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# Design, synthesis, and cytotoxicity evaluation of new 2,4-disubstituted quinazolines as potential anticancer agents

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**ABSTRACT**

A series of new 2, 4-disubstituted quinazolines were synthesized by an analog design approach. The synthesis of title compounds (**3a–f**, **4a–c**, **5a–c,** and **6a–c**) was achieved from the corresponding key intermediates 2-(pyridin-3-yl) quinazolin-4(3H)-one(**2a**), 2-(pyridin-3-yl) quinazolin-4(3*H*)-one (**2b**) and 2-(pyrazin-2-yl)quinazolin-4(3*H*)-one (**2c**) with appropriate amines. The synthesized compounds were characterized by the spectral studies. All the synthesized compounds were evaluated for *in vitro* anticancer activity employing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay against human adenocarcinoma (HT-29), breast cancer (MDA-231), and Ehrlich ascites carcinoma cell lines. Among the tested compounds, **5a** has a significant anticancer activity (5.33 µM/ml) against the human adenocarcinoma cell line. Other compounds have shown a moderate anticancer activity against the tested cell lines.

## INTRODUCTION

Cancer continues to be a major health concern in all over the world outpacing the cardiac diseases. It stands in the first position in mortality rate due to distinct factors. Even though many major advancements have been made in the management of cancer chemotherapy for some patients, there is a need for continued effort in the identification of efficacious novel anticancer agents with minimal side effects (Eckhardt, 2002; El-Azab *et al*., 2010). The contemporary approaches have been focused mainly on the design of ideal anticancer agents, which eradicate the cancer cells without involving the normal tissues. Inappropriately, none of the reported drugs meet this criterion.

Quinazolines are being an excellent reservoir of bioactive substances and have exhibited a wide spectrum of biological activities, especially antiproliferative (Cao *et al*., 2015; Govindaraj *et al*., 2009; Ho *et al*., 2002; Raffa *et al*., 2004; Rajveer *et al*., 2010). The stable nature of the quinazoline nucleus has encouraged the medicinal chemists to bring molecular modifications to this nucleus to synthesize new potential medicinal agents (Ghorab *et al*., 2016). It is evident from the literature that the quinazoline derivatives are effective as epidermal growth factor receptor (EGFR) inhibitors (Barbosa *et al*., 2014; Dissoki *et al*., 2007; Fernandes *et al*., 2007; Li *et al*., 2012; Mishani *et al*., 2004; Zhao *et al*., 2013; Zhang *et al*., 2014). The EGFR is a transmembrane glycoprotein, which belongs to the erbB family of closely related cell membrane receptors that include EGFR (erbB-1 or HER1), erbB-2 (HER2), erbB-3 (HER3), and erbB-4 (HER4). The expression and overexpression of EGFR are observed in many human solid tumors including breast, ovarian, non-small cell lung (NSCLC), colorectal, and head and neck cancers. In support of their important role in tumor biology, an EGFR activation may assist in tumor growth by increasing cell proliferation, motility, adhesion, invasive capacity, and blocking apoptosis. Due to their multidimensional role in the progression of cancer, EGFR is emerged as an attractive drug target for anticancer therapy (Seshacharyulu *et al*., 2012).

Recently, quinazolines were reported as versatile template molecules for the inhibition of a wide range of tyrosine kinases. Among these, EGFR is more widely studied target. Gefitinib, a small-molecule inhibitor from this class, was first to be approved for treating NSCLC refractory before chemotherapeutic intervention (Cohen *et al*., 2003). In view

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of the above observations and continuation of the research program on aiming to synthesize biologically active heterocycles for anticancer potentials (Singh *et al*., 2013), we report the design, synthesis, and anticancer activity evaluation of new 2, 4-disubstituted quinazolines.

## MATERIALS AND METHODS

The synthesized compounds were checked for their melting point by an open glass capillary method and are uncorrected. The KBr pellet technique was adopted to record IR spectra using Shimadzu FT-IR 8400-S spectrophotometer. $^{1}$H Nuclear Magnetic Resonance (NMR) and $^{13}$C NMR spectra were recorded in AMX-400 NMR spectrophotometer at 400 MHz, in which Dimethyl sulfoxide DMSO-$_{d6}$ was used as solvent and tetramethylsilane as an internal standard. The chemical shifts were expressed in δ ppm. Mass spectra were recorded in the Shimadzu LC-MS-2010A instrument by electrospray ionization technique.

### *Synthesis of 2-phenylquinazolin-4(3H)-one (2a)*

An equimolar quantity of anthranilic acid (0.1 mol) and benzamide (0.1 mol) was taken in a glass mortar, mixed well, and then transferred into a round bottom flask and were heated at 130˚C for 5 hours, a solvent-free reaction. The reaction progress was monitored by Thin Layer Chromatography (TLC). Then, the reaction mixture was poured on cold water and stirred. The solid obtained was filtered, washed with sodium bicarbonate solution (10%) to remove unreacted acid, dried, and recrystallized from ethanol to afford the compound (**2a**). A similar procedure was followed to synthesize **2b** and **2c** using pyridine-3-carboxamide and pyrazine-2-carboxamide, respectively.

**2-phenylquinazolin-4(3H)-one (2a)**

Yield: 58%. mp 136°C–138°C. IR (KBr) cm$^{-1}$:1582 (C=N), 1,680 (C=O), 3,071 (C-H), 3,500 (N-H); $^{1}$H NMR (DMSO-*d6*, δ ppm): 7.26–8.33 (m, 5H, ArH), 8.1 (s, 1H, NH); $^{13}$C NMR (DMSO-*d6*, δ ppm): 120.9, 122.4, 126.1 (2), 127.4, 128.7, 128.8, 128.9 (2), 130.2, 133.5, 151.3, 159.0, (C=N), 161.0 (C=O); LC-MS m/z: 222.24 (M$^{+}$).

**2-(pyridin-3-yl)quinazolin-4(3H)-one (2b)**

Yield: 69%. mp 142°C–143°C. IR (KBr) cm$^{-1}$: 1,584 (C=N), 1,681 (C=O), 3,070 (C-H), 3,510 (N-H); $^{1}$H NMR (DMSO-*d6*, δ ppm): 7.62–8.04 (m, 4H, ArH), 8.2 (s, 1H, NH), 7.58–9.07 (m, 4H, ArH); $^{13}$C NMR (DMSO-*d6*, δ ppm): 120.9, 122.4, 123.9, 127.4, 128.0, 128.8, 133.5, 134.1, 147.1, 148.4, 151.3, 159.0, (C=N),161.0(C=O); LC-MS m/z: 223.23 (M$^{+}$).

**2-(pyrazin-2-yl) quinazolin-4(3H)-one (2c)**

Yield: 77%. mp 149°C–150°C. IR (KBr) cm$^{-1}$: 1,582 (C=N), 1,585 (C=N), 1,683 (C=O), 3,073 (C-H), 3512 (N-H); $^{1}$H NMR (DMSO-*d6*, δ ppm): 7.64–8.02 (m, 3H, ArH), 8.4 (s, 1H, NH), 8.76–9.34 (m, 2H, ArH); $^{13}$C NMR (DMSO-*d6*, δ ppm): 120.9, 122.4, 127.4, 128.8, 133.5, 141.2, 143.3, 144.3, 145.7, 147.1, 159.8, (C=N), 160.8 (C=O); LC-MS m/z: 224.22 (M$^{+}$).

### *Synthesis of compounds N-(4-methoxyphenyl)-2-phenylquinazolin-4-amine (3a)*

2-phenylquinazolin-4(3H)-one (0.01 mol), 4-methoxybenzenamine (0.01 mol), and zinc chloride (1.0 g) in a 15 ml of dimethyl formamide were taken in a round bottom flask and were refluxed at 150˚C for 48 hours. The reaction progress was monitored by TLC. Then, the reaction mixture was cooled and poured on ice cubes. The solid obtained was collected by filtration, washed with dichloromethane, and recrystallized from ethanol to afford the compound **3a**. A similar procedure was followed to synthesize **3b–f, 4a–c, 5a–c,** and **6a–c** using appropriate 2-substituted quinazolin-4(3H)-one with arylamide/aryl hydrazide/phenylhydrazine.

**N-(4-methoxyphenyl)-2-phenylquinazolin-4-amine (3a)**

Yield: 51%. mp 177°C–178°C. IR (KBr) cm$^{-1}$: 1,649 (C=C), 1,483 (C=N), 3,340 (N-H); $^{1}$H NMR (DMSO-*d6*, δ ppm): 3.83 (s, 3H, $OCH_3$), 9.86 (s, 1H, NH), 7.12–7.65 (m, 2H, ArH of methoxyphenyl), 7.78–8.26 (m, 4H, quinazoline), 7.56–8.58 (m, 5H, ArH); $^{13}$C NMR (DMSO-*d6*, δ ppm): 55.9, 115.1(2), 116.2, 117.3 (2), 120.1, 126.5, 127.5 (2), 128.8, 128.9, 129.3 (2), 130.7, 133.7, 135.4, 150.0, 150.7, 161.0, 170.0; LC-MS m/z: 327.38 (M$^{+}$).

**N-(4-methoxyphenyl)-2-(pyridin-3-yl)quinazolin-4-amine (3b)**

Yield: 45%. mp 164°C–165°C; IR (KBr) cm$^{-1}$: 1,649 (C=C), 1,483 (C=N), 3,336 (N-H); $^{1}$H NMR (DMSO-*d6*, δ ppm): 3.83 (s, 3H, $OCH_3$), 9.96 (s, 1H, NH), 7.02–7.55 (m, 2H, ArH of methoxyphenyl), 7.58–8.16 (m, 4H, quinazoline), 7.57–9.24 (m, 4H, pyridine); $^{13}$C NMR (DMSO-*d6*, δ ppm): 55.9, 115.1 (2), 117.3 (2), 116.2, 120.1, 124.0, 126.5, 128.9, 133.0, 133.7, 134.1,135.4, 148.0, 149.1, 150,0, 150.7, 161.0, 170.0; LC-MS m/z: 328.37 (M$^{+}$).

**N-(4-methoxyphenyl)-2-(pyrazin-2-yl)quinazolin-4-amine (3c)**

Yield: 57%. mp 159°C–160°C; IR (KBr) cm$^{-1}$: 1,649 (C=C), 1,275 (C-O), 1,535 (C=N), 3,350 (N-H);$^{1}$H NMR (DMSO-*d6*, δ ppm): 3.92 (s, 3H, $OCH_3$), 10.24 (s, 1H, NH), 7.58–7.87 (m, 2H, ArH of methoxyphenyl), 7.68–8.26 (m, 4H, quinazoline), 7.45–7.48 (m, 3H, pyrazine); $^{13}$C NMR (DMSO-*d6*, δ ppm): 55.9, 115.1 (2), 116.2, 117.3 (2), 120.1, 126.5, 128.9, 133.7, 135.4, 141.2, 142.7, 144.1, 144.5, 150,0, 150.7, 161.0, 170.0; LC-MS m/z: 329.36 (M$^{+}$).

**N-(4-fluorophenyl)-2-phenylquinazolin-4-amine (3d)**

Yield: 51%. mp 194°C–195°C; IR (KBr) cm$^{-1}$: 1,644 (C=C), 1,276 (C-O), 1,483 (C=N), 3,333 (N-H); $^{1}$H NMR (DMSO-*d6*, δ ppm): 10.25 (s, 1H, NH), 7.41–7.46 (m, 2H, ArH of fluorophenyl), 7.68–8.36 (m, 4H, quinazoline), 7.61–8.35 (m, 5H, ArH); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 116.3(2), 117.9(2), 120.1, 126.5, 127.5 (2), 128.8, 128.9, 129.3 (2), 130.7, 133.7, 138.7, 150.0, 152.9, 61.0, 170.0; LC-MS m/z: 315.38 (M$^{+}$).

**N-(4-fluorophenyl)-2-(pyridin-3-yl) quinazolin-4-amine (3e)**

Yield: 58%. mp 209°C–210°C; IR (KBr) cm$^{-1}$: 1,644 (C=C), 1,480 (C=N), 3,334 (N-H); $^{1}$H NMR (DMSO-*d6*, δ ppm): 9.92 (s, 1H, NH), 7.41–7.61 (m, 2H, ArH of fluorophenyl), 7.68–8.26 (m, 4H, quinazoline), 7.67–9.14 (m, 4H, pyridine); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 116.3 (2), 117.9 (2), 120.1, 124.0, 126.5, 128.9, 133.0, 133.7, 134.1, 138.7, 148.0, 149.1, 150,0, 152.9, 161.0, 170.0; LC-MS m/z: 316.33 (M$^{+}$).

**N-(4-fluorophenyl)-2-(pyrazin-2-yl) quinazolin-4-amine (3f)**

Yield: 59%. mp 181°C–182°C; IR (KBr) cm$^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,343 (N-H); $^{1}$H NMR (DMSO-*d6*, δ ppm):

9.96 (s, 1H, NH), 7.36–7.51 (m, 2H, ArH of fluorophenyl), 7.56–8.26 (m, 4H, quinazoline), 8.66–9.44 (m, 3H, pyrazine); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 116.3 (2), 117.9(2), 120.1, 126.5, 128.9, 133.7, 138.7, 141.2, 142.7, 144.5, 144.7, 150.0, 152.9, 161.0, 170.0; LC-MS m/z: 317.32 ($M^+$).

**4-chloro-N'-(2-phenylquinazolin-4-yl) benzohydrazide (4a)**

Yield: 60%. mp 189°C–190°C; IR (KBr) $cm^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,128 (N-H), 550 (C-Cl), 1,640 (C=O); 1H NMR (DMSO-*d6*, δ ppm): 9.91 (s, 1H, NH), 10.27 (d, 1H, CONH), 7.16–7.87 (m, 2H, ArH of chlorobenzene), 7.56–8.79 (m, 4H, quinazoline), 7.51–8.38 (m, 5H, phenyl); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 120.1, 126.5, 127.5 (2), 128.8, 128.9 (3), 129.0(2), 129.3 (2), 130.7, 132.3, 133.7, 137.7, 150.0, 161.0, 164.9, 170.0; LC-MS m/z: 374.82 ($M^+$).

**4-chloro-N'-(2-(pyridin-3-yl) quinazolin-4-yl) benzohydrazide (4b)**

Yield: 58%. mp 119°C–220°C; IR (KBr) $cm^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,353 (N-H), 550 (C-Cl), 1,640 (C=O); $^1$H NMR (DMSO-*d6*, δ ppm): 9.96 (s, 1H, NH), 8.0 (d, 1H, CONH), 7.66–7.96 (m, 2H, chlorobenzene), 7.59–8.26 (m, 4H, quinazoline), 7.26–8.69 (m, 4H, pyridyl); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 120.1, 124.0, 126.5, 128.9 (3), 129.0 (2), 132.3, 133.0, 133.7, 134.1, 137.7, 148.0, 149.1, 150.0, 161.0, 164.9, 170.0; LC-MS m/z: 375.08 ($M^+$).

**4-chloro-N-(2-(pyrazin-2-yl) quinazolin-4-yl) benzohydrazide (4c)**

Yield: 46%. mp 200°C–201°C; IR (KBr) $cm^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,353 (N-H), 550 (C-Cl), 1,640 (C=O); $^1$H NMR (DMSO-*d6*, δ ppm): 10.03 (s, 1H, NH), 8.0 (d, 1H, CONH), 7.57–7.87 (m, 2H, chlorobenzene), 7.68–8.26 (m, 4H, quinazoline), 8.86–9.24 (m, 3H, pyrazine); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 120.1, 126.5, 128.9 (3), 129.0 (2), 132.3, 133.7, 137.7, 141.2, 142.7, 144.5, 144.7, 150,0, 161.0, 164.9, 170.0; LC-MS m/z: 376.08 ($M^+$).

**N-(2-phenylquinazolin-4-yl) isonicotinohydrazide (5a)**

Yield: 59%. mp 237°C–238°C; IR (KBr) $cm^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,354 (N-H), 1,640 (C=O); $^1$H NMR (DMSO-*d6*, δ ppm): 10.21 (s, 1H, NH), 8.0 (d, 1H, CONH), 7.71–8.59 (m, 4H, pyridyl), 7.68–8.26 (m, 4H, quinazoline), 7.31–8.38 (m, 5H, ArH); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 120.1, 122.8 (2), 126.5, 127.5 (2), 128.8, 128.9, 129.3 (2), 130.7, 133.7, 140.9, 149.8 (2), 150,0, 161.0, 164.9, 170.0; LC-MS m/z: 341.10 ($M^+$).

**N-(2-(pyridin-3-yl) quinazolin-4-yl) isonicotinohydrazide (5b)**

Yield: 60%. mp 249°C–250°C; IR (KBr) $cm^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,354 (N-H), 1,640 (C=O); $^1$H NMR (DMSO-d6, δ ppm): 9.98 (s, 1H, NH), 8.0 (d, 1H, CONH), 7.26–8.49 (m, 4H, pyridyl), 7.68–8.36 (m, 4H, quinazoline); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 120.1, 122.8 (2), 124.0, 126.5, 128.9, 133.0, 133.7, 134.1, 140.9, 148.0, 149.1, 149.8 (2), 150.0, 161.0, 164.9, 170.0; LC-MS m/z: 342.35 ($M^+$).

**N-(2-(pyrazin-2-yl) quinazolin-4-yl) isonicotinohydrazide (5c)**

Yield: 58%. mp 264°C–265°C; IR (KBr) $cm^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,348 (N-H), 1,640 (C=O); $^1$H NMR (DMSO-*d6*, δ ppm): 9.98 (s, 1H, NH), 10.07 (d, 1H, CONH), 7.61–8.59 (m, 2H, pyridyl), 7.61–7.86 (m ,4H, quinazoline), 7.92–8.44 (m, 3H, pyrazine); $^{13}$C NMR (DMSO-*d6*, δ ppm): 116.2, 120.1, 122.8 (2), 126.5, 128.9, 133.7, 140.9, 141.2, 142.7, 144.5, 144.7, 149.8 (2), 150.0, 161.0, 164.9, 170.0; LC-MS m/z: 343.34 ($M^+$).

**2-phenyl-4-(2-phenylhydrazinyl) quinazoline (6a)**

Yield: 53%. mp 311°C–312°C; IR (KBr) $cm^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,259 (N-H); $^1$H NMR (DMSO-*d6*, δ ppm): 10.21 (s, 1H, NH), 8.2 (d, 1H, NH), 6.99–7.47 (m, 5H, ArH), 7.58–8.26 (m, 4H, ArH of quinazoline), 7.31–8.38 (m, 5H, phenyl); $^{13}$C NMR (DMSO-*d6*, δ ppm): 113.2 (2), 116.2, 119.2, 120.1, 126.5, 127.5 (2), 128.8, 128.9, 129.3 (4), 130.7, 133.7, 150.0, 151.0, 161.0, 170.0; LC-MS m/z: 312.37 ($M^+$).

**4-(2-phenylhydrazinyl)-2-(pyridin-3-yl) quinazoline (6b)**

Yield: 54%. mp 297°C–298°C; IR (KBr) $cm^{-1}$: 1,646 (C=C), 1,484 (C=N), 3,359 (N-H); $^1$H NMR (DMSO-*d6*, δ ppm): 10.23 (s, 1H, NH), 8.0 (d, 1H, NH), 6.87–7.47 (m, 3H, ArH of phenyl), 7.59–8.26 (m, 4H, ArH of quinazoline), 7.36–8.49 (m, 4H, ArH, pyridine); $^{13}$C NMR (DMSO-*d6*, δ ppm): 113.2 (2), 116.2, 119.2, 120.1, 124.0, 126.5, 127.5 (2), 128.9, 129.3 (2), 133.0, 133.7, 134.1, 148.0, 149.1, 150.0, 161.0, 170.0; LC-MS m/z: 313.36 ($M^+$).

**4-(2-phenylhydrazinyl)-2-(pyrazin-2-yl) quinazoline (6c)**

Yield 56%. mp 73°C–74°C; IR (KBr) $cm^{-1}$: 1,644 (C=C), 1,483 (C=N), 3,333 (NH); $^1$H NMR (DMSO-*d6*, δ ppm): 9.97 (s, 1H, NH), 8.0 (d, 1H, NH), 6.97–7.36 (m, 3H, ArH of phenyl), 7.48–8.26 (m, 4H, ArH of quinazoline), 8.86–9.24 (m, 2H, ArH of pyrazine); $^{13}$C NMR (DMSO-*d6*, δ ppm): 113.2 (2), 116.2, 119.2, 120.1, 124.0, 126.5, 127.5 (2), 128.9, 129.3 (2), 133.0, 133.7, 134.1, 148.0, 149.1, 150.0, 161.0, 170.0; LC-MS m/z: 314.34 ($M^+$).

***Cytotoxicity assay***

The cell viability of HT-29 (human adenocarcinoma), MDA-231 (breast cancer), and Ehrlich ascites carcinoma (EAC) cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Alley *et al*., 1988; Singh *et al*., 2013). The cells were placed at 3,000 per well in 96-well plates and were incubated for 24 hours in Dulbecco's Modified Eagle medium contained with 10% fetal bovine serum in 5% $CO_2$ at 37°C. After, the concentrations of 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2, and 1 µg/ml of compounds were added to the wells and were cultured for 72 hours. Then, the MTT solution was added (10 µl/well) to the above, and the cells were again incubated further at 37°C for 4 hours. Cell lysates were added to the well (100 µl/well) and kept overnight incubation, and the absorbance value at 570 nm was detected employing ELISA reader. Each

concentration was tested in triplicates. The % cytotoxicity was calculated using the following formula.

$$\% \text{ cytotoxicity} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

A graph of % cytotoxicity on the *Y*-axis and the concentration of test compound on the *X*-axis was extrapolated for the determination of cytotoxicity ($IC_{50}$).

## RESULTS AND DISCUSSION

### *Design strategy*

It was proposed to develop 2, 4-disubstituted quinazolines as new structural lead with the hope of achieving potent chemotherapeutic agents in reducing the viability of the cancerous cells. The molecular modifications were made on the quinazoline nucleus primarily focused at the fourth position with the substitution of aryl moiety through amine or amide linkage and at the second position with aryl/heteroaryl substitutions that would improve the lipophilicity (Fig. 1).

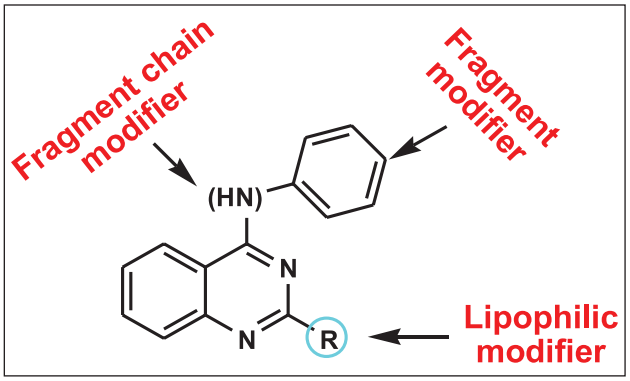
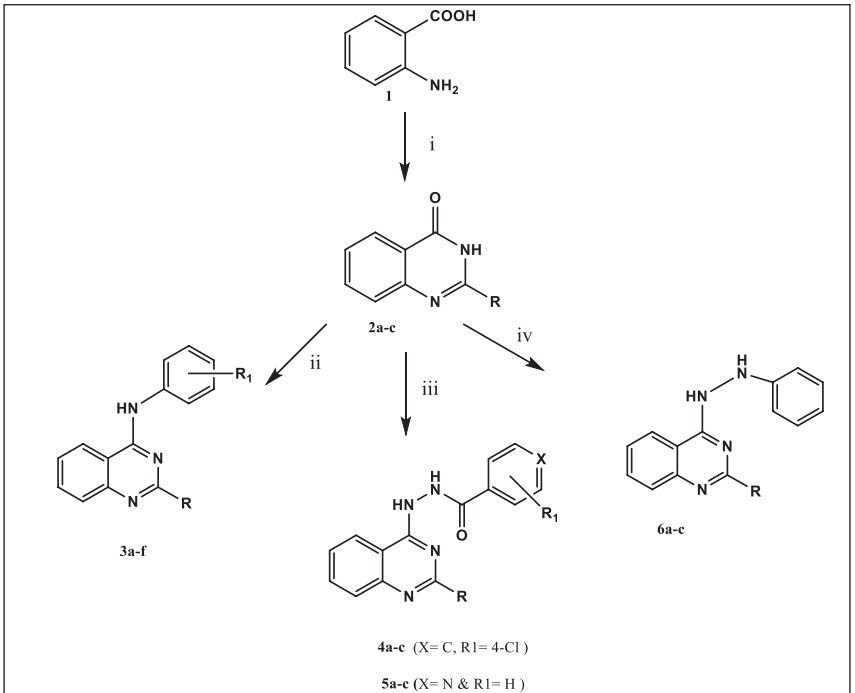



**Figure 1.** Design strategy for the new quinazoline derivatives.

### *Chemistry*

The title compounds 2, 4-disubstituted quinazoline derivatives (**3a–f**, **4a–i**, **5a–c,** and **6a–c**) were conveniently synthesized from the corresponding key intermediates 2-phenylquinazolin-4(3H)-one (**2a**), 2-(pyridin-3-yl) quinazolin-4(3H)-one (**2b**) and 2-(pyrazin-2-yl)quinazolin-4(3H)-one (**2c**) as per synthetic methodology as shown in Figure 2. The intermediates **2a–c** were synthesized from the anthranilic acid with an appropriate aryl/heteroaryl amide.

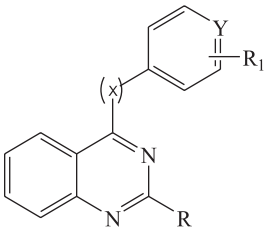
The physical characterization data of the synthesized compounds are shown in Table 1. The structures of all the synthesized compounds were characterized based on IR, $^{1}$HNMR, $^{13}$CNMR, and Liquid Chromatography-Mass Spectrometry (LCMS) data. The spectral data are consistent with the proposed structures. The IR spectrum of 2-phenylquinazolin-4(3H)-one (**2a**) exhibits characteristic carbonyl absorption band at 1,680 $cm^{-1}$ and N-H at 3,350 $cm^{-1}$. In the $^{1}$H NMR spectrum of the compound **2a**, a singlet at 8.1 ppm integrates for a NH proton of the quinazoline ring, and the aromatic protons resonate as a complex multiplet between 7.26 and 8.33 ppm. These corresponding proton shifts indicate the formation of compound **2a**. The IR spectrum of N-(4-methoxyphenyl)-2-(pyrazin-2-yl) quinazolin-4-amine (**3a**) exhibits characteristic C=N absorption band at 1,535 $cm^{-1}$ and N-H at 3,350 $cm^{-1}$. The $^{1}$HNMR spectrum of the **3a** exhibits a singlet at 3.92 ppm integrates for




**Reagents and conditions**: i) Arylamide, 150 $^{o}$C, 5 h ii) Arylamine, $ZnCl_2$, DMF, 150 $^{o}$C, 48 h iii) Aryl acid hydrazide/ Isoniazid, $ZnCl_2$, DMF, 150 $^{o}$C, 48 h iv) Phenyl hydrazine, $ZnCl_2$, DMF, 150 $^{o}$C, 48 h.

**Figure 2.** Scheme of synthesis for the title compounds.

**Table 1**. *In vitro* cytotoxicity of the synthesized quinazolines.




| Compounds | R | X | R1 | Y | HT-29 | EAC | MDA-231 |
|---|---|---|---|---|---|---|---|
| | | | | | | $IC_{50}$ µM/ml | |
| 3a | Phenyl | NH | 4- $OCH_3$ | C | 46.69 | 381.34 | 176.94 |
| 3b | 3-Pyridyl | NH | 4- $OCH_3$ | C | 81.06 | 459.02 | 233.35 |
| 3c | Pyrazyl | NH | 4- $OCH_3$ | C | 87.41 | 446.07 | 139.75 |
| 3d | Phenyl | NH | 4-F | C | 33.46 | 287.61 | 81.14 |
| 3e | 3-Pyridyl | NH | 4-F | C | 30.88 | 312.34 | 47.12 |
| 3f | Pyrazyl | NH | 4-F | C | 285.96 | 307.88 | 282.17 |
| 4a | Phenyl | NHNHCO | 4-Cl | C | 92.43 | 480.74 | 123.04 |
| 4b | 3-Pyridyl | NHNHCO | 4-Cl | C | 33.49 | 239.62 | 139.01 |
| 4c | Pyrazyl | NHNHCO | 4-Cl | C | 91.75 | 334.04 | 66.43 |
| 5a | Phenyl | NHNHCO | H | N | 5.33 | 566.15 | 615.83 |
| 5b | Pyridyl | NHNHCO | H | N | 95.20 | 373.85 | 69.53 |
| 5c | Pyrazyl | NHNHCO | H | N | 176.53 | 830.32 | 982.68 |
| 6a | Phenyl | NHNH | H | C | 75.54 | 435.88 | 82.56 |
| 6b | Pyridyl | NHNH | H | C | 384.98 | 628.49 | 599.58 |
| 6c | Pyrazyl | NHNH | H | C | 746.15 | 607.57 | 598.66 |
| 5-Fluorouracil | | - | - | - | 1.23 | 1.76 | 2.92 |

the protons of $OCH_3$ of the aromatic ring. The pyrazine ring protons appear as multiplet between 7.45 and 7.48 ppm and a singlet resonate at 10.24 ppm for NH proton. The aromatic protons appear as a complex multiplet between 7.58 and 7.87 ppm. These data indicate the formation of **3a**. The IR spectrum of 4-chloro-N'-(2-phenylquinazolin-4-yl)benzohydrazide (**4c**) exhibits characteristic C=N absorption band at 1,483 $cm^{-1}$ and N-H at 3,128 $cm^{-1}$. In the $^1$HNMR spectrum of **4c**, a singlet at 9.91 ppm integrates for a proton of NH attached to quinazoline. A singlet peak for NH adjacent to a carbonyl group appears at 10.27 ppm. The aromatic protons appear as a complex multiplet between 7.16 and 8.79 ppm. These data indicate the formation of **4c**. The IR spectrum of N'-(2-(pyrazin-2-yl) quinazolin-4-yl)isonicotinohydrazide (**5c**) exhibits the characteristic C=N absorption band at 1,483 $cm^{-1}$ and N-H at 3,348 $cm^{-1}$. The $^1$HNMR spectrum exhibits a singlet peak at 9.98 ppm integrates for NH proton attached to the quinazoline ring. The protons of pyrazine ring appear between 7.43 and 8.69 ppm. A broad singlet peak for NH proton appears at 10.07 ppm. The aromatic protons appear as a complex multiplet between 7.61 and 8.44 ppm. The data indicate the formation of **5c.** The IR spectrum of 2-phenyl-1-(2-phenylquinazolin-4-yl) hydrazine (**6a**) exhibits C=N absorption band at 1,483 $cm^{-1}$ and N-H at 3,259 $cm^{-1}$. The $^1$HNMR spectrum **6a** exhibits the characteristic peaks at 8.2 and 10.21 ppm integrates for two protons of NH. The aromatic protons appear as a complex multiplet between 6.99 and 8.38 ppm. These data confirm the formation of **6a**.

***Cytotoxicity evaluation***

The anticancer activity of all the newly synthesized compounds was measured by microculture tetrazolium assay (MTT) assay against HT-29 (human adenocarcinoma), EAC, and MDA-231 (breast cancer) cells involving 5-fluorouracil as a positive control (1.23, 1.76, and 2.92 µM/ml, respectively). The cytotoxicity ($IC_{50}$) details of the new compounds are shown in Table 1**.** The cytotoxicity ($IC_{50}$) was analyzed by linear regression of the concentration-response curves of each compound. The synthesized compounds show better inhibitory activity toward HT-29 and MDA-231 cell lines, especially HT-29 cell lines. Compounds show an inhibitory action on HT-29, MDA-231, and EAC in the range of 5.33–746, 47.12–982.68, and 239.62–830 µM/ml, respectively. From the cytotoxicity activity of 3 series compound against HT-29 cell lines, it is evident that the role of phenyl/pyridyl ring as R substituent and 4-fluoro derivative as $R_1$ is crucial for activity. Compound **3e** is the most active in three series, which supports the above data. Compound 4 series had 4-chloro substitution at $R_1$ having lesser activity than three series, except the compound **4c**. Hence, chain linker difference might also influence the activity. However, controversy lies in five series that **5a**, which had phenyl ring as R and hydrogen at $R_1$ substitution with -NH-NH-C=O linker (same as that of four series), is highly potent among all synthesized compounds which m/z: show $IC_{50}$ value of 5.33 µM/ml. This may due to some diverse interaction with the receptor pocket. In the case of MDA-231 cell lines, the cytotoxicity of compounds is slightly related to the presence of pyridyl, pyrazyl ring at R, and

4F derivative at R1 and –NH as a linker. However, linearity in a relationship is not as much in HT-29 cell lines.

## CONCLUSION

In summary, 2, 4-disubstituted quinazolines were designed by substituting linkers (-NH-, -NHNH-, and -NHNHCO-) between the fourth position of the quinazoline moiety and aryl/ heteroaryl ring system with the hope of achieving enhanced anticancer profiles. Fifteen 2, 4-designed compounds were synthesized in good yield and were evaluated for *in vitro* anticancer activity against HT-29, EAC, and MDA-231cell lines. Among the tested compounds, **5a** has shown a significant anticancer activity against HT-29 cell lines. Among the linkers attached, compound **5a** has -NHNHCO- group between fourth positions of the quinazoline moiety, and pyridinyl ring is emerged as a promising anticancer candidate. Exploring molecular modification and inhibitory activity of compound **5a** on epidermal growth factor receptor would give a better insight into its anticancer potential.

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## CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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## REFERENCES

Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res, 1988; 48:589–601.

Barbosa ML, Lima LM, Tesch R, Sant Anna CM, Totzke F, Kubbutat MH, Schächtele C, Laufer SA, Barreiro EJ. Novel 2-chloro-4-anilino-quinazoline derivatives as EGFR and VEGFR-2 dual inhibitors. Eur J Med Chem, 2014; 71:1–14.

Cao SL, Feng YP, Jiang YY, Liu SY, Ding GY, Li RT. Synthesis and in vitro antitumor activity of 4(3*H*)-quinazolinone derivatives with dithiocarbamate side chains. Bioorg Med Chem Lett, 2015; 15:1915–7.

Cohen MH, Williams GA, Sridhara R, Chen G, Pazdur R. FDA drug approval summary: gefitinib (ZD1839) tablets. Oncologist, 2003; 8:303–6.

Dissoki S, Aviv Y, Laky D, Abourbeh G, Levitzki A, Mishani E. The effect of the [18F]-PEG group on tracer qualification of [4-(phenylamino)-quinazoline-6-YL]-amide moiety—an EGFR putative irreversible inhibitor. Appl Radiat Isot, 2007; 65:1140–51.

Eckhardt S. Recent progress in the development of anticancer agents. Curr Med Chem, 2002; 2:419–39.

El-Azab AS, Al-Omar MA, Abdel-Aziz AA, Abdel-Aziz NI, el-Sayed MA, Aleisa AM, Sayed-Ahmed MM, Abdel-Hamide SG. Design, synthesis, and biological evaluation of novel quinazoline derivatives as potential antitumor agents: Molecular docking study. Eur J Med Chem, 2010; 45:4188–98.

Fernandes C, Oliveira C, Gano L, Bourkoula A, Pirmettis I, Santos I. Radioiodination of new EGFR inhibitors as potential SPECT agents for molecular imaging of breast cancer. Bioorg Med Chem, 2007; 15:3974–80.

Ghorab MM, Alsaid MS, El-Gazzar MG, Higgins M, Dinkova-Kostova AT, Shahat AA. Synthesis and biological evaluation of novel 2- phenylquinazoline-4-amine derivatives: identification of 6-phenyl-8Hbenzo[g]quinazolino[4,3-]quinazolin-8-one as a highly potent inducer of NAD(P)H quinone oxidoreductase 1. J Enzyme Inhib Med Chem, 2016; 31:34–9.

Govindaraj Y, Sathyamoorthy, Karthikeyan V, Melanaphuru V, Agrahari V, Gupta S, Nema RK. Synthesis and in vivo anticancer screening of 2-{[bis-(2-chloroethyl) amino] methyl} -6, 8-dinitro-1-(4-substituted ethyl)-1h-quinazolin-4-one derivatives. Academic J Cancer Res, 2009; 2:73–7.

Ho N, Harapanhalli RS, Dahman BA, Chen K, Wang K, Adelstein SJ, Kassis AI. Synthesis and biologic evaluation of a radioiodinated quinazolinone derivative for enzyme mediated insolubilization therapy. Bioconjug Chem, 2002; 3:357–64.

Li S, Guo C, Sun X, Li Y, Zhao H, Zhan D, Lan M, Tang Y. Synthesis and biological evaluation of quinazoline and quinoline bearing 2,2,6,6-tetramethylpiperidine-N-oxyl as potential epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors and EPR bio-probe agents. Eur J Med Chem, 2012; 49:271–8.

Mishani E, Abourbeh G, Rozen Y, Jacobson O, Laky D, Ben David I, Levitzki A, Shaul M. Novel carbon-11 labeled 4-dimethylamino-but-2-enoic acid [4-(phenylamino)-quinazoline-6-yl]-amides potential PET bioprobes for molecular imaging of EGFR-positive tumors. Nucl Med Biol, 2004; 31:469–76.

Raffa D, Edler MC, Daidone G, Maggio B, Merikech M, Plescia S, Schillaci D, Bai R, Hamel E. Synthesis, cytotoxicity, and inhibitory effects on tubulin polymerization of a new 3-heterocyclo substituted 2-styrylquinazolinones. Eur J Med Chem, 2004; 39:299–304.

Rajveer C D, Kumaraswamy S, Sudharani S, Stephen R. Synthesis of some 6-bromo quinazolinone derivatives for their pharmacological activities. Int J Pharm Bio Sci, 2010; 1:1–10.

Seshacharyulu P, Ponnusamy MP, Haridas D, Jain M, Ganti AK, Batra SK. Targeting the EGFR signalling pathway in cancer therapy. Expert Opin Ther Targets, 2012;16:15–31.

Singh R, Pujar G, Purohit M, Chandrashekar VM. Synthesis, in vitro cytotoxicity, and antibacterial studies of new asymmetric bis-1,2,4-triazoles. Med Chem Res, 2013; 22:2163–73.

Zhang Y, Huang YJ, Xiang HM, Wang PY, Hu DY, Xue W, Song BA, Yang S. Synthesis, and anticancer activities of 4-(4-substituted piperazin)-5,6,7-trialkoxy quinazoline derivatives. Eur J Med Chem, 2014; 78:23–34.

Zhao F, Lin Z, Wang F, Zhao W, Dong X. Four-membered heterocycles-containing 4-anilino-quinazoline derivatives as epidermal growth factor receptor (EGFR) kinase inhibitors. Bioorg Med Chem Lett, 2013; 23:5385–8.

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