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In vitro evaluation of a series of Azone analogs as dermal penetration enhancers. II. (Thio)amides

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Summary

The sorption promoting ability of nine Azone (*N*-dodecylazacycloheptan-2-one) analogs was tested against the model drug, hydrocortisone 21-acetate using a hairless mouse skin model in vitro. The synthesis of these compounds is presented. The enhancers were applied in propylene glycol, 1 h prior to the application of the steroid which was applied as a saturated suspension in the same vehicle. All but enhancers **3–5**, **8**, and **9** were applied at 0.4 M. The remaining enhancers (all solids) were applied at their respective saturation solubilities. Flux, receptor concentrations, and skin accumulation of hydrocortisone acetate were measured over 24 h and compared with controls (no enhancer) and three model enhancers: Azone (*N*-dodecylazacycloheptan-2-one), 2-pyrrolidinone, and *N*-methyl-2-pyrrolidinone. Pre-treatment of skin with the Azone analogs markedly increased penetration and skin retention of the steroid. The greatest enhancement of flux was observed for **2**, where flux increased 53.8-fold over control and 2.76-fold over Azone; receptor concentrations were 35.37-fold and skin retentions 1.6-fold higher than control. Compound **1** gave the greatest skin retention enhancement ratio (ER) (2.2 over control) of the series, while 2-pyrrolidinone produced an ER of 3.2, and Azone 1.5 compared with controls at an ER of 1.0.

Introduction

One of the principal roles of the stratum corneum, the dead outer layer of the skin, is to act as a barrier to the inward transport of topically contacting substances, as well as the outward transport of water. One of the ways of overcoming this barrier property is to apply agents known as penetration enhancers (sorption promoters, accelerants). Examples include a wide range of compounds: alkanols (Hori et al., 1992; Kim et al., 1992), Azone (*N*-dodecylazacycloheptan-2-one) (Stoughton, 1983; Vaidyanathan et al., 1987), Azone analogs (Okamoto, et al., 1988, 1991), alkyl *N*,*N*-dialkyl-substituted aminoacetates (Wong et al., 1989), and terpenes (Williams and Barry, 1991). Several attempts have been made to relate structure to the activity of dermal enhancers since this would facilitate selection of

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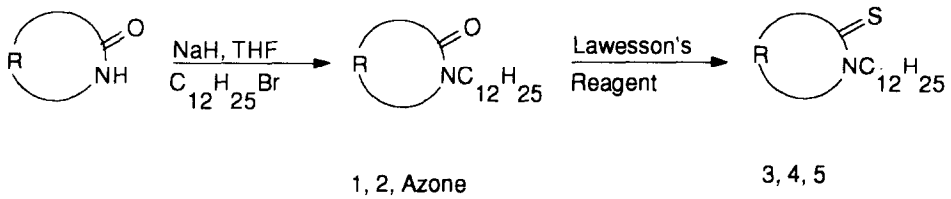


Scheme 1.

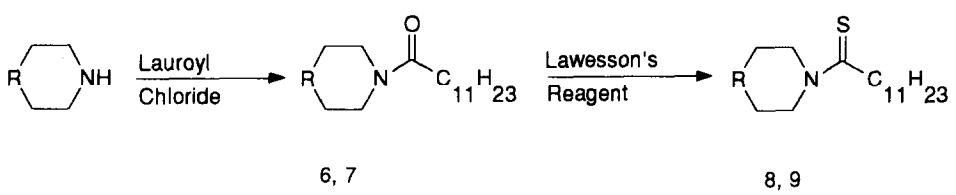
chemicals to be screened as potential accelerants (Okamoto et al., 1988; Hori et al., 1990). This study involves production of a series of amide and thioamide analogs of Azone. While many of the amides and corresponding thioamides synthesized for the current investigation have been patented as penetration enhancers **(1–6)** (Minaskanian and Peck, 1990), no data on their relative efficacies has been disclosed. As part of our continuing effort to develop structure-activity relationships for Azone analogs these compounds were tested in vitro for their ability to enhance the absorption of the model drug hydrocortisone 21-acetate using hairless mouse skin and a diffusion cell technique.

The authors have previously presented data on twelve Azone analog enhancers and this is a continuation of the work (Michniak et al., 1993b). The same parameters were used with respect to model drug, vehicle, and diffusion cell technique, allowing comparisons in enhancer activities to be made. The structures of the Azone analogs are presented below in Schemes 1 and 2.

Materials

All chemicals were purchased from Aldrich Chemical Co. in the highest available purity, except hydrocortisone 21-acetate, hydrocortisone, polyoxyethylene 20-cetyl ether and propylene glycol which were obtained from Sigma Chemical Co. Baxter Diagnostics, Inc. supplied reagent grade solvents, except for methanol and acetonitrile which were HPLC grade. All were used as received except tetrahydrofuran which was distilled over metallic sodium immediately prior to use.

Male hairless mice strain SKHl (hr/hr), 8 weeks old, were obtained from Charles River Laboratories, Inc., Wilmington, MA.



Compounds	R
1, 3	$-(CH_2)_3-$
2, 4	$-(CH_2)_4-$
Azone, 5	$-(CH_2)_5-$
6, 8	-O-
7, 9	$-(CH_2)_2-$

Scheme 2.

TABLE 1

Effect of enhancers on flux, 24 h receptor concentrations, and steroid skin contents of hydrocortisone 21-acetate

Enhancer in P.G. [a,e]	m.p. (°C)	L [b] (h)	Flux (μM cm^2 h^{-1})	E.R.$_{flux}$ [f]	Q_{24} [c] (μM)	E.R.$_{Q_{24}}$ [f]	S.C. (HCA) [d] (μg g^{-1})	S.C. (HC) [d] (μg g^{-1})	E.R.$_{S.C.}$ (HCA and HC)
None ($n = 8$)	–	1.16 ± 0.32	0.045 ± 0.016	1.00	0.751 ± 0.250	1.00	285.2 ± 21.6	ND	1.0
Azone ($n = 5$)	–	0.73 ± 0.09	0.878 ± 0.251	19.51	28.760 ± 4.624	38.30	410.6 ± 34.4	9.9 ± 2.5	1.5
2-Pyr ($n = 5$)	–	1.27 ± 0.05	0.207 ± 0.072	4.60	6.541 ± 1.629	8.71	900.1 ± 22.9	4.3 ± 0.9	3.2
Me-Pyr ($n = 5$)	–	1.00 ± 0.14	0.210 ± 0.091	4.67	3.329 ± 1.446	4.43	442.8 ± 18.4	9.7 ± 0.5	1.6
1 ($n = 5$)	–	0.84 ± 0.12	1.712 ± 0.151	38.04	25.371 ± 2.179	33.78	624.5 ± 16.9	9.5 ± 1.2	2.2
2 ($n = 5$)	–	0.73 ± 0.34	2.421 ± 0.194	53.80	26.564 ± 3.199	35.37	431.0 ± 20.4	12.1 ± 2.4	1.6
3 ($n = 5$)	42 –42.5	0.51 ± 0.14	0.617 ± 0.188	13.71	8.640 ± 1.399	11.50	598.7 ± 19.9	6.7 ± 1.1	2.1
4 ($n = 5$)	37.5–39	0.39 ± 0.15	0.385 ± 0.195	8.56	6.529 ± 1.986	8.69	571.8 ± 20.7	2.0 ± 0.9	2.0
5 ($n = 5$)	33.5–34	0.59 ± 0.05	0.195 ± 0.055	4.33	2.259 ± 0.564	3.00	541.3 ± 15.6	ND	1.9
6 ($n = 5$)	–	0.54 ± 0.08	1.180 ± 0.129	26.22	22.039 ± 2.066	29.35	274.5 ± 17.5	6.9 ± 1.1	1.0
7 ($n = 5$)	–	0.80 ± 0.11	1.531 ± 0.206	34.02	14.307 ± 1.708	19.05	412.3 ± 20.6	9.9 ± 2.2	1.5
8 ($n = 5$)	54 –55	0.81 ± 0.12	0.094 ± 0.015	2.09	4.391 ± 0.099	5.85	245.2 ± 21.9	ND	0.9
9 ($n = 5$)	42.5–43	0.68 ± 0.24	0.340 ± 0.196	7.56	5.091 ± 1.151	6.78	272.8 ± 10.2	ND	1.0

[a] P.G., propylene glycol; 2-Pyr, (2-pyrrolidinone); Me-Pyr, (*N*-methyl-2-pyrrolidinone). [b] L, lag time. [c] Q_{24}, receptor concentration after 24 h. [d] S.C., skin content of hydrocortisone 21-acetate (HCA) and hydrocortisone (HC) (metabolite); ND, not detected. [e] Saturation solubilities (M) at 32 ± 0.5°C of **3** = 0.179; **4** = 0.187; **5** = 0.163; **8** = 0.069; **9** = 0.090. [f] E.R., enhancement ratio calculated as permeation parameter after enhancer treatment divided by the corresponding parameter from control (control = 1.00).

Methods

Synthesis

The amides (**1**, **2**, Azone (*N*-dodecylazacycloheptan-2-one)) were prepared by treatment of the lactam (0.1 mol) with NaH (0.1 mol) for 2 h followed by treatment with lauryl bromide (0.1 mol) and heating under reflux for 12 h to effect *N*-alkylation. Excess NaH was neutralized with ethanol. The amides (**6**, **7**) were prepared by treatment of the amine (0.2 mol) with lauroyl chloride (0.1 mol) followed by heating under reflux for 12 h. Workup involved filtration of the precipitated NaBr or amine salt, evaporation of solvent in vacuo and elution of the product by medium pressure liquid chromatography (MPLC) with a 1 part hexane:1 part ethyl acetate mobile phase.

The thioamides were prepared by treatment of the amide (**3–5**, **8**, **9**) with Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3-dithio-2,4-diphosphetane 2,4-disulfide) in toluene at room temperature (Pederson et al., 1979). The reaction was followed by TLC until completion. Workup included evaporation of toluene in vacuo followed by elution of the product by MPLC with a 1 part hexane:1 part ethyl acetate mobile phase.

Synthetic characterization

The melting points were determined on an Electrothermal apparatus and are uncorrected. Infrared spectra were recorded on a Beckman Acculab 4 spectrophotometer either neat or in dichloromethane solution and the ^{1}H-NMR spectra were obtained on a Bruker AM 300 NMR spectrometer. Spectroscopic data agreed with assigned structures in all cases. Elemental analyses were conducted by Atlantic Microlabs, Atlanta, GA and were within $\pm 0.4\%$ of theoretical for all compounds. TLC was performed using Merck pre-coated silica gel plates type 60 and visualized using UV light or iodine vapor. All compounds were purified by MPLC with 32–63 μm 'flash' silica gel obtained from Selecto, Inc., Kennesaw, GA.

Permeability experiments

The flux and skin retention of hydrocortisone 21-acetate in hairless mouse skin was measured using a previously described in vitro technique (Michniak et al., 1993a,b). Unoccluded modified Franz diffusion cells containing full-thickness dorsal mouse skins (3.14 cm^2 diffusional area) were maintained at 37 ± 0.5°C. The receptor phase (12 ml) consisted of isotonic phosphate buffer (pH 7.2) with 0.1% v/v of 36% aqueous formaldehyde as preservative (Sloan et al., 1991) and 0.5% w/v polyoxyethylene 20-cetyl ether as solubilizer (Chien, 1982), and was stirred continuously at 600 rpm.

Following a hydration period of 1.5 h, the donor side of each skin was covered with 5 μl enhancer solution (0.4 M enhancer in propylene glycol). Solid enhancers (**3–5**, **8** and **9**) were applied at their respective saturation solubilites (Table 1). This ensured maximum flux of the compound into the skin. The enhancer solution was left on the skin for 1 h and was not washed off prior to drug application.

At this time, 0.03 M hydrocortisone 21-acetate suspension in 500 μl propylene glycol was placed on each skin. Samples (100 μl) were withdrawn from the receptor phase over 24 h, the withdrawn volume being immediately replaced by 100 μl of warm diffusion buffer. Analysis of each subsequent sample was corrected for any previous samples removed for analysis. Each experiment was repeated five times.

After 24 h, the skins were removed, washed and homogenized in a fixed volume of methanol, as described previously (Michniak et al., 1993b). The average skin weight was 0.1105 ± 0.0862 g ($n = 35$). Samples were filtered, centrifuged if required and frozen at -80°C prior to analysis by HPLC. Recovery experiments were also performed and yielded $> 95\%$ recovery of the original donor concentration of hydrocortisone acetate. HPLC analyses were performed as described previously (Michniak et al., 1993b).

Data analysis

For each skin, the cumulative corrected amounts of steroid permeating (μM) were plotted against time (h). The x-intercept of the extrapolated linear region gave the lag time. The slope of this linear portion of the graph gave values of maximum flux at steady state ((μM cm^2

h^{-1}). The effectiveness of the enhancer was determined by comparing flux, 24 h receptor concentrations (μM) and steroid skin retention ($\mu g\ g^{-1}$) for enhancer treated and untreated skins, using the enhancement ratio:

$$ER = \frac{\text{skin parameter from enhancer treated skin}}{\text{skin parameter from control}}$$

Data treatment consisted of analysis of variance (ANOVA) followed by a least significant difference test (LSD) if the ANOVA indicated that a difference existed (Daniel, 1987; Bolton, 1990). The level of significance (α) was selected to be 0.05. All parameters in Table 1 are expressed as means ± S.D.

Results and Discussion

All novel enhancers tested showed some degree of activity. Table 1 summarizes the data on flux, 24 h receptor concentrations, and whole skin steroid content after 24 h. A typical permeation profile of steroid concentration (μM) against time for several enhancers and control is presented in Fig. 1. With the more active enhancers steady state was not maintained in the final sampling periods, as depicted by the plateauing of the

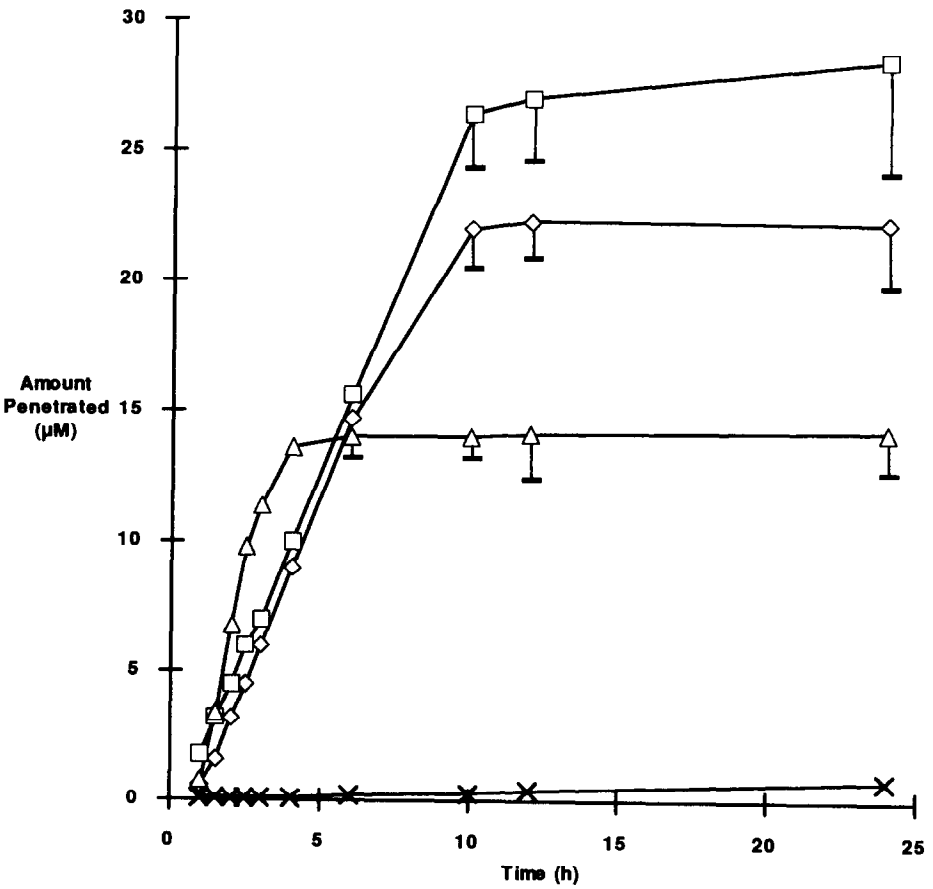


Fig. 1. Cumulative amount (μM) of hydrocortisone acetate penetrated through hairless mouse skin over 24 h. Pretreatment with 0.4 M enhancer: (□) Azone, (◇) **6**, (△) **7** and (×) controls.

graphs in Fig. 1. This may have been due to a number of factors including depletion of the donor, evaporation of the vehicle, some unknown enhancer effect on the skin or high receptor concentrations. For this reason Table 1 presents steady state flux values in addition to the 24 h receptor concentrations.

Compound **2** showed the highest enhancement ratio (ER) for flux of 53.80 compared with 19.51 for Azone. Skin steroid contents and 24 h receptor concentrations were similar for both compounds. The highest skin retentions were shown by 2-pyrrolidinone (ER 3.2), **1** (ER 2.2), **3** (ER 2.1) and **4** (ER 2.0). Both **3** and **4** were solids, and were applied at saturation solubility ([**3**] = 0.179 M; [**4**] = 0.187 M). The flux of Azone, 2-pyrrolidinone, *N*-methyl-2-pyrrolidinone, **1–7** and **9** were all significantly higher compared with control ($p < 0.01$). Receptor concentrations at 24 h were significantly higher for all compounds tested ($p < 0.05$) as were skin steroid contents for 2-pyrrolidinone, *N*-methyl-2-pyrrolidinone, **1–5** and **7** ($p < 0.01$). Even though the skin retentions were high, fluxes for 2-pyrrolidinone, **3**, and **4** were much lower, a trend also reflected by 24 h receptor concentrations (Q_{24}). This difference may be important since such enhancers may be useful for local topical, rather than for systemic (transdermal) delivery. Further studies will be made to evaluate the extent of stratum corneum fluidization and therefore, possible mechanism of action of these 'drug skin-retaining' compounds (Knutson et al., 1990; Barry, 1991; Bouwstra et al., 1992). Many of the enhancers used in products today produce high fluxes, which also increase skin retention, but the concept of increasing skin retention but maintaining lower systemic delivery of a drug is a fairly novel concept. Azone is a typical example of a transdermal enhancer, with ER flux values of 19.51, Q_{24} ER of 38.30 but skin steroid content only increased by 1.5-fold. Other such enhancers include propylene glycol which acts primarily as a solvent for the drug, and its permeation can be enhanced by Azone (Hoelgaard and Møllgaard, 1985). The mechanism of increased skin retention with some of our compounds, may be more complex and may involve the formation of a reservoir of the steroid in some manner, i.e., it may be model drug specific (Okamoto et al., 1991). Steroids have been known to form epidermal reservoirs due to the physiochemical nature of drug solubility and diffusion within the stratum corneum under both occluded and non-occluded conditions (Woodford et al., 1977a,b; Barry, 1983). Other concentrations of enhancers need to be tested as well, to examine whether the retention is concentration related (Lambert et al., 1989).

Another aim of this study was to relate the chemical structural changes in the enhancers to activity. The size of the ring structure did not affect the extent of skin retention. For example, high skin retentions were obtained with hexahydro-2-oxo-1*H*-azepine-1-acetic acid tetradecyl ester (ER 7.6) and *N*-decyldihydro-1,4-oxazepine-5,7-dione (ER 5.2), seven-membered ring compounds which were examined in a previous study (Michniak et al., 1993b). In the present study, however, seven-membered ring compounds such as Azone (ER 1.5) and **7** (ER 1.5) show lower skin retention. In addition, there was no correlation between five- and six-membered ring enhancers and the ER for skin retention. This conclusion is supported by previous studies of other Azone derivatives (Okamoto et al., 1988).

Several Azone analogs containing thione groups have been patented (Minaskanian and Peck, 1988). We synthesized compounds **3–5** as corresponding sulfur-containing enhancers to Azone, **1** and **2**. These possessed five- (**3**), six- (**4**) or seven-membered (Azone) rings with a thione moiety. All three thione enhancers showed less activity with the exception of skin retention. Compound **3** had a similar skin retention to **1** (ER 2.1 and 2.2, respectively), and **4** (ER 2.0) had a better skin retention than **2** (ER 1.6). Introduction of a thione group on the alkyl side chain produced a dramatic lowering in flux. Compound **9** had an ER for flux of 7.56, while **7** had an ER of 34.02; **8** had an ER_{flux} of 2.09, and **6** had and ER_{flux} of 26.22. It should be noted that both **8** and **9** were solids applied at saturation solubility in propylene glycol, hence in this vehicle, maximum flux of enhancer was occurring. Higuchi (1978) suggested that solute penetration from its saturated solution is maximal and independent of

the vehicle used. It is even possible that all Azone analog activity may be enhanced by propylene glycol which is known to act as a 'carrier solvent' (Hoelgaard and Møllgaard, 1985).

There was some correlation between increasing maximum solubility of enhancers in propylene glycol and activity, especially with **8** and **9**, and **5** and **3**. Otherwise, there was no relationship between maximum solubility and activity of the solid enhancers. Wong et al. (1989) found that inclusion of a cyclic structure in an enhancer (alkyl *N*,*N*-disubstituted aminoacetates), can decrease effectiveness if compared with the equivalent long chain compound. We intend to investigate whether the same phenomenon will be observed with our Azone analog enhancers. Also, we chose to keep the side chain length for this enhancer series constant (C12) since this seems to be an optimal chain length for several enhancers such as fatty acids and fatty alcohols (Cooper, 1984; Aungst, 1989), and some Azone derivatives (Okamoto et al., 1988).

In summary, the highest skin steroid content was observed for 2-pyrrolidinone, ER 3.2 compared with control and ER 2.1 compared with Azone. The structure-activity relationship of the enhancers based on flux data was **1** > **3**, **2** > **4**, Azone > **5**; **2** > **1** > Azone; **7** > Azone; and **7** > **6**, **7** > **9**. No correlation was found between flux values and skin steroid contents. From the results obtained in this and a previous study (Michniak et al., 1993b), it is concluded that some newly designed penetration enhancers related to Azone, have the potential to enhance the percutaneous penetration of hydrocortisone 21-acetate. Some compounds were superior to Azone in enhancing skin retention of this steroid. Caution is advisable, however, in extrapolating these enhancement ratios from hairless mouse to human skin.

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