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Synthesis and Antitumor Evaluation of Some 5-fluorouracil Derivatives

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ABSTRACT

The present work is designed to synthesize some new derivatives of 5-Fluorouracil (5-FU) and evaluation of their antitumor activity in comparison with the reference drug 5-FU. A series of five 5-FU derivatives (1-5) were synthesized. Characterization of all compounds was done by elemental analysis (C, H and N), Fourier Transform Infrared (FTIR) spectroscopy and other physicochemical properties. All the newly synthesized compounds were tested for their in vitro antitumor activity; two cell lines were used for the evaluation, Human Cervix Carcinoma Cell Line (HeLa) and Human Breast Carcinoma Cell Line (AMJ13).

Keywords: 5-Fluorouracil, Antitumor activity, 2-Aminobenzothiazole

INTRODUCTION

5-Fluorouracil (5-FU) is an antimetabolite of the pyrimidine analogue type, which is widely used in the treatment of solid tumors, such as colorectal, gastric tract and liver carcinomas [1-3]. Unfortunately, the clinical uses of 5-FU are greatly limited by its short plasma half-life, poor tumor affinity, myelosuppression, and strong intestinal toxicity. Consequently, numerous research have focused on the discovery of suitable carrier-linked prodrugs, in which 5-FU is conjugated with several carriers including glucose, peptides, and biodegradable polymers such as polysaccharides, liposomes, etc. [4-10].

Numerous modifications of 5-FU structure have been employed to improve its pharmacological and pharmacokinetic properties, among which tegafur (1b), carmofur (1c) and floxuridine (1d) have been frequently used clinically with increased activity, selectivity, metabolic stability, absorption and lower toxicity (Figure 1) [11]. Other research efforts have focused on modifications at N1 or N3 positions, such as nucleoside analogues like FdUMP [12] or conjugation with peptides [13] amino acids [14] glucose [15], other strategies include incorporation of 5-FU into synthetic or natural macromolecules, for example pectin-5-FU [16], porphyran-5-FU [17] and folic acid-5-FU [18] were associated with significant antitumor activity.

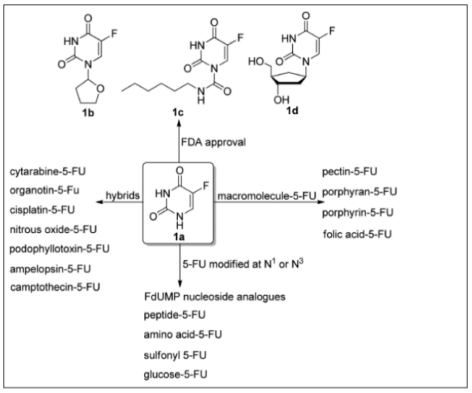


Figure 1: 5-FU and its derivatives

On the other hand various benzothiazole derivatives received much attention due to unique structure and their uses as anticancer agents [19,20]. Benzothiazoles are bicyclic ring system with multiple applications. A number of 2-aminobenzothiazole derivatives were intensively studied, as the 2-amino benzothiazole scaffold is a promising class in medicinal chemistry [19,21] and reported cytotoxic effect on cancer cells [21]. In recent years, combination chemotherapy with agents possessing different mechanisms of action is one of the methods that are being adopted to treat cancer. Therefore, a single molecule containing more than one pharmacophore, each with different mode of action could be beneficial for the treatment of cancer.

MATERIALS AND METHODS

5-FU was purchased from Ampla Pharmaceutical Inc. USA, 2-aminobenzothiazole was purchased from Sigma-Aldrich USA, the solvents and all other chemicals were purchased from Riedel-De Haen Germany. Melting points (°C, uncorrected) were measured in open glass capillaries using a Barnstead 9001 Electrothermal melting point apparatus. Infrared spectra (ν, cm^{-1}) were recorded on a Shimadzu FT/IR-330E, Fourier Transform Infrared Spectrometer using KBr discs. Elemental analyses (C, H and N) were in full agreement with the proposed structures. Monitoring the reactions and checking the purity of the final products were carried out by Thin Layer Chromatography (TLC) using silica gel precoated aluminum sheets (60 F254, Merck) and visualization with ultraviolet light (UV) at 365 and 254 nm. Crystal violet cell viability assay was conducted on 96-well plates (Santa Cruze Biotechnology, USA), Crystal violet stain was purchased from Sigma-Aldrich USA.

Anticancer screening

All the newly synthesized compounds were tested at the Iraqi biotechnology Company, Baghdad, Iraq. Two cell lines were used for the evaluation, Human Cervix Carcinoma Cell Line (HeLa) and Human Breast Carcinoma Cell Line (AMJ13) (Figure 2). The reference drug used was 5-FU. The activity of the samples and the reference drug was assayed under identical conditions at concentrations of 12.5, 25 and 50 µg/ml.

To determine the cell killing effect of the tested compounds, crystal violet cell viability assay was conducted on 96-well plates (Santa Cruze Biotechnology, USA), HeLa and breast cancer cell lines were seeded at 7000-10000 cells/well after 24 h or confluent monolayer is achieved (Figure 3), Cells were treated with tested compounds in the following dilutions from (12.5 ug) ml/ml, (25 ug/ml) and (50 µg/ml). Cell viability was measured at 72 h (Figure 4). Of exposure by removing the medium, adding 50 µl of crystal violet stain and incubating for 2 h at 37°C. After removing the stain, it washed with PBS. The absorbency was determined on a micro plate reader (Assyshitech, Austria) at 550 nm (test wavelength); the assay was performed in triplicate. Endpoint parameters that are calculated for the HeLa and Breast cancer cell lines include the Proliferation rate=(B/A) × 100 and inhibiting rate of cell growth (GI) (the percentage of cytotoxicity) was calculated as (A-B)/A × 100, Where, A is the mean optical density of untreated wells and B is the optical density of treated wells.

Synthesis of Ethyl P-aminobenzoate (EPAB) (Compound A)

P-amino benzoic acid (10 g, 0.073 mol) was dissolved in 80 ml of absolute ethanol in around bottom flask then 4 ml of concentrated H_2SO_4 added slowly and the mixture was refluxed for 2 h at 60°C on water bath, the flask then cooled for few minutes, and the mixture then neutralized by the addition of 150 ml of 10% Na_2CO_3 solution. The solution was Filtered and the precipitate collected and recrystallized from rectified spirit and dried in desiccator under vacuum to give white crystals; Yield 73%; m.p. 86-88°C; IR (KBr) ν_{max} (cm^{-1}): 3341, 3221 (NH_2), 1683 (C=O), 1640 (C=C), 1175 (C-O). Anal. calcd. for $C_9H_{11}NO_2$ (165.19): C, 65.44; H, 6.71; N, 8.48. Found: C, 65.23; H, 6.56; N, 8.86.

Synthesis of 2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (Compound 1)

5-FU (6.5 g, 50 mmol) and potassium hydroxide (5.6 g, 100 mmol) was dissolved in water (20 ml) then a solution of chloroacetic acid (7.16 g, 75 mmol) in water (15 ml) was added and the resulting mixture was stirred at room temperature for 30 min. The pH value of the reaction mixture was adjusted to 10 by the dropwise addition of a 10% aqueous potassium hydroxide solution. The mixture was then refluxed for 2 h, cooled and acidified by the addition of concentrated HCl to pH 5.5, cooled at 4°C for 2 h, the crystals were isolated by suction and the solution was further acidified to pH 2 by the addition of concentrated HCl and cooled at 4°C for 2 h. The crystals were isolated by suction. The product then dissolved in saturated solution of sodium bicarbonate and reprecipitating with concentrated HCl to produce white needles; Yield 65%; m.p. 275-277°C; IR (KBr): 3169 (NH), 2951-2733 (broad) (OH), 1717 (C=C of uracil), 1705 (C=O) 1683, 1666 (C=O). Anal. calcd. for $C_6H_5FN_2O_4$ (188.11): C, 38.31; H, 2.68; F,10.10; N, 14.89. Found: C, 38.18; H, 2.76; F, 10.33; N, 14.45.

Synthesis of ethyl 4-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)benzoate (Compound 2)

To a stirred solution of compound 1 (0.5 g, 2.66 mmol) in (20 ml) of Dimethylformamide (DMF), (0.44 g, 2.66 mmol) of compound A was added and the mixture was cooled down to (-10°C) then (0.72 g, 5.32 mmol) of 1-Hydroxybenzotriazole (HOBt) and (0.55 g, 2.66 mmol) of N,N-Dicyclohexylcarbodiimide (DCC) were added with stirring which was continued for 2 days at 0 C^0 and then at room temperature for 5 days. The reaction mixture was evaporated and the residue redissolved in chloroform from which the N,N-Dicyclohexyl Urea (DCU) was filtered off. The clear filtrate washed twice with 5% sodium bicarbonate solution, 0.1 N HCl, once with distilled water. The chloroform layer was dried with anhydrous magnesium sulfate and evaporated under vacuum; the resulted product was collected, recrystallized from Chloroform: Ether (1:1), to give pale green crystals. Yield 72%; m.p. 215-217°C; IR (KBr): 3339 (NH), 3174 (NH) 2954, 2856 (CH), 1718 (C=C of uracil), 1695, 1670 (C=O). Anal. calcd. for $C_{15}H_{14}FN_3O_5$ (335.29): C, 53.73; H, 4.21; F, 5.67; N, 12.53. Found: C, 53.62; H, 4.33; F, 5.58; N, 12.34.

Synthesis of 4-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)benzoic acid (Compound 3)

To a stirred solution of compound 2 (0.4 g, 1.19 mmol) in a mixture of Ethanol: DMF (4:1) kept at 18-22°C, an aqueous solution of 1 N NaOH (3 ml) was added drop wise over period of 30 min. The mixture was stirred for additional 5 h. Then the solvents evaporated and the residue was dissolved in water and acidified with 1 N HCl to get pH 1. After cooling a precipitate was appeared this was separated by filtration, washed on filter paper with water and recrystallized from ethanol. Light brown powder; yield 67%; m.p. 224-226°C; IR (KBr): 3325 (NH), 3132 (NH), 2981-2825 (broad) (OH), 1718 (C=C of uracil) 1689 (C=O), 1666 (C=O). Anal. calcd. for $C_{13}H_{10}FN_3O_5$ (307.23): C, 50.82; H, 3.28; F, 6.18; N, 13.68. Found: C, 50.76; H, 3.19; F, 6.07; N, 13.84.

Synthesis of N-(benzo[d]thiazol-2-yl)-4-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)benzamide (Compound 4)

To a stirred solution of compound 1 (1 g, 5.32 mmol) in (20 ml) of DMF, (0.8 g, 5.32 mmol) of 2-aminobenzothiazole was added and the mixture was cooled down to (-10°C) then (1.46 g, 10.64 mmol) of HOBt and (1.1 g, 5.32 mmol) of DCC were added with stirring which was continued for 2 days at 0°C and then at room temperature for 5 days. The procedure then continued as in the synthesis of compound 2.

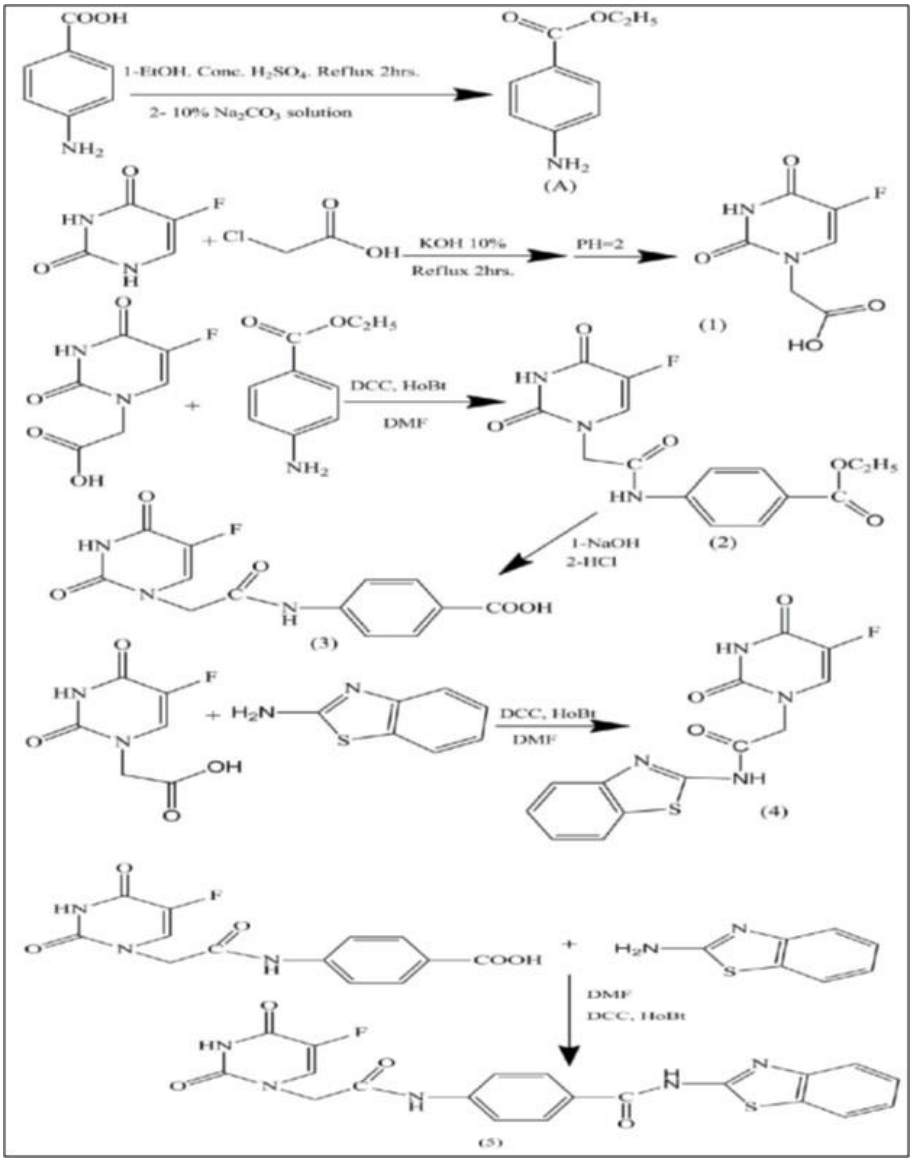
Dark brown powder, yield 62.5% m.p. 194-196°C; IR (KBr): 3327 (NH), 3124 (NH), 1716 (C=C of uracil), 1681 (C=O), 1666 (C=O), 1627 (C=N). Anal. calcd. for $C_{13}H_9FN_4O_3S$ (320.30): C, 48.75; H, 2.83; F, 5.93 N, 17.49; S, 10.01. Found: C, 48.68; H, 2.77; F, 6.08; N, 17.86; S, 10.09.

Synthesis of N-(benzo[d]thiazol-2-yl)-2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (Compound 5)

To a stirred solution of compound 3 (1.25 g, 4 mmol) in (20 ml) of DMF, (0.6 g, 4 mmol) of 2-aminobenzothiazole was added and the mixture was cooled down to (-10°C) then (1.1 g, 8 mmol) of HOBt and (0.83 g, 4 mmol) of DCC were added with stirring which was continued for 2 days at 0°C and then at room temperature for 5 days. The procedure then continued as in the synthesis of compound 2. To give off-white powder, yield 64%, m.p. 335-337°C; IR (KBr): 3327 (NH), 3157 (NH), 1719 (C=C of uracil), 1682 (C=O), 1672 (C=O), 1629 (C=N). Anal. calcd. for $C_{20}H_{14}FN_5O_4S$ (439.42): C, 54.67; H, 3.21; F, 4.32; N, 15.94; S, 7.3. Found: C, 54.73; H, 3.28; F, 4.39; N, 16.12; S, 7.37.

RESULTS AND DISCUSSION

The synthetic pathway of the intermediate and final target compounds are shown in Scheme 1 (Tables 1 and 2).



Scheme 1: The synthetic pathway of the intermediate and final target compounds

Table 1: Effect of compounds 2, 3, 4 and 5 on breast carcinoma cell line

Concentration	5-FU (F)	compound 2	Compound 3	compound 4	compound 5
12.5 ug/ml	42.24	79.18	97.55	94.48	108.45
25 ug/ml	73	70.6	98.98	103.87	97.35
50 ug/ml	74.5	40	64.08	67.35	89.79

Table 2: Effect of compounds 2, 3, 4 and 5 on cervix carcinoma (HeLa) cell line

Concentration	5-FU (F)	Compound 2	compound 3	compound 4	compound 5
12.5 ug/ml	60	100	108	105	102
25 ug/ml	35	95	116	96	104
50 ug/ml	8	25	94	77	81

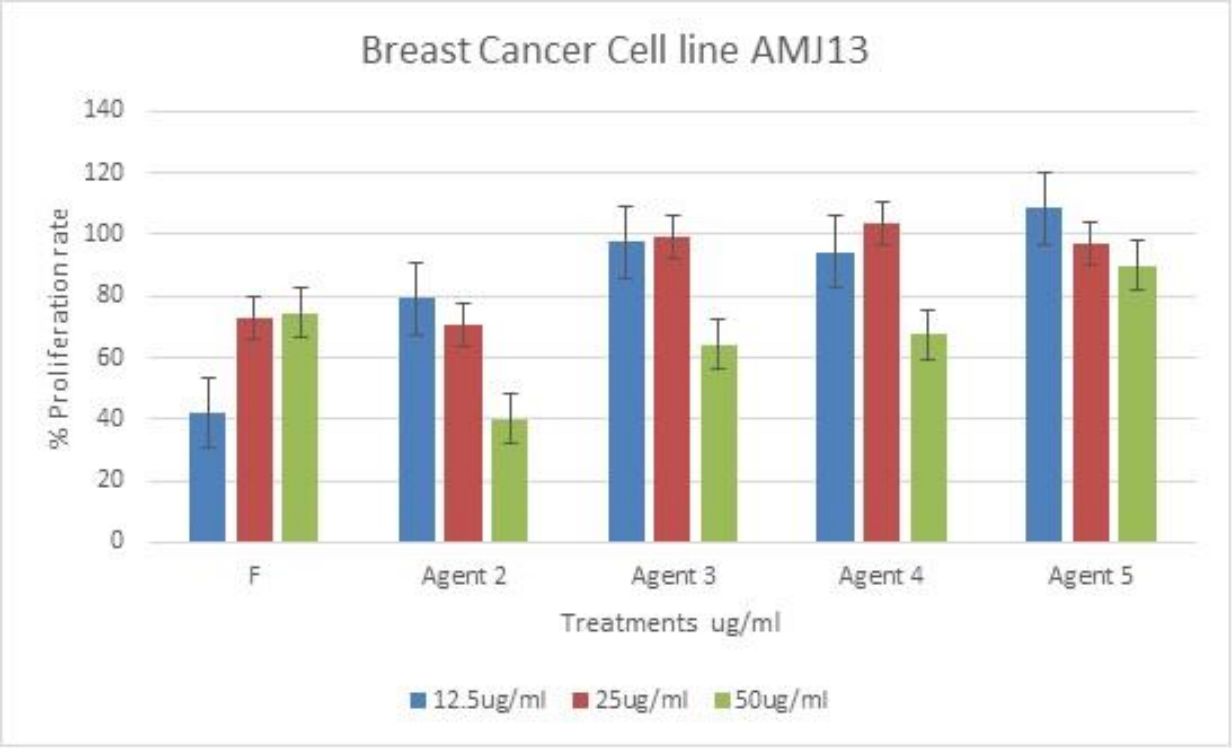


Figure 2: Antiproliferative effect of the tested compounds against breast cancer cells growth *in vitro*

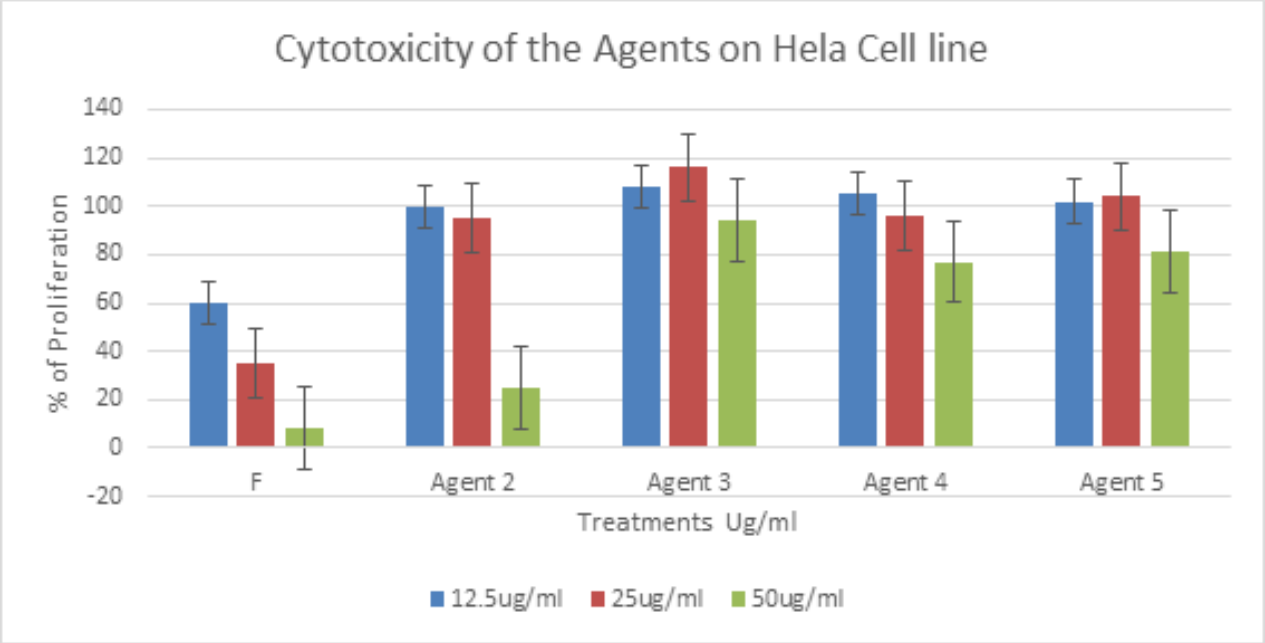


Figure 3: Antiproliferative effect of the tested compounds against HeLa cells growth *in vitro*

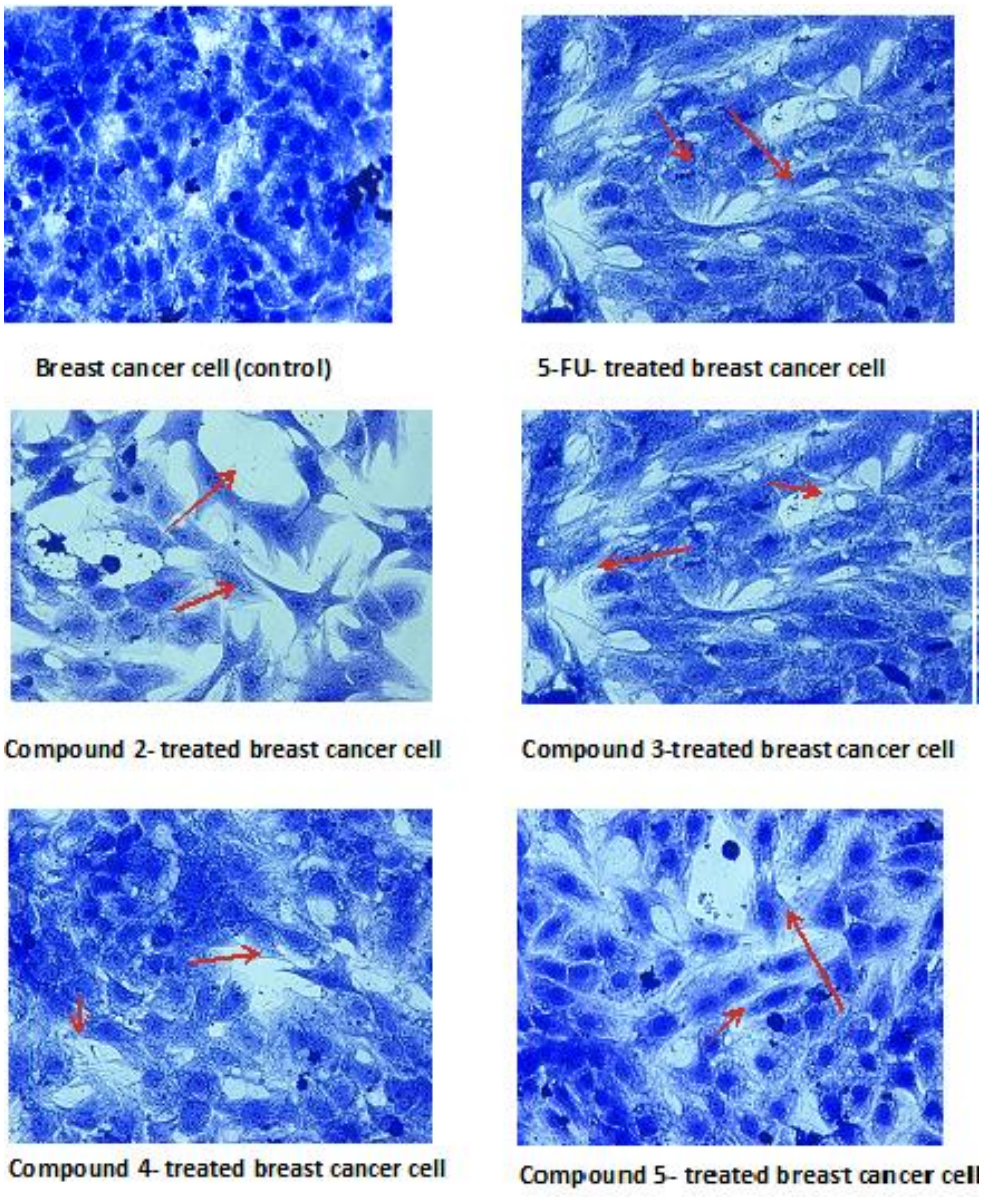


Figure 4: Changes to cell size and granularity distribution of treated cells. Photomicrograph of the treated cells was acquired along with the untreated control cells after 72 h. Treated cells showed shrinkage compared to the untreated control cells as shown by arrow mark

CONCLUSION

In conclusion, a series of 5-fluorouracil derivatives were synthesized and some compounds were found to be active against tumor cell lines *in vitro*. At high concentrations (50 µg/ml) compounds 2, 3 and 4 were more effective than 5-FU against breast carcinoma cell lines, compound 2 also was more effective than 5-FU at (25 µg/ml) against breast carcinoma cell lines At the same time, all the tested compounds were less effective than 5-FU against (HeLa) cell lines, This might be attributed to the difficult hydrolysis of the target compounds to give free 5-FU at the physiological pH conditions.

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