ELSEVIER

International Journal of Pharmaceutics 161 (1998) 169–178

international journal of pharmaceutics

In vitro evaluation of azone analogs as dermal penetration enhancers
V. Miscellaneous compounds

B.B. Michniak [a,*], M.R. Player [b], D.A. Godwin [1,c], C.C. Lockhart [d], J.W. Sowell [a]

[a] *Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of South Carolina, Columbia, SC 29208, USA*
[b] *Section on Biomedicinal Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA*
[c] *The University of New Mexico, College of Pharmacy, 2502 North Marble NE, Albuquerque, NM 87131, USA*
[d] *Bristol-Myers Squibb, Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA*

Received 29 July 1997; received in revised form 6 October 1997; accepted 7 October 1997

Abstract

Dermal penetration enhancers were evaluated (14) using diffusion cell techniques, hairless mouse skin and hydrocortisone as the model drug. The following were synthesized: 1-dodecanoylpiperidine (**1**), 1-dodecanoylpyrrolidine (**2**), 1-dodecanoyl-2-piperidinone (**3**), 1-dodecanoyl-2-pyrrolidinone (**4**), 2-decylcyclohexanone (**5**), 2-decylcyclopentanone (**6**), 4-(dodecanoyl)-thiomorpholine (**7**), *N*,*N*-didodecylacetamide (**8**) and *N*-dodecyltricyclo [3.3.1.1^{3,7}]decane-1-carboxamide (**11**). *N*-Acetylcaprolactam (**9**), 4-acetylmorpholine (**10**) and *N*-dodecylpyrrolidinone (**13**) were purchased. The syntheses of Azone, *N*-(1-oxododecyl)morpholine (**12**) and *N*-dodecyl-2-piperidinone (**14**) have been reported previously. Enhancers were applied at 0.4 M in propylene glycol (PG) (or as a suspension) to mouse skin. Hydrocortisone (0.03 M in PG) was applied 1 h following enhancer treatment. Controls (no pretreatment) yielded 24 h diffusion cell receptor concentrations (Q_{24}) of 9.93 ± 3.15 μM and model drug skin retention of 26.1 ± 5.6 μg g^{-1}. Compound **7** yielded a high Q_{24} of 208.18 ± 39.52 μM. The highest skin retention was observed with **6** of 566.7 ± 39.7 μg g^{-1}. Azone gave values of 218.96 ± 47.84 μM for Q_{24} and 294.9 ± 66.7 μg g^{-1} for skin retention. Compounds **13** and **14** gave Q_{24} values of 274.44 ± 50.90 and 220.21 ± 59.63 μM and skin retention values of 226.5 ± 51.8 and 259.0 ± 62.2 μg g^{-1}, respectively. © 1998 Elsevier Science B.V.

Keywords: Percutaneous absorption; Enhancers; Hairless mouse skin; Hydrocortisone; Steroids

* Corresponding author. Tel.: +1 803 7777832; fax: +1 803 7772971; e-mail: Michniak@phar2.pharm.sc.edu
[1] A Fellow of the American Foundation for Pharmaceutical Education.

PII S0378-5173(97)00349-9

1. Introduction

Initial research into dermal penetration enhancers focused on simple molecules which disrupted the stratum corneum (SC) and were usually non-drug specific keratolytic agents which destroyed the barrier integrity of the SC membrane. Recently, there has been a rapid expansion in our knowledge concerning the SC barrier and function as well as the mechanisms by which enhancers exert their actions. This has led to a more organized approach to the design of novel, reversibly-acting, non-toxic compounds. Examples of some of these agents include terpenes (Moghimi et al., 1996), Azone analogs (Michniak et al., 1993a,b, 1994a,b), phospholipids (Yokomizo, 1996), alkyl *N*,*N*-disubstituted amino acetates (Büyütktimkin et al., 1993), clofibric acid amides and esters (Michniak et al., 1993c), pirothiodecane (Yano et al., 1995), oxazolidinones (Rajadhaksha and Pfister, 1996) and n-pentyl-*N*-acetylprolinate (Harris et al., 1995).

Penetration enhancers have been graded by several investigators. Lambert et al. (1993) divided these agents into simple fatty acids and alcohols, those that act as mainly solvents and hydrogen bond acceptors (dimethylsulfoxide, DMSO) and weak surfactants containing a polar head group (Azone). Using Hildebrand solubility parameters, another group classified enhancers as either polar or non-polar (Pfister et al., 1990). Hori et al. (1990) plotted organic versus inorganic values for enhancers and found that two distinct groups existed. One area of the plot contained agents such as DMSO, ethanol, PG and the other Azone, lauryl alcohol and oleic acid.

This investigation is a continuation of an examination of structure-activity relationships in several series of compounds related to Azone (laurocapram). Data have been published for cyclic and acyclic enhancer activities using a standard testing procedure (Michniak et al., 1993a,b, 1994a, 1995).*N*-dodecyl-2-pyrrolidinone, *N*-(1-oxododecyl)morpholine and *N*-dodecyl-2-piperidinone were active enhancers for hydrocortisone 21-acetate (Michniak et al., 1993b). The most active acyclic enhancer was *N*-dodecyl-*N*-(2-methoxyethyl)acetamide.

Several studies with Azone and *N*-alkyl analogues of Azone suggest that these compounds interact with the SC and change the barrier properties of this skin layer. The greatest activities and barrier disruption were recorded for compounds with alkyl chains between C8 and C16 (Bouwstra and Bodde, 1995).

The C12 alkyl side chain of Azone corresponds to the dimensions of the cholesterol skeleton, leading to the possible disruption of ceramide–cholesterol or cholesterol–cholesterol interactions within the SC by this enhancer. Molecular modeling studies involving electrostatic interactions of Azone with skin lipids have also been described (Brain et al., 1993). Additional mechanisms, however, also play an important role in enhancer activity and include solubility, interaction with proteins in the skin, partitioning, concentration effects and choice of accompanying model drug/vehicle(s) and formulations (Parks et al., 1997; Pellet et al., 1997).

This present study involved 14 novel enhancer compounds using Azone as the standard enhancer for comparison. The model drug chosen was a polar steroid, hydrocortisone and the vehicle was PG.

2. Materials

All chemicals were purchased from Aldrich Chemical Company in the highest available purity. *N*-Acetylcaprolactam (**9**) and 4-acetylmorpholine (**10**) were obtained from Fluka, Ronkonkoma, NY. Sigma supplied hydrocortisone, polyoxyethylene-20-cetyl ether and PG. *N*-dodecyl-2-pyrrolidinone (**13**) was obtained from Aldrich. VWR Scientific, supplied reagent grade solvents, except for methanol and acetonitrile, which were HPLC grade. Male hairless mice, strain SKH1 (h/h), 8 weeks old, were supplied by Charles River Laboratories, Wilmington, MA.

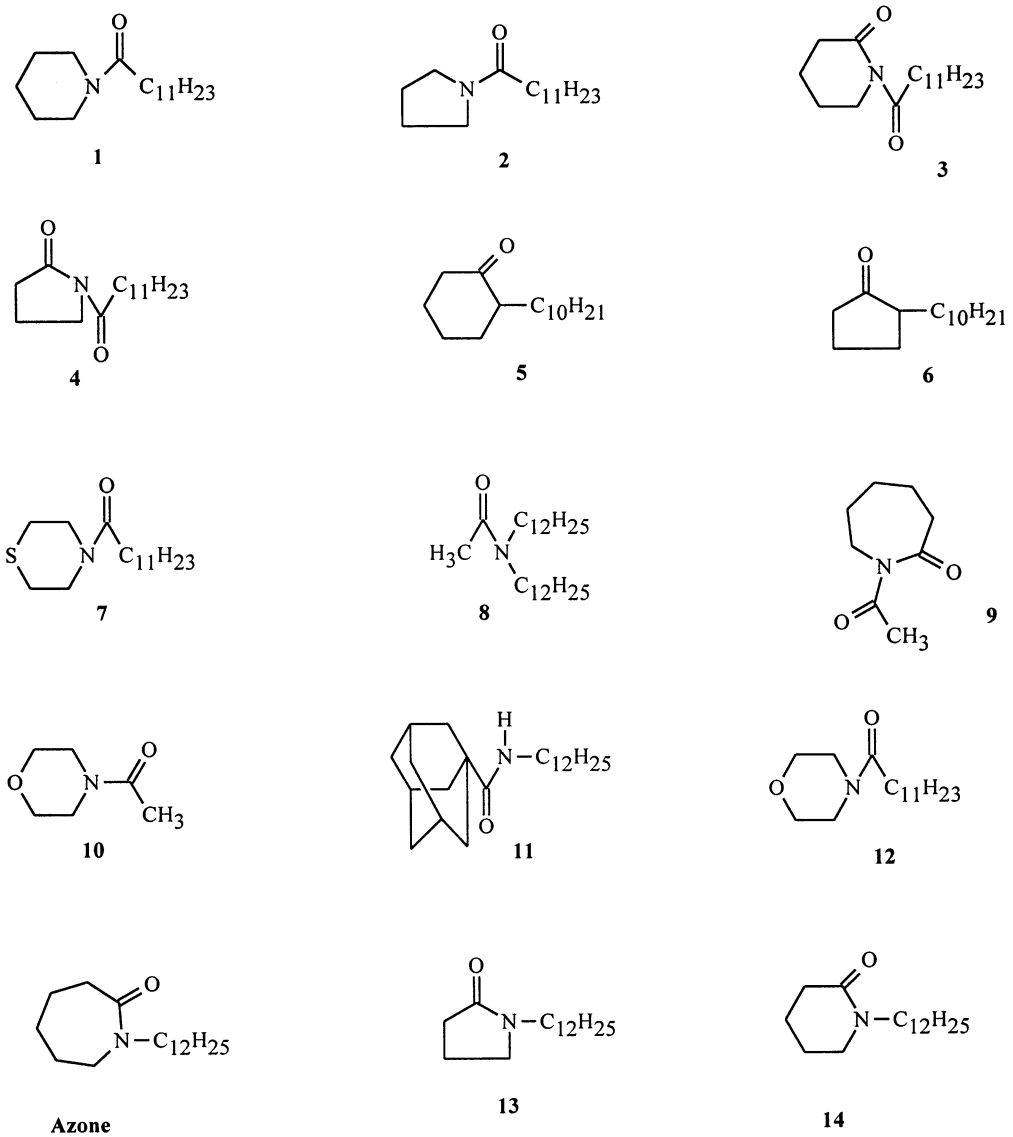


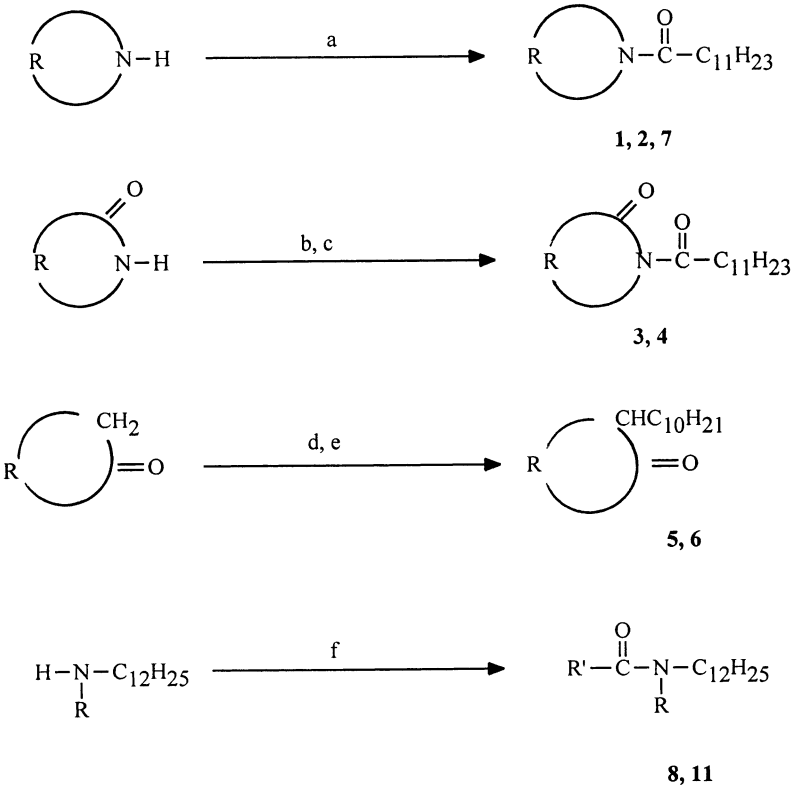
Fig. 1. Structures of enhancers **1–14.**

3. Methods

3.1. Enhancer synthesis

The chemical structures of compounds **1–12** tested in this study are presented in Fig. 1. Azone, compounds **12** and **14** were synthesized according to previously published methods (Michniak et al., 1993b,c).

Piperidine, pyrrolidine and thiomorpholine were converted into their corresponding dodecanoylamides **1**, **2** and **7**, by reaction with lauroyl chloride. Imides **3** and **4** were prepared from 2-piperidinone and 2-pyrrolidone, respectively, by treating the lactams with sodium hydride in THF, followed by acylation of the sodium salt with lauroyl chloride. Compounds **5** and **6** were previously reported (Nikishin et al., 1961; Tanchuk et



Scheme 1. (a) $C_{11}H_{23}COCl$, CH_2Cl_2. (b) NaH, THF. (c) $C_{11}H_{23}COCl$. (d) $[(CH_3)_3Si]_2NK$, THF. (e) $C_{10}H_{21}Br$. (f) R^1COCl or $(R^1CO)_2O$.

al., 1980), but were synthesized for this study by a modification of the original procedures. The potassium enolates of cyclohexanone and cyclopentanone were alkylated with decyl bromide to yield **5** and **6**, respectively. The alkyl ketones obtained were identical to those previously reported. Acetylation of didodecylamine provided compound **8** and acylation of dodecylamine with tricyclo [3.3.1.1^{3,7}] decane-1-carbonyl chloride afforded compound **11** (Scheme 1, Table 1).

*3.1.1. 1-Dodecanoylpiperidine (**1**)*

A solution of lauroyl chloride (1.10 g, 5 mmol) in methylene chloride (5 ml) was added dropwise to piperidine (0.85 g, 10 mmol) in methylene chloride (50 ml) at 0°C. The solution was stirred at ambient temperature for 3 h, then washed consecutively with hydrochloric acid (1 N), sodium hydroxide (1 N) and brine. The organic layer was dried over anhydrous magnesium sulfate and the solvent removed in vacuo. The product was obtained by column chromatography with hexanes/ethyl acetate (9:1) mobile phase as a clear viscous oil in 77% yield; ^{1}H-NMR $CDCl_3$ δ 0.83 (t, 3H, $-CH_3$), 1.21–1.59 (complex m, 24H, $-CH_2-$), 2.27 (t, 2H, $-COCH_2-$), 3.37, 3.48 (two broad s, 4H, $-CH_2N-$).

3.1.1.1. Analysis. $C_{17}H_{33}NO$ requires C, 76.34; H, 12.44 and N, 5.24. Found: C, 76.03; H, 12.36 and N, 5.37.

*3.1.2. 1-Dodecanoylpyrrolidine (**2**)*

The procedure for the synthesis and purification of Compound **1** was utilized to yield a viscous, colorless oil in 51% yield; ^{1}H-NMR ($CDCl_3$) δ 0.85 (t, 3H, $-CH_3-$), 1.23–1.90 (complex m, 22H, $-CH_2-$), 2.22 (t, 2H, $-COCH_2-$), 3.41 (m, 4H, $-CH_2N-$).

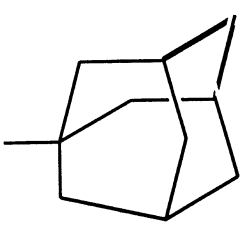
3.1.2.1. Analysis. $C_{16}H_{31}NO$ requires C, 75.83; H, 12.33 and N, 5.52. Found: C, 75.61; H, 12.33 and N, 5.43.

*3.1.3. 4-Dodecanoylthiomorpholine (**7**)*

The procedure for the synthesis and purification of Compound **1** was employed to yield white crystals in 70% yield, m.p. 36.5–37.0°C; ^{1}H-NMR ($CDCl_3$) δ 0.81 (t, 3H, $-CH_3$), 1.19–1.55 (complex m, 18H, $-CH_2-$), 2.24 (t, 2H, $-COCH_2-$), 2.54 (m, 4H, $-CH_2SCH_2-$), 3.68–3.81 (two broad s, 4H, $-CH_2NRCH_2-$).

3.1.3.1. Analysis. $C_{16}H_{31}NOS$ requires C, 67.31; H, 10.94 and N 4.91. Found: C, 67.04; H, 10.87 and N, 4.97.

Table 1
The ^{1}H-NMR spectra were obtained on a Brüker AM 300 NMR spectrometer in $CDCl_3$ solution and the resulting spectroscopic data agreed with assigned structures in all cases

Compound number	R	R^1
1	$-(CH_2)_5-$	—
2	$-(CH_2)_4-$	—
7	$-(CH_2)_2S(CH_2)_2-$	—
3	$-(CH_2)_4-$	—
4	$-(CH_2)_3-$	—
5	$-(CH_2)_4-$	—
6	$-(CH_2)_3-$	—
8	$-C_{12}H_{25}$	$-CH_3$
11	–H	

The TLC were performed with Baker silica plates, type Si250F. Column chromatography was performed with E.M. Science Silica Gel type 60, 40–63 μM. Elemental analyses were performed on all novel compounds by Atlantic Microlabs, Atlanta, GA and the reported values were within $\pm$0.4% of the theoretical value for all compounds.

*3.1.4. 1-Dodecanoyl-2-piperidinone (**3**)*

A solution of 2-piperidone (0.99 g, 10 mmol) in anhydrous THF (30 ml) was treated with sodium hydride (0.24 g, 10 mmol) and stirred at room temperature for 2 h. The suspension was cooled to 0°C and lauroyl chloride (2.2 g, 10 mmol) added. After stirring at room temperature for 12 h, the desired product was obtained by column chromatography with hexanes/ethyl acetate (4:1) mobile phase. The imide was obtained as a viscous, colorless oil in 22% yield; ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H, $-CH_3$), 1.23–1.81 (m, 22H, $-CH_2-$), 2.33 (t, 2H, $-COCH_2-$), 2.52 (t, 2H, $-CH_2CO-$), 3.67 (m, 2H, $-CH_2N-$).

3.1.4.1. Analysis. $C_{17}H_{31}NO_2$ requires C, 72.55; H, 11.10 and N, 4.98. Found: C, 72.38; H, 11.19 and N, 4.78.

*3.1.5. 1-Dodecanoyl-2-pyrrolidinone (**4**)*

The procedure for the synthesis and purification of Compound **3** was utilized to yield the imide as a viscous, colorless oil in 36% yield; ^{1}H-NMR ($CDCl_3$) δ 0.84 (t, 3H, $-CH_3$), 1.19–1.51 (m, 18H, $-CH_2-$), 1.99 (m, 2H, ring $-COCH_2\underline{CH_2}CH_2N-$), 2.52 (t, 2H, $-COCH_2-$), 2.80 (t, 2H, $-CH_2CO-$) 3.76 (m, 2H, $-CH_2N-$).

3.1.5.1. Analysis. $C_{16}H_{29}NO_2$ requires C, 71.87; N, 10.93 and N, 5.24. Found: C, 71.61; H, 10.93 and N, 5.04.

*3.1.6. 2-Decylcyclohexanone (**5**)*

Cyclohexanone (0.98 g, 10 mmol) in freshly distilled THF (25 ml) was stirred at 0°C while potassium bis(trimethylsilyl)amide (1.99 g, 10 mmol) was added. After 5 min at room temperature, 1-bromodecane (2.21 g, 10 mmol) was added dropwise. After the addition was complete, the orange-colored mixture was stirred for 1 h at room temperature and THF removed in vacuo. The residue was partitioned between ethyl acetate and water and the organic layer was consecutively washed with aqueous sodium hydroxide, hydrochloric acid and brine. The desired product was obtained by column chromatography with hexanes/ethyl acetate (9:1) mobile phase as a clear, viscous oil in 12.5% yield; ^{1}H-NMR

($CDCl_3$) δ 0.95 (t, 3H, $-CH_3$), 1.2–2.0 (complex m, 27H, $-CH_2-$).

*3.1.7. 2-Decylcyclopentanone (**6**)*

The procedure for the synthesis and purification of Compound **5** was utilized to yield the desired product in 17% yield; ^{1}H-NMR ($CDCl_3$) δ 0.95 (t, 3H, terminal CH_3), 1.1–2.2 (complex m, 25H, $-CH_2-$).

*3.1.8. N,N-didodecylacetamide (**8**)*

A solution of didodecylamine (1.64 g, 4.6 mmol), pyridine (0.367 g, 4.6 mmol) and acetic anhydride (0.47 g, 4.6 mmol) in acetonitrile (75 ml) was refluxed with stirring for 5 h. The solvent was removed in vacuo, the residue dissolved in ethyl acetate and the organic layer was consecutively washed with aqueous sodium hydroxide (1 N), hydrochloric acid (1 N), and brine. The desired product was obtained by column chromatography with hexanes/ethyl acetate (9:1) mobile phase in 74.5% yield. ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 6H, $-CH_3$), 1.23–1.51 (m, 40H, $-CH_2-$), 2.06 (s, 3H, $-COCH_3$), 3.15–3.29 (m, 4H, $-N-CH_2-$).

3.1.8.1. Analysis. $C_{26}H_{53}NO$ 0.25 H_2O requires C, 78.03; H, 13.47 and N, 3.50. Found: C, 78.22; H, 13.47 and N, 3.52.

*3.1.9. N-dodecyltricyclo [3.3.1.1^{3,7}]decane-1-carboxamide (**11**)*

A solution of dodecylamine (2 g, 11 mmol) and pyridine (0.87 g, 11 mmol) in acetonitrile (50 ml) was treated with 1-adamantanecarbonyl chloride (2.14 g, 11 mmol) in acetonitrile (25 ml). After the addition was complete, the mixture was refluxed for 2 h, diluted with water and the solid collected. Recrystallization from methanol yielded the desired product as a solid, m.p. 77.5–78.0°C. ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, 3H, terminal CH_3), 1.23–1.82 (m, 33H, $-CH_2-$and –CH–), 2.01–2.15 (m, 2H, $-NHCH_2\underline{CH_2}-$), 3.19 (m, 2H, $-NHCH_2-$), 5.54 (s, 1H, amide NH).

3.1.9.1. Analysis. $C_{23}H_{41}NO$ requires C, 79.48; H, 11.89 and N, 4.03. Found: C, 79.44; H, 11.88 and N, 4.09.

3.2. Permeability experiments

Full-thickness dorsal skins were obtained from 6–8-week-old male hairless mice (SKH1, Charles River Laboratories, Wilmington, MA). These skins were placed in donor compartments of modified Franz diffusion cells (Vangard, Neptune, NJ) with exposed areas of 3.14 cm^2. The receptors (12 ml) were filled with isotonic phosphate buffer (pH 7.2) containing 0.1% v/v of 36% aqueous formaldehyde as preservative and 0.5% w/v polyoxyethylene-20-cetyl ether as solubilizer (Chien, 1982; Sloan et al., 1991). The receptors were maintained at 37 ± 0.5°C and stirred continuously at 600 rpm. Skins were allowed to hydrate for 1.5 h prior to experimentation.

Following this hydration period, 8 μl enhancer in PG was spread uniformly over each skin at a concentration of 0.4 M. Enhancers **1–10**, **12–14** and Azone were liquids. Compound **11** was a solid (m.p. 77–78.5°C and solubility in PG of 0.03 M at 32 ± 0.5°C). The solid enhancer was applied as a suspension in PG. A suspension of hydrocortisone in PG (80 μl/cell, solubility 0.03 M at 32 ± 0.5°C) was applied to each skin and the cells were occluded with Parafilm® 1 h later. The skins were not washed prior to hydrocortisone application. Controls consisted only of model drug application in PG and no pretreatment of skin ($n = 5$). Samples (300 μl) were withdrawn from the receptor over 24 h. Analysis of subsequent samples was corrected for all previous samples removed. The receptor was replenished each time with 300 μl diffusion buffer.

At 24 h, skins were removed, washed in methanol (100 ml) for 5 s ($\times 3$), weighed and homogenized using a Kinematica GmbH tissue homogenizer (Switzerland).

Extraction methods and recoveries have been reported previously (Sasaki et al., 1991; Michniak et al., 1993c). The skin extracts and receptor samples were analyzed for hydrocortisone using HPLC. The HPLC method utilized a Perkin–Elmer system which consisted of an ISS 100 automatic sampling system, a solvent environment control (SEC-4), a Series 410 LC pump and a LC-235 diode array detector. The HPLC was run by an Epson III + computer with Ω peak integra-

Table 2
Effect of enhancers **1–12** and Azone on steroid skin retention and skin permeation parameters

Enhancer in PG[a,b] ($n=5$)	L[c] (h)	$P\times10^4$ (cm h^{-1})[g]	ER_P^f	Q_{24} (μM)[d]	ER_{Q24}^f	SC (HC)[e] ($\mu g\ g^{-1}$)	ER_{SC}^f
None	3.85 ± 2.50	0.51 ± 0.25	1.0	9.93 ± 3.15	1.0	26.1 ± 5.6	1.0
Azone	1.39 ± 0.06	21.52 ± 4.86	42.2	218.96 ± 47.84	22.1	294.9 ± 66.7	11.3
1	0.48 ± 0.12	9.63 ± 3.29	18.9	145.87 ± 25.24	14.7	167.7 ± 49.0	6.4
2	0.89 ± 0.25	23.18 ± 8.42	45.5	154.96 ± 24.15	15.6	540.9 ± 120.1	20.7
3	0.42 ± 0.11	4.39 ± 1.22	8.6	76.05 ± 15.67	7.7	315.9 ± 47.7	12.1
4	1.78 ± 0.22	6.50 ± 2.09	12.8	99.73 ± 19.30	10.1	279.6 ± 57.9	10.7
5	1.30 ± 0.21	5.25 ± 1.68	10.3	78.31 ± 14.18	7.9	199.3 ± 42.1	7.6
6	1.26 ± 0.19	4.18 ± 1.30	8.2	66.64 ± 11.76	6.7	566.7 ± 39.7	21.7
7	0.34 ± 0.24	16.80 ± 6.95	32.9	208.18 ± 39.52	21.0	550.3 ± 196.3	21.1
8	0.71 ± 0.31	4.69 ± 1.22	9.2	88.63 ± 16.12	8.9	120.8 ± 50.1	4.6
9	0.10 ± 0.09	9.29 ± 2.86	18.2	45.81 ± 13.00	4.6	168.3 ± 56.5	6.5
10	1.37 ± 0.19	0.69 ± 0.12	1.4	12.73 ± 3.81	1.3	143.4 ± 9.8	5.5
11	1.51 ± 0.52	2.57 ± 0.47	5.0	19.78 ± 5.00	2.0	142.7 ± 58.5	5.5
12	3.02 ± 0.38	28.74 ± 11.62	56.4	149.53 ± 33.24	15.1	226.7 ± 37.4	8.7
13	0.64 ± 0.72	30.63 ± 6.15	60.1	274.44 ± 50.90	27.6	226.5 ± 51.8	8.7
14	0.96 ± 0.11	24.65 ± 5.67	48.3	220.21 ± 59.63	22.2	259.0 ± 62.6	9.9

[a] PG, propylene glycol.
[b] All $n=5$. Melting point of **11**: 77.5–78°C.
[c] L, lag time.
[d] Q_{24}, receptor concentration after 24 h.
[e] SC, skin content of hydrocortisone (HC).
[f] ER, enhancement ratio calculated as permeation parameter after enhancer treatment divided by corresponding parameter from control.
[g] P, permeability coefficient.

tion software designed by Perkin–Elmer. All samples were analyzed using a reverse phase C_{18} column (Rainin Microsorb MV®, 25 cm, 5 μm). Hydrocortisone was detected at 242 nm with a retention time of approximately 4.5 min using a mobile phase of 40:60 acetonitrile:water at 1 ml/min^{-1} and ambient temperature. Testing of linearity of the calibration graph from 1.0 to 100 $\mu g\ ml^{-1}$ revealed a correlation coefficient of 0.9960. Intraday and interday variability were 7.2 and 8.0%, respectively.

Results were expressed as lag time (h), permeability coefficient P (cmh^{-1}), receptor concentration after 24 h (Q_{24}, μM) and skin content of hydrocortisone ($\mu g\ g^{-1}$). Enhancement ratios (ER) were calculated using controls equal to 1.00. All parameters were reported as means $\pm$ SD. Data treatment involved analysis of variance (ANOVA) followed by a least significant difference test (LSD) if the ANOVA indicated that a difference existed. The level of significance was 0.05 (Bolton, 1990).

4. Results and discussion

The data obtained are presented in Table 2. Receptor concentrations at 24 h (Q_{24}) for controls were 9.93 ± 3.15 μM and hydrocortisone skin content was 26.1 ± 5.6 $\mu g\ g^{-1}$ ($n=5$). Azone increased Q_{24} values to 218.96 ± 47.84 and skin content to 294.9 ± 66.7 $\mu g\ g^{-1}$ ($n=5$). However, enhancers **2**, **6** and **7** all exhibited significantly higher skin contents compared with Azone: 540.0 ± 120.1 $\mu g\ g^{-1}$ for **2**, 566.7 ± 39.7 $\mu g\ g^{-1}$ for **6** and 550.3 ± 196.3 $\mu g\ g^{-1}$ for **7** ($P<0.05$). The respective Q_{24} values for these enhancers were: 154.96 ± 24.15 μM, 66.64 ± 11.76 μM and 208.18 ± 39.52 μM. All compounds tested (**1–12**) were effective enhancers and showed higher Q_{24} values and skin contents compared with controls ($P<0.05$). The least effective enhancer for Q_{24} was **10** with a Q_{24} of 12.73 ± 3.81 μM. Skin content for **10** was 143.4 ± 9.8 $\mu g\ g^{-1}$. The least effective enhancer for skin content was **8** with 120.8 ± 50.1 $\mu g\ g^{-1}$. Q_{24} data for **8**: 88.63 ± 16.12 μM.

N-dodecyl-2-pyrrolidinone (**13**) had a Q_{24} of 274.44 ± 50.90 μM and a skin content of 226.5 ± 51.8 μg g^{-1}. *N*-dodecyl-2-piperidinone (**14**) Q_{24} was 220.21 ± 59.63 μM and skin content of 259.0 ± 62.6 μg g^{-1}. Lag times for these two compounds were 0.64 ± 0.72 and 0.96 ± 0.11 h, respectively, and P values ($\times 10^{-4}$): 30.63 ± 6.15 and 24.65 ± 5.67 cm h^{-1}, respectively.

Comparing Azone, **13** and **14**, the most effective enhancer for Q_{24} and P values was **13**, suggesting that the presence of the pyrrolidinone structure increased activity. It is of interest to note that **9** was devoid of the long dodecyl side chain of Azone and although activity was decreased, it was not lost (ER_P for **9** was 18.2, ER_{Q24} 4.6 and ER_{SC} 6.5). Likewise, **8** was an acyclic structure which still showed mild enhancing activity (ER_P 9.2, ER_{Q24} 8.9 and ER_{SC} of 4.6). It seems that for activity to be present (even if mild), a cyclic structure is not necessary, neither is the presence of a long alkyl side chain. However, these do increase activity if present in the enhancer structure. This is illustrated well by compounds **10** and **12**, where the long *N*-alkyl side chain in **12** significantly increased ER_P from 1.4 to 56.4 ($P < 0.05$). ER_{Q24} increased from 1.3 to 15.1 ($P < 0.05$) and ER_{SC} from 5.5 to 8.7.

Compounds **1** and **2** differed in their ring size and Q_{24} for **1** was 145.87 ± 25.24 μM and for **2** 154.96 ± 24.15. P values for **2** were 2.4-fold higher and skin content 3.2-fold higher than for **1**. This is in contrast to the situation with **13** and **14**, where the piperidinone analog had less activity as illustrated by the P and Q_{24} values. However, skin contents were higher for **14**.

Comparing 1-dodecanoylpiperidine (**1**) with *N*-dodecyl-2-piperidinone, the former produced lower Q_{24} and skin contents (145.87 ± 24.24 μM and 167.7 ± 49.0 μg g^{-1}). Likewise, comparing 1-dodecanoylpyrrolidine (**2**) with *N*-dodecyl-2-pyrrolidinone, the former produced a lower Q_{24} but a higher skin content (154.96 ± 24.15 μM and 540.9 ± 120.1 μg g^{-1}). This suggested that decreased activity was observed when the carbonyl group was present as an amide rather than as a lactam.

Compounds **3** and **4** (a piperidinone and a pyrrolidinone) were less active than **1** and **2** (a piperidine and a pyrrolidine). The ER_P for **3** and **4** were 8.6 and 12.8, respectively, compared with ER_P for **1** and **2** of 18.9 and 45.5, respectively. Comparing **3** and **4** with **14** and **13** suggested that the presence of a dodecyl side chain leads to increased transdermal enhancer activity, but decreased skin contents. ER_{Q24} for **3** was 7.7 and for **14** was 22.2. ER_{Q24} for **4** was 10.1 and for **3** was 27.63. The presence of the carbonyl in the side chain versus the ring was an important factor in controlling the relative activity of the enhancers. It is interesting that **3** and **4**, which have carbonyls in both positions, showed the least activity with respect to permeability, suggesting that the polarity of the headgroup may be important in controlling specific interaction with the skin.

Comparing **2** and **4** showed higher activity with **2** (ER_P for **2**: 45.5; ER_{Q24} 15.6; ER_{SC} 20.7 and ER_P for **4**: 12.8; ER_{Q24} 10.1; ER_{SC} 10.7). In the same manner, **3** produced the following values: ER_P 8.6, ER_{Q24} 7.7 and ER_{SC} 12.1. Compound **1** corresponding ER values were: 18.9, 14.7 and 6.4. These data showed higher activity again in the 1-dodecanoyl piperidine. The skin content, however, was lower than that of compound **3**.

Comparing compounds **7** and **1** showed that the presence of sulfur in the ring caused an increase in transdermal activity ($P < 0.05$). Compound **12** had an oxygen in place of the sulfur and this lead to a higher ER_P of 56.4, compared to ER_P for **7** of 32.9, however, Q_{24} values and skin content values were lower in **12**.

Several thioamides have been patented previously and Michniak et al. (1995) have also reported on the activity of several such compounds using hydrocortisone 21-acetate as a model drug (Miniskanian and Peck, 1990; Michniak et al., 1993b).

In general, the thione compounds showed less activity than the equivalent parent compound with no sulfur. For example, Azone, *N*-dodecyl-2-pyrrolidinone and *N*-dodecyl-2-piperidinone were more active than *N*-dodecyl-hexahydro-2-thio-1*H*-azepine, *N*-dodecyl-piperidine-2-thione and *N*-dodecyl-pyrrolidine-2-thione. Replacement of sulfur by oxygen resulted in increased activity. For example, comparing *N*-(1-oxododecyl)hexahydro-1*H*-azepine and *N*-(1-thiododе-

cyl)hexahydro-1*H*-azepine. ER_{Q24} for the former was 19.05 and for the latter 6.78 (Michniak et al., 1993b).

It seems that thiomorpholines, however, may have increased activity (compare **7** and **1**), and replacement with oxygen caused a further enhancement in activity (compare **1**, **7** and **12**).

It must be noted that both permeability coefficients and 24 h receptor concentrations are provided in Table 2, since with the more active enhancers, the donor was depleted faster and by 24 h steady-state was lost as evidenced by the presence of a plateau in the flux graphs. This could have been overcome by using an infinite rather than finite dose in the donor cell. However, finite doses more closely resemble clinical use of topicals and have been used by the authors in previous screening of enhancer compounds. Values of P were calculated using initial steady-state portions of the graph.

2-Decylcyclopentanone (**6**) with a shorter alkyl side chain and no nitrogen compared with *N*-dodecyl-2-pyrrolidinone produced a lower Q_{24} value of 66.64 ± 11.76 μM, but a higher skin content of 566.7 ± 39.7 $\mu g\ g^{-1}$. The shorter alkyl chain length analog of *N*-dodecyl-2-piperidinone with no nitrogen, 2-decylcyclohexanone (**5**) produced a lower Q_{24} and skin content: (78.31 ± 14.18 μM and 199.3 ± 42.1 $\mu g\ g^{-1}$). Compound **11** had steric bulk on the head group and this decreased activity: ER_P 5.0, ER_{Q24} 2.0 and ER_{SC} 5.5.

In a previous investigation of irritancy using a histopathological technique on mice in vivo and testing Azone and enhancers **13** and **14**, relative irritation scores were 9.0 for Azone, 26.0 for **13** and 24.0 for **14**. Cyclic enhancers were less irritating than acyclics tested (Phillips and Michniak, 1995). PG was found not to be irritant. Clinical studies on humans support the fact that Azone is tolerated well and irritation is observed only when high concentrations of Azone are used (Allan, 1995).

In conclusion, compounds **2**, **7**, **12**, **13** and **14** seem to be the most promising enhancers for future development.

Acknowledgements

The authors would like to acknowledge the following: NSF # CHE-8411172, NIH # S10-RR02424-1 for the provision of Brücker AM300, American Foundation for Pharmaceutical Education for a fellowship for D.A.G., and the South Carolina Law Enforcement Division, Columbia, SC., for use of HPLC equipment.

References

Allan, G., 1995. Azone®. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers. CRC Press, Boca Raton, FL, pp. 129–136.

Bolton, S., 1990. Pharmaceutical Statistics: Practical and Clinical Applications. Dekker, New York, pp. 117–181, 262–302.

Bouwstra, J.A., Bodde, H.E., 1995. Human SC barrier impairment by N-alkylazacycloheptanones: a mechanistic study of drug flux enhancement, Azone® mobility and protein and lipid perturbation. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers. CRC Press, Boca Raton, FL, pp. 137–164.

Brain, K.R., Hadgraft, J., Walters, K.A., 1993. Significance of electrostatic interactions in the modification of the permeability of ceramide membranes. In: Brain, K.R., James, V.J., Walters, K.A. (Eds.), Prediction of Percutaneous Penetration. STS, Cardiff, pp. 166–173.

Büyütktimkin, S., Büyüktimkin, N., Rytting, J.H., 1993. Synthesis and enhancing effect of dodecyl 2-(*N*,*N*-dimethylamino) propionate on the transepidermal delivery of indomethacin clonidine and hydrocortisone. Pharm. Res. 10, 1632–1637.

Chien, Y.W., 1982. Novel Drug Delivery Systems: Fundamentals, Developmental Concepts, Biomedical Assessments. Dekker, New York, pp. 551–553.

Harris, W.T., Tenjarla, S.N., Holbrook, J.M., Smith, J., Mead, C., Entrekin, J., 1995. n-Pentyl *N*-acetylprolinate. A new skin penetration enhancer. J. Pharm. Sci. 84, 640–642.

Hori, M., Satoh, S., Maibach, H.I., 1990. Classification of penetration enhancers: a conceptual diagram. J. Pharm. Pharmacol. 42, 71–72.

Lambert, W.J., Kudlar, R.J., Hollard, J.M., Curry, J.T., 1993. A biodegradable transdermal penetration enhancer based on *N*-(2-hydroxyethyl)-2-pyrrolidone. I. Synthesis and characterization. Int. J. Pharm. 45, 181–192.

Michniak, B.B., Chapman, J.M., Seyda, K.L., 1993c. Facilitated transport of two model steroids by esters and amides of clofibric acid. J. Pharm. Sci. 82, 214–219.

Michniak, B.B., Player, M.R., Chapman, J.M. Jr., Sowell, J.W. Sr., 1994b. Azone analogs as penetration enhancers: effect of different vehicles on hydrocortisone acetate skin permeation and retention. J. Control. Release 32, 147–154.

Michniak, B.B., Player, M.R., Chapman, J.M. Jr., Sowell, J.W. Sr., 1993a. In vitro evaluation of a series of Azone analogs as dermal penetration enhancers: I. Int. J. Pharm. 91, 85–93.

Michniak, B.B., Player, M.R., Fuhrman, L.C., Christensen, C.A., Chapman, J.M. Jr., Sowell, J.W. Sr., 1993b. In vitro evaluation of a series of Azone analogs as dermal penetration enhancers: II. (Thio)amides. Int. J. Pharm. 94, 203–210.

Michniak, B.B., Player, M.R., Fuhrman, L.C., Christensen, C.A., Chapman, J.M. Jr., Sowell, J.W. Sr., 1994a. In vitro evaluation of a series of Azone analogs as dermal penetration enhancers: III. Acyclic amides. Int. J. Pharm. 110, 231–239.

Michniak, B.B., Player, M.R., Godwin, D.A., Phillips, C.A., Sowell, J.W. Sr., 1995. In vitro evaluation of a series of Azone analogs as dermal penetration enhancers: IV. Amines. Int. J. Pharm. 116, 201–209.

Miniskanian, G., Peck, J.V., 1990. US Patent Application, 199 801; Chem. Abstr., 113, 158682k.

Moghimi, H.R., Williams, A.C., Barry, B.W., 1996. A lamellar matrix model for SC intercellular lipids IV. Effects of terpene penetration enhancers on the permeation of 5-fluorouracil and oestradiol through the matrix. Int. J. Pharm. 145, 49–59.

Nikishin, G.I., Somov, G.V., Petrov, A.D., 1961. Free radical addition of cyclopentanone and cyclohexanone to α-olefins and allyl ethers. Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk., pp. 2065–2071; through Chem. Abstr., 56 (1962) 7155.

Parks, T.M., Cleek, R.L., Bunge, A.L., 1997. Chemical release from topical formulations across synthetic membranes: infinite dose. J. Pharm. Sci. 86, 187–192.

Pellet, M.A., Castellano, S., Hadgraft, J., Davis, A.F., 1997. The penetration of supersaturated solutions of piroxicam across silicone membranes and human skin in vitro. J. Control. Release 46, 205–214.

Pfister, W.R., Dean, S., Hsieh, S.T., 1990. Permeation enhancers compatible with transdermal drug delivery systems. I. Selection and formulation considerations. Pharm. Tech. 8, 132–140.

Phillips, C.A., Michniak, B.B., 1995. Topical application of Azone analogs to hairless mouse skin: a histopathological study. Int. J. Pharm. 125, 63–71.

Rajadhaksha, V.J., Pfister, W.R., 1996. Oxazolidinones: optimizing delivery of active ingredients in skin care products. Drug Cosmet. Ind., March, 36–107.

Sasaki, H., Kojima, M., Mori, Y., Nakamura, J., Shibasaki, J., 1991. Enhancing effect of pyrrolidone derivatives on transdermal penetration of 5-fluorouracil, triamcinolone acetonide, indomethacin and flurbiprofen. J. Pharm. Sci. 80, 533–538.

Sloan, K.B., Beall, H.D., Weimar, W.R., Villanueva, R., 1991. The effect of receptor phase composition on the permeability of hairless mouse skin in diffusion cell experiments. Int. J. Pharm. 73, 97–104.

Tanchuk, A., Yu, V., Kotenko, S.I., Voloshchuk, T.P., 1980. Synthesis of 6-hydroxy acids and 4-alkylbutyrolactones. Ukr. Khim. Zh., 46 (7), 763–767; through Chem. Abstr., 93, 238795h.

Yano, T., Shimozono, Y., Manako, T., Fukuda, K., Yahiro, S., Kanji, N., 1995. Profile of pirotiodecane (HPE-101): a new potential penetration enhancer. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers. CRC Press, Boca Raton, FL, pp. 233–244.

Yokomizo, Y., 1996. Effects of phospholipids on the percutaneous penetration of drugs through the dorsal skin of the guinea pig, in vitro. 3. The effects of phospholipids on several drugs having different polarities. J. Control. Release 42, 217–228.