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Analysing molecular polar surface descriptors to predict blood-brain barrier permeation

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Abstract: Molecular polar surface (PS) descriptors are very useful parameters in prediction of drug transport properties. They could be also used to investigate the blood-brain barrier (BBB) permeation rate for various chemical compounds. In this study, a dataset of drugs (n = 19) from various pharmacological groups was studied to estimate their potential properties to permeate across the BBB. Experimental logBB data were available as steady-state distribution values of the *in vivo* rat model for these molecules. Including accurate calculation of the electrostatic potential maps, polar surface descriptors, such as a two-dimensional polar surface area (2D-PSA), topological polar surface area (TPSA) and three-dimensional polar surface area or polar area (3D-PSA; PA) were measured and analysed. We report the strong correlation of these descriptors with logBB values for the prediction of BBB permeation using the linear partial least squares (PLS) fitting technique. The

3D-PSA descriptor showed the best fit to logBB values with $R^2 = 0.92$ and RMSD = 0.29 (p-value < 0.0001). The obtained results demonstrate that all descriptors bear high predictive powers and could provide an efficient strategy to envisage the pharmacokinetic properties of chemical compounds to permeate across the BBB at an early stage of the drug development process.

Keywords: polar surface descriptors; blood-brain barrier; logBB; P-glycoprotein.

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1 Introduction

Permeation of active drugs across the vascular brain endothelium into the central nervous system (CNS) is controlled by the blood-brain barrier (BBB). The BBB separates the bloodstream from the brain. Characteristics of the BBB are the restriction of paracellular substance permeation across the endothelium by intercellular tight junctions, the lack of cellular fenestrae and reduced pinocytosis (van Bree et al., 1992; Hirase et al., 1997). In contrast to several endogenous hydrophilic nutrients (glucose, amino acids, etc...), which are transported by carrier-mediated mechanisms across the brain endothelium, the BBB prevents the entry into the CNS of the majority of polar drugs (Geldenhuys et al., 2010). An important parameter for BBB penetration is the rate at which a molecule passes through a lipid membrane. Highly lipophilic chemical agents can penetrate the BBB easily by passive diffusion, whereas less lipophilic agents cannot (Hansch et al., 1987). Therefore, most of the drug compounds have a mixture of lipophilic-hydrophilic properties that allow them to partition between lipid and aqueous phases and to reach their site of action. Usually, these compounds have low molecular weight to permeate across the BBB (Fu et al., 2008).

Herewith, if a molecule is not sufficiently polar, it will not be able to leave the membrane to return to the aqueous environment. Thus, overall molecular polarity plays an obvious role: if the substance is too polar, the lipid may not be able to drag the molecule from the aqueous solvent. In this case, some molecular quantities, such as polar surface (PS) descriptors are of key interest to medicinal chemists to predict the BBB permeation fate for different drug-like chemical compounds. The descriptors commonly used to account for polarity are the so-called polar surface descriptors like two-dimensional polar surface area (2D-PSA), topological polar surface area (TPSA) and three-dimensional polar surface area or polar area (3D-PSA, PA) (Clark, 1999; Kelder et al., 1999; Ertl et al., 2000; Kim et al., 2011). The last has to be calculated with time-consuming quantum-mechanical methods usually providing better results (Dove, 2008). All of these parameters are interpretable, numerically stable and suitable for good correlation with experimental transport data, such as logBB (Kelder et al., 1999). However, one has to consider the fact that some molecular compounds are substrates for efflux transporters, such as P-glycoprotein (P-gp). Therefore, the access of these molecules to the brain is very restricted (Osterberg and Norinder, 2000; Ooms et al., 2002; Ward et al., 2004; Gunes et al., 2008; Thiel-Demby et al., 2009). Some of the molecular descriptors (2D-PSA, TPSA) were defined to be numerical properties that can be calculated from the connection table representation of a molecule (e.g. elements, formal charges and bonds, but not atomic coordinates). This type of descriptor is not dependent on the 3D conformation of a molecule and is mostly suitable for analysis of huge molecular databases and drug high-throughput screening (Ertl et al., 2000). Therefore, 2D-PSA as well as TPSA descriptors are widely used medicinal chemistry metrics for the optimisation of a drug's ability to penetrate the BBB. The calculations of 3D-PSA, for example, are time-consuming and require highly specialised software to construct and optimise the molecular surface itself.

We report the prediction of the BBB permeation by analysing polar surface descriptors of diverse CNS-active/inactive compounds to find a strong correlation of these descriptors with logBB values using the linear partial least squares (PLS) fitting technique. In the case of effective CNS-acting drugs, the understanding of their permeation mechanism through the BBB is pivotal to filter potential leads and to estimate and diminish the various neurotoxic side-effects.

2 Computational methods

A good *in silico* model is based on good data sets; therefore, all necessary information regarding the chemical structures and experimental logBB values for 19 diverse drugs was taken from the literature (Kelder et al., 1999). All data sets contained the blood/ brain concentration ratios taken as steady-state distribution values of rat model measurements as:

$$\log BB = \log\left(C_{brain}/C_{blood}\right)$$

The logBB ranged from –2.0 to 1.0 (Table 1) were either brain-penetrating (logBB > 0.5), or had moderate permeation (logBB from 0.0 to 0.5), or possessed little ability to cross the blood-brain barrier (logBB > –0.3), or demonstrated very little permeation (logBB < –0.3).

The three-dimensional structure of the corresponding molecules was built and minimised using the Spartan'10 general purpose molecular modelling tool. 2D-PSA (Spartan'10 calculated descriptor) was mainly defined as the area associated with oxygen, nitrogen and hydrogen attached to these polarising atoms (Hehre, 2003).

Table 1 Calculated polar surface descriptors for 19 CNS-active/inactive drugs

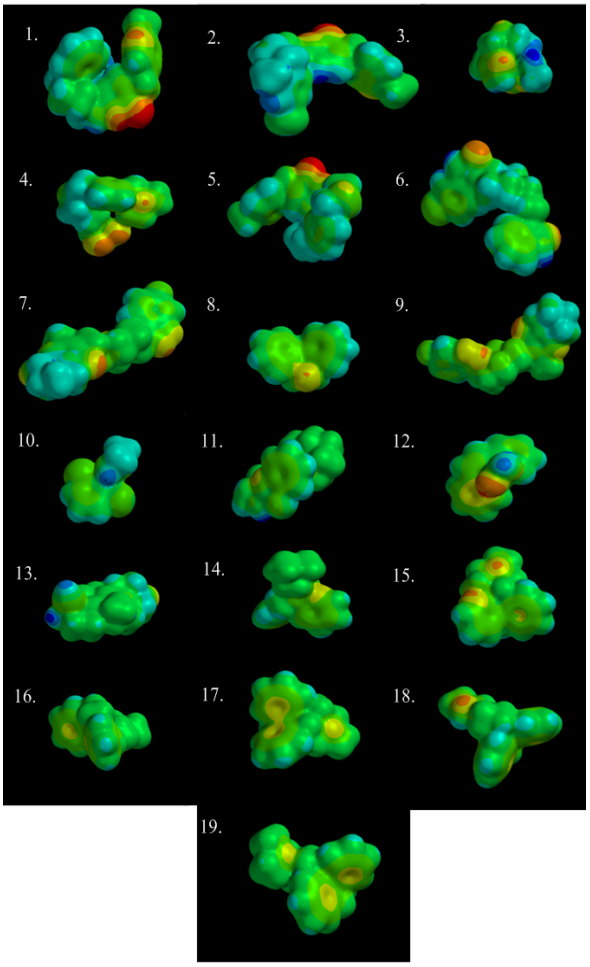
Compound	*MW*	*logBB*	*PSA*	*TPSA*	*PA*	*W2*	*P-gp*
1. icotidine	378.52	–2.00	49.52	75.61	79.53	744.25	+ [11]
2. temelastine	442.40	–1.88	47.79	79.27	91.20	813.25	+ [12]
3. cimetidine	252.39	–1.42	59.14	88.89	65.34	632.63	+ [13]
4. ranitidine	413.60	–1.23	77.95	96.50	71.55	698.38	+ [13]
5. lupitidine	425.96	–1.06	47.55	82.70	81.89	803.50	n.a
6. domperidone	426.54	–0.78	57.98	67.92	62.71	893.88	+ [14]
7. 9-hydroxyrisperidone	426.54	–0.67	63.36	82.17	56.75	1116.13	+ [15]
8. 10,11-epoxycarbamazepine	252.29	–0.33	48.25	58.86	45.19	568.75	– [29]
9. risperidone	410.54	–0.02	44.28	61.94	51.72	883.63	+ [15]
10. carbamazepine	236.29	0.00	36.76	46.33	34.85	479.88	– [30]
11. clonidine	230.11	0.11	29.98	36.42	34.40	360.00	– [7]
12. zolantidine	381.59	0.14	27.77	37.39	42.61	649.13	n.a
13. tribolone	312.49	0.40	33.57	37.30	36.09	689.63	n.a
14. pyrilamine	285.43	0.49	15.11	28.60	17.79	545.25	– [31]
15. mirtazapine	265.39	0.53	10.86	19.37	15.56	443.75	n.a [32]
16. amitriptyline	277.44	0.98	2.54	3.24	11.85	410.00	+ [33]
17. mianserin	264.40	0.99	4.00	6.48	16.62	375.00	n.a [34]
18. desipramine	266.42	1.00	12.90	15.27	13.07	435.63	– [35]
19. imipramine	280.45	1.00	3.26	6.40	15.77	366.00	– [7]

Notes: n.a. = not available, '+' means P-gp substrate, '–' means non-P-gp substrate.

Similar to 2D-PSA, the topological polar surface area descriptor was calculated using group contributions, applying the build-in module in the MOE2009 software, to approximate the polar surface area from atomic connectivity using topological information only. TPSA of a molecule was defined as the surface sum over all polar atoms and is based simply on the summation of polar fragments representing tabulated surface contributions, i.e. bonding patterns of a molecule (Ertl et al., 2000).

The Merck Molecular Force Field (MMFF) molecular mechanics was used to refine and minimise molecular geometry. To accurately analyse the charge distribution of a molecule, a potential energy describing the electrostatic interactions between charged particles was calculated. Density functional theory (DFT), single-point energy calculations were performed with 6-31G* basis set to build electrostatic potential maps for all the molecules (Figure 1). These maps for each molecule were used to reconstruct its three-dimensional polar surface area (3D-PSA) in order to build the electrostatic potential at locations on a particular surface, most commonly a surface of electron density corresponding to overall molecular size (Csizmadia, 1976; Petrucci et al., 2007; Oxtoby et al., 2008). The map was defined as an area for which the absolute value of the electrostatic potential exceeded 100 kJ/mol default parameter.

Figure 2 Electrostatic potential maps (3D-PSA) for 19 CNS-active/inactive diverse chemical compounds. The numbers in the figure correspond to the compound numbers in Table 1. Electrostatic potential maps fit roughly to conventional space-filling models and overall sizes, and shapes were that of the electron densities. The colours indicate the value of the electrostatic potential. Red colours designate areas of negative potential (where a positive charge is most likely to be attracted), while blue colours depicted the areas of positive potential (see online version for colours)



Descriptor of hydrophilic regions, such as hydrophilic volume (W2) was computed from molecular fields using the Volsurf+ molecular modelling software. Hydrophilic W2 descriptor described the molecular envelope, which is accessible to water molecules and attractively interacts with them. The volume of this envelope varies with the level of interaction energies. For W2 descriptor, polarisability and dispersion force parameters were comprised within the range of –0.2 to –1.0 kcal/mol.

Finally, the root mean square difference (RMSD), an indication of the average error in the analysis; the square of the correlation coefficient (R2), an indication of the quality of fit of all the data to a straight line (clear correlation of descriptors versus experimental logBB values) were also calculated and compared for all the descriptors. The experimental logBB were plotted against the calculated molecular surface descriptors; linear partial least squares technique was used to perform a fit to both sets of variables.

3 Results and discussion

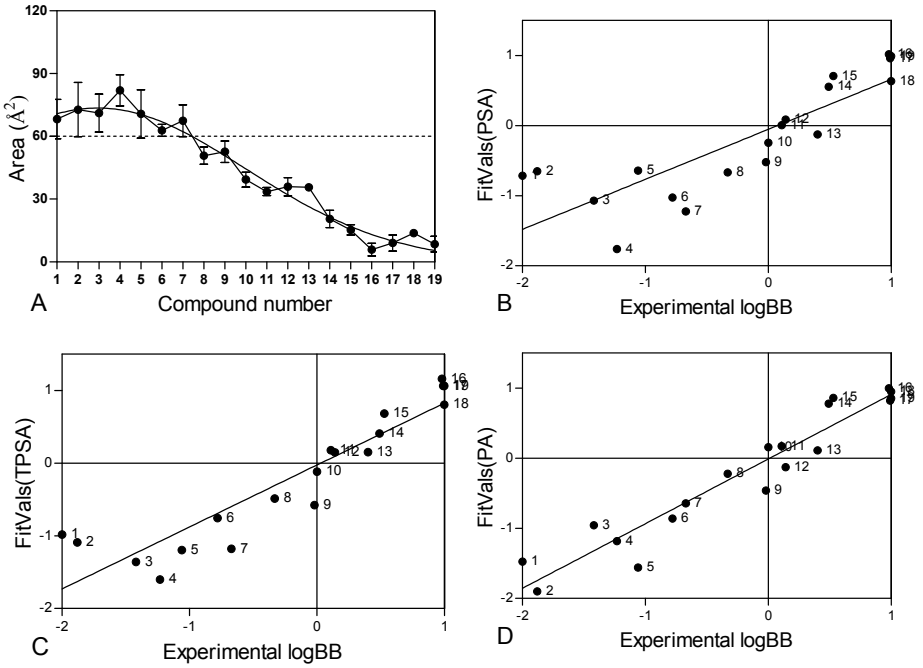
First, we did accurate calculations of the electrostatic potential maps for each molecule. Then, the PS descriptor methodology has been implemented by using published data for logBB properties. Specific descriptors were calculated by different modelling software packages for the molecular dataset ($n = 19$). The results of correlation studies are summarised in Table 1, where calculated polar surface descriptors and molecular weights for drug molecules are given. Considering the role of efflux transporters, such as P-glycoprotein (P-gp) in the drug permeation of the BBB, the information about the P-gp substrate specificity was assigned for each chemical compound. The corresponding electrostatic potential maps are presented in Figure 1.

From Figure 2A it can be concluded that BBB non-penetrants (compounds 1–7) represent higher total area deviations (SD = 3.09) for all calculated descriptors than BBB penetrants (compounds 10–19) with SD = 1.18. The polar surface area parameter was the main factor contributing to increased standard deviation rate. The serious disadvantage of using PSA as well as TPSA is the inability to distinguish the activity differences of chemical compounds differing only in their non-polar groups. Moreover, using the TPSA descriptor, it would be problematic to analyse the influence of positional charges of functional groups (Prasanna and Doerksen, 2009). In our study, we also calculated the computationally more expensive 3D descriptors (PA) to improve this mode. However, overall distribution patterns for PS descriptors remain characteristic. In other words, for molecules to penetrate the blood-brain barrier an area less than 60 $Å^2$ (Figure 2A, threshold depicted as a dashed line) is usually needed; and molecules with a polar surface area of greater than 120–140 $Å^2$ tend to be poor in permeating cell membranes (Palm et al., 1997).

It is well known that multidrug efflux transporter pumps like P-gp could limit a variety of medications from reaching their therapeutic targets thereby preventing or minimising pharmacological effects (Mizuno et al., 2003). By using the P-gp knockout mouse model, Wang and co-authors have demonstrated that some anti-psychotic drugs, such as risperidone are P-gp substrates and their entry into the brain is dramatically limited by P-gp efflux transporter in the BBB (Wang et al., 2004; Doran et al., 2005). However, anti-epileptic compound, 10,11-epoxycarbamazepine, and risperidone, both have in addition, as the calculation shows, a mean area value below the 60 $Å^2$ (50.77 $Å^2$

and 52.65 Å^2) indicating BBB permeability. For the last compound, the difference in logBB distribution from its 9-hydroxy metabolite (9-hydroxyrisperidone) could be explained by the higher area value of the metabolite (67.43 Å^2).

Figure 2 Comparing the overall polar surface descriptors against each other. The numbers in the figure correspond to the compound numbers in Table 1; the descriptors are highly correlated. There are BBB– and BBB+ clusters defined by the area threshold (60 Å) (A). Plots of experimental logBB values versus calculated fit variables (FitVals) of polar surface descriptors are shown (B–D)



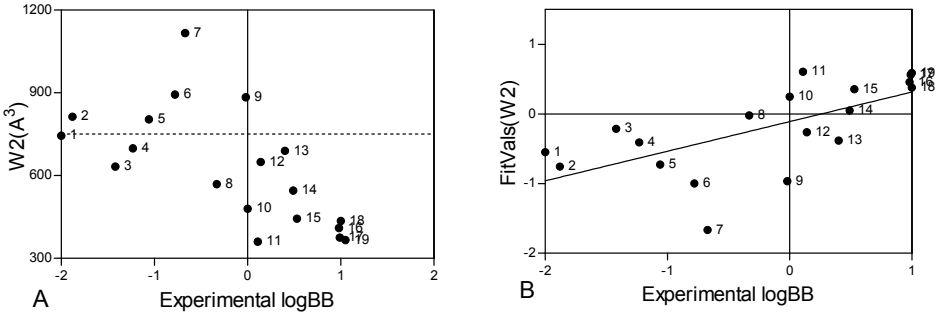
Furthermore, 10,11-epoxycarbamazepine is a major metabolite of carbamazepine, which is known to be a cytochrome P4503A4 (CYP3A4) but not a P-gp substrate (Owen et al., 2001). Initially, carbamazepine was extensively catalysed by CYP3A4 via the oxidative degradation pathway, mainly in the liver (Kerr et al., 1994).

However, in spite of previously shown data, those compounds with logBB around –0.5 might still be considered in vivo experiments to be BBB crossing (Crivori et al., 2000).

The linear partial least squares fitting provided high correlation coefficients of calculated descriptors vs. experimental logBB data, namely: $R^2 = 0.71$, RMSD = 0.54 for PSA; $R^2 = 0.85$, RMSD = 0.39 for TPSA and $R^2 = 0.92$, RMSD = 0.29 for PA descriptor with the p-value < 0.0001 indicating the highly predictive properties of the introduced descriptors for drug permeation across the BBB. The RMSD values are an estimate of the absolute error prediction for a property: the smaller RMSD parameter and the closer correlation coefficient is to a unity – the better the fit (Figure 2B-D). Although the R^2 deviation from calculated descriptor distribution modes indicates the different predictive power of analysed descriptors, the predictive power is also influenced by experimental difficulties, for instance, when the concentration of the drug in the brain is low.

For comparison, we calculated the hydrophilic volume descriptor W2. In general, hydrophilic volume descriptors are known to be highly correlative to logBB values (Cruciani et al., 2000; Molecular Discovery, 2002; Braiuca et al., 2007). The W2-logBB plot (Figure 3A) reports chemical substances as both BBB crossing or non-crossing compounds; and W2 vs. logBB curve fitting provided R^2 = 0.42 because of too large RMSD (0.77) with the *p*-value = 0.0026 (Figure 3B). It is evident that the scatter observed in this plot further supports the conclusion that W2 is less reliable for BBB permeation prediction than PS descriptors.

Figure 3 Relationships between experimental logBB and calculated hydrophilic volume (W2) descriptor (A) and its fit values (B). The correlation clearly shows that W2 increases when the brain penetration decreases. The numbers in the figure correspond to the compound numbers in Table 1



Overall, when W2 is larger than 750 Å^3 (Figure 3A, dashed line), it is unlikely that compounds will penetrate the BBB. Hence, compounds with W2 exceeding this threshold are considered to be non-penetrants. This threshold shows a distinct separation of compounds into BBB penetrants (compounds 10–19) and non-penetrants (compounds 1–9) with three exceptions (two H2-histamine receptor antagonists: cimetidine and ranitidine, and 10,11-epoxycarbamazepine). In the case of H2-histamine receptor antagonists; it was already shown that their permeability is pH-dependent as well as controlled by their P-glycoprotein substrate specificity. Similarly, their brain distribution is mediated primarily by P-gp efflux mechanism (Dudley and Brown, 1996).

For more accurate predictions using PS descriptors, the basic assumption has to be that drugs penetrate across the BBB by passive diffusion. However, it is clear from the previously shown graphs that icotidine, temelastine, ranitidine, domperidone, 9-hydroxyrisperidone and risperidone were depicted as outliers because of their P-gp substrate activity (Osterberg and Norinder, 2000; Ooms et al., 2002; Ward et al., 2004; Gunes et al., 2008; Thiel-Demby et al., 2009). Surprisingly, amitriptyline was reported as P-gp substrate with a high logBB value. In this case, it could be hypothesised that amitriptyline might be transported into the brain by unknown influx transport mechanism, which exhibit higher transport rates than its efflux by P-gp at the BBB.

4 Conclusion

In order to be effective as therapeutic agents, centrally acting drugs must cross the BBB, and entry into the brain is a complex phenomenon, which depends on a multiplicity of factors.

Therefore, it is important to mention that intramolecular H-bond formations in drug molecules could dramatically change the BBB permeation properties of the chemical compounds, which were not included in the calculations. The intramolecular H-bond could reduce polarity considerably and increase the lipophilicity of the chemical substance. This problem will be even more pronounced for structurally flexible molecules with great abilities to generate multiple conformational isoforms. Therefore, the BBB permeability of drugs could be improved in the future by lowering the overall hydrogen-bonding ability of a drug compound via facilitating intramolecular hydrogen bonding. This could be achieved by protecting a molecule with non-polar groups, or by synthesising less polar prodrugs (Young et al., 1988).

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