AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Synthesis of 10 - {(P-Methyl Anilino) - Methyl} - 7, 8, 9 – Substituted – 1 - Carboxyl and 7 / 3 - Substituted – 2 / 8 - Chloro Phenothiazines as Potential Antimicrobial Agents

Nilesh Y. Jadhav[1*], Shivakumar M. Hipparagi[2], M. Bhagavan Raju[3]

1. Research Scholar, JNTU Hyderabad, HSBPVT's GOI College of Pharmacy, Department of Pharmaceutical Chemistry, Kashti, Ahmednagar, Maharashtra, India 414701

2. KLEUniversity's College of Pharmacy, Rajajinagar, Bangalore, Karnataka, India 560010

3. Gland Institute of Pharmaceutical Sciences, Shivampeth, Hyderabad, Andhra Pradesh, India502313

ABSTRACT

Two series of substituted phenothiazine derivatives were synthesized in order to obtain better antimicrobial agents. The titled compounds 3a, 3b, 3c, 3d, 3e, 3f and 3g, 3h, 3i, 3j were obtained by using o-chlorobenzoic acid and m-dichlorobenzene as starting material respectively. These compounds were synthesized according to Ullmann and Mannich base reaction. The structures of all these compounds were confirmed by spectral analysis. The titled compounds were screened for antibacterial and antifungal activities. Except 3e all compounds have shown good antibacterial activity against gram-positive and gram-negative bacteria. However, all these compounds have shown good antifungal activity against *A. niger*. The compound 3g has shown greater antifungal activity than the standard.

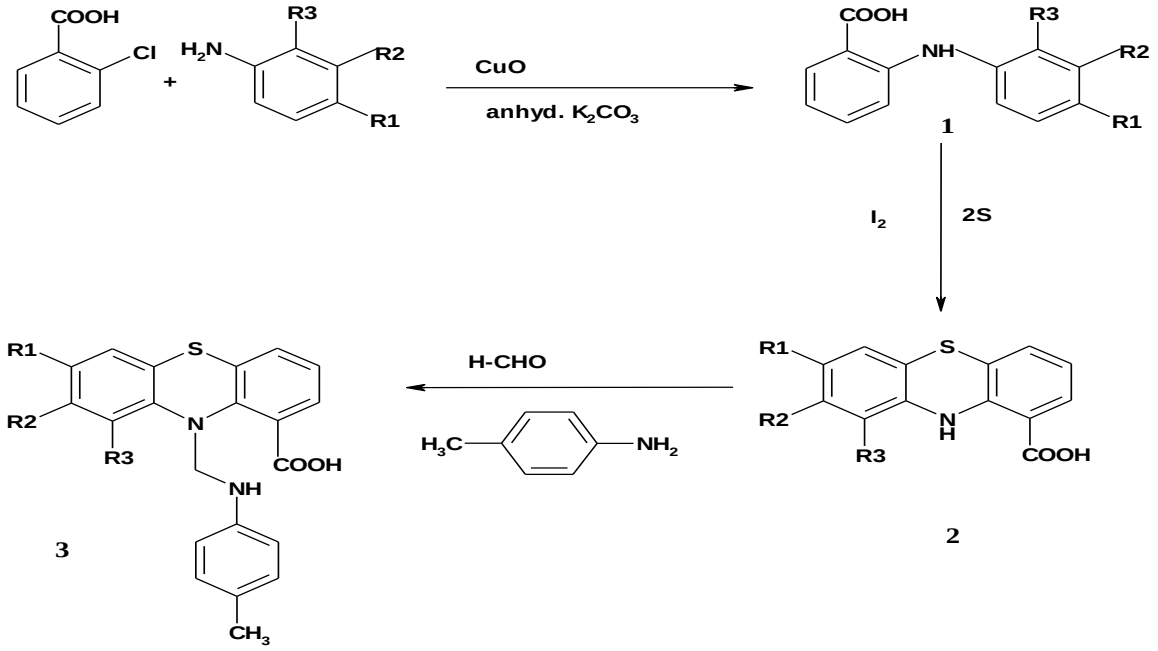
Keywords: Phenothiazine; o-Chlorobenzoic acid; Antibacterial; Ullmann; Mannich.

*Corresponding Author Email: masternilesh@rediffmail.com
Received 06 June 2015, Accepted 23 June 2015

Please cite this article as: Jadhav N *et al*., Synthesis of 10 - {(P-Methyl Anilino) - Methyl} - 7, 8, 9 – Substituted – 1 - Carboxyl and 7 / 3 - Substituted – 2 / 8 - Chloro Phenothiazines as Potential Antimicrobial Agents. American Journal of PharmTech Research 2015.

INTRODUCTION

Phenothiazine derivatives constitute an important class of compounds possessing number of biological activities including anthelmintic[1], antihistaminic[2], analgesic[3], antiinflammarotry[4], tranquilizers[5,7], antimicrobial activities[6]. It has been reported that slight variation in its structure can lead to different biological activities. In phenothiazine ring the substituents at positions 2 and 10 modify pharmacological activity. Substitution at 3 position can improve activity over non substituted compounds but not as significantly as substitutions at the 2 position. Phenothiazines have been proved to be successful chemotherapeutic agents also. The aim of the present investigation was to synthesize two series of ten substituted phenothiazine compounds in order to obtain better antimicrobial agents. We report here the synthesis and antimicrobial activity of N -10 substituted phenothiazine derivatives containing carboxylic group at 1 position (scheme 1) and chloro group at 2 / 8 position (scheme 2).



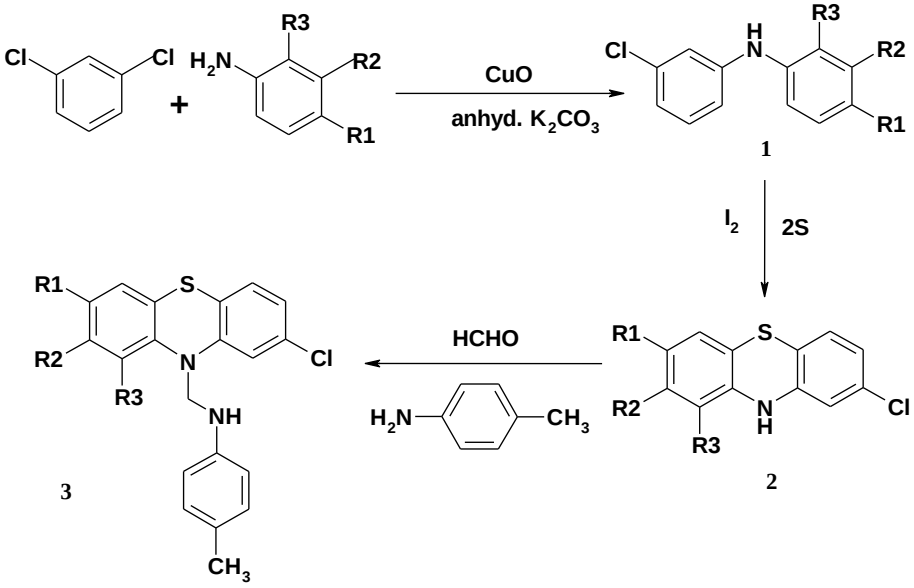
Compounds 3a - 3f

Scheme 1: Synthesis of 10 - {(p-methyl anilino) - methyl} - 7, 8, 9 – substituted – 1 – carboxyl phenothiazines 3a – 3f

MATERIALS AND METHOD

All the chemicals used were of synthetic grade and procured from Loba Chemie Pvt. Ltd., Research-Lab, Ranbaxy fine Chemicals Ltd., S.D. fine-Chem Ltd., India. Melting points of compounds were taken in open capillary tubes and are uncorrected. IR spectra were recorded on SHIMADZU FT-IR – 8400 spectrophotometer using KBr pellet. ^{1}H NMR spectra were recorded

on a VARIAN MERCURY YH – 300 spectrometer (300 MHz) using DMSO – d6, $CDCl_3$ and $CDCl_3$ + DMSO – d6 mixture as a solvent. Mass spectra were recorded on GCMS QP 5050 SHIMADZU (Gas Chromatograph Mass Spectrometer). Thin layer of chromatography was performed using plate coated with Silica gel $GF_{254}LR$ of 0.25 thicknesses. The mobile phase used for all compound was benzene, methanol, ammonia in the ratio of 75:25:0.25 respectively. The spots were located by subjecting the plates to iodine chamber.



Compounds 3g -3j

Scheme 2: Synthesis of 10 - {(p-methyl anilino) - methyl} – 7 / 3 - substituted – 2 / 8 - chloro phenothiazines 3g – 3j

MATERIALS AND METHODS

All the compounds were synthesized by conventional method. **Scheme 1** shows the synthetic route to the target compounds **(3a-3f)**. The target compounds **3a-3f** were synthesized by Ullmann and Mannich base reaction. Ullmann reaction is used to prepare symmetrical and unsymmetrical biaryls (step1)[8].

Step 1. 2 – Carboxyl – 2', 3', 4' - substituted diphenylamine

A mixture of 2- Chlorobenzoic acid (0.01 mole); 2, 3, 4 – substituted aniline (0.01 mole); were condensed using water bath for nearly about 2 hrs. in the presence of CuO and anhydrous K_2CO_3. The intermediate 1 was collected and recrystallised from hot ethanol.

Step 2. 7, 8, 9 - Substituted - 1- carboxyl phenothiazines

A mixture of 2 – carboxy – 2', 3', 4' – substituted diphenylamine (0.01 mole) and sulphur (0.02 mole) were condensed in the presence of iodine as a catalyst using water bath for nearly about 2

hrs. The product was cooled and poured into crushed ice, filtered, air dried and recrystallised from dioxane.

Step 3. [10- {[(aryl) – amino] – methyl} – 7, 8, 9 – substituted – 1 – carboxyl phenothiazines]

A mixture of 7, 8, 9 substituted – 1- carboxyl phenothiazines (0.01 mole), formaldehyde (2.0 ml), 4 – methyl aniline (0.01 mole), hydrochloric acid (5 ml) and dioxane (25 ml) was refluxed for 5 hr in a oil – bath or sand bath. The cold reaction mixture was filtered and filtrate is poured directly into ice water. Then the isolated product was collected from the ice – water and wet solid was air-dried and recrystallised from dioxane.

Scheme 2 shows the synthetic route of the title compounds **3g – 3j.** These compounds were synthesized as per the procedure described in scheme 1 using m-dichlorobenzene instead of o-chlorobenzoic acid as the starting material. The first step was carried out using sand bath. All the compounds were obtained in acceptable yield.

Corresponding R_1, R_2, R_3 Values of synthesized compounds 3a-3j (i.e. Scheme 1 and 2) are shown in Table 1 and physicochemical parameters are shown in Table 2.

Table 1: Corresponding R_1, R_2, R_3 Values of synthesized compounds 3a-3j (Scheme 1 and 2)

Sr. No.	Corresponding R_1, R_2, R_3 Values			Code Assigned
	Nature of R_1	Nature of R_2	Nature of R_3	
1	F	H	H	3a
2	SO_3H	H	H	3b
3	NO_2	H	H	3c
4	H	NO_2	H	3d
5	H	H	NO_2	3e
6	Cl	H	H	3f
7	OCH_3	H	H	3g
8	Cl	H	H	3h
9	NO_2	H	H	3i
10	SO_3H	H	H	3j

Table 2: Physicochemical Parameters of Synthesized Compounds (3a-3j)

Compounds	Melting Point	R_f value	% Yield
3a	108^0C	0.72	58
3b	103^0C	0.80	72
3c	101^0C	0.60	60
3d	98^0C	0.58	52
3e	125^0C	0.55	48
3f	80^0C	0.64	46
3g	87^0C	0.70	65
3h	95^0C	0.62	58
3i	90^0C	0.71	51
3j	92^0C	0.45	55

Table 3: Antibacterial and antifungal activity of 3a-3j compounds

Sr.No.	Compounds	A*	B*	C*	D*	E*	F*	G*
1	3a	16	35	21	16	24	19	19
2	3b	19	24	19	10	18	17	20
3	3c	17	20	19	12	20	16	15
4	3d	16	24	14	14	18	15	23
5	3e	15	23	6	14	21	10	18
6	3f	19	24	19	14	14	18	20
7	3g	21	25	24	17	22	22	26
8	3h	20	24	18	14	17	23	22
9	3i	16	20	24	16	23	21	20
10	3j	18	22	18	15	15	22	17
11	Ofloxacin	25	45	32	24	30	26	NA
12	Cefadroxil	NA	40	NA	NA	28	NA	NA
13	Griseofulvin	NA	NA	NA	NA	NA	NA	25
14	Control	0	0	0	0	0	0	0

A = *E. coli* **B** = *P. vulgaris* **C** = *S.aureus* **D** = *K. pneumoniae* **E** = *S. typhi* **F** = *B. subtilis* **G** = *A.niger***.**

***= Zone of inhibition in mm**

Antibacterial activity[9, 10]

Antibacterial activity studies were carried out by cup plate method against the micro-organisms, *E. Coli, P. vulgaris, S. aureus, K. pneumoniae, S. typhi, B. subtilis*. The nutrient agar medium was prepared in the distilled water (28 g in 1000 ml) then it was sterilized in the autoclave. In the mean while plate and pipettes were sterilized in the hot air oven. Then the sterilized hot medium was poured in petri plates and kept for solidify for half hour. Then the suspension of microbial culture was poured into to petriplates and spread on the plate evenly. The well was prepared. To those well 0.1 ml solution of compounds to be tested was added aseptically at a concentration of 50 µg/ml and the plates were kept for incubation at 37^0C for 24 hr in an incubator. The zone of inhibition was measured after 24 hr in mm. Ofloxacin and Cefadroxil were used as standard.

Antifungal activity[11]

The antifungal activity of the compounds was tested against *A.niger* using nutrient agar medium. The sterilized (autoclaved at 120°c for 30min) medium (40°-50°c) was inoculated with the suspension of microorganism and poured into petriplates to give a depth of 3-4 mm. The test compounds at the concentration of 50µg/ml in DMSO was placed on the medium. The plates were pre incubated for 1hr at room temperature and incubated at 37°c for 48 hrs for antifungal activity. Griseofulvin in the same concentration was used as a standard. The zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

All the synthesized compounds 3a – 3j were screened for antibacterial activity against the micro-organisms, *E. Coli, P. vulgaris, S. aureus, K. pneumoniae, S. typhi, B. subtilis* and antifungal activity against *A.niger*. The results obtained are expressed in Table1 and discussed graphically as shown in figures 1-7.

7-Fluoro – 10 – {[(4- methylphenyl) amino] methyl} – 10H – phenothiazine – 1- carboxylic acid (3a)

A mixture of o-chlorobenzoic acid (0.01mole), p-fluoroaniline (0.01mole) was condensed using water bath for nearly about 2 hours in the presence of CuO and anhydrous K_2CO_3 by Ullmann reaction. The intermediate 1 was collected and recrystallized from hot ethanol. This intermediate 1 (0.01mole) and sulphur (0.02mole) were condensed in the presence of iodine as catalyst using water bath for nearly about 1 to 2 hours. The product was cooled and poured into crushed ice, filtered, to yield intermediate 2. It was air-dried and recrystallised from dioxane. A mixture of intermediate 2 (0.01mole), formaldehyde (2 ml), 4-methyl aniline (0.01mole), hydrochloric acid (5ml) and dioxane (25 ml) was refluxed for 5 hours in oil bath or sand bath. The cold reaction mixture was filtered and filtrate was poured directly into ice water. The isolated product was collected from the ice water and wet solid was air-dried and recrystallised from dioxane (3a). The yield was 58%, m.p. 108 -109°C, R_f 0.72,

IR (cm^{-1}): 3058.89 (aromatic C-H stretch), 1508 (C=N stretch), 653 (C-S stretch), 3278 (N-H stretch), 1664.45 (>C=O stretch, COOH), 1336 (aromatic N-H stretch), 3278-3255 (aromatic N-H stretch),

^{1}H NMR, δ: 2.32 (s, 3H, CH_3), 3.32 (s, 2H, CH_2), 4.03(s, 3H-OCH_3), 6.73-8.04 (m 7H, 1H, Ar-H and NH).

10 – {[(4 – methylphenyl) amino] methyl} – 7 – sulfo – 10H – phenothiazine – 1- carboxylic acid (3b)

The title phenothiazine -1- carboxylic acid was prepared by using o-chlorobenzoic acid and sulphanilic acid as described for **3a.** The yield was 72%, m.p. 102 -103°C, R_f 0.80, IR (cm^{-1}): 3039.60 (Aromatic C-H stretch), 3218.97 (N-H stretch), 2918.10 (aliphatic C-H stretch), 649.97 (C-S-C stretch sym), 1662.52 (>C=O stretch, COOH), 1037.63 (C-O-C stretch sym), 1338.51 (asym. S (=O) $_2$ stretch). ^{1}H NMR, δ: 2.36 (s, 3H, CH_3), 3.51 (s, 1H, CH_2), 4.04 (s, 3 H, -OCH_3), 6.70-8.03 (m, 7H, 1H Ar-H and NH), MS calc for $C_{21}H_{18}N_2O_5S_2$, molecular weight 442.51: (*m/z*): 443 $[M + H]^+$.

10 – {[(4- methylphenyl) amino] methyl} – 7 – nitro – 10H – phenothiazine – 1 – carboxylic acid (3c)

This compound was prepared by using o-chlorobenzoic acid and p-nitroaniline as described for **3a.** The yield was 60%, m.p. 100 -101°C, R_f 0.60, IR (cm^{-1}): 1521.73 [sym (Ar NO_2) N= O stretch], 1319.22 [sym. (Ar NO_2) N=O stretch], 1662.52 (>C=O stretch, COOH), 1595.02 (C=C ring stretch), 3352.05 (N-H stretch), ^1H NMR, δ: 2.38 (s, 3H, CH_3); 3.31 (s, 2H, CH_2), 6.74-8.03 (m,1H-7H,Ar-H and NH) 2.38 (s, 3H, CH_3); 3.31 (s, 2H, CH_2), 6.74-8.03 (m, 7H, 1H Ar-H and NH).

10 – {[(4- methylphenyl) amino] methyl} – 8 – nitro – 10H – phenothiazine – 1 – carboxylic acid (3d)

This compound was prepared by using o-chlorobenzoic acid and m-nitroaniline as described for **3a.** The yield was 52%, m.p. 97 -98°C, R_f 0.58, IR (cm^{-1}): 1531.37 [asym. (Ar. NO_2) N= O stretch], 1348.15 [sym. (Ar. NO_2) N=O stretch], 1664.45 (>C=O stretch, -COOH), 3039.60 (aromatic C-H stretch), 3371 (N-H stretch), ^1H NMR, δ: 2.39 (s, 3H, CH_3); 3.30 (s, 2H, CH_2), 6.75-8.02 (m, 7H, 1H, Ar-H and NH).

10 – {[(4- methylphenyl) amino] methyl} – 9 – nitro – 10H – phenothiazine – 1 – carboxylic acid (3e)

This compound was prepared by using 0-chlorobenzoic acid and o-nitroaniline as described for **3a.** The yield was 48%, m.p. 124 -125°C, R_f 0.55, IR (cm^{-1}): 1523.66 [asym (Ar. NO_2) N= O stretch], 1344.29 [sym (Ar. NO_2) N=O stretch], 1664.45 (>C=O stretch, -COOH), 3060.82 (aromatic C-H stretch), 3371 (N-H stretch), ^1H NMR, δ: 2.33 (s, 3H, CH_3); 3.02 (s, 2H, CH_2), 6.83-8.15 (m, 7H, 1H, Ar-H and NH).

7 – Chloro – 10 – {[(4 – methylphenyl) amino] methyl} – 10 H – phenothiazine – 1 – carboxylic acid (3f)

This compound was prepared by using 0-chlorobenzoic acid and p-chloroaniline as described for **3a.** The yield was 46%, m.p. 79 -80°C, R_f 0.64, IR (cm^{-1}): 1652.52 (C=O stretch), 1583.09 (C=C stretch), 3340.48 (N-H stretch), 1209.26 (C-O-C stretch asym), 623.55 (C-S-C stretch), ^1H NMR, δ: 2.32 (s, 3H, CH_3); 3.48 (s, 2H, CH_2), 6.90-7.98 (m, 7H, 1H, Ar-H and NH).

N – [(2 – Chloro – 7 – methoxy – 10H – phenothiazine – 10 – yl) methyl] – N – (4 - methylphenyl) amine (3g)

This compound was prepared according to scheme 2 using m-dichlorobenzene and 4-methoxy aniline as starting material. The yield was 65%, m.p. 86 -87°C, R_f 0.70, IR (cm^{-1}): 1600.81 (C=C

ring stretch), 1253.64 (C-O-C (stretch asym), 1027.99 (C-O-C stretch sym), 3338.55 (N-H stretch), 1128.28 (Ar. Cl stretch), ^{1}H NMR, δ: 2.35 (s, 3H, CH_3); 3.17 (s, 2H, CH_2), 6.58-7.96 (m, 7H, 1H, Ar-H and NH).

N – [(2, 7 – dichloro – 10H – phenothiazine – 10 yl) methyl] – N – (4 – methylphenyl) amine (3h)

This compound was prepared by using m-dichlorobenzene and p-chloroaniline. The yield was 58%, m.p. 94 -95°C, R_f 0.62, IR (cm^{-1}): 1591.16 (C=C ring stretch), 3346.27 (N-H stretch), 3047.32 (aromatic C-H stretch), 634.54 (C-S-C stretch), 1097.42 (aromatic, Cl stretch), ^{1}H NMR, δ: 2.397 (s, 3H, CH_3); 3.696 (s, 2H, CH_2), 6.789-8.457 (m, 7H, 1H, Ar-H and NH).

N – [(2 – Chloro – 7 – nitro – 10 H – phenothiazine – 10 yl) methyl] – N- (4 – methylphenyl) amine (3i)

This compound was prepared by using m-dichlorobenzene and p-nitroaniline. The yield was 51%, m.p. 89 -90°C, R_f 0.71, IR (cm^{-1}): 1494.75 [asym (Ar. NO_2) stretch], 1303.97 [sym (Ar. NO_2) stretch], 1593.09 (C=C ring stretch), 3064.68 (aromatic C-H stretch). ^{1}H NMR, δ: 2.32 (s, 3H, CH_3); 3.30 (s, 2H, CH_2), 6.63-8.40 (m, 7H, 1H, Ar-H and NH).

8 – Chloro – 10 – {[(4- methylphenyl) amino] methyl} – 10H phenothiazine – 3- sulphonic acid (3j)

This compound was prepared by using m-dichlorobenzene and sulphanilic acid. The yield was 51%, m.p. 89 -90°C, R_f 0.71, IR (cm^{-1}): 1160 (sym S $(=O)_2$ stretch), 600 (C-S-C stretch), 1500.50 [asym (Ar. NO_2) stretch), 1600 (C=C ring stretch), 3090.40 (aromatic C-H stretch). ^{1}H NMR, δ: 2.319 (s, 3H, CH_3); 3.284 (s, 2H, CH_2), 6.754-8.894 (m, 7H, 1H, Ar-H and NH).

Antibacterial activity

The figure 1 shows that analogues 3b, 3g, 3h, 3f have been found to possess good antibacterial activity against *E. coli*, other analogues have shown moderate activity. The figure 2 shows that only analogue 3a has been found to possess good antibacterial activity against *P.vulgaris* while other analogues 3b – 3j have shown moderate antibacterial activity. The figure 3 shows that analogues 3a, 3g, 3i have been found to possess good antibacterial activity against *S. aureus*. Other analogues except 3e possess moderate antibacterial activity. The figure 4 shows that analogues 3a, 3g, 3i have been found to possess good antibacterial activity against *K.pneumoniae*. Analogues 3a, 3e, 3h, 3f have been found to be equally effective. The figure 5 shows that analogues 3a, 3c, 3e, 3g, 3i have been found to possess good antibacterial activity against *S.typhi*. Analogues 3b and 3d have been found to be equally effective while other analogues have shown moderate activity. The

figure 6 shows that 3h analogue found to be most effective against *B. subtilis*. Analogues 3g and 3j have been found to be equally effective. The 3e analogue has shown less activity among all these compounds.

From all above figures it is clear that 3e analogue has shown little activity against all types of microorganisms.

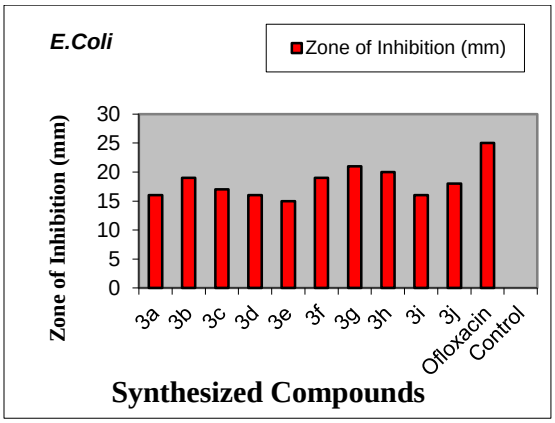


Figure 1. Graph of antibacterial activity against *E.coli*

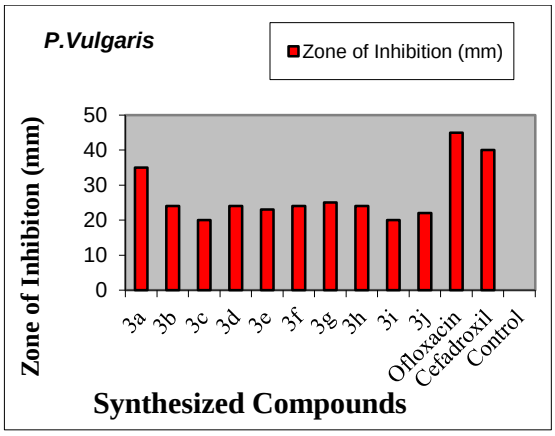


Figure 2. Graph of antibacterial activity against *P.vulgaris*

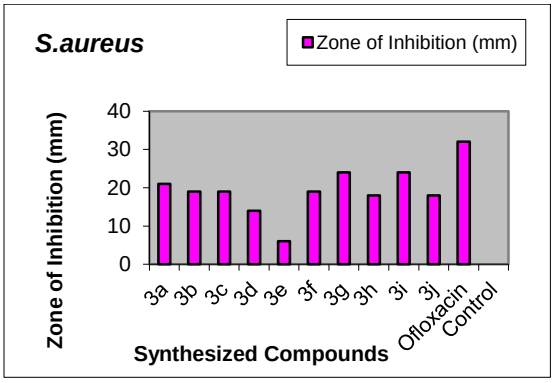


Figure 3. Graph of antibacterial activity against *S.aureus*

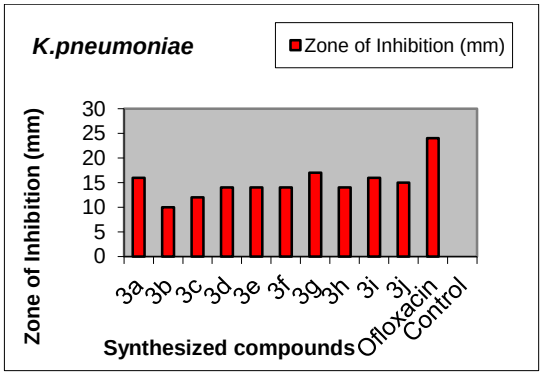


Figure 4. Graph of antibacterial activity against *K.pneumoniae*.

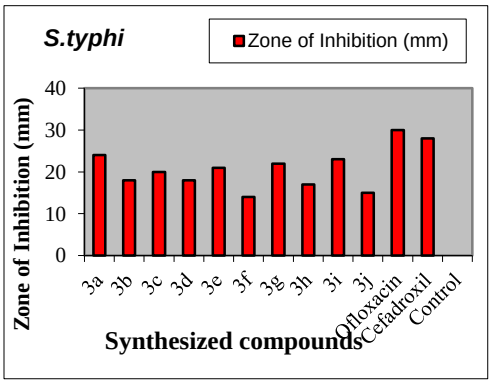


Figure 5. Graph of antibacterial activity against *S.typh*

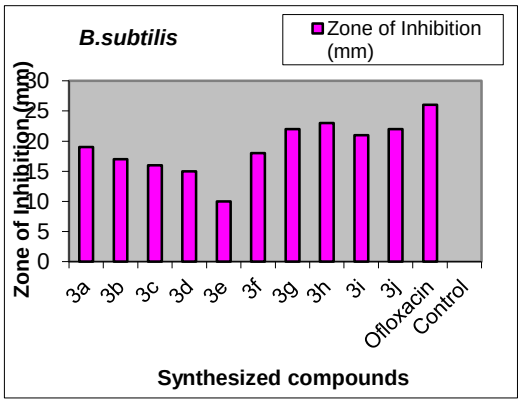


Figure 6. Graph of antibacterial activity against *B.subtilis*

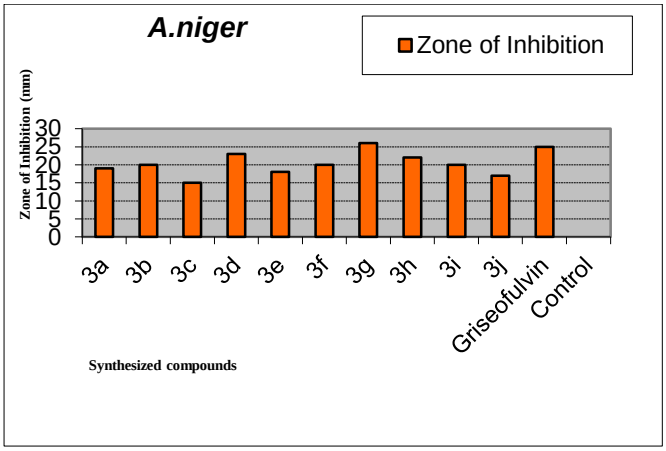


Figure 7. Graph of antifungal activity against *A.niger*

Antifungal activity

All analogues 3a-3j were screened for antifungal activity against *A. niger*. The results obtained were expressed graphically as shown in figure 7. The compound 3g have been found to possess greater antifungal activity than standard Griseofulvin. The compounds 3d, 3h have also found to possess comparable activity to that of standard Griseofulvin. The compounds 3b, 3f, 3i were found to be equally effective. The compounds 3a, 3c, 3e, 3j have shown moderate activity.

CONCLUSION

Two series of N_{10} substituted 1- carboxylic acid and 2 / 8 – chloro phenothiazines have been synthesized by Ullmann and Mannich base reaction. The analogues were obtained by substituting the positions 7, 8 and 9. All these compounds were screened for antibacterial and antifungal activities. In antibacterial activity, except 3e, all the synthesized compounds have shown good antibacterial activity against the gram- positive and gram-negative microorganisms. From these studies it is concluded that in 1-carboxyl phenothiazine series (scheme1) the electron-withdrawing group at R1 influences gram-negative activity. The halogens fluorine and chlorine are better at position R1. In 2 / 8- chloro series (scheme 2) the electron donating methoxy group at R1 (3g) influences gram-positive activity. However the 2, 7- dichloro analogue (3h) has also found to be better effective against both the types of microorganisms. Further all these compounds have also shown good antifungal activity against *A. niger* and 3g have been found to possess greater antifungal activity than Griseofulvin.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. S.S. Shyale, Principal, HSBPVT's GOI College of Pharmacy, Kashti, India for providing all the necessary facilities for the work. The authors are also thankful to KLEUniversity's College of Pharmacy, Bangalore and Science and Technology Park, Savitribai

Phule Pune University, India for I.R., N.M.R and Mass Spectroscopy facilities. The authors are also thankful to Mr.Vikramsinh B. Pachpute, Secretary, HSBPVT's Group of institutions, Kashti and management of Gland Institute of Pharmaceutical Sciences, Hyderabad for their valuable guidance.

REFERENCES

1. Britton J. W. Anthelmintic activity. Vet; 1941; 31; Chem Abstr; 1941; 35: 7540v.
2. Reid WB, Wright JB, Kolloff H.G, Hunter JH. Histamine Antagonists. II. N-(Pyrrolidylalkyl)-phenothiazines. J Amer chem Soc 1948; 70: 3100–3102.
3. Meiermed I., Monatsschr. Analgesic activity.1954: 8,397; Chem Abstr; 1954; 48: 10225.
4. Ducomum P., Lehmann S. D. (Univ. Montreal). Anti-inflammatory effect of the combination of irgapyrin and phenothiazine derivatives in experimental arthritis. Rev. Can. biol., 1952; 11:298-300, Chem Abstr; 1953; 47:1292f.
5. Gupta R. R. (ed.). Phenothiazines and 1, 4-Benzothiazines: Chemical and Biomedical Aspects. Elsevier Science Publishers; Amsterdam; 1988: 992.
6. Radadiya VR, Purohit DM, Patolia VN. Synthesis and antimicrobial activity of 10 N-{[(Aryl)-amino]-methyl}-3-methoxy-10,10a -dihydro-4a-H-phenothiazine-9-carboxylic acid. Ind J Chem 2005; 44 B: 1112-1114.
7. Samson- Belei D, Bacu E, Andrei M, Petrovanu M, Roum. Biotechnol. lett., 6 (Monte Carlo analysis of magnetic after effect 2001);501-506.
8. March J. (ed.). Advanced Organic Chemistry. The Ullmann Reaction. 4th ed., A Wiley-Interscience Publication John Wiley & Sons; 2004: 665-666.
9. Cappuccing JG, Sherman N. Microbiology A Laboratory Manual. Chemical Agents of Control: Chemotherapeutic Agents- Antimicrobial Susceptibility Test Procedure. International Student ed., 253-255.
10. Government of India. Ministry of Health & Family Welfare. Indian Pharmacopoeia Vol. II. The Controller of Publication, New Delhi; 1996: A-105.
11. Gillespie S. H. Medical Microbiology-Illustrated. Butterworth Heinemann London; 1994: 234 - 237.