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RESEARCH ARTICLE

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Synthesis and cytotoxic activity of certain benzothiazole derivatives against human MCF-7 cancer cell line

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A new series of benzothiazole has been synthesized as cytotoxic agents. The new derivatives were tested for their cytotoxic activity toward the human breast cancer MCF-7 cell line against cisplatin as the reference drug. Many derivatives revealed good cytotoxic effect, whereas four of them, **4, 5c, 5d,** and **6b**, were more potent than cisplatin, with IC_{50} values being 8.64, 7.39, 7.56, and 5.15 μM compared to 13.33 μM of cisplatin. The four derivatives' cytotoxic activity was accompanied by regulating free radicals production, by increasing the activity of superoxide dismutase and depletion of intracellular reduced glutathione, catalase, and glutathione peroxidase activities, accordingly, the high production of hydrogen peroxide, nitric oxide, and other free radicals causing tumor cell death as monitored by reduction in the synthesis of protein and nucleic acids. Most of the tested compounds showed potent to moderate growth inhibitory activity; in particular, compound **6b** exhibited the highest activity suggesting it is a lead compound in cytotoxic activity.

KEYWORDS
anticancer, antioxidant, benzothiazole, cytotoxic, MCF-7

1 | INTRODUCTION

Cancer is continuing to be a major health problem in developing as well as undeveloped countries.[1] It is a leading cause of mortality worldwide accounting for almost 13% of all deaths.[2] Among all types of cancer, lung, breast, colorectal, stomach, and prostate cancers are the underlying causes for the majority of cancer deaths.[2] In addition, a single "cure" for cancer has been proven elusive because there are more than 100 different types of cancer present.[3]

On the other hand, excessive unbalanced production of reactive oxygen species (ROS) has been well established to be associated with many pathological conditions including cancer and inflammation.[4] DNA has proven to be an important target in cancer therapy. The planar structure of DNA intercalating drugs can strongly bind to DNA resulting in the death of cancer cells.[5]

Although many classes of drugs are being used for the treatment of breast cancer, as axitinib, methotrexate, raloxifene, and doxorubicin, the need for more potent selective antitumor agents is still not precluded[6] (Figure 1).

The benzothiazole ring system shows diversity of biological activities as anxiolytic, antiallergic, cardiovascular, antidiabetic, antipsychotic, and antioxidant effect.[7–12] It was found that some benzothiazole derivatives possess anticancer activity.[13–16] For example, pyrazolobenzothiazoles **I** were recorded as active anticancer against human breast cancer cell lines.[17] Further derivatives **II** showed cytotoxicity and growth inhibitory activity on both breast and liver cancer cell lines.[18] Recently, compound **III** showed anticancer activity against HeLa cell line[19] (Figure 2).

Accordingly, the effect of the newly synthesized compounds on the activities of the free-radical-metabolizing

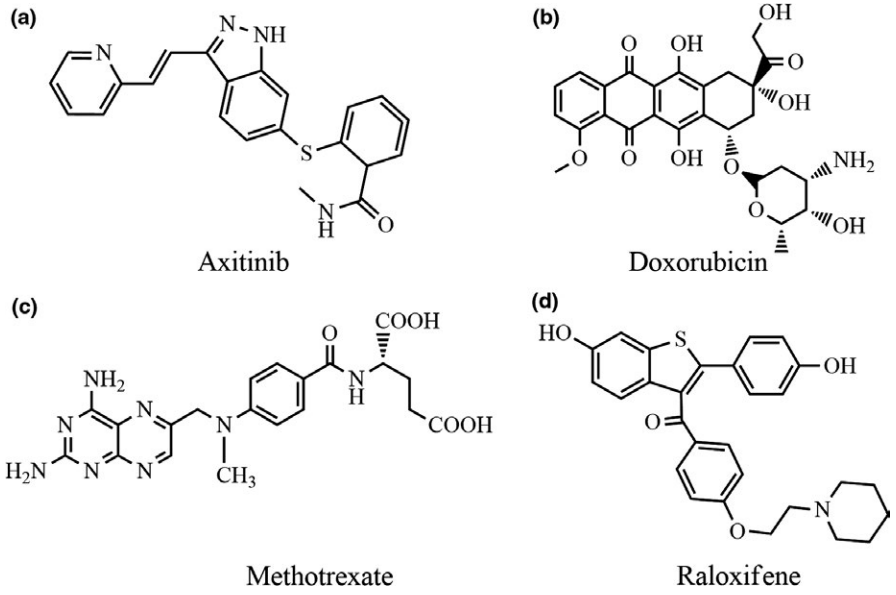


FIGURE 1 Drugs approved for breast cancer treatment (a) Axitinib, (b) Doxorubicin, (c) Methotrexate and (d) Raloxifene

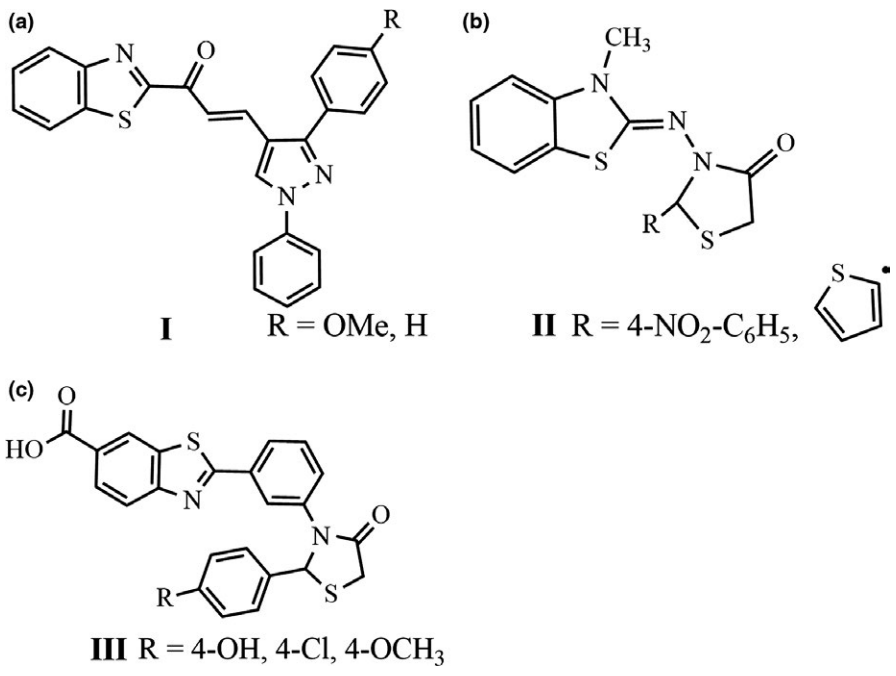


FIGURE 2 Structures of some reported benzothiazole derivatives having cytotoxic and antitumor activity (a) I, (b) II and (c) III

enzymes, as well as the levels of the oxidative stress parameters in MCF-7 cells, was estimated.

2 | METHODS AND MATERIALS

2.1 | Chemistry

All reagents were commercially available and were used without further purification. Melting points were recorded on a Griffin apparatus and are uncorrected. Microanalyses for C, H, and N were carried out at the micro-analytical center, Al-Azhar University, Egypt. IR spectra were recorded on Shimadzu IR 435 spectrophotometer in KBr disk, Faculty of Pharmacy, Cairo University, Egypt, and values were represented in υ cm^{-1}. ^{1}H-NMR spectra were obtained on a Mercury-300 BB MHz spectrophotometers using TMS as an internal reference, and shift values were recorded in ppm on δ scale, microanalytical center, Cairo University, and Bruker Ascend 400 MHz, Faculty of Pharmacy, Cairo University, Egypt. ^{13}C-NMR spectra were recorded on Varian Gemini 300 MHZ spectrophotometer using TMS as internal standard, and chemical shift values were recorded in ppm on δ scale, Main Defence Chemical Laboratory, El Khanka, Al-Qalyubiyah, Egypt, and Bruker Ascend 400 MHz, Faculty of Pharmacy, Cairo University, Egypt. Mass spectra were run on GCMS-QP2010 Plus spectrometer, Microanalytical

center, Cairo University, Egypt. Progress of the reaction and the purity of products were monitored by TLC precoated aluminum sheet silica gel (Merck 60F 254) and was visualized by UV lamp (C_6H_6/$(CH_3)_2O$ 9:1) and ($CHCl_3$/CH_3OH 9:1).

2-(1,3 benzothiazol-2-yl)acetonitrile (1) was synthesized according to reported procedure.[20]

2.2 | General procedure of synthesis of compounds (2a–d)

Phenylisothiocyanate (0.77 g, 0.57 mmol) was added portion wise to a well-stirred and ice-cooled suspension of powdered potassium hydroxide (0.644 g, 1.14 mmol) and 2-(1,3 benzothiazol-2-yl)acetonitrile **(1)** (1 g, 0.57 mmol) in dry *N,N*-dimethylformamide (10 ml). The mixture was stirred at room temperature for three hours and cooled to 0°C, and then, appropriate chlorinated compound 0.57 mmol [either 1,2-dichloroethane (0.56 g) or oxalyl dichloride (0.72 g) or chloroacetyl chloride (0.64 g) or 2-chloropropanoyl chloride (0.72 g)] was added slowly. After stirring for 24 hr at room temperature, ice/water mixture was added and the separated solid was filtered, washed with water (2 × 15 ml), dried, and crystallized from ethanol.

2.2.1 | 2-(1,3 benzothiazol-2-yl)-2-(3-phenyl(1,3)thiazolidin-2-ylidene)aceto-nitrile (2a)

Bronze crystals; (1.2 g, 0.357 mmol), yield: 62.76%; m.p: 165–167°C; IR (cm^{-1}): 3,057 (CH aromatic), 2,920 (CH aliphatic), 2,185 (C≡N); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 3.40 (t, 2H, $J = 7.5$ Hz, thiazolidine-C-$_5$), 4.26 (t, 2H, $J = 7.5$ Hz, thiazolidine-C_4-H), 7.33 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.38–7.49 (m, 5H, phenyl-H), 7.77 (d, 1H, $J = 7.5$ Hz, benzothiazole-C_7-H), 7.95 (d, 1H, $J = 8.1$ Hz, benzothiazole-C_4-H); ^{13}CNMR-DEPT(DMSO-*d6*-400 MHz, ppm): 28.46 (thiazolidine-C_5), 40.28 (C-$_{CN}$), 60.25 (thiazolidine-C_4), 121.18 (benzothiazole-$C_{5,6}$), 122.12 (phenyl-C_4, CN), 124.19 (phenyl-$C_{3,5}$), 124.96 (phenyl-$C_{2,6}$), 125.87 (benzothiazole-$C_{4,7}$), 126.00 (benzothiazole-C_{1a}), 126.70 (phenyl-C_1), 127.20 (benzothiazole-C_{3a}), 128.66 (benzothiazole-C_2), 129.66 (thiazolidine-C_2); MS (*m/z*): 337.90 (M^++2, 2.30%), 336.90 (M^+ +1, 11.99%), 335.95 (M^+, 25.12), 334.95 (M^+, 100%); Anal. Calcd. for $C_{18}H_{13}N_3S_2$ (335.45): C, 64.45, H, 3.91, N, 12.53. Found: C, 64.52, H, 3.97, N, 12.61.

2.2.2 | 2-(1,3 benzothiazol-2-yl)-2-(4,5-dioxo-3-phenyl(1,3)thiazolidin-2-ylidene) acetonitrile (2b)

Pale brown crystals; (1.29 g, 0.354 mmol), yield: 62.31%; m.p: 208–210°C; IR(cm^{-1}): 3,059 (CH aromatic), 2,194 (C≡N), 1,700, 1,680 (2 C=O); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 7.29 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.41–7.52 (m, 5H, phenyl-H), 7.66 (d, 1H, $J = 7.5$ Hz, benzothiazole-C_7-H), 7.86 (d, 1H, $J = 7.5$ Hz, benzothiazole-C_4-H); ^{13}CNMR(DMSO-d6-300 MHz, ppm): 78.85 (C-$_{C\equiv N}$), 113.60 (C≡N), 117.39 (benzothiazole-$C_{5,6}$), 122.21 (phenyl-C_4), 123.77 (phenyl-$C_{3,5}$), 125.33 (phenyl-$C_{2,6}$), 126.52 (benzothiazole-$C_{4,7}$), 127.11 (phenyl-C_1), 128.18 (benzothiazole-C_{1a}), 129.14 (benzothiazole-C_{3a}), 138.37 (benzothiazole-C_2), 140.12 (thiazolidine-C_2), 168.52 (thiazolidine-C_4), 186.59 (thiazolidine-C_5); MS (*m/z*): 363.90 (M^+, 0.77%), 216.85 ($C_{10}H_5N_2S_2$, 100%); Anal. Calcd. for $C_{18}H_9N_3O_2S_2$ (363.41): C, 59.49, H, 2.50, N, 11.56. Found: C, 59.62, H, 2.48, N, 17.81.

2.2.3 | 2-(1,3 benzothiazol-2-yl)-2-(5-oxo-3-phenyl(1,3)thiazolidin-2-ylidene) acetonitrile (2c)

This product was obtained as goldenrod crystals; (1.57 g, 0.432 mmol), yield: 78.89%, m.p: 188–190°C; IR(cm^{-1}): 3,093 (CH aromatic), 2,974 (CH aliphatic), 2,194 (C≡N), 1,720 (C=O); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 4.17 (s, 2H, thiazolidine-C_4-H), 7.17 (t, 1H, phenyl-C_4-H), 7.41 (t, 2H, phenyl-$C_{3,5}$-H), 7.51–7.58 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.66 (d, 2H, $J = 8.1$ Hz, phenyl-$C_{2,6}$-H), 7.87–7.92 (dd, 1H, $J = 7.5$, 8.1 Hz, benzothiazole-C_7-H), 8.07 (d, 1H, $J = 8.7$ Hz, benzothiazole-C_4-H); ^{13}CNMR(DMSO-*d6*-300 MHz, ppm): 78.98 (thiazolidine-C_4, C-$_{C\equiv N}$), 114.32 (C≡N), 121.69 (phenyl-C_4), 124.85 (phenyl-$C_{3,5}$), 126.68 (phenyl-$C_{2,6}$), 129.45 (benzothiazole-$C_{5,6}$), 130.63 (benzothiazole-$C_{4,7}$), 132.84 (phenyl-C_1), 134.82 (benzothiazole-C_{1a}), 152.97 (benzothiazole-C_{3a}), 163.83 (benzothiazole-C_2), 164.33 (thiazolidine-C_2), 173.08 (thiazolidine-C_5); MS (*m/z*): 350.85 (M^+ +1, 5.75%), 349.90 (M^+, 12.6%), 275.95 ($C_{12}H_{10}N_3OS_2$, 100%); Anal. Calcd. for $C_{18}H_9N_3O_2S_2$ (363.41): C, 61.87, H, 3.17, N, 12.03. Found: C, 61.95, H, 3.21, N, 12.11.

2.2.4 | 2-(1,3 benzothiazol-2-yl)-2-(4-methyl-5-oxo-3-phenyl(1,3)thiazolidin-2-ylidene) acetonitrile (2d)

Dark brown crystals; (1.6 g, 0.44 mmol), yield: 78.31%, m.p: 180–182°C; IR(cm^{-1}): 3,059 (CH aromatic), 2,981 (CH aliphatic), 2,196 (C≡N), 1,734 (C=O); ^{1}H-NMR (DMSO-*d6*-400 MHz, ppm): 1.69 (d, 3H, $J = 7.24$ Hz, CH_3-H), 4.42 (q, 1H, $J = 7.12$, 7.16 Hz, thiazolidine-C_4-H), 7.17 (t, 1H, phenyl-C_4-H), 7.32–7.38 (t, 2H, phenyl-C-$_{3,5}$-H), 7.48 (d, 2H, $J = 10$ Hz, phenyl-$C_{2,6}$-H), 7.51–7.57 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.93 (dd, 1H, $J = 7.7$, 8.12 Hz, benzothiazole-C_7-H), 8.05 (dd, 1H, $J = 7.8$ Hz, benzothiazole-C_4-H); Anal. Calcd. for $C_{19}H_{13}N_3OS_2$ (363.46): C, 62.79, H, 3.61, N, 11.56. Found: C, 62.92, H, 3.65, N, 11.72.

2.3 | General procedure of synthesis of compounds (3,4)

A mixture of **2b** (1 g, 0.27 mmol) and either phenylenediamine (0.29 g, 0.27 mmol) or 2-aminothiophenol (0.33 g, 0.27 mmol) was heated under reflux in glacial acetic acid (15 ml) for 10 hr. After cooling, the reaction mixture was poured onto ice-cold water and stirred for 10 min, and the separated solid was filtered, dried, and crystallized from ethanol.

2.3.1 | 2-(1,3 benzothiazol-2-yl)-2-(3-phenylthiazolo[4,5-b]quinoxalin-2(3H)-ylidene) acetonitrile (3)

It was obtained as black crystals; (0.80 g, 0.183 mmole), yield: 68.08%, m.p: 223–225°C; IR(cm^{-1}): 3,057 (CH aromatic), 2,200 (C≡N), 1,541 (C=N); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 7.28 (t, 3H, phenyl-$C_{3,4,5}$-H), 7.38 (t, 4H, benzothiazole-$C_{5,6}$-H, quinoxaline-$C_{6,7}$-H), 7.65 (d, 2H, $J = 8.1$ Hz, phenyl-$C_{2,6}$-H), 7,94 (d, 4H, $J = 7.5$ Hz, benzothiazole-$C_{4,7}$-H, quinoxaline-$C_{5,8}$-H); MS (*m/z*): 435.90 (M^+, 0.28%), 79.90 ($C_4H_4N_2$, 100%); Anal. Calcd. for $C_{24}H_{13}N_5S_2$ (435.52): C, 66.19, H, 3.01, N, 16.08. Found: C, 66.34, H, 2.97, N, 16.21.

2.3.2 | 2-(1,3 benzothiazol-2-yl)-2-(3-phenyl-3,3a-Dihydro-thiazolo[4,5-b](1,4) benzothiazin-2-ylidene) acetonitrile (4)

Black crystals; (0.74 g, 0.162 mmole), yield: 60.30%, m.p: 202–204°C; IR (cm^{-1}): 3,059 (CH aromatic), 2,918 (CH aliphatic), 2,181(C≡N), 1,537(C=N); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 2.82 (s, 1H, thiazole-C_4-H) 7.25 (t, 3H, phenyl-$C_{3,4,5}$-H), 7.43 (t, 4H, benzothiazole-$C_{5,6}$-H, benzothiazine-$C_{6,7}$-H), 7.66 (d, 2H, $J = 7.8$ Hz, phenyl-$C_{2,6}$-H), 7.93 (d, 4H, $J = 7.5$ Hz, benzothiazole-$C_{4,7}$-H, benzothiazine-$C_{5,8}$-H); MS (*m/z*): 455.20 (M^++1, 20.15%), 454.20 (M^+, 24.38%), 62.00 (CH_4NS, 100%); Anal. Calcd. for $C_{24}H_{14}N_4S_3$ (454.59): C, 63.41, H, 3.10, N, 12.32. Found: C, 63.47, H, 3.08, N, 12.51.

2.4 | General procedure of synthesis of compounds (5a–e)

A mixture of **2c** (1 g, 0.28 mmol), appropriate aldehyde (0.28 mmol) [either 4-hydroxybenzaldehyde (0.34 g), 4-chlorobenzaldehyde (0.39 g), 3-hydroxybenzaldehyde (0.349 g), salicylaldehyde (0.34 g) or furaldehyde (0.26 g)], and acetic acid (1 ml) in absolute ethanol (20 ml) was heated under reflux for 10 hr. The reaction mixture was cooled, and the separated solid was filtered, dried, and crystallized from ethanol.

2.4.1 | 2-(1,3 benzothiazol-2-yl)-2-(4-(4-hydroxybenzylidene)-5-oxo-3-phenyl-thiazolidin -2-ylidene)acetonitrile (5a)

Yellow crystals; (1.08 g, 0.198 mmole), yield: 85.10%, m.p: >300°C; IR(cm^{-1}): 3,360–3,200 (OH), 3,070 (CH aromatic), 2,200 (C≡N), 1,707 (C=O); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 7.03 (d, 2H, $J = 8.7$ Hz, aldehyde-$C_{3,5}$-H), 7.42 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.55–7.65 (m, 5H, phenyl-H), 7.69 (d, 2H, $J = 8.7$ Hz, aldehyde-$C_{2,6}$-H), 7.83 (s, 1H, =CH), 8.07 (d, 1H, $J = 7.2$ Hz, benzothiazole-C_7-H), 8.18 (d, 1H, $J = 7.8$ Hz, benzothiazole-C_4-H), 10.47 (s, 1H, OH, D_2O exchangeable); ^{13}CNMR (DMSO-d6-300 MHz, ppm): 79.00 (C-$_{C\equiv N}$), 114.27 (aldehyde-$C_{3,5}$), 116.50 (benzothiazole-$C_{5,6}$), 121.97 (C≡N), 122.33 (phenyl-C_4), 124.26 (phenyl-$C_{3,5}$), 125.05 (phenyl-$C_{2,6}$), 126.73 (phenyl-C_1), 129.42 (aldehyde-$C_{1,2,6}$), 130.76 (C=C), 132.9 (benzothiazole-$C_{4,7}$), 133.20 (benzothiazole-C_{1a}), 134.50 (thiazolidine-C_4), 153.05 (thiazolidine-C_2), 156.43 (benzothiazole-C_{3a}), 160.36 (benzothiazole-C_2), 163.22 (aldehyde-C_4), 166.22 (thiazolidine-C_5); Anal. Calcd. for $C_{25}H_{15}N_3O_2S_2$ (453.54): C, 66.21, H, 3.33, N, 9.27. Found: C, 66.29, H, 3.37, N, 9.39.

2.4.2 | 2-(1,3 benzothiazol-2-yl)-2-(4-(4-chlorobenzylidene)-5-oxo-3-phenyl-thiazolidin-2-ylidene)acetonitrile (5b)

Ocher crystals; (1.05 g, 0.22 mmole), yield: 79.54%, m.p: >300°C; IR(cm^{-1}): 3,024 (CH aromatic), 2,196 (C≡N), 1,705 (C=O); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 7.45 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.55–7.65 (m, 5H, phenyl-H), 7.72 (d, 2H, $J = 8.7$ Hz, aldehyde-$C_{3,5}$-H), 7.83 (d, 2H, $J = 8.7$ Hz, aldehyde-$C_{2,6}$-H), 7.92 (s, 1H, =CH), 8.08 (d, 1H, $J = 7.8$ Hz, benzothiazole-C_7-H), 8.15 (d, 1H, $J = 7.8$ Hz, benzothiazole-C_4-H); Anal. Calcd. for $C_{25}H_{14}ClN_3OS_2$ (471.98): C, 63.62, H, 2.99, N, 8.90. Found: C, 63.68, H, 2.96, N, 9.02.

2.4.3 | 2-(1,3 benzothiazol-2-yl)-2-(4-(3-hydroxybenzylidene)-5-oxo-3-phenyl-thiazolidin-2-ylidene)acetonitrile (5c)

Yellow crystals; (0.93 g, 0.205 mmole), yield: 73.28%, m.p: >300°C; IR(cm^{-1}): 3,530–3,200 (OH), 3,026 (CH aromatic), 2,194 (C≡N), 1,708 (C=O); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 6.98 (d, 1H, $J = 8.1$ Hz, aldehyde-C_4-H), 7.23 (s, 1H, aldehyde-C_2-H), 7.25 (d, 1H, $J = 7.5$ Hz, aldehyde-C_6-H), 7.48 (t, 1H, aldehyde-C_5-H), 7.59–7.62 (t, 2H, benzothiazole-$C_{5,6}$-H) 7.63–7.67 (m, 5H, phenyl-H), 7.82 (s, 1H, =CH), 8.09 (d, 1H, $J = 0.6$ Hz, benzothiazole-C_7-H), 8.14 (d, 1H, $J = 7.5$ Hz, benzothiazole-C_4-H), 9.99 (s, 1H, OH, D_2O exchangeable); MS (*m/z*): 454.80 (M^++1, 8.69%), 453.80 (M^+, 19.33%), 274.90 ($C_{12}H_9N_3OS_2$, 100%); Anal. Calcd. for

$C_{25}H_{15}N_3O_2S_2$ (453.54): C, 66.21, H, 3.33, N, 9.27. Found: C, 66.33, H, 3.37, N, 9.38.

2.4.4 | 2-(1,3 benzothiazol-2-yl)-2-(4-(2-hydroxybenzylidene)-5-oxo-3-phenyl-thiazolidin -2-ylidene)acetonitrile (5d)

Brown crystals; (0.67 g, 0.147 mmole), yield: 52.79%, m.p: 260–262°C; IR(cm^{-1}): 3,400–3,120 (OH), 3,040 (CH aromatic), 2,196 (C≡N), 1,718 (C=O); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 7.05 (d, 1H, J = 8.1 Hz, aldehyde-C_3-H), 7.15 (t, 1H, aldehyde-C_5-H), 7.40 (t, 1H, aldehyde-C_4-H), 7.43 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.55–7.63 (m, 5H, phenyl-H), 7.92 (d, 1H, J = 8.1, aldehyde-C_6-H), 8.04 (d, 1H, J = 9.6 Hz, benzothiazole-C_7-H), 8.07 (s, 1H, =CH), 8.13 (d, 1H, J = 7.5 Hz, benzothiazole-C_4-H), 10.67 (s, 1H, OH, D_2O exchangeable); Anal. Calcd. for $C_{25}H_{15}N_3O_2S_2$ (453.54): C, 66.21, H, 3.33, N, 9.27. Found: C, 66.31, H, 3.38, N, 9.35.

2.4.5 | 2-(1,3 benzothiazol-2-yl)-2-((4-(furan-2-yl)methylene)-5-oxo-3-phenyl-thiazolidin -2-yli-dene)acetonitrile (5e)

Copper crystals; (0.70 g, 0.163 mmole), yield: 58.50%, m.p: >300°C; IR(cm^{-1}): 3,035 (CH aromatic), 2,196 (C≡N), 1,705 (C=O); ^{1}H-NMR (DMSO-*d6*-300 MHz, ppm): 6.85 (t, 1H, aldehyde-C_4-H), 7.24 (d, 1H, J = 3.3 Hz, aldehyde-C_3-H), 7.44 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.58–7.64 (m, 5H, phenyl-H), 7.76 (s, 1H, =CH), 8.09 (d, 2H, J = 8.4 Hz, benzothiazole-C_7-H, aldehyde-C_5-H), 8.27 (d, 1H, J = 1.5 Hz, benzothiazole-C_4-H); ^{13}CNMR(DMSO-*d6*-300 MHz, ppm): 76.06 ($C_{-C\equiv N}$), 113.92 (aldehyde-C_4), 114.26 (aldehyde-C_3), 118.35 (benzothiazole-$C_{5,6}$), 119.80 (phenyl-C_4, C≡N), 122.03 (phenyl-$C_{3,5}$), 125.12 (phenyl-$C_{2,6}$), 126.78 (phenyl-C_1), 129.42 (benzothiazole-$C_{4,7}$,C=C), 129.76 (benzothiazole-C_{1a}), 130.76 (aldehyde-C_5), 133.50 (thiazolidine-C_2), 133.80 (aldehyde-C_2), 134.75 (benzothiazole-C_{3a}), 149.84 (thiazolidine-C_4, benzothiazole-C_2), 153.03 (C=O); MS (*m/z*): 429.85 (M^++2, 1.33%), 428.85 (M^++1, 5.84%), 427.85 (M^+, 12.39%), 124.00 ($C_7H_8O_2$, 100%); Anal. Calcd. for $C_{23}H_{13}N_3O_2S_2$ (427.50) C, 64.62, H, 3.07, N, 9.83. Found: C, 64.73, H, 3.12, N, 10.01.

2.5 | General procedure of synthesis of compounds (6a–g)

An ice-cold solution of various diazonium salts [prepared from appropriate amine (0.11 mmol) either 3-amino-1*H*-pyrazol-5(4*H*)-one (0.113 g), 3-amino-1-phen yl-1*H*-pyrazol-5(4*H*)-one (0.2 g), 4-amino-1,2-dihydro-1,5-dimethyl-2-phenylpyrazol-3-one (0.23 g), 4-hydroxyaniline (0.124 g),4-chloroaniline (0.146 g),3-hydroxyaniline (0.124 g) or 3-methylaniline (0.122 g)], concentrated hydrochloric acid (0.11 mmol), and sodium nitrite (0.11 mmol) in water (3 ml)] was added to a chilled solution of **2c** (0.11 mmol) and sodium hydroxide (0.11 mmol) in water (3 ml). The reaction mixture was maintained at −5°C with continuous stirring for 30 mins and then acidified with glacial acetic acid till pH 5–5.5. The resulting solid was filtered, washed with water, dried, and crystallized from ethanol.

2.5.1 | 4-[2-(1H-pyrazol-5(4H)-one-3-yl) diazen-1-yl]-5-oxo-3-phenylthiazo-lidin-2-ylidene -2-1,3 benzothiazol-2-yl-acetonitrile (6a)

Brick red crystals; (0.41 g, 0.089 mmole), yield: 81.18%, m.p: >300°C; IR(cm^{-1}): 3,431(NH), 3,032 (CH aromatic), 2,953 (CH aliphatic), 2,196 (C≡N) 1,718, 1,700 (2 C=O); ^{1}H-NMR (DMSO-*d6*-400 MHz, ppm): 2.48 (s, 2H, pyrazole-C_4-H), 3.16 (s, 1H, thiazolidine-C_4-H), 6.11 (s, 1H, NH, D_2O exchangeable), 7.14 (t, 1H, phenyl-C_4-H), 7.30 (d, 2H, J = 8.5 Hz, phenyl-$C_{2,6}$-H), 7.41 (t, 2H, phenyl-$C_{3,5}$-H), 7.51 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.92 (d, 1H, J = 7.48 Hz, benzothiazole-C_7-H), 8.01 (dd, 1H, J = 8 Hz, benzothiazole-C_4-H); Anal. Calcd. for $C_{21}H_{13}N_7O_2S_2$ (459.50) C, 54.89, H 2.85, N 21.34. Found: C, 54.97, H, 2.81, N, 21.49.

2.5.2 | 4-[2-(1-phenyl-1H-pyrazol-5(4H)-one-3-yl)diazen-1-yl]-5-oxo-3-phenyl-thiazo-lidin-2-ylidene-2-1,3 benzothiazol-2-yl-acetonitrile (6b)

Brick red crystals; (0.44 g, 0.082 mmole), yield: 74.68%, m.p: 140–142°C, IR(cm^{-1}): 3,061 (CH aromatic), 2,926 (CH aliphatic), 2,198 (C≡N), 1,701, 1,690 (2 C=O); ^{1}H-NMR (DMSO-*d6*-400 MHz, ppm): 2.48 (s, 2H, pyrazole-C_4-H), 3.16 (s, 1H, thiazolidine-C_4-H), 6.11 (s, 1H, NH, D_2O exchangeable), 7.14 (t, 1H, phenyl-C_4-H), 7.30 (d, 2H, J = 8.5 Hz, phenyl-$C_{2,6}$-H), 7.41 (t, 2H, phenyl-$C_{3,5}$-H), 7.51 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.92 (d, 1H, J = 7.48 Hz, benzothiazole-C_7-H), 8.01 (dd, 1H, J = 8 Hz, benzothiazole-C_4-H); Anal. Calcd. for $C_{27}H_{17}N_7O_2S_2$ (535.60) C, 60.55, H, 3.20, N, 18.31. Found: C, 60.75, H, 3.24, N, 18.83.

2.5.3 | 4-[2-(1,2-Dihydro-1,5-dimethyl-2-phenylpyrazol-3-one-4-yl)diazen-1-yl]-5-ox-3-phenylthiazolidin-2-ylidene-2-1,3 benzothiazol-2-yl-acetonitrile (6c)

Dark red brown crystals; (0.45 g, 0.079 mmole), yield: 72.58%, m.p: 170–172°C; IR(cm^{-1}): 3,032 (CH aromatic), 2,974 (CH aliphatic), 2,196 (C≡N), 1,718, 1,700 (2 C=O); ^{1}H-NMR (DMSO- *d6*-400 MHz, ppm): 2.68 (s, 3H, CH_3-H), 3.15 (s, 3H, N-CH_3-H), 3.44 (s, 1H, thiazolidine-C_4-H),7.34

(t, 2H, benzothiazole-$C_{5,6}$-H), 7.40–7.43 (m, 5H, phenyl-H), 7.50–7.60 (m, 5H, amine-phenyl-H), 7.92 (d, 1H, $J = 8$fz, benzothiazole-C_7-H), 8.05 (d, 1H, $J = 8$ Hz, benzothiazole-C_4-H); ^{13}CNMR (DMSO- *d6*-400 MHz, ppm): 19.03 (CH_3), 37.30 (N- CH_3), 56.48 ($C_{-C\equiv N}$), 112.48 (pyrazolone-C_4), 120.16 (thiazolidine-C_4), 121.34 (C≡N), 122.08 (benzothiazole-$C_{5,6}$), 122.85 (phenyl-$C_{2,6}$), 124.03 (phenyl-C_4), 124.26 (phenyl-$C_{3,5}$), 124.58 (amine-phenyl-$C_{2,6}$), 125.46 (amine-phenyl-C_4), 126.71 (amine-phenyl-$C_{3,5}$), 128.56 (benzothiazole-$C_{4,7}$), 129.07 (benzothiazole-C_{1a}), 129.41 (phenyl-C_1), 129.48 (amine-phenyl-C_1), 129.77 (thiazolidine-C_2), 129.86 (benzothiazole-C_{3a}), 132.99 (benzothiazole-C_2), 153.85 (pyrazolone-C_5), 164.42 (pyrazolone-C_3), 170.29 (thiazolidine-C_5); Anal. Calcd. for $C_{29}H_{21}N_7O_2S_2$ (563.65) C, 61.80, H, 3.76, N, 17.39. Found: C, 61.94, H, 3.82, N, 17.51.

2.5.4 | 4-[2-(4-hydroxyphenyl)diazen-1-yl]-5-oxo-3-phenylthiaazolidin-2-ylidene-2-1,3 benzothiazol-2-yl-acetonitrile (6d)

Dark brick red crystals; (0.39 g, 0.083 mmole), yield: 75.58%, m.p: 82–84°C; IR (cm^{-1}): 3,529–3,155 (OH), 3,016 (CH aromatic), 2,956 (CH aliphatic), 2,187 (C≡N), 1,720 (C=O); ^{1}H-NMR (DMSO- *d6*-400 MHz, ppm): 3.45 (s, 1H, thiazolidine-C_4-H), 6.94 (d, 2H, $J = 6$ Hz, amine-$C_{2,6}$-H), 7.33 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.46–7.63 (m, 5H, phenyl-H), 7.82 (d, 2H, $J = 6$ Hz, amine-$C_{3,5}$-H), 7.91 (dd, 1H, $J = 6$ Hz benzothiazole-C_7-H), 8.05 (d, 1H, $J = 7.5$ Hz, benzothiazole-C_4-H), 9.24 (s, 1H, OH, D_2O exchangeable); ^{13}CNMR (DMSO- *d6*-400 MHz, ppm): 66.56 ($C_{-C\equiv N}$), 113.66 (thiazolidine-C_4), 114.84 (C≡N), 116.80 (amine-phenyl-$C_{3,5}$), 120.64 (benzothiazole-$C_{5,6}$), 121.23 (phenyl-$C_{2,6}$), 122.55 (phenyl-C_4), 123.45 (phenyl-$C_{3,5}$), 124.57 (amine-phenyl-$C_{2,6}$), 125.81 (benzothiazole-$C_{4,7}$), 127.21 (benzothiazole-C_{1a}), 128.66 (phenyl-C_1), 129.17 (amine-phenyl-C_1), 129.96 (thiazolidine-C_2), 130.14 (benzothiazole-C_{3a}), 153.46 (amine-phenyl-C_4), 167.63 (benzothiazole-C_2), 173.63 (thiazolidine-C_5), Anal. Calcd. for $C_{24}H_{15}N_5O_2S_2$ (469.54) C, 61.39, H 3.22, N, 14.92. Found: C, 61.31, H, 3.25, N, 15.08.

2.5.5 | 4-[2-(4-chlorophenyl)diazen-1-yl]-5-oxo-3-phenylthiaazolidin-2-ylidene-2-1,3 benzothiazol-2-yl-acetonitrile (6e)

Brick red crystals; (0.40 g, 0.081 mmole), yield: 74.51%, m.p: 87–89°C; IR(cm^{-1}): 3,032 (CH aromatic), 2,983 (CH aliphatic), 2,196 (C≡N), 1,720 (C=O); ^{1}H-NMR (DMSO-*d6*-400 MHz, ppm): 3.48 (s, 1H, thiazolidine-C_4-H), 7.11 (d, 2H, $J = 4$ Hz, amine-$C_{3,5}$-H), 7.19 (d, 2H, $J = 4$ Hz, amine-$C_{2,6}$-H), 7.36 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.41–7.49 (m, 5H, phenyl-H), 7.80 (d, 1H, $J = 7.5$ Hz, benzothiazole-C_7-H), 7.95 (d, 1H, $J = 8$ Hz, benzothiazole-C_4-H); Anal. Calcd. for $C_{24}H_{14}ClN_5OS_2$ (487.98) C, 59.07, H, 2.89, N, 14.35. Found: C, 59.22, H, 2.83, N, 14.52.

2.5.6 | 4-[2-(3-hydroxyphenyl)diazen-1-yl]-5-oxo-3-phenylthiaazolidin-2-ylidene-2-1,3 benzothiazol-2-yl-acetonitrile (6f)

Brick red crystals; (0.38 g, 0.08 mmol), yield: 73.64%, m.p: >300°C; IR(cm^{-1}): 3,535–3,143 (OH), 3,016 (CH aromatic), 2,926 (CH aliphatic), 2,218 (C≡N), 1,722 (C=O); ^{1}H-NMR (DMSO- *d6*-400 MHz, ppm): 3.34 (s, 1H, thiazolidine-C_4-H), 6.94 (s, 1H, amine-C_2-H), 7.15 (d, 1H, $J = 6$ Hz, amine-C_6-H), 7.33 (m, 5H, phenyl-H), 7.40 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.51 (t, 1H, amine-C_4-H), 7.56 (d, 1H, $J = 4.8$ Hz, amine-C_5-H), 7.80 (d, 1H, $J = 6.2$ Hz, benzothiazole-C_7-H), 7.91(d, 1H, $J = 6.8$ Hz, benzothiazole-C_4-H), 9.23 (s, 1H, OH, D_2O exchangeable); Anal. Calcd. for $C_{24}H_{15}N_5O_2S_2$ (469.54) C, 61.39, H, 3.22, N, 14.92. Found: C, 61.52, H, 3.27, N, 15.11.

2.5.7 | 4-[2-(3-methylphenyl)diazen-1-yl]-5-oxo-3-phenylthiazolidin-2-ylidene-2-1,3 benzothiazol-2-yl-acetonitrile (6g)

Brick red crystals; (0.41 g, 0.0812 mmole), yield: 72.40%, m.p: 82–84°C; IR(cm^{-1}): 3,032 (CH aromatic), 2,953 (CH aliphatic), 2,212 (C≡N), 1,720 (C=O); ^{1}H-NMR (DMSO-*d6*-400 MHz, ppm): 2.35 (s, 3H, CH_3-H), 3.70 (s, 1H, thiazolidine-C_4-H), 6.94 (s, 1H, amine-C_2-H), 6.94 (d, 1H, $J = 8$ Hz, amine-C_4-H), 7.31 (m, 5H, phenyl-H), 7.50 (t, 2H, benzothiazole-$C_{5,6}$-H), 7.56 (t, 1H, amine-C_5-H), 7.60 (d, 1H, $J = 7.72$ Hz, amine-C_6-H), 7.94 (d, 1H, $J = 8.6$ Hz, benzothiazole-C_7-H), 8.08 (d, 1H, $J = 7.72$ Hz, benzothiazole-C_4-H); Anal. Calcd. for $C_{24}H_{15}N_5O_2S_2$ (469.54) C, 64.22, H, 3.66, N, 14.98. Found: C, 64.30, H, 3.69, N, 15.13.

2.6 | Cytotoxic activity studies

Cytotoxic activity studies were conducted at Ain Shams University, Faculty of Pharmacy, Pharmacology Department and National Research Centre, Biochemistry Department, Division of Genetic Engineering and Biotechnology.

All compounds were tested using sulfo-rhodamine B (SRB) assay for cytotoxic activity against breast carcinoma cell line (MCF-7).

2.7 | Measurement of potential cytotoxicity by SRB assay against cisplatin

Cytotoxicity was determined using SRB method as previously described by Skehan et al.[21] The compounds were tested against cisplatin. Exponentially growing cells were collected using 0.25% trypsin–EDTA and seeded in 96-well plates at (10^4 cells/well) in RPMI-1640-supplemented

medium. After 24 hr, cells were incubated for 72 hr with various concentrations of the tested compounds. Following 72-hr treatment, the cells were fixed with 10% trichloroacetic acid for 1 hr at 4°C. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air-dried for 24 hr, and the dye was solubilized with Tris–HCl for five min. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA). IC_{50} values were calculated subject to the equation of Boltzmann sigmoidal concentration–response curve using the nonlinear regression fitting models.

2.8 | Antioxidant status assays

2.8.1 | Antioxidant enzyme assays

The cells in culture medium were treated with 20 µl of 1/10 of IC_{50} values of the compounds and then incubated for 24 hr at 37°C, in a humidified 5% CO_2 atmosphere. The MCF-7 cells were harvested and homogenates were prepared in saline using a tight pestle homogenizer until complete cell disruption for further biochemical analysis. The supernatant obtained after centrifugation of cell homogenates at 3,000 g was used for determination of activities of enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) as described by Paglia and Valentine,[22] Aebi,[23] and Marklund and Marklund[24]

2.8.2 | Oxidative stress assays

The levels of hydrogen peroxide (H_2O_2), nitric oxide (NO), and reduced glutathione (GSH) were determined by methods of Wolf,[25] Montgomery and Dymock,[26] and Ellman,[27]

2.8.3 | Estimation of nucleic acids and protein

Nucleic acids (DNA and RNA) and total protein were precipitated and measured in cell homogenates. Total DNA was extracted and assayed according to the method described by Zhou et al.[28] Total RNA was extracted and assayed according to the method adopted from the method provided by Hybaid/AGS (Germany), and total cellular protein was assayed by the method of Lowry and associates.[29]

2.9 | Statistical analysis

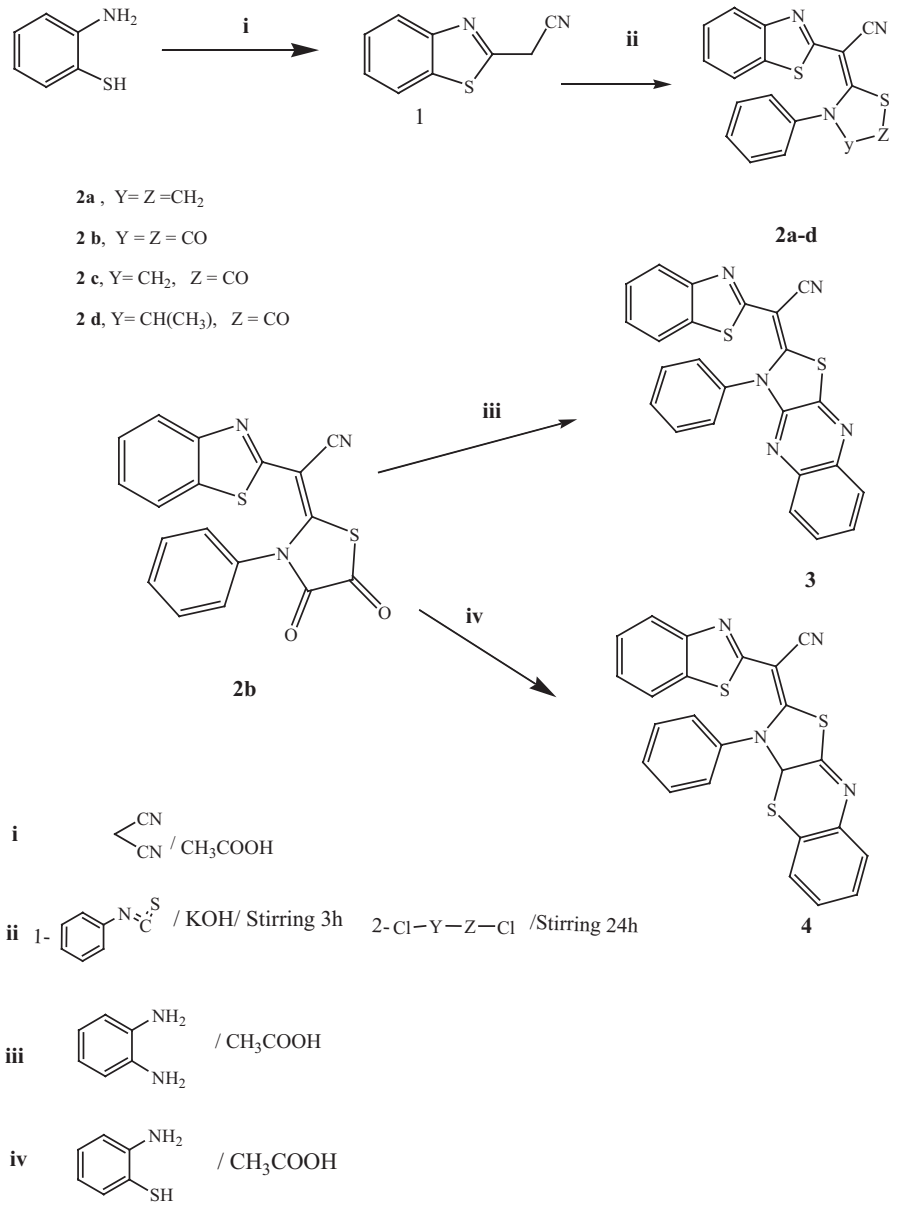
The results were reported as Mean ± standard error (*SE*) for at least four times. Statistical differences were analyzed according to one-way ANOVA test followed by Student's *t* test wherein the differences were considered to be significant at $p < .05$.

3 | RESULTS AND DISCUSSION

3.1 | Chemistry

The synthesis of new benzothiazole derivatives was achieved through Schemes 1 and 2. The structure of the newly synthesized compounds was confirmed by elemental analysis and spectral data (IR, ^{1}HNMR, ^{13}CNMR, and MS). First 2-(1,3 benzothiazol-2-yl) acetonitrile **(1)** was synthesized as starting material by stirring a mixture of *o*-aminothiophenol and malononitrile in absolute ethanol and glacial acetic acid at room temperature.[20] Preparation of new compounds **2a–d** was achieved following a previously reported procedure for analogous compounds,[30] in which compound **1** was treated with phenylisothiocyanate and potassium hydroxide in dimethylformamide followed by the addition of appropriate acid chloride (chloroacetyl chloride, 3-chloropropionyl chloride, 2-chloropropionyl chloride, and oxalyl chloride). The produced derivatives gave good yield (62%–87%). The structure of which was proven by the appearance of CH aliphatic absorption band at 2,920 cm^{-1} for **2a** and its ^{1}HNMR spectrum showed the appearance of two triplet signals at δ 3.40 and 4.26 ppm with $J = 7.50$ Hz corresponding to thiazolidine-$C_{-5,4}$-H. On the other hand, the structure of **2b** was proven by the IR spectrum which declared the appearance of 2 (C=O) absorption bands at 1,700, 1,680 cm^{-1}, where the ^{1}HNMR spectrum revealed the presence of multiplet signal at δ 7.41–7.52 ppm equivalent to aromatic protons. The two C=O groups were confirmed by the ^{13}CNMR spectrum, which exhibited two signals at δ 168.52 and 186.59 ppm. Furthermore, 3c IR declared CH aliphatic absorption band at 2,974 cm^{-1} and (C=O) band at 1,720 cm^{-1}. The ^{1}HNMR spectrum showed the appearance of singlet signal at δ 4.17 ppm equivalent to thiazolidine-C_4-H. Finally, the IR spectrum of **2d** revealed CH aliphatic absorption band at 2,981 cm^{-1} and (C=O) band at 1,734 cm^{-1}. The ^{1}HNMR spectrum declared the presence of doublet signal at δ 1.69 ppm for the CH_3-H with $J = 7.24$ Hz and quartet at δ 4.42 ppm for the thiazolidine-C_4-H with $J = 7.12, 7.16$ Hz, where the phenyl protons appeared as two triplet signals at δ 7.17 and 7.32–7.38 ppm for C_4-H and $C_{3,5}$-H, respectively, while the phenyl-$C_{2,6}$-H was detected as doublet signal at δ 7.48 ppm with $J = 10.00$ Hz.

It was reported that 1,4-diazines and 1,4-thiazines were synthesized via the condensation of 1,2-diamines with 1,2-dicarbonyl compounds or 2-aminothiophenol with maleic anhydride.[31,32] In our study, cyclization was performed by the condensation of the dicarbonyl derivative **2b** with phenylene diamine and 2-aminothiophenol producing compounds **3** and **4**, whose structures were proven by disappearance of the carbonyl group bands at 1,700 and 1,680 cm^{-1} and detection of C=N band at 1,541 cm^{-1}. The ^{1}HNMR

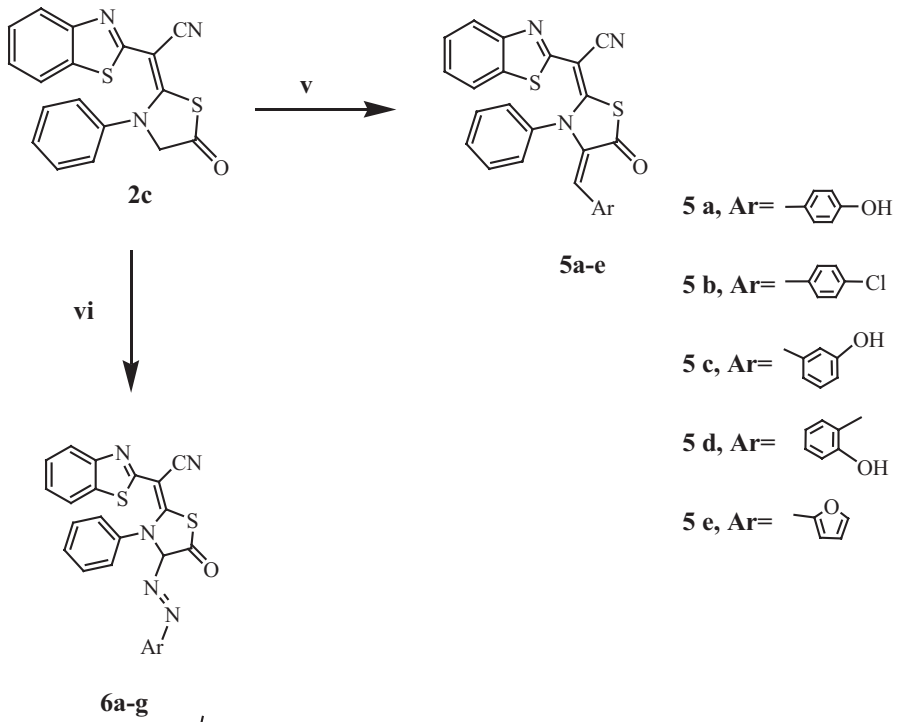


SCHEME 1 Synthesis protocol. General synthesis of compounds **2a–2d and 3,4**

spectrum of **3** showed triplet signal at δ 7.38 ppm of both benzothiazole-$C_{5,6}$-H and quinoxaline-$C_{6,7}$-H, while doublet signal at δ 7.94 ppm with $J = 7.50$ Hz was assigned to benzothiazole-$C_{4,7}$-H and quinoxaline-$C_{5,8}$-H, and that of **4** showed the appearance of singlet signal at δ 2.82 ppm due to the thiazole-C_4-H, triplet signal at δ 7.43 ppm of both benzothiazole-$C_{5,6}$-H and benzothiazine-$C_{6,7}$-H, while doublet signal at δ 7.93 ppm with $J = 7.50$ Hz was assigned to both benzothiazole-$C_{4,7}$-H and benzothiazine-$C_{5,8}$-H.

Moreover, the new derivatives **5a–e** were prepared following the previously reported procedure[33] by acid-catalyzed reaction of compound **2c** with different aldehydes (4-hydroxybenzaldehyde, 4-chlorobenzaldehyde, 3-hydroxybenzaldehyde, salicylaldehyde, and furaldehyde) in absolute ethanol. Although two isomers were supposed to be produced by this procedure, only one isomer of **5a–e** was separated, which was confirmed by the appearance of singlet signal in ^{1}HNMR at 7.83, 7.92, 7.82, 8.07, and 7.76 for **5a–e**.

The new compounds **6a–g** were synthesized following Shabaan et al.'s procedure.[34] The diazotized compounds were synthesized by reaction of the active methylene of compound **2c** with various amines (3-amino-1*H*-pyrazol-5(4*H*)-one, 3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)-one, 4-amino-1,2-dihy -dro-1,5-dimethyl-2-phenylpyrazol-3-one, 4-hydroxyaniline, 4-chloroaniline, 3-hydro-xyaniline, and 3-methylaniline).The newly synthesized compounds' structure was confirmed by the added groups from the diazotized amines where **6a** IR spectrum revealed the presence of (NH) group band at 3,431 cm^{-1} and two (C=O) bands at 1,718 and 1,700 cm^{-1}, while the ^{1}H-NMR spectrum showed singlet signal at δ 2.48 ppm corresponding to pyrazole-C_4-H and D_2O exchangeable singlet signal was appeared at δ 6.11 ppm for the NH proton. **6b**



SCHEME 2 Synthesis protocol. General synthesis of compounds **5a–e and 6a–g**

showed 2 (C=O) absorption bands at 1,701 and 1,690 cm^{-1}, and singlet signal was detected at the ^{1}HNMR spectrum at δ 3.34 ppm equivalent to pyrazole-C_4-H. Moreover, **6c** IR revealed 2 (C=O) absorption bands at 1,718 and 1,700 cm^{-1} and its ^{1}HNMR spectrum revealed two singlet signals at δ 2.68 and 3.15 ppm representing CH_3-H and N-CH_3-H. (OH) absorption band was detected at 3,529–3,155 cm^{-1} for compound **6d.** Hence, **6e** ^{1}HNMR spectrum exhibited two doublet signals at δ 7.11 and 7.19 ppm corresponding to the amine-$C_{3,5}$-H and amine-$C_{2,6}$-H with $J = 4.00$ Hz revealing the *p*-substitution. Compound **6f** IR spectrum revealed (OH) absorption band at 3,535–3,143 cm^{-1}, which was detected at the ^{1}HNMR spectrum as singlet D_2O exchangeable signal at δ 9.23 ppm. Finally, compound **6g** IR spectrum showed (C=O) absorption band at 1,720 cm^{-1}. The ^{1}HNMR spectrum showed singlet signal at δ 2.35 ppm for the CH_3-H.

3.2 | Cytotoxic effect

All the newly synthesized compounds were tested for their cytotoxic activity against human breast cancer cell line (MCF-7) compared to cisplatin (Figure 3)**.** The results are

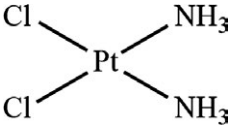


FIGURE 3 Structure of cisplatin

shown in Table 1. It was noticed that most of the compounds were found to possess good cytotoxic activity, four of which revealed potent effect, so submitted to further assays to elucidate the mechanism by which the prepared compounds **4, 5c, 5d,** and **6b** exerted their cytotoxic activities. Some structural features that are important for the explanation of their cytotoxic effects showed that the coupling with aldehydes at C-4 of the thiazolidine ring contributes better activity for most derivatives specifically those bearing hydroxyl group. Moreover, the coupling with the diazotized amines provided **6b** showing the highest activity among all derivatives. On the other hand, compounds **3** and **4** although similar in structure but the added sulfur atom in **4** may be the cause for its better activity.

TABLE 1 The cytotoxic effect of prepared compounds against human breast MCF-7 cancer cell line as measured with SRB assay against cisplatin

No.	IC_{50} in μM
Cisplatin	13.33
2a	47.12
2b	30.39
2c	37.71
2d	48.99
3	24.83
4	8.64
5a	16.61
5b	103.1
5c	7.39
5d	7.56
5e	34.55
6a	130.9
6b	5.15
6c	56.14
6d	31.16
6e	34.98
6f	553.1
6g	32.18

3.3 | Antioxidant effect

We estimated the activities of the free-radical-metabolizing enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), the levels of the oxidative stress parameters, including hydrogen peroxide (H_2O_2), nitric oxide (NO), and reduced glutathione (GSH) in MCF-7 cells treated with the prepared compounds and the effect of these compounds on the levels of total protein and nucleic acids.

As shown in Table 2, general treatment of the cells with different compounds and cisplatin (at the 1/10 of IC_{50} values as safe dose) resulted in a significant increase in the activity of SOD and level of H_2O_2 higher than those of the control cells, accompanied with a significant depletion in the activity of CAT, GSH-Px, and the level of GSH These changes

TABLE 2 Effect of treatment with the prepared compounds on the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), as well as the levels of reduced glutathione (GSH), and hydrogen peroxide (H_2O_2) in MCF-7-treated cells

Treatment (μg/ml)	SOD (U/mg protein)	CAT (U/mg protein)	GSH-Px (U/mg protein)	GSH (nmol/mg protein)	H_2O_2 (nmol/mg protein)
Control (DMSO)	43.30 ± 4.70	8.30 ± 0.88	10.00 ± 1.20	46.30 ± 5.00	18.90 ± 2.10
Cisplatin	122.80 ± 14.45[a]	3.66 ± 0.37[a]	5.37 ± 0.60[a]	25.00 ± 2.85[a]	50.20 ± 5.50[a]
4	130.00 ± 14.80[a]	3.50 ± 0.45[a]	5.12 ± 0.60[a]	24. 00 ± 2.75[a]	50.80 ± 6.90[a]
5c	150.75 ± 17.00[a,b]	2.90 ± 0.36[a,b]	4.11 ± 0.50[a]	18. 90 ± 2.00[a,b]	62.00 ± 6.70[a,b]
5d	136.00 ± 15.12[a,b]	3.00 ± 0.32[a]	4.80 ± 0.60[a]	20. 40 ± 2.70[a,b]	60.50 ± 7.00[a,b]
6b	160.00 ± 16.90[a,b]	2.11 ± 0.20[a,b]	3.90 ± 0.42[a,b]	15. 60 ± 1.80[a,b]	66.00 ± 7.20[a,b]

Data are expressed as means ± *SE* of four separate experiments.
[a,b]Significantly different from control and cisplatin groups respectively at ($p < .05$).

TABLE 3 Effect of prepared compounds on the level of total protein, nucleic acids (RNA and DNA), and nitric oxide (NO) in MCF-7-treated cells

Compounds	Protein (μg/10^6 cells)	RNA (μg/10^6 cells)	DNA (μg/10^6 cells)	NO (μmol/mg protein)
Control (DMSO)	145.00 ± 15.30	18.60 ± 1.90	9.80 ± 1.10	2.20 ± 0.24
Cisplatin	65.70 ± 7.50[a]	4.60 ± 0.50[a]	4.80 ± 0.54[a]	4.60 ± 0.50[a]
4	58.40 ± 6.20[a]	4.20 ± 0.55[a]	4.70 ± 0.55[a,b]	4.40 ± 0.49[a]
5c	45.60 ± 5.00[a,b]	3.40 ± 0.40[a,b]	3.65 ± 0.45[a,b]	5.50 ± 0.65[a,b]
5d	48.80 ± 5.60[a,b]	3.72 ± 0.42[a,b]	3.90 ± 0.45[a,b]	5.30 ± 0.70[a]
6b	36.50 ± 4.20[a,b]	3.00 ± 0.35[a,b]	3.20 ± 0.33[a]	5.90 ± 0.63[a,b]

The values are expressed as mean ± *SE* of four separate experiments.
[a,b]Significantly different from control and cisplatin groups respectively at ($p < .05$).

were in the order of **6b > 5c > 5d > 4 > cisplatin**, which is in accordance with the order of cytotoxic activity of the tested compounds, indicating an increase in the cellular levels of reactive oxygen species. These results stated that the antitumor effect of the present compounds might be exerted at least partly by production of reactive oxygen species. As shown in Table 3, the level of total protein and nucleic acids was significantly lower than that of control, while the level of NO was significantly higher in MCF-7 cells treated with the compounds, compared to control cells. The highest activity was found for compound **6b**, which resulted in the highest SOD activity, H_2O_2, low activities of CAT, GSH-Px, and GSH levels than the other tested compounds which showed the highest antitumor activity (Table 3).

4 | CONCLUSION

In conclusion, the present results suggested that the synthesized compounds possess good cytotoxic activity compared to cisplatin. Compounds **4, 5c, 5d,** and **6b** reveal significant cytotoxic activity comparable to the activity of commonly used anticancer drug, cisplatin, exerting their cytotoxic activity by increasing the activity of superoxide dismutase and depleting intracellular reduced glutathione, catalase, glutathione peroxidase activities, accompanied with high production of hydrogen peroxide, nitric oxide, and other free radicals causing death of tumor cells, as monitored by reduction in the synthesis of protein and nucleic acids. The results reveal that these compounds may come as potent cytotoxic agent(s).

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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