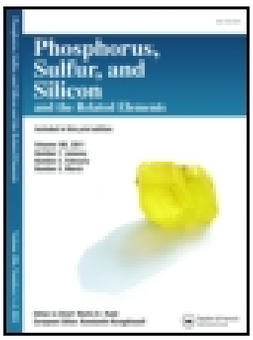

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Synthesis and biological evaluation of 5-methyl-4-phenyl thiazole derivatives as anticancer agents

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ABSTRACT

New *N*-(5-methyl-4-phenylthiazol-2-yl)-2-(substituted thio)acetamides were synthesized and studied for their anticancer activity. The title compounds were procured by reacting 2-chloro-*N*-(5-methyl-4-phenylthiazol-2-yl)acetamide with some mercapto derivatives. The structural elucidation of the compounds was performed by ^{1}H-NMR, ^{13}C-NMR and LC-MS/MS spectral data and elemental analyses. The synthesized compounds were investigated for their antitumor activities against A549 human lung adenocarcinoma cells and NIH/3T3 mouse embryoblast cell line for determining their selective cytotoxicity. 2-[(1-methyl-1*H*-tetrazol-5-yl)thio]-*N*-(5-methyl-4-phenylthiazol-2-yl)acetamide (**4c**) showed high selectivity, and whose IC_{50} value was determined as 23.30 ± 0.35 μM and >1000 μM against A549 human lung adenocarcinoma cells and NIH/3T3 mouse embryoblast cell lines, respectively. 2-[(1-Methyl-1*H*-imidazol-2-yl)thio]-N-(5-methyl-4-phenyl thiazol-2-yl)acetamide (**4a**) and 2-[(1-Methyl-1*H*-tetrazol-5-yl)thio]-N-(5-methyl-4-phenyl thiazol-2-yl)acetamide (**4c**) exhibited the highest apoptosis percentage among those tested, but not as high as the standard, cisplatin.

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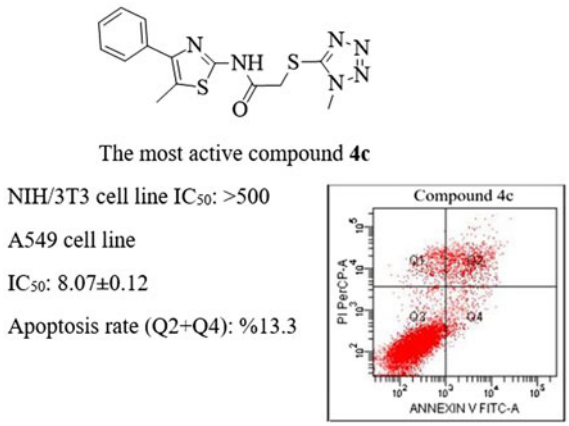
Azoles; anticancer activity; thiazole; apoptosis

GRAPHICAL ABSTRACT



Introduction

Cancer has been described as a complex disease which includes over 100 various diseases caused by the uncontrolled mitosis of the body's cells and that evolves in time.[1] Even though cancer can progress in virtually any of the body's tissues, and each type of cancer has its unique features, the essential processes that produce cancer are fairly similar in all forms of the disease.[2,3]

In recent years, anticancer therapies have made extraordinary progress after the approval of some small molecule inhibitors. But the incidence of cancer continuously rises, and the misdiagnosis or the misapplicaiton of treatments makes the patient's condition worse in many cases. Therefore, the control of malignancies is a massive concern.[4–8] About 12.7 million cancer cases and 7.6 million cancer deaths were reported worldwide in 2008. Generally, 56% of the cancer cases and 64% of the deaths due to cancer were in economically developing countries. Lung cancer in males is one of the commonly diagnosed cancer. Also, lung cancer is the leading cause of the death for each sex in both economically developed and developing countries. In 2035, the estimated death number due to lung cancer may be about 2.9 million cases, and the estimated

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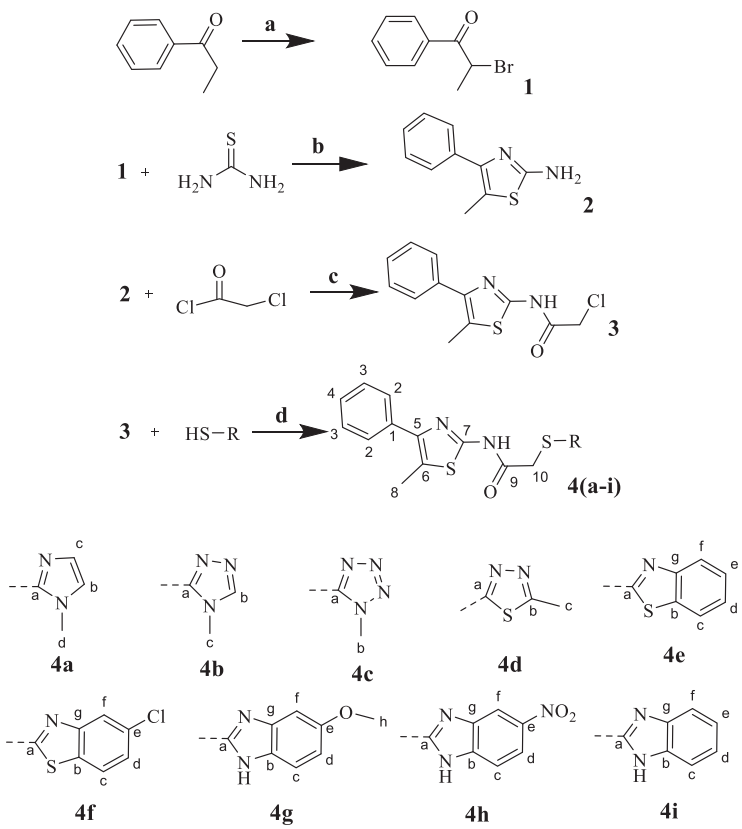

Scheme 1. The synthesis diagram of the compounds **4a–4i.** *Reagents and conditions*: **(a)** Bromine, CH_3COOH, HBr **(b)** EtOH, r.t., 24 h; **(c)** TEA, THF, $ClCOCH_2Cl$, 0–5 °C, then r.t 3 h; **(d)** Acetone, Potassium carbonate, rt.

new lung cancer cases is expected to be over 3.3 million with reference to cancer data of 2012.[9–11]

Cytotoxic chemotherapy agents have powerful anticancer activity and they are used in many treatments. Although there are several active agents used clinically against tumors, most chemotherapy protocols do not effectively increase the patient survival. Even though many patients respond to treatment well at the beginning, they go on to develop resistance against the chemotherapeutic which can cause the treatment to fail with a poorer prognosis. These issues are announced by several reports that stated the resistance developing against the therapeutic agents.[12–18] Thus, a goal of researchers is to execute the selective inactivation of the DNA in tumor cells with the agents which have small structure.[19–21]

Among various heterocyclic rings, the thiazole ring represents a leading molecule in medicinal chemistry and its derivatives have been used in different studies. Recently activity studies of thiazole ring and derivatives have focused on various biological properties such as antifungal,[22–24] antibacterial,[25] antiviral,[26–28] analgesic,[29] anticancer,[30] antihypertensive,[31] and antiproliferative[32,33] activities. Additionally, the thiazole ring plays an important role in many anticancer pathways such as inhibition of tyrosine kinase,[34] peroxisome proliferator activated receptor gamma (PPARy),[35,36] tumor necrosis factor (TNF-α),[37] inhibition of phosphatidylinositol 3-kinase (PI3K),[38,39] and inhibition of c-Jun N-terminal kinase stimulatory phosphatase-1 (JSP-1).[40] Furthermore, recent clinically used anticancer drugs tiazofurin and BMS-354825;[41,42] antidiabetic drug troglitazone[43] possess a thiazole nucleus. Moreover, the anticancer activity of some synthesized thiazole derivatives was accrued *in vitro* against A549 human lung adenocarcinoma cells in previous studies.[44–46]

Also, azole derivatives like imidazole, triazole, tetrazole, thiadiazole, benzimidazole and benzothiazole have been utilized for their high anticancer activities in earlier studies.[47–54] In these study, the anticancer activity of the 1,5-disubstituted tetrazole moiety has been reported. Here, the researchers have found a way that breaking the resistance of some cancer cell lines, such as A549-T12 which is a cell line with an α-tubulin mutation with increased resistance to Taxol.[55–58]

Based on the above information, we synthesized some new 5-methyl-4-phenyl thiazole derivatives in this work. There are nine compounds (**4a–4i**), and we investigated their anticancer activity against A549 human lung adenocarcinoma cells and NIH/3T3 mouse embryoblast cell line for determining their selective cytotoxicity. Also, apoptotic cell deaths caused by the most cytotoxic compounds were determined.

Results and discussion

Chemistry

In this study, we synthesized nine new compounds which included the *N*-(5-methyl-4-phenylthiazol-2-yl)acetamide nucleus in their core structures. The synthesis reaction was

performed via four steps. In the first step, propiophenone and bromine were reacted in acetic acid to get 2-bromo-1-phenylpropanone (**1**).[59] Then compound **1** and thiourea was reacted at room temperature for ring closure to obtain 2-amino-4-phenyl-5-methylthiazole (**2**).[60] Compound (**2**) was acetylated with chloroacetyl chloride pursuant to method.[61] Finally, the obtained product 2-chloro-*N*-(5-methyl-4-phenylthiazol-2-yl)acetamide (**3**) was reacted with 2-mercapto azole derivatives to gain the final products *N*-(5-methyl-4-phenylthiazol-2-yl)-2-(substituted mercapto)acetamide (**4a–4i**) as shown in **Scheme 1**. All the synthesized compounds were characterized by analytical and spectral data.

The ^{1}H-NMR spectra of compounds showed signals at δ 2.45–2.47 ppm (CH_3) for 5-methyl thiazole protons which were singlet peaks. Acetyl protons were observed at δ 3.95–4.48 ppm (CH_2) as a singlet and a broad singlet peak seen at δ 12.38–12.78 ppm indicated the acetamide N–H proton. The appearance of a pair of singlet, doublets, triplets and/or multiplets at δ 6.75–8.57 ppm was due to the aromatic protons of the aromatic rings. The ^{13}C-NMR spectra of compounds showed signals at δ 35.20–37.41 ppm for methylene carbon (CH_2), at δ 12.32–12.34 ppm for 5-methyl thiazole carbon (CH_3), at δ 94.89–165.93 ppm for aromatic carbon and at δ 165.88–170.00 for carbonyl (C = O) carbon. M + 1 peaks in LC-MS/MS spectra were in agreement with the calculated molecular weight of the target compounds (**4a–4i**). Elemental analysis results for C, H and N elements were in agreement to the calculated values for the synthesized compounds.

Anticancer activity

The cytotoxicity assays and determination of IC_{50} doses of cisplatin and all compounds against A549 and NIH/3T3 cell were performed by MTT assay (as shown in Table S1 Supplemental Materials). The formazan formation was quantified spectrophotometrically at 540 nm using a microplate reader. Relating to the assay results, all compounds have showed high anticancer activity. Additionally, compounds **4a–4f** displayed stronger activity than cisplatin against A549 cell line.

Compounds **4a, 4b, 4c, 4d, 4e** and **4f** were significantly more active than the reference drug (cisplatin). Among them, compounds **4a, 4c** and **4e** were determined to be 7 times more active than cisplatin whereas compound **4b** was found to be 5 times more active than cisplatin. At most compounds **4d** and **4f** were found 15 times more active than cisplatin. whereas compounds **4g** and **4h** exhibited lower activity than cisplatin. The IC_{50} values of synthesized compounds and reference drug were given in **Table S 1**.

When evaluated according to the toxicity of the compounds, compound **4c** showed highly cytotoxic effect against A549 cells (IC_{50}= 23.30 ± 0.35 μM) and it did not show cytotoxic activity against NIH/3T3 cells (IC_{50}>1000 μM). Similarly, compounds **4b**, **4d** and **4f** showed selective cytotoxicity against A549 cells. On the other hand, for compounds **4a, 4e** and **4h,** the difference between the concentrations was close, but still the required IC_{50} value for A549 cells is smaller than the required IC_{50} value for NIH/3T3. It means that the required cytotoxic doses were found to be nontoxic against NIH/3T3 cells. On the contrary, compound **4g** was determined with nonselective profile which displayed cytotoxicity against both of the tumor and normal cell lines (IC_{50}=353.22 ± 12.18 μM against A549 and IC_{50}=77.15 ± 9.84 μM against NIH/3T3), which indicates that the required therapeutic dose was found to be cytotoxic against NIH/3T3 cells.

After determination of the most cytotoxic compounds, their apoptotic effect was investigated. The apoptotic effects of compounds **4a–4f** were analyzed for A549 human lung adenocarcinoma based on Annexin V-PI binding capacities in flow cytometry as to their IC_{50} values. Early and late apoptotic rates of compounds were given in Table S2 (Supplemental Materials) as percentages. Also, early and late apoptotic rates by flow cytometric analysis against both cell lines portrait were shown in Figure S28. Especially, compounds **4b**, **4e** and **4f** killed a high percentage of A549 cells by the way of necrosis. On the contrary, compounds **4a** and **4c** have killed A549 cells by the way of apoptosis.

Results of ADME parameters and rule of five

Log P values were found between 1.99-5.30. Drug-likeness model scores (DLMS) were found between −0.30 and 1.05. Especially, the DLM score for the most active compounds **4a, 4b, 4c, 4d, 4e** and **4f** were found 0.86, 1.05, 0.69, 0.55, 0.54 and 0.73. The number of hydrogen bond donor (HBD) was stated for **4g**, **4h** and **4i** as 2 and for the rest of them as 1. Also, the number of hydrogen bond acceptor (HBA) was determined as maximal 7 for all compounds. Molecular volume was calculated between 285.75 and 346.66 A^3. All findings are in accord with the range according to Lipinski's rule of five. Even if there is no exact finding in practice, these results are in agreement with the active compounds. Additionally, these findings are making them oral anticancer agents. Thus, it may be said that synthesized compounds may have a good pharmacokinetic profile. All findings were shown in Table S3.

These results point out that 2-substituted-*N*-(5-methyl-4-phenylthiazol-2-yl)acetamide nucleus showed good anticancer activity. The highest activity by the way of apoptosis was observed when the functional group substitutions are smaller such as in compounds **4a–4d**. The compounds bearing a benzothiazole nucleus were found to be more effective than the compounds bearing a benzimidazole nucleus. Besides, the presence of an electron withdrawing group substitution on the benzothiazole or the absence of any substitution on the benzimidazole increased the activity. But their selective apoptotic anticancer activity was established to be inadequate compared to the five-membered ring systems.

In general, compounds **4a** (1-methyl-1*H*-imidazol-2-yl) and **4c** (1-methyl-tetrazol-2-yl) were found to be more suited as chemotherapeutic agents against A549 human lung adenocarcinoma cells. Most particularly, compound **4c** was superior to **4a** due to apoptosis rates and can be considered

for several reasons. Firstly, compound **4c** has smaller molecular volume than the others, and it has low molecular weight. Also, it has low partition constant (Log P = 1.99). All these parameters can positively affect the solubility. In fact, one of the major influences may be breaking the developed resistance by 1,5-disubstituted tetrazole moiety.[55] Therefore compound **4c** showed a potent anti-lung cancer activity as it may increase the chance of breaking a drug resistance development.

Experimental

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). All melting points (m.p.) were determined by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were uncorrected. All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: ^{1}H NMR (nuclear magnetic resonance) Bruker DPX 300 FT-NMR spectrometer, ^{13}C NMR, Bruker DPX 75 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) M + 1 peaks were determined by Shimadzu 8040 LC/MS/MS system (Shimadzu, Tokyo, Japan). Elemental analyses were performed on a Leco 932 CHNS analyzer (Leco, Michigan, USA). The Supplemental Materials file contains sample ^{1}H and ^{13}C NMR Spectra for products **4** (Figures S1–S27).

Materials and methods

General synthesis of 2-bromo-1-phenylpropane (1)[59]

In a flask, propiophenone (9.39 g, 0.07 mol) was dissolved in acetic acid and hydrobromic acid was added as a catalyst. The bromine (13.42 g, 0.084 mol) was dissolved in acetic acid. Reaction temperature was set to 0–5 °C. The bromine solution from the dropping funnel was carefully added dropwise, considering the color change. The mixture was stirred at room temperature until the reaction is finished which was checked by TLC. After the reaction was completed, the mixture was poured into ice-water and the reaction mixture was neutralized with saturated $NaHCO_3$ solution. Extraction was performed with dichloromethane (DCM) (3 × 100 mL). Combined organics was dried over Na_2SO_4 and evaporated *in vacuo* to give the title product.

Synthesis of 2-amino-4-phenyl-5-methylthiazole (2)[60]

2-Bromo-1-phenylpropanone (12.78 g, 0.06 mol) and thiourea (4.57 g, 0.06 mol) were dissolved in ethanol and mixed at room temperature for 24 h. The completion of the reaction was controlled by the TLC. The mixture was filtered and obtained the raw product.

Synthesis of 2-chloro-N-(5-methyl-4-phenylthiazol-2-yl) acetamide (3)[61]

In an ice bath, 2-amino-4-phenyl-5-methylthiazole (40 mmol) was dissolved in tetrahydrofuran (THF) and triethylamine (48 mmol) was added into the flask. 2-Chloroacetylchloride (48 mmol) was dissolved in THF and the solution was added to the dropping funnel. This solution was added dropwise gradually to the flask. After the dropping is over the mixture was stirred at room temperature for 3 h. The completion of the reaction was monitored with the help of TLC. The reaction solvent was evaporated, and the crude product was washed with water and filtered.

Synthesis of N-(5-methyl-4-phenylthiazol-2-yl)-2-(substituted thio) acetamide derivatives (4a–i)

2-Chloro-*N*-(5-methyl-4-phenylthiazol-2-yl)acetamide and equal mole 2-mercapto aryl derivatives were reacted in the presence of potassium carbonate in acetone. The completion of the reaction was monitored with the help of TLC. From the reaction mixture, the solution was evaporated and treated with water to yield the final products. The final products (**4a–4i**) were recrystallized from ethanol.

2 -[(1-Methyl-1H-imidazol-2-yl)thio]-N-(5-methyl-4-phenylthiazol-2-yl)acetamide (4a)

m. p. 157-159 °C, Yield: 73%, light-brown powder, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.46 (s, 3H, CH_3), 3.60 (s, 3H, imidazole-CH_3), 3.95 (s, 2H, CH_2), 6.95 (d, J = 1.20 Hz, H, Ar-H), 7.27 (d, J = 1.20 Hz, H, Ar-H), 7.31–7.38 (m, H, Ar-H), 7.42–7.47 (m, 2 H, Ar-H), 7.61-7.62 (m, H, Ar-H), 7.65 (d, J = 1.45 Hz, H, Ar-H), 12.39 (brs, H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.32 (C-8), 33.47 (C-d), 37.41 (C-10), 121.78 (C-6), 124.16 (C-b), 127.74 (C-2), 128.42(C-4), 128.84 (C-3), 129.13 (C-c), 135.27 (C-1), 144.66 (C-5), 144.81 (C-a), 154.12 (C-7), 167.28 (C-9). For $C_{16}H_{16}N_4OS_2$ calculated: Elem. Anal.: 55.79% C, 4.68% H, 16.27% N, found: 55.78% C, 4.67% H, 16.28% N. HRMS (m/z): $[M+1]^+$ calculated 345.0838; found 345.0830.

N-(5-Methyl-4-phenylthiazol-2-yl)-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetamide (4b)

m. p. 193–195 °C, Yield: 80%, off-white powder, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.46 (s, 3H, CH_3), 3.60 (s, 3H, triazole-CH_3), 4.12 (s, 2H, CH_2), 7.32–7.37 (m, H, Ar-H), 7.43–7.47 (m, 2H, Ar-H), 7.62–7.65 (m, 2H, Ar-H), 8.57 (s, H, Ar-H), 12.38 (brs, H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.32 (C-8), 31.32 (C-c), 36.69 (C-10), 121.84 (C-6), 127.76 (C-2), 128.42 (C-4), 128.85 (C-3), 135.25 (C-1), 144.70 (C-5), 146.81 (C-b), 148.77 (C-a), 154.09 (C-7), 166.63 (C-9). For $C_{15}H_{15}N_5OS_2$ calculated: Elem. Anal.: 52.16% C; 4.38% H, 20.27% N. found: 52.17% C, 4.39% H, 20.26% N. HRMS (m/z): $[M+1]^+$ calculated 346.0783; found 346.0780.

2 -[(1-Methyl-1H-tetrazol-5-yl)thio]-N-(5-methyl-4-phenylthiazol-2-yl)acetamide (4c)

m. p. 196-168 °C, Yield: 82%, brown solid, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.47 (s, 3H, CH_3), 3.99 (s, 3H, CH_3), 4.35 (s, 2H, CH_2), 7.36 (brs, H, Ar-H), 7.46 (brd, J = 4.05 Hz, 2H, Ar-H), 7.64 (brs, 2H, Ar-H), 12.48 (H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.33 (C-8), 34.20 (C-b), 36.61 (C-10), 121.95 (C-6), 127.89 (C-2), 128.43(C-4), 128.86 (C-3), 135.22 (C-1), 144.75 (C-5), 153.55 (C-a), 154.03 (C-7), 165.88 (C-9). For $C_{14}H_{14}N_6OS_2$ calculated: Elem. Anal.: 48.54% C, 4.07% H, 24.26% N, found: 48.53% C, 4.08% H, 24.27% N. HRMS (m/z): $[M+1]^+$ calculated 347.0743; found 347.0730.

2 -[(5-Methyl-1,3,4-thiadiazol-2-yl)thio]-N-(5-methyl-4-phenylthiazol-2-yl)acetamide (4d)

m. p. 204-206 °C, Yield: 76%, off-white solid, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.47 (s, 3H, CH_3), 2.67 (s, 3H, thiadiazole-CH_3), 4.35 (s, 2H, CH_2), 7.32–7.38 (m, H, Ar-H), 7.43–7.48 (m, 2H, Ar-H), 7.63–7.66 (m, 2H, Ar-H), 12.47 (brs, H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.33 (C-8), 15.66 (C-c), 37.08 (C-10), 121.90 (C-6), 127.78 (C-2), 128.43 (C-4), 128.86 (C-3), 135.24 (C-1), 144.73 (C-5), 154.09 (C-7), 164.36 (C-b), 166.11 (C-a), 166.38 (C-9). For $C_{15}H_{14}N_4OS_3$ calculated: Elem. Anal.: 49.70% C, 3.89% H, 15.46% N, found: 49.71% C, 3.88% H, 15.45% N. HRMS (m/z): $[M+1]^+$ calculated 363.0403; found 363.0403.

2 -(Benzothiazol-2-ylthio)-N-(5-methyl-4-phenylthiazol-2-yl)acetamide (4e)

m. p. 139-141 °C, Yield: 78%, brown powder, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.46 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 7.34-7.39 (m, 2H, Ar-H), 7.43-7.49 (m, 3H, Ar-H), 7.64-7.67 (m, 2 H, Ar-H), 7.81 (d, J = 7.57 Hz, H, Ar-H), 8.01-8.04 (m, H, Ar-H), 12.54 (brs, H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.33 (C-8), 36.69 (C-10), 121.62 (C-c or C-f), 121.92 (C-6), 122.39 (C-c or C-f), 125.07 (C-d or C-e), 126.91 (C-d or C-e), 127.78 (C-2), 128.44 (C-4), 128.87 (C-3), 135.25 (C-1), 135.30 (C-b), 144.73 (C-5), 152.94 (C-g), 154.13 (C-7), 166.10 (C-a) and (C-9). For $C_{19}H_{15}N_3OS_3$ calculated: Elem. Anal.: 57.41% C, 3.80% H, 10.57% N, found: 57.40% C, 3.81% H, 10.58% N. HRMS (m/z): $[M+1]^+$ calculated 398.0450; found 398.0440.

2 -[(5-Chlorobenzothiazol-2-yl)thio]-N-(5-methyl-4-phenylthiazol-2-yl)acetamide (4f)

m. p. 165-166 °C, Yield: 79%, light brown powder, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.46 (s, 3H, CH_3), 4.48 (s, 2H, CH_2), 7.33–7.38 (m, H, Ar-H), 7.41–7.49 (m, 3H, Ar-H), 7.63–7.67 (m, 2H, Ar-H), 7.88 (d, J = 2.00 Hz, H, Ar-H), 8.07 (d, J = 8.59 Hz, H, Ar-H), 12.55 (brs, H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.34 (C-8), 36.79 (C-10), 121.05 (C-c or C-f),121.95 (C-6), 123.85 (C-c or C-f), 125.07 (C-d), 127.79 (C-2), 128.43 (C-4), 128.87(C-3), 131.72 (C-e), 134.10 (C-b), 135.23 (C-1), 144.75 (C-5), 153.83 (C-g), 154.11 (C-7), 165.93 (C-a), 169.09 (C-9). For $C_{19}H_{14}ClN_3OS_3$ calculated: Elem. Anal.: 52.83% C, 3.27% H, 9.73% N, found: 52.84% C, 3.28% H, 9.74% N. HRMS (m/z): $[M+1]^+$ calculated 432.0060; found 432.0059.

2 -[(5-Methoxy-1H-benzimidazol-2-yl)thio]-N-(5-methyl-4-phenylthiazol-2-yl)acetamide (4g)

m. p. 94-96 °C, Yield: 73%, brown powder, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.45 (s, 3H, CH_3), 3.75 (s, 3H, O-CH_3), 4.31 (s, 2H, CH_2), 6.75 (dd, J_1=2.45 Hz, J_2=8.72 Hz, H, Ar-H), 6.96 (d, J = 2.35 Hz, H, Ar-H), 7.32-7.37 (m, 2H, Ar-H), 7.43-7.48 (2H, Ar-H), 7.63-7.66 (m, 2H, Ar-H), 11.31 (brs, H, benzimidazole-NH), 12.41 (brs, H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.33 (C-8), 35.43 (C-10), 55.92 (C-h), 94.89 (C-f), 110.31 (C-c or C-d), 111.01 (C-c or C-d), 121.72 (C-6), 127.73 (C-2), 128.42 (C-4), 128.57 (C-b), 128.84 (C-3), 133.52 (C-g), 135.31 (C-1), 144.65 (C-5), 148.73 (C-a), 154.33 (C-7), 155.80 (C-e), 167.03 (C-9). For $C_{20}H_{18}N_4O_2S_2$ calculated: Elem. Anal.: 58.52% C, 4.42% H, 13.65% N, found: 58.53% C, 4.41% H, 13.66% N. HRMS (m/z): $[M+1]^+$ calculated 411.0944; found 411.0928.

N-(5-Methyl-4-phenylthiazol-2-yl)-2-[(5-nitro-1H-benzimidazol-2-yl)thio]acetamide (4h)

m. p. 191-193 °C, Yield: 72%, deep brown powder, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.45 (s, 3H, CH_3), 4.40 (s, 2H, CH_2), 7.13–7.37 (m, H, Ar-H), 7.43–7.48 (m, 2H, Ar-H), 7.57 (d, J = 8.86 Hz, H, Ar-H), 7.63–7.66 (m, 2H, Ar-H), 8.02 (dd, J_1=2.29, J_2=8.86, H, Ar-H), 8.29 (d, J = 2.19, H, Ar-H), 11.88 (brs, H, benzimidazole-NH), 12.78 (brs, H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.33 (C-8), 35.20 (C-10), 110.77 (C-f), 113.98 (C-c), 117.59 (C-d), 121.79 (C-6), 127.75 (C-2), 128.42 (C-4), 128.84 (C-3), 135.27 (C-1), 140.67 (C-g), 142.21 (C-e), 144.68 (C-5), 145.37 (C-b), 154.19 (C-7), 157.23 (C-a), 166.75 (C-9). For $C_{19}H_{15}N_5O_3S_2$ calculated: Elem. Anal.: 53.64% C, 3.55% H, 16.46% N, found: 53.65% C, 3.56% H, 16.45% N. HRMS (m/z): $[M+1]^+$ calculated 426.0689; found 426.0673.

2 -[(1H-benzimidazol-2-yl)thio]-N-(5-methyl-4-phenylthiazol-2-yl)acetamide (4i)

m. p. 122-124 °C, Yield: 70%, light brown powder, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 2.45 (s, 3H, CH_3), 4.36 (s, 2H, CH_2), 7.10–7.13 (m, 3H, Ar-H), 7.43–7.48 (m, 4H, Ar-H), 7.63-7.66 (m, 2H, Ar-H), 12.64 (brs, 2H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 12.34 (C-8), 35.29 (C-10), 109.93 (C-c), 121.94 (C-6), 122.75 (C-d), 127.73 (C-2), 128.42 (C-4), 128.84 (C-3), 132.72 (C-b), 135.32 (C-1), 144.66 (C-5), 149.92 (C-a), 154.35 (C-7), 170.00 (C-9). For $C_{19}H_{16}N_4OS_2$ calculated: Elem. Anal.: 59.98% C, 4.24% H, 14.73% N, found: 59.99% C, 4.25% H, 14.72% N. HRMS (m/z): $[M+1]^+$ calculated 381.0838; found 381.0838.

Biochemistry

Cell viability analysis

MTT method was performed to measure the cytotoxicity of the tested compounds against NIH/3T3 and A549 cell lines according to the reported data.[62] The A549 and NIH/3T3 cells were cultured at a density of 5×10^3 cells per well in flat bottomed 96-well plates with various concentrations (50-1000 μM) at the same time cisplatin was used as control drug and they incubated 24 h. After incubation, MTT powder (5 mg/mL) dissolved in phosphate-buffered saline (PBS) was added to each well (20 μL). 2-4 h later, the medium was removed from the plate and 100 μL of DMSO was added to each well to dissolve the dye and kept for 10 min. The cells were measured at 540 nm using microtiter plate reader (BioTek Instruments ELx808IU, USA). Cell viability was calculated as a percentage and compared with the control cells. Each concentration was repeated in three wells and half-maximal inhibitory concentration 50 (IC_{50}) values were defined as the drug concentrations that reduced absorbance to 50% of control values.

The determination of early/late apoptosis by flow cytometry

The A549 and 3T3 cells were seeded at 10^5 cells/mL per well in six well plates at 37 °C in a humidified atmosphere containing 5% CO_2 in air. Then, cells were treated with cisplatin and active compounds at IC_{50} doses for 24 h. The A549 and 3T3 cells were harvested and washed twice with ice-cold PBS and resuspended in 100 μL of binding buffer. A volume of 5 μL (5 μg/mL) of Annexin V-FITC and PI were added to the cells and incubated for 15 min in the dark at room temperature (20–25 °C). Then, 400 μL of binding buffer was added to the mixture samples and analyzed with a flow cytometer (BD FACS Aria Cell Sorter flow cytometry, BD Biosciences).[63]

Data sources

GLOBOCAN extrapolates incidence, mortality, and prevalence of cancer globally. Incidence data are acquired from population-based cancer registries (PBCR). Although the quality of information from less developed countries is often considered limited compared with that from more developed countries, PBCR are a key source of information on the local scale and profile of cancer and are critical in developing and evaluating cancer control programs.[10] Statistical data obtained from GLOBOCAN 2012, developed by the IARC, were used.[11] Furthermore, the incidence and mortality rates were calculated by using the simple percentage method.

ADME parameters

It has become indispensable to important some parameters because of increasing chemical compounds and the development of modern medicinal chemistry in recent years. The head of these parameters is the pharmacokinetic properties of the compounds. Particularly, several preliminary tests such as state the ADME (absorption, distribution, metabolism, excretion) properties are becoming more important due to the occur of many new compounds which are resulting from the original compounds synthesis studies that accelerated at the end of the 1990s. We want to learn these properties of the new compounds; thus, we can work up a connection relationship between molecular structure and their properties.[64,65]

Predictions of ADME properties of the obtained compounds **4a–4i** were calculated by the online Molinspiration property program[66] and the Molsoft software.[67]

Conclusions

In summary, we have synthesized novel 2-[substituted thio]-*N*-(5-methyl-4-phenylthiazol-2-yl) acetamide derivatives (**4a–4i**) and evaluated their anticancer activity against A549 lung adenocarcinoma cells in this study. In general, all compounds (**4a–4i**) have shown to possess an anticancer activity. Mainly, compounds **4a–4f** have acted against A549 lung adenocarcinoma cells in low concentrations. According to apoptosis rates and IC_{50} values, we indicate that compound **4c** (1-methyl-1*H*-tetrazol-5-yl) has been stated both selective and the most active compound against A549 lung adenocarcinoma cells.

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Disclosure statement

The authors confirm that this article content has no conflict of interest.

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