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SYNTHESIS, *In-vitro* CYTOTOXICITY AND IN SILICO INVESTIGATIONS OF QUINAZOLINONE INTEGRATED CHALCONES: AS NOVEL POTENTIAL DUAL-TARGETING ANTICANCER AGENTS TO TREAT LUNG CANCER AND COLORECTAL CANCER

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ABSTRACT

The present study focuses on the synthesis of some 2-methoxyphenylquinazolin-4-one incorporated chalcone hybrids to evaluate their cytotoxic potential by MTT assay, and their affinity to bind with T790M mutated epidermal growth factor receptor (EGFR; protein data bank Id: 5Y9T) and G12D mutated Kirsten rat sarcoma (K-RAS; protein data bank Id:4EPT) by molecular docking (auto dock-4) by employing validated docking parameters. Against the lung cancer cells (A549), except C7 (IC_{50}: 48.22 µg/ml), the other title compounds exhibited more cytotoxicity (IC_{50} 16.88µg/ml to 33.98µg/ml) than the erlotinib (reference). Against the colorectal cancer cells, the compound C4 (IC_{50}: 9.74 µg/ml) exhibited more cytotoxicity than the reference (IC_{50}: 16.13 µg/ml). For the normal cell lines (Vero), the compound C1 (IC_{50} 27.88µg/ml) is less toxic to normal cells, than the reference. The title compounds can be a boon for the development of smart anticancer drugs with dual target virtue.

Keywords: Quinazolinone, Chalcone, Cytotoxicity, Molecular Docking, EGFR, K-RAS, Anticancer.
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INTRODUCTION

Lung cancer and colorectal cancer, two of the deadliest malignancies in the world, are among the most often diagnosed cancers in both men and women.[1,2] The review of the literature demonstrated the enormous therapeutic potential of the quinazolinone/quinazoline scaffold.[3,4] Mirgany *et al.* described derivatives of 3-phenylquinazolin-4-one as powerful anticancer agents.[5] For the discovery of anticancer drugs, the molecular hybridization approach is helpful.[6,7] Various research papers support the integration of chalcone structural motifs with different heterocyclic rings (e.g. thiazole, quinoline, benzimidazole, pyrazole, etc).[8-11] EGFR is over-expressed in lung and colorectal cancer, and it is one of the important factors which results in a low survival rate.[12,13] EGFR inhibitors have been created to improve the prognosis and, to get rid of severe toxicities linked with conventional chemotherapeutics.[14] Currently, EGFR inhibitors are used clinically to treat NSCLC and mCRC.[15] The Ist generation EGFR inhibitors are not effective to treat NSCLC associated with T790M mutated EGFR.[16] The 2nd generation EGFR inhibitors in preclinical models showed potential effectiveness against T790M mutated EGFR, however, owing to their dose-limiting toxicity they were not successful in clinical trials.[16,17] RAS is a master component in the EGFR signaling pathways. Mutations in RAS make it persistently active and unresponsive to 'EGFR- initiated signals', which is one of the primary reasons EGFR-targeted therapy fails.[18] The KRAS mutation, which occurs in mCRC (40% of the cases), is the most common of these genetic modifications. The G12D mutation type is the most prevalent and, as a result, one of the most

strategic and relevant targets for drug discovery.[18,19] The current work focuses on in-vitro cytotoxic screening of quinazolinone-integrated chalcone ligands on lung cancer cells (A549), colorectal cancer cells (Caco2), and normal cells (Vero) and, in-silico investigations to evaluate ligand's potential to inhibit mutated EGFR and, mutated K-RAS. Hence, the current work's objective is to find potential anticancer agents that possess the virtue of inhibiting two important biological targets involved in lung and, colorectal cancer.

EXPERIMENTAL

Material and Methods Employed

The synthesis/ commercial grade chemical reagents/organic solvents were used in the synthesis work.TLC plates coated with silica gel F254 were used to monitor organic reactions and to determine Rf values using the solvent system (30% ethyl acetate + 70% hexane). The melting points are observed by the open capillary method. The characterization of the title compounds was accomplished using the mass spectrometry ESI+ (Waters USA: Xevo G2-S Q Tof), ^{1}H-NMR (ECS Zeol spectrometer 400MHz), ^{13}C-NMR spectroscopy (ECS Zeol spectrometer 400MHz) and, FTIR-spectroscopy (spectrometer Alpha-E BRUKER). The microplate reader (Bio-Rad 550) was used to observe absorbance in the MTT assay. The NCCS in Pune (India) provided the cell lines. The 3D structure building of ligands and minimization of their energy was carried out by using Marvin Sketch version 18.23. The docking target proteins were the T790M mutated EGFR (5Y9T) and G12D mutated K-RAS (4EPT), accessed from the protein data bank (PDB). For molecular docking studies, AutoDock 4.0 MGL tools were employed. Discovery studio visualizer 2017 R2 (Dassault Systèmes) was used to observe the ligand-protein interactions. UCSF Chimera was used for the RMSD determination.

Chemical Synthesis of Compound 1

Anthranilic acid (0.01 mol) solution prepared in dry pyridine (30 ml) was chilled using an ice bath followed by the drop-wise addition of 4-methoxybenzoyl chloride (0.021 mol). The mixture was subjected to stirring for 15 minutes in an ice bath, followed by 1 hr room temperature stirring. The mixture obtained was kept aside for 40 minutes with occasional shaking and, then added to cold water (nearly 200 ml), and the 10% $NaHCO_3$ (aq.) was added till the effervescence got subsided. The solid mass formed was separated by filtration and was subjected to washing with cold water until the pyridine odor vanished. After being dried, the white solid precipitate was recrystallized from ethanol(Scheme-1, Fig.-1).[20-22]

Chemical Synthesis of Compound 2

4-Aminoacetophenone and compound **1** were heated under reflux using glacial acetic acid as a solvent (Fig.-1). The resulting solution was allowed to attain room temperature and was added to cold water. The solid mass thus formed was collected by filtration, and washed with cold water, and methanol. The dried white product was recrystallized twice with methanol (Scheme-1, Fig.-1).[22,23]

Chemical Synthesis of Title Compounds (C1 – C8)

To compound **2** (0.004 mol), 3 ml 10% sodium hydroxide (aq.), and around 6 ml of ethanol were added, and the resulting mixture was subjected to stirring for 10 minutes at room temperature. Then, aromatic aldehyde was added dropwise (0.004mol). Till the completion of the reaction, the stirring was continued.[24] In the end, crushed ice was added, and the resulting solid was collected by filtration, and to remove alkalinity water washing of the separated solid was done. The products were recrystallized using glacial acetic acid(Scheme-1, Fig.-1).

In vitro Cytotoxic Evaluation (MTT Assay)

The cell lines were centrifuged and diluted with DMEM to get 1.0 x10^5 cells per ml. 100 µl of the resulting suspension of cells was added to each well of the microtiter plate such that each well contained 10^4 cells (approximately). The centrifugation was done after 24 hours, and different sample concentrations (100 µl) that were prepared in a maintenance medium, were introduced. For 48 hours at 37 °C in a CO2 environment (5%) the plates were incubated, followed by the addition of 20 µl of MTT solution (2 mg per ml) in MEM-PR (MEM without phenol red). The microtiter plates were incubated for

2 hours at 37˚C in a CO_2 environment (5%). To dissolve the formed formazan 100µl of DMSO was used. The absorbance readings were observed at 540nm.[25]

Molecular Docking Studies

Preparation of Target Proteins

The 3D- structures (X-ray diffraction) of the target macromolecules were retrieved in .pdb format. Water molecules, and their respective complexed ligands, were removed from Discovery Studio Visualizer. The target proteins were made ready for docking runs by merging the hydrogens (non-polar) and addition of the Kollman charges, and their respective .pdb files were converted into pdbqt format files.[26,27]

Preparation of Autodock Type Ligands

Gradient optimization function was used to minimize the ligands' energy using MMFF94 force field. The 3D structures of the ligands were stored in .pdb format. By using auto dock-4 all the ligands were made ready for docking run by assigning all the ligand's atoms as AD4 type, merging hydrogens (non-polar), and inclusion of Gasteiger charges. The ligand structure files were saved in. pdbqt file format.[26,27]

Validation of Docking Experiment

The co-crystallized ligands naquotinib and GDP were separated from their macromolecules 5Y9T and 4EPT, respectively, using Discovery Studio Visualizer, and stored in .pdb file format. The co-crystallized conformers were made ready for docking runs with the same protocol as aforementioned for the ligands. Using different grid parameters, the separated co-crystallized ligands were docked with their respective targets to get the docked poses of the separated conformers having a minimum RMSD value to their respective reported co-crystallized conformers. The grid parameters that generated the lowest RMSD values were chosen for title compounds docking.[28]

Docking Launch

For macromolecule 5Y9T

To make the grid parameter file (.gpf), the 3-dimensional grid box was positioned at the center of the 5Y9T, and along all three coordinates 90 grid points were chosen, with 0.375 A° spacing. Autogrid-4 of MGL tools was used to generate a grid log file (.glg) of the ligand under study, from its .gpf file. The genetic algorithm approach was opted for preparing the docking parameter file (.dpf) of the ligand under study. The number of generations for picking the worst individual was fixed to 10, and cluster analysis with an elitism value equal to 1 was chosen. Docking was launched by employing the .dpf file of the ligand and each launch was set for one hundred independent runs. The docking launch generated a docking log file (.dlg) of the docking study.[22,27]

Macromolecule 4EPT

The protocol employed was the same as aforementioned for the target 5Y9T, but, for this target along all three coordinates, 100 grid points were chosen.[22]

Docking Investigations

The docking data regarding free binding energy (BE) and inhibition constant (KI) were obtained from the ligand's .dlg files. The complex of the conformer with its target macromolecule was stored as a .pdb file and was viewed to know the interactions involved.

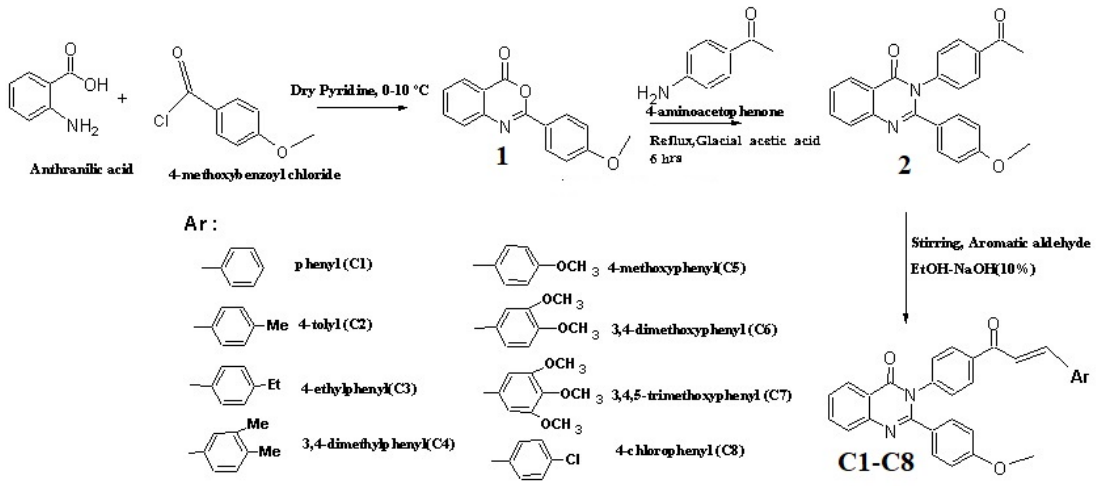
RESULTS AND DISCUSSION

Synthetic Chemistry

The title compounds were synthesized as described in scheme-1 (Fig.-1). All the title compounds are satisfactorily characterized.

2-(4-methoxyphenyl)-3-(4-((E)-3-phenylacryloyl)phenyl)quinazolin-4(3H)-one(C1): Molecular formula: $C_{30}H_{22}N_2O_3$; Molecular Weight: 458.50728; Light yellow solid; Melting point: 205-208^{0}C; Reaction time: 8 hours; Rf : 0.392; Yield:47.21%; MS(ESI+): 459.50728 (calculated) , 459.5073(found); IR-ATR (cm^{-1},ν): 1650.17, 1585.02,1500.30, 1443.10, 1397.39, 1370.97, 1307.33, 1238.16, 1211.15, 1167.37, 1026.26, 827.08, 754.07; ^{1}H-NMR (DMSO-deuterated) δ ppm:

8.32(doublet,1H, Aromatic),8.12(doublet, 2H, Aromatic),7.88(doublet, 2H,Aromatic),7.85-7.78 (multiplet, 6H,Aromatic and ethylenic) 7.65(doublet,1H, COCH=CH),7.53 (triplet,1H,Aromatic),7.39-7.36 (multiplet,3H,Aromatic), 7.20(triplet,1H,Aromatic),7.00(doublet,2H,Aromatic), 3.74(singlet,3H,CH_3); ^{13}C-NMR (DMSO-deuterated)δ ppm: 188.17(1C, CO of chalcone), 168.30(1C, N=C-N site of quinazolin-4-one), 164.68(1C, CO of quinazolin-4-one), 162.69(1C, Ar), 144.00(1C, C=C of chalcone), 143.72(1C, Ar), 139.21(1C,Ar), 135.25(1C, Ar), 133.47(1C, Ar), 132.89(1C, Ar), 131.00(1C, Ar), 130.16(2C, Ar),129.6(1C,Ar), 129.53(2C, Ar), 129.40(2C, Ar), 129.31(2C, Ar), 127 (1C,Ar), 123.65(1C, Ar), 122.45(1C, Ar), 122.07(1C, C=C of chalcone), 120.66(2C, Ar), 119.94(1C, Ar), 114.57(2C, Ar), 55.94 (1C, methyl of OCH3).



Scheme-1 and Fig.-1: Synthesis of the title compounds (C1–C8)

2-(4-methoxyphenyl)-3-(4-((E)-3-p-tolylacryloyl)phenyl)quinazolin-4(3H)-one(C2)
Molecular formula: $C_{31}H_{24}N_2O_3$; Molecular weight: 472.5339; Light yellow solid; Melting point: 210-213^{0}C; Reaction time: 8 hours 15 minutes; Rf: 0.405; Yield: 43.15%; Mass spectra (ESI+): 473.5339(calculated), 473.5407(found); IR-ATR (cm^{-1} ,ν): 1649.36, 1583.28, 1499.10, 1213.98, 1166.10, 995.46,803.04,749.42; ^{1}H-NMR (DMSO-deuterated) δ ppm**:** 8.33 (doublet,1H, Aromatic), 8.13 (doublet, 2H, Aromatic), 7.93(doublet,2H,Aromatic), 7.86 (doublet,1H, COCH=CH), 7.85(doublet,2H,Aromatic), 7.83 (doublet,1H,Aromatic),7.71(doublet,2H,Aromatic), 7.65(doublet,1H, COCH=CH), 7.56 (triplet,1H,Aromatic), 7.22 (triplet,1H,Aromatic), 7.03(doublet,2H,Aromatic),7.02 (doublet,2H,Aromatic), 3.76(singlet,3H,CH_3 of OCH_3), 2.29 (singlet,3H,CH_3); ^{13}C-NMR(DMSO-deuterated) δ ppm: 188.13(1C, CO of chalcone),168.27(1C, N=C-N site of quinazolin-4-one),164.69(1C, CO of quinazolin-4-one),162.69(1C, Ar),144.08(1C, C=C of chalcone), 143.63(1C, Ar), 141.09 (1C,Ar),139.16(1C, Ar), 133.57(1C, Ar), 132.88(1C, Ar),132.53(1C, Ar), 130.10(2C, Ar), 130.03(2C, Ar), 129.6(1C,Ar), 129.54(2C, Ar), 129.36(2C, Ar), 127(1C, Ar), 123.74(1C, Ar), 123.67(1C, Ar),122.11(1C, C=C of chalcone), 120.64 (2C, Ar), 119.93(1C, Ar), 114.59(2C, Ar), 55.96 (1C, methyl of OCH3), 21.59 (1C, CH_3).

3-(4-((E)-3-(4-ethylphenyl)acryloyl)phenyl)-2-(4-methoxyphenyl)quinazolin-4(3H)-one (C3)
Molecular formula: $C_{32}H_{26}N_2O_3$; Molecular weight: 486.5604; Light yellow solid; Melting point: 215-218 ^{0}C; Reaction time: 8 hours 15 minutes; Rf : 0.418; Yield: 42.26%**;** Mass spectra (ESI+): 487.5604(calculated), 487.5607 (found); IR-ATR (cm^{-1} ,ν): 1648.65, 1583.91, 1498.45, 1310.10, 1216.95,1163.09,997.63,825.05,748.54 ; ^{1}H-NMR (DMSO-deuterated) δppm : 8.33 (doublet,1H,Aromatic), 8.13(doublet,2H, Aromatic),7.87 (doublet,2H,Aromatic), 7.84-7.82(multiplet,4H, Aromatic and ethylenic),7.73(doublet,2H, Aromatic), 7.55(triplet,1H,Aromatic), 7.65(doublet,1H, COCH=CH), 7.24-7.20(multiplet,3H, Aromatic), 7.01(doublet,2H,Aromatic),3.75(singlet,3H, CH_3 of methoxy), 2.5(multiplet,2H, CH_2 of CH_2CH_3), 1.11(triplet,3H, CH_3 of CH_2CH_3); ^{13}C-NMR(DMSO-

deuterated)δ ppm: 188.16(1C, CO of chalcone), 168.30(1C, N=C-N site of quinazolin-4-one),164.70(1C, CO of quinazolin-4-one), 162.71(1C, Ar), 147.311(1C, Ar),144.11(1C, C=C of chalcone), 143.65(1C, Ar), 139.20(1C, Ar), 133.59(1C, Ar),132.89(1C, Ar), 132.81(1C, Ar), 130.12(2C, Ar), 129.64(1C, Ar), 129.55(2C, Ar), 129.46(2C, Ar), 128.87(2C, Ar), 127.01(1C, Ar), 123.70(1C, Ar), 123.67(1C, Ar), 122.10(1C, C=C of chalcone), 121.46(1C, Ar), 120.66(2C,Ar), 114.60(2C,Ar), 55.96 (1C, methyl of OCH3), 28.64 (1C,CH_2 of ethyl), 15.86 (1C, CH_3 of ethyl).

2-(4-methoxyphenyl)-3-(4-((E)-3-(3,4-dimethylphenyl)acryloyl)phenyl)quinazolin-4(3H)-one(C4)
Molecular formula:$C_{32}H_{26}N_2O_3$; Molecular weight: 486.5604; Light yellow solid; Melting point: 212-216^{0}C; Reaction time: 8 hours 15 minutes; Rf : 0.418;Yield: 41.33%; Mass spectra (ESI+): 487.5604(calculated), 487.5605(found); IR-ATR (cm^{-1} ,ν): 1645.94, 1591.2,1506.95, 1442.48, 1404.83, 1309.58, 1248.84, 1176.76, 1026.12,902.44,835.40,754.94; ^{1}H-NMR (DMSO-deuterated) δ ppm: 8.32(doublet,1H,Aromatic), 8.12(doublet,2H, Aromatic), 7.86(doublet,2H,Aromatic), 7.83(doublet,1H,Aromatic), 7.82(doublet,1H, COCH=CH), 7.81(doublet, 2H, Aromatic), 7.6(doublet,1H, COCH=CH),7.57-7.50(multiplet, 3H, Aromatic), 7.22(triplet,1H,Aromatic), 7.15(doublet,1H,Aromatic), 7.02(doublet,2H,Aromatic) 3.75(singlet,3H, CH_3 of methoxy), 2.2(singlet,3H,methyl), 2.19(singlet,3H,methyl); ^{13}C-NMR(DMSO-deuterated)δ ppm: 188.09(1C, CO of chalcone), 168.28 (1C, N=C-N site of quinazolin-4-one), 164.70(1C, CO of quinazolin-4-one), 162.70(1C, Ar), 144.31(1C, C=C of chalcone), 143.63(1C, Ar), 140.01(1C, Ar), 139.15(1C, Ar),137.39(1C, Ar), 133.60(1C, Ar), 132.85(1C, Ar), 130.52(1C, Ar), 130.10(2C, Ar), 129.64(1C, Ar), 129.55(2C, Ar), 129.54(1C,Ar), 127.22(1C, Ar), 127 (1C, Ar), 123.78(1C, Ar), 123.68(1C, Ar),123.67(1C,Ar), 122.13(1C, C=C of chalcone), 121.14(1C, Ar), 120.63(2C, Ar), 114.60(2C, Ar), 55.97(1C, methyl of OCH3), 19.98 (1C, methyl), 19.80(1C, methyl).

2-(4-methoxyphenyl)-3-(4-((E)-3-(4-methoxyphenyl)acryloyl)phenyl)quinazolin-4(3H)-one(C5)
Molecular formula: $C_{31}H_{24}N_2O_4$; Molecular weight: 488.5333; Yellow solid; Melting point: 212-215^{0}C; Reaction time: 9 hours; Rf: 0.250;Yield: 40.52%; Mass spectra (ESI+): 489.5333(Calculated), 489.5331 (found); IR-ATR (cm^{-1}, ν): 1648.06, 1582.61, 1500.78, 1291.80, 1211.61, 1163.88, 1025.94, 820.05, 746.72;^{1}H-NMR (DMSO-deuterated) δ ppm: 8.31(doublet,1H, A romatic),8.09 (doublet,2H, Aromatic), 7.84(doublet,2H, Aromatic), 7.83(doublet,2H, Aromatic), 7.8(doublet,1H, Aromatic), 7.75(doublet,2H, Aromatic), 7.73(doublet,1H, COCH=CH), 7.62(doublet,1H, COCH=CH), 7.5(triplet,1H, Aromatic), 7.19(triplet,1H, Aromatic), 7(doublet,2H, Aromatic), 6.92(doublet,2H, Aromatic),3.73(merged, singlet,6H, two CH_3 of twmethoxysxy). ^{13}C-NMR(DMSO-deuterated)δ ppm: 188.02(1C, CO of chalcone), 168.27(1C, N=C-N site of quinazolin-4-one), 164.68(1C, CO of quinazolin-4-one),162.69(1C, Ar), 161.78(1C, Ar), 144.00(1C, C=C of chalcone), 143.49(1C,Ar), 139.20(1C, Ar), 133.75(1C, Ar), 132.88(1C, Ar), 131.21(2C, Ar), 130.01(2C, Ar), 129.62(1C, Ar), 129.52(2C, Ar), 127.88(1C, Ar), 127(1C, Ar), 123.64(1C, Ar), 123.64(1C, Ar), 122.05(1C, C=C of chalcone), 120.63(2C, Ar), 119.91(1C, Ar), 114.83(2C, Ar), 114.58(2C,Ar), 55.95(1C, CH3 of methoxy), 55.86(1C,CH3 of methoxy).

2-(4-methoxyphenyl)-3-(4-((E)-3-(3,4-dimethoxyphenyl)acryloyl)phenyl)quinazolin-4(3H)-one(C6)
Molecular formula: $C_{32}H_{26}N_2O_5$; Molecular weight: 518.5592;Yellow solid; Melting point: 225-228^{0}C; Reaction time: 9 hours; Rf: 0.152;Yield: 39.71%;Mass spectra (ESI+): 519.5592 (Calculated) , 519.5584 (found) ; IR-ATR (cm^{-1} ,ν): 1646.06, 1577.76, 1509.87, 1445.03, 1320.90, 1232.30, 1177.75, 1128.15, 1025.69,831.64,755.37; ^{1}H-NMR (DMSO-deuterated) δ ppm: 8.33(doublet, 1H,Aromatic), 8.14(doublet,2H,Aromatic), 7.88(doublet,2H,Aromatic), 7.86(doublet,2H,Aromatic), 7.83(doublet,1H,Aromatic),7.78 (doublet,1H, COCH=CH), 7.63(doublet,1H, COCH=CH), 7.55(triplet,1H,Aromatic), 7.48(singlet,1H,Aromatic), 7.32(doublet,1H,Aromatic), 7.23(triplet,1H,Aromatic), 7.03(doublet,2H,Aromatic), 6.95(doublet,1H,Aromatic), 3.75 (singlet,3H,methoxy), 3.78 (singlet,3H,methoxy), 3.80 (singlet,3H,methoxy); ^{13}C-NMR (DMSO-deuterated)δ ppm: 188.00(1C, CO of chalcone),168.27(1C, N=C-N site of quinazolin-4-one), 164.68(1C, CO of quinazolin-4-one), 162.69(1C, Ar), 151.70(1C, Ar), 149.49(1C, Ar), 144.54(1C, C=C of chalcone), 143.52(1C, Ar), 139.16(1C, Ar), 133.74(1C, Ar), 132.87(1C, Ar), 130.04(2C, Ar), 129.62(1C, Ar), 129.52(2C, Ar), 128.1(1C,Ar), 126.99(1C, Ar), 124.44(1C, Ar), 123.72(1C, Ar), 123.67(1C,Ar),

122.09(1C, C=C of chalcone), 120.60 (2C, Ar), 119.94 (1C, Ar) 114.59 (2C, Ar), 111.99(1C, Ar), 111.07(1C, Ar), 56.21(1C,CH_3 of methoxy), 56.07(1C,CH_3 of methoxy)., 55.95(1C,CH_3 of methoxy).

2-(4-methoxyphenyl)-3-(4-((E)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)quinazolin-4(3H)-one(C7)

Molecular formula: $C_{33}H_{28}N_2O_6$; Molecular weight: 548.5852; Light yellow solid; Melting point: 241-245^{0}C; Reaction time: 9 hours; Rf: 0.125; Yield: 42.46%; Mass spectra (ESI+): 549.5852 (Calculated), 549.5862 (found); IR-ATR (cm^{-1} ,ν):1656.52, 1588.67, 1509.15, 1445.22, 1315.20, 1228.07, 1175.36, 1127.85, 1026.15,904.16,816.79,754.09; ^{1}H-NMR (DMSO-deuterated) δ ppm: 8.3(doublet,1H,Aromatic), 8.14(doublet,2H,Aromatic), 7.88(doublet,2H,Aromatic),7.85(d,2H,Aromatic), 7.84(doublet,1H, COCH=CH), 7.82(doublet,1H,Aromatic),7.6(doublet,1H, COCH=CH), 7.55(triplet,1H,Aromatic), 7.23(triplet,1H,Aromatic), 7.16 (singlet,2H,Aromatic), 7.03(doublet,2H,Aromatic), 3.79(singlet, 6H, two CH_3 of two methoxy), 3.75(singlet,3H,methoxy), 3.64(s,3H,methoxy);^{13}C-NMR(DMSO-deuterated)δ ppm: 188.07(1C, CO of chalcone), 168.29(1C, N=C-N site of quinazolin-4-one), 164.71(1C, CO of quinazolin-4-one), 162.71(1C, Ar), 153.60(2C, Ar), 144.55(1C, C=C of chalcone), 143.70(1C, Ar), 140.13(1C, Ar),139.12(1C, Ar), 133.54(1C, Ar), 132.89(1C, Ar), 130.82(1C, Ar), 130.19(2C, Ar), 129.64(1C, Ar), 129.55(2C, Ar), 127 (1C, Ar), 123.86(1C, Ar), 123.72(1C, Ar), 122.17(1C, C=C of chalcone), 121.59(1C, Ar), 120.60(2C, Ar), 114.61(2C,Ar), 106.95(2C, Ar), 60.64(1C,CH_3 of methoxy), 56.62(2C, CH_3 of methoxy), 55.98(1C,CH_3 of methoxy).

3-(4-((E)-3-(4-chlorophenyl)acryloyl)phenyl)-2-(4-methoxyphenyl)quinazolin-4(3H)-one (C8)

Molecular formula $C_{30}H_{21}ClN_2O_3$; Molecular weight 492.9523; Light yellow solid; M.Pt: 218-220^{0}C; Reaction completion time:7 hours 30 minutes; Rf: 0.319; Yield: 43.52%;Mass spectra (ESI+): 493.9523(calculated), 493.9524(found); IR-ATR (cm^{-1} ,ν,):1651.28, 1585.80, 1495.34, 1308.94, 1245.56, 1166.61, 998.02, 819.56,749.34; ^{1}H-NMR (DMSO-deuterated) δ ppm: 8.4(doublet,1H,Aromatic), 8.09(doublet,2H, Aromatic), 7.9(doublet,2H,Aromatic), 7.86(doublet,2H, Aromatic), 7.85(doublet,1H, Aromatic), 7.81(doublet,2H,Ar-H), 7.8(doublet,1H, COCH=CH), 7.62 (doublet,1H, COCH=CH), 7.5(triplet,1H, Aromatic), 7.38(doublet, 2H, Aromatic), 7.17(triplet,1H,Aromatic), 6.97(doublet, 2H, Aromatic),3.74(singlet,3H,methoxy); ^{13}C-NMR(DMSO-deuterated)δ ppm: 187.96(1C, CO of chalcone), 168.37(1C, N=C-N site of quinazolin-4-one), 164.59(1C, CO of quinazolin-4-one), 162.67(1C, Ar), 144.53(1C, C=C of chalcone), 143.68(1C, Ar),139.55(1C, Ar),135.55(1C, Ar), 134.15(1C, Ar), 133.41(1C, Ar), 132.88(1C, Ar), 130.83(2C, Ar), 130.09(2C, Ar), 129.6(1C,Ar),129.43(2C, Ar), 129.34(2C, Ar), 127.01(1C, Ar), 123.29(1C, Ar), 123.08(1C, Ar), 122.87(1C, C=C of chalcone), 120.67(2C, Ar), 119.88(1C, Ar), 114.45(2C, Ar), 55.85(1C,CH_3 of methoxy).

***In-vitro* Cytotoxicity Evaluation**

Table -1 represents the outcomes of in vitro cytotoxicity evaluation using the MTT method.

Table-1: Cytotoxicity (IC_{50} μg/ml)

Code of Compound	A549	Caco2	Vero
C1	21.06	16.40	27.88
C2	20.38	24.96	17.89
C3	16.88	17.24	21.27
C4	16.88	9.74	16.04
C5	22.81	16.73	14.21
C6	33.98	43.20	24.22
C7	48.22	26.64	22.79
C8	19.87	39.62	14.79
Erlotinib[22] (Reference)	44.41	16.13	25.78

Against the A549 cell lines, except C7 (IC_{50}: 48.22 μg/ml), the other compounds exhibited more cytotoxicity with the IC_{50} (μg/mol) values ranging from 16.88 to 33.98, than the reference. For lung

cancer cells, the most potent cytotoxic compounds are C3 and C4. Against the Caco2 cell lines, the compound C4 (IC_{50}: 9.74 µg/ml) exhibited more cytotoxicity than the reference and is the most potent among all. For the Vero cell lines, the compound C1 exhibited lesser cytotoxicity (27.88µg/ml) to normal cells than the reference (25.78µg/ml).

Molecular Docking Investigations

Docking Validation

The cocrystallized ligand naquotinib's conformation and its post-docked conformation had an RMSD of 1.585 A^0 while the RMSD between the cocrystallized GDP and its redocked pose was found to be 1.365 A^0 (Fig.-2). It demonstrates that the docking pose generated in the docking experiments had a close resemblance with their respective co-crystallized conformers.

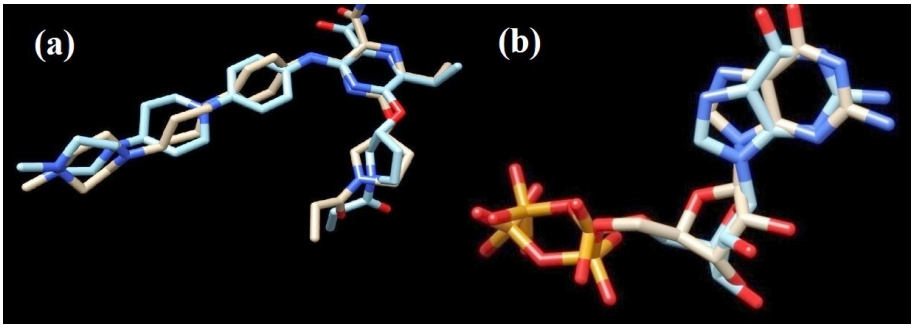


Fig.-2: (a) Superimposed Co-Crystallized Naquotinib Conformer (blue) and its Redocked Pose (Yellow) (b) Superimposed Co-Crystallized GDP Conformer (Blue) and its Redocked Pose (Yellow)

Docking Analysis (with 5Y9T and 4EPT)

All the studied compounds complexed at the catalytic site of the targets 5Y9T and 4EPT (Fig.-3), demonstrating their affinity for the respective catalytic sites of the targets.[29,30]

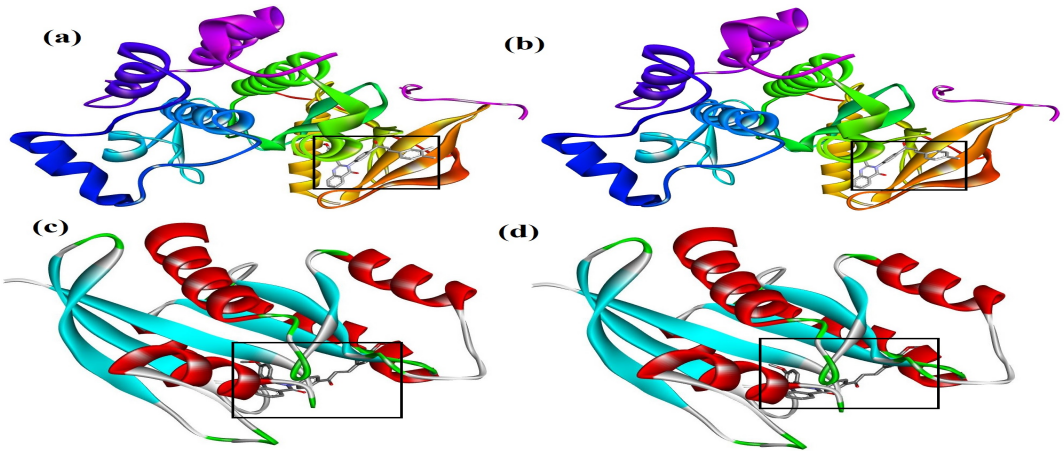


Fig.-3: (a) C4-5Y9T Complex (b) C2-5Y9T Complex (c) C6-4EPT Complex (d) C5-4EPT Complex

The results of the docking study for the molecular target 5Y9T and 4EPT are compiled in Table-2. The compounds C6 and C4 were found as the most potent inhibitors for 4EPT and 5Y9T, respectively.

Molecular docking studies with 5Y9T (Fig.-4) reveal that chalcone carbonyl 'O' is an important structural site of the ligands for hydrogen bond formation with MET793 residue. While the '4-methoxy phenyl' substituent of the quinazolin-4-one ring is pivotal for Pi-anion type polar interaction with ASP855 amino acid of 5Y9T. Molecular docking investigations with 4EPT(Fig.-4) reveal that N1 of quinazolin-4-one is a pivotal structural site for hydrogen bond formation with ARG68. While the '4-methoxy phenyl'

substituent of the quinazolin-4-one ring is pivotal for Pi-cation type polar interactions with ARG68 as well as with HIS95 residues.

Table-2: Docking Investigations for Target 5Y9T and 4EPT

Ligand	Free binding energy (kJ/mol)	Inhibition constant KI nano-mol	Docking rank	H- bond(s) (Classical)	H-bond(s) interaction amino acids (A^0)	Other polar interactions between amino acids and non-polar interactions between amino acids	Total Interacting amino acids
C1	-38.33	191.88	8	1	CYS797(2.85)	GLY796, LEU718, LEU844, VAL726, MET790, ASP855	7
C2	-41.34	57.3	2	1	MET793 (1.97)	VAL726, PHE723, ASP855, MET766, MET790, PRO794, LEU718, ALA743, LEU844	10
C3	-40.92	67.34	3	1	MET793(1.82)	PHE723, VAL726, ASP855, MET790, MET766, PRO794, LEU718, LEU844, ALA743	10
C4	-43.14	27.94	1	1	MET793(1.86)	PHE723, VAL726, LEU844, ALA743, LEU718, PRO794, LEU792, LYS728, MET790, MET766, ASP855	12
C5	-39.20	134.41	7	2	LYS745(1.87), CYS797(2.92)	GLY796, LEU718, LEU844, VAL726, ASP855	7
C6	-39.75	108.6	6	2	MET793(2.31),THR854(2.96)	LEU718, LEU844, VAL726, PHE723,ASP855, MET790, PRO794	9
C7	-40.29	86.86	5	4	LYS716(2.03), LYS716(2.62),GLY796(3.05), THR854(3.04)	LEU718, GLY719, PHE723, MET790, VAL726, ASP855, LYS728	10
C8	-40.58	78.23	4	2	MET793(2.53),THR854(2.93)	LEU718, LEU844, GLY719, VAL726,PHE723, ASP855, LEU792, LYS728, PRO794	11

For Target 4EPT							
C1	-41.92	45.03	7	1	ARG68(1.89)	ARG68, ALA59, TYR96, HIS95	4
C2	-42.89	30.43	6	3	SER17(1.10), ASN116(2.58),LYS117(2.9 8)	PHE28, ALA146, LYS147, LYS16, ALA59, PRO34, GLY15, ALA18	11
C3	-43.56	23.44	4	1	ASN116(5.54)	LYS117, LYS147, ALA146, ALA18, PHE28, GLY15, LYS16, ALA59	9
C4	-44.14	18.6	3	NIL	NIL	PRO34, ALA59, ALA18, PHE28, LYS117, ALA146, LEU120, LYS147	8
C5	-44.14	18.55	2	1	ARG68(1.89)	ALA11, TYR96, ARG68, HIS96, GLU91, HIS94	6
C6	-45.10	12.55	1	1	ARG68(1.85)	TYR96, ALA59, ARG68, HIS95, HIS94, GLU91	6
C7	-36.99	333.46	8	3	ARG68(2.31), LYS16(2.11), LYS88(1.99)	ALA11, GLY12, TYR96	6
C8	-42.97	29.83	5	1	ARG68(1.85)	HIS94, TYR96, ALA59, ARG68, HIS95, TYR64	6

According to a literature review, there is still a serious need to treat CRC with RAS mutation and, NSCLC with mutant EGFR. The '3-methyl quinazolinone integrated chalcones' (IC_{50}: 5.5 to 8.5 μM), and 4-aminoquinazoline-chalcone hybrids (IC_{50}:0.10 to 0.19 μM) have been reported as potent anticancer agents.[31,32] The 3-methylquinazolinone derivatives (IC_{50} : 0.01-0.54μM) and 2-phenoxymethyl-quinazolin-4-one analogues(IC_{50} : 0.047 to 2.71μM) were described as potent inhibitors of wild-type EGFR.[33,34] The EGFR inhibitors of III generation had raised hope to treat NSCLC patients with EGFR mutation(T790M), but they are also associated with treatment-related severe toxicities, therefore such NSCLC patients are still devoid of better treatment.[35] Certain quinazoline compounds have been described as KRAS-G12C inhibitors.[36] The AMG 510 (sotorasib) is an FDA-approved drug that substantiates that targeting KRAS (G12C) in its GDP-bound state can be a promising strategy for developing innovative anti-KRAS treatments.[19,37]In the current study, K-RAS (G12D mutation) was docked with the title ligands, in its GDP-bound conformation. The outcomes of the in vitro studies reflect that the title compounds are potent anticancer agents. Docking investigations imply that the studied compounds have a good affinity for the catalytic sites of both the target macromolecules. Hence, the present work enlightens the scope of the development of anticancer agents with dual/multiple targeting virtues and is an effort to address a serious need to treat CRC with RAS mutation, and NSCLC with mutant EGFR.

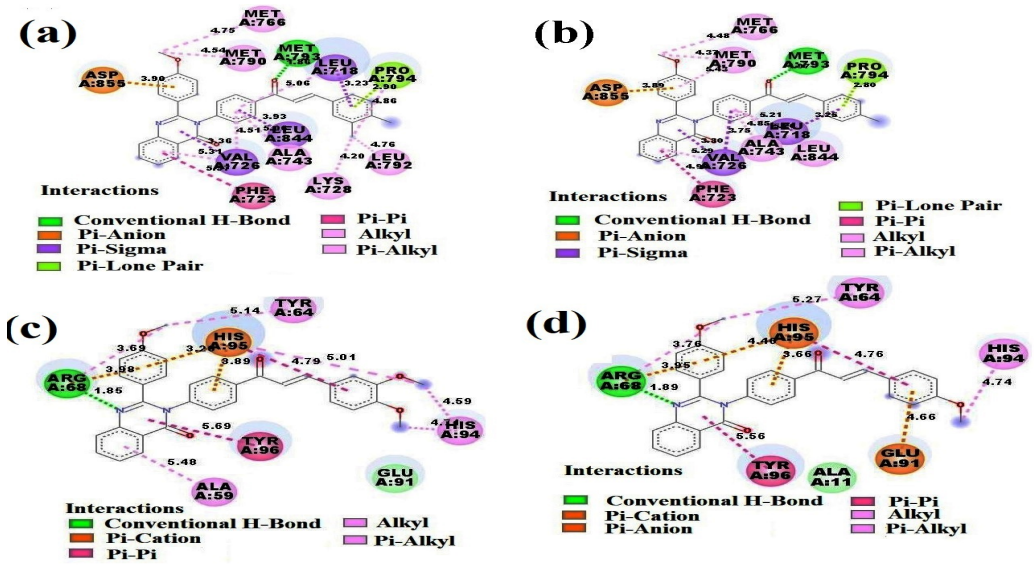


Fig.-4: (a) C4-5Y9T Complex Interactions (b) C2-5Y9T Complex Interactions (c) C6 -4EPT Complex Interactions (d) C5-4EPT Complex Interactions

CONCLUSION

In vitro cytotoxicity, and in silico screening concludes that the 2nd and 3rd positions of the quinazolin-4-one scaffold are pivotal for aryl ring substitutions for anticancer activity. The hydrophobic alkyl substituents on the β-phenyl ring (w.r.t chalcone carbonyl) enhance the cytotoxicity against the lung cancer cells as well as against colorectal cancer cells. The current research may therefore serve as a springboard for the creation of novel, low-molecular-weight medicinal compounds with multiple targeting virtues to combat lung and colorectal cancers associated with EGFR and K-RAS mutations.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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