In Silico ADME Prediction: Data, Models, Facts and Myths

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Abstract: A critical review of a very recent work in the field of in silico ADME prediction is presented with emphasis on the work published during the period 2000-2002, and several other review articles are mentioned in order to offer a broader view of the field. We find that not much progress has been made in developing robust and predictive models, and that the lack of accurate data, together with the use of questionable modeling end-points, has greatly hindered the real progress in defining generally applicable models.

Due to the largely empirical nature of QSAR/QSPR approaches, general and truly predictive models for complex phenomena, such as absorption and clearance, may still be chimeric. The development of local models for use within focused chemical series may be the most appropriate way of utilizing in silico ADME predictions, once experience and data have been gained on a given project and/or structural class.

Keywords: ADME, distribution, solubility, permeability, in silico, excretion, metabolism.

INTRODUCTION

The pressures on the drug industry to limit the time and expense of drug development, and the realization that quite a few hurdles are there due to non-optimal Absorption, Metabolism, Distribution and Excretion (ADME) properties, have brought into focus high-throughput ADME properties screening methods and their in silico counterparts. Examples of experimental screens are represented by the Caco-2 [1,2], MDCK [3] and PAMPA [4] screening approaches for membrane permeability, as well as by various solubility screening methods largely based on turbidimetric end-points. Both of these aspects contribute to absorption.

The last 5-10 years have seen a "combinatorial explosion" of hardware and high throughput experimental methods, aimed at determining ADME properties in a medium to high-throughput fashion, and of software tools aimed at their computation. The resulting questions of what tools are most useful and what level of accuracy should be sought are open-ended, and the subject of ongoing debate. The answer, in a multidimensional and highly regulated and complex area of research, such as drug discovery, cannot be definitive and will depend on the property sought, the stage at which the screen is used, and the intended use of the data. It is difficult, but not redundant, to speculate on the "human factor", i.e., the differing views among researchers in the field, which cross national, industrial and academic borders, and there will be greatly differing views on speed vs. accuracy even within a research organization. However, these differing views are in turn reflected by the quality of the data available for database, QSAR and general computational efforts.

It is the data quality, in our opinion, what is mostly lacking in these efforts, as a plethora of models have been developed through the use of similar "ready" data sets from one author to the other, without reference to or a thorough analysis of the original literature. This is a practice to be discouraged because it will contribute to error propagation, and will not show any significant improvement in any set of descriptors. A slightly better r^2 value, obtained for a more recent model, is hardly a reason for claiming an improvement over a previous model. Test sets are often not used or they are very small, leaving the reader wondering about the ruggedness and predictive ability of the model. These aspects severely limit progress and multiply the plethora of "preferred" descriptors without the possibility of real discrimination between models and statistical tools. A recent book published by Todeschini and Consonni [5] provides an excellent source of information and references with 1800 descriptors examined, and more are continuously developed or revisited while only limited efforts are devoted to improving the size and quality of data sets (see articles by Petrauskas *et al.*, Tetko and Caron *et al.* in this issue).

Furthermore, a choice of the model is often made based on the intended application, and they can be broadly divided in "fast scoring" models that do not lend themselves to physical interpretation and could hardly be used to modify the structural class of interest, and methods that do allow some physically intuitive and quali-quantitative interpretation and may be used, albeit with some difficulty, to modify structural feature of drug compounds. The latter kind may also help shed light on the biochemical and physiological mechanisms underlying the property of interest. Obviously, the choice is often a matter of the stage at which the model is being used but, in our opinion, preference should be given to methods amenable to some physical interpretation.

In this work, we have examined all four aspects of ADME, and have generally considered the period comprised between 2000 and 2002, with some more recent work appearing in the literature in the very early part of 2003. Many more articles have appeared between the first and subsequent drafts of this work, and a search through the

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1389-5575/03 $41.00+.00 © 2003 Bentham Science Publishers, Ltd.

literature is likely to yield several new publications on this general topic every 2 to 4 weeks. We have included several other recent reviews that have appeared in the literature on the general topic of in silico ADME prediction [6-11], and on specific aspects of it [12-16]. It is hoped that this would offer an even more comprehensive historical view of the field.

Finally, we note that the tables presented offer an "abstract" of the data and method considered by the original authors, and that the format chosen should constitute a useful "at a glance" comparison tool.

ABSORPTION

Wessel and Mente [17], in a recent review article, concluded that the lack of quality data had precluded any real advance in absorption (defined as the actual uptake of drug by the intestinal capillaries) prediction. Furthermore, they noted, numerous efforts aimed at predicting log P_{oct} had appeared, while distribution (aside from blood-brain barrier permeation), metabolism, and excretion were being largely ignored. We believe these statements are still largely true to date.

Passive absorption is a very complex phenomenon [18], the prediction of which encompasses the knowledge of solid state (lattice energy) and solution properties (solvation by water) of a compound, its lipophilicity and H-bonding properties as well as its acid-base properties. These components of passive absorption need to be balanced, since a compound that is permeable may not be well absorbed, owing to its poor solubility. The reverse is true for a soluble but poorly permeable (i.e., unable to cross the lipoidal intestinal membrane) compound. However, the former case seems to be more the rule in our experience, and Curatolo [19] points out that a very soluble compound may still overcome a poor permeability across intestinal membranes, owing to the fact that the gradient between the apical (lumen) to the basolateral (blood capillaries) sides of the intestinal membrane is very high. As a general observation, and the SAR for the target protein notwithstanding, we would submit that solubility, perhaps due to an upward shift in size and lipophilicity of molecules [20] may be a wider problem than permeability, in drug research. Furthermore, the prediction of permeability is often complicated by a plethora of active efflux or influx transporters we are just beginning to understand and which may be coupled, as in the case of P-gp (P-glycoprotein) and CYP450 3A4, with metabolic barriers. The question may be asked whether or not a data set takes into account these phenomena, and whether or not the prediction will be able to account for them as well.

The models considered are summarized in Table 1, and are discussed in more detail below, with a particular interest in the presence or absence of test sets, size and quality of the data set as well as, of course, the type of descriptors.

Solubility

Solubility modeling has been the subject of many efforts described recently [15,16], and some of the most recent published models are reviewed here in detail, while other models, not discussed in detail in the text, are included in Table 1 (see also the article by Petrauskas *et al.* in this issue). Notably, the Yalkowski's AQUASOL [21] and the PhysProp [22] databases have been widely used (see article by Tetko in this issue), and the range of data utilized in most approaches has spanned, expressed as the logarithm of the molar solubility (log S), 10 to 12 orders of magnitude. This practice may result in artificially high correlation coefficients, and may be detrimental if a fairly accurate value in a meaningful range is desired. However, this is an essentially constant theme in the literature, as the examples discussed and the data reported in Table 1 will reveal.

In some cases a critical evaluation of the data used has been performed, and high quality in-house data have been used by scientists in an industrial pharmaceutical setting [23] with a description of whether or not they were equilibrium data. In other cases, there was no mention of data selection criteria that, although arguably arbitrary, constitute a necessary filter for data quality.

In general, however, there is a gross under-appreciation for the fact that the determination of aqueous equilibrium solubility data is far from being a trivial task, while the modeling literature freely uses data of questionable value, in terms of range, accuracy and structural classes, i.e., using drug-like and non-drug-like structures. The latter, in our opinion, is an aspect of paramount importance. Models derived on data sets largely comprised of simple? molecules will invariably encounter formidable challenges when used to predict the properties of drug-like molecules. Furthermore, we note that the question of the charge state of a solute is often not explicitly addressed in discussing the origin of data sets, while all models refer to a neutral state.

Gao [23] used 24 molecular descriptors and principal component regression algorithm to derive a QSPR model for aqueous solubility. A set of 930 compounds was used as a training set and a set of 249 compounds as a test set, most of the data taken from AQUASOL database and literature. Some in-house measurements were added to enhance the set with drug-like molecules, since a larger diversity of compounds in a training set generally enhances the applicability of a model to different classes of compounds. The model resulted in impressive statistics with $r^2 = 0.91$ and RMSE = 0.49 log S units, where RMSE has the usual meaning of root mean square error, i.e., a measure of the random error not captured by the regression parameters. This work addresses the use of drug-like compounds, but still employs a very wide data range, and such aspect brings into question the data accuracy in the very low ranges.

A fairly limited data set also lacking the structural complexity we would deem useful for drug discovery applications was reported by Klamt *et al.* [24]. These authors appropriately discussed the problems associated with the modeling of the free energy of melting (fusion), which is relevant to crystalline solids, but they reported a very expensive computational scheme, using a training set of 150 compounds, a good one-half of which are non drug-like and/or liquid compounds. Furthermore, in a test set comprised of 24 drug compounds, 6 of them were part of the training set, and 5 other compounds were congeneric β-blockers. It is apparent that these are hardly well balanced

training and test sets, at least for application to drug-like molecule solubility prediction, and they illustrate the issues raised above in terms of data range, number and nature of the compounds used.

Klopman and Zhu [25] also compiled a data set for their group contribution model from several literature sources with some attention to the elimination of low molecular weight compounds and salts, but the set cannot be regarded as drug-like. Furthermore, as with any group contribution model, the calculation may not be accurate due to the lack of fragments present in a new compound and not present in the data set used.

Huuskonen [26] reported a fully computational model for the solubility prediction based on 30 topological descriptors using multilinear regression analysis (MLR) and artificial neural network (ANN) approach (see also article by Migliavacca in this issue). A final data set of 1297 compounds (884 used as a training set) was taken from the AQUASOL database of University of Arizona [21] and the PhysProp database [22]. Yan and Gasteiger [27], in addition to presenting their work, also reported a comparison of the prediction power of several methods based on Huuskonen data set and Yalkowsky's "21 compounds" test set. They concluded that the prediction results via ANN by their model are similar to those of Huuskonen [26] and Tetko [28], while multilinear regression analysis results were superior in Huuskonen's model. These authors [27] also expressed the need, echoed by several other authors, for good quality data, showing a keen awareness of one of the most crucial problem in ADME (or any) modeling efforts. We note that Huuskonen's models yielded good results on a 413-compound test set, but they appear to be too complicated for use in guiding chemists in drug discovery. This aspect illustrates another issue related to the choice of modeling approach that is, the interpretability of the results, as we discussed in the Introduction section.

Liu and So [29] suggested a simplified model using only seven physically meaningful 1D and 2D descriptors and an ANN approach (N=1033 for the training set) to achieve an r = 0.87 and s = 0.87 for the test set of 108 drug-like compounds. Comparison of their model to the commercial program QMPR+^{TM} (see below) showed that the two approaches had essentially the same predictive ability. However, a standard deviation close to one unit (on a molar log S scale) is probably too large to allow a confident prediction in the region of interest for drug development, which could reasonably be defined by - 6 < log S < -2 (see below).

Butina and Gola [30] recently reported that the PhysProp database is comprised, for the vast majority, of computed values and it includes experimental solubility data on reactive molecules. They removed 70% of its data, paring the database down to about 3200 compounds, prior to the development of their model. This is a commendable effort since the PhysProp database has been widely cited (and used) by several authors. However, even when selections and data checking procedures are adopted, as in this case, the question of the relevance of log S values well below - 6 may be asked, together with questions on the accuracy of the determination of solubility for (very insoluble) compounds in the range of log S - 7 to -12.

A possible answer, or at least a step toward a clear discussion of this point, came from Engkvist and Wrede [31]. These authors developed a model using a wide range of values but performed their testing using only compounds at or above log S of - 6. It may be recalled that Lipinski [20] put the minimum required solubility for a drug, having an average dose (1 mg/kg) and average intestinal permeability, at 52 μg/mL. In molar terms that value is equivalent to a log S of – 4, for a compound having a molecular weight near 500 Da. Thus, it may be advisable and more useful to develop models using only drug compounds and in a much narrower range, in the hope to achieve better predictions in a range of much higher importance for actual drug discovery and development work. Engkvist and Wrede took the route of checking the World Drug Index and Available Chemical Directory databases in order to calculate, using their model, the percentage of molecules having a solubility value below 52 μg/mL and reported values of 35 and 49%, respectively, for the two databases.

We would also like to discuss the model recently developed by Raevski [32], which uses a data set of about 1500 compounds (for solubility) and relies on a non-linear fit, calculating an "increment" solubility via an "increment polarizability and H-bonding" values, determined by comparing the properties of the unknown compound with a nearest neighbor of known solubility. This approach obviously relies on *ad hoc* databases pre-populated with analogs of the compounds of interest, which may be thought of as viable during the candidate seeking stage. However, at that stage a chemist would have identified a small number of compounds, and the experimental profiling of their crystalline form would then be generally sought, in order to select a candidate. Furthermore it is not clear, from the test set shown, how many neighbors would actually be needed and whether "more is better".

McFarland [33] reported on the use of HYBOTPLUS software for calculations of the most important descriptors for the solubility estimations. A set of 24 drug-like compounds with carefully determined aqueous solubility values was used for all correlations. The signs of the coefficients of some of the parameters do not seem physically reasonable, and the data set is very small, even though comprised of high quality measurements.

A very recent article, by Manallack *et al.* [34] reported a solubility classification method based on a threshold of 0.1 mg/mL, to discriminate between soluble and poorly soluble compounds. We think that this threshold is very sensible and "practically oriented" and it should find application in a drug discovery setting. Furthermore, the issues of data quality, excessive range and choice of models, as illustrated so far, seem to encourage the development of a "threshold" over "continuum" methods. The former could of course be "derived" from the latter ones.

We note, in closing, that a very high log P_{oct} value (> 5 or 6) is generally a sufficient indication of the low solubility of a compound (roughly log S < - 6), at least for neutral compounds and even for low melting compounds, on the basis of the well-known Yalkowski's solubility equation, which takes into consideration log P_{oct} and melting point. On that basis, a computed log P_{oct}, perhaps averaged over several and trusted log P_{oct} models, and some sensible

Table 1. Comparison of Models for the Prediction of Solubility, Caco-2 Cells Permeability and Absorption

Property	Set	Data range (N)	Descriptors	Approach	Reference
Solubility (log S)	Mostly Drug-like	-11.6 to 4.75 (930)	24: 2D and 3D descriptors	MLR	23
Solubility (log S)	Drug/Organic	-10.8 to 1.56 (150)	3: QM	MLR	24
Solubility (log S)	Drug/Organic	-10.7 to 1 (est.) (1168)	118 parameters	Group Contribution Method	25
Solubility (log S)	Drug/Organic	-11.6 to 1.58 (884)	30: E-state indices, topological	MLR and ANN	26
Solubility (log S)	Drug/Organic	-11.6 to 1.58 (797)	32: 3D descriptors (RDF code) plus 8 additional descriptors	MLR; Back-propagation neural network	27
Solubility (log S)	Drug/Organic	-11.6 to 1.58 (1291)	33: E-state indices	NN	28
Solubility (log S)	Drug/Organic	-11.6 to 1.58 (1033)	7: 1D and 2D descriptors	ANN	29
Solubility (log S)	Drug/Organic	-10.5 to 2 (3042)	63: Topological, physico-chemical	NN	31
Solubility (log S)	Drug/Organic	n/a (1502)	3: polarizability and H-bonding	Non linear model	32
Solubility Classification	Drug-like	Above or below 0.1 mg/mL (788)	20: BCUT 3D metrics	Consensus neural network	34
Solubility (log S)	Drug/Organic	-10.5 to 2 (522, 1038)	6-16: Topological, geometric, charge	NN	35
Solubility (log S)	Drug/Organic	-10.8 to 2.06 (150)	5: molecular descriptors MC simulations	MLR	36
Caco-2 (log P_{app})	Mostly drug	-6.96 to - 3.88 (73)	24: E-state, topological	GA-PLS	37
Caco-2 (log P_{app})	Mostly drug	-6.96 to -3.88 (87)	5: Polarizability, H-bonding	MLR	38
Caco-2 (log P_{app})	Mostly drug	-6.60 to – 4.48 (30)	1-6: MD, topological, surface area, charge	MLR (MI-QSAR)	39
Artificial membrane Permeability rate (log nm/s)	Drug-like	-1 to 3.30 (3061)	6: PSA, H-bonding, Clog P_{oct}, # of rotatable bonds	Classification	40
%BA	Drug-like	0 to 100 (1117)	As above	Classification	40
%FA (and Caco-2 log P_{app})	Drugs	0 to 100 (210)	9: QM, electrotopological, H-bonding, Clog P_{oct}	PLS	41
FA	Drugs	0.2 to 0.99 (31)	1: H-bonding	Non linear model	32
% FA	Drugs	3 to 100 (28, test set)	IDEA™ v. 2.0 and Gastroplus™ v. 3.1.0	Comparative analysis	42
% Absorbed	Drugs	0 to 100 (180)	3: Solvation parameters	MLR	44
Caco-2 (log P_{app})	Drugs	-7.43 to -4.01 (27)	9: QM, electrotopological, H-bonding, Clog P_{oct}	PLS	45
% Bioavailability	Mostly drugs	Class 1: ≤ 20 Class 4: ≥ 80 (232)	18: LogD, H-bonding, metabolic pathways-based	Classification	46
% Absorbed	Drugs	1 to 100 (417)	36: structural descriptors and # of hydrogen bond donors	Mod. Group contribution method	49

judgment on its quality and use, may be all that is needed for library design.

Intestinal Permeability, Bioavailability and Fraction Absorbed

Table 1 also shows a variety of recent approaches to the prediction of intestinal permeability, bioavailability and fraction absorbed [37-49]. Caco-2 cells permeability data are still a major target property for modeling, despite substantial inter- and even intra-laboratory variability in the data, as reported by several authors [1-2]. In some cases [37-38], authors have chosen not to average the data reported for different compounds using a set comprising 87 structures with 129 permeability values. Some of these values, however, are significantly different for a given compound especially considering that the total range covered by the data is usually 3 orders of magnitude.

Yamashita *et al.* [37] used a similar set of compounds (73 structures for 110 permeability values) and genetic

algorithm-PLS statistics. The statistics seem better than in similar work reported by the same group [38], but the number of parameters used is also five-fold larger than in the other approach. No independent test sets were reported in either case, and the underlying theme of paucity of data may have been the reason for neglecting this important practice. The scarcity of data, however, superimposed to the high variability of Caco-2 determinations, should suggest caution in attempting to develop models aimed at reproducing very complex phenomena, such as intestinal absorption and/or permeability, if a reasonably thorough test of a model cannot be performed.

Kulkarni and Hopfinger [39] reported a more insightful model utilizing a membrane interaction-QSAR approach (termed MI-QSAR) and using three classes of descriptors. The computation of some of them, however, is based on MD techniques and is fairly complex and time consuming, thus severely limiting the throughput. At any rate, it could yield some insight into potential differences in the behavior of lead compounds, but does not seem useful for library design or otherwise massive computational screening stages. $F(H_2O)$, the aqueous solvation free energy, was found to be a significant parameter in all 6 equations reported, using an increasing number of descriptors, and a reasonably good coefficient of determination ($r^2 = 0.75$) was reported, using this parameter as the sole descriptor. The training set, as in the vast majority of the reported literature in the field, was very small (30 compounds) although we note that an independent test set was used in this case. A particular finding of this work was the consideration of the conformational flexibility of the molecules, which led these authors to indicate this parameter as being positively correlated with intestinal permeability (increase in permeability with an increase in flexibility) while other authors [40] derived an opposite conclusion, although their end point was not permeability, but the bioavailabity (%BA) *in vivo*.

Veber *et al.* [40] have examined both bioavailability (expressed as %BA, N =1117) and permeability rates (N =3061) of proprietary compounds through artificial membranes. They performed a "quartile" classification and their work seems to conclusively show that a measure of molecular flexibility, the number of rotatable bonds, affects both target properties, and it is likely to have a negative impact on passive permeability rather than on solubility and/or active transport phenomena. The fairly large proprietary data sets and the discussion provided by the authors offer a solid basis to attribute relevance to these findings, and a classification model may be all that is needed and/or all that is attainable, considering the "noise" of the experimental end points.

Stenberg *et al.* [41] have reported a study focusing attention primarily on the prediction of polar surface area, as a primary (or sole) determinant of fraction absorbed and/or permeability. Their approach involves rather costly QM calculations, coupled with the use of molecular mechanics calculations and fragmental parameters. The proposed approach was named partitioned total surface area (PTSA) but the very small data set, in terms of either fraction absorbed or apparent permeability (N = 27), does not show any superiority of this over other models.

Raevski [32] adopted a non-linear model based on hydrogen-bonding characteristic of solutes, in an attempt to predict the fraction absorbed. Most compounds in this data set, as it would be expected when using drugs, are very highly absorbed. This author, as in the previously discussed solubility model, adopted the use of "nearest neighbors" to improve the quality of the prediction. In general, the use of a larger rather than smaller number of nearest neighbors improves the prediction as it may be expected. However, from compound to compound, as in the case of some steroids in the data set, it is not always true that 3 "neighbors" would be better than 1 in improving the prediction. In addition, this type of approach would require prior knowledge of the behavior of analogs, at which point enough knowledge may have been gathered by the scientists in the project to make the use of this (or any) model superfluous.

Parrott and Lave [42] reported an interesting comparison of two commercially available software packages: GastroPlus™ v. 3.1.0 and IDEA™ v. 2.0. The performance of either package does not seem impressive, in our opinion, on the small set of well-studied molecules used, when the fraction absorbed was the target property. The authors pointed out that a comparison of RMSE (see definition on page 8) values might not be appropriate, when different sets are used, but we fail to discern appreciable improvement over the work of Wessel and Jurs [43] and of Zhao *et al.* [44]. Other issues involved input and output format, batch processing capabilities and ease of use. One of the conclusions offered was that GastroPlus™ seems to be a tool for trained and frequent users while IDEA™, albeit simpler to use, is somewhat restricted in its applicability to multiple compound batches and has limited functionality. It is also interesting to note that even when experimental permeability and solubility data were used as input the performance did not seem to improve.

Zhao *et al.* [44] reported several correlations between the % dose absorbed intestinally, and the well-known Abraham's solvation parameters, calculated via the program ABSOLV® (see also article by Caron *et al.* in this issue). The data sets used vary between 31 and 180 compounds, and the correlations reported pointed toward the importance of H-bonding donor and acceptor capability of the compounds, together with the importance of volume. The data span the 0 to 100% of dose absorbed range, and they seem well researched with 271 original references provided altogether. We comment that the latter aspect is highly commendable, since a plethora of authors provide only references for data sets that may have been taken, in turn, from several other authors.

Most recently, Bergstrom *et al.* [45] have reported an absorption classification based on molecular surface properties (PTSA, see also ref. [41]) but the data set was very small, in terms of solubility and Caco-2 permeability. Furthermore, the solubility of some compounds had to be determined in water-methanol mixtures and then extrapolated to 0% organic solvent. Nevertheless, the data quality was higher than in most cases and a seemingly promising approach was resulted from this study. PTSAs were deemed to be, as quoted by the authors, "a rapid and transparent alternative descriptors in property based drug design".

It is worth mentioning that most of these studies found volume to be an important parameter, while Veber *et al.* [40] did not find that the highly correlated molecular weight is an all important parameter pointing out, rather, that the number of rotatable bonds is a more discriminating factor. The data set used by Veber *et al.* is much larger than any other set reported, although based on rat data owing to its proprietary nature, while the data for commercial drugs generally consider human absorption, but on a much more limited range of compounds. It would be useful, as Veber *et al.* commented in closing, to have other proprietary data sets analyzed and reported, along the same lines. Data range, number of points, and the accuracy of the determinations, especially in dealing with highly complex phenomena, such as intestinal absorption, should be scrutinized carefully as different sets may yield different answers.

Yoshida and Topliss [46] analyzed a set comprising 232 compounds, with reported bioavailability. The parameter set included some "reactivity" indicator variables, such as the presence of hydrolytically sensitive or readily oxidized groups. These authors used the *Or*dered *Mu*lticategorical *C*lassification Method using the *s*implex technique (ORMUCS) to achieve, with seemingly good results, a classification of the compounds in 4 classes of different bioavailability. The inclusion of "stability variables" however, is of questionable applicability as it would not be easy to judge, in an "objective" fashion and without a subjective intervention, the rate or even the probability of intestinal degradation for a wide variety of "unknown" structures.

A further example of the application of a computed H-bonding value came from the work of Rey *et al.* [47], who used the Molecular Hydrogen Bonding Potential (MHBP) on a set of 20 previously reported compounds. The data set seems to be overlapping with the one used by Bergstrom *et al.* [45] and it encompasses several analogs. Only the donor potential seemed to be correlated with absorption, (%FA) through a sigmoidal relationship, while the acceptor potential was not found to be a significant parameter, not yielding a "recognizable" sigmoidal relationship. This seems to be at odds with intuitive approaches such as the "rule of five" [48], which allows for the influence of both donor and acceptors, although it emphasizes the donor over the acceptor character of a molecule via a lower "threshold" (5 vs. 10 counts) for an alert. And it seems to differ from the recognized importance of PSA, which would by definition include both donor and acceptor groups. Furthermore, all the well-absorbed molecules could be found in the lower third of the acceptor MHBP scale shown and only two or three of them, in that range, showed a poor %FA. The use of MHBP may also not be trivial, as the authors warned, due to the strong directional character of H-bonding and it will also have to be validated on a much larger data set.

We would like to close this section by observing that no major improvement, in large part due to the paucity of reliable and available data, has been achieved over earlier models and it may be unlikely for a truly predictive and widely applicable in silico model to appear, in the near future, also considering the plethora of influx and efflux phenomena we are only beginning to discover and understand. The "rule of five" [48], a very simple and widely cited and implemented model, still seems to hold quite well in revealing, in a semi-quantitative and very intuitive fashion, the parameters that are important for passive intestinal absorption. In contrast most of the work reported attempts to discover "new" parameters that are likely to be, at best, a different side of the same coin, and generally capture the same property (or group of properties) under a different name.

The work of Veber *et al.* [40] may be viewed as an exception to the issues mentioned earlier in this review and illustrated by the reported literature. Veber's work represents a step in the right direction, at least from an industrial discovery perspective, in terms of the size of the data sets used, the exclusive drug-like nature of the compounds, and points to flexibility as an important determinant of passive permeability.

DISTRIBUTION

The distribution of drugs in the body could be divided, for the purpose of examining predictive approaches, in three major areas represented by blood-brain barrier (BBB) permeability, plasma protein binding (ppb), and volume of distribution (VD or VD_{ss}). It is apparent that all of them are very important aspects of the pharmacokinetic (PK) profile of a drug, as they help establish the dosage regimen, the effective (free) plasma concentration, and the likelihood of blood-brain barrier crossing, which is important for CNS targets, but also to help predict the possibility of CNS side effects for non-CNS drugs. However, BBB permeability prediction approaches account for the vast majority of the computational efforts in the "D" of ADME, especially after the seminal experimental work of Young *et al.* [50], and as brought out by a recent review published by Norinder [12].

BBB Permeability: The Modeling and Limitations of Log BB

We center our discussion on some of the most recent attempts aimed at predicting BBB permeability, and we note that almost all efforts have focused on the use of the ratio of concentration in whole brain vs. blood (or plasma), whether at equilibrium or pseudo-equilibrium conditions, which was expressed as log C_{brain}/log C_{blood} and reported as log BB. These aspects, related to the nature of the experimental data, are relevant, and clearly impact on what data will eventually be available for modeling efforts and on what data are deemed most useful for BBB permeability prediction and CNS activity.

Bonate [51] has discussed several of the methods used in the determination of brain uptake of a solute, and our view is that an "optimal" and well-accepted method may still be some time away. We are not aware of reasonably large bodies of publicly available data, other than log BB, and we suspect this to be a consequence of differing opinions among CNS medicinal chemist, biologists and drug metabolism scientists, and perhaps a more direct consequence of historical views on the subject.

One "pragmatic" argument in favor of the use of log BB data has been the fact that the vast majority of

determinations have been performed, historically and in most recent times, using brain tissues homogenates with corrections (in some cases) for drug in the vascular spaces of the brain. To the best of our knowledge these determinations have been helpful in assessing BBB permeability, despite their experimental challenges and potential for error, and have generally correlated well with the final outcome of whether a drug was crossing or not crossing the BBB, with permeable drugs having log BB values well above - 0.1.

However, a recent report by Kalvass and Maurer [52] discusses the pitfalls of using the total brain/total plasma ratio, as in log BB, especially when they are used as a continuum value and in a quantitative way. An apparently higher log BB obtained for a given compound may not indicate that the "useful" (free) concentration is indeed higher than for another compound having a lower log BB. Once the respective fractions unbound in plasma and brain are considered the compounds may not differ at all. A detailed discussion of the affinities of drug compounds for phospholipids and other tissue components is beyond the scope of this review, but relevant references can be found in the work just cited.

Other authors [53] have proposed the use of CSF/free plasma concentration ratios, but it could be argued that this value is not a reflection of true blood-brain barrier permeability, given the important physicochemical, physiological and metabolic differences between the CSF-blood and brain-blood barriers.

The most valuable alternative may be the use of microdialysis determination [54] of the drug concentration in the brain extracellular fluids (ECF) and the use of this value in the ratio over free plasma concentration. The use of this method, however, is far from being trivial in terms of drug recovery from the dialysate, but it may be the method of choice if the experimental challenges could be solved.

Last, but not least, it should be recognized that all the computational techniques have so far assumed passive diffusion and equilibrium conditions between brain and blood. An increasingly larger number of active transporters are being discovered and identified, with some of them more and some of them less compound or class specific, and much more needs to be learned about their localization and specificity [55]. At the moment, no computational technique is capable of handling, on a routine basis, these active transport phenomena, although large deviations, between an "accepted" model and experimental findings may offer some insight on the involvement of transporters for a given structural class of compounds.

Table 2 shows a comparison of the most recent approaches reported for the prediction of blood-brain permeability, although the compilation is far from being exhaustive. It is readily apparent that, except for the work reported by Engkvist *et al.* [56], the data sets are fairly small. This is not surprising, considering the difficulty of generating good quality log BB data, if that is the desired property, and a reasonable guess would put the number of data points well below 1,000 for any proprietary data set owned by a company. Engkvist *et al.* reported a very nice classification scheme based on substructures with a rather crude, yet intuitively correct, assignment of charges at physiological pH. The target property, however, was not a measure of passive diffusion but a broad classification in CNS+ (active) and CNS- (inactive) compounds. This "measure" cannot be taken as being equivalent to log BB (or any other measure of brain or CSF uptake), as it involves an activity component against different targets and active transport liabilities, although it may sound attractive as an "all encompassing" measure of "success" for CNS activity. The data set used may also bias the predictivity of the model toward known "CNS scaffolds" and yield a "CNS-" prediction for any novel structure and target which may be pursued, thus limiting the exploration of potentially novel structures and targets.

An interesting observation, which emerges with respect to the charge state of a solute is that, in the literature, the addition of a variable based on the protonation state of a molecule was not found to be relevant at all for the prediction of log BB. The data sets used by Engkvist are much larger than any other reported so far, and that may be part of the explanation, although doubts still remain upon the relevance of the charge state of a molecule with respect to its BBB permeation and/or CNS activity.

Several authors [57-63] have used nearly identical or significantly overlapping sets of data, with similar or identical range of log BB value and tested, on those grounds, various descriptors and statistical approaches. Platts *et al.* [57] discussed the development of a model based on solvation parameters (linear free energy relationships, LFERs, see also article by Petrauskas *et al.* in this issue) and used a set of 148 molecules, while the set of Rose *et al.* [58] comprised 102 molecules. In all cases these sets include very simple and often gaseous compounds, and most other authors have used data sets of about 50 compounds.

The question of the importance of log P_{oct} as a measure of lipophilicity to be applied to the BBB permeability prediction may be asked, since seemingly ambiguous results populate the literature. The work of Young *et al.* [50] showed that this parameter (experimentally determined) was not correlated with log BB, for a small series of 20 compounds. However, a significant correlation was found with Δlog P (log P_{oct} – log P_{cyc}) for the same set. These results do not account for any charge on the basic nitrogen atoms present, and may have been biased by the size and nature of the data set, although Δlog P is generally considered a measure of H-bonding ability of a solute.

Iyer *et al.* [59] included clog P_{oct} in 5 out of 6 models presented in his work, but clog P_{oct} only accounted for 7% of the variance in a MLR correlation, while the polar surface area (PSA) accounts for almost 70% of the variance as a single parameter. The same point is presented indirectly by Keseru *et al.* [60] and more directly by Platts *et al.* [57]. Keseru *et al.* showed that solvation free energy (or PSA) is a better descriptor than log P_{oct}, and Platts *et al.* showed that a comparison of LFERs developed to model log P_{oct} and log BB do not show a high degree of similarity. Some of these results may also point to the fact that PSA may be likely correlated with clog P_{oct} and the use of both parameters may be redundant at best.

Platts and co-workers also pointed out that the small data set used by Young *et al.* (N = 20) in developing their correlation might have biased the results, in comparison to a

much larger data set (N =148) they used to develop their LFERs. In direct contrast with Engkvist *et al.* [56], they also found that an indicator variable was significant for compounds containing COOH groups, but a similar indicator variable for basic amines was found not to be significant. Once again, the apparent "fluctuations" in the findings and the significance attributed to some of the variables examined seem very much dependent on the data set and target property used.

Kaznessis *et al.* [61] did not include a computed clog P_{oct} in the model they developed but compared some of the parameters derived from their Monte Carlo (MC) simulations in water to the corresponding ones used by other authors. They found, for example, that a good correlation was obtained with the same set and log P_{oct} value used by Feher *et al.* [62], when their MC log P_{oct} was used as the computed log P_{oct} parameter. They also "confirmed" the findings of Kaliszan *et al.* [63] by using MC log P_{oct} and molecular weight. However, a model based on the two descriptors, polar surface area and molecular volume, as reported by van de Waterbeemd and Kansy [64], and cast by Kaznessis *et al.* using the corresponding MC parameters, did not show a very good performance.

The elegant work of Crivori *et al.* [65] (see also the article by Migliavacca in this issue) relied on a training set of 229 compounds, divided in BBB+ and BBB- compounds on the basis of their permeability, as opposed to CNS activity. This approach used 72 descriptors generated via VolSurf in a seemingly automated procedure, and on neutral compounds. These authors constructed a predictive PLS model, which was able to discriminate between the two groups, although BBB- were predicted with more difficulty, likely because of metabolic and efflux phenomena, which are very difficult to model. This work underscores, once again, the importance of PSA and H-bonding ability of a solute in the passive diffusion of molecules across the BBB, but also the "distribution" and "density" of such properties in a solute. Furthermore these authors comment that a conformational search has only a modest effect over the properties calculated by a single (optimized) conformation.

This work, not yielding a quantitative continuum model, may be useful in helping to partition the compounds into two classes (BBB+ and BBB-). However, as we discussed above, no quantitative differentiation of brain penetration among compounds in each class can be made, until their respective free plasma and brain concentration is known [52].

The use of polar surface area (PSA) and H-bonding parameters, calculated from reasonable single conformations, is likely to be the avenue to pursue, provided that large enough sets of accurate values are used to yield a more

Table 2. In-Silico Models for the Prediction of BBB Permeability and Plasma Protein Binding

Property	Set	Data range (N)	Descriptors	Approach	Reference
CNS activity (active/inactive)	Drugs (WDI)	CNS+ /CNS-[a] (3678 /5000)	92: atom types (NN)	NN; substructure	56
BBB permeability (log BB)	Drug/Organic	-1.82 to 1.64 (148)	6: solvation parameters plus 1 indicator variable	MLR	57
BBB permeability (log BB)	Drug/Organic	-2.15 to 1.44 (102)	3: electrotopological	MLR	58
BBB permeability (log BB)	Drug/Organic	-2.15 to 1.04 (56)	1-6: PSA plus other 5 descriptors	MLR (MI-QSAR)	59
BBB permeability (log BB)	Drug/Organic	-2.00 to 1.04 (55)	1: solvation free energy	LR	60
BBB permeability (log BB)	Drug/Organic	-1.82 to 1.04 (76)	3: H-bond donor, acceptor and MW	MLR	61
BBB permeability (log BB)	Drug/Organic	-2.00 to 1.04 61 -1.82 to 1.44 (test set)	3: PSA, solvated H-bond acceptors, log P_{oct}	MLR	62
BBB permeability (Permeable/ impermeable)	Drugs	BBB+/BBB-[b] (229)	72: VolSurf descriptors	PCA; PLS	65
BBB permeability (log BB)	Drug/Organic	-2.14 to 1.04 (55)	3: PSA, lipoaffinity and MW	MLR; NN	66
Plasma protein binding (% Bound)	Drugs	4 to 99.4 (154)	7: biophores 24: modulators	Group recognition/expert system	67
Plasma protein binding (Affinity, $logk_a$)	Drugs	2.4 to 7.9 (151)	62: TPR[c] 149: DFP[d]	PLS	68
Plasma protein (Affinity, logK'hsa)	Drugs	-2.69 to 1.34 (94)	5-6: Clog P, topological, PSA	MLR	69

a. CNS active or CNS inactive compounds. b. BBB permeable or impermeable compounds. c. Topological PhaRmacophore. d. Daylight Fingerprints.

complete understanding of the important parameters. The development of "continuum models" may not be necessary, at least for a "first line" separation between BBB+ and BBB- compounds. Local models may be developed to assist the search for a suitable candidate or back-up molecule, once experience has been gained with the project and its SAR limitations. This may be especially true if "unusual" targets or "unusual CNS scaffolds" would be pursued.

Plasma Protein Binding Modeling

We are aware of only a few computational approaches describing the prediction of ppb [67-71], and some of these approaches examined exclusively the binding to human serum albumin (HSA) [67-69]. This protein is the most abundant protein in plasma (40 g/L or 600 μM), but it is not the sole determinant of ppb, especially for certain classes of drugs, and these approaches thus seem restrictive even though they may lend themselves to more detailed analyses and offer access to actual binding constants.

Saiakhov [67] described a model based on the grouping of f_u values in classes of strongly bound ($f_b > 32$ %), low binding affinity ($f_b \leq 25$ %) and not bound ($f_b < 19$ %) compounds. All of these data are based on affinity for human serum albumin (HSA), and while acidic compounds generally bind strongly to HSA, this parameter is certainly not the sole determinant of ppb for neutral and basic drugs. At any rate, this classification seems inadequate to answer the questions related to PK issues in the "upper region" since the lowest f_u threshold used ($f_u < 68\%$) is very far from a "useful" range, where a seemingly slight variation in % of free drug would have great impact in the PK profile of a compound. In fact, only 8 out of 154 drugs reported have $f_b \geq 98$ % (or $f_u \leq 2\%$) in this data set, and the classification, based on "biophores" and "modulators", does not lend itself to a straightforward medicinal chemistry interpretation.

Kratochwil *et al.* [68] considered a much larger range of affinities, with some compounds reaching an f_b of 99.9 %, and discussed the issues related to the affinity (micromolar or sub-micromolar) of drugs toward HSA. However, their model was built on the assumption that the HSA is the only binding protein, and on the existence of a 1:1 complex upon binding. They used topological pharmacophores coupled with a PLS approach, to model an affinity constant rather than the % bound. No consideration is given to the protonation state of a molecule, in their model. Furthermore the use of HSA affinity as the sole binding parameter, coupled with the assumption of a 1:1 complex between HSA and drug detracts, in our opinion, from the value of this approach. It should also be noted that almost half of the entire data set was comprised of acidic compounds, which are known to bind tightly to HSA, but which represent only a "minority" fraction of the generally known drug compounds, and which can be roughly estimated to be 15-20%. Also, we note this work seemed to indicate that log D_{oct} at pH 7.4 did not correlate with the HSA binding, although only small subsets of acidic or basic compounds were used. We comment that the log D_{oct} method developed and reported in their work does not seem robust enough to handle a wide variety of compounds. Even though the determinations were made on commercial drugs, many compounds could not be determined, as reported by the authors.

Colmenarejo *et al.* [69] developed QSAR models for binding affinities to HSA using a diverse set of 95 drugs. The binding constants to HSA were experimentally determined by using high-performance affinity chromatography and expressed as logK'hsa. As discussed above, HSA is neither the sole nor the most important binding protein for a wide variety of compounds, and the use of chromatographic affinity data, as opposed to dialysis experiments, is questionable. Furthermore, while Clog P_{oct} was found to be the most important parameter in a variety of models built, and in the two global models reported, it is puzzling to note that specific structural classes, in univariate models, were each correlated with the target property by a different single parameter. Only in one case was Clog P_{oct} selected and, in several cases, parameters that were not part of the global models were instead reported as the dominant parameter for that class.

Mager and Jusko [70] reported an MLR model, which was based on 11 steroid molecules, and they attempted to predict VD_{ss} and f_u, as well, for the same data set. The data set is very small and it is not diverse enough to allow any estimation of its predictive usefulness. Furthermore, some of the coefficients found (e.g., for log P_{oct}) seem to yield a negative sign while it would be intuitive to expect a positive correlation, when modeling properties, such as VD_{ss}. Furthermore, the value of the intercept is very large (VD_{ss} in liters) for both the volume of distribution and f_u models, although the negative coefficient of the calculated log P_{oct} parameter, in the latter model, seems physically reasonable.

Hunt [71] described his SIMCA approach [72] (see also the article by Migliavacca in this issue), yielding a classification based on 459 compounds divided in 6 classes, and ranging from a ppb value of < 14% to > 96%. Once again, it is usually the compounds in the upper regions that need a more accurate prediction, but these are also the compounds for which the experimental determination (especially at or above 99%) is most challenging. Hunt's data sets comprised several hundreds compounds with PK data reported from human studies, mostly from generally available public sources. These data were also used for further PK modeling (see below).

The accurate prediction of plasma protein binding, given the possibility of multiple binding sites for a given protein (e.g., albumin), and the fact that a single protein cannot generally exclusively account for the total binding, remains elusive, and it may be too complex to model from computed parameters only. However, it is unlikely that a library would be optimized for plasma protein binding, while simpler parameters (e.g., log P_{oct}) may suffice in "controlling", to some extent, undesired properties like low solubility, high metabolic clearance, etc., which generally plague high lipophilicity compounds.

On the question of the importance of lipophilicity in modeling ppb, and expressed as log D_{oct} or log P_{oct} (see also article by Caron *et al.* in this issue), we note some very different views between Kratchowil *et al.* [68] and Colmenarejo *et al.* [69]. The first group of authors specifically argues that this parameter (expressed as

experimental log D_{oct} at pH 7.4) is not relevant for ppb, while Colmenarejo *et al.* argue, on the contrary, that it is, albeit expressed as Clog P_{oct} in this case. We find hard to believe that, although one should not expect an exact or even exceedingly similar balance of forces between ppb and log P_{oct} (or log D_{oct}), the latter parameter would not have strong relevance to ppb modeling. Once again, the use of heterogeneous sets of data and questionable choices in terms of data sets may have yielded vastly different conclusions.

Volume of Distribution: Challenges and Current Status

The prediction of volume of distribution, a very useful proportionality constant, offers a great challenge since a "continuum" prediction method, rather than a classification bin as reported by Hunt [71], is desirable for the calculation of $t_{1/2}$. However, many factors are involved in this composite parameter, owing to the relative affinity of a compound for a variety of different tissues and organelles and efflux and uptake phenomena. Therefore, it is probably not surprising that recent experimental work [73-75] has focused on the possibility of bypassing *in vivo* measurements, relying on experimentally determined physicochemical properties (i.e., log D_{oct}, pK_a) [75] or other *in vitro* determinations, such as tissue-plasma partition coefficients [73-74]. At the same time only limited attempts, aiming at the direct prediction of the volume of distribution at steady state (VD_{ss}) via computed parameters and using a set of diverse drugs, have been reported by Hunt [71] as a classification, and by Lombardo *et al.* [76], as a continuum model, albeit limited to basic and neutral drugs, in the latter case. The modeling of acidic compounds, which are highly bound to plasma proteins and therefore very often yield VD_{ss} values < 1 L/kg, was only reported by Hunt in his classification method. The direct and fairly accurate prediction of VD_{ss} from computed parameters remains a challenge.

The work reported so far has not shown that general computational methods capable of handling complex phenomena like volume of distribution are in sight, when a continuum value would be needed in order to calculate $t_{1/2}$. A notable exception may be represented by the work of Hunt [71], where the "direct" binning of $t_{1/2}$ values may be useful and yield an acceptable first pass prediction of $t_{1/2}$ (see Excretion). A more accurate prediction of $t_{1/2}$ would require the knowledge of clearance and volume of distribution of drugs.

METABOLISM

Metabolism, one of the primary factors influencing excretion (see next section), has long been the target of computational models. Clearly there are several distinct metabolism related end-points amenable to modeling. And although most efforts to date have focused on modeling cytochrome P450 (CYP450) mediated metabolic stability and CYP450 inhibition, there are now examples of other drug metabolism end-points for which computational models have been derived [77-80] including glucuronidation and enzymatic hydrolysis.

Recent review articles [17, 81] have very logically classified computational approaches to predictive drug metabolism along the lines of i) protein structure based methods, ii) statistical methods, also known as quantitative structure-metabolism relationships, iii) pharmacophore based methods and, finally, iv) rule-based expert systems. These classifications are very useful to facilitating our discussion. However, there are growing numbers of studies that are not easily partitioned into a single class. As we will illustrate, some of the most successful modeling efforts have relied on a combination of methods [81-84], but the general theme of the paucity of data is maintained throughout the "M" of ADME, together with a marked model "specialization".

Protein based models have been most effective for visualizing and rationalizing potential sites of metabolism on the test substrate. Most, if not all, of the current protein structure based metabolism models are homology models derived from publicly available CYP450 crystal structures. These models are primarily used to examine specific substrate protein interactions in an attempt to rationalize observed metabolic transformations.

Dai *et al.* [85] have published a review of the major concepts and current approaches of molecular modeling CYP450s. This review focused on structure-based CYP450 models to increase the understanding of CYP450 action.

Ridderstrom *et al.* [86] have applied the GRID/CPCA methodology to map the active sites of CYP 2C8, 2C9, 2C18, and 2C19 homology models. These models are reported to be the first derived from the only publicly available mammalian P450 crystal structure, CYP2C5. The GRID calculations were conducted using 10 probes that cover hydrophobic, steric, hydrogen bond acceptor, and hydrogen bond donor interactions. Analysis of the resulting GRID fields yields an inverse pharmacophore model for 2C9.

Lewis [87] has recently summarized his labs efforts in building homology models for 1A1, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4 and 4A11. It is reported that all of the selected substrates are shown to fit within the corresponding enzymes' active sites in a manner that is consistent with experimental results. Each of these protein models was based on sequence homology to with CYP102. The substrates were then interactively docked to the corresponding homology model.

There have been several studies published that rely on combinations of protein structure, 3D pharmacophore methods and molecular orbital calculations. Wang and Halpert [88] published a combined pharmacophore and homology model for CYP2B6. Catalyst pharmacophores were derived for a set of 16 structurally diverse 2B6 substrates. A homology model of 2B6 based on the 2C5 crystal structure is also reported. The pharmacophore model was used in conjunction with the homology model to predict the K_m values of substrates in a test set of 5 compounds.

Molecular orbital calculations have been successfully incorporated into the combined pharmacophore and homology model. de Groot *et al.* [84] reported a 2D6 model derived using 40 substrates using a combination of protein modeling, pharmacophore and molecular orbital calculations. It was acknowledged by these authors that the combination of modeling techniques yielded much more satisfying results that any of the techniques used individually.

Ekins, De Groot, and Jones [89] have published an excellent mini-review of pharmacophore and three-dimensional QSAR P450 models. The review describes in detail the development of the impact of the combined approach in increasing our understanding of P450 active sites. The authors also describe in detail key pharmacophoric features for the most relevant drug metabolizing CYP450s. The review highlights how the computational approaches have helped in understanding substrate and inhibitor binding to CYPs 1A2, 2B6, 2C9, 2D6, and 3A4.

Although pharmacophore models are typically presented in the context of protein structure based homology models, there are published examples of pharmacophore models used alone to predict 2B6 [90] and 3A4 [91] substrates. The published 2B6 pharmacophore model was constructed using 16 known substrates and the 3A4 model was constructed using 38 known substrates. K_m was the experimentally determined end-point in each case. Work from this lab has also been the subject of a review article [92].

Statistically based quantitative structure metabolism relationships have been reviewed by Lewis *et al.* [93-94]. The models presented typically contain log P, size, and some combination of molecular orbital derived properties, such as HOMO, LUMO, ionization potential and/or dipole moment. These models, although simple to implement and fairly interpretable, are of limited value due to the relatively small, homologous data sets from which they were derived. Jones *et al.* [95] have published a very simple, yet highly predictive, model for aromatic oxidation and hydrogen atom abstraction.

The models described to this point have all been derived to either provide a more detailed understanding of CYP structure or to predict potential sites of metabolism on a substrate. Another area of great importance and hence one that has also received the attention of in silico methods is the inhibition of CYPs. CYP inhibition can be a primary factor in considering drug-drug interactions and potential drug safety issues. The majority of the published models for CYP inhibition rely less on protein structure and more on QSAR methods. An exception is the work of Afzelius *et al.* [96] in which they derived a model for predicting 2C9 inhibition based on their previously described homology model. The docking program GOLD was used to select conformers to use in the 3D-QSAR analysis. Principal component analysis and PLS were then used to build the final 3D-QSAR model. These authors have also reported a discriminant and quantitative PLS model of 2C9 inhibition using the alignment independent GRIND descriptors without the consideration of the homology model. Ekins *et al.* have published models for 3A4 [97] and 2C9 [98] inhibition using both the catalyst pharmacophores and a method referred to as PSL MS-WHIM. In each case predictive models for a relatively diverse set of molecules were obtained. Poso *et al.* [99] have used CoMFA and GOLPE/GRID descriptors to derive 2A5 and 2A6 inhibition models for a series of substituted coumarins. CoMFA was used, as part of a 3D-QSAR study to model 2C9 inhibition, by Rao *et al.* [100]. In this work a set of 14 structurally diverse compounds was successfully predicted. Multilinear regression and neural networks were employed by Moon *et al*. [101] to derive a QSAR model for 1A2 inhibition potential of a series of flavonoid derivatives. It is clear from the number of published studies that the 3D QSAR and pharmacophore methods are proving to be effective techniques for developing CYP inhibition models.

Each of the aforementioned approaches has been shown to be useful for predicting metabolic stability, metabolic regioselectivity, and inhibitory potential. There are additional efforts directed at predicting the metabolic pathways. The tools that have evolved to predict metabolic pathways are primarily based on the codification of expert knowledge, or on the statistical analysis of the occurrences of a given biotransformation in a metabolic pathway database, and Hawkins [102] has highlighted the usefulness of drug metabolism databases for these purposes. This very recent study describes the probabilistic scoring of metabolic transformations contained in a metabolic pathway database. These probabilistic scores are then used in conjunction with the authors previously described pharmacophore/field based methods.

Expert system rule based systems round out our discussion of in silico methods. The commercially available tools MetabolExpert [103], META [104], and Meteor [105] each rely on a set of rules for predicting metabolic pathways. More information on these commercial packages may be obtained from the reported web sites.

The literature contains a diverse array of methods for predicting drug metabolism endpoints. And in fact, the notion of "drug metabolism" as a computational target does not take into account the complexity of the various factors affecting this term. As we have seen, a variety of methods, QSAR to Structure Based Drug Design, have been used to model the literature data sets. In each case the resulting model displays some promise of predictive performance as long as the model is applied within the context of its derivation, and that is an obvious limitation since a wider application is generally not possible. Future efforts will undoubtedly focus on deriving unified, more general models hopefully on the basis of larger data sets.

EXCRETION

Excretion (clearance) is the process by which the body eliminates the xenobiotics (i.e., “foreign” or “extraneous” compounds) and most of the transformations of these compounds take place in the liver, although this organ is not the exclusive site of metabolism. Another important route of excretion, whether for a metabolite or the parent compound, is renal clearance. Generally, renal clearance is associated with small and hydrophilic compounds, and these properties are often in contrast with the ability of crossing the BBB (for CNS drugs) and/or the general ability to cross membranes and reach an intracellular target.

These processes, however, are not just simple diffusive ones, but have important active components and these aspects enhance the difficulties encountered by the modeling efforts. Most drugs are metabolized by the liver, which leaves a fairly small number of drugs for which renal clearance is important. This may be thought, at least in part, as a consequence of the upward lipophilicity and MW shift [20,48] largely due to combinatorial synthetic techniques,

Table 3. In-Silico Models for the Prediction of Metabolism

Property	Set	Data range (N)	Descriptors	Approach	Reference
Metabolism (Biotransform. regioselectivity)	Drugs	Data mining metabolic transformation database	Fragment queries, ALMOND	Statistical analysis, 3D QSAR	81
Metabolism (CYP2C9 biotransform. regioselectivity)	Drugs	Sites of metabolism (27 substrates)	Pharmacophore, homology, molecular orbital calculations	Combined methods	82
Metabolism (CYP2D6 biotransform. regioselectivity)	Drugs	Sites of metabolism (40 substrates)	Pharmacophore, homology, molecular orbital calculations	Combined methods	84
Metabolism (CYP2B6 Affinity)	Drugs	1.3 to 9700 μM, Ki (16)	Catalyst Homology Model	3D-QSAR Protein Model	88
Metabolism (CYP2B6 affinity)	Drugs	1.28 to 17,700 μM, Km (16)	MS-WHIM Catalyst	3D-QSAR	90
Metabolism (CYP3A4 affinity)	Drugs	0.35 to 5600 μM, KM (38)	Catalyst	3D-QSAR	91
Metabolism (Microsomal rates and clearance)	Drugs/Organic	Multiple models	Surface areas, molecular orbital calculations, log P	MLR	93
Metabolism (P450 substrates and inhibitors)	Drugs	Multiple models	Surface areas, molecular orbital calculations, log P	MLR	94
Metabolism (CYP2C9 Inhibition)	Drugs	0.5 to 250 μM, Ki (21)	GRIND, ALMOND, GoMSIA, GOLPE	3D-QSAR PCA, PLS	96
Metabolism (CYP3A4 Inhibition)	Drugs	1.8 to 700 μM, Ki (14)	MS-WHIM Catalyst	3D-QSAR	97
Metabolism (CYP2C9 Inhibition)	Drugs	3.5 to 95 μM, Ki (9) 0.1 to 50 μM, Ki (29) 12.9 to 250 μM, Ki (13)	MS-WHIM Catalyst	3D-QSAR	98
Metabolism (CYP2A5 & CYP2A6 Inhibition)	Cumarin derivatives	0.46 to 5.7 pIC50 (23)	CoMFA, GOLPE, GRID	3D-QSAR	99
Metabolism (CYP2C9 Inhibition)	Drugs/drug-like	0.1 to 48 μM, Ki (14)	CoMFA Homology Model	3D-QSAR Protein Model	100
Metabolism (CYP1A2 Inhibition)	Flavonoid derivatives	0. 2 to 600 μM, IC50 (19)	Hammett constant, HOMO, LUMO, π coeff	MLR, NN	101
Metabolism (CYP2C9 Inhibition)	Drugs	0.5 to 245 μM, Ki (29)	Homology Model, Volsurf, GRID	3D QSAR Docking	106

since an increase in lipophilicity generally brings about an increase in potency, the latter being still the dominating parameter. Eventually, the size and lipophilicity of candidates will have to be optimized, together with other parameters, but only a few drugs are so hydrophylic and small to be excreted unchanged renally.

We are not aware of many computational approaches specifically aimed at predicting urinary excretion (renal clearance), and this subject typically involves a discussion of lipophilicity (log D_{oct}), pK_a and the presence of electron-withdrawing groups [53] for the affinity of a drug for renal transporters and/or the rate of its glomerular filtration. Very recently, two interesting attempts have surfaced and will be briefly discussed in this section.

As reported in the case of VD_{ss} and f_u, Hunt [71] has adopted a similar approach, involving a classification via SIMCA of 451 and 466 compounds, for clearance and urinary excretion, in 6 and 5 classes, respectively. These classes (bins) range from < 1 mL/min/kg (class 1) to > 16 mL/min/kg (class 6) for clearance, and from < 1% (class 1) to > 70% (class 5) for urinary excretion. A reasonably good prediction was observed for a small, yet independent test set, but the question may be asked about the Discovery or Development stages where these models would be useful,

and we are reluctant to accept the idea that library design should (or would) consider such "specific" models, as opposed to more general parameters. Thus they may find use at later stages, but the project team involved with a particular set of leads or therapeutic area may decide to develop more local models, in the early stages of a project, and/or simply rely on experimental data when the project is in a candidate seeking mode and experience has been gained with the class of compounds and the optimization of a candidate.

Similarly, the model of Mager and Jusko [70] seems inadequate for general use, but in this case the authors stated that it was developed as a "local" model to answer specific questions on a certain class of steroids. However, even within the constraint of its "local" use we find difficult to rationalize why a Connolly molecular surface area, used as the sole parameter in modeling CL (L/h), and obviously yielding very similar computed values for all steroids, would provide a reasonable and predictive model. The intercept seems very large, in fact much larger than any of the values reported in the training set (9 to 84 L/h) with an absolute value of 265.

CONCLUSION

It is apparent, from this review and other work recently published, that two main themes emerge: one is represented by the accuracy of the data, and the other by the intrinsically empirical nature of QSAR/QSPR approaches. Although we have not specifically mentioned the use of high-throughput data, we would like to comment that while they provide a reasonable experimental answer, useful for early discovery work, they do not seem suitable for general and predictive modeling work. They may be useful when a very crude "yes-no" answer is sought, but may be misleading if not used carefully. More generally, and as reiterated in the course of the present discussion, it is the data quality and the structural and parameter diversity of the compounds used that are of paramount importance.

We are of the opinion that smaller data sets comprising accurate determinations are better suited for predictive modeling than larger ones comprised of much less accurate data points. The generation of accurate data and their analysis is by no means a trivial task, as it is time consuming, expensive and tedious, but we maintain that it is the only approach possible to discern, among the (over) abundance of descriptors and approaches available, useful parameters and statistically sound techniques. In many cases similar data sets yielded contrasting findings, owing primarily to the heterogeneity of the data used, and it is rare to see detailed analysis and comments on the experimental protocols and their potential shortcomings, other than generic comments on "experimental errors" and "heterogeneity" of data sources.

The definition of what constitutes a "large" or a "small" data set is of course, a matter of endless debate, and there are very different views in the field, on the basis of the desired "target" accuracy and preferred approaches. Our view is that a few hundreds compounds, coupled with high accuracy in the determination of the target properties and supported by a judicious choice of property and chemistry space occupancy, as in the work of Gao *et al.* [23], should be preferred to thousands of less accurate data points. The latter approach may nevertheless be used to develop models for a library design stage. And, even at that stage, simpler models as the "rule of 5" [48] or other criteria proposed by Veber *et al.* [40] may suffice in most cases. We would concur with Bergstrom *et al.* [45] who point out that the lack of data remains a major issue and that several hundreds accurate data points would be needed. We would like to stress the word "accurate", even though the assembly of such data set(s) would be by no means rapid or inexpensive.

Among the criteria used for compound selection, drug-likeness should be considered very important as well (see also the article by Migliavacca in this issue). Especially at the inception of a medicinal chemistry project, the team of scientists involved should devote every effort to the generation of good quality data, with replicate measurements, if possible, focusing on local models since general and all encompassing models may be hard to find for the complex phenomena underlying ADME.

The empirical nature of QSAR/QSPR approaches (see also the article by Petrauskas *et al.* in this issue) may, ultimately, dictate the use of fairly "local" models since it would be hard to extrapolate predictions outside the range of parameters used in the training set. Even when broad data ranges are used, there will be exceptions due to the "non-equilibrium" and complex nature of many ADME phenomena, such as active transport, specific or unspecific binding phenomena, and the synergistic action of different metabolic and transport enzymes. These aspects should not discourage the use of correlations and the search for useful insights on ADME properties, but they should point toward the complexity of the phenomena scientists attempt to model and suggest caution in extrapolation and generalization of models. This is particularly true when the data sources are quite heterogeneous and other QSPR models (e.g., Clog P_{oct} models) were used to derive parameters used, in turn, in the correlation sought.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Drs. T. S. Maurer, S. Mente and M. D. Miller (PGRD, Groton Laboratories) for a critical reading of the manuscript and useful comments. In particular we are indebted to Dr. T. S. Maurer for several discussions and for some of the references provided.

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