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# DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NOVEL β- CARBOLINE DERIVATIVES

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**ABSTRACT**

A series of 51 compounds [N-(substituted-benzylidene)-β-carboline-3-carbohydrazide derivatives (Barbosa et al., 2011)] having significant inhibitory activity against cancer cell lines was selected and the presented biological activity (in micromolar concentration) of those compounds were conveniently converted into Log (1/IC50) values (molar) for carrying out QSAR analysis against anti-cancer activities using Chemoffice Ultra version 7.0.1, from Cambridge software corporation. The values of related parameters of all the molecules were calculated after effective energy minimization through MM2, MOPAC force fields provided by Chem3D Ultra 7.0.1

**Key words:** QSAR, β-Carboline, Drug Design, Anti-Cancer, Cell Lines.

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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.[1] 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.[2]

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. [3] 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. [4]

Drug designing is reckoned as a multi-dimensional practice which entails pharmacologists, biologists, biochemists, chemists and many others. [5] 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.[6]

The discovery of a lead compound is assumed to be the most complicated aspect of the drug scheming process.[7] 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.

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QSAR basically involves implication of methods to establish correlation between structural descriptors of selected compounds with their activities.[8] These structural descriptors are deciphered empirically or with the help of computation principles and they may include various parameters which account for hydrophobic properties, topological properties, electronic properties, and steric effects for a compound.[9] In general chemical dimensions and biological assays are the activities which are used in QSAR to compare with its descriptors. QSAR approaches are presently being purposeful in many fields allied to drug design. In the decade of 1890, Charles Overton of Zurich University and Hans H. Meyer of Marburg University, who were autonomously operational, distinguished that the lipophilicity of organic compounds had a great proportional influence on their toxicity. Hammett's equation for QSAR utilized electronic properties as the structural descriptors for compounds. [10] But when investigators attempted to apply Hammett-type relationships for biological systems various difficulties were observed, this indicated that the other structural descriptors were also pretty much necessary for QSAR analysis (Borman, 1990).

Chemotherapy, or the use of chemical agents for treatment of diseases, is considered a mainstream approach in the present scenario. [11] 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 focussed 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.[12]

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.[13] A great deal of current efforts has been focusing on the design and development of various antimicrobial drugs. [14] 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 [15] 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

### 2-D QSAR process

**Biological activity conversion:**

The observed potency ($IC_{50}$ values) against renal cancer cell lines for all 26 compounds were altered from micromolar concentration to molar concentration and then putting these $IC_{50}$ values for renal cell lines from the reported series [N-(substituted-benzylidene)-$\beta$-carboline-3-carbohydrazide derivatives [16] in the equation (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.

**Structure build-up & 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.[17] These structures were then saved in MDL (.mol) format and therefore the energy minimization process was carried out 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 least value for root mean square gradient to be 0.100.

**Property Calculation:**

The descriptive properties of all these compounds were simultaneously computed using Chem3D ultra. Subsequently, all these calculated values of properties obtained were arranged in Microsoft Excel 2007 sheet and were subjected to the statistical software recognized as VALSTAT software.[18] 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, non-Van der waal forces, Molecular topological index, Shape attribute, Shape coefficient, Stretch energy, Stretch-bend energy, Torsion energy, van der waal force, Sum of valence degrees.[19]

**QSAR Model Development:**

Training set and Test set selection was automatically carried out by the VALSTAT software through randomized selection. The training set of compounds were used for development/ preparation of suitable models whereas the test set of compounds were used for cross checking/cross validation of the various models developed through training set.

The compounds which were selected by the software for training set were and the compounds selected for test set w

The QSAR model was fabricated using Sequential Linear Multiple Regression method.

The Internal validation of the best developed model was carried out using Leave-One-Out method

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

$$Q^2 = 1 - \frac{PRESS}{\sum_{i=1}^{N} (Z_i - Z_m)^2}$$

…….. (1.13)

Where PRESS = predicted residual sum of squares,
$Z_i$ = activity for training set,
$Z_m$ = mean observed value, corresponding to the mean of the values for each cross-validation group

Stepwise multiple liear regression was carried out to build up QSAR model

The statistically significant equations were considered as best model.

An Inter-Correlation matrix between all parameters was developed and it is mentioned in the **Table A2.** The observed, calculated, predicted and residual activity values for training set of compounds are mentioned in the **Table A1**. And the predicted, observed and predicted residual activity for test set of compounds is given in **Table A4**.

***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 $37^0$C, 5% CO2, 95% air and 100% relative humidity for 48 hours. Triplicate was maintained and the medium containing without samples were served as control. [20]

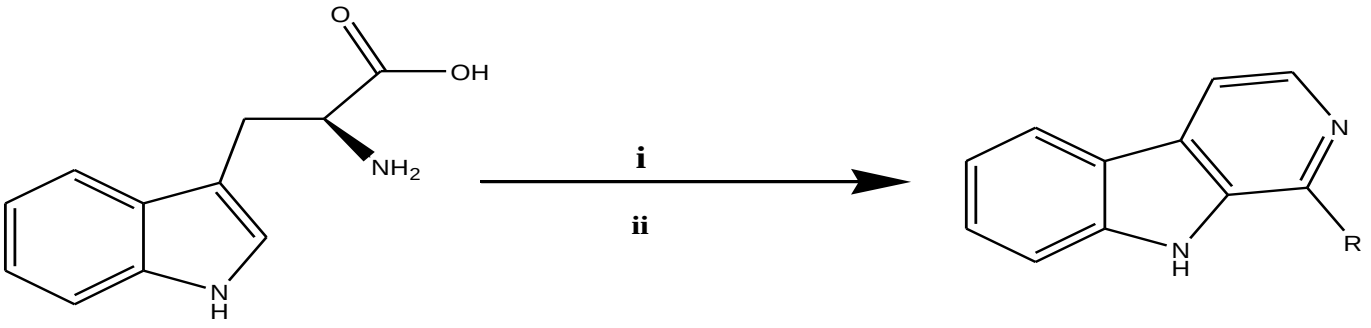
After 48 hours, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at $37^0$C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. [21] The % cell inhibition was determined using the following formula.

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

Nonlinear regression graph was plotted between % Cell inhibition and Log10 concentration and IC50 was determined using GraphPad Prism software. [22]

**SCHEME FOR SYNTHESIS OF β-CARBOLINE DERIVATIVES**

**The synthesis of derivatives was performed as per the following scheme. Synthetic route for compounds 1a – 1k.**




**1** **1a-1k**

**Reagents and conditions:**

i)Aliphatic aldehydes,0.5M $H_2SO_4/H_2O$,room temperature,
OR Aromatic aldehydes, HOAc, reflux. ii),$K_2CrO_7$,HOAc/$H_2O$,reflux.

**Table 1: Structures of β-carboline Derivatives.**

| | | | |
|---|---|---|---|
| **1a** | H | **1b** | $CH_3$ |
| **1c** | $CH_3CH_2$- | **1d** | $CH_3CH_2CH_2$- |
| **1e** | $(CH_3)_2CH$- | **1f** | $C_6H_5$ |
| **1g** | m-nitro-$C_6H_5$ | **1h** | o-nitro-$C_6H_5$ |
| **1i** | p-anisyl-$C_6H_5$ | **1j** | 4-hydroxy-3-methoxyphenyl |
| **1k** | 2-hydroxyphenyl | | |

**RESULTS AND DISCUSSION**

**QSAR Models and Statistical data**

The QSAR models developed using VALSTAT are mentioned below:

BA = [3.80861(±0.57417)] +LogP [0.431076 (± 0.101695)] +BE [-0.0376416 (±0.0165881)] +ChDi [0.455172(± 0.225939)]-------- (1)

N = 21, r = 0.780219, $r^2$=0.608742, $r^2$adj=0.539696, variance=0.152946, std=0.391084, QF=1.99502, PE=0.0569197, F=8.81652, FIT=0.881652, LOF=5.8502, AIC=0.224921, $Q^2$ = 0.461691, pred $r^2$ = 0.32913, Spress= 0.458726, SDEP = 0.412732

BA = [4.17537 (± 0.605439)] +LogP [0.305977 (± 0.1277)] +BE [-0.0477708(± 0.0171435)] +MTI [2.15155e-005 (± 1.15673e-005)] ----------(2)

N = 21, r = 0.772846, $r^2$ = 0.59729, $r^2$adj = 0.526224, variance = 0.157423, std = 0.396766, QF = 1.94786, PE = 0.0585857, F = 8.40467, FIT = 0.840467, LOF = 6.02143, AIC = 0.231504, $r^2$ pred = 0.259422, $Q^2$ = 0.440109, Spress = 0.467832, SDEP = 0.420925

BA = [2.29594 (± 0.830433)] +MOREF [0.355035 (± 0.0783638)] +BE [-0.0683345 (± 0.0206988)] +SE [-0.00578236 (± 0.00299006)] ----(3)

N = 21, r = 0.769929, $r^2$ = 0.59279, $r^2$adj = 0.520929, variance = 0.159182, std = 0.398976, QF = 1.92976, PE = 0.0592403, F = 8.24917, FIT = 0.824917, LOF = 6.08872, AIC = 0.234091, $r^2$ pred = 0.357582, $Q^2$ = 0.418511, Spress = 0.47677, SDEP = 0.428967

BA = [1.97921 (± 0.894114)] +MWt [0.0113072 (± 0.0025127)] +BE [-0.0685135 (± 0.0208124)] +SE [-0.00666666 (± 0.00313194)] -----------(4)

N = 21, r = 0.767966, $r^2$ = 0.589772, $r^2$adj = 0.517378, variance = 0.160362, std = 0.400452, QF = 1.91775, PE = 0.0596795, F = 8.14678, FIT = 0.814678, LOF = 6.13385, AIC = 0.235827, $r^2$ = 0.310834, $Q^2$ = 0.396642, Spress = 0.485652, SDEP = 0.436959

BA = [3.7067 (± 0.598276)] +LogP [0.355948 (± 0.117565)] +PMOIX [0.00022756 (± 0.000135149)] +BE [-0.0482381 (± 0.0175505)] -----------(5)

N = 21, r = 0.764597, $r^2$ = 0.584609, $r^2$ adj = 0.511304, variance = 0.16238, std = 0.402964, QF = 1.89743, PE = 0.0604305, F = 7.97509, FIT = 0.797509, LOF = 6.21104, AIC = 0.238794, $r^2$ pred = 0.406848, $Q^2$ = 0.407322, Spress = 0.481335, SDEP = 0.433074

Among the above given models' model number 1 showed two compounds (compounds no. 8, compound no. 34) as outliers. [23-25] Therefore, these compounds were selectively taken out of the test set and the new optimized model having 19 training set compounds was generated.

This model was selected as the best model, and it is mentioned as follows. [26]

BA = [3.72949 (± 0.364258)] +LogP [0.497787 (± 0.0659259)] +BE [-0.0431053 (± 0.0108995)] +ChDi [0.369448 (± 0.143659)]

N = 19, r = 0.913222, $r^2$ = 0.833974, $r^2$ adj = 0.800769, variance = 0.0609463, std = 0.246873, QF = 3.69916, PE = 0.0253927, F = 25.1157, FIT = 2.84645, LOF = 2.29183, AIC = 0.0790826, $r^2$ pred = 0.337368, $Q^2$ = 0.757384, Spress = 0.298432, SDEP = 0.265163

Where, n = No. of training set of compounds, r = correlation coefficient, std = Standard Error for Regression, F-ratio = F-ratio between variation of calculated and observed value, $q^2$ = cross validated $r^2$.[27] Out of above five models, model number 1 was selected after removing the outliers as the best model on the basis of high $q^2$, $r^2$, and low standard error < 0.3 values.[28-30] Figure A2 shows graph between predicted and observed values of test set compounds, and Figure A1 shows a graph between calculated values of training set of compounds. Table A4 shows the activity values obtained for test set compounds after internal validation.

## CONCLUSION

A series of 51 compounds [N-(substituted-benzylidene)-$\beta$-carboline-3-carbohydrazide derivatives (Barbosa et al., 2011)] having significant inhibitory activity against cancer cell lines was selected and the presented biological activity (in micromolar concentration) of those compounds were conveniently converted into Log ($1/IC_{50}$) values (molar) for carrying out QSAR analysis against anti-cancer activities using Chemoffice Ultra version 7.0.1, from Cambridge software corporation.

The values of related parameters of all the molecules were calculated after effective energy minimization through MM2, MOPAC force fields provided by Chem3D Ultra 7.0.1.

The best QSAR model obtained was taken into consideration on the basis of high $Q^2$ value, which reveals that in order to increase the biological activity, the properties like LogP, and Charge-dipole energy should be increased, whereas Bending energy which is showing a negative value in the equation should be decreased. Thus, it is concluded that the biological activity will be increased if substituents that bring about changes in the molecule as mentioned above are attached to it.

As per the given QSAR data, a new series of 1-substituted β-carboline derivatives (1a-1k) were synthesized having increased LogP value.

These title compounds containing seven different substituents at C-1 were screened for their invitro anti-cancer and Anti-microbial activity. Most of the test compounds were found to exhibit significant anti-cancer activity. Among the substituents at C-1, isopropyl substituent showed maximum potency, while n-propyl substituent showed equipotent activity remaining substituents exhibited least activity when compare to other substituents.

The order of activity at C-1 is as understood by QSAR factor (Compound 1e) was found to be the most active agent which showed highest percentage of cell inhibition against all the cancer cell lines in the minimum concentration, which have *isopropyl* group at the 1st position in the β-carboline nucleus. Hence this molecule can be selected as a lead molecule of the present study for further exploitation.

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