The Effect of Penetration Enhancers on Drug Delivery through Skin: A QSAR study

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Abstract

Skin penetration enhancers are used to allow formulation of transdermal delivery systems for drugs that are otherwise insufficiently skin permeable. A full understanding of the mode of action could be beneficial for the design of potent enhancers and for the choice of the enhancer to be used in topical formulation of a special drug. In this study, the structural requirements of penetration enhancers have been investigated using the Quantitative Structure-Activity Relationship (QSAR) technique. Activities of naturally occurring terpenes, pyrrolidinone and N-acetylprolinate derivatives on the skin penetration of 5-fluorouracil, diclofenac sodium, hydrocortisone, estradiol, and benazepril have been considered. The resulting QSARs indicated that for 5-fluorouracil and diclofenac sodium less hydrophobic enhancers were the most active. More precisely, molecular descriptors in the corresponding QSARs indicated the possible involvement of intermolecular electron donor-acceptor interactions. This was in contrast to the skin permeation promotion of hydrocortisone, estradiol, and benazepril by enhancers, where a linear relationship between enhancement activity and n-octanol/water partition coefficients of enhancers was evident. The possible mechanisms of penetration enhancement as suggested by the QSARs will be discussed.

1. Introduction

The transdermal route offers several advantages over other routes for the delivery of drugs with systemic activity. These include the ease of use and withdrawal, in case of side effects, and avoidance of first-pass metabolism. However, skin is resistant to the permeation of most external chemicals and drugs. Physical and chemical methods have been implemented in order to increase absorption of drugs through skin [1]. The chemical methods involve incorporation of specific chemicals in topical drug formulations in order to increase the penetration of drug. The penetration enhancers facilitate the absorption of penetrant through the skin by temporarily increasing the permeability of the skin. Some of the important penetration enhancers as classified by Sinha and Kaur [2] are terpenes and terpenoids, pyrrolidinones, fatty acids and esters, sulfoxides, alcohols and glycerides, and miscellaneous enhancers including phospholipids, cyclodextrin complexes, amino acid derivatives, lipid synthesis inhibitors, clofibric acid, dodecyl-N,N-dimethylamino acetate, and enzymes. Because of their widely different chemical structures, it is likely that the enhancers act by more than one mechanism and that their precise enhancer activity will depend on the physicochemical properties of the penetrant as well as the enhancer [3]. Yu *et al.* [4], in a study of oleic acid-induced transdermal diffusion pathways, showed that the mechanism of oleic acid chemical enhancer action depends on the physicochemical properties of the model drug.

The design of skin penetration enhancers would be facilitated by an understanding of their mode of action within the target tissue. Furthermore, it would be beneficial to the choice of the enhancer to be used in topical formulation of a certain drug, as the enhancing activities of enhancers towards different drugs are different. In this study,

the structural requirements for enhancement activities towards different drugs have been explored using the QSAR technique. Three chemical classes of enhancers, namely, terpenes, N-acetylprolinate esters and pyrrolidinone derivatives were investigated. Terpenes are naturally occurring volatile oils that appear to be promising candidates for use as clinically acceptable enhancers [5]. They have been reported to have good toxicological profiles, high percutaneous enhancement abilities, and low cutaneous irritancy at low concentrations [6]. Pyrrolidinones have recently become of interest to the pharmaceutical industry as penetration enhancers [7] and 2-pyrrolidinone-5-carboxylic acid is a component of the natural moisturizing factors in the skin [8]. N-acetylprolinate esters have been synthesized by Tenjarla *et al.*. [9] and have been characterised as novel penetration enhancers.

2. Material and Methods

2.1 Transdermal penetration enhancement data

The enhancers were selected on the basis that the number of enhancers whose activities towards a special drug have been measured under the same conditions was enough to construct a QSAR. The minimum number of observations (enhancers) for a single variable QSAR is five [10] but a higher number of chemicals will add to the robustness of the model.

a) Terpene penetration enhancers:

Figure 1 shows the molecular structure of the terpene enhancers used in this study. The enhancing activities of terpenes towards 5-fluorouracil, 5FU [11], hydrocortisone, HC [12], diclofenac sodium, DFS [13] and oestradiol [14] were used as the biological

response. Penetration-enhancing activities of terpenes were expressed as enhancement ratios (ER). The enhancement ratio for 5-FU and oestradiol is the permeability coefficient of the saturated solution of drug in water after terpene treatment (incubation with the pure terpene for 12 hours) divided by the permeability coefficient before terpene treatment through excised abdominal human skin [15]. The enhancement ratio for DFS and HC is the ratio of the permeability coefficient with enhancer to that obtained with control formulation without terpene. DFS was formulated as carbopol gel containing propylene glycol with the terpene concentration of 1% (w/w) and the penetration was measured in abdominal rat skin [13]. HC was formulated as HPMC gels containing ethanol, water and glycerol with 2% terpene [12] and hairless mouse skin was used as barrier. The enhancement ratios are listed in Table 1. In QSAR analyses throughout the paper logarithm of the ratio is used.

[FIGURE 1 HERE]

[TABLE 1 HERE]

b) Pyrrolidinone derivatives

The transdermal penetration-enhancing abilities of 16 pyrrolidinone derivatives (Figure 2) towards HC have been measured using hairless mouse skin *in vitro*. Skins were pretreated for 1 h with the enhancer in propylene glycol before application of the drug also in propylene glycol. Enhancement ratios have been reported for permeability coefficient (ER (k_P)), and 24-h receptor concentration (ER_{Q24}) [16]. The enhancement ratios are tabulated in Table 2.

[FIGURE 2 HERE]

[TABLE 2 HERE]

c) N-acetylprolinate esters

The series consists of N-acetylprolinate esters with the alkyl side chain lengths of 5-18 carbon atoms and Azone (Figure 3). The enhancement activities towards HC and benazepril have been measured *in vitro* using full thickness hairless mouse skin. Saturated drug solutions in propylene glycol with or without enhancers (5% (w/v)) have been used as the donor phase [9] and the enhancement ratios for permeability coefficients were reported (Table 3).

[FIGURE 3 HERE]

[TABLE 3 HERE]

2.2. Structural descriptors

The structures of the enhancers were generated and optimised using the COSMIC force field and the molecular mechanical descriptors were obtained using the NEMESIS software. The software was distributed by Oxford Molecular Ltd (Oxford, UK), Oxford Molecular was incorporated into Accelrys Inc. and the software packages are available through Accelrys Inc. The descriptors consisted of solvent accessible surface area, and the highest and the lowest electrostatic potential on the surface. The MNDO Hamiltonian in MOPAC 7.0 (QCPE, Department of Chemistry, Indiana University, 800 East Kirkwood Ave., Bloomington) was used for further minimisation of the structure and calculation of molecular orbital descriptors. These consisted of atomic charges, dipole moment, molecular weight, energies of the highest occupied and the lowest unoccupied molecular orbitals, as well as electrophilic and

nucleophilic superdelocalisability indices. Log P was calculated using the ACD / log D software (Advanced Chemistry Development Incorporated, Ontario, Canada). Molecular connectivity and molecular shape indices, as well as the atom level and bond electrotopological state indices were calculated by MOLCONN-Z software version 3.15 (Hall Associates, Quincy, MA). Molar volume, energy of vaporization and solubility parameter were calculated by a group contribution method [17].

2.3. Development of QSARs

Stepwise regression analysis was used to determine statistically significant relationships between structural parameters and the penetration enhancer activity. The statistical analyses were performed using the MINITAB statistical software (version 13.1, MINITAB Inc.). In order to minimise the risk of chance correlations the maximum p-value for a descriptor to be included in equations was set at 0.10 and maximum number of descriptors in equations was lower than one fifth of the number of observations. Furthermore, the correlation between log ER and log P was explored by linear regression analysis and the resulting equation was reported for each dataset. The following statistical criteria of the models were noted: n the number of observations, r^2 the squared of the correlation coefficient, s the standard deviation, F the Fisher statistic and the P value.

3. Results

3.1. Enhancement activity of terpenes towards 5FU

Following equation was resulted for the 26 terpene enhancers:

$$\text{Log ER} = 0.138\ (\pm 0.26) - 5.79\ (\pm 0.95)\ q^{-} - 0.46\ (\pm 0.13)\ E_V/10^4 \qquad (1)$$

$N = 26 \quad s = 0.329 \quad r^2 = 0.627 \quad F = 19.3 \quad P < 0.0005$

In equation 1, q^- is the lowest atomic charge in the molecule and E_V is the free energy of vaporisation. Although q^- is an electrostatic parameter explaining electrostatic interactions, it has been shown that it can also model hydrogen bonding in QSAR equations, with 'low' q^- values (high negative charges) leading to 'high' ability to accept hydrogens in hydrogen-bonding interactions [18, 19]. Therefore the negative slope of q^- in equation 1 can indicate that increasing hydrogen-bonding-acceptor ability increases the enhancement ratio towards 5FU. The correlation with q^- indicates that ketones, ethers and alcohols are better enhancers than are hydrocarbons. This can be shown by calculating the mean and standard deviation of ER values for alcohols, ethers, ketones and hydrocarbons as 0.97(±0.30), 1.24(±0.61), 1.18(±0.35) and 0.29(±0.13), respectively. The equation also shows that terpenes with lower energies of vaporization are better penetration enhancers than are those possessing higher energies of vaporisation. Cyclic ethers and alcohols possess the lowest and the highest E_V respectively. Thus a lower enhancement ratio for alcohols and the highest ER for cyclic ethers will be expected. This is evident in the scatter plot between the observed log ER and log ER calculated by equation 1 (Figure 4). Cyclohexene oxide is an outlier from equation 1 and its exclusion from regression analysis improves the correlation considerably ($r^2 = 0.762$).

[FIGURE 4 HERE]

Linear regression between log ER and partition coefficient resulted in equation 2:

$$\text{Log ER}_{5FU} = 1.49\ (\pm 0.18) - 0.148\ (\pm 0.04)\ \log P \qquad (2)$$

$n = 26 \quad s = 0.428 \quad r^2 = 0.341 \quad F = 12.4 \quad P = 0.002$

Table 4 shows the equations obtained for the enhancement activities of different chemical groups. Note that the structural descriptors (selected by stepwise regression) in these equations are different. In other words different structural features are controlling the enhancement capabilities of each chemical class. In equations 3, $S_N^=$ is the average of nucleophilic superdelocalisability indices for carbon atoms with double bonding. Nucleophilic superdelocalisability index for an atom is the sum of squares of the coefficients of atomic orbitals in each molecular orbital divided by the energy of that molecular orbital calculated for the unoccupied molecular orbitals [20]. The indices have been widely used in QSAR studies and are especially useful for modelling of intermolecular interactions [21]. Equation 3 may indicate that an intermolecular electron donor-acceptor interaction is involved in the enhancement process by hydrocarbons.

In equation 4, log MW (logarithm of molecular weight) shows that smaller alcohol molecules with a higher number of double bonds are better penetration enhancers. X0 in this equation is the difference between simple and valence corrected zero-order connectivity indices calculated by MOLCONN-Z software; therefore values of X0 show the presence of heteroatoms or double (or triple) bonds. As all the alcohols under the study have only one heteroatom (oxygen), the higher X0 values in this series correspond to those molecules containing higher numbers of double bonds.

Presence of q^- in equation 5 and 6 indicates that a higher negative charge on the oxygen atoms of ethers and ketones increases the enhancement activities.

[TABLE 4 HERE]

3.2. Enhancement activities of terpenes towards HC

Stepwise regression analysis indicated that log P was the most significant descriptor of enhancement ratio of terpenes towards HC:

$$\text{Log ER} = 0.719\ (\pm 0.09) + 0.153\ (\pm 0.03)\ \log P \qquad (7)$$

$N = 12 \quad s = 0.089 \quad r^2 = 0.760 \quad F = 31.6 \quad P = 0.000$

Comparing equations 7 and 2 reveals different structural requirements for terpenes to enhance penetration of hydrocortisone or 5FU; a high lipophilicity of terpenes will increase ER towards HC, while it will reduce ER towards 5FU. El-Kattan *et al.*. [12] suggested that the higher thermodynamic activity was responsible for the higher enhancement activity of hydrocarbon terpenes towards HC in the gel formulation.

3.3. Enhancement activities of terpenes towards DFS

Arellano and co-workers [13] investigated the enhancing effect of some terpenes on the *in vitro* percutaneous absorption of DFS from carbopol gels containing propylene glycol. The terpenes were from the chemical classes of hydrocarbons, alcohols, ketones and oxides. Unfortunately the thermodynamic activities of different terpenes were not equal as they were used at 1% w/w concentration in the gels. Assuming similar solubilities for the terpenes in the gel formulae, the enhancement ratio, which is the ratio of the k_P value with enhancer to that obtained with control gel, was analysed.

$$\log \text{ER} = 0.297\ (\pm 0.18) + 0.017\ (\pm 0.006)\ ESP^{+} \qquad (8)$$

$n = 8 \quad s = 0.2977 \quad r^2 = 0.554 \quad F = 7.4 \quad P = 0.034$

ESP^+ is the highest electrostatic potential on the solvent accessible surface of the molecules. This parameter describes the electrostatic intermolecular interactions (including hydrogen bonding) [19]. Therefore, equation 8 shows the positive effect of hydrogen bonding donor ability on the enhancement activity towards DFS. Correlation with log P is statistically insignificant (P = 0.17).

3.4. Enhancement activities of terpenes towards ES

Pretreatment of human epidermal membranes with terpenes results in a change in the permeability towards oestradiol [14]. Stepwise regression analysis of the enhancement ratios against the structural parameters of the enhancers resulted in the following QSAR:

$$\log ER = 0.743\ (\pm 0.30) - 0.206\ (\pm 0.03)\ S(I) - 2.91\ (\pm 1.5)\ q^- \qquad (9)$$

$n = 12 \quad s = 0.232 \quad r^2 = 0.853 \quad F = 26.1 \quad P = 0.000$

In equation 9, S(I) is the highest electrotopological state index in a molecule. Electrotopological indices encode information about both the topological environment of that atom and the electronic interactions due to all other atoms in the molecule; they may also be considered as measures of atomic electronic accessibility [22]. The ranking of different chemical classes of the terpenes with increasing S(I) values is hydrocarbons, ethers, alcohols and ketones. Hence increasing S(I) values correspond to the decreasing log ER values. In other words, hydrocarbons are the most potent enhancers and alcohols and ketones are the weakest. However, within the chemical classes, those with lower q^- value have a higher activity.

Menthone is an outlier from this equation and its deletion improves the equation:

$\log ER = 0.686\ (\pm 0.24) - 0.248\ (\pm 0.03)\ S(I) - 4.08(\pm 1.33)\ q^-$ (10)

$n = 11 \quad s = 0.189 \quad r^2 = 0.906 \quad F = 38.7 \quad P = 0.000$

There is no correlation between the enhancement ratios and log P for this set (P = 0.801).

3.5. Enhancement activities of pyrrolidinone derivatives towards HC

Stepwise regression analysis was performed for the enhancement ratios of permeability coefficient (k_p, cm/h) and receptor concentration at 24 h (Q_{24}, μM). This resulted in the following QSARs:

$\text{Log ER } (k_p) = -0.281\ (\pm 0.11) + 1.23\text{E-}5\ (\pm 0.18\text{E-}5)\ (SA)^2$ (11)

$N = 16 \quad r^2 = 0.773 \quad s = 0.30 \quad F = 47.7 \quad P = 0.000$

$\text{Log ER } (Q_{24}) = -0.083\ (\pm 0.06) + 0.84\text{E-}5\ (\pm 0.11\text{E-}5)\ (SA)^2$ (12)

$N = 16 \quad r^2 = 0.809 \quad s = 0.18 \quad F = 59.4 \quad p = 0.000$

In equations 11 and 12, SA is accessible surface area of pyrrolidinone derivatives. The relationships of log ER(k_p) and log ER(Q_{24}) with SA^2 indicates that larger pyrrolidinone derivatives are better enhancers of hydrocortisone penetration. Surface area is often correlated with the hydrophobicity of molecules, and in this pyrrolidinone series the correlation between SA^2 and log P has an r^2 value of 0.809. The correlation of log ER (k_p) with log P was also explored and a weak positive correlation resulted:

Log ER (k_p) = 0.114 (±0.10) + 0.172 (±0.04) log P (13)

N = 16 r^2 = 0.621 s = 0.38 F = 23.0 p = 0.000

3.6. Enhancement activities of N-acetylprolinate esters towards HC

Enhancement ratios for permeability coefficient (ER (k_p)), diffusion coefficient (ER (D)) and membrane vehicle partition coefficient (ER (K_m)) were analysed using stepwise regression analysis. The results showed that HDNAP (Figure 3) was an outlier from correlations. This could be due to the methods used for skin permeation studies using this enhancer. Unlike other enhancers, HDNAP was not soluble in propylene glycol, and therefore ethanol was used as a cosolvent [9]. Although a different control containing the same amount of ethanol was used for ER calculation, ethanol might have induced a synergistic effect with HDNAP. The synergy between ethanol and some other enhancers has been reported previously [23]. The following QSARs were obtained from stepwise regression analyses for the remainder of the enhancers:

Log ER (k_p) = - 1.76 (±1.23) + 1.21 (±0.48) log SA (14)

n = 7 s = 0.101 r^2 = 0.560 F = 6.4 P = 0.053

Log ER (K_m) = 1.31 (±0.19) - 0.103 (±0.035) log P (15)

n = 7 s = 0.181 r^2 = 0.629 F = 8.5 P = 0.033

log ER (D) = - 0.19 (±0.16) + 0.158 (±0.03) log P (16)

n = 7 s = 0.154 r^2 = 0.845 F = 27.3 P = 0.003

The correlation between log ER (k_p) and log P is:

Log ER (k_p) = 1.13 (±0.12) + 0.0452 (±0.02) log P (17)

n = 7 s = 0.111 r^2 = 0.461 F = 4.3 P = 0.093

The positive coefficient of log P in equations 16 and 17, and the negative coefficient in equation 15, indicate that the positive relationship between enhancement of k_P and log P of the enhancers is due to the increased ER of drug diffusion to the skin by enhancers with higher lipophilicity and not due to the increased partitioning.

3.7. Enhancement activities of N-acetylprolinate esters towards benazepril

For the reasons explained in section 3.5, HDNAP was an outlier in the QSARs and its exclusion resulted in the following QSARs from stepwise regression:

Log ER (k_p) = - 0.552 (±0.46) + 0.314 (±0.09) log P (18)

n = 7 s = 0.438 r^2 = 0.728 F = 13.4 P = 0.015

Log ER (K_m) = - 0.397 (±0.52) + 0.296 (±0.10) log P (19)

n = 7 s = 0.493 r^2 = 0.653 F = 9.4 P = 0.028

The positive correlation between log ER (D) and log P was not statistically significant (P = 0.23) and is not presented here. The higher coefficient of log P in equation 18 compared with that in equation 19 shows the effect of increased diffusion in higher lipophilicity enhancers.

4. Discussion

This study presents QSARs for enhancement ratios of skin penetration of penetrants by various chemical enhancers. The aim was to find the structural requirements of

chemicals in order to act as skin penetration enhancers of different drugs. Due to different procedures used for skin permeation studies, including the animal source of skin, the enhancer concentration, and the solvent, it was not possible to combine the ERs from different experiments. The enhancement ratios of terpenes towards 5FU depend mainly on the hydrogen bonding characteristic of the enhancers (equation 1). Moghimi *et al.* [11] suggested that terpenes might increase the permeation of 5-fluorouracil through the stratum corneum as a result of a molecular complex formation between the drug and the enhancer. The equation shows that an electron donor-acceptor interaction could be involved in the facilitated transport, which may or may not be between the drug and the enhancer. Likewise, the QSARs obtained for different chemical classes of terpenes (Table 4, equations 3-6) involve descriptors indicative of possible intermolecular interactions: In equation 3 the nucleophilic superdelocalisability index of double bonding carbon atoms could be an indicator of charge transfer interactions. Equation 4 shows that a higher number of double bonds (indicated by a high X0) in smaller (low molecular weight) alcohol molecules have a higher enhancing potency. Moreover, equations 5 and 6 involve correlation of ER with the most negative atomic charge, which is often an indicator of hydrogen bonding acceptor ability [19].

Hydrophobicity has a negative effect on the enhancement activity of terpenes towards 5FU (equation 2). This is in contrast with the positive correlation observed between log P and ER of terpenes, pyrrolidinones, and N-acetylprolinates, towards HC (equations 7, 13 and 17, respectively), and ER of N-acetylprolinates towards benazepril (equation 18). This is also in contrast with previous findings that suggest a positive or parabolic correlation between hydrophobicity and ER. Among these are the study of Aungst *et al.* [24] indicating a parabolic effect of alkyl chain lengths of

enhancers of naloxone penetration through human skin *in vitro*, and the parabolic relationship observed between the enhancement ratio of ketoprofen percutaneous absorption and octanol/water partition coefficient of cyclohexanol derivatives as the enhancers [25]. However, a number of studies indicate that the enhancing effect of an enhancer depends on actual the permeation pathway of the drug [26, 4, 27]. Accordingly, the activity of an enhancer is related to the structure of the drug as well as that of the enhancer. There are several suggested mechanisms (action sites) involved in the penetration enhancement activities of various enhancers. These have been summarised by Barry [28, 29] as the lipid-protein-partitioning theory. According to this theory, accelerants may act by one or more of the three main mechanisms. They may alter the lipid domain of the stratum corneum, may interact with the protein components, or may increase partitioning of the model drug or the coadministered vehicles, or of water into the skin. The alteration of the lipid domain occurs by fluidisation of the stratum corneum lipids. Figure 5 shows the chemical structures and some properties of the penetrants used in this study. It can be seen that 5FU is much more hydrophobic than are HC and benazepril. Therefore, the different structural characteristics of enhancers required for the promotion of 5FU, HC and benazepril transport might be due to a different mechanism by which the drug moves across the SC. Another explanation could be the molecular complex formation between 5FU and the terpenes as suggested by Moghimi *et al.* [11].

[FIGURE 5 HERE]

For estradiol, although there was no statistically significant correlation between log ER and log P, equation 9 shows that hydrophilic alcohols and ketones are weak and

hydrophobic hydrocarbons strong penetration enhancers. Considering the high hydrophobicity of estradiol (log D = 4.13), this follows the argument made earlier. Enhancement of DFS penetration, on the other hand, shows a positive relationship with the maximum electrostatic potential on the surface of the terpene enhancers (equation 8), suggesting a higher activity for the alcohols in comparison with ethers, ketones and hydrocarbons. Furthermore, there is no significant correlation with log P. It can be seen in Figure 5 that log D for DFS is lower than that for estradiol, but, it is slightly higher than that for benazepril. Considering, for the latter drug, the positive relationship of enhancement activities of N-acetylprolinate esters with hydrophobicity, a similar correlation is expected for the enhancers of the more hydrophobic DFS. However, it should be noted that the type of enhancers, the animal speices used for the penetration study and the experimental procedures (concentration, solvent, etc) are different for the two series of enhancers. Moreover, the pK_a value used for the calculation of log D at pH 7.4 is experimentally measured for DFS but is estimated for benazepril. Therefore, it may be that the acidity has been overestimated, leading to an underestimated log D for benazepril.

A final note that is worth stressing is that the skin types used for some of the penetration studies are not human skin: In the study of the effect of tepenes on DFS penetration abdominal rat skin has been used; for the studies on the effects of terpenes on the penetration of HC, as well as pyrrolidinone derivatives and N-acetyl prolinate esters the barrier used is hairless mouse skin. Therefore the results of QSAR analyses for penetration enhancement through human skin might be different.

Conclusion

Structural requirements for penetration enhancement of several penetrants with varying lipophilicities by three groups of enhancers were studied using QSAR technique. The resulting QSARs for enhancement towards different drugs incorporated different structural descriptors suggesting involvement of different mechanisms. For 5-fluorouracil and diclofenac sodium molecular descriptors in the corresponding QSARs indicated the possible involvement of intermolecular electron donor-acceptor interactions. Effect of log P of enhancers on the enhancement ratio was contradictory for different drugs. It ranged from a negative effect for 5-fluorouracil to a positive effect for hydrocortisone. The QSARs could shed some light on the mechanism of drug penetration.

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Table 1. Penetration enhancement activities of terpenes towards 5-fluorouracil (5FU), hydrocortisone (HC), diclofenac sodium (DFS) and oestradiol (ES)

No.	Terpene	ER			
		5FU [a]	HC [b]	DFS [c]	ES [d]
1	(+)-β-Cedrene	2.7	-	-	-
2	(-)-trans-Caryophyllene	2.0	-	-	-
3	1R-(+)-α-Pinene	1.2	28.4	-	3.09
4	(+)-Limonene	2.1	-	3.53	3.75
5	(+)-Longifolene	1.7	-	-	-
6	(-)-Guaiol	3.8	-	-	-
7	(+)-Aromadendrene	2.5	-	-	-
8	Safrole	5.0	-	-	-
9	(+)-Cedrol	4.6	13.1	-	-
10	R-(-)-Carvone	12.0	-	-	0.10
11	(+)-Limonene oxide	11.0	-	-	1.61
12	Cyclopentene oxide	31.0	18.7	-	-
13	(-)-Menthone	38.0	-	3.07	0.36
14	Cyclohexene oxide	2.4	-	-	1.42
15	(-)-α-Pinene oxide	14.0	10.1	-	1.90
16	1R-(-)-Fenchone	7.8	14.5	1.87	-
17	1,8-Cineole	94.0	-	1.39	4.40
18	7-Oxabicyclo [2.2.1] heptane	92.0	-	-	4.93
19	Phytol	3.4	-	-	-
20	Farnesol	14.0	35.3	-	-
21	Nerolidol	23.0	-	13.60	-
22	(-)-Carveol	20.0	-	-	0.42
23	(-)-α-Bisabolol	8.4	16.9	-	-
24	Geraniol	18.0	13.3	18.97	-
25	α-Terpineol	9.4	11.3	-	0.33
26	(+)-Terpinen-4-ol	10.0	-	-	0.45
27	Verbenone	-	11.5	-	-
28	Thymol	-	11.0	4.74	-
29	Cymene	-	-22.9	-	-
30	Menthol	-	-	10.63	-
31	3-Carene	-	-	-	4.36
32	Pulegone	-	-	-	0.34
33	Piperitone	-	-	-	0.17
34	Ascaridole	-	-	-	4.75

[a] data taken from Moghimi *et al.* [11]; [b] data taken from El-Kattan *et al.* [12]; [c] data taken from Arellano *et al.* [13]; [d] data taken from Williams and Barry [14].

Table 2. Penetration enhancement activities of pyrrolidinone derivatives

No.	ER (k_p)	ER (Q_{24})
1	5.4	5.2
2	42.0	23.0
3	0.6	1.2
4	0.9	1.0
5	1.2	1.1
6	0.8	1.3
7	1.4	1.3
8	3.9	1.4
9	0.8	1.2
10	0.4	1.8
11	0.7	1.1
12	1.4	1.4
13	41.0	11.0
14	0.9	1.1
15	1.0	1.2
16	1.9	2.0

Table 3. Penetration enhancement activities of N-acetylprolinate esters

Compound	Hydrocortisone			Benazepril		
	ER (k_p)	ER (K_m)	ER (D)	ER (k_p)	ER (K_m)	ER (D)
PNAP	14.4	7.65	1.94	1.2	1.56	0.92
ONAP	17.7	14.96	1.24	1.0	1.06	0.91
DNAP	18.2	6.60	2.86	4.5	5.44	0.80
UNAP	30.6	6.70	4.89	40.1	53.75	0.70
DDNAP	34.3	8.46	4.41	23.7	28.63	0.78
HDNAP	13.8	4.07	1.80	6.1	3.02	1.98
Oleyl-NAP	27.1	2.14	14.79	40.6	35.25	1.11
Azone	22.0	3.68	6.90	67.7	82.44	0.79

Table 4. QSARs obtained for different chemical classes of terpenes

No.	Chemical group	Equation	n	r^2	s	F	P
3	Hydrocarbons	Log ER = 31.8 (±8.37) - 120 (±31.8) $S^{=}_{N}$	6	0.780	0.067	14.2	0.02
4	Alcohols	Log ER = 3.77 (±0.55) - 1.59 (±0.23) log MW + 0.931 (±0.09) X0	10	0.958	0.070	79.0	0.00
5	Ethers	Log ER = - 3.72 (±1.9) – 18.2 (±7.1) q^{-}	7	0.566	0.438	6.5	0.05
6	Ethers & ketones	Log ER = - 3.58 (±1.7) - 17.4 (±6.2) q^{-}	10	0.496	0.394	7.8	0.02

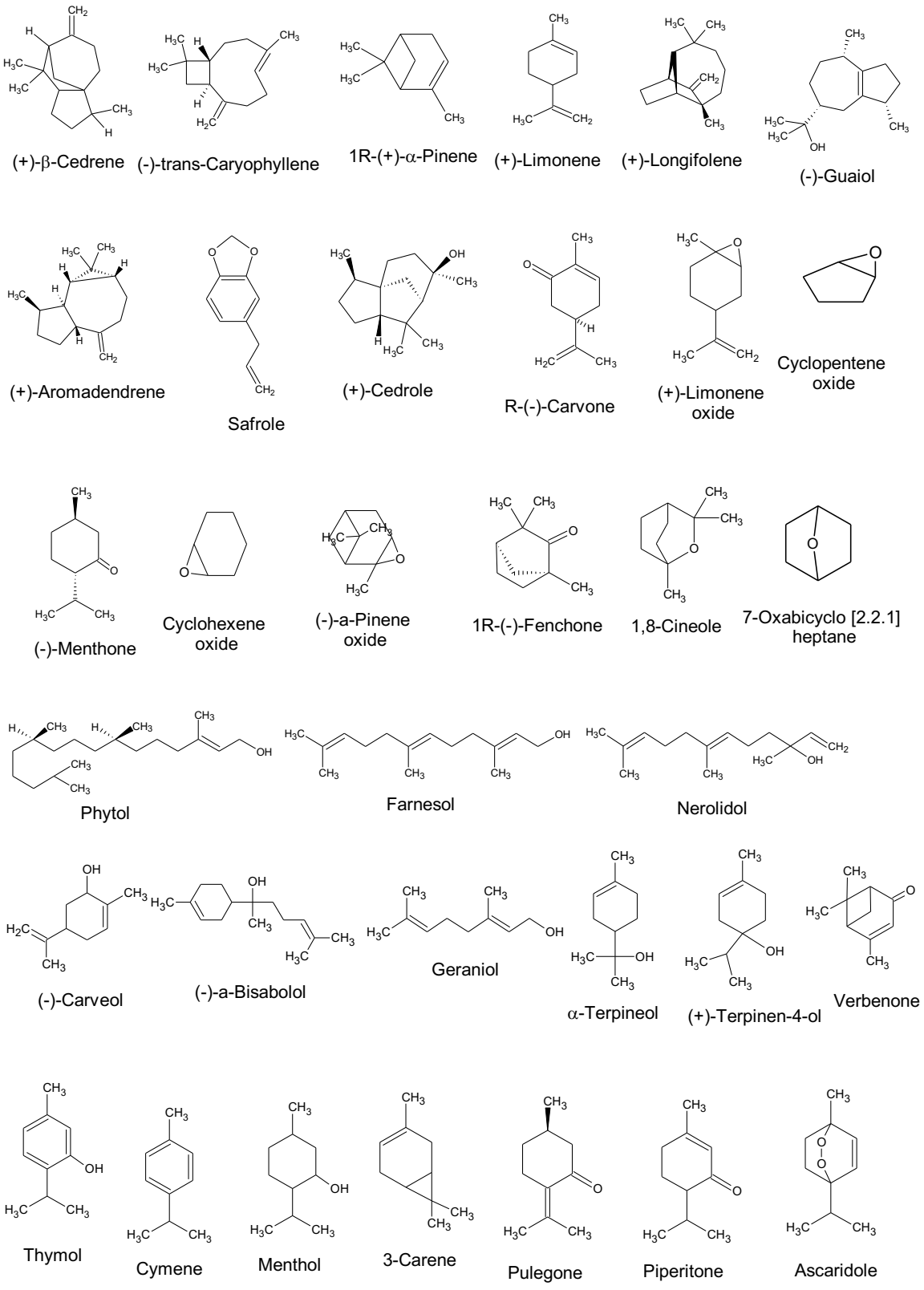


Figure 1. Chemical structures of terpenes.

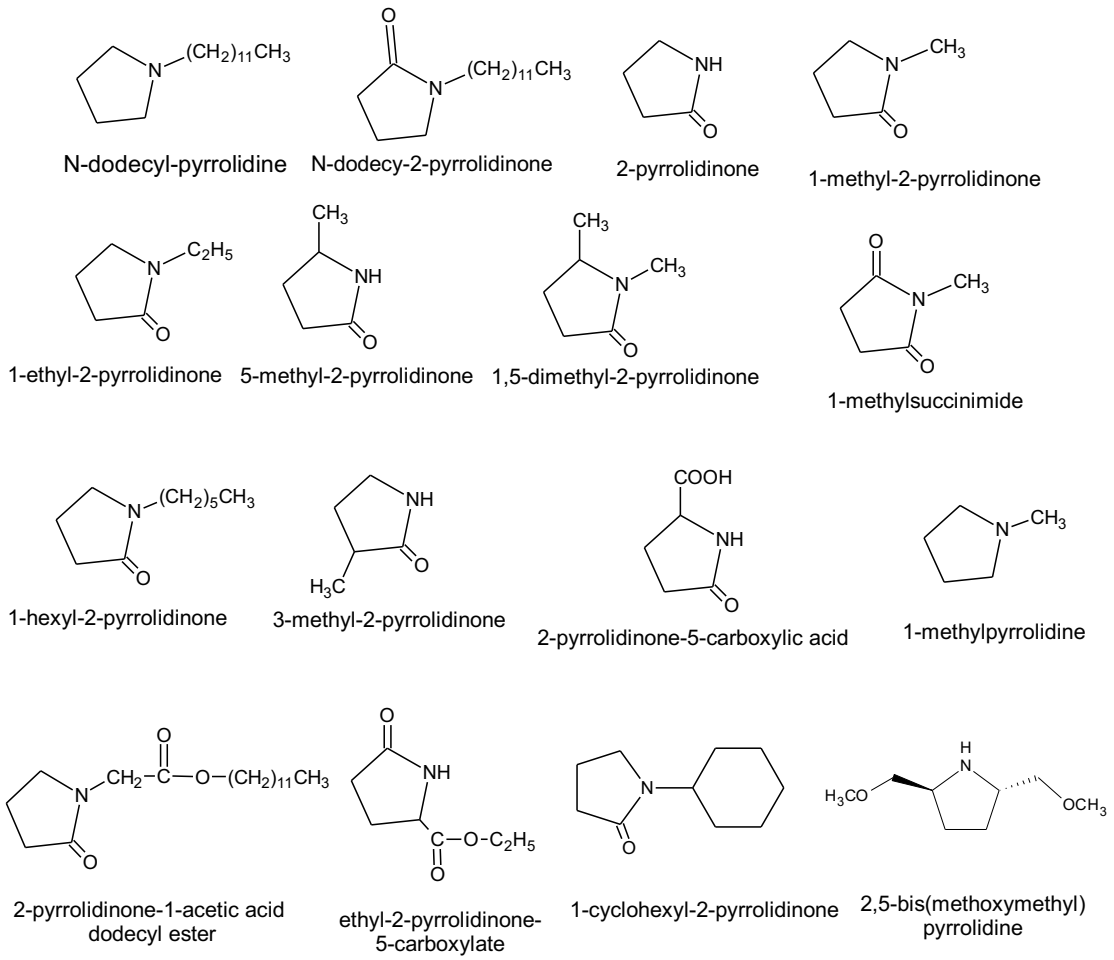
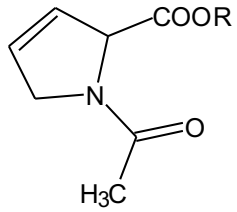
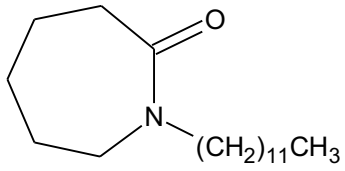


Figure 2. Chemical structures of pyrrolidinone derivatives.



N-Acetylprolinate
(Compounds 1-7)

No.	Compound	R
1	n-Pentyl-N-acetylprolinate (PNAP)	$-C_5H_{11}$
2	n-Octyl-N-acetylprolinate (ONAP)	$-C_8H_{17}$
3	n-Decyl-N-acetylprolinate (DNAP	$-C_{10}H_{21}$
4	n-Undecyl-N-acetylprolinate (UNAP)	$-C_{11}H_{23}$
5	n-Dodecyl-N-acetylprolinate (DDNAP	$-C_{12}H_{25}$
6	n-Hexadecyl-N-acetylprolinate (HDNAP)	$-C_{16}H_{33}$
7	9-Octadecenyl-N-acetylprolinate (Oleyl-NAP)	$-C_{18}H_{35}$



Compound 8:
1-Dodecylazacycloheptan-2-one
(Azone)

Figure 3. Chemical structures of N-acetylprolinate esters and Azone.

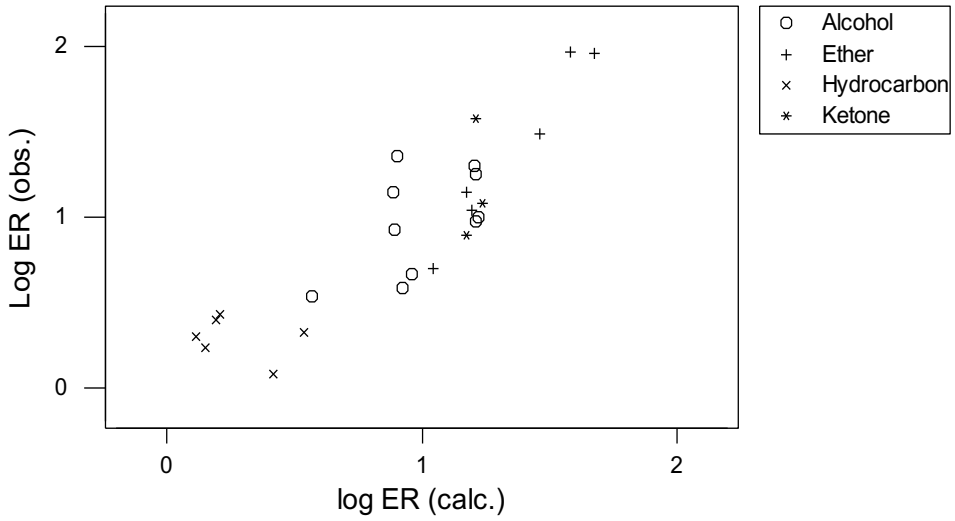
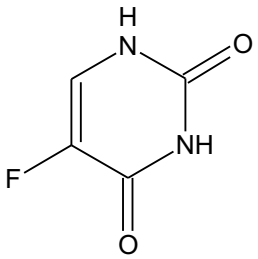
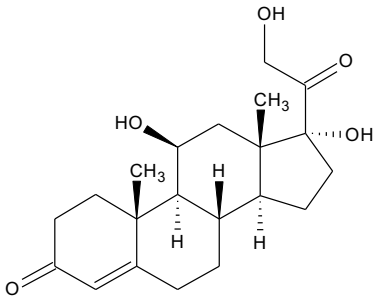


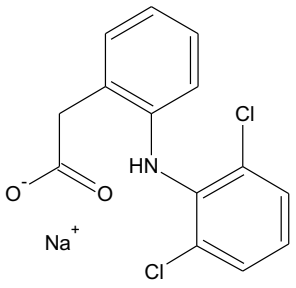
Figure 4. Scatter plot between observed log ER and the log ER calculated using equation 1.



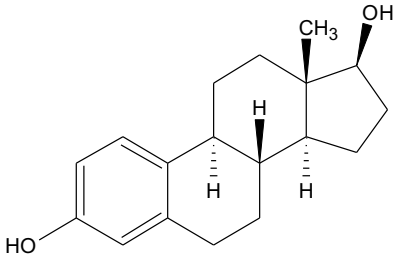
5-FU
log P = -0.78
log D = -0.90
pKa = 7.86



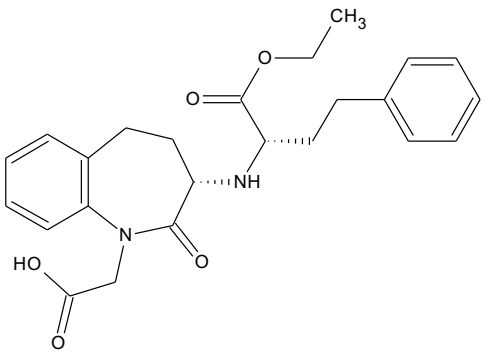
HC:
log P = 1.43
log D = 1.43
pKa = 12.48



DFS:
log P = 4.06
log D = 0.95
pKa = 4.01



ES:
log P = 4.13
log D = 4.13
pKa = 10.27



Benazepril:
log P = 3.86
log D = 0.46
pKa (acid) = 3.73
pKa (base) = 4.55

Figure 5. Chemical structures of drugs (penetrants) together with some of the physicochemical properties calculated by ACD/log D Suite.