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Prediction of Caco-2 cell permeability using partial least squares multivariate analysis

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The permeability across Caco-2 cell monolayers of structurally diverse compounds were predicted using computed molecular descriptors and multivariate Partial Least Squares (PLS). The molecular descriptors including log polarization, log solvent accessible surface area, hydration energy, heat of formation, and dipole moment were calculated with Hyperchem and ChemPlus QSAR programs for Windows. Other physicochemical properties such as hydrogen acceptor for oxygen atoms, hydrogen acceptor for nitrogen atoms, hydrogen bond donors, hydrogen bond forming ability, molecular weight, and log distribution coefficient were also used as descriptors. Cross validation with internal test set and prediction with external test set indicated the usefulness of the derived model for Caco-2 cell permeability. Hydrogen bonding is one of the important factors associated with permeability. While increased logD and hydration energy facilitate permeability, an increased dipole moment of molecules has a negative effect on permeability.

1. Introduction

The use of Caco-2 cell monolayers has increased in popularity as a surrogate marker for *in vivo* intestinal permeability in humans [1–3]. The apparent permeability in Caco-2 cells shows good correlation with *in vivo* human absorption and can be used to predict absorption of compounds regardless of transport mechanism, transcellular, paracellular or carrier-mediated [4]. The oral absorption can also be predicted by *in vivo* animal models, however, these methods are more expensive, time-consuming and sometimes controversial. Thus, at present, the Caco-2 cell permeability model is generally used as a screen for selection of new chemical entities for drug discovery and development programs. The ability to predict Caco-2 cell permeability therefore has a scientific value. The relationship between Caco-2 cellular permeability and physicochemical properties [5–7], molecular surface properties [8] and capacity factors from immobilized artificial membrane (IAM) columns [9] has been reported.

For drug design, it is of a great value if the optimum physicochemical properties for new drug structures can be predicted earlier using a computational method. Thus it is not necessary to synthesize new compounds for testing the required physicochemical properties. The calculation and prediction of Caco-2 cell permeability using Molsurf parametrization has been previously reported [6], however, small size of data set (only 17 compounds) was examined in that study. In this paper, the relationship between the Caco-2 cell permeability and molecular properties calculated by computational methods were investigated using Partial Least Squares or Projections to Latent Structures (PLS) multivariate analysis. Fifty-one structurally diverse compounds with a variety of physicochemical characteristics and different transport routes (transcellular and paracellular transports) were examined.

2. Investigations, results and discussion

The log of Caco-2 cell permeability coefficient, logD [5], physicochemical and calculated molecular parameters of all compounds are summarized and presented in Table 1.

The goodness-of-fit of multivariate model can be expressed as a root mean square error (RMSE)

$$\mathrm{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}$$

where $\hat{y}_i$ and y_i represent the calculated and the experimental value of Caco-2 cell permeability coefficient, respectively, and N is a number of samples. The value of RMSE is an indication of the average error in the analysis for each set.

The statistical results obtained for the PLS model 1 (training set 1) and model 2 (training set 2) are summarized in Table 2. The optimum number of PCs for both models is three. It can be observed that the better values of correlation coefficient, RMSEs for calibration and validation are obtained for model 1. The predictability using PLS model 1 is also better since the RMSE for prediction of the same samples (external test set 2) is lower as shown in Table 2. The calculated and predicted results of PLS analyses of models 1 and 2 were shown in Table 3. Plots of experimental vs. calculated/prediction permeabilities for models 1 and 2 are shown in the Fig.

Yazdanian et al. recently investigated the correlation of distribution coefficients in three solvent systems and Caco-2 cell permeability of these 51 compounds [5]. It was reported that good correlation could not be obtained with simple mathematics. In this study, the PLS analyses of the same data set provided both good correlation between physicochemical properties and Caco-2 permeability and also prediction of permeability by removing some compounds (1, 3, 6, 9, 35, 45) considered as outliers. It is noteworthy that the Caco-2 permeability of these compounds are quite low ranging from −6.29 to −6.72 (log scale). Compounds with high $\log P_{caco\text{-}2}$ values are better predicted. The predictive ability of model 1 with RMSEs of 0.414 and 0.404 (test sets for 22 and 6 samples, respectively) are comparable to that reported by Norinder et al. (RMSE = 0.409, test set for 8 samples) [6]. The extended model (model 2) also provides a parallel predictability with the RMSE for prediction of 0.550.

A good model for the prediction of human skin permeability coefficients has been reported to be derived by the

Table 1: Caco-2 cell permeability coefficient, distribution coefficient, physicochemical and molecular parameters of compounds

No	Compound	HAO	HAN	HD	HB	MP	log polar	logSAS	Hyd E	HoF	μ	MW	$logP_{caco-2}$	log D
1	Acebutalol	8	2	3	13	121.00	1.56	2.61	−7.31	−163.57	4.26	336.40	−6.29	−0.09
2	Acetylsalicylic acid	8	0	1	9	135.00	1.24	2.27	−4.92	−141.74	1.78	180.20	−5.04	−2.25
3	Acyclovir	6	5	4	15	256.75	1.32	2.37	5.76	−72.91	5.32	225.20	−6.60	−0.35
4	Alprenolol	4	1	2	7	–	1.47	2.50	−7.07	−57.55	1.02	249.30	−4.60	1.38
5	Aminopyrine	2	3	0	5	108.00	1.42	2.41	−0.96	23.37	4.17	231.30	−4.44	0.63
6	Atenolol	6	2	4	12	147.00	1.47	2.51	−13.48	−115.63	2.07	266.30	−6.28	−1.29
7	Bremazocine	4	1	2	7	–	1.56	2.54	−5.17	−65.80	2.40	315.46	−5.10	1.66
8	Caffeine	4	4	0	8	238.00	1.28	2.31	−2.25	−49.37	3.90	194.20	−4.51	0.02
9	Chlorothiazide	8	3	3	14	342.75	1.29	2.37	−16.47	−87.09	5.00	295.70	−6.72	−1.15
10	Chlorpromazine	0	2	0	2	–	1.56	2.51	−1.02	48.62	2.48	318.90	−4.70	1.86
11	Cimetidine	0	6	3	9	142.00	1.43	2.47	−15.11	77.12	8.44	252.30	−5.86	−0.36
12	Clonidine	0	3	2	5	130.00	1.36	2.34	−7.39	29.01	3.07	230.10	−4.66	0.78
13	Corticosterone	8	0	2	10	182.00	1.57	2.56	−7.86	−184.26	4.12	346.50	−4.67	1.78
14	Desipramine	0	2	1	3	–	1.52	2.49	−2.70	43.32	1.39	266.40	−4.61	1.57
15	Dexamethasone	10	3	3	16	269.50	1.60	2.58	−10.13	−241.90	3.64	392.50	−4.91	2.16
16	Diazepam	2	2	0	4	125.50	1.49	2.45	−2.44	28.95	3.37	284.80	−4.48	2.58
17	Dopamine	4	1	4	9	–	1.22	2.26	−18.62	−70.57	2.58	153.20	−5.03	−0.80
18	Estradiol	4	0	2	6	217.50	1.50	2.47	−5.44	−98.40	2.28	272.40	−4.77	2.24
19	Ganciclovir	8	5	5	18	250.00	1.37	2.42	−22.01	−119.56	7.53	255.20	−6.42	−0.10
20	Griseofulvin	12	0	0	12	220.00	1.53	2.53	−4.65	−183.53	4.37	352.80	−4.44	2.47
21	Hydrochlorothiazide	8	3	4	15	274.00	1.30	2.39	−14.58	−101.43	5.07	297.70	−6.29	−0.12
22	Hydrocortisone	10	0	3	13	212.50	1.58	2.56	−10.74	−224.16	4.32	362.50	−4.85	1.48
23	Indomethacin	8	1	1	10	162.00	1.56	2.55	−9.06	−112.25	2.08	357.70	−4.69	1.00
24	Labetalol	6	2	5	13	–	1.57	2.57	−15.87	−97.62	5.20	328.41	−5.03	1.24
25	Mannitol	12	0	6	18	167.00	1.19	2.30	−26.26	−274.76	3.38	182.20	−6.42	−2.65
26	Meloxicam	8	3	2	13	254.00	1.50	2.51	−13.42	−56.57	5.30	351.40	−4.71	0.03
27	Methyl scopolamine	8	0	1	9	–	1.52	2.53	−7.60	64.52	14.12	318.50	−6.16	−1.14
28	Metoprolol	6	1	2	9	–	1.48	2.52	−6.83	−119.92	2.89	267.40	−4.63	0.51
29	Nadolol	8	1	4	13	130.00	1.53	2.55	−13.53	−177.60	2.00	309.40	−5.41	0.68
30	Nevirapine	2	4	1	7	248.00	1.46	2.44	−3.75	50.12	2.73	266.30	−4.52	1.81
31	Nicotine	0	2	0	2	–	1.29	2.30	−1.30	20.13	2.56	162.20	−4.71	0.41
32	Phencyclidine	0	1	0	1	46.25	1.48	2.45	1.08	−4.77	0.90	243.38	−4.61	1.31
33	Phenytoin	4	2	2	8	296.50	1.44	2.41	−7.56	−18.85	3.00	252.30	−4.57	2.26
34	Pindolol	4	2	3	9	172.00	1.45	2.47	−10.94	−51.33	3.90	248.30	−4.78	0.19
35	Pirenzepine	4	5	1	10	–	1.58	2.56	−4.60	−8.02	3.29	351.41	−6.36	−0.46
36	Piroxicam	8	3	2	13	199.00	1.48	2.50	−12.33	−63.35	3.22	331.40	−4.45	−0.07
37	Progesterone	4	0	0	4	129.50	1.56	2.54	1.40	−108.25	4.17	314.50	−4.63	3.48
38	Propranolol	4	1	2	7	96.00	1.50	2.49	−7.26	−52.33	2.26	259.30	−4.66	1.55
39	Ranitidine	4	3	2	9	68.50	1.52	2.55	−12.20	0.72	9.09	314.40	−6.31	−0.12
40	Salicylic acid	6	0	2	8	158.00	1.13	2.15	−11.89	−113.08	1.00	138.10	−4.66	−1.44
41	Scopolamine	8	1	1	10	80.00	1.50	2.51	−8.62	−97.38	2.51	303.40	−4.93	0.21
42	Sucrose	22	0	8	30	–	1.45	2.52	−31.52	−476.74	1.63	342.30	−5.77	−3.34
43	Sulfasalazine	10	4	3	17	242.50	1.57	2.58	−25.18	−71.15	4.60	398.39	−6.52	−0.42
44	Telmisartan	4	4	1	9	–	1.78	2.74	−10.09	30.56	7.45	514.60	−4.82	2.41
45	Terbutaline	6	1	4	11	120.50	1.39	2.43	−17.65	−133.87	1.19	225.30	−6.33	−1.07
46	Testosterone	4	0	1	5	155.00	1.52	2.50	−2.05	−111.15	3.80	288.40	−4.60	2.91
47	Timolol	6	4	2	12	–	1.51	2.55	−8.81	−110.07	4.34	316.42	−4.89	0.03
48	Uracil	4	2	2	8	335.00	1.00	2.06	−6.73	−67.79	3.99	112.10	−5.37	−1.11
49	Urea	2	2	4	8	132.70	0.73	1.89	−11.89	−41.04	4.07	60.10	−5.34	−1.64
50	Warfarin	8	0	1	9	161.00	1.52	2.49	−8.60	−105.97	3.37	308.30	−4.68	0.64
51	Zidovudine	8	5	3	16	109.00	1.40	2.43	−26.95	−69.04	0.81	268.25	−5.16	−0.58

HAO: number of hydrogen bond acceptor oxygen atoms; HAN: number of hydrogen bond acceptor nitrogen atoms; HD: number of hydrogen bond donor atoms; HB: hydrogen bond forming ability; MP: melting point (°C); log polar: log polarizability ($°A^3$); logSAS: log solvent accessible surface area ($°A^2$); HydE: hydration energy (kcal/mole); HoF: heat of formation (kcal/mole); μ: dipole moment (debye); MW: molecular weight; $logP_{caco-2}$: log Caco-2 cell permeability coefficient (cm/sec); logD: log distribution coefficient

inclusion of the melting point as an independent variable [10]. It was therefore included as an additional descriptor (model 4). The same training set for model 4 and model 3 (without melting point variable) consists of 29 compounds (2, 4, 6, 8–19, 21–25, 27–31, 33–34, 36–37). There is no advantage to include this additional variable in the model, since the correlation coefficient, RMSEs for calibration and validation of models 3 and 4 are comparable (Table 2).

The regression coefficients of PLS models are shown in Table 4. The important variables influencing the model are hydrogen bonding, logD, dipole moment and hydration energy. The sign for number of hydrogen bond acceptor oxygen and nitrogen atoms (HAO and HAN) and donor (HD) atoms is negative, however, that for hydrogen bond forming ability (HB) is positive. Since HB is the sum of HAO, HAN and HD, the sign may be wrong due to the interaction of these variables. Because the better correlation is obtained with all hydrogen bond parameters (HAO, HAN, HD, HB) and correlation among parameters is not a problem for PLS analysis, hydrogen bond parameters were all used as variables. To verify the effect of hydrogen bond on permeability, PLS analyses were performed without HB variable; negative signs still obtained for HAO, HAN and HD. Nevertheless, PLS analyses without HAO, HAN and HD gave either a negative or a positive sign for HB depending on the samples. The negative effect of hy-

Table 2: Statistical parameters of the derived PLS models

Model	r-c	r-v	No. of PCs	Nc	RMSEC	RMSEcv	Np1	RMSEp1	Np2	RMSEp2
1	0.960	0.881	3	17	0.142	0.241	22	0.414	6	0.404
2	0.893	0.825	3	38	0.276	0.348			6	0.550
3	0.904	0.846	2	29	0.273	0.343				
4	0.903	0.836	2	29	0.275	0.353				

r-c: calibration correlation coefficient; r-v: validated correlation coefficient; No. of PCs: number of principle components; Nc: number of compounds in the training set; RMSEC: root mean square error of calibration; RMSEcv: root mean square error of validation; Np1: number of compounds in the external test set 1; RMSEp1: root mean square error for external test set 1; Np2: number of compounds in the external test set 2; RMSEp2: root mean square error for external test set 2.

drogen bonding was observed by Norinder et al. [6, 11] and Abraham et al. [12]. However, Lien and Gao reported that increased hydrogen bonding could have either a positive or a negative effect on skin permeability, depending on the experiments [13]. So the hydrogen bonding may be able to provide either a positive or a negative effect on Caco-2 cell permeability for this data set [5] which includes compounds transported via both transcellular and paracellular routes. Paracellularly, compounds are transported by water drag through the tight junctions; hydrogen bond forming ability of compounds with water might facilitate the permeation. However, transcellular transport of

Table 3: Experimental, calculated, and predicted permeability values over Caco-2 cells

Compound		Model 1			Model 2	
	Exp[a]	Calc[b]	Pred[c]	Pred[d]	Calc[e]	Pred[f]
Acetylsalicylic acid	−5.04	−4.93			−4.88	
Alprenolol	−4.60	−4.63			−4.60	
Aminopyrine	−4.44		−4.86		−4.90	
Bremazocine	−5.10		−4.68		−4.71	
Caffeine	−4.51		−4.93		−4.95	
Chlorpromazine	−4.70	−4.56			−4.61	
Cimetidine	−5.86	−5.90			−6.17	
Clonidine	−4.66		−5.06		−5.07	
Corticosterone	−4.67	−4.76			−4.85	
Desipramine	−4.61		−4.61		−4.58	
Dexamethasone	−4.91	−4.76			−4.92	
Diazepam	−4.48		−4.56		−4.60	
Dopamine	−5.03	−5.26			−5.26	
Estradiol	−4.77		−4.52		−4.49	
Ganciclovir	−6.42		−5.95		−6.19	
Griseofulvin	−4.44	−4.58			−4.70	
Hydrochlorothiazide	−6.29		−5.75		−5.90	
Hydrocortisone	−4.85		−4.91		−5.03	
Indomethacin	−4.69		−4.65		−4.78	
Labetalol	−5.03		−5.29		−5.47	
Mannitol	−6.42	−6.33			−6.31	
Meloxicam	−4.71		−5.30			
Methyl scopolamine	−6.16			−6.05		−6.49
Metoprolol	−4.63		−4.82		−4.85	
Nadolol	−5.41	−5.30			−5.31	
Nevirapine	−4.52		−4.36		−4.48	
Nicotine	−4.71	−4.76			−4.67	
Phencyclidine	−4.61		−4.17		−4.11	
Phenytoin	−4.57	−4.82			−4.83	
Pindolol	−4.78		−4.85		−4.97	
Piroxicam	−4.45			−4.88		−5.11
Progesterone	−4.63		−4.08		−4.12	
Propranolol	−4.66		−4.62		−4.63	
Ranitidine	−6.31		−5.49		−5.83	
Salicylic acid	−4.66	−4.66			−4.62	
Scopolamine	−4.93	−4.60			−4.73	
Sucrose	−5.77			−6.29		−6.41
Sulfasalazine	−6.52		−5.60		−5.99	
Telmisartan	−4.82			−4.86		−5.35
Testosterone	−4.60	−4.69			−4.65	
Timolol	−4.89			−5.51		−5.61
Uracil	−5.37	−5.33			−5.25	
Urea	−5.34			−5.70		−5.60
Warfarin	−4.68		−4.70		−4.81	
Zidovudine	−5.16	−5.23			−5.44	

[a]log values of experimental data taken from reference 5. [b]calculated from model 1. [c]predicted external test set 1 from model 1. [d]predicted external test set 2 from model 1. [e]calculated with model 2. [f]predicted external test set 2 from model 2.

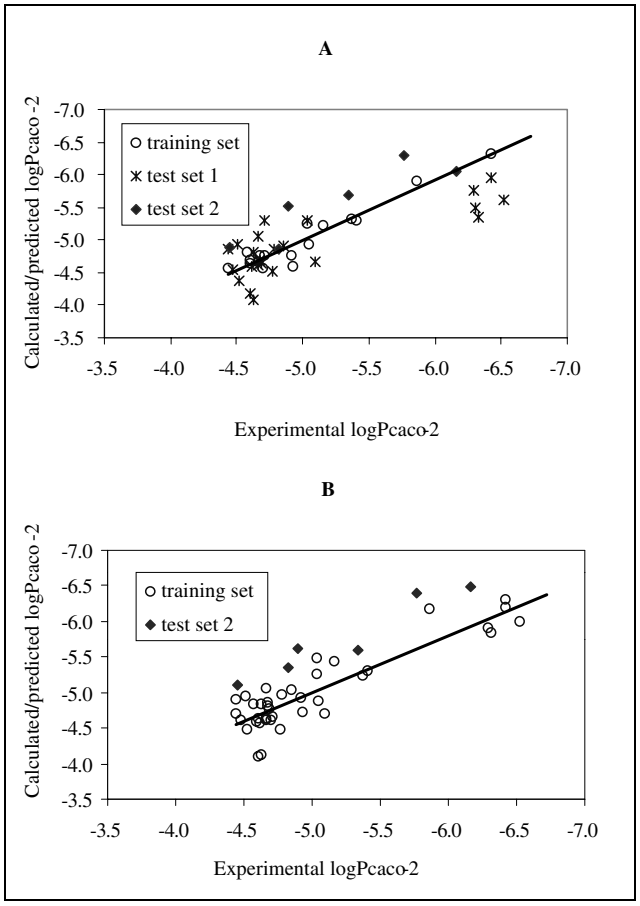

Fig.: Plots of experimental vs. calculated/predicted permeability over Caco-2 cell monolayers for (A) model 1, (B) model 2

compounds might be unfavorable if compounds have a strong hydrogen bond forming ability with cell constituents. As expected, increased logD has a positive effect on the permeability. While increased hydration energy facilitates permeability, an increased dipole moment of molecules has a negative effect on permeability.

These PLS models with the descriptors of solvent accessible surface area (logSAS), logD, polarizability (logpolar), hydration energy, heat of formation, dipole moment, molecular weight, hydrogen acceptor for oxygen and nitrogen atoms, hydrogen bond donors, and hydrogen bond forming ability provide good predictability for Caco-2 cell permeability. These descriptors can be electronically calculated so the derived PLS models should be useful for optimization of structure properties relationship of new design chemicals.

3. Experimental

3.1. Conformational analysis and molecular property calculation

The hyperchem program package [14] was used to build and calculate the optimum molecular structures of investigated compounds. The MM+ molecular mechanics force field was first run to get close to the optimized geometry. Molecular mechanics calculations treat atoms as Newtonian particles interacting through a potential energy function. The potential energies depend on bond lengths, bond angles, torsion angles, and nonbonded interactions (including van der Waals forces, electrostatic interactions, and hydrogen bonds). In these calculations, the forces on atoms are functions of atomic position. The conformation obtained from molecular mechanics was subjected to a refined geometry optimization using PM3 semiempirical quantum chemistry. Semiempirical calculations solve the Schrödinger equation to describe the electronic properties of atoms and molecules. Semiempirical methods make many simplifications, calculating only for valence electrons; neglecting the integrals for certain interactions; using standard, non-optimized, electron orbital basis functions. Dipole moment and heat of formation which represent basic molecular properties were obtained as the results of this calculation. The ChemPlus QSAR properties program [15] was used for further calculation of other molecular properties such as hydration energy, polarizability, and solvent accessible surface area (van der Waals surface). The hydrogen bond-forming ability of the whole molecule is the sum of maximum hydrogen bond numbers of the various functional groups and was calculated as described by Xia et al. [16].

3.2. Statistical analysis

The relationship between the experimentally determined Caco-2 cell permeability values ($\log P_{caco\text{-}2}$) and the descriptors was determined using the PLS1 regression. The software package used for conducting PLS calibration and prediction was Unscrambler [17]. Calibration was done using a set of samples of known permeability and original variables. In this way, the descriptor matrix X (*m, n*) and permeability matrix Y (*m, k*), where *m*, *n* and *k* are calibration samples, original variables, and permeability, respectively, are obtained. PLS methodology breaks down matrices X and Y into their latent variables.

$$X = T_x L_x + E_x$$

$$Y = T_y L_y + E_y$$

Table 4: Regression coefficients of PLS models

Descriptor	Model 1	Model 2	Model 3	Model 4
log solvent accessible surface area	−0.0127	−0.0264	−0.0234	−0.0358
logD	0.3491	0.2652	0.1790	0.1843
log polarizability	0.0789	0.0726	0.0542	0.0429
Hydration energy	0.2201	0.2603	0.2850	0.2862
Heat of formation	0.0693	−0.0231	0.0380	0.0293
Dipole moment	−0.3384	−0.3608	−0.2433	−0.2329
Molecular weight	0.0402	−0.0835	−0.0559	−0.0573
Number of hydrogen bond acceptor oxygen atoms	−0.0287	−0.0600	−0.1053	−0.0950
Number of hydrogen bond acceptor nitrogen atoms	−0.1760	−0.1500	−0.1624	−0.1568
Number of hydrogen bond donor atoms	−0.3420	−0.2351	−0.2959	−0.2912
Hydrogen bond forming ability	0.3570	0.2285	0.1057	0.1141
Melting point	–	–	–	−0.0497

where T_x (*m, a*) and T_y (*m, a*) are the score matrices, L_x (*a, n*) and L_y (*a, k*) the loading matrices, and E_x (*m, n*) and E_y (*m, k*) the residual matrices, *a* being the number of principal components or factors. By relating T_x and T_y, a diagonal relation matrix F is obtained.

$$T_y = T_x F + E$$

Matrix F is used in the prediction step to estimate the permeability from the descriptor values x_0 of the sample:

$$y_0 = x_0(T'_y X)' F L_y$$

Determining the number of principal components (PC) required is the most important step in implementing multivariate calibration. Model components are typically extracted in such a way that most information is conveyed by the first principal component (PC), then the second PC, and so on. At a certain point, the variation modeled by any new PC is mostly noise. The optimum number of PCs, providing useful modeling information, but avoiding overfitting, was chosen using the Unscrambler's criterion. It employs the number of PCs resulting in the first local minimum in the residual variances vs. PC plot.

Prior to PLS processing, all variables were centered and also scaled (standardization weighting technique, where the weight is the variable's standard deviation, 1/Sdev) in order to give all variables the same variance, i.e. 1. This gives all variables the same chance to influence the estimation of the components. The calibration model was obtained by the full cross-validation method in which only one sample at a time is kept out of the calibration and used for prediction. This is repeated in a way that all samples are kept out once. The residual variance can then be determined from the prediction residuals. The advantage of PLS analysis over other methods is that it performs particularly well when the various variables express common information, i.e. when there is a large amount of correlation, or even collinearity.

3.3. Data set

In this study, the experimental values for the Caco-2 cell permeability and distribution coefficients (D) for the 51 data set compounds were taken from Yazdanian et al. [5].

A calibration set 1 (training set 1) was composed of 17 compounds as indicated in Table 3. It was selected to cover compounds that span the variations both in variable descriptors and $\log P_{caco\text{-}2}$. The outliers which showed up having a large leverage as well as a high residual were removed to make a better model. The remaining compounds were used as an external test set to assess the predictive ability of the model. The expanded data set (training set 2) consisted of 38 compounds and was listed in Table 3. This data set was used to explore the correlation of a large sample set and also the predictability of the model.

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