International Journal of Pharmaceutics, 55 (1989) 91–97
Elsevier

IJP 01856

Research Papers

Enhanced delivery of nalidixic acid through human skin via acyloxymethyl ester prodrugs

Hans Bundgaard [1], Niels Mørk [1] and Annie Hoelgaard [2]

The Royal Danish School of Pharmacy, Departments of [1] Pharmaceutical Chemistry and [2] Pharmaceutics, Copenhagen (Denmark)

(Received 20 March 1989)
(Accepted 10 April 1989)

Key words: Nalidixic acid; Dermal delivery; Prodrug; Diffusion cell; Skin penetration; Nalidixic acid ester

Summary

Nalidixic acid has recently been suggested as a potentially useful agent for the treatment of psoriasis. In an attempt to improve the dermal delivery characteristics of nalidixic acid various esters of the compound were synthesized and assessed as prodrug forms for the parent drug. The esters studied include the methyl ester, a glycolamide ester and several acyloxylmethyl esters. Whereas the former two esters were only partly converted to the parent drug during diffusion through human skin samples, the acyloxymethyl derivatives were completely converted by enzymatic hydrolysis. Among the latter derivatives the butyryloxymethyl and the isobutyryloxymethyl esters showed a 5–6-fold enhanced delivery of nalidixic acid from both polar and apolar vehicles relative to application of nalidixic acid itself. The enhanced transport was suggested to be a result of the increased water and lipid solubilities of the ester derivatives in comparison to the parent drug.

Introduction

Using primary cultures of human epidermal keratinocytes as a model system, Bohr et al. (1986) have recently screened a large number of growth inhibitory compounds for their relative effects on DNA replication, DNA repair and protein synthesis as well as for direct damage to DNA in an attempt to identify an optimal agent for the treatment of hyperproliferative skin disorders, notably psoriasis. Among the various compounds tested the antibiotic topoisomerase II inhibitors, novobiocin and nalidixic acid, appeared to be of particular interest since they caused no detectable damage to the DNA and were strong inhibitors of DNA replication, but had only slight inhibitory effects on DNA repair. These properties render the compounds potentially interesting drugs for antiproliferative therapy. In a subsequent pilot clinical study (Bohr et al., 1987) with direct topical application of these compounds to localized plaques on patients with psoriasis vulgaris, a clinical improvement was observed in 6 of the 7 patients treated for a period of 3 weeks. The formulations used of nalidixic acid and novobiocin were 2–5% aqueous suspensions and, as also confessed by the authors (Bohr et al., 1987), such formulations may not be optimal as regards delivery of the agents to their site of action in the skin.

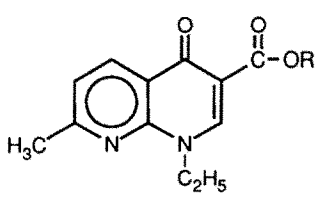
Based upon the promising results obtained by Bohr and coworkers we have examined the pro-

Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

drug approach to enhance the dermal delivery of nalidixic acid, 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-napthyridine-3-carboxylic acid (**I**). The physicochemical properties of nalidixic acid were considered suboptimal for an efficient topical absorption: it shows a high melting point (225–231° C) and has both a relatively poor lipid and water solubility (Grubb, 1979). As discussed by Sloan (1989), several studies have demonstrated biphasic solubility as being an important determinant of flux across skin. The pK_a of the carboxylic acid function of nalidixic acid is 6.1 (Vincent et al., 1981) so even at neutral pH, the compound is only partly ionized and hence only slightly water-soluble (Staroscik and Sulkowska, 1971).

In the prodrug approach, which in the past few years has been increasingly used to optimize the dermal delivery of a variety of drugs (for reviews, see Hadgraft, 1985; Sloan, 1989), the physicochemical properties of the drug are changed in order to increase the diffusion rate through the skin, primarily the stratum corneum. When a local effect is desired as in the present case, a prerequisite for success in use of prodrugs is further that reconversion of the prodrug into the parent drug occurs in the skin.

The present paper describes the synthesis of various esters of nalidixic acid (II–VII), and the evaluation of their abilities to increase the dermal delivery of the parent drug.



I	R = H
II	R = -CH_3
III	R = -$CH_2CON(C_2H_5)_2$
IV	R = -CH_2OCOCH_3
V	R = -$CH_2OCOC_2H_5$
VI	R = -$CH_2OCOC_3H_7$
VII	R = -$CH_2OCOCH(CH_3)_2$

Materials and Methods

Chemicals

Nalidixic acid and its sodium salt were purchased from Sigma Chemical Company, St. Louis. Acyloxymethyl chlorides were prepared as described by Waranis and Sloan (1987).

*Preparation of the esters **II–VIII***

*Nalidixic acid methyl ester (**II**).* Methyl iodide (0.31 ml, 5 mmol) was added to a mixture of nalidixic acid sodium salt (1.27 g, 5 mmol) in *N,N*-dimethylformamide (10 ml) and the mixture stirred at 80° C for 4 h. Water (50 ml) was added to the cooled reaction mixture and the mixture extracted with ethyl acetate (2 × 50 ml). The combined extracts were washed with a 2% aqueous solution of sodium carbonate and water, dried over anhydrous sodium sulphate and evaporated in vacuo. The solid residue obtained was recrystallized from ethyl acetate-petroleum ether to yield 0.9 g of the title compound, m.p. 153–154° C, rep. m.p. 154° C (Rufer et al., 1977).

*Nalidixic acid N,N-diethylglycolamide ester (**III**).* A mixture of nalidixic acid sodium salt (1.27 g, 5 mmol), sodium iodide (75 mg, 0.5 mmol) and *N,N*-diethyl-2-chloroacetamide (0.82 ml, 6 mmol) in *N,N*-dimethylformamide (10 ml) was stirred at 50° C for 18 h. Water (50 ml) was added and the mixture extracted with ethyl acetate (2 × 75 ml). The extracts were washed with a 2% aqueous solution of sodium carbonate and water, dried over anhydrous sodium sulphate and evaporated in vacuo. The residue obtained solidified after a few hours and was recrystallized from ethyl acetate–ethanol–petroleum ether to give 1.1 g of the title compound, m.p. 142–143° C. *Anal.*: Calc. for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.66; H, 6.87; N, 12.01.

*Nalidixic acid acetoxymethyl ester (**IV**).* A mixture of nalidixic acid sodium salt (3.8 g, 15 mmol), sodium iodide (0.23 g, 1.5 mmol) and acetoxymethyl chloride (1.73 ml, 16 mmol) in *N,N*-dimethylformamide (22 ml) was stirred at 80° C for

5 h. Water (100 ml) was added and the mixture extracted with ethyl acetate (2 × 100 ml). The combined extracts were washed with a 2% aqueous solution of sodium carbonate (2 × 50 ml) and water, dried and evaporated in vacuo. The solid residue obtained was recrystallized from ethanol–water, yielding 2.6 g of ester **IV**, m.p. 120–121°C. *Anal.*: Calc. for $C_{15}H_{16}N_2O_5$: C, 59.21; H, 5.30; N, 9.21. Found: C, 59.16; H, 5.35; N, 9.19.

Other acyloxymethyl esters (**V–VIII**) were prepared by the same procedure.

Nalidixic acid propionyloxymethyl ester (V): m.p. 105–106°C (from ethanol–water). *Anal.*: Calc. for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.45; H, 5.67; N, 8.76.

Nalidixic acid butyryloxymethyl ester (VI): m.p. 104–105°C (from chloroform–petroleum ether). *Anal.*: Calc. for $C_{17}H_{20}N_2O_5$: C, 61.43; H, 6.07; N, 8.43. Found: C, 61.36; H, 6.10; N, 8.40.

Nalidixic acid isobutyryloxymethyl ester (VII): m.p. 105–106°C (from chloroform–petroleum ether). *Anal.*: Calc. for $C_{17}H_{20}N_2O_5$: C, 61.43; H, 6.07; N, 8.43. Found: C, 61.49; H, 6.03; N, 8.53. The ^{1}H-NMR and UV spectral data of the esters II–VIII were consistent with their structures.

Determination of solubilities and partition coefficients

The solubilities of nalidixic acid and the esters II–VIII were determined in duplicate in water, isopropyl myristate and other solvents at 21 ± 1°C by placing excess amounts of the compounds in 2–5 ml of the solvent. The mixtures were rotated on a mechanical spindle for 24 h and filtered. The concentrations of the compounds in their saturated solutions were determined by the HPLC methods described below. No degradation of the esters took place during the solubility determination as revealed by HPLC analysis.

The partition coefficients of the compounds were determined at 21°C in a 1-octanol–water system. The concentration of the compounds in the aqueous phase before and after partitioning was determined by HPLC analysis and the partition coefficients calculated as described before (Bundgaard et al., 1986).

Permeability–metabolism studies using excised human skin

Whole abdominal human skin obtained under autopsy from two donors was used. The skin was stored at −18°C and was allowed to thaw gradually at room temperature before use. All subcutaneous fat was removed and the skin cut into pieces. The excised skin was mounted in open diffusion cells of the same type as those used by Franz (1975); they have an available diffusion area of 1.8 cm^2. The dermal side of the skin was exposed to the receptor medium (7.5 ml of 0.05 M isotonic phosphate buffer solution of pH 7.2). The receptor phase was stirred magnetically and was kept at a constant temperature of 37°C with a circulating water bath.

The compounds were applied as suspensions (100 μl). The suspensions were allowed to stir for 24 h prior to application to the skin surface. At appropriate intervals samples of 2 ml were removed from the receptor phase and replaced with fresh buffer. The samples were stored at −20°C until they were analyzed for nalidixic acid and ester content by HPLC as described below. The permeation studies of each compound in each vehicle were done in duplicate or triplicate, the data obtained being reproducible within ±15%.

HPLC analysis of nalidixic acid and its esters

Nalidixic acid and its esters were determined by HPLC using a system consisting of a Waters pump model 510, a variable-wavelength UV-detector Waters type Lambda Max model 481 operated at 258 nm and a Rheodyne 7125 injection valve with a 20-μl loop. A Waters Radial-PAK column (100 × 8 mm) packed with NOVA-PAK C 18 (4 μm particles) and supplied with a precolumn packed with μBondapak C 18 (10 μm particles) (Waters) was eluted at ambient temperature with a mobile phase consisting of aceto-nitrile–methanol–water–85% phosphoric acid (40 : 10 : 50 : 1 v/v), the flow rate being 1.5 ml/min.

Under these conditions nalidixic acid and its esters showed the following retention times: **I**, 4.2 min; **II**, 3.8 min; **III**, 4.0 min; **IV**, 3.6 min; **V**, 5.0 min; **VI**, 7.5 min; **VII**, 7.3 min. Thus, the system enabled the separation and simultaneous de-

termination of nalidixic acid and its various esters. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

Hydrolysis of nalidixic acid esters

The rate of hydrolysis of the ester derivatives II–VII was studied in sodium carbonate and sodium hydroxide buffer solutions (pH 10–12) with an ionic strength (μ) of 0.5 at 37 °C as well as in human plasma diluted to 80% with 0.02 M phosphate buffer (pH 7.4) at 37 °C. The reactions were initiated by adding 50 μl of a stock solution of the compounds in acetonitrile to 5 ml of preheated buffer or plasma solution in screw-capped testtubes, the final concentrations of the compounds being about 10^{-4} M. The solutions were kept in a water bath at 37 °C and at appropriate intervals samples were taken and analyzed for remaining ester and nalidixic acid formed by the HPLC method described above. For the analysis of the plasma solutions, the samples (250 μl) taken were deproteinized by mixing with 500 μl of a 2% solution of zinc sulphate in water–methanol (1 : 1 v/v). After centrifugation for 2 min at 13,000 rpm, 20 μl of the clear supernatant was chromatographed.

Pseudo-first-order rate constants for the hydrolysis of the esters were determined from the slopes of linear plots of the logarithm of residual derivative against time.

Results and Discussion

Solubility data

The solubilities of nalidixic acid and its esters **II–VII** in water and isopropyl myristate are shown in Table 1. It can be seen that all the esters showed both improved water and lipid solubility over that of the parent compound. This increase in solubility is accompanied by a marked decrease in melting point. The high melting point of nalidixic acid may probably be ascribed to intramolecular hydrogen bonding as depicted in Scheme 1. By esterifying the carboxylic acid group such hydrogen bonding is no longer possible which helps to decrease the crystal lattice forces present in the acid. As seen from Table 1 most ester derivatives are also more lipophilic than the parent acid in terms of partition coefficients between octanol and water.

TABLE 1

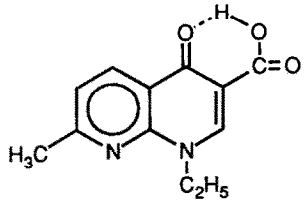
Melting points, solubilities and partition coefficients of nalidixic acid and various ester prodrugs

Compound	m.p. (°C)	log P [a]	Solubility (mg/ml)	
			In water	In IPM
I	225–231	1.51	0.024	0.11
II	154–155	1.23	0.49	0.78
III	142–143	1.43	12.8	0.19
IV	120–121	1.21	0.99	0.45
V	105–106	1.70	0.47	1.24
VI	104–105	2.20	0.38	3.85
VII	105–106	2.17	0.32	3.56

[a] P is the partition coefficient between water and octanol.

Enzymatic and chemical lability of the esters

As stated in the introduction an important feature of prodrugs to be used as dermal delivery forms is the ability to be converted to the active parent drug in the skin. It is well known that whole skin, particularly the epidermis, contains many highly active enzyme systems including esterases (Pannatier et al., 1978; 1981; Møllgaard et al., 1982; Bucks, 1984; Guy et al., 1987). As an indication of the susceptibility of the present series of nalidixic acid esters to undergo bioconversion by esterases in the skin their stability in the presence of human plasma was examined at 37 °C. The kinetic data obtained are shown in Table 2. Both the simple methyl ester (II) and the *N*,*N*-diethylglycolamide ester (III) proved to be highly resistant to cleavage by plasma enzymes. The behavior of the latter ester was surprising since such



Scheme 1.

Scheme 2.

an ester of various other carboxylic acids has been found to be hydrolyzed very rapidly in human plasma solutions, mainly by virtue of cholinesterase enzymes (Bundgaard and Nielsen, 1987, 1988; Nielsen and Bundgaard, 1988). Apparently, the structural surroundings of the carboxylic groups in nalidixic acid makes the enzymatic attack on the ester carbonyl moiety difficult.

In contrast to these esters, the *O*-acyloxymethyl derivatives **IV–VII** are quite easily hydrolyzed by plasma enzymes, the half-lives in 80% human plasma being 8–18 min (Table 2). The hydrolysis of these double esters takes most likely place as depicted in Scheme 2. The first step in the hydrolysis is enzymatic cleavage of the terminal and sterically more unhindered ester grouping with formation of a highly unstable hydroxymethyl ester which rapidly dissociates to the parent nalidixic acid and formaldehyde. As reviewed by Bundgaard (1985) this double ester principle has already been utilized for a variety of carboxylic acids in the design of enzymatically labile prodrug derivatives.

TABLE 2

Half-lives of hydrolysis of various nalidixic acid esters at 37 °C

Ester	Half-life	
	pH 7.4 buffer [a]	80% human plasma
II	3.1×10^3 h	> 100 h [b]
III	3.6×10^3 h	> 100 h [b]
IV	87 h	18.2 min
V	89 h	8.9 min
VI	114 h	7.8 min
VII	130 h	15.5 min

[a] These values were estimated from rate data obtained at pH 10–12. In this pH range the rate of hydrolysis was found to be directly proportional to the hydroxide ion activity indicating specific base-catalyzed hydrolysis of the ester moiety.
[b] Less than 5% degradation occurred after 24 h.

Some data for the non-enzymatic hydrolysis of the nalidixic acid esters are also shown in Table 2. It is readily seen that at the conditions used in the skin diffusion experiments no significant non-enzymatic cleavage of the esters should occur.

Skin permeation

Excised human skin was used to examine the dermal delivery of nalidixic acid and various ester prodrugs. Each compound was studied in both a polar (i.e., water) and an apolar (i.e., isopropyl myristate) vehicle. Suspensions of the compounds in the vehicles were applied in order to keep a constant driving force for diffusion and to provide the maximum flux attainable which is the property to be optimized in dermal drug delivery.

For each diffusion experiment the cumulative amounts (in μmol) of nalidixic acid measured in the receptor phase divided by the surface area of the diffusion cell (1.8 cm^2) were plotted against the time of sampling. Some typical plots are shown in Fig. 1. The steady-state fluxes were obtained from the slopes of the linear portions of such plots. The permeability coefficients (K_p) for the steady-state delivery of nalidixic acid were obtained by dividing the steady-state fluxes by the corresponding solubilities of the compounds in the vehicle applied (equivalent μmol of nalidixic acid/ml). The steady-state fluxes and permeability coefficients for the delivery of nalidixic acid as such and by the ester prodrugs are given in Table 3.

In the case of the acyloxymethyl esters (**IV–VII**), only nalidixic acid was found in the receptor

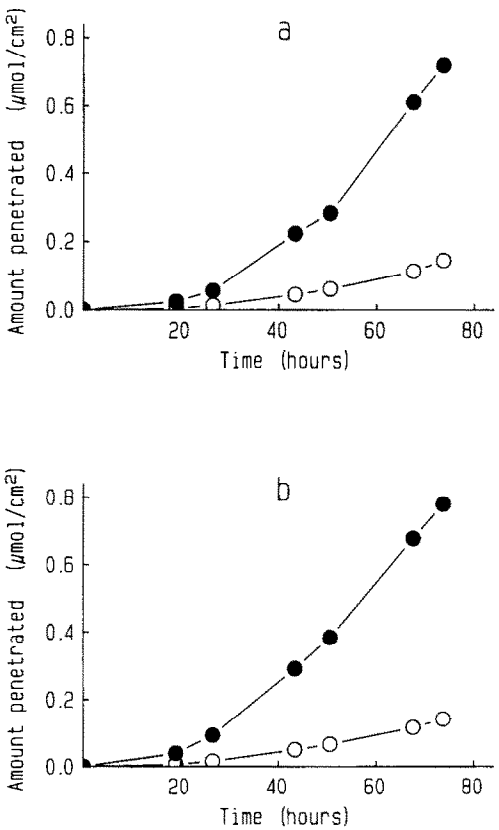


Fig. 1. Permeability of nalidixic acid (**I**) and nalidixic acid butyryloxymethyl ester (**VI**) through human skin as amount of nalidixic acid appearing in the receptor phase as a function of time from saturated solutions of compound **I** (○) and **VI** (●) in water (a) and isopropyl myristate (b).

TABLE 3

Fluxes and permeability coefficients (K_p) for steady-state phase of delivery of nalidixic acid through human skin from isopropyl myristate (IPM) and water

Compound	Flux (μmol/cm²/h) ×10³		K_p (cm/h) ×10³	
	IPM	Water	IPM	Water
I	3.2	3.2	6.7	31
II [a]	10	9.7	3.2	4.9
III [a]	13	0.54	24	0.015
IV	10	2.3	6.8	0.71
V	5.0	3.1	1.3	2.1
VI	16	16	1.4	14
VII	18	6.5	1.7	68

[a] These compounds appeared in the receptor phase both as nalidixic acid and as intact ester. The flux values given were calculated in terms of total nalidixic acid equivalents.

phase, indicating an efficient enzymatic hydrolysis of these esters during the transport through the skin. The methyl ester (**II**) and the *N,N*-diethylglycolamide ester (**III**) were present both as intact ester and as nalidixic acid in the receptor phase. In the case of the methyl ester, $65 \pm 15\%$ was present as the intact ester at the various sampling times whereas the corresponding figure for the ester **III** was $50 \pm 12\%$. These different abilities of the esters to undergo cutaneous hydrolysis are seen to be in accordance with the relative stabilities of the compounds in human plasma as reported above. It is of interest to note, however, that although the esters II and III showed no appreciable plasma-catalyzed hydrolysis the skin enzyme-mediated hydrolysis of the esters during the diffusion experiment was significant, albeit incomplete.

The results obtained from the diffusion experiments show that it is possible to improve significantly the dermal delivery of nalidixic acid via prodrugs. The butyryloxymethyl (**VI**) and isobutyryloxymethyl (**VII**) ester prodrugs exhibited the highest delivery rate, with a steady-state rate of skin permeation of nalidixic acid 5–6 times that of nalidixic acid itself delivered from IPM. When delivered from water the butyryloxymethyl ester also afforded a 5-fold higher flux relative to nalidixic acid itself. The increased solubility of the prodrug in the vehicles combined with expected concomitant increase in solubility in the skin must be responsible for the higher fluxes, since the ability of the ester to partition into the skin is part of the driving force for diffusion. The results obtained are in harmony with the general trend observed (Sloan, 1989) for various prodrugs designed to enhance skin penetration that the most effective derivatives are the ones that combine an increased lipid solubility and an increased water solubility over the parent drug.

An attempt was made to optimize the penetration of nalidixic acid and the ester **VI** through formulation in a vehicle consisting of propylene glycol containing 5% of the absorption enhancing agent Azone® (1-dodecylazacycloheptan-2-one) (Stoughton, 1982). The solubilities of nalidixic acid and ester **VI** in this vehicle were found to be 1.01 and 63.4 mg/ml, respectively. For nalidixic acid a

steady-state flux of 2.1×10^{-2} μmol/cm^2/h was observed whereas the ester **VI** showed a flux of 9.5×10^{-2} μmol/cm^2/h. Thus, whereas this vehicle provides a marked increase in the rate of delivery for nalidixic acid relative to the IPM and water vehicles, it also improves the delivery of nalidixic acid from the prodrug **VI**.

In conclusion, the butyryloxymethyl or isobutyryloxymethyl nalidixic acid ester has been shown to function as prodrug forms capable of increasing the topical delivery of nalidixic acid from both polar, medium polar and apolar vehicles. Considering the potential use of the butyryloxymethyl ester derivative as a dermal delivery form of nalidixic acid for treatment of psoriasis, it is interesting to note that one of the pro-moieties split off from the derivative upon enzymatic hydrolysis in the skin, butyric acid, also has been found to be an agent capable of inhibiting DNA replication and at the same time being without inhibitory effect on the DNA repair process (Bohr et al., 1986). Thus, further studies of the nalidixic acid butyryloxymethyl ester as an anti-psoriasis agent are warranted.

References

Bohr, V., Abel, E.A., Farber, E.M. and Hanawalt, P., Topical treatment of psoriasis with the topoisomerase inhibitors novobiocin and nalidixic acid: a pilot study. *Arch. Dermatol. Res.*, 279 (1987) 147–150.

Bohr, V., Mansbridge, J. and Hanawalt, P., Comparative effects of growth inhibitors on DNA replication, DNA repair, and protein synthesis in human epidermal keratinocytes. *Cancer Res.*, 46 (1986) 2929–2935.

Bucks, D.A.W., Skin structure and metabolism: relevance to the design of cutaneous therapeutics. *Pharm. Res.*, 1 (1984) 148–153.

Bundgaard, H., Design of prodrugs: bioreversible derivatives for various functional groups and chemical entities. In H. Bundgaard (Ed.), *Design of Prodrugs*, Elsevier, Amsterdam, 1985, pp. 1–92.

Bundgaard, H., Falch, E., Larsen, C., Mosher, G.L. and Mikkelson, T.J., Pilocarpine prodrugs. II. Synthesis, stability, bioconversion, and physicochemical properties of sequentially labile pilocarpic acid diesters. *J. Pharm. Sci.*, 75 (1986) 775–783.

Bundgaard, H. and Nielsen, N.M., Esters of *N*,*N*-disubstituted 2-hydroxyacetamides as a novel highly biolabile prodrug type for carboxylic acid agents. *J. Med. Chem.*, 30 (1987) 451–454.

Bundgaard, H. and Nielsen, N.M., Glycolamide esters as a novel biolabile prodrug type for non-steroidal anti-inflammatory carboxylic acid drugs. *Int. J. Pharm.*, 43 (1988) 101–110.

Franz, T.J., Percutaneous absorption. On the relevance of in vitro data. *J. Invest. Dermatol.*, 64 (1975) 190–195.

Grubb, P.E., Nalidixic acid. In K. Florey (Ed.), *Analytical Profiles of Drug Substances, Vol. 8*, Academic Press, New York, 1979, pp. 371–397.

Guy, R.H., Hadgraft, J. and Bucks, D.A.W., Transdermal drug delivery and cutaneous metabolism. *Xenobiotica*, 17 (1987) 325–342.

Hadgraft, J., Prodrugs and skin absorption. In H. Bundgaard (Ed.), *Design of Prodrugs*, Elsevier, Amsterdam, 1985, pp. 271–289.

Møllgaard, B., Hoelgaard, A. and Bundgaard, H., Prodrugs as drug delivery systems. XXIII. Improved dermal delivery of 5-fluorouracil through human skin via *N*-acyloxymethyl prodrug derivatives. *Int. J. Pharm.*, 12 (1982) 153–162.

Nielsen, N.M. and Bundgaard, H., Glycolamide esters as biolabile prodrugs of carboxylic acid agents. Synthesis, stability, bioconversion and physicochemical properties. *J. Pharm. Sci.*, 77 (1988) 285–298.

Pannatier, A., Jenner, P., Testa, B. and Etter, J.-C., The skin as a drug-metabolizing organ. *Drug Metab. Rev.*, 8 (1978) 319–343.

Pannatier, A., Testa, B. and Etter, J.-C., Enzymatic hydrolysis by mouse skin homogenates: structure–metabolism relationships of para-nitrobenzoate esters. *Int. J. Pharm.*, 8 (1981) 167–174.

Rufer, C., Kessler, H.-J. and Schwarz, K., Chinoloncarbonsäuren. V. Azaanaloge heterocyclisch substituierte 1-Äthyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-carbonsäuren. *Eur. J. Med. Chem. Chim. Ther.*, 12 (1977) 27–29.

Sloan, K.B., Prodrugs for dermal delivery. *Adv. Drug Del. Rev.*, 3 (1989) 67–101.

Staroscik, R. and Sulkowska, J., A study of the acid-base equilibrium of nalidixic acid. *Acta Polon. Pharm.*, 28 (1971) 601–606.

Stoughton, R.B., Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one (Azone). *Arch. Dermatol.*, 188 (1982) 474–477.

Vincent, W.R., Schulman, S.G., Midgley, J.M., van Oort, W.J. and Sorel, R.H.A., Prototropic and metal complexation equilibria of nalidixic acid in the physicochemical pH region. *Int. J. Pharm.*, 9 (1981) 191–198.

Waranis, R.P. and Sloan, K.B., Effects of vehicles and prodrug properties and their interactions on the delivery of 6-mercaptopurine through skin: bisacyloxymethyl-6-mercaptopurine prodrugs. *J. Pharm. Sci.*, 76 (1987) 587–595.