STRUCTURE-BASED MULTITARGETED MOLECULAR DOCKING ANALYSIS OF PYRAZOLE-CONDENSED HETEROCYCLES AGAINST LUNG CANCER

JAINEY P. JAMES1*, AISWARYA T. C.1, SNEH PRIYA1, DIVYA JYOTHI1, SHESHAGIRI R. DIXIT2

1Nitte (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGS MIPS), Deralakatte, Mangaluru 575018, Karnataka, India, 2Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru 570015, Karnataka, India

Email: jaineyjames@nitte.edu.in

Received: 02 Aug 2021, Revised and Accepted: 03 Sep 2021

ABSTRACT

Objective: The significant drawbacks of chemotherapy are that it destroys healthy cells, resulting in adverse effects. Hence, there is a need to adopt new techniques to develop cancer-specific chemicals that target the molecular pathways in a non-toxic fashion. This study aims to screen pyrazole-condensed heterocycles for their anticancer activities and analyse their enzyme inhibitory potentials EGFR, ALK, VEGFR and TNKS receptors.

Methods: The structures of the compounds were confirmed by IR, NMR and Mass spectral studies. The in silico techniques applied in this study were molecular docking and pharmacophore modeling to analyze the protein-ligand interactions, as they have a significant role in drug discovery. Drug-likeness properties were assessed by the Lipinski rule of five and ADMET properties. Anticancer activity was performed by in vitro MTT assay on lung cancer cell lines.

Results: The results confirm that all the synthesised pyrazole derivatives interacted well with the selected targets showing docking scores above 5 kcal/mol. Pyrazole 2e interacted well with all the four lung cancer targets with its stable binding mode and was found to be potent as per the in vitro reports, followed by compounds 3d and 2d. Pharmacophore modeling exposed the responsible features responsible for the anticancer action. ADMET properties reported that all the compounds were found to have properties within the standard limit. The activity spectra of the pyrazoles predicted that pyrazolopyridines (2a-2e) are more effective against specific receptors such as EGFR, ALK and Tankyrase.

Conclusion: Thus, this study suggests that the synthesised pyrazole derivatives can be further investigated to validate their enzyme inhibitory potentials by in vivo studies.

Keywords: Lung Cancer, Pyrazolopyridines, Pyrazolopyridines, Molecular docking, Pharmacophore modeling, Anticancer activities

INTRODUCTION

Lung cancer is one of the leading causes of cancer mortality in men and women, [1, 2] responsible for 1.6 million deaths. Non-small-cell lung cancers (NSCLCs), including large-cell carcinoma, adenocarcinoma, and squamous cell carcinoma, contribute approximately 80-85% of lung cancer.

The major shortcoming of lung cancer chemotherapy is that it causes damages to normal cells, causing surplus adverse effects. Therefore targeted therapies [3] are needed to target only cancer cells, avoiding injuries to the healthy cells. One of the novel methods adopted in lung cancer therapy is developing cancer-specific compounds that can attack the molecular signalling pathways, thus creating non-toxic substances. The significant targets of paramount importance for lung cancers are EGFR (Epidermal growth factor receptor) [4, 5] ALK (Anaplastic lymphoma tyrosine kinase) [6, 7] BRF (B-raf murine sarcoma viral oncogene homolog B1) [8, 9] VEGFR receptors (Vascular endothelial growth factor) [10, 11], and Wnt signalling pathway [12].

The EGFR receptor is recognized as a significant anticancer target. It belongs to the ErbB (epidermal growth factor receptor) tyrosine kinase family and is expressed at high levels on the surface of some cancer cells. The inhibition of EGFR plays a crucial role in angiogenesis, tumor suppression, and metastasis [13].

In anaplastic non-Hodgkin's lymphoma, the ALK gene was first described as a driver mutation. Dysregulated ALK expression is now an identified driver mutation in nearly twenty different human malignancies. The dysregulated ALK expression is now recognized as the driver mutation, including 4-9% of NSCLC [14].

One of the critical mediators promoting the angiogenesis process is VEGFR, as it has a prominent role in maintaining the vascular supply within the tumour. Its increased levels are a confirmary factor in diverse human cancers, including NSCLC [15]. The Wnt signaling pathway is another potential target for lung cancer. Effective pharmacological inhibitors of the Wnt pathway have only recently become available. The tankyrase (TNKS), a poly-ADP-ribose polymerase (PARP) enzyme, was the critical mediator of Wnt signaling by the screens for small molecular antagonists of the Wnt pathway. Hence, using the targets mentioned above as partial agonists/antagonists can show promising treatment strategies [16].

The approved therapeutic EGFR inhibitors are gefitinib, erlotinib, afatinib, osimertinib, dacomitinib [17], and ALK inhibitors crizotinib, alectinib, brigatinib, lorlatinib [18], VEGFR inhibitors are axitinib, bevacizumab, sorafenib [19] etc.

Nitrogen-containing heterocycle-pyrazole has a vital role in the development of cancer therapies. The anticancer activity of these compounds is by the inhibition of different types of proteins, receptors and enzymes, which has a crucial role in cell division. Condensed pyrazole rings such as pyrazolopyrimidines, pyrazolopyridines and pyranopyrazole are known for their anticancer properties [20], and the available drugs with these core moieties are depicted in fig. 1.

An extension of previous works on pyrazole scaffolds [21, 22] and in silico studies [23, 24], we have performed an analysis to screen the inhibitory potency of synthesized pyrazole fused derivatives on various targets EGFR, ALK, VEGFR and TNKS by employing molecular docking and pharmacophore modelling techniques.

MATERIALS AND METHODS

Most of the chemicals were purchased from Sigma Aldrich, and further purification was not required. Melting points were determined by the capillary method and were uncorrected. Shimadzu Perkin Ekmer 8201
Preparation of pyrazolopyrimidines (2a-2e) and pyrazolopyridines (3a-3e)

A solution of 0.01 mole of malonitrile/diethyl malonate and different pyrazole carbonitrile derivatives (0.01 mole) was prepared in sodium ethoxide and ethanol, which was refluxed for eight hours. The solution was concentrated, and the obtained residue was filtered, washed with ice-cold water [25].

Molecular docking and binding free energy calculation

Based on the literature, the selected targets for lung cancers are EGFR, ALK, VEGFR and tankyrase and their crystal structures (PDB ID: 4WKQ) [27], ALK (PDB ID: 4225) [28], VEGFR (PDB ID: 4AG8) [29], TNKS (PDB ID: 4WSS) [30] were awaited from the protein data bank. The downloaded proteins were minimized by Protein Preparation Wizard, using the OPLS-2005 force field of Schrodinger software. The designed fused-pyrazoles were prepared by LigPrep application (Schrodinger, 2019-4) [26] and were used for docking. The minimized protein was employed to generate the grid, and the grid box was developed by applying default parameters. Glide-XP (extra precision) [31] was used for molecular docking computations. The binding free energy MMGBSA (Molecular Mechanics, Generalized Born Model and Solvent Accessibility) dGbind (kcal/mol), between the receptor and ligands, were calculated by the Prime module (Schrodinger, 2019-4) [26]. The docking scores and the 2D and 3D conformations were generated to analyse further the affinities and binding interactions of the selected ten fused-pyrazole molecules.

In vitro anticancer study by MTT assay

We procured A-549 (Human small-cell lung carcinoma) cell culture from National Centre for Cell Sciences (NCCS), Pune, India. Ten available drugs with pyrazolopyrimidines and pyrazolopyridines moiety

Fig. 1: Available drugs with pyrazolopyrimidines and pyrazolopyridines moiety

Drug-likeness, ADMET property and prediction of activity spectral studies

The compounds were screened for drug-likeness properties by checking with the Lipinski Rule of five [32] and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) property prediction by the QikProp tool [26]. The features considered for ADMET studies are the following: QLogHERG, QPlogCaco Caco-2 cell permeability, QPlogKhsa, Percent Human Oral Absorption. Further, to validate them as appropriate drug candidates, an online tool, prediction of activity spectra for substances (PASS), was used, which evaluate the biological activity based on their structural data [33]. This tool gives the values for the probability of activity (Pa) and inactivity (Pi) by comparing more than 300 pharmacological effects and biochemical mechanisms of compounds.
compounds were incubated with different concentrations (25, 50, 100, 200 µM) to screen the cytotoxic activity of the compounds against human small-cell lung carcinoma (A-549). The cell viability was then determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after 24 h of incubation. Percent inhibition was calculated from the absorbance as % growth inhibition [34].

**RESULTS**

**Chemistry**

The fused pyrazole derivatives were synthesized from substituted aminopyrazoles cyclising with malononitrile and diethyl malonate to yield pyrazolopyridines and pyrazolopyrimidines. IR, NMR and mass spectroscopic techniques were used to confirm the structures (table 1).

| S. No. | Compound code | Structure | IR (KBr, cm⁻¹) | ¹H NMR (400 MHz, DMSO, δ/ppm) | LC-MS (m/z) |
|--------|---------------|-----------|----------------|-------------------------------|-------------|
| 1.     | 2a            | ![Structure_2a](image) | 1625.78 (C=N), 1583.61 (C=C), 3463.56 and 3296.45 (NH₂), 2213.54 (CN) | 3.67 (s, 2H, CH₂), 7.74 (s, 2H, NH₂), 8.19 (s, 1H, CH), 7.51-7.60 (m, 5H, Ar-H) | (M⁺) 250   |
| 2.     | 2b            | ![Structure_2b](image) | 1666.38 (C=N), 1592.32 (C=C), 3423.76 and 3265.45 (NH₂), 2219.34 (CN), 767.97 (C-Cl) | 3.12 (s, 2H, CH₂), 7.68 (s, 2H, NH₂), 8.33 (s, 1H, CH), 7.52-7.61 (m, 4H, Ar-H) | (M⁺) 284   |
| 3.     | 2c            | ![Structure_2c](image) | 1635.89 (C=N), 1593.82 (C=C), 3421.45 and 3288.32 (NH₂), 2214.43 (CN), 786.23 (C-Cl) | 3.31 (s, 2H, CH₂), 7.58 (s, 2H, NH₂), 8.35 (s, 1H, CH), 7.53-7.60 (m, 4H, Ar-H) | (M⁺) 284   |
| 4.     | 2d            | ![Structure_2d](image) | 1646.32 (C=N), 1594.69 (C=C), 3408.08 and 3269.67 (NH₂), 2245.76 (CN), 732.43 (C-Cl) | 3.52 (s, 2H, CH₂), 7.64 (s, 2H, NH₂), 8.42 (s, 1H, CH), 7.54-7.63 (m, 4H, Ar-H) | (M⁺) 284   |
| 5.     | 2e            | ![Structure_2e](image) | 1654.21 (C=N), 1591.81 (C=C), 3414.34 and 3285.67 (NH₂), 2249.76 (CN), 11.52 (s, 1H, OH) | 3.61 (s, 2H, CH₂), 7.73 (s, 2H, NH₂), 8.39 (s, 1H, CH), 7.57-7.64 (m, 4H, Ar-H) | (M⁺) 268   |
| 6.     | 3a            | ![Structure_3a](image) | 1653.08 (C=N), 1588.63 (C=C), 3444.54 and 3281.32 (NH₂), 2243.76 (CN), 1447.93 (C-F) | 3.34 (s, 2H, CH₂), 7.78 (s, 2H, NH₂), 8.41 (s, 1H, CH), 7.55-7.59 (m, 5H, Ar-H), 11.52 (s, 1H, OH) | (M⁺) 298   |
| 7.     | 3b            | ![Structure_3b](image) | 1687.24 (C=N), 1589.11 (C=C), 3451.65 and 3256.78 (NH₂), 2234.31 (CN), 778.98 (C-F) | 3.51 (s, 2H, CH₂), 7.62 (s, 2H, NH₂), 8.37 (s, 1H, CH), 7.52-7.58 (m, 4H, Ar-H), 11.23 (s, 1H, OH) | (M⁺) 332   |
| 8.     | 3c            | ![Structure_3c](image) | 1632.58 (C=N), 1589.31 (C=C), 3454.44 and 3268.91 (NH₂), 2247.04 (CN), 778.12 (C-CI) | 3.11 (s, 2H, CH₂), 7.55 (s, 2H, NH₂), 8.39 (s, 1H, CH), 7.37-7.57 (m, 4H, Ar-H), 11.05 (s, 1H, OH) | (M⁺) 332   |
| 9.     | 3d            | ![Structure_3d](image) | 1651.32 (C=N), 1598.12 (C=C), 3464.07 and 3256.17 (NH₂), 2241.75 (CN), 756.76 (C-CI) | 3.52 (s, 2H, CH₂), 7.77 (s, 2H, NH₂), 8.47 (s, 1H, CH), 7.50-7.56 (m, 4H, Ar-H), 11.41 (s, 1H, OH) | (M⁺) 332   |
| 10.    | 3e            | ![Structure_3e](image) | 1643.13 (C=N), 1591.32 (C=C), 3401.67 and 3239.31 (NH₂), 2208.89 (CN), 1432.76 (C-F) | 3.12 (s, 2H, CH₂), 7.67 (s, 2H, NH₂), 8.31 (s, 1H, CH), 7.48-7.60 (m, 4H, Ar-H), 11.41 (s, 1H, OH) | (M⁺) 316   |
Molecular docking

The docking and binding free energy scores obtained from their respective receptor targets, EGFR, ALK, VEGFR, and TNKS, confirmed the molecular interactions. The co-crystallised structures of gefitinib, ceritinib, axitinib, 3J1, which are active against lung cancer with the corresponding PDB IDs 4WKQ, 4Z55, 4AG8 and 4W5S, were obtained and found to have docking scores-8.80 kcal/mol, -11.36 kcal/mol, -14.41 kcal/mol, and -13.95 kcal/mol respectively; and their binding free energies are -95.15 kcal/mol, -100.94 kcal/mol, -123.86 kcal/mol and -101.34 kcal/mol towards their respective receptors EGFR, ALK, VEGFR and TNKS (table 1). The RMSD values of the crystallised structures showed RMSD values as 1.231 Å, 1.321 Å, 1.412 Å, 1.114 Å, respectively, which validated the docking results.

All the ten pyrazole derivatives screened for lung cancer targets exhibited docking values above 5 kcal/mol. The top pyrazole derivatives were 2e, 2d and 3d towards EGFR, ALK, VEGFR and TNKS. Their docking scores and binding affinity were given in Table 2. In these top evaluations, 2e showed the best docking conformation with docking scores -7.75, -7.23, -8.52 and -8.31 kcal/mol and binding free energy -53.42, -77.78, and -63.67 kcal/mol against, followed by pyrazoles 2d (-7.70,-7.13,-8.47,-8.22 kcal/mol) and 3d (-7.51,-7.20,-8.30,-8.01 kcal/mol) EGFR, ALK, VEGFR and TNKS respectively (table 2).

![Docking scores](image)

Table 2: Structures, docking score and MMGBSA dG bind of reference compounds

| Reference compounds available in PDB | Structures | Target receptors | Docking scores | MMGBSA dG Bind |
|--------------------------------------|------------|-----------------|----------------|----------------|
| Gefitinib                            | 4WKQ       | -8.806          | -95.15         |
| Ceritinib                            | 4Z55       | -11.362         | -100.94        |
| Axitinib                             | 4AG8       | -14.414         | -123.86        |
| 3J1                                  | 4W5S       | -13.953         | -101.34        |

To validate the chemical interactions, the analysis of co-crystall conformation are as follows: in enzyme EGFR, the common amino acids that make interactions with standard gefitinib and pyrazole derivatives are Gln 791, Thr 790 and Thr 854 (hydrophobic); Met 793 (hydrogen bond); Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 745, Met 766, Leu 788 (polar). Further, the common amino acids for the standard ceritinib and pyrazole derivatives that make interactions with enzyme ALK are Asn 1254; polar interactions are Leu 1122, Val 1130, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Val 1180 and hydrogen bond with the same amino acid Met 1199. Similarly, for the enzyme, VEGFR, the common amino acids for axitinib and pyrazole derivatives that bond by hydrophobic interactions is Asn 923 and polar interactions are Cys 919, Phe 918, Val 916, Leu 1035, Ala 866, Val 899, Phe 1047, Cys 1045, Leu 840, Val 848. In the case of the TNKS enzyme, for the ligand 3J1 and pyrazoles, the amino acids that frequently make hydrophobic interactions are Ser 1221, His 1194, Ser 1186; Tyr 1224, Tyr 1213, Phe 1214, Ala 1215, Phe 1188, Pro 1187; polar interactions are Ala 1215, Phe 1214, Tyr 1213, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224.

The finest docking conformations were also examined to reveal the primary interacting amino acid residues in the active pockets of EGFR, ALK, VEGFR and TNKS (table 3 and fig 2-5).

Table 3: Docking score and MMGBSA dG bind of pyrazole derivatives

| Compounds | Docking scores (in kcal/mol) | MMGBSA dG bind (in kcal/mol) |
|-----------|-----------------------------|-----------------------------|
|           | EGFR-4WKQ | ALK-4Z55 | VEGFR-4AG8 | TNKS-4W5S | EGFR-4WKQ | ALK-4Z55 | VEGFR-4AG8 | TNKS-4W5S |
| 2a        | -6.11     | -6.75    | -6.50     | -6.64     | -49.30    | -50.17    | -59.46     | -56.49    |
| 2b        | -6.08     | -6.04    | -6.17     | -7.96     | -48.88    | -55.08    | -52.09     | -56.23    |
| 2c        | -5.25     | -5.39    | -6.55     | -7.43     | -54.55    | -54.42    | -59.13     | -66.61    |
| 2d        | -7.70     | -7.13    | -8.47     | -8.23     | -54.62    | -48.27    | -69.69     | -67.16    |
| 2e        | -7.75     | -7.23    | -8.52     | -8.31     | -62.77    | -53.42    | -77.78     | -63.67    |
| 3a        | -6.97     | -7.04    | -7.83     | -7.06     | -61.04    | -53.37    | -68.07     | -58.26    |
| 3b        | -6.35     | -5.91    | -7.72     | -7.02     | -60.63    | -57.81    | -85.72     | -69.41    |
| 3c        | -7.44     | -7.06    | -7.66     | -7.94     | -65.99    | -59.02    | -81.08     | -79.86    |
| 3d        | -7.51     | -7.20    | -8.30     | -8.01     | -63.06    | -55.07    | -78.91     | -73.87    |
| 3e        | -7.21     | -6.75    | -7.48     | -7.91     | -60.18    | -50.9     | -70.68     | -64.4     |
### Table 4: Molecular interactions of reference compounds with the active site of protein

| Reference compounds | Protein and PDB IDs | Nature of interactions | Amino acids on active sites with |
|---------------------|---------------------|------------------------|---------------------------------|
| Gefitinib | EGFR-4WKQ | Hydrophobic Interaction, Polar Interactions | Gln 791, Thr 790, Thr 854, Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Ile 744, Ile 789, Leu 788, Leu 777, Met 766, Pro 794, Phe 795, Leu 788, Met 793, Cx 797 |
| Ceritinib | ALK-4Z5S | Hydrophobic Interaction, Polar Interactions | Asn 1254, Leu 1122, Val 1130, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Ala 1200, Val 1180, Met 1199, Glu 1197, Lys 1150 |
| Axitinib | VEGFR-4AG8 | Hydrophobic Interaction, Polar Interactions | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047, Val 867, Val 914, Leu 889, Asp 1046, Glu 885, Glu 917 |
| 3J1 | TNKS 1-4W5S | Hydrophobic Interaction, Polar Interactions | Tyr 1203, Ile 1228, Met 1207, Tyr 1224, Tyr 1213, Phe 1214, Ala 1215, Phe 1188, Pro 1187 |

### Table 5: Molecular interactions of the pyrazole derivatives with the active site of protein

| Compounds | Protein and PDB IDs | Nature of Interactions | Amino acids on active sites |
|-----------|---------------------|------------------------|-----------------------------|
| 2a | EGFR-4WKQ | Hydrophobic Interaction, Polar Interactions | Gln 791, Thr 790, Thr 854, Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Ile 744, Ile 789, Leu 788, Leu 777, Met 766, Met 793 |
|          | ALK-4Z5S | Hydrophobic Interaction, Polar Interactions | Asn 923, Cys 919, Phe 918, Val 1035, Ala 866, Val 899, Phe 1047, Cys 1045, Leu 840, Val 848 |
|          | VEGFR-4AG8 | Hydrophobic Interaction, Polar Interactions | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Phe 1047, Cys 1045, Leu 840, Val 848 |
|          | TNKS-4W5S | Hydrophobic Interaction, Polar Interactions | Ser 1221, Hid 1184, Ser 1186, Hid 1201 |
| 2b | EGFR-4WKQ | Hydrophobic Interaction, Polar Interactions | Gln 791, Thr 790, Thr 854, Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Ile 744, Ile 789, Leu 788, Leu 777, Met 766, Met 793 |
|          | ALK-4Z5S | Hydrophobic Interaction, Polar Interactions | Asp 855, Leu 1122, Val 1130, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Val 1180, Ala 1126 |
|          | VEGFR-4AG8 | Hydrophobic Interaction, Polar Interactions | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Phe 1047, Cys 1045, Leu 840, Val 848 |
|          | TNKS-4W5S | Hydrophobic Interaction, Polar Interactions | Ser 1221, Hid 1184, Ser 1186, Hid 1201, Tyr 1213, Hid 1201 |

161
| Compounds | Protein and PDB IDs | Nature of Interactions | Amino acids on active sites |
|-----------|-------------------|-----------------------|---------------------------|
| 2c        | EGFR-4WKQ         | Pi-PiStacking         | Hid 1184, Tyr 1224        |
|           |                   | Hydrophobic Interaction| Gln 791, Thr 790, Thr 854 |
|           |                   | Polar Interactions    | Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Ile 744, Leu 788, Met 766, Phe 856 |
|           |                   | H-Bond                | Met 793                   |
|           |                   | Halogen Bonding       | Leu 788, Lys 745, Ala 743 |
|           | ALK-4Z55          | Hydrophobic Interaction| Leu 1122, Val 1130, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Val 1180 |
|           |                   | Polar Interactions    | Gln 919, Val 916, Leu 1035, Ala 866, Val 899, Phe 1047, Cys 1045, Leu 840, Val 848, Val 914, Val 915, Val 867, Leu 889, Val 898, Ile 1044 |
|           |                   | H-Bond                | Leu 1122, Val 1130, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Val 1180 |
|           |                   | Halogen Bonding       | Gln 1197                  |
|           | VEGFR-4AG8        | Hydrophobic Interaction| Ser 1221, Hid 1184, Ser 1186, Hid 1201 |
|           |                   | Polar Interactions    | Phe 1214, Tyr 1213, Met 1207, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Ile 1212, Ala 1202 |
|           |                   | H-Bond                | Hid 1201, Tyr 1213        |
|           |                   | Halogen Bonding       | Ser 1221, Gly 1185        |
| 2d        | EGFR-4WKQ         | Hydrophobic Interaction| Thr 790, Thr 854          |
|           |                   | Polar Interactions    | Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Met 766, Pro 794 |
|           |                   | H-Bond                | Cys 797, Met 793          |
|           |                   | Halogen Bonding       | Cys 745                   |
|           | ALK-4Z55          | Hydrophobic Interaction| Leu 1122, Val 1130, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Val 1180 |
|           |                   | Polar Interactions    | Gln 919, Thr 926          |
|           |                   | H-Bond                | Cys 919                   |
|           |                   | Halogen Bonding       | Asp 1046                  |
|           | VEGFR-4AG8        | Hydrophobic Interaction| Asn 923, Thr 926          |
| 2e        | EGFR-4WKQ         | Hydrophobic Interaction| Asn 923, Thr 926          |
|           |                   | Polar Interactions    | Gln 919, Thr 790          |
|           |                   | H-Bond                | Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Met 766, Pro 794 |
|           |                   | Halogen Bonding       | Cys 797, Met 793          |
|           | ALK-4Z55          | Hydrophobic Interaction| Leu 1122, Val 1130, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Val 1180 |
|           |                   | Polar Interactions    | Leu 1122, Val 1130, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Val 1180 |
|           |                   | H-Bond                | Ala 1126, Lys 1150, Hid 1124 |
|           |                   | Halogen Bonding       | Cys 919                   |
|           | VEGFR-4AG8        | Hydrophobic Interaction| Cys 919, Thr 926          |
|            |                   | Polar Interactions    | Asn 923, Thr 926          |
| 3a        | EGFR-4WKQ         | Hydrophobic Interaction| Ser 1221, Hid 1184, Ser 1186, Hid 1201 |
|           |                   | Polar Interactions    | Ala 1215, Phe 1214, Tyr 1213, Met 1207, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Tyr 1203 |
|           |                   | H-Bond                | Tyr 1224                  |
|           |                   | Halogen Bonding       | Hid 1201, Tyr 1213        |
|           | ALK-4Z55          | Hydrophobic Interaction| Ala 1215, Phe 1214, Tyr 1213, Met 1207, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Ala 1202, Phe 1188, Ile 1212 |
|           |                   | Polar Interactions    | Hid 1184, Tyr 1224        |
|           |                   | H-Bond                | Hid 1201, Tyr 1213        |
|           | VEGFR-4AG8        | Hydrophobic Interaction| Ser 1221, Hid 1184, Ser 1186, Hid 1201 |
| 3b        | EGFR-4WKQ         | Hydrophobic Interaction| Ala 1215, Phe 1214, Tyr 1213, Met 1207, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Phe 1188, Ala 1202, Ile 1212, Phe 1214 |
|           |                   | Polar Interactions    | Hid 1201, Tyr 1213        |
|           |                   | H-Bond                | Hid 1184, Hid 1201        |

References:

- P. Jam ... et al. Int J App Pharm, Vol 13, Issue 6, 2021, 157-169.
| Compounds | Protein and PDB IDs | Nature of Interactions | Amino acids on active sites |
|-----------|--------------------|------------------------|-----------------------------|
| ALK-4Z55  |                    | H-Bond                 | Met 793, Glu 791            |
|           |                    | Halogen Bonding        | Lys 745                     |
|           |                    | Hydrophobic Interaction|                            |
|           |                    | Polar Interactions     |                            |
|           |                    |                        | Leu 1122, Met 1199, Leu 1198, Leu 1256, Leu 1196, Val 1180, Ala 1148, Val 1180, Ala 1200 |
|           |                    | H-Bond                 | Met 1199                     |
|           |                    | Hydrophobic Interaction| Asn 923                     |
|           |                    | Polar Interactions     | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047, Val 867, Val 914, Leu 889 |
|           |                    | H-Bond                 | Cys 919                     |
|           |                    | Pi-PiStacking          | Phe 1047                     |
|           |                    | Pi Cation              | Lys 868                      |
| TNKS-4W5S |                    | Hydrophobic Interaction| Ser 1221, His 1184, Ser 1186, His 1201 |
|           |                    | Polar Interactions     | Ala 1215, Phe 1214, Tyr 1213, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Ala 1202, Ile 1212, Phe 1188, Phe 1183 |
|           |                    | Halogen Bonding        | Tyr 1224, Tyr 1213           |
|           |                    | Hydrophobic Interaction| Gln 791, Thr 790, Thr 854   |
|           |                    | Polar Interactions     | Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Met 766, Leu 788, Pro 794 Met 793 |
|           |                    | H-Bond                 | Asn 1254                     |
|           |                    | Hydrophobic Interaction| Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047, Val 867, Val 914, Leu 889 |
|           |                    | Polar Interactions     | Cys 919                     |
|           |                    | Pi-PiStacking          | Phe 1047                     |
|           |                    | Pi Cation              | Lys 868                      |
| 3c EGFR-4WKQ |                 | Hydrophobic Interaction| Ser 1221, His 1184, Ser 1186, His 1201 |
|           |                    | Polar Interactions     | Phe 1214, Tyr 1213, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Ile 1212, Phe 1188, Phe 1197, Ile 1192 |
|           |                    | H-Bond                 | Gly 1185, Ser 1221           |
|           |                    | Halogen Bonding        | Tyr 1224                     |
|           |                    | Pi-PiStacking          | Thr 790, Thr 854             |
|           |                    | Pi Cation              | Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Met 766, Pro 794, Phe 795 Met 793 |
|           |                    | Halogen Bonding        | Asn 1254                     |
|           |                    | Polar Interactions     | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047 |
|           |                    | H-Bond                 | Cys 919, Leu 840             |
|           |                    | Halogen Bonding        | Asp 1046                     |
|           |                    | Pi-PiStacking          | --                           |
|           |                    | Pi Cation              | --                           |
| 3d EGFR-4WKQ |                 | Hydrophobic Interaction| Thr 926, Asn 923             |
|           |                    | Polar Interactions     | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047 |
|           |                    | H-Bond                 | Cys 919, Leu 840             |
|           |                    | Halogen Bonding        | Asp 1046                     |
|           |                    | Pi-PiStacking          | --                           |
|           |                    | Pi Cation              | --                           |
| VEGFR-4AG8 |                    | Hydrophobic Interaction| Ser 1221, His 1184, Ser 1186, His 1201 |
|           |                    | Polar Interactions     | Ala 1215, Phe 1214, Tyr 1213, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Ile 1212, Phe 1188, Phe 1197, Ile 1192, Ala 1191 |
|           |                    | Pi-PiStacking          | Tyr 1224                     |
| 3e EGFR-4WKQ |                 | Hydrophobic Interaction| Gln 791, Thr 790, Thr 854   |
|           |                    | Polar Interactions     | Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Met 766, Leu 788, Phe 794 Met 793 |
|           |                    | H-Bond                 | Asn 1254                     |
|           |                    | Halogen Bonding        | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047 |
|           |                    | Pi-PiStacking          | Met 793                      |
|           |                    | Pi Cation              | --                           |
| 3f EGFR-4WKQ |                 | Hydrophobic Interaction| Ser 1221, His 1184, Ser 1186, His 1201 |
|           |                    | Polar Interactions     | Ala 1215, Phe 1214, Tyr 1213, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Ile 1212, Phe 1188, Phe 1197, Ile 1192, Ala 1191 |
|           |                    | H-Bond                 | Gly 1185, Ser 1221           |
|           |                    | Halogen Bonding        | Tyr 1224                     |
|           |                    | Pi-PiStacking          | Thr 790, Thr 854             |
|           |                    | Pi Cation              | Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Met 766, Pro 794, Phe 795 Met 793 |
|           |                    | Halogen Bonding        | Asn 1254                     |
|           |                    | Polar Interactions     | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047 |
|           |                    | H-Bond                 | Cys 919, Leu 840             |
|           |                    | Halogen Bonding        | Asp 1046                     |
|           |                    | Pi-PiStacking          | --                           |
|           |                    | Pi Cation              | --                           |
| ALK-4Z55  |                    | Hydrophobic Interaction| Ser 1221, His 1184, Ser 1186, His 1201 |
|           |                    | Polar Interactions     | Ala 1215, Phe 1214, Tyr 1213, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Ile 1212, Phe 1188, Phe 1197, Ile 1192, Ala 1191 |
|           |                    | H-Bond                 | Ser 1186                     |
|           |                    | Halogen Bonding        | Tyr 1224                     |
|           |                    | Pi-PiStacking          | Thr 790, Thr 854             |
|           |                    | Pi Cation              | Leu 718, Leu 844, Met 793, Leu 792, Val 726, Ala 743, Met 766, Leu 788, Phe 794 Met 793 |
|           |                    | Halogen Bonding        | Asn 1254                     |
|           |                    | Polar Interactions     | Phe 921, Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047 |
|           |                    | H-Bond                 | Cys 919, Leu 840             |
|           |                    | Halogen Bonding        | Asp 1046                     |
|           |                    | Pi-PiStacking          | --                           |
|           |                    | Pi Cation              | --                           |
| Compounds     | Protein and PDB IDs | Nature of Interactions | Amino acids on active sites |
|---------------|---------------------|------------------------|-----------------------------|
| VEGFR–4AG8    |                     | Pi Cation              | --                         |
|               |                     | Hydrophobic Interaction|                            |
|               |                     | Polar Interactions     |                            |
|               |                     | H-Bond                 | Cys 919, Phe 918, Leu 1035, Val 916, Ala 866, Val 899, Cys 1045, Leu 840, Val 848, Phe 1047 |
|               |                     | Halogen Bonding        | --                         |
|               |                     | Pt-PiStacking          | Phe 1047                   |
|               |                     | Pi Cation              | --                         |
| TNKS–4W5S     |                     | Hydrophobic Interaction| Ser 1221, His 1184, Ser 1186, His 1201 |
|               |                     | Polar Interactions     | Ala 1215, Phe 1214, Tyr 1213, Tyr 1203, Ile 1228, Pro 1187, Tyr 1224, Ile 1212, Phe 1188, Phe 1197, Ile 1192, Ala 1191 |
|               |                     | H-Bond                 | Ser 1186                   |
|               |                     | Halogen Bonding        | --                         |
|               |                     | Pt-PiStacking          | Tyr 1224                   |
|               |                     | Pi Cation              | --                         |

![Fig. 2: Molecular docking (a) 2D (b) 3D interactions of pyrazolopyrimidine 2e with 4WKQ](image1)

![Fig. 3: Molecular docking (a) 2D (b) 3D interactions of pyrazolopyrimidine 2e with 4Z55](image2)

![Fig. 4: Molecular docking (a) 2D (b) 3D interactions of pyrazolopyrimidine 2e with 4AG8](image3)
Pharmacophore hypothesis generation and modeling

The results of all featured pharmacophore hypotheses are in table 6. DHRRR_1 is having the best survival score of 5.1979 in this study, which consists of one hydrophobic group (H), one hydrogen bond donor (D), and three aromatic rings (R). The distances between the sites in the common pharmacophore hypothesis DHRRR_1 are given in fig. 6 (a-b) and table 7.

Table 6: Score hypothesis

| Hypothesis ID | Survival score | Site score | Vector score | Volume | Selectivity |
|---------------|----------------|------------|--------------|--------|-------------|
| DHRRR_1       | 4.753541       | 0.967441   | 0.897639     | 0.77978 | 1.331332    |
| DHRRR_2       | 4.742687       | 0.966377   | 0.897544     | 0.77513 | 1.323101    |
| ADRR_1        | 4.638511       | 0.966887   | 0.898106     | 0.77694 | 1.198658    |
| ADRR_2        | 4.622626       | 0.970017   | 0.90864      | 0.69058 | 1.18903     |
| DHRRR_1       | 5.197949       | 0.90054    | 0.861293     | 0.81263 | 2.021416    |
| DHRRR_2       | 5.164714       | 0.882849   | 0.854915     | 0.80952 | 2.015368    |
| ADHRR_1       | 5.000595       | 0.864909   | 0.861097     | 0.81365 | 1.858875    |
| ADHRR_2       | 4.968628       | 0.864643   | 0.858113     | 0.80704 | 1.836308    |
| ADHRR_3       | 4.915803       | 0.853138   | 0.86407      | 0.81378 | 1.754002    |
| ADHRR_4       | 4.904614       | 0.868992   | 0.865852     | 0.81378 | 1.754002    |
| ADHRR_5       | 4.898018       | 0.851548   | 0.85963      | 0.80743 | 1.77342     |
| ADHRR_6       | 4.893939       | 0.846438   | 0.886796     | 0.78132 | 1.77319     |
| ADHRR_7       | 4.881754       | 0.844744   | 0.859158     | 0.81478 | 1.761005    |
| DHRR_5        | 4.529072       | 0.70509    | 0.955143     | 0.68846 | 1.481023    |
| DHRR_1        | 4.712077       | 0.988026   | 0.852928     | 0.77352 | 1.492433    |
| DHRR_2        | 4.684691       | 0.934964   | 0.862256     | 0.786716| 1.498695    |
| DHRR_3        | 4.682611       | 0.917492   | 0.865663     | 0.781164| 1.51623     |
| DHRR_4        | 4.620457       | 0.867853   | 0.852025     | 0.78364 | 1.514955    |
| DHRRR_3       | 4.901226       | 0.663248   | 0.935309     | 0.69000 | 2.016060    |

Table 7: Distances between different sites of model DHRRR_1

| S. No. | Site 1 | Site 2 | Distance |
|--------|--------|--------|----------|
| 1.     | H8     | D6     | 5.12     |
| 2.     | H8     | R11    | 3.16     |
| 3.     | H8     | R9     | 5.09     |
| 4.     | H8     | R10    | 6.53     |
| 5.     | D6     | R11    | 3.41     |
| 6.     | D6     | R9     | 4.57     |
| 7.     | D6     | R10    | 8.34     |
| 8.     | R11    | R9     | 2.15     |
| 9.     | R11    | R10    | 5.12     |
| 10.    | R11    | H8     | 3.16     |
| 11.    | R11    | D6     | 3.41     |
| 12.    | R9     | R10    | 3.97     |
| 13.    | R9     | H8     | 5.09     |
| 14.    | R9     | D6     | 4.57     |
| 15.    | R9     | R11    | 2.15     |
| 16.    | R10    | H8     | 6.53     |
| 17.    | R10    | D6     | 8.34     |
| 18.    | R10    | R11    | 5.12     |
| 19.    | R10    | R9     | 3.97     |
DHRRR_1

Fig. 6: a) Pharmacophore hypothesis DHRRR_1 b) Distances in the pharmacophore hypothesis DHRRR_1

Table 8: Physicochemical and ADMET properties of pyrazole derivatives

| S. No. | Compounds | MW      | Log P | donorHB | Accep HB | PSA     | QPlogHERG | QPlogCaco | QPlogKhsa | Percent human oral absorption |
|--------|-----------|---------|-------|---------|----------|---------|-----------|-----------|-----------|------------------------------|
| 1.     | Ceritinib | 577.743 | 4.838 | 2       | 9.75     | 119.60  | -7.51     | 54.85     | 1.146     | 73.44                        |
| 2.     | Axitinib  | 386.47  | 4.721 | 2       | 4.5      | 74.60   | -6.76     | 861.39    | 0.728     | 100                          |
| 3.     | Gefitinib | 446.90  | 4.314 | 1       | 7.7      | 61.21   | -7.10     | 1044.67   | 0.351     | 100                          |
| 4.     | Ceritinib | 332.36  | 2.438 | 2       | 6.7      | 19.74   | -7.51     | 54.85     | 1.146     | 73.44                        |
| 5.     | Axitinib  | 250.26  | 1.41  | 2       | 5        | 89.79   | -5.27     | 236.60    | -0.209    | 77.689                       |
| 6.     | Gefitinib | 284.70  | 1.897 | 2       | 5        | 89.76   | -5.22     | 236.90    | -0.108    | 80.553                       |
| 7.     | Gefitinib | 284.70  | 1.897 | 2       | 5        | 89.76   | -5.22     | 236.90    | -0.108    | 80.553                       |
| 8.     | Gefitinib | 284.70  | 1.897 | 2       | 5        | 89.76   | -5.22     | 236.90    | -0.108    | 80.553                       |
| 9.     | Gefitinib | 268.25  | 1.638 | 2       | 5        | 89.79   | -5.15     | 237.07    | -0.173    | 79.045                       |
| 10.    | Ceritinib | 298.30  | 3.232 | 1       | 3        | 104.22  | -5.70     | 299.71    | 0.471     | 90.197                       |
| 11.    | Axitinib  | 332.74  | 3.679 | 1       | 3        | 105.31  | -5.61     | 282.56    | 0.597     | 92.346                       |
| 12.    | Gefitinib | 332.74  | 3.728 | 1       | 3        | 104.21  | -5.62     | 299.50    | 0.591     | 93.095                       |
| 13.    | Gefitinib | 332.74  | 3.728 | 1       | 3        | 104.22  | -5.62     | 299.53    | 0.591     | 93.099                       |
| 14.    | Gefitinib | 316.29  | 3.468 | 1       | 3        | 104.23  | -5.58     | 299.43    | 0.515     | 91.575                       |

Table 9: PASS prediction of anticancer properties

| S. No. | Compounds | Activity                                      | Pa    |
|--------|-----------|-----------------------------------------------|-------|
| 1.     | 2a        | Antineoplastic (melanoma)                      | 0.155 |
|        |           | Antineoplastic antimetabolite                  | 0.108 |
|        |           | Epidermal growth factor receptor kinase inhibitor | 0.142 |
|        |           | ALK inhibitor                                  | 0.107 |
|        |           | Tankyrase inhibitor                            | 0.254 |
| 2.     | 2b        | ALK inhibitor                                  | 0.101 |
|        |           | Antineoplastic (melanoma)                      | 0.139 |
|        |           | Tankyrase inhibitor                            | 0.174 |
|        |           | Epidermal growth factor receptor kinase inhibitor | 0.133 |
| 3.     | 2c        | Epidermal growth factor receptor kinase inhibitor | 0.138 |
|        |           | ALK inhibitor                                  | 0.100 |
|        |           | Tankyrase inhibitor                            | 0.182 |
| 4.     | 2d        | Tankyrase inhibitor                            | 0.192 |
|        |           | Epidermal growth factor receptor kinase inhibitor | 0.146 |
|        |           | ALK inhibitor                                  | 0.108 |
| 5.     | 2e        | Tankyrase inhibitor                            | 0.275 |
|        |           | ALK inhibitor                                  | 0.115 |
|        |           | Epidermal growth factor receptor kinase inhibitor | 0.145 |
| 6.     | 3a        | Antineoplastic (melanoma)                      | 0.148 |
|        |           | Antineoplastic antimetabolite                  | 0.113 |
|        |           | ALK inhibitor                                  | 0.097 |
|        |           | Antileukemic                                   | 0.205 |
| 7.     | 3b        | Antineoplastic (multiple myeloma)              | 0.269 |
|        |           | Antineoplastic (melanoma)                      | 0.136 |
|        |           | ALK inhibitor                                  | 0.094 |
| 8.     | 3c        | Antileukemic                                   | 0.152 |
|        |           | ALK inhibitor                                  | 0.093 |
| 9.     | 3d        | Antineoplastic (multiple myeloma)              | 0.223 |
|        |           | ALK inhibitor                                  | 0.098 |
| 10.    | 3e        | Antineoplastic antimetabolite                  | 0.102 |
|        |           | ALK inhibitor                                  | 0.104 |
|        |           | Antileukemic                                   | 0.186 |
|        |           | Tankyrase inhibitor                            | 0.175 |
Drug likeness, ADMET and prediction of activity spectral studies

The synthesized ten pyrazoles have good drug likeness properties, as shown in table 8. We evaluated the physicochemical properties to fit into the Lipinski rule of five, which is a way to determine if they are orally bioavailable. The compounds have shown no violations for the Lipinski rule of 5. Their ADMET properties were analysed, and reported that all the compounds checked were found to have all the properties within the standard limit (table 8). The activity spectra for anticancer activity of the pyrazoles were predicted to find out the inhibitory effect on the particular enzymes (table 9). The compounds bearing pyrazolopyridines (2a-2e) are more effective against specific receptors such as EGFR, ALK and tankyrase.

### In vitro anticancer study by MTT assay

The results of the cytotoxicity studies were presented in table 10. Compound 2e, at the highest concentration, 200 \( \mu \text{M} \), exhibited the most increased activity, which was 92% cytotoxic in nature and compounds 2d and 3d showed moderate cell growth inhibition around 80%. On correlating with their docking scores, these compounds have excellently interacted with the four lung cancer targets. Thus, the results interpret that the synthesized derivatives might inhibit any of the four targets discussed and exert their anticancer action. On further analysis of the top interacted pyrazole 2e, they have maximum interaction with the VEGFR receptor, which proves their mechanism.

| S. No. | Compound ID | % Cytotoxicity Concentration (µM) |
|-------|-------------|----------------------------------|
| 1.    | 2a          | 88 18 33 48                      |
| 2.    | 2b          | 15 32 48 71                      |
| 3.    | 2c          | 13 31 44 69                      |
| 4.    | 2d          | 17 34 51 84                      |
| 5.    | 2e          | 40 58 78 92                      |
| 6.    | 3a          | 09 20 30 45                      |
| 7.    | 3b          | 17 34 51 78                      |
| 8.    | 3c          | 08 19 36 53                      |
| 9.    | 3d          | 11 26 51 85                      |
| 10.   | 3e          | 10 22 38 56                      |

### DISCUSSION

We found that the pyrazole condensed derivatives interacted with four lung cancer targets EGFR, ALK, VEGFR and TNKS, and their cytotoxicity action was proved against lung cancer. The compound 2e was the most active in both Caco-2 and HepG2 studies, followed by 3d and 2d. Top compound 2e interacted with the VEGFR receptor excellently with stable binding mode and affinity. The best pharmacophore hypothesis, DHHRR 1 reveals the importance of the hydrogen bond donors, hydrophobic and aromatic groups which is essential for the anticancer action. Thus, validating the hydrogen bonds, hydrophobic groups and pi-pi interactions, which were showed by molecular docking. As per the cytotoxicity studies, the anticancer action of the compounds 2e, 3d and 2d might be due to the introduction of electron-withdrawing fluorine and chlorine atoms in the benzene ring attached to the pyrazole ring.

Lung cancer development is stimulated by specific signaling pathways produced by receptors such as EGFR, ALK, VEGFR and TNKS. Much research has been performed to prove the anticancer efficacy of pyrazolopyrimidines on lung cancer [35], and some reported their inhibitory potentials on specific targets such as EGFR [36], VEGFR [37], tankyrase inhibitors [38] etc. We have screened the anticancer action by in vitro studies using A549 cell lines as a preliminary evaluation. Some reports are interfering in EGFR [39, 40] /VEGFR [41] /ALK [42] /Not [43, 44] pathways inhibits the proliferation of A549 cell lines, and with this proof, we have carried the MTT assay. Cucurbitacin [39] and diazole [40] have been reported in proliferation inhibition in A549 cells by interfering VEGFR signaling pathway. A study was performed to evaluate the TNKS small molecule inhibitor XAV939 on the proliferation and migration of lung adenocarcinoma A549 cells and found that XAV939 intervention inhibited A549 cell proliferation [43]. Determination of the appropriate target should be performed by analysing the enzyme antagonistic potential further, authenticating the mechanism of inhibition.

### CONCLUSION

The synthesized pyrazole derivatives interacted well with the selected lung cancer targets-EGFR, ALK, VEGFR and TNKS; with their docking scores above 5 kcal/mol equivalent with their standards. The molecular interactions are based on various parameters such as glide score, binding free energy, polar interactions, hydrophobic interactions, and hydrogen bond interactions. Further, the in vitro results exhibit compounds 2e as the best anti-lung cancer agents followed by 3d and 2d, which was in agreement with their docking results. ADMET properties reported that all the compounds were found to have properties within the standard limit. The activity spectra of the pyrazoles predicted that pyrazolopyridines (2a-2e) are more effective against specific receptors such as EGFR, ALK and Tankyrase. Thus, this study suggests that the synthesized pyrazole derivatives can be further investigated to validate their enzyme inhibitory potentials by in vivo studies.

### ACKNOWLEDGEMENT

We acknowledge Nitte Deemed to be University, Mangaluru, for the funding to carry out this project (University Research Grant No. NUFRI/2017/06/16). Also, thank to the authorities of the NGSIM Institute of Pharmaceutical Sciences, Mangaluru and NGSIM IPS CADD lab for providing requirements for this work. Thanks to CUSAT, Cochin for NMR, Mysore University, Mysuru for Mass and Yenepoya Research Centre for anticancer studies.

### AUTHORS CONTRIBUTIONS

All authors have contributed equally.

### CONFLICT OF INTERESTS

The authors declare no conflict of interest, financial or otherwise.

### REFERENCES

1. Prabhu VV, Devaraj N. Epidermal growth factor receptor tyrosine kinase: A potential target in the treatment of non-small-cell lung carcinoma. J Environ Pathol Toxicol Oncol. 2017;36(2):151-8. doi: 10.1615/JEnvironPatholToxicolOncol.2017018341. PMID 2017018341.
2. Alferrez D, Wilkinson RW, Watkins J, Poulsom R, Mandir N, Wedge SR, Pyrah IT, Smith NR, Jackson L, Ryan AJ, Goodlad RA. Dual inhibition of VEGFR and EGFR signaling reduces the incidence and size of intestinal adenomas in Apc(Min/+ ) mice. Mol Cancer Ther. 2008;7(8):590-8. doi: 10.1158/1535-7163.MCT-07-0433. PMID 18347145.
3. Antonicelli A, Cafarotti S, Indini A, Galli A, Russo A, Cesario A, Loco CO, FM, Russo P, Mainini AF, Bonifati LG, Nosotti M, Santambrogio L, Margaritotta S, Granone PM, Dufly AE. EGFR-targeted therapy for non-small cell lung cancer: focus on EGFR.
oncogenic mutation. Int J Med Sci. 2013;10(3):320-30. doi: 10.7150/ijms.4609. PMID 23423768.

4. Castanon E, Martin P, Rolfo C, Fusco JP, Genieros L, Legasi J, Santisteban M, Gil-Bazo I. Epidermal growth factor receptor targeting in non-small cell lung cancer: reviewing different strategies against the same target. Curr Drug Targets. 2014;15(4):1273-83. doi: 10.2174/1389450141412692935. PMID 25511613.

5. Jänne PA, Yang JCH, Kim DW, Planchard D, Hye Y, Ramalingam SS, Ahn MJ, Kim SW, Su WC, Horn L, Haggstrom D, Felp E, Kim JH, Freuwer F, Cantarini M, Brown KH, Dickinson PA, Ghiorgihi S, Ransom M, AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. N Engl J Med. 2015;372(18):1699-99. doi: 10.1056/NEJMoa1411817. PMID 25923549.

6. Gerber DE, Minna JD. ALK inhibition for non-small cell lung cancer: from discovery to therapy in record time. Cancer Cell. 2016;18(6):549-51. doi: 10.1016/j.ccr.2010.11.033. PMID 21516280.

7. Sang J, Acquaviva J, Friedland IC, Smith DL, Sequeira M, Zhang C, Jiang Q, Xue L, Lovly CM, Jimenez JP, Shaw AT, Doebbe RC, He S, Bates RC, Camrade DR, Morris SW, Irby J, Proia DA. Targeted inhibition of the molecular chaperone Hsp90 overcomes ALK inhibitor resistance in non-small cell lung cancer. Cancer Discovery. 2013;3(4):430-43. doi: 10.1158/2159-8290.CD-12-0440. PMID 23533265.

8. Nguyen-Ngc T, Bouchaab H, Adjie AA, Peters S. BRAF alterations as therapeutic targets in non-small-cell lung cancer. J Thorac Oncol. 2015;10(10):1396-403. doi: 10.1097/JTO.0000000000000464. PMID 26361799.

9. Gautchi O, Milia J, Cabarro BP, Bhutgen MV, Bese B, Smit EF, Wolf J, Peters S, Früh M, Koebeler D, Ouhnou Y, Schuler M, Curioni-Fontecedo A, Huret B, Kerjuam M, Michels S, Pall G, Gothschild S, Schmid-Bindert G, Scheffler M, Veillon R, Wannesson L, Diedholm J, Zalcman G, Billerey C, Vannier T. Targeted therapy for patients with BRAF-mutant lung cancer: results from the European EURAF cohort. J Thorac Oncol. 2015;10(10):1451-7. doi: 10.1097/10.2200000000000265. PMID 26200454.

10. Feng Y, Hu J, Ma J, Feng K, Zhang X, Yang S, Wang W, Zhang J, Zhang Y. RNA-mediated silencing of VEGF-C inhibits non-small-cell lung cancer progression by simultaneously down-regulating the CXCR4, CCR7, VEGFR-2 and VEGFR-3-dependent axes-induced ERK, p38 and AKT signalling pathways. Eur J Cancer. 2011;47(15):2533-63. doi: 10.1016/j.ejca.2011.05.006. PMID 21680174.

11. Villaruz LC, Socinski MA. The role of anti-angiogenesis in non-small-cell lung cancer: an update. Curr Oncol Rep. 2015;17(6). doi: 10.1007/s11912-015-00129-0. PMID 26200454.

12. Yang J, Chen J, He J, Li J, Shi J, Cho WC, Liu X. Wnt signaling as potential therapeutic target in lung cancer. Expert Opin Ther Targets. 2016;20(8):999-1015. doi: 10.1517/14728222.2016.1154941. PMID 26982052.

13. Sigismund S, Avanzato D, Lanzetti L. Emerging functions of the EGFR in cancer. Mol Oncol. 2018;12(1):3-20. doi: 10.1002/1876-0261.12155. PMID 29124875.

14. Shackelford RE, Vora M, Mayhall K, Cotelingam J. ALK rearrangements and testing methods in non-small cell lung cancer: a review. Genes Cancer. 2014;5(1-2):1-14. doi: 10.1089/gec.2013.0056. PMID 24955213.

15. Alevizakos M, Kalsas S, Syrigos KN. The VEGF pathway in lung cancer. Cancer Chemother Pharmacol. 2013;72(6):1169-81. doi: 10.1007/s00280-013-2289-3. PMID 24085262.

16. Riffel H, Lord CJ, Ashworth A. Tankyrase-targeted therapeutics: expanding opportunities in the PARP family. Nat Rev Drug Discovery. 2012;11(12):923-36. doi: 10.1038/nrd3868. PMID 23197039.

17. Santarpia M, Ligouris A, Karachalou N, Gonzalez-Cao M, Daffina MG, D’Aveni A, Marabelli G, Altavilla G, Rosell R, Osinsmitnib in the treatment of non-small cell lung cancer: design, development and place in therapy. Lung Cancer (Auckl). 2017;8:109-25. doi: 10.2147/LCCT.S196444. PMID 28860885.

18. Descoutt Perol M, Rousseau-Bussac G, Planchard D, Menneret B, Wislez M, Cortot A, Guissier F, Galland L, Dö P, Schott R, Dansin E, Arrondeaux J, Aubiac J, Chouaid C. Brigitatinib in patients with ALK-positive advanced non-small-cell lung cancer pretreated with sequential ALK inhibitors: A multicentric real-world study (BRIGALK study). Lung Cancer. 2020;109:1016-10. doi: 10.1016/j.jclincan.2019.08.010. PMID 31491676.

19. Martellini E, Troiani T, Morgillo F, Rodolico G, Vitagliano D, Morelli MP, Tuccillo C, Vecchione L, Capasso A, Ordinura M, De Vita F, Eckhardt SG, Santoro M, Berrino L, Ciardelli F. Synergistic antitumor activity of soralenib in combination with epidermal growth factor receptor inhibitors in colorectal and lung cancer cells. Clin Cancer Res. 2016;20(20):4990-5001. doi: 10.1158/1078-0432.CCR-10-0923. PMID 28081084.

20. Shukla P, Sharma A, Fageria L, Chowdhury R. Novel spiro/non-spiro pyrazolone derivatives: eco-friendly synthesis, in vitro anticancer activity, DNA binding, and in-silico docking studies. Curr Biochem Compt. 2019;15(2):257-67. doi: 10.2174/1734071213666170828165512.

21. Pj J KB. Synthesis. in silico physicochemical properties and biological activities of some pyrazolones. Asian J Pharm Clin Res. 2017;10(4):456-9. doi: 10.22159/ajpcr.2017.v10i4.17093.
activity of organic compounds. Russ Chem Bull. 2016;65(2):384-93. doi:10.1007/s11172-016-1310-6.

34. Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. J Immunol Method. 1986;89(2):271-7. doi: 10.1016/0022-1759(86)90366-6.

35. El-Kalayoubi SA. Synthesis and anticancer evaluation of some novel pyrimido[5,4-e][1,2,4]triazines and pyrazolo[3,4-d]pyrimidine using DMF-DMA as methylating and cyclizing agent. Chem Cent J. 2018;12(1):64. doi: 10.1186/s13065-018-0424-3, PMID 29796716.

36. Ismail NSM, Ali EMH, Ibrahim DA, Serya RAT, Abou El Ella DA. Pyrazolo[3,4-d]pyrimidine-based scaffold derivatives targeting kinases as anticancer agents. Future J Pharm Sci. 2016;2(1):20-30. doi: 10.1016/j.fjps.2016.02.002.

37. Sun N, Ji H, Wang W, Zhu Q, Cao M, Zang Q. Inhibitory effect of dexamethasone on residual Lewis lung cancer cells in mice following palliative surgery. Oncol Lett. 2017;13(1):356-62. doi: 10.3892/ol.2016.5422, PMID 28123567.

38. Chedid M, Eissa HO, Engler TA, Furness KW, Woods TA, Wrobleski AD. US Patent No. 9,624,218. Washington, DC: US Patent and Trademark Office; 2017.

39. Zhang J, Song Y, Liang Y, Zou H, Zuo P, Yan M, Jing S, Li T, Wang Y, Li D, Zhang T, Wei Z. Cucurbitacin Ila interferes with EGFR-MAPK signaling pathway leads to proliferation inhibition in A549 cells. Food Chem Toxicol. 2019 Oct 1;132:110654. doi: 10.1016/j.fct.2019.110654.

40. Vinod Prabhu V, Elangoovan P, Niranjali Devaraj S, Sakthivel KM. Targeting apoptosis by 1, 2-diazole through regulation of EGFR, Bcl-2 and CDK-2 mediated signaling pathway in human non-small cell lung carcinoma A549 cells. Gene. 2018 Dec 30;679:352-9. doi: 10.1016/j.gene.2018.09.014, PMID 30218747.

41. Shi L, Zhang S, Wu H, Zhang L, Dai X, Hu J, Xue J, Liu T, Liang Y, Wu G. MIR-200c increases the radiosensitivity of non-small-cell lung cancer cell line A549 by targeting the VEGF-VEGFR2 pathway. PLOS ONE. 2013 Oct 30;8(10):e78344. doi: 10.1371/journal.pone.0078344, PMID 24205206.

42. Li C, Zheng X, Han Y, Lv Y, Lan F, Zhao J. XAV939 inhibits the proliferation and migration of lung adenocarcinoma A549 cells through the WNT pathway. Oncol Lett. 2018 Jun 1;15(6):8973-82. doi: 10.3892/ol.2018.8491, PMID 29805633.

43. Li P, Zhao S, Hu Y. SFRP2 modulates non-small cell lung cancer A549 cell apoptosis and metastasis by regulating mitochondrial fission via Wnt pathways. Mol Med Rep. 2019 Aug 1;20(2):1925-32. doi: 10.3892/mmr.2019.10393, PMID 31257495.