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Journal of Molecular Graphics and Modelling 22 (2004) 507–517
Journal of Molecular Graphics and Modelling
www.elsevier.com/locate/JMGM

Towards integrated ADME prediction: past, present and future directions for modelling metabolism by UDP-glucuronosyltransferases

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Accepted 4 March 2004

Available online 27 April 2004

Abstract

Undesirable absorption, distribution, metabolism, excretion (ADME) properties are the cause of many drug development failures and this has led to the need to identify such problems earlier in the development process. This review highlights computational (in silico) approaches that have been used to identify the characteristics of ligands influencing molecular recognition and/or metabolism by the drug-metabolising enzyme UDP-gucuronosyltransferase (UGT). Current studies applying pharmacophore elucidation, 2D-quantitative structure metabolism relationships (2D-QSMR), 3D-quantitative structure metabolism relationships (3D-QSMR), and non-linear pattern recognition techniques such as artificial neural networks and support vector machines for modelling metabolism by UGT are reported. An assessment of the utility of in silico approaches for the qualitative and quantitative prediction of drug glucuronidation parameters highlights the benefit of using multiple pharmacophores and also non-linear techniques for classification. Some of the challenges facing the development of generalisable models for predicting metabolism by UGT, including the need for screening of more diverse structures, are also outlined.

Keywords: UDP-glucuronosyltransferase; UGT; ADME; QSAR; QSMR; Pharmacophore; Metabolism; Support vector machine; Modelling

1. Introduction

Drug discovery and development are expensive and time-consuming processes. Recognition by the pharmaceutical industry that undesirable absorption, distribution, metabolism and excretion (ADME) properties of new drug candidates are the cause of many clinical phase drug development failures has resulted in a paradigm shift to identify such problems early in the drug discovery process [1]. Thus, in vitro approaches are now widely used to investigate the ADME properties of new chemical entities and, more recently, computational (in silico) modelling has been investigated as a tool to optimise selection of the most suitable drug candidates for development.

Hepatic metabolism is the primary elimination mechanism for the majority of drugs, and indeed non-drug xenobiotics, in humans. Cytochrome P450 (CYP), a heme monooxygenase, and UDP-glucuronosyltransferase (UGT) are quantitatively the most important functionalisation (Phase I) and conjugation (Phase II) enzymes involved in drug and chemical metabolism. CYP and UGT are together responsible for the elimination of more than 90% of drugs cleared by the liver and additionally contribute to xenobiotic biotransformation in extrahepatic tissues (e.g. gastrointestinal tract). The versatility of both CYP and UGT arises from the existence of multiple enzyme forms ('isoforms'), which tend to differ in terms of substrate selectivity, patterns of drug–drug interactions, regulation and genetic polymorphism. Given these characteristics of CYP and UGT, prediction of drug metabolism parameters at the qualitative and quantitative levels assumes major importance in drug discovery and for dosage optimisation of established drugs. Identification of the isoform(s) responsible for the metabolism of any given drug, a process referred to reaction phenotyping, together with knowledge of isoform regulation, genetic polymorphism and drug interactions allows prediction of those factors likely to alter elimination and hence response in vivo [2–4]. Quantitative prediction most commonly involves scaling of the kinetic parameters (viz. K_m and V_{max}) for metabolite formation to in vivo hepatic clearance [8,10,14]. While in vitro and in silico approaches

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doi:10.1016/j.jmgm.2004.03.011

are finding increasing acceptance for prediction of the dispositional characteristics of drugs cleared by CYP-catalysed biotransformation, the application of these strategies to drugs metabolised by UGT has received less attention.

UGT catalyses the covalent linkage, or conjugation, of glucuronic acid to substrate bearing a suitable functional group (typically OH, CO_2H, NR_2) according to a second order nucleophilic substitution mechanism [5]. As noted above, UGT exists as an enzyme 'superfamily'. cDNAs encoding 18 UGT proteins have been isolated to date and these have been classified in two families, UGT1 and UGT2, based on amino acid sequence identity [6]. Of the various human UGTs, substrate selectivites now approach interpretable levels for UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17. Available evidence suggests that the individual UGT isoforms tend to exhibit unique substrate selectivities, but there is often a significant overlap of substrate profiles between isoforms [5,7]. In comparison to CYP, UGT structure–function relations are poorly understood. An X-ray crystal structure of a UGT is not yet available and few structurally or catalytically relevant amino acids have been identified.

This review highlights computational (in silico) approaches that have been used to identify the characteristics of ligands influencing molecular recognition and/or metabolism by UGT. An assessment of the utility of in silico approaches for the qualitative and quantitative prediction of drug glucuronidation parameters is also presented, together with an outline of some challenges facing the development of generalisable models for predicting metabolism by UGT.

2. Past

2.1. Non-human UGT modelling

Initial UGT modelling was performed using a relatively small number of datasets [8–14] derived from testing UGT enzyme activity towards simple chemical structures in laboratory animals. In a pioneering study, Bray et al. [8] reported an empirically derived structure–metabolism relationship (SMR) for the glucuronidation of 14 substituted phenols in the rabbit in vivo. Analysis of approximate velocity constants showed that a group of nine low molecular weight phenols had higher velocity constants than a second group of five phenols with higher molecular weights. Furthermore, these authors observed that the velocity constant did not seem to be related to the electronic influence of the substituent for *para*-substituted phenols. Little further was reported until Hansch et al. [15,16] demonstrated that the rate of glucuronidation of benzoic acids and primary aliphatic alcohols in rat and rabbit showed a parabolic dependence on the partition coefficient of the chemical between octanol and water ($\log P$). Schaefer et al. [17] subsequently investigated a series of substituted phenols and showed that substrate hydrophobicity was linearly correlated with rate of glucuronidation although the electronic constant ($\sigma-$) did not seem to play an important role despite the nucleophilic substitution mechanism involved.

The field did not progress until the late 1980s to early 1990s when research was spurred by an increasing realisation in the pharmaceutical industry of the importance of drug metabolism in the development process. This was also facilitated by technological advances in the field of molecular biology and improved assay techniques for measuring microsomal drug-metabolising enzyme activities. Expression of a cDNA encoding rat UGT1A6 in COS-7 cells enabled Jackson et al. [18] to screen the glucuronidation of more than 20 compounds to elucidate the substrate selectivity of this isoform. Restricted specificity towards planar phenols was demonstrated. An empirical SMR based on the observation that molecular 'thickness' needed to be <4 Å and the molecule length <6.3 Å to ensure effective glucuronidation was also reported. This study assumes particular importance as it was the first to investigate structural features of the substrates of an individual UGT isoform. SMRs developed using data generated from tissue microsome activities are usually of limited utility as there may be a number of different isoforms contributing to metabolism, which leads to a 'hybrid' representation of the chemical features influencing metabolism.

Mercier et al. [19] remodelled an early dataset [12–14] comprising 27 primary, secondary and tertiary alcohols tested for glucuronidation in the rabbit. Global, fragment and topological descriptors were used to determine the capacity of alcohols to undergo glucuronidation and three 'rules of thumb' were reported. The nature of the hydroxyl (OH) group was shown to be important. The glucuronidation of secondary and tertiary alcohols was comparable and an order of magnitude higher than that of primary alcohols. Furthermore, branching on primary alcohols and chain-lengthening up to a threshold of six carbon atoms increased the likelihood of glucuronidation. This work represents the first reported use of topological descriptors for modelling metabolism by UGT. Kim [20] also reviewed and remodelled a number of datasets at this time, confirming that much of the variation in rate of glucuronidation in rats and rabbits can be accounted for by the lipophilic properties of the substrates. In addition to the relevance of molar refractivity and standard substituent effects such as steric bulk, a parabolic dependence on $\log P$, with an optimum $\log P = 2$, was highlighted.

In 1992, the first of a number of reports that focused on classifying the metabolic conversion of a chemical appeared. Ghauri et al. [21] reported the relative conjugation 14 chemicals with glucuronic acid and glycine in the rat, and assessed the utility of several pattern recognition techniques for modelling the data. Three compounds were predominantly glucuronidated and 11 were predominantly conjugated with glycine. Principal component maps were used to develop a structure–metabolism model capable of separating the classes. Of the 39 primarily semi-empirical

molecular orbital-derived physicochemical descriptors, 10 allowed separation; the two most important parameters were partial atomic charge *meta* to the carboxylic acid and electrophilic superdelocalisability at same position. This work highlights that powerful electronic effects influence, and sometimes dominate, the route of metabolism.

Yin et al. [22] probed the effect of electron withdrawing and donating substituents on the glucuronidation rate of phenols using a preparation of partially purified rat liver UGT. Electron donation increased the rate of conjugation, consistent with nucleophilic attack via an S_N2 mechanism. There was a good correlation ($r = 0.88$) between Hammett $\sigma-$ values and log V_{max} difference (relative to parent). Inclusion of the hydrophobicity parameter π improved the correlation ($r = 0.98$). The strong $\sigma-$ correlation further highlights the importance of electronic influences and demonstrates that reactivity of the nucleophile is an important determinant of the rate of reaction. A hypothetical transition state was also proposed, involving axial orientation of glucuronic acid so as to maximise overlap between the oxygen of the phenol and the antibonding orbital of the anomeric sugar carbon.

In the mid-1990s two more reports applied the methodology highlighted by Ghauri et al. for classification of metabolic fate. Urinary excretion of sulfate and glucuronide conjugates of 16 substituted phenols in the rat were investigated [23] and classified using a number of semi-empirical molecular orbital-derived physicochemical descriptors. In addition to classification, linear regression techniques were used to derive quantitative structure–metabolism relationships (QSMR) capable of quantifying the relative excretion of sulfate and glucuronide conjugates in urine. The urinary excretion of glycine and glucuronide conjugates of 24 substituted benzoic acids in the rat were further investigated [24], and both classification and regression were performed. Percentage excretion of parent chemical was additionally modelled. The highest occupied molecular orbital (HOMO) energy, log *P* and electrophilic superdelocalisability on the aromatic ring were highlighted as important for delineating the classes. This study was extended to include a further 22 benzoic acids [25], further demonstrating the importance of log *P*, volume, ellipsoid dimensions, and partial atom charges.

The first three-dimensional QSMR (3D-QSMR) study was reported by Said et al. [26] and examined a series of 18 compounds (structurally related to phenolphthalein and a triphenylalkylcarboxylic acid) as inhibitors of rat liver bilirubin UGT (i.e. UGT1A1) using comparative molecular-field analysis (CoMFA), with IC_{50} values as the index of inhibition. The model allowed good prediction of inhibitory potency. The relative contribution of steric (43%) and electrostatic fields (57%) and their distribution allowed inference regarding binding site geometry. A subsequent study by this group [27] demonstrated potent, competitive inhibition of rat liver bilirubin glucuronidation by derivatives of 7,7,7-triphenylheptanoic acid. The relevance of pK_a was additionally demonstrated, with less acidic chemicals showing better inhibition and suggesting that absence of ionisation was important for inhibition. A similar effect of pK_a was observed using rat UGT2B1 [28], which metabolised phenols (unionised pK_a 9–13) effectively, but analogous carboxylic acids (p$K_a \sim 4$) poorly. Molecular modelling was used to highlight the 3D structural analogy between the inhibitors and bilirubin, which comprised aromatic (hydrophobic) groups in a triangular arrangement of dimensions 6.5, 4.6 and 4.4 Å. Naydenova et al. [29] reported a Free-Wilson QSAR study using the measured inhibitory activity of "transition-state" analogues based on oligopeptide derivatives of uridine. The study outlined the significance of a lipophilic residue linked to uridine by a minimum length of five atoms. A recent review of natural and synthetic inhibitors of UDP-glucuronosyltransferase has been published [30].

The rate of glucuronidation of 42 catechols (1,2-dihydroxyphenols) by rat liver microsomes was recently modelled using PLS regression [31] in order to determine the factors that direct catechol metabolism via different pathways and hence govern their biotransformation. Fourteen substructure descriptors were used, in addition to three physicochemical descriptors (log *P*, pK_a, molecular volume), however since a number of the descriptors are related only to catechols, the model can only be used to predict the metabolism of this chemical class. Significant descriptors for the rat liver microsomal UGT QSMR included hydrophobicity/volume, pK_a (parabolic around 8–9), hydrogen bonding and steric effects. Twelve substructure descriptors were the most relevant, with carboxyl group position and log *P*/volume exerting the strongest influence. The log *P*/volume term may reflect diffusion of substrates to the active site, while pK_a probably relates to mechanism of catalysis.

2.2. Human UGT modelling

Computational modelling of human xenobiotic glucuronidation has occurred only in the last decade or so. Kinetic constants (K_m and V_{max}) for the glucuronidation and sulfation of 24 monosubstituted phenolic substrates were analysed by Temellini et al. [32]. It was noted that an alkyl group in the *ortho* position increased K_m. Furthermore, a methyl or ethyl in any position increased the rate of glucuronidation compared to phenol. Higher V_{max} values were observed for *para* substitution than for *ortho*, while bulky *ortho* substituents impaired glucuronidation. More recently, the glucuronidation of propofol (2,6 diisopropyl, an intravenous anaesthetic, and a number of 2,5-(*o*,*m*-) and 2,6-(*o*,*o*-) disubstituted analogues were investigated using both human and rat microsomes [33]. In both species, K_m values for 2,5 disubstituted analogues were lower than for 2,6 disubstituted although V_{max} values associated with 2,5-substitution were higher than for 2,6-substitution. This highlights that 2,5-substitution leads to higher intrinsic clearance (V_{max}/K_m), information that could potentially be

used in the design of new anaesthetic agents with modified pharmacokinetic properties. Investigation of the glucuronidation of zidovudine and a number of nucleoside analogues by human liver microsomes demonstrated that nucleoside hydrophobicity correlates strongly with binding affinity (measured as K_i), although there was no correlation with V_{max} [34].

The cloning of human UGT cDNAs throughout the 1990s allowed expression of the individual isoforms in mammalian cell lines, an undertaking which has enormous implications for development of useful models of drug and chemical metabolism by UGT. Vashishtha et al. [35] have recently published QSMR for the glucuronidation of eight 1-substituted imadazoles by recombinant human UGT isoforms. These represent the only QSMR for glucuronides not linked through an oxygen atom, as imidazole glucuronidation results in a quaternary ammonium linkage. Testing against many of the known recombinant human UGT isoforms showed that UGT1A4 exhibited highest activity. Non-linear least-squares regression was used to develop models for prediction of K_m and V_{max}. There was a linear, inverse relationship between V_{max} and log P, and both the K_m and V_{max} models showed a linear relationship with pK_a. Correlation between activity and pK_a has been demonstrated previously, and is presumably related to the reaction mechanism of glucuronidation (i.e. S_N2). Delocalisation of electron density by the strongly electron-withdrawing nitro-substituted analogue, highlighted in this study by the absence of glucuronidation, is consistent with the catalytic mechanism again highlighting that the availability of lone pair of electrons on the substrate is important. It was also hypothesised that UGT1A3 and UGT1A4 selective substrates might be developed based on the observation that UGT1A3 preferentially glucuronidates aliphatic nitrogens at pH 8.4, whereas UGT1A4 apparently preferentially glucuronidates aromatic nitrogens at pH 7.4. The eight imidazoles were subsequently screened for glucuronidation by liver microsomes from four different species, including humans, in order to assess interspecies difference, which was significant (8–18-fold) [36]. This variation highlights the challenge in extrapolating QSMR from animal to human UGTs and the need to conduct studies with human isoforms wherever possible. As with the recombinant isoforms, lipophilic, electronic and steric descriptors were investigated; K_m correlated significantly with lipophilicity while V_{max}/K_m correlated with electronic substituent properties and pK_a.

QSMR that predict the K_m and V_{max} values for the glucuronidation of 24 simple 4-substituted phenols [37] by human UGT1A6 and UGT1A9 have also been developed. Molecular surface (MS-WHIM) and atomic descriptors (AT-WHIM) were used with a genetic function algorithm for model development. Eleven directional and six non-directional descriptors accounted for molecular surface properties of the compounds. Of the 24 compounds screened, all were glucuronidated by UGT1A9, but only 10 were metabolised by UGT1A6. K_m values for the UGT1A6 substrates tended to be higher than those observed for UGT1A9 substrates. As UGT1A6 substrate size (assessed as molecular volume) decreased, the V_{max} increased; that is the turnover of bulky phenols was low. The UGT1A6 K_m model included four descriptors associated with hydrogen-bond donation, positive molecular electrostatic potential, atomic mass and atomic partial charge. While also including atomic partial charge, the UGT1A9 K_m model contained descriptors associated with hydrogen-bond acceptors, negative molecular electrostatic potential and atomic polarisability. The authors acknowledged the limited range of K_m values (∼1 log order) modelled in this work.

2.3. Summary

A number of issues are apparent from assessment of the previously published studies, the most apparent being the challenge of combining the knowledge in an integrated manner. Despite the lack of integration, some of the studies highlight common factors. Not surprisingly, a range of descriptors is important for molecular recognition and catalysis by UGT and the descriptor-type is dependant on the parameter being modelled (e.g. K_m or V_{max}). Log P commonly occurs in models [38], mostly associated with K_m since lipophilicity is primarily associated with substrate binding (a low energy process) rather than catalysis [39]. Consistent with chemical intuition, electronic descriptors are most commonly associated with rate measures (e.g. V_{max}). In particular, the electronic nature of the nucleophile is important, a point highlighted in studies of substituent effects and by the significance of pK_a in models.

Assessment of the modelling techniques used indicates that both classification and regression models provide useful, indeed complementary, information. Despite the relevance of classification, few examples exist in the UGT literature. Only linear modelling techniques have previously been attempted. Thus non-linearity in datasets is difficult to assess other than through simple parabolic dependencies, and significant limitations may exist considering the complex nature of the processes being modelled. Moreover, no comparison between a range of modelling techniques and descriptor combinations using a single dataset has been attempted. The predominant use of liver microsomes generally precludes direct linkage of the relationships back to a particular isoform or active site. Data cannot be pooled due to significant interlaboratory variability in the assessment of UGT activity. Measures of V_{max} are particularly challenging as they differ widely depending on the level of UGT isoform expression and cannot currently be related to the amount of active enzyme, unlike the spectrophotometric measurement of CYP. Both K_m and V_{max} need to be modelled for each dataset in order to assess catalytic competency as intrinsic clearance (i.e. V_{max}/K_m), and little or no characterisation of conjugative regioselectivity on polynucleophilic substrates

exists. Finally, most modelling has been performed using animal data, which is not appropriate for translation to humans in most cases. Despite these caveats, the modelling of UGT ligands is feasible and certainly merits further investigation.

Many of the published studies aimed to assess the suitability of data modelling and pattern recognition techniques for determining chemical properties associated with metabolism by UGT. However, it is apparent that the concepts, approaches and descriptors outlined above are suitable, but only for certain endpoints. The appropriateness to the field as a whole of the endpoints being modelled clearly remains to be assessed. While it would appear that 'local' models can be developed and these are suitable for lead optimisation of analogues, the paradigm shift in the pharmaceutical industry to assess ADME properties earlier, for the purposes of choosing leads with suitable properties capable of reducing late-stage attrition [1] demands that models adopt a more 'global' nature, capable of prediction from diverse datasets [40,41]. Hence, the major bottleneck for the evolution of UGT modelling toward this endpoint is undoubtedly the chronic data shortfall that exists currently. However, even within the framework of data currently available, a number of avenues exist for the assessment and application of more advanced modelling approaches with more diverse datasets. Hence, the goal of recent research in this laboratory was to use human UGT isoform-selective data to investigate a range of modelling techniques and descriptors previously not assessed with UGT. The process included the development and use of more structurally diverse datasets with wider ranges of activity measures, and assessment of a broader range of techniques including pharmacophore elucidation and 3D-QSMR in addition to 2D-QSMR. Furthermore, we aimed to apply the latest linear and non-linear pattern recognition techniques for development of QSMR, but also for classification, and to incorporate more diverse descriptors accounting for the underlying mechanism and alignment rules appropriate to UGT. These studies aimed to generate predictive and interpretable models that can be used in a number of ways to help design and choose molecules with the desired metabolic stability that are metabolised by isoforms least affected by genetic polymorphism and drug–drug interactions. A summary of the principal results and conclusions from our assessments follow.

3. Present

3.1. 2D-QSMR

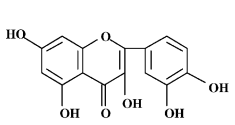
Initial modelling in this laboratory focused on substrates for UGT1A1 [42], UGT1A4 [43] and UGT1A9, each of which has been reported to glucuronidate structurally diverse compounds. As highlighted previously, evidence exists that linear regression techniques provide useful mathematical approaches for 2D-QSMR, and a number of 2D-descriptors show relevance. However, the size and diversity of the training sets used to generate models have generally been limited. Hence, we employed structurally diverse datasets (~24 molecules) spanning 3 log orders of K_m (or the surrogate, K_i) to assess how the increase in diversity would affect the 2D-QSMR modelling process [42,43]. The K_i values for the UGT1A1 substrates were determined in this laboratory, while the UGT1A4 K_m dataset was derived from data published by Green and Tephly [44,45]. Both datasets gave models with reasonable to good predictivity as assessed by independent test-set and cross-validation approaches as shown in Table 1.

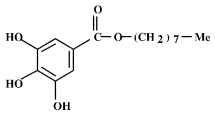
This established that more diverse datasets could be modelled, and in light the success of a UGT1A9 2D-QSMR developed previously for simple phenols [37], a more structurally diverse dataset of UGT1A9 substrates was assembled and their K_i values determined using the same procedure as reported previously for UGT1A1 [42]. The chemical structures and K_i values (μM) for the UGT1A9 dataset is shown in Fig. 1.

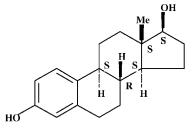
The same variable selection, partial-least-square (PLS) regression and principal component analysis (PCA) approaches reported for the UGT1A1 and UGT1A4 substrates were applied to the UGT1A9 dataset. The leave-one-out cross-validation r^2 statistic (<0.3) indicated that the model generated was not useful for predicting the K_i (or K_m) of other substrates. Further exploration of the dataset showed that there were no descriptors with significant linear correlation to the pK_i, when multiple testing was taken into account. This was in marked contrast to the UGT1A1 and UGT1A4 models, where many individual descriptors were strongly correlated ($r > 0.7$) with pK_i. This presumably reflects either the relatively small size of the dataset or the existence of significant

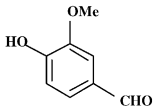
Table 1
Salient features of the UGT1A1, UGT1A4 and UGT1A9 models

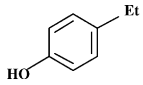
	UGT1A1	UGT1A4	UGT1A9
Common-features pharmacophore	1 Glucuronidation, 2 Hydrophobic	1 Glucuronidation, 2 Hydrophobic	1 Glucuronidation, 2 hydrophobic, 1 H-bond acceptor
2D-QSAR			
r^2	0.92	0.80	No model
CV r^2	0.92	0.73	No model
Pharmacophore-based 3D QSAR r^2	0.87	0.88	No model
Molecular-field-based 3D-QSAR r^2	0.71	0.73	No model

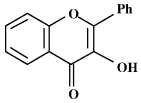

Quercetin
($K_{i,app}$ = 1 μM)

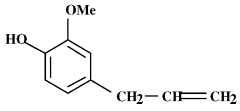

Octylgallate
($K_{i,app}$ = 1.5 μM)

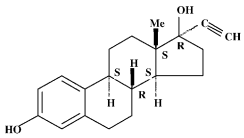

Estradiol
($K_{i,app}$ = 1.5 μM)

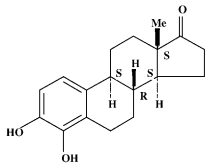

Vanillin
($K_{i,app}$ = 2 μM)

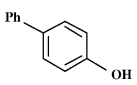

4-Ethylphenol
($K_{i,app}$ = 3.5 μM)

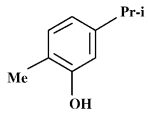

3-Hydroxyflavone
($K_{i,app}$ = 3.5 μM)

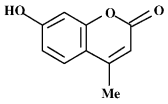

Eugenol
($K_{i,app}$ = 4.5 μM)

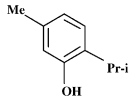

Ethinylestradiol
($K_{i,app}$ = 5.5 μM)

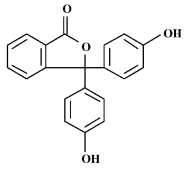

4-Hydroxyestrone
($K_{i,app}$ = 9 μM)

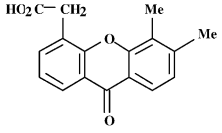

4-Hydroxybiphenyl
($K_{i,app}$ = 10 μM)

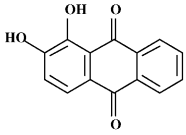

Carvacrol
($K_{i,app}$ = 11 μM)

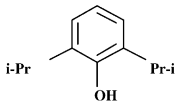

4-Methylumbelliferone
($K_{i,app}$ = 12 μM)

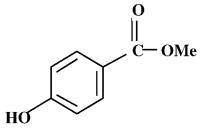

Thymol
($K_{i,app}$ = 13 μM)

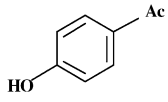

Phenolphthalein
($K_{i,app}$ = 25 μM)

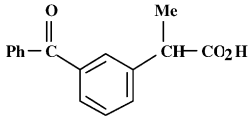

Dimethylxanthenone acetic acid (DMXAA)
($K_{i,app}$ = 25 μM)

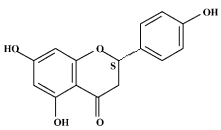

Alizarin
($K_{i,app}$ = 32 μM)

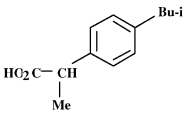

Propofol
($K_{i,app}$ = 57 μM)

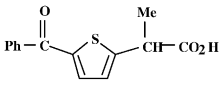

4-Hydroxybenzoic acid methyl ester
($K_{i,app}$ = 113 μM)


4-Acetylphenol
($K_{i,app}$ = 115 μM)


Ketoprofen
($K_{i,app}$ = 216 μM)


Naringenin
($K_{i,app}$ = 219 μM)


Ibuprofen
($K_{i,app}$ = 288 μM)


Tiaprofenic acid
($K_{i,app}$ = 417 μM)

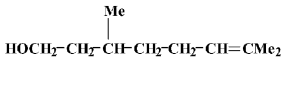

Citronellol
($K_{i,app}$ = 737 μM)

Fig. 1. The chemical structures and K_i values (μM) for the UGT1A9 dataset.

non-linearity in the relationship between descriptors and pK_i.

3.2. Pharmacophores

Pharmacophores represent a configuration of structural features associated with biological activity (in this case metabolism by an individual UGT isoform), and represent one of the most intuitive 3D-descriptors. A striking absence of any attempt to develop pharmacophores for UGT substrates is evident from the review of previous UGT modelling reports. This is somewhat surprising, given the widespread use of pharmacophore elucidation in general drug discovery and in particular its application to the other major drug-metabolising enzyme cytochrome P450 (CYP) [46]. Common-features pharmacophores for the UGT1A1 [42] and UGT1A4 [43] datasets were generated using the program Catalyst, with the aid of a novel, user-defined 'glucuronidation' feature that allowed overlay of the substrate conjugation sites. Use of a 'site of reaction' feature in pharmacophores of enzyme substrates allows a chemically sensible alignment that may better represent the catalytic binding mode. Interestingly the UGT1A1 and UGT1A4 pharmacophores share a striking similarity in core features; the site of glucuronidation is invariably adjacent to a hydrophobic region, with another hydrophobic domain located 6–8 Å from the site of conjugation [47] (see Fig. 2). Using the UGT1A9 dataset published in this report, and the same pharmacophore approach used with UGT1A1/UGT1A4, a UGT1A9 pharmacophore has also been developed. Fig. 2 shows the UGT1A9 pharmacophore in addition to the previously reported UGT1A1 and UGT1A4 pharmacophores. Although the core features recur, the chemical phenotype of UGT1A9 substrates differs from that of UGT1A1/UGT1A4 substrates through the additional presence of a hydrogen-bond acceptor (HBA) region near the outermost hydrophobic region. These core features may represent the molecular basis for the overlapping substrate selectivities observed between UGT1A family isoforms.

3.3. 3D-QSMR

Determination of a pharmacophore represents an early, but significant, step towards the understanding of a given receptor–ligand binding event. However, while satisfaction of the pharmacophore criteria by a molecule is necessary for a binding interaction to occur, steric and electronic influences on the binding event may nevertheless be ignored by this approach. Thus, a 3D-QSAR modelling approach that more specifically accounts for such influences could complement the pharmacophore approach well.

The UGT1A1 and UGT1A4 common-features pharmacophores were used as alignment rules for one such molecular-field-based approach, a self organising molecular-field analysis (SOMFA), which is a variant of CoMFA [42,43]. Both alignment rules gave moderately predictive models, though not as predictive as the 2D-QSMR (see Table 1). An alternate 3D-QSAR approach is available through the Hypogen module of Catalyst. The Hypogen algorithm searches for a 3D arrangement of chemical features to explain trends and variations of activity with chemical structure, and can be used to quantitatively predict the binding of chemicals by an enzyme or receptor. The 'fit' of the chemicals to the pharmacophore features (i.e. how closely the features of each chemical can match the features of the pharmacophore) can then be linearly correlated to the activity of the chemicals. Application of this approach with UGT1A1 and UGT1A4 yielded moderately predictive models also (see Table 1), although the alignments provided little catalytic interpretation since shared sites of conjugation were not overlaid. Using the UGT1A9 dataset described above, no satisfactorily predictive model could be developed, perhaps for reasons related to multiple binding modes that are elaborated further in the following section.

3.4. Classification

Use of computational methods for identification of the UGT isoform(s) responsible for the metabolism of any given drug (in silico reaction phenotyping), potentially provides a facile and economic alternative to in vitro approaches [48]. In vitro procedures for reaction phenotyping normally involve the integration of data from human liver microsomes (although hepatocyte suspensions may also be used) and recombinant human enzymes. In silico reaction phenotyping, however, utilises pattern recognition techniques to elucidate a set of chemical properties (descriptors) associated with the binding and metabolism of substrates by an enzyme. Developing datasets for each isoform by combining known substrates with carefully selected and relevant non-substrate data has the potential to provide valuable information when classification algorithms are used to probe explicit differences between the two sets.

A comprehensive database of all reported substrates and non-substrates of 12 human UGT isoforms has been compiled in this laboratory to investigate the utility of various classification techniques for UGT reaction phenotyping. The isoforms investigated are UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17. Partial-least-squares discriminant analysis (PLS-DA) in addition to an artificial neural network (ANN) and a Support Vector Machine (SVM) classification algorithm, which both potentially provide greater flexibility and generalisation performance [49], were compared using 2D-chemical descriptors generated for substrates and non-substrates [50]. The SVM algorithm generally outperformed ANN and PLS-DA showing excellent (>80% accuracy) predictability for five isoforms and good (63–80% accuracy) predictability for the other seven, confirming the potential value of this approach. The variability between isoforms probably reflects differences in the size, structural diversity and quality of the datasets, and confirms the inherent complexity

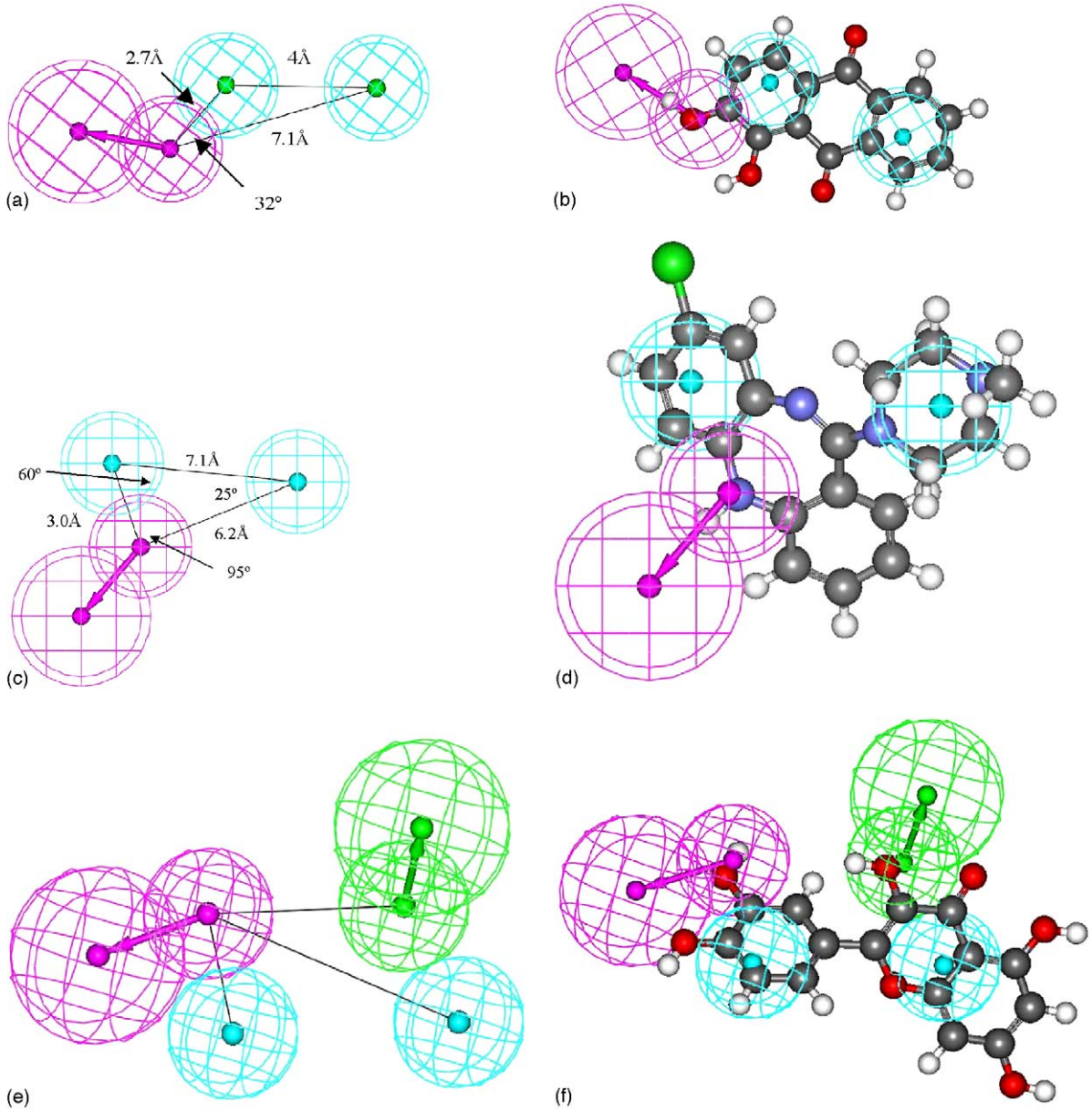


Fig. 2. UGT1A1 (panel a), UGT1A4 (panel c) and UGT1A9 (panel e) common-features pharmacophores. The cyan, green and purple spheres represent a hydrophobic feature, hydrogen-bond acceptor and glucuronidation feature, respectively. Bold arrows show the direction of lone-pair electron donation. Panels (b), (d) and (f) show alizarin, clozapine and quercetin mapped to the respective UGT1A1, UGT1A4 and UGT1A9 pharmacophores.

of defining relationships for enzymes with broad and overlapping selectivities. This work represents the first detailed assessment of SVM algorithms for ADME prediction, although an SVM has recently been successfully applied to classifying drug CNS-permeability for a single dataset [51].

In addition to good predictivity, it is desirable for a model to have a degree of interpretability, in order to allow translation of chemical characteristics directly to the molecular design and optimisation process. Recent studies in this laboratory investigated whether multiple pharmacophores, which allow simple interpretation and encompass the chemical features relevant to each of the possible binding modes, would prove useful for classification of UGT substrates [52]. Single, common-features pharmacophores require the assumption that all ligands share a common binding mode [53]. However, this assumption clearly does not hold for many drug-metabolising enzymes, which often generate multiple metabolites from a single chemical presumably due to multiple binding modes. While the field of drug-metabolism prediction is a novel application for such ‘pharmacophore fingerprints’, successful application of this concept has been reported in other fields of drug discovery [54,55]. Indeed multiple pharmacophores

have also been utilised to characterise molecular recognition by a number of xenobiotic binding proteins [56–58]. Employing concepts developed in these reports and incorporating the 'site of metabolism' pharmacophore concept developed earlier, pattern recognition techniques were used to select subsets of pharmacophores associated with substrates and non-substrates of the 12 UGT isoforms in our database. The models were more intuitive but marginally less predictive than classification using 2D-descriptors. The ease of interpretability associated with these models greatly increases the potential for the design of ligands with either enhanced or reduced affinity for particular UGT isoforms. Furthermore, both the 2D-descriptor and pharmacophore-fingerprint-based classifications should be amenable to high-throughput virtual screening paradigms, well suited to ADME property design process increasingly incorporated in drug discovery.

It was also observed that a number of the pharmacophores selected as important included simple chemical features. Further analysis demonstrated significant isoform-related differences in the prevalence of nucleophilic functional groups (e.g. phenol, hydroxyl, carboxyl, imidazole, and primary, secondary and tertiary amine function) in substrates [52]. These simple, intuitive features may be used for classification with equal or better accuracy than approaches using 2D-descriptors or pharmacophore 'fingerprints'. This method is likely to prove most valuable when conjugative regioselectivity data, which has generally been characterised poorly in the past, is included. Advances in the prediction of CYP regioselectivity using quantum chemical descriptors [59,60] is in no small part due to the availability of training data derived from thorough characterisation of metabolite regioselectivity. Diligence in the characterisation of conjugative regioselectivity is required in order to realise similar advances with in silico UGT models.

3.5. Future directions

The successful adoption of in silico approaches for modelling CYP-catalysed biotransformation has progressed in parallel with the increasing availability of CYP isoform substrate and inhibitor selectivities, and the development of such models has also been aided by access to homology models of human CYP's. However, the development of models for predicting metabolism and for characterising structural features of substrates for UGT isoforms is less advanced relative to CYP. Only recently has the substrate profile of UGT isoforms begun to approach an interpretable level, and an X-ray crystal structure is not yet available. Nevertheless, it seems certain that the modelling techniques outlined in this review will find increasing application to UGT given the significance of this enzyme for drug elimination.

In a recent review Van de Waterbeemd and Gifford [1] note, "Good predictive models for ADMET parameters depend crucially on selecting the right mathematical approach, the right molecular descriptors for the particular ADMET endpoint, and a sufficiently large set of experimental data relating to this endpoint. . . . In particular, more needs to be learnt about how the size of the training set influences the choice of the most capable model." The limited UGT kinetic data currently available lends itself primarily to the development of 'local' models and the linear 2D-QSMR and classification approaches described here seem well suited to this application. Increasingly, however, there is a requirement for 'global' or generalisable ADMET models [1,40]. Given the inherent complexity of the physiological processes involved and the vastness of the chemical space to be investigated, sophisticated pattern recognition techniques and large, quality datasets will undoubtedly be required to generate 'globally' predictive models in future. Work in this laboratory suggests that linear techniques and 'reductionist' alignment-based approaches do not account for the promiscuous nature of drug-metabolising enzymes and show mixed success with more structurally diverse datasets. However, increased dataset size in addition to the application of non-linear pattern recognition techniques or use of multiple pharmacophores can result in significant progress towards generalisable and interpretable models. The crucial requisite for advancement of the field, however, is the development and publication of datasets of increased size and structural diversity, a requirement mirrored across the ADME prediction field as a whole [1]. Future assessment of the 'chemical diversity space' that has been screened against UGT should also highlight the areas where increased screening will serve most usefully.

The other major challenge for UGT modelling is to link the complementary roles of ligand-based and protein-based modelling. Most critical in this pursuit is obtaining a representation of the 3D structure of UGTs, either by homology modelling or by meeting the challenge of crystallising human UGTs. The docking of ligands into protein structures has aided determination of reaction phenotype and binding affinities of ligands for CYP [61] and such techniques are anticipated to translate effectively to UGTs. Increasingly, advancements in crystallisation technology and high-throughput capacity are being focused toward membrane-bound enzymes, including human CYP. Homology modelling of UGT's may also be realised by reference to the increasing studies of the structural biology of other glycosyltransferases [62].

More generally, robust assessment of models is essential and comparison between pattern recognition techniques for datasets of given sizes and diversity will serve as a solid grounding from which to further advance UGT modelling. Vigilance in defining the required endpoint of the model, and increased understanding and consideration of the underlying mechanisms of the metabolic processes involved in molecular recognition and catalysis will ensure increasingly useful models.

Acknowledgements

This work was funded by a grant from the National Health and Medical Research Council of Australia. MJS is the recipient of an Australian Postgraduate Award.

References

[1] H. Van de Waterbeemd, E. Gifford, ADMET in silico modelling: towards prediction paradise? Nat. Rev. Drug Discov. 2 (2003) 192–204.
[2] J.O. Miners, M.E. Veronese, D.J. Birkett, In vitro approaches for the prediction of human drug metabolism, Ann. Rep. Med. Chem. 29 (1994) 307–316.
[3] A.D. Rodrigues, Integrated cytochrome P450 reaction phenotyping. Attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes, Biochem. Pharmacol. 57 (1999) 465–480.
[4] J.O. Miners, Hitchhiking the technology bandwagon: the evolution of drug metabolism, Clin. Exp. Pharmacol. Physiol. 29 (2002) 1040–1044.
[5] A. Radominska-Pandya, P.J. Czernik, J.M. Little, E. Battaglia, P.I. Mackenzie, Structural and functional studies of UDP-glucuronosyltransferases, Drug Metab. Rev. 31 (1999) 817–899.
[6] P.I. Mackenzie, I.S. Owens, B. Burchell, K.W. Bock, A. Bairoch, A. Belanger, The UDP-glycosyltransferase gene superfamily—recommended nomenclature update based on evolutionary divergence, Pharmacogenetics 7 (1997) 255–269.
[7] R.H. Tukey, C.P. Strassburg, Human UDP-glucuronosyltransferases: metabolism, expression, and disease, Ann. Rev. Pharmacol. Toxicol. 40 (2000) 581–616.
[8] H.G. Bray, B.G. Humphris, W.V. Thorpe, K. White, P.B. Wood, Kinetic studies of the metabolism of foreign organic compounds. III. Conjugation of phenols with glucuronic acid, Biochem. J. 52 (1952) 416–419.
[9] G.J. Mulder, A.B. Van Doorn, A rapid NAD+-linked assay for microsomal uridine diphosphate glucuronyltransferase of rat liver and some observations on substrate specificity of the enzyme, Biochem. J. 151 (1975) 131–140.
[10] H.P. Illing, D. Benford, Observations on the accessibility of acceptor substrates to the active centre of UDP-glucuronosyltransferase in vitro, Biochim. Biophys. 429 (1976) 768–779.
[11] G.J. Mulder, J.H.N. Meerman, Glucuronidation and sulfation in vivo and in vitro: selective inhibition of sulfation by drugs and deficiency of inorganic sulfate, in: Proceedings of the Conjugation Reactions of Drug Biotransformations, 1978, pp. 389–397.
[12] I.A. Kamil, J.N. Smith, R.T. Williams, Detoxication. L. Isolation of methyl and ethyl glucuronides from the urine of rabbits receiving methanol and ethanol, Biochem. J. 54 (1953) 390–392.
[13] I.A. Kamil, J.N. Smith, R.T. Williams, Detoxication. XLVII. Formation of ester glucuronides of aliphatic acids during the metabolism of 2-ethylbutanol and 2-ethylhexanol, Biochem. J. 53 (1953) 137–140.
[14] I.A. Kamil, J.N. Smith, R.T. Williams, Detoxication. XLVI. Metabolism of aliphatic alcohols. The glucuronic conjugation of cyclic aliphatic alcohols, Biochem. J. 53 (1953) 129–136.
[15] C. Hansch, E.J. Lien, F. Helmer, Structure–activity correlations in the metabolism of drugs, Arch. Biochem. Biophys. 128 (1968) 319–330.
[16] C. Hansch, J.M. Clayton, Lipophilic character and biological activity of drugs. II. Parabolic case, J. Pharm. Sci. 62 (1973) 1–21.
[17] M. Schaefer, I. Okulicz-Kozaryn, A. Batt, G. Siest, V. Loppinet, Structure–activity relationships in glucuronidation of substituted phenols, Eur. J. Med. Chem. 16 (1981) 461–464.
[18] M.R. Jackson, S. Fournel-Gigleux, D. Harding, B. Burchell, Examination of the substrate specificity of cloned rat kidney phenol UDP-glucuronyltransferase expressed in COS-7 cells, Mol. Pharmacol. 34 (1988) 638–642.
[19] C. Mercier, V. Fabart, Y. Sobel, J.E. Dubois, Modeling alcohol metabolism with the DARC/CALPHI system, J. Med. Chem. 34 (1991) 934–942.
[20] K.H. Kim, Quantitative structure–activity relationships of the metabolism of drugs by uridine diphosphate glucuronosyltransferase, J. Pharm. Sci. 80 (1991) 966–970.
[21] F.Y. Ghauri, C.A. Blackledge, R.C. Glen, B.C. Sweatman, J.C. Lindon, C.R. Beddell, I.D. Wilson, J.K. Nicholson, Quantitative structure–metabolism relationships for substituted benzoic acids in the rat. Computational chemistry, NMR spectroscopy and pattern recognition studies, Biochem. Pharmacol. 44 (1992) 1935–1946.
[22] H.Q. Yin, G. Bennett, J.P. Jones, Mechanistic studies of uridine diphosphate glucuronosyltransferase, Chemico-Biol. Interact. 90 (1994) 47–58.
[23] E. Holmes, B.C. Sweatman, M.E. Bollard, C.A. Blackledge, C.R. Beddell, I.D. Wilson, J.C. Lindon, J.K. Nicholson, Prediction of urinary sulphate and glucuronide conjugate excretion for substituted phenols in the rat using quantitative structure–metabolism relationships, Xenobiotica 25 (1995) 1269–1281.
[24] B.C. Cupid, C.R. Beddell, J.C. Lindon, I.D. Wilson, J.K. Nicholson, Quantitative structure–metabolism relationships for substituted benzoic acids in the rabbit: prediction of urinary excretion of glycine and glucuronide conjugates, Xenobiotica 26 (1996) 157–176.
[25] B.C. Cupid, E. Holmes, I.D. Wilson, J.C. Lindon, J.K. Nicholson, Quantitative structure–metabolism relationships (QSMR) using computational chemistry: pattern recognition analysis and statistical prediction of phase II conjugation reactions of substituted benzoic acids in the rat, Xenobiotica 29 (1999) 27–42.
[26] M. Said, J.C. Ziegler, J. Magdalou, A. Elass, G. Vergoten, Inhibition of bilirubin UDP-glucuronosyltransferase—a comparative molecular field analysis (CoMFA), Quant. Struct. Act. Relat. 15 (1996) 382–388.
[27] M. Said, E. Battaglia, A. Elass, V. Cano, J.C. Ziegler, A. Cartier, M.H. Livertoux, G. Vergoten, S. Fournel-Gigleux, J. Magdalou, Mechanism of inhibition of rat liver bilirubin UDP-glucuronosyltransferase by triphenylalkyl derivatives, J. Biochem. Mol. Toxicol. 12 (1998) 19–27.
[28] M. Pritchard, S. Fournel-Gigleux, G. Siest, P.I. Mackenzie, J.A. Magdalou, Recombinant phenobarbital-inducible rat liver UDP-glucuronosyltransferase (UDP-glucuronosyltransferase 2B1) stably expressed in V79 cells catalyzes the glucuronidation of morphine, phenols, and carboxylic acids, Mol. Pharmacol. 45 (1994) 42–50.
[29] Z.G. Naydenova, K.C. Grancharov, D.K. Alargov, E.V. Golovinsky, I.M. Stanoeva, L.D. Shalamanova, I.K. Pajeva, Inhibition of UDP-glucuronosyltransferase by 5′-*o*-amino acid and oligopeptide derivatives of uridine—structure–activity relationships, Z. Naturforsch. 53 (1998) 173–181.
[30] K. Grancharov, Z. Naydenova, S. Lozeva, E. Golovinsky, Natural and synthetic inhibitors of UDP-glucuronosyltransferase, Pharmacol. Ther. 89 (2001) 176–181.
[31] L. Antonio, J.P. Grillasca, J. Taskinen, E. Elovaara, B. Burchell, M.H. Piet, B. Ethell, M. Ouzzine, S. Fournel-Gigleux, J. Magdalou, Characterization of catechol glucuronidation in rat liver, Drug Metab. Dispos. 30 (2002) 199–207.
[32] A. Temellini, M. Franchi, L. Giuliani, G.M. Pacifici, Human liver sulphotransferase and UDP-glucuronosyltransferase: structure–activity relationship for phenolic substrates, Xenobiotica 21 (1991) 171–177.
[33] M. Shimizu, Y. Matsumoto, M. Tatsuno, M. Fukuoka, Glucuronidation of propofol and its analogs by human and rat liver microsomes, Biol. Pharm. Bull. 26 (2003) 216–219.

[34] A. Resetar, D. Minick, T. Spector, Glucuronidation of 3′-azido-3′-deoxythymidine catalyzed by human liver UDP-glucuronosyltransferase. Significance of nucleoside hydrophobicity and inhibition by xenobiotics, Biochem. Pharmacol. 42 (1991) 559–568.
[35] S.C. Vashishtha, E.M. Hawes, G. McKay, D.J. McCann, Quaternary ammonium-linked glucuronidation of 1-substituted imidazoles: studies of human UDP-glucuronosyltransferases involved and substrate specificities, Drug Metab. Dispos. 29 (2001) 1290–1295.
[36] S.C. Vashishtha, E.M. Hawes, D.J. McCann, O. Gosheh, L. Hogg, Quaternary ammonium-linked glucuronidation of 1-substituted imidazoles by liver microsomes: interspecies differences and structure–metabolism relationships, Drug Metab. Dispos. 30 (2002) 1070–1076.
[37] B.T. Ethell, S. Ekins, J.B. Wang, B. Burchell, Quantitative structure activity relationships for the glucuronidation of simple phenols by expressed human UGT1A6 and UGT1A9, Drug Metab. Dispos. 30 (2002) 734–738.
[38] C. Hansch, Comparative QSAR: understanding hydrophobic interactions, Class. Three-dimens. QSAR Agrochem. 606 (1995) 254–262.
[39] B. Testa, P. Crivori, M. Reist, P.A. Carrupt, The influence of lipophilicity on the pharmacokinetic behavior of drugs: concepts and examples, Perspect. Drug Discov. Des. 19 (2000) 179–211.
[40] P. Buchwald, N. Bodor, Computer-aided drug design: the role of quantitative structure–property, structure–activity and structure–metabolism relationships (QSPR, QSAR, QSMR), Drugs Future 27 (2002) 577–588.
[41] H. Van de Waterbeemd, High-throughput and in silico techniques in drug metabolism and pharmacokinetics, Curr. Opin. Drug Discov. Dev. 5 (2002) 33–43.
[42] M.J. Sorich, P.A. Smith, R.A. McKinnon, J.O. Miners, Pharmacophore and quantitative structure activity relationship modeling of UDP-glucuronosyltransferase 1A1 (UGT1A1) substrates, Pharmacogenetics 12 (2002) 635–645.
[43] P.A. Smith, M.J. Sorich, R.A. McKinnon, J.O. Miners, Pharmacophore and quantitative structure activity relationship (QSAR) modelling: complementary approaches to the rationalisation and prediction of UDP-glucuronosyltransferase 1A4 (UGT1A4) substrate selectivity, J. Med. Chem. 46 (2002) 1617–1626.
[44] M.D. Green, C.D. King, B. Mojarrabi, P.I. Mackenzie, T.R. Tephly, Glucuronidation of amines and other xenobiotics catalyzed by expressed human UDP-glucuronosyltransferase 1A3, Drug Metab. Dispos. 26 (1998) 507–512.
[45] M.D. Green, T.R. Tephly, Glucuronidation of amines and hydroxylated xenobiotics and endobiotics catalyzed by expressed human Ugt1.4 protein, Drug Metab. Dispos. 24 (1996) 356–363.
[46] M.J. de Groot, S. Ekins, Pharmacophore modeling of cytochromes P450, Adv. Drug Del. Rev. 54 (2002) 367–383.
[47] P.A. Smith, M.J. Sorich, R.A. McKinnon, J.O. Miners, In silico insights: chemical and structural characteristics associated with UDP-glucuronosyltransferase (UGT) substrate selectivity, Clin. Exp. Pharmacol. Physiol. 30 (2003) 836–840.
[48] J.O. Miners, P.A. Smith, M.J. Sorich, R.A. McKinnon, P.I. Mackenzie, Predicting human drug glucuronidation parameters: application of in vitro and in silico modelling approaches, Ann. Rev. Pharmacol. Toxicol. 44 (2004) 1–25.
[49] D. Winkler, The broader applications of neural and genetic modelling methods, Drug Discov. Today 6 (2001) 1198–1199.
[50] M.J. Sorich, R.A. McKinnon, J.O. Miners, P.A. Smith, Comparison of linear and non-linear classification algorithms for the prediction of chemical metabolism by UDP-glucuronosyltransferase isoforms, J. Chem. Inform. Comp. Sci. 43 (2003) 2019–2024.
[51] S. Doniger, T. Hofmann, J. Yeh, Predicting CNS permeability of drug molecules: comparison of neural network and support vector machine algorithms, J. Comput. Biol. 9 (2002) 849–864.
[52] M.J. Sorich, J.O. Miners, R.A. McKinnon, P.A. Smith, Multiple pharmacophores for the investigation of human UDP-glucuronosyltransferase isoform substrate selectivity, Mol. Pharm. 65 (2004) 301–308.
[53] A.K. Ghose, J.J. Wendoloski, Pharmacophore modelling—methods, experimental verification and applications, Perspect. Drug Discov. Des. 9–11 (1998) 253–271.
[54] M.J. McGregor, S.M. Muskal, Pharmacophore fingerprinting. 1. Application to QSAR and focused library design, J. Chem. Inf. Comput. Sci. 39 (1999) 569–574.
[55] J.S. Mason, A.C. Good, E.J. Martin, 3-D pharmacophores in drug discovery, Curr. Pharmacol. Des. 7 (2001) 567–597.
[56] A. Garrigues, N. Loiseau, M. Delaforge, J. Ferte, M. Garrigos, Characterization of two pharmacophores on the multidrug transporter P-glycoprotein, Mol. Pharmacol. 62 (2002) 1288–1298.
[57] J.E. Penzotti, M.L. Lamb, E. Evensen, P.D.J. Grootenhuis, A computational ensemble pharmacophore model for identifying substrates of P-glycoprotein, J. Med. Chem. 45 (2002) 1737–1740.
[58] R.E. Watkins, S.M. Noble, M.R. Redinbo, Structural insights into the promiscuity and function of the human pregnane X receptor, Curr. Opin. Drug Discov. Dev. 5 (2002) 150–158.
[59] M.J. de Groot, M.J. Ackland, V.A. Horne, A.A. Alex, B.C. Jones, A novel approach to predicting P450 mediated drug metabolism. CYP2D6 catalyzed *N*-dealkylation reactions and qualitative metabolite predictions using a combined protein and pharmacophore model for CYP2D6, J. Med. Chem. 42 (1999) 4062–4070.
[60] J.P. Jones, M. Mysinger, K.R. Korzekwa, Computational models for cytochrome P450: a predictive electronic model for aromatic oxidation and hydrogen atom abstraction, Drug Metab. Disp. 30 (2002) 7–12.
[61] G.M. Keseru, A virtual high throughput screen for high affinity cytochrome P450cam substrates. Implications for in silico prediction of drug metabolism, J. Comput.-aided Mol. Des. 15 (2001) 649–657.
[62] Y.N. Hu, S. Walker, Remarkable structural similarities between diverse glycosyltransferases, Chem. Biol. 9 (2002) 1287–1296.