Indian Journal of Heterocyclic Chemistry
Vol. 32 - Number 03 (Jul-Sep 2022) 393-399
**DocID:** https://connectjournals.com/01951.2022.32.393

ISSN (Print) : 0971-1627
ISSN (Online) : 2456-4311

# Rationale Design, Synthesis, Cytotoxicity Evaluation and Molecular Docking Studies of 3-Chloro-4-aryl-1-(phenazin-7-yl) Azetidin-2-ones Analogues

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**ABSTRACT** 2-phenazinamines (3-chloro-4-aryl-1-(phenazin-7-yl) azetidin-2-ones) possess a broad spectrum of biological activities. They were reported as antineoplastic agents. Hence it is of great interest to design newer 2- phenazinamine derivatives (3-chloro-4-aryl-1-(phenazin-7-yl) azetidin-2-ones) by molecular docking studies to evaluate their anticancer potential. The 2-phenazinamine derivatives (**4a-e**) were prepared with the reduction of 2, 4-dinitrodiphenylamine by adding tin chloride and catalytic agents (nitrobenzene and magnesium sulfate) fallowed by cyclization process, The *in-vitro* cytotoxic activity of all compounds (**4a-e**) carried on K562 human chronic myelogenous leukemia cell line by employing 3-(4,5-dimethylthiazol-2-4yl)-2,5-diphenyl tetrazolium bromide assay method. Molecular docking studies were done by using docking study for the assessment of their anticancer potential. All five compounds (**4a-e**) showed the best and lowest the binding energy scores among all of the ligands. Compound 4c showed binding energy of −7.99. Amongst the two series of 2- phenazinamines.

**KEY WORDS** Anticancer, Drug design, K562, Phenazinamines

**How to cite this article:** Sonwane, G., Kale, M., Khan, S. Rationale Design, Synthesis, Cytotoxicity Evaluation and Molecular Docking Studies of 3-Chloro-4-aryl-1-(phenazin-7-yl) Azetidin-2-ones Analogues, *Indian J. Heterocycl. Chem.*, **2022**, *32*, 393–399. (*DocID: https://connectjournals.com/01951.2022.32.393*)

## INTRODUCTION

Since the past few years, there is a surge to develop anticancer agents because cancer has been now considered a veritable health issue. All over the world and it is also one of the important causes of mortality.[1] Thus, in the earlier, investigators have been facing problem to find viable clinical approaches for the management of cancer and to look for novel antineoplastic agents.[2] Aside from the utility of surgical operations and radiation therapy in past years, chemotherapy remains a significant alternative for the treatment of cancer in the clinical.[3] Unfortunately, it is faced with the problem that most of the chemotherapeutic agents lack tumor specificity.[4] However, current anticancer research is utilizing the known fact that the malignant cells are endogenous and almost lacking in specific metabolic properties.[5] Enzyme or protein plays important role in the development of cancer.[6] Docking strategies can trap this particular enzyme or protein, which is responsible for these diseases condition.[7] Due to the reason of this specificity, target (enzyme) specific drugs play an important role. The phosphorylation of tyrosine residues in turn causes a change in the function of the protein that they are contained in Phosphorylation at tyrosine residues controls a wide range of properties in proteins.[8] Furthermore, Tyrosine kinases cascade the signal transduction to the nucleus cytoplasm via cell membrane where the genes are modified. Finally, transmutations will cause some aminoalkanoic acid kinases to become constitutively active, an eternal purposeful state that will contribute to the initiation or progression of cancer.[9]

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**Table 5: The dockings study performed in research laboratory clearly indicates the better binding efficacy of phenazinamine derivatives in comparison to the standard drug doxorubicin**

| Sr. No | Standard Compound | Test Compound |
|---|---|---|
| Compound Name | Doxorubicin | 4c |
| PBD Code | 3IK | 3IK |
| Enzyme | BCR | BCR |
| Binding Energy | -8.79 | -8 |
| Ligand Efficiency | -0.24 | -0.3 |
| Inhibitory Constant | 359.85 | 1.37 |
| Internal Energy | -10.67 | -8.73 |
| VDW Energy | -8.74 | -7.37 |
| Electrostatic Energy | -1.93 | -1 |
| Total Internal Energy | -1.23 | -0.98 |
| Torsional Energy | 2.09 | 0.89 |
| Unbounded Energy | -1.02 | -0.89 |
| Hydrogen Bonding | 1 | 1 |

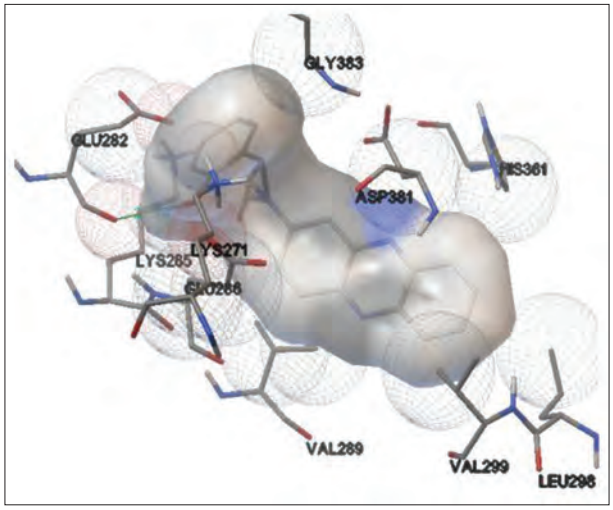


**Figure 5: Docking validation structures 4 series (4c) against 3IK3**

multiplication rate and cell suitability when metabolic occasions lead to necrosis. The assay was conducted using the K562 cancer cell line. The lines were prepared and cryopreserved in ampoule using reagents such as DMSO, which preserves the cells during freezing. The frozen ampoule was bought to at temperature 37°C by slow agitation and transferred into the water bath. It was rapidly warm up until it liquefied. The solution was centrifuged at 3000 r.p.m with normal saline for 10 min to remove the DMSO. The saline was discarded and an aliquot was used for further experiments.[18] Cells from specific cell lines in the log period of development were trypsinized and cell viability was checked through hemocytometer. Density was adjusted in suitable medium and inoculated in multiwall-plates (usually 96 well microtiter plates). These cells were preserved at temperature 37°C in 5% $CO_2$/95% humidified air for 3 days with different concentrations. MTT was added (5 mg/ml) into each well and incubated for 4 h [**Figures 3 and 5**].[19]

## Antimitotic activity

The results of antimitotic assay are tabulated in Table 4. The model used for this activity was *A. cepa* root tip meristem model[18] in which onion bulbs were cleaned and kept with root tips were kept into the beaker containing distilled water till the tips grew up to 2–3 cm. Then these bulbs were expelled from the water and put on a layer of tissue paper to remove excess of water. The solutions were divided into five groups viz., the first group served as control (DMSO) 0.6 ml and distilled water volume adjusted to 600 ml), the second group (synthesized compounds) of 4 series 4a, 4b, 4c, 4d, 4e (10 µg/mL). The selection criteria of these mentioned compounds of all the series were done on the basis of results of toxicity assessment studies and antioxidant activity and the fifth group methotrexate (10 µg/mL) was used as a standard drug. The grown root tips were dipped into these solutions mentioned above and were stored at temperature 25 ± 2°C for 96 h of direct daylight. The test sample was changed day by day with new ones. The length of roots developed during incubation (recently showing up roots excluded), root number and the mitotic index were recorded after 96 h. The percent of root growth inhibition was calculated by, percent of root growth inhibition = Control – Test × 100. The $EC_{50}$ value) was calculated by plotting treatment concentration vs mean of root length as percentage of the water control group. $ID_{50}$ values of the synthetic derivatives for antimitotic activity are tabulated in.[20,21]

## Molecular docking

The phenazinamine derivatives were further subjected to docking studies for exploring their potential as Bcr-Abl tyrosine kinase inhibitors using the Autodock 4.2 version. Auto Dock helps the ligand to dock into cavity of action site. 2D structures of the active ligands were drawn using chemdraw software (2D Ultra 8.0), converted into 3D, and saved in PDB format and it was optimized using semi-empirical method called MOPAC.[22] The structure of Bcr-Abl tyrosine kinase (PDB id: 3IK3) was downloaded from RCSC Protein Data Bank [**Figure 5**]. Polar hydrogen atoms and water were added, which did not participate in interactions. The results were found in the form of the most favorable free energy of binding in kcal/mol. PYMOL was used for the binding interactions between protein and ligands. OSIRIS property explorer was to predict the molecular properties of the designed compounds as mentioned in **Table 5**.[23]

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*Received: 20 Aug 2019; Accepted: 20 Apr 2021*