DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW 2-AZETEDINONE - QUINOLINE CONJUGATES AS ANTI-CANCER AGENTS

Pradeep Kumar Y[1], Siva Sanker Reddy L*

[1]Research Scholar, Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Anantapur, Ananthapuramu, A.P., 515002.
*Professor, Department of Pharmaceutical Analysis & Chemistry, Santhiram College of Pharmacy, NH40, Nandyal, A.P., 518112.

Corresponding Author:
Dr. L. Siva Sanker Reddy,
Professor,
Department of Pharmaceutical Analysis & Chemistry,
Santhiram College of Pharmacy,
NH40, Nandyal,
Andhra Pradesh, 518112.
Phone: +919885697242
E-mail: shiva_s_rl@yahoo.co.in

ABSTRACT:

A Series of New (E)-1-(4-(((2-chloro-5,6,7-trimethylquinolin-3-yl)methylene)amino)-phenyl) ethan-1-one (5a-o) were designed, synthesized, characterized by respective spectral data and evaluated for anti cancer activity. Most of the synthesized compounds showed good in vitro inhibitory activities against MDA-MB-231, HeLa, MCF-7**.** Among the compounds **5j** and **5n** having 3,4,5-trimethoxy, 2,6-diflouro, substituents on the aromatic ring attached at the stereogenic center have shown equal potency to that of cisplatin with IC_{50} values were found to be the potent antiproliferative agents against MDA-MB-231, HeLa, MCF-7**.** cellline with an IC_{50} value with 0.72±0.04 µM, 1.39±0.02 µM, 2.02±0.28 µM and 0.78±0.08 µM, 1.31±0.02 µM, 2.62±0.12 µM respectively. Standard drug cisplatin exhibited IC_{50} values of 0.54±0.05 and 0.95±0.02 and 1.32±0.22 against MDA-MB-231, HeLa, MCF-7**.** Compounds 5j, 5n were showed lower cytotoxic property in normal cell lines. Docking studies of all the molecules disclosed close hydrogen bond interactions with the binding site.

Keywords: 2-Azetidinones; anti-proliferative effect; apoptosis; Molecular docking.

1. Introduction:

According to the GLOBOCAN 2020 estimations, cancer is the second most common cause of death worldwide. In 2020, there were 19.3 million fresh cases of cancer worldwide. Among these cases, breast cancer in females made for 11.7% of the total [1]. Obesity, hereditary disposition, alcohol consumption, tobacco use, exposure to radiation, and postmenopausal hormone therapy are recognized factors that can cause breast cancer. BRCA1, BRCA2, and PALB-2 mutations are often seen in breast tumors as a result of genetic inheritance. Multiple cellular growth regulatory mechanisms have been found and thoroughly examined for their involvement in cancer. The most prominent pathways include the PI3K-AKT-mTOR (PAM) pathway, the Wnt/b-catenin signalling pathway, JAK/STAT pathway, Notch signalling pathway, Hedgehog pathway, and Hippo pathway [2-3]. These pathways are not only involved in the onset and advancement of the illness, but they are also linked to the metastasis of cancer and the resistance to treatment with therapeutic drugs. There is a constant need for improved chemotherapeutic drugs that are more effective, responsive, and specific in treating tumors. This is because the risk of drug resistance remains a persistent concern throughout the treatment process [4]. Moreover, the risk of recurrence once the treatment ceases always exists. The currently used drugs work against a variety of pathways and hence, a cocktail of drugs is therefore recommended, so as to delay the occurrence of drug resistance and to mask low sensitivity of individual agents [5-6]. The epidermal growth factor receptor (EGFR) and herceptin both belong to the epidermal growth factor receptor family. EGFR is known for its ability to enhance cellular motility and invasion. Therefore, these receptors are considered significant targets for anticancer treatment in breast cancer, as well as being prominent in non-small cell lung carcinomas. EGFR mutations have been detected in both hereditary and spontaneous breast cancers [7-8]. EGFR-targeted therapy is currently being studied as a potential therapeutic method for causing cell death in breast cancer cells. Several compounds that specifically target tyrosine kinase are now being studied at different stages for their potential utility as inhibitors of the EGFR protein. Erlotinib, gefitinib, and lapatinib are molecules that have the quinazoline nucleus as their pharmacophore. Additionally, substituted pyrrolopyrimidines are being investigated as a potential pharmacophore for tyrosine kinase inhibitory action [9]. The medicinal chemist has been intrigued by the usage of subunits of pharmacologically active compounds in recent years to create new molecules with a wider spectrum of biological activities. The investigation of these small molecules, known as "privileged scaffolds," offers the advantage of having extensively studied interactions with biological systems. As a result, accurate predictions can be made regarding their ability to interact with newly investigated receptors [10]. Nitrogen-containing heterocycles are a prevalent form of privileged structures seen in a wide range of medications. The added presence of nitrogenous heterocyclic moieties helps the modification of properties such as polarity, lipophilicity, solubility, and hydrogen bonding ability in bioactive molecules. This, in consequently, improves the ADMET properties of lead molecules and drugs. These N containing heterocycles offer the advantage of hydrogen bonding to the target site [11-14].

The synthesis of the quinoline nucleus is straightforward, and it has advantageous "drug-like" characteristics, making its a attractive scaffold for medicinal chemists. The presence of a

nucleus is seen in several efficacious pharmacological compounds exhibiting anticancer properties. Irinotecan, Topotecan, and Belotecan are drugs that suppress the activity of topoisomerase enzymes. Foretinib, Cabozantinib, Lenvatinib, Bosutinib, and Neratinib are inhibitors of tyrosine kinases. Primaquine is also a tyrosine kinase inhibitor. Chloroquine is an antimalarial drug, Saquinavir is an antiviral drug, and Bedaquiline is an antitubercular drug. Quinoline derivatives have been shown to possess multiple advantages such as anticancer activity [19-21].

The compound 2-azetidinone is an unique structure that serves as the central ring in several antibacterial medications, such as penicillins, cephalosporins, nocardicins, and b-lactamase inhibitors. Furthermore, this structure is also seen in the antihyperlipidemic drug ezetimibe and the reduced form of 2-azetidinone, namely the oxetan ring, which plays a crucial role in the anti-cancer action of paclitaxel and docetaxel. Scientific evidence has shown that derivatives of 2-azetidinones possess a wide range of bioactivities, including but not limited to anticancer, antiproliferative, antioxidant, and CNS activities. Molecules synthesized using this ring structure have shown promising anticancer properties against both drug-sensitive and drug-resistant cell types in laboratory experiments. Therefore, the advantages of these small molecules were combined in order to build inhibitors that are expected to have efficacy against EGFR in this investigation [32-37].

Molecular Design:

Ghorab et al. (2014) designed and synthesized of 2-methyl-N-(7-trifluoromethyl)quinolin-4-yl)quinolin-3-amine **(I)** and were reported almost twice to thrice as potent as doxorubicin .The result of in vitro studies strongly recommended that the 4-amino, 7-substituted-quinoline derivatives possess antiproliferative activity based on a 4-aminoquinoline scaffold. The result of in vitro studies strongly recommended that the 4-amino, 7-substituted-quinoline derivatives possess antiproliferative activity.

R. Kayarmar et al . (2017) designed and synthesized of 3-Chloro-4-(4-chlorophenyl)-1-[(1-isobutyl-1H-imidazo[4,5-c]quinolin-4-yl)amino] azetidin-2-one **(II)** are showed most potent cytotoxic activity against HeLa cancer cell line, with IC_{50} values in the range of 11.44–11.77 lg/ml against the tested cancer cell line.

R. Khanam et al. (2018) reported the Piperazine scaffolds or 2-azetidinone pharmacophores have been reported to show anti-cancer activities. Among all, the compound N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-2-(4-phenylpiperazin-1-yl)-acetamide **(III)** remarkably inhibited the growth of HeLa cells in a concentration dependent manner having IC_{50} value of 29.44 ± 1.46 µg/ml.

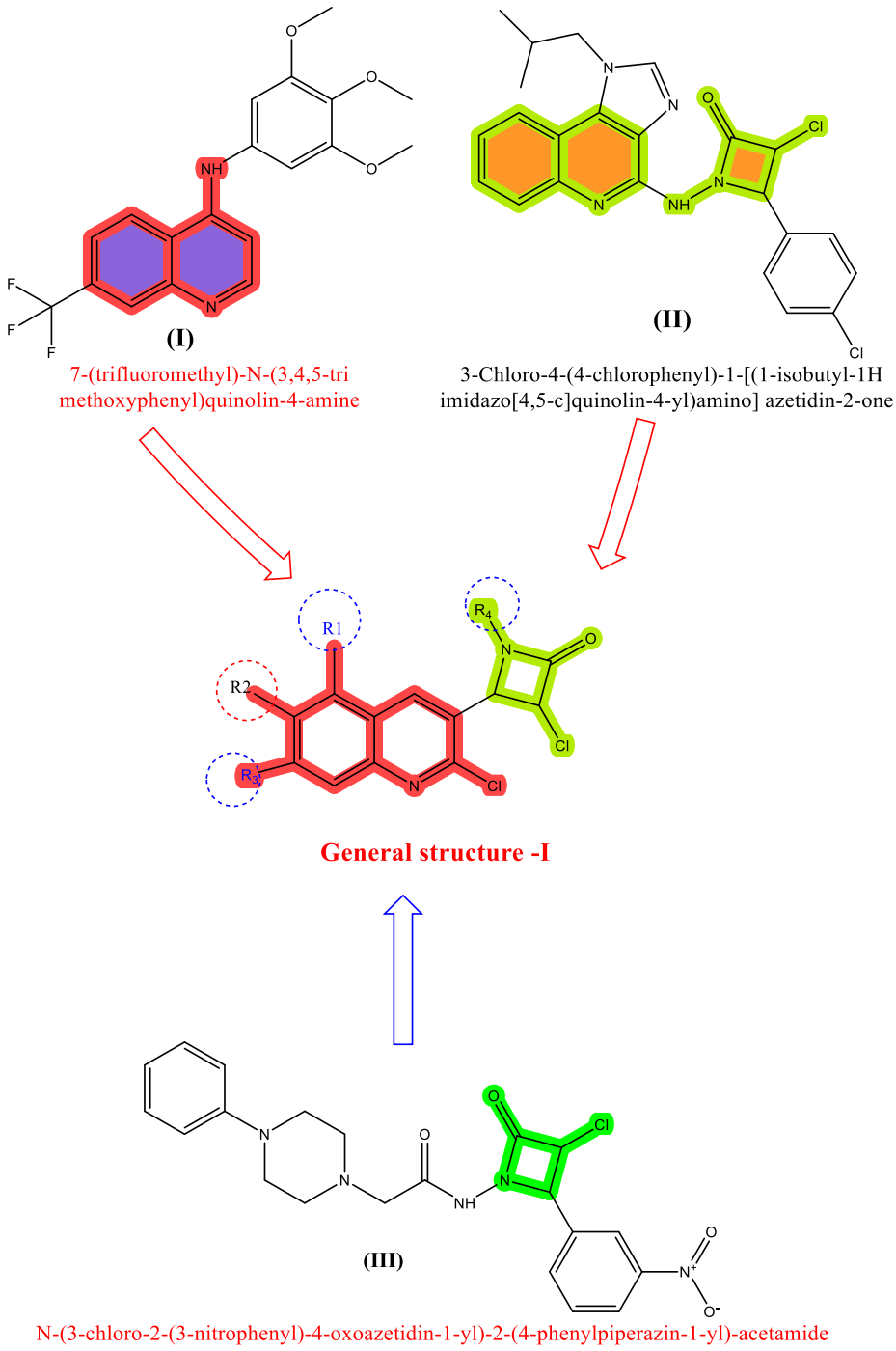


Figure 1. Design of New 2-azatedinone and quinoline derivatives

2.0. Materials and Methods:

2.1 Chemistry:

The chemicals, including standard drugs and solvents, were obtained from Sigma-Aldrich, HiMedia, Bangalore, India, and other sources. They were utilized without any further purification, except for liquid aldehydes, which were processed using normal processes before

being employed. The melting points of all the compounds were measured using the VEEGO VMP-D Digital melting point device, and the measurements were recorded in open capillary tubes.The FTIR spectra were obtained using the JASCO FTIR 4100 series instrument, using KBr pellets as the sample medium. The resulting spectra are shown in units of cm-1.The 1H NMR and 13C NMR spectra were obtained using a BRUKER-II 400 spectrophotometer, which operates at 400 MHz for 1H NMR and 100 MHz for 13C NMR. TMS was used as the internal standard. Pre-coated thin-layer chromatography (TLC) plates were used to assess the purity of the chemicals. The spots on the plates were made visible by exposing them to iodine vapors and ultraviolet light. The elements tests were conducted using a CHN-VarioElico Micro elemental analyzer. The determination of biochemical parameters was performed using readily available test kits from Sigma-Aldrich.

2.2. Synthetic Procedure:

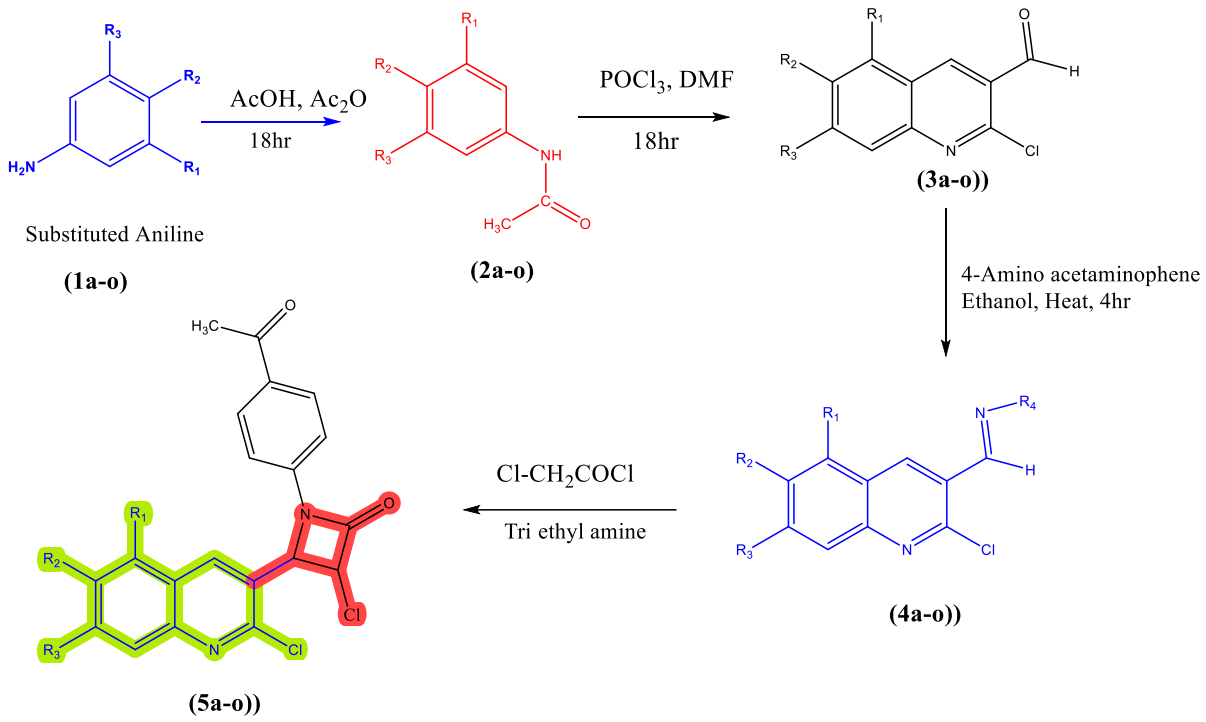
2.2.1. Synthetic Procedure of N-(3,4,5-tri-substituted-phenyl)acetamide (2a-o):

A mixture of substituted anilines (1a-o, 1.0 mmol) and acetic acid and acetic anhydride (1.0 mmol) was mixed in 10 mL of dry ethanol at room temperature with stirring. The mixture was then refluxed for 18 hours. The progression of the reaction can be observed using thin-layer chromatography (TLC) after the reaction mixture was filtered to collect the solid product. The filter was washed twice with dichloromethane to dissolve the reaction product. The solvent was removed and compound N-(3,4,5-tri-substituted-phenyl)acetamide **(2a-o)** was collected, and purified by using column chromatography. The combined organic extracts were evaporated to give colorless to yellow solids.

2.2.2. Synthetic procedure of 2-chloro-5,6,7-trisubstituted-quinoline-3-carbaldehyde (3a-o): N-(3,4,5-tri-substituted-phenyl)acetamide (**2**, 10 mmol) (4a-o, 20 mmol) were dissolved in DMF and allowed to addition of $POCl_3$ by using syringe and stirred at room temperature for 18 hours. On completion of the reaction mixture was diluted with cold water (15 mL) and extracted with pet. ether (3 x 25 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography (hexanes :Ethyl acetate, (9:1)) to afford pure 2-chloro-5,6,7-trisubstituted-quinoline-3-carbaldehyde (3a-o) in 80-90% yield.

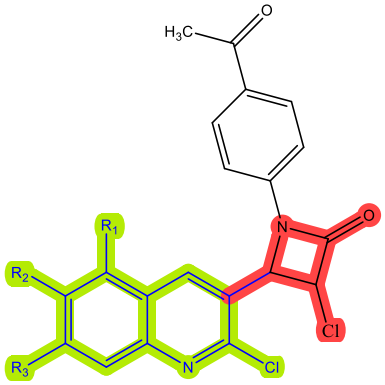
2.2.3. Synthetic procedure of (E)-1-(4-(((2-chloro-5,6,7-trimethylquinolin-3-yl)methylene)amino)phenyl)ethan-1-one (5a-o):

To a well stirred solution of various 2-chloro-5,6,7-trisubstituted-quinoline-3-carbaldehyde (3a-o) (1.8 mmol) in Chloro acetyl chloride at ambient temperature allowed stirring for 10 min. After this triethylamine was added slowly at 0°C, followed by chloro-acetyl chloride in toluene and allowed to stir with increasing temperature to 40°C until completion of reaction (6-8 h). Reaction was monitored by thin layer chromatography. After completion of the reaction, cool it to room temperature, diluted with ethyl acetate, washed with brine solution, and dried over Na_2SO_4 and purified by column chromatography to get the (E)-1-(4-(((2-chloro-5,6,7-trimethylquinolin-3-yl)methylene)amino)phenyl)ethan-1-one derivatives (5a-o).



Scheme-1: 4(E)-1-(4-(((2-chloro-5,6,7-trimethylquinolin-3-yl)methylene)amino)-phenyl) ethan-1-one (5a-o).

Table-1: Physical data of 4(E)-1-(4-(((2-chloro-5,6,7-trimethylquinolin-3-yl)methylene)amino)-phenyl) ethan-1-one (5a-o).



General structure-I

Com.	R1	R2	R3	M. Form	M.Wt	M.P	Rf*	% Yield
5a	H	H	H	$C_{20}H_{14}Cl_2N_2O_2$	385	165-167	0.5	60
5b	Cl	H	H	$C_{20}H_{13}Cl_3N_2O_2$	419	150-152	0.8	45

5c	Br	H	H	$C_{20}H_{13}BrCl_2N_2O_2$	461	160-162	0.7	65
5d	F	H	H	$C_{20}H_{13}Cl_2FN_2O_2$	403	140-142	0.8	50
5e	NO2	H	H	$C_{20}H_{13}Cl_2N_3O_4$	429	138-140	0.6	78
5f	CH3	H	H	$C_{21}H_{16}Cl_2N_2O_3$	415	128-130	0.3	32
5g	H	H	OCH3	$C_{21}H_{16}Cl_2N_2O_3$	414	160-162	0.4	45
5h	H	H	Br	$C_{20}H_{13}BrCl_2N_2O_2$	462	140-142	0.7	78
5i	H	H	Cl	$C_{20}H_{13}Cl_3N_2O_2$	419	155-157	0.9	68
5j	OCH3	OCH3	OCH3	$C_{23}H_{20}Cl_2N_2O_5$	475	146-148	0.5	71
5k	CH3	CH3	H	$C_{22}H_{18}Cl_2N_2O_2$	454	201-203	0.5	65
5l	Cl	Cl	H	$C_{20}H_{12}Cl_4N_2O_2$	454	180-182	0.8	74
5m	Br	Br	H	$C_{20}H_{12}Br_2Cl_2N_2O_2$	543	210-212	0.6	52
5n	F	F	H	$C_{20}H_{12}Cl_2F_2N_2O_2$	421	146-148	0.8	45
5o	H	C2H5	H	$C_{22}H_{18}Cl_2N_2O_2$	413	139-141	0.5	50

***Mobile phase: hexane: ethyl acetate**

4.0. Characterization of compounds :

4.1.1. 1-(4-acetylphenyl)-3-chloro-4-(2-chloroquinolin-3-yl)azetidin-2-one (5a)

Compound 5a obtained as white solid (yield 60%), m. p.165-167°C. ^{1}H NMR (400MHz DMSO, δ ppm): 7.80-7.85(d, 2H, *J*= 8.0 Hz, Ar-H), 7.77-7.79 (d, 2H, *J*= 4.0 Hz, Ar-H), 7.45-7.49 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.30-7.35 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.30-7.34 (d, 1H, Ar-H), 7.24-7.30 (m, 2H, Ar-H), 4.351-4.354 (d, 2H), 2.533 (s, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 160.10, 159.21, 155.10, 154.17, 148.10, 138.10, 135.20, 130.12, 129.08, 127.12, 125.24, 124.18, 123.01, 120.25, 116.52, 101.28, 60.21, 35.04. MASS spectrum m/z: 387.18 $[M+2]^+$; Calc.for $C_{20}H1_4Cl_2N_2O_2$; CHN: C, 62.36; H, 3.66; N, 7.27; Found: C, 62.30; H, 3.60; N, 7.25; IR (KBr, cm^{-1}): 3085.33 (C-H, Aromatic), 2940.12 (C-H, Aliphatic), 1732.10 (C=O**),** 1595.15 (C=C, Aromatic), 1184.14 (C-O).

4.1.2. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-5-methylquinolin-3-yl)azetidin-2-one (5b)

Compound 5b obtained as cream solid (yield 45%), m. p.150-152°C. ^{1}H NMR (400MHz DMSO, δ ppm): 7.92-7.94 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.86-7.88 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.76-7.79 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.63-7.36 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.32-7.34 (d, 1H, Ar-H), 7.20-7.24 (m, 1H, Ar-H), 4.355 (d, 2H), 2.58 (s, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 164.10, 160.24, 159.10, 158.12, 150.10, 149.10, 145.10, 139.12, 136.08, 130.10, 127.20, 125.18, 124.01, 122.25, 116.52, 101.28, 58.20, 34.01. MASS spectrum m/z: 419 $[M]^+$, 421 $[M+2]^+$, 423 $[M+4]^+$; Calc. for $C_{20}H_{13}Cl_3N_2O_2$: C, 63.17; H, 4.04; N, 7.02; Found: C, 63.10; H, 4.01; N, 7.04; IR (KBr, cm^{-1}): 3063.33 (C-H, Aromatic), 2940.12 (C-H, Aliphatic), 1710.10 (C=O**),** 1593.15 (C=C, Aromatic), 1180.14 (C-O).

4.1.3. 1-(4-acetylphenyl)-4-(5-bromo-2-chloroquinolin-3-yl)-3-chloroazetidin-2-one (5c)

Compound 5c obtained as brownish solid (yield 65%), m.p.160-162°C. ^{1}H NMR (400MHz DMSO, δ ppm): 7.98-8.01 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.94-7.96 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.50-

7.53 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.39-7.42 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.30-7.32 (d, 2H, Ar-H), 7.20-7.24 (m, 1H, Ar-H), 5.29 (d, 1H), 2.58 (s, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 161.11, 159.21, 158.10, 154.18, 149.10, 145.10, 137.10, 135.18, 131.08, 130.18, 129.27, 128.20, 127.18, 126.25, 116.52, 101.28, 61.01, 40.08. MASS spectrum m/z: 461.10 $[M]^+$, 463.15 $[M+2]^+$, 465.15 $[M+2]^+$ Calc. for $C_{20}H_{13}BrCl_2N_2O_2$; CHN: C, 51.71; H, 2.80; N, 6.05; Found: C, 51.76; H, 2.82; N, 6.04; IR (KBr, cm^{-1}): 3058.33 (C-H, Aromatic), 2932.10 (C-H, Aliphatic), 1712.18(C=O**),** 1590.25 (C=C, Aromatic), 1182.18 (C-O).

4.1.4. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-5-fluoroquinolin-3-yl)azetidin-2-one (5d)

Compound 5d obtained as yellow solid (yield 50%), m.p.150-152°C. ^{1}H NMR (400MHz DMSO, δ ppm): 7.88-7.90 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.65-7.67 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.43-7.51 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.33-7.36 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.32-7.34 (d, 1H, Ar-H), 7.21-7.25 (m, 1H, Ar-H), 4.315 (s, 1H, NH), 3.14-3.18 (m, 1H, *J*= 4.0 Hz), 1.64-1.66 (d, 2H, CH_3). ^{13}C NMR (100MHz, DMSO): 161.11, 160.10, 158.10, 154.12, 146.18, 139.10, 135.10, 130.12, 129.08, 128.10, 127.20, 125.18, 124.01, 122.25, 115.50, 101.20, 58.20, 34.01. MASS spectrum m/z: 403.10 $[M]^+$, 405 $[M+2]^+$. Calc. for $C_{20}H_{13}Cl_2FN_2O_2$: C, 59.50; H, 3.20; N, 6.90; Found: C, 59.57; H, 3.25; N, 6.95; IR (KBr, cm^{-1}): 3084.13 (C-H, Aromatic), 2954.12 (C-H, Aliphatic), 1716.10 (C=O**),** 1590.15 (C=C, Aromatic), 1189.18 (C-O).

4.1.5. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-5-nitroquinolin-3-yl)azetidin-2-one (5e)

Compound 5e obtained as cream solid (yield 78%), m. p. 138-140°C. ^{1}H NMR (400MHz DMSO, δ ppm): 8.01 (s, 1H, amide NH), 7.85-7.87 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.70-7.72 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.56-7.59(t, 1H, *J*= 4.0 Hz, Ar-H), 7.30-7.35 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.30-7.34 (d, 1H, Ar-H), 7.23-7.31 (m, 1H, Ar-H), 4.21 (s, 1H, NH), 3.20-3.25 (m, 1H, *J*= 4.0 Hz), 1.80-1.82 (d, 2H, CH_3). ^{13}C NMR (100MHz, DMSO): 160.15, 158.25, 154.15, 149.10, 148.10, 138.10, 135.20, 130.12, 129.18, 128.10, 126.20, 125.18, 124.01, 122.25, 116.52, 101.28, 56.21, 40.02. MASS spectrum m/z: 431.18 $[M+2]^+$. Calc. for $C_{20}H_{13}Cl_2N_3O_4$; CHN: C, 55.83; H, 3.05; N, 9.77; Found: C, 55.80; H, 3.04; N, 9.70; IR (KBr, cm^{-1}): 3080.20 (C-H, Aromatic), 2948.05 (C-H, Aliphatic), 1712.10 (C=O**),** 1585.10 (C=C, Aromatic), 1174.12 (C-O).

4.1.6. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-5-methylquinolin-3-yl)azetidin-2-one (5f)

Compound 5f obtained as white solid (yield 32%), m. p.128-130°C. ^{1}H NMR (400MHz DMSO, δ ppm): 7.85-7.87 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.72-7.74 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.46-7.48 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.33-7.36 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.32-7.34 (d, 1H, Ar-H), 7.20-7.24 (m, 1H, Ar-H), 4.355 (d, 2H), 2.32 (s, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 161.11, 160.12, 157.10, 154.12, 146.18, 139.10, 135.10, 132.12, 129.15, 128.19, 126.10, 125.18, 123.52, 120.25, 118.18, 101.28, 60.20, 41.01. MASS spectrum m/z: 415 $[M]^+$, 417 $[M+2]^+$. Calc. for $C_{21}H_{16}Cl_2N_2O_3$: C, 63.17; H, 4.04; N, 7.02; Found: C, 63.17; H, 4.04; N, 7.07; IR (KBr, cm^{-1}): 3080.33 (C-H, Aromatic), 2949.12 (C-H, Aliphatic), 1720.10 (C=O**),** 1593.15 (C=C, Aromatic), 1184.14 (C-O).

4.1.7. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-7-methoxyquinolin-3-yl)azetidin-2-one (5g)

Compound 5g obtained as white solid (yield 45%), m.p.160-162°C. ^{1}H NMR (400MHz DMSO, δ ppm): 7.90-7.92 (m, 2H, *J*= 8.0 Hz, Ar-H), 7.86-7.88 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.60-7.63 (m, 2H, *J*= 4.0 Hz, Ar-H), 7.40-7.42 (d, 1H, Ar-H), 7.30-7.34 (m, 1H, Ar-H), 5.31-5.35 (d, 2H), 2.55 (s, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 160.15, 159.15, 158.18, 157.10, 145.10, 140.10, 138.10, 136.18, 130.08, 128.18, 127.27, 125.20, 123.18, 120.25, 115.12, 100.20, 60.01, 42.14. MASS spectrum m/z: 414.10 $[M]^+$, 416.10 $[M+2]^+$. Calc. for $C_{21}H_{16}Cl_2N_2O_3$; CHN: C, 60.74; H, 3.88; N, 6.75; Found: C, 60.70; H, 3.81; N, 6.71; IR (KBr, cm^{-1}): 3062.30 (C-H, Aromatic), 2950.18 (C-H, Aliphatic), 1720.10(C=O**),** 1594.20 (C=C, Aromatic), 1180.10 (C-O).

4.1.8. 1-(4-acetylphenyl)-4-(7-bromo-2-chloroquinolin-3-yl)-3-chloroazetidin-2-one (5h)
Compound 5h obtained as yellow solid (yield 78%), m.p.140-142°C. ^{1}H NMR (400MHz DMSO, δ ppm): 7.90-7.92 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.68-7.70 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.42-7.46 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.33-7.38 (m, 2H, Ar-H), 7.32-7.34 (d, 1H, Ar-H), 5.34-5.36 (d, 2H), 2.55 (s, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 161.08, 160.12, 159.10, 158.12, 140.18, 139.10, 135.10, 130.12, 129.08, 128.10, 127.20, 125.18, 124.01, 122.25, 115.50, 101.20, 56.20, 40.01. MASS spectrum m/z: 464.15 $[M]^+$, 466.12 $[M+2]^+$. Calc. for $C_{20}H_{13}BrCl_2N_2O_2$: C, 51.76; H, 2.82; N, 6.04; Found: C, 51.70; H, 2.80; N, 6.05; IR (KBr, cm^{-1}): 3065.10 (C-H, Aromatic), 2980.10 (C-H, Aliphatic), 1701.18 (C=O**),** 1594.14 (C=C, Aromatic), 1180.10 (C-O).

4.1.9. 1-(4-acetylphenyl)-3-chloro-4-(2,7-dichloroquinolin-3-yl)azetidin-2-one (5i)
Compound 5i obtained as cream solid (yield 68%), m. p.155-157°C. ^{1}H NMR (400MHz DMSO, δ ppm): 7.82-7.83 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.75-7.77 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.61-7.63(t, 1H, *J*= 4.0 Hz, Ar-H), 7.42-7.45 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.30-7.34 (d, 1H, Ar-H), 7.23-7.31 (m, 1H, Ar-H), 5.432-5.46 (d, 2H), 2.56 (s, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 161.25, 159.25, 156.15, 149.10, 148.10, 138.10, 135.20, 130.12, 129.18, 128.10, 126.20, 125.18, 124.01, 122.25, 116.52, 101.28, 56.21, 40.02. MASS spectrum m/z: 347.10 $[M+H]^+$. Calc. for $C_{20}H_{13}Cl_3N_2O_2$; CHN: C, 57.24; H, 3.12; N, 6.67; Found: C, 57.20; H, 3.10; N, 6.60; IR (KBr, cm^{-1}): 3078.10 (C-H, Aromatic), 2950.15 (C-H, Aliphatic), 1710.20 (C=O**),** 1580.10 (C=C, Aromatic), 1170.12 (C-O).

4.2.0. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-5,6,7-trimethoxyquinolin-3-yl)azetidin-2-one (5j): Compound 5j obtained as white solid (yield 71 %), m. p. 146-148 °C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 7.864-7.917(m, J=9.6 Hz, 2H, Ar-H), 7.469-7.568 (m, 2H, Ar-H), 7.375-7.397 (d, J=4.8Hz, 1H, Ar-H), 6.377 (s, 1H, Ar-H), 5.346-5.349(d, 2H), 3.755 (s, 3H, OCH_3), 3.801 (s, 6H,OCH_3). ^{13}C NMR (100MHz, $CDCl_3$): 173.14, 169.12, 158.45, 132.17, 132.17, 130.28, 130.16, 129.65, 128.47, 126.34, 126.12, 123.12, 119.35, 110.26, 66.54, 41.06. MASS spectrum m/z: 475.15$[M]^+$, 477.12$[M+2]^+$, 479.31$[M+H]^+$. Calc. for $C_{23}H_{20}Cl_2N_2O_5$; CHN: C, 58.12; H, 4.24; N, 5.89; Found C, 58.120; H, 4.20; N, 5.80; IR (KBr, cm^{-1}): 3087.19 (C-H, Aromatic), 2923.40 (C-H, Aliphatic), 1613.80(C=O), 1586.04 (C=C, Aromatic), 1244.10 (C-O).

4.2.1. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-5,6-dimethylquinolin-3-yl)azetidin-2-one (5k): Compound 5k obtained as yellow solid (yield 65 %), m. p. 202-204 °C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 7.897-7.899 (d, J=9.6 Hz, 2H, Ar-H), 7.626-7.628 (t, J=8.8Hz, 1H, Ar-H), 7.373-7.395 (d, J=8.8 Hz, 3H, Ar-H), 6.378 (d, 2H, Ar-H), 5.321-5.323 (d, 2H), 3.797(s, 3H, CH_3), 3.750 (s, 6H, CH_3). ^{13}C NMR (100MHz, $CDCl_3$): 161.50, 159.30, 158.15, 148.65, 135.65, 130.25, 129.85, 129.32, 128.16, 126.32, 125.45, 123.12, 117.12, 102.16, 61.28, 58.32, 40.12. MASS spectrum m/z: 413.11$[M]^+$, 415.05 $[M+2]^+$. Calc. for $C_{22}H_{18}Cl_2N_2O_2$; CHN: C, 63.93; H, 4.39; N, 6.78; Found C, 63.90; H, 4.34; N, 6.78; IR (KBr, cm^{-1}): 3062.10 (C-H, Aromatic), 2958.15 (C-H, Aliphatic), 1726.80(C=O), 1535.75 (C=C, Aromatic), 1278.19 (C-O).

4.2.2. 1-(4-acetylphenyl)-3-chloro-4-(2,5,6-trichloroquinolin-3-yl)azetidin-2-one (5l) : Compound 5l obtained as yellow solid (yield 35 %), m. p.190-192°C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 8.11 (s, 1H, amide NH), 7.865-7.919 (m, 2H, Ar-H), 7.473-7.555 (m,2H, Ar-H), 7.316-7.428 (m, 2H, Ar-H), 5.351-5.352 (d, 2H), 3.803(s, 3H, CH_3). ^{13}C NMR (100MHz, $CDCl_3$): 162.14, 157.81, 152.64, 142.08, 132.54, 131.08, 129.35, 129.09, 128.56, 128.24, 125.58, 125.25, 124.18, 116.04, 101.28, 60.21, 57.40, 48.58, 38.04, 36.24, 29.29, 25.07, MASS spectrum m/z: 454$[M]^+$, 456 $[M+2]^+$Calc. for $C_{20}H_{12}Cl_4N_2O_2$; CHN: C, 52.90; H, 2.66; N, 6.17; Found C, 52.94; H, 2.60; N, 6.10; IR (KBr, cm^{-1}): 3050.15 (C-H, Aromatic), 2945.61 (C-H, Aliphatic), 1710.32 (C=O), 1590.12 (C=C, Aromatic), 1258.15(C-O).

4.2.3. 1-(4-acetylphenyl)-3-chloro-4-(5,6-dibromo-2-chloroquinolin-3-yl)azetidin-2-one (8m): Compound 5m obtained as white solid (yield 52 %), m. p. 210-212°C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 7.896-7.898 (d, J=9.6 Hz, 2H, Ar-H), 7.628-7.631 (t, J=8.8Hz, 1H, Ar-H), 7.377-7.379 (d, J=8.8 Hz, 3H, Ar-H), 5.321-5.325 (d, 2H), 3.797(s, 3H, CH_3), ^{13}C NMR (100MHz, $CDCl_3$): 160.50, 158.30, 157.10, 150.61, 145.65, 142.25, 139.85, 135.32, 130.16, 126.32, 125.45, 123.12, 117.12, 102.16, 61.28, 58.32, 40.12. MASS spectrum m/z: 543$[M]^+$, 545 $[M+2]^+$, 547 $[M+4]^+$. Calc. for $C_{20}H_{12}Br_2Cl_2N_2O_{24}$; C, 44.24; H, 2.23; N, 5.16; Found C, 44.20; H, 2.20; N, 5.10. IR (KBr, cm^{-1}): 3058.12 (C-H, Aromatic), 2950.10 (C-H, Aliphatic), 1716.80(C=O), 1538.75 (C=C, Aromatic), 1270.19 (C-O).

4.2.4. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-5,6-difluoroquinolin-3-yl)azetidin-2-one (5n)

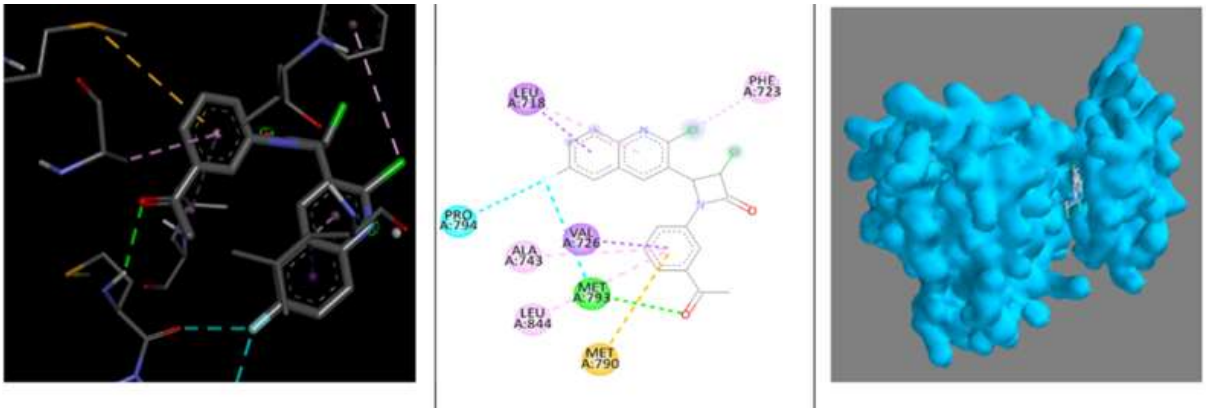
Compound 5n obtained as cream white solid (yield 45 %), m. p. 143-145°C.^{1}H NMR (400MHz $CDCl_3$, δ ppm): 7.8903-7.8934 (d, 2H, J= 8.0 Hz,Ar-H), 7.7476-7.7651(m, 1H,J= 8.0 Hz,Ar-H), 7.625-7.651 (d, 1H, Ar-H), 7.5493-7.552 (t, 1H, J= 4.0 Hz,Ar-H), 7.4010-7.4038 (t, 1H, J= 4.0 Hz,Ar-H), 7.3006-7.3587(m, 1H, J= 8.0 Hz,Ar-H), 5.351-5.353 (d, 2H), 2.55 (s, 3H,CH3), ^{13}C NMR (100MHz, $CDCl_3$): 170.01, 168.72, 130.28, 130.94, 129.86, 129.62, 127.29, 127.23, 125.41, 125.22, 124.34, 123.96, 109.86, 65.35, 40.60. MASS spectrum m/z: 421 $[M]^+$, 423 $[M+2]^+$, 425 $[M+4]^+$. Calc. for $C_{20}H_{12}Cl_2F_2N_2O_2$; CHN: C, 57.03; H, 2.87; Cl, 16.83; N, 6.65; Found C, 57.02; H, 2.80; Cl, 16.80; N, 6.60; IR (KBr, cm^{-1}): 3086.15(C-H, Aromatic), 2922.83 (C-H, Aliphatic), 1614.52(C=O**)**, 1549.75(C=C, Aromatic), 1246.00 (C-N).

4.2.5. 1-(4-acetylphenyl)-3-chloro-4-(2-chloro-6-ethylquinolin-3-yl)azetidin-2-one(5o): Compound 5o obtained as orange solid (yield 50 %), m. p. 139-141°C.^{1}H NMR (400MHz DMSO, δ ppm): 8.10-8.12 (d, 1H, J= 8.0 Hz,Ar-H), 7.82-7.84 (d, 1H, J= 8.0 Hz,Ar-H), 7.72-7.73 (d, 1H, J= 4.2 Hz,Ar-H), 7.48-7.51 (t, 1H, J= 4.0 Hz,Ar-H), 7.30-7.33 (t, 1H, J= 4.0 Hz,Ar-H), 7.27-7.29 (d, 1H, J= 8.0 Hz,Ar-H), 6.69(s, 1H, Ar-H), 6.62-6.64(d, 1H, J= 8.0 Hz,Ar-H), 5.30-5.32 (d, 2H), 3.19-3.22 (t, 3H, J= 4.0 Hz,), 2.70-2.76(m, 2H), 2.55(s, 3H, CH3). ^{13}C NMR (100MHz, DMSO): 195.6, 168.6, 163.3, 156.8, 148.2, 132.5, 129.6, 129.3, 128.2, 128.1, 128.0, 126.8, 126.4, 123.8, 123.2, 123.1, 122.8, 118.6, 109.4, 52.1, 38.4, 35.6, 32.8, 30.5, 23.1, 14.9; MASS spectrum m/z: 434.28$[M+H]^{+}$Calc. for $C_{22}H_{18}Cl_2N_2O_2$; CHN: C, 63.93; H, 4.39; N, 6.78; Found C, 63.91; H, 4.15 N, 6.70; IR (KBr, cm^{-1}): 3082.10 (C-H, Aromatic), 2975.15 (C-H, Aliphatic), 1720.12(C=O**)**, 1541.20 (C=C, Aromatic), 1150.68(C-O).

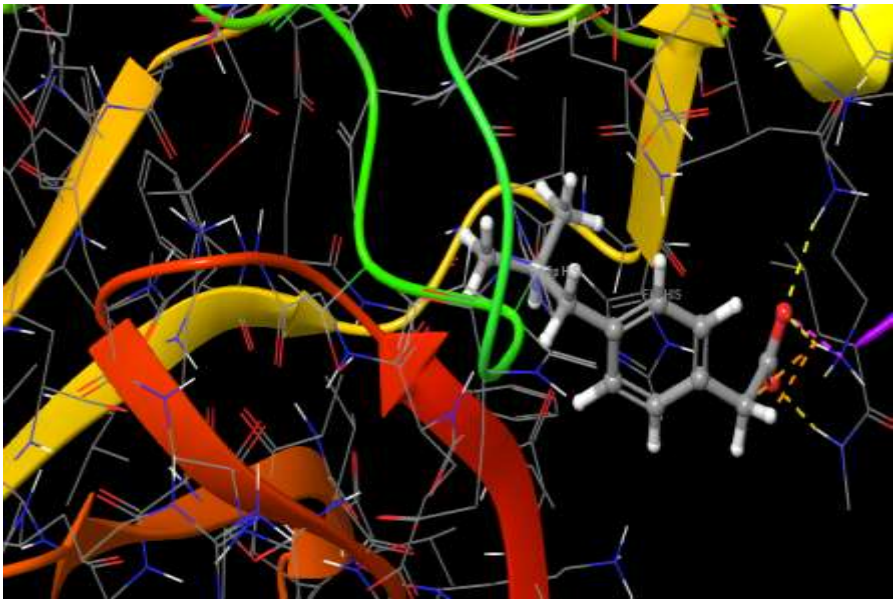
5.0. Molecular Docking:

Molecular docking investigations on (E)-1-(4-(((2-chloro-5,6,7-trimethylquinolin-3-yl)methylene)amino)-phenyl) ethan-1-one (5a-o) were conducted using Schrödinger software (Schrödinger, Version 2023-4) installed on an Intel Xenon W 3565 processor and Ubuntu enterprise (version 14.04) as an operating system. The ligands were drawn using ChemDraw 18.0. The use of XP Visualizers (Schrödinger, Version 2023-4). The results were analyzed.

Schrodinger software (Version 2023-4; Schrodinger) (Glide module). The ligands used as inputs for docking were sketched by using ChemDraw software. Ligands were prepared by using OPLS3e force field in Ligprep (Dizdaroglu et al. 2020) (Version 2023-3, Schrodinger) was used to carry out, the docking studies This minimization helps to assign bond orders, Addition of the hydrogens to the ligands. The generated output file containing the best conformations of the ligands was used for docking studies. Protein was prepared by using the protein preparation wizard (Dizdaroglu et al. 2020) (Version 2023-3, Schrodinger). Charges were assigned to the protein after addition of hydrogen atoms Generated Het states using epik at pH 7.2. The protein was pre-processed refined, modified by analyzing workspace. Atoms which are non-significant were excluded from the crystal structure. Finally, the protein was optimized by using OPLS3e force filed. A receptor grid was generated around the cocrystal ligand (X-ray pose of the ligand in the protein). Ligand centroid was selected to generate grid box, and Vander Waal radius of receptor atoms was scaled to 1.00 Å having a partial atomic charge of 0.25. From the output, The best-docked structure was determined using Glide docking score. Poses of the generated output of ligands after docking was analyzed by the help of XP Visualizer (Version 2023-3, Schrodinger). The results are presented in **Tables-2** and **Figures-1** and **2**.



Docking poses of compound 5a in the active site of human EGFR kinase protein



Docking poses of compound 5j in the active site

Binding Energies (Kcal/mol), No. of HBs and Binding Sites

S.No	Compound	Docking score of MCF-7 (6ENV)	Docking score of HELA (7OZR)	Docking score of MDA-MB-231 (6VJ3)
1	5a	9.971	8.782	9.971
2	5b	7.707	6.723	7.707
3	**5c**	5.057	3.415	5.057
4	5d	-5.146	-3.714	-5.146
5	5e	-6.861	-5.395	-6.861
6	5f	7.401	-6.711	7.401
7	5g	-5.381	-3.484	-5.381
8	5h	-4.712	-3.489	-4.712
9	5i	-3.942	-3.526	-3.942
10	**5j**	**-9.386**	**-9.475**	**-9.386**
11	5k	-7.386	-5.241	-7.386
12	5l	-5.534	-5.224	-5.534

13	5m	-8.270	-8.252	-7.270
14	5n	**-8.270**	**-8.252**	**-7.270**
15	5o	4.401	-4.711	4.401
16	Cocrystal Ligand	-4.122	-4.321	-4.122

1

6.0. Anti-cancer activity:

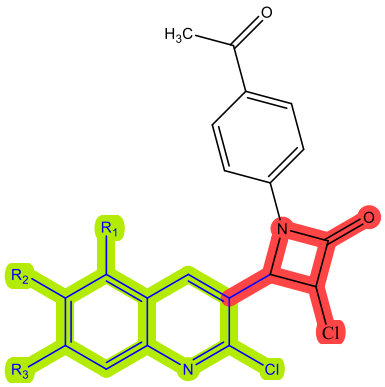
6.1.1. Biological assay

Cultures and cell lines Human breast cancer cell line (MDA-MB-231), cervical carcinoma cell line (HeLa), the liver cancer cell line (SMMC-7721) and normal hepatocyte cell line (QSG-7701) were maintained in Dulbecco Modified Eagle Medium (DMEM) containing 4.0 mM L-Glutamine and 4500 mg/L Glucose supplemented with 10% (v/v) foetal bovine serum (FBS) and 100 U/mL penicillin/streptomycin at 37°C in humidified atmosphere of 5% CO2 and 95% air.

6.1.2. MTT assay

MDA-MB-231, Hela, and SMMC-7721 cell lines were seeded onto 96-well plates for 100 lL and treated with varied doses of synthetic chemicals for 72 h before being incubated for 3-4 h at 37°C. The purple formazan crystals formed by viability can be dissolved in 100 lL with DMSO. The plates were quantified by measuring their OD at a wavelength of 540 nm after being gently spun for 5 minutes. The identical experimental condition was offered for all substances, and each concentration was replicated in three wells. The percentage of cells that survive was determined to be 50%, and the data were represented as IC50 values with standard deviations. As a positive control, cisplatin was also evaluated.

Table 3: anti-cancer activity of (E)-1-(4-(((2-chloro-5,6,7-trimethylquinolin-3-yl) methylene) amino)-phenyl) ethan-1-one (5a-o) derivative



General structure-I (5a-o)

Com.	R1	R2	R3	M. Form	MDA-MB-231 IC_{50} (μM)	HELa IC_{50} (μM)	SMMC-7721 IC_{50} (μM)
5a	H	H	H	$C_{20}H14Cl_2N_2O_2$	6.20±0.02	5.27±0.02	7.62±0.02
5b	Cl	H	H	$C_{20}H_{13}Cl_3N_2O_2$	3.12±0.04	2.02±0.08	5.95±0.03
5c	Br	H	H	$C_{20}H_{13}BrCl_2N_2O_2$	3.58±0.02	3.78±0.04	6.26±0.03
5d	F	H	H	$C_{20}H_{13}Cl_2FN_2O_2$	3.05±0.02	3.62±0.02	6.45±0.03
5e	NO2	H	H	$C_{20}H_{13}Cl_2N_3O_4$	4.80±0.02	5.35±0.03	6.38±0.03
5f	CH3	H	H	$C_{21}H_{16}Cl_2N_2O_3$	2.36±0.03	2.26±0.03	5.62±0.02
5g	H	H	OCH3	$C_{21}H_{16}Cl_2N_2O_3$	3.35±0.02	3.45±0.03	4.35±0.03
5h	H	H	Br	$C_{20}H_{13}BrCl_2N_2O_2$	2.08±0.03	2.38±0.03	5.26±0.03
5i	H	H	Cl	$C_{20}H_{13}Cl_3N_2O_2$	2.90±0.02	7.64±0.02	3.45±0.03
5j	OCH3	OCH3	OCH3	$C_{23}H_{20}Cl_2N_2O_5$	**0.72±0.04**	**1.39±0.02**	**2.02±0.28**
5k	CH3	CH3	H	$C_{22}H_{18}Cl_2N_2O_2$	**0.72±0.04**	**1.39±0.02**	**2.02±0.28**
5l	Cl	Cl	H	$C_{20}H_{12}Cl_4N_2O_2$	1.35±0.03	3.24±0.03	2.58±0.24
5m	Br	Br	H	$C_{20}H_{12}Br_2Cl_2N_2O_2$	1.68±0.02	1.65±0.02	3.27±0.12
5n	F	F	H	$C_{20}H_{12}Cl_2F_2N_2O_2$	**0.78±0.08**	**1.31±0.02**	**2.62±0.12**
5o	H	C2H5	H	$C_{22}H_{18}Cl_2N_2O_2$	1.12±0.05	2.95±0.03	3.26±0.13
Cisplatin			-----	-----	0.54±0.05	0.95±0.02	1.32±0.22

[a]Data are expressed as (mean±SD in μM, n=3.

7.2.1. In vitro cytotoxic assay and Results & discussions:

The in vitro cytotoxic activity of derivatives **5a-o** were evaluated by MTT assay against human breast cancer cell line (MDA-MB-231), cervical carcinoma cell line (HeLa), hepatocarcinoma cell line (SMMC-7721).The anticancer drug cisplatin was coassayed as the positive control. All tested compounds were dissolved in DMSO and the stock solutions were diluted by DMEM medium before treatment of the cultured cells. The IC_{50} values of the tested compounds against four cell lines are shown in Table 2. As illustrated in Table 2, the tested compounds displayed varying degrees of cytotoxic activity against the three cancer cell lines. Generally, these derivatives showed the strongest activities against HeLa cells, then MDA-MB-231 cells, and were least active to Mcf-7 cells. Concerning different derivatives, it was found that compounds 5a-o with various aldehydes exhibited potent cytotoxic activities against MDA-MB-231 and HeLa cells at low lM levels and moderate activities against Mcf-7 cells. Compounds 5k and 5m showed strong cytotoxicity against MDA-MB-231 and HeLa cells, respectively. while compounds 5a, 5b, 5c were showed mild or no cytotoxicity against three cancer cell lines. It is worth noting that compound 5j exhibited the most potent anticancer activity against all three cancer cells at low lM to nM range (IC_{50}: 0.12 ± 0.01, 0.08 ± 0.01 and 0.34 ± 0.03 lM, respectively), stronger than positive control cisplatin. In addition, compound 4d was less cytotoxic to normal hepatocyte cells (QSG-7701) with IC50 value of 10.76 ± 0.72 lM, which indicated a high selectivity of cytotoxicity (134.5) between cancer cells and normal hepatocyte cells. Hence, compound 5j was selected for further investigations on its anticancer mechanisms.

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