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Synthesis and Cytotoxic Activity Evaluation of Novel Piperazine-quinoline Derivatives on Brest cancer Cell Lines

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ABSTRACT

*A series of new piperizine-quinoline derivatives (**RB1-10**) were designed and synthesized from 4-(bromomethyl)quinolin-2(1H)-one and arylpiperazines. The structure of newly synthesized compounds were characterized by spectral data's and screened for their cytotoxic activity against barest cancer cell lines. The compound **RB1** with trifluoromethoxy substitution on aryl group showed moderate activity.*

Keywords: 4-(Bromomethyl)quinolin-2(1*H*)-one, aryl-piperazine, cytotoxicity, biological assays, anti-cancer.

INTRODUCTION

Nitrogen containing heterocyclic molecules constitutes the largest portion of chemical entities, which are part of many natural products, fine chemicals, and biologically active pharmaceuticals.

In the field of six membered heterocyclic structures piperazine & quinolinone pharmacophores have significant therapeutic values in medicinal chemistry.

Piperazine is a broad class of heterocyclic symmetrical aliphatic molecule consists of a six-membered ring containing two nitrogen atoms at opposite positions in the ring. There are many important biologically active molecules which contain piperazine moiety such are Ranolazine [1] and Trimetazidine [2] (antianginals drugs), Amoxapine [3], Befuraline [4], Buspirone [5], Flesinoxan [6], Ipsapirone [7], Nefazodone [8] (Antidepressant drugs), Niaprazine [9] (Antihistamine drug), Imatinib [10] (used to treat certain cancers), Antrafenine [11] (analgesic and anti-inflammatory drug) meta-chlorophenylpiperazine [12] (psychoactive drug), Olanzapine [13] (atypical antipsychotic drug)

Quinolinone represents another most important class of heterocyclic molecule possessing wide spectrum of biological activities. Also, they have occupied a unique place in the medicinal and biological chemistry due to their diverse pharmacological displays as antitumor [14], antimicrobial [15], antibacterial [16] HIV-1 integrase inhibitors [17], antifungal [18], herbicidal [19]. antineoplastic [20], gastric, antiulcer and antischistosomal agents [21]. They are also useful intermediates in the manufacture of azo dyestuffs that can be used for dyeing both naturally occurring and synthetic fibers [22].

These properties of piperazines and quinolinones with numerous pharmacological and physiological activities encouraged us to develop new scaffold of molecules to synthesize novel compounds. The development of new and efficient methodologies for synthesis of potentially bioactive piperazine-quinolineone derivatives is important.

Compounds of the nature shown in figure 1 revealed the framework of piperazine-quinolinone pharmacophoric component systems.

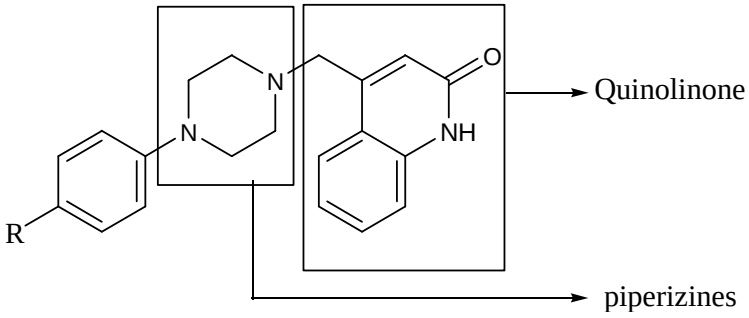


Figure-1: Piperazine-quinoline scaffold

Currently cancer is one of the most deadly diseases and requires chemotherapy for the treatment [23]. Chemotherapeutic compounds are drugs that are used to destroy cancer cells. The study of destroying the cancer cell is called cytotoxicity. The cytotoxicity study is important to know about the toxic nature of compounds on cell lines. In this research we choose breast cancer lines for our studies against piperizine-quinoline scaffold molecules. For preliminary research we choose substituted and un-substituted aryl moieties attached to piperazine-quinolinone pharmacophores. The synthesised new molecules are characterized by spectral data's like mass, IR, NMR and physical constants. The synthesised new molecules were subjected to pharmacological studies.

MATERIALS AND METHODS

All chemicals used for the synthesis were of reagent grade and were procured from Sigma Aldrich Chemical Co, Bangalore; SDFCL, Mumbai; and the intermediates were prepared as per the known literature procedure. NMR spectra were recorded on 400 MHz Varian-AS NMR spectrometer using TMS as an internal standard. IR spectra were recorded by using PerkinElmer Spectrum 100 Series FT-IR spectrometer. Mass spectra were recorded on Agilent 1200 Series LC/MSD VL system. Melting points were determined by using Buchi melting point B-545 instrument and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) using pre-coated silica 60 F_{254}, 0.25 mm aluminum plates (Merck). The crude compounds were crystallized with appropriate solvents.

Preparation of aryl-piperazines: General procedure [24]:

The slurry of amine (0.1 mol) and bis(2-chloroethylamine) hydrochloride (0.13 mol) in sulfolane (3 volumes) were heated to 150 °C for 15 hours, after completion of reaction, cooled to 30 °C and diluted with acetone (6 volumes) and further cooled to 0 °C, maintained for 5h to precipitate the desired product. The product was filtered, slurry washed with acetone (1 volume), and dried at 60 °C under vacuum for 8 h to yield aryl-piperazine hydrochlorides.

Table-1: Aryl-piperizine hydrochlorides

Sl. No	Amine	Product	Yield	MP °C
1	4-(Trifluoromethoxy)aniline	1-[4-(Trifluoromethoxy)phenyl]piperazine Hydrochloride	55 %	192-195
2	4-Methyl aniline	1-(4-Methylphenyl)piperazine Hydrochloride	79 %	214-216
3	4-chloroaniline	1-(4-chlorophenyl)piperazine Hydrochloride	82 %	276-278
4	2,4-difluoroaniline	1-(2,4-Difluorophenyl)piperazine Hydrochloride	78 %	207-212
5	3-Chloroaniline	1-(3-chlorophenyl)piperazine Hydrochloride	59 %	235-237
6	2-Isopropyl-6-methyl aniline	[2-Methyl-6-isopropylphenyl]piperazine hydrochloride	49 %	223-225
7	2-Fluoro-3-chloro aniline	1-(3-Chloro-2-fluorophenyl)piperazine Hydrochloride	55 %	237-239
8	2,3-Dichloroaniline	1-(2,3-Dichlorophenyl)piperazine Hydrochloride	82 %	245-247
9	4-Methoxy aniline	1-(4-Methoxyphenyl)piperazine. Hydrochloride	80 %	248-250
10	2-Fluoro aniline	1-(2-Fluorophenyl)piperazine Hydrochloride	83 %	187-189

Preparation of aryl-piperazine-quinolinones Rb(1-10): General procedure:

The mixture of 4-(bromomethyl)quinolin-2(1H)-one (**4**) (10.0 mmole), substituted piperazines (**3a-j**) (11.0 mmol), potassium carbonate (30.0 mmole) and tetrabutyl ammonium bromide (0.1g) in N,N-dimethyl formamide (20 mL) were heated to 100-105 °C for 8 h. The reaction progress was monitored by TLC, after completion of reaction, cooled to 25-30 °C, quenched with ice water (100 mL), stirred the mass for 30 min. The precipitated product was filtered, washed with water and dried to get crude compound. The crude compound was crystallized from isopropanol to get pure compounds. **RB(1-10)**.

4-({4-[4-(Trifluoromethoxy)phenyl]piperazin-1-yl}methyl)quinolin-2(1H)-one (RB1). Off-white solid; Yield: 62.2 %; Mp: 225-227 ^{o}C; MS: m/z=404.1 (M+1); IR (KBr) cm^{-1}: 2962, 1666, 1396, 1064; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.48-2.55 (dd, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.14-3.30 (dd, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.71 (s, 2H,C**H$_2$**), 6.52 (s, 1H, **Ar-H**), 6.96-6.98 (m,2H, **Ar-H**), 7.14-7.16 (m,3H, **Ar-H**), 7.29-7.31 (m, 1H, **Ar-H**), 7.44-7.46 (m, 1H, **Ar-H**) 7.90-7.92 (m, 1H, **Ar-H**), 11.66 (s,1H, **NH**). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 40.63 (2C), 48.60 (2C), 59.06, 151,91, 116.64 (2C), 119.09 (2C), 119.44, 121.61, 121.90, 122.24, 125.54, 130.63, 139.52, 140.93, 147.58, 150.48, 162.02.

4-{[4-(4-Methylphenyl)piperazin-1-yl]methyl}quinolin-2(1H)-one (RB2). White solid; Yield: 43.2 %; Mp=255-257 ^{o}C; MS: m/z=334.1 (M+1); IR (KBr) cm^{-1}: 3001, 2955, 28216, 1975, 1658; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.17 (S, 3H, C**H$_3$**), 2.48-2.59 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.05-3.30 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.70 (s, 2H,-C**H$_2$**-), 6.51 (s, 1H, **Ar-H**), 6.79-6.81 (m, 2H, **Ar-H**), 6.98-7.0 (m,2H, **Ar-H**), 7.13-7.15 (m,1H, **Ar-H**), 7.17-7.28 (m, 1H, **Ar-H**), 7.30-7.44 (m, 1H, **Ar-H**), 7.90-7.92 (d, 1H, **Ar-H**), 11.65 (s,1H, **NH**). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 20.48, 49.24 (2C), 53.31 (2C), 59.17, 115.98, 116.16 (2C), 119.09 (2C), 121.58, 121.91, 125.56, 128.08, 129.79, 130.63, 139.49, 147.68, 149.38, 162.02.

4-{[4-(4-chlorophenyl)piperazin-1-yl]methyl}quinolin-2(1H)-one (RB3). Brown solid; Yield: 43.2 %; Mp=248-252 ^{o}C; MS: m/z=354.1 (M+1); IR (KBr) cm^{-1}: 3063, 2931, 1718, 1658, 1504; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.63-2.69 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 2.98-3.00 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.71 (s, 2H,C**H$_2$**), 6.51 (s, 1H, **Ar-H**), 6.95-7.17 (m, 5H, **Ar-H**), 7.28-7.30 (m, 1H, **Ar-H**), 7.44-7.50 (m, 1H, **Ar-H**), 7.90-7.92 (d, 1H, **Ar-H**), 11.65 (s,1H, **NH**).

4-{[4-(2,4-difluorophenyl)piperazin-1-yl]methyl}quinolin-2(1H)-one (RB4). Off-white solid; Yield: 70.4 %; Mp=252-257 ^{o}C; MS: m/z=356.1 (M+1); IR (KBr) cm^{-1}: 3064, 2930, 1720, 1657, 1591; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.48-2.61 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 2.80-2.87 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.72 (s, 2H,C**H$_2$**), 6.51 (s, 1H, **Ar-H**), 6.95-6.98 (m, 1H, **Ar-H**), 7.01-7.04 (m,2H, **Ar-H**), 7.05-7.12 (m,2H, **Ar-H**), 7.13-7.18 (m, 1H, **Ar-H**), 7.28-7.30 (m, 1H, **Ar-H**), 7.90-7.92 (m, 1H, **Ar-H**), 11.65 (s,1H, **NH**).

4-{[4-(3-chlorophenyl)piperazin-1-yl]methyl}quinolin-2(1H)-one (RB5). Off-white solid; Yield: 75.4 %; Mp=244-247 ^{o}C; MS: m/z=354.1 (M+1); IR (KBr) cm^{-1}: 3063, 2947, 1720, 1658, 1558; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.42-2.51 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 2.78-2.89 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.71 (s, 2H,C**H$_2$**), 6.51 (s, 1H, **Ar-H**), 6.87-6.88 (m, 1H, **Ar-H**), 6.99-7.09 (m,2H, **Ar-H**), 7.04-7.17 (m,2H, **Ar-H**), 7.19-7.20 (m, 1H, **Ar-H**), 7.28-7.30 (m, 1H, **Ar-H**), 7.91-7.96 (m, 1H, **Ar-H**), 11.65 (s,1H, **NH**).

4-[4-(2-Isopropyl-6-methyl-phenyl)-piperazin-1-ylmethyl]-1H-quinolin-2-one (RB6): White solid; Yield: 50.4 %; Mp=376.1 ^{o}C; MS: m/z=389.1 (M+1); IR (KBr) cm^{-1}: 3063, 2955, 2800, 1651, 1442; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.14-1.15 (d, 6H, isopropyl two C**H$_3$**), 2.28 (s, 3H, C**H$_3$**), 2.56-2.66 (m, 4H, -C**H$_2$**-N-C**H$_2$**), 2.89-2.91 (m, 4H, -C**H$_2$**-N-C**H$_2$**), 3.45-3.50 (m, 1H, Isopropyl- C**H**), 3.74 (s, 2H, Benzylic C**H$_2$**), 6.56 (s, 1H, Ar-H), 6.92-6.94 (d, 1H, Ar-H), 7.00-7.08 (m., 2H, Ar-H), 7.18-7.22 (t, 1H, **Ar-H**), 7.31-7.33 (d, 1H, Ar-**H**), 7.47-7.51 (t, 1H, Ar-**H**), 7.94-7.96 (d, 1H, Ar-**H**), 11.65 (s, 1H, **NH**).

-{[4-(3-Chloro-2-fluorophenyl)piperazin-1-yl]methyl}quinolin-2(1H)-one (RB7). Off-white solid; Yield: 72.2 %; Mp=254-258 ^{o}C; MS: m/z=372.1 (M+1); IR (KBr) cm^{-1}: 3064, 2958, 2811, 1658, 1481; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.48-2.62 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.03-3.29 (dd, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.72 (s, 2H,C**H$_2$**), 6.51 (s, 1H, **Ar-H**), 6.98-6.99 (m, 1H, **Ar-H**), 7.01-7.08 (m,2H, **Ar-H**), 7.10-7.14 (m,1H, **Ar-H**), 7.16-7.18 (m, 1H, **Ar-H**), 7.28-7.30 (m, 1H, **Ar-H**), 7.90-7.92 (m, 1H, **Ar-H**), 11.65 (s,1H, **NH**).

4-{[4-(2,3-dichlorophenyl)piperazin-1-yl]methyl}quinolin-2(1H)-one (RB8): White solid; Yield: 55.4 %; Mp=266-268 ^{o}C; MS: m/z=389.1 (M+1); IR (KBr) cm^{-1}: 3063, 2955, 2816, 1658, 1481; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.47-2.60 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 2.80-2.86 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.71 (s, 2H,C**H$_2$**), 6.51 (s, 1H, **Ar-H**), 6.85-6.98 (m, 1H, **Ar-H**), 7.01-7.08 (m,2H, **Ar-H**), 7.05-7.19 (m,2H, **Ar-H**), 7.14-7.18 (m, 1H, **Ar-H**), 7.28-7.30 (m, 1H, **Ar-H**), 7.90-7.95 (m, 1H, **Ar-H**), 11.65 (s,1H, **NH**).

4-{[4-(4-methoxyphenyl)piperazin-1-yl]methyl}quinolin-2(1H)-one (RB9). White solid; Yield: 72.9 %; Mp=249-252 ^{o}C; MS: m/z=350.1 (M+1); IR (KBr) cm^{-1}: 2947, 2816, 1658, 1512; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.48-2.59 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.00-3.30 (dd, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.65 (s, 3H, C**H$_3$**), 3.70 (s, 2H,C**H$_2$**), 6.51 (s, 1H, **Ar-H**), 6.77-6.79 (m, 2H, **Ar-H**), 6.85-6.87 (m,2H, **Ar-H**), 7.14-7.17 (m,1H, **Ar-H**), 7.28-7.30 (m, 1H, **Ar-H**), 7.44-7.46 (m, 1H, **Ar-H**), 7.90-7.92 (d, 1H, **Ar-H**), 11.65 (s,1H, **NH**).

4-{[4-(2-fluorophenyl)piperazin-1-yl]methyl}quinolin-2(1H)-one (RB10). Off-White solid; Yield: 72.9 %; Mp=254-258 ^{o}C; MS: m/z=338.1 (M+1); IR (KBr) cm^{-1}: 2947, 2854, 1658, 1558; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.62-2.71 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.00-3.29 (d, 4H, -C**H$_2$**-N-C**H$_2$**-), 3.72 (s, 2H,C**H$_2$**), 6.51 (s, 1H, **Ar-H**), 6.94-

7.18 (m, 5H, **Ar-H**), 7.28-7.30 (m, 1H, **Ar-H**), 7.45-7.48 (m, 1H, **Ar-H**), 7.90-7.92 (d, 1H, **Ar-H**), 11.65 (s,1H, **NH**).

Biological activity:

The cytotoxic activities of newly synthesized compounds were studied by MTT assay method [25]. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. This viability assay is based on the color change of the MTT molecule into insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells when it is exposed to viable cells. The water insoluble formazan can be solubilized using DMSO. Measurement of the absorbance, which is proportional to the number of viable cells, and comparison to untreated controls, enables assessment of the cell growth inhibition capabilities of the compound tested.

Procedure:

Breast cancer cell lines (MDA-MB-231) were grown in T25 flasks and trypsinized after 70-80% confluent growth and checked for the viability by trypan blue dye exclusion method. Cells (50,000 cells / well) were seeded in a 96 well plate and incubated for 24 hrs at 37 °C in a humidified (5 % CO_2) incubator. Compounds were tested from 0-100 µM [2 fold variations] in RPMI media without FBS are incubated for 24 hr. After incubation with compounds, the media is removed from the wells and MTT (100 µl /well, 0.5mg/mL) is added. Post incubation with MTT reagent for 3 to 4 hours, the media is removed from the wells and formazan is solubilized with 100 µl of DMSO and absorbance at 590 nm is recorded. The percentage inhibition was calculated by using the following formula.

$$\% \text{ Inhibition} = 100 - \frac{\text{Sample}}{\text{Control}} \text{X } 100$$

All experiments were performed in triplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells. The results were tabulated in table-1.

Table 1. Cytotoxicity of tested compounds against Breast Cancer cells (MDA-MB-231)

Test Compounds	Concentration (µM)	Absorbance 590nm	% Inhibition (n=3)
0	0.0	0.392	0.00
RB1	100	0.192	51.05
RB2	100	0.328	16.21
RB3	100	0.278	29.25
RB4	100	0.343	12.53
RB5	100	0.351	10.71
RB6	100	0.333	15.05
RB7	100	0.343	12.53
RB8	100	0.351	10.72
RB9	100	0.282	28.05
RB10	100	0.350	10.68

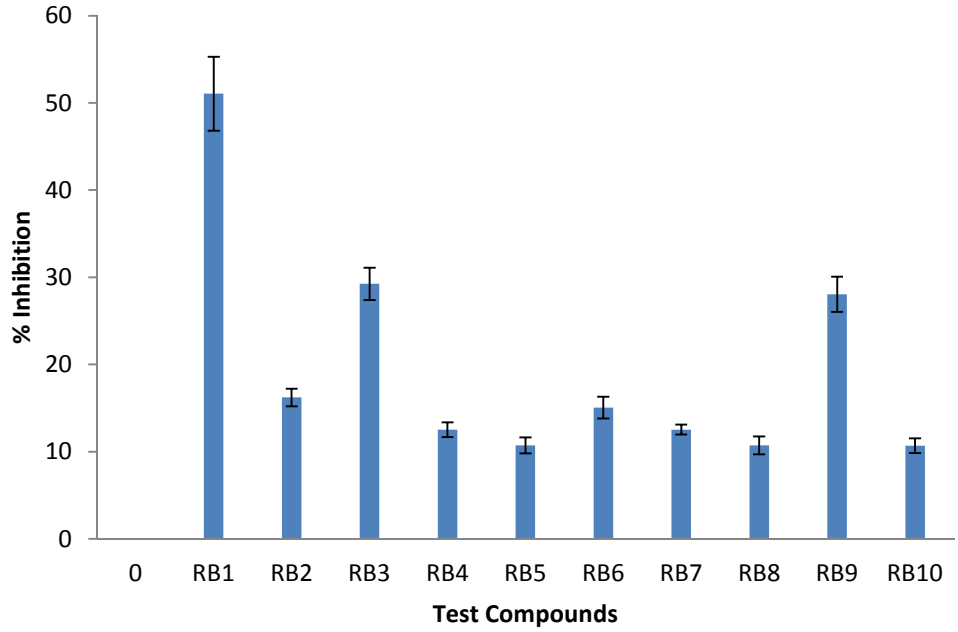
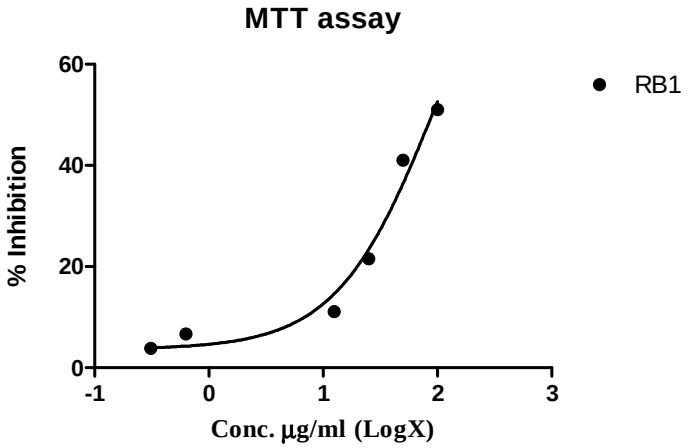


Fig.1 Cytotoxicity of test compounds against Breast Cancer (MDA-MB-231 cells)

From the results it is shown that the compound **RB1** shown some inhibition, and assayed for for IC_{50} value at different concentratin and shown in table-2.

Table2. Cytotoxicity of RB1 against Breast Cancer cells

Compound	Concentration (µM/ml)	Absorbance 590nm	% Inhibition	IC_{50}
Control	0.0	0.392	0.00	
RB1	0.31	0.377	3.85	98.34µM
	0.63	0.366	6.69	
	12.5	0.349	11.05	
	25	0.307	21.52	
	50	0.231	41.02	
	100	0.192	51.05	



	RB1
log(inhibitor) vs. response	
Best-fit values	
BOTTOM	100.8
TOP	3.637
LOGIC50	1.993
IC50	98.34
Span	-97.20

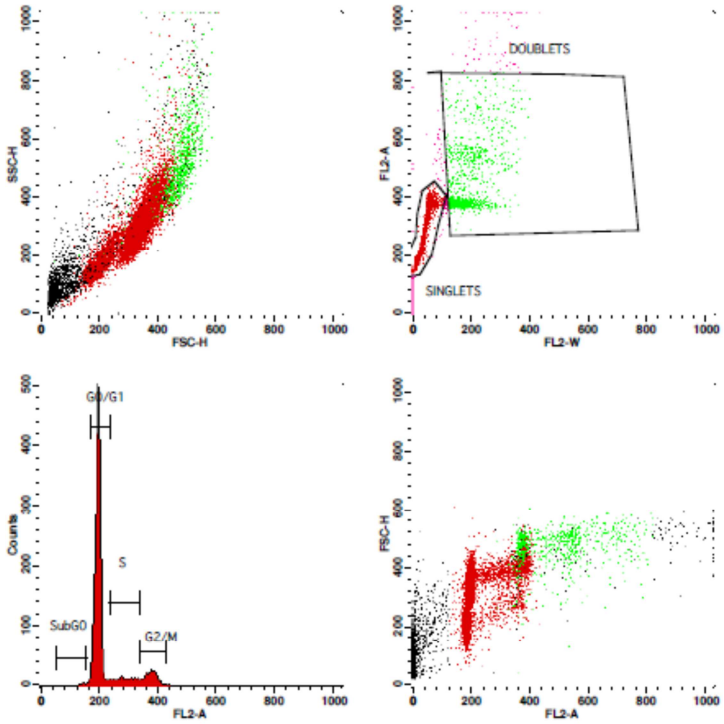
Fig.2 Cytotoxicity of test compounds against Breast Cancer (MDA-MB-231 cells)

From the tested compounds **RB1** to **RB10**, it is shown that, **RB1** has showed some moderate cytotoxicity on MDA-MB-231 breast cancer cell line with IC_{50} value of 98.34 µM. These results suggest that RB1 could have some cytotoxic potential and taken further for cell cycle studies.

Cell Cycle Analysis

One of the earliest applications of flow cytometry was the measurement of DNA content in cells [26]. This analysis is based on the ability to stain the cellular DNA in a stoichiometric manner. A variety of dyes are available to serve this function, all of which have high binding affinities for DNA. The location to which these dyes bind on the DNA molecule varies with the type of dye used. The most common DNA binding dye in use today is the blue-excited dye Propidium Iodide (PI). PI is an intercalating dye which binds to DNA and double stranded RNA (and is thus almost always used in conjunction with RNase to remove RNA). When diploid cells which have been stained with a dye that stochiometrically binds to DNA are analyzed by flow cytometry, a "narrow" distribution of fluorescent intensities is obtained [27].

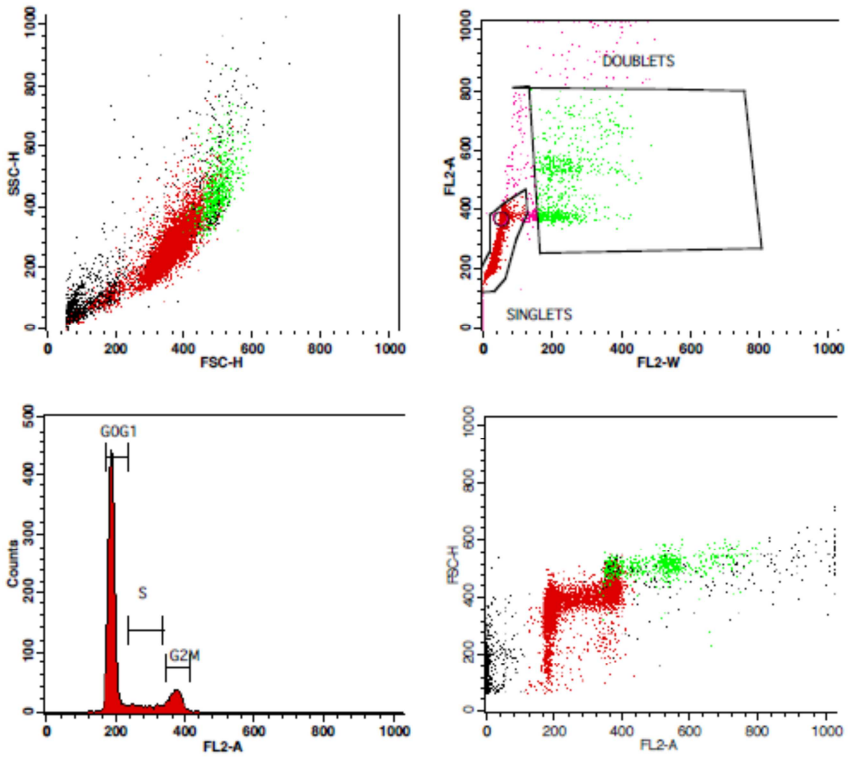
Cells (1 x10^6) were cultured in a 6-well plate containing 2 ml of complete DMEM media. After 24h of incubation, cells were treated with or without 100 µM of test compounds; 20µM Colchicine as positive control, and 1% DMSO as control in 1 ml / well of serum-free DMEM media and incubated for 24 h. After 24 h of treatment, cells were collected and pelleted the cells by centrifuging at 1500 rpm for 5 minutes at room temperature and discarded the supernatant. Resuspended the cells pellet gently in 1XPBS. Discarded the supernatant liquid and resuspended pellet in 200 µl of 1X PBS and fix overnight in a 2 ml of fixing solution (70% ethanol). After overnight fixing, centrifuged at 4000 rpm for 10 min at 4 °C and discarded the supernatant. Pelletted cells were washed two times with 2 ml of cold 1XPBS. Later, cells were incubated for 15 min at room temperature in 500 µl of propidium iodide (PI) solution containing 0.05 mg/ml PI and 0.05 mg/ml RNase A in PBS. The percentage of cells in various stages of cell cycle in compounds treated and un-treated populations were determined using FACS Caliber (BD Biosciences, San Jose, CA). The effect of compound **RB1** on cell cycle in MDA-MB-231 cells as analyzed by flow cytometry are depicted in figures below.



Histogram Statistics

Marker	Left, Right	Events	% Gated	% Total	Mean	CV
All	0, 1023	10000	100.00	75.64	216.76	25.94
SubG0	54, 154	36	0.36	0.27	142.81	4.57
G0/G1	172, 234	8308	83.08	62.84	194.41	4.28
S	237, 336	694	6.94	5.25	288.15	9.97
G2/M	341, 427	860	8.60	6.51	374.71	4.47

Fig 3. Cell cycle analysis of untreated control MDA-MB-231 cells



Histogram Statistics

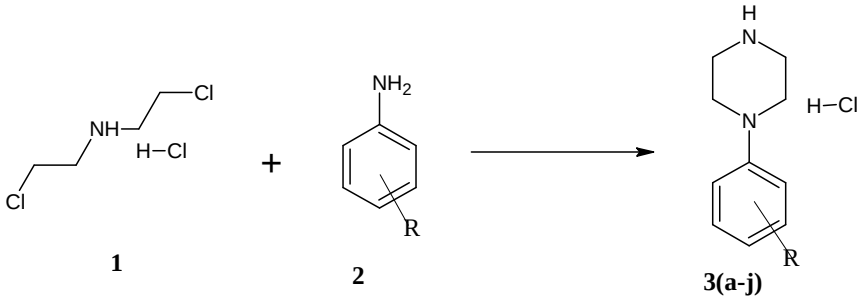
Marker	Left, Right	Events	%Gated	%Total	Mean	CV
All	0, 1023	10035	100.00	82.34	222.90	29.19
G0G1	172, 234	7790	77.63	63.92	191.50	4.60
S	237, 336	805	8.02	6.61	285.65	10.54
G2M	344, 412	1257	12.53	10.31	372.49	3.78

Fig 4. Cell cycle analysis of RB1 (100 µM) on MDA-MB-231 cells

RESULTS AND DISCUSSION

Chemistry:

The major starting material for the synthesis of piperazine-quinoline derivatives are 4-(bromomethyl)quinolin-2(1H)-one and aryl-piperazines which were prepared by the known literature procedure.

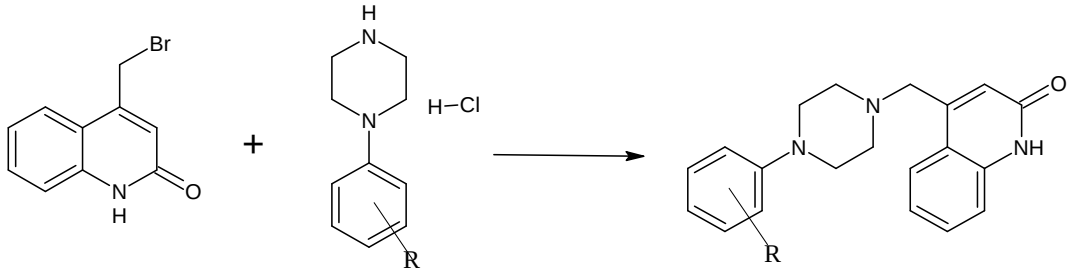


1 **2** **3(a-j)**

R=3a=4-trifluoromethoxy, 3b=4-methyl, 3c= 4-Chloro,
3d=2,3-difluoro, 3e= 3-Chloro, 3f=2-isopropyl-6-methyl,
3g=2-fluoro-3-chloro, 3h= 2,3-dichloro, 3i=4-methoxy,
3j=2-fluoro

Scheme-1: Preparation of aryl-piperazine hydrochlorides

Different anilines were cyclized with bis(2-chloroethylamine) hydrochloride in presence of sulfolane as solvent at 150 °C for 15 h. The reactions were monitored by TLC and the isolated compounds were characterized with respect to MP and IR spectra's. Compounds melting points were matched with literature and the IR match with the functional groups.



Scheme-2: Preparation of Aryl-piperizine-quinolines RB(1-10)

Aryl-piperazines were reacted with 4-(bromomethyl)quinolin-2(1H)-one (Commercially available) compound in presence of base potassium carbonate, N,N-dimethylformamide as solvent and tetrabutylammonium bromide (TBAB) as phase transfer catalyst. The isolated compounds were characterized by their spectra's. The NMR spectra of all the compounds showed the two doublets at 2.5 to 2.8 ppm correspond to the two sets of methylene groups of piperzines and a peak at around 3.7 for benzylic methylene group. All the aromatic protons correspond to their multiplicities and matches with the structure. IR shows the absorption at around 1760 corresponds to amide keto group and the aliphatic hydrogen's at around 2900, confirms the function groups.

Biological activity:

The cytotoxicity study for the synthesized compounds against Breast Cancer (MDA-MB-231) cells shows that the compounds are not so active except **RB-1,** which has functional group trifluoromethoxy on aryl moiety showed 51 % inhibition with an IC_{50} of 98.34 µM. Compound with chloro and methoxy groups in para position on aryl shows 29.25 % and 28.05 % inhibition, and the other compounds showed around 10-15 % inhibition. The results shows that the compounds are not highly toxic towards the cell lines and are not considered has cytotoxic compounds. Cell cycle studies for the compound **RB-1** by flow cytometry shows that with only 12.53% of cells at G2M phase of cell cycle compared to 8.60 % in untreated control, is not significantly affected at 100 µM concentration. This result suggests that **RB-1** has not affected on cell cycle arrest and at the same time it does not induce the apoptosis.

CONCLUSION

In this article we report the synthesis of (**RB1-10**), new piperazine-quinoline heterocyclic moieties, starting from commercially available anilines and 4-(bromomethyl)quinolin-2(1H)-one. Investigation of their cytotoxicity revealed that compound **RB-1** with trifluoromethoxy substitution on aryl ring was moderately active, although it was significantly less than that of untreated control, the fact that the compounds prepared in this study are chemically unrelated to the current medication suggests that the further work is clearly warranted.

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