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Visit Journal at <https://www.jacsdirectory.com/jacs>Novel Pyrrolopyrimidine Based Dual Heterocyclic Hybrids as Antiproliferative Agents: Synthesis, *In-vitro* Assessment, Docking, and ADME ExplorationNagaraju Madala^{1,2}, Vishal Sharma^{3,*}, Srilalitha Vinnakota⁴¹Centre for Chemical Sciences and Technology, University College of Engineering Science and Technology, Jawaharlal Nehru Technological University, Hyderabad – 500 085, Telangana, India.²Medicinal Chemistry Division, VSN LABS, IDA Cherlapally, Phase-V, Hyderabad – 500 051, Telangana, India.³Discovery Chemistry Division, Jubilant Biosys Ltd, Knowledge Park II, Greater Noida – 201 306, Uttar Pradesh, India.⁴Dept. of Chemistry, Faculty of Science and Technology, ICFAI Foundation for Higher Education, Dontanpally, Hyderabad – 501 203, Telangana, India.

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ABSTRACT

The present work reports the synthesis of novel pyrrolo[2,3-*d*]pyrimidine and 1,3,4-oxadiazole hybrids bearing 1,2,3-triazole substituents, efficiently obtained via CuAAC click reactions. The results indicated that 1,2,3-triazole hybrids **5b** and **5g** demonstrated the highest cytotoxicity against A-549 and HCT-116 cells with IC₅₀ values of 6.12±1.15 μM and 8.01±1.72 μM and 5.20±0.99 μM and 6.60±1.90 μM, respectively, compared with the reference drug doxorubicin (5.40±1.75 μM for A-549 and 6.60±1.23 μM for HCT-116). Molecular docking revealed the structural basis for this activity, showing compounds **5b**, **5e** and **5g** strong binding to key target like EGFR mutant in lung cancer pocket of (PDB code: 8d76) via binding affinities ranging from –7.49 to –7.79 kcal/mol. The *in-silico* ADME/Tox analysis confirmed the drug-likeness of all the prepared compounds, indicating their potential for oral bioavailability and overall therapeutic efficacy. Collectively, these properties distinguish **5b**, **5e** and **5g**, positioning it as the most potent and selective anticancer lead.

1. Introduction

One of the biggest problems facing contemporary medicine and public health is cancer, which has a major negative impact on people's health, social lives, and economies all around the world [1]. In the near future, cancer is predicted to overtake cardiovascular disease as the leading cause of mortality worldwide. In 112 countries, cancer is the leading or secondary cause of death before the age of 70, according to World Health Organization (WHO) estimates for 2019 [2]. Additionally, 134 nations have cancer as their leading or principal cause of death, according to a 2020 report from the International Agency for Research on Cancer (IARC) [3]. The burden of cancer is expected to rise by more than 60% over the next 20 years, adding to the burden on people, communities, and healthcare systems [4]. It is estimated that 35 million new cases of cancer will occur by 2050 [5]. Lung and colon cancer rates are 11.4% and 18.0%, respectively, according to GLOBOCAN 2020 statistics. Furthermore, according to the WHO, about 4 million people worldwide were diagnosed with lung or colon cancer in 2020. These cancers claimed the lives of almost 2.7 million people [6]. Creating novel medications with two or more bioactive components or combining two or more pharmacophores is one potential tactic to combat cancer cells' resistance to treatment.

Bioactive substances, both synthetic and natural, especially heterocyclic systems, are crucial for the creation of novel scaffolds in medicinal chemistry [7]. Because of its nitrogen-rich structure, which permits a variety of intermolecular interactions like hydrogen bonding and coordination with biological targets, the 1,2,3-triazole ring is a highly adaptable heterocyclic scaffold [8]. Numerous biological pathways have been demonstrated to be modulated by compounds containing this moiety, leading to a wide range of pharmacological actions, such as antibacterial, antifungal [9], antioxidant [10], antitubercular [11], antidepressant [12], antimalarial [13], anti-inflammatory [14], antiviral [15], and most notably anticancer effects [16, 17]. In parallel with the extensive interest in 1,2,3-triazole-based scaffolds, several clinically established drugs, including cefatrizine and tazobactam, incorporate this heterocycle within their molecular architectures and are widely applied as

antibiotics in clinical practice [18]. Oxadiazoles are novel heterocyclic compounds with substantial applications in synthetic and medicinal chemistry [19]. Among the isomers, 1,3,4-oxadiazoles play an important role in medicinal chemistry and display a wide range of biological actions, such as anticancer [20], antibacterial [21], anti-tuberculosis [22], antimalarial [23], anticonvulsant [24], antidiabetic [25], and anti-inflammatory [26]. In addition, various medications having a 1,3,4-oxadiazole core are available in the market, including raltegravir (antiretroviral drug), furamizole (antibiotic drug), nesapidil (antiarrhythmic drug), zibotentan (anticancer agent), fenadiazole (hypnotic drug), and tiodazosin (adrenergic receptor antagonist).

Because nitrogen-containing heterocycles can establish hydrogen bonds, hydrophobic effects, Van der Waals forces, dipole-dipole interactions, and π -stacking interactions with biological targets, they make up over 80% of FDA-approved commercial medications [27]. The majority of nucleotides contain the pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) scaffold, a special heterocyclic system. Plants, DNA and RNA, and synthetic derivatives that are essential to biology fall under this category [28]. According to pharmacological research, pyrrolo[2,3-*d*]pyrimidines have a variety of bioactivities, such as kinase inhibitory [35], anticancer [29], antibacterial [30], antifungal [31], antitumor [32], antiviral [33], and anti-inflammatory [34]. However, because of their potent UV-blue fluorescence, pyrrolo[2,3-*d*]pyrimidine analogues have lately been employed as fluorescent functional materials [36]. Many drugs that inhibit tyrosine kinases, such as ribociclib, ruxolitinib, tofacitinib, and baricitinib, have a pyrrolo[2,3-*d*]pyrimidine core in their skeleton and have received FDA approval [37].

In light of this, we have persisted in our attempts to create novel anticancer medicines in order to reduce the cytotoxic side effects while enhancing their pharmacological profile and therapeutic performance. We devised a logical plan to substitute a 1,3,4-oxadiazole and 1,2,3-triazole bioisostere for the pyrrolo[2,3-*d*]pyrimidine carboxylic acid functional group. It is expected that this alteration will maintain the compound's mechanism of action while improving its pharmacokinetic and pharmacodynamic characteristics. Along with doing *in silico* docking analysis, ADME (absorption, distribution, metabolism, and excretion) evaluations, we also assessed the *in vitro* anticancer activity against lung and colon cancer cell lines.

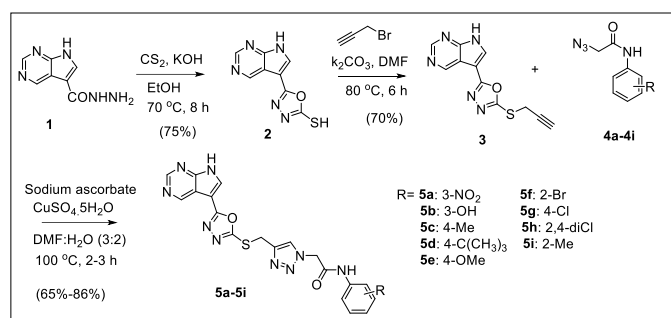
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2. Results and Discussion

2.1 Chemistry

Pyrrolo[2,3-*d*]pyrimidine derivatives were synthesized starting from 7H-pyrrolo[2,3-*d*]pyrimidine-5-carbohydrazide, which was prepared using previously reported methods using 2-fluoro-5-nitrobenzaldehyde. The 5-(7H-pyrrolo[2,3-*d*]pyrimidin-5-yl)-1,3,4-oxadiazole-2-thiol (**2**) was synthesized by treating with 7H-pyrrolo[2,3-*d*]pyrimidine-5-carbohydrazide (**1**), carbon disulphide, and potassium hydroxide in ethanol to obtain the cyclized oxadiazole product **3**. Then, propargylation reaction with produced oxadiazole and propargyl bromide through an *S*-alkylation reaction subsequently accompanied by the removal of HBr molecule. To obtain the matching phenyl acetamides, were obtained by the refluxing for five hours using a -stirred solution of corresponding 2-chloro-*N*-phenylacetamides and sodium azide in potassium carbonate in acetone. Specifically, the CuAAC reaction of **3** with corresponding 2-azido-*N*-phenylacetamide derivatives in DMF:H₂O (3:2), using a catalytic amount of CuSO₄·5H₂O and Sodium ascorbate, resulted in the formation of the corresponding 1,2,3-triazole compounds **5a-i** (Scheme 1) (65–86%).



Scheme 1 Synthetic route for final oxadiazolyl-triazole **5a-5i**

The synthesized compounds were confirmed with the help of ¹H NMR, ¹³C NMR, and mass spectrometry. The ¹H nuclear magnetic resonance spectrum of **5a** exhibited two broad singlets at δ 10.70 and 9.80 ppm, were assigned amide-NH and pyrrole-NH. The multiple peaks observed at δ 8.56–6.85 ppm correspond to aromatic protons of the triazole compound **5a**. The two singlets appeared at 5.10 belongs to methylene in acetamide and at 4.35 δ ppm indicates the methylthio carbon. The two singlets appeared at 8.91 and 7.68 δ ppm corresponding to pyrimidine and triazole ring protons. The carbonyl and pyrimidine ring carbons are represented at 169.1, 151.6, and 147.3 δ ppm in the ¹³C NMR spectrum. Two peaks at 57.3 and 36.4 δ ppm belong to the acetamide and methylthio carbons, whereas the remaining aromatic carbons resonance signals appeared at 143.1–111.3 ppm. In the mass spectrum, compound **5a** produced a molecular ion peak at *m/z* 479.8 (*M*+1)⁺ to confirm the structure. In the ¹H NMR spectra of **5e**, the multiple peaks observed at δ 8.91–7.25 ppm correspond to aromatic protons of the phenyl ring. The singlet at δ 3.35 ppm was assigned to the methoxy protons attached to the phenyl group. In the ¹³C NMR spectrum of compound **5e**, the resonance signals at δ 168.7 and 147.4 ppm were shown to the pyrimidine and triazole moiety. The chemical shift at δ 158.5 ppm was assigned to the carbon atom of the methoxy-substituted phenyl ring, while the singlet peak at δ 60.1 ppm matches the methoxy group itself. Further confirmation was obtained from the mass spectrum, which exhibited a peak at *m/z* 464.1 (*[M+1]*⁺), consistent with the chemical formula C₂₀H₁₇N₉O₃S.

2.2 Anticancer Activity

One of the main characteristics of cancer is unchecked cell growth, which results in the development of tumor masses by avoiding the system of programmed cell death. Using tiny chemicals to limit cancer cells' ability to proliferate, migrate, and invade, ultimately leading to cell death, is one promising method of cancer treatment. In this work, we tested a library of synthetic small compounds for anticancer activity against lung cancer cells A-549, NCI-H1975, and HCT-116, HT-29 colorectal. MTT assay [38] was used for all synthesized compounds **5a-i**, and Table 1 shows the IC₅₀ values of doxorubicin (DXN) as a reference. One important indicator of a compound's potency is the IC₅₀ value, which shows the concentration required to inhibit 50% of cell viability. The IC₅₀ values were computed using GraphPad Prism software, and results were expressed as mean ± SEM and the corresponding data are presented in Table 1. Compound (**5g**) had the highest anticancer impact on colon cancer among the studied chemicals, indicating a considerable reduction of cell growth. With IC₅₀

values ranging from 5.20±0.99 to 8.60±1.05 μM, compounds (**5b**) and (**5e**) were more potent against breast cancer than other compounds. Compound **5e** had the most anticancer activity among the synthesized pyrrolo[2,3-*d*]pyrimidines on the A549 cell line (90.80% inhibition, IC₅₀=5.80±1.18 μM), closely followed by **5b** (86.45%, 6.12±1.15 μM) and **5g** (84.90%, 6.60±1.90 μM) (Fig. 1). Compared to other analogues, these compounds showed IC₅₀ values of 5.80 μM and 6.60 μM, demonstrating good inhibitory activity against breast cancer. While 2-bromophenyl substituent **5f** recorded the lowest activity (IC₅₀=43.20 ±1.11 μM), indicating a comparatively lesser efficacy, compounds **5d** and **5i** also displayed modest IC₅₀ values (15.30±1.08–20.12±1.55 μM).

Table 1 Cytotoxicity effects of the pyrrolo[2,3-*d*]pyrimidine linked triazole compounds on lung and colon cancer cell lines

Entry	Lung cancer cell lines				Colon cancer cell lines			
	A-549	NCI-H1975			HCT-116	HT-29		
	IC ₅₀ (μM)	SD	IC ₅₀ (μM)	SD	IC ₅₀ (μM)	SD	IC ₅₀ (μM)	SD
5a	29.40	0.94	27.10	1.10	NC		NC	
5b	6.12	1.15	7.05	0.88	8.01	1.72	8.60	1.05
5c	NC		22.50	0.73	NC		28.85	1.09
5d	15.30	1.08	NC		12.60	0.80	NC	
5e	5.80	1.18	8.27	2.10	8.20	1.10	6.30	1.07
5f	43.20	1.11	NC		NC		40.50	1.11
5g	6.60	1.90	7.50	1.04	5.20	0.99	9.30	0.89
5h	NC		30.80	1.10	22.20	1.09	NC	
5i	20.12	1.55	24.08	1.06	33.80	1.50	38.33	1.13
DXN	6.60	1.23	7.19	0.88	5.40	1.75	6.80	1.35

DXN: Doxorubicin was used as reference drug. All the biological experiments were conducted in triplicate and the results are shown to be the mean values of three independent experiments. Biological activities were shown to be *n* = 3, ±SD = 3. NC, noncytotoxic. IC₅₀>50 μM denoted as noncytotoxic; The most potent compounds are shown in bold. (*P*≤0.05) significantly different using the unpaired *t*-test in GraphPad prism 10.6.1

Compound **5b**, incorporating a 3-hydroxyphenyl core linked to a 1,2,3-triazole acetamide substituent, displayed a complementary pattern of activity, showing potent effects against NCI-H1975 (IC₅₀=7.05±0.88 μM), HCT-116 colon cancer (IC₅₀=8.01±1.72 μM), and HT-29 colon cancer (IC₅₀=8.60±1.05 μM), consistent with selective targeting of rapidly proliferating or metabolically active cells. In contrast, excellent activity was observed against NCI-H1975 breast cancer cells (IC₅₀=8.27±2.10 and 7.50±1.04 μM), suggesting differences in cellular uptake, target engagement, or pathway susceptibility. Within the same series, **5a**, **5c** and **5i**, comprising a pyrrolo[2,3-*d*]pyrimidine core conjugated to a 1,2,3-triazole bearing a 3-nitro, 4-methyl and 2-methyl phenyl substituents, showed moderate activity against NCI-H1975 lung cancer cells (IC₅₀=27.10±1.10, 22.50±0.73 and 24.08±1.06 μM, respectively). In contrast, **5e** and **5g** displayed pronounced cytotoxicity, with IC₅₀ values 8.20±1.10 to 5.20±0.99 μM toward HCT-116 colon cancer cells. It was discovered that compounds **5b**, **5e**, and **5g** have a highly significant anticancer profile against HT-29 cells. Compared to DXN, an anticancer drug with an IC₅₀ of 6.80±1.35 μM, their inhibitory concentration readings of 8.60±1.05, 6.30±1.07, and 9.30±0.89 μM suggested greater efficacy. On colon HT-29 cells, the produced triazole-linked pyrimidines (**5a** and **5h**) did not exhibit any appreciable anticancer activity. Compounds **5f** and **5i** showed strong anticancer activity against HT-29, with IC₅₀ values of 40.50±1.11 and 38.33±1.13 μM, respectively. With IC₅₀ values of 12.60±0.80 and 22.20±1.09 μM, respectively, the additional dual heterocyclics, **5d** and **5h**, showed moderate anticancer potential. These compounds IC₅₀ values demonstrated that substitution was well tolerated. Conversely, all other substances exhibited a moderate level of efficacy against the four cancer cell lines.

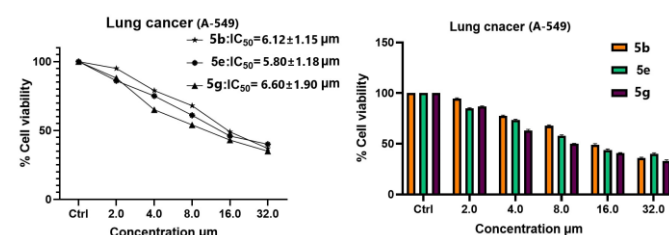


Fig. 1 Graph represents for % cell viability of 1,2,3-triazolyl oxadiazole compounds. Anticancer effects of compounds **5b**, **5e** and **5g** with IC₅₀ values in μg mL⁻¹ (Left side). Cell viability (%) was accessed from A-549 lung cancer with concentrations 2.0, 4.0, 8.0, 16.0, and 32.0 μM of compounds **5b**, **5e** and **5g** (right side). For this assay, cell viability was assessed using the MTT assay. Data are expressed as mean ± standard deviation (SD); (*n*=3). (**p*<0.05; ***p*<0.01; ****p*<0.001)

2.3 In-Silico Studies

2.3.1 Docking Analysis

All the synthesized compounds were docked into the EGFR mutant in lung cancer pocket of (PDB code: 8d76) [39, 40]. The results (Table 2) show that the selected compounds exhibited favorable binding affinities, with docking scores ranging from -7.49 to -7.79 kcal/mol. The triazole compounds (**5b**, **5e** and **5g**, which displayed the strongest lung cancer cell viability activity *in vitro*, with IC₅₀ values of 35.67 and 46.44 μ M, respectively, showed comparatively low binding energies of -7.49 to -7.79 kcal/mol. These results suggest that these ligands (**5b**, **5e** and **5g**) may interact more effectively with the lung cancer active site, comprising the hydrophobic cavity and the hydrophilic side pocket. Docking reports of **5b** showed three extensive H-bond interactions, one is triazole with the CysA:797 (2.16 Å), second one is hydroxy with TyrA:998 (1.98 Å) and third one is hydroxy with ProA:794 [1.77 Å] residues. It is known that the π -sigma bond between an pyrrolo[2,3-*d*]pyrimidine and the backbone of LeuA:718 (3.78 Å), a distinguishing feature of the binding mode of this class of inhibitors (Fig. 2). π -Donor H bond interactions are critically important in drug design, as they play a key role in determining the binding affinity, specificity, and overall effectiveness of a drug. Additionally, the oxadiazole and pyrimidine ring establishes π -Donor H bond and CH bonds with GlyA:796 3.45, ProA:794 3.98, and CysA:797 (2.16 Å), enabling selective interaction with the lung pocket. Derivative **5b** also showed a π -alkyl interaction with AlaA:743, LeuA:792, LeuA:844, ValA:726, highlighting the complementarity between the ligand's hydrophobic regions and the lipophilic side pocket of lung cancer active site. Notably, compound **5a** forms a van der Waals interactions with MetA:793, GluA:804, PheA:795, TyrA:801, and HisA:805 residues, located at the entrance of the active-site cavity and known to play a key role in substrate orientation.

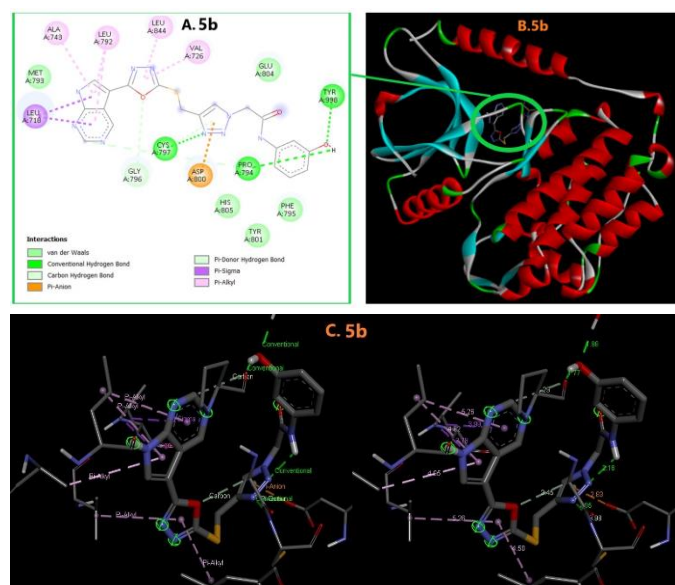


Fig. 2 A. 2D and B. 3D representations of ligand-protein interactions within the binding pocket for compound **5b** (PDB: 8d76). In 2D visualization, green denote hydrogen bond acceptor or donor residues, and other colors represent non-covalent interactions such as hydrophobic, aromatic, or electrostatic contacts. C. types of bond and bond distances between ligand and protein (8d76)

The molecule **5e** interacts particularly well with the active site of 8d76 protein, exhibiting a variety of interactions with different residues and distances. 1,3,4-Oxadiazole ring also formed key π -anion and π -sulphur

bonds with residues AspA:800, CysA:797 at distance 4.41 and 3.59 Å. The important H-bond was identified between the pyrrole linker and GluA:804 (1.90 Å) and amide linker and MetA:793 (2.52 Å). Additional π -alkyl interactions were established between the triazole and p-methoxy phenyl ring with TyrA:998, LeuA:792, LeuA:718, and LeuA:844 at distance ranging from 4.28-5.26 Å. These molecular features may explain the highest docking score and significant anticancer activity observed in the *in vitro* assay.

Table 2 Analysis of the optimal docking pose, including atomic contact energy values (kcal/mol), number of H-bonds, hydrophobic contacts, and interacting residues in the active site of EGFR mutant in lung cancer (PDB: 8d76)

Ligand	Target protein	ΔG (kcal/mol)	No of H-bonds	Binding residues forming H-bonds	Binding residues forming hydrophobic bonds
5b	8d76	-7.79	3	CysA:797, TyrA:998, ProA:794	GlyA:796, ProA:794, LeuA:781, AlaA:743, LeuA:792, LeuA:844, ValA:726, MetA:793, GluA:804, PheA:795, TyrA:801, HisA:805
5e	8d76	-7.77	2	GluA:804, MetA:793	AspA:800, CysA:797, TyrA:998, LeuA:792, LeuA:718, LeuA:844, GlyA:719, LysA:728, LysA:716, ValA:726, GlyA:796, AlaA:743, PheA:795

2.3.2 In-Silico Pharmacokinetic Analysis

The five-letter acronym for absorption, distribution, metabolism, excretion, and toxicity (ADME-T) was used for the powerful scaffolds in order to evaluate the aspects of their drug-likeness. This investigation aims to verify the medication's potency and examine certain features of drug similarity. An extensive ADME analysis was carried out to evaluate the pharmacokinetic behavior and drug similarity of the discovered compounds. The factors of interest, such as oral bioavailability, solubility, lipophilicity, and toxicity, were predicted using the SwissADME and pkCSM web servers [41–43]. Based on the information gathered from the docking simulation experiments, the bioavailability properties of the eight most active compounds are shown in Table 3. Lipinski's rule of five states that a compound is deemed drug-like if it has a molecular weight of 500 Daltons or less, less than five hydrogen bond donors, a logP value of no more than five, and fewer than ten hydrogen bond acceptors. The findings demonstrate that several compounds have high lipophilicity values (XLOGP>5) and exceed the permitted molecular weight threshold of 500 g mol⁻¹ (except from **5f**), leading to violations of the Lipinski rule. Oral bioavailability and water solubility may be negatively impacted by certain physicochemical characteristics. These compounds have strong water solubility, as indicated by the calculated ESOL logS values (-3.22 to -4.10). Greater intestine absorption is indicated by a TPSA value below 140 Å², but insufficient cell membrane penetration is often indicated by a polar surface area above 140 Å². In general, compounds with a TPSA of less than 60 Å² have good bioavailability and can cross the blood-brain barrier. According to the data, **5a**, **5b**, and **5e** ligands have high TPSA (211.42, 185.83, and 174.83), which leads in poor drug bioavailability and absorption. The findings verify that every other derivative, with the exception of **5f** and **5h**, complies with Lipinski's principles. These compounds are further supported by the Veber, Ghose, Egan, Golden, and Muegge guidelines, which state that molecules having ten or less rotatable bonds and a TPSA of 140 Å² or less are regarded as drug-like.

Table 3 The molecular descriptor and parameters of Lipinski's rule for investigated compounds calculated using server [SwissADME]

S.No.	5a	5b	5c	5d	5e	5f	5g	5h	5i
MWt	478.09	449.10	447.12	489.17	463.12	511.02	467.89	501.03	447.12
nHA	14	12	11	11	12	11	8	11	11
nHD	2	3	2	2	2	2	2	2	2
nRot	9	8	8	9	9	8	8	8	8
Flexibility	0.321	0.296	0.296	0.333	0.333	0.296	0.297	0.296	0.296
TPSA (Å ²)	211.42	185.83	165.60	165.60	174.83	165.60	165.60	165.60	165.60
LogS	-3.87	-3.22	-3.25	-4.10	-3.49	-3.88	-4.01	-3.96	-3.54
LogP	1.81	1.45	1.83	3.58	1.82	2.06	2.63	2.85	2.19
LogD	2.39	2.24	2.48	3.54	2.55	2.77	2.96	3.32	2.81
Lipinski	Yes/1	Yes/1	Yes/1	Yes/1	Yes/1	Yes/2	Yes/1	Yes/2	Yes/1
Veber	No/1	No/1	No/1	No/1	No/1	No/1	No/1	No/1	No/1
Muegge	No/1	No/1	No/1	No/1	No/1	No/1	No/1	No/1	No/1

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3. Experimental Methods

3.1 Materials and Methods

The melting point of all titled compounds was determined on the Casiae–Siamia device and is uncorrected. Commercially available solvents and reagents were distilled before use from supplier. ^1H -NMR and ^{13}C -NMR spectra were recorded on a Bruker AV 400 MHz and 100 MHz spectrometer, respectively, using solvent $\text{DMSO}-d_6$ and tetramethyl silane (TMS) as an internal standard; chemical shift is shown in (δ) ppm, and coupling constant (J) was expressed in Hz. Thin-layer chromatography (TLC) was performed on aluminium sheets covered with silica gel 60F254, and the spots are visualised with UV light at 256 nm. Pure compounds were isolated using flash column chromatography with silica gel Merak (100–200 Mesh). ESI-Mass was recorded using an Agilent 6230B time of flight (TOF) instrument.

3.2 Preparation of 5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazole-2-thiol (**2**)

To a stirred solution of 7H-pyrrolo[2,3-d]pyrimidine-5-carbohydrazide (**1**) (2.5 g, 0.014 mol) in ethanol (25 mL), potassium hydroxide (1.6 g, 0.028 mol) was slowly added with continuous stirring between 0 °C and 5 °C. Subsequently, carbon disulphide (3.2 g, 0.042 mol) was added slowly over 10–15 min at same temperature. After warming to refluxing temperature, the reaction mixture was stirred for a further 8 h. Thin-layer chromatography was used to monitor the reaction's development (20% methanol in chloroform). After the end, the reaction mixture was poured into the crushed ice water, neutralized with dil. HCl and stirred until all solids had dissolved. Dichloromethane (2 x 50 mL) was used to extract the crude mixture, followed by water (100 mL) and brine (20 mL) that were used to wash the organic layer, which was then dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was further processed and purified by flash column chromatography with a solvent system of 20% ethyl acetate in hexane elute pure; compound **2** yield (75%), a brown solid, mp. 185 °C–187 °C.

3.3 Preparation of 2-(Prop-2-yn-1-ylthio)-5-(7H-pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazole (**3**)

Compound **2** (2.0 g, 0.009 mol, 1 equiv), was suspended in *N,N*-dimethylformamide (20 mL) in a stirred round-bottom flask, and potassium carbonate (1.5 g, 0.010 mol) was added under a nitrogen atmosphere for 10 min. Propargyl bromide (1.4 g, 0.011 mol) was so added while stirring. The reaction mixture was then given six hours to reflux. TLC was later used to track the reaction process. After the solvent was eliminated by rotary evaporation, the crude product was added to 150 mL of water, acidified with diluted HCl, and extracted using dichloromethane (2 x 50 mL). *S*-alkylated-1,3,4-oxadiazole (**3**) was obtained as a brown solid when the combined organic layers were dried over anhydrous Na_2SO_4 , concentrated by decreased pressure, and purified by flash column chromatography on silica gel (20% EtOAc/hexanes). Yield (70%). mp. 192 °C–194 °C.

3.4 General Procedure for the Preparation of 2-Azido-*N*-Phenylacetamide Derivatives (**4a-i**)

Triethyl amine, aromatic amine, and chloroacetyl chloride were dissolved in DCM and agitated for three hours at room temperature. The equivalent pure product was obtained by crystallizing the crude solid in ethanol once the reaction was finished. After adding the proper sodium azide and chloroacetamide derivative to 20 mL of ethanol, the reaction mixture was agitated for 8–9 hours at 70 °C. After the reaction was finished, the liquid was diluted with dichloromethane and cleaned with brine and water. The crude residue was recrystallized from ethanol after the mixed organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*.

3.5 General Procedure for the Preparation of Final Triazolyl Pyrrolo[2,3-d]pyrimidine Derivatives (**5a-j**)

A mixture of 2-(prop-2-yn-1-ylthio)-5-(7H-pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazole (**3**) (1.5 g, 0.005 mol) and copper sulphate pentahydrate (1.1 g, 0.004 mol) were added in a mixture of DMF: H_2O (3:2) (15 mL). Subsequently, an appropriate 2-azido-*N*-phenylacetamide derivative (**4a**, 1.9 g, 0.008 mol) and g of sodium ascorbate (1.0 g, 0.004 mol) were introduced into the reaction at room temperature. The reaction mixture was stirred in a nitrogen atmosphere for two to three hours at 100

°C. The reaction mixture was cooled to room temperature once the reaction (which was tracked by TLC) was finished, and the solvent was subsequently evaporated under low pressure. After adding the reaction mixture to water, it was extracted using dichloromethane (2 x 50 mL) and brine (2 x 20 mL). The mixed organic layers were concentrated under low pressure after being dried over anhydrous Na_2SO_4 . After the residue was purified by flash column chromatography and eluted with ethyl acetate/petroleum ether, the corresponding 1,2,3-triazole derivative **5a** (yield) was obtained as a yellow solid.

3.6 Spectroscopic Data

3.6.1 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-*N*-(3-nitrophenyl)acetamide (**5a**)

Yellow solid, mp. 194–196 °C. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 10.70 (1H, brs, NH), 9.80 (1H, brs, NH), 8.91 (1H, s, Ar-H), 8.56 (1H, s, Ar-H), 8.27 (1H, s, Ar-H), 7.68 (1H, s, triazole-H), 7.60 (1H, d, $J=10.6$ Hz, Ar-H), 7.17 (1H, d, $J=10.6$ Hz, Ar-H), 7.10 (1H, t, $J=10.4$ Hz, Ar-H), 6.85 (1H, s, Ar-H), 5.10 (2H, s, NCH_2), 4.35 (2H, s, SCH_2). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ : 169.1, 163.5, 151.6, 147.3, 143.1, 140.5, 138.4, 133.3, 129.9, 129.2, 123.9, 120.8, 120.1, 113.2, 111.3, 57.3, 36.4. Mass (m/z): 479.8 [$\text{M}+1$] $^+$.

3.6.2 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-*N*-(3-hydroxyphenyl)acetamide (**5b**)

Off white solid, mp. 200–202 °C. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 10.72 (1H, brs, NH), 10.08 (1H, brs, OH), 9.67 (1H, brs, NH), 8.69 (1H, s, Ar-H), 8.43 (1H, s, Ar-H), 8.09 (1H, s, triazole-H), 7.90 (1H, t, $J=10.4$ Hz, Ar-H), 7.68 (1H, d, $J=10.6$ Hz, Ar-H), 7.41 (1H, s, Ar-H), 7.13 (1H, d, $J=10.6$ Hz, Ar-H), 7.07 (1H, s, Ar-H), 5.22 (2H, s, NCH_2), 4.49 (2H, s, SCH_2). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ : 166.4, 164.6, 158.5, 151.2, 149.1, 147.3, 133.3, 132.4, 129.7, 127.5, 123.5, 120.9, 120.1, 113.2, 111.3, 58.6, 34.5. Mass (m/z): 450.7 [$\text{M}+1$] $^+$.

3.6.3 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-*N*-(*p*-tolyl)acetamide (**5c**)

Off white solid, mp. 164–166 °C. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 10.41 (1H, brs, NH), 9.70 (1H, brs, NH), 8.97 (1H, s, Ar-H), 8.64 (1H, s, Ar-H), 7.73 (1H, s, triazole-H), 7.58 (2H, d, $J=10.5$ Hz, Ar-H), 7.30 (2H, d, $J=10.5$ Hz, Ar-H), 6.75 (1H, s, Ar-H), 5.30 (2H, s, NCH_2), 4.55 (2H, s, SCH_2), 2.20 (3H, s, ArCH_3). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ : 169.2, 165.8, 151.3, 146.8, 139.5, 135.7, 133.2, 131.7, 129.6, 129.3, 127.2, 123.9, 120.0, 111.1, 51.6, 35.6, 26.4. Mass (m/z): 448.3 [$\text{M}+1$] $^+$.

3.6.4 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-*N*-(4-(*tert*-butyl)phenyl)acetamide (**5d**)

Off white solid, mp. 158–160 °C. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 10.85 (1H, brs, NH), 10.13 (1H, brs, NH), 9.08 (1H, s, Ar-H), 8.69 (1H, s, Ar-H), 7.84 (1H, s, triazole-H), 7.67 (2H, d, $J=10.2$ Hz, Ar-H), 7.37 (2H, d, $J=10.2$ Hz, Ar-H), 6.84 (1H, s, Ar-H), 5.42 (2H, s, NCH_2), 4.66 (2H, s, SCH_2), 1.64 (9H, s, CH_3). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ : 173.1, 166.2, 151.6, 149.3, 146.8, 133.5, 133.3, 129.7, 127.1, 123.9, 120.6, 120.0, 116.4, 113.2, 111.1, 59.8, 36.0, 34.4, 30.7. Mass (m/z): 490.6 [$\text{M}+1$] $^+$.

3.6.5 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-*N*-(4-methoxyphenyl)acetamide (**5e**)

Light brown solid, mp. 165–167 °C. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 9.63 (1H, brs, NH), 9.30 (1H, brs, NH), 8.91 (1H, s, Ar-H), 8.62 (1H, s, Ar-H), 8.07 (1H, s, triazole-H), 7.86 (2H, d, $J=10.2$ Hz, Ar-H), 7.68 (2H, d, $J=10.2$ Hz, Ar-H), 7.25 (1H, s, Ar-H), 5.04 (2H, s, NCH_2), 4.35 (2H, s, SCH_2), 3.34 (3H, s, OCH_3). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ : 168.7, 165.5, 158.5, 151.6, 147.4, 132.9, 132.8, 129.2, 127.5, 123.7, 120.0, 111.3, 60.1, 50.8, 36.3. Mass (m/z): 464.1 [$\text{M}+1$] $^+$.

3.6.6 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-*N*-(2-bromophenyl)acetamide (**5f**)

Brown solid, mp. 150–152 °C. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 9.86 (1H, brs, NH), 9.11 (1H, brs, NH), 8.58 (1H, s, Ar-H), 8.23 (1H, s, Ar-H), 7.71 (1H, d, $J=10.6$ Hz, Ar-H), 7.50 (1H, s, triazole-H), 7.31 (1H, d, $J=10.6$ Hz, Ar-H), 7.15 (1H, t, $J=10.6$ Hz, Ar-H), 7.09 (1H, d, $J=10.6$ Hz, Ar-H), 6.71 (1H, s, Ar-H), 4.75 (2H, s, NCH_2), 3.76 (2H, s, SCH_2). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) δ : 168.6, 164.3, 151.2, 146.9, 141.2, 139.5, 135.5, 131.0, 130.3, 129.4, 127.6, 123.7, 119.8, 110.9, 59.2, 36.7. Mass (m/z): 512.3 [$\text{M}+1$] $^+$.

3.6.7 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-

yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-chlorophenyl)acetamide (**5g**)

White solid, mp. 168–170 °C. ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 10.53 (1H, brs, NH), 9.53 (1H, brs, NH), 8.70 (1H, s, Ar-H), 8.31 (1H, s, Ar-H), 7.60 (1H, s, triazole-H), 7.41 (2H, d, *J*=10.2 Hz, Ar-H), 7.07 (1H, s, Ar-H), 6.99 (2H, d, *J*=10.2 Hz, Ar-H), 5.23 (2H, s, NCH₂), 4.50 (2H, s, SCH₂). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 163.2, 158.0, 151.6, 149.3, 137.6, 133.5, 131.0, 129.9, 125.6, 123.8, 120.5, 120.0, 114.8, 113.2, 111.4, 56.1, 33.1. Mass (*m/z*): 468.4 [M+1]⁺.

3.6.8 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-

yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2,4-dichlorophenyl)acetamide

(**5h**)

White solid, mp. 175–177 °C. ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 10.12 (1H, brs, NH), 9.82 (1H, brs, NH), 9.12 (1H, s, Ar-H), 8.70 (1H, s, Ar-H), 8.02 (1H, s, Ar-H), 7.84 (1H, d, *J*=10.6 Hz, Ar-H), 7.59 (1H, s, triazole-H), 7.31 (1H, d, *J*=10.6 Hz, Ar-H), 6.94 (1H, s, Ar-H), 5.37 (2H, s, NCH₂), 4.75 (2H, s, SCH₂). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 170.3, 165.6, 158.5, 151.9, 147.4, 138.7, 135.6, 133.2, 131.8, 130.2, 129.7, 129.1, 123.5, 120.6, 111.6, 56.5, 36.4. Mass (*m/z*): 503.1 [M+1]⁺.

3.6.9 2-(4-(((5-(7H-Pyrrolo[2,3-d]pyrimidin-5-yl)-1,3,4-oxadiazol-2-

yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(*o*-tolyl)acetamide (**5i**)

Off white solid, mp. 150–152 °C. ¹H NMR (DMSO-*d*₆, 400 MHz) δ: 10.19 (1H, brs, NH), 9.51 (1H, brs, NH), 8.76 (1H, s, Ar-H), 8.41 (1H, s, Ar-H), 7.83 (1H, s, triazole-H), 7.60 (1H, d, *J*=10.6 Hz, Ar-H), 7.29 (1H, d, *J*=10.6 Hz, Ar-H), 7.19 (1H, t, *J*=10.4 Hz, Ar-H), 7.11 (1H, t, *J*=10.4 Hz, Ar-H), 6.78 (1H, s, Ar-H), 5.10 (2H, s, NCH₂), 4.26 (2H, s, SCH₂), 2.30 (3H, s, ArCH₃). ¹³C NMR (DMSO-*d*₆, 100 MHz) δ: 171.4, 164.3, 154.5, 151.7, 134.4, 130.3, 129.9, 129.4, 128.9, 123.9, 120.1, 114.5, 111.0, 54.3, 36.4, 22.6. Mass (*m/z*): 448.7 [M+1]⁺.

3.7 Anticancer Activity

Cell lines HCT-116, HT-29 (colorectal cancer), and A-549, NCI-H1975 (lung cancer) were obtained from the clinical laboratory at National Centre for Cell Sciences (NCCS), Pune, India. All cell lines were cultured in DMEM medium, supplemented with 10% fetal bovine serum, 1% glutamine, and penicillin/streptomycin in a humidified atmosphere containing 5% CO₂ at 37 °C.

Cell viability is tested using Trypan blue staining. In the MTT assay, the yellow dye MTT that dissolves in water are changed by mitochondrial reductase into a purple formazan that is non-soluble. The water-insoluble formazan produced is solubilized in a solubilization buffer, and absorbances are detected.

In 96-well plates, the cells were cultivated at a density of 5 × 10³ cells/well using 100 μL medium. The cells were cultured for 24, 48, and 72 h after being exposed to different concentrations of etodolac and its derivatives (2, 4, 8, 16, 32, 50, and 100 μM). 10 μL of the MTT labelling reagent (final concentration 0.5 mg/mL) were added to each well at the end of the incubation periods, and the wells were incubated for an additional 4 h.

Next, each well received 100 μL of solubilization buffer (10% SDS in 0.01 M HCl) and was overnight incubated, and the absorbance of formazan products was evaluated at 550 nm using a multi-mode microplate reader (Synergy H1, Biotec Instruments Inc., Vermont, USA). The results were normalized relative to the absorbances of calculated percent concentrations of DMSO. All tests involved three replicates with three independent experiments.

3.8 Docking Analysis

Using Auto Dock Vina, molecular docking was performed to determine the binding affinity of the protein-ligand complexes, with binding energies reported in kcal/mol.

The docking process includes developing a grid box that completely covers the target protein's active site, with grid dimensions set to (x=40, y=40, z=40) and the grid center coordinates placed at (x=-1.7890, y=37.6273, z=66.2371 of PDB ID: 8d76). The virtual molecular docking was carried out using Auto Dock Vina version 1.5.7.

Biovia Discovery Studio Visualizer 2021 was used to visualize and analyze protein-ligand interactions, showing each ligand accurately docked into the designated active site of the protein (8d76). The compounds with the strongest interactions with the target protein were those with favorable binding scores, as indicated by the lowest (most negative) binding energies.

4. Conclusion

Hybridization of the 1,2,3-triazole nucleus with the 1,3,4-oxadiazole ring was designed to enhance the biological potential of both moieties, as each is known to exhibit diverse pharmacological properties. A novel series of triazole-oxadiazole linked pyrrolo[2,3-d]pyrimidine hybrid derivatives (**5a–i**) was synthesized through a three-step click reaction pathway and structurally confirmed by various spectroscopic (¹H-NMR, ¹³C-NMR, and mass) analyses. The compounds **5b** and **5e** demonstrated selective cytotoxicity, showing IC₅₀ values of 6.12±1.15 μM against A-549 and 6.30±1.07 μM against HT-29 cells, while maintaining notably higher toxicity than DXN (IC₅₀=6.60±1.23 μM). Molecular docking studies confirmed strong binding affinities with the EGFR mutant in lung cancer-associated protein 8d76, stabilized through key hydrogen bonds (e.g., with TyrA:998, GluA:804, MetA:793, and AspA:800) and hydrophobic interactions, including π-sigma stacking with LeuA:718. Compound **5g** was found to be the most advantageous lung and colon active site by ADME profiling, and molecular docking, which corroborated the experimental results. Based on computational and experimental evaluations, this integrated approach identifies molecules as selectively anticancer candidate scaffolds with a favorable selectivity profile, indicating the need for additional lead optimization.

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