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Combining high-throughput pharmacokinetic screens at the hits-to-leads stage of drug discovery

David J.M. Spalding, Andrew J. Harker and Martin K. Bayliss

Profound technological advances in the drug discovery process have led to the identification of an increasingly large number of promising compounds at the hits-to-leads stage. Higher-throughput pharmacokinetic screens have therefore been developed to enhance the tractability of selected leads and minimize the risk of failure in the later stages of drug development.

David J.M. Spalding
Andrew J. Harker and
***Martin K. Bayliss**
BioMet Discovery 1
BioMet Division
GlaxoWellcome R&D
Park Road, Ware
Herts, UK SG12 0DP
*tel: +44 1920 883619
fax: +44 1920 884374
e-mail: mkb2621@
glaxowellcome.co.uk

▼ In response to the high cost of drug development and the drive to reduce the high rate of attrition of lead compounds, the pharmaceutical industry has had to revolutionize its approach to drug discovery over recent years. These changes have resulted in the production of very large numbers of pharmacologically active compounds with the potential to further increase production several-fold[1,2]. The advent of combinatorial synthesis, miniaturization and automation has provided the opportunity for the medicinal chemist to generate diverse libraries, mapping large areas of both chemical and pharmacological space. High-throughput screening (HTS) robots that are capable of measuring the pharmacological activity of tens of thousands of compounds are meeting the challenge presented to biologists by this revolution in chemistry[2]. These profound technological advances, made at the earliest stages of the drug discovery process, have therefore enabled lead-identification groups to deliver increasing numbers of promising hits for evaluation. This has applied significant pressure to the evaluation processes that are performed when selecting tractable lead series to enter the lead-optimization phase. Traditionally, the hits-to-leads part of the process has focussed solely on aspects of potency and selectivity, with little input from functions associated with development criteria. The benefits of obtaining early knowledge regarding the pharmacokinetic properties of diverse templates are now being realized. The potential exists to reduce the time in lead optimization through the judicious choice of compounds with the best balance of properties. Consequently, the scientists involved in assessing pharmacokinetics during the lead-identification and -optimization phases have to deal with larger numbers of diverse compounds at an earlier stage in the process.

Historically, inappropriate pharmacokinetic properties have been a major reason for the failure of compounds in the later stages of development[3]. This was largely owing to an inability to rectify poor pharmacokinetic characteristics present in many lead series accepted for lead optimization. It is therefore necessary to bring the 'traditional' low-throughput activities of drug metabolism into the higher-throughput arena of the lead-identification stages in order to provide information and create options for lead-optimization teams. The approaches that have been developed, and that continue to evolve to meet this challenge, are the focus of this review.

In silico screening

In addition to the problem of generating larger numbers of molecules to be tested, the high-throughput combinatorial approach poses the problem that novel compounds that are produced in the libraries are not necessarily drug-like in their physicochemical properties. Recent experience at

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Pfizer (Groton, CT, USA) has shown a gradual increase in the average molecular weight and lipophilicity of novel compounds over the past decade[4]. Furthermore, as discussed by Curatolo[5], the non-aqueous solvents used in HTS remove any selection pressure against poorly soluble leads, resulting in the problem having to be addressed at the lead-optimization stage. At this stage, it can be much more difficult to make structural changes to the molecule while maintaining the desired intrinsic activity. There is, therefore, a need for methods by which to screen the large number of compounds produced in the early discovery stages for the high solubility and moderate lipophilicity required for absorption, balanced with appropriate metabolic stability and the ability to cross other membranes such as the blood–brain barrier.

There is a great deal of interest in the development and application of computational models to determine the drug-likeness of a molecule because of their cost-effectiveness, speed, high-throughput and the fact that they do not require physical samples for their application[6,7]. Where discussing the combined approach of *in silico*, *in vitro* and *in vivo* screening utilized at GlaxoWellcome, models used within the authors' laboratories will be presented, together with a brief review of alternative approaches in the literature. In particular, methods used to predict intestinal absorption and blood–brain barrier penetration will be reviewed, in addition to ongoing work to predict the metabolic stability of new chemical entities (NCEs). A recent review by Clark and Pickett[7] provided a comprehensive overview of state-of-the-art approaches to computational methods.

An initial filter for new compounds that will remove those that are, on the basis of their chemical structure, likely to be unsuitable for further progression, is a very valuable tool to reduce the number of compounds undergoing evaluation. At GlaxoWellcome, a method based on a genetic algorithm has been evaluated and successfully used to filter out unsuitable compounds before further testing[8]. This approach involves calculation of weights that describe the differential occurrence of molecular properties (such as molecular weight and hydrogen bond donors and/or acceptors) that are present in drug-like molecules in the World Drug Index (WDI) (www.daylight.com/daycgi/wdi), compared with a database of compounds assumed to be non-drug-like[9]. Taking this a stage further, the utility of neural nets has been evaluated in this area by workers from Vertex[10] (Cambridge, MA, USA) and BASF (Ref. 11) (Ludwigshafen, Germany) with very promising results.

Prediction of intestinal absorption

The oral absorption of a drug is a function of its solubility and dissolution in the aqueous contents of the gastrointestinal tract and its ability to traverse the gastrointestinal membrane. It is obvious, therefore, that the physicochemical properties of the molecule are important determinants of its permeability. Two approaches are used at GlaxoWellcome to improve absorption based on physicochemical properties, which are derived from empirical analysis of databases of absorbed compounds.

The first approach that is utilized was first proposed by Lipinski and co-workers[4] at Pfizer and is termed the 'Rule of Five'. This rule was derived from the physicochemical properties of 2,245 drugs from the WDI that were believed to have entered Phase II trials and be orally absorbed. In this system, oxygen and nitrogen atoms are classified as hydrogen bond acceptors and N-H or O-H groups as hydrogen bond donors. The calculated octanol/water partition coefficient, ClogP, is derived at GlaxoWellcome using Daylight software (Daylight Information Systems, Mission Viejo, CA, USA). However, the MlogP (based on atom contributions to the overall logP, unlike ClogP which is derived by molecular fragment contribution) derived by Moriguchi and co-workers[12] can also be used. In the application of this rule, a fail flag is raised when a molecule satisfies any two of the following conditions:

- number of hydrogen bond donors >5
- number of hydrogen bond acceptors >10
- ClogP >5
- Molecular weight >500

It is recognized that the rule is not applicable to molecules that are substrates for transporters.

As a secondary measure of likely absorption, a qualitative model derived by Hill and co-workers[13] is used in conjunction with the Rule of Five. This model is based upon the knowledge that the lipophilicity and size of a molecule are important factors in determining the absorption of compounds by passive diffusion. A plot of the intrinsic lipophilicity of a molecule, taking into account its degree of ionization at physiological pH (ClogD), against the calculated molecular refraction (CMR), a measure of molecular size, gives a distribution of compounds as illustrated in Fig. 1. The distribution of molecules can be divided into four quadrants. In quadrants one and three, molecules are of sufficient lipophilicity to be absorbed via the transcellular route (i.e. through the membrane). In quadrant two, molecules are small and hydrophilic and are likely to be absorbed via the paracellular route, that is, by means of the tight junctions between cells rather than traversing the membrane itself. In quadrant four, there are very few orally absorbed molecules as they are too large to be absorbed via the paracellular route and too hydrophilic to be absorbed via the transcellular route. Thus, the likelihood of absorption can be determined by plotting these parameters for a set of diverse molecules that are potential pharmacological hits of unknown absorption, and assessing in

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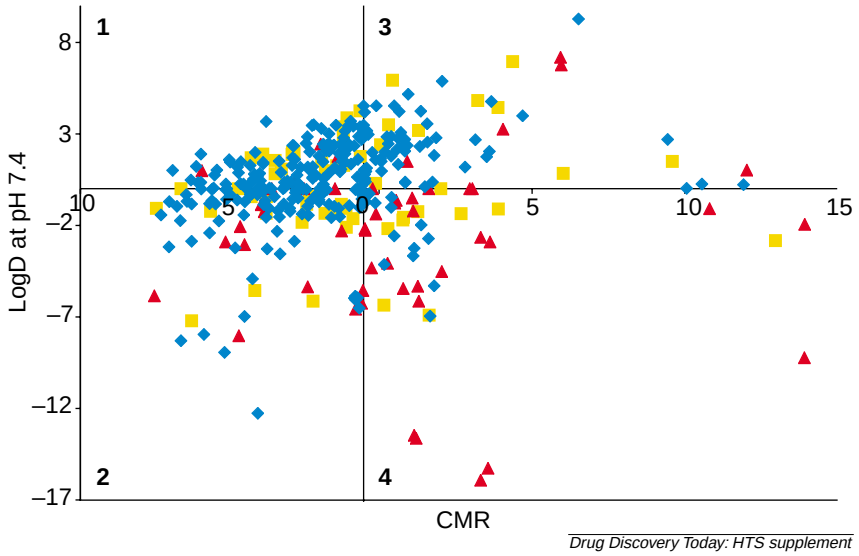

Figure 1. LogD versus calculated molecular refraction (CMR) plot illustrating the distribution for drugs of good (blue diamonds), intermediate (yellow squares) and poor (red triangles) absorption. Drugs that fall in the lower right quadrant tend to have poor absorption and those in the upper and lower left quadrants can be absorbed by transcellular and paracellular absorption, respectively.

which quadrant they lie. Moreover, it is also possible to determine which of the physicochemical properties require modification to obtain the desired absorption profile. This approach has been used to design virtual molecules that aid in the design and selection of hits for further evaluation, and to direct lead-optimization.

An alternative approach, which takes further the concept of the role of molecular size, is the use of polar surface area (PSA) as a predictor of absorption[14–16]. Early work by Van de Waterbeemd and colleagues[14] derived a quantitative structure–property relationship to describe the apparent permeability through the monolayer of Caco2 cells (an *in vitro* model of absorption) based on PSA and molecular weight. It has subsequently been shown that PSA correlates with human fractional absorption[15] and human effective permeability[16]. The use of PSA as a predictor of absorption has great potential in molecular design and deserves greater attention in the future with respect to evaluation of pharmacokinetic tractability.

Prediction of blood–brain barrier penetration

The blood–brain barrier, as the name indicates, represents a significant barrier to the entry of drugs into the brain and central nervous system (CNS)[17]. A knowledge of the physicochemical parameters that determine the ability to cross this membrane is important in terms of helping to predict which molecules have the potential to cross into the brain (e.g. a CNS target). Alternatively, knowledge of which structural features will preclude crossing of this membrane is also desirable for drugs with a peripheral mechanism of action, where structural modifications could be made to reduce the risk of centrally mediated side effects.

One approach to gaining this knowledge has been to use discriminant analysis on chemically diverse compound sets containing compounds with high and low/no CNS activity to derive topological descriptors of CNS penetration[18,19]. This approach has had some success and Van de Waterbeemd and colleagues[19] have proposed that, for brain penetration, the molecular weight should be <450 and PSA <90 Å.

An alternative approach, which has found favour within the pharmaceutical industry, is prediction of the penetration measure LogBB, which is defined as the ratio of the steady-state concentrations of drug in the brain and drug in the blood. This method was derived by Abraham and co-workers in 1995 (Ref. 20), who devised a general solvation equation to describe the distribution of a solute in a given system.

A modification of this equation has been used within the authors' laboratories to classify compounds. The model has been validated using appropriate *in vivo* data and can categorize molecules into those having low, medium or high CNS penetration. Those categorized as low can be discarded if CNS penetration is required, or positively selected if CNS penetration is an undesirable characteristic. After validation for a particular series of structural analogues, the model has been used to predict likely CNS penetration and could be applied to virtual libraries and molecular templates of chemical diversity to prioritize future chemical synthesis.

More recently, Clark[21] published a simple two-variable equation for LogBB, based on PSA and the calculated octanol/water partition coefficient. These parameters are quickly and easily calculable and have the advantage of allowing the chemist to alter readily the molecular structure of a potential hit to achieve the desired degree of penetration. Although this equation correlated well with the data set evaluated, further application is required to evaluate fully the potential of this approach.

A shortcoming of all these *in silico* models is their failure to take into account the influence of P-glycoprotein and other transporters involved in blood–brain barrier permeation. The currently limited understanding of the structure–activity relationship (SAR) of P-glycoprotein substrates is clearly an important area for further investigation.

Prediction of metabolic stability

Achieving optimal bioavailability requires the correct balance between intrinsic absorbability and metabolic stability. *In silico*

models that would enable prediction of metabolic stability represent a useful adjunct to the absorption methods already described. Knowledge in this area is growing and we are beginning to understand the structural features of molecules that determine their susceptibility to metabolic attack. It is now clear that the lipid solubility of a compound, its size and shape and the presence and position of functional groups in the compound all contribute to how well it is metabolized, because they determine how the molecule fits into the active site of the cytochrome P450 enzyme[22–24]. In an attempt to produce predictive models, scientists within the pharmaceutical industry and academia are focussing not only on enzyme properties, but also on understanding substrate properties using metabolic SARs (Ref. 22), physicochemical properties[23], *ab initio* and molecular orbital geometry calculations of radical stability[24,25] and the active-site structures of human P450s obtained using nuclear magnetic resonance (NMR) spectroscopy and homology modelling[22,26]. Definitive models have yet to be published, but this remains an area of keen interest with significant potential to influence the selection of the most appropriate chemical templates.

In summary, *in silico* tools enable the drug metabolism scientist to be proactive in rationalizing changes in chemical structure, and the effects on absorption and disposition of a diverse set of molecular templates. Furthermore, the introduction of informatics at this early stage offers the possibility of identifying undesirable features of molecular templates and provides suggestions for redirecting them to a more tractable area of chemical space. Validated models also allow the identification of subsets of molecules for subsequent *in vitro* or *in vivo* screening, further reducing the time in the hits-to-leads phase.

In vitro screening

In terms of throughput and cost, *in vitro* screens represent an intermediate level of filtering between *in silico* and *in vivo* and their value in the drug metabolism toolkit has been recognized[27–29]. The controlled environment of the *in vitro* situation means that the multiple factors influencing pharmacokinetics and drug metabolism *in vivo* can be investigated as single components. This can be seen as an advantage in that it enables a complex problem to be broken down into its constitutive parts, and SARs for tractable templates can potentially be independently established around permeability and metabolism. However, it also means that the permeability and metabolism determined *in vitro* are not subject to the full range of influences experienced *in vivo*. For this reason, although acceptance or rejection criteria can be applied to *in vitro* screens, they are more commonly regarded as ranking tools, used to prioritize compounds for relatively slower-throughput *in vivo* investigations. As with *in silico* screens, confidence in the validity of the screens has to be established with targeted *in vivo* experiments for each chemical series under investigation. *In vitro* screens have the advantage that human tissues can be used, thus providing early data on potential hits in the target species, and cross-species comparisons can be made, which could assist in the selection of an appropriate species for future *in vivo* studies.

In vitro studies: permeability

In vitro models of membrane permeability have largely utilized cell monolayers – most commonly, Caco-2 cells, which are derived from human colon adenocarcinoma, or Madin-Darby canine kidney cells (MDCK)[30–32]. In terms of using this screen to help forecast human oral bioavailability, Caco-2 cells might seem to be the more appropriate, being derived from humans and being from the gut. However, MDCK cells are significantly quicker to grow, and little difference in permeability has been demonstrated using large test sets of compounds[32]. The throughput of this screen has been increased with the advent of trans-well (and, subsequently, miniaturized) technology, in combination with LC–MS analytical methodologies. The screen is compatible with automated dispensing and sampling technologies by virtue of growing the cells in trans-well or miniaturized formats. Furthermore, the selectivity of mass spectrometric detection has allowed the development of fast, generic, short-column, digital gradient chromatographic methods, capable of processing a sample every 2 min. The output from this screen is an apparent permeability value (Papp) – a measurement of the rate of transfer from the apical to basal side of the monolayer. Comparison of *in vitro* with *in vivo* data has been used to establish statistically a Papp value above which permeation *in vivo* is likely to be high. Any judgement regarding lower Papp values is less clear cut however, because this is a measure of rate rather than extent, and there could be transport mechanisms operating *in vivo* that are not present *in vitro*. Thus, it is possible for compounds with low *in vitro* Papp values to be well absorbed *in vivo*. However, bearing these considerations in mind, the screen has been used successfully to filter out potential 'hits' with inappropriate absorption characteristics before testing *in vivo*.

In vitro studies: metabolism

Microsomal preparations from human liver are commonly used to investigate metabolic stability *in vitro* because they can be stored frozen for many months with little drop-off in activity and provide an assessment of likely stability in the target species[28,29]. They also lend themselves to automation, and can be performed in 96-well format with LC–MS analysis as already described. Such screening systems are now used routinely throughout the pharmaceutical industry. One of the major shortcomings of using liver microsomes or a similar enzyme source, is the inconsistency between preparations and the instability, absence or cofactor requirements of some enzymes in

the preparation. Typically, differences in enzyme activity between different preparations are monitored by the inclusion of a standard compound or compounds in each batch. Expressing the metabolic turnover of the unknowns as a ratio of the turnover of a standard enables relatively small variations in enzyme activity. Using such a system, we can generate metabolic stability data routinely on hits emerging from an HTS campaign in three days, where this might once have taken several weeks using conventional methods. Metabolic stability is generally of greater importance in lead optimization than it is during the hits-to-leads stage. However, these data not only provide a benchmark for subsequent improvement where required, but are also useful in the selection of the most appropriate *in vivo* 'tools' from among the hits. Although not otherwise optimized, relatively metabolically stable hits can be used *in vivo* to validate the target or the animal model if either are novel.

In vitro preparations have also been used to investigate the ability of drugs to induce cytochrome P450, usually by exposing human hepatocytes to the compound of interest in primary culture[33]. This is reliant on the supply of suitable human material that is not always routinely available. Recently, the human pregnane X receptor was cloned and shown to be activated by known inducers of CYP3A4 (Ref. 34). This offers great potential as a screen for likely inducers of the major form of P450 in humans, which could be used at the hit- and/or lead-identification stage to eliminate those templates likely to cause significant drug–drug interactions.

In summary, now that high-throughput *in vitro* screens of permeability and drug metabolism are well established, greater attention is likely to be paid to the development of more specialized *in vitro* screens. Blood–brain-barrier permeation, substrates for P-glycoprotein and other transporter mechanisms, and metabolic processes not covered by the microsomal screen are likely targets. A template that is a substrate for a transporter could be used in the optimization process depending on the target, and thus such screens would provide further information about the value of these templates.

In vivo screening

Significant progress has been made in developing *in silico* and *in vitro* models to help ensure that candidate templates are optimized in terms of their absorption, distribution to the target receptor, and pharmacokinetics. However, determining whether a compound meets these criteria is often best answered by carrying out an *in vivo* pharmacokinetic study. Traditionally, such studies have involved administration of single compounds to laboratory animals to calculate parameters such as clearance, half-life, volume of distribution and oral bioavailability. Inevitably, such studies are resource intensive, both in terms of animals and in the amount of compound required, and are also low-throughput because of the need for individually tailored analytical methods. There is also a moral impetus to improve efficiency in this area. Against this background, many researchers have challenged conventional thinking and investigated the utility of combining a number of compounds into one-dose solution for a single administration to animals[35–38]. This technique is now commonly used within the pharmaceutical industry. The use of these 'cassette dosing' or 'N-in-1' protocols has recently had a significant impact at the hits-to-leads stage, where a range of diverse hits can be analysed quickly to identify the most appropriate templates for chemical modification.

Instrumental to the success of both high-throughput *in vitro* and *in vivo* approaches have been the concurrent advances in analytical technology and automation that have occurred over the past five years and the subsequent changes in working practices within industrial drug metabolism departments. The early studies using cassette dosing carried out by Toon and Rowland employed HPLC with ultraviolet detection[39]. The assay of even a single compound in biological matrices such as plasma can be difficult owing to interference from endogenous materials. Obviously, the administration of compounds in cassettes complicates these problems even further by increasing the demand for selectivity of detection, because the multiple compounds and/or their metabolites might co-elute and interfere with each other. In addition, a sensitive assay is required because the dose of each individual compound needs to be low to avoid drug–drug interactions and overdosing of the animal. It was noted during early cassette-dosing experiments using HPLC with ultraviolet detection that the time saved by using cassette dosing was being consumed by the time taken to develop sufficiently selective methods.

The full potential of cassette dosing and *in vitro* screens has been realized by the coupling of HPLC to MS, in particular the multiple reaction monitoring (MRM) technique afforded by tandem mass spectrometry (LC–MS/MS)[40]. In MRM, an ion of the intact analyte is isolated in the first quadrupole of the mass spectrometer, before being fragmented with gas in a collision cell. A specific fragment ion is then isolated and monitored in the third quadrupole. The selectivity is high because it is a multi-step process. Thus, although endogenous compounds having the same parent ion molecular weight as the analyte might be selected in the first quadrupole, the probability that these interfering substances will share a common fragment ion with the analyte is very small. Additionally, sensitivity is excellent with MRM because the levels of 'chemical noise' are very low. For these reasons, LC–MS/MS has become a very powerful tool for enhancing the analysis of mixtures of compounds and has now become the analytical tool of choice[40].

Combining high-throughput pharmacokinetic screens at the hits-to-leads stage

In an HTS campaign, the number of compounds tested is commonly in the order of 250,000. At the average hit rate the

number of compounds that require evaluation runs into hundreds. This continues to represent the main challenge, which is to identify the most tractable templates containing the most appropriate developability features using the standard techniques of drug metabolism. The real value of the screens already described in the identification and exemplification of 'hits' and potential leads lies in combining their powers of resolution to decrease the number of compounds that need to progress to the *in vivo* stages of assessment. For example, a recent focussed screen gave rise to 150 diverse compound templates with acceptable activity against the target. The leads were then evaluated for acceptable oral bioavailability and pharmacokinetics by investigating metabolic stability *in vitro*, gastrointestinal permeability *in vitro* and intravenous cassette dosing. These screens rapidly determined that absorption was not an issue for this series, but that there was a high plasma clearance for the majority of the compounds that was attributable to metabolism. A number of diverse compounds was rapidly identified that had reasonable membrane permeation, stability in human microsomes and plasma clearances of less than liver blood flow, which were progressed to testing in discrete pharmacokinetic studies. In summary, the project had several chemically diverse templates with and without pharmacological activity but with good developability characteristics to progress into lead optimization for further modification.

The principles applied to the identification of hit molecules can be applied in a similar way to the optimization of monomers. A recent example from the authors' laboratories involved the screening of three core libraries of 1,200 compounds using *in silico* models, which predicted that some 80 compounds met the project developability criteria by having appropriate CNS penetration. Furthermore, a number of these molecules was also predicted to have good oral absorption. Using this information, the project chemistry team was able to prioritize rationally the sequence in which the libraries were synthesized.

Conclusion

In response to the increased number of compounds that now require evaluation in the early hits-to-leads stage of drug discovery, the more traditional methods of drug metabolism and pharmacokinetics have had to be modified and applied earlier in the process. The advent of *in silico* models to predict diverse chemical templates with inappropriate physicochemical properties for absorption and brain penetration, combined with high-throughput *in vitro* assays and cassette dosing *in vivo*, represents a powerful means of selecting the hits with the highest potential for successful optimization. However, these tools only represent a start. There is pressure to use the increased amount of data generated by these screens to help us gain a greater understanding of the processes underlying the handling of drugs in the body and further reduce the rate of candidate attrition. In particular, the role of transporters in drug disposition will represent a new challenge for discovery scientists in the future. It is highly probable, therefore, that screen development and evolution will remain a focus in the pharmaceutical industry.

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References

1 Gordon, E.M. *et al*. (1996) Strategy and tactics in combinatorial organic synthesis. Applications to drug discovery. *Acc. Chem. Res*. 29, 144–154

2 Berman, J. and Howard, R.J. (1998) Combinatorial drug discovery: concepts. In *Combinatorial Chemistry and Molecular Diversity in Drug Discovery* (Gordon, E. and Kerwin, J., eds), pp. 447–452, John Wiley & Sons

3 Prentis, R.A. *et al*. (1988) Pharmaceutical innovation by seven UK pharmaceutical companies (1964–1985). *Br. J. Pharmacol*. 25, 387–396

4 Lipinski, C.A. *et al*. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev*. 23, 3–25

5 Curatolo, W. (1998) Physical chemical properties of oral drug candidates in the discovery and exploratory development settings. *Pharm. Sci. Technol. Today* 1, 387–393

6 Walters, W.P. *et al*. (1999) Recognising molecules with drug-like properties. *Curr. Opin. Chem. Biol*. 3, 384–387

7 Clark, D.E. and Pickett, S.D. (2000) Computational methods for the prediction of 'drug-likeness'. *Drug Discov. Today* 5, 49–58

8 Hann, M. *et al*. (1999) Strategic pooling of compounds for high throughput screening. *J. Chem. Inf. Comput. Sci*. 39, 897–902

9 Gillet, V.J. *et al*. (1998) Identification of biological activity profiles using substructure analysis and genetic algorithms. *J. Chem. Inf. Comput. Sci*. 38, 165–179

10 Ajay, A. *et al*. (1998) Can we learn to distinguish between drug-like and non-drug-like molecules. *J. Med. Chem*. 41, 3314–3324

11 Sadowski, J. and Kubinyi, H. (1998) A scoring scheme for discriminating between drugs and non-drugs. *J. Med. Chem*. 41, 3325–3329

12 Moriguchi, I. *et al*. (1992) Simple method of calculating octanol/water partition coefficient. *Chem. Pharm. Bull*. 40, 127–130

13 Hill, A.P. *et al*. (1994) Oral delivery of 5-HT_{1D} receptor antagonists: towards the discovery of 311C90, a novel anti-migraine agent. *Headache* 34, 308

14 van de Waterbeemd, H. *et al*. (1996) Estimation of Caco-2 cell permeability using calculated molecular descriptors. *Quant. Struct.-Act. Relatsh*. 15, 480–490

15 Palm, K. *et al*. (1997) Polar molecular surface properties predict the intestinal absorption of drugs in humans. *Pharm. Res*. 14, 568–571

16 Winiwarter, S. *et al*. (1998) Correlation of human jejunal permeability (*in vivo*) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. *J. Med. Chem*. 41, 4939–4949

17 Audus, K.L. *et al*. (1992) Brain uptake of drugs: the influence of chemical and biological factors. *Adv. Drug Res*. 23, 3–53

18 Basak, S.C. *et al*. (1996) Predicting blood–brain barrier transport of drugs: A computational approach. *Pharm. Res*. 13, 775–778

19 van de Waterbeemd, H. *et al.* (1998) Estimation of blood–brain barrier crossing of drugs using molecular size and shape, and H-bonding descriptors. *J. Drug Targeting* 6, 151–165

20 Abraham, M.H. *et al*. (1995) Hydrogen bonding. 36. Determination of blood brain distribution using octanol-water partition coefficients. *Drug Des. Discov.* 13, 123–131

21 Clark, D.E. (1999) Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 2. Prediction of blood–brain barrier penetration. *J. Pharm. Sci*. 88, 815–821

22 Lewis, D.F.V., ed. (1996) *Cytochrome P-450: Structure, Function and Mechanism*, Taylor & Francis

23 Lewis, D.F.V. (2000) Structural characteristics of human P450s involved in drug metabolism: QSAR and lipophilicity profiles. *Toxicology* 144, 197–203

24 Lightfoot, T. *et al*. (2000) Regioselective hydroxylation of debrisoquine by cytochrome P4502D6: implications for active site modelling. *Xenobiotica* 30, 219–233

25 Ackland, M.J. (1993) Correlation between site specificity and election frontier values in the metabolic hydroxylation of biphenyl, di-aromatic and CYP2D6 substrates: a molecular modelling study. *Xenobiotica* 23, 1135–1144

26 Modi, S. *et al*. (1996) A model for human cytochrome P-450 2D6 based on homology modelling and NMR studies of substrate binding. *Biochemistry* 35, 4540–4550

27 Rodriguez, A.D. (1997) Preclinical drug metabolism in the age of high-throughput screening: an industrial perspective. *Pharm. Res*. 14, 1504–1510

28 Tarbit, M.H. and Berman, J. (1998) High throughput approaches for evaluating absorption, distribution, metabolic and excretion properties of lead compounds. *Curr. Opin. Chem. Biol*. 2, 411–416

29 Dickens, M. and Eddershaw, P.J. (1999) Advances in *in vitro* drug metabolism screening. *Pharm. Sci. Technol. Today* 2, 13–19

30 Stewart, B.H. *et al*. (1995) Comparison of intestinal permeabilities determined in multiple *in vitro* and *in situ* models: relationship to absorption in humans. *Pharm. Res*. 12, 693–699

31 Rubas, W. *et al*. (1996) Flux measurements across Caco-2 monolayers may predict transport in human large intestinal tissue. *J. Pharm. Sci*. 85, 165–169

32 Irvine, J.D. *et al*. (1999) MDCK (Madin-Darby Canine Kidney) cells: a tool for membrane permeability screening. *J. Pharm. Sci*. 88, 28–33

33 Silva, J.M. *et al*. (1998) Refinement of an *in vitro* model for cytochrome P450 induction. *Drug Metab. Dispos*. 26, 490–496

34 Lehmann, J.M. *et al*. (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Invest*. 102, 1016–1023

35 Adkinson, K.K. *et al*. (1996) Multiple in one dosing: increasing compound throughput in whole animal pharmacokinetics. *Pharm. Res*. (Suppl.)13, S486

36 Berman, J. *et al*. (1997) Simultaneous pharmacokinetic screening of a mixture of compounds in the dog using API LC/MS/MS analysis for increased throughput. *J. Med. Chem*. 40, 827–829

37 Olah, T.V. *et al.* (1997) The simultaneous determination of mixtures of drug candidates by liquid chromatography/atmospheric pressure chemical ionization mass spectrometry as an *in vivo* drug screening procedure. *Rapid Commun. Mass Spectrom.* 11, 17–23

38 Watt, A.P. *et al.* (2000) Approaches to higher-throughput pharmacokinetics (HTPK) in drug discovery. *Drug Discov. Today* 5, 17–24

39 Toon, S. and Rowland, M. (1983) Structure activity relationships among the barbiturates in the rat. *J. Pharmacol. Exp. Ther*. 225, 752–763

40 Korfmacher, W.A. *et al*. (1997) HPLC-API/MS/MS: A powerful tool for integrating drug metabolism into the drug discovery process. *Drug Discov. Today* 2, 532–537