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N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-phenylacetamido)acetamide derivatives as potential anticancer agents: Synthesis and cytotoxicity evaluation

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ABSTRACT

*According to the rising statistics of cancer cases, the discovery of novel anticancer drugs is a critical issue in current medical research. Besides, drug resistance and the incidence of severe adverse effects are the logical reasons for the discovery of new antineoplastic agents. According to the positive background that has been observed for 1,3-thiazole derivatives as potential anticancer drugs, we decided to synthesize a new series of 1,3-thiazole-based cytotoxic agents. MTT assay, activation of caspase 3, capability for reduction of the mitochondrial membrane potential (MMP), and production potency of reactive oxygen species (ROS) were investigated. Some of the tested compounds demonstrated potent cytotoxic activity and also caspase 3 activation, MMP reduction, and ROS generation. Three cancerous cell lines namely Hela (cervical cancer), A549 (lung carcinoma), and U87 (glioblastoma) were applied to perform the MTT assay. Diverse moieties with different electronic features were substituted on the phenyl ring to reveal the structure-activity relationships of the target compounds **8a-8o**. Hela (cervical cancer), A549 (lung carcinoma), and U87 (glioblastoma) were utilized as cancerous cells to explore the cytotoxicity via MTT assay. Hela and U87 cells were more sensitive to the tested compounds and A549 was more resistant to the tested derivatives. Compound **8a** with ortho chlorine moiety on the phenyl ring was the most active derivative against Hela cells (IC_{50} = 1.3±0.14 µM). All evaluated derivatives rendered lower activity against A549 than doxorubicin as a standard anticancer drug. Only some of the evaluated compounds showed more cytotoxicity against U87 than doxorubicin. Furthermore, caspase 3 activation, mitochondrial membrane potential (MMP), and reactive oxygen species (ROS) generation were also investigated. Fortunately, some of the tested compounds were also active in these tests. In conclusion, the current 1,3-thiazole derivatives could be suggested as potential anticancer lead compounds.*

KEYWORDS: *Synthesis, 1,3-Thiazole, Phenylacetamide, Cytotoxicity, Caspase 3*

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INTRODUCTION

The word cancer implies a range of disorders containing solid tumors and hematological cancers. In Western countries, cancer is the second leading cause of death after cardiovascular diseases and nowadays it has converted to one of the major health problems. The diversity of the tumor types and the resemblance of the cancerous cells to the normal cells have confined the development of novel anticancer drugs [1-3]. The incidence of cancer cases is increasing and it is estimated that it will reach 19.3 million by 2025 [4–6]. The currently in use anticancer medications have some limitations such as selectivity deficiency for cancerous cells and acquired resistance to the administered drugs [5–10]. Manifestation of adverse effects such as nausea, vomiting, and myelosuppression is another limitation of the marketed drugs. Hence, discovering new anticancer agents devoid of the mentioned limitation is a goal in current medicinal chemistry research.

1,3-Thiazole as a five-membered aromatic ring contains sulfur as well as nitrogen atoms. 1,3-Thiazole heterocycle as a known chemical structure has represented different pharmacological activities such as antibacterial (cefixime, sulfathiazole), antiparasitic (nitazoxanide), antifungal (ravuconazole), antiviral (ritonavir) and anti-inflammatory (meloxicam) [11–16]. Besides, 1,3-thiazole ring could be found in the chemical structure of thiamin (Vitamin B_1). Furthermore, the 1,3-thiazole has been shown significant anticancer effects in market-launched drugs like dasatinib, ixabepilone, and dabrafenib (**Fig. 1**) [11, 16].

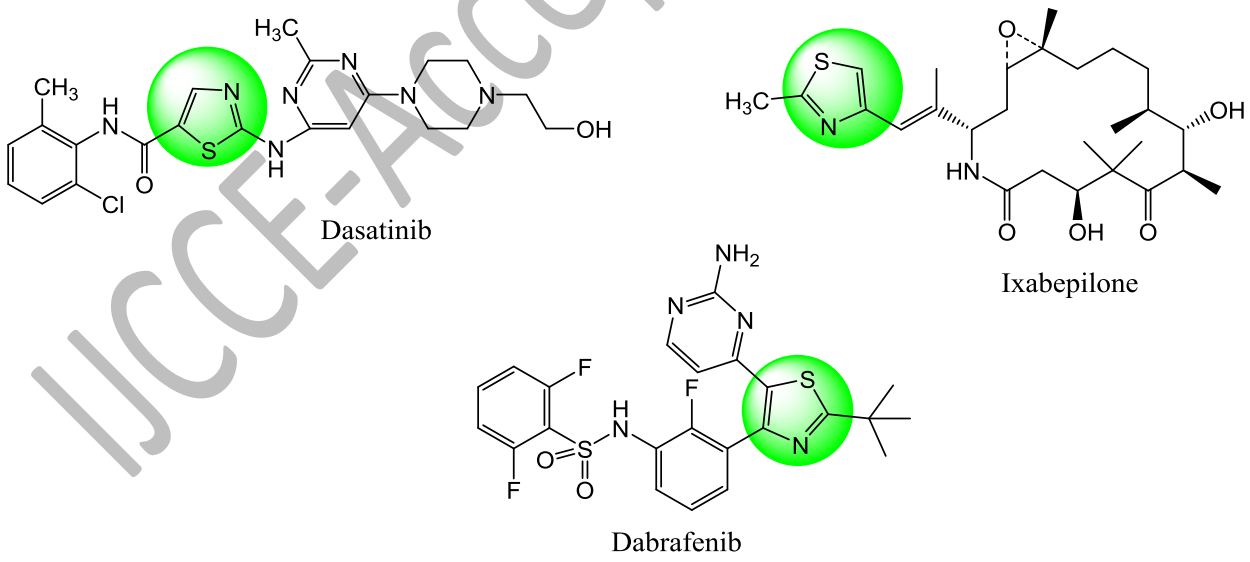


Fig. 1. Dasatinib, ixabepilone and dabrafenib as 1,3-thiazole containing anticancer drugs.

Several mechanisms containing DNA binding property and intercalation, apoptosis induction, antiangiogenic activity, tyrosine kinase, and acetylcholinesterase inhibition have been reported for the responsibility of the anticancer activity of 1,3-thiazole derivatives [10, 14, 17].

As exhibited promising response from the 1,3-thiazole derived compounds as anticancer agents [18–24] and in continuation of our previous research about the 1,3-thiazole derivatives with anticancer potential **(Fig. 2)** [25–29], we embarked on the development of novel anticancer chemical structure containing 1,3-thiazole core and the respective anticancer effectiveness was investigated *in vitro*. Besides, some probable mechanisms responsible for cytotoxicity such as production of reactive oxygen species (ROS), activation of caspase 3, and decline of the mitochondrial membrane potential (MMP) were also explored.

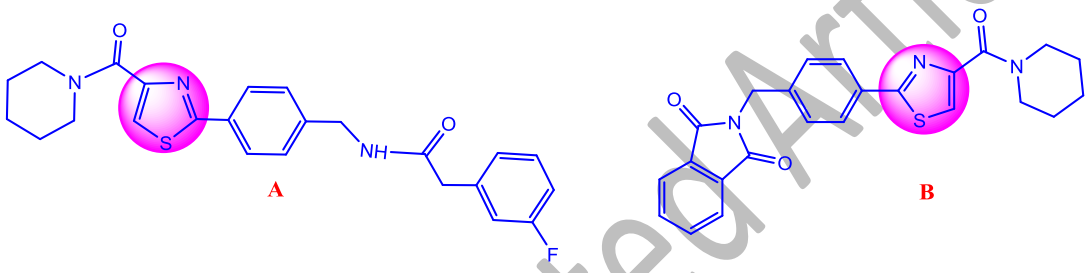


Fig. 2. Structures of compounds A and B as 1,3-thiazole derivatives with anticancer effects that were reported previously.

For the design of the target compounds **8a-8o**, a hybridization approach was implemented **(Fig. 3)**. According to the previous reports about the positive impact of 4-(4-chlorophenyl)thiazole-2-amides as well as phenylacetamides bearing thiazole moiety as cytotoxic agents, the target compounds **8a-8o** were designed [18, 25]. We hope that the new compounds possess better cytotoxic activity.

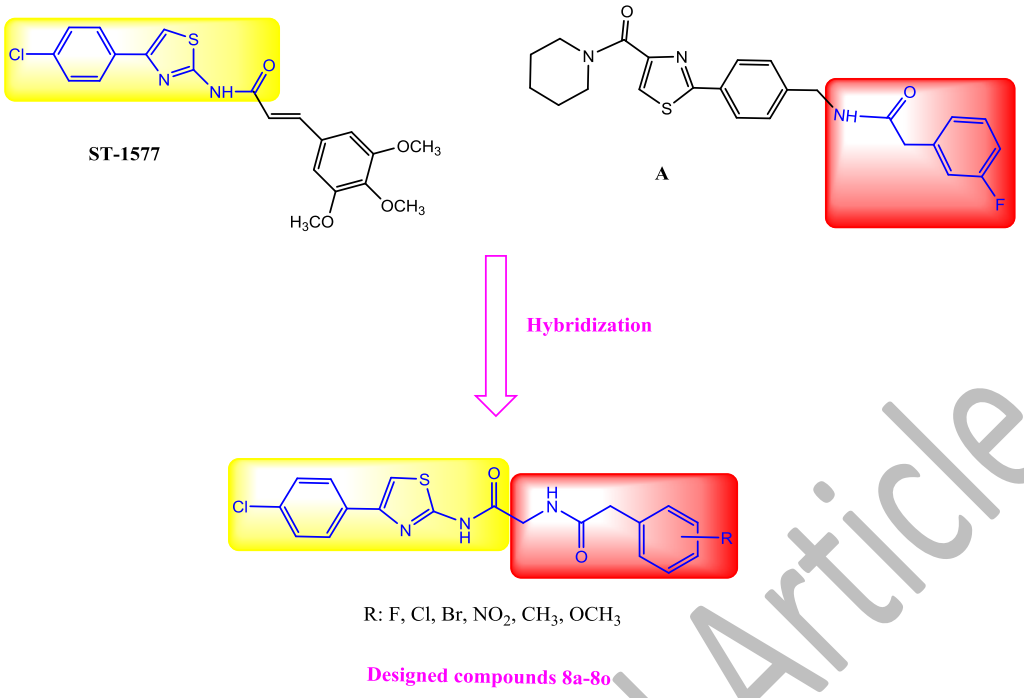


Fig. 3. The hybridization strategy for design of new compounds 8a-8o.

MATERIALS AND METHODS

Chemistry

The common chemical vendors such as Merck and Sigma-Aldrich were considered to prepare the requisite chemical reagents, solvents and the respective material for synthesis. Thin layer chromatography (TLC) sheets were ordered from Merck supplier. All synthesized substances were purified using silica gel with 70-230 mesh (Merck). A Brucker 250 MHz instrument was utilized for acquisition of the ^{1}HNMR and ^{13}CNMR spectra. Tetramethylsilane (TMS) was used as standard internally and the expression of the chemical shifts as δ (ppm) were presented. Potassium bromide salt was applied for preparation of the corresponding disk for each of the synthesized compounds to perform the IR spectroscopy. A spectrophotometer with the Shimadzu 470 brand was accessible for this end. The measurement of the melting points for all of the synthesized derivatives was carried out on an electrothermal analyzer apparatus. Mass spectroscopy was implemented at 70 eV on a Finigan TSQ-70 spectrometer (USA). The needed materials for cell culture and biological tests were purchased from the Iranian vendors. The cancerous cell lines supplied by Pasteur Institute of Iran.

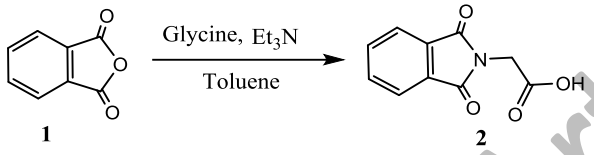
Table 1. Properties of intermediate and final compounds.

Compound	*R*	*MW (g/mol)*	*mp (˚C)*	*Chemical formula*	*Yield (%)*
2	*-*	*205*	*115*	$C_{10}H_7NO_4$	*76*
4	*-*	*233*	*95*	C_8H_6BrClO	*86*
5	*-*	*210*	*164*	$C_9H_7ClN_2S$	*92*
6	*-*	*397*	*273*	$C_{19}H_{12}ClN_3O_3S$	*53*
7	*-*	*267*	*172*	$C_{11}H_{10}ClN_3OS$	*46*
8a	*2-Cl*	*419*	*206*	$C_{19}H_{15}Cl_2N_3O_2S$	*46*
8b	*3-Cl*	*419*	*201*	$C_{19}H_{15}Cl_2N_3O_2S$	*53*
8c	*4-Cl*	*419*	*201*	$C_{19}H_{15}Cl_2N_3O_2S$	*61*
8d	*2-F*	*403*	*203*	$C_{19}H_{15}ClFN_3O_2S$	*37*
8e	*3-F*	*403*	*160*	$C_{19}H_{15}ClFN_3O_2S$	*43*
8f	*4-F*	*403*	*154*	$C_{19}H_{15}ClFN_3O_2S$	*55*
8g	*2-OCH_3*	*415*	*180*	$C_{20}H_{18}ClN_3O_3S$	*35*
8h	*3-OCH_3*	*415*	*180*	$C_{20}H_{18}ClN_3O_3S$	*31*
8i	*4-OCH_3*	*415*	*174*	$C_{20}H_{18}ClN_3O_3S$	*42*
8j	*2-OH*	*401*	*171*	$C_{19}H_{16}ClN_3O_3S$	*63*
8k	*3-OH*	*401*	*103*	$C_{19}H_{16}ClN_3O_3S$	*55*
8l	*2-NO_2*	*430*	*128*	$C_{19}H_{15}ClN_4O_4S$	*33*
8m	*4-NO_2*	*430*	*138*	$C_{19}H_{15}ClN_4O_4S$	*41*
8n	*4-Br*	*462*	*148*	$C_{19}H_{15}BrClN_3O_2S$	*49*
8o	*2-CH_3*	*399*	*192*	$C_{20}H_{18}ClN_3O_2S$	*52*

Synthesis of 2-(1,3-Dioxoisoindolin-2-yl)acetic acid **(2)**

The synthesis of compound **(2)** was carried out according to the literature (**Scheme 1**) [30].

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 4.3 (s, 2H, -CH_2-), 7.70 (dd, 2H, $H_{5,6}$-Phthalimide), 7.85 (dd, 2H, $H_{4,7}$-Phthalimide), 11.97 (brs, -COOH). IR (KBr, cm^{-1}) ῡ: 3468 (OH, acid), 3155, 2989, 2939, 1705 (C=O, acid). MS (*m/z*, %): 205 (M^+, weak), 160 (100), 133 (20), 104 (40), 76 (35), 50 (20).



Scheme 1. Synthetic pathway for compound 2.

Synthesis of 2-Bromo-1-(4-chlorophenyl)ethan-1-one **(4)**

Using a flask with flat bottom shape, 30 ml of methanol was used as solvent and 10 g (64.9 mmol) of 4′-chloroacetophenone derivative and 11.49 g (64.9 mmol) of *N*-bromosuccinimide (NBS) were reacted. In addition, 1.12 g (6.49 mmol) *p*-toluene sulfonic acid (PTSA) was applied to this reaction container. The mixture was refluxed for 20 h at 60 °C and the thin layer chromatography (TLC) was used to detect the completion of the reaction. Methanol was removed utilizing a rotary evaporator instrument. The residue was treated by *n*-hexane for more purification [31].

1HNMR (CDCl3, 250 MHz) δ (ppm): 4.43 (s, 2H, -CH_2-Br), 7.50 (d, 2H, *J* = 8.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.95 (d, 2H, *J* = 8.5 Hz, $H_{3,5}$-4-chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3086, 3062, 2951, 2854, 1693, 1589, 1485, 1396, 1195, 1091, 991, 810.

Synthesis of 4-(4-Chlorophenyl)thiazol-2-amine **(5)**

4 g (18.6 mmol) of the obtained compound **(4)** was refluxed with 1.41 g (18.6 mmol) of thiourea in absolute ethanol (40 ml) for 8 h. Then, the reaction was cooled with addition of the crushed ice and subsequently, the afforded sediment solid was separated. The organic layer was evaporated and a creamy powder was collected and recrystallized in ethanol [22, 31].

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 4.10 (brs, NH_2), 7.26 (s, 1H, H_5-thiazole), 7.53 (d, 2H, *J* = 8.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.73 (d, 2H, *J* = 8.5 Hz, $H_{3,5}$-4-chlorophenyl). IR (KBr, cm^{-1}) ū: 3387, 3263 (stretch, NH_2). MS (*m/z*, %): 212 (M^++2, 210 (M^+, 100), 168 (30), 133 (12), 89 (12), 45 (10).

Synthesis of N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(1,3-dioxoisoindolin-2-yl)acetamide **(6)**

In a flask with the flattened shape on the bottom, 8.78 g (42.8 mmol) of compound **(2)**, 10 g (42.8 mmol) of compound **(5)**, 8.82 g (42.8 mmol) dicyclohexylcarbodiimide (DCC) and 5.78 g (42.8 mmol) hydroxybenzotriazole (HOBt) were dissolved in 100 ml of tetrahydrofuran (THF). The reaction performance was continued for 1 h in a bath containing ice. The subsequent step was overnight stirring at 25°C. The product formation was checked using thin layer chromatography (TLC) and turning the reaction off was occurred. After this, the formed dicyclohexylurea (DCU) sediment was removed by filtration. Removal of the THF solvent was take into account under reduced pressure. The extraction of the intended product was implemented by ethyl acetate/water mixture. After omitting the aqueous phase, organic phase was washed by brine (tree times) and dried over anhydrous sodium sulfate. Filtration of the sodium sulfate was carried out and evaporation of the THF was done. A creamy powder was achieved that triturated with *n*-hexane and diethyl ether (Et_2O) [32].

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 4.57 (s, 1H, -CH_2-), 7.46 (d, 2H, *J* = 8 Hz, $H_{2,6}$-4-chlorophenyl), 7.69 (s, H_5-thiazole), 7.89 (m, 6H, aromatic), 12.73 (brs, NH). IR (KBr, cm^{-1}) ū: 3298 (stretch, NH), 1712 (stretch, C=O). MS (*m/z*, %): 397 (M^+, 5), 291 (20), 272 (70), 236 (20), 228 (20), 211 (20), 205 (90), 198 (30), 188 (60), 180 (40), 160 (100), 133 (20), 104 (20).

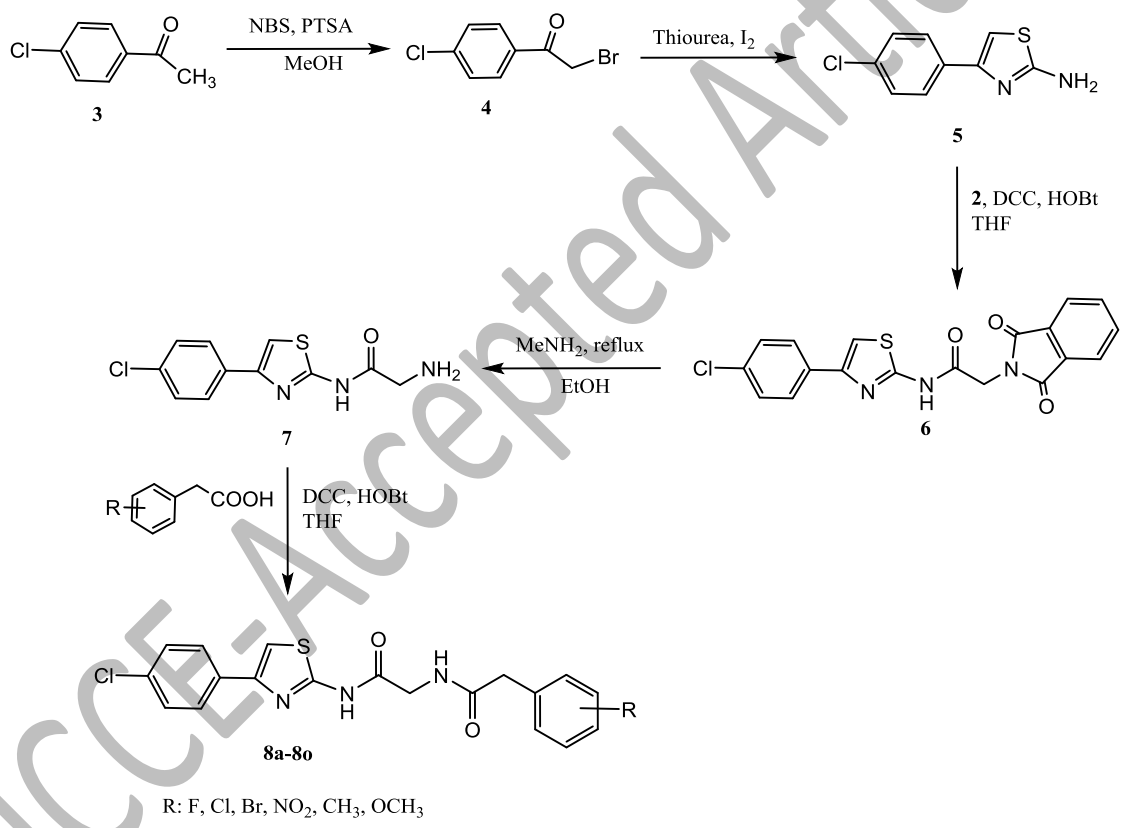
Synthesis of 2-Amino-N-(4-(4-chlorophenyl)thiazol-2-yl)acetamide **(7)**

Refluxing condition was utilized for the reaction of 10 g (25.2 mmol) of compound **6** with 19.5 ml (252.0 mmol) of methylamine 40%. The reaction was performed in absolute ethanol (100 ml). The refluxing was continued for 8 h and the final point was determined using TLC. The solvent was removed by rotatory evaporator and then ethyl acetate/water was used for extraction. Watery phase was ignored and the ethyl acetate phase was washed three turns by brine. *n*-Hexane and diethyl ether (Et_2O) were applied for washing and more purification. The obtained pale yellow solid was free of any impurities and therefore transferred to the subsequent reaction without excessive purification process [25].

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.64 (s, 2H, -CH_2-), 3.46, 4.21 (brs, NH_2), 6.70 (brs, amidic NH), 7.100 (s, 1H, H_5-thiazole), 7.35 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.73 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl). IR (KBr, cm^{-1}) ū: 3325, 3194 (stretch, NH_2), 3062 (stretch, C-H), 2927, 2850 (stretch, C-H), 1674 (stretch, C=O), 1627, 1566 (bend, NH). MS (*m/z*, %): 267 (M^+, weak), 262 (20), 236 (40), 223 (30), 210 (100), 168 (60), 160 (75), 141 (40), 133 (30), 110 (45), 98 (75), 90 (30), 77 (40), 55 (80), 41 (40).

General procedure for the synthesis of compounds **8a-8o**

0.2 g (0.75 mmol) of the obtained amino derivatives (**7)** that afforded in the previous step was reacted with equimolar quantities of appropriate phenylacetic acid derivative, DCC, and HOBt in THF (20 ml) in a bath contained the crushed ice. This step was lasted for 1 h. Subsequently, the stirring of the reaction medium was kept on for about 20 h duration at room temperature. The filtration was carried out for removal of DCU byproduct and THF was eliminated under vacuum condition of the evaporator. Ethyl acetate was used for extraction. The organic layer was washed three folds by brine and dryness was done using anhydrous sodium sulfate. Finally, the ethyl acetate was removed under reduced pressure. Column chromatography was applied for purification (Petroleum/ethyl acetate; 70:30) (**Scheme 2**) [32].



Scheme 2. Synthetic pathway for compounds 8a-8o.

2-(2-Chlorophenyl)-*N*-(2-((4-(4-chlorophenyl)thiazol-2-yl)amino)-2-oxoethyl)acetamide **(8a)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.47 (s, 2H, Ph-C$\underline{H}_2$-), 4.10 (s, 2H, -CO-C$\underline{H}_2$-NH-), 6.95 (brs, -CH_2-CON$\underline{H}$-), 7.21 (s, 1H, H_5-thiazole), 7.12-7.47 (m, 4H, 2-chlorophenyl), 7.60-7.90 (m, 4H, 4-chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 3035 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 420 (M^+, weak), 221 (20), 173 (25), 141 (25), 138 (50), 120 (100), 91 (70), 82 (70), 56 (40).

2-(3-Chlorophenyl)-*N*-(2-((4-(4-chlorophenyl)thiazol-2-yl)amino)-2-oxoethyl)acetamide **(8b)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.45 (s, 2H, Ph-C$\underline{H}_2$-), 4.14 (s, 2H, -CO-C$\underline{H}_2$-NH-), 6.88 (brs, -CH_2-CON$\underline{H}$-), 7.21 (s, 1H, H_5-thiazole), 7.08-7.38 (m, 4H, 3-chlorophenyl), 7.60-7.90 (m, 4H, 4-chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3035 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 420 (M^+, 15), 221 (35), 173 (15), 141 (40), 138 (30), 120 (100), 91 (55), 82 (50), 56 (65).

2-(4-Chlorophenyl)-*N*-(2-((4-(4-chlorophenyl)thiazol-2-yl)amino)-2-oxoethyl)acetamide **(8c)** ^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.45 (s, 2H, Ph-C$\underline{H}_2$-), 4.17 (s, 2H, -CO-C$\underline{H}_2$-NH-), 7.10-7.33 (m, 5H, aromatic), 7.65 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.88 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3035 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 420 (M^+, 10), 221 (40), 173 (20), 141 (55), 138 (30), 120 (100), 91 (35), 82 (50), 56 (15).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(2-fluorophenyl)acetamido)acetamide **(8d)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.45 (s, 2H, -PhCH_2-), 4.14 (s, 2H, -COC$\underline{H}_2$NH-), 6.75 (brs, CH_2-N$\underline{H}$CO-), 7.15 (m, 2H, 2-fluorophenyl), 7.35 (m, 2H, 2-fluorophenyl), 7.62 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.87 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3035 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 403 (M+, 10), 242 (25), 173 (65), 145 (25), 137 (25), 123 (50), 95 (55), 91 (90), 82 (100).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(3-fluorophenyl)acetamido)acetamide **(8e)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.44 (s, 2H, Ph-CH_2-), 4.35 (s, 2H, Ph-CH_2-), 6.84 (brs, -N$\underline{H}$-CO-CH_2-), 7.04 (d, 2H, *J* = 8.5 Hz, aromatic), 7.10 (s, 1H, H_5-thiazole), 7.35 (d, 2H, *J* = 8.5 Hz, aromatic), 7.68 (t, 2H, *J* = 8.5 Hz, aromatic), 7.84 (d, 2H, *J* = 8.5 Hz, aromatic), 10.23 (brs, thiazole-NH). ^{13}CNMR ($CDCl_3$, 62 MHz) δ (ppm): 42.8 (-$\underline{C}H_2$-Ph), 49.3 (-$\underline{C}H_2$-NH-), 108.3 (C_5-thiazole), 111.3 (C_4-3-fluorophenyl), 114.7 (C_2-3-fluorophenyl), 116.5 (C_6-3-fluorophenyl), 118.5 ($C_{2,6}$-4-chlorophenyl), 124.4 ($C_{3,5}$-4-chlorophenyl), 125.2 (C_5-3-fluorophenyl), 127.4 (C_1-4-chlorophenyl), 128.9 (C_4-4-chlorophenyl), 130.6 (C_1-3-fluorophenyl), 139.7 (C_4-thiazole), 143.1 (C_3-3-fluorophenyl), 147.2 (C_2-thiazole), 158.0 (thiazole-NH-$\underline{C}$O-), 169.3 (Ph-CH_2-$\underline{C}$O-). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 3039 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1689 (stretch, C=O). MS (*m/z*, %): 403 (M+, weak), 242 (40), 173 (50), 145 (15), 137 (35), 123 (60), 95 (35), 91 (80), 82 (100).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(4-fluorophenyl)acetamido)acetamide **(8f)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.76 (s, 2H, Ph-CH_2-), 4.41 (s, 2H, -CO-CH_2-NH-), 6.80 (brs, -CH_2NHCO-), 7.04 (m, 2H, 4-fluorophenyl), 7.34 (m, 2H, 4-fluorophenyl), 10.54 (brs, -thiazole-NH). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3043 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1689, 1627 (stretch, C=O). MS (*m/z*, %): 403 (M+, 15), 242 (35), 173 (25), 145 (25), 137 (15), 123 (35), 95 (20), 91 (65), 82 (100).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(2-methoxyphenyl)acetamido)acetamide **(8g)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.43 (s, 2H, Ph-CH_2-), 3.81 (s, 2H, -CH_2-NH-), 3.84 (s, 3H, -OCH_3), 6.80 (brs, -CH_2-NH-), 6.89-7.10 (m, 2H, 2-methoxyphenyl), 7.31 (m, 1H, 2-methoxyphenyl), 7.63 (d, 1H, *J* = 7.5 Hz, H_6-2-methoxyphenyl), 7.68 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.79 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl), 10.54 (brs, thiazole-NH). ^{13}CNMR ($CDCl_3$, 62 MHz) δ (ppm): 33.8 (-CH_2-Ph), 49.2 (-CH_2-NH-), 55.5 (-OCH_3), 109.0 (C_5-thiazole), 110.6 (C_3-2-methoxyphenyl), 118.3 (C_5-2-methoxyphenyl), 121.5 (C_1-2-methoxyphenyl), 124.9 (C_4-2-methoxyphenyl), 126.2 ($C_{2,6}$-4-chlorophenyl), 127.3 ($C_{3,5}$-4-chlorophenyl), 128.8 (C_1-4-chlorophenyl), 129.3 (C_6-2-methoxyphenyl), 131.4 (C_4-4-chlorophenyl), 142.8 (C_4-thiazole), 146.5 (C_2-2-methoxyphenyl), 148.2 (C_2-thiazole), 155.3 (thiazole-NH-CO-), 157.0 (Ph-CH_2-CO-). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3035 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 415 (M^+, 10), 358 (15), 210 (55), 168 (20), 148 (55), 121 (100), 78 (10).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(3-methoxyphenyl)acetamido)acetamide **(8h)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.46 (s, 2H, Ph-CH_2-), 3.74 (s, 2H, -CH_2-NH-), 3.80 (s, 3H, -OCH_3), 6.66 (brs, -CH_2-NH-), 6.84 (m, 1H, H_4-3-methoxyphenyl), 7.01 (s, 1H, H_2-3-methoxyphenyl), 7.12-7.37 (m, 2H, 3-methoxyphenyl), 7.64 (d, 2H, *J* = 8 Hz, $H_{2,6}$-4-chlorophenyl), 7.68 (s, 1H, H_5-thiazole), 7.76 (d, 2H, *J* = 8 Hz, $H_{3,5}$-4-chlorophenyl), 10.10 (brs, thiaole-NH-). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3039 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 415 (M^+, 10), 358 (25), 210 (45), 168 (35), 148 (60), 121 (100), 78 (25).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(4-methoxyphenyl)acetamido)acetamide **(8i)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.47 (s, 2H, Ph-CH_2-), 3.74 (s, 2H, -CH_2-NH-), 3.82 (s, 3H, -OCH_3), 4.22 (-CONH-CH_2-), 6.91 (d, 2H, *J* = 8 Hz, $H_{3,5}$-4-methoxyphenyl), 7.09 (s, 1H, H_5-thiazole), 7.17 (d, 2H, *J* = 8 Hz, $H_{2,6}$-4-methoxyphenyl), 7.35 (d, 2H, *J* = 8 Hz, $H_{2,6}$-4-chlorophenyl), 7.69 (d, 2H, *J* = 8 Hz, $H_{3,5}$-4-chlorophenyl), 9.40 (brs, thiazole-NH-). ^{13}CNMR ($CDCl_3$, 62 MHz) δ (ppm): 42.4 (-CH_2-Ph), 49.2 (-CH_2-NH-), 55.3 (-OCH_3), 108.2 (C_5-thiazole), 114.5 ($C_{3,5}$-4-methoxyphenyl), 125.0 (C_1-4-methoxyphenyl), 127.4 ($C_{2,6}$-4-chlorophenyl), 128.9 ($C_{3,5}$-4-chlorophenyl), 130.6 ($C_{2,6}$-4-methoxyphenyl), 132.8 (C_1-4-chlorophenyl), 133.8 (C_4-4-chlorophenyl), 148.6 (C_4-thiazole), 157.0 (C_4-4-methoxyphenyl), 158.2 (C_2-thiazole), 159.3 (thiazole-NH-CO-), 169.4 (Ph-CH_2-CO-). IR (KBr,

cm^{-1}) ῡ: 3329 (stretch, NH), 3039 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1689, 1627 (stretch, C=O). MS (*m/z*, %): 415 (M^+, weak), 358 (15), 210 (60), 168 (15), 148 (80), 121 (100), 78 (15).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(2-hydroxyphenyl)acetamido)acetamide **(8j)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 2.83 (s, 2H, Ph-CH_2-), 3.39 (s, 2H, Ph-CH_2-), 4.34 (brs, OH), 6.67 (brs, -CH_2-NHCO-), 6.78-7.15 (m, 4H, 2-hydroxyphenyl), 7.38 (s, H_5-thiazole), 7.63 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.79 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl). ^{13}CNMR ($CDCl_3$, 62 MHz) δ (ppm): 33.8 (-CH_2-Ph), 49.3 (-CH_2-NH-), 106.5 (C_5-thiazole), 110.8 (C_3-2-hydroxyphenyl), 118.09 (C_5-2-hydroxyphenyl), 120.1 (C_1-2-hydroxyphenyl), 125.1 ($C_{2,6}$-4-chlorophenyl), 126.2 (C_4-2-hydroxyphenyl) 126.9 ($C_{3,5}$-4-chlorophenyl), 127.3 (C_1-4-cholorophenyl), 130.2 (C_6-2-hydroxyphenyl), 131.0 (C_4-4-cholorophenyl), 142.4 (C_4-thiazole), 149.3 (C_2-2-hydroxyphenyl), 152.2 (C_2-thiazole), 157.1 (thiazole-NH-CO-), 162.8 (Ph-CH_2-CO-). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 3039 (stretch, C-H, aromatic), 3300 (stretch, O-H, broad), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 401 (M^+, weak), 383 (10), 236 (15), 210 (60), 168 (20), 141 (10), 110 (20), 98 (30), 83 (20), 55 (100), 41 (95).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(3-hydroxyphenyl)acetamido)acetamide **(8k)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.39 (s, 2H, Ph-CH_2-), 3.71 (s, 2H, -CO-CH_2-NH-, 4.65 (brs, OH), 5.95 (brs, -CH_2-NHCO-), 6.65-6.96 (m, 2H, 3-hydroxyphenyl), 7.02 (s, 1H, H_5-thiazole), 7.12 (d, 1H, *J* = 8 Hz, 3-hydroxyphenyl), 7.31 (d, 2H, *J* = 8 Hz, $H_{2,6}$-4-chlorophenyl), 7.61 (m, 2H, *J* = 8 Hz, $H_{3,5}$-4-chlorophenyl), 9.15 (brs, thiazole-NH-). ^{13}CNMR ($CDCl_3$, 62 MHz) δ (ppm): 43.5 (-CH_2-Ph), 49.3 (-CH_2-NH-), 108.3 (C_5-thiazole), 114.8 (C_4-3-hydroxyphenyl), 117.5 (C_2-3-hydroxyphenyl), 120.8 (C_6-3-hydroxyphenyl), 126.1 ($C_{3,5}$-4-chlorophenyl), 127.0 ($C_{2,6}$-4-chlorophenyl), 128.9 (C_5-3-hydroxyphenyl), 129.6 (C_1-4-cholorophenyl), 130.1 (C_4-4-cholorophenyl), 134.0 (C_1-3-hydroxyphenyl), 135.8 (C_4-thiazole), 149.0 (C_3-3-hydroxyphenyl), 151.5 (C_2-thiazole), 157.5 (thiazole-NH-CO-), 169.5 (Ph-CH_2-CO-). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3059 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 401 (M^+, 15), 236 (10), 210 (45), 168 (20), 110 (20), 98 (15), 83 (30), 55 (100), 41 (75).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(2-nitrophenyl)acetamido)acetamide **(8l)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.18 (s, 2H, Ph-CH_2-), 4.01 (s, 2H, -CH_2-NH), 5.96 (brs, NH), 7.03 (s, H_5-thiazole), 7.34 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.42-7.60 (m, 2H, 2-nitrophenyl), 7.70 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl), 8.00 (d, 2H, *J* = 7.5 Hz, H_6-2-nitrophenyl), 8.10 (d, 2H, *J* = 7.5 Hz, H_3-2-nitrophenyl), 10.87 (brs, thiazole-NH). ^{13}CNMR ($CDCl_3$, 62 MHz) δ (ppm): 33.8 (-CH_2-Ph), 40.9 (-CH_2-NH-), 108.4 (C_5-thiazole), 111.0 (C_3-2-nitrophenyl), 119.1 (C_4-2-nitrophenyl), 123.2 ($C_{2,6}$-4-chlorophenyl), 125.1 ($C_{3,5}$-4-chlorophenyl), 125.5 (C_1-2-

nitrophenyl), 127.4 (C_6-2-nitrophenyl), 128.4 (C_1-4-cholorophenyl), 129.0 (C_4-4-cholorophenyl), 133.6 (C_5-2-nitrophenyl), 142.0 (C_2-2-nitrophenyl), 148.7 (C_4-thiazole), 157.2 (C_2-thiazole), 157.0 (thiazole-NH-CO-), 167.3 (Ph-CH_2-CO-). IR (KBr, cm^{-1}) ῡ: 3263 (stretch, NH), 3101, 3062 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1705 (stretch, C=O), 1519 (stretch, asymmetric, NO_2), 1350 (stretch, symmetric, NO_2). MS (*m/z*, %): 430 (M^+, 10), 137 (60), 120 (30), 107 (60), 90 (30), 58 (100), 41 (20).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(4-nitrophenyl)acetamido)acetamide **(8m)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.65 (s, 2H, Ph-CH_2-), 3.90 (s, 2H, -CH_2-NH-), 6.81 (brs, -NH-CO), 7.12 (s, 1H, H_5-thiazole), 7.37 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.45 (d, 2H, *J* = 7.75 Hz, $H_{2,6}$-4-nitrophenyl), 7.69 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl), 8.19 (d, 2H, *J* = 7.75 Hz, $H_{3,5}$-4-nitrophenyl), 10.45 (brs, thiazole-NH). ^{13}CNMR ($CDCl_3$, 62 MHz) δ (ppm): 43.2 (-CH_2-Ph), 49.3 (-CH_2-NH-), 108.4 (C_5-thiazole), 119.0 ($C_{3,5}$-4-nitrophenyl), 119.5 ($C_{2,6}$-4-chlorophenyl), 123.9 ($C_{3,5}$-4-chlorophenyl), 127.4 ($C_{2,6}$-4-nitrophenyl), 128.9 (C_1-4-cholorophenyl), 130.2 (C_4-4-cholorophenyl), 134.4 (C_1-4-nitrophenyl), 142.4 (C_4-thiazole), 143.0 (C_4-4-nitrophenyl), 147.2 (C_2-thiazole), 157.0 (thiazole-NH-CO-), 169.8 (Ph-CH_2-CO-). IR (KBr, cm^{-1}) ῡ: 3271 (stretch, NH), 3086 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1685, 1643 (stretch, C=O), 1512 (stretch, asymmetric, NO_2), 1346 (stretch, symmetric, NO_2). MS (*m/z*, %): 430 (M^+, weak), 137 (75), 120 (15), 107 (40), 90 (50), 58 (100), 41 (40).

2-(4-Bromophenyl)-*N*-(2-((4-(4-chlorophenyl)thiazol-2-yl)amino)-2-oxoethyl)acetamide **(8n)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 3.64 (s, 2H, Ph-CH_2-), 3.73 (s, 2H, -CH_2-NH-), 6.45 (brs, -CH_2-NH-), 7.07 (s, 1H, H_5-thiazole), 7.17 (d, 2H, *J* = 8 Hz, $H_{2,6}$-4-chlorophenyl), 7.36 (d, 2H, *J* = 8 Hz, $H_{3,5}$-4-bromophenyl), 7.46 (d, 2H, *J* = 8 Hz, $H_{3,5}$-4-bromophenyl), 7.63 (d, 2H, *J* = 8 Hz, $H_{3,5}$-4-chlorophenyl), 10.73 (brs, thiazole-NH). ^{13}CNMR ($CDCl_3$, 62 MHz) δ (ppm): 42.5 (-CH_2-Ph), 49.3 (-CH_2-NH-), 108.4 (C_5-thiazole), 118.5 (C_4-4-bromophenyl), 125.0 ($C_{2,6}$-4-chlorophenyl), 126.5 ($C_{3,5}$-4-chlorophenyl), 127.4 ($C_{2,6}$-4-bromophenyl), 129.5 (C_1-4-bromophenyl), 131.1 (C_1-4-chloroophenyl), 131.6 ($C_{3,5}$-4-bromophenyl), 132.2 (C_4-4-chlorophenyl), 148.6 (C_4-thiazole), 158.2 (C_2-thiazole), 159.3 (thiazole-NH-CO-), 168.4 (Ph-CH_2-CO-). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3039 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1689, 1627 (stretch, C=O). MS (*m/z*, %): 263 (M^+, weak), 408 (30), 212 (90), 211 (40), 210 (100), 169 (70), 89 (70).

N-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(2-(*o*-tolyl)acetamido)acetamide **(8o)**

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.93 (s, 3H, -CH_3), 3.43 (s, 2H, Ph-CH_2-), 3.81 (s, 2H, -CH_2-NH-), 6.82 (brs, -CH_2-NH-), 7.10-7.35 (m, 4H, 2-methylphenyl), 7.65 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.84 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3035 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1627 (stretch, C=O). MS (*m/z*, %): 399 (M^+, weak), 305 (5), 237 (30), 210 (30), 160 (30), 105 (70), 77 (40), 55 (100).

Cell culture

The cancerous cell lines were obtained from Pasteur Institute (Tehran, Iran) and maintained in Dulbecco's modified Eagle's medium DMEM0 which was supplemented with 10% fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml). The cells were incubated at 37 °C in a 5% CO_2 atmosphere. The cells were seeded at a concentration of 5×10^5 in a 25 cm^2 flask containing 4 ml of complete culture medium.

MTT assay

Cellular toxicities of the synthesized compounds were evaluated in 3 cancer cell lines Hela, A549, and U87 using the methylthiazoltetrazolium bromide (MTT) method. Briefly, cells culture was carried out in a 96-well plate. The concentration of 6×10^3 cells/well was achieved. The solutions of the synthesized substances were made with concentration 10^{-9}-10^{-3} M in dimethyl sulfoxide (DMSO) in which the final concentration of DMSO was 0.1 % in the medium. After 24 hours, the incubation of the cells was implemented with various concentrations of target compounds **8a-8o** that lasted 24 h. Cells were washed with PBS and then incubated with a solution of 0.5 mg/ml MTT for 3 h at 37°C. After removal of the medium blue formazan crystals are dissolved in 200 µl DMSO in a 5-minute period. A microplate reader manufactured by Biotek Instrument Inco. was utilized to investigate the inhibitory potency of the tested compounds. So, the measurement was done at λ = 450 nm. Calculations of inhibition were based on the following equation: %Inhibition of growth = (1 − absorbance of treated cells/ absorbance of control cells) × 100. The half-maximal inhibitory concentrations (IC_{50}) were calculated after performing a linear regression analysis using the GraphPad prism 6.0 (GraphPad Software, CA, USA) [25].

Caspase-3 assay

The test procedure to assess the activity of selected compounds on caspase-3 activity was done with accordance to the protocol that provided by manufacturer's company. This test is based on the detection of *p*-nitroanilide (pNA) which is cleaved from its precursor DEVD-pNA by caspase 3. Briefly 5× 105 cells were cultured in 6-well plates and were incubated with the IC_{50} concentration of chemicals for 24 hours. Then the cells were washed with PBS, and lysed with 60 µL of lysis buffer while they were kept on ice. Centrifuging of the cell lysate was carried out in a period of 5 min at 4°C. Equal amounts (10 µL) of supernatant were added to the reaction buffer which contained the caspase 3 substrate. The incubation of the reaction was done for 2 h in 37°C and then the amount of pNA was measured at 405 nm according to the intensity of the light. The percentage of caspase activity was calculated based on the comparison of the absorbance of each compound with a drug-free control [25].

Measurement of mitochondrial membrane potential (MMP)

The changes in the permeability of the mitochondrial membrane can start the apoptosis. This event can decline the potential of the mitochondrial membrane due to the application of rhodamine 123 as fluorescent dye. This cationic and cell-permeant dye is trapped by active mitochondria. Cellular apoptosis diminished the mitochondrial membrane potential and subsequently the release of the rhodamine 123 from the mitochondria that led to the lowering of the intracellular fluorescence intensity. According to the IC_{50} concentration of the synthesized compounds, the cells were incubated, washed with PBS, reacted with rhodamine 123. This step lasted for 30 min and performed at 37°C. After the incubation of cells with IC_{50} concentration of the compounds they were washed with PBS and treated with rhodamine 123 for 30 min at 37°C. Then, a second washing process was applied with PBS. The intensity of the fluorescence was determined at wavelengths 488 nM and 520 nM for excitation and emission respectively [33].

Determination of intracellular reactive oxygen species (ROS)

Oxidative stress was assessed by measuring reactive oxygen generation using the fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA). When oxidized this molecule becomes a fluorochrome which can be traced using the excitation and emission wavelengths of 485 nm and 530 nm, respectively. Cells are seeded (4×10^5) in 6-well plates and incubated with the IC_{50} concentrations of each agent for 24 h. Then cells are washed with PBS and incubated for 30 minutes with 30 µl DCF-DA at 37 °C. Next cells were lysed with Triton X-100 and 100 µl of the lysate was transferred to a 96-well plate for fluorescence detection using the microplate reader (BioTek, H1M, USA) [34].

RESULTS AND DISCUSSION

Cytotoxicity

Anticancer assessment of the synthesized 1,3-thiazole was taken into account *in vitro*. Three cancerous cell lines namely Hela (cervical cancer), A549 (lung carcinoma) and U87 (glioblastoma) were applied to perform the MTT assay. Various moieties with different electronic properties were substituted on the phenyl part to reveal the structure-activity relationships about the synthesized derivatives. Hela and U87 cells were more sensitive to the tested compounds and A549 was more resistant to these derivatives.

According to **Table 2**, compound **8a** was the most active derivative against Hela cells (IC_{50} = 1.3±0.14 µM). In this derivative, chlorine moiety that has substituted at *ortho* position induced a noticeable raising in activity. Compound **8c** with *para* positioning of the chlorine atom also demonstrated a robust anticancer activity towards Hela cells (IC_{50} = 2.8±0.48 µM), but a significant decrease was observed while positioning of the chlorine changed to *meta*. Amongst the fluorinated derivatives, compound **8f** (2-F) displayed superior activity than other fluorinated derivatives (IC_{50} = 2.25±0.4 µM). All of the mentioned potent compounds **8a**, **8c,** and **8f** exhibited

better cytotoxicity than doxorubicin (IC_{50} = 3.8±0.31 µM) as a reference drug towards Hela cells. None of the methoxylated derivatives (**8g**, **8h**, **8i**) showed favorable cytotoxicity against Hela cells. It could be proposed that electron-donating moieties such as methoxy, hydroxyl, and methyl have detrimental effects on the cytotoxicity of these derivatives. Only compound **8j** (2-OH) possessed better potency (IC_{50} = 8.5±0.34 µM) compared to other derivatives with electron-donating property against Hela cells.

Table 2. Cytotoxicity results (IC_{50}±SEM, µM) of final compounds 8a-8o using MTT assay.

Compound	*R*	*Hela*	*A549*	*U87*
8a	*2-Cl*	*1.3±0.14*	*47±3*	*50±9*
8b	*3-Cl*	*17±0.16*	*28±0.36*	*25±1.6*
8c	*4-Cl*	*2.8±0.48*	*50±4*	*44±2.2*
8d	*2-F*	*16.7±0.61*	*50±4*	*55±4*
8e	*3-F*	*10.3±0.28*	*41±3*	*47±3*
8f	*4-F*	*2.3±0.4*	*36.5±3*	*12±0.7*
8g	*2-OCH_3*	*32±0.64*	*25±1.2*	*46±1.5*
8h	*3-OCH_3*	*50±9*	*45±2*	*31±3*
8i	*4-OCH_3*	*30±4*	*48±4*	*40±4*
8j	*2-OH*	*8.5±0.34*	*22.5±0.5*	*22±1.8*
8k	*3-OH*	*33±0.76*	*33±2*	*37±2.6*
8l	*2-NO_2*	*30±3*	*26±5*	*37.5±5*
8m	*4-NO_2*	*6±0.41*	*35±3*	*33±4*
8n	*4-Br*	*29±3*	*50±0.45*	*22±0.95*
8o	*2-CH_3*	*55±1.17*	*43±2*	*33±2*
Doxorubicin	-	*3.8±0.31*	*17±2*	*31.3±3*

All investigated compounds exerted inferior activity against A549 in comparison with doxorubicin. Some of the tested derivatives showed higher cytotoxicity towards U87. Compound **8b** (3-Cl) demonstrated better activity (IC_{50} = 25±1.6 µM) than doxorubicin. It seems that electron-withdrawing feature of the chlorine not be a helpful item for cytotoxicity against U87 cells. As a lower electron withdrawing effect performed at position *meta* lower cytotoxicity was deduced. Enhancement of lipophilicity at position 3 of the phenyl ring by chlorine may also be another beneficial parameter for cytotoxicity against U87 cells. The Electron withdrawing effect exerted by compound **8f** due to settlement of the fluorine atom at *para* site produced a significant boost in potency. The electron-receiving effect of this moiety was not effective for the improvement of cytotoxicity at positions *ortho* and *meta*. As obtained results for Hela and A549 cell lines, methoxylated derivatives were not capable of exhibiting of remarkable cytotoxic activity. All compounds bearing electron-donating substituents displayed lower potency than doxorubicin except for compound **8j** (IC_{50} = 22±1.8 µM) with hydroxyl moiety at position

ortho. Compound **8n** that was substituted with a bromine atom also rendered stronger cytotoxicity than doxorubicin against U87.

The cytotoxicity of the potent compounds (8 of selected derivatives) was tested against PC12 cells as a healthy cell line and obtained results were provided in **Table 3** and supplementary file. Fortunately, the potent derivatives only in high concentrations caused cytotoxic effects on normal cells. Compounds **8a** and **8b** as chlorinated derivatives at positions *ortho* and *meta* respectively, demonstrated more safety for normal cells compared to compound **8c** as *para* substituted congener. Compound **8d** as *ortho* fluorinated derivative exhibited superior safety amongst the other fluorinated compounds. Compound **8j** as a hydroxylated derivative was also a very safe compound and did not render any significant cytotoxicity toward normal cells. The detrimental effect of the compound **8m** as a nitrated compound was also negligible on healthy cell line. Despite the presence of the nitro group as a susceptible moiety for inducing the general toxicity in this compound, the corresponding cytotoxic effect was not observed on normal cells for it. It's likely the nitrated derivative **8m** produces the cytotoxic effect via the pathways that consist of abnormality in cancerous cells. This statement could be also proposed for other safe compounds that are mentioned above.

Table 3. Cytotoxicity results (IC_{50}) of potent compounds against healthy cell line.

Compound	*R*	IC_{50} (µM)
8a	*2-Cl*	-
8b	*3-Cl*	-
8c	*4-Cl*	67.33
8d	*2-F*	-
8e	*3-F*	44.44
8f	*4-F*	60
8j	*2-OH*	-
8m	*4-NO_2*	67.33

Caspase 3 activation

According to **Fig. 4**, eight of synthesized derivatives were investigated for caspase 3 activity. Fortunately, four out of eight tested compounds were capable to activate the caspase 3. As caspase 3 is a pivotal factor for proceeding apoptosis, the active derivatives could be regarded as likely apoptosis inducer. Compounds **8c** (4-Chlorine) and **8e** (3-Fluorine) were the best activators for caspase 3. These compounds displayed slightly lower enhancement in caspase activity than doxorubicin. Besides, compounds **8d** (2-F) and **8m** (4-NO_2) were also remarkable caspase activators, but their capability was less than doxorubicin to activate this enzyme. Totally, electron withdrawing moieties such as Cl, F and NO_2 may induce better capability in synthesized compounds to activate the caspase 3. This reality was observed noticeably when chlorine and nitro introduced at position *para*. Electronegativity of the substituents may have role in producing this result. But the obtained results declined when the electron withdrawing moieties like Cl and F moved to the *ortho* position. On the other hand, the fluorine did not cause a significant increase in caspase activity when applied at position *para* but better at position *meta*. The mentioned point about fluorine at position *para* was paradoxical compared to Cl and NO_2 at this position. It could be

concluded that electronegativity is not the unique parameter for increasing the caspase 3 activity at position *para* or generally on the phenyl ring. Certainly, the other features of the chlorine atom like lipophilicity or may be electrostatic interactions of the nitro group have a critical role in raising the caspase 3 activity. The low steric effect of the fluorine atom due to the same size to the hydrogen atom as well as the capability for hydrogen bonding interaction may be the main reason for the effectiveness of this substituent at positions *ortho* and *meta*.

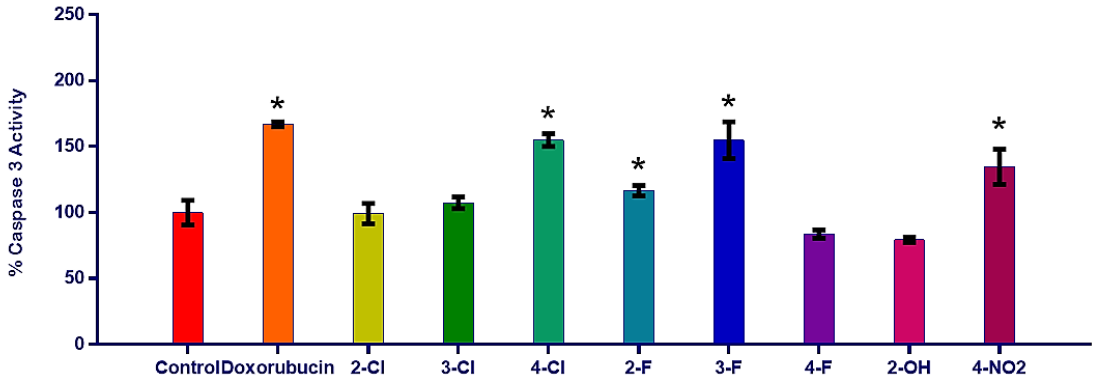


Fig. 4. Activation of caspase 3 by some selected potent compounds.

Mitochondrial membrane potential (MMP)

The measurement of mitochondrial membrane potential (MMP) was carried out and the obtained results were presented in **Fig. 4**. The corresponding test was done for eight selected compounds and four of them demonstrated a significant decline in MMP. The MMP reduction is a marker of apoptosis induction via intrinsic pathway. Fortunately, the compounds that caused caspase 3 activation (**Fig. 3**) were also active in MMP reduction. Compound **8m** that bearing the nitro moiety at *para* site was the best compound in this series for reducing the mitochondrial membrane potential. Compounds **8f** (4-F) and **8m** (4-NO_2) demonstrated more MMP reduction than the reference drug doxorubicin. According to **Fig. 5**, it's obvious that electron-withdrawing moieties (Cl, F, NO_2) cause a beneficial impact on the potency of the tested compound for MMP reduction when utilized at position *para*. The electron-withdrawing effect was not an effective factor when the mentioned moieties moved to the position *ortho*. It means that the electron-withdrawing effects of these moieties are not the lone responsible factors for caspase 3 activation at position *para*. Probably, the electrostatic charge of the nitro group, the hydrogen bonding capability of the fluorine or lipophilicity of the chlorine atom may have an outstanding role in this observation.

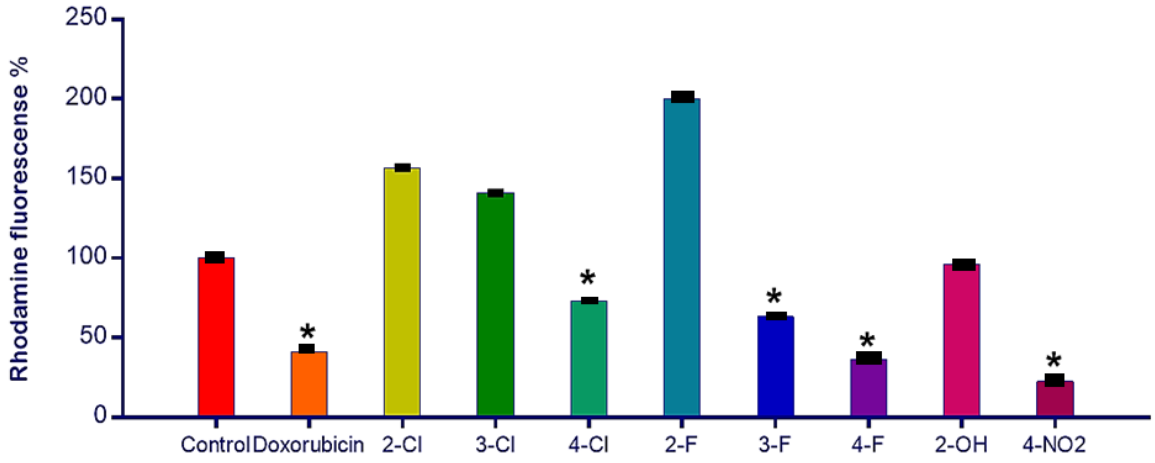


Fig. 5. Mitochondrial membrane potential (MMP) according to the percent of rhodamine fluorescence in potent compounds.

ROS production

Apoptosis inducing activity and promoting the cytotoxic effects could be dependent on the capability for generation of reactive oxygen species. The capability of generation of ROS was explored for eight of the selected potent derivatives and the corresponding results were reported as **Fig. 5**. Seven out of eight tested compounds showed remarkable potency for the generation of ROS compared to control. All of the electron-withdrawing groups (Cl, F, NO_2) caused a strong enhancement in potentiality for ROS generation. The tested compounds are capable of ROS generation and may induce the apoptotic pathway consequently promoting the anticancer activity through this mechanism. An exceptional outcome was afforded for compound **8c** (4-Cl) in this test. Interestingly, compounds **8e** (3-F) and **8j** (2-OH) showed higher capability for ROS generation than doxorubicin as seen in **Fig. 6**, compound 8j as hydroxylated derivative was the best ROS generator in these series. Susceptibility of the hydroxyl group for conversion to the related quinone analog could be suggested as a likely mechanism for the high ability of this moiety in ROS production.

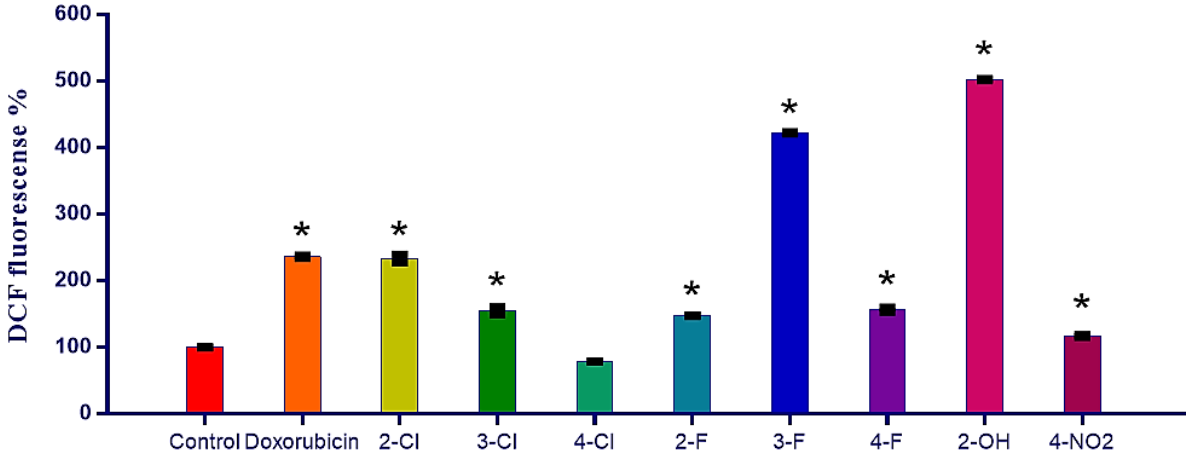


Fig. 6. Generation of reactive oxygen species (ROS) according to the percent of DCF fluorescence.

CONCLUSIONS

According to the positive background of the anticancer activity of the 1,3-thiazole derivatives, a novel series of 1,3-thiazole-based anticancer agents were synthesized and their corresponding anticancer effects were assessed *in vitro*. Some investigated compounds demonstrated superior activity than doxorubicin against Hela and U87 cells. In conclusion, the studied compounds could be proposed as potential lead compounds for developing new anticancer drugs.

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REFERENCES

[1] S.M. El-Messery, G.S. Hassan, F.A.M. Al-Omary, H.I. El-Subbagh, Substituted thiazoles VI. Synthesis and antitumor activity of new 2-acetamido- and 2 or 3-propanamido-thiazole analogs, *European Journal of Medicinal Chemistry*, **54:** 615-625 (2012). doi:10.1016/j.ejmech.2012.06.013.

[2] F. Azmian Moghadam, H. Kefayati, M. Evazalipour, S. Ghasemi, **Design, Synthesis, Biological Evaluation, and Docking Study of Novel 4-Anilinoquinazolines Derivatives as Anticancer Agents**, *Iranian Journal of Chemistry and Chemical Engineering*, 41(2): 353-367 (2022). DOI: **10.30492/IJCCE.2020.132389.4271**.

[3] M.K. MOHAMMADI, S. BOHLULI, A. JAVID, H. TAVAKKOLI, A. GHORBANPOUR; N. RAZZAGHI ASL, **UNVEILING THE FULL POTENTIAL OF 2-ARYL-1H-PHENANTHRO [9,10-D] IMIDAZOLES AS CYTOTOXIC AGENTS VS AGS, HEPG2 AND MCF-7 CELL LINES.** *IRANIAN JOURNAL OF CHEMISTRY AND CHEMICAL ENGINEERING*, 41(2): 455-463 (2022). DOI: **10.30492/IJCCE.2021.116590.3821**

[4] S. Chang, Z. Zhang, X. Zhuang, J. Luo, X. Cao, H. Li, Z. Tu, X. Lu, X. Ren, K. Ding, New thiazole carboxamides as potent inhibitors of Akt kinases, *Bioorganic & Medicinal Chemistry Letter*, **22(2):** 1208-1212 (2012). doi:10.1016/j.bmcl.2011.11.080.

[5] G. Charitos, D.T. Trafalis, P. Dalezis, C. Potamitis, V. Sarli, P. Zoumpoulakis, C. Camoutsis, Synthesis and anticancer activity of novel 3,6-disubstituted 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives, *Arabian Journal of Chemistry*, **12(8):** 4784-4794 (2016). doi:10.1016/j.arabjc.2016.09.015.

[6] S. Li, S. Xu, Y. Tang, S. Ding, J. Zhang, S. Wang, G. Zhou, C. Zhou, X. Li, Synthesis, anticancer activity and DNA-binding properties of novel 4-pyrazolyl-1,8-naphthalimide derivatives, *Bioorganic & Medicinal Chemistry Letter*, **24(2):** 586-590 (2014). doi:10.1016/j.bmcl.2013.12.014.

[7] R. Sadashiva, D. Naral, J. Kudva, S. Madan Kumar, K. Byrappa, R. Mohammed Shafeeulla, M. Kumsi, Synthesis, structure characterization, in vitro and in silico biological evaluation of a new series of thiazole nucleus integrated with pyrazoline scaffolds, *Journal of Molecular Structure*, **1145:** 18-31 (2017). doi:10.1016/j.molstruc.2017.05.066.

[8] S. Mirza, S. Asma Naqvi, K. Mohammed Khan, U. Salar, M.I. Choudhary, Facile synthesis of novel substituted aryl-thiazole (SAT) analogs via one-pot multi-component reaction as potent cytotoxic agents against cancer cell lines, *Bioorganic Chemistry*, **70:** 133-143 (2017). doi:10.1016/j.bioorg.2016.12.003.

[9] A.R. Ali, E.R. El-Bendary, M.A. Ghaly, I.A. Shehata, Synthesis, in vitro anticancer evaluation and in silico studies of novel imidazo[2,1-b]thiazole derivatives bearing pyrazole moieties, *European Journal of Medicinal Chemistry*, **75:** 492-500 (2017). doi:10.1016/j.ejmech.2013.12.010.

[10] G. Turan-Zitouni, M.D. Altlntop, A. Özdemir, Z.A. Kaplanclkll, G.A. Çiftçi, H.E. Temel, Synthesis and evaluation of bis-thiazole derivatives as new anticancer agents, *European Journal of Medicinal Chemistry*, **107:** 288-294 (2016). doi:10.1016/j.ejmech.2015.11.002.

[11] A. Ayati, S. Emami, A. Asadipour, A. Shafiee, A. Foroumadi, Recent applications of 1,3-thiazole core structure in the identification of new lead compounds and drug discovery, *European Journal of Medicinal Chemistry*, **97:** 699-718 (2015). doi:10.1016/j.ejmech.2015.04.015.

[12] F. Cagide, F. Borges, L.R. Gomes, J.N. Low, Synthesis and characterisation of new 4-oxo-N-(substituted-thiazol-2-yl)-4H-chromene-2-carboxamides as potential adenosine receptor ligands, *Journal of Molecular Structure*, **1089:** 206-215 (2015). doi:10.1016/j.molstruc.2015.02.009.

[13] N. Siddiqui, M.F. Arshad, W. Ahsan, M.S. Alam, Thiazoles: A Valuable Insight into the Recent Advances and Biological Activities, *International Journal of Pharmaceutical Sciences & Drug Research*, **1(3):** 136-

143 (2009). doi:10.1002/jemt.20664.

[14] M.S. Al-Saadi, H.M. Faidallah, S.A.F. Rostom, Synthesis and biological evaluation of some 2,4,5-trisubstituted thiazole derivatives as potential antimicrobial and anticancer agents, *Archiv Der Pharmazie*, (Weinheim). **341(7):** 424-434 (2008). doi:10.1002/ardp.200800026.

[15] A. Rouf, C. Tanyeli, Bioactive thiazole and benzothiazole derivatives, *European Journal of Medicinal Chemistry*, **97:** 911-927 (2015). doi:10.1016/j.ejmech.2014.10.058.

[16] H. He, X. Wang, L. Shi, W. Yin, Z. Yang, H. He, Y. Liang, Synthesis, antitumor activity and mechanism of action of novel 1,3-thiazole derivatives containing hydrazide-hydrazone and carboxamide moiety, *Bioorganic & Medicinal Chemistry Letter*, **26(14):** 3263-3270 (2016). doi:10.1016/j.bmcl.2016.05.059.

[17] E.B. Veale, J.E. O'Brien, T. McCabe, T. Gunnlaugsson, The synthesis, N-alkylation and epimerisation study of a phthaloyl derived thiazolidine, *Tetrahedron*, **64(28):** 6794-6800 (2008). doi:10.1016/j.tet.2008.04.097.

[18] D. Vogt, J. Weber, K. Ihlefeld, A. Brüggerhoff, E. Proschak, H. Stark, Design, synthesis and evaluation of 2-aminothiazole derivatives as sphingosine kinase inhibitors, *Bioorganic & Medicinal Chemistry*, **22(19):** 5354-5367 (2014). doi:10.1016/j.bmc.2014.07.044.

[19] T. Prashanth, P. Thirusangu, B.R. Vijay Avin, V. Lakshmi Ranganatha, B.T. Prabhakar, S.A. Khanum, Synthesis and evaluation of novel benzophenone-thiazole derivatives as potent VEGF-A inhibitors, *European Journal of Medicinal Chemistry*, **87:** 274-283 (2014). doi:10.1016/j.ejmech.2014.09.069.

[20] G.S. Hassan, S.M. El-Messery, F.A.M. Al-Omary, H.I. El-Subbagh, Substituted thiazoles VII. Synthesis and antitumor activity of certain 2-(substituted amino)-4-phenyl-1,3-thiazole analogs, *Bioorganic & Medicinal Chemistry Letter*, **22(20):** 6318-6323 (2012). doi:10.1016/j.bmcl.2012.08.095.

[21] K.Z. Łączkowski, J. Anusiak, M. Świtalska, K. Dzitko, J. Cytarska, A. Baranowska-Łączkowska, T. Plech, A. Paneth, J. Wietrzyk, J. Białczyk, Synthesis, molecular docking, ctDNA interaction, DFT calculation and evaluation of antiproliferative and anti-Toxoplasma gondii activities of 2,4-diaminotriazine-thiazole derivatives, *Medicinal Chemistry Research*, **27:** 1131-1148 (2018). doi:10.1007/s00044-018-2136-6.

[22] R. Romagnoli, P.G. Baraldi, M.K. Salvador, M.E. Camacho, D. Preti, M.A. Tabrizi, M. Bassetto, A. Brancale, E. Hamel, R. Bortolozzi, G. Basso, G. Viola, Synthesis and biological evaluation of 2-substituted-4-(3',4',5'-trimethoxyphenyl)-5-aryl thiazoles as anticancer agents, *Bioorganic & Medicinal Chemistry*, **20(24):** 7083-7094 (2012). doi:10.1016/j.bmc.2012.10.001.

[23] A.R. Ali, E.R. El-Bendary, M.A. Ghaly, I.A. Shehata, Novel acetamidothiazole derivatives: Synthesis and in vitro anticancer evaluation, *European Journal of Medicinal Chemistry*, **69:** 908-919 (2013). doi:10.1016/j.ejmech.2013.08.021.

[24] A. Reichelt, J.M. Bailis, M.D. Bartberger, G. Yao, H. Shu, M.R. Kaller, J.G. Allen, M.F. Weidner, K.S. Keegan,

J.H. Dao, Synthesis and structure-activity relationship of trisubstituted thiazoles as Cdc7 kinase inhibitors, *European Journal of Medicinal Chemistry*, **80:** 364-382 (2014). doi:10.1016/j.ejmech.2014.04.013.

[25] T.A. Farghaly, W.A. Al-Hasani, M.H. Ibrahim, M.H. Abdellattif, Z.A. Abdallah, Design, Synthesis, Anticancer Activity and Docking Studies of Thiazole Linked Phenylsulfone Moiety as Cyclin-Dependent Kinase 2 (CDK2) Inhibitors, *Polycyclic Aromatic Compounds*, **43(6):** 5001-5020 (2023). doi.org/10.1080/10406638.2022.2097715.

[26] Finiuk, N., Zelisko, N., Klyuchivska, O., Yushyn, I., Lozynskyi, A., Cherniienko, A., Manko, N., Senkiv, J., Stoika, R. and Lesyk, R., Thiopyrano [2, 3-*d*] thiazole structures as promising scaffold with anticancer potential. *Chemico-Biological Interactions*, **368:** 110246 (2022). doi.org/10.1016/j.cbi.2022.110246.

[27] Ibrahim Taha Radwan, Ahmed H.M. Elwahy, Ahmed F. Darweesh, Marwa Sharaky, Noha Bagato, Hanem Fathy Khater, Mostafa E. Salem, Design, synthesis, docking study, and anticancer evaluation of novel bis-thiazole derivatives linked to benzofuran or benzothiazole moieties as PI3k inhibitors and apoptosis inducers, *Journal of Molecular Structure*, **1265:** 133454 (2022), https://doi.org/10.1016/j.molstruc.2022.133454.

[28] M.S. Raghu, H.A. Swarup, T. Shamala, B.S. Prathibha, K. Yogesh Kumar, Fahd Alharethy, M.K. Prashanth, Byong-Hun Jeon, Design, synthesis, anticancer activity and docking studies of novel quinazoline-based thiazole derivatives as EGFR kinase inhibitors, *Heliyon*, **9(9):** e20300 (2023), doi.org/10.1016/j.heliyon.2023.e20300.

[29] I. Ivasechko, A. Lozynskyi, J. Senkiv, P. Roszczenko, Y. Kozak, N. Finiuk, O. Klyuchivska, N. Kashchak, N. Manko, Z. Maslyak, D. Lesyk, A. Karkhut, S. Polovkovych, R. Czarnomysy, O. Szewczyk, A. Kozytskiy, O. Karpenko, D. Khyluk, A. Gzella, K. Bielawski, A. Bielawska, P. Dzubak, S. Gurska, M. Hajduch, R. Stoika, R. Lesyk, Molecular design, synthesis and anticancer activity of new thiopyrano[2,3-*d*]thiazoles based on 5-hydroxy-1,4-naphthoquinone (juglone), *European Journal of Medicinal Chemistry*, **252:** 115304 (2023), doi.org/10.1016/j.ejmech.2023.115304.

[30] S. H. L. Kok, R. Gambari, C. H. Chui, M. C. W. Yuen, E. Lin, R. S. M. Wong, F. Y. Lau, G. Y. M. Cheng, W. S. Lam, S. H. Chan, K. H. Lam, C. H. Cheng, P. B. S. Lai, M. W. Y. Yu, F. Cheung, J. C. On Tang, A. S. C. Chan, Synthesis and anti-cancer activity of benzothiazole containing phthalimide on human carcinoma cell lines, *Bioorganic & Medicinal Chemistry*, **16(7):** 3626-3631 (2008), doi.org/10.1016/j.bmc.2008.02.005.

[31] S.S. Arbuj, S.B. Waghmode, A.V. Ramaswamy, Photochemical α-bromination of ketones using *N*-bromosuccinimide: a simple, mild and efficient method, *Tetrahedron Lett*, **48(8):** 1411-1415 (2007). doi:10.1016/j.tetlet.2006.12.101.

[32] M. Khajouei, A. Mohammadi-Farani, A. Moradi, A. Aliabadi, Synthesis and evaluation of anticonvulsant activity of (*Z*)-4-(2-oxoindolin-3-ylideneamino)-*N*-phenylbenzamide derivatives in mice, *Research in Pharmaceutical Sciences*, **13(3):** 262-272 (2018). doi:10.4103/1735-5362.228956.

[33] M. Wang, Y. Ruan, Q. Chen, S. Li, Q. Wang, J. Cai, Curcumin induced HepG2 cell apoptosis-associated mitochondrial membrane potential and intracellular free Ca^{2+} concentration, *European Journal of Pharmacology*, 650(1) 41-47 (2011). doi:10.1016/j.ejphar.2010.09.049.

[34] D. J. Choi, S. Kim, J. W. Choi, Y. I. Park, Neuroprotective effects of corn silk maysin via inhibition of H_2O_2-induced apoptotic cell death in SKNMC cells, *Life Sciences*, 109(1) (2014) 57-64. doi:10.1016/j.lfs.2014.05.020.