In silico prediction of blood–brain barrier permeation

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This review examines the progress that is being made towards the *in silico* prediction of brain permeation. Following a brief introduction to the blood–brain barrier, the datasets currently available for *in silico* modeling are discussed. Recent developments in *in silico* models of brain permeation are summarized in the context of the current state of the art in prediction accuracy. An analysis of recent models is presented, focusing on what such models reveal about the molecular properties that determine brain permeation. The review concludes by presenting the current key issues in this area of research, noting in particular, the paucity of brain permeation data available for modeling. Finally, possible future directions are suggested.

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▼ The recognition that 'poor ADME properties' are one of the major reasons for the failure of drug candidates in the pre-clinical or clinical phases of development has driven a revolution in drug discovery research in recent years. Historically, the 'R' function of R&D has focused almost exclusively on producing drug candidates with excellent potency and selectivity for the biological target in question, leaving the 'D' function with the difficult, if not impossible, task of 'fixing' the pharmacokinetic properties of the compound while retaining potency and selectivity [1]. By the early 1990s, this paradigm was leading to an unacceptably high attrition rate for drug candidates that, in turn, was causing the cost of drug discovery to spiral upwards with a direct impact on the profits of pharmaceutical companies.

Thus, from the mid-1990s, under the weight of this economic pressure, the paradigm began to change. It was realized that to reduce attrition rates at later stages, compounds that would make poor candidates needed to be identified and eliminated much earlier in the research process. 'Fail fast, fail cheap' became every research manager's watchword. To spot the 'no-hopers' among early stage candidates, a battery of *in vitro* ADME screens was implemented almost universally. It seemed that, almost overnight, 'Caco-2' and 'microsomes' became part of the lingua franca of the drug discovery scientist, as these early screens for permeability and metabolic stability were incorporated into research programmes. In parallel to these *in vitro* technologies, considerable effort was also invested in the development of computational (or '*in silico*') approaches to the prediction of ADME properties in the hope of identifying poor compounds, perhaps even before they were synthesized.

Owing to the overwhelming preference for oral administration, much of the attention of both the *in vitro* and *in silico* approaches was naturally focused on the prediction of a compound's likely ability to cross the intestinal mucosa separating the gut from the bloodstream. However, there is another physiological barrier that is of concern to drug designers – the blood-brain barrier (BBB) – and it is the prediction of a compound's ability to cross this that is the subject matter of this review.

Introduction to the blood-brain barrier

As its name suggests, the blood–brain barrier separates the brain and central nervous system (CNS) from the bloodstream. Clearly, for the great majority of drugs aimed at CNS targets, this barrier must be crossed for a therapeutic effect to be exerted, the only exceptions being compounds delivered by invasive or intranasal routes. Conversely, for non-CNS targets, passage across the BBB could lead to undesirable side effects and so should be minimized. One of the distinguishing features of the blood–brain barrier is the presence of high-resistance tight junctions between the brain capillary endothelial cells that form the barrier [2]. These present a highly effective impediment – it has been estimated that the BBB prevents the uptake of >98% of all potential

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neurotherapeutics by the brain - and make the BBB much less 'leaky' than the intestinal epithelium. Consequently, paracellular transport of compounds across the barrier is effectively hindered and thus, most compounds entering the brain by passive diffusion will travel by the transcellular route. Apart from passive diffusion, compounds might also traverse the BBB by means of a variety of catalyzed transport systems that can carry compounds into the brain (carrier-mediated transport, receptor-mediated transcytosis) or out of the brain (active efflux). In the case of transport into the brain, a growing number of systems have been discovered, including transporters for amino acids, monocarboxylic acids, organic cations, hexoses, nucleosides, and peptides. In terms of transport out of the brain and into the blood, some of the key efflux transporters are P-glycoprotein (P-gp) and other multidrug-resistance proteins such as BMDP (brain multidrug-resistance protein) [3–5].

Here, the *in silico* prediction of BBB permeation by passive diffusion will be considered. At present, there are insufficient data for modeling of active processes, although this should become feasible as further knowledge of these systems is acquired.

Datasets available for *in silico* modeling

Datasets of sufficient size and quality are required to build predictive models. For the prediction of BBB permeation, there are various types of dataset that are available.

Surrogate measures

To assemble large datasets for modeling from databases of known drugs, some workers have chosen to equate a compound's activity (CNS^+) or inactivity (CNS^-) against a CNS target with its brain permeation. The underlying assumption of this approach is that CNS^+ compounds penetrate the BBB, whereas CNS^- compounds do not. The first assumption is evidently true, although the mechanism of passage across the BBB can vary, depending on the compound; in particular, some compounds might be substrates for active transport mechanisms. The second assumption is less reliable because non-permeation of the BBB is not the only reason for lack of observed CNS activity. For instance, compounds might be rapidly metabolized or effluxed from the brain by systems such as P-gp [3–5], or might simply be inactive against the relevant molecular target in the brain. So, while CNS activity implies BBB permeation, CNS inactivity does not necessarily mean that a compound does not penetrate the brain.

In vitro *measures*

The most popular *in vitro* model for the BBB is the Bovine Brain Microvessel Endothelial Cell (BBMEC) system. When these endothelial cells are co-cultured with primary astrocytes, it is possible to observe characteristics that are normally associated with the BBB *in vivo*, for example, the presence of tight junctions, efflux mechanisms and transporters. This is reflected in the fact that good correlations between *in vitro* and *in vivo* brain permeation data can be obtained [6]. However, few publicly available collections of such *in vitro* data are currently available and none of those that are available have yet formed the basis for any published *in silico* models. Furthermore, it is likely that BBMEC results will suffer from inter-laboratory variation because the assay uses primary cells, with attendant donor to donor variability, and there is, as yet, no standard methodology to run the assay. Such variation could make it difficult, if not impossible, to combine BBMEC data from different sources reliably.

In vivo *measures*

One *in vivo* measure of brain permeation is logPS, which gives an indication of the permeability of the BBB to a compound. LogPS is measured using a short-duration vascular perfusion method, from which a permeability–surface area product is calculated (hence logPS, a measure of the rate of transfer of the compound from the blood to the brain). LogPS values for a set of 18 compounds have been published and used in an initial modeling study [7], but as with *in vitro* measures, there are still too few publicly available data for this measure to receive wide attention from modelers.

The most commonly used type of *in vivo* data for *in silico* prediction is logBB, which is defined as the ratio of the steady-state concentrations of a compound in the brain to those in the blood (logBB = log [brain]/[blood]). Over the years, data from different sources have been compiled and have been used widely. It is worth noting, however, that even the largest compilation of logBB data comprises only ~150 compounds [8]. Clearly, this number cannot be representative of all chemical, or even drug-like, space.

Types of *in silico* model available and current leaders in prediction accuracy

Based on either CNS^+/CNS^- or logBB datasets, various kinds of *in silico* prediction methods have been developed over the last decade or so (reviewed in Refs [9–11]). Here, some recent developments are highlighted.

Rules of thumb

At the simplest level, several 'rules of thumb' have emerged from studies of brain permeation data that have been conducted in recent years. Such rules give simple guidance concerning the molecular properties that favour brain

permeation. Norinder and Haeberlein [9] propose two very simple rules:

Rule 1: if the sum of nitrogen and oxygen (N + O) atoms in a molecule is five or less, then the molecule has a high chance of entering the brain.

This rule is so simple that it actually requires no computation at all!

Rule 2: if ClogP – (N + O) > 0, then logBB is likely to be positive. In this rule, ClogP denotes the logarithm of the octanol–water partition coefficient (P) of a compound, as computed by the Daylight ClogP program (http://www.daylight.com). P is the ratio of the concentration of the compound in octanol to the concentration of the compound in water.

Other studies have suggested the following guidelines for compounds intended to enter the brain:

Rule 3: for good brain permeation, the polar surface area (PSA) of the compound should be below a certain limit. The PSA is a measure of a molecule's hydrogen-bonding capacity and is commonly calculated by summing the contributions to the molecular surface area from oxygen and nitrogen atoms and hydrogens attached to oxygen and nitrogen atoms. Two differing limits have been proposed: van de Waterbeemd *et al.* suggest a limit of 90 $Å^2$ [12], whereas Kelder *et al.* have a lower limit of 60–70 $Å^2$ [13].

Rule 4: molecular weight (MW) should be kept below 450 to facilitate brain permeation [12].

Rule 5: a logD value in the range 1–3 is recommended [12]. For a compound with one or more ionisable centres, at any given pH, there will be a mixture of species present in solution. By analogy to logP, logD is the logarithm of the distribution coefficient (D) of a compound. D is the ratio of the sum of the concentrations of all species of the compound in octanol to the sum of the concentrations of all species of the compound in water. For neutral compounds, logD is equal to logP.

From a comparative study, it has been suggested that, overall, compared to non-CNS drugs, CNS drugs tend to be more lipophilic, more rigid, have fewer hydrogen-bond donors, fewer formal charges (particularly negative charges), and lower PSA (< 80 $Å^2$) [14].

Classification approaches

At the next level of complexity in brain permeation prediction are classification approaches. Starting from training sets containing compounds classified as CNS^+/CNS^-, several groups have developed models that seek to predict to which class a previously unseen compound will belong.

The training sets used for generating these classifiers could be based on CNS activity data, as described earlier. Alternatively, some scientists use logBB data, simply defining a cut-off logBB value (zero is a common choice) above which a compound is designated as CNS^+ and below which it is designated CNS^-. It is worth noting that an early study, upon which subsequent scientists in the field have often drawn, combined CNS permeation data from different measurements (e.g. logBB and brain perfusion experiments) into single CNS^+/CNS^- sets [15]. Thus, the datasets in some studies might be intrinsically non-homogeneous. In recent studies of this kind, various molecular representations and classification methods have been employed to generate models from training sets. These models are then challenged with compounds that are not used in training – 'external' test sets – to assess their predictive capabilities. A summary of the recent work in this area, together with the results obtained, is given in Table 1.

From these results, several conclusions can be drawn. First, the prediction accuracy on the test sets is good, particularly when predicting CNS^+, for which the accuracy is better than 80% in all cases (it should be noted that the CNS^+ test set used in [20] comprised only 11 compounds, hence the 100% accuracy should be set in that context). This level of accuracy would certainly seem to be sufficient for these methods to be applied in the design of combinatorial libraries or the selection of compounds for screening against CNS targets. Second, it is interesting to observe that, in general, accuracy of prediction of CNS^+ exceeds that for CNS^-; this might well be a consequence of the difficulty in being certain that a CNS^- compound does not actually penetrate the brain, as discussed earlier.

QSAR models based on logBB data

At the top of the hierarchy of brain permeation models are QSAR models, based on logBB data. During the past ten years or so, many such models have been reported (recently reviewed by Norinder and Haeberlein [9]). Invariably, the models have been built using what has become known as the 'Abraham' dataset (originally compiled and recently augmented by Mike Abraham's group at University College, London [8]). It is worth noting that, over time, errors have crept into both the chemical structures and logBB data that are reported in some publications. Thus, when embarking on a new project to generate a logBB QSAR model, the structures and data to be used should be checked carefully, with reference to the primary literature being made whenever possible.

Some recent, diverse and illustrative approaches have combined Multiple Linear Regression or Partial Least Squares with a variety of molecular descriptors to generate logBB models. The results of these efforts are summarized in Table 2. Perhaps the most striking conclusion that can be

Table 1. Summary of recent classification models for brain permeation

Training set	Molecular descriptors	Classification method	Test set prediction accuracy (%)		Refs
			CNS^+	CNS^-	
15,000 CNS^+, 50,000 CNS^-, based on activity designations in MDDR and CMC databases	1D (molecular weight, counts of hydrogen-bond donors etc), 2D substructural keys	Bayesian neural network	92	71	[16]
110 compounds with CNS^+/CNS^- designations based on antinociceptive activity	Volsurf descriptors	PCA, discriminant PLS	90	65	[17]
3500 CNS^+ from Cipsline database, 3500 CNS^- from Sigma-Aldrich catalogue	Unity 2D fingerprints	Feed-forward neural network	84	87	[18]
179 CNS^+, 145 CNS^-	Computed molecular properties, e.g. molecular weight, lipophilicity	Support vector machine	83	80	[19]
3678 CNS^+, 5000 CNS^- from WDI database	Substructural fragments	Substructural analysis	100	67	[20]

Abbreviations: PCA, principal component analysis; PLS, partial least squares

drawn from these data is that, despite the great diversity of molecular descriptors employed and despite the variations in the composition of the training sets, the predictive performance of the models is more or less the same – ~0.4 log units. This seems like a large error in comparison to the range of logBB determined by experiment (approximately –2 to +1.5, i.e. ~3.5 log units), but it should be remembered that the experimental error in logBB measurements can be around 0.3 log units, and so this value provides a limit to the accuracy of *in silico* methods. What this apparent convergence might indicate is that current logBB QSAR models have reached their optimum use, given the currently available data for training and testing.

What do logBB QSAR models tell us?

An examination of the descriptors (and their associated coefficients) that feature in the logBB QSAR models (summarized in Table 2), provides an insight into the molecular properties that determine brain permeation, a brief discussion of which follows.

Hydrogen bonding

All of the models contain a descriptor relating to polarity or hydrogen bonding capacity, for example, PSA [24,25], number of hydrogen-bond donors and acceptors [21] or hydrogen-bond acidity/basicity [8]. In all cases, these descriptors correlate negatively with logBB; in other words, increasing hydrogen-bonding strength or capacity generally leads to reduced brain permeation. This reflects the observation that highly polar/strongly hydrogen-bonding compounds do not permeate membranes easily.

Lipophilicity

Four of the six models contain a descriptor that relates to lipophilicity, for example, ClogP [24] or the Volsurf hydrophobic descriptors [22]. Such descriptors correlate positively with logBB, that is, increasing lipophilicity tends to increase brain permeation. Again, this is in keeping with the fact that lipophilic compounds tend to traverse membranes more easily than do hydrophilic ligands. Interestingly, the Volsurf model [22] suggests that lipophilicity has less of an impact than polarity in determining brain permeation.

Molecular size

Two of the models include a molecular volume term [8,21], and in both cases this has a positive coefficient (i.e. increasing molecular volume seems to be correlated with increasing brain permeation). At first glance, this finding seems to be in conflict with a general understanding of diffusion (classically, according to the Stokes-Einstein relation, the diffusion coefficient of an object is inversely related to its radius). However, the Stokes-Einstein relation only refers to diffusion in a homogeneous solution; it is not clear whether it applies to blood–brain barrier permeation, particularly when this is measured as logBB, which is an equilibrium, or at least, quasi-equilibrium property. Furthermore, it has been noted that because cohesive solvent–solvent (usually water–water) interactions must be

Table 2. Summary of recently published logBB QSAR models

Molecular descriptors	n_{train}	r^2_{train}	s_{train}	n_{test}	Test set predictive performance	Refs
Abraham solute descriptors	148	0.75	0.34	74	r^2_{test} = 0.72, s = 0.38 (mean of 2 test sets)	[8]
Molecular properties derived from Monte Carlo simulations in water	76	0.94	0.17	4	RMS = 0.48	[21]
Volsurf	79	0.76	N/A	N/A	q^2_{train} = 0.65	[22]
Electrotopological state indices	102	0.66	0.45	20	RMS = 0.38, MAE = 0.32	[23]
Computed molecular properties including membrane-interaction descriptors	56	0.85	-	7	MAE = 0.39	[24]
Computed molecular properties from Cerius^2 and ACD/Labs software	48	0.84	0.19	17	r^2_{test} = 0.68, MAE = 0.41	[25]

n_{train} = the number of compounds in the training set
r^2_{train}, s_{train}, and q^2_{train} = the r^2 value, the standard deviation and the cross-validated r^2 value, respectively, for the model derived from the training set
n_{test} = the number of compounds in the test set used to assess the model's predictive power
r^2_{test} = the r^2 value for test set predictions
Abbreviations: RMS, Root Mean Square; MAE, Mean Absolute Error of prediction on the test set compounds.

disrupted to make a solute-accommodating cavity, there is a driving force proportional to molecular volume that pushes hydrophobic solutes into the less polar phase [26].

Molecular charge

Only one of the models considers molecular charge explicitly [25], drawing the following conclusions:

(1) Possession of a positive charge at pH 7–8 tends to favour brain permeation. (This needs to be qualified by the observation of Fischer *et al.*, that molecules with a pK_a value of >10 do not cross the blood–brain barrier by passive diffusion [27]. The parameter pK_a (-log K_a) refers to the propensity of a compound to donate a proton, as measured by its acid ionization constant, K_a. An acidic compound with a pK_a value of <4 will be fully ionized at physiological pH (7.4), as will a basic compound with a pK_a value of >10). Initially, this conclusion appears to be at variance with the pH-partition hypothesis, which assumes that only neutral species partition into membranes. However, it has been shown that positively charged species can enter membranes to a greater degree than suggested by the pH-partition hypothesis [28,29]. Additionally, compounds possessing a tertiary amine moiety (a feature of several CNS drugs) show a higher degree of brain permeation than would be expected from their apparent pK_a value (~8), possibly because their ionized form is destabilized in the membrane, resulting in an effective decrease in pK_a values [25].

(2) The presence of a negative charge at pH ≤5 correlates negatively with logBB. Again, this is in keeping with previous observations that molecules with pK_a values of <4 are poor at penetrating the brain [27]. This is further reflected by the presence of a carboxylic acid indicator variable (with a negative coefficient) in the model reported by Platts *et al.* [8].

Overall, these two conclusions support the fact that acids are generally poorer than bases at permeating membranes [29].

Molecular shape and flexibility

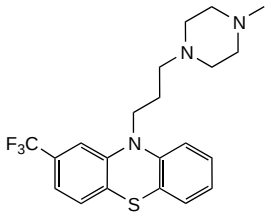
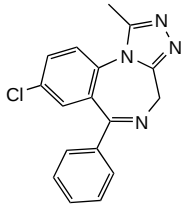
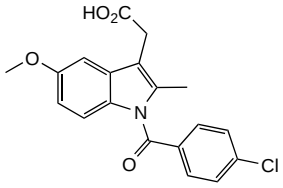
The roles of molecular shape and flexibility in governing brain permeation are less clear from the models considered in Table 2. Two of the models suggest that spherical-shaped molecules are preferable to rod-shaped molecules for brain permeation [22,25]. However, the model based on the electrotopological state indices indicates that increased molecular branching correlates negatively with logBB – an apparent contradiction to this conclusion.

Only one model studied molecular flexibility and concluded that increased solute flexibility correlates positively with logBB [24]. This seems to be at odds with a recent study that investigated the role of molecular flexibility in permeation rate through artificial membranes [30]. The conclusion of this work was that increasing permeation rate was actually paralleled by decreasing rotatable bond count. Possible resolutions of this conflict are discussed in [24].

Examples

To illustrate the aforementioned discussions with some brief examples, Table 3 collates three compounds that span

Table 3. Three illustrative compounds showing effects of various molecular properties on brain permeation.

Compound	Structure	LogBB (from [23])	Key calculated physicochemical property values	
Trifluoroperazine		1.44	MW	407.5
			PSA[a]	15.9
			N + O count	3
			ClogP[b]	5.19
			ACDlogD (pH 7)[c]	4.04
			p*K*a (most basic)[c]	7.82
Alprazolam		0.04	MW	308.8
			PSA	49.5
			N + O count	4
			ClogP	2.21
			ACDlogD (pH 7)	2.50
			p*K*a (most basic)	2.39 +/– 0.40
Indomethacin		–1.26	MW	357.8
			PSA	77.9
			N + O count	5
			ClogP	4.18
			ACDlogD (pH 7)	0.30
			p*K*a (most acidic)	4.17 +/– 0.20

[a]PSA, polar surface area, is calculated by an in-house script
[b]ClogP is calculated using v4.0 of the Daylight software
[c]ACDlogD and the pK_a calculations are those provided in the CAS Scifinder entries for the compounds

the range of brain permeation, together with several computed physicochemical properties.

Trifluoroperazine is a good brain permeator (logBB = 1.44) and an examination of the calculated properties shows that the MW, PSA and logD are within the guidelines as explained earlier. Also, the Norinder 'rule' of ClogP – (N + O) gives a value of 2.19, which predicts a logBB >0. The pK_a value of the most basic nitrogen is 7.82, which is likely to be favourable for brain permeation in the light of the known effect of molecular charge.

Alprazolam appears to partition almost equally between the brain and the blood, as evidenced by a logBB value of almost zero. A look at the computed properties seems to indicate that the balance between hydrogen-bonding and lipophilicity is not ideal for this compound, compared to trifluoroperazine, for example. This is borne out by the value of ClogP – (N + O), which is -1.79.

Finally, indomethacin is a poor brain permeator, with a logBB of < -1. This statistic is largely due to the presence of the carboxylic acid moiety, which causes the compound to be completely ionized at physiological pH, and which is reflected in its low logD value. In addition, the hydrogen-bonding/lipophilicity balance for this compound is also tilted against brain penetration.

Conclusions and future directions

Over recent years, there has been a great deal of work seeking to generate predictive models for brain permeation. The few datasets that are available have been studied using a variety of molecular descriptors and statistical methods. State-of-the-art approaches seem able to achieve >80% correct classifications based on CNS^+/CNS^- data and predictions on small logBB test sets that approach experimental error (0.3–0.4 log units). Helpful insights into the molecular determinants of passive blood–brain barrier permeation have also been gained. In particular, the crucial role of hydrogen-bonding/polarity has been brought to light. This improved understanding can be applied within ongoing drug discovery programmes, if only, perhaps, in a qualitative manner.

Looking to the future, the most fundamental need is for more data, both *in vitro* and *in vivo*, upon which the next generation of predictive models can be built. As highlighted earlier, the largest logBB dataset consists of only ~150 compounds, of which some are not at all 'drug-like'. Clearly, the small size of these training sets will limit the general applicability of any models that are derived from them. Also, for increased effectiveness, brain permeation models will need to account for the role of active transport

and efflux systems, as more data on these become available. Finally, *in silico* modeling will need to embrace the more information-rich data emerging from the increasing use of non-invasive methods of measuring the distribution of compounds within the brain, such as Positron Emission Tomography [31–33].

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