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Synthesis And Biological Screening of Some New N-Substituted Thiocarbamido 2-Chlorophenothiazines

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Abstract:
A series of synthesis of new N- substituted thiocarbamido derivatives of 2-chlorophenothiazines using various substituted thiourea and synthesized compounds were characterized by IR, ^{1}H NMR, and Mass spectra. Final compounds [2(a-f)] have been evaluated for their antibacterial and antioxidant screening. Some of the compounds showed promising antimicrobial, antifungal and antioxidant activities.

Keywords: Substituted 2-chlorophenothiazine, Antibacterial activity, Antifungal activity, Antioxidant activity

1.1INTRODUCTION

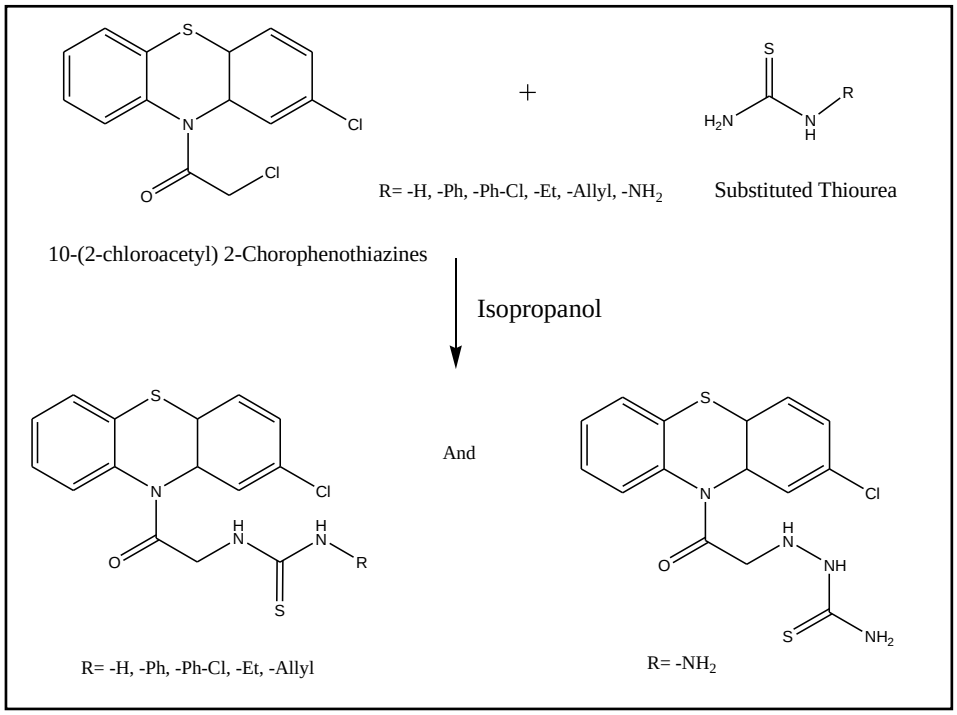
Medicinal chemistry comprises designing and synthesizing new compounds with subsequent evaluation of their biological activities to set the new assumption as the basis for further compound design and synthesis.[1] Phenothiazine represents one of the deserved intermediate for this study. Phenothiazines are amongst the most frequently encountered heterocycles in compounds of biological interest. They have been shown to possess a broad spectrum of biological activity depending on their structure. Infect, it constitutes the largest group of psychoactive clinically used compounds, phenothiazine derivatives possess several other biological activities including antibacterial[2-3], antifungal, antiproliferative[4], antipsychotic[5-6], anti-inflammatory[7-8] and antitumor activities[9-10]. Like urea, thiourea is a useful organic molecule with a wide range of applications. The only difference is that thiourea has a sulphur atom instead of the oxygen atom. Because sulphur and oxygen have different electronegativity levels, urea and thiourea have quite different characteristics. Thiourea is therefore an adaptable chemical for use in organic synthesis. In the realm of medicinal chemistry, derivatives of urea, thiourea, and thiosemicarbazide are essential because they control a variety of pharmacological properties. A review of the literature indicates that urea and thiourea compounds exhibited a wide range of biological activities, including analgesic, HDL-raising, antiviral, and anti-HIV effects[11].

Tayade *et al.* Synthesised newly Cyanoamidinothio carbamides and evaluated Antimicrobial Activities[12]. Aswale *et al.* synthesized thi ocarbamidoace to phenone by an interaction of 2-hydroxy-3-nitro-5-methyl-α-bromoacetophenone with substituted thiourea[13]. Waghmare *et al*. reported Interactions of (2E)-1-(4-chlorophenyl)-3-(3,4 dimethoxyphenyl) prop-2-en-1-one with various substituted thioureas such as thiourea, N-phenylthiourea, 2-chlorophenylthiourea, 3-chlorophenylthiourea and 4- chlorophenyl thiourea in presence of isopropanol as a medium and all synthesized compounds screened against various microorganisms such as gram-positive *Staphylococcus aureus, gram-negative Escherichia coli*[14]. Patil *et al.* successfully synthesized 1-(4-Hydroxy-6-methyl-pyrimidino)-3-substituted thiocarbamides and antimicrobial activities of these newly synthesized compounds were carried out by MIC method[15]. Synthesis of N-(7-substitutedthiocarbamidoquoniline-4-yl)-N, N-diethyl- pentane-1,4-diamines from N-(7-chloroquinoline-4-yl)-diethyl-pentane-1,4-diamine and substituted thiourea was reported[16].

Synthesis of 1-substituted-3-(4-pyridinoimino) thiocarbamides from substitutedthiourea was reported[17].
pH metric, conductometric and refractometric study of various 5-substitutedthiocarbamido-1-naphthol were carried out in laboratory[18-22].
Synthesis of 4-amino-5-substitutedthiocarbamido-N-[2-(diethylamino)- ethyl] -*o*-anisamides from 4-amino-5-chloro-N-[2-(diethyl-amino)- ethyl]-*o*-anasimide and substituted thiourea was reported[23].
Green synthesis of 1-formamidino-3-substitutedformamidinothiocarbamides from cyanogunadine and thiocarbamides were also mentioned in literature[24].
Spectrophotometric measurements of substituted thiocarbamidona phthols were also reported[25].
Synthesis of 2-chloro-11-(piperazin-1-yl) dibenzo[b,f]-[1,4]oxazepines from substituted thiourea was reported[26].
Recently in this laboratory, a series of (2*E*)-1-(4-substitutedthiocarba- midophenyl)-3-(3,4-dimethoxyphenyl) prop-2-en-1-ones was synthesized by interactions of (2*E*)-1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl) prop-2-en-1-one and different thiourea, some of them showed good biological activities[27].
By the known literature in this laboratory synthesize a starting material 10-(2-chloroacetyl) phenothiazines from 2- Chlorophenothiazine and Chloroacetylchloride which is confirmed by IR, NMR data and Melting point

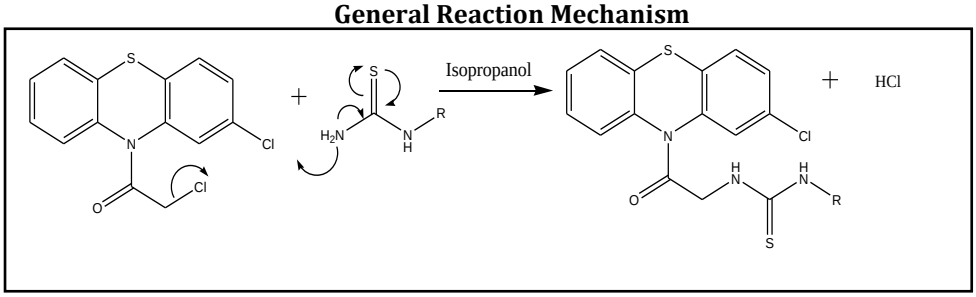
1.2PRESENT WORK

The synthesis of biologically relevant 2-Chlorophenothiazine derivatives has been of considerable interest for its appreciable role in medicinal chemistry. Hence in our present research work we have synthesize the Literature survey showed that, yet interactions of N-(2-chloroacetyl) phenothiazines and various thiourea are still lacking, hence it was thought interesting to investigate interactions of 10-(2-chloroacetyl) 2-Chorophenothiazines with substituted thiourea in isopropanol medium to obtain a novel series of 10-substituted thiocarbamido 2-chlorophenothiazines [II(a-f)] by somewhat suitable and eco-friendly method (Scheme-1) and synthesized compounds were characterized by IR, ^{1}H NMR, and Mass spectra. Final compounds **[2(a-f)]** have been evaluated for their antibacterial, antioxidant and anti-cancer screening.



Scheme I: - Synthesis of 10-substituted thiocarbamido 2-chlorophenothiazines [2(a-f)]

General Reaction Mechanism



1.3: RESULTS AND DISCUSSION

Formation of 10-substituted thiocarbamido derivatives of 2-chlorophenothiazine [2(a-f)] was confirmed based on elemental analysis, IR, NMR and Mass spectra. The IR spectra of compound show absorption in the range 3329–3340 cm^{-1} due to the presence N–H group, 1679-1704 cm^{-1} due to the presence C=O group, 1426-1484 cm^{-1} due to the presence C=S group and 1104-1172 cm^{-1} due to the presence C-N bending vibration group.

The ^{1}H NMR spectrum of all synthesized compound showed a singlet at δ (8.71-8.78) due to the N-H protons, singlet at δ (3.37-4.14) due to the -CH2 protons, and multiplate at δ (6.66-7.81) due to the aromatic region, in compound 2a showed δ (5.21), δ (5.85) and δ (3.91) due to the -CH_2, -C=H and -CH_2 protons, in compound 2d showed δ (4.58), δ (4.12) due to the - CH_2 and -CH_3 protons, in compound 2e showed δ (1.97) due to the N-H proton and in compound 2e and 2f -NH_2 protons are invisible.

Further evidence for the formation of compounds [2(a-f)] was obtained by recording the mass spectra. The mass spectrum of compounds [2(a-f)] showed a molecular ion peak at m/z 389.9, 425.9, 460.4, 377.0, 351.8, 364.8, and 351.8 which is in conformity with the molecular formula.

It gives Lassigne's positive test for nitrogen and sulphur. Synthesized Products was desulphurized by alkaline plumbite solution indicating that sulphur is present in open chain and formation of wine red colour by added sodium nitroprusside solution indicating that ketonic group is present.
The Rf value was found to be 0.54, by using Hexane+Ethyl acetate solvent 9:1 on silica Gel-G having layer thickness 0.3 mm.
All synthesized compounds screened their antimicrobial and antioxidant activity showed predominant activity.

1.4: EXPERIMENTAL SECTION

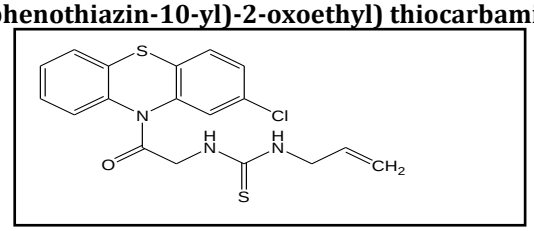
All chemicals were purchased from Aldrich and TCI, Mumbai (India), and were used without further purification. The melting points were determined in open capillaries using a Toshniwal melting point apparatus and are uncorrected. The IR spectra were recorded in the solid state as a KBr suspension on a Perkin– Elmer spectrum one FT-IR spectrophotometer and ^{1}H NMR spectra were obtained in DMSO-d^6 on a Brucker 400 MHz instrument using TMS as an internal standard (chemical shifts in δ, ppm), Mass spectra on a LCQ Advantage Thermo Finnigen spectrometer. Elemental analysis was performed on Carlo Erba 1108 analyzer.

General procedure for the preparation of 10-substituted thiocarbamido 2-chlorophenothiazines [2(a-f)]:

Equimolar quantities of 10-(2-chloroacetyl) 2-Chorophenothiazines and substituted thiourea was taken in isopropanol and the reaction mixture was refluxed for about 3 hours at 80°C. During refluxing homogeneous reaction mixture was obtained and formation product was confirmed by TLC. It was then allowed to cool at room temperature then water was added to it and extracted with dichloromethane (DCM). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to get certain product. All synthesized products were recrystallized by ethanol.

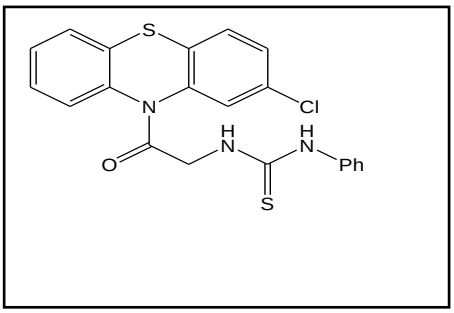
1.5: CHARACTERIZATION DATA

1.5.1:-1-allyl-3-(2-(2-chloro-10H-phenothiazin-10-yl)-2-oxoethyl) thiocarbamide (2a)



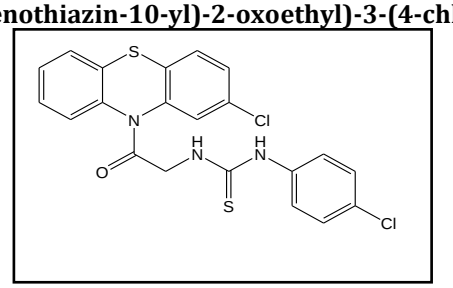
In 100 ml round bottom flask a reaction mixture of 10-(2-chloroacetyl) 2-Chorophenothiazines (0.0048 M, 1.5gm) and allyl thiourea (0.0058M, 0.67gm) was taken in isopropanol (60 ml) and the reaction mixture was refluxed for about 3 hours at 80°C. During refluxing homogeneous reaction mixture was obtained and formation product was confirmed by TLC. It was then allowed to cool at room temperature then water was added to it and extracted with dichloromethane (DCM). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to get certain product. All synthesized products were recrystallized by ethanol.
M.F.: $C_{18}H_{16}ON_3S_2Cl$, Light Brown, Yield: 70% elemental analysis: calculated (Found) C: 55.45, H: 4.14, N: 10.78, S: 16.45 M.P.: > 300 °C, (Lit 40 > 280 °C)
IR (KBr):.3382.92 (-NH stretching), 3065.02 (Ar-H stretching), 1468.86 (N-C=S stretching), 1575.91 (Ar-C=C stretching), 1679.11 (C=O stretching) and 1108.15 (N-C=S bending).
1H NMR (DMSO-*d6*, 400 MHz): δ 8.712 (s, 1H, -NH), 6.65-7.02 (m, 7H, Ar-H), 5.21 (d, allyl -CH_2), 5.85 (q, allyl CH), 4.12 (s, 1H, -NH) and 3.9 (s, carbonyl -CH_2).
LC-MS m/z (ES+): m/z: 313.30 $[M+H]^+$ Base peak: 197.30 (100%), 233.20 (25%)

1.5.2:-1-(2-(2-chloro-10H-phenothiazin-10-yl)-2-oxoethyl)-3-phenyl thiocarbamide (2b)



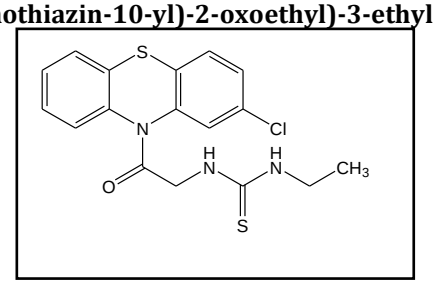
In 100 ml round bottom flask a reaction mixture of 10-(2-chloroacetyl) 2-Chorophenothiazines (0.0048 M, 1.5gm) and phenyl thiourea (0.0058M, 0.88gm) was taken in isopropanol (60 ml) and the reaction mixture was refluxed for about 3 hours at 80°C. During refluxing homogeneous reaction mixture was obtained and formation product was confirmed by TLC. It was then allowed to cool at room temperature then water was added to it and extracted with dichloromethane (DCM). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to get certain product. All synthesized products were recrystallized by ethanol.
M.F.: $C_{21}H_{16}ON_3S_2Cl$, Dark Brown, Yield: 84% elemental analysis: calculated (Found) C: 59.21, H: 3.79, N: 9.86, S: 15.06 M.P.: > 300 °C, (Lit 40 > 280 °C)
IR (KBr):.3337.92 (-NH stretching), 3023.23 (Ar-H stretching), 1477.54 (N-C=S stretching), 1580.73 (Ar-C=C stretching), 1681.04 (C=O stretching) and 1170.84 (N-C=S bending).
1H NMR (DMSO-*d6*, 400 MHz): δ 8.761 (s, 1H, -NH), 6.65-7.02 (m, 12H, Ar-H), 4.12 (s, 1H, -NH) and 3.34 (s, carbonyl $-CH_2$).
LC-MS m/z (ES+): m/z: 442.42 $[M+H]^+$ Base peak: 269.30 (100%), 212.30 (75%)

1.5.3:-1-(2-(2-chloro-10H-phenothiazin-10-yl)-2-oxoethyl)-3-(4-chlorophenyl) thiocarbamide (2c)



In 100 ml round bottom flask a reaction mixture of 10-(2-chloroacetyl) 2-Chorophenothiazines (0.0048 M, 1.5gm) and p-chlorophenyl thiourea (0.0058M, 0.89gm) was taken in isopropanol (60 ml) and the reaction mixture was refluxed for about 3 hours at 80°C. During refluxing homogeneous reaction mixture was obtained and formation product was confirmed by TLC. It was then allowed to cool at room temperature then water was added to it and extracted with dichloromethane (DCM). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to get certain product. All synthesized products were recrystallized by ethanol.
M.F.: $C_{21}H_{15}ON_3S_2Cl_2$, Dark Brown, Yield: 90% elemental analysis: calculated (Found) C: 54.78, H: 3.29, N: 9.13, S: 13.93 M.P.: > 300 °C, (Lit 40 > 280 °C)
IR (KBr):.3336.99 (-NH stretching), 3033.19 (Ar-H stretching), 1484.29 (N-C=S stretching), 1574.95 (Ar-C=C stretching), 1681.04 (C=O stretching) and 1172.71 (N-C=S bending).
1H NMR (DMSO-*d6*, 400 MHz): δ 8.81 (s, 1H, -NH), 6.66-7.81 (m, 11H, Ar-H), 4.02-3.99 (s, 1H, -NH) and 4.15 (s, carbonyl $-CH_2$).
LC-MS m/z (ES+): m/z: 460.89 $[M+H]^+$ Base peak: 280.25(100%), 337.25 (80%)

1.5.4:-1-(2-(2-chloro-10H-phenothiazin-10-yl)-2-oxoethyl)-3-ethyl `thiocarbamide (2d)



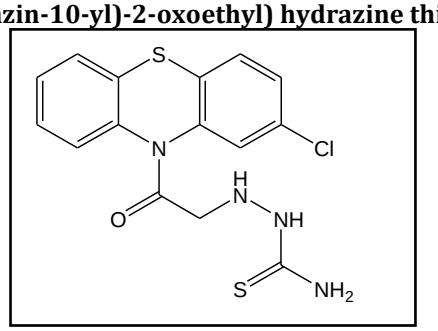
In 100 ml round bottom flask a reaction mixture of 10-(2-chloroacetyl) 2-Chorophenothiazines (0.0048 M, 1.5gm) and ehyl thiourea (0.0058M, 0.53gm) was taken in isopropanol (60 ml) and the reaction mixture was refluxed for about 3 hours at 80°C. During refluxing homogeneous reaction mixture was obtained and formation product was confirmed by TLC. It was then allowed to cool at room temperature then water was added to it and extracted with dichloromethane (DCM). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to get certain product. All synthesized products were recrystallized by ethanol.

M.F.: $C_{17}H_{16}ON_3S_2Cl$, Light Brown, Yield: 80% elemental analysis: calculated (Found) C: 54.03, H: 4.27, N: 11.12, S: 16.97 M.P.: > 300 °C, (Lit 40 > 280 °C)
IR (KBr):.3339.89 (-NH stretching), 3065.98 (Ar-H stretching), 1461.19 (N-C=S stretching), 1583.63 (Ar-C=C stretching), 1685.86 (C=O stretching) and 1130.33 (N-C=S bending).

1H NMR (DMSO-*d6*, 400 MHz): δ 8.72 (s, 1H, -NH), 6.65-7.81 (m, 7H, Ar-H), 4.56-3.4.53 (s, 1H, -NH), 4.28-4.26 (q, 2H, CH_2), 1.12 (t, 3H, CH_3) and 3.89 (s, carbonyl -CH_2).

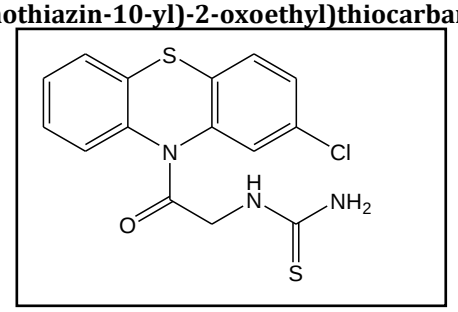
LC-MS m/z (ES+): m/z: 377.78 $[M+H]^+$ Base peak: 289.30 (100%), 131.25 (50%)

1.5.5:-2-(2-(2-chloro-10H-phenothiazin-10-yl)-2-oxoethyl) hydrazine thiocarbamide (2e)



In 100 ml round bottom flask a reaction mixture of 10-(2-chloroacetyl) 2-Chorophenothiazines (0.016 M, 5gm) and thiosemicarbazide (0.019M, 1.78gm) was taken in isopropanol (60 ml) and the reaction mixture was refluxed for about 3 hours at 80°C. During refluxing homogeneous reaction mixture was obtained and formation product was confirmed by TLC. It was then allowed to cool at room temperature then water was added to it and extracted with dichloromethane (DCM). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to get certain product. All synthesized products were recrystallized by ethanol.
M.F.: $C_{15}H_{13}ON_4S_2Cl$, Light Brown, Yield: 80% elemental analysis: calculated (Found) C: 49.38, H: 3.59, N: 15.36, S: 17.58 M.P.: 121°C (Known Lit.)
IR (KBr):.3329.15 (-$NHNH_2$ stretching), 2998.59 (Ar-H stretching), 1459.31 (N-C=S stretching), 1590.97 (Ar-C=C stretching), 1704.06 (C=O stretching) and 1151.19 (N-C=S bending).
1H NMR (DMSO-*d6*, 400 MHz): δ 8.71 (s, 2H, -NH-NH), 6.66-7.08 (m, 7H, Ar-H), 1.89-1.91 (s, 2H, -NH_2) and 3.37 (s, carbonyl -CH_2).
LC-MS m/z (ES+): m/z: 368.25 $[M+H]^+$ Base peak: 132.10.25(100%), 233.05 (60%)

1.5.6:-1-(2-(2-chloro-10H-phenothiazin-10-yl)-2-oxoethyl)thiocarbamide (2f)



In 100 ml round bottom flask a reaction mixture of 10-(2-chloroacetyl) 2-Chorophenothiazines (0.0048 M, 1.5gm) and thiourea (0.0058M, 0.45gm) was taken in isopropanol (60 ml) and the reaction mixture was refluxed for about 3 hours at 80°C. During refluxing homogeneous reaction mixture was obtained and formation product was confirmed by TLC. It was then allowed to cool at room temperature then water was added to it and extracted with dichloromethane (DCM). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to get certain product. All synthesized products were recrystallized by ethanol.
M.F.: $C_{15}H_{12}ON_3S_2Cl$, Light green, Yield: 70% elemental analysis: calculated (Found) C: 51.50, H: 3.46, N: 12.01, S: 18.33 M.P.: 178-182°C
IR (KBr):.3340.85 (-$NHCSNH_2$ stretching), 3065.98 (Ar-H stretching), 1465.96 (N-C=S stretching), 1583.63 (Ar-C=C stretching), 1682.06 (C=O stretching) and 1104.29 (N-C=S bending).
1H NMR (DMSO-*d6*, 400 MHz): δ 8.76 (s, 1H, -NH), 6.65-7.09 (m, 7H, Ar-H) and 3.19 (s, carbonyl -CH_2).
LC-MS m/z (ES+): m/z: 352.84 $[M+H]^+$ Base peak: 231.85(100%), 291.80 (70%)

1.6 BIOLOGICAL ACTIVITY

1.6.1 Antimicrobial and Antifungal Studies

The newly synthesized compounds were screened for their antibacterial and antifungal activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* bacterial strains at 50µl and 100µl concentration by disc diffusion method[28-29].

Tetracycline and Griseofulvin used for standard drug for antibacterial and antifungal screening and treat similar condition for comparison. Solvent and growth controls were prepared and kept. Zones of inhibition and minimum inhibitory concentrations (MICs) were noted. The results of antibacterial and antifungal studies are given in Table 1.

Table 1: Antibacterial and Antifungal data

Compound no.	*Staphylococcus aureus*		*Escherichia coli*		*Candida albicans*	
	50µl	**100µl**	**50µl**	**100µl**	**50µl**	**100µl**
2a	18	23	-	-	15	22
2b	-	9	-	-	-	13
2c	-	9	-	-	9	14
2f	19	22	-	-	12	18
TC 50 µl	27		18		16	

"-" Indicates bacteria are resistant to the compounds at concentration >100 µl
TC = Tetracycline inhibition diameter in mm

From fig. 1 and above evaluation of antibacterial and antifungal activity data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compounds **2a** and **2f** showed good activity against *Staphylococcus aureus* and *Candida albicans* and compounds **2b** and **2c** showed moderate activity against *Staphylococcus aureus* at 100 µl concentrations also compounds **2b** and **2c** showed moderate activity against *Candida albicans.* All compounds are resistant to *Escherichia coli* bacterial strains.

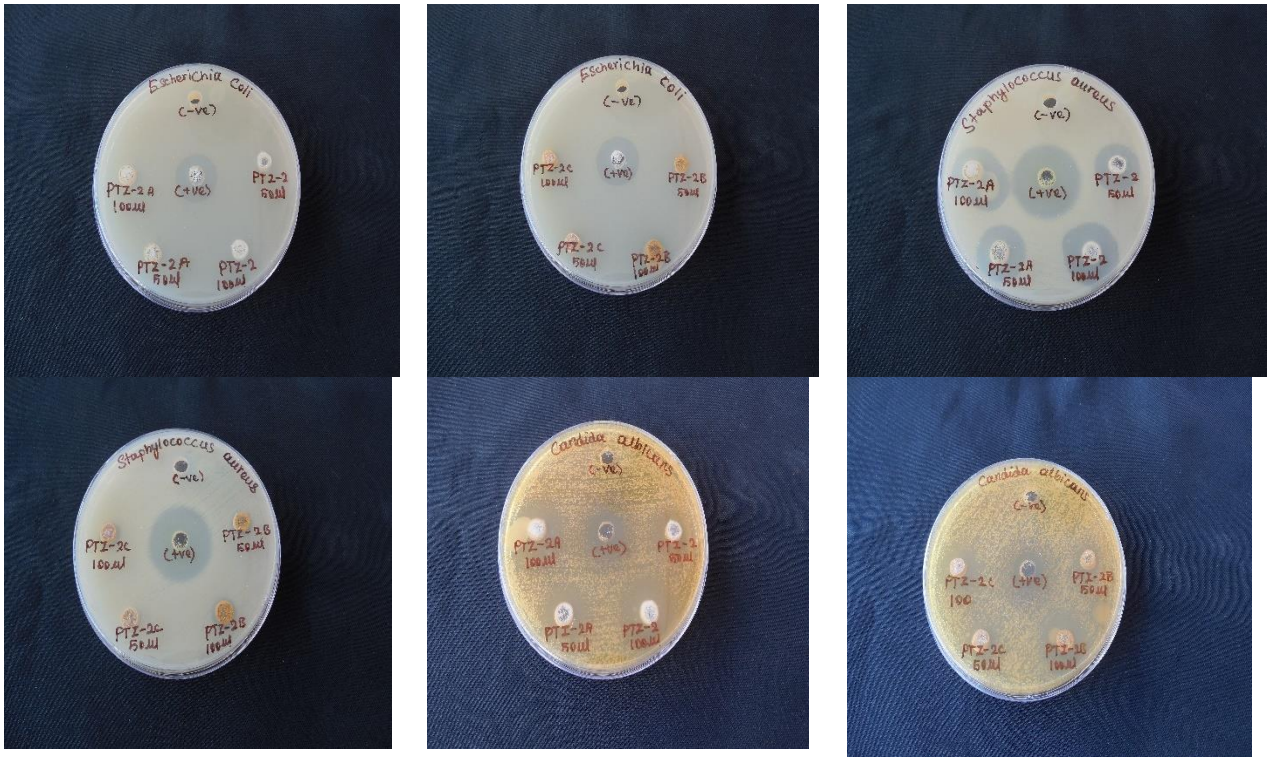


Fig 1: Antimicrobial and Antifungal evaluation of compounds 2a, 2b, 2c and 2f against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*

1.6.1 Antioxidant studies (by DPPH method)

The antioxidant activity of synthesized compounds was determined by e free radical scavenging activity[30]. Several concentrations of the derivatives were prepared and the strength in inhibiting free radicals of DPPH was tested by coupling with their own free radicals, ascorbic acid was used as a standard. The DPPH radical scavenging capability was calculated using the following equation,% inhibition = (ABS control – ABS test) /ABS control × 100
The percentage antioxidant activity (% inhibition) was extrapolated against concentration of the compound and IC_{50} was determined graphically. The results are tabulated in Table 2

Compound no.	Concentration	%I	IC_{50}	Compound no.	Concentration	%I	IC_{50}
2b	10	13.84	39	2d	10	-1.64	120.65
	20	31.42			20	1.91	
	40	51.28			40	8.74	
	60	54.83			60	16.12	
	80	71.86			80	35.06	
	100	72.13			100	41.44	
2c	10	15.48	64.58	2f	10	-1.32	81.33
	20	19.03			20	8.31	
	40	44.72			40	18.42	
	60	46.45			60	33.22	
	80	54.55			80	49.18	
	100	57.56			100	56.00	
Standard (**Ascorbic acid**)	10	50.10	9.98				
	20	53.43					
	40	60.86					
	60	67.14					
	80	65.62					
	100	69.05					

From fig. 2 and the results summarized in table 2 the compound **2b** and **2c** showed strong activity due to presence of electron withdrawing substituent are present. Compound **2f** showed moderate activity due to presence of free amine group and compound **2d** showed less activity due to presence of electron donating substituent.

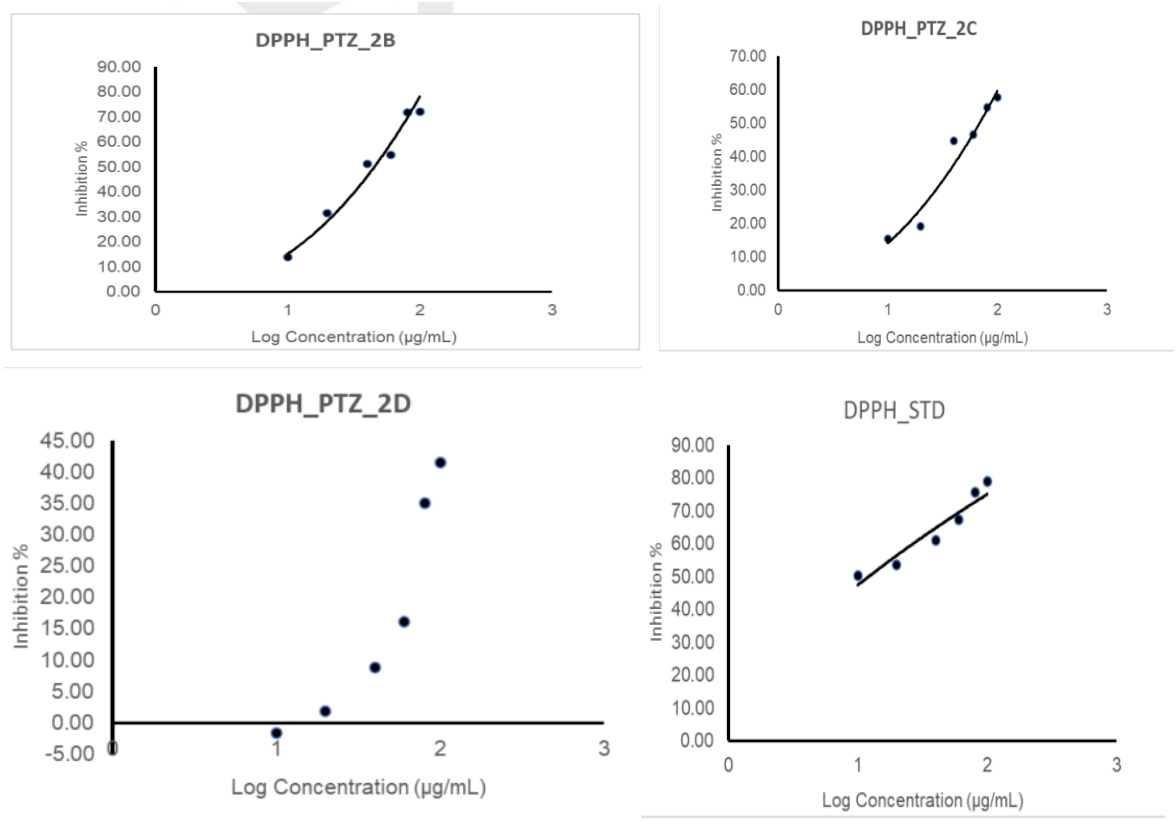


Fig 2: Graph plotted between the percentage growth inhibition and log concentration of compounds 2b, 2c, 2d and standard (Ascorbic acid)

1.6 CONCLUSION

In Summary, we have reported a facile and convenient route for the synthesis of 10-substituted thiocarbamido 2-chlorophenothiazines [II(a-f)] having allyl, phenyl, 4-chlorophenyl, ethyl, hydrazine as novel N-substituted 2-chlorophenothiazines. In the present work simple workup procedure, short reaction time as compared to other synthetic routes, no need of catalysts, and good to excellent yields of final products. All the synthesized compounds have been thoroughly characterized by IR, ^{1}H NMR, and Mass spectroscopy. Synthesized compounds screened their antimicrobial and antifungal studies compounds 2a and 2f showed strong activity and antioxidant study compound 2b and 2c showed strong activity due to presence of electron withdrawing substituent.

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Conflict of interest No potential conflict of interest was reported by the authors.

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