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RESEARCH ARTICLE

Synthesis and antitumor activity evaluation of new 2-(4-aminophenyl)benzothiazole derivatives bearing different heterocyclic rings

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Abstract

Twenty-five new *N*-[4-(benzothiazole-2-yl)phenyl]acetamide derivatives bearing different heterocyclic ring systems were synthesized using 2-(4-aminophenyl)benzothiazole structure as a pharmacophoric group. Final compounds were screened for their potential antitumor activity *in vitro* against approximately 60 human tumor cell lines derived from nine neoplastic diseases at National Cancer Institute, USA. 2-(4-Aminophenyl)benzothiazole structure was prepared by the reaction of 4-aminobenzoic acid and 2-aminothiophenol in polyphosphoric acid using microwave irradiation. After acetylation reaction, amide compounds **2a** and **2b** were obtained, which were then reacted with 2-mercapto(benz)imidazole/benzothiazole/benzoxazole derivatives in acetone with the presence of potassium carbonate to gain final compounds (**3**–**27**). Among all tested compounds, compound **10**, namely *N*-[4-(benzothiazole-2-yl)-3-chlorophenyl]-2-[(benzimidazole-2-yl)thio]acetamide, and compound **16**, namely *N*-[4-(benzothiazole-2-yl)phenyl]-2-[(1,5-diphenyl-1*H*-imidazole-2-yl)thio]acetamide, were found to be of considerable anticancer activity against some cancer cell lines.

Keywords

2-(4-aminophenyl)benzothiazole, anticancer activity, antitumor activity, synthesis

History

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Introduction

2-(4-Aminophenyl)benzothiazole structure is known with high antitumor activity since 1996[1–5]. Unexpectedly, it was found that, 2-(4-aminophenyl)benzothiazole derivatives inhibit cancer cell growth with nanomolar scale against a large panel of human cancer cell lines particularly against breast, colon and ovarian cell lines in *in vitro* anticancer screening program of the National Cancer Institute (NCI) with a characteristic biphasic dose–response relationship[6,7]. Up to present, scientists Shi and Bradshaw have a series of studies on antitumor activity of some benzothiazole derivatives[8–14]. First, the original lead compound 2-(4-aminophenyl)benzothiazole (CJM 126, NSC34445), which was originally prepared as a synthetic intermediate in a program of screening for tyrosine-kinase inhibitors, was found to possess selective *in vitro* activity against MCF-7 breast carcinoma cell line. This discovery was followed by the identification of the 2-(4-amino-3-methylphenyl)benzothiazole (DF 203, NSC 674495) and 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203, NSC 703786) and the evaluation of the analogue compounds with more potent and diverse activities[8–14].

Phortress (NSC 710305, dihydrochloride salt of the lysylamide prodrug of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203)), the fluorinated water-soluble pro-drug, which has been synthesized to address formulation and bioavailability issues related to the desired parenteral administration[15–19], was then chosen for phase 1 clinical trials in Britain in 2004 (Figure 1)[20]. The mechanism of action involves formation of reactive intermediates that can bind covalently to DNA and can be metabolized only by sensitive cancer cell lines[21]. Conversely, in insensitive cell lines, neither retaining nor metabolization occurs, thereby selective antitumor properties appear due through to metabolism[22–26].

Motivated by the above observations and extending our previous study[27], we planned to synthesize new 2-(4-aminophenyl)benzothiazole derivatives including (benz)imidazole/benzoxazole/benzothiazole heterocyclic ring systems and to evaluate their antitumor activity against nine cancer types comprised of approximately 60 cell lines.

Experimental section

The synthesis of 2-(4-aminophenyl)benzothiazole derivatives carried out by using Milestone microwave reaction apparatus (Milestone, Monroe, CT). Melting points were determined by using an Electrothermal IA9000 digital melting point apparatus (Electrothermal, Essex, UK). Spectroscopic data were recorded on the following instruments: a Bruker Tensor 27 IR spectrophotometer (Bruker Bioscience, Billerica, MA); a ^{1}H-NMR (nuclear magnetic resonance) Bruker DPX-400 FT-NMR spectrometer (Bruker Bioscience, Billerica, MA), and a mass spectrometry (MS) Agilent 1100 MSD spectrometer (Agilent Technologies,

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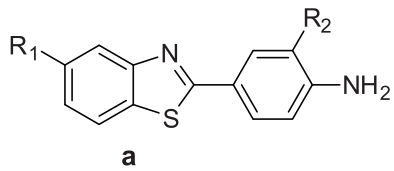
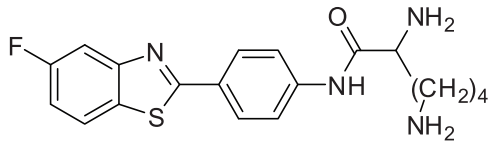
Figure 1. Chemical structures of some 2-(4-aminophenyl)benzothiazoles (a) and Phortress (b).



a

	R_1	R_2
CJM 126	H	H
DF 203	H	CH_3
5F 203	F	CH_3



b

Palo Alto, CA). Elemental analyses were performed in a Perkin Elmer EAL 240 elemental analyzer (Perkin-Elmer, Norwalk, CT) for C, H and N, and the results were found within 0.4% of the theoretical values.

2-(4-Aminophenyl)benzothiazole (1a)/2-(4-amino-2-chlorophenyl)benzothiazole (1b)

A mixture of 5 mL (46 mmol) 2-aminothiophenol, (46 mmol) 4-aminobenzoic acid/4-amino-2-chlorobenzoic acid and 300 mL polyphosphoric acid (PPA) was irradiated at 350 watt in a Microwave Organic Synthesis Apparatus for 30 min. The hot mixture was cooled, poured into ice-water and neutralized with sodium hydroxide solution. The precipitate formed was filtered and washed with water. **1a** = 82% yield; m.p. 155–157 °C. **1b** = yield 75%; m.p. 100–101 °C [7].

N-[4-(Benzothiazole-2-yl)phenyl]-2-chloroacetamide (2a)/ N-[4-(benzothiazole-2-yl)-3-chlorophenyl]-2-chloroacetamide (2b)

2-(4-Aminophenyl)benzothiazole/2-(4-amino-2-chlorophenyl)-benzothiazole (31.2 mmol) was solved in 100 mL tetrahydrofuran and 10 mL dimethylformamide. After 5.5 mL (37.4 mmol) triethylamine was added to the solution, 3 mL (37.4 mmol) chloroacetyl chloride was dropped into the solution for one hour. Then the mixture was poured into water, the precipitate formed was filtered. **2a** = yield 86%; m.p. 232–234 °C [9]. **2b** = yield 82%; m.p. 95–98 °C.

General procedure for the synthesis of N-[4-(benzothiazole-2-yl)phenyl]-2-[(substituted (benz)imidazole/thiazole/ oxazole-2-yl)thio]acetamide derivatives (3–27)

A mixture of *N*-[4-(benzothiazole-2-yl)phenyl]-2-chloroacetamide (1.65 mmol, 0.5 g), appropriate 2-mercapto derivatives (1.98 mmol) and K_2CO_3 (1.98 mmol, 0.3 g) in acetone was stirred for three hours. After TLC, acetone was evaporated, and the precipitate was treated with water and filtered. Dry product recrystallized from DMSO/alcohol.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[(benzimidazole-2-yl)thio]acetamide (3)

IR (KBr)ν_{max} (cm^{-1}): 3250 (N–H), 3067 (aromatic C–H), 2885 (aliphatic C–H), 1674 (C=O), 1541–1450 (C=C, C=N), 1342–1032 (C–N). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.33 (s, 2H, CH_2), 7.14 (m, 2H, Ar-H), 7.44 (m, 3H, Ar-H), 7.54 (dt, *J*: 8.24 Hz, *J*: 8.22 Hz, 1H, Ar-H), 7.81 (d, *J*: 8.74 Hz, 2H, Ar-H), 8.04 (d, *J*: 8.06 Hz, 1H, Ar-H), 8.07 (d, *J*: 6.91 Hz, 2H, Ar-H), 8.12 (d, *J*: 7.78 Hz, 1H, Ar-H), 10.87 (s, 1H, NH), 12.71 (s, 1H, benzimidazole NH). MS (ES^+): 417.1 (100%) M + 1, 418.1 (27.1%) M + 2, 419.1 (12.8%) M + 3. Anal Calcd for $C_{22}H_{16}N_4OS_2.3H_2O$: C: 56.15%, H: 4.71%, N: 11.91%; found C: 56.40%, H: 4.58%, N: 11.86%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[(5-chlorobenzimidazole-2-yl)thio]acetamide (4)

IR (KBr)ν_{max}(cm^{-1}): 3321 (N–H), 3053 (aromatic C–H), 2868 (aliphatic C–H), 1689 (C=O), 1556–1463 (C=C, C=N), 1313–1053 (C–N). ^{1}H-NMR (400 MHz)(DMSO-d_6) δ (ppm): 4.32 (s, 2H, CH_2), 7.13 (dd, *J*: 8.49 Hz, 1H, Ar-H), 7.43–7.47 (m, 2H, Ar-H), 7.51 (d, *J* = 1.94 Hz, 1H, Ar-H), 7.54 (dt, *J*: 7.64 Hz, *J*: 7.62 Hz, 1H, Ar-H), 7.80 (d, *J*: 7.89 Hz, 2H, Ar-H), 8.03 (d, *J*: 8.02 Hz, 1H, Ar-H), 8.08 (d, *J*: 7.86 Hz, 2H, Ar-H), 8.13 (d, *J*: 7.90 Hz, 1H, Ar-H), 11.10 (s, 1H, NH), 12.70 (s, 1H, benzimidazole NH). MS (ES^+): 451.5 (100%) M + 1, 452.5 (27%) M + 2, 453.5 (43%) M + 3. Anal Calcd for $C_{22}H_{15}ClN_4OS_2.H_2O$: C: 56.34%, H: 3.65%, N: 11.95%; found C: 56.51%, H: 3.42%, N: 11.68%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[(5-methylbenzimidazole-2-yl)thio]acetamide (5)

IR (KBr)ν_{max} (cm^{-1}): 3240 (N–H), 3049 (aromatic C–H), 2864 (aliphatic C–H), 1693 (C=O), 1548–1437 (C=C, C=N), 1316–1028 (C–N). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.38 (s, 3H, CH_3), 4.31 (s, 2H, CH_2), 6.96 (t, *J*: 7.82 Hz, 1H, Ar-H), 7.25 (m, 1H, Ar-H), 7.40 (m, 2H, Ar-H), 7.54 (dt, *J*: 8.11 Hz, *J*: 8.09 Hz, 1H, Ar-H), 7.81 (d, *J*: 8.69 Hz, 2H, Ar-H), 8.08 (d, 2H *J*: 8.64 Hz, Ar-H), 8.13 (d, *J*: 7.82 Hz, 1H, Ar-H), 8.30 (d, *J*: 8.04 Hz, 1H, Ar-H), 10.90 (s, 1H, NH), 12.60 (1H, s, imidazole NH). MS (ES^+): 431 (100%) M + 1, 432 (25%) M + 2, 433 (12%) M + 3. Anal Calcd for $C_{23}H_{18}N_4OS_2.H_2O$:C:61.59%, H: 4.49%, N: 12.49%; found C: 61.36%, H: 4.35%, N: 12.59%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[(benzothiazole-2-yl)thio]acetamide (6)

IR (KBr) ν_{max} (cm^{-1}): 3290 (N–H), 3176 (aromatic C–H), 3053 (aliphatic C–H), 1674 (C=O), 1556–1429 (C=C, C=N), 1313–1056 (C–N). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.45 (s, 2H, CH_2), 7.38 (dt, *J*: 8.15 Hz, *J*: 8.14 Hz, 1H, Ar-H), 7.46 (m, 2H, Ar-H), 7.54 (dt, *J*: 8.22 Hz, *J*: 8.21 Hz, 1H, Ar-H), 7.83 (m, 3H, Ar-H), 8.03 (d, *J*: 8.13 Hz, 2H, Ar-H), 8.08 (d, *J*: 7.90 Hz, 2H, Ar-H), 8.13 (d, *J*: 7.90 Hz, 1H, Ar-H), 10.80 (s, 1H, NH). MS (ES^+): 434 (100%) M + 1, 435 (27%) M + 2, 436 (17%) M + 3.

Anal Calcd for $C_{22}H_{15}N_3OS_3.3H_2O$: C: 54.19%, H: 4.34%, N: 8.62%; found C: 54.07%, H: 4.39%, N: 8.56%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[(5-chlorobenzothiazole-2-yl)thio]acetamide (7)

IR (KBr) ν_{max} (cm^{-1}): 3242 (N–H), 3174 (aromatic C–H), 2951 (aliphatic C–H), 1683 (C=O), 1545–1423 (C=C, C=N), 1332–1115 (C–N). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.46 (s, 2H, CH_2), 7.44 (m, 2H, Ar-H), 7.54 (dt, *J*: 8.23 Hz, *J*: 8.21 Hz, 1H, Ar-H), 7.81 (d, *J*: 8.72 Hz, 2H, Ar-H), 7.90 (d, *J*: 2.05 Hz, 1H, Ar-H), 8.03 (d, *J*: 8.04 Hz, 1H, Ar-H), 8.07 (t, *J*: 7.22 Hz, 3H, Ar-H), 8.13 (d, *J*: 7.94 Hz, 1H, Ar-H), 10.80 (s, 1H, NH). MS (ES^+): 468.5 (100%) M + 1, 469.5 (27%) M + 2, 470.5 (49%) M + 3. Anal Calcd for $C_{22}H_{14}ClN_3OS_3.H_2O$: C: 54.37%, H: 3.32%, N: 8.65%; found C: 54.30%, H: 3.19%, N: 8.81%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[(benzoxazole-2-yl)thio]acetamide (8)

IR (KBr) ν_{max} (cm^{-1}): 3325 (N–H), 3051 (aromatic C–H), 2912 (aliphatic C–H), 1674 (C=O), 1593–1467 (C=C, C=N), 1312–1024 (C–N, O–C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.50 (s, 2H, CH_2), 7.32–7.36 (m, 2H, Ar-H), 7.45 (dt, *J*: 7.58 Hz, *J*: 7.56 Hz, 1H, Ar-H), 7.54 (dt, *J*: 6.84 Hz, *J*: 6.83 Hz, 1H, Ar-H), 7.63–7.69 (m, 2H, Ar-H), 7.82 (d, *J*: 8.74 Hz, 2H, Ar-H), 8.03 (d, *J*: 2.68 Hz, 1H, Ar-H), 8.09 (d, *J*: 8.77 Hz, 2H, Ar-H), 8.14 (d, *J*: 7.82 Hz, 1H, Ar-H), 10.82 (1H, s, NH). MS (ES^+): 418 (100%) M + 1, 419 (20%) M + 2, 420 (14%) M + 3. Anal Calcd for $C_{22}H_{15}N_3O_2S_2.4H_2O$: C: 53.97%, H: 4.70%, N: 8.58%; found C: 54.06%, H: 4.68%, N: 8.51%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[(5-methylbenzoxazole-2-yl)thio]acetamide (9)

IR (KBr) ν_{max} (cm^{-1}): 3261 (N–H), 3057 (aromatic C–H), 2900 (aliphatic C–H), 1697 (C=O), 1548–1429 (C=C, C=N), 1349–1028 (C–N, C–O). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 7.14 (d, *J*: 7.22 Hz, 1H, Ar-H), 7.45 (m, 2H, Ar-H), 7.55 (m, 2H, Ar-H), 7.81 (d, *J*: 8.73 Hz, 2H, Ar-H), 8.04 (d, *J*: 8.01 Hz, 1H, Ar-H), 8.08 (d, *J*: 6.07 Hz, 2H, Ar-H), 8.13 (d, *J*: 7.93 Hz, 1H, Ar-H), 10.80 (s, 1H, NH). MS (ES^+): 432 (100%) M + 1, 433 (26%) M + 2, 434 (13.2%) M + 3. Anal Calcd for $C_{23}H_{17}N_3O_2S_2.H_2O$: C: 61.45%, H: 4.26%, N: 9.35%; found C: 61.32%, H: 4.09%, N: 9.61%.

N-[4-(Benzothiazole-2-yl)-3-chlorophenyl]-2-[(benzimidazole-2-yl)thio]acetamide (10)

IR(KBr) ν_{max} (cm^{-1}): 3434 (N–H), 3051 (aromatic C–H), 2986 (aliphatic C–H), 1677 (C=O), 1599–1497 (C=C, C=N), 1326–1045 (C–N). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.34 (s, 2H, CH_2), 7.13 (m, 2H, Ar-H), 7.45 (m, 2H, Ar-H), 7.50 (dt, *J*: 8.15 Hz, *J*: 8.14 Hz, 1H, Ar-H), 7.58 (dt, *J*: 8.30 Hz, *J*: 8.28 Hz, 1H, Ar-H), 7.68 (dd, *J*: 2.15 Hz, *J*: 2.11 Hz, 1H, Ar-H), 8.04 (d, *J*: 2.10 Hz, 1H, Ar-H), 8.10 (d, *J*: 7.96 Hz, 1H, Ar-H), 8,19 (d, *J*: 7.47 Hz, 1H, Ar-H), 8.28 (d, *J*: 8.72 Hz, 1H, Ar-H), 10.99 (s, 1H, NH), 12.68 (s, 1H, benzimidazole NH). MS (ES^+): 451 (100%) M + 1, 452 (24%) M + 2, 453 (40%) M + 3. Anal Calcd for $C_{22}H_{15}ClN_4OS_2.1/4H_2O$: C: 58.02%, H: 3.30%, N: 12.31%; found C: 57.92%, H: 3.28%, N: 12.28%.

N-[4-(Benzothiazole-2-yl)-3-chlorophenyl]-2-[(5-chlorobenzimidazole-2-yl)thio]acetamide (11)

IR (KBr) ν_{max} (cm^{-1}): 3290 (N–H), 3054 (aromatic C–H), 2929 (aliphatic C–H), 1677 (C=O), 1596–1454 (C=C, C=N), 1334–1021 (C–N). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.32 (s, 2H, CH_2), 7.13 (dd, *J*: 8.49 Hz, 1H, Ar-H), 7.43–7.47 (m, 1H, Ar-H), 7.49 (dt, *J*: 7.62 Hz, *J*: 7.60 Hz, 1H, Ar-H), 7.52 (dt, *J*: 8.15 Hz, *J*: 8.14 Hz, 1H, Ar-H), 7.58 (dt, *J*: 8.30 Hz, *J*: 8.28 Hz, 1H, Ar-H), 7.68 (dd, *J*: 2.15 Hz, *J*: 2.11 Hz, 1H, Ar-H), 8.04 (d, *J*: 2.10 Hz, 1H, Ar-H), 8.10 (d, *J*: 7.96 Hz, 1H, Ar-H), 8.19 (d, *J*: 7.47 Hz, 1H, Ar-H), 8.28 (d, *J*: 8.72 Hz, 1H, Ar-H), 10.99 (s, 1H, NH), 12.68 (s, 1H, benzimidazole NH). MS (ES^+): 485 (100%) M + 1, 486 (23%) M + 2, 487 (71.7%) M + 3. Anal Calcd for $C_{22}H_{14}Cl_2N_4OS_2.1/2H_2O$: C: 53.44%, H: 3.04%, N: 11.34%; found C: 53.57%, H: 3.22%, N: 11.52%.

N-[4-(Benzothiazole-2-yl)-3-chlorophenyl]-2-[(5-methylbenzimidazole-2-yl)thio]acetamide (12)

IR (KBr) ν_{max} (cm^{-1}): 3238 (NH), 3071 (aromatic C–H), 2917 (aliphatic C–H), 1672 (C=O), 1600–1457 (C=C, C=N), 1342–1034 (C–N). ^{1}H-NMR (400 MHz)(DMSO-d_6) δ (ppm): 2.38 (s, 3H, CH_3), 4.31 (s, 2H, CH_2), 6.96 (t, *J*: 7.82 Hz, 1H, Ar-H), 7.25 (m, 1H, Ar-H), 7.40 (m, 1H, Ar-H), 7.50 (dt, *J*: 8.15 Hz, *J*: 8.14 Hz, 1H, Ar-H), 7.58 (dt, *J*: 8.30 Hz, *J*: 8.28 Hz, 1H, Ar-H), 7.68 (dd, *J*: 2.15 Hz, *J*: 2.11 Hz, 1H, Ar-H), 8.04 (d, *J*: 2.10 Hz, 1H, Ar-H), 8.10 (d, *J*: 7.96 Hz, 1H, Ar-H), 8,19 (d, *J*: 7.47 Hz, 1H, Ar-H), 8.28 (d, *J*: 8.72 Hz, 1H, Ar-H), 10.99 0 (s, 1H, NH), 12.68 (s, 1H, benzimidazole NH). MS (ES^+): 465.5 (100%) M + 1, 466.5 (25.2%) M + 2, 467.5 (32%) M + 3. Anal Calcd for $C_{23}H_{17}ClN_4OS_2.H_2O$: C: 57.19%, H: 3.96%, N: 11.60%; found C: 57.28%, H: 3.75%, N: 12.02%.

N-[4-(Benzothiazole-2-yl)-3-chlorophenyl]-2-[(benzothiazole-2-yl)thio]acetamide (13)

IR (KBr) ν_{max} (cm^{-1}): 3249 (NH), 3049 (aromatic C–H), 2945 (aliphatic C–H), 1690 (C=O), 1602–1457 (C=C, C=N), 1343–1021 (C–N). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm):4.47 (s, 2H, CH_2), 7.38 (t, *J*: 7.85 Hz, 1H, Ar-H), 7.50 (m, 2H, Ar-H) 7.59 (t, *J*: 8.08 Hz, 1H, Ar-H), 7.69 (dd, *J*: 1.92 Hz, *J*: 1.99 Hz, 1H, Ar-H), 7.85 (d *J*: 8.02 Hz, 1H, Ar-H), 8.04 (m, 2H, Ar-H), 8.10 (d, *J*: 8.07 Hz, 1H, Ar-H), 8.19 (d, *J*: 7.99 Hz, 1H, Ar-H), 8.29 (d, *J*: 8.71 Hz, 1H, Ar-H), 10.94 (s, 1H, NH). MS (ES^+): 468 (100%) M + 1, 469 (24%) M + 2, 470 (52%) M + 3, 471 (14%) M + 4. Anal Calcd for $C_{22}H_{14}ClN_3OS_3$: C: 56.46%, H: 3.03%, N: 8.98%; found C: 56.38%, H: 3.01%, N: 8.93%.

N-[4-(Benzothiazole-2-yl)-3-chlorophenyl]-2-[(benzoxazole-2-yl)thio]acetamide (14)

IR (KBr) ν_{max} (cm^{-1}): 3232 (NH), 3066 (aromatic C–H), 2961 (aliphatic C–H), 1666 (C=O), 1589–1457 (C=C, C=N), 1325–1018 (C–N, O–C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.50 (s, 2H, CH_2), 7.32–7.36 (m, 2H, Ar-H), 7.63–7.69 (m, 2H, Ar-H), 7.70 (dt, *J*: 8.15 Hz, *J*: 8.14 Hz, 1H, Ar-H), 7.78 (dt, *J*: 8.30 Hz, *J*: 8.28 Hz, 1H, Ar-H), 7.88 (dd, *J*: 2.13 Hz, *J*: 2.08 Hz, 1H, Ar-H), 8.04 (d, *J*: 2.10 Hz, 1H, Ar-H), 8.10 (d, *J*: 7.96 Hz, 1H, Ar-H), 8,19 (d, *J*: 7.47 Hz, 1H, Ar-H), 8.28 (1H, d, *J*: 8.72 Hz, Ar-H), 10.99 (s, 1H, NH). MS (ES^+): 451.5 (100%) M + 1, 452.5 (25.7%) M + 2, 453.5 (42.8%) M + 3. Anal Calcd for $C_{22}H_{14}ClN_3O_2S_2$: C: 58.47%, H: 3.12%, N: 9.30%; found C: 58.54%, H: 3.01%, N: 9.14%.

N-[4-(Benzothiazole-2-yl)-3-chlorophenyl]-2-[(5-methylbenzoxazole-2-yl)thio]acetamide (15)

IR (KBr) ν_{max} (cm^{-1}): 3247 (NH), 3064 (aromatic C–H), 2963 (aliphatic C–H), 1687 (C=O), 1527–1345 (C=C, C=N), 1350–1076 (C–N, O–C). ^{1}H-NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 7.14 (d, *J*: 7.22 Hz, 1H, Ar-H), 7.45 (m, 2H, Ar-H), 7.50 (dt, *J*: 8.15 Hz, *J*: 8.14 Hz, 1H, Ar-H), 7.58 (dt, *J*: 8.30 Hz, *J*: 8.28 Hz, 1H, Ar-H), 7.68 (dd, *J*:

2.15 Hz, *J*: 2.11 Hz, 1H, Ar-H), 8.04 (d, *J*: 2.10 Hz, 1H, Ar-H), 8.10 (d, *J*: 7.96 Hz, 1H, Ar-H), 8.19 (d, *J*: 7.47 Hz, 1H, Ar-H), 8.28 (d, *J*: 8.72 Hz, 1H, Ar-H), 10.99 (s, 1H, NH). MS (ES^+): 466.5 (100%) M + 1, 467.5 (27.8%) M + 2, 468.5 (45.1%) M + 3. Anal Calcd for $C_{23}H_{16}ClN_3O_2S_2$: C: 59.28%, H: 3.46%, N: 9.02%; found C: 59.32%, H: 3.43%, N: 9.04%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[(1,5-diphenyl-1H-imidazole-2-yl)thio]acetamide (16)

IR (KBr) ν_{max} (cm^{-1}): 3248 (N–H), 3032 (aromatic C–H), 2943 (aliphatic C–H), 1694 (C=O), 1604–1482 (C=C, C=N), 1333–1015 (C–N). ^1H NMR (400 MHz)(DMSO-d_6) δ (ppm): 4.10 (2H, s, CH_2), 7.09 (d, *J*: 7.20 Hz, 2H, Ar-H), 7.24 (m, 3H, Ar-H), 7.33 (m, 2H, Ar-H), 7.37 (s, 1H, Ar-H), 7.50 (m, 5H, Ar-H), 7.79 (d, *J*: 8.68 Hz, 2H, Ar-H), 8.03 (d, *J*: 8.04 Hz, 1H, Ar-H), 8.07 (d, *J*: 8.64 Hz, 2H, Ar-H), 8.13 (d, *J*: 8.01 Hz, 1H, Ar-H), 10.71 (s, 1H, NH). MS (ES^+): 519.1 (100%) M + 1, 520.1 (36%) M + 2, 521.1 (14%) M + 3. Anal Calcd for $C_{30}H_{22}N_4OS_2.1/2H_2O$: C: 68.31%, H: 4.36%, N: 10.61%; found C: 68.19%, H: 4.16%, N: 10.59%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1,5-bis(4-methylphenyl)-1H-imidazole-2-yl]thio]acetamide (17)

IR (KBr) ν_{max} (cm^{-1}): 3259 (N–H), 3034 (aromatic C–H), 2921 (aliphatic C–H), 1693 (C=O), 1602–1481 (C=C, C=N), 1315–1028 (C–N). ^1H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.69 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 4.08 (s, 2H, CH_2), 6.78 (d, *J*: 6.69 Hz, 2H, Ar-H), 7.06 (t, *J*: 8.81 Hz, *J*: 8.78 Hz, 4H, Ar-H) 7.28 (d, *J*: 8.88 Hz, 3H, Ar-H), 7.47 (dt, *J*: 7.53 Hz, *J*: 7.53 Hz, 1H, Ar-H), 7.59 (dt, *J*: 7.79 Hz, *J*: 7.67 Hz, 1H, Ar-H), 7.81 (d, *J*: 8.78 Hz, 2H, Ar-H), 8.15 (m, 4H, Ar-H), 10.71 (s, 1H, NH). MS (ES^+): 547.1 (100%) M + 1, 548.1 (39%) M + 2, 549.1 (18%) M + 3. Anal Calcd for $C_{32}H_{26}N_4OS_2$: C: 70.30%, H: 4.79%, N: 10.25%, found: C: 70.36%, H: 4.78%, N: 10.27%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1,5-bis(4-methoxyphenyl)-1H-imidazole-2-yl]thio]acetamide (18)

IR (KBr) ν_{max} (cm^{-1}): 3270 (N–H), 3054 (aromatic C–H), 2957 (aliphatic C–H), 1696 (C=O), 1604–1413 (C=C, C=N), 1321–1005 (C–N). ^1H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.69 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.04 (s, 2H, CH_2), 6.82 (d, *J*: 6.88 Hz, 2H, Ar-H), 7.02 (t, *J*: 8.69 Hz, *J*: 8.69 Hz, 4H, Ar-H) 7.22 (d, *J*: 8.84 Hz, 3H, Ar-H), 7.44 (dt, *J*: 7.53 Hz, *J*: 7.53 Hz, 1H, Ar-H), 7.53 (dt, *J*: 7.67 Hz, *J*: 7.67 Hz, 1H, Ar-H), 7.78 (d, *J*: 8.77 Hz, 2H, Ar-H), 8.10 (m, 4H, Ar-H), 10.70 (s, 1H, NH). MS (ES^+): 579.1 (100%) M + 1, 580.1 (39%) M + 2, 581.1 (18%) M + 3. Anal Calcd for $C_{32}H_{26}N_4O_3S_2.H_2O$: C: 64.41%, H: 4.73%, N: 9.39%, found: C: 64.29%, H: 4.54%, N: 9.37%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-(4-methylphenyl)-5-(4′-methoxyphenyl)-1H-imidazole-2-yl]thio]acetamide (19)

IR (KBr) ν_{max} (cm^{-1}): 3263 (N–H), 3055 (aromatic C–H), 2921 (aliphatic C–H), 1696 (C=O), 1604–1456 (C=C, C=N), 1334–1017 (C–N). ^1H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.35 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2), 6.82 (d, *J*: 8.82 Hz, 2H, Ar-H), 7.03 (d, *J*: 8.76 Hz, 2H, Ar-H), 7.18 (d, *J*: 8.23 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 7.28 (d, *J*: 8.28 Hz, 2H, Ar-H), 7.44 (dt, *J*: 7.56 Hz, *J*: 7.58 Hz, 1H, Ar-H), 7.54 (dt, *J*: 8.17 Hz, *J*: 8.28 Hz, 1H, Ar-H), 7.79 (d, *J*: 6.77 Hz, 2H, Ar-H), 8.07 (m, 4H, Ar-H), 10.72 (s, 1H, NH). MS (ES^+): 563.1 (100%) M + 1, 564.1 (39%) M + 2, 565.1 (18%) M + 3. Anal Calcd for $C_{32}H_{26}N_4O_2S_2.2H_2O$: C: 64.19%, H: 5.05%, N: 9.36%, found: C: 64.13%, H: 4.98%, N: 9.32%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-(4-methoxyphenyl)-5-(4′-methylphenyl)-1H-imidazole-2-yl]thio]acetamide (20)

IR (KBr) ν_{max} (cm^{-1}): 3258 (N–H), 3054 (aromatic C–H), 2933 (aliphatic C–H), 1696 (C=O), 1614–1423 (C=C, C=N), 1318–1063 (C–N). ^1H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.37 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 4.07 (s, 2H, CH_2), 6.86 (d, *J*: 8.82 Hz, 2H, Ar-H), 7.06 (d, *J*: 8.82 Hz, 2H, Ar-H), 7.19 (d, *J*: 8.23 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 7.29 (d, *J*: 8.29 Hz, 2H, Ar-H), 7.46 (dt, *J*: 7.58 Hz, *J*: 7.58 Hz, 1H, Ar-H), 7.55 (dt, *J*: 8.19 Hz, *J*: 8.32 Hz, 1H, Ar-H), 7.80 (d, *J*: 6.84 Hz, 2H, Ar-H), 8.09 (m, 4H, Ar-H), 10.75 (s, 1H, NH). MS (ES^+): 563.1 (100%) M + 1, 564.1 (39%) M + 2, 565.1 (18%) M + 3. Anal Calcd for $C_{32}H_{26}N_4O_2S_2$: C: 68.30%, H: 4.66%, N: 9.96%, found: C: 68.33%, H: 4.68%, N: 9.86%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-(4-methylphenyl)-5-phenyl-1H-imidazole-2-yl]thio]acetamide (21)

IR (KBr) ν_{max} (cm^{-1}): 3261 (N–H), 3055 (aromatic C–H), 2921 (aliphatic C–H), 1694 (C=O), 1602–1480 (C=C, C=N), 1318–1056 (C–N). ^1H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.36 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 7.11 (d, *J*: 7.49 Hz, 2H, Ar-H), 7.20 (m, 5H, Ar-H), 7.29 (d, *J*: 8.20 Hz, 2H, Ar-H), 7.35 (s, 1H, Ar-H), 7.44 (dt, *J*: 7.58 Hz, *J*: 7.1 Hz, 1H, Ar-H), 7.53 (dt, *J*: 7.63 Hz, *J*: 7.69 Hz, 1H, Ar-H), 7.79 (d, *J*: 8.74 Hz, 2H, Ar-H), 8.05 (m, 3H, Ar-H), 8.13 (d, *J*: 7.95 Hz, 1H, Ar-H), 10.72 (s, 1H, NH). MS (ES^+): 533.1 (100%) M + 1, 534.1 (39%) M + 2, 535.1 (18%) M + 3. Anal Calcd for $C_{31}H_{24}N_4OS_2$: C: 69.90%, H: 4.54%, N: 10.52%, found: C: 69.63%, H: 4.32%, N: 9.95%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-(4-methoxyphenyl)-5-phenyl-1H-imidazole-2-yl]thio]acetamide (22)

IR (KBr) ν_{max} (cm^{-1}): 3260 (N–H), 3053 (aromatic C–H), 2933 (aliphatic C–H), 1697 (C=O), 1605–1473 (C=C, C=N), 1345–1032 (C–N). ^1H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.67 (s, 3H, OCH_3), 4.11 (s, 2H, CH_2), 7.19 (d, *J*: 7.53 Hz, 2H, Ar-H), 7.25 (m, 5H, Ar-H), 7.45 (d, *J*: 8.36 Hz, 2H, Ar-H), 7.53 (s, 1H, Ar-H), 7.62 (dt, *J*: 7.12 Hz, *J*: 7.58 Hz, 1H, Ar-H), 7.55 (dt, *J*: 7.69 Hz, *J*: 7.65 Hz, 1H, Ar-H), 7.79 (d, *J*: 8.74 Hz, 2H, Ar-H), 8.05 (m, 3H, Ar-H), 8.15 (d, *J*: 7.93 Hz, 1H, Ar-H), 10.74 (s, 1H, NH). MS (ES^+): 549.1 (100%) M + 1, 550.1 (33%) M + 2, 551.1 (17%) M + 3. Anal Calcd for $C_{31}H_{24}N_4O_2S_2$: C: 67.86 %, H: 4.41%, N: 10.21%, found: C: 68.05%, H: 4.61%, N: 10.01%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-phenyl-5-(4′-methylphenyl)-1H-imidazole-2-yl]thio]acetamide (23)

IR (KBr) ν_{max} (cm^{-1}): 3258 (N–H), 3055 (aromatic C–H), 2920 (aliphatic C–H), 1695 (C=O), 1602–1481 (C=C, C=N), 1331–1017 (C–N). ^1H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.36 (s, 3H, CH_3), 4.08 (s, 2H, CH_2), 7.11 (d, *J*: 7.38 Hz, 2H, Ar-H), 7.22 (m, 5H, Ar-H), 7.3 (d, *J*: 8.06 Hz, 2H, Ar-H), 7.35 (s, 1H, Ar-H), 7.45 (dt, *J*: 7.52 Hz, *J*: 7.29 Hz, 1H, Ar-H), 7.54 (dt, *J*: 7.19 Hz, *J*: 7.67 Hz, 1H, Ar-H), 7.78 (d, *J*: 8.79 Hz, 2H, Ar-H), 8.03 (d, *J*: 7.94 Hz, 1H, Ar-H), 8.07 (d, *J*: 8.76 Hz, 2H, Ar-H), 8.13 (d, *J*: 7.64 Hz, 1H, Ar-H), 10.71 (s, 1H, NH). MS (ES^+): 533.1 (100%) M + 1, 534.1 (33%) M + 2, 535.1 (17 %) M + 3. Anal Calcd for $C_{31}H_{24}N_4OS_2.3/2H_2O$: C: 66.55%, H: 4.83%, N: 10.01%, found: C: 66.51%, H: 4.67%, N: 9.98%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-phenyl-5-(4′-methoxyphenyl)-1H-imidazole-2-yl]thio]acetamide (24)

IR (KBr) ν_{max} (cm^{-1}): 3257 (N–H), 3054 (aromatic C–H), 2929 (aliphatic C–H), 1694 (C=O), 1604–1499 (C=C, C=N),

1342–1054 (C–N). ^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.68 (s, 3H, OCH_3), 4.08 (s, 2H, CH_2), 6.80 (d, *J*: 8.84 Hz, 2H, Ar-H), 7.02 (d, *J*: 8.80 Hz, 2H, Ar-H), 7.30 (m, 2H, Ar-H), 7.35 (s, 1H, Ar-H), 7.48 (m, 5H, Ar-H), 7.79 (d, *J*: 8.75 Hz, 2H, Ar-H), 8.07 (m, 4H, Ar-H), 10.74 (s, 1H, NH). MS (ES^+): 549.1 (100%) M + 1, 550.1 (33%) M + 2, 551.1 (17 %) M + 3. Anal Calcd for $C_{31}H_{24}N_4O_2S_2.H_2O$: C: 65.70%, H: 4.62%, N: 9.89%, found: C: 65.61%, H: 4.47%, N: 9.82%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-phenyl-5-(4′-fluorophenyl)-1H-imidazole-2-yl]thio]acetamide (25)

IR (KBr) ν_{max} (cm^{-1}): 3252 (N–H), 3055 (aromatic C–H), 2929 (aliphatic C–H), 1688 (C=O), 1602–1498 (C=C, C=N), 1316–1027 (C–N). ^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.10 (s, 2H, CH_2), 7.12 (m, 4H, Ar-H), 7.33 (m, 3H, Ar-H), 7.50 (m, 5H, Ar-H), 7.79 (d, *J*: 8.71 Hz, 2H, Ar-H), 8.05 (m, 3H, Ar-H), 8.13 (d, *J*: 7.96 Hz, 1H, Ar-H), 10.71 (s, 1H, NH). MS (ES^+): 537.1 (100%) M + 1, 538.1 (37%) M + 2, 539 (14%) M + 3. Anal Calcd for $C_{30}H_{21}FN_4OS_2.2H_2O$: C: 62.96%, H: 4.40%, N: 9.78%, found: C: 62.91%, H: 4.66% H, N: 9.74%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-(4-methylphenyl)-5-(4′-fluorophenyl)-1H-imidazole-2-yl]thio]acetamide (26)

IR (KBr) ν_{max} (cm^{-1}): 3268 (N–H), 3056 (aromatic C–H), 2920 (aliphatic C–H), 1699 (C=O), 1603–1497 (C=C, C=N), 1347–1030 (C–N). ^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.35 (s, 3H, CH_3), 4.07 (s, 2H, CH_2), 6.83 (d, *J*: 8.72 Hz, 2H, Ar-H), 7.05 (d, *J*: 8.73 Hz, 2H, Ar-H), 7.21 (d, *J*: 8.14 Hz, 2H, Ar-H), 7.27 (s, 1H, Ar-H), 7.32 (d, *J*: 8.29 Hz, 2H, Ar-H), 7.45 (dt, *J*: 7.59 Hz, *J*: 7.59 Hz, 1H, Ar-H), 7.54 (dt, *J*: 8.18 Hz, *J*: 8.28 Hz, 1H, Ar-H), 7.83 (d, *J*: 6.94 Hz, 2H, Ar-H), 8.09 (m, 4H, Ar-H), 10.77 (s, 1H, NH). MS (ES^+): 550.1 (100%) M + 1, 551.1 (39%) M + 2, 552.1 (18%) M + 3. Anal Calcd for $C_{31}H_{23}FN_4OS_2$: C: 67.61%, H: 4.21%, N: 10.17%, found: C: 67.63%, H: 4.28%, N: 10.26%.

N-[4-(Benzothiazole-2-yl)phenyl]-2-[[1-(4-methoxyphenyl)-5-(4′-fluorophenyl)-1H-imidazole-2-yl]thio]acetamide (27)

IR (KBr) ν_{max} (cm^{-1}): 3269 (N–H), 3056 (aromatic C–H), 2958 (aliphatic C–H), 1698 (C=O), 1609–1459 (C=C, C=N), 1336–1012 (C–N). ^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.79 (s, 3H, OCH_3), 4.08 (s, 2H, CH_2), 7.03 (d, *J*: 8.85 Hz, 2H, Ar-H), 7.14 (m, 4H, Ar-H), 7.25 (d, *J*: 8.85 Hz, 2H, Ar-H), 7.33 (s, 1H, Ar-H), 7.44 (t, *J*: 7.28 Hz, *J*: 7.59 Hz, 1H, Ar-H), 7.53 (t, *J*: 7.16 Hz, *J*: 7.43 Hz, 1H, Ar-H), 7.79 (d, *J*: 8.7 Hz, 2H, Ar-H), 8.07 (m, 4H, Ar-H), 10.72 (s, 1H, NH). MS (ES^+): 566.1 (100%) M + 1, 567.1 (37%) M + 2, 568 (14%) M + 3. Anal Calcd for $C_{31}H_{23}FN_4O_2S_2$: C: 65.71%, H: 4.09%, N: 9.89%, found: C: 65.81%, H: 4.23% H, N: 9.79%.

Anticancer activity

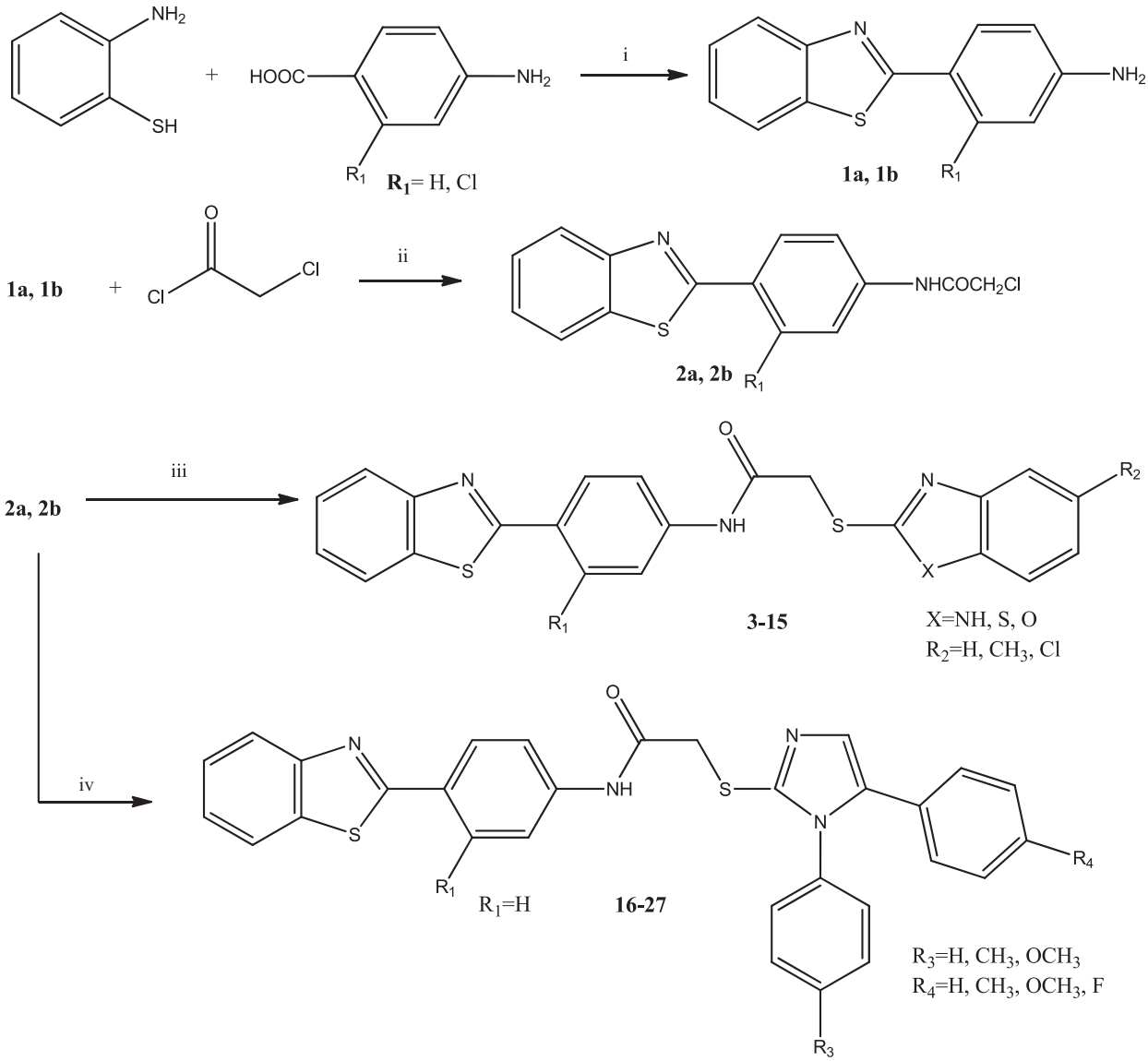
The cyctotoxic and/or growth inhibitory effects of the compounds were evaluated *in vitro* against approximately 60 human tumor cell lines derived from nine neoplastic diseases, namely: leukemia (L), non-small cell lung cancer (NSCLC), colon cancer (CC), central nervous system cancer (CNSC), melanoma (M), ovarian cancer (OC), renal cancer (RC), prostate cancer (PC) and breast cancer (BC) at the NCI, Bethesda, USA. The *in vitro* screening program was based upon the use of multiple panels of 60 human tumor cell lines, against which the compounds were tested at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The percentage growth was evaluated spectrophotometrically against controls not treated with test agents. A 48-h continuous drug exposure protocol was followed, and a sulforhodamine B protein assay was used to estimate cell growth[28].

Results and discussions

Final compounds were synthesized with a three-step synthetic procedure (Scheme 1) and some characteristics of the compounds were given in Table 1. In first step, compounds **1a** and **1b** were prepared *via* PPA mediated oxidative condensation of 2-aminothiophenol and 4-aminobenzoic acid/4-amino-2-chlorobenzoic acid in microwave conditions (30 min, 125 °C). The obtained amino compounds (**1a** and **1b**) were reacted with chloroacetyl chloride with triethylamine in THF and DMF to produce the halides (**2a** and **2b**). Finally, compounds **3–15** were obtained by the reaction of *N*-[4-(benzothiazole-2-yl)phenyl]-2-chloroacetamide **(2a)**/*N*-[4-(benzothiazole-2-yl)-3-chlorophenyl]-2-chloroacetamide (**2b**) and various 2-mercaptobenzimidazole/benzothiazole/benzoxazole derivatives; compounds **16–27** were obtained by the reaction of *N*-[4-(benzothiazole-2-yl)phenyl]-2-chloroacetamide (**2a**) and 2-mercapto-(1,5-substituted phenyl)imidazole derivatives in a mild reaction condition. The structures of the final compounds were elucidated by using spectral data. In the IR spectra of the compounds (**3–27**), characteristic amide carbonyl functions were observed at 1666–1699 cm^{-1} region. The NMR spectra of all final compounds exhibited singlet peaks resulting from resonances of the acetamide residue assigned to –S–CH_2-protons at 4.04–4.50 ppm, and N-H protons at 10.70–11.10 ppm, respectively. In the spectrum of the compounds including benzimidazole-2-thione moiety (**3–5**, **10–12**) 1H singlet peaks were assigned at about 12.60–12.71 ppm belonging to the benzimidazole -NH proton. For the other compounds, the same protons appeared in multiplets, because of overlapping with aromatic protons. In the mass spectra of the compounds, M + 1 peaks agreed well with the calculated molecular weight of the target compounds.

All final compounds (**3–37**) were offered NCI, USA for testing their anticancer activity according to *in vitro* drug screening protocol of the institute. Compounds **3**, **6**, **8**, **10**, **11**, **12**, **16**, **18** and **20** were selected by NCI for 60 human tumor cell lines' anticancer screening test at single dose assay. *In vitro* single-dose anticancer assay was performed in full NCI 60 cell panel representing L, NSCLC, CC, CNSC, M, OC, RC, PC, and BC. Results were given as percentage growth of the tumor cells, which were treated with selected compounds (Table 2).

As can be seen in Table 2, according to the mean values compounds **3**, **10** and **16** exhibited strong antitumor activity with a percentage growth below 73%. In fact, compound **10** has attracted attention with a value of 10.19%, which is significantly lower than other compounds growth percentage values. Compound **3**, which had growth percentages below 40%, showed strong inhibitory activity especially, against A498, HOP-92, MDA-MB-468 and SK-MEL-5 cell lines. However, it inhibited leukemia cells' growth beside RC, non small lung cancer, BC and M cancer cells evaluating mean values of the cancer diseases. Compound **16** also displayed high antitumor activity against MDA-MB-468, MALME-3M, A549 and U251 cell lines with growth percentages below 25%. Addition to noticeable activity against BC, M, non small lung cancer and CNSC cells, compound **16** showed observable antitumor activity against CC with a mean percentage of 61.28%. In regard to compound **10**, cell lines HCT-116 with −90.17%, SF-539 with −88.57%, 786–0 with −72.88% growth percentages belonging to NSCLC, CNSC and RC have come into prominence among the other growth percentage results. Furthermore, with respect to mean values, OC (−10.92%) and CNCS (−12.11%) were found as the most susceptible cancer types against same compound. Other tested compounds **6** and **11**



Scheme 1. Reagents and conditions; (i) PPA, MW irradiation, 30 min; (ii) Et_3N, THF and DMF; (iii) K_2CO_3, acetone and 2-mercapto-5-substituted (benz)imidazole/benzothiazole/benzoxazoles; and (iv) K_2CO_3, acetone and 2-mercapto-1,5-(substituted phenyl)imidazoles.

Table 1. Some characteristics of the compounds **3–27**.

C	R_1	R_2	R_3	R_4	X	m.p. (°C)	Yield
3	H	H	–	–	NH	239	76
4	H	5-Cl	–	–	NH	235	72
5	H	5-CH_3	–	–	NH	227	69
6	H	H	–	–	S	166	79
7	H	5-Cl	–	–	S	160	63
8	H	H	–	–	O	186	65
9	H	5-CH_3	–	–	O	209	64
10	Cl	H	–	–	NH	240	64
11	Cl	5-Cl	–	–	NH	228	72
12	Cl	5-CH_3	–	–	NH	262	71
13	Cl	H	–	–	S	202	70
14	Cl	H	–	–	O	103	69
15	Cl	5-CH_3	–	–	O	95	73
16	H	–	H	H	–	244	73
17	H	–	CH_3	CH_3	–	116	76
18	H	–	OCH_3	OCH_3	–	204	78
19	H	–	CH_3	OCH_3	–	135	74
20	H	–	OCH_3	CH_3	–	225	70
21	H	–	CH_3	H	–	195	71
22	H	–	OCH_3	H	–	227	73
23	H	–	H	CH_3	–	196	72
24	H	–	H	OCH_3	–	125	72
25	H	–	H	F	–	202	71
26	H	–	CH_3	F	–	128	73
27	H	–	OCH_3	F	–	228	79

showed moderate activity with the growth percentage range of between 90 and 100%, whereas compounds **8**, **12**, **18** and **20** displayed weak antitumor activity with the percentage above 100%.

Compounds **10** and **16** were further selected for NCI full panel five dose assay at 10-fold dilutions of five different concentrations (0.01, 0.1, 1, 10 and 100 μM). Mean $\log_{10}$ GI50 values obtained from the NCI's *in vitro* disease-oriented human tumor cell lines for compounds **10** and **16** on nine cancer disease at five concentrations were listed in Table 3. Dose response curves of compound **10** were also given by nine different graphic indicating tested cancer types (Figure 2). From the results, Table 3,

Table 2. Anticancer activity of some compounds as growth %.

Compounds	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	Mean
3	59.17	67.30	80.51	81.28	78.74	74.23	70.52	67.19	63.91	72.19
6	78.52	87.95	96.18	84.04	106.56	94.45	90.07	81.03	78.39	90.70
8	102.56	100.04	109.42	89.84	113.71	98.74	96.33	98.38	87.58	100.43
10	48.15	5.54	12.29	−12.11	12.19	−10.92	17.28	26.28	15.97	10.19
11	90.85	96.70	98.87	96.72	102.76	100.97	94.96	91.50	94.04	97.08
12	88.71	106.92	106.70	103.65	108.0	107.64	102.98	119.17	102.83	106.06
16	76.61	72.91	61.28	71.51	64.61	72.63	84.12	74.22	65.53	71.45
18	104.27	103.33	107.16	102.68	109.03	104.83	106.15	108.42	75.14	102.39
20	108.63	110.50	106.90	105.37	110.35	111.75	107.91	104.77	93.08	107.17

Table 3. Mean $\log_{10}$GI50 values of compounds 10, 16 and control anticancer agents.

Comp.	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	MG_MID
10	>−4.00	−5.42	−5.29	−5.51	−5.41	−5.55	−5.49	−5.55	−5.40	−5.35
16	>−4.00	−5.04	>−4.00	−5.25	−5.0	−4.90	−4.78	>−4.00	−5.32	−4.87
A	−5.48	−5.17	−5.11	−5.12	−5.08	−5.18	−4.99	−4.49	−4.79	−5.09
B	−6.39	−6.20	−6.14	−6.18	−6.08	−6.45	−6.17	−6.41	−6.05	−6.20

A: Melphalan and B: Cisplatin.

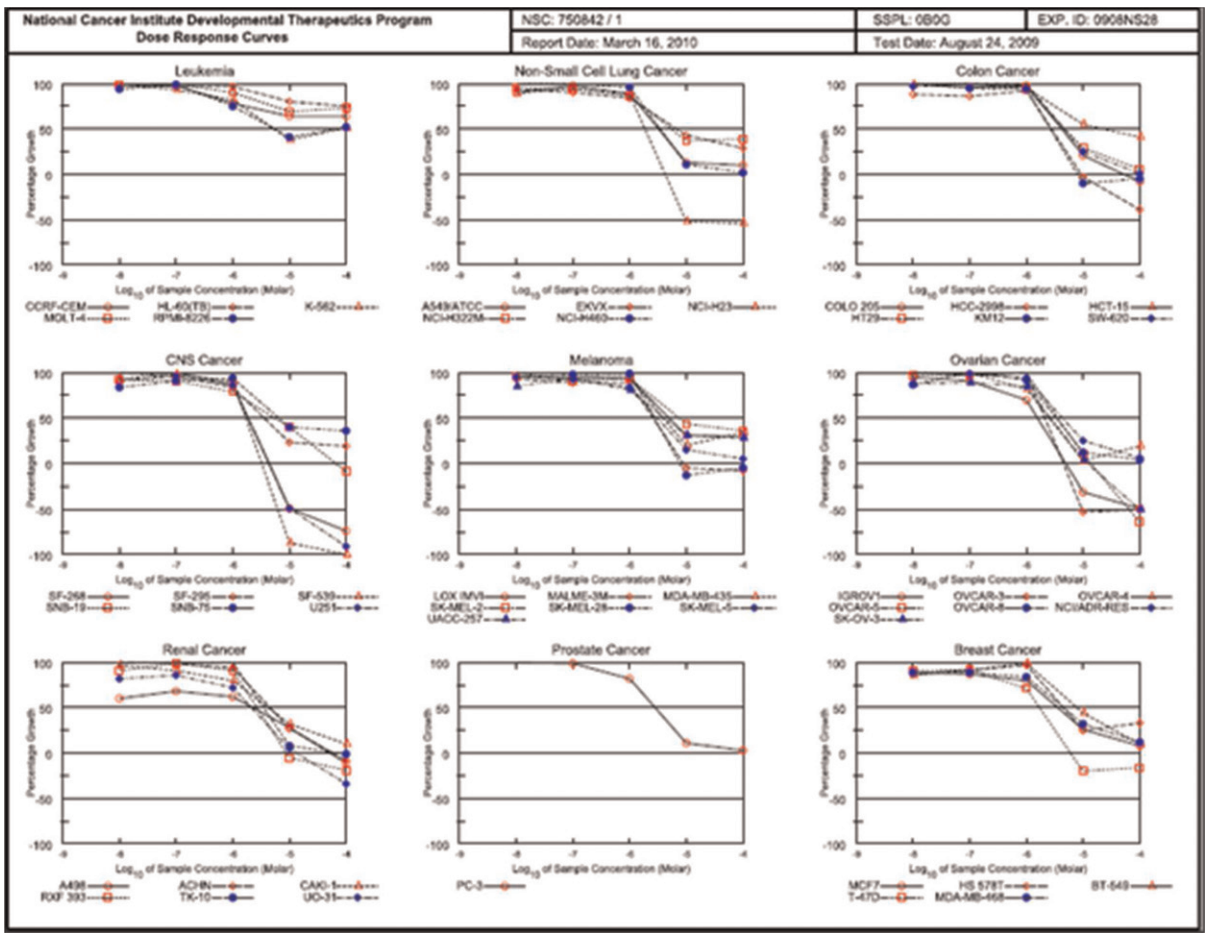


Figure 2. Dose-response curves of the compound 10 against tested cancer types.

compound **10** showed higher antitumor activity than standard drug melphalan and lower activity than cisplatin in all tested cancer types except leukemia. A considerable activity has also been shown by compound **16** although not up to the standard drugs. The test method states that the compounds having $\log_{10}$GI50 values greater than −4 were considered as inactive. Accordingly, both of the tested compounds (**10** and **16**) were found inactive against leukemia and compound **16** was found inactive against colon and PCs.

The structure of the final compounds differ from each other due to the heterocyclic rings ((benz)imidazole/benzothiazole/benzoxazole) bonded to acetyl group with a thioester linkage. Furthermore, compounds **9**–**15** vary from compounds **3**–**9** due to including chlorine atom on phenyl ring at second position of the

benzothiazole structure. Imidazole including compounds namely *N*-[4-(benzothiazole-2-yl)phenyl]-2-[(1,5-diphenyl-1*H*-imidazole-2-yl)thio]acetamide derivatives (**16**–**27**) have methyl, methoxy and fluoro substituents on phenyl groups at the first and fifth positions of the imidazole ring. Among the nine tested compounds, imidazole and non-substituted benzimidazole including compounds (**3**, **10** and **16**) possessed higher activity. Compound **10** (10.19% growth inhibition) including benzimidazole ring and also 2-chloro substitution on phenyl ring showed the highest activity compared with compounds **3** (72.19%) and **16** (71.15%). The decreasing activity according to the heterocyclic rings can be arranged as benzimidazole $\geq$ imidazole $>$ benzothiazole $>$ benzoxazole.

Conclusion

The synthesis of new 2-(4-aminophenyl)benzothiazole derivatives bearing (benz)imidazole, benzoxazole and benzothiazole heterocyclic ring systems and evaluation their antitumor activity have been investigated and reported, in this study. Compound **10**, namely *N*-[4-(benzothiazole-2-yl)-3-chlorophenyl]-2-[(benzimidazole-2-yl)thio]acetamide, and compound **16**, namely *N*-[4-(benzothiazole-2-yl)phenyl]-2-[(1,5-diphenyl-1*H*-imidazole-2-yl)thio]acetamide, exhibited strong antitumor activity against various cancer diseases and even more compound **10** has been observed to possess comparable $\log_{10}$GI50 values with standard drugs.

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Declaration of interest

The authors have declared no conflict of interest.

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References

1. Hu W-P, Chen Y-K, Liao C-C, et al. Synthesis, and biological evaluation of 2-(4-aminophenyl)benzothiazole derivatives as photosensitizing agents. Bioorg Med Chem 2010;18:6197–207.
2. Choi S-J, Park HJ, Lee SK, et al. Solid phase combinatorial synthesis of benzothiazoles and evaluation of topoisomerase II inhibitory activity. Bioorg Med Chem 2006;14:1229–35.
3. Novak M, Chakraborty M. Reactions of a putative metabolite of the model antitumor drug 2-(4-aminophenyl) benzothiazole with purines and pyrimidines. J Phys Org Chem 2011;24:960–8.
4. Tasler S, Müller O, Wieber T, et al. *N*-substituted 2′-(aminoaryl)benzothiazoles as kinase inhibitors: hit identification and scaffold hopping. Bioorg Med Chem Lett 2009;19:1349–56.
5. Shi D-F, Bradshaw TD, Wrigley S, et al. Antitumor benzothiazoles. 3. Synthesis of 2-(4-aminophenyl)benzothiazoles and evaluation of their activities against breast cancer cell lines *in vitro* and *in vivo*. J Med Chem 1996;39:3375–84.
6. Tzanopoulou S, Pirmettis IC, Patsis G, et al. Synthesis, characterization, and biological evaluation of M(I)(CO)3(NNO) complexes (M) Re, 99mTc) conjugated to 2-(4-aminophenyl)benzothiazole as potential breast cancer radiopharmaceuticals. J Med Chem 2006;49:5408–10.
7. Hutchinson I, Bradshaw TD, Matthews CS, et al. Antitumour benzothiazoles. Part 20: 3′-Cyano and 3′-alkynyl-substituted 2-(4′-aminophenyl)benzothiazoles as new potent and selective analogues. Bioorg Med Chem Lett 2003;13:471–4.
8. Bradshaw TD, Wrigley S, Shi D-F, et al. 2-(4-Aminophenyl)benzothiazoles: novel agents with selective profiles of *in vitro* anti-tumour activity. Brit J Cancer 1998;77:745–52.
9. Chua M-S, Shi D-F, Wrigley S, et al. Antitumor benzothiazoles. 7. Synthesis of 2-(4-acylaminophenyl)benzothiazoles and investigations into the role of acetylation in the antitumor activities of the parent amines. J Med Chem 1999;42:381–92.
10. Bradshaw TD, Shi D-F, Schultz RJ, et al. Influence of 2-(4-aminophenyl)benzothiazoles on growth of human ovarian carcinoma cells *in vitro* and *in vivo*. Brit J Cancer 1998;78:421–9.
11. Kashiyama E, Hutchinson I, Chua M-S, et al. Antitumor benzothiazoles. 8. Synthesis, metabolic formation, and biological properties of the C- and N-oxidation products of antitumor 2-(4-aminophenyl)benzothiazoles. J Med Chem 1999;42:4172–84.
12. Bradshaw TD, Stevens MFG, Westwell AD. The discovery of the potent and selective antitumour agent 2-(4-amino-3-methylphenyl)-benzothiazole (DF 203) and related compounds. Curr Med Chem 2001;8:203–10.
13. Shi D-F, Bradshaw TD, Chua M-S, et al. Antitumour benzothiazoles. Part 15: The synthesis and physicochemical properties of 2-(4-aminophenyl)benzothiazole sulfamate salt derivatives. Bioorg Med Chem Lett 2011;11:1093–5.
14. Leong C-O, Gaskell M, Martin EA, et al. Antitumour 2-(4-aminophenyl)benzothiazoles generate DNA adducts in sensitive tumour cells *in vitro* and *in vivo*. Brit J Cancer 2003;88:470–7.
15. Hutchinson I, Jennings SA, Vishnuvajjala BR, et al. Antitumor benzothiazoles. 16. Synthesis and pharmaceutical properties of antitumor 2-(4-aminophenyl)benzothiazole amino acid prodrugs. J Med Chem 2002;45:744–7.
16. Mortimer CG, Wells G, Crochard J-P, et al. Antitumor benzothiazoles. 26.1 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW 610, NSC 721648), a simple fluorinated 2-arylbenzothiazole, shows potent and selective inhibitory activity against lung, colon, and breast cancer cell lines. J Med Chem 2006;49:179–85.
17. Chakraborty M, Jin KJ, Glover SA, Novak M. Characterization of the 4-(benzothiazol-2-yl)phenylnitrenium ion from a putative metabolite of a model antitumor drug. J Org Chem 2010;75:5296–304.
18. Racane L, Tralic-Kulenovic V, Kitson RP, Karminski-Zamola G. Synthesis and antiproliferative activity of cyano and amidino substituted 2-phenylbenzothiazoles. Monatsh Chem 2006;137:1571–7.
19. Racane L, Stojkovic R, Tralic-Kulenovic V, Karminski-Zamola G. Synthesis and antitumor evaluation of novel derivatives of 6-amino-2-phenylbenzothiazoles. Molecules 2006;11:325–33.
20. Bradshaw TD, Westwell AD. The development of the antitumour benzothiazole prodrug, Phortress, as a clinical candidate. Curr Med Chem 2004;11:1241–53.
21. Brantley E, Trapani V, Alley MC, et al. Fluorinated 2-(4-amino-3-methylphenyl)benzothiazoles induce CYP1A1 expression, become metabolized, and bind to macromolecules in sensitive human cancer cells. Drug Metab Dispos 2004;32:1392–401.
22. Hutchinson I, Chua M-S, Browne HL, et al. Antitumor benzothiazoles. 14.1 Synthesis and *in vitro* biological properties of fluorinated 2-(4-aminophenyl)benzothiazoles. J Med Chem 2001;44:1446–55.
23. Tzanopoulou S, Sagnou M, Paravatou-Petsotas M, et al. Evaluation of Re and (99m)Tc complexes of 2-(4′-aminophenyl)benzothiazole as potential breast cancer radiopharmaceuticals. J Med Chem 2010;53:4633–41.
24. Fichtner I, Monks A, Hose C, et al. The experimental antitumor agents Phortress and Doxorubicin are equiactive against human-derived breast carcinoma xenograft models. Breast Cancer Res Tr 2004;87:97–107.
25. Kamal A, Reddy KS, Khan MNA, et al. Synthesis, DNA-binding ability and anticancer activity of benzothiazole/benzoxazole–pyrrolo[2,1-c][1,4]benzodiazepine conjugates. Bioorg Med Chem 2010;18:4747–61.
26. Racane L, Tralic-Kulenovic V, Pavelic SK, et al. Novel diamidino-substituted derivatives of phenyl benzothiazolyl and dibenzothiazolyl furans and thiophenes: synthesis, antiproliferative and DNA binding properties. J Med Chem 2010;53:2418–32.
27. Tay F, Yurttaş L, Demirayak Ş. Synthesis of some N-[4-(benzothiazole-2yl) phenyl]-2-aryloxyacetamide derivatives and their anticancer activities. J Enzyme Inhib Med Chem 2012;27:515–20.
28. Boyd MR. Status of the NCI preclinical antitumor drug discovery screen. In: DeVita Jr VT, Hellman S, Rosenberg SA, eds. Cancer: principles and practice of oncology updates, vol. 3, no. 10. Philadelphia: JPLippincott; 1989:1–12.