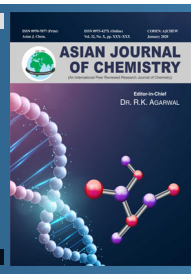
Asian Journal of Chemistry; Vol. 32, No. 10 (2020), 2617-2623

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22837



Synthesis and *in vitro* Cytotoxicity Study of Novel 4-Substituted Quinazolines Encompassed with Thiazolidinone and Azetidinone

AKASH JORI, SHESHAGIRI R. DIXIT and GURUBASAVRAJ V. PUJAR*

Department of Pharmaceutical Chemistry, J.S.S. College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru-570015, India

*Corresponding author: Fax: +91 821 2548359; E-mail: gvpujar@jssuni.edu.in

Received: 26 May 2020; *Accepted*: 18 July 2020; *Published online*: 25 September 2020; AJC-20079

A series of quinazolines encompassed with thiazolidinone and azetidinone have been synthesized and evaluated for their antioxidant, anticancer and DNA binding studies. All the synthesized compounds were characterized by IR, ^{1}H & ^{13}C NMR and mass spectra. Antioxidant activity was carried out using % free radical scavenging by DPPH assay. Compounds **4b**, **5b** and **5d** have shown better antioxidant activity (60, 67 and 66%, respectively) among the tested compounds. Compounds having % free radical scavenging activity more than 55% were evaluated for anticancer activity by MTT assay towards cell lines A-549 (lung carcinoma) and MDA-231 (human breast cancer). Results revealed that the tested compounds exhibited moderate to low anticancer activity. Further, DNA binding activity was studied by absorption titration method for all the synthesized compounds, and compound **5b** showed a good binding constant of 70.05 and % hyperchromicity of 82.93%.

Keywords: Quinzolines, Thiazolidinones, Azetidinones, *in vitro* anticancer activity, MTT assay.

INTRODUCTION

Today, cancer is a growing problem in developed and undeveloped countries and one of the principal causes of fatality around the globe [1]. Many researchers have focused to work on the development of newer anticancer agents by various approaches and among them one of most commonly employed strategies of new drug design is molecular exploitation involving the efforts to coalesce group having comparable activity or by eliminating or substituting new moiety to a parent lead compound. Structural variation brings about new physical, biological and chemotherapeutic properties in the lead molecule [2]. The improvement of multifunctional remedial tools, *i.e.* a drug candidate to interact with manifold distorted pathogenetic pathways for the multifactorial mechanistic nature of cancer cells, is of current interest.

A nitrogen-rich heterocyclic compound characterizes a distinctive category of chemicals with wide variety of biological activities and has been modified to the design novel pharmaceutically active compounds [3,4]. Among nitrogen containing heterocyclic compounds, quinazolines are known for their wide spectrum of biological activities like, anti-inflammatory [5], fungicidal [6], antituberculosis [7], antimalarial [8] and antiviral [9]. Several clinically useful drugs such as doxazosinmesylate, quinoxalinephthalazine, cinnoline, methotrexate, diproqualone, gefitinib possess quinazoline moiety. The anticancer activity of quinazoline derivatives is one of the most important properties because of their behaviour as a multi target molecules [10], some of the derivatives causes the polymerization of tubulin after interaction [11]. Numerous quinazolines impress apoptosis inducers or influence acute phase in the cell cycle [12], apart from these they are inhibitors of dihydrofolate reductase [13], topoisomerase I [14], checkpoint kinase [15], and protein kinase [16] such as gefitinib. In view of these findings on quinazolines, herein the synthesis, characterization of novel quinazoline moiety linked with thiazolidin-4-ones and azetidin-2-one and evaluated for *in vitro* antioxidant, anticancer activities and DNA binding studies is proposed.

EXPERIMENTAL

The reagents used for synthesis were of laboratory grade and solvents of analytical grade. The melting point of the synthesized compounds was determined in open capillary method

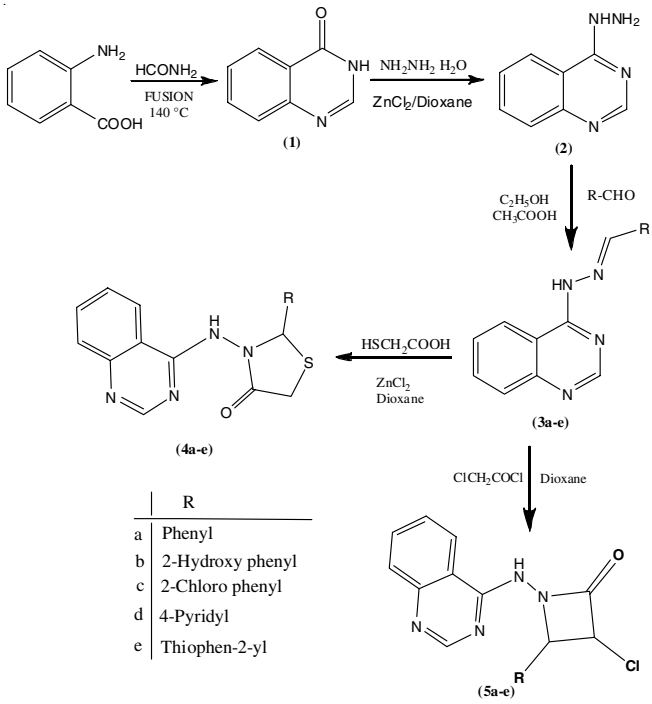
and are uncorrected. A periodic monitoring of the reaction was made through TLC with the solvent system toluene:ethyl acetate (3:7). IR spectra were recorded on Shimadzu FT-IR 8400-S spectrophotometer by KBr pellet technique and are expressed in cm^{-1}. UV absorbance was recorded using Shimadzu-1800 UV Spectrophotometer. ^1H NMR spectra were recorded on Bruker 400 MHz FT-NMR spectrophotometer using DMSO (d_6) and $CDCl_3$ as the solvent and TMS as internal standard (δ ppm). Mass spectra were recorded on GC-MS with electron impact ionization technique.

General procedure for the synthesis of quinazoline-4(3*H*)-one (1): An equimolar quantity of anthranilic acid (0.01 mol) and formamide (0.01 mol) were fused together at 140 ºC for 2 h. Further, the resulting reaction mixture was cooled to room temperature and extracted with ethyl acetate. The organic layer was treated with sodium bicarbonate to remove the excess of anthranilic acid and the organic layer was evaporated to dryness under vacuum to obtain solid product, dried and recrystallized from ethanol to get quinazolin-4(3*H*)-one (**1**) [17].

General procedure for the synthesis of 1-(quinazolin-4-yl) hydrazine (2): 0.01 mol of quinazoline-4(3*H*)-one (**1**), hydrazine hydrate (0.02mol) and zinc chloride (1.0 g) were fused at 150 ºC for 8 h. Then the reaction mixture was poured in dichloromethane with stirring and solid product formed was filtered, washed with cold water to remove the excess of zinc chloride, dried and recrystallized from ethanol to gave 1-(quinazolin-4-yl) hydrazine (**2**). Yield: 72%, m.p.: 132-136 ºC, m.w.: 160. FT-IR (KBr, ν_{max}, cm^{-1}): 3450 (NH *str.*); 2924 (Ar-H); 1620 (Ar-C=C); 1543 (C=N). ^1H NMR (400 MHz, DMSO-d_6) (δ, ppm): 9.01 (s, 1H, C_2-H), 7.38 (m, 2H, C_6, C_7-H), 6.98 (m, 2H, C_5, C_8-H), 5.42 (m, 3H, C_4-H and 2-NH), 4.20 (s, 2H, -NH_2). Mass (GC-MS) *m/z* = 161 (M+1)

General procedure for the synthesis of (*E*)-4-(2-benzylidenehydrazinyl)quinazoline (3a): To an equimolar concentration solution of 1-(quinazolin-4-yl)hydrazine (**2**), substituted benzaldehyde in ethanol and catalytic amount of glacial acetic acid was added. Then the mixture was refluxed for 2-4 h on water bath. The completion of reaction was monitored by TLC. The excess of solvent was distilled off and then remaining residue was poured in to ice cold water. The separated solid was filtered, washed and recrystalized from ethanol to give 2-benzylidene-1-(quinazolin-4-yl)hydrazine (**3a**) (**Scheme-I**). A similar procedure was followed to synthesize other quinazoline Schiff bases (**3b-e**) using appropriate aldehydes. Yield: 60%, m.p.: 140-143 ºC, m.w.: 248. FT-IR (KBr, ν_{max}, cm^{-1}): 3039 (Ar-H); 1689 (N=CH); 1620 (Ar C=C), 1311 (C-N). ^1H NMR (400 MHz, DMSO-d_6): (δ, ppm) 10.48 (s, 1H, NH), 8.40 (s, 2H, quinazoline C_2-H and N=CH), 7.80-8.20 (m, 5H, quinazoline C_5, C_7, C_8-H and ph C_2, C_6-H), 7.50-7.65 (m, 4H, quinazoline C_6-H and ph C_3, C_4, C_5-H). ^{13}C NMR (100 MHz, DMSO-d_6): (δ, ppm) 170, 157, 150, 145, 134, 130, 129, 128, 126, 117. Mass (GC-MS) *m/z* = 249 (M+1).

4-(2-(2-Hydroxybenzylidene)hydrazinyl)quinazoline (3b): Yield: 58%, m.p.: 220-222 ºC, m.w.: 264. FT-IR (KBr, ν_{max}, cm^{-1}): 3600 (OH); 3424 (NH); 3008 (Ar-H); 1612 (N=CH). ^1H NMR (400 MHz, DMSO-d_6): (δ, ppm) 11.40 (s, 2H, -OH and NH), 8.54 (s, 2H, N=Ch and quinazoline C_2-H), 7.90-8.25 (m, 3H, quinazoline C_5, C_7, C_8-H), 6.95-7.68 (m, 5H, quinazoline C_6-H and 2-OHph C_3, C_4, C_5, C_6-H). ^{13}C NMR (100 MHz, DMSO-d_6): (δ, ppm) 170, 156, 150, 144, 133, 129, 127, 115. Mass (GC-MS) *m/z* = 265 (M+1).



Scheme-I

4-(2-(2-Chlorobenzylidene)hydrazinyl)quinazoline (3c): Yield: 65%, m.p.: 155-158 ºC, m.w.: 282. FT-IR (KBr, ν_{max}, cm^{-1}): 3448 (NH), 3039 (Ar-H), 1620 (N=CH), 1556 (Ar C=C), 1306(C-N), 748 (Cl). ^1H NMR (400 MHz, DMSO-d_6): (δ, ppm) 10.66 (s, 1H, NH), 8.50 (s, 1H, quinazoline C_2-H), 8.10-8.28 (m, 2H, quinazoline C_8-H and N=CH), 7.24-7.86 (m, 7H, quinazoline C5, C6, C7-H and 2-Clph C_3, C_4, C_5, C_6-H). Mass (GC-MS) *m/z* = 283 (M+1).

4-(2-(Pyridin-4-ylmethylene)hydrazinyl)quinazoline (3d): Yield: 63%, m.p.: 200-202 ºC, m.w.: 249. FT-IR (KBr, ν_{max}, cm^{-1}): 3425 (NH); 3047 (Ar-H); 1612(N=CH); 1530 (Ar C=C); 1311(C-N). ^1H NMR (400 MHz, DMSO-d_6): (δ, ppm) 10.50 (s, 1H, NH), 8.66 (s, 2H, pyridine C_2, C_6-H), 8.40 (s, 2H, N=CH and quinazoline C_2-H), 7.60-8.08 (m, 6H, pyridine C_2, C_6-H and quinazoline C_5, C_6, C_7, C_8-H).

4-(2-(Thiophen-2-ylmethylene)hydrazinyl)quinazoline (3e): Yield: 62%, m.p.: 170-173 ºC, m.w.: 254. FT-IR (KBr, ν_{max}, cm^{-1}): 3386 (NH), 3070 (Ar-H), 1612 (N=CH), 1535 (Ar C=C), 1311 (C-N), 1265 (C=S). ^1H NMR (400 MHz, DMSO-d_6): (δ, ppm) 10.52 (s, 1H, NH), 8.66 (s, 1H, quinazoline C_2-H), 8.25 (s, 2H, quinazoline C_8-H and N=CH), 7.80-7.88 (m, 3H, thiophen C_5-H and quinazoline C_5, C_7-H), 7.60 (s, 2H, thipohen C_3-H and quinazoline C_6-H), 7.24 (s, 1H, thiophen C_4-H).

General procedure for the synthesis of 2-phenyl-3-(quinazolin-4-yl amino)thiazolidin-4-one (4a): A mixture of 0.01 mol of 2-benzylidene-1-(quinazolin-4-yl) hydrazine (**3a**) and thioglycolic acid (0.02 mol) in presence of zinc chloride and 1,4-dioxane were refluxed for 12 h. The completion of

reaction was monitored by TLC. After completion, reaction mass was poured onto ice cold water. The formed crude product was filtered, washed with water and recrystallized from ethanol to give 2-phenyl-3-(quinazolin-4-ylamino)thiazolidin-4-ones (**4a**). A similar procedure was followed to synthesize other 2-substituted-3-(quinazolin-4-ylamino) thiazolidin-4-ones (**4b-e**) using appropriate Schiff base. m.p.: 118-120 ºC. Yield: 62%, m.w.: 322. FT-IR (KBr, ν_{max}, cm^{-1}): 3630 (NH str), 2996 (Ar-H), 1637 (C=O), 1565 (Ar C=C), 1232 (C-S), 830 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 8.40 (s, 2H, quinazoline C_2-H and -NH), 7.83 (s, 2H, quinazoline C_5, C_7-H), 7.48 (s, 1H, quinazoline C_6-H), 7.38 (m, 5H, Ar-H), 6.02 (s, 1H, thiazolidinone C_2-H), 3.75 (m, 2H, thiazolidinone C_4-H). ^{13}C NMR (400 MHz, DMSO-d_6): (δ, ppm) 168, 155, 148, 140, 131, 129, 128, 126, 115, 66, 36. Mass (GC-MS) *m/z* = 323 (M+1).

2-(2-Hydroxyphenyl)-3-(quinazolin-4-ylamino)thiazolidin-4-one (4b): Yield: 60%, m.p.: 205-208 ºC, m.w.: 338. FT-IR (KBr, ν_{max}, cm^{-1}): 3630 (OH), 3416 (NH), 3045 (Ar-H), 1620 (C=O), 1582 (N=CH), 1572 (Ar C=C), 1240 (C-S), 856 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 9.50 (s, 1H, OH), 8.45 (s, 2H, quinazoline C_2-H and -NH), 8.10 (s, 1H, quinazoline C_8-H), 7.75 (s, 2H, quinazoline C_5, C_7-H), 7.40 (s, 1H, quinazoline C_6-H), 7.11 (s, 2H, 2-OHph C_4, C_6-H), 6.80 (s, 2H, 2-OHph C_3, C_5-H), 5.80 (s, 1H, thiazolidinone C_2-H), 3.75 (s, 2H, thiazolidinone C_4-H). Mass (GC-MS) *m/z* = 339 (M+1).

2-(2-Chlorophenyl)-3-(quinazolin-4-ylamino)thiazolidin-4-one (4c): Yield: 60%, m.p.: 136-138 ºC, m.w.: 356. FT-IR (KBr, ν_{max}, cm^{-1}): 3676 (NH), 3049 (Ar-H), 1682 (C=O), 1487 (Ar C=C), 1271 (C-S), 854 (C-N), 748 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 8.50 (s, 1H, quinazoline C_2-H), 8.25 (s, 1H, NH and quinazoline C_8-H), 7.90-7.94 (s, 2H, quinazoline C_5, C_7-H), 7.65-7.62 (m, 2H, 2-OHph C_3-H and quinazoline C_6-H); 7.11-7.18 (m, 3H, quinazoline C_4, C_5, C_6-H), 5.76 (s, 1H, thiazolidinone-C_2-H); 3.55-3.61 (m, 2H, thiazolidinone-CH_2). ^{13}C NMR (400 MHz, DMSO-d_6): (δ, ppm) 170, 157, 150, 133, 131, 128, 127, 125, 117, 105, 60, 36. Mass (GC-MS) *m/z* = 355 (M-1).

2-(Pyridin-4-yl)-3-(quinazolin-4-ylamino)thiazolidin-4-one (4d): Yield: 70%, m.p.: 180-182 ºC, m.w.: 323. FT-IR (KBr, ν_{max}, cm^{-1}): 3446 (NH), 3068 (Ar-H), 1680 (C=O), 1541 (Ar C=C), 1269 (C-S), 840 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 8.65-8.50 (m, 3H, pyridine C_2, C_6-H and quinazoline C_2-H), 8.25 (s, 2H, quinazoline C_8-H and NH), 7.80-7.84 (m, 2H, quinazoline C_5, C_7-H), 7.30-7.60 (m, 3H, pyridine C_3, C_5-H and quinazoline C_6-H), 6.02 (s, 1H, thiazolidinone C_2-H), 3.75-3.80 (m, 2H, thiazolidinone C_4-H). Mass (GC-MS) *m/z* = 323 (M^+).

3-(Quinazolin-4-ylamino)-2-(thiophen-2-yl)thiazolidin-4-one (4e): Yield: 70%, m.p.: 154-157 ºC, m.w.: 328. FT-IR (KBr, ν_{max}, cm^{-1}): 3402(NH), 3043 (Ar-H), 1610 (C=O), 1552 (Ar C=C), 1234 (C-S), 830 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 8.50 (s, 1H, quinazoline C_2-H), 8.25-8.30 (m, 2H, quinazoline C_8-H and NH), 7.35-7.80 (m, 4H, quinazoline C_5, C_6, C_7-H and thiophene C_5-H), 6.80 (s, 2H, thiophene C_3, C_4-H), 5.80 (s, 1H, thiazolidinone C_2-H), 3.75-3.80 (m, 2H, thiazolidinone C_4-H).

General procedure for the synthesis of 3-chloro-4-phenyl-1-(quinazolin-4-ylamino)azetidin-2-one (5a): An equimolar quantity of 2-benzylidene-1-(quinazolin-4-yl) hydrazine (**3a)** and chloroacetyl chloride were dissolved in minimum quantity of 1,4-dioxane was stirred for 48 h at room temperature. The completion of reaction was monitored by TLC. The solid obtained was filtered, dried and recrystallized from absolute ethanol to give 3-chloro-4-phenyl-1-(quinazolin-4-ylamino)azetidin-2-one (**5a**). A similar procedure was followed to synthesize other 3-chloro-4-substituted-1-(quinazolin-4-ylamino)-azetidin-2-one (**5b-e**) using appropriate Schiff bases. Yield: 70%, m.p.: 120-122 ºC, m.w.: 324. FT-IR (KBr, ν_{max}, cm^{-1}): 3345 (NH str), 3030 (Ar-H), 1676(C=O), 1538 (Ar C=C), 1350 (C-N), 698 (Cl). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 8.50 (s, 1H, quinazoline C_2-H), 8.25-8.30 (m, 2H, quinazoline C_8-H and NH), 7.67-7.52 (m, 3H, quinazoline C_5, C_6, C_7-H), 7.20-7.38 (m, 5H, Ar-H), 5.30 (s, 1H, azetidinone C_3-H), 4.99 (s, 1H, azetidinone C_4-H). Mass (GC-MS) *m/z* = 323 (M-1).

3-Chloro-4-(2-hydroxyphenyl)-1-(quinazolin-4-ylamino)azetidin-2-one (5b): Yield: 60%, m.p.: 210-212 ºC, m.w.: 340. FT-IR (KBr, ν_{max}, cm^{-1}): 3400 (NH), 3043 (Ar-H), 1650 (C=O), 1550 (Ar C=C), 790 (C-N), 704 (Cl). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 10.03 (s, 1H, OH), 8.40 (s, 1H, quinazoline C_2-H), 7.80-7.99 (m, 5H, quinazoline C_5, C_6, C_7, C_8-H and NH), 6.80-7.15 (m, 4H, 2-OHph C_3, C_4, C_5, C_6-H), 5.30 (s, 1H, azetidinone C_3-H), 4.99 (s, 1H, azetidinone C_4-H). Mass (GC-MS) *m/z* = 340 (M^+).

3-Chloro-4-(2-chlorophenyl)-1-(quinazolin-4-ylamino)azetidin-2-one (5c): Yield: 60%, m.p.: 130-132 ºC, m.w.: 358. FT-IR (KBr, ν_{max}, cm^{-1}): 3446 (NH), 3070 (Ar-H), 1664 (C=O), 1585 (Ar C=C), 1271 (C-N), 700 (Cl). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 8.50 (s, 1H, quinazoline C_2-H), 8.23 (s, 2H, NH and quinazoline C_8-H), 7.91-7.58 (m, 4H, quinazoline C_5, C_6, C_7-H and 2-Clph C_3-H), 7.18-7.22 (m, 3H, 2-Clph C_4, C_5, C_6-H), 5.30 (s, 1H, azetidinone C_3-H), 4.99 (s, 1H, azetidinone C_4-H). Mass (GC-MS) *m/z* = 358 (M^+).

3-Chloro-4-(pyridin-4-yl)-1-(quinazolin-4-ylamino)-azetidin-2-one (5d): Yield: 60%, m.p.: 173-175 ºC, m.w.: 325. FT-IR (KBr, ν_{max}, cm^{-1}): 3445 (NH), 3070 (Ar-H), 1635 (C=O), 1535 (Ar C=C), 1284 (C-N), 794 (Cl). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 8.55-8.50 (m, 3H, quinazoline C_2-H and pyridine C_2, C_6-H), 8.23 (s, 2H, NH and quinazoline C_8-H), 7.91 (s, 2H, quinazoline C_5, C_7-H), 7.30-7.52 (m, 3H, pyridine C_3, C_5-H), 5.30 (s, 1H, azetidinone C_3-H), 4.99 (s, 1H, azetidinone C_4-H). ^{13}C NMR (100 MHz, DMSO-d_6): (δ, ppm) 170, 157, 148, 132, 128, 126, 123, 115, 68, 65. Mass (GC-MS) *m/z* = 325 (M^+).

3-Chloro-1-(quinazolin-4-ylamino)-4-(thiophen-2-yl)-azetidin-2-one (5e): Yield: 70%, m.p.: 165-168 ºC, m.w.: 330. FT-IR (KBr, ν_{max}, cm^{-1}): 3331 (NH), 2922 (Ar-H), 1633 (C=O), 1568 (Ar C=C), 779 (C-N), 704 (Cl). ^{1}H NMR (400 MHz, DMSO-d_6): (δ, ppm) 8.55-8.50 (m, 1H, quinazoline C_2-H), 8.23 (s, 2H, NH and quinazoline C_8-H), 7.88 (s, 2H, quinazoline C_5, C_7-H), 7.30-7.52 (m, 2H, quinazoline C_6-H and thiophen C_5-H), 7.10 (s, 2H, thiophen C_3, C_4-H), 5.30 (s, 1H, azetidinone C_3-H), 4.99 (s, 1H, azetidinone C_4-H). ^{13}C NMR (100 MHz, DMSO-d_6): (δ, ppm) 170, 165, 157, 148, 132, 129, 128, 127, 115, 68, 65. Mass (GC-MS) *m/z* = 331 (M+1).

Biological activity

***in vitro* DPPH radical scavenging assay:** All the synthesized molecules were analyzed for antioxidant activity using DPPH assay method. To 1 mL of synthesized compounds (40 µg/mL) mixed with 1 mL DPPH solution (40 µg/mL) in methanol and incubated at 37 ºC for 30 min and the control was prepared as above without sample was used for the baseline correction. The absorption of each solution was measured at 517 nm [18]. Experiment was performed in triplicate.

$$\text{Radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where control absorbance is the measurement of DPPH radical solution without compound and sample absorbance is the measurement of DPPH radical solution with the compound. Ascorbic acid (40 µg/mL) solution was used as a standard for the comparison of antioxidant activity.

MTT based cytotoxicity activity: Cellular conversion of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide] into a formazan product [19] was used to evaluate cytotoxic activity (IC_{50}) of the compounds against A549 (lung adenocarcinoma) and MDA 231 (breast cancer) cell-line up to concentrations ranging from 31.25-500 µg/mL using Promega Cell Titer 96 non-radioactive cell proliferation assay [20] with cisplatin as the positive control. The IC_{50} values are the averages ± SEM of three independent experiments.

A-549 (Lung carcinoma) and MDA-231 (breast cancer) cell lines were plated 5000-10,000 cells/well in 100 µL was seeded into the wells of 96 well plate. The plates were incubated overnight in a humidifier air atmosphere at 37 ºC with 5% CO_2. After overnight incubation, 100 µL of the compounds in DMSO were added to each well containing media and missed thoroughly at 150 rpm for 5 min. The plates were incubated further 48 h to allow the drug to produce effect. After completion of the treatment, 20 µL MTT solution was added to each well and mixed well at 150 rpm for 5 min followed by 3 h incubation. After incubation, the media was then replaced with 200 µL DMSO and the absorbance of each well was measured at 570 nm. For each compound, the concentration causing 50% cell growth inhibition (IC_{50}) compared with the control was calculated from concentration response curves by regression analysis.

DNA binding activity by absorption titration: The spectrometric titration was conducted by UV spectrophotometer at room temperature. The CT-DNA was dissolved in double distilled de-ionized water with 50 mM NaCl and dialyzed against buffer solution for 2 days. Its concentration was determined by absorption spectrometry at 340 nm using a molar extinction coefficient 6600 M^{-1} cm^{-1}. The ratio $A_{325}/A_{355} > 1.80$ was used as an indication of a protein-free DNA. Absorption titration was performed at a fixed concentration of drugs (10 µM) in a sodium phosphate buffer (20 mM sodium phosphate, 150 mM NaCl, pH 74). Small aliquots of concentrated CT-DNA (5 mM) were added into the solution at final concentrations from 0 to 100 mM and stirred for 5 min before measurement. The parameters, hypochromicity and binding constant were found from the absorption spectra. The intrinsic binding constant (K_i) for a given complex with DNA was obtained from a plot of $D/\Delta\varepsilon_{app}$ *versus* D according to the following equation:

$$\frac{D}{\Delta\varepsilon_{app}} = \frac{D}{\Delta\varepsilon} + \frac{1}{\Delta\varepsilon \times K}$$

where D = concentration of DNA in base molarities.

$$\Delta\varepsilon_{app} = |\varepsilon_a - \varepsilon_f| \text{ and } \Delta\varepsilon = |\varepsilon_b - \varepsilon_f|$$

where ε_a and ε_f are respective extinction coefficient of the complex in the presence and absence of DNA. The apparent extinction coefficient ε_a is obtained by calculating A_{obs}/[Drug]. The data were fitted to the equation with a slope equal to $1/\Delta\varepsilon$ and Y-intercept equal to $1/(\Delta\varepsilon \times K)$. The intrinsic binding constant (K_i) is determined from the slope of Y-intercept. The percentage hypochromism was calculated as follows [21]:

$$\text{Hypochromism (\%)} = \frac{\varepsilon_{free} - \varepsilon_{bound}}{\varepsilon_{free}} \times 100$$

RESULTS AND DISCUSSION

A series of quinazoline encompassed with thiazolidinones (**4a-e**) and azetidinones (**5a-e**) were synthesized using synthetic procedure as per **Scheme-I**. The precursor, quinazoline-4(3*H*)-one (**1**) was synthesized by fusing anthranilic acid with formamide which follows Niementowski reaction, further the formation of hydrazide (**2**) was straight forward by reacting it with hydrazine hydrate in presence of $ZnCl_2$ and 1,4-dioxane. Hydrazide derivative (**2**) was then made to react with different aldehydes in presence of ethanol and AcOH to yield quinazoline Schiff bases (**3a-e**). Schiff bases (**3a-e**) were then made to react with thioglycolic acid and chloroacetyl chloride to respectively yield thiazolidinones (**4a-e**) and azetidinones (**5a-e**) and compounds were characterized.

FTIR spectrum of compound **3a**, as a representative of Schiff bases, showed absorption band at 1689 cm^{-1} indicates the presence of -N=CH- of Schiff base, further the structure of compound **3a** was confirmed by ^{1}H NMR, which showed a singlet peak at 10.48 ppm representing the proton of -NH group and a singlet peak appeared at 8.40 ppm indicating the presence of quinazoline C_2-H and imine group (-N=CH-) and mass spectra confirms the formation of compound **3a**, a molecular ion peak at 249 (M+1).

FTIR spectrum of compound **4c**, showed absorption band at 3676 cm^{-1} attributed to the presence of -NH, further presence of carbonyl and C-S group were confirmed by the peaks at 1682 and 1271 cm^{-1}, respectively. Further the structure of compound **4c** was confirmed by ^{1}H NMR which showed the presence of 2 protons of thiazolidinone-CH_2 as a multiplet between δ 3.55-3.61 ppm and a singlet for a proton of thiazolidinone-CH was observed at 5.76 ppm. Further, mass spectra showed a molecular ion peak at 355 (M-1).

FTIR spectrum of compound **5c** showed absorption band at 3446 cm^{-1}, which attributed to the -NH stretching and a peak at 1664 cm^{-1} for carbonyl group. Further, structural **5c** was confirmed by ^{1}H NMR which showed a singlet peaks at 5.30 ppm and 4.99 ppm indicating C_3 & C_4 protons, respectively of azetidinone. Further, mass spectra showed a molecular ion peak at 355 (M+).

Biological activity

***in vitro* DPPH radical scavenging assay:** *in vitro* DPPH radical scavenging assay was performed spectrophotometrically with ascorbic acid as positive control. The activity of quinazoline derivatives was compared with standard ascorbic acid (Table-1). The synthesized compounds showed significant free radical scavenging activity ranging from 51-67% while standard, ascorbic acid has 91% (Fig. 1). Compounds **4b**, **5b**, **5c** and **5d** have shown good antioxidant activity than the rest of the compounds. The compounds exhibited more than 55% of radical scavenging activity were selected for *in vitro* anticancer activity by MTT assay method. This test was performed to understand the radical scavenging activity of the synthesized compounds as this activity is associated with anti-proliferative property of molecules.

TABLE-1
ANTIOXIDANT ACTIVITY DATA OF THE SYNTHESIZED COMPOUNDS

Compounds	% Free radical scavenging activity at 40 µg/mL
4a	51
4b	64
4c	57
4d	59
4e	56
5a	52
5b	67
5c	60
5d	66
5e	58
Ascorbic acid (std)	91

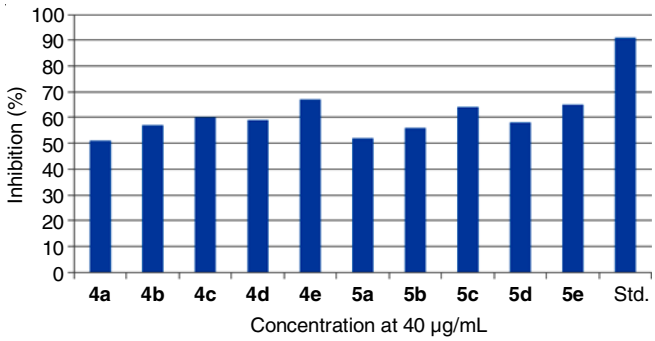


Fig. 1. *in vitro* free radical scavenging activity of the synthesized compounds

MTT based cytotoxicity activity: The selected synthesized compounds (**4b**, **4c**, **4d**, **4e**, **5b**, **5c**, **5d** and **5e**) were evaluated for *in vitro* anticancer activity against two human cancer cell lines, respectively A-549 (lung carcinoma) and MDA-231 (human breast cancer). The percentage cytotoxicity for the tested compounds was calculated as per the MTT standard protocol using cisplatin as standard drug. The IC_{50} values reported in Table-2 indicates that thiazolidinone derivatives are comparatively shows less significant activity whereas the azitidinone derivatives in particular compound **5b** exhibited better cytotoxic activity against all the tested cell lines.

DNA binding activity: The binding properties of all the synthesized compounds were evaluated by UV absorptiometry

TABLE-2
% CYTOTOXICITY ACTIVITY OF THE TESTED COMPOUNDS

Compound	A-549 (Lung carcinoma)	MDA-231 (Breast cancer)
Control	–	–
Cisplatin	9.9	8.0
4b	153.20	149.06
4c	144.08	138.39
4d	142.91	146.19
4e	134.77	142.91
5b	127.25	137.40
5c	137.77	145.14
5d	129.25	139.41
5e	134.73	145.37

method using CT-DNA. This study was performed to study the intercalation of molecule with DNA as they are analogues of quinaolines. To examine this aspect, the intercalation of synthesized compounds with duplex DNA was examined by monitoring the changes in the UV-visible spectra of quinazoline analogues upon addition of CT-DNA. The DNA binding properties of the compounds were studied by monitoring the change in the absorption spectra of the quinazoline analogues upon addition of CT-DNA.

As shown in Figs. 2-4, the absorption spectra of compounds **4b**, **5b** and **5d**, respectively in the absence and presence of varying amount of CT-DNA. The percentage hypochromism was found to be 52.46, 73.54 and 82.93. Similarly, the absorption spectra of all the compounds were also obtained. The binding of the compounds to duplex DNA led to strong decrease in the absorption intensities in the absorption maxima. The progressive addition of DNA leads to strong hypochromism in the absorption intensity. The half reciprocal plots for binding of compounds **4b**, **5b** and **5d** with CT-DNA are shown in Figs. 5-7. The binding constants (K_i) of synthesized compounds were in the following order **4a > 4d > 4e > 4b > 4c > 5a > 5b > 5e > 5c > 5d**. The tested compounds showed good to moderate DNA binding activity ranging from 49.31 to 80. The % hyperchromicity and DNA binding constant value are given in Table-3.

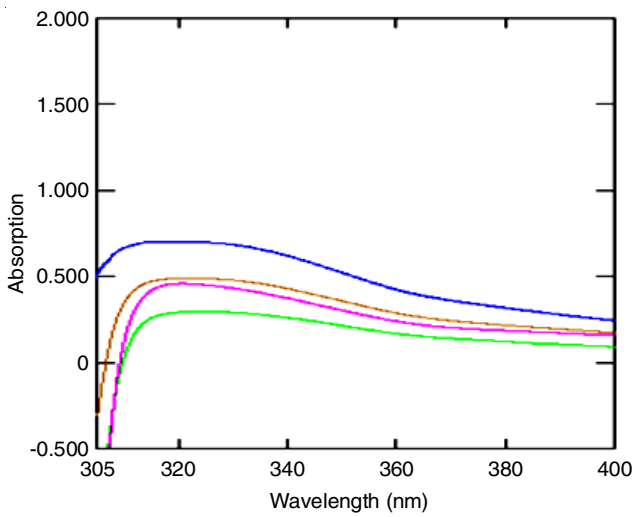


Fig. 2. Absorption titration of **4b** at 10 µM in 20 mM sodium phosphate buffer with 150 mM NaCl at increasing CT-DNA concentration

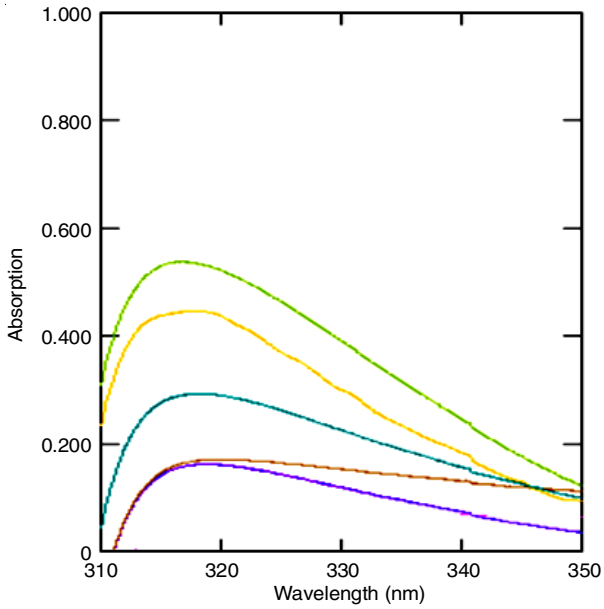


Fig. 3. Absorption titration of **5b** at 10 µM in 20 mM sodium phosphate buffer with 150 mM NaCl at increasing CT-DNA concentration

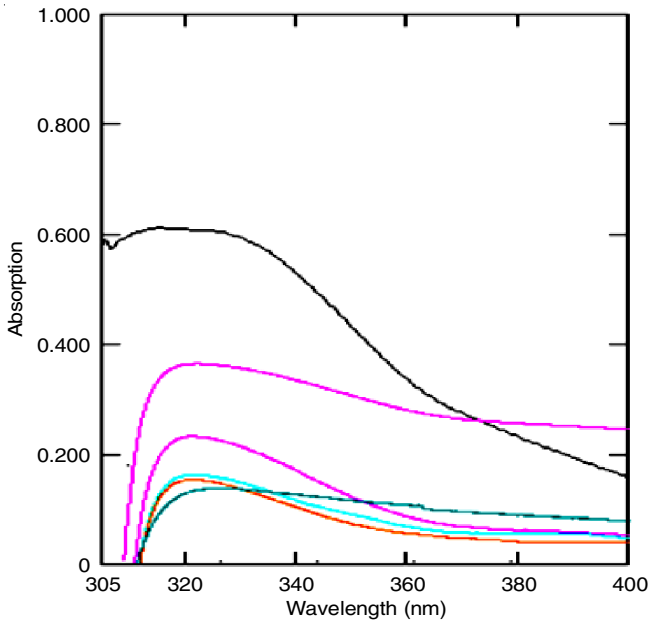


Fig. 4. Absorption titration of **5d** at 10 µM in 20 mM sodium phosphate buffer with 150 mM NaCl at increasing CT-DNA concentration

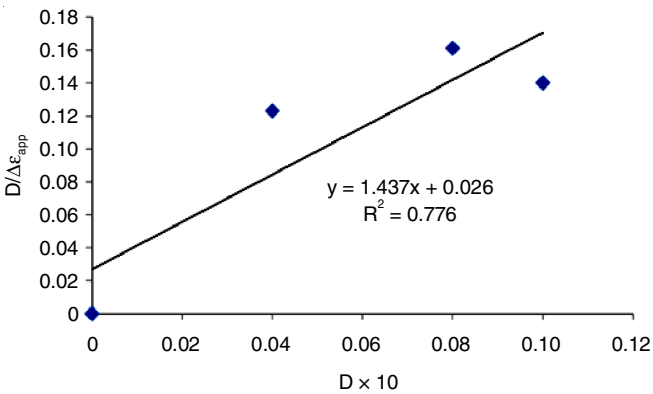


Fig. 5. Half reciprocal plot for binding of **4b** with CT-DNA

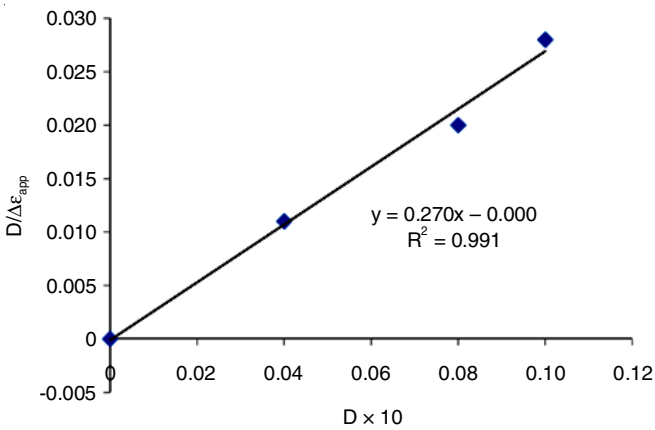


Fig. 6. Half reciprocal plot for binding of **5b** with CT-DNA

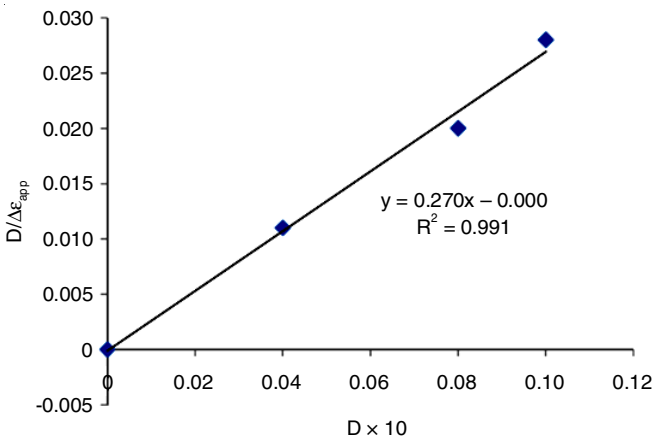


Fig. 7. Half reciprocal plot for binding of **5d** with CT-DNA

TABLE-3
DNA BINDING RESULTS OF SYNTHESIZED COMPOUNDS

Compound	% Hyperchromicity	K_i
4a	51.32	49.31
4b	53.12	56.28
4c	52.46	55.32
4d	48.54	52.34
4e	51.62	54.23
5a	68.32	62.56
5b	82.93	70.05
5c	71.98	63.54
5d	73.54	68.75
5e	67.89	64.55

Conclusion

The present study was focused on the synthesis and characterization of new quinazolines encompassed with thiazolidiones and azetidinones with the hope of developing new chemical entities serving as potential lead for anticancer agents. The antioxidant, anticancer and DNA binding studies were also evaluated. The results of antioxidant activity of the compounds **4b**, **5b** and **5d** (64, 67 and 66%, respectively @ 40 µg/mL) showed a better % free radical scavenging activity then other synthesized compounds as compared to standard, ascorbic acid has 91% @ 40 µg/mL. The *in vitro* cytotoxic potential of the compounds were tested against two human cancer cell lines namely, A-549 lung carcinoma and MDA-231 breast carcinoma

by using MTT based assay method. Compound **5b** was found to be more active than other compounds in comparison with standard, cisplatin. Further, DNA binding assay was studied to understand the possible role of cytotoxic nature of the compounds by DNA affinity and studies indicated that compound **5b** have showed better binding constant among all the synthesized compounds. From the results, it was observed that in azetidinone, 4th position is substituted with hydroxy phenyl group shows a better activity as compared to the substitution with unsubstituted phenyl ring or heterocyclic compounds, which showed comparatively less active. In case of thiazolidinones, 2nd position of ring is substituted with unsubstituted aromatic ring or heterocyclic ring decreases the activity as compared to substitution with hydroxyphenyl ring.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. R.L. Siege, K.D. Miller and A. Jemal, *CA Cancer J. Clin.*, **66**, 7 (2016); https://doi.org/10.3322/caac.21332
2. J. Baselga and S.M. Swain, *Nat. Rev. Cancer*, **9**, 463 (2009); https://doi.org/10.1038/nrc2656
3. A. Gomtsyan, *Chem. Heterocycl. Compd.*, **48**, 7 (2012); https://doi.org/10.1007/s10593-012-0960-z
4. M.J. Camarasa, ed.: J.J. Li, Heterocyclic Chemistry in Drug Discovery. Wiley & Sons: Hoboken, N.J., pp. 8-16 (2014).
5. A.A. Abdel-Aziz, L.A. Abou-Zeid, K.E. ElTahir, M.A. Mohamed, M.A. Abu El-Enin and A.S. El-Azab, *Bioorg. Med. Chem.*, **24**, 3818 (2016); https://doi.org/10.1016/j.bmc.2016.06.026
6. C.K. Ryu, Y.H. Kim, H.A. Im, J.Y. Kim, J.H. Yoon and A. Kim, *Bioorg. Med. Chem. Lett.*, **22**, 500 (2012); https://doi.org/10.1016/j.bmcl.2011.10.099
7. A. Kamal, B.V. Reddy, B. Sridevi, A. Ravikumar, A. Venkateswarlu, G. Sravanthi, J.P. Sridevi, P. Yogeeswari and D. Sriram, *Bioorg. Med. Chem. Lett.*, **25**, 3867 (2015); https://doi.org/10.1016/j.bmcl.2015.07.057
8. A.K. Bhattacharjee, M.G. Hartell, D.A. Nichols, R.P. Hicks, B. Stanton, J.E. van Hamont and W.K. Milhous, *Eur. J. Med. Chem.*, **39**, 59 (2004); https://doi.org/10.1016/j.ejmech.2003.10.004
9. Z. Wang, M. Wang, X. Yao, Y. Li, J. Tan, L. Wang, W. Qiao, Y. Geng, Y. Liu and Q. Wang, *Eur. J. Med. Chem.*, **53**, 275 (2012); https://doi.org/10.1016/j.ejmech.2012.04.010
10. S. Shagufta and I. Ahmad, *MedChemComm*, **8**, 871 (2017); https://doi.org/10.1039/C7MD00097A
11. A. Kamal, E.V. Bharathi, J.S. Reddy, M.J. Ramaiah, D. Dastagiri, M.K. Reddy, A. Viswanath, T.L. Reddy, T.B. Shaik, S.N. Pushpavalli and M.P. Bhadra, *Eur. J. Med. Chem.*, **46**, 691 (2011); https://doi.org/10.1016/j.ejmech.2010.12.004
12. M. Zahedifard, F. Lafta Faraj, M. Paydar, C. Yeng Looi, M. Hajrezaei, M. Hasanpourghadi, B. Kamalidehghan, N. Abdul Majid, H. Mohd Ali and M. Ameen Abdulla, *Sci. Rep.*, **5**, 11544 (2015); https://doi.org/10.1038/srep11544
13. S.T. Al-Rashood, I.A. Aboldahab, M.N. Nagi, L.A. Abouzeid, A.A. Abdel-Aziz, S.G. Abdel-Hamide, K.M. Youssef, A.M. Al-Obaid and H.I. El-Subbagh, *Bioorg. Med. Chem.*, **14**, 8608 (2006); https://doi.org/10.1016/j.bmc.2006.08.030
14. S. Taliani, I. Pugliesi, E. Barresi, S. Salerno, C. Marchand, K. Agama, F. Simorini, C. La Motta, A.M. Marini, F.S. Di Leva, L. Marinelli, S. Cosconati, E. Novellino, Y. Pommier, R. Di Santo and F. Da Settimo, *J. Med. Chem.*, **56**, 7458 (2013); https://doi.org/10.1021/jm400932c
15. T.P. Matthews, A.M. Jones and I. Collins, *Expert Opin. Drug Discov.*, **8**, 621 (2013); https://doi.org/10.1517/17460441.2013.788496
16. O. Cruz-Lopez, A. Conejo-Garcia, M. C. Nunez, M. Kimatrai, M. E. Garcia-Rubino, F. Morales, V. Gomez-Perez and J. M. Campos, *Curr. Med. Chem.*, **18**, 943 (2011); https://doi.org/10.2174/092986711794940824
17. S.J. von Niementowski, *J. Prakt. Chem.*, **51**, 564 (1894); https://doi.org/10.1002/prac.18950510150
18. S.M. Roopan, T. Maiyalagan and F.N. Khan, *Can. J. Chem.*, **86**, 1019 (2008); https://doi.org/10.1139/v08-149
19. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983); https://doi.org/10.1016/0022-1759(83)90303-4
20. L.L. Gundersen, J. Nissen-Meyer and B. Spilsberg, *J. Med. Chem.*, **45**, 1383 (2002); https://doi.org/10.1021/jm0110284
21. K.H. Chandramouli, K.N. Thimmaiah, A. Chandrashekar and C.J.M. D'Souza, *Nucleos. Nucleot. Nucleic Acids*, **23**, 1639 (2004); https://doi.org/10.1081/NCN-200031461