Synthesis and antimicrobial evaluation of novel 3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one derivatives

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ABSTRACT
A novel series of potentially biologically active 3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one derivatives (5a-5k) have been synthesized by the condensation-cyclization reaction of 4,6-diphenyl-6H-1,3-thiazin-2-amine, aromatic aldehyde and thioglycolic acid in polypropylene glycol at 110°C temperature. The structure of the newly synthesized compounds has been established on the basis of their spectral data and elemental analysis. The antimicrobial activity of the synthesized compounds were tested in vitro against the sensitive organisms Staphylococcus aureus, Bacillus subtilis as a Gram positive bacteria and Escherichia coli, Pseudomonas aeruginosa as a Gram negative bacteria and two pathogenic fungal strains Candida albicans, Aspergillus niger by using the disc diffusion method. The detailed synthesis, spectroscopic data, and antimicrobial screening of synthesized compounds were reported.

INTRODUCTION
Nitrogen-containing heterocycles are undoubtedly one of the most important targets in organic chemistry. They are widely distributed in natural products and in pharmaceutical agents, and numerous studies for their chemistry and synthesis have been reported (Attanasi et al., 2008) and Sulfur-containing heterocyclic ring systems, such as thiazine derivatives have shown a great potential in pharmaceutical research (Snick et al., 2013). Thiazine derivatives, a versatile pharmacophore, has been the subject of great interest due to its wide range of biological activities such as antimicrobial and anti-diabetic (Faidallah et al., 2011; Adly et al., 2012), anti-histaminic (Arya et al., 2012), antibacterial and antifungal (Tandon et al., 2006; Zia-ur Rehman et al., 2009; Ganorka et al., 2013), phagocytic activity of human neutrophils (Barros-Garcia et al., 2011), antagonistic (Galanski et al., 2006), potassium channel-opening agents (Erker et al., 2000), antioxidant (Smith et al., 1951) analgesic and anti-inflammatory, (Chia et al., 2008; Tozkoparan et al., 2002) anti-tuberculosis (Koketsua et al., 2002), antitumor (Wei et al., 2012), antihelmintic and insecticidal (Smith et al., 1942), nitric oxide synthase inhibitor (Tung-Mei et al., 2005), Smooth Muscle Relaxants (Schreder et al., 2000), antimycobacterial (Indumathi et al., 2009), urokinase inhibitors (Tanaka et al., 1998). The derivatives of thiazine act as myocardial calcium channel modulators (Budriesi et al., 2002).

Among pharmacologically important heterocyclic compounds, thiazolidinone derivatives have been known to possess a wide range of biological activities such as antimicrobial (Bhaskar et al., 2008; Sah et al., 2012; Ramachandran et al., 2011; Bhatt et al., 2012) anti-HIV(Rawal et al., 2007), antifungal, antibacterial (Nagaraj et al., 2012; Patel et al., 2011; Omar et al., 2010; Vicini et al., 2006), antihyperglycemic (Datar et al., 2012), anti-inflammatory (Rakha et al., 2011) and antitubercular (Samadiya et al., 2013). Owing to the biological significance of these two classes of compounds and in continuation of our ongoing study on antimicrobial agents (Prasad et al., 2011). Hence, considerable efforts have been carried out for the synthesis of a combined molecular framework that involves these two different chromophores.

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Thus a novel series of potentially biologically active 3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one derivatives has been synthesized by the condensation and cyclization reaction of 4,6-diphenyl-6H-1,3-thiazin-2-amine, aromatic aldehyde and thioglycolic acid in polypropylene glycol and evaluated their antimicrobial activity.

**EXPERIMENTAL SECTION**

The melting points were recorded on electrothermal apparatus and are uncorrected. The purity of the compounds was checked by TLC on pre-coated SiO₂ gel (HF254, 200 mesh) aluminum plates (E Merck) using hexane and ethyl acetate visualized in iodine chamber. IR spectra were recorded in KBr on a perkin-Elmer model-983. 1HNMR spectrum recorded on Varian Mercury 300MHz instrument using CDCl₃, DMSO-d₆ as solvent (chemical shift in δ ppm), using TMS as internal standard. Elemental analysis was performed on a Heraus CHN analyzer and was within the ±0.5% of the theoretical values.

**Preparation of (2E)-1,3-diphenylprop-2-en-1-one derivatives (3a-3b)**

Equimolar quantities of benzaldehyde / anisaldehyde (0.01mol) and acetophenone (0.01 mol) were dissolved in minimum amount of ethanol. Sodium hydroxide solution (0.02 mol) was added and the mixture stirred for 2hr until the entire mixture becomes very cloudy. Then the reaction mixture was poured slowly into ice water with constant stirring and kept in refrigerator for 24 hours. The precipitates obtained was filtered, washed with cold water and recrystallized from ethanol to give compounds 3a-3b. The completion of the reaction was monitored by TLC.

**Preparation of 4,6-diphenyl-6H-1,3-thiazin-2-amine derivatives (4a-4b)**

A mixture of chalcone (3a -3b) (0.02mol), thiourea (0.02mol) were dissolved in ethanol or NaOH (25ml) was stirred about 2-3 hours with a magnetic stirrer. This was then poured into 400ml of cold water with constant stirring for an hour & then kept in refrigerator for 24 hours. The separated solid was filtered, washed and recrystallized from ethanol. The completion of the reaction was monitored by TLC.

**Preparation of 3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one (5a-5k)**

A mixture of 4,6-diphenyl-6H-1,3-thiazin-2-amine (0.01 mol), aldehyde (0.02 mol), and thioglycolic acid (0.03mol) in PPG ~ 2000 (2 ml) was heated at 110°C for 4-11 h. After completion of the reaction as indicated by TLC, the reaction mixture was diluted with hexane, and the precipitated product was filtered. In the case of oily products, the hexane layer was decanted, and sticky material was dissolved in ethyl acetate (20 ml). The solution was washed well with saturated NaHCO₃ solution (15 ml) 3 times followed by water (15 ml) 3 times. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the crude compound. The product was purified by column chromatography on silica gel using 2-4 % MeOH in benzene as eluent. In addition, the hexane layer was evaporated under reduced pressure to recover PPG which can be recycled.

**Spectral characterization and elemental analysis of synthesized compounds (5a-5k)**

3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(4-methoxyphenyl) thiazolidin-4-one (5a)

IR.(KBr,cm⁻¹): 3070(Ar-H), 1695 (>C=O), 1627, 1476 (>C=C), 1250 (C-N), 1210 (C-O); 1HNMR (300 MHz, CDCl₃, DMSO-d₆ ppm): 5.41 (d,1H,CH), 6.37(d,1H,CH), 6.62(d,1H,Ar-H), 7.64(d,1H,Ar-H), 8.04 (d,1H,Ar-H) 7.33-7.55 (m,3H,Ar-H), 5.83(s,1H,CH), 3.85(s,2H,CH₂), 3.81(s,3H,OCH₃); Anal. C₉H₇N₂O₆S₂; Calculated: C 68.09; H,4.84; N,6.11; O,6.92; S,13.98. Found: C,67.88; H,4.63; N,5.91; O,6.79; S,13.68.

2-(4-chlorophenyl)-3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)thiazolidin-4-one (5b)

IR.(KBr,cm⁻¹): 3070(Ar-H), 1695 (>C=O), 1627, 1476 (>C=C), 1250(C-N), 765(C-Cl); 1HNMR (300 MHz,CDCl₃, DMSO-d₆ ppm): 5.40(d,1H,CH), 6.39(d,1H,CH), 7.23(d,1H,Ar-H), 6.95 (d,1H,Ar-H), 8.04(d,1H,Ar-H) 7.33-7.55 (m,3H,Ar-H), 5.81(s,1H,CH), 3.85(s,2H,CH₂); Anal. C₉H₇ClN₂O₆S₂; Calculated: C,64.85;H,4.14; Cl,7.66; N,6.05; O,3.46; S,13.85. Found: C,64.63;H,3.97; Cl,7.43; N,5.91; O,3.26; S,13.61.

2-(2-chlorophenyl)-3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-1,3-thiazolidin-4-one (5c)

IR.(KBr,cm⁻¹): 3070(Ar-H), 1698 (>C=O), 1627, 1476 (>C=C), 1250(C-N), 773(C-Cl); 1HNMR (300 MHz,CDCl₃, DMSO-d₆ ppm): 5.39 (d,1H,CH), 6.36(d,1H,CH), 7.39(d,1H,Ar-H), 6.91 (m,3H,Ar-H), 7.04(d,1H,Ar-H) 7.33-7.55 (m,3H,Ar-H), 5.83(s,1H,CH), 3.85(s,2H,CH₂); Anal. C₉H₇ClN₂O₆S₂; Calculated: C,64.85;H,4.14; Cl,7.66; N,6.05; O,3.46; S,13.85. Found: C,64.61;H,3.92; Cl,7.46; N,5.91; O,3.26; S,13.59.

3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(4-hydroxyphenyl) thiazolidin-4-one (5d)

IR.(KBr,cm⁻¹): 3071(Ar-H), 1696 (>C=O), 1623, 1475 (>C=C), 1250(C-N),1225(C-O); 1HNMR (300 MHz, CDCl₃, DMSO-d₆ ppm): 5.40(d,1H,CH), 6.39(d,1H,CH), 6.55(d,1H,Ar-H), 7.47(d,1H,Ar-H), 8.04(d,1H,Ar-H) 7.33-7.55 (m,3H,Ar-H), 5.81(s,1H,CH), 3.83(s,2H,CH₂); Anal. C₉H₇NO₃S₂; Calculated: C,67.54; H,4.53; N,6.30; O,7.20; S,14.43. Found: C,67.24; H,4.21; N,6.15; O,7.05; S,14.23.

3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-phenylthiazolidin-4-one (5e)

IR.(KBr,cm⁻¹): 3067(Ar-H), 1685 (>C=O), 1615,1475 (>C=C), 1249(C-N); 1HNMR (300 MHz,CDCl₃, DMSO-d₆ ppm): 5.40(d,1H,CH), 6.39(d,1H,CH),7.33-7.49(m,10H,Ar-H), 8.04 (d,1H,Ar-H) 7.33-7.55(m,3H,Ar-H), 5.83(s,1H,CH), 3.85
(s,2H,CH); Anal.C_{3}H_{5}N_{2}O_{2}S_{2}; Calculated:C,70.06; H, 4.70; N,6.54; O,3.73; S,14.96. Found: C,69.90; H,4.47; N,6.23; O,3.37; S,14.57.

3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(2-nitrophenyl)thiazolidin-4-one(5f)
IR(KBr,cm\(^{-1}\)):3071(Ar-C-H), 1694(C=O), 1623,1475 (C=C<), 1250(C-N),1547,1353(N=O); 1HNM R(300MHZ,CDCl\(_{3}\), DMSO-\(d_{6}\),ppm): 5.41(d,1H,CH), 6.38(d,1H,CH), 7.65(d,1H,Ar-H), 7.37(m,3H,Ar-H), 8.04(d,1H,Ar-H),7.55(m,3H,Ar-H), 5.84(s,1H,CH),3.87(s,2H,CH\(_{2}\)); Anal.C_{19}H_{16}N_{2}O_{2}S \_2; Calculated: C,63.41; H,4.04; O,9.96; S,13.34.

3- (6- (4-methoxyphenyl) -4-phenyl-6H-1,3-thiazin-2-yl)-2-phenylthiazolidin-4-one(5g)
IR(KBr,cm\(^{-1}\)):3071(Ar-C-H), 1691(C=O), 1622, 1475(C=C<), 1250(C-N), 1215(C-O), 1547,1353(N=O); 1HNM R(300MHZ, CDCl\(_{3}\), DMSO-\(d_{6}\),ppm): 5.41(d,1H,CH), 6.37(d,1H,CH), 7.33-7.49 (m,5H,Ar-H), 8.04(d,1H, Ar-H),6.39(d,1H,Ar-H), 6.97(d,1H,Ar-H),7.33-7.55 (m,3H,Ar-H), 5.82(s,1H,CH),3.85(s,2H,CH\(_{2}\) ), 3.81 (s,3H,CH\(_{3}\)); Anal. C_{29}H_{24}N_{2}O_{2}S\_2; Calculated: C,68.09; H,4.84; N,6.11; O,6.98 S,13.98. Found: C,67.89; H,4.53; N,5.90; O,6.55 S,13.59.

2- (4-methoxyphenyl)-3-(6- (4-methoxyphenyl)-4-phenyl-6H-1,3-thiazin-2-yl)thiazolidin-4-one(5h)
IR(KBr,cm\(^{-1}\)):3075(Ar-H), 1696(C=O), 1631, 1476 (C=C<), 1250(C-N), 1219,1210(C-O); 1HNM R(300MHZ, CDCl\(_{3}\), DMSO-\(d_{6}\),ppm): 5.41(d,1H,CH), 6.39(d,1H,CH), 7.43(d,1H,Ar-H),  8.04(d,1H, Ar-H), 6.39(d,1H, Ar-H), 6.97(d,1H,Ar-H), 7.33-7.55 (m,3H,Ar-H), 5.82(s,1H,CH), 3.85(s,2H,CH\(_{2}\) ), 3.81 (s,3H,CH\(_{3}\)); Anal. C_{29}H_{24}N_{2}O_{2}S\_2; Calculated: C,66.37; H,4.95; N,5.73; O,9.82; S,13.12. Found: C,66.14; H,4.62; N,5.47;O,9.61;S,12.91.

2- (4-chlorophenyl)-3-(6- (4-methoxyphenyl)-4-phenyl-6H-1,3-thiazin-2-yl)thiazolidin-4-one(5i)
IR(KBr,cm\(^{-1}\)):3081(Ar-H), 1698 (C=O), 1635, 1476 (C=C<), 1250(C-N), 1217(C-O),775(C-Cl); 1HNMR (300MHZ, CDCl\(_{3}\), DMSO-\(d_{6}\),ppm): 5.41(d,1H,CH), 6.37(d,1H,CH), 7.25(d,1H,Ar-H), 6.94 (d, 1H,Ar-H), 8.04(d,1H,Ar-H) 6.38 (d,1H,Ar-H), 6.95 (d,1H,Ar-H), 7.33-7.55(m,3H,Ar-H), 5.82 (s,1H,CH),3.85(s,2H,CH\(_{2}\) ), 3.81 (s,3H,CH\(_{3}\)); Anal. C_{29}H_{22}Cl_{2}N_{2}O_{2}S\_2; Calculated: C,63.34; H,4.29; Cl,17.19;N,5.68;O,6.49;S,13.01. Found: C,63.11; H,4.05; Cl,7.00;N,5.37;O,6.29;S,12.90.

2-(4-hydroxyphenyl)-3-(6-(4-methoxyphenyl)-4-phenyl-6H-1,3-thiazin-2-yl)thiazolidin-4-one(5j)
IR(KBr,cm\(^{-1}\)):3365 (O-H), 3076(Ar-H), 1693(C=O), 1633, 1476 (C=C<), 1250(C-N), 1211(C-O); 1HNMR (300MHZ, CDCl\(_{3}\), DMSO-\(d_{6}\),ppm): 5.41(d,1H,CH), 6.37(d,1H,CH), 6.55(d,1H,Ar-H),7.47 (d,1H,Ar-H),8.04(d,1H,Ar-H)6.38(d,1H,Ar-H), 6.95(d,1H,Ar-H), 7.33-7.55(m,3H,Ar-H), 5.82 (s,1H, CH), 3.85(s,2H,CH\(_{2}\)), 3.81(s,3H,CH\(_{3}\)); Anal. C_{29}H_{23}N_{2}O_{2}S\_2; Calculated: C,60.80; H,4.20; N,5.83; O,12.17;S,12.73. Found: C,61.87; H,3.97; N,8.07;O,12.49;S,12.27.

Antimicrobial activity
The synthesised compounds (5a-5k) were screened for their in vitro antimicrobial activity by using disc diffusion method (Osman et al., 2012). Antibacterial activity was screened against two gram positive bacteria Staphylococcus aureus (ATCC 9144), Bacillus subtilis (ATCC 6399) and two gram negative bacteria Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 17933) by measuring the zone of inhibition on agar plates at concentrations 100 μg/mL. Antifungal activity was screened against Candida albicans (ATCC 10231), Aspergillus niger (ATCC 6275) by measuring the zone of inhibition on agar plates at concentrations 100 μg/mL and reported in Table-2.

Disc diffusion method
Nutrient agar and Potato Dextrose Agar plates were employed as culture medium and dimethylformamide was used as solvent control for antimicrobial activity. Ciprofloxacin and Flucanazole were used as standard for antibacterial and antifungal activities respectively.

Preparation of microbial suspension
The bacterial and fungal strains were subculture at 37°C for six hrs in the corresponding medium of three successive days. These suspensions were used to inoculate the antibiograms.

Preparation of the biograms
The agar disc diffusion method was performed on each of the tested substance solution in dimethylformamide. Filter paper discs were impregnated with 1 ml of the solution and placed on the inoculated plates. These plates after standing at 4°C for 2 hours were incubated at 37°C for 24 hours. Ciprofloxacin and Flucanazole were used as standard drugs for antibacterial and antifungal activities respectively. The diameters of the inhibition zones were measured in millimeters and were reported in table no. 2.
RESULTS AND DISCUSSION

Compounds (5a-5k) are readily obtained in 70–86% yields by the condensation and cyclization reaction of 4,6-diphenyl-6H-1,3-thiazin-2-amine, aromatic aldehyde (Prasad et al., 2011) and thioglycolic acid in polypropylene glycol at 110°C temperature. Initially, we attempted the synthesis of 3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(4-methoxyphenyl)thiazolidin-4-one derivatives by the reaction of 4,6-diphenyl-6H-1,3-thiazin-2-amine, aromatic aldehyde and thioglycolic acid at 110°C in polyethylene glycol (PEG), as many organic transformations and multi-component reaction are reported in polyethylene glycol, but surprisingly, no product formation was observed even after 24 h of the reaction. However, the reaction proceeds well in PPG is possibly due to its immiscibility with water, which helps in the removal of a water molecule from the reaction mixture during the formation of thiazolidin-4-one ring. In addition, PPG is an eco-friendly solvent and associated with many advantages, such as low cost, less toxicity, efficient recyclability, easy work-up, and miscibility with a wide range of organic solvents. IR spectra of all the compounds (5a–5k) showed an absorption band at 1685–1698 cm⁻¹ due to carbon–oxygen double bond, typical of the stretching vibrations of the carbon–nitrogen single bond. No peaks were found due to starting material amino or aldehydic functionalities. ¹H NMR spectra of all the compounds showed the broad singlets due CH-N protons and a singlets due to CH₃ protons. Our further object, pharmacological point of view we plan to synthesize combined molecular framework that involves these two different chromophores. All the synthesized compounds were tested for their antimicrobial activity using Ciprofloxacin and Fluconazole as standard drugs. The antibacterial activity are shown in Table 2. The Compounds 5b,5c,5d,5i,5j exhibited good activity against gram-positive bacteria S. aureus, B. subtilis and gram-negative bacteria E. coli, P. aeruginosa. While other compounds 5a,5e,5f,5g,5h,5k exhibited moderate to poor activity against the tested microorganisms, compared to standard drug. The antifungal activity are shown in Table 2. The Compounds 5a,5b,5c,5g,5i,5k showed good activity against C. albicans, A. niger. While the remaining compounds 5d,5e,5f,5j,5k, exhibited moderate to poor activity as compared to standard drugs Ciprofloxacin and Fluconazole.

Table 1: Physical data of compounds (5a-5k).

<table>
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<tr>
<th>Comp.</th>
<th>M.P. °C</th>
<th>Yield</th>
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<tr>
<td>5a</td>
<td>85</td>
<td>76</td>
</tr>
<tr>
<td>5b</td>
<td>77</td>
<td>63</td>
</tr>
<tr>
<td>5c</td>
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<td>5j</td>
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</tr>
<tr>
<td>5k</td>
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<td>50</td>
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</table>

Table 2: Antimicrobial activity of Synthesized Compounds.

<table>
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<tr>
<th>Comp. (100µg/ml)</th>
<th>S. Aureus</th>
<th>B. Subtilis</th>
<th>E. Coli</th>
<th>P. aeruginosa</th>
<th>C. albicans</th>
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CONCLUSION

In conclusion, we have demonstrated the synthesis of novel series of potentially biologically active 3-(4,6-diphenyl-6H-1,3-thiazin-2-yl)-2-(4-methoxyphenyl)thiazolidin-4-one derivatives by the reaction of 4,6-diphenyl-6H-1,3-thiazin-2-amine, aromatic aldehyde and thioglycolic acid in polypropylene glycol at 110°C temperature then characterization and in-vitro antimicrobial evaluations. The results reveal that some of the compounds of the series exhibited promising antibacterial and antifungal activity compared to standard drugs.

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