Section A-Research paper
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Molecular Docking study of N-substituted Quinazoline Derivatives for theirEnzyme inhibition & Cytotoxic Activity

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Abstract

The presence of biologically active substances and natural products, quinazolinones are preferred themes. Therefore, an important research goal in organic and medicinal chemistry has been the development of effective methods for the synthesis of quinazoline derivatives. Molecular docking is frequently employed in contemporary drug design to comprehend drug-receptor interaction. The twelve N- substituted Quinazoline derivatives were synthesized in the current work. Using spectrum analysis, the newly synthesized compounds' structures were characterized. All synthetic compounds were subjected to molecular modelling to determine their propensity for binding to the 2FVD protein (Cyclin Dependent Kinase 2 (CDK2) with inhibitor of diaminopyrimidine). The cyclin-dependent kinases (CDKs) and their cyclin partners play a crucial role in controlling the cell cycle. Since dysregulation of CDKs occurs often in many human cancer cells, pharmacological inhibition of CDKs with small molecules has the potential to be a successful technique for the treatment of cancer as a hypothesised mode of their anticancer activity. The data obtained from the molecular docking was strongly correlated with that obtained from the biological screening which revealed that; compounds 1,6,7,2,12, and 8 showed the highest binding affinities towards 2 FVD protein in the range of -8.9 to - 8.4 kcal/mol than standard i.e. (4-Amino-2-{[1-(Methyl sulfonyl) Piperidin-4-yl]Amino}Pyrimidin-5-yl)(2,3-Difluoro-6 Methoxyphenyl) Methanone (LIA) which is to - 8.4 kcal/mol. The results showed that the most effective chemicals could be used in the development of more potent analogues for anticancer therapy in the future.

Key words: N-substituted, Quinazoline, Cytotoxicity, Molecular Docking, 2FVD protein

1. Introduction

In medicinal chemistry, quinazolines are notable for their broad range of antibacterial, antifungal, [1,2,3,4,5,6] anti-inflammatory, [7,8] antimalaria,[9] anti-HIV,[10] antiviral, [10,11] and antituberculosis [12] effects. Globally, the issue of bacterial resistance to

present medications is getting worse. The synthesis of novel quinazolinone compounds with strong antibacterial activity has been the subject of extensive research. By interacting with the cell wall and DNA structures, these compounds have antibacterial effects, especially against gram positive types of bacteria and fungus. [13] Structure activity relationship studies of quinazolinone derivatives in various literatures have revealed that substitution at positions 2 and 3, existence of halogen atom at 6 and 8 positions and substitution (mainly amine or substituted amine) at 4th position of the quinazolinone ring can improve their antimicrobial activities [14-17] During the last decade lots of anticancer drugs with quinazoline structure have been discovered. Gefitinib (Iressa) and erlotinib are important examples of this group which were introduced to the market as anticancer agents [18]

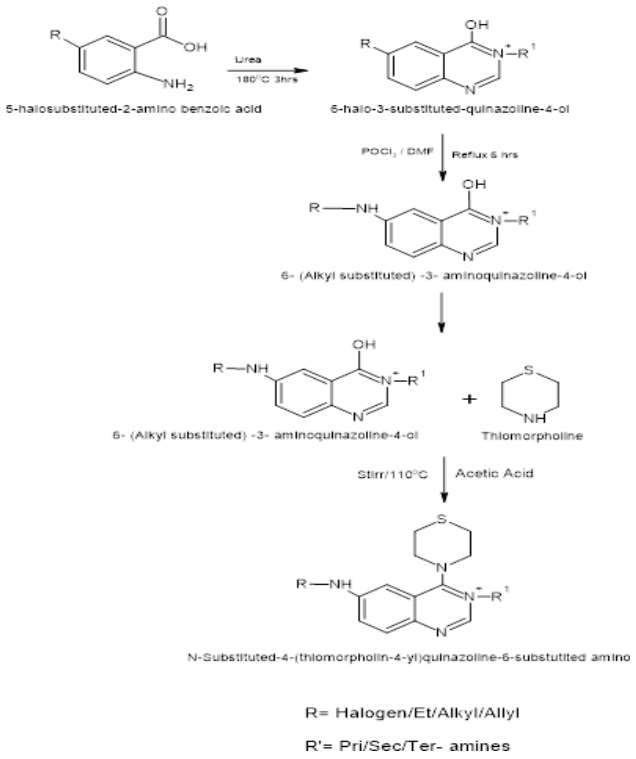
2. Experimental

2.1. Materials and methods

By using the open capillary approach, melting points were identified. A JASCO FT/IR-4100 spectrophotometer was used to record the IR spectra (in KBr pellets). TMS was used as the internal standard while recording the 1H NMR and 13C NMR spectra on a Bruker (400 MHz).Values for chemical shift are expressed in d (ppm) scales. A JEOL JMS-D 300 spectrometer operating at 70 eV was used to record the mass spectra. Thin layer chromatography (TLC) was used on aluminium sheets coated in silica gel (silica gel 60 F254) purchased from Merck to determine whether the reaction had fully completed. Without additional filtration, reagents and solvents of commercial quality were used. Chemicals from Aldrich Chemical Company are bought and utilised without additional purification, including 5-halosubstituted-2-amino benzoic acid, POCl3, DMF, and thiomorpholine.

2.2. General procedure for the synthesis of new derivatives of N-Substituted quinazoline-4-ol

The mixture of 5-halosubstituted-2-amino benzoic acid and urea was refluxed for3 hrs at 180^0c then obtained product filtered and reflux with POCL3 for 6 hrs. obtained compound treated with thiomorpholine in the presence of acetic acid



Scheme 1 Synthetic route for the compounds

2.3 In silico molecular docking studies

Twelve N-substituted Quinazoline Derivative chemicals and the Cyclin Dependent Kinase 2 (CDK2) protein, as shown in Figures 1 through 12, were predicted to interact in silico using Autodocking version 1.5.6 software techniques (https://vina.scripps.edu) [19]. Ligplot+ 2.2 and Maestro 12.5.139 were used for visualisation. The SDF file was converted to PDB format using Open Babel 3.1.1, which was acquired at http://openbabel.org [20]. On a Windows 10 computer, the computations were done.

2.3.1 Preparation **of Ligand:**

Each compound's structure was examined for connection errors in bond order after the ligands were drawn in ChemDraw Ultra 6.0 (a component of the ChemOffice suite) and assigned suitable 2D orientation. The total drug score was predicted using in silico using OSIRIS, an ADMET-based Java library layer and wholly internal drug discovery informatics system that offers reusable cheminformatics capability [21]. Using the Dundee PRODRG2 server, the molecules' energy was reduced [22]. Then, in order to perform the docking simulation, the energy-minimized compounds were read as input for AutoDock 1.5.6. [23].

2.3.2. Preparation of Protein:

Protein data bank (PDB) (http://www.rcsb.org) was used to extract the protein's three-dimensional (3D) structure. For the purpose of preparing the extracted protein, Ligplot+ 2.2 and Maestro 12.5.139 were used. The hetero-atoms and all water molecules that contributed to

the improvement in resolution were removed. Watch for ligand groups, protein groups, and active sites.

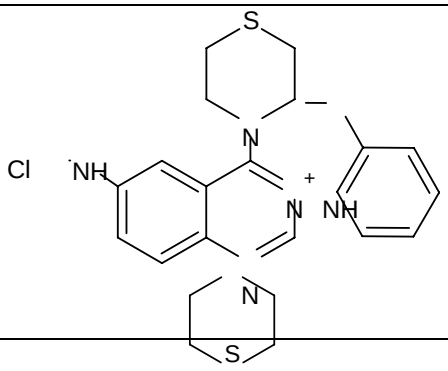
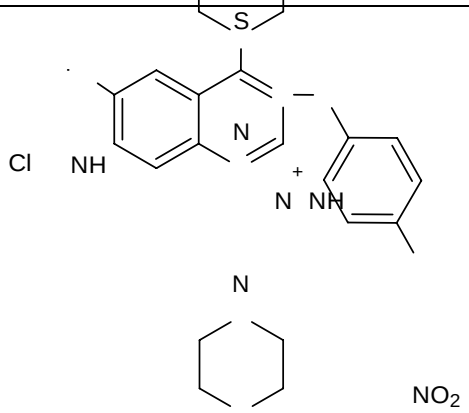
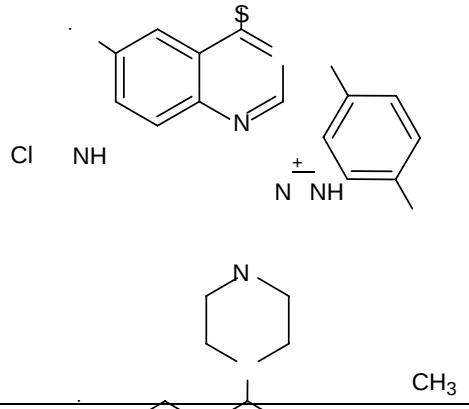
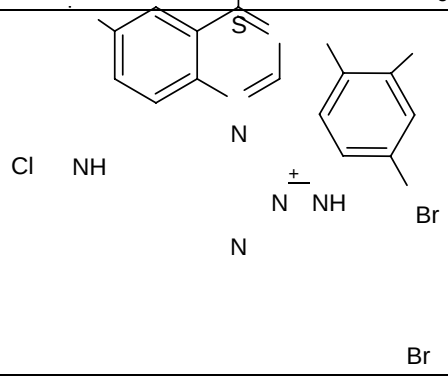
2.3.3 Target and Ligand Optimization

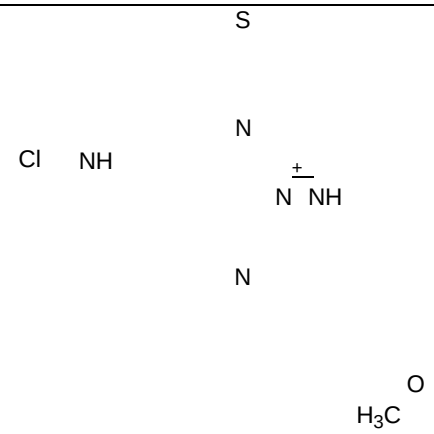
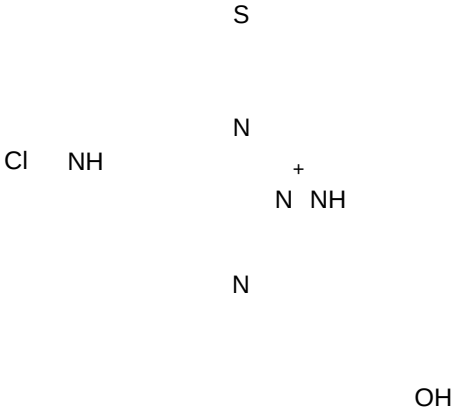
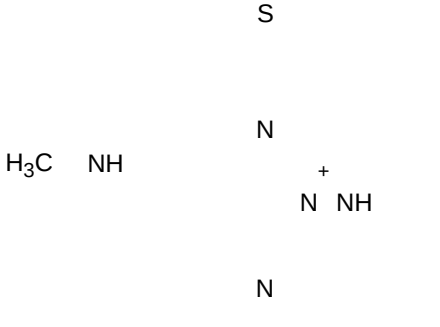
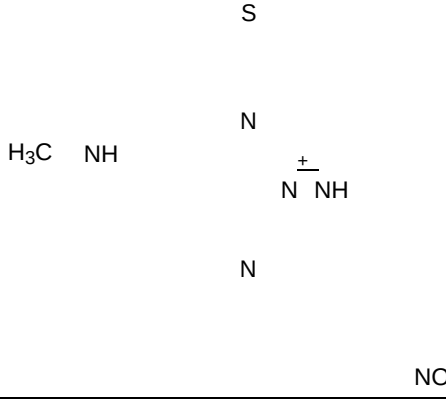
In molecular docking, an optimization technique was used to locate an appropriate binding pose of a ligand against a protein target to demonstrate high binding affinity. The docking accuracy is determined in a dynamic way using this algorithm. Autodock Vina (version 1.5.6) was used to find an appropriate binding pose, then Ligplot+ 2.2 and Maestro 12.5.139 were used for visualization.[24]

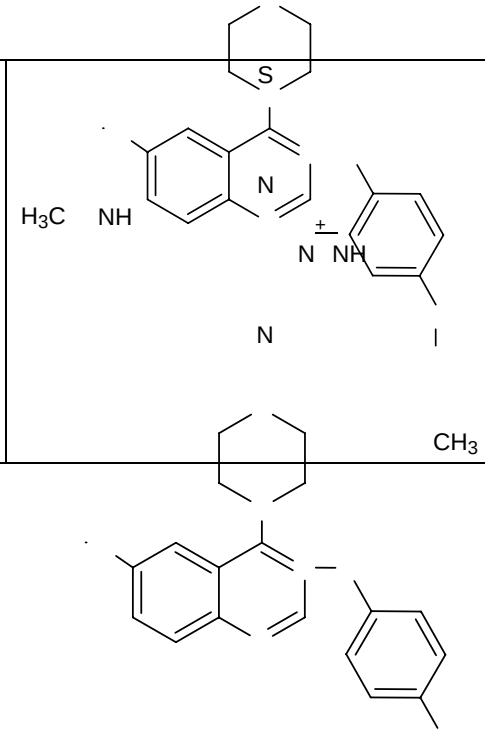
3 Result and Discussion:

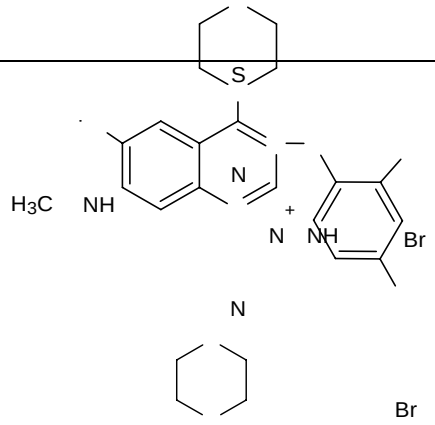
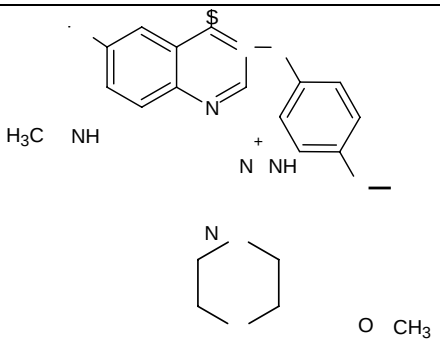
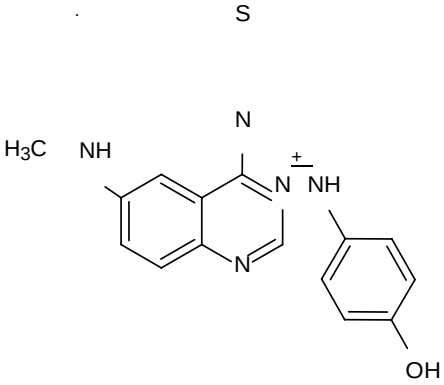
The mixture of 5-halosubstituted-2-amino benzoic acid and urea was refluxed for3 hrs at 180^0c then obtained product filtered and reflux with POCL3 for 6 hrs. obtained compound treated with thiomorpholine in the presence of acetic acid by substituting different alkyl group different N-Substituted derivatives are synthesized as shown in Table no 1. The synthecized derivaties are characterized using IR, 1H NMR, 13C NMR, mass spectra and TLC.

Table 1: Different N-Substituted quinazoline-4-ol Derivatives

Sr. No	Structure	Mole. Formula	Chemical Name	Molecular Weight
1.		$C_{18}H_{19}ClN_5S$	3-anilino-6-(chloroamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**372**
2.		$C_{18}H_{18}ClN_6O_2S$	6-(chloroamino)-3-(4-nitroanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**417**
3.		$C_{19}H_{21}ClN_5S$	6-(chloroamino)-3-(4-methylanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**386**
4.		$C_{18}H_{27}Br_2ClN_5S$	6-(chloroamino)-3-(2,4-dibromoanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**530**

5.		$C_{19}H_{21}ClN_5OS$	6-(chloroamino)-3-(4-methoxyanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**402**
6.		$C_{18}H_{19}ClN_5OS$	6-(chloroamino)-3-(4-hydroxyanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**388**
7.		$C_{19}H_{22}N_5S$	3-anilino-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**352**
8.		$C_{18}H_{21}N_6O_2S$	6-(methylamino)-3-(4-nitroanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**397**

9.		$C_{20}H_{24}N_5S$	6-(methylamino)-3-(4-methylanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**366**

10.		$C_{19}H_{20}Br_2N_5S$	3-(2,4-dibromoanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**510**
11.		$C_{20}H_{24}N_5OS$	3-(4-methoxyanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**382**
12.		$C_{19}H_{22}N_5OS$	3-(4-hydroxyanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium	**368**

3.1 Characterization of synthesized compounds

3.1.1. 3-anilino-6-(chloroamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 1673, 1607, 1556, 1484, 1370, 1321, 1178, 1154, 1089, 1020, 879, 776, 696;
^{1}H NMR: δ 3.41-3.59 (8H, 3.49 (*J* = 16.7, 6.8, 2.2 Hz), 3.52 (*J* = 13.5, 6.8, 2.2 Hz)), 6.95 (1H, tt, *J* = 7.8, 1.2 Hz), 7.13 (2H, dtd, *J* = 8.2, 1.2, 0.5 Hz), 7.28 (2H, dddd, *J* = 8.2, 7.8, 1.4, 0.5 Hz), 7.54-7.66 (2H, 7.59 (*J* = 3.7, 0.5 Hz), 7.60 (*J* = 3.7, 1.8 Hz)), 8.41 (1H, *J* = 0.4 Hz), 8.73 (1H, dd, *J* = 1.8, 0.5 Hz).^{13}C NMR: δ 27.3 (2C, s), 49.1 (2C, s), 113.0 (2C, s), 113.7 (1C, s), 117.7 (1C, s), 126.9 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 128.8 (1C, s), 134.4 (1C, s), 139.5 (1C, s), 145.8 (1C, s), 147.3 (1C, s), 155.4 (1C, s). M/Z 372.89, M+: 372.10, M-: 372.10, [M+H]+: 373.11, [M+H]-: 373.11, [M-H]+: 371.09, [M-H]-: 371.09 C(57.98%) H(5.14%) Cl(9.51%) N(18.78%) S(8.60%)

3.1.2. 6-(chloroamino)-3-(4-nitroanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 3088, 3054, 1670, 1613, 1570, 1485, 1371, 1325, 1275, 1155, 1014, 956, 880, 775
^{1}H NMR: δ 3.41-3.59 (8H, 3.49 (*J* = 16.7, 6.8, 2.2 Hz), 3.51 (*J* = 13.5, 6.8, 2.2 Hz)), 7.32 (2H,

J = 8.4, 2.2, 0.5 Hz), 7.54-7.67 (2H, 7.60 (*J* = 6.1, 0.5 Hz), 7.61 (*J* = 6.1, 1.8 Hz), 8.13 (2H, *J* = 8.4, 1.8, 0.5 Hz), 8.53 (1H, d, *J* = 0.4 Hz), 8.74 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 27.3 (2C, s), 49.1 (2C, s), 113.7 (1C, s), 117.7-117.8 (3C, 117.7 (s), 117.7 (s), 125.0 (2C, s), 126.9 (1C, s), 128.8 (1C, s), 134.4 (1C, s), 139.5 (1C, s), 145.8 (1C, s), 147.2-147.4 (2C, 147.3 (s), 147.3 (s)), 155.4 (1C, s).M/Z 417.89, M+: 417.08, M-: 417.09, [M+H]+: 418.09, [M+H]-: 418.09, [M-H]+: 416.08, [M-H]-: 416.08 C(51.73%) H(4.34%) Cl(8.48%) N(20.11%) O(7.66%) S(7.67%)

3.1.3. 6-(chloroamino)-3-(4-methylanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 3058, 2361, 1668, 1602, 1509, 1486, 1364, 1320, 1216, 1013, 885, 784, 744, 699 ^{1}H NMR: δ 2.21 (3H, s), 3.41-3.59 (8H, 3.49 (*J* = 16.7, 6.8, 2.2 Hz), 3.52 (*J* = 13.5, 6.8, 2.2 Hz)), 6.96-7.15 (4H, 7.02 (*J* = 8.1, 1.4, 0.5 Hz), 7.09 (*J* = 8.1, 1.4, 0.5 Hz)), 7.53-7.65 (2H, 7.59 (*J* = 3.7, 1.8 Hz), 7.59 (*J* = 3.7, 0.5 Hz)), 8.41 (1H, d, *J* = 0.4 Hz), 8.64 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 21.3 (1C, s), 27.3 (2C, s), 49.1 (2C, s), 113.7 (1C, s), 117.4 (2C, s), 117.7 (1C, s), 126.9 (1C, s), 128.8 (1C, s), 129.6 (2C, s), 134.4 (1C, s), 139.5 (1C, s), 141.5 (1C, s), 145.8 (1C, s), 147.3 (1C, s), 155.4 (1C, s). M/Z 386.92, M+: 386.11, M-: 386.12, [M+H]+: 387.12, [M+H]-: 387.12, [M-H]+: 385.11, [M-H]-: 385.11 C(58.98%) H(5.47%) Cl(9.16%) N(18.10%) S(8.29%)

3.1.4 6-(chloroamino)-3-(2,4-dibromoanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 1655, 1608, 1503, 1400, 1377, 1270, 1222, 1143, 1115, 1016, 837, 784 ^{1}H NMR: δ 3.41-3.59 (8H, 3.50 (*J* = 16.7, 6.8, 2.2 Hz), 3.52 (*J* = 13.5, 6.8, 2.2 Hz), 7.35-7.67 (5H, 7.41 (*J* = 8.5, 0.5 Hz), 7.45 (*J* = 8.5, 1.7 Hz), 7.52 (*J* = 1.7, 0.5 Hz), 7.59 (*J* = 6.1, 0.5 Hz), 7.61 (*J* = 6.1, 1.8 Hz)), 8.41 (1H, d, *J* = 0.4 Hz), 8.74 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 27.3 (2C, s), 49.1 (2C, s), 113.6-113.8 (2C, 113.6 (s), 113.7 (s)), 117.7-117.8 (2C, 117.7 (s), 117.7 (s)), 119.6 (1C, s), 126.9 (1C, s), 128.8 (1C, s), 131.7 (1C, s), 134.4 (1C, s), 134.5 (1C, s), 139.4-139.6 (2C, 139.5 (s), 139.5 (s)), 147.3 (1C, s), 155.4 (1C, s). M/Z 530.68, M+: 527.92, M-: 527.92, [M+H]+: 528.93, [M+H]-: 528.93, [M-H]+: 526.91, [M-H]-: 526.91 C(40.74%) H(3.23%) Br(30.11%) Cl(6.68%) N(13.20%) S(6.04%)

3.1.5. 6-(chloroamino)-3-(4-methoxyanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 1660, 1608, 1510, 1479, 1367, 1267, 1221, 1160, 1142, 1023, 889, 843, 808 ^{1}H NMR: δ 3.41-3.59 (8H, 3.49 (*J* = 16.7, 6.8, 2.2 Hz), 3.52 (*J* = 13.5, 6.8, 2.2 Hz) 3.76 (3H, s), 6.63 (2H, *J* = 8.8, 2.7, 0.6 Hz), 7.42 (2H, *J* = 8.8, 1.4, 0.6 Hz), 7.54-7.66 (2H, 7.59 (*J* = 3.7, 0.5 Hz), 7.61 (*J* = 3.7, 1.8 Hz), 8.41 (1H, d, *J* = 0.4 Hz), 8.64 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 27.3 (2C, s), 49.1 (2C, s), 56.0 (1C, s), 113.7 (1C, s), 114.5 (2C, s), 117.7-117.8 (3C, 117.7 (s), 117.7 (s)), 126.9 (1C, s), 128.8 (1C, s), 134.4 (1C, s), 139.5 (1C, s), 145.8 (1C, s), 147.3 (1C, s), 155.4 (1C, s), 159.8 (1C, s).M/Z 402.92, M+: 402.11, M-: 402.11, [M+H]+: 403.12, [M+H]-: 403.12, [M-H]+: 401.10, [M-H]-: 401.10 C(56.64%) H(5.25%) Cl(8.80%) N(17.38%) O(3.97%) S(7.96%)

3.1.6 6-(chloroamino)-3-(4-hydroxyanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 1667, 1606, 1493, 1474, 1370, 1244, 1169, 1022, 833, 771, 697

^{1}H NMR: δ 3.41-3.59 (8H, 3.49 (*J* = 16.7, 6.8, 2.2 Hz), 3.52 (*J* = 13.5, 6.8, 2.2 Hz), 6.72 (2H, *J* = 8.8, 2.6, 0.5 Hz), 7.43 (2H, *J* = 8.8, 1.7, 0.5 Hz), 7.54-7.66 (2H, 7.59 (*J* = 3.7, 0.5 Hz), 7.61 (*J* = 3.7, 1.8 Hz)), 8.41 (1H, d, *J* = 0.4 Hz), 8.73 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 27.3 (2C, s), 49.1 (2C, s), 113.7 (1C, s), 115.2 (2C, s), 117.7-117.8 (3C, 117.7 (s), 117.7 (s)), 126.9 (1C, s), 128.8 (1C, s), 134.4 (1C, s), 139.5 (1C, s), 145.8 (1C, s), 147.3 (1C, s), 155.4 (1C, s), 157.4 (1C, s). M/Z 388.89, M+: 388.09, M-: 388.09, [M+H]+: 389.10, [M+H]-: 389.10, [M-H]+: 387.09, [M-H]-: 387.09 C(55.59%) H(4.92%) Cl(9.12%) N(18.01%) O(4.11%) S(8.25%)

3.1.7 3-anilino-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 1681, 1606, 1491, 1472, 1374, 1320, 1235, 1168, 1149, 1019, 837 ^{1}H NMR: δ 3.00 (3H, s), 3.36-3.58 (8H, 3.44 (*J* = 13.4, 6.8, 2.2 Hz), 3.49 (*J* = 16.6, 6.8, 2.2 Hz)), 6.95 (1H, t, *J* = 7.8, 1.2 Hz), 7.12 (2H, *J* = 8.2, 1.2, 0.5 Hz), 7.28 (2H, *J* = 8.2, 7.8, 1.4, 0.5 Hz), 7.45 (1H, *J* = 3.9, 0.5 Hz), 7.67 (1H, *J* = 3.9, 1.8 Hz), 8.40 (1H, d, *J* = 0.4 Hz), 8.59 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 27.3 (2C, s), 30.2 (1C, s), 49.1 (2C, s), 113.0 (2C, s), 113.7 (1C, s), 117.7 (1C, s), 126.9 (1C, s), 127.8 (1C, s), 128.2 (2C, s), 128.8 (1C, s), 134.4 (1C, s), 138.7 (1C, s), 145.8 (1C, s), 147.3 (1C, s), 155.4 (1C, s). M/Z 352.47, M+: 352.15, M-: 352.15, [M+H]+: 353.16, [M+H]-: 353.16, [M-H]+: 351.15, [M-H]-: 351.15 C(64.74%) H(6.29%) N(19.87%) S(9.10%)

3.1.8. 6-(methylamino)-3-(4-nitroanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 2989, 1831, 1674, 1601, 1502, 1476, 1407, 1378, 1282, 1245, 1216, 1112, 1022, 970, 854 ^{1}H NMR: δ 3.00 (3H, s), 3.32-3.58 (8H, 3.40 (*J* = 13.4, 6.8, 2.2 Hz), 3.49 (*J* = 16.6, 6.8, 2.2 Hz)), 7.32 (2H, *J* = 8.4, 2.2, 0.5 Hz), 7.45 (1H, *J* = 3.9, 0.5 Hz), 7.68 (1H, *J* = 3.9, 1.8 Hz), 8.13 (2H, *J* = 8.4, 1.8, 0.5 Hz), 8.48-8.65 (2H, 8.53 (d, *J* = 0.4 Hz), 8.59 (*J* = 1.8, 0.5 Hz)). ^{13}C NMR: δ 27.3 (2C, s), 30.2 (1C, s), 49.1 (2C, s), 113.7 (1C, s), 117.7-117.8 (3C, 117.7 (s), 117.7 (s)), 125.0 (2C, s), 126.9 (1C, s), 128.8 (1C, s), 134.4 (1C, s), 138.7 (1C, s), 145.8 (1C, s), 147.2-147.4 (2C, 147.3 (s), 147.3 (s)), 155.4 (1C, s). M/Z 397.47, M+: 397.14, M-: 397.14, [M+H]+: 398.15, [M+H]-: 398.15, [M-H]+: 396.13, [M-H]-: 396.13 C(57.41%) H(5.33%) N(21.14%) O(8.05%) S(8.07%)

3.1.9 6-(methylamino)-3-(4-methylanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 3009, 1901, 1674, 1604, 1493, 1468, 1367, 1245, 1169, 1018, 927, 820, 773, 650 ^{1}H NMR: δ 2.21 (3H, s), 3.00 (3H, s), 3.37-3.58 (8H, 3.45 (*J* = 13.4, 6.8, 2.2 Hz), 3.49 (*J* = 16.6, 6.8, 2.2 Hz), 6.96-7.15 (4H, 7.02 (*J* = 8.1, 1.4, 0.5 Hz), 7.09 (*J* = 8.1, 1.4, 0.5 Hz), 7.45 (1H, , *J* = 3.9, 0.5 Hz), 7.66 (1H, *J* = 3.9, 1.8 Hz), 8.40 (1H, d, *J* = 0.4 Hz), 8.59 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 21.3 (1C, s), 27.3 (2C, s), 30.2 (1C, s), 49.1 (2C, s), 113.7 (1C, s), 117.4 (2C, s), 117.7 (1C, s), 126.9 (1C, s), 128.8 (1C, s), 129.6 (2C, s), 134.4 (1C, s), 138.7 (1C, s), 141.5 (1C, s), 145.8 (1C, s), 147.3 (1C, s), 155.4 (1C, s). M/Z 366.50, M+: 366.17, M-: 366.17, [M+H]+: 367.18, [M+H]-: 367.18, [M-H]+: 365.16, [M-H]-: 365.16 C(65.54%) H(6.60%) N(19.11%) S(8.75%)

3.1.10 3-(2,4-dibromoanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 2945, 1667, 1606, 1508, 1473, 1365, 1249, 1172, 1018, 974, 875, 826, 770, 694

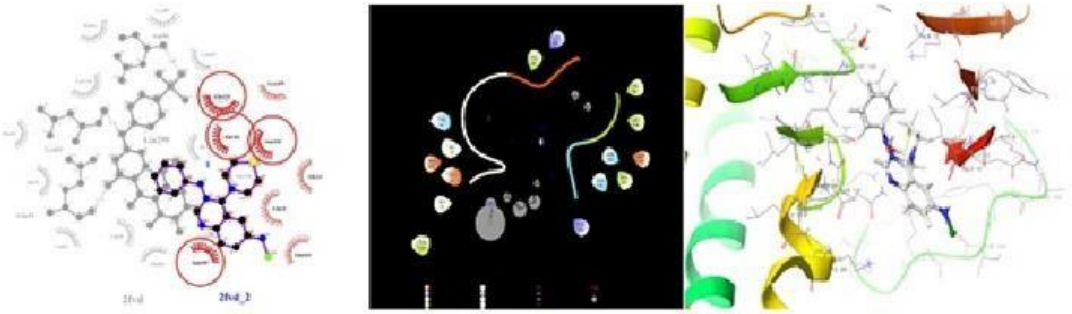
^{1}H NMR: δ 2.99 (3H, s), 3.41-3.58 (8H, 3.49 (*J* = 16.6, 6.8, 2.2 Hz), 3.50 (*J* = 13.4, 6.8, 2.2 Hz)), 7.34-7.57 (4H, 7.41 (*J* = 8.5, 0.5 Hz), 7.45 (*J* = 8.5, 1.7 Hz), 7.45 (*J* = 3.9, 0.5 Hz), 7.52 (*J* = 1.7, 0.5 Hz)), 7.68 (1H, *J* = 3.9, 1.8 Hz), 8.41 (1H, d, *J* = 0.4 Hz), 8.59 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 27.3 (2C, s), 30.2 (1C, s), 49.1 (2C, s), 113.6-113.8 (2C, 113.6 (s), 113.7 (s)), 117.7-117.8 (2C, 117.7 (s), 117.7 (s)), 119.6 (1C, s), 126.9 (1C, s), 128.8 (1C, s), 131.7 (1C, s), 134.4 (1C, s), 134.5 (1C, s), 138.7 (1C, s), 139.5 (1C, s), 147.3 (1C, s), 155.4 (1C, s). M/Z 510.26, M+: 507.97, M-: 507.98, [M+H]+: 508.98, [M+H]-: 508.98, [M-H]+: 506.97, [M-H]-: 506.97 C(44.72%) H(3.95%) Br(31.32%) N(13.72%) S(6.28%)

3.1.11 -(4-methoxyanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 2983, 2355, 1674, 1608, 1503, 1479, 1378, 1285, 1216, 1173, 1115, 1017, 972, 830, 804 ^{1}H NMR: δ 3.00 (3H, s), 3.36-3.58 (8H, 3.44 (*J* = 13.4, 6.8, 2.2 Hz), 3.49 (*J* = 16.6, 6.8, 2.2 Hz)), 3.76 (3H, s), 6.63 (2H, *J* = 8.8, 2.7, 0.6 Hz), 7.36-7.50 (3H, 7.42 (*J* = 8.8, 1.4, 0.6 Hz), 7.45 (*J* = 3.9, 0.5 Hz)), 7.67 (1H, *J* = 3.9, 1.8 Hz), 8.40 (1H, d, *J* = 0.4 Hz), 8.59 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 27.3 (2C, s), 30.2 (1C, s), 49.1 (2C, s), 56.0 (1C, s), 113.7 (1C, s), 114.5 (2C, s), 117.7-117.8 (3C, 117.7 (s), 117.7 (s)), 126.9 (1C, s), 128.8 (1C, s), 134.4 (1C, s), 138.7 (1C, s), 145.8 (1C, s), 147.3 (1C, s), 155.4 (1C, s), 159.8 (1C, s). M/Z 382.50, M+: 382.16, M-: 382.17, [M+H]+: 383.17, [M+H]-: 383.17, [M-H]+: 381.16, [M-H]-: 381.16 C(62.80%) H(6.32%) N(18.31%) O(4.18%) S(8.38%)

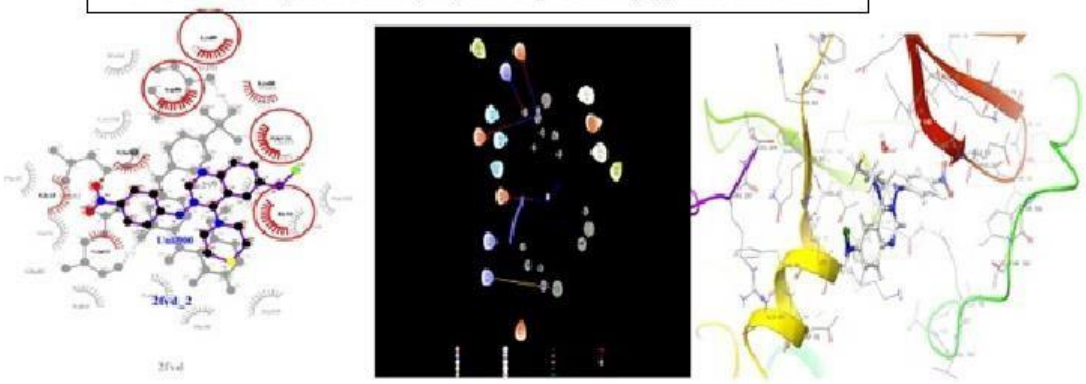
3.1.12 3-(4-hydroxyanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium: IR (KBr, cm^{-1}) *v*: 1658, 1609, 1508, 1473, 1466, 1379, 1325, 1248, 1172, 1142, 1026, 835, 825, 807, 645 ^{1}H NMR: δ 3.00 (3H, s), 3.36-3.58 (8H, 3.44 (*J* = 13.4, 6.8, 2.2 Hz), 3.49 (*J* = 16.6, 6.8, 2.2 Hz)), 6.72 (2H, *J* = 8.8, 2.6, 0.5 Hz), 7.37-7.50 (3H, 7.43 (*J* = 8.8, 1.7, 0.5 Hz), 7.45 (*J* = 3.9, 0.5 Hz)), 7.67 (1H, *J* = 3.9, 1.8 Hz), 8.40 (1H, d, *J* = 0.4 Hz), 8.59 (1H, *J* = 1.8, 0.5 Hz). ^{13}C NMR: δ 27.3 (2C, s), 30.2 (1C, s), 49.1 (2C, s), 113.7 (1C, s), 115.2 (2C, s), 117.7-117.8 (3C, 117.7 (s), 117.7 (s)), 126.9 (1C, s), 128.8 (1C, s), 134.4 (1C, s), 138.7 (1C, s), 145.8 (1C, s), 147.3 (1C, s), 155.4 (1C, s), 157.4 (1C, s). M/Z 368.47, M+: 368.15, M-: 368.15, [M+H]+: 369.16, [M+H]-: 369.16, [M-H]+: 367.14, [M-H]-: 367.14 C(61.93%) H(6.02%) N(19.01%) O(4.34%) S(8.70%)

3.2. In silico molecular docking studies:

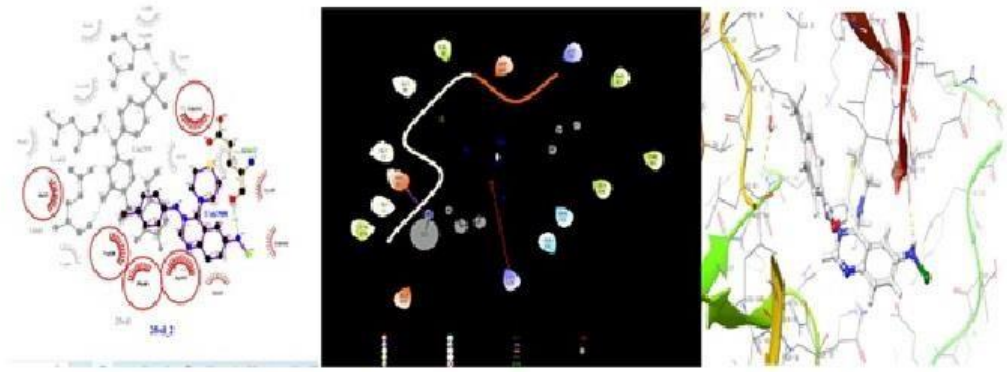
The N-substituted quinazoline-4-ol derivatives' potential as anticancer agents might be investigated using a variety of protein architectures. For the molecular docking investigation, we have chosen the Cyclin Dependent Kinase 2 (CDK2) protein and obtained it from the RCSB protein data library. One of the key methods used in drug development is molecular docking, which accurately predicts binding affinities, amino acid involvements, and protein-ligand complexes. [25-27]



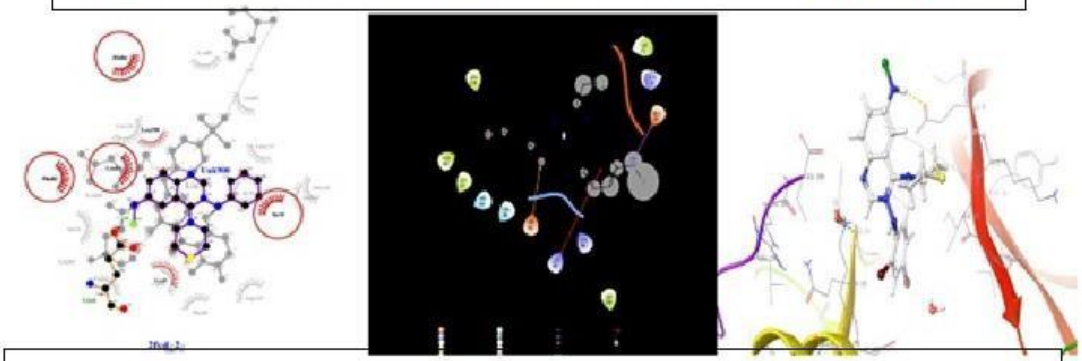
1. 3-anilino-6-(chloroamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



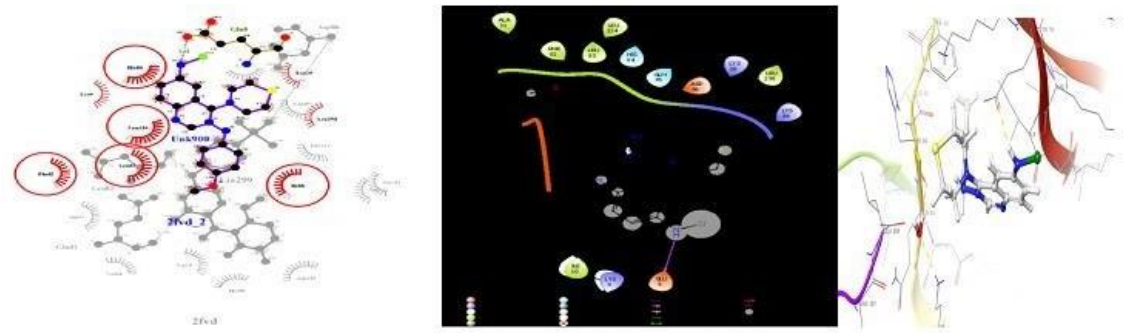
2. 6-(chloroamino)-3-(4-nitroanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



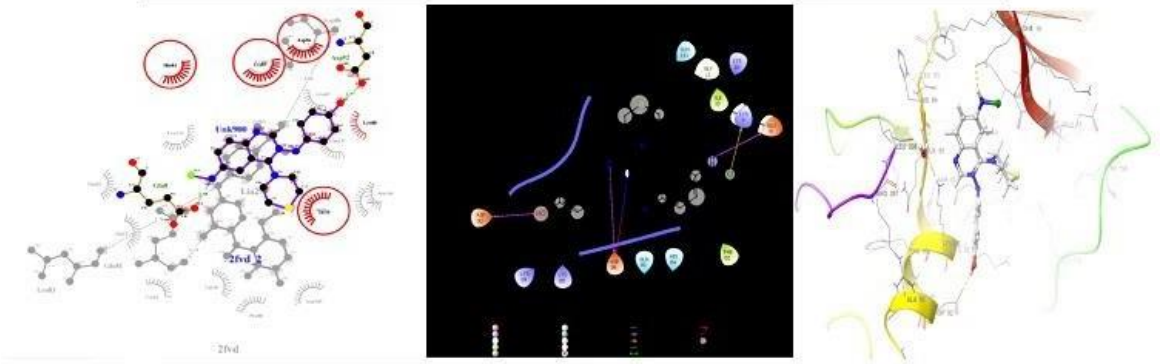
3. 6-(chloroamino)-3-(4-methylanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



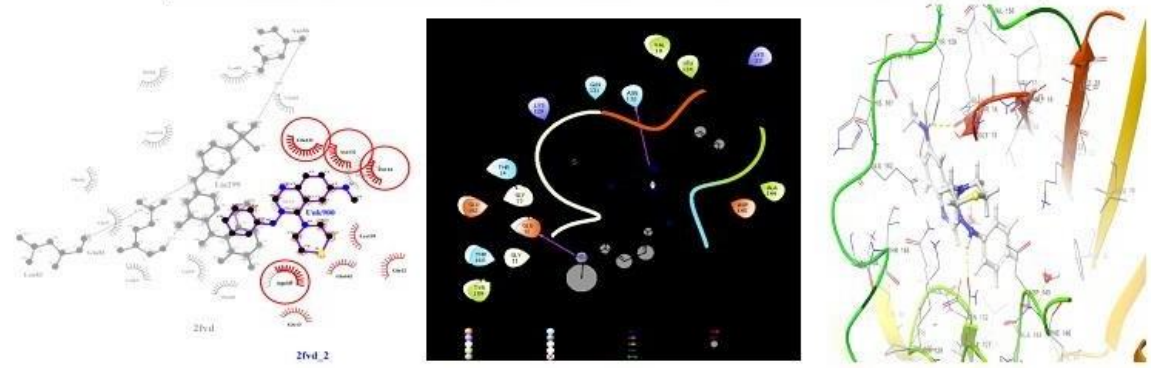
4. 6-(chloroamino)-3-(2,4-dibromoanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



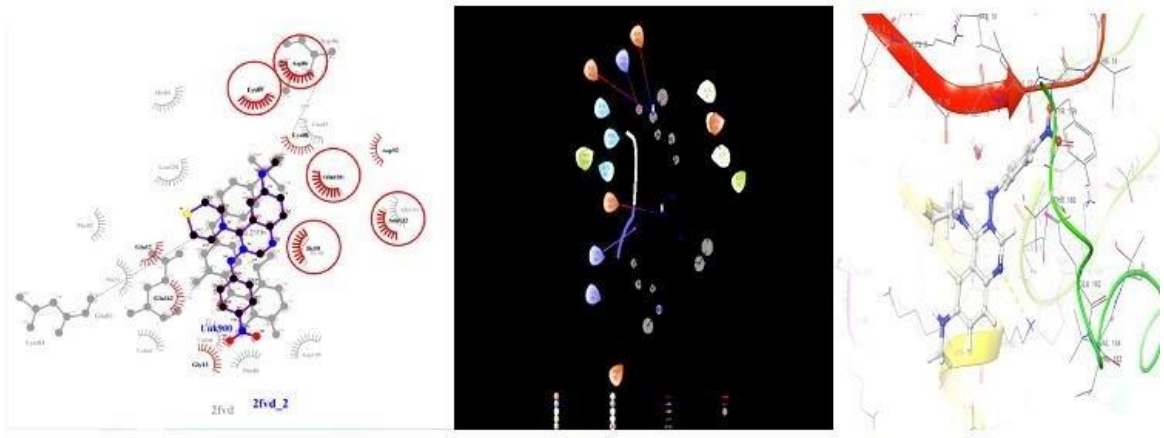
5. 6-(chloroamino)-3-(4-methoxyanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



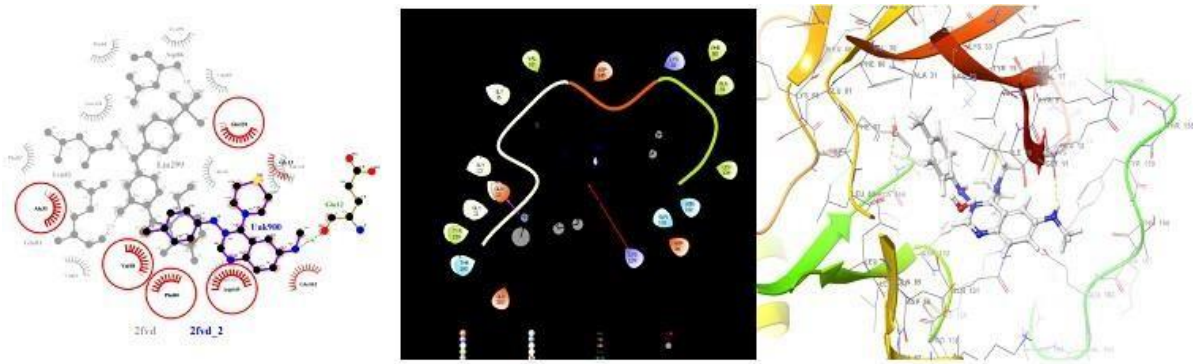
5. 6-(chloroamino)-3-(4-methoxyanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



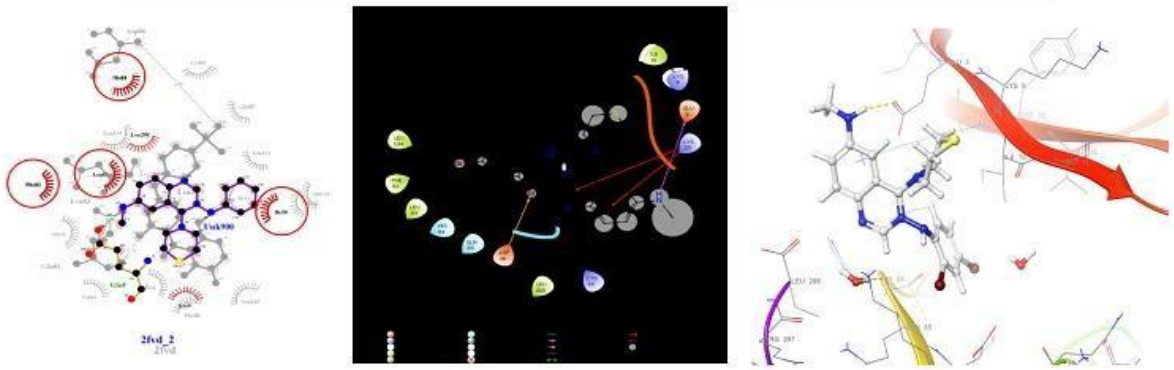
7. 3-anilino-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



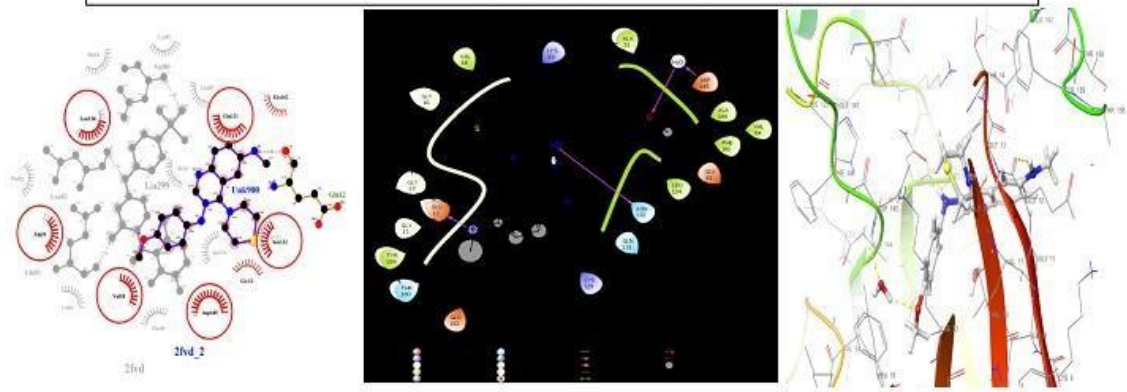
8. 6-(methylamino)-3-(4-nitroanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



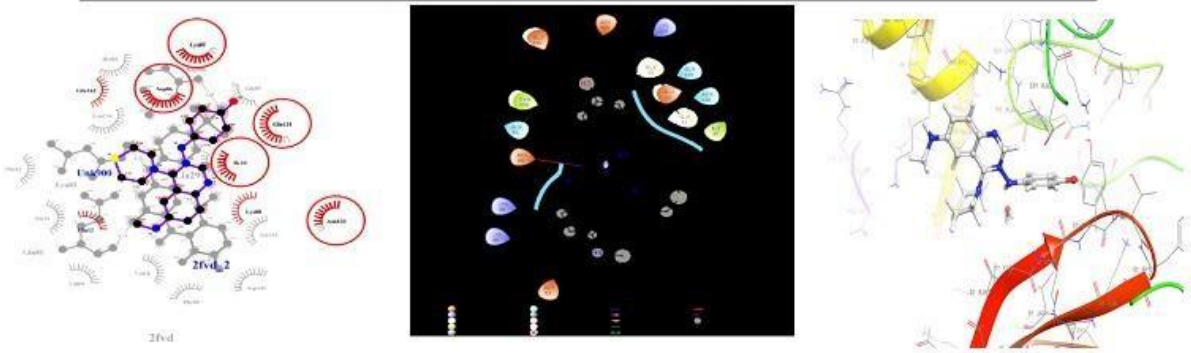
9. 6-(methylamino)-3-(4-methylanilino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



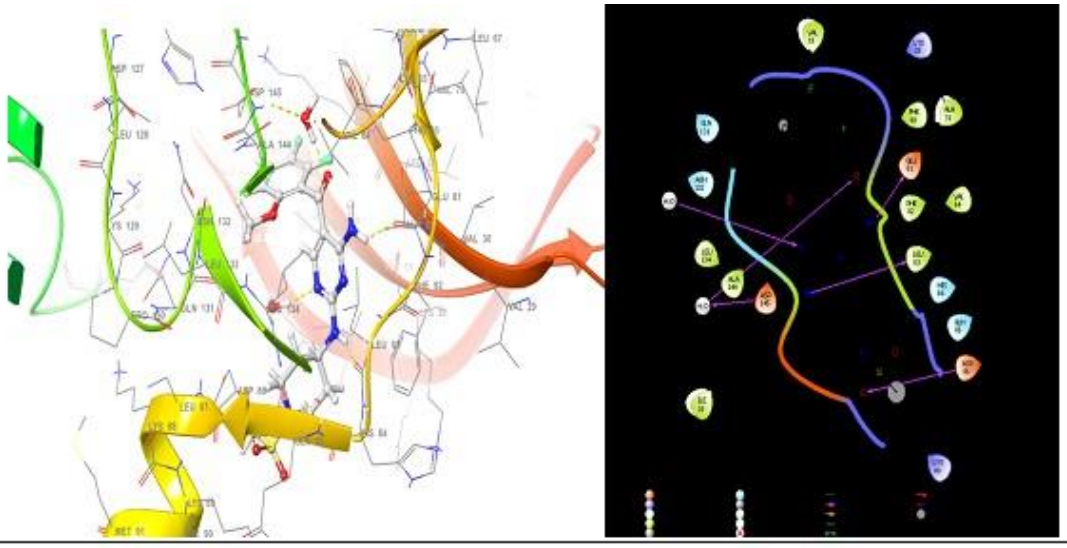
10. 3-(2,4-dibromoanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



11.3 -(4-methoxyanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



12. 3-(4-hydroxyanilino)-6-(methylamino)-4-(thiomorpholin-4-yl)quinazolin-3-ium



Std. - (4-AMINO-2-{[1-(METHYLSULFONYL)PIPERIDIN-4-YL]AMINO}PYRIMIDIN-5-YL)(2,3-DIFLUORO-6-METHOXYPHENYL)METHANONE (LIA)

As compare to standard (LIA) all synthesized compound have good anticancer activity in range of -7.5 to -8.6 kcal/ mol. The data obtained from the molecular docking was strongly correlated with that obtained from the biological screening which revealed that; compounds 1,6,7,2,12, and 8 showed the highest binding affinities towards 2 FVD protein in the range of -8.9 to - 8.4 kcal/mol than standard i.e. (4-Amino-2-{[1-(Methylsulfonyl)Piperidin-4-yl]Amino}Pyrimidin-5-yl)(2,3-Difluoro-6 Methoxyphenyl) Methanone (LIA) which is to - 8.4 kcal/Mol.The binding affinities are as shown in table no. 2.The compound 1 showed highest binding affinity i.e. -8.9 and showed hydrophobic interaction amino acid ALA at bond distance of 3.57. and hydrogen bond with amino acid GLU at distance 2.92 the compound 6 showed -8.7 binding affinity and hydrophobic interaction with amino acid LYS at distance of 3.54. compound 7 showed same binding affinity (-8.7) with hydrophobic interaction with amino acid ALA at distance 3.59. The compound 2 showed binding affinity -8.6 and hydrophobic interaction with amino acid LYS at bond distance of 3.54. and hydrogen bond with amino acid ASP at distance 3.54, and with amino acid LYS at distance 2.92. The compound 12 showed binding affinity of -8.5. and hydrophobic interaction with amino acid LYS at distance 3.58 and with GLN at distance 3.99. hydrogen bond with amino acid LYS & ASP at distance 2.83 & 3.16 respectively.

Table 2: Different synthesized derivatives with their Binding Affinities and interactions against protein 2 FVD

Compound	Type of Interaction	Binding Affinities	Residue	Amino Acid	Distance
1.	Hydrophobic Interactions	-8.9	144A	ALA	3.57
	Hydrogen Bonds		12A	GLU	2.92
			12A	GLU	2.86

2.	Hydrophobic Interactions	-8.6	88A	LYS	3.54
	Hydrogen Bonds		86A	ASP	3.49
			88A	LYS	2.92
3.	Hydrophobic Interactions	-8.3	31A	ALA	3.47
			80A	PHE	3.66
			145A	ASP	3.58
	Hydrogen Bonds		12A	GLU	2.71
			12A	GLU	2.08
			131A	GLN	3.68
4.	Hydrophobic Interactions	-8.1	10A	ILE	3.76
			82A	PHE	3.60
	Hydrogen Bonds		8A	GLU	2.98
			298A	LEU	3.92
	Water Bridges		89A	LYS	3.18
5.	Hydrophobic Interactions	-7.9	10A	ILE	3.84
			82A	PHE	3.44
			134A	LEU	3.88
	Hydrogen Bond		8A	GLU	1.88
			84A	HIS	2.87
6.	Hydrophobic Interactions	-8.7	88A	LYS	3.80
			89A	LYS	3.75
	Hydrogen Bond		8A	GLU	2.01
			86A	ASP	2.03
			89A	LYS	3.25
			92A	ASP	1.63
	Water Bridges		85A	GLN	3.93
7.	Hydrophobic Interactions	-8.7	144A	ALA	3.59
	Hydrogen Bond		12A	GLU	2.72
			12A	GLU	2.75
			131A	GLN	3.14
			132A	ASN	2.08

8.	Hydrophobic Interactions	-8.4	88A	LYS	3.52
	Hydrogen Bond		86A	ASP	3.45
			88A	LYS	2.77
9.	Hydrophobic Interactions	-8	18A	VAL	3.96
			31A	ALA	3.43
			80A	PHE	3.59
			145A	ASP	3.57
	Hydrogen Bond		12A	GLU	2.69
			12A	GLU	2.01
			131A	GLN	3.70
10.	Hydrophobic Interactions	-7.7	10A	ILE	3.76
			82A	PHE	3.60
	Hydrogen Bond		8A	GLU	2.01
			298A	LEU	3.19
	Water Bridge		89A	LYS	3.26
11.	Hydrophobic Interactions	-7.5	134A	LEU	3.97
			145A	ASP	3.51
	Hydrogen Bond		12A	GLU	2.34
			12A	GLU	2.00
			132A	ASN	1.84
12.	Hydrophobic Interactions	-8.5	88A	LYS	3.58
			131A	GLN	3.99
	Hydrogen Bond		88A	LYS	2.83
			145A	ASP	3.16
STD	Hydrophobic Interactions	-8.4	18A	VAL	3.81
			31A	ALA	3.74
			82A	PHE	4.00
			134A	LEU	3.81
			134A	LEU	3.58
			145A	ASP	3.56
	Hydrogen Bond		81A	GLU	2.05
			83A	LEU	2.82
			83A	LEU	1.86
			86A	ASP	2.19

			89A	LYS	2.61
	Salt Bridges		86A	ASP	4.50

Conclusion:

The ability of each synthetic substance to bind to the 2FVD protein (Cyclin Dependent Kinase 2 (CDK2) with inhibitor of diaminopyrimidine) was evaluated using molecular modelling. The data obtained from the molecular docking was strongly correlated with that obtained from the biological screening which revealed that; compounds 1,6,7,2,12, and 8 showed the highest binding affinities towards 2 FVD protein in the range of -8.9 to - 8.4 kcal/mol than standard i.e. (4-Amino-2-{[1-(Methyl sulfonyl) Piperidin-4-yl] Amino} Pyrimidin-5-yl)(2,3-Difluoro-6 Methoxyphenyl) Methanone (LIA) which is to - 8.4 kcal/mol. The outcomes demonstrated the potential for future development of more potent analogues for anticancer therapy.

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