Research in Pharmaceutical Sciences, October 2018; 13(5): 450-459
Received: September 2017
Accepted: February 2018

School of Pharmacy & Pharmaceutical Sciences
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Original Article

Synthesis and cytotoxic evaluation of novel quinazolinone derivatives as potential anticancer agents

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Abstract

Nitrogen-rich heterocyclic compounds represent a unique class of chemicals with especial properties and have been modified to design novel pharmaceutically active compounds. In this study, a series of novel quinazolinone derivatives with substituted quinoxalindione were synthesized in two parts. In the first part, 6-(4-amino-3-methylphenoxy)quinoxaline-2,3(1H,4H)-dione was prepared from para-amino -m-crozol in 5 steps. In the next part, 2-alkyl-4H-benzo[d][1,3]oxazin-4-one derivatives were obtained from antranilic acid. Then reaction of 6-(4-amino-3-methylphenoxy)quinoxaline-2,3(1H,4H)-dione with 2-alkyl-4H-benzo[d][1,3]oxazin-4-one derivatives resulted in the production of final componds. The structures of synthesized compounds were confirmed by IR and ^{1}H-NMR. Cytotoxic activity of the compounds were evaluated at 0.1, 1, 10, 50 and 100 µM concentrations against MCF-7 and HeLa cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Almost all new compounds showed cytotoxic activity in both cell lines. Among tested compounds, 11g displayed the highest cytotoxic activity against both cell lines.

Keywords: Cytotoxicity; Quinazolinone; Quinoxalindione.

INTRODUCTION

Today, cancer is a growing problem in undeveloped and developing countries and a major cause of death in the world (1). Many researchers have focused their works on finding new anticancer agents. Nitrogen-rich heterocyclic compounds represent a unique class of chemicals with broad spectrum of biological activities and have been modified to design novel pharmaceutically active compounds (2,3). Among N-containing heterocyclic compounds, we focused on quinazoline, quinoxaline and their derivatives because of their biological activities.

A large number of therapeutic capacities of quinazoline derivatives including anti-inflammatory (4), antibacterial (5), antifungal (6), anti-viral (7), anti-tuberculosis (8), antimalarial (9), and anticancer (10) have so far been distinguished. The anticancer activity of quinazoline derivatives is one of the most important properties of these compounds because they behave as multi target molecules (11). Some derivatives interact with tubulin and affect its polymerization (12). Several quinazolines impress apoptosis inducers or influence acute phase in the cell cycle (13). Others are dihydrofolate reductase inhibitors (14), topoisomerase I inhibitors (15), checkpoint kinase inhibitors (16), and protein kinase inhibitors (17) such as gefitinib.

Quinoxaline as like as Quinazoline is N-containing heterocyclic compound with especial therapeutic activities of anti-inflammatory (18), antifungal (19), antibacterial (20), antileishmanial (21), anti-herpetic (22) anti-HIV (23) and anticancer (24). Quinoxaline derivatives have anticancer activity with various mechanisms including topoisomerase inhibition (25), DNA cleaving (26), arresting the G1 phase and kinase inhibition (27).

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Access this article online

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DOI: 10.4103/1735-5362. 236838

A lot of proteins and enzymes have been recognized to play important roles in human carcinogenesis. Kinases control the survival, growth, proliferation, and apoptosis of cells and are known as the promising target for designing novel anticancer drugs (28).

Recent studies suggest that protein kinase inhibitors are usually cytotoxic and can widely be used in anticancer drug design and drug discovery (29). In recent years, quinazolines and quinoxaline derivatives have been identified as potent inhibitors of different protein kinases with significant cytotoxic activities.

The successful researches based on hybrid drug design (30) encouraged us to design and synthesis of novel quinazolinone with quinoxalindione substituent as potentially new anticancer agents.

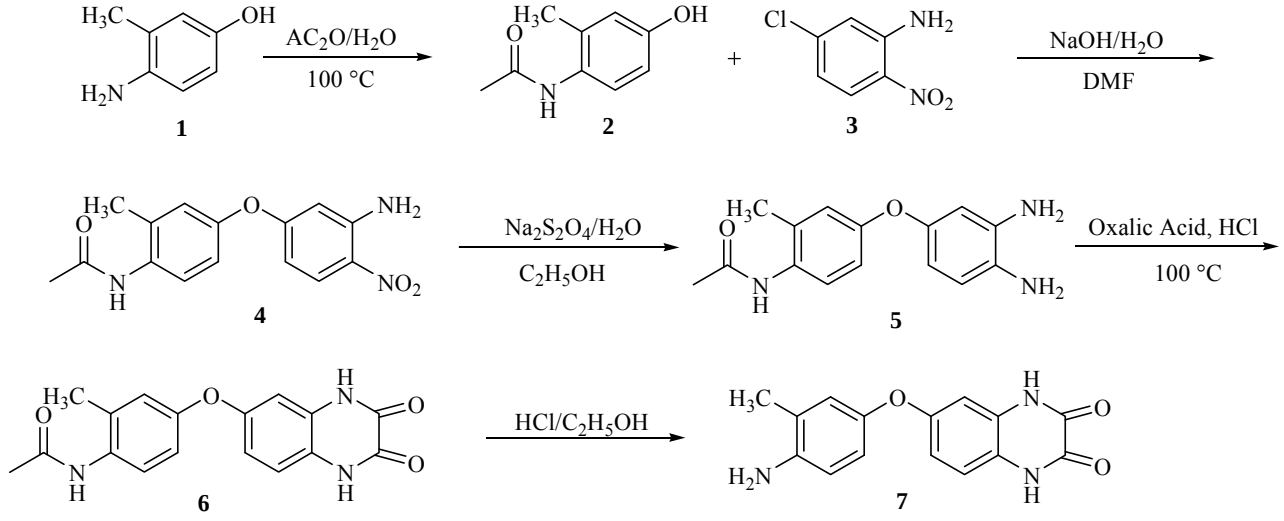
MATERIALS AND METHODS

Instrumentation

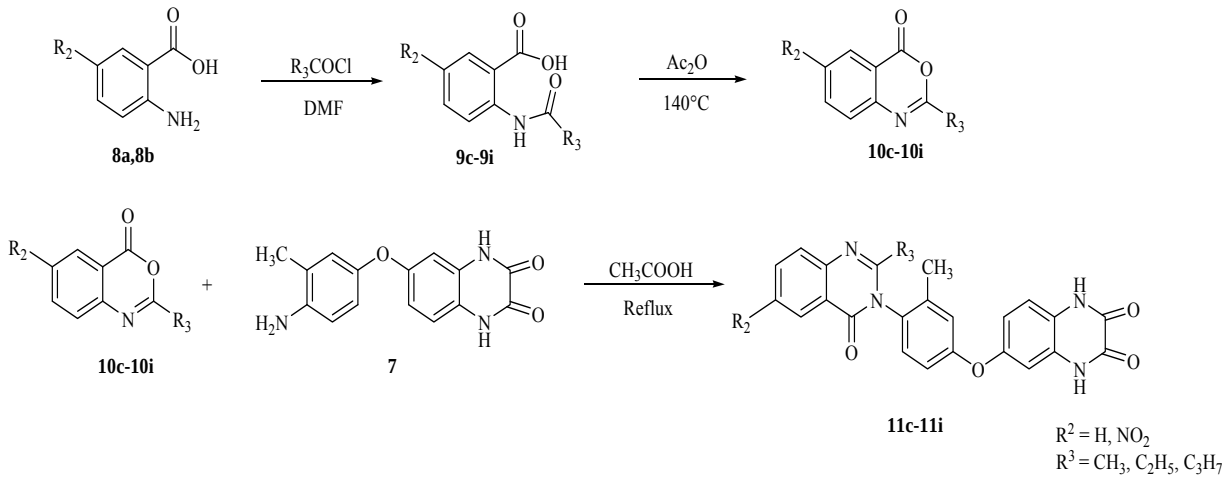
All chemicals used in this research were procured either from Merck (Germany) or Sigma (USA) companies. Melting points were assigned in open capillaries using Electrothermal 9200 melting point apparatus (Germany) and the infrared (IR) spectra were recorded as KBr pellets using a WQF-510 ratio recording FTIR spectrometer (China). (Proton nuclear magnetic resonance) ^{1}H-NMR spectra were taken in deuterated dimethyl sulfoxide (DMSO-d_6) as solvent on Bruker 400 MHz spectrometers (Germany) using tetramethylsilane as an internal standard and all chemical shifts are given in δ scale (ppm). DMSO-d_6 was purchased from Mesbah Energy Company (Iran).Reactions were followed by thin-layer chromatography (TLC) and visualization on TLC was achieved by ultraviolet light spectroscopy.

Preparation of compounds

The final products were synthesized in two stages. In the first stage, the 6-(4-amino-3-methylphenoxy)quinoxaline-2,3(1H,4H)-dione (compound **7**) was produced in 5 steps (scheme 1). (a) N-(4-hydroxy-2-methyl-phenyl)acetamide (compound **2**) was obtained by reacting para-amino-m-crozol (compound **1**) with acetic anhydride, (b) N-(4-(3-amino-4-nitrophenoxy) -2- methylphenyl) acetamide (compound **4**) was prepared by treatment of compound **2** with 5-chloro-2-nitro-aniline (compound **3**), (c) N-(4-(3,4-diaminophenoxy)-2-methylphenyl)acetamide (compound **5**) was synthesized by reacting of compound 4 with sodium dithionite in refluxing ethanol, (d) N-(4(2,3-dioxo-1,2,3,3 - tetrahydroquinoxalin-6-yloxy)-2-methylphenyl)acetamide (compound **6**) was prepared by treatment of compound **5** with oxalic acid, and (e) 6-(4-amino-3-methylphenoxy)quinoxaline-2,3(1H,4H) -dione (compound **7**) was obtained by treating of compound **6** with HCl.



Scheme 1. Synthetic route for the preparation of compound **7**.



Scheme 2. Synthetic route for the preparation of compounds **11c-11i**.

In the second stage, 6-(3-methyl-4-(2-alkyl-4-oxoquinazolin-3(4H)-yl)phenoxy)quinoxaline-2,3(1H,4H)-dione derivatives **11c-11i** were prepared in 3 steps. (Scheme 2). In the first step N-alkyl antranilic acid derivatives **9c-9i** were synthesized by treating of antranilic acid (**8a, 8b**) with aliphatic acyl chloride in the presence of dimethylformamide (DMF). In the second step 2-alkyl-4H-benzo[d][1,3]oxazin-4-one derivatives **10c-10i** were obtained by cyclization of compounds **9c-9i** in the presence of acetic anhydride, and in the third step 6-(3-methyl-4- (2-alkyl-4-oxoquinazolin- 3 (4H)-yl) phenoxy) quinoxaline-2,3(1H,4H) -dione derivatives **11c-11i** were obtained by reacting of compounds **10c-10i** with compound **7**. The structures of synthesized compounds were confirmed by IR and ^{1}H-NMR.

Biological activity assessments

The novel synthesized compounds were assessed for their cytotoxic activity against MCF-7 and HeLa cell lines by rapid colorimetric assay using 3-(4,5-dimethyl-thiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT). The tested cell lines were obtained from the National Cell Bank of Iran. Cell lines were cultivated in Roswell Park Memorial Institute (RPMI) 1640 containing 100 units/mL penicillin and 100 µg/mL streptomycin and supplemented with heat-inactivated 10% fetal bovine serum (FBS) in a humidified environment at 37 °C with 5% CO_2. After 2-3 subcultures, cells were seeded in a 96-well plate at a concentration of 5×10^4 cells/µL and incubated for 24 h. Then the cells were treated with various concentrations of the synthesized compounds (final concentrations of the compounds were 0.1, 1, 10, 50, 100 µM).

Doxorubicin was used as the positive control (final concentration 7.7 µM) and the wells containing DMSO (1%) was purchased from Merck Company (Germany) and cell suspension was regarded as the negative control.

The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 48 h. After 48 h of treatment, 20 µL of MTT dye (5 mg/mL) was added to each well and kept for another 3 h at the same condition. After incubation, the media was removed and 150 µL DMSO was added to each well for dissolving the formazan crystal, and the absorbance was recorded at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader (BioTek, USA). Each assay was carried out at least three times at three different days, and the results of the experiment were summarized in Figs. 1, 2 and Table 1. Cell viability was calculated using following formula:

$$Cell\ survaival\ (\%) = \frac{Well\ absorbance - blank\ absorbance}{Control\ absorbance - blank\ absorbance} \times 100$$

IC_{50} values were determined by plotting the cell viability against compound concentrations. All statistical analyzes were performed with the SPSS Statistics 18.

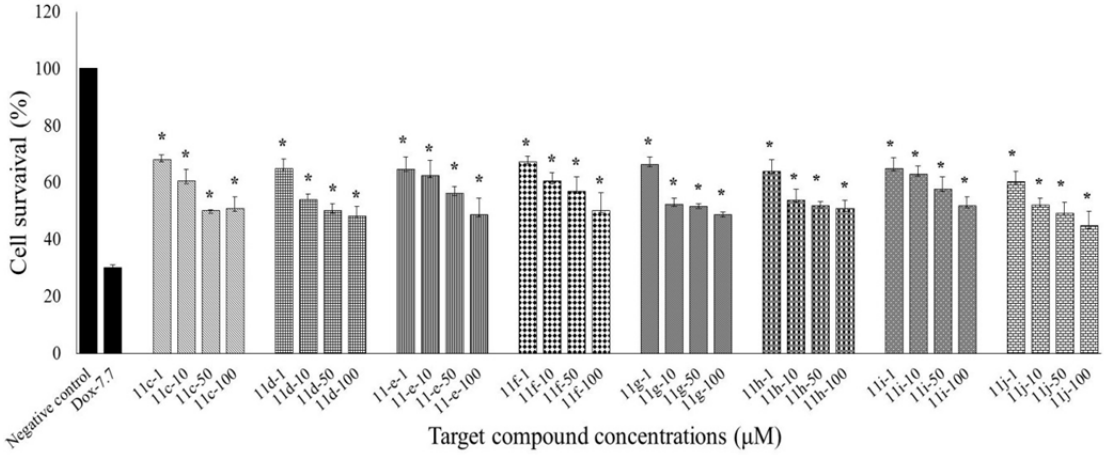


Fig. 1. Cytotoxic effects of compounds (**11c-11j**) on HeLa cell line following exposure to different concentrations (μM) of compounds (**11c-11j**). Cell viability was assessed using the MTT method. Data are presented as mean ± SD of cell survival compared to negative control (cell survival of 100%), **P* < 0.05, n = 3.

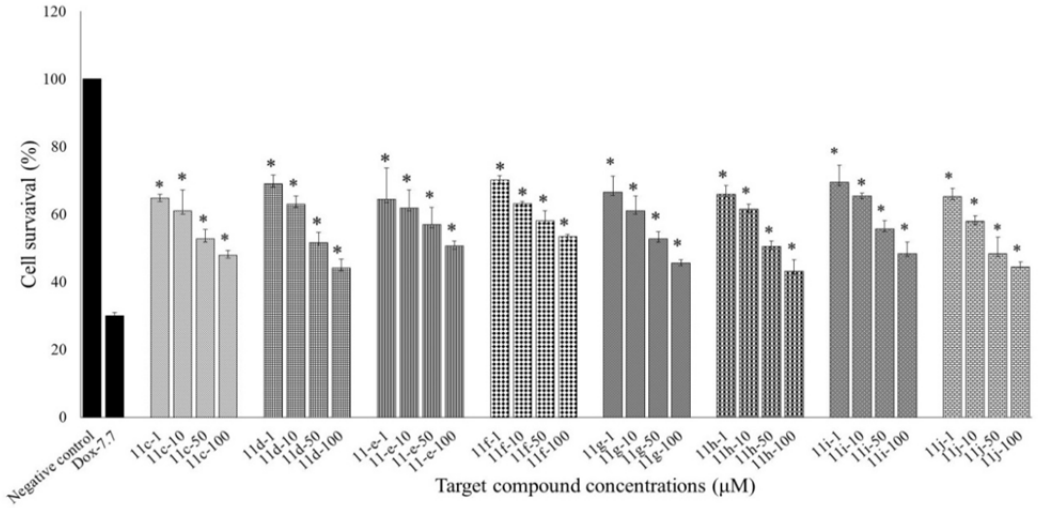
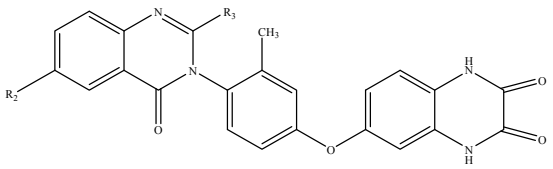


Fig. 2. Cytotoxic effects of compounds (**11c-11j**) on MCF-7 cell line following exposure to different concentrations (μM) of compounds (**11c-11j**). Cell viability was assessed using the MTT method. Data are presented as mean ± SD of cell survival compared to negative control (cell survival of 100%). **P* < 0.05, n = 3.

Table 1. IC_{50} values (μM) of compounds **11c-11j** against MCF-7 and HeLa cell lines using MTT assay.



Compound	R^2	R^3	HeLa	MCF-7
11c	H	Methyl	50	50
11d	H	Ethyl	50	50
11e	H	Isopropyl	100	100
11f	H	Propyl	100	> 100
11g	NO_2	Methyl	10	50
11h	NO_2	Ethyl	50	50
11i	NO_2	Isopropyl	100	100
11j	NO_2	Propyl	50	50
Doxorubicin			3.56	3.12

Statistical analysis

One-way analysis of variance (ANOVA) followed by LSD post hoc test were used for data analysis. All results were expressed as mean ± SEM. $P < 0.05$ was considered statistically significant.

RESULTS

Details of preparation procedures of synthesized compounds

*N-(4-hydroxy-2-methylphenyl)acetamide (**2**)*

A mixture of para-amino -m-crozol (compound **1**) (23.6 g, 191 mmol), water (58 mL) and acetic anhydride (21.2 mL) was stirred for 2 h in 100 °C. The progress of reaction was monitored by TLC. Then the reaction mixture was cooled to room temperature and put in ice bath. After formation, crystals were filtered and washed with cold water.

*N-(4-(3- amino-4 - nitrophenoxy) - 2 - methyl phenyl)acetamide (**4**)*

A mixture of NaOH (1.6 g, 40 mmol), water (3 mL), DMSO (12 mL) and N-(4-hydroxy-2-methylphenyl)acetamide (compound **2**) (6.6 g, 40 mmol) stirred for 30 min. 5-chloro-2-nitro-aniline (compound **3**) (6.88 g, 40 mmol) was added to the mixture and the mixture was stirred overnight at 100 °C in an oil bath. The progress of reaction was followed by TLC. After completion of the reaction, the mixture was allowed to cool down to room temperature. Then cold water (20 mL) was added and the precipitate was filtered.

*N-(4- (3,4 - diaminophenoxy) - 2 - methyl phenyl)acetamide (**5**)*

N- (4- (3-amino-4-nitrophenoxy)-2-methyl-phenyl)acetamide (compound **4**) (7.05 g, 23.5 mmol) was dissolved in ethanol (200 mL) and water (70 mL). Then sodium dithionite (12.4 g, 70 mmol) was added and the mixture of reaction was refluxed with intense stirring until the yellow color of mixture converted to brown. Then the reaction mixture was made cool to room temperature. The reaction mixture was filtered and evaporated to condense. The mixture was extracted with water and ethyl acetate. The organic layer was dried by magnesium sulfate and solvent was evaporated to afford the desired product.

*N-(4(2,3- dioxo-1,2,3,3- tetrahydroquinoxalin-6-yloxy)-2-methylphenyl)acetamide (**6**)*

Oxalic acid (90 mg, 1 mmol) was added to water (1 mL) and the mixture was heated at 100 °C until oxalic acid was dissolved and HCl 37% (1 mL) was added to the solution. Finally N-(4-(3,4-diaminophenoxy)-2-methyl-phenyl)acetamide (compound **5**) (274 mg, 1 mmol) was added. The progress of reaction was followed by TLC. After the completion of the reaction, the mixture was put in ice bath. The solid precipitate was filtered and washed with cold water.

*6-(4-amino-3-methylphenoxy)quinoxaline - 2,3 (1H,4H)-dione (**7**)*

N-(4(2,3-dioxo-1,2,3,3-tetrahydroquinoxalin-6-yloxy)-2-methylphenyl)acetamide (compound **6**) (700 mg, 2.15 mmol) was added to ethanol/HCl (2 M ,50 mL). The reaction mixture was refluxed for 2 days. The progress of reaction was followed by TLC. After completion of the reaction, the reaction mixture was put in ice bath and ammonia (30%) was slowly added to reach basic pH. The solid precipitate was filtered and washed with cold water.

General procedure for synthesis of N-Acyl antranilic acid drivatives (9c-9i)

Antranilic acid (compound **8a, 8b**) (10 mmol) was added to DMF (5 mL). Then aliphatic acyl chloride (10.1 mmol) was added dropwise to the solution. The reaction mixture was stirred for 3 h at room temperature. Then water (40 mL) was added to the reaction mixture and the mixture was stirred for 1 h. The reaction mixture was filtered and precipitated product was washed with cold water.

General procedure for synthesis of 2-alkyl-4H-benzo [d][1,3] oxazin-4-one derivatives (10c-10i)

N-acyl antranilic acid (compound **9c-9i**) (2.5 mmol) was added to acetic anhydride (2 mL) and the reaction mixture was heated at 140 °C. The progress of reaction was

monitored by TLC. After the completion of the reaction, the excess of acetic anhydride was removed at reduced pressure. Then the afforded product was quickly cooled and the product was triturated with N-hexane to solidify.

General procedure for synthesis of 6-(3-methyl -4- (2-alkyl-4- oxoquinazolin-3 (4H)-yl)phenoxy)quinoxaline-2,3(1H,4H)-dione derivatives (11c-11i)

6-(4-amino-3-methylphenoxy)quinoxaline-2,3(1H,4H)-dione (compound **7**) (1.5 mmol) and 2-alkyl-4H- benzo[d][1,3] oxazin-4-one (compound **10c-10i**) (2 mmol) were refluxed in glacial acetic acid for 6 h. The progress of reaction was followed by TLC. After the completion of the reaction, the acetic acid was evaporated by a rotary evaporator. Then the residue was washed with hot isopropanol to dissolve byproduct and the desired product was filtered and washed with isopropanol.

6- (3- methyl-4- (2- methyl-4- oxoquinazolin-3(4H)-yl) phenoxy) quinoxaline-2,3(1H,4H)-dione (***11c***)

Greenish yellow powder (Yield: 50%), Mp >300 °C. IR (KBr, cm^{-1}) v_{max} = 3431(NH), 1712, 1685 (C=O), 1610 (C=N) 1471 (C=C). ^{1}H-NMR (400 MHz-DMSO-d_6) δ: 2.06 (3H, s, CH_3), 2.09 (3H, s, CH3), 6.93 (1H, d, *J* = 2.8 Hz, H13), 6.98 (1H, dd, *J* = 8.8 Hz, *J* = 2.8 Hz, H15), 7.04 (1H, dd, *J* = 8.8 Hz, *J* = 2.8 Hz, H23), 7.14 (1H, d, *J* = 2.8 Hz, H19), 7.25 (1H, d, *J* = 8.8 Hz, H22), 7.46 (1H, d, *J* = 8.8 Hz, H16), 7.60 (1H, td, *J* = 8 Hz, *J* = 0.8 Hz, H2), 7.75 (1H, d, *J* = 8 Hz, H6), 7.93 (1H, td, *J* = 8 Hz, *J* = 1.2 Hz, H1), 8.19 (1H, dd, *J* = 8, *J* = 1.2 Hz, H3), 12.02 (2H, NH).

6-(4- (2- ethyl-4-oxoquinazolin- 3(4H)-yl) -3-methylphenoxy)quinoxaline-2,3(1H,4H)-dione ***(11d)***

Greenish yellow powder (Yield: 40%), Mp > 300 °C. IR (KBr, cm^{-1}) v_{max} = 3431(NH), 1701 (C=O), 1608 (C=N), 1473 (C=C). ^{1}H-NMR (400 MHz-DMSO-d_6) δ (ppm): 1.17 (3H, t, *J* = 7.2 Hz, CH_3), 1.98 (3H, s, CH_3), 2.31 (2H, m, CH_2), 6.88 (1H, d, *J* = 2.8 Hz, H13), 6.92 (1H, dd, *J* = 8.8 Hz, *J* = 2.8 Hz, H15), 6.97 (1H, dd, *J* = 8.8 Hz, *J* = 2.8 Hz, H23), 7.08 (1H, d, *J* = 2.8 Hz, H19), 7.20 (1H, d, *J* = 8.8 Hz, H22), 7.38 (1H, d, *J* = 8.8 Hz, H16), 7.54 (1H, td, *J* = 8Hz, *J* = 1.2 Hz, H2), 7.72 (1H, d, *J* = 8 Hz, H6), 7.67 (1H, td, *J* = 8 Hz, *J* = 1.2 Hz, H1), 8.13 (1H, dd, *J* = 8 Hz, *J* = 1.2 Hz, H3), 11.96 (2H, NH).

6-(4-(2-isopropyl-4- oxoquinazolin -3(4H)-yl)-3- methylphenoxy) quinoxaline-2,3(1H,4H)-dione ***(11e)***

Greenish yellow powder (Yield: 45%), Mp > 300 °C. IR (KBr cm^{-1}) v_{max} = 3415(NH), 1692 (C=O), 1635 (C=N), 1494 (C=C). ^{1}H-NMR (400 MHz-DMSO-d_6) δ: 1.21 (3H, d, *J* = 6.4 Hz, CH_3), 1.24 (3H, d, *J* = 6.4 Hz, CH_3), 2.03 (3H, s, CH_3), 2.15 (1H, m, CH), 6.86 (2H, m, H13, H15), 7.00 (1H, dd, *J* = 8.4 Hz, *J* = 2.4 Hz, H23), 7.11 (1H, d, *J* = 2.4 Hz, H19), 7.18(1H, d, *J* = 8.4 Hz, H22), 7.45 (1H, d, *J* = 8.4 Hz, H16), 7.59 (1H, t, *J* = 8 Hz, H2) 7.77 (1H, d, *J* = 8 Hz, H6), 7.92 (td, *J* = 8 Hz, *J* = 1.2 Hz, H1), 8.18 (1H, dd, *J* = 8 Hz, *J* = 1.2 Hz, H3), 9.30 (2H, NH).

6-(3- methyl-4-(4-oxo-2- propylquinazolin-3(4H)-yl) phenoxy) quinoxaline -2,3(1H,4H)-dione ***(11f)***

Greenish yellow powder (Yield: 60%), Mp > 300 °C. IR (KBr, cm^{-1}) v_{max} = 3473 (NH), 1695 (C=O), 1608 (C=N), 1470 (C=C). ^{1}H-NMR (400 MHz-DMSO-d_6) δ (ppm): 0.92 (3H, t, *J* = 7.2 Hz, CH_3), 1.76 (2H, HEx, *J* = 7.2 Hz, CH_2), 2.03 (3H, s, CH_3), 2.33 (2H, m, *J* = 7.2 Hz, CH_2), 6.94 (1H, m, H13), 6.98 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, H15), 7.03 (1H, dd, ,*J* = 8.4 Hz, *J* = 2.8 Hz, H23), 7.14 (1H, d, *J* = 2.8 Hz, H19), 7.25 (1H, d, *J* = 8.4 Hz, H22), 7.43 (1H, d, *J* = 8.8 Hz, H16) 7.59 (1H, t, *J* = 8 Hz, H2), 7.66 (1H, d, *J* = 8 Hz, H6), 7.92 (1H, td, *J* = 8 Hz, *J* = 1.2 Hz, H1), 8.18 (1H, dd, *J* = 8 Hz, *J* = 1.2 Hz, H3), 12.03(2H, NH).

6-(3- methyl -4-(2- methyl-6 -nitro-4-oxoquinazolin -3(4H)-yl)phenoxy)quinoxaline-2,3(1H,4H)-dione ***(11g)***

Greenish yellow powder (Yield: 50%), Mp > 300 °C. IR (KBr, cm^{-1}) v_{max} = 3419 (NH), 1709 (C=O), 1616 (C=N), 1475 (C=C), 1531, 1342 (NO_2). ^{1}H-NMR (400 MHz-DMSO-d_6) δ (ppm): 2.09 (3H, s, CH_3), 2.26 (3H, s,

CH_3),6.94 (1H, s, H13), 6.98 (1H, d, *J* = 8.4 Hz, H23), 7.07 (1H, d, *J* = 8.8 Hz, H15), 7.16 (1H, s, H19), 7.26 (1H, d, *J* = 8.4 Hz, H22), 7.51 (1H, d, *J* = 8.8 Hz, H16), 7.95 (1H, d, *J* = 8.8 Hz, H6,), 8.67 (1H, d, *J* = 8.8 Hz, H1), 8.88 (1H, s, H3), 12.04 (2H, NH).

*6-(4-(2- ethyl-6-nitro-4- oxoquinazolin -3(4H)-yl)-3- methylphenoxy)quinoxaline-2,3(1H,4H)-dione (**11h**)*

Greenish yellow powder (Yield: 55%), Mp > 300 °C. IR (KBr) cm^{-1}: 3424 (NH), 1697 (C=O), 1610 (C=N), 1470 (C=C), 1521, 1341 (NO_2). ^{1}H-NMR (400 MHz-DMSO-d_6) δ (ppm): 1.25 (3H, t, CH_3, *J* = 7.6 Hz), 2.06 (3H, s, CH_3), 2.43 (2H, m, *J* = 7.6 Hz, CH_2), 6.95 (1H, d, *J* = 2.4 Hz, H13), 6.99 (1H, dd, *J* = 8.4 Hz, *J* = 2.4 Hz, H15), 7.06 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, H23), 7.15 (1H, d, *J* = 2.4 Hz, H19), 7.25 (1H, d, *J* = 8.8 Hz, H22), 7.49 (1H, d, *J* = 8.4 Hz, H16), 7.98 (1H, d, *J* = 8.8 Hz, H6), 8.66 (1H, dd, *J* = 8.8 Hz, *J* =2.4 Hz, H1) 8.89 (1H, d, *J* = 2.4 Hz, H3), 12.02 (2H, NH).

*6-(4- (2- isopropyl -6-nitro -4- oxoquinazolin -3(4H) -yl)-3- methylphenoxy) quinoxaline-2,3(1H,4H)-dione (**11i**)*

Greenish yellow powder (Yield: 50%), Mp > 300 °C. IR (KBr cm^{-1}) v_{max} = 3461 (NH), 1694 (C=O), 1616 (C=N), 1490 (C=C), 1573, 1345 (NO_2). ^{1}H-NMR (400 MHz-DMSO-d_6) δ (ppm): 1.24 (3H, d, *J* = 6.4 Hz, CH_3), 1.27 (3H, d, *J* = 6.4 Hz, CH_3), 2.06 (3H, s, CH_3), 2.62 (1H, m, *J* = 6.4 Hz, CH), 6.96 (1H, d, *J* = 2.4 Hz, H13,), 7.00 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, H15), 7.06 (1H, dd, *J* = 8.4 Hz, *J* = 2.8 Hz, H23), 7.16 (1H, d, *J* = 2.8 Hz, H19), 7.26 (1H, d, *J* = 8.4 Hz, H22), 7.53 (1H, d, *J* = 8.8 Hz, H16), 7.97 (1H, d, *J* = 8.8 Hz, H6), 8.66 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, H1), 8.88 (1H, d, *J* = 2.4 Hz, H3,), 12.04 (2H, NH).

*6-(3- methyl -4- (6-nitro -4- oxo-2 -propyl-quinazolin- 3(4H)-yl) phenoxy) quinoxaline-2,3(1H,4H)-dione (**11j**)*

Greenish yellow powder (Yield: 50%) Mp > 300 °C. IR (KBr) cm^{-1}: 3417 (NH), 1694 (C=O), 1617 (C=N), 1480 (C=C), 1531, 1337 (NO_2). ^{1}H-NMR (400 MHz-DMSO-d_6) δ (ppm): 0.95 (3H, t, *J* = 7.2 Hz, CH_3), 1.78 (2H, CH_2), 2.06 (3H, s, CH_3), 2.39 (2H, m, CH_2), 6.96 (1H, d, *J* = 2.4 Hz, H13), 6.99 (1H, dd, *J* = 8 Hz, *J* = 2.4 Hz, H15), 7.06 (1H, dd, *J* = 8.8 Hz, *J*= 2.4 Hz, H23), 7.15 (1H, d, *J* = 2.4 Hz, H19), 7.26 (1H, d, *J* = 8.8 Hz, H22), 7.48 (1H, d, *J* = 8, Hz, H16), 7.96 (1H, d, *J* = 8.8 Hz, H6), 8.65 (1H, dd, *J* = 8.8 Hz, *J* = 2.8 Hz, H1), 8.88 (1H, d, *J* = 2.8 Hz, H3) 12.03 (2H, NH).

DISCUSSION

In this project, we synthesized new derivatives of quinazolinone with substituted quinoxalindione at position 3 (Fig. 3). The first part of our work was producing amine bearing quinoxalinedione moiety (compound **7**) from para-amio-m-crosol (compound **1**) as a starting material. Treatment of para-amino-m-crosol (compound **1**) with acetic anhydride gave the protected compound **2** which was acylated. Nucleophilic displacement of the Cl of 5-chloro-2-nitro-aniline (compound **3**) with compound **2** gave compound **4**. Reduction of nitro group in compound **4** with sodium dithionite gave compound **5** with two amino groups in ortho position. Cyclization of compound **5** with oxalic acid gave quinoxalindione (compound **6**). At the end of this part, deprotection of amide group of compound 6 with HCl afforded compound **7**.

In the next part, for synthesizing quinazolinone, antranilic acid (compounds **8a, 8b**) was treated with appropriate aliphatic acyl halides to afford compounds **9c-9j** through nucleophilic substitution reaction.

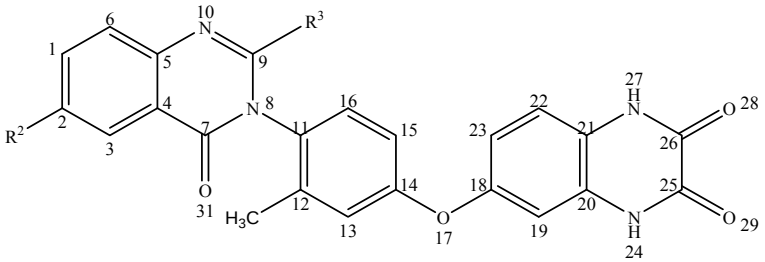


Fig. 3. General structure of final compounds.

Benzoxazine (compounds **10c-10j**) was synthesized from intra molecular reaction of nitrogen and carboxylic acid moiety of compounds **9c-9j** in the presence of acetic anhydride. To synthesize the final products (compounds **12c-12j**), benzoxazine (compounds **10c-10j**) were reacted with compound **7** through nucleophilic substitution mechanism.

The synthesized compounds were screened for their *in vitro* cytotoxic activity against MCF-7 and HeLa cell lines using MTT assay. The results are summarized and represented graphically in Figs. 1, 2, and Table 1. In Figs. 1 and 2, the concentration of 0.1 µM of synthesized compounds is not shown because the cell survival was more than 70%. Almost all new compounds showed cytotoxic activity at 50 to 100 µM concentrations in both cell lines except compound **11f** which was not cytotoxic against MCF-7 cell line. In the most of previous researches, quinazolinone derivatives and quinoxaline derivatives possesing halogen substituents showed reasonable cytotoxic activity (31,32). Ahmed and Belal designed and synthesized 2-(furan-2-yl)-4-oxoquinazolin-3-phenyl derivatives hybridized with 2-imino-pyrane and evaluated their cytotoxicity against HEPG-2, HCT116 and MCF-7 cells. Cytotoxic activity revealed the influence of p-Cl-phenyl moiety through the significant increasing of the anticancer activity against HCT116 and MCF-7 cell lines (33). Although our synthesized compounds did not have halogen substitutes, they displayed acceptable cytotoxic activities.

In previous studies, significant cytotoxic activities of compounds with quinazoline and quinoxaline moieties have been attributed to their capability of polar, van der waals interaction and hydrogen band formation with receptors. Higher flexibility of these compounds is another reason for their better interaction with active site of the receptors (30).

In our previous team works, diaryl urea derivatives bearing quinoxalindione moiety displayed great cytotoxic activity. Urea as a more flexible moiety might be responsible for higher activity of this series of the compounds compared to that of more rigid amid derivatives presented here (34).

According to the cytotoxicity evaluation performed here, compound **11g** had the best activity against HeLa cells with IC_{50} value 10 µM. It seems that withdrawing effect of nitro group at 2 position could probably improve cytotoxic activity. Compounds **11e** and **11f** with propyl and isopropyl substitutions showed lowest cytotoxic activity against both cell lines probably because of their increased lipophilicity (35).

CONCLUSION

In summary, the novel derivatives of quinazolinone with substituted quinoxalindione at position 3 were synthesized in several steps and tested for their *in vitro* cytotoxic activities against MCF-7 and HeLa cell lines. The cytotoxic evaluation of synthesized derivatives on both MCF-7 and HeLa cell lines demonstrated that compounds with propyl and isopropyl substitutes were less potent than others, and compound **11g** with nitro substituent showed best cytotoxic activity against HeLa cell line.

ACKNOWLEDGEMENTS

The content of this paper is extracted from the M.Sc thesis (No. 395457) submitted by Safoora Poorirani which was financially supported by the Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

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