**ABSTRACT**

Objective: Cancer is a group of disease characterized by uncontrolled growth of cells. The objective of the study includes the in silico designing of benzoxazole bearing azetidinone derivatives as Vascular Endothelial Growth Factor 2 in cancer.

Methods: In silico design of proposed derivatives was conducted using tools such as AutoDock Vina, ACD Lab ChemSketch ver. 12.0, Prediction of Activity Spectra for Substances online, molinspiration, and Swiss ADME. The derivatives obeying Lipinski’s Rule of Five in accordance with molinspiration were selected for docking studies.

Results: The data obtained from molinspiration revealed that the designed derivatives have physical and chemical properties meant for an orally bioavailable drug. From the docking studies derivatives BT1 and BT5 showed high docking score which indicate that these derivatives possess high affinity and high polar interaction towards protein 4DBN.

Conclusion: The designed benzoxazole bearing azetidinone derivatives were found to possess good binding affinity and good interaction in the binding pocket of the target 4DBN. Therefore, these derivatives are expected to exhibit good anticancer property with minimal side effects.

Keywords: Cancer, Vascular endothelial growth factor 2, Docking, AutoDock vina, Sorafenib.

INTRODUCTION

Cancer is a disease caused by an uncontrolled growth of abnormal cells. It is the second leading cause of death globally. The major types of cancers are sarcoma, melanoma, leukemia, lymphoma, and carcinomas. Tumor cells do not have any programming so that they do not provide any physiological function [1-3].

Benzoxazole and its derivatives constitute an important class of heterocycles in drug discovery. These derivatives show anti-bacterial, anti-fungal, anti-histaminic, and anti-cancer properties [4-6]. Azetidinones are the important scaffold with anti-tubercular, anti-HIV, anti-inflammatory activity etc. [7].

Vascular endothelial growth factor (VEGF) signaling pathway creates a vital role in governing tumor angiogenesis. Inhibition of the signaling pathway is considered as an effective therapeutic target for tumor angiogenesis inhibition and successive tumor growth. VEGF receptor (VEGFR) is a well-known target for many antineoplastic drugs including Sorafenib and Axitinib [8,9].

METHODS

ACD Lab ChemSketch ver. 12.0

ACD Lab ChemSketch ver. 12.0 is a software program helpful in the drawing and naming of chemical structures of various organic compounds. It gives information about characteristics such as calculation of molecular descriptors which include molecular weight, Molar volume, surface tension, Parachor, Polarizability, and Refractive index.

Molinspiration

It is a free web tool for the calculation of different molecular properties needed in QSAR, which includes log P, number of rotatable bonds, number of hydrogen bond acceptors, number of hydrogen bond donors, and number of violations. The oral bioavailability of synthesized derivatives can be predicted by these molecular descriptors which come under Lipinski Rule of Five. Molinspiration also help to predict the bioactivity score for the most important drug targets.

Prediction of activity spectra for substances (PASS) online

It is an online software for the prediction of biological activities. The results can be obtained with a list of over 4000 kinds of pharmacological activities consisting of Pa and Pi values arranged in their descending order of differences.

- If Pa>0.7, the substance is very likely to exhibit activity and chance of being an analog of a known pharmaceutical agent
- If 0.5<Pa>0.7, the substance is likely to exhibit activity and substance is unlike known pharmaceutical agent
- If Pa<0.5, substance unlikely to exhibit activity and a chance of being a new chemical entity.

Swiss ADME

Swiss ADME is a web tool with free access to the physicochemical, pharmacokinetic, and similar properties of powerful molecules. It produce predictive models using various methods such as BOILED-Egg (Fig. 1), log P, and bioavailability Radar (Fig. 2). In BOILED-Egg model, the white region denotes high probability of passive absorption by the gastrointestinal tract and yolk region is for high probability of brain penetration. In bioavailability radar, the pink area represents the optimal range for each property (Lipophilicity: XLOGP3 between ~0.7 and +5.0, size: Mol Wt. between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Ao, solubility: log S not higher than 6 etc. The parameters are tabulated in Table 1.

Protein data bank

Protein data bank is a resource for various proteins and macromolecules. Each entry in PDB is represented by a PBD ID, which
Protein preparation can be done by using Pymol where the protein is obtained from Protein Data Bank. This protein structure can be cleaned by various commands like remove < >resn < > HOH (for removing water) remove < >resn < >DSN (for detergents), remove < > resn < >IRE and also small molecules to be eliminated. Finally hydrogen atom should be added to the protein structure.

Ligand preparation

The 2D chemical structure of ligands was drawn using ACD Lab Chemsketch ver. 12.0 and generated smiles notation. This smiles notation is being converted into 3D PDB format with the help of freely accessible Corina Online Software.

Docking by autodock vina

Docking was performed using PyRx software program. Selected derivatives were loaded into navigation platform. The cleaned protein was converted into macromolecules. The docking procedure was done by clicking Vina wizard start button and adjusting the grid size. The docking scores were obtained which are shown in the Table 2.

Table 2: Molecular descriptors of derivatives derived using ACD Lab Chemsketch V 12.0

| Compound code | Parachor (cm$^3$) (±6.0) | Molar volume (cm$^3$) (±5.0) | Polarizability (10^{-24}) (±5.0) | Molar refractivity (±0.4) | Surface tension (Dyne/cm) (±5.0) | Refractive index (±0.03) |
|---------------|--------------------------|-------------------------------|---------------------------------|--------------------------|-------------------------------|--------------------------|
| BT 1          | 756.1                    | 256.4                         | 40.03                           | 100.98                   | 75.6                          | 1.717                    |
| BT 2          | 793.3                    | 267.2                         | 41.94                           | 105.80                   | 77.6                          | 1.722                    |
| BT 3          | 771.3                    | 253.2                         | 40.63                           | 102.51                   | 86.0                          | 1.743                    |
| BT 4          | 794.4                    | 272.1                         | 41.86                           | 105.51                   | 72.6                          | 1.703                    |
| BT 5          | 813.2                    | 267.4                         | 42.42                           | 107.01                   | 85.4                          | 1.732                    |
| Standard      |                          |                               |                                 |                          |                               |                          |

**Fig. 1:** Boiled egg model of BT1

**Fig. 2:** Bioavailability radar diagram of BT1

**Fig. 3:** Structure of vascular endothelial growth factor receptor 2 (PDB ID- 4DBN)

is a four-character unique identifier called PDB ID, for example, 4DBN. Fig. 3 (Crystal Structure of the Kinase domain of Human B-raf with a [1,3] thiazolo [5,4-b] pyridine derivative).

Molecular docking

Molecular docking studies are the computational techniques used to determine the interaction of two molecules and to find out the best orientation of ligand which would form a complex with the intended receptor. PyRx and PyMol are the two programs which are used for this purpose. PyMol is generally adopted for protein preparation and their visualization whereas PyRx provide a better platform for docking of ligand with receptor [10].

Protein preparation

Protein preparation can be done by using Pymol where the protein is obtained from Protein Data Bank. This protein structure can be cleaned by various commands like remove < >resn < > HOH (for removing water) remove < >resn < >DSN (for detergents), remove < > resn < >IRE and also small molecules to be eliminated. Finally hydrogen atom should be added to the protein structure.

Ligand preparation

The 2D chemical structure of ligands was drawn using ACD Lab Chemsketch ver. 12.0 and generated smiles notation. This smiles notation is being converted into 3D PDB format with the help of freely accessible Corina Online Software.
Visualization and analysis

The hydrogen bond, hydrophobic bond, and pi-pi interactions were analyzed using PyMol molecular graphic system. PyMOL can be used to develop a well-defined 3D image of small molecules, biological macromolecules such as proteins [11].

RESULTS

Molecular descriptors

The evaluation of molecular descriptors was done using ACD Lab Chemsketch ver. 12.0 is shown in Table 2.

**Molinspiration**

Molinspiration analysis is used to calculate the physicochemical parameters and to analyze Lipinski’s Rule of Five. The results are shown in Table 3.

**PASS online software**

In this study, PASS online was performed. The derivatives showed good anti-cancer properties which is tabulated in Table 4.

**Prediction of ADME properties**

Pharmacokinetic properties evaluated through Swiss ADME, the data were obtained are tabulated in Table 1.

**Molecular docking**

The docking analysis was performed through PyMol and PyRx software programs and the results are shown in Table 5 and the figures are shown in Fig. 4.
CONCLUSION
The present study scientifically revealed the in silico design, ADME prediction, and docking studies to predict anticancer activity. We have selected five benzoxazole substituted azetidinone derivatives and all the compounds showed good molecular properties. Based on the analysis of Lipinski rule of five, all the derivatives passed the rule of five and therefore these compounds were further preceded to pharmacokinetic and docking studies. PASS online predicted anti-cancer activity for those derivatives. Swiss ADME studies resulted with all the five derivatives to be orally bioavailable and they do not cross BBB. From docking scores, we can conclude that the designed benzoxazole substituted azetidinone derivatives are found to have good interaction in binding pocket of target 4DBN, derivatives possess good anticancer activity with high binding affinity. So these compounds are expected to possess good anti-cancer property with minimal side effects.

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AUTHORS’ CONTRIBUTIONS
The 1st and 2nd author contributed to the entire work and drafted the manuscript and the 3rd author participated in docking studies.

CONFLICTS OF INTEREST
The authors confirm that this article content has no conflicts of interest.

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