Rapid Communication
CORAL: Quantitative Structure–Activity Relationship Models for Estimating Toxicity of Organic Compounds in Rats

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Abstract: For six random splits, one-variable models of rat toxicity (minus decimal logarithm of the 50% lethal dose [pLD50], oral exposure) have been calculated with CORAL software (http://www.insilico.eu/coral/). The total number of considered compounds is 689. New additional global attributes of the simplified molecular input line entry system (SMILES) have been examined for improvement of the optimal SMILES-based descriptors. These global SMILES attributes are representing the presence of some chemical elements and different kinds of chemical bonds (double, triple, and stereochemical). The "classic" scheme of building up quantitative structure–property/activity relationships and the balance of correlations (BC) with the ideal slopes were compared. For all six random splits, best prediction takes place if the aforementioned BC along with the global SMILES attributes are included in the modeling process. The average statistical characteristics for the external test set are the following: $n = 119 \pm 6.4$, $R^2 = 0.7371 \pm 0.013$, and root mean square error = 0.360 ± 0.037.

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Key words: balance of correlations; oral rat toxicity; QSAR; SMILES

Introduction

Quantitative structure–property/activity relationships (QSPR/QSAR) are tools for prediction of an endpoint for substances that have not been examined experimentally.[1–11]

It is a typical concept that satisfactory QSPR/QSAR model should be adequate for both the training and the external test sets. However, there are cases when a modest (or even poor) model for the training set is accompanied by a satisfactory model for the external test set. It is important that, however, this result should be reproduced in several probes involved in the building up of the model, and the result should be reproduced for a group of splits into the training and test sets.[12,13]

The QSPR/QSAR approaches are criticized in the literature[14–16] due to the frequent absence of the external test[17] and

also because depending on the split of data into training and test sets the outcome could be satisfactory or unacceptable. Basak et al.[18] have suggested the definition "inflated" in case of QSAR models which are unreliable for making predictions for chemicals similar to those used to calibrate the model. Indeed, the success of the result can be dependent on the split of the compounds between training, calibration and test sets.

The possible way to avoid the inflated correlations[18] is first, the consideration of a group of splits, and second, the reliability of the QSPR/QSAR models should be considered as more important quality than their precision.[12,13]
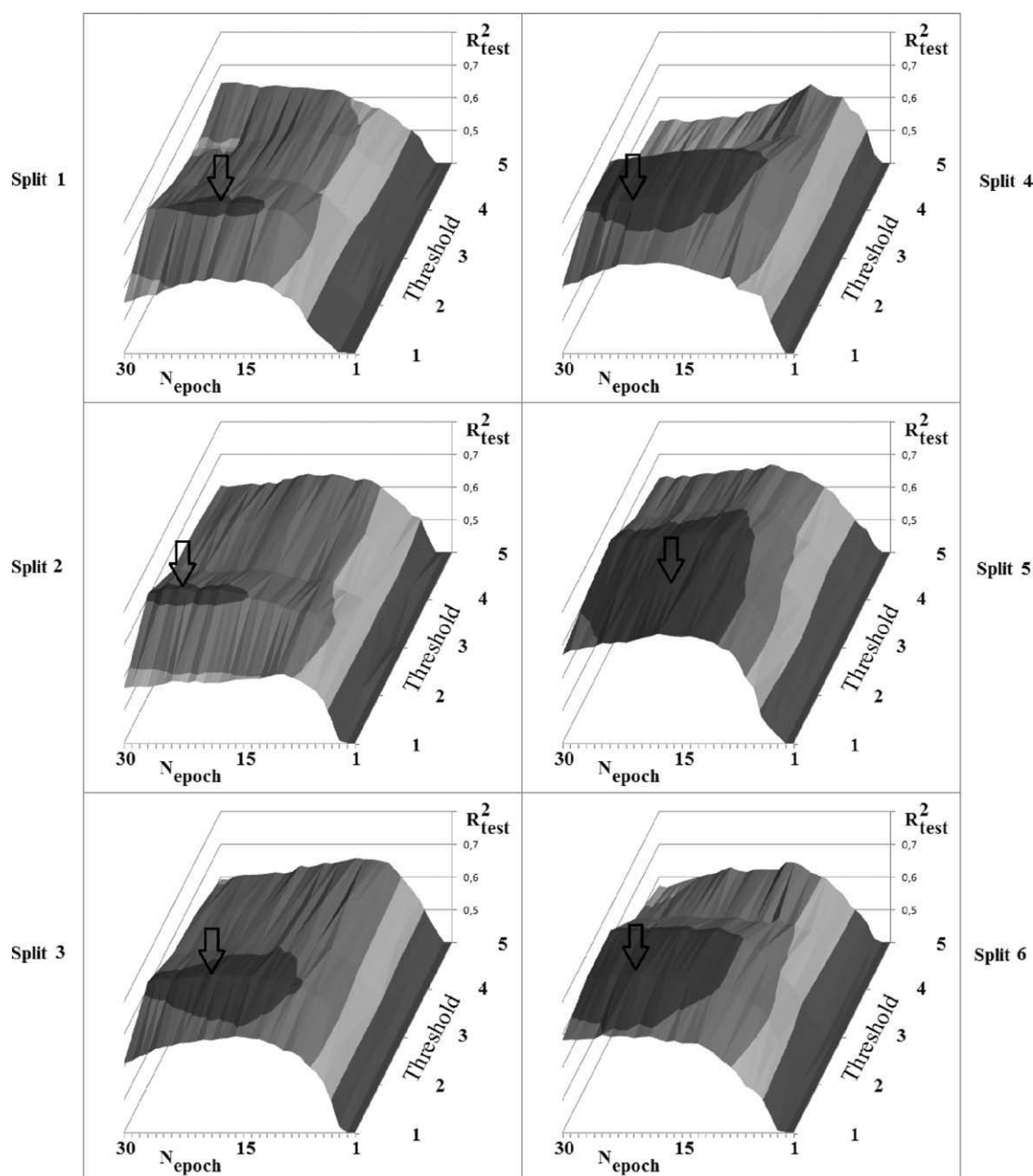

Figure 1. The correlation coefficients between the pLD50 experimental and the pLD50 which are calculated with eq. (1) for the external test set as the mathematical function [eq. (3)] for six random splits. The places of maximums of R^2_{test} are shown by pointers.

The aim of this study is the building up and the estimation of QSAR models for the oral rat toxicity of organic compounds, according to aforementioned principles. The list of organic compounds for this study was selected as a collection of substances that are usually considered in the literature related to QSPR/QSAR for various endpoints, such as toxicology,[19–24] carcinogenic potency,[25] hepatocarcinogenic toxicity,[26] toxicity of pesticides,[27] and aquatic toxicity.[28]

Method

Data

Rat toxicity data (LD50, in mg/kg, oral exposure) were taken from the U.S. Library of Medicine.[29] The $\log_{10}[1/\text{LD50}] = $ pLD50 has been used as endpoint. Total number of compounds is 689. Six random splits into the subtraining, calibration, and test sets were examined.

Table 1. Definitions of the BOND, NOSP, and HALO Attributes.

=	#	@		Comments
Calculation of the BOND index				
0	0	0		There are no double, triple, or stereochemical bonds.
0	0	1		The molecule contains only stereochemical bonds.
0	1	0		The molecule contains only triple bonds.
0	1	1		The molecule contains triple and stereochemical bonds.
1	0	0		The molecule contains only double bonds.
1	0	1		The molecule contains double bonds and stereochemical bonds.
1	1	0		The molecule contains double and triple bonds.
1	1	1		The molecule contains double, triple, and stereochemical bonds.
N	**O**	**S**	**P**	**Comments**
Calculation of the NOSP index				
0	0	0	0	Nitrogen, oxygen, sulfur, and phosphorus are absent.
0	0	0	1	The molecule contains only phosphorus.
0	0	1	0	The molecule contains only sulfur.
0	0	1	1	The molecule contains sulfur and phosphorus.
0	1	0	0	The molecule contains only oxygen.
0	1	0	1	The molecule contains oxygen and phosphorus.
0	1	1	0	The molecule contains oxygen and sulfr.
0	1	1	1	The molecule contains oxygen, sulfur, and phosphorus.
1	0	0	0	The molecule contains only nitrogen.
1	0	0	1	The molecule contains nitrogen and phosphorus.
1	0	1	0	The molecule contains nitrogen and sulfur.
1	0	1	1	The molecule contains nitrogen, sulfur, and phosphorus.
1	1	0	0	The molecule contains nitrogen and oxygen.
1	1	0	1	The molecule contains nitrogen, oxygen. and phosphorus.
1	1	1	0	The molecule contains nitrogen, oxygen, and sulfur.
1	1	1	1	The molecule contains nitrogen, oxygen, sulfur, and phosphorus
F	**Cl**	**Br**		**Comments**
Calculation of the HALO index				
0	0	0		Fluorine, chlorine, and bromine are absent.
0	0	1		The molecule contains only bromine.
0	1	0		The molecule contains only chlorine.
0	1	1		The molecule contains chlorine and bromine.
1	0	0		The molecule contains only fluorine.
1	0	1		The molecule contains fluorine and bromine.
1	1	0		The molecule contains fluorine and chlorine.
1	1	1		The molecule contains fluorine, chlorine, and bromine.

Table 2. Definition of the ATOMPAIRs, that is, Situations which are Defined by Presence (Absence) of Seven Chemical Elements.

	Cl	Br	N	O	S	P
F	++++F−−−Cl==	++++F−−−Br==	++++F−−−N===	++++F−−−O===	++++F−−−S===	++++F−−−P===
Cl		++++Cl−−Br==	++++Cl−−N===	++++Cl−−O===	++++Cl−−S===	++++Cl−−P===
Br			++++Br−−N===	++++Br−−O===	++++Br−−S===	++++Br−−P===
N				++++N−−−O===	++++N−−−S===	++++N−−−P===
O					++++O−−−S===	++++O−−−P===
S						++++S−−−P===

For example, ++++Cl−−−Br== indicates that both chlorine and bromine are present in the molecular structure (without taking into account the numbers of these in molecule).

Table 3. Statistical Quality of the Models.

Split	Thershold	N_{epoch}	N_{act}	Training set				Calibration set			Test set			
				n	R^2	RMSE	F	n	R^2	RMSE	n	R^2	RMSE	R_m^2
Version 1. Classic scheme without the global attributes														
1	4	19	470	563	0.7506	0.461	1689				126	0.4952	0.431	0.4031
2	2	30	684	582	0.7976	0.411	2285				107	0.5801	0.418	0.4453
3	4	15	469	564	0.7233	0.477	1469				125	0.5766	0.472	0.5306
4	4	19	462	567	0.7465	0.453	1664				122	0.5285	0.562	0.3715
5	5	21	417	571	0.7302	0.469	1540				118	0.6087	0.465	0.5504
6	4	28	464	572	0.7596	0.445	1801				117	0.4977	0.530	0.3435
				Subtraining set				Calibration set			Test set			
Version 2. Balance of correlations without the global attributes														
1	2	18	566	344	0.7094	0.521	835	219	0.8290	0.362	126	0.5815	0.391	0.4889
2	2	30	582	369	0.7180	0.500	934	213	0.8855	0.332	107	0.5590	0.413	0.4777
3	3	14	447	347	0.6623	0.550	677	217	0.7969	0.392	125	0.5851	0.462	0.5771
4	2	26	558	351	0.7428	0.479	1008	216	0.8273	0.362	122	0.6019	0.508	0.4541
5	3	17	448	357	0.6857	0.526	774	214	0.8091	0.403	118	0.5822	0.490	0.5086
6	2	22	564	362	0.7266	0.490	957	210	0.8565	0.347	117	0.5177	0.497	0.4062
				Training set				Calibration set			Test set			
Version 3. Classic scheme with the global attributes														
1	721	721	721	563	0.8113	0.401	2413				126	0.6347	0.371	0.5295
2	567	567	567	582	0.8228	0.384	2694				107	0.6780	0.367	0.5476
3	572	572	572	564	0.8170	0.388	2509				125	0.6720	0.430	0.5549
4	567	567	567	567	0.8142	0.388	2476				122	0.6535	0.479	0.4917
5	456	456	456	571	0.7586	0.443	1788				118	0.7245	0.386	0.6997
6	709	709	709	572	0.8176	0.387	2556				117	0.7094	0.380	0.6052
				Subtraining set				Calibration set			Test set			
Version 4. Balance of correlations with the global attributes														
1	2	19	610	344	0.7662	0.468	1120	219	0.8759	0.304	126	0.7237	0.315	0.6388
2	2	26	625	369	0.7725	0.449	1246	213	0.9073	0.277	107	0.7327	0.315	0.6736
3	2	21	634	347	0.7688	0.455	1147	217	0.8922	0.284	125	0.7412	0.382	0.6265
4	2	24	601	351	0.7816	0.442	1249	216	0.8863	0.300	122	0.7399	0.420	0.5688
5	2	17	621	357	0.7723	0.448	1204	214	0.8738	0.313	118	0.7610	0.363	0.7276
6	2	22	607	362	0.7726	0.447	1223	210	0.8907	0.317	117	0.7239	0.366	0.6560

The N_{act} is the number of SMILES attributes classified as not rare; RMSE is the root mean square error; F is the Fischer F-ratio; R_m^2 is the measure of the predictability according to Roy and Roy[38]: a model is satisfactory if $R_m^2 > 0.5$.

Descriptors

Optimal simplified molecular input line entry system (SMILES)-based descriptors were calculated by the Monte Carlo method using the CORAL software.[30] The same options for the calculation of the optimal SMILES-based descriptors were used for the six splits (Fig. 1). This approach generates the following models

$$pLD50 = C_0 + C_1 \, DCW(Threshold) \quad (1)$$

where DCW(Threshold) is the optimal SMILES-based optimal descriptor calculated as

$$DCW(Threshold) = \alpha^* \sum CW(\mathbf{S_k}) + \beta^* \sum CW(\mathbf{SS_k})$$
$$+ \gamma^* \sum CW(\mathbf{SSS_k}) + x^* CW(BOND) + y^* CW(NOSP)$$
$$+ z^* CW(HALO) + w^* CW(ATOMPAIR) \quad (2)$$

The coefficients α, β, γ, x, y, z, and w can be either 1 or 0. One indicates that the SMILES attribute is involved in the calculation of the DCW(Threshold) and zero indicates that the SMILES attribute is not involved. $\mathbf{S_k}$ is the SMILES atoms, that is, one symbol or two symbols which should be examined as a concatence, for example, 'Cl', 'Br', 'As', and 'Na'. The $\mathbf{SS_k}$ and $\mathbf{SSS_k}$ are the SMILES attributes or combinations of two or three SMILES atoms, respectively. The $\mathbf{S_k}$, $\mathbf{SS_k}$, and $\mathbf{SSS_k}$ are local SMILES attributes. In fact, these are substrings of the total SMILES string. If a SMILES is the sequence of SMILES atoms ABCDE, then one can show the $\mathbf{S_k}$, $\mathbf{SS_k}$, and $\mathbf{SSS_k}$ as the following:

- 'A', 'B', 'C,' 'D,' and 'E' are examples of the $\mathbf{S_k}$;
- 'AB', 'BC', 'CD', and 'DE' are examples of the $\mathbf{SS_k}$;
- 'ABC', 'BCD', and 'CDE' are examples of the $\mathbf{SSS_k}$.

However, having two SMILES atoms AB, one can obtain two versions of the SMILES attribute AB or BA. To avoid this uncertainty, one can use ASCII code for definition of only one
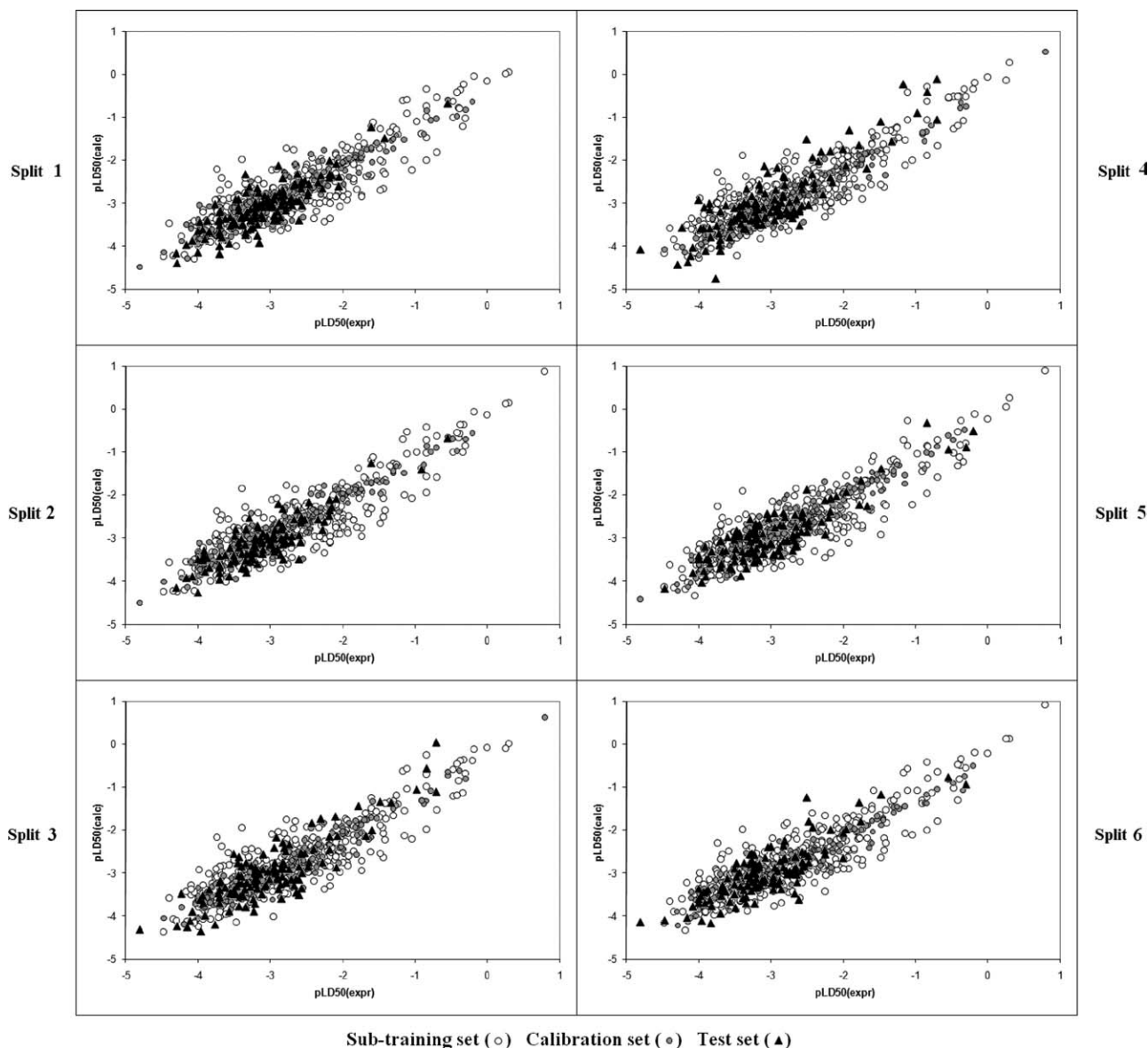
Figure 2. Graphical representation of the best QSAR models of pLD50 which are calculated with the balance of correlations with ideal slopes for six random splits. In eq. (2) the following options have been used: $\alpha = 1$, $\beta = 1$, $\gamma = 1$, $x = 1$, $y = 1$, $z = 1$, and $w = 1$.

possibility (e.g., AB without BA). The same solution is used for selection of only one of two combination of three atoms (e.g., ABC without CBA).

In addition to local attributes, CORAL can involve global SMILES attributes, which are a characteristic of SMILES string in whole. Table 1 contains definitions of the global SMILES attributes BOND, NOSP, and HALO.[31] Table 2 shows scheme for calculation of the global SMILES attribute ATOMPAIR.

The correlation coefficient between pLD50 and DCW(Threshold) is a mathematical function of the correlation weights of the SMILES attributes. By the Monte Carlo method, one can

calculate the correlation weights that produce the maximum of the correlation coefficient for the training set, assuming that this correlation will also take place for the external test set.

There are two basic approaches of the CORAL modeling. The first is the "classic" scheme based on the split of all substances into the training and test sets. In this case, the maximum of the correlation coefficient for the training set is the target of the Monte Carlo optimization.[30] The second is the balance of correlations (BC) which is based on split into three sets: sub-training, calibration, and test sets.[32–36] In the case of the BC, the calibration plays role of "a preliminary" test set. In this case, the maximum of BC = $[R + R' - \mathrm{abs}(R–R')]dR$ is the target of

Table 4. The Checking of the Models with Randomization.

Probe of Y-scrambling	R_r^2					
	Split 1	Split 2	Split 3	Split 4	Split 5	Split 6
1	0.0594	0.1221	0.0881	0.1001	0.1669	0.1901
2	0.1983	0.0975	0.1343	0.1439	0.1514	0.0699
3	0.1233	0.1173	0.2038	0.1765	0.1865	0.1289
4	0.0827	0.1765	0.0630	0.0756	0.1328	0.1694
5	0.1152	0.2621	0.1369	0.1884	0.1364	0.1781
6	0.1335	0.1914	0.0530	0.1196	0.0611	0.2195
7	0.1898	0.1789	0.2419	0.1426	0.0853	0.2219
8	0.1785	0.0951	0.1222	0.0863	0.1416	0.0679
9	0.1086	0.1767	0.1721	0.1339	0.1092	0.1246
10	0.1085	0.1153	0.0733	0.1063	0.1872	0.1548
$\overline{R_r^2}$	0.1298	0.1336	0.1115	0.1306	0.1617	0.1162
$^cR_p^2$	0.7046	0.7025	0.7109	0.7134	0.6867	0.7121

The $\overline{R_r^2}$ is average for 10 probes of the Y-scrambling.[39] The $^cR_p^2$ (calculated by $^cR_p^2 = R\sqrt{R^2 - \overline{R_r^2}}$) should be larger than 0.5.

the Monte Carlo optimization: the R and R' are correlation coefficients for the subtraining and calibration sets, respectively; dR is an empirical parameter.

The improved version of the BC is the BC with the ideal slopes (IS).[37] The BC with IS was used in this study. The basic idea of the BC with IS is taking into account the difference between C_0 and C_1 [see eq. (1)] of the training set and C_0' and C_1' of the calibration set. The additional component of the target function is IS = $[(C_0 + C_0') + (C_1 + C_1')]dC$. The maximum of the BC together with the minimum of the IS are components of the target function, in the case of the BC with IS. dC is an empirical parameter.

The BC, in general, and the BC with IS, in particular, can improve the predictability of a model in comparison with the classic scheme.

If the total number of SMILES attributes is redundant, one can obtain the inflated correlation,[18] which is high for the training set (the case of classic scheme) or for the subtraining and calibration sets (the case of the BC) but it will be modest or even poor correlation for the external test set. In the literature, this situation is named the "overtraining."[16]

In the case of the CORAL, there are two ways to try to avoid the overtraining. The first way is to select an adequate threshold for classification of SMILES attributes into two categories: rare and not rare. The correlation weights for the rare (in the subtraining set) SMILES attributes should be fixed as zero, and consequently, the rare attributes will not influence the building up of the model. The reduction of the number of SMILES attributes can lead to improvement in the predictability of the model. The second way is to select an adequate number of epochs of the Monte Carlo optimization: if the number of epochs is too large, one can obtain "excellent" statistics for training but poor statistics for the external test set. If the optimization is stopped with a correlation (for the training set) which is not inflated, it can be a reasonable correlation for the test set.

Results and Discussion

The building up of the QSAR model for pLD50 was carried out in two phases. The first phase is the definition of the best threshold and the number of epochs (N_{epoch}) of the Monte Carlo optimization. The best models were calculated with the best combination of the threshold (from one to five) and the N_{epoch} (from 1 to 30) for the correlation coefficient of the external test set, which is a mathematical function of these two parameters:

$$R_{test}^2 = F(\text{Threshold}, N_{epoch}) \qquad (3)$$

These calculations were carried out in four versions:

- Version 1 was based on the classic scheme without global SMILES attributes.
- Version 2 was based on the BC with IS without the global attributes.
- Version 3 was based on the classic scheme with the global attributes.
- Version 4 was based on the BC with IS with the global attributes.

Table 3 contains the statistical characteristics of the best models obtained in the versions 1–4. Figure 1 gives the graphical representation of the functions (i.e., R_{test}^2) calculated with eq. (3) for six splits. Figure 2 shows the best models for these six splits graphically.

The developed QSAR models were further validated using the randomization technique (Y-scrambling)[39] to examine their robustness. This validation is the following. There are two columns pEC50$_{calc}$ and pEC50$_{expr}$. One probe of the randomization is the random shuffling of 100 pEC50$_{calc}$ without any change in the column of pEC50$_{expr}$. This check was carried out for the subtraining set. A model can be considered as robust if $^cR_p^2 > 0.5$.[39] Table 4 contains the results of this checking. One can see that all six models are robust according to this criterion.[39]

Lists of the splits that are examined in this study together with the CAS numbers, SMILES, and numerical data on the pLD50 are available (i.e., it can be downloaded) on the Internet[30] as the folder "Example 5." Supporting Information section contains screenshots of the calculations with CORAL software together with the comparison of the classic scheme and the BC for six random splits into the subtraining, calibration, and test sets.

Conclusions

All represented data are averages for a series of runs of the CORAL software. There is the satisfactory reproducing of the described results obtained by the Monte Carlo technique.

For all six random splits, using the global SMILES attributes BOND, NOSP, HALO, and ATOMPAIR, the software above leads to improvement in the predictability of the QSAR models of the pLD50. For all six random splits, the BC gives the improvement in the predictability of the QSAR models in comparison with the classic scheme.

Acknowledgments

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