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# Synthesis and biological evaluation of 1,3,5-triazine based chalcones and their derivatives

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## ABSTRACT

Chalcones, 2,4-bis-(4'-chlorophenylamino)-6-[4'-{3''-(substituted phenyl/ 2'''- thienyl/2'''-furanyl/cinnamyl)-2''-propenon-1''-yl}phenyl amino]-*s*-triazines**(6a-d)** have been prepared from ketone **(5)** based on *s*-triazine nucleus. These chalcones **(6a-d)** on cyclisation with guanidine nitrate in presence of alkali, malononitrile in presence of ammonium acetate and hydrazine hydrate in presence of glacial acetic acid give the corresponding aminopyrimidines **(7a-d)**, cyanopyridines **(8a-d)** and acetylpyrazolines **(9a-d)** respectively. The structures of newely synthesized compounds have been confirmed on the basis of elemental analysis, IR and $^1$H NMR spectral data. They are also tested for their antibacterial activity.



## KEYWORDS

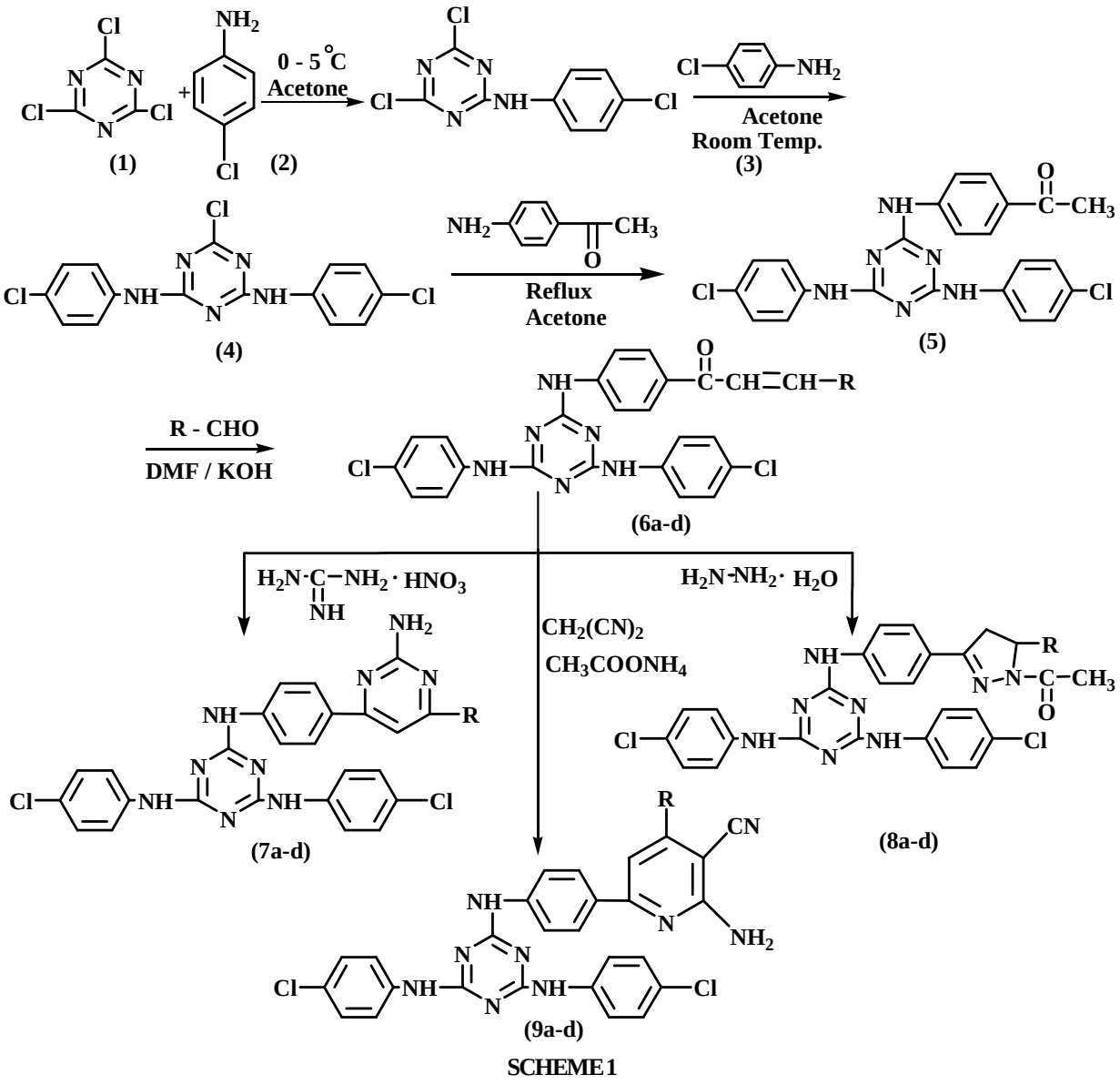
Aminopyrimidines;
Cyanopyridines;
Acetylpyrazolines;
Antibacterial activity.

## INTRODUCTION

The synthesis of α,β-unsaturated carbonyl compounds is one of the main structural component in various naturally occurring and biologically essential substances[1]. Moreover, α,β-unsaturated ketones are important intermediates in many addition reactions due to carbonyl group at the β-position[2]. Several strategies for the synthesis of these systems based on the formation of carbon-carbon bond have been reported and among them the direct Aldol condensation and Claisen-Schimdt condensation still occupy prominent position[3]. It is well known that most natural or synthetic chalcones are highly active with extensive pharmaceutical and medicinal application[4]. Recently, chalcones have been used as anti-AIDS[5], antimalarial[6], anti-inflammatory[7], antibacterial[8] and antitumor[9] agents.

Pyrimidine derivatives have been reported to possess a variety of biological activity such as analgesic[10], antihypertensive[11], antipyretic[12], antiviral[13] and anti-inflammatory[14]. Many of the pyrimidine derivatives are reported to possess potential CNS depressant properties[15]. The chemistry of pyridines and their derivatives has gained increasing attention because pyridine ring substituted with amino and cyano groups may be having good biologically active properties such as antibacterial[16], antifungal[17], antitubercular[18] and analgesic[19] activities. Pyrazoline derivatives have been found to be anticancer[20], antitubercular[21] and antibacterial[22] activities. It has been reported that introduction of acetyl group at 1st-position enhances the molluscicidal[23] activity as well as increases the stability of pyrazolines.

In continuation of our work on chalcones and their derivatives[24-26], we have synthesized 2,4-bis-(4'-chlorophenylamino)-6-[4'-{3''-(substituted phenyl/ 2'''- thienyl/ 2'''- furanyl/ cinnamyl)-2''-propenon-1''-




**SCHEME 1**

yl}phenyl amino]-*s*-triazines **(6a-d)** according to Claisen-Schmidt condensation by using ketone **(5)** with different aromatic aldehydes. Further these chalcones **(6a-d)** on cyclisation with guanidine nitrate in presence of alkali, malononitrile in presence of ammonium acetate and hydrazine hydrate in presence of glacial acetic acid give the corresponding aminopyrimidines **(7a-d)**, cyanopyridines **(8a-d)** and acetylpyrazolines **(9a-d)** respectively. The IR spectra of compound **(6a)** revealed the presence of(–C=O) group by exhibiting a strong absorption at 1652cm$^{-1}$. The IR spectrum of compounds **(7a)** and **(8a)** showed the absorption at 3409cm$^{-1}$ and 3413cm$^{-1}$ which indicate the presence of (-$NH_2$) group. A strong absorption at 1568 cm$^{-1}$ due to the presence of (–C=N) group in compounds **(9a)**. The $^1$H NMR spectrum of **(6a)** displayed doublet at δ 6.90 due to (-CO-CH=) proton. A singlet appeared at δ5.18 and δ5.25 due to the (–$NH_2$) protons presence in **(7a)** and **(8a)** respectively. The $^1$H NMR spectrum of **(9a)** showed a sharp singlet at δ 2.45 due to (–$COCH_3$) protons.

## Antibacterial activity

All the synthesized compounds were screened for their antibacterial activity by using agar diffusion method[27] against *S.aureus*(MTCC 96), *B.subtilis*

(MTCC 441) Gram positive and *E.coli*(MTCC 443), *S.paratyphi-B*(MTCC 733) Gram negative bacteria in nutrient agar medium. The sterilized agar media [2.4 % (w/v) agar-agar, 5%(w/v) NaCl, 3%(w/v) peptone, pH(6.8 to 7.0)] was poured into petridishes and allowed to solidify. On the surface of the media microbial suspension was spread over the agar plates to solidify. A stainless steel cylinder (pre-sterilized) was used to bore the cavities. All the synthesized compounds (100µg/ml) in DMF were placed serially in the cavities, with the help of micropipette. It is then allowed to diffuse for 10 minutes in refrigerator. The plates were incubated at 37°C for 24 hours. After incubation the diameter of zone of inhibition was measured in mm. Under similar conditions controlled experiment was carried out by using Ciprofloxacin as standard drug for comparison.

## EXPERIMENTAL

All the melting points were determined in an open capillary and are uncorrected. The IR spectra were recorded on Perkin-Elmer 237 spectrophotometer. $^1$H NMR spectra on a Bruker Avance DPX 300 MHz spectrometer with $CDCl_3$ as a solvent and TMS as internal reference. Purity of the compounds were checked on TLC using silica gel-G.

### Preparation of 2-(4'-chlorophenylamino)-4,6-dichloro-*s*-triazine (3)

4-Chloroaniline **(2)** (0.01mole) was added slowly to cyanuric chloride **(1)** (0.01 mole) in acetone (30 ml) with constant stirring for 4 hours at 0-5ºC. Sodium carbonate solution was added to neutralize HCl evolved during the reaction. Finally the content was poured into crushed ice. The solid separated out was filtered, washed with water and recrystallised from alcohol to give **(3)**.

Yeild 94%; m.p. 238°C.

### Preparation of 2,4-bis-(4'-chlorophenylamino)-6-chloro-*s*-triazine (4)

4-Chloroaniline (0.01mole) was added slowly to 2-(4'-chlorophenylamino)-4,6-dichloro-*s*-triazine **(3)** (0.01mole) in acetone (35ml) with constant stirring for 6-hours at room temperature. Sodium carbonate solution was added to neutralize HCl evolved during the reaction. Finally the content was poured into crushed ice. The solid separated out was filtered, washed with water and recrystallised from alcohol to give **(4)**.

Yield 87%; m.p. 212°C; IR (KBr, cm$^{-1}$): 813 (C-N, *s*-triazine), 770 (C-Cl); $^1$H NMR ($CDCl_3$): δ7.00 to 7.75(m, 8 Ar-H and 2 NH). Anal.Calcd. for $C_{15}H_{10}N_5Cl_3$: C,49.11; H,2.73; N,19.10. Found: C,49.13; H,2.70; N,19.12.

### Preparation of 2,4-bis-(4'-chlorophenylamino)-6-(4'-acetylphenylamino)-*s*-triazine (5)

4-Aminoacetophenone (0.01mole) and 2,4-bis-(4'-chlorophenylamino)-6-chloro-*s*-triazine **(4)** (0.01mole) were dissolved in acetone(40ml). The reaction mixture was refluxed for 6 hours. Periodically sodium carbonate solution was added to neutralize HCl evolved during the reaction. Finally the reaction mixture was cooled and the content was poured into crushed ice. The solid separated out was filtered, washed with water and recrystallised from alcohol to give **(5)**.

Yield 82%; m.p. 195°C; IR(KBr, cm$^{-1}$): 1662(C=O), 818(C-N, *s*-triazine), 773 (C-Cl); $^1$H NMR($CDCl_3$): δ2.54(s, 3H, -$COCH_3$), δ7.26 to 8.41(m, 12 Ar-H and 3 NH). Anal. Calcd. for $C_{23}H_{18}N_6Cl_2$: C,59.35; H,3.87; N,18.06. Found: C,59.36; H,3.85; N,18.09.

### Preparation of 2,4-bis-(4'-chlorophenylamino)-6-[4'-{3''-(3''',4''',5'''-trimethoxyphenyl)-2''-propenon-1''-yl}-phenylamino]-*s*-triazine (6a).

2,4-Bis-(4'-chlorophenylamino)-6-(4'-acetyl phenylamino)-*s*-triazine **(5)**(0.01 mole) was dissolved in DMF(30ml) and 40% KOH in distilled water was added to it. Then 3,4,5-trimethoxy benzaldehyde (0.01mole) was added with constant stirring at room temperature. After 24 hours the reaction mixture was poured into crushed ice and neutralize with HCl. The product separated out was filtered, washed with water and recrystllised from alcohol to give **(6a)**.

Yield 78%; m.p. 153°C; IR (KBr, cm$^{-1}$):1652 (C=O), 1026 (C-O-C), 815 (C-N, *s*-triazine), 757(C-Cl); $^1$H NMR ($CDCl_3$): δ3.74(s, 6H, m-$OCH_3$), δ3.84(s, 3H, p-$OCH_3$), δ7.30(d, 1H, -CO-CH=), δ7.10 to 7.81(m, 14 Ar-H and 3 NH), δ8.05 (d, 1H, Ar-CH=). Anal. Calcd. for $C_{33}H_{28}N_6O_4Cl_2$: C,61.59; H,4.35; N,13.06. Found: C,61.62; H,4.33; N,13.08.

### Preparation of 2,4-bis-(4'-chlorophenylamino)-6-

**TABLE 1 : Physical and analytical data of compounds (6a-d), (7a-d), (8a-d) and (9a-d)**

| No. | R | M.P. °C | Yield (%) | M.F. | Found (calcd)% | | |
|---|---|---|---|---|---|---|---|
| | | | | | C | H | N |
| 6a | 3,4,5-Trimethoxyphenyl | 153 | 78 | $C_{33}H_{28}N_6O_4Cl_2$ | 61.62(61.59) | 4.33(4.35) | 13.08(13.06) |
| 6b | 2-Furanyl | 137 | 72 | $C_{28}H_{20}N_6O_2Cl_2$ | 61.90(61.88) | 3.70(3.68) | 15.49(15.47) |
| 6c | 2-Thienyl | 148 | 73 | $C_{28}H_{20}N_6OSCl_2$ | 60.13(60.11) | 3.55(3.58) | 15.06(15.03) |
| 6d | Cinnamyl | 174 | 70 | $C_{32}H_{24}N_6OCl_2$ | 66.30(66.32) | 4.18(4.15) | 14.54(14.51) |
| 7a | 3,4,5-Trimethoxyphenyl | 160 | 72 | $C_{34}H_{29}N_9O_3Cl_2$ | 59.84(59.82) | 4.27(4.25) | 18.49(18.48) |
| 7b | 2-Furanyl | 170 | 66 | $C_{29}H_{21}N_9OCl_2$ | 59.78(59.79) | 3.58(3.61) | 21.67(21.65) |
| 7c | 2-Thienyl | 165 | 68 | $C_{29}H_{21}N_9SCl_2$ | 58.21(58.19) | 3.54(3.51) | 21.09(21.07) |
| 7d | Cinnamyl | 159 | 63 | $C_{33}H_{25}N_9Cl_2$ | 64.06(64.08) | 4.06(4.05) | 20.36(20.39) |
| 8a | 3,4,5-Trimethoxyphenyl | 170 | 71 | $C_{36}H_{29}N_9O_3Cl_2$ | 61.21(61.19) | 4.13(4.11) | 17.82(17.85) |
| 8b | 2-Furanyl | 183 | 67 | $C_{31}H_{21}N_9OCl_2$ | 61.36(61.39) | 3.49(3.47) | 20.76(20.79) |
| 8c | 2-Thienyl | 123 | 68 | $C_{31}H_{21}N_9SCl_2$ | 59.84(59.81) | 3.36(3.38) | 20.24(20.26) |
| 8d | Cinnamyl | 187 | 62 | $C_{35}H_{25}N_9Cl_2$ | 65.45(65.42) | 3.90(3.89) | 19.66(19.63) |
| 9a | 3,4,5-Trimethoxyphenyl | 157 | 70 | $C_{35}H_{32}N_8O_4Cl_2$ | 60.06(60.09) | 4.60(4.58) | 16.00(16.02) |
| 9b | 2-Furanyl | 176 | 69 | $C_{30}H_{24}N_8O_2Cl_2$ | 60.13(60.10) | 4.03(4.01) | 18.72(18.70) |
| 9c | 2-Thienyl | 238 | 68 | $C_{30}H_{24}N_8OSCl_2$ | 58.57(58.54) | 3.93(3.90) | 18.24(18.21) |
| 9d | Cinnamyl | 143 | 67 | $C_{34}H_{28}N_8OCl_2$ | 64.27(64.25) | 4.39(4.41) | 17.66(17.64) |

**[4'-{2''-amino-6''-(3''',4''',5'''-trimethoxy phenyl)-pyrimidine-4''-yl}-phenylamino]-*s*-triazine (7a)**

A mixture of 2,4-bis-(4'-chlorophenylamino)-6-[4'-{3''-(3''',4''',5'''-trimethoxy phenyl)-2''-propenon-1''-yl}-phenylamino]-*s*-triazine**(6a)** (0.01mole) in 25ml dioxane, guanidine nitrate (0.01mole) and 40% KOH in distilled water (2ml) was refluxed for 10 hours. Then the reaction mixture was cooled and poured into crushed ice. The product separated out was filtered, washed with water and recrystallised from alcohol to give **(7a)**.

Yield 72%; m.p. 160°C; IR (KBr, $cm^{-1}$): 3380 (-$NH_2$), 1613(C=N, pyrimidine moiety), 1021(C-O-C), 819(C-N, *s*-triazine), 770(C-Cl); $^1$H NMR($CDCl_3$): δ3.74 (s, 6H, m-$OCH_3$), δ3.84(s, 3H, p-$OCH_3$), δ 5.18(s, 2H, -$NH_2$), δ 7.0 to 8.12(m, 15 Ar-H and 3 NH). Anal. Calcd. for $C_{34}H_{29}N_9O_3Cl_2$: C,59.82; H,4.25; N,18.48. Found: C,59.84; H,4.27; N,18.49.

**Preparation of 2,4-bis-(4'-chlorophenylamino)-6-[4'-{2''-amino-3''-cyano-4''-(3''',4''',5'''-trimethoxyphenyl)-pyridine-6''-yl}-phenylamino]-*s*-triazine (8a)**

A mixture of 2,4-bis-(4'-chlorophenylamino)-6-[4'-{3''-(3''',4''',5'''-trimethoxy phenyl)-2''-propenon-1''-yl}-phenylamino]-*s*-triazine **(6a)** (0.01mole) in 50ml alcohol, malononitrile (0.01mole) and ammonium acetate (0.02mole) was refluxed for 10 hours. Then the reaction mixture was cooled and poured into crushed ice. The product separated out was filtered, washed with water and recrystallised from alcohol to give **(8a)**.

Yield 71%; m.p. 170°C; IR(KBr, $cm^{-1}$): 3383(-$NH_2$), 2213(C=N, pyridine moiety), 1027 (C-O-C), 815(C-N, *s*-triazine), 774(C-Cl); $^1$H NMR($CDCl_3$): δ 3.72 (s, 6H, m-$OCH_3$), δ3.83 (s, 3H, p-$OCH_3$), δ 5.25 (s, 2H, -$NH_2$), δ 7.0 to 8.0 (m, 15 Ar-H and 3 NH). Anal. Calcd. for $C_{36}H_{29}N_9O_3Cl_2$: C,61.19; H,4.11; N,17.85. Found: C,61.21; H,4.13; N,17.82.

**Preparation of 2,4-bis-(4'-chlorophenylamino)-6-[4'-{1"-acetyl- 5''-(3''',4''',5'''-trimethoxy phenyl)-2''-pyrazoline-3''-yl}phenylamino]-*s*-triazine (9a)**

A mixture of 2,4-bis-(4'-chlorophenylamino)-6-[4'-{3''-(3''',4''',5'''-trimethoxy phenyl)-2''-propenon- 1''-yl}-phenylamino]-*s*-triazine **(6a)** (0.01mole) in 20ml glacial aceticacid, hydrazine hydrate (0.01mole) was refluxed for 8 hours. Then the reaction mixture was cooled and poured into crushed ice. The product separated out was filtered, washed with water and recrystallised from alcohol to give **(9a)**.

Yield 70%; m.p. 157°C; IR (KBr, $cm^{-1}$): 1568 (C=N, pyrazoline moiety), 1030 (C-O-C), 813(C-N, *s*-triazine), 764(C-Cl); $^1$H NMR ($CDCl_3$): δ 2.45 (s, 3H, -$COCH_3$), δ3.04 (dd, 1H, -$CH_A$), δ3.45(dd, 1H, -$CH_B$), δ 3.72(s, 6H, m-$OCH_3$), δ3.83(s, 3H, p-$OCH_3$), 5.62(dd, 1H, -CH), 6.90 to 7.70 (m, 14 Ar-

**TABLE 2: Antimicrobial activity of compounds (6a-d), (7a-d), (8a-d) and (9a-d)**

| No. | R | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | *S.aureus* | *B.subtilis* | *E.coli* | *S.paratyphi-B* |
| 6a | 3,4,5-Trimethoxyphenyl | 10 | 17 | 15 | 19 |
| 6b | 2-Furanyl | 12 | 16 | - | 20 |
| 6c | 2-Thienyl | 10 | - | 16 | 16 |
| 6d | Cinnamyl | 12 | 18 | - | 18 |
| 7a | 3,4,5-Trimethoxyphenyl | 10 | 14 | 19 | 21 |
| 7b | 2-Furanyl | 14 | 16 | 16 | 18 |
| 7c | 2-Thienyl | 13 | - | 18 | 20 |
| 7d | Cinnamyl | - | 15 | 18 | 20 |
| 8a | 3,4,5-Trimethoxyphenyl | 22 | 13 | - | - |
| 8b | 2-Furanyl | 12 | 16 | 18 | 20 |
| 8c | 2-Thienyl | 10 | 15 | 18 | 21 |
| 8d | Cinnamyl | 11 | 16 | 19 | 20 |
| 9a | 3,4,5-Trimethoxyphenyl | - | 17 | 16 | 16 |
| 9b | 2-Furanyl | 12 | 16 | - | 21 |
| 9c | 2-Thienyl | - | 17 | - | 18 |
| 9d | Cinnamyl | 10 | 15 | 12 | 20 |
| Standard drug | Ciprofloxacin | 22 | 20 | 20 | 18 |

H and 3 NH). Anal. Calcd. for $C_{35}H_{32}N_8O_4Cl_2$: C,60.09; H,4.58; N,16.02. Found: C,60.06; H,4.60; N,16.00.

## CONCLUSION

By visualizing activity data, it is observed that compound **(8a)** exhibit significant activity against *S.aureus*. Compound **(6d)** showed moderately active against *B. subtilis*. In case of *E.coli* **(7a)**, **(7c)**, **(7d)**, **(8b)**, **(8c)** and **(8d)** showed good activity. compounds **(6a)**, **(6b)**, **(6d)**, **(7a)**, **(7b)**, **(7c)**, **(7d)**, **(8b)**, **(8c)**, **(8d)**, **(9b)**, **(9c)** and **(9d)** showed remarkable activity against *s.paratyphi-B*. All the antibacterial data are represented in TABLE 2.

From the above data, it can be concluded that most of the compounds showed promising activity against Gram negative bacteria in comparison of Gram positive bacteria.

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