Dihydroquinazolinones as Potential Antiproliferative and Tumor Inhibiting agents

Kereyagalhally H Narasimhamurthy, Hanumappa Ananda, Kothanahally S SharathKumar, Kanchugarakoppal S Rangappa*
Department of Studies in Chemistry, Manasagangotri, University of Mysore, India
Submission: July 11, 2016; Published: July 27, 2016
*Corresponding author: Kanchugarakoppal S Rangappa, Department of Studies in Chemistry, Manasagangotri, University of Mysore, Mysuru-570006, India, Tel: +91-821-2419662; Fax: +91-821-2500846 Email: rangappaks@chemistry.uni-mysore.ac.in

Abstract

2,3dihydroquinazolinone 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: 2,3dihydroquinazolinone; 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

Quinazolinones are nitrogen containing heterocycles and found in various naturally occurring alkaloid with wide range of biological activity [1,2] among different substituted quinazolinones, dihydroquinazolinones have been identified as potential anticancer agents, major reports are concerned with dihydroquinazolinones as inhibitors of tubulin depolymerisation [3-5] and as poly(ADP-ribose) polymerase-1 inhibitor [6]. Apart from this, dihydroquinazolinones exhibited different biological activities like antibacterial, [7] anticonvulsant, [8] antiemetic and gastrointestinal motility enhancing agents, [9] IMPDH inhibitors, [10] antifibrillatory activity, [11] antiinflammatory, [13,14] antitubercular activities, [15] potential inhibitors of SIRT1, [16] Antileishmanial agents [17] and antitumor agents [18].

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-dibromomethylenamines 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 ¹H 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 ¹³C NMR the carbonyl carbon C4 of the 2,3 dihydroquinazolinones ring appeared at 162.9-163.8 delta.

**Structures of molecules**

![Chemical structures](image)

**Supplementary Experimental Section**

**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 1x10⁵ 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 (5x10⁴ 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, Terrissian College, Mysuru. Tumor cell suspensions were dissolved in phosphate buffer saline (PBS), counted and re-injected (1×10^6 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 μM) 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 μM, respectively of tested compound 3j.

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

| Comp. code | Cell line IC50 in μM |
|-----------|-----------------------|
|           | HeLa | MCF7 | K562 |
| 3a        | 48.2 | 52.4 | 42.1 |
| 3b        | 39.7 | 42.3 | 39.4 |
| 3c        | 29.3 | 29.8 | 38.7 |
| 3d        | 32.4 | 35.6 | 49.7 |
| 3e        | 39.6 | 37.8 | 53.2 |
| 3f        | 49.1 | 49.1 | 53.2 |
| 3g        | 36.5 | 32.4 | 52.3 |
| 3h        | 29.8 | 49.8 | 62.1 |
| 3i        | 38.7 | 52.3 | 38.7 |
| 3j        | 17.1 | 31.5 | 25.3 |

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 integrity 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 μM 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. B&D) Compound 3j; damaged or broken cells showing fragmented DNA resulting in red fluorescence.](image-url)
Hence further we carried out in vivo tumor regression studies for the potent compound 3j (30 mg/kg) on mice bearing mouse breast adenocarcinoma tumor cells (EAC). The histopathology studies showed that morphology and cellular construction of the tissues (Figure 2A and B) was unaltered by the compound 3j treatment. Cell proliferations as well as multifocal area of necrosis and enumerable number of apoptotic cells were observed following treatment with compound 3j.

Experimental Details

Reactions were monitored by TLC using pre coated 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: 185-186°C; IR (KBr) \( \nu_{\text{max}} \): 364.2135. 

HRMS (ESI) calcd for [C114H115N4O6+H+] 375.3957, found 375.3954.

2-(4-tert-butylphenyl)-6-chloro-2,3-dihydroquinazolin-4(1H)-one (3b) [22]: Light yellow solid; M.P: 180-182°C; IR (KBr) \( \nu_{\text{max}} \): 3328.91, 3257.55, 2929.67, 1741.60, 1612.38 cm\(^{-1}\). 

HRM (ESI) calcd for [C114H115N4O6+H+] 302.3498, found 302.3495.

2-(3,4,5-trimethoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (3c) [22]: White solid; M.P: 208-210°C; IR (KBr) \( \nu_{\text{max}} \): 3193.9, 3068.53, 2923.88, 2854.45, 2813.95, 1646.38, 1604.57 cm\(^{-1}\). 

HRMS (ESI) calcd for [C114H115N4O6+H+] 363.2055, found 363.2055.
8.45 (s, 1H), 8.11-8.09 (d, J=8.4, 1H), 7.95-7.93 (d, J=8Hz, 1H), 7.89-7.84 (t, J=1.46, 1H), 7.62-7.56 (m, 4H), 7.43 (s, 1H), 7.36-7.33 (m, 1H), 6.8-6.79 (d, J=2Hz, 1H), 6.69-6.67 (dd, J=2Hz, 1H), 5.86 (s, 1H) ppm; 13C NMR (100 MHz, DMSO-d6): δ 162.63, 155.48, 149.53, 148.66, 142.04, 138.94, 137.81, 137.37, 137.21, 129.32, 128.35, 127.1, 126.54, 125.99, 120.29, 117.04, 113.62, 66.07 ppm; MS(ESI): m/z= 335.787, HRMS (ESI) calcd for [C_{10}H_{15}ClN,O+H^+] 336.7949, found 336.7945.

7-chloro-2-(2,5-dimethoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (3i) [22]: Light yellow solid; M.P: 197-198 0C; IR (KBr) v\textsubscript{max} 3330.84, 3288.4, 3234.4, 3060.82, 2962.46, 2867.95, 1643.24, 1608.52 cm\textsuperscript{-1}; 1H NMR (400 MHz, DMSO-d6): δ 8.33 (s, 1H), 7.94-7.76 (m, 4H), 7.66-7.62 (dd, J=2Hz, 1H), 7.29-7.22 (m, 2H), 6.81-6.68 (d, J=1.2Hz, 1H), 6.57-6.72 (dd, J=1.8Hz, 1H), 6.1 (s, 1H) ppm; 13C NMR (100 MHz, DMSO-d6): δ 162.91, 152.85, 150.36, 148.71, 137.74, 129.58, 129.21, 124.74, 116.92, 116.31, 115.3, 113.34, 112.24, 61.07, 55.99, 55.36 ppm; MS(ESI): m/z= 318.754, HRMS (ESI) calcd for [C_{14}H_{16}ClN,O+H^+] 319.7628, found 319.7628.

2-(4-tert-butylphenyl)-7-chloro-2,3-dihydroquinazolin-4(1H)-one (3j) [22]: Light yellow solid; M.P: 110-112 0C; IR (KBr) v\textsubscript{max} 315.8172, found 315.8170.

2-(2-bromo-5-flurophenyl)-7-chloro-2,3-dihydroquinazolin-4(1H)-one 3j was shown more red fluorescence scored as dead or apoptotic cells. Hence the compound2-(2-bromo-5-flurophenyl)-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-dihydroquinazolin-4(1H)-ones opens a new avenue for combating cancer.

Acknowledgement

We thankful to Prof. Suguna Rao, Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru for helping in in vivo studies. KSR is grateful to DST, CSIR, for the financial assistance under different projects. No. DST/INT/S-A/20-05/2011-12, Dated 10-01-12/INT/India-Korea/152-2011-12, Dated 13.09.2011.No. 01 (2434) /10/ EMR-II, Dated 28.12.2010. IFCPAR Sanction No. IFC/4303-1-2010 dated 22/12/2010. KINH & KSS is grateful to UGC, RFMS Govt. of India for Research fellowship. A.H. thanks ICMR for SRF (No 45/54/2013-PHA/BMS, Dated: 08.12.2014) for research fellowship.

References

1. Liverton NJ, Armstrong DJ, Claremon DA, Remy DC, Bakhun J, et al. (1998) Nonpeptide glycophorin L/IIIA inhibitors: Substituted quinazolinediones and quinazolinones as potent fibrinogen receptor antagonists. Bioorg & Med Chem Lett 8(5): 483-486.
2. de Laslze SR, Quagliato CS, Greenlee WJ, Patchett AA, Chang RSL, et al. (1993) A potent orally active balanced affinity angiotensin II AT1 antagonist and AT2 binding inhibitor. J Med Chem 36(21): 3207-3210.
3. Hour MJ, Huang LJ, Kuo SC, Xia Y, Bastow K, et al. (2006) 6-Alkylamino-2,3-Dihydro-2-(5-nitro-2-benzoxazepinyl)-quinazolinones and Related Compounds: Their Synthesis, Cytotoxicity and Inhibition of Tubulin Polymerization. J Med Chem 43(23): 4479-4487.
4. Kamal A, Bharathi EV, Reddy JS, Ramaiah M, Dastagir D, et al. (2011) Synthesis and biological evaluation of 3,5-diaryl isoazolone/isoazole linked 2,3-dihydroquinazoline hybrids as anticancer agents. J Med Chem 46(2): 691-703.
5. Chingio GM, Paige M, Grindrod S, Hamel E, Dakshnamurthy S, et al. (2008) Asymmetric Synthesis of 2,3-Dihydro-2-arylquinazolin-4-ones: Methodology and Application to a Potent Fluorescent Tubulin Inhibitor with Anticancer Activity. J Med Chem 51(15): 4620-4631.
6. Iwashita A, Tojo N, Matsaura S, Yamazaki S, Kamiyo K, et al. (2004) A novel and potent poly(ADP-ribose) polymerase-1 inhibitor, FR247304 (5-chloro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone) attenuates neuronal damage in in vitro and in vivo models of cerebral ischemia. J Pharma Exp Ther 310(2): 425-436.
7. Alaimo RJ, Russell HE (1972) Antibacterial 2,3-Dihydro-2-(5-nitro-2-thienyl)quinazolin-4(1H)-ones. J Med Chem 15(3): 335-336.
8. Bajaj K, Srivastava VK, Kumar A (2003) Newer substituted benzoaxepinyl-quinazolines as potent antipsychotic and anticonvulsant agents. Arzneimittel-Forschung 53(7): 480-485.
9. Baldazzi C, Barbanti M, Basaglia R, Benelli A, Bertolini A, et al. (1996) A new series of 6-chloro-2,3-dihydro-4(1H)-quinazolinone derivatives as antiepileptic and gastrointestinal motility enhancing agents. Arzneimittel-Forschung 46(9): 911-918.

10. Birch HL, Buckley GM, Davies N, Dyke HJ, Frost EJ, et al. (2005) Novel 7-methoxy-6-oxo-1,5-yl-2,3-dihydro-1H-quinazolin-4-ones as IMPDH inhibitors. Bioorg Med Chem Lett 15(23): 5335-5339.

11. Bonola G, Da Re P, Magistretti MJ, Massarani E, Setnikar I (1968) 1-Aminoacyl-2,3-dihydro-4(1H)-quinazolinone derivatives with choleretic and antiarrhythmic activity. J Med Chem 11(6): 1136-1139.

12. Buyuktimkin S, Buschauer A, Schunack W (1991) [Quinazolinones. 18. Synthesis and H1/H2-antihistaminic action of omega-[2-aryl-2,3-dihydro-4(1H)-quinazolinone-1-yl]alkyl substituted ureas and cyanoguanidines]. Arch Pharm (Weinheim) 324(5): 291-295.

13. Khalil MA, Soliman R, Farghaly AM, Bekhit AA (1994) Non-steroidal anti-inflammatory agents: novel pyrazolyl-1,2-oxazolyl and 1,3-diazinyl derivatives of 4(3H)-quinazolinones. Arch Pharm (Weinheim) 327(1): 27-30.

14. Manivannan E, Chaturvedi SC (2012) Analogue-based design, synthesis and docking of non-steroidal anti-inflammatory agents. Part 2: Methyl sulfonyl/methyl sulfinyl substituted 2,3-diaeryl-2,3-dihydro-1H-quinazolin-4(1H)-ones: their in vitro evaluation against chorismate mutase. Tetrahedron Lett 54(6): 495-501.

15. Rambabu D, Kiran Kumar S, Yogi Sreenivas B, Sanda S, Kandale A, et al. (2013) Ultrasound-based approach to spiro-2,3-dihydroquinazolin-4(1H)-ones: their in vitro evaluation against chorismate mutase. Bioorg Med Chem 20(24): 7119-7127.

16. Rambabu D, Raja G, Yogi Sreenivas B, Serrapu GP, Lalith Kumar K, et al. (2013) Spiro heterocycles as potential inhibitors of SIRT1: Pd/C-mediated synthesis of novel N-indolyloxymethyl spiroindoline-3,2'-quinazolines. Bioorg Med Chem Lett 23(5): 1351-1357.

17. Sharma M, Chauhan K, Shishhare R, Vishwakarma P, Suthar MK, et al. (2013) Discovery of a new class of natural product-inspired quinazolinone hybrid as potent antileishmanial agents. J Med Chem 56(11): 4374-4392.

18. Roopan SM, Nawaz Khan F, Jin JS, Senthil Kumar R (2011) An efficient one-pot-three component cyclocondensation in the synthesis of 2-(2-chloroquinolin-3-yl)-2,3-dihydroquinazolin-4(1H)-ones: potential antitumor agents. Res Chem Intermed 37(8): 919-927.

19. Sharath Kumar KS, Swaroop TR, Harsha KB, Narasimhamurthy KH, Rangappa KS, et al. (2012) T3P®-DMSO mediated one pot cascade protocol for the synthesis of 4-thiazolidinones from alcohols. Tetrahedron Letters 53(42): 5619-5623.

20. Narasimhamurthy KH, Chandrappa S, Kumar KSS, Swaroop TR, Rangappa KS, et al. (2013) Synthetic Utility of Propylyphosphonic Anhydride & ndash; DMSO Media: An Efficient One-pot Three-component Synthesis of 2-Arylquinolines. Chemistry Letters 42(9): 1073-1075.

21. Girish YR, Kumar KSS, Mudddegowda U, Lokanath NK, Rangappa KS, et al. (2014) ZrO2 2-supported Cu (ii)-β-cyclodextrin complex: construction of 2, 4, 5-trisubstituted-1, 2, 3-triazoles via azide–chalcone oxidative cycloaddition and post-triazole alkylation. RSC Advances 4(99): 55800-55806.

22. Narasimhamurthy KH, Chandrappa S, Sharath Kumar KS, Harsha KB, Rangappa KS, et al. (2014) Easy access for the synthesis of 2-aryl-2,3-dihydroquinazolin-4(1H)-ones using gem-dibromomethylarenes as synthetic aldehyde equivalent. RSC Advances 4(65): 34479-34486.

23. Srinivas V, Mohan CD, Baburajeev CP, Rangappa S, Jagadish S, et al. (2015) Synthesis and characterization of novel oxazines and demonstration that they specifically target cyclooxygenase 2. Bio org Med Chem Lett 25(15): 2931-2936.

24. Rangappa KS, Kumar KSS, Mohan CD, Jagadish S, Rakesh KS, et al. (2015) Synthesis and acetylcholinesterase/butyrylcholinesterase inhibition activity of arecoline, 4-thiazolidine and piperidine based conjugates. A J Pharma Clin Res 8(1).

25. Sharath Kumar KS, Hanumappa A, Hegde M, Narasimhamurthy KH, Raghavan SC, et al. (2014) Synthesis and antiproliferative effect of novel 4-thiazolidinone, pyridine and piperazine-based conjugates on human leukemic cells. E J Med Chem 81(0): 341-349.