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In silico modeling of protein tyrosine phosphatase 1B inhibitors with cellular activity

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Abstract—Protein tyrosine phosphatase 1B (PTP1B) is a potential drug target for the treatment of Type 2 diabetes and obesity. The design of PTP1B inhibitors as therapeutic agents has been hampered mostly owing to their poor cell permeability and oral bioavailability. In the present study, we investigated the cellular activity of PTP1B inhibitors in relation to the 3D structure using classical VolSurf analysis. A model based on the VolSurf descriptors for a set of 80 compounds of PTP1B inhibitors, half of which display cellular activity, was analyzed using the principal components analysis (PCA) approach. The PCA model was applied to predict the cellular activities of an external data set of 40 PTP1B inhibitors and satisfactory results were obtained. Further partial least squares (PLS) analysis revealed useful information about the behavior of the Volsurf descriptors in predicting the cell permeability and pharmacokinetic properties of PTP1B inhibitors. In silico ADME studies provide a valuable tool in the development of effective PTP1B inhibitors as drug candidates.

Protein tyrosine phosphatases (PTPases) are signaling transducing enzymes that play vital roles in regulating diverse cellular processes including cell growth, proliferation, and differentiation, metabolism, immune response, and cell–cell adhesion.[1,2] Human protein tyrosine phosphatase 1B (PTP1B), the first characterized PTPase, has attracted intensive research because of its involvement in the insulin signaling cascade as a major negative regulator of insulin signaling.[3] The compelling transgenic experiments have demonstrated that it plays a key regulatory role in modulating both insulin sensitivity and resistance to weight gain, indicating that PTP1B is a potential therapeutic target for the treatment of both Type 2 diabetes and obesity.[4,5] A large number of PTP1B inhibitors have been developed over the last decade in an effort to design potent and selective compounds as drug candidates (for reviews, see Refs. 6–9). These inhibitors typically incorporate a charged pTyr mimetic to achieve strong binding to the highly conserved and polarized active site of PTPases. The commonly used pTyr mimetics include phosphonates, carboxylic acid, sulfamic acid, difluoromethylphosphonates (DFMP), oxalylaminobenzoic acid (OBA), *O*-carboxymethyl salicylic acid, etc. (Fig. 1). Unfortunately, most of these multiple-charged phosphate-mimicking components have proven difficult to develop into effective drugs due to their low cell permeability and oral bioavailability.

Intense efforts have been made in recent years on the design of PTP1B inhibitors to improve the cellular activity. Figure 2 shows some PTP1B inhibitors with good cell permeability and bioavailability. A series of DFMP inhibitors substituted with a deoxybenzoin side chain was developed at Merck, which displayed strong PTP1B inhibition and cellular activity.[10] Compound **17** with an *ortho*-bromo substituent on the phenyl ring proved to be orally bioavailable and showed glucose-lowering activity in rat models.[10] Liljebris et al. investigated bioisosteric replacements for the carboxylic acid moiety of *O*-carboxymethyl salicyclic acid with an *ortho*-tetrazole unit (compound **18**). The monocarboxylic acid analogue revealed significantly higher Caco-2 cell permeability as compared to all previous compounds.[11] Researchers at Wyeth-Ayerst discovered a series of novel PTP1B inhibitors utilizing a neutral pTyr moiety azolidinedione. Some of these azolidinedione derivatives (compound **19**) normalize plasma glucose and insulin levels in diabetic mouse models.[12] The orally active pyrimidotrizineridamine-based compounds (compound **20**) discovered at Roche display excellent bioavailability in the ob/ob

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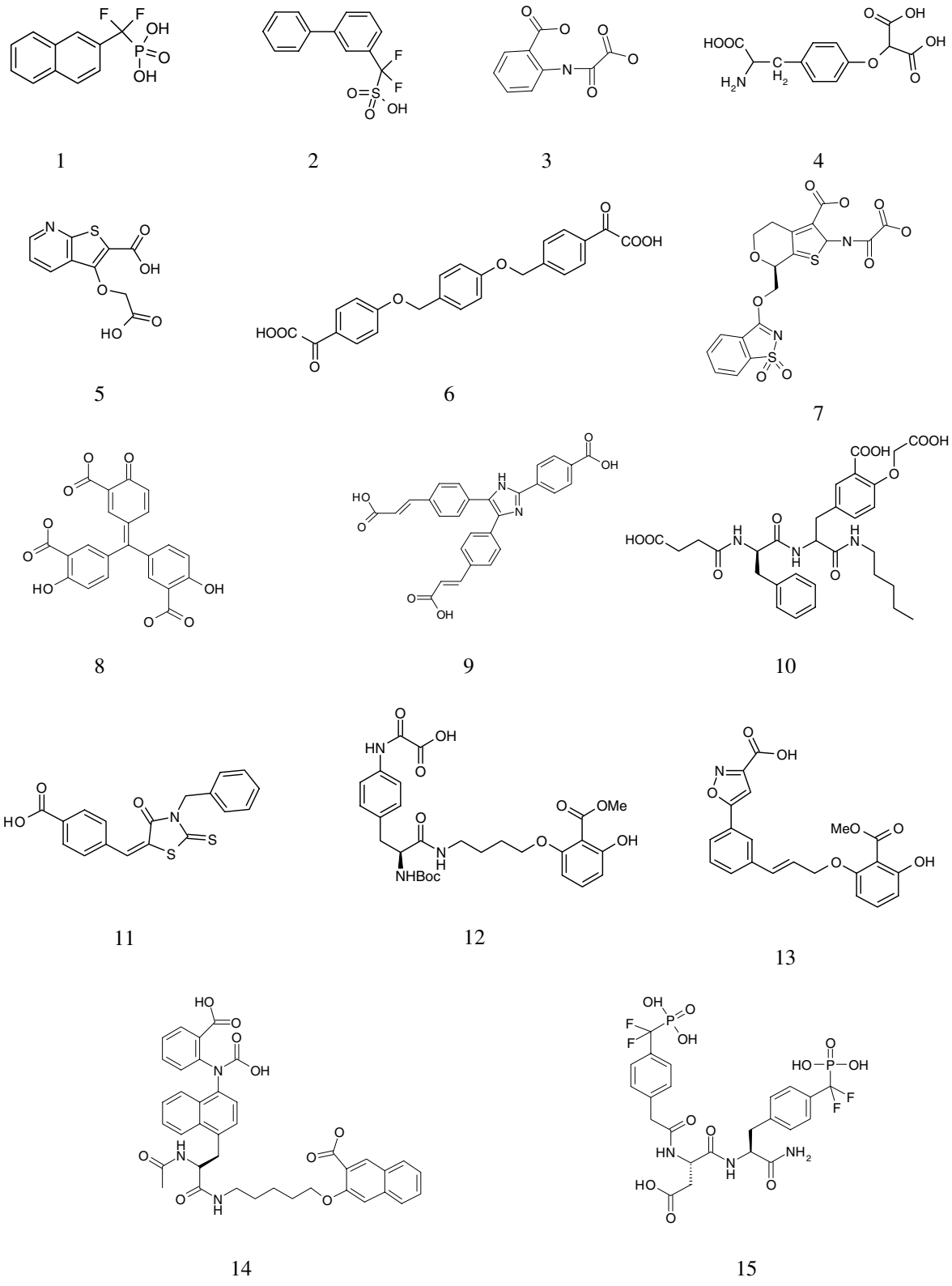

Figure 1. Representative compounds of PTP1B inhibitors with poor cell permeability.

mouse model.[13] Interestingly, the two classes of allosteric PTP1B inhibitors reported so far, the pyridazine analogues (compound **21**) and benzbromarone derivatives (compound **22**), possessed good cellular activities and increased insulin-stimulated phosphorylation of the insulin receptor.[14,15]

In this study, we investigated the relationship between the cellular activity and the 3D structure of PTP1B inhibitors using classical VolSurf analysis.[16] The VolSurf approach is widely used in ADME (absorption, distribution, metabolism, elimination) modeling of druglike compounds to guide early drug development.

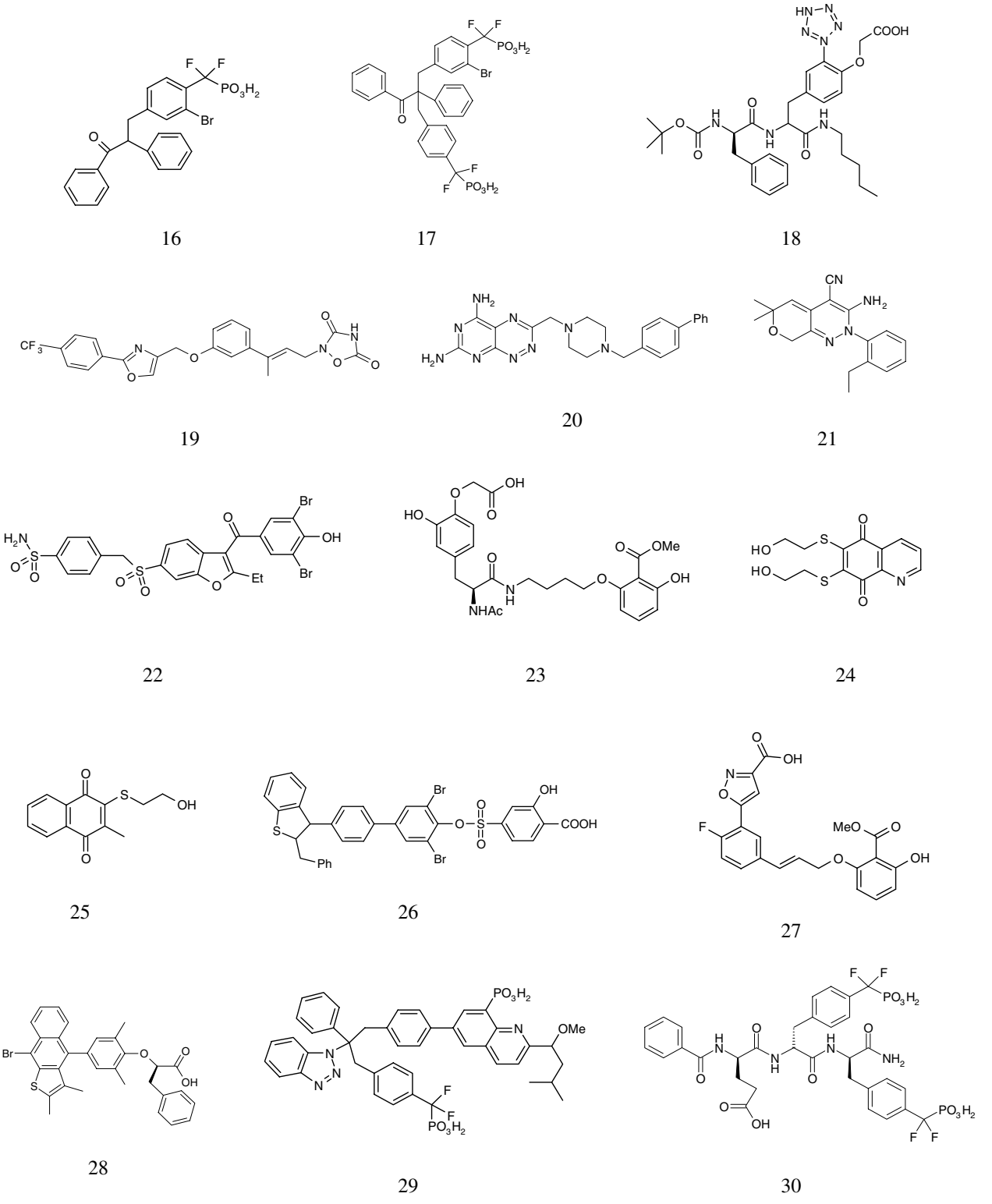

Figure 2. Representative compounds of PTP1B inhibitors with cellular activity.

Molecular descriptors calculated by the VolSurf program have been applied to model pharmacokinetic parameters, for example, passive permeability through the gastrointestinal tract or through the blood–brain barrier (BBB).[17,18] VolSurf descriptors quantify steric, hydrophobic, and hydrogen bond interactions between model compounds and the target molecule to describe the 3D molecular fields. Since these interactions are the same as those involved in the ligand–receptor binding, VolSurf descriptors could potentially be relevant to the process of cellular permeation and are useful to model the pharmacokinetic parameters.

The principal components analysis (PCA) was conducted for a set of 80 compounds of PTP1B inhibitors selected from the literature, half of which display good or

moderate cellular activities in vitro or in vivo. A total of 78 VolSurf descriptors were calculated using the VolSurf 4.0 program with two probes (OH2 and DRY) and the data matrix was analyzed using PCA method. Five significant principal components (PCs) were found by cross-validation technique (Table 1). The first two principal components explained about 52% of the total variance of the matrix. The score plot for the first two PCs is shown in Figure 3. The compounds with cellular activity are in red color, and the cellular-inactive compounds are in black. Clearly, the model distinguished the two classes of compounds very well, with only a few outliers that cannot be classified properly. Such a result is quite remarkable because the model was obtained merely from the structures and the molecular descriptors, and no activity input was given in the PCA analysis. The results indicate that the VolSurf descriptors, which are specifically designed to model the pharmacokinetic properties and membrane permeation of drug compounds, are efficient in the prediction of cellular activity for PTP1B inhibitors.

To test the predictive capacity of the PCA model, 40 compounds were selected as external testing data set and the cellular activities were predicted by projecting the VolSurf descriptors into the PCA model made with the training set. The predicted result is shown in Figure 4, which is PC1 versus PC2 score plot and the compounds are colored according to their cellular activity. Overall, the prediction is quite satisfactory, with a correct classification of more than 80% of compounds with cellular activity, and 90% accuracy of prediction for the compounds with poor cellular activity.

Table 1. Summary of principal components analysis (PCA)

Components	XVarEXP	XAccum
1	29.50	29.50
2	21.56	51.06
3	9.75	60.80
4	7.51	68.32
5	4.42	72.75

XVarExp, Percentage of X-matrix variance explained by that component; XAccum, accumulative percentage of the X-matrix variance explained by the model.

Inspection of the mispredicted compounds revealed useful information on the different behavior of cellular activity related to the 3D structure. Compound **23** demonstrates a high level of membrane penetration.[19] However, it was predicted with poor cellular permeability by the PCA model. In fact, compound **23** forms a lactone between the 2-hydroxy group and the oxamic acid. The equilibrium between the lactone and acid forms make it possible to circumvent the poor cell permeability of most of other oxamic acid compounds. Because our prediction was based on the acid form, the predicted result of poor cellular permeability for the acid form may be in fact correct. It is interesting to note that the naphthoquinone derivative compounds **24** and **25** differ from most of other PTP1B inhibitors. It is possible that these compounds cross the membrane with a different mechanism from others (e.g., active transporter or fast efflux). The significant outlier is compound **30**, which is a tripeptide-based DFMP PTP1B inhibitor and exhibits good permeability in the Sf9 cell-based assay.[10,20] The failure of prediction of this compound is probably due to the limitation of the statistical model, which was trained mainly by non-peptide small molecules.

Two cell-impermeable compounds were mispredicted (compounds **11** and **13**, Fig. 1). Interestingly, compound

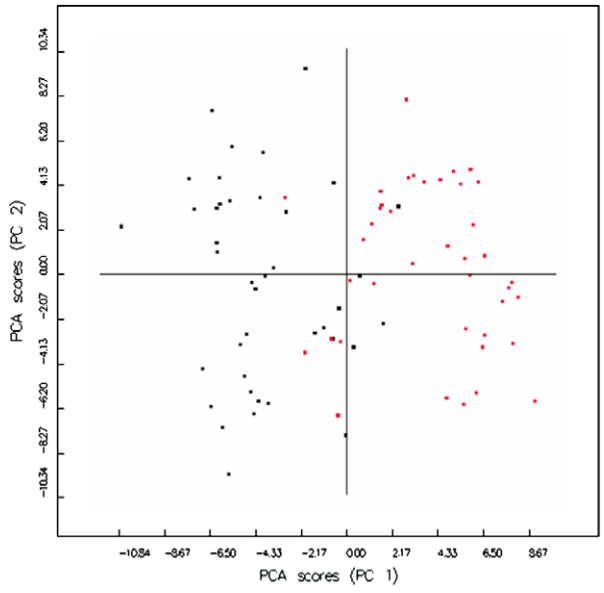

Figure 3. PCA score plot for the training data set of 80 compounds. The cellular-active compounds are in red color, and cellular-inactive compounds are in black.

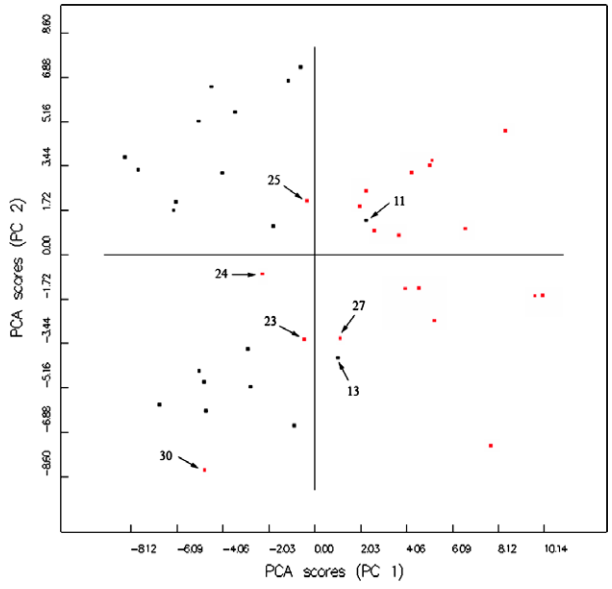

Figure 4. PCA predictions for the test set of compounds. The cellular-active compounds are in red color, and cellular-inactive compounds are in black. Some of the mispredicted compounds are labeled in the plot.

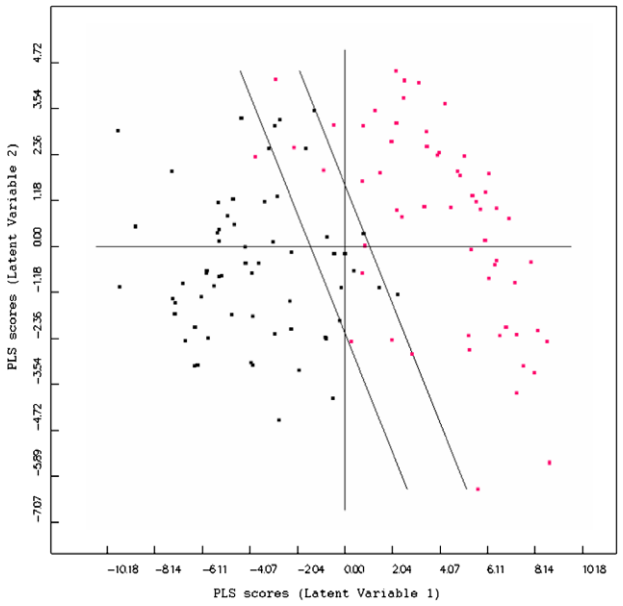

Figure 5. PLS score plot for the global model (training and test data sets combined). A confidence interval is shown in the plot.

13 is structurally close to compound **27**, which displayed permeability and was predicted correctly by the PCA model (Fig. 4). The difference between these two compounds is that a fluorine replaces hydrogen in compound **13**, and the increased cell permeability of compound **27** is most likely caused by the increased lipophilicity of a fluorine effect.[21] The model appears not able to explain the biological behavior resulting from the subtle structural difference. It is worth noting that there are some limitations with the statistic model, for example, it was trained by a relatively small chemical space due to the lack of experimental data; the cellular activity was measured by different groups, and even with different methods. Therefore, it is expected that some compounds were not predicted correctly due to the limitations of the statistic model, such as compound **11** mispredicted in the testing data set.

To gain more insights into the cellular activity of PTP1B inhibitors related to the 3D structure, partial least squares (PLS) discriminant analysis was carried out by combining the training set and the test set of 120 compounds. In the PLS analysis, the cellularly active compounds were assigned to a categorical score 1, and the cellularly inactive compounds were assigned to a categorical score −1. The PLS score plot of the resulting model is shown in Figure 5. The PLS model discriminated well between the two classes of compounds. The result appears to be better than the PCA model because the PLS model was obtained by adding biological information. The model was evaluated by cross-validation technique, giving 0.57 of q^2 and 0.65 unit of SDEP (standard deviation of error of prediction error). The confidence interval is also shown in Figure 5.

The coefficient plot of the PLS model shows the contribution of VolSurf descriptors to the cell permeability. As shown in Figure 6, the polar descriptors such as hydrophilic regions and the capacity factors, which refer to polar water-accessible surface areas and polar interactions per surface unit, are inversely correlated with cell

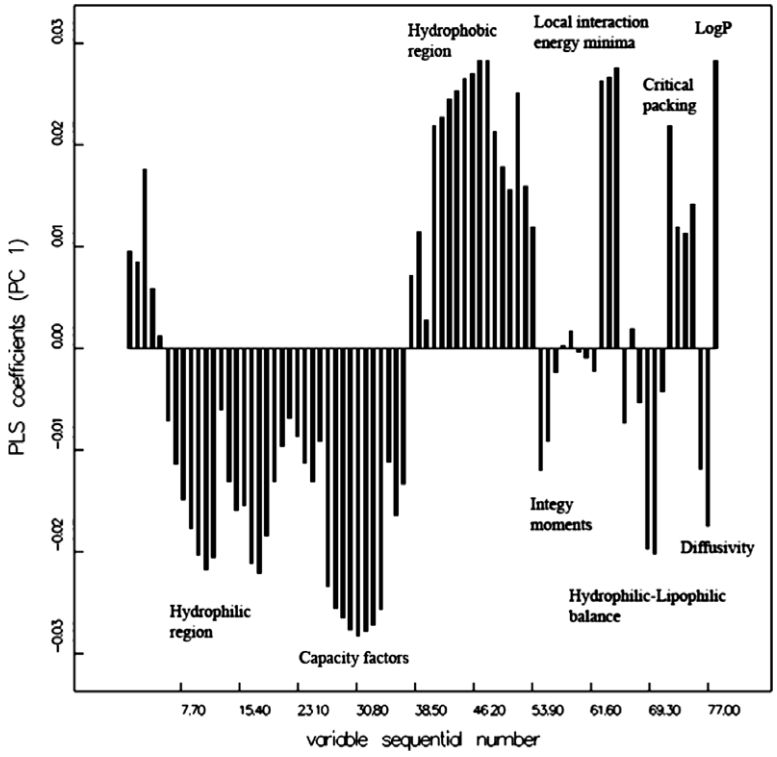

Figure 6. PLS coefficient plot of the model for the correlation of VolSurf descriptors.

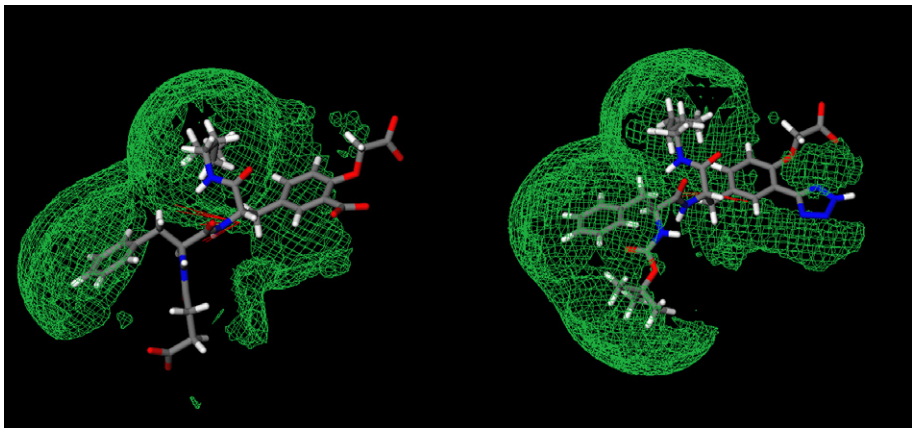

Figure 7. Grid 3D molecular fields of compound **10** (left) and compound **18** (right) calculated with a DRY probe. The arrows represent the integy moment's pattern.

permeability. This result indicates that cell permeability decreases when the polar surface and other polar factors increase such as the charge distribution and electron lone pairs. Integy moment measures the unbalance between the center of mass of a molecule and the position of the hydrophilic or hydrophobic center, but it appears less important. As expected, the descriptors of hydrophobic interactions and LogP are strongly correlated with the cell permeability. This is consistent with experimental observation. In addition, the critical packing and local interaction energy minima are also important descriptors to the cell permeability.

VolSurf descriptors can be projected back into the original 3D grid map to help interpretation. Figure 7 shows a comparison of the GRID 3D molecular fields of two closely related compounds **10** and **18** but with different cell permeability. Both compounds belong to the class of *O*-carboxymethyl salicyclic acids. One carboxylate moiety of compound **10** is replaced with a tetrazole unit, and another N-terminal carboxyl of compound **10** is replaced with a butyloxycarbonyl group. The contour around the molecules represents the hydrophobic regions calculated with a DRY probe. The arrows represent the vectors of the integy moments. The hydrophobic regions of compound **18** are significantly larger and well surrounded as compared to those of compound **10** because of the replacement of the charged groups. Notably, the integy moments of these two compounds are significantly different. As the size of these hydrophobic regions is strongly correlated with cell permeability, the larger region gave an explanation on the increased cell permeability of compound **18**.

In summary, using classical VolSurf analysis, reliable and predictive PCA and PLS models were obtained for the cellular activity of PTP1B inhibitors in relation to their 3D structures. The PCA model was used to predict the cellular activity of an external data set of 40 PTP1B inhibitors and satisfactory results were obtained. The predictive capacity of these in silico models reveals useful information about the cell permeability and 3D molecular structures of PTP1B inhibitors, providing a valuable tool in virtual screening for druglike lead compounds for the treatment of Type 2 diabetes and obesity.

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