdermal products is primarily hindered by the low permeability of the skin. To overcome this barrier effect, numerous new chemicals have been synthesized as potential permeation enhancers for transdermal drug delivery. In this review, we presented an overview of the investigations in this field, and further implications on selection or design of suitable permeation enhancers for transdermal drug delivery were also discussed.

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## 1. Introduction

For decades, utilization of skin as a route for delivering drugs has been an attracting alternative to conventional methods including injections and tablets. Its advantages include the avoidance of pain, elimination of hepatic first-pass metabolism, sustained drug release for a prolonged period, easy use and withdrawal in case of side effects. However, the major limitation for transdermal drug delivery system (TDDS) is also the skin itself. As the outmost layer of the epidermis, the stratum corneum (SC) provides an outstanding barrier towards the absorption of chemical and biological toxins [1]. It has a unique hierarchical structure, which is filled with multiple lipid bilayers and the embedded corneocytes [2,3]. In theory, there are two main permeation routes through the SC, first the intracellular route across the corneocytes as well as the lipid matrix, and second the intercellular route via the lipid domains between the corneocytes. The lipid phase is continuous throughout the SC, and therefore the penetrating substances must interact with this phase whether they penetrate transcelluarly or intercelluarly.

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**Table 1 – Ceramide analogs with different polar heads.[a]**

| Polar head | Structure | No. | $R_1$ | $R_2$ | ER (IPM) | ER (W) | Ref. |
|---|---|---|---|---|---|---|---|
| Amino acid |  | 1 | H | $C_{11}H_{23}$ | 1.11 | 12.50 | 15 |
| | | 2 | $CH_2OH$ | $C_{11}H_{23}$ | 1.21 | 2.74 | |
| | | 3 | H | $(CH_2)_7CH=CH(CH_2)_7CH_3$ | 1.49 | 1.26 | |
| Dipeptide |  | 4 | H | H | 1.09 | 2.28 | 15 |
| | | 5 | $CH_2OH$ | $CH_2OH$ | 1.07 | 1.26 | |
| L-serine + maleic acid |  | 6 | $CH_2OH$ | – | 1.45 | 1.36 | 15 |

[a] The enhancing activity of these compounds was studied *in vitro*, using water (W) and isopropyl myristate (IPM) as the donor vehicle, human cadaver skin as the transdermal barrier and theophylline as the model drug.

In order to facilitate the passage of molecules through the SC, transdermal permeation enhancers have been extensively studied. Up to date, more than 360 chemicals have been demonstrated to enhance the skin permeability [4], including terpenes, sulphoxides, laurocapram, pyrrolidones, fatty acid and fatty alcohol, alcohol and glycol, surfactants, urea, and so on. However, as a result of their incompatibility in the formulation or local irritation issues, few to date have been routinely incorporated in the currently marketed transdermal products [5]. Therefore, exploration of chemicals to safely improve the skin permeability remains an intensive area of transdermal research.

There have been several reviews discussing the enhancement activity of the classic permeation enhancers that are commonly used in the transdermal products [5–8]. However, a detailed introduction of those potential candidates newly developed is still lacking. Therefore, this paper provides a review on the current researches in this field, which might be helpful the selection or development of a suitable absorption promoter in TDDS.

## 2.     Novel permeation enhancers

### 2.1.     Ceramide analogs

The lipids in the SC consist of ceramides, cholesterol and fatty acids, as well as a small quantity of cholesterol sulfate [9,10]. It has been known that ceramides are the main components responsible for the barrier effect of the SC. Structurally, they are amphiphilic molecules, containing two long hydrocarbon tails and an acylamide polar head [11,12]. Vávrová et al. believed that certain structure similarity might exist between

enhancers and ceramides, so that the enhancer molecules could insert themselves between the hydrophobic tails of the ceramide bilayers and weaken the continuity of the lipid barrier [13]. In their study, a series of ceramide analogs (Table 1) with two kinds of polar heads based on L-serine and glycine were respectively synthesized, and the relationship between the properties of the polar heads and enhancement activity was discussed. The result indicated that although these compounds were inactive in isopropyl myristate (IPM), significant enhancement effects were observed when they were used in the water (W) suspensions. Especially, compound **1** with the simplest glycine group as the polar head structure was the most potent one, and its promoting activity was significantly higher than compound **2**, an L-serine based derivative which had a hydroxyl group in the polar head. Furthermore, these two compounds were both more active than their respective analogs with dipeptide as the polar heads, which were shown by compound **4** and **5**. These results led to a hypothesis that the increasing size and H-bonding ability would decrease the activity of the ceramide analogs, and it might account for the little enhancing activity of compound **6**, which possessed a large polar head with three hydroxyl groups. In addition, the study also suggested that the introduction of a double bond into the hydrocarbon chain did not contribute much to the activity of the present ceramide analog, which was demonstrated by the compound **3**.

To further examine the aforementioned hypothesis, ceramide analogs (Table 2) with six different chain lengths (compound **7–12**) and eight different polar head groups (compound **7, 13, 15–20**) were synthesized [14]. When analogs of the same chain length were compared, the best enhancement effect was observed with compound **7** and

**Table 2 — Ceramide analogs with different polar head and hydrophobic chain length.[a]**

| Polar head | Structure | No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | ER (W) | Ref. |
|---|---|---|---|---|---|---|---|---|
| Amino acid | | 7 | $C_{12}H_{25}$ | $C_{11}H_{23}$ | H | — | 4.53 | 16 |
| | | 8 | $C_{11}H_{23}$ | $C_{10}H_{21}$ | H | | 2.26 | |
| | | 9 | $C_{10}H_{21}$ | $C_9H_{19}$ | H | | 3.19 | |
| | | 10 | $C_9H_{19}$ | $C_8H_{17}$ | H | | 2.16 | |
| | | 11 | $C_8H_{17}$ | $C_7H_{15}$ | H | | 2.35 | |
| | | 12 | $C_{12}H_{25}$ | $(CH_2)_7CH{=}CH(CH_2)_7CH_3$ | H | | 0.45 | |
| | | 13 | $C_{12}H_{25}$ | $C_{11}H_{23}$ | $CH_2OH$ | | 1.24 | |
| | | 14 | $C_{12}H_{25}$ | $(CH_2)_7CH{=}CH(CH_2)_7CH_3$ | $CH_2OH$ | | 0.45 | |
| Dipeptide | | 15 | $C_{12}H_{25}$ | $C_{11}H_{23}$ | H | H | 1.00 | 16 |
| | | 16 | $C_{12}H_{25}$ | $C_{11}H_{23}$ | H | $CH_2OH$ | 0.94 | |
| | | 17 | $C_{12}H_{25}$ | $C_{11}H_{23}$ | $CH_2OH$ | $CH_2OH$ | 0.94 | |
| Amino acid + maleic acid | | 18 | $C_{12}H_{25}$ | $C_{12}H_{25}$ | H | — | 6.64 | 16 |
| Amino acid + tartaric acid | | 19 | $C_{12}H_{25}$ | $C_{12}H_{25}$ | H | — | 1.04 | 16 |
| | | 20 | $C_{12}H_{25}$ | $C_{12}H_{25}$ | $CH_2OH$ | — | 0.83 | |

[a] The enhancing activity of these compounds was studied *in vitro*, using water (W) as the donor vehicle, porcine ear skin as the transdermal barrier and theophylline as the model drug.

compound **18**. These two compounds respectively had the polar head with the smallest size and that with the lowest H-bonding ability, which further confirmed the previous hypothesis that decreasing the size or H-bonding ability of the polar heads of ceramide analogs would contribute to their transdermal enhancement activity. Considering the fact that the polar head of compound 18 is relatively large, comparable to that of compound **15—17** which were inactive as permeation enhancers, it was concluded that H-bonding might be more significant than the polar head size or other factors. In addition, the optimum chain length of the ceramide analogs was found to be 12 carbons (compound **7**), which might be due to its favorable interaction with the intercellular skin lipids.

### 2.2.    Azone analogs

Azone (1-dodecylazacycloheptan-2-one or laurocapram, compound **21**, Table 3) is the first molecule specially designed as a skin permeation enhancer [10]. It has been demonstrated to enhance the transdermal absorption of a wide variety of drugs [15]. Azone probably exerts its permeation enhancing effects mainly via the interaction with the lipid domains of the SC. The 'soup spoon' structure would make it more likely to partition into the bilayer lipids, where Azone might exist dispersed or in separated domains to disrupt the organized lipid packing [5].
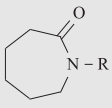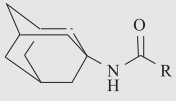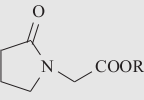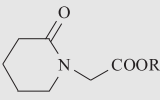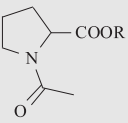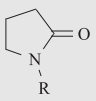
Although FDA has not approved for its use in pharmaceutical products for its slight side effects, Azone is recorded in the Chinese Pharmacopoeia and widely used in China. To improve its properties, hundreds of Azone like compounds have been synthesized, which provide a huge amount of information about the molecular design for Azone analogs [16].

The structure of Azone can be divided into two parts, viz. the hydrophobic chain and the polar cycle head (the heterocycle and α carbon on the hydrophobic chain). Change of the chain length was the common structural modification to the hydrophobic tails. By contrast, the modifications of the polar head group was complex, and usually carried out by alternation of heterocyclic ring size, isosteric replacements of N, O, and C atoms, removal of function groups, addition of other polar groups onto the α carbon of the hydrophobic chain [16]. Since the data was too large to easily pick out the information of interest, only key structural requirement for Azone like compounds were summarized here. To avoid the inter-laboratory differences such as sample types and solvent systems for the enhancers, activity of Azone analogs were compared using the data from one research group.

#### 2.2.1.    The optimal hydrocarbon chain length
The enhancement activities of enhancers always increased as the length of the hydrophobic chains increased, and when the

**Table 3 – The optimal chain lengths for Azone analogs with different polar heads.**

| Polar head | No. | The optimal chain length | Ref. |
|---|---|---|---|
| | 21[a] | $C_{12}H_{25}$ | 17 |
| | 22[b] | $C_{10}H_{21}$ | 18 |
| | 23[c] | $C_{10}H_{21}$ | 19 |
| | 24[c] | $C_{14}H_{29}$ | 19 |
| | 25[d] | $C_{11}H_{23}$, $C_{12}H_{25}$ | 20 |
| | 26[e] | $C_8H_{17}$ | 21 |

[a] Human stratum corneum *in vitro*, pretreated with enhancers in the solution of propylene glycol, desglycinamide arginine vasopressin as a model drug [17].
[b] Rabbit skin *in vivo*, treated with petrolatum ointments containing salicylic acid [18].
[c] Hairless mouse skin *in vitro*, treated with enhancer solution of propylene glycol containing hydrocortisone 21-acetate as a model drug [19].
[d] Hairless mouse skin *in vitro*, treated with solution of propylene glycol saturated with benazepril and hydrocortisone as model drugs, as well as 5% (w/v) enhancers [20].
[e] Hairless mouse skin *in vitro*, treated with an enhancer/PBS solution containing β-estradiol, corticosterone and hydrocortisone as model drugs [21].
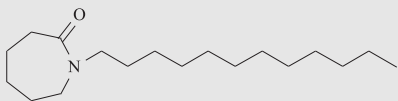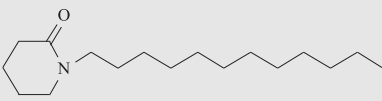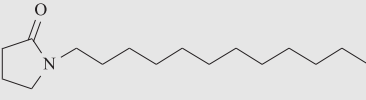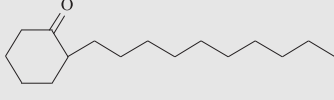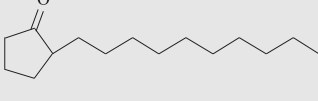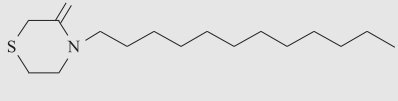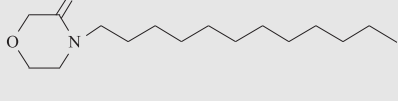
length of the chain was longer than a certain limit, the activities rather decreased [17]. This kind of parabolic dependence was frequently observed in the study of structure-activity relationship for amphiphilic permeation enhancer. The optimal hydrocarbon chain length for Azone analogs were always observed between 8 and 14 carbons, which would vary according to their different polar heads (Table 3). The action of an enhancer was considered to be dependent on the balance between its hydrophilic and lipophilic nature, and therefore different length of alkyl chain was required for best incorporation of the enhancer with different polar heads into the skin lipid bilayers. This effect would produce a free space within the lipid lamellae, and thereby producing lateral

fluidization of the tightly packed lamellar lipids. In addition, these compounds could also form separate phases within the lipid lamellae, providing a more permeable shortcut for penetrating compounds.

### 2.2.2. Heterocyclic ring size

Michniak et al. compared the transdermal promoting activity of Azone and its analogs containing five or six member cycle. As shown in Table 4, the presence of five-member cycle (compound **28**) increased the activity, while six (compound **27**) and seven member cycle (Azone) analogs exhibited lower activity [22]. The H-bonding net formed between the head groups is accepted as an important factor in stabilizing the lipid bilayers [23]. Azone analogs with a small polar head might be expected to be situated well into the polar lipid domain, thus disrupting the ordered organization of the lipid layers.

**Table 4 – Azone analogs with different polar cycle size and heteroatoms on the polar head.[a]**

| No. | Structure | ER |
|---|---|---|
| Azone | | 22.1 |
| 27 | | 22.2 |
| 28 | | 27.6 |
| 29 | | 7.9 |
| 30 | | 6.7 |
| 31 | | 21.0 |
| 32 | | 15.1 |

[a] The enhancing experiments were performed *in vitro*, using hairless mouse skin as the transdermal barrier, which was first pretreated with these compounds in the solution of propylene glycol (PG), and then treated with the suspension of hydrocortisone.

### 2.2.3. The heteroatoms in the polar head

The effect of the heteroatom in the polar head of Azone analogs was investigated in the study [22]. As shown in Table 4, removal of the nitrogen atom in the polar heads of compound **27** and **28** resulted in a significantly decrease in the promoting activity, which were demonstrated by compound **29** and **30**. By contrast, in the polar cycle heads of compound **27** and **28**, introduction of another heteroatom generated compounds with better promoting properties, i.e. compound **31** and **32**. These findings demonstrated that the heteroatom in the polar head was essential for the activity of Azone analogs.

### 2.2.4. The carbonyl in polar head

Hadgraft et al. evaluated the modifying effects of Azone and its several analogs on the transdermal absorption of metronidazole (Table 5) [23]. Moving the carbonyl group of Azone from the cycle head to its α carbon of the alkyl chain gave the product of compound **32**, which showed a comparable enhancement effect in comparison of Azone. However, isosteric substitution of carbonyl oxygen in Azone by sulfur led to a dramatically decreasing activity, which was demonstrated by compound **33**. Additionally, when the carbonyl group of Azone was reduced into the alkyl, its enhancing activity also significantly decreased, as shown by compound **34** [24]. Therefore, it appeared that the carbonyl in polar head was needed for activity.

## 2.3. Menthol derivatives

Since its introduction, terpenes have attracted much interest as potential transdermal permeation enhancers [25,26]. Of these compounds, menthol (compound **35**, Table 6) represents a traditional, and arguably, the most common one that has been known as a lipid disordered agent to disrupt the molecular organization of the bilayer lipids in SC [9,27−29]. In spite of its enhancement activity and relatively low skin irritation, application of menthol is often hindered by its undesirable odor and high volatility. Therefore, many derivatives of menthol have been synthesized in the identification of promising candidates with suitable properties for application in TDDS.
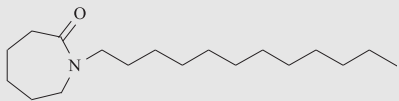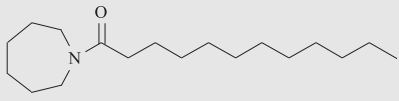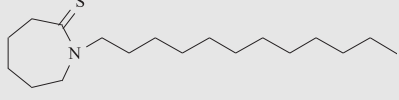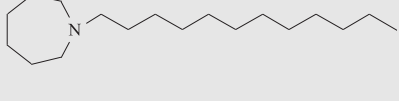
### 2.3.1. Etherified derivatives

Obata et al. first found that an ether derivative of menthol, O-ethylmenthol (compound **36**, Table 6), had an excellent activity toward the in vivo percutaneous absorption of ketoprofen alcoholic hydrogels through the rabbit skin [30−33]. Based on this finding, a series of mono- and disubstituted derivatives of cyclohexane with an O-ethyl group were synthesized and evaluated for their absorption enhancement activity [34]. As shown in Table 6, in the list of mono-substituted derivatives (compound **37**−**54**), most of them acted better than O-ethylmenthol, except for methyl-substituted products (compound **37**, **43**, **49**). Among the C-2 substituted compounds (compound **37**−**42**), compound **40** which has an isopropyl group showed the highest activity. Among the C-3 substituted compounds (compound **43**−**48**), isopropylated or butylated derivatives (compounds **46** and **47**) showed the highest activity. Among the C-4 substituted compounds (compounds **49-54**), compound **52** which also has an isopropyl group showed the highest activity. In the list of the di-substituted derivatives (compound **55**−**70**), all of them had a similar or better effect than MET. The study also suggested that the irritation potency of these derivatives correlated well with their enhancing activity, and both of them were parabolically dependent with the log P of the compounds. As an exception, 1-O-ethyl-3-butylcyclohexanol (OEBC, compound **47**) was proved to be a safe and prominent candidate that promoted the transdermal absorption of ten different model drugs, yet exhibiting rather low irritation [35]. In addition, thiomenthol derivatives (compound **71-83**, Table 7) were also synthesized for more effective and safer permeation enhancer candidates. However, severe skin irritation was produced by these compounds, although some of them had strong promoting activity [36].

### 2.3.2. Esterified derivatives

Zhao et al. employed menthol as a lead compound and synthesized O-acylmenthol derivatives (Table 8) with a series of saturated fatty acid (C2−C18) and pharmaceutical excipient acids [37,38]. Indomethacin, ketoprofen, lidocaine, isosorbide dinitrate and 5-fluorouracil were selected as model drugs based on their different physicochemical properties. In the case of saturated fatty acid esters, most of them exhibit good enhancing effect on the transdermal absorption of indomethacin, ketoprofen and isosorbide dinitrate, among which compound **88** and **89** respectively produced the greatest enhancing activity towards indomethacin and isosorbide dinitrate. For ketoprofen, compounds with shorter acylchains, such as compound **85** and **86** generally provided the best enhancing effect. However, most of these compounds had no enhancing activity towards 5-fluorouracil and
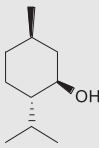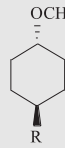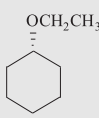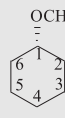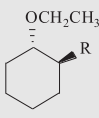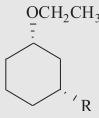
**Table 5 − Alternation of the carbonyl group in the polar head of Azone analogs.**

| No. | Structure | ER |
|---|---|---|
| Azone |  | 6.7[a]/19.51[b] |
| 32 |  | 6.4[a] |
| 33 |  | 1.4[a] |
| 34 |  | 15.33[b] |

[a] Human skin in vitro, treated with ethanolic solution containing 1% enhancer and 5 μmol/ml metronidazole as a model drug.
[b] Hairless mouse skin in vitro, treated with propylene glycol containing enhancers and hydrocortisone 21-acetate as a model drug.

**Table 6 – Structural modification on O-ethylmenthol.[a]**

| Structure | No. | R | Structure | No. | R |
|---|---|---|---|---|---|
| (menthol, OH) | 35 | – | (OCH2CH3, R) | 49 | $CH_3$ |
|  |  |  |  | 50 | $CH_2CH_3$ |
|  |  |  |  | 51 | $CH_2CH_2CH_3$ |
|  |  |  |  | 52 | $CH(CH_3)_2$ |
|  |  |  |  | 53 | $CH_2(CH_2)_2CH_3$ |
|  |  |  |  | 54 | CH2(CH2)3CH3 |
| (OCH2CH3) | 36 | – | (numbered ring, OCH2CH3) | 55 | 2,2-di $CH_3$ |
|  |  |  |  | 56 | 3,3-di $CH_3$ |
|  |  |  |  | 57 | 4,4-di $CH_3$ |
| (OCH2CH3, R) | 37 | $CH_3$ |  | 58 | 2,3-di $CH_3$ |
|  | 38 | $CH_2CH_3$ |  | 59 | 2,4-di $CH_3$ |
|  | 39 | $CH_2CH_2CH_3$ |  | 60 | 2,5-di $CH_3$ |
|  | 40 | $CH(CH_3)_2$ |  | 61 | 2,6-di $CH_3$ |
|  | 41 | $CH_2(CH_2)_2CH$ |  | 62 | 3,4-di $CH_3$ |
|  | 42 | $CH_2(CH_2)_3CH$ |  | 63 | 3,5-di $CH_3$ |
| (OCH2CH3, R) | 43 | $CH_3$ |  | 64 | 2-$CH_3$, 4-$CH_2CH_3$ |
|  | 44 | $CH_2CH_3$ |  | 65 | 3-$CH_3$, 4-$CH_2CH_3$ |
|  | 45 | $CH_2CH_2CH_3$ |  | 66 | 5-$CH_3$, 2-$CH_2CH_3$ |
|  | 46 | $CH(CH_3)_2$ |  | 67 | 2-$CH_3$, 4-$CH(CH_3)_2$ |
|  | 47 | $CH_2(CH_2)_2CH$ |  | 68 | 2-$CH_3$, 5-$CH(CH_3)_2$ |
|  | 48 | $CH_2(CH_2)_3CH$ |  | 69 | 3-$CH_3$, 4-$CH(CH_3)_2$ |
|  |  |  |  | 70 | 3-$CH_3$, 5-$CH(CH_3)_2$ |

[a] The enhancing activity of these compounds was studied *in vivo* using rat skin as the transdermal barrier, and ketoprofen hydrogels as the model drug.

lidocaine, except for compound **89** and **94**. In the case of pharmaceutical excipient acids (lactic acid, cinnamic acid, salicylic acid and oleic acid), all of the compounds except for compound **99** promoted the transdermal absorption of isosorbide dinitrate very well. Compound **98** and **100** exhibited good promoting effect for ketoprofen. However, for 5-fluorouracil and lidocaine, only compound **97** exhibited good acti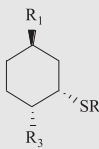vity, and for indomethacin, only compound **100** had enhancing effectiveness. The *in vivo* results obtained from the patches containing these compounds as permeation enhancers were in good agreement with the plasma concentration predicted form the *in vitro* data [39]. The study also revealed that the drug lipophilicity had a significant impact on the promoting activity of the O-acylmenthol derivatives, and a parabolic relationship between flux and log $K_{o/w}$ of the drugs was observed. In recent investigations, the O-acylmenthol derivatives were demonstrated their enhancing activity towards several drugs with different physiochemical properties,

**Table 7 – Thiomenthol derivatives.[a]**

| Structure | No. | R | | | |
|---|---|---|---|---|---|
| (ring with $R_1$, $SR_2$, $R_3$) | 71 | $R_1$: H | $R_2$: $CH_3$ $R_2$: $CH_2CH_3$ $R_2$: $CH_2CH_3$ | | $R_3$: H |
|  | 72 | $R_1$: H $R_1$: $CH_3$ | $R_2$: $CH_2CH_3$ | | $R_3$: H |
|  | 73 | $R_1$: H | $R_2$: $CH_2CH_3$ | | $R_3$: $CH(CH)_3$ |
|  | 74 | $R_1$: H | $R_2$: CH2CH3 | | $R_3$: $CH_2CH_2CH_2CH_3$ |
|  | 75 | $R_1$: H | $R_2$: $CH_3$ | | $R_3$: $CH(CH)_3$ |
|  | 76 | $R_1$: $CH_3$ | $R_2$: H | | $R_3$: CH2CH2CH3 |
|  | 77 | $R_1$: $CH_3$ | $R_2$: $CH_2CH_3$ | | $R_3$: $CH(CH)_3$ |
|  | 78 | $R_1$: H | $R_2$: $CH_2CH_3$ | | $R_3$: $CH(CH)_3$ |
|  | 79 | $R_1$: $CH_3$ | $R_2$: $CH_2CH_3$ | | $R_3$: $CH_3$ |
|  | 80 | $R_1$: $CH_3$ | $R_2$: CH2CH3 | | $R_3$: $CH_2CH_3$ |
|  | 81 | $R_1$: H | $R_2$: $CH_2CH_3$ | | $R_3$: H |
|  | 82 | $R_1$: $CH_3$ | $R_2$: $CH_3$ $R_2$: $CH_2CH_3$ $R_2$: $CH_2CH_3$ | | $R_3$: $CH_2CH_3$ |
|  | 83 | $R_1$: H | $R_2$: $CH_2CH_3$ | | $R_3$: $CH_3$ |

[a] The enhancing activity of these compounds was studied *in vivo* using rat skin as the transdermal barrier and ketoprofen hydrogels as the model drug.

**Table 8 — Ester derivatives based on l-menthol.[a]**

| Structure | No. | R | ER[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | LD | IM | 5-FU | ISDN | KP |
| | Menthol | — | 0.84 | 0.78 | 0.51 | 1.10 | 1.09 |
| | 84 | $CH_3$ | 0.96 | 1.97 | 0.35 | 0.50 | 1.30 |
| | 85 | $CH_2CH_3$ | 1.21 | 2.01 | 0.40 | 1.38 | 1.87 |
| | 86 | $(CH_2)_2CH_3$ | 1.20 | 0.05 | 0.23 | 0.95 | 1.81 |
| | 87 | $(CH_2)_3CH_3$ | 0.90 | 1.21 | 0.44 | 1.12 | 1.43 |
| | 88 | $(CH2)_4CH_3$ | 1.00 | 0.77 | 0.71 | 1.91 | 1.82 |
| | 89 | $(CH_2)_5CH_3$ | 1.58 | 3.70 | 0.63 | 1.47 | 1.59 |
| | 90 | $(CH_2)_6CH_3$ | 1.18 | 0.53 | 0.11 | 1.51 | 1.79 |
| | 91 | $(CH_2)_7CH_3$ | 1.03 | 2.09 | 0.98 | 1.27 | 1.69 |
| | 92 | $(CH_2)_8CH_3$ | 0.95 | 1.78 | 0.53 | 1.73 | 1.25 |
| | 93 | $(CH_2)_{10}CH_3$ | 1.26 | 1.13 | 0.52 | 1.09 | 1.32 |
| | 94 | $(CH_2)_{12}CH_3$ | 0.72 | 0.78 | 1.34 | 1.22 | 1.39 |
| | 95 | $(CH_2)_{14}CH_3$ | 0.71 | 0.78 | 1.11 | 1.43 | 1.35 |
| | 96 | $(CH_2)_{16}CH_3$ | 0.73 | 0.75 | 0.20 | 1.00 | 1.44 |
| | 97 | | 3.17 | 0.04 | 3.74 | 1.49 | 0.44 |
| | 98 | | 0.84 | 1.13 | 0.71 | 1.29 | 1.94 |
| | 99 | | 1.08 | 0.85 | 0.13 | 1.07 | 1.27 |
| | 100 | | 0.92 | 1.66 | 0.81 | 1.67 | 1.84 |

[a] The enhancing activity was studied *in vitro* using IPM as the donor vehicle and rat skin as the transdermal barrier.

including daphnetin [40] and granisetron [41]. The mechanism for the enhancer action is still being studied.

### 2.4. Transkarbams

Esters of amino acids were often synthesized as biodegradable permeation enhancers because of their safe and well-known metabolites. Doležal et al. first found that an acyclic Azone analog, i.e. dodecyl-6-aminohexanoate (DDEAC, Fig. 1A), had a markedly higher activity and a lower toxicity than Azone [42]. However, it was indicated that the behavior of the enhancer did not correspond to that of an amino ester molecule. DDEAC would react with the carbon dioxide by the free amino group and form another two-chain enhancer with a carbamate salt

forming its polar head, termed as Transkarbam 12 (T12), which was suggested to be responsible for the enhancing effect [43]. T12 has been demonstrated to be an effective and biodegradable skin permeation enhancer. In addition, lots of efforts have been made to explore its structure-activity relationship by structural modification at its three molecular sites, which was illustrated in Fig. 1B.

#### 2.4.1. Carbamate salt
The authors believed that the carbamate salt (Part I) in the polar head of T12 played an essential role in its transdermal enhancement activity. To examine this hypothesis, a series of T12 derivatives with different polar heads (Table 9) were synthesized and studied, including the analogs with carbonic
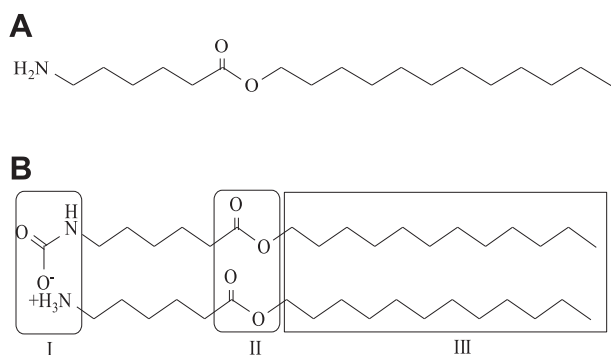
**A**



**B**



**Fig. 1 – Chemical structures of DDEAC (A) and T12 (B). Structural modifications of T12 were carried out mainly at three molecular sites as indicated in its chemical structure.**

and carbamic acid esters in the polar heads, which were shown by compound **101–114** [44]. The results showed that although in aqueous suspension some of them were slightly more effective than T12; however, none of them could be comparable to T12 neither in PG/W nor IPM systems. Therefore, it was clear that the transdermal enhancing ability of T12 was closely related to the carbamate group, i.e. carbamic acid

salt. A further study revealed that the possible mechanisms for T12 action could be that $CO_2$ was released from the polar moiety under the mildly acidic condition of the skin, causing the disruption of the highly ordered lipid lamellae. Additionally, its degradation product, protonated dodecyl-6-aminohexanoate (DDEAC) was also an active enhancer [45].
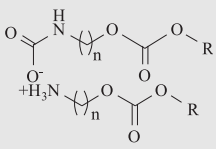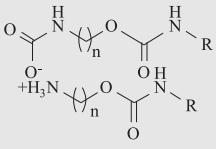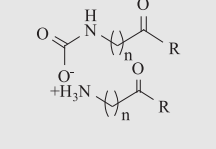
### 2.4.2. Ester linkage

The modifications of ester bonds (Part II) in the molecule of T12 were also taken into consideration and the corresponding carbonate (compound **115–120**), carbamate (compound **121–130**), ketone (compound **131–132**), amide (compound **133–140**), alkane (compound **141**) analogs were synthesized, which were shown in Table 10 [46,47]. In IPM suspension, analogs of amides (compound **133–140**) and compound **119** were comparable to that of T12. However, in PG/W vehicle, the activity of T12 was much better than all the synthesized compounds. The best chain length was further investigated among the amide analogs. As expected, a parabolic relationship was obtained with maximum at nonyl derivative (compound **137**) in both vehicles and branching of the hydrocarbon chain decreased the activity dramatically. Based on these observations, it was found that ester bonds in T12 were indispensible for its enhancement effect.

| Table 9 – T12 analogs based on carbonic and carbamic acid esters.[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Structure | NO. | $n$ | R1 | R2 | R3 | X | ER[a] | | |
| | | | | | | | W | PG/W (6:4) | IPM |
| T12 | 101 | — | — | — | — | — | 1.90 | 22.80 | 6.56 |
|  | 102 | 4 | H | $C_{10}H_{21}$ | $C_2H_5$ | NH | 3.37 | 1.36 | 1.75 |
| | 103 | 4 | H | $C_{10}H_{21}$ | $C_{12}H_{25}$ | NH | 1.13 | 2.66 | 1.48 |
| | 104 | 2 | $CH_3$ | $C_{12}H_{25}$ | $C_2H_5$ | NH | 1.48 | 1.45 | 1.77 |
| | 105 | 2 | $CH_3$ | $C_{12}H_{25}$ | $C_{12}H_{25}$ | NH | 1.70 | 1.11 | 1.15 |
| | 106 | 4 | H | $C_{12}H_{25}$ | $C_2H_5$ | O | 2.40 | 2.13 | 1.07 |
| | 107 | 4 | H | $C_{12}H_{25}$ | $C_{12}H_{25}$ | O | 2.49 | 1.13 | 1.29 |
|  | 108 | 3 | — | — | — | NH | 0.91 | 3.29 | 1.23 |
| | 109 | 3 | — | — | — | O | 1.76 | 3.70 | 1.39 |
| | 110 | 4 | — | — | — | O | 0.80 | 1.24 | 1.49 |
|  | 111 | — | — | — | $C_2H_5$ | — | 1.83 | 1.52 | 1.01 |
| | 112 | — | — | — | $C_{12}H_{25}$ | — | 0.77 | 0.47 | 0.57 |
|  | 113 | 3 | — | — | — | — | 2.27 | 2.63 | 1.29 |
| | 114 | 4 | — | — | — | — | 1.12 | 1.02 | 1.07 |

[a] The enhancing activity of these compounds was studied *in vitro*, using porcine ear skin as the transdermal barrier, and theophylline as the model drug. Water (W), isopropyl myristate (IPM) and propylene glycol/water (PG/W) was respectively used as the different donor vehicles.

## Table 10 – Ketone, amide, and alkane analogs of T12.[a]

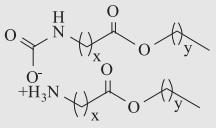| Structure | NO. | n | R | ER[a] W | PG/W (6:4) | IPM |
|---|---|---|---|---|---|---|
|  | T12 | — | — | 1.90 | 22.80 | 6.56 |
| | 115 | 5 | $C_8H_{17}$ | 4.1 | 12.4 | 2.2 |
| | 116 | 5 | $C_{10}H_{21}$ | 5.1 | 11.8 | 5.5 |
| | 117 | 5 | $C_{12}H_{25}$ | 2.5 | 7.4 | 2.9 |
| | 118 | 6 | $C_8H_{17}$ | 4.6 | 7.1 | 3.4 |
| | 119 | 6 | $C_{10}H_{21}$ | 4.7 | 7.9 | 9.7 |
| | 120 | 6 | $C_{12}H_{25}$ | 1.6 | 8.4 | 4.0 |
|  | 121 | 4 | $C_8H_{17}$ | 1.6 | 3.2 | 4.8 |
| | 122 | 4 | $C_9H_{19}$ | 1.9 | 6.3 | 5.6 |
| | 123 | 4 | $C_{10}H_{21}$ | 2.1 | 5.6 | 3.6 |
| | 124 | 4 | $C_{11}H_{23}$ | 1.5 | 2.9 | 2.5 |
| | 125 | 5 | $C_{10}H_{21}$ | 1.5 | 5.3 | 2.6 |
| | 126 | 5 | $C_{11}H_{23}$ | 1.0 | 2.3 | 2.2 |
| | 127 | 5 | $C_{12}H_{25}$ | 1.2 | 1.7 | 1.7 |
| | 128 | 6 | $C_{10}H_{21}$ | 1.2 | 4.3 | 3.1 |
| | 129 | 6 | $C_{11}H_{23}$ | 1.5 | 3.7 | 1.7 |
| | 130 | 6 | $C_{12}H_{25}$ | 1.3 | 1.9 | 0.8 |
|  | 131 | 5 | $C_{11}H_{23}$ | — | 3.8 | 2.6 |
| | 132 | 5 | $C_{12}H_{25}$ | | 3.4 | 1.1 |
|  | 133 | 5 | $C_6H_{13}$ | — | 3.8 | 4.9 |
| | 134 | 5 | $C_7H_{15}$ | — | 3.8 | 6.8 |
| | 135 | 5 | Octane-2-yl | — | 2.2 | 5.2 |
| | 136 | 5 | $C_8H_{17}$ | — | 5.0 | 7.0 |
| | 137 | 5 | $C_9H_{19}$ | — | 8.6 | 8.6 |
| | 138 | 5 | $C_{10}H_{21}$ | — | 6.0 | 6.7 |
| | 139 | 5 | $C_{12}H_{25}$ | — | 2.2 | 5.1 |
| | 140 | 5 | $C_{18}H_{37}$ | — | 0.9 | 1.3 |
| | 141 | 5 | $C_{12}H_{25}$ | — | 1.8 | 1.2 |
|  | | | | | | |

[a] The enhancing activity of these compounds was studied *in vitro*, using porcine ear skin as the transdermal barrier, and theophylline as the model drug. Water (W), isopropyl myristate (IPM) and propylene glycol/water (PG/W) was respectively used as the different donor vehicles.
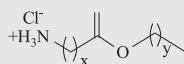
## Table 11 – T12 isomers with varying ester positions.[a]

| Structure | No. | x | y |
|---|---|---|---|
|  | 142 | 2 | 14 |
| | 143 | 3 | 13 |
| | 144 | 4 | 12 |
| | 145 | 5 | 11 |
| | 146 | 6 | 10 |
| | 147 | 7 | 9 |
| | 148 | 9 | 7 |
| | 149 | 11 | 5 |

[a] The enhancing activity of these compounds was studied *in vitro*, using porcine ear skin as the transdermal barrier, propylene glycol/water (PG/W) as the donor vehicle and theophylline as the model drug.

In addition, a series of T12 isomers with varying ester positions (compound **142–149**, Table 11) were studied on their enhancing activity in a latest investigation [48]. The result suggested that the activity of these derivatives highly depended on the positions of the ester bonds, and the optimum performance was observed when the distance of the ester group from the carbamate polar head reached 6–7 carbons (compound **146–147**), permitting no longer or shorter. An intramolecular hydrogen bond between the ester carbonyl and N–H of transkarbams might lead to the formation of a pseudo cyclic head similar to that of Azone, which would be one possible explanation of this result [49]. The study also suggested that the ammonium salt (ammonium chlorides) formed upon decomposition of the carbamate was also an active enhancer species (compound **150–157**, Table 12), though not as effective as the corresponding Transkarbams.

**Table 12 − Ammonium chloride analogs of T12.[a]**

| Structure | No. | x | y |
|---|---|---|---|
| | 150 | 2 | 14 |
| | 151 | 3 | 13 |
| | 152 | 4 | 12 |
| | 153 | 5 | 11 |
| | 154 | 6 | 10 |
| | 155 | 7 | 9 |
| | 156 | 9 | 7 |
| | 157 | 11 | 5 |

[a] The enhancing activity of these compounds was studied *in vitro*, using porcine ear skin as the transdermal barrier, propylene glycol/water (PG/W, 60/40) as the donor vehicle and theophylline as the model drug.
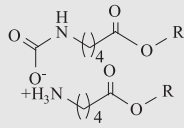
**Table 14 − TXA derivatives with different hydrocarbon chain.[a]**

| Structure | No. | R | ER[a] Water | ER[a] IPM |
|---|---|---|---|---|
| | Azone | − | 2.2 | 0.9 |
| | 170 | $C_8H_{17}$ | 1.6 | 2.6 |
| | 171 | $C_{10}H_{21}$ | 1.4 | 4.9 |
| | 172 | $C_{11}H_{23}$ | 1.3 | 2.9 |
| | 173 | $C_{12}H_{25}$ | 4.3 | 3.2 |

[a] The enhancing activity of these compounds was studied *in vitro*, using human cadaver skin as the percutaneous barrier, water (W) and isopropyl myristate (IPM) as different donor vehicles, theophylline as the model drug.

### 2.4.3. Hydrophobic tails

Transkarbams with terminal methyl or ethyl branching in Part III (compound **153−169**, Table 13) were also prepared and their promoting activity was evaluated by comparison with that of their linear analogs (compound **158−162**) [50]. Both of these two kinds of transkarbams exhibited a parabolic relationship between the chain length and the permeation enhancing activity. The best enhancement effect was observed at around 10 carbons in the alkanol moiety in both series, namely compound **160** and **165**. No significant difference could be observed between the linear group and the methyl-branched group with the same chain length, such as compound **161** and **166**. In the case of the ethyl-branched analogs, the activity of compound **169** did not change significantly by comparison with its linear analog (compound **162**), however, compound **168** showed a lower activity than its linear analog, compound **160**.
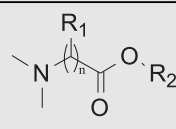
### 2.5. TXA Derivatives

Tranexamic acid (TXA) is known to improve the skin barrier homeostasis as well as the skin condition as a whole. Based on the studies of T12, a series of TXA derivatives (compound

**Table 13 − Transkarbams analogs with terminal methyl or ethyl branching.[a]**

| Structure | No. | R | ER[a] |
|---|---|---|---|
| | 158 | Octyl | 10.35 |
| | 159 | Nonyl | 16.28 |
| | 160 | Decyl | 33.60 |
| | 161 | Undecyl | 28.86 |
| | 162 | Dodecyl | 20.02 |
| | 163 | 7-Methyloctyl | 11.41 |
| | 164 | 8-Methylnonyl | 23.28 |
| | 165 | 9-Methyldecyl | 26.34 |
| | 166 | 10-Methylundecyl | 26.12 |
| | 167 | 11-Methyldodecyl | 18.97 |
| | 168 | 8-Ethyldecyl | 23.19 |
| | 169 | 10-Ethyldodecyl | 18.08 |

[a] The enhancing activity of these compounds was studied *in vitro*, using porcine ear skin as the transdermal barrier, propylene glycol/water (PG/W, 60/40) as the donor vehicle and theophylline as the model drug.

**170−173**, Table 14) were synthesized as permeation enhancers, which were expected to be metabolized into TXA in viable epidermis, and hereby restore the skin barrier function [51]. The structure of TXA is similar to 6-aminohexanoic acid, which constitutes the polar head of T12. The only difference between transkarbams and TXA derivatives is that TXA possesses a cyclohexane ring instead of the aliphatic chain.

TX12 (compound **173**) showed an excellent activity both in aqueous and IPM donor vehicle, which was almost two times higher than that of Azone. However, as a result of its relative rigidity of the cyclohexane cycle and energetically unfavorable change of the conformation, its activity was still lower than T12. Degradation of the carbamate salt of TX12 was verified in a slightly acidic environment by FTIR. Enzymolysis of the pertinent ester bond by porcine esterase was monitored by TLC and HPLC for qualitative and quantitative investigation, proving the release of TXA in the skin. In consequence, easier and faster recovery of the skin would be expected as a result of a favorable influence of TXA on skin repair process.

### 2.6. DDAIP And DDAK

DDAIP (dodecyl 2-(dimethylamino) propanoate, compound **174**, Table 15), was a potent and biodegradable enhancer that had excellent transdermal enhancing activity towards several

**Table 15 − DDAK and its derivatives.[a]**

| Structure | No. | n | R1 | R2 |
|---|---|---|---|---|
| | 174 | 1 | $CH_3$ | $CH_{12}H_{25}$ |
| | 175 | 2 | H | $CH_{12}H_{25}$ |
| | 176 | 3 | H | $CH_{12}H_{25}$ |
| | 177 | 4 | H | $CH_{12}H_{25}$ |
| | 178 | 5 | H | $CH_{12}H_{25}$ |
| | 179 | 7 | H | $CH_{12}H_{25}$ |
| | 180 | 5 | H | $C_8H_{17}$ |
| | 181 | 5 | H | $C_9H_{19}$ |
| | 182 | 5 | H | $C_{10}H_{21}$ |
| | 183 | 5 | H | $C_{11}H_{23}$ |

[a] The enhancing activity of these compounds was studied *in vitro*, using porcine skin as the transdermal barrier, propylene glycol/water (PG/W, 60/40) as the donor vehicle, and theophylline as the model drug.

types of drugs, including indomethacin, clonidine, hydrocortisone and adefovir [52–55]. DDAK (compound **178**, Table 15), was synthesized by combination of the ionizable dimethylamino polar head from DDAIP and 5-carbon linking group from T12 [56,57]. The only difference between DDAK and DDAIP lies in the linking group, in which DDAK has a longer chain length of five carbon atoms while DDAIP has a branched linking group. To compare these two compounds in detail, four model drugs, including theophylline, hydrocortisone, adefovir and indomethacin were selected to evaluate the activity of the enhancers. The results revealed that DDAK exhibited significantly better activity than DDAIP for theophylline, hydrocortisone and adefovir, while DDAIP was only better for indomethacin. Subsequently the effect of different linking chain length was studied with compound **175–179**, which were also shown in Table 15. An optimum linking chain length ($n = 4–6$) was suggested to play a significant role in the enhancing activity of DDAK and its derivatives. By comparison of DDAIP and its linear isomer (compound **175**, Table 15), it was found that the branching linking group played a negative effect on the activity of DDAIP [53]. DDAK analogs with different alky chains were also prepared (compound **178** and **180–183**, Table 15) and their promoting effect on theophylline was studied. The most potent enhancement effect was provided by compound **183**. The relationship between the chain length and the enhancement effect has a bell-like shape [58].

### 2.7.    *Natural essential oils*

Currently, considerable research is now in progress on the use of natural essential oils as potential permeation enhancers to improve the drug permeation through the skin [59]. Up to date, many natural oils, such as niaouli oil [60], eucalyptus oil [61], peppermint oil [62], chuanxiong oil [63], turpentine oil [64], chlorophytum borivilianum oil [65], sweet basil oil [66] and so on, have all been demonstrated their potential ability in transdermal permeation enhancement for a number of hydrophilic and lipophilic drugs.

Natural oils from plants are mixtures of many diverse and unique chemical compounds. Their constitutions are complex and variable, depending on the growing place, season and extraction process of the origin plants. Therefore, how to clarify the action of natural oils remains a great challenge for the researchers in the field of natural skin permeation enhancers. Gradual separation might provide a promising method to elucidate the individual effect of each component in the natural oils [67]. Additionally, this work would also contribute to the formulation of certain multi-component mixtures acting more efficiently and reproducibly with respect to the natural essential oils [60]. Mechanistic study revealed that natural essential oils, such as tulsi oil, eucalyptus oil, clove oil and black cumin oil increased the percutaneous absorption of carvedilol by extraction of lipids from SC as well as by loosening the hydrogen bonds between ceramides subsequently leading to fluidization of the lipid bilayers [61]. In addition, further research is still desirable to scale up the natural oils for transdermal enhancement use and implement manufacturing of final dosage forms on commercial scale [68].

## 3.    Mechanisms of permeation enhancement and selection of an enhancer

The mechanisms of action of permeation enhancers are complex. According to the lipid protein partitioning (LPP) theory proposed by Barry, enhancers would act by one or more of the three main mechanisms: (a) disruption of the highly ordered structure of the lipids in SC, (b) interaction with the epidermal keratin and (c) improvement in partitioning of the drug into the skin [69]. Actions on the intercellular keratin might result in the denaturation of the protein, which was an irreversible biological process [5]. Enhancers targeting the keratin in SC would also lead to great irritation to the skin [70]. Therefore, the skin irritation of a permeation enhancer would likely be more reversible if it only interacted with the intercellular lipid of the SC. In terms of lipid disruptors, they often fall into two categories: ones that exact the lipids from the SC, labeled "extractors," and ones that partition into the SC lipid bilayers exhibiting a fluidizing effect on the lipid bilayers, labeled "fluidizers". Generally, the lipid fluidizers regularly outperform the lipid extractors for their little effect on the normal constitution of the SC. Karande et al. introduced two equations (1) and (2) to assess the safety and efficacy of the lipid fluidizers and the lipid extractors [4]. In the equations, the values of ER and IP respectively represent the enhancement ratio and irritation potential of a CPE, and their ratio indicates the quality to be an ideal permeation enhancer. Obviously, for the lipid extractors, ER/IP is bound by a theoretical limit of one. However, no theoretical upper limit is evident in the case of the lipid fluidizers, which indicated their efficacy and safety as potential transdermal permeation enhancers.

$$\left.\frac{ER}{IP}\right|_{extractors} \alpha \frac{\delta_p}{\sqrt{\delta_p^2 + \delta_h^2 + \delta_d^2}} \tag{1}$$

$$\left.\frac{ER}{IP}\right|_{fluidizers} \alpha \text{Log P}\left(\frac{\delta_p}{\delta_h}\right) \tag{2}$$

Amphiphilicity was an important determinant for the enhancing activity of the lipid fluidizers. In general, most of the above mentioned permeation enhancers have the similar structures with the SC lipids, i.e. containing a polar head and one or two hydrophobic chains. The long chains of the amphiphilic enhancers enable themselves to be inserted into the intercellular lipids of the SC, and their polar heads could interact with the polar lipid region via H-bonds and Van der Waals force. The dual effects would effectively disturb the orderly organization of the lipid packing, thus increasing the diffusion of drugs with low selectivity [71]. From the analysis of the above listed materials, there is usually an optimum length of the hydrocarbon chain ($C_n \geq 7$) that is neither branching nor cyclization to obtain the best promoting activity. Alternatively, the changes of polar head also affect the enhancing activity, likely by modifying its ability of H-bonding with the intercellular lipids.

Although LPP theory suggested a general scheme for the mechanisms of permeation enhancement, it could not explain the specific effectiveness of an enhancer towards one

or a certain kind of drugs. It is likely that the precise enhancer activity will depend on the physicochemical properties of the enhancer as well as the penetrant, and the possible complex formation between drugs and enhancers might be of particular importance in disruption of the transdermal barrier. For example, Drakulić et al. has introduced a modeling method to prove the formation of complexes between different drugs and terpenes, and indicated their different behavior in the process of transdermal permeation [72].

Most of the current studies have been aimed at gaining better insights into the relationship between the structures of the enhancers and their effectiveness in transdermal enhancement. However, it has been confirmed that there is no evident correlation between the permeation enhancement effect and the skin irritation [73]. Studies of structure-irritation relationship and mechanisms of the irritation of the enhancer are important as well [74–76].

## 4.    Conclusion

The paper has given an overview of the investigations on the design of novel permeation enhancers for transdermal drug delivery systems. The list of these materials in the paper is clearly not exhaustive, but represents the general strategies involving in the identification of agents that can facilitate drug delivery through the skin barrier. At the same time further studies are also needed in the areas of skin irritation and acting mechanisms of the skin permeation enhancers.

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