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A recursive-partitioning model for blood–brain barrier permeation

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Summary

A series of bagged recursive partitioning models for log(BB) is presented. Using a LGO-CV, three sets of physical property descriptors are evaluated and found to have Q^2 values of 0.51 (CPSA), 0.53 (Ro5x) and 0.53 (MOE). Extrapolating these models to Pfizer chemical space is difficult due to P-glycoprotein (P-gp) mediated efflux. Low correlation coefficients for this test set are improved ($R^2 = 0.39$) when compounds known to be P-gp substrates or statistical extrapolations are removed. The use of simple linear models for specific chemical series is also found to improve the correlation over a limited chemical space.

Introduction

The distribution of therapeutic compounds between brain and blood is an important component in the design of CNS-active drugs. The experimental determination of the brain–blood partition ratio is difficult and expensive since it involves the direct measurement of the drug concentration in the brain and blood of laboratory animals. For this reason, it is desirable to predict the brain–blood distribution ratio of complex molecules from physicochemical parameters and parameters derived from molecular structures [1]. A variety of approaches have been examined which generally rely on few or no experimentally determined parameters. Several of these are reviewed here [1–10], and a number of recent reviews are also available [11–14].

Young et al. [1] determined the correlation between log (C_{brain}/C_{blood}) and ΔlogP (octanol-cyclohexane) ($r = 0.83$, $s = 0.44$) for a series of 20 structurally diverse histamine H_2 antagonists. Van der Waterbeemd and Kansy [9] modified this correlation by substituting the computed term hydrophobic fraction of the van der Waals surface area ($r = 0.835$), thus eliminating the need to include any experimentally determined partition coefficients. Abraham et al. [2, 10] considered a larger data set of 57 compounds permitting for the construction of what may have been the first general model for brain–blood partitioning based only upon empirically determined fragment descriptors. In support of this point, they demonstrated the similarity of the coefficients between two ''bunches/clusters'' of compounds while iterating that a ''satisfactory general regression'' was only possible upon combination of the two sets.

Subsequent to Abraham's important work, a large number of papers have been published that explore various aspects of molecular representation (descriptors) and statistical modeling methods. Most studies have built upon Abraham's initial study and in many cases used essentially the same set of data to build QSAR models. Lombardo et al. [3] used computed free energies of solvation ($r = 0.82$, $s = 0.41$). In a different study, MolSurf descriptors resulted in an internally cross-validated model, $q^2 = 0.782$. These authors concluded that hydrogen bonding descriptors as well

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as descriptors relating to the polarizability of electrons (i.e. groups containing conjugated and aromatic structures as well as large halogens) are important [15]. These same authors then developed a simpler system for the calculation of log(BB) using simple "rule-of-five" type descriptors available via ACD/Labs commercial software [16]. Topological descriptors of the sort developed by Kier and Hall have also been used to construct a PLS-QSAR model [17, 18]. The latter study used an expanded data set of 106 compounds collected from a variety of other log(BB)-related QSAR studies.

Despite the diversity of calculable descriptors used, there seems to be a general consensus that the polar surface area of the molecule appears to be the most important descriptor for the prediction of passive diffusion into the brain [5, 6]. The first article to make this claim established a predictive and simple QSAR relation between the dynamic polar surface area, obtained from time-averaged molecular dynamics simulations and brain penetration [6]. Here, brain penetration was found to decrease with increasing polar surface area. Analysis of 776 CNS and 1590 non-CNS drugs showed an apparent cutoff at approximately 120 $\AA^2$, above which drugs stand little chance of passively diffusing into the brain. Subsequent studies have shown that further improvement may be obtained by adding a log P term to polar surface area QSAR equations [5]. Using the C log P octanol–water partition calculation yields a simple equation with log(BB) ($r = 0.876$).

Finally, Platts et al.[4] revisited the problem and included plasma-brain data from Salminen. The new model reported therein demonstrates comparable accuracy to that seen on smaller data sets ($r^2 = 0.745$, $s = 0.343$) even during a leave-20%-out cross validation procedure ($r^2 = 0.733$, $s = 0.356$). In addition, they noted that a specific "indicator" descriptor to flag carboxylic acid functionalities appears to be very important, while a similar descriptor to flag basic moieties is not required.

Two research groups have approached the problem by asking a more general question: can CNS drugs be identified prospectively based on current data sets of CNS active drugs? One group trained a neural network to classify CNS actives and inactives using simple chemical descriptors such as molecular weight, number of donors/acceptors as well as 166 2-dimensional fragment-based descriptors [19]. Here the MDDR database, which includes 15,000 CNS active drugs and 50,000 CNS inactives, was used as a training set for the neural network. The neural network was able to identify 92% of a validation set of 275 compounds with known CNS activity.

In related work, the VolSurf program was presented as means of classifying drugs as being CNS active and inactive [20]. The VolSurf program takes 3-dimensional molecular structures and calculates 1-dimensional descriptors derived from the 3-dimensional GRID interaction fields. In addition, some simple "rule-of-five" like descriptors are also included. The program then performs a principal-components analysis (PCA) and a partial least squares model (PLS). For the training set compounds, the first principal component is shown to separate CNS actives and inactives, although a high false-positive rate was found when applied to an external validation set. This lack of accuracy is rationalized as being due to the multiple mechanisms that may stop a drug from entering the brain, such as metabolism and efflux processes.

Other methods, ranging from those using molecular dynamics derived descriptors [7] to support vector machine algorithms [8] have been applied, all with reported success. In those, as well as in almost all of the studies summarized here, a single general theme may be seen: CNS drugs must have a fair degree of hydrophobicity in order cross the blood–brain barrier by passive diffusion. This attribute is reflected in a number of the models primary descriptors: polar surface area, free energy of solvation, as well as various hydrogen-bonding descriptors. These descriptors are presumably well correlated with each other and reflect the general differences between the environment of the central nervous system and that of the circulatory system. Interestingly only Crivori et al. [20] reported any difficulty in model validation that could possibly be caused by relatively difficult to model efflux and/or metabolic mechanisms. Only recently has the transport out of the brain by P-gp considered [21] explicitly as part of the modeling process.

The work we report here builds upon the successes achieved by these earlier works and attempts to further examine the severity of efflux-related mechanisms. We employ a bagged recursive partitioning statistical model to examine the relationship between several different sets of physical–chemical properties and brain–blood dis-

tribution for 190 different compounds. The data set used in this work is very comprehensive, as we have added many data points from the clinical literature in addition to internally measured brain–blood data. This expanded set allows us to examine the issue initially raised by Abraham [2] regarding the effects of bunching and the ability to develop ''general'' models from physical property descriptors. The relative absence of serious discussion regarding efflux issues undoubtedly reflects the inherent difficulties involved in realistic modeling of what is a multitude of very specific mechanisms. Prior to presenting our modeling results, we therefore first give some data regarding the efflux liability of internal Pfizer compounds and some discussion as to how we believe this may affect subsequent modeling attempts. We also briefly examine the concerns related to the use of whole-brain homogenates/plasma vs. the seemingly preferred CSF (cerebrospinal fluid) exposure vs. the use of unbound fraction (in brain and plasma), and their potentially negative impact on drug discovery. We then present our statistical modeling results and discuss the ability of our ''general'' model to extrapolate to our Pfizer data sets. Of particular interest is the role that different descriptor sets play with respect to making model ''general'' or not. We conjecture that simple descriptor sets are more likely to provide a ''general'' model, although overall accuracy remains relatively poor as strong variations in standard error still exist depending upon the chemical scaffold. When available, a preferable alternative would be to construct models based upon series-specific chemical data.

The organization of the remainder of the paper is as follows. In part A of the Methods section, we discuss the data, where they came from and some comment on the quality of various sources. We feel that this section is of particular importance not only to the interpretation of the results we reach in this paper, but also as a guide for subsequent studies that may choose to use this set of data as a basis for statistical modeling. Our modeling methodology and details of descriptor calculation are given in parts B and C of the Methods section. The Results and Discussion is divided into four parts. Part A contains a presentation of brain–blood ratios for whole-brain homogenate measurements provided by various Pharmacodynamic and Drug Metabolism groups at Pfizer Groton Laboratories. Of particular interest is the graphical comparison between data taken from wild-type mice to that from the P-gp knock-out mice, which will serve as the basis of our discussion of efflux issues. Part B then turns to a similar but brief discussion of CSF issues. The main results of this study are contained in part C, where we describe the results of our statistical modeling and provide details concerning how differences in descriptor set can impact the ability of a given model to predict new chemical entities and thus guide discovery efforts. A summary of the main results of this work along with ideas for future directions is provided in the conclusions section. There is also a supplemental information section included that provides a SMILES representation of each molecule in the training set as well as the brain–blood ratios and relevant annotations.

Methods

A. Data collection

The data used in this work can be divided into three broad classes. In-house wild-type and knock-out (mdr 1a -/-) mouse data, in-house rat data and literature data, largely from rats. The latter data was obtained via literature screening and analysis, to try to discern possible experimental problems, affinity of a compound for influx or efflux transporters and lack of data reproducibility especially when two or more sources were available for comparison. This set is indexed by CAS number in Table 1, which also includes the logBB values, original references and a designation of drug class. We have generally kept the ''threshold'' of variability at 0.3–0.5 logBB units as a criterion for acceptance of two data points from different sources and for their averaging. In many cases, it was reassuring to see a much higher convergence between data from different authors. No allowance was made, however, for differences between B/P (brain–plasma) and B/B (brain–blood). In very few cases, for example in the case of acebutolol and alprenolol, post-mortem human data were deemed reliable, and care was exercised in trying to establish whether redistribution of a compound among tissues had occurred. In almost all cases we have used original references as the source of data and

Table 1. List of drugs and their log(brain/plasma) values.

#	CAS #	NAME	Class	logBB	Ref.[a]
1	101-40-6	Propylhexedrine	Adrenergic	1.08	[44]
2	23830-88-8	TZ-10	Adrenergic	0.16	[45]
3	59465-39-3	TZ-11	Adrenergic	0.38	[45]
4	4201-26-7	TZ-12	Adrenergic	−0.87	[45]
5	4201-22-3	TZ-13	Adrenergic	−0.65	[45]
6	4794-83-6	TZ-14	Adrenergic	−1.3	[45]
7	138474-57-4	TZ-15	Adrenergic	−1.89	[45]
8	36318-56-6	TZ-17	Adrenergic	−1.39	[45]
9	21571-08-4	TZ-18	Adrenergic	0.47	[45]
10	38941-33-2	TZ-19	Adrenergic	0.58	[45]
11	4205-93-0	TZ-2	Adrenergic	0.33	[45]
12	40065-09-6	TZ-21	Adrenergic	0.41	[45]
13	65936-23-4	TZ-28	Adrenergic	−0.28	[45]
14	59465-54-2	TZ-3	Adrenergic	−0.2	[45]
15	4205-90-7	Y-G6 (Clonidine)	Adrenergic	0.19	[1, 45]
16	50679-08-8	Terfenadine	Allergy	0.64	InH
17	153439-40-8	Fexofenadine (Allegra)	Allergy	−0.98	[46]
18	103-90-2	Acetaminophen	Analgesic	−0.74	[47]
19	50-78-2	Acetylsalicylic acid	Analgesic	−1.30	[48-49]
20	60-80-0	Antipyrine	Analgesic	−0.07	[50]
21	76-57-3	Codeine	Analgesic	0.54	[51]
22	156154-71-1	DM44 (ENAMINONE)	Analgesic	−1.00	[52]
23	156154-42-6	DM5 (ENAMINONE)	Analgesic	−0.96	[52]
24	156164-76-6	DM49 (ENAMINONE)	Analgesic	−0.17	[52]
25	69-72-7	Salicylic acid	Analgesic	−1.1	[53]
26	15687-27-1	Ibuprofen	Analgesic	−0.18	[54]
27	56-54-2	Quinidine	Antiarrhythmic	0.33	[55]
28	99-66-1	Valproic Acid	Anticonvulsant	−0.84	[56]
29	54910-89-3	Fluoxetine	Antidepressant	1.08	InH, [57, 58]
30	54739-18-3	Fluvoxamine	Antidepressant	0.79	[57]
31	79559-97-0	Sertraline	Antidepressant	1.6	InH
32	53179-11-6	Loperamide	Antidiareheal	0.77	InH
33	91161-71-6	Terbinafine	Antifungal	0.08	[59]
34	73590-58-6	Omeprazole	Antiulcerative	−0.82	[60]
35	132235-73-5	2',3'-Dideoxy-3'-hydroxymethylcytidine	Antiviral	−0.79	[61, 62]
36	7481-89-2	2',3'-Dideoxycytidine (Zalcitabine)	Antiviral	−1.5	[61]
37	161814-49-9	Amprenavir (KVX 478)	Antiviral	−0.56	[63]
38	69655-05-6	Didanosine	Antiviral	−1.28	[64]
39	150378-17-9	Indinavir	Antiviral	−0.72	[63, 65]
40	159989-64-7	Nelfinavir (AG−1341)	Antiviral	−0.93	[63]
41	127779-20-8	Saquinavir (Ro 31-8959)	Antiviral	−0.86	[63]
42	30516-87-1	Zidovudine	Antiviral	−0.72	[65]
43	129618-40-2	Nevirapine	Antiviral	0	[65]
44	56-29-1	Hexobarbital	Barbiturate	−0.31	[66]
45	151-83-7	Methohexital	Barbiturate	−0.06	[67]
46	76-73-3	Secobarbital	Barbiturate	0.20	InH
47	76-75-5	Thiopental	Barbiturate	−0.45	[68]
48	59468-90-5	1-Hydroxymidazolam	Benzodiazepine	−0.07	[69]
49	59468-85-8	4- Hydroxymidazolam	Benzodiazepine	−0.03	[69]

Table 1. Continued.

#	CAS #	NAME	Class	logBB	Ref.[a]
50	28981-97-7	Alprazolam	Benzodiazepine	−0.04	[69]
51	84379-13-5	Bretazenil	Benzodiazepine	−0.09	[70]
52	22316-47-8	Clobazam	Benzodiazepine	0.35	[69]
53	22316-55-8	Desmethylclobazam	Benzodiazepine	0.36	[69]
54	1088-11-5	Desmethyldiazepam	Benzodiazepine	0.61	[69, 71]
55	439-14-5	Diazepam	Benzodiazepine	0.56	[69, 71]
56	78755-81-4	Flumazenil	Benzodiazepine	−0.29	[70]
57	1622-62-4	Flunitrazepam	Benzodiazepine	0.06	[69]
58	122384-14-9	L-663581 (FG 8205)	Benzodiazepine	−0.3	[72]
59	128246-10-6	M1 L-663581	Benzodiazepine	−1.34	[72]
60	130073-36-8	M2 L-663581	Benzodiazepine	−1.82	[72]
61	59467-70-8	Midazolam	Benzodiazepine	0.32	[69, 70]
62	604-75-1	Oxazepam	Benzodiazepine	0.55	[69, 71]
63	99632-94-7	RO19-4603	Benzodiazepine	−0.25	[70]
64	28911-01-5	Triazolam	Benzodiazepine	0.6	[69, 71]
65	37517-30-9	Acebutolol	Beta-Blocker	−0.15	[73]
66	13655-52-2	Alprenolol	Beta-Blocker	−0.23	[73]
67	29122-68-7	Atenolol	Beta-Blocker	−1.0	[47, 74]
68	63659-18-7	Betaxolol	Beta-Blocker	0.39	[73]
69	51384-51-1	Metoprolol	Beta-Blocker	1.15	[74]
70	525-66-6	Propranolol	Beta-Blocker	1.58	[44, 55, 74]
71	3930-20-9	Sotalol	Beta-Blocker	−0.28	[75]
72	120014-06-4	Donepezil (Aricept)	Cholinergic	0.89	[76]
73	357-70-0	Galantamine	Cholinergic	0.32	[77]
74	364079-69-6	LU-201640	Cholinergic	0.3	
75	123441-03-2	Rivastigmine (ENA-713)	Cholinergic	0.88	[78]
76	142852-50-4	Zanapezil (TAK-147)	Cholinergic	1.14	[78]
77	91374-21-9	Ropinirole (SKF-101468)	Dopamine	0.25	[5]
78	187281-31-8	Pfizer Compound 1	GPCR-NK1	0.48	InH
79	147116-67-4	Pfizer Compound 2	GPCR-NK1	0.46	InH
80	161105-56-2	Pfizer Compound 3	GPCR-NK1	−0.89	InH
81	135095-42-0	Pfizer Compound 4	GPCR-NK1	0.37	InH
82	145148-39-6	Pfizer Compound 5	GPCR-NK1	0.87	InH
83	145741-90-8	Pfizer Compound 6	GPCR-NK1	0.98	InH
84	145877-52-7	Pfizer Compound 7	GPCR-NK1	0.63	InH
85	145742-16-1	Pfizer Compound 8	GPCR-NK1	0.92	InH
86	145742-01-4	Pfizer Compound 9	GPCR-NK1	0.96	InH
87	145742-22-9	Pfizer Compound 10	GPCR-NK1	0.88	InH
88	145742-21-8	Pfizer Compound 11	GPCR-NK1	0.41	InH
89	145741-95-3	Pfizer Compound 12	GPCR-NK1	−0.15	InH
90	160502-69-2	Pfizer Compound 13	GPCR-NK1	−1	InH
91	160502-85-2	Pfizer Compound 14	GPCR-NK1	−0.42	InH
92	161499-49-6	Pfizer Compound 15	GPCR-NK1	0.36	InH
93	163257-90-7	Pfizer Compound 16	GPCR-NK1	−0.22	InH
94	163257-80-5	Pfizer Compound 17	GPCR-NK1	0.48	InH
95	164352-88-9	Pfizer Compound 18	GPCR-NK1	−0.37	InH
96	190275-53-7	Pfizer Compound 19	GPCR-NK1	0.49	InH
97	163257-64-5	Pfizer Compound 20	GPCR-NK1	0.11	InH
98	163257-86-1	Pfizer Compound 21	GPCR-NK1	0.85	InH

Table 1. Continued.

#	CAS #	NAME	Class	logBB	Ref.[a]
99	163257-88-3	Pfizer Compound 22	GPCR-NK1	0.32	InH
100	163257-84-9	Pfizer Compound 23	GPCR-NK1	1.26	InH
101	163257-85-0	Pfizer Compound 24	GPCR-NK1	0.62	InH
102	77086-21-6	MK-801	GPCR-NMDA	1.11	[79]
103	52-26-6	Morphine	GPCR-Opiod	−0.16	[51]
104	151581-23-6	Apaxifylline	GPCR-AA1A	−1.4	[80]
105	222722-65-8	Compound 13	GPCR-AA1A	−0.23	[80]
106	210880-09-4	Compound 17	GPCR-AA1A	−1	[80]
107	141060-39-1	Compound 28	GPCR-AA1A	0.38	[80]
108	202646-80-8	Compound 32	GPCR-AA1A	0.06	[80]
109	210879-61-1	Compound 5	GPCR-AA1A	−0.31	[80]
110	5638-76-6	Betahistine (B14)	Histamine	−0.3	[81]
111	6304-27-4	B15	Histamine	−0.06	[81]
112	18453-07-1	B16	Histamine	−0.42	[81]
113	61887-92-1	B19	Histamine	−1.3	[81]
114	83881-51-0	Cetirizine (Zyrtec)	Histamine	−1.30	[82]
115	43170-96-3	SKF 71473 (B20)	Histamine	−1.4	[81]
116	68-88-2	Hydroxyzine	Histamine	0.18	[83]
117	86181-42-2	Temelastine (SKF93944)	Histamine	−1.87	[84]
118	2507-81-5	Y-G 19	Histamine	−0.18	[1]
119	51481-61-9	Cimetidine (Y-G1)	Histamine	−1.42	[1]
120	69014-14-8	Tiotidine (Y-G10, ICI 125211)	Histamine	−0.82	[1]
121	112598-26-2	Y-G12	Histamine	−1.17	[1]
122	52378-66-2	Y-G13	Histamine	−2.15	[1]
123	54856-23-4	Y-G14	Histamine	−0.3	[1]
124	112598-28-4	Y-G15 (SKF-94445)	Histamine	−0.67	[1]
125	112598-30-8	Y-G16	Histamine	−0.66	[1]
126	112598-32-0	Y-G17	Histamine	−0.12	[1]
127	7120-01-6	Y-G2	Histamine	−0.04	[1]
128	72801-60-6	YG−20	Histamine	−1.15	[1]
129	72801-74-2	Y-G22	Histamine	−1.57	[1]
130	72801-63-9	Y-G23 (ICI 127032)	Histamine	−1.54	[1]
131	74188-86-6	Y-G24	Histamine	−1.12	[1]
132	112598-43-3	Y-G25	Histamine	−0.73	[1]
133	112598-45-5	Y-G26	Histamine	−0.27	[1]
134	112598-49-9	Y-G29	Histamine	−0.28	[1]
135	71351-79-6	Icotidine (Y-G3, SKF 93319)	Histamine	−2	[1]
136	78273-74-2	Y-G30	Histamine	−0.46	[1]
137	112598-52-4	Y-G31 (SKF-94826)	Histamine	−0.24	[1]
138	87078-26-0	Y-G34	Histamine	−0.02	[1]
139	104076-40-6	Y-G36 (SKF-94674)	Histamine	0.69	[1]
140	104076-45-1	Y-G37	Histamine	0.44	[1]
141	145459-26-3	Y-G4 (SKF 93619)	Histamine	−1.3	[1]
142	104076-38-2	Zolantidine (Y-G41, SKF-95282)	Histamine	0.14	[1]
143	104076-32-6	Y-G42	Histamine	0.22	[1]
144	83903-06-4	Lupitidine (Y-G5, SKF93479)	Histamine	−1.06	[1]
145	59-33-6	Y-G7 (Mepyramine Maleate)	Histamine	0.49	[1, 84]
146	113-52-0	Y-G8 (Imipramine HCl)	Tricyclic Amine	1.17	[1, 55, 85]
147	66357-35-5	Y-G9 (Ranitidine)	Histamine	−1.23	[1]

Table 1. Continued.

#	CAS #	NAME	Class	logBB	Ref.[a]
148	82626-48-0	Zolpidem	Hypnotic	−0.54	[86]
149	203321-88-4	Compound A	Muscarinic	−0.89	[87]
150	133099-04-4	Darifenacin	Muscarinic	−0.62	[87]
151	51-34-3	Scopolamine	Muscarinic	0.23	[87]
152	117-89-5	Trifluperazine	Neuroleptic	1.38	[88]
153	10457-90-6	Bromperidol	Neuroleptic	1.38	[89]
154	1063-55-4	Butaperazine-Maleate	Neuroleptic	0.83	[90]
155	50-53-3	Chlorpromazine	Neuroleptic	0.84	[90]
156	2095-20-7	Desmonomethylpromazine	Neuroleptic	0.59	[89, 91]
157	146-56-5	Fluphenazine-HCl	Neuroleptic	1.53	[90]
158	52-86-8	Haloperidol	Neuroleptic	1.34	[90]
159	32672-69-8	Mesoridazine-Besylate	Neuroleptic	−0.01	[90]
160	2095-17-2	Nor-2-Chlorpromazine	Neuroleptic	0.97	[89]
161	1225-64-5	Nor-Chlorpromazine	Neuroleptic	1.37	[89]
162	10538-32-6	Northioridazine	Neuroleptic	0.75	[89]
163	85650-56-2	Asenapine (ORG-5222)	Neuroleptic	1.03	[6]
164	210821-63-9	ORG-12962	Neuroleptic	1.64	[6]
165	142494-12-0	ORG-13011	Neuroleptic	0.16	[6]
166	128915-56-0	ORG-30526	Neuroleptic	0.39	[6]
167	129234-06-6	ORG-32104	Neuroleptic	0.52	[6]
168	198968-25-1	ORG-34167	Neuroleptic	0	[6]
169	135928-30-2	ORG-4428	Neuroleptic	0.82	[6]
170	104535-64-0	SKF-89124	Neuroleptic	−0.43	[5]
171	167782-15-2	BMS-184111 HCl	Neuroleptic	0.74	[92]
172	53-60-1	Promazine HCl	Neuroleptic	0.67	[91, 93]
173	146-21-4	Promazine-Sulfoxide	Neuroleptic	−0.48	
174	14759-06-9	Sulforidazine	Neuroleptic	0.18	[89]
175	50-52-2	Thioridazine	Neuroleptic	0.34	[90]
176	207390-15-6	Bromocytisine	Nicotinic	−0.05	InH
177	486-56-6	Cotinine	Nicotinic	0.04	[94, 95]
178	485-35-8	Cytisine	Nicotinic	−1.09	[96]
179	54-11-5	Nicotine	Nicotinic	0.56	[95-97]
180	494-97-3	Nor-nicotine	Nicotinic	0.32	[95]
181	34911-55-2	Bupropion (Zyban)	Smoking	1.40	[98]
182	5630-53-5	Tibolone	Steroid	0.4	[6]
183	58-08-2	Caffeine	Stimulant	0.01	[99–102]
184	83-67-0	Theobromine	Stimulant	−0.29	[100]
185	58-55-9	Theophylline	Stimulant	−0.38	[100, 102, 103]
186	1977-15-7	2-OH-Desm.Imipramine	Tri.Anti.Depr	0.53	[85]
187	50-48-6	Amitriptyline	Tri.Anti.Depr	0.89	[104]
188	50-47-5	Desipramine	Tri.Anti.Depr	0.9	[55, 85, 105]
189	2095-95-6	Desmethyldesipramine	Tri.Anti.Depr	0.96	[85, 105]
190	24219-97-4	Mianserine	Tri.Anti.Depr	0.99	[6]
191	85650-52-8	Mirtazapine	Tri.Anti.Depr	0.53	[6]
Not Used[b]					
193	58-15-1	Aminopyrine	Analgesic	0.04	[101, 106]
194	15687-27-1	Ibuprofen	Analgesic	−0.18	[54]
195	53-86-1	Indomethacin	Analgesic	−1.26	[106]
213	50-33-9	Phenylbutazone	Analgesic	−0.58	[50, 53]

Table 1. Continued.

#	CAS #	NAME	Class	logBB	Ref.[a]
218	487-54-7	Salicyluric Acid	Analgesic		[48]
198	298-46-4	Carbamazepine	Anticonvulsant	0.00	[107]
199	36507-30-9	Carbamazepine 10,11-epox.	Anticonvulsant	−0.34	[107]
214	57-41-0	Phenytoin	Anticonvolsant	−0.035	[53]
221	21489-20-3	Talsupram (LU 5-003)	Antidepressant	0.22	[108]
204	86386-73-4	Fluconazole	Antifungal	−0.22	[109]
207		Itraconazole	Antifungal		[61]
208	65277-42-1	Ketoconazole	Antifungal	−0.63	[110]
216	106266-06-2	Risperidone	Antipsychotic	−0.11	[86, 111]
217	144598-75-4	Risperidone (9-OH)	Antipsychotic	−1.22	[86, 111]
219	3056-17-5	Stavudine	Antiviral		[112]
193	52-43-7	Allobarbital	Barbiturate	−0.22	[68]
195	57-43-2	Amobarbital	Barbiturate	0	[68]
196	57-44-3	Barbital	Barbiturate	−0.25	[68]
202	52-31-3	Cyclobarbital	Barbiturate	−0.301	[68]
211	143-81-7	Pentobarbital	Barbiturate	0.1	[68]
212	50-06-6	Phenobarbital	Barbiturate	−0.12	[68]
215	6673-35-4	Practolol	Beta-blocker	−0.55	[93]
220	1684-40-8	Tacrine	Cholinergic	1.16	[113]
200	529-38-4	Cocaethylene	Cocaine		[114]
201	50-36-2	Cocaine	Cocaine		[114]
203	57808-66-9	Domperidone	Dopamine	−0.78	[115]
209	18717-72-1	Nor-cocaine	Cocaine		[114]
197	519-09-5	Benzoylecgonine	Stimulant		[114]
210	611-59-6	Paraxanthine	Stimulant	0.57	[100]

[a]'InH' refers to values generated from in-house measurements that are disclosed for the first time here. Experimental designs represent those employed in several different drug discovery programs and should be taken as similar to those found on page 331 of Kalvass and Maurer [41] or page 167 of Doran et al. [86].
[b]"Not Used" refers to values for which we could not determine the accuracy. This could be due to inconsistent values between references (i.e. values for Risperidone cited in references [86] and [111] are very different, and we were unable to determine the source of the discrepancy), or inconsistent methodologies (i.e. CSF/plasma values).

comments, rather than secondary references. These criteria, of course, do not provide ultimate assurance of data reliability, but do constitute a necessary, albeit arguable, filter toward the lowering of errors encountered. Furthermore, they allow an additional measure of control, by allowing other authors to consult and consider the accuracy of the original reports, as well as the quality of the data used in the present work. All the original references are provided and the compounds that may have been identified as possible efflux substrates are identified.

B. Statistical analysis

The R program [22] was used to generate bagged decision tree models using the "rpart" library. This methodology creates a number of decision tree models and takes the average of those models as the predicted value. In all cases reported here, 25 trees were used in each "bagged" tree model. The multiple linear regression model (MLR) for the rule-of-five (Ro5) data set was also performed in R with the "lm" command.

We prefer the recursive partitioning method for three primary reasons. First, overfitting is reduced [22]. While the overfitting of one tree may occur, overfitting 25 trees is relatively less likely. Second, the standard deviation of the 25 predictions provides a quick assessment of the confidence of the prediction. For instance, if a new compound is tested and the model reports that the logBB predicted is 0.7 ± 0.3, this may be considered a good prediction, while a logBB prediction of

0.7 ± 0.6 may be considered a relatively poor prediction. Finally, descriptor selection is done automatically [22].

Three separate statistical levels are reported. First, a training set model is generated for each descriptor set. Here all 190 compounds are used to train a bagged decision tree model. The same compounds are then predicted and the ability of the model to predict those compounds is evaluated. The second level reported here is the leave-group-out cross validation (LGO-CV). In this case, 10 separate bagged decision tree models are generated. For each model, 25% of the literature compounds are selected randomly using the "rbinom" function in R. A model is trained on the remaining 75% of the data set and the selected 25% is used as a test set. The correlation coefficient reported, Q^2, is the average correlation coefficient for 10 randomly selected test sets. This averaging was done because Q^2 varies by about 0.10 unit from one randomly selected test set to the next. Finally, using the training model of the 190 literature compounds, all internal Pfizer compounds were used as a test set. Many of the proprietary Pfizer compounds are from active drug discovery projects and their structures are not disclosed here. We provide the statistical analysis to share our experiences as they relate to data extrapolation and the important consideration of transporter assessment.

Two additional statistical techniques have also been used to examine descriptor importance and to detect outliers. Relative variable importance was determined from summing the number of times a particular chemical descriptor was chosen as a split-point in one of the bagged decision trees. Here we presume that descriptors that are chosen more often as splits are determined by the model to be more relevant to the observed variable.

The hat matrix was used as a means for estimating "extrapolated" descriptor strings in the Pfizer data sets. The hat matrix is defined as

$$H = X(X^T X)^{-1} X^T \quad (1)$$

where X is an N (number of observations) by D (number of descriptors) matrix and may be used as a projection matrix for subsequent test sets in a linear model. The initial H matrix is defined by the training set. The diagonal elements of H indicate the effect of a given observation. These values (termed leverage values) indicate whether or not the descriptor values for a given observation (in this case a new compound) are far from the main body of the data. For our purposes a high *leverage value* for a test compound indicates that the particular observation is distant from the centre of the X observations (i.e. the training set) and is thus designated as a potential extrapolation. We determine this by comparing the leverage value of a test compound to the leverage values of the training set. If the test compound has a leverage value that is less than or greater than the minimum and maximum training set leverage values, respectively, then an outlier, hence an extrapolation, is assumed.

C. Descriptor calculations

Three descriptor sets were examined in this study: the Charge Polar Surface Area (CPSA) descriptors [23], the Rule-of-Five (Ro5) descriptors [24], and a set of descriptors from the MOE computer program [25]. In the course of our work, we also evaluated the Volsurf descriptors [26, 27] and the Electrotopological State (E-state) descriptors [28]. However, the results we achieved for these last two descriptor sets were generally poor, possibly due to inconsistent methodology compared with the original reference. For these two sets, it is likely that some differences exist between the specific descriptors calculated here and those reported by blood–brain barrier models found in the literature [20, 25, 29]. Due to lack of direct reproducibility with the original references and for the sake of brevity the results obtained using these programs will not be reported here.

The CPSA descriptors used for this study are similar to those defined by Stanton and Jurs [23] and recently used to examine the brain/plasma endpoint. These descriptors are calculated by first using the Corina program [30] to provide a uniform 3-dimensional translation for all molecules. The internal version of the CPSA program then uses AMSOL [31] and the CM1A method for calculating partial atomic charges [32, 33]. SAVOL2 [34] is used for generating the partial solvent accessible surface areas and molecular

surface areas/volumes. A number of mathematical permutations of charge summations/differences of different surface areas are then used as descriptors of the molecules. A table defining these descriptors is included in the Supplemental Information section of this paper and is available upon request.

The extended Rule-of-Five (Ro5x) descriptor set used here contains a number of extra descriptors in addition to the well-known "Rule of Five" [24]. These include an estimation of the Andrew's binding energy [35], the number of rotatable bonds, CLOGP [36], rule-of-five violations, rule-of-five alerts [24], TPSA [37], and a flag for the presence of positive and negative ionizable groups, as well as all of the Moriguchi parameters: MlogP, PRX, POL, UB, NO and CX [38]. All of these have been calculated using an internal SPL (Sybyl Programming Language) programs with the exception of CLOGP, and TPSA. This set includes a total of 16 descriptors.

The MOE [39] descriptor set includes all of the molecular VSA (van der Waals surface area) descriptors. These "VSA-type" descriptors subdivide the molecular surface area of the molecules based upon property type using three properties: SMR (molecular refractivity), SlogP (logP) and PEOE (partial electrostatic charges) [40]. In addition, the TPSA (topological polar surface area), molecular weight, logP (o/w), counts for aromatic, acidic and basic atoms are included, as well as total van der Waals surface areas corresponding to various atomic properties (acids, bases, hydrogen bond donors, etc.) have been included as calculated by the commercially available MOE program [39].

Results and discussion

A. Efflux issues

Before considering the ability of various descriptor-based statistical models to predict the brain/plasma ratio, we first consider the underlying mechanisms that determine drug disposition and their relative impact upon the observed variable. Most models attempting to correlate calculable physical properties with B/P ratios make the assumption that the observed brain/plasma ratio reflects the ability of the drug to partition from an aqueous-like environment to a lipid-like environment. This assumption is supported by numerous empirical correlations between observed brain/plasma ratios and calculated estimates of a drugs lipophilicity (ClogP) or hydrophilicity (polar surface area).

Generally, drugs that are suspected to have anomalously low brain/plasma ratios due to P-gp-mediated efflux from the brain are flagged as outliers and not included in quantitative correlations. The issue of the transporter-mediated efflux raises important questions: what is the impact on overall brain permeation from efflux mechanisms? What is the variation of this effect from one chemical series to the next?

Figure 1 shows the correlation of brain/plasma ratios observed between P-gp ko-mice and brain/plasma from wild-type mice for a series of 250 compounds measured at Pfizer. The three panels all show the same set of data and differ only in the shading of the data points that refer to different target types. Compounds made for a specific kinase inhibitor project are shaded dark on panel

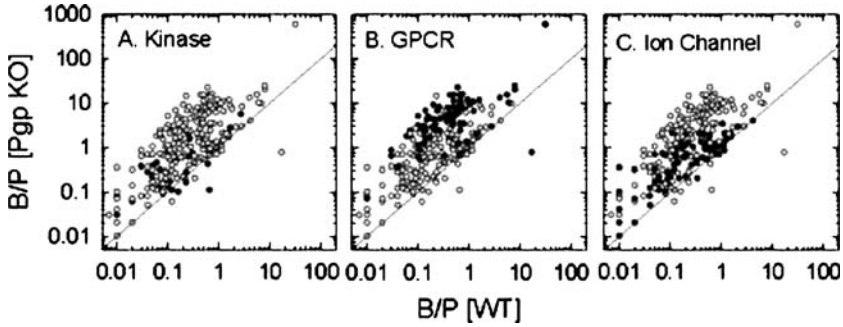

Figure 1. Comparison of measured P-gp knock out (KO) and wild-type (WT) brain/plasma ratios. Panels "A", "B" and "C" all have the same data plotted and differ only by the shading. Black data points in Panel "A" refer only to compounds from a program with a kinase target, while all other data are shaded grey. Panels "B" and "C" follow this convention with darkened symbols for a GPCR and Ion Channel targets.

A, while potential GPCR ligands and ion-channel ligands are shaded dark on panels B and C respectively. Although we have arbitrarily partitioned the data set by the gene-family of the target, a very limited number of distinct chemical scaffolds comprise these sets. The ratio of [brain/plasma]knock-out/[brain/plasma]wild-type (referred to KO/WT) gives an *in vivo* measure of the P-gp liability of a given compound. The average KO/WT ratio is 8.14. Only 90 out of the 230 compounds have KO/WT ratios less than 3.0. From this perspective, approximately 60% of all compounds tested have substantial (KO/WT > 3) P-gp-mediated efflux issues. This percentage may not accurately reflect all of the compounds being made for CNS projects at Pfizer, Groton since KO experiments are typically only performed on compounds that are suspected of having some P-gp-mediated efflux.

The implication of the series-dependent P-gp-liability for statistical modeling of novel chemical series is important. It suggests that B/P ratios cannot be predicted accurately until the P-gp liability is well characterized. As we shall see in the next section, extrapolating to new chemical series remains a major hurdle for just this reason. Unfortunately, no rapid *in vitro* methods have been established that may act as a surrogate measure for the KO/WT ratio.

One strategy that may turn out to perform well in the future is to have two statistical models: one that predicts [brain/plasma]wild-type and one that predicts [brain/plasma]knock-out data. The ability of the models to extrapolate to a novel series will remain a question. However, a model built solely on the knock-out data will at least attempt to provide an answer to the question "do these compounds passively diffuse into the brain?"

B. CSF

Another major issue related to building statistical models for "brain penetration" based upon continuous-valued brain/plasma ratios are their relevance, or lack of relevance with respect to *in vivo* CNS activity. A number of sources have argued towards measuring CSF or ECF levels in the brain as opposed to using whole-brain homogenates [41, 42]. The primary idea behind this argument is that measurements of whole-brain homogenates include concentrations of drug bound to non-specific brain tissues. Statistical models based upon this number would then drive SAR towards excessively lipophilic compounds with relatively low receptor availability and therefore lower than expected *in vivo* activity. This argument assumes that the receptor active sites are themselves exposed to ECF. Based upon a small but growing number of crystal structures in historically CNS-related gene families [43], and the observed low solvent exposure of these active sites one may argue that the assumption that high ECF concentrations leads to high receptor occupancy is not necessarily valid. In any case, lack of sizable ECF or CSF data sets precludes statistical modeling efforts at this stage.

C. Descriptor comparison

Of particular interest to us is the ability of the brain/plasma statistical model to predict virtual compounds that either have not been made or have not yet been tested. To assess the predictive ability of the models, we have presented a number of quantitative measures. First, a training set model has been generated for each descriptor set. These models use all of the literature data (190 compounds), train a bagged tree model and predict all of the compounds using this model. This measure accounts for the model's ability to reproduce the values from the training set. A set of correlation plots of predicted vs. observed log(BB) values is shown in Figure 2, and basic statistics for these models are given in Table 2. For all descriptor sets studied, these models produce typical R^2 and RMSE values [2–4, 18, 29, 116, 117]. The lowest observed R^2 value is for the simple descriptor set comprising of the "augmented" Rule-of-Five descriptor set, which also has the highest RMSE. Using this same descriptor set, a much higher quality of model can be found using the recursive partitioning statistical method compared to the multiple linear regression. A leave-group-out cross validation (LGO-CV) is also reported in Table 2. In this case, 10 separate bagged tree models are generated. For each bagged model 25% of the literature compounds are selected randomly using the "rbinom" function in *R*. A model is trained on the remaining 75% of the data set and the selected 25% are used as a test set. The

Table 2. List of drugs and their log(brain/plasma) values.

Model	Literature set		LGO-CV		Pfizer data	Percentage extrapolation[c]
	R^2	RSE	$Q^{2(a)}$ (STDV)	RSE (STDV)	$Q^{2(b)}$	
CPSA	0.83	0.35	0.51 (0.09)	0.58 (0.06)	0.19	13.1
Ro5_tpsa	0.77	0.40	0.53 (0.10)	0.58 (0.07)	0.27	1.7
Ro5-MLR	0.54	0.57	0.52 (0.07)	0.58 (0.06)	0.19	1.7
MOE	0.80	0.38	0.53 (0.08)	0.57 (0.06)	0.22	13.7

[a]Q^2 values represent average correlation coefficients for 10 test sets. Each of the 10 test sets was generated by randomly selecting 25% data points from the literature set (190 compounds). Numbers in parenthesis indicate the standard deviation of the Q^2 values for the 10 test sets.
[b]Q^2 values represent the correlation coefficients of Pfizer compounds tested using a training set model of literature compounds.
[c]Refers to the percentage of the test set that falls outside the bounds of the training set as defined by the leverage values computed using the Hat Matrix analysis.

correlation coefficient reported, Q^2, is the average correlation coefficient for 10 randomly selected test sets. All recursive-partitioning models have a drop-off of at least ~0.24 units when compared to the training set R^2. All physical property-based models (CPSA, Ro5, and MOE) have a very similar range of accuracy ($R^2 = 0.51$–0.53) with the simple Ro5 and MOE-rpart model giving the best average Q^2 of 0.53. These Q^2 values are lower than others reported in literature using a leave-one-out method. It is our feeling that this methodology gives a slightly more realistic picture of the ability of these models to predict novel chemical series. Finally, using the training model of the 190 literature compounds, all internal Pfizer compounds were tested. These compounds were collected from eight separate CNS-related projects. For our purposes, the R^2 values reported for this set represent a true test set. The percentage coverage column refers to the percentage of compounds in the set that is within the training set descriptor space for that set of descriptors based upon the hat matrix determination of whether or not a compound is an extrapolation. Compared to the LGO-CV Q^2, the R^2 values reported for this set show a further decline. The extended rule-of-five descriptor set demonstrates the best ability to extrapolate from the literature training set to the Pfizer test set. Considering that the average KO/WT ratio of the Pfizer compounds is 8.14, and that the model has no method of determining the Pgp-liability of the compounds, these results are not surprising and underscore the difficulty of predicting compounds with P-gp-mediated efflux.

Given that all of the descriptor sets analyzed here may be considered "physical property" descriptors, it is interesting that their apparent ability to extrapolate to new chemical series is as different as it is. To better understand the difference in performance between seemingly similar physical-property based descriptor sets, we have examined the construction of the individual partitioning trees. Figure 3 shows the relative variable importance determined from summing the number of times a particular chemical descriptor was chosen as a split-point in one of the 25 bagged decision tree models. Here, we presume that descriptors that are chosen more often as split points are more relevant to the observed variable. The right panel of Figure 3 shows the relative variable importance for rule-of-five descriptors. Our findings are consistent with previously observed results by Clark [5]. The TPSA descriptor that is calculated from a fragment-based VdW polar surface area [37] is the most prominent descriptor split along with ClogP. Interestingly, the TPSA descriptor is used less often when included in the much larger CPSA descriptor pool. A number of the fractional permutations of the CPSA set appear at or near the top in terms of number of splits. For instance, the FNSA3 descriptor, defined as $\sum (SA \times q^-)/SA^1$ is the most prevalent split.

Although the CPSA descriptors provide a much more detailed picture of the physical chemical properties of the molecules, when combined with a recursive partitioning algorithm this set performs poorly when predicting new compounds. One plausible explanation is that including many more permutations of fractional CPSA

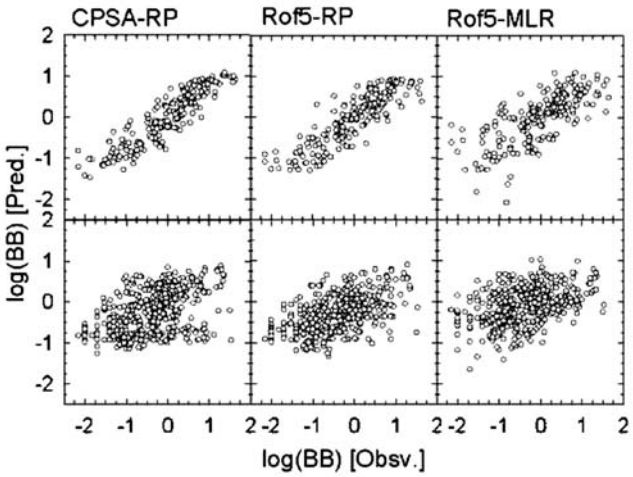

Figure 2. Comparison of predicted and observed log(BB) values. CPSA-RP refers to predicted values generated using the CPSA descriptors and a recursive partitioning model. Rof5-RP refers to predicted values generated using the Rof5 descriptors and a recursive partitioning model. Rof5-MLR refers to predicted values generated using the Rof5 descriptors and a multiple linear regression model. The top panels compare predicted and observed values for the literature training set, while the bottom panels compare predicted and observed values for the in-house Pfizer test set.

produces a "fingerprint-like" descriptor string that is overly descriptive and thus not as transferable to different chemical scaffolds. To address this issue, we have evaluated the descriptor strings using the hat matrix as defined in the methods section. Test compounds with hat matrix values lower than the hat matrix minimum or higher than the hat matrix maximum may be considered outliers relative to the training set descriptor space. Of the 350 Pfizer compounds examined, 46 were determined to be outliers when defined using the CPSA descriptors, 48 were determined to be outliers when defined using the MOE descriptor set and six were determined to be outliers when defined using the Rof5 descriptor set. The description of modeled compounds as a set of "bunches" or "clusters" used by Abraham is very useful. The analysis presented here helps to establish whether a not a new compound falls into an existing "cluster". For the Rof5 descriptor set, it may be that new chemical entities have a much higher chance of looking

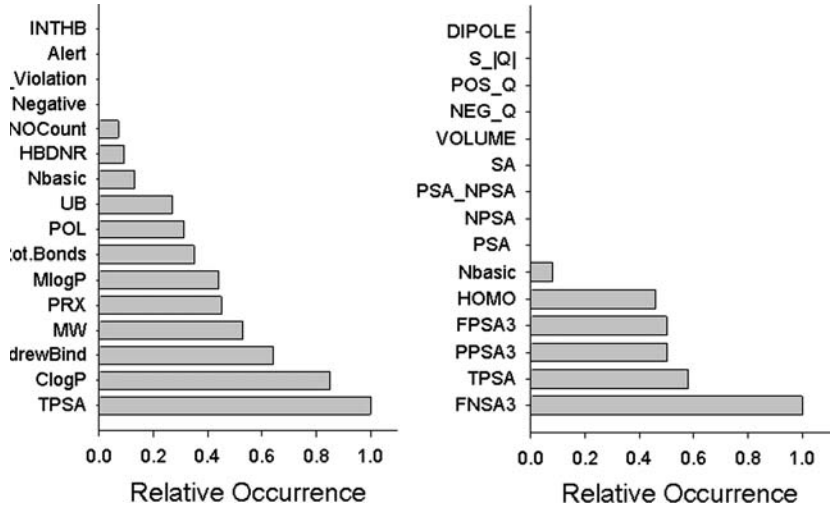

Figure 3. Evaluation of descriptor importance for all Rof5 descriptors (left panel) and selected CPSA descriptors (right panel).

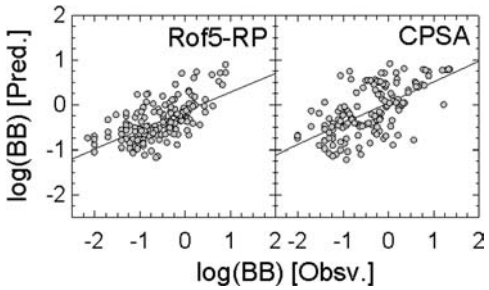

Figure 4. Comparison of predicted and observed log(BB) values using a reduced test set which includes only data from compounds that are not statistical extrapolations and have measured KO/WT ratios less than 10.

similar to an existing cluster since the properties used to describe the molecules are more general. In this regard it makes some sense that they tend to be predicted more accurately by using this set of relatively simple descriptor strings.

There are two possible reasons why the Pfizer internal compounds are predicted relatively poor. The first is the apparent efflux liability and the second is the presence of statistical extrapolations. How well does the model predict observed log(BB) if the both of these effects are removed? Figure 4 shows the correlation plot of the Rof5 (panel A) and CPSA (panel B) models for compounds with known KO/WT ratios less than 10 without compounds that are statistical extrapolations. Comparing these correlation plots to the bottom plots of Figure 2 demonstrates that the correlation does improve with the removal of known extrapolations. The observed R^2 values are 0.39 ($s = 0.43$) for CPSA and 0.39 ($s = 0.33$) for Rof5. Once extrapolations are removed, both models appear to be equally predictive. The advantage of using the Rof5 descriptor set is only that there are far fewer statistical extrapolations, so the model may be correctly applied to a nearly all of the test set compounds.

D. Extending the rule of five

This is not the first time that rule-of-five or similarly simple chemical descriptors have been used when modeling log(BB). In addition to past QSAR studies using polar surface area [5, 6], free energies of solvation [3, 118] and other simple physical properties [2, 4, 29], there also exists a "CNS-Rule of Five" [14]. Not as well-known as the original Rule of Five [24], this version of the rule of five offers a similar set of simple property cut-offs: MW < 400, hydrogen-bond acceptors < 6, hydrogen-bond donors < 2 and neutral or basic with a p*K*a between 7.5 and 10.5. Noticeably absent from this list is a revised term for logP.

This simple analysis suffers from a similar weakness as other models that separate compounds into CNS active and inactive classes. That is, compounds that either do not have a basic center or possess a non-traditional CNS scaffold will generally be filtered-out, thus hindering potential use for non-traditional CNS targets (for example, [119, 120]). Nevertheless, it underscores the usefulness of focusing on simple chemical descriptors. In practice, it is often more useful from both a predictive and interpretive standpoint to build 'local models' based upon small sets of compounds belonging to a similar chemical scaffold using a simple set of descriptors. Figure 5 demonstrates three correlations that may be found using historic and/or in-house data sets for a few focused chemical series. What

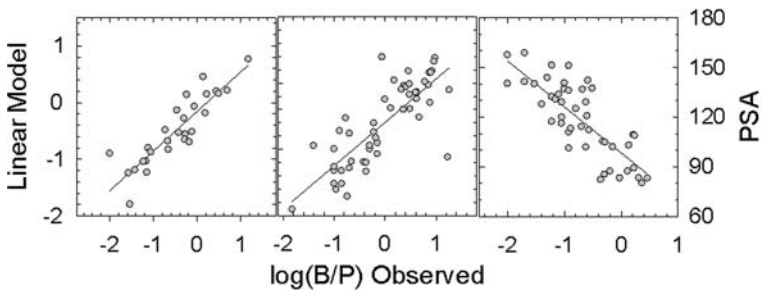

Figure 5. Comparison of observed log(B/P) values with either simple linear models or physical properties. Left and middle panels refer to simple models for Histamine and NK-1, generated using equations 2 and 3 in the text. The panel on the right refers to the simple correlation (equation 4) that log(B/P) values share with the TPSA parameter for a set of compounds targeting a kinase.

is surprising in this analysis is that the three models have an assorted reliance upon the descriptors used to model them:

Histamine Data Set:
$\log(B/P) = 0.06 - 0.014^{*}PSA + 0.16^{*}ClogP$ (2)
$n = 28, R^2 = 0.79$

NK-1 Data Set:
$\log(B/P) = 1.55 - 0.009^{*}MW + 0.41^{*}ClogP$ (3)
$n = 51, R^2 = 0.73$

Kinase Data Set:
$\log(B/P) = 2.04 - 0.023^{*}PSA$ (4)
$n = 47, R^2 = 0.65$

Here, the NK-1 data set shows relatively little correlation with PSA. Presumably due to the P-gp-efflux component to the data, MW rather than PSA contributes more to the ability of these particular compounds to partition into the brain. Additionally, from a design perspective, these models have a much greater intuitive appeal since these parameters all are relatively easy to calculate and modulate.

Conclusions

A series of bagged recursive partitioning models for log(BB) are presented. Three descriptor types are compared using a LGO-CV as well as an external data set of proprietary Pfizer compounds. Pfizer compounds are typically difficult to predict, primarily due to P-gp-mediated efflux. Low correlation coefficients for this test set are improved when compounds known to be P-gp-substrates or statistical extrapolations are removed. The use of simple linear models for specific chemical series can also improve the correlation over a limited chemical space. Of the general use models studied here, the Ro5_tpsa recursive partitioning model appears to extrapolate the best to 'new' series. However, the overall correlation is poor, and only incrementally better than other recursive partitioning models built using the MOE and CPSA descriptor sets.

The most useful future direction would most likely be either a model of the brain/plasma ratio generated from knock-out mice or a model of the KO/WT ratio. The latter would have the added benefit of canceling-out some of questions arising from non-specific plasma protein binding and binding to non-specific brain tissues since these factors (assuming they are the same in KO and WT species) would cancel out. Considering the difficulty of predicting effect of Pgp-mediated efflux from WT data alone and the tremendous shift this may cause in the observed WT brain/plasma ratios (i.e. Figure 1), this model would most likely be a more useful tool for the prediction of *in vivo* activity. However, it would only explain P-gp-mediated efflux mechanisms, which although predominant, are not the only cause of efflux from the brain.

Notes

1. SA is the total solvent-accessible-surface area, SA^{-} is the corresponding negative contribution.

Acknowledgements

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