

# Synthesis and docking studies of 1,3,4-oxadiazole, keto pyrazole, pyrrole and lactam derivatives of Thienopyrimidines as EGFR tyrosine kinase inhibitors

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## ABSTRACT

Derivatives of thieno[3,2-d]pyrimidines were synthesized from a starting material 4-(4-(methylamino)thieno[3,2-d]pyrimidin-2-yl) benzohydrazide and thoroughly characterized by using various spectroscopic techniques such as Infra Red, <sup>1</sup>H Nuclear Magnetic Resonance, <sup>13</sup>C Nuclear Magnetic Resonance and Mass spectrometry. The study aimed to explore the potential of these derivatives as anti-cancer agents targeting the epidermal growth factor receptor (EGFR), a primary target in cancer development. The synthesized compounds underwent molecular docking using Auto dock tools to assess the interaction with EGFR. The EGFR tyrosine kinase obtained from the Protein Data Bank was used for docking studies. Among all the four compounds subjected to docking, compound 3 exhibited the highest binding energy (-10.73 kcal/mol) with EGFR.

## KEYWORDS

Thienopyrimidines, EGFR, Anti-cancer activity, Docking, Protein kinases

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## INTRODUCTION

Cancer is a global health problem that is increasingly prevalent and leading to high mortality rates. The development and progression of cancer cells involve the activity of certain enzymes known as protein kinases.<sup>1</sup> As a result, protein kinase inhibitors gained significant importance in cancer treatment, targeting pathways that address various cellular communication issues. Protein kinases are a prime focus in clinical oncology due to their pivotal role in signal transduction pathways, contributing to metastasis and drug resistance. The development of kinase inhibitors as anticancer drugs remains key research area to improve selectivity, safety and efficacy.

Among the chemical scaffolds utilized in drug development, the thienopyrimidine scaffold is widely employed. Thienopyrimidine-containing compounds share structural and isoelectronic characteristics with purines,<sup>1</sup> making them attractive for pharmaceutical drug production. Thienopyrimidines have demonstrated diverse pharmacological properties, including anti-bacterial,<sup>2,3</sup> anti-viral, anti-cancer,<sup>4,5</sup> anti-fungal,<sup>6,7</sup> anti-inflammatory and anti-protozoal activities.<sup>8,9</sup> Figure 1 illustrates several thienopyrimidine-containing drugs with varying biological activity profiles.

For instance, Relugolix (TAK-385) has potential to treat prostate carcinoma; DDP-225 aims to treat gastrointestinal tract diseases and irritable bowel syndrome. Pictilisib (GDC-0941) is used to treat advanced solid tumors. Additionally, Olmutinib is an approved drug that inhibits epidermal growth factor receptor (EGFR) and is utilized in the treatment of small cell lung cancer.

In summary, due to the escalating global impact of cancer, effective treatment strategies and early diagnosis are crucial. Inhibiting protein kinases and other relevant targets have become essential approaches in cancer treatment. Thienopyrimidine drugs with their diverse pharmacological properties have shown promise in the development of various drugs, especially, anti-cancer drugs.

Scheme 1 outlines the synthesis of several compounds using different reagents and conditions. Compound 1 was treated with triethyl orthoformate (2) in the presence of p-TSA (p-toluene sulfonic acid),

in ethanol solvent at 90°C for 1 hour under microwave irradiation to afford pure target compound 3. Compound 1 was reacted with pentane-2,4-dione 4(1,3-diketone) in the presence of P-TSA in ethanol solvent at 90°C for 1hour under microwave irradiation to achieve pure target compound 5. Compound 1 was also treated with hexane-2,5-dione (6) in the presence of P-TSA in ethanol solvent at 90°C for 1 hour under microwave irradiation to give compound 7. Further, Compound 1 was condensed with 4-chlorobenzaldehyde (8) in the presence of P-TSA in ethanol solvent at 90°C for 1hour under microwave irradiation to afford pure target compound 9. Finally, compound 9 was reacted with chloroacetyl chloride (10) in DMF (dimethyl formamide) solvent and was heated at 120 °C for 24 hours to afford pure target compound 11.

The reactions use p-TSA as a catalyst and microwave irradiation to accelerate the reaction and reduce the reaction time. The solvents used are ethanol and DMF. The temperatures and reaction times are also specified for each step.

## EXPERIMENTAL

All solvents and chemicals were obtained commercially, from Sigma-Aldrich and used without further purification. All the reactions were

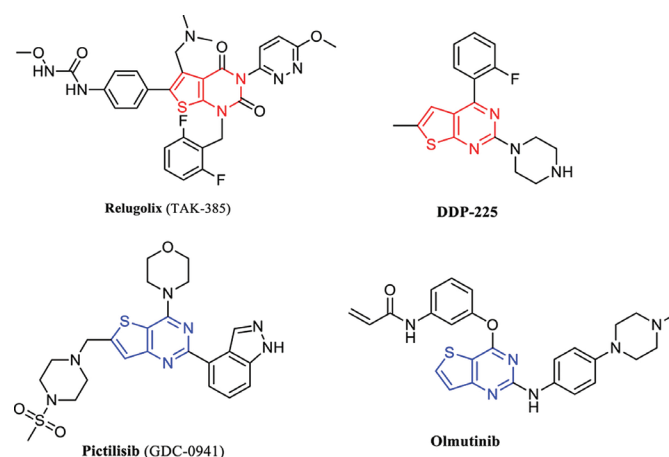
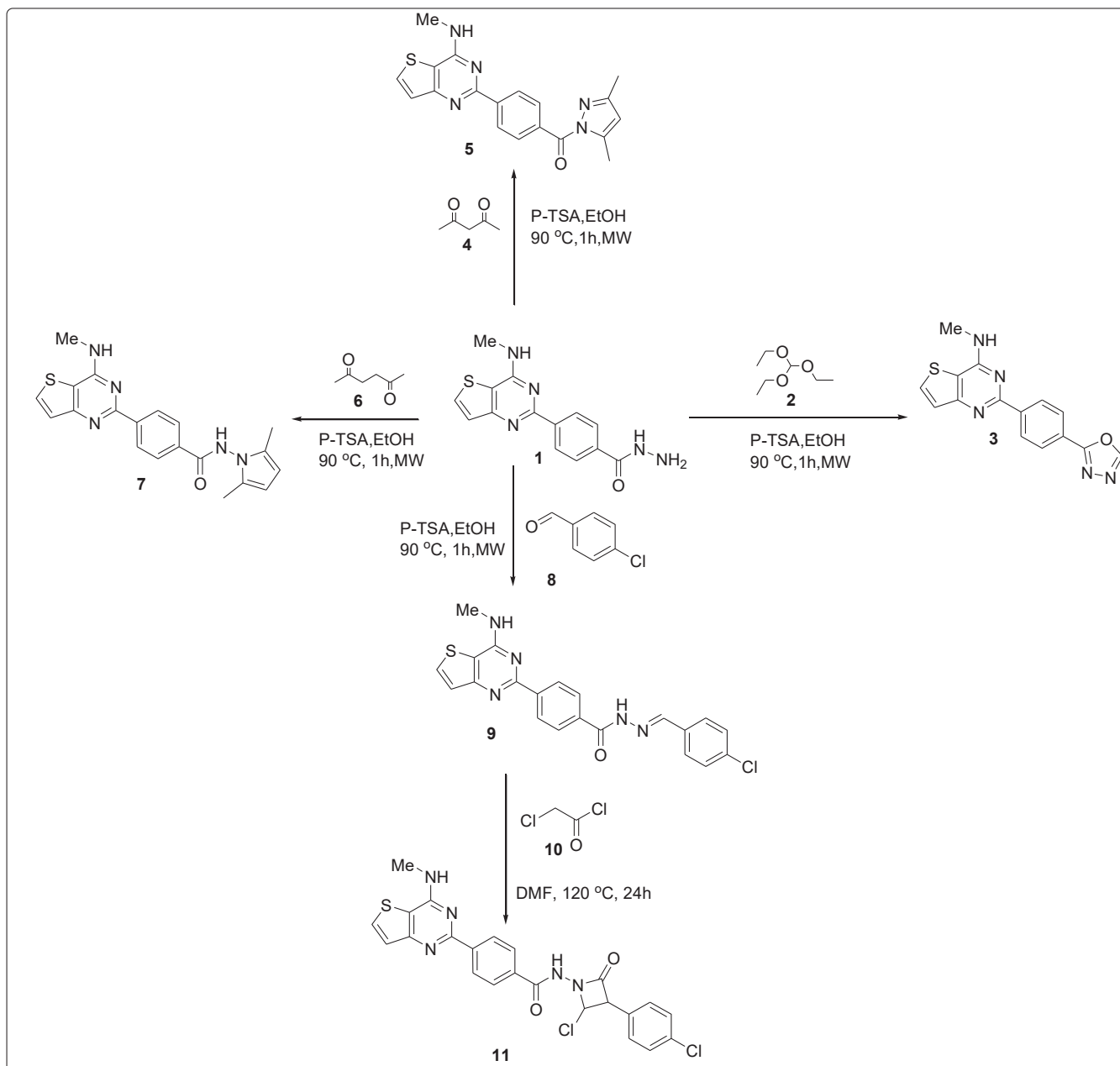


Figure 1: Thienopyrimidines based drug molecules

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Scheme 1: Synthesis of hydrazone derivatives of Thienopyrimidines

monitored by TLC on Merck Kieselgel 60 F524, by UV light and/or spraying a 5%  $\text{H}_2\text{SO}_4$  in Ethanol followed by heating. Column chromatography was performed on Silica Gel 60 (60–120 mesh). Melting points were determined in open capillary tubes on a Gallenkamp MFB-595 apparatus. Element analysis was carried out with Thermofinnigan CHNS analyzer. IR spectra were recorded with Shimadzu FTIR 8400s spectrophotometer.  $^1\text{H}$  NMR spectra were recorded on a Bruker 500 MHz NMR spectrometer. Mass spectra were recorded by Shimadzu mass spectrometer.

#### General procedure for Synthesis of 1,3,4-oxadiazole, keto pyrazole, pyrrole and hydrazone derivatives of Thienopyrimidines (3,5,7&9)

To a solution of 4-(4-(methylamino)thieno[3,2-d]pyrimidin-2-yl)benzohydrazide (**1**) (100 mg, 0.33 mmol) in ethanol (4 mL) was added corresponding tri ethyl orthoformate or diketone or aldehyde (0.36 mmol) followed by p-TSA (catalyst) and irradiated to 90 °C for 1h in microwave oven. After the completion, reaction mixture was poured into ice water (4 mL), filtered and the obtained precipitate was washed with ethyl acetate to afford compounds **3,5,7&9**.

#### 2-(4-(1,3,4-oxadiazol-2-yl)phenyl)-N-methylthieno[3,2-d]pyrimidin-4-amine (**3**)

Yield: 91%; Off white solid; m.p: 220–225°C; IR (KBr):  $\nu_{\text{max}}$  3440, 3314, 3082, 1665, 1601, 1539, 1389, 1345, 1182, 1110, 1039, 949, 902, 858, 793, 712, 645, 563, 527;  $^1\text{H}$  NMR (500 MHz, DMSO):  $\delta$  9.401 (s, 1H), 8.657–8.640 (d,  $J$  = 8.5 Hz, 2H), 8.182–8.166 (d,  $J$  = 8 Hz, 4H), 7.503–7.492 (d,  $J$  = 5.5 Hz, 1H), 3.152–3.143 (d,  $J$  = 4.5 Hz, 3H); ESI-MS:  $m/z$ , 310.11 ( $\text{M}+\text{H}^+$ );

#### (3,5-dimethyl-1H-pyrazol-1-yl)(4-(4-(methylamino)thieno[3,2-d]pyrimidin-2-yl)phenyl)methanone (**5**)

Yield: 88%; off white solid; m.p: 222–227°C; IR (KBr):  $\nu_{\text{max}}$  3270, 3151, 3064, 2930, 2890, 1927, 1812, 1708, 1597, 1541, 1504, 1427, 1384, 1336, 1269, 1189, 1044, 970, 908, 857, 788, 704, 658, 609, 568, 529;  $^1\text{H}$  NMR (400 MHz, DMSO):  $\delta$  8.578–8.557 (d,  $J$  = 8.4 Hz, 2H), 8.156–8.142 (d,  $J$  = 5.6 Hz, 1H), 7.993–7.972 (d,  $J$  = 8.4 Hz, 3H), 7.501–7.488 (d,  $J$  = 5.21 Hz, 1H), 6.307 (s, 1H), 3.142–3.131 (d,  $J$  = 4.4 Hz, 3H), 2.591 (s, 3H), 2.183 (s, 3H);  $^{13}\text{C}$  NMR (500 MHz, DMSO):  $\delta$  13.47–13.87, 27.24, 111.327, 113.932, 124.686, 126.932, 130.795, 133.196, 134.126,

141.975, 144.429, 151.697, 157.383, 158.787, 159.887, 167.731; ESI-MS:  $m/z$ , 364.24 (M+H)<sup>+</sup>;

### N-(2,5-dimethyl-1H-pyrrol-1-yl)-4-(4-(methylamino)thieno[3,2-d]pyrimidin-2-yl)benzamide(7)

Yield: 91%; Off white solid; m.p: 214-219°C; IR (KBr):  $\nu_{\max}$  3364, 3263, 3188, 2923, 2857, 1814, 1731, 1674, 1620, 1598, 1535, 1497, 1388, 1349, 1271, 1172, 1116, 1038, 1011, 905, 760, 690, 647, 565; <sup>1</sup>H NMR (500 MHz, DMSO):  $\delta$  11.377 (s, 1H), 8.573-8.557 (d,  $J$  = 8 Hz, 2H), 8.223-8.213 (d,  $J$  = 5 Hz, 1H), 8.114-8.098 (d,  $J$  = 8 Hz, 2H), 7.519-7.508 (d,  $J$  = 5.5 Hz, 1H), 7.480-7.464 (d,  $J$  = 8 Hz, 1H), 7.116-7.101 (d,  $J$  = 7.5 Hz, 1H), 5.729 (s, 2H), 3.168 (s, 3H), 2.286 (s, 1H), 2.067 (s, 6H); ESI-MS:  $m/z$ , 378.28 (M+H)<sup>+</sup>.

### Synthesis of (E)-N'-(4-chlorobenzylidene)-4-(4-(methylamino)thieno[3,2-d]pyrimidin-2-yl)benzohydrazide (9)

Yield: 87%; White solid; m.p: 282-290°C; IR (KBr):  $\nu_{\max}$  3332, 3242, 3052, 2928, 2856, 1649, 1582, 1535, 1492, 1388, 1347, 1275, 1047, 822, 652,  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (400 MHz, DMSO):  $\delta$  12.01 (s, 1H), 8.59-8.49 (m, 3H), 8.14-8.13 (d,  $J$  = 5.2 Hz, 1H), 8.052-8.032 (d,  $J$  = 8 Hz, 2H), 7.963-7.952 (d,  $J$  = 4.4 Hz, 1H), 7.795-7.775 (d,  $J$  = 8 Hz, 2H), 7.556-7.483 (m, 3H), 3.14-3.13 (s,  $J$  = 4.4 Hz, 3H); <sup>13</sup>C NMR (400 MHz, DMSO): 18.5, 27.2, 56.0, 113.8, 124.6, 127.5-127.7, 128.4-128.9, 133.1-133.4, 134.3-134.6, 141.4, 146.4, 157.3, 158.9, 159.9, 163.2; ESI-MS:  $m/z$ , 422.2 (M+H)<sup>+</sup>; HRMS (ESI):  $m/z$  calcd for C<sub>21</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>SCl [M + H]<sup>+</sup>: 422.0842; found: 422.0825.

### N-(2-chloro-3-(4-chlorophenyl)-4-oxoazetidin-1-yl)-4-(4-(methylamino)thieno[3,2-d]pyrimidin-2-yl)benzamide (11)

To a stirred solution of (E)-N'-(4-chlorobenzylidene)-4-(4-(methylamino)thieno[3,2-d]pyrimidin-2-yl)benzohydrazide (9) (100 mg, 1 eq.) in DMF (2 mL) was added TEA (2 eq.), chloro acetylchloride 10 (10 eq.) as drop wise and heated to 120°C for 36 h. After completion, solvent was evaporated to get crude residue. crude residue was poured in to ice water basified with aq. Na<sub>2</sub>CO<sub>3</sub> solution, extracted with 10% MeOH in DCM (10 mL X 3 Times). Combined extracts were washed with water followed by brine solution, dried the organics over anhydrous Na<sub>2</sub>SO<sub>4</sub> filtered and evaporated to get the crude product. The crude product was purified by column chromatography eluted

with 8% MeOH in DCM to afford 11 as yellow solid. Yield: 87%; pale yellow solid; m.p: 228-233°C; <sup>1</sup>H NMR (500 MHz, DMSO):  $\delta$  8.607-8.590 (d,  $J$  = 6.5, 2H), 8.145-8.134 (d,  $J$  = 5.5, 1H), 8.134-7.973 (d,  $J$  = 8, 2H), 7.959 (s, 1H), 7.956-7.934 (m,  $J$  = 5.5, 4H), 7.485-7.475 (d,  $J$  = 5, 2H), 7.301 (s, 1H), 4.779-4.751 (d,  $J$  = 1.5, 1H), 4.673-4.644 (d,  $J$  = 1.5, 1H), 3.119 (s, 3H); <sup>13</sup>C NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  27.26, 28.96, 41.89, 91.50, 113.94, 124.35-124.59, 126.84, 128.22, 128.72-128.98, 133.29, 134.71-134.82, 155.32, 157.37, 158.53, 162.61; ESI-MS:  $m/z$ , 498.10 (M+H)<sup>+</sup>.

## RESULTS AND DISCUSSION

### Molecular docking studies

Molecular docking investigations were conducted using Auto dock tools focusing on the epidermal growth factor receptor (EGFR) as the primary target. EGFR protein, a cell-surface receptor, is a crucial target for the development of anti-cancer agents<sup>10</sup> and plays a significant role in the ductal development of the mammary gland.<sup>11</sup> Over expression of EGFR is associated with various cancers.<sup>12</sup> The protein, with the PDB ID 4HJ0EGFR tyrosine kinase, was obtained from the Protein Data Bank.<sup>13,14</sup>

Among the four compounds subjected to docking, compound 3 exhibited the highest binding energy measuring -10.73 kcal/mol with EGFR. It formed a single hydrogen bond with the amino acid residue LEU838, with bond length 1.95 Å. Further, compound 3 exhibited  $\pi$ - $\pi$  stacking with the PHE699 residue. Compound 1 displayed second highest binding energy (-9.51 kcal/mol) and formed a hydrogen bond with the ASP831 amino acid residue, with a bond length of 2.15 Å. Similarly, nitrogen atom of amide bond in compound 4 established a hydrogen bond with THR830 residue, measuring bond length of 1.96 Å. Lastly, compound 2 has exhibited the lowest binding energy (-7.59 kcal/mol) among the four compounds and formed two hydrogen bonds. The first bond had a bond length of 1.96 Å with LYS721 residue while the second bond had a bond length 2.13 Å respectively with ASP831 residue (Figure 2-5 and Table 1).

## CONCLUSION

A series of derivatives including 1,3,4-oxadiazole, keto pyrazole, pyrrole and hydrazone derivatives of thieno[3,2-d]pyrimidines were successfully synthesized and purified using column chromatography.

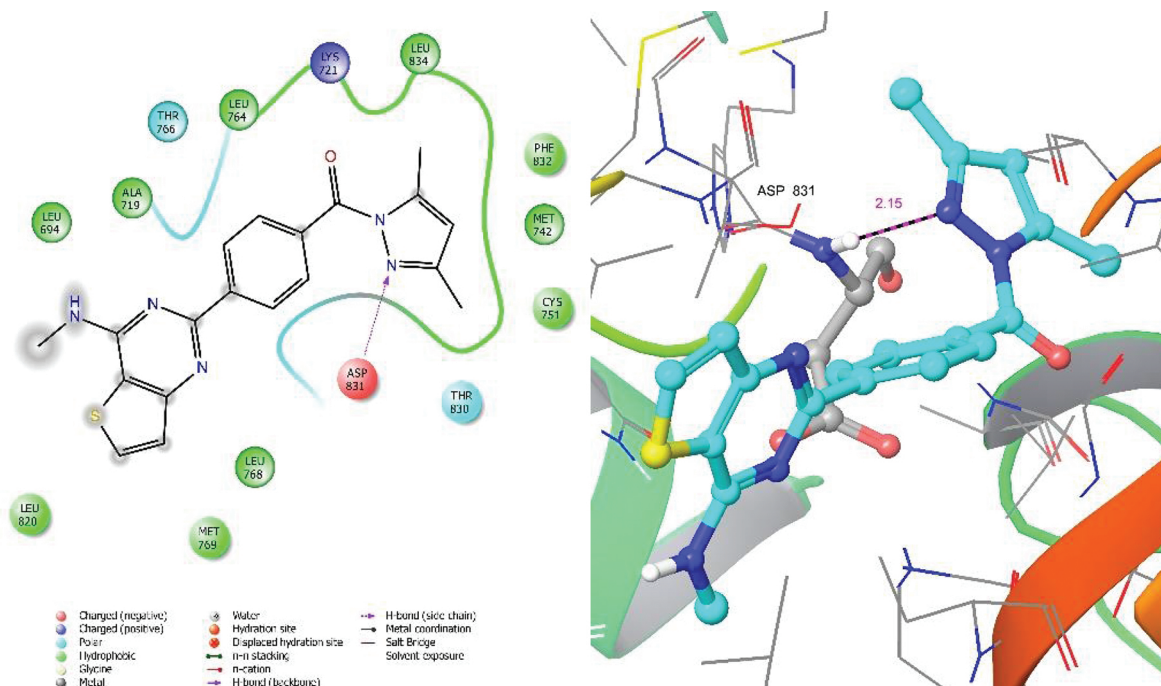


Figure 2: 2D and 3D ligand interactions of compound 1 with protein PDB ID 4HJ0

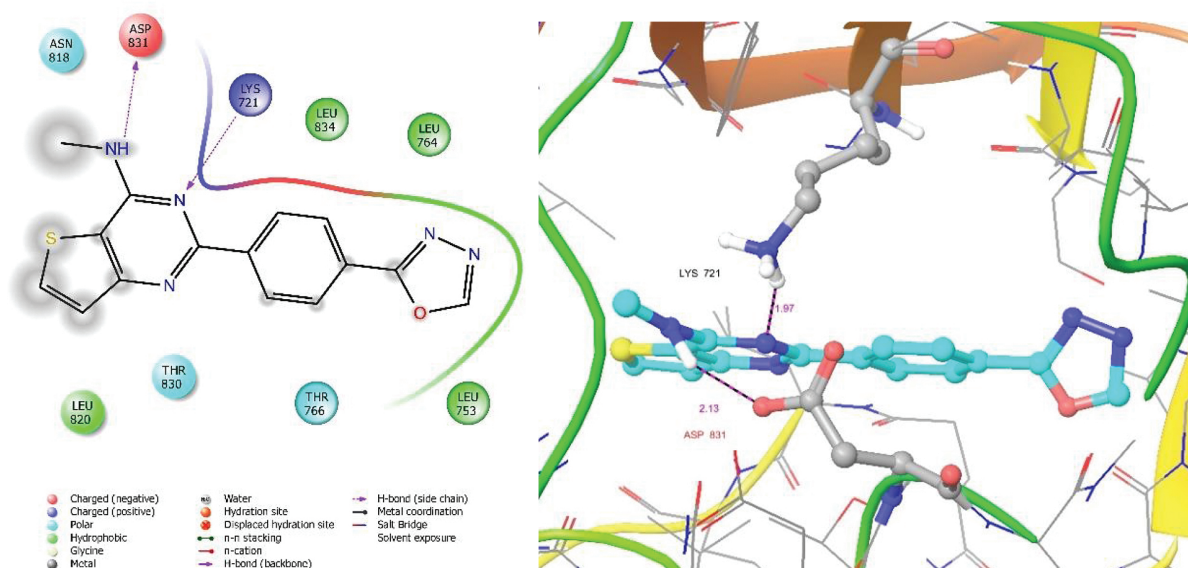


Figure 3: 2D and 3D ligand interactions of compound 2 with protein PDB ID 4HJO

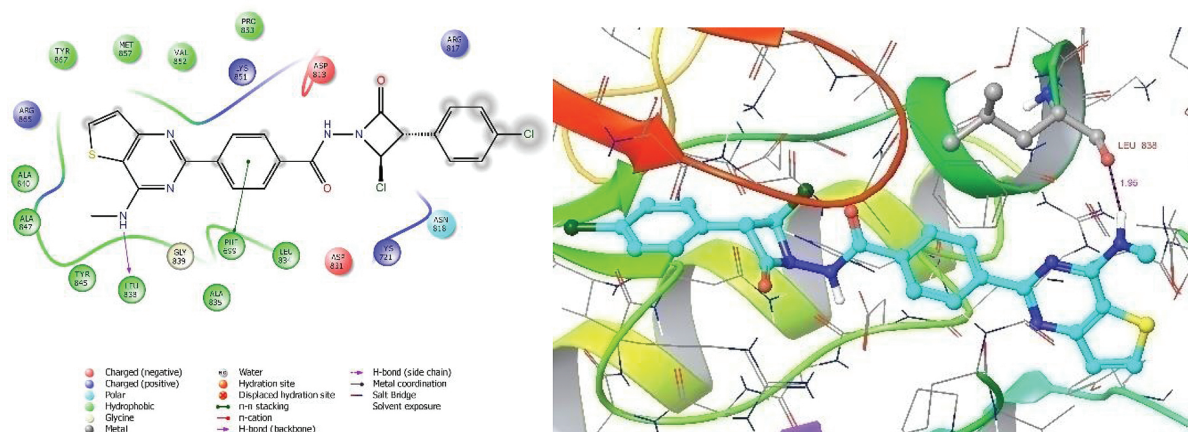


Figure 4: 2D and 3D ligand interactions of compound 3 with protein PDB ID 4HJO

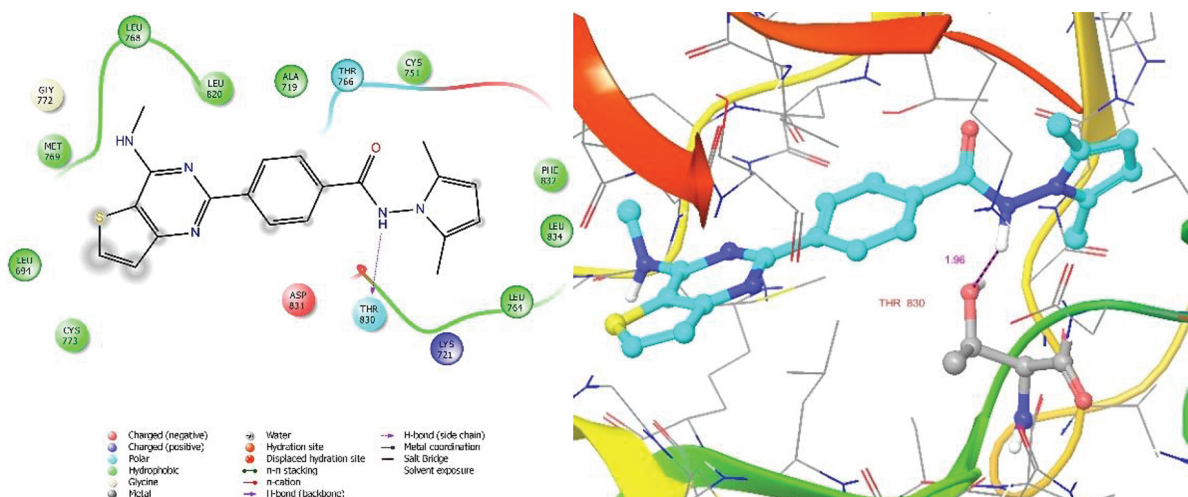


Figure 5: 2D and 3D ligand interactions of compound 4 with protein PDB ID 4HJO

Table 1. Molecular docking interaction parameters of compounds (1 to 4) with the epidermal growth factor receptor (PDB ID-4HJO)

Compound Entry	Binding Energy (kcal/mol)	Inhibition Constant	No. of hydrogen bonds	Residues involved in hydrogen bonding (bond length in Å)
1	-9.51	107.57 nM	1	ASP831(2.15)
2	-7.59	2.73 mM	2	LYS721(1.97), ASP831(2.13)
3	-10.73	13.57 nM	1	LEU838(1.95)
4	-8.88	307.87 nM	1	THR830(1.96)

Their melting points were determined to assess their purity. Additionally, elementary analysis was performed on all four final compounds to determine the elemental composition accurately. To further characterize the synthesized products, spectroscopic techniques such as IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and Mass were employed.

In pursuit of potential anti-cancer agents, the binding affinity of these derivatives was also investigated using molecular docking through Auto dock tools with a focus on the epidermal growth factor receptor (EGFR) which plays a pivotal role in cancer development. The study helps in the development of new anti-cancer agents. Among the four compounds subjected to docking, compound **3** exhibited the highest binding energy measuring -10.73 kcal/mol with EGFR.

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