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Research Article

"QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP (QSAR) ANALYSIS OF OLIVACINE DERIVATIVES AS TOPOISOMERASE INHIBITORS"

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ABSTRACT

Quantitative Structure Activity Analysis was carried out on 9-o-substituted dervative of 9-hydroxy-5,6-dimethyl-6H-pyrido[4,3-b] carbazole-1-carboxylic acid (2-(dimethyl-mino)ethyl) amide and their 10- and 11- methyl analogues using TSAR in order to determine the structural features responsible for their activity. The Multiple Linear Analysis yielded a stastically significant model.The mode shown a good r^2 0.821468 and r^2CV 0.762943.The result obtained from the present study indicates that electronic and steric descriptors play major role in topoisomerase II inhibitory activity. In case of electronic descriptors, with an increase in Dipole Moment Descriptor value and Bond Dipole Moment value, there is an increase in biological activity and with an increase in Polarization Descriptor value, biological activity decreases and biological activity increases with an increase in Verloop B1–B5 parameters which are steric descriptors.

Keywords: QSAR – Quantitative Structure Activity Relationship, TSAR – Tool for Structure Activity Relationship, pIC50 – Negative logarithim, HOMO – Highly occupied molecular orbital, R^2cv - Cross Validated Squared Correlation coefficient.

INTRODUCTION

Cancer is not a disease but a complex group of disease, which affects different organs systems of the body. It is one of the major diseases of the present world, with one in four persons in developed countries is expected to get cancer in their lifetime. Of the new cases each year, more than 50% occur in developing countries, with six to nine million cases occurring in India[1]. Effective treatment of cancer results from the destruction of the cancer cells, which is a direct result of the cytotoxicity of drugs against highly proliferative cells. Most of the clinically available anticancer drugs interfere with DNA function to exert their cytotoxic activity. DNA topoisomerase inhibitors represent an important class of anticancer drugs.

Topoisomerases play an important role in the modulation of DNA topology and are necessary for realeasing tortional stress generated in DNA during processes such as replication, transcription, recombination and chromosome seggregation. There are two different types of topoisomerase; type I which catalyses the cleavage and re-ligation reactions in S-phase, and type II which catalyses proper decatenation and segregation during G2/M phase[2].

Topoisomerase II that plays critical roles in many DNA processes, including maintainence of the structure of chromosome as well as chromosome segregation. In order to carry out its important physiological functions, topoisomerase II creates and rejoins double-stranded breaks in the genetic material. Thus, the enzyme is not only necessary for cell survival, but also has the capacity to fragment the genome. Topoisomerase II-mediated DNA breaks are sequestered within a covalent enzyme-DNA complex. Normally, these "cleavage complexes" are present at low levels and are tolerated by the cell[3]. However, conditions which significantly increase the physiological concentration or life-time of topoisomerase II DNA cleavage complexes lead to chromosomal translocations and other mutagenic events, and can induce cell death pathways. The potentially lethal aspect of enzyme mechanism has been exploited by number of topoisomerases II inhibitors.These inhibitors are catalytic topoisomerase II inhibitors and topoisomerse II poison.

Catalytic topoisomerase II inhibitors are a heterogeneous group of compounds that might interfere with the binding between DNA and topoisomerase II, stabilize non-covalent DNA topoisomerase II complexes whereas topoisomerase II poisons act by trapping the enzyme in the cleavable complex which in turn leads to accumulation of truncated DNAs in the cell, therefore transforming the enzyme into a cellular poison. Topoisomerase II poisons include DNA intercalators such as anthracyclines[4-9] and non-intercalators such as epipophyllotoxins[10-13] and also DNA minor-groove binders such as distamycin and netropsin.

As the number of active derivatives increase, the formulation of a useful SAR becomes increasingly difficult[14].Thus, molecular models should be developed that can better interpret pharmacological data and predict novel biologically active compounds. Ligand based methods such as phamacophore mapping and quantitative structure activity relationships (QSAR) are frequently used to develop predictive correlations between ligand structure and activity. Many different approaches in QSAR have been developed during the past few decades. Modern methods are characterized by the use of multiple descriptors of chemical structure combined with the application of both linear and non-linear optimization approaches, and a strong emphasis on rigorous model validation to afford robust and predictive QSAR models.

In the present study we are reporting Quantitative Structure Activity Relationship analysis of 9-o-substituted dervative of 9-hydroxy-5,6-dimethyl-6H-pyrido[4,3-b] carbazole-1- carboxylic acid (2-(dimethyl-mino)ethyl)amide and their 10- and 11- methyl analogues.

MATERIAL AND METHOD

A series of Olivacine derivatives[15] were taken from the literature having in-vitro inhibitory concentration expressed as IC_{50} in (table1).These values were converted into inverse of logarithim and were used as biological dependent parameter. Six compounds (22, 35, 38, 49, 54 and 56) were excluded from the study as their IC_{50} values were not reported.

The structures of molecules were sketched using TSAR visualizer and were loaded to the worksheet of TSAR 3.3 version. The series had three major substituents which were then defined using define substituents option provided by TSAR worksheet toolbar. The structures and substituents were then converted into 3-dimensional molecular structures. With the help of Corina make 3-D option charges were derived through charge-2 derived option and were further subjected to optimization using Cosmic-optimized 3D option.

To develop predictive QSAR models the molecules were divided into training set of 36 molecules and test set of 7 molecules. The training set was used to develop multiple linear regression models while the test set helped to determine predictive capabilities of the model. Molecular descriptors were then calculated for whole molecule and their substituents. Total 282 descriptors were calculated which included Molecular Indices, Molecular Attributes and Vamp electrostatics property descriptors. Pair wise correlation analysis of the calculated descriptors was then performed and the descriptors having inter-correlation co-efficient above 0.5 were discarded. The models obtained had descriptors which were independent to each

other and were highly correlated with the biological activity. The seven independent molecular descriptors namely Dipole moment-Y component, Vamp Dipole Z-component, Vamp polarization-XX, Verloop B1 (substituent 1), Bond dipole moment (substituent 2), Bond dipole moment (substituent 3) obtained were used to perform the Multiple Linear Regression Analysis[16] and to predict the robustness of the model.

During Multiple Linear Regression analysis Vamp Dipole Z-component was excluded from the model, owing to its low t-test value. The model thus obtained with the remaining six descriptors showed good correlation co-efficient (r^2). Five compounds (5, 18, 35, 37 and 57) were identified as outliers and were then deleted to obtain model with lowest prediction error. The statistical significance of multiple linear regression equation was tested on the basis of cross validation regression coefficient and Fisher's ratio (F test).

Regression of the test set was obtained by using evaluate fuction tool and a graph was plotted between actual activity and predicted acivity. Thus, in the above mentioned way, the test set was used to check the predictive capability of the model

RESULT AND DISCUSSION

The 2D QSAR analysis of Olivacine derivatives was carried out using T-SAR. Total six descriptors were used to analyse the effect on biological activity.With an increase in Dipole Moment Y-Component value and Bond Dipole Moment, there is an increase in biological activity and with an increase in Vamp Polarization XX value, biological activity decreases.

The Dipole Moment descriptor [17] is an electronic descriptor that indicates the strength and orientation behavior of a molecule in an electrostatic field. This descriptor reflects global polarity of the molecule. The higher biological activities of the compounds 23 and 33 are reflected by their high dipole moment value as compared to componds 40, 41 and 43 which have low value of dipole moment.

Bond Dipole Moment[18] refers to the charge separation resulting from the unequal sharing of the electrons in a chemical bond as a bond dipole. The greater the difference in electro negativities of the bonded atoms, the greater is the bond dipole. Enhanced biological activity of compound 23 can be explained by its high bond dipole moment (subs 2) value as compared to compounds 14 and 19 that have low value of bond dipole moment (subs 2). Likewise; influence of bond dipole moment (subs 3) descriptor can be explained.

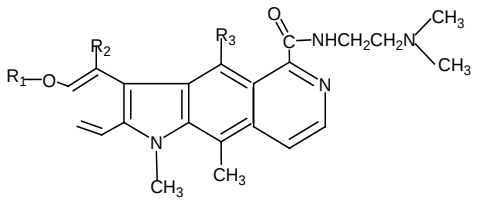
Polarization descriptor[19] refers to the relative tendency of a charge distribution, like the electron cloud of an atom or molecule, to be distorted from its normal shape by an external electric field, which can be caused by the presence of a nearby ion or dipole. Compound 1, 4 and 13 are more biologically active than 17, 41 and 47; as former compounds have less polarization values than later.

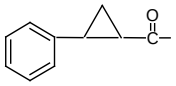
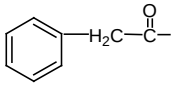
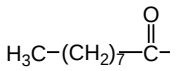
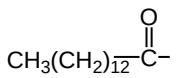
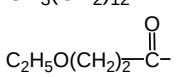
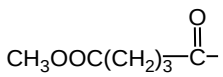
HOMO Descriptor[20] reflects the relative reactivity of different molecules. The molecules with higher HOMO donate their electrons more easily. Thus, they are more reactive and behave as better nucleophile. In this series molecule 58 has high HOMO thus it is highly reactive molecule.

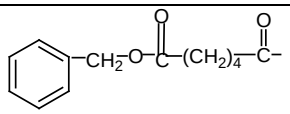
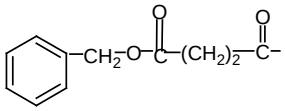
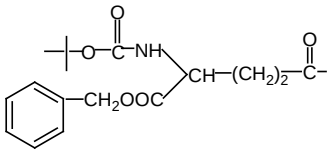
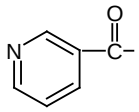
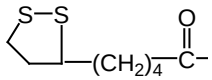
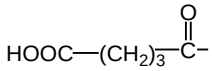
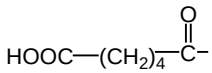
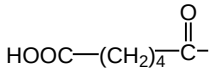
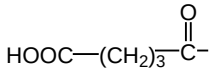
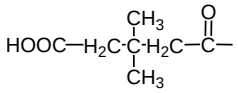
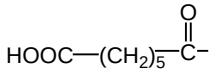
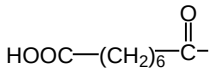
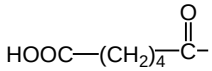
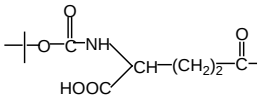
The Verloop parameters[21] are a set of multidimensional steric descriptors that define a box that can be used to characterize the shape and volume of the substituent, which are very important in explaining the steric influence of substituents in the interaction of organic compounds with macromolecular drug receptors. The Verloop B1–B5 parameters describe the width of the substituent in the direction perpendicular to L.The width of the substituent is more in compound 58 than compounds 1,4 and 13 which have hydrogen as a substituent. Thus, the verloop B_1 (subs 1) value of compound 58 is high as compared to compounds 1, 4 and 13.

The actual and predicted activity of training and test set are shown in (Table 2) and (Table 3) respectively; (Figure 1) shows plots of the actual versus the predicted $LogIC_{50}$ values for the training set molecules and (Figure 2) shows plots of the actual versus the predicted $LogIC_{50}$ values for the test set molecules.

Table 1: Structures of inhibitors used for 2D QSAR analysis with the corresponding biological activity



Comds	R_1	R_2	R_2	IC_{50} (B16)
1	H	H	H	5.4
4	H	H	H	3.4
5	H	CH_3	CH_3	0.2
13		H	H	6.1
14		H	H	14.1
15		H	H	22.3
16		H	H	20.8
17		H	H	95.3
18		H	H	3.0
19		H	H	7.1

20		H	H	58.4
21		H	H	9.9
22		H	H	
23		H	H	2.3
24		H	H	14.0
25		H	H	14.8
26		CH_3	CH_3	6.4
27		H	H	6.3
28		H	H	1.4
29		H	H	3.7
30		H	H	3.2
31		H	H	3.1
32		H	H	5.0
33		H	H	4.0
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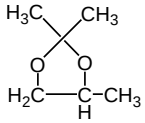
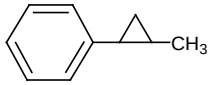
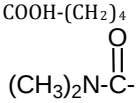
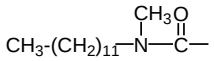
35		H	H	
36		H	H	279.3
37	$HO\text{-}CH_2CHOH\text{-}CH_2$	H	H	954.5
38	$C_2H_5OOC\text{-}(CH_2)_4$	H	H	
39	$COOH\text{-}(CH_2)_4$	H	H	3466.0
40		H	H	2666.7
41		H	H	3256.0

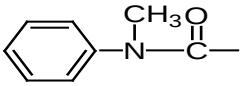
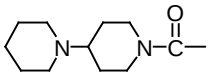
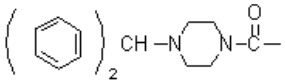
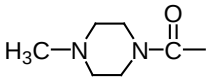
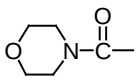
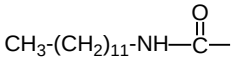
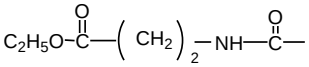
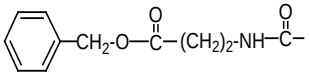
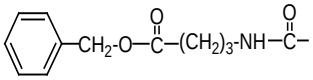
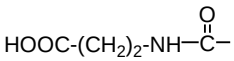
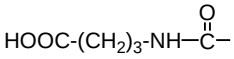
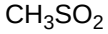
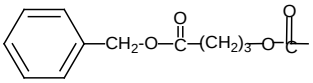
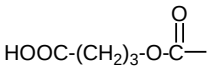
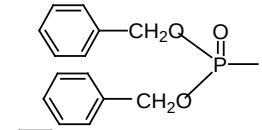
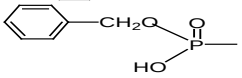
42		H	H	1374.0
43		H	H	2689.0
44		H	H	589.9
45		H	H	2471.3
46		H	H	1766.5
47		H	H	2299.5
48		H	H	5.7
49		H	H	
50		H	H	29.4
51		H	H	78.7
52		H	H	45.7
53		H	H	782.1
54		H	H	
55		H	H	4.8
56		H	H	
57		H	H	511.0
58		H	H	4.1

Table 2: Actual value verses Predicted value for training set

Compounds	Actual Value	Predict Value
1	-0.732	-0.206379
4	-0.5314	-0.546745
13	-0.7853	-1.00249
15	-1.3483	-1.20092
16	-1.318	-1.86768
17	-2.2907	-1.96768
20	-1.76641	-1.38512
21	-0.9956	-1.1879
23	-0.3617	-0.866214
25	-1.17026	-1.20963
26	-0.8061	-0.334926
27	-0.7993	-1.24803
28	-0.1461	-0.608407
30	-0.50514	-1.09983
31	-0.49136	-0.625178

32	-0.6989	-0.965524
33	-0.602	-1.08753
36	-2.446	-2.25905
40	-3.4259	-3.67481
41	-3.51268	-3.66444
42	-3.1379	-3.28886
43	-3.4295	-3.94869
45	-3.3929	-2.61219
46	-3.2471	-2.43665
47	-3.3616	-2.37889
48	-0.75587	-0.747605
50	-1.4683	-2.09679
51	-1.8959	-1.17876
52	-1.6599	-0.830677
55	-0.68124	-1.09403
58	-0.61278	-0.267474

Table 3: Actual value verses Predicted value for test set

Compounds	Actual Value	Predict Value
14	-1.1492	-2.18581
19	-0.851	-0.708939
24	-1.1461	-0.930445
29	-0.5682	-0.37297
34	-2.52322	-2.07291
39	-3.5398	-2.61224
44	-2.7707	-2.90961

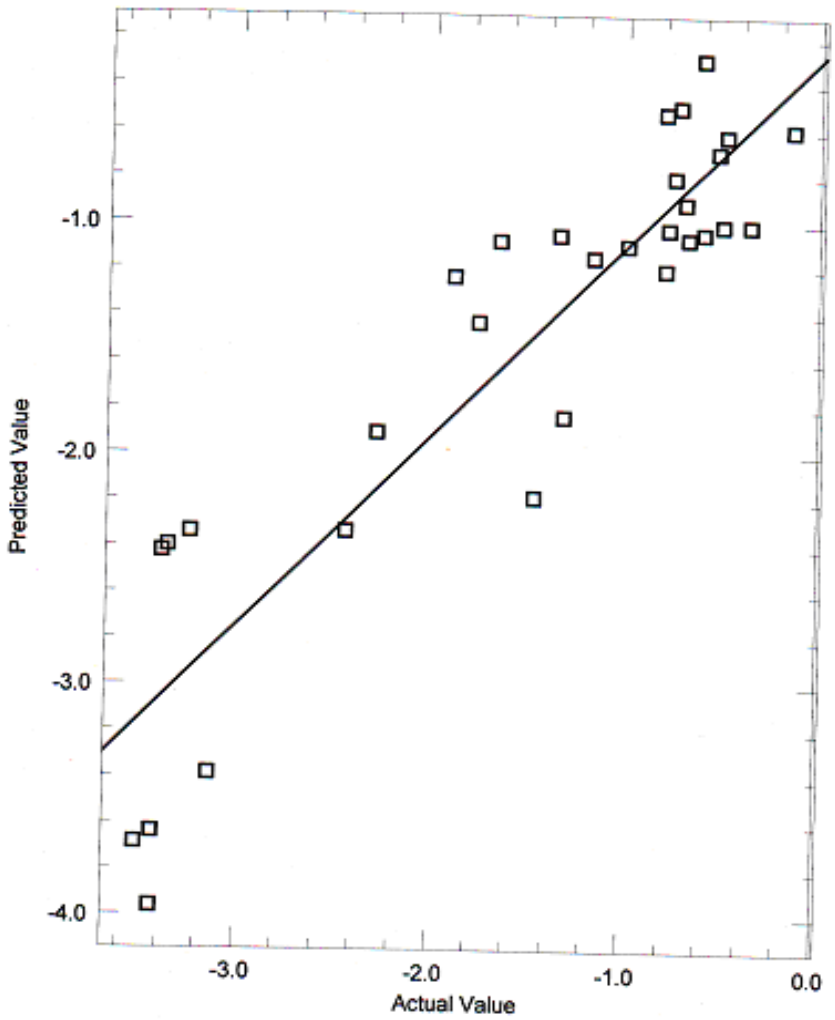


Fig. 1: Graph of actual value verse predicted value for the training set.

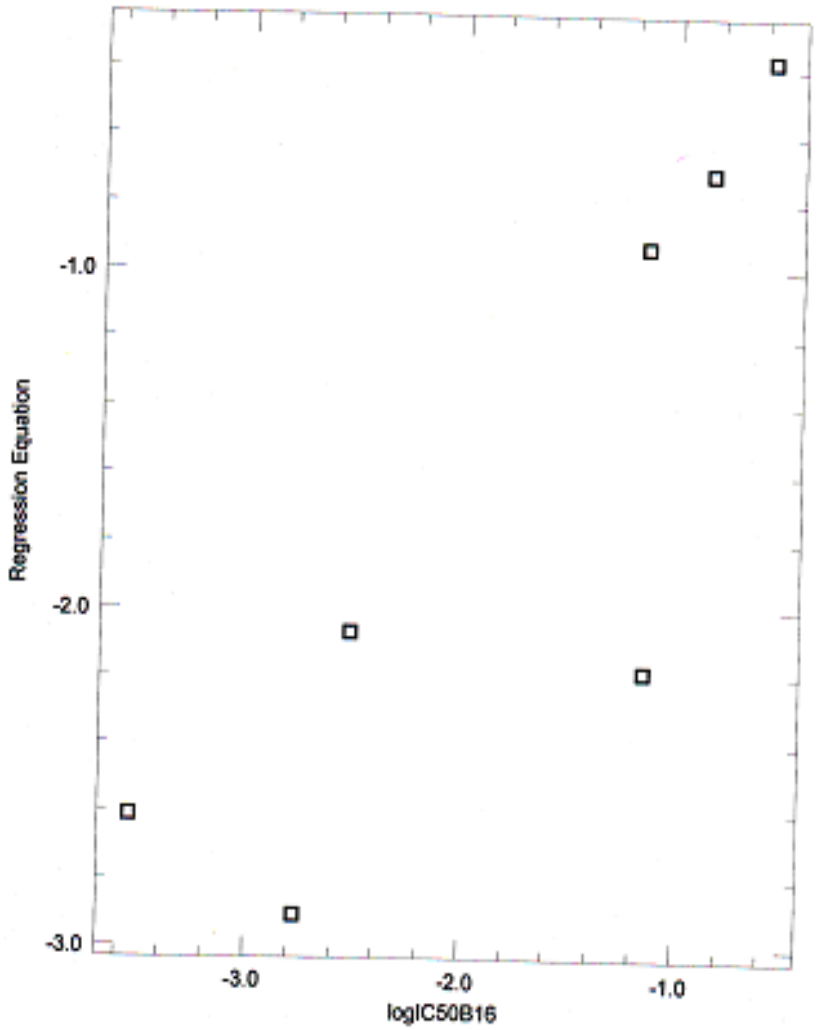


Fig. 2: Graph between Actual value and Predicted value for test set.

The QSAR model with high statistical significance is represented by the following equation-

Original Data: Y = 0.20659542*X1 – 15.504414*X2 - 0.057770062*X3 -2.0899754*X4 – 0.78609687*X5 – 4.0481453*X6 – 112.89805

Where, X1 is Dipole Moment Y – Component, X2 is Vamp HOMO Component, X3 is Vamp Polarization XX, X4 is Verloop B1 (subs 1), X5 is Bond Dipole Moment (subs 2), X6 is Bond Dipole Moment (subs 3)

Standardized Data: Y = 0.62473649*S – 0.9070394*S2 – 0.49245805*S3 – 0.4121269*S4 – 0.065637141*S5 – 0.46948788*S6 – 1.5605206

Multiple regression analysis for training set is summarized in (Table 3). The value obtained for non cross-validated correlation coefficient was 0.821468, which clearly indicates goodness of the fit. The model exhibited the value of r^2 CV of 0.762943, which reflects the ratio of variance explained by the model and the variance due to the error in the regression. A high value of F-test indicates that the model is statistically significant.

CONCLUSION

A QSAR analysis using Olivacine derivatives was successfully carried out to build a statistically significant model possessing a good correlative and predictive capability for topoisomerase II inhibition. The result obtained from MLR equation can be used to design potent inhibitors of topoisomerase II as anticancer agents.

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