Research Paper Chemistry

Molecular Docking and Cytotoxic Activity of 1, 8-naphthyridine derivatives in Human Lung Cancer

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ABSTRACT

The Naphthyridine family seeks an attention due to its antibacterial and anticancer properties.In present study the effect of 9-methoxy benzo[b][1,8] Naphthyridin-2(1H)-one has been synthesized and tested for its potential anticancer property against lung cancer . The proteins ALK, EGFR, KRAS , RET and ROSI which are responsible for causing lung cancer , the compound has shown strong binding property against the proteins , tested in docking analysis in which RET protein had showed highest activity with reference to the results obtained through Insilico analysis .Invitro antitumor activity has been investigated using A549 cell line.The result shown that the compound has produced maximum cell death of about 75%. Hence with the results of the study, 9-methoxy benzo[b][1,8] Naphthyridin-2(1H)-one has shown to have a considerable property against lung cancer.These findings suggest that 1,8-naphthyridine derivatives are a promising class of compounds in cancer research.

KEYWORDS Insilico, Invitro, 1,8 naphthyridine, Molecular Docking

INTRODUCTION

Naphthyridine derivatives have received significant attention due to their exceptionally broad spectrum of biological activity.It was recently found that 1,8-naphthyridine derivative vosaroxin was found to have potential anticancer activity ; it is currently subjected to clinical development.[1]1,8-Naphthyridines based synthesized compound known to possess antibacterial,[2-3] antimycobacterial[4], antitumor[5], anti-inflammatory[6] ,antiplatele[7] gastric antisecretary[8] antiallergic [9] local anaesthetic[10] and benzodiazepine receptor activity[11] were been reported. The skeleton of 1,8-Naphthyridine is present in many compounds that have been isolated from natural substance with great importance due to their broad spectrum of biological activities[12] . These compounds have been investigated as potential anticancer agents and several compounds are part of clinical trials [13-14]. Lung cancer, one of the most frequently diagnosed cancers in the world, is characterized with relatively high morbidity and mortality[15] . Chemotherapy is recognized to be the main therapeutic way to delay tumor growth. However, the overall survival remains poor. Therefore, there is an urgent need to identify effective drugs for the treatment of lung cancer. The role of *In silico* chemistry is emerging in drug design and discovery. In an effort to find lead compounds at lower cost and greater speed, computational chemistry methods have focused on developing fast and highly efficient molecular docking methods for virtual screening .To find potent anticancer agents, we have synthesized 1,8-Naphthyridine derivatives and tested them by means of cytotoxicity.The most active compound resulting from this selection was characterized for its anticancer properties.Gene therapy provides a novel method for the prevention and treatment of cancer but the clinical application of gene therapy is restricted, mainly because of the absence of an efficient and safe gene delivery system. For recent years number of new potential lung cancer gene alteration have been characterized, including ALK, BRAF, EGFR, KRAS, MEK1, RET and ROS1. These selected proteins known to cause lung cancer were been evaluated for Insilico analysis of anticancer property of the synthesized compound .Invitro antitumour activity has been performed using lung adeno carcinoma cell line (A549 cell line).to find potent anticancer agents

INSILICO ANALYSIS

(a) PROTEIN

The proteins which are responsible for causing lung cancer are ALK (Anaplastic lymphoma kinase), BRAF (serine/threonine protein kinase), EGFR (Epidermal growth factor receptor), KRAS (Kirsten rat sarcoma virus) MEK1 (MAP kinase 1), RET (proto-oncogene located on chromosome 10) and ROS1 (Receptor tyrosine kinase) respectively has been used for the Insilico analysis

(b) LIGAND

The Ligand 9-methoxy benzo [b] [1,8] Naphthyridin-2(1H)-one was drawn from the sketcher controls are modeled from the ChemDoodle desktop application. This ChemDoodle desktop application was immediately comfortable drawing structure and export to the SDF file.

MOLECULAR DOCKING

In the current study program molecular docking server[16] has been used to compute the free binding energy (ΔG) of docked complexes. 2D coordinates of the 9-methoxy benzo [b] [1,8] Naphthyridin-2(1H)-one and the protein was submitted in PDB format with default parameters. Gasteiger partial charges were added to the ligand atoms. Nonpolar hydrogen atoms were merged and rotatable bonds were defined. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools[17]. The grid points and spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method[18]. Each docking experiment was derived from 25 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

MEASUREMENT OF POTENTIAL CYTOTOXICITY BY MTT ASSAY.

Human lung adeno carcinoma cell line (A549) was grown in Dulbecco's modifications of eugal's medium with L-glutamine & 4.5g/l glucose supplemented with fetal bovine serum 100 units/ml of penicillin G and 0.1 mg/ml of streptomycin sulfate in a humidified atmosphere of a 5% CO_2 at 37°C. The monolayer cell culture was trypsinized and the cell count was adjusted to 3 lakh cells/ ml using medium containing 10% new-

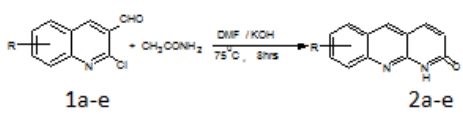
born calf serum. Pre incubate cells at a concentration of 1× 10^6 cells/ml in culture medium for 3 h at 37°C and 6.5% CO_2. The cells were seeded at a concentration of 5× 104 cells/well in 100 µl culture medium and incubated at 37°C in 5 % CO_2 incubator for 24 hrs. After 24 hours, when the monolayer formed, the supernatant was flicked off and added previously diluted with media of 100µl of different concentrations of test extract in microtitre plates and kept for incubation at 37°C in 5 % CO_2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 10µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37oC in in atmosphere of 5% CO_2 incubator. The supernatant was removed and 100 µl of Isopropanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at 590 nm with a reference filter of 620 nm. The percentage cell growth inhibition or percentage Cytotoxicity was calculated by following formula:

$$\% \text{ of inhibition} = \frac{\text{Control O.D - Dose O.D}}{\text{Control O.D}} \times 100$$

Inhibition concentration (IC_{50}) was evaluated by plotting graph with concentration (ug) of Gallic acid at X axis and % of inhibition at Y axis.

RESULT AND DISCUSSION

The ligand 9-methoxy benzo [b] [1,8] naphthyridin -2 (1H) –one (Fig.1) was synthesized by oxidizing of potassium hydroxide to react with 2- chloro - 3- formyl quinoline and acetamide



1a-e 2a-e

2a:R= 7-CH_3 , **2b**: R= 8-CH_3 , **2c**:R=6,9- CH_3 , **2d**:R= 7, 9-CH_3, **2e**: R= 9-OCH_3

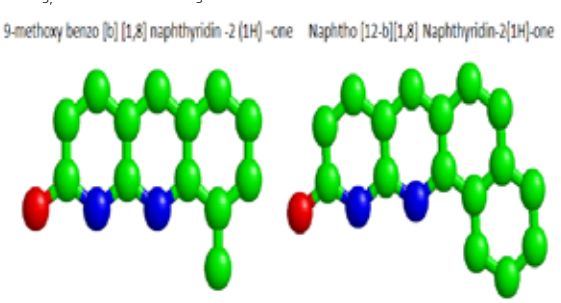


Figure1. *Synthesized ligand of 9-methoxy benzo [b] [1,8] naphthyridin -2 (1H) –one*

Studies on the chemistry of protein binding drugs like ligand are of ongoing interest due to their promising functions and biological activities, including their anti cancer properties and ability to regulate gene expression. Hence in the present study,the desired compound were synthesized and tested against lung cancer. Molecular docking is one of the tools to resolve this problem for Insilico analysis which paves the way for invitro and invivo analysis. Table.1 shows the binding energy for 9-methoxy benzo [b] [1,8] Naphthyridin-2(1H)-one with various human lung cancer protein such as ALK, BRAF, EGFR, KRAS,MEK1, RET, ROS1are listed below. Binding energy of the compounds were calculated using the following formula, Binding energy= A+B+C-D.Where, A denotes final intermolecular energy + van der Walls energy (vdW) + hydrogen bonds + desolvation energy + electrostatic energy (kcl/mol), B denotes final total internal energy (kcl/mol), C denotes torsional free energy (kcl/mol), D denotes unbound system's energy (kcal/mol). The synthesized compound has effective binding formation for except BRAF and MEK.. The protein ALK (-6.05kcl/mol), EGFR (-5.85 kcl/mol), KRAS (6.03 kcl/mol), RET (-5.63 kcl/mol) and ROS1 (-5.67 kcl/mol). In addition other parameters like inhibition constant (Ki) also determined. Inhibition constant is directly proportional to binding energy. RET protein shows highest binding energy of 74.93uM and lowest for MEK1 of 8.39uM, remaining listed in table1. The effective interaction of responsible hydrophobic amino acids and hydrogen bond binding amino acids also listed in table1 and figure1. The lowest binding energy and higher inhibition constant conclude that synthesized compound have a more active for lung cancer therapy.

Protein	Free Energy of Binding	Est Inhibition Constant Ki	Hydrogen Bond	Hydrophobic
ALK	-6.05	37.00µM	MET119, GLU119	LEU112, LEU125, LEU119, ALA114
BRAF	-6.39	20.55µM	CYS532	ILE463, VAL471, ALA481, LEU514
EGFR	-5.85	51.21µM	ASP855	VAL726, LEU788, MET766, ALA743
KRAS	-6.03	37.98µM	ASP119	PHE28, ALA146, LEU120, ALA18
MEK1	-6.93	8.39µM	MET146	LEU197, LEU74, MET143, ALA95
RET	-5.63	74.93µM	SER904, ASP898	TYR928, VAL899, VAL871
ROS1	-5.67	69.29µM	MET202	VAL195, LEU196, LEU202, LEU208, ALA197, LEU201

Table1. *Ligand binding factors of lung cancer protein*.

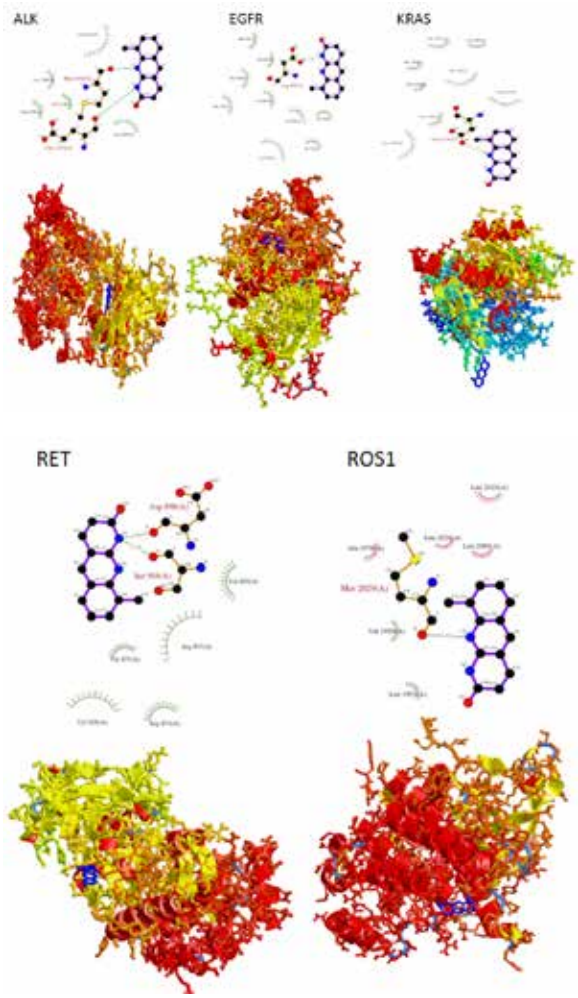


Figure2. *Molecular docking of ligand from selected protein molecules.*

INVITRO STUDIES

The cytotoxicity of various concentrations of the 9-methoxy benzo [b] [1,8] Naphthyridin-2(1H)-one were measured using the MTT assay. Concentrations of compound were 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0 and 100.0µg/mL and result are represented as Cell viability graph. To determine the cytotoxicity effect of the compound on A549 cells, IC50 value of compound was calculated from the cell viability graph as seen in Fig(3.b). IC 50 value of compound was found

49.24µg/mL for A549 cells. It can be concluded from this result that significant activity on A549 independant manner. The activity increases by increasing the dose.

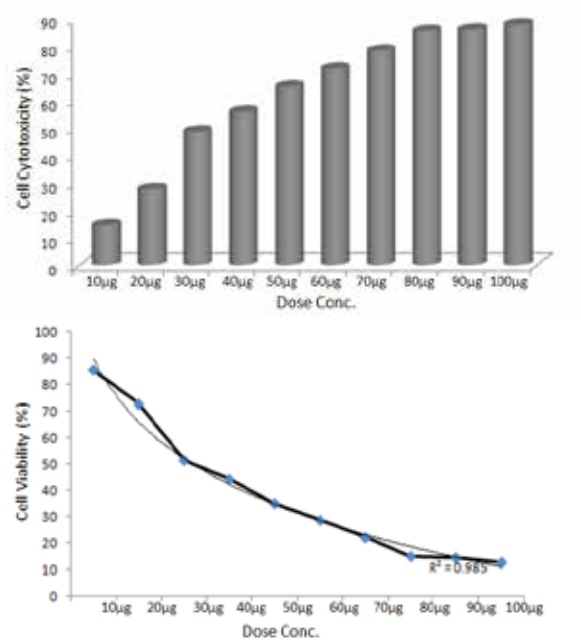


Figure3. MTT Assay for A549 cell a) % of cell cytotoxicity b) % of cell viability

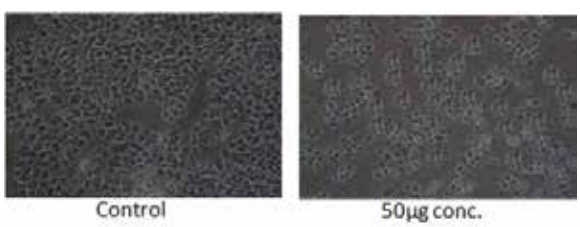


Figure4. Microscopic observation of A549 cell viability at 50µg concentration.

CONCLUSION

Overall, our study suggests that the 1,8-naphthyridine derivatives presented here have medicinal values and the basic framework of this class of heterocyclic compounds is an attractive template for the identification of novel potential antitumor agents. Among all the tested compounds, **2e** was found to have the highest inhibitory activity against A549 Cell lines with IC value of 49.24µg/mL. Further investigation in this area are currently under way of invivo action.

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