Vol 10| Issue 2| 2020 | 183-188.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

International Journal of
Current Pharmaceutical & Clinical Research

www.ijcpcr.com

DESIGN, DEVELOPMENT OF 2D-QSAR AND IN SILICO PHARMACOLOGICAL EVALUATION OF SOME NOVEL β CARBOLINE ANALOGUES

Ravi Nirala*, Amit Nayak

RKDF College of Pharmacy Near Ruchi Lifeescape, Jatkhedi, Misrod, Bhopal, Madhya Pradesh-462026, India.

ABSTRACT

A series of 51 compounds [N-(substituted-benzylidene)- -carboline-3-carbohydrazide derivatives 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. The best QSAR model obtained was taken into consideration on the basis of high Q2 value, which reveals that in order to increase the biological activity, the properties like Log P, 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 (1a1k) 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.

Key words: β-carboline, 2D-QSAR, Log P, Energy Minimization, Biological Activity.

INTRODUCTION

β-carboline heterocyclic ring containing derivatives are very much popular due to their broadspectrum activities. β-carboline (9H-pyrido-[3,4-b] indole) which is also renowned as norharmane is a nitrogen enclosing heterocyclic compound. It is also the archetype of a group of compounds identified as β-carbolines. These compounds are said to belong to the set of indole alkaloids and constitute a pyridine ring that is merged to an indole frame (Encyclopedia of psychoactive plants, 2005). The structural arrangement of molecules of β-carboline is analogous to the structure of tryptamine, in which the ethylamine chain is joined to the indole ring through an extra carbon atom, to generate a three-ring arrangement. Biological synthesis of these molecules is assumed to track this path from related tryptamines (Minguez et al., 2011). Eight plant families are recognized to articulate 64 diverse varieties of β-carboline alkaloids. The alkaloidal content varies in different species like, the seeds of Peganum harmala contain 0.16% (Baiget et al., 2006) to 5.9% (González et al., 2010) β-carboline alkaloids.

Corresponding Author: -. Ravi Nirala

As a consequence of the existence of β-carbolines in the cuticle of scorpions, their crust is well-known to incandesce when exposed to definite wavelengths of ultraviolet light such as that formed by a blacklight (Stachel et al., 1999). A group of β-carboline derivatives, expressed as eudistomins were extorted from ascidians (marine tunicates-family Ascidiacea), like Ritterella sigillinoides (Lake et al., 1989), Lissoclinum fragile (Badre et al., 1994) and Pseudodistoma aureum (Carroll et al., 1998).

β-carboline act as precursor for synthesis of number of biological structures. The important biological and biophysical properties of β-carboline derivates have considerable current interest for their study.

β-Carboline alkaloids are widely distributed in nature including various plants, foodstuffs, marine creatures, insects, mammals as well as human tissues and body fluids. Numerous representatives of this family show various biological activities [3–5]. The fascinating diversity of structures and medicinal potential [6] inherent in them encourage several researchers to deal with the synthesis of β-carboline containing natural products and their synthetic derivatives [2, 4, 7–9]. Several commercial drugs or drug candidates such as vinpocetine, brovincamine, abecarnil, cipargamin, tadalafil, reserpine and lurbinectedin contain this unique framework.

Natural products have a protracted history as therapeutics for broad range of diseases. Indelible co-evolution between biological communities and humans has tried to explain the baffle of biological significance of natural products in humans and other species [1-7]. Many chemists and biologists in both industrial and academic sector have commenced and proved clinical potentiality of natural compounds as a prolific source of chemical inspiration for the evolution of new drugs. The impact of plant-derived drugs on mankind become enormous in the recent days and is proved by the development of plant-derived drugs such as vinblastine, 4 vincristine, paclitaxel, quinine, etoposide, artemisinin, teniposide, morphine, and the camptothecin derivatives topotecan and irinotecan. Even though, natural products derived from microbial origin have made significant contribution, marine derived natural products are also having an increasing impact on the treatment of human disease, particularly as anticancer agents [8-10]. Evolution of semi-synthetic modifications of natural products as a source of bioactivelead compounds to improve drug-likeness and clinical utility is one of the transitions taken an advanced role in drug discovery and drug development. Hence, further research regarding the development of new chemotherapeutic agents that are more effectively combat cancer is an active area of research in medicinal chemistry [11-13].

EXPERIMENTAL

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)- -carboline-3-carbohydrazide derivatives (Barbosa et al., 2011)] in the equation (Log $1/IC_{50}$). Although the series presented a total of 51 compounds, but about twentyfive compounds which were shown having the IC50 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. 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. 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.

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 24 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 3, 8, 11, 12, 13, 15, 16, 21, 22, 23, 26, 27, 31, 34, 38, 42, 44, 47, 48, 53, 54. And the compounds selected for test set were 4, 6, 20, 33, 46.

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

Where PRESS = predicted residual sum of squares, Zi = activity for training set, Zm = 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.

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.132 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.138,140Eliminating 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.141 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.135-136 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.

Prediction of physicochemical properties and drug likeliness

The Mol inspiration online property calculation toolkit was used to calculate the molecular properties of synthesized compounds like TPSA, number of rotatable bonds (n-ROTB), molecular weight (MW), molecular volume (MV), number of hydrogen donor (n-OHNH), acceptor atoms (n-ON) and violations of Lipinski's rule of five.

A Topological polar surface area (TPSA) is one of the descriptors used to predict the passive transportation of molecule through membranes. It helps to determine the kinetics of molecule with crossing the intestine and 39 blood-brain barrier (BBB). The Absorption percentage (%ABS) was calculated using a formula: %ABS = 109-(0.345 x TPSA).

Lipinski's "Rule of- Five" helps to make a distinction between drug like and non-drug like molecules.It is used for the prediction of probability of success or failure due to drug likeliness for molecules should obey with 2 or more of the following rules:

1. Molecular mass should be less than 500 Dalton
2. High lipophilicity (expressed as LogP less than 5)
3. Hydrogen bond donors should be < 5
4. Hydrogen bond acceptors should be < 10
5. Molar refractivity should lie between 40-130

Prediction of ADME and related properties

Main elements of pharmacokinetics like absorption, distribution, metabolism and excretion, were predicted with PreADMET web tool (https://preadmet.bmdrc.kr/). PreADMET is an online tool, used for prediction of ADME and toxicity data to develop drug-like library by means of in silico method. It makes use of Caco2-cell (heterogeneous human epithelial colorectal adenocarcinoma cell lines) model for the prediction of oral drug absorption at the same time as human intestinal absorption model for oral and trans-dermal drug absorption prediction. Distribution is predicted through experimental distribution coefficients at pH 7.4 in octanol/water, the ionization constant of the compounds and calculated plasma-protein-binding data. It in addition Predicts the solubility of each compound in water at 25oC.143 CYP2D6 is a member of the cytochrome P450 mixed-function oxidase system. It is one of the most significant enzymes involved in the metabolism of xenobiotics. Predominantly CYP2D6 is responsible for the metabolism and elimination of about 25% of clinically used drugs, by the process referred to as O-demethylation.

In-silico prediction of toxicity issues

About ~20–40% of drug failures in research and drug development may be due to their toxicity concerns. Modern software packages are having main emphasis on carcinogenicity and mutagenicity, at the same time as some packages do also comprise models such as teratogenicity, irritation, sensitization, immunotoxicology, neurotoxicity, QT prolongation, hepatotoxicity and phospholipidosis. Toxicity prediction by Pre-ADMET tool is based on the mutagenicity of Ames test, rodent carcinogenicity assays and hERG inhibition. hERG (the human Ether-à-go-go- 40 Related Gene) potassium channels are crucial for normal electrical activity in the heart. Inhibition of this channel by any drug may result in fatal disorder called long QT syndrome and for that reason hERG inhibition study is imperative to establish the safety of drug candidate in drug designing.

Assessment of drug likeliness and prediction of ADMET

All the 11 synthesized compounds which showed best yield were selected to determine drug likeliness, ADME prediction and toxicity prediction study. Lipinski's "Rule of- Five" were calculated to determine the drug likeliness and bioavailability of the ligands. The calculated properties of all compounds were shown the non-violation of Lipinski's rule and further they were selected for the prediction of ADME and toxicity profile. The pharmacokinetics properties and toxicity prediction of all the selected compounds were analyzed with the help of Pre ADMET software.

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.

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.

Methods for determining antitumor activity: -

The MTT/MTS in vitro cell proliferation assay is one of the most widely used assays for evaluating preliminary anticancer activity of both synthetic derivatives and natural products and natural product extracts.

Herein, we describe the methods for performing both in vitro MTT/MTS cytotoxicity and kinase enzyme inhibition assays. These are two of the most useful anticancer screening techniques available that are relatively economical and can be easily and routinely performed in the laboratory to characterize anticancer activity.

Both assays are highly versatile and can be modified to test against targeted disease processes by using specific kinase enzymes or cell lines.

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, 1.25ml HEPES was added and volume was made up to 50ml 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.

RESULTS AND DISCUSSION

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. 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.

CONCLUSION

A series of 51 compounds [N-(substituted-benzylidene)- -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.
- The best QSAR model obtained was taken into consideration on the basis of high Q2 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 (1a1k) were synthesized having increased LogP value.

REFERENCES

1. Hill RA. Annual Reports on the Progress of Chemistry, Section B: Organic Chemistry. 108, 2012, 131-146.
2. Hoffman A, *et al*. The Encyclopedia of Psychoactive Plants: Ethnopharmacology and its Applications. South Paris: Park Street Press, 2005.
3. Jean YT, *et al.* Synthetic biology for pharmaceutical drug discovery. *Drug Des Devel Ther.* 9, 2015, 6285-6302.
4. John Smith H. Introduction to the Principles of Drug Design and Action. 4th Edition. CRC Press, New York, 2006; 78-80.
5. Johnson P J. Metronidazole and Drug Resistance Parasitol. Today , 9, 1993, 183-186.
6. Jug K, et al. Quantitative measures of aromaticity for mono-, bi-, and tricyclic penta- and hexaatomic heteroaromatic ring systems and their interrelationships. *Chem Rev.* 101(5), 2001, 1421.
7. Katritzy AR. Handbook of Heterocyclic Chemistry. 1st ed., Pergamom Press, New York, 1985; 3-7.
8. Kawasaki T, *et al.* Simple indole alkaloids and those with a nonrearranged monoterpenoid unit. *Natural Product Reports*. 22, 2005, 761-793
9. Kenakin T. A guide to drug discovery: predicting therapeutic value in the lead optimization phase of drug discovery. *Nat Rev Drug Discov*. 2, 2003, 429– 438.
10. Knowles J, *et al.* A guide to drug discovery: Target selection in drug discovery. *Nat Rev Drug Discov*. 2(1), 2003, 63-9.
11. Kochanowska-Karamyan AJ, *et al.* Marine indole alkaloids: Potential new drug leads for the control of depression and anxiety. *Chemical Reviews*. 110, 2010, 4489-4497
12. Kohli DV, *et al.* 2D-QSAR studies of some 1, 3, 4-thiadiazol-2-yl-azetidin-2-one as antimicrobial agents. *Dig J Nano Biostru* 4, 2009, 339-347.
13. Kozikowski A. Comprehensive Heterocyclic Chemistry, *Pergamom Press*, 1, 1984, 567.
14. Kumar R, *et al.* Synthesis and cytotoxicity evaluation of (tetrahydro- -carboline)-1,3,5- triazine hybrids as anticancer agents. *J Med Chem* 45, 2010, 2265-2276.
15. Kunied T, *et al.* The chemistry of heterocyclic compounds. *International Journal of Pharmaceutical Sciences and Research, Palmer B.* 2002, 175.
16. Lake RJ, *et al.* Eudistomins from the new zealand ascidian Ritterella sigillinoides. *Aust J Chem* 42, 1989, 1201–1206
17. Laronze M, *et al.* Synthesis and anticancer activity of new pyrrolocarbazoles and pyrrolo- - carbolines. *Bioorg Med Chem* 13, 2005, 2263-2283.
18. Li Y, *et al.* a -carboline anti-cancer drug, inhibits the CDK activity of budding yeast. *Cancer Biol Ther* 8, 2007, 1193-1199.
19. Lombardino JG, *et al.* The role of the medicinal chemist in drug discovery – then and now. *Nat Rev Drug Discov*. 3, 2004, 853–862.
20. Ma C, *et al*. Synthesis and cytotoxic evaluation of 1-carboxamide and 1-amino side chain substituted -carbolines. *J Med Chem* 45, 2010, 5513-5519.
21. Mansoor TA, *et al.* Tabernines A−C. ß-Carbolines from the leaves of Tabernaemontana elegans. *Journal of Natural Products.* 72, 2009, 1147-1150
22. Maw GN, *et al.* Design synthesis and biological activity of β-caroline based type-5 phosphodiesterase inhibhitors. *Bioorganic & Medicinal Chemistry Letters.* 13, 2003, 1425-1428
23. Mishra Bhuwan B, *et al.* Cyclo-Release Strategy in Solid-Phase Combinatorial Synthesis of Heterocyclic Skeletons. *Advances in Heterocyclic Chemistry*. 107, 2012, 41-99.
24. Mohammad Asif. A Mini Review: Biological Significances of Nitrogen Hetero Atom Containing Heterocyclic Compounds. *International Journal of Bioorganic Chemistry*. 2(3), 2017, 146-152.
25. Mojdeh Yousefian Langroudi. Review of Studies Focused on Heterocyclic Compounds Containing a Heteroatom and Aromaticity Evaluation Methods. *BEPLS*. 3(7), 2014, 108-115.

26. Mojdeh Yousefian Langroudi. Review of Studies Focused on Heterocyclic Compounds Containing A Heteroatom and Aromaticity Evaluation Methods. *Indian J Sci Res*. 7(1), 2014, 724-731.
27. Molina P, *et al.* An iminophosphorane-mediated efficient synthesis of alkaloid Eudistomin U of marine origin. *Tetrahedron Letters*. 36, 1995, 3581-3582
28. Molina P, *et al.* Iminophosphorane-mediated syntheses of the fscaplasyn alkaloid of marine origin and nitramarine. *Tetrahedron Letters*. 35, 1994, 8851-8854.
29. Molnar M, *et al.* Effective synthesis of 6-substituted 7H-tetrazolo [5,1-b][1,3,4] thiadiazines via a one-pot condensation/nitrosation/azide-tetrazole tautomerism reaction sequence. *Heterocycl Commun*. 23(1), 2017, 1-8.
30. Mukhtyar. S. Saini, *et al.* A Review: Biological Significances of Heterocyclic Compounds. *IJPSR*. 4 (3), 2013, 66-77.
31. Mwense M, *et al.* QSAR approach for mixture toxicity prediction using independent latent descriptors and fuzzy membership functions. *SAR QSAR Environ Res* 17, 2006, 53–73.
32. Newman D J, *et al*. Natural products as sources of new drugs over the last 25 years. *J Nat Prod.* 70, 2007, 461–477.