IJPSR (2022), Volume 13, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 November 2021; received in revised form, 24 June 2022; accepted, 25 June 2022; published 01 July 2022

IN-SILICO MOLECULAR DOCKING STUDIES, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL FURAN-AZETIDINONE HYBRIDS AS POTENTIAL ANTI-CANCER AGENTS

Aiswarya Raju * and Judy Jays

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ramaiah University of Applied Sciences, Bangalore - 560054, Karnataka, India.

Keywords:

Furan, Azetidinone, Molecular docking, Anticancer

Correspondence to Author:
Aiswarya Raju

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ramaiah University of Applied Sciences, Bangalore - 560054, Karnataka, India.

E-mail: aiswaryaraju86@gmail.com

ABSTRACT: Furan and Azetidinone have been reported to possess various therapeutic activities, including anti-microbial and anti-cancer activity. Increasing drug resistance and high toxicity to the existing drugs have dictated the need to develop novel, effective agents with different scaffolds. With the perspective of identifying novel anti-cancer molecules, an approach was made to hybridize the different heterocyclic moieties. Thirtynovel Furan-Azetidinone hybrids were subjected to *in-silico* molecular docking studies to determine the novel molecules' affinity for the anti-cancer targets. The four most promising derivatives were selected and synthesized based on docking scores. Condensation of ethyl cyanoacetate and *o*-toluidine at 160-190^{0}C yielded 2-cyano-N-2(methylphenyl) acetamide (1). The reaction between (1) and benzoin yielded 2-amino-N-(2-methylphenyl) 4, 5-diphenylfuran-3- carboxamide (2). A series of Schiff bases ARJJ03 (1-2), were synthesized from (2) by refluxing it with various substituted aromatic aldehydes in ethanol using concentrated sulphuric acid as the catalyst. The title compounds ARJJ04 (1-2) were obtained by the cyclization of ARJJ03 (1-2) with Chloroacetyl chloride in 1, 4-dioxane in the presence of triethylamine. The synthesized derivatives were characterized by the UV, IR, NMR, and Mass spectral data. Investigation of *in-vitro* anti-cancer activity of compounds [ARJJ04 (1-2)] has been performed by MCF-7 cell line using MTT Assay.

INTRODUCTION: Cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths. Lung, prostate, colorectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(7).2759-64

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(7).2759-64

More than 70% of cancer deaths occurred in low- and middle-income countries. It is predicted that by 2030, two in five people globally will face a cancer diagnosis during their lifetime, and gains against infections and other conditions have led to increased life expectancy.

It is high time to accelerate global cancer control through prevention, diagnosis, treatment and management, palliative care, and surveillance. As effective cancer control is delayed every year, the response becomes more expensive, and the preventable loss of life increases. Drug resistance and high toxicity are the major challenges in cancer therapy; hence, new drugs with good activity and

minimum toxicity are required. Hence it is necessary to design and develop novel anti-cancer drugs [2-3]. 2-Azetidinones are the common structural feature in antibiotics. It possesses various pharmacological actions such as antibacterial, anti-cancer, antifungal, anti-tubercular, and anti-convulsant [4-7].

Furan plays an important role in the field of medicinal chemistry. Furan derivatives display anti-hypertensive, analgesic, ant-inflammatory, antibacterial, antifungal and antitumor properties [8].

Literature review reveals that the anti-cancer potency of Furan-Azetidinone hybrids was not explored. Keeping this view enormous biological potential of both the moieties, novel furan-azetidinone hybrids were designed and synthesized to obtain more potent anti-cancer drugs.

MATERIALS AND METHODS: *In-silico* molecular docking studies of Compounds were performed using PyRx by Auto Dock vina 8.0. The chemicals and the reagents required for synthesizing and evaluating the proposed compounds are procured from Sigma -Aldrich, CDH, Himedia, Loba chemicals, Merck, *etc*. Melting points of the synthesized compounds were determined by Thiel's melting point apparatus (open capillary method) and the compounds exhibited sharp melting points.

The purity of the compounds was ascertained by thin-layer chromatography using silica gel-G as the stationary phase and appropriate solvents were used for the mobile phase. The spots were visualized using an iodine chamber and UV detector.

The λ max value of the synthesized compounds was done on Shimadzu 1900. The IR spectra of the synthesized compounds were recorded on FTIR Shimadzu 8400s in the range of 400-4000 using a diffraction reflectance system, and the values are reported in cm^{-1}.

Mass spectra of synthesized compounds were recorded on Water's Synapt Xs HDMS using the ESI (Electrospray ionization) mode. The ^{1}H NMR spectra of synthesized compounds were recorded on a Brucker spectrometer operating at 400MHz using dimethyl sulphoxide (DMSO-d6) as solvent.

EXPERIMENTAL:

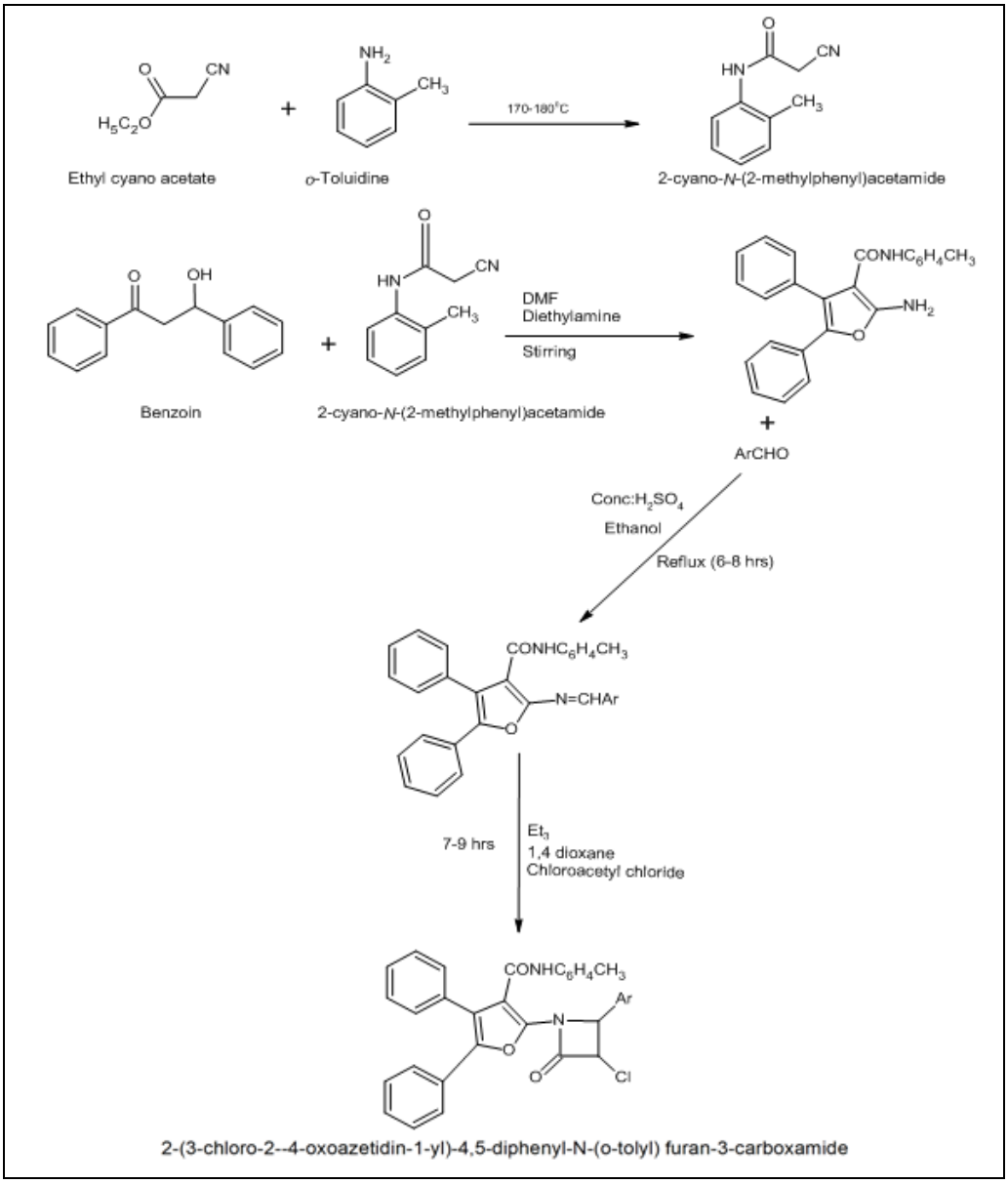
***In-silico* Molecular Docking Studies:** Molecular Docking is an effective and competent tool for *in-silico* screening. It plays an important and ever-increasing role in rational drug design. The 2D structures of thirty-five title compounds were drawn and 3D structures were generated using chem draw software.

BIOVIA Discovery Studio Visualizer software was used to combine all the ligands into a single file; the ligands were optimized for bond lengths and angles by applying the AMBER force field and saved in the sdf format. The crystal structure of the selected eleven protein targets was downloaded from the protein data bank (PDB) (https://www.rcsb.org/) and used for docking studies.

This online tool allows us to explore and analyze the structures or compare any protein in the PDB archive, including support for rigid-body and flexible alignments. The retrieved protein structure was processed by removing the co-crystallized ligand, crystal water molecules and all the other heteroatoms using Pymol. Missing amino acids were corrected, and unwanted amino acid residues were removed using the Swiss PDB viewer (SPDBV4.10).

The mode of binding and interaction of the selected target proteins with individual ligands was performed using Auto Dock vina in PyRx 0.8 software. Docking was performed to obtain possible conformation and orientation for the ligand at the active site.

The protein was loaded in PyRx software, creating a PDBQT file containing proteins' structure with Hydrogens in all polar residues. The docking site on protein-ligand was defined by establishing a grid box with dimensions. The best conformation was chosen with the lowest binding scores. The complex ligand-protein interaction was prepared using Pymol software. 2D and 3D interactions were visualized by Maestro module 11.7 of Schrodinger. The protein targets selected for docking studies were Breast cancer type 1(PDBID: 1JNX), vascular endothelial growth factor receptor 2 (PDBID: 3EWH), human HER2 (PDBID: 1N8Z)



ARJJ04 (1) 4-CHLORO, ARJJ04 (2) 3 -NITRO
SCHEME 1: SYNTHESIS OF FURAN-AZETIDINONE HYBRIDS

Procedure for Synthesis of 2-cyano-N-2(methylphenyl) Acetamide: A mixture of *o*-Toluidine 0.1mol (10.4 mL) and ethyl cyanoacetate 0.1 mol (11.3 mL) were taken in a conical flask, mixed well and heated at 160- 190 °C for 4 hrs. The reaction mixture was left at room temperature overnight. The solid product obtained was washed with ethanol and dried in air. It was purified by recrystallization using Isopropyl alcohol. A white crystalline solid was obtained. Percentage Yield, 82.90%; melting point 115°C; Rf value 0.76; Molecular formula $C_{10}H_{12}N_2O$; Molecular Weight 163; IR(KBR): 3159 (NH str, amide); 3053 (Ar CH, str); 2979(CH str); 2260 (CN str); 1677 (C=O, str);1528,1433 (Ar C=C ring str).

Procedure for Synthesis of 2-amino-N-(2-methylphenyl) 4, 5 – diphenyl – furan – 3 - carboxamide: Diethyl amine (13.8g, 0.13mol) was added dropwise over a period of 30 minutes to a mixture of benzoin (10.6g, 0.05mol) and methyl phenyl cyanoacetamide (8.0g, 0.05mol) in dimethylformamide (30mL) at 0-5 °C. The reaction mixture was stirred at room temperature for 16-18 hours. The resulting mixture was added slowly with stirring to 100 mL of ice-cold water and left at room temperature for 1 hour. The solid obtained was filtered, washed with water (3x10mL), and recrystallized from methanol. Percentage Yield, 51.5%; melting point 150°C; Rf value 0.57;

Molecular formula $C_{24}H_{20}N_2O_2$; Molecular Weight,176; IR(KBR): 3282 (-NH str); 3060 (Ar C-H str); 1621(C=O str of amide); 1524, (Ar C=C ring str); 1251 (C-N str, aromatic amines); 1134 (C-O-C str); 709 (Substituted phenyl rings); MS ESI m/e= found 175 (M+) calculated: 175.

Procedure for synthesis of 2-((benzylidene) amino)-4,5-diphenyl-N-furan-3carboxamide (ARJJ03(1)-ARJJ03(2)): To a mixture of 2-amino-N-(2-methylphenyl)4, 5-diphenylfuran-3-carboxamide (0.01mol) and the appropriate aromatic aldehyde (0.01mol) in absolute ethanol (30 ml) and a few drops of concentrated sulphuric acid was added. The reaction mixture was refluxed for 8-10 hrs. Recrystallization of the compounds was done with ethanol. Percentage Yield, 71.3%; melting point 125°C; Rf value 0.71; Molecular formula $C_{30}H_{22}N_2O_2$; Molecular Weight 365; IR (KBR cm-1): 3288 (NH str of CONH); 3031(Ar CH, str); 1683 (C=O str of amide);1610 (imine C=N str); 1450,1469,1505 (Ar C=C ring str); 1230 (C-O str); 1184 (C-O-C str); 682 (Substituted phenyl rings).

Procedure for synthesis of 2-(3-chloro-2--4-oxoazetidin-1-yl) - 4, 5 – diphenyl – N - (o-tolyl) furan-3-carboxamide (ARJJ04(1)-ARJJ04(2)): Chloroacetyl chloride (0.01 mol) was added drop wise to a well stirred solution of substituted Schiff bases (0.01mol) and 1, 4-dioxane (25ml). The reaction mixture was kept for 1 hr stirring and refluxing from 8-12 hrs. After completion of reaction the mixture was poured into crushed ice and filtered, dried. Recrystallization was carried out by using absolute ethanol.

Physicochemical Data and Spectral Analysis of the Synthesized Furan-Azetidinones: 2-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-4,5-diphenyl-N- (o-tolyl) furan-3-carboxamide ARJJ04(1). Percentage Yield, 62.16%; melting point 164 °C; Rf value 0.64; Molecular formula, $C_{30}H_{22}N_2O_2Cl_2$; Molecular Weight 564; IR (KBR): 1618 Ar (C=C str), 1799 (C=O str), 3037 (SP^2 CH str), 2879 (SP3 CH str), 1427 (CH_2 bend), 1357 (CH3 bend),3481 (N-H str),1544 (N-H bend)794 (C-Cl str),1145 (Ar Cl str); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 5.3 (1H, CH), 6.9 (1H, CH), 7.2-7.6 (19H, Ar), 7.9 (1H, NH); MS (ESI): m/z = found 569 [M+2]; cald. 567.

2-(3-chloro – 2 - (3-nitrophenyl) – 4 - oxoazetidin-1-yl)-4, 5 – diphenyl – N - (o - tolyl) furan-3-carboxamide ARJJ04 (2). Percentage Yield, 62.16%; melting point 164 °C; Rf value 0.64; Molecular formula, $C_{33}H_{24}O_5N_3Cl$; Molecular Weight 564; IR (KBR):3191 (amide N-H str), 3033 (Ar C-H str), 2943 (C-H str), 1770 (lactam C=O str), 1686 (amide C=O str), 1596,1492 (Ar C=C ring str), 1548,1384 (N-O str), 1157 (C-O-C str), 711 (Ar-Cl str); ^{1}H NMR (400 MHz, DMSO) δ (ppm): 7.42 (4H,Ar),7.26 (4H, Ar),7.11 (2H,Ar), 7.38 (2H,Ar), 4.5 (2H, Ar) 7.9 (1H, NH);MS (ESI): m/z = found 579 [M+]; cald. 578.

Anti-Cancer Activity:

Preparation of Primary Stocks of test Compounds: The primary stocks of the test compounds (20, 10, 5, 2.5, 1.25, 0.625, 0.3125 mM) were prepared in DMSO.

Preparation of MTT Reagent: 5mg/mL of primary MTT stock was prepared in PBS and stored at -20^{o}C until use.

Assay Procedure: The growth medium was removed from the culture flask. Cells were washed with 5mL of sterile PBS. Then the PBS was removed, and 1mL of trypsin EDTA was added and kept at 37oC for 5 min or until the cells detached from the surface of the flask. After detachment of cells, 5mL of complete medium containing FBS was added to inactivate the trypsin, and the cells were mixed to obtain a single-cell suspension. Then the cells were collected into a sterile 15mL falcon tube and centrifuged at 1500 rpm for 5 min. The supernatant was discarded, the cell pellet was resuspended in a fresh culture medium and proceeded for cell counting. Cells were seeded (5000 cells/well- 200µL/well) into sterile 96 well culture plates and incubated overnight at 37 °C, 5% CO_2, 95% air, and 85 % relative humidity. After overnight incubation, the cell culture medium was removed and replenished with 200 µL of fresh culture medium containing test compounds at different final concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.5625 µM, n=3) and incubated in CO_2 incubator for 48 hours. After 48 hrs, the media was removed and washed the cells with PBS, and MTT reagent was added with a final concentration of 0.5 mg/mL and incubated at 37°C until farmazan crystals were observed (3 hours). Then the MTT

reagent was removed, the formazan crystals were dissolved with DMSO and the plate was read at 570nm on microplate reader.

RESULTS AND DISCUSSION: Molecular docking of the thirty ligands with different protein targets was performed. To understand the binding affinity and interactions of Furan-Azetidinone analogues, ligands were docked into the active site of proteins. The docking score for the derivatives against the selected target proteins ranged from -3.629 to -11.23. Based on molecular docking studies promising compounds against targets of protein were selected. The title compounds [ARJJ04 (1)-ARJJ04 (2)] have shown significant results for the docking with proteins at their active site. Compound ARJJ04 (9) (3-Nitro derivative) with the docking score -11 showed the best inhibition for the enzyme among the derivatives of anti-cancer targets. The nitro group of the ligand has formed H-bond with ALA: 61, while phenyl rings showed pi-pi stacking interactions with GLU: 58 at the protein's active site. The docking score for many derivatives was better than the docking score for the standard drugs.

TABLE 1: DOCKING STUDIES OF COMPOUNDS WITH POTENTIAL ACTIVITY

Serial Number	Compound Code (Ar)	Docking Score	Interactions
1	ARJJ04(1)	-11	ALA-61, GLU-58, ARG-84, ILE-86, VAL-101, ILE-102
2	ARJJ04(2)	-10.9	GLU-201, PHE-204, LEU-205, ARG-198, HIS-143,

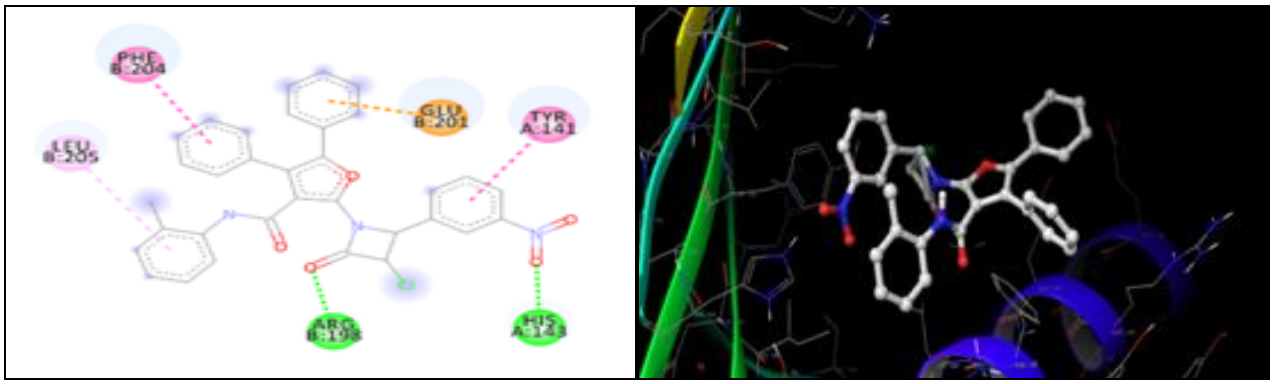


FIG. 1: 2D AND 3D DOCKED POSES OF ARJJ04 (2) AT THE ACTIVE SITE OF BRCA1

Based on molecular docking results, ten furan-azetidinone hybrids were synthesized by the described method, resulting in good product yields. Condensation of ethyl cyanoacetate and *ortho*-toluidine at 160-190°C yielded 2-cyano-N-2(methylphenyl) acetamide (1).

The reaction between (1) and benzoin yielded 2-amino-N-(2-methylphenyl)4, 5-diphenylfuran-3-carboxamide (2). A series of Schiff bases ARJJ03(1-2), were synthesized from 2-amino-N-(2-methylphenyl)4, 5-diphenylfuran-3- carbo-xamide (2) by refluxing it with various substituted aromatic aldehydes in ethanol using concentrated sulphuric acid as the catalyst. The title compounds 2-(3-chloro-2--4-oxoazetidin-1-yl)-4, 5-diphenyl-N-(o-tolyl) furan-3-carboxamide ARJJ04 (1-2) were obtained by the cyclization of ARJJ03 (1-4) with Chloroacetyl chloride in 1,4-dioxane in the presence of triethylamine. Melting points were determined by the open capillary method and were uncorrected. The purity of the compounds was checked by TLC using different solvent systems. The compounds' physical constants and spectral data are given in the experimental section. The structures of the title compounds were confirmed by IR, ^{1}H NMR and mass spectral data.

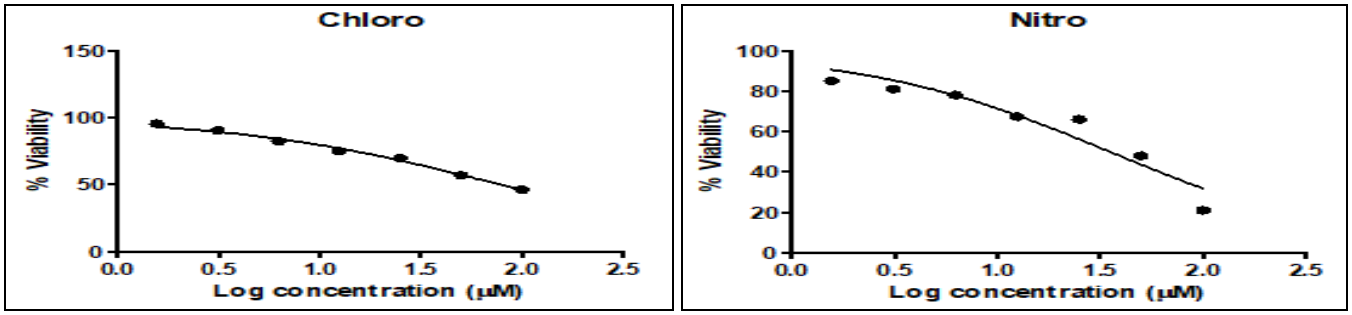
The intermediate 2-amino furan exhibits a broad peak, doublet at 3282 (-NH, str of H-bonded NH_2 group); while intermediate ARJJ03 is confirmed by the disappearance of the doublet and appearance of peaks at 1610 (imine C=N str) and [M]+ peak at 479 which is the also the base peak indicating the stability of the Schiff's base.

The presence of β-lactam ring in the title compounds were confirmed by the appearance of IR peaks at 1770 (lactam C=O str) and the NMR signals at δ 5.5 (1H, CH-N) and δ 6.6-6.9 (1H, CH-Cl) of azetidinone ring. The aromatic protons were observed in the usual region as multiplets between

δ 7.1- 7.8. All the compounds have shown the M+ peak, which is also the base peak indicating that the molecular ion is quite stable. Based on the docking score, two compounds were subjected to anti-cancer activity.

TABLE 2: MCF-7 CELL LINE RESULT

Serial No.	Compound	IC_{50}(µm)
1	ARJJ04(1) (Chloro)	78.22
2	ARJJ04(2) (Nitro)	35.20



GRAPH 1: GRAPHICAL REPRESENTATION OF MCF-7 CELL LINE AGAINST ARJJ04 (1) AND ARJJ04(2)

The anti-cancer activity of newly synthesized scaffolds [ARJJ04 (1-2)] has been evaluated for the MCF-7 cell line using an MTT assay. The IC_{50} values of compounds were measured at the concentration of 0.5 mg/ml. ARJJ04 (1) showed the lowest IC_{50} value (35.2µm) compared to ARJJ04(1). Lower the value of IC_{50}, the higher the potency of the drug. Therefore, ARJJ04 (2) exhibited reasonable anti-cancer activity against the MCF-7 cell line.

CONCLUSION: The objective of the present work was to design, synthesize, characterize and evaluate the biological activity of some novel Furan-Azetidinone hybrids. The *in-silico* molecular docking studies were carried out for the thirty compounds to predict the possible mechanism of action against the antimicrobial targets. ARJJ04 (2) (3-nitro derivative) showed the highest docking score against anti-cancer target.

The structures of the title compounds were characterized and confirmed with the help of spectral analysis and physical data. All the synthesized compounds have exhibited moderate anti-cancer activity. From this study, it may be concluded that the two different derivatives of Furan-Azetidinone have shown reasonable anti-cancer activity.

ACKNOWLEDGMENT: Nil

CONFLICTS OF INTEREST: The authors declare no conflict of interest

REFERENCES:

1. Neu HC: The crisis in antibiotic resistance. Science 1992; 257(5073): 1064-73.
2. World Health Organization: Antimicrobial resistance: global report on surveillance. World Health Organization 2014.
3. World Health Organization: Antimicrobial resistance. Weekly Epidemiological epidemiology hebdomadaire. 2000; 75(41).
4. Chalise SR: Biodiversity measures to summarize antibiotic resistance (Doctoral dissertation, Memorial University of Newfoundland).
5. Davies J and Davies D: Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews 2010; 74(3): 417-33.
6. Toraskar M, Kulkarni V and Kadam V: Azetidinone: A bioactive moiety. J Pharm Res 2010; 3: 169-73.
7. Mehta PD, Sengar NP and Pathak AK: 2-Azetidinone–a new profile of various pharmacological activities. European J of Medicinal Chem 2010; 45(12): 5541-60.
8. Banerjee R, Kumar HK and Banerjee M: Medicinal significance of furan derivatives: a review. International J of Res in Phytochemistry and Pharma 2015; 5(3): 48-57.
9. McRae JA and Kuehner AL: The condensation of benzoin and benzyl with ethylcynoacetate Am Chem Soc 1930; 52(8): 3377–8217.
10. Blount JF, Coffen DL and Katonak DA: Synthesis of some 2-amino furans from cyano acetone enolate and their rearrangement to 3-cyano pyrroles with ammonia. J Org Chem 1978; 43(20): 3821–4 18.
11. Ertuzun Vedia: The Synthesis of 2-amino furan from a nitrile bearing active α-methylene and hydroxyketone and investigation of its properties. Commun Fac Sci Univ Ank Serie B 1986; 32-41.

How to cite this article:
Raju A and Jays J: *In-silico* molecular docking studies, synthesis and biological evaluation of novel furan-azetidinone hybrids as potential anticancer agents. Int J Pharm Sci & Res 2022; 13(7): 2759-64. doi: 10.13040/IJPSR.0975-8232.13(7). 2759-64.