# Synthesis, *in vitro* anticancer and antioxidant activity of thiadiazole substituted thiazolidin-4-ones

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A series of novel 5-alkyl/aryl thiadiazole substituted thiazolidin-4-ones were synthesized by a two-step process. In the first step, 5-alkyl/aryl substituted 2-amino-thiadiazoles were synthesized, which on reaction with substituted aromatic aldehydes and thioglycolic acid in the presence of dicyclohexylcarbodiimide afforded thia-zolidin-4-ones. All the compounds were synthesized in fairly good yields and their structures were confirmed by spectral and physical data. The title compounds were screened for *in vitro* anti-proliferative activity on human breast adenocarcinoma cells (MCF-7) by MTT assay. Most of the derivatives showed an $IC_{50}$ less than 150 µmol $L^{-1}$. Among the compounds tested, 2-(2-nitrophe-nyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3f**), 2-(3-fluorophenyl)-3-(5-methyl-1,3,4-thiadiazol-2--yl)-thiazolidin-4-one (**3b**), and 2-(4-chlorophenyl)-3--(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3c**) were found to be the most active derivatives with $IC_{50}$ values of 46.34, 66.84, and 60.71 µmol $L^{-1}$, respectively. Antioxidant studies of all the synthesized compounds were carried out by diphenylpicrylhydrazyl (DPPH) as-say. Among the compounds tested, 2-phenyl-3-(5-styryl--1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3s**) elicited supe-rior antioxidant activity with $IC_{50}$ of 161.93 µmol $L^{-1}$.

*Keywords*: thiadiazole, thiazolidin-4-ones, anticancer, an-tioxidant

Reactive oxygen species (ROS) induced oxidative stress is believed to be a primary causative factor of various inflammatory diseases. ROS has been linked with cancer, cor-onary heart disease, and neurodegenerative diseases, and their presence in the body causes damage to the DNA of cells. Antioxidants exert their effects by scavenging or preventing the generation of ROS, which can protect the formation of free radicals and retard the progress of many chronic diseases, including cancer, inflammation, and car-

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diovascular diseases (1). Despite significant progress achieved in anticancer therapy, high systemic toxicity and drug resistance remain a major challenge for contemporary medicine in the management of cancers. Chemotherapy causes severe side-effects, which may be due to its cytotoxic effect on normal cells. Therefore, it is important that anticancer drugs display antiproliferative and cytotoxic activity in tumor cells without affecting normal tissues. Taking all the above mentioned facts into account, the development of novel antioxidant chemotherapeutics is being pursued.

Thiazolidin-4-one is a versatile scaffold for designing potential bioactive agents. Thiazolidin-4-one derivatives have been reported for broad spectrum of biological activities such as antioxidant (2), anticancer (3, 4), anti-inflammatory (5, 6), antimicrobial (7, 8), anti-HIV (9, 10), antiviral (11), anticonvulsant (12, 13), and antihypertensive (14) activities. Mechanisms of thiazolidin-4-one and related heterocycles for anticancer activity may be associated with their affinity to anticancer biotargets, such as non-membrane protein tyrosine phosphatase (SHP-2) (15), JNK-stimulating phosphatase-1 (JSP-1) (16) tumor necrosis factor TNF-$\alpha$ (17), antiapoptotic biocomplex Bcl-X$_L$-BH3 (18) and integrin $\alpha_v\beta_3$ (19), *etc*. On the other hand, azoles are proven chemotherapeutic agents. Among the azoles, 1,3,4-thiadiazole and its derivatives are well known for its *in vitro* and *in vivo* anticancer activity (20, 21). The 1,3,4-thiadiazoles induce early-phase apoptosis in human non-small lung cancer A549 cells through down-regulation of Bcl-XL and up-regulation of Bax expression (22). The 1,3,4-thiadiazoles also inhibit Akt/protein kinaze B (PKB) of cancer cells (22). In addition, these compounds have also exhibited anti-angiogenic activity in the nude mice angiogenesis model (22).

Since 1,3,4-thiadiazole and thiazolidin-4-one moieties are biologically proven anticancer and antioxidant pharmacophores and substitution in these scaffolds may further enhance their activity, prompted us to undertake this problem. Moreover, the combination of two pharmacophores in a single molecule is a well established hypothesis for synthesis of more active drugs with dual activity (23). Also, single molecule acting on multiple targets is better drug candidate compared to drug combinations, as administration of single drug will have more predictable pharmacokinetic and pharmacodynamic properties and improved patient compliance (23). Thus, a series of novel 5-alkyl/aryl thiadiazole substituted thiazolidine-4-ones were synthesized and evaluated for their antioxidant and anticancer activity.

## EXPERIMENTAL

Melting points were determined by using a melting point apparatus (Shital Scientific Industries, India) and were not corrected. Infrared spectra were obtained on a Shimadzu FT-IR-8310 (Shimadzu, Japan) using potassium bromide discs. $^1$H NMR spectra were recorded on a Bruker 400 MHz spectrometer (Bruker, Germany). Chemical shifts are reported in parts per million ($\delta$) units relative to an internal standard of tetramethylsilane. Mass spectra were recorded on a GC-MS QP 5050 (Shimadzu). Microanalysis was done on a Perkin-Elmer model 2400 CHN analyzer (USA). The purity of all compounds was established by a single spot on TLC plates (Merck, Germany). Iodine vapour was used as developing agent. The solvent system used for TLC was *n*-hexane/ ethyl acetate (3:7).

*General procedure for synthesis of 2-amino-5-alkyl-1,3,4-thiadiazoles (**2a,b**)*

Required fatty acid (0.15 mol), concentrated sulfuric acid (25 mL) and thiosemicarbazide (0.15 mol) were slowly heated to 80–90 °C on a thermostatically controlled water bath for 7 h. After cooling the contents were poured onto crushed ice. The acid was neutralized with 10 % ammonia solution. The crude precipitate which separated was filtered and washed several times with distilled water and dried. Final product was recrystallized from hot water (24).

*General procedure for synthesis of 2-amino-5-aryl-1,3,4-thiadiazoles (**2c-m**)*

Aromatic aldehyde (0.2 mol) in warm alcohol (300 mL) was added to a solution of thiosemicarbazide (0.2 mol) in hot water (300 mL). It was mixed slowly with continuous stirring. The product separated was filtered off and recrystallized from 50 % aqueous ethanol. Thiosemicarbazone (0.005 mol) obtained was suspended in 300 mL distilled water in a 1000 mL beaker. Ferric chloride solution (0.15 mol in 300 mL distilled water) was added to it. The contents were heated and maintained at 80–90 °C for 1 h, then it was filtered hot. A mixture of citric acid (0.11 mol) and sodium citrate (0.05 mol) was added to the filtrate and stirred. After cooling, the whole solution was taken in a bigger vessel (to account for the increase in volume) and neutralized with 10 % aqueous ammonia. The precipitate separated out was filtered and recrystallized from 25 % aqueous ethanol (24).

*General procedure for synthesis of 2-aryl-3-(5-alkyl/aryl-1,3,4-thiadiazol-2-yl)- -thiazolidin-4-ones (**3a-s**)*

A mixture of appropriate heterocyclic amine (1.0 mmol), *i.e.* synthesized thiadiazole derivatives (**2a-m**) and aromatic aldehyde (2.0 mmol) were stirred in tetrahydrofuran under ice-cold conditions for 5 min, followed by the addition of thioglycolic acid (3.0 mmol). After 5 min, dicyclohexylcarbodiimide (DCC) (1.2 mmol) was added to the reaction mixture at 0 °C and the reaction mixture was stirred for additional 50 min at room temperature to complete the reaction. The precipitated dicyclohexylurea was filtered off; the filtrate was concentrated to dryness under reduced pressure. Deionized water was added to the residue and the mixture was extracted with chloroform. The organic layer was successively washed with 5 % aqueous sodium hydrogen carbonate and dried over anhydrous sodium sulphate. The crude solid obtained on evaporation of the solvent under reduced pressure was purified by column chromatography (*n*-hexane/ethyl acetate, 5:5) and further by recrystallization in methanol to yield a crystalline product (25).

In vitro *antioxidant screening*

Free radical scavenging capacity of test compounds was studied by diphenylpicrylhydrazyl (DPPH) scavenging assay (26). The assay was carried out in a 96-well microtitre plate. DPPH solution was prepared by dissolving 3.96 mg of DPPH in 50 mL of methanol to give a concentration of 200 μmol $L^{-1}$. Stock solution (5000 μg $mL^{-1}$ in DMSO) of test compounds was suitably diluted with DMSO to get final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.81 μg $mL^{-1}$. Each test compound was added to each well separately in triplicate. DPPH solution was added to each well of test

compounds. Negative control wells were loaded with 100 µL of DMSO and 100 µL of DPPH solution each. Sample blank (200 µL of test compound in 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.81 µg mL$^{-1}$ concentration, dissolved in DMSO, no DPPH added) and control blank (200 µL of methanol without DPPH) were also performed. The plates were incubated at 37 °C for 30 min without exposing to light and the absorbance of each solution was measured. Ascorbic acid was used as a reference compound. Results of antioxidant assay are expressed as mean $IC_{50}$ ± SEM (Table III).

## In vitro *anticancer activity in cultured cells by MTT assay*

All the synthesized compounds were tested for *in vitro* anticancer activity against human breast adenocarcinoma cells (MCF-7 cells) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (27). Exponentially growing MCF-7 cells were harvested from a 75-cm$^2$ tissue culture flask and a stock cell suspension (1x10$^5$ cell mL$^{-1}$) was prepared. A 96-well flat bottom tissue culture plate was seeded with 2 x 10$^3$ cells in 0.1 mL of Eagle's minimum essential medium (MEM medium) supplemented with 10 % fetal bovine serum (FBS) and allowed to attach for 24 h. After 24 h of incubation cells were treated with 7.5, 15, 30, and 60 µg mL$^{-1}$ of test compounds. The cells in the control group received only the medium containing 0.2 % DMSO. Each treatment was performed in triplicate. After the treatment for 48 h, drug containing medium was removed and cells were washed with 200 µL of phosphate buffered saline (PBS). To each well, 100 µL of MTT reagent (1 mg mL$^{-1}$ MTT dissolved in MEM medium) was added and incubated for 4 h at 37 °C. After 4 h of incubation the plate was inverted on tissue paper to remove the MTT reagent. To solubilize formazan crystals in the wells, 100 µL of DMSO was added to each well. The optical density (absorbance) was measured and percentage inhibition or percentage cell death was calculated. Cisplatin was used as a reference compound. Results of MTT assay are expressed as as mean $IC_{50}$ ± SEM (Table IV).

RESULTS AND DISCUSSION

## *Chemistry*

Thiadiazole substituted thiazolidin-4-one derivatives were synthesized by two-step process. In the step one, 2-amino-5-aryl/alkyl-1,3,4-thiadiazoles (**2a-m**) were synthesized (Scheme 1), while in the step two, one pot reaction of 2-amino-5-aryl/alkyl-1,3,4--thiadiazoles with different aryl aldehydes and thioglycolic acid in the presence of dicyclohexylcarbodiimide afforded thiadiazole substituted thiazolidin-4-ones (**3a-s**) (Scheme 1). In the initial step of reaction, amino group nitrogen attacks the carbonyl carbon of aldehyde function and forms imine. Then thiol nucleophile attacks the imine carbon followed by intramolecular cyclization with loss of water resulting in the formation of thiazolidin-4-one. The last step is crucial for the high yield of thiazolidin-4-ones. The dehydrating agent DCC accelerates the intramolecular cyclization process and increases the yield of the reaction as well.
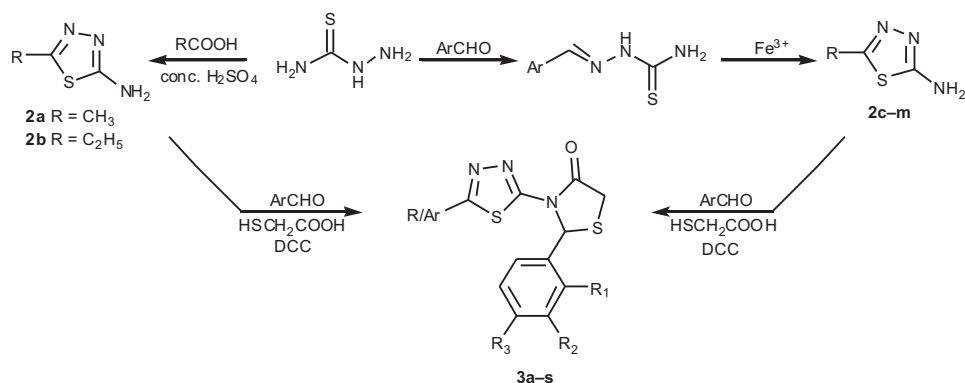
| Compd. | R | $R_1$ | $R_2$ | $R_3$ | Compd. | Ar | $R_1$ | $R_2$ | $R_3$ |
|--------|-----|-------|-------|-------|--------|-------------------|-------|-------|-------|
| **3a** | $CH_3$ | H | H | H | **3i** | $C_6H_5$ | H | H | H |
| **3b** | $CH_3$ | H | F | H | **3j** | $o$-F-$C_6H_5$ | H | F | H |
| **3c** | $CH_3$ | H | H | Cl | **3k** | $m$-F-$C_6H_5$ | H | H | H |
| **3d** | $CH_3$ | Br | H | H | **3l** | $p$-F-$C_6H_5$ | H | H | H |
| **3e** | $CH_3$ | H | Br | H | **3m** | $m$-Cl-$C_6H_5$ | H | H | H |
| **3f** | $CH_3$ | $NO_2$ | H | H | **3n** | $p$-Cl-$C_6H_5$ | H | H | H |
| **3g** | $CH_3$ | H | H | $NO_2$ | **3o** | $o$-Br-$C_6H_5$ | H | H | H |
| **3h** | $C_2H_5$ | H | H | H | **3p** | $m$-Br-$C_6H_5$ | H | H | H |
| | | | | | **3q** | $o$-$NO_2$-$C_6H_5$ | H | H | H |
| | | | | | **3r** | $o$-$OCH_3$-$C_6H_5$ | H | H | H |
| | | | | | **3s** | -CH=CH-$C_6H_5$ | H | H | H |

Scheme 1

Spectral data of synthesized compounds (Table II) along with physical and analytical data (Table I) confirmed the successful synthesis of thiadiazole substituted thiazolidin-4-ones (**3a-s**). The FT-IR (KBr pellet) spectra of compounds exhibited peaks corresponding to C=O and C=N functions. IR spectra of all thiadiazole substituted thiazolidin-4-ones showed C=N stretching between 1605 and 1634 $cm^{-1}$. The carbonyl group of synthesized compounds appeared at 1671–1700 $cm^{-1}$. Proton NMR spectra of all the synthesized compounds exhibited double doublet peaks between 3.84 and 4.34 ppm ($J =$ 15.6–16.4 Hz) corresponding to two methylene protons (5-$H_A$ and 5-$H_B$) of the thiazolidin-4-one moiety, which confirms the formation of thiazolidinone ring. The 5-$H_A$ proton appeared more downfield than the 5-$H_B$ proton due to its proximity to the 4-one (ketonic) group. The methine (–C–H–) proton of cyclized thiazolidin-4-one moiety appeared as a singlet between 6.69–6.99 ppm. Methyl group present in the compounds (**3a-g**) appeared as singlet between 1.26–1.31 ppm. Aromatic protons of all synthesized compounds appeared between 7.20 and 7.98 ppm. Mass spectra of all compounds showed corresponding molecular ion ($M^+$) peaks in addition to the characteristic fragmen-

*Table I. Physical and analytical data of synthesized compounds*

| Compd. | Mol. formula ($M_r$) | M. p. (ºC) | Yield (%) | Elemental analysis calcd./found (%) | | |
|---|---|---|---|---|---|---|
| | | | | C | H | N |
| **3a** | $C_{12}H_{11}N_3OS_2$ (277.03) | 190–192 | 52 | 51.96/51.80 | 4.00/4.05 | 15.15/15.14 |
| **3b** | $C_{12}H_{10}FN_3OS_2$ (295.02) | 140–142 | 45 | 48.80/48.54 | 3.41/3.38 | 14.23/14.32 |
| **3c** | $C_{12}H_{10}ClN_3OS_2$ (311.81) | 180–183 | 43 | 46.22/46.00 | 3.23/3.35 | 13.48/13.55 |
| **3d** | $C_{12}H_{10}BrN_3OS_2$ (356.26) | 180–181 | 40 | 40.46/40.75 | 2.83/2.68 | 11.79/11.85 |
| **3e** | $C_{12}H_{10}BrN_3OS_2$ (356.26) | 179–181 | 52 | 40.46/40.80 | 2.83/2.74 | 11.79/11.95 |
| **3f** | $C_{12}H_{10}N_4O_3S_2$ (322.36) | 150–151 | 52 | 44.71/45.05 | 3.13/3.42 | 17.38/17.65 |
| **3g** | $C_{12}H_{10}N_4O_3S_2$ (322.36) | 160–163 | 54 | 44.71/44.98 | 3.13/3.45 | 17.38/17.25 |
| **3h** | $C_{13}H_{13}N_3OS_2$ (291.39) | 184–185 | 66 | 53.58/53.95 | 4.50/4.89 | 14.42/14.21 |
| **3i** | $C_{17}H_{13}N_3OS_2$ (339.43) | 182–183 | 55 | 60.15/59.91 | 3.86/3.75 | 12.38/12.15 |
| **3j** | $C_{17}H_{12}FN_3OS_2$ (357.43) | 220–222 | 61 | 57.13/56.85 | 3.38/3.12 | 11.76/11.97 |
| **3k** | $C_{17}H_{12}FN_3OS_2$ (357.43) | 190–191 | 68 | 57.13/56.98 | 3.38/3.78 | 11.76/11.85 |
| **3l** | $C_{17}H_{12}FN_3OS_2$ (357.43) | 200–202 | 72 | 57.13/57.45 | 3.38/3.65 | 11.76/11.45 |
| **3m** | $C_{17}H_{12}ClN_3OS_2$ (373.88) | 175–176 | 64 | 54.61/54.96 | 3.24/3.38 | 11.24/11.46 |
| **3n** | $C_{17}H_{12}ClN_3OS_2$ (373.88) | 178–179 | 56 | 54.61/54.75 | 3.24/3.54 | 11.24/11.74 |
| **3o** | $C_{17}H_{12}BrN_3OS_2$ (418.33) | 160–161 | 66 | 48.81/49.05 | 2.89/2.99 | 10.04/10.18 |
| **3p** | $C_{17}H_{12}BrN_3OS_2$ (418.33) | 168–170 | 64 | 48.81/48.98 | 2.89/2.75 | 10.04/10.29 |
| **3q** | $C_{17}H_{12}N_4O_3S_2$ (384.43) | 210–211 | 69 | 53.11/53.26 | 3.15/3.53 | 14.57/14.89 |
| **3r** | $C_{18}H_{15}N_3O_2S_2$ (369.46) | 190–193 | 57 | 58.52/58.94 | 4.09/4.25 | 11.37/11.58 |
| **3s** | $C_{19}H_{15}N_3OS_2$ (365.47) | 200–201 | 64 | 62.44/62.15 | 4.14/4.15 | 11.50/11.59 |

tation peaks. Chloro and bromo substituted compounds displayed M+2 peaks in addition to the molecular ion peaks (M$^+$). Compounds possessing chloro group exhibited M$^+$ to M+2 peak height ratio of 3:1, while bromo group possessing compounds showed M$^+$ to M+2 peak height ratio of 1:1. Most compounds showed notable peaks at *m/z* 104 and 77 for molecular ions $C_6H_5CN^+$ and $C_6H_5^+$, respectively.

## In vitro *antioxidant activity by DPPH assay*

Among the compounds tested for antioxidant activity, 2-phenyl-3-(5-styryl-1,3,4--thiadiazol-2-yl)-thiazolidin-4-one (**3s**) exhibited the highest antioxidant activity with the *IC*$_{50}$ value of 161.93 µmol L$^{-1}$, while *IC*$_{50}$ of reference compound ascorbic acid was found to be 22.99 µmol L$^{-1}$. Other moderately active compounds, 2-(2-bromophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3d**) and 2-(2-nitrophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3f**) showed the *IC*$_{50}$ values of 254.67 and 252.05 µmol L$^{-1}$, respectively. Substitution of styryl ring at 5th position of 1,3,4-thiadiazol-2-yl moiety imparted maximum activity (**3s**), while, replacement of styryl ring with phenyl ring (**3i**), substituted phenyl ring (**3j-r**) or methyl group (**3a-h**) resulted in the loss of ac-

*Table II. Spectral data of synthesised compounds*

| Compd. | IR ($\nu$, cm$^{-1}$) | $^1$H NMR ($\delta$, ppm) (DMSO-$d_6$) | ESI-MS |
|---|---|---|---|
| **3a** | 3050 (Ar-H), 1698 (C=O), 1625 (C=N), 1573 (C=C) | 1.26 (s, 3H of CH$_3$), 3.84 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.10 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.69 (s, 1H, -CH of thiazolidinone ring), 7.20–7.35 (m, 5H, Ar-H) | 277 M$^+$ (20), 222 (25), 104 (100), 77 (30) |
| **3b** | 3040 (Ar-H), 1685 (C=O), 1614 (C=N), 1583 (C=C) | 1.28 (s, 3H of CH$_3$), 3.94 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.21 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.78 (s, 1H, -CH of thiazolidinone ring), 7.28–7.45 (m, 4H, Ar-H) | 295 M$^+$ (20), 196 (25), 104 (100) |
| **3c** | 3060 (Ar-H), 1687 (C=O), 1634 (C=N), 1596 (C=C) | 1.29 (s, 3H of CH$_3$), 3.98 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.26 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.88 (s, 1H, -CH of thiazolidinone ring), 7.29–7.49 (m, 4H, Ar-H) | 312 M$^+$ (30), 314 M+2 (10), 211 (25), 104 (100) |
| **3d** | 3049 (Ar-H), 1695 (C=O), 1612 (C=N), 1591 (C=C) | 1.28 (s, 3H of CH$_3$), 3.92 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.20 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.76 (s, 1H, -CH of thiazolidinone ring), 7.25–7.45 (m, 4H, Ar-H) | 356 M$^+$ (30), 358 M+2 (30), 255 (25), 104 (100) |
| **3e** | 3060 (Ar-H), 1687 (C=O), 1614 (C=N), 1571 (C=C) | 1.28 (s, 3H of CH$_3$), 3.85 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.15 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.75 (s, 1H, -CH of thiazolidinone ring), 7.20–7.33 (m, 4H, Ar-H) | 356 M$^+$ (25), 358 M+2 (30), 255 (20), 104 (100) |
| **3f** | 3070(Ar-H), 1677 (C=O), 1624 (C=N), 1585 (C=C) | 1.31 (s, 3H of CH$_3$), 3.99 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.29 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.86 (s, 1H, -CH of thiazolidinone ring), 7.30–7.55 (m, 4H, Ar-H) | 322 M$^+$ (10), 276 (20), 223 (25), 200 (100), 104 (50) |
| **3g** | 3075 (Ar-H), 1678 (C=O), 1622 (C=N), 1585 (C=C) | 1.30 (s, 3H of CH$_3$), 3.94 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.16 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.81 (s, 1H, -CH of thiazolidinone ring), 7.29–7.48 (m, 4H, Ar-H) | 322 M$^+$ (05), 276 (15), 104 (100) |
| **3h** | 3050(Ar-H), 1696 (C=O), 1626 (C=N), 1576(C=C) | 1.25 (t, 3H, -CH$_3$ side chain), 2.99 (q, 2H, -CH$_2$ of side chain), 4.03 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.29 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.72 (s, 1H, -CH of thiazolidinone ring), 7.25–7.40 (m, 4H, Ar-H) | 291 M$^+$ (20), 178 (15), 214 (20), 104 (100), 77 (25) |
| **3i** | 3032 (Ar-H), 1686 (C=O), 1625 (C=N), 1576 (C=C). | 4.09 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.32 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.80 (s, 1H, -CH of thiazolidinone ring), 7.22–7.92 (m, 10H, Ar-H) | 339 M$^+$ (40), 269 (40), 161 (20), 104 (100), 77(50) |
| **3j** | 3045 (Ar-H), 1696 (C=O), 1610 (C=N), 1566 (C=C) | 4.12 (dd, 1H, 5-H$_B$ of thiazolidinone ring), 4.34 (dd, 1H, 5-H$_A$ of thiazolidinone ring), 6.85 (s, 1H, -CH of thiazolidinone ring), 7.28–7.97 (m, 9H, Ar-H) | 357 M$^+$ (20), 262 (40), 179 (20), 104 (100) |

| | | | |
|---|---|---|---|
| **3k** | 3049 (Ar-H), 1692 (C=O), 1615 (C=N), 1566 (C=C) | 3.92 (dd, 1H, 5-$H_B$ of thiazolidinone ring), 4.15 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.79 (s, 1H, -CH of thiazolidinone ring), 7.25–7.75 (m, 9H, Ar-H) | 357 M$^+$ (25), 262 (30), 179 (20), 104 (100) |
| **3l** | 3035 (Ar-H), 1676 (C=O), 1605 (C=N), 1576 (C=C) | 4.14 (dd, 1H, 5-$H_B$ of thiazolidinone ring), 4.29 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.81 (s, 1H, -CH of thiazolidinone ring), 7.26–7.87 (m, 9H, Ar-H) | 357 M$^+$ (20), 262 (35), 179 (25), 104 (100) |
| **3m** | 3030 (Ar-H), 1694 (C=O), 1616 (C=N), 1578 (C=C) | 3.85 (dd, 1H, 5-$H_B$ of thiazolidinone ring), 3.98 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.70 (s, 1H, -CH of thiazolidinone ring), 7.28–7.75 (m, 9H, Ar-H) | 374 M$^+$ (25), 376 M+2 (10), 338 (40), 194 (20), 104 (100), 77 (20) |
| **3n** | 3033 (Ar-H), 1692 (C=O), 1619 (C=N), 1578 (C=C) | 3.89 (dd, 1H, 5-$H_B$ of thiazolidinone ring), 4.17 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.71 (s, 1H, -CH of thiazolidinone ring), 7.33–7.88 (m, 9H, Ar-H) | 374 M$^+$ (25), 376 M+2 (10), 338 (35), 194 (25), 104 (100), 77 (30) |
| **3o** | 3025 (Ar-H), 1672 (C=O), 1610 (C=N), 1588 (C=C) | 3.95 (dd, 1H, 5-$H_B$ of thiazolidinone ring), 4.27 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.82 (s, 1H, -CH of thiazolidinone ring), 7.41–7.92 (m, 9H, Ar-H) | 418 M$^+$ (20), 420 M+2 (20), 238 (40), 104 (100), 77 (30) |
| **3p** | 3030 (Ar-H), 1683 (C=O), 1615 (C=N), 1598 (C=C) | 3.91 (dd, 1H, 5-$H_B$ of thiazolidinone ring), 4.14 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.78 (s, 1H, -CH of thiazolidinone ring), 7.39–7.88 (m, 9H, Ar-H) | 418 M$^+$ (25), 420 M+2 (25), 238 (30), 104 (100), 77 (40) |
| **3q** | 3020 (Ar-H), 1671 (C=O), 1625 (C=N), 1577 (C=C) | 3.86 (dd, 1H, 5-$H_B$ of thiazolidinone ring), 4.03 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.85 (s, 1H, -CH of thiazolidinone ring), 7.24–7.72 (m, 9H, Ar-H) | 384 M$^+$ (15), 206 (40), 104 (100), 77 (10) |
| **3r** | 3070 (Ar-H), 1695 (C=O), 1610 (C=N), 1598 (C=C), 1100 (C-O) | 3.75 (s, 3H, -OCH$_3$), 4.12 (dd, 1H, 5-$H_B$ of thiazolidinone ring), 4.33 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.99 (s, 1H, -CH of thiazolidinone ring), 7.29–7.98 (m, 9H, Ar-H) | 369 M$^+$ (10), 262 (10), 191 (35), 104 (100), 77 (10) |
| **3s** | 1700 (C=O), 1627 (C=N), 1578 (C=C) | 4.05 (dd, 1H, 5-$H_B$ of thiazolidinone ring) 4.24 (dd, 1H, 5-$H_A$ of thiazolidinone ring), 6.56 (d, 2H, CH=CH), 6.90 (s, 1H, -CH of thiazolidinone ring), 7.22–7.68 (m, 10H, Ar-H) | 365 M$^+$ (10), 187 (35), 104 (100), 77 (10) |

tivity. Presence of methyl group at 5th position of 1,3,4-thiadiazol-2-yl moiety with 2-bromo (**3d**) or 2-nitro (**3f**) substituted phenyl ring at 2nd position of thiazolidin-4-one moiety imparted moderate activity, while, fluoro, chloro, and ethyl substitutions (**3b**, **3c**, and **3h**, respectively) on the phenyl ring of thiazolidin-4-one scaffold were inactive or decreased the activity. Hence, it can be concluded that styryl substituted 1,3,4-thiadiazol-2-yl moiety with no substitution on 2-phenyl ring of thiazolidin-4-one moiety (**3s**) was the most optimum substitution condition for antioxidant activity.

| Table III. Antioxidant activity of synthesised compounds | | Table IV. Cytotoxic activity of synthesized compounds | |
|---|---|---|---|
| Compd. | $IC_{50}$ (µmol $L^{-1}$)[a] | Compd. | $IC_{50}$ (µmol $L^{-1}$)[a] |
| **3a** | > 600 | **3a** | 173.88 ± 0.18 |
| **3b** | > 600 | **3b** | 66.84 ± 0.13 |
| **3c** | > 600 | **3c** | 60.71 ± 0.26 |
| **3d** | 254.67 ± 2.6 | **3d** | 89.04 ± 0.11 |
| **3e** | 337.17 ± 3.4 | **3e** | 100.18 ± 0.11 |
| **3f** | 252.05 ± 3.2 | **3f** | **46.34 ± 0.15** |
| **3g** | 336.46 ± 2.6 | **3g** | 76.99 ± 0.09 |
| **3h** | 532.28 ± 4.5 | **3h** | 142.08 ± 0.24 |
| **3i** | 461.57 ± 4.1 | **3i** | 150.55 ± 0.23 |
| **3j** | 401.34 ± 2.7 | **3j** | 88.77 ± 3.07 |
| **3k** | 389.42 ± 2.8 | **3k** | 154.66 ± 0.11 |
| **3l** | 336.29 ± 3.1 | **3l** | 144.61 ± 0.25 |
| **3m** | 345.51 ± 3.2 | **3m** | 158.15 ± 0.16 |
| **3n** | 351.15 ± 3.7 | **3n** | 81.15 ± 0.13 |
| **3o** | 336.29 ± 3.4 | **3o** | 100.45 ± 0.07 |
| **3p** | 413.17 ± 3.0 | **3p** | 77.50 ± 2.80 |
| **3q** | > 600 | **3q** | 104.93 ± 3.17 |
| **3r** | 350.09 ± 3.9 | **3r** | 134.06 ± 0.22 |
| **3s** | 161.93 ± 2.0 | **3s** | 94.21 ± 0.14 |
| Ascorbic acid | 22.99 ± 0.18 | Cisplatin | 10.56 ± 0.10 |

[a] $IC_{50}$ – 50 % inhibitory concentration. Mean ± *SEM*, *n* = 3.

[a] $IC_{50}$ – 50 % inhibitory concentration. Mean ± *SEM*, *n* = 3.

## In vitro *anticancer activity by MTT assay*

Anticancer assay results showed that compounds 2-(3-fluorophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3b**), 2-(4-chlorophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3c**), and 2-(2-nitrophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3f**) were the most potent cytotoxic agents with $IC_{50}$ values of 66.84, 60.71, and 46.34 µmol $L^{-1}$, respectively. $IC_{50}$ of reference drug cisplatin was found to be 10.56 µM. Other compounds that showed $IC_{50}$ values below 100 µmol $L^{-1}$ were 2-(2-bromophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3d**), 2-(4-nitrophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3g**), 2-phenyl-3-(5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)-thiazolidin-4-ones (**3j**), 2-phenyl-3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-thiazolidin-4-ones (**3n**), 2-phenyl-3-(5-(3-bromophenyl)-1,3,4-thiadiazol-2-yl)-thiazolidin-4-ones (**3p**), and 2-phenyl-3-(5-styryl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-ones (**3s**). It was observed that presence of 3-fluoro (**3b**), 4-chloro (**3c**), and 2-nitro groups (**3f**) at phenyl ring of thiazolidin-4-one moiety confer maximum activity, while bromo substitutions on phenyl ring (**3d**, **3e**) resulted into the decreased activity. Also, removal of halogen atoms at 2-phenyl ring (**3a**) or incorporation of ethyl group at 2-phenyl

ring (**3h**) caused further loss of activity. The styryl ring (**3s**) and halogen substituted phenyl ring (**3j**, **3n**, and **3p**) at the 5th position of 1,3,4-thiadiazol-2-yl moiety exhibited fairly good anticancer activity. Phenyl (**3i**) or 2-methoxyphenyl (**3r**) groups at the 5th position of 1,3,4-thiadiazol-2-yl moiety were found to be inactive. Structure activity relationship (SAR) study revealed that 3-fluorophenyl, 4-chlorophenyl, and 2-nitrophenyl substitutions at the 2nd position of thiazolidin-4-one ring (**3b**, **3c**, and **3f,** respectively) were more favorable than substitutions at the 5th position of 1,3,4-thiadiazol-2-yl moiety (**3i-s**) for imparting anticancer activity.

## CONCLUSIONS

Test compound 2-phenyl-3-(5-styryl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3s**) was found to be the most potent antioxidant with the $IC_{50}$ value of 161.93 μmol L$^{-1}$. The presence of styryl ring at thiadiazol-2-yl moiety was critical for the antioxidant property. In the *in vitro* anticancer activity, most of the compounds exhibited $IC_{50}$ values below 150 μM. The SAR study of tested compounds revealed that substitution of 3-fluorophenyl (**3b**), 4-chlorophenyl (**3c**), and 2-nitrophenyl (**3f**) at the 2nd position of thiazolidin-4-one moiety produced the most potent cytotoxic agents that exhibited $IC_{50}$ below 70 μmol L$^{-1}$ (**3b**, **3c**, and **3f**). Cytotoxic potential of (2-(3-fluorophenyl)-3-(5-methyl-1,3,4-thiadiazol--2-yl)-thiazolidin-4-one (**3b**), 2-(4-chlorophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3c**) and 2-(2-nitrophenyl)-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thiazolidin-4-one (**3f**) was comparable to that of the clinically used anticancer drug cisplatin. Further modification in thiadiazole and thiazolidin-4-one scaffolds with intramolecular rearrangement of styryl, nitro and halogen groups on the both moieties and introduction of new substituents preferably containing styryl, nitro, and halogen groups may give potent thiadiazole substituted thiazolidin-4-ones.

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