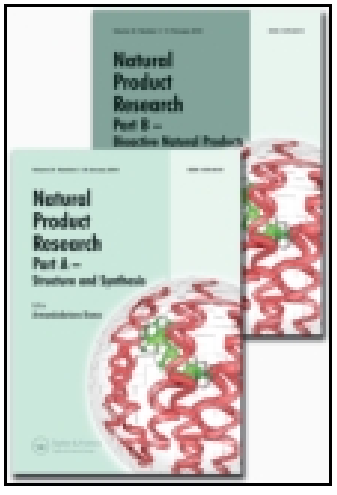

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Piperidine-mediated synthesis of thiazolyl chalcones and their derivatives as potent antimicrobial agents

P. Venkatesan [a] & T. Maruthavanan [b]
[a] Department of Chemistry , Mahendra Institute of Technology , Namakkal 637503 , India
[b] Department of Chemistry , Sona College of Technology , Salem 636005 , India
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Piperidine-mediated synthesis of thiazolyl chalcones and their derivatives as potent antimicrobial agents

P. Venkatesan[a]* and T. Maruthavanan[b]

[a]*Department of Chemistry, Mahendra Institute of Technology, Namakkal 637503, India;*
[b]*Department of Chemistry, Sona College of Technology, Salem 636005, India*

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A series of new thiazolyl chalcones, 1-[2-amino-4-methyl-1,3-thiazol-5-yl]-3-aryl-prop-2-en-1-one were prepared by piperidine mediated Claisen–Schmidt condensation of thiazolyl ketone with aromatic aldehyde. These chalcones on cyclisation gave 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-*4H*-pyridine-3-carbonitrile and 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-*4H*-pyran-3-carbonitrile. The result showed that the compounds exhibited marked potency as antimicrobial agents.

Keywords: piperidine; chalcone; cyanopyridine; cyanopyran; antimicrobial agent

1. Introduction

Chalcone (1,3-diaryl-2-propen-1-one) derivatives are an important group of secondary metabolites found ubiquitously in the plant kingdom. These are well-known intermediates towards the synthesis of the variety of heterocyclic compounds (Lokhande, Sakate, Taksande, & Navghare, 2005; Lorenz, Shahjahan Kabir, & Cook, 2010) and also exhibit various biological activities such as antibacterial (Venkatesan & Sumathi, 2010), antifungal (Lahtchev, Batovska, Parushev, Ubiyvovk, & Sibirny, 2008), antiviral (Cheenpracha, Karalai, Ponglimanont, Subhadhirasakul, & Tewtrakul, 2005), anti-inflammatory (Won et al., 2005), antitumour (Rao et al., 2004), antioxidant (Vaya et al., 1997), and insect antifeedant (Simmonds, Blaney, Delle Monache, & Marini Bettolo, 1990) and also act as tyrosinase inhibitors (Khatib et al., 2005). The complex pharmacological activities together with the easiest synthetic method by Claisen–Schmidt condensation have attracted considerable interest towards the synthesis of novel chalcones as potent microbial agents (Dimmock, Elias, Beazely, & Kandepu, 1999).

As thiazolidine moiety seems to be a potential pharmacophore in various pharmacologically active agents (Nora de Souza & Almeida, 2003; Zia-ur-Rehman, Choudary, & Ahmad, 2005), we have synthesised chalcones with a thiazolidine moiety and heterocyclised to cyanopyridine and cyanopyran derivatives as possible antimicrobial agents.

*Corresponding author. Email: venkatesanps@yahoo.co.in

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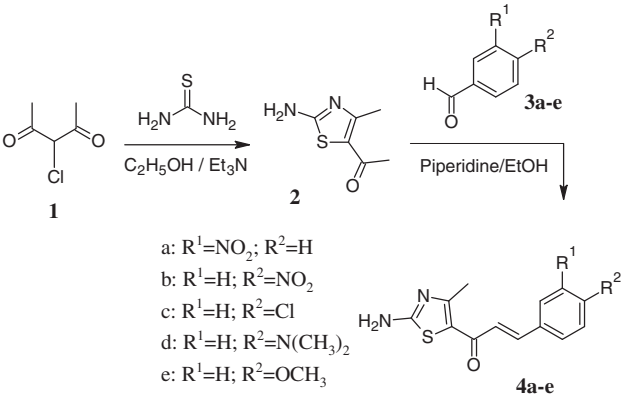
2. Results and discussion

2.1. *Chemistry*

In this study, 1-(2-amino-4-methyl-1,3-thiazol-5-yl)ethanone (**2**) was synthesised by the cyclisation of 3-chloroacetylacetone (**1**) with thiourea in accordance with the method described in the literature (Sayed, Raslan, Khalil, & Dawood, 1999). In the Claisen–Schmidt condensation of chalcone synthesis, strong alkali reduces the yield of the target adduct and makes its purification difficult due to undesirable side reactions such as multiple condensation, polymerisation and rearrangement (Devitt, Timoney, & Vickars, 1961). So, we used piperidine instead of a strong alkali for the synthesis of 1-[2-amino-4-methyl-1,3-thiazol-5-yl]-3-aryl-prop-2-en-1-one (**4a–e**) as shown in scheme 1, which gives a considerably better yield.

The IR spectra of compounds **4a–e** showed the absorption band at 1620–1628 cm^{-1} for C=O group and 1610–1612 cm^{-1} for C=C group, indicating the presence of α, β-unsaturated carbonyl group. The ^{1}H-NMR spectra gave two doublets at δ 7.60–7.64 ppm and δ 8.20–8.28 ppm with the coupling constant, $J = 15.2$–15.8 Hz. It is evident that the olefinic protons at C_α and C_β positions of α, β-unsaturated carbonyl group are arranged in *trans* configuration. Moreover, N–H asymmetric stretching absorption band appeared around 3324–3354 cm^{-1}, and symmetric stretching absorption band around 3402–3488 cm^{-1} indicating the presence of $-NH_2$ group. The C=N and CH=CH ring stretching absorption bands appeared around 1596–1582 cm^{-1} and 1498–1494 cm^{-1}, respectively, supporting the aromaticity of thiazole ring. In addition, C–N stretching absorption band got displayed at around 1173–1176 cm^{-1}. ^{13}C-NMR spectra of compounds **4a–e** exhibited chemical shifts at δ 121.1–121.3 ppm (C_α), δ 148.2–148.3 ppm (C_β) and δ 187.1–189.2 ppm (>C=O), indicating the presence of α, β-unsaturated carbonyl groups of chalcone. The methyl group on thiazole ring resonated at δ 28.1–28.9 ppm. In addition, the signals at δ 166.2–166.6 ppm, δ 152.1–152.2 ppm and δ 112.3–112.8 ppm were assigned to C_2, C_3 and C_5 of thiazole ring, respectively. The entire spectral data and elemental analysis results are in agreement with the proposed structure of chalcones (**4a–e**) as expected, and they are given in the experimental part.

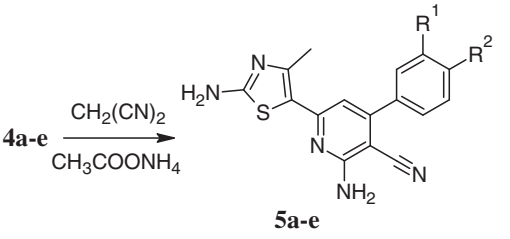


Scheme 1. Synthesis of 1-(2-amino-4-methyl-1,3-thiazol-5-yl)ethanone **(2)** and substituted thiazolyl chalcone **(4a–e)**.

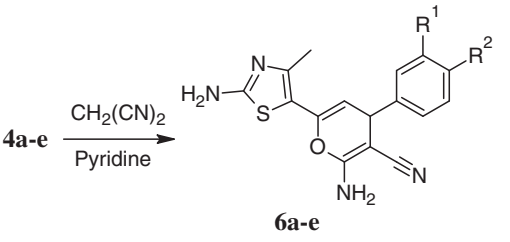
2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-*4H*-pyridine-3-carbonitrile (**5a–e**) was prepared by heterocyclisation of thiazolyl chalcones (**4a–e**) with the ethanolic solution of malononitrile in the presence of ammonium acetate. Similarly, 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-*4H*-pyran-3-carbonitrile (**6a–e**) were prepared by the heterocyclisation of thiazolyl chalcones (**4a–e**) with the ethanolic solution of malononitrile in the presence of pyridine. The synthetic route of compounds **5a–e** and **6a–e** are in accordance with the method described in the literature (Vyas et al., 2009), and are outlined in schemes 2 and 3, respectively. The signals for α, β-unsaturated carbonyl group were not observed in the spectral data of compounds **5a–e** and **6a–e**. The formations of a new heterocyclic ring in compounds **5a–e** and **6a–e** are evident. IR spectra of compounds **5a–e** revealed the presence of $-NH_2$ stretching absorption bands in the region of 3462–3474 cm^{-1} and 3326–3356 cm^{-1} and compounds **6a–e** also gave absorption bands at 3456–3476 cm^{-1} and 3342–3352 cm^{-1} for the same. The stretching absorption band of about 2216–2232 cm^{-1} corresponding to the –C≡N group was observed for both the compounds **5a–e** and **6a–e**. The ^{1}H-NMR spectra gave an additional broad singlet at about δ 5.67–5.84 ppm, in compounds **5a–e** and a broad singlet at about δ 5.67–5.84 ppm in compounds **6a–e**, indicating the formation of $-NH_2$ group in both the compounds **5a–e** and **6a–e**. The singlet around δ 6.32–6.74 ppm was assigned to pyridine-H of compounds **5a–e**. Similarly, two doublets around δ 6.11–6.16 ppm and δ 4.14–4.32 ppm with a coupling constant of about $J = 3.04$–3.09 Hz was assigned to pyran-H of compounds **6a–e**. In the Mass spectral fragmentation of compounds **5a–e** and **6a–e**, loss of –CN group was observed by lowering the m/z 26 from its molecular ion peak (M^+), indicating the presence of CN group in the heterocyclic ring of compounds **5a–e** and **6a–e**. The entire spectral data and elemental analysis reports are consistent with the structure of compounds **5a–e** and **6a–e** as expected, and they are given in the experimental part.

2.2. *Pharmacology*

The antimicrobial activity of the new synthesised compounds **4a–e**, **5a–e** and **6a–e** was evaluated using the agar diffusion method (Shrinivasan, Nathan, Suresh, & Lakshmana Perumalsamy, 2001). The *in vitro* antibacterial activity was evaluated against various pathogenic bacteria *viz*., *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*) and *Salmonella typhi* (*S. typhi*). Similarly, the *in vitro* antifungal activity was evaluated against *Aspergillus niger*



Scheme 2. Synthesis of cyanopyridine **(5a–e)**.



6a-e

Scheme 3. Synthesis of cyanopyran **(6a–e)**.

Table 1. Antibacterial activity of compounds **4a–e**, **5a–e** and **6a–e**.

	Diameters of zone inhibition in mm (MIC value, $\mu g\,mL^{-1}$)			
Compounds	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*
4a	–	–	–	–
4b	< 10 (50)	11 ± 0.8 (6.25)	–	–
4c	–	13 ± 1.2 (12.5)	–	–
4d	< 10 (50)	–	–	–
4e	–	–	–	–
5a	–	< 10 (50)	–	< 10 (50)
5b	12 ± 1.1 (12.5)	–	13 ± 1.4 (12.5)	–
5c	< 10 (50)	12 ± 1.3 (6.25)	–	–
5d	16 ± 1.2 (25)	–	–	–
5e	–	–	–	14 ± 1.2 (6.25)
6a	–	–	< 10 (50)	< 10 (50)
6b	14 ± 1.2 (25)	16 ± 0.8 (25)	–	< 10 (50)
6c	18 ± 1.0 (12.5)	14 ± 1.3 (12.5)	–	18 ± 0.9 (12.5)
6d	14 ± 1.2 (6.25)	–	–	–
6e	15 ± 0.7 (12.5)	16 ± 1.1 (25)	12 ± 1.2 (25)	< 10 (50)
[a]Cip	25–28 (6.25)	19–23 (6.25)	23–29 (6.25)	19–22 (6.25)

Note: [a]Cip, Ciprofloxacin.

(*A. niger*), *Aspergillus flavus* (*A. flavus*), *Penicillium chrysogenum* (*P. chrysogenum*) and *Fusarium moniliforme* (*F. moniliforme*). The results of antibacterial and antifungal screening of the newly synthesised compounds are given in Tables 1 and 2, respectively. Among the newly synthesised chalcones, the compound **6c** showed good activity (zone of inhibition up to 17–19 mM at the concentration of 12.5 $\mu g\,mL^{-1}$) against *S. aureus* and *S. typhi* and the compound **6e** showed good activity (zone of inhibition 16–18 mM at concentration of 6.25–12.5 $\mu g\,mL^{-1}$) against *A. niger*, *A. flavus* and *F. moniliforme*. Also, the compound **4b** showed moderate activity against *A. flavus*, and **4c** showed moderate activity against *A. niger* and *P. chrysogenum* (zone of inhibition < 10 mM at the concentration of 50 $\mu g\,mL^{-1}$). The chalcones **4a–e** were inactive against most of the fungal strains. However, the compounds **5a–e** and **6a–e** showed remarkable antifungal activity. Similarly, **6c** showed good activity (zone of inhibition up to 16 mM at the concentration of 12.5 $\mu g\,mL^{-1}$) against *A. niger* and *A. flavus* but no activity against *F. moniliforme*.

Table 2. Antifungal activity of compounds **4a–e**, **5a–e** and **6a–e**.

Compounds	Diameter of zone inhibition in mm (MIC value, $\mu g\,mL^{-1}$)			
	A. niger	*A. flavus*	*P. chrysogenum*	*F. moneliforme*
4a	–	–	–	–
4b	–	< 10 (50)	–	–
4c	< 10 (50)	–	< 10 (50)	–
4d	–	–	–	–
4e	–	–	–	–
5a	–	–	–	–
5b	–	< 10 (50)	–	14 ± 1.2 (25)
5c	15 ± 1.0 (6.25)	12 ± 1.2 (25)	13 ± 1.1 (12.5)	< 10 (50)
5d	< 10 (50)	< 10 (50)	–	–
5e	–	–	< 10 (50)	14 ± 1.1 (6.25)
6a	12 ± 0.9 (6.25)	–	–	< 10 (50)
6b	15 ± 0.8 (12.5)	14 ± 1.2 (25)	–	13 ± 1.2 (25)
6c	16 ± 1.1 (12.5)	16 ± 1.1 (12.5)	12 ± 1.2 (25)	< 10 (50)
6d	–	–	< 10 (50)	14 ± 1.2 (25)
6e	17 ± 1.2 (6.25)	16 ± 1.3 (12.5)	14 ± 1.3 (25)	16 ± 0.9 (12.5)
[a]Flu	20–23 (6.25)	19–24 (6.25)	20–25 (6.25)	19–24 (6.25)

Note: [a]Flu, Fluconazole.

The mode of the antimicrobial action of chalcones has long been believed to be due to their reaction with important thiol groups of essential enzymes *via* Michael addition at the ketovinyl double bond (Opletalova, Ricicarova, Sedivy, Meltrova, & Krivakova, 2000). Also, *p*-electron withdrawing group will increase the electrophilicity of C-β and thus facilitate the nucleophilic attack of the cellular thiol groups (Batovska et al., 2009). Interestingly, the antimicrobial activity was found to be good in compounds **4b**, **5b** and **6b** rather than **4a**, **5a** and **6a**, which is due to *p*-NO_2 substitution. Compounds **5c** and **6c**, which are carrying *p*-Cl substituent, exhibited moderate to good antibacterial and antifungal activity against most of the test strains. Similarly, compound **6e** showed almost good activities due to the presence of *p*-OCH_3 substitution. In general, microbial activities were increased remarkably on heterocyclisation of thiazolyl chalcones **(4a–e)** to cyanopyridine **(5a–e)** and cyanopyran **(6a–e)** derivatives. As nitrogen-containing heterocyclic compound is generally known to possess antimicrobial activity due to the formation of hydrogen bonding with the active site of an enzyme (Li et al., 1995), it is believed that the $-NH_2$ group and –CN group of cyanopyridine/cyanopyran ring systems may act as the active centre.

3. Experimental

3.1. *General*

All the common chemicals, 3-chloroacetylacetone (**1**) and substituted aryl aldehyde (**3a–e**), were obtained from Sigma–Aldrich chemicals. The melting points of the synthesised compounds were determined in open glass capillaries

and were uncorrected. UV spectra were recorded using Perkin Elmer 402 UV-Vis spectrophotometer. IR spectra were recorded on Perkin Elmer 577 IR spectrophotometer using KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Brucker 300 MHz NMR spectrometer in $CDCl_3$ with tetramethylsilane as the internal standard and the chemical shifts were reported in ppm scale. Mass spectra were studied using Finnigan MAT 8230 mass spectrometer. Elemental analysis was done on Vario EL-III elemental analyser and the analysed reports were within $\pm 0.4\%$ of the theoretical values. The purity of the compounds was checked by thin layer chromatography on silica gel 60 F_{254} (Merck) and spots were developed using iodine vapour or ultraviolet light.

3.2. *General procedure for synthesis of 1-[2-amino-4-methyl-1,3-thiazol-5-yl]-ethanone (2)*

To a stirred solution of thiourea (15.2 g, 0.2 mol) in absolute ethanol (75 mL) containing a catalytic amount of triethylamine, a solution of 3-chloroacetylacetone (26.9 g, 0.2 mol) in 10 mL of absolute ethanol was added dropwise. After complete addition, the mixture was stirred at room temperature for 30 min and then refluxed for 5 h. The reaction mixture was cooled, and a solid was filtered off, washed with cold ethanol, dried and finally recrystallised from DMF to get the compound **2** with 65% yield. m.p. 275°C (lit m.p. 274–276°C) (Sayed et al., 1999), ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.78 (s, 3H, CH_3 thiazole), 2.35 (s, 3H, CH_3 acetyl) and 8.48 (br s, 2H, NH_2 thiazole).

3.3. *General procedure for synthesis of 1-(2-amino-4-methyl-1, 3-thiazol-5-yl)-3-aryl-prop-2-en-1-one (4a–e)*

To a mixture of 1-[2-amino-4-methyl-1,3-thiazol-5-yl]ethanone (0.01 mol) and arylaldehyde (0.01 mol) in ethanol (50 mL), piperidine (1 mL) was added and refluxed. After the completion of reaction, which was monitored by TLC, ethanol was distilled off and residue was poured on ice water (100 mL). It was kept overnight in the refrigerator. The resulting solid was collected by filtration, washed with distilled water and recrystallised from methanol to get the corresponding thiazolyl chalcone (**4a–e**).

3.3.1. *1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(3-nitrophenyl)prop-2-en-1-one (4a)*

Pale yellow solid, yield 1.62 g (56%), m.p. 148–150°C, λ_{max} ($CHCl_3$, nm): 268, 322. IR (KBr, cm^{-1}): 3468 and 3354 (NH_2), 3184 (ArCH), 1620 (C=O), 1497 (CH=CH), 1596 (C=N), 1546 and 1424 ($ArNO_2$) and 1175 (C–N str). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.48 (3H, s, CH_3 thiazole), 8.82 (2H, br s, NH_2 thiazole) and 7.41–8.12 (6H, m, ArH + CH=CH). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 187.1, 166.2, 152.1, 144.2, 148.2, 129.3, 124.7, 122.2, 121.1, 112.3 and 28.9. *m/z*: 290 ($M^+ + 1$). Anal. Calcd for $C_{13}H_{11}N_3O_3S$ (289.31): C, 53.97%; H, 3.83%; and N, 14.52%, Found: C, 53.94%; H, 3.81%; and N, 14.54%.

3.3.2. *1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(4-nitrophenyl) prop-2-en-1-one (4b)*

Pale yellow solid, yield 1.88 g (65%), m.p. 146–148°C, λ_{max} ($CHCl_3$, nm): 286, 348. IR (KBr, cm^{-1}): 3488 and 3324 (NH_2), 3168 (ArCH), 1620 (C=O), 1494 (CH=CH), 1552 and 1424 ($ArNO_2$), 1592 (C=N) and 1174 (C–N str). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.51 (3H, s, CH_3 thiazole, 8.86 (2H, br s, NH_2 thiazole) and 7.36–8.24 (6H, m, ArH + CH=CH). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 187.9, 166.6, 155.3, 152.1, 148.3, 128.4, 125.1, 122.2, 121.1, 112.6 and 28.2. *m/z*: 290 ($M^+ + 1$). Anal. Calcd for $C_{13}H_{11}N_3O_3S$ (289.31): C, 53.97%; H, 3.83%; and N, 14.52%, Found: C, 54.01%; H, 3.82%; and N, 14.54%.

3.3.3. *1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(4-chlorophenyl) prop-2-en-1-one (4c)*

Colourless solid, yield 2 g (72%), m.p. 146–148°C, λ_{max} ($CHCl_3$, nm): 262, 366. IR (KBr, cm^{-1}): 3402 and 3336 (NH_2), 3164 (ArCH), 1626 (C=O), 1494 (CH=CH), 1584 (C=N), 1176 (C–N str) and 724 (ArCl). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.51 (3H, s, CH_3 thiazole, 8.68 (2H, br s, NH_2 thiazole), 7.28–8.42 (6H, m, ArH + CH=CH). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 188.2, 166.4, 156.2, 152.2, 148.2, 126.8, 124.4, 122.2, 121.3, 112.8 and 28.6. *m/z*: 280 ($M^+ + 1$). Anal. Calcd for $C_{13}H_{11}ClN_2OS$ (278.75): C, 56.01%; H, 3.96%; and N, 10.05%, Found: C, 56.06%; H, 3.97%; and N, 10.02%.

3.3.4. *1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(4-dimethylaminophenyl) prop-2-en-1-one (4d)*

Pale yellow solid, yield 1.67 g (58%), m.p. 128–130°C, λ_{max} ($CHCl_3$, nm): 272, 382. IR (KBr, cm^{-1}): 3486 and 3324 (NH_2), 3168 (ArCH), 1620 (C=O), 1498 (CH=CH), 1582 (C=N) and 1173 (C–N str). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.51 (3H, s, CH_3 thiazole, 8.84 (2H, br s, NH_2 thiazole), 7.12–8.36 (6H, m, ArH + CH=CH) and 2.84 (6H, s, $N(CH_3)_2$). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 189.2, 166.6, 158.3, 152.1, 148.3, 128.6, 125.2, 122.2, 121.1, 112.6, 44.6 and 28.1. *m/z*: 288 ($M^+ + 1$). Anal. Calcd for $C_{15}H_{17}N_3OS$ (287.38): C, 62.73%; H, 5.95%; and N, 14.66%, Found: C, 62.69%; H, 5.96%; and N, 14.62%.

3.3.5. *1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(4-methoxyphenyl) prop-2-en-1-one (4e)*

Colourless solid, yield 1.81 g (66%), m.p. 136–138°C, λ_{max} ($CHCl_3$, nm): 286, 386. IR (KBr, cm^{-1}): 3448 and 3326 (NH_2), 3186 (ArCH), 1628 (C=O), 1498 (CH=CH), 1596 (C=N), 1232 and 1028 (C–O str), 1174 (C–N str). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.52 (3H, s, CH_3 thiazole, 8.68 (2H, br s, NH_2 thiazole), 7.21–8.32 (6H, m, ArH + CH=CH) and 3.81 (3H, s, $ArOCH_3$). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 188.6, 166.2, 157.9, 152.1, 148.2, 127.4, 124.8, 122.2, 121.2, 112.8, 53.8 and 28.5. *m/z*: 275 ($M^+ + 1$). Anal. Calcd for $C_{14}H_{14}N_2O_2S$ (274.34): C, 61.32%; H, 5.12%; and N, 10.24%, Found: C, 61.29%; H, 5.14%; and N, 10.21%.

3.4. *General procedure for synthesis of 2-amino-6-(2-amino-4-methyl-1, 3-thiazol-5-yl)-4-aryl-4H-pyridine-3-carbonitrile derivatives (5a–e)*

The thiazolyl chalcone, (**4a–e**) (0.01 mol), malononitrile (0.66 g, 0.01 mol) and ammonium acetate (6.16 g, 0.08 mol) in ethanol (95%, 25 mL) were refluxed on a water bath for 4–10 h. The progress of reaction was monitored by TLC at the appropriate time intervals. The solution was poured on to crushed ice and kept aside. The precipitate thus separated was collected by filtration and crystallised from ethanol.

3.4.1. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(3-nitrophenyl)-4H-pyridine-3-carbonitrile (5a)*

Yellow solid, (0.94 g (25.5%), m.p. 216–218°C, λ_{max} ($CHCl_3$, nm): 262, 374. IR (KBr, cm^{-1}): 3462 and 3342 (NH_2), 3182 (ArCH), 2216 (C≡N), 1581 (C=N), 1512 (CH=CH), and 1546 and 1374 ($ArNO_2$). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.51 (3H, s, CH_3 thiazole), 8.42 (2H, br s, NH_2 thiazole), 6.74 (1H, s, H pyridine), 5.67 (2H, br s, NH_2 pyridine) and 7.43–8.06 (4H, m, ArH). *m/z*: 353 ($M^+ + 1$). Anal. Calcd for $C_{16}H_{12}N_6O_2S$ (352.37): C, 54.52%; H, 3.44%; and N, 23.86%, Found: C, 54.54%; H, 3.43%; and N, 23.85%.

3.4.2. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-nitrophenyl)-4H-pyridine-3-carbonitrile (5b)*

Yellow solid, yield 0.96 g (26.1%), m.p. 172–174°C, λ_{max} ($CHCl_3$, nm): 282, 386. IR (KBr, cm^{-1}): 3468 and 3338 (NH_2), 3188 (ArCH), 2224 (C≡N), 1579 (C=N), 1514 (CH=CH) and 1552 and 1354 ($ArNO_2$). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.50 (3H, s, CH_3 thiazole), 8.62 (2H, br s, NH_2 thiazole), 6.68 (1H, s, H pyridine), 5.72 (2H, br s, NH_2 pyridine) and 7.24–8.08 (4H, m, ArH). *m/z*: 353 ($M^+ + 1$). Anal. Calcd for $C_{16}H_{12}N_6O_2S$ (352.37): C, 54.54%; H, 3.43%; and N, 23.85%, Found: C, 54.58%; H, 3.44%; and N, 23.79%.

3.4.3. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-chlorophenyl)-4H-pyridine-3-carbonitrile (5c)*

Yellow solid, yield 0.94 g (26.3%), m.p. 158–160°C, λ_{max} ($CHCl_3$, nm): 274, 378. IR (KBr, cm^{-1}): 3474 and 3326 (NH_2), 3154 (ArCH), 2218 (C≡N), 1582 (C=N), 1518 (CH=CH), and 726 (ArCl). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.55 (3H, s, CH_3 thiazole), 8.64 (2H, br s, NH_2 thiazole), 6.72 (1H, s, H pyridine), 5.84 (2H, br s, NH_2 pyridine), and 7.28–8.01 (4H, m, ArH). *m/z*: 343 ($M^+ + 1$). Anal. Calcd for $C_{16}H_{12}ClN_5S$ (341.82): C, 56.22%; H, 3.54%; and N, 20.49%, Found: C, 56.28%; H, 3.53%; and N, 20.42%.

3.4.4. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-dimethylaminophenyl)-4H-pyridine-3-carbonitrile (5d)*

Yellow solid, yield 0.99 g (27.1%), m.p. 234–236°C, λ_{max} ($CHCl_3$, nm): 276, 384. IR (KBr, cm^{-1}): 3462 and 3356 (NH_2), 3176 (ArCH), 2224 (C≡N), 1581 (C=N) and 1512 (CH=CH). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.42 (3H, s, CH_3 thiazole), 8.68

(2H, br s, NH_2 thiazole), 6.32 (1H, s, H pyridine), 5.82 (2H, br s, NH_2 pyridine), 7.23–8.23 (4H, m, ArH) and 2.84 (6H, s, $ArCH_3$). *m/z*: 351 ($M^+ + 1$). Anal. Calcd for $C_{18}H_{18}N_6S$ (350.44): C, 61.69%; H, 5.18%; and N, 23.98%, Found: C, 61.65%; H, 5.19%; and N, 23.96%.

3.4.5. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-methoxyphenyl)-4H-pyridine-3-carbonitrile (5e)*

Yellow solid, yield 0.91 g (27.1%), m.p. 196–198°C, λ_{max} ($CHCl_3$, nm): 282, 394. IR (KBr, cm^{-1}): 3474 and 3336 (NH_2), 3176 (ArCH), 2216 (C≡N), 1578 (C=N), and 1514 (CH=CH). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.39 (3H, s, CH_3 thiazole), 8.68 (2H, br s, NH_2 thiazole), 6.41 (1H, s, H pyridine), 5.78 (2H, br s, NH_2 pyridine), 7.48–8.12 (4H, m, ArH), and 3.81 (3H, s, $ArOCH_3$). *m/z*: 338 ($M^+ + 1$). Anal. Calcd for $C_{17}H_{15}N_5OS$ (337.4): C, 60.52%; H, 4.48%; and N, 20.76%, Found: C, 60.48%; H, 4.47%; and N, 20.71%.

3.5. *General procedure for synthesis of 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-4H-pyran-3-carbonitrile derivatives (6a–e)*

The thiazolyl chalcone, (**4a–e**) (0.01 mol) and malononitrile (0.66 g, 0.01 mol) dissolved in dry pyridine (15 mL) were refluxed for 4–10 h. The progress of reaction was monitored by TLC at appropriate time intervals. The solution was poured on to crushed ice, neutralised with diluted HCl and kept aside. The precipitate thus separated was collected by filtration and crystallised from ethanol.

3.5.1. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(3-nitrophenyl)-4H-pyran-3-carbonitrile (6a)*

Yellow solid yield, 1.01 g (27.2%), m.p. 164–166°C, λ_{max} ($CHCl_3$, nm): 290, 376. IR (KBr, cm^{-1}): 3476 and 3351 (NH_2), 3166 (ArCH), 2232 (C≡N), 1586 (C=N), 1514 (CH=CH) and 1532 and 1328 ($ArNO_2$). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.23 (3H, s, CH_3 thiazole), 8.96 (2H, br s, NH_2 thiazole), 6.12 (1H, d, $J = 3.04$ Hz, H pyran), 4.28 (1H, d, $J = 3.04$ Hz, H pyran), 5.92 (2H, br s, NH_2 pyran) and 7.38–7.86 (4H, m, ArH). *m/z*: 356 ($M^+ + 1$). Anal. Calcd for $C_{16}H_{13}N_5O_3S$ (355.37): C, 54.08%; H, 3.69%; and N, 19.71%, Found: C, 54.06%; H, 3.68%; and N, 19.69%.

3.5.2. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-nitrophenyl)-4H-pyran-3-carbonitrile (6b)*

Yellow solid, yield 0.99 g (26.7%), m.p. 192–194°C, λ_{max} ($CHCl_3$, nm): 266, 384. IR (KBr, cm^{-1}): 3458 and 3348 (NH_2), 3174 (ArCH), 2218 (C≡N), 1582 (C=N), 1516 (CH=CH), 1532 and 1366 ($ArNO_2$). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.22 (3H, s, CH_3 thiazole), 8.98 (2H, br s, NH_2 thiazole), 6.16 (1H, d, $J = 3.09$ Hz, H pyran), 4.32 (1H, d, $J = 3.09$ Hz, H pyran), 5.96 (2H, br s, NH_2 pyran), and 7.18–7.92 (4H, m, ArH). *m/z*: 356 ($M^+ + 1$). Anal. Calcd for $C_{16}H_{13}N_5O_3S$ (355.37): C, 54.08%; H, 3.69%; and N, 19.71%, Found: C, 54.04%; H, 3.67%; and N, 19.74%. Anal. Calcd for $C_{16}H_{13}N_5O_3S$ (355.37): C, 54.08%; H, 3.69%; and N, 19.71%, Found: C, 54.04%; H, 3.67%; and N, 19.74%.

3.5.3. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-chlorophenyl)-4H-pyran-3-carbonitrile (6c)*

Yellow solid, yield 1.02 g (28.3%), m.p. 162–164°C, λ_{max} ($CHCl_3$, nm): 272, 386. IR (KBr, cm^{-1}): 3464 and 3352 (NH_2), 3182 (ArCH), 2224 (C≡N), 1588 (C=N), 1514 (CH=CH), 728 (ArCl). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.23 (3H, s, CH_3 thiazole), 8.92 (2H, br s, NH_2 thiazole), 6.11 (1H, d, $J = 3.08$ Hz, H pyran), 4.28 (1H, d, $J = 3.08$ Hz, H pyran), 5.92 (2H, br s, NH_2 pyran), and 7.18–7.98 (4H, m, ArH). *m/z*: 346 ($M^+ + 1$). Anal. Calcd for $C_{16}H_{13}ClN_4OS$ (344.82): C, 55.73%; H, 3.8%; and N, 16.25%, Found: C, 55.75%; H, 3.81%; and N, 16.27%.

3.5.4. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-(dimethylamino-phenyl)-4H-pyran-3-carbonitrile (6d)*

Yellow solid, yield 1.02 g (27.6%), m.p. 138–140°C, λ_{max} ($CHCl_3$, nm): 276, 382. IR (KBr, cm^{-1}): 3456 and 3342 (NH_2), 3164 (ArCH), 2226 (C≡N), 1588 (C=N) and 1512 (CH=CH). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.23 (3H, s, CH_3 thiazole), 8.96 (2H, br s, NH_2 thiazole), 6.12 (1H, d, $J = 3.08$ Hz, H pyran), 4.14 (1H, d, $J = 3.08$ Hz, H pyran), 5.88 (2H, br s, NH_2 pyran), 7.12–7.92 (4H, m, ArH) and 2.83 (6H, s, $ArCH_3$). *m/z*: 354 ($M^+ + 1$). Anal. Calcd for $C_{18}H_{19}N_5OS$ (353.44): C, 61.17%; H, 5.42%; and N, 19.81%, Found: C, 61.19%; H, 5.43%; and N, 19.79%.

3.5.5. *2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-methoyphenyl)-4H-pyran-3-carbonitrile (6e)*

Yellow solid, yield 0.93 g (27.3%), m.p. 158–160°C, λ_{max} ($CHCl_3$, nm): 284, 374. IR (KBr, cm^{-1}): 3464 and 3348 (NH_2), 3178 (ArCH), 2224 (C≡N), 1583 (C=N) and 1512 (CH=CH). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.22 (3H, s, CH_3 thiazole), 8.89 (2H, br s, NH_2 thiazole), 6.11 (1H, d, $J = 3.08$ Hz, H pyran), 4.22 (1H, d, $J = 3.08$ Hz, H pyran), 5.82 (2H, br s, NH_2 pyran), 7.38–7.52 (4H, m, ArH) and 3.61 (3H, s, $ArOCH_3$). *m/z*: 341 ($M^+ + 1$). Anal. Calcd for $C_{17}H_{16}N_4O_2S$ (340.4): C, 59.98%; H, 4.74%; N, 16.46%, Found: C, 59.92%; H, 4.75%; and N, 16.48%.

3.6. *Procedure for antimicrobial activity*

3.6.1. *Disc diffusion method*

The antimicrobial activity of newly synthesised compounds was evaluated using the agar diffusion method. Briefly, a 24/48 h-old culture of selected bacteria/fungi was mixed with sterile physiological saline (0.85%), and the turbidity was adjusted to the standard inoculum of Mac-Farland scale 0.5 [$\sim 10^6$ colony-forming units (CFU) per millilitre]. Petri plates containing 20 mL of Mueller Hinton agar (MHA, Hi-Media) were used for all the bacteria tested. Fungi was cultured in Sabouraud's dextrose agar (SDA)/potato dextrose agar (PDA) (Hi-Media) and were purified by single-spore isolation technique. The inoculum was spread on the surface of the solidified media and Whatman number 1 filter paper discs (6 mm in diameter) impregnated with the test compound (20 µL/disc) were placed on the plates. Ciprofloxacin (5 µg/disc, Hi-Media) was used as positive control for bacteria. Fluconazole (10 µg/disc, Hi-Media) was used as positive control for fungi. A paper disc impregnated with

dimethyl sulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24 h at 37°C and the fungal culture was incubated for 72 h at 25°C. The inhibition zone diameters were measured in millimeters. All the tests were performed in triplicate and the average was taken as the final reading.

3.6.2. *Determination of MIC*

Solutions of the test compounds ciprofloxacin and fluconazole were prepared in DMSO at a concentration of 100 $\mu g\,mL^{-1}$. From the stock solution, serial dilutions of the compounds ($50, 25, \ldots, 3.12\,\mu g\,mL^{-1}$) were prepared to determine the minimum inhibitory concentration (MIC). All determinations were done in triplicates and the average was taken as final reading. The standard antibiotics, ciprofloxacin ($100\,\mu g\,mL^{-1}$) for bacteria and fluconazole ($100\,\mu g\,mL^{-1}$) for fungi, were used as positive controls and 100 mL of DMSO used as a negative control. At the end of the incubation period, the MIC values were determined.

4. Conclusion

A series of thiazolyl chalcones were synthesised by piperidine-mediated Claisen–Schemidt condensation. From these, a series of cyanopyridine and cyanopyran derivatives were prepared and they showed better antibacterial and antifungal activity than that of thiazolyl chalcone. Predominantly, cyanopyrane derivatives showed excellent antimicrobial activities. In addition, 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-chlorophenyl)-*4H*-pyran-3-carbonitrile (**6c**) and 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-methoy-phenyl)-*4H*-pyran-3-carbonitrile (**6e**) were found to be the most active antibacterial agent and antifungal agent, respectively.

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