Efficient synthesis and antibacterial screening of 2,6-diamino-6-phenyl pyrimidine-5-carbonitrile derivatives

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ABSTRACT:

One-pot three component synthesis of 2,6-diamino-6-phenyl pyrimidine-5-carbonitrile derivatives by the condensation of substituted Aromatic aldehydes, malononitrile and guanidine hydro-chloride in ethanol using iron acetylacetonate $Fe(acac)_3$ catalyst. The products have been assayed for their antimicrobial screening against Gram+ve and Gram-ve bacteria. Some of the products showed moderate activity when compared with known standard drug viz. penicillin at the same concentration 50µgm/ml. Spectroscopic tequeniques are very good tools for the identification of compounds. The structures have been confirmed by 1H NMR, ^{13}C-NMR, IR, and Mass spectral data.

KEYWORDS: Aromatic aldehydes, Malononitrile, Guanidine Hydrochloride, Iron Acetylacetonate, Antimicrobial screening.

INTRODUCTION:

Multicomponent reactions (MCRs), by virtue of their convergence, productivity, facile execution and generally high yields, have attracted much attention in the area of combinatorial chemistry [1–3]. Heterocycles are of significant biological and pharmaceutical importance and play vital roles in drug discovery process. Among these, the multicomponent synthesis of polyfunctionalized heterocyclic compounds has become more challenging in organic and medicinal chemistry [4–6]. Pyrimidines are one of the biologically substantial scaffolds due to their wide range of biological and pharmaceutical properties such as antihypertensive [7], antimicrobial [8, 9], antitumor [10], antimalarial [11], antioxidant [12] and protein kinase inhibitors [13]. Pyrimidines having amino group at positions 2 and 4 of the ring have been reported to exhibit application in Alzheimer's disease treatment [14]. Moreover, pyrimidine derivatives have played a significant role in agriculture as crop protection agents and in electro-optics as building blocks for calamitic liquid crystals [15]. Due to the valuable biological significance of pyrimidine compounds, the synthesis of pyrimidine derivatives has received considerable attention. Until today, a number of methods have been reported for the synthesis of pyrimidine rings by the use of 1,3-binucleophilic centers such as guanidine, amidines, urea, and thiourea [16–20] in the presence of different catalysts such as sodium acetate [21], Bi(NO3)3.5H2O [22], NaOH [23, 24], CuO microspheres [25], K_2CO_3 and tetra butyl ammonium bromide [26].

LITERATURE REVIEW:

Herein, with the aim to improve more efficient synthetic procedures, reduce the number of separate reaction steps, minimize by-products and in continuation of our efforts to the preparation of heterocyclic compounds [27-28], we sought to design, synthesize and evaluate antimicrobial activity of pyrimidine derivatives through MCRs. Herein, we report synthesis of 2,6-diamino-6-phenyl pyrimidine-5-carbonitrile derivatives by using commercially available aromatic aldehydes, malononitrile and guanidine hydrochloride in ethanol at 80^0C, as shown in Scheme 1.

MATERIALS and METHOD:

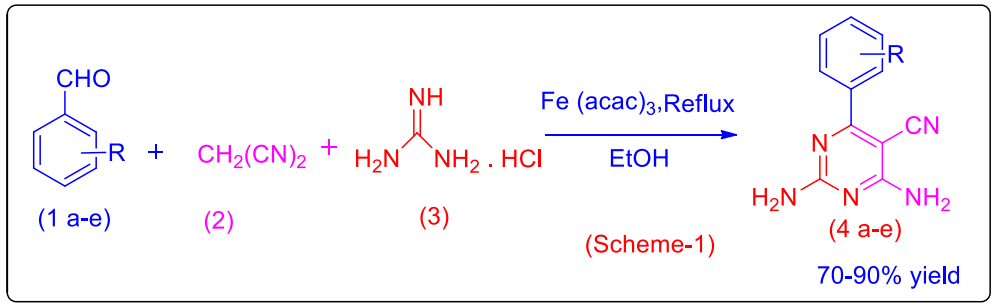
All the chemicals were obtained from Sigma Aldrich and Thomas Baker. These chemicals were applied without extra purification procedures. The reactions were carried out in dried glassware. The chemicals of analytical grade were procured from commercial sources and used as such without further purification. Open capillary tubes were used for melting points of isolated synthesized compounds and are uncorrected. Perkin-Elmer FTIR spectrophotometer was used for IR (KBr) spectra of compounds. Mass spectral data were recorded on liquid chromatography mass spectrometer (Shimadzu 2010Ev) using ESI probe. The ^{1}H and ^{13}C NMR spectra were recorded on spectrometer at 400MHz using TMS as an internal standard.

General procedure for the synthesis of 2,6-diamino-6-phenyl pyrimidine-5-carbonitrile derivatives (4a-e):

The equimolar mixture of aromatic aldehyde, (10 mmol), malononitrile (10 mmol) and $Fe(acac)_3$ (15 mole%) in 20 ml ethanol was stirred mechanically for at least 10 min, then guanidine hydrochloride (10 mmol) was added to the above reaction mixture and reaction mixture was refluxed till completion of reaction as monitored by TLC. After the

completion of reaction, the reaction mixture was poured into ice-cooled water and neutralized by 1:1 HCl to get the desired product. The separated solid was filtered, washed with little distilled water to remove acid. Finally, the crude product was purified by recrystallisation from ethanol to get pure product in almost quantitative yield (Scheme 1).

RESULT AND DISCUSSION:



We initially focused on optimization reaction condition. The reaction mixture of aromatic aldehyde, malononitrile and guanidine hydrochloride was refluxed independently in ethanol using $Fe(acac)_3$15 mole % as an efficient and novel catalyst, It was considered as a model reaction (**Scheme-1**) for investigating the effectiveness of different polar and non polar solvent using catalytic amount of $Fe(acac)_3$15 mmol %. Solvent optimization clearly noted that ethanol is the best solvent for the desired transformation due to fast reaction rate and high yield (Table1, entry 6). The other polar protic solvents gives moderate yield (Table1, entry 5), while other aprotic solvent like Acetonitrile, DMF, THF, DCM displayed slow reaction rates leading lower yield (Table-1, entry 1-4). We have carried out the model reaction using different stoichiometric amount of catalyst. The catalyst screening result are summarized in Table 2. It was observed that the excellent yield was achieved by using of Fe $(acac)_3$ 15 mole % (Table 2, entry 6).

Further investigating the influence of different parameters on the model reaction, we turned our attention towards the 2,6-diamino-6-phenyl pyrimidine-5-carbonitrile derivatives**(4 a-e)** using reaction of aromatic aldehyde **(1)**, malononitrile**(2),** and guanidine hydrochloride **(3),** was refluxed independently in ethanol using $Fe(acac)_3$15 mole % and the result are summarized in Table-3. With the both electron-poor and electron-rich benzaldhydes (Table-3, entries 1-5), the corresponding 2,6-diamino-6-phenyl pyrimidine-5-carbonitrile derivatives**(4a-e)** were obtained to excellent yields. These synthesized products **(4a-e)** were characterized from IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopic technique and elemental analysis.

TABLE-1: Optimization of the reaction conditions using different solvents[a]

Entry	Solvent	Reaction Time (h)	Yield (%)[b]
1	Acetonitrile	3.0	40
2	DCM	3.0	50
3	DMF	3.0	55
4	THF	3.0	62
5	Ethylene glycol	3.0	75
6	Ethanol-water	3.0	90

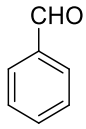
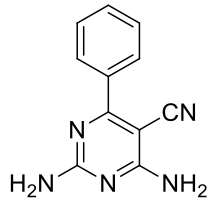
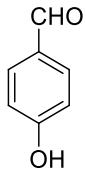
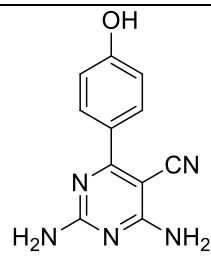
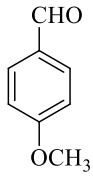
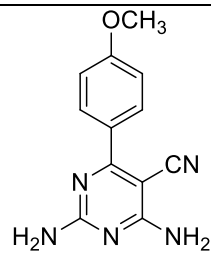
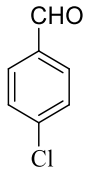
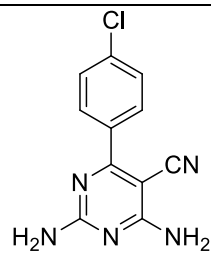
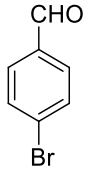
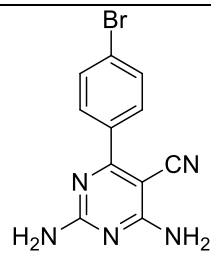
[a]***Reaction conditions:*** Aromatic aldehyde (10 mmol), Malononitrile (10 mmol) and guanidine hydrochloride (10 mmol) was refluxed.
[b] Isolated yields.

TABLE 2: Optimization Study for the amount Fe $(acac)_3$[a]

Entry	Catalyst (mole %)	Temperature (^{0}C)	Reaction Time (h)	Yield %[b]
1	01	80	3.0	45
2	02	80	3.0	50
3	05	80	3.0	55
4	08	80	3.0	60
5	10	80	3.0	70
6	15	80	3.0	90
7	20	80	3.0	92

[a]***Reaction conditions:*** Aromatic aldehyde (10 mmol), Malononitrile (10 mmol) and guanidine hydrochloride (10 mmol) was refluxed.
[b] Isolated yields.

Table 3: Aromatic aldehyde, Malononitrile and guanidine hydrochloride for the synthesis of **(4a-e)**[a]

Entry	Aldehyde (1a-e)	Products (4 a-e)	Time (h)	Yield (%)[b]	M.P. (^{0}C)
1		 **3a**	3.0	85	240-242
2		 **3b**	3.0	80	260-262
3		 **3c**	3.0	75	236-238
4		 **3d**	3.0	70	264-266
5		 **3e**	3.0	90	260-262

[a]***Reaction conditions:*** (1) (10 mmol), (2) (10 mmol), (3) (10 mmol) and ethanol in Fe $(acac)_3$ 15 mole % were refluxed.
[b] Isolated yield

Spectral Analysis:
2,4-diamino-6-phenyl pyrimidine-5-carbonitrile (4a):

M.P. 240-242^{0}C; Yield 85%; IR (KBr, *v*max, cm^{-1}), 3410, 3380, 3122, 2223; ^{1}H NMR (400MHz, DMSO-d$_6$, ppm) δ 7.04-7.38 (brs, 4H, 2NH$_2$), δ 7.40-7.80 (m, 5H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d$_6$, ppm): *δ* 170.4, 168.6, 166.2, 135.6., 130.1, 129.4, 128.0, 116.4, 78.7; EI-MS (m/z: RA %): 211 (M$^{+\cdot}$, 100%). Elemental analysis Calculated data for $C_{11}H_9N_5$; C, 62.55; H, 4.29; N, 33.16; Found: C, 62.50; H, 4.36; N, 33.10.

2,4-diamino-6-(4-hydroxyphenyl)pyrimidine-5-carbonitrile (4b):

M.P. 260-262^{0}C; Yield 80%; IR (KBr, *v*max, cm^{-1}), 3395, 3374, 3256, 3022, 2230; ^{1}H NMR (400MHz, DMSO-d$_6$, ppm) δ 7.20-7.50 (brs, 4H, 2NH$_2$), δ 4.62 (s, 1H, OH), δ 7.14 (d, 2H, Ar-H), δ 7.68 (d, 2H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d$_6$, ppm): *δ* 168.2, 167.4, 165.8, 155.6., 129.2, 118.0, 115.6, 78.9, 52.6; EI-MS (m/z: RA %): 227 (M$^{+\cdot}$, 100%). Elemental analysis Calculated data for $C_{11}H_9N_5O$; C, 58.14; H, 3.99; N, 30.82; Found: C, 58.10; H, 3.90; N, 30.88.

2,4-diamino-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile (4c):

M.P. 236-238^{0}C; Yield 75%; IR (KBr, *v*max, cm^{-1}), 3436, 3390, 3110, 2910, 2218; ^{1}H NMR (400MHz, DMSO-d$_6$, ppm) δ 7.10-7.70 (brs, 4H, 2NH$_2$), δ 3.78 (s, 1H, CH$_3$), δ 6.90 (d, 2H, Ar-H), δ 7.40 (d, 2H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d$_6$, ppm): *δ* 169.4, 167.6, 166.2, 160.3., 128.1, 120.0, 117.1, 79.2; EI-MS (m/z: RA %): 242 (M$^{+\cdot}$, 100%). Elemental analysis Calculated data for $C_{12}H_{11}N_5O$; C, 59.74; H, 4.60; N, 29.03; Found: C, 59.70; H, 4.60; N, 29.10.

2,4-diamino-6-(3-chlorophenyl)pyrimidine-5-carbonitrile (4d):

M.P. 264-266^{0}C; Yield 70%; IR (KBr, *v*max, cm^{-1}), 3466, 3374, 3145, 2215; ^{1}H NMR (400MHz, DMSO-d$_6$, ppm) δ 7.04-7.62 (brs, 4H, 2NH$_2$), δ 7.50 (d, 2H, Ar-H), δ 7.78 (d, 2H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d$_6$, ppm): *δ* 170.2, 168.6, 167.5, 151.3., 130.1, 128.4, 119.2, 78.7; EI-MS (m/z: RA %): 245 (M$^{+\cdot}$, 100%). Elemental analysis Calculated data for $C_{11}H_8N_5Cl$; C, 53.78; H, 3.28; N, 28.51; Found: C, 53.80; H, 3.30; N, 28.20.

2,4-diamino-6-(4-bromophenyl)pyrimidine-5-carbonitrile (4e):

M.P. 260-262^{0}C; Yield 90%; IR (KBr, *v*max, cm^{-1}), 3472, 3360, 3130, 2290; ^{1}H NMR (400MHz, DMSO-d$_6$, ppm) δ 7.12-7.55 (brs, 4H, 2NH$_2$), δ 7.70 (d, 2H, Ar-H), δ 7.94 (d, 2H, Ar-H); ^{13}C NMR (400 MHz, DMSO–d$_6$, ppm): *δ* 168.4, 167.5, 166.2, 134.7., 132.8, 122.5, 115.4, 79.4; EI-MS (m/z: RA %): 289 (M$^{+\cdot}$, 100%). Elemental analysis Calculated data for $C_{11}H_8N_5Br$; C, 45.58; H, 2.84; N, 24.20; Found: C, 45.54; H, 2.78; N, 24.14.

Antimicrobial Activity:

We have used the *Agar* well diffusion method for assessment of the antimicrobial activity of newly synthesized compounds. On Muller-Hinton agar medium zone of inhibition were observed and zone diameter was recorded in mm against specific test microorganisms.

The synthesized compounds were accessed antimicrobial activity particularly antibacterial and antifungal. The antibacterial activity against Gram positive Staphylococcus aureus bacteria and Gram negative bacteria are Escherichia coli, Proteus vulgaris using standard drugs are Penicillin and Streptomycin. The antifungal activity screened against Aspergillus fumigatus, Aspergillus niger using Nystatin as standard drug.

The synthesized compounds **4b, 4c and 4e** showed good antibacterial activity against **Staphylococcus aureus** as compaired to standard drugs Penicillin and Streptomycin. The compounds **4a, 4b and 4e** showed good antibacterial activity against **Escherichia colias** compaired to standard drugs Penicillin and Streptomycin. The synthesized compounds **4b, 4d and 4e** showed good zone of inhibition against **Proteus vulgaris** as compaired to Penicillin and Streptomycin.

The synthesized compounds **4b, 4d and 4e** showed good zone of inhibition against **Aspergillus fumigatus** as compaired to Nystatin. The synthesized compounds **4c, 4d and 4e** shows good zone of inhibition against **Aspergillus niger** as compaired to Nystatin.

TABLE 4: Antimicrobial activity of tested compounds (4 a-e).

Sr. No.	Compounds	Zone of inhibition* (mm)				
		Bacterial Species			Fungal Species	
		Gram positive	Gram negative		*Aspergillusf umigatus*	*Aspergillus niger*
		Staphylococcus aureus	*Escherichia coli*	*Proteus vulgaris*		
01	**4a**	12	14	10	10	ND
02	**4b**	16	18	16	16	12
03	**4c**	18	12	10	10	20
03	**4d**	16	10	16	16	18
05	**4e**	20	18	14	18	14
Ref*	Penicillin	26 mm	40 mm	18 mm	-----	-----
	Streptomycin	40 mm	35 mm	34 mm	----	-----
	Nystatin (50 µgm/ml)	-----	-----	-----	40 mm	28 mm

ND= Not detected zone of inhibition under experimental condition.

CONCLUSION:

We have developed an operationally simple, eco-friendly, inexpensive and efficient, synthesis of tetramethyl-hexahydro-1*H*-xanthene-1,8(2*H*)-dione derivatives. The procedure offers several advantages including improved yields, cleaner reactions and low cost which makes it a useful and attractive strategy in view of economic and environmental advantages. Furthermore these compounds were evaluated for their antibacterial activity. Some of the compounds showed good activity against gram positive and gram negative bacterial strains.

ACKNOWLEDGMENTS:

Authors are grateful to Principal, Yeshwant Mahavidyalaya, Nanded for providing laboratory facilities, SRTMUN for sanctioning MRP (APDS/Uni.MRP/Sci.and technology-hem./2019-20/2819, UGC, New Delhi (File no.41-230/2012) (SR) Vishnu chemical Hyderabad, The Director, CSIR-IICT, Hyderabad for providing spectra.

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