

## Synthesis and cytotoxic evaluation of some new 3-(2-(2-phenylthiazol-4-yl) ethyl)-quinazolin-4(3H) one derivatives with potential anticancer effects

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### Abstract

Quinazolinones are a group of heterocyclic compounds that have important biological activities such as cytotoxicity, anti-bacterial, and anti-fungal effects. Thiazole-containing compounds have also many biological effects including antitumor, antibacterial, anti-inflammatory, and analgesic activities. Due to significant cytotoxic effects of both quinazoline and thiazole derivatives, in this work a group of quinazolinone-thiazol hybrids were prepared and their cytotoxic effects on three cell lines were evaluated using MTT assay. Compounds **A3**, **A2**, **B4**, and **A1** showed highest cytotoxic activities against PC3 cell line. Compounds **A3**, **A5**, and **A2** were most active against MCF-7 and **A3**, **A5**, and **A6** showed good cytotoxic effect on HT-29 cell line. According to the results, **A3** efficiently inhibited all cell growth tested in a dose dependent manner. The IC<sub>50</sub> of **A3** was 10 M, 10 μM, and 12 μM on PC3, MCF-7, and HT-29 cells, respectively.

**Keywords:** Quinazolinone; Thiazole; Cytotoxic

### INTRODUCTION

Cancer is a life threatening health problem in developing and undeveloped countries. Although great progress in the treatment of this disease is made with respect to the problems associated with drug resistance, more research is essential for discovery of new anticancer agents. Combining two or more pharmacophores into one molecule is an approach to discover new targets. Therefore, there is more than one pharmacophore in a single molecule, each with a different mechanism of action, which can be effective for cancer treatment. Hybrid pharmacophores may be attached to different locations in the active site leading to elimination of drug resistance. Also, this method can reduce anticancer side effects (1,2).

Quinazoline and their derivatives are structural units for 150 natural alkaloids isolated from a number of families of the plant kingdom, from microorganisms and animals (3).

There are many reports on biological activities of synthetic and natural quinazolines including sedative (4), anticonvulsant (4-6), anti-inflammatory (4,7), antitumor (4,8), antibacterial (4,9-11), antifungal (5,6), antitubercular (5,7,9,12), antimalarial (10,13), antiviral (5,7), anti-HIV (4,9-11,14), and antihyperlipidemic activities (15,16).

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Website: <http://rps.mui.ac.ir>

DOI: 10.4103/1735-5362.212046

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Some drugs have been synthesized with quinazoline structure such as chloroqualone (antitussive), diproqualone (analgesic) (17), gefitinib, lapatinib (anticancer) (1), piriqualone (anticonvulsant) (18), doxazocin (antihypertensive) (19), prazosin (antihypertensive) (20), trimetrexate (antibacterial), thymitaq (anticancer) (21) and raltitrexed (anticancer) (22).

Thiazole-containing compounds also have valuable biological activities such as antitumor, anti-inflammatory, analgesic, antibacterial, and antifungal effects (23-26). Thiazole, an important heterocyclic ring, is widely used in anticancer drug development. Several anticancer agents containing thiazole moiety have been discovered, like tiazofurin and bleomycin. Ritonavir (anti-HIV), meloxicam (anti-inflammatory), nizatidine (antipeptic ulcer), and penicillin (antibiotic) are some examples of other thiazole compounds with biological activities (23,27). Due to the valuable cytotoxic effects of both thiazole and quinazoline compounds, in this work a group of quinazolinone-thiazole hybrids were synthesized and their antiproliferative activities were determined using tumor cells in culture.

## MATERIALS AND METHODS

### Instrumentation

All starting materials, reagents, and solvents were purchased from commercial suppliers like Merck (Germany) and Aldrich (USA) companies. The purity of the synthesized compounds was proved by thin layer chromatography (TLC) using various solvents. Merck silica gel 60 F254 plates were applied for analytical TLC. <sup>1</sup>H-NMR spectra were recorded using a Bruker 400 MHz spectrometer (Germany), and chemical shifts are expressed as  $\delta$  (ppm) with tetramethylsilane as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (Japan) (potassium bromide disks). Melting points were determined using electrothermal melting point analyzer apparatus and are uncorrected. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. All cell lines were purchased from Pasteur Institute of Iran.

### Preparation of compounds

To produce 3-(2-(2-phenylthiazol-4-yl)ethyl)-quinazolin-4(3H)-one derivatives, the primary amine **G** was synthesized through a five step procedure. In the first step, 4-phthalimido-2-butanone (**B**), was prepared through the addition of methyl vinyl ketone to phthalimide (**A**). In the second step, 1-bromo-4-N-phthalimido-2-butanone (**C**) was synthesized by bromination of the methyl group of compound **B**. Nucleophilic substitution of thiobenzamide (**E**) to the brominated intermediate (**C**) resulted in compound **F** which was reacted with hydrazine hydrate and deprotected to produce the 2-phenyl-4-(2-aminoethyl) thiazole **G** (1). A group of benzoxazinones with different substituents at position 2 were synthesized. Reaction of the primary amine **G** with these benzoxazinones yielded the final compounds as presented in Fig. 1. The structure of final compounds are presented in Figs. 2 and 3.

### Cell culture conditions

PC3 (human prostate cancer cell line), MCF7 (human breast adenocarcinoma cell line), and HT-29 (human colon adenocarcinoma cell line) cells were maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO<sub>2</sub>. PC3, MCF7, and HT-29 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM-F12) with 10% v/v fetal bovine serum, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin. The medium was changed every two to three days and sub-cultured when the cell population density reached to 70-80% confluence. Cells were seeded at an appropriate density according to each experimental design (28).

### Cytotoxicity assay

HT-29, MCF-7, and PC-3 cells were seeded in triplicate in 96-well tissue culture plates (15  $\times$  10<sup>3</sup> cells/well) and incubated overnight. Doxorubicin was used as positive control and the wells containing DMSO (1%) and cell suspension was regarded as the negative control. The blank wells were consisted of 200  $\mu$ L of the culture medium. Cells were treated with different concentrations of the derivatives (0-275  $\mu$ M).

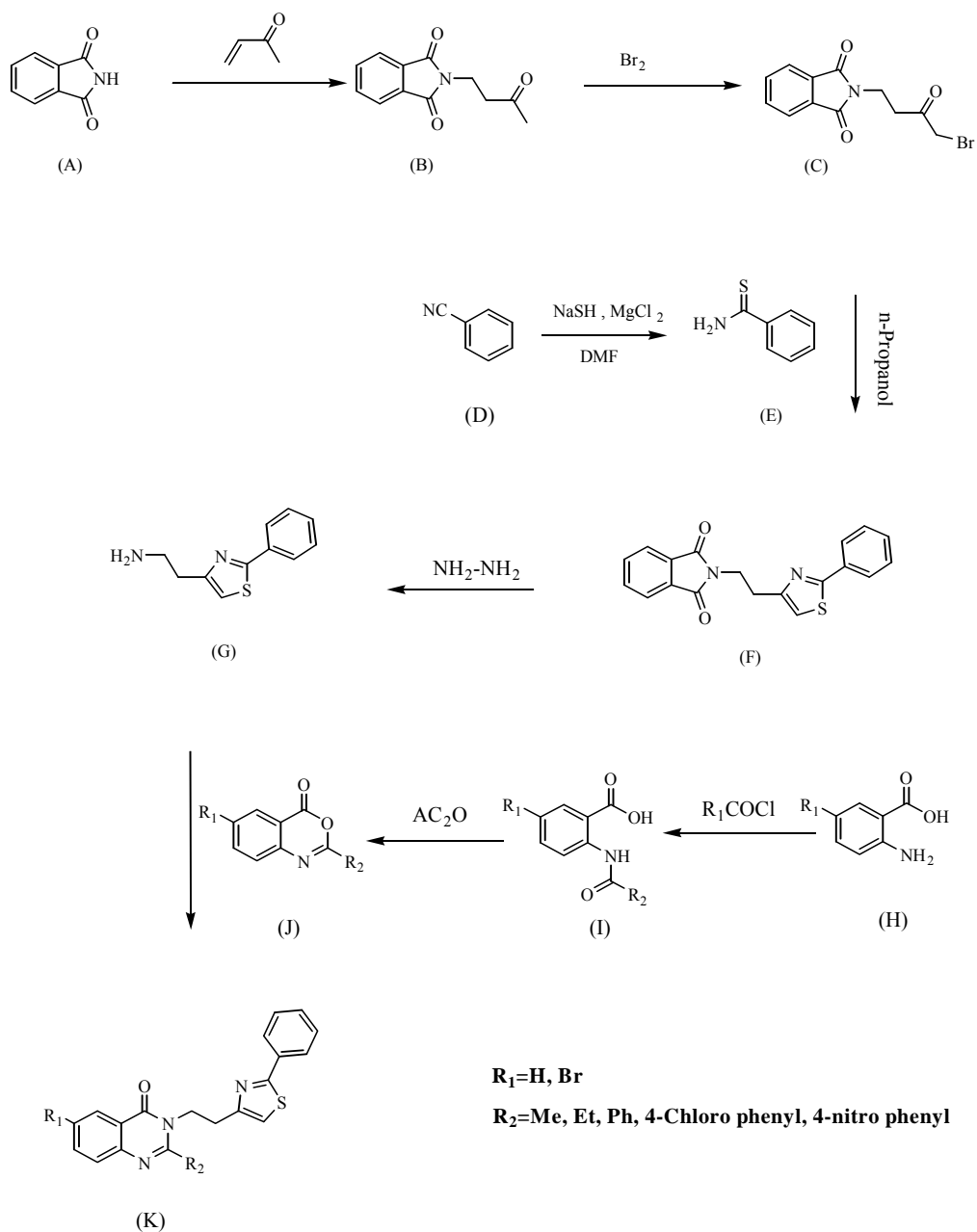


Fig. 1. General reaction scheme for preparation of the final compounds.

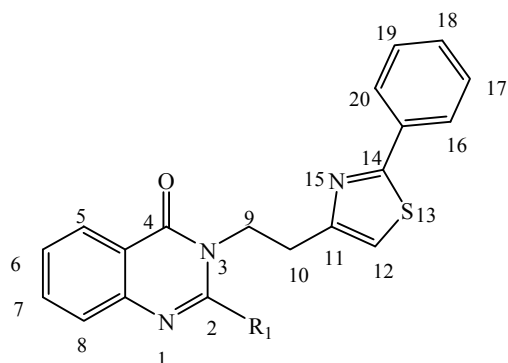
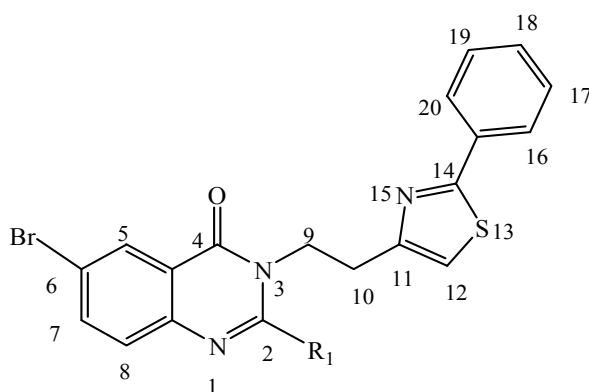


Fig. 2. General structure of final compounds (group A).



**Fig. 3.** General structure of final compounds (group B).

The microplates were further incubated for 48 h. To evaluate cell survival, each well was then incubated with 20  $\mu$ L of MTT solution (5 mg/mL in PBS) for 3 h and the media in each well was replaced with 200  $\mu$ L of DMSO and pipetted up and down to dissolve the formazan crystals.

The absorbance of each well was measured at 540 nm using an ELISA reader. Each experiment was repeated three times. The percentage of cell viability was calculated using the following formula:

*Survival (%) =*

$$\frac{\text{Well absorbance} - \text{Blank absorbance}}{\text{Control absorbance} - \text{Blank absorbance}} \times 100$$

IC<sub>50</sub> values were calculated by plotting the log<sub>10</sub> of percent cell viability against compound concentrations (28,29).

## RESULTS

### *Details of preparation procedures and chemistry of synthesized compounds*

#### *N-Acyl anthranilic acid (I)*

Acyl chloride (0.37 mol) was added dropwise to a mixture of compound **H** (0.25 mol) in dimethyl formamide (125 mL) at such rate that the temperature of the mixture did not rise above 40 °C.

The mixture was stirred at room temperature for at least an additional 3 h. Completion of the reaction was determined by TLC and the mixture was poured into water (1 L) and stirred for 1 h.

The precipitated product was collected by filtration, washed with cold water, and dried under reduced pressure yielding **I** as a white powder (50-70%).

#### *2-Substituted-3,1-benzoxazin-4-one (J)*

Compound **I** (0.125 mol) was dissolved in acetic anhydride (90 mL) and slowly heated to 170-180 °C in a round-bottom flask equipped with a magnetic stirrer bar and a claisen-distillation head. Completion of the reaction was confirmed by TLC, and the produced acetic acid was distilled under reduced pressure. The residue was then cooled and product was washed by n-hexan to give compound **J** as yellow crystals (65-80%).

#### *3-(2-(2-phenylthiazol-4-yl) ethyl) -quinazolin-4 (3H)-one derivatives*

To prepare 3-(2-(2-phenylthiazol-4-yl)ethyl)-quinazolin-4(3H)-one derivatives, 0.5 mmol of related benzoxazine was refluxed with 1 mmol of amine **G** in chloroform (5 mL) for 6-7 h. After completion of the reaction, chloroform was evaporated under reduced pressure and the residue was treated with ethylene glycol (2 mL) and NaOH pellets (0.003 g) in a flask equipped with a claisen-distillation head.

The mixture was reheated to 130-140 °C for 5 h. After completion of the reaction, the clear solution was allowed to cool to room temperature and kept overnight to precipitate which was then crystallized from 2-propanol to obtain final products.

**2-methyl-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (A1)**

Yield: 55%. m.p 119 °C, (Found: M 347, C<sub>20</sub>H<sub>17</sub>N<sub>3</sub>OS requires 347), IR (KBr, cm<sup>-1</sup>) v<sub>max</sub> = 1671, 1595, 1474. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.60 (3H, s, H-CH<sub>3</sub>:R<sub>1</sub>), 3.29 (2H, t, J = 6.8 Hz, H-C<sub>10</sub>), 4.51 (2H, t, J = 6.8 Hz, H-C<sub>9</sub>), 6.93 (1H, s, H-C<sub>12</sub>), 7.4-7.5 (4H, m, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>, H-C<sub>6</sub>), 7.60 (1H, d, J = 7.2 Hz, H-C<sub>8</sub>), 7.70-7.76 (1H, m, H-C<sub>7</sub>), 7.88-7.94 (2H, m, H-C<sub>16</sub>, H-C<sub>20</sub>), 8.28 (1H, dd, J = 6.4 Hz, J = 1.6 Hz, H-C<sub>5</sub>).

**2-ethyl-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (A2)**

Yield: 48%. m.p 122 °C, (Found: M 361, C<sub>21</sub>H<sub>19</sub>N<sub>3</sub>OS requires 361), IR (KBr, cm<sup>-1</sup>) v<sub>max</sub> = 1671, 1593, 1473. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.34 (3H, t, J = 7.5, CH<sub>3</sub>:R<sub>1</sub>), 2.81 (2H, q, J = 7.5 Hz, CH<sub>2</sub>:R<sub>1</sub>), 3.28 (2H, t, J = 7.5 Hz, H-C<sub>10</sub>), 4.51 (2H, t, J = 7.5 Hz, H-C<sub>9</sub>), 6.94 (1H, s, H-C<sub>12</sub>), 7.40-7.50 (4H, m, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>, H-C<sub>6</sub>), 7.62-7.67 (1H, m, H-C<sub>8</sub>), 7.70-7.76 (1H, m, H-C<sub>7</sub>), 7.87-7.96 (2H, m, H-C<sub>16</sub>, H-C<sub>20</sub>), 8.26-8.31 (1H, m, H-C<sub>5</sub>).

**3-(2-(2-phenylthiazol-4-yl)ethyl)-2-propylquinazolin-4(3H)-one (A3)**

Yield: 35%. m.p 184 °C, (Found: M 375, C<sub>22</sub>H<sub>21</sub>N<sub>3</sub>OS requires 375), IR (KBr, cm<sup>-1</sup>) v<sub>max</sub> = 3093, 2873, 1678, 1523. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 0.95 (3H, t, J = 7.2 Hz, CH<sub>3</sub>:R<sub>1</sub>), 1.75 (2H, Hex, J = 7.2 Hz, CH<sub>2</sub>:R<sub>1</sub>), 2.66 (2H, t, J = 7.6 Hz, CH<sub>2</sub>:R<sub>1</sub>), 3.21 (2H, t, J = 7.2 Hz, H-C<sub>10</sub>), 4.44 (2H, t, J = 7.2 Hz, H-C<sub>9</sub>), 6.86 (1H, s, H-C<sub>12</sub>), 7.30-7.40 (4H, m, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>, H-C<sub>6</sub>), 7.56 (1H, d, J = 8.0 Hz, H-C<sub>8</sub>), 7.65 (1H, t, J = 5.6 Hz, H-C<sub>7</sub>), 7.80-7.90 (2H, m, H-C<sub>16</sub>, H-C<sub>20</sub>), 8.20 (1H, d, J = 5.6 Hz, H-C<sub>5</sub>).

**2-phenyl-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (A4)**

Yield: 50%. m.p 140 °C, (Found: M 409, C<sub>25</sub>H<sub>19</sub>N<sub>3</sub>OS requires 409), IR (KBr, cm<sup>-1</sup>) v<sub>max</sub> = 1672, 1605, 1472. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 3.17 (2H, t, J = 7.2 Hz, H-C<sub>10</sub>), 4.46 (2H, t, J = 7.2 Hz, H-C<sub>9</sub>), 6.72 (1H, s, H-C<sub>12</sub>), 7.24-7.42 (8H, m, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>, H-C<sub>6</sub>, H-C<sub>8</sub>, 3H-Ph:R<sub>1</sub>), 7.52 (1H, t, J = 7.2 Hz, H-C<sub>7</sub>), 7.66-7.80 (4H, m, H-C<sub>16</sub>, H-C<sub>20</sub>, 2H-Ph:R<sub>1</sub>), 8.36 (1H, d, J = 8.0 Hz, H-C<sub>5</sub>).

**2-(4-chlorophenyl)-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (A5)**

Yield: 36%. m.p 170 °C, (Found: M 443, C<sub>25</sub>H<sub>18</sub>ClN<sub>3</sub>OS requires 443), IR (KBr, cm<sup>-1</sup>) v<sub>max</sub> = 3120, 2920, 1678, 1566. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 3.10 (2H, t, J = 6.4 Hz, H-C<sub>10</sub>), 4.41 (2H, t, J = 6.4 Hz, H-C<sub>9</sub>), 6.67 (1H, s, H-C<sub>12</sub>), 7.22-7.28 (4H, m, 4H-ClPh:R<sub>1</sub>), 7.30-7.38 (4H, m, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>, H-C<sub>6</sub>), 7.46 (1H, t, J = 6.8 Hz, H-C<sub>7</sub>), 7.58-7.64 (2H, m, H-C<sub>16</sub>, H-C<sub>20</sub>), 7.69-7.73 (1H, m, H-C<sub>8</sub>), 8.30 (1H, t, J = 6.8 Hz, H-C<sub>5</sub>).

**2-(4-nitrophenyl)-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (A6)**

Yield: 30%. m.p 145 °C, (Found: M 454, C<sub>25</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>S requires 454), IR (KBr, cm<sup>-1</sup>) v<sub>max</sub> = 3329, 3066, 1674, 1600. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 3.05 (2H, t, J = 6.8 Hz, H-C<sub>10</sub>), 3.75 (2H, t, J = 6.4 Hz, H-C<sub>9</sub>), 6.99 (1H, s, H-C<sub>12</sub>), 7.05 (1H, t, J = 6.4 Hz, H-C<sub>18</sub>), 7.34-7.40 (2H, m, H-C<sub>17</sub>, H-C<sub>19</sub>), 7.50 (1H, t, J = 5.6 Hz, H-C<sub>7</sub>), 7.66 (1H, d, J = 6.8 Hz, H-C<sub>8</sub>), 7.83-7.90 (2H, m, H-C<sub>16</sub>, H-C<sub>20</sub>), 8.14 (2H, d, J = 8.4 Hz, 2H-NO<sub>2</sub>Ph:R<sub>1</sub>), 8.28 (2H, d, J = 8.8 Hz, 2H-NO<sub>2</sub>Ph:R<sub>1</sub>), 8.32-8.38 (1H, m, H-C<sub>6</sub>), 8.76 (1H, d, J = 8.4 Hz, H-C<sub>5</sub>).

**6-bromo-2-methyl-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (B1)**

Yield: 75%. m.p 156 °C, (Found: M 425, C<sub>20</sub>H<sub>16</sub>BrN<sub>3</sub>OS requires 425), IR (KBr, cm<sup>-1</sup>) v<sub>max</sub> = 3390, 2922, 1670, 1593. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 2.44 (3H, s, CH<sub>3</sub>:R<sub>1</sub>), 3.21 (2H, t, J = 6.8 Hz, H-C<sub>10</sub>), 4.42 (2H, t, J = 6.8 Hz, H-C<sub>9</sub>), 6.85 (1H, s, H-C<sub>12</sub>), 7.32-7.42 (5H, m, H-C<sub>16</sub>, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>, H-C<sub>20</sub>), 7.72 (1H, d, J = 6.4 Hz, H-C<sub>8</sub>), 7.8-7.86 (1H, m, H-C<sub>7</sub>), 8.33 (1H, d, J = 2.4 Hz, H-C<sub>5</sub>).

**6-bromo-2-ethyl-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (B2)**

Yield: 55%. m.p 125 °C, (Found: M 439, C<sub>21</sub>H<sub>18</sub>BrN<sub>3</sub>OS requires 439), IR (KBr, cm<sup>-1</sup>) v<sub>max</sub> = 3298, 3062, 1670, 1508. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 1.25 (3H, t, J = 7.2 Hz, CH<sub>3</sub>:R<sub>1</sub>), 2.71 (2H, q, J = 7.2 Hz, CH<sub>2</sub>:R<sub>1</sub>), 3.19 (2H, t, J = 7.2 Hz, H-C<sub>10</sub>), 4.43 (2H, t, J = 7.2 Hz, H-C<sub>9</sub>), 6.67 (1H, s, H-C<sub>12</sub>), 7.30-7.40 (3H, m, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>), 7.44 (2H, d, J = 8.8 Hz, H-C<sub>16</sub>, H-C<sub>20</sub>), 7.72 (1H, d, J = 6.4 Hz, H-C<sub>8</sub>), 7.81-7.83 (1H, m, H-C<sub>7</sub>), 8.33 (1H, d, J = 2.4 Hz, H-C<sub>5</sub>).

**6-bromo-2-phenyl-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (B3)**

Yield: 40%. m.p 137 °C, (Found: M487, C<sub>25</sub>H<sub>18</sub>BrN<sub>3</sub>OS requires 487), IR (KBr, cm<sup>-1</sup>)  $\nu_{\max}$  = 3321, 3059, 1678, 1083. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 3.08(2H, t, J = 6.8 Hz, H-C<sub>10</sub>), 4.40 (2H, t, J = 7.2 Hz, H-C<sub>9</sub>), 6.65 (1H, s, H-C<sub>12</sub>), 7.30-7.40 (6H, m, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>, Ph-3H:R<sub>1</sub>), 7.50 (2H, d, J = 8.8 Hz, H-C<sub>16</sub>, H-C<sub>20</sub>), 7.59 (2H, d, J = 6.4 Hz, Ph-2H:R<sub>1</sub>), 7.76 (1H, d, J = 6.4 Hz, H-C<sub>8</sub>), 7.83-7.87 (1H, m, H-C<sub>7</sub>), 8.41 (1H, d, J = 2.0 Hz, H-C<sub>5</sub>).

**6-bromo-2-(4-chlorophenyl)-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (B4)**

Yield: 36%. m.p 195 °C, (Found: M 521, C<sub>25</sub>H<sub>17</sub>BrClN<sub>3</sub>OS requires 521), IR (KBr, cm<sup>-1</sup>)  $\nu_{\max}$  = 3066, 2924, 1643, 1516, 1091. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 3.22 (2H, t, J = 6.4 Hz, H-C<sub>10</sub>), 3.75 (2H, t, J = 6.4 Hz, H-C<sub>9</sub>), 6.86 (1H, s, H-C<sub>12</sub>), 7.30-7.40 (5H, m, H-C<sub>16</sub>, H-C<sub>17</sub>, H-C<sub>18</sub>, H-C<sub>19</sub>, H-C<sub>20</sub>), 7.70-7.80 (4H, m,

Cl-Ph-4H:R<sub>1</sub>), 7.84-7.90 (2H, m, H-C<sub>7</sub>, H-C<sub>8</sub>), 8.64 (1H, d, J = 8.8 Hz, H-C<sub>5</sub>).

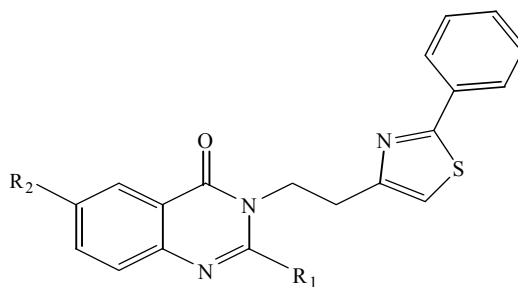
**6-bromo-2-(4-nitrophenyl)-3-(2-(2-phenylthiazol-4-yl)ethyl)quinazolin-4(3H)-one (B5)**

Yield: 62%. m.p 185 °C, (Found: M 532, C<sub>25</sub>H<sub>17</sub>BrN<sub>4</sub>O<sub>3</sub>S requires 532), IR (KBr, cm<sup>-1</sup>)  $\nu_{\max}$  = 3309, 3066, 1685, 1585. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 3.04 (2H, t, J = 6.0 Hz, H-C<sub>10</sub>), 3.75 (2H, t, J = 6.4 Hz, H-C<sub>9</sub>), 6.98 (1H, s, H-C<sub>12</sub>), 7.30-7.50 (4H, m, H-C<sub>16</sub>, H-C<sub>17</sub>, H-C<sub>19</sub>, H-C<sub>20</sub>), 7.60 (1H, d, J = 6.8 Hz, H-C<sub>18</sub>), 7.75-7.90 (2H, m, NO<sub>2</sub>-Ph-2H:R<sub>1</sub>), 8.11 (2H, d, J = 8.8 Hz, NO<sub>2</sub>-Ph-2H:R<sub>1</sub>), 8.28 (1H, d, J = 8.8 Hz, H-C<sub>8</sub>), 8.34-8.40 (1H, m, H-C<sub>7</sub>), 8.69 (1H, d, J = 8.8 Hz, H-C<sub>5</sub>).

**Antiproliferative effects of the derivatives**

Results of MTT assay for evaluation of cytotoxic effects of the fractions are listed in Table 1.

**Table 1.** The IC<sub>50</sub> ( $\mu$ M) of tested compounds against PC-3, MCF-7, and HT-29 cancer cell lines.



Cell line compound	R (R1/R2)	PC3, IC <sub>50</sub> ( $\mu$ M)	MCF-7, IC <sub>50</sub> ( $\mu$ M)	HT-29, IC <sub>50</sub> ( $\mu$ M)
A1	Me/H	13	15	34
A2	Et/H	11	12.5	27
A3	Pro/H	10	10	12
A4	Phenyl/H	127	25	44
A5	4-Chlorophenyl/H	16	12	12
A6	4-Nitrophenyl/H	16	16	12
B1	Me/Br	75	28	85
B2	Et/Br	34	52	51
B3	Ph/Br	16	15	140
B4	4-Chlorophenyl/Br	11	28	25
B5	4-Nitrophenyl/Br	25	20	60
Doxorubicin	-	3.7	7.2	5.6

## DISCUSSION

In this study, quinazolinones as biologically active compounds were conjugated with another well-known moiety (thiazole ring) in a multi-step reaction procedure to produce interesting novel compounds. Next, all synthesized compounds were tested for their cytotoxic effects on three human carcinoma cell lines including MCF-7, HT-29, and PC-3.

The primary synthesis of quinazolinones may be performed by cyclization of benzene substrates which have appropriate substituents. This could be achieved by substitution of proper groups on COOH or NH<sub>2</sub> of antranilic acid or its derivatives to provide one or more of the ring atoms required to complete the pyrimidine ring. Modification of appropriate derivatives of other heterocyclic systems such as benzodiazepines is another procedure for preparation of quinazolinones.

In another synthetic method, 3,1-benzoxazin-4-one was used to prepare quinazolinones. This procedure has been used extensively to make a large number of 4(3H)-quinazolinones with good yields. In these reactions almost any primary amine may be added to 3,1-benzoxazin-4-one to achieve overall replacement of ring-O by ring-N with the formation of 4(3H)-quinazolinone. Preparation of benzoxazinone has been reported in several literatures using different methods. It can be produced via one or two step(s) procedures using anthranilic acid or its derivatives as starting materials in high yields.

Benzoxazinones are highly reactive and should be used immediately after preparation. In this study benzoxazinones were prepared by a two steps procedure.

The synthesized 4(3H)quinazolinones in this study contained 4-ethyl-2-phenylthiazole group on position 3 of quinazolinone structure. For preparation of these quinazolinones, a primary amine containing thiazole (compound **G**, Fig. 1) was synthesized.

The most practical method to prepare thiazoles is Hantzsch reaction which involves the condensation of  $\alpha$ -haloketones and thiourea or thioamides in refluxing alcohol. Phthalimide as an NH<sub>2</sub>-synthon was used here for the preparation of the amine. Application

of phthalimide in Gabriel synthesis for preparation of primary amines is well documented. After alkylation, the resulting alkyl phthalimide is reacted with hydrazine. The desired primary amine could be generated by reacting with hydrazine hydrate. Consequently phthalazine as a stable cyclic product is formed and precipitated.

The reaction of 2-phenyl-4-(2-aminoethyl)thiazole (compound **G**) with different benzoxazinones resulted in the production of new 4(3H)quinazolinones (Fig. 1). The results of the *in vitro* experiments revealed that the compounds **A3**, **A2**, **B4**, and **A1** showed the highest cytotoxic activities against PC3 cell line. Compounds **A3**, **A5**, and **A2** potentially inhibited proliferation of MCF-7 cell line. Moreover, in the HT-29, **A3**, **A5**, and **A6** are able to inhibit strongly growth of the cells. In the next study, more details and methods will be used to explore the molecular mechanisms that mediated the cytotoxic effects of the compounds.

## CONCLUSION

According to the results, **A3** efficiently inhibited the growth of all cell tested in a dose dependent manner. The IC<sub>50</sub> of **A3** was 10 M, 10  $\mu$ M, and 12  $\mu$ M in PC3, MCF-7, and HT-29 cells, respectively. The IC<sub>50</sub> values for doxorubicin as positive control was 3.7, 7.2, and 5.6 in PC3, MCF-7, and HT-29 cell lines, respectively. None of the compounds were found as effective as doxorubicin. According to cytotoxic results, presence of Br in position 6 resulted in reduction of cytotoxicity as well as nitro group in another group of similar quinazolinones (1), so it can be concluded that substitution on position 6 is not beneficial for cytotoxicity in this group of quinazolinones.

## ACKNOWLEDGEMENTS

Authors appreciate the Research Council of Kermanshah University of Medical Sciences for financial support of this project.

## REFERENCES

1. Hosseinzadeh L, Aliabadi A, Kalantari M, Mostafavi A, Rahmani Khajouei M. Synthesis and cytotoxicity

- evaluation of some new 6-nitro derivatives of thiazole-containing 4-(3H)-quinazolinone. *Res Pharm Sci.* 2016;11(3):210-218.
- Solomon VR, Hu C, Lee H. Design and synthesis of anti-breast cancer agents from 4-piperazinylquinoline: a hybrid pharmacophore approach. *Bioorg Med Chem.* 2010;18(4):1563-1572.
  - Tiwari AK, Singh VK, Bajpai A, Shukla G, Singh S, Mishra AK. Synthesis and biological properties of 4-(3H)-quinazolinone derivatives. *Eur J Med Chem.* 2007;42(9):1234-1238.
  - Mhaske SB, Argade NP. The chemistry of recently isolated naturally occurring quinazolinone alkaloids. *Tetrahedron.* 2006;62(42):9787-9826.
  - Gursoy A, Unal B, Karali N, Otuk G. Synthesis, characterization and primary antimicrobial activity evaluation of 3-Phenyl-6-methyl-4-(3H)-quinazolinone-2-yl-mercaptoacetic acid arylidenehydrazides. *Turk J Chem.* 2005;29(3):233-245.
  - Mourad AFE, Aly AA, Farag HH, Beshr EA. Microwave assisted synthesis of triazoloquinazolinones and benzimidazoquinazolinones. *Beilstein J Org Chem.* 2007;3(11):1-5.
  - Zhou Y, Murphy DE, Sun Z, Gregor VE. Novel parallel synthesis of N-(4-oxo-2-substituted-4H-quinazolin-3-yl)-substituted sulfonamides. *Tetrahedron Lett.* 2004;45(43):8049-8051.
  - Dinakaran M, Selvam P, DeClercq E, Sridhar SK. Synthesis, antiviral and cytotoxic activity of 6-bromo-2, 3-disubstituted-4 (3H)-quinazolinones. *Biol Pharm Bull.* 2003;26(9):1278-1282.
  - Raghavendra NM, Thampi P, Gurubasavarajaswamy PM, Sriram D. Synthesis and antimicrobial activities of some novel substituted 2-Imidazolyl-N-(4-oxoquinazolin-3 (4H)-yl)-acetamides. *Chem Pharm Bull (Tokyo).* 2007;55(11):1615-1619.
  - Laddha S, Wadodkar SG, Meghal SK. Studies on some biologically active substituted 4(3H)-quinazolinones. Part 1. Synthesis, characterization and anti-inflammatory-antimicrobial activity of 6, 8-disubstituted 2-phenyl-3-[substituted-benzothiazol-2-yl]-4(3H)-quinazolinones. *Arkivoc.* 2006;11:1-20.
  - Pandeya SN, Sriram D, Nath G, De Clercq E. Synthesis, antibacterial, antifungal and anti-HIV evaluation of Schiff and Mannich bases of isatin derivatives with 3-amino-2-methylmercaptoquinazolin-4 (3H)-one. *Pharm Acta Helv.* 1999;74(1):11-17.
  - Nanda AK, Ganguli S, Chakraborty R. Antibacterial activity of some 3-(Arylideneamino)-2-phenylquinazolinone-4(3H)-ones: synthesis and preliminary QSAR studies. *Molecules.* 2007;12(10):2413-2426.
  - Philipova I, Dobrikov G, Krumova K, Kaneti J. Convenient synthesis of some 2 substituted 4 (3H) quinazolinone derivatives. *J Heterocyclic Chem.* 2006;43:1057-1063.
  - Gursoy A, Karali N. Synthesis and primary cytotoxicity evaluation of 3-[[3-phenyl-4 (3H)-quinazolinone-2-yl) mercaptoacetyl] hydrazono]-1H-2-indolinones. *Eur J Med Chem.* 2003;38(6):633-643.
  - Ghabrial SS, Gaber HM. Dipolar cycloaddition reactions with quinazolinones: a new route for the synthesis of several annelated pyrrolo and pyridazino quinazolinone derivatives. *Molecules.* 2003;8(5):401-410.
  - Refaie FM, Esmat AY, Gawad SM, Ibrahim AM, Mohamed MA. The antihyperlipidemic activities of 4 (3H) quinazolinone and two halogenated derivatives in rats. *Lipids Health Dis.* 2005;4:22.
  - Shcherbakova I, Balandrin MF, Fox J, Ghatak A, Heaton WL, Conklin RL. 3H-Quinazolin-4-ones as a new calcilytic template for the potential treatment of osteoporosis. *Bioorg Med Chem Lett.* 2005;15(6):1557-1560.
  - Liu JF, Lee J, Dalton AM, Bi G, Yu L, Baldino CM, *et al.* Microwave-assisted one-pot synthesis of 2, 3-disubstituted 3H-quinazolin-4-ones. *Tetrahedron Lett.* 2005;46:1241-1244.
  - Piller LB, Davis BR, Cutler JA, Cushman WC, Wright Jr JT, Williamson JD, *et al.* Validation of heart failure events in the antihypertensive and lipid lowering treatment to prevent heart attack trial (ALLHAT) participants assigned to doxazosin and chlorthalidone. *Curr Control Trials Cardiovasc Med.* 2002;3(1):10.
  - Akazome M, Yamamoto J, Kondo T, Watanabe Y. Palladium complex-catalyzed intermolecular reductive N-heterocyclization: novel synthesis of quinazolinone derivatives from 2-nitrobenzaldehyde or 2-nitrophenyl ketones with formamide. *J Organomet Chem.* 1995;494(1-2):229-233.
  - Al-Omary FA, Abou-zeid LA, Nagi MN, Habib el-SE, Abdel-Aziz AA, El-Azab AS, *et al.* Nonclassical antifolates. Part 2: Synthesis, biological evaluation, and molecular modeling study of some new 2, 6-substituted-quinazolin-4-ones. *Bioorg Med Chem.* 2010;18(8):2849-2863.
  - Cao SL, Feng YP, Jiang YY, Liu SY, Ding GY, Li RT. Synthesis and *in vitro* antitumor activity of 4(3H)-quinazolinone derivatives with dithiocarbamate side chains. *Bioorg Med Chem Lett.* 2005;15(7):1915-1917.
  - Bharti SK, Nath G, Tilak R, Singh SK. Synthesis, antibacterial and anti-fungal activities of some novel Schiff bases containing 2, 4-disubstituted thiazole ring. *Eur J Med Chem.* 2010;45(2):651-660.
  - Khalil AM, Berghot MA, Gouda MA. Synthesis and antibacterial activity of some new thiazole and thiophene derivatives. *Eur J Med Chem.* 2009;44(11):4434-4440.
  - Pandeya SN, Sriram D, Nath G, DeClercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of schiff and mannich bases derived from isatin derivatives and N-[4-(4'-chlorophenyl) thiazol-2-yl] thiosemicarbazide. *Eur J Pharm Sci.* 1999;9(1):25-31.
  - Cukurovali A, Yilmaz I, Gur S, Kazaz C. Synthesis, antibacterial and antifungal activity of some new thiazolyldhydrazone derivatives containing 3-



- substituted cyclobutane ring. Eur J Med Chem. 2006;41(2):201-207.
27. Li J, Xu Z, Tan M, Su W, Gong XG. 3-(4-(Benzo[d]thiazol-2-yl)-1-phenyl-1H-pyrazol-3-yl) phenyl acetate induced Hep G2 cell apoptosis through a ROS-mediated pathway. Chem Biol Interact. 2010;183(3):341-348.
28. Luo Y, Xiao F, Qian S, Lu W, Yang B. Synthesis and *in vitro* cytotoxic evaluation of some thiazolylbenzimidazole derivatives. Eur J Med Chem. 2011;46(1):417-422.
29. Saghaie L, Sadeghi-Aliabadi H, Ashaehshoar M. Synthesis, analysis and cytotoxic evaluation of some hydroxypyridinone derivatives on HeLa and K562 cell lines. Res Pharm Sci. 2013;8(3):185-195.