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# Discriminant analysis as a tool to identify compounds with potential as transdermal enhancers

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## Abstract

Structure–activity relationships were sought for 73 enhancers of hydrocortisone permeation from propylene glycol across hairless mouse skin. Enhancers had chain lengths (CC) from 0 to 16 carbon atoms, 1 to 8 H-bonding atoms (HB), molecular weight 60 to 450, log P (calculated) −1.7 to 9.7 and log S (calculated) −7.8 to 0.7. These predictive properties were chosen because of their ready availability. Enhancement ratio (ER) was defined as hydrocortisone transferred after 24 h relative to control. Values for the ER ranged from 0.2 to 25.3. Multiple regression analysis failed to predict activity; ER values for the 'good' enhancers (ER > 10) were underestimated. Simple guidelines suggested that high ER was associated with CC >12 and HB 2–5. This was refined by multivariate analysis to identify significant predictors. Discriminant analysis using CC, HB, and molecular weight correctly assigned 11 of the 12 'good' enhancers (92%). The incorrectly assigned compound was a known, idiosyncratic Br compound. Seventeen of the 61 'poor' enhancers (28%) were incorrectly assigned but four could be considered marginal (ER > 8). The success of this simple approach in identifying potent enhancers suggested its potential in predicting novel enhancer activity.

## Introduction

The usefulness of transdermal delivery is restricted by the efficiency of the skin barrier which resides in the uppermost layer of the skin, the stratum corneum. Various methods have been used to enhance percutaneous absorption, including the co-formulation of enhancer chemicals, where the second enhancer may be the formulation vehicle, such as ethanol or propylene glycol. Incorporation of certain chemicals into the drug delivery vehicle may lead to the enhancement of drug release and a more rapid clinical response. Such chemicals have been variously labelled penetration enhancers, accelerants or sorption promoters. Katz & Poulsen (1972) proposed that the ideal penetration enhancer should be pharmacologically inert, non-toxic, irritating or allergenic, able to provide immediate onset of penetration enhancement upon application, and allow the skin to recover its barrier function immediately and fully upon removal. They went on to list that it should be compatible with a wide range of drugs and excipients, be able to solubilize drugs, and be compliant, inexpensive, odourless, colourless and tasteless, and therefore cosmetically acceptable.

There are many classes of enhancers reported in the literature (Ghosh et al 1997). One classification is based on: those compounds that enhance co-administered drug concentrations across and within the skin; those that enhance transdermal permeation alone, leaving little drug within the skin layers by the time the enhancer stops exerting its action; those that enhance local concentrations within the skin layers and therefore have more use for topical delivery of drugs; and those that retard drug permeation into and across the skin membrane (Asbill & Michniak 2000). The phenomenon of chemical enhancement by co-formulated chemicals was reviewed by Shah (1994). Shah proposed that penetration enhancers may cause stratum corneum lipid fluidization, increase diffusivity within the skin, optimize the thermodynamic activity of the drug in the vehicle and the skin, enable formation of a drug depot within the skin layers, and/or affect the partitioning of the drug/vehicle into the skin and across the skin layers. The final enhancer used in a therapeutic product will need to undergo extensive testing for efficacy and toxicity, but

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the initial development is typically in-vitro work carried out on a range of proposed enhancers. Even this is expensive and time consuming and as such the number of compounds that can be tested is small.

The same fundamental problem underlies all pharmaceutical development and relationships between chemical properties and activity are sought to focus effort to maximize the chance of success. Multiple regression analyses are often used to identify and quantify relationships (quantitative structure–activity relationships, QSARs) between an effect and a set of accessible molecular properties. These have been applied to the prediction of permeability coefficients and flux (Pugh & Hadgraft 1994; Potts & Guy 1995; Abraham et al 1997; Cronin et al 1999; Pugh et al 2000; Buchwald & Bodor 2001; Lim et al 2002; Moss & Cronin 2002) since the pioneering paper of Potts & Guy (1992). However, while these models describe the physicochemical properties of a molecular structure that influence its skin permeability from a saturated aqueous solution, they do not take into account the nature of a formulation, and how that may affect the prediction of permeability (Moss et al 2002). Further, the issue of a specific QSPR (quantitative structure–property relationship) to predict the effect of penetration enhancers is complicated by the vast range of chemicals that will actively promote increased percutaneous absorption. Penetration enhancers fall into a wide range of chemical classes and may exert their function by a wide range of mechanisms. These have been comprehensively reviewed by Williams & Barry (2004).

Previously, QSARs have been used to investigate the enhancement effects associated with a range of penetration enhancers (Ghafourian et al 2004). It was determined that, due to a large range of structural differences, a single, generic QSAR could not be developed. Rather, different mechanisms of enhancement (reflected in the choice of descriptors) were proposed for each different chemical class of enhancer and for drugs with different physicochemical properties. With QSPRs for skin absorption, there is usually an assumption of linearity of response, although this may be overcome by the use of quadratic or power terms in modelling data (Monod et al 1965; Garg et al 2003; Hansch et al 2001, 2003). Although such mathematical rigour seems desirable it may be illusory from the viewpoint of the formulator, since the least-squares equation is determined by mean values of the predictors, and is little influenced by rare, extreme values. It therefore describes – often quite accurately – the 'mediocre' compounds, whilst it is the outliers with exceptionally high activity that are often of most interest (Magnusson et al 2004). An alternative approach is to identify guidelines for a simple 'pass/fail' for rapid screening, as used by Lipinski et al (1997) to predict intestinal absorption. We believe that this approach may allow differentiation between discrete chemical classes of penetration enhancers, and as such may offer several qualitative advantages to QSPR-based models.

The advent of user-friendly statistical packages enables the increasingly rapid and straightforward application of appropriate tests. The suite of tests grouped under the description of multivariate analysis enables quantification of the relationships within a set of predictors. The relationships are described fundamentally by principal components analysis, which identifies the various groupings of predictors that determine the variation in the dataset. Discriminant analysis, which assigns a novel compound to one of a set of predetermined levels of activity, is probably the most useful in the context of the current discussion. Therefore, discriminant analysis was used in this study to assign chemical penetration enhancers as exerting potentially 'good' or 'poor' effects on skin permeability, with the boundary set at 10-fold enhancement, chosen as being a sufficiently large effect to be of practical use.

## Methods

Data for the in-vitro enhancement of hydrocortisone transfer over 24 h from propylene glycol solutions of enhancers across hairless mouse skin were extracted from six papers published by Michniak's research group (Godwin et al 1997, 1998; Michniak et al 1998; Kim et al 1999, 2001; Strekowski et al 1999). They offer the opportunity to study a dataset with as low a level of experimental variation as can be expected, with skin strain, temperature, donor and receptor phase standardized. The propylene glycol vehicle may well affect the skin barrier over 24 h but its effect should be constant throughout the dataset. The main objective of this paper was to demonstrate that a multivariate method such as discriminant analysis may be superior to the more traditional regression analysis for finding a QSAR. We recognized that the values for the enhancement ratio (ER) may not accurately predict the equivalents for human skin, yet felt it likely that the best enhancers in mouse were more likely to be effective in man.

Compounds were coded as they had been in the original papers and the key is provided at the bottom of Table 1. The enhancement ratio was defined as the amount crossing the skin in 24 h relative to control. Data for 73 compounds were thus available with a high degree of standardization of experimental procedure. Mean values were used for the two enhancers with duplicated results: azone and S, S-dimethyl-N-(4-bromobenzoyl)iminosulfurane.

A range of calculable molecular features were considered as predictors. These included the molar refractivity, solvatochromic quantities $\alpha$, $\beta$, $\pi^*$ and $V_x$ of Kamlet et al (1983) calculated from the tables of Abraham (1993) and Abraham & Platts (2001), and the Hildebrand and 3-dimensional Hansen solubility parameters calculated by the group contribution methods (Fedors 1974; Van Krevelen & Hoftyzer 1976). None of these proved to be successful and this report was therefore limited to a discussion of the five most successful predictors.

The carbon numbers (CC) of chain lengths were noted. Some compounds had ring structures as part, or all, of the side chain, and a CC component of 3 was assigned for this feature. Hydrogen bonding numbers (HB) were assigned on the basis of each atom capable of H-bonding. Thus COOH is assigned a value of 3, comprising 1 H-donor hydrogen atom and 2 H-acceptor oxygens. When the numbers of donors and acceptors were used separately,

**Table 1** Physicochemical properties of the enhancers

| Compound | Name | Michniak Code | HB | CC | MW | log P | log S | ER | Misclassified discriminant analysis |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1-Methyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam | 1.1 | 4 | 1 | 210.28 | 0.242 | −0.335 | 0.80 | |
| 2 | 1-Hexyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam | 1.2 | 4 | 6 | 280.41 | 2.690 | −2.290 | 2.10 | |
| *3* | *1-Decyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam* | *1.3* | *4* | *10* | *336.52* | *4.682* | *−3.945* | *8.80* | $P = 0.58$ |
| 4 | 1-Dodecyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam | 1.4 | 4 | 12 | 364.57 | 65.688 | −4.460 | 11.00 | |
| 5 | 1-Tetradecyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam | 1.5 | 4 | 14 | 392.63 | 6.432 | −4.970 | 18.60 | |
| *6* | *1-Hexadecyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam* | *1.6* | *4* | *16* | *420.68* | *7.382* | *−5.455* | *9.60* | $P = 0.92$ |
| 7 | Methyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam-1-acetate | 1.7 | 6 | 1 | 268.31 | 0.156 | −0.890 | 0.60 | |
| 8 | Hexyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam-1-acetate | 1.8 | 6 | 6 | 338.45 | 2.706 | −2.605 | 1.00 | |
| 9 | Octyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam-1-acetate | 1.9 | 6 | 8 | 366.50 | 3.704 | −3.505 | 2.20 | |
| 10 | Decyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam-1-acetate | 1.10 | 6 | 10 | 394.55 | 4.648 | −4.160 | 4.00 | |
| 11 | Dodecyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam-1-acetate | 1.11 | 6 | 12 | 422.61 | 5.652 | −4.720 | 9.10 | |
| *12* | *Tetradecyl-3-(2-oxo-1-pyrrolidine)-ϵ-caprolactam-1-acetate* | *1.12* | *6* | *14* | *450.66* | *6.394* | *−5.290* | *9.60* | $P = 0.59$ |
| 13 | 1-Dodecanoylpiperidine | 2.1 | 2 | 11 | 267.46 | 5.650 | −4.560 | 14.70 | |
| 14 | 1-Dodecanoylpyrrolidinone | 2.2 | 2 | 11 | 253.43 | 5.190 | −4.325 | 15.60 | |
| *15* | *1-Dodecanoyl-2-piperidinone* | *2.3* | *3* | *11* | *281.44* | *5.360* | *−4.165* | *7.70* | $P = 0.77$ |
| 16 | 1-Dodecanoyl-2-pyrrolidinone | 2.4 | 3 | 11 | 267.41 | 4.946 | −3.895 | 10.10 | |
| *17* | *2-Decylcyclohexanone* | *2.5* | *1* | *10* | *238.41* | *6.058* | *−5.475* | *7.90* | $P = 0.90$ |
| 18 | 2-Decylcyclopentanone | 2.6 | 1 | 10 | 224.39 | 5.770 | −5.230 | 6.70 | $P = 0.90$ |
| 19 | 4-(Dodecanoyl)-thiomorpholine | 2.7 | 3 | 11 | 285.49 | 5.422 | −4.490 | 21.00 | |
| 20 | N, N-didodecylacetamide | 2.8 | 2 | 12 | 395.71 | 9.684 | −6.550 | 8.90 | $P = 0.91$ |
| 21 | N-acetylcaprolactam | 2.9 | 3 | 1 | 155.20 | 0.580 | −0.285 | 4.60 | |
| 22 | 4-Acetylmorpholine | 2.10 | 3 | 1 | 129.16 | −0.598 | 0.645 | 1.30 | |
| 23 | N-dodecyltricyclo[3.3.1.1$^{3,7}$]decane-1-carboxamide | 2.11 | 3 | 12 | 347.00 | 6.750 | −5.160 | 2.00 | $P = 0.83$ |
| 24 | N-(1-oxododecyl)morpholine | 2.12 | 3 | 11 | 269.43 | 4.488 | −3.620 | 15.10 | |
| 25 | N-dodecylpyrrolidinone | 2.13/3.1 | 2 | 12 | 253.43 | 5.494 | −4.410 | 25.30* | |
| 26 | N-dodecyl-2-piperidinone | 2.14 | 2 | 12 | 267.46 | 5.820 | −4.620 | 22.20 | |
| 27 | 2-Ppyrrolidinone-1-acetic acid dodecyl ester | 3.2 | 4 | 12 | 311.46 | 5.308 | −4.105 | 11.00 | |
| 28 | N-dodecyl-pyrrolidine | 3.3 | 1 | 12 | 239.44 | 6.492 | −5.450 | 5.20 | $P = 0.95$ |
| 29 | 2-Pyrrolidinone | 3.4 | 3 | 0 | 85.11 | −0.658 | 0.595 | 1.20 | |
| 30 | 1-Methyl-2-pyrrolidinone | 3.5 | 2 | 1 | 99.13 | −0.328 | 0.685 | 1.00 | |
| 31 | 5-Methyl-2-pyrrolidinone | 3.6 | 3 | 1 | 99.13 | −0.164 | 0.340 | 1.30 | |
| 32 | 1,5-Dimethyl-2-pyrrolidinone | 3.7 | 2 | 2 | 113.16 | 0.148 | 0.425 | 1.30 | |
| 33 | 1-Ethyl-2-pyrrolidinone | 3.8 | 2 | 2 | 113.16 | 0.228 | 0.445 | 1.10 | |
| 34 | 2-Pyrrolidinone-5-carboxylic acid | 3.9 | 6 | 0 | 129.12 | −1.102 | −0.065 | 1.10 | |
| 35 | (±)-3-Methyl-2-pyrrolidinone | 3.10 | 3 | 1 | 99.13 | −0.188 | 0.375 | 1.80 | |
| 36 | Ethyl (R)-(−)-2-pyrrolidinone-5-carboxylate | 3.11 | 5 | 2 | 157.17 | −0.226 | −0.130 | 1.10 | |
| 37 | 1-Cyclohexyl-2-pyrrolidinone | 3.12 | 2 | 2 | 167.25 | 1.756 | −1.020 | 1.20 | |
| 38 | 1-Methylpyrrolidine | 3.13 | 1 | 1 | 85.15 | 0.720 | 0.385 | 1.40 | |
| 39 | 1-Methylsuccinimide | 3.14 | 3 | 1 | 113.12 | −0.688 | 0.465 | 1.40 | |
| 40 | 1-Hexyl-2-pyrrolidinone | 3.15 | 2 | 6 | 169.27 | 2.276 | −1.365 | 1.20 | $P = 0.57$ |
| 41 | (R,R)-(−)-2,5-bis(methoxymethyl)pyrrolidinone | 3.16 | 4 | 1 | 159.23 | 0.214 | −0.013 | 2.00 | |
| 42 | Urea | 4.1 | 7 | 0 | 60.06 | −1.692 | 0.565 | 1.50 | |

*(Cont.)*

**Table 1.** (*Cont.*)
Physicochemical properties of the enhancers

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 43 | 1-Dodecylurea | 4.2 | 6 | 12 | 228.38 | 4.466 | −3.300 | 2.80 | $P=0.55$ |
| 44 | 1-Dodecyl-3-methylurea | 4.3 | 5 | 12 | 242.40 | 4.908 | −3.295 | 1.80 | $P=0.65$ |
| 45 | 1-Dodecyl-3-methylthiourea | 4.4 | 5 | 12 | 258.47 | 5.180 | −4.310 | 5.30 | $P=0.64$ |
| 46 | 1,3-Didodecylurea | 4.5 | 5 | 12 | 396.70 | 9.484 | −6.850 | 1.90 | $P=0.61$ |
| 47 | 1,3-Didodecylthiourea | 4.6 | 5 | 12 | 412.77 | 9.702 | −7.810 | 1.60 | $P=0.61$ |
| 48 | 1,3-Diphenylurea | 4.7 | 5 | 2 | 212.25 | 2.842 | −3.370 | 2.00 | |
| 49 | 1,3-Diphenylthiourea | 4.8 | 5 | 2 | 228.32 | 2.752 | −4.330 | 3.70 | |
| 50 | 1-Dodecyl-3-phenylurea | 4.9 | 5 | 12 | 304.48 | 6.675 | −3.940 | 1.10 | $P=0.62$ |
| 51 | 1-Dodecyl-3-phenylthiourea | 4.10 | 5 | 12 | 320.54 | 6.614 | −5.085 | 3.40 | $P=0.61$ |
| 52 | S,S-dimethyl-N-(benzoyl)iminosulfurane | 5a | 2 | 1 | 181.26 | 2.263 | −2.540 | 0.74 | |
| 53 | S,S-dimethyl-N-(4-chlorobenzenesulfonyl)iminosulfurane | 5b | 2 | 1 | 215.70 | 2.750 | −3.050 | 1.44 | |
| *54* | *S,S-dimethyl-N-(4-bromobenzoyl)iminosulfurane* | *5c* | *2* | *1* | *260.15* | *2.738* | *−2.830* | *23.12* | $P=0.91$ |
| 55 | S,S-dimethyl-N-(4-nitrobenzoyl)iminosulfurane | 5d | 4 | 1 | 226.26 | 2.175 | −3.610 | 0.77 | |
| 56 | S,S-dimethyl-N-benzenesulfonyliminosulfurane | 6.1 | 3 | 1 | 217.31 | 2.007 | −2.180 | 0.48 | |
| 57 | S,S-dimethyl-N-(2-nitrobenzenesulfonyl)iminosulfurane | 6.2 | 5 | 1 | 262.31 | 1.960 | −3.280 | 1.84 | |
| 58 | S,S-dimethyl-N-(3-nitrobenzenesulfonyl)iminosulfurane | 6.3 | 5 | 1 | 262.31 | 1.958 | −3.320 | 0.95 | |
| 59 | S,S-dimethyl-N-(4-nitrobenzenesulfonyl)iminosulfurane | 6.4 | 5 | 1 | 262.31 | 1.968 | −3.330 | 70.68 | |
| 60 | S,S-dimethyl-N-(*o*-tolylsulfonyl)iminosulfurane | 6.5 | 3 | 1 | 231.34 | 2.373 | −2.340 | 1.30 | |
| 61 | S,S-dimethyl-N-(*p*-tolylsulfonyl)iminosulfurane | 6.6 | 3 | 1 | 231.34 | 2.492 | −2.460 | 0.93 | |
| 62 | S,S-dimethyl-N-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane | 6.7 | 5 | 1 | 275.35 | 2.117 | −2.940 | 0.19 | |
| 63 | S,S-dimethyl-N-(4-chlorobenzenesulfonyl)iminosulfurane | 6.8 | 3 | 1 | 251.76 | 2.600 | −2.900 | 0.40 | |
| 64 | S,S-dimethyl-N-(4-nitro-1-naphthyl)iminosulfurane | 6.11 | 3 | 1 | 248.31 | 3.575 | −4.920 | 1.28 | |
| 65 | S,S-dimethyl-N-(4-cyano-1-naphthyl)iminosulfurane | 6.12 | 2 | 1 | 228.32 | 3.490 | −5.690 | 1.16 | |
| 66 | S,S-dimethyl-N-(5-nitro-2-pyridyl)iminosulfurane | 6.13 | 4 | 1 | 199.23 | 1.540 | −2.860 | 9.03 | |
| 67 | S,S-dimethyl-N-(2-methyl-4-nitrophenyl)iminosulfurane | 6.14 | 3 | 1 | 212.27 | 2.880 | −3.490 | 1.17 | |
| 68 | S,S-dimethyl-N-(4-nitrophenyl)iminosulfurane | 6.15 | 3 | 1 | 198.25 | 2.520 | −3.320 | 1.47 | |
| 69 | S,S-dimethyl-N-(4-phenylazophenyl)iminosulfurane | 6.16 | 3 | 2 | 257.36 | 3.775 | −4.060 | 2.81 | |
| 70 | S,S-dimethyl-N-[4-[(4,6-dimethylpyrimidine-2yl) aminosulfonyl]phenyl]iminosulfurane | 6.17 | 7 | 1 | 338.45 | 1.777 | −3.830 | 0.83 | |
| 71 | S,S-dimethyl-N-(2,4,6-trichlorophenyl)iminosulfurane | 6.18 | 1 | 1 | 256.58 | 4.292 | −4.690 | 2.21 | |
| 72 | S,S-dimethyl-N-[4-[(5-methoxypyrimidin-2yl) aminosulfonyl]phenyl]iminosulfurane | 6.19 | 8 | 1 | 340.43 | 1.495 | −4.300 | 70.72 | |
| Azone 73 | Azone, laurocapram | 1, 2 | 2 | 12 | 281.48 | 6.254 | −4.850 | 13.85** | |

Compounds are identifiable by their Michniak codes, e.g. compound 6.12 is the compound coded 12 in paper 6. Paper codes: 1,6. Kim et al (2001, 1999); 2. Michniak et al (1998); 3,4. Godwin et al (1997, 1998); 5. Strekowski et al (1999). *Mean value of 27.6 and 25.3: papers 2 and 3. **Mean value of 5.6 and 22.1: papers 1 and 2. Compounds misclassified by the discriminant analysis are *italicized* and the probability of the assignment shown in the last column.

the analyses were less successful, so this simplified approach only was reported. Molecular weights and calculated values of octanol/water (P) and solubility (S) expressed as molarity were obtained from Virtual Computational Chemistry Laboratory (http://146.107.217.178/). This gave values of log P and log S, calculated by five and two different procedures, respectively. Mean values were taken, with any anomalous results for log P being excluded. There were no experimental reports for log P and log S to compare or validate the predicted values generated.

All data were analysed with Minitab (version 14, Minitab Inc.) using standard statistical procedures, as described in detail previously (Pugh et al 2000; Magnusson et al 2004). The conditions are briefly summarized as follows.

Ideally all the variables used as predictors should not be correlated with one another, so that each contributes independently to the outcome. In practice this is unattainable and a cut off value of the correlation coefficient, r, was taken as 0.7. This means that less than 0.5 ($r^2 = 0.49$) of the variation in one predictor is attributable to another.

Analyses are therefore reported for the three 'independent' variables CC, HB and molecular weight. (See Results section.)

The $r^2$ values in the regression analyses were adjusted for degrees of freedom. Contour maps used the 'Area' option in Minitab and the contour levels were set at ER intervals of 5. Standardized (Z-transformed) values of variables were used in all multivariate analyses. Principal component analyses used the correlation matrix option and plots of the first two principal component scores were made to identify groupings of 'good' (ER > 10) enhancers. Cluster analysis used the single linkage and Euclidean distance options, as this showed the different clusters to best effect.

Discriminant analysis was used to decide whether a 'novel' compound was more likely to belong to the set of 'good' or 'poor' predictors. The boundary was set at ER = 10 as being a reasonable degree of enhancement to merit further investigation of a compound. Essentially the properties of a novel compound (CC, HB, molecular weight) were compared with the means for the good and bad subsets to see which it more closely resembled. The distance measure was chosen as the linear discriminant function and assignments used the cross-validation subcommand. Here, each compound in the dataset was excluded in turn and assigned to a group as a novel compound on the basis of the remaining compounds, so that its properties did not bias the analysis.

## Results and Discussion

### Correlations coefficients (r) amongst variables (Table 2)

As shown in Table 2, log P was highly correlated with log S and both correlated with molecular weight. Molecular weight, CC and HB had intraset correlation coefficients $< 0.7$, showing that $< 0.5$ of variation in any one was explained by variation in any other. These three simple, precise properties were therefore sufficiently independent to use as predictor variables.

**Table 2** Correlation coefficients (r) amongst variables

| | HB | CC | MW | log P |
|---|---|---|---|---|
| CC | 0.02 | | | |
| MW | 0.36 | 0.67 | | |
| log P | 0.08 | 0.86 | 0.80 | |
| log S | 0.04 | 0.66 | 0.82 | 0.91 |

HB, H-bonding atoms; CC, chain lengths; MW, molecular weight.

### Regression analysis

Use of log ER, rather than ER, gave statistically better regressions, and only these results were described in detail. The low *P*-values for HB and CC in equation 1 suggested that they were significant predictors, whilst molecular weight (MW) contributed little to the prediction and may be omitted without affecting the regression (equation 2).

$$\log \mathrm{ER} = 0.326 - 0.0756\ \mathrm{HB} + 0.0677\ \mathrm{CC} - 0.000072\ \mathrm{MW} \quad (1)$$

*P* values 0.015 0.008 <0.001 0.915

$r^2 = 0.53$

$$\log \mathrm{ER} = 0.318 - 0.0770\ \mathrm{HB} + 0.0668\ \mathrm{CC} \quad (2)$$

*P* values 0.004 0.002 <0.001

$r^2 = 0.54$

The low $r^2$ values showed that the regression was poor, and when fitted values were plotted against experimental ER values (Figure 1) the good enhancers were greatly underestimated.

In any structure–activity relationship the most desirable result is a mathematical relationship that relates outcome to a set of readily accessible molecular features. Usually this is achieved by a multiple regression analysis using linear or quadratic predictors. Whilst this may be realistic when the response relies on a single, well-defined mechanism, such as the interaction of a drug with a receptor site, it may well not apply to the poorly understood and quite possibly multiple mechanisms that determine enhancement. The barrier to transdermal diffusion within the stratum corneum is generally accepted as being an ordered array of bipolar lipids, and enhancement could

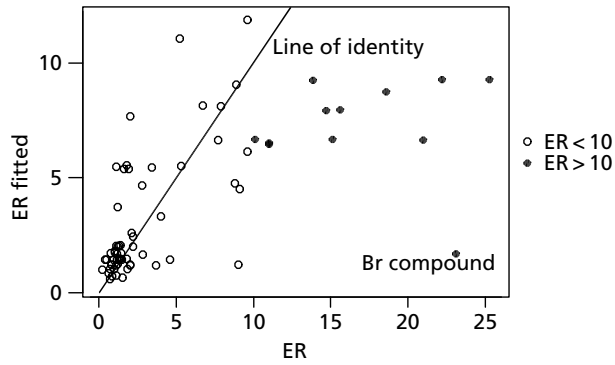


**Figure 1** Fitted ER values from the regression equation log ER = 0.326 − 0.077*HB + 0.0668*CC. All 'high activity' enhancers were under-estimated. Note that the Br compound (number 54 in Table 1) was very much underestimated by the regression.

conceivably involve various mechanisms such as interaction with polar and/or non-polar domains or simply destruction of the array by a solvent effect. Under these highly complex circumstances it is perhaps unsurprising that regression analysis completely failed to predict the activity of effective enhancers. The multivariate analyses used as an alternative make no assumption about the mathematical form of the activity–structure relationships.

## Contour mapping

Minitab has the facility to plot contour levels of a response on a two dimensional plot of predictor variables. Figure 2 showed the levels of ER using CC and HB as the plotting variables. Whilst no great level of precision could be inferred from this diagram, it indicated clearly that high enhancement activity was generally associated with CC > 12 and HB 3 or 4. The data were well scattered over the plotted area, and it must be remembered that some points represented several results since CC and HB were integers.

## Principal components and cluster analysis

An alternative approach was to define a boundary value separating 'good' and 'poor' enhancers. This was set at ER 10 as being a level of pharmaceutical interest. Our aim in this study was to find a set of predictors that would enable the good enhancers to be isolated from the others. The choice of predictors could be aided by principal components analysis, which detects relationships called principal components that account for the data variation in a table (matrix).

Principal components analysis used the correlation matrix for the CC, HB, and molecular weight dataset. Fifty-nine percent of the variation was contained in the first principal component and 33% in the second, showing that these two components gave a good two-dimensional spread of molecular features. The scores of the first and second principal components for individual compounds are plotted in Figure 3, which shows that the good enhancers, with the exception of the anomalous Br compound, were confined to one quadrant of the plot. This suggested that good enhancers could be identified by a combination of CC, HB and molecular weight. The presence of poor enhancers in the same area suggested that some poor enhancers might be falsely predicted as good.

This could be illustrated by cluster analysis, which separates the dataset into groups with similar combinations of properties. A dendrogram (Figure 4) connects pairs of compounds showing their level of similarity as the ordinate, and it may be used to indicate clustering of compounds on the basis of shared molecular properties. Using CC, HB, and molecular weight it could be seen that the set of compounds could be divided into two large subsets, and all the 'good' enhancers – except the anomalous Br compound – were in the same cluster.

## Discriminant analysis

To re-capitulate, it appeared that the variation in the properties of the compounds could be adequately described by a combination of the variations in their

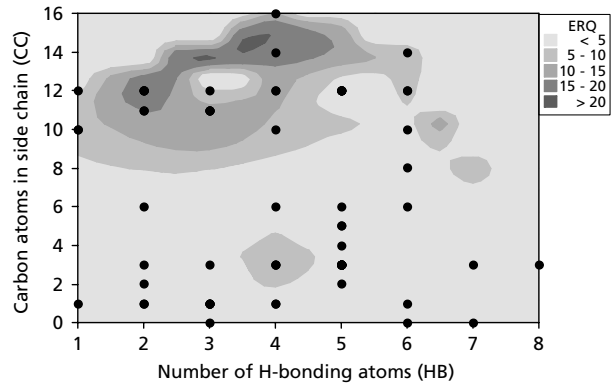


**Figure 2** Contour plots of enhancement ratio (ER). The general trend for good enhancers to have reasonably long side chains and polar head groups with fewer than 5 H-bonding groups is apparent. Many points represent more than one compound.

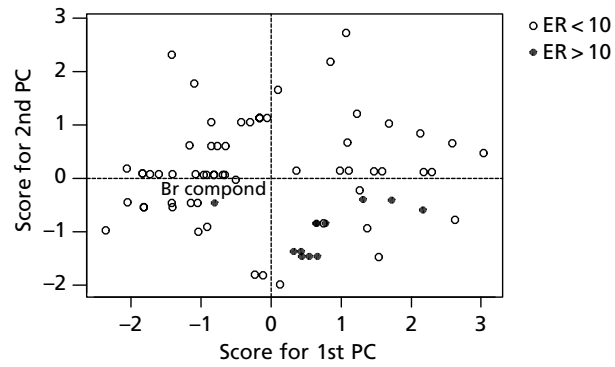


**Figure 3** Plot of the first and second principal components (PC) of the molecular weight, CC, HB values. There is segregation of compounds with ER values > 10. Note than the Br compound (number 54 in Table 1) was separated from the other good enhancers.

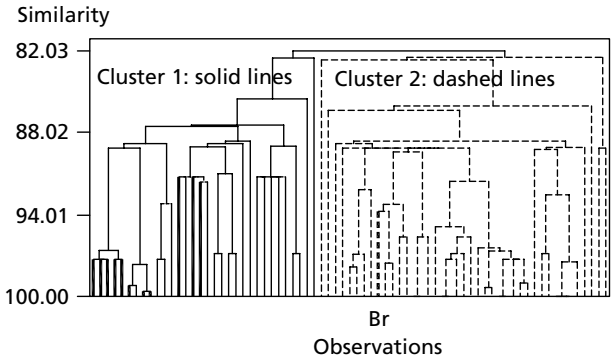


**Figure 4** Dendrogram from cluster analysis based on CC, HB, and molecular weight. Most compounds with ER > 10 (thick lines) were in cluster 1, indicating that these three molecular features should predict high ER with few false negatives. Some poor enhancers were in cluster 1, suggesting false positives when predicting a poor enhancer. Note the Br compound was in cluster 2.

HB, CC and molecular weight values. It remained to find a technique that enabled us to decide whether a novel compound would be a good or poor enhancer on the basis of its HB, CC and molecular weight values.

The method we used was discriminant analysis. The basis is that it calculates the means of the three properties for the good and poor enhancers. These two points can be visualized as being plotted on a three-dimension graph. The corresponding point for the novel compound is then added to the 3-D plot and its distance from the two mean points calculated. It is assigned to the group with the lower distance of separation. Furthermore the probability of its belonging to either group can be calculated by comparing the two distances. The procedure can of course be applied to any number of predictors and groups. Discriminant analysis theoretically works best when distinguishing outcomes (here ER) amongst groups of data containing similar numbers of data values and which are individually normally distributed. This is often unrealistic in practice and the boundary between 'good' and 'bad' enhancers was set at ER 10 on the basis that a 10-fold enhancement in drug absorption was a realistic basis for enhancer development. We used the cross-validation option in the Minitab package so that each of the 73 compounds could be treated as a novel compound and assigned to the good or poor groupings as determined by the remaining 72 compounds. Misclassified results were reported, with the probability values for belonging to the assigned subset. The results are summarized in Table 3.

**Table 3** Classifications from discriminant analysis with cross-validation

| Assigned group | True group | |
|---|---|---|
| | Poor | Good |
| Poor | 44 | 1 |
| Good | 17 | 11 |
| Total | 61 | 12 |
| Correct | 44 | 11 |
| Proportion | 0.72 | 0.92 |

n = 73; n correct = 55; proportion correct = 0.75.

## Classifications from discriminant analysis with cross-validation (Table 3)

As shown in Table 3, 17/61 (28%) false positives were returned for 'poor' enhancers, although six had an interesting degree of activity, with ER > 5. Additional guidance to the reliability of the results is given by the probability of correct group assignment (Table 1, final column).

Of much greater importance was the level of success in identifying good enhancers. Only one compound was misclassified as poor. This was the remarkable Br compound (number 54 in Table 1) reported by Strekowski et al (1999). It exhibits an ER approximately 20-times greater than its Cl (number 53) and $NO_2$ (number 57) analogues (Kim et al 1999; Strekowski et al 1999). While this type of analysis can identify broad trends, it cannot – in common with any such approach – hope to identify the truly exceptional, particularly if a different mechanism of action may be involved. It may be that such a large departure from the discriminant analysis results and its appearance in the top left quadrant of the principal component plot indicated a different mechanism of action, and suggested that it could be the lead compound for a set of brominated compounds.

It is recognized that the approach has been applied to the enhancement effect on a single drug. The effectiveness of an enhancer may vary with the physicochemical properties of the drug as measured by its log P value, and Williams & Barry (2004) recently reviewed the state of thinking regarding the enhancement of percutaneous absorption. We are currently examining how far our approach can be extended for a general prediction of activity.

## Conclusion

Multivariate regression analysis failed completely to relate ER to physicochemical features. From a pharmaceutical development perspective, all the good enhancers (ER > 10) were seriously under-predicted. Contour plotting of ER values showed that high activity was associated with carbon chain length >12 and 3 or 4 H-bonding atoms. Principal components analysis suggested that the variation in molecular properties of the dataset could be described by the variations in CC, HB and molecular weight. Cluster analysis showed that good enhancers were clustered together on the basis of these three simple, precisely known predictors, and discriminant analysis using them successfully assigned all except one of the good enhancers. This was the exceptional Br compound (number 54 in Table 1) reported previously (Kim et al 1999; Strekowski et al 1999). We consider that the procedure is sufficiently reliable to identify potential transdermal enhancers for in-vitro screening.

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