"STRUCTURE BASED DRUG DISCOVERY, DOCKING, MODELLING, SYNTHESIS AND ANTICANCER SCREENING OF SOME NOVEL QUINOLINE DERIVATIVES"

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ABSTRACT

A new series of (2-(substituted-phenyl) quinoline-4-yl) (3-(substituted phenyl)-5-phenyl-1H-pyrazol-1-yl) methanone derivatives was carried out docking modelling and synthesized. Purity was checked by TLC and chemical structures of synthesized compounds were elucidated by their IR, ^{1}HNMR, MS analysis data. The synthesized compounds were screened for anticancer activity by using cell line MCF-7 (Human breast cancer cell line) correlate with docking modelling.

Key words: IR, MS, ^{1}HNMR, Auto Dock Vina softwere, Docking, Modelling, Quinoline, MCF-7, Anticancer,

INTRODUCTION

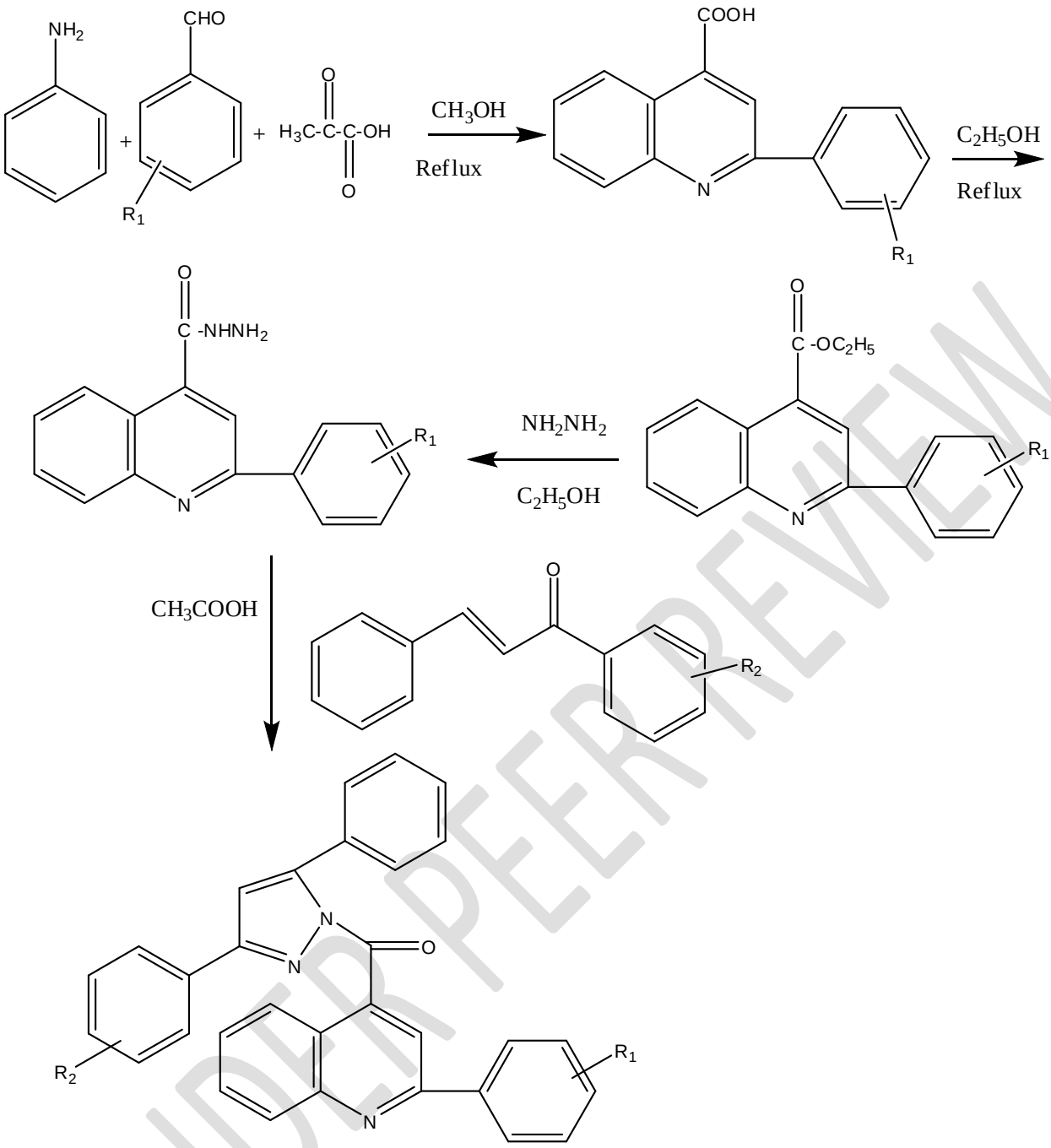
Cancer is an abnormal uncontrollable cell cycle disease characterized by the rapid proliferation of normal cells. Cancer has been ranked as the second leading cause of death all over the world, preceded only by cardiovascular diseases.[1,2] Chalcone moieties and quinoline play an important role in medicinal chemistry, especially in the identification and development of potential anticancer agents. The multi target approach or hybridization is considered as a promising strategy in drug design and discovery. Hybridization may improve the affinity and potency while simultaneously decreasing the resistance and or side effects. The conjugation of quinolines with chalcones has been a promising approach to the identification of potential anticancer agents. In this

article, the quinolone chalcone hybrids with potential anticancer activity have been reviewed. This class of compounds might be helpful for the design, discovery and development of new and potential multi-target anticancer agentsor drugs.[3]The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines or primary patient cells. In this chapter the protocol of the assay is described including important considerations relevant for each step of the assay as well as its limitations and possible applications.[4]In medicinal chemistry quinoline and pyrazole derivatives have been very well known for their therapeutic applications. The development of a new synthetic methodology forsynthesis of compounds containing quinoline and pyrazole continues to be an active and exciting area of research in pharmaceutical chemistry. Quinolinecompounds play an important role in designing new classes of structural entities of medicinal importance with potentially new mechanisms of action. Quinoline is nitrogen containing heterocyclic compound[5].The biological activity of these quinoline derivatives depends not only on the bicyclic hetero-aromatic pharmacophore but, also on the nature of the peripheral substituent and their spatial relationship.Various quinoline compounds can be prepared by *Skraup* synthesis using series of different oxidizing agents[6]. The pyrazole function is quite stable and has inspired chemist to utilize this stable fragment in bioactive moieties to synthesize new compounds possessing biological activity[6,7].

EXPERIMENTAL

In this research work, the melting points of the synthesized organic compounds were determined by open capillary tube and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel G as stationary phase and a combination of benzene: chloroform as mobile phase. The IR spectra of intermediate as well as final derivatives were recorded on Fourier Transform Infrared Spectrophotometer on JASCO FTIR 4100 spectrophotometer by using KBR powder. The (^{1}H NMR) spectra of the representative compounds were recorded at on Varian NMR 300 MHz spectrophotometer using TMS as an internal standard and chloroform as a solvent.

Image 1: Scheme of synthesis



(1a)Synthesis of 2-Phenylquinolin- 4 carboxylic acid :

The mixture of substituted aromatic aldehydes (0.01mole), aniline (0,01mole) and pyruvic acid (0.01 mole) in methanol (100ml) was refluxed in a 250 ml of round bottom flask for 1-3 hours. Reaction was monitored by TLC using ethyl acetate: acetone (2:1) as solvent and iodine vapours as visualising agent, after completion of reaction 50 ml of warm water was added and solution was allowed to cool. The precipitated solid was filtered washed with aqueous methanol (10ml) and recrystallized from methanol[5,6].

(1b)Synthesis of 2-Phenylquinolin-4-carboxylate (A):

2-Phenylquinolin- 4 carboxylic acid added ethanol for was refluxed in a 250 ml of round bottom flask for 1-2 hours Reaction was monitored by TLC using ethyl acetate: acetone (2:1) as solvent and iodine vapours as visualising agent, after completion of reaction the solid was filtered washed with aqueous methanol (10ml) and recrystallized from methanol,dried

(2)Synthesis of 2-Phenylquinolin-4-carbohydrazide(B):

2-Phenylquinolin- 4 carboxylateand hydrazine hydrate in 1:1 portion was mixed in methanol (30ml) and refluxed for 4-6 hours. Reaction was monitored by TLC using ethyl acetate: acetone (2:1) as solvent. The excess of methanol was removed by distillation on cooling the product, the acid hydrazide separated out; it was filtered, dried and recrystallized from methanol, dried.

(3) Synthesis of chalcones or 1-phenyl-3 substituted phenyl propene 1-one (c): A solution of 10% NaOH and rectified spirit was taken in Erlenmeyer flask provided with mechanical stirrer. The flask was immersed in a bath of crushed ice, acetophenone (0.83ml, 0.43mol) was poured and stirring was started, substituted aromatic aldehydes (0.43mol) was then added. Temperature of the mixture was kept within 15 to 30 °C. Stirring was continued until the mixture becomes so thick that stirring is no longer effective and then reaction mixture was left in a refrigerator overnight. The product was filtered, washed with cold water until the washing are neutral to litmus and recrystallized from methanol. This substance should be handled with great care.

(4) Synthesis of (2-(substituted-phenyl) quinoline-4-yl) (3-(substituted phenyl)-5-phenyl-1H-pyrazol 1-yl) methanone derivatives(d):

A mixture of substituted chalcone (0.01mol) and 2-Phenylquinolin- 4 carbohydrazide (0.01mol) was refluxed in acetic acid (20 ml) for 8-16 hours. The reaction mixture was monitored by TLC using benzene: chloroform (2:1) as solvent. After completion of reaction, the mixture was cooled and obtained solid was filtered, recrystallized frommethanol,n-hexane,Chloroform,pet.ether.

DOCKING MODELLING:

The rational design of new chemical entities intended for use as drugs can be based on several methods. For the optimization of binding to the molecular target, structure based design has been very successful. However, a good drug has not only high and selective affinity for its target; it should also have appropriate pharmacokinetic and bio pharmaceutics properties[8]. The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of a protein is called molecular docking. Molecular docking is an efficient tool for investigating receptor-ligand interactions and for virtual screening which plays a key role in rational drug design, especially when the crystal structure of a receptor or enzyme is available. It is widely accepted that drug activity is obtained through the molecular binding of ligand to receptor which is commonly a protein. In their binding conformations, the molecules exhibit geometric and chemical complementarily, both of which are essential for successful drug activity[9]. The grid based docking is a rigid and exhaustive docking method. In this method, after unique conformers of the ligand are generated, the receptor cavity of interest is chosen by the user and a grid is generated around the cavity (default grid interval size 1 Å). Cavity points are found and the centre of mass of the ligand is moved to each cavity point. All rotations of ligand are

scanned at each cavity point where ligand is placed (step size of rotation could be typically 100-150 as an example). For each rotation a pose of the ligand is generated and the corresponding bumps are checked for each pose of ligand. The dock score is calculated for each valid pose (determined by the cut off criteria fed by user in terms of max no of allowed bumps) and the pose of the ligand with the best score is given as output to user[10]. Docking study of the title compounds was done on Auto dock Vina and then this enzyme structure was used further for docking purpose.

MTT Assay Experimental Procedure;
Cell line: MCF-7 (Human breast cancer cell line)

1. CO_2 Incubator- Thermo Fisher,USP
2. Multimode micro plate reader- Bene Sphera E21 Avantor USP
3. Refrigeraterated centrifuge- Eppendorf Germany
4. Cell : MCF-7 (Human breast cancer cell line) NCCS Pune.
5. MTT.(3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazoliumbromide)).
6. Fetabovineserum (Gibco,Invitrogen) (CatNo.-10270106)
7. Trypsin.
8. Penicillin.
9. DMEM With high glucose (CatNo.-11965-092).
10. Antibiotic- Antimycotic 100X solution (Thermo fisher scientific)- (CatNo.-1524006)

Cells were incubated at a concentration of $1x10^4$cells/ml in culture medium for 24h at 37°c and 5%CO_2. Cells were seeded at a concentration (70microml)10^4cells/well in 100µl culture medium and 100 µl sample of 1 to 10 (1000µg/ml)in to micro plates respectively (tissue culture grade and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.Cell cultures were incubated for 24h at 37°c and 55CO_2 in CO_2 incubator (Thermo scientific BB150). After incubation, the medium was completely removed and added 20 µl of MTT reagent (5mg/min PBS). After addition of MTT, cells incubated for 4hrs at 37°c in CO_2incubator.Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark coloured formazan by visible cells only. After removing the medium completely.Added 200 µl of DMSO (kept for 10min.) and incubate at 37°c (wrapped with aluminium foil). Triplicate samples were analyzed by measuring the absorbance of each sample by a microplatereader (Benesphera E21) at wavelength 550nm.

Result and discussion:

Table (1): Physical properties of synthesized compounds (4a-4v).

Sr. No.	Compound	R_1	R_2	Mol. Formula	Mol.Wt. (gm)	% yield	M.P. (^{0}C)
1	4a	Ar-2Cl	Ar	$C_{31}H_{20}N_3OCl$	485	65	151-156
2	4b	Ar	Ar-4OCH_3	$C_{32}H_{23}N_3O_2$	481	65	163-170
3	4c	Ar	Ar -2Br	$C_{31}H_{20}ON_3Br$	529	70	166-171
4	4d	Ar	Ar-4OH	$C_{31}H_{21}O2N_3$	467	68	164-170
5	4e	Ar-3NO_2	Ar-2Br	$C_{31}H_{19}N_4O_3Br$	575	70	150-155
6	4f	Ar-2Cl	Ar-5Cl	$C_{31}H_{19}N_3OCl_2$	519	69	153-158
7	4g	Ar-2Cl	Ar-4NH_2	$C_{31}H_{21}N_4OCl$	500	68	158-163
8	4h	Ar	Ar	$C_{31}H_{21}N_3O$	451	70	161-166

4a) IR(KBr) cm^{-1}: 3244(N-H); 3144,2975(Ar-C-H); 1730(C=O); 1644(Ar-C=C); 773(C-H-Ar).

4b) IR(KBr) cm^{-1}: 3338(N-H); 2924(Ar-C-H); 1623(N=C):1646(C=O); 1677(Ar-C=C); 1317(C-N):1233(Ar-O-C):

4c) IR(KBr) cm^{-1}: 3388(N-H); 3067(Ar-C-H); 1592(C=O); 924(C-H-Ar).

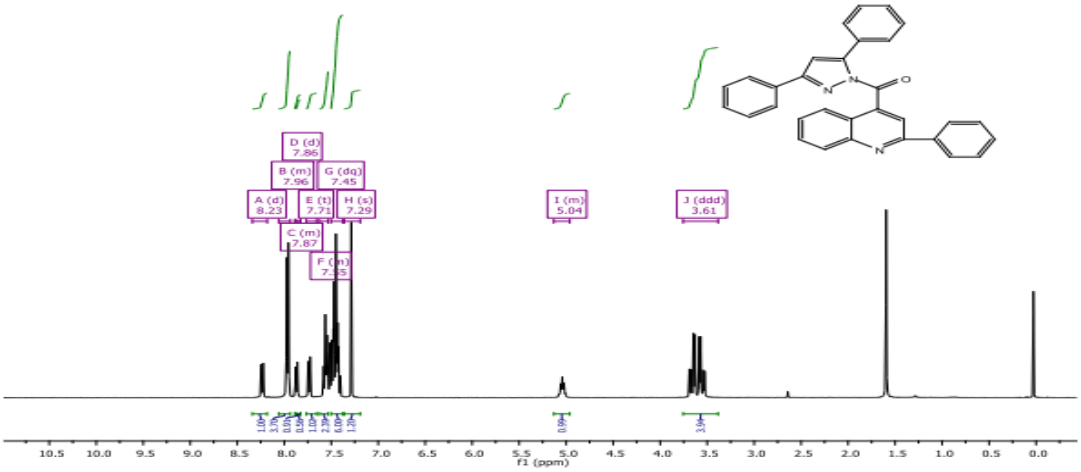
4d) IR(KBr) cm^{-1}: 3319(O-H); 2924(Ar-C-H); 1617.02(N=C)1641(C=O); 1671.02(Ar-C=C); 1113(C-N):754(C-H-Ar).

4e) IR(KBr) cm^{-1}: 3250(N-H); 3120,2982(Ar-C-H); 1730(C=O); 1644(Ar-C=C); 1094(C-N):780(C-H-Ar).

4f) IR(KBr) cm^{-1}: . 3237, 3126 (N-H); 2982(Ar-CH); 1724(C=O); 1644(NH-C=O); 826(C-Cl).

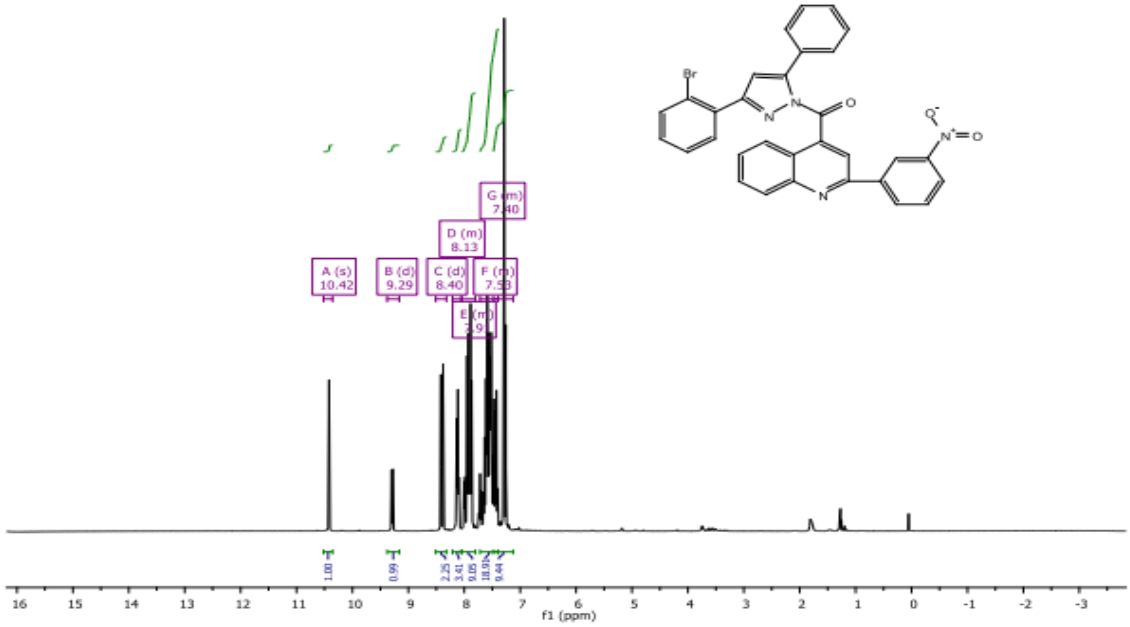
4g) IR(KBr) cm^{-1}: 3215(N-H); 3113 (=C-H); 2982(C-H); 1703(C=O); 1651(C=N); 1088 (C-N): 773(C-H-Ar).

4h) IR(KBr) cm^{-1}: : 3402(N-H); 3055 (=C-H); 1700(C=O); 1574(C=C); 705(C-H-Ar).



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 8.4 Hz, 1H), 8.06 – 7.92 (m, 4H), 7.92 – 7.78 (m, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.60 – 7.51 (m, 3H), 7.49 – 7.39 (m, 6H), 7.29 (s, 1H), 5.14 – 4.96 (m, 1H), 3.61 (ddd, J = 23.3, 17.0, 6.8 Hz, 4H).

Image 2 (3,5-diphenyl-1*H*-pyrazol-1-yl)(2-phenylquinolin-4-yl)methanone



^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (s, 5H), 9.29 (d, J = 8.6 Hz, 5H), 8.40 (d, J = 15.8 Hz, 11H), 8.21 – 8.05 (m, 17H), 8.05 – 7.80 (m, 45H), 7.72 – 7.39 (m, 94H), 7.48 – 7.13 (m, 47H).

Image 3 (3-(2-bromophenyl)-5-phenyl-1*H*-pyrazol-1-yl)(2-(3-nitrophenyl)quinolin-4-yl) methanone

Mass Interpretation

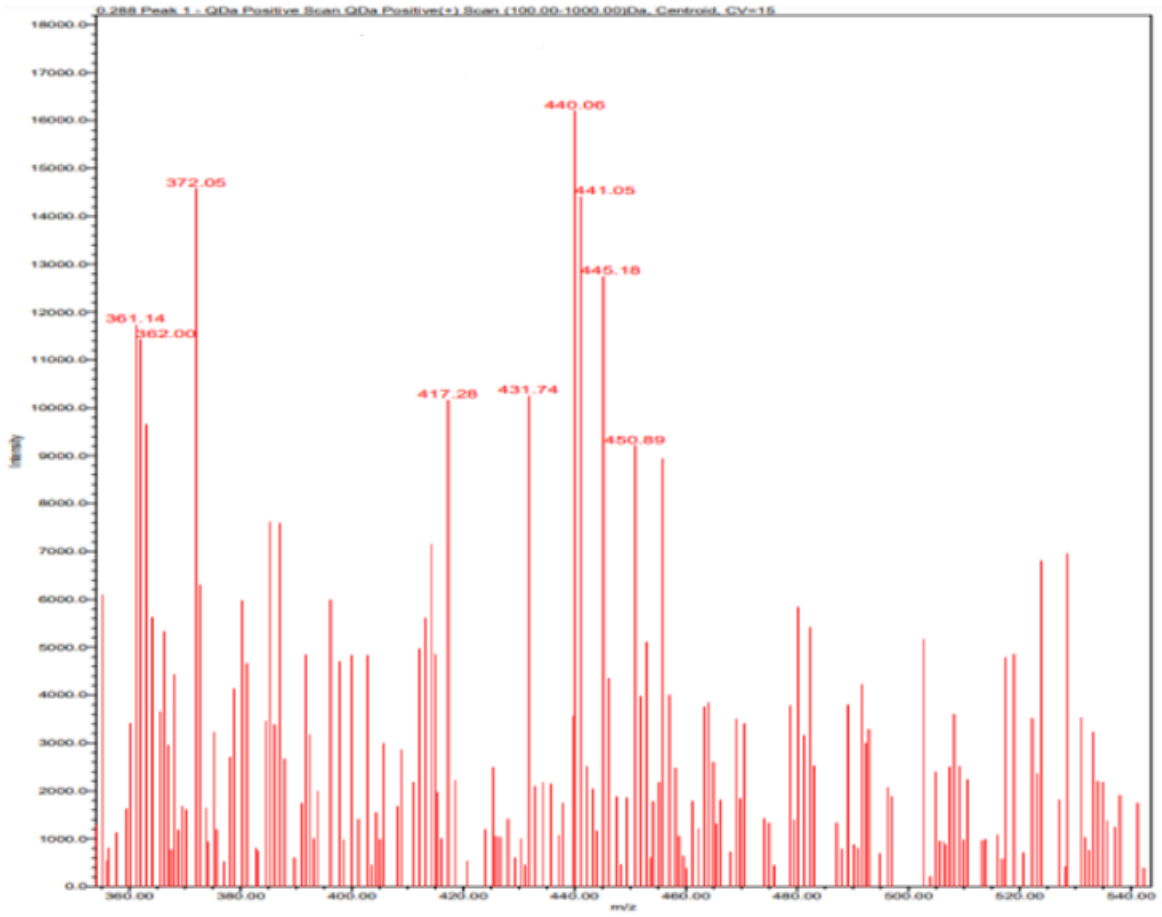


Image 4: Mass spectrum of (3,5-diphenyl-1*H*-pyrazol-1-yl)(2-phenylquinolin-4-yl)methanone

450.89(100 %, base peak); 445.18(32.85%); 441.05(75.07%); 440.06(45.7%); 431.74(60.0%); 417.28(91.4%); 372.08(37.14%);362:361.14

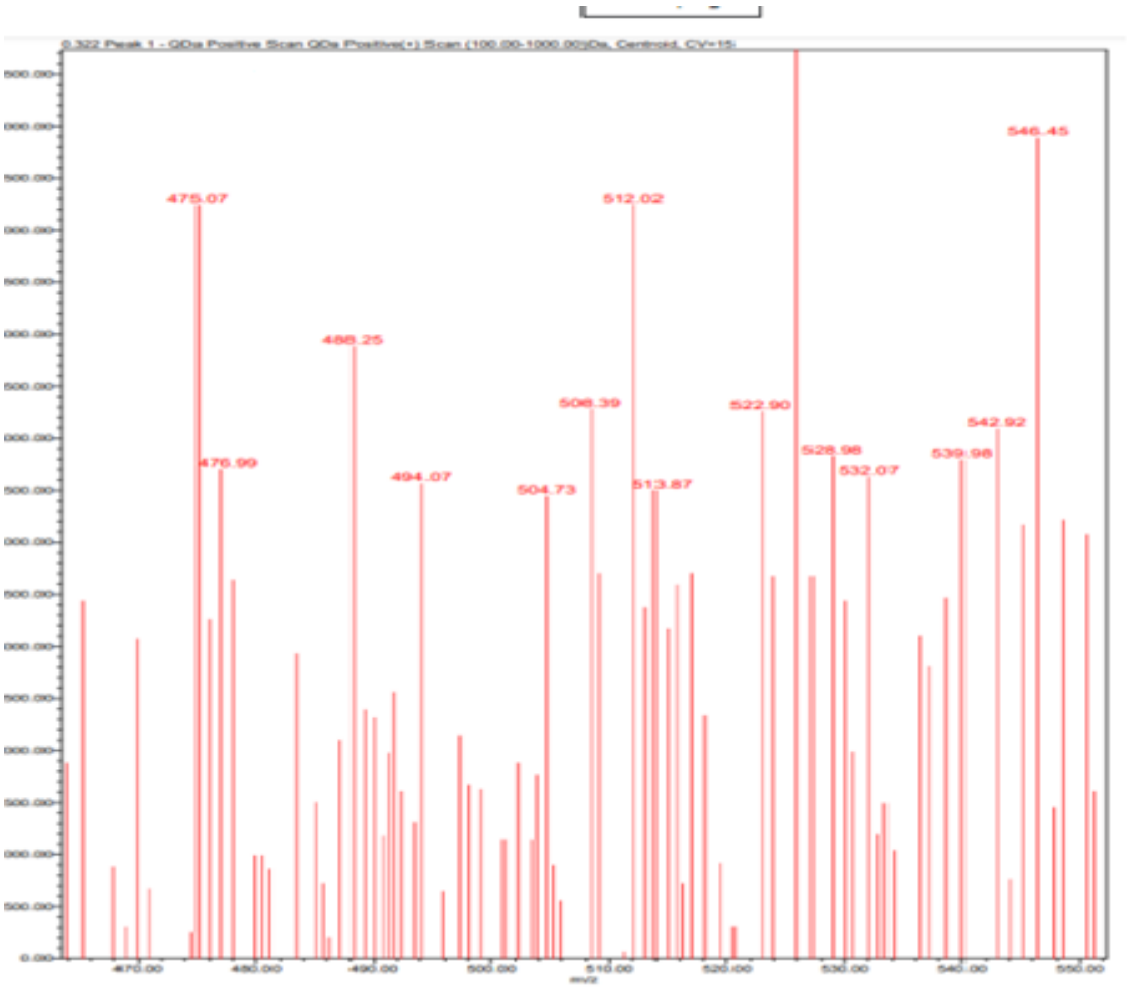


Image 5: Mass spectrum of (3-(3-chlorophenyl)-5-phenyl-1H-pyrazol-1-yl)(2-(2-chlorophenyl)quinoline-4yl)methanone

546.45(100 %, base peak); 542.92(32.85%); 539.98(75.07%); 532.07(45.7%); 528.08(60.0%); 522.90(91.4%); 513.87(37.14%);512.02:508.39 :504.73:494.07:488.25:476.99:475.07

Docking Modelling Image

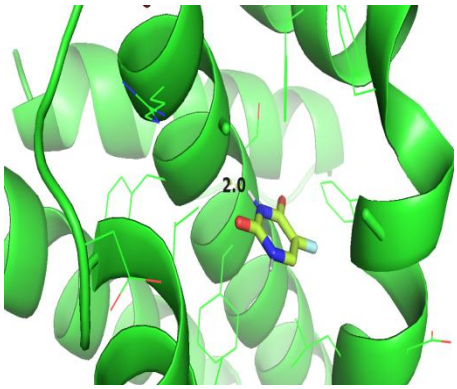


Image :6 5-Fluorouracil

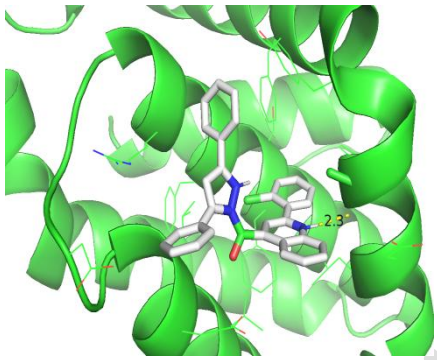


Image :7 4a

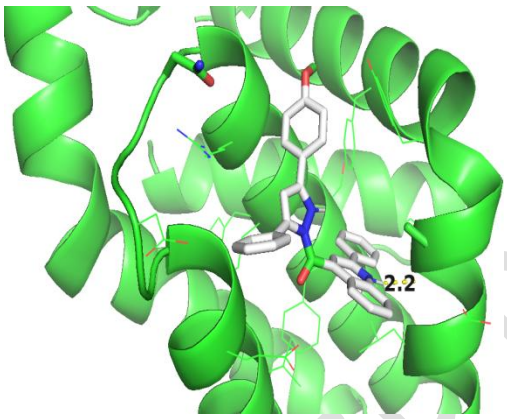


Image :8 4b

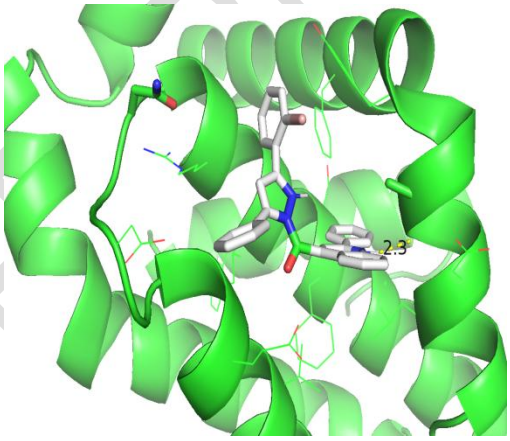


Image :9 4c

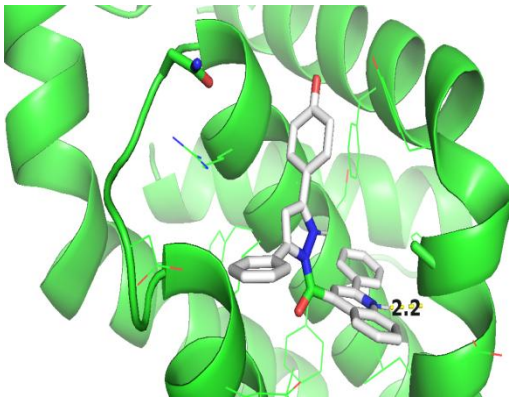


Image 10 4d

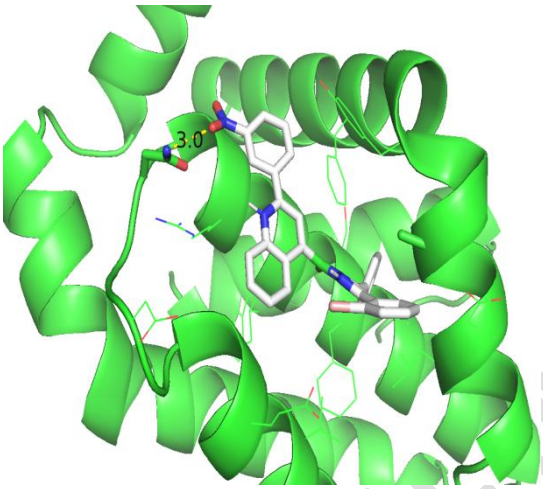


Image 11 4e

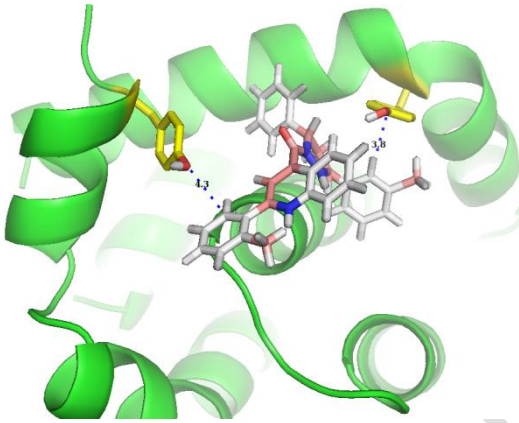


Image 12 4f

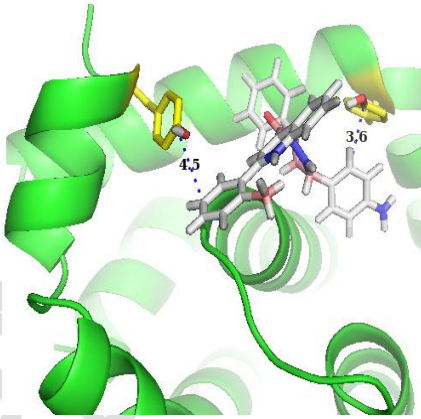


Image 13 4g

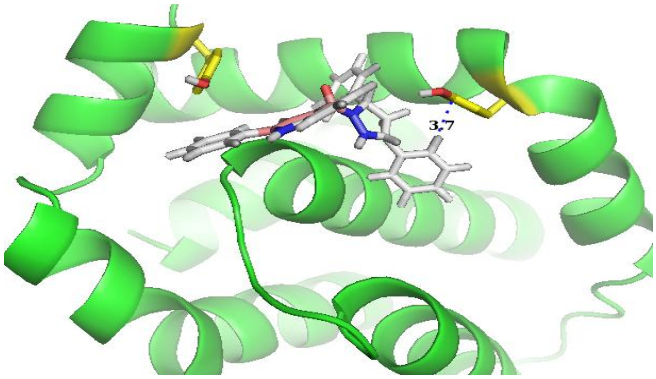


Image 14 4h

In the mid-2000s, Abbott Laboratories developed a novel inhibitor of Bcl-2, Bcl-xL and Bcl-w, known as ABT-737. This compound is part of a group of BH_3 mimetic small molecule inhibitors (SMI) that target these Bcl-2 family proteins.. *In vitro* studies showed that primary cells from patients with B-cell malignancies are sensitive to 5FU, ABT-737.ABT-737 does not directly induce apoptosis; it enhances the effects of apoptotic signals.

Table (2): Docking score of substituted quinoline derivatives (pdbcode)

Sr. No.	**Compound**	**Binding Affinity (Kcal/mol)**
1	**Control**	-
2	**5FU**	-9.1
3	4a	-10.9
4	4b	-10.3
5	4c	-10.3
6	4d	-10.3
7	4e	-10.1
8	4f	-9.5
9	4g	-9.4
10	4h	-8.9

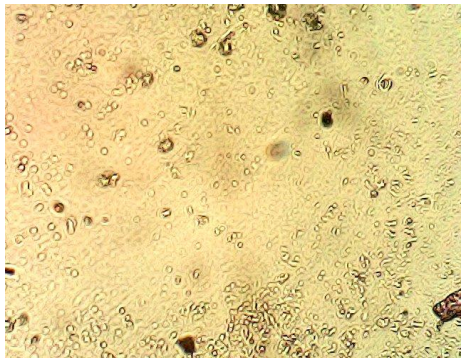


Image 15 5-Fluorouracil

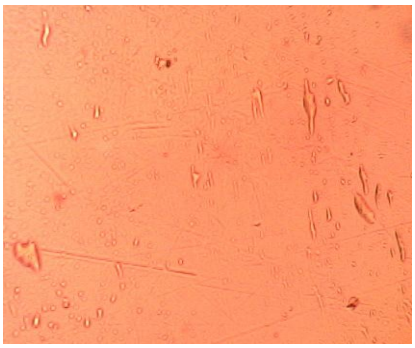


Image 16 4a

Table (3): Percentage inhibition of synthesized substituted quinoline derivatives.

Sr. No.	Synthesis Compound	Binding Affinity (Kcal/mol)	% Inhibition		
			10	30	100
1	**Control**	-	-	-	-
2	**5FU**	**-9.1**	**62.02**	**68.75**	**75.22**
3	4a	-10.9	58.62	61.80	64.05
4	4b	-10.3	57.42	58.22	58.75
6	4c	-10.3	46.28	51.59	57.29
5	4d	-10.3	42.83	50.13	51.98
7	4e	-10.1	43.89	44.16	48.14
8	4f	-9.5	40.18	42.57	46.81
9	4g	-9.4	39.52	42.70	46.00
10	4h	-8.9	30.76	35.14	38.72

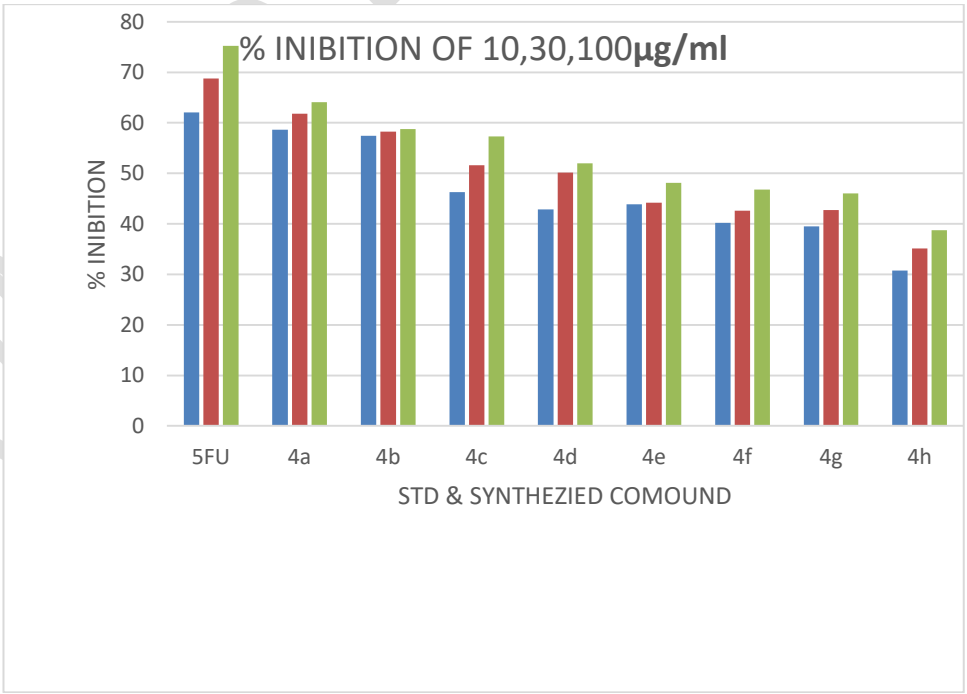


Image **16:** Graphical representation of the anticancer activity of synthesized Substituted quinoline derivatives.

CONCLUSION:

The synthesized compounds are solids and having melting point in the range 150-200 °C. The physical properties of the compounds synthesized are given in table 1.Among the various synthetic approaches followed in past research works on quinoline the easiest method i.e. three component cyclocondensation of aromatic aldehyde, purvic acid and aniline in presence of ethanol was followed for synthesis of ester. The synthesized quinoline ester was reacted with hydrazine hydrate to obtain the quinoline carbohydrazide. The final quinoline derivatives were synthesized by reaction of quinoline carbohydrazidewith chalcones in acetic acid. The compounds formed were confirmed by physical and spectral data. All the compounds were subjected to anticancer activity by MTT assay. The MCF-7 (Human breast cancer cell line) was tested. Comparison was done with the standard 5FU drug. The results of the anticancer activity are given in table 3.The compounds correlate with docking, modelling and activity 4a have showed greater as compared to other derivatives. 4b,4c,4d moderate activity and 4e,4f,4g and 4h show good activity according to 30 and 100 **µg/ml** as compared to std. 5-Fluro uracil drug.

COMPETING INTERESTS DISCLAIMER:

No competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ABBREVIATIONS:

MCF-7-Michigan Cancer Foundatio-7, TLC-Thin Layer Chromatography, IR-Infra red, MS-Mass Spectrum. ^{1}HNMR-Proton Nuclear Magnetic Resonance, JASCO FTIR-Fourier Transfer Infra red, KBR-Potassium Bromide ,TMS-Tetramethylsilane,CO_2 –Carbon Dioxide, USP-United State Pharmacopeia. DMEM-Dulbecco's Modified Eagle Medium, DMSO- Dimethylsulfoxide , Bcl-2- B-cell lymphoma 2, Bcl-xL- B-cell lymphoma-extra large.

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