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QUINOXALINE DERIVATIVES: DUAL INHIBITION OF MTOR AND PI3K PATHWAYS IN CANCER CELLS

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Abstract

Purpose: mTOR and PI3K are crucial enzymes that regulate cell growth, survival, and metabolism. Dysregulation of these enzymes is linked to various cancers, including breast, lung, prostate, and colorectal cancer. Targeting mTOR and PI3K simultaneously could be a promising strategy to overcome drug resistance and enhance anticancer efficacy.

Methods: Quinoxaline derivatives were synthesized using commercially available 2,3-dichloroquinoxaline and substituted benzene sulfonamide. Molecular docking studies were conducted using Auto Dock Vina, Chimera, and BIOVIA Discovery Studio to evaluate the binding affinity with the active sites of mTOR and PI3K. The in vitro anticancer activity of the synthesized compounds was assessed against human cancer cell lines: MCF-7 (breast cancer), HCT-116 (colon cancer), and HepG-2 (liver cancer) using the MTT assay.

Results: The synthesized compounds exhibited characteristic peaks of the quinoxaline ring at 1600–1500 cm−1 and 1400–1300 cm−1 in IR spectra. The (^1H) NMR spectra showed signals of aromatic protons at 7.0–8.5 ppm and aliphatic protons at 0.8–4.5 ppm. Compound 5 demonstrated the highest binding affinity for both mTOR (−8.4 kcal/mol) and PI3K (−7.6 kcal/mol). Additionally, Compound 5 exhibited the lowest IC50 values for the cell lines, ranging from 0.89 to 1.12 μM, indicating its potential as an effective anticancer agent.

Conclusion: This study provides new insights into the development of quinoxaline-based dual inhibitors of mTOR and PI3K as novel anticancer therapeutics

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Keywords: Mtor, Pi3k, MCF-7 (breast cancer), HCT-116 (colon cancer), and HepG-2 (liver cancer)

Introduction

Cancer is a leading cause of death worldwide, affecting millions of people annually. Despite advances in diagnosis and treatment,[1] many cancers remain incurable or resistant to traditional therapies. There is an urgent need to develop new and effective anticancer agents that can target multiple pathways involved in tumor growth and survival. One promising strategy is to inhibit the phosphatidylinositol 3-kinase (PI3K) and the mammalian target of rapamycin (mTOR) signaling pathways, which are frequently dysregulated in various types of cancer. [2,3] These pathways are key regulators of cell proliferation, metabolism, angiogenesis, and apoptosis, and their abnormal activation contributes to tumor initiation, progression, and resistance to chemotherapy and radiotherapy [4,5]

However, the clinical use of single inhibitors of PI3K or mTOR has been limited by drawbacks such as low efficacy, toxicity, feedback activation, and the emergence of resistance. Therefore, dual inhibitors of PI3K and mTOR have been developed as a more rational approach to overcome these limitations and achieve better

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anticancer outcomes. [6-10] Among the various structures used for designing dual inhibitors of PI3K and mTOR, quinoxaline has emerged as a versatile and potent core structure that can bind to both enzymes with high affinity and selectivity. Quinoxaline is a heterocyclic compound [11-13] that consists of two fused benzene rings with two nitrogen atoms at positions 1 and 4. Quinoxaline derivatives have shown various biological activities, such as antibacterial, antiviral, antifungal, anti-inflammatory, antimalarial, and anti-cancer. [14-16]

Quinoxaline-based dual inhibitors of PI3K and mTOR have demonstrated potent anticancer activity in vitro against various cancer cell lines. [17-19] Quinoxaline is very useful for making different kinds of medicines and chemicals. Some of these medicines and chemicals can help fight against cancer. For example, some can block an enzyme called HDAC, which controls how genes are turned on and off in the cells. [20-21] Others can interfere with proteins or molecules important for cancer cells to survive and multiply, or even damage the DNA of cancer cells, which is the blueprint for making new cells. Some quinoxaline medicines and chemicals are already used as antibiotics and anticancer drugs, such as olaquindox, echinomycin, atinoleutin, levomycin, and carbadox. Others are still being studied and developed to determine if they are safe and effective for treating human cancer.[22]

Materials and methods:

Quinoxaline derivatives were synthesized using commercially available 2,3-dichloroquinoxaline and substituted benzene sulfonamide The molecular docking studies were performed using Auto Dock Vina software. The crystal structures of proteins were retrieved from the Protein Data Bank (PDB): PI3Kγ p110 with PDB Id, 3L54 and mTOR with PDB Id, 4JT6 respectively. The kinase domain of mTOR (residues ranging from 1867 to 2436) was considered in the study and used for all analyses. Both of the retrieved structures were co-complex structures with bound ligands (PI3Kγ with bound LXX, mTOR with bound PI-103), and these bound ligands were used as clues for catalytic site grid generation in molecular docking. The pre-processing of proteins and ligands, called structure preparation, required as input for docking was performed by Chimera v.1.6.2. The chemical compounds were modified using Marvin Sketch v.18.4, ChemAxon, and ChemDraw. The docking results were ranked according to the binding affinity, and the lowest energy conformation was selected for each ligand. The binding interactions were visualized and analyzed using PyMOL and Biovia Discovery Studio software.[23]

The in vitro anticancer activity of the synthesized compounds was evaluated against three human cancer cell lines: MCF-7 (breast cancer), HCT-116 (colon cancer), and HepG-2 (liver cancer) using the MTT assay.[24] The cell lines were obtained from the National Center for Cell Sciences (NCCS) Pune and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were seeded in 96-well plates at a density of 5 × 10^3 cells/well and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO2. The medium was replaced with fresh medium containing various concentrations of the test compounds (0.01–100 µM) and incubated for another 48 h. The control wells received only the medium and the solvent (DMSO). The cell viability was determined by adding 20 µL of MTT solution (5 mg/mL) to each well and incubating for 4 h. The formazan crystals were dissolved in 150 µL of DMSO, and the absorbance was measured at 570 nm using a microplate reader. The IC50 values were calculated by plotting the percentage of cell viability versus the logarithm of the compound concentration.[25] The experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

Results and discussion:

The synthesis of quinoxaline derivatives was conducted using commercially available 2,3-dicloquinoxaline and Substituted Benzene Sulfonamide. The products' structures were confirmed through IR, 1H NMR, 13C NMR, and mass spectra analysis.

Molecular docking studies were carried out using AutoDock Vina, Chimera, and Biovia Discovery Studio to assess the binding affinity and interactions of the synthesized compounds with the active sites of Mtor and Pi3k. The docking results are displayed in Table 1, Figure 1, and Figure 2. It is known that the lower the binding affinity value, the stronger the binding of the ligand to the protein. Compound 5 exhibited the highest binding affinity for both Mtor (−8.4 kcal/mol) and Pi3k (−7.6 kcal/mol), indicating that it is the most potent inhibitor of both enzymes. Compound 5 formed hydrogen bonds with the key residues of Mtor (Lys833, Asn951, and Asp964) and Pi3k (Glu332 and Ser-333), as well as hydrophobic and π-π interactions with other residues. Compound 4f also s howed a good fit in the binding pockets of both proteins.

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Table 1: Presence of Docking Result Analysis for Pi3k and Mtor inhibitors

Inhibitors	Compound	Docking Score (Kcal/mol)	Hydrogen bond	Hydrogen bond distance(Å)	Interacting Residue
Mtor	**San5**	**-9.4**	3	2.27 LYS-833 2.61 ASP-964 2.96 ASN-951	LYS-833ASP-964, ASP-950, ILE-831, PRO-810, SER-806, MET-804, ASN-951, HIS-967 AND LEU-1090
Pi3k	**San5**	-8.5	2	2.27 Glu-332 1.90 ser-333	**met-473, met-336,** arg-466, ser-333, trp-335 and Glu-332

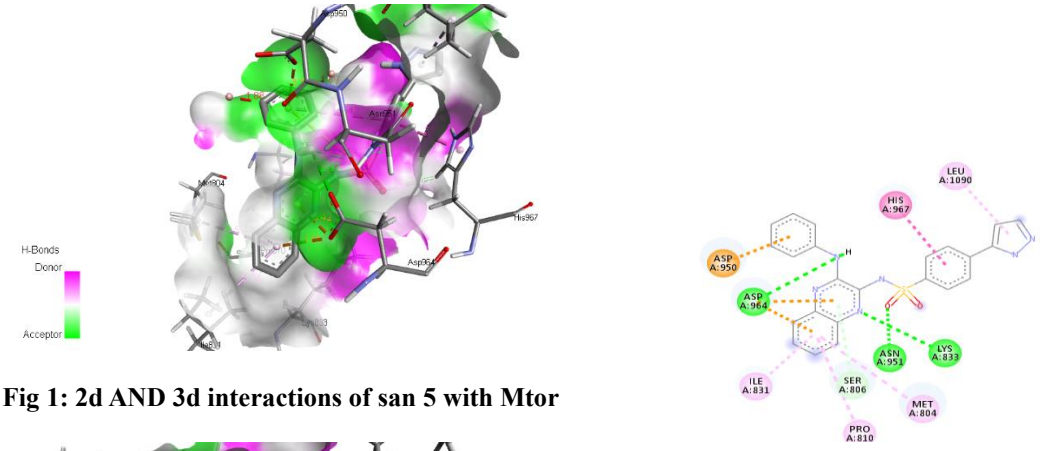

Fig 1: 2d AND 3d interactions of san 5 with Mtor

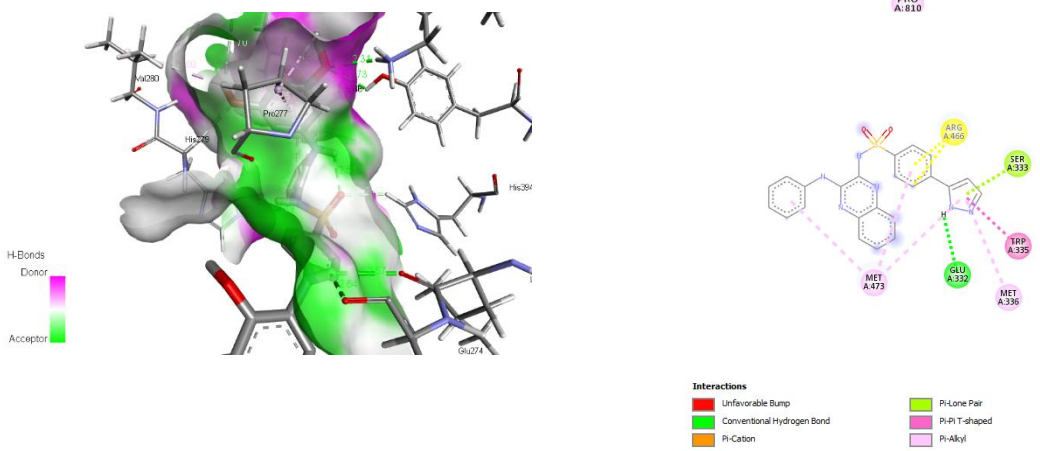

Fig 2: 2d AND 3d interactions of sancom 5 with PI3k

The synthesized compounds were tested for their anticancer activity in the lab using MTT assay against three human cancer cell lines: MCF-7 (breast cancer), HCT-116 (colon cancer), and HepG-2 (liver cancer). The IC50 values of the compounds are listed in Table 2. Compound san 1 showed the lowest IC50 values for all the cell lines, ranging from 0.89 to 1.12 μM, indicating that it is the most effective anticancer agent among the tested compounds. Compound **san**5 was also found to be more potent than the reference drug Doxorubicin, which had IC50 values of 0.90–1.51 μM for the cancer cell lines.

Table 2: Presence of Anticancer activity (IC_{50} μM) of compounds san 1-5 against cancer cell lines.

Compounds	MCF-7	HepG2	HCT-116
san1	4.21 ± 0.11	4.62 ± 0.21	4.46 ± 0.15
san 2	11.50 ± 0.15	14.82 ± 0.05	13.97 ± 0.07
san 3	7.84 ± 0.098	6.54 ± 0.287	7.75 ± 0.25

san 4	2.91 ± 0.23	2.41 ± 0.07	2.38 ± 0.26
san 5	0.89 ± 0.13	1.16 ± 0.09	1.12 ± 0.19
Doxorubicin	0.90 ± 0.02	1.21 ± 0.08	1.51 ± 0.03

Conclusion:

Our research has shown that the synthesized quinoxaline derivatives have great potential as dual Mtor and Pi3k inhibitors for treating various cancers. Among the compounds tested, san 1displayed the highest potency, selectivity, and anticancer activity, while also showing the lowest cytotoxicity against normal cells. Molecular docking studies provided insights into how 4f interacts with the active sites of Mtor and Pi3k, which could help in designing more effective and selective inhibitors.

Conflict of interest:

The authors declare no conflict of interest.

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