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Original Article

Synthesis and cytotoxic evaluation of some novel quinoxalinedione diarylamide sorafenib analogues

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Abstract

A series of novel sorafenib analogues containing a quinoxalinedione ring and amide linker were synthesized. A total of 9 novel compounds in 6 synthetic steps were synthesized. Briefly, the amino group of p-aminophenol was first protected which then followed by O-arylation with 5-chloro-2-nitroaniline to provide compound **d**. Reduction of the nitro group of compound **d** and cyclization of the diamine group of compound **e** with oxalic acid afforded compound **f** which on deacetylation yeilded compound **g**. Then compound **g** was reacted with different acyl halides to afford the target compounds **1h-1p**. Chemical structures of synthesized compounds were confirmed by ^{1}H NMR and FT-IR analysis. All compounds were evaluated at 1, 10, 50 and 100 µM concentrations for their cytotoxicity against HeLa and MCF-7 cancer cell lines. Some of the compounds showed good cytotoxic activity, especially compounds **1i** and **1k-1n** with the IC_{50} values of 19, 16, 22, 18, and 16 µM against MCF-7 cell line and 20, 18, 25, 20, and 18 µM against HeLa cell line, respectively.

Keywords: Cytotoxicity; Sorafenib; Quinoxalinedione; Amide

INTRODUCTION

Cancer, one of the most serious illnesses, considered the second leading cause of death worldwide after cardiovascular diseases. According to the World Health Organization (WHO) reports, more than 13 million dying of cancer worldwide are awaiting to happen in 2030 (1-3).

Many research articles have lately reported the potential antiproliferative activity of diarylureas and diarylamides against some cancer cell lines. Thus far, with the same origin nine drugs including sorafenib, sunitinib, pazopanib, axitinib, vandetanib, regorafenib, lenvatinib, nintedanib, and cediranib have been approved for cancer therapy. (4-7).

Sorafenib (Nexavar) is an example of anticancer diarylurea approved by the U.S. Food and Drug Administration (FDA) for therapy of advanced renal cell carcinoma and advanced hepatocellular carcinoma (8). It is also exposed to clinical experiments for other cancer types such as metastatic colorectal, brain, leukemia, breast, glioblastoma, advanced gastric, cervical, thyroid, non-small cell lung cancer, prostate, bladder and neuroendocrine cancers. Sorafenib prevents tumor growth by the inhibition of C-RAF and B-RAF serine/threonine kinase activities and tumor angiogenesis by canceling vascular endothelial growth factor receptor (VEGFR) and platelet derived growth factor receptors signaling. However, therapy at full dose (800 mg/day) induces a number of side effects which emphasize on the urgent need to optimize the effectiveness and reduce the side effects of sorafenib (9).

In this research, based on previous structure activity relationships (SARs) of sorafenib and its derivatives, our main correction focused on replacement of N-methylpicolinamide group of sorafenib with quinoxalindione (10) (Fig. 1).

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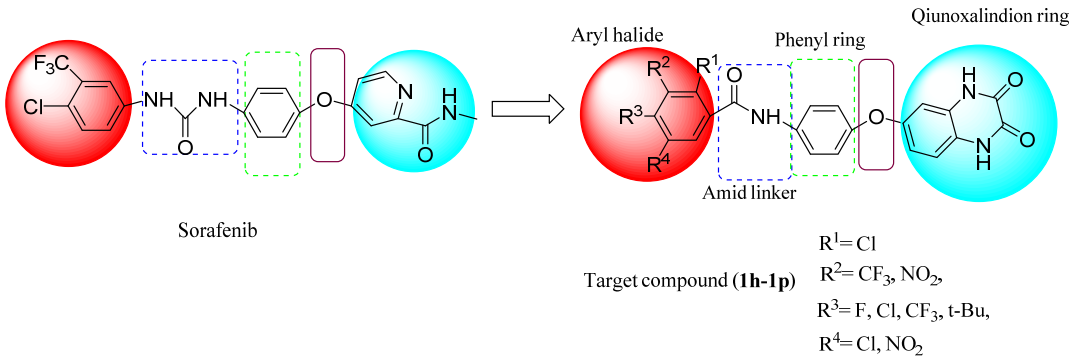


Fig. 1. The design concepts of the targeted compounds.

Nitrogen atoms in the N-methylpicolinamide moiety of sorafenib are necessary for binding to ATP-binding pocket of kinase. Quinoxalindione is an aromatic heterocycle containing two nitrogen atoms in its structure which is comparable with N-methylpicolinamide moiety in terms of size and polarity (11,12). Replacing the urea linker with an amide maintains hydrogen bonding with VEGFR-2 while reduce the flexibility of compounds (Fig. 1). Quinoxaline and its derivatives has broad spectrum of pharmacological activities such as anti-HIV, anti-inflammatory, and anti-cancer activity. Therefore, considerable biological activities of quinoxalindione derivatives make it a good moiety in drug design (13-16).

In this work, the structure of novel sorafenib analogues containing a quinoxalindione ring with amide linker were synthesized and confirmed by proton nuclear magnetic resonance (^{1}H NMR) and Fourier transform infrared (FT-IR) (Fig. 1). Cytotoxic effects of compounds on MCF-7 (17) and HeLa (18) cell lines were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method.

MATERIALS AND METHODS

Instrumentation

All starting materials, reagents, and solvents were purchased from commercial suppliers of Merck (Germany) and Aldrich (USA) companies. The purity of the prepared compounds was proved by thin layer chromatography (TLC) using various solvents of different polarities. Proton nuclear magnetic resonance (^{1}H NMR) spectra were taken in deuterated dimethyl sulfoxide (DMSO-d_6) as a solvent on Bruker 400 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. All chemical shifts are given in δ scale (ppm). Infrared (IR) analyses (KBr disks) were done using a Bruker Fourier transform infrared spectroscopy (FT-IR) instrument (Bruker Bioscience, Billerica, MA, USA).

Details of preparation procedures and chemistry of synthesized compounds

*Synthesis of N-(4-hydroxyphenyl)acetamide (**b**)*

para-amino phenol (a) (229 mmol, 25 g), water (68 mL) and acetic anhydride (29 mL, 263 mmol) were stirred for 2 h in 100 ºC to produce a uniform brown solution. The container was placed in an ice bath and when the crystals were formed, filtered and washed with cold water.

*Synthesis of N-(4-(3-amino-4-nitrophenoxy) phenyl)acetamide (**d**)*

N-(4-hydroxyphenyl)acetamide (b) (32.98 mmol, 4.98 g), was added to NaOH (33 mmol, 1.32 g), water (3 mL) and DMSO (11 mL) the mixture was stirred for 30 min. 5-chloro-2-nitro-aniline (**c**) (29.82 mmol, 5.13 g) was added to the mixture and stirred overnight at 100 °C in oil bath. The reaction mixture was poured onto aqueous NaOH (2 M) to form a solid, which was collected by filtration, washed with water and dried to give compound **d**.

*Synthesis of N-(4-(3,4-diaminophenoxy) phenyl)acetamide (**e**)*

N-(4-(3-amino-4-nitrophenoxy) phenyl) acetamide (d) (19.51 mmol, 5.6 g) was

dissolved in ethanol (140 mL) and water (55 mL). Then sodium dithionite (14 g, 80 mmol) was added and the mixture of the reaction was refluxed with intense stirring until the yellow color of mixture converted to brown. Then the reaction mixture was cooled down 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 the solvent was evaporated to afford the desired product.

*Synthesis of N-(4-((2,3-dioxo-1,2,3,4-tetra-hydroquinoxalin-6-yl)oxy)phenyl)acetamide **(f)***

A mixture of oxalic acid (1 mmol), water (1 mL) and HCl (1.5 mL, 0.75 M) was heated to 100 °C. Then N-(4-(3,4-diaminophenoxy)-2-methylphenyl)acetamide (e) (1 mmol, 0.257 g) was added and stirred for 2 h. The progress of the reaction was monitored by TLC until the reaction was complete. The reaction mixture was put in ice bath and the solid precipitate (compound **f**) was filtered and washed with water.

*Synthesis of 6-(4-aminophenoxy)-1,4-dihydro-quinoxaline-2,3-dione (**g**)*

A mixture of N-(4(2,3-dioxo-1,2,3,3-tetrahydroquinoxalin-6-yloxy)-2-methylphenyl) acetamide (compound **f**) (2800 mg, 8.99 mmol), 125 mL ethanol/HCl (3 M) was refluxed for 2 days. The progress of the reaction was monitored by TLC until the reaction was completed. The reaction mixture was put in ice bath and the pH was adjusted to 8 by ammonia solution. The solid precipitate (compound **g**) was filtered and washed with water.

General procedure for synthesis of target compounds (1h-1p)

6-(4-aminophenoxy)-1,4-dihydroquinoxaline-2,3-dione(45 mg, 0.169 mmol) and diisopropylethylamine (35 µL, 0.203 mmol) were mixed in dry tetrahydrofuran (THF) (5.0 mL) and appropriate acyl halide (0.203 mmol) was added. This mixture was refluxed for 24 h. After cooling to room temperature and the solvent was removed in vacuo. To the obtained oily residue was added chloroform (2 mL) and stirred for 5 min to form a solid. The precipitate was filtered with dichloromethane, ethyl acetate, and diethyl ether to give the target compounds **1h-1p**.

Cell culture

Two cell lines were used in this study including Michigan Cancer Foundation-7 (MCF-7) and Henrietta Lacks (HeLa). Cell lines were obtained from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). The cells were cultured in RPMI 1640 (Gibco, Scotland) supplemented with 10% fetal bovine serum (FBS) (Gibco, Scotland) and 100 Units/mL penicillin-G and 100 µg/mL streptomycin at 37 °C in humidified air containing 5 % CO2. 4, 5-dimethylthiazole-2-yl, 2, 5-diphenyl tetrazolium (MTT) was from Merck (Germany). RPMI1640, Dulbecco's phosphate buffered saline (DPBS), and penicillin-G/streptomycin were products of Biosera (Ringmer, UK).

MTT assay

MCF-7 and HeLa cells were seeded in 96-well plate at a density of 5×10^4 cell/mL, respectively and incubated for 24 h. Doxorubicin was used as the positive control and the wells containing DMSO (1%) and cell suspension was regarded as the negative controls. Then 20 µL of various concentrations of target compounds (1, 10, 50, 100 µM) were added and plates were incubated for 48 h. After the treatment period, 20 µL of MTT 0.5% (Merck, Germany) was added and further incubated for 3 h. Then supernatant of each well was replaced with 150 µL of DMSO (Merck, Germany) to dissolve the formed formazan crystals. The absorbances were determined at 570 nm with an enzyme-linked immunosorbent assay (ELISA) plate reader (BioTek, USA). Each assay was carried out three times at three different days, and the results of the experiment were summarized in Fig. 2 and Table 1 (23-25). Percent cell survival was calculated as follows:

$$Cell\ survaival\ (\%) = \frac{Well\ absorbance - blank\ absorbance}{Control\ absorbance - blank\ absorbance} \times 100 \quad (1)$$

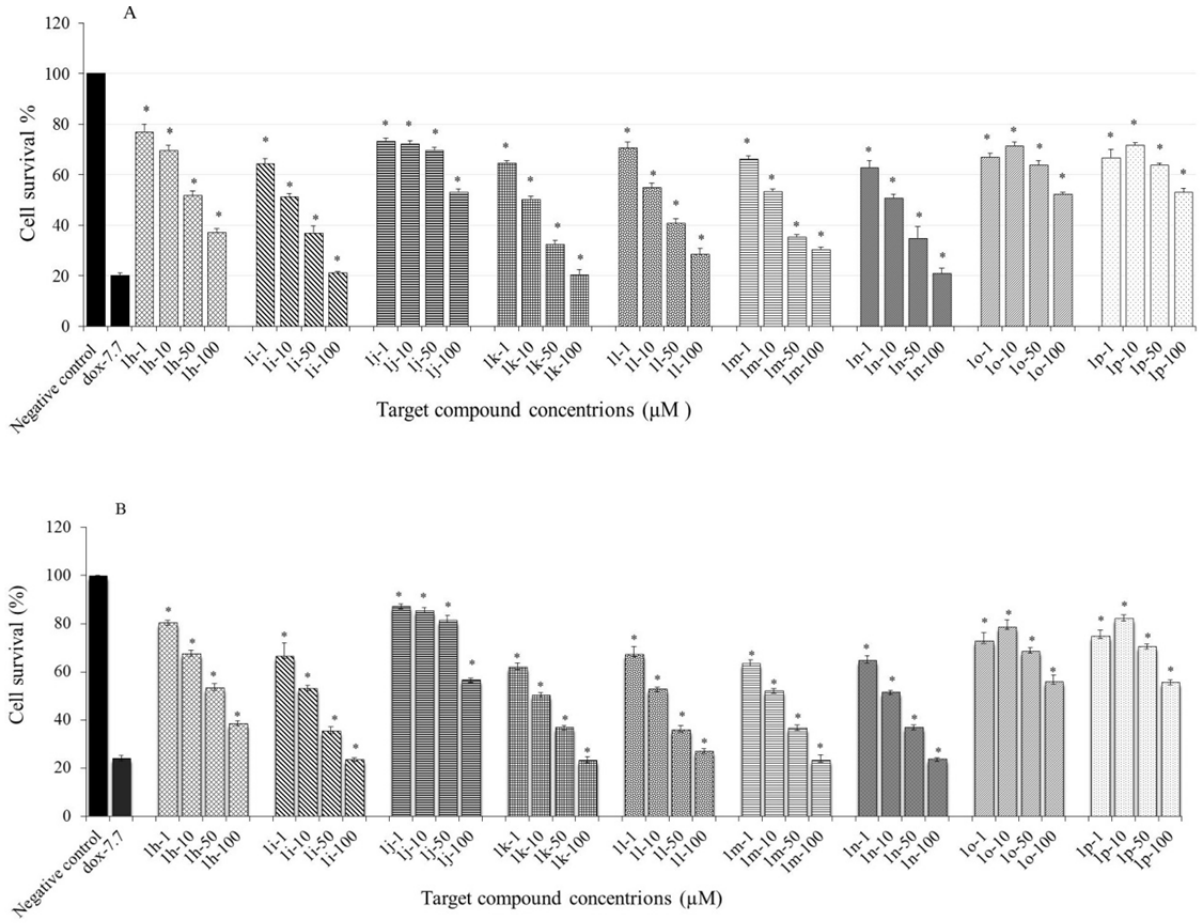
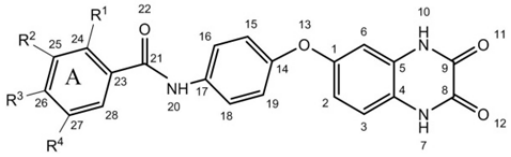


Fig. 2. Cytotoxic effects of compounds **1h-1p** on MCF-7 (A) and HeLa (B) cell lines following exposure to different concentrations (μM) of compounds **1h-1p**. Doxorubicin (dox) was used as the positive control (7.7 μM). Data are presented as mean ± SD of cell survival compared to negative control. **P* < 0.05, n = 9.

Table 1. The IC_{50} (μM) of tested compounds against MCF-7 and HeLa cancer cell lines



Target compound	R^1	R^2	R^3	R^4	IC_{50} (μM)	
					MCF-7	HeLa
1h	H	H	H	H	53.05 ± 1.71	55.92 ± 1.75
1i	H	CF3	H	H	19.20 ± 0.99	20.86 ± 1.30
1j	H	H	t-Bu	H	>100	>100
1k	H	CF3	F	H	16.87 ± 1.04	18.12 ± 0.86
1l	H	H	Cl	H	22.26 ± 1.50	25.72 ± 0.76
1m	Cl	H	H	Cl	18.52 ± 0.88	20.88 ± 1.14
1n	H	H	CF3	H	16.94 ± 1.54	18.15 ± 1.80
1o	H	H	NO2	H	>100	>100
1p	H	NO2	H	NO2	>100	>100

RESULTS

Synthesis of compounds

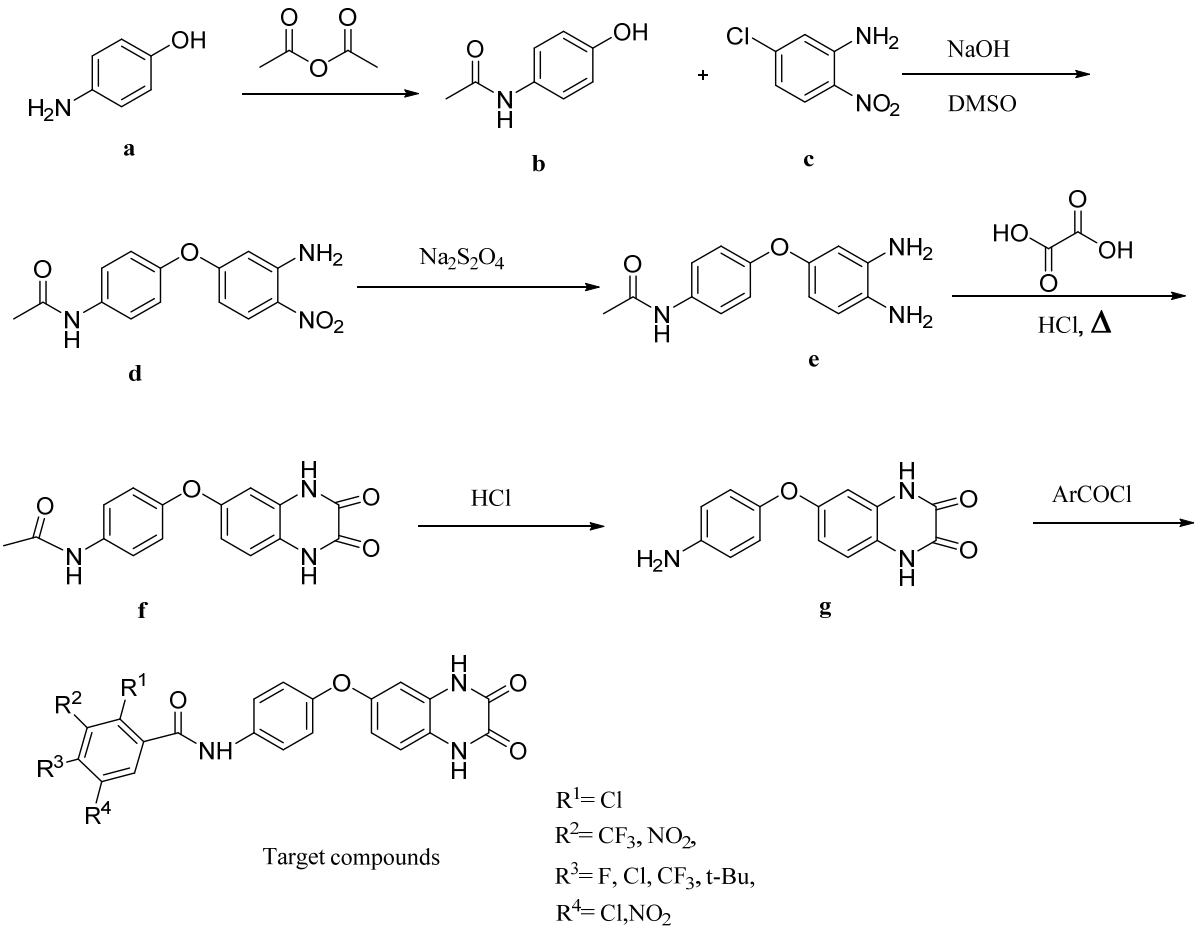
To achieve the target derivates (**1h-1p**), the general synthetic steps in Scheme 1 were adopted. The amine group of compound **a** was protected with acetic anhydride. Compound **b** was reacted with 5-chloro-2-nitroaniline **c** in DMSO in the presence of NaOH to provide compound **d** whose nitro group was reduced with $Na_2S_2O_4$ to provide diamine **e**. Cyclization of compound **e** with oxalic acid gave quinoxalindione (compound **f**). At the end, deprotection of amide group of compound **f** with HCl/ethanol gave rise to compound **g**. This was then reacted with the appropriate acyl halides to give the target products **1h-1p**.

*N-(4-((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)oxy)phenyl)-3-(trifluoromethyl)benzamide (**1i**)*

Yield: 70%. Mp > 300 °C. IR (KBr, cm^{-1}) ῡ: 3283 (NH), 1693 (C=O), 1658 (C=O), 1502, 1530 (C=C). ^{1}H NMR (400 MHz, DMSO) δ (ppm): 6.82 (1H, d, J = 2.4 Hz, H6), 6.87 (1H, dd, J = 8.8 Hz, 2.4 Hz, H2), 7.11 (2H, d, J = 8.8 Hz, H15,19), 7.19 (1H, d, J = 8.4 Hz, H3), 7.85 (3H, m, H16,18,27), 8.03 (1H, d, J = 7.6 Hz, H28), 8.33 (2H, m, H24, 26), 10.56 (1H, s, NH20), 11.81 (1H, s, NH7), 11.97 (1H, s, NH10).

*N-(4-((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)oxy)-2-methylphenyl)benzamide (**1h**)*

Yield: 55%. Mp > 300 °C. IR (KBr, cm^{-1}) ῡ: 3250 (NH), 1695 (C=O), 1648 (C=O), 1501, 1517 (C=C). ^{1}H NMR (400 MHz, DMSO) δ (ppm): 6.84 (1H, d, J = 2.4 Hz, H6), 6.88 (1H, dd, J = 8.8 Hz, 2.4 Hz, H2), 7.12 (2H, d, J = 8.8 Hz, H15,19), 7.21 (1H, d, J = 8.4 Hz, H3), 7.61 (2H, m, H25,27), 7.68 (1H, m, H26), 7.88 (2H, d, J = 8.8 Hz, H16,18), 8.03 (2H, m, H24,28), 10.37 (1H, s, NH20), 11.90 (1H, s, NH7), 11.98 (1H, s, NH10).



Scheme 1. General procedure for preparation of the target compounds.

*4-(tert-butyl)-N-(4-((2,3-dioxo-1,2,3,4-tetra-hydroquinoxalin-6-yl)oxy)phenyl) benzamide **(1j)***

Yield: 65%. Mp > 300 °C. IR (KBr, cm^{-1}) ῡ: 3159 (NH), 1697 (C=O), 1648 (C=O), 1501, 1541 (C=C). ^{1}HNMR (400 MHz, DMSO) δ (ppm): 1.40 (9H, s, t-Bu), 6.83 (1H, d, J = 2.4 Hz, H6), 6.88 (1H, dd, J = 8.8 Hz, 2.4 Hz, H2), 7.11 (2H, d, J = 8.8 Hz, H15,19), 7.20 (1H, d, J = 8.4 Hz, H3), 7.63 (2H, d, J = 8.4 Hz, H25,27), 7.87 (2H, d, J = 8.8 Hz, H16,18), 7.92 (2H, d, J = 8.4 Hz, H24,28), 10.31 (1H, s, NH20), 11.91 (1H, s, NH7), 12.00 (1H, s, NH10).

*N-(4-((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)oxy)phenyl)-4-fluoro-3-(trifluoromethyl) benzamide (**1k**)*

Yield: 70%. Mp > 300 °C. IR (KBr, cm^{-1}) ῡ: 3184 (NH), 1693 (C=O), 1622 (C=O), 1504, 1546 (C=C). HNMR (400 MHz, DMSO) δ (ppm): 6.83 (1H, d, J = 2.4Hz, H6), 6.88 (1H, dd, J = 8.8 Hz, 2.4 Hz, H2), 7.12 (2H, d, J = 8.8 Hz, H15,19), 7.20 (1H, d, J = 8.4 Hz, H3), 7.78 (1H, d, J = 9.6 Hz, H27), 7.83 (2H, d, J = 8.8 Hz, H16,18), 8.42 (2H, m, H24,28), 10.58 (1H, s, NH20), 11.91 (1H, s, NH7), 11.99 (1H, s, NH10).

*4-chloro-N-(4-((2,3-dioxo-1,2,3,4-tetrahydro quinoxalin-6-yl)oxy)phenyl)benzamide (**1l**)*

Yield: 75%. Mp > 300 ºC. IR (KBr, cm^{-1}) ῡ: 3147 (NH), 1691 (C=O), 1625 (C=O), 1504, 1541 (C=C). HNMR (400MHz, DMSO) δ (ppm): 6.83 (1H, d, J = 2.4 Hz, H6), 6.88 (1H, dd, J = 8.8 Hz, 2.4 Hz, H2), 7.12 (2H, d, J = 8.8 Hz, H15,19), 7.20 (1H, d, J = 8.4 Hz, H3), 7.70 (2H, d, J = 8.8 Hz, H16,18), 7.86 (2H, d, J = 8.8 Hz, H24,28), 8.06 (2H, d, J = 8.8 Hz, H25,27), 10.45 (1H, s, NH20), 11.91 (1H, s, NH7), 12.00 (1H, s, NH10).

*2,5-dichloro-N-(4-((2,3-dioxo-1,2,3,4-tetra hydroquinoxalin-6-yl)oxy)phenyl)benzamide (**1m**)*

Yield: 65%. Mp > 300 ºC. IR (KBr, cm^{-1}) ῡ: 3184 (NH), 1689 (C=O), 1635 (C=O), 1500, 1525 (C=C). ^{1}H NMR (400 MHz, DMSO) δ (ppm): 6.82 (1H, d, J = 2.4 Hz, H6), 6.88 (1H, dd, J = 8.8 Hz, 2.4 Hz, H2), 7.13 (2H, d, J = 8.8 Hz, H15,19), 7.20 (1H, d, J = 8.4 Hz, H3), 7.70 (2H, d, J = 8.8 Hz, H16,18), 7.78 (1H, s, H28), 7.81 (2H, m, H25,26), 10.70 (1H, s, NH20), 11.89 (1H, s, NH7), 11.99 (1H, s, NH10).

*N-(4-((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)oxy)phenyl)-4-(trifluoromethyl)benzamide (**1n**)*

Yield: 70%. Mp > 300 ºC. IR (KBr, cm^{-1}) ῡ: 3184 (NH), 1684 (C=O), 1630 (C=O), 1504, 1527 (C=C). ^{1}H NMR (400 MHz, DMSO) δ (ppm): 6.82 (1H, d, J = 2.4 Hz, H6), 6.88 (1H, dd, J = 8.8 Hz, 2.4 Hz, H2), 7.11 (2H, d, J = 8.8 Hz, H15,19), 7.19 (1H, d, J = 8.4 Hz, H3), 7.85 (2H, d, J = 8.8 Hz, H16,18), 7.98 (2H, d, J = 8 Hz, H24,28), 8.21 (2H, d, J = 8 Hz, H25,27), 10.70 (1H, s, NH20), 11.88 (1H, s, NH7), 11.97 (1H, s, NH10).

*N-(4-((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)oxy)phenyl)-4-nitrobenzamide (**1o**)*

Yield: 75%.Mp >300 ºC.IR (KBr, cm^{-1}) ῡ: 3174 (NH), 1684 (C=O), 1626 (C=O), 1504, 1523 (C=C). ^{1}H NMR (400 MHz, DMSO) δ (ppm): 6.83 (1H, d, J = 2.4 Hz, H6), 6.88 (1H, d, J = 8.8 Hz, 2.4 Hz, H2), 7.12 (2H, d, J = 8.8 Hz, H15,19), 7.20 (1H, d, J = 8.4 Hz, H3), 7.86 (2H, d, J = 8.8 Hz, H16,18), 8.26 (2H, d, J = 8.8 Hz, H24,28), 8.44 (2H, d, J = 8.8 Hz, H25,27), 10.69 (1H, s, NH20), 11.90 (1H, s, NH7), 11.99 (1H, s, NH10).

*N-(4-((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)oxy)phenyl)-3,5-dinitrobenzamide (**1p**)*

Yield: 70%. Mp >300 ºC. IR (KBr, cm^{-1}) ῡ: 3167 (NH), 1684 (C=O), 1628 (C=O), 1502, 1539 (C=C). ^{1}H NMR (400 MHz, DMSO) δ (ppm): 6.85 (1H, d, J = 2.4 Hz, H6), 6.883 (1H, dd, J = 8.8 Hz, 2.4 Hz, H2), 7.14 (2H, d, J = 8.8 Hz, H15,19), 7.21 (1H, d, J = 8.4 Hz, H3), 7.88 (2H, d, J = 8.8 Hz, H16,18), 9.07 (1H, t, J = 2 Hz, H26), 9.24 (2H, d, J = 2 Hz, H24,28), 11.01 (1H, s, NH20), 11.92 (1H, s, NH7), 11.99 (1H, s, NH10)

Biological evaluation

All the synthesized compounds were tested for cytotoxic activity against HeLa and MCF-7 cell lines by MTT assay. The IC_{50} values

represent the mean of three independent experiments each performed in triplicate. The IC_{50} values of all compounds are given in Table 1. The results indicated that most compounds exhibited moderate to good activities against MCF-7 and HeLa cell lines. Compounds **1i** and **1k-1n** showed the best activity with the IC_{50} values of 19, 16, 22, 18, and 16 μM against MCF-7 cell line and 20, 18, 25, 20 and 18 μM against HeLa cell line, respectively. Compounds **1j, 1o,** and **1p** showed the lowest activity against two cancer cell lines with IC_{50} values more than 100 μM.

Analysis of variance (ANOVA) showed that the cytotoxic effects of all prepared target compounds at all concentrations were significant in comparison with the negative control ($P < 0.05$), (Fig.2).

DISCUSSION

In this project, a total of 9 novel compounds in 6 synthetic steps were synthesized. Briefly, the amino group of p-aminophenol was first protected followed by O-arylation of N-(4-hydroxyphenyl) acetamide with 5-chloro-2-nitroaniline to provide N-(4-(3-amino-4-nitrophenoxy)phenyl) acetamide. Reduction of the nitro group of N-(4-(3-amino-4-nitrophenoxy)phenyl) acetamide and cyclization of diamine N-(4-(3,4-diaminophenoxy)phenyl) acetamide with oxalic acid afforded compound N-(4-((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)oxy)phenyl) acetamide which on deacetylation gave compound 6-(4-aminophenoxy)-1,4-dihydroquinoxaline-2,3-dione. Then 6- (4-aminophenoxy) -1,4-dihydroquinoxaline-2,3-dione were reacted with different acyl halides to give the target compounds **1h-1p**.

The synthesized compounds were screened for their *in vitro* cytotoxic activities against MCF-7 and HeLa cell lines using MTT assay. The results are summarized in Table 1 and has shown graphically in Fig. 2.

In the most of previous researches, quinoxaline derivatives possessing halogen substituents showed reasonable cytotoxic activity (26). Kingkan and Valery designed and synthesized 1-(2-((4-methoxyphenyl) amino)-3-oxo-3,4 dihydroquinoxalin-6-yl)-3-phenylurea and 1-(2-((4-chlorophenyl) amino)-3-oxo-3,4 dihydroquinoxalin-6-yl)-3-phenylurea and evaluated their cytotoxicity against HeLa and MCF-7 cells. Cytotoxic activity revealed the influence of p-Cl-phenyl and p-OCH3- phenyl moieties through the considerable increasing of the anticancer activity against HeLa and MCF-7 cell lines (12). Considerable cytotoxic activities of compounds with quinoxalindion moieties have been attributed to their capability of polar and hydrogen band formation with receptors. Higher flexibility of these compounds is another reason for their better interaction with active site of the receptors (27).

In our previous works, diaryl urea derivatives bearing quinoxalindione moiety displayed great cytotoxic activity. Replacing the urea linker with an amide maintained hydrogen bonding while reduced the flexibility of derivatives presented here (28). Careful verification of the relations between structure and the data of tested activities revealed the following points.

Compound **1h**, the parent compound, exhibited moderate toxicity whit IC_{50} values of 53 μM against MCF-7 cell line and 55 μM against HeLa cell line. Substitution of lipophilic groups such as trifluoromethyl ($-CF_3$) at 3-position or 4-position or fluorine (F) in 4-position or chlorine (Cl) in 4-position or 2,5-position of the A ring led to increased cytotoxic activity in compounds **1i** and **1k-1n** with IC_{50} values of 19, 16, 22, 18 and 16 μM against MCF-7 cell line and 20, 18, 25, 20 and 18 μM against HeLa cell line, respectively, compared to the unsubstituted compound **1h**.

Introduction of F group at the para position along with CF3 at the meta position increased activities of compound **1k** in comparison with the compound without F group **1i**. On the other hand, different positions of A ring indicate the susceptibility to the size of the substituent, since the bulky substituent such as NO_2 or t-Bu groups, without considering electron-donating or electron-withdrawing properties significantly reduced the cytotoxicity of compounds **1j, 1o,** and **1p** with IC_{50} values more than 100 μM compared to the small substituent F, Cl, CF_3 in compounds **1i** and **1k-1n**.

CONCLUSION

In the present study, a series of novel sorafenib analogs containing a quinoxalinedione ring with amide linker were synthesized. Chemical structures of synthesized compounds confirmed by ^{1}H-NMR and FT-IR analysis. All compounds were evaluated at 1, 10, 50, and 100 µM concentrations for their cytotoxicity against HeLa and MCF-7 cancer cell lines. Compounds **1i** and **1k-1n** showed good cytotoxic activity with the IC_{50} values of 19, 16, 22, 18, and 16 µM against MCF-7 cell line and 20, 18, 25, 20, and 18 µM against HeLa cell line, respectively; which substitution of lipophilic and electron-withdrawing groups such as CF_3, F, and Cl in the A ring led to increased activity cytotoxicity.

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