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Research article

Cytotoxic activity and molecular docking studies of 9*H*-pyrido[3,4-b] indole derivatives

Alivelu Samala*[1,3], Srinivasa Murthy M[2], Krishna Mohan Gottumukkala[3]

[1]Department of Pharmaceutical Chemistry, Holy Mary Institute of Technology and Science-College of Pharmacy, Bogaram-Ghatkesar Rd, Kondapur, Telangana, India, 501301.
[2]Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical sciences, Near Ramoji Film City, Deshmukhi, Yadadri-Bhuvanagiri, Telangana, India, 508284.
[3]Centre for Pharmaceutical Sciences, Inst of Science and Technology, JNT University, Hyderabad, Kukatpally, Telangana, India, 500085.

***Corresponding Author: Alivelu Samala**

ABSTRACT

In the current study, we report the in vitro cytotoxic activity and molecular docking studies of a 9*H*-Pyrido[3,4-b]indole derivatives. Molecular docking studies were performed to determine the protein-ligand interactions. All the compounds were docked with EGFR tyrosine kinase and the data reveals that all the synthesized compounds have good binding affinity ranging from -4.46 to -3.01 Kcal/mol. Further, Cytotoxic activity was tested by MTT assay against human breast cancer cell lines (MCF7) and human cervical cancer cell lines (HeLa), using Cisplatin as a standard drug. All the tested compounds showed IC_{50} values in the range of 12.81±0.43 µg/ml to 31.27±0.23 µg/ml and 20.43±0.25 µg/ml to 37.28±1.02 µg/ml against MCF7 cell lines and Hela cell lines respectively.

Keywords: Cytotoxic activity, Molecular docking, 9*H*-Pyrido[3,4-b]indole, EGFR tyrosine kinase, MTT assay.

INTRODUCTION

One of the leading causes of mortality and morbidity across the world is cancer. It has a major impact on society. More than 27% deaths are caused by cancer worldwide every year. Around 20 million people are affected by cancer annually and average 9.5 million cancer deaths have been observed each year worldwide [1]. Current management of cancers is based on various principles ranging from chemotherapy to radiotherapy and also extends to surgical management depending on type and severity. Although plethora of research has been done in this area, yet a lot of challenges have been faced by researchers in order to address this devastating clinical problem. One such challenge that has led to failure of the early success of research in this area is 'drug resistance'.

The main complication of drug resistance is highly proliferating extrinsic as well as intrinsic factors. The research of drug resistance in cancer is mainly focused on the cellular resistance which is primarily accounted by the genetic makeup of the cancerous cells that may develop mutations because of the toxic chemotherapy in case of sensitive tumors and secondly, due to the adaptive response such as increased expression of the therapeutic target and activation of alternative compensatory signaling pathways. The primary method for identifying mechanisms of drug resistance is to subject the surviving cancer cells to the cytotoxic drugs and then identify the altered genes using various cellular and molecular biology techniques.
Consequently, three major mechanisms of drug resistance were identified i.e. the decreased uptake of hydrophilic anticancer drugs such as nucleoside

analogs, folate antagonists and cisplatin that require membrane transport proteins to enter cell; second, increased energy dependent efflux of hydrophobic drugs out of the cells; and third, changes in cellular events such as alterations in cell cycle, reduced apoptosis, increased repair of DNA damage and altered metabolism of drug eventually, decrease the capacity of cytotoxic drugs to kill the cancer cells. The problem of resistance leads to the remission of cancer, thereby increasing the burden on society as well as patient's mental health [2-3].

A combination therapy is the current status of cancer therapeutics as it prevents development of resistance and is more effective than a single drug. Cancer progenitor cells are often drug resistant and may lead to remission, hence the elimination of such progenitor should be the next step towards cancer elimination [3]. It is indispensable to continue research in the direction of understanding the underlying mechanisms of cancer drug resistance and to identify therapies that can treat cancers without causing threat of resistance. Meanwhile various new modalities can be explored further by using existing drugs. A 9*H*-Pyrido[3,4-b]indole (β-Carboline) alkaloid was isolated in 1841 from the plant species *Peganum harmala*, obtained from the region of Middle East, North Africa and Northwest China, are used as abortifacient emmenagogue however, in some region of South America, they are utilized as hallucinogenic drink or snuffs [4]. Chemically, β-Carbolines are a prototype of indole alkaloids containing tricyclic pyrido[3,4-*b*]indole ring in their structure. These alkaloids are primarily categorized on the basis of nitrogen containing six membered ring. Various reported analogs contain different substituents on the Pyrido ring or indole ring in these tricyclic compounds [5]. The major uses of these compounds were observed in the treatment of alimentary canal cancer [6], which has helped these compounds to be identified as major scientific leads in the synthesis of various anticancer agents [7-8]. Major bioactive components of various carbolines alkaloids with saturated or unsaturated tricyclic ring system were isolated and characterized from numerous terrestrial plants [9, 10]. β-Carbolines also possess broad range of biological activities including sedative [11], anxiolytic [12], hypnotic [13], anticonvulsant [14], antitumor [15], antiviral [16], antihypertensive [17], antibacterial [18]. Currently, various reports are being published on using β-Carbolines promising candidates for the treatment of cancer [18]. In the present scenario *In silico* studies plays an important role in drug design due to its ability to predict the binding conformation of small molecule ligands to the appropriate target binding site, considering its importance present article reports the molecular docking studies and cytotoxic activity of title compounds.

MATERIALS AND METHODS

All of the reagents were purchased from commercial suppliers. Synthesis and antibacterial activity of a series of title compounds were previously reported [19].

Molecular Docking studies

Accession of ligand and protein

To execute molecular docking studies, at first, the ligand structures of 9*H*-Pyrido[3,4-b]indole derivatives (PID1-PID12) were generated using Chem Draw 12.0 software in which 2D structure was converted into 3D structure and energy minimization was carried by using MM2 tools in Chem Draw software for each ligand. The structures were saved as .sdf format then changed into.pdb format to .pdbqt format by using PyMOL and Autodock Tools 1.5.7 respectively. The crystal structure of Epidermal growth factor receptor tyrosine kinase (PDB ID:1M17) with resolution of 2.60A° was accessed from protein data bank (http:/www.rcsb.org). For preparation of protein, water molecules were deleted, polar hydrogen's were added and kollman united atom charges were assigned by using Autodock Tools1.5.7 and saved as .pdbqt format. Molecular docking was carried out by Autodock Vina 1.5.7. Receptor-ligand interactions were visualized by using discovery studio 2021 and PyMol softwares

Evaluation of cytotoxic activity

Pyrido-indole derivatives with high binding affinity were tested for their cytotoxic activity in vitro against human breast cancer cell lines (MCF7) and human cervical cancer cell lines (HeLa) by using MTT assay [20]. Cell viability of test compounds were determined on the basis of measurement of in vitro growth inhibition of cell lines by cell mediated reduction of tetrazolium salt to form water insoluble formazan crystals. Briefly, the exponential growing cells were harvested and plated ($1X10^4$) in 96-well microtiter plates and grown for a period of 24 h. The cells were treated with different concentrations of test compounds and incubated for 48 h. Later, the cells were incubated again for 3 h with 250 µg mL^{-1} of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. After incubation, the medium was replaced with 100 µL of DMSO and the absorbance was measured at 570 nm. The IC_{50} values of the compounds were calculated from the dose response curves. Cisplatin was used as a standard drug. Each experiment was performed in triplicate and the IC_{50} values were expressed in mean ± SEM.

RESULTS AND DISCUSSIONS

Molecular docking studies

Molecular docking studies were performed to determine the protein-ligand interactions. All the 10 compounds (PID1-PID10) were docked into the active site of Epidermal growth factor receptor tyrosine kinase to gain more insight on their mode of binding. Docking results revealed that all the synthesized compounds displayed good binding affinity to the target enzyme ranging from -4.46 to -3.01 Kcal/mol (Table 1). Among the docked compounds, PID8 showed highest binding affinity for 1M17. Figure 1 shows the 3D and 2D receptor interactions of the PIDs.

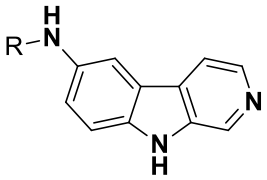
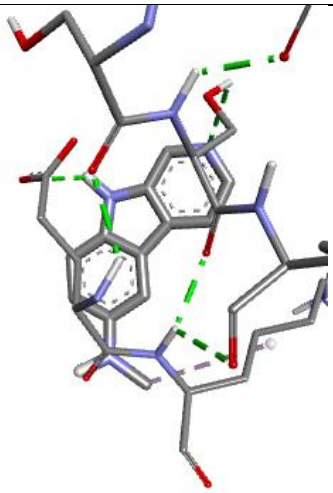
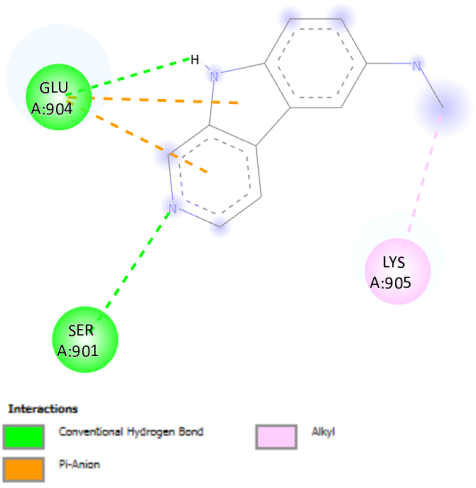
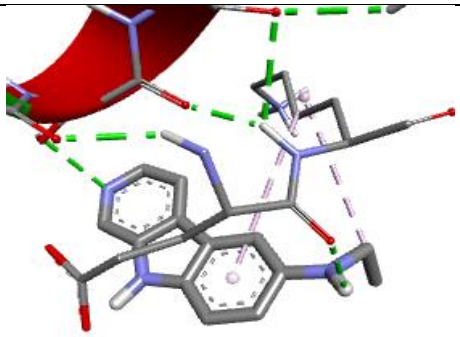
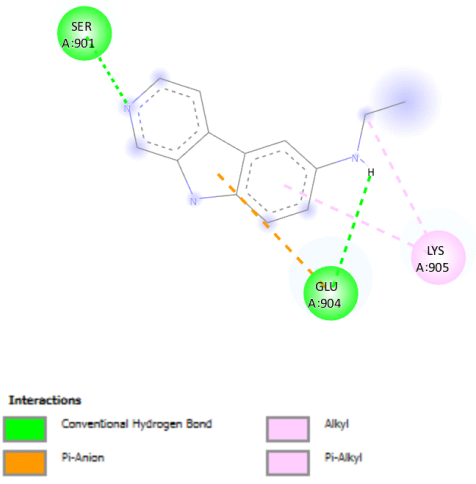
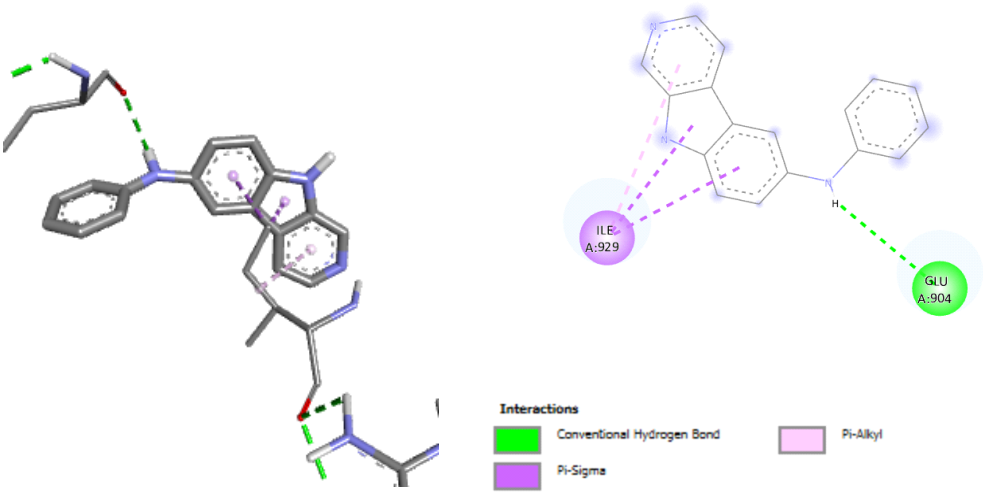


Table 1: Molecular Docking with EGFR protein (PID1-PID10)

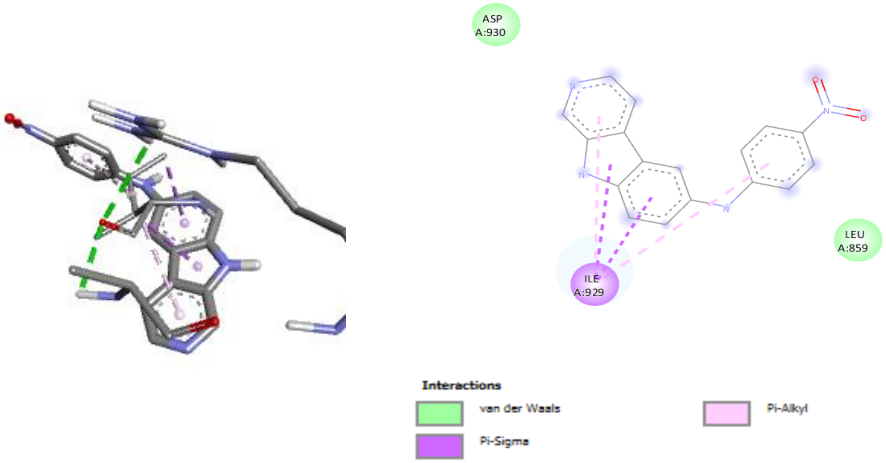
Compound	R	Dock Score(K cal/mol)	No of H-bonds	Interacting amino acids	Hydrogen bond distance (Å)
PID1	$-CH_3$	-3.75	2	GLU 904 SER 901	2.96 2.88
PID2	$-CH_2CH_3$	-3.51	2	GLU 904 SER 901	2.43 2.77
PID3	$-CH_2CH_2CH_3$	-3.01	1	GLU 904	1.95
PID4	$-CH(CH_3)_2$	-3.25	2	GLU 904 SER 901	3.07, 2.71 2.84
PID5	$-CH_2CH_2CH_2CH_3$	-3.16	1	SER 901	2.85
PID6	$-CH_2CH(CH_3)_2$	-3.17	1	SER 901	2.91
PID7	$-C_6H_5$	-4.30	1	GLU 904	2.87
PID8	$-C_6H_4$ (4-NO_2)	-4.46	0	-	-
PID9	$-C_6H_4$ (4-CH_3)	-3.33	1	GLU 904	2.35
PID10	Furfuryl	-4.12	1	SER 901	2.83

Compound	3D interactions	2D interactions
PID1		
PID2		

PID7



PID8



PID10

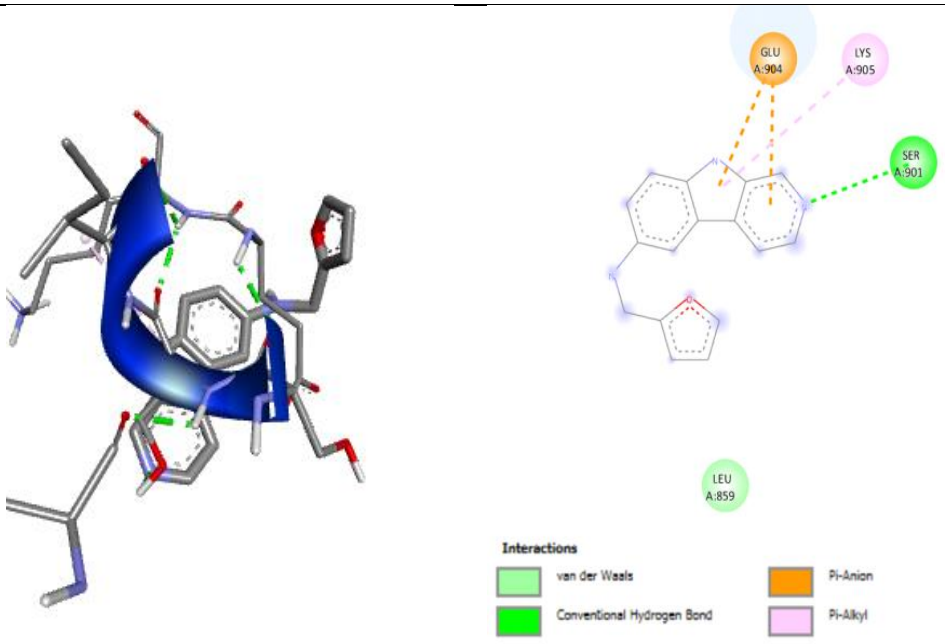


Fig 1: 3D and 2D receptor interactions of the Pyrido-indole derivatives

In vitro Cytotoxic activity

Novel Pyrido-indole derivatives were evaluated for cytotoxcicity against human breast cancer cell lines (MCF7) and human cervical cancer cell lines (HeLa) by MTT assay, using cisplatin as standard drug. Results (Table 2) proposed that both MCF7 cell lines and HeLa cell lines were susceptible to the evaluated compounds. Tested compounds showed IC_{50} values in the range of 12.81±0.43 µg/ml to 31.27±0.23 µg/ml against MCF7 cell lines and 20.43±0.25 µg/ml to 37.28±1.02 µg/ml against HeLa cell lines. Among the tested compounds, PID1 and PID2 showed highest activity with IC_{50} values 12.81±0.43 µg/ml and 16.25±0.15 µg/ml against MCF7 cell lines respectively. Remaining all other compounds showed moderate activity against both the cell lines.

Table 2: Cytotoxic Activity of 9*H*-Pyrido[3,4-b]indole derivatives against MCF7 and HeLa Cell lines

Compound No.	-R	IC_{50} values (Mean ± SEM) µg/ml[a]	
		MCF7[b]	HeLa[c]
PID1	$-CH_3$	12.81 ± 0.43	25.03 ± 0.26
PID2	$-CH_2CH_3$	16.25±0.15	20.43 ± 0.25
PID7	$-C_6H_5$	29.74 ± 1.42	25.91 ± 0.63
PID8	$-C_6H_4$ (4-NO_2)	31.27 ± 0.23	26.62 ± 0.41
PID10	Furfuryl	29.72 ± 1.82	37.28 ± 1.02
Standard (Cisplatin)	-	1.85±0.23	1.02±0.03

[a]Cytotoxic concentration or compound concentration required to reduce viability of MCF7 & and HeLa cell lines by 50% using MTT assay.
[b] Breast cancer cell lines, [c] Cervical Cancer cell lines

CONCLUSION

In silico molecular docking studies of new 6-Substituted pyrido-indole derivatives (PIDs) were performed with protein 1M17. Results revealed that all the compounds had a good binding affinity. Further, in vitro cytotoxic studies showed good to moderate cytotoxic activity. Specifically, presence of alkyl groups on amine nitrogen attached to Pyrido-indole ring at 6th position (PID1 & PID2) showed good activity than aryl groups containing compounds (PID7 & PID8).

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