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ARTICLE

Synthesis of some 5-arylidene-2-(4-acetamidophenylimino)-thiazolidin-4-one derivatives and exploring their breast anticancer activity

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Abstract

Ten 2-(4-acetamidophenylimino)-5-arylidenethiazolidin-4-one derivatives **6a-k** were synthesized and evaluated for their anticancer activity against MCF-7 cell line (breast adenocarcinoma). The synthetic approach involves cyclocondensation of *N*,*N*′-bis(4-acetamidophenyl)-thiourea **(3)** with ethyl bromoacetate in ethanol and sodium acetate to furnish the 2-(4-acetamidophenylimino)-4-thiazolidinone derivative **4**, which underwent Knoevenagel condensation reaction with some substituted aldehydes to afford the targeted 2-(4-acetamidophenylimino)-5-arylidenethiazolidin-4-ones **6a-k**. The 4-chlorobenzylidene-thiazolidin-4-one compound **6h** exhibited strong inhibitory effect on the growth of breast cancer cell with IC_{50} (58.33 ± 1.74μM), very close to that of the reference drug doxorubicin (IC_{50} 48.06 ± 0.36μM).

1 | INTRODUCTION

Sulfur and nitrogen heterocycles are very important due to their physicochemical properties, particularly in the sense of developing new drugs and materials.[1–4] Especially, thiazolidin-4-one–containing compounds have gained significant attention since they were promising scaffolds in the area of medicinal chemistry.[5,6] The literature survey of thiazolidin-4-ones demonstrated a wide range of pharmacological properties, including antimicrobial,[7,8] anti-inflammatory,[9,10] antioxidant,[11,12] antitubercular,[13,14] antidiabetic,[15] human immunodeficiency virus (HIV) inhibitors,[16] antihypertensive,[17] anticonvulsant,[18] antihepatitis,[19] antiproliferative,[20] and anticancer activities.[21–24] Published data showed that anticancer activity of thiazolidin-4-ones may be correlated with their sensitivity to various targets including non-membrane protein tyrosine phosphatase (SHP-2), JNK-stimulating phosphatase-1 (JSP-1), tumor necrosis factor (TNF-α), anti-apoptotic biocomplex Bcl-XL-BH3, and inregrin.[25] Among the thiazolidinone scaffolds, 5-alkenylthiazolidinones are in recent decades, one of the most promising molecules in the anticancer drug discovery process. They have displayed significant antitumor activity against a variety of cancer cells such as breast cancer, lung cancer, and leukemia.[26] Considering the biological importance of 4-thiazolidinones as anticancer agents (Figure 1), the present work focuses on the design and synthesis of 2-(4-acetamidophenylimino)-2-arylidene-4-thiazolidin-4-ones and the evaluation of their anticancer activity.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The nucleophilic addition 4-aminoacetanilide **(1)** to phenyl isothiocyanate proceeded as previously described in the literature[27] in boiling ethanol to furnish *N*-(4-acetamidophenyl)-*N*′-phenylthiourea **(2)** (Scheme 1). Reaction of unsymmetrical thiourea derivative **2** with 4-aminoacetanilide **(1)** affected transamination through

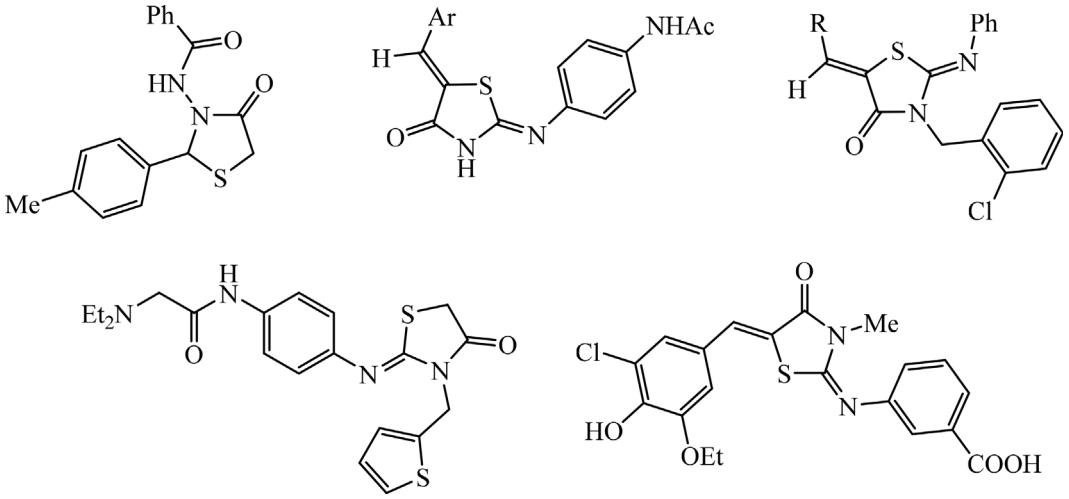
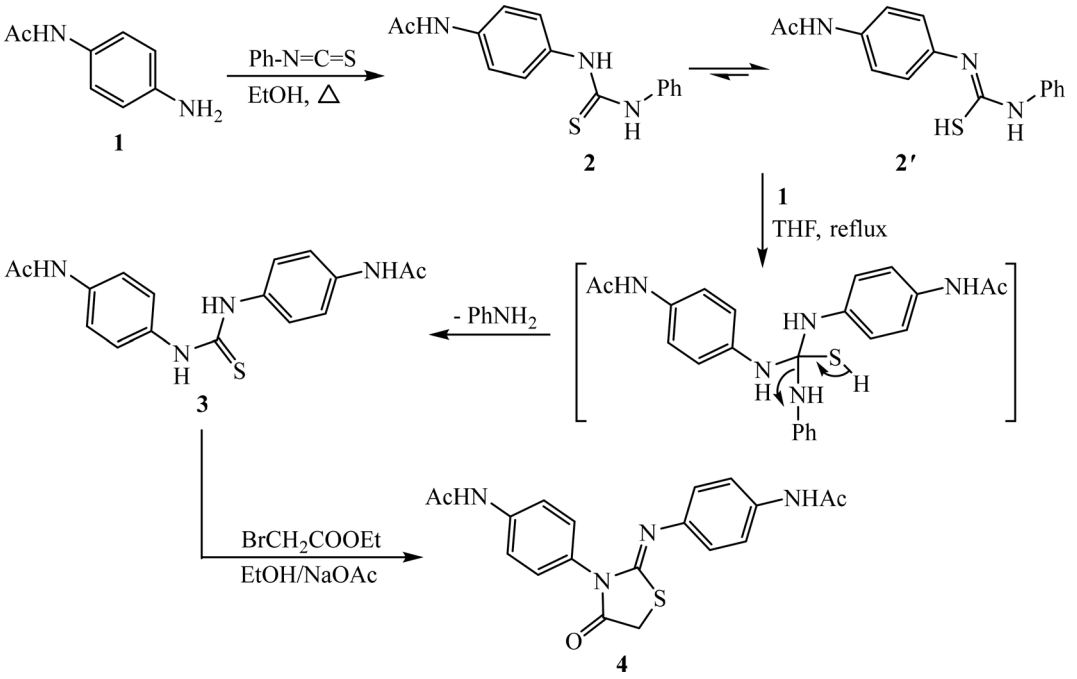
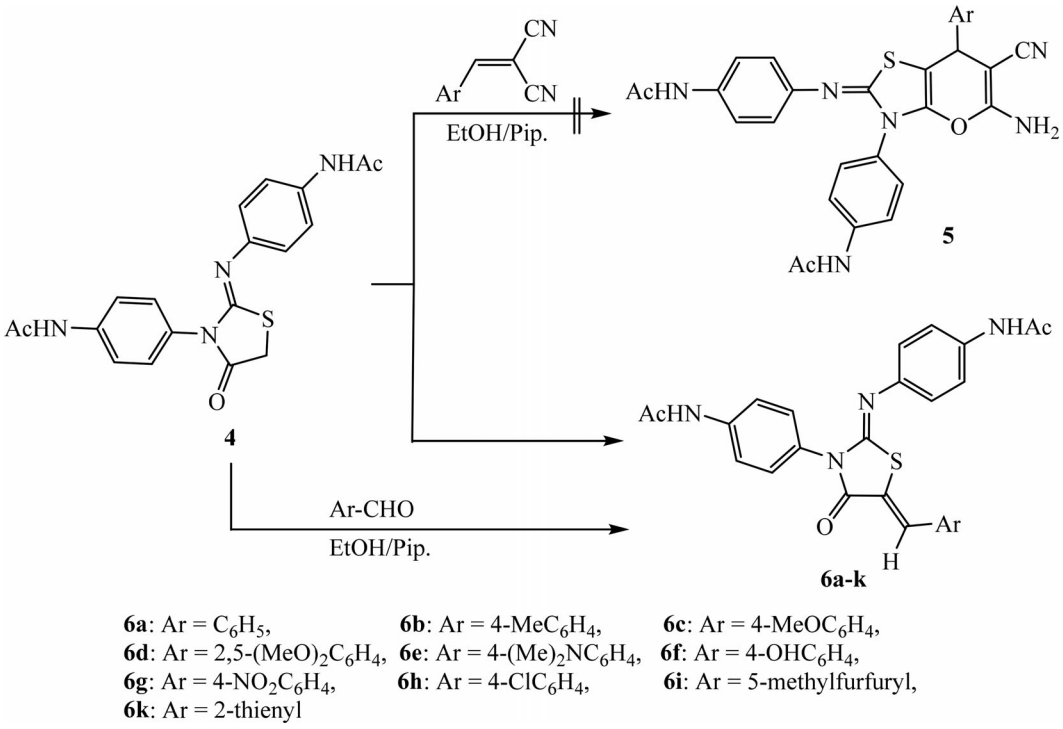


FIGURE 1 Selected examples of potent anticancer thiazolidine-4-ones



SCHEME 1 Synthesis of 2-(4-acetamidophenylimino)-4-thiazolidinone derivative **4**

replacement the aniline moiety by 4-aminoacetanilide and produced the symmetric thiourea, *N*,*N*′-bis (4-acetamidophenyl)-thiourea **(3)**. Heterocyclization of **3** with ethyl bromoacetate has been successfully achieved in ethanol containing fused sodium acetate to afford the precursor, 3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one **(4)**. The infrared (IR) spectrum of **4** clearly demonstrated absorptions at 3362, 3327, and 1705 cm^{-1} to indicate the presence of two NH and carbonyl (C═O) of thiazolidine ring, respectively. The ^{1}H NMR spectrum showed two singlet signals for six protons of two methyl groups at 2.05 and 2.07 ppm and singlet for two protons of methylene group (thiazolidinone ring) at 4.13 ppm. The aromatic protons (AA`BB` system) resonated as four doublet signals at down-fielded chemical shift 6.79, 7.28, 7.50, and 7.66 ppm. In addition, the singlet signals at 9.88 and 10.10 ppm indicated the two protons of acetamide moieties (MeCONH–). The ^{13}C NMR spectrum clearly demonstrated the signals of carbon atoms related to the methylene and carbonyl part (thiazolidine-4-one ring) at 32.70 and 171.73 ppm, respectively.



SCHEME 2 Synthesis of 2-(4-acetamidophenylimino)-5-arylidenethiazolidin-4-one derivatives **6a-k**

In an attempt to prepare the pyranothiazole derivatives **5** through treatment of thiazolidine-4-one derivative **4** with arylidene-malononitrile derivatives in hot ethanol containing drops of piperidine, the reaction did not afford our target pyranothiazoles **5**, and the product was assigned as the 5-arylidenethiazolidin-4-ones **6** (Scheme 2). The synthesis of all 5-arylidenethiazolidin-4-one derivatives **6** was performed by heating the 2-(4-acetamidophenylimino)-thiazolidine-4-one derivative **4** with different aromatic aldehydes (particularly benzaldehyde, 4-methylbenzaldehyde, 4-methoxybenzaldehyde, 2,5-dimethoxybenzaldehyde, 4-*N*,*N*-dimethylaminobenzaldehyde, 4-hydroxybenzaldehyde, 4-nitrobenzaldehyde, 4-chlorobenzaldehyde, 5-methylfurfural, and 2-thiophenecarboxaldehyde) in ethanol containing five drops of piperidine (Scheme 2). The chemical structures of 5-arylidenethiazolidin-4-one derivatives **6** were secured based on their compatible spectroscopic (IR and NMR) data. The IR spectrum of **6c** (as an example) clearly demonstrated absorptions at 3289 and 3175 cm^{-1} for the N–H groups along with the characteristic carbonyl absorptions at 1714 and 1658 cm^{-1}. The ^{1}H NMR signals of **6c** were presented at their expected regions, singlet signals resonated at 2.03, 2.06, and 3.78 ppm due to the protons of three methyl groups. The aromatic protons appeared as six double signals at 6.90, 7.07, 7.41, 7.53, 7.56, and 7.70 ppm. The olefinic proton was observed as singlet at 7.74 ppm. The protons of NH groups were observed as singlet signals at 9.95 and 10.14 ppm. The ^{13}C NMR spectrum of **6c** displayed signals for the aliphatic carbon atoms in the expected regions, 23.93 (CH_3), 24.01 (CH_3), and 55.40 ppm (OCH_3). The signals for aromatic carbon atoms were indicated by their chemical shifts, 114.90 (2C), 119.28 (2C), 119.91 (2C), 121.13 (2C), 125.88, 128.82 (2C), 129.59, 130.32, 131.85 (2C), 136.22, 139.46, and 150.56 ppm. The signal at 118.12 ppm indicated the fifth carbon of thiazolidinone ring. The signal of olefinic carbon was observed at 143.09 ppm. The signal of imine group (C═N) was observed at 160.63 ppm. In addition, the signals of carbonyl groups were resonated at 165.81 (C═O, amide), 168.13 (C═O, amide), and 168.53 ppm (C═O, thiazolidinone ring).

2.2 | In vitro anticancer activity

Breast cancer is the most commonly occurring cancer in women and the second leading cause of cancer death in women. The chance that a woman will die from breast cancer is about one in 38 (about 2.6%). Based on American Cancer Society's estimates for 2019, approximately 268 600 new cases of invasive breast cancer will be diagnosed in women, and about 41 760 women will die from breast cancer.[28,29] Thus, it is imperative to search for new alternatives to breast cancer prevention agents. The aim of the present investigation is to explore the anticancer activity of the chemically synthesized 2-(4-acetamidophenylimino)-

TABLE 1 In vitro cytotoxic activity of the synthesized 2-(4-acetamidophenylimino)-thiazolidine-4-ones against MCF-7 cell line

Compound	IC_{50}, μM
Doxorubicin	48.06 ± 0.36
3	216.19 ± 8.47
4	213.74 ± 8.63
6a	192.89 ± 9.14
6b	138.51 ± 6.19
6c	147.92 ± 5.40
6d	166.88 ± 7.35
6e	139.39 ± 9.35
6f	147.13 ± 8.23
6g	75.92 ± 4.07
6h	58.33 ± 1.74
6i	101.26 ± 4.64
6k	95.79 ± 5.88

5-arylidene-thiazolidin-4-ones **6a-k** against breast cancer (MCF-7). Accordingly, the anticancer activity of the new thiazolidin-4-one derivatives was assessed using MTT assay.[30–32]

The inhibitory effect of the synthesized thiazolidin-4-ones on breast cancer cells was studied at different concentrations (12.5, 25, 50, and 100 μg/mL). IC_{50} values for the investigated thiazolidine-4-ones **4** and **6a-k** are listed in Table 1 and compared with standard anticancer drug doxorubicin. The SAR was generated by cyclization of thiourea derivative **3** into its corresponding thiazolidine-4-one **4** that did not improve the anticancer activity. Introduction of benzylidene and substituted benzylidene moieties to the thiazolidine-4-one ring encouraged an increase the anticancer activity. The obtained results indicated that the significance of the potency relates to the type of substituents on the phenyl ring hybridized with the thiazolidinone ring. It was observed that the presence of electron donating groups (methyl, methoxy, dimethylamino, and hydroxyl) at the benzylidene moiety of compounds **6b-f** resulted into moderate activity, their IC_{50} values ranged from 138.51 to 166.88μM. The most potent activity was gained by the 4-chlorobenzylidene-thiazolidinone hybrid **6h**, its IC_{50} (58.33 ± 1.74μM) was very close to that obtained by the reference doxorubicin (IC_{50} = 48.06 ± 0.36μM). The next potent hybrid was 4-nitrobenzylidene-thiazolidinone hybrid **6g**, which display anticancer activity with IC_{50} = 75.92 ± 4.07μM. The presence of the chlorine or nitro substituents on the phenyl ring yields an electron withdrawing effect. Even so, the halogen atom increased the lipophilicity of the molecules, which is considered as potentially responsible for enhancement of the anticancer activity of the thiazolidinones **6**. Furthermore, the replacement of the phenyl ring with different heterocyclic rings such as; 5-methyl-2-furyl and 2-thienyl derivatives **6i** and **6k** did not enhance the anticancer activity. However, it encouraged an increase in the anticancer activity and contributed to a decrease in the IC_{50} value from 192.89μM to 101.26 and 95.79μM, respectively.

Upon treatment of MCF-7 cells with various concentrations of **6h** at (12.5, 25, and 50 μg/mL), no appreciable changes were observed. At higher concentration of **6h** at (100 μg/mL), marked changes in the cell morphology, were evident after 24 hours of incubation (Figure 2). MCF-7 cells were smaller, distorted, shrunken, and had condensed nuclei.

3 | CONCLUSION

A new series of 2-(4-acetamidophenylimino)-5-arylidenethiazolidin-4-ones have been synthesized and characterized by IR, ^{1}H- and ^{13}C-NMR tools. All synthesized thiazolidin-4-ones were subjected to anticancer evaluation against breast cancer growth by MTT technique using doxorubicin as a standard drug. The best activity was obtained by 4-chlorobenzylidene-thiazolidinone derivative **6h** (IC_{50} = 58.33 ± 1.74μM).

4 | EXPERIMENTAL

All melting points (uncorrected) were measured on Gallenkamp electric melting point apparatus. IR spectra were determined on Nicolete IS10 spectrometer (KBr discs). NMR spectra were measured in DMSO-d_6 as a solvent at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) on JEOL's NMR spectrometer. Elemental analyses (C, H, and N) were determined on Perkin-Elmer 2400 analyzer. Anticancer activity evaluation was performed at the Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt.

4.1 | Synthesis of *N*,*N*′-bis (4-acetamidophenyl)-thiourea (3)

N-(4-Acetamidophenyl)-*N*′-phenylthiourea **(2)** (2.85 g, 10 mmol) was suspended in 20 mL tetrahydrofuran (THF), and then 4-aminoacetanilide (1.50 g, 10 mmol) was added. The reaction mixture was refluxed for 7 to 8 hours. The solid that obtained upon cooling was filtered and dried to afford *N*,*N*′-bis(4-acetamidophenyl)-thiourea **(3)**.

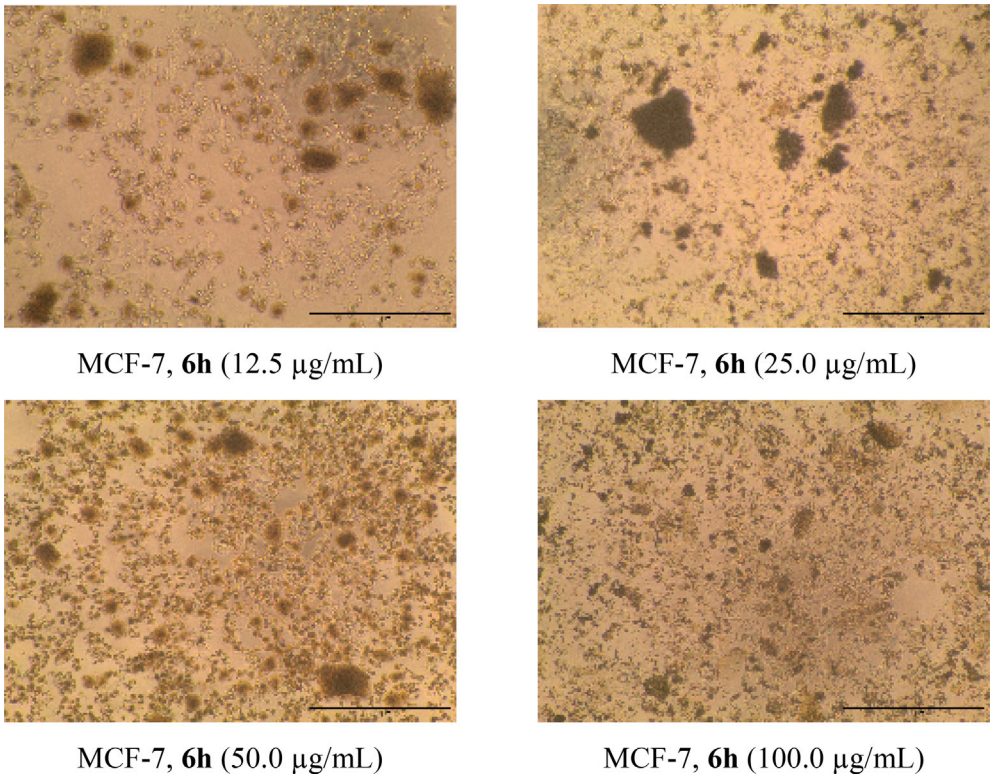


FIGURE 2 Treatment of MCF-7 cells with various concentrations of **6h**

Gray crystals; yield 71%; m.p. = 248-250°C. IR (υ/cm^{-1}): 3297, 3219 (N–H), 1663 (C═O). ^{1}H NMR (DMSO-d_6) δ: 2.05 (s, 6H, 2CH$_3$), 7.36 (d, J = 8.4 Hz, 4H, Ar–H), 7.54 (d, J = 8.4 Hz, 4H, Ar–H), 9.58 (s, 2H, 2NH), 9.94 (s, 2H, 2NH). Anal. Calcd. for $C_{17}H_{18}N_4O_2S$ (342): C, 59.63; H, 5.30; N, 16.36%. Found: C, 59.77; H, 5.33; N, 16.42%.

4.2 | Synthesis of 3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (4)

To a suspension of thiourea derivative **3** (1.71 g, 5 mmol) and fused sodium acetate (1.0 g) in 30 mL ethanol, ethyl bromoacetate (5 mmol) was added. The reaction components were refluxed for 3-4 hours, and then cooled in air to 25°C. The precipitate that formed was filtered, and recrystallized from ethanol/DMF mixture (5:1) to afford the conforming thiazolidin-4-one derivative **4**.

White crystals; yield 75%; m.p. = 288-290°C. IR (υ/cm^{-1}): 3362, 3327 (N–H), 1705, 1675 (C═O), 1636 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.00 (s, 3H, CH$_3$), 2.05 (s, 3H, CH$_3$), 4.13 (s, 2H, CH$_2$), 6.79 (d, J = 8.5 Hz, 2H, Ar–H), 7.28 (d, J = 9.0 Hz, 2H, Ar–H), 7.50 (d, J = 9.0 Hz, 2H, Ar–H), 7.66 (d, J = 8.0 Hz, 2H,Ar–H), 9.88 (s, 1H, NH), 10.10 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 23.93, 24.01, 32.7, 119.28 (2C), 119.79 (2C), 120.93 (2C), 128.76 (2C), 129.87, 135.78, 139.29, 143.22, 155.79, 168.04, 168.52, 171.73. Anal. Calcd. for $C_{19}H_{18}N_4O_3S$ (382): C, 59.67; H, 4.74; N, 14.65%. Found: C, 59.52; H, 4.79; N, 14.56%.

4.3 | General procedure for the synthesis of 3-(4-acetamidophenyl)-2-phenylimino-5-(4-substitutedbenzylidene)thiazolidin-4-one derivatives 6a-k

Thiazolidine-4-one derivative **4** (0.76 g, 2 mmol) was dissolved in hot ethanol 30 mL, and then the appropriate aldehyde, namely, benzaldehyde, 4-methylbenzaldehyde, 4-methoxybenzaldehyde, 2,5-dimethoxybenzaldehyde, 4-*N*, *N*-dimethylaminobenzaldehyde, 4-hydroxybenzaldehyde, 4-nitrobenzaldehyde, 4-chlorobenzaldehyde, 5-methylfurfural, and 2-thiophenecarboxaldehyde (2 mmol), and five drops of piperidine was added. The reaction components were refluxed for 3 to 4 hours. After cooling to 25°C, the precipitate that formed was picked up by filtration and recrystallized from acetic acid.

4.3.1 | 5-(Benzylidene)-3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (6a)

Golden crystals; yield 77%; m.p. = 288-289°C. IR (υ/cm^{-1}): 3352, 3322 (N–H), broad centered at 1678

(C═O), 1624 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 6.90 (d, J = 9.0 Hz, 2H, Ar–H), 7.41-7.51 (m, 5H, Ar–H), 7.57 (d, 4H, J = 8.5 Hz, Ar–H), 7.70 (d, J = 8.5 Hz, 2H, Ar–H), 7.78 (s, 1H, CH═C), 9.95 (s, 1H, NH), 10.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 23.96, 24.03, 119.31 (2C), 119.92 (2C), 121.14 (2C), 121.34, 128.84 (2C), 129.33 (2C), 129.52, 129.85 (2C), 130.02, 130.28, 133.38, 136.33, 139.53, 142.93, 150.34, 165.66, 168.17, 168.58. Anal. Calcd. for $C_{26}H_{22}N_4O_3S$ (470): C, 66.37; H, 4.71; N, 11.91%. Found: C, 66.47; H, 4.66; N, 11.95%.

4.3.2 | 5-(4-Methylbenzylidene)-3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (6b)

Yellow crystals; yield 80%; m.p. = 315-317°C. IR (υ/cm^{-1}): 3347, 3315 (N–H), broad centered at 1677 (C═O), 1623 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 6.90 (d, J = 8.0 Hz, 2H, Ar–H), 7.31 (d, J = 7.5 Hz, 2H, Ar–H), 7.41 (d, J = 8.5 Hz, 2H, Ar–H), 7.46 (d, J = 7.5 Hz, 2H, Ar–H), 7.57 (d, J = 8.5 Hz, 2H, Ar–H), 7.70 (d, J = 8.5 Hz, 2H, Ar–H), 7.75 (s, 1H, CH═C), 9.95 (s, 1H, NH), 10.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 21.11, 23.97, 24.05, 119.32 (2C), 119.94 (2C), 120.07, 121.15 (2C), 128.55 (2C), 129.58, 129.93 (4C), 130.41, 130.63, 136.32, 139.52, 140.21, 142.99, 150.40, 165.74, 168.18, 168.59. Anal. Calcd. for $C_{27}H_{24}N_4O_3S$ (484): C, 66.92; H, 4.99; N, 11.56%. Found: C, 66.84; H, 4.95; N, 11.50%.

4.3.3 | 5-(4-Methoxybenzylidene)-3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (6c)

White crystals; yield 86%; m.p. = 310-311°C. IR (υ/cm^{-1}): 3289, 3175 (N-H), 1714, 1658 (C═O), 1632 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.90 (d, J = 9.0 Hz, 2H, Ar–H), 7.07 (d, J = 8.0 Hz, 2H, Ar–H), 7.41 (d, J = 9.0 Hz, 2H, Ar–H), 7.53 (d, J = 8.5 Hz, 2H, Ar–H), 7.56 (d, J = 9.0 Hz, 2H, Ar–H), 7.70 (d, J = 9.0 Hz, 2H, Ar–H), 7.74 (s, 1H, CH═C), 9.95 (s, 1H, NH), 10.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 23.93, 24.01, 55.40, 114.90 (2C), 118.12, 119.28 (2C), 119.91 (2C), 121.13 (2C), 125.88, 128.82 (2C), 129.59, 130.32, 131.85 (2C), 136.22, 139.46, 143.09, 150.56, 160.63, 165.81, 168.13, 168.53. Anal. Calcd. for $C_{27}H_{24}N_4O_4S$ (500): C, 64.78; H, 4.83; N, 11.19%. Found: C, 64.91; H, 4.88; N, 11.08%.

4.3.4 | 5-(2,5-Dimethoxybenzylidene)-3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (6d)

Yellow crystals; yield 82%; m.p. = 301-302°C. IR (υ/cm^{-1}): 3287, 3258 (N–H), 1721, 1668 (C═O), 1641 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.02 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.89 (d, J = 6.0 Hz, 2H, Ar–H), 7.05 (s, 2H, Ar–H), 7.27 (s, 1H, Ar–H), 7.41 (d, J = 6.5 Hz, 2H, Ar–H), 7.56 (d, J = 6.0 Hz, 2H, Ar–H), 7.69 (d, J = 6.5 Hz, 2H, Ar–H), 7.90 (s, 1H, CH═C), 9.95 (s, 1H, NH), 10.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 23.95, 24.01, 55.49, 56.13, 112.84, 114.25, 115.93, 119.29 (2C), 119.81 (2C), 120.91, 121.16 (2C), 121.96, 122.59, 124.75, 128.80 (2C), 129.49, 136.32, 139.50, 142.85, 150.37, 152.10, 165.56, 168.13, 168.56. Anal. Calcd. for $C_{28}H_{26}N_4O_5S$ (530): C, 63.38; H, 4.94; N, 10.56%. Found: C, 63.54; H, 4.88; N, 10.47%.

4.3.5 | 5-(4-(Dimethylamino) benzylidene)-3-(4-acetamidophenyl)-2-(4-acetamidophenyl-imino)-thiazolidin-4-one (6e)

Orange crystals; yield 75%; m.p. > 330°C. IR (υ/cm^{-1}): 3299, 3217 (N–H), 1699 (C═O), 1635 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.96 (s, 6H, –N(CH_3)$_2$), 6.79 (d, J = 9.0 Hz, 2H, Ar–H), 6.89 (d, J = 8.0 Hz, 2H, Ar–H), 7.39 (d, J = 8.5 Hz, 4H, Ar–H), 7.56 (d, J = 8.0 Hz, 2H, Ar–H), 7.65 (s, 1H, CH═C), 7.69 (d, J = 8.5 Hz, 2H, Ar–H), 9.94 (s, 1H, NH), 10.13 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 23.92, 24.01, 39.50, (2C), 112.06 (2C), 113.64, 119.25 (2C), 119.89 (2C), 120.32, 121.17 (2C), 128.83 (2C), 129.78, 131.39, 131.83 (2C), 136.07, 139.32, 143.35, 151.02, 151.12, 166.00, 168.08, 168.49. Anal. Calcd. for $C_{28}H_{27}N_5O_3S$ (513): C, 65.48; H, 5.30; N, 13.64%. Found: C, 65.58; H, 5.26; N, 13.75%.

4.3.6 | 5-(4-Hydroxybenzylidene)-3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (6f)

Yellow crystals; yield 89%; m.p. > 330°C. IR (υ/cm^{-1}): 3464 (O-H), 3352, 3296 (N–H), broad centered at 1678 (C═O), 1624 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 6.90 (d, J = 9.0 Hz, 2H, Ar–H), 7.42 (d, J = 8.5 Hz, 3H, Ar–H and OH), 7.50 (t, J = 7.5 Hz, 2H, Ar–H), 7.57 (d, J = 8.0 Hz, 4H, Ar–H), 7.70 (d, J = 8.5 Hz, 2H, Ar–H), 7.78 (s, 1H, CH═C), 9.95 (s, 1H, NH), 10.14 (s, 1H,

NH). ^{13}C NMR (DMSO-d_6) δ: 23.97, 24.05, 119.32 (2C), 119.93 (2C), 121.15 (2C), 121.35, 128.85 (2C), 129.35 (2C), 129.52, 129.86 (2C), 130.03, 130.29, 133.39, 136.34, 139.54, 142.94, 150.35, 165.68, 168.18, 168.59. Anal. Calcd. for $C_{26}H_{22}N_4O_4S$ (486): C, 64.18; H, 4.56; N, 11.52%. Found: C, 64.07; H, 4.58; N, 11.44%.

4.3.7 | 5-(4-Nitrobenzylidene)-3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (6g)

Brown crystals; yield 71%; m.p. > 330°C. IR (υ/cm^{-1}): 3294 (N–H), 1716, 1666 (C═O), 1637 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 6.91 (d, J = 7.5 Hz, 2H, Ar–H), 7.44 (d, J = 8.5 Hz, 2H, Ar–H), 7.57 (d, J = 7.5 Hz, 2H, Ar–H), 7.71 (d, J = 8.0 Hz, 2H, Ar–H), 7.82 (d, J = 7.5 Hz, 2H, Ar–H), 7.89 (s, 1H, CH═C), 8.31 (d, J = 8.0 Hz, 2H, Ar–H), 9.96 (s, 1H, NH), 10.15 (s, 1H, NH). Anal. Calcd. for $C_{26}H_{21}N_5O_5S$ (515): C, 60.57; H, 4.11; N, 13.58%. Found: C, 60.47; H, 4.06; N, 13.51%.

4.3.8 | 5-(4-Chlorobenzylidene)-3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (6h)

Yellow crystals; yield 73%; m.p. = 308-309°C. IR (υ/cm^{-1}): 3291 (N–H), 1713, 1673 (C═O), 1635 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 6.90 (d, J = 7.5 Hz, 2H, Ar–H), 7.42 (d, J = 7.5 Hz, 2H, Ar–H), 7.57 (broad s, 6H, Ar–H), 7.70 (d, J = 7.5 Hz, 2H, Ar–H), 7.78 (s, 1H, CH═C), 9.95 (s, 1H, NH), 10.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 23.94, 24.01, 119.30 (2C), 119.92 (2C), 121.10 (2C), 122.11, 128.80 (2C), 128.91, 129.39 (2C), 131.49 (2C), 132.27, 134.52, 136.35, 139.54, 142.86, 150.02, 165.54, 168.15, 168.54. Anal. Calcd. for $C_{26}H_{21}ClN_4O_3S$ (504): C, 61.84; H, 4.19; N, 11.09%. Found: C, 61.71; H, 4.12; N, 11.00%.

4.3.9 | 5-((5-Methylfuran-2-yl) methylene)-3-(4-acetamidophenyl)-2-(4-acetamidophenyl-imino)-thiazolidin-4-one (6i)

Orange crystals; yield 87%; m.p. > 330°C. IR (υ/cm^{-1}): 3328 (N–H), broad centered at 1673 (C═O). ^{1}H NMR (DMSO-d_6) δ: 2.02 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 6.35 (d, J = 2.0 Hz, 1H, furan-H4), 6.88 (d, J = 8.5 Hz, 2H, Ar–H), 6.95 (d, J = 3.0 Hz, 1H, furan-H3), 7.39 (d, J = 8.5 Hz, 2H, Ar–H), 7.52 (s, 1H, CH═C), 7.56 (d, J = 8.5 Hz, 2H, Ar–H), 7.68 (d, J = 8.5 Hz, 2H, Ar–H), 9.94 (s, 1H, NH), 10.13 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 13.88, 23.96, 24.03, 110.19, 116.29, 117.20, 119.24 (2C), 119.57, 119.88 (2C), 121.11 (2C), 128.83 (2C), 129.62, 136.15, 139.41, 142.97, 148.33, 151.11, 156.98, 165.56, 168.14, 168.55. Anal. Calcd. for $C_{25}H_{22}N_4O_4S$ (474): C, 63.28; H, 4.67; N, 11.81%. Found: C, 63.38; H, 4.73; N, 11.73%.

4.3.10 | 5-(Thiophen-2-ylmethylene)-3-(4-acetamidophenyl)-2-(4-acetamidophenylimino)-thiazolidin-4-one (6k)

Orange crystals; yield 81%; m.p. > 330°C. IR (υ/cm^{-1}): 3311 (N–H), broad centered at 1675 (C═O), 1626 (C═N). ^{1}H NMR (DMSO-d_6) δ: 2.03 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 6.91 (d, J = 8.5 Hz, 2H, Ar–H), 7.23-7.25 (dd, J = 5.0, 3.5 Hz, 1H, thiophene-H4), 7.42 (d, J = 8.5 Hz, 2H, Ar–H), 7.57 (d, J = 9.0 Hz, 2H, Ar–H), 7.64 (d, J = 3.0 Hz, 1H, thiophene-H3), 7.70 (d, J = 9.0 Hz, 2H, Ar–H), 7.90 (d, J = 5.0 Hz, 1H, thiophene-5), 8.06 (s, 1H, CH═C), 9.96 (s, 1H, NH), 10.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 23.9, 24.03, 118.80, 119.29 (2C), 119.95 (2C), 121.14 (2C), 123.77, 128.82 (3C), 129.61, 132.48, 133.92, 136.34, 137.48, 139.50, 143.05, 150.03, 165.45, 168.18, 168.57. Anal. Calcd. for $C_{24}H_{20}N_4O_3S_2$ (476): C, 60.49; H, 4.23; N, 11.76%. Found: C, 60.71; H, 4.31; N, 11.64%.

4.4 | Cytotoxicity assay

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan.[30] All the procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in DMEM medium for MCF-7, 1% antibiotic-antimycotic mixture (10 000 U/mL potassium penicillin, 10 000 μg/mL streptomycin sulfate and 25 μg/mL amphotericin B) and 1% L-glutamine at 37°C under 5% CO_2. Cells were batch cultured for 10 days, then seeded at concentration of 10×10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at

37°C for 24 hours under 5% CO_2 using a water-jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media were aspirated, fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100, 50, 25, and 12.5 μg/mL). After 48 hours of incubation, medium was aspirated, 40 μL MTT salt (2.5 μg/mL) were added to each well and incubated for further 4 hours at 37°C under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 μL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. A positive control, which composed of 100 μg/mL was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions.[31,32]

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t* test by SPSS 11 program. Dimethyl sulfoxide (DMSO) is the vehicle used for dissolution of plant extracts, and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula: ([Reading of extract/Reading of negative control] − 1) × 100.

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