



## Design, Synthesis and Biological Evaluation of Novel Thiazolidinone Derivatives

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In present study, the novel thiazolidinone derivatives were designed and synthesized. The formation of the target compounds was confirmed by the elemental analyses, FT-IR, mass spectroscopy and <sup>1</sup>H NMR spectroscopy. These derivatives were subjected to antibacterial and antioxidant activities. The antibacterial results revealed that these derivatives were selectively active against Gram-negative *Pseudomonas aeruginosa* bacteria. Also, these derivatives displayed a good DPPH radical scavenging effect. *Drosophila melanogaster* flies were used for evaluating the toxic impact of novel thiazolidinone derivatives.

**Keywords:** Thiazolidinone, Vanillin, Antibacterial activity, Antioxidant activity, *Drosophila melanogaster*.

### INTRODUCTION

One of the serious health threats which are responsible for the majority of hospital acquired infections is bacterial infections [1]. Usually, antibiotics destroy the bacteria without harming the host and help in improving public health [2]. Development of resistance by the bacteria against the existing antibiotics is one of the important issues in the treatment of diseases. To overcome this crisis, the searching of novel compounds with antibiotic activity remains a challenge for researchers [3]. Hence, it is necessary to modify the existing drugs which emerge as a new pharmacophore with more potency to defeat antibiotic resistant mechanism [4].

The loss of equilibrium between the antioxidative defense of cells and oxidants due to the excess oxidants results in oxidative stress [5]. Numerous diseases as well as aging are associated with disturbances in an organism's redox balance [6]. The risk of cancer, as well as neurological and cardiovascular pathologies, is reduced by intake of antioxidants [7]. The anti-oxidants that exhibit DPPH radical scavenging activity are reported to possess anticancer, anti-inflammatory and anti-ageing activities [8].

The molecules bearing heteroatoms especially nitrogen, sulphur and oxygen, constantly recognized by chemists over the years due to diverse biological applications. One among such compounds is thiazolidinones which contains sulfur, nitrogen and carbonyl groups. For many decades, this moiety proved to possess varied biological applications which include anticonvulsant [9], antiarthritic [10], antimicrobial [11], anticancer [12], antiparasitic [13], tyrosine kinase inhibitor [14], anti-mycobacterial [15], antioxidant [16] and antidiabetic [17] activities. Also, this derivative behaves as non-nucleoside HIV reverse transcriptase inhibitors [18].

Vanillin has different functional groups, like aldehyde, ether and hydroxyl attached to an aromatic ring. Vanillin which is extracted from vanilla is known for its flavour and fragrance [19]. It is also known to exhibit outstanding pharmacological activity such as antimicrobial [20], antioxidant [21], antiviral [22], anticancer [23], antifungal [24], etc. Also, prevention of amyloid aggregation and inhibition of AChE is observed in vanillin derivatives [25]. The promising activity exhibited by these molecules prompted us to explore new conjugates by the amalgamation of thiazolidinones and vanillin to search for possible hybrid molecules with antibacterial and antioxidant activity.

*Drosophila* is widely used as a genetic model to study the toxicity effect of different chemicals. *Drosophila* and mammals share many biological and physiological properties in common. The disease genes show 75% sequence similarity between humans and *Drosophila*. The short life cycle of only 10-12 days at room temperature and the ease of maintaining them in the lab has resulted in *Drosophila* widely being used as an *in vivo* model. In addition, they offer many genetic tools to study gene regulations and other genetic effects [26]. In the current study, wild-type *Drosophila melanogaster* flies are used for evaluating the toxic impact of novel thiazolidinone derivatives. Two biological assays, survival and climbing assays were performed.

## EXPERIMENTAL

The reagents used for the synthesis of this series were analytical grade and purchased from S.D. Fine, Sigma-Aldrich and Spectrochem, which were used directly without further purification. The determination of melting point was done by open capillary method and are uncorrected. JEOL, JNM-ECZ-400/L1, 400 MHz instrument was used record <sup>1</sup>H NMR. The chemical shift values were stated in parts per million (ppm) and coupling constants in Hertz (Hz). TMS was used as internal standard. FT (ATR)-IR absorption spectra were recorded on Shimadzu in the range 4000-400 cm<sup>-1</sup>. Mass spectra were recorded on Shimadzu LCMS-8030 and are uncorrected. Thin layer chromatography on a silica coated aluminium sheet was used to check the completion of reaction and for checking the purity. Ethylacetate and hexane was used as mobile phase.

**Synthesis of substituted 2-(4-formyl-2-methoxyphenoxy)-N-phenylacetamide (2a-c):** The intermediates **1a-c** and **3a-e** were synthesized according to the reported procedure [27]. To a stirred solution of vanillin (0.01 mol) in DMF (10 mL), added K<sub>2</sub>CO<sub>3</sub> (0.011 mol) with stirring at room temperature. To this solution added substituted phenylacetamides (0.01 mol) and heated to reflux for 3 h. The completion of the reaction was monitored by TLC. After the completion of the reaction, the reaction mass was poured into crushed ice, the precipitate formed was filtered, washed with water, dried and taken for the next step without any purification.

**General procedure for the synthesis of target compounds (5a-o):** The preparation of intermediates **4a-c** was carried out according to the literature procedure [27]. The target compounds were obtained by refluxing intermediate **2a-c** (0.01 mol) and **4a-c** (0.01 mol) in refluxing ethanol (10 mL) in the presence of the catalytic amount of piperidine for 12 h and cooled at room temperature. The solid obtained was filtered and washed with ethanol. Later the crude product was recrystallized in dimethylformamide.

**(Z)-N-(2-Ethyl-6-methylphenyl)-2-(4-((2-((4-fluorophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)acetamide (5a):** Yield 95%; m.p.: 240-242 °C; Pale brown solid; FT-IR (ATR,  $\nu_{\max}$ , cm<sup>-1</sup>): 3226 (N-H), 1658 (C=O), 1508 (C=N), 1151 (C-F); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 11.47 (bs, 1H, N-H), 8.97 (s, 1H, amide N-H), 7.65 (s, 1H, =C-H), 7.18-7.23 (m, 4H, Ar-H), 7.12-7.16 (m, 3H, Ar-H), 7.07-7.09 (m, 2H, Ar-H), 4.78 (s, 2H, CH<sub>2</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 2.50-2.56 (2H, CH<sub>2</sub>), 2.16 (s, 3H, CH<sub>3</sub>), 1.11

(t, 3H, *J* = 7.6 Hz, CH<sub>3</sub>); ESI-MS (*m/z*): 520.15 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>28</sub>H<sub>26</sub>N<sub>3</sub>O<sub>4</sub>FS: C, 64.73 (64.75); H, 5.04 (5.07); N, 8.09 (8.10).

**(Z)-2-(4-((2-((4-Chlorophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)-N-(2-ethyl-6-methylphenyl)acetamide (5b):** Yield 94%; m.p.: 250-252 °C; Pale brown solid; FT IR (ATR,  $\nu_{\max}$ , cm<sup>-1</sup>): 3230 (N-H), 1720 (C=O), 1521 (C=N), 860 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 11.45 (bs, 1H, N-H), 8.99 (s, 1H, amide N-H), 7.68 (s, 1H, =C-H), 7.16-7.21 (m, 4H, Ar-H), 7.10-7.14 (m, 3H, Ar-H), 7.05-7.07 (m, 2H, Ar-H), 4.75 (s, 2H, CH<sub>2</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 2.52-2.58 (2H, CH<sub>2</sub>), 2.17 (s, 3H, CH<sub>3</sub>), 1.13 (t, 3H, *J* = 7.6 Hz, CH<sub>3</sub>); ESI-MS (*m/z*): 536.14 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>28</sub>H<sub>26</sub>N<sub>3</sub>O<sub>4</sub>ClS: C, 62.74 (62.76); H, 4.89 (4.91); N, 7.84 (7.87).

**(Z)-N-(2-Ethyl-6-methylphenyl)-2-(2-methoxy-4-((2-((3-nitrophenyl)amino)-4-oxothiazol-5(4H)-ylidene)-methyl)phenoxy)acetamide (5c):** Yield 91%; m.p.: 210-212 °C; Pale brown solid; FT-IR (ATR,  $\nu_{\max}$ , cm<sup>-1</sup>): 3288 (N-H), 1718 (C=O), 1587 (C=N), 1532 (NO<sub>2</sub> asymmetric), 1343 (NO<sub>2</sub> symmetric); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 11.98 (bs, 1H, N-H), 8.97 (s, 1H, amide N-H), 7.95 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.85 (s, 1H, Ar-H), 7.68 (s, 1H, =C-H), 7.56 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.10-7.22 (m, 6H, Ar-H), 4.78 (s, 2H, CH<sub>2</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 2.12 (s, 3H, CH<sub>3</sub>), 1.05 (t, 3H, *J* = 6.0 Hz, CH<sub>3</sub>); ESI-MS (*m/z*): 547.17 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>28</sub>H<sub>26</sub>N<sub>4</sub>O<sub>6</sub>S: C, 61.53 (61.54); H, 4.79 (4.80); N, 10.25 (10.28).

**(Z)-N-(2-Ethyl-6-methylphenyl)-2-(2-methoxy-4-((2-((2-methyl-5-nitrophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)phenoxy)acetamide (5d):** Yield 96%; m.p.: 192-194 °C; Pale yellow solid; FT-IR (ATR,  $\nu_{\max}$ , cm<sup>-1</sup>): 3307 (N-H), 1680 (C=O), 1595 (C=N), 1510 (NO<sub>2</sub> asymmetric), 1336 (NO<sub>2</sub> symmetric); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 12.01 (bs, 1H, N-H), 8.93 (s, 1H, amide N-H), 7.93 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.83 (s, 1H, Ar-H), 7.66 (s, 1H, =C-H), 7.54 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.08-7.20 (m, 6H, Ar-H), 4.76 (s, 2H, CH<sub>2</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 2.30 (s, 3H, CH<sub>3</sub>), 2.14 (s, 3H, CH<sub>3</sub>), 1.08 (t, 3H, *J* = 6.0 Hz, CH<sub>3</sub>); ESI-MS (*m/z*): 561.18 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>29</sub>H<sub>28</sub>N<sub>4</sub>O<sub>6</sub>S: C, 62.13 (62.15); H, 5.03 (5.05); N, 9.99 (9.98).

**(Z)-2-(4-((2-((3,4-Dimethylphenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)-N-(2-ethyl-6-methylphenyl)acetamide (5e):** Yield 92%; m.p.: 184-186 °C; Pale brown solid; FT-IR (ATR,  $\nu_{\max}$ , cm<sup>-1</sup>): 3258 (N-H), 1710 (C=O), 1542 (C=N); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>,  $\delta$  ppm): 11.15 (bs, 1H, N-H), 8.97 (s, 1H, amide N-H), 7.79 (s, 1H, =C-H), 7.23-7.32 (m, 3H, Ar-H), 7.05-7.10 (m, 2H, Ar-H), 6.84-6.93 (m, 3H, Ar-H), 4.72 (s, 2H, CH<sub>2</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 2.50-2.56 (2H, CH<sub>2</sub>), 2.24 (s, 3H, CH<sub>3</sub>), 2.23 (s, 3H, CH<sub>3</sub>), 2.15 (s, 3H, CH<sub>3</sub>), 1.09 (t, 3H, *J* = 7.6 Hz, CH<sub>3</sub>); ESI-MS (*m/z*): 530.21 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>30</sub>H<sub>31</sub>N<sub>3</sub>O<sub>4</sub>S: C, 68.03 (68.05); H, 5.09 (5.10); N, 7.93 (7.95).

**(Z)-2-(4-((2-((4-Fluorophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)-N-(3-(trifluoromethyl)phenyl)acetamide (5f):** Yield 95%; m.p.: 190-192 °C; Off-brown solid; FT-IR (ATR,  $\nu_{\max}$ , cm<sup>-1</sup>): 3275 (N-H), 1713

(C=O), 1525 (C=N), 1115 (C-F); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.10 (bs, 1H, N-H), 10.00 (s, 1H, amide N-H), 8.04 (s, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.61 (s, 1H, =C-H), 7.56 (t, 1H, *J* = 7.89 Hz, Ar-H), 7.42-7.47 (m, 3H, Ar-H), 7.00-7.25 (m, 5H, Ar-H), 4.76 (s, 2H, CH<sub>2</sub>), 3.85 (s, 3H, OCH<sub>3</sub>); ESI-MS (*m/z*): 546.13 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>26</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>SF<sub>4</sub>: C, 57.25 (57.24); H, 3.51 (3.53); N, 7.70 (7.72).

**(Z)-2-(4-((2-((4-Chlorophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)-N-(3-(trifluoromethyl)phenyl)acetamide (5g):** Yield 94%; m.p.: 194-196 °C; Off-brown solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3308 (N-H), 1714 (C=O), 1582 (C=N), 1120 (C-F), 854 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.42 (bs, 1H, N-H), 10.10 (s, 1H, amide N-H), 8.07 (s, 1H, Ar-H), 7.74 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.62 (s, 1H, =C-H), 7.53 (t, 1H, *J* = 7.89 Hz, Ar-H), 7.40-7.43 (m, 2H, Ar-H), 6.87-7.13 (m, 6H, Ar-H), 4.77 (s, 2H, CH<sub>2</sub>), 3.84 (s, 3H, OCH<sub>3</sub>); ESI-MS (*m/z*): 562.08 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>26</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>SClF<sub>3</sub>: C, 55.57 (55.54); H, 3.41 (3.42); N, 7.48 (7.45).

**(Z)-2-(2-Methoxy-4-((2-((3-nitrophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)phenoxy)-N-(3-(trifluoromethyl)phenyl)acetamide (5h):** Yield 90%; m.p.: 218-220 °C; Pale brown solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3298 (N-H), 1708 (C=O), 1597 (C=N), 1530 (NO<sub>2</sub> asymmetric), 1340 (NO<sub>2</sub> symmetric), 1130 (C-F); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.25 (bs, 1H, N-H), 10.03 (s, 1H, amide N-H), 8.05 (s, 1H, Ar-H), 7.82 (s, 1H, Ar-H), 7.75 (d, 1H, *J* = 7.89 Hz, Ar-H), 7.65 (s, 1H, =C-H), 7.59 (t, 1H, *J* = 7.89 Hz, Ar-H), 7.55 (d, 1H, *J* = 8.24 Hz, Ar-H), 7.38 (d, 1H, *J* = 7.7 Hz, Ar-H), 6.93-7.30 (m, 5H, Ar-H), 4.65 (s, 2H, CH<sub>2</sub>), 3.83 (s, 3H, OCH<sub>3</sub>); ESI-MS (*m/z*): 573.53 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>26</sub>H<sub>19</sub>N<sub>4</sub>O<sub>6</sub>SF<sub>3</sub>: C, 54.55 (54.53); H, 3.35 (3.33); N, 9.79 (9.77).

**(Z)-2-(2-Methoxy-4-((2-((2-methyl-5-nitrophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)phenoxy)-N-(3-(trifluoromethyl)phenyl)acetamide (5i):** Yield 94%; m.p.: 230-232 °C; Pale yellow solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3308 (N-H), 1714 (C=O), 1582 (C=N), 1459 (NO<sub>2</sub> asymmetric), 1338 (NO<sub>2</sub> symmetric), 1134 (C-F); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.40 (bs, 1H, N-H), 10.00 (s, 1H, amide N-H), 8.07 (s, 1H, Ar-H), 7.73 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.65 (s, 1H, =C-H), 7.58 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.52 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.40 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.10-7.20 (m, 5H, Ar-H), 4.68 (s, 2H, CH<sub>2</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 2.19 (s, 3H, CH<sub>3</sub>); ESI-MS (*m/z*): 587.12 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>27</sub>H<sub>21</sub>N<sub>4</sub>O<sub>6</sub>SF<sub>3</sub>: C, 55.29 (55.25); H, 3.61 (3.60); N, 9.55 (9.53).

**(Z)-2-(4-((2-((3,4-Dimethylphenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)-N-(3-(trifluoromethyl)phenyl)acetamide (5j):** Yield 92%; m.p.: 210-212 °C; Pale yellow solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3298 (N-H), 1678 (C=O), 1531 (C=N), 1118 (C-F); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.39 (bs, 1H, N-H), 10.01 (s, 1H, amide N-H), 8.06 (s, 1H, Ar-H), 7.85 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.63 (s, 1H, =C-H), 7.56 (t, 1H, *J* = 8.0 Hz, Ar-H), 7.41 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.12-7.24 (m, 6H, Ar-H), 4.77 (s, 2H, CH<sub>2</sub>), 3.89 (s, 3H, OCH<sub>3</sub>), 2.26 (s, 3H, CH<sub>3</sub>), 2.25 (s, 3H, CH<sub>3</sub>); ESI-MS (*m/z*): 556.15 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>28</sub>H<sub>24</sub>N<sub>3</sub>O<sub>4</sub>SF<sub>3</sub>: C, 60.53 (60.51); H, 4.35 (4.30); N, 7.56 (7.53).

**(Z)-N-(2-Chlorobenzyl)-2-(4-((2-((4-fluorophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)acetamide (5k):** Yield 92%; m.p.: 200-202 °C; Off-brown solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3278 (N-H), 1701 (C=O), 1528 (C=N), 1115 (C-F), 780 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.65 (bs, 1H, N-H), 8.05 (bs, 1H, amide N-H), 7.68 (s, 1H, =C-H), 7.35-7.47 (m, 5H, Ar-H), 7.17-7.32 (m, 4H, Ar-H), 7.07-7.17 (m, 2H, Ar-H), 4.65 (s, 2H, CH<sub>2</sub>), 4.49 (s, 2H, benzyl-H), 3.85 (s, 3H, OCH<sub>3</sub>); ESI-MS (*m/z*): 526.10 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>26</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>SClF: C, 59.37 (59.39); H, 4.02 (4.05); N, 7.99 (7.98).

**(Z)-N-(2-Chlorobenzyl)-2-(4-((2-((4-chlorophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)acetamide (5l):** Yield 95%; m.p.: 198-200 °C; Pale yellow solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3412 (N-H), 1666 (C=O), 1514 (C=N), 750 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.67 (bs, 1H, N-H), 8.07 (bs, 1H, amide N-H), 7.65 (s, 1H, =C-H), 7.32-7.44 (m, 5H, Ar-H), 7.15-7.29 (m, 4H, Ar-H), 7.05-7.11 (m, 2H, Ar-H), 4.64 (s, 2H, CH<sub>2</sub>), 4.47 (s, 2H, benzyl-H), 3.83 (s, 3H, OCH<sub>3</sub>); ESI-MS (*m/z*): 542.00 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>26</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>SCl<sub>2</sub>: C, 57.57 (57.59); H, 3.90 (3.93); N, 7.75 (7.77).

**(Z)-N-(2-Chlorobenzyl)-2-(2-methoxy-4-((2-((3-nitrophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-phenoxy)acetamide (5m):** Yield 90%; m.p.: 220-222 °C; Pale brown solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3414 (N-H), 1668 (C=O), 1510 (NO<sub>2</sub> asymmetric), 1346 (NO<sub>2</sub> symmetric), 744 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.93 (bs, 1H, N-H), 8.03 (d, 1H, amide N-H), 7.68 (s, 1H, =C-H), 7.32-7.41 (m, 4H, Ar-H), 7.22-7.28 (m, 4H, Ar-H), 7.08-7.11 (m, 3H, Ar-H), 4.63 (s, 2H, CH<sub>2</sub>), 4.47 (d, 2H, benzyl-H), 3.83 (s, 3H, OCH<sub>3</sub>); ESI-MS (*m/z*): 553.10 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>26</sub>H<sub>21</sub>N<sub>4</sub>O<sub>6</sub>SCl: C, 56.47 (56.49); H, 3.83 (3.85); N, 10.13 (10.15).

**(Z)-N-(2-Chlorobenzyl)-2-(2-methoxy-4-((2-((2-methyl-5-nitrophenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-phenoxy)acetamide (5n):** Yield 96%; m.p.: 198-200 °C; Pale yellow solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3315 (N-H), 1698 (C=O), 1590 (C=N), 1522 (NO<sub>2</sub> asymmetric), 1340 (NO<sub>2</sub> symmetric), 860 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.85 (bs, 1H, N-H), 8.07 (d, 1H, amide N-H), 7.64 (s, 1H, =C-H), 7.30-7.42 (m, 4H, Ar-H), 7.24-7.29 (m, 4H, Ar-H), 7.09-7.12 (m, 3H, Ar-H), 4.63 (s, 2H, CH<sub>2</sub>), 4.48 (d, 2H, benzyl-H), 3.87 (s, 3H, OCH<sub>3</sub>), 2.20 (s, 3H, CH<sub>3</sub>); ESI-MS (*m/z*): 567.10 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>27</sub>H<sub>23</sub>N<sub>4</sub>O<sub>6</sub>SCl: C, 57.19 (57.20); H, 4.09 (4.11); N, 9.88 (9.90).

**(Z)-N-(2-Chlorobenzyl)-2-(4-((2-((3,4-dimethylphenyl)amino)-4-oxothiazol-5(4H)-ylidene)methyl)-2-methoxyphenoxy)acetamide (5o):** Yield 92%; m.p.: 178-180 °C; Off-brown solid; FT-IR (ATR, *v*<sub>max</sub>, cm<sup>-1</sup>): 3250 (N-H), 1699 (C=O), 1550 (C=N), 865 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>, δ ppm): 11.63 (bs, 1H, N-H), 8.06 (bs, 1H, amide N-H), 7.64 (s, 1H, =C-H), 7.27-7.49 (m, 3H, Ar-H), 7.21-7.23 (m, 3H, Ar-H), 6.68-6.93 (m, 4H, Ar-H), 4.61 (s, 2H, CH<sub>2</sub>), 4.48 (d, 2H, benzyl-H), 3.85 (s, 3H, OCH<sub>3</sub>), 2.25 (s, 3H, CH<sub>3</sub>), 2.24 (s, 3H, CH<sub>3</sub>); ESI-MS (*m/z*): 536.13 [M+H]<sup>+</sup>; Anal. calcd. (found) % for C<sub>28</sub>H<sub>26</sub>N<sub>3</sub>O<sub>4</sub>SCl: C, 62.74 (62.76); H, 4.89 (4.90); N, 7.84 (7.86).

**Antibacterial activity:** The antibacterial activity of the compounds was screened preliminarily by the disc diffusion method [28]. Briefly, the synthetic compounds were loaded into the sterile paper discs and these were placed on the pre-seeded Mueller-Hinton (Himedia, Mumbai) agar plates. Overnight culture of test microorganisms in broth (turbidity adjusted to 0.5 McFarland unit) was used for the seeding. Ciprofloxacin discs (6 mm, 30 µg/disc) was used as a positive control for bacteria. Then, the bacterial plates were incubated at 37 °C for 24 h and fungal plates for three days at 30 °C. The diameters of the inhibition zones (in mm) were measured after the incubation.

**Determination of minimum inhibitory concentration (MIC):** MIC of the synthetic compounds against *Pseudomonas aeruginosa* was performed as reported method [28]. A serial two-fold dilution of the synthetic compounds was prepared in a 96-well microtiter plate (Tarsons, U bottom) over the range 1.25-0.019 mg/mL. Each of the wells was inoculated with  $1 \times 10^8$  of the inoculum. The plates were incubated at 37 °C for 24 h. Appropriate growth controls and sterility controls were maintained. The bacterial growth was assessed by measuring the turbidity after the incubation.

**DPPH free radical scavenging activity:** The free radical scavenging activity of the title compounds was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A solution of DPPH in methanol (0.2 mM) was prepared and 100 µL of this solution was added to various concentrations of title compounds at the concentrations of 10, 20, 30, 40, 50 µg/mL. After 30 min, absorbance was measured at 517 nm and the scavenging activity was calculated as a percentage of radical reduction. Ascorbic acid was used as the reference material [29].

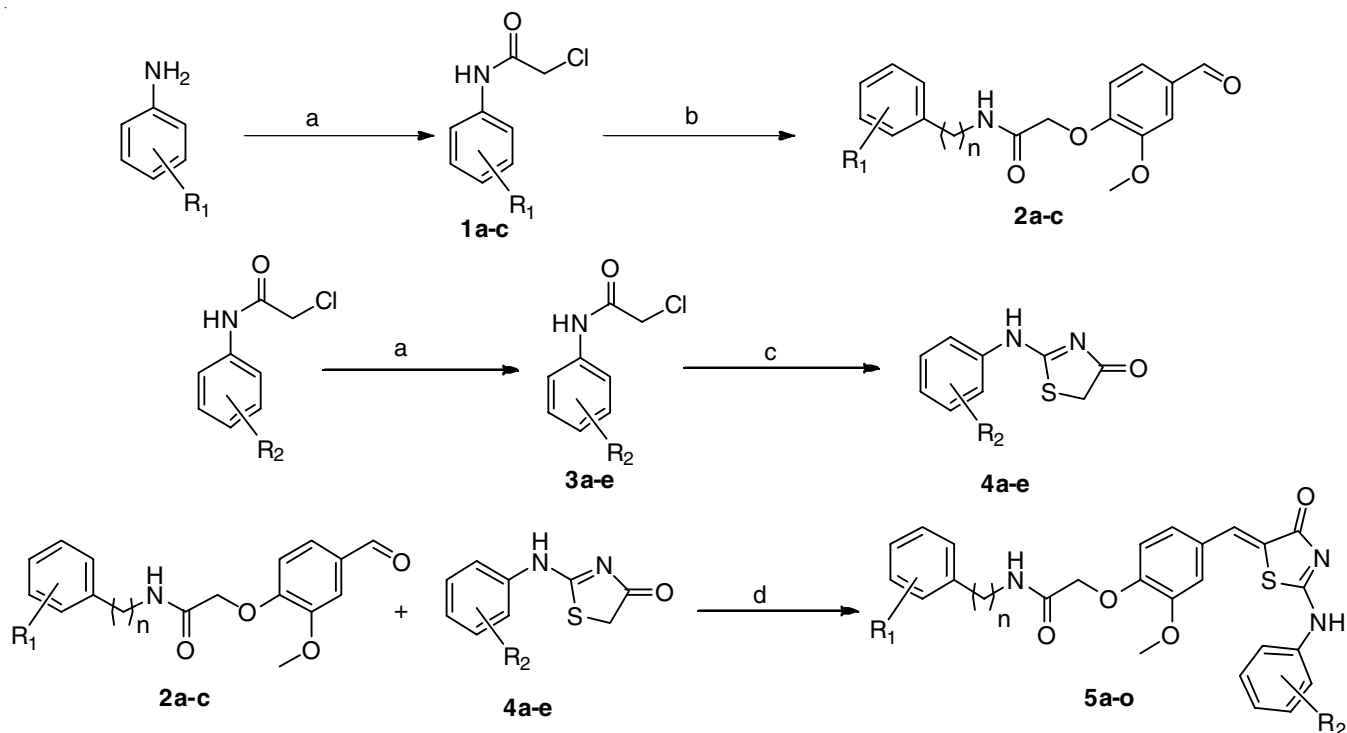
$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

**Survival assay:** The wild type Canton-S (CS) (*Drosophila* Stock Center, Mysore University, India) were maintained on wheat cream-agar medium at 25 °C with relative humidity of 65-70 % and 12:12 h light-dark cycle. One-day old female flies were isolated and fed with compound **5j** and **5n** prepared in 5% yeast solution. The flies were observed for its mortality rate and the food vials were checked daily to count the number of dead flies. The flies that escaped and those that stuck to the food were excluded from the count. The analysis was carried out for 20 days and a survival curve was plotted to compare the statistical significance between the control and treatment groups.

**Climbing assay:** Twenty days old control and compound **5j** and **5n** treated flies were transferred in 25 mL tube containing a mark at 20 mL. The two ends of the tube were closed using parafilm with minute holes for air exchange. The flies were tapped down 2-3 times to ensure that all the flies were at the bottom of the tube. The flies were then allowed to climb for 20 cm and the number of flies that crossed the mark in 1 min was counted. This procedure was repeated three times and percentage value was used for plotting final graph.

## RESULTS AND DISCUSSION

The general synthetic pathway to achieve title compounds is depicted in **Scheme-I**. Initially, substituted amines in DMF were treated with chloroacetyl chloride at room temperature for 4-6 h to yield respective chloroacetamides (**1a-c** and **3a-e**). Now, the first key intermediate thiazolidine-4-one (**4a-e**) was



**Scheme-I:** Synthesis of target compounds **5a-o**; Reagents and conditions: (a) chloroacetyl chloride, DMF, RT; (b)  $K_2CO_3$ , DMF, reflux, 3 h; (c)  $NH_4SCN$ , ethanol, reflux 10 h; (d) piperidine, ethanol, reflux, 12 h (details of  $R_1$  and  $R_2$  are given in Table-1)

achieved by refluxing chloroacetamides (**3a-e**) with  $\text{NH}_4\text{SCN}$  in ethanol for 10 h. The second key intermediate 2-(4-formyl-2-methoxyphenoxy)-*N*-substituted phenylacetamides (**2a-c**) was formed by reacting vanillin with potassium carbonate in DMF, followed by the addition of chloroacetamides (**1a-c**). The reaction mixture was then heated to reflux for 1 h. In final step, target compounds (**5a-o**) were achieved by clubbing two key scaffolds employing Knoevenagel condensation using piperidine as a catalyst. The formation of the target molecules (**5a-o**) was confirmed by elemental analyses, mass, FT-IR and  $^1\text{H}$  NMR spectroscopies.

Now considering the final compound **5j**, in its FT-IR spectrum the absorption band observed at  $3298\text{ cm}^{-1}$  corresponds to N-H stretching vibration. The characteristic C=O absorption band was observed at  $1678\text{ cm}^{-1}$  and the C=N absorption band at  $1531\text{ cm}^{-1}$ . The C-F stretching absorption was observed at  $1118\text{ cm}^{-1}$ . Now in its  $400\text{ Hz}$   $^1\text{H}$  NMR spectrum a broad singlet at  $\delta 11.39\text{ ppm}$  corresponds to N-H proton and amide N-H proton was resonated at  $\delta 10.01\text{ ppm}$ . A distinct singlet observed at  $\delta 8.06\text{ ppm}$  corresponds to 2<sup>nd</sup> proton of 3-trifluoromethyl phenyl-acetamide moiety. A doublet with coupling constant  $J = 8.0\text{ Hz}$  resonated at  $\delta 7.85\text{ ppm}$  corresponds to the 6<sup>th</sup> proton of 3-trifluoromethyl phenylacetamide moiety. The singlet at  $\delta 7.63\text{ ppm}$  appeared at a downfield (high chemical shift) for exocyclic proton (=C-H) indicating that the exocyclic double bond exists in *Z*-configuration with respect to thiazolidinone ring. Further, a triplet and a doublet at  $\delta 7.56$  and  $7.41\text{ ppm}$  with coupling constants  $J = 8.0$  and  $7.6\text{ Hz}$  correspond to the 5<sup>th</sup> and 4<sup>th</sup> protons of 3-trifluoromethyl phenylacetamide moiety respectively. The remaining six protons of the aromatic ring appeared as a multiplet at  $\delta 7.12\text{-}7.24\text{ ppm}$ . The  $\text{CH}_2$  protons were observed at  $\delta 4.77\text{ ppm}$  as a singlet. The two methyl groups and methoxy group were resonated at  $\delta 2.26, 2.25$  and  $3.89$

ppm, respectively. The mass spectrum showed protonated molecular ion peak at  $556.15$  in conformity with its molecular formula  $\text{C}_{28}\text{H}_{24}\text{N}_3\text{O}_4\text{SF}_3$ .

### Biological studies

**Antibacterial activity:** The antibacterial activity of the title compounds (**5a-o**) was undertaken using disc diffusion method over Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Chromobacterium violaceum*) and Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus mutans*). Initially, antibacterial activity was evaluated in terms of zone of inhibition (ZOI) against chosen bacterial strain by disc diffusion method and results are shown in Table-1. The antibacterial screening data found that the newly synthesized compounds were selectively active against *P. aeruginosa* bacterial strain. Compounds **5a-o** showed comparatively good activity against *P. aeruginosa*. The compounds which possessed electron-withdrawing substituents on thiazolidinone exhibited as the most active compounds when compared to the electron-donating group on thiazolidinone. Also, substituents present on phenylacetamides fairly affected the activity. The compounds with electron-donating on phenylacetamides showed better activity than compounds with inductively electron-withdrawing groups. Overall, electron withdrawing groups on thiazolidinone and electron-donating substituents on phenylacetamides showed good activity among the series. The antibacterial activity was measured in terms of zone of inhibition which was in the range of 12-18 mm. Among the series, compounds **5d** and **5n** showed better activity which had an electron-withdrawing group on thiazolidinone. On replacement of electron-withdrawing to the electron-donating group on thiazolidinone ring (**5b, 5e, 5g, 5j, 5l, 5o**), the activity deteriorated. The minimum concentration of antibacterial agent

TABLE-1  
ANTIBACTERIAL SCREENING AND DPPH RADICAL SCAVENGING  
ACTIVITY DATA OF THE SYNTHESIZED COMPOUNDS (**5a-o**)

| Compound                                  | R <sub>1</sub>                                     | R <sub>2</sub>                       | n | Zone of inhibition <sup>a</sup> (mm) at 1 mg/mL |                  |                  |                      |                     | MIC<br>( $\mu\text{g/mL}$ ) | IC <sub>50</sub> <sup>b</sup><br>( $\mu\text{g/mL}$ ) |
|-------------------------------------------|----------------------------------------------------|--------------------------------------|---|-------------------------------------------------|------------------|------------------|----------------------|---------------------|-----------------------------|-------------------------------------------------------|
|                                           |                                                    |                                      |   | <i>E. coli</i>                                  | <i>S. aureus</i> | <i>S. mutans</i> | <i>P. aeruginosa</i> | <i>C. violaceum</i> |                             |                                                       |
| <b>5a</b>                                 | 2-C <sub>2</sub> H <sub>5</sub> -6-CH <sub>3</sub> | F                                    | 0 | NA                                              | NA               | NA               | 17±0.2               | NA                  | 250                         | 46.31±1.07                                            |
| <b>5b</b>                                 | 2-C <sub>2</sub> H <sub>5</sub> -6-CH <sub>3</sub> | Cl                                   | 0 | NA                                              | NA               | NA               | 12±0.5               | NA                  | 500                         | 46.83±2.14                                            |
| <b>5c</b>                                 | 2-C <sub>2</sub> H <sub>5</sub> -6-CH <sub>3</sub> | NO <sub>2</sub>                      | 0 | NA                                              | NA               | NA               | 17±0.6               | NA                  | 250                         | 46.01±1.62                                            |
| <b>5d</b>                                 | 2-C <sub>2</sub> H <sub>5</sub> -6-CH <sub>3</sub> | 2-CH <sub>3</sub> -5-NO <sub>2</sub> | 0 | NA                                              | NA               | NA               | 18±0.2               | NA                  | 250                         | 38.73±1.63                                            |
| <b>5e</b>                                 | 2-C <sub>2</sub> H <sub>5</sub> -6-CH <sub>3</sub> | 3,4-(CH <sub>3</sub> ) <sub>2</sub>  | 0 | NA                                              | NA               | NA               | 12±0.4               | NA                  | 500                         | 40.88±3.8                                             |
| <b>5f</b>                                 | 3-CF <sub>3</sub>                                  | F                                    | 0 | NA                                              | NA               | NA               | 15±0.2               | NA                  | 500                         | 32.17±7.25                                            |
| <b>5g</b>                                 | 3-CF <sub>3</sub>                                  | Cl                                   | 0 | NA                                              | NA               | NA               | 13±0.5               | NA                  | 250                         | 35.26±1.16                                            |
| <b>5h</b>                                 | 3-CF <sub>3</sub>                                  | NO <sub>2</sub>                      | 0 | NA                                              | NA               | NA               | 15±0.3               | NA                  | 250                         | 25.13±4.17                                            |
| <b>5i</b>                                 | 3-CF <sub>3</sub>                                  | 2-CH <sub>3</sub> -5-NO <sub>2</sub> | 0 | NA                                              | NA               | NA               | 16±0.4               | NA                  | 250                         | 24.21±3.72                                            |
| <b>5j</b>                                 | 3-CF <sub>3</sub>                                  | 3,4-(CH <sub>3</sub> ) <sub>2</sub>  | 0 | NA                                              | NA               | NA               | 14±0.5               | NA                  | 500                         | 23.86±1.59                                            |
| <b>5k</b>                                 | 2-Cl                                               | F                                    | 1 | NA                                              | NA               | NA               | 17±0.2               | NA                  | 250                         | 30.40±4.56                                            |
| <b>5l</b>                                 | 2-Cl                                               | Cl                                   | 1 | NA                                              | NA               | NA               | 13±0.4               | NA                  | 500                         | 30.65±2.23                                            |
| <b>5m</b>                                 | 2-Cl                                               | NO <sub>2</sub>                      | 1 | NA                                              | NA               | NA               | 17±0.5               | NA                  | 250                         | 37.93±2.98                                            |
| <b>5n</b>                                 | 2-Cl                                               | 2-CH <sub>3</sub> -5-NO <sub>2</sub> | 1 | NA                                              | NA               | NA               | 19±0.3               | NA                  | 250                         | 36.75±5.25                                            |
| <b>5o</b>                                 | 2-Cl                                               | 3,4-(CH <sub>3</sub> ) <sub>2</sub>  | 1 | NA                                              | NA               | NA               | 14±0.5               | NA                  | 500                         | 51.45±0.91                                            |
| Ciprofloxacin<br>(30 $\mu\text{g}$ /disc) |                                                    |                                      |   | 26±0.6                                          | 25±0.3           | 28±0.5           | 23±0.8               | 27±0.5              | < 32                        | 23.75±5.03                                            |

<sup>a</sup>The zone of inhibition was expressed as an average from three experiments  $\pm$  standard deviation; NA = no activity.

<sup>b</sup>Ascorbic acid was used as a standard antioxidant; Each value is expressed as mean  $\pm$  SD of three replicates.

required to inhibit bacterial growth (MIC  $\mu\text{g/mL}$ ) was determined for entire derivatives. Most of the compounds showed MIC value at 250  $\mu\text{g/mL}$  and ciprofloxacin was used as a standard (Table-1).

**Antioxidant activity:** The most frequently used method for determining the reducing property of the compound is the DPPH assay. A purple stable free radical, DPPH $^{\bullet}$  (2,2-diphenyl-1-picrylhydrazyl) reacts with hydrogen donor which gives the reduced form of DPPH, indicated by a colour change from purple to pale yellow. All the derivatives **5a-o** were screened for their antioxidant activity (Table-1).

The radical scavenging activity with varying concentrations *viz.* 10, 20, 30, 40 and 50  $\mu\text{g/mL}$  were analyzed for the synthesized compounds (**5a-o**). The results revealed that the scavenging activity increased with an increase in concentration. Ascorbic acid was used as a standard and results are expressed in  $\text{IC}_{50}$  and shown in Table-1. Among the synthesized compounds (**5a-o**), molecule **5j** showed excellent activity with  $\text{IC}_{50}$  value 23.86  $\mu\text{g/mL}$ . It is worth noticing that compounds with inductively electron withdrawing substituents on phenylacetamides showed better activity than compounds with electron donating substituents.

**Survival assay and climbing assay:** In order to assess the toxicity of **5j** and **5n**, survival (Fig. 1) and climbing (Fig. 2) assays were performed with newly hatched female flies. It was seen that for a period of approximately 20 days, the survival curves for control and treatment groups did not exhibit any significant difference. Compounds **5j** and **5n** has no significant effect on the climbing ability of the flies. Hence, these results indicate the compounds were non-toxic.

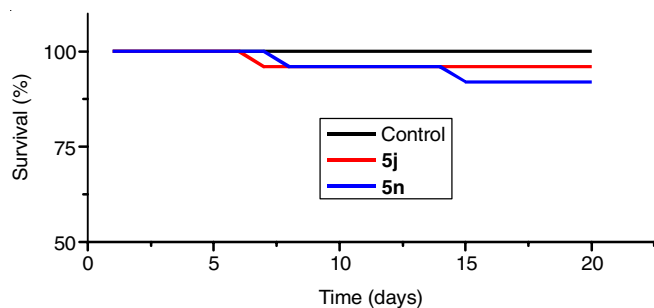


Fig. 1. Survival assay of compounds **5j** and **5n**

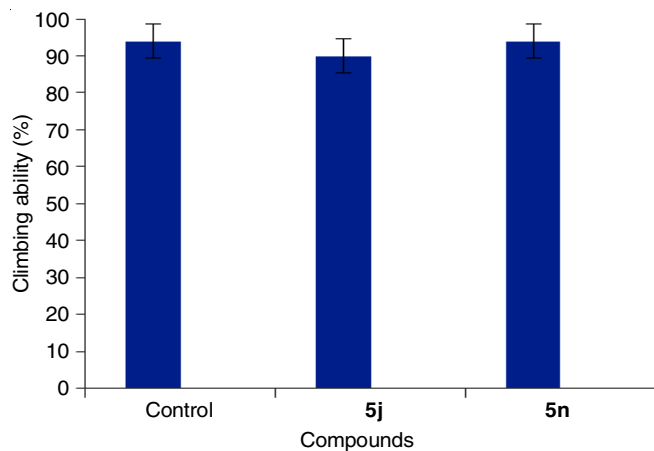


Fig. 2. Climbing assay of compounds **5j** and **5n**

## Conclusion

Novel thiazolidinone derivatives were synthesized in good yield and screened for their antibacterial and antioxidant activities. The biological assay revealed that these derivatives were selective towards *Pseudomonas aeruginosa* bacteria among the chosen bacterial strain. Among the series, compounds **5d** and **5n** showed potent antibacterial activity. Further, compound **5j** showed excellent DPPH radical scavenging activity with  $\text{IC}_{50}$  value 23.86  $\mu\text{g/mL}$ . The survival and climbing assay revealed that potent compounds **5j** and **5n** were non-toxic in nature.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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