Synthesis, spectroscopic characterization and antimicrobial activity of 5-arylidene-2-substituted-1,3-thiazol-4-one

Prajwal L. Lobo¹*, Boja Poojary², D. Jagadeesh prasad² and Nalilu Suchetha Kumari⁴

¹Department of Chemistry, Maharani’s Science College for Women, Bangalore-560001, Karnataka, India
²Department of Chemistry, Mangalore University, Mangalagangothri-574199, Karnataka, India.
³Department of Biochemistry, K.S. Hegde Medical Academy, Deralakatte-574162, Karnataka, India.

ABSTRACT

Various substituted 5-arylidene-2-substituted-1,3-thiazol-4-one derivatives 3a-h were synthesized in good yield by the reaction of methyl thiocyanatoacetate 1 with various secondary amines to afford 2-substituted 1,3-thiazol-4(5H)-one which on further reaction with various aryl aldehydes afforded the title compounds 3a-h. All structures of the newly synthesized compounds were elucidated by elemental analyses, spectral data and a few by single crystal XRD study and screened for their antimicrobial activity.

Key words: methylthiocyanatoacetate, thiazole, secondary amine, chair conformation

INTRODUCTION

The development of simple synthetic routes for widely used organic compounds from readily available reagents is one of the major challenges in organic synthesis. Heterocycles form the basis of a variety of pharmaceutical compounds [1]. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules [2,3]. Thiazolidine template is one such privileged structure fragments in modern medicinal chemistry considering its broad pharmacological spectrum and affinity for various biotargets of these class heterocyclic compounds [4]. Therefore, the synthesis of combinatorial libraries of these compounds was elaborated [5-8]. The thiazolidinone derivatives are well known in medicinal and biological chemistry due to their diverse pharmacological displays as anti-inflammatory, antimicrobial [9, 10], antitumor [11], anticonvulsant [12], analgesic [13], anticancer properties. The biological and synthetic significance, places this group (thiazolidinone) in an important position in medicinal chemistry research. As a part of our continuing interest in biologically active thiazolidinone derivatives and on the basis of the assumption that a benzylidene moiety at the 5-position of the 4-thiazolidinone is necessary for the antimicrobial activity, we are reporting a simple root for the synthesis of novel thiazolidinones starting from ethyl chloro acetate and secondary amines. In this contribution, results of synthesis, spectroscopic studies and antimicrobial activity of thiazolidinone derivatives are presented.

MATERIALS AND METHODS

The melting points were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. The ¹H-NMR spectra were recorded (CDCl₃) on a BRUKER AVANCE II - 400 (400 MHz) spectrometer using TMS as an internal standard. The DART-MS was
recorded on a JEOL-ACCUTOF JMS-T100LC mass spectrometer having a DART source. Dry helium was used with 4LPM flow rate for ionization at 350°C. Elemental analysis (CHNS) was performed on the CHNS Elementar Vario EL III. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates. All the spectral data of newly synthesized compounds are consistent with proposed structure and microanalysis within ±0.3 of the calculated values. The synthetic strategies adopted to obtain the target compounds are depicted in Scheme 1.

Scheme 1: Synthesis of 5-arylidene-2-substituted-1,3-thiazol-4-ones

X=O, CH₂, N-CH₃

R= 4-OCH₃, 2,4-Cl₂, 3,4,5-(OCH₃)₂, 3,4,5-(OCH₃)₃

General procedure for the synthesis of thiocyanatoacetate (1)
An equimolar mixture of sodium thiocyanate and methylchloroacetate in acetonitrile was stirred and heated to approximately 30°C and held at this temperature for 20 hours. The precipitated NaCl is filtered and acetonitrile was removed by rotary evaporation (the concentrate contained approximately 10% of acetonitrile). The crude product was washed with water. The separated organic layer was dried over anhydrous Na₂SO₄. After Na₂SO₄ was filtered off, the product methyl thiocyanatoacetate was used as such for the second step without further purification.

General procedure for the synthesis of 2-substituted-1,3-thiazol-4(5H)-one (2a-c)
A mixture of thiocyanatoacetate (0.01mol), secondary amine (0.01mol) and acetic acid in ethanol was refluxed for 30 hours. The course of the reaction was monitored by TLC. After cooling the reaction mixture the precipitate formed was filtered, washed and recrystallised from tetrahydrofuran.

General procedure for the synthesis of 5-benzylidene-2-substituted-1,3-thiazol-4(5H)-one (3a-h)
To a mixture of 2-substituted-1,3-thiazol-4(5H)-one (0.01mole), appropriate aromatic aldehyde (0.01mole), anhydrous sodium acetate (0.8g) and glacial acetic acid (10ml) was heated under reflux for 1.5 hours. The reaction mixture was cooled to room temperature and poured to ice water. The separated solid was filtered, washed with water, recrystallised from tetrahydrofuran.

2-(Morpholin-4-yl)-5-(3,4,5-trimethoxybenzylidene)-1,3-thiazol-4(5H)-one (3b)
IR (KBr, ν_max cm⁻¹): 3059 (Ar-H), 2946 (C-H), 1684 (C=O), 1567 (C=C); ¹H NMR (400MHz, CDCl₃, δ ppm): 3.65 (t, 2H, J = 4.9 Hz, CH₂ of morpholine), 3.69 (s, 6H, OCH₃), 3.79 (s, 3H, OCH₃), 3.80-3.85 (m, 4H, (CH₂)₂N of morpholine), 4.09 (t, 2H, J = 4.9 Hz, CH₂ of morpholine), 7.00 (s, 2H, Ar-H), 8.06 (s, 1H, =CH); DART MS (m/z): 364.01 (M⁺); Anal. for C₁₇H₂₀N₂O₅S: C 56.03, H 5.53, N 7.69; Found: C 56.07, H 5.55, N 7.72.

2-(Piperidin-1-yl)-5-(3,4,5-trimethoxybenzylidene)-1,3-thiazol-4(5H)-one (3e)
IR (KBr, ν_max cm⁻¹): 3054 (Ar-H), 2948 (C-H), 1682 (C=O); ¹H NMR (400MHz, CDCl₃, δ ppm): 1.78 (s, 6H, CH₃ of piperidine), 3.56 (m, 2H, CH₂ of piperidine), 3.66 (s, 6H, OCH₃), 3.81 (s, 3H, OCH₃), 4.04-4.06 (m, 2H, CH₂ of piperidine), 7.01 (s, 2H, Ar-H), 7.98 (s, 1H, =CH); DART MS (m/z): 362.1 (M⁺); Anal. for C₁₈H₂₂N₂O₄S: C 59.65, H 6.12, N 7.73; Found: C 59.68, H 6.15, N 7.77.

5-(2,4-Dichlorobenzylidene)-2-(4-methylpiperazin-1-yl)-1,3-thiazol-4(5H)-one (3f)
IR (KBr, ν_max cm⁻¹): 3058.4 (Ar-H), 2942 (C-H), 1685 (C=O), 1613 (C=N), 1562 (C=C), 772 (C-Cl); ¹H NMR (400 MHz, CDCl₃, δ ppm): 2.27 (s, 3H, CH₃), 2.44-2.47 (m, 4H, CH₂ of piperazine), 3.26 (t, 2H, J = 5.2 Hz, CH₃ of piperazine), 3.82 (t, 2H, J = 5.2 Hz, CH₂ of piperazine), 7.31-7.35 (dd, 1H, Ar-H, J = 8.4 Hz and 2.4 Hz), 7.50 (d, 1H, Ar-H, J = 2.4 Hz), 7.52 (d, 1H, Ar-H, J = 8.4 Hz), 8.0 (s, 1H, =CH); DART MS (m/z): 356.07 (M⁺); Anal. for C₁₅H₁₅Cl₂N₂O₃S: C 50.57, H 4.24, N 11.79; Found: C 50.60, H 4.26, N 11.82.
IR (KBr, v_max cm⁻¹): 3062 (Ar-H), 2939 (C-H), 1687 (C=O), 1564 (C=C); ¹H NMR (400 MHz, CDCl₃, δ ppm): 2.27 (s, 3H, CH₃), 2.44-2.48 (m, 4H, CH₂ of piperazine), 3.26 (t, 2H, CH₂ of piperazine, J = 5.2 Hz), 3.82 (t, 2H, CH₂ of piperazine, J = 5.2 Hz), 3.94 (s, 6H, OCH₃), 6.93 (d, 1H, J = 8.5 Hz, Ar-H), 7.05 (d, 1H, J = 2.0 Hz, Ar-H), 7.15 (dd, 1H, J = 8.5 Hz, J = 2.0 Hz, Ar-H), 7.98 (s, 1H, =CH); DART MS (m/z) : 377.01 (M⁺); Anal. for C₇H₈N₂O₃S: C 58.77, H 6.09, N 12.09; Found: C 58.79, H 6.12, N 12.12.

RESULTS AND DISCUSSION

The synthetic route for the target compounds is outlined in scheme 1. Target Compounds (3a-h) were prepared by the above method by taking sodium thiocyanate and ethyl chloroacetate as the starting material. The final product was obtained in two steps. In the first step the thioycyanatoacetate formed is treated with secondary amines in alcohol media in the presence of a few drops of glacial acetic acid, to bring about the cyclisation, to form the 2-substituted thiazolone. In the second step equimolar quantities of thiazolone formed in the first step and suitable aromatic aldehyde were refluxed in glacial acetic acid in the presence of anhydrous sodium acetate to obtain the desired compound (3a-h) in good yield. The chemical structures of the synthesized compounds were established by spectroscopic (FT-IR, ¹H NMR, mass), elemental analysis and X-ray crystallographic data. The spectral data are reported in the experimental section. The elemental analysis, yield, molecular weight and melting point data of the newly synthesized compounds are given in Table 1.

The IR spectrum of 3a showed a characteristic C=O absorption peak at 1667 cm⁻¹. Absorption bands at 2958.7 cm⁻¹ and 2846.7 cm⁻¹ were due to C-H stretching vibrations. A band at 1280.1 cm⁻¹ was due to C-O stretching. The 400 MHz ¹H NMR spectra of 3a showed two characteristic triplets at δ 3.53 and δ 3.99 integrating for methylene protons of morpholine with coupling constant J = 5.2 Hz. A characteristic multiplet integrating for four protons of morpholine was observed at δ 3.75 - 3.81. The methylene proton of thiazoline ring appeared as a singlet at δ 3.97. Further, the DART mass spectrum of 3a confirmed the formation of the compound with m/z 186 in conformity with its molecular formula C₇H₈N₂O₃S.

The IR spectrum of 3b showed a strong absorption band at 3059 cm⁻¹ for Ar-H, 2939 cm⁻¹ and 2858 cm⁻¹ for C-H, 1681 cm⁻¹ for C-O, 1552 cm⁻¹ for C=C and 769 cm⁻¹ for C-Cl. The 400 MHz ¹H NMR spectrum of 3b displayed a broad singlet at δ 7.17 for the four protons of morpholine ring thus integrating for four protons. Two multiplets for N-H protons of piperidine

Table 1. Characterization data of compounds 3a-h.

<table>
<thead>
<tr>
<th>Comp</th>
<th>X</th>
<th>R</th>
<th>Mol. formula</th>
<th>Mol. wt</th>
<th>m.p (°C)</th>
<th>Yield</th>
<th>Analysis Found (Calculated) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>O</td>
<td>2,4-Cl₂</td>
<td>C₇H₈N₂Cl₂O₃S</td>
<td>344.22</td>
<td>140-142</td>
<td>90</td>
<td>58.77 (58.81) 6.09(6.11) 12.09 (12.13)</td>
</tr>
<tr>
<td>3b</td>
<td>O</td>
<td>3,4,5-(OCH₃)₂</td>
<td>C₇H₈N₂O₄S</td>
<td>364.41</td>
<td>178-180</td>
<td>67</td>
<td>58.77 (58.81) 6.09(6.11) 12.09 (12.13)</td>
</tr>
<tr>
<td>3c</td>
<td>CH₂</td>
<td>4-OCH₃</td>
<td>C₇H₈N₂O₃S</td>
<td>302.39</td>
<td>192-194</td>
<td>89</td>
<td>58.77 (58.81) 6.09(6.11) 12.09 (12.13)</td>
</tr>
<tr>
<td>3d</td>
<td>CH₂</td>
<td>2,4-Cl₂</td>
<td>C₇H₈Cl₂N₂O₃S</td>
<td>342.32</td>
<td>198-200</td>
<td>86</td>
<td>58.77 (58.81) 6.09(6.11) 12.09 (12.13)</td>
</tr>
<tr>
<td>3e</td>
<td>CH₂</td>
<td>3,4,5-(OCH₃)₂</td>
<td>C₇H₈N₂O₃S</td>
<td>362.44</td>
<td>172-174</td>
<td>64</td>
<td>58.77 (58.81) 6.09(6.11) 12.09 (12.13)</td>
</tr>
<tr>
<td>3f</td>
<td>N-CH₂</td>
<td>2,4-Cl₂</td>
<td>C₇H₈Cl₂N₂O₃S</td>
<td>357.27</td>
<td>174-176</td>
<td>80</td>
<td>58.77 (58.81) 6.09(6.11) 12.09 (12.13)</td>
</tr>
<tr>
<td>3g</td>
<td>N-CH₂</td>
<td>3,4,5-(OCH₃)₂</td>
<td>C₇H₈N₂O₃S</td>
<td>347.43</td>
<td>196-198</td>
<td>69</td>
<td>58.77 (58.81) 6.09(6.11) 12.09 (12.13)</td>
</tr>
<tr>
<td>3h</td>
<td>N-CH₂</td>
<td>3,4,5-(OCH₃)₂</td>
<td>C₇H₈N₂O₃S</td>
<td>377.45</td>
<td>190-192</td>
<td>61</td>
<td>58.77 (58.81) 6.09(6.11) 12.09 (12.13)</td>
</tr>
</tbody>
</table>
were observed at $\delta$ 3.56-3.58 and $\delta$ 4.02-4.05 each integrating for two protons. One of the aromatic protons resonated as a doublet of doublet at $\delta$ 7.29-7.32 with coupling constant $J = 8.4$ Hz and $J = 2.4$ Hz. One more aromatic proton between two Cl atoms resonated as a doublet at $\delta$ 7.48 with coupling constant $J = 2.4$ Hz. The third aromatic proton was observed at $\delta$ 7.52 as a doublet with coupling constant $J = 8.4$ Hz. A singlet observed at $\delta$ 8.039 was due to benzylidene proton. Further, DART mass spectrum of 3d showed the M+1 molecular ion peak at m/z 341.05 in conformity with its molecular formula C$_{15}$H$_{12}$N$_2$Cl$_2$OS.

Crystal Structure Analysis
The structure of (5E)-(4-Methoxybenzylidene)-2-(piperidin-1-yl)-1,3-thiazol-4(5H)-one (3e) and (5E)-5-(2,4-Dichlorobenzylidene)-2-(piperidin-1-yl)-1,3-thiazol-4(5H)-one (3d), is confirmed by single crystal X-ray study [14,15,16].

Pharmacological activity
Antibacterial activity
All the newly synthesized compounds were screened for their antibacterial activity against four bacterial strains, viz., Staphylococcus aureus (ATTC-25923), Escherichia coli (ATTC-25922), Pseudomonas aeruginosa (ATTC-27853) and Klebsiella pneumoniae (recultured) species by disc diffusion method [17]. The discs measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 discs were dispensed to each crew of plates. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1h. The incubation was carried out at 37 °C for 24 h. Ampicillin was used as a standard drug. Amphotericin B has 16-22 mm inhibition length. The minimum inhibitory concentrations (MIC) of each compound were determined.

The antibacterial study revealed that the compound 3a, 3b, 3d and 3f containing dichloro or trimethoxy substituted phenyl group showed excellent activity towards all the four bacterial strains. The compounds 3c, 3e, 3h showed moderate activity towards all the four bacterial strains. The significant activity of these compounds may be attributed to the presence of pharmacologically active groups 2, 4-Cl, capped bottles and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using DMF. 1mL containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Amphotericin B has 16-22 mm inhibition length. The minimum inhibitory concentrations (MIC) of each compound were determined.

The MIC values were evaluated at a concentration range 1.56 – 25 µg mL$^{-1}$. The MIC values were evaluated at a concentration range 1.56 – 25 µg mL$^{-1}$.

Antifungal activity
The compounds screened for antibacterial activity were also tested for their antifungal activity against Penicillium marneffei (recultured), Trichophyton mentagrophytes (recultured), Aspergillus flavus (NCIM No.524) and Aspergillus fumigates (NCIM No.902) by serial plate dilution method [18]. Sabourands agar media was prepared by dissolving peptone (1g), D-glucose (4g) and agar (2g) in distilled water (100ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty ml of agar media was poured in to each petri dishes. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1h. Using an agar punch wells were made in to each well labeled. A control was also prepared in triplicate and maintained at 37 °C for 3-4 days. Activity of each compound was compared with itraconazole as standard. Itraconozole has a MIC value of 0.03-16 micro g mL$^{-1}$. The minimum inhibitory concentrations of each compound were determined.
The study revealed that the compound 3a, 3d and 3f showed very good activity against all the four fungal strains. The structure of these compounds contains biologically active 2, 4-Cl₂ attached to the phenyl ring. The compound 3c, 3e, 3g showed moderate activity against all the four fungal strains. The results are presented in Table 3.

Table 3. Antifungal activity of the compounds 3a-h

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC (in µg mL⁻¹) and zone of inhibition (mm) in parentheses</th>
<th>P. marneffei (recultured)</th>
<th>T. mentagrophytes</th>
<th>A. flavus (NCIM No.524)</th>
<th>A. fumigates (NCIM No.902)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>6.25 (16-20) 6.25 (16-20) 6.25 (16-20) 6.25 (16-20)</td>
<td>6.25 (16-20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>25 (&lt; 10) 25 (&lt; 10) 25 (&lt; 10) 25 (&lt; 10)</td>
<td>25 (&lt; 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>12.5 (11-15) 12.5 (11-15) 12.5 (11-15) 12.5 (11-15)</td>
<td>25 (&lt; 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3d</td>
<td>6.25 (16-20) 6.25 (16-20) 6.25 (16-20) 6.25 (16-20)</td>
<td>6.25 (16-20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3e</td>
<td>12.5 (11-15) 12.5 (11-15) 12.5 (11-15) 12.5 (11-15)</td>
<td>6.25 (16-20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3f</td>
<td>6.25 (16-20) 6.25 (16-20) 12.5 (11-15) 6.25 (16-20)</td>
<td>6.25 (16-20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3g</td>
<td>12.5 (11-15) 12.5 (11-15) 12.5 (11-15) 12.5 (11-15)</td>
<td>12.5 (11-15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3h</td>
<td>25 (&lt; 10) 25 (&lt; 10) 25 (&lt; 10) 25 (&lt; 10)</td>
<td>25 (&lt; 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itraconozole</td>
<td>6.25 (25-33) 6.25 (23-27) 1.56 (22-30) 6.25 (30-40)</td>
<td>6.25 (25-33)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The MIC values were evaluated at a concentration range 1.56 – 25 g mL⁻¹.*

CONCLUSION

This study reports the successful synthesis of the title compounds. The crystal structure of the compound (3c and 3d) revealed that the piperidine ring adopts a chair conformation. An intramolecular C—H — S hydrogen bond stabilizes the molecular structure and generates an S(6) ring motif. In the crystal, molecules are linked into a tape along the c axis by intermolecular C—H — O hydrogen bonds. The antimicrobial activity study showed that 5-benzylidene-2-substituted thiazolones containing 2,4-dichlorophenyl ring and trimethoxy phenyl rings showed very good antibacterial activity and compounds having 2,4-dichlorophenyl ring showed very good antifungal activity against all the tested microbial species.

Acknowledgments

The authors are thankful to UGC for the financial assistance and to the Head, SAIF, CDRI, Lucknow, the Chairman, Indian institute of science, Bangalore and the Head, USIC, Mangalore University for providing mass, ¹H NMR and IR spectral data.

REFERENCES

[16] CCDC 841125 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/ data_request/cif.