IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 1, March 2026
Impact Factor: 8.2

Synthesis and Biological Evaluation of Some Phenothiazine Derivatives

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Abstract: *As we all know that in this modern era, humans are the most troubled due to health problems. Keeping in mind the health problems, the name phenothiazine comes to mind, which is known for many medicinal properties since 1930. The phenothiazine structure and its derivatives have been important in pharmacology and biomedicine since its discovery in the early stage of organic dye chemistry. Phenothiazine provides the most valuable template used to create a variety of derivatives that can be used targeting different biological processes. Phenothiazine was being used since the early 1930s, whether it was its antibiotic property or anti-helmintic property. Phenothiazine-based drugs began to be used as antihistaminic and by the end of the 1950s, it had become popular for its sedative and antipsychotic properties. This page seeks to present all available data regarding phenothiazine-based medications and summarizes research on phenothiazine's synthesis ,physical and biological property and also structural conformation.*

Keywords: Phenothiazine, synthesis of phenothiazine derivative, physical property, structural conformation , Biological screening

I. INTRODUCTION

Medicinal Chemistry is a multidisciplinary subject involving Organic chemistry, Pharmacology, Biochemistry, Physiology, Microbiology, Toxicology, Genetics and Computer modelling . Its explain the design and production of compounds that can be used for prevention, treatment or cure of human or animal diseases. More recently the IUPAC have recommended the following Description for the Medicinal chemistry term; “Medicinal chemistry is a chemistry based discipline, also involving aspects of biological, medical and Pharmaceutical science. It is concerned with the invention, discovery, design, identification and preparation of biologically active compounds, the study of their metabolism, the interpretation of their mode of action at the molecular level and construction of structure activity relationship.

Medicinal chemistry covers the following stages:

- In the first stage new active substance or drugs are identified and prepared from natural sources by Organic chemical reactions or biotechnological processes. They are known as lead molecules.
- The second stage is optimization of lead molecule to improve potency, selectivity and to reduce toxicity.
- The next stage involves optimization of synthetic route for bulk production and modification of pharmacokinetic and pharmaceutical properties of active substance to render it clinically useful.

1. NUCLEUS INTRODUCTION

Phenothiazine are a group chemical compounds known for their tricyclic ring structure these compounds have wide ranging applications from being used as antipsychotic medication to dyes and pesticides .

DOI: 10.48175/568

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Their unique structure and properties make phenothiazine versatile and important in various fields. Phenothiazine has a diverse range of properties that make them valuable in different applications. Some key properties include their ability to act as dopamine receptor antagonist, which is why they are commonly as antipsychotic medication they also exibit antioxidant , antiviral ,anticancer , antitussive, antiemetic, antibacterial, antinflamatory etc. properties making them potentially useful in treating various condition additional phenothiazine have light sensitive properties which have led to their usage in dyes and pigments. Many studies conducted recently indicate that anti-schizophrenic phenothiazine medications can achieve their therapeutic benefits and avoid extra-pyramidal side effects by blocking dopamine synaptic receptor sites in the brain. By inhibiting the brain's dopamine receptors, phenothiazine functions as a dopamine receptor antagonist. For nearly four decades, antipsychotics containing phenothiazine are used for clinical management of psychiatric illnesses. Fluphenazine and chlorpromazine, together with haloperidol, are still listed as the three essential medications for the treatment of psychotic illnesses in the World Health Organization's (WHO) 2009

2. DERIVATIVES OF PHENOTHIAZINE

Although several phenothiazine derivatives have been shown to have biological activity, phenothiazine moieties are still receiving a lot of study because of their many medical applications. For example, **Chlorpromazine**

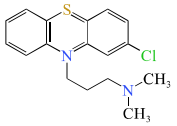
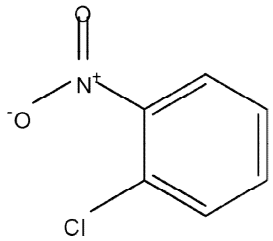


Figure 1: General structure for Chlorpromazine

The first phenothiazine derivative to develop is chlorpromazine, which is linked to the C-3 carbon of the N, Ndimethylpropanamine component and substituted with ring nitrogen at position 10. This drug is prescribed and monitored for the psychosis management , manic-depression disorder, and acute psychosis.It blocks the receptors in the forebrain that are important for neuronal transmission. According to some recent research, drugs may be used for a variety of purposes, including the treatment of oral cancer in humans

3. INTRODUCTION TO STARTING MATERIAL

Orthochloro nitrobenzene



O chloronitrobenzene

Chemical name = **1-chloro 2-nitro benzene**
Mol.formula = $C_6H_4ClNO_2$
Mol.weight = *157.55446g/mol*

Physical description

Colour - Yellow to green crystals with the monoclinic
Odour - Characteristic/aromatic
Boiling point - 246^0c / 475^0F
Melting point - 33^0c/90-91^0F
Solubility - Soluble in alcohol,benzene,ether

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Very soluble in acetone,pyridine,

Density - 1.4g/cm^2

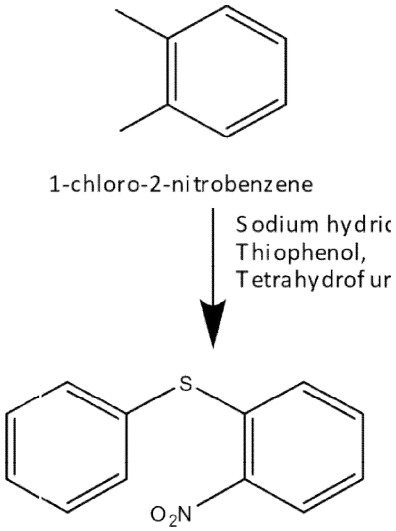
Synthesis;

- Nitro chlorobenzene is a typically synthesized by nitration of chlorobenzene in the presence of sulfuric acid.
- This reaction affords a mixture of isomers using an acid ratio of 30/56/14, the product mixture typically 34/36% 2-nitro chlorobenzene.

Reactions;

Alkylation and electrophilic aromatic substitution can occur at the chlorinated carbon center, and a diverse array of reactions can be carried out using the nitro group. 2-Nitro chlorobenzene can be reduced to the 2 – chloroaniline with Fe/Hcl mixture the Bechamp reduction.

4. SCHEME OF WORK



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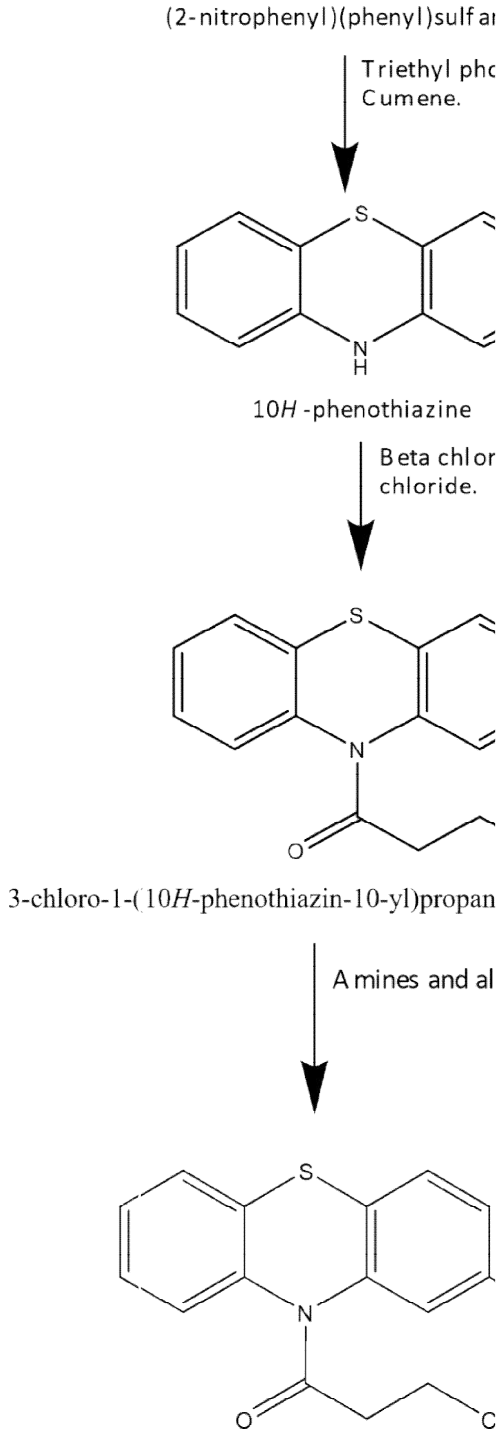

3-chloro-1-(10H-phenothiazine-10yl)-3-arylaminopropane-1-one

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5. EXPERIMENTAL WORK

5.1. PROCEEDURE

STEP:1

SYNTHESIS OF (2-NITRO PHENYL) (PHENYL) SULFONE .

A mixture of o-chloro-7 nitro benzene (0.1 mole) , tetrahydrofuran (0.1 mole) ,sodium hydride (0.1 mole)& ethanol (70 ml) reflux for 4 hrs.

After completion of reaction , excess of solvent is remove under reduced pressure.

The solution is washed with 0.1N NaoH.

The reaction mixture is poured into crushed ice ,The product is wash with water repeatedly, dried & recrystalline from ethanol.

STEP: 2

SYNTHESIS OF PHENOTHIAZINE.

Equimolar quantities of compound-1 and tri ethylamine were reflux in ethanol using pyridine as catalyst for 8 hr.

The solution mixture is concentrated and poured into crushed ice .The product is washed with water repeatedly, dried and recrystallized from ethanol.

STEP: 3

SYNTHESIS OF 3-CHLORO-1-(10H-PHENOTHIAZINE-10-YL) PROPANE-1-ONE.

Compound-2 (0.1 ml) in ethanol (30ml) , 2 ml of hydrazine hydride is added to the mixture is added 50ml of 1,2-dicloroethane .

The mixture is maintained under reflux for 8 hrs.

After cooling, The mixture is poured in ice and solid formed is collected by filtration, washed with cold water and recrystallized with ethanol.

STEP: 4

SYNTHESIS OF 3-CHLORO-1-(10 H PHENOTHIAZINE-10 YL)-3-ARYL AMINO PROPANE1ONE

Compound-3 (0.1mole) in 20 ml of ethanol .it is added on equimolar amount of aromatic or aliphatic amines in presence of acetic acid.

The mixture is maintained and reflux of 4hr then the reaction mixture is poured in cold water and precipitate is formed and wash with ethanol recrystallization in ethanol and DMF

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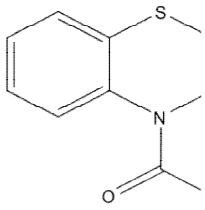
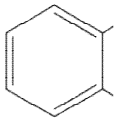
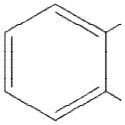
5.2. LIST OF COMPOUNDS SYNTHESIZED

Table No.1

S.No	Compound code	Struc
1.	S_1	 4-(10-(3-chloropro ylamino)benzoicac
2.	S_2	 3-chloro-1-(2-(2-hy yl)propan-1-one
3.	S_3	 3-chloro-1-(2-(2-ni yl)propan-1-one

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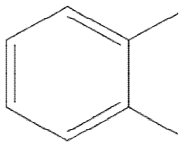
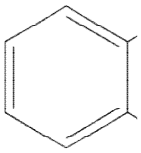
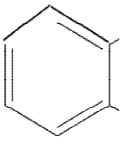
4.	S_4	  1-(2-benzyl-10*H*-p
5.	S_5	 3-chloro-1-(2-ethy
6.	S_6	 3-chloro-1-(2-(fura 10yl)propan-1-one

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6. PHYSICAL CHARACTERIZATION

All the synthesized compounds are to be characterized by following methods:

- **Melting point**

Melting point will be measured in open-end capillary tube method by electrically heating melting point apparatus

- Solubility

At room temperature solubility of newly synthesized compound will be determined

- **Paper Chromatography**

The purity of new compound will be analyzed and reaction completion will be checked by paper chromatography by measuring Rf value of product

- **Analytical characterization**

IR Spectroscopy
NMR Spectroscopy

6.1.Solubility

At the room temperature, solubility of all synthesized compound was determined and shown in Table No.2

Table No:2

S.NO	COMPOUND CODE	SOLUBILITY
1.	Compound 1	MeOH, EtOH, DMSO, DMF
2.	Compound 2	MeOH, EtOH, DMSO, DMF
3.	Compound 3	MeOH, EtOH, DMSO, DMF
4.	S_1	MeOH, EtOH, DMSO, DMF
5.	S_2	MeOH, EtOH, DMSO, DMF
6.	S_3	MeOH, EtOH, DMSO, DMF
7.	S_4	MeOH, EtOH, DMSO, DMF
8.	S_5	MeOH, EtOH, DMSO, DMF
9.	S_6	MeOH, EtOH, DMSO, DMF

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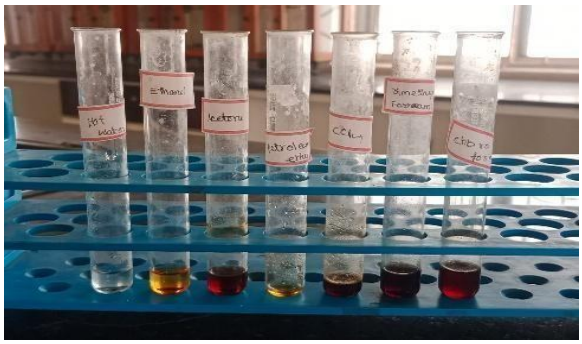

6.2. Thin Layer Chromatography

The purity of all synthesized compounds was monitored on paper chromatography.

Absorbent used : Whatt'smann filter paper

Mobile phase = Isopropanol ; Distilled water (6;4)

Detecting technique : Iodine chamber

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Table No.3

S.No	Compound Code	R_f value
1.	Compound 1	0.89
2.	Compound 2	0.85
3.	Compound 3	0.91
4.	S^1	0.90
5.	S_2	0.89
6.	S_3	0.84
7.	S_4	0.80
8.	S_5	0.90
9.	S_6	0.92

7. STRUCTURAL CONFIRMATION

SPECTRAL ANALYSIS

The objective of the spectral analysis is to confirm the chemical structures of the synthesized compounds and the various functional groups in the final compounds. The IR spectra of starting compound and intermediates were taken to confirm the changes at the reactive functional groups and then the final compounds were confirmed by IR, NMR and Mass spectroscopy.

The Infra Red spectroscopy was performed with KBr on Perkin Elmer Spectrum Version 10.03.07 instrument.

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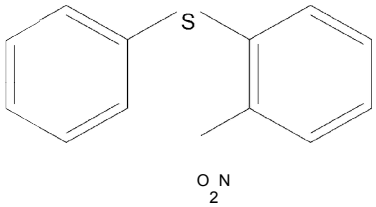
Compound-I



IUPAC Name : (2-nitrophenyl)(phenyl)sulfane IR interpretation

I.R Spectral data (KBr discs) (in Cm^{-1})	
C=N str.	1697.45
C=C str.	1541.36
C-H str.	3107.26

8. BIOLOGICAL SCREENING

8.*1. ANTIOXIDANT ACTIVITY*

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidations reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reaction by being oxidized themselves. Hydrogen peroxide scavenging activity is one of the methods for determining antioxidant *in-vitro* active. **8.1.1. Hydrogen peroxide (H_2O_2) Scavenging Activity**

Reagents

Hydrogen peroxide

Methanol

Phosphate buffer saline(PH-7.4)

Standard: **-** Ascorbic acid

Principle

All the compound and the standard are dissolved in methanol and the various concentration of sample ranging from 50-250µgm/ml) was prepared using methanol in different 10ml volumetric flasks. To each solution 2ml hydrogen peroxide (2ml) was be added and the volume made 10ml with phosphate buffer saline (PH-7.4). A control solution was prepared with methanolic solution in phosphate buffer saline without hydrogen peroxide solution. The absorbance at 230nm was recorded using a UV-visible spectrophotometer against blank samples. The percentage inhibition of Hydrogen peroxide scavenging activity will be calculated using the following formula

$$\% \text{ inhibition} = \frac{\text{Abs.control-Abs.of test}}{\text{Abs. control}} \times 100$$

Hydrogen peroxide Scavenging Effect (% Inhibition) Of Compounds (S_1-S_6)

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Table No: 4

Compounds	50µg/ml	100µg/ml	150µg/ml	200µg/ml	250µg/ml
S_1	48	69	62	80	90
S_2	40	49	63	55	82
S_3	59	68	82	71	78
S_4	53	57	62	74	87
S_5	51	41	60	84	79
S_6	45	66	75	84	88
STD	64	68	78	84	89

9. RESULTS AND DISCUSSION

Synthesis

The present study report the synthesis of phenothiazine derivatives nucleophillic substitution of 1-chloro nitro benezene with sodium hydride and thiophenol was carried out stepwise at different temperature by various amines and aldehydes. The first step involves substitution of thiophenol and the next by cumene. The final phenothiazine derivative in the synthesized compound 3 was replaced by various amine and aldehyde groups. Since the report regarding this compound suggest a phenothiazine posses a good bioactive moiety.

Physical characterization

Melting points of the synthesized compound was taken in open capillary tubes and was uncorrected and were found to be in the range 105-125˚C.

TLC was performed using precoated silica gel plates of 0.25mm thickness. Eluents used were chloroform, methanol (3:7) spots were visualized in U.V. light.

At room temperature solubility of newly synthesized compounds were determined by various organic solvents and it was found that all compounds were freely soluble in Methanol, Ethanol, DMSO and DMF.

Structural confirmation

The Infra red spectroscopy was performed with KBr on Perkin Elmer Spectrum Version 10.03.07 instrument. Presence of stretching in the range of 1500 cm^{-1} to 1800 cm^{-1} indicating the presence of C=N functional group. C=O stretching between at 1300 cm^{-1} to 1700 cm^{-1}.

^{1} H NMR spectroscopy was recorded on Bruker 400 MZs Avance. ^{1}H NMR the chemical shifts were reported as parts per million downfield from tetra methyl silane and solvent used as DMSO. Presence of chemical shift in the range 7.02-7.49 (m, 6H, CH), 6.76 - 6.86 (d, 6H, CH)

In-vitro Anti-oxidant activity

All the compounds were subjected to *in-vitro* anti-oxidant activity using ascorbic acid as a standard by two methods i.e. by Hydrogen peroxide scavenging method and nitric oxide radical scavenging method. Antioxidant activity revealed that all the synthesized compounds have shown significant antioxidant activity when compared with that of standard drug. The compounds S2 showed more activity as compared to the other derivatives.

In-vitro Anti-inflammatory activity

The synthesized compounds were subjected to *in-vitro* anti-inflammatory activity using HRBC membrane stabilizing method. The method involves the stabilization of human red blood cell membrane by hypotonicity induced membrane

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lysis. The prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the drug. The compound S3 showed better activity as compared to the standard diclofenac. Rest of the compounds showed less activity.

II. CONCLUSION

In the present study certain phenothiazine derivatives were synthesized and characterized by IR, ^{1}HNMR, and MASS spectroscopy. All the synthesized compounds show characteristic absorption peaks -in IR, ^{1}HNMR spectra. Expected molecules in (m+) fragments were observed for the entire compounds in mass spectra.

The synthesized compounds were subjected to various biological evaluations. The compounds were evaluated for anti-oxidant and anti-inflammatory studies revealed that the substitution of different aromatic amines and aldehydes to parent phenothiazine nucleus show the moderate activity.

Anti oxidant activity revealed that all the synthesized compounds have showed significant antioxidant activity when compared with that of standard by Hydrogen peroxide and Nitric oxide scavenging methods.

Anti inflammatory activity revealed that all the synthesized compounds have showed significant activity when compared with that of standard HRBC membrane stabilization methode.

REFERENCES

1.Ilango K, Valentina P, " Text Book of Medicinal Chemistry", Keerthi Publishers, Chennai 2007; 1st Edition : pp No 1-2.

2.Surendra Nath Pandeya "Text Book of Medicinal Chemistry" by S G Publisher Varanasi 2004; Third Edition: 1-6.

3.K Pandey*,Seema, R Pathak, "Synthesis of 1(p-arylidenophenyl)-2-oxo-4-methylpyrido[b] phenothiazines", Indian Journal of Chemistry, September 1999,volume-38B,Page.no.11111113.

4.Madhukar S Chande*, Vijay V Sugdare, " Novel Synthesis of fused ring [1,4]benzothiazines/[1,4] benzoxazines and related heterocycles ", Indian Journal of Chemistry, January 2000,volume39B,Page.no.65-67.

5.Rajini Guptha& R R Guptha*,Vibha Srivastav, "Synthesis of 4H-1,4-benzothiazines", Indian Journal of Chemistry, March 2000, volume-39B, Page.no.223-224.

6.D BhaskarReddy*,N Chandrasekhar Babu & A Padmaja, "Synthesis of 1-Aroyl-2styrylsulfonylethenes ; A source for dithianes,thiazines and thiaselenananes", Indian Journal of Chemistry, June2000, volume-39B,Page.no.406-409.

7.Mithun Rudrapal, S. J. K.; Jadhav, A. G. Drug Repurposing (DR): An Emerging Approach in Drug Discovery. In Drug Repurposing - Hypothesis, Molecular Aspects and Therapeutic Applications; Badria, F. A., Ed.; IntechOpen: London, United Kingdom, 2020; pp 3– 22.

8.Rácz, B.; Spengler, G. Repurposing antidepressants and phenothiazine antipsychotics as efflux pump Inhibitors in cancer and infectious diseases. Antibiotics 2023, 12 (1), 137, DOI: 10.3390/antibiotics12010137

9.Lopes, R. M.; Souza, A. C. S.; Otręba, M.; Rzepecka-Stojko, A.; Tersariol, I. L.; Rodrigues, T. Targeting autophagy by antipsychotic phenothiazines: potential drug repurposing for cancer therapy. Biochem. Pharmacol. 2024, 222, 116075, DOI: 10.1016/j.bcp.2024.116075 10.eleń, M.; Otto-Ślusarczyk, D.; Morak-Młodawska, B.; Struga, M. Novel tetracyclic azaphenothiazines with the quinoline ring as new anticancer and antibacterial derivatives of chlorpromazine. Int. J. Mol. Sci. 2024, 25 (8), 4148, DOI: 10.3390/ijms25084148

11.Phenothiazine Derivatives and Their Impact on the Apoptosis Processes: A Review Michał Otręba 1, Łukasz Marek 2, Piotr Paduszyński 3, Jerzy Stojko 2, Anna Rzepecka-Stojko . , PMID: 40926568,PMCID: PMC12668872,DOI: 10.1002/jat.4921.2026 Jan;46(1):42-60.doi: 10.1002/jat.4921. Epub 2025 Sep 10.

12.2023 Jun 15:492:153528. doi: 10.1016/j.tox.2023.153528. Epub 2023 Apr 29. Phenothiazine derivatives and their impact on the necroptosis and necrosis processes. A review Michał Otręba 1, Jerzy Stojko 2, Anna Rzepecka-Stojko 3