Research Article

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A preliminary investigation of anticancer activity of novel benzothiazole derivatives against A549 lung carcinoma cell line

Yeni benzotiyazol türevlerinin A549 akciğer karsinomu hücre hattına karşı antikanser etkinliğinin ilk basamak araştırması

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Abstract

Objective(s): In this study, it was aimed to synthesize new chemotherapeutic agents based on known antiproliferative properties of benzothiazol-2-amine moiety. The antitumor activity of the newly synthesized compounds was determined against A549 lung cancer cell lines.
Methods: Eighteen compounds were obtained by two steps synthetic route. The anticancer potency of the compounds were detected using MTT assay and flow cytometric analysis on A549 cell line. Some physicochemical properties of the compounds were calculated, virtually.
Results: Compounds 15 and 18 showed the highest cytotoxic activity even than cisplatin. Also, it was determined that compound 18 caused 19.1% (early and late) apoptosis whereas cisplatin caused 21.6% (early and late).
Conclusion: Considering the calculated virtual data, eighteen new compounds were found within the boundaries of Lipinski rule of five to be an oral drug. Besides, the most potent compounds 15 and 18 were detected that both have 1-methylbenzimidazole structure, and also methoxy and ethoxy substituents on benzothiazole ring.

Keywords: Benzothiazole; Synthesis; Lung cancer; Cytotoxicity; Apoptosis.

Özet

Amaç: Bu çalışmada, benzotiyazol-2-amin kalıntısının bilinen antiproliferatif özelliklerinden yola çıkılarak yeni kemoterapötik ajanların sentezlenmesi amaçlanmıştır. Yeni sentezlenen bileşiklerin antitümör etkinliği, A549 akciğer kanseri hücre dizisine karşı belirlenmiştir.
Yöntemler: On sekiz bileşik iki aşamalı sentetik yolla elde edilmiştir. Bileşiklerin antikanser potansiyeli A549 hücre dizisinde MTT yöntemi ve akış sitometrisi analizi kullanılarak tespit edilmiştir. Bileşiklerin bazı fizikokimyasal özellikleri sanal olarak hesaplanmıştır.
Bulgular: Bileşiklerden **15** ve **18**, sisplatinden daha yüksek sitotoksik aktivite göstermiştir. Ayrıca sisplatinin %21.6'lık (erken ve geç) apoptoza sebep olurken, bileşik **18**'in %19.1 (erken ve geç) apoptoza neden olduğu belirlenmiştir.
Sonuç: Sanal olarak hesaplanan veriler göz önüne aldığında, on sekiz yeni bileşiğin Lipinsiki'nin oral ilaç olabilmek için gerekli beş kuralının sınırları içerisinde olduğu belirlenmiştir. Ayrıca en aktif bileşikler olan **15** ve **18**'in, her ikisinin de 1-metilbenzimidazol yapısına sahip oldukları ve benzotiyazol halkasında metoksi ve etoksi sübstitüentleri taşıdıkları belirlenmiştir.

Anahtar kelimeler: Benzotiyazol; Sentez; Akciğer kanseri; Sitotoksisite; Apoptoz.

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Introduction

Lung cancer is the most common cause of cancer-related death which represents 29% of all cancer deaths worldwide. Among its types, non-small cell lung cancer (NSCLC) is higher than eighty percent of all lung cancer diseases [1, 2]. Chemotherapy provides symptomatic treatment and modest improvement in survival in advanced non-small cell lung cancer [3, 4]. However, recent literature indicate that newer chemotherapeutic agents extend life span and relieve symptoms in all stages of lung cancer, such as NSCLC [5].

Benzothiazoles have been known to have a large scale of biological activities including antimalarial, antitubercular, antihelmintic, anticonvulsant, analgesic, anti-inflammatory, antidiabetic and antitumor activities [6–8]. The diversity of biological activity varies according to the alkyl, aryl group or any other functional groups at the second position of benzothiazole ring. It has been reported that the benzothiazole structure modified with an aryl group and imidazole ring can affect inhibition of the growth of certain cancer cell lines [9–11]. In various studies, new benzothiazole compounds were focused on substituting 2-aminobenzothiazoles, 2-arylbenzothiazoles and benzothiazole-2-thiol as functional groups [12–17]. Among them, a large number of 2-aminobenzothiazoles are generated one of the privileged part of medicinal chemistry which are also extensively studied [18, 19]. Related compounds were reported to possess cytotoxic properties on different cancer cells which were comparable to cisplatin [20]. Besides, combination of 2-aminobenzothiazoles with other heterocycles is a well-known approach to design new drug like molecules, which allows achieving new pharmacological profile, action, toxicity lowering [21].

Considering these literature, we have designed novel benzothiazole derivatives including different heterocyclic rings, imidazole, triazole and benzimidazole. Compounds have been studied to determine their anticancer potency against A549 tumor cell line (human lung carcinoma cell) compared with cisplatin.

Materials and methods

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points were determined by MP90 digital melting point apparatus (Mettler Toledo, OH) and were uncorrected. Spectroscopic data were recorded on the following instruments: Bruker Tensor 27 IR spectrophotometer; ^{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 LC/MS ITTOF system (Shimadzu, Tokyo, Japan). Elemental analyses were performed in a Perkin Elmer EAL 240 elemental analyser for C, H, N, O and S.

Synthesis of the compounds

General method for the synthesis of 2-chloro-*N*-(6-substituted benzothiazol-2-yl)acetamides (1–6)

A solution of 2-amino-6-substituted benzothiazol (1g, 6 mmol) in 250 mL of tetrahydrofuran was prepared and triethylamine (1.1 mL, 7.2 mmol) was added. Chloroacetyl chloride (0.64 mL, 7.2 mmol) in 5 mL of tetrahydrofuran was added dropwise in ice bath. After completion of dropping, the resulting mixture was stirred for 1 h at room temperature and evaporated to dryness. The residue was washed with water, dried and recrystallized from ethanol to afford the pure compounds **1–6**.

General method for the synthesis of 2-[(1*H*-benzimidazol-2-yl)thio]-*N*-(6-substituted benzo[d] thiazol-2-yl)acetamides (7–12) and 2-[(1-methyl-1*H*-benzimidazol-2-yl)thio]-*N*-(6-substituted benzothiazol-2-yl)acetamides (13–18)

For the preparation of compounds **13–18**, 2-[(1*H*-benzimidazol-2-yl)thio]-*N*-(6-substituted benzothiazol-2-yl) acetamides (**7–12)** were employed as key intermediates. Compounds **7–12** were synthesized using appropriate 2-chloro-*N*-(6-substituted benzothiazol-2-yl)acetamide (**1–6**) as starting materials. Compounds (**1–6**) (0.5 g, 2 mmol) and 1*H*-benzimidazole-2-thiol (**A**) (2 mmol) were stirred at room temperature for 5 h in acetone (40 mL) with the presence of potassium carbonate (2.4 mmol). Acetone was evaporated and the product was washed with water, dried and recrystallized from ethanol to give **7–12**. After this procedure, compounds **7–12** (2 mmol) and methyl iodide (2 mmol) were dissolved in acetone (40 mL) and stirred at room temperature for 12 h with potassium hydroxide (10 mmol). After evaporation of acetone, the residue was washed, dried and recrystallized from ethanol to synthesize compounds **13–18**.

General method for the synthesis of 2-[(1-methyl-1*H*-imidazol-2-yl)thio]-*N*-(6-substituted benzothiazol-2-yl) acetamides (19–24) and 2-[(4-methyl-4*H*-1,2,4-triazol-3-yl)thio]-*N*-(6-substituted benzothiazol-2-yl)acetamides (25–30)

A mixture of appropriate 2-chloro-*N*-(6-substituted benzothiazol-2-yl)acetamide (**1–6**) (0.5 g, 2 mmol), 2-mercaptobenzimidazole (**A**)/1-methyl-1*H*-imidazole-2-thiol (**B**)/4-methyl-3-mercapto-1,2,4-triazole (**C**) (2 mmol) and potassium carbonate (2.4 mmol) were stirred at room temperature for 6 h in 40 mL of acetone. The solvent was evaporated under reduced pressure, the product was washed, dried and recrystallized from ethanol to give compounds **7–12** and **19–30**. Compounds derived from benzimidazole was then methylated with methyl iodide/KOH reagent to gain **13–18**.

***N*-(Benzothiazol-2-yl)-2-[(1-methyl-1*H*-benzimidazol-2-yl)thio]acetamide (13)** Yield 69%, m.p. 222–226°C. IR (cm^{-1}): υ_{max} 3396 (N–H), 3061 (aromatic C–H), 2974 (aliphatic C–H), 1699 (C=O), 1598–1440 (C=N and C=C), 1255 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 4.33 (3H, s, NCH_3), 5.08 (2H, s, $COCH_2$), 7.75–7.81 (2H, m, Ar-H), 7.87–7.93 (1H, m, Ar-H), 8.01–8.12 (3H, m, Ar-H), 8.35 and 8.38 (1H, dd, J_1 = 2.3 Hz, J_2 = 8.0 Hz, Ar-H), 8.55 and 8.58 (1H, dd, J_1 = 2.3 Hz, J_2 = 7.8 Hz, Ar-H), 13.36 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 31.06 (NCH_3), 36.55 ($CO\underline{C}H_2$), 110.65, 118.59, 121.72, 122.64, 122.78, 122.82, 124.72, 127.25, 132.53, 137.91, 143.64, 168.39 (C=O). For $C_{17}H_{14}N_4OS_2$ calculated: 57.61% C, 3.98% H, 15.81% N, 4.51% O, 18.09% S; found: 57.69% C, 3.99% H, 15.77% N, 4.50% O, 18.15% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{17}H_{14}N_4OS_2$: 355.0682; found 355.0683.

***N*-(6-Methylbenzothiazol-2-yl)-2-[(1-methyl-1*H*-benzimidazol-2-yl)thio]acetamide (14)** Yield 71%, m.p. 212–216°C. IR (cm^{-1}): υ_{max} 3392 (N–H), 3055 (aromatic C–H), 2974 (aliphatic C–H), 1689 (C=O), 1606–1444 (C=N and C=C), 1172 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 2.39 (3H, s, CH_3), 3.73 (3H, s, NCH_3), 4.47 (2H, s, $COCH_2$), 7.12–7.26 (3H, m, Ar-H), 7.49 and 7.51 (2H, dt, J_1 = 1.8 Hz, J_2 = 8.4 Hz, Ar-H), 7.65 (1H, d, J = 8.3 Hz, Ar-H), 7.74 (1H, s, Ar-H), 12.70 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 21.44 (CH_3), 30.47 (NCH_3), 35.94 ($CO\underline{C}H_2$), 110.03, 117.99, 120.75, 121.79, 122.03, 122.17, 127.96, 132.07, 133.59, 137.30, 143.04, 146.95, 151.21, 157.34, 167.64 (C=O). For $C_{18}H_{16}N_4OS_2$ calculated: 58.67% C, 4.38% H, 15.21% N, 4.34% O, 17.40% S; found: 58.60% C, 4.36% H, 15.17% N, 4.33% O, 17.37% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{18}H_{16}N_4OS_2$: 369.0838; found 369.0831.

***N*-(6-Methoxybenzothiazol-2-yl)-2-[(1-methyl-1*H*-benzimidazol-2-yl)thio] acetamide (15)** Yield 70%, m.p. 213–216°C. IR (cm^{-1}): υ_{max} 3356 (N–H), 3062 (aromatic C–H), 2972 (aliphatic C–H), 1683 (C=O), 1598–1465 (C=N and C=C), 1261–1056 (C–N and C–O). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.74 (3H, s, NCH_3), 3.79 (3H, s, OCH_3), 4.46 (2H, s, $COCH_2$), 7.02 and 7.05 (1H, dd, J_1 = 2.6 Hz, J_2 = 8.8 Hz, Ar-H), 7.11–7.23 (2H, m, Ar-H), 7.50 (2H, d, J = 7.9 Hz, Ar-H), 7.56 (1H, d, J = 2.6 Hz, Ar-H), 7.66 (1H, d, J = 8.9 Hz, Ar-H), 12.64 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 30.47 (NCH_3), 35.87 ($CO\underline{C}H_2$), 56.07 (OCH_3), 105.19, 110.03, 115.47, 117.99, 121.74, 122.03, 122.17, 133.25, 137.31, 143.04, 151.22, 156.13, 156.67, 167.46 (C=O). For $C_{18}H_{16}N_4O_2S_2$ calculated: 56.23% C, 4.19% H, 14.57% N, 8.32% O, 16.68% S; found: 56.32% C, 4.20% H, 14.60% N, 8.30% O, 16.72% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{18}H_{16}N_4O_2S_2$: 385.0787; found 385.0780.

***N*-(6-Chlorobenzothiazol-2-yl)-2-[(1-methyl-1*H*-benzimidazol-2-yl)thio]acetamide (16)** Yield 68%, m.p. 257–261°C. IR (cm^{-1}): υ_{max} 3358 (N–H), 3064 (aromatic C–H), 2976 (aliphatic C–H), 1687 (C=O), 1589–1440 (C=N and C=C), 1230 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.73 (3H, s, NCH_3), 4.33 (2H, s, $COCH_2$), 7.11–7.21 (2H, m, Ar-H), 7.30 and 7.33 (1H, dd, J_1 = 2.2 Hz, J_2 = 8.6 Hz, Ar-H), 7.46–7.52 (2H, m, Ar-H), 7.58 (1H, d, J = 8.6 Hz, Ar-H), 7.91 (1H, d, J = 2.2 Hz, Ar-H), 12.84 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 30.38 (NCH_3), 38.27 ($CO\underline{C}H_2$), 109.84, 117.90, 121.13, 121.24, 121.84, 121.92, 125.90, 126.36, 134.37, 137.23, 143.22, 148.91, 152.25, 170.33 (C=O). For $C_{17}H_{13}ClN_4OS_2$ calculated: 52.50% C, 3.37% H, 9.12% Cl, 14.41% N, 4.11% O, 16.49% S; found: 52.63% C, 3.38% H, 9.10% Cl, 14.36% N, 4.12% O, 16.53% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{17}H_{13}ClN_4OS_2$: 389.0292; found 389.0288.

***N*-(6-Fluorobenzothiazol-2-yl)-2-[(1-methyl-1*H*-benzimidazol-2-yl)thio]acetamide (17)** Yield 70%, m.p. 233–238°C. IR (cm^{-1}): υ_{max} 3325 (N–H), 3070 (aromatic C–H), 2970 (aliphatic C–H), 1697 (C=O), 1604–1448 (C=N and C=C), 1149 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.74 (3H, s, NCH_3), 4.48 (2H, s, $COCH_2$), 7.10–7.23 (2H, m, Ar-H), 7.29 (1H, t, J = 9.1 Hz, Ar-H), 7.50 (2H, d, J = 8.1 Hz, Ar-H), 7.76–7.80 (1H, m, Ar-H), 7.87 and 7.90 (1H, dd, J_1 = 2.7 Hz, J_2 = 8.7 Hz, Ar-H), 12.79 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 30.48 (NCH_3), 35.87 ($CO\underline{C}H_2$), 108.51, 108.87, 110.04, 114.61, 114.93, 117.99, 122.04, 122.18, 122.32, 133.10, 133.25, 137.31, 143.03, 145.72, 149.80, 151.17, 157.56, 158.21, 160.74, 167.90 (C=O). For $C_{17}H_{13}FN_4OS_2$ calculated: 54.82% C, 3.52% H, 5.10% F, 15.04% N, 4.30% O, 17.22% S; found: 54.72% C, 3.51% H, 5.11% F, 15.03% N, 4.29% O, 17.25% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{17}H_{13}FN_4OS_2$: 373.0588; found 373.0576.

***N*-(6-Ethoxybenzothiazol-2-yl)-2-[(1-methyl-1*H*-benzimidazol-2-yl)thio]acetamide (18)** Yield 75%, m.p. 220–225°C. IR (cm^{-1}): υ_{max} 3318 (N–H), 3087 (aromatic C–H), 2942 (aliphatic C–H), 1682 (C=O), 1608–1467 (C=N and C=C), 1183 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 1.34 (3H, t, J = 6.9 Hz, $OCH_2C\underline{H}_3$), 3.74 (3H, s, NCH_3), 4.05 (2H, q, J = 6.9, $OC\underline{H}_2CH_3$), 4.46 (2H, s, $COCH_2$), 7.00 and 7.03 (1H, dd, J_1 = 2.6 Hz, J_2 = 8.8 Hz, Ar-H), 7.12–7.23 (2H, m, Ar-H), 7.48–7.54 (3H, m, Ar-H), 7.65 (1H, d, J = 8.8 Hz, Ar-H), 12.63 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 15.15 ($OCH_2\underline{C}H_3$), 30.47 (NCH_3), 35.88 ($CO\underline{C}H_2$), 64.05 ($O\underline{C}H_2$) 105.83, 110.03, 115.81, 117.99, 121.73, 122.03, 122.17, 133.24, 137.31, 143.04, 151.21, 155.89, 156.10, 167.45 (C=O). For $C_{19}H_{18}N_4O_2S_2$ calculated: 57.27% C, 4.55% H, 14.06% N, 8.03% O, 16.09% S; found: 57.38% C, 4.53% H, 14.04% N, 8.05% O, 16.10% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{19}H_{18}N_4O_2S_2$: 399.0944; found 399.0931.

***N*-(Benzothiazol-2-yl)-2-[(1-methyl-1*H*-imidazol-2-yl)thio]acetamide (19)** Yield 70%, m.p. 164–167°C. IR (cm^{-1}): υ_{max} 3361 (N–H), 3053 (aromatic C–H), 2970 (aliphatic C–H), 1687 (C=O), 1600–1446 (C=N and C=C), 1280 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.61 (3H, s, NCH_3), 4.03 (2H, s, $COCH_2$), 6.97 (1H, d, J = 1.1 Hz, Ar-H), 7.28 (1H, d, J = 1.1 Hz, Ar-H), 7.31 (1H, t, J = 7.6 Hz, Ar-H), 7.44 (1H, t, J = 7.6 Hz, Ar-H), 7.76 (1H, d, J = 8 Hz, Ar-H), 7.98 (1H, d, J = 7.9 Hz, Ar-H), 12.73 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 33.46 (NCH_3), 37.57 ($CO\underline{C}H_2$), 121.09, 122.21, 124.10, 124.18, 124.55, 126.63, 129.10, 131.93, 139.62, 148.96, 158.19, 168.43 (C=O). For $C_{13}H_{12}N_4OS_2$ calculated: 51.30% C, 3.97% H, 18.41% N, 5.26% O, 21.07% S; found: 51.25% C, 3.96% H, 18.46% N, 5.24% O, 21.02% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{13}H_{12}N_4OS_2$: 305.0525; found 305.0509.

***N*-(6-Methylbenzothiazol-2-yl)-2-[(1-methyl-1*H*-imidazol-2-yl)thio]acetamide (20)** Yield 72%, m.p. 180–182°C. IR (cm^{-1}): υ_{max} 3338 (N–H), 3053 (aromatic C–H), 2922 (aliphatic C–H), 1697 (C=O), 1608–1456 (C=N and C=C), 1261 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 2.41 (3H, s, CH_3), 3.61 (3H, s, NCH_3), 4.02 (2H, s, $COCH_2$), 6.97 (1H, d, J = 1.2 Hz, Ar-H), 7.23–7.28 (2H, m, Ar-H), 7.64 (1H, d, J = 8.3 Hz, Ar-H), 7.76 (1H, s, Ar-H), 12.65 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 21.43 (CH_3), 33.45 (NCH_3), 37.55 ($CO\underline{C}H_2$), 120.72, 121.78, 122.63, 123.78, 124.17, 124.53, 127.55, 127.95, 129.08, 132.08, 133.57, 139.65, 146.93, 157.30, 168.27 (C=O). For $C_{14}H_{14}N_4OS_2$ calculated: 52.81% C, 4.43% H, 17.60% N, 5.02% O, 20.14% S; found: 52.93% C, 4.41% H, 17.54% N, 5.03% O, 20.17% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{14}H_{14}N_4OS_2$: 319.0682; found 319.0659.

***N*-(6-Methoxybenzothiazol-2-yl)-2-[(1-methyl-1*H*-imidazol-2-yl)thio]acetamide (21)** Yield 64%, m.p. 168–170°C. IR (cm^{-1}): υ_{max} 3350 (N–H), 3062 (aromatic C–H), 2966 (aliphatic C–H), 1685 (C=O), 1598–1462 (C=N and C=C), 1276–1056 (C–N and C–O). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.61 (3H, s, NCH_3), 3.81 (3H, s, OCH_3), 4.01 (2H, s, $COCH_2$), 6.97 (1H, d, J = 1.2 Hz, Ar-H), 7.02 and 7.05 (1H, dd, J_1 = 2.6 Hz, J_2 = 8.8 Hz, Ar-H), 7.27 (1H, s, Ar-H), 7.57 (1H, d, J = 2.4 Hz, Ar-H), 7.65 (1H, d, J = 8.8 Hz, Ar-H), 12.59 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 33.45 (NCH_3), 37.50 ($CO\underline{C}H_2$), 56.07 (OCH_3), 105.19, 115.44, 121.70, 124.17, 129.09, 133.26, 139.65, 143.05, 156.12, 156.65, 168.11 (C=O). For $C_{14}H_{14}N_4O_2S_2$ calculated: 50.28% C, 4.22% H, 16.75% N, 9.57% O, 19.18% S; found: 50.37% C, 4.23% H, 16.70% N, 9.59% O, 19.22% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{14}H_{14}N_4O_2S_2$: 335.0631; found 335.0625.

***N*-(6-Chlorobenzothiazol-2-yl)-2-[(1-methyl-1*H*-imidazol-2-yl)thio]acetamide (22)** Yield 69%, m.p. 201–203°C. IR (cm^{-1}): υ_{max} 3354 (N–H), 3051 (aromatic C–H), 2972 (aliphatic C–H), 1687 (C=O), 1593–1451 (C=N and C=C), 1269 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.60 (3H, s, NCH_3), 4.03 (2H, s, $COCH_2$), 6.96 (1H, d, J = 1.2 Hz, Ar-H), 7.27 (1H, d, J = 1.1 Hz, Ar-H), 7.44 and 7.46 (1H, dd, J_1 = 2.4 Hz, J_2 = 8.6 Hz, Ar-H), 7.74 (1H, d, J = 8.6 Hz, Ar-H), 8.12 (1H, d, J = 2.1 Hz, Ar-H), 12.82 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 33.46 (NCH_3), 37.51 ($CO\underline{C}H_2$), 121.93, 122.30, 124.19, 126.98, 128.15, 133.64, 139.58, 147.87, 159.06, 168.65 (C=O). For $C_{13}H_{11}ClN_4OS_2$ calculated: 46.08% C, 3.27% H, 10.46% Cl, 16.54% N, 4.72% O, 18.93% S; found: 45.97% C, 3.26% H, 10.49% Cl, 16.50% N, 4.73% O, 18.90% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{13}H_{11}ClN_4OS_2$: 339.0136; found 339.0124.

***N*-(6-Fluorobenzothiazol-2-yl)-2-[(1-methyl-1*H*-imidazol-2-yl)thio]acetamide (23)** Yield 75%, m.p. 189–193°C. IR (cm^{-1}): υ_{max} 3323 (N–H), 3059 (aromatic C–H), 2964 (aliphatic C–H), 1689 (C=O), 1608–1452 (C=N and C=C), 1257 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.60 (3H, s, NCH_3), 4.03 (2H, s, $COCH_2$), 6.96 (1H, d, J = 1.2 Hz, Ar-H), 7.24–7.31 (2H, m, Ar-H), 7.73–7.78 (1H, m, Ar-H), 7.86 and 7.89 (1H, dd, J_1 = 2.7 Hz, J_2 = 8.7 Hz, Ar-H), 12.76 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 33.45 (NCH_3), 37.49 ($CO\underline{C}H_2$), 108.47, 108.83, 114.57, 114.90, 122.14, 122.26, 124.18, 129.10, 133.11, 133.26, 139.61, 145.69, 157.55, 158.18, 158.21, 160.73, 168.52 (C=O). For $C_{13}H_{11}FN_4OS_2$ calculated: 48.43% C, 3.44% H, 5.89% F, 17.38% N, 4.96% O, 19.89% S; found: 48.51% C, 3.44% H, 5.88% F, 17.33% N, 4.95% O, 19.95% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{13}H_{11}FN_4OS_2$: 323.0431; found 323.0414.

***N*-(6-Ethoxybenzothiazol-2-yl)-2-[(1-methyl-1*H*-imidazol-2-yl)thio]acetamide (24)** Yield 70%, m.p. 123–126°C. IR (cm^{-1}): υ_{max} 3354 (N–H), 3068 (aromatic C–H), 2980 (aliphatic C–H), 1668 (C=O), 1604–1458 (C=N and C=C), 1251–1058 (C–N and C–O). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 1.34 (3H, t, J = 6.9 Hz, $OCH_2C\underline{H}_3$), 3.60 (3H, s, NCH_3), 4.00 (2H, s, $COCH_2$), 4.06 (2H, q, J = 6.96, $OC\underline{H}_2CH_3$), 6.96 (1H, d, J = 1.2 Hz, Ar-H), 7.00 and 7.02 (1H, dd, J_1 = 2.6 Hz, J_2 = 8.8 Hz, Ar-H), 7.27 (1H, d, J = 1.2 Hz, Ar-H), 7.54 (1H, d, J = 2.5 Hz, Ar-H), 7.63 (1H, d, J = 8.9 Hz, Ar-H), 12.57 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 15.15 ($OCH_2\underline{C}H_3$), 33.45 (NCH_3), 37.51 ($CO\underline{C}H_2$), 64.04 ($O\underline{C}H_2$) 105.83, 115.78, 121.69, 124.17, 129.09, 133.24, 139.64, 142.97, 155.88, 156.08, 168.10 (C=O). For $C_{15}H_{16}N_4O_2S_2$ calculated: 51.70% C, 4.63% H, 16.08% N, 9.18% O, 18.40% S; found: 51.84% C, 4.62% H, 16.11% N, 9.15% O, 18.46% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{15}H_{16}N_4O_2S_2$: 349.0787; found 349.0788.

***N*-(Benzothiazol-2-yl)-2-[(4-methyl-4*H*-1,2,4-triazol-3-yl)thio]acetamide (25)** Yield 68%, m.p. 227–232°C. IR (cm^{-1}): υ_{max} 3336 (N–H), 3049 (aromatic C–H), 2926 (aliphatic C–H), 1683 (C=O), 1597–1440 (C=N and C=C), 1253 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.61 (3H, s, NCH_3), 4.21 (2H, s, $COCH_2$), 7.31 (1H, t, J = 7.6 Hz, Ar-H), 7.44 (1H, t, J = 7.7 Hz, Ar-H), 7.76 (1H, d, J = 7.9 Hz, Ar-H), 7.98 (1H, d, J = 7.3 Hz, Ar-H), 8.57 (1H, s, Ar-H), 12.64 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 31.30 (NCH_3), 36.86 ($CO\underline{C}H_2$), 121.14, 122.23, 124.15, 126.66, 131.91, 146.84, 148.81, 148.97, 158.12, 167.72 (C=O). For $C_{12}H_{11}N_5OS_2$ calculated: 47.20% C, 3.63% H, 22.93% N, 5.24% O, 21.00% S; found: 47.30% C, 3.62% H, 22.88% N, 5.25% O, 20.95% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{12}H_{11}N_5OS_2$: 306.0478; found 306.0469.

***N*-(6-Methylbenzothiazol-2-yl)-2-[(4-methyl-4*H*-1,2,4-triazol-3-yl)thio]acetamide (26)** Yield 63%, m.p. 244–247°C. IR (cm^{-1}): υ_{max} 3361 (N–H), 3062 (aromatic C–H), 2912 (aliphatic C–H), 1670 (C=O), 1610–1456 (C=N and C=C), 1257 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 2.41 (3H, s, CH_3), 3.61 (3H, s, NCH_3), 4.20 (2H, s, $COCH_2$), 7.24 and 7.27 (1H, dd, J_1 = 1.3 Hz, J_2 = 8.3 Hz, Ar-H), 7.64 (1H, d, J = 8.2 Hz, Ar-H), 7.76 (1H, s, Ar-H), 8.57 (1H, s, Ar-H), 12.58 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 21.44 (CH_3), 31.30 (NCH_3), 36.85 ($CO\underline{C}H_2$), 120.72, 121.80, 127.98, 132.05, 133.63, 146.83, 148.82, 157.24, 167.56 (C=O). For $C_{13}H_{13}N_5OS_2$ calculated: 48.88% C, 4.10% H, 21.93% N, 5.01% O, 20.08% S; found: 48.74% C, 4.09% H, 21.99% N, 5.00% O, 20.02% S. HRMS (*m/z*): $[M+H]^+$ calculatedfor $C_{13}H_{13}N_5OS_2$: 320.0634; found 320.0622.

***N*-(6-Methoxybenzothiazol-2-yl)-2-[(4-methyl-4*H*-1,2,4-triazol-3-yl)thio]acetamide (27)** Yield 67%, m.p. 227–232°C. IR (cm^{-1}): υ_{max} 3404 (N–H), 3018 (aromatic C–H), 2927 (aliphatic C–H), 1683 (C=O), 1614–1469 (C=N and C=C), 1261–1165 (C–N and C–O). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.61 (3H, s, NCH_3), 3.81 (3H, s, OCH_3), 4.19 (2H, s, $COCH_2$), 7.02 and 7.05 (1H, dd, J_1 = 2.6 Hz, J_2 = 8.9 Hz, Ar-H), 7.57 (1H, d, J = 2.6 Hz, Ar-H), 7.65 (1H, d, J = 8.9 Hz, Ar-H), 8.57 (1H, s, Ar-H), 12.51 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 31.30 (NCH_3), 36.79 ($CO\underline{C}H_2$), 56.08 (OCH_3), 105.20, 115.48, 121.75, 133.24, 143.05, 146.83, 148.83, 156.05, 156.68, 167.39 (C=O). For $C_{13}H_{13}N_5O_2S_2$ calculated: 46.55% C, 3.91% H, 20.88% N, 9.54% O, 19.12% S; found: 46.67% C, 3.90% H, 20.85% N, 9.51% O, 19.17% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{13}H_{13}N_5O_2S_2$: 336.0583; found 336.0575.

***N*-(6-Chlorobenzothiazol-2-yl)-2-[(4-methyl-4*H*-1,2,4-triazol-3-yl)thio]acetamide (28)** Yield 76%, m.p. 263–266°C. IR (cm^{-1}): υ_{max} 3377 (N–H), 3078 (aromatic C–H), 2968 (aliphatic C–H), 1678 (C=O), 1600–1444 (C=N and C=C), 1257 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.62 (3H, s, NCH_3), 4.23 (2H, s, $COCH_2$), 7.47 (1H, d, J = 6.8 Hz, Ar-H), 7.76 (1H, d, J = 7.2 Hz, Ar-H), 8.13 (1H, s, Ar-H), 8.58 (1H, s, Ar-H), 12.76 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 31.30 (CH_3), 36.81 ($CO\underline{C}H_2$), 121.94, 122.33, 127.00, 128.20, 133.61, 146.84, 147.85, 148.80, 159.01, 167.97 (C=O). For $C_{12}H_{10}ClN_5OS_2$ calculated: 42.41% C, 2.97% H, 10.43% Cl, 20.61% N, 4.71% O, 18.87% S; found: 42.35% C, 2.97% H, 10.45% Cl, 20.57% N, 4.69% O, 18.92% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{12}H_{10}ClN_5OS_2$: 340.0088; found 340.0074.

***N*-(6-Fluorobenzothiazol-2-yl)-2-[(4-methyl-4*H*-1,2,4-triazol-3-yl)thio]acetamide (29)** Yield 60%, m.p. 257–260°C. IR (cm^{-1}): υ_{max} 3307 (N–H), 3062 (aromatic C–H), 2922 (aliphatic C–H), 1676 (C=O), 1602–1454 (C=N and C=C), 1161 (C–N). ^{1}H-NMR (300 Mhz, DMSO-d_6, ppm) δ 3.60 (3H, s, NCH_3), 4.21 (2H, s, $COCH_2$), 7.29 (1H, t, J = 9.1 Hz, Ar-H), 7.74–7.79 (1H, m, Ar-H), 7.87 and 7.90 (1H, dd, J_1 = 2.7 Hz, J_2 = 8.7 Hz, Ar-H), 8.57 (1H, m, Ar-H), 12.68 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 31.30 (NCH_3), 36.78 ($CO\underline{C}H_2$), 108.50, 108.86, 114.62, 114.95, 122.20, 122.32, 133.08, 133.23, 145.69, 146.84, 148.80, 157.57, 158.13, 160.76, 167.83 (C=O). For $C_{12}H_{10}FN_5OS_2$ calculated: 44.57% C, 3.12% H, 5.88% F, 21.66% N, 4.95% O, 19.83% S; found: 44.67% C, 3.11% H, 5.90% F, 21.60% N, 4.96% O, 19.79% S. HRMS (*m/z*): $[M+H]^+$ calculated for $C_{12}H_{10}FN_5OS_2$: 324.0384; found 324.0362.

***N*-(6-Ethoxybenzothiazol-2-yl)-2-[(4-methyl-4*H*-1,2,4-triazol-3-yl)thio]acetamide (30)** Yield 64%, m.p. 215–218°C. IR (cm^{-1}): υ_{max} 3385 (N–H), 3055 (aromatic C–H), 2970 (aliphatic C–H), 1676 (C=O), 1614–1462 (C=N and C=C), 1259–1041 (C–N and C–O). ^{1}H-NMR (300 Mhz, DMSO-d_6,

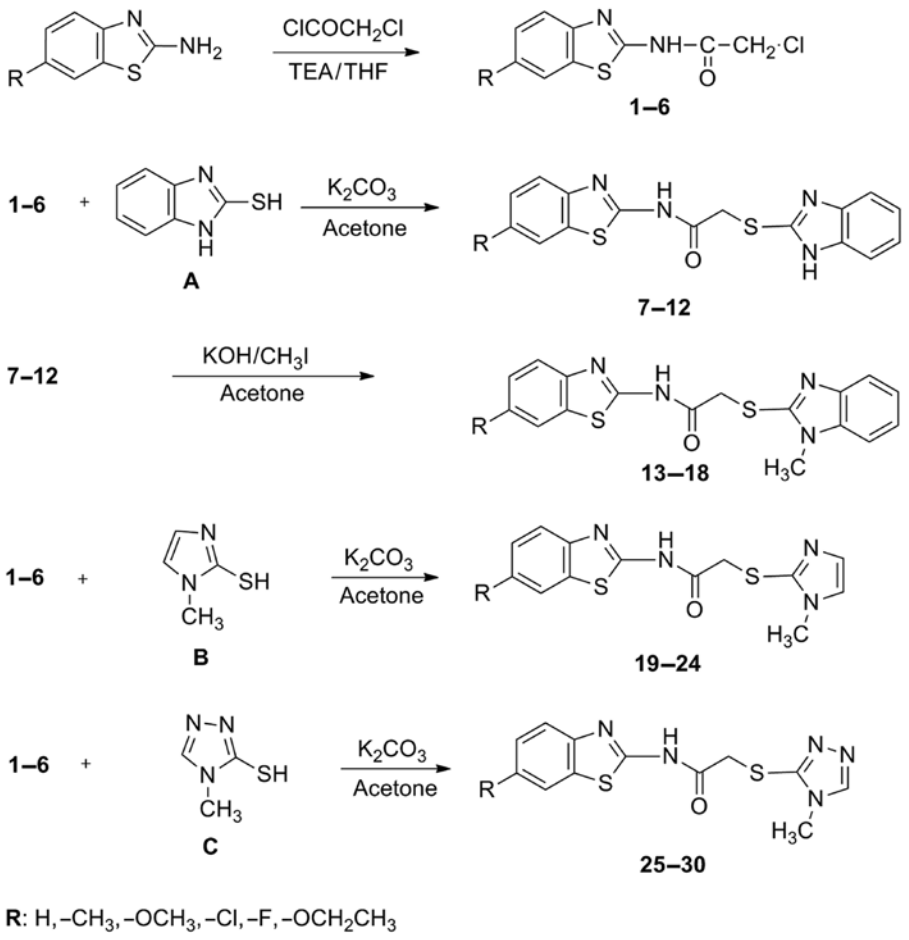


Figure 1: The synthetic route for the preparation of compounds **1–30**.

ppm) δ 1.34 (3H, t, J = 6.9 Hz, $OCH_2C\underline{H}_3$), 3.60 (3H, s, NCH_3), 4.06 (2H, q, J = 7 Hz, $OC\underline{H}_2CH_3$), 4.18 (2H, s, $COCH_2$), 7.00 and 7.03 (1H, dd, J_1 = 2.3 Hz, J_2 = 8.9 Hz, Ar-H), 7.54 (1H, d, J = 2.5 Hz, Ar-H), 7.64 (1H, d, J = 8.8 Hz, Ar-H), 8.57 (1H, s, Ar-H), 12.50 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 15.16 ($OCH_2\underline{C}H_3$), 31.30 (CH_3), 36.81 ($CO\underline{C}H_2$), 64.06 ($O\underline{C}H_2$) 105.85, 115.82, 121.75, 146.84, 155.91, 167.38 (C=O). For $C_{14}H_{15}N_5O_2S_2$ calculated: 48.12% C, 4.33% H, 20.04% N, 9.16% O, 18.35% S; found: 48.00% C, 4.32% H, 19.98% N, 9.18% O, 18.31% S. HRMS (m/z): $[M+H]^+$ calculated for $C_{14}H_{15}N_5O_2S_2$: 350.0740; found 350.0730.

Biochemistry

MTT assay

MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was carried out to determine cytotoxicity of the compounds against A549 (human lung adenocarcinoma cells) cell line according to the reported data [22, 23]. A549 cells were cultured in 96-well flat-bottom plates at 37°C for 24 h (2 x 10^4 cells per well). After 24 h drug incubation, 20 µL MTT solution (5 mg/mL MTT powder in PBS) was added to each well and incubated for 2 h in the same conditions. Formazan crystals were dissolved in 200 µL DMSO and the absorbance was read by ELISA reader (OD570 nm). The percentage of viable cells was calculated based on the medium control. All experiments were performed at least three times.

Annexin V/ PI staining

FACS-Aria I (Becton Dickinson USA) instrument was used to detect apoptosis ratios using the Annexin V-FITC Apoptosis Detection Kit from BD, Pharmingen, according to the manufacturer's instruction. A549 cells in normal life cycle, cells treated with IC_{50} concentrations of selected compounds (**15**, **18**, **22**, **23** and **26**) and cells treated with cisplatin (24 h incubation) were

Table 1: IC_{50} (μg/mL)[a] values of the compounds against A549 cell line.

Compounds	A549
3a	>500
3b	>500
3c	10.67 ± 2.02
3d	236.67 ± 73.34
3e	>500
3f	9.0 ± 1.0
4a	466.67 ± 41.63
4b	256.67 ± 11.54
4c	256.67 ± 83.86
4d	46.67 ± 2.87
4e	31.33 ± 7.57
4f	nt[b]
5a	>500
5b	64.33 ± 6.03
5c	103.33 ± 5.77
5d	95.33 ± 5.03
5e	136.67 ± 5.77
5f	153.33 ± 15.27
Cisplatin	21.0 ± 2.0

[a]Cytotoxicity of the compounds. Incubation for 24 h. The values represent mean ± standard deviation of triplicate determinations.
[b]nt, Not tested.

Table 2: Percentage of typical quadrant analysis of Annexin V FITC/ Propidium Iodide flow cytometry of A549 cells treated with compounds **15**, **18**, **22**, **23**, **26** and Cisplatin.

Groups	Early apoptotic cells (%)	Late apoptotic cells (%)	Viable cells (%)
Control (untreated)	1.9	0.1	96.7
15	6.4	0.9	81.1
18	18.1	1.0	71.7
22	2.0	0.0	84.2
23	0.3	0.1	95.4
26	8.2	3.2	82.7
Cisplatin	17.0	4.6	37.0

A549 cells were cultured for 24 hours in medium with compounds **15**, **18**, **22**, **23**, **26** and Cisplatin at IC_{50} values. At least 10.000 cells were analyzed per sample, and quadrant analysis was performed.

applicated to the system. The results were analyzed by using FACS Express software and represented as percentage of normal and apoptotic cells at various stages [24]. The percentage of apoptotic cells was determined from the number of cells in sub-G1 phase, representing fragmented cell vesicles. The four areas in the diagrams represent for necrotic cells (Q1, positive for PI and negative for annexin/ FITC, left square on the top), live cells (Q3, negative for annexin and PI, left square at the bottom), late apoptotic or necrotic cells (Q2, positive for annexin and PI, right square on the top) and apoptotic cells (Q4, negative for PI and positive for annexin, right square at the bottom), respectively.

Results and discussion

Chemistry

The preparation of final compounds was performed according to the reactions outlined in Figure 1. The starting compounds **1–6** were synthesized via acetylation of 2-amino-6-substituted benzothiazole with chloroacetyl chloride. The bimolecular nucleophilic substitution (S_N2) reaction between 2-chloro-*N*-(6-substituted benzothiazol-2-yl)acetamides (**1–6**) and appropriate thiol compound **A**, **B** and **C** gave 2-[(1*H*-benzimidazol-2-yl)thio]-*N*-(6-substituted benzothiazol-2-yl)acetamides **(7–12)**, 2-[(1-methyl-1*H*-imidazol-2-yl)thio]-*N*-(6-substituted benzothiazol-2-yl) acetamides (**19–24)** and 2-[(4-methyl-4*H*-1,2,4-triazol-3-yl) thio]-*N*-(6-substituted benzothiazol-2-yl)acetamides (**25–30)**, respectively. *N*-methylation of compounds **7–12** with methyl iodide in acetone provided 2-[(1-methyl-1*H*-benzimidazol-2-yl)thio]-*N*-(6-substituted benzothiazol-2-yl) acetamide **(13–18)**.

Structure clarifications of the final compounds (**13–30**) were carried out through FT-IR, ^{1}H-NMR, ^{13}C-NMR and HRMS spectroscopic data and elemental analysis. Characteristic stretching absorptions of C=O and N-H bonds were observed in the region of 1699–1668 cm^{-1} and 3404–3307 cm^{-1}, respectively. The stretching bands for C=N and C=C were recorded at about 1614–1440 cm^{-1}. In the ^{1}H-NMR spectra, the signal due to methylene and NH protons of acetamide (-$NHCOCH_2$) moiety appeared as singlet peaks at 4.00–5.08 ppm and 12.50–13.36 ppm, respectively. N-CH_3 protons of 1*H*-imidazole, 1*H*-triazole and 1*H*-benzimidazole rings were observed as singlet peaks about 3.60–4.33 ppm. All the other aromatic and aliphatic protons were observed at the expected regions. In the ^{13}C-NMR spectra, the signals belonging to N-CH_3 and the methylene carbons of -$COCH_2$ moiety were assigned at between 30.38–33.46 ppm and 35.87–38.27 ppm, respectively. The carbonyl carbons were recorded at between 167.39 and 170.33 ppm. HRMS spectra of all the compounds showed that M + 1 peaks were in agreement with the calculated molecular weight of the final compounds (**13–30**). Elemental analysis results for C, H, and N elements were satisfactory within ±0.4% calculated values of the compounds.

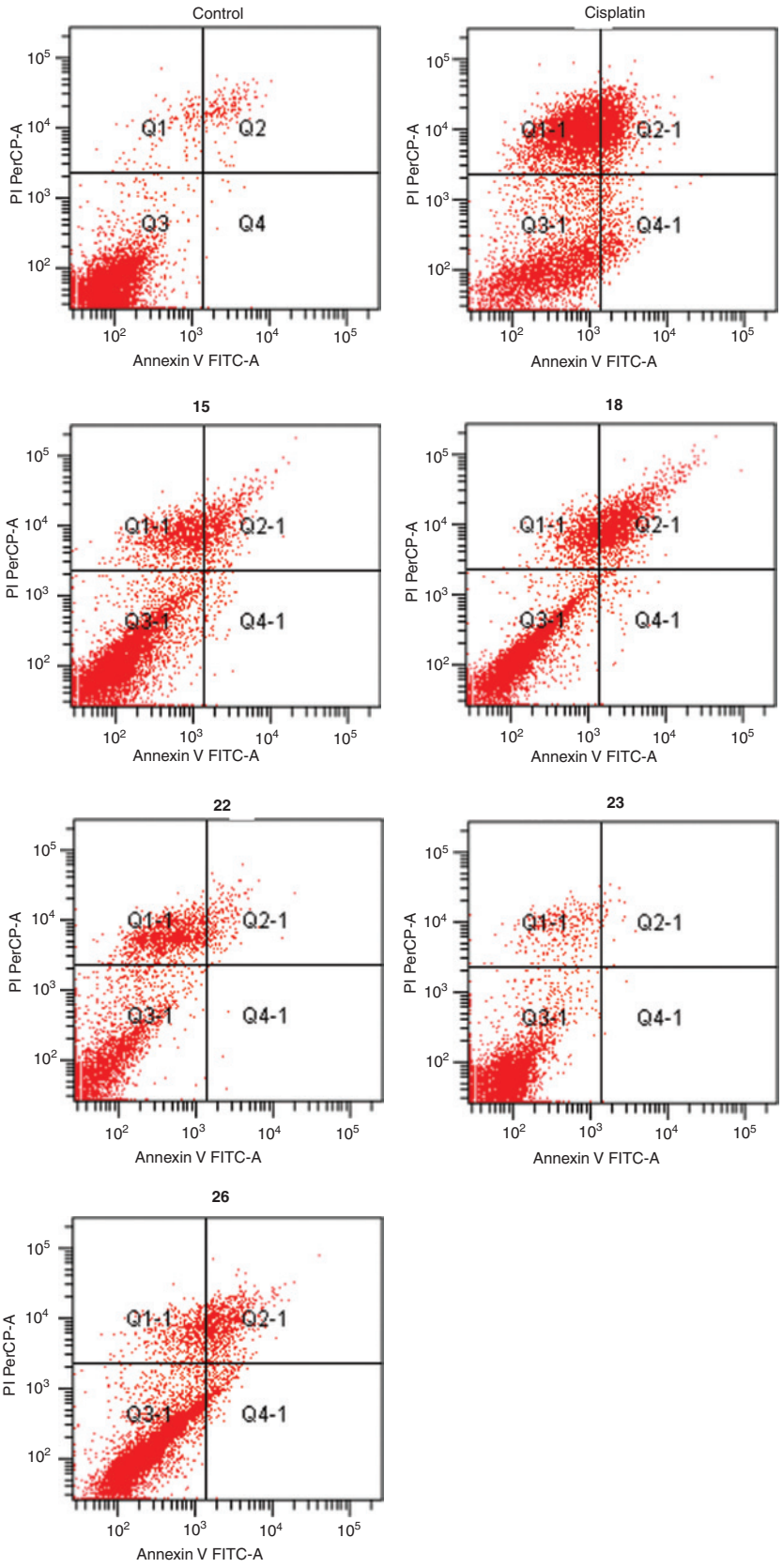
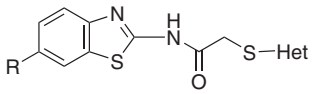


Figure 2: Flow cytometric diagrams of the A549 cell line as control group, cells treated with cisplatin and cell treated with compounds **15**, **18**, **22**, **23** and **26.**

Table 3: In silico physicochemical parameters of the compounds **13–30**.



13–30

Comp	R	Hetetocyclic ring	Log P	TPSA	MW	nON	nOHNH	nrotb	Volume
13	H	1-CH_3 benzimidazole	3.78	59.82	354.46	5	1	4	294.08
14	CH_3	1-CH_3 benzimidazole	4.20	59.82	368.49	5	1	4	310.64
15	OCH_3	1-CH_3 benzimidazole	3.81	69.05	384.49	6	1	5	319.62
16	Cl	1-CH_3 benzimidazole	4.43	59.82	388.90	5	1	4	307.62
17	F	1-CH_3 benzimidazole	3.92	59.82	372.45	5	1	4	299.01
18	OC_2H_5	1-CH_3 benzimidazole	4.18	69.05	398.51	6	1	6	336.43
19	H	1-CH_3 imidazole	2.27	59.82	304.40	5	1	4	250.09
20	CH_3	1-CH_3 imidazole	2.70	59.82	318.43	5	1	4	266.65
21	OCH_3	1-CH_3 imidazole	2.31	69.05	334.43	6	1	5	275.63
22	Cl	1-CH_3 imidazole	2.93	59.82	338.85	5	1	4	263.62
23	F	1-CH_3 imidazole	2.41	59.82	322.39	5	1	4	255.02
24	OC_2H_5	1-CH_3 imidazole	2.68	69.05	348.45	6	1	6	292.44
25	H	1-CH_3 triazole	1.74	72.71	305.39	6	1	4	245.93
26	CH_3	1-CH_3 triazole	2.16	72.71	319.42	6	1	4	262.49
27	OCH_3	1-CH_3 triazole	1.77	81.94	335.41	7	1	5	271.48
28	Cl	1-CH_3 triazole	2.39	72.71	339.83	6	1	4	259.47
29	F	1-CH_3 triazole	1.88	72.71	323.38	6	1	4	250.86
30	OC_2H_5	1-CH_3 triazole	2.15	81.94	349.44	7	1	6	288.28

Log P, log octanol/water partition coefficient; MW, Molecular weight; TPSA, total polar surface area; nON, no of hydrogen acceptors; nOHNH, no. of hydrogen donors; nrotb, no of rotatable bonds were calculated using molinspiration calculation of molecular properties toolkit.

Biochemistry

The synthesized compounds were screened for their in vitro antiproliferative activity against A549 non-small cell lung cancer cell line. The results were represented in Table 1 as in inhibition concentration (IC_{50}). The compounds were displayed cytotoxic profile within ranges 9.0 ± 1.0 μg/mL–500 μg/mL whereas standard drug showed cytotoxicity at 21.0 ± 2.0 μg/mL concentration. Compounds **15** and **18** exhibited highest antiproliferative activity with IC_{50} values 10.67 ± 2.02 and 9.0 ± 1.0 μg/mL which were lower even than cisplatin. These two compounds both have 1-methylbenzimidazole moiety, and also methoxy and ethoxy substituents on benzothiazole ring. This extraordinary result was not seen in the other members of this group, that IC_{50} values were not calculated for compounds **13**, **14** and **17** at the highest tested concentration. Among compounds containing 1-methylimidazole heterocyclic ring, compounds **22** and **23** exhibited remarkable cytotoxicity with IC_{50} of 46.67 ± 2.87 and 31.33 ± 7.57 μg/mL, respectively. Other compounds with the same ring showed poor activity with concentration higher than 256.67 μg/mL. Compounds bearing 1-methyltriazole showed moderate activity IC_{50} values between 64.33 and 153.33 μg/mL.

Five compounds (**15**, **18**, **22**, **23** and **26**) showed higher cytotoxicity, were studied further in the flow cytometry to detect apoptotic cell death ratios that they caused. Compounds and cisplatin were performed to the system at IC_{50} concentrations. The results of the Annexin V/PI staining protocol were summarized in Table 2 and the diagrams were represented in Figure 2. The survival of A549 cell line was confirmed and viable cells were found 96.7% for control group. Early, late apoptotic and viable cell ratios were determined as 17.0, 4.6, 37.0% for cisplatin treated tumor cells survived with a ratio of 37.0%. None of the compounds did not reach cisplatin's potency. Only compound **18** caused 19.1% (early and late) apoptosis totally whereas standard drug had 21.6% (early and late). Also, compound **26** exhibited half apoptotic potency of cisplatin.

Some physicochemical characteristics were calculated virtually and demonstrated in Table 3. For all compounds, log octanol/water partition coefficient, molecular weight, total polar surface area, number of hydrogen acceptors, number of hydrogen donors, number of rotatable bonds were calculated using Molinspiration Calculation of Molecular Properties toolkit. According to Lipinski rule of five [25] and its variations; an oral drug has log P lower than five, hydrogen bond donors lower than five, hydrogen

bond acceptors lower than 10, molecular mass less than 500 daltons, polar surface area equal to or less than 140 $Å^2$ and number of rotable bonds less than 10. Eighteen new compounds were found within the boundaries of this rule and they may be oral drugs considering the data.

Conclusion

Compounds **(15)**, namely, *N*-(6-methoxybenzothiazol-2-yl)-2-[(1-methyl-1*H*-benzimidazol-2-yl)thio] acetamide and (**18**), namely, *N*-(6-ethoxybenzothiazol-2-yl)-2-[(1-methyl-1*H*-benzimidazol-2-yl)thio] acetamide were found as the most potent compounds with lowest IC_{50} values 10.67 and 9.0 μg/mL against A549 lung cancer cell line. Among these two compounds, **18** caused 19.1% (early and late) apoptosis which was very close to cisplatin caused. Compounds were all detected to be an oral drug and some of them were found to be a potential antitumor agent. In further studies, new and similar compounds are planned to be sythesized for developing this issue. Besides, promising results are expected to be obtained with increased number of cell lines.

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

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