Synthesis of 2, 3-Dihydro-1,4-naphthaquinone Derivatives for Targeting the Altered Cancer Cells Metabolism

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Abstract

A new four derivatives of 2,3-Dihydro-1,4-naphthaquinone were synthesized as possible bioreductive prodrugs for 5-fluorouracil (5-Fu), Mercaptopurine (6-MP), N-acetyl cysteine (NAC) and 3-bromopyrovic acid (3-BPA) to selectively deliver the drugs into the cancer cells and these are: 2-(((5-fluoro-1,2-dihydropyrimidin-4-yl)-3-hydroxy-naphthalene-1,4-dione)(compound A), 2-(((9H-purin-6-ylthio)-3-hydroxynaphthalene-1,4-dione) (compound B) acetamido-3-((3methyl-1,4-dihydro-naphthalen-2-yl)methylthio)propanoic acid (compound C) and hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl-3-bromo-2-oxopropanoate (compound D). Their chemical structures were characterized by H\textsuperscript{1} NMR, IR spectroscopy and elemental microanalysis. The in vitro antitumor activity tests against Hep-2 human larynx cancer cell line indicated that compound A, B, C and D have significant anticancer activities when compared with 5-Fu.

Keywords: Anticancer, prodrug, 2, 3-Dihydro-1, 4-naphthaquinone, bromopyrovic acid, N-acetyl cysteine; 5-fluorouracil; 6-Mercaptopurine.
1. Introduction

Thiopurines (6-MP) are used in the maintenance therapy of acute lymphoblastic leukemia and it also displays activity against acute and chronic myelogenous leukemias\(^1,2\). The clinical use of the thiopurines against solid tumors has been limited by severe bone marrow toxicity\(^3\). 5-Fu is used for gastrointestinal, pancreas, breast, ovary, and colorectal cancer. The use of 5-fluorouracil accompanied by several disadvantages including severe adverse effects, drug resistance, limitation of uses, and variable bioavailability\(^4\). (NAC) is the acetylated derivative of the amino acid L-cysteine\(^5\). Recently, animal and human studies have shown NAC to be a powerful antioxidant and potential therapeutic agent in the treatment of cancer\(^6,7\). Research has shown NAC to have potential as a chemo preventive agent and as a treatment in certain types of cancer\(^8\). Including lung, skin, head and neck, breast, and liver cancer. In vitro studies have demonstrated that NAC to be directly anti-mutagenic and anti-carcinogenic, it is also inhibits the mutagenicity of certain compounds in vivo\(^9\). (3-BPA) is a pyruvate-lactate analogue, and strong alkylating agent\(^10\). Recently, it was found to be potent inhibitor of glycolysis and oxidative phosphorylation by targeting hexokinase II enzyme (HK II), this enzyme is the most important enzyme in the maintaining the high rate of glycolytic energy production\(^11\). By inhibition this enzyme, ATP depletion, and consequently cells death will occur. 3-bromopyruvate (3-BPA) show excellent antitumor activity against different types of cancer cells, especially against Hepatocellular carcinoma\(^12\). One promising aspect for improving the distribution of anticancer drugs appears to be the prodrug approach by which the pharmacokinetic patterns and ultimately therapeutic success can be obtained through introduction of promoieties with the ideal physiochemical properties.

Targeted prodrug design represents a new strategy for directed and efficient drug delivery. Particularly, targeting the prodrugs to a specific enzyme or a specific membrane transporter, or both, has potential as a selective drug delivery system in cancer chemotherapy\(^13\). Hypoxia distinguish tumor cells, especially those solid tumor cells, from normal cells. In vitro studies have demonstrated that NAC to be directly anti-mutagenic and anti-carcinogenic, it is also inhibits the mutagenicity of certain compounds in vivo\(^9\). (3-BPA) is a pyruvate-lactate analogue, and strong alkylating agent\(^10\). Recently, it was found to be potent inhibitor of glycolysis and oxidative phosphorylation by targeting hexokinase II enzyme (HK II), this enzyme is the most important enzyme in the maintaining the high rate of glycolytic energy production\(^11\). By inhibition this enzyme, ATP depletion, and consequently cells death will occur. 3-bromopyruvate (3-BPA) show excellent antitumor activity against different types of cancer cells, especially against Hepatocellular carcinoma\(^12\). One promising aspect for improving the distribution of anticancer drugs appears to be the prodrug approach by which the pharmacokinetic patterns and ultimately therapeutic success can be obtained through introduction of promoieties with the ideal physiochemical properties.

Targeted prodrug design represents a new strategy for directed and efficient drug delivery. Particularly, targeting the prodrugs to a specific enzyme or a specific membrane transporter, or both, has potential as a selective drug delivery system in cancer chemotherapy\(^13\). Hypoxia distinguish tumor cells, especially those solid tumor cells, from normal cells, thus presenting new opportunities for selective cancer treatment\(^14\). The rationale employed in the design and development of hypoxia-selective agents targeting tumor cells is that hypoxia-selective prodrugs are able to release the active cytotoxic agents upon reduction under hypoxic conditions, thus minimizing the systemic toxicity. The efficacy of cancer chemotherapy is frequently hampered by a low side effects and improve its efficacy and therapeutic index\(^15\). Recently bio reductive prodrugs strategies have been developed to improve this index by selective delivery of highly cytotoxic drugs to the tumor cells themselves. A key advance in this approach has been the principle of conversion of a specially designed nontoxic prodrug to a cytotoxic drug by an enzymatic addition of one (1e−) or two (2e−) electrons, to initiates the formation of reactive species, these enzymes contained within the tumor cells themselves, on the tumor cell surface, or in the tumor cell microenvironment e.g. thioredoxin1 enzymes\(^16\). In view of these observations, four new derivatives of 2,3-Dihydro-1,4 naphthaquinone have been designed, synthesized and characterized.

2. Results and Discussion

2.1 The chemistry

The designated compounds were synthesized according to schemes (1 to 3). A series of intermediates were synthesized as precursors of the target compounds by several experimental steps. The intermediates I was synthesized by reacting the acetic anhydride with sodium peroxide in dry ether using the method described previously\(^17\). According scheme 1. This afforded diacetyl peroxide I. This diacetate peroxide I was reacted with 1,4 naphthaquinone in glacial acetic acid\(^18\) whereupon new intermediate II were obtained. In the following step II allowed to react with hydrogen peroxide\(^19\) leading to the formation of III. The III was allowed to reacted with sulphoric acid\(^20\) gave IV. Then IV was reacted with N-bromosuccinamide\(^21\) leading to the formation of V.
Scheme 1: The synthesis of intermediates (I, II, III, IV, V).

\[
\text{Na}_2\text{O}_2 + \text{acetic anhydride} \xrightarrow{\text{stirring 0}^\circ\text{C}} \text{H}_3\text{C} = \text{O} - \text{CH}_3 + 2 \text{CH}_3\text{COONa}
\]

1/4 naphthaquinone

\[
\overset{100^\circ\text{C}}{\text{H}_2\text{O}_2/\text{Na}_2\text{CO}_3} \rightarrow \overset{\text{Br}}{\text{Benzoyl peroxide}}
\]

\[\text{Compound A, B and C were obtained from the reaction of V with sodium salt of 5-fluorouracil, triethylamine salt of 6-mercaptopurine or potassium salt of acetyl cysteine (scheme 2) and the reactions are nucleophilic substitution reaction.}^{(22, 23)}\]
Scheme 2: The synthesis of compound A, B and C.

The reaction of V with sodium pyruvate \(^{(24)}\) and then with sodium bromide \(^{(25)}\) lead to formation of compound D (scheme 4).
Scheme 3: The synthesis of compound D

The chemical structures of these newly synthesized compound were confirmed by IR, ¹H-NMR spectral measurements and elemental microanalysis and were in good agreement with the proposed structures. The IR spectra of II showed stretching absorption bands 2962 and 2920 cm⁻¹, attributed to the C-H of methyl group. The stretching absorption band of C=O of α-β unsaturated ketone, function appeared in the 1668 and 1658 cm⁻¹ region. The bands appearing at 1668 and 1658 cm⁻¹ were for the C=C of aromatic function. In the IR spectra of III the stretching vibration of epoxy methane were found at 2995 cm⁻¹. Methyl adjacent to epoxy ring at 1402 cm⁻¹. The IR spectra of IV indicated the presence stretching vibration of Broad band of O-H at 3338 cm⁻¹. The IR spectra of V showed stretching absorption band of C-Br at 634. However, compounds A,B,C and D exhibited IR and ¹H-NMR spectra consistent with their assigned structures, described in details in the Experimental section. The IR spectrum of compound A displayed the presence of the general characteristic absorption bands: NH stretching of 2° amide appeared at 3209 cm⁻¹, C=O strong stretching vibration of 2° amide. Compound B showed bands at 3427 cm⁻¹ for NH stretching of secondary amine and at 1589 cm⁻¹ for C=N stretching vibration of purine. Compound C displayed the NH stretching of 2° amide at 3209 cm⁻¹ and carboxyl C=O at 1720. While compound D showed C=O strong asymmetric stretching vibration of ester at 1732 cm⁻¹.

In the ¹H-NMR of compound A, the following peaks appeared: 10.6 (s, 1H, -OH), 8.0 (s, 1H, C-H), 7.6-7.9 (m, 4H, Ar-H), 4.2 (s, 2H, CH₂). The compound B showed the following characteristic peaks: 13.2 (d, 1H, NH of purine), 8.6 (d, 1H, CH), 8.4 (s, 1H, CH). Compound C showed the following characteristic peaks: 12(s, 1H, COOH), 8.3 (d, 1H, NH), 4.7 (q, 1H, CH), 1.8 (s, 3H, CH₃). While compound D showed the following characteristic peaks: 5.1 (s, 2H, CH₂-Br).

Elemental microanalysis results were within ± 0.5% of the theoretical values and in good agreement with the proposed chemical structures. The detailed data for all compounds are given in the experimental section.
2.2. Anticancer Activities

The *in vitro* antitumor activities of the newly synthesized compounds (A, B, C and D) were evaluated against Hep-2 human larynx cancer cell line by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) \(^{(26)}\) with 5-FU (Tables 1-3).

Table 1: effects of different concentrations of 5- Fu and compound A, B, C and D on growth rate of Hep-2 human larynx cancer cell line after 24 hrs.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>IR% At Different Concentration of Compound A and 5-Fu</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>7.5µM</td>
</tr>
<tr>
<td>A</td>
<td>74</td>
</tr>
<tr>
<td>B</td>
<td>65.1</td>
</tr>
<tr>
<td>C</td>
<td>58.7</td>
</tr>
<tr>
<td>D</td>
<td>58.3</td>
</tr>
<tr>
<td>5-Fu</td>
<td>72.3</td>
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</table>

Table 2: effects of different concentrations of 5- Fu and compound A, B, C and D on growth rate of Hep-2 human larynx cancer cell line after 48 hrs.

<table>
<thead>
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<th>Comp.</th>
<th>IR% At Different Concentration of Compound A and 5-Fu</th>
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<tr>
<td></td>
<td>7.5µM</td>
</tr>
<tr>
<td>A</td>
<td>54.7</td>
</tr>
<tr>
<td>B</td>
<td>65.4</td>
</tr>
<tr>
<td>C</td>
<td>53.3</td>
</tr>
<tr>
<td>D</td>
<td>60.3</td>
</tr>
<tr>
<td>5-Fu</td>
<td>55.2</td>
</tr>
</tbody>
</table>

Table 3: effects of different concentrations of 5- Fu and compound A, B, C and D on growth rate of Hep-2 human larynx cancer cell line after 72 hrs.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>IR% At Different Concentration of Compound A and 5-Fu</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>7.5µM</td>
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<tr>
<td>A</td>
<td>60.3</td>
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<tr>
<td>B</td>
<td>65.2</td>
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<td>C</td>
<td>55.9</td>
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<tr>
<td>D</td>
<td>51.2</td>
</tr>
<tr>
<td>5-Fu</td>
<td>74.3</td>
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</table>
According to the results shown in the tables 1 to 3 the synthesized Compounds (A, B, C and D) exhibited marked cytotoxic effects against Hep-2 human larynx cancer cell line at six different concentrations and all exposures times which were 24,48 and 72 hrs. as compared to 5-Fu.

3. Experimental

3.1. General

Melting points were determined in open capillary tubes on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded (in KBr) on a Buck M500 model IR spectrophotometer and the wave numbers are given in cm\(^{-1}\). The \(^1\)H-NMR spectra were recorded in DMSO-d6 solutions on Perkin Elmer 60 MHz spectrometer at ambient temperature. Chemical shifts were recorded in parts per million (ppm) and were referenced to tetramethylsilane. Elemental microanalyses were determined on a Carlo-Erba analyzer type 1106. Purity of the synthesized compounds was checked by TLC aluminium sheets coated with silica gel 60 F254 (0.2 mm thickness) and by melting points. 1,4-naphthaquinone and Sodium pyruvate were pushed from BDH, while Fluorouracil, 6-mercaptopurine and acetylcysteine were kind gift from Fluka. All solvents and reagent were Analar grade.

3.2. Chemical Methods

3.2.1 Synthesis of diacetyl peroxide (I):

Acetic anhydride (6gm, 65.01 mmole) and (25 ml) of dry ether were placed in an Erlenmeyer flask placed in an ice path, the mixture was stirred until the internal temperature of the flask about 0 C\(^0\), (2.5 gm, 32.05 mmole) of sodium peroxide was added in one portion. Cold water then added drop wise to the stirred reaction mixture unit all the sodium peroxide had been dissolved. The ether layer was separated from water and the water layer was extracted twice with ether. The organic layers was combined and washed with 1 % sodium bicarbonate solution, the ether solution was separated and added water on magnesium sulphate ,the ether solution was chilled in ice-isopropyl alcohol over-night and titrated. Yield: 60%.

3.2.2 Synthesis of 2-methyl naphthalene-1, 4-dione (compound II):

A suspension of 1,4 naphthaquinone (0.5gm, 3.16 mmole) in 20 ml of glacial acetic acid was heated to about 100 C\(^0\), with stirring, when the solid was dissolved, ethereal solution of compound I (0.3 gm, 3.16 mmole) was added very slowly through a dropping funnel with stem extending nearly to the bottom of the flask. Evolution of carbon dioxide bubbles appear soon and the solution changed to yellow color, the addition was completed after 1 hour, heating continue for additional half an hour. The solvent was removed by vacuum and the residue dissolved in ether, washed with 1% sodium bicarbonate solution. The ether layer was separated, dried over anhydrous magnesium sulphate, and the solvent evaporated in vacuo to give a yellow crystalline powder, which recrystallized from ether-benzene mixture. Yield: 65%, m.p. 103–105 °C; IR (\(v, cm^{-1}\)): 2962 and 2920 of C-H symmetric stretching vibration of methyl group, 1668 and 1658 of C=O symmetric stretching vibration of aromatic, 1624, 1587 and 1433 of C=O C stretching vibration of aromatic.

3.2.3 Synthesis of 1-\(\alpha\)-methyl naphtho 2, 3boxirene-2, 7(1aH, 7aH)-dione (compound III):

Compound II (1gm, 5.81 m mole) was dissolved in (12ml) of warm Ethanol and left a side while a second solution of anhydrous sodium carbonate(0.25 gm, 2.34 mmole) dissolved in 5ml of water and a cold 30% hydrogen peroxide solution(1ml, 8.8mmole) was prepared, and added to the first solution. The yellow color of quinone was disappeared immediately. The flask was cooled with shaking in an ice path. (40ml) water was added. A white precipitate was formed, filtered and washed with water until the pink color of the filtrate was disappeared, the precipitate dried and recrystallized from methanol to give compound III. Yield: 91%, m.p. 90–92 °C; IR (\(v, cm^{-1}\)): 12995 of C=O stretching vibration of epoxy methane, 1701 and 1695 of C=O strong symmetric stretching vibration of \(\alpha\)-\(\beta\) unsaturated ketone, 1593 and 1448 of C=C symmetric stretching vibration of aromatic, 1402 of Methyl adjacent to epoxy ring and 1246 of 8 micron band symmetric stretching of epoxy ring.

3.2.4 Synthesis of 3-methyl naphthalene-2-hydroxy-1, 4-dione (compound IV):

Cold and concentrated sulphuric acid (5 ml) was added slowly to dry compound III (1gm, 5.31 m mole) in flask, with stirring until a deep red homogenous solution was formed, the solution was stirred for 15 minute and then cooled in an ice path. Then 20 ml of distilled water was added slowly to the red solution, yellow precipitate was formed, the mixture was extracted with 25 ml of each ether and benzene, the organic layer was separated and dried over anhydrous magnesium sulphate. The solvent was evaporated to about 10 ml and filtered. The precipitate was collected to give compound IV. Yield: 88%, m.p. 170–171 °C; IR (\(v, cm^{-1}\)): 3338 of Broad band of O-H asymmetric stretching vibration associated, 2999 of C-H stretching vibration of methyl group, 1660 and 1649 of C=C symmetric stretching vibration of unsaturated ketone and 1645, 1587and1454 of C=C stretching vibration of aromatic.

3.2.5 Synthesis of 2-(bromo methyl)-3-hydroxy naphthalene-1, 4-dione (compound V):

Compound IV (1gm, 5.31 mmole) was dissolved in 20ml of dry dichloromethane with stirring, N-bromosuccinimide (0.94gm, 5.31 mmole) and a catalytic amount (0.01gm) of benzoyl peroxide was added in portions, the resulting solution was refluxed for 2.5 hours. The solvent was evaporated, and the residue was dissolved in ether, filtered to remove succinimide, the filtrate was evaporated. The residue was triturated with petroleum ether and dried, to give crystals of
3.2.6 Synthesis of 2-((5-fluoro-1, 2-dihydropyrimidin-4-yl)oxy)methyl)-3-hydroxy naphthalene-1,4-dione (compound A):

To a stirred suspension of sodium 5-fluorouracil (1gm, 6.57mmole) in 20ml of dry acetone, a solution of compound V (1.75gm, 6.57mmole) in 10ml of dry acetone was added, the mixture was heated to gent flow for 18 hours, then cooled, evaporate the solvent and the residue was dissolved in water: ethyl acetate(50:50) mixture(30ml). The organic layer was separated, and the aqueous layer with ether acetate, the combined organic layer was dried with magnesium sulphate . The solvent was evaporated, to give compound A. Yields: 65%, m.p. 232-233°C. Anal.% calcd. For C15H16FN3O2S(316.24): C:56.97, H: 2.87, N:8.86.found: C: 57.21, H: 2.89,N:8.89. IR ( ν, cm−1): 3209 of NH stretching of 2nd amide,2929 and 2835 of CH2 stretching vibration, 1691 of C=O strong stretching vibration of α-unsaturated ketone,1683 of C=O strong stretching vibration of 2nd amide and 1141 and 1645, 1587and1545 of C=C stretching vibration of aromatic. H-NMR δ (ppm): 10.6 (s, 1H, -OH), 8.0(s, 1H, C-H), 7.6-7.9 (m, 4H, Ar-H), 4.2 (s, 2H, CH2).

3.2.6 Synthesis of 2-((9H-purin-6-ylthio)methyl)-3-hydroxy naphthalene-1, 4-dione (compound B):

6-mercaptopurine mono hydrate(1gm,5.88mmole) was suspended in(20 ml) of D.W. Triethyl amine(0.84ml, 6 mmole) was added, and the resulted clear solution was stirred at room temperature for 10 minute, a solution of compound V (1.57gm, 5.88mmole) in 5ml of THF was added slowly with continuous stirring, after of completion of addition, the solution was stirred for 3 hours, and a precipitate was formed, filtered ,dried and recrystallized from ethanol-water, to give compound B. Yields: 60%, m.p. 214-217 °C (decomposed). Anal.% calcd. For C15H16FN3O2S(338.34): C:56.80, H: 2.98, N:16.58, S:9.48; found:C: 56.93, H:3.00, N: 16.60, S:9.51. IR ( ν, cm−1): 3427 of NH stretching of secondary amine,3329 of O-H strong stretching vibration of hydroxyl group,2929 and 2825 of C-H stretching vibration of CH2, 1691 of C=O strong stretching vibration of α-unsaturated ketone,1589 of C=N stretching of secondary amine and 678 of C=C stretching vibration . H-NMR δ (ppm):13.2 (d, 1H. NH of purine), 10.6 (s,1H, -OH),8.6 (d, 1H, CH), 8.4 (s (1H, CH),7.6-6.9(m, 4H, Ar-H),3.6 (s, 2H, CH2).

3.2.7 Synthesis of acetimido-3-((3-methyl-1, 4-dihydropnaphthalen-2-yl) methylthio) propanoic acid (compound C):

N-acetylcysteine (0.5gm,3.08 m mole) was dissolved in (15ml) of DMF ,and powdered KOH(0.345gm,6.16 mmole) was added, and the mixture was stirred at room temperature for 30minute,then compound V(0.82 gm, 3.08 mmole) in 10 ml DMF was added slowly with stirring. After completion of addition, the stirring continue for 2 hrs. and acidified with dilute HCl to pH 5 then 50 ml of cold D.w was added and the resulting precipitate was extracted by ethyl acetate. The ethyl acetate layer was separated and washed with water; the water layer was extracted with ethyl acetate. The combined organic layer was dried over anhydrous s magnesium sulphate and evaporated, to give compound C. Yield: 65%, m.p. 140-141°C (decomposed). Anal.% calcd. For C15H16FN3O2S(347.39): C:56.78, H:4.93, N:4.03, S:9.28; found:C:56.83, H:4.94, N:4.07, S:9.28. IR ( ν, cm−1): 3209 for NH stretching of 2nd amide,2929 and 2835 for C-H stretching vibration,2298-3200 for Broad O-H stretching, 1720 for carbonyl C=O, 1691 of C=O strong stretching vibration of α-unsaturated ketone,1589 of C=N stretching of purine and 678 of C=C stretching vibration . H-NMR δ (ppm):12.0 (s, 1H, -COOH), 10.6 (s,1H, -OH),8.3 (d, 1H, NH),7.6-6.9(m, 4H, Ar-H), 4.7 (q, 1H, CH), 3.11(s, 1H, CH), 3.00 (d, 2H, CH3), 1.8 (s, 3H, CH3). H-NMR 5 (ppm):

3.2.8 Synthesis of (3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl 2-oxopropanoate (compound VI):

To a stirred suspension of sodium pyruvate (1gm, 9.09 g mole) in10ml of DMSO, a solution of (2.42g, 9.09mmole)of compound V in 7 ml of DMSO was added ,and the mixture was stirred at room temperature for 2 hours, then 40 ml of cooled water was added. The resulted precipitate was extracted with ethyl acetate, the ethyl acetate layer separated and washed with water twice. The combined ethyl acetate layer was dried with magnesium sulphate , and the solvent was evaporated, to give compound VI. Yield: 70%, oily material; IR ( ν, cm−1):3404 for O-H stretching vibration of hydroxyl group, 2985 and 2850 of C-H stretching vibration of methylene, 1724 for C=O strong symmetric stretching vibration of ester, 1691 of C=O strong stretching vibration of α-unsaturated ketone,1589and 1458 for C=C stretching vibration of aromatic. H-NMR δ (ppm): 10.6 (s,1H, -OH), 8.0(s, 1H, C-H), 7.6-7.9 (m, 4H, Ar-H),5.1 (s, 2H, CH2-Br), 4.8(s, 2H, CH2-O-)}
3.3. Anticancer Activities

Cancer cell line utilized in this study was obtained from Biotechnology Center/ Al-Nahrain University, Baghdad, Al-Jadiya and was maintained in RPMI-1640 supplemented with 10% FCS. The *in vitro* antitumor activities of the compounds by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2-Htetrazolium bromide (MTT) method and the data were analyzed by 2-way analysis of variance with ANOVA. The data were represented as mean ± SD. The level of significance (p <0.05) was used for analysis of variance in all result.

References


