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Synthesis of new 7-benzothiazol-2-yl quinolone derivatives as antitumor agents

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ABSTRACT

*In this work, 3-benzothazol-2-yl-phenylamine(**1**)was synthesized through the reaction of 2-aminothiophenol and 3-aminobenzoic acid using polyphosphoric acid as dehydrating agent, and used as start for the preparation of the target compounds **IV and V**. Benzothiazolyl-phenylamine**1**was reacted with ethoxymethylene diethyl malonate ester (EMME) to afford compound II which was thermally cyclized in diphenyl ether to give 7-benzothizol-2-ylquinolone III. Benzaothiazolylquinolone IV was synthesized from the reflux of 7-benzothizol-2-ylquinolone III with $POCl_5$. The nucleophillic substitution of chloride anion of 7-benzothizol-2-ylchloroquinolone IVwith p-toluidine was preceded by using anhydrous potassium carbonate in DMF. Compounds IV and V were screened for antitumor activity against breast carcinoma cell line (MCF-7). The IC 50% of compounds IV and V were 0.066 and 0.056 umol/mL respectively and showed high activity in comparison to 0.065 umol/mL standard A . The structure of the compounds **IV and V** was confirmed using IR, NMR, mass spectroscopy and elemental analysis.*

Keywords: quinolone, Benzothiazole, MCF7, Breast cancer

INTRODUCTION

Quinolone derivatives have an exploitable source of new anticancer agents, which might also help addressing side-toxicity and resistance[1].Also, benzothiazolesor benzoxazoles containing compounds were found to have strong cytotoxic activity CNS cancer cell line (SNB-75)[2-8]. Benzothiazole containing compound A (diagram) have been showed anticancer activity against various cell lines[9&10].In addition, new synthesized compounds containing benzothiazole linked to quinolone showed anticancer and antimicrobial activities [11].

In order to overcome the side effects and develop potent tumor growth inhibitors as novel anticancer agents, we designed and synthesized a novel quinolone derivatives through

a) Substitution at quinolone bybenzothiazol-2-yl moiety (which has anticancer activity)[7-11]at 7 position of quinolone
b) Maintain the main structure core of quinolone which similar to Voreloxin (B) (anticancer quinolone) and doxorubicin (C) (anticancer agent)[12].
c) Substitution of carboxyl group at 3-postion by ester to increase the lipophilicity of the new compounds[3].
d) Substitution of the carbonyl group at 4-position by chloride or 4-methylphenylamino
e) Over all incorporation of benzothiazole and quinolone in one scaffold structure (Figure 1 and 2)

Figure 1

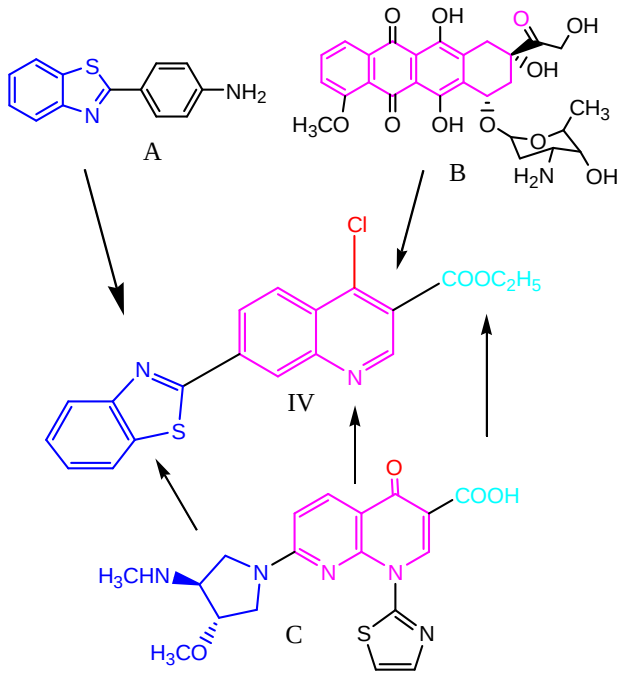
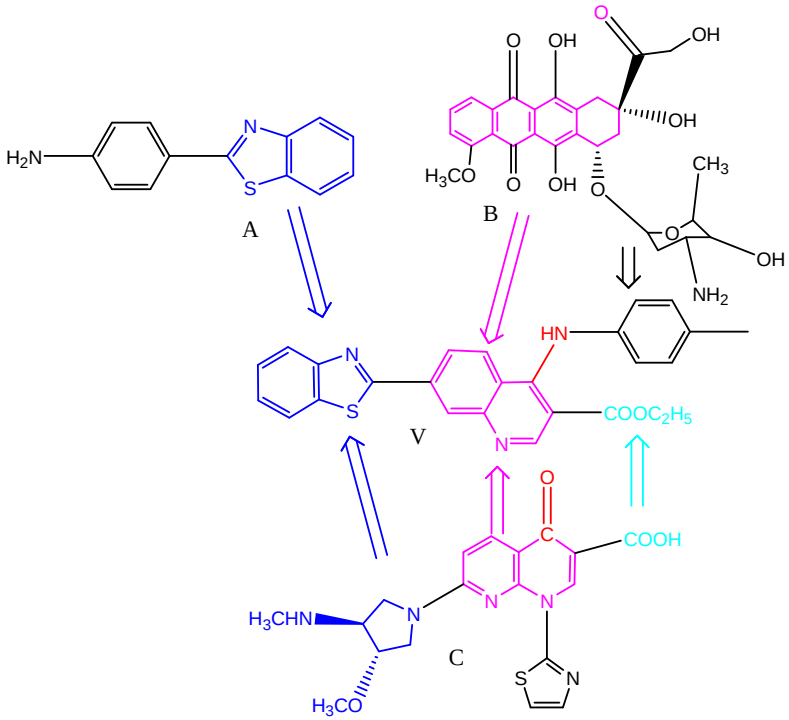
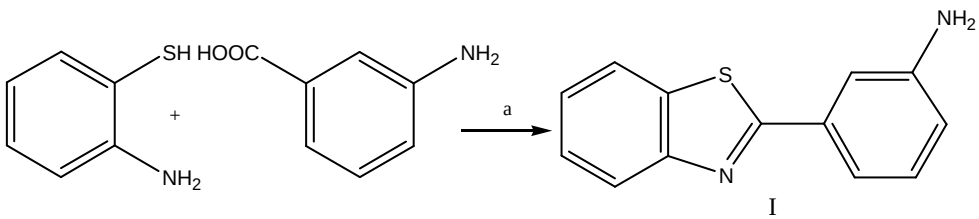


Figure 2



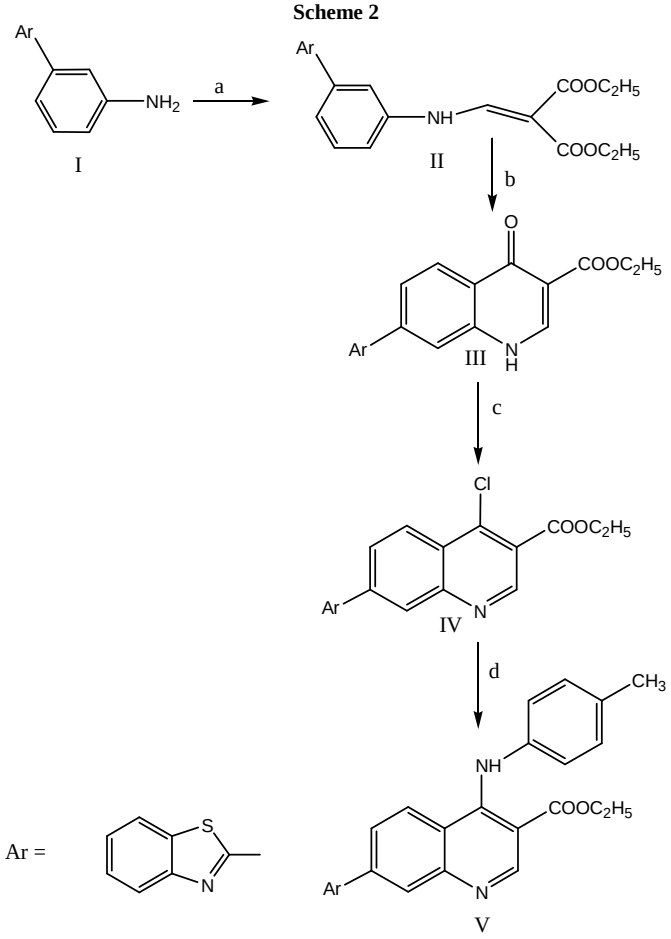
The target compounds were prepared through Scheme 1 and Scheme 2

Scheme 1



a) Reflux in PPA (poly phosphoric acid) for 4h

Scheme 2



a). EMME , ethanol , reflux for 4h b) diphenyl ether, reflux for 1 h c) $POCl_5$ reflux for 12h d) P-toluidine, anhydrous potassium carbonate, DMF, reflux for 24h.

The new compounds were synthesized according schemes 1 and II.

EXPERIMENTAL SECTION

3.1. Chemistry

General

Melting points were determined on a Graffin apparatus and were uncorrected. Element analyses (C, H, and N) were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Micro analytical unit of Cairo University, Egypt. All compounds were within + 0.4% of the theoretical values. IR spectra were determined as KBr discs on Shimadzu IR 435 Spectrophotometer and values were represented in cm^{-1}. ^1H NMR and ^{13}CNMR spectra were carried out on a Bruker 400 and 100 MHz NMR Spectrophotometer respectively in Beni-Suef University, BeniSuef, Egypt, using (Bruker, Munich, Germany) in DMSO-d_6 as a solvent, TMS as internal standard and chemical shifts were recorded in ppm on δ scale. Mass spectra were run on Hewlett Packard 5988 Spectrometer, Micro analytical center, Cairo University, Egypt. Progress of the reactions was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel MERCK 60 F 254 that were visualized by UV lamp.

3.1.1 Procedure for the synthesis of 2-[(3-benzothiazol-2ylphenylamino)-methylene] malonic acid diethyl ester (II)

A well stirred mixture of 3-benzothiazol-2-yl-phenylamine (1)(2.26g, 0.01mol) and ethoxymethylene-malonic acid diethyl ester (EMME) (2.16 g, 0.01mol) in ethanol (30 ml) was heated under reflux for 4h. The reaction mixture was cooled, filtered, washed with ethanol and crystalized from hot ethanol to give compound II.

Yield: 53%; yellow crystal mp: 135^0C;IR (cm^{-1}): 3432.67 (NH), 3059.51 (CH aromatic), 2977.55, 2933.2(CH aliphatic), 1690.3 (C=O), 1643(C=O); ^1H NMR (DMSO-d_6) δ ppm 1.25 (t, H, *J*= 6.8 Hz , CH_3),1.29(t, H, *J*= 6.8 Hz , CH_3) 4.15(q, 2H, *J*= 6.8 Hz , CH_2), 4.23(q, 2H, *J*= 6.8 Hz , CH_2)7.43-7.47(m, 1H, ArH), 7.52-7.58(m, 3H,ArH), 8.03-8.15 (m, 4H,ArH), 8.47(d, 1H, , *J*= 13.6 Hz, CH=C) , 10.79(d, 1H, *J*= 13.6 Hz, NH, D_2O exchangeable), ^{13}C NMR (DMSO-d_6) δ ppm 14.61,14.7,60.21,60.40,95.31,118.36,122.76,123.13,125.88,127.15, 129.13, 129.16,

134.79, 142.33, 150.30, 154.03, 165.35, 167.02, 167.51.Anal.Calcd. For $C_{21}H_{20}N_2O_4S$: C, 63.62; H, 5.08; N, 7.07. Found: C, 63.50; H, 5.20; N, 7.10 .

3.1.2 Procedure for the synthesis of 7-(benzothiazol-2-yl)-4-oxo-1,4dihydroquinoline-3-carboxylic acid ethyl ester **(III)**.
The suspension of compounds **1I** (3.96g, 0.01mol) in diphenyl ether (30 mL) was heated under reflux for 1h. The reaction mixture was cooled and filtered. The obtained precipitate was washed with diethyl ether(4x30mL).The precipitated solid was dried and crystallized from DMF/ethanol.

Yield: 53%; yellowish white powder mp: >300 ^{0}C; IR (cm^{-1}): 3417.24 (NH), 3057.58 (CH aromatic), 2931.27, 2904.27(CH aliphatic), 1705.69 (C=O, ester),1673.91(C=O, ketone) 1614(N=C); ^{1}H NMR (DMSO-d_6) δ ppm 1.30 (t, 3H, *J*= 6.8 Hz , CH_3) 4.25(q, 2H, *J*= 6.8 Hz , CH_2), 7.01(d, 1H, , *J*= 8 Hz ArH), 7.37.-8.12(m, 3H,ArH), 8.19 (d, 1H,*J*= 8 Hz, ArH), 8.42-8.63(m, 2H,, ArH) ,8.79(s, 1H. N-CH=C) 12.61 (s, 1H, NH, D_2O exchangeable),; MS *m/z*: 352 [(M+2)$^{+\cdot}$, 2.33%], 351 [(M+1) $^{+\cdot}$, 6.96%], 350 [(M) $^{+\cdot}$, 30%]; 304 [100%]. Anal.Calcd. For $C_{19}H_{14}N_2O_3S$: C, 65.13; H, 4.03; N, 9.99. Found: C, 65.20; H, 4.10; N, 8.10 .

3.1.3 Procedure for the synthesis of 7-benzothiazol-2-yl-4-chloroquinoline-3-carboxylic acid ethyl ester **(IV)**.
A mixture of 7-(benzothiazol-2-yl)-4-oxo-1,4 dihydroquinoline-3-carboxylic acid ethyl ester (III, 3.69g, 0.01mol) and $POCl_5$ (30ml) was heated under reflux for 12h. The reaction mixture was poured into ice cooled water (200g) and stirred for 1h.The sodium carbonate solution (10%) was added until thereaction mixture became basic to litmus paper. The separated solid was filtered, washed with water and crystallized from DMF to yield compound IV.

Yield: 60%; greyish white powder;mp: >300 ^{0}C; IR (cm^{-1}): 3046.96 (CH aromatic), 2960.2, 2904.27(CH aliphatic), 1709.59 (C=O, ester), 1673.91(C=O, ketone); ^{1}H NMR (DMSO-d_6) δ ppm 1.32 (t, 3H, *J*= 5.6 Hz , CH_3) 4.21(q, 2H, *J*= 5.6 Hz , CH_2), 7.44-7.95(m, 3H, ArH), 8.04.-8.32(m, 2H,ArH), 8.74 (s, 1H, ArH), ,8.88(s, 1H. N-CH=C); ^{13}CNMR (DMSO-d_6) δ ppm 14.97, 59.12, 111.38, 112.24, 116.24, , 118.26, 122.75, 123.07, 125.48, 125.71, 127.06, 127.25, 128.24, 134.76, 140.24, 150.23, 157.23, 154.26, 174.74; MS *m/z*: 370 [(M+2)$^{+\cdot}$, 39.85%], 369 [(M+1) $^{+\cdot}$, 24.39%], 368 [(M) $^{+\cdot}$, 100%]; . Anal.Calcd. For $C_{19}H_{13}ClN_2O_2S$: C, 61.87; H, 3.55; N, 7.60. Found: C, 61.90; H, 3.60; N, 7.80 .

3.1.4 Procedure for the synthesis of 7-benzothiazol-2-yl-4-P-tolylamino-quinoline-3-carboxylic acid ethyl ester (V).
To a solution of 7-benzothiazol-2-yl-4-chloroquinoline-3-carboxylic acid ethyl ester (IV, 3.68g, 0.01 mol.)and anhydrous potassium carbonate in DMF (30 mL), 4-methylaniline (1.07g, 0.01mol.) was added .The reaction mixture was heated under reflux for 24h. The reaction mixture was poured into ice cooled water (100mL) .The obtained solid was filtered and crystallized from DMF to yield compound IV.

Yield: 60%; grey powder; mp: >300 ^{0}C; IR (cm^{-1}): 3053.73 (CH aromatic), 2975.62 (CH aliphatic), 1705.73 (C=O, ester), 1619.91(N=C); ^{1}H NMR (DMSO-d_6) δ ppm 1.39 (t, 3H, *J*= 6.8 Hz , CH_2 CH_3), 2.13(s,3H, CH_3), 4.22(q, 2H, *J*= 6.8 Hz , CH_2), 6.96-7.54 (m, 6H, ArH),7.73-7.79 (m, 2H,ArH), 8.05-8.27 (m, 2H, ArH), ,8.71(s, 1H.ArH), 8.86(s, 1H. N-CH=C), 10.21(s, NH);^{13}CNMR(DMSO-d_6) δ ppm,18.28 , 21.26, 49.23, 115.44, 118.25, 119.45, 122.73, 123.12, 127.11, 128.22, 129.70, 130.42, 132.24, 134.77, 140.32, 142,22, 154.18, 155.22, 166.64 ; MS *m/z*: 441 [(M+2)$^{+\cdot}$, 0.39%], 440 [(M+1) $^{+\cdot}$, 1.10%], 439 [(M) $^{+\cdot}$, 3.31%], 304[100%]; . Anal.Calcd. For $C_{26}H_{21}N_3O_2S$: C, 71.05; H, 4.82; N, 9.56. Found: C, 71.10; H, 4.70; N, 9.50 .

3.2 Biological evaluation

3.2.1 Anticancer screening

Materials

Human tumor cell lines:

Breast carcinoma cell lines (MCF-7) used in this study were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.) through the Tissue Culture Unit of the Egyptian Organization for Biological Products and Vaccines, Vacsera, (Giza, Egypt). The tumor cell lines were maintained and processed at Center for Genetic Engineering, Al-Azhar University, Cairo, Egypt.

Chemicals

Dimethylsulphoxide (DMSO), Dulbecco's Modified Eagle Medium (DMEM), trypan blue, Fetal Bovine Serum, Penicillin/ Streptomycin antibiotic and Trypsin- EDTA was purchased from Sigma Aldrich Chemical Co. (Mo, U.S.A). Tris buffer was obtained from Applichem, Germany. All chemicals and reagents used in this study are of highest analytical grade.

Methods

Preparation of test compounds:

The tested derivatives **IV** and **V** were dissolved in dimethylsulfoxide (DMSO) as a stock stored at -20°C. Different concentrations of the compounds 0, 6.25, 12.5, 25, 50 and 100 µg/ml in culture medium were used.

Preparatory steps prior to cytotoxicity investigation*:*

Maintenance of MCF-7 in the laboratory, cryopreservation of cells, collection of cells by trypsinization and determination and counting of viable cells are performed according to the methods of Abdelgawad et al.[13]and Ahmed et al.[14]

Determination of potential cytotoxicity of the synthesized derivatives MCF-7.

The cytotoxicity was carried out using Sulphorhodamine-B (SRB) assay following the method reported by Vichai and Kirtikara[15].

Cells of MCF-7 cell lines are seeded in 16 well microtiter plates at a confluence of 1000-2000 cells/well, 100 µl/well. After 24 h, cells will be incubated for 72 h with graded concentrations from drugs (0, 6.25, 12.5, 25, 50 and 100 µg/ml). DMEM containing 10% foetal calf serum, 1% sodium pyruvate, 100 U/ml penicillin and 100 mg/ml streptomycin was used as culture medium and incubated at37 ^{o}C and 5% CO_2. At the end of the incubation, the medium is discarded. The cells are fixed with 150 µl cold trichloroacetic acid 10% final concentration for 1 hour at 4 ^{0}C. The plates were washed with distilled water using automatic washer (Tecan, Germany) and stained with 50 µl 0.4 % SRB dissolved in 1 % acetic acid for 30 minutes at room temperature in dark. The plates were washed with 1 % acetic acid to remove unbound dye and air-dried for 24 h. The dye was solubilized with 150 µl/well of 10 mMtris base (PH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 490 nm using an ELISA microplate reader. The mean background absorbance was automatically subtracted and mean values of each derivative and compound A concentration was calculated. The experiment was repeated 3 times. The percentage of cell survival was calculated by using the following formula,

Surviving percent = [O.D. (treated cells)/O.D. (control cells)] x100.

The IC_{50} values (the concentrations of derivatives required to produce 50% inhibition of cell growth) were also calculated using linear trend line equation.

RESULTS AND DISCUSSION

2.1 Chemistry

In this manuscript, the synthesis of new compounds containing quinolone and benzothiazole moieties was made. The antitumor benzothiazole nucleus was merged with quinolone nucleus. 7-Benzothiazol-2-yl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester(**III)**was prepared and employed for the preparation of the target compounds IV and V.

3-Benzothiazol-2-yl-phenylamine (1) was reacted with ethoxymethylene-malonic acid diethyl ester (EMME) in ethanol to give compound II . IR spectroscopy of compound II showed the carbonyl group at 1690.3 cm^{-1}. ^{1}H NMR showed the effect on the intramolecular of hydrogen bond in the spectrum of compound II through the appearance of the two equivalent ethyl groups in different positions at ppm 1.25 or 1.29 for CH_3 groups and 4.15 or 4.23 for CH_2 also in ^{13}CNMR proved the same explanation as the aliphatic CH appeared at ppm 14.61 or 14.7, for CH_3 groups and at 60.21 or 60.40 for CH_2groups (figure 3) .

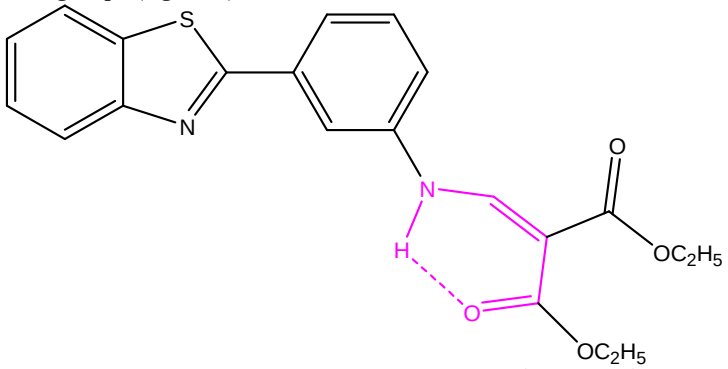


Figure (1) The intramolecular hydrogen bond in compound II and formation of sex membered ring

The 7-(benzothiazol-2-yl)-4-oxo-1,4 dihydroquinoline-3-carboxylic acid ethyl ester (II) was synthesized through thermal cyclization of 2-[(3-benzothiazol-2ylphenylamino)-methylene] malonic acid diethyl ester (II) using diphenyl ether.
The structure of the quinolone III was confirmed by using, NMR which showed the disappearance of one ethyl , also IR spectrum showed two peaks corresponding to the two carbonyl groups of ester and ketone. Also in mass spectrum , the molecular ion peak appeared at [$M^{\cdot+}$] equivalent to 350 in percentage 30% and [M+2] appeared as a result of presence of sulphur. The chloroquinolone IV was prepared through the reflux of quinolone II with $POCl_5$.

The HNMR of the prepared chloroquinolone IV showed disappearance of NH peak and deshielding of proton as a result of –I (inductive effect) of chloride. In the IR spectrum of compound IV, the carbonyl of ketone and NH peakswere absent. Additionally, the mass spectrum of compound **IV** reveled molecular ion peaks at *m/z*368 and 370 corresponding to $[M]^{+\cdot}$and $[M+2]^{+\cdot}$, respectively in ratio of 3:1 (Cl pattern).

The nucleophillic substitution of chloroquinolone IV with p-toluidine in DMF in presence of anhydrous potassium carbonate results in the formation of 7-benzothiazol-2-yl-4-P-tolylamino-quinoline-3-carboxylic acid ethyl ester **(V)**.The structure of compound V was confirmed through ^{1}HNMR and mass spectrum. The HNMR of compound showed the appearance of new band at ppm 2.13 equivalent to 3H of tolyl group and increase the integration of aromatic proton in comparing to compound IV. Also in mass spectrum, the molecular ion peak appeared at m/z 439 at intensity 3.31% and M+2 ion appeared which indicated the presence of sulphur in its structure.

2.2 Anticancer activity

Data are showing the anti-proliferative effects of the tested derivatives on (MCF-7) cell line in table 1 (Figures 1 and 2). Derivatives IV or V produced a marked decrease in the percentage survivability of MCF-7 by increasing the dose of derivatives (0 to 100 µmol/ml). Based on the values of IC_{50}, the IC 50% of compounds IV, V and the slandered 2-(4-aminophenyl)benzothiazole are 0.066 µmol/ml , 0.056 and 0.065 respectively. (table1)

Table 1: Anti-proliferative effect of quinolone derivatives IV, V and slandered on breast carcinoma cell lines (MCF-7) *in vitro*

Compound no	**IC50** µg/ml	**IC50** µmol/ml
IV	24.6	0.066
V	25	0.056
Standard A	14.69	0.065

CONCLUSION

The synthesized derivatives exhibited strong cytotoxic activity at MCF-7 in vitro. **Compound V** seemed to have the most potent cytotoxic effect.

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