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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL PHENOTHIAZINE DERIVATIVES

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Abstract : New compounds were synthesized containing novel hydantoin derivatives and tested for their antimicrobial activity against gram positive and gram negative strains. The scheme was adopted for the synthesis of phenothiazine derivatives is done via thionation of aryl amines. Total five compounds were synthesized and characterization was done using various physical and spectral techniques such as Thin Layer Chromatography, Melting point ,Solubility determination, UV spectroscopy and FTIR spectroscopy and [1]HNMR spectroscopy. All the synthesized compounds were biologically evaluated for in-vitro antimicrobial activity against gram positive and gram negative bacteria by well diffusion method. The in-vitro evaluation of the synthesized compounds was performed by taking gram positive bacteria *Staphylococcus Aureus* and gram negative bacteria *Escherichia Coli*. The antibacterial activity of phenothiazine derivatives showed significant zone of inhibition against both bacterias. Thus from the study it is concluded that these new hydration derivatives could serve as effective antimicrobial agents.

IndexTerms **– phenothiazine, diphenylamine , antimicrobial activity, antibacterial activity.**

INTRODUCTION

Heterocyclic chemistry is a vast and expanding area of chemistry because of the obvious applications of compounds derived from heterocyclic rings in pharmacy, medicine, agriculture and other fields. The chemistry of heterocyclic compounds is as relevant as that of aliphatic or aromatic compounds. Their study is of great interest both from the theoretical as well as practical standpoint[1]. The versatility of heterocycles has been known for a century since their direct involvement in natural products. Particularly, the nitrogen-containing heterocycles (N-heterocycles) have proven ubiquitous structural features and pivotal roles in medicinal chemistry. Amongst the various N-heterocycles, indole motifs have received significant attention due to their presence in proteins, amino acids, bioactive alkaloids, and drugs.

Phenothiazines (heterocyclic ring system consisting of two benzene rings ortho-fused to 1,4-thiazine ring) and their analogs constitute an important class of bioactive heterocycles. Phenothiazine was first prepared by Bernthsen in 1883 in the course of proof of structure studies on Lauth's violet and methylene blue. Since then it has played an important role in dye chemistry as the parent compound of the thiazine dyes[2]. The chemistry of phenothiazine derivatives has since assumed considerable importance due to the diversity and number of compounds synthesized and the many products exhibiting extremely important therapeutic properties. Phenothiazine and related compounds have been reported to possess various diverse biological activities including neuroleptic, tranquilizer, antifungal, antimicrobial, anti-tumor, antiparkinson, anti-bacterial, anti-malarial, CNS depressant, analgesic, antiviral, antipyretic, anti-inflammatory, anti-tubercular and anti-histamine properties.[3] The present investigation was to synthesize some novel phenothiazine derivatives and their purification and characterization was performed. Later on biologically evaluated for antimicrobial activities.

NEED OF THE STUDY

Massive use of antibiotics has resulted in the emergence of resistance against them, which is another problem affecting public health. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella spp*, coagulase negative *Staphylococcus*, *Shigella*, *Enterococcus sp.* And *Escherichia coli* are amongst some of the main bacteria with multidrug resistance and are included in the category of community and hospital acquired pathogens. This has resulted in thestrong demand of new antibiotics by consumers against pathogens and an interest has been developed by the scientific community for using herbal medicines with antimicrobial properties.[3,4] Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. During the last few decades, it has been possible to combat the bacterial and fungal infections through major

improvement in early detection techniques, and newly developed antibiotics. Nevertheless, many of the currently used antimicrobials often lead to toxicity and undesirable side effects, or drug-drug interactions. The problem of antibiotic resistance has become particularly alarming due to the emergence of multi-drug resistant strains exhibiting simultaneous resistance to two or more classes of antibiotics. Some of these microbes initially caused nosocomial infections among the immunosuppressed or chronically ill patients in the hospitals.[5] More recently they have spread to the community, causing illness in previously healthy and otherwise non-vulnerable patients, as the available treatments were rather unresponsive as well as expensive. Therefore, the challenge is to develop better understanding of the genetic mechanisms of resistance, control the indiscriminate use of antibiotics, and find newer drugs against microbial diseases[5,6].

RESEARCH ENVISAGED

Antibiotics have been found to be one of humankind's most imperative weapons in combating microbial infections. Although there are highly effective antibiotics to cure nearly all major infectious diseases, such health benefits have come under threat, not only because many of these possess toxicity but also due to emergence of antibiotic-resistant bacteria. In the clinical world, consideration of retardation of pathogenic bacteria towards the available antibiotic was becoming a major worldwide problem as many bacterial pathogens have already established resistance against them.[7] The research on the development of new antimicrobial agents has mainly focused on two aspects like increasing potent bacterial antigen drugs and the appearance of new bacteria pathogen. The main aspect of synthesizing effective drugs is their structural characteristic and the rate of activity. Initially, there has been consideration of a heterocyclic compound as a parent compound to synthesize the effective antibacterial drug. The assessment of those vast antimicrobial literature data admits that heterocyclic compound has played a vital role in the clinical field[8]. Therefore, in the field of pharmacology, heterocycles are very popular for their unique controlling properties within a drug, such as a solubility, lipophilicity, and polarity, and are also being investigated several times for the discovery of desired active drugs. The fascinating biological significance of antibacterial pathogens has led to the urgency for drug discovery and the synthesis of new antimicrobial compounds.

Phenothiazines possess a wide spectrum of pharmacological/biological activities and their several derivatives are in clinical use. Phenothiazines are electron donors and therefore can form charge transfer complex with metal ions and with electron accepting molecules. The charge transfer complexes of phenothiazines play an important role in biological activities. This study was designed to develop some novel phenothiazine derivatives for the purpose of screening its antimicrobial activity against some gram positive and gram negative bacteria.

RESEARCH METHODOLOGY

Materials And Methods: Chemicals used in the synthesis of title compound described were purchased from SD fine chem limited 71-A, M I G Colony, Indore, Madhya Pradesh. All the other chemical and distilled water were used of laboratory grade. The melting point of all the synthesized compounds was carried out via Thiel's tube method and was uncorrected. For structural characterization of synthesized compound, the IR spectra will recorded in Perkin Spectrum BX spectrophotometer. The confirmation of reaction at every step were observe by TLC, using silica gel plates (silica gel 60 F254) in a developing solvent system of n-hexane:ethyl acetate (9:1) and the spots were visualized with the help of UV chamber and iodine vapors or sulfuric acid (30%v/v) and Rf value were determined. 1H NMR of the synthesized compound showing best antimicrobial activity was performed by the Fourier Transform Nuclear Magnetic Resonance spectroscopy, Model AVNACENEO500 Ascend Bruker BioSpin International AG[58].

Synthesis via thionation of aryl amines: Phenothiazine is prepared by a ring closure reaction called thionation of arylamines. It is prepared by fusing diphenyl amine with sulphur with the rapid evolution of hydrogen sulphide. In a beaker Diphenylamine (2g), Sulphur (364mg), and Anhydrous Calcium Chloride (284 mg) are melted together. The reaction sets 140-150°C with the rapid evolution of hydrogen sulfide; by lowering the temperature, a few degrees the reaction can be slackened. When the reaction has moderated, the temperature is raised to 160°C for a time. The melt, when cool is ground up and extracted first with water and then with dilute alcohol the residue consists of almost pure phenothiazine it can be recrystallized from alcohol**.** All the compounds were synthesized with same method.

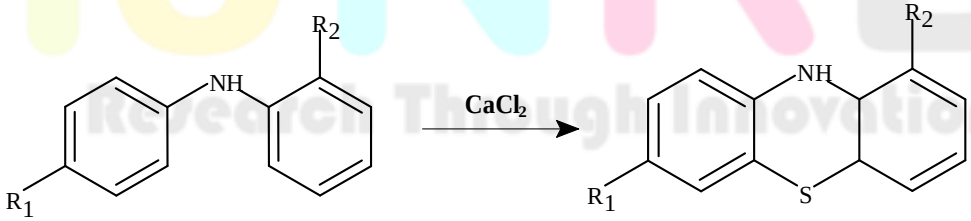
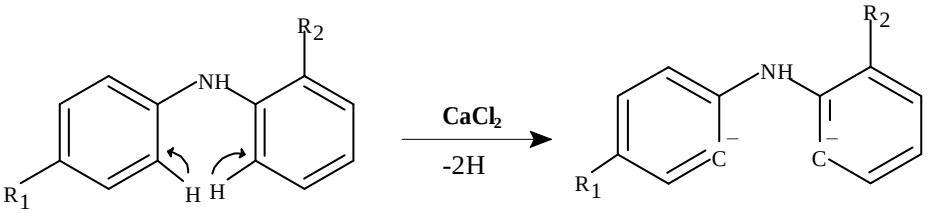


Fig: General reaction scheme

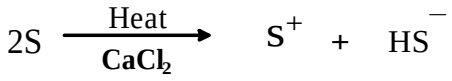
(where R_1 and R_2 varied alkyl or aryl substituent**)**

Reaction mechanism: The following reaction mechanism is used to synthesize all the derivative compound.

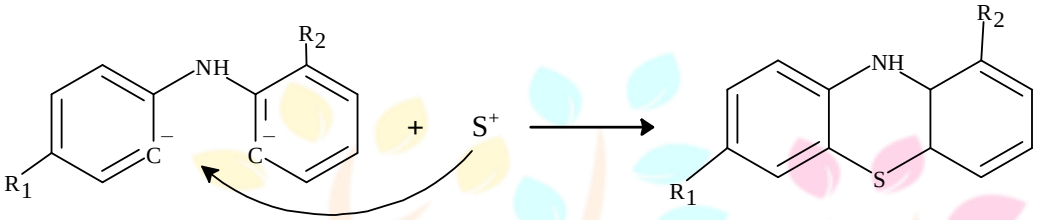
Step 1: Dehydrogenation of diphenylamine



Step 2: Generation of electrophile



Step 3: Evoluation of hydrogen sulfide



Step 4: Attack of electrophile

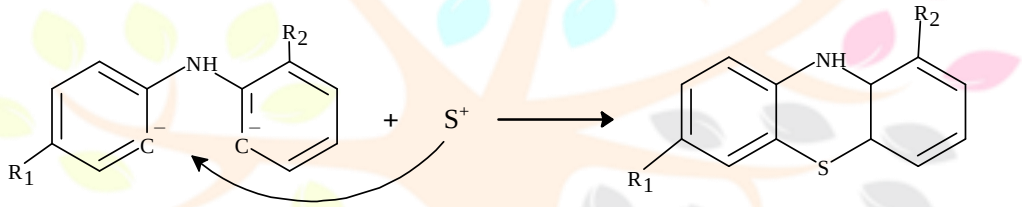
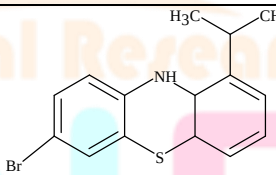
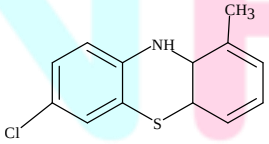
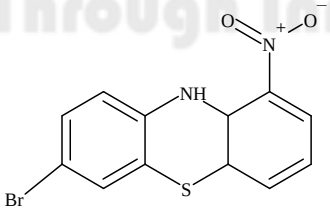
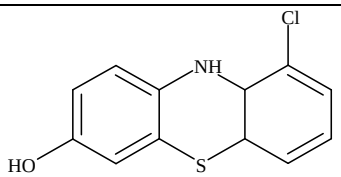


Table 01. Proposed structure phenothiazine derivatives.

S. No.	R_1	R_2	Proposed Structure	Final Phenothiazine Derivatives IUPAC Name
1	Bromo 	Iso-propyl 		7-bromo-1-(propan-2-yl)-10*H*-phenothiazine
2	Chloro 	Methyl 		7-chloro-1-methyl-10*H*-phenothiazine
3	Bromo 	Nitro 		7-bromo-1-nitro-10*H*-phenothiazine
4	Hydroxy 	Chloro 		1-chloro-10*H*-phenothiazin-7-ol

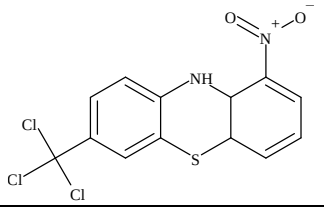
5	Trichloro-methyl 	Nitro 		1-nitro-7-(trichloromethyl)-10*H*-phenothiazine

Table 02. Physicochemical data of the compounds.

Compound Code	Yield (%)	Melting Point	R_f value
P1	42.56%	180-182°C	0.65
P2	47.37%	217-219°C	0.60
P3	36.84%	241-243°C	0.68
P4	51.83%	233-235°C	0.54
P5	49.73%	262-264°C	0.48

P1: 7-bromo-1-(propan-2-yl)-10*H*-phenothiazine
UV-Visible : λ_{max} is found to be 292.56 nm.

FT-IR (KBr cm^{-1}): 3396.45 cm^{-1} (N-H stretching), 1659.32 cm^{-1} (C-C stretching), 1723.27 cm^{-1} (C=O stretching), 3054.67 cm^{-1} (C–H stretching), 1534.64 cm^{-1} (C-C bending), 729.05 cm^{-1} (C-H rock).

P2: 7-chloro-1-methyl-10*H*-phenothiazine
UV-Visible : λ_{max} is found to be 296.54 nm.

FT-IR (KBr cm^{-1}): 3430.90 cm^{-1} (N-H stretching), 3094.34 cm^{-1} (aromatic C–H stretching), 2945.34 cm^{-1} (alkane C-H stretching), 1540.45 cm^{-1} (aromatic C–C stretching), 1495.05 cm^{-1} (alkane C-H bending), 950.05 cm^{-1} (aromatic C-H 'oop'), 554.96 cm^{-1} (C-Cl stretching).

P3: 7-bromo-1-nitro-10*H*-phenothiazine
UV-Visible : λ_{max} is found to be 241.23 nm .

FT-IR (KBr cm^{-1}): 3357.71 cm^{-1} (N-H stretching), 2834.53 cm^{-1} (C–H stretching), 3754.29 cm^{-1}, 1291.76 cm^{-1} (C–N stretching), 1420.36 cm^{-1} (C-C stretching), 3071.68 cm^{-1} (=C-H stretching).

P4: 1-chloro-10*H*-phenothiazin-7-ol
UV-Visible : λ_{max} is found to be 221.96 nm.

FT-IR (KBr cm^{-1}): 3360.45 cm^{-1} (N-H stretching), 3062.32 cm^{-1} (C–H stretching), 3496.24 cm^{-1} (O-H stretching), 1262.29 cm^{-1} (C–N stretching), 1545.35 cm^{-1} (C-C stretching), 559.24 cm^{-1} (C-Cl stretching), 1424.39 cm^{-1} (C-H bending).

P5: 1-nitro-7-(trichloromethyl)-10*H*-phenothiazine
UV-Visible : λ_{max} is found to be 295.67 nm.

FT-IR (KBr cm^{-1}): 3214.45 cm^{-1} (N-H stretching), 3089.44 cm^{-1} (C–H stretching), 1515.23 cm^{-1} (N-O stretching), 1000.12 cm^{-1} (=C-H bending), 724.53 cm^{-1} (C-H rock).

^{1}H NMR (500MHz, $CDCL_3$): δ 7.58 (d, J = 5.5, 1H -N(=O)=O group), 7.52 (m, 1H, 1H -C(Cl) (Cl) (Cl) group), 7.18 (d, J = 8, 1H =C-H-C-H=C-CCl_3), 6.78(s, 1H -N-H), 3.22 (s, 1H (=C-H) of 1-benzene) 2.07 (s, 1H H-C=C-H).

BIOLOGICAL EVALUATION

Antibacterial Activity:
The antimicrobial assay of all the synthesized compound was performed by Well Diffusion method. To evaluate the effect of synthesized compound against both gram+ve and gram-ve organism strains representing of each taken. These are *Escherichia coli* and *Staphylococcus aureus.* Nutrient media used for bacterial strains, stock culture of the microbial strains were prepared from original lyophilized strains using standard method. The nutrient agar media was prepared separately by standard method, and inoculum of 1 gram+ve and 1 gram -ve was prepared, test organism were incubated in 10 ml Nutrient broth. Then, 10 mg of standard (Ofloxacin) was taken with 1 ml solvent (distilled water) to make 10mg/10ml solution. The bacterial suspension was standardized to 108 CFU/ml of bacteria and kept into the shaker. Then, 50µl of the inoculum from the broth (containing 108 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate. The agar plate was inoculated by spreading the inoculum with a sterile spreader, over the entire sterile agar surface. Four wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with different concentration (25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml) of sample and another plate well was filled with 50µl of standard drug respectively. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed

for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well and compared to that of conventional compound. All the compounds showed comparable activities as that of Standard Ofloxacin (mm) against both bacterias.[1-nitro-7-(trichloromethyl)-10H-phenothiazine] showed best ZOI (Zone of Inhibition) of 32mm and 35mm in diameter at 100 µg/ml concentration against gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*E.coli*) which is found to be more prominent to that of standard _____(gram +ve) _____ mm and Ofloxacin (gram -ve) _____mm.

Antimicrobial activity of all compounds against *E.coli*

Compound code	Different concentrations			
	25 µg/ml	**50 µg/ml**	**75 µg/ml**	**100 µg/ml**
P1	6mm	6.6mm	7.2mm	8.2mm
P2	6.4mm	6.2mm	7.7mm	8.5mm
P3	7.3mm	8.2mm	9.5mm	10.3mm
P4	7.6mm	9.6mm	10.8mm	12.6mm
P5	7.5mm	8.4mm	13.2mm	13.7mm

Table 03: Compound code, Diameter in mm Inhibition.

Antimicrobial activity of all compounds against *Staphylococcus aureus*

Compound Code	Different concentrations			
	25 µg/ml	**50 µg/ml**	**75 µg/ml**	**100 µg/ml**
P1	6mm	6.2mm	7mm	8mm
P2	6.2mm	6.5mm	7.5mm	8.2mm
P3	7.4mm	8.4mm	9.2mm	10.5mm
P4	7.8mm	9.8mm	10.5mm	12.7mm
P5	8mm	8.8mm	13mm	13.9mm

Table 04: Compound code, Diameter in mm Inhibition.

IV. RESULTS AND DISCUSSION

The present study was aimed to synthesized, and characterized some novel phenothiazine derivatives as an anti-microbial agent. The scheme was adopted for the synthesis of phenothiazine derivatives is thionation of aryl amines. Total five compounds were synthesized and percentage yield was calculated. Characterization was done using various physical and spectral techniques such as Thin Layer Chromatography, Melting point, Solubility determination, UV-Visible Spectroscopy and Fourier Transform Infrared Spectroscopy. Thin Layer Chromatography of all the synthesized compounds was performed on silica gel plate and n-hexane: ethyl acetate was used as mobile phase, R_f values of all the synthesized compounds were calculated. The compounds were synthesized successfully and their structure were confirmed using FT-IR and ^{1}HNMR. In FT-IR spectra of compounds, the characteristic peak of N-H stretching, C-H stretching, N-O stretching and C-H bending were observed at 3214.45 cm^{-1} , 3089.44 cm^{-1}, 1515.23 cm^{-1}, & 1000.12 cm^{-1},respectively confirmed the structure of title compounds. Compounds (H1-H5) were screened for antibacterial activity against one-gram positive bacteria *Staphylococcus aureus* and one gram negative bacteria *E.coli*. The antibacterial activity of all derivatives showed the significant zone of inhibition against *E.coli*, and *Staphylococcus aureus*. Thus from the study it is concluded that these new derivatives could serve as effective antimicrobial agents, where P5 [1-nitro-7-(trichloromethyl)-10*H*-phenothiazine] have shown their antimicrobial activity less then 12mm against microbial strain. The activity of P5 was found to be good activity.

CONCLUSION

This study aims to synthesize novel phenothiazine derivatives, characterize their chemical and physical properties, and evaluate their activity as an antimicrobial agent. Among all the phenothiazine derivatives synthesized, compound P51-nitro-7-(trichloromethyl)-10*H*-phenothiazine showed greater zone of inhibition of 13.7mm and 13.9 mm against both the bacterial strains. The result obtained from this study could lead to the development of new drugs for the treatment of microbial infections.

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