been formed. The resulting co-crystals were subjected to standard dissolution and solubility testing. High-throughput methods were also used to make these co-crystals at differing ratios (including the ratio of the manually produced crystal), for comparison and to explore their potential for co-crystal manufacture.

Results DSC and XRPD analysis confirmed that co-crystals were formed, except with the combination of nicotinamide and benzamide with fumaric acid. Dissolution tests indicated that benzamide co-crystals (unlike nicotinamide co-crystals) had an enhanced dissolution profile compared with the pure API, although the solubility of nicotinamide and benzamide in co-crystal form was unchanged. Finally, high-throughput methods were found to produce co-crystals at the differing ratios, including the same co-crystals that were produced by the manual method.

Conclusions This study has demonstrated that co-crystals can be made manually and via high-throughput methods, and can offer opportunities for modification of biopharmaceutical properties such as dissolution rate. This raises the potential to enhance the bioavailability of active pharmaceutical ingredients.

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Chemistry

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Prediction of carcinogenicity of diverse chemical substances by a support vector machine

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Objectives The ability to assess the toxicity of a chemical depends on the available information on the compound and/or its related compounds. Among chemicals currently in commerce, very few are ascertained on their toxicity, and reliable data on the carcinogenicity are very limited, especially for pharmaceutical chemicals. Therefore, attempts on the basis of quantitative structure–activity relationship (QSAR) models for estimating carcinogenicity have been performed (Benigni 2003, Tanabe et al 2005). The support vector machine (SVM) technique (Chen et al 2004) was applied to develop a QSAR model that relates the structures of diverse chemicals to their carcinogenicity in this study. Compared with traditional regression and neural network methods, SVMs have some advantages, including global optimum, good generalization ability and simple implementation.

Methods The carcinogenicity dataset used in the Predictive Toxicology Challenge 2000–2001 contest (Helma et al, http://www.informatik.uni-freiburg.de/~ml/ptc/) on 454 diverse chemicals was employed to develop the SVM model to compare its predictability with that of our previous artificial neural network (ANN) model (Tanabe et al 2005). The chemical carcinogenicity data consist of two discrete values (carcinogenic or non-carcinogenic). For the SVM modelling, LIBSVM ver.2.85 software (Chang and Lin, http://www.csie.ntu.edu.tw/~cjlin/libsvm/) was used for regression. Molecular descriptors calculated from the three-dimensional geometries of the compounds alone with Fujitsu Project-Leader were used to represent the molecules.

Results The relationship between experimental carcinogenicity data and 37 descriptors taking into account size, shape, electronic structure and hydrophobicity of the molecules was analysed with the SVM and ANN. The architecture of the ANN is a fully connected three-layer design with the error-back-propagation algorithm. For both methods, models were optimized using a cross-validation test for the training dataset, and their performances were evaluated using the test dataset. The training of ANNs took several months using seven computers to solve the problems, such as over-training, over-fitting and local minima, while SVM gave a predictability of 74% just comparable with that by ANN, in a much shorter computation time. The prediction accuracy was higher than the best predictability value of 71% reported in the literature for the same dataset (Helma et al, http://www.informatik.uni-freiburg.de/~ml/ptc/).

Conclusions The SVM, a novel machine learning approach, was applied to the structure–carcinogenicity problem based on the information on molecular structure alone. From the analysis of the results obtained, a non-linear model using SVM produced a better model with good predictive ability than the artificial neural network approach. The prediction results were quite good and reasonable under the present uncertainties of the experimental animal carcinogenic data.

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Novel phenothiaziniums as putative photo-antiviral agents

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Objectives The use of photosensitizers in viral eradication, for example as a topical treatment, or in the disinfection of blood products, has been a clinical reality for over 10 years. However, the efficacy of the photosensitizers employed is less than optimal, usually from the point of view of viral targeting (Wainwright 2005). Methylene blue (MB) is a standard photosensitizer currently employed in viral eradication in blood plasma. The aim of this work was firstly to synthesize novel MB derivatives having one of the original dialkylamino groups replaced by a side chain containing an aromatic ring, and secondly to examine the effect of this substitution on the photosensitizing behaviour and DNA-targeting of the resulting compounds. Substitution patterns in the aromatic side chain were chosen to allow an investigation of any electronic effects on activity and photoproperties.

Methods Phenothiazinium tetraiodide was treated with either diethylamine or di-*n*-propylamine in methanol to furnish the corresponding 3-*N*,*N*-dialkylamino-phenothiazinium tri-iodide. Further reaction with benzylamine derivatives produced the target 3-*N*,*N*-dialkylamino-7-(subs)benzylaminophenothiazinium iodides. Singlet oxygen generation on illumination, and DNA-intercalating activity of the compounds, were carried out spectrophotometrically, relative to the standard photosensitizer MB.

Results Ten new derivatives were obtained by the above procedure, pure chromatography/mass spectrometry. The compounds exhibited intense light absorption in the long-wavelength red region (≈650 nm), which would allow their use in red blood cell fractions or whole blood samples (i.e. at longer wavelengths than haem). Each derivative was shown to produce singlet oxygen in similar yield to that of MB under the same conditions. Each derivative also exhibited a large bathochromic shift when mixed with DNA, demonstrating strongly intercalative behaviour, in a similar fashion to that of dimethyl methylene blue (DMMB), which is known to intercalate much more efficiently than MB (Table 1).

Conclusions The novel derivatives satisfied performance criteria regarding long-wavelength light absorption and singlet oxygen production. Significantly increased DNA intercalation suggests that the anti-viral activities may be higher than that of MB and these are now undergoing cellular testing.

Table 1 Photoproperties and DNA binding of the derivatives

Derivative	Relative yield of $^1O_2$	λ_{max} (H_2O, nm)	λ_{max} + DNA (H_2O, nm)	Shift (nm)
MB (NMe_2/NMe_2)	1.00	665	669	+4
DMMB (NMe_2/NMe_2)	0.55	647	661	+14
$NEt_2/NHCH_2Ph$	0.69	652	666	+14
$NEt_2/NHCH_2(4\text{-}C_6H_4Cl)$	0.71	650	662	+12
$NEt_2/NHCH_2(4\text{-}C_6H_4F)$	0.53	653	665	+12
$NEt_2/NHCH_2(4\text{-}C_6H_4OMe)$	0.54	654	665	+11
$NEt_2/NHCH_2(4\text{-}C_6H_4Me)$	0.57	645	659	+14
$NPr^n_2/NHCH_2Ph$	0.47	658	670	+12
$NPr^n_2/NHCH_2(4\text{-}C_6H_4Cl)$	1.23	656	669	+13
$NPr^n_2/NHCH_2(4\text{-}C_6H_4F)$	0.46	656	666	+10
$NPr^n_2/NHCH_2(4\text{-}C_6H_4OMe)$	0.90	658	669	+11
$NPr^n_2/NHCH_2(4\text{-}C_6H_4Me)$	0.38	658	668	+10

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Molecular modelling of a lipid bilayer and the use of the bilayer in the rationalization of a small range of percutaneous enhancers

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Objectives The lipid bilayer represents the most important barrier to molecules entering or leaving a cell. Furthermore, the epidermal structure (in particular the intercellular space) in the skin is also made up of similar bilayer structures which have been under extensive investigation in an attempt to enhance percutaneous absorption by drug substances through the use of skin-penetration enhancers. The ability to formulate compounds which disrupt the barrier and hence enhance the

penetration of drug substances is a key issue for drug delivery and a clear understanding of the mode of action of compounds able to disrupt the lipid structures would greatly improve the further design of better skin-penetration enhancers. However, the lipid bilayer has been extremely difficult to model. Here we report the development of an initial model of the bilayer (using ceramide to create the bilayer as this has been shown to make up to 18% of the lipid within the epidermis and intercellular space), using which we have modelled the mode of action of a number of compounds that have been shown previously to aid drug delivery through the skin.

Methods In the modelling of the lipid bilayer, we considered the use of ceramide (in particular, ω-hydroxyacyl sphingosine) as the lipid mimic. As such, the monolayer was initially created within the CaChe molecular modelling program and each pair of molecule's interactions minimized (using MM3 parameters). As each pair was minimized, the next pair were added and again minimized. Once the monolayer had been created (consisting of some 10×8 molecules), the lower part of the bilayer was then created and minimized (again using MM3 parameters) and the outer surface locked. The penetration enhancers to be studied (e.g. dimethyl sulfoxide, Brij 76T, decylmethyl sulphoxide and *cis*-9-octadecanoic acid) were also produced within CaChe, initially minimized and then inserted into the bilayer, following which the full structure was minimized and the energy of the bilayer with and without the enhancer was determined so as to elucidate the impact of the different enhancers.

Results The minimization of the ceramide molecules resulted in the formation of an interdigitized bilayer with a total span of approximately 80 Å. The incorporation of different penetration enhancers caused different effects, as would be expected; that is, we discovered that the use of Brij 76T resulted in the space between the ceramide molecules increasing. Indeed, the whole molecule was found to fit within the bilayer such that the molecule crossed the span of the bilayer resulting in an overall energy increase of 1631.45 kcal/mol. Our results further support the previous hypotheses that compounds are able to embed themselves within the structure of the bilayer and as a result disrupt the overall structure of the homogenous nature of the bilayer, thereby enhancing the movement of compounds through the creation of 'micro-channels'.

Conclusions We have provided the initial model for the overall structure of the lipid bilayer and using this the mode of action of a range of penetration enhancers may be elucidated.

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Inhibition of the hydroxysteroid dehydrogenase family of enzymes: an investigation into the specificity of a range of benzyl imidazole-based compounds

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Objectives The enzyme 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) is currently under investigation in the treatment of androgen-dependent prostate cancer. We have previously designed, synthesized and subsequently evaluated a series of compounds based upon benzyl imidazole (Owen et al 2006, Patel et al 2006). Whilst the compounds were equipotent to the standard compound, ketoconazole (KTZ), the inhibitory profile of the compounds against other enzymes was not known. In an effort to determine their specificity, we considered the inhibitory activity of the previously synthesized compounds against two forms of 17β-hydroxysteroid dehydrogenase (17β-HSD); namely, type 1 (17β-HSD1; responsible for the conversion of oestrone to oestradiol) and type 3 (17β-HSD3; responsible for the conversion of androstenedione to testosterone), as well as 3β-hydroxysteroid dehydrogenase (3β-HSD; responsible for the conversion of dehydroepiandrosterone to androstenedione). Here, we report the initial results of our study into the biochemical evaluation (and therefore the determination of the specificity) of the 4-substituted (e.g. F, Cl, Br, I, Me, etc.) benzyl imidazole-based compounds previously reported by us, compared with the three HSD enzymes.

Methods For the current investigation, the target compounds were synthesized using the methodology previously reported by us (Owen et al 2006, Patel et al 2006). The reactions proceeded in good yield (ranging from 40 to 85%) and no major problems were encountered. Biochemical evaluation of the synthesized compounds (final concentration 100 μM) was undertaken using a literature assay procedure using rat testes homogenate and radiolabelled substrates (Lota et al 2006).

Results The results of the biochemical evaluation of the previously reported compounds against the three HSD enzymes show that the compounds were, in general, equipotent to the standard compound, namely KTZ, and which was found to possess 25, 23 and 34% inhibitory activity against 17β-HSD1, 17β-HSD3 and 3β-HSD respectively. However, a small number of compounds were found to possess greater inhibitory activity than KTZ; in particular, the most potent was found to be 4-fluorobenzyl imidazole, which was found to possess 68, 41 and 31% inhibitory activity against 17β-HSD1, 17β-HSD3 and 3β-HSD respectively under similar assay conditions. Further consideration of the inhibitory activity observed against the family of HSD enzymes shows that these compounds possess selectivity between 17β-HSD1 and 17β-HSD3, while possessing extremely poor inhibitory activity against 3β-HSD. For example, 4-bromobenzyl imidazole was found to be a relatively potent inhibitor of the lyase component (possessing an IC50 of 6.8 μM in comparison with 53.4 μM against the 17α-hydroxylase) of $P450_{17\alpha}$ and is found to possess approximately 49% inhibitory activity against 17β-HSD1 and approximately 33% inhibitory activity against 3β-HSD; however, no inhibitory activity was observed against 17β-HSD3.

Conclusions The results suggest that inhibitors of $P450_{17\alpha}$ designed using a benzyl imidazole template possess good selectivity against their target enzyme and are therefore good lead compounds in the design of novel inhibitors of $P450_{17\alpha}$.

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Drug Delivery

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Design and physico-chemical characterization of a novel drug-delivery system for photodiagnosis and photodynamic therapy of colorectal neoplasias

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Objectives Serious cellular abnormalities in the colorectal region are a leading cause of morbidity and mortality in industrialized countries, with an estimated 300000 new cases and 200000 related deaths annually in Europe and the USA. The development of technologies to improve the detection process or enhance treatment would be a welcome addition to current treatment methods. Two such promising procedures are photodynamic therapy and photodiagnosis. The techniques rely on specific accumulation of photosensitizer in a neoplastic lesion with the former therapy used to bring about selective destruction and the latter only making it more conspicuous upon fluorescent emission. Administration of 5-aminolevulinic acid (ALA) leads to selective accumulation of the photosensitizer protoporphyrin IX in neoplastic tissue. However, systemic administration of ALA is associated with significant side effects. In this study, we aimed to design and characterize a novel drug-delivery system that may be of use in photodiagnosis and photodynamic therapy of colorectal neoplasias.

Methods ALA-loaded, poly(ethylene glycol) (PEG) discs were prepared using three PEG molecular weights (1000, 6000 and 10000 Da) and subsequently characterized using friability measurements and differential scanning calorimetry. Drug-release studies were also performed using modified Franz diffusion cells. ALA was quantified by high-performance liquid chromatography (HPLC) employing fluorescence detection, as described previously (Donnelly et al 2006) following derivatization with acetyl acetone and formaldehyde.

Results The disc-shaped delivery system was mechanically robust, as judged by friability measurements. Calorimetric analysis confirmed that low concentrations of ALA (1% w/w) were dispersed completely throughout the PEG matrix, but higher concentrations (5 and 10% w/w) formed crystalline suspensions. The molecular weight of the PEG determined the melting temperature, with PEG 1000 being suitable for melting around body temperature. The drug-release kinetics was shown to be a function of both molecular weight and drug loading. Although the higher-molecular-weight PEG discs were resistant to surface erosion arising from an aqueous receptor phase, this effect was counterbalanced by more rapid and complete release when the ALA loading was increased. The lowest loading used (1% w/w) produced incomplete release, often not exceeding 30% of the total amount of drug.

Conclusions There is little doubt that photodynamic technology could make a significant and important impact in allowing expedient diagnosis or treatment of early-stage lesions without the need for surgery or toxic chemotherapy regimens. In this respect, ALA has many advantages over preformed photosensitizers in both diagnosis and treatment of lesions of the gastrointestinal tract, especially considering its high selectivity for neoplastic cells over normal tissue. The preliminary studies carried out here suggest that dosage forms prepared from poly (ethylene glycol) may be suitable for delivery of ALA to the colorectal region for photodynamic therapy or photodiagnosis of colorectal cancer. Further work is planned to formulate the system in the form of coated tablets to allow for colon-specific delivery after oral administration. Moreover, rectal delivery is to be optimized by shaping the dosage forms as suppositories.

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