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Use of Volsurf approach for pharmacokinetic optimization of β-carboline derivatives as antitumor agents

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The β-carboline derivatives are a large group of naturally-occurring and synthetic alkaloids which have a wide spectrum of biological and pharmaceutical properties. The newly developed procedure called VolSurf has been used to explore a significant correlation between the 3D molecular interaction fields (MIF) and physicochemical and pharmacokinetic properties of a set of 30 β-carboline compounds acting as antitumor agents. In general terms, VolSurf generates a limited set of quantitative numerical descriptors from MIFs by calculating the volume or the surface of the interaction contours. These descriptors that encode the information content from the chosen probes are simple to interpret from a chemistry point of view. The aim of this approach is to allow the analysis of a large number of quantitative descriptors by using chemometric tools such as partial least squares (PLS) and principle component analysis (PCA). The PLS model gave statistically significant results with R^2 and Q^2 values of 0.92 and 0.72, respectively. The ability of the model was validated by an external test set of 10 compounds, which gave $R^2_{(pred)}$ of 0.85. The VolSurf model developed here identifies hydrophobicity as the major physicochemical parameters responsible for the antitumor activity of the β-carboline derivatives towards HepG2 cell lines.

Keywords: β-carboline; HePG2; Volsurf; Pharmacokinetic properties

1. INTRODUCTION

The β-carboline alkaloids which are originally isolated from the medicinal plant *Peganum Harmala*, exert a wide spectrum of pharmaceutical properties [1, 2]. During recent years numerous β-carboline derivatives bearing various substituents at positions 1, 3, 7, and 9 have been synthesized that all have a thricylic planar system with different degrees of aromaticity. Different biochemical and pharmacological effects of these compounds like anxiolytic, antimicrobial [3], antiviral [4], sedative [5], antithrombotic [6] and antiparasitic [7] effects are due to presence, location and nature of the substituents. These are also associated with nurological diseases such as Paikinson's disease [8]. Moreover, a large series of β-carboline have shown high affinity for several receptors such as benzodiazepine (BZ) [9], 5-hydroxytryptamine (5-HT) [10], imidazoline [11], and dopamine (DA) 12].

Recently, β-carboline alkaloids have been characterized as a class of potential antitumor agents that apoptosis in HepG2 cells induced by β-carbolines is the main cause of antitumor activity of these compounds [13-18]. The VolSurf procedure generates a set of molecular descriptors in order to quantify steric, hydrophobic and hydrogen bond interactions between drugs and their receptors [19]. The procedure starts with 3D molecular interaction fields generated from the interactions of different probes like water probe (OH2), hydrophobic probe (DRY), and ionic probes with the target molecule. In the next step the 3D descriptors convert to 2D descriptor called VolSurf

descriptors, which refer to molecular size and shape, to hydrophilic and hydrophobic regions and the balance between them [20]. The present study is dedicated to select the most appropriate descriptors according to the type of 3D features via VolSurf procedure in order to drive chemometric models for a dataset of β-carboline derivatives.

2. EXPERIMENTAL

2.1. Data set

The data set was adopted from the work of Rihui Cao et al [21] in which they reported the cytotoxic potential of a number of synthesized β-carbolines against a panel of human tumor cell lines. The VolSurf approach was applied to obtain a 3D-QSAR model for the potency of 40 from total 47 β-carboline derivatives which have significant pIC_{50} toward HepG2 human tumor cell lines (Table 1). A statistical subset selection was made using most descriptive compound (MDC) [22] method in which the compounds are weighted according to their population density. The complete molecular set was split into two different sets involving: training and test set. The training set is composed of 30 compounds which were used to adjust the parameters of the models. The corresponding pIC_{50} values which are listed in Table 1 range from 3.63 for the most weakly compound (**45**) to a value of 5.8 for the most potent compound (**57**) and cover a spectrum of approximately 3-log units. The 3D QSAR model derived was successfully validated by using a test set of 10 similar compounds, with comparable pIC_{50}.

2.2. VolSurf approach

The VolSurf program has been extensively used to calculate a set of 2D descriptors from the 3D maps of molecular interaction field (MIF) related to pharmacokinetic properties of drugs. The principles of this procedure have been described elsewhere [23, 24] in more detail, but it is worth mentioning about whole procedure briefly.

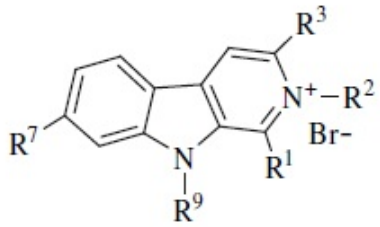
The strategy for the VolSurf calculation consist in two main steps: firstly the 3D molecular interaction fields are calculated from the interaction of water (OH2) probe simulating solvation and desolvation, the hydrophobic (DRY) probe representing the hydrophobicity, and the carbonyl oxygen (O) and amide nitrogen (N1) probes representing, the hydrogen bond acceptor and donor, respectively, around the target molecule. Then the **Vol**ume and **Surf**ace descriptors are obtained at different energy levels [25]. Thus during this procedure all relevant information contained in Grid maps are transferred from 3D to 2D descriptors, that are alignment-independent and are only marginally influenced by conformational sampling [20]. Some of these descriptors are easily interpretive because they can be projected back into the original 3D-Grid maps from which they are obtained.

Among all kinds of descriptors produced and used in 3D-QSAR methods, the VolSurf descriptors are well suited for modeling some permeation properties, and also to describe some ADME properties of drug compounds[26].

2.3. Model construction and validation

The 3D structures of dataset were constructed and minimized in SYBYL7.3 molecular modeling package (Tripos Inc., St. Louis, USA). Geometries of the molecules were optimized by using the Tripos force field with a distance dependent dielectric and the Powell conjugate gradient algorithm with a convergence criterion of 0.01kcal/mol. Partial atomic charges were calculated using the Gasteiger-Hückel method. From the 3D structures, molecular descriptors were calculated using the VolSurf+ 1.0.4 program. All these calculations were carried out on Linux workstation.

Table 1. Structures and potencies toward HepG2 tumor cell lines of compounds 1-40.



Comp.	Substituents					pIC_{50}	pIC_{50}
	R_1	R_2	R_3	R_7	R_9	(exp.)	(cal.)
1	3,4,5-trimethoxyphenyl	_	$CO_2C_2H_5$	H	H	3.78	3.75
3	3,4,5-trimethoxyphenyl	_	CO_2H	H	*n*-C_4H_9	3.68	3.74
4	3,4,5-trimethoxyphenyl	_	$CONH(CH_2)_2OH$	H	*n*-C_4H_9	4.06	4
11	H	_	$CONH(CH_2)_2NH_2$	H	*n*-C_4H_9	4.08	4
12	H	_	$CONH(CH_2)_6NH_2$	H	*n*-C_4H_9	4.02	4.24
13[a]	CH_3	_	$CONH(CH_2)_2OH$	H	*n*-C_4H_9	3.85	3.67
14	CH_3	_	$CONH(CH_2)_2NH_2$	H	*n*-C_4H_9	4.66	4.62
15[a]	CH_3	_	$CONH(CH_2)_2NH_2$	H	$CH_2C_6H_5$	4.46	4.34
17	H	_	$CONH(CH_2)_2NH_2$	H	$CH_2C_6H_5$	3.98	3.84
18[a]	H	_	$CONH(CH_2)_6NH_2$	H	$CH_2C_6H_5$	4.23	3.94
21[a]	CH_3	_	CH_2OH	H	*n*-C_4H_9	3.89	3.87
23	CH_3	_	CHO	H	*n*-C_4H_9	3.84	3.71
29	CH_3	$CH_2C_6H_5$	H	H	H	4.16	4.08
30	CH_3	$(CH_2)_3C_6H_5$	H	H	H	4.48	4.53
31	CH_3	$CH_2C_6H_5$	$CO_2C_2H_5$	H	H	4.28	4.03
32	CH_3	$CH_2C_6H_5$	H	OCH_3	H	4.26	4.17
33	H	*n*-C_4H_9	H	H	H	4.03	3.97
34[a]	H	$CH_2C_6H_5$	H	H	H	4.11	4.1
35	H	$(CH_2)_3C_6H_5$	H	H	H	4.35	4.48
40	CH_3	_	H	OH	C_2H_5	3.87	4.14
41[a]	CH_3	_	H	OH	*n*-C_4H_9	4.09	4.16
42	CH_3	_	H	OH	*i*-C_4H_9	3.94	4.02
43	CH_3	_	H	OH	$(CH_2)_3C_6H_5$	4.55	4.45
44	CH_3	_	H	OC_2H_5	C_2H_5	4.16	4.36
45	CH_3	_	H	$OCH_2C_6F_5$	C_2H_5	3.63	3.59
46[a]	CH_3	_	H	OC_2H_5	*n*-C_4H_9	4.36	4.28

Continued Table 1.

Comp.	R_1	R_2	R_3	R_7	R_9	pIC_{50} (exp.)	pIC_{50} (cal.)
47	CH_3	_	H	$OCH(CH_3)_2$	*n*-C_4H_9	4.52	4.66
48	CH_3	_	H	OC_4H_9	*n*-C_4H_9	4.81	4.48
49	CH_3	_	H	$OC_{10}H_{21}$	*n*-C_4H_9	3.90	3.91
50	CH_3	_	H	OC_4H_9	*i*-C_4H_9	4.92	4.73
51	CH_3	_	H	$OCH_2C_6H_5$	*i*-C_4H_9	4.65	4.6
52[a]	CH_3	_	H	$OCH(CH_3)_2$	$(CH_2)_3C_6H_5$	4.84	4.34
53	CH_3	_	H	OC_8H_{17}	$(CH_2)_3C_6H_5$	3.98	4.18
54[a]	CH_3	_	H	$OCH_2C_6H_5$	$(CH_2)_3C_6H_5$	4.80	4.22
55	CH_3	_	H	$OCH_2C_6F_5$	$(CH_2)_3C_6H_5$	3.83	4.14
56	CH_3	$CH_2C_6H_5$	H	OC_2H_5	C_2H_5	4.84	4.92
57	CH_3	$CH_2C_6H_5$	H	$OCH_2C_6F_5$	C_2H_5	5.80	5.56
58	CH_3	$CH_2C_6H_5$	H	OC_4H_9	*i*-C_4H_9	5.74	5.91
59[a]	CH_3	$CH_2C_6H_5$	H	$OCH_2C_6F_5$	*i*-C_4H_9	5.72	5.66
60	CH_3	$CH_2C_6H_5$	H	OC_8H_{17}	$(CH_2)_3C_6H_5$	5.41	5.33

In the present study, the water (OH2), hydrophobic (DRY), carbonyl oxygen (O), and amide nitrogen (N1) probes were used, resulting in 123 descriptors. Since there is no variable selection implemented in the VolSurf+ program, in order to extract the more informative descriptors and build a predictive model, a variable selection techniques namely genetic algorithm (GA)[27] performed in the MATLAB (version 7.6.0., Math Works, Inc.) was applied that yields 23 out of total 123 descriptors. The GA-PLS analysis resulted in a model with six latent variables and a correlation coefficient of 0.92 and a standard deviation of the error of calculation (SDEC) of 0.15. Also, the validation of this model using cross-validation method and an external test set shows Q^2_{loo} =0.72 of the validated model and the R^2_{Pred} for the external test set was 0.85. The statistical parameters of the PLS analysis resulted in a six-latent-variables (LV) are given in Table 2. The plot of experimental vs. predicted values of pIC_{50} in Fig.1 proves the good quality of PLS model obtained.

Table 2. The statistical results of the PLS model developed for the series of β-carboline alkaloids.

N[a]	R^2[b]	$Q^2_{(loo)}$[c]	SDEC[d]	SDEP[e]
6	0.92	0.70	0.15	0.30

[a] optimum number of latent variables
[b] noncross-validated correlation coefficient
[c] cross-validated correlation coefficient
[d] standard deviation of error of calculation
[e] standard deviation of error of prediction

3. RESULTS AND DISCUSSION

The PLS coefficient plot (Fig. 2) shows the contribuation of all 23 VolSurf descriptors selected by GA to explain the PLS model, is reported in. The GRID molecular interaction fields (MIFs) around an active (**58**), and an inactive (**45**) is shown in Fig. 3. The colored areas around the molecules are the Grid fields produced by the molecule: green for DRY probe and cyan for OH2 probe, red for O and blue for N1 probe. As can be seen from Fig. 3 for the most inactive compounds red and blue color that represent O and N1 probes and refer to H-bond acceptor and H-bond donor respectively are dominant in Grid maps of inactive compounds e.g. **3** and **45**. Also for the most active compounds like **57** and **58** DRY probe that hydrophobic effects has high impact on activity. It is worth mentioning that all compounds have hydrophobic effects due to their three-ring system, but the active compounds have more hydrophobic moieties like phenyl ring in 2-benzyl substituent for compounds **57** and **58**.

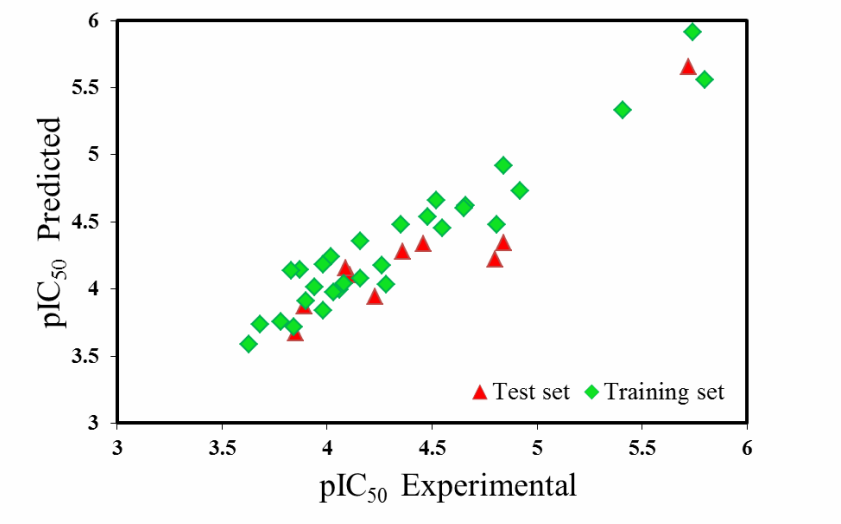


Fig. 1. Plot of Experimental vs. Predicted pIC_{50} values for 3D-QSAR model.

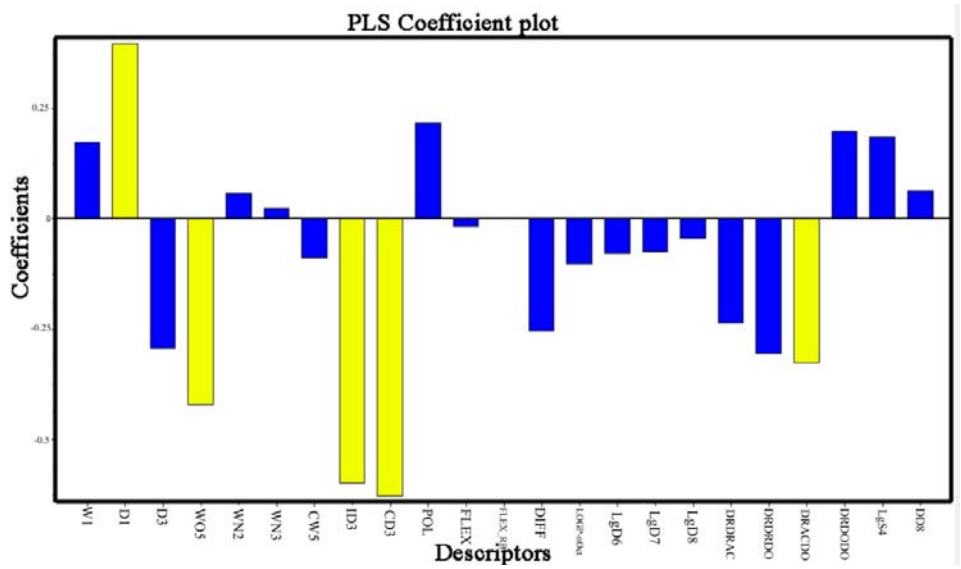


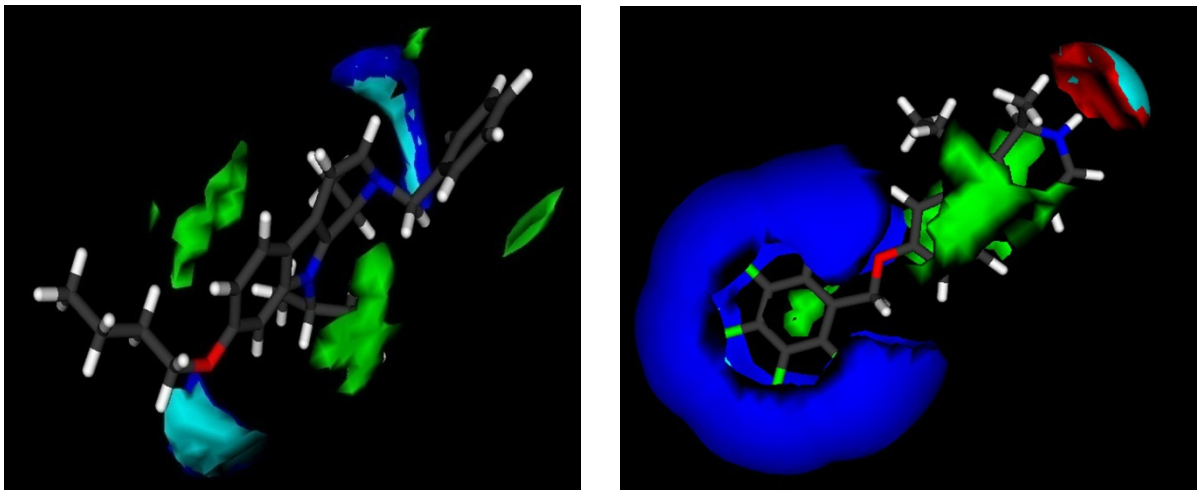
Fig. 2. PLS coefficient plots for 3D-QSAR model. Direct and reverse correlation with the activity are indicated with positive and negative PLS coefficients, respectively. Bars with the most intensive height in the PLS plots have the most profound impact on the model obtained.

The five most significant VolSurf descriptors extracted by GA feature selection procedure and used as PLS input are: Capacity factor (CD3), hydrophobic regions (D1), H-bond donor volumes (WO5), hydrophobic integy moment (ID3), and 3D-pharmacophoric descriptors (DRACDO), that the activity particularly increases with high values of D1, and the rest are inversely related to the antitumor activity of β-carboline derivatives.

Capacity factor (CD3): which is measure of amount of hydrophobic regions per surface unit is an important factor and according to PLS coefficient plot is detrimental for activity. Hydrophobic regions (D1): it can be defined as the molecular envelope generating attractive hydrophobic interactions. These interactions were calculated by using a special probe called DRY. According to PLS coefficient plot the activity is directly proportional to the value of this descriptor which is in agreement with Gird maps of active and inactive compounds. High values of this descriptor for active compounds like **57** and **58**, and low values for inactive compounds like **3** and **45** are in complete agreement with the PLS coefficient plot for the correlation of Volsurf descriptors with β-carboline antitumor activity (Fig. 2). We can deduce from the plot that activity is directly proportional to the higher values of D1.

The green zones around molecules represent the hydrophobic regions. From the field maps it is clear that hydrophobic regions of **45** are larger than **58**, thus it means there is a clear concentration of hydrophobic regions in only one part of the compound **45** as an inactive one, whereas the lower integy moments in case of **58** indicates that the polar moieties are distributed throughout the molecule and the resultant barycenter is close to the centre of this compound as a active one. All these results suggest that the value of this descriptor correlate inversely with the activity of compounds which is evidently shown in PLS coefficient plot.

H-bond donor volumes (WO5): Fig. 3 shows the visual comparison of Grid 3D molecular fields of active compound **58** and inactive compound **45** calculated with O (carbonylic oxygen) probe. The red zones around molecules represent the H-bond donor fields, which may be defined as the molecular envelope generating attractive H-donor interaction. Since this descriptor is inversely proportional to activity it is expected result that there is no H-bond donor fields around the active compounds.



Compound 58 Compound 45

Fig. 3. GRID Molecular Interaction Fields (MIFs) around compound **58** as an active one, and compound **45** as an inactive one.

Hydrophobic Integy moments (ID3): this descriptor indicates the unbalance between the molecular center of mass and the barycenter of polar moieties (in this case hydrophobic regions) were measured with integy moments. High values of integy moments means clear concentration of hydrophobic regions in only one part of the inactive compounds, and low values of integy moment

in inactive compounds means that the hydrophobic interaction sites are either close to the center of mass or they balance at opposite ends of the molecules.

3D-pharmacophoric descriptors (DRACDO): VolSurf+ 3D pharmacophoric descriptors are generated from all possible triplets of distances between the atoms (points) of structures which are classified as hydrophobic (DR), H-bond donor (DO) and H-bond acceptor (AC). The descriptor DRACDO, being pharmacophoric in nature, shows a negative correlation with the activity of the compounds. Most of these descriptors mainly represent hydrophobicity as the major physicochemical parameter influencing on the antitumor activity of β-carboline derivatives. To summarize the importance of different VolSurf descriptors, it can be concluded that hydrophobic interaction contribute more than do other descriptors

4- CONCLUSION

The VolSurf approach was applied to a set of 40 β-carboline derivatives acting as antitumor compounds. It is straightforward to recognize the most relevant physicochemical properties characterizing the β-carboline derivatives in this study using the VolSurf descriptors that are lattice-independent and have clear chemical meaning. The method produced a reliable in silico model, based on four MIFs, which is able to predict the affinity of β-carboline derivatives towards the HepG2 tumor cell lines. Actually, the 3D QSAR analysis confirmed the crucial role of hydrophobicity on antitumor activity in these compounds. The results show that Volsurf approach is highly efficient in predicting the biological activities and the characterization of descriptors related pharmacokinetic behaviour of these compounds revealed some novel perspectives for designing new lead compounds.

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