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Molecular hybridation based design and synthesis of novel quinoline derivatives as anti-cancer agents

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Abstract:
A Series of Newsubstituted 4-(quinolin-4-ylamino)benzohydrazide derivatives were designed, synthesized, characterized by respective spectral data and evaluated for anti cancer activity. Most of the synthesized compounds showed good anti-cancer activityagainst MDA-MB-231, HeLa, SMMC-7721 cell lines**.** Among the compounds **8m** and **8k** having 2,6-dihydroxy, 3,4,5-trimethoxy 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, SMMC-7721cellline 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 µMrespectively. Standard drug cisplatin exhibited IC_{50} values of 0.54±0.05 and 0.95±0.02 and 1.32±0.22 againstMDA-MB-231, HeLa, SMMC-7721**.** Compounds 8a, 8b were showed lower cytotoxic property in normal celllines. Docking studies of all the molecules disclosed close hydrogen bond interactions with the binding site.

Keywords: Cancer, Antioxidants, anti-inflammatory, hepatic cells, anti-proliferative activity, cisplatin

1.0. Introduction:

Cancer is the leading cause of death and a major impediment to extending life spans around the world. In 2020, 19.3 million cancer cases were registered, and around 10 million victims died as a result of cancer.[1]It is the most life-threatening disease, characterized by uncontrolled cell

development that spreads through the body. Various cancer causes have been hypothesized, including genetic aspects. [2] It also occurs due to the life style factor or habits of human beings like smoking tobacco[3], consuming alcohol[4], and eating red meat[5] etc., A few kinds of cancer are also caused by Helicobacter pylori [6],HPV[7]and EBV infections [8]. Anticancer medications are used to keep cancer cells from growing and replicating; if this occurs, the tumor cells usually die. As a result, a number of studies have been conducted to develop a more effective anti-cancer medicine. However, it is a constant process for making significant progress in developing a medicine with no side effects. [9]In the recent past, heterocyclic compounds have been used to treat a wide range of diseases as an efficient pharmacophore. Quinoline is one of the heterocyclic chemicals that works well as a scaffold for drugs development. Quinoline is an aromatic heterocyclic organic molecule with the chemical formula C_9H7N. Its double-ring structure is created when the benzene ring and pyridine fuse at two nearby carbon atoms.[10]
Quinoline's ability to attract electrons through resonance makes it an essential component of medicinal chemistry. This bicyclic aromatic molecule has poor tertiary base characteristics and is an electron-deficient system. It shows substitutions that are both nucleophilic and electrophilic, and pyridine also shows both kinds of substitutions. The clinical observation demonstrates that even a human breathing in 0.001 ppm of quinoline for eight hours was completely safe. This is in stark contrast to benzene, which even at lower concentrations causes a variety of biological diseases, including cancer.[11]

Design:

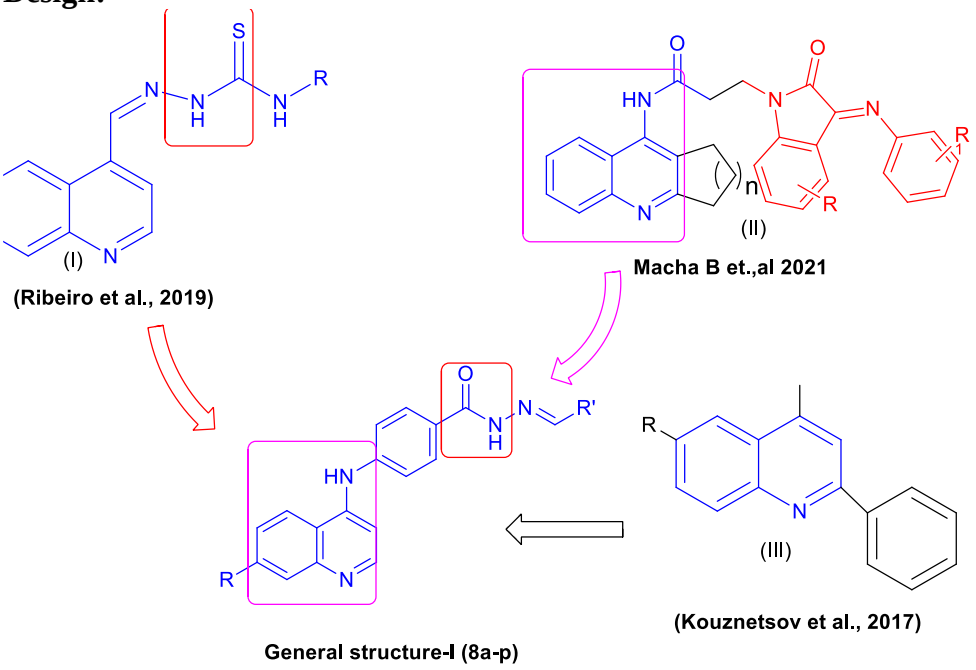


Figure 1. Design of New quinoline derivatives

Ribeiro et al., 2019[12]reported A new 4-quinoline-thiosemicarbazone molecule demonstrated the ability to bind both BSA and DNA. The carbothioamide compound N-(4-ethylphenyl)-2-(quinoline-4-ylmethylene) hydrazine was chosen for study because it showed a higher Kb and Ksv value for binding to both DNA and BSA, suggesting a potential DNA intercalation mechanism. Compound (I) was an interfacial antitumoral inhibitor since it partially inhibited topos-II and had a reduced IC_{50} value (0.82 lm) for MCF 7 cell lines. The reduction of the acridine ring to a quinoline ring did not interfere with the anticancer activity, indicating that substitution patterns are essential for biomolecule contact affinity.

Kouznetsov et al., 2017[13]synthesized 4-methyl-2-(2-,3- and 4-pyridinyl) quinolones, which were evaluated for their anticancer activity against human tumor cell lines, including SKBR3, MCF7, PC3, and HeLa. The cytotoxicity of these compounds has been investigated in vitro using human skin fibroblasts as noncancer cells. 4-methyl-2-(3-pyridinyl)quinoline distinguishes out as a viable approach for anticancer drugs against prostate carcinoma due to its non-specific cytotoxicity and high selectivity for PC3 cells (IC50 = 4.40-l m).

Macha B et al., 2021[14]synthesized of new cycloalkyl ring fused quinolines as multifunctional agents for Alzheimer's treatment and anti-oxidant activity.

The quinoline derived compounds, and their activity toward various cancer has been discussed in detail, in a nutshell, the synthesis involved in each compound is demonstrated. Plenty of quinoline-bearing compounds were developed and screened for anticancer activity.

In view of the above, it is planned to synthesize **New quinoline derivatives** as shown in**General Structure-I** and evaluate them for their anticancer activities by standard protocol.

2.0. NEED FOR THE PRESENT WORK

Bosutinib, Camptothecin, Lenvatinib, Cabozantinib, Topotecan, Irinotecan, and a few more quinoline derivatives are now being investigated as possible medications for a range of cancer therapies. Several recently described compounds derived from quinolines exhibit increased anticancer efficacy, even though these medications provide a greater potential for treating cancer. The main problems with all the synthetic compounds include normal cell damage, limited efficacy, and drug resistance. In our opinion, in order to commercialize quinoline molecules, the researchers must overcome these challenges. Therefore, since the current study will help with the synthesis of novel anticancer medications that are targeted at diverse human tumors, we predict that pharmaceutical scientists and the drug discovery community would benefit from it. The survey above makes clear that numerous novel quinoline compounds have been created as anticancer drugs. Among the heterocyclic compounds with anticancer properties and potential applications in other diseased diseases, novel quinoline derivatives have been investigated the most. Therefore, creating novel quinoline derivatives connected by hydrazine bridges, as illustrated in General Structure-I, is deemed valuable containing some electron-withdrawing/electron-releasing substituents on both new quinoline derivatives.

3.0. Materials and Methods:

3.1.Chemistry:

Melting points were measured with the VEEGO VMP-D Digital Melting Point equipment using open capillary tubes. The powdered compounds' ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER-II 400 (400 MHz NMR, 13C NMR 100 MHz) spectrophotometer using TMS as an internal reference. The FTIR spectra were recorded using KBr on a JASCO FTIR 4100 series and are reported in cm-1. On TLC plates that had already been coated, the compounds' purity was examined using silica G as the stationary phase, iodine fumes, and ultraviolet light as the

visualizing agent. Sigma-Aldrich, Hi Media, Bangalore, India, and other suppliers provided all chemicals, including common medications and solvents. Using kits from Sigma-Aldrich, the biochemical parameters were evaluated.

3.1.1. Synthesis of 4-chloroquinoline derivatives (3a-i):

A solution of 4-hydroxyquinoline derivatives**(1a-i)** (0.020 mol) and phosphorus oxychloride**(2)** (30 mL) was stirred at 120 °C for 12 h. After cooling the mixture at room temperature the mixture was dried and the residue thus obtained was treated with 10% NaOH solution was adjusted pH to 8–9 and extracted with dichloromethane (DCM, 3 × 30 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuum to get the colorless liquid.

3.1.2. Synthesis of ethyl 4-(quinolin-4-ylamino)benzoate derivatives (5a-i):

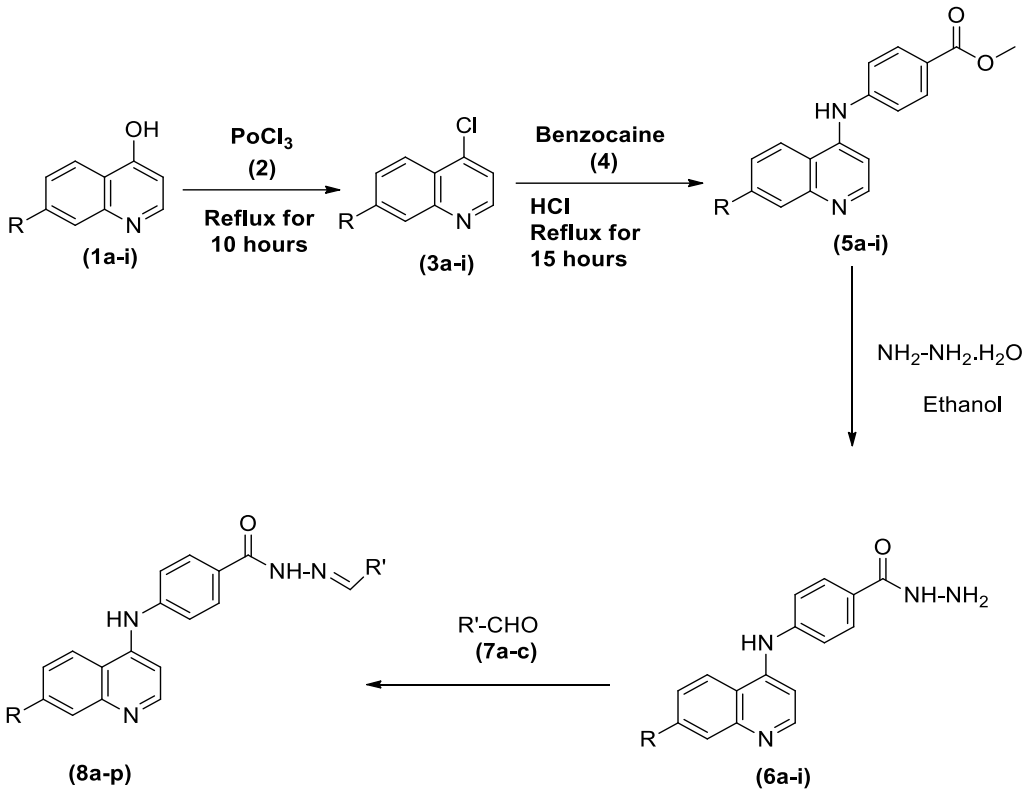
The synthesized 4-chloroquinoline derivatives**(3a-i)** (10.010 mol), benzocaine(**4**) (0.010 mol), n-butanol (35 mL), and 2 drops of concentrated hydrochloric acid were added to the dry 100 mL round bottom flask, and the reaction mixture was refluxed for 15 h. Then, the mixture was cooled to room temperature and the resulting solid was filtered off, washed with Hexane, and dried to get yellowish solid.

3.1.3. Synthesis of 4-(quinolin-4-ylamino) benzohydrazide derivatives(6a-i):

A suspension of ethyl 4-(quinolin-4-ylamino) benzoate derivatives**(5a-i)** (0.010 mol) and hydrazine hydrate (20 mL) in ethanol (15 mL) was stirred and refluxed for 24 h. The reaction mixture was then cooled to room temperature, and the resulting solid was filtered, washed with waterand dried to get white solid.

3.1.4. General procedure for the synthesis of compounds (8a-8p)

A mixture of the appropriate substituted aldehydes **(7a-c)** (0.2 mol) and the 4-(quinolin-4-ylamino) benzohydrazide derivatives**(6a-i)** (0.2 mmol) in absolute ethanol (5 mL) containing two drops of trifluoroacetic acid was stirred and refluxed for 8 h. Then, the reaction mixture was cooled to room temperature.Volatilities were removed under vacuum to yield the title compounds (**8a-8p**) which were purified over silica.



8a= R=H; R'=CH_3 8b= R=Cl; R'= CH_3

8c= R=Br; R'=CH_3 8d= R=F; R'= CH_3

8e= R=CH_3 ; R'=CH_3 8f= R=Cl ; R'= CH_3-CH_2-CH_3

8g= R=Br ; R'= CH_3-CH_2-CH_3 8h= R=F ; R'= CH_3-CH_2-CH_3

8i= R=CH_3 ; R'= CH_3-CH_2-CH_3 8j= R=H; R'= 3,4,5-trimethoxy phenyl

8k= R=Cl; R'= 3,4,5-trimethoxy phenyl 8l= R=Br; R'= 3,4,5-trimethoxy phenyl

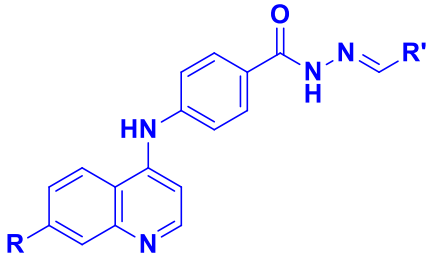
8m= R=F ; R'= 3,4,5-trimethoxy phenyl 8n= R=H; R'= 2,6-di hydroxy phenyl

8n= R=Cl; R'= 2,6-di hydroxy phenyl 8o= R=Br; R'= 2,6-di hydroxy phenyl

8p= R=F; R'= 2,6-di hydroxy phenyl

Scheme: Synthesis of compounds 8a-8p

Table1: Physical data of New substituted 4-(quinolin-4-ylamino)benzohydrazide derivatives



General structure-I (8a-p)

Com.	R	R'	M. Form	M.Wt	M.P	Rf*	% Yield
8a	H	CH_3	$C_{18}H_{16}N_4O$	304	168-170	0.6	60
8b	Cl	CH_3	$C_{18}H_{15}ClN_4O$	338	150-152	0.9	75
8c	Br	CH_3	$C_{18}H_{15}BrN_4O$	382	128-130	0.5	55
8d	F	CH_3	$C_{18}H_{15}FN_4O$	322	165-167	0.8	55
8e	CH_3	CH_3	$C_{19}H_{18}N_4O$	318	190-192	0.8	70
8f	Cl	CH_3-CH_2CH_2-	$C_{18}H_{15}ClN_4O$	366	198-200	0.8	52
8g	Br	CH_3-CH_2CH_2-	$C_{20}H_{19}BrN_4O$	410	138-140	0.4	50
8h	F	CH_3-CH_2CH_2-	$C_{20}H_{19}FN_4O$	350	160-162	0.7	50
8i	CH_3	CH_3-CH_2CH_2-	$C_{21}H_{22}N_4O$	346	202-204	0.9	71
8j	H	3,4,5Trimethoxy phenyl	$C_{26}H_{24}N_4O_4$	457	172-174	0.5	48
8k	Cl	3,4,5Trimethoxy-phenyl	$C_{26}H_{23}ClN_4O_4$	490	230-232	0.5	45
8l	Br	3,4,5Trimethoxy-phenyl	$C_{26}H_{23}BrN_4O_4$	535	190-192	0.8	50
8m	F	3,4,5Trimethoxy phenyl	$C_{26}H_{23}FN_4O_4$	474	201-202	0.6	65
8n	H	2,4 dihydroxyl phenyl	$C_{23}H_{18}N_4O_3$	398	165-167	0.4	81
8o	Cl	2,4 di hydroxyl phenyl	$C_{23}H_{17}ClN_4O_3$	381	157-159	0.5	35
8p	F	2,4 di hydroxyl phenyl	$C_{23}H_{17}FN_4O_3$	416	148-150	0.3	48

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

4.0.Characterization of compounds:

4.1.1. N'-ethylidene-4-(quinolin-4-ylamino)benzohydrazide (8a)

Compound **8a** obtained as white solid (yield 60%), m. p.168-170°C. ^{1}H NMR (400MHz DMSO, δ ppm): 8.15-8.19 (m, 3H, Ar-H), 7.88-7.90 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.76-7.78 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.48-7.50 (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), 4.351 (s, 1H, NH), 3.21(m, 1H, *J*= 4.0 Hz), 1.86 (d, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 162.15, 158.25, 156.15, 150.17, 148.10, 138.10, 135.20, 130.12, 129.08, 127.12, 126.24, 125.18, 124.01, 122.25, 116.52, 101.28, 38.04. MASS spectrum m/z: 305.18 $[M+H]^+$Calc. for $C_{18}H_{16}N_4O$; CHN: C, 71.04; H, 5.30; N, 18.41; Found: C, 71.09; H, 5.35; N, 18.48; IR (KBr, cm^{-1}): 3084.33 (C-H, Aromatic), 2948.12 (C-H, Aliphatic), 1706.10 (C=O**),** 1593.15 (C=C, Aromatic), 1180.14 (C-O).

4.1.2. (E)-4-((7-chloroquinolin-4-yl)amino)-N'-ethylidenebenzohydrazide (8b)

Compound 8b obtained as cream solid (yield 75%), m. p.150-152°C. ^{1}H NMR (400MHz DMSO, δ ppm): 8.02-8.06 (d, 2H, Ar-H), 7.86-7.88 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.62-7.64 (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 (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): 160.11, 159.20, 157.10, 154.12, 146.18, 139.10, 135.10, 130.12, 129.08, 128.10, 127.20, 125.18, 124.01, 122.25, 116.52, 101.28, 58.20, 34.01. MASS spectrum m/z: 338.18 $[M]^+$, 340.20 $[M+2]^+$. Calc. for $C_{18}H_{15}ClN_4O$: C, 71.04; H, 5.30; N, 18.41; Found: C, 71.09; H, 5.35; N, 18.48; IR (KBr, cm^{-1}): 3084.33 (C-H, Aromatic), 2948.12 (C-H, Aliphatic), 1706.10 (C=O**),** 1593.15 (C=C, Aromatic), 1180.14 (C-O).

4.1.3. (E)-4-((7-bromoquinolin-4-yl)amino)-N'-ethylidenebenzohydrazide (8c):

Compound 8c obtained as brownish solid (yield 55%), m.p.128-130°C. ^{1}H NMR (400MHz DMSO, δ ppm): 8.45-8.51 (m, 4H, Ar-H), 7.92-7.94 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.80-7.82 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.53-7.58 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.39-7.42 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.30-7.32 (d, 1H, Ar-H), 7.20-7.24 (m, 1H, Ar-H), 4.29 (s, 1H, NH), 3.09 (s, 1H, *J*= 4.0 Hz), 1.32 (d, 2H, CH_3). ^{13}C NMR (100MHz, DMSO): 160.11, 158.20, 157.10, 155.18, 145.10, 140.10, 138.10, 136.18, 130.08, 129.18, 128.27, 126.20, 124.18, 123.25, 116.52, 101.28, 62.01, 40.04. MASS spectrum m/z: 382.10 $[M]^+$, 384.15 $[M+2]^+$. Calc. for $C_{18}H_{15}BrN_4O$; CHN: C, 56.41; H, 3.95; N, 14.62; Found: 56.40; H, 3.90; N, 14.10; 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. (E)-N'-ethylidene-4-((7-fluoroquinolin-4-yl)amino)benzohydrazide (8d)

Compound 8d obtained as yellow solid (yield 55%), m.p.165-167°C. ^{1}H NMR (400MHz DMSO, δ ppm): 8.17-8.23 (m, 3H, Ar-H), 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, 3H, Ar-H), 4.315 (s, 1H, NH), 3.18 (s, 1H), 1.66 (s, 2H, CH_3). ^{13}C NMR (100MHz, DMSO): 162.11, 160.20, 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: 322.10 $[M]^+$, 324.20 $[M+2]^+$. Calc. for $C_{18}H_{15}FN_4O$: C, 67.07; H, 4.69; F, 5.89; N, 17.38; Found: C, 71.09; H, 5.35; N, 18.48; IR (KBr, cm^{-1}): 3081.13 (C-H, Aromatic), 2950.12 (C-H, Aliphatic), 1716.10 (C=O**),** 1590.15 (C=C, Aromatic), 1189.18 (C-O).

4.1.5. (E)-N'-ethylidene-4-((7-methylquinolin-4-yl)amino)benzohydrazide (8e)

Compound 8e obtained as cream solid (yield 70%), m. p.190-192°C. ^{1}H NMR (400MHz DMSO, δ ppm): 8.28-8.32 (m, 3H, Ar-H), 7.89-7.91 (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 (s, 1H), 1.80 (s, 3H, 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: 319.18 $[M+H]^+$. Calc. for $C_{19}H_{18}N_4O$; CHN: C, 71.68; H, 5.70; N, 17.60; Found: C, 71.09; H, 5.35; N, 17.18; 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. (E)-N'-butylidene-4-((7-chloroquinolin-4-yl)amino)benzohydrazide (8f)

Compound 8f obtained as white solid (yield 52%), m. p.198-200°C. ^1H NMR (400MHz DMSO, δ ppm): 8.23-8.29 (m, 3H, Ar-H), 7.82-7.89 (d, 2H, *J*= 8.0 Hz, Ar-H), 7.60-7.64 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.48-7.51 (t, 1H, *J*= 4.0 Hz, Ar-H), 7.33-7.36 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.32-7.34 (d, 2H, Ar-H), 7.20-7.24 (m, 2H, Ar-H), 4.358 (s, 1H, NH), 3.14 (s, 1H, *J*= 4.0 Hz), 1.66 (s, 2H). ^{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: 366.18 $[M]^+$, 368.20 $[M+2]^+$. Calc. for $C_{18}H_{15}ClN_4O$: C, 71.04; H, 5.30; N, 18.41; Found: C, 71.09; H, 5.35; N, 18.48; IR (KBr, cm^{-1}): 3084.33 (C-H, Aromatic), 2948.12 (C-H, Aliphatic), 1706.10 (C=O**),** 1593.15 (C=C, Aromatic), 1180.14 (C-O).

4.1.7. (E)-4-((7-bromoquinolin-4-yl)amino)-N'-butylidenebenzohydrazide (8g)

Compound 8g obtained as white solid (yield 50%), m.p.138-140°C. ^1H NMR (400MHz DMSO, δ ppm): 8.07-8.15 (m, 3H, Ar-H), 7.90-7.92 (m, 2H, *J*= 8.0 Hz, Ar-H), 7.80-7.82 (d, 1H, *J*= 4.0 Hz, Ar-H), 7.50-7.53 (m, 2H, *J*= 4.0 Hz, Ar-H), 7.30-7.32 (d, 1H, Ar-H), 7.20-7.24 (m, 1H, Ar-H), 4.31 (s, 1H, NH), 3.10-3.12 (m, 1H, *J*= 4.0 Hz), 1.38-1.40 (d, 2H, CH_3). ^{13}C NMR (100MHz, DMSO): 162.15, 159.15, 158.18, 156.10, 145.10, 140.10, 138.10, 136.18, 130.08, 129.18, 128.27, 126.20, 124.18, 123.25, 115.12, 100.20, 60.01, 42.14. MASS spectrum m/z: 410.10 $[M]^+$, 412.10 $[M+2]^+$. Calc. for $C_{20}H_{19}BrN_4O$; CHN: C, 58.40; H, 4.66; N, 13.62; Found: C, 58.45; H, 4.61; N, 13.60; 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. (E)-N'-butylidene-4-((7-fluoroquinolin-4-yl)amino)benzohydrazide (8h)

Compound 8h obtained as yellow solid (yield 50%), m.p.160-162°C. ^1H NMR (400MHz DMSO, δ ppm): 8.21-8.24 (m, 4H, Ar-H),, 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), 4.34 (s, 1H, NH), 3.18-3.24 (m, 1H), 1.34-1.36 (d, 3H, CH_3). ^{13}C NMR (100MHz, DMSO): 163.08, 162.12, 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: 350.15 $[M]^+$, 352.12 $[M+2]^+$. Calc. for $C_{20}H_{19}FN_4O$: C, 68.56; H, 5.47; N, 15.99; Found: C, 68.50; H, 5.41; N, 15.90; 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. (E)-N'-butylidene-4-((7-methylquinolin-4-yl)amino)benzohydrazide (8i)

Compound 8i obtained as cream solid (yield 71%), m. p.202-204°C. ^1H NMR (400MHz DMSO, δ ppm): 8.28-8.32 (d, J=8.6 Hz, 2H, Ar-H),7.82-7.83 (m, 4H, 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 (d, 2H, *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 (s, 1H), 1.80-1.82 (s, 1H). ^{13}C NMR (100MHz, DMSO): 161.25, 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.MASS spectrum m/z: 347.10 $[M+H]^+$. Calc. for $C_{21}H_{22}N_4O$; CHN: C, 72.81; H, 6.40; N, 16.17;; Found: C, 72.80; H,

6.45; N, 16.27; 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. (E)-4-(quinolin-4-ylamino)-N'-(3,4,5-trimethoxybenzylidene)benzohydrazide (8j)

Compound 8j obtained as white solid (yield 48%), m. p. 172-174 °C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 8.21-8.23 (d, J=8.6 Hz, 2H, Ar-H), 7.867-7.891(d, J=9.6 Hz, 2H, Ar-H), 7.526-7.563 (t, J=8.8Hz, 2H, Ar-H), 7.462-7.499 (d, J=14.8Hz, 2H, Ar-H), 7.373-7.395 (d, J=8.0 Hz, 2H, Ar-H), 6.378 (s, 2H, Ar-H), 4.351 (s, 1H, NH), 3.797(s, 3H, OCH_3), 3.750 (s, 6H,OCH_3). ^{13}C NMR (100MHz, $CDCl_3$): 160.58, 158.38, 156.05, 148.65, 135.65, 130.25, 129.85, 129.32, 128.16, 126.32, 125.45, 123.12, 117.12, 102.16, 48.12. MASS spectrum m/z: 457.15$[M+H]^+$. Calc. for$C_{26}H_{23}ClN_4O_4$; CHN: C, 68.41; H, 5.30; N, 12.27 ; Found C, 68.41; H, 5.30; N, 12.27. IR (KBr, cm^{-1}): 3086.15 (C-H, Aromatic), 2922.83 (C-H, Aliphatic), 1722.81(C=O), 1549.75 (C=C, Aromatic), 1269.79 (C-O).

4.2.1.(E)-4-((7-chloroquinolin-4-yl)-amino)-N'-(3,4,5-trimethoxybenzylidene) benzohydrazide (8k): Compound 8k obtained as yellow solid (yield 45%), m. p. 230-232 °C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 8.11-8.18 (m, 3H, Ar-H), 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), 4.321 (s, 1H), 3.797(s, 3H, OCH_3), 3.750 (s, 6H,OCH_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, 40.12. MASS spectrum m/z: 490.11$[M]^+$, 492.05 $[M+2]^+$. Calc. for $C_{26}H_{23}ClN_4O_4$; CHN: C, 63.61; H, 4.72; N, 11.41; Found C, 63.51; H, 4.70; N, 11.45. 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 (E)-4-((7-bromoquinolin-4-yl)amino)-N'-(3,4,5-trimethoxybenzylidene) benzohydrazide (8l): Compound 8lobtained as yellow solid (yield 50%), m. p.190-192°C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 8.235-8.321 (d, 2H, Ar-H), 7.865-7.919 (m, 2H, Ar-H), 7.473-7.555 (m, 2H, Ar-H), 7.316-7.428 (m, 2H, Ar-H), 4.351 (s, 1H), 3.803(s, 6H, OCH_3), 3.757 (s, 3H, OCH_3) 1.258 (s, 1H, Aliphatic); ^{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: 535$[M]^+$, 537 $[M+2]^+$Calc. for $C_{26}H_{23}BrN_4O_4$; CHN: C, 58.33; H, 4.33; N, 10.46; Found C, 58.33; H, 4.33; N, 10.46; 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.(E)-4-((7-fluoroquinolin-4-yl)amino)-N'-(3,4,5-trimethoxybenzylidene)benzohydrazide (8m): Compound 8m obtained as white solid (yield 65%), m. p. 201-202°C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 7.864-7.917 (m, 3H, Ar-H), 7.531-7.568 (t, J=8.8Hz, 2H, Ar-H), 7.469-7.506 (t, J=8.8 Hz, 2H, Ar-H),7.375-7.397 (d, J=8.8Hz, 1H, Ar-H), 6.377 (s, 2H, Ar-H), 5.439 (s, 1H, NH), 3.801(s, 3H, OCH_3), 3.755 (s, 6H,OCH_3), 1.067(s, 1H, aliphatic). ^{13}C NMR (100MHz, $CDCl_3$): 168.65, 161.15, 142.08, 132.54, 131.08, 129.35, 129.09, 128.56, 128.14, 126.88, 125.27, 124.48, 114.02, 42.48.MASS spectrum m/z: 474.11$[M]^+$, 475.05 $[M+2]^+$. Calc. for $C_{26}H_{23}FN_4O_4$; C, 65.81; H, 4.89; N, 11.81; Found C, 65.80; H, 4.81; N, 11.85. IR (KBr, cm^{-1}):3438.01 (NH), 3085.98 (C-H, Aromatic), 2974.62(C-H, Aliphatic), 1618.70(C=O), 1585.10 (C=C, Aromatic), 981.35 (C-Cl).

4.2.4. (E)-N'-(2,6-dihydroxybenzylidene)-4-(quinolin-4-ylamino)benzohydrazide (8n)

Compound 8nobtained as cream white solid (yield 81%), m. p. 165-167°C. ^{1}H NMR (400MHz $CDCl_3$, δ ppm): 8.26-8.28 (d, 2H, J= 8.0 Hz, Ar-H), 7.90-7.92 (d, 2H, J= 8.0 Hz,Ar-H), 7.70-7.72(t, 1H, J= 8.0 Hz, Ar-H), 7.40-7.43 (m, 3H, J= 4.0 Hz,Ar-H), 7.29-7.31 (d, 1H, J= 4.0

Hz,Ar-H), 7.26-7.28 (3, 3H, J= 8.0 Hz,Ar-H), 5.35 (s, 1H, NH), 3.21 (s, 1H, J= 4.0 Hz), 2.72 (s, 1H, OH), 1.74 (S, 1H, OH). ^{13}C NMR (100MHz, $CDCl_3$):196.2, 170.4, 168.1, 158.2, 145.2, 138.2, 129.5, 129.3, 128.2, 128.1, 128.0, 126.8, 126.4, 123.8, 123.2, 123.1, 122.8, 118.6, 109.4, 41.23; MASS spectrum m/z: 399$[M+H]^+$Calc. for $C_{23}H_{18}N_4O_3$; CHN: C, 76.22; H, 5.45; N, 3.29; O, 15.04; Found C, 76.20; H, 5.40; N, 3.32; O, 15.10. IR (KBr, cm^{-1}): 3416.21 (NH), 3068.52(C-H, Aromatic), 2980.20 (C-H, Aliphatic), 1716.34(C=O**),** 1535.12(C=C, Aromatic), 1135.18(C-O).

4.2.5. (E)-4-((7-chloroquinolin-4-yl)amino)-N'-(2,6-dihydroxybenzylidene)benzohydrazide (8o) Compound **8o**obtained as orange solid (yield 35%), m. p. 157-159°C.^{1}H NMR (400MHz DMSO, δ ppm): 8.835-8.843 (d, 2H, J= 8.0 Hz, Ar-H), 8.431-8.524 (m, 3H, J= 8.0 Hz,Ar-H), 7.92-7.94 (d, 2H, J= 4.2 Hz, Ar-H), 7.48-7.51 (t, 2H, J= 4.0 Hz, Ar-H), 7.30-7.33 (d, 1H, J= 4.0 Hz, Ar-H), 7.27-7.29 (m, 3H, J= 8.0 Hz, Ar-H), 4.96 (s, 1H, NH), 4.546 (s, 1H), 3.541(s, 1H), 1.745 (s, 1H,aliphatic). ^{13}C NMR (100MHz, DMSO): 168.6, 163.3, 158.8, 149.2, 136.5, 129.3, 128.0, 126.8, 126.4, 123.8, 123.2, 123.1, 122.8, 118.6, 109.4, 38.41. MASS spectrum m/z: 434.28$[M+2]^+$Calc. for $C_{23}H_{17}ClN_4O_3$; CHN: C, 75.90; H, 5.14; N, 3.40; O, 15.55; Found C, 75.84; H, 5.18; N, 3.45; O, 15.60.IR (KBr, cm^{-1}): 3451.21 (NH), 3082.10 (C-H, Aromatic), 2975.15 (C-H, Aliphatic), 1720.12(C=O**)**, 1541.20 (C=C, Aromatic),654 (C-Cl).

4.2.6. (E)-N'-(2,6-dihydroxybenzylidene)-4-((7-fluoroquinolin-4-yl)amino)benzohydrazide (8p) Compound **8p** obtained as white solid (yield 48%), m. p. 148-150°C.^{1}H NMR (400MHz DMSO, δ ppm): 8.821-8.843 (d, 2H, J= 8.0 Hz, Ar-H), 8.228-8.290 (m, 3H, Ar-H), 7.808-7.846 (t, 2H, J= 8.0 Hz, Ar-H), 7.697-7.737 (t, 2H, J= 8.2 Hz, Ar-H), 7.550-7.582 (m, 2H, Ar-H), 7.430-7.455 (d, 2H, Ar-H), 4.829 (s, 1H, NH), 4.082 (s, 1H, OH), 3.505(s, 1H, OH), 1.278(s, 1H, aliphatic). ^{13}C NMR (100MHz, DMSO): 165.53, 158.96, 152.72, 143.90, 137.00, 135.53, 134.92, 131.24, 128.89, 126.73, 124.41, 120.92, 119.82, 117.79, 52.7, 42.48. MASS spectrum peak m/z: 418 $[M+2]^+$ Calc. for $C_{23}H_{17}FN_4O_3$; CHN: C, 75.55; H, 4.82; N, 3.52; O, 16.10; Found: C, 75.58; H, 4.86; N, 3.50; O, 16.08.IR (KBr, cm^{-1}): 3332.71 (NH), 3053.61 (C-H, Aromatic), 2968.88(C-H, Aliphatic), 1615.87(C=O), 1538.75 (C=C, Aromatic), 1270.19 (C-O).

5.0. Molecular Docking[15]:

Schrödinger software (Schrödinger, Version 2019-1) was installed on an Intel Xenon W 3565 processor running Ubuntu enterprise (version 14.04) in order to do molecular docking studies of all the produced chemicals. ChemDraw 18.0 was used to draw the ligands. With the aid of Schrödinger's (2019-1) XP Visualizer. The outcomes were studied.

Schrodinger software (Glide module) (Schrodinger, Version 2019-1). The ligands that were used as docking inputs were drawn using ChemDraw. The OPLS3e force field in Ligprep (Version 2019-1, Schrodinger) was used to create ligands. Hydrogens have been added to the ligands, and docking studies were employed to help assign bond placing an order. For docking investigations, the output file that was generated and contained the optimal ligand conformations was utilized. The protein preparation wizard (Dizdaroglu et al. 2020) (Version 2019-1, Schrodinger) was utilized to prepare the protein. After hydrogen atoms were added, the protein was given charges. Het states were produced at pH 7.2 utilizing epik. The pre-processed, refined protein had been modified by workspace analysis. Non-significant atoms have been extracted out of the crystal structure. Finally, OPLS3e force filed was used to improve the protein. In the immediate vicinity of the cocrystal ligand—the ligand's X-ray position within the protein—a receptor grid was produced. The centroid of the ligand was chosen to create the grid box, and the receptor atoms' Vander Waal radius was adjusted to 1.00 Å with a partial atomic charge of 0.25. Using the Glide docking score, the output was processed to identify the best-

docked structure. Poses of the ligands' generated output during docking were evaluated with the aid of XP Visualizer (Schrodinger, Version 2019-1), have been listed in **Tables 2 and Figures 1 and 2** present results.

6.0. Results and discussion

6.1. Chemistry:

N′-substituted methylene-4-(quinoline-4-amino)benzoylhydrazides**(8a-p)**were obtainedby the reaction of 7- Substituted 4-Hydroxyquinoline **(1a-i)** was firstly halogenated to form 7-Substituted 4-chloroquinoline **(3a-i)** using phosphorus oxychloride (**2)**. Ethyl 4-(quinolin-4-ylamino)benzoate**(5a-i)** was then prepared from benzocaine **(4)** by N-alkylation using 4-chloroquinoline **(3a-i)** in the presence of hydrochloric acid as a catalyst in refluxing butyl alcohol. Compound **(5a-i)** was treated with hydrazine hydrate**(6)** in refluxing ethanol, followed by condensation with different substituted aldehydes **(7a-c)** to give the corresponding target compounds **(8a-p)**. All the new target compounds were characterized by ^{1}H NMR, ^{13}C NMR, and electrospray ionization mass spectrometry (ESI-MS).

The FTIR spectra of intermediates **3a-i** showed absorption bands between 3400-3300 cm^{-1} due to the asymmetric and symmetric stretching vibrations of primary amine group and also ^{1}H NMR spectrum of the same compounds showed a broad peak at δ ~8.0-8.10 which indicates the presence of amide. FTIR spectra of compounds **6a-c**, **7a-c** and **8a-c** showed carbonyl stretching vibration around 1700 cm^{-1}, whereas band for secondary amine was located at ~3200 cm^{-1}. The secondary amidic NH resonance of **6a-c**. FTIR spectrum of all final compounds **8a-p** indicates the presence of amidic carbonyl group showing a band at 1700-1600 cm^{-1} and also the absorption band of secondary NH of amide was found at ~3200 cm^{-1}. ^{1}H NMR spectrum of all the compounds showed resonance for NH proton at around δ value 8.01-8.09 ppm and all the alkenyl protons in the range of 4.01-4.08 and aromatic protons in the range of 7.0-8.0 ppm. The ESI mass spectrum of all the compounds showed peaks at relevant M+H m/z and complemented the FTIR &^{1}H NMR spectra for the confirmation of expected structures of all the compounds. The Physical data of the final compounds has been listed in **Table 1**.

6.2. Results and discussions:

Investigation performed in vitro on synthetic compounds showed that they exhibited anti-cancer potential. Out of all the compounds tested, compound 8m demonstrated the most promising results. In order to gain insight into the binding manner of synthetic compounds within the binding pockets of SMMC-7721, MDA-MB-231, and HeLa, these results prompted us to carry out docking investigations. The Schrodinger package's LigPrep was used to further prepare the ligand structures once they were all developed with maestro. Protein structures have been obtained from the Protein Data Bank (PDB ID: MDA-MB-231-6NZ8 and HeLa -6I2I, SMMC-7721-7LBM), and the Protein Preparation Wizard in the Schrodinger software was used to make the necessary corrections to the protein structure. Using the Glide docking software, docking investigations were carried out. The cocrystal ligand was docked to validate the docking methodology, and the resulting RMSD of the docked conformation and cocrystal ligand posture were found to be 0.6. have been listed in **Table 2, Figures 1 and 2**, and HeLa -6I2I, SMMC-7721-7LBM include a list of the binding interactions of drugs with these proteins.Docking study was performed on binding poses of synthesized compounds with SMMC-7721have shown that these molecules bind well within binding pocket of enzyme. Among the all synthesized molecules, compound with potent anti-cancer activity **8m,** has shown the highest binding score(-**8.270**and-**8.326** and-**6.252** against MDA-MB-231 andSMMC-7721 and HeLa). In superimposed

pose of **8m,** with cocrystal ligand, aldehyde and quinoline ring was coinciding with skeleton of cocrystal ligand as depicted in **Figure-2.**

Table 2: Binding Energies (Kcal/mol), No. of HBs and Binding Sites

S.No	Compound	Docking score of MDA-MB-231 (6NZ8)	Docking score ofSMMC-7721 (7LBM)	Docking score ofHELA (6I2I)
1	8a	9.971	9.321	8.782
2	8b	7.707	7.214	6.723
3	8c	5.057	4.125	3.415
4	8d	-5.146	-4.325	-3.714
5	8e	-6.861	-6.784	-5.395
6	8f	7.401	5.654	-6.711
7	8g	-5.381	-4.124	-3.484
8	8h	-4.712	-5.145	-3.489
9	8i	-3.942	-4.578	-3.526
10	8j	-7.386	-4.862	-5.475
11	**8k**	**-7.386**	**-6.895**	**-5.241**
12	8l	-5.534	-4.451	-5.224
13	**8m**	**-8.270**	**-8.326**	**-6.252**
14	8n	-4.352	-5.321	-4.721
15	8o	4.401	4.321	-4.711
16	8p	-4.122	4.321	-4.321
16	Cocrystal Ligand	-5.787	-5.255	-9.068

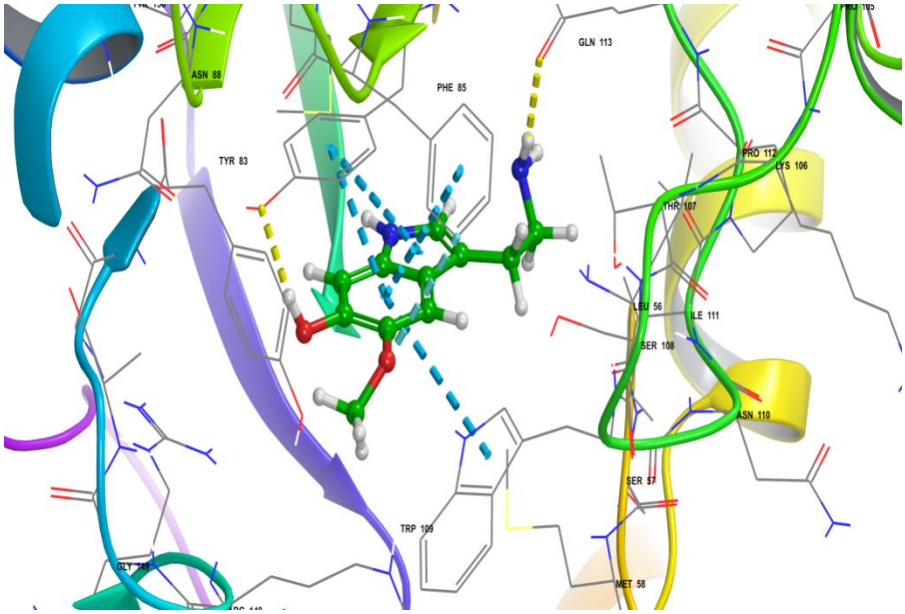


Figure-1: Binding poses and interactions of compound **8m** to the binding sites of target protein SMMC-7721 receptor (7LBM)

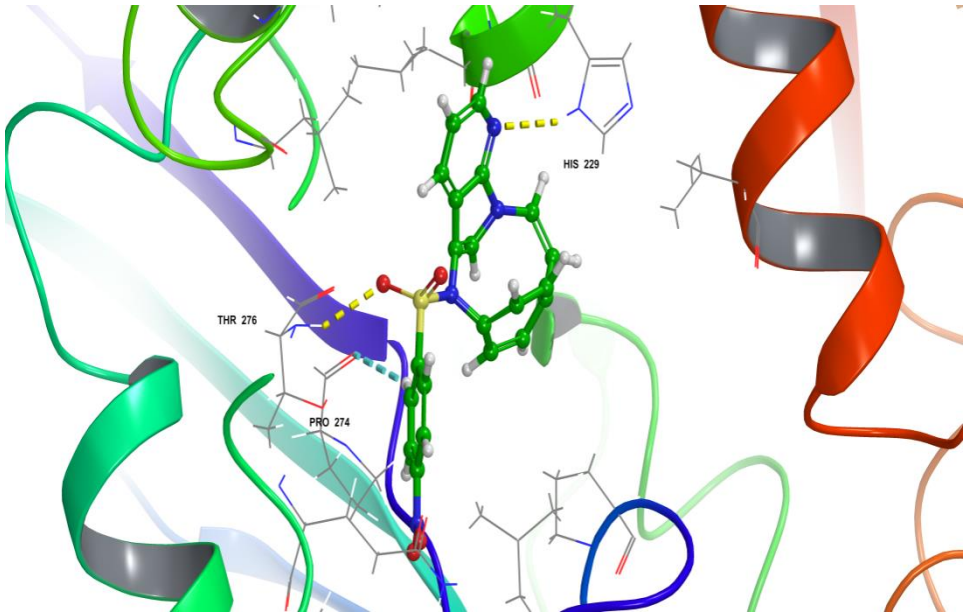


Figure-2: Binding poses and interactions of compound **8m** to the binding sites of target protein **MDA-MB-231** receptor (3HY3)

7.0. Anti cancer activity:

7.1.1. Biological assay

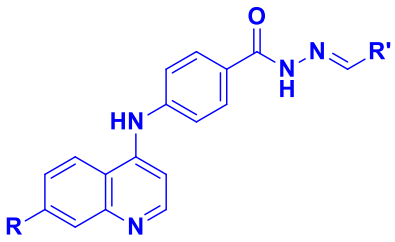
Cultures and cell lines Human breast cancer cell line (MDA-MB-231), cervical carcinoma cell line (HeLa), the liver cancer cell line (SMMC-7721) and normal hepatocyte cell line (QSG-7701) were maintained in Dulbecco Modified Eagle Medium (DMEM) containing 4.0 mM L-

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% CO_2 and 95% air.

7.1.2. MTT assay[16]

New quinoline derivatives was evaluated by using (3-4, 5- dimethylthiazol-2-yl)-2, 5–diphenyltetrazolium bromide (MTT) assay. The MTT test works by converting tetrazolium soluble salt into formazan crystals using live cells' mitochondrial NAD and NADH-dehydrogenases. Human breast cancer cell lines MDA-MB-231, HeLa, and SMMC-7721 cell lines and primary mammary epithelial control cells MEpiC were plated in a 96-well plate at a density of 5×103 cells per well. Cells were treated with the synthesized compounds (8a-o) at a range of concentrations from 0.1, 0.3, 1, 3, 10, 30, 100 µM for 48 h at 37 ˚C in a humidified CO_2 incubator followed by removal of the supernatant medium and addition of MTT solution (0.5 mg/mL). MTT-added cells were incubated for 4 hours at 37 ˚C in a humidified circumstance. The medium is then removed and allowed to dry at room temperature in the dark. The generated formazan crystals were then dissolved with DMSO, and the end product was measured using a microplate spectrophotometer. (Perkin Elmer Enspire, Germany) .Cisplatin , was used as positive control for all the experiments. The cytotoxicity was expressed as the concentration of sample that inhibited 50% of cell growth (IC_{50}).

Table-3: anti-cancer activity of New substituted 4-(quinolin-4-ylamino)benzohydrazide derivatives



General structure-I (8a-p)

Com.	R	R’	M. Form	MDA-MB-231 IC_{50} (µM)	HELa IC_{50} (µM)	SMMC-7721 IC_{50} (µM)
8a	H	CH_3	$C_{18}H_{16}N_4O$	6.20±0.02	5.27±0.02	7.62±0.02
8b	Cl	CH_3	$C_{18}H_{15}ClN_4O$	3.12±0.04	2.02±0.08	5.95±0.03
8c	Br	CH_3	$C_{18}H_{15}BrN_4O$	3.58±0.02	3.78±0.04	6.26±0.03
8d	F	CH_3	$C_{18}H_{15}FN_4O$	3.05±0.02	3.62±0.02	6.45±0.03
8e	CH_3	CH_3	$C_{19}H_{18}N_4O$	4.80±0.02	5.35±0.03	6.38±0.03
8f	Cl	CH_3-CH_2CH_2-	$C_{18}H_{15}ClN_4O$	2.36±0.03	2.26±0.03	5.62±0.02
8g	Br	CH_3-CH_2CH_2-	$C_{20}H_{19}BrN_4O$	3.35±0.02	3.45±0.03	4.35±0.03

8h	F	CH_3-CH_2CH_2-	$C_{20}H_{19}FN_4O$	2.08±0.03	2.38±0.03	5.26±0.03
8i	CH_3	CH_3-CH_2CH_2-	$C_{21}H_{22}N_4O$	2.90±0.02	7.64±0.02	3.45±0.03
8j	H	3,4,5Trimethoxyphenyl	$C_{26}H_{24}N_4O_4$	1.50±0.08	5.64±0.02	2.27±0.12
8k	Cl	3,4,5Trimethoxy-phenyl	$C_{26}H_{23}ClN_4O_4$	**0.72±0.04**	**1.39±0.02**	**2.02±0.28**
8l	Br	3,4,5Trimethoxy-phenyl	$C_{26}H_{23}BrN_4O_4$	1.35±0.03	3.24±0.03	2.58±0.24
8m	F	3,4,5Trimethoxy phenyl	$C_{26}H_{23}FN_4O_4$	**0.78±0.08**	**1.31±0.02**	**2.62±0.12**
8n	H	2,4 di hydroxyphenyl	$C_{23}H_{18}N_4O_3$	2.92±0.05	3.01±0.02	4.35±0.23
8o	Cl	2,4 di hydroxyl phenyl	$C_{23}H_{17}ClN_4O_3$	1.12±0.05	2.95±0.03	3.26±0.13
8p	F	2,4 di hydroxyl phenyl	$C_{23}H_{17}FN_4O_3$	1.68±0.02	1.65±0.02	3.27±0.12
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 Results& discussions:

The in vitro cytotoxic activity of derivatives **8a-p** 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 used as the standard. All tested compounds were dissolved in DMSO and the stock solutions were diluted by DMEM medium before evaluation of the cultured cells. The IC_{50} values of the tested compounds against four cell lines are shown in Table 3. 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 SMMC-7721 cells. Concerning different derivatives, it was found that compounds **8a-p** with various aldehydes exhibited potent cytotoxic activities against MDA-MB-231 and HeLa cells at low lM levels and moderate activities against SMMC-7721 cells. Compounds 8k and 8m showed strong cytotoxicity against MDA-MB-231 and HeLa cells, respectively. Compounds 8j and 8l displayed moderate activities against MDA-MB-231 and HeLa cells, while compounds 8a, 8b, 8c were showed mild or no cytotoxicity against three cancer cell lines. It is worth noting that compound 8k exhibited the most potent anticancer activity against all three cancer cells at low lM toµM range (IC_{50}: 0.78±0.08, 1.31±0.02 and 2.62±0.12 µM, respectively. In addition, compound 8a was less cytotoxic to (MDA-MB-231), cervical carcinoma cell line (HeLa), hepatocarcinoma cell line (SMMC-7721) with IC_{50} value of 6.20±0.02, 5.27±0.02 and 7.62±0.02µM, respectively. Hence, compound 8k was selected for further investigations on its anticancer mechanisms.

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