Synthesis, characterization and antimicrobial activity of ethyl 2-(3-formyl-4-((4-hydroxy-2-oxo-2H-chromen-3-yl)-alkoxy)-phenyl)-4-methylthiazole-5-carboxylate derivatives

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During the present study, a number of substituted thiazole derivatives have been synthesized by the reaction of 4-hydroxy-benzothioamide and ethyl-2-chloro acetooacetate to give ethyl 2-(4-hydroxyphenyl)-4-methylthiazole-5-carboxylate. The latter reacts with PPA, HMTA and acetic acid to yield ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylate. These compounds further react with di-bromo alkane and 4-hydroxy coumarin to give the final thiazole derivatives. These are characterized by elemental analysis, IR and \(^{1}H\) NMR spectra, and have been screened for their antimicrobial activity and found to have significant effect against the tested microorganisms. Some of the synthesized thiazole derivatives are found to exhibit promising activity.

**Keywords:** Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylate, ethyl 2-(4-hydroxyphenyl)-4-methylthiazole-5-carboxylate, coumarin, antimicrobial activity

In the living organisms, molecules that possess sulphur atom are very important. Thiazole is one of the important class among them, which is a heterocyclic compound having one sulphur atom. Thiazole is also one of the important members of five membered aromatic heterocyclic ring compounds\(^1\)\(^2\),\(^3\)\(^4\). Considering the importance of thiazole nucleus, many scientists have focused their research on this nucleus. Commercially important thiazoIolium salts large numbers of dyes are derived. Wide varieties of applications of 2-amino thiazoles include dyes\(^5\)\(^6\), fungicides\(^7\)\(^8\), accelerators in rubber vulcanization\(^9\)\(^10\), and antioxidants\(^11\)\(^12\). From thiazoIolium salts large numbers of dyes are derived. Wide varieties of applications of 2-amino thiazoles have been used in the fields of pharmaceuticals, agriculture, photography and related activities\(^2\)\(^3\)\(^4\). Thiazole derivative are found to possess antibacterial\(^11\)\(^14\)\(^17\), fungicidal\(^15\)\(^18\)\(^19\), anti-inflammatory\(^20\)\(^21\), antihelmintic\(^22\), antitubercular\(^23\)\(^24\), anti-HIV\(^25\)\(^26\), herbicidal\(^27\)\(^28\) and antiviral\(^29\) activities.

**Results and Discussion**

The thiazole derivative was prepared from 4-hydroxy-benzothioamide which was reacted with ethyl-2-chloro acetoacetate to form ethyl 2-(4-hydroxyphenyl)-4-methylthiazole-5-carboxylate \( \text{I} \). This was reacted with PPA and HMTA to form ethyl 2-(3-formyl-4-hydroxy phenyl)-4-methylthiazole-5-carboxylate \( \text{II} \) (Scheme I). The ethyl 2-(3-formyl-4-hydroxy phenyl)-4-methylthiazole-5-carboxylate \( \text{II} \) was reacted with di-bromo alkane in the presence of base to form ethyl 2-(4-(2-bromo-alkoxy)-3-formylphenyl)-4-methylthiazole-5-carboxylates \( \text{III-VI} \). The crystallization of ethyl 2-(4-(2-bromo-alkoxy)-3-formylphenyl)-4-methylthiazole-5-carboxylates \( \text{III-VI} \) was carried out in presence of IPA at 45-50°C to obtain pure compound. The ethyl 2-(4-(2-bromo-alkoxy)-3-formylphenyl)-4-methylthiazole-5-carboxylates \( \text{III-VI} \) was carried out from IPA at 45-50°C to obtain pure compound. The IR spectra of compounds \( \text{III-VI} \) showed a broad band in the region 2872.10-2980.12 cm\(^{-1}\) due to the C-H groups. The C-H bending vibrations are observed as a sharp medium to strong band at 1327.00 cm\(^{-1}\) in all compounds. The C-H linkage of the six-membered ring caused a weak and sharp absorption band at 700-750 cm\(^{-1}\) in all the compounds. The C=O group is observed as a strong medium to strong band at 1650-1710 cm\(^{-1}\) in these compounds. Further, \(^{1}H\) NMR spectra exhibited multiplets in the region \( \delta \) 7.04-8.21 for 7 aromatic protons (4 aromatic protons of coumarin and 3 aromatic protons of benzene) \( \text{IV} \). Ten protons present in –CH\(_2\) of compound \( \text{IV} \) are found to resonate as multiplets at \( \delta \) 1.29-4.38 (alkane). One proton present in –OH of compound \( \text{IV} \) is found to resonate as a singlet at \( \delta \) 5.67. Six protons present in –CH\(_3\) of compound \( \text{IV} \) are found to resonate as triplets at \( \delta \) 1.20-1.21 (thiazole) and singlet at \( \delta \) 2.74 (thiazole). One proton of the –CHO group is observed to resonate as a singlet at \( \delta \) 10.50 as a singlet compound \( \text{V} \). The results given in Table I show the antibacterial activity as compared to standard Warfarin at MIC 300 µg against \textit{Pseudomonas aeruginosa}. The activity of all compounds was found higher but maximum was...
shown by compounds 4 and 6, against *Escherichia coli*, at 300 µg. The activity of all compounds was higher but maximum activity was exhibited by compounds 6 and 10. Whereas, against *Bacillus subtilis* at 300 µg, activity of all compounds was higher but maximum activity was for compound 6 and 8 against *Staphylococcus aureus* at 300 µg. All compounds exhibited higher activity but maximum activity was for compound 6 and 10. The results of antifungal activity are shown in Table I along with comparison of standard at MIC 10 µg against *Candida albicans* at 40 µg of Flucanazole. Activity of all compounds was found less. From the above results, it may be concluded that antibacterial activity of all reported compounds was very good but antifungal activity was not so good compared to standard.

**Experimental Section**

Melting points were determined using Stuart SMP10 MP apparatus and are uncorrected. The homogeneity
was checked by TLC. The IR spectra were obtained with a 8400 FT-IR-435 spectrometer in KBr pellets. $^1$H NMR spectra ($\delta$, ppm) were recorded in DMSO-$d_6$ solutions on a Bruker-Avance 400 MHz spectrometer using TMS as internal reference. Mass spectra was recorded on Waters Micro mass Q-Tof Micro having range of 4000 amu in quadrupole and 20000 amu in ToF. Elemental analysis was performed on ECS 4010 Elemental Combustion System.

**Antimicrobials activity (MIC)**

The anti-bacterial activity of the synthesized compounds was tested against two gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using nutrient agar medium. The antifungal activity of the compounds was tested against *Candida albicans* using sabouraud dextrose agar medium.$^{30}$ The sterilized medium was inoculated (1 mL/100 mL of medium) with the suspension ($10^5$cfu mL$^{-1}$) of the micro-organism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds (100 $\mu$g/disc) was placed on the solidified medium. The plates were pre-incubated for 1 hour at RT and incubated at 37°C for 24 and 48 hrs for antibacterial and anti-fungal activities, respectively. Niconmaculam (100 $\mu$g/disc) and Flucanazole (100 $\mu$g/disc)
were used as standard for anti-bacterial and anti-fungal activities respectively. The results are presented in Table I.

**Preparation of ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylate, 2**

4-Hydroxybenzo-thioamide (1.0 mmol), IPA (10 mL) and ethyl-2-chloro acetate (1.1 mmol) was heated to 75°C. After completion of reaction, the mixture was poured into water, filtered and washed with water to neutral pH and recrystallized from IPA to give the compound 1. The ethyl 2-(4-hydroxyphenyl)-4-methyl-thiazole-5-carboxylate 1 (1.0 mmol) after adding PPA (10.0 mmol) was heated to 75°C and then HMTA (2.6 mmol) added at 75°C. After the completion of reaction, the reaction mass was poured onto aqueous acetic acid solution and stirred for a few minutes at RT and the obtained product was filtered and washed with water at RT. The final intermediate ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylate 2 was purified by recrystallization from IPA.

1: Off white crystals, m.p. 184-87°C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 9.88 (s, 1H, Ar-OH), 6.84-7.79 (m, 4H, Ar-H), 4.27-4.32 (q, 2H, CH\(_2\)), 2.68 (s, 3H, CH\(_3\)), 1.34-1.37 (t, 3H, CH\(_3\)).

2: Light yellow crystals, m.p. 96-100°C. IR (KBr): 2981.90-3053.42 (OH), 1710.92 (C=O), 1440.87-1415.80 (CH\(_2\)), 806.27-833.28 cm\(^{-1}\) substituted benzene; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 11.29(s, 1H, Ar-CHO), 9.98 (s, 1H, Ar-OH), 7.06-8.24 (m, 3H, Ar-H), 4.33-4.38 (q, 2H, CH\(_2\)), 2.77(s, 3H, CH\(_3\)), 1.37-1.41 (t, 3H, CH\(_3\)).

**Preparation of ethyl 2-(3-formyl-4-(2-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethoxy) phenyl)-4-methylthiazole-5-carboxylate, 6**

Ethyl 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylate 2 (1.0 mmol), dimethyl formamide (4 mL) as a solvent media and potassium carbonate (1.05 mmol) was taken in RBF and heated to 75°C for 15 to 20 min.

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**Table I — Antimicrobial activity data of synthesized compounds**

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<th>Compounds and standard</th>
<th>E. coli 200µg</th>
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<th>P. aeruginosa 200µg</th>
<th>300µg</th>
<th>B. subtilis 200µg</th>
<th>300µg</th>
<th>S. aureus 200µg</th>
<th>300µg</th>
<th>C. albicans 200µg</th>
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and then dibromo ethane (2.0 mmol) solution in dimethyl formamide (1 mL) was added and reaction continued for 4-5 hr. The completion of reaction was checked by TLC. Heating was stopped after the completion of reaction and reaction mixture was gradually cooled to RT. It was filtered and washed with dimethyl formamide (1 mL). The reaction mass was collected and poured onto water (20 mL), stirred for 30 min, filtered and washed with water. The product ethyl 2-(4-(2-bromoethoxy)-3-formylphenyl)-4-methylthiazole-5-carboxylate 5 was purified by recrystallization from IPA at RT.

4-Hydroxy coumarin (1.0 mmol) in dimethyl formamide (3 mL) as a solvent media and potassium carbonate (2.05 mmol) was heated at 85°C for 15 to 20 min. Then, solution of ethyl 2-(4-(2-bromoethoxy)-3-formylphenyl)-4-methylthiazole-5-carboxylate (1.0 mmol) in DMF (2 mL) was added into the reaction mixture slowly at 85°C and the mass heated for further 6 hr. The completion of reaction was checked by TLC and heating was stopped and reaction mixture was gradually cooled to RT. The reaction mass was pumped into water (20 mL) stirred for 30 min, filtered and washed with water. The product ethyl 2-(3-formyl-4-(2-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethoxy) phenyl)-4-methylthiazole-5-carboxylate 6 was purified by recrystallization from IPA.

5: Off white crystals, m.p. 143-48°C. IR (KBr): 2980.12 (CH), 1712.85 (C=O), 1604.83 (C=C), 1431.23 (CH₃), 578.66 cm⁻¹ (C-Br); H NMR (400 MHz, DMSO-d₆): δ₁H 10.53 (s, 1H, Ar-CHO), 7.03-8.36 (m, 3H, Ar-H), 3.73-4.50 (m, 6H, CH₂), 2.78 (s, 3H, CH₃), 1.37-1.40 (t, 3H, CH₃).

6: Off white crystals, m.p. 123-26°C. IR (KBr): 2872.10-2980.12 (OH), 1707.06 (C=O), 1392.65-1431.23 (CH₃), 763.84 cm⁻¹ (substituted benzene); H NMR (400 MHz, CDCl₃): δ₀H 10.50 (s, 1H, Ar-CHO), 7.04-8.21 (m, 7H, Ar-H), 5.67 (s, 1H, OH), 2.32-4.38 (m, 6H, CH₂), 2.73 (s, 3H, CH₃), 1.34 (t, 3H, CH₃). Anal. Calcd for C_{25}H_{21}NO_{3}S (479.1): C, 62.62; H, 4.41; N, 2.92. Found: C, 62.68; H, 4.44; N, 3.03%. MS: m/z 481.4 (M⁺).

NMR spectra of remaining intermediates and compounds are as follows:

3: Off white crystals, m.p.154-59°C. IR (KBr): 2985.12 (CH), 1710.85 (C=O), 1600.63 (C=C), 1435.20(CH₃), 575.65 cm⁻¹(C-Br); H NMR (400 MHz, DMSO-d₆): δ₁H 10.49 (s, 1H, Ar-CHO), 7.05-8.35 (m, 3H, Ar-H), 4.37-4.87 (q, 4H, CH₂), 2.76 (s, 3H, CH₃), 1.34-1.37 (t, 3H, CH₃).

4: Off white crystals, m.p. 119-23°C. IR (KBr): 2949.26-3074.63 (CH), 1708.99 (C=O), 1448.58-1431.23 (CH₃), 815.92 cm⁻¹(substituted benzene); H NMR (400 MHz, CDCl₃): δ₀H 10.50 (s, 1H, Ar-CHO), 7.04-8.21 (m, 7H, Ar-H), 5.61 (s, 1H, OH), 4.23-4.32 (q, 4H, CH₂), 2.72 (s, 3H, CH₃), 1.23-1.24 (t, 3H, CH₃). Anal. Calcd for C_{25}H_{21}NO_{3}S (465.1): C, 61.93; H, 4.11; N, 3.01%. Found: C, 61.85; H, 4.14; N, 3.15%. MS: m/z 466.1 (M⁺).

7: Off white crystals, m.p. 132-36°C. IR (KBr): 2976.26 (CH), 1710.92 (C=O), 1604.83 (C=C), 1435.23 (CH₃), 586.66 cm⁻¹(C-Br); H NMR (400 MHz, DMSO-d₆): δ₀H 10.49 (s, 1H, Ar-CHO), 7.05-8.35 (m, 3H, Ar-H), 2.10-4.37 (m, 8H, CH₂), 2.76 (s, 3H, CH₃), 1.32-1.38 (t, 3H, CH₃).

8: Off white crystals, m.p. 115-18°C. IR (KBr): 2949.26-3074.63 (CH), 1708.99 (C=O), 1448.58-1431.23 (CH₃), 815.92 cm⁻¹(substituted benzene); H NMR (400 MHz, CDCl₃): δ₀H 10.50 (s, 1H, Ar-CHO), 7.04-8.21 (m, 7H, Ar-H), 5.65 (s, 1H, OH), 1.89-4.28 (m, 8H, CH₂), 2.74 (s, 3H, CH₃), 1.20-1.21 (t, 3H, CH₃). Anal. Calcd for C_{25}H_{21}NO_{3}S (493.5): C, 63.27; H, 4.70; N, 2.84%. Found: C, 63.20; H, 4.65; N, 2.90%. MS: m/z 494.2 (M⁺).

9: Off white crystals, m.p. 122-25°C. IR (KBr): 2976.26 (CH), 1710.92 (C=O), 1606.76 (C=C), 1431.23 (CH₃), 520.80 cm⁻¹(C-Br); H NMR (400 MHz, DMSO-d₆): δ₀H 10.49 (s, 1H, Ar-CHO), 7.05-8.35 (m, 3H, Ar-H), 2.04-4.37 (m, 10H, CH₂), 2.76 (s, 3H, CH₃), 1.40 (t, 3H, CH₃).

10: Off white crystals, m.p. 106-110°C. IR (KBr): 2949.26-3074.63 (CH), 1708.99 (C=O), 1448.58-1431.23 (CH₃), 815.92 cm⁻¹ (substituted benzene); H NMR (400 MHz, CDCl₃): δ₀H 10.50 (s, 1H, Ar-CHO), 7.04-8.21 (m, 7H, Ar-H), 5.67 (s, 1H, OH), 1.29-4.38 (m, 10H, CH₂), 2.74 (s, 3H, CH₃), 1.20-1.21 (t, 3H, CH₃). Anal. Calcd for C_{25}H_{21}NO_{3}S (507.1): C, 63.89; H, 4.96; N, 2.76. Found: C, 63.8; H, 5.06; N, 2.79%. MS: m/z 508.1 (M⁺).

11: Off white crystals, m.p. 117-20°C. IR (KBr): 2980.6 (CH), 1716.90 (C=O), 1609.76 (C=C), 1435.33 (CH₃), 560.80 cm⁻¹(C-Br); H NMR (400 MHz, DMSO-d₆): δ₀H 10.49 (s, 1H, Ar-CHO), 7.05-8.35 (m, 3H, Ar-H), 2.01-4.37 (m, 12H, CH₂), 2.76 (s, 3H, CH₃), 1.36-1.40 (t, 3H, CH₃).

12: Off white crystals, m.p. 103-105°C. IR (KBr): 2949.26-3074.63 (CH), 1708.99 (C=O), 1448.58-1431.23 (CH₃), 815.92 cm⁻¹(substituted benzene); H NMR (400 MHz, CDCl₃): δ₀H 10.50 (s, 1H, Ar-CHO), 7.04-8.21 (m, 7H, Ar-H), 5.68 (s, 1H, OH), 1.29-4.38 (m, 12H, CH₂), 2.74 (s, 3H, CH₃), 1.20-1.21 (t, 3H, CH₃). Anal.
Calcd for C$_{29}$H$_{27}$NO$_3$S (521.5): C, 64.48; H, 5.22; N, 2.69. Found: C, 64.41; H, 5.24; N, 2.61%. MS: m/z 522.4 (M+).

**Conclusion**

The results of synthesized thiazole derivatives which were screened for antibacterial activity exhibited very good antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. All thiazole derivatives showed less antifungal activity against *Candida albicans*. The final compounds 4 and 6 exhibited good activity against *Pseudomonas aeruginosa*. The compounds 6 and 10 exhibited good activity against *Escherichia coli*. The compounds 6 and 8 exhibited good activity against *Bacillus subtilis* and 6 and 10 showed good activity against *Staphylococcus aureus*. The activity data are presented in Table I.

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**References**