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SYNTHESIS AND STUDY OF MALONONITRILE BASED PYRIMIDINES AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Pyrimidine serves as a crucial intermediate compound in the synthesis of heterocyclic compounds, which are significant due to their medicinal applications. The exploration of heterocyclic systems is of considerable interest for both their medicinal properties and synthetic potential. These compounds are vital in the development of new pharmacologically active motifs. Chalcones play a key role in the synthesis of pyrimidine derivatives.

In this study, chalcones were synthesized through a one-pot condensation process involving 4-chloroaniline and 1-(5-hydroxynaphthalen-1-yl) ethenone, followed by further condensation with various aromatic aldehydes. The resulting chalcones (designated **A1-A15**) were then refluxed with malononitrile to produce a range of pyridine derivatives (designated **B1-B15**). The synthesized compounds were characterized using ^{1}HNMR, ^{13}CNMR, IR, and MASS

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spectrometry, and were evaluated for antimicrobial activity against both Gram-positive and Gram-negative bacteria.

Keywords: Chalcones, Pyrimidine, Malononitrile, Spectroscopy, Antimicrobial Activity.

INTRODUCTION

Molecules that contain both a carbon-oxygen double bond and a carbon-carbon double bond often share functional group properties. In unsaturated carbonyl compounds, these double bonds are conjugated, meaning the carbon-oxygen double bond and the carbon-carbon double bond are separated by a single carbon-carbon bond. Chalcones are a specific type of unsaturated ketone characterized by two aromatic rings linked by a highly electrophilic three-carbon unsaturated carbonyl system. They typically display a nearly planar or linear arrangement, as depicted in Figure 1 **[1, 2]**.

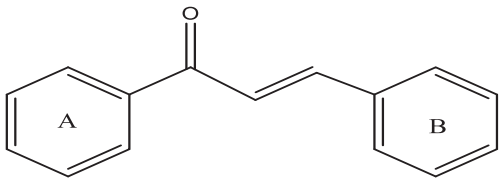

Figure 1 Chalcone Structure

Unsaturated carbonyl compounds, important in synthetic and medicinal chemistry, serve as key components in creating heterocyclic and biodynamic systems. Chalcones, a category of bichromophoric molecules linked by a keto-vinyl chain, are notable natural flavonoids with diverse biological activities **[3]**. Their activity arises from the reactive unsaturated keto functional group. Found predominantly in vibrant flowers, chalcones show various effects: 3,4-methylenedioxychalcones exhibit anticonvulsant properties **[4]**, halo-substituted methylenedioxychalcones have uterotropic, estrogenic, and anti-fertility effects **[5]**, and 3,4-dimethoxychalcones display antibacterial activity **[6]**. Chalcones with different halo, nitro, or amino substitutions can exhibit antibacterial **[7]**, antiviral **[8]**, antifungal **[9, 10]**, and anti-allergic effects, with activity enhanced by hydroxyl substitutions at specific ring positions **[11]**.

In Chinese medicine, isoprenyl chalcone sophradin from the root of *Sophara subprotstrata* and its analogues have been identified as effective anti-ulcer agents **[12]**. The 2',4'-dihydroxy-3'-methoxychalcone affects spore germination in Pitysaranima calomelaons **[13]**. Additionally, mecinarone, a benzofuranylehalcone, shows vascular and cardiac effects **[14]**. Furthermore, the 2',4'-dihydroxy-3',6'-dimethoxychalcone, which is isolated from Polygonum Senegalese, exhibits molluscicidal activity **[15]**. Heterocyclic compounds are cyclic organic molecules containing at least one heteroatom such as Nitrogen, Sulphur, Oxygen, Phosphorous, Silicon, Boron, or Selenium in addition to carbon and hydrogen. Nitrogen, Oxygen, and Sulphur are the most common heteroatoms.

This study aims to synthesize chalcones **A1-A15** through the condensation of 4-chloroaniline with 1-(5-hydroxynaphthalen-1-yl) ethenone, further treatment with aromatic aldehydes. Chalcones designated **A1-A15** will then be refluxed with malononitrile to produce pyridine derivatives **B1-B15**. The synthesized compounds will be characterized using ^{1}HNMR, ^{13}CNMR, IR and mass spectrometry techniques.

MATERIALS AND METHODS

Reagents and Chemicals

All chemicals utilized were of laboratory reagent grade and were used as received without additional purification. The materials included various aldehydes, malononitrile, 4-chloroaniline, 1-(5-hydroxynaphthalen-1-yl) ethenone, ethanol, and potassium hydroxide.

Externals

Melting points were measured using the open capillary method and are reported without correction. The ^{1}HNMR and ^{13}CNMR spectra were obtained using a Bruker Avance 400 spectrometer, operating at 400 MHz for ^{1}HNMR and 100 MHz for ^{13}CNMR, with solutions in DMSO-d6. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent. FT-IR spectra were recorded on a Shimadzu FT-IR 8400 spectrometer using KBr discs, with results expressed in wavenumbers (cm^{-1}). Mass spectra (ESI-MS) were acquired on a Shimadzu LCMS-2010 spectrometer. Carbon, hydrogen, and nitrogen contents

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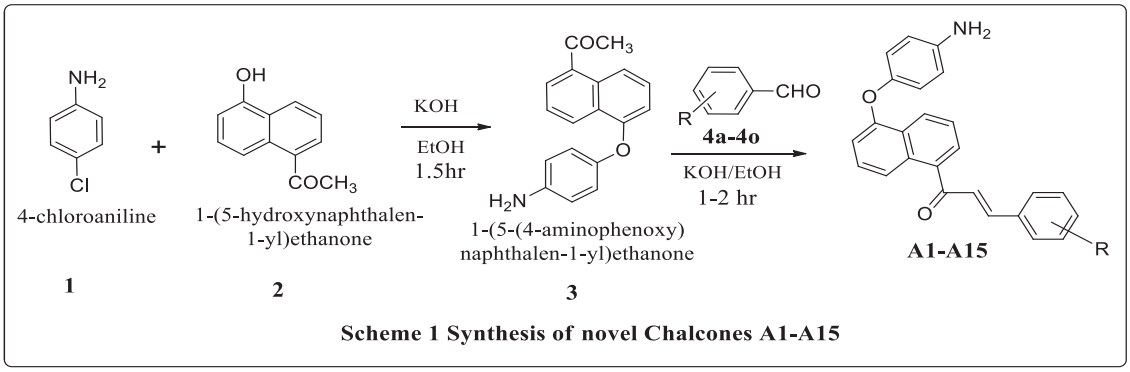
were analyzed using a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer. Thin-layer chromatography (TLC) was employed to monitor all reactions.

Method of Synthesis

Synthesis of Chalcones A1-A15

1-(5-(4-Aminophenoxy)naphthalen-1-yl)ethanone was synthesized by combining 4-chloroaniline (0.01 mol) and 1-(5-hydroxynaphthalen-1-yl)ethenone (0.01 mol) in a 250 ml round-bottom flask. Ethanol (30 ml) was added to the mixture while maintaining a temperature below 25°C and stirring continuously. Potassium hydroxide (0.01 mol) was introduced, and the mixture was refluxed for 1-2 hours. After refluxing, the mixture was cooled to room temperature and then poured into crushed ice. The resulting solid was isolated by filtration and recrystallized from ethanol. The resulting product, 1-(5-(4-aminophenoxy)naphthalen-1-yl)ethenone (0.01 mol), was then reacted with an aromatic aldehyde (0.01 mol) in the presence of ethanol and KOH to produce Chalcones **A1-A15** (**Scheme 1**).



Scheme 1 Synthesis of novel Chalcones A1-A15

Condensation of chalcones with Malononitrile

The chalcones synthesized in the previous section were refluxed with malononitrile in the presence of potassium hydroxide and ethanol to yield pyridines **B1-B15**. This reaction was carried out over a period of 1-2 hours. The progress and completion of the reaction were monitored using thin-layer chromatography (TLC) (**Scheme 2**).

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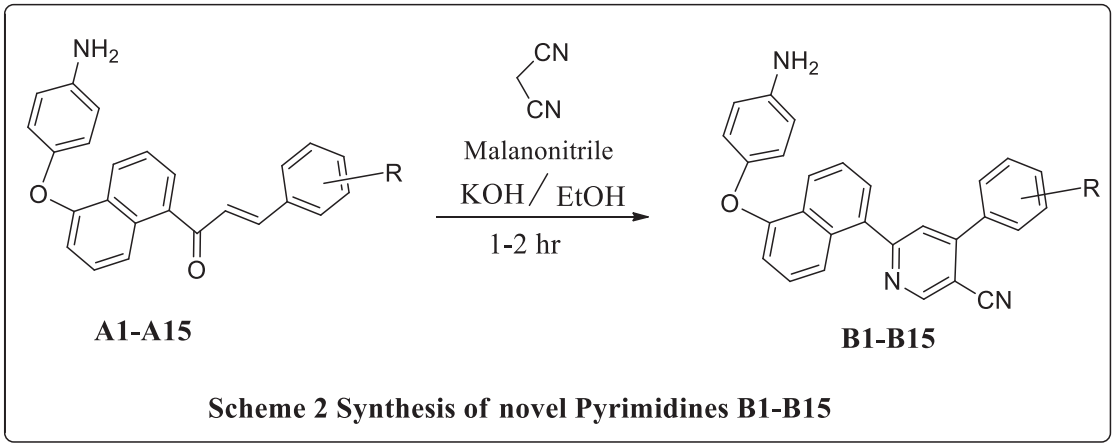
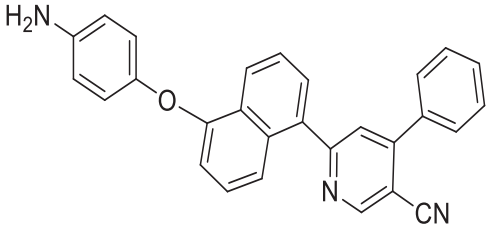


Scheme 2 Synthesis of novel Pyrimidines B1-B15

Characterization

The compound **B1** from the series is used as a representative example. In the ^{1}HNMR spectrum, the distinctive signals corresponding to each proton and functional group are well-detailed, based on their shielding and deshielding effects. The aromatic protons of the compound appear in the downfield region, with chemical shifts ranging from approximately 6 to 8 ppm. The spectroscopic data for **B1**, including ^{1}HNMR, ^{13}CNMR, IR, and mass spectrometry, are provided below.

Compound code: B1	
Molecular formula: $C_{28}H_{19}N_3O$	
M. P. (°C):	268
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:	5.6 (2H, s, -NH_2),) 6.4-8.6 (17H, Ar-H, m).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:	117.5, 127.5, 128.1, 129.3, 130.1, 131.4, 131.9, 135.2, 140.2, 143.6, 145.2, 147.2, 151.8, 153.6, 155.1, 156.8.

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IR cm^{-1} (KBr):	3542, 3432, 3049, 2240, 1644, 1614, 1592, 1569, 744.
Mass (M+1):	413.1
Elemental analysis:	Calculated (%): C: 81.34; H: 4.63; N: 10.16 Found (%): C: 81.20; H: 4.70; N: 10.20

RESULT AND DISCUSSION

Table 1 presents the various condensation products obtained from the reaction of 4-chloroaniline with 1-(5-hydroxynaphthalen-1-yl)ethenone, followed by further condensation with different aromatic aldehydes. The chalcones A1-A15 that were produced were then refluxed with malononitrile to yield various pyridine derivatives B1-B15. The data clearly shows that compounds containing electron-withdrawing groups were synthesized in shorter reaction times compared to those with electron-donating groups. Specifically, compounds B10-B12, which have electron-withdrawing groups, were synthesized in 3.5 hours, whereas compounds B5 and B6, which have electron-donating groups, required 5 hours. Notably, very good yields were achieved for aldehydes with electron-withdrawing groups, especially those with nitro groups.

Table 1 Characteristic data showing synthesis of pyrimidine B1-B15

Sr. No.	Compounds Code	R	Reaction Time[a] (hr)	% Yiled[b]	M. P. of Compounds
1	**B1**	-H	4	79	268
2	**B2**	4-OH	4.5	78	256
3	**B3**	3-OH	4.5	78	245

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4	**B4**	2-OH	4.5	76	248
5	**B5**	2- OCH_3	5	81	233
6	**B6**	4-OCH_3	5	81	240
7	**B7**	2-Cl	3.5	84	242
8	**B8**	4-Cl	3.5	85	254
9	**B9**	3-Cl	3.5	84	263
10	**B10**	2-NO_2	3.5	90	237
11	**B11**	4-NO_2	3.5	90	233
12	**B12**	3-NO_2	3.5	88	227
13	**B13**	3-Br	3.5	86	221
14	**B14**	2- Br	3.5	86	261
15	**B15**	4- Br	3.5	85	241

[a]Reaction is monitored by TLC, [b]Isolated yield

ANTIMICROBIAL ACTIVITY

Preparation of Media:

Five grams of peptone, three grams of metal extract, five grams of NaCl, and fifteen grams of agar-agar peptone were mixed in one liter of distilled water and heated until all components were dissolved. The medium was then autoclaved at 15 pounds of pressure and 125°C for 20 minutes. After cooling to 45°C, 20 ml of the medium was poured into sterilized Petri dishes, and the pH was adjusted to between 7.0 and 7.5.

The culture for the organism was prepared in nutrient broth made by dissolving the following in distilled water:

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1. Beef extract: 10 g
2. Peptone: 10 g
3. Sodium chloride: 5 g

After sterilizing the nutrient broth, it was used for culturing. The culture was incubated at 37°C. Using a swab, the culture was spread over the agar plates, and 5 mm diameter paper discs were prepared and sterilized in the autoclave. Solutions of the test compound were applied to these discs with a micropipette, and the discs were dried to eliminate the solvent. The sterile test compound-coated discs were then placed on the culture media in the Petri dishes. The discs were pressed onto the media, and the dishes were incubated for 24 hours at 37°C. After incubation, the zones of inhibition were measured.

Experimental Data of Antimicrobial Study.

Table 2 Antibacterial Activities of *compounds B1-B15*

Samples	*S.aureus* (+Ve)	*B.megaterium* (+Ve)	*E.coli* (-Ve)	*P.vulgaris* (-Ve)
B1	7	4	3	6
B2	5	6	9	7
B3	7	5	8	**9**
B4	4	4	5	5
B5	9	**10**	8	7
B6	8	5	5	7
B7	6	5	6	**9**
B8	9	8	9	3
B9	**10**	**10**	8	7

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B10	8	3	6	7
B11	8	6	5	8
B12	**10**	8	8	**9**
B13	9	7	**10**	5
B14	7	6	7	5
B15	7	6	8	6
Ampicillin	**15**	**14**	**16**	**12**
Gentamycin	**16**	**15**	**14**	**16**

(I) Against *Staphylococcus aureus*:

The compounds B9 and B12 exhibited the highest activity, with a zone of inhibition of 10.0 mm, while compound B4 showed the least activity, with a zone of inhibition of 4.0 mm.

(II) Against *Bacillus megaterium*:

Compounds B5 and B9 displayed maximum activity, achieving a zone of inhibition of 10.0 mm, whereas compounds B1 and B4 had the minimum activity, with a zone of inhibition of 4.0 mm.

(III) Against *Escherichia coli*:

The highest activity was observed in compound B13, which had a zone of inhibition of 10.0 mm (similar to the standard drug), while compound B1 demonstrated the lowest activity, with a zone of inhibition of 3.0 mm.

(IV) Against *Proteus vulgaris*:

Compounds B3, B7, and B12 showed maximum activity, each with a zone of inhibition of 9.0 mm, while compound B8 had the minimum activity, with a zone of inhibition of 3.0 mm.

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CONCLUSION

In summary, the highly functionalized pyridines B1-B15 were successfully synthesized from various chalcones A1-A15. It was noted that compounds with electron-withdrawing groups were produced in shorter reaction times compared to those containing electron-donating groups. Each synthesized compound was thoroughly characterized using various spectroscopic techniques. The compounds exhibited good to moderate activity against both Gram-positive and Gram-negative bacteria.

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