Dihydroquinazolinones as Potential Antiproliferative and Tumor Inhibiting agents

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Abstract

2,3-dihydroquinazolinone derivatives were screened for their antiproliferative activity, among all the synthesized molecules compound 3j with electron withdrawing substituents on phenyl ring displayed considerable cytotoxicity against HeLa, MCF7 and K562 cell lines with an IC50 value of 17.1, 31.5 and 25.3 µM, respectively. Further we carried out live dead fluorescence dye assay using calcein-AM and ethidium homodimer staining results further validate the capacity of compound 3j it induces apoptosis in HeLa cells. In addition, the potent compound 3j tested for tumor regression studies in Swiss albino mouse. Both in vitro and in vivo results revealed the significant antiproliferative activity of compound 3j in tumor cell lines and tumor tissue showed multifocal areas of necrosis and numerous number of apoptotic cells.

Graphical Abstract

Keywords: 2,3-dihydroquinazolinone; Apoptosis; HeLa

Abbreviations: HeLa: Human Cervical Adeno Carcinoma Cell Line; ESI: Electrospray Ionization; NMR: Nuclear Magnetic Resonance; TLC: Thin Layer Chromatography; M.P: Melting Point; CML: chronic myelogenous leukemia; PBS: Phosphate Buffer Saline

Introduction


From the past few years we are engaged in developing new synthetic routes [19-21] for the synthesis of different heterocycles and recently we have reported novel method for the synthesis of structurally diversified 2,3-dihydroquinazolinones derivatives by using gem-dibromomethylarenes as synthetic benzaldehyde equivalent (Scheme 1). [22] Staying on our efforts to identify the new target molecules [23,24] and considering the pharmacological activities of dihydroquinazolinones, we have screened most of those derivatives against different cancer cell lines.

Scheme 1: Synthesis of 2-(2-bromo-5-fluorophenyl)-7-chloro-2,3-dihydroquinazolin-4(1H)-one.

All the synthesized compounds (3a-3j) were characterized by IR, 1H NMR, 13C NMR and GCMS. The IR spectra showed a characteristic
peak at 2964-2969 cm\(^{-1}\) for C-H stretching & 1650-1654 cm\(^{-1}\) corresponds to carbonyl group of 2,3 dihydroquinazolinones. Further, the \(^1\)H NMR spectra of all the compounds showed singlet around δ 7.88-8.05 & 5.5-6.2 due to the NH protons of 2,3 dihydroquinazolinones moiety and in the \(^{13}\)C NMR the carbonyl carbon C4 of the 2,3 dihydroquinazolinones ring appeared at 162.9-163.8 delta.

**Structures of molecules**

![Structures of molecules](image)

**Supplementary**

**Experimental Section**

The human chronic myelogenous leukemia (CML) cell line K562, human cervical adeno carcinoma cell line HeLa and breast adeno carcinoma cell line MCF7 used for the screening of newly synthesized compounds. To evaluate the anti proliferation MTT and trypan blue dye exclusion assays, MTT assay were employed as described earlier. Further, live dead assay and in vivo tumor regression analysis were also performed in order to understand the method of apoptosis after treatment with compound 3j.

**Cell lines and culture**

Human cell lines K562, HeLa and MCF7 cells were purchased from National Center for Cell Science, Pune, India. Cells were grown in RPMI 1640 & DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL of Penicillin, and 100 mg of streptomycin/mL and incubated at 37°C in a humidified atmosphere containing 5% CO2.

**Trypan blue dye exclusion assay**

The effect of 3(a-j) on cell viability of K562, HeLa cells was determined by Trypan blue dye exclusion assay. Cells were seeded at a density of 1x10^5 cells/ml cultured for 24 h and synthesized compounds were added at a concentration of 10, 20, 40 and 80 µM DMSO (Sigma Aldrich, USA) treated cells were used as vehicle control. Cells were harvested after 48 h and suspended in 0.4% Trypan blue (Sigma Aldrich, USA) and the viable cells were counted using haemocytometer.

**Cytotoxicity assay**

Cell multiplying was further assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay which is based on the ability of viable cells to metabolize a yellow tetrazolium salt to violet formazan. Exponentially growing K562, HeLa and MCF7 cells (5x10^4 cells/well) were plated 24 well plates and incubated with 10, 20, 40 and 80 µM of compounds 3a-3j, cells were harvested after 48 h of treatment and incubated with MTT (0.5 mg/mL) at 37 ºC in 96 well plate. The blue MTT formazan precipitate was then solubilized in detergent (50% final concentration of N, N-dimethyl formamide and 10% of sodium dodecyl sulfate). Absorbance was measured at 570 nm using ELISA plate reader. The mean absorbance of culture medium was used as the blank and was subtracted. All measurements were performed in triplicate.

**Live –dead viability assay**

The determination of live –dead assay allowed to assess the viable and nonviable cells. Calcein-AM indicates intracellular esterase activity and the ethidiumhomodimer indicates membrane integrity. The tested compound 3j was exposed for 48h, the test compound was removed and the wells washed with phosphate buffer saline. For live-dead staining add 200 µl of 2 µM Ethidiumhomodimer, 0.5 µM Calcein–AM for 45 minutes in a moist dark chamber at room temperature. After incubation cells were immediately viewed with a fluorescence microscope at 485nm excitation and 515nm emission wavelength. The non fluorescent
Calcein-AM is converted into green in colour by intracellular esterase predicts active cell metabolism. Ethidium homodimer is excludes viable cells but permeates broken cell membranes, binds to DNA and results red in colour.

**In vivo studies**

**Animal models and in vivo**

Swiss albino female mice weighing 22 to 25 g were housed under standard laboratory conditions with food and water ad libitum. All procedures for animal experimentation used were approved by the Institutional Animal Ethics Committee (IAEC), DOS in Zoology, University of Mysore, India in accordance with the CPCSEA guidelines for laboratory animal facility (Approval no UOM/IAEC/10/1012.Dated 10/11/2012).

**EAC induced tumor treated with compound 3j**

The antitumor activity and efficacy of the compound 3j tested against EAC cells in vivo. EAC cells were the generous gift from Dr. Shankar, Department of Biotechnology, Terrisian College, Mysuru. Tumor cell suspensions were dissolved in phosphate buffer saline (PBS), counted and re-injected (1×10⁶ cells/animal) to the right thigh of experimental animal for development of solid tumor as described. Control and treated groups consisted of six mice each.

**Group 1:** Tumor control (Vehicle Control).

**Group 2:** Tumor treated with 30 mg/kg of compound 3j dissolved in 5% methylcellulose and diluted with distilled water.

After 9 days of tumor induction started compound 3j treatment every alternative day (8 doses) and continued maintained animals up to 30 days, at the end of the 30th day from tumor implantation animals from each group were sacrificed and the tumor tissue was collected and fixed with neutral buffer saline for histopathology studies.

**Histopathology**

The animals sacrificed on 30th day of the experiment. The tissue samples were collected in 10 per cent neutral buffered formalin for histopathological examination. The tissues were processed by the routine paraffin embedding technique and sections of 4 micron thicknesses were cut using a microtome and subjected to routine hematoxylin and eosin (H & E) staining. Each section was evaluated by inverted microscopy and images were captured (Carl Zeiss, Germany).

**Results and Discussion**

The 2-aryl- 2,3-dihydro quinazolin-4(1H)-ones (3a-3j) molecules were screened for their antiproliferative activity against various human cancer cell lines such as HeLa (Human cervical adenocarcinoma cell line), MCF7 (Breast adenocarcinoma cell line) and K562 (Chronic myelogenous leukemia) it induces cytotoxicity upon treatment with various concentration (1, 25, 50 and 100 μΜ) of the compounds (Table 1). MTT assay [25] showed the considerable amount of inhibition in HeLa, MCF7 and K562 is 17.1, 31.5 and 25.3 μΜ, respectively of tested compound 3j.

**Table 1: Antiproliferative activity of 2-Aryl- 2,3-dihydro quinazolin-4(1H)-ones.**

<table>
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<th>Comp. code</th>
<th>Cell line IC₅₀ in μM</th>
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The compound 3j induces cell death in HeLa cells, we carried out livedead fluorescent dye assay using calcein-AM and ethidium homodimer staining. This assay is very useful to determine to check cytotoxicity resulting from interaction of the cells with cytotoxic agents. Calcein-AM stains live cells with an intact membrane and gives green fluorescence and ethidiumhomodimer stains dead cells or cells whose membrane intergrity is damaged are distinguished by a bright red fluorescence, which indicated the amount of cell death caused by compound 3j in HeLa cells (Figure 1A and B). These results further validate the capacity of compound 3j induces cell death in HeLa (Human cervical cancer) cells.

**Figure 1:** Livedead assay on HeLa cells was treated with of 20 μΜ of compound.

A) Metabolically active confluent layer of HeLa cells showing stained with calcein –AM into green fluorescence.

B) Vehicle control cells unstained with ethidium homodimer.

C&D) Compound 3j; damaged or broken cells showing fragmented DNA resulting in red fluorescence.
**Experimental Details**

Reactions were monitored by TLC using precoated sheets of silica gel G/UV-254 of 0.25 mm thickness (Merck 60F254) using UV light for visualization. The melting points were determined on Selaco melting point apparatus and are uncorrected. 1H and 13C NMR spectra were recorded on an NMR spectrometer operating at 400 and 100 MHz, respectively, using the residual solvent peaks as reference relative to SiMe4. Mass spectra were recorded using electrospray ionization (ESI) mass spectrometry. The C, H and N analysis were performed using CE-400 CHN analyzer. Infrared spectra were recorded on Shimadzu FT-IR model 8300 spectrophotometer.

2-(5-phenylpyridin-2-yl)-2,3-dihydroquinazolin-4(1H)-one (3a) [22]: Light yellow solid; M.P: 180-182 0C; IR (KBr) v\textsubscript{max} 3142.78, 3066.61, 2929.67, 1612.33 cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-d\textsubscript{6}): δ 8.65-8.64 (d, J=4.8Hz, 1H), 8.36 (s, 1H), 7.54-7.53 (d, J=2.4Hz, 1H), 7.29-7.24 ((m, 2H), 6.82-6.77 (m, 4H), 5.72 (s, 1H), 3.67 (s, 4H), 3.64 (s, 8H) ppm; 13C NMR (100 MHz, DMSO-d\textsubscript{6}) δ 162.49, 152.76, 146.73, 134.60, 131.58, 127.41, 127.12, 125.07, 124.98, 120.86, 104.96, 104.80, 97.99, 65.92, 55.74, 55.34 ppm; MS (ESI): m/z = 352.307, HRMS (ESI) calcld for [C\textsubscript{14}H\textsubscript{13}N\textsubscript{2}O\textsubscript{2}]+ 349.7885 found 349.7885.

6,7-dimethoxy-2-(4-(trifluoromethyl)phenyl)-2,3-dihydroquinazolin-4(1H)-one (3b) [22]: Light yellow solid; M.P: 185-186 0C; IR (KBr) v\textsubscript{max} 3184.26, 3066.61, 2929.67, 1666.38, 1610.45 cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-d\textsubscript{6}) δ 8.66-8.64 (d, J=4.4Hz, 1H), 8.36 (s, 1H), 7.54-7.53 (d, J=2.4Hz, 1H), 7.29-7.24 ((m, 2H), 6.82-6.77 (m, 4H), 5.72 (s, 1H), 3.67 (s, 4H), 3.64 (s, 8H) ppm; 13C NMR (100 MHz, DMSO-d\textsubscript{6}) δ 162.40, 151.13, 146.73, 134.60, 131.58, 127.41, 127.12, 125.07, 124.98, 120.86, 104.96, 104.80, 97.99, 65.92, 55.74, 55.34 ppm; MS (ESI): m/z = 374.387, HRMS (ESI) calcld for [C\textsubscript{14}H\textsubscript{13}F\textsubscript{3}N\textsubscript{2}O\textsubscript{2}]+ 375.3957 found 375.3954.

7-bromo-2-(3,4,5-trimethoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (3c) [22]: Light yellow solid; M.P: 197-198 0C; IR (KBr) v\textsubscript{max} 3274.90, 3197.96, 2964.39, 2929.67, 1654.81, 1620.09 cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-d\textsubscript{6}) δ 8.18 (s, 1H), 7.13 (s, 1H), 6.85 (s, 2H), 6.7 (s, 1H), 6.4 (s, 1H), 5.64 (s, 1H), 3.78 (s, 3H) ppm; 13C NMR (100 MHz, DMSO-d\textsubscript{6}) δ 163.44, 153.93, 146.60, 143.07, 141.58, 127.74, 127.54, 125.18, 125.15, 125.04, 109.74, 106.62, 97.99, 65.92, 55.74, 55.34 ppm; MS (ESI): m/z = 393.3157 found 393.3155.

6-chloro-2-(3,4,5-trimethoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (3d) [22]: Light yellow solid; M.P: 195-196 0C; IR (KBr) v\textsubscript{max} 3237.90, 3176.37, 2964.39, 2929.67, 1654.81, 1620.09 cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-d\textsubscript{6}) δ 8.09-8.07 (d, J=8Hz, 1H), 7.88-7.84 (tt, J=1.6Hz, 1H), 7.62-7.58 (m 3H), 7.35-7.32 (1H), 7.26-7.22 (tt, J= 8.4Hz,1H), 7.15 (s, 1H), 6.76-6.74 (d, J= 8Hz,1H), 6.69-6.62 (tt, J=8Hz,1H), 5.8 (s, 1H) ppm; 13C NMR (100 MHz, DMSO-d\textsubscript{6}) δ 163.52, 155.57, 149.54, 147.76, 142.41, 138.8, 137.2, 133.3, 127.34, 127.22, 126.45, 122.68, 120.29, 117.13, 114.97, 114.42, 66.18 ppm; MS (ESI): m/z = 301.122, HRMS (ESI) calcld for [C\textsubscript{14}H\textsubscript{13}ClN\textsubscript{2}O\textsubscript{2}]+ 302.3498, found 302.3495.

2-(4-tert-butylphenyl)-6-chloro-2,3-dihydroquinazolin-4(1H)-one (3e) [22]: Light yellow solid; M.P: 180-182 0C; IR (KBr) v\textsubscript{max} 3328.91, 3257.55, 2929.67, 1741.60, 1612.38 cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-d\textsubscript{6}) δ 8.38 (s, 1H), 7.53-7.52 (d, J=2.4Hz, 1H), 7.42-7.38 (m, 4H), 7.27-7.24 (m, 2H), 6.75-6.73 (d, J=8.4Hz, 1H), 5.73 (s, 1H), 1.26 (s, 9H) ppm; 13C NMR (100 MHz, DMSO-d\textsubscript{6}) δ 162.40, 151.13, 146.62, 138.18, 132.96, 126.59, 126.35, 126.20, 125.11, 120.61, 116.31, 116, 66.27, 34.28, 31.04 ppm; MS(ESI): m/z = 314.119. HRMS (ESI) calcld for [C\textsubscript{16}H\textsubscript{16}ClN\textsubscript{2}O\textsubscript{2}]+ 315.8172 found 315.8170.

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References


Conclusion

In conclusion, the cytotoxicity assay was used for preliminary screening of 2-aryl-2,3-dihydro quinazolin-4(1H)-ones. Among all the screened compounds, compound 3j was found to be active against human cancer cell lines at lower concentrations. Further live/dead fluorescent cell assay showed more red fluorescence scored as dead or apoptotic cells. Hence the compound 2-(bromo-5-fluorophenyl)-7-chloro-2,3-dihydroquinazolin-4(1H)-one 3j was subjected to in vivo studies EAC tumor models. In control, tumor section showed solid arrangement of neoplastic cells infiltrating between adjacent tissues. Tumor tissue treated with compound 3jshowing multifocal area of necrosis and apoptotic cells. Hence, 2-aryl-2,3-dihydro quinazolin-4(1H)-ones opens a new avenue for combating cancer.

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