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Percutaneous penetration enhancement activity of aromatic *S*,*S*-dimethyliminosulfuranes

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Abstract

The activity of three series of iminosulfuranes (classes I–III) as potential transdermal penetration enhancers was investigated. These dimethyl sulfoxide (DMSO) related compounds were synthesized from activated DMSO by trifluoroacetic anhydride. Structure confirmation was accomplished by ^{1}H NMR, and ^{13}C NMR spectroscopy and elemental analysis prior to in vitro testing. Hydrocortisone (HC) was used as a model drug, and the effect of the iminosulfuranes on the penetration of HC through hairless mouse skin was evaluated. All enhancers tested were applied to the skin as saturated suspensions in propylene glycol to ensure their maximum thermodynamic activity. Three compounds, *S*,*S*-dimethyl-*N*-(4-bromobenzoyl)iminosulfurane (**9**), *S*,*S*-dimethyl-*N*-(5-nitro-2-pyridyl)iminosulfurane (**13**), and *S*,*S*-dimethyl-*N*-(4-phenylazaphenyl)iminosulfurane (**16**) showed statistically significant activity quantitated by amounts of model drug permeated through the skin in 24 h (Q_{24}), and flux values, compared to control (propylene glycol without enhancer). Highest Q_{24} and flux values were obtained for **9**: 996.2 ± 192.5 μg/cm^2 and 42.9 ± 7.5 μg/cm^2 per h, respectively. All arylsulfonyl substituted compounds showed lower or similar enhancement activity when compared to control. *S*,*S*-dimethyl-*N*-(benzenesulfonyl)iminosulfurane (**1**), *S*,*S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl)iminosulfurane (**7**), and *S*,*S*-dimethyl-*N*-(4-chlorobenzenesulfonyl)iminosulfurane (**8**) decreased the permeation of HC significantly ($P < 0.05$). It is possible that these agents work as retardants under these experimental conditions. None of the enhancers tested showed significant skin model drug retention, suggesting that these compounds could be useful for increasing systemic rather than local drug delivery.

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

1. Introduction

The skin has attracted much attention as an alternative route for administering systemically active drugs, but its potential use is often hindered by poor tissue permeability, predominantly at-

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tributed to the outermost layer of the skin, the stratum corneum (SC). This layer provides a protective barrier that prevents the loss of physiologically essential substances and limits the diffusion of potentially toxic chemicals from the external environment into the body. Different methods have been implemented to overcome the barrier property of the SC (Sloan et al., 1984; Mezel, 1985; Morimoto et al., 1992; Chang and Banga, 1998). One of the more widely used techniques utilizes chemical penetration enhancers (Smith and Maibach, 1995). Ideally, an enhancer should be chemically and pharmacologically inert, nontoxic, non-irritant and non-allergenic, have a rapid and reversible onset of action, be potent in low concentrations, compatible with the formulation ingredients, and cosmetically acceptable (Chattaraj and Walker, 1995). Of all the compounds published to date few meet these criteria and the search for more active enhancers continues (Rajadhyaksha and Pfister, 1996). Some of the penetration enhancers reported in the literature include alcohols, amides, surfactants, terpenes, alkanones, organic acids, and sulfoxides. Enhancers may promote drug permeation across the skin by a variety of mechanisms. Williams and Barry (1991) hypothesized that enhancers may act by one or a combination of the following: imparting disorganization to the highly ordered intercellular lipid of the SC, thus increasing the paracellular diffusivity, interaction with the intracellular proteins to enhance the transcellular permeation through the corneocytes, and/or increasing the partitioning of the drug in the SC.

Numerous published articles describe the possible mechanisms and uses of various enhancers. However, in spite of major interest in this field, few penetration enhancers have been made commercially available. One of the earliest and widely investigated penetration enhancers is dimethyl sulfoxide (DMSO) (Williams and Barry, 1992; Chattaraj and Walker, 1995). It is a dipolar aprotic solvent that is miscible in both aqueous and polar organic solvents; therefore, it may be easily incorporated into pharmaceutical formulations. DMSO has been tested as a penetration enhancer using a wide variety of drugs including steroids (Maibach and Feldman, 1967), narcotics (Collom and Wink, 1968), and salicylates (Shen et al., 1976). Although DMSO has been found to be a potent enhancer, high concentrations, usually greater than 60%, are required to produce an enhancement effect. Unfortunately, at this high concentration, DMSO has been shown to produce irreversible skin damage, and to cause side effects such as erythema and wheals (Kligman, 1965). This makes it undesirable for further development as a transdermal penetration enhancer.

Like DMSO, *N*,*N*-dimethylacetamide (DMAC) and *N*,*N*-dimethylformamide (DMF) are also aprotic solvents. Although less effective than DMSO, both DMAC and DMF have been shown to enhance the permeation of griseofulvin (Munro and Stoughton, 1965), lidocaine (Akerman et al., 1979), caffeine (Southwell and Barry, 1983), and hydrocortisone (HC) (Barry and Woodford, 1982) in vitro. In a study of the effect of homologs of DMSO, decyl methyl sulfoxide (DCMS) has been shown to be an effective penetration enhancer. DCMS has been reported to enhance the delivery of both hydrophilic and lipophilic drugs, including HC (Sekura and Scala, 1972; Barry and Woodford, 1982), and progesterone (Sekura and Scala, 1972; Barry and Woodford, 1982), methotrexate (Cooper, 1982), 5-fluorouracil (Touitou and Abed, 1985), and naloxone (Aungst et al., 1986). The major advantage of DCMS over DMSO is that DCMS can be used at concentrations as low as 0.1% (Touitou and Abed, 1985).

Studies involving the structure activity relationships of several groups of enhancers showed that the presence of a cyclic structure in the molecule plays a major role in determining the activity of the enhancers. For example, cyclic amides such as Azone® (1-dodecyl-azacycloheptan-2-one), pyrrolidone derivatives, and cyclic ureas were synthesized and their enhancing activites were investigated. Recently, a group of compounds possessing a cyclic sulfoxide group as the polar and a long alkyl group as the non-polar group were prepared and their enhancing effects were studied (Aoyagi et al., 1992). These studies showed that by increasing the substituted alkyl chain length from C_8 to C_{16}, the lipophilicity increased and hence the activity of the enhancers.

In this study, novel transdermal penetration enhancers were synthesized by introducing modifications to the DMSO molecule where the oxygen atom of the DMSO was replaced by a nitrogen atom, which in turn was substituted with an arylsulfonyl, aroyl or aryl group. Like DMSO, iminosulfuranes are polar aprotic compounds. However, the polarity of iminosulfuranes can additionally be modulated by placing diverse substituents at the aromatic core of the molecule. The lipophilicity of iminosulfuranes is greater than that of DMSO, suggesting an increased permeability of these agents with the SC. Additionally, since DMSO is known to cause skin irritant at high concentrations, these compounds were designed to be used at lower concentrations (below 10%), which would be predicted to result in less skin irritation. These cyclic derivatives were tested for their enhancement activities using hairless mouse skin and HC as a model drug in vitro.

2. Materials and methods

2.1. Chemicals

DMSO, propylene glycol (PG), and the model drug, HC, were purchased from Sigma (St. Louis, MO). Acetonitrile, methanol, and water were of HPLC grade and purchased from EM Science (New Briggs, NJ) Male hairless mice, strain SKH1, 8 weeks old, were supplied by Charles River Laboratories (Wilmington, MA). All synthetic reagents were obtained from Aldrich (Milwaukee, WI). Compounds **1–19** were synthesized with purity of 99% as assessed by elemental analysis, ^{1}H NMR, and ^{13}C NMR.

2.2. Synthesis of Iminosulfuranes ***1–19***

Iminosulfuranes are conveniently synthesized by activation of DMSO by the reaction with an electrophilic reagent followed by treatment of the resultant active complex (Varkey et al., 1974; Sharma et al., 1975) with an amino derivative such as arylsulfonamide, arylamide or aniline (Scheme 1). In the authors' hands the best results were obtained by using trifluoroacetic anhydride as the activating agent, and the general method is given below. Compounds **1**, **4**, **6**, that had been obtained previously by using sulfur trioxide for activation of DMSO (Varkey et al., 1974), were also synthesized in this work by using this general procedure.

All reactions were conducted in a glassware that was dried in an oven at 140°C, assembled hot, and cooled in a stream of high purity nitrogen. A positive pressure of nitrogen was maintained during the reactions. DMSO was dried with molecular sieves 3A and dichloromethane was distilled over phosphorus pentoxide immediately before use.

2.2.1. General procedure

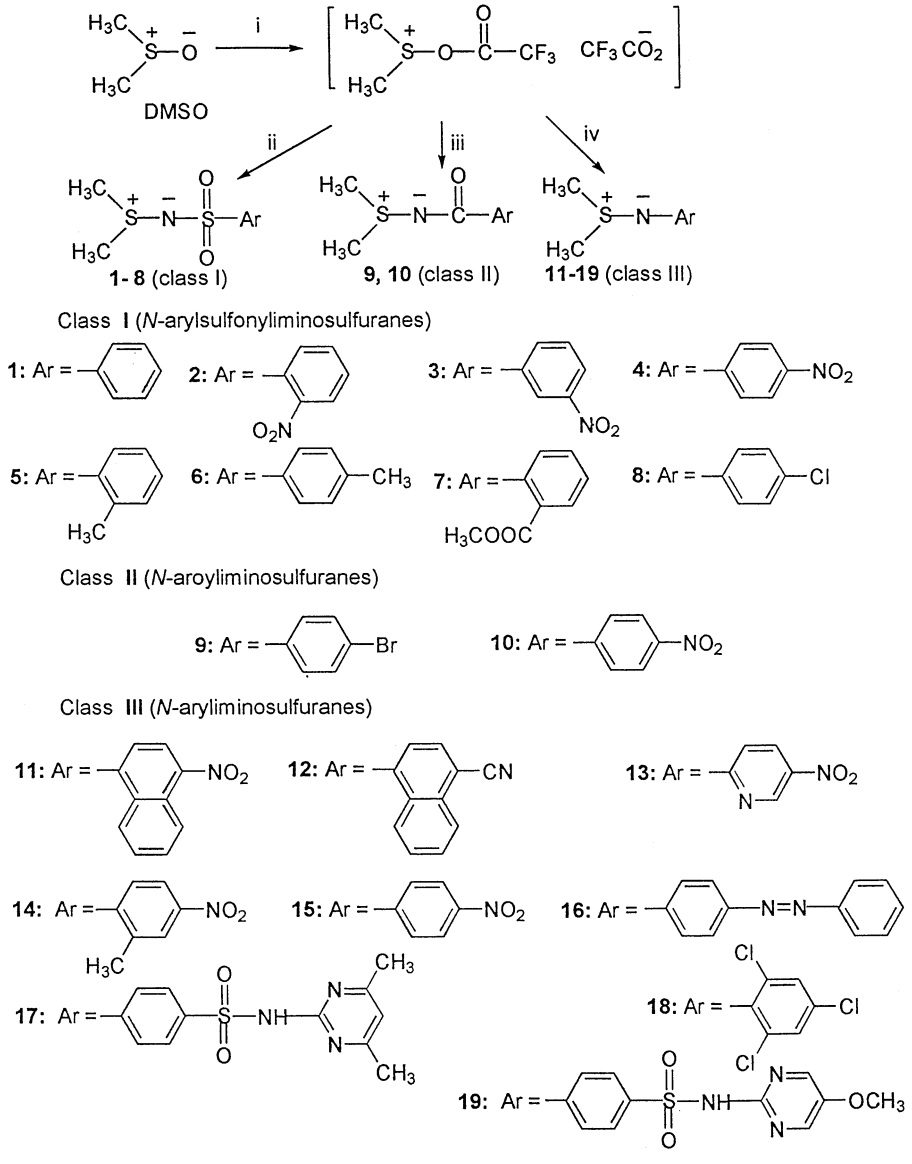
A solution of DMSO (1.3 ml, 18 mmol) in dichloromethane (5 ml) was stirred at −60°C and treated dropwise with trifluoroacetic anhydride (1.4 ml, 10 mmol) at such a rate that the temperature did not rise above −50°C (*caution-exothermic*). The resultant mixture containing a white precipitate was treated dropwise with a solution of a sulfonamide, a benzamide or an aniline (10 mmol) in dichloromethane/DMSO (1:1, 4 ml) maintaining the temperature below −50°C. Upon completion of the addition (about 10 min) the white precipitate disappeared and a clear solution was then stirred at −50°C for an additional 1 h. Quenching with aqueous sodium hydroxide (10%, 5 ml) was followed by extraction with dichloromethane (2×20 ml). The extract was washed with water, dried ($MgSO_4$), concentrated, and the residue was crystallized from a solvent shown below for each individual product.

*2.2.1.1. S,S-Dimethyl-N-benzenesulfonyliminosulfurane (**1**).* This compound was obtained from benzenesulfonamide: yield 85%, mp 130–132°C [lit. mp 129–131°C, (Sharma et al., 1975)].

*2.2.1.2. S,S-Dimethyl-N-(2-nitrobenzenesulfonyl)-iminosulfurane (**2**).* This compound was obtained from 2-nitrobenzenesulfonamide: yield 75%; mp 164–166°C (from methanol/diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (s, 6 H), 7.61 (m, 3 H), 8.18 (d, $J = 8.4$ Hz, 1 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 36.4, 123.6, 130.4,

131.8, 132.2, 137.2, 147.6. *Analysis*. $C_8H_{10}N_2O_4S_2$. 1/2 H_2O requires C, 35.42; H, 4.08 and N, 10.32. Found: C, 35.54; H, 3.77 and N, 10.31.

*2.2.1.3. S,S-Dimethyl-N-(3-nitrobenzenesulfonyl)-iminosulfurane (**3**).* This compound was obtained from 3-nitrobenzenesulfonamide: yield 85%; mp 136–138°C (from diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.78 (s, 6 H), 7.68 (t, $J = 8.0$ Hz, 1 H), 8.24 (d, $J = 8.0$ Hz, 1 H), 8.35 (t, 1 H), 8.72 (s, 1 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 36.1, 121.4, 126.0, 130.2, 132.0, 146.1, 148.1. *Analysis*. $C_8H_{10}N_2O_4S_2$. requires C, 36.63; H, 3.84 and N, 10.67. Found: C, 36.66; H, 3.74 and N, 10.57.



Scheme 1.

*2.2.1.4. S,S-Dimethyl-N-(4-nitrobenzenesulfonyl)-iminosulfurane (**4**).* This compound was ob-tained from 4-nitrobenzenesulfonamide: yield 87%; mp 184–186°C [lit. mp 184–185°C, (Sharma et al., 1975)].

*2.2.1.5. S,S-Dimethyl-N-(o-tolylsulfonyl)iminosulfurane (**5**).* This compound was obtained from *o*-tolylsulfonamide: yield 53%; mp 153–155°C (from dichloromethane/diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.68 (s, 6 H), 6.69 (s, 3 H), 7.25 (m, 2 H), 7.37 (t, $J = 8.0$ Hz, 1 H), 7.98 (d, $J = 8.0$ Hz, 1 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 20.5, 35.9, 125.6, 127.8, 131.5, 132.1, 137.0, 141.9. *Analysis*. $C_9H_{13}NO_2S_2$. requires C, 46.73; H, 3.37 and N, 6.06. Found: C, 46.40; H, 5.76 and N, 6.05.

*2.2.1.6. S,S-Dimethyl-N-(p-tolylsulfonyl)iminosulfurane (**6**).* This compound was obtained from *p*-tolylsulfonamide: yield 76%; mp 158–161°C [lit. mp 156–158°C, (Varkey et al., 1974)].

*2.2.1.7. S,S-Dimethyl-N-(2-methoxycarbonylbenzenesulfonyl)iminosulfurane (**7**).* This compound was obtained from 2-(methoxycarbonyl)benzenesulfonamide: yield 87%; mp 111–113°C (from dichloromethane/diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71 (s, 6 H), 3.93 (s, 3 H), 7.50 (m, 3 H), 8.02 (d, $J = 5.2$ Hz, 1H), 8.72 (s, 1 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 36.0, 53.1, 128.2, 128.4, 130.4, 131.1, 131.4, 142.0, 169.0. *Analysis*. $C_{10}H_{13}NO_4S_2$. requires C, 43.62; H, 4.80 and N, 5.08. Found: C, 43.53; H, 4.95 and N, 5.07.

*2.2.1.8. S,S-Dimethyl-N-(4-chlorobenzenesulfonyl)iminosulfurane (**8**).* This compound was obtained from 4-chlorobenzenesulfonamide: yield 80%; mp 114–116°C [lit. mp 110–113°C, (Varkey et al., 1974)].

*2.2.1.9. S,S-Dimethyl-N-(4-bromobenzoyl)iminosulfurane (**9**).* This compound was obtained from 4-bromobenzamide: yield 78%; mp 105–107°C (from methanol/pentane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.78 (s, 6 H), 7.47 (d, $J = 8.4$ Hz, 2 H), 7.95 (d, $J = 8.4$ Hz, 2 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 32.0, 125.4, 130.2, 131.0, 135.3, 176.0. *Analysis*. C_9H_{10} BrNOS**.** 1/2 H_2O requires C, 40.16; H, 4.10 and N, 5.20. Found: C, 40.32; H, 3.66 and N, 5.17.

*2.2.1.10. S,S-Dimethyl-N-(4-nitrobenzoyl)iminosulfurane (**10**).* This compound was obtained from 4-nitrobenzamide: yield 80%; mp 224–226°C [lit. mp 217–218°C, (Sharma et al., 1975)].

*2.2.1.11. S,S-Dimethyl-N-(4-nitro-1-naphthyl)-iminosulfurane (**11**).* This compound was obtained from 4-nitro-1-naphthylamine: yield 93%; mp 182–183°C (from dichloromethane/diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.82 (s, 6 H), 6.56 (d, $J = 8.8$ Hz, 1 H), 7.44 (t, $J = 8.0$ Hz, 1 H), 7.65 (t, $J = 8.0$ Hz, 1 H), 8.45 (d, $J = 8.8$ Hz, 1 H), 8.64 (d, $J = 8.0$ Hz, 1 H), 9.02 (d, $J = 8.0$ Hz, 1 H). *Analysis*. $C_{12}H_{12}$ N_3O_2S requires C, 58.04 and H, 4.87. Found: C, 57.96 and H, 4.87.

*2.2.1.12. S,S-Dimethyl-N-(4-cyano-1-naphthyl)-iminosulfurane (**12**).* This compound was obtained from 4-amino-1-naphthalenecarbonitrile: yield 83%; mp 162–163°C (from dichloromethane/diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.74 (s, 6 H), 6.53 (d, $J = 8.8$ Hz, 1 H), 7.52 (m, 3 H), 8.0 (m, 1 H), 8.50 (m, 1 H). *Analysis*. $C_{13}H_{12}$ N_2S requires C, 68.39 and H, 5.30. Found: C, 68.42 and H, 5.35.

*2.2.1.13. S,S-Dimethyl-N-(5-nitro-2-pyridyl)iminosulfurane (**13**).* This compound was obtained from 2-amino-5-nitropyridine: yield 83%; mp 165–166°C (from dichloromethane/diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.79 (s, 6 H), 6.53 (d, $J = 9.4$ Hz, 1 H), 8.02 (dd, $J = 9.4$ and 2.7 Hz, 1 H), 8.9 (d, $J = 2.7$ Hz, 1 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 32.9, 112.9, 131.5, 134.7, 146.1, 168.8. *Analysis*. C_7H_3 N_3O_2S requires C, 42.20, H, 4.55 and N, 21.10. Found: C, 41.85, H, 4.37 and N, 20.97.

*2.2.1.14. S,S-Dimethyl-N-(2-methyl-4-nitrophenyl)iminosulfurane (**14**).* This compound was obtained from 2-methyl-4-nitroaniline: yield 90%; mp 138–139°C (from dichloromethane/diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27 (s, 3

H), 2.71 (s, 6 H), 7.10 (d, $J = 8.3$ Hz, 1 H), 7.43 (dd, $J = 8.3$ and 2.2 Hz, 1 H), 7.53 (d, $J = 2.2$ Hz, 1 H). *Analysis*. $C_9H_{12}N_2O_2S$ requires C, 50.92 and H, 5.70 and. Found: C, 51.03 and H, 5.65.

*2.2.1.15. S,S-Dimethyl-N-(4-nitrophenyl)imino-sulfurane (**15**).* This compound was obtained from 4-nitroaniline: yield 56%; mp 162–164°C [lit. mp 166–167°C, (Sharma et al., 1975)].

*2.2.1.16. S,S-Dimethyl-N-(4-phenylazophenyl)-iminosulfurane (**16**).* This compound was obtained from 4-phenylazoaniline: yield 62%; mp 144–147°C (from dichloromethane/diethyl ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.48 (s, 6 H), 7.30 (d, $J = 8.4$ Hz, 2 H), 7.50 (m, 3 H), 7.90 (d, $J = 8.4$ Hz, 2 H), 7.93 (d, $J = 8.4$ Hz, 2 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 40.3, 114.8, 122.3, 125.2, 129.8, 141.1, 145.6, 149.5, 152.8.

*2.2.1.17. S,S-Dimethyl-N-[4-[(4,6-dimethylpyri-midin - 2 - yl)aminosulfonyl]phenyl]imino - sulfurane (**17**).* This compound was obtained from sulfamethazine: yield 60%; mp 149–151°C (from dichloromethane/diethyl ether); ^{1}H NMR (400 MHz, DMSO) δ 2.25 (s, 3 H), 2.68 (s, 3 H), 3.29 (s, 6 H), 6.62 (d, $J = 8.8$ Hz, 2 H), 6.74 (s, 1 H), 7.56 (d, $J = 8.8$ Hz, 1 H), 10.99 (bs, exchangeable with D_2O, 1 H); ^{13}C NMR (300 MHz, DMSO) δ 23.1, 34.3, 95.0, 113.7, 115.2, 123.7, 129.8, 156.7, 167.2. *Analysis*. $C_{14}H_{18}N_4O_2S_2$ requires C, 49.70; H, 5.36 and N, 16.55. Found: C, 49.30; H, 5.18 and N, 16.20.

*2.2.1.18. S,S-Dimethyl-N-(2,4,6-trichlorophenyl)-iminosulfurane (**18**).* This compound was obtained from 2,4,6-trichloroaniline: yield 83%; mp 165–166°C (from dichloromethane/diethyl ether and traces of trifluoroacetic acid); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.51 (s, 6 H), 7.37 (s, 2 H), 9.60 (bs, exchangeable with D_2O, 1 H). *Analysis*. $C_8H_8Cl_3N$. CF_3COOH requires C, 32.31; H, 2.40 and N, 3.76. Found: C, 32.59; H, 2.29 and N, 3.64.

*2.2.1.19. S,S-Dimethyl-N-[4-[(5-methoxypyri-midin - 2 - yl)aminosulfonyl]phenyl]imino - sulfurane (**19**).* This compound was obtained from 5-methoxysulfadiazine: yield 59%; mp 177–179°C (from methanol/diethyl ether and traces of trifluoroacetic acid); ^{1}H NMR (400 MHz, DMSO) δ 3.24 (s, 6 H), 3.80 (s, 3 H), 7.22 (d, $J = 8.4$ Hz, 2 H), 7.93 (d, $J = 8.4$ Hz, 2 H), 8.29 (s, 2 H), 10.10 (bs, exchangeable with D_2O, 1 H), 11.20 (bs, exchangeable with D_2O, 1 H). *Analysis*. $C_{13}H_{16}N_4O_3S_2$. CF_3COOH requires C, 39.66; H, 3.80 and N, 12.33. Found: C, 39.55; H, 4.09 and N, 12.30.

2.3. In vitro skin penetration studies

Male hairless mouse skins were mounted in Franz diffusion cells (Permegear, Riegelsville, PA, USA), with diffusional areas of 0.64 cm^2 and a receptor compartment volume of 5.1 ml. The receptor compartment was filled with isotonic phosphate buffer (pH 7.2) containing 0.1% v/v of 36% aqueous formaldehyde as preservative. Receptor solution temperature was maintained at 37 ± 0.5°C and was constantly stirred at 600 rpm. Skins were allowed to hydrate for 1 h prior to experimentation. Following this hydration period, 8 μl saturated suspension of each enhancer in PG was applied to each skin. Following a 1 h pretreatment period, 16.2 μl of a saturated suspension of HC in PG was applied per cell (HC solubility in PG at 32 ± 0.5°C was 0.03 M), without removing the enhancer (Michniak et al., 1998). All cell donors were occluded with a triple layer of Parafilm. At predetermined times, 300 μl samples were taken from the receptor compartment over 24 h and were immediately replaced by the same volume of a fresh buffer solution. The samples were kept frozen at −70°C prior to HPLC analysis. The amount of drug withdrawn was corrected in the subsequent calculations of cumulative amount penetrated. After 24 h of sampling, the skins were removed from the cells and washed briefly in methanol. The skins were then homogenized in 4ml methanol using a Kinematica GmbH tissue homogenizer and passed through C_{18} Sep Pak® cartridges (Michniak et al., 1993a). All samples were analyzed using HPLC using previously published methods (Michniak et al., 1993b).

2.4. HPLC analysis of HC

Concentrations of HC in the samples were determined using a Hewlett Packard 1100 HPLC system with diode array and a C_{18}-Microsorb column (15 cm × 4.6 mm; 5 μm) at an ambient temperature. The mobile phase was a mixture of CH_3CN: H_2O (40:60, V/V) and injection volume was 40 μl. HC was detected at 242 nm. The flow rate was 1ml/min and the retention time of HC was 3.5 min. Calibration of HC in the samples was determined using an external standard technique (Michniak et al., 1993b).

2.5. HPLC analysis and solubility of compounds *9* and *13*

The solubility in PG for compounds **9** and **13** was determined by HPLC. After equilibrating excess compound in PG (48 h, at 32 ± 0.3°C), the suspension was centrifuged. The supernatant was diluted and analyzed with an HP 1050 HPLC equipped with a 79852A quaternary pump, a 79853C variable wavelength detector, and a 795855A programmable autosampler. Detection was at 260 and 380 nm for compounds **9** and **13,** respectively. Twenty μl of each sample was injected into a Microsorb MV™ C_{18} 5 μm 100 Å column. The mobile phase used was acetonitrile:water at a ratio of 5:5 (V/V) and acetonitrile:water at a ratio of 4:6 (V/V) for compounds **9** and **13**, respectively. The same HPLC method was used as described in Section 2.4 to analyze skin permeation of both compounds **9** and **13** using CH_3CN: H_2O (4:6, V/V) as the mobile phase and 40 μl as injection volume.

2.6. Data analysis

The permeation parameters of HC were calculated by plotting the cumulative corrected amounts (μg/cm^2) of HC permeated through the skin versus time (h). Calculating the skin penetration parameters was based on the assumption that the amount of enhancer applied on the skin is small, so there will be a minimal effect on HC solubility. In addition, the stability of HC was evaluated in the presence of iminosulfuranes using PG as a solvent at 37 ± 0.5°C. HC remained stable over the 24 h experimentation period as shown by no significant change in concentration of HC measured using HPLC. The slope of the linear portion of the graph provided maximum flux values (J) at steady state (μg/cm^2 per h). The lag time was determined by extrapolating the linear portion of the curve to the X-axis. Enhancement ratio (ER) for each skin parameter (J, cumulative amount of HC permeated after 24-h (Q_{24}), and model drug skin content) were calculated by using following (Goodman and Barry, 1988):

$$\mathrm{ER} = \frac{\text{skin parameters for enhancer treated skin}}{\text{skin parameters for control}}$$

Statistical analysis of the data were done using analysis of variance (ANOVA, $\alpha = 0.05$). A least significant different test (LSD) was followed if the ANOVA indicated that a difference existed.

3. Results and discussion

The compounds tested may be divided into three classes based on their structures. Class-I compounds (**1**–**8**) are those having a benzenesulfonyl group attached to the nitrogen atom of the backbone structure [$N^{(-)}$–$S^{(+)}$–$(CH_3)_2$]. Class-II compounds (**9** and **10**) are N-aroyliminosulfuranes and class-III iminosulfuranes (**11**–**19**) are N-aryl derivatives.

The permeation parameters for all compounds are shown in Table 1. All results are expressed as means ± standard deviations. A control experiment was run without enhancer but using PG as the vehicle. In addition, the activity of DMSO was also investigated. DMSO, which was applied as 0.4 M concentration in PG (equivalent to 0.36%), showed no significant activity when compared with control ($P > 0.05$). A number of articles have reported the concentration dependent behavior of DMSO therefore experiments with different DMSO concentrations were conducted to examine if the same phenomenon would be observed under the experimental conditions (Stoughton, 1965; Maibach and Feldman, 1967; Franz et al., 1995). Concentration dependent be-

Table 1
The effect of the dimethyl (arylimino) sulfuranes on percutaneous permeation parameters of hydrocortisone through hairless mouse skin

Enhancer in PG[a] ($n^b = 5$)	T_{lag}[c] (h)	Q_{24}[d] (μg/cm^2)	ER_{Q24}[g]	J[e] (μg/cm^2 per h)	ER_J[g]	SC[f] (μg/g)	ER_{SC}[g]
None	3.08 ± 0.65	43.09 ± 5.19	1.00	2.04 ± 0.24	1.00	1059.9 ± 78.6	1.00
DMSO	2.62 ± 0.50	45.46 ± 12.07	1.06	2.79 ± 0.32	1.37	381.6 ± 96.8	0.36
1	3.68 ± 0.16	20.66 ± 0.77	0.48	1.01 ± 0.06	0.50	685.9 ± 258.6	0.65
2	3.77 ± 0.93	79.31 ± 9.01	1.84	3.93 ± 0.43	1.93	1156.0 ± 384.5	1.09
3	2.21 ± 1.24	41.08 ± 12.50	0.95	1.88 ± 0.50	0.92	1354.1 ± 193.7	1.28
4	8.76 ± 1.03	29.20 ± 10.10	0.68	1.91 ± 0.67	0.94	1160.7 ± 786.4	1.10
5	1.31 ± 0.79	56.17 ± 18.16	1.30	3.24 ± 0.25	1.59	1019.5 ± 675.4	0.96
6	4.72 ± 2.84	39.98 ± 17.23	0.93	2.99 ± 0.58	1.47	713.3 ± 112.2	0.67
7	0.73 ± 0.15	8.38 ± 2.66	0.19	0.24 ± 0.02	0.12	809.6 ± 346.2	0.76
8	2.35 ± 1.10	17.38 ± 1.42	0.40	1.01 ± 0.06	0.50	878.1 ± 232.9	0.83
9	1.50 ± 0.70	996.22 ± 192.46	23.12	42.91 ± 7.47	21.03	1583.7 ± 284.9	1.49
10	6.43 ± 0.99	33.18 ± 6.53	0.77	1.79 ± 0.32	0.88	1736.4 ± 628.8	1.64
11	4.25 ± 0.69	56.39 ± 13.92	1.28	2.74 ± 0.66	1.34	187.3 ± 46.6	0.18
12	1.97 ± 0.85	50.15 ± 25.23	1.16	2.25 ± 0.43	1.10	233.2 ± 43.2	0.22
13	1.23 ± 0.90	389.05 ± 61.18	9.03	22.34 ± 0.1	10.95	1070.9 ± 419.3	1.01
14	5.48 ± 1.22	50.29 ± 12.24	1.17	2.63 ± 0.54	1.29	427.0 ± 96.4	0.40
15	3.08 ± 2.36	63.24 ± 13.66	1.47	2.44 ± 0.35	1.20	445.2 ± 14.6	0.42
16	3.08 ± 1.36	121.52 ± 54.3	2.81	8.02 ± 0.68	3.93	176.0 ± 59.3	0.17
17	5.00 ± 0.79	35.90 ± 8.77	0.83	2.88 ± .55	1.41	1542.0 ± 618.1	1.45
18	1.47 ± 0.76	95.03 ± 8.11	2.21	4.30 ± 0.22	2.11	1140.2 ± 183.9	1.08
19	4.91 ± 0.94	30.86 ± 2.63	0.72	1.63 ± 0.11	0.80	742.0 ± 346.9	0.70

[a] PG, propylene glycol.
[b] $n = 5$ or more.
[c] T_{lag}, Lag time.
[d] Q_{24}, receptor concentration after 24 h.
[e] J, flux.
[f] SC, skin content of hydrocortisone (HC).
[g] ER, enhancement ratio calculated as parameter following enhancer treatment divided by corresponding parameter from control.

havior of DMSO was observed and the maximum activity was recorded in the range of between 40 and 60% (ER_{flux} and ER_{Q24} were 5.73 and 5.44, respectively).

Eq. (1) shows that T_{lag} is directly proportional to the square of the membrane thickness (l), and inversely related to the diffusion coefficient (D). In this study assuming that the membrane thickness does not change, calculating T_{lag} provides information on the mechanism of action of enhancers, since changes in T_{lag} reflect changes in the diffusivity of HC across the skin. The data provided in Table 1 shows that there is no relationship between the activity of these compounds and their effect of $T_{lag,}$ for example compounds **9** and **13**, which showed the highest enhancement reduced T_{lag} by only 2-fold. However, compound **7** that showed the least enhancement activity reduced T_{lag} by 4-fold. The effect of these compounds on T_{lag} might be as a result of a combined effect on the membrane fluidity and HC partitioning into the skin.

$$T_{lag} = l^2/6D. \quad (1)$$

For class-I compounds, all except **2** and **5** produced lower or similar Q_{24} and flux values compared to control. Compounds **2** and **5** showed mild enhancement activity with ER_{flux} and ER_{Q24} 1.5–2 compared to the control ($P > 0.05$). Whereas the other compounds apparently retarded the permeation of HC through hairless mouse skin (**1**, **7**, and **8**), or had no activity. It seems that the position of substitution on the phenyl ring affected enhancer activity, where the substitution on the *ortho*-position (**2** and **5**) was more effective than the substitution on the *para*-

position (**4** and **6**). For example, permeation decreased from 3.93 ± 0.43 to 1.91 ± 0.67 μg/cm^2 per h when the same functional group was substituted from *ortho* to *para* position in compounds **2** and **4**. According to Hadgraft, some agents can make the SC more impermeable or decrease the partitioning of drug into the skin by imparting order to the skin lipids. Such agents are referred to as retardants and they prevent penetrate sufficiently to elicit systemic effects (Hadgraft et al., 1996). An effective retardant is useful for preventing absorption of agents such as the mosquito repellents, pesticides, and sunscreens. When compared with other compounds in the same class, **1**, **7**, and **8** showed significantly lower flux, Q_{24}, and skin content values than those of control ($P < 0.05$). However, the retarding effects of these compounds may be specific to experimental conditions and model drug used. Therefore further investigation with different drugs in different vehicle systems will be performed. Permeation profiles for compound **1**, **7**, **8**, and control are shown in Fig. 1.

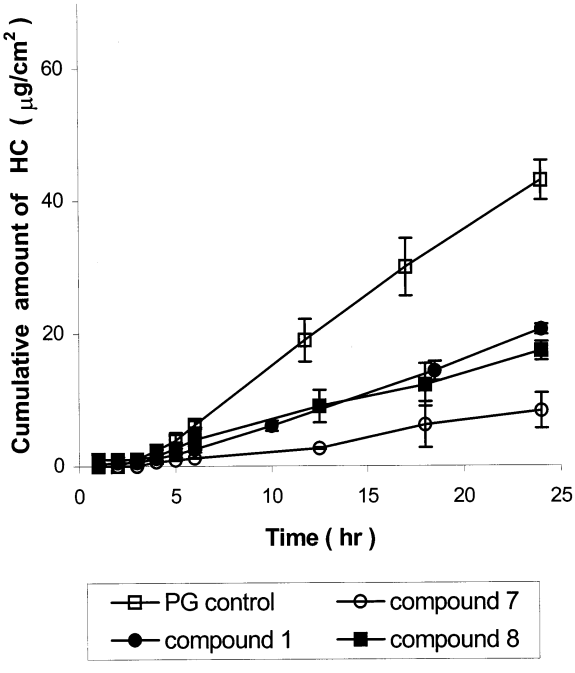


Fig. 1. Cumulative amount of hydrocortisone (HC) penetrated through male hairless mouse skin over 24 h for compound **1**, **7**, **8**, and control.

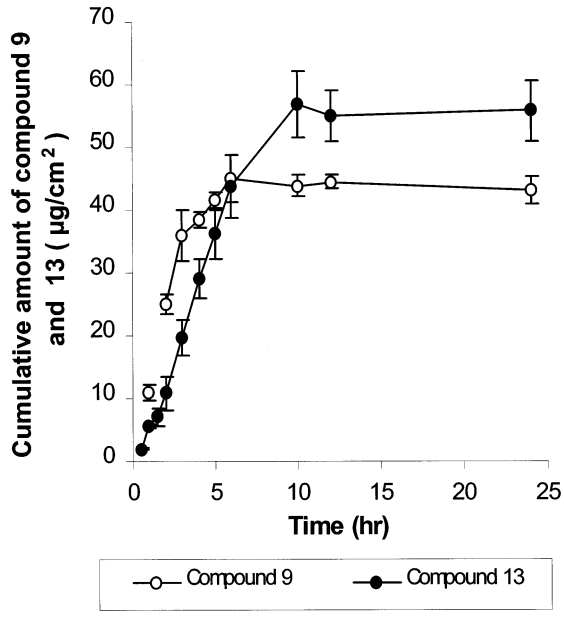


Fig. 2. In vitro release of compound **9** and **13** through male hairless mouse skin over 24 h.

For the class-II compounds, a 4-bromophenyl derivative **9**, showed statistically higher flux and Q_{24} values compared to those of the control ($P < 0.05$). Moreover, this iminosulfurane produced the highest activity of the all enhancers tested in this study ($ER_{Q24} = 23$, and $ER_J = 21$). Sulfurane **13** (class-III) containing a 4-nitro-2-pyridyl group, yielded the second highest activity with an ER_{Q24} of 9.03 and an ER_J of 10.95. Compound **16** was also relatively active. The enhancement activity of these compounds was higher than the maximum enhancement activity of DMSO. HPLC analytical methods were developed for compounds **9** and **13**. The skin penetration was determined for both of these agents. The corresponding permeation profiles are shown in Fig. 2. The solubilities for compounds **9** and **13** at 32 ± 0.5°C in PG were 0.183 and 0.076 M, respectively. No degradation of compounds **9** and **13** was observed during the 24 h experiment and maximum penetration was achieved in 10 h for both compounds. In the case of complex structures (**18** and **19**), compound **18** showed statistically significant enhancement effect, while compound **19** produced lower flux, Q_{24}, and skin content values than those of control.

Stoughton (1965) demonstrated that DMSO rapidly forms a SC reservoir of HC in human skin. Application of 40% of DMSO incorporated into a [^{14}C]HC alcohol solution and a cream base for different time intervals, resulted in a 10-fold increase in the skin content of the drug after just 30 min. Whereas increasing the contact time of the drug with the skin had a minor effect on the skin content. The retained radioactivity persisted in the skin for approximately two weeks. The retained drug was removed immediately by complete tape stripping of the SC, which indicated that DMSO had established a SC drug reservoir. In this study on hairless mouse skin, this phenomenon was not observed even at higher concentrations of DMSO (100%). Based on previously published studies (Sasaki et al., 1991; Michniak et al., 1993a), the recovery from skin homogenate by Sep Pak® elution was more than 95%. Therefore, low skin content results obtained in this study are thought to be due to the thickness of the hairless mouse SC or to a SC composition difference, rather than to a low extraction of HC from the homogenate. With respect to skin content, compounds **11**, **12**, and **16**, produced the lowest skin content values and ER_{SC} was 0.18, 0.22, and 0.17, respectively.

It is thought that much of the unique properties of DMSO, are related to its characteristic polar O–S bond, which also enables the compound to interact strongly with water by forming hydrogen bonds. This interaction with water is thought to be one of the key elements in the mechanism of action of DMSO as a penetration enhancer. It is thought that DMSO at low concentration displaces protein bound water resulting in a more solvated environment, and at high concentrations it displaces water surrounding the polar head groups of intercellular lipids of the SC leading to less packing of the lipid hydrocarbon tails (Franz et al., 1995). In the present study, the polarity of S–O bond was modified by changing oxygen to nitrogen substituted with different cyclic functions. Due to the similarity in the structure of the basic backbone between these compounds and DMSO, it was expected that these compounds may exert their action by similar mechanisms. However considering the low enhancement activity and skin content of class-I compounds, containing a highly polar sulfonyl functionality, it seems that the possible mode of action of these compounds might be different from that of DMSO.

It is well known that human skin is much less permeable than hairless mouse skin, and enhancers may have different behavior in different skin models (Fuhrman et al., 1997). Further studies are needed in order to assess the activity and toxicity of these compounds in a less permeable model such as human skin. In conclusion, compounds **9** and **13** showed higher Q_{24} values and flux values compared with controls ($P < 0.05$), although both compounds had low solubility in PG (0.076 and 0.183 M, respectively). Highest Q_{24} and flux values were obtained for compound **9** with 996.22 ± 192.46 μg/cm^2 and 42.91 ± 7.47 μg/cm^2 per h, respectively. It is of interest to note that compounds **1**, **7**, and **8** act as retardants under the described experimental conditions.

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