

Synthesis and cytotoxic evaluation of some derivatives of triazole-quinazolinone hybrids

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Abstract

Triazoles and quinazolinones are important heterocyclic structures with diverse biological properties including cytotoxic, antibacterial, antifungal and anticonvulsant activities. Due to valuable cytotoxic effects of both triazole and quinazolinone derivatives, in this study a series of quinazolinone-triazole hybrids were synthesized in a multiple-step reaction procedure. 3-Amino-quinazolinone derivatives were treated with chloroacetyl chloride in the presence of dichloromethane/triethylamine to afford 2-chloro -N-(4-oxo-2-quinazolin-3(3H)-yl) acetamide derivatives. The reaction of resultants with 4-methyl-4H-1, 2, 4-triazole-3-thiol in dry acetone and potassium carbonate led to the formation of final products. Synthesized compounds were evaluated for their cytotoxic effects against MCF-7 and Hela cell lines using MTT colorimetric assay. Amongst tested compounds, **6a** showed the highest cytotoxic activity against MCF7 cell line at all tested concentrations while compounds **6b** and **6c** indicated mild cytotoxic effects against Hela cell line at highest tested concentration reducing cell viability about 40%. The IC₅₀ values of tested compounds revealed that the MCF-7 is more susceptible to the compound **6a**.

Keywords: Cytotoxicity; Hybrid; Quinazolinone; Triazole.

INTRODUCTION

Cancer, as a major health problem, has been identified by high proliferation of abnormal cells. Although there have been great advances in the treatment of cancer, due to the complications associated with clinical use of anticancer drugs such as drug resistance, side effects, and systemic toxicity, discovering various chemical structures with high efficacy and minimum undesirable side effects is very important (1). Quinazolinone as an important pharmacophore in medicinal chemistry has drawn much attention due to its wide range of biological effects, which include antibacterial, antifungal, anticancer, anti-inflammatory, and antihypertensive activities (2-7). Different mechanisms such as inhibition of the DNA repair enzyme, inhibition of epidermal growth factor receptor (EGFR), thymidylate enzyme inhibition and inhibitory effects for tubulin polymerize have been proposed for anticancer

activities of quinazolinone-based structures (8,9). Triazole is another important heterocyclic structure, which can be incorporated in different templates to improve their activities (10). This five membered ring heterocycle is available with three nitrogen atoms in the form of two isomers 1,2,3-triazole and 1,2,4-triazole. Biological activities of 1,2,4-triazole such as antibacterial, antifungal, antiviral, anticancer, anticonvulsant, and anti-tubercular have been explored in many investigations (11-14). The known systems fused to triazoles are pyridines, pyridazines, quinazolines, pyrazines and triazines (15). Triazoles have been linked to quinazolinone structure to synthesis novel hybrid structure with different biological effects (15,17). Triazoloquinazolines constitute an important class of compounds in medicinal chemistry (15).

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In view of valuable cytotoxic effects of both triazoles and quinazolinones and in continuation of our previous research on quinazolinone derivatives (2-9), it seemed most interesting to synthesize derivatives of quinazolinone bearing triazole group as enhancing moiety for cytotoxic activity.

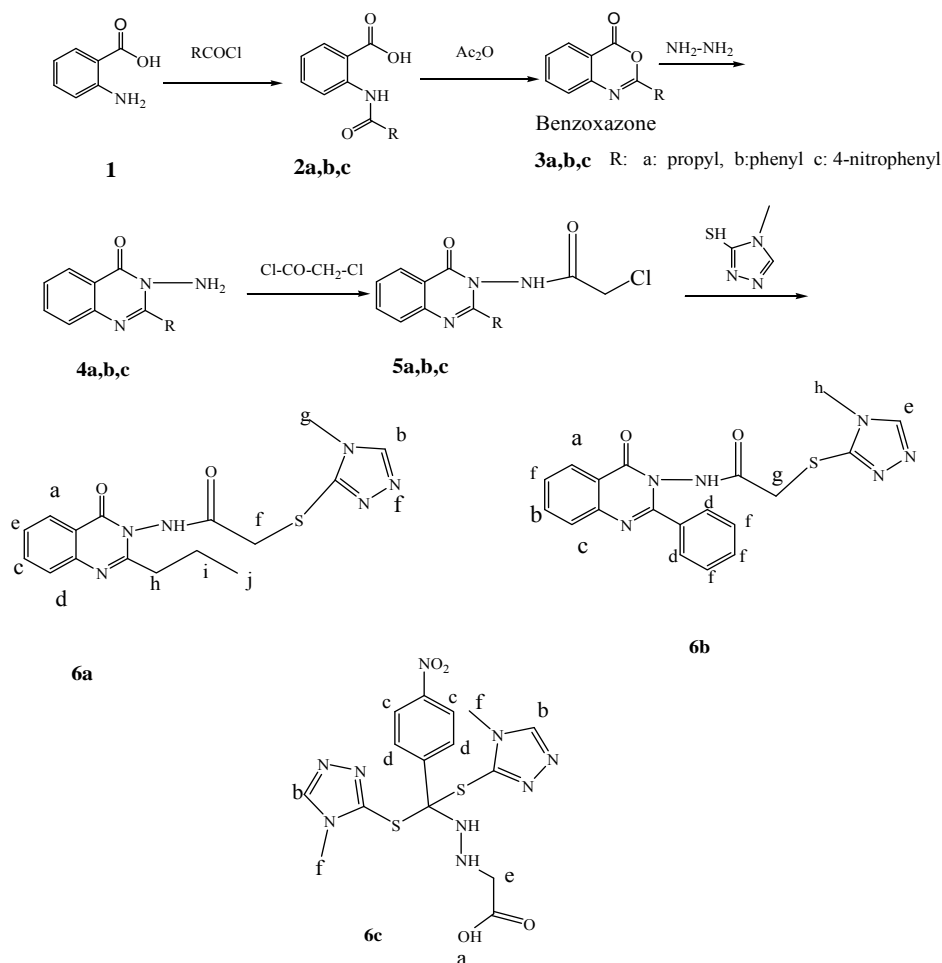
MATERIALS AND METHODS

All starting materials, reagents, and solvents were purchased from commercial suppliers Merck (Germany) and Aldrich (USA) companies. Merck silica gel 60 F₂₅₄ plates (Germany) were applied for analytical thin layer chromatography (TLC) (Germany). Proton nuclear magnetic resonance (HNMR) spectra were recorded using a Bruker 400 MHz (Germany). Spectrometer and chemical shifts are expressed as ppm with tetramethylsilane (TMS) as internal standard.

IR (KBr discs) spectra were recorded with a WQF-510 FT-IR spectrophotometer (China). Melting points were determined using electrothermal 9200 melting point apparatus (England) and are uncorrected. All cell lines were supplied from Pasteur Institute of Iran (Tehran, I.R. Iran).

General procedure for synthesis of compounds

3-Amino-quinazolinone derivatives were prepared according to the procedures shown in Scheme 1 (18). Then quinazolinone derivatives were treated with chloroacetyl chloride in the presence of dichloromethane/triethylamine to afford the 2-chloro -N-(4-oxo-2-quinazolin3 (3H)-yl) acetamide derivatives. Final products were synthesized by the reaction of 2-chloro -N-(4-oxo-2-quinazolin3 (3H)-yl) acetamide derivatives with 4-methyl-4-H-1, 2, 4-triazole-3-thiol in dry acetone and potassium carbonate (19,20).



Scheme 1. Synthesis of the target compounds (6a-6c).

Cytotoxicity assay**Sample and culture media preparation**

MCF-7 (breast cancer) and HeLa (cervical cancer) cells were purchased from pasture institute of Iran (Tehran, I.R. Iran) and maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO₂. Cells were cultured in RPMI 1640 completed with 5% v/v fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. After 2-3 subcultures, 180 µL of the cell suspension (5 × 10⁴ cells/mL) were seeded in 96-well plates and incubated for 24 h.

The stock solutions of compounds (10 mM, 1 mL) were prepared in DMSO and diluted with the medium to obtain 10, 100, 1000 µM concentrations.

After 24 h incubation, cells were treated with different concentrations of the derivatives (1, 10, 100 µM) for 48 h. Doxorubicin was used as positive control. Then 20 µL of MTT solution (5 mg/mL in phosphate buffer solution) was added to each well and incubated for 4 h. Afterwards, the media in each well was gently replaced with 150 µL DMSO to dissolve formazan crystals. The absorbance of each well was measured at 540 nm using an ELISA plate reader. Each experiment was repeated three times. Analysis of variance (ANOVA) followed by Tukey test was used to determine the differences between various groups. The significance level was set at $P < 0.05$. Cell viability was calculated using following equation:

$$\text{Cell Survival (\%)} = \frac{\text{Mean absorbance in drug treated wells} - \text{mean absorbance in blank}}{\text{Mean absorbance in negative control wells} - \text{mean absorbance in blank}} \times 100$$

RESULTS**Synthesis of 2-amido-benzoic acid derivatives (2a, 2b, 2c)**

Acyl chloride (butyryl chloride, benzoyl chloride, and 4-nitrobenzoyl chloride) (0.045 mol) was added dropwise to a solution of (0.04 mol) anthranilic acid (**1**) in dimethyl formamide (35 mL) and stirred at room temperature for 3 h. The mixture was poured into water and the precipitate was collected by filtration, washed with water, and dried under reduced pressure to give **2a-c** (Scheme 1).

Synthesis of benzoxazone derivatives (3a, 3b, 3c)

Compound **2a-c** (0.01 mol) was dissolved in acetic anhydride (30 mL) and heated for 1 h with vigorous stirring. The solvent was removed by distillation under reduced pressure to give **3a-c** (Scheme 1).

Synthesis of 3-aminoquinazolinone derivatives (4a, 4b, 4c)

A mixture of benzoxazone derivatives **3a-c** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol was refluxed for 3 h. After the reaction was completed, the mixture was cooled and the separated solid was collected by filtration and recrystallized from ethanol or isopropanol.

3-Amino-2-propylquinazolin-4(3H)-one (4a)

¹HNMR δ: (400 MHz; CDCl₃), 8.14 (1H, dd, $J = 8\text{ Hz}$, $J = 4\text{ Hz}$, H^a), 7.65 (1H, t, $J = 8\text{ Hz}$, H^b), 7.58 (1H, d, $J = 8\text{ Hz}$, H^c), 7.35 (1H, t, $J = 8\text{ Hz}$, H^d), 4.8 (2H, s, NH₂), 2.92 (2H, t, $J = 8\text{ Hz}$, CH₂^e), 1.77 (2H, m, CH₂^f), 1.0 (3H, t, $J = 8\text{ Hz}$, CH₃^g) (Fig. 1)

3-Amino-2-phenylquinazolin-4(3H)-one (4b)

¹HNMR δ: (400 MHz; CDCl₃), 8.26 (1H, dd, $J = 8\text{ Hz}$, $J = 4\text{ Hz}$, H^a), 7.71-7.74 (4H, m, H^b), 7.44-7.47 (4H, m, H^c), 4.94 (2H, s, NH₂) (Fig1).

3-Amino-2-(4-nitrophenyl)quinazolin-4(3H)-one (4c)

¹HNMR δ: (400 MHz; CDCl₃), 8.30 (2H, d, $J = 8\text{ Hz}$, H^a), 8.28 (1H, d, $J = 8\text{ Hz}$, H^b), 7.95 (2H, d, $J = 8\text{ Hz}$, H^c), 7.7-7.8 (2H, m, H^d, H^e), 7.5 (1H, t, $J = 8\text{ Hz}$, H^f), 4.8 (2H, s, NH₂) (Fig 1).

Synthesis of 2-chloro-N-(4-oxo-2-quinazolin-3(3H)-yl)acetamide derivatives (5a, 5b, 5c)

3-Amino-quinazolinone derivatives **4a-4c** (0.01 mol) was dissolved in dry dichloromethane (20 mL). To this mixture, triethylamine (0.01 mol) and chloroacetylchloride (0.01 mol) were added and stirred at room temperature for 30 min. Then the reaction mixture was poured in to ice-water and extracted with dichloromethane and ethyl acetate. The extracted ethyl acetate was washed with 3 % sodium bicarbonate solution and dried over anhydrous magnesium sulfate, which upon evaporation afforded the product **5a-5c**.

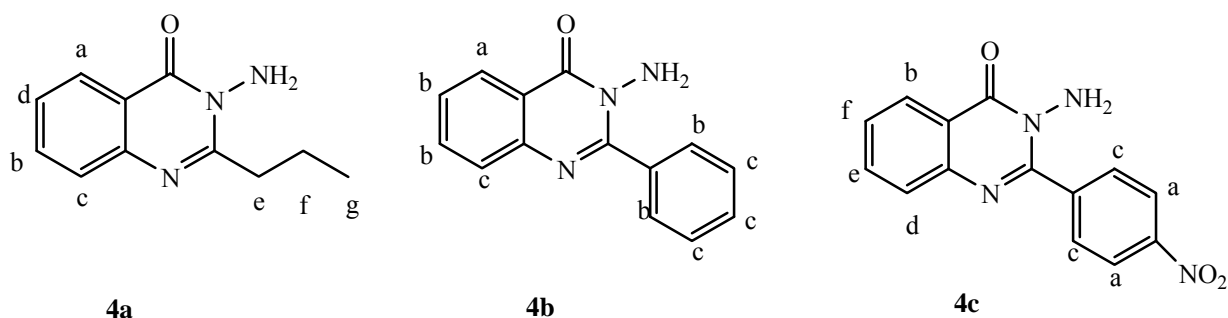


Fig. 1. 3-Aminoquinazolinone derivatives (**4a**, **4b**, **4c**).

Synthesis of quinazolinone-triazole hybrid derivative (**6a**, **6b**, **6c**)

A mixture of 2-chloro -N-(4-oxo-2-quinazolin-3(4H)-yl) acetamide derivatives **5a-5c** (0.01 mol), 4-methyl-4H-1,2,4-triazole-3-thiol (0.01 mol) and anhydrous potassium carbonate (0.01 mmol) was refluxed in dry acetone (20 mL) for 6 h. The reaction mixture was filtered while hot and the residue was washed with hot acetone. The organic solution was concentrated and purified by preparative thin layer chromatography.

2-(4-Methyl-4H-1,2,4-triazol-3-ylthio)-N-(4-oxo-2-propylquinazolin-3(4H)-yl) acetamide (**6a**)

Yield: 30%, m.p. 128-129 °C, IR ν_{\max} , 3428 (NH), 2844 (C-H), 1696 (C=O) cm^{-1} . $^1\text{H NMR}$ δ : (400 MHz; CDCl_3), 10 (b, NH), 8.05-8.15 (2H, m, H^a , H^b), 7.65 (1H, t, $J = 8$ Hz, H^c), 7.58 (1H, d, $J = 8$ Hz, H^d), 7.32 (1H, t, $J = 8$ Hz, H^e), 4.14-4.17 (1H, d, $J = 12$ Hz, CH_2^f), 3.82-3.85 (1H, d, $J = 12$ Hz, CH_2^f), 3.62 (3H, s, CH_3^g), 2.65 (2H, m, $J = 8$ Hz, CH_2^h), 1.73 (2H, m, CH_2^i), 0.93 (3H, t, $J = 8$ Hz, CH_3^j).

2-(4-Methyl-4H-1,2,4-triazol-3-ylthio)-N-(4-oxo-2-phenylquinazolin-3(4H)-yl) acetamide (**6b**)

Yield: 33%, m.p. 190-191 °C, IR ν_{\max} , 3353 (NH), 3050 (C-H, Ar), 1690 (C=O) cm^{-1} . $^1\text{H NMR}$ δ : (400 MHz; DMSO), 8.27 (1H, d, $J = 8$ Hz, H^a), 8.0 (1H, t, $J = 8$ Hz, H^b), 7.84 (1H, d, $J = 8$ Hz, H^c), 7.68-7.72 (3H, m, H^d , H^e), 7.54-7.61 (4H, m, H^f), 7.15 (1H, s, NH), 4.23-4.26 (1H, d, $J = 12$ Hz, CH_2^g), 4.18-4.21 (1H, d, $J = 12$ Hz, CH_2^g), 3.61 (3H, s, CH_3^h).

2-(2-bis(4-methyl-4H-1,2,4-triazol-3-nitrophenyl)methylhydrazinyl)acetic acid (**6c**)

Yield: 35%, m.p. 208-209 °C, IR ν_{\max} , 3200 (NH), 2993 (CH), 1691 (C=O), 1536, 1345 (NO_2) cm^{-1} . $^1\text{H NMR}$ δ : (400 MHz; DMSO), 10.62 (1H, s, H^a), 9.39 (2H, s, H^b), 8.38 (2H, d, $J = 8$ Hz, H^c), 8.22 (2H, d, $J = 8$ Hz, H^d), 4.32 (2H, s, H^e), 4.23 (1H, NH), 3.86 (1H, NH), 3.75 (6H, s, H^f).

Cytotoxic effects of the derivatives (**6a-c**)

The cytotoxicity of compounds were evaluated against HeLa and MCF7 cell lines at different concentrations of 1, 10, and 100 μM using MTT assay. Results are shown in Figs. 2, 3, and Table 1.

The obtained results from cytotoxic tests against HeLa cell line indicated that all three compounds **6a-c** exhibited significant cytotoxic effects ($P < 0.05$ at 1 μM and $P < 0.01$ at 10 and 100 μM) in comparison with the negative control. Although significant differences between different compounds in the same concentrations were not observed (**6a-6c**), compounds **6b** and **6c** at highest tested concentrations demonstrated mild cytotoxic effects against HeLa cell line.

Cytotoxic assessment also indicated that compounds **6b** and **6c** had significant cytotoxic activities against MCF7 cell line ($P < 0.05$ at 1 μM and $P < 0.01$ at 10 and 100 μM) compared to the negative control. Compound **6a**, however, showed more considerable cytotoxicity ($P < 0.01$ at 10 μM , 1 μM and $P < 0.001$ at 100 μM) against MCF7 cell line compared to the negative control.

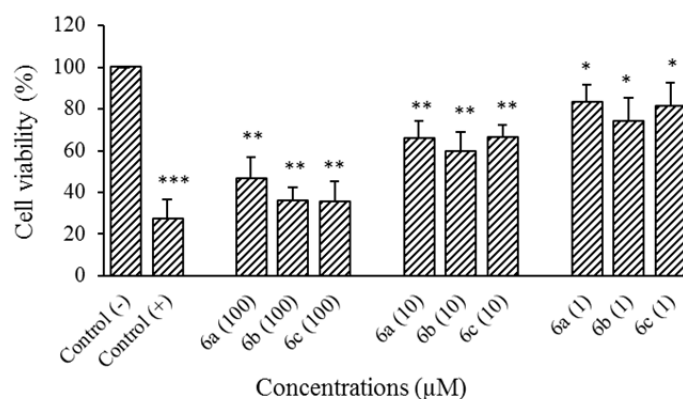


Fig. 2. Cytotoxic effect of compounds **6a-6c** on HeLa cells following exposure to different concentrations (1, 10, and 100 μM). Data are presented as mean \pm SD, (SD for negative control is 0.06), $n = 3$. * Shows significant differences ($*P < 0.05$ and $**P < 0.01$) in comparison with negative control group; doxorubicin used as positive control.

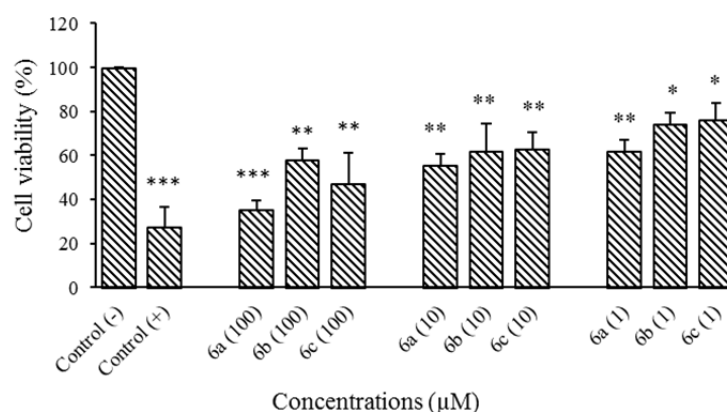


Fig. 3. Cytotoxic effect of compounds **6a-6c** on MCF7 cells following exposure to different concentrations (1, 10, and 100 μM). Data are presented as mean \pm SD, (SD for negative control is 0.06), $n = 3$. * Shows significant differences ($*P < 0.05$, $**P < 0.01$, and $***P < 0.001$) in comparison with negative control group; doxorubicin used as positive control.

Table 1. The IC_{50} (μM) of tested compounds against MCF7 and HeLa cell lines.

Target compounds	R	MCF-7 cells	HeLa cells
6a	Propyl	38.385 ± 4.5	85.58 ± 9
6b	Phenyl	159.42 ± 8.5	49.30 ± 7.5
6c	Nitro phenyl	87.77 ± 9	54.11 ± 7.4

DISCUSSION

Quinazolinone are important scaffolds for new drug development with diverse biological properties particularly anticancer activity. While many of the naturally bioactive quinazolinones have rigid structures, the synthetic derivatives such as 2 or 4-substituted are more flexible (21). It is also reported that quinazolinone derivatives that contain aromatic rings or some nitrogenated heterocycles in their structures are potential

antitumor agents (5,22,23). 2-(2thieno)-6-iodo-3-phenylamino-3,4-dihydro-quinazolin - 4-one, 3-benzyl-2-cinnamylthio - 6 - (methyl or nitro)quinazolin-4(3H)-ones, 2-(2-Aminothiazol-5-ylthio)-3-phenyl -6-methyl-quinazolin-4(3H)-ones and 2- (4 - chlorophenyl)-6-nitro-3-(2 - (2phenylthiazole - 4 - yl)ethyl)quinazolin - 4(3H)one are derivatives whose anticancer activities are mediated via dihydrofolate reductase (DHFR) inhibition (8,24-26).

Triazole is a unique template which can conjugate to different heterocyclic to improve

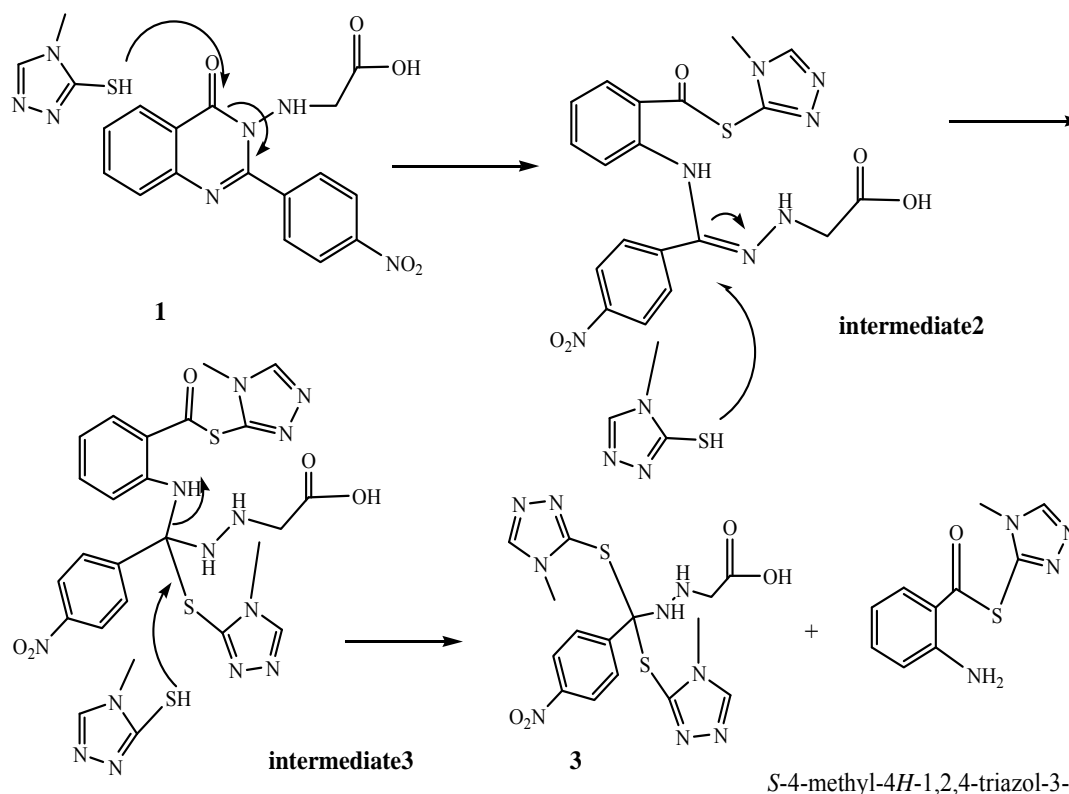
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their activities (10,27). The known systems fused to triazoles are pyridines, pyridazines, quinazolines, pyrazines and triazines(15). Hybrid structures of quinazolinone-triazole have been reported with anticancer (28), antibacterial (15,16,29), anti-malaria (30) and anticonvulsant (31) activities.

In this study, 3-aminoquinazolinone-4-(3H)-one derivatives were synthesized in three steps starting from anthranilic acid. Fairly unstable compounds **5a-5c** were prepared by the reaction between 3-aminoquinazolinone-4-(3H)-one derivatives and chloroacetyl chloride in dry dichloromethane in the presence of triethylamine. Final compounds **6a-6c** were obtained through the nucleophilic displacement of the chloride with thiol of 4-methyl-4H-1,2,4-triazole-3-thiol using acetone and potassium carbonate in reflux condition. Compound **6c** was produced according to proposed mechanism in Scheme 2. Reaction between 3-aminoquinazolinone-4-(3H)-one derivatives and chloroacetyl chloride in dry dichloromethane in the presence of

triethylamine produced compound **1** as an undesirable product. Triazole acted as a nucleophile and attacked the carbonyl group of molecule **1**, which resulted in the ring opening to afford the intermediate **2**. Subsequently two molecules of triazole attacked to the C=N and carbon of intermediate **2** and **3**, respectively. Exiting the derivative of anthranilic acid-triazole from the intermediate **3** produced compound **6c** (Scheme 2).

Among tested compounds, **6a** showed the highest cytotoxic activity against MCF7 cell line at all tested concentrations while compounds **6b** and **6c** indicated mild cytotoxic effects against Hela cell line at highest tested concentrations. The IC₅₀ values of tested compounds revealed that the MCF-7 is more susceptible to the compound **6a**. Substitution of propyl group at 2 position of quinazolinone improved the cytotoxic activity against MCF7 due to electronic effect. The cell growth inhibitory activity of the compounds may be attributed to substituent property at position 2 of quinazolinone ring.



Scheme 2. The probable mechanism to produce compound **6c**.

CONCLUSIONS

In this study, quinazolinones as a pharmacophore structure were conjugated with triazole ring in a multi-step procedure. The final compounds were tested against two cell lines. Compound **6a** showed the highest cytotoxic activities against MCF7 cell line. Compounds **6b** and **6c** indicated mild cytotoxic effects against HeLa cells at highest tested concentrations.

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