Jyothi A et.al IJPPA, 2018; VOL-02(01); 31-36

Original Research Article
ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

INTERNATIONAL JOURNAL OF PHARMACY AND PHARMACEUTICAL ANALYSIS

"SYNTHESIS AND ANTICANCER ACTIVITY OF SOME PYRAZOLONE DERIVATIVES: DESIGN AND DOCKING"

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ABSTRACT: A total of twelve pyrazolone nucleus-based derivatives **(PA1-PA6 & PC1-PC6)** were synthesized by a condensation of 4-acyl-5-pyrazolones with aromatic amines, and the aromatic aldehydes added to the parent moiety. All the synthesized carried out their insilico study as well as screened for their cytotoxicity activity. Among all the insilico score of the twelve compounds, the two (**PA2 & PC3**) were screened with the DLA cells to find out the cytotoxicity. They have shown a moderate activity at the concentration of 150 µg/ml due to the presence of electron with drawing groups 2, 4-dinitro and nitro moieties.

Key Words: Pyrazolone, Schiff base, Benzylidene, Anticancer activity, Molecular Docking

INTRODUCTION

The pyrazolone derivatives are organic compounds used as intermediates for synthesizing pharmaceuticals. It is an important nitrogen containing five-membered heterocyclic compound. The pyrazolone function is quite stable and has inspired chemists to utilize this stable fragment in bioactive moieties to synthesis new compounds possessing biological activities[1] Most of the therapeutic agents are synthesized with the help of pyrazolone nucleus. During recent years there have been some important developments in the biological activities of pyrazolone nucleus. These compounds have more beneficial significance in the field medicinal chemistry due to their remarkable pharmacological potentialities.[2] These compounds exhibit remarkable analgesic[3], antitubercular[4], antifungal[5], antibacterial[6], anti-inflammatory, antioxidant and antitumor activities[7]. Due to their easier preparation and rich biological activity, pyrazolone framework plays an essential role and represents an interesting template for combinatorial and medicinal chemistry.[8]

Pyrazolones are pharmacophores of numerous compounds display activities such as analgesic and antipyretic (propylphenazone, phenazone, metamizole etc.), anti-cancer (TELIN), anti-ischemic (edaravone), and anti-anxiolytic. Pyrazolones are gaining importance, especially in drug discovery

ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

programs towards cerebral ischemia and cardiovascular diseases. Due to its diverse pharmacological properties, the chemistry of pyrazolones is gaining attention, and there have been numerous novel methodologies reported recently. We describe here in our research findings in this area. The increasing clinical importance of anticancer drugs has emphasized the toxicity inherent in their use, the evaluation and modification of the untoward effects have become a matter of some concern. Here in this research work the pyrazolone activity as anticancer agent has been preliminary explored by treating the DLA cells. Cancer is now becoming a main area of concern in human life. Cancer is a major health issue, despite the advancement in the healthcare sector. The present-day research is aimed at identifying synthetic and natural molecules to be used in the prevention or treatment of cancer. Chemotherapy is considered as the most effective method, among many other methods prevalent, to treat cancer.[9]

Molecular docking was performed on a diverse set series of pyrazolone derivatives and identified a lead molecule from the set of databases. The poses and docked scores for the respective lead molecules were compared with the standard scaffold and have been reported. The enzyme used for the insilico study of cancer are PIK3. Phosphoinositide-3-kinase (PI3K). The phosphoinositide 3-kinase (PI3K) pathway, a critical signal transduction system linking oncogenes and multiple receptor classes to many essential cellular functions, is perhaps the most commonly activated signaling pathway in human cancer. This pathway, thus presents both an opportunity and a challenge for cancer therapy. Even as inhibitors that target PI3K isoforms and other major nodes in the pathway, including AKT and mTOR reach clinical trials, major issues remain.[10]

EXPERIMENTAL

Cytotoxicity studies: The Dalton's ascites lymphoma (DLA) cell lines are maintained at the Amala Cancer Research Institute and propagated into transplantable tumors in the peritoneal cavity of female Swiss albino mice. The freshly aspirated cells from the mouse peritoneum were washed with phosphate-buffered saline (PBS) under sterile conditions and their concentration was determined using a hemocytometer before transplantation. DLA cell lines were maintained in mice models.[11] The cells were aspirated, washed thrice in normal saline counted using a haemocytometer and cell suspension of 1million cells/ml was prepared. One ml of this suspension was injected into peritoneal cavity of swiss albino mice. Lung fibroblast - Mouse L929 cell lines[12] were used for long term in vitro cytotoxicity experiments. Short term in vitro cytotoxicity assay by trypan blue dye exclusion technique. Any compound, which is cytotoxic to cells, inhibits the cell proliferation and kills the cells. Trypan blue has the ability to penetrate into the dead cells and give it a blue color. This method gives an exact number of dead and viable cells.[13]

The cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tubes containing only cell suspension. These assay mixtures were incubated for 3h at 370 ˚C and then 1ml of trypan blue was added after incubation and the number of dead cells was counted using a haemocytometer (**Fig 1)**.

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IP INDEX Impact Factor is 2.608

Percentage cytoxicity = (No.of dead cells / Total no. of cells) × 100

MATERIALS AND METHODS: In the present research study, the molecular docking methodology was implemented by autodock 1.5.6 and MGL tools 1.5.6 packages (ADT; The Scripps Research Institute, Molecular Graphics Laboratory, 10550 North Torrey Pines Road, CA, 92037). Construction and the energy minimization were done with Chemdraw ultra 8.0 and chem3D ultra 8.0 (Cambridge soft. Com, 100cambridge park drive, Cambridge, MA 02140, USA). In this study, autodock 1.5.6 were used to establish a legend-based computer modeling algorithm for the prediction of binding energy and calculation of inhibition constants of the designed antiviral scaffold with the NS5 protein. The hardware used for this study with the Lenovo brand and the processor of Intel (R) Pentium (R) CPU N3710 @ 1.60 GHZ with 4.00 GB RAM, by 64 bit operating systems.

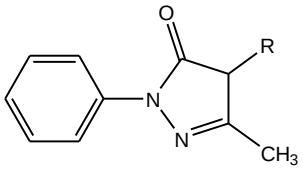
Preparation of enzyme

Crystallographic model of PIK3 gamma inhibitor protein **(3l54)** was retrieved from www.pdb.org. Within pdb format, therefore the docking tool can assess it. Macromolecule preparation was finished with the marginal options accessible within the autodock tools 1.5.6. Water molecules were deleted, hydrogens were added using polar, assigned AD4 type atoms, Gasteiger charges were added and eventually saved it as macromolecule.

Preparation of ligand: All the designed ligands were built, 3D optimized and energy minimized using ChemDraw Ultra 8.0 version and saved in pdb formats, which is compatible input file for the autodock tool. The ligands were then imported in ADT for assigning charge and torsion. Finally, it is saved as ligand.

Docking methodology

In the current docking methodology, the torsional freedom of the protein was determined and saved it as a pdbqt format for the further docking procedure. The receptor grids developed by using 60×60×60 grid points in xyz (32.563,18.812,-16.457 respectively) with grid spacing of 0.375Å. The parameter file was saved as protein.gpf and .glg file was developed by autogrid4. Docking parameter was created with the parameters of the Lamarckian genetic algorithm was used for all molecular docking simulations. Population size of 150, 10 GA runs, GA crossover rate of 0.8 and genetic mutation of 0.02 with a maximum number of generations of 27000 were set as the parameters. The parameter file was saved it as ligand.dpf and docking simulations were done using autodock4. Analyze option in autodock 1.5.6 was used to analyze the docking. Finally, the scoring of binding energies determines the good molecule, lesser the energy, better the conformation.



RESULTS AND DISCUSSION

The insilico study reveals that the designed pyrazolone derivatives have anticancer effect on the phosphoinositide 3-kinase inhibitor. These are family of enzymes involved in the cellular functions such as cell growth, proliferation, differentiation, motility, and intracellular trafficking which inturn is included in cancer [14]. The autodock, docking study gives a result that all the

ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

designed molecules are having activity against the enzyme group PIK3. The autodock results between the interaction of PI3K gamma inhibitor with the designed pyrazolone moieties has given an outcome, that the -8.51K cal/Mol and 573.8 µM for the **PC3.** On the other hand, the Schiff base derived pyrazolone **PA2** nucleus shows a binding energy of -8.42 Kcal/Mol and 67.55 µM as inhibition constant.

Thus, based on the above study result these two samples were given for the cytotoxicity studies in the DLA cell lines. The result of this study pointed out that both the derivatives have the activity but the Schiff base derivative of pyrazolone has better activity than the benzylidene based pyrazolone nucleus (**Figure 2**).

CONCLUSION

It was also noticed that the compound showed a moderate cytotoxicity effect on the tumor cell lines (DLA) at its lower concentration. Cancer still remains a main threat to human health; it is a major cause of death worldwide. Therefore, it is the peak time for the development of highly efficous, and less toxic antitumor drugs for the betterment of humanity. The Schiff base substituted pyrazolone nucleus was found to possess a moderate cytotoxicity effect. While the benzylidene based pyrazolone have activity, but not so effective. The research reveals that the modification of the same nucleus with nitrogen bases would increase the antineoplastic activity well in advance in near future.

Table 1. Percentage cytotoxicity studies

Concentration (µg/ml)	Percentage cell death (%)	
	PA2	PC3
10	4	0
20	10	2
50	19	7
100	25	14
150	38	30

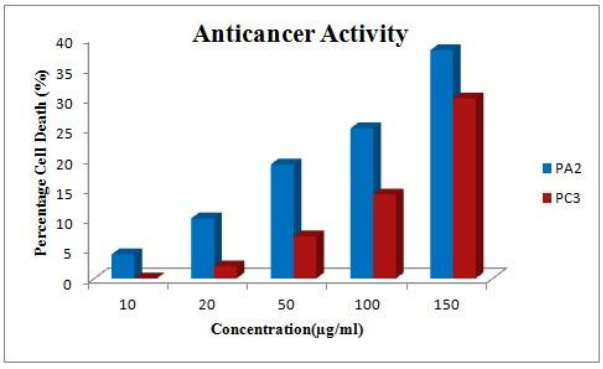
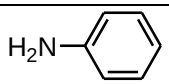
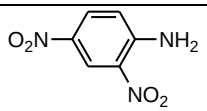
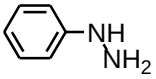
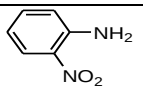
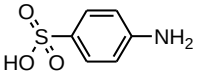
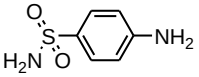
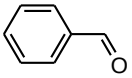
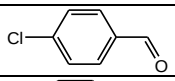
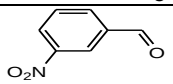
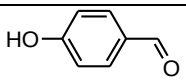
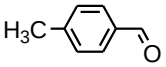
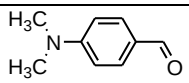


Fig 1: Anticancer activity of PA2 & PC3 Against DLA cell lines

ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

Table 2: Docking results of designed derivatives

Sl. No	Compd. Code	R	Binding energy (Kcal/Mol)	Inhibition constant	Hydrogen
1.	**PA1**		-6.63	13.72	0
2.	**PA2**		**-8.42**	**673.55***	**1**
3.	**PA3**		-6.55	15.71	2
4.	**PA4**		-8.39	714.02*	0
5.	**PA5**		-7.76	2.04	1
6.	**PA6**		-6.7	12.29	1
7.	**PC1**		-8.25	900.13*	0
8.	**PC2**		-7.76	2.06	1
9.	**PC3**		***-8.51***	***573.8****	***0***
10.	**PC4**		-7.92	1.57	0
11.	**PC5**		-8.07	8.07	0
12.	**PC6**		-6.48	17.82	1
13.	**Standard**	-	-7.17	5.56	0
* Nanomole					

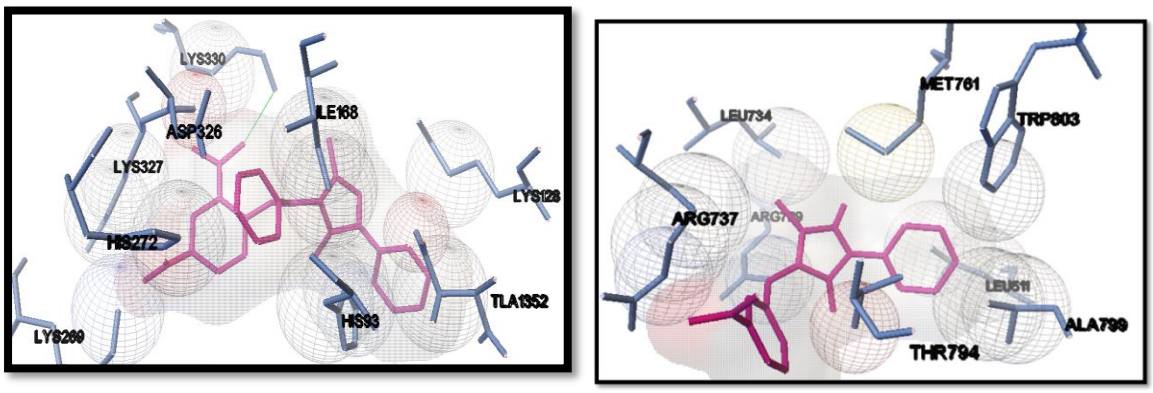


Figure 2: Docking pose of PA2 & PC3 against PIK3 enzyme.

ISSN: 2394-1618
IP INDEX Impact Factor is 2.608

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