

RESEARCH ARTICLE

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

Leyla Yurttaş<sup>1</sup>, Funda Tay<sup>2</sup>, and Şeref Demirayak<sup>3</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey, <sup>2</sup>Department of Chemistry, Faculty of Arts & Sciences, Eskişehir Osmangazi University, Eskişehir, Turkey, and <sup>3</sup>Department of Pharmaceutical Chemistry, School of Pharmacy, Medipol University, İstanbul, Turkey

### 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.

### Introduction

2-(4-Aminophenyl)benzothiazole structure is known with high antitumor activity since 1996<sup>1–5</sup>. 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<sup>6,7</sup>. Up to present, scientists Shi and Bradshaw have a series of studies on antitumor activity of some benzothiazole derivatives<sup>8–14</sup>. 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<sup>8–14</sup>.

Phortress (NSC 710305, dihydrochloride salt of the lysylamide prodrug of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole

### Keywords

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

### History

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(5F 203)), the fluorinated water-soluble pro-drug, which has been synthesized to address formulation and bioavailability issues related to the desired parenteral administration<sup>15–19</sup>, was then chosen for phase I clinical trials in Britain in 2004 (Figure 1)<sup>20</sup>. 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<sup>21</sup>. Conversely, in insensitive cell lines, neither retaining nor metabolism occurs, thereby selective antitumor properties appear due through to metabolism<sup>22–26</sup>.

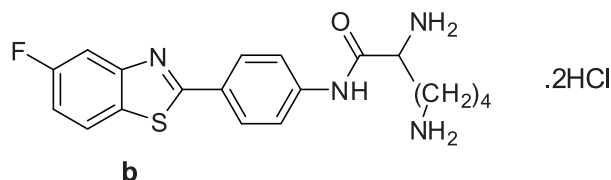
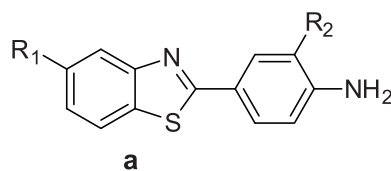
Motivated by the above observations and extending our previous study<sup>27</sup>, we planned to synthesize new 2-(4-aminophenyl)benzothiazole derivatives including (benz)imidazole/benzoxazole/benzothiazole heterocyclic ring systems and to evaluate their antitumor activity against nine cancer types comprised 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 <sup>1</sup>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,

Address for correspondence: Leyla Yurttaş, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir 26470, Turkey. Tel: +902223350580/3783. E-mail: lyurttas@anadolu.edu.tr

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



	R <sub>1</sub>	R <sub>2</sub>
CJM 126	H	H
DF 203	H	CH <sub>3</sub>
5F 203	F	CH <sub>3</sub>

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<sub>2</sub>CO<sub>3</sub> (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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3250 (N–H), 3067 (aromatic C–H), 2885 (aliphatic C–H), 1674 (C=O), 1541–1450 (C=C, C=N), 1342–1032 (C–N). <sup>1</sup>H-NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.33 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 417.1 (100%) M + 1, 418.1 (27.1%) M + 2, 419.1 (12.8%) M + 3. Anal Calcd for C<sub>22</sub>H<sub>16</sub>N<sub>4</sub>OS<sub>2</sub>·3H<sub>2</sub>O: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3321 (N–H), 3053 (aromatic C–H), 2868 (aliphatic C–H), 1689 (C=O), 1556–1463 (C=C, C=N), 1313–1053 (C–N). <sup>1</sup>H-NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.32 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 451.5 (100%) M + 1, 452.5 (27%) M + 2, 453.5 (43%) M + 3. Anal Calcd for C<sub>22</sub>H<sub>15</sub>ClN<sub>4</sub>OS<sub>2</sub>·H<sub>2</sub>O: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3240 (N–H), 3049 (aromatic C–H), 2864 (aliphatic C–H), 1693 (C=O), 1548–1437 (C=C, C=N), 1316–1028 (C–N). <sup>1</sup>H-NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 2.38 (s, 3H, CH<sub>3</sub>), 4.31 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 431 (100%) M + 1, 432 (25%) M + 2, 433 (12%) M + 3. Anal Calcd for C<sub>23</sub>H<sub>18</sub>N<sub>4</sub>OS<sub>2</sub>·H<sub>2</sub>O: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3290 (N–H), 3176 (aromatic C–H), 3053 (aliphatic C–H), 1674 (C=O), 1556–1429 (C=C, C=N), 1313–1056 (C–N). <sup>1</sup>H-NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.45 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 434 (100%) M + 1, 435 (27%) M + 2, 436 (17%) M + 3.

Anal Calcd for  $C_{22}H_{15}N_3OS_3 \cdot 3H_2O$ : 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)  $\nu_{max}$  ( $cm^{-1}$ ): 3242 (N–H), 3174 (aromatic C–H), 2951 (aliphatic C–H), 1683 (C=O), 1545–1423 (C=C, C=N), 1332–1115 (C–N).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 4.46 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 468.5 (100%) M + 1, 469.5 (27%) M + 2, 470.5 (49%) M + 3. Anal Calcd for  $C_{22}H_{14}ClN_3OS_3 \cdot H_2O$ : 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)  $\nu_{max}$  ( $cm^{-1}$ ): 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).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 4.50 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 418 (100%) M + 1, 419 (20%) M + 2, 420 (14%) M + 3. Anal Calcd for  $C_{22}H_{15}N_3O_2S_2 \cdot 4H_2O$ : 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)  $\nu_{max}$  ( $cm^{-1}$ ): 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).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 2.40 (s, 3H, CH<sub>3</sub>), 4.15 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 432 (100%) M + 1, 433 (26%) M + 2, 434 (13.2%) M + 3. Anal Calcd for  $C_{23}H_{17}N_3O_2S_2 \cdot H_2O$ : 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)  $\nu_{max}$  ( $cm^{-1}$ ): 3434 (N–H), 3051 (aromatic C–H), 2986 (aliphatic C–H), 1677 (C=O), 1599–1497 (C=C, C=N), 1326–1045 (C–N).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 4.34 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 451 (100%) M + 1, 452 (24%) M + 2, 453 (40%) M + 3. Anal Calcd for  $C_{22}H_{15}ClN_4OS_2 \cdot 1/4H_2O$ : 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)  $\nu_{max}$  ( $cm^{-1}$ ): 3290 (N–H), 3054 (aromatic C–H), 2929 (aliphatic C–H), 1677 (C=O), 1596–1454 (C=C, C=N), 1334–1021 (C–N).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm):

4.32 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 485 (100%) M + 1, 486 (23%) M + 2, 487 (71.7%) M + 3. Anal Calcd for  $C_{22}H_{14}Cl_2N_4OS_2 \cdot 1/2H_2O$ : 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)  $\nu_{max}$  ( $cm^{-1}$ ): 3238 (NH), 3071 (aromatic C–H), 2917 (aliphatic C–H), 1672 (C=O), 1600–1457 (C=C, C=N), 1342–1034 (C–N).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 2.38 (s, 3H, CH<sub>3</sub>), 4.31 (s, 2H, CH<sub>2</sub>), 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 (s, 1H, NH), 12.68 (s, 1H, benzimidazole NH). MS (ES<sup>+</sup>): 465.5 (100%) M + 1, 466.5 (25.2%) M + 2, 467.5 (32%) M + 3. Anal Calcd for  $C_{23}H_{17}ClN_4OS_2 \cdot H_2O$ : 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)  $\nu_{max}$  ( $cm^{-1}$ ): 3249 (NH), 3049 (aromatic C–H), 2945 (aliphatic C–H), 1690 (C=O), 1602–1457 (C=C, C=N), 1343–1021 (C–N).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 4.47 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 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)  $\nu_{max}$  ( $cm^{-1}$ ): 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).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 4.50 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 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)  $\nu_{max}$  ( $cm^{-1}$ ): 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).  $^1H$ -NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 2.40 (s, 3H, CH<sub>3</sub>), 4.15 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 466.5 (100%) M + 1, 467.5 (27.8%) M + 2, 468.5 (45.1%) M + 3. Anal Calcd for C<sub>23</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>2</sub>S<sub>2</sub>: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3248 (N–H), 3032 (aromatic C–H), 2943 (aliphatic C–H), 1694 (C=O), 1604–1482 (C=C, C=N), 1333–1015 (C–N). <sup>1</sup>H NMR (400 MHz)(DMSO-d<sub>6</sub>)  $\delta$  (ppm): 4.10 (2H, s, CH<sub>2</sub>), 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<sup>+</sup>): 519.1 (100%) M + 1, 520.1 (36%) M + 2, 521.1 (14%) M + 3. Anal Calcd for C<sub>30</sub>H<sub>22</sub>N<sub>4</sub>OS<sub>2</sub>·1/2H<sub>2</sub>O: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3259 (N–H), 3034 (aromatic C–H), 2921 (aliphatic C–H), 1693 (C=O), 1602–1481 (C=C, C=N), 1315–1028 (C–N). <sup>1</sup>H NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 2.69 (s, 3H, CH<sub>3</sub>), 2.75 (s, 3H, CH<sub>3</sub>), 4.08 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 547.1 (100%) M + 1, 548.1 (39%) M + 2, 549.1 (18%) M + 3. Anal Calcd for C<sub>32</sub>H<sub>26</sub>N<sub>4</sub>OS<sub>2</sub>: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3270 (N–H), 3054 (aromatic C–H), 2957 (aliphatic C–H), 1696 (C=O), 1604–1413 (C=C, C=N), 1321–1005 (C–N). <sup>1</sup>H NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 3.69 (s, 3H, OCH<sub>3</sub>), 3.78 (s, 3H, OCH<sub>3</sub>), 4.04 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 579.1 (100%) M + 1, 580.1 (39%) M + 2, 581.1 (18%) M + 3. Anal Calcd for C<sub>32</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub>·H<sub>2</sub>O: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3263 (N–H), 3055 (aromatic C–H), 2921 (aliphatic C–H), 1696 (C=O), 1604–1456 (C=C, C=N), 1334–1017 (C–N). <sup>1</sup>H NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 2.35 (s, 3H, CH<sub>3</sub>), 3.69 (s, 3H, OCH<sub>3</sub>), 4.05 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 563.1 (100%) M + 1, 564.1 (39%) M + 2, 565.1 (18%) M + 3. Anal Calcd for C<sub>32</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>·2H<sub>2</sub>O: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3258 (N–H), 3054 (aromatic C–H), 2933 (aliphatic C–H), 1696 (C=O), 1614–1423 (C=C, C=N), 1318–1063 (C–N). <sup>1</sup>H NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 2.37 (s, 3H, CH<sub>3</sub>), 3.68 (s, 3H, OCH<sub>3</sub>), 4.07 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 563.1 (100%) M + 1, 564.1 (39%) M + 2, 565.1 (18%) M + 3. Anal Calcd for C<sub>32</sub>H<sub>26</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3261 (N–H), 3055 (aromatic C–H), 2921 (aliphatic C–H), 1694 (C=O), 1602–1480 (C=C, C=N), 1318–1056 (C–N). <sup>1</sup>H NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 2.36 (s, 3H, CH<sub>3</sub>), 4.09 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 533.1 (100%) M + 1, 534.1 (39%) M + 2, 535.1 (18%) M + 3. Anal Calcd for C<sub>31</sub>H<sub>24</sub>N<sub>4</sub>OS<sub>2</sub>: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3260 (N–H), 3053 (aromatic C–H), 2933 (aliphatic C–H), 1697 (C=O), 1605–1473 (C=C, C=N), 1345–1032 (C–N). <sup>1</sup>H NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 3.67 (s, 3H, OCH<sub>3</sub>), 4.11 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 549.1 (100%) M + 1, 550.1 (33%) M + 2, 551.1 (17%) M + 3. Anal Calcd for C<sub>31</sub>H<sub>24</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3258 (N–H), 3055 (aromatic C–H), 2920 (aliphatic C–H), 1695 (C=O), 1602–1481 (C=C, C=N), 1331–1017 (C–N). <sup>1</sup>H NMR (400 MHz) (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 2.36 (s, 3H, CH<sub>3</sub>), 4.08 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 533.1 (100%) M + 1, 534.1 (33%) M + 2, 535.1 (17 %) M + 3. Anal Calcd for C<sub>31</sub>H<sub>24</sub>N<sub>4</sub>OS<sub>2</sub>·3/2H<sub>2</sub>O: 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)  $\nu_{\max}$  (cm<sup>-1</sup>): 3257 (N–H), 3054 (aromatic C–H), 2929 (aliphatic C–H), 1694 (C=O), 1604–1499 (C=C, C=N),

1342–1054 (C–N).  $^1\text{H}$  NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 3.68 (s, 3H, OCH<sub>3</sub>), 4.08 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 549.1 (100%) M + 1, 550.1 (33%) M + 2, 551.1 (17%) M + 3. Anal Calcd for C<sub>31</sub>H<sub>24</sub>N<sub>4</sub>O<sub>2</sub>S<sub>2</sub>·H<sub>2</sub>O: 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)  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3252 (N–H), 3055 (aromatic C–H), 2929 (aliphatic C–H), 1688 (C=O), 1602–1498 (C=C, C=N), 1316–1027 (C–N).  $^1\text{H}$  NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 4.10 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 537.1 (100%) M + 1, 538.1 (37%) M + 2, 539 (14%) M + 3. Anal Calcd for C<sub>30</sub>H<sub>21</sub>FN<sub>4</sub>OS<sub>2</sub>·2H<sub>2</sub>O: 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)  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3268 (N–H), 3056 (aromatic C–H), 2920 (aliphatic C–H), 1699 (C=O), 1603–1497 (C=C, C=N), 1347–1030 (C–N).  $^1\text{H}$  NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 2.35 (s, 3H, CH<sub>3</sub>), 4.07 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 550.1 (100%) M + 1, 551.1 (39%) M + 2, 552.1 (18%) M + 3. Anal Calcd for C<sub>31</sub>H<sub>23</sub>FN<sub>4</sub>OS<sub>2</sub>: 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)  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3269 (N–H), 3056 (aromatic C–H), 2958 (aliphatic C–H), 1698 (C=O), 1609–1459 (C=C, C=N), 1336–1012 (C–N).  $^1\text{H}$  NMR (400 MHz) (DMSO- $d_6$ )  $\delta$  (ppm): 3.79 (s, 3H, OCH<sub>3</sub>), 4.08 (s, 2H, CH<sub>2</sub>), 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<sup>+</sup>): 566.1 (100%) M + 1, 567.1 (37%) M + 2, 568 (14%) M + 3. Anal Calcd for C<sub>31</sub>H<sub>23</sub>FN<sub>4</sub>O<sub>2</sub>S<sub>2</sub>: C: 65.71%, H: 4.09%, N: 9.89%, found: C: 65.81%, H: 4.23% H, N: 9.79%.

### Anticancer activity

The cytotoxic 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<sup>-4</sup> to 10<sup>-8</sup> M. The percentage growth was evaluated spectrophotometrically against controls not treated with test agents. A 48-h continuous

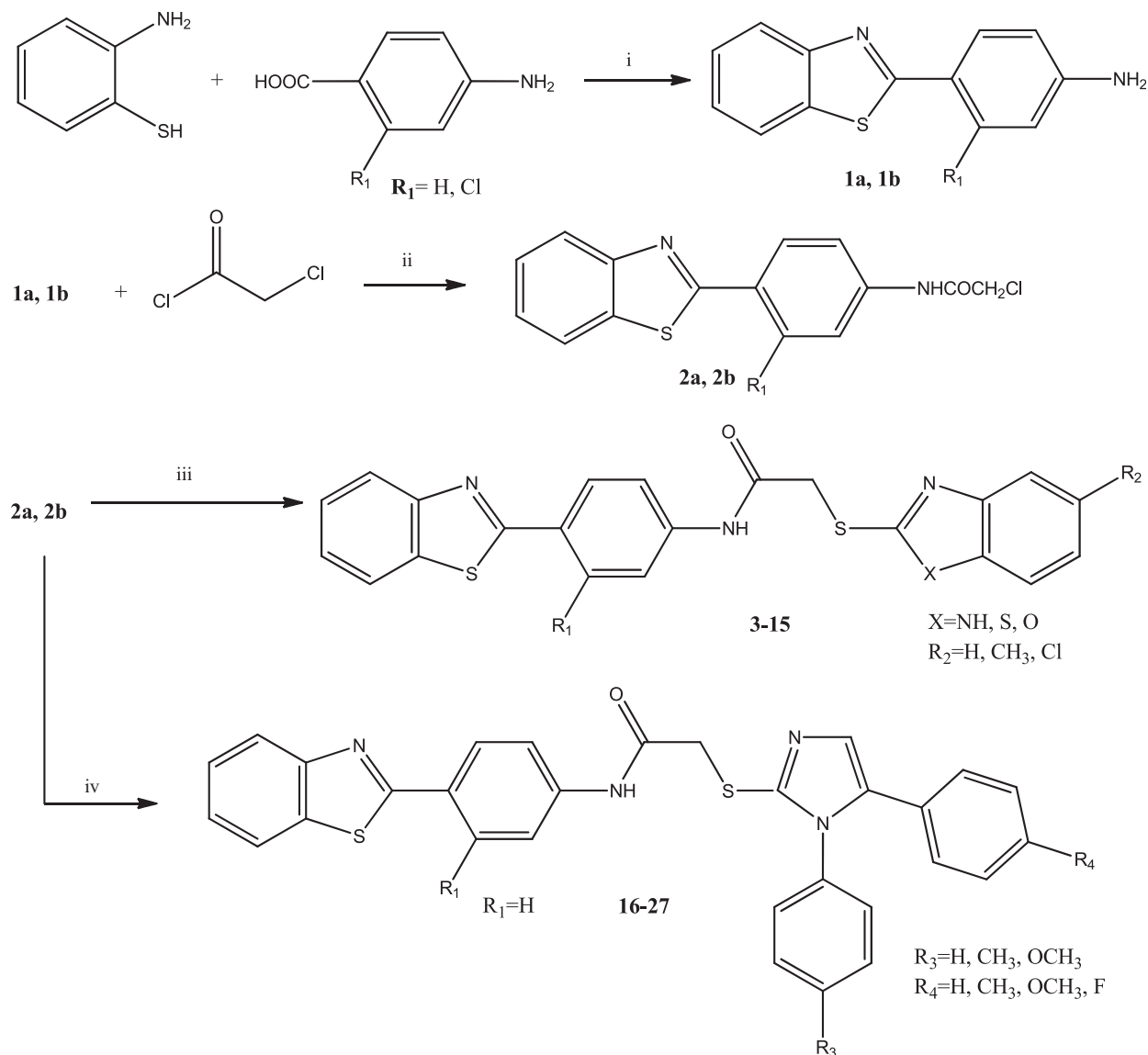
drug exposure protocol was followed, and a sulforhodamine B protein assay was used to estimate cell growth<sup>28</sup>.

### 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<sup>-1</sup> region. The NMR spectra of all final compounds exhibited singlet peaks resulting from resonances of the acetamide residue assigned to –S–CH<sub>2</sub>–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 CNSC (–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)  $\text{Et}_3\text{N}$ , THF and DMF; (iii)  $\text{K}_2\text{CO}_3$ , acetone and 2-mercapto-5-substituted (benz)imidazole/benzothiazole/benzoxazoles; and (iv)  $\text{K}_2\text{CO}_3$ , 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. ( $^\circ\text{C}$ )	Yield
<b>3</b>	H	H	–	–	NH	239	76
<b>4</b>	H	5-Cl	–	–	NH	235	72
<b>5</b>	H	5- $\text{CH}_3$	–	–	NH	227	69
<b>6</b>	H	H	–	–	S	166	79
<b>7</b>	H	5-Cl	–	–	S	160	63
<b>8</b>	H	H	–	–	O	186	65
<b>9</b>	H	5- $\text{CH}_3$	–	–	O	209	64
<b>10</b>	Cl	H	–	–	NH	240	64
<b>11</b>	Cl	5-Cl	–	–	NH	228	72
<b>12</b>	Cl	5- $\text{CH}_3$	–	–	NH	262	71
<b>13</b>	Cl	H	–	–	S	202	70
<b>14</b>	Cl	H	–	–	O	103	69
<b>15</b>	Cl	5- $\text{CH}_3$	–	–	O	95	73
<b>16</b>	H	–	H	H	–	244	73
<b>17</b>	H	–	$\text{CH}_3$	$\text{CH}_3$	–	116	76
<b>18</b>	H	–	$\text{OCH}_3$	$\text{OCH}_3$	–	204	78
<b>19</b>	H	–	$\text{CH}_3$	$\text{OCH}_3$	–	135	74
<b>20</b>	H	–	$\text{OCH}_3$	$\text{CH}_3$	–	225	70
<b>21</b>	H	–	$\text{CH}_3$	H	–	195	71
<b>22</b>	H	–	$\text{OCH}_3$	H	–	227	73

(continued)

Table 1. Continued

C	$R_1$	$R_2$	$R_3$	$R_4$	X	m.p. ( $^\circ\text{C}$ )	Yield
<b>23</b>	H	–	H	$\text{CH}_3$	–	196	72
<b>24</b>	H	–	H	$\text{OCH}_3$	–	125	72
<b>25</b>	H	–	H	F	–	202	71
<b>26</b>	H	–	$\text{CH}_3$	F	–	128	73
<b>27</b>	H	–	$\text{OCH}_3$	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  $\mu\text{M}$ ). 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
<b>3</b>	59.17	67.30	80.51	81.28	78.74	74.23	70.52	67.19	63.91	72.19
<b>6</b>	78.52	87.95	96.18	84.04	106.56	94.45	90.07	81.03	78.39	90.70
<b>8</b>	102.56	100.04	109.42	89.84	113.71	98.74	96.33	98.38	87.58	100.43
<b>10</b>	48.15	5.54	12.29	-12.11	12.19	-10.92	17.28	26.28	15.97	10.19
<b>11</b>	90.85	96.70	98.87	96.72	102.76	100.97	94.96	91.50	94.04	97.08
<b>12</b>	88.71	106.92	106.70	103.65	108.0	107.64	102.98	119.17	102.83	106.06
<b>16</b>	76.61	72.91	61.28	71.51	64.61	72.63	84.12	74.22	65.53	71.45
<b>18</b>	104.27	103.33	107.16	102.68	109.03	104.83	106.15	108.42	75.14	102.39
<b>20</b>	108.63	110.50	106.90	105.37	110.35	111.75	107.91	104.77	93.08	107.17

Table 3. Mean log<sub>10</sub>GI50 values of compounds 10, 16 and control anticancer agents.

Comp.	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	MG_MID
<b>10</b>	>-4.00	-5.42	-5.29	-5.51	-5.41	-5.55	-5.49	-5.55	-5.40	-5.35
<b>16</b>	>-4.00	-5.04	>-4.00	-5.25	-5.0	-4.90	-4.78	>-4.00	-5.32	-4.87
<b>A</b>	-5.48	-5.17	-5.11	-5.12	-5.08	-5.18	-4.99	-4.49	-4.79	-5.09
<b>B</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.

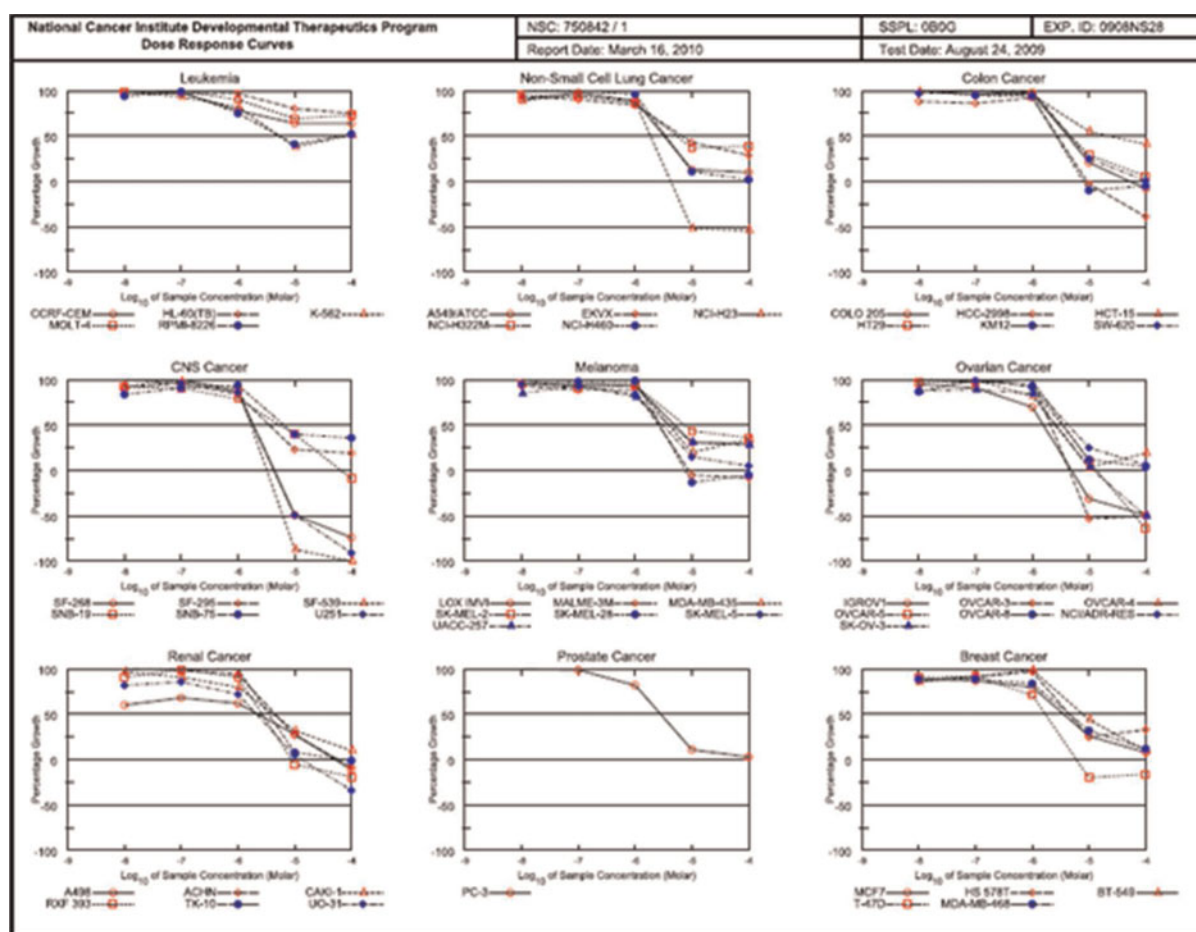


Figure 2. Dose-response curves of the compound 10 against tested cancer types.

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<sub>10</sub>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<sub>10</sub>GI50 values with standard drugs.

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## Declaration of interest

The authors have declared no conflict of interest.

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