Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : http://ijpsdr.com/index.php/ijpsdr

Research Article

1,2,3-Triazole Carboxamide Derivatives as Novel Prospective Anticancer Agents: Synthesis, Characterization and *In-silico* Studies

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ARTICLE INFO

Article history:
Received: 07 January, 2024
Revised: 09 February, 2024
Accepted: 18 February, 2024
Published: 30 March, 2024

Keywords:
1,2,3-Triazole, Anticancer, MTT assay, EGFR, CDK-4.

DOI:
10.25004/IJPSDR.2024.160211

ABSTRACT

The ongoing and persistent endeavor to discover effective new anticancer medications remains a steadfast objective. Presently, this research focuses on exploring novel substituted derivatives of 1,2,3-triazole carboxamide. A set of novel derivatives of 1,2,3-triazole carboxamide(5a-5n) were successfully synthesized with yields ranging from satisfactory to excellent. These compounds underwent characterization using various analytical methods, such as proton nuclear magnetic resonance (^{1}H NMR), Carbon^{13} nuclear magnetic resonance (^{13}C NMR), and mass spectrometry. Their cytotoxic potential against four cancer cell lines-HeLa, PANC-1, HCT-116, and A-549 was evaluated *in-vitro*. Compounds 5j, 5i, 5m, and 5f displayed significant anticancer activity. Molecular docking experiments were conducted on the synthesized compounds, revealing strong binding interactions with the active sites of EGFR and CDK4-Cyclin D3. However, out of all the derivatives tested, namely 5i, 5j, 5g, 5f, and 5h, it was observed that these compounds displayed a favorable binding affinity towards both the EGFR and the CDK4-cyclin D3 active site. The results of the study suggest that the synthesized compounds have potential as agents for cancer therapy. Furthermore, further alterations to the structure of triazole-carboxamide derivatives could lead to the development of effective anticancer drugs.

Introduction

Cancer poses a substantial global health challenge due to its intricate nature and the potential for life-threatening consequences stemming from uncontrolled cellular proliferation.[1] Chemotherapy is a widely recognized and validated approach that utilizes potent pharmaceutical agents to specifically target and eliminate proliferating malignant cells in various regions of the body. Although chemotherapy is known for its efficacy, it can have detrimental effects on healthy cells. The necessity to create new anticancer treatments arises from the inherent danger of chemotherapy causing harm to healthy cells.[2] Although chemotherapy is successful in targeting cancer cells, its non-selective nature leads to inadvertent damage to healthy tissues, resulting in adverse reactions that undermine both the well-being of patients and the effectiveness of their treatment. Targeted therapies aim to selectively target cancer cells while preserving the integrity of healthy tissues, therefore reducing the occurrence of side effects and enhancing the accuracy of treatment.[3]

The utilisation of the 1,2,3-triazole framework has been recognized as a very adaptable and auspicious foundational component in the development of novel anticancer medicines. The molecular flexibility of this compound permits the integration of different functional groups, facilitating the creation of compounds with enhanced characteristics.[4] The triazole ring acts as a bio isostere in pharmaceutical chemistry, substituting conventional moieties.[5] The inhibitory effects of 1,2,3-triazole derivatives on many cancer-related targets, including as EGFR,[6,7] VEGFR,[8-10] thymidine

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

phosphorylase,[11, 12] topoisomerase,[13, 14] aromatase,[15] and tubulin polymerization,[16, 17] have been highlighted in recent reports (Fig. 1). The above investigation's findings underscore these moieties' capacity to selectively interfere with critical pathways implicated in the proliferation of cancerous cells. In addition, these interventions present the potential to surmount drug resistance, a substantial obstacle encountered in the realm of cancer therapy.[18] Certain derivatives demonstrate advantageous pharmacokinetic characteristics, hence increasing their viability as viable anticancer medicines in clinical environments.[19]

Based on the aforementioned findings, a compelling rationale emerges for synthesizing and evaluating 1,2,3-triazole-4-carboxamide derivatives in the pursuit of novel anticancer therapeutics. The structural versatility, bioisosteric properties, multifaceted inhibitory actions, and advantageous pharmacokinetic attributes collectively position these compounds as promising subjects for continued investigation and advancement in the anticancer drug discovery landscape. The research aimed to explore the possibility of utilizing these compounds as agents for combating cancer. Furthermore, the investigation employed molecular docking methodologies to examine the molecular interactions between the synthesized derivatives and the targets EGFR and CDK-4. The objective was to acquire a deeper understanding of the underlying mechanisms involved.

Materials And Methods

No additional purification procedures were conducted on the synthetic-grade compounds or solvents used in this study; all were procured from Sigma-Aldrich, Bangalore, India. Merck-precoated aluminum TLC plates with silica gel 60 F254 were employed for reaction monitoring with Ethyl acetate: Hexane (1:10 v/v) used as mobile phase for all the synthetic steps. Melting points were determined using Remi electronic melting point equipment. The ^{1}H and ^{13}C-NMR spectra were recorded on a BRUKER DRX instrument, with chemical shift values reported relative to tetramethyl silane as the internal standard in parts per million (ppm). Various splitting patterns were denoted by letters: "s" for singlet, "d" for doublet, "t" for triplet, "q" for quartet, and "m" for multiplet. High-resolution mass spectrometry (HRMS) spectra were obtained using a Waters Xevo Q-Tof Mass spectrometer.

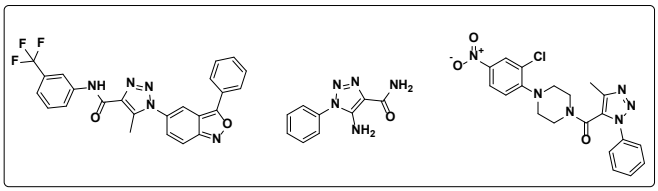

Fig. 1: Structures of some 1H-1,2,3-triazole-4-carboxamide derivatives with anticancer activity

Synthesis of 1H-1,2,3-Triazole-4-carboxamide Derivatives (5a-5n)

Procedure

The scheme of synthesis for 1H-1,2,3-triazole-4-carboxamide derivatives (5a-5n) displayed in Fig. 2.

- *Synthesis of substituted 1H-1,2,3-triazole-4-carbaldehyde (3)*

α-haloacrolein (1) (0.20 mmol) and Alkyl azide (2) (0.13 mmol) were reacted in the presence of 200 L of solvent ($DMSO/H_2o$) (0.65 M) at room temperature for 36 hours to obtain substituted 1H-1,2,3-triazole-4-carbaldehyde (3).[20]

- *Conversion of 1H-1,2,3-triazole-4-carbaldehyde (3) to ethyl 1,5-dimethyl-1H-1,2,3-triazole-4-carboxylate (4)*

Iodine (1.2 mmol) and K_2CO_3 (3.0 mmol) were added to a solution of 1H-1,2,3-triazole-4-carbaldehyde (3) (1 mmol) and ethanol (1.05 mmol) in an inert atmosphere. The resulting mixture was agitated at room temperature until the iodine colour almost disappeared. The mixture was quenched by adding water (20 mL), Et_2O (5 mL), and standard aq Na_2SO_3 (0.5 mL) at 0°C after 22 hours at the same temperature. The mixture was subsequently extracted three times with Et_2O. Ethyl 1,5-dimethyl-1H-1,2,3-triazole-4-carboxylate (4) was produced by washing the organic layer with brine and drying it over Na_2SO_4. To produce a pure final product, the product was, if necessary, purified by flash column chromatography on silica gel (hexane-EtOAcZ10:1).[21]

- *Synthesis of 1H-1,2,3-triazole-4-carboxamide derivatives (5a-5n)*

In order to catalyze the reaction, indium triiodide (20 mol%) was freshly produced by stirring indium and iodine in THF. Ethyl 1,5-dimethyl-1H-1,2,3-triazole-4-carboxylate (1) (1-mmol) was heated in an oil bath at a temperature of 110 to 120°C in a primary amine (2 cm^3). Following the completion of the reaction, the reaction mixture was extracted with ether, and the ether extract was subsequently washed with 1N aqueous HCl to remove excess brine or amine before being dried over Na_2SO_4. The crude substance that remained after the solvent evaporated

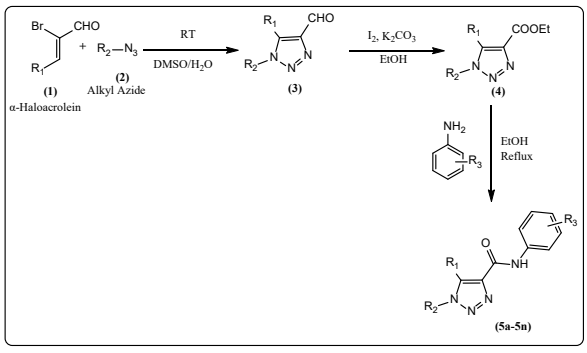

Fig. 2: Scheme of synthesis for 1,2,3-triazole carboxamide derivatives

was refined into pure amide by recrystallizing it from an ether-petroleum ether (60-80°C) solvent mixture.[22]

Molecular Docking Studies

The EGFR (6LUD) and CDK-4 (7SJ3) domain X-ray crystal structures were obtained from the Protein Data Bank. The Protein Preparation Wizard tool within the Schrödinger software was employed to add hydrogen atoms and assign bond orders in the 3D structure of the protein. Chiral ligands were prepared and their 3D structures optimized using the LigPrep module in Schrödinger software, utilizing the OPLS 2005 force field. Receptor sites for 6LUD and 7SJ3 were analyzed using the SITEMAP ANALYSIS TOOL in Maestro 11.8, followed by grid creation using Schrödinger suite's grid generation tool. The XP Glide score, incorporating binding interaction energy, van der Waals energy, electrostatic potential energy, and strain energy assessments, was calculated during molecular docking using Glide's extra-precision docking modes (Glide XP). Ligand binding to the active sites of EGFR and CDK-4 was studied using the Schrödinger Maestro interface.[23]

Cytotoxicity Assay

The American Type Cell Culture Collection provided non-small cell lung (A-549) and colorectal (HCT-116) and pancreatic (PANC-1) cancer cells, and the cells were grown in RPMI 1640 media with 1% penicillin-streptomycin, 1% L-glutamine and 10% heat-inactivated fetal bovine serum. Human embryonic kidney (HEK-293) cells were collected from the American Type Cell Culture Collection and grown in Eagle's minimum essential medium with the addition of 1% penicillin-streptomycin, 1% L-glutamine, and 10% heat-inactivated fetal bovine serum. At 37 °C, all cells were incubated in a 5% CO_2 humidified environment.

Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the cytotoxic effects of all synthesized compounds (5a–5n) were assessed. In 96-well plates, cells were seeded at a density of $1x10^4$ cells/well in 100 µL culture medium and grown in a carbon dioxide incubator (37°C, 5% CO_2). In a serum-free medium, compounds (5a–5n) were diluted to the necessary quantities before being supplied to the wells corresponding to the vehicle control. Following the incubation periods, 10 µL MTT was added to each well, and the plates underwent an additional 4 hours of incubation at 37°C. The medium was then removed, the formazan crystals were dissolved in 100 mL of dimethyl sulfoxide (DMSO), and the absorbance was measured with a spectrophotometer at 570 nm wavelength.[24]

Results And Discussion

Chemistry

5a: 1-methyl-N-phenyl-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-79%, m.p- 256–258°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.47 (s, 1H), 7.72–7.67 (m, 2H), 7.36–7.29 (m, 2H), 7.09 (tt, *J* = 7.1, 1.4 Hz, 1H), 4.13 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 161.85, 140.26, 137.48, 128.78, 126.61, 123.80, 120.22, 36.60. HRMS: m/z: For $C_{10}H_{10}N_4O$ ([M + H]+): 203.2168, found 203.2163.

5b: 1-methyl-N-(m-tolyl)-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-72%, m.p- 219–220°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.54 (s, 1H), 7.46–7.42 (m, 1H), 7.42–7.38 (m, 1H), 7.18 (t, *J* = 7.7 Hz, 1H), 6.87 (ddt, *J* = 7.7, 2.0, 0.9 Hz, 1H), 4.05 (s, 3H), 2.35 (d, *J* = 1.0 Hz, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.63, 141.16, 138.74, 137.94, 128.85, 126.50, 124.57, 119.99, 117.82, 36.06, 20.69. HRMS: m/z: For $C_{11}H_{12}N_4O$ ([M + H]+): 217.2402, found 217.2402.

5c: 1-methyl-N-(p-tolyl)-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-76%, m.p- 217–218°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.40 (s, 1H), 7.47–7.41 (m, 2H), 7.30–7.25 (m, 2H), 4.06 (s, 3H), 2.33 (d, *J* = 0.7 Hz, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 161.40, 139.20, 135.80, 133.11, 129.41, 126.61, 120.39, 37.51, 21.20. HRMS: m/z: For $C_{11}H_{12}N_4O$ ([M + H]+): 217.2304, found 217.2306.

5d: N-(4-methoxyphenyl)-1-methyl-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-81%, m.p- 204–205°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.52 (s, 1H), 7.66–7.61 (m, 1H), 7.61–7.55 (m, 1H), 6.95–6.90 (m, 2H), 4.02 (s, 3H), 3.73 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.61, 155.80, 141.67, 134.35, 127.53, 122.50, 114.28, 54.32, 37.00. HRMS: m/z: For $C_{11}H_{12}N_4O_2$ ([M + H]+): 233.2403, found 234.2409.

5e: N-(-aminophenyl)-1-methyl-1H-1,2,3-triazole-4-carboxamide

Pale Yellow solid, Yield-69%, m.p- 229–230°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.52 (s, 1H), 7.50–7.44 (m, 2H), 6.64–6.58 (m, 2H), 5.10 (s, 2H), 4.19 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 160.89, 144.35, 141.16, 133.40, 128.04, 121.86, 115.46, 35.77. HRMS: m/z: For $C_{10}H_{11}N_5O$ ([M + H]+): 218.2229, found 218.2305.

5f: N-(4-hydroxyphenyl)-1-methyl-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-67%, m.p- 238–239°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.78 (s, 1H), 8.40 (s, 1H), 7.34–7.28 (m, 2H), 6.81–6.76 (m, 2H), 4.05 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.34, 152.61, 141.45, 133.40, 127.82, 122.62, 115.54, 35.77. HRMS: m/z: For $C_{10}H_{10}N_4O_2$ ([M + H]+): 219.2195, found 219.2198.

5g: N-(4-fluorophenyl)-1-methyl-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-70%, m.p- 187–188°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.60 (s, 1H), 7.54–7.47 (m, 2H), 7.18–7.10 (m, 2H), 4.14 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.34, 141.16, 135.07 (d, *J* = 2.6 Hz), 127.32, 122.59 (d, *J* = 8.7 Hz), 116.05, 115.87, 36.06. HRMS: m/z:

For $C_{10}H_9FN_4O$ ([M + H]+): 221.2105, found 221.2108.

5h: N-(4-chlorophenyl)-1-methyl-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-73%, m.p- 266–267°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.41 (s, 1H), 7.65–7.59 (m, 2H), 7.42–7.36 (m, 2H), 4.08 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 161.40, 140.94, 135.80, 129.07, 128.00, 125.57, 121.85, 35.77. HRMS: m/z: For $C_{10}H_9ClN_4O$ ([M + H]+): 237.6534, found 237.6532.

5i: N-(4-bromophenyl)-1-methyl-1H-1,2,3-triazole-4-carboxamide

Pale Brown solid, Yield-68%, m.p- 249–251°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.21 (s, 1H), 8.54 (s, 1H), 7.68–7.62 (m, 2H), 7.52–7.46 (m, 2H), 4.05 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.34, 141.16, 138.04, 133.10, 127.32, 123.16, 118.54, 35.77. HRMS: m/z: For $C_{10}H_9BrN_4O$ ([M + H]+): 282.1079, found 282.1076.

5j: 1-methyl-N-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-77%, m.p- 145–147°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.54 (s, 1H), 7.85 (dq, J = 7.6, 1.4 Hz, 2H), 7.81–7.75 (m, 2H), 4.10 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.61, 140.94, 139.20, 126.84–126.52 (m), 125.22 (d, J = 1.3 Hz), 120.17 (q, J = 3.7 Hz), 37.00. HRMS: m/z: For $C_{11}H_9F_3N_4O$ ([M + H]+): 271.2048, found 271.2048.

5k: N-(3,4-dichlorophenyl)-1-methyl-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-71%, m.p- 303–304°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.38 (s, 1H), 7.87 (d, J = 2.0 Hz, 1H), 7.54 (dd, J = 8.0, 1.8 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 4.08 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.34, 141.45, 138.48, 131.03, 130.52, 127.32, 125.87, 121.50, 120.66, 35.77. HRMS: m/z: For $C_{10}H_8Cl_2N_4O$ ([M + H]+): 272.1024, found 272.1023

5l: N-(3,4-difluorophenyl)-1-methyl-1H-1,2,3-triazole-4-carboxamide

White solid, Yield-66%, m.p- 151–153°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.54 (s, 1H), 7.48–7.34 (m, 3H), 4.18 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 161.54, 140.09, 136.42 (dd, J = 14.9, 3.3 Hz), 126.50, 117.74 (dd, J = 7.2, 3.9 Hz), 117.06 (dd, J = 21.3, 6.6 Hz), 108.85 (dd, J = 23.0, 6.1 Hz), 36.60. HRMS: m/z: For $C_{10}H_8F_2N_4O$ ([M + H]+): 239.2017, found 239.1012.

5m: 1-methyl-N-(4-nitrophenyl)-1H-1,2,3-triazole-4-carboxamide

Brown solid, Yield-75%, m.p- 292–293°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.54 (s, 1H), 8.29–8.23 (m, 2H), 7.91–7.85 (m, 2H), 4.08 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 161.76, 143.47, 142.31, 140.25, 126.50, 125.46, 118.80, 36.60. HRMS: m/z: For $C_{10}H_9N_5O_3$ ([M + H]+): 248.2113, found 248.2113.

5n: 1-methyl-N-(3-nitrophenyl)-1H-1,2,3-triazole-4-carboxamide

Brown solid, Yield-74%, m.p- 286–287°C,^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.59 (s, 1H), 8.61 (t, J = 2.3 Hz, 1H), 8.47 (s, 1H), 8.05 (ddd, J = 8.0, 2.0, 1.0 Hz, 1H), 7.79 (ddd, J = 8.0, 2.5, 0.9 Hz, 1H), 7.62 (t, J = 8.0 Hz, 1H), 4.18 (s, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ 162.13, 149.42, 141.16, 140.43, 130.65, 126.86, 126.50, 117.62, 114.82, 37.51. HRMS: m/z: For $C_{10}H_9N_5O_3$ ([M + H]+): 248.2109, found 248.2106."

Molecular Docking

All the triazole-carboxamide compounds under investigation showed docking scores between −3.04 and −4.932 kcal/mol against EGFR (6LUD) and −4.016 and −6.031 kcal/mol against CDK4-Cyclin D3 (PDB ID 7SJ3). The docking scores for the triazole-carboxamide compounds 5j and 5g were equivalent to those for the co-crystallized ligands osimertinib and abemaciclib. As is displayed in Table 1 these triazole-carboxamide derivatives were incorporated into the EGFR (PDB ID 6LUD) and CDK4-Cyclin D3 (PDB ID 7SJ3) active sites *via* interactions with the amino acid residues, which were hydrophobic, H-donor, and H-acceptor.

Molecular docking of synthesized compounds against EGFR (PDB ID 6LUD) protein

Compounds 5j and 5i showed the highest binding affinities, demonstrating binding energy values of −4.932 and −4.648 Kcal/mol against the active site of the EGFR protein (Table 1). Derivative 5i showed one H-bond with MET 793 and hydrophobic interactions with PRO 794, MET 793, LEU 792, MET 790, ALA 743, VAL 726, LEU 1001, LEU 718, and LEU 844 (Fig. 2). While compound 5j displayed one H-bond with MET 793 and hydrophobic interactions with PHE 795, PRO 794, MET 793, LEU 792, MET 790, LEU 718, LEU 844, VAL 726, and ALA 743 amino acid residues (Fig. 3).

Molecular docking of synthesized compounds against CDK4-Cyclin D3 (PDB ID 7SJ3) protein

Compounds 5f and 5g showed the highest binding affinities, demonstrating binding energy values of −5.854 and 6.031 Kcal/mol against the active site of the CDK4-Cyclin D3 protein (Table 1). Derivative 5f formed two H-bonds

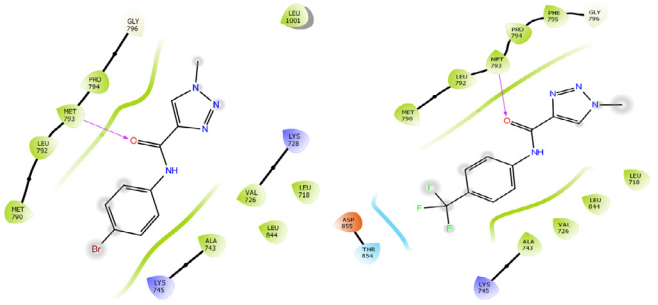

Fig. 3: Molecular interactions of compound 5i and 5j with the EGFR (6LUD) active site

Table 1: Results of molecular docking studies and MTT assay of synthesized 1,2,3-triazole carboxamide derivatives (5a-5n)

Compound	*R*	*Docking Scores*		*Non-small cell lung cancer line (A-549)*	*Colorectal cancer cell line (HCT-116)*	*Pancreatic cancer cell line (PANC-1)*	*Cervical cancer cell line (HeLa)*	*Human embryonic kidney cell line (HEK-293)*
		6LUD	*7SJ3*					
5a	H	-3.583	-4.752	17.712 ± 1.02	15.132 ± 0.42	18.384 ± 0.24	19.164 ± 0.61	44.7 ± 1.4
5b	CH_3	-3.983	-4.016	25.608 ± 0.61	29.54 ± 0.20	38.064 ± 0.40	29.676 ± 0.14	53.1 ± 0.25
5c	4-CH_3	-4.501	-4.515	12.612 ± 0.42	15.25 ± 1.52	20.328 ± 0.21	17.736 ± 1.08	49.116 ± 1.08
5d	4-OCH_3	-4.203	-4.579	22.224 ± 1.08	22.44 ± 0.24	34.704 ± 0.17	22.56 ± 1.45	53.52 ± 1.02
5e	2-NH_2	-3.623	-4.546	30.276 ± 1.02	25.26 ± 1.08	24.432 ± 0.42	28.368 ± 0.24	49.5 ± 0.20
5f	4-NH_2	-4.356	-5.854	12.31 ± 0.24	20.052 ± 1.29	9.984 ± 0.19	14.16 ± 1.62	48.468 ± 0.32
5g	4-OH	-4.308	-6.031	23.7 ± 0.61	20.66 ± 1.03	23.412 ± 1.08	24.096 ± 1.6	45.54 ± 0.61
5h	4-F	-3.04	-5.004	21.612 ± 0.12	23.52 ± 1.02	23.28 ± 0.61	19.224 ± 0.61	45.216 ± 1.08
5i	4-Cl	-4.648	-4.988	8.364 ± 0.24	12.72 ± 0.61	17.8164 ± 1.02	7.704 ± 0.32	37.824 ± 0.42
5j	4-Br	-4.932	-4.932	8.532 ± 1.02	6.132 ± 0.11	14.856 ± 0.10	12.468 ± 1.02	38.604 ± 0.40
5k	4-CF_3	-4.263	-4.386	31.74 ± 0.86	21.66 ± 0.32	24.864 ± 0.14	26.844 ± 0.17	47.544 ± 0.27
5l	2,4-Cl	-4.197	-5.143	27.324 ± 1.29	22.81 ± 1.02	33.516 ± 0.82	27.72 ± 0.32	42.432 ± 0.24
5m	3-NO_2	-4.527	-4.632	12.54 ± 0.22	20.05 ± 0.27	9.984 ± 1.29	14.16 ± 1.48	48.468
5n	4-NO_2	-4.295	-4.494	23.892 ± 1.08	21.85 ± 0.61	31.867 ± 1.54	22.924 ± 0.42	53.52 ± 0.61
Osimertinib		-5.952	------	------	------	------	------	------
Abemaciclib		------	-7.541	------	------	------	------	------
Doxorubicin (reference standard)				1.12 ± 0.89	1.76 ± 1.05	1.84 ± 0.24	1.93 ± 1.49	2.85 ± 1.05

with VAL 96 and LYS 35 and hydrophobic interactions with TYR 17, VAL 20, ALA 33, ALA 157, VAL 72, PHE 93, VAL 96, LEU 147, and ILE 12 (Fig. 3). Derivative 5g displayed one H-bond with VAL 96 and six hydrophobic interactions with VAL 20, PHE 93, ALA 33, VAL 96, LEU 147, and ILE 12 (Fig. 4).

Among all the synthesized compounds, electron-withdrawing groups bearing compounds 5i (4-Cl) and 5j (4-Br) exhibited maximum binding affinity against the EGFR (PDB ID 6LUD) protein compared to electron-donating groups bearing compounds. While, electron-donating as well as electron-withdrawing groups bearing compounds 5g (4-OH), 5f (4-NH_2), and 5h (4-F) demonstrated good binding affinity against CDK4-Cyclin D3 (PDB ID 7SJ3).

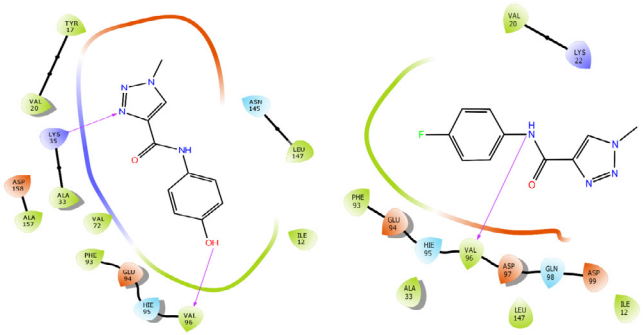

Fig. 4: Molecular interactions of compound 5f and 5g with the CDK4-Cyclin D3 (7SJ3) active site

Cytotoxicity Assay

The IC_{50} values of the 1,2,3-triazole carboxamide derivatives (compounds 5a-5n) were displayed in Table 1. The synthesized compounds' cytotoxicity was evaluated against four tumor cell lines (HeLa, PANC-1, HCT-116, and A-549) and one normal human embryonic kidney cell line (HEK-293) *via* MTT assay. As mentioned in Table 1, compounds 5i, 5j, 5f, and 5m showed significant inhibitory activities against all the four cell lines tested under this study such as A-549, HeLa, HCT-116, and PANC-1 cell lines. However, when compared with the reference standard, doxorubicin, all the synthesized molecules displayed lower inhibitory activity.

When tested against A-549 cell lines, the synthesized compounds displayed numerous inhibitory activities. Among all the compounds, 5i and 5j displayed maximum inhibitory potential, with IC_{50} values of 8.364 ± 0.24 µg/mL and 8.532 ± 1.02 µg/mL respectively. However, the IC_{50} values of these compounds were significantly lower than the reference doxorubicin IC_{50} value 1.12 ± 0.89 µg/mL. The compounds such as 5a (IC_{50} = 17.712 ± 1.02 µg/mL), 5c (IC_{50} =12.612 ± 0.42 µg/mL), 5f (IC_{50} =12.31 ± 0.24 µg/mL), 5m (IC_{50} =12.54 ± 0.22 µg/mL) displayed moderate inhibitory potential against A-549 cell lines. While substances 5b (IC_{50} =25.608 ± 0.61 µg/mL), 5d (IC_{50} =22.224 ± 1.08 µg/mL), 5e (IC_{50} =30.276 ± 1.02 µg/mL), 5g (IC_{50} =23.7 ± 0.61 µg/mL), 5h (IC_{50} =21.612 ± 0.12 µg/mL), 5k (IC_{50} =31.74 ± 0.86 µg/mL), 5l (IC_{50}

=27.324 ± 1.29 µg/mL), and 5n (IC_{50} =23.892 ± 1.08 µg/mL) demonstrated lower inhibitory potential.

The MTT assay results of 1,2,3-triazole carboxamide derivatives (5a-5n) on the HCT-116 cell lines demonstrated that derivative 5j (IC_{50}=6.132 ± 0.11 µg/mL) displayed the highest inhibitory potential, followed by 5i (IC_{50}=12.72 ± 0.61 µg/mL). Compounds 5a (H, IC_{50}=15.132 ± 0.42 µg/mL), 5c (4-CH_3, IC_{50}=15.2544 ± 1.52 µg/mL), 5f (4-NH_2, IC_{50} =20.052 ± 1.29 µg/mL), 5g (4-OH, IC_{50} = 20.664 ± 1.03 µg/mL) and 5m (3-NO_2, 20.052 ± 0.27 µg/mL) exhibited moderate inhibitory activity. Compounds 5b (CH_3, IC_{50} = 29.5428 ± 0.20 µg/mL), 5d (4-OCH_3, IC_{50} = 22.44 ± 0.24 µg/mL), 5e (2-NH_2, IC_{50}=25.26 ± 1.08 µg/mL), 5h (4-F, IC_{50}=23.52 ± 1.02 µg/mL), 5k (4-CF_3, IC_{50}=21.66 ± 0.32 µg/mL), 5l (2,4-Cl, IC_{50}=22.812 ± 1.02 µg/mL) and 5n (4-NO_2, IC_{50}=21.857 ± 0.61 µg/mL) exhibited relatively low inhibitory activity.

Compounds 5f (4-NH_2, IC_{50}=9.984 ± 0.19 µg/mL), 5m (3-NO_2, IC_{50}=9.984 ± 1.29 µg/mL), 5a (H, IC_{50}=18.384 ± 0.24 µg/mL), 5c (4-CH_3, IC_{50}=20.328 ± 0.21 µg/mL), 5j (4-Br, IC_{50}=14.856 ± 0.10 µg/mL) and 5i (4-Cl, IC_{50}=17.8164 ± 1.02 µg/mL) when tested against PANC-1 cell lines exhibited maximum inhibitory effect. The IC_{50} values of the aforementioned compounds were relatively lower than the doxorubicin IC_{50} value, i.e., 1.84 ± 0.24 µg/mL. Certain substances 5b (CH_3, IC_{50}=38.064 ± 0.40 µg/mL), 5d (4-OCH_3, IC_{50}=22.44 ± 0.24 µg/mL), 5e (2-NH_2, IC_{50}=24.432 ± 0.42 µg/mL), 5g (4-OH, IC_{50}=23.412 ± 1.08 µg/mL), 5h (4-F, IC_{50}=23.28 ± 0.61 µg/mL), 5k (4-CF_3, IC_{50}=24.864 ± 0.14 µg/mL), 5l (2,4-Cl , IC_{50}=33.516 ± 0.82 µg/mL), and 5n (4-NO_2, IC_{50}=31.867 ± 1.54 µg/mL) displayed lowest inhibitory efficacy against PANC-1 cell lines.

The synthesized compounds MTT assay results against HeLa revealed that derivatives 5i (4-Cl, IC_{50}=7.704 ± 0.32 µg/mL), 5f (4-NH_2, IC_{50}=14.16 ± 1.62 µg/mL), 5j (4-Br, IC_{50}=12.468 ± 1.02 µg/mL), 5m (3-NO_2, IC_{50}=14.16 ± 1.48 µg/mL), 5c (4-CH_3, IC_{50}=17.736 ± 1.08 µg/mL), 5a (H, IC_{50}=19.164 ± 0.61 µg/mL), and 5h (4-F, IC_{50}=19.224 ± 0.61 µg/mL) exhibited maximum inhibitory potential. The lowest inhibitory potential was demonstrated by compounds 5b (CH_3, IC_{50}=29.676 ± 0.14 µg/mL), 5d (4-OCH_3, IC_{50}=22.56 ± 1.45 µg/mL), 5e (2-NH_2, IC_{50}=28.368 ± 0.24 µg/mL), 5g (4-OH, IC_{50}=24.096 ± 1.6 µg/mL), 5k (4-CF_3, IC_{50}=26.844 ± 0.17 µg/mL), 5l (2,4-Cl, IC_{50}=27.72 ± 0.32 µg/mL), and 5n (4-NO_2, IC_{50}=22.924 ± 0.42 µg/mL). The standard drug doxorubicin (1.93 ± 1.49 µg/mL) exhibited a significantly greater inhibitory effect than the synthesized compounds.

Compound 5i (4-Cl) with an IC_{50} value of 8.364 ± 0.24 µg/mL showed maximum cytotoxicity against A-549 cell lines, followed by compound 5j (4-Br) with an IC_{50} value of 8.532 ± 1.02 µg/mL. Against HCT-116 cell lines, derivative 5j (4-Br) exhibited the highest cytotoxicity with an IC_{50} value 6.132 ± 0.11 µg/mL. Molecules 5f (4-NH_2) and 5m (3-NO_2) displayed potent cytotoxicity against PANC-1 cell lines with IC_{50} values of 9.984 ± 0.19 and 9.984 ± 1.29 µg/mL, respectively. Compound 5i (4-Cl) with an IC_{50} value of 7.704 ± 0.32 µg/mL displayed potent cytotoxicity against the HeLa cell line. All the synthesized compounds demonstrated lesser cytotoxicity against HEK-293 cell lines. Against all the cancer cell lines, compounds 5j and 5i displayed potent cytotoxicity compared to all other compounds.

From the above results, we can conclude that electron-withdrawing groups (4-Br, 4-Cl, 3-NO_2, and 4-NH_2) substituted compounds 5j, 5i, 5m, and 5f demonstrated potent cytotoxicity against all the cancer cell lines employed in the study than the electron-donating groups. A good correlation exists between molecular docking and cytotoxicity.

Conclusion

In conclusion, a series of novel 1,2,3-triazole carboxamide derivatives (5a-5n) were synthesized with moderate to good yields. Comprehensive characterization was performed using ^{1}H-NMR, ^{13}C-NMR, and mass spectral analysis for all compounds. The *in-vitro* evaluation against four cancer cell lines HeLa, PANC-1, HCT-116, and A-549 revealed promising cytotoxic potential. 5j, 5i, 5m, and 5f had significant anticancer activity among all the synthesized compounds. Molecular docking studies of the synthesized compounds have revealed that all the compounds showed strong binding interactions with the EGFR and CDK4-Cyclin D3 active sites. However, among all the derivatives, 5i, 5j, 5g, 5f, and 5h exhibited good binding affinity against EGFR and the CDK4-Cyclin D3 active site. The results suggest that the synthesized compounds show potential as candidates for cancer therapy. Further exploration into structural modifications of triazole-carboxamide derivatives could lead to the development of promising anticancer agents.

Acknowledgement

Authors acknowledge the technical support provided by Rx Innovation Pvt Ltd, Hyderabad, India in the completion of the current research work. For current research work, all the spectra (^{1}H-NMR, ^{13}C-NMR and MASS) and *in-vitro* cytotoxic activity were performed at Rx Innovation Pvt Ltd, Hyderabad, India. The authors are also thankful to the support provided by TRR college of Pharmacy, Hyderabad for providing the Schrödinger 2018 suite for molecular docking studies.

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HOW TO CITE THIS ARTICLE: Sharma DK, Singh S, Bikshapathi DVRN, Kandagatla S. 1,2,3-Triazole Carboxamide Derivatives as Novel Prospective Anticancer Agents: Synthesis, Characterization and *In-silico* Studies. Int. J. Pharm. Sci. Drug Res. 2024;16(2):213-219. **DOI:** 10.25004/IJPSDR.2024.160211