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Role of complexes formation between drugs and penetration enhancers in transdermal delivery

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ABSTRACT

The use of chemical penetration enhancers (CPE) is growing due to their ability to improve drug delivery through the skin. A possible mechanism of penetration enhancement could involve the complex formation between drug and components in the pharmaceutical formulation, thus altering the physicochemical properties of the active substance. Here, modelling studies indicate that hydrocarbon and oxygen-containing terpenes (penetration enhancers) could form complexes with drugs. Satisfactory correlations have been obtained between the predicted molecular properties of enhancers and their enhancement effects.

1. Introduction

Transdermal drug delivery offers a variety of advantages over oral and intravenous dosage, such as sustained release directly to the blood stream over a long period of time, bypass of the gastrointestinal and hepatic elimination pathways, high patient compliance, and easily administered dosage form that is portable and inexpensive.

Skin penetration enhancers are used to optimize formulation of transdermal delivery system for drugs that are otherwise insufficiently skin-permeable. Numerous compounds have been evaluated for penetration enhancing activity (William and Barry, 2004). Because of their diverse 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 (Hadgraft and Walters, 1993; Yu et al., 2003; Hadgraft, 2004).

The design of skin penetration enhancers (PE) would be facilitated by an understanding of their mode of action within the target tissue. Since the same enhancer can have different effects on permeability of different drugs, rationalization of a penetration mechanism of a selected enhancer for a particular drug would be beneficial for the right choice of components in a transdermal formulation. It was also noted that molecular simulations can be used in the design of pharmaceutical formulations as a useful tool in the optimization of transdermal delivery systems (Hadgraft, 2004).

Terpenes represent one of the favourable penetration enhancer groups due to their low toxicity and irritability profile. Different chemical structures can be found in this chemical group. There are two main types: non-polar hydrocarbons and oxygen-containing molecules. The latter can have epoxy, hydroxyl, keto, carboxyl or ester functionalities as hydrogen bond acceptor (HBA) or hydrogen bond donor (HBD) groups. Consequently, different modes of enhancing permeability are possible, making terpenes suitable enhancers for both hydrophilic and hydrophobic permeants (Hori et al., 1991; Morimoto et al., 1993; Takayama et al., 1993; Priborsky et al., 1992).

The structural requirements of penetration enhancers have been investigated using quantitative structure activity relationship (QSAR) approach, and results suggested the involvement of different mechanisms for enhancing the permeation of different drugs (Ghafourian et al., 2004). A simple QSAR model has also been developed for the prediction of permeation enhancement effects of large set of terpenes for Haloperidol and other drugs with similar physicochemical properties (Kang et al., 2007). However, these studies do not explain why several terpenes suppress the transdermal delivery of Estradiol (enhancement ratio, ER < 1), while having an ability to enhance the transdermal delivery of other drugs in this study

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(ER > 1). Furthermore, the molecular modelling was used to investigate a mechanism of permeation enhancement of Zidovudine by some oxygen containing terpenes (Narishetty and Panchagnula, 2004). The permeation enhancement was explained by the disruption of the hydrogen bond network of stratum corneum due to the intermolecular interactions with HBD and HBA groups of terpenes and stratum corneum. However, the same explanation cannot apply to hydrocarbon terpenes without HBD or HBA groups, so an alternative mechanism has to be considered.

The effect of terpenes on the permeation of a drug depends on the physicochemical properties of both permeant and enhancer molecules. In our previous molecular modelling study, we proposed that interactions between small molecules might play an important role in overcoming biological barriers in MDR bacteria and cancer cells (Zloh et al., 2004; Zloh and Gibbons, 2007). Hence, investigation of possible complex formation between permeants and terpenes is of particular interest.

Here, we have applied molecular modelling to investigate the role of molecular interactions between terpenes with drugs: 5-fluorouracil (5FU), hydrocortisone (HC), estradiol (ES) and diclofenac sodium (DFS), aimed to rationalize the mode of their action in overcoming the skin barrier.

2. Computational methods

The drug permeability data summarized in Ghafourian et al. (2004) was chosen to investigate the relationship between molecular properties and enhancer potencies of terpenes, ensuring that data in the table corresponds to the data in the original publications. Originally, the permeability enhancements of 5-fluorouracil, hydrocortione, diclofenac sodium and estradiol in the presence of terpenes were reported in the literature (Moghimi et al., 1998; El-Kattan et al., 2000; Arellano et al., 1996; Williams and Barry, 1991). The enhancement ratio was reported as the permeation coefficient through the pretreated mammal's stratum corneum of a drug with applied enhancer divided by the permeability coefficient of a drug without enhancer.

The examined set of terpenes included *alpha*-pinene (**1**), 3-carene (**2**), limonene (**3**), 7-oxabicyclo[2.2.1]heptane (**4**), *alpha*-pineneoxide (**5**), ascaridole (**6**), carveol (**7**), (*R*)-carvone (**8**), 1,8-cineole (**9**), cyclohexeneoxide (**10**), limonenoxide (**11**), menthone (**12**), piperitone (**13**), pulegone (**14**), terpinen-4-ol (**15**), terpineol (**16**), cedrene (**17**), longifolene (**18**), *trans*-caryophyllene (**19**), bisabolol (**20**), cedrole (**21**), cyclopenteneoxide (**22**), farnesol (**23**), fenchone (**24**), geraniol (**25**), guaiol (**26**), nerolidol (**27**), phytol (**28**), safrole (**29**), aromadendrene **(30)**, verbenone (**31**), thymol (**32**), cymene (**33**) and menthol (**34**) (Fig. 1).

Various experimental procedures were used for the determination of enhancement abilities of terpenes for four drugs; therefore four sets of data were treated separately. The enhancer potencies (EP) of terpenes toward estradiol and 5-fluorouracyl were examined using only enhancer molecular properties. This was in agreement with experimental procedures for the determination of EP for these two drugs, i.e. the stratum corneum (SC) was initially pretreated by enhancers (terpenes), followed by drug dissolution in an appropriate solvent and its application to a prepared SC. The enhancer changed SC prior to application of drugs; the EP values should reflect these changes when the drug is applied.

Hydrocortisone and diclofenac sodium were used in formulations that comprise approximately two molar equivalents of enhancer and one molar equivalent of drug. Consequently the molecular properties of complexes between two molecules of enhancers and one molecule of drug were used as descriptors.

The initial structures of drugs and terpenes were sketched using ChemDraw Ultra 7.0.1, converted into 3D structures and saved as mol2 files by Chem3D Ultra 7.0.1 (ChembridgeSoft). These structures were imported into Maestro v7.5 (Schrodinger), atom and bond types were adjusted and minimized with the MMFFs force field parameters (Cramer and Truhlar, 1995). The generalized born solvent accessibility (GB/SA) continuum solvent model for H_2O, 1-octanol and $CHCl_3$ (Still et al., 1990), implemented in MacroModel (Mohamadi et al., 1990), was used to simulate a solvent environment, with a constant dielectric function ($\varepsilon = 1$). An extended non-bonded cutoff (van der Waals: 8 Å; electrostatics: 20 Å) was used.

Complex formation between a drug and enhancer molecule was evaluated using conformational search and torsional sampling (MCMM) with a generation of up to 2000 different conformations. The energy cut off was generally set to $\Delta E = 30$ kJ/mol above the lowest energy conformation.

The free energy MINTA calculations (Kolossvary, 1997) of single molecules and complexes were used to predict the relative binding energies of different drugs to a given enhancer by using a thermodynamic cycle. Molecular lipophilicity potential (MLP), calculated by projection of the Broto–Moreau atomic constants on the molecular surface (Gaillard et al., 1994), was used for visual comparison of lipophilic properties of the same complexes in different environments (i.e. solvent models).

All quantitative correlations were simple multiple linear regressions (MLR) derived by the BILIN program (Kubiniyi, 1977). The most stable conformations of drugs, enhancers (**1**–**34**) and drug–enhancer complexes were used for calculations of all molecular properties (descriptors) namely: surface areas (total (SA), polar (PA) and apolar (AA)) calculated using a probe of 1.4 Å, volume (*V*), virtual log *P* (3D dependent property), log *P* obtained by Broto fragmentation method (2D dependent property) as well as the molecular interaction fields (MIF) minima obtained using four probes (H_2O – water; DRY – hydrophobic probe; N1 – hydrogen bond donor; O – hydrogen bond acceptor).

Molecular properties were evaluated using VegaZZ 2.0.8 software (Pedretti et al., 2002, 2004). The MIFs were obtained by GRID22b (Goodford, 1985; Goodford, 2006), allowing flexible parts of molecules to move under the influence of the probe (MOVE = 1) and using grid resolution of 0.5 Å (NPLA = 2). Results of GRID calculations were analyzed using BIOCUBE (Ermondi et al., 2006). Only the numerical values of descriptors included in final correlations are shown in the main text; all other data is enclosed as Supplementary material.

3. Results and discussions

3.1. Complex formation and molecular properties of complexes

Initially, the influence of different solvent environments on conformation and molecular properties of complexes between drugs and PE was considered. A possibility of complex formation between terpenes and drug molecules was examined by torsional conformational search implemented in Macromodel. Although the terpenes disrupt the hydrogen bond network of phospholipid head groups (Narishetty and Panchagnula, 2004), a drug molecule still has to diffuse from an outer environment through different layers of SC, which inherently have different properties.

The nature of solvent in transdermal formulations affects the interactions between drug and SC lipids as it was shown for the interaction between fatty acids and intracellular lipids (Wang et al., 2004). Therefore, different environments were considered during conformational search by selecting different solvent models available within Macromodel for GB/SA calculations.

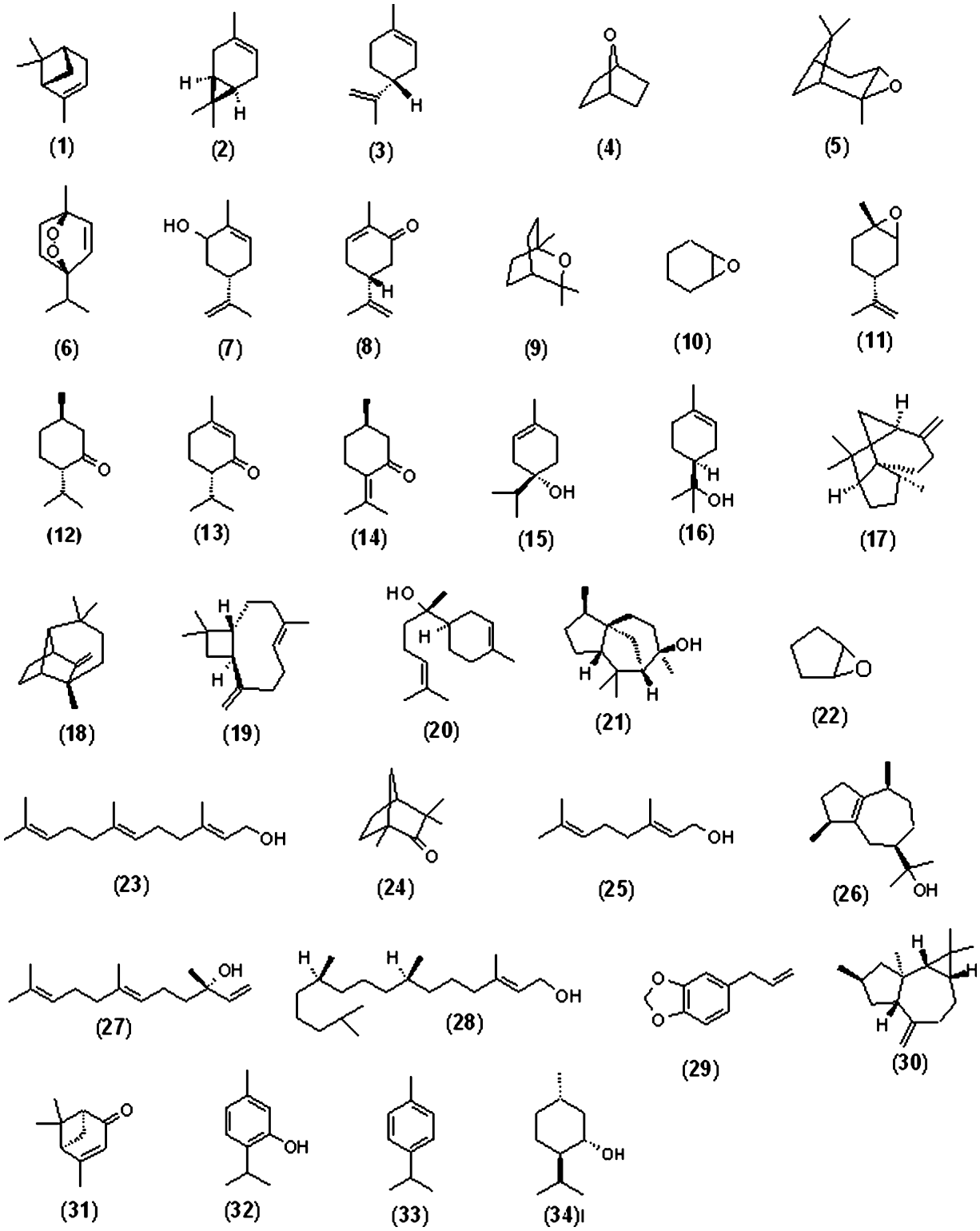

Fig. 1. Structures of **1**–**34** used in this study.

Structures of over 60 complexes (1:1 drug–enhancer) were calculated for three solvent environments (water, 1-octanol and $CHCl_3$). In an attempt to overcome the differences in the setup of experimental methods that were used to determine the effects of terpene on drugs permeability, the possibility of different molar ratios of complexes (1:2 drug–enhancer) were considered for HC and DFS in a water environment.

The free energies of complexes and isolated molecules were estimated by MINTA calculations. The free energies of interactions were calculated as the difference between free energies of complexes and free energies of corresponding single molecular species. It was found that the interaction energies were generally negative, indicating that a complex formation between drugs and terpenes is possible. Interactions were more favourable for oxygen-containing

terpenes, since those molecules could form hydrogen bonds with drugs.

We have analyzed the interactions that can be formed between drugs and terpenes in different solvents and how those interactions can influence different molecular properties, in particular those that can affect the transport through the skin. For example, molecular lipophilicity potential can differentiate the same molecular composition in various 3D arrangements, obtained by conformational search in three applied solvent models. The complexes formed between 5FU and two different enhancers (1,8-cineole and *alpha*-pinene) are shown in Fig. 2. Both molecules potentiate permeability, however the 5FU permeability in the presence of 1,8-cineole is about 90 times higher than the permeability of 5FU in the presence of *alpha*-pinene. If the complex formation is considered, the MLP surface changes in different solvents due to the different spatial arrangement that the two molecules can adopt in the complex (plots of MLP surface for these complexes are shown in Supplementary material). The MLP increases as the polarity of the solvent decreases, due to the rearrangement of the 5FU in respect to the enhancer. This may mimic the behaviour of the complex as it travels through the stratum corneum.

The different behaviour of two complexes in different solvents may contribute towards different enhancement abilities. For example, the shape of 5FU–1,8–cineole complex does not change significantly with the solvent change. However, the 5FU changes the orientation in respect to the *alpha*-pinene significantly when moving from less polar 1-octanol to non-polar $CHCl_3$.

The moderate potentiator for 5FU, (*R*)-carvone, does not alter shape by the change of the solvent from 1-octanol to $CHCl_3$. However, the complex adopts a different shape in water. That might be a reason for the lower potentiation ability of (*R*)-carvone compared to that of 1,8-cineole, although both are oxygen-containing terpenes and both have a hydrogen-bonding ability. These differences in permeation enhancement for two oxygen-containing terpenes cannot be distinguished by a reported QSAR model (Ghafourian et al., 2004).

Similar behaviour is observed for the complexes formed between ES and its most potent enhancer (7-oxybicyclo[2.2.1]heptane) shown in Fig. 3a. The enhancement

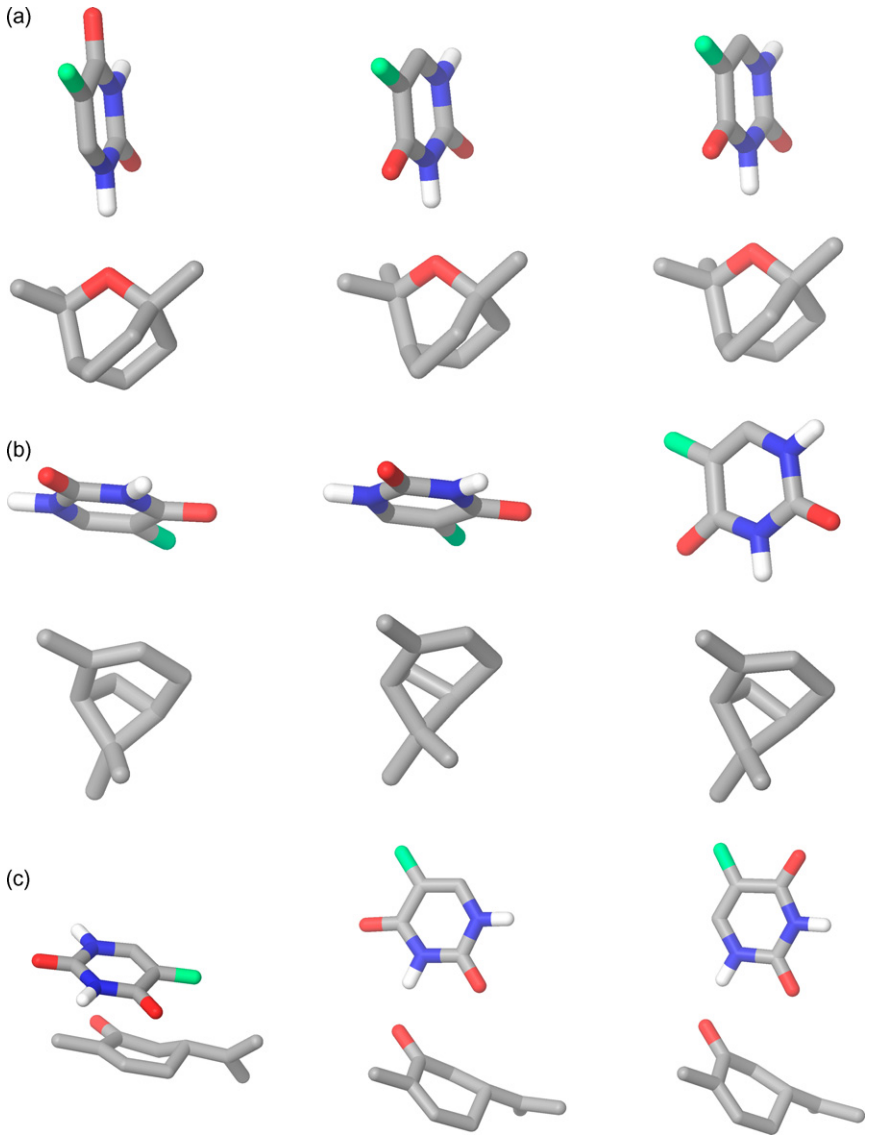

Fig. 2. Most stable conformations of the complexes that 5FU could form with (a) its most potent enhancer, 1,8-cineole (**9**), in water (left), 1-octanol (center) and $CHCl_3$ (right); (b) its least potent enhancer, *alpha*-pinene (**1**), in water (left), 1-octanol (center) and $CHCl_3$ (right) and (c) (*R*)-carvone **8** in water (left), 1-octanol (center) and $CHCl_3$ (right).

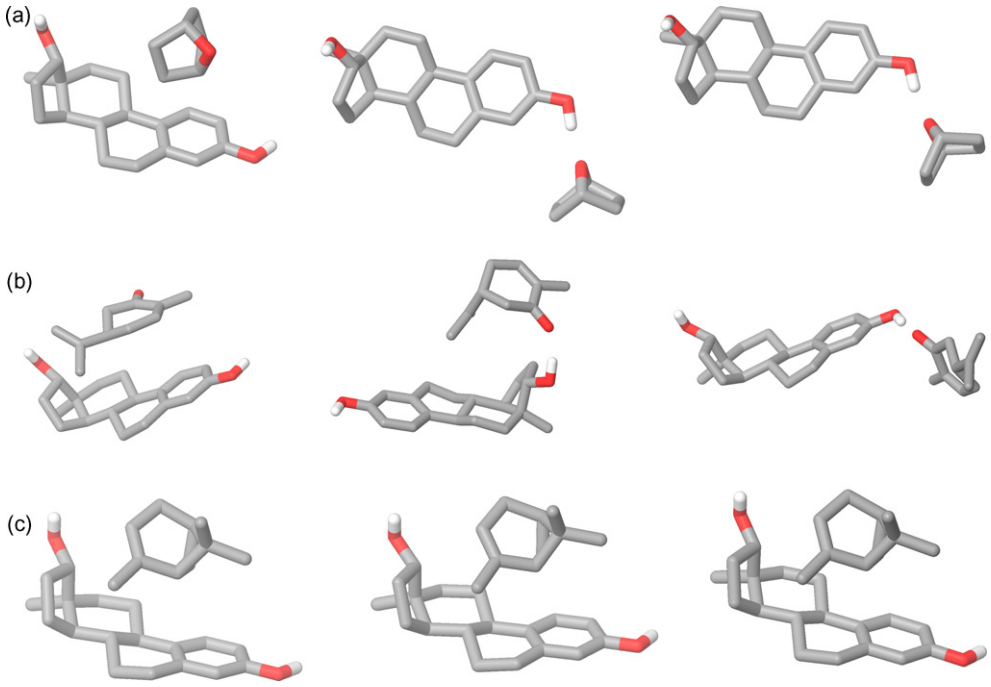

Fig. 3. Most stable conformations of the complexes that ES could form with (a) its most potent enhancer, 7-oxabicyclo[2.2.1]heptane (**4**), in water (left), 1-octanol (center) and $CHCl_3$ (right); (b) its least potent enhancer, (*R*)-carvone (**8**), in water (left), 1-octanol (center) and $CHCl_3$ (right) and (c) *alpha*-Pinene (**1**) in water (left), 1-octanol (center) and $CHCl_3$ (right).

ratio is relatively small (ER = 4.9), again the complex has to rearrange significantly during transport through the stratum corneum, which may affect the enhancement potency. For comparison, the complexes between ES and (*R*)-carvone that suppresses drug permeability is also shown in Fig. 3b. The ER for the (*R*)-carvone is 0.1, indicating that the permeability of ES in presence of (*R*)-carvone is 10 times lower than the permeability of ES on its own. In this case, the molecules of the complex in water were arranged in such a way to minimize exposure of hydrophobic parts to the polar environment of aqueous solution. For this complex to travel through the stratum corneum, even more drastic rearrangements are needed, which are unlikely to occur spontaneously. Consequently, complex might not penetrate the hydrophilic interface to access the hydrophobic environment of the SC.

In comparison, (*R*)-carvone is a moderate enhancer for 5FU (ER = 12.0), where such drastic rearrangement does not occur. If the ability to disrupt the SC is the only factor to affect permeability of a drug, (*R*)-carvone should have similar enhancement potencies for both drugs and consequently it would not diminish the permeability of the estradiol.

Furthermore, *alpha*-pinene is a weakest enhancer for 5FU and yet it is a potent enhancer for ES. The molecular complexes of ES with *alpha*-pinene in different solvents are very similar and do not require rearrangement during the transport (Fig. 3c). *alpha*-Pinene also does not have hydrogen bond donor or acceptor groups and does not have the ability to disrupt SC in the same way as oxygen containing terpenes, but it can improve the lipid solubility of ES through complexation.

The 2:1 mixtures of terpenes and drugs (HC and DFS) indicate different types of complexes were formed and different properties play a part in permeation enhancement. Therefore more strict analysis of complex properties were required to explain the ability to enhance HC and DFS permeation.

Table 1
Descriptors used for derivation of Eq. (1) and values of experimental (log ER Exper.) and calculated (log ER Calculated) relative enhancer potencies for ES

Comp. no.	Compound name	(SA/V)	log *P* Broto	DRY[a]	log ER Exper.	log ER Calculated
1	*alpha*-Pinene	2.250	2.529	−2.231	0.490	0.570
2	3-Carene	2.289	2.529	−2.182	0.639	0.688
3	Limonene	2.313	2.257	−2.520	0.574	0.418
4	7-Oxabicyclo-[2.2.1]heptane	2.655	0.719	−1.704	0.693	0.627
5	*alpha*-Pineneoxide	2.127	2.101	−2.048	0.279	0.073
6	Ascaridole	2.176	2.660	−1.926	0.677	0.610
7	Carveol	2.237	0.904	−2.491	−0.377	−0.633
8	(*R*)-Carvone	2.247	0.619	−2.489	−1.000	−0.794
9	1,8-Cineole	2.111	2.967	−1.748	0.643	0.730
10	Cyclohexeneoxide	2.607	0.719	−2.082	0.152	0.340
11	Limonenoxide	2.266	1.829	−2.493	0.207	0.039
12	Menthone	2.132	2.111	−2.662	−0.444	−0.185
13	Piperitone	2.190	1.145	−2.602	−0.770	−0.641
14	Pulegone	2.265	0.856	−2.645	−0.469	−0.665
15	Terpinen-4-ol	2.179	1.499	−2.197	−0.347	−0.259
16	Terpineol	2.148	1.499	−2.462	−0.481	−0.452

[a] kcal/mol for DRY probe minima.

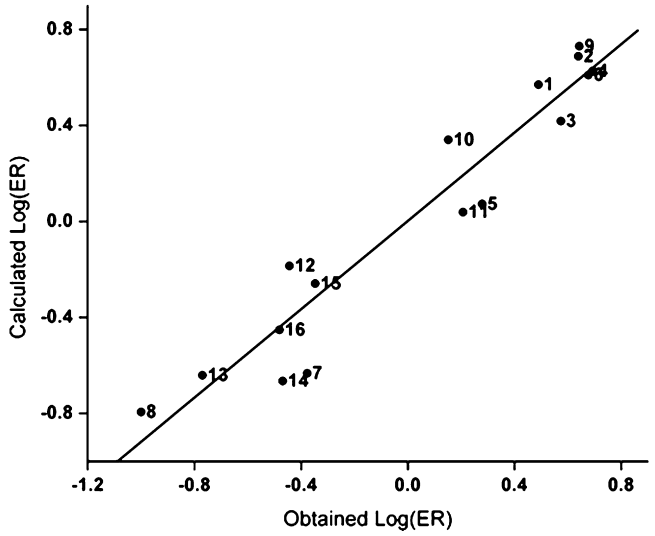

Fig. 4. The correlation between calculated and experimental log ER for the set of terpenes that enhanced the permeability of estradiol. The calculated values were obtained using Eq. (1).

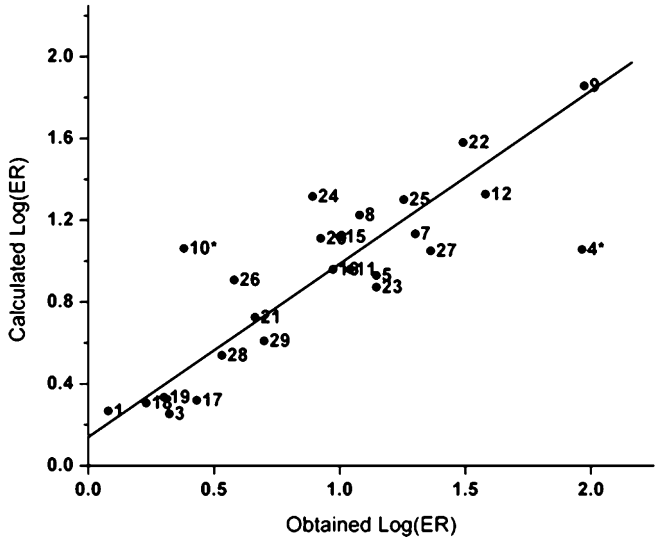

Fig. 6. The correlation between calculated and experimental log ER for the set of terpenes that enhanced the permeability of 5-fluorouracil. The calculated values were obtained using Eq. (2).

Visual and qualitative analysis of complexes formed in different environments can aid the rational explanation of the improvement of transdermal delivery by using terpenes as PEs. The more rigorous mathematical treatment of these observations was carried out, which supports the above-analyzed findings of complex formation importance for permeation enhancement.

3.2. Quantitative treatment of permeation enhancement data

3.2.1. Enhancing the transdermal delivery of estradiol

The correlation obtained for ER data of estradiol was obtained using the set of 16 applied enhancers. The descriptor set that appears in the correlation comprises surface area to volume ratio (SA/V), Broto log *P* and energy minima of hydrophobic probe (DRY) (Eq. (1), Table 1). The correlation shown in Eq. (1) is graphically represented in Fig. 4:

$$\log \mathrm{ER} = 2.457\,(\pm 0.92) \times (\mathrm{SA/V}) + 0.649(\pm 0.18) \times \log P + 0.449(\pm 0.41) \times \mathrm{DRY} - 5.599(\pm 2.95)$$

$$(n = 16;\ r = 0.959;\ s = 0.181;\ F = 46.354;\ Q^2 = 0.858;\ s_{\mathrm{PRESS}} = 0.242) \quad (1)$$

The most potent enhancers, **4** and **6**, have rigid structures and egg-like shape. The high weight of SA/V term probably reflects the importance of the ovality of molecules. The DRY probe minima should in a way differentiate the energies of interactions between enhancers and apolar parts of either stratum corneum or estradiol. Even though this term has the lowest weight, it appears that DRY minima value between −1.7 and −2.0 kcal/mol are optimal for desired potency. More importantly, the terpenes that have a very high negative DRY minima value could potentially form complexes with estradiol through strong hydrophobic interaction. The DRY probe MIF surfaces of most potent **4** and **6** and the least potent **8** are shown in Fig. 5.

3.2.2. Enhancing the transdermal delivery of 5FU

The largest set studied includes 26 enhancers for the permeability of 5-fluorouracyl (Table 2). The numerical values of descriptors for 24 compounds used to obtain an acceptable correlation (Eq. (2)) were given in Table 2. The agreement between experimental and calculated log ER values is shown in Fig. 6.

$$\log \mathrm{ER} = 151.6(\pm 102) \times (\mathrm{PA/SA})^2 - 51.24(\pm 17.2) \times (\mathrm{PA/SA}) + 9.284(\pm 5.05) \times (\mathrm{PA/V}) - 0.489(\pm 0.15) \times \mathrm{H2O} - 0.252(\pm 0.26), \quad (\mathrm{PA/SA})_{\mathrm{OPTIMUM}} = 0.17(0.11/0.41)$$

$$(n = 24;\ r = 0.923;\ s = 0.203;\ F = 27.154;\ Q^2 = 0.767;\ s_{\mathrm{PRESS}} = 0.255). \quad (2)$$

Enhancers' potencies show parabolic dependencies of polar to total surface area ratio, having optimal value between 0.11 and 0.41. Polar area to volume ratio has a lower weight in Eq. (2). Both PA/SA and

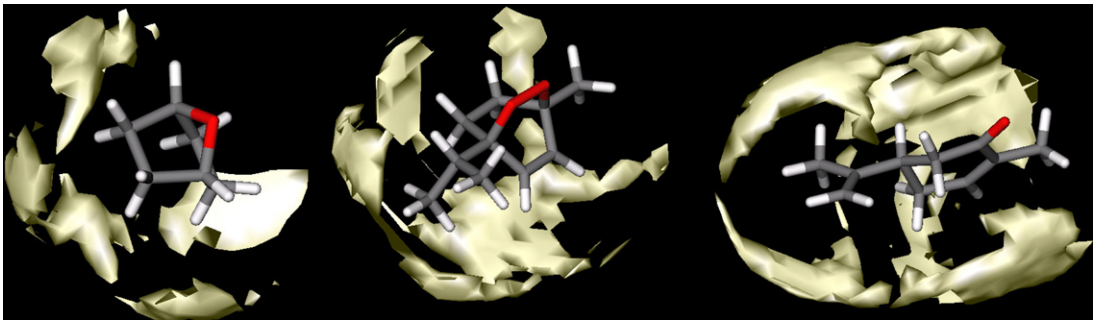

Fig. 5. Comparisons of DRY MIF probe surfaces on −0.53 kcal/mol for terpenes **4**, **6** and **8**.

Table 2
Descriptors used for derivation of Eq. (2) and values of experimental (log ER Exper.) and calculated (log ER Calculated) relative enhancer potencies for 5FU

Comp. no.	Compound name	PA/SA	PA/V	H2O[#]	log ER Exper.	log ER Calculated
1	*alpha*-Pinene	0	0	−1.094	0.079	0.283
3	Limonene	0	0	−1.066	0.322	0.270
4[a]	7-Oxabicyclo[2.2.1] heptane	0.1	0.264	−5.035	1.964	1.053
5	*alpha*-Pinene-oxide	0.054	0.114	−5.000	1.146	0.926
7	Carveol	0.117	0.262	−5.879	1.301	1.135
8	(*R*)-Carvone	0.106	0.238	−6.128	1.079	1.226
9	1,8-Cineole	0.017	0.036	−5.325	1.973	1.859
10[a]	Cyclohexeneoxide	0.114	0.296	−4.982	0.380	1.061
11	Limoneneoxide	0.068	0.153	−5.246	1.041	0.950
12	Menthone	0.084	0.179	−6.442	1.580	1.325
15	Terpinen-4-ol	0.078	0.17	−5.869	1.000	1.122
16	*alpha*-Terpineol	0.098	0.211	−5.780	0.973	0.967
17	Cedrene	0	0	−1.199	0.431	0.335
18	Longifolene	0	0	−1.173	0.230	0.322
19	*trans*-Caryophyllene	0	0	−1.229	0.301	0.349
20	Bisabolol	0.064	0.127	−5.806	0.924	1.108
21	Cedrole	0.084	0.151	−5.757	0.663	0.730
22	Cyclopenteneoxide	0.132	0.372	−5.113	1.491	1.579
23	Farnesol	0.111	0.231	−5.728	1.146	0.873
24	Fenchone	0.096	0.211	−6.395	0.892	1.312
25	Geraniol	0.141	0.325	−5.607	1.255	1.296
26	Guaiol	0.080	0.147	−5.994	0.580	0.915
27	Nerolidol	0.077	0.158	−5.891	1.362	1.049
28	Phytol	0.092	0.156	−5.667	0.531	0.536
29	Safrole	0.136	0.326	−4.098	0.699	0.613
30	Aromadendrene	0	0	−1.163	0.398	0.317

[#] kcal/mol for H2O probe minima.
[a] Omitted from equation derivation.

PA/V terms have numerical values lower than 0.5. This shows that all enhancers are to a significant extent apolar molecules, with polar surfaces that can associate with 5FU, which may play a significant role in the enhancement of transdermal delivery. The presence of MIF H_2O probe minima that have both HBA and HBD ability in Eq. (2) additionally supports this observation. All molecules that have MIF H2O probe minima on or below −5.00 kcal/mol have good enhancer potencies. This probably accounts for strong H-bonding with 5FU, a small rigid molecule having **a** HBA and HBD close to each other.

The MIF's H2O minima of the most potent enhancers **9** and **12** on −5.00 kcal/mol and the least potent **1** on −0.850 kcal/mol are shown in Fig. 7. Compounds **4** and **10** are outliers. The presence of **4** as an outlier could not be rationally explained within the frame of the present study. The reported ER value of **10** in the original article could potentially be wrong, since the previous QSAR study (Ghafourian et al., 2004) also classified **10** as an outlier.

3.2.3. *Enhancing the transdermal delivery of hydrocortisone*

The correlation of permeability enhancement for hydrocortisone is given in Eq. (3). The formation HC–enhancer complexes (1:2) were considered for 11 terpenes. Numerical values of descriptors for HC–terpene complexes that appears in the correlation are given in Table 3. The correlation is parabolic in respect to N1 MIF probe minima and Eq. (3) also includes Virtual log *P*. The correlation between experimental and calculated log ER values is given

Table 3
Descriptors used for derivation of Eq. (3) and values of experimental (log ER Exper.) and calculated (log ER Calculated) relative enhancer potencies for HC

Comp. no	Compound name	N1[a]	Virtual log *P*	log ER Exper.	log ER Calculated
3	Limonene	−6.579	3.478	1.450	1.396
8	(*R*)-Carvone	−6.769	1.887	1.117	1.187
9	1,8-Cineole	−6.116	7.633	1.160	1.208
12	Menthone	−6.802	3.491	1.270	1.306
15	Terpinen-4-ol	−6.994	3.071	1.050	0.999
16	Terpineol	−6.276	2.634	1.130	1.061
24[b]	Fenchone	−6.315	4.442	1.004	1.285
25	Geraniol	−6.385	2.452	1.230	1.188
27	Nerolidol	−6.731	4.612	1.550	1.464
31	Verbenone	−6.785	1.801	1.060	1.166
32	Thymol	−7.049	4.573	1.040	1.028
33	Cymene	−6.379	4.972	1.360	1.413

[a] kcal/mol for N1 probe minima.
[b] Omitted for equation derivation.

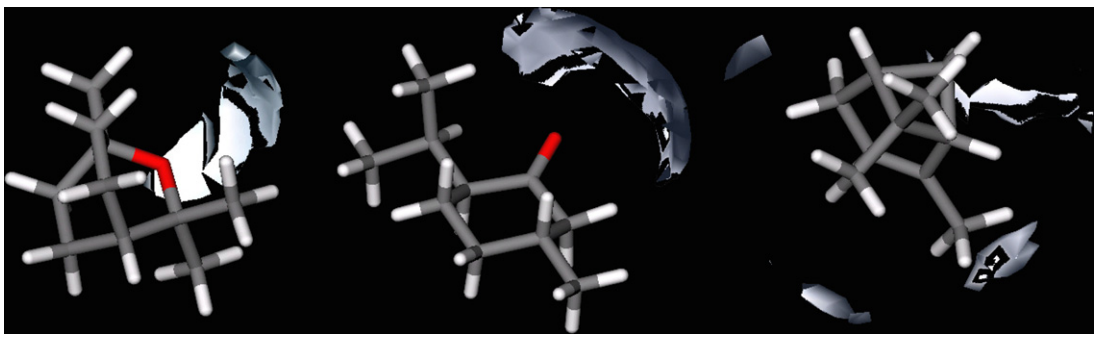

Fig. 7. The H_2O MIF probe surfaces on −5.00 kcal/mol for **9**, **12** and on −0.850 kcal/mol for **1**.

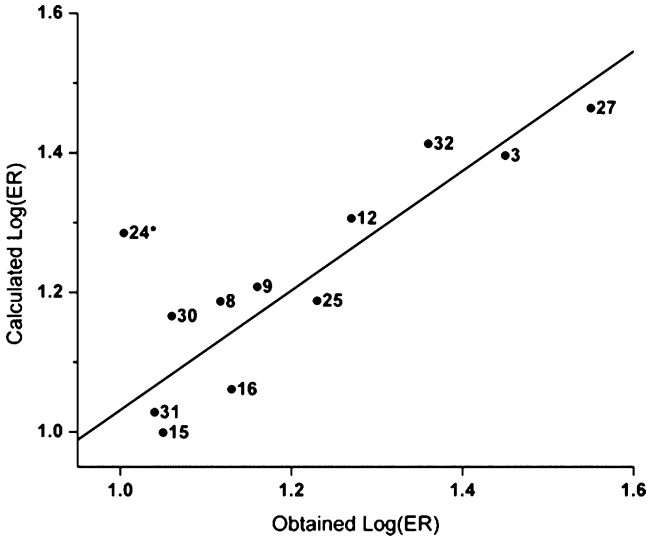

Fig. 8. The correlation between calculated and experimental log ER for the set of terpenes that enhanced the permeability of hydrocortisone. The calculated values were obtained using Eq. (3).

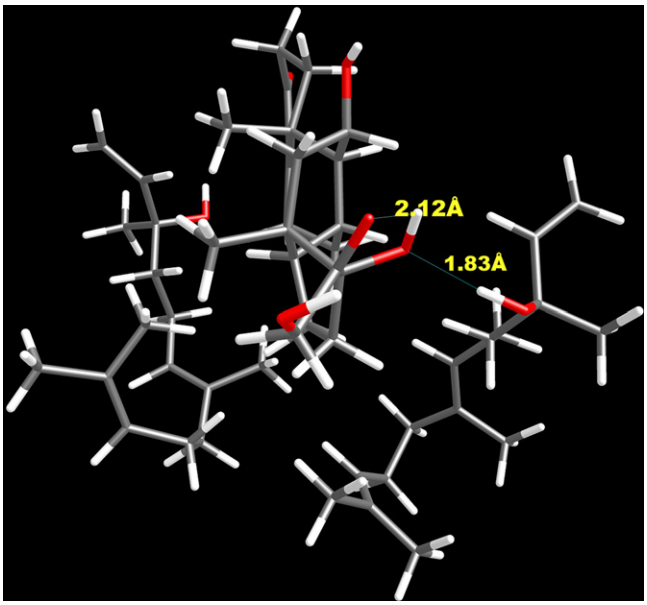

Fig. 9. The lowest energy conformation of HC–nerolidol complex (1:2).

in Fig. 8.

$$\log \mathrm{ER} = -2.388(\pm 0.920) \times [N1]^2 - 31.540(\pm 12.200) \times N1 + 0.092(\pm 0.047) \times \text{Virtual } \log P - 103.1(\pm 40.4),$$

$$[N1]_{\mathrm{OPTIMUM}} = -6.61(-6.65/-6.55) \quad (n = 11;\ r = 0.925;\ s = 0.078;\ F = 13.879;\ Q^2 = 0.539;\ s_{\mathrm{PRESS}} = 0.139) \qquad (3)$$

The parabolic term of N1 minima values show that there is an optimal value for hydrogen bonding ability of studied drug–enhancer complexes. This value is close to MIF N1 minima of most potent enhancers, i.e. drug–enhancer complexes **27** and **3**. N1 probe is a standard HBD probe. Although, virtual log *P* term has a significantly lower weighting, the lipophilicity of HC–enhancer complexes plays an important role for permeation enhancement.

The hydrocortisone molecule comprises two keto groups (HBA) located on opposite sides of molecules. Additionally, the two of three hydroxyl groups (HBD/HBA), separated by one carbon from one of keto group, can attenuate its HBA ability. The most potent HC enhancer **1** has one OH group and in our 3D model that group of one of enhancer molecules forms H-bond with the OH group of HC (Fig. 9).

This H-bond network can guarantee the safe transport of (at least) 1:1 drug/enhancer complexes from a formulation mixture through the skin. The rest of the HBA ability of the drug is free to make hydrogen bonds with compatible moieties of stratum corneum. Furthermore, both enhancer molecules have a bent shape in an HC–nerolidol complex (Fig. 9). Nerolidol (**27**) molecules are large and flexible enough to shield whole hydrocortisone molecule by formation of intramolecular hydrophobic interactions as seen in modelled complexes. Obviously, bent shape of both nerolidol molecules in this complex is a consequence of the well-known tendency of apolar molecules to hide apolar surfaces from the polar surroundings.

Secondly, the calculated ratios between molecular properties used as descriptors in this study show that all properties except the surface area, apolar area, volume and Virtual log *P* for complexes applies equally as with the free drug. The surface area of complexes are about 1.5–2 times higher, the volumes are about two times higher, than in free drug, but Virtual log *P* values are 30–110 times higher. This probably accounts for high enhancing abilities of purely hydrophobic enhancers such as **3** and **33**.

Consequently, complexation of HC with studied terpenes increase lipophilicity of complexes to a huge extent leaving the HBA and HBD ability of drugs on approximately the same level. In this way drugs benefit on a property that allows interactions with hydrophobic parts of SC (changed by enhancer or not) and retaining the same HBA/HBD ability as uncomplexed drug that allows interactions with polar parts of SC. The complex **24** is an outlier and we could not offer an explanation for this. The N1 probe MIF surfaces at −4.93 kcal/mol for most potent enhancers

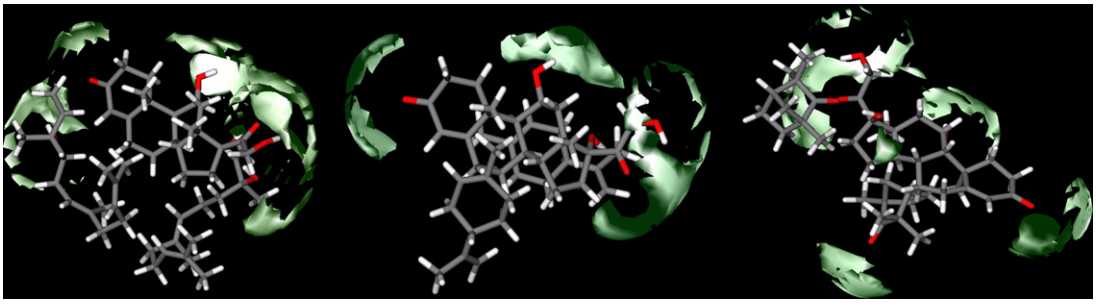

Fig. 10. Comparison of N1 MIF probe surfaces for **27**, **3** and **24** terpene–HC complexes depicted on −4.93 kcal/mol.

Table 4
O probe minima descriptor used for derivation of Eq. (4) to describe the effects of terpenes on transdermal delivery of DFS, and values of experimental relative enhancer potencies (log ER Exper.)

Comp. no.	Compound name	O[a]	log ER Exper.
3	Limonene	−5.746	0.548
9	1,8-Cineole	−4.827	0.143
12	Menthone	−4.736	0.487
24	Fenchone	−4.956	0.272
25	Geraniol	−6.705	1.278
27	Nerolidol	−6.500	1.134
32	Thymol	−5.132	0.676
34	Menthol	−5.593	1.027

[a] kcal/mol for O probe minima.

(drug–enhancer complexes) **27** and **3**, and the least potent **24** (outlier) are shown in Fig. 10.

3.2.4. *Enhancing the transdermal delivery of diclofenac sodium*

The smallest set included in the present study comprises eight diclofenac sodium–enhancer complexes (Table 4). Accordingly, a low number of studied complexes allow derivation of statistically valid correlations using one descriptor only. All complexes are built *in silico* using acid (diclofenac–DF) in place of its sodium salt due to problems experienced with modelling the salt. Nevertheless, the linear dependences of log ER vs. O probe MIF minima are observed.

$$\log \mathrm{ER} = -0.478(\pm 0.27) \cdot \mathrm{O} - 1.944(\pm 1.48) \quad (n = 8;\ r = 0.874;$$
$$s = 0.217;\ F = 19.336;\ Q^2 = 0.645;\ s_{\mathrm{PRESS}} = 0.265) \qquad (4)$$

The log ER vs. O minima show almost straight lines for **9**, **24**, **3**, **27** and **25** and for **12**, **32** and **34** separately (Fig. 11).

Even structurally dissimilar, enhancers **27**, **34** and **32** are ranked as second, third and fourth in respect to enhancer potencies (Fig. 11). Fig. 12a depicts O probe MIF on −4.56 kcal/mol of the complex between diclofenac and nerolidole (2:1).

The carboxyl–OH bridges of two nerolidol molecules are available to create a H-bond network that allows a significant association of molecules, and probably decreases HBD abilities of DF. One of Nerolidol–OH is free for "external" H-bonding, as well as the NH group of DF diphenylamino moiety. Two nerolidole molecules are additionally associated by hydrophobic interactions of their hydrocarbon termini.

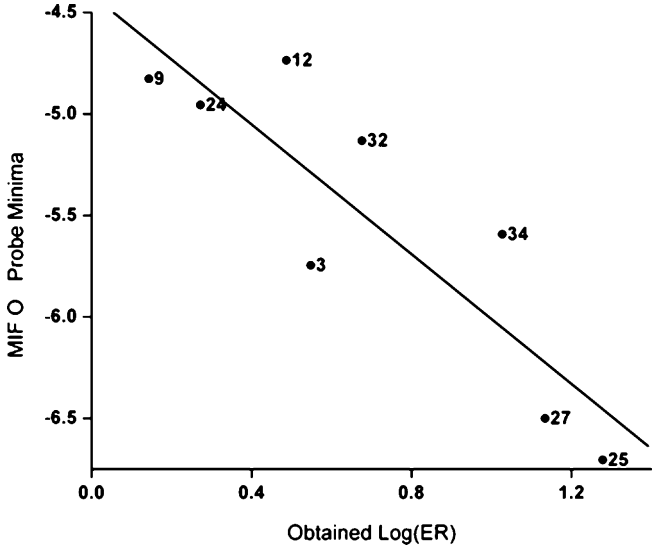

Fig. 11. The line of best fit for the correlation between MIF O probe minima and log ER of DFS.

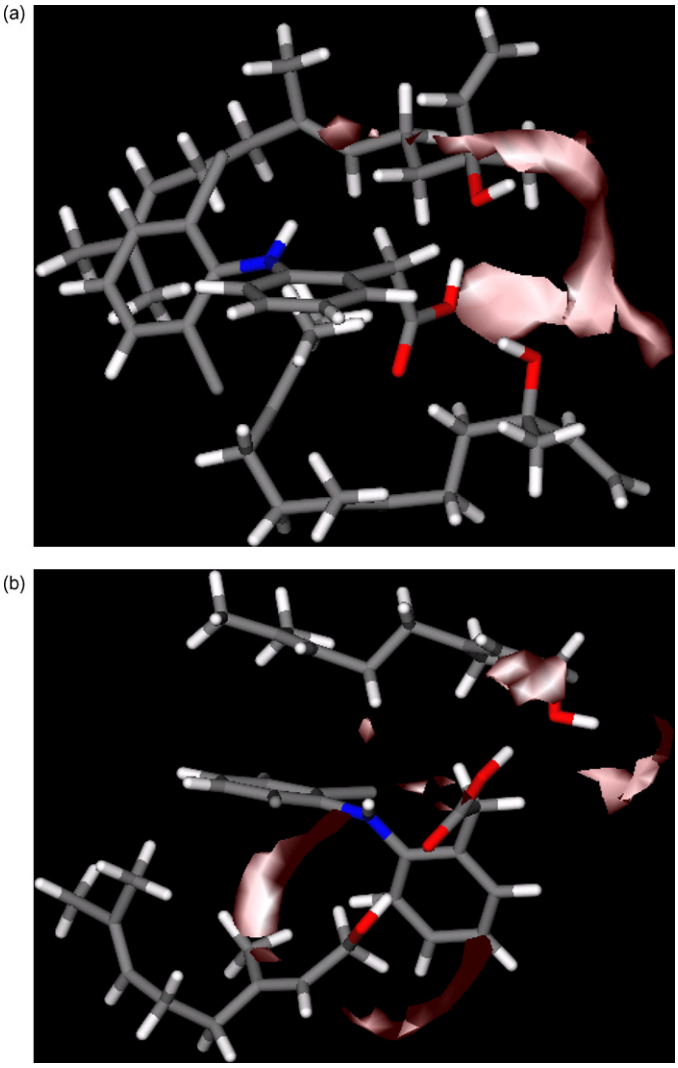

Fig. 12. The MIF O probe surfaces of DF complexed with nerolidol (**27**) (a) and geraniol (**25**) (b).

A very similar situation can be observed for two most active and structurally similar enhancers, nerolidol and geraniol. Both enhancers' hydrocarbon termini form hydrophobic interactions with terminal DF phenyl group. Fig. 12b present MIF on −4.48 kcal/mol of the complex between diclofenac and geraniol (2:1).

4. Conclusions

Enhanced potencies of terpenes and structurally related compounds have been investigated using molecular modelling. The computational study of interactions of teprenes with 5FU, ES, DFS and HC showed that the formation and properties of the complexes between terpenes and these drugs was dependant on the environmental properties. It was proposed that H bonding is involved in interactions between oxygen-containing terpenes and all drugs, while hydrocarbon terpenes were supposed to interact by donor/acceptor interactions, van der Waals forces and HBD–π interactions. Good correlations between the ER of the enhancers with properties derived either from the modelled complexes, or from the structures of the examined terpenes have been obtained.

Using MIFs minima obtained by H_2O probe (HBD/HBA), DRY probe (hydrophobic), N1 probe (HBD) and O probe (HBA), surface

areas, volumes and Virtual log *P*'s of enhancers' molecules or their complexes with drugs, the enhancer potencies can be qualitatively described and more important, a rational for different potencies of molecules can be proposed to an acceptable level.

Our modelling studies indicate that the complexation between drugs and permeability enhancers can potentially play a role in transdermal delivery. Further experimental data is needed to develop a modelling system for the optimization of composition formulations for transdermal delivery.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2008.06.032.

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