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Similarity-Based Descriptors (SIBAR) as Tool for QSAR Studies on P-Glycoprotein Inhibitors: Influence of the Reference Set

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Abstract

Polyspecific proteins, such as the cytochrome P450 enzyme family, the hERG potassium channel and the ABC-type multidrug efflux pumps ABCB1, ABCC1 and ABCG2 are increasingly recognised as playing a major role in bioavailability and toxicity of drugs. Although considerable efforts have been undertaken to establish *in silico* tools for predicting drug–protein interactions, especially in the field of ABC pumps, general applicable models are still rare. We recently showed that similarity-based descriptors are a versatile tool for prediction of ABCB1 (P-glycoprotein, P-gp) inhibitory activity. These descriptors are based on the calculation of similarity values between the compounds of the training set to a group of reference set compounds. The similarity values are subsequently used as independent variables in QSAR analyses. Within this paper, we address the influence of the reference set on the predictive ability of QSAR models for a set of 412 inhibitors of the multidrug efflux pump ABCB1. Four different reference sets were designed comprising highly diverse, drug-like compounds (A), a subset of the training set compounds (B), a set of manually selected ABCB1 inhibitors (C) and low molecular weight chemicals (D). Our results indicate that a combination of high diversity and an interaction of the reference compounds with the biological target is beneficial for yielding good models. The reference dataset tailored to the specific problem (the biological target) scored best in predicting the biological activity of compounds from an external test set.

1 Introduction

Within the past decade, high-throughput prediction of ADMET properties (Absorption-Distribution-Metabolism-Elimination-Toxicity) became increasingly important in the drug discovery and development process. Several key proteins involved in absorption, metabolism and toxicity have been identified and shown to exhibit polyspecific (promiscuous) ligand recognition patterns [1]. These include the Cytochrome P450 enzyme complex, the human ether-a-go-go-related potassium channel (hERG) and several transport proteins from the ABC superfamily (ATP-binding cassette). The latter comprise multidrug efflux transporters such as P-glycoprotein (P-gp, ABCB1), the Multidrug Resistance-Related Protein (MRP1, ABCC1) and the Breast Cancer-Related Protein (BCRP, MXR, ABCG2) [2]. Although for these proteins several methods have been described to properly predict the ligand–protein interaction within homologous series of compounds, generally applicable models are still rare. Those published mainly utilise pharmacophore modelling [3–5], field-based descriptors such as VolSurf and GRIND [6, 7] and non-linear methods [8]. In light of our QSAR studies on propafenone-type inhibitors of ABCB1, we recently demonstrated the successful application of Similarity-based Descriptors (SIBAR) for ABCB1 inhibitors [9]. These descriptors are based on the calculation of similarity values between compounds under consideration and a set of reference compounds. Thus, each compound is described by a similarity-based fingerprint which is used as input vector in PLS analyses. In contrast to conventional QSAR approaches, where the only degree of freedom lies in the choice of appropriate descriptors, SIBAR offers a second possibility to adapt to the given problem. Assuming that compounds similar to active ones show a higher likelihood of being also active, the reference set might be tailored to the respective target protein by including both active and inactive compounds. Within this paper, we address the in-

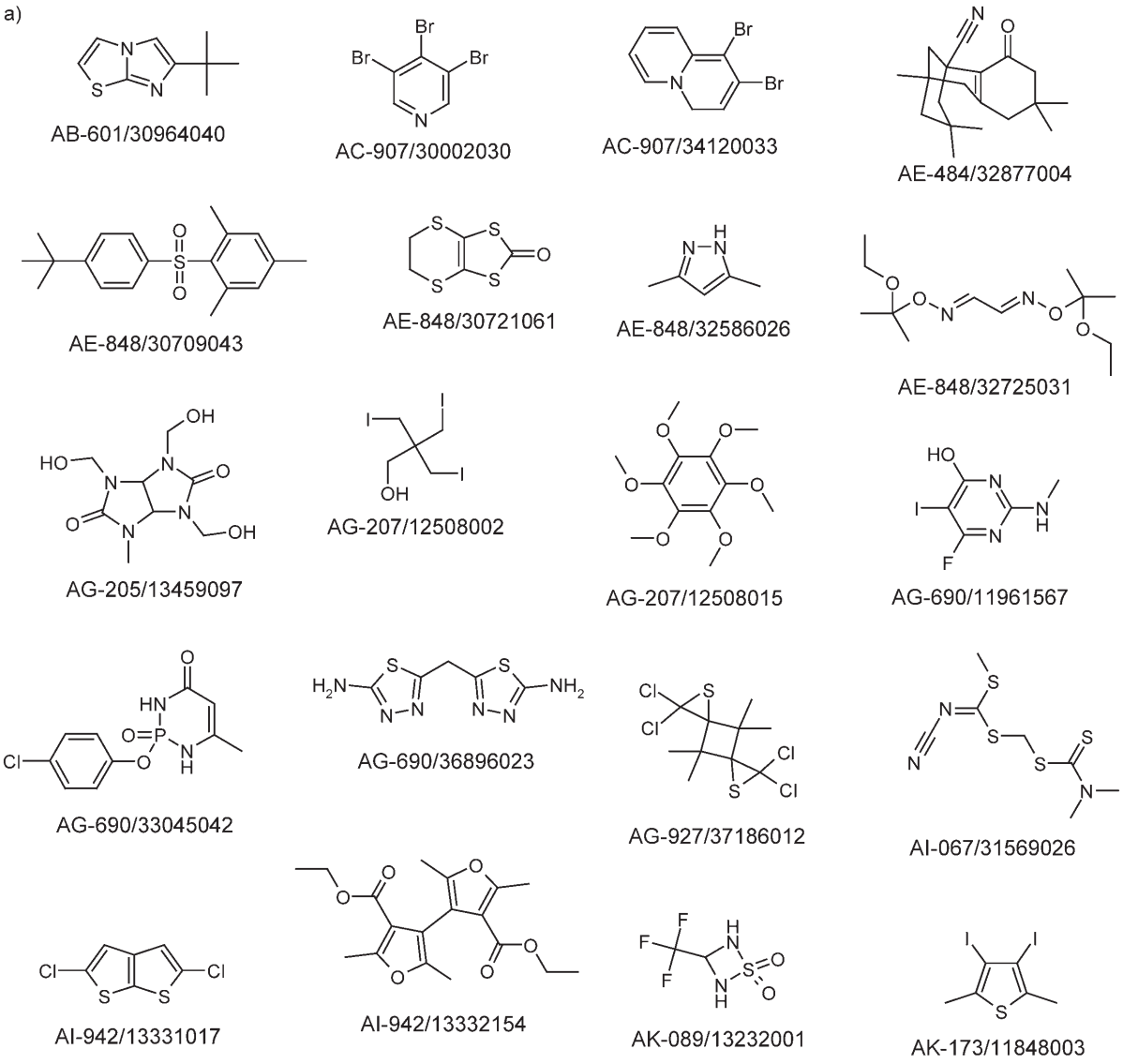


Figure 1. a) Chemical structures of the compounds in reference set A. b) Chemical structures of the compounds in reference set B. c) Chemical structures of the compounds in reference set C. d) Chemical structures of the compounds in reference set D.

fluence of the composition of the reference set on the predictivity of PLS models for a set of inhibitors of the multidrug efflux pump ABCB1.

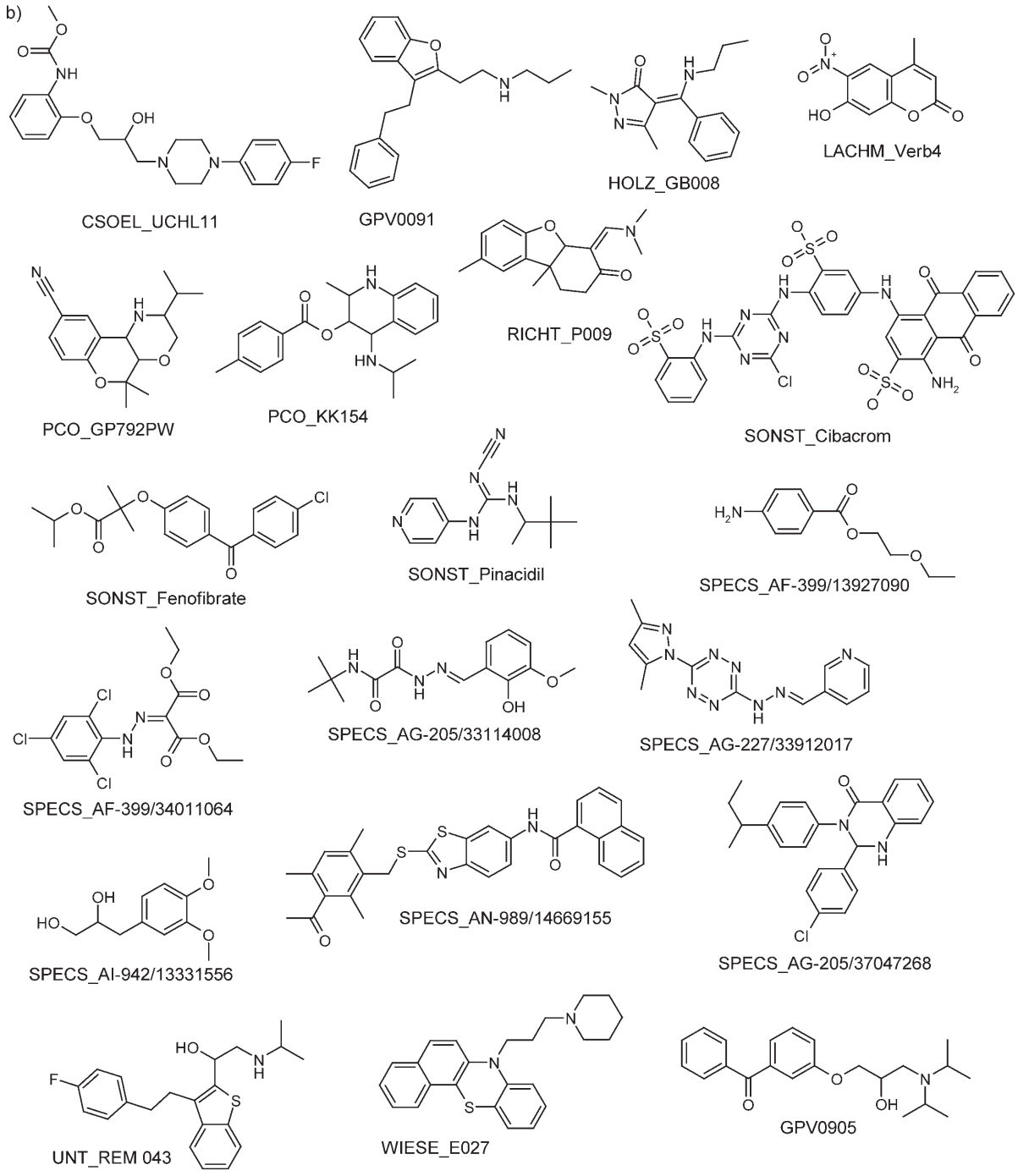
2 Methods

2.1 Datasets Used

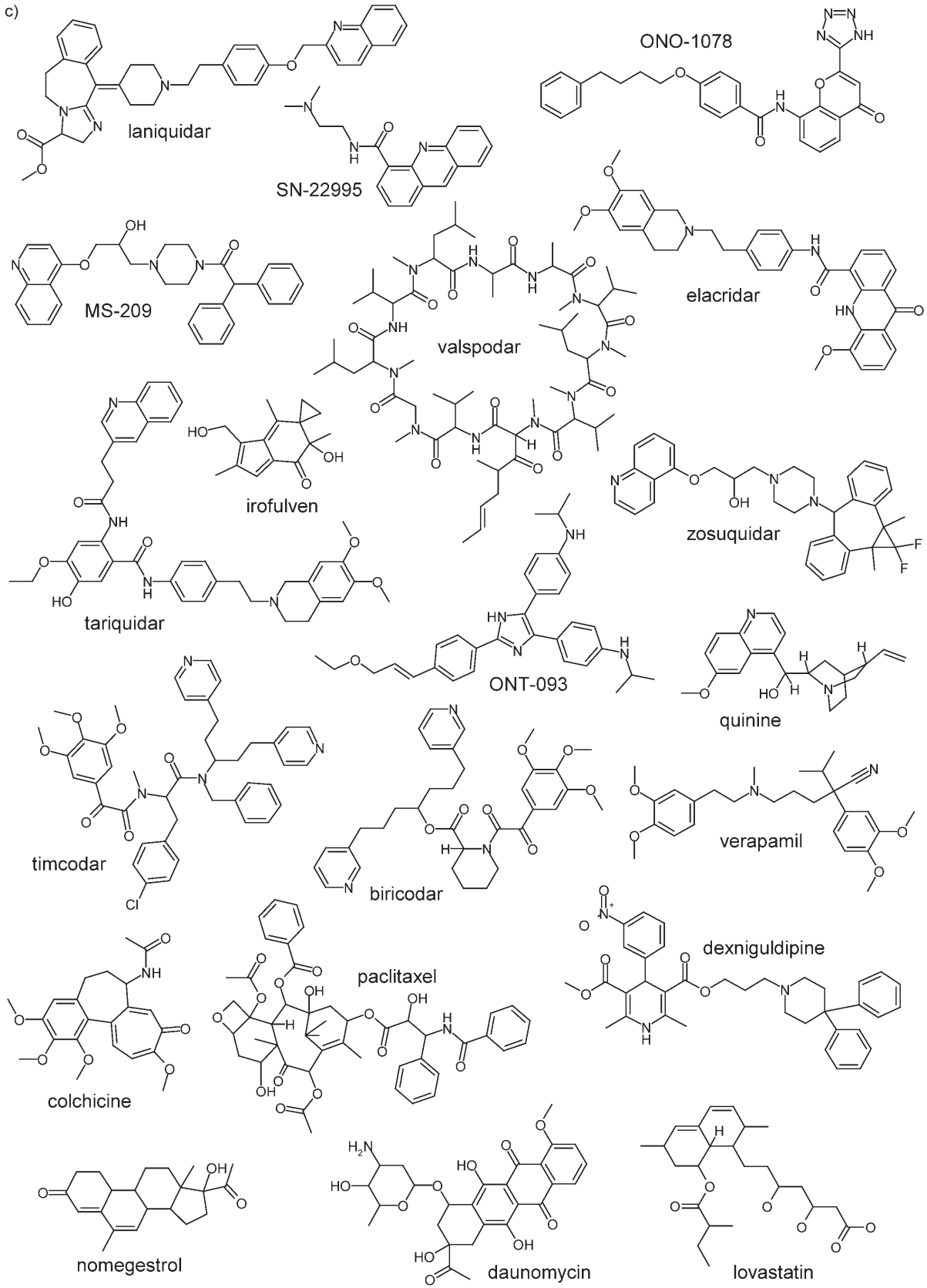
All calculations are performed on our in-house database of P-gp inhibitors comprising 412 compounds. This database consists of 275 propafenone-type inhibitors [10] and 116 compounds comprising benzopyranones [11], indanones [12], benzofuranes [13] and indazoles [14]. Additionally, we included a set of 21 structurally diverse molecules which have been obtained by virtual screening of the SPECS compound library [15]. Compounds were drawn with ISIS-DRAW and a database was set up using ACCORD for EXCEL (Accelrys Inc., San Diego). The 2-D to 3-D conversion was performed using CORINA (Molecular Networks, Germany) and the structures were imported into MOE (Molecular Operating Environment, Chemical Computing Group), followed by calculation of PEOE charges and energy minimisation (forcefield: MMFF94x; gradient: 0.001). The database was split up into two subsets *via* random selection: a 293 compounds training set and a test set (TS) consisting of 119 molecules.

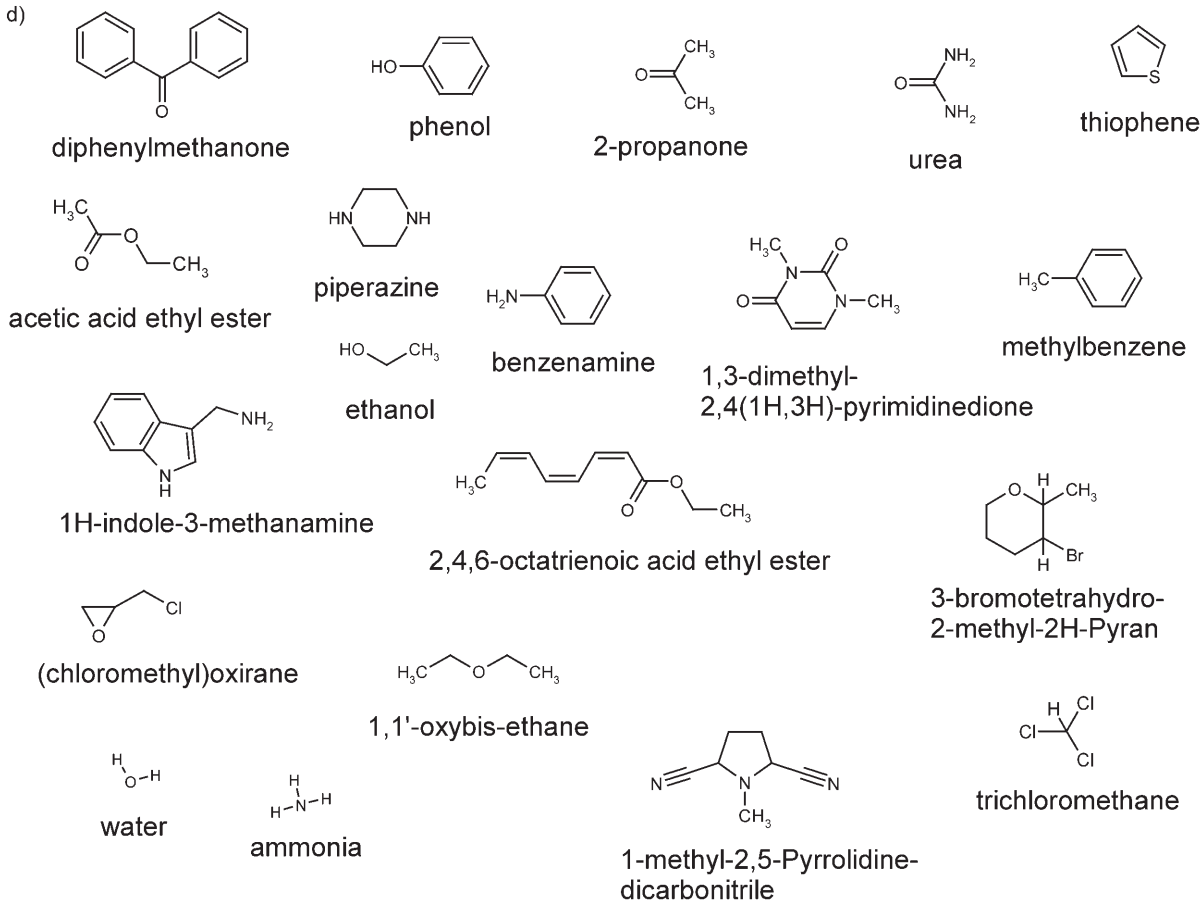
2.2 Reference Sets Used

Reference sets A – D (Ref_A to Ref_D) comprise 20 diverse compounds, respectively. Being aware of the fact



that similarity is always relative to an established reference, we used different similarity measures for the selection of the reference compounds. Ref_A is identical to those we used in our first paper on SIBAR [9]. It was selected from the SPECS library by applying the SELECTOR module as implemented in SYBYL (Tripos Inc.) to select a maximum diverse subset using UNITY fingerprints and the Tanimoto index as similarity measure. In order to obtain Ref_B, we performed diversity selection on our in-house database with MOE's DIVERSE SUBSET MODULE on basis of the Tanimoto coefficient using MACCS Structural Keys. Supported by the findings of Ghuloum *et al.* [21] that basis sets (equivalent to our reference sets) chosen by picking molecules at random allowed sufficient encoding of the molecular properties, we generated Ref_C and Ref_D by handpicking. Ref_C was handpicked at random from P-gp substrates/inhibitors known from the literature and Ref_D was handpicked from a list





of simple chemicals. Chemical structures of the compounds of reference sets A – D are shown in Figures 1a – d.

2.3 Descriptors Used

In total, 31 2-D and 3-D molecular descriptors were calculated using MOE. They are related to the ones used in our previous work on SIBAR [9]. A detailed list of descriptors used and their explanation is given in Table 1. Each descriptor was subjected to scaling to unit variance using the values obtained for the 293 training set compounds. For the reference and TSs, scaling was performed by applying the respective mean value and standard deviation as calculated for the training set.

2.4 The SIBAR Approach

SIBAR values are based on calculation of similarity, based on Euclidean distance. For each compound of the training set, a similarity value is calculated to every compound of a reference set. This leads to a similarity fingerprint with the number of bins equal to the number of reference compounds used (Figure 2).

The workflow for calculation of the SIBAR values is outlined as follows: (1) selection of a reference compound set, (2) calculation of a set of descriptors for both the training set and the reference set and (3) calculation of the SIBAR values D (Eq. 1) *via* calculation of Euclidean distances between the i reference compounds and the j compounds of interest (training set and TS) using k molecular descriptors, X_k

$$D(i,j) = \sqrt{\sum \left(X_{ik} - X_{jk}\right)^2}$$

2.5 Model Generation and Validation

QSAR models were generated *via* Partial Least-Squares analysis (PLS) as implemented in MOE. A first estimate of the predictive capabilities of the models was obtained *via* Leave-One-Out (LOO) cross-validation within the training set. To further assess the predictive power of the models, biological activities of the compounds of the TS were predicted and correlated with the respective observed activities.

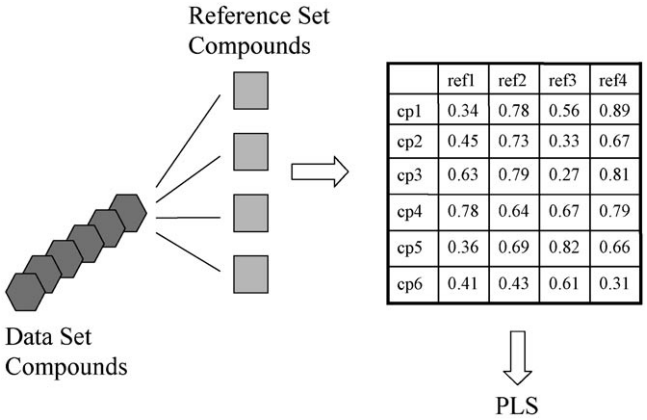
2.6 Calculation of the Diversity of the Reference Sets

To compare the diversity of the four reference sets, the Euclidean distances from each compound of a reference set to the other remaining compounds of this dataset were cal-

Table 1. Molecular descriptors used and their explanation (adapted from MOE tutorial, Chemical Computing Group).

Descriptor variable	Explanation
Diameter	
Radius	
WeinerPath	Wiener path number
WeinerPol	Wiener polarity number
a_IC	Total atom information content
Weight	Molecular weight
Zagreb	Zagreb index
PEOE_VSA_HYD	Total hydrophobic van der Waals surface area
PEOE_VSA_POL	Total polar van der Waals surface area
E	Value of the potential energy
E_ang	Angle bend potential energy
E_ele	Electrostatic component of the potential energy
E_sol	Solvation energy
E_str	Bond stretch potential energy
E_strain	Local strain energy
E_tor	Torsion (proper and improper) potential energy
E_vdw	Van der Waals component of the potential energy
Kier1	First kappa shape index
Bpol	Sum of the absolute value of the difference between atomic polarisabilities of all bonded atoms in the molecule
Pmi	Principle moment of inertia
Vsa_acc	Approximation to the sum of VDW surface areas of pure hydrogen bond acceptors
Vsa_don	Approximation to the sum of VDW surface areas of pure hydrogen bond donors
Vsa_hyd	Approximation to the sum of VDW surface areas of hydrophobic atoms
Vsa_other	Approximation to the sum of VDW surface areas of atoms typed as 'other'
Vsa_pol	Approximation to the sum of VDW surface areas of polar atoms
ASA	Water accessible surface area
VSA	Van der Waals surface area
TPSA	Polar surface area
Vdw_area	Area of van der Waals surface
Vdw_vol	Van der Waals volume
Vol	Van der Waals volume

culated ($n \times n$ similarity matrix). Consequently, for a given number of n reference compounds (20), we obtained $n-1$ (19) similarity values for every reference compound. Thus, we obatained $n^*(n-1)/2$ (190) similarity values for every reference set (excluding the 0.00 Euclidean distances between the compounds themselves and values that were double determined). The mean value of these Euclidean distances was used as an overall measure of self-similarity within this reference set (Table 4).



	ref1	ref2	ref3	ref4
cp1	0.34	0.78	0.56	0.89
cp2	0.45	0.73	0.33	0.67
cp3	0.63	0.79	0.27	0.81
cp4	0.78	0.64	0.67	0.79
cp5	0.36	0.69	0.82	0.66
cp6	0.41	0.43	0.61	0.31

Figure 2. Workflow for the SIBAR approach; cp: training set compound; sv: similarity value; PLS: partial least squares.

2.7 ABCB1 Inhibitory Activity

The pharmacological activity of the compounds was measured in a zero trans efflux protocol using daunorubicin as the fluorochrome [16]. Briefly, multidrug resistant CCRF vcr1000 cells were incubated with daunorubicin and the time-dependent decrease in mean cellular fluorescence was measured in the absence and presence of various concentrations of the modulator. EC_{50} values were calculated from the concentration–response curve of efflux first order rate constants (V_{max}/K_m) plotted as a function of the modulator concentration. Thus, the effect of different modulators on the transport rate is measured in a direct functional assay.

3 Results and Discussion

The basic underlying principle of structure–activity relationship studies assumes that compounds similar to biolog-

ically active ones should also be active and *vice versa.* Thus, besides the use of classical descriptors, QSAR approaches based on similarity measures have also been reported in the literature. Most of them use $n \times n$ similarity matrices [17] as input vector.

Very interesting in this context is the Chemical Global Positioning System (ChemGPS) developed by Oprea. Selection of a set of satellite structures with extreme values of standard descriptors and a set of representative drugs (core structures) allowed to develop a unique mapping device for the drug-like chemical space [18]. Furthermore, the combination of ChemGPS and VolSurf allowed a pharmacokinetically based mapping of compounds with respect to permeability and solubility [19]. The concept of similarity is also underlying the LASSOO approach of Villar and coworkers [20]. The LASSOO algorithm intends to prioritise compounds that are most similar to a specified set of favourable target molecules (*i.e.* actives) and, at the same time, very different from compounds that reside in this set. Moreover, Ghuloum *et al.* used molecular hashkeys based on molecular surface similarity [21]. The latter inspired us to explore the concept of using similarity values as independent variables in QSAR equations. Within SIBAR, similarity values between training set compounds and a set of reference compounds are calculated and subsequently used as a similarity-based fingerprint. Also in this case, the basic assumption is that compounds similar to actives have a higher likelihood of being also active than those more similar to inactives. Thus, the composition of the reference set might be of high importance for the predictivity of the QSAR models obtained. Within this paper, we systematically investigated the influence of the reference set both for LOO runs and prediction of a TS for a set of inhibitors of the multidrug efflux pump P-gp (ABCB1).

3.1 Influence of the Reference Set on the Predictive Power of the Models

Table 2 summarises the r^2 and r^2_{CV} values for the LOO cross-validation runs for 1–10 principal components (PCs). Subsequently, we chose for each descriptor set the model with the optimum relation of high r^2_{CV} values and a low number of PCs.

Final models are given in Table 3 which shows the predictive power of the models obtained both with the MOE descriptors alone and when applying SIBAR. For the training set, SIBAR in all cases performed slightly better in LOO cross-validation procedures as when using directly the MOE descriptors for deriving the models (0.66–0.69 *vs.* 0.64). Differences of the r^2_{CV} values between the models generated on basis of different reference sets are marginal. In case of the TS, a different picture can be seen. Only in case of reference sets A and B, SIBAR led to better r^2 values in comparison to the use of classical descriptors (0.66 and 0.70 *vs.* 0.62). However, whereas r^2_{CV} values in the LOO runs were rather similar, for the TS r^2 values ranged from 0.59 to 0.70, with reference set B yielding the highest value. This indicates that at least for external prediction of P-gp inhibitory activity the composition of the reference set significantly influences the predictive ability of the model.

Table 2. r^2/r^2_{cv} values for PLS models using 1–10 PCs; final models are highlighted in bold letters.

No. PCs	MOE	SIBAR			
		Ref_A	Ref_B	Ref_C	Ref_D
1	0.59/0.58	0.43/0.42	0.40/0.38	0.49/0.48	0.45/0.44
2	0.66/0.64	0.59/0.58	0.64/0.63	0.66/0.65	0.57/0.53
3	**0.67/0.64**	0.64/0.61	0.67/0.65	0.68/0.66	0.63/0.61
4	0.68/0.63	0.66/0.64	0.69/0.67	**0.68/0.67**	0.64/0.60
5	0.69/0.64	0.68/0.65	**0.70/0.68**	0.69/0.67	0.65/0.62
6	0.69/0.64	0.70/0.67	0.71/0.68	0.70/0.67	0.66/0.63
7	0.70/0.64	**0.71/0.69**	0.71/0.68	0.71/0.67	**0.69/0.66**
8	0.70/0.65	0.72/0.69	0.71/0.68	0.71/0.67	0.69/0.63
9	0.71/0.65	0.73/0.70	0.72/0.67	0.72/0.67	0.70/0.63
10	0.71/0.65	0.73/0.70	0.72/0.66	0.72/0.67	0.71/0.65

No. PCs: number of PCs; MOE: MOE descriptors used; SIBAR: SIBAR descriptors used.

Table 3. QSAR models with/without SIBAR descriptors.

Descriptors	No. PCs	Ref. set	r^2_{CV}	r^2(TS)
MOE	3	–	0.64	0.62
SIBAR	7	Ref_A	0.69	0.66
SIBAR	5	Ref_B	0.68	0.70
SIBAR	4	Ref_C	0.67	0.59
SIBAR	7	Ref_D	0.66	0.62

No. PCs: number of PCs for the final model; Ref. set: reference set used; r^2_{CV}: cross-validated correlation coefficient; r^2(TS): correlation coefficient for prediction of a TS.

3.2 Diversity of the Reference Set

On principle, the SIBAR approach may suffer from an insufficient diversity of the reference set with respect to the training set. To address this issue, both a Principal Component Analysis (PCA, as implemented in MOE) of the SIBAR values and calculation of the mean similarity values within each reference set was performed (Table 4). As outlined, the SIBAR values generally show a higher degree of intercorrelation than the original descriptors. Obviously, application of the SIBAR-concept results in a potential loss of information. SIBAR values obtained from reference set B, which gave highest predictive power, also showed lowest degree of intercorrelation. Ref_D, which is composed of 20 hand-picked chemicals, exhibits the lowest degree of diversity. Interestingly, although 99.89% of the variance is represented in five PCs, the highest r^2_{cv} is obtained with seven PCs. As outlined in a plot of the first two PCs, these small molecules are located beyond the chemical space of the training set compounds (Figure 3d).

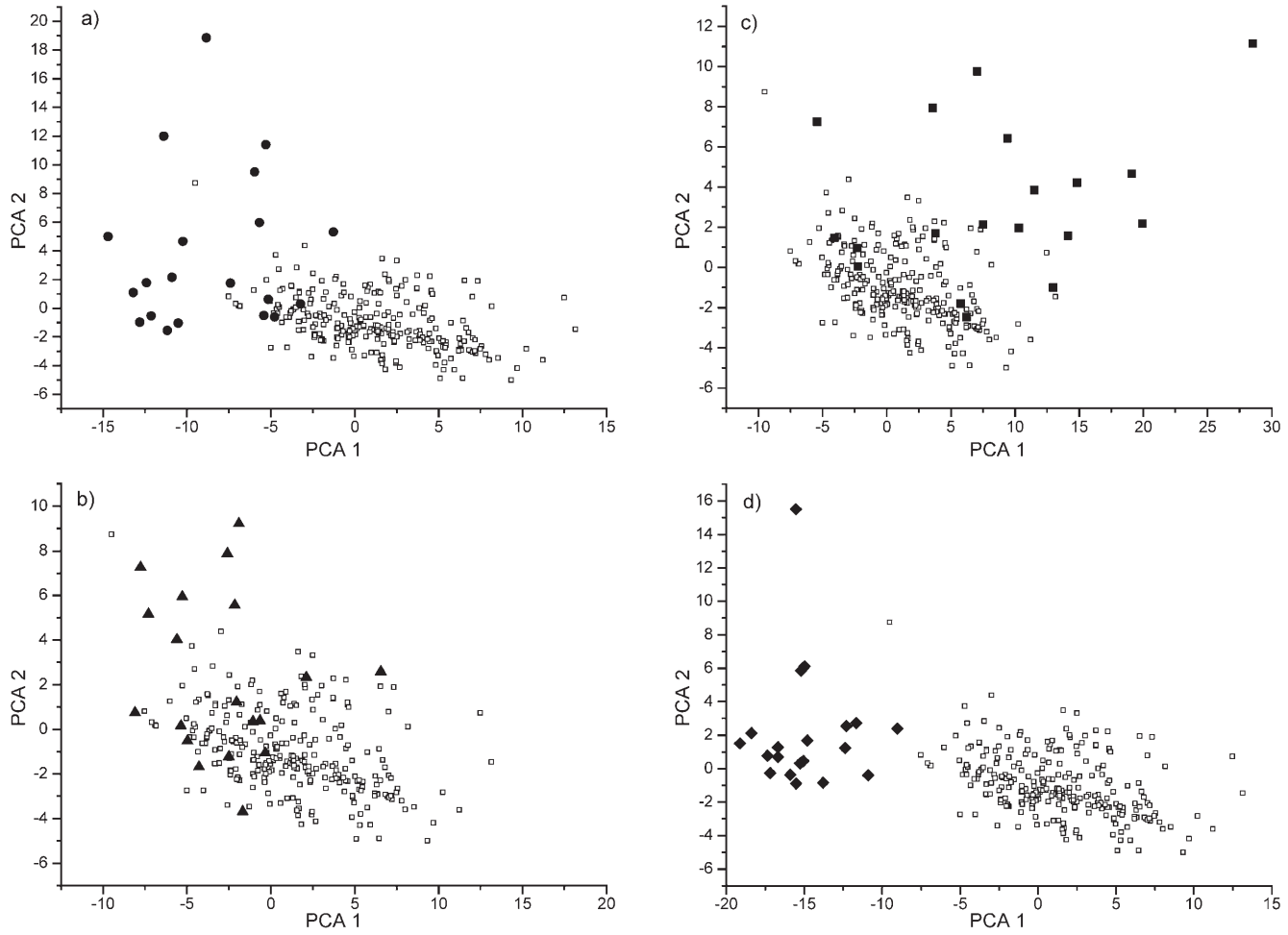


Figure 3. (a – d) Plot of the first two PCs obtained in a PCA analysis of the MOE descriptors showing the relative position of reference sets A – D to the compounds of the training dataset. The training dataset compounds are drawn as open squares, Ref_A compounds as full circles (a), Ref_B compounds as full triangles (b), Ref_C compounds as full squares (c) and Ref_D compounds as full diamonds (d). In case of Ref_B and Ref_C, the plot was obtained by deleting the two outlier cibachrom and valspodar from the dataset.

Thus, Ref_D corresponds to a scenario signifying a low diversity and almost no structural relationship to the training set compounds. The low diversity of Ref_D is further supported by an analysis of the mean Euclidean distances (μ) within the four reference sets used (Table 4), where Ref_D indeed exhibits the lowest value (10.32). The highest mean Euclidean distance corresponds to Ref_C (21.16), which is a set of 20 manually selected P-gp ligands. However, it seems that differences between the mean Euclidean distances do not correlate with the predictive ability of the models obtained. Ref_B, which showed highest predictive power for the external TS, is in the middle range of the values (12.93).

3.3 Composition of the Reference Set

Besides the diversity, also the structural and/or functional relationship of the reference set to the training set compounds might also be of major interest. Figures 3a – d show a plot of the first two PCs, exemplifying the relative position of the four reference sets to the training set. Figures were obtained from the scaled MOE descriptors. Ref_A and Ref_D are composed of compounds which are functionally unrelated to P-gp. In both datasets, they are predominantly lying beyond the chemical space of the training set (Figures 3a and 3d). In contrast, Ref_B and Ref_C are composed of target-related structures. Compounds from Ref_B were extracted from the in-house library of P-gp ligands by means of maximum diversity. With exception of cibachrom, they are well located within the chemical space of the training set (Figure 3b). Use of Ref_B for external prediction indeed gave the highest r^2 values, indicating that a combination of adequate diversity and a relationship to the biological target is beneficial for obtaining predictive models. To check whether a reference compound which is clearly located beyond the chemical

Table 4. PCA and mean Euclidean distances of training and reference sets.

No. PCs	MOE	Sibar_Ref_A	Sibar_Ref_B	Sibar_Ref_C	Sibar_Ref_D
1 comp.	53.15	89.26	73.34	74.46	98.33
2 comp.	64.65	94.81	87.13	87.54	99.05
3 comp.	72.97	97.20	95.60	93.17	99.47
4 comp.	78.96	98.72	96.85	96.63	99.78
5 comp.	83.62	99.16	97.97	98.27	99.89
μ	–	15.50	12.93	21.16	10.32

Upper part: PCA of the 31 MOE descriptors and the 20 SIBAR values for each reference set, respectively; the variance (in %) after variance test of 1 – 5 PCs is shown. Lowest line: mean euclidean distance (μ) within reference sets A – D.

space of both training and reference compounds influences the quality of the PLS models, cibachrom was eliminated from Ref_B and the SIBAR model was established on basis of only 19 reference compounds. Both r^2_{cv} and r^2 for the TS did not show any change. This also happens when eliminating valspodar from Ref_C.

Interestingly, Ref_C, which consists of P-gp ligands and exhibits the highest μ-value, gave only an r^2 value of 0.59 for the TS. However, this is mainly due to two severe outliers in the observed *versus* predicted plot of the TS, which only appear when calculating the SIBAR fingerprint for Ref_C: AG-227/33912017 [$\log(1/EC_{50})_{obs} = -2.20$ *vs.* $\log(1/EC_{50})_{pred} = -5.53$] and AG-205/33114008 [$\log(1/EC_{50})_{obs} = -2.01$ *vs.* $\log(1/EC_{50})_{pred} = -5.19$]. When excluding these two outliers, the r^2 value for TS calculated on the basis of the model obtained from Ref_C increases to 0.63, whereas the values for the other models change only slightly (MOE: 0.60; Ref_A: 0.66; Ref_B: 0.69; Ref_D: 0.63). Now all the reference sets perform (slightly) better in predicting the TS, whereby Ref_B still outperforms all the other models (0.69).

4 Conclusions

Within this paper, we studied the influence of the reference set on the predictive ability of QSAR models using SIBAR applied on a dataset of 412 inhibitors of the multidrug efflux pump ABCB1. In LOO cross-validation runs, the composition of the reference set only to a minor extent influenced the quality of the models. However, in the case of external predictions the choice of an appropriate reference set became increasingly important. Our results demonstrate that a combination of high diversity and a relation of the compounds to the biological target is beneficial for obtaining good models. However, it has to be pointed out that, in this study, the overall performance of SIBAR was more or less equal to those of classical descriptors and that differences in r^2 values are not considered as statistically significant. This might be due either to a suboptimal reference set or to the descriptors used for calculating the respective SIBAR values. For the latter, preliminary results obtained in our group indicate that, for a small set of compounds, use of shape similarity values remarkably improved the results. With respect to an optimal reference set, two strategies might be applied. One is to look for a generally valid, globally applicable reference set by following the ChemGPS approach, the other would be to apply a genetic algorithm or to use other established methods for variable selection for selecting the reference compounds out of the respective training set. However, the latter might be limited to the chemical space of the training set.

We already showed that the SIBAR concept does not work with the steroid dataset widely used for validating new QSAR methods. This indicates that the primary applicability of SIBAR might be in the field of promiscuous targets and antitargets, where the chemical space of the ligands is highly diverse and general models are rare. Undoubtedly, additional studies utilising both different ways of calculating similarity and other polyspecific proteins are needed to further explore the SIBAR approach. This concept is currently under active investigation and will also be extended to other polyspecific proteins.

Acknowledgements

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