

## N-Phenyl-2-p-tolylthiazole-4-carboxamide derivatives: Synthesis and cytotoxicity evaluation as anticancer agents

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### ABSTRACT

**Objective(s):** According to the prevalence of neoplastic diseases, there is a deep necessity for discovery of novel anticancer drugs in the field of medicinal chemistry. In the current study, a new series of phenylthiazole derivatives (compounds **4a-4f**) was synthesized and their anticancer activity was assessed *in vitro*.

**Materials and Methods:** All synthesized derivatives were evaluated towards three human cancerous cell lines of SKNMC (Neuroblastoma), Hep-G2 (Human hepatocarcinoma) and MCF-7 cell (Breast cancer) using MTT assay and obtained values (IC<sub>50</sub> ± SD) were compared with doxorubicin.

**Results:** Unfortunately, none of the synthesized compounds showed superior activity than doxorubicin against cancerous cell lines. MCF-7 cell line was the most resistant cell line against tested compounds. Compounds **4c** with *para* nitro (IC<sub>50</sub> = 10.8 ± 0.08 μM) and **4d** with *meta* chlorine (IC<sub>50</sub> = 11.6 ± 0.12 μM) moieties exerted the highest cytotoxic effects towards SKNMC and Hep-G2 cell lines respectively.

**Conclusion:** A new series of phenylthiazole derivatives were synthesized and their anticancer activity was assessed against cancerous cell lines. More structural modifications and derivatization is necessary to achieve to the more potent compounds.

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### Introduction

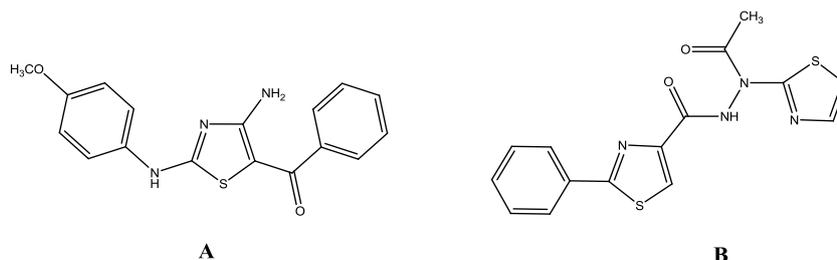
Cancer is a disease in which the control of cell growth and proliferation is lost in one or more cells and finally leading either to a solid tumor such as breast, prostate cancer or to a liquid cancer like hematological cancers (Leukemia, Lymphoma). It is one of the leading causes of death throughout the world and currently the main therapeutic options involve surgery, chemotherapy and radiotherapy. Chemotherapy involves the use of low molecular weight drugs (small molecules such as methotrexate, cisplatin, doxorubicin, etc.) to selectively destroy tumor cells or at least prevent their proliferation (1, 2). Cancer may affect people at all ages, even fetuses, but risk for the more common varieties tends to increase with age. Cancer causes about 13% of all deaths. According to the American Cancer Society, 7.6 million people died from cancer in the world

during 2007. In fact, cancer is the second leading cause of death in the Western World (3-5).

The survey for identification and discovery of novel chemical structures that can act as more effective and reliable anticancer agents is still a major challenge to medicinal chemists. Despite of the important advances achieved over recent decades in the research and development of various anticancer drugs, current anticancer drugs still have major limitations such as drug resistance, lack of selectivity and unwanted side effects (bone marrow depression, nausea and vomiting, etc.). Hence, there is a strong demand for the discovery and development of effective new cancer therapies devoid of mentioned limitations (7-11).

Thiazoles have been exhibited a broad spectrum of pharmacological activities and found in some drugs currently in the market such as famotidine

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**Figure 1.** Structures of two thiazole containing compounds. Compound **A**: Antioxidant (free radical scavenger). Compound **B**: An anticancer agent

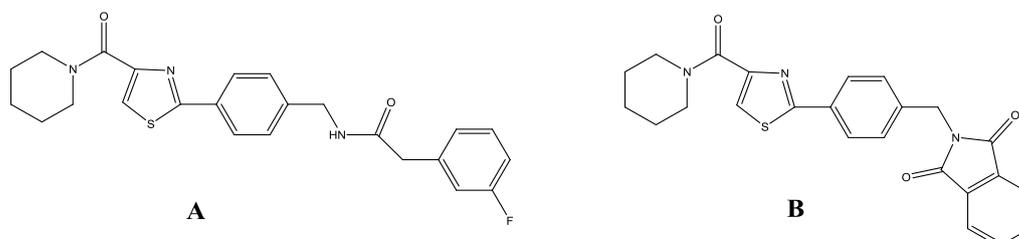
(Antacid, H<sub>2</sub> blocker), third generation cephalosporins (ceftriaxone, etc.), sulfathiazole (antibacterial agent). A thiazole ring is also found naturally in the essential vitamin B<sub>1</sub> (thiamine). Thiazole is an aromatic five membered heterocyclic ring containing nitrogen and sulfur atoms. The recent reports have been declared the applications of thiazoles in drug design and development of novel therapeutic agents. Thiazole ring as part of diverse chemical structures have been exerted various biological activities such as anticancer, anti-inflammatory, antimicrobial, antifungal, antitubercular, neuroprotective, antioxidant, diuretic, anticonvulsant and etc (Figure 1) (12-14).

Recently we reported a new series of thiazole and phenylthiazole derivatives with potential anticancer properties (Figure 2) (15-19). In addition, it was revealed that the activation of caspase-3 and induction of apoptosis maybe the probable mechanism for anticancer activity of these compounds. In the present study, design and synthesis of new analogs of previous phenylthiazole derivatives was done. In facts, according to the Figure 3, Compound 7 was demonstrated favorable cytotoxic activity against T47D (breast cancer cell line) in the previous study (18). This result encouraged us to explore the role of the amidic residue in this molecule. Hence, piperidine moiety of compound 7 was replaced with aniline derived aromatic amides and their anticancer activity was assessed against three cancerous cell lines *in vitro*.

## Materials and Methods

### Chemistry

All chemical reagents, solvents and starter materials were prepared from commercial companies like Merck,



**Figure 2.** Structures of some phenylthiazole derivatives with potential anticancer activity reported in previous projects. Compound **A**: Cytotoxic agent via caspase-3 activation and inducer of apoptosis (13, 14). Compound **B**: Cytotoxic agent probably through tyrosine kinase inhibition (15)

Acros and Sigma-Aldrich. <sup>1</sup>H NMR spectra acquisition was done using Bruker 400 MHz in deuterated solvents such as DMSO-d<sub>6</sub> and CDCl<sub>3</sub>. The spectra were recorded in δ (ppm) with tetramethylsilane (TMS) as internal standard. Potassium bromide (KBr) was prepared for IR spectra acquisition. IR spectra were obtained by Shimadzu 470 spectrophotometer. MS spectra of synthesized compounds were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Thin layer chromatography (TLC) as Merck silica gel 60 F<sub>254</sub> plates was applied for investigation of the reaction end and checking the completion of the reaction. Column chromatography was performed on Merck silica gel (70-230 mesh) for purification of intermediate and final compounds. Melting points of intermediate and final compounds were determined using electrothermal 9001 melting point analyser apparatus on open capillary tubes.

### 4-Methylbenzothioamide (2)

In a flat bottom flask, 3 g (25.6 mmol) of *p*-toluonitrile and 8.55 ml (25.6 mmol) of ammonium sulfide 20% were mixed in 50 ml of *N,N*-dimethylformamide as solvent and reaction mixture was stirred at room temperature for 5 hr. A yellow solid was precipitated after the addition of crushed ice to the reaction mixture. The precipitated solid was filtered and washed with cold water and *n*-hexane (13). mp: 160°C, Yield: 96%. <sup>1</sup>H NMR (δ, CDCl<sub>3</sub>): NMR (400 MHz, CDCl<sub>3</sub>): δ 2.39 (s, 3H, -CH<sub>3</sub>), 7.21 (d, 2H, *J* = 8 Hz, phenyl), 7.79 (d, 2H, *J* = 8 Hz, phenyl), 7.6 (brs, NH<sub>2</sub>). IR (KBr, cm<sup>-1</sup>): 3375, 3278, 3159, 2920, 2854, 1624, 1415, 1323, 879, 821. MS (*m/z*, %): 151 (M<sup>+</sup>, 55), 135 (35), 91 (100).

**2-p-Tolylthiazole-4-carboxylic acid (3)**

The obtained thioamide derivative **2** (2 g, 13.2 mmol), was treated with bromopyruvic acid (3.32 g, 19.9 mmol) and calcium carbonate (CaCO<sub>3</sub>, 3.31 g, 33.1 mmol) in 50 ml dry ethanol. The reaction medium was stirred under argon atmosphere at room temperature for 30 hr. The end of the reaction was determined by thin layer chromatography (TLC). Then, the ethanol was evaporated under reduced pressure using rotary evaporator apparatus. The obtained product was recrystallized from EtOH (18). mp: 123°C, Yield: 82%. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 2.4 (s, 3H, CH<sub>3</sub>), 7.25 (d, 2H, *J* = 8 Hz, C<sub>3</sub>-H, C<sub>5</sub>-H phenyl), 7.9 (d, 2H, *J* = 8 Hz, C<sub>2</sub>-H C<sub>6</sub>-H phenyl), 8.14 (s, 1H, C4-H thiazole). IR (KBr, cm<sup>-1</sup>): 3277 (OH, Stretch), 3078 (CH, Stretch, Aromatic), 2919 (CH, Stretch, Asymmetric, Aliphatic), 2858 (CH, Stretch, Symmetric, Aliphatic), 1721 (C=O, Stretch), 1603 (C=C, Stretch, Aromatic), 1521, 1454 (C=C, Stretch, Aromatic), 1403, 1342, 1244 (C-O, Stretch), 809. MS (*m/z*): 219 (100, M<sup>+</sup>), 175 (22), 134 (30), 119 (95), 115(75), 91 (40), 56 (32).

**General procedure for the synthesis of compounds 4a-4f**

In a flat bottom flask 200 mg (0.91 mmol) of 2-p-Tolylthiazole-4-carboxylic acid (**3**), 174 mg (0.91 mmol) *N*-ethyl-*N'*-dimethylaminopropyl carbodiimide (EDC) and 123 mg (0.91 mmol) hydroxybenzotriazole (HOBt) were mixed in 25 ml acetonitrile solvent (CH<sub>3</sub>CN). The prepared mixture was stirred at room temperature for 30 min and then, equimolar quantity of appropriate aniline derivative was added. Stirring was continued for 24 hr. Thin layer chromatography (TLC) was applied to determine the reaction end. Then, acetonitrile was evaporated under reduced pressure and EtOAc/H<sub>2</sub>O was added to the residue. Aqueous phase was discarded and the organic phase was washed two times by sodium bicarbonate 5%, sulfuric acid 2% and brine. Anhydrous sodium sulfate was added for drying and then, its removal was carried out by filtration. Finally, ethyl acetate was evaporated using rotary evaporator apparatus. Diethyl ether (Et<sub>2</sub>O) and *n*-hexane was utilized for washing the obtained precipitate (21, 22).

***N*-(3-Chlorophenyl)-2-p-tolylthiazole-4-carboxamide (4a)**

mp: 88°C, Yield: 52%, IR (KBr, cm<sup>-1</sup>)  $\bar{\nu}$ : 3363 (N-H, Stretch), 3120 (C-H, Stretch, Aromatic), 2924 (C-H, Aliphatic, Stretch, Asymmetric), 2858 (C-H, Aliphatic, Stretch, Asymmetric), 1689 (C=O, Stretch), 1589 (C=C, Aromatic), 1535 (N-H, Bend), 1485 (C=C, Aromatic). MS (*m/z*, %): 330 (M<sup>+</sup>+2, 15), 328 (M<sup>+</sup>, 47), 245 (40), 202 (65), 174 (5), 135 (15), 119 (100), 91 (65), 65 (10).

***N*-(4-Chlorophenyl)-2-p-tolylthiazole-4-carboxa-****mid (4b)**

mp: 120°C, Yield: 53%, <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 2.4 (s, 3H, -CH<sub>3</sub>), 7.38 (d, 2H, *J* = 8 Hz, H<sub>3,5</sub>-4-Methylphenyl), 7.42 (d, 2H, *J* = 8 Hz, H<sub>2,6</sub>-4-Methylphenyl), 7.87 (d, 2H, *J* = 8 Hz, H<sub>2,6</sub>-4-Chlorophenyl), 7.92 (d, 2H, *J* = 8 Hz, H<sub>3,5</sub>-4-Chlorophenyl), 8.47 (s, 1H, H<sub>5</sub>-thiazole), 10.38 (s, NH). IR (KBr, cm<sup>-1</sup>)  $\bar{\nu}$ : 3348 (N-H, Stretch), 3120 (C-H, Stretch, Aromatic), 2924 (C-H, Aliphatic, Stretch, Asymmetric), 2858 (C-H, Aliphatic, Stretch, Asymmetric), 1658 (C=O, Stretch), 1597 (C=C, Stretch, Aromatic), 1535 (N-H, Bend), 1496 (C=C, Stretch, Aromatic). MS (*m/z*, %): 330 (M<sup>+</sup>+2, 22), 328 (M<sup>+</sup>, 50), 245 (25), 202 (80), 174 (10), 135 (10), 119 (100), 91 (40), 65 (15).

***N*-(2-Nitrophenyl)-2-p-tolylthiazole-4-carboxamide (4c)**

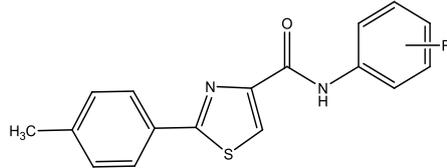
mp: 114°C, Yield: 40%, IR (KBr, cm<sup>-1</sup>)  $\bar{\nu}$ : 3425 (N-H, Stretch), 3105 (C-H, Stretch, Aromatic), 2924 (C-H, Aliphatic, Stretch, Asymmetric), 2858 (C-H, Aliphatic, Stretch, Symmetric), 1697 (C=O, Stretch), 1504 (NO<sub>2</sub>, Stretch, Asymmetric), 1342 (NO<sub>2</sub>, Stretch, Symmetric). MS (*m/z*, %): 339 (M<sup>+</sup>, 60), 322 (35), 202 (100), 174 (10), 135 (5), 119 (45), 91 (55).

***N*-(3-Nitrophenyl)-2-p-tolylthiazole-4-carboxamide (4d)**

mp: 157°C, Yield: 33%, <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 2.46 (s, 3H, -CH<sub>3</sub>), 7.43 (d, 2H, *J* = 8 Hz, H<sub>3,5</sub>-4-Methylphenyl), 7.77 (t, *J* = 8 Hz, 2H, H<sub>5</sub>-3-Nitrophenyl), 8.05 (d, 1H, *J* = 8 Hz, H<sub>4</sub>-3-Nitrophenyl), 8.12 (d, 2H, *J* = 8 Hz, H<sub>2,6</sub>-4-Methylphenyl), 8.31 (d, 1H, *J* = 8 Hz, H<sub>6</sub>-4-Nitrophenyl), 8.59 (s, 1H, H<sub>5</sub>-thiazole), 8.97 (s, 1H, H<sub>2</sub>-3-Nitrophenyl), 10.78 (s, NH). IR (KBr, cm<sup>-1</sup>)  $\bar{\nu}$ : 3375 (N-H, Stretch), 3109 (C-H, Aromatic), 2920 (C-H aliphatic, Stretch, Asymmetric), 2858 (C-H aliphatic, Stretch, Symmetric), 1693 (C=O, Stretch), 1593 (C=C, Stretch, Aromatic), 1527 (NO<sub>2</sub>, Stretch, Asymmetric), 1465 (C=C, Stretch, Aromatic), 1350 (NO<sub>2</sub>, Stretch, Asymmetric). MS (*m/z*, %): 339 (M<sup>+</sup>, 75), 322 (25), 202 (100), 174 (15), 135 (15), 119 (25), 91 (20).

***N*-(4-Nitrophenyl)-2-p-tolylthiazole-4-carboxamide (4e)**

mp: 124°C, Yield: 33%, <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ: 2.46 (s, 3H, -CH<sub>3</sub>), 6.67 (d, 2H, *J* = 8 Hz, H<sub>3,5</sub>-4-Methylphenyl), 7.45 (d, 2H, *J* = 8 Hz, H<sub>2,6</sub>-4-Methylphenyl), 8.25 (t, 2H, *J* = 8 Hz, H<sub>2,6</sub>-4-Nitrophenyl), 8.36 (t, 2H, *J* = 8 Hz, H<sub>3,5</sub>-4-Nitrophenyl), 8.62 (s, 1H, H<sub>5</sub>-thiazole), 10.84 (s, NH). IR (KBr, cm<sup>-1</sup>)  $\bar{\nu}$ : 3367 (NH, Stretch), 3113 (CH, Stretch, Aromatic), 2924 (CH, Stretch, Asymmetric, Aliphatic), 2854 (CH, Stretch, Symmetric, Aliphatic), 1685 (C=O, Stretch), 1600 (C=C, Aromatic), 1543 (NH, Bend), 1477 (C=C,

**Table 1.** Cytotoxicity results (IC<sub>50</sub> ± SD, μM) of compounds **4a-4f** against cancerous cell lines


| Compounds   | R                         | SKNMC      | HEP-G2     | MCF-7    |
|-------------|---------------------------|------------|------------|----------|
| 4a          | <i>o</i> -NO <sub>2</sub> | 16.2±1.001 | 12.7±0.023 | >25      |
| 4b          | <i>m</i> -NO <sub>2</sub> | 15.3±1.12  | 18.5±1.04  | >25      |
| 4c          | <i>p</i> -NO <sub>2</sub> | 10.8±0.08  | 14.1±1.52  | >25      |
| 4d          | <i>m</i> -Cl              | 25.1±0.71  | 11.6±0.12  | >25      |
| 4e          | <i>p</i> -Cl              | 17.8±1.42  | 22.3±1.89  | >25      |
| 4f          | <i>p</i> -F               | 16.2±0.005 | 14.7±1.004 | >25      |
| Doxorubicin | -                         | 5.7±0.02   | 5.8±1.01   | 8.7±0.15 |

Aromatic). MS (*m/z*, %): 339 (M<sup>+</sup>, 35), 322 (15), 202 (100), 174 (65), 135 (5), 119 (55), 91 (15).

#### ***N*-(4-Fluorophenyl)-2-*p*-tolylthiazole-4-carboxamide (4f)**

mp: 107°C, Yield: 31%, <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>) δ: 2.36 (s, 3H, -CH<sub>3</sub>), 7.20 (d, 2H, *J* = 8 Hz, H<sub>3,5</sub>-4-Methylphenyl), 7.35 (d, 2H, *J* = 8 Hz, H<sub>2,6</sub>-4-Methylphenyl), 7.90 (t, 2H, *J* = 8 Hz, H<sub>2,6</sub>-4-Fluorophenyl), 8.00 (t, 2H, *J* = 8 Hz, H<sub>3,5</sub>-4-Fluorophenyl), 8.45 (s, 1H, H<sub>5</sub>-thiazole), 10.32 (s, NH). IR (KBr, cm<sup>-1</sup>)  $\bar{\nu}$ : 3356 (N-H, Stretch), 3120 (C-H, Stretch, Aromatic), 2924 (C-H aliphatic, Stretch, Asymmetric), 2854 (C-H aliphatic, Stretch, Symmetric), 1662 (C=O, Stretch), 1612 (C=C, Stretch, Aromatic), 1512 (N-H, Bend), 1462 (C=C, Stretch, Aromatic). MS (*m/z*, %): 312 (M<sup>+</sup>, 5), 229 (30), 205 (15), 176 (5), 119 (100), 91 (40), 65 (20).

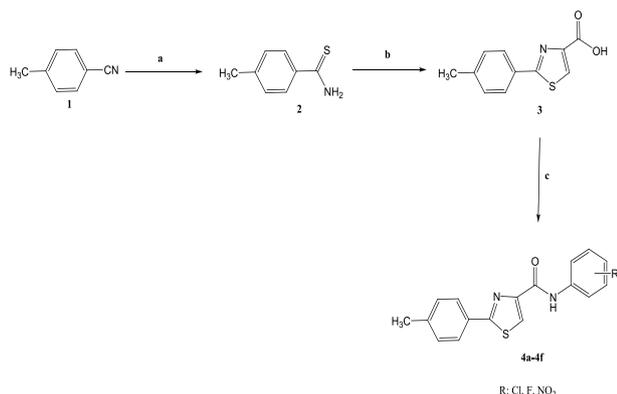
#### *Cytotoxicity assay*

Synthesized derivatives of 1,3,4-thiadiazole (compounds **4a-4f**) were tested for cytotoxic activity at 0.1-25 μM concentration in three human cancer cell lines of SKNMC (Neuroblastoma), Hep-G2 (Human hepatocarcinoma) and MCF-7 cell (Breast cancer). Cells were purchased from the Pasteur Institute of Iran. Cells from different cell lines were seeded in 96-well plates at the density of 8000-10,000 viable cells per well and incubated for 24 hr to allow cell attachment. The cells were then incubated for another 24 hr (depends to cell cycle of each cell line) with various concentrations of compounds **4a-4f**. Cells were then washed in PBS, and 20 μl of MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution (5 mg/ml) were added to each well. An additional 4 hr of

incubation at 37°C were done, and then the medium was discarded. Dimethyl sulfoxide (60 μl) was added to each well, and the solution was vigorously mixed to dissolve the purple tetrazolium crystals. The absorbance of each well was measured by plate reader (Anthous 2020; Austria) at a test wavelength of 550 nm against a standard reference solution at 690 nm. The amount of produced purple formazan is proportional to the number of viable cells (21, 22).

#### **Results**

A new series of phenylthiazole analogs were synthesized (Scheme 1) and their chemical structures were characterized by spectroscopic methods containing <sup>1</sup>H NMR, MS and IR. *p*-Toluonitrile (compound **1**) was treated with ammonium sulfide to form the corresponding thioamide derivative (compound **2**). The reaction was carried out in DMF solvent at room temperature. After completion, the crushed ice was added and yellow precipitate was filtered. The related product was afforded with high yield (96%). The obtained powder was treated with *n*-hexane and diethyl ether. Obtained thioamide derivative was refluxed with bromopyruvic acid in ethanol solvent to form the 1,3-thiazole ring with an acidic residue (compound **3**). Compound **3** was utilized to prepare the intended derivatives **4a-4f**. Compound **3** was mixed with EDC and HOBT in acetonitrile solvent. The reaction mixture was stirred for 30 min at room temperature and appropriate aniline derivative was added to the mixture. Stirring was continued for 24 hr to complete the reaction. The melting points of all synthesized derivatives were measured using melting point analyzer by open capillary tube. Among the final compounds, compound **4d** with *meta* nitro substituent



**Scheme 1.** Synthetic pathway for preparation of compounds **4a-4f**. Reagents and conditions: **a)** Ammonium sulfide, DMF, rt, **b)** Bromopyruvic acid, CaCO<sub>3</sub>, EtOH, rt, **c)** EDC, HOBt, appropriate aniline derivative, CH<sub>3</sub>CN, rt, 24 hr

showed the highest melting point (157°C), whereas compound **4a** with *meta* chlorine moiety exerted the lowest melting point (88°C).

The MTT assay was utilized for evaluation of *in vitro* cytotoxic potency. Three human cancerous cell lines consisting SKNMC (Neuroblastoma), Hep-G2 (Human hepatocarcinoma) and MCF-7 (Breast cancer) cell line using MTT assay and obtained values (IC<sub>50</sub> ± SD) were compared with doxorubicin (Table 1). Unfortunately, none of the synthesized compounds showed superior activity than doxorubicin against cancerous cell lines. MCF-7 cell line was the most resistant cell line against tested compounds. Compounds **4c** with *para* nitro (IC<sub>50</sub> = 10.8 ± 0.08 μM) and **4d** with *meta* chlorine (IC<sub>50</sub> = 11.6 ± 0.12 μM) moieties exerted the highest cytotoxic effects towards SKNMC and Hep-G2 cell lines respectively.

## Discussion

### Structure activity relationship

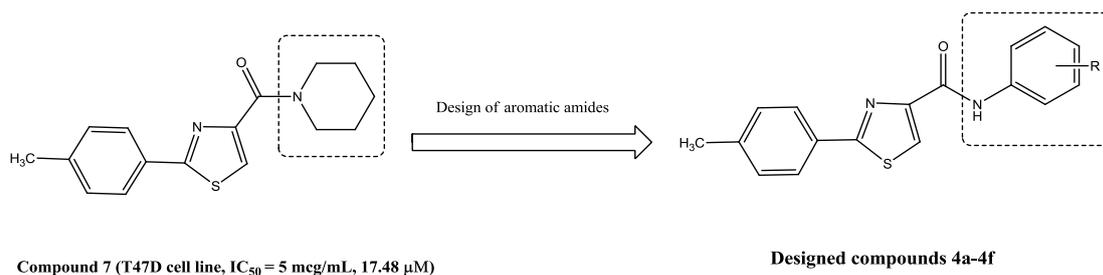
According to the **Table 1**, compound **4c** with *para* nitro moiety exerted the highest cytotoxic activity (*P* = 0.023) among nitro containing derivatives against SKNMC cell line (IC<sub>50</sub> = 10.8 ± 0.08). Whereas, substitution of the nitro moiety at position *meta* of the phenyl ring caused a detrimental effect for *in vitro* anticancer potency towards this cell line. In fact, compound **4b** demonstrated the lowest cytotoxic activity (IC<sub>50</sub> = 15.3 ± 1.12) in comparison with compounds **4a** and **4c**. It is also notable to state that compound **4c** was also the most potent compound against SKNMC cell line compared to other compounds (*P* < 0.05). Substitution of chlorine atom

at positions *meta* and *para* of the phenyl ring exhibited a similar trend like nitro moiety. In the other words, chlorine moiety showed a better activity against SKNMC cell line while introduced at position *para* in compound **4e** (*P* = 0.041). Compound **4f** with *para* positioning of fluorine moiety rendered an equal potency like compound **4a**. In Hep-G2 cell line compound **4d** showed a remarkable activity in comparison with other compounds (IC<sub>50</sub> = 11.6 ± 0.12) (*P* < 0.05). But, it provided an inferior potency than doxorubicin (IC<sub>50</sub> = 5.8 ± 1.01). *Ortho* position of the phenyl ring was the best position for nitro substituent to exert its cytotoxic activity against Hep-G2 cell line as observed in compound **4a**. Whereas, *para* positioning of the chlorine atom on the phenyl ring led to a significant decreased the anticancer activity in this series (compound **4d**, IC<sub>50</sub> = 22.3 ± 1.89). MCF-7 cell line was the most resistant cell line to all tested derivatives **4a-4f**. Unfortunately, none of the tested compounds rendered acceptable cytotoxic effect up to 25 μM concentration towards this cell line.

Generally, comparison of the cytotoxicity results of synthesized compounds with compound **7** (Table 1, Figure 3) demonstrated that replacement of the cyclohexyl moiety with aniline moiety caused an increase in activity. In the other words, in the previous study compound **7** exhibited inhibitory concentrations equal to 17.48 μM. But, the most of the tested compounds in the current study exerted superior potency compared to compound **7**. This obtained results stated that an aromatic moiety such as phenyl ring could be a better choice for substitution than aliphatic substituent like cyclohexyl residue.

## Conclusion

Totally a new series of phenylthiazole derivatives were synthesized and their anticancer activity was assessed *in vitro*. MCF-7 cell line was the most resistant cell line to the tested compounds, whereas SKNMC and HEP-G2 exhibited an averaged susceptibility to cytotoxic effect in MTT assay. Compound **4c** with *para* nitro moiety rendered the best anticancer activity against SKNMC cell line. Probably the electron withdrawing property of this moiety has a critical role in its cytotoxic potency towards SKNMC cell line. In the other words, electron withdrawing effect of the nitro residue is maximum at *para* position of the phenyl ring. In Hep-G2 cell line compound **4d** with *meta* chlorine moiety exerted a remarkable activity in comparison with other compounds. It is likely that electron withdrawing as well as lipophilicity of the chlorine atom at position 3 of the phenyl ring enhance the cytotoxic effect against this cell line.



**Figure 3.** Structures of compound 7 reported in the previous study (T47D,  $IC_{50} = 5 \text{ }\mu\text{g/ml}$ ) and designed compounds 4a-4f

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## Conflict of interest

The authors declared no conflict of interest.

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