Development of Quantitative Structure−Property Relationship Models for Early ADME Evaluation in Drug Discovery. 2. Blood-Brain Barrier Penetration

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A new molecular lipoaffinity descriptor was introduced in this paper to account for the effect of molecular hydrophobicity on blood-brain barrier penetration. The descriptor was defined based on Kier and Hall's atom-type electrotopological state indices. Its evaluation requires 2-D molecular bonding information only. A multiple linear regression equation using this descriptor and molecular weight reproduces the experimental logBB values of 55 training set compounds and 11 test set compounds satisfactorily with statistical parameters nearly identical to the best models based on polar surface area and ClogP. The results indicate that the lipoaffinity descriptor defined in this paper may be a significant descriptor for molecular transport properties across lipid bilayers.

INTRODUCTION

The ability of synthesizing large numbers of diverse combinatorial chemistry libraries and the recent advances in automated high throughput screening (HTS) techniques have reduced significantly the time required for identifying a lead candidate with desired potency against a biological target. As a result, the bottleneck of faster drug discovery seems shifting toward optimizing lead candidates so as to resulting in adequate ADME (absorption, distribution, metabolism, and excretion) properties. It was pointed out by Prentis et al.[1] that between 1964 and 1985, 66% of new chemical entities were withdrawn from further development in seven large British pharmaceutical companies. A similar trend was found in the United States. Between 1964 and 1989 only one in six new chemical entities nominated to the investigational new drug status eventually became a marketed drug.[2] It was shown that in addition to unproved efficiency, toxicity, and adverse reactions, inadequate pharmacokinetic properties resulted in nearly 40% of withdrawals of drug leads from further development.[3] Apparently fast evaluation of ADME properties in the early stages of drug discovery will save both time and expense. However due to the complex nature of these properties and the time-consuming experimental procedures involved, these properties are not apt to experimental high throughput screening.

It has been suggested that computational models for reliable prediction of ADME properties are promising as early screening tools for drug candidates and for designing more successful combinatorial libraries. In the preceding article,[4] we summarized that to serve this purpose, a computational model should possess the following desirable features: (1) it should be inexpensive, because to be a high throughput screening tool it is expected to process a large number of compounds in a short period of time; (2) it should not use any experimentally determined parameters because reliable experimental parameters may not be available for many compounds in early stages of drug discovery; and (3) preferably it should not use 3-D molecular descriptors, because reliable 3-D molecular structures of condensed phases are usually not available in early phase of drug development. Models possessing these desirable features may not be as accurate as those not limited by these features. However, if high accuracy is required one can fine-tune predictions with more elaborate models for smaller subsets of candidates that have passed early screening.

A good example that exemplifies the great utility of a predictive computational model in drug discovery is a model for predicting blood-brain barrier (BBB) penetration. The BBB is a complex cellular system consisting of endothelial cells of the brain capillaries. It plays the role of maintaining the homeostasis of the central nervous system (CNS) by separating the brain from the systemic blood circulation. In searching for drugs targeted at CNS diseases, the ideal candidates must be able to penetrate BBB effectively. On the other hand, peripherally acting drugs must have limited ability to cross BBB to avoid adverse CNS side effects. Experimental determination of BBB penetration is, however, very difficult, time-consuming, expensive, and not suitable for screening large compound libraries. A reliable and easily applicable computational model for predicting BBB permeation of drug candidates in the early stages of drug discovery will therefore have a significant impact on drug research and development.

Building predictive models of BBB penetration has been the subject of many studies. The first purely computational approach was perhaps that of Kansy and van de Waterbeemd[5] who developed a QSPR model based on a training set of 20 compounds using polar surface area and molecular volume as descriptors. However, subsequent application of their equation to compounds outside their training set showed that the model was not satisfactory.[6]

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Abraham and co-workers[7,8] developed two successful linear models based on a training set of 57 compounds. The descriptors they used include $\log P_{oct}$ (partition coefficient between octanol and water), excess molar refraction, dipolarity/polarizability, hydrogen bonding acidity, hydrogen bonding basicity, and McGowan molecular volume. A potential problem of their models is that the descriptors are not easy to calculate for structurally diverse drug candidates.

Following these pioneering work, more studies were published[9−15] in the past couple of years using molecular descriptors that are easier to calculate such as molecular volume, surface area, shape, topological indices, $\log P_{oct}$, etc. Most of these studies use some conformation-dependent and/or experimentally determined descriptors and therefore do not conform to the desirable features stated above. The recent studies of Kelder et al.[11] and Clark[14] stand out. Using polar surface area (PSA) as the only descriptor, Kelder et al. arrived at the following simple equation for a training set of 45 compounds:[11]

$$\log BB = -0.0322PSA + 1.33,$$
$$n = 45,\ r^2 = 0.84, F = 229$$

A similar equation was also developed by Clark who based on a training set of 55 compounds derived the following results:[14]

$$\log BB = -0.0156(0.001)PSA + 0.548(0.048),$$
$$n = 55,\ r^2 = 0.71,\ s = 0.41,\ F = 128$$

These equations, while very impressive, cannot distinguish the difference in BBB penetration of hydrocarbon compounds, because by definition PSA is molecular surface area contributed by polar (i.e. nitrogen and oxygen) atoms only. In an effort to account for hydrophobic contributions, Clark introduced $\log P_{oct}$ as an additional descriptor.[14] As the experimental values of logP of many compounds are not readily available, he used values calculated by the ClogP program,[16] a fragment contribution based method, and by the MlogP program,[17] a rule based method of logP estimation. Generally speaking, ClogP is perhaps the most popular and reliable method of logP prediction. However a major problem of ClogP prediction was missing fragment parameters which resulted in a roughly 25% fail rate in Lipinski's experience[18] (Biobyte announced that a new version of the program, ClogP 4.0, has no missing fragments.) MlogP does not suffer from this problem, but it is usually less reliable than ClogP. The difference in the performance of these two logP predictors is manifested in the following models Clark developed:[14]

$$\log BB = -0.148(0.001)PSA + 0.152(0.036)ClogP + 0.139(0.073)$$
$$n = 55,\ r^2 = 0.79,\ s = 0.35,\ F = 95.8$$

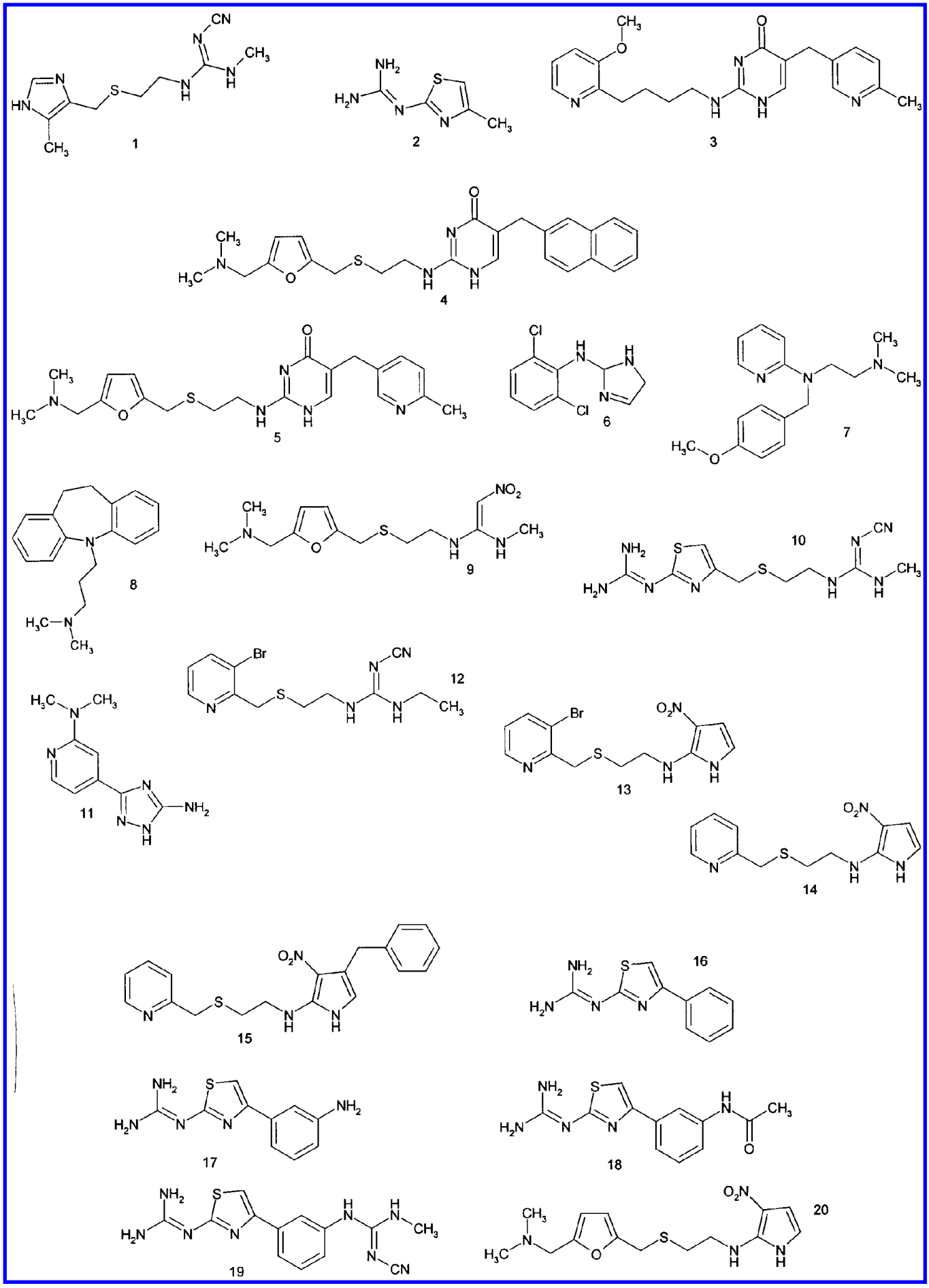
$$\log BB = -0.145(0.001)PSA + 0.172(0.022)MlogP + 0.131(0.033)$$
$$n = 55,\ r^2 = 0.77,\ s = 0.37,\ F = 86.0$$

The statistical parameters of these two models show significant improvement of the two-descriptor models over the one-descriptor model, indicating hydrophobicity is a significant contributing factor to logBB. Its contribution should, therefore, be taken into account in developing predictive models. There is, however, conflicting evidence concerning whether logP is a good hydrophobicity descriptor for BBB permeation.[19−21] For example, Young et al. found[20] no clear

Table 1. Experimental and Predicted logBB of the Training Set Compounds[a]

compd	LA	TPSA	$\log BB_{expt}$	$\log BB_{MLR}$[b]	$\log BB_{ANN}$[c]
1	4.959	88.89	−1.42	−0.89	−0.87
2	2.849	77.30	−0.04	−0.58	−0.65
3	8.275	92.80	−2.00	−1.11	−1.08
4	12.243	74.16	−1.30	−0.44	−0.73
5	9.775	87.05	−1.06	−0.95	−1.02
6	6.339	36.42	0.11	−0.14	0.03
7	9.302	28.60	0.49	0.32	0.46
8	11.022	6.48	0.83	1.00	0.98
9	6.655	86.26	−1.23	−0.97	−0.93
10	5.525	125.15	−0.82	−1.35	−1.25
11	4.027	83.73	−1.17	−0.69	−0.71
12[d]	8.302	73.10	−2.15	−0.68	
13	7.748	86.53	−0.67	−1.05	−1.00
14	5.600	86.53	−0.66	−0.95	−0.90
15	8.730	86.53	−0.12	−0.82	−0.90
16	5.456	77.30	−0.18	−0.33	−0.39
17	4.776	103.32	−1.15	−0.74	−0.85
18	5.322	106.40	−1.57	−1.02	−1.03
19	5.615	137.51	−1.54	−1.35	−1.30
20	6.756	90.02	−1.12	−1.04	−0.99
21	9.878	90.02	−0.73	−0.92	−1.02
22	7.315	90.02	−0.27	−0.86	−0.90
23	8.133	89.77	−0.28	−0.69	−0.82
24	8.221	41.57	−0.46	−0.13	0.00
25	10.389	41.57	−0.24	−0.04	0.00
26	7.247	32.70	−0.02	−0.02	0.17
27	10.349	37.39	0.69	0.25	0.30
28	10.575	37.39	0.44	0.27	0.30
29	12.696	37.39	0.14	0.48	0.38
30	10.714	50.53	0.22	−0.06	−0.13
butanone	1.768	17.07	−0.08	−0.03	−0.10
benzene	3.588	0.00	0.37	0.57	0.55
3-methylpentane	4.590	0.00	1.01	0.84	0.76
3-methylhexane	5.309	0.00	0.90	0.95	0.85
2-propanol	1.417	20.23	−0.15	−0.02	−0.13
2-methylpropanol	2.038	20.23	−0.17	0.05	−0.02
2-methylpentane	4.593	0.00	0.97	0.84	0.76
2,2-dimethylbutane	4.606	0.00	1.04	0.85	0.77
1,1,1-trifluoro-2-chloroethane	4.081	0.00	0.08	0.29	0.40
1,1,1-trichloroethane	5.037	0.00	0.40	0.47	0.58
diethyl ether	2.532	9.23	0.00	0.23	0.21
enflurane	4.899	9.23	0.24	−0.15	0.09
ethanol	0.847	20.23	−0.16	−0.07	−0.21
fluroxene	4.054	9.23	0.13	0.20	0.30
halothane	5.656	0.00	0.35	−0.02	0.28
heptane	5.318	0.00	0.81	0.95	0.85
hexane	4.597	0.00	0.80	0.84	0.77
isoflurane	5.003	9.23	0.42	−0.11	0.12
methane	0.000	0.00	0.04	−0.04	−0.11
methylcyclopentane	4.322	0.00	0.93	0.77	0.71
nitrogen[e]	0.000	47.58	0.03	−0.18	
pentane	3.876	0.00	0.76	0.74	0.67
propanol	1.410	20.23	−0.16	−0.02	−0.13
propanone	1.297	17.07	−0.15	−0.04	−0.14
teflurane	4.822	0.00	0.27	−0.14	0.12
toluene	4.233	0.00	0.37	0.64	0.64
trichloroethene	4.843	0.00	0.34	0.43	0.54

[a] Structures of these compounds were presented in Figure 1. [b] Multilinear regression model, eq 3. [c] Artificial neural network model. [d] Outlier, excluded from the training set. [e] Excluded from the training set due to too few features.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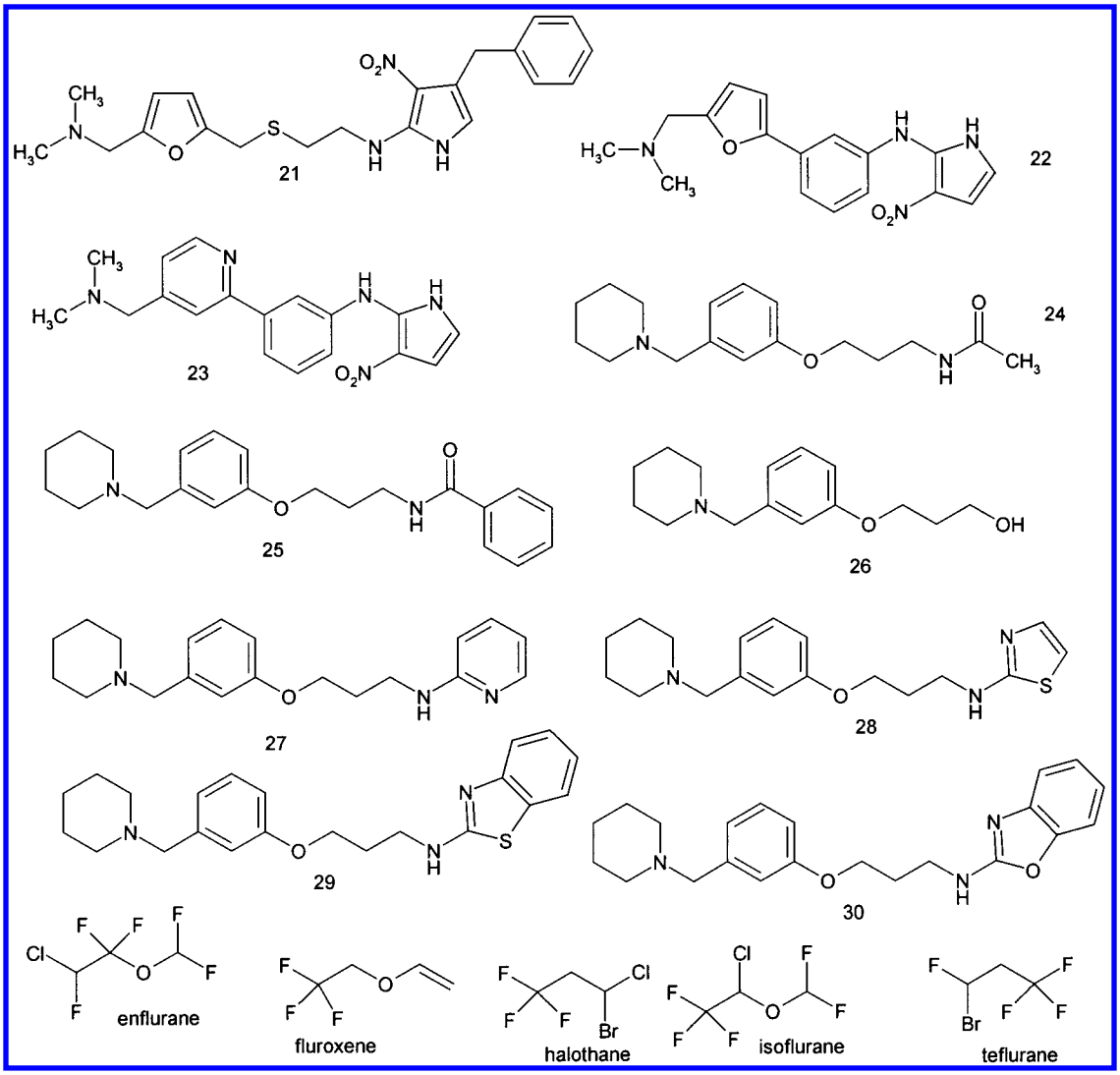

Figure 1. Structures of compounds 1−30 and less familiar compounds from a training set[14] (Clark, D. E. *J. Pharm. Sci.* Copyright © 1999. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.).

correlation between the blood-brain uptake for 20 histamine H_2 receptor antagonists and $\log P_{oct}$. Similar finding was also presented by Seelig et al.[21]

There have been some efforts seeking a better hydrophobicity descriptor for drug transport properties. Based on the consideration that in order to be transported (by passive transcellular route) across the lipid bilayer, a molecule must pass through the outer hydrated polar part of the bilayer as well as through the much more hydrophobic membrane interior, one naturally would think that an ideal parameter should be similar or related to $\log P_{oct}$. Young et al. investigated the performance of $\log P_{cyclohexane-water}$ partition coefficient and found that the difference ($\Delta\log P$) between $\log P_{oct}$ and $\log P_{cyclohexane-water}$ correlates better with the logBB of 20 histamine H_2 receptor antagonists.[20] On the other hand, Buur et al. found that $\log D_{oct}$, not $\Delta\log P$, could be correlated with the permeability of 5-fluorouracil prodrugs in Caco-2 monolayers.[22] Similarly, ter Laak et al. found[23] that the brain permeability of a series of structurally diverse histamine H_1 receptor antagonists was better explained by $\log D_{oct}$ rather than by $\Delta\log P$. At present it seems there is no general consensus as to what is the best hydrophobicity descriptor for drug transport properties.

In this article, we like to present the results of our recent study defining a new hydrophobicity descriptor and developing a simple predictive model of BBB penetration for early screening in drug discovery.

DETAILS OF MODEL DEVELOPMENT

I. Data Sets. The quality of a quantitative structure−property relationship (QSPR) model depends strongly on the size and quality of the training set used. Since many complex physical processes are involved in drug transport (such as passive transcellular, passive paracellular, active carrier-mediated transcellular routes), and different experimental conditions and measurements may be involved, it is not an easy task to compile a diverse and internally consistent data set of experimental BBB permeation. Instead of collecting and critically evaluating available experimental data to build a new training set, we decided to use a popular data set of BBB penetration in this study. The training set of Abraham et al., consisting of 57 compounds,[8] was used in this study. Clark[14] used the same set of compounds but removed two outliers (compound 12 in Table 1 and nitrogen), as his training set. These compounds were also the training compounds of Norinder et al.,[10] Luco,[13] and Lombardo et

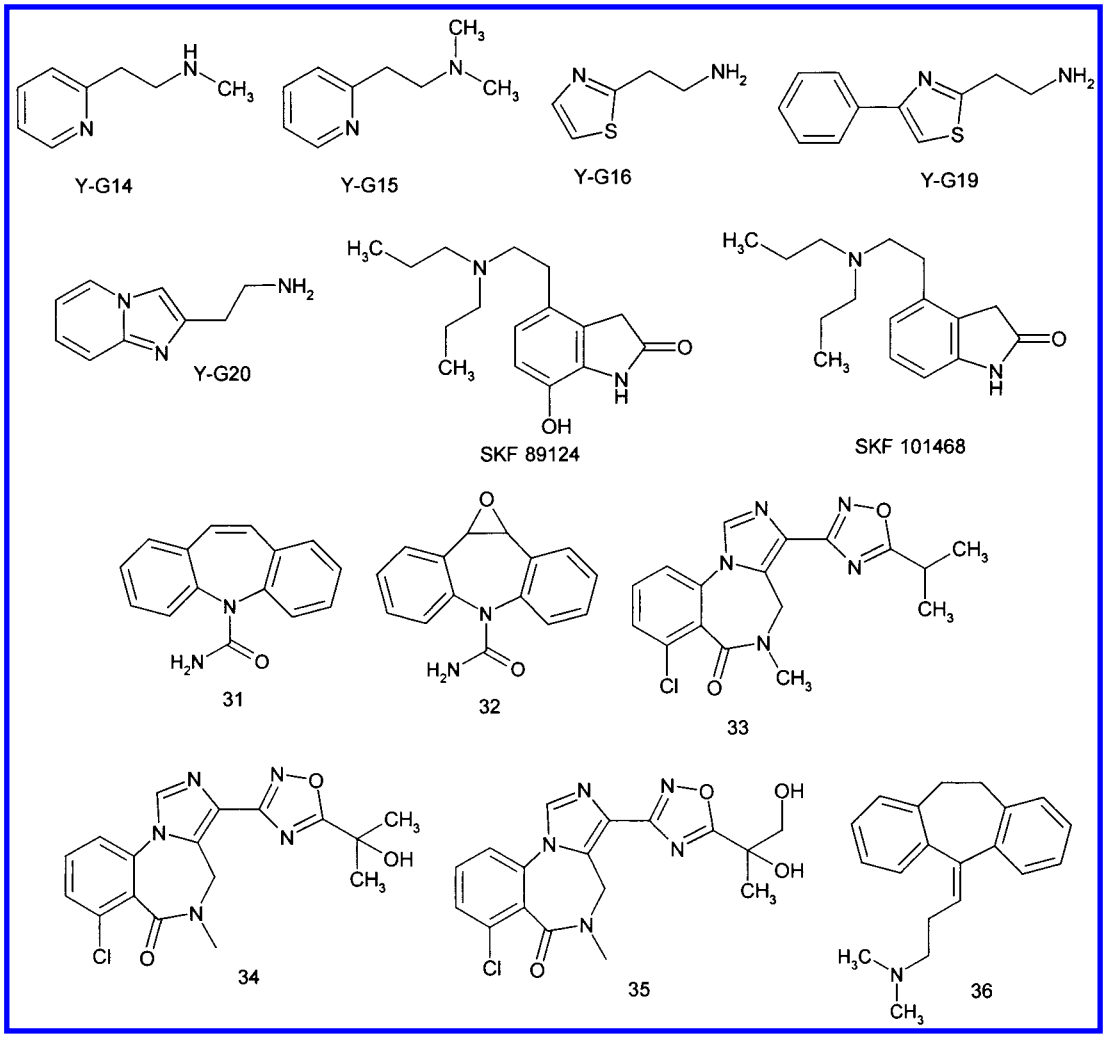

Figure 2. Structures of test set compounds[14] (Clark, D. E. *J. Pharm. Sci.* Copyright © 1999. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.).

al.[9] The structures of the training set compounds are presented in Figure 1. The experimental and predicted logBB data of these compounds are given in Table 1.

To examine the reliability of the QSPR model developed, 13 compounds were selected as our test set. Seven of the compounds were used as a test set by Abraham et al.[8] The other six compounds were the test set of Lombardo et al.[9] The structures of these compounds are presented in Figure 2, and their logBB values are given in Table 2. The BBB permeation of both the training and test set compounds were assumed to be predominately passive transport.

II. Descriptor Selection. 1. Hydrophilicity Descriptor. Due to the physical nature of the lipid bilayer, a successful model of drug transport must include descriptors accounting for both molecular hydrophilicity and hydrophobicity. The most obvious measure of hydrophilicity of an organic compound is its ability to form hydrogen bonds. To form an effective hydrogen bond, a molecule should have highly electronegative atoms (oxygen, nitrogen, etc.) that are exposed on the molecular surface so that they are accessible to other hydrogen bonding atoms. Based on this consideration, a reasonable measure of hydrogen bonding capacity is polar surface area (PSA, molecular surface area contributed by polar atoms, i.e., atoms capable of hydrogen bonding such as nitrogen and oxygen, etc.). Indeed it has been shown recently that PSA is a very significant descriptor for drug transport properties such as human intestinal permeation[24−27] and blood-brain barrier penetration.[5,11,14]

Table 2. Experimental and Predicted logBB of the Test Compounds[a]

compd	LA	TPSA	$\log BB_{expt}$	$\log BB_{MLR}$[b]	$\log BB_{ANN}$[c]
Y-14	4.374	24.92	−0.30	0.20	0.27
Y-G15	5.389	16.13	−0.06	0.41	0.52
Y-G16	3.491	38.91	−0.42	−0.03	−0.02
Y-G19	6.463	38.91	−1.30	0.20	0.30
Y-G20	5.024	43.32	−1.40	0.16	0.21
SKF89124	6.892	52.57	−0.43	−0.46	−0.35
SKF101468	8.046	32.34	0.25	0.14	0.31
31	6.519	48.02	0.00	−0.16	−0.06
32	5.455	58.86	−0.34	−0.71	−0.59
33	7.538	77.05	−0.30	−1.13	−0.99
34	6.522	97.28	−1.34	−1.68	−1.29
35	5.215	117.51	−1.82	−2.34	−1.49
36	10.931	3.24	0.85	1.00	1.00

[a] Structures of these compounds were presented in Figure 2. [b] Multiple linear regression model, eq 3. [c] Artificial neural network model.

By definition, PSA evaluation requires 3-D molecular conformation information. It therefore violates the desirable features stated in the Introduction. Very recently a new protocol to generate PSA based solely on molecular topological (atom connectivity) information was proposed by Ertl

et al.[28] The procedure calculates PSA from 2-D molecular bonding information only. The result was termed topological polar surface area (TPSA). Comparison between PSA and TPSA of a large number of organic compounds indicates that TPSA and PSA are practically identical.[28] However, because only 2-D molecular information is needed in the evaluation of TPSA, it conforms to our desirable features, and its evaluation is 2−3 orders of magnitude faster than the evaluation of PSA. In the present study, we choose to use TPSA as our hydrophilicity descriptor.

2. Hydrophobicity Descriptor. The need of an adequate hydrophobicity descriptor for drug transport properties is clear. Poor correlation between logBB and $\log P_{oct}$ indicates that $\log P_{oct}$, as an overall hydrophobicity measure which bundles hydroaffinity (contributed by polar atoms) and lipoaffinity, is unsatisfactory for this purpose. On the other hand, good correlation between PSA and logBB suggests that separating hydrophilic and lipophilic contributions in $\log P_{oct}$ into separate hydroaffinity descriptor (PSA in this case) and lipoaffinity descriptor may work better. Following the idea of PSA, we examined the correlation between logBB and nonpolar surface area and total surface area, but no apparent correlation was discovered.

Recently a new model of $\log P_{oct}$ prediction was published by Huuskonen et al.[29] On the basis of 36 atom-type electrotopological state (E-state) indices and molecular weight, they developed the following multilinear regression model[29]

$$\log P_{oct} = \sum(a_i S_i) - 0.015\mathrm{MW} - 0.765 \qquad (1)$$

$$n = 1754,\ r^2 = 0.82,\ \mathrm{rms} = 0.62,\ r^2_{loo} = 0.81,\ \mathrm{rms}_{loo} = 0.64$$

where a_i is linear regression coefficient, S_i is E-state index, n is the number of compounds in the training set, r^2_{loo} is the squared correlation coefficient of leave-one-out cross validation, and rms_{loo} is the root-mean-square deviation of the leave-one-out cross validation. We like this model because it uses 2-D topological descriptors only; therefore, it conforms to the desirable features of an early screening tool. It also indicates clearly the polar and nonpolar contributions to $\log P_{oct}$. In the search of a lipoaffinity descriptor that is not bundled with hydroaffinity, we decide to define, according to the above equation, a lipoaffinity descriptor (LA) as

$$\mathrm{LA} = \sum(a_i S_i), \text{ where } i \neq \mathrm{N} \text{ and } \mathrm{O}$$

That is, we define the contribution to $\log P_{oct}$ from atoms other than nitrogen and oxygen as LA. It includes contributions mainly from carbon atoms and atoms of low hydrogen bonding capacity such as Cl and F as well as atoms of high polarizability such as P, S, Br, I, etc. The linear regression coefficients, a_i, can be considered as scaling factors or weights that make the contributions to LA from different atom types compatible (Table 3). Since LA is defined on the basis of topological E-state indices, its evaluation is fast, and no molecular 3-D information is needed.

3. Molecular Weight. It may not be very obvious why molecular weight (MW) was chosen as a descriptor in many QSAR studies. We believe this is mainly due to the fact that MW correlates very well with molecular size (volume and surface area). MW also correlates with polarizability because the higher the MW, the more electrons there are. The importance of including a molecular size descriptor is obvious as the rate of passive paracelluar transport depends strongly on molecular size. However we like to avoid using molecular volume as a descriptor, because its evaluation involves 3-D geometry optimization and therefore violates the desirable features we like to have. Several topological descriptors also correlate strongly with molecular size; however, none of them is as simple and easy to understand as molecular weight. In the present study, we choose MW as a descriptor with the hope that it encodes sufficient molecular volume and polarizability information.

Table 3. Coefficients (a_i) for the Calculation of Lipoaffinity Descriptor

symbol	atom type	coeff[a]	symbol	atom type	coeff[a]
SsCH3	−CH_3	0.432	SaaaC	aaCa	0.334
SdCH2	=CH_2	0.318	SssssC	>C<	0.245
SssCH2	−CH_2−	0.482	SsF	−F	0.137
StCH	≡CH	0.123	SsSH	−SH	0.309
SdsCH	=CH−	0.280	SdS	=S	0.314
SaaCH	aCHa	0.299	SssS	−S−	0.561
SsssCH	>CH−	0.425	SaaS	aSa	0.821
SddC	=C=	0.333	SsCl	−Cl	0.307
StsC	≡C−	0.125	SsBr	−Br	0.755
SdssC	=C<	−0.141	SsI	−I	1.616
SaasC	saCa	0.200			

[a] The values are taken from ref 29.

Regression Methods. To build a statistically sound QSPR model of BBB permeation, we first used the Stepwise Multiple Linear Regression method in the Cerius2 program from MSI.[30] Recognizing the fact that there might be higher-order dependencies of logBB on the descriptors, we also developed a nonlinear model based on artificial neural network. A three-layered, fully connected neural network was trained with a logistic $f(x) = 1/(1 + e^{-x})$ activation function for both the hidden and output nodes. All three descriptors, TPSA, LA, and MW were used as input nodes. Two hidden neurons were used, resulting in a 3:2:1 network architecture.

RESULTS AND DISCUSSIONS

1. Multiple Linear Regression. Our preliminary calculations indicate that compound 12 (Table 1), which was found to be an outlier in several previous studies,[14] is indeed problematic for our QSPR predictions. We therefore excluded it from the training set. We also discarded nitrogen, as Clark did,[14] from the training set because it has too few features. Thus the training set is reduced to 55 compounds.

To test the performance of different descriptors, we first carried out a simple linear regression of the 55 training set compounds using TPSA as the only descriptor. The resulting equation and statistics are

$$\mathrm{logBB} = 0.524(0.081) - 0.0153(0.0014)\mathrm{TPSA} \qquad (2)$$

$$n = 55,\ r^2 = 0.694,\ r^2_{loo} = 0.670,\ s = 0.42,\ F = 120.3$$

Compared to the results of Clark[14] on the same training set

$$\mathrm{logBB} = 0.548 - 0.0156\mathrm{PSA},$$
$$n = 55,\ r^2 = 0.707,\ s = 0.41,\ F = 128.4$$

the slight difference supports the conclusion of Ertl et al. that practically, TPSA and PSA are nearly identical. Since there is virtually no loss of performance, clearly TPSA is an attractive and inexpensive alternative to PSA.

Next, we applied the stepwise multiple linear regression to the training set providing TPSA, LA, and MW as the descriptors with the hope of building a model that takes into account of hydroaffinity, lipoaffinity, and molecular size effects. However, the calculation failed to generate a statistically sound linear equation using all three descriptors. Instead the following linear model was generated:

$$\log BB = 0.138(0.104) - 0.0112(0.0008)MW + 0.364(0.033)LA \quad (3)$$

$$n = 55,\ r^2 = 0.790,\ r^2_{loo} = 0.763,\ s = 0.35,\ F = 97.7$$

Compared to Clark's best model:[14]

$$\log BB = 0.139 - 0.0148PSA + 0.152ClogP,$$
$$n = 55,\ r^2 = 0.787,\ s = 0.35,\ F = 95.8$$

The statistical parameters are nearly identical. However the calculation of MW is much easier and faster than the calculation of PSA, and the calculation of LA is simpler than the calculation of ClogP, as it is derived from topological indices only therefore does not have missing parameter problems. Huuskonen's equation based on which LA is defined is published nonproprietary work, the use of which does not involve license expense.

For comparison, we also attempted forcing TPSA, MW, and LA into one linear equation by a multiple linear regression, resulting in the following equation:

$$\log BB = 0.181(0.109) - 0.00876(0.00205)MW + 0.296(0.061)LA - 0.00412(0.00315)TPSA \quad (4)$$

$$n = 55,\ r^2 = 0.797,\ r^2_{loo} = 0.760,\ s = 0.35,\ F = 66.6$$

Compared to eq 3, eq 4 has insignificant improvements in r^2 and s values but also slight deterioration in r^2_{loo} and significant drop in F statistics. Due to the decrease in F value and based on parsimony principle, we believe eq 4 is less attractive than eq 3. However, it is enlightening to inspect the contribution of each descriptor in eq 4. Since MW correlates with molecular size, the negative sign of its coefficient indicates indeed that larger molecules have lower permeation rates. The positive coefficient of LA indicates that lipoaffinity enhances permeation rate, and the negative coefficient of TPSA is in agreement with the intuition that hydrophilicity reduces permeation rate.

To examine whether the hypothesis that bundling hydroaffinity and lipoaffinity into a single $\log P_{oct}$ parameter is inferior to separating them, we made multiple linear regression using MW and $H\log P_{oct}$, the 1-octanol/water partition coefficient calculated by eq 1 (Huuskonen's simple $\log P_{oct}$ model[29]), as descriptors. The results are presented below:

$$\log BB = -0.0454(0.1261) - 0.00673(0.00056)MW + 0.470(0.050)H\log P_{oct} \quad (5)$$

$$n = 55,\ r^2 = 0.741,\ r^2_{loo} = 0.710,\ s = 0.39,\ F = 74.3$$

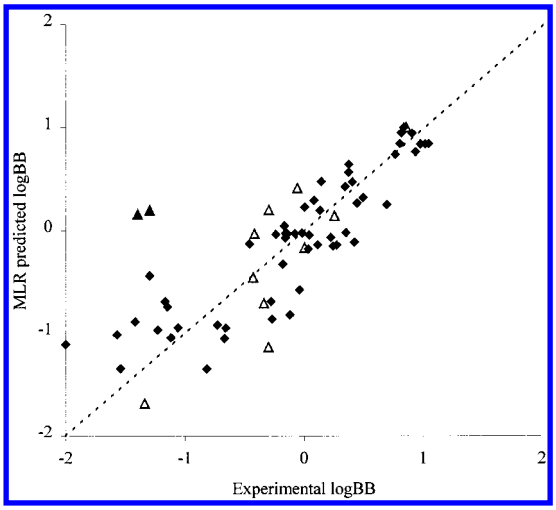

Figure 3. Experimental and MLR predicted logBB of the training set compounds (filled diamond) and test set compounds (unfilled and filled triangles). The two filled triangles are results of compounds Y-G19 and Y-G20 which are apparently outliers. The dotted line represents a perfect correlation between experimental and predicted logBB values.

Comparing the performance of eqs 5 and 3, it is clear that $H\log P_{oct}$ is indeed inferior to LA as a descriptor of BBB permeation.

Finally, we explored the possibility that MW may enhance the performance of eq 2 by a multiple linear regression using TPSA and MW as descriptors. The results are given below:

$$\log BB = 0.423(0.116) - 0.0171(0.0020)TPSA + 0.000822(0.000679)MW \quad (6)$$

$$n = 55,\ r^2 = 0.703,\ r^2_{loo} = 0.663,\ s = 0.42,\ F = 61.4$$

Comparing r^2_{loo}, s, and F statistics of eqs 2 and 6, clearly eq 6 is inferior to eq 2.

Judging from the statistical parameters of the linear eqs 2−6, the best linear model of BBB permeation is clearly eq 3. To ensure the results of eq 3 are not due to chance correlation, we carried out a randomization test. In the test, we randomly assigned the 55 experimental logBB data to the compounds followed by multilinear regression using MW and LA as descriptors. If significant correlation exists between the descriptors and the randomized logBB, the significance of eq 3 diminishes. We performed 50 such randomization runs. The mean values and standard deviations of correlation coefficients of training (R_t) and leave-one-out cross-validation (R_{cv}) values of the 50 randomization runs are 0.17 ± 0.10 and -0.23 ± 0.29, respectively. These values are much lower than the corresponding R_t (0.89) and R_{cv} (0.87) values for the linear regression on the original data set. The significant difference between the results using real and randomized data indicates that the likelihood of chance correlation is very low in this model. The experimental logBB and the predicted values based on eq 3 for the training and test set compounds are presented in Tables 1 and 2, respectively. Also presented in these tables are the descriptor values of TPSA and LA. The experimental and calculated results are also presented graphically in Figure 3. For the 55 training set compounds, the squared correlation coefficient and standard deviation between the predicted and experimental logBB are 0.790 and 0.35, respectively. However for

the 13 test set compounds, the corresponding values are 0.419 and 0.60. These are much worse statistics. Inspecting the results in Table 2, we find that for most of the 13 test set compounds, the deviations between the predicted and experimental logBB values are smaller or close to 0.35, but two compounds, Y-G19 and Y-G20, show significantly higher deviations (1.50 and 1.56, respectively). As shown in Figure 3, these two compounds are clearly outliers of our regression model. Unusual high deviations for these two compounds are also found in all previous studies, such as the studies of Abraham et al. (deviations of 1.16 and 0.83, respectively),[8] Luco (1.26 and 1.11, respectively),[13] and Clark (1.06 and 0.87, respectively).[14] They were also identified as outliers by the QikProp program of Schrödinger Inc.[31] Excluding the two compounds, the squared correlation coefficient and standard deviation between the predicted and experimental logBB are 0.838 and 0.30, respectively, in line with the corresponding parameters of the training set compounds.

Since the descriptors in eq 3 are MW and LA, it seems that the equation accounts for mainly the effect of lipoaffinity on BBB permeation. We feel that this is not completely correct. As the atom-type E-state indices are defined considering atoms in a molecular field. It actually takes into account of the effects of neighboring atoms on the atom under consideration. Therefore unlike PSA or TPSA which are contributed by polar atoms only, LA has contributions from both polar and nonpolar atoms. On the other hand, since eq 2 uses TPSA as the only descriptor, it truly accounts for the effect of hydroaffinity only on BBB permeation. In practice, we feel it is preferable to have a single model that takes into account of all these effects. However the restriction imposed by a linear equation and the limited experimental data prevented us from developing a statistically sound equation that takes into account of all the effects explicitly. We believe that when reliable experimental data of more diverse compounds become available, more predictive model may be developed. For current practical applications, an alternative to achieve more reliable prediction may be to make predictions with both eqs 1 and 3, the average of the two predictions may be more reliable.

2. Artificial Neural Network Analysis. In an effort to explore nonlinear dependencies of logBB on the descriptors and try to bring all three descriptors (TPSA, LA, and MW) into one model, we performed artificial neural network analysis on the training and test set compounds. With a 3:2:1 architecture, the network was trained with the 55 training set compounds, and predictions were made for the 13 test set compounds. The results are also presented in Tables 1 and 2 and graphically in Figure 4. For the training set compounds, the squared correlation coefficient and standard deviation between the neural network predicted and experimental logBB values are 0.808 and 0.30, respectively. They represent a slight improvement over the two-descriptor linear eq 3. Compounds Y-G19 and Y-G20 are again predicted to be outliers as shown in Figure 4. Excluding these two compounds, the squared correlation coefficient and standard deviation between the neural network predicted and experimental logBB values of the 11 test compounds are 0.806 and 0.35, respectively. To ensure again that the results of the neural network analysis are not due to chance correlation, we performed a randomization test of 50 randomized neural

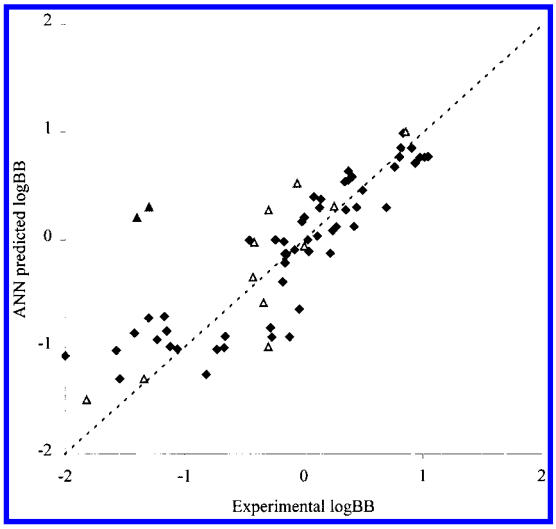

Figure 4. Experimental and ANN predicted logBB of the training set compounds (filled diamond) and test set compounds (unfilled and filled triangles). The two filled triangles are results of compounds Y-G19 and Y-G20 which are apparently outliers. The dotted line represents a perfect correlation between experimental and predicted logBB values.

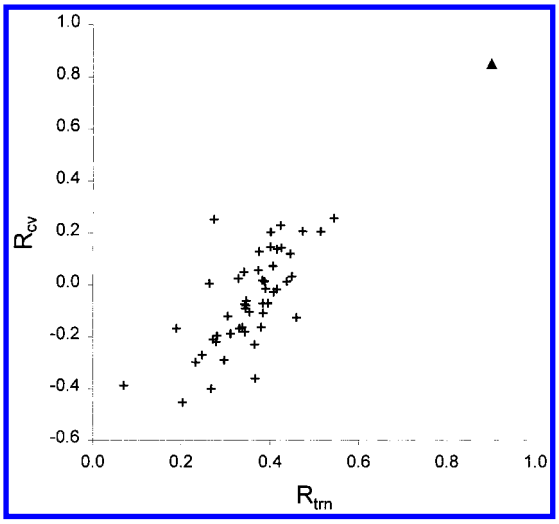

Figure 5. Scatter plot of R_{cv} versus R_t for the true (triangle ▲) and randomized (cross +) neural network training.

network training. Results of the randomization test are presented in Figure 5. The R_t and R_{cv} values of the true neural network training are 0.90 and 0.85, respectively. The mean values of R_t and R_{cv} of the 50 randomization runs are 0.35 and −0.06, respectively. Their corresponding standard deviations from the mean values are 0.08 and 0.18, respectively. The results indicate that the neural network model is also due to genuine instead of chance correlation. In most cases, neural network models outperform linear regression models in fitting training set data. However in the current case, the performance of the linear regression and neural network are nearly the same. Considering that there are 11 variables controlled by the network, the result seems unusual. To understand this behavior, a neural network monitoring scheme was used. We first trained the neural network the normal way. After the training, variation of calculated logBB on changing values (from minimum to maximum) of one descriptor was observed while keeping the other descriptors at the mid values of their respective ranges. This procedure was repeated for all three descriptors. The results, logBB

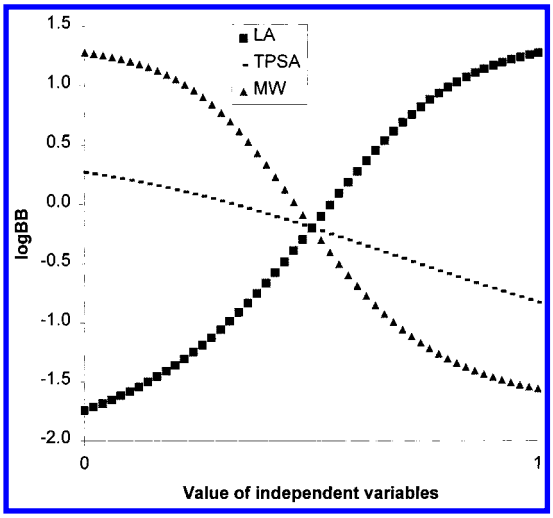

Figure 6. logBB as a function of individual descriptors in the neural network model. The value of each descriptor runs from minimum (represented by 0 in the *x*-axis) to maximum (represented by 1 in the *x*-axis) while keeping the other descriptors at their respective mid values.

versus descriptor values, are presented in Figure 6. The figure shows that in a fairly wide range around the mid descriptor values, the descriptors are in reasonably good linear correlation with logBB. This may explain the nearly identical performance of the linear regression and neural network models.

SUMMARY

In this study, we introduced a new molecular lipoaffinity descriptor, based on atom-type electrotopological state indices, to account for the effect of molecular hydrophobicity on blood-brain barrier penetration. The descriptor was defined according to Huuskonen's multiple linear regression equation of $\log P_{oct/water}$ but excluding contributions from strong hydrogen bonding atoms (nitrogen and oxygen). A statistically sound multiple linear regression equation based on the lipoaffinity descriptor and molecular weight was developed which reproduced the experimental logBB data of 55 training set compounds with $r^2 = 0.79$, $r^2_{loo} = 0.76$, $s = 0.35$, and $F = 97.8$. The simple linear model predicts the logBB values of 11 structurally diverse test set compounds satisfactorily with $r^2 = 0.84$ and $s = 0.30$. These statistical parameters are nearly identical to those of Clark's best model based on polar surface area and ClogP. However, evaluation of polar surface area (involving 3-D conformation search and geometry optimization) and ClogP (licensing required) is less convenient and more time-consuming than evaluation of MW and the lipoaffinity descriptor.

Compared to several recent QSPR studies of BBB permeation on the similar data set, the merit of the current study is that only a few carefully chosen physically meaningful descriptors were employed in the current study. The predictivity of our simple model is at least as good as the best models reported in the literature.

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