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2D-QSAR ANALYSIS: DESIGN DEVELOPMENT AND EVALUATION OF BETA-CARBOLINE ANALOGUES

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ABSTRACT

The best model designed exposed that the Log P values and Charge-Dipole energy of the molecules are positively associated to the biological activity whereas the Bending energy values showed negative relationship. The best model developed also shows a greater control of Log P values and Charge-Dipole energy on biological activity than bending energy. Therefore, one should keep in mind that only those groups which impart the above mentioned changes must be attached to the molecules for escalating the biological activity. This study may prove to be helpful in further studies related with the synthesis of newer potent derivatives of β-carboline.

Key words: Beta-Carboline, LogP, QSAR, Descripter, Hansch Analysis.

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INTRODUCTION

Medicinal Chemistry is generally considered as the branch of science whose prime objective is to discover as well as carry out the design of novel and therapeutically active chemicals and then forge them into useful medicines. This field had its instigation when physicians along with chemists and pharmacists became successful in isolating and further purifying medicinally active principles of tissues from animals as well as plants and from microorganism and their fermented products in upcoming years. During the latter part of 20th century, the field of medicinal chemistry, which had organic chemistry, biology and some areas of physics, extended new branches and potential into some of the emerging topics at that time like biomedicine, molecular pharmacology and molecular biology. Diseases which originate from protozoa or various spirochetes respond to chemotherapeutic agents, thus a huge deal of curiosity developed for synthetic chemicals which were able to restrain the rapid reproduction of microbes and facilitated host organism to cope up with them.

Drug designing is reckoned as a multi-dimensional practice which entails pharmacologists, biologists, biochemists, chemists and many others. It may be considered that a chemist acquires a central role in this process related with the invention of novel compounds, which may have medicinally beneficial effects.

The discovery of a lead compound is assumed to be the most complicated aspect of the drug scheming process. Once a lead compound for a novel therapeutically vigorous drug has been revealed, it is additionally subjected to effectual toxicological studies so that its worth and protection can be thoroughly evaluated before the instigation of its clinical trials. Thus, one can realize that the term “drug designing” symbolize mainly tiresome as well as pioneering hard work to build up new drug molecules on a sane basis. Chemotherapy, or the use of chemical agents for treatment of diseases, is considered a mainstream approach in the present scenario. A major advantage of chemotherapy is its ability to treat widespread or metastatic infections. In past few decades, the chemistry of β-carboline nucleus has focused significant consideration for the development of newer compounds due to its effective biological importance.

All these β-carboline containing compounds associated with multiple therapeutic activities such as antitumor,

antitubercular, antimalarial, anticonvulsant, anthelmintic, analgesic, anti-inflammatory, antifungal and topical carbonic anhydrase inhibitor. Some of β-carboline based compounds were also found with therapeutic importance for the treatment of diabetes. On the basis of literature, it concluded with noteworthy aimed to synthesize the some new β-carboline derivatives which have potent anti-microbial activity with minimum side effects and toxicity. A great deal of current efforts has been focusing on the design and development of various antimicrobial drugs. Kenji Suzuki et al., (2018), GianPrimahana et al., (2020), Franciele C. Savariz., (2010), Zhibin Li et al., (2015), Hongjian Song et al., (2014) and many others have reported novel derivatives of β–carboline showing significant anti-microbial activity but no molecular modeling studies are available for Novel 1 Substituted Phenyl-3-[3-alkylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] b-Carboline Derivatives (Franciele C. Savariz et al., 2010).

Therefore, in the present study a series of N-(substituted-benzylidene)-β-carboline-3-carbohydrazide derivatives (Barbosa et al., 2011) was selected for QSAR modeling. Newly designed compounds selected on the basis of the best model will be synthesized followed by their anti-microbial activity evaluation.

MATERIALS & METHODS

ADME and toxicity predictions

Pre-clinical ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) research is at the moment a loom used for challenging and screening for development of drugs at an early stage of the drug discovery process. The sooner that flawed candidate drugs can be recognized and eliminated, the less time and cost needed for extensive testing and approval processes necessarily required for launching a new drug. Eliminating candidate drugs for reasons of their toxicity preceding to the clinical trials reduces the on the whole investment in risk and time in bringing successful drugs to the market, for this reason this is a smart option for numerous drug discovery companies.

- Absorption (how much and how fast, often referred to as bioavailability)
- Distribution (where the drug is distributed in body, how fast and how extensive)
- Metabolism (how fast, what mechanism, what metabolite is formed, and whether metabolites are toxic)
- Elimination (how fast, which route)
- Toxicity (Toxicity profile of drug molecule)

In designing of new compounds, ADMET information plays an imperative role. Outcomes of ADMET information can influence the selection to proceed with synthesis of newer drugs. At this stage, computational approaches are the solitary option to get the information, though it can also be accepted that the predictions are not just right at this point.

The relationships between important ADME parameters and molecular structure and properties with deep understanding, has been used to develop in silico models that allow the early assessment of several ADME properties.

Amongst other main issues, we go to predict properties to get the information concerning dose size and dose frequency, for instance oral absorption, bioavailability, brain penetration, clearance and volume of distribution.

Antitumor Activity

The highly reliable, colorimetric based assay is readily performed on a wide range of cell lines. This assay gives an indication of whole cell cytotoxicity; however, to determine the exact molecular target further assays need to be performed. 49

Of these, kinase inhibition assays are also one of the most widespread enzyme inhibition screening assays performed. Kinases are enzymes that play a key role in a number of physiological processes and their inhibitors have been found to exhibit anticancer activity against various human cancer cell lines. 71

Procurement of cell line

All the work on cell lines (3LL, MCF-7, BGC-823, QGY-7701) with passage number 45 was performed in Sapience Bioanalytical Research Laboratory, Bhopal (M.P).

Media Preparation

20-30 ml of MEM media was poured in centrifuge. To this 10ml of bovine serum, 0.5 ml antibiotic solution, 1.25 ml HEPES was added and volume was made up to 50 ml by appropriate media. All chemicals were Mixed and stored at 208 0C (for up to 4 weeks)

Sub culturing cells

Above solution was taken and the media was removed and wash with PBS. After this PBS was Removed and 1ml trypsin-EDTA solution was added. The flask was incubated at 370C in CO2 incubator.

In-vitro anticancer screening

All the work on cell lines (3LL, MCF-7, BGC-823, QGY-7701) with passage number 45 was performed in Sapience Bioanalytical Research Laboratory, Bhopal (M.P.). The cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). For screening experiment, the cells were seeded into 96-well plates in 100µl of medium containing 5 % FBS, at plating density of 10,000 cells/well and incubated at 37 0C, 5 % CO2, 95 % air and 100 % relative humidity for 24 hours prior to addition of samples. The samples were solubilized in Dimethyl sulfoxide and diluted in serum free medium. After 24 hours, 100 µl of the medium containing the samples at various concentration (eg; 0.063, 0.125, 0.25,

0.5, 1.0 mM etc…) was added and incubated at 370C, 5% CO2, 95% air and 100% relative humidity for 48 hours. Triplicate was maintained and the medium containing without samples were served as control. 129

After 48 hours, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 370C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula. 75

% cell Inhibition = 100 – {(sample) / Abs (control)} × 100

IC50 values for compounds (1a-1k) on related cell lines are mentioned in Table 6.10, and Cell Line Pictures for most active Compounds (1c, 1d, 1e, and 1k) are mentioned in Fig.6.48, Fig.6.49, Fig.6.50, Fig.6.51.

A series of β-carboline derivatives was selected from a reported article which presented the synthesis of novel derivatives of this compound and tested their anti-cancer potential against various cancer cell lines (Barbosa et al., 2011). Structure build-up, physico-chemical property determination, and sequential multiple regression analysis was performed on the reported series.

Biological Activity Calculation:

The observed potency (IC_{50} values) against renal cancer cell line (786-O) for all 26 compounds were altered from micromolar concentration to molar concentration and subsequently these values for renal cell lines from the reported series [N-(substituted-benzylidene)β-carboline-3-carbohydrazide derivatives (Barbosa et al., 2011)] were used to derive the biological activity values in the form of (Log $1/IC_{50}$). Although the series presented a total of 51 compounds, but about twenty-five compounds which were shown having the IC_{50} values greater than 100 micromolar concentration (>100) were eliminated because their IC_{50} values were not exactly defined. These structures along with their activity (Log $1/IC_{50}$) values are mentioned in the Table 1.

Table 1: 3-(carbohydrazide substituted) -β-carboline derivatives with their experimental activities.

Cpd	R_1	R_2	R_3	BA
3	3-NO_2	C_6H_5	H	5.425
4	3-NO_2	4-$N(CH_3)C_6H_5$	H	7.398
6	3-NO_2	2-ClC_6H_5	H	4.769
8	4-OCH_3	C_6H_5	H	4.027
11	4-OCH_3	2-ClC_6H_5	H	5.560
12	4-OH	4-$OCH_3C_6H_5$	H	4.208
13	4-OH	C_6H_5	H	5.365
15	4-OH	4-$NO_2C_6H_5$	H	5.019
16	4-OH	2-ClC_6H_5	H	5.492
20	H	4-$NO_2C_6H_5$	H	4.527
21	H	2-ClC_6H_5	H	5.665
22	4-NO_2	4-$OCH_3C_6H_5$	H	4.851
23	4-NO_2	C_6H_5	H	4.524
26	4-NO_2	2-ClC_6H_5	-	5.560
27	4-OCH_3	Cyclohexyl	-	4.731
31	4-OH	Cyclohexyl	-	4.825
33	4-NO_2	Cyclohexyl	-	4.138
34	4-OCH_3	Cyclohexyl	-	4.610
38	2-Cl	Cyclopentyl	-	4.013
42	2-Cl	CH_3	-	4.933
44	H	CH_3	CH_3	4.794
46	3-OH, 4-OCH_3	CH_3	CH_3	4.155
47	4-OH	CH_3	CH_3	4.615
48	4-$N(CH_3)_2$	CH_3	CH_3	4.576
53	3-NO_2	4-$N(CH_3)_2C_6H_5$	-	5.906
54	4-OCH_3	C_6H_5	-	5.857

Table 2: Experimental, calculated, predicted activity values for training set compound

Compound	Observed	Calculated	Residual	Predicted	Pred_Residual
3	5.42481	5.45187	-0.02706	5.46998	-0.045168
11	5.5986	5.34112	0.257479	5.30671	0.291889
12	4.20775	4.69608	-0.48833	4.77606	-0.568312
13	5.36452	5.12146	0.243056	5.10425	0.260266
15	5.01863	4.99608	0.022554	4.98876	0.029874
16	5.49214	5.4208	0.071344	5.41219	0.079954
21	5.66555	5.77724	-0.11169	5.81323	-0.147684
22	4.85109	4.66209	0.188999	4.63038	0.220709
23	4.52462	5.01469	-0.49007	5.13963	-0.615011
26	5.56067	5.37762	0.183047	5.33636	0.224307
27	4.73166	4.82166	-0.09	4.83041	-0.098754
31	4.82507	4.84394	-0.01887	4.8452	-0.020132
38	4.01305	4.04024	-0.02719	4.06502	-0.051971
42	4.93293	5.20844	-0.27551	5.22993	-0.297001
44	4.79425	4.64326	0.150994	4.61525	0.179004
47	4.61475	4.30385	0.310901	4.22267	0.392081
48	4.57659	4.59239	-0.0158	4.59421	-0.01762
53	5.90658	5.92131	-0.01473	5.93595	-0.029372
54	5.85699	5.72611	0.130875	5.6048	0.252185

Table 3: Experimental, predicted activity values of test set of compounds

Cpd	Observed	Predicted	Pred residual
20	4.52739	5.36056	-0.83317
6	4.768785	5.23678	-0.467995
4	7.39794	5.48049	1.91745
33	4.137869	4.71912	-0.581251
46	4.154902	3.97681	0.178092

Figure 1: Graph between experimental and calculated activity values for training set of compounds

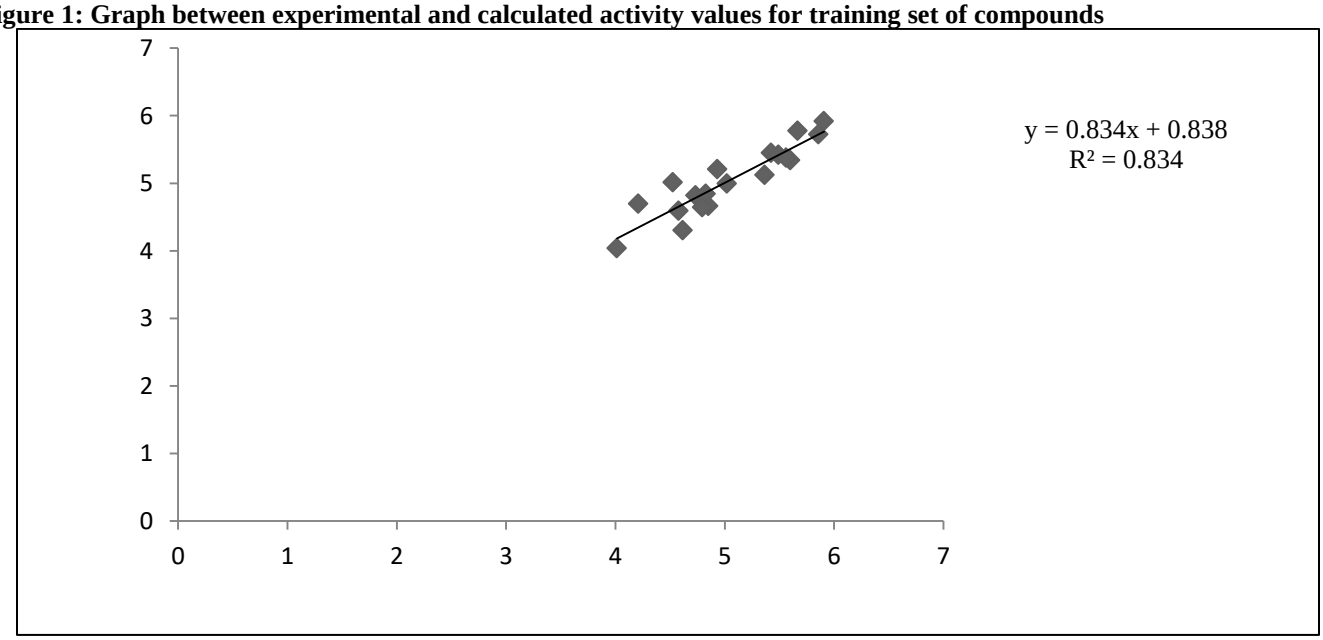


Figure 2: Graph between experimental and predicted activity values for training set of compounds.

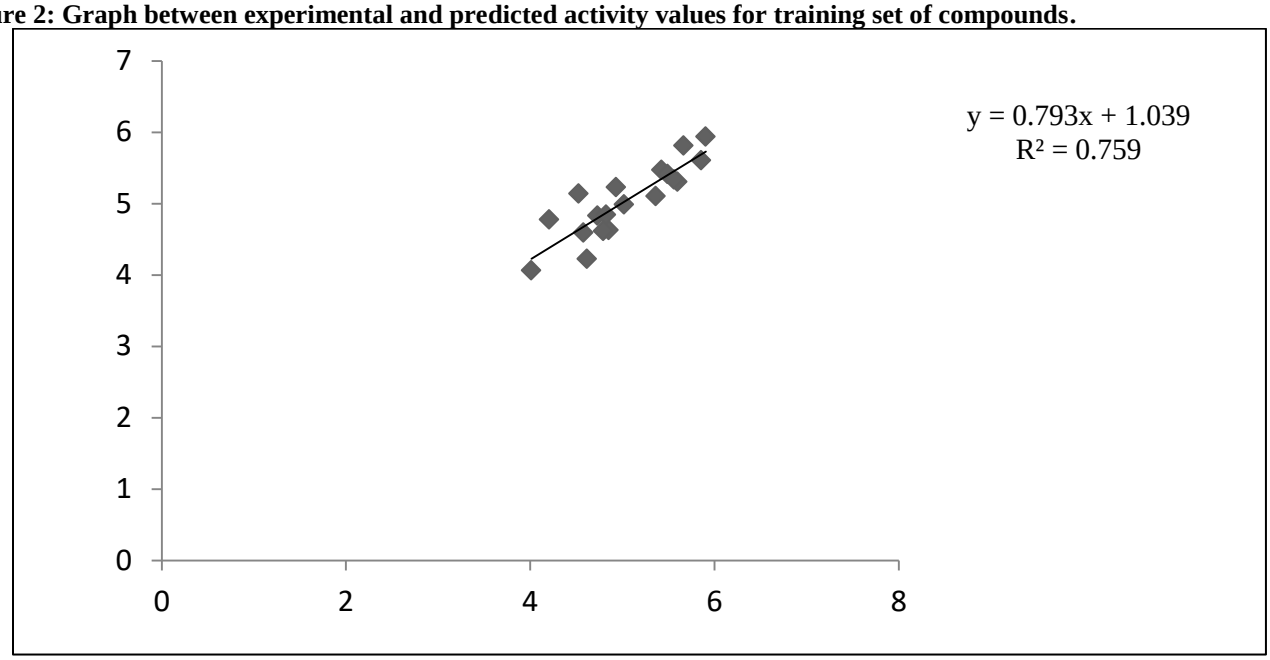
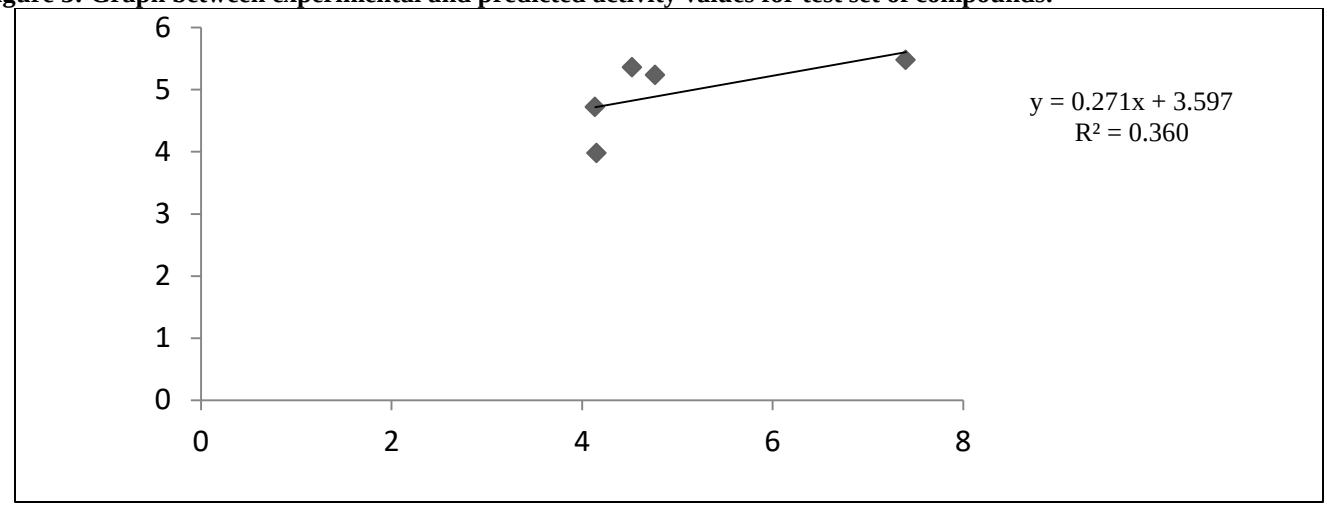


Figure 3: Graph between experimental and predicted activity values for test set of compounds.



RESULTS AND DISCUSSION

Structure Building & Energy Minimization:

The structures of the remaining twenty-six compounds were fabricated by means of Chemdraw Ultra 7.0.1 of Chemoffice Ultra 7.0.1 suite software, which is a product of Cambridge soft corporation, U.S.A. These structures were then saved in MDL (.mol) format which is followed by energy minimization using Chem3D ultra 7.0.1 by the means of MM2 (Molecular Mechanics) force fields and followed by MOPAC-Closed shell(AM-1) pro force fields using 0.100 as root mean square gradient.

Physico-Chemical Property Calculation: The properties of all these compounds were simultaneously computed using Chem3D ultra. Subsequently, all these calculated properties were arranged in Microsoft Excel 2007 sheet and subjected to the statistical software VALSTAT. The different properties of the molecules computed were log P, connolly accessible area, connolly molecular area, connolly solvent accessible volume, molecular weight, ovality, principle moment of inertia X, Y, Z, molecular refractivity, partition coefficient, bending energy, charge-dipole energy, dipole-dipole energy, molecular topological index, shape attribute, shape coefficient, stretch energy, stretch-bend energy, bending energy, torsion energy, van der waal forces, sum of valence degrees.

QSAR Model Development:

Dataset of compounds was separated into training and test set which was randomly carried out by VALSTAT software. The compounds which were selected by the

software for training set were 3, 8, 11, 12, 13, 15, 16, 21, 22, 23, 26, 27, 31, 34, 38, 42, 44, 47, 48, 53, 54 and for test set were 4, 6, 20, 33, 46. The training set of compounds was used for development of suitable models whereas the test set of compounds was used for cross validation of the various models developed through training set.

The QSAR model was fabricated using Sequential Linear Multiple Regression method. An Inter-Correlation matrix between all parameters was developed and it is mentioned in the Table 2. The observed, calculated, predicted and residual activity values for training set of compounds are mentioned in the Table 3. Figure 1 shows a graph between experimental and calculated values of training set of compounds. Figure 2 shows graph between predicted and experimental values of training set compounds. The predicted, experimental and predicted residual activity for test set of compounds is given. Figure 3 shows graph between predicted and experimental values of test set compounds.

Model Validation

The developed models were validated using following methods-

- External Validation
- Internal Validation (Leave-one-out method)

The Cross-validated regression coefficient value was calculated by the following formula.

$$Q^2 = 1 - \frac{PRESS}{\sum_{i=1}^{N} (Z_i - Z_m)^2} \quad \text{…….. (1.13)}$$

Where PRESS = predicted residual sum of squares,

Z_i = activity for training set,

$\mathbf{Z_m}$ = mean observed value, corresponding to the mean of the values for each cross-validation group.

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