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SYNTHESIS OF PHENOTHIAZINE DERIVATIVES FROM 3-AMINONAPHTHALENE-2-THIOL AND ITS ANTIMICROBIAL STUDY

Gadhavi Ritu Sanjaykumar

Department of Chemistry, Monark University, Ahmedabad

Chandani Halpani

Department of Chemistry, Monark University, Ahmedabad

ABSTRACT

Synthesis of novel Phenothiazine was carried out by refluxing a mixture of 3-aminonaphthalene-2-thiol and 2-hydroxynaphthalene-1,4-dione in ethanol as a solvent, with a few drops of HCl as a catalyst. The resulting products were further modified using malononitrile and various aldehydes to produce a library of compounds. All synthesized compounds were characterized using ^{1}H NMR, ^{13}C NMR, IR, and mass spectroscopic techniques. The antimicrobial activities of these compounds were assessed using the open disc method against Gram-positive bacteria, including Staphylococcus aureus and Bacillus megaterium, as well as Gram-negative bacteria such as Escherichia coli and Proteus vulgaris.

KEYWORDS: Phenothiazine, Malononitrile, Antimicrobial activities, 3-aminonaphthalene-2-thiol, Aldehydes and Spectroscopy.

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1. Introduction

Quinazolinones and their derivatives serve as basic components for over 150 naturally occurring alkaloids found in plants, animal, and microorganism. Recently, there has been a significant surge of interest among biologists and chemists regarding the synthesis and biological activities of quinazolinone derivatives. Heterocyclic compounds are particularly valuable in the pharmaceutical and agrochemical sectors due to their potent physiological properties, leading to a wide range of applications.

Phenothiazines represent a crucial class of nitrogen-containing heterocycles that hold substantial significance in both chemistry and biology **[1, 2]**. These compounds form the foundation for various antitumor agents, fungicides, insecticides, herbicides, and receptor antagonists **[3-7]**. Additionally, they are utilized in dye production **[8]**, as building blocks for organic semiconductors **[9]**, chemically controllable switches **[10]**, cavitands **[11]**, DNA cleaving agents **[12]**, dehydroannulenes **[13]**, electrical-photochemical materials **[14-15]**, and as inhibitors of mild steel corrosion **[16]**. Several phenothiazine derivatives are naturally occurring, produced by bacteria such as Pseudomonas spp., Streptomyces spp., and Pantoea agglomerans. These natural compounds have been linked to the virulence and competitive fitness of their producing organisms **[17-18]**.

Various synthetic strategies have been devised for creating substituted phenothiazines **[19]**. However, some of these methods face challenges such as low product yields, harsh reaction conditions, extended reaction times, and complicated isolation procedures.

Present study describes the synthesis of novel phenothiazines from readily available starting materials such as 3-aminonaphthalene-2-thiol and 2-hydroxynaphthalene-1,4-dione and modified prepared product using malononitrile and various aldehydes to produced library of compounds and give good antimicrobial activities.

2. Materials & Methods

2.1 Reagents

All types of reagents used are LR grade and utilized as received without any purification. Reagents used are aromatic aldehydes, 3-aminonaphthalene-2-thiol, 2-hydroxynaphthalene-1,4-dione, Malononitrile, HCl and ethanol.

2.2 Experimental

The Proton NMR study was conducted using a Bruker Avance-400 instrument, while a 100 MHz frequency instrument was employed for the ^{13}CNMR analysis, with chemical shift values reported in parts per million (ppm). For the infrared spectral study, an ABB Bomem Inc. FT-IR 3000 spectrophotometer was utilized, and the data were expressed in cm^{-1}. Mass spectral analysis was performed using a Shimadzu LCMS-2010. Additionally, the composition measurements were carried out with a Perkin Elmer-2400 Series II CHNS/O elemental analyzer.

2.3 Method of Synthesis

Synthesis of Novel Phenothiazine A1-A19

Phenothiazine was synthesized by reacting 3-aminonaphthalene-2-thiol (1mmol) and 2-hydroxynaphthalene-1,4-dione (1.1mmol) in the presence of few drops of HCl under 10 ml ethanol as solvent by refluxing mixture for 30-40 minutes. After completion of reaction, product obtained was further treated with malononitrile (1 mmol) and 1 mmol of aromatic aldehyde using 10ml ethanol. Warm the mixture for 40-50 minutes under ethanol to obtained phenothiazines. Reaction completion checked using TLC (**Scheme 1**).

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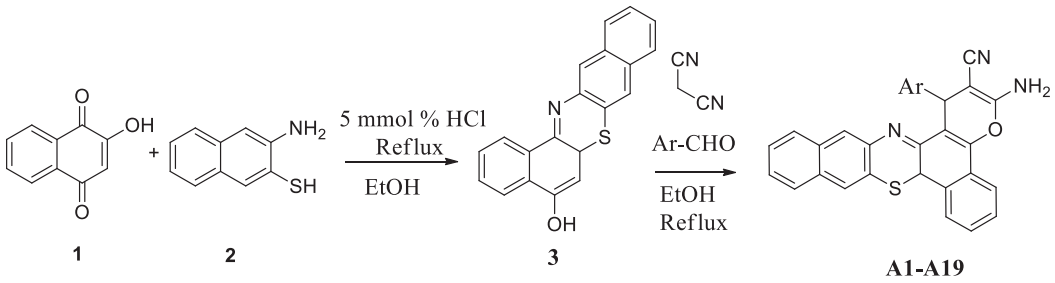

Scheme 1 Synthesis of Phenothiazines A1-A19

3. Result and Discussion

From the Table 1 show the various condensation product obtained by reaction of 3-aminonaphthalene-2-thiol and 2-hydroxynaphthalene-1,4-dione followed by treatment with malononitrile and aromaticaldehydes. The results indicate that compounds containing electron-withdrawing groups are synthesized in a shorter reaction time compared to those with electron-donating groups. Specifically, compounds A7-A15, which feature electron-withdrawing groups, were synthesized in 1.3 hours, while compounds A16 and A17, which possess electron-donating groups, took 1.6 hours to synthesize.

Table-1 Synthetic data for Synthesis of Phenothiazines A1-A19.

Sr. No.	Code	R	Time (Hr)	% Yield[b]	Melting Point (°C)
1	**A1**	-H	1.3	77	222
2	**A2**	4-OH	1.4	76	261
3	**A3**	3-OH	1.6	75	251
4	**A4**	2-OH	1.6	77	254
5	**A5**	2- OCH_3	1.6	80	241
6	**A6**	4-OCH_3	1.6	74	244

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7	**A7**	2-Cl	1.3	84	244
8	**A8**	4-Cl	1.3	82	261
9	**A9**	3-Cl	1.3	77	254
10	**A10**	2-NO_2	1.3	84	230
11	**A11**	4-NO_2	1.3	84	211
12	**A12**	3-NO_2	1.3	82	214
13	**A13**	3-Br	1.3	85	251
14	**A14**	2- Br	1.3	84	241
15	**A15**	4- Br	1.3	84	231
16	**A16**	3, 4-$(OCH_3)_2$	1.6	77	224
17	**A17**	3,4,5-$(OCH_3)_3$	1.6	75	231
18	**A18**	2-furfuryl[c]	1.3	85	241
19	**A19**	2-Thineyl[c]	1.3	80	220

[a]Reaction is monitored by TLC, [b]Isolated yield&[c]Names of aldehyde groups

4. Antimicrobial Activity

4.1 Preparation of Media:

For assessing bacterial activity, nutrient agar was utilized, prepared as follows: 5 g of peptone, 3 g of metal extract, 5 g of NaCl, and 15 g of agar-agar were combined in one liter of distilled water and heated until all components dissolved. The medium was then autoclaved at 15 psi and 125°C for 20 minutes. After cooling to 45°C, 20 ml was poured into sterilized Petri dishes, with the pH adjusted to between 7.0 and 7.5.

The bacterial culture was prepared in nutrient broth, which consists of:

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

Following sterilization of the nutrient broth, it was used for culturing. The culture was incubated at 37°C. Using a swab, the culture was evenly spread across the agar plates. Sterilized paper discs, 5 mm in diameter, were prepared and placed in the agar. A solution of the test compound was applied to these discs using a micropipette, and the discs were allowed to dry to remove any solvent. The sterile discs coated with the test compound were then placed on the agar medium, pressed down gently, and the Petri dishes were incubated for 24 hours at 37°C. After incubation, the zones of inhibition were measured.

4.2 Experimental Data of Antimicrobial Study.

Table 1.2 Antibacterial Activities of Compounds A1-A19

Sample Code	*S. aureus* (+ve)	*B. megaterium (+ve)*	*E. Coli* (-ve)	*P. vulgaris* (-ve)
A1	5	8	7	6
A2	6	7	8	8
A3	7	9	11	9
A4	4	4	4	7
A5	10	11	8	6
A6	6	10	7	10
A7	6	9	11	4
A8	10	8	4	8
A9	12	10	7	11

A10	4	8	4	10
A11	6	9	9	4
A12	12	7	10	9
A13	11	8	6	11
A14	9	7	8	8
A15	8	9	6	6
A16	9	9	5	10
A17	10	11	11	11
A18	11	11	12	11
A19	11	12	8	11
Ampicillin	**15**	**14**	**17**	**18**
Gentamycin	**16**	**15**	**14**	**16**

Antibacterial Activity Results

I. Against Staphylococcus aureus:

The compound (A9) exhibited the highest activity, with a zone of inhibition measuring 12.0 mm. In contrast, compounds (A4) and (A10) showed the lowest activity, both with a zone of inhibition of 4.0 mm.

II. Against Bacillus megaterium:

Maximum activity was observed in compound (A19), which also had a zone of inhibition of 12.0 mm. The lowest activity was recorded for compound (A4), with a zone of inhibition of 4.0 mm.

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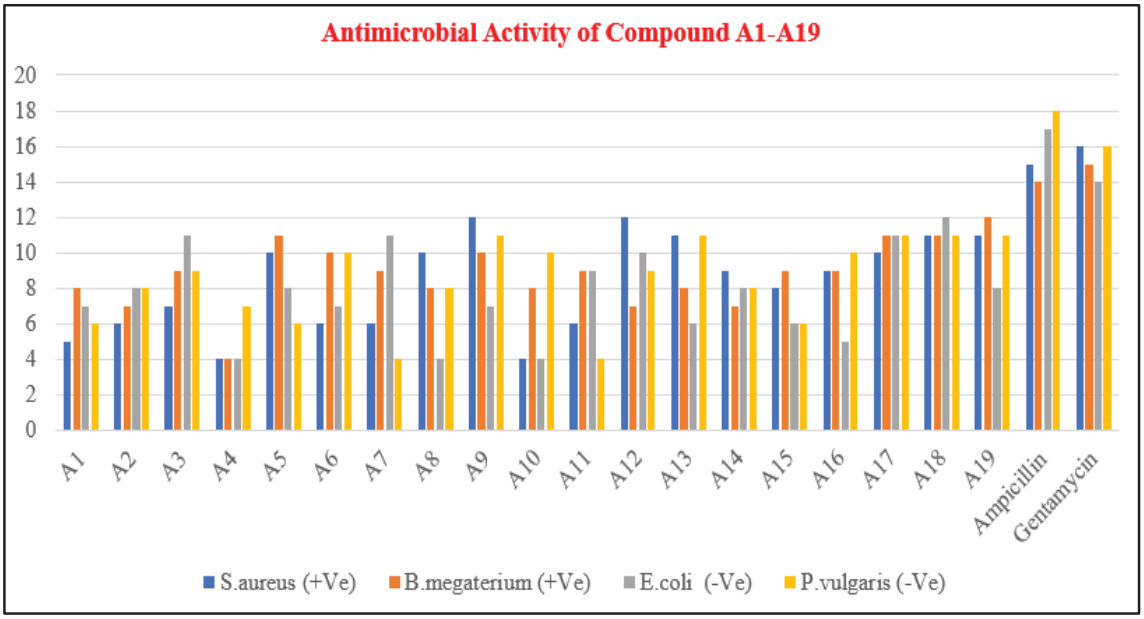
III. Against Escherichia coli:

Compound (A18) demonstrated the highest activity, with a zone of inhibition of 12.0 mm. The least activity was noted for compounds (A4), (A8), and (A10), each showing a zone of inhibition of 4.0 mm.

IV. Against Proteus vulgaris:

Compounds (A9), (A13), (A17), (A18), and (A19) all displayed maximum activity, each with a zone of inhibition of 12.0 mm, comparable to the standard drug. Conversely, compounds (A7) and (A11) showed the minimum activity, with a zone of inhibition of 4.0 mm.



5. Characterization

Compound A1 from the series is chosen as the representative compound. In the ^{1}HNMR spectrum, the distinctive signals corresponding to each proton and functional group are well characterized based on shielding and deshielding effects. The signal for the aromatic protons of the compound appears in the downfield region, with a chemical shift value around 6 to 8 ppm. Below are the ^{1}HNMR, ^{13}CNMR, IR, and mass spectroscopic data for compound A1.

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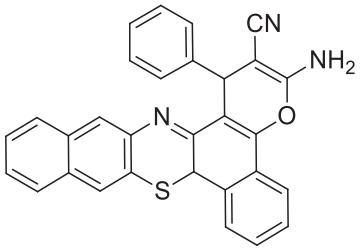
Code of Compound: A1



Mol. Formula: $C_{30}H_{19}N_3OS$

M. P. (°C): 222

^{1}HNMR (400 MHz, $CDCl_3$) δ ppm:	2.5 (s, 2H, -NH_2group), 3.3 (s, 1H, CH Proton), 3.5 (s, 1H, CH Proton), 6.86-8.30 (15H, Ar-H, complex).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:	62.4, 120.0, 128.9, 129.4, 130.3, 131.6, 140.2, 140.6, 141.8, 143.6, 144.1, 160.2.
IR cm^{-1} (KBr):	3311, 3120, 2950, 2150, 1610, 1592, 1569, 744.
Mass (M+1):	469.1
Elemental analysis:	**Calculated (%):** C 76.74; H 4.08; N 8.95. **Found (%) :** C 76.80; H 4.11; N 8.90

6. Summary

In summary one can say that highly functionalized derivatives of phenothiazines are synthesized using 2-hydroxynaphthalene-1,4-dione and 3-aminonaphthalene-2-thiol. Library of compounds were prepared by using various aromatic aldehydes with different functional groups. All the compounds show moderate to good activity against gram positive and gram negative bacteria.

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