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Synthesis, Characterization and Antibacterial Screening of Amino Cyanopyridines Analogues

S. N. Lakhekar[1], G. B. Rahatikar[2], Seema habib[3], B. D. Kalyankar[4], M. A. Baseer[5]

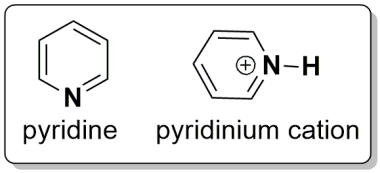
[1]Assistant Professor, Dept. of Chemistry, Chintamani College of Arts and Science, Gondpipri, Dist-Chandrapur-(MS), India
[2]Assistant Professor, Dept. of Chemistry, Late Babasaheb Deshmukh Gorthekar Arts, Commerce and Science College, Umari Dist-Nanded (MS), India
[3]Assistant Professor, Dept. of Chemistry, G. M. Momin Women College, Bhiwandi Dist-Thane -(MS), India
[4]Assistant Professor, Dept. of Chemistry, Chintamani College of Science, Pombhurna, Dist-Chandrapur-(MS), India
[5*]Professor, Dept. of Chemistry, Yeshwant College, Nanded, Dist-Nanded (MS), India

Abstract:
The new series of amino cyanopyridine derivatives were prepared from mixture of Chalcones **(2.3a-i),** malononitrile and ammonium acetate in ethanol. all the newly synthesized compounds were evaluated for their antibacterial action in vitro against gram +ve bacteria S. aureus, B. subtilis and gram -ve bacteria P. aeruginosa, E. coli by agar well diffusion method. The tested compounds **3.1a** showed good activity against two bacterial strain viz. *E. coli*, *P. aeruginosa* and the compound **3.1b** showed good antibacterial activity against all bacterial strain w.r.t penicillin as a std. drig while remaining compounds showed moderate to poor activity. The chemical structures of the compounds were proved by IR, 1H NMR, Mass, C^{13} NMR spectrometric data.

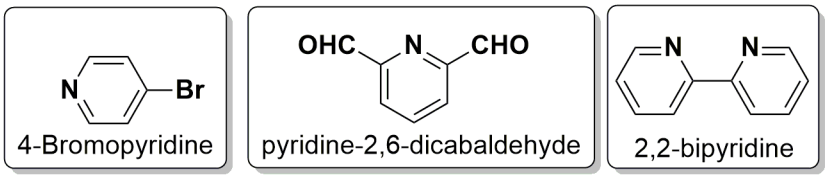
keywords: amino cyanopyridine, chalcone, malononitrile, antibacterial activity.

1. Introduction

Pyridine (azabenzene/azine/azinine) is a six-membered basic heterocyclic organic compound with the formula C_5H_5N. It's a colourless, flammable, diamagnetic, water-soluble liquid with a foul fishy odour**.** The molecule is electron deficient due to the electronegative nitrogen in the pyridine ring. As a result, it enters electrophilic aromatic substitution reactions less readily than benzene derivatives. Pyridine, like imines and carbonyls, is more susceptible to nucleophilic substitution with nucleophiles. By reacting with acids, pyridine forms the pyridinium cation, a positively charged aromatic polyaromatic ion that is isoelectronic to benzene with one methine group (=CH) replaced by a nitrogen atom.



The pyridine ring can be found in a variety of natural products, including vitamins such as nicotinic acid (niacin), nicotin amide (niamide), pyridoxine (vitamin-B_6), alkaloids, nicotin (from tobacco), pipeline (from black pepper), nucleotides such as diphosphopyridine nucleotide (DNP) and triphosphopyridine nucleotide (TPN), isoniazide (antitubercular), coramine (respiratory stimulants), a and p-eucane (local anaesthetic), dimerol (analgesic) and 2, 2-bipyridyl (analytical reagent) etc. Pyridine derivatives, on the other hand, are frequently found in biomolecules such as niacin, pyridoxine, pyridine nucleotides, and alkaloids. In everyday life, trace amounts of pyridine can be found in fried chicken[1], potato chips[2], black tea[3], saliva of gingivitis patients[4], Beaufort cheese[5], roasted coffee [6]. Pyidine was extracted from coal tar or obtained as a byproduct of coal gasification.



The pyridine, class of heterocyclic compounds is extremely important due to their multipurpose biological and pharmacological activities. Pyridine derivative are found to exhibit good antimicrobial (antibacterial, antifungal) [7-17], antioxidant [18-19], antitubercular[20], antiulcer[21], anti HIV[22], Herbicidal[23], cytotoxicity or antitumor or anticancer[24-25], anti-malerial [26], anti-inflammatory[27], analgesic anticonvulsant[28-29], antiproliferative[30], cardiotic[31], aurora kinase inhibitor[32] activities.

Pyridine derivatives have played an important role in the history of heterocyclic chemistry as important pharmacophores and starting materials in organic chemistry and medicinal chemistry. In the pharmaceutical industry, cyanopyridines are important intermediates in the synthesis of nicotinamide, nicotinic acid, and isonicotinic acid. Cyanopyridines have grown in importance in organic synthesis over the last few decades because they are among the most versatile organic synthetic intermediates. Because of their significant and diverse biological and pharmacological activities, cyanopyridines are a very interesting class of compounds.

The purpose of present article to synthesize the new amino cyanopyridines and evaluate their antibacterial activities.

2. Present work and method.

We thought it would be useful to synthesise a new series of amino cyanopyridines and examine their antibacterial activities because of the significant role played by basic moiety as antibacterial agent. In the present investigation, pyidines were synthesised using a conventional method by reacting, α, β unsaturated ketones (chalcones) **(2.3a-i)** with malononitrile and ammonium acetate in ethanol as a reaction solvent. **(scheme 3a, 3b)**

3. Experimental

3.1: General procedure for synthesis of substituted amino cyanopyridines (Scheme 3a).

A mixture of Chalcone **(2.3a-i),** malononitrile and ammonium acetate in ethanol was refluxed for 7-8 hours monitored by TLC using silicagel aluminium plate in eluent system of pet ether and ethyl acetate (7:3). The spots were visualized in an ultraviolet light chamber at λ=254-266nm. After completion of the reaction, the reaction mixture was cooled at room temperature and poured in crushed ice or cold water (100 ml). The separated solid was filtered, washed with ethanol then dried and recrystallized from ethanol. **(3.1a-i)**

structures of synthesized compounds were confirmed by spectral analysis (IR, ^{1}HNMR, MS and ^{13}C NMR).

3.1a Synthesis of 2-amino-4-(4-chlorophenyl)-6-(3,5-dibromo-2-hydroxyphenyl) nicotinonitrile (3.1a).

A mixture of (*E*)-3-(4-Chlorophenyl)-1-(3,5-dibromo-2-hydroxypheny) pro-2-en-1-one. **(2.3a)** (0.001mol), malononitrile (0.001mol) and ammonium acetate (0.001mol) in ethanol 20 ml was refluxed for 7-8 hrs. Reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled at room temperature and transfered in ice cold water (100 ml). Filtered to separate solid product i.e 2-amino-4-(4-chlorophenyl)-6-(3,5-dibromo-2-hydroxyphenyl) nicotine nitrile **(3.1a)** washed and after drying recrystallized from ethanol.

Similarly, remaining compounds of this series **(3.1b-i)** were prepared by same procedure. The substitution pattern and physical data of synthesized pyridine compounds are tabulated as in the **Table 1.** and **2.**

Scheme 3a: Synthesis of substituted Amino cyanopyridine. (3.1a-i)

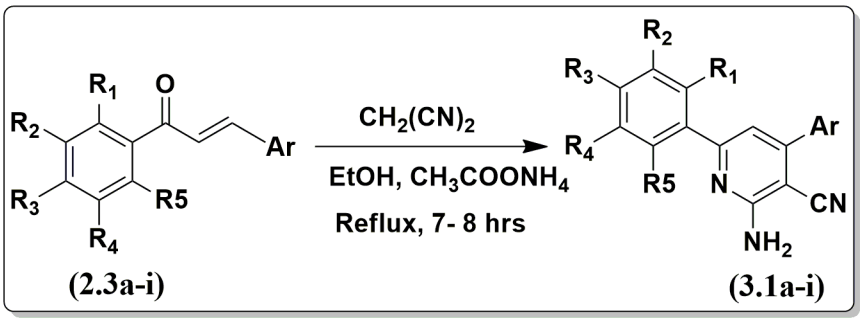


Table. No. 1: Substitution pattern of synthesized Amino cyanopyridines (3.1a-i).

Entry	Product	R_1	R_2	R_3	R_4	R_5	Ar
1	**3.1a**	OH	Br	H	Br	H	-4-Chloro Phenyl
2	**3.1b**	OH	Br	H	Br	H	-4-Bromo Phenyl
3	**3.1c**	OH	Br	H	Br	H	-thiophenyl
4	**3.1d**	H	Br	OH	Br	H	-4-Chloro Phenyl
5	**3.1e**	H	Br	OH	Br	H	-4-Bromo Phenyl
6	**3.1f**	H	Br	OH	Br	H	-thiophenyl
7	**3.1g**	OH	Br	OH	Br	H	-4-Chloro Phenyl

8	**3.1h**	OH	Br	OH	Br	H	-4-Bromo Phenyl
9	**3.1i**	OH	Br	OH	Br	H	-thiophenyl

Table no. 2: The Physical data of synthesized amino cyanopyridines.

Entry	Product	M/F &M/W	Yield %	M.P. (°C)	Color
1	**3.1a**	$C_{18}H_{10}Br_2ClN_3O$ (479)	67	174-176	White solid
2	**3.1b**	$C_{18}H_{10}Br_3N_3O$ (524)	62	167-169	White solid
3	**3.1c**	$C_{16}H_9Br_2N_3OS$ (451)	72	164-166	Cream like solid
4	**3.1d**	$C_{18}H_{10}Br_2ClN_3O$ (479)	70	146-148	White solid
5	**3.1e**	$C_{18}H_{10}Br_3N_3O$ (524)	77	157-159	White solid
6	**3.1f**	$C_{16}H_9Br_2N_3OS$ (451)	69	128-130	White solid
7	**3.1g**	$C_{18}H_{10}Br_2ClN_3O_2$ (495)	75	170-172	Cream like solid
8	**3.1h**	$C_{18}H_{10}Br_3N_3O_2$ (540)	64	160-162	White solid
9	**3.1i**	$C_{16}H_9Br_2N_3O_2S$ (467)	67	174-176	Pale yellow solid

3.2. Antibacterial Activity

In the present investigations, the antibacterial activity (table no. 3) of newly synthesized above said derivatives were evaluated against gram-positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria like, *Escherichia coli*, *Pseudomonas aeruginosa* [33-36] using disc diffusion method at 50 µg/mL concentration.[37-38]

Table 3. Antibacterial activity of Amino Cyanopyridine Compounds (3.1a-i)

Sample	Diameter of zone of inhibition (mm)			
	Gram +ve Bacteria		Gram –ve Bacteria	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
3.1a	20	19	26	27
3.1b	24	21	23	24
3.1c	ND	15	13	ND
3.1d	18	ND	19	22

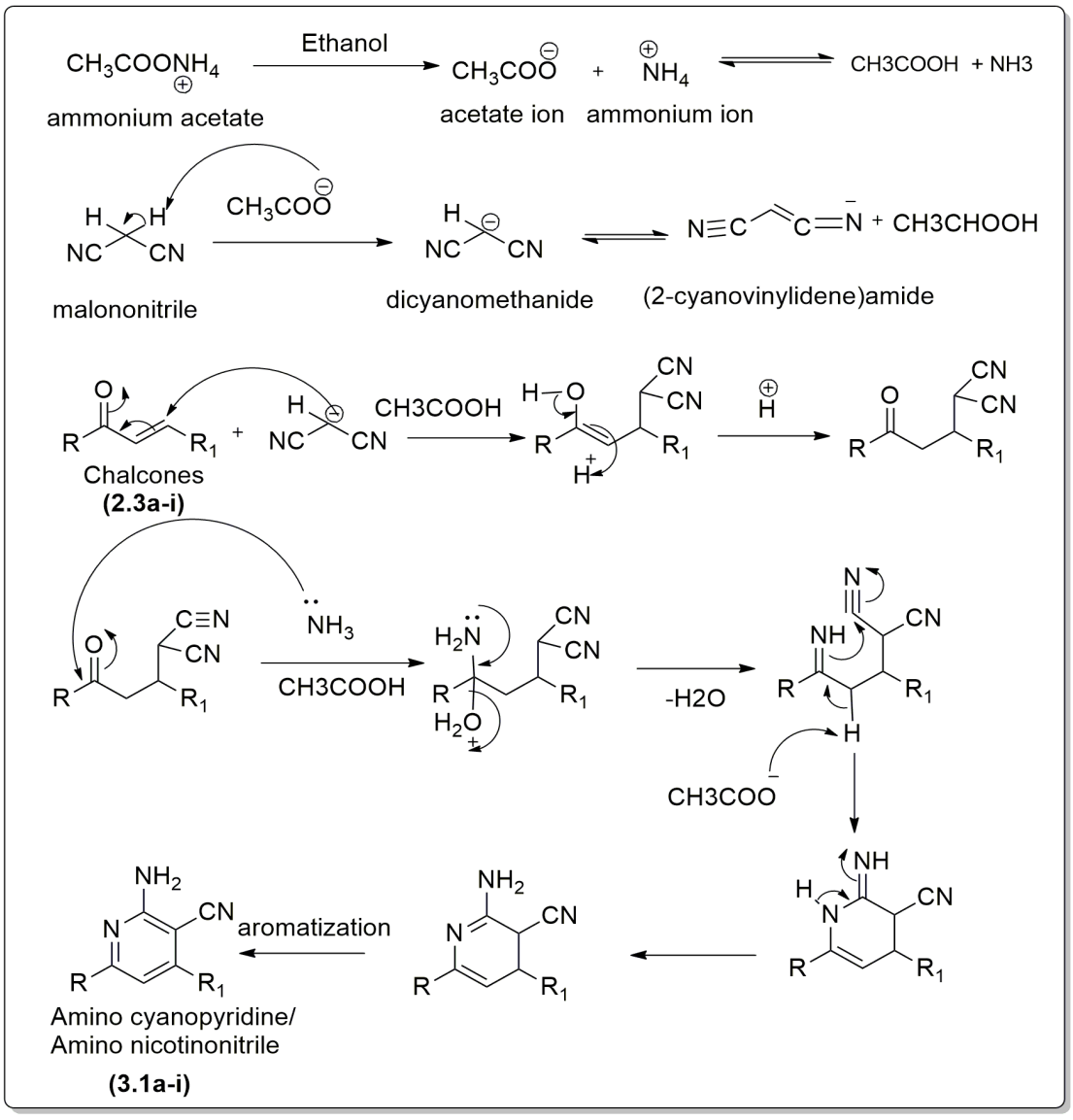
3.1e	19	17	22	ND
3.1f	ND	10	10	ND
3.1g	ND	12	18	15
3.1h	11	15	ND	16
3.1i	09	ND	ND	13
Penicillin (Std. Drug)	28 mm	26 mm	30 mm	32 Mm

N.D: Not Detected

4. Mechanism

The following mechanism seems to be possible for the condensation of :

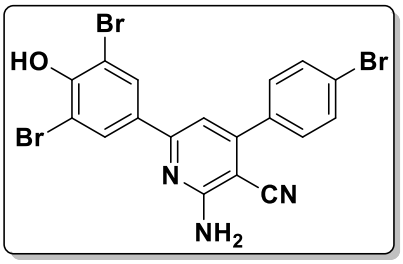
1. Chalcones with malononitrile and ammonium acetate.



Mechanism of Amino cyanopyridine

5. Spectral data of Selected compounds

Compound No. 3.1e:



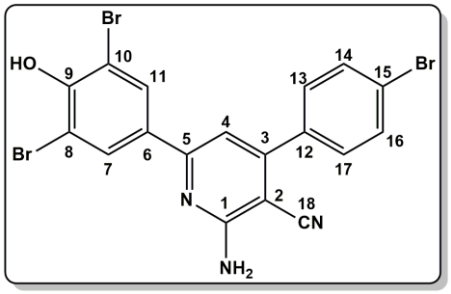
2-amino-4-(4-bromophenyl)-6-(3,5-dibromo-4-hydroxyphenyl)nicotinonitrile.

IR (KBr) cm^{-1} **:**3444 (-OH str.), 3367(-NH str.), 2908-3074 (arom. C-H str.), 2214 (-CN str.), 1581 (C=N str.) 1396, 1477, 1546, (arom. ring C=C str.), 559-678 (C-Br str.)

^{1}H NMR (CDCl$_3$) (δ ppm) **:**11.80 (s, 1H, -OH, D_2O exch.), 7.74 (s, 2H, Ar- H), 7.41- 7.59 (m, 5H, Ar-H), 6.7 (s, 2H, -NH_2, D_2O exch.)

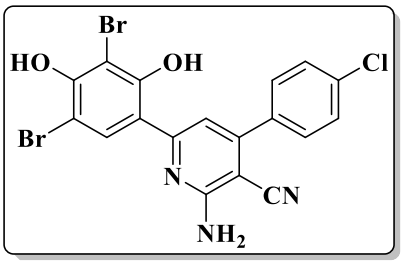
MS (m/z) **:** 523 (M-1)

^{13}C NMR CDCl$_3$ (ppm):



The total no. of carbon atoms in compound (**3.1e)** are eighteen but carbon no. [8, 10], [7, 11], [13, 17] and [14, 16] of Aromatic ring are equivalent in nature, hence it shows fourteen peaks in its ^{13}C NMR spectrum. The peak at 148 ppm (C_9) shows the presence of C-OH carbon of aromatic ring. The peak at 119 ppm (C_{18}) due to cyanide carbon atom (-CN). The peak appeared at 159 ppm (C_5) and 162 ppm (C_1) are due to imine carbon (C=N) atom and C-NH_2 carbon of pyridine ring respectively. The values appeared at 155 (C_3), 138 (C_{12}), 135 (C_7 & C_{11}), 134 (C_6), 132 (C_{14} & C_{16}), 130 (C_{13} & C_{17}), 121 (C_{15}), 118 (C_8 & C_{10}), 109 (C_4) and 87 (C_2) ppm due to aromatic carbons.

Compound No. 3.1g:



2-amino-4-(4-chlorophenyl)-6-(3,5-dibromo-2,4-dihydroxy phenyl)nicotinonitrile

IR (KBr) cm^{-1} **:**3409 (-OH str.), 3352(-NH str.), 2214 (-CN str.), 1589 (C=N str.) 1415, 1469, 1542 (arom. ring C=C str.), 574-644 (C-Br str.), 759 (C-Cl str.)

^{1}H NMR (CDCl$_3$) (δ ppm) **:**13.33 (s,1H, OH, D_2O exch.**),** 12.70 (s, 1H, OH**,** D_2O exch.**),** 7.89 (s, 1H, CH of Pyridine), 7.37-7.45 (m,4H, Ar-H), 6.75 (s, 2H, NH_2**,** D_2O exch.**)**

M.S. (m/z) **:**495 (M^+)

6. Result and Discussion

IR Spectra:

The IR spectra of synthesized substituted amino cyanopyridine **(3.1e, 3.1g)** showed the absorption bands in the region of 1589 1581 cm^{-1} due to –C=N str. The absorption band between 3444-3409 cm^{-1} is due to OH stretching and 2214 cm^{-1} is due to –CN stretching. The absorption band between 3074-3069 cm^{-1} is due to Aromatic C-H stretching. The aromatic C=C str. observed in the region 1546-1396 cm^{-1}. Beside these band between 569-678 cm^{-1} due to strong C-Br stretching. Band in the region of 759 cm^{-1} due to strong -C-Cl stretching. The IR spectrum of the compounds **(3.1e, 3.1g)** showed the absorption bands in the region of 3367-3352 cm^{-1} due to NH str. All these observations are in agreement with those observed earlier.[11-16]

^{1}H NMR spectra:

^{1}H NMR spectra of synthesized substituted amino cyanopyridine **(3.1e, 3.1g)** revealed that a singlet signal in the range of **δ** 6.7 ppm corresponds to -NH_2 proton. Aromatic Proton appeared as singlet signal for 2H and 1H proton at **δ** 7.74 and **δ** 7.89 ppm respectively. Aromatic Proton appeared as multiplet signal for 5H and 4H proton at **δ** 7.41-7.59 and 7.37-7.45 ppm respectively. Phenolic (-OH) proton appeared as a singlet in between δ 11.80-13.33 ppm. All such type of peaks observed were according to the reported literature[30] values. [11-16]

Mass spectra:

The mass spectra of synthesized substituted amino cyanopyridine **(3.1e, 3.1g)** products demonstrated that molecular ion peak which associate with their molecular weight of the compounds.

^{13}C NMR spectra:

Carbon-13NMR is a nuclear magnetic resonance spectroscopy application used to recognize carbon atoms in chemical structure elucidation in organic chemistry. It only detects the ^{13}C carbon isotope, which has a natural abundance of only 1.1%. Normally, the chemical shift range is 0 to 220 ppm. The number of peaks represents the number of C types.

In synthesised substituted amino cyanopyridine compound **(3.1e),** The peak at 148 ppm (C_9) shows the presence of C-OH carbon of aromatic ring. The peak at 119 ppm (C_{18}) due to cyanide carbon atom (-CN). The peak appeared at 159 ppm (C_5) and 162 ppm (C_1) are due to imine carbon (C=N) atom and C-NH_2 carbon of pyridine ring respectively, which are the strong evidence for the formed products. The values appeared at 155 (C_3), 138 (C_{12}), 135 (C_7 & C_{11}), 134 (C_6), 132 (C_{14} & C_{16}), 130 (C_{13} & C_{17}), 121 (C_{15}), 118 (C_8 & C_{10}), 109 (C_4) and 87 (C_2) ppm due to aromatic carbons.^{13}C NMR spectrum of **(3.1e)** showed that the fourteen number of peaks in the spectrum typically corresponds to the eighteen number of various equivalent and nonequivalent carbon. The chemical shifts (δ) of carbon signals give some clues about the characteristics of each environment.

A series of amino cyanopyridine derivatives **(3.1a-i),** were screened for antibacterial activity **(Table no. 6.3)** and we have found that due to the presence of hydroxyl group at ortho, electron withdrawing group i.e halogen (-Br) at meta, (-Cl) at para position of phenyl ring responsible for increasing the antibacterial activity of the compounds **3.1a** showed good activity against two bacterial strain viz. *E. coli, P. aeruginosa* and the compound **3.1b** showed good antibacterial activity against all bacterial strain .

7. Conclusion

It has been found that 2-amino-3-cyanopyridine is a crucial biologically active framework with a wide range of physiological actions. All of the modifications were completed using conventional techniques, which resulted in significant time savings and good to better yields. The structures of the newly synthesized compounds were confirmed by spectral data The tested compounds **3.1a** showed good activity against two bacterial strain viz. *E. coli*, *P. aeruginosa* and the compound **3.1b** showed good antibacterial activity against all bacterial strain w.r.t penicillin as a std. drug.

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