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in silico Modeling of Curcumin Based Sulfonamides Inhibitors of the Human *trans*-Membrane Carbonic Anhydrase Isozyme, hCA IX by CoMSIA

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ABSTRACT

Carbonic anhydrases, hCAs IX and XII are applied as the markers of progression of the disease in many oxygen deficient tumours and their specially manoeuvred inhibition is directly related to containing the growth of both primary tumours and tumour growth of secondary nature. Ligand-based quantitative structure-activity relationship (QSAR) studies were carried out on curcumin related, sulphonamide derivatives as inhibitors of human *trans*-membrane carbonic anhydrase isozyme, hCA IX by comparative molecular field similarity analysis (CoMSIA) implemented through the SYBYL package. The capacity of the model to predict coveted compound was evaluated using test set of three compounds. The best model created was found to be of choice as it showed a r^2 value of 0.811 and a cross validated coefficient q^2 value of 0.617 in tripos CoMSIA hydrophobic region. Results of the present study indicated that hydrophobic region factors play an important role in carbonic anhydrase hCA IX inhibition for compounds.

KEYWORDS

3D-QSAR, CoMSIA, Carbonic anhydrase inhibitor, Sulfonamides, Isozyme hCA IX.

INTRODUCTION

Carbonic anhydrases are a family of metalloenzymes, which catalyze the carbon dioxide hydration/dehydration reaction and are classified into seven genetically different families namely, α, β, γ, δ, ζ, η and θ [1-3]. All carbonic anhydrases, found in humans (hCAs) fall under alpha category of these enzymes. In all, fifteen different types of isoforms of hCAs have been identified and characterized among which twelve are catalytically active *viz.* hCAs I-IV, VA, VB, VI, VII, IX, XII-XIV. These enzymes are found in majority of tissues and organs of living beings and catalyze a wide variety of physiological processes therein. Deregulated formation or abnormal activity of hCAs can culminate in a health hazard, tumour formation being one such risk amongst others [1,4-7]. Apart from this, hCAs IX and XII are used as agents for identifying the level of spread the disease in case of many tumours in oxygen deficient environment and restricting their activity by binding to some external agent is found to bring about reduction in growth of primary and secondary tumours [1]. Carbonic anhydrase inhibitors (CAIs) can also be classified into different groups on the basis of their mode of binding to the active site

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of the enzyme. Amongst these, the zinc-binders are found to be most effective and are also the most studied ones for the drug design purpose [8]. In this class, sulphonamides occupy an important place as regards their readiness to bind to the zinc which is a consequence of a definite combination of interactions that take place between this moiety and the zinc ion and the structures lying in its vicinity [8].

Amongst various natural products examined, curcumin has been found to be an important carbonic anhydrase inhibitor [9-12]. Curcumin is the main ingredient of the typical kitchen spice, turmeric, the *Curcuma longa.* Curcumin is a symmetric β-diketone, that is, methane in which two of the hydrogens are replaced by feruloyl groups. Curcumin and its analogues and 4′-(phenylurenyl) chalcones have been found to perform as CAIs [13,14]. Therefore, curcumin based sulphonamide derivatives are a new class of CAIs that act against isoforms I, II, IX and XII. Nocentini & Supuran [4] have carried out the work on this topic which is summarized as follows.

A number of curcumin based sulphonamide derivatives were prepared from chalcones and 4-sulphamoyl benzaldehyde, using Claisen-Schmidt condensation reaction. Different sulphonamides produced were evaluated for their inhibition capacity towards four isoforms, *viz.* hCA I, II, IX and XII by means of stopped-flow carbon di oxide hydration assay and a comparative study of their activity was made *vis-a-vis* the activity of the standard CA inhibitor (CAI) acetazolamide. An interesting trend of inhibitory activities was noticed with regard to all these isoforms of carbonic anhydrases (CAs) [15].

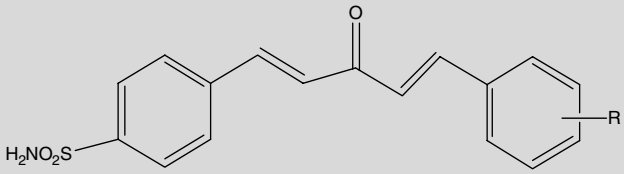
3D Quantitative structure-activity relationship (3D QSAR) investigations play an important role in designing new drugs. The comparative molecular similarity analysis (CoMSIA) used for 3D-QSAR methodology correlates biological activity of a series of molecules with 3D shape and their hydrophobic characteristics. Efforts are being made continuously to develop new, potent CA IX inhibitors for treatment of cancerous activity efficiently. QSAR relates numerical properties of molecular structure to the biological activity of the molecule by a mathematical model. The term QSPR is also coined sometimes when a property other than biological activity is under consideration. The QSAR study works out parameters of compounds that govern their biological activity and throws light on their mechanism of action. These two attributes of QSAR are of immense help in modifying structures of existing compounds, which acts as a tool in designing new drugs with desired curative value.

EXPERIMENTAL

Calibration set: Nocentini & Supuran [4] have reported results of inhibition study against hCA IX with a series of aromatic sulphonamides. A set of ten sulphonamides and their inhibitory activities against the hCA IX are represented in Table-1. The enzyme inhibition data, represented as K_I values in nanomolar (nM) range were transformed into "A" where A = log (2300/K_I). These values were taken as the dependent variable for the three dimensional-QSAR investigation at hand (Table-1) and were found between 0 and 3 for molecules under consideration.

Validation set (test set): Cross validation is a widely used and strongly recommended technique for checking the quality of a regression model. Here, we have taken up the QSAR study with a validation set (test set) and reduced calibration set (training set). The test set was selected from the homogenized calibration set. The test set for the present study has been selected on the basis of the hierarchical clustering technique. In cluster analysis, objects are arranged in different groups. In the present study, molecules having ranks 4, 7 and 9 comprise the test set and the rest are part of the training set. The test set of seven molecules (25% of the database) incorporates all the features and covers the activity range of the entire dataset. Inhibitory activity (A) values and compounds selected for the training and test sets are shown in Tables 2 and 3.

TABLE-1
STRUCTURAL DETAIL AND CA hCA IX INHIBITORY ACTIVITIES (IN nM AND A = log 10000/K_I), ESTIMATED ACTIVITIES OF THE CALIBRATION SET MOLECULES 1-19



Compd. No.	R	hCA IXKi (nm)	Obs. A
1	-4$(OCH_3)_2$	2.3	3.000
2	-4OCH_3	8.6	2.480
3	-4OCH_3, 6OCH_3	7.5	2.486
4*	-3OCH_3, 6OCH_3	8.4	2.437
5	-3OCH_3, 5OCH_3	2.3	3.000
6	-4OCH_3, 5OCH_3, 6OCH_3	2.4	2.981
7*	-2OCH_3, 4OCH_3, OCH_3	2.4	2.981
8	-3OCH_3, 4OCH_3, OCH_3	7.8	2.469
9*	-4Cl	87.3	1.427
10	-4OCH_3, 6F	61.0	1.576

TABLE-2
OBSERVED AND ESTIMATED VALUES OF CA hCa IX INHIBITORY ACTIVITY (A) FOR THE MOLECULES USED IN THE TRAINING AND TEST SET FOR CoMSIA (TRIPOS REGION)

Compd. No.	Obs.	Est.
1	3.000	2.950
2	2.480	2.592
3	2.486	2.479
5	3.000	2.974
6	2.981	2.922
8	2.469	2.548
10	1.576	1.529
4	2.437	1.711
7	2.981	2.397
9	1.427	1.723

Compd. No. **4**, **7**, **9** used as test set.

Template selection and alignment: Selection of template conformation is an important step in developing 3D-QSAR models. Here, compound No. 1 was chosen as the molecular template because of its importance as a lead structure. This apart, it is also a good inhibitor of hCA IX. Out of three different methods of alignment in the CoMFA *viz.* multifit, atomfit and database, we have switched over to database alignment for the purpose of present study.

CoMSIA: A distance dependent Gaussian-type functional form is introduced in CoMSIA, which can obviate singularities

TABLE-3
SUMMERY OF 3D-ASAR ANALYSIS ON CA IX INHIBITORS OF CoMFA ON TRIPOS STANDARD REGION

PLS statistics	Calibration set (all compounds)	Training set
q^2 (leave –one out cross-validated predicted power of model r^2_{CV})	0.617	0.582
R^2(correlation coefficient squared of PLS analysis)	0.811	0.982
N (optimum number of components obtained from cross-validated PLS analysis and the same used in final non cross-validated analysis)	1	5
SEE (standard error of estimate)	0.196	0.096
F-test value (F-value)	34.344	55.008
Hydrophobic	70%	–

at the atomic positions and changes of potential energy for those grids which are close to the surface. The CoMSIA procedure requires hardly any definition of cut-off limits. This apart, the contour maps of the relative spatial contributions of different fields can be improved in CoMSIA, which is forthright for interpretation in terms of separate property fields. Just like the CoMFA procedure, a 3D-QSAR model can be summarized with the help of CoMSIA approach.

CoMSIA model: Investigations for CA IX inhibitors through CoMSIA studies were performed using QSAR module of the SYBYL 7.0 programme. The five similarity index fields of CoMSIA available with the SYBYL *viz.* steric, hydrophobic, electrostatic, hydrogen-bond donor and hydrogen-bond acceptor were calculated at lattice points using a common probe atom of 100 pm (1 Å) radius as also the 2.4 CoMSIA model.

The CoMSIA fields with observed biological activity (A) were included in a molecule's spread sheet and partial least square (PLS) [16] method was applied to generate 3D-QSAR models. The PLS algorithm following the leave-one-out [17] cross validation method was applied to pick optimum number of components. It was followed by assessment of the statistical importance of each model. The cross-validation PLS analyses were carried out with a column filters value of 2.0. The cross-validated coefficient, q^2 was worked out with the help of the equation:

$$\in (Y_{predicted} - Y_{observed})^2$$
$$q^2 (r^2_{cv}) = 1 \qquad (1)$$
$$\in (Y_{observed} - Y_{mean})^2$$

where $Y_{predicted}$, $Y_{observed}$ and Y_{mean} are predicted, actual and mean values of the target inhibitory activity (A), respectively.

The optimum number of components was chosen, which gave less standard error of prediction and high r^2_{cv}. In addition, the r^2_{cv} and number of components, the conventional correlation coefficient r^2 and its standard error were also computed for the model. The predictive r^2 (r^2_{pred}) value was calculated using the following equation:

$$r^2_{pred} = \frac{SD - PRESS}{SD} \qquad (2)$$

where SD is the sum of squared deviation between biological activity of the test set and the mean activity of training set molecules and PRESS is the sum of squared deviation between the actual and the predicted activity values for every molecule in the test set. The CoMFA results were graphically interpreted by field contribution maps, using the stDEV * COEFF: field type. The used statistical method does not identify outlier molecules in a calibration.

RESULTS AND DISCUSSION

Hydrophobicity of a molecule, electronic effects and steric effects are directly related to the structure of a compound and these affect the physico-chemical properties and biological activity of the compound concerned. It is the force or the corresponding energy that operates between two or more non-polar solutes in water and arises from dispersive and electrostatic forces and the consequent entropic factor. Simply put, hydrophobicity is the tendency to fear or repel water and hydrophobic substances are generally soluble in non-polar solvents and sparingly soluble in water. Investigations through CoMSIA study involve construction of hydrophobic similarity index fields and hydrophobic contour maps are drawn as shown in Fig. 1. The yellow polyhedral shows that hydrophobic substituents are 'good' for increasing the potency while hydrophilic substituents are beneficial to the activity at regions of white contours. A yellow area encircles the $–OCH_3$ group making the compound most active on this side, which means that it is favourable for a hydrophobic centre.

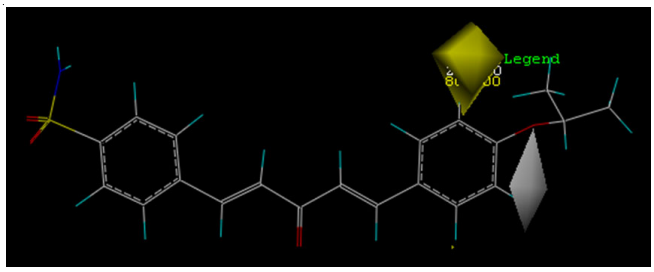


Fig. 1.

CoMSIA 3D-QSAR analysis: The leave-one-out (LOO) cross-validated PLS analysis of the best model gave rise to a cross-validated value (q^2) of 0.617 at one component suggesting that the model was a useful tool for predicting hCA IX inhibitory activity. The correlation coefficient (r^2) value of 0.811 between the calculated and experimental activities of non-cross validated experiment with standard error of 0.196 suggests that the results are in consonance with the experimental results to the extent of 90%. The statistical parameters of CoMSIA study of calibration set compounds are summarized in Table-3. On the basis of above observations, the best CoMSIA model obtained with database alignment was then picked up for further investigation. The observed and estimated inhibitory activity (A) values of the calibration set are depicted

in Table-1. The correlation of observed *vs.* estimated inhibitory activity of the training set and test set are presented in Fig. 2.

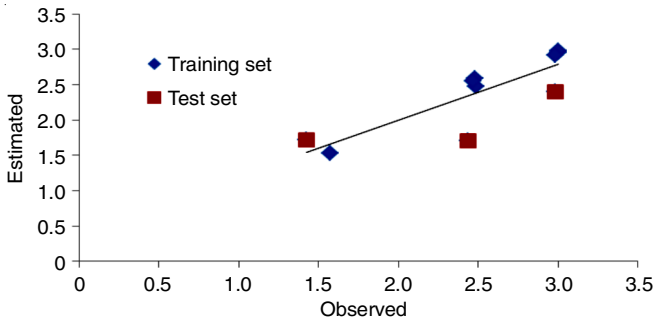


Fig. 2. Observed *versus* estimated inhibitory activity (A) of training set and test set

Predictive ability of CoMFA models: The predictive power of a CoMSIA model is assessed on the basis of the parameter, q^2, the cross-validated leave-one-out correlation coefficient, which describes in quantitative terms the predictive ability of the model. Models with q^2 value greater than 0.5 are supposed to have good predictive ability. A test set is required to validate again the predictive power of a CoMFA model through the predictive r^2 value (prd-r^2). In the presence of test set, we obtained the 3D-QSAR CoMFA model for the training set (for 7 Training set and 3 test set molecules): results predicted are summarized in Table-3.Thus, the 3D-QSAR model is found suitable for designing new hCA IX inhibitor(s). The observed and estimated inhibitory activity (A) values of the training set and test set are shown in Table-2. It is observed that the estimated values in the test set (validation set) are close to the hCA IX experimental inhibitory values and has ordered the molecules in a sequence similar enough to the real one. The correlation between observed and estimated inhibitory activities of the training and test set is presented in Fig. 2.

The contour plot representations of CoMSIA results for hCA IX inhibitors are presented in Fig. 1, taking the most active compound **1** as a reference. The contour plots are supposed representation of the lattice points and the difference in the field values is associated with difference in the affinity to bind to a receptor. The absence of lattice points is not indicative of absence of influence on biological activity by a particular substructure. There is a probability that all the compounds considered for present study exert the same steric and/or hydrophobic effect in a certain area. Although CoMSIA contour maps cannot take place of receptor maps, they offer useful ground to draw inferences.

Conclusion

In conclusion, the 3D-QSAR analysis using CoMSIA platform has been successfully applied to a set of recently synthesized hCA IX inhibitor analogue. The contour plots offer useful information on relationship between structural features and inhibition attributes and also present vivid picture of main chemical features underneath the significant inhibitory activity.

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