



Synthesis and antibacterial screening of some 2-(*p*-substituted benzylidene)-3-(5-ethyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-ones

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ABSTRACT

A series of 2-(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones were synthesized by the reaction of Schiff's bases of *N*-(*p*-substituted benzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amines with thioglycolic acid in 1, 4-dioxane as solvent and studied for their *in vitro* antibacterial activity. Reaction of thiosemicarbazide with propanoic acid in presence of concentrated sulphuric acid led to the formation of 5-ethyl 1, 3, 4-thiadiazole-2-amines which on further reaction with different *p*-substituted benzaldehydes yielded the compounds Schiff's bases of *N*-(*p*-substituted benzylidene)-5-ethyl-1,3, 4-thiadiazole-2-amines which is further reaction with thioglycolic acid in presence of small amount of zinc chloride in 1,4 dioxane as solvent gave title compounds. These compounds were characterized by spectral analysis. All the synthesized compounds were evaluated for their *in vitro* for their antibacterial activity against two Gram positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and their minimum inhibitory concentration (MIC) were determined.

Keywords: Thiazolidinone, Schiff's base, antibacterial, minimum inhibitory concentration (MIC).

INTRODUCTION

Heterocyclic ring systems containing compounds continue to attract considerable interest due to their many biological activities. A unique place is occupied by five membered in the natural and synthetic organic chemistry. Thiazolidinones are derivatives of thiazolidine which belong to important groups of heterocyclic compounds. Depending upon the presence of the carbonyl group at different position thiazolidinones are of different type such as 2, 4, and 5-position known as 2-thiazolidinones, 4-thiazolidinones, and 5-thiazolidinones. Among these thiazolidinone, 4-thiazolidinones shows greater biological activity [1]. The excess use of chemotherapeutic agents for the treatment of infectious diseases leads to the development of microbial resistance to existing drugs. The development of resistance to the major classes of antibacterial drugs is recognized as a serious health problem. The hunt for novel antibacterial agents with different mode of actions will always remain an important and challenging task [2]. Compounds containing thiazolidinone nucleus have been reported as antimicrobials [3-6], anti-inflammatory [7-8] and anti-tuberculosis [9]. These reports including our ongoing research program in the field of synthesis and antimicrobial activity of medicinally important compounds [10-15] inspired us to undertake the synthesis of some 2-(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones. The synthesized compounds were characterized on the basis of IR and ¹H NMR spectral data. All the compounds were screened for their *in vitro* antibacterial activity against two Gram positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) respectively and their minimum inhibitory concentration (MIC) were also determined.

EXPERIMENTAL SECTION

All the chemical and reagents used were of analytical grade and all the reaction were monitored by thin layer chromatography (TLC) using silica gel G as stationary phase, different solvent systems as mobile phase and iodine vapors as detecting agent. Melting points of the compounds were determined in open capillary tube by Decible Melting Point Apparatus and were uncorrected. Proton NMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer using tetra-methyl silane as internal standard. Infrared Spectra were recorded by Perkins Elmer IR spectrophotometer using KBr pellets.

Synthesis of N-(*p*-substituted benzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amines

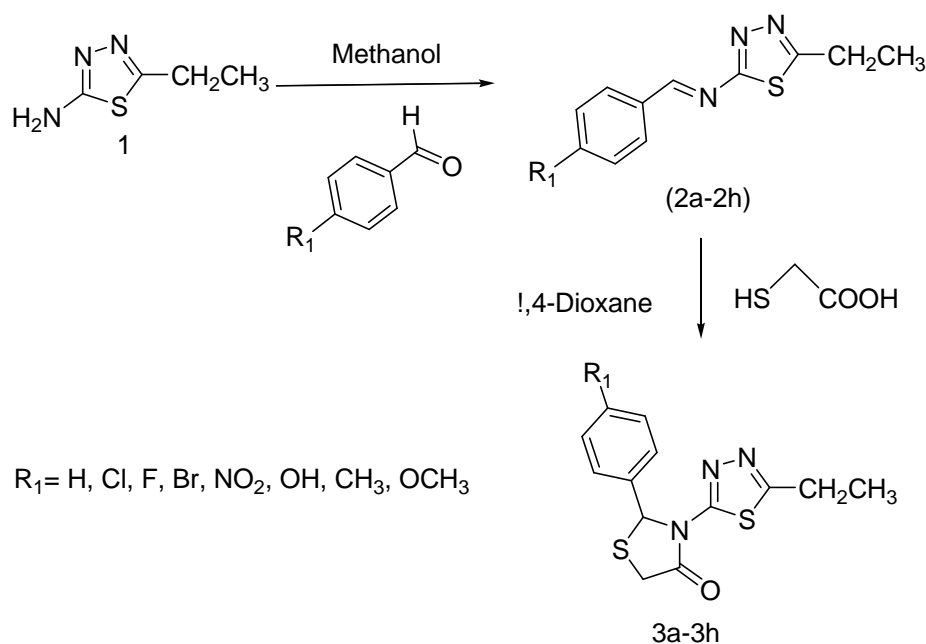
Synthesis of 2-amino-5-ethyl-1,3,4-thiadiazole was carried out by the reported method [16]. N-(*p*-substituted benzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amines were synthesized according to the method [17] from equimolar quantity of 2-amino-5-ethyl-1,3,4-thiadiazoles (0.06 mol) and aromatic aldehydes (0.06 mol) in 30 mL of methanol and heated at 60-70 °C on water bath for 4 hrs in presence of few drops of glacial acetic acid. The crude products were obtained after removal of methanol under reduced pressure. The products were recrystallized from methanol.

Synthesis of 2--(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl)-thiazolidin-4-one

These were prepared according to the method [17] by taking thioglycolic acid (0.04 mol) and Schiff's bases (N-(*p*-substituted benzylidene)-5-ethyl-1,3,4-thiadiazole-2-amines) (0.02mol) in 30 mL of 1,4-dioxane and heated at 70-80 °C in presence of catalytic amount of anhydrous zinc chloride on water bath for 7 hrs. The product was cooled, poured into cold water, filtered and recrystallized from rectified spirit.

Characterization of the compounds was accomplished by the spectral (IR, ¹HNMR, ¹³C NMR) means as well as elemental analysis. Purity of the compounds was checked by TLC. The results of elemental analysis were in good agreement with the calculated values.

All products were obtained in good yield. Physicochemical characterization of the synthesized compounds is given in **Tables 1**.



Scheme: Synthesis of 2-(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-one

Table 1: Physicochemical data of 2-(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones

| Compound | R | Molecular Weight | Molecular Formula | M.P. (°C) | % Yield |
|-----------|------------------|------------------|--|-----------|---------|
| 3a | H | 291.39 | C ₁₃ H ₁₃ N ₃ OS ₂ | 238-240 | 65.3 |
| 3b | CH ₃ | 305.42 | C ₁₄ H ₁₅ N ₃ OS ₂ | 130-132 | 70.4 |
| 3c | OH | 307.39 | C ₁₃ H ₁₂ N ₃ O ₂ S ₂ | 150-152 | 69.7 |
| 3d | OCH ₃ | 321.42 | C ₁₄ H ₁₅ N ₃ O ₃ S ₂ | 139-141 | 77.7 |
| 3e | Cl | 325.84 | C ₁₃ H ₁₂ ClN ₃ OS ₂ | 173-175 | 72.5 |
| 3f | Br | 370.29 | C ₁₃ H ₁₂ BrN ₃ OS ₂ | 146-148 | 63.4 |
| 3g | F | 309.38 | C ₁₃ H ₁₂ FN ₃ OS ₂ | 209-211 | 68.4 |
| 3h | NO ₂ | 336.39 | C ₁₃ H ₁₂ N ₄ O ₃ S ₂ | 189-193 | 70.2 |

Spectral data of the title compounds.**3-(5-ethyl-1, 3, 4-thiadiazol-2-yl)-2-phenylthiazolidin-4-one (3a)**

IR (KBr, cm⁻¹): 3069 (aromatic C–H *str.*), 1682 (C=O), 1675 (C=N), 1597, 1436, 1397 (C=C ring *str.*), 1029 (N–N), 800 (*p*-di-substituted benzene), 644 (C–S–C); ¹H NMR (DMSO, *δ*, ppm): 8.12 (s, 1H, CH), 7.01–7.14 (m, 4H, ArH), 5.66 (s, 1H, SCHN), 3.45–3.68 (s, 2H, SCH₂), 2.20–2.24 (q, 2H, CH₂), 1.21–1.25 (t, 3H, CH₃); ¹³C NMR (DMSO, *δ*, ppm): 172.95, 170.12, 165.32, 140.21, 129.34, 125.62, 124.32, 68.53, 34.28, 27.65, 14.72

2-(4-methylphenyl)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-one (3b)

IR (KBr, cm⁻¹): 3087 (aromatic C–H *str.*), 1681 (C=O), 1652 (C=N), 1597, 1438, 1355 (C=C ring *str.*), 1323 (Ar–CH₃), 1032 (N–N), 811 (*p*-di-substituted benzene), 646 (C–S–C); ¹H NMR (DMSO, *δ*, ppm): 8.10 (s, 1H, CH), 7.05–7.16 (m, 4H, ArH), 5.18 (s, 1H, SCHN), 3.61–3.71 (s, 2H, SCH₂), 2.45–2.51 (s, 3H, CH₃), 2.33–2.37 (q, 2H, CH₂), 1.1–1.5 (t, 3H, CH₃); ¹³C NMR (DMSO, *δ*, ppm): 174.43, 170.13, 163.80, 134.29, 134.29, 121.37, 27.66, 68.70, 33.61, 18.17.

2-(4-hydroxyphenyl)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-one (3c)

IR (KBr, cm⁻¹): 3300 (Ar–OH), 3077 (aromatic C–H *str.*), 1687 (C=O), 1675 (C=N), 1557, 1458, 1345 (C=C ring *str.*), 1039 (N–N), 815 (*p*-di-substituted benzene); 650 (C–S–C), ¹H NMR (DMSO, *δ*, ppm): 8.10 (s, 1H, CH), 7.06–7.20 (m, 4H, ArH), 5.38 (s, 1H, SCHN), 4.9 (s, 1H, OH), 3.65–3.51 (s, 2H, SCH₂), 2.47–2.51 (q, 2H, CH₂), 0.94–0.98 (t, 3H, CH₃); ¹³C NMR (DMSO, *δ*, ppm): 174.21, 169.53, 166.35, 154.26, 132.54, 129.63, 113.24, 73.25, 34.10, 22.35, 15.03.

2-(4-methoxyphenyl)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-one (3d)

IR (KBr, cm⁻¹): 3084 (aromatic C–H *str.*), 1689 (C=O), 1670 (C=N), 1597, 1430, 1395 (C=C ring *str.*), 1329 (Ar–OCH₃), 1020 (N–N), 809 (*p*-di-substituted benzene), 648 (C–S–C); ¹H NMR (DMSO, *δ*, ppm): 8.09 (s, 1H, CH), 7.02–7.19 (m, 4H, ArH), 5.50 (s, 1H, SCHN), 3.38–3.50 (s, 2H, SCH₂), 3.74–3.80 (s, 3H, OCH₃), 2.24–2.28 (q, 2H, CH₂), 0.99–1.03 (t, 3H, CH₃); ¹³C NMR (DMSO, *δ*, ppm): 172.54, 164.25, 162.35, 156.32, 133.24, 130.25, 112.20, 53.24, 73.26, 36.23, 25.14, 14.32.

2-(4-chlorophenyl)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-one (3e)

IR (KBr, cm⁻¹): 3059 (aromatic C–H *str.*), 1689 (C=O), 1670 (C=N), 1587, 1456, 1377 (C=C ring *str.*), 1088 (Ar–Cl), 1039 (N–N), 817 (*p*-di-substituted benzene), 649 (C–S–C); ¹H NMR (DMSO, *δ*, ppm): 8.09 (s, 1H, CH), 7.04–7.06 (m, 4H, ArH), 4.98 (s, 1H, SCHN), 3.57–3.65 (s, 2H, SCH₂), 2.41–2.45 (q, 2H, CH₂), 1.50–1.54 (t, 3H, CH₃); ¹³C NMR (DMSO, *δ*, ppm): 173.26, 165.58, 159.36, 140.21, 131.34, 130.34, 126.21, 74.48, 31.42, 27.16, 15.03.

2-(4-bromophenyl)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-one (3f)

IR (KBr, cm⁻¹): 3099 (aromatic C–H *str.*), 1694 (C=O), 1654 (C=N), 1587, 1446, 1357 (C=C ring *str.*), 1072 (Ar–Br), 1019 (N–N), 803 (*p*-di-substituted benzene), 642 (C–S–C); ¹H NMR (DMSO, *δ*, ppm): 8.09 (s, 1H, CH), 7.07–7.17 (m, 4H, ArH), 5.47 (s, 1H, SCHN), 3.61–3.65 (s, 2H, SCH₂), 2.25–2.29 (q, 2H, CH₂), 1.1–1.5 (t, 3H, CH₃); ¹³C NMR (DMSO, *δ*, ppm): 174.32, 164.25, 159.86, 140.23, 132.54, 124.32, 129.63, 71.15, 31.25, 27.45, 15.23.

2-(4-fluorophenyl)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-one (3g)

IR (KBr, cm⁻¹): 3069 (aromatic C–H *str.*), 1697 (C=O), 1677 (C=N), 1597, 1466, 1337 (C=C ring *str.*), 1323 (Ar–F), 1030 (N–N), 817 (*p*-di-substituted benzene), 640 (C–S–C); ¹H NMR (DMSO, *δ*, ppm): 8.10 (s, 1H, CH), 7.07–7.19 (m, 4H, ArH), 4.40 (s, 1H, SCHN), 3.43–3.70 (s, 2H, SCH₂), 2.31–2.35 (q, 2H, CH₂), 1.2–1.6 (t, 3H, CH₃); ¹³C NMR (DMSO, *δ*, ppm): 172.47, 165.49, 165.49, 158.58, 138.29, 131.59, 116.68, 68.56, 28.65, 14.42.

2-(4-nitrophenyl)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-one (3h)

IR (KBr, cm^{-1}): 3064 (aromatic C–H *str.*), 1692 (C=O), 1667 (C=N), 1598, 1460, 1335 (C=C ring *str.*), 1327 (Ar–NO₂), 1035 (N–N), 814 (*p*-di-substituted benzene), 649 (C–S–C); ¹H NMR (DMSO, *d*₆, δ ppm): 8.06 (s, 1H, CH), 7.03-7.18 (m, 4H, ArH), 5.48 (s, 1H, SCHN), 3.35-3.49 (s, 2H, SCH₂), 2.31-2.35 (q, 2H, CH₂), 1.5-1.9 (t, 3H, CH₃); ¹³C NMR (DMSO, *d*₆, δ ppm): 170.34, 164.28, 158.35, 149.36, 146.32, 131.25, 121.35, 75.28, 36.54, 26.32, 15.23.

Antibacterial activity

All the title compounds were screened for their *in vitro* antibacterial activity against two Gram positive strains, *i.e.*, *Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 96) and two Gram negative strains, *i.e.*, *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453) respectively. Ciprofloxacin was used as the standard drug for the present study. Serial two fold dilution technique was used for the study of antibacterial activity [17]. A stock solution (10 $\mu\text{g/ml}$) of all the title compounds and standard drug was prepared in dimethyl sulfoxide. Sterilized double strength nutrient broth (DSNB) was used as a growth media. The stock solution was serially diluted by DSNB aseptically to give concentrations of 5.0–0.01 $\mu\text{g/ml}$ into a series of sterilized culture tubes. All the tubes were inoculated by bacterial strain. The inoculum's size was approximately 10⁶ colony forming units (CFU/ml). The inoculated tubes were incubated for 24 h at 37(\pm 1) °C. After 24 h, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound. The MIC for the title compounds and the standard drug, *i.e.*, ciprofloxacin are presented in Table 2.

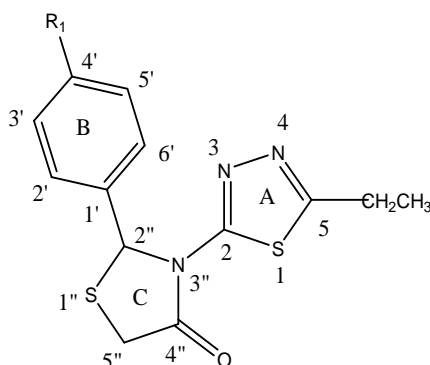
Table 2: Antibacterial activity of 2-(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones

| Compound | Minimum inhibitory concentration (MIC $\mu\text{g/ml}$) | | | |
|----------------------|--|-------------------------------|---------------------------|----------------------------------|
| | <i>S. aureus</i> MTCC 3160 | <i>B. subtilis</i> MTCC 16 | <i>E. coli</i> MTCC 40 | <i>P. aeruginosa</i> MTCC 424 |
| 3a | 0.75 | 0.75 | 0.65 | 0.65 |
| 3b | 0.85 | 0.85 | 0.75 | 0.75 |
| 3c | 0.55 | 0.55 | 0.45 | 0.50 |
| 3d | 0.85 | 0.85 | 0.85 | 0.85 |
| 3e | 0.65 | 0.60 | 0.70 | 0.75 |
| 3f | 0.65 | 0.60 | 0.50 | 0.55 |
| 3g | 0.40 | 0.40 | 0.40 | 0.40 |
| 3h | 0.45 | 0.45 | 0.40 | 0.40 |
| Ciprofloxacin | 0.15 | 0.12 | 0.01 | 0.25 |

RESULTS AND DISCUSSION**Chemistry**

The syntheses of 2-(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones were achieved following the steps outlined in the scheme. Reaction of compound **1** with different *p*-substituted benzaldehydes in presence of few drops of glacial acetic acid furnished the Schiff's bases *i.e.*, N-(*p*-substituted benzylidene)-5-ethyl-1,3, 4-thiadiazole-2-amines (**2**). The reaction of compound (**2**) with thioglycollic acid in presence of small amount of zinc chloride in 1,4 dioxane as solvent gave title compounds. All the compounds were obtained in good yield. All the compounds were characterized by spectral analysis.

The IR spectra showed a band for (C–S–C) *stretching* vibrations near 640-655 cm^{-1} and (N–N) *stretching* vibrations were observed near 1018-1039 cm^{-1} . The (C=N) *stretching* vibrations were observed in range of 1652-1684 cm^{-1} . The aromatic C–H *stretching* was observed in range of 3026-3098 cm^{-1} and aromatic O–H *stretching* in range of 3300-3307 cm^{-1} . The *bending* vibrations for *p*-di-substituted benzene appeared in the range of 800-819 cm^{-1} . In case of ¹H NMR the signals for ethyl protons at position five of 1, 3, 4 thiadiazole appeared signals as quartet and triplet in range of 2.20 to 2.51 δ (ppm) and 0.9 to 1.9 δ (ppm) for methyl and methylene protons respectively. A singlet was shown for aromatics methoxy and methyl protons at 3.62 to 3.70 δ (ppm) and 2.33 to 2.39 1.21-1.78 δ (ppm) respectively. The imine proton was appeared as singlet in range 8.06-8.19 δ (ppm). The chemical shift value for aromatic protons was observed in the range of 7.00-7.88 δ (ppm) and appeared as multiplet. In the Schiff's bases, imine proton was appeared as singlet in range 8.06-8.19 δ (ppm). In case of ¹³C NMR, The chemical shift value for carbon (N=CH) were observed in range 159.58-174.52 δ (ppm) in schiff's base. Ethyl showed signals in aliphatic region in the range of 34.28 δ (ppm) – 15.03 δ (ppm).. In the ring A the chemical shift value were observed for C₂ and C₅ in range of 153.50-166.35 δ (ppm), 140.25-172.95 δ (ppm) respectively. The signals for aromatic carbons in ring B were detected in range of 130.12-168.24 δ (ppm), 128.35-168.24 δ (ppm), 114.56-134.58 δ (ppm), 128.36-136.91 δ (ppm) for C'₁, C'₄, (C'₃ and C'₅), (C'₂ and C'₆) respectively. The chemical shift value in ring C for carbon C''₅ and C''₄ were observed in range of 29.02-39.34 δ (ppm) and 168.15-176.08 δ (ppm) respectively.



Antibacterial activity

All the synthesized title compounds were screened for their *in vitro* antibacterial activity against two Gram positive bacterial strains *i.e.*, *Bacillus subtilis* (MTCC 16) and *Staphylococcus aureus* (MTCC 3160) and two Gram negative bacterial strains *i.e.*, *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 424) respectively and their minimum inhibitory concentration (MIC) were determined. The activity data in the **table 2** shows that all the title compounds were found to be active against all the bacterial strains used in this study. However, they showed more activity against the Gram negative than the Gram positive bacterial strains. The minimum inhibitory concentration (MIC) of the title compounds **3a-h** were found to be 0.85-0.40 $\mu\text{g/ml}$ and 0.80-0.40 $\mu\text{g/ml}$ against Gram positive and Gram negative bacterial strains respectively. The MICs of the title compounds containing electron withdrawing groups like fluoro, chloro, bromo or nitro were found somewhat less than the compounds containing electron releasing groups like methyl and methoxy. The reference standard ciprofloxacin inhibited Gram negative bacteria *viz.*, *E. coli* and *P. aeruginosa* at a MIC of 0.01 $\mu\text{g/ml}$ and 0.25 $\mu\text{g/ml}$ respectively whereas against Gram positive bacteria *viz.*, *S. aureus* and *B. subtilis* MIC was found to be 0.15 $\mu\text{g/ml}$ and 0.12 $\mu\text{g/ml}$ respectively. The results of the MIC for the standard drug, ciprofloxacin, against the bacterial strains used were found to be within the range as reported in the literature [18-20].

CONCLUSION

Present study describes the synthesis of a series of 2-(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones. The compounds were characterized by spectral techniques such as IR, proton NMR and ^{13}C NMR spectra. All the title compounds were screened for their *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive) and *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative) and their minimum inhibitory concentration (MIC) were determined. The results of antibacterial activity showed that compounds containing electron withdrawing groups *e.g.*, chloro, bromo, fluoro or nitro were found to be more active than the compounds containing electron releasing groups such as methyl and methoxy. These results suggest that some more compounds using different aliphatic acids and hetero-aromatic aldehydes or ketones should be synthesized and screened for their antibacterial activity to explore the possibility of 2-(*p*-substituted benzylidene)-3-(5-ethyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones as a new series of antibacterial.

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REFERENCES

- [1] FC Brown; *Chemical rev.* **1961**, 61 (5), 463-521
- [2] R Sharma; CL Sharma; B Kapoor, *Indian J. Med. Sci.*, **2005**, 59, 120.
- [3] K Vadodari; D Ladva; V R Shah and AR Parikh, *Australian Journal of Basic and Applied Sciences*, **2013**, 7(9), 327-330.
- [4] RR Mishra; KS Nimavat; KB Vyas, *Rasyan J Chem.* **2012**, Vol. 5 (2), 214-219.
- [5] R Sharma; P Samadhiya; SD Srivastava; S K Srivastava, *J. Serb. Chem. Soc.*, **2012**, 77 (1), 17-26.
- [6] PR Kumar; M M S Yadav; K K Murali; ST Rao, *E-Journal of Chemistry*, **2006**, Vol. 3 (10), 44-48.
- [7] P Gangwar; P Sharma, B Shrivastava, Sharma J, Sharma S, *IJCTPR*. **2013**, Vol. 1(3), 181-184.
- [8] KC Asati; S K Srivastav and S D Srivastav, *Indian Journal of Chemistry*. **2006**, Vol. 45B, 526-531.

- [9] HH Parekh; K A Parikh; AR Parikh, *Journal of Sciences, Islamic Republic of Iran*, **2004**, 15(2), 143-148.
- [10] NB Pate; FM Shaikh, *Journal of Sciences, Islamic Republic of Iran*, 2010, 21(2), 121-129.
- [11] TM Bhagat; DK Swamy; SG Badne; SV Kuberkar, *Rasayan J. Chem.*, **2011**, Vol.4 (1), 24-28.
- [12] RS Lodhi; SD Srivastav, *Indian Journal of Chemistry*. **1997**, Vol. 39B, 947-950.
- [13] N Jain; DP Pathak; P Mishra; S Jain, *J. Iran. Chem. Soc.*, **2009**, 6, 77.
- [14] A Goyal, S Jain, *Der Pharma Chemica*. **2012**, 4 (1), 234.
- [15] A Goyal; N Jain; S Jain; *Der Pharmacia Sinica*, **2011**, 4 (4), 112.
- [16] V Jatav; SK Jain; SK Kashaw; P Mishra, *Indian Journal of Pharmaceutical Sciences*. **2006**, 68 (3), 360.
- [17] A Deep; S Jain; and PC Sharma; *Acta Poloniae Pharmaceutica-Drug Research*, **2010**, 67 (1), 63-67.
- [18] JG Cappucino; N Sherman, *Microbiology: A Laboratory Manual*, Addison Wesley Publisher, San-Francisco CA, **1999**, 263.
- [19] Bauernfeind; *J. Antimicrob. Chemother*, **1997**, 40, 639.
- [20] A A Hoogkamp-Korstanje; *J. Antimicrob. Chemother*. **1997**, 40, 427.
- [21] D J Weber; SM Saviteer; WA Rutala; CA Thomann, *Antimicrob. Agents Chemother.*, **1988**, 32, 642.