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-637 503, India
[b]Department of Chemistry, Sona College of Technology, Salem-636 005, India
*E-mail: venkatesanps@yahoo.co.in

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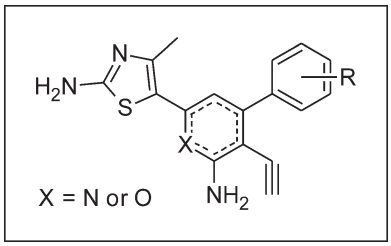

A series of new thiazolyl chalcones, 1-[2-amino-4-methyl-1, 3-thiazol-5-yl]-3-aryl-prop-2-en-1-one were prepared by piperidine mediated Claisen-Schmidt condensation of thiazolyl ketone with substituted aromatic aldehyde. These chalcones on cyclization gave 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-*4H*-pyridine-3-carbonitrile and 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-*4H*-pyran-3-carbonitrile. The results showed that this skeletal framework exhibited marked potency as antimicrobial agents. The most active antibacterial agent was 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-chlorophenyl)-*4H*-pyran-3-carbonitrile while 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-methoyphenyl)-*4H*-pyran-3-carbonitrile appeared to be the most active antifungal agent.

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INTRODUCTION

The chalcone (1,3-diaryl-2-propen-1-one) derivative is 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 [1]. In addition, the chalcone exhibits a various biological activities such as antibacterial [2], antifungal [3], antiviral [4], anti-inflammatory [5], antitumor [6], antioxidant [7], insect antifeedant [8], and act as tyrosinase inhibitors [9]. The complex pharmacological activities together with the easiest synthetic method by Claisen-Schmidt condensation has solicited considerable interest towards the synthesis of novel chalcone as a potent microbial agents [10].

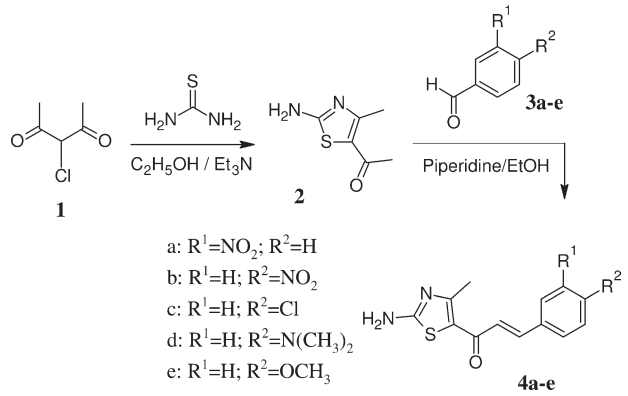
As thiazolidine moiety seems to be a potential pharmacophore in various pharmacologically active agents [11], we have synthesized chalcones with a thiazolidine moiety and heterocyclized to cyanopyridine and cyanopyran derivatives as possible antimicrobial agents, which could furnish better therapeutic results.

RESULT AND DISCUSSION

1-(2-Amino-4-methyl-1,3-thiazol-5-yl)ethanone **(2)** was synthesized by cyclization of 3-chloroacetylacetone **(1)** with thiourea in accordance with the method described in literature [12]. 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, polymerization, and rearrangement [13]. In this work, therefore, we used piperidine instead of 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 considerable 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 ^1H 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 evident that the olefinic protons at C_α and C_β position 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 band 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 displayed around 1173–1176 cm^{-1}. ^{13}C NMR spectra of compounds **4a–e** exhibited chemical shifts at

Scheme 1

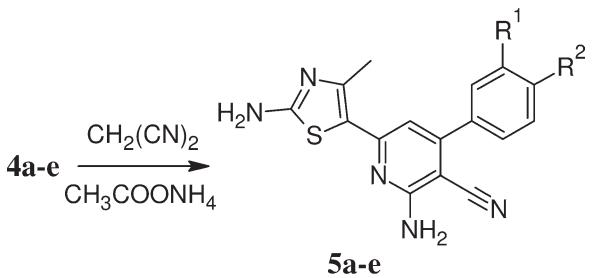


δ 121.1–121.3 ppm (C_α), δ 148.2–148.3 ppm (C_β), and δ 187.1–189.2 ppm (=C=O) indicating the presence of α,β-unsaturated carbonyl groups of chalcone. The methyl group on thiazole ring resonated at δ 28.1–28.9 ppm. In addition, the signals at δ 166.2–166.6 ppm, δ 152.1–152.2 ppm, δ 112.3–112.8 ppm were assigned to C_2, C_3, and C_5 of thiazole ring. 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 experimental part.

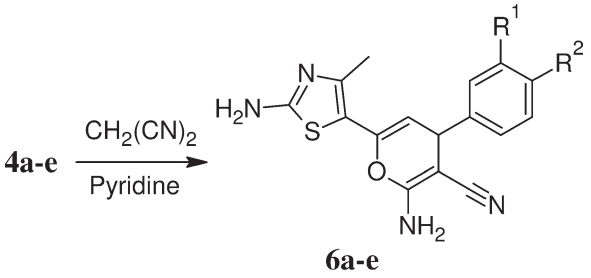
2-Amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-*4H*-pyridine-3-carbonitrile **(5a–e)** was prepared by heterocyclization of thiazolyl chalcones **(4a–e)** with the ethanolic solution of malononitrile in presence of ammonium acetate. Similarly, 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-4H-pyran-3-carbonitrile **(6a–e)** were prepared by heterocyclization of thiazolyl chalcones **(4a-e)** with the ethanolic solution of malononitrile in presence of pyridine. The synthetic route of compounds **5a–e** and **6a–e** are in accordance with the method described in the literature [14], and are outlined in Scheme 2 and Scheme 3, respectively.

The signals for α, β–unsaturated carbonyl group was not observed in the spectral data of compounds **5a–e** and **6a–e**. It is evident that the formations of a new heterocyclic ring in compounds **5a–e** and **6a–e**. IR spectra of compounds **5a–e** revealed the presence of —NH_2 stretching absorption bands in the region of

Scheme 2



Scheme 3



3462–3474 cm^{-1} and 3326–3356 cm^{-1} and compounds **6a–e** also gave the absorption band at 3456–3476 cm^{-1} and 3342–3352 cm^{-1} for the same. The stretching absorption band 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 additional broad singlet about δ 5.67–5.84 ppm in compounds **5a–e** and broad singlet about δ 5.67–5.84 ppm in compounds **6a–e** indicating 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 coupling constant 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^+), which 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 experimental part.

Antimicrobial activities. The antimicrobial activity of new synthesized compounds **4a–e**, **5a–e,** and **6a–e** was evaluated using the agar diffusion method [15]. The *in vitro* antibacterial activity was evaluated against various pathogenic bacteria (Gram-positive and Gram-negative) 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* (*A. niger*), *Aspergillus flavus* (*A. flavus*), *Penicillium chrysogenum* (*P. chrysogenum*), and *Fusarium moniliforme* (*F. moniliforme*). The results of antibacterial and antifungal screening of the new synthesized compounds are given in Table 1.

The compound 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-chlorophenyl)-4*H*-pyran-3-carbonitrile **(6c)** was showed good activity (zone of inhibition up to 17–19 m*M* at the concentration of 12.5 μg/mL) against *S. aureus* and *S. typhi*. The compounds, 1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(3-nitrophenyl)prop-2-en-1-one **(4a)** and

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

Compd	Diameter of zone inhibition in mm (MIC value, μg mL^{-1})							
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*	*A. niger*	*A. flavus*	*P. chrysogenum*	*F. moneliforme*
4a	–	–	–	–	–	–	–	–
4b	<10 (50)	11 ± 0.8 (6.25)	–	–	–	<10 (50)	–	–
4c	–	13 ± 1.2 (12.5)	–	–	<10 (50)	–	<10 (50)	–
4d	<10 (50)	–	–	–	–	–	–	–
4e	–	–	–	–	–	–	–	–
5a	–	<10 (50)	–	<10 (50)	–	–	–	–
5b	12 ± 1.1 (12.5)	–	13 ± 1.4 (12.5)	–	–	<10 (50)	–	14 ± 1.2 (25)
5c	<10 (50)	12 ± 1.3 (6.25)	–	–	15 ± 1.0 (6.25)	12 ± 1.2 (25)	13 ± 1.1 (12.5)	<10 (50)
5d	16 ± 1.2 (25)	–	–	–	<10 (50)	<10 (50)	–	–
5e	–	–	–	14 ± 1.2 (6.25)	–	–	<10 (50)	14 ± 1.1 (6.25)
6a	–	–	<10 (50)	<10 (50)	12 ± 0.9 (6.25)	–	–	<10 (50)
6b	14 ± 1.2 (25)	16 ± 0.8 (25)	–	<10 (50)	15 ± 0.8 (12.5)	14 ± 1.2 (25)	–	13 ± 1.2 (25)
6c	18 ± 1.0 (12.5)	14 ± 1.3 (12.5)	–	18 ± 0.9 (12.5)	16 ± 1.1 (12.5)	16 ± 1.1 (12.5)	12 ± 1.2 (25)	<10 (50)
6d	14 ± 1.2 (6.25)	–	–	–	–	–	<10 (50)	14 ± 1.2 (25)
6e	15 ± 0.7 (12.5)	16 ± 1.1 (25)	12 ± 1.2 (25)	<10 (50)	17 ± 1.2 (6.25)	16 ± 1.3 (12.5)	14 ± 1.3 (25)	16 ± 0.9 (12.5)
Ciprofloxacin	25–28 (6.25)	19–23 (6.25)	23–29 (6.25)	19–22 (6.25)	–	–	–	–
Fluconazole	–	–	–	–	20–23 (6.25)	19–24 (6.25)	20–25 (6.25)	19–24 (6.25)

1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one **(4e)** were inactive against all test bacterial strain. However, most of the compounds **5a–e** and **6a–e** were showed moderate to good activity with MIC value in the range of 6.25–25 μg/mL) in DMSO.

Among the new synthesized chalcones, compound 1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(4-nitrophenyl)-prop-2-en-1-one **(4b)** was showed moderate activity against *A. flavus*, and 1-(2-amino-4-methyl-1,3-thiazol-5-yl)-3-(4-chlorophenyl)prop-2-en-1-one **(4c)** was showed moderate activity against *A. niger* and *P. chrysogenum* (zone of inhibition <10 m*M* at the concentration of 50 μg/mL). The chalcones **4a–e** were inactive against most of the fungal strain. However, the compounds **5a–e** and **6a–e** were showed remarkable antifungal activity. The compound 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-methoyphenyl)-4*H*-pyran-3-carbonitrile **(6e)** was showed good activity (zone of inhibition 16–18 m*M* at concentration of 6.25–12.5 μg/mL) against *A. niger*, *A. flavus,* and *F. moniliforme*. Similarly, 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-chlorophenyl)-4*H*-pyran-3-carbonitrile **(6c)** showed good activity (zone of inhibition up to 16 mM at the concentration of 12.5 μg/mL) against *A. niger* and *A. flavus* but no activity against *F. moniliforme*.

The mode of the antimicrobial action of chalcones has long been believed, due to their reaction with important thiol groups of essential enzymes via Michael addition at the ketovinyl double bond [16]. According to Daniela Batovska *et al*. [17], *p*-electron withdrawing group will increase the electrophilicity of C-β and thus facilitate the nucleophilic attack of the cellular thiol groups. Interesting, 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. However, nitro substitution has not shown significant antimicrobial activity against most of the test strains.

Compounds **5c** and **6c,** which carried *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, on heterocyclization of thiazolyl chalcones **(4a–e)** to cyanopyridine **(5a–e)** and cyanopyran **(6a–e)** derivatives, both antibacterial and antifungal activities were increased remarkably. 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 [18], it is believed that the —NH_2 group and —CN group of cyanopyridine/cyanopyran ring systems may act as the active center.

It can be concluded that the cyanopyridine and cyanopyrane derivatives meet stereochemical and electronic requirements of the target in a better way than chalcone, and hence

exhibited good antibacterial and antifungal activity. 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)** acts as the most active antibacterial agent and 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-methoy-phenyl)-*4H*-pyran-3-carbonitrile **(6e)** appeared to be the most active antifungal agent.

EXPERIMENTAL

General. All the common chemicals, 3-chloroacetylacetone (**1**) and substituted aryl aldehyde (**3a–e**) were obtained from Sigma-Aldrich chemicals (India). Melting points of synthesized 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 analyzer and analyzed reports were within ± 0.4% of the theoretical values. The purity of the compounds was checked by thin layer chromatography on silica gel 60 F_{254} (Merck, India) and spots were developed using iodine vapour or ultraviolet light.

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 drop wise. After complete addition, the mixture was stirred at room temperature for 30 min and then refluxed for 5 h. The reaction mixture was cooled, a solid filtered off, washed with cold ethanol, dried, and finally recrystallized from DMF to get the compound **2** with 65 % yield. mp 275°C [lit mp 274–276°C], ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78 (s, 3H, CH_3 thiazole), 2.35 (s, 3H, CH_3 acetyl), 8.48 (br s, 2H, NH_2 thiazole).

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 recrystallized from methanol to give corresponding thiazolyl chalcone **4a–e**.

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

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

1-(2-Amino-4-methyl-1,3-thiazol-5-yl)-3-(4-chlorophenyl)prop-2-en-1-one (4c). Colorless solid, yield 2 g (72%), mp 146–148°C; UV ($CHCl_3$): 262, 366 nm; *ir*: 3402 and 3336 (NH_2), 3164 (ArCH), 1626 (C=O), 1494 (CH=CH), 1584 (C=N), 1176 (C—N str), 724 cm^{-1} (ArCl); ^{1}H NMR ($CDCl_3$): δ 2.51 (3H, s, CH_3 thiazole, 8.68 (2H, br s, NH_2 thiazole), 7.28–8.42 ppm (6H, m, ArH + CH=CH); ^{13}C NMR ($CDCl_3$): δ 188.2, 166.4, 156.2, 152.2, 148.2, 126.8, 124.4, 122.2, 121.3, 112.8, 28.6 ppm; ms: *m*/*z* 280 (M^+ + 1); Anal. Calcd for $C_{13}H_{11}ClN_2OS$ (278.75): C, 56.01; H, 3.96; N, 10.05%, Found: C, 56.06; H, 3.97; N, 10.02%.

1-(2-Amino-4-methyl-1,3-thiazol-5-yl)-3-(4-dimethylamino-phenyl)prop-2-en-1-one (4d). Pale yellow solid, yield 1.67 g (58%), mp 128–130°C; UV ($CHCl_3$): 272, 382 nm; *ir* (KBr, cm^{-1}): 3486 and 3324 (NH_2), 3168 (ArCH), 1620 (C=O), 1498 (CH=CH), 1582 (C=N), 1173 cm^{-1} (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), 2.84 ppm (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, 28.1 ppm; ms: *m*/*z* 288 (M^+ + 1); Anal. Calcd for $C_{15}H_{17}N_3OS$ (287.38): C, 62.73; H, 5.95; N, 14.66%, Found: C, 62.69; H, 5.96; N, 14.62%.

1-(2-Amino-4-methyl-1,3-thiazol-5-yl)-3-(4-methoxyphenyl)-prop-2-en-1-one (4e). Colorless solid, yield 1.81 g (66%), mp 136–138°C; UV ($CHCl_3$): 286, 386 nm; *ir*: 3448 and 3326 (NH_2), 3186 (ArCH), 1628 (C=O), 1498 (CH=CH), 1596 (C=N), 1232 and 1028 (C—O str), 1174 cm^{-1} (C—N str); ^{1}H NMR ($CDCl_3$): 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), 3.81 ppm (3H, s, $ArOCH_3$); ^{13}C NMR ($CDCl_3$): δ 188.6, 166.2, 157.9, 152.1, 148.2, 127.4, 124.8, 122.2, 121.2, 112.8, 53.8, 28.5 ppm; ms: *m*/*z* 275 (M^+ + 1); Anal. Calcd for $C_{14}H_{14}N_2O_2S$ (274.34): C, 61.32; H, 5.12; N, 10.24%, Found: C, 61.29; H, 5.14; N, 10.21%.

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 crystallized from ethanol.

2-Amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(3-nitro-phenyl)-4H-pyridine-3-carbonitrile (5a). Yellow solid, (0.94 g (25.5%), mp 216–218°C; UV ($CHCl_3$): 262, 374 nm; *ir*: 3462 and 3342 (NH_2), 3182 (ArCH), 2216 (C≡N), 1581 (C=N), 1512 (CH=CH), 1546 and 1374 cm^{-1} ($ArNO_2$); ^{1}H NMR ($CDCl_3$): 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), 7.43–8.06 ppm (4H, m, ArH); ms: *m/z* 353 (M^+ + 1); Anal. Calcd for $C_{16}H_{12}N_6O_2S$ (352.37): C, 54.52; H, 3.44; N, 23.86%, Found: C, 54.54; H, 3.43; N, 23.85%.

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%), mp 172–174°C; UV ($CHCl_3$): 282, 386 nm; *ir*: 3468 and 3338 (NH_2), 3188 (ArCH), 2224 (C≡N), 1579 (C=N), 1514 (CH=CH), 1552 and 1354 cm^{-1} ($ArNO_2$); ^1H NMR ($CDCl_3$): 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), 7.24-8.08 ppm (4H, m, ArH); ms: *m/z* 353 (M^+ + 1); Anal. Calcd for $C_{16}H_{12}N_6O_2S$ (352.37): C, 54.54; H, 3.43; N, 23.85%, Found: C, 54.58; H, 3.44; N, 23.79%.

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%), mp 158–160°C; UV ($CHCl_3$): 274, 378 nm; *ir*: 3474 and 3326 (NH_2), 3154 (ArCH), 2218 (C≡N), 1582 (C=N), 1518 (CH=CH), 726 cm^{-1} (ArCl); ^1H NMR ($CDCl_3$): δ 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), 7.28–8.01 ppm (4H, m, ArH); ms: *m/z* 343 (M^+ + 1); Anal. Calcd for $C_{16}H_{12}ClN_5S$ (341.82): C, 56.22; H, 3.54; N, 20.49%, Found: C, 56.28; H, 3.53; N, 20.42%.

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%), mp 234–236°C; UV ($CHCl_3$): 276, 384 nm; *ir*: 3462 and 3356 (NH_2), 3176 (ArCH), 2224 (C≡N), 1581 (C=N), 1512 cm^{-1} (CH=CH); ^1H NMR ($CDCl_3$): 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), 2.84 ppm (6H, s, $ArCH_3$); ms: *m/z* 351 (M^+ + 1); Anal. Calcd for $C_{18}H_{18}N_6S$ (350.44): C, 61.69; H, 5.18; N, 23.98%, Found: C, 61.65; H, 5.19; N, 23.96%.

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%), mp 196–198°C; UV ($CHCl_3$): 282, 394 nm; *ir*: 3474 and 3336 (NH_2), 3176 (ArCH), 2216 (C≡N), 1578 (C=N), 1514 cm^{-1} (CH=CH); ^1H NMR ($CDCl_3$): 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), 3.81 ppm (3H, s, $ArOCH_3$); ms: *m/z* 338 (M^+ + 1); Anal. Calcd for $C_{17}H_{15}N_5OS$ (337.4): C, 60.52; H, 4.48; N, 20.76%, Found: C, 60.48; H, 4.47; N, 20.71%.

General procedure for synthesis of 2-amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-aryl-4*H*-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 on a water bath for 4–10 h. The progress of reaction was monitored by TLC at appropriate time interval. The solution was poured on to crushed ice, neutralized with dil. HCl and kept aside. The precipitate thus separated was collected by filtration and crystallized from ethanol.

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%), mp 164–166°C; UV ($CHCl_3$): 290, 376 nm; *ir* (KBr, cm^{-1}): 3476 and 3351 (NH_2), 3166 (ArCH), 2232 (C≡N), 1586 (C=N), 1514 (CH=CH), 1532 and 1328 cm^{-1} ($ArNO_2$); ^1H NMR ($CDCl_3$): 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), 7.38–7.86 ppm (4H, m, ArH); ms: *m/z* 356 (M^+ + 1); Anal. Calcd for $C_{16}H_{13}N_5O_3S$ (355.37): C, 54.08; H, 3.69; N, 19.71%, Found: C, 54.06; H, 3.68; N, 19.69%.

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%), mp 192–194°C; UV ($CHCl_3$): 266, 384 nm; *ir* (KBr, cm^{-1}): 3458 and 3348 (NH_2), 3174 (ArCH), 2218 (C≡N), 1582 (C=N), 1516 (CH=CH), 1532 and 1366 cm^{-1} ($ArNO_2$); ^1H NMR ($CDCl_3$): 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), 7.18-7.92 ppm (4H, m, ArH); ms: *m/z* 356 (M^+ + 1); Anal. Calcd for $C_{16}H_{13}N_5O_3S$ (355.37): C, 54.08; H, 3.69; N, 19.71%, Found: C, 54.04; H, 3.67; N, 19.74%.

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%), mp 162–164°C; UV ($CHCl_3$): 272, 386 nm; *ir* (KBr, cm^{-1}): 3464 and 3352 (NH_2), 3182 (ArCH), 2224 (C≡N), 1588 (C=N), 1514 (CH=CH), 728 cm^{-1} (ArCl); ^1H NMR ($CDCl_3$): 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), 7.18–7.98 ppm (4H, m, ArH); ms: *m/z* 346 (M^+ + 1); Anal. Calcd for $C_{16}H_{13}ClN_4OS$ (344.82): C, 55.73; H, 3.8; N, 16.25%, Found: C, 55.75; H, 3.81; N, 16.27%.

2-Amino-6-(2-amino-4-methyl-1,3-thiazol-5-yl)-4-(4-dimethylaminophenyl)-4H-pyran-3-carbonitrile (6d). Yellow solid, yield 1.02 g (27.6%), mp 138–140°C; UV ($CHCl_3$): 276, 382 nm; *ir*: 3456 and 3342 (NH_2), 3164 (ArCH), 2226 (C≡N), 1588 (C=N), 1512 cm^{-1} (CH=CH); ^1H NMR ($CDCl_3$): 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), 2.83 ppm (6H, s, $ArCH_3$); ms: *m/z* 354 (M^+ + 1); Anal. Calcd for $C_{18}H_{19}N_5OS$ (353.44): C, 61.17; H, 5.42; N, 19.81%, Found: C, 61.19; H, 5.43; N, 19.79%.

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%), mp 158–160°C; UV ($CHCl_3$): 284, 374 nm; *ir*: 3464 and 3348 (NH_2), 3178 (ArCH), 2224 (C≡N), 1583 (C=N), 1512 cm^{-1} (CH=CH); ^1H NMR ($CDCl_3$): 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), 3.61 ppm (3H, s, $ArOCH_3$); ms: *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; N, 16.48%.

Procedure for antimicrobial activity. The antimicrobial activity of newly synthesized 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 MacFarland scale 0.5 [10^6 colony forming units (CFU) per milliliter]. Petri plates containing 20 mL of Mueller Hinton Agar (MHA; Hi-Media) were used for all the bacteria tested. Fungi were cultured in Sabouraud's dextrose agar (SDA)/potato dextrose agar (PDA; Hi-Media, India) and were purified by single spore isolation technique. The inoculum was spread on the surface of the solidified media and Whatman no. 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 dimethylsulfoxide (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 final reading.

Determination of MIC. Solutions of the test compounds, ciprofloxacin and fluconazole were prepared in DMSO at a concentration of 100 μg/mL. From the stock solution, serial dilutions of the compounds (50, 25, ... 3.12 μg/mL) were prepared to determine the MIC. All determinations were done in triplicates and the average was taken as final reading. The standard antibiotic, ciprofloxacin (100 μg/mL) for bacteria and fluconazole (100 μg/mL) 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.

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