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Combined Micellar Liquid Chromatography Technique and QSARs Modeling in Predicting the Blood–Brain Barrier Permeation of Heterocyclic Drug-like Compounds

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Abstract: The quantitative structure–activity relationship (QSAR) methodology was used to predict the blood–brain permeability (log *BB*) for 65 synthetic heterocyclic compounds tested as promising drug candidates. The compounds were characterized by different descriptors: lipophilicity, parachor, polarizability, molecular weight, number of hydrogen bond acceptors, number of rotatable bonds, and polar surface area. Lipophilic properties of the compounds were evaluated experimentally by micellar liquid chromatography (MLC). In the experiments, sodium dodecyl sulfate (SDS) as the effluent component and the ODS-2 column were used. Using multiple linear regression and leave-one-out cross-validation, we derived the statistically significant and highly predictive quantitative structure–activity relationship models. Thus, this study provides valuable information on the expected properties of the substances that can be used as a support tool in the design of new therapeutic agents.

Keywords: micellar chromatography; QSARs; log *BB*; lipophilicity; heterocyclic drug-like compounds



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1. Introduction

The development of new drugs with desired properties is a tedious, laborious, time-consuming, and expensive process. Quantitative structure–activity relationship (QSAR) methods should be useful tools on this complicated path. The approach is based on the assumption that biological (pharmacokinetic) properties of structurally similar compounds can be quantitatively described by mathematical models. In addition, these models should predict with good probability the activity of structural analogs not yet synthesized. Although the establishment of QSAR models involves a number of steps and conditions, such as use of reliable and accurate input data, selection of relevant descriptors, and use of appropriate software and validation of the suggested model, the advantages of the QSAR methodology are not in doubt [1–8]. First of all, it reduces overhead costs, decreases the time of obtaining positive results, reduces animal testing, and respects the principles of Green Chemistry [5].

A crucial factor for a drug candidate is transport through the blood–brain barrier (BBB). Satisfactory transport through the BBB is an essential prerequisite for a potential drug to affect the central nervous system. However, to avoid side effects, the agents that act peripherally should not cross the BBB. In both cases, the permeability of the BBB must be known and should be evaluated at the earliest possible stage of testing [3–5,9–12].

The uptake of a compound into the brain is a complex process [13–16]. However, it is known that moderately lipophilic drugs can cross the BBB by passive diffusion [17–21]. A measure of the ability of a compound to penetrate the BBB is defined as the log *BB*, which is the ratio of the concentration of the drug molecules in the brain to the concentration in the blood at equilibrium [3]. The traditional method of measurement, requiring animal or human testing, is time-consuming, expensive, and complicated. For this reason, it is unacceptable in screening, and the indirect methods for log *BB* prediction are highly desired. QSAR methods combining in vitro and in silico techniques have become very attractive alternatives to in vivo testing [9]. Generally, QSAR models are functions of a molecule's structure, electronic properties, and lipophilicity. More or less sophisticated models using new descriptors selection have been proposed [22–32]. As polarity descriptors are used polar surface area (*PSA*) or topological polar surface area (*TPSA*) [33,34], the numbers of hydrogen bond donors (*HBD*) and hydrogen bond acceptors (*HBA*) [4,35,36]. Molecular size is characterized by molar weight, polarizability parameter, parachor, refractivity, molar volume, etc. [7,10]. Lipophilicity is usually evaluated using the log *P*_{o/w} parameter describing solute partitioning between water and *n*-octanol.

For many years, various liquid chromatography techniques have been known and accepted to predict the lipophilic properties of organic substances, especially bioactive ones, as an alternative for the shake-flask technique. The theoretical bases are Collander-type equations [37], confirming good relationships between log *P*_{o/w} (the logarithm of partition coefficient in the *n*-octanol/water system) and chromatographic parameters. In particular, the log *k*_w parameter corresponding to the retention of the solute in water as the mobile phase is considered as the lipophilicity descriptor [37–42].

Chromatographic techniques assessing the lipophilic properties of substances contribute to the development of QRAR (quantitative retention–property relationship) and/or QSAR (quantitative structure–activity relationship) models for predicting the penetration of substances through the BBB. There are different reversed-phase liquid chromatography techniques, both planar and column [37–45], used in this type of research. Chromatography with stationary phases that imitate biological partitioning systems, such as an artificial membrane, phases with immobilized lipids, albumin, cholesterol, ceramides, or liposomes, allow not only the prediction of the lipophilic properties [37–45] but also the behaviors of solutes in real biological systems (bounding to serum albumin, skin permeation, blood–brain barrier permeability, intestinal absorption, concentration of unbound form in blood, and others) [46–51].

Similar possibilities are offered by micellar liquid chromatography (MLC) using surfactants as components of the mobile phase. MLC is using a surfactant solution above the critical micellization concentration (*cmc*). Under these conditions, the micelles form the so-called micellar pseudophase in the bulk phase. The surrounding bulk water or aqueous–organic mixture contains surfactant monomers in a concentration approximately equal to the *cmc*. Moreover, surfactant monomers modify the surface phase as a result of the hydrophobic interactions between the tail of the surfactant and the alkyl chain grafted to the carrier surface of the stationary phase. Molecular interactions present in this system, i.e., solute association with the polar head of the surfactant, solute penetration into the micelle core, and solute interactions with adsorbed surfactant and alkyl chains, affect retention by three different equilibria, which are (1) the solute distribution between the micelle (micellar pseudophase) and the bulk phase, (2) the solute partition between the stationary phase modified by the surfactant and the bulk phase, and (3) the direct transfer of solute molecules between the surfactant-modified surface and the micelle [52–56].

The effect of the concentration of the surfactant in the effluent on the retention of the solute can be described by Foley's equation [57], where the following relationship exists between the retention parameter, *k*, and the concentration of the surfactant in the effluent:

$$\frac{1}{k} = \frac{1}{k_m} + \frac{K_{AM}}{k_m} [M] \quad (1)$$

where $[M]$ is the total concentration of surfactant in the mobile phase minus the critical micellization concentration, cmc , K_{AM} is the constant that describes solute–micelle binding, and k_m is the solute retention parameter at zero micellar concentration, i.e., at surfactant monomer concentration equal to cmc . The K_{AM} and k_m parameters can be evaluated from the slope and intercept of experimental $1/k$ vs. $[M]$ relationships. Equation (1) describes a linear dependence with decreasing retention as the micelle concentration increases. This equation is valid for aqueous solutions of surfactant or mobile phases with the same concentrations of the organic modifier. The micellar retention parameter, $\log k_m$, is considered analogous to the $\log k_w$ value evaluated in RPLC. Thus, this parameter is considered as a lipophilicity descriptor, and Equation (1) is a simple way to achieve the indirect determination of the lipophilic properties of compounds. It is postulated that retention in micellar chromatography depends on the hydrophobic (lipophilic), electronic, and steric features of the compounds in a similar way as many pharmacokinetic phenomena. Additional similarity is indicated by the fact that the phospholipids, cholesterol, fatty acids, and triglycerides that are present in the extracellular and intracellular fluids also form micelles with proteins.

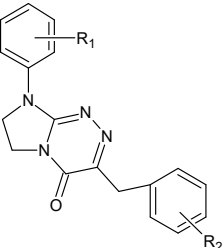
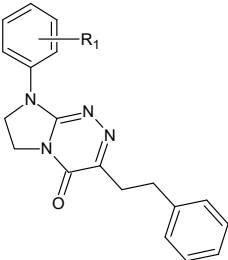
All the compounds (1–65) [58–74] investigated in present studies have been designed and synthesized in our laboratory. Their molecular structures—confirmed by spectroscopic methods in previous studies [58–64,66–71]—are presented in Table 1. The purity and homogeneity of all the compound samples (1–65) have been proven [75]. These heterocyclic molecules may be of pharmaceutical importance due to their promising anticancer [58–68,70], analgesic [58,59,69,71], antiviral, and antihemolytic [65] activities as well as their medical applicability [61–63,67–69,73,74], high purity, and thermal stability [72]. Compounds belonging to the particular structurally related classes, e.g., I (1–6), III (12–17), IV (18–28), V (34, 37–40), and VII (61–65), have been recognized as anticancer agent candidates, especially in the treatment of human cancers of the cervix, breast (1–6, 12–17, 18–28, and 61–65) [60–64], lung, ovary (1–6 and 61–65) [60–62], pharynx, tongue (1–6) [65], and in the therapy of human multiple myeloma (12–17, 34, 37–40) [63,64,70]. Simultaneously, most of these molecules — as promising drug candidates — proved to be low in toxicity to normal cells, including erythrocytes [60–68,70]. Compounds 1–3 were found to be safer for the early life stages of zebrafish than an antiviral agent acyclovir, while molecules 3–6 were equally safe as this pharmaceutical [65]. Additionally, all these heterocycles (1–6) showed a protective effect on oxidatively stressed red blood cells, which was stronger or comparable to that of ascorbic acid. It has been shown that molecule 6 is antivirally active, too [65]. Furthermore, many compounds with low in vivo toxicity (8–11, 32, 34, 39, 42, 48, 51, and 53) were found to be analgesic active, with three structures (8, 42, and 51) showing the strongest antinociceptive activity in the acetic acid-induced writhing test in mice [58,59,69,71]. The first two analytical procedures for quantifying the pharmacologically relevant molecules (15 and 39) in solution and in serum samples have recently been developed and disclosed [73,74].

In our investigation, for the first time, the lipophilic properties of all the compounds (1–65) were experimentally determined by micellar liquid chromatography (MLC). The micellar lipophilicity parameters from MLC (as an experimental in vitro technique) and in silico data were combined with the aim of building the most satisfactory models for the prediction of the BBB permeation of 65 heterocyclic drug-like compounds. The linear quantitative structure–activity relationship models were produced using multiple linear regression on a database that consisted of different lipophilicity, polarity, electronic, and molecular size descriptors. The predictive ability of the developed models was validated by leave-one-out cross-validation (LOOCV).

Table 1. Heterocyclic molecules (1–65) belonging to the particular classes (I–VII).

Class	General Structure	No	R ₁	R ₂
I		1	R ₁ = H	—
		2	R ₁ = 4-CH ₃	—
		3	R ₁ = 2-Cl	—
		4	R ₁ = 3-Cl	—
		5	R ₁ = 4-Cl	—
		6	R ₁ = 3,4-Cl ₂	—
II		7	R ₁ = H	—
		8	R ₁ = 4-CH ₃	—
		9	R ₁ = 4-OCH ₃	—
		10	R ₁ = 4-OC ₂ H ₅	—
		11	R ₁ = 4-Cl	—
III		12	R ₁ = H	—
		13	R ₁ = 4-CH ₃	—
		14	R ₁ = 4-OCH ₃	—
		15	R ₁ = 3-Cl	—
		16	R ₁ = 4-Cl	—
		17	R ₁ = 3,4-Cl ₂	—
IV		18	R ₁ = H	—
		19	R ₁ = 2-CH ₃	—
		20	R ₁ = 4-CH ₃	—
		21	R ₁ = 2,3-(CH ₃) ₂	—
		22	R ₁ = 2-OCH ₃	—
		23	R ₁ = 4-OCH ₃	—
		24	R ₁ = 2-Cl	—
		25	R ₁ = 3-Cl	—
		26	R ₁ = 4-Cl	—
		27	R ₁ = 3,4-Cl ₂	—
		28	R ₁ = 2,6-Cl ₂	—
V		29	R ₁ = H	—
		30	R ₁ = 2-CH ₃	—
		31	R ₁ = 3-CH ₃	—
		32	R ₁ = 4-CH ₃	—
		33	R ₁ = 2-OCH ₃	—
		34	R ₁ = 4-OCH ₃	—
		35	R ₁ = 4-OC ₂ H ₅	—
		36	R ₁ = 2,3-(CH ₃) ₂	—
		37	R ₁ = 2-Cl	—
		38	R ₁ = 3-Cl	—
		39	R ₁ = 4-Cl	—
		40	R ₁ = 3,4-Cl ₂	—

Table 1. Cont.

Class	General Structure	No	R ₁	R ₂
VI		41	R ₁ = H	R ₂ = H
		42	R ₁ = H	R ₂ = 2-Cl
		43	R ₁ = H	R ₂ = 3-Cl
		44	R ₁ = H	R ₂ = 4-Cl
		45	R ₁ = 4-CH ₃	R ₂ = H
		46	R ₁ = 4-CH ₃	R ₂ = 4-CH ₃
		47	R ₁ = 4-CH ₃	R ₂ = 3-CH ₃
		48	R ₁ = 4-CH ₃	R ₂ = 2-Cl
		49	R ₁ = 4-CH ₃	R ₂ = 3-Cl
		50	R ₁ = 4-CH ₃	R ₂ = 4-Cl
		51	R ₁ = 4-OC ₂ H ₅	R ₂ = H
		52	R ₁ = 4-OC ₂ H ₅	R ₂ = 4-CH ₃
		53	R ₁ = 4-OC ₂ H ₅	R ₂ = 2-Cl
		54	R ₁ = 4-OC ₂ H ₅	R ₂ = 3-Cl
		55	R ₁ = 4-OC ₂ H ₅	R ₂ = 4-Cl
		56	R ₁ = 2-CH ₃	R ₂ = 2-Cl
		57	R ₁ = 4-Cl	R ₂ = H
		58	R ₁ = 4-Cl	R ₂ = 2-Cl
		59	R ₁ = 4-Cl	R ₂ = 3-Cl
		60	R ₁ = 4-Cl	R ₂ = 4-Cl
VII		61	R ₁ = H	—
		62	R ₁ = 4-CH ₃	—
		63	R ₁ = 2-Cl	—
		64	R ₁ = 4-Cl	—
		65	R ₁ = 3,4-Cl ₂	—

2. Results

Chromatographic retention parameters (k) were calculated according to the following relationship:

$$k = \frac{t_R - t_M}{t_M} \quad (2)$$

where t_R and t_M are retention times for a given solute and an unretained compound, respectively.

To describe the effect of surfactant concentration on solutes retention, we applied the Foley equation and four effluents with different SDS concentrations: 0.1, 0.105, 0.11, and 0.12 mol L^{−1}. The obtained results are presented in Figure 1 as the $1/k$ vs. $[M]$ relationships (Equation (1)) for five chosen compounds. The parameters of Equation (1) for all compounds tested, together with coefficients of determination R^2 , are presented in Table 2.

Physico-chemical parameters characterizing the investigated compounds, i.e., the logarithm of the partition coefficient ($\log P_{o/w}$) in the *n*-octanol/water system, the numbers of hydrogen bond donors (HBD), acceptors (HBA), and rotatable bonds (NRB), molecular weight (MW), topological polar surface area (TPSA), polarizability (α), and parachor (P), are presented in Table 3. Also included are the $\log BB$ parameters. All the values were evaluated in silico from molecular structures (ACD Percepta software). The $\log BB^*$ parameters from Table 3 were calculated in our previous studies [75], using the equation derived for 23 structurally similar compounds with known experimental $\log BB$ values. The relationship between both, $\log BB$ and $\log BB^*$, parameters is linear and very good ($R^2 = 0.9010$).

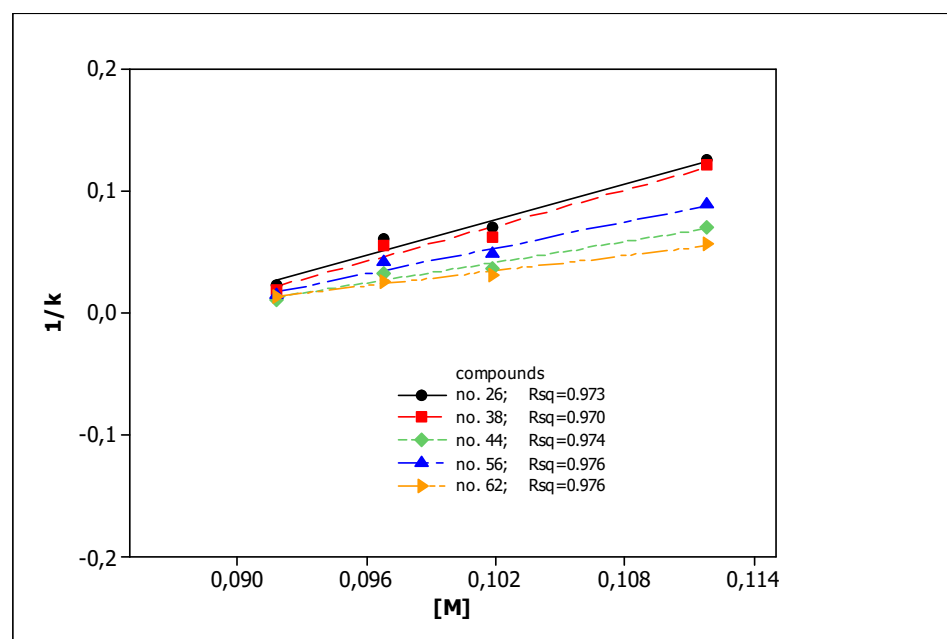


Figure 1. The $1/k$ vs. $[M]$ relationships obtained for compounds 26, 38, 44, 56, and 62 from MLC measurements.

Table 2. Coefficients of determination and parameters of Equation (1) obtained for the particular compounds.

No	$1/k_m$	K_{AM}/k_m	R^2	No	$1/k_m$	K_{AM}/k_m	R^2
1	−0.765	9.066	0.9178	34	−0.329	3.829	0.9615
2	−0.586	6.826	0.9530	35	−0.321	3.685	0.9604
3	−0.758	8.907	0.9110	36	−0.432	5.006	0.9593
4	−0.528	6.157	0.9700	37	−0.425	4.933	0.9442
5	−0.518	6.028	0.9553	38	−0.425	4.870	0.9606
6	−0.406	4.722	0.9748	39	−0.288	3.326	0.9717
7	−1.046	12.573	0.9124	40	−0.214	2.470	0.9692
8	−0.785	9.294	0.9592	41	−0.354	4.119	0.9753
9	−1.163	13.801	0.9618	42	−0.289	3.322	0.9764
10	−0.876	10.274	0.9477	43	−0.225	2.944	0.9724
11	−0.707	8.316	0.9759	44	−0.246	2.822	0.9741
12	−0.760	8.902	0.9302	45	−0.257	2.976	0.9378
13	−0.568	6.593	0.9592	46	−0.206	2.361	0.9705
14	−0.565	7.520	0.9932	47	−0.190	2.198	0.9728
15	−0.509	5.898	0.9696	48	−0.199	2.272	0.9694
16	−0.503	5.825	0.9732	49	−0.213	2.448	0.9695
17	−0.395	4.569	0.9734	50	−0.174	2.001	0.9666
18	−0.599	7.079	0.9622	51	−0.295	3.399	0.9704
19	−0.597	6.995	0.9375	52	−0.211	2.441	0.9718
20	−0.410	4.836	0.9776	53	−0.227	2.606	0.9684
21	−0.594	6.814	0.9473	54	−0.285	3.202	0.9441
22	−0.458	5.274	0.9638	55	−0.191	2.203	0.9636
23	−0.474	5.694	0.9695	56	−0.309	3.549	0.9763
24	−1.131	12.985	0.9193	57	−0.540	6.358	0.9690
25	−0.692	7.917	0.9672	58	−0.180	2.073	0.9521
26	−0.420	4.868	0.9733	59	−0.185	2.118	0.9609
27	−0.639	7.247	0.9662	60	−0.157	1.820	0.9465
28	−0.461	5.307	0.9593	61	−0.241	2.856	0.9792
29	−0.382	4.464	0.9659	62	−0.175	2.051	0.9765
30	−0.451	5.916	0.9656	63	−0.193	2.343	0.9682
31	−0.301	3.477	0.9657	64	−0.151	1.802	0.9488
32	−0.287	3.322	0.9707	65	−0.132	1.550	0.9358
33	−0.479	5.531	0.9187				

Table 3. Characteristics of the tested compounds.

No	log (k_m/K_{AM})	log <i>BB</i>	log <i>BB</i> * [75]	<i>TPSA</i> Å ²	<i>HBD</i>	<i>HBA</i>	<i>NRB</i>	<i>MW</i> g mol ^{−1}	α Å ³	<i>P</i> m ³ mol ^{−1}
1	−0.957	0.117	0.21	48.27	0	5	2	242.28	27.55	497.81
2	−0.834	0.305	0.32	48.27	0	5	2	256.30	29.30	528.90
3	−0.950	0.226	0.26	48.27	0	5	2	276.72	29.37	526.66
4	−0.789	0.270	0.30	48.27	0	5	2	276.72	29.37	526.66
5	−0.780	0.209	0.24	48.27	0	5	2	276.72	29.37	526.66
6	−0.674	0.360	0.34	48.27	0	5	2	311.17	31.20	555.51
7	−1.099	−0.243	−0.16	74.57	0	7	4	286.29	30.27	561.26
8	−0.968	−0.051	−0.04	74.57	0	7	4	300.31	32.02	592.35
9	−1.140	−0.293	−0.28	83.80	0	8	5	316.31	32.57	611.52
10	−1.012	−0.167	−0.20	83.80	0	8	6	330.34	34.40	650.13
11	−0.920	−0.151	−0.12	74.57	0	7	4	320.73	32.09	590.11
12	−0.949	−0.132	−0.10	74.57	0	7	5	300.31	32.09	604.35
13	−0.819	0.055	0.02	74.57	0	7	5	314.38	33.85	635.45
14	−0.876	−0.167	−0.20	83.80	0	8	6	330.34	34.40	599.87
15	−0.771	0.029	0.00	74.57	0	7	5	334.76	33.92	630.96
16	−0.766	−0.033	−0.05	74.57	0	7	5	334.76	33.92	650.13
17	−0.660	0.117	0.05	74.57	0	7	5	369.20	35.74	628.72
18	−0.850	0.038	0.06	61.41	0	6	2	280.31	30.82	628.72
19	−0.845	0.225	0.17	61.41	0	6	2	294.34	33.57	657.57
20	−0.684	0.225	0.17	61.41	0	6	2	294.34	32.57	578.01
21	−0.833	0.417	0.29	61.41	0	6	2	308.37	34.33	609.11
22	−0.722	0.006	−0.04	70.64	0	7	3	310.34	33.12	597.18
23	−0.755	0.003	−0.05	70.64	0	7	3	310.34	33.12	597.18
24	−1.113	0.141	0.11	61.41	0	6	2	314.75	32.64	575.77
25	−0.899	0.194	0.16	61.41	0	6	2	314.75	32.64	575.77
26	−0.687	0.130	0.09	61.41	0	6	2	314.75	32.64	575.77
27	−0.860	0.281	0.19	61.41	0	6	2	349.20	34.47	604.62
28	−0.725	0.297	0.21	61.41	0	6	2	349.20	34.47	604.62
29	−0.650	0.227	0.15	48.27	0	5	2	290.32	33.92	604.97
30	−0.772	0.407	0.26	48.27	0	5	2	304.35	35.68	636.07
31	−0.541	0.407	0.26	48.27	0	5	2	304.35	35.68	636.07
32	−0.521	0.407	0.26	48.27	0	5	2	304.35	35.68	636.07
33	−0.743	0.188	0.05	57.50	0	6	3	320.35	36.23	655.23
34	−0.583	0.172	0.04	57.50	0	6	3	320.35	36.23	655.23
35	−0.566	0.298	0.11	57.50	0	6	4	334.41	38.05	693.84
36	−0.699	0.594	0.38	48.27	0	5	2	318.37	37.43	667.16
37	−0.693	0.331	0.20	48.27	0	5	2	324.76	35.75	633.82
38	−0.682	0.376	0.25	48.27	0	5	2	324.76	35.75	633.82
39	−0.522	0.319	0.19	48.27	0	5	2	324.76	35.75	633.82
40	−0.393	0.465	0.29	48.27	0	5	2	359.21	37.57	662.67
41	−0.615	0.341	0.22	48.27	0	5	3	304.35	35.75	643.58
42	−0.521	0.459	0.28	48.27	0	5	3	338.82	37.57	672.43
43	−0.469	0.459	0.28	48.27	0	5	3	338.82	37.57	672.43
44	−0.451	0.459	0.28	48.27	0	5	3	338.82	37.57	672.43
45	−0.474	0.524	0.33	48.27	0	5	3	318.41	37.57	674.68
46	−0.373	0.712	0.45	48.27	0	5	3	332.44	39.26	705.77
47	−0.342	0.712	0.45	48.27	0	5	3	332.44	39.26	705.77
48	−0.356	0.635	0.38	48.27	0	5	3	352.85	39.26	703.53
49	−0.389	0.635	0.38	48.27	0	5	3	352.85	39.33	703.53
50	−0.302	0.635	0.38	48.27	0	5	3	352.85	39.33	703.53
51	−0.531	0.424	0.19	57.50	0	6	5	348.44	39.88	732.46
52	−0.387	0.602	0.37	57.50	0	6	5	362.47	41.63	763.55
53	−0.416	0.526	0.23	57.50	0	6	5	382.88	41.70	761.31
54	−0.505	0.526	0.23	57.50	0	6	5	382.88	41.70	761.31
55	−0.343	0.526	0.22	57.50	0	6	5	382.88	41.70	761.31
56	−0.550	0.635	0.38	48.27	0	5	3	352.85	39.33	703.53
57	−0.803	0.440	0.27	48.27	0	5	3	338.82	37.57	672.43

Table 3. Cont.

No	$\log(k_m/K_{AM})$	$\log BB$	$\log BB^*$ [75]	$TPSA$ $\text{\AA}^2$	HBD	HBA	NRB	MW g mol^{-1}	α $\text{\AA}^3$	P $\text{m}^3 \text{mol}^{-1}$
58	−0.317	0.551	0.31	48.27	0	5	3	373.27	39.40	701.28
59	−0.326	0.551	0.31	48.27	0	5	3	373.27	39.40	701.28
60	−0.260	0.551	0.31	48.27	0	5	3	373.27	39.40	701.28
61	−0.456	0.459	0.29	48.27	0	5	4	318.37	37.58	682.19
62	−0.312	0.651	0.41	48.27	0	5	4	332.40	39.33	713.29
63	−0.370	0.567	0.34	48.27	0	5	4	352.82	39.40	711.04
64	−0.256	0.551	0.33	48.27	0	5	4	352.82	39.40	711.04
65	−0.190	0.701	0.43	48.27	0	5	4	387.26	41.22	739.89

3. Discussion

3.1. Chromatographic Data

In our previous studies [75], all the compounds presented in Table 1 were analyzed using RP HPLC with three different stationary phases imitating biological partitioning: ODS (octadecylsilyl), IAM (artificial immobilized membrane), and Cholester (immobilized cholesterol). As lipophilicity descriptors, $\log k_w$ parameters were used, describing solute retention in the system with 100% aqueous mobile phase, calculated by linear extrapolation. Presently, to mimic biodistribution, we have used the micellar chromatography technique with SDS as the mobile phase component and the Foley equation to describe solute retention. Very good linear relationships ($R^2 > 0.9$) were obtained for all tested compounds (Figure 1, Table 2), confirming that the Foley equation correctly describes the retention of solutes in the tested chromatographic systems. Unfortunately, due to the strong retention of the tested compounds, the intercepts for all equations are negative. This is inconsistent with the physico-chemical interpretation of the regression coefficient of this equation: the intercept is equal to the reciprocal of k_m , i.e., the retention factor in the system in which the concentration of unbound surfactant ($[M]$) in the effluent is equal to zero. This value may in no case be less than zero. For this reason, we decided to use $\log(k_m/K_{AM})$ values calculated from the slopes of Equation (1) as micellar lipophilicity descriptors of the compounds. The rationale is that both parameters (k_m and K_{AM}) characterize lipophilic properties of solutes: their affinity to the stationary phase modified by the surfactant (k_m) and binding to the micelles (K_{AM}). Moreover, in our previous research [76] on the group of pesticides, we compared both micellar parameters ($\log k_m$ and $\log K_{AM}$), obtaining a very good rectilinear relationship with $R^2 = 0.9724$. To assess the correctness of our deductive reasoning, we examined the correlation between $\log(k_m/K_{AM})$ values and partition coefficients $\log P_{o/w}$ obtained *in silico* from molecular structures of compounds, commonly accepted as lipophilicity descriptors. They were compared with analogous relationships for other chromatographic lipophilicities ($\log k_w$) evaluated for ODS, IAM, and Cholester columns. The graphs presented in Figure 2 show the correct (direct proportion) relationships with moderate fit ($R^2 > 0.6$) but the best one for the micellar parameter ($R^2 = 0.7980$). The correlations between different chromatographic parameters considered as lipophilicity descriptors ($\log k_w$ and $\log(k_m/K_{AM})$) are also moderate— R^2 in the range 0.6002–0.6990. The above relationships confirm that micellar parameters can be considered as lipophilicity descriptors.

3.2. In Silico Data

The compounds investigated have *in silico* $\log BB$ values in the range −0.293–0.712 (Table 3), and they penetrate the blood–brain barrier better ($\log BB > 0$) or weaker ($\log BB < 0$). Without more in-depth research, it is impossible to decide which may be CNS-active. It is clear that CNS activity requires BBB permeation, but some drugs that are not CNS-active may still pass through the BBB and show no activity because they do not interact with any CNS targets. Similarly, some drugs with an expected peripheral site of action may pass through the BBB, leading to undesirable side effects on the CNS. Molar weights of

compounds range from 242.28 to 387.26 g mol⁻¹ (Table 3) and meet the rule formulated by Lipiński and coworkers (The Rule of 5, Ro5) [77], i.e., $MW \leq 500$ g mol⁻¹ or one of the “Rules of Thumb” proposed by Clark [78] ($MW \leq 450$ g mol⁻¹) for brain permeation by drugs and clinical candidates. The numbers of hydrogen bond acceptors $HBA \leq 10$, and the numbers of hydrogen bond donors $HBD \leq 5$. HBDs also fulfill Ro5. The investigated molecules are bases: $HBD = 0$ for all compounds [75], with HBA ranging from five to eight (Table 2). Moreover, the polar surface areas, described by $TPSA$ values, are in the range 48.27–57.50 Å², which meets the next principle given by Clark [78]. For good brain permeation, the polar surface area of the compound should be below a certain limit. In the literature on the subject, there are two differing limits: 90 Å² suggested by van de Waterbeemd et al. [36] and a lower limit of 60–70 Å² proposed by Kelder et al. [33]. The test substances (except compounds from groups II and III) satisfy both limits. Table 3 also provides values of parachor (P) ranging from 497.81 to 763.55 m³ mol⁻¹ and polarizability (α) ranging from 27.55 to 41.70 Å³.

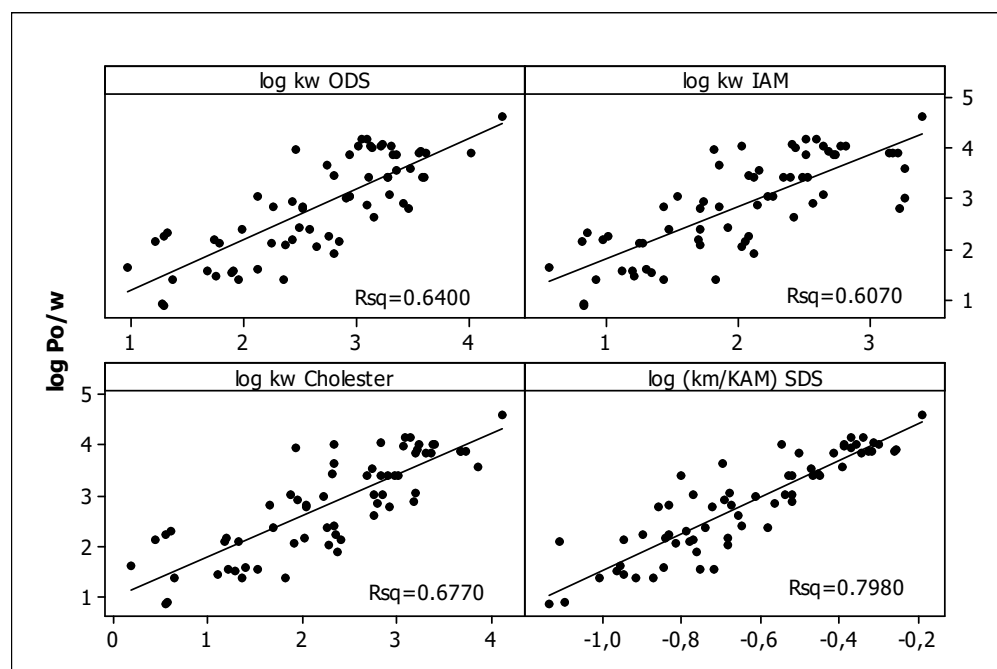


Figure 2. The relationships between $\log P_{o/w}$ and $\log k_w$, ODS, $\log k_w$, IAM, $\log k_w$, Cholesterol [75], and $\log (k_m/K_{AM})$ values.

In our procedure, parameters characterizing the lipophilic, structural, and electronic properties of molecules, i.e., micellar parameter ($\log k_m/K_{AM}$), MW , $TPSA$, HBA , α , and P , will be used as independent variables. To ensure the variables have a minimal impact on each other (to keep the principle of orthogonality), we checked for similarities among them. The results are presented in Figure 3. Here, we can see three groups of strongly correlated descriptors: (I) including $TPSA$ and HBA (99.62%) characterizing the polar nature of the molecule and its ability to form hydrogen bonds; (II) consisting of polarizability, parachor, and molar weight (93.16%) related to the size of the molecule; and a single-element group (III) containing NRB describing molecule flexibility.

3.3. Establishment of Quantitative Structure–Activity Relationships

The establishment of QSAR models involves the use of reliable and accurate input data, selection of relevant descriptors, use of appropriate software, and validation of the suggested model [79,80]. Our models have involved descriptors characterizing solutes' lipophilicity (micellar $\log (k_m/K_{AM})$ values), polarity ($TPSA$, HBA), flexibility (NRB), and size (MW , α , P). We used *in silico* (HBA , NRB , MW , $TPSA$, α , P , $\log BB$, and $\log BB^*$)

and in vitro ($\log k_m/K_{AM}$) data. The models were produced using the multiple linear regression (MLR) technique on a database that consisted of 65 recently discovered drug-like compounds. The linear quantitative structure–activity relationships (QSARs) were presented for the modeling of $\log BB$ values. The developed models were validated by leave-one-out cross-validation (LOOCV).

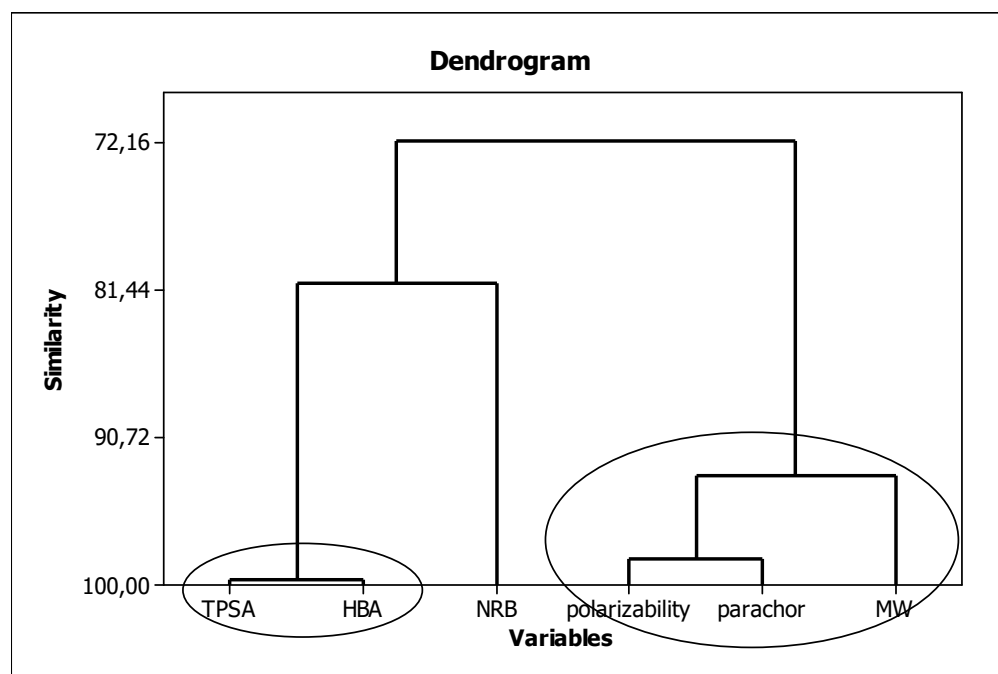


Figure 3. Similarities between the in silico molecular descriptors.

Validation is a necessary step to establish the quality of a QSAR model [81–83]. In our investigations, traditional validation metrics were applied: the mean squared error (MSE), the coefficient of determination (R^2), the determination coefficient adjusted (R^2_{adj}), and the determination coefficient predicted (R^2_{pred}). R^2_{adj} is used to compare the goodness-of-fit for regression models that contain differing numbers of independent variables while R^2_{pred} determines how well a regression model makes predictions. These coefficients (R^2 , R^2_{adj} , R^2_{pred}) have values between zero and one, and the closer to one, the more accurate the model. The MSE is used to assess the predictive ability and accuracy of the model, and models with small MSE values yield more highly reliable predictions. The derived models were compared and assessed by leave-one-out cross-validation (LOO), and the resulting determination coefficient (Q^2_{LOO}) and $PRESS$ were calculated (Table 4). $PRESS$ is a good estimate of the real prediction error of the model. It assesses a model's predictive ability and, in general, the smaller the $PRESS$ value, the better the model's predictive ability [84]. The calculated global $PRESS$ value must be lower than the sum of the squares of the response values of the total observations (SS). This proves that the developed models predict better than chance [83]. A reasonable QSAR model should have Q^2_{LOO} values greater than 0.6 or the ratio of $PRESS/SS$ smaller than 0.4 [84]. QSAR models are only valid in the domain they were validated [85] so the determination of applicability domain (AD) is of great importance [86]. AD is a space of (physico-chemical) information, on which the model has been developed and for which it is applicable to make predictions for new compounds. In the present work, we used the leverage approach (Williams plot) where the warning leverage, h^* , was calculated according to:

$$h^* = \frac{3(p+1)}{n} \quad (3)$$

where n is the total number of samples in, and p is the number of descriptors involved in the correlation [87].

Table 4. Statistics of the established models M1-M12, M5*, M11*, and M12*: the coefficient of determination (R^2 , Q^2), the determination coefficient adjusted (R^2_{adj}), the determination coefficient predicted (R^2_{pred}), the predicted residual error sum of squares ($PRESS$), the variance inflation factor (VIF), the sum of squared differences from the mean (SS), the mean squared error (MSE), F -value, p -value; * the highest value.

Model	R^2	R^2_{adj}	R^2_{pred}	$PRESS$	VIF^*	SS	MSE	F	p	Q^2_{LOO}	$PRESS_{LOO}$
M1: log BB vs. ($\log(k_m/K_{AM})$, $TPSA$, NRB , α)	0.9202	0.9149	0.9088	0.3889	5.3	4.2636	0.0057	173.0	0.00000	–	–
M2: log BB vs. ($\log(k_m/K_{AM})$, $TPSA$, NRB , P)	0.9070	0.9008	0.8931	0.4556	4.7	4.2636	0.0066	146.3	0.00000	–	–
M3: log BB vs. ($\log(k_m/K_{AM})$, $TPSA$, NRB , MW)	0.8943	0.8873	0.8799	0.5119	5.0	4.2636	0.0075	129.9	0.00000	–	–
M4: log BB vs. ($\log(k_m/K_{AM})$, HBA , NRB , α)	0.9260	0.9210	0.9150	0.3625	5.4	4.2636	0.2774	187.6	0.00000	–	–
M5: log BB vs. ($\log(k_m/K_{AM})$, HBA , NRB , P)	0.9109	0.9049	0.8971	0.4385	4.7	4.2636	0.0063	153.3	0.00000	0.9109	0.4385
M6: log BB vs. ($\log(k_m/K_{AM})$, HBA , NRB , MW)	0.8914	0.8841	0.8764	0.5268	4.8	4.2636	0.0077	123.1	0.00000	–	–
M7: log BB^* vs. ($\log(k_m/K_{AM})$, $TPSA$, NRB , α)	0.8523	0.8425	0.8310	0.3247	5.3	1.9212	0.0047	86.6	0.00000	–	–
M8: log BB^* vs. ($\log(k_m/K_{AM})$, $TPSA$, NRB , P)	0.8508	0.8409	0.8297	0.3271	4.7	1.9212	0.0048	85.5	0.00000	–	–
M9: log BB^* vs. ($\log(k_m/K_{AM})$, $TPSA$, NRB , MW)	0.8529	0.8430	0.8324	0.3219	5.0	1.9212	0.0047	86.9	0.00000	–	–
M10: log BB^* vs. ($\log(k_m/K_{AM})$, HBA , NRB , α)	0.8682	0.8595	0.8489	0.2904	5.4	1.9212	0.0042	98.8	0.00000	–	–
M11: log BB^* vs. ($\log(k_m/K_{AM})$, HBA , NRB , P)	0.8659	0.8569	0.8466	0.2941	4.7	1.9212	0.0043	96.8	0.00000	0.8659	0.2947
M12: log BB^* vs. ($\log(k_m/K_{AM})$, HBA , NRB , MW)	0.8662	0.8572	0.8473	0.2933	4.8	1.9211	0.0043	97.1	0.00000	0.8662	0.2933
M5*: log BB vs. ($\log(k_m/K_{AM})$, HBA , P)	0.9087	0.9042	0.8994	0.4290	4.7	4.2636	0.2178	202.4	0.00000	0.9087	0.4290
M11*: log BB^* vs. ($\log(k_m/K_{AM})$, HBA , P)	0.8656	0.8590	0.8513	0.2858	4.7	1.9212	0.0042	131.0	0.00000	0.8656	0.2858
M12*: log BB^* vs. ($\log(k_m/K_{AM})$, HBA , MW)	0.8661	0.8595	0.8516	0.2852	4.1	1.9212	0.0042	131.5	0.00000	0.8515	0.2852

We searched for relationships between solute property (SP), i.e., $\log BB$, and its lipophilicity, molar size, and flexibility descriptors, that is:

$$\log SP = a_0 + a_1 \text{lipophilicity} + a_2 d_I + a_3 d_{II} + a_4 d_{III} \quad (4)$$

where a_0 – a_4 are regression coefficients, and d_I , d_{II} , and d_{III} are molecular descriptors from group I, II, and III, respectively.

Accounting for possible combinations of independent variables (descriptors from groups I, II, and III) for both parameters ($\log BB$ and $\log BB^*$), twelve models denoted as M1–M6 (for $\log BB$) and M7–M12 (for $\log BB^*$) have been obtained. Table 4 contains the R^2 , R^2_{adj} , R^2_{pred} , $PRESS$, and VIF (variance inflation factors) values calculated for these models, which became the basis for their preliminary evaluation leading to the selection of the most promising ones. Very high R^2 values (>0.8) indicate that all M1–M12 equations are very good for modeling the data included (good fit of dataset). All R^2_{pred} values are >0.6 , indicating high predictive ability of the models. The decrease in the value of R^2_{adj} compared to the values of R^2 ranges from 0.0053 to 0.0099 units. High predictive abilities of the models are also confirmed by small $PRESS$ values, which are in the range 0.2904–0.5268. Moreover, the ratios of $PRESS/SS$ are smaller than 0.4. Variance inflation factors VIF should not exceed five to ensure that descriptors are moderately correlated. Some VIF values presented in Table 4 are equal to five or are slightly higher. For this reason, the M1, M3, M4, M7, M9, and M10 models were excluded from further analysis. Of the remaining six models, the most favorable were those with the highest R^2 and R^2_{pred} values and the lowest $PRESS$. The analysis of the calculated statistical parameters leads to the M5, M11, and M12 models as the most satisfactory. The models selected are as follows:

$$\text{M5: } \log BB = 0.253(0.232) + 0.198(0.091) \log(k_m/K_{AM}) - 0.160(0.023) HBA - 0.019(0.016) NRB + 0.002(0.000) P; \quad (5)$$

$$\text{M11: } \log BB^* = 0.842(0.191) + 0.106(0.075) \log(k_m/K_{AM}) - 0.148(0.019) HBA - 0.004(0.012) NRB + 0.0004(0.000) P; \quad (6)$$

$$\text{M12: } \log BB^* = 0.911(0.149) + 0.103(0.075) \log(k_m/K_{AM}) - 0.158(0.020) HBA + 0.002(0.011) NRB + 0.001(0.000) MW \quad (7)$$

Figures 4–6 present graphical results of LOO cross-validation of models M5, M11, and M12. The plots present in segments B illustrate the standard coefficients of the equations of selected models (Equations (5)–(7)). They explain both the direction and strength of the impact of a given descriptor on the calculated biological parameter. The correlations shown in segments A illustrate the relationships between the actual and predicted response, i.e., between $\log BB$ or $\log BB^*$ values from Table 3, and these predicted by the QSAR models were developed (Equations (5)–(7)). The applicability domain (AD) was also evaluated and visualized as the Williams plots (segments C). The results proved that the obtained models are valid within the domain for which they were developed.

The results (Figures 4B, 5B and 6B) indicate that the NRB parameter seems to be of negligible importance in the case of BBB permeation. Therefore, it was checked whether the omission of this descriptor would affect the evaluation of the derived models. The rationale is to capture the most important properties of compounds and build them into the QSAR model without overfitting the data. The results are presented as the following QSAR equations:

$$\text{M5*}: \log BB = 0.406(0.196) + 0.181(0.091) \log(k_m/K_{AM}) - 0.180(0.016) HBA + 0.002(0.000) P; \quad (8)$$

$$\text{M11*}: \log BB^* = 0.874(0.159) + 0.102(0.074) \log(k_m/K_{AM}) - 0.152(0.013) HBA + 0.0004(0.000) P; \quad (9)$$

$$\text{M12*}: \log BB^* = 0.903(0.139) + 0.108(0.069) \log(k_m/K_{AM}) - 0.156(0.013) HBA + 0.001(0.000) MW \quad (10)$$

The analysis of statistics of the above models, i.e., lower R^2_{adj} , R^2_{pred} , and $PRESS$ values (Table 4), indicates that the new models (omitting the NRB) are better for predicting

substance permeation through the BBB. The new models were also cross-validated, and the results are presented graphically in Figures 7–9.

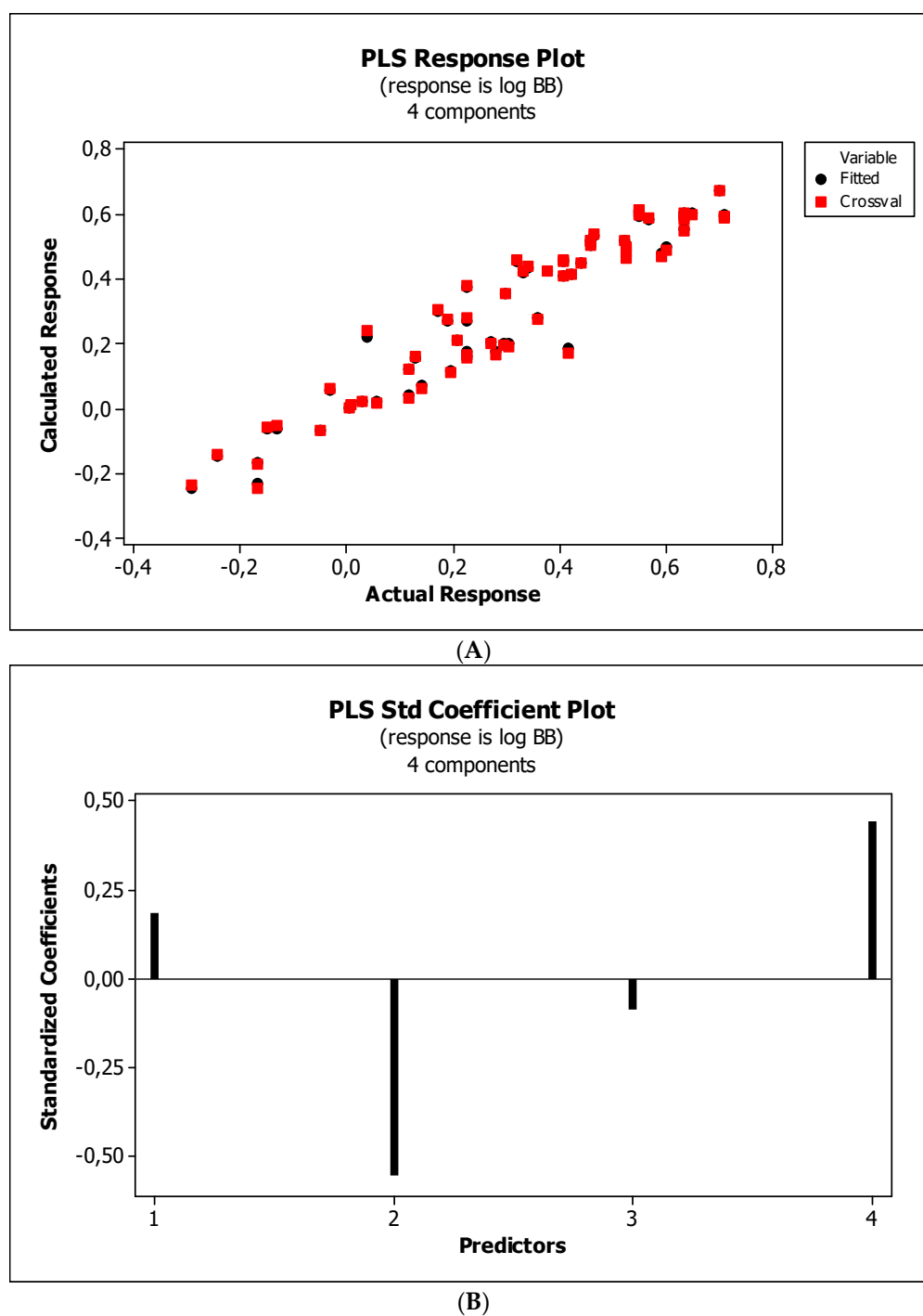


Figure 4. Cont.

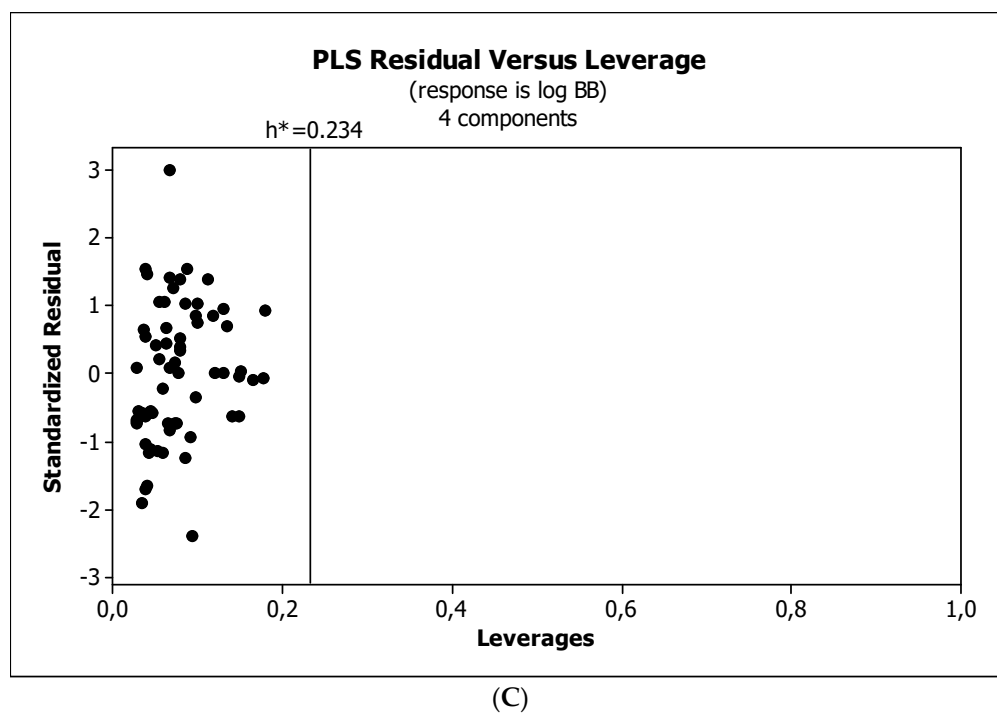


Figure 4. (A) Model M5: the response plot. (B) Model M5: the standardized coefficients plot. (C) Model M5: Williams plot.

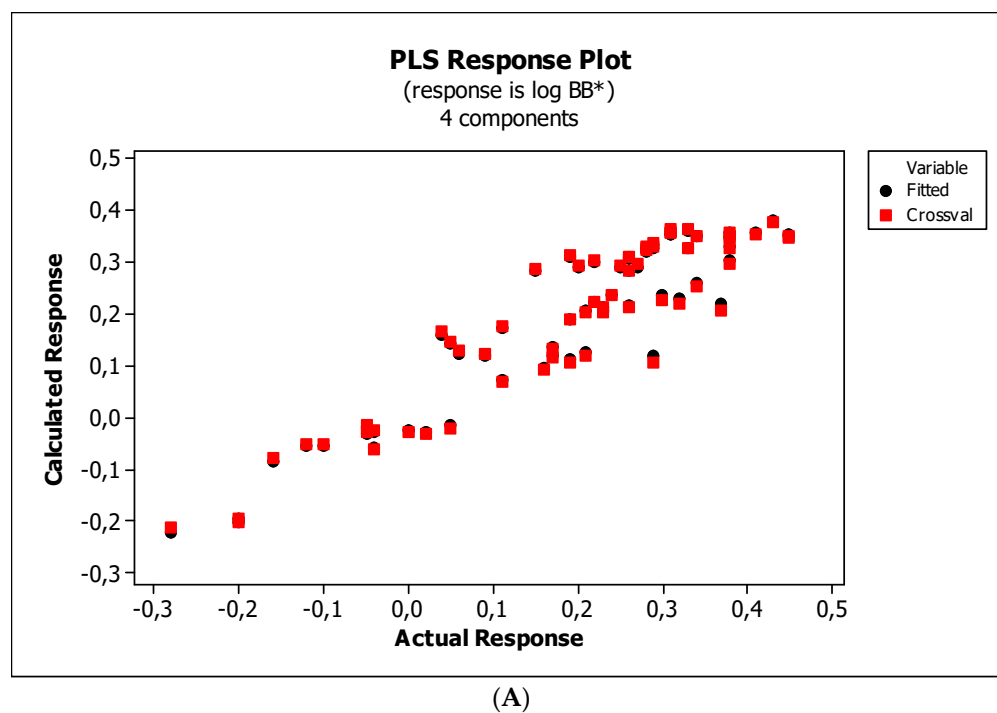


Figure 5. Cont.

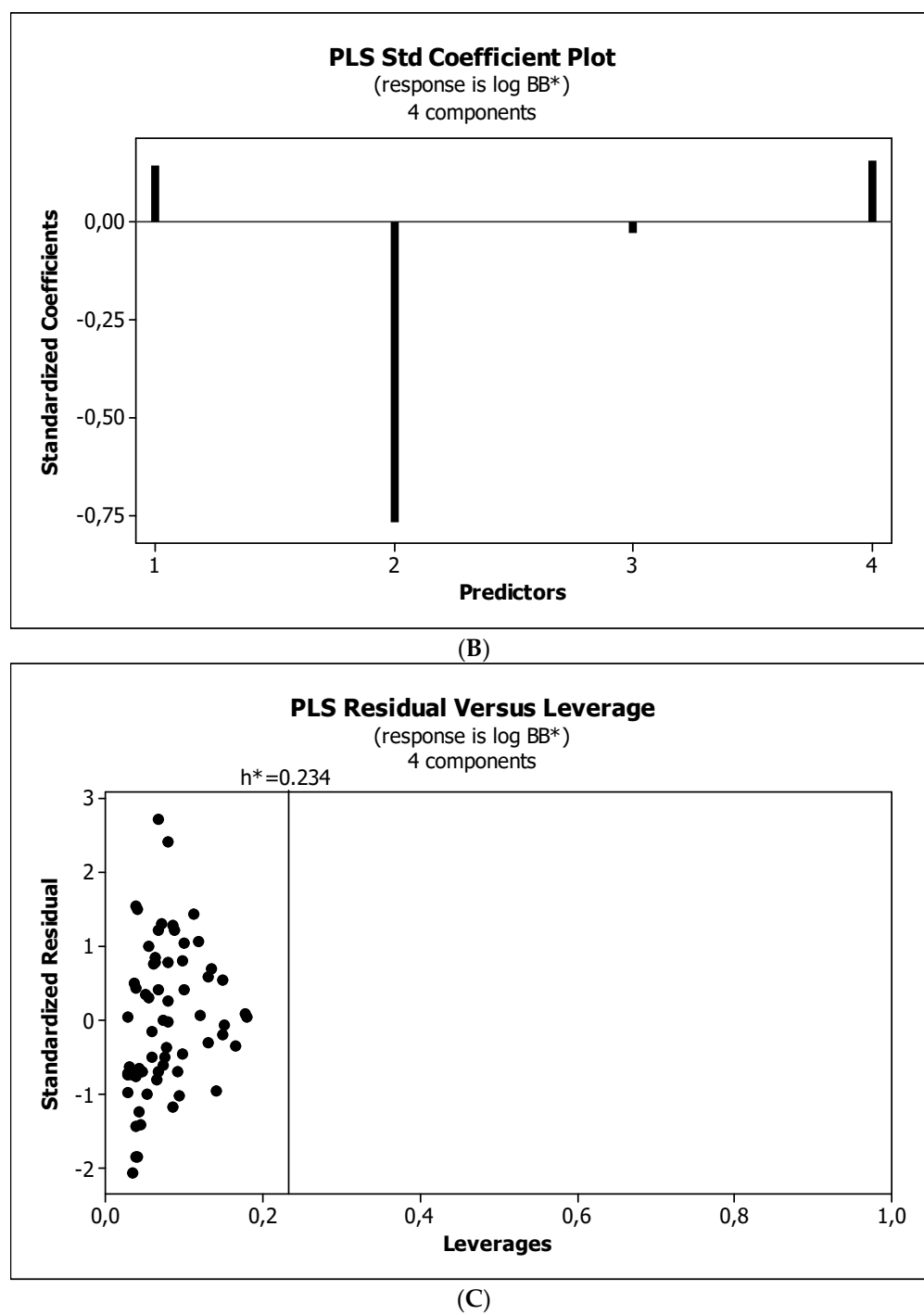


Figure 5. (A) Model M11: the response plot. (B) Model M11: the standardized coefficients plot. (C) Model M11: Williams plot.

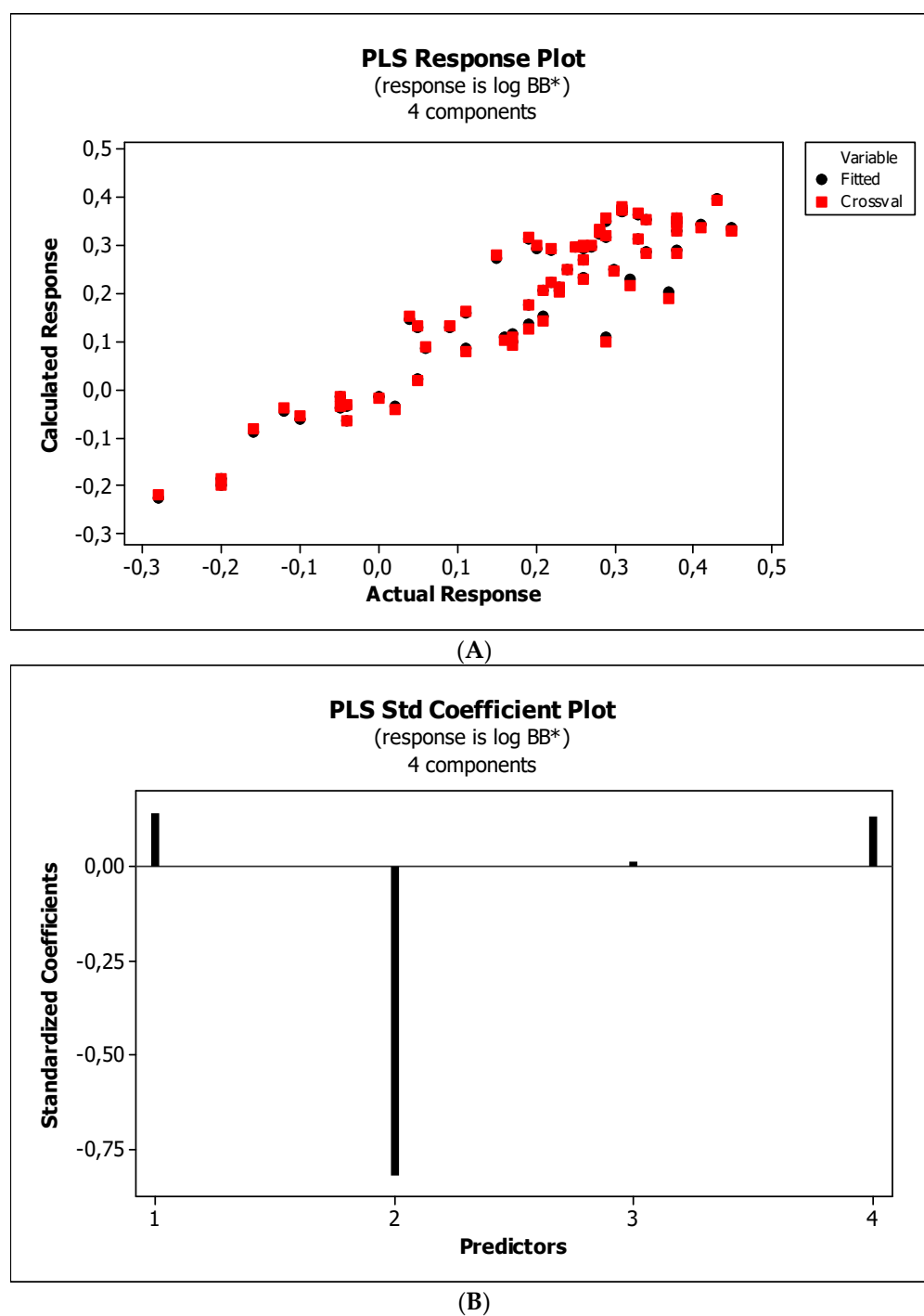


Figure 6. Cont.

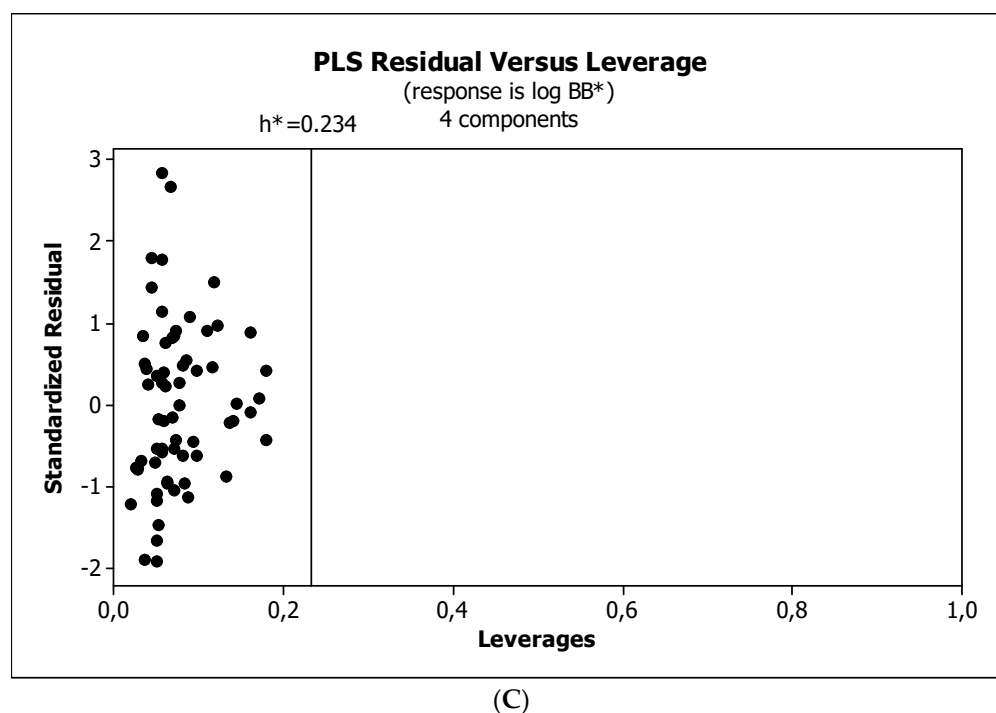


Figure 6. (A) Model M12: the response plot. (B) Model M12: the standardized coefficients plot. (C) Model M12: Williams plot.

3.4. Interpretation of Descriptors

3.4.1. Lipophilicity

All derived models predict an increase in the BBB permeation with an increase of the substance lipophilicity. Thus, lipophilic compounds have a greater BBB permeability than hydrophilic. However, this influence is not indicated as dominant. Lipophilicity is undoubtedly an important parameter affecting BBB penetration, and it is the base parameter used in different QSARs modeling. Generally, transport of small molecules through membranes occurs via passive diffusion: a molecule dissolves in the phospholipid bilayer, diffuses across it, and then dissolves in the aqueous solution at the other side of the membrane. This process is closely related to the lipophilicity of the molecule: it cannot be too lipophilic, because it will not dissolve in the aqueous environment surrounding the bilayer on both sides; however, it also cannot be too hydrophilic, because it will not penetrate the lipid bilayer. Compounds investigated in this research are moderately lipophilic. Their lipophilicity, as assessed by $\log P_{o/w}$, ranges from 0.868 to 4.638, for which a positive effect on $\log BB$ is always expected.

3.4.2. HBA

Our results indicate *HBA* as the dominant factor affecting the $\log BB$ values. As the number of hydrogen bond acceptors in the molecule increases, its ability to permeate the BBB decreases. Solute polarity and the ability to form hydrogen bonds increase its solubility in the aqueous environment of the membrane, and highly polar molecules do not easily enter the hydrophobic environment of the BBB. In most QSARs models, the dominant descriptor of molecules' polarity is the polar surface area. Clark [78] and Kelder et al. [33] presented linear regression between $\log BB$ and *PSA* only, for a group of 45 drugs. In our models, this parameter was also used (M1-M3 and M9), but due to the statistical evaluation the models including *HBA* turned out to be more accurate. However, it should be noted that the *TPSA* values of the test compounds meet the requirements for active substances. The highest *TPSA* value is $83.80 \text{ \AA}^2$, and the number of hydrogen bond acceptors *HBA* does not exceed 10.

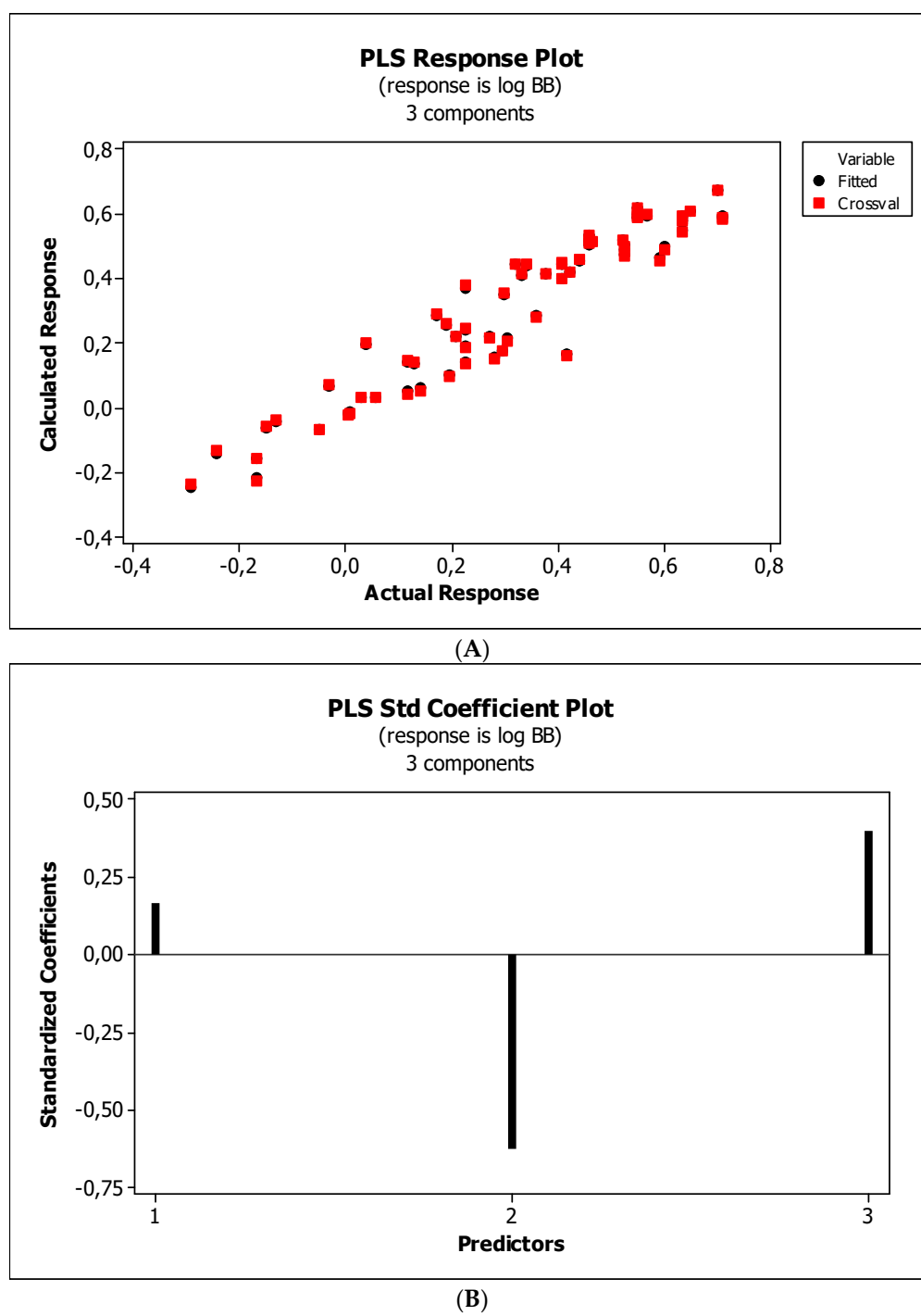


Figure 7. Cont.

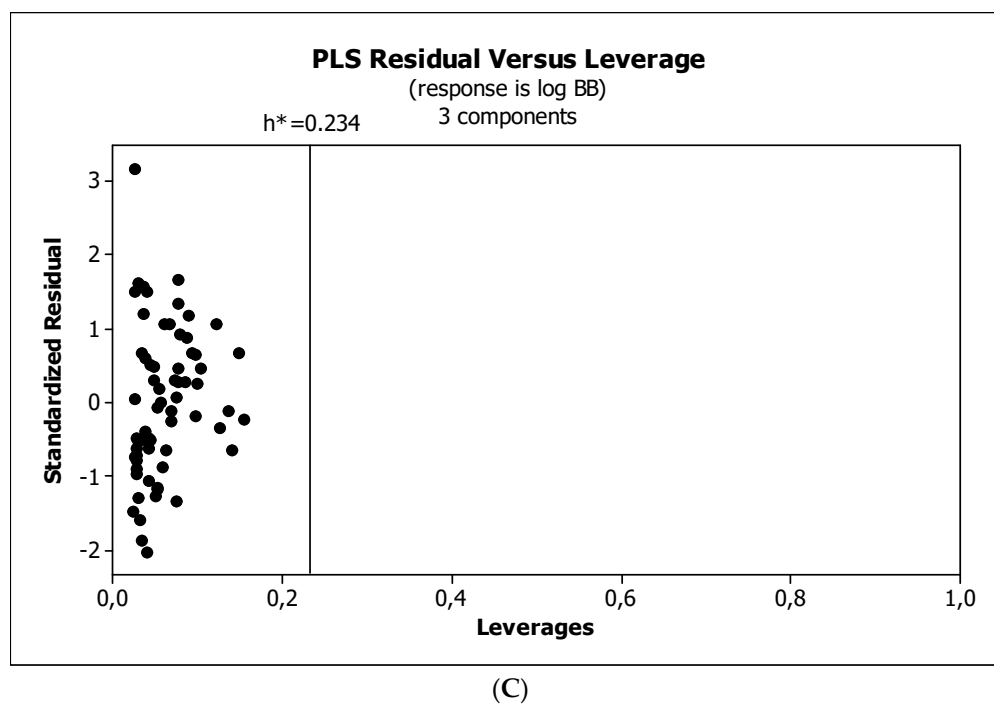


Figure 7. (A) Model M5*: the response plot. (B) Model M5*: the standardized coefficients plot. (C) Model M5*: Williams plot.

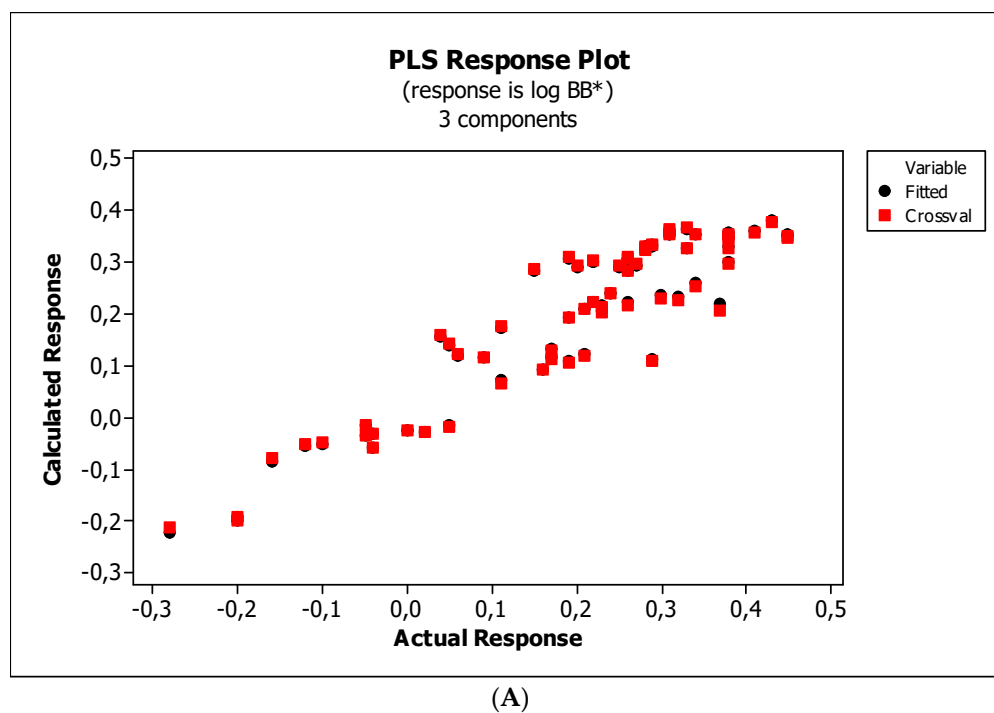


Figure 8. Cont.

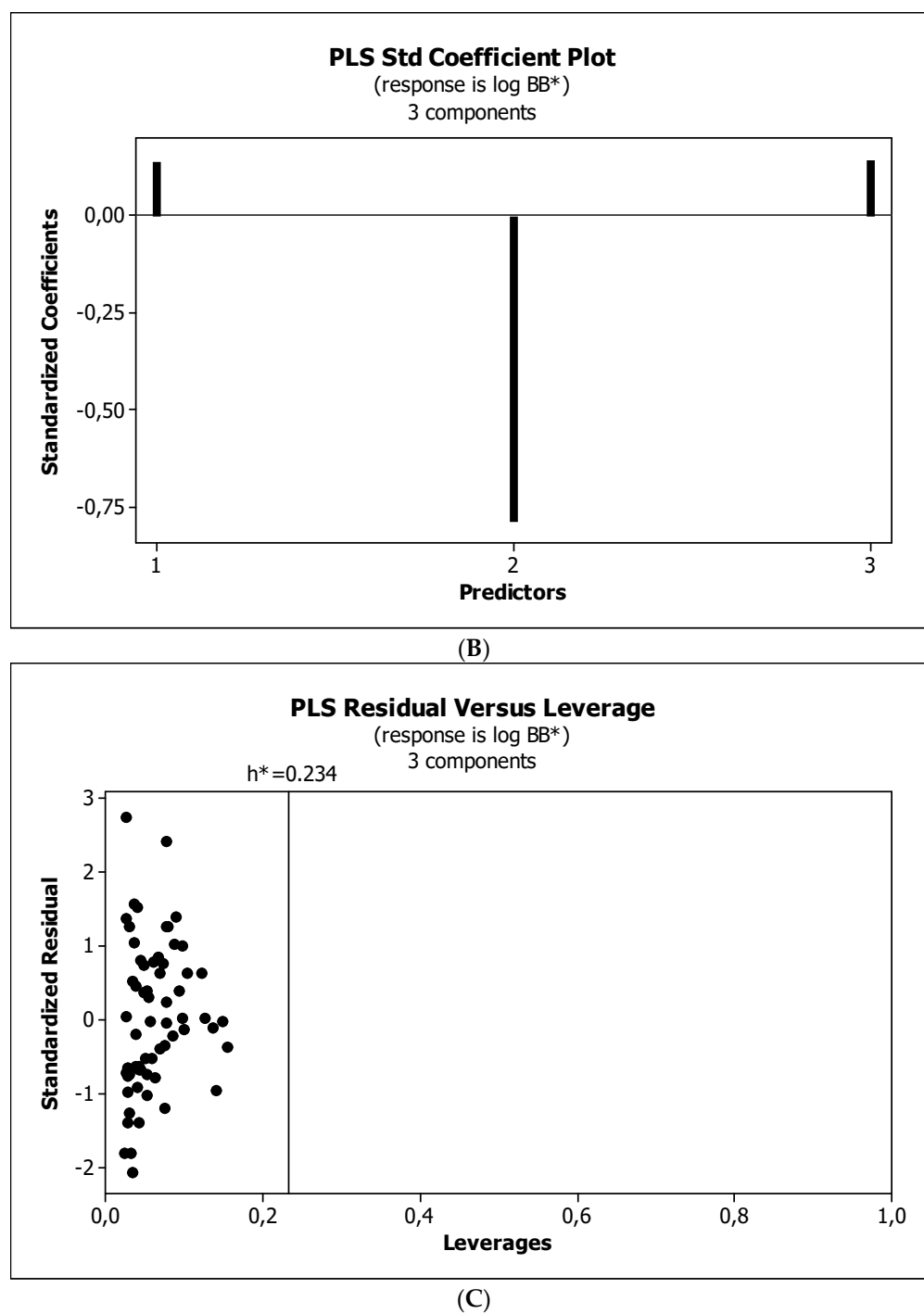


Figure 8. (A) Model M11*: the response plot. (B) Model M11*: the standardized coefficients plot. (C) Model M11*: Williams plot.

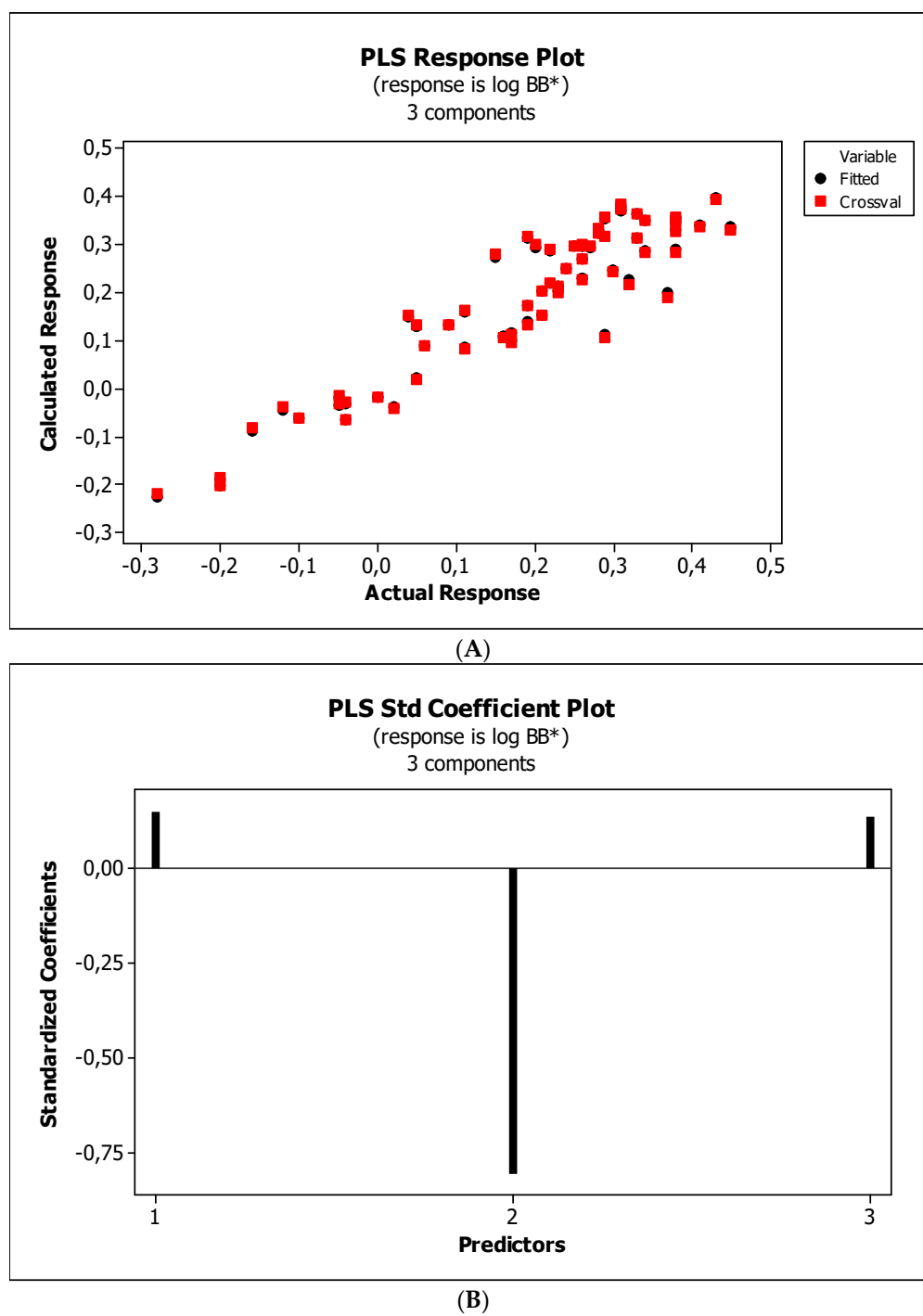


Figure 9. Cont.

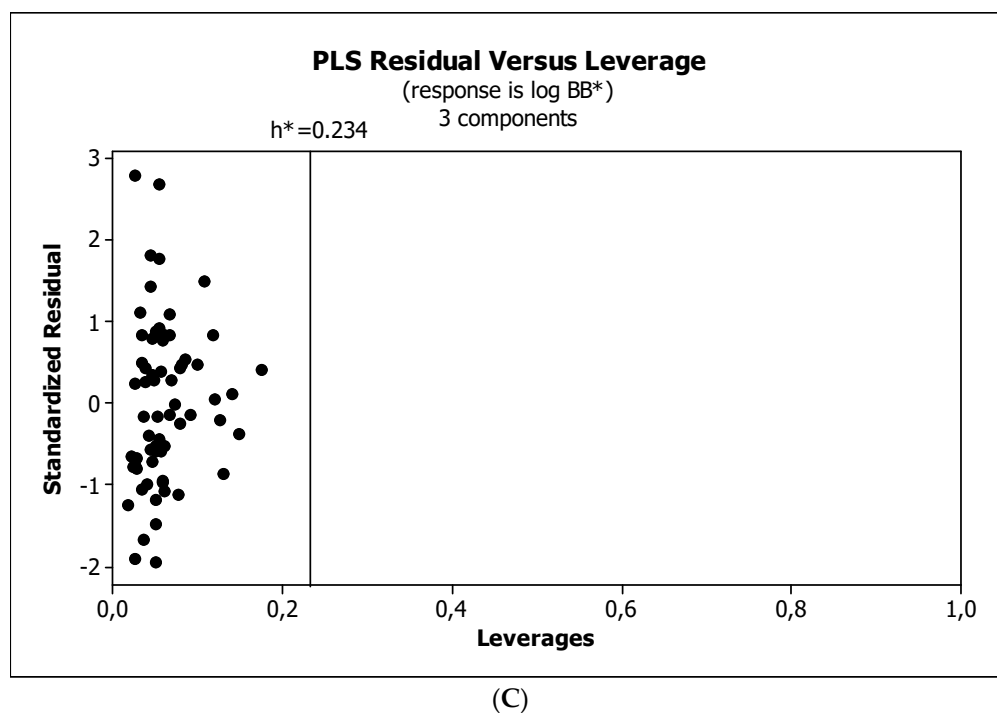


Figure 9. (A) Model M12*: the response plot. (B) Model M12*: the standardized coefficients plot. (C) Model M12*: Williams plot.

3.4.3. Molecular Size

In this study, three descriptors for molecular size were proposed, i.e., molecular weight MW , polarizability α , and parachor P . In selected models (M5*, M11*, and M12*), MW and α and P turned out to be the most appropriate. The effect of molecular size on the BBB permeation ($\log BB$ and $\log BB^*$ values) is similar to that observed for lipophilicity, i.e., with the increase of MW or P values, the permeation of the test substances through the BBB increases. This is in contrast to the results obtained by different researchers who noticed the negative effect of molecule size on compounds' permeation through biological membranes [88–90]. The positive effect of molecular size on the $\log BB$ (and $\log BB^*$) values observed herein could be explained by the partition mechanism of this process. Similarly, Platts et al. [91] obtained the positive effect of molecular size on the permeability through the skin. This relationship is a reflection of the correlation between the size of the molecule and its lipophilicity. It is especially important in the case of low molecular weights ($MW < 400 \text{ mg mol}^{-1}$), such as those in the presented research. Kouskoura et al. [12] noted that moderately increased molecular weight of the compound guarantees that its lipophilicity is sufficient to dissolve in the phospholipid bilayer and enter the BBB via passive diffusion. The same effect was observed in our previous research [92].

3.4.4. Flexibility

Research reveals that blood–brain barrier partitioning is governed not only by solute lipophilicity and polarity but also solute flexibility, solute–membrane flexibility, and solute–membrane binding. In general, ease in traversing the membrane depends on the flexibility of a compound. Limited flexibility can be considered as a merit, and higher flexibility can be proved to be a demerit [93]. The increase in BBB penetration of the solute with increasing solute flexibility was described by Iyer et al. [3]. Veber et al. [94] have found that increasing solute molecular flexibility (measured by the number of rotatable bonds) promotes a decrease in oral bioavailability. It suggests a parabolic relationship between $\log BB$ and molecular flexibility. That is, some “amount” of flexibility enhances $\log BB$, but too much flexibility will diminish $\log BB$. Our research (Figures 4B, 5B and 6B) indicates a slight and ambiguous (positive for M12 and negative for M5 and M11 models) influence of the

NRB value on the penetration of the tested substances through the blood–brain barrier. The numbers of rotatable bonds calculated for tested compounds are in the range from two to six, with average value equal to 3.2. Probably, in the case of the investigated molecules, these values are close to the maximum of the aforementioned parabolic relationship. Ultimately, NRB was considered a negligible factor and omitted in subsequent QSAR models.

4. Materials and Methods

4.1. Reagents and Materials

Isopropanol and acetonitrile (both HPLC grade), as well as sodium dodecyl sulfate SDS (for synthesis), were supplied from Merck (Lublin, Poland). Anhydrous citric acid ($C_6H_8O_7$) and disodium phosphate (Na_2HPO_4)—both pure—were purchased from POCh (Lublin, Poland). Deionized water was produced using the Direct-Q3 UV system (Millipore, Warsaw, Poland).

4.2. Instrumental

Shimadzu Vp (Shimadzu, Izabelin, Poland) liquid chromatographic system was used in the measurements. It was equipped with LC 10AT pump, SPD 10A UV–Vis detector, SCL 10A system controller, CTO-10 AS chromatographic oven, and Rheodyne injector valve with a 20 μ L loop. A Spherisorb ODS-2 column, 125 $\times$ 4 mm i.d., 5 μ m (Merck, Lublin, Poland) was applied as the stationary phase.

4.3. Chromatographic Conditions

As the mobile phases buffered, SDS mixtures (0.10; 0.105, 0.11, and 0.12 mol L^{−1}) with 7% (v/v) addition of isopropanol were used. The buffer was prepared from 0.01 mol L^{−1} solutions of disodium phosphate and citric acid, and the pH 7.4 value was fixed before mixing with an organic modifier. The flow rate was 1 mL min^{−1}. Solutes samples were dissolved in acetonitrile—c.a. 0.005 mg mL^{−1}. The compounds were detected under UV light at λ_{max} 254 nm. All measurements were carried out at 25 °C. The dead time values were measured from non-retained compound (e.g., sodium chloride) peaks. All reported *k* values are the average of at least three independent measurements.

4.4. In Silico Calculations

Molecular weight (MW), topological polar surface area (TPSA), polarizability (α), parachor (*P*), the numbers of hydrogen bond donors (HBD), acceptors (HBA), and rotatable bonds (NRB), the partition coefficient in the *n*-octanol/water system ($\log P_{o/w}$), and the $\log BB$ values were calculated by ACD/Percepta software, version 1994–2012 (ACD/Labs, Advanced Chemistry Development, Inc., Toronto, ON, Canada).

4.5. Statistical Analysis

Linear regression (LR), multiple linear regression (MLR), and leave-one-out cross-validation (LOOCv) were conducted using the statistical software Minitab 16.2.4.0, version 1991–2004 (Minitab Inc., State College, PA, USA).

5. Conclusions

The linear quantitative structure–activity relationship models are presented for the modeling and prediction of the BBB permeation of heterocyclic drug-like molecules with promising activity. The models were produced using the multiple linear regression technique on a database that consisted of 65 recently discovered compounds. Among the different lipophilicity, polarity, electronic, and molecular size descriptors that were considered as inputs to the model, four variables were selected, i.e., micellar parameter characterizing the solutes lipophilicity $\log(k_m/K_{AM})$, the number of hydrogen bond acceptors HBA connected with polarity, and parachor or molecular weight (MW), describing the molecular size. The rationale was to combine in the model in vitro (micellar lipophilicity parameters $\log(k_m/K_{AM})$) and in silico (α , MW, *P*) data. The accuracy of the proposed

MLR models was illustrated using LOO cross-validation. The predictive ability of the developed models was found to be satisfactory and could be used for designing a similar set of heterocyclic compounds.

Our research confirmed that solute polarity is one of the most important properties affecting the BBB permeation. The increase of *HBA* values decreases the log *BB* (or log *BB**) values. In the QSARs models established in our studies, the number of *HBA*s was indicated as the dominant factor. The log *BB* (as well as log *BB**) values increase with micellar (chromatographic) log (k_m/K_{AM}) parameters. This means that more lipophilic drugs have a greater BBB permeability than less lipophilic. This is not the same as their CNS activity, because some compounds that are CNS-inactive may still pass through the BBB and show no activity because they do not interact with any CNS targets. Molecule size descriptors (*MW*, *P*) also increase the penetration of tested substances through the blood–brain barrier. Generally, polarity, lipophilicity, and molecular size are the most important characteristics of the investigated substances in modeling the permeation of the blood–brain barrier. The results obtained for our heterocyclic compounds indicate that less polar, more lipophilic, and bigger-size molecules partition more readily into the brain.

Our investigation shows the undoubted advantages of micellar chromatography which, combined with computational techniques, enable the prediction of BBB permeation with a high probability. This is an important achievement, especially in screening new potential drugs. It allows the reduction of unethical and expensive animal testing and respects the principles of Green Chemistry.

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