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CYTOTOXITY OF MARINE ALGAL STEROIDS IN HELA CELLS – 2D & 3D QSAR APPROACH

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ABSTRACT

The steroids of the brown alga *Turbinaria conoides* were recognized as having mild-to-moderate cytotoxicity in HeLa cell, as it is being investigated towards determination of toxicity with various drugs. The cytotoxicity in HeLa cells was expressed in terms of 50% cytotoxic concentration (CC_{50}). These oxygenated steroids exhibited cytotoxicity against HeLa cells with CC_{50} values ranging from 60.9 µg/mL to >100 µg/mL. To analyse for their cytotoxicity, Quantitative structure activity relationship (QSAR) study was performed. It was done by multiple regression analysis with simulated annealing method of partial least square (PLS) model.

A PLS model (r^2 = 0.90, q^2 = 0.80, pred_r^2 = 0.96) was used as a base of consensus prediction of cytotoxicity. QSAR studies indicated the carbon atom (T_C_O_4: 12%) and any atom (T_2_2_7: 15%) away from the oxygen and double bond respectively, atomic valence connectivity index-order 0 (chiV0: 50%) and distance between most hydrophobic and hydrophilic point on the VanderWaals surface (XAMostHydrophobicHydrophilic distance: 20%) correlating well with mild cytotoxicity while the partial charges of the molecules (dipole moment: 25%) contributing moderate cytotoxicity.

The 3D QSAR model further described that less bulkier substituent is required at steric site S_2441. Thus it can be concluded from the present study that cytotoxicity can be achieved by modifying the aromatic ring and preferring less bulky group to reduce steric hindrance.

KEY WORDS

Turbinaria conoides, oxygenated steroids, cytotoxicity, QSAR

INTRODUCTION

Turbinaria conoides[1] Kutzing, commonly named Agar-agar Lesong is a brown alga belonging to the family Sargassaceae. *Turbinaria* and other members of the family Sargassaceae are inedible, due to the concentration of polyphenolic substances based upon the polymerization of phloroglucinol[2]. A number of sterols have been isolated from the genus *Turbinaria*. Oxygenated steroids of algae have been reported to possess cytotoxic properties[3-7]. The ethyl acetate extract of *Turbinaria conoides* and its oxygenated fucosterols have been reported for their cytotoxicity[8]. Traditionally, it is used to cure children's fever and as fertilizer, insect repellent and antibacterial[9].

Although a variety of brown algae are known, the isolation and development of natural compounds from new species to get new biologically active natural products is of considerable interest.

The main success of the QSAR method is the possibility to estimate the characteristics of new chemical compounds without the need to synthesize and test them. This analysis represents an attempt to relate structural descriptors of compounds with their physicochemical properties and biological activities.

This is widely used for the prediction of physicochemical properties in the chemical, pharmaceutical, and environmental spheres. This method included data collection, molecular descriptor selection, correlation model development, and finally model evaluation. QSAR studies have predictive ability and simultaneously provide deeper insight into mechanism of drug receptor interactions.

In view of the above and in continuation of our studies on the cytotoxic properties of the oxygenated steroids isolated from Turbinaria conoides as well as on correlation of molecular properties with activity, the objective of this investigation was to study the usefulness of QSAR in the prediction of the cytotoxic activity of oxygenated derivatives. Partial Least Square Regression & Simulated annealing method models have been developed as a mathematical equation which can relate chemical structure to the cytotoxic activity.

2. MATERIALS AND METHODS:

2.1. ALGAL MATERIAL:

Turbinaria conoides was collected from Salin Munthal, Gulf of Mannar, Bay of Bengal, Ramanathapuram district, Tamil Nadu, India and voucher specimen was deposited at Marine algal research station, Mandapam camp, Tamil Nadu, South India. It was authenticated by K. Eswaran, Scientist, Marine algal research station, India.

2.2. TRAINING AND TEST COMPOUNDS:

Steroids (A-H) were isolated from the ethyl acetate extract as per the procedure reported[8] and steroids (I-K) from the cyclohexane extract of Turbinaria conoides[10]. All the steroids were characterized by spectral analyses. Acyclovir and Brivudine were used as positive controls for the screening **(Table 1).**

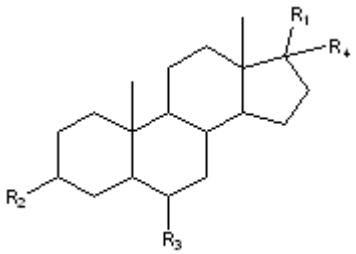
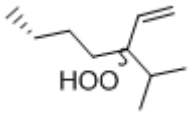
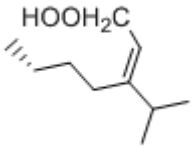
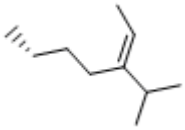
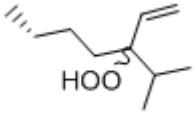
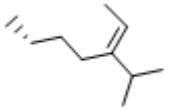
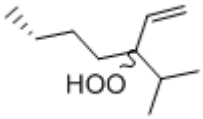
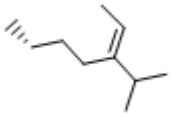
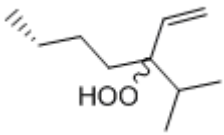
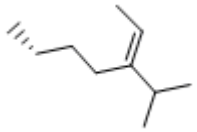
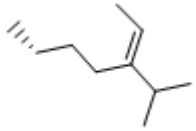
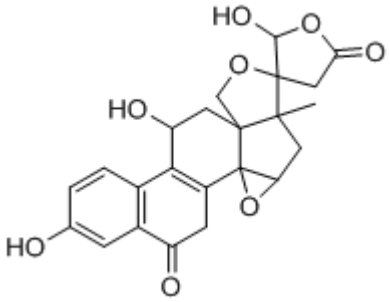


Table 1
Structure of training & test# set molecules

Compound/ Drug	Double bonds	R_1	R_2	R_3	R_4
A	C5-C6		OH	H	H
B	C5-C6		OH	H	H
C	C4-C5		CO	H	H
D	C4-C5		CO	H	H
E	C4-C5		CO	H	H
F#	C4-C5		CO	CO	H
G	C4-C5		CO	OH	H
H#	C4-C5		CO	OH	H

I	C4-C5 C7-C8		OH	OH	OH
J	C5-C6		OH	H	H
K[#]					
L	Acyclovir				
M	Brivudine				

3. RESULTS AND DISCUSSION

In this study, various oxygenated steroids of Turbinaria conoides **(Table 1)** were evaluated for *in vitro* cytotoxic activity against HeLa Cells**.**

The Observed vs Predicted values of test & training set molecules are summarized in **Table 2**. The results revealed that the predicted values of all the oxygenated derivatives exhibited cytotoxic activity. In the second step, we focused our efforts on developing the QSAR models of compounds A-K as cytotoxic agents. A set of ten molecules and a set of three molecules (Compounds F, H and K) were used for model generation. Cytotoxic activity data was used as a dependent variable in the QSAR study. Different physicochemical, steric, electronic, and structural molecular descriptors were used as independent variables and were correlated with cytotoxicity.

Developing a QSAR model requires a diverse set of data, and, thereby a large number of descriptors have to be considered. Descriptors are numerical values that encode different structural features of the molecules. Selection of a set of appropriate descriptors from a large number of them requires a method, which is able to discriminate between the parameters. Correlation matrix was performed on all descriptors by using Vlife MDS Statistical Software. The analysis of the matrix revealed five descriptors in the developed model which were of utmost importance and were contributing most for cytotoxicity. The details of the five descriptors are given in **Table 3**.

Table 2
Observed Vs Predicted values of Compounds/ Drugs

Compound/Drug	Observed	Predicted
A	0.4740	0.434900
B	0.4083	0.428158
C	0.4026	0.419881

D	0.4080	0.414673
E	0.4050	0.392318
F	0.4102	0.401061
G	0.4053	0.396678
H	0.4648	0.469374
I	0.5000	0.509189
J	0.4608	0.447167
K	0.5000	0.506210
L	0.3283	0.334770
M	0.3477	0.345530

Table 3
Details of the observed descriptors

S.no.	Name of the descriptor	Detail
1	T_C_O_4	Any carbon atom which is four bonds away from oxygen
2	chiV0	Descriptor signifies atomic valence connectivity index (order 0)
3	T_2_2_7	Any atom which is seven bonds away from a double bond
4	XA – Distance between most hydrophobic and hydrophilic point	The hydrophobic and hydrophilic groups present in the molecule
5	Dipole Moment	Calculated from the partial charges of the molecule

3.1. MODEL FOR 2D ANALYSIS:

The Uni-column statistics revealed a combination of statistically significant results through the training and test sets. The maximum of the test was less than that of training set. The minimum of the test was greater than that of the training set. The result showed that the test set is interpolative i.e. derived within the minimum-maximum range of the training set. The mean in the test set was higher than the training set which showed the presence of relatively more active molecules as compared to the inactive once. Also the similar standard deviation in both the sets indicates that the spread in both the set with their respective means was comparable (**Table 4 and Table5**).

Table 4
Uni-Column Statistics for training & test set molecules

Column name	Average	Max	Min	Std Dev	Sum
Training set	0.4205	0.5000	0.3283	0.0551	4.2047
Test set	0.4205	0.5000	0.4050	0.0548	1.3103

Table 5
Selected descriptors & their values:

Compound	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
A	444.6983	467.899	3	2	7.413	52.55713	7	21.277	0	6.090438	2.862732
B	444.6983	468.006	3	2	7.4146	52.55713	5	21.277	0	5.551889	2.917847
C	410.6836	444.037	1	0	8.1531	50.84574	2	20.59812	0	7.119873	2.709318
D	442.6824	462.105	3	1	7.6212	52.02306	7	21.16069	0	4.769145	3.650724
E	424.6672	446.875	2	0	7.3321	50.89282	7	20.79926	0	6.98517	4.447415
F	456.666	464.641	4	1	6.8002	52.09715	12	21.36183	0	6.736965	4.819774
G	426.683	452.203	2	1	7.1239	51.42792	7	20.91558	0	4.322265	4.306602
H	458.6818	470.753	4	2	6.592	52.62995	12	21.47814	0	4.214136	2.070458
I	225.2072	184.929	4	3	1.6128	20.0232	3	8.381372	0	4.356858	2.398875
J	333.1387	232.821	4	3	0.2011	26.43704	6	10.4447	0	4.748932	2.947155
K	442.6824	460.42	3	3	5.9507	52.34123	10	21.19465	1	5.885433	2.038473
Acyclovir	412.6995	449.694	1	1	7.9449	51.38202	2	20.71443	0	9.95269	1.633436
Brivudine	426.4229	355.539	5	3	1.0651	45.43563	29	16.88252	2	10.36843	4.777566

I. Mass II. Volume III. H-Acceptor count IV. H-Doner count V. slogp VI. Polarizability VII. T_C_O_4 VIII. chiV0 IX.T_2_2_7 X. XAMostHydrophobicHydrophilicDistance XI. Dipole moment

Partial Least Square Regression method was employed to obtain q^2 value of 0.8011, where the following descriptors T_2_2_7 (15%), T_C_O_4 (12%), $chiV_0$ (50%) and XAMostHydrophobicHydrophilic distance (20%) were detrimental for cytotoxicity while dipole moment (25%) influenced cytotoxicity. Compounds F, H and K were taken as training set while all the rest were used as test set. Regression was performed using Simulated Annealing Method with seven as the value of degree of freedom. r^2 value equal to 0.9016 proved that the developed model is highly statistically significant which was further proved by the F-test value of 32.05.

3.2. MODEL FOR 3D ANALYSIS:

Out of several models built, the best model was selected to interpret the results. The model was built using advanced kNN Method with nearest neighbor value equal to two. Further, forward & backward distance based option best suited the model building keeping the value of the degree of freedom at eight. Unicolumn statistics was same as that of 2D model. Training set size was three while test set was kept at ten. The model which was obtained by using variable selection method showed that the steric interaction plays a major role in determining biological activity. It can also be noted that descriptor S_2441 had been selected in the generated model using variable selection method implying the significant role of this steric field interaction for structure activity relationship.

The q^2 value of 0.6096 proved the usefulness of the internal model validation as well as the external model validation value ($pred\_r^2$ = 0.8407) correctly predicts the activity from 60%~84% for the training & test set respectively.

The descriptor range (S_2441) -0.0011 to -0.0011 is a negative value. The negative range indicated that negative steric potential is favorable for the increase in the activity and hence less bulky substituent is preferred in that region.

Figure 1
Observed Vs Predicted activity values for training and test set molecules

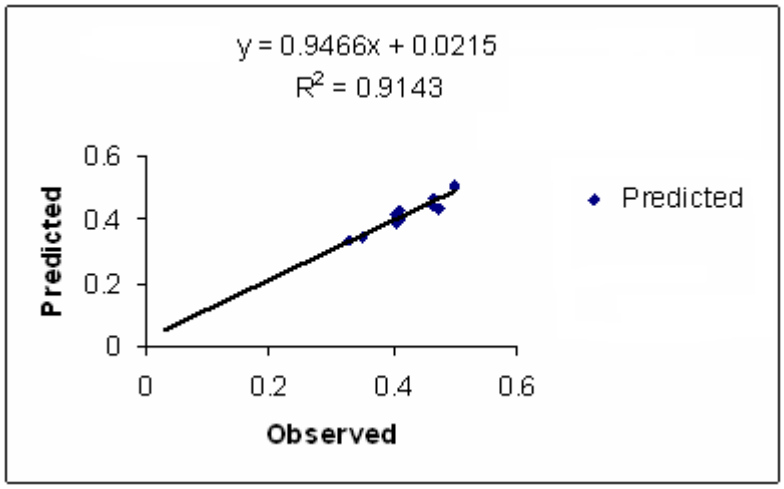
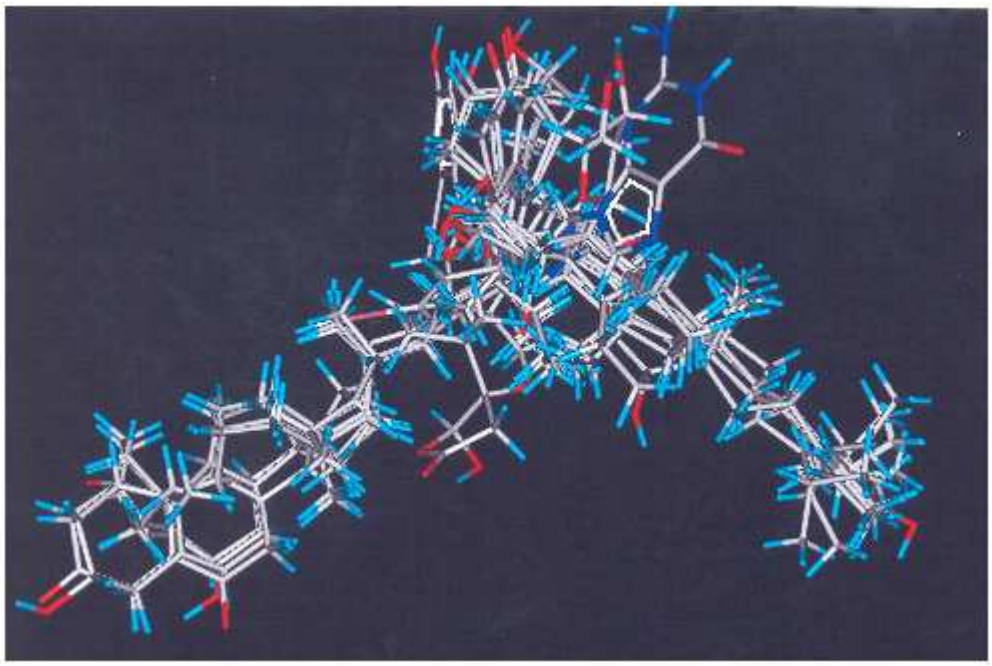


Figure 2
Common template of the training and train set molecules



4. CONCLUSION

From the 2DQSAR studies it was quite clear that the carbon atom (T_C_O_4: 12%) and any atom (T_2_2_7: 15%) away from the oxygen and double bond respectively, atomic valence connectivity index-order 0 (chiV0: 50%) and distance between most hydrophobic and hydrophilic point on the VanderWaals surface (XAMostHydrophobicHydrophilic distance: 20%) correlated well with mild cytotoxicity while the partial charges of the molecules (dipole moment: 25%) contributing moderate cytotoxicity. These five descriptors described most useful relevance to cytotoxicity.

The 3D QSAR model further described that less bulkier substituent is required at steric site S_2441. For the statistical analysis the multiple linear regression analysis technique was used. Thus it can be concluded from the present study that cytotoxicity can be achieved by the modification in the aromatic ring i.e. a para-hydroxyl group must be substituted and a hydroxyl group may be attached at 4^{th} carbon atom of the steroidal rings, while to reduce steric hindrances a lesser bulkier group must be used.

2D & 3D QSAR analyses were to study the quantitative effects of the molecular structure of the isolated oxygenated steroids of *Turbinaria conoides* to improve their cytotoxicity. Accurate mathematical models were developed for predicting the cytotoxity of this class of compounds. The validity of the models was established by the determination of suitable statistical parameters. From the established QSAR models, close agreement between observed and predicted values were obtained. The low residual activity and high cross-validated r^2 values were observed and they indicated the predictive ability of the developed QSAR models.

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