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Research Article

Synthesis and Antimicrobial potential of Newly synthesized Dinitrophenothiazine derivatives

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ABSTRACT

A series of substituted dinitrophenothiazine derivatives have been synthesized successfully for their antimicrobial activity through the chemical reactions of different substituted chloronitrobenzne derivatives with different nitro aromatic amine derivatives. Formation of all the compounds have been checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. The compounds have been mainlycharacterized by IR and ^{1}H NMR spectral data. The compounds were evaluated for their antimicrobial activity on both gram positive as well as on gram negative strains of bacteria. The compounds third (C) exhibited most effective and potent antimicrobial activities among allthe newly synthesized derivatives. Although the total seven compounds have been give rise to good antibacterial activity when compare to the standard drug like levofloxacin, ciprofloxacin, chloramphenicol and amoxicillin etc. The *in-vitro* antimicrobial activity is compared with known standard drug at the same concentration. Then the antimicrobial activity was determined according to their zone of inhibition when compared with the standard drug and further discussed according to the SAR studies of newly synthesized compounds.

Keywords: Dinitrophenothiazine, Diphenylether, Thionation and Antimicrobial potential.

INTRODUCTION

Phenothiazine is also known as thiodiphenylamine. When two benzene nucleus are fused with 2, 3 and 5, 6 position of thiazine is called phenothiazine. A.Bernthsen has synthesized phenothiazine by the reaction of diphenylamine and sulphur in the presence of catalytic amount of iodine particles in 1883.Phenothiazine have synthesized by condensation of 2-chlorobenzoic acid with aniline by Ullman reaction in the presence of anhydrous K_2CO_3 and CuO[1, 2].

There are also numerous methods for synthesizing substituted phenothiazine derivatives. These are given below[3-6]

1. By treating the diphenylamine with sulphur.
2. By reacting diphenylamine with thionyl chloride.
3. By reacting 2 amino thiophenol with 2, 4, 6-trinitro chlorobenzene in presence of sodium hydroxide solution.
4. Treating 2-aminothiophenol with 2, 5-dinitro chlorobenzene in presence of sodium acetate solution.

5. Reaction of 2-amino-2'iodo-4,4-dinitro diphenyl sulphide in presence of cuprous iodide and sodium carbonate.
6. Reacting phenothiazine in glacial acetic acid with sodium nitrite.
7. By reacting 3-nitrophenothiazine in glacial acetic acid with hydrogen chloride.
8. By treating of 3, 7-dinitro phenothiazine in dil. HCl with tin/zinc metal.
9. By oxidative cyclisation of 2-amino-4-chloro-3-methyl benzene thiol with 2-halo/2, 6-dibromo/chloro nitrobenzene.
10. By the oxidative cyclisation of 2-amino-4-chloro-3-methyl benzene thiol with 2-halo/2, 6-dibromo/chloro nitro benzene.
11. By the reaction of 2-formamido-5'-methoxy-2'-nitro-4-trifluoromethyl diphenyl sulphide in presence of ethanolic sodium hydroxide.
12. By the reaction of 3, 4-dichloro-2-formamido-2'-nitro-4'-bromo diphenylsulphide in presence of ethanolic potassium hydroxide.

Phenothiazine possesses diverse types of biological activities as well as pharmacological activities and therapeutic properties which are like antimicrobial activity, anthelmintic activity, bactericidal activity, antidepressant activity, antitumor activity, anticancer activity, antitubercular activity, antileprotic activity, Lipid peroxidation inhibitors, cytoprotective activity, antitrypnosomal activity, sedative activity, anti-inflammatory activity, tranquillizers, antipsychotropic activity, antiviral activity, antidiabetic activity, antimalarial activity.[7, 8]

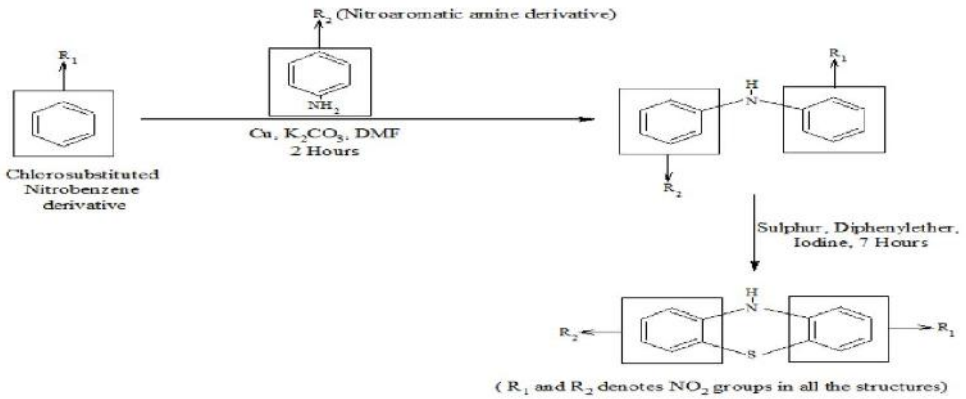
Phenothiazine derivatives have been reported as a valuable human medicine in the treatment of several disorders like different psychotic disorders, Parkinson's disease etc.

All the newly synthesized compounds were screened for *In-vitro* antibacterial activity against different microorganisms namely *Staphylococcus aureus* (Gram +Ve) and *Escherichia coli* (Gram-Ve). All the compounds subjected for antimicrobial activity and was performed by different concentrations and compared with known antibacterial drug like ciprofloxacin etc. The zone of inhibition was recorded by using zone reader in millimeter (mm).

Here in this method there is done synthesis of several dinitrophenothiazine derivatives based on Ullman reaction of chloronitrobenzene derivatives with different nitroaromatic amine derivatives. The yield of the products is good. In this study the use of dimethylformamide as a solvent in the Ullman reaction possess numerous benefits like condensation between nucleophile (aromatic amine) and chloro nitrobenzene takes place within a short time. The purity and yield of the product are in good agreement. The completion of the reaction is mainly checked by the TLC data. The thionation of the intermediate products in diphenylether gives the desired phenothiazine derivatives. After completion of the reaction resulting dinitrophenothiazine derivatives were crystallized from toluene-acetone solvent. The chemical structure of the newly synthesized compounds were characterized by their spectroscopic data like IR, NMR etc. which are reported in results and discussion sections.[9, 17, 18]

In this research article synthetic methods were investigated and all the components were screened for their *in-vitro* biological activity, like antimicrobial activity towards gram positive as well as gram negative bacterial strains at different concentrations. The biological activity of the newly synthesized compounds was compared with different standard drug.[12]

Preparation of the Compounds-
Chemical Scheme:



EXPERIMENTAL WORK (Synthesis of the compound, their characterization and antimicrobial potential)

Melting points were determined by capillarytube with the help of melting point apparatus. Formation of the compounds have been checked by TLC on silica gel-G plates of 0.5 mm thickness and the spots were located by iodine. The compounds were characterized mainly on the basis of IR and NMR spectroscopic data.

Step I

A mixture of chloro nitrobenzene derivative with nitroaromatic amine derivatives are treated together in the presence of Copper powder, anhydrous potassium carbonate taking dimethylformamide as a solvent on an oil bath and refluxed for 2hours. Finally, the mixture was filtered to remove the residue of potassium carbonate and copper powder. Then the residue was washed with hot DMF. The filtrate so obtained was poured into ice cold water to afford the solid product. The solid products so obtained was filtered then dried in air and recrystallized from toluene.[1, 10]

Step II

Now the mixture of dinitrodiphenylamine derivative was treated with sulphur powder and iodine in the solvent diphenylether and it was refluxed for 7 hours. The reaction mixture was distilled to remove excess solvent. On cooling the reaction mixture that is dinitrophenothiazine derivative separated out as an orange crystalline mass. This was recrystallized from a toluene-acetone mixture to give the final newly synthesized dinitrophenothiazine derivatives.[1, 11]

ANTIMICROBIAL ACTIVITY OF THE NEWLY SYNTHESIZED COMPOUNDS

The newly synthesized dinitrophenothiazine derivatives were successfully tested for their antimicrobial activity. The compounds are able to produce significant antibacterial activity. The various strains of bacteria were taken and the newly synthesized compounds were tested against the gram positive as well as gram negative strains of the bacteria.[12, 13]

The minimum inhibitory concentration (MIC) was determined by using Disc Diffusion method by measuring the zone of inhibition according to the designed protocol. All the compounds were dissolved in Dimethylsulphoxide (DMSO) which was previously tested for the antibacterial activity and found to have no given antibacterial activity.[14]

All the newly synthesized compounds were screened *in-vitro* for their antimicrobial activity against a variety of bacterial strains (Gram+Ve and Gram–Ve) such as ***Staphylococcus aureus, Staphylococcus pyogenes, Bacillus subtilis and Escherichia coli***. The MIC of the compounds was defined as the lowest concentration at which there was 80% inhibition of growth compared with the growth for a drug free control. Inhibition of zone size for the standard drug is maximum at ≤50µg/ml.[15, 19, 20]

The discs were also previously sterilized and then used. The diameters of the taken discs were 6mm and they were impregnated with the known concentration of the compounds and finally inoculated in the prepared agar media.[16, 21]

RESULTS AND DISCUSSION

All the seven novel dinitrophenothiazine derivatives were synthesized by Ullman coupling of chloronitrobenzene derivative and nitroaromatic amine derivatives taking copper powder as a catalyst and taking anhydrous potassium carbonate in presence of dimethylformamide as a solvent. The dinitrophenylamine derivatives formed, were further treated with sulphur and iodine in presence of diphenylether as a solvent. After the formation of the compounds they were properly recrystallized by recrystallization and column chromatography. The structure of the synthesized compounds was characterized on the basis of their spectral data mainly IR and NMR. The newly synthesized compounds showed mild to potent antimicrobial activity.

Among the seven synthesized compounds the compound C showed antimicrobial activity up to maximum extent as shown by the standard drug.

The significant outcome of the study is the emergence of the different dinitrophenothiazine derivatives as promising antimicrobial agents. An extension of the study in future may contribute to the development of useful antimicrobial agents of this series of compounds.

The parent dinitrophenothiazine derivatives are good antimicrobial agents against several strains of the bacteria, including ***Staphylococcus aureus, Staphylococcus pyogenes, Bacillus subtilis and Escherichia coli.*** The newly synthesized dinitrophenothiazine derivatives showed no activity below 50µg/ml but give rise to noticeable activity at≥50µg/ml concentration.[12, 21]

Table 1
PHYSICAL DATA OF THE COMPOUNDS

Compounds	R^1	R^2	Molecular formula	Melting Range(0^0C)	% yield
Compound A	1 NO_2	7 NO_2	$C_{12}H_7N_3O_4S$	207-209	56.2
Compound B	1 NO_2	6 NO_2	$C_{12}H_7N_3O_4S$	211-213	55.6
Compound C	2 NO_2	7 NO_2	$C_{12}H_7N_3O_4S$	216-218	67.4
Compound D	3 NO_2	6 NO_2	$C_{12}H_7N_3O_4S$	210-212	60.2
Compound E	3 NO_2	7 NO_2	$C_{12}H_7N_3O_4S$	206-208	59.2
Compound F	3 NO_2	8 NO_2	$C_{12}H_7N_3O_4S$	214-216	68.7
Compound G	3 NO_2	9 NO_2	$C_{12}H_7N_3O_4S$	205-207	64.2

Explanation of the R_1 and R_2-These explain the position of the nitro substitutions on the final prepared phenothiazine nucleus.
In compound A 1 NO_2 and 7 NO_2 denotes the nitro substitutions on the main phenothiazine derivatives. Simply it is 1, 7 dinitrophenothiazine. In compound B 1 NO_2 and 6 NO_2 denotes the nitro substitutions on the main phenothiazine derivatives. Simply it is 1, 6 dinitrophenothiazine. In compound C 2 NO_2 and 7 NO_2 denotes the nitro substitutions on the main phenothiazine derivatives. Simply it is 2, 7dinitrophenothiazine. In compound D 3 NO_2 and 6 NO_2 denotes the nitro substitutions on the main phenothiazine derivatives. Simply it is 3, 6 dinitrophenothiazine. In compound E 3 NO_2 and 7NO_2 denotes the nitro substitutions on the main phenothiazine derivatives. Simply it is 3, 7 dinitrophenothiazine. In compound F 3 NO_2 and 8NO_2 denotes the nitro substitutions on the main phenothiazine derivatives. Simply it is 3, 8 dinitrophenothiazine. In compound G 3NO_2 and 9NO_2 denotes the nitro substitutions on the main phenothiazine derivatives. Simply it is 3, 9 dinitrophenothiazine.

Table 2
IR SPECTRA & ^{1}HNMR SPECTRAL DATA OF COMPOUNDS

Compounds	IR (KBr pellets) cm^{-1}	^{1}HNMR
Compound A	3481-------N-H stretch 3361----------N-H Asym. stretch 3082 (C-H stretch aromatic) 1631 (C= C stretch) 1481(C-S-stretch), 1300 (O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz)7.93-7.91(brs, 2H-H6&8), 7.38-7.37-(s,1H-H-2), 7.35-7.13 (s,1H-H-3),7.11-7.10(s,1H-H4)6.99-6.97(s,1H-H-9)&3.36(brd hump1H-NH)
Compound B	3431---NH stretch 3332-------- N-H Asym. stretch 3099 (C-H stretch aromatic) 1602 (C= C stretch) 1447 (C-S-stretch),1344(O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz)8.23-7.21(s, 1H-H2), 7.73-7.70-(s,1H-H-7), 7.36-7.35 (s,1H-H-3),7.35-7.29(s,1H-H4),7.27-7.25(s,1H-H-8),7.23-7.21(s,1H-H-9)&3.34(brd hump1H-NH)
Compound C	3431---NH stretch 3332-------- N-H Asym. stretch 3099 (C-H stretch aromatic) 1620 (C= C stretch) 1479 (C-S-stretch),1346(O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz)8.23-8.21(brs, 2H-H1&3), 7.73-7.71-(brs,2H-H-6&8), 7.35-7.29 (s,1H-H-4),7.27-7.25(s,1H-H-9) & 3.34(brd hump1H-NH)
Compound D	3481---NH stretch 3361-------- N-H Asym. stretch 3050 (C-H stretch aromatic) 1631 (C= C stretch) 1471 (C-S-stretch),1298(O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz)8.81-8.64(brs, 2H-H2&4), 8.62-7.96-(s,1H-H-7), 7.92-7.90 (s,1H-H-1),6.69-6.58(s,1H-H-8,6.56(s,1H-H-9), &3.40 (brd hump1H-NH)
Compound E	3457---NH stretch 3354-------- N-H Asym. stretch 3224 (C-H stretch aromatic) 1606 (C= C stretch) 1465 (C-S-stretch), 1309(O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz)7.93-7.91(brs, 2H-H2&4),7.38-7.36(brs,2H-H-6&8)7.34-7.13(s,1H-H-1), 7.11-7.09 (s,1H-H-9), &3.36(brd hump1H-NH)
Compound F	3774---NH stretch 3369-------- N-H Asym. stretch 3097 (C-H stretch aromatic) 1614 (C= C stretch) 1350 (C-S-stretch), 1157(O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz)8.85-8.81(brs, 2H-H2&4),8.81-8.80(brs,2H-H7&9)8.64-8.62-(s,1H-H-1), 7.96-7.94 (s,1H-H-6), &3.32(brd hump1H-NH)
Compound G	3431---NH stretch 3329-------- N-H Asym. stretch 3076 (C-H stretch aromatic) 1625 (C= C stretch) 1438 (C-S-stretch), 1157(O-N-O stretch)	^{1}HNMR(DMSO-D_6-400MHz)8.24-8.22(brs, 2H-H2&4),7.74-7.72(s,1H-H-8)7.35-7.29(s,1H-H-1), 7.27-7.25 (s,1H-H-6),7.23-7.21(s,1H-H-7)&3.28(brd hump1H-NH)

Table 3
Antibacterial activity against *Staphylococcus aureus* (Gram +Ve)

S No	Compounds	Diameter of zone of inhibition(mm)			
		Different concentrations of the compounds			
		50 µg/ml	100µg/ml	150µg/ml	200µg/ml
1	Compound A	7	6	8	9
2	Compound B	11	12	11	13
3	Compound C	13	16	22	17
4	Compound D	8	7	9	10
5	Compound E	11	10	12	13
6	Compound F	10	12	10	16
7	Compound G	11	10	12	17
Standard drug	Levofloxacin	12	16	24	19

Zone of Inhibition-Zone of inhibition is expressed in mm. The standard drug shows zone of inhibition at 24 mm at 150µg/ml. Zone of inhibition below 10 mm denotes no antibacterial activity. Zone of inhibition at 10-15 mm denotes mild antibacterial activity. Further zone of inhibition at15-20 mm denotes the moderate antibacterial activity. Similarly zone of inhibition at more than 20 mm denotes potent antibacterial activity.

Standard drug: Levofloxacin
Standard drug shows significant antibacterial action at doses at ≤50µg/ml and better antibacterial activity at other remaining doses.
In all the above seven compounds, the Compound C shows maximum antibacterial action at doses 150 µg/ml and mild to moderate antibacterial action at other remaining doses. Compound A does not show antibacterial activity at doses 50 µg/ml, 100 µg/ml, and 150 µg/ml and at 200 µg/ml. Compound B shows mild antibacterial action at doses 50 µg/ml, 100 µg/ml, 150µg/ml and 200 µg/ml.
Compound D does not show antibacterial action at doses50 µg/ml, 100 µg/ml, 150 µg/ml, but shows mild antibacterial action at200µg/ml.
Compound E shows mild antibacterial action at all the doses. Compound F shows moderate antibacterial activity at 200µg/ml and mild antibacterial action at other remaining doses.
Compound G shows mild antibacterial action at all the doses but shows moderate antibacterial action at 200 µg/ml.

Table 4
Antibacterial activity against *Staphylococcus pyogenes* (Gram+Ve)

S No	Compounds	Diameter of zone of inhibition(mm)			
		Different concentrations of the compounds			
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
1	Compound A	8	6	10	7
2	Compound B	11	10	12	14
3	Compound C	10	15	20	18
4	Compound D	7	10	8	12
5	Compound E	11	8	12	14
6	Compound F	11	10	14	11
7	Compound G	10	12	10	11
Standard drug	Ciprofloxacin	11	15	22	18

Standard Drug: **Ciprofloxacin**
Zone of Inhibition-Zone of inhibition is expressed in mm. The standard drug shows zone of inhibition at 22 mm at 150µg/ml. Zone of inhibition below 10 mm denotes no antibacterial activity. Zone of inhibition at 10-15 mm denotes mild antibacterial activity. Further zone of inhibition at 15-20 mm denotes the moderate antibacterial activity. Similarly zone of inhibition at more than 20 mm denotes potent antibacterial activity. Standard drug shows significant antibacterial activity at doses at doses at ≤50µg/ml.
Compound C shows maximum antibacterial action at doses 150 µg/ml, mild to moderate antibacterial action at other remaining doses. Compounds A does not show antibacterial action at doses at 50 µg/ml,100 µg/ml and 200 µg/ml but shows mild antibacterial action at 150 µg/ml. Compound B shows mild antibacterial action at all the mentioned doses. Compound D does not show antibacterial action at 50 µg/ml and 150 µg/ml but exhibits mild antibacterial action at 100 µg/ml and 200 µg/ml respectively. Compound E does not show antibacterial action at 100 µg/ml but shows mild antibacterial action at all other remaining doses. Compound F shows mild antibacterial action at all the given doses. Similarly Compound G also exhibits mild antibacterial action at all the given doses.

Table 5
Antibacterial activity against *Bacillus subtilis* (Gram+Ve)

S No	Compounds	Diameter of zone of inhibition(mm)			
		Different concentrations of the compounds			
		50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
1	Compound A	7	8	11	9
2	Compound B	10	12	11	13
3	Compound C	11	17	21	19
4	Compound D	9	11	10	12
5	Compound E	10	12	14	11
6	Compound F	9	10	11	14
7	Compound G	10	11	14	10
Standard drug	Chloramphenicol	10	16	24	16

Standard drug: Chloramphenicol
Zone of Inhibition-Zone of inhibition is expressed in mm. The standard drug shows zone of inhibition at 24 mm at 150µg/ml. Zone of inhibition below 10 mm denotes no antibacterial activity. Zone of inhibition at 10-15 mm denotes mild antibacterial activity. Further zone of inhibition at 15-20 mm denotes the moderate antibacterial activity. Similarly zone of inhibition at more than 20 mm denotes potent antibacterial activity.
Compounds C shows significant antibacterial action at doses at doses at 150 µg/ml also mild to moderate antibacterial action at other reaming doses. Compound A does not show antibacterial action at 50 µg/ml, 100 µg/ml and 200 µg/ml, but shows mild antibacterial action at 150 µg/ml. Compound B shows mild antibacterial action at all the doses. Compound D does not show antibacterial action at 50 µg/ml but shows mild antibacterial action at other remaining doses. Compound E shows mild antibacterial action at all the mentioned doses.Compound F does not show antibacterial action at 50 µg/ml but possesses mild antibacterial action at other remaining doses.Compound G possesses mild antibacterial action at all the doses.

Table-6
Antibacterial activity against *Escherichia coli* (Gram –Ve)

S No	Compounds	Diameter of zone of inhibition (mm)			
		Different concentration of the compounds			
		50 µg/ml	100µg/ml	150µg/ml	200µg/ml
1	Compound A	7	9	12	8
2	Compound B	10	12	14	11
3	Compound C	10	16	22	17
4	Compound D	8	10	9	12
5	Compound E	10	13	12	11
6	Compound F	7	9	12	10
7	Compound G	10	15	11	12
Standard drug	Amoxicillin	11	15	22	14

Standard drug: Amoxicillin
Zone of Inhibition-Zone of inhibition is expressed in mm. The standard drug shows zone of inhibition at 22mm at 150µg/ml. Zone of inhibition below 10 mm denotes no antibacterial activity. Zone of inhibition at 10-15 mm denotes mild antibacterial activity. Further zone of inhibition at 15-20 mm denotes the moderate antibacterial activity. Similarly zone of inhibition at more than 20 mm denotes potent antibacterial activity.
Compound C significant antibacterial action at dose 150 µg/ml and mild antibacterial action at other remaining doses.
Compound A does not show antibacterial action at doses at 50 µg/ml, 100 µg/ml and 200 µg/ml but shows mild antibacterial action at doses at 150 µg/ml. Compound B shows mild antibacterial action at all the mentioned doses. Compound D does not show mild antibacterial action at doses 50 µg/ml, 150 µg/ml but shows mild antibacterial action at 100 µg/ml and 200 µg/ml respectively. Compound E shows mild antibacterial action at all the mentioned doses. Compound F does not show antibacterial action at 50 µg/ml and 100 µg/ml but possesses mild antibacterial action at 150 µg/ml and 200 µg/ml respectively. Compound G shows moderate antibacterial action at100 µg/ml but shows mild antibacterial action at other remaining doses.

CONCLUSION

It is concluded from the chemical scheme that it is appropriate and efficient method for the synthesis of substituted dinitrophenothiazine derivatives with excellent yield that have been developed. Other related libraries are under investigation. In conclusion the newly synthesized dinitrophenothiazine derivatives mainly showed wide spectrum of antimicrobial activity exhibiting an equal inhibition of the growth of bacteria. The some compounds possessing activity almost equal to the reference drug like levofloxacin, ciprofloxacin, chloramphenicol and amoxicillin. Some newly synthesized compounds

mainly the compound C showed potent antibacterial activity up to great extent at 150µg/ml nearly equal to the known reference drug like levofloxacin, ciprofloxacin, chloramphenicol and amoxicillin. The compound C showed equal growth inhibition to the standard drug Amoxicillin (22 mm) against *Escherichia coli* (gram negative bacteria).Similarly the mainly the compound C also possesses nearly equal growth inhibition when compared to the standard drug against other bacterial strains.

It can be stated that these newly synthesized compounds are promising new antibacterial agents in treating microbial infections. The present result is worth noticing because in recent years increasing rates of antimicrobial resistance among community for treating microbial infections. It is observed that introduction of a chloro group at para position increases the activity exceedingly then ortho and meta position. So finally it is concluded that the newly synthesized compounds were evaluated for antimicrobial activity and the results are compared with the known standard drugs. The newly synthesized compounds showed prominent action on gram positive as well as on gram negative bacteria showed mild to moderate antimicrobial potential but some compounds mainly the compound C possesses potent antimicrobial activity.

By the conclusion we can also plan to do further studies on these dinitrophenothiazine series of compounds for their numerous types of biological activities besides the antimicrobial activity.

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