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Synthesis and antimicrobial studies of some new *s*-triazine based chalcones, acetylpyrazolines and aminopyrimides

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ABSTRACT

Some new chalcones, 2-4 bis (tetrahydro - 1,4-oxazine)-6-[4'-{3"-(substituted phenyl) -2"-propenon-1"-yl} phenyl amino]-*s*-triazine (6a-f) have been achieved by the reaction between 2-4-(bis- tetrahydro- 1,4-oxazine) -6-(4'-acetylphenylamino)-*s*-triazine (5) and different aromatic aldehydes, which on cyclisation with hydrazine hydrate in presence of acetic acid give acetyl pyrazolines (7a-f). Chalcones (6a-f) on cyclisation with guanidine hydrochloride in presence o f alkali give aminopyrimidines (8a-f). The characterization of newly synthesised compounds has been done on the basis of IR, ^{1}H NMR spectral data as well as elemental analysis. All the synthesised compounds have been screened for their antimicrobial activity.

Introduction

The *s*-triazine based chalcones and their derivatives have their own importance in heterocyclic chemistry due to their good biological activities. The presence of a reactive α, β unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity [1]. In recent years a variety of chalcones have been reviewed for their anticancer chemoprevenive and mutagenic as well as antiviral, insecticidal and enzyme inhibitory properties [2,3]. A number of chalcones having hydroxy, alkoxy groups in different position have been reported to possess antibacterial [4], antiulcer [5], antifungal [6], antioxidant [7], vasodilatory [8], antimitotic [9], antimalarial [10] and antileshmanial [11] activities etc… . Pyrazolines are well known and important nitrogen-containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. They have several prominent effects, such as cerebroprotective [12], cardiovascular [13-14], Parkinson & Alzheimer [15] and antiamoebic properties [16]. Nitrogen heterocycles have received a great deal of attention in the literature as a result of their role as pharmacophores of great historical significance. which are those annelated to a pyrimidine ring, are of paramount importance because of their wide range of biological [17] and pharmaceutical applications (i.e.,bronchodilators, vasodilators) and their antiallergic, cardiotonic, antihypertensive and hepatoprotective activities [18]. Some of them have shown properties as antitumour, antibacterial, analgesic and CNS depressants [19] activities etc… . In continuation of our work [20-24], the scope for further studies on chalcones and its derivatives, we herein report some novel chalcones (**6a-f**), acetyl pyrazolines (**7a-f**) and aminopyrimidines (**8a-f**). The synthesised compounds were ascertained from spectral analysis.

Materials and Methods

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 Varian Gemini 400 MHz spectrometer with $CDCl_3$ as a solvent and TMS as internal reference. The chemical shifts are expressed in parts per million (ppm) downfield from the internal standard and signals are quoted as *s* (singlet), *d* (doublet) and *m* (multiplate). Thin Layer Chromatography (TLC) analytical separation was conducted with Silica Gel 60 F-254 (Merck) plates of 0.25mm thickness eluted with visualized with UV (254nm) or iodine to check the purity of the synthesised compounds.

General procedure for the compounds (3), (4) and (5). compounds (3), (4) and (5) werw prepared by the reported method [25]

Preparation of 2-4 bis (tetrahydro- 1,4-oxazine) 6- [4'- { 3''-(3'''-methoxyphenyl) -2''-propenon-1''-yl } phenyl amino]-*s*-triazine (6a).

Compound **(5)** (0.01 mole) was dissolved in DMF (30 ml) and 3-methoxybenzaldehyde (0.01 mole) ware added to it. Then solution of KOH (5 ml of 40%) was added to the reaction mixture with constant stirring at room temperature. The progress of the reaction was monitored on TLC plate. After completion, the reaction mixture was poured into crushed ice and neutralise with HCl. The product separated out was filtered, washed with water and dried. The product separated out was filtered, washed with water, dried and recrystallised from alcohol.

Similarly, the remaining compounds **(6b-f)** were prepared by this method. Their physical data are given in **Table-1**

Compound (6a) : IR (KBr) cm^{-1}: 1649 (-C=O), 1220 (C-O-C, ether), 806 (C-N, *s*-triazine), 786 (=CH). ^{1}H NMR ($CDCl_3$) : 3.52 (t, 8H, oxazine ring), 3.63 (t, 8H, oxazine ring), δ 3.92 (s, 3H, m-OCH_3), δ 6.70 (s, 1H, -NH), δ 7.1 - 7.8 (m, 8H, Ar-H), δ 8.40 (d, lH, -CO-CH=), δ 8.7 (d, 1H, Ar-CH=). Anal. Calcd. for

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$C_{27}H_{30}N_6O_4$: C, 64.53; N, 16.72; H, 6.01 Found : C, 64.35; N, 16.55; H, 6.00%.

Compound (6b) : IR (KBr) cm^{-1}: 1639 (-C=O), 1545 (C-NO_2), 1250 (C-O-C, ether), 801 (C-N, *s*-triazine), 797 (=CH). ^1H NMR ($CDCl_3$) : 3.42 (t, 8H, oxazine ring), 3.55 (t, 8H, oxazine ring), δ 6.77 (s, 1H, -NH), δ 7.2 - 7.8 (m, 8H, Ar-H), δ 8.33 {d, lH, -CO-CH=), δ 8.62 (d, 1H, Ar-CH=). Calcd. for $C_{26}H_{27}N_7O_5$: C, 60.34; N, 18.95; H, 5.25 Found : C, 60.30; N, 18.55; H, 5.28%.

Compound (6c) : IR (KBr) cm^{-1}: 1619 (-C=O), 1234 (C-O-C, ether), 809 (C-N, *s*-triazine), 820 (=CH). ^1H NMR ($CDCl_3$) : 3.39 (t, 8H, oxazine ring), 3.50 (t, 8H, oxazine ring), δ 3.89 (s, 6H, m-OCH_3), δ 4.09 (s, 3H, p-OCH_3), δ 6.70 (s, 1H, -NH), δ 7.1 - 7.8 (m, 7H, Ar-H), δ 8.23 (d, lH, -CO-CH=), δ 8.52 (d, 1H, Ar-CH=). Anal. Calcd. for $C_{29}H_{34}N_6O_6$: C, 61.91; N, 14.94; H, 6.09 Found : C, 61.94; N, 14.95; H, 6.02%.

Compound (6d) : IR (KBr) cm^{-1}: 1638 (-C=O), 1259 (C-O-C, ether), 800 (C-N, *s*-triazine), 793 (=CH), 594 (-C-Br). ^1H NMR ($CDCl_3$) : 3.45 (t, 8H, oxazine ring), 3.60 (t, 8H, oxazine ring), δ 6.57 (s, 1H, -NH), δ 7.0 - 8.1 (m, 8H, Ar-H), δ 8.41 {d, lH, -CO-CH=), δ 8.81 (d, 1H, Ar-CH=). Anal. Calcd. for $C_{26}H_{27}BrN_6O_3$: C, 56.63; N, 15.24; H, 4.93 Found : C, 56.60; N, 15.21; H, 4.91%.

Compound (6e) : IR (KBr) cm^{-1}: 1622 (-C=O), 1244 (C-O-C, ether), 806 (C-N, *s*-triazine), 802 (=CH), 786 (C-Cl). ^1H NMR ($CDCl_3$) : 3.39 (t, 8H, oxazine ring), 3.50 (t, 8H, oxazine ring), δ 6.70 (s, 1H, -NH), δ 7.1 - 7.8 (m, 13H, Ar-H), δ 8.23 (d, lH, -CO-CH=), δ 8.52 (d, 1H, Ar-CH=). Anal. Calcd. for $C_{32}H_{32}N_6O_4$: C, 68.07; N, 14.88; H, 5.71 Found : C, 68.05; N, 14.80; H, 5.73%.

Compound (6f) : IR (KBr) cm^{-1}: 1682 (-C=O), 1257 (C-O-C, ether), 802 (C-N, *s*-triazine), 818 (=CH). ^1H NMR ($CDCl_3$) : 3.29 (t, 8H, oxazine ring), 3.30 (t, 8H, oxazine ring), δ 6.70 (s, 1H, -NH), δ 7.1 - 7.9 (m, 7H, Ar-H), δ 8.29 (d, lH, -CO-CH=), δ 8.50 (d, 1H, Ar-CH=). Anal. Calcd. for $C_{24}H_{26}N_6O_4$: C, 62.33; N, 18.17; H, 5.66 Found : C, 62.35; N, 18.20; H, 5.69%.

Preparation of 2,4-bis-(tetrahydro-1,4-oxazine)-6-[4''-{1''-acetyl-5''-(3'''-methoxyphenyl)-2''-pyrazoline-3''-yl} phenylamino]-*s*-triazine (7a)

Compound **(6a)** (0.01 mol) in minimum amount of glacial acetic acid and hydrazine hydrate (0.015 mol, 0.75g) ware refluxed for 4 hours. The progress of the reaction was monitored on TLC plate. After completion, the reaction mixture was cooled and poured into crushed ice and the product separated out was filtered, washed with water and recrystallised from alcohol to give **(7a)**.

Similarly, the remaining compounds **(7b-f)** were prepared by this method. Their physical data are given in **Table-1**

Compound (7a) : IR (KBr) cm^{-1}: 3272 (-NH), 1650 (C=O), 1613 (C=N, pyrazoline moiety), 1039 (C-O-C). ^1H NMR ($CDCl_3$) δ ppm : δ 2.5 (s, 3H, -COC$\underline{H}_3$), 3.0 (d, 1H, -C$\underline{H}$a-CH-), 3.6 (d, 1H, -C$\underline{H}$b-CH-), δ 3.58 (t, 8H, oxazine ring), δ 3.78 (t, 8H, oxazine ring), 3.7 (s, 3H, m-OC$\underline{H}_3$), 5.8 (dd , 1H, -C$\underline{H}$-CH_2), 6.8 to 7.8 (m, 9H, 8Ar-$\underline{H}$ and 1-N$\underline{H}$). Anal. Calcd. for $C_{29}H_{34}N_8O_4$: C, 62.35; N, 20.06; H, 6.13 Found : C, 62.32; N, 20.04; H, 6.11%.

Compound (7b) : IR (KBr) cm^{-1}: 3275 (-NH), 1640 (C=O), 1610 (C=N, pyrazoline moiety), 1075 (C-NO_2), 1045 (C-O-C). ^1H NMR ($CDCl_3$) δ ppm : δ 2.5 (s, 3H, -COC$\underline{H}_3$), 3.0 (d, 1H, -C$\underline{H}$a-CH-), 3.6 (d, 1H, -C$\underline{H}$b-CH-), δ 3.58 (t, 8H, oxazine ring), δ 3.78 (t, 8H, oxazine ring), 5.8 (dd , 1H, -C$\underline{H}$-CH_2), 6.8 to 7.8 (m, 9H, 8Ar-$\underline{H}$ and 1-N$\underline{H}$). Anal. Calcd. for $C_{28}H_{31}N_9O_5$: C, 58.63; N, 21.98; H, 5.45 Found : C, 58.60; N, 21.94; H, 5.43%.

Compound (7c) : IR (KBr) cm^{-1}: 3260 (-NH), 1666 (C=O), 1611 (C=N, pyrazoline moiety), 1032 (C-O-C). ^1H NMR ($CDCl_3$) δ ppm : δ 2.5 (s, 3H, -COC$\underline{H}_3$), 3.0 (d, 1H, -C$\underline{H}$a-CH-), 3.4 (d, 1H, -C$\underline{H}$b-CH-), δ 3.58 (t, 8H, oxazine ring), δ 3.60 (t, 8H, oxazine ring), 3.7 (s, 6H, m-OC$\underline{H}_3$), 3.79 (s, 3H, p-OC$\underline{H}_3$), 5.8 (dd , 1H, -C$\underline{H}$-CH_2), 6.8 to 7.8 (m, 7H, 6Ar-$\underline{H}$ and 1-N$\underline{H}$). Anal. Calcd. for $C_{31}H_{38}N_8O_6$: C, 60.18; N, 18.11; H, 6.19 Found : C, 60.20; N, 18.12; H, 6.18%.

Compound (7d) : IR (KBr) cm^{-1}: 3273 (-NH), 1640 (C=O), 1615 (C=N, pyrazoline moiety), 598 (C-Br), 1043 (C-O-C). ^1H NMR ($CDCl_3$) δ ppm : δ 2.54 (s, 3H, -COC$\underline{H}_3$), 3.1 (d, 1H, -C$\underline{H}$a-CH-), 3.5 (d, 1H, -C$\underline{H}$b-CH-), δ 3.66 (t, 8H, oxazine ring), δ 3.78 (t, 8H, oxazine ring), 5.9 (dd , 1H, -C$\underline{H}$-CH_2), 6.8 to 7.8 (m, 9H, 8Ar-$\underline{H}$ and 1-N$\underline{H}$). Anal. Calcd. for $C_{28}H_{31}BrN_8O_3$: C, 55.36; N, 18.44; H, 5.14 Found : C, 55.40; N, 18.47; H, 5.16%.

Compound (7e) : IR (KBr) cm^{-1}: 3270 (-NH), 1645 (C=O), 1605 (C=N, pyrazoline moiety), 1040 (C-O-C). ^1H NMR ($CDCl_3$) δ ppm : δ 2.49 (s, 3H, -COC$\underline{H}_3$), 3.19 (d, 1H, -C$\underline{H}$a-CH-), 3.45 (d, 1H, -C$\underline{H}$b-CH-), δ 3.60 (t, 8H, oxazine ring), δ 3.72 (t, 8H, oxazine ring), 5.89 (dd , 1H, -C$\underline{H}$-CH_2), 6.7 to 7.9 (m, 14H, 13Ar-$\underline{H}$ and 1-N$\underline{H}$). Anal. Calcd. for $C_{34}H_{36}N_8O_4$: C,65.79; N, 18.05; H, 5.85 Found : C,65.80; N, 18.08; H, 5.89%.

Compound (7f) : IR (KBr) cm^{-1}: 3260 (-NH), 1666 (C=O), 1611 (C=N, pyrazoline moiety), 1032 (C-O-C). ^1H NMR ($CDCl_3$) δ ppm : δ 2.4 (s, 3H, -COC$\underline{H}_3$), 3.09 (d, 1H, -C$\underline{H}$a-CH-), 3.38 (d, 1H, -C$\underline{H}$b-CH-), δ 3.56 (t, 8H, oxazine ring), δ 3.67 (t, 8H, oxazine ring), 5.7 (dd , 1H, -C$\underline{H}$-CH_2), 6.8 to 8.6 (m, 9H, 8Ar-$\underline{H}$ and 1-N$\underline{H}$). Anal. Calcd. for $C_{28}H_{31}N_9O_5$: C, 58.63; N, 21.98; H, 6.10 Found : C, 58.60; N, 21.89; H, 6.08%.

Preparation of 2,4-bis-tetrahydro- 1,4-oxazine-6-[4'-{2"-amino-6"-(3'''-methoxyphenyl)- pyrimidin-4"-yl} phenyl amino]-*s*-triazine(8a)

Compound **(6a)** (0.01 mol) was dissolved in alcohol (25 ml) and guanidine hydrochloride (0.01 mol) were added to it. Then solution of KOH (5 ml of 40%) was added to the reaction mixture and refluxed for 10 hours. The reaction mixture was cooled, poured into crushed ice and product separated out was filtered, washed with water, dried and recrystallised from alcohol to give **(7a)**.

Similarly, the remaining compounds **(8b-f)** were prepared by this method. Their physical data are given in **Table-1.**

Compound (8a) : IR (KBr) cm^{-1}: 3398 (-NH_2), 1575 (C=N), 806 (C-N, *s*-triazine), ^1H NMR ($CDCl_3$) : δ 3.85 (s, 3H, m-OCH_3), δ 3.58 (t, 8H, oxazine ring), δ 3.78 (t, 8H, oxazine ring), 5.1 (s, 2H, -NH_2), 6.85 (1H, *s*, -CH=), 6.90 to 8.15 (m, 9H, 8Ar-H and 1H, -NH). Anal. Calcd. for $C_{28}H_{31}N_9O_3$: C, 62.09; N, 23.27; H, 5.77. Found : C, 62.07; N, 23.25; H, 5.76%.

Compound (8b) : IR (KBr) cm^{-1}: 3382 (-NH_2), 1564 (C=N), 1075 (C-NO_2), 800 (C-N, *s*-triazine), ^1H NMR ($CDCl_3$) : δ 3.46 (t, 8H, oxazine ring), δ 3.69 (t, 8H, oxazine ring), 5.4 (s, 2H, -NH_2), 6.82 (1H, *s*, -CH=), 6.90 to 8.15 (m, 9H, 8Ar-H and 1H -NH). Anal. Calcd. for $C_{27}H_{28}N_{10}O_4$: C, 58.27; N, 25.17; H, 5.07. Found : C, 58.24; N, 25.15; H, 5.03%.

Compound (8c) : IR (KBr) cm^{-1}: 3398 (-NH_2), 1575 (C=N), 806 (C-N, *s*-triazine), ^1H NMR ($CDCl_3$) : δ 3.75 (s, 6H, m-OCH_3), δ 3.80 (s, 3H, p-OCH_3), δ 3.50 (t, 8H, oxazine ring), δ 3.75 (t, 8H, oxazine ring), 5.23 (s, 2H, -NH_2), 6.80 (1H, *s*, -CH=), 6.90 to 8.15 (m, 7H, 6H, Ar-H and 1H, -NH). Anal. Calcd. for $C_{30}H_{35}N_9O_5$: C, 58.89; N, 20.95; H, 5.86. Found : C, 58.90; N, 20.98; H, 5.89%.

Table-1 The physical data of synthesis ed compounds (6a-f), (7a-f) and (8a-f)

Compounds	R	M.P.	% Yield
6a	3-Methoxyphenyl	118-122 °C	75
6b	3-Nitrophenyl	216-220 °C	69
6c	3,4,5-Trimethoxyphenyl	121-126 °C	76
6d	4-Bromophenyl	110-115 °C	69
6e	3-Phenoxyphenyl	111-115 °C	66
6f	2-Furanayl	145-150 °C	72
7a	3-Methoxyphenyl	155-160 °C	70
7b	3-Nitrophenyl	256-261 °C	72
7c	3,4,5-Trimethoxyphenyl	142-147 °C	59
7d	4-Bromophenyl	148-152 °C	68
7e	3-Phenoxyphenyl	118-123 °C	55
7f	2-Furanayl	275-281 °C	51
8a	3-Methoxyphenyl	146-151 °C	65
8b	3-Nitrophenyl	270-275 °C	59
8c	3,4,5-Trimethoxyphenyl	157-162 °C	67
8d	4-Bromophenyl	127-132 °C	58
8e	3-Phenoxyphenyl	135-141 °C	49
8f	2-Furanayl	145-150 °C	64

Table 2 – Antibacterial and antifungal activity data of compounds 7(a-f), 8(a-f) and 9(a-f).

Compound	Minimal bactericidal concentration µg/ml				Minimal fungicidal concentration µg/ml		
	Gram positive		Gram negative				
	S.aureus MTCC-96	*S.pyogenus* MTCC-442	*E. coli* MTCC-443	*P. aerug* MTCC-1688	*C. albicans*MTCC-227	*A. niger* MTCC-282	*A. clavatus*MTCC-1323
7a	200	250	200	250	500	1000	1000
7b	125	125	500	250	1000	250	250
7c	500	500	62.5	100	1000	500	1000
7d	100	125	125	250	500	1000	1000
7e	200	250	200	250	250	500	500
7f	250	250	250	250	1000	1000	1000
8a	125	250	200	200	500	250	500
8b	200	200	100	200	500	500	500
8c	500	500	200	200	1000	250	500
8d	250	250	200	200	1000	500	500
8e	500	500	125	125	1000	1000	500
8f	250	250	250	250	250	>1000	>1000
9a	100	125	250	250	500	1000	1000
9b	500	500	200	100	1000	>1000	>1000
9c	100	125	500	500	1000	>1000	>1000
9d	500	500	500	500	>1000	250	500
9e	100	62.5	250	250	1000	500	1000
9f	250	250	200	200	250	>1000	>1000
Ampicillin	250	100	100	100	-	-	-
Griseofulvin	-	-	-		500	100	100

Compound (8d) : IR (KBr) cm^{-1}: 3380 (-NH_2), 1562 (C=N), 575 (C-Br), 808 (C-N, *s*-triazine), ^1H NMR ($CDCl_3$) : δ 3.46 (t, 8H, oxazine ring), δ 3.69 (t, 8H, oxazine ring), 5.4 (s, 2H, -NH_2), 6.80 (1H, *s*, -CH=), 6.90 to 8.15 (m, 9H, 8Ar-H and 1H, -NH). Anal. Calcd. for $C_{27}H_{28}BrN_9O_2$: C, 54.92; N, 21.35; H, 4.78. Found : C, 54.94; N, 21.37; H, 4.80%.

Compound (8e) : IR (KBr) cm^{-1}: 3375 (-NH_2), 1562 (C=N), 803 (C-N, *s*-triazine), ^1H NMR ($CDCl_3$) : δ 3.46 (t, 8H, oxazine ring), δ 3.69 (t, 8H, oxazine ring), 5.4 (s, 2H, -NH_2), 6.80 (1H, *s*, -CH=), 6.90 to 8.15 (m, 14H, 13Ar-H and 1H, -NH). Anal. Calcd. For $C_{33}H_{33}N_9O_3$: C, 65.66; N, 20.88; H, 5.51. Found : C, 65.67; N, 20.90; H, 5.53%.

Compound (8f) : IR (KBr) cm^{-1}: 3398 (-NH_2), 1575 (C=N), 806 (C-N, *s*-triazine), ^1H NMR ($CDCl_3$) : δ 3.50 (t, 8H, oxazine ring), δ 3.75 (t, 8H, oxazine ring), 5.23 (s, 2H, -NH_2), 6.89 (1H, *s*, -CH=), 6.90 to 8.15 (m, 8H, 7Ar-H and 1H, -NH). Anal. Calcd. for $C_{25}H_{27}N_9O_3$: C, 59.87; N, 25.53; H, 5.43. Found : C, 59.89; N, 25.56; H, 5.44%.

Results and Discussion

Minimum inhibitory concentration (MIC) of all the synthesised compounds have been screened by Broth dilution method [26] against four different strains, viz. Gram positive bacteria (*S. aureus* MTCC 96 and *S. pyogenes* MTCC 442) and Gram negative bacteria (*E.coli* MTCC 443 and *P. aeruginosa* MTCC 1688) and compared with standard drug : Ampicillin. Antifungal activity against *C. albicans* MTCC 227, *A. niger* MTCC 282 and *A. clavatus* MTCC 1323 organisms was determined by same method and compared with standard drug : Griseofulvin.

Antibacterial activity

In Gram positive bacterial strains compounds **7a, 7b, 7d, 7e, 8a, 8b, 9a, 9c** and **9e** showed good to very good activity (25 – 150 µg/ml) against *S. aureus* ; where as compound **9e** showed very good activity (62.5 – 100 µg/ml) against *S. pyogenes* compared with Ampicillin. In Gram negative bacterial strains : The result shows that compounds **7c** showed very good activity (25 – 125 µg/ml) against *E. coli* ; compounds **7c** and **9b** showed good activity (50 – 100 µg/ml) against *P. aeruginosa*. All others compounds show moderately active or less active against all bacterial strains.

Antifungal activity

From the screening results **(Table – 2)**, compounds **7e, 8f** and **9f** showed very good activity against *C. albicans*, while Compounds **7a, 7d, 8a, 8b** and **9a** showed good activity against *C. albicans* compared with Griseofulvin. Rest of the compounds show moderately active or less active against all bacterial strains.

Conclusion

From the results of antibacterial and antifungal activity; it can be concluded that the compounds bearing -OCH_3 and -Br group are more potent than the remaining compounds. They showed comparatively good antibacterial as well as antifungal activity.

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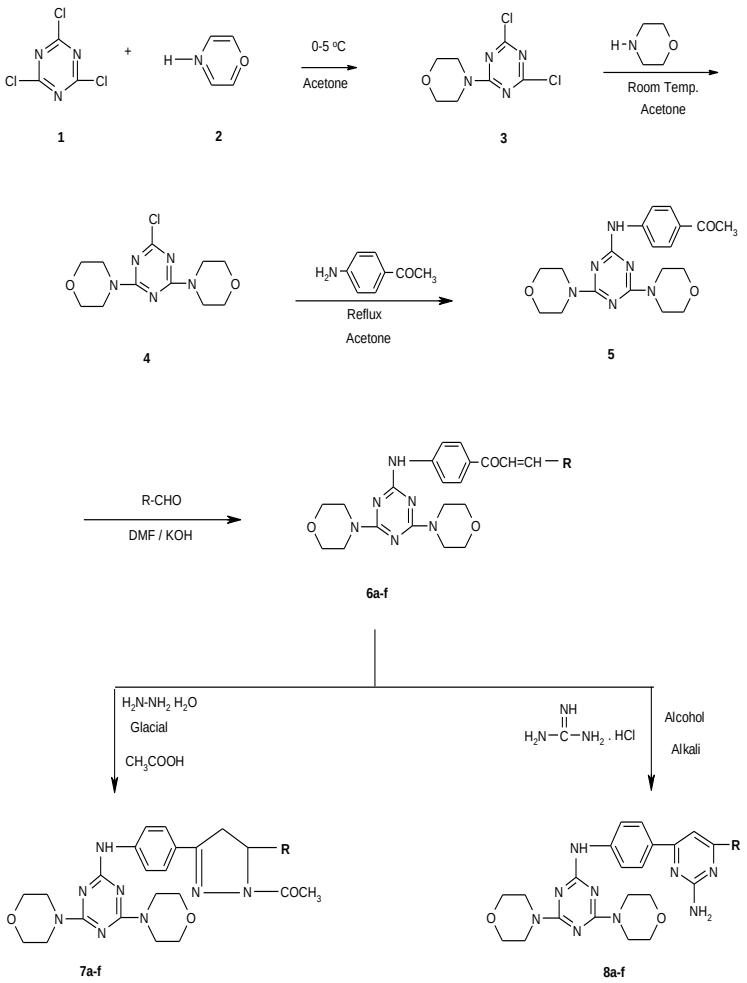

SCHEME -1

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