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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 4–SUBSTITUTED -1-(4-SUBSTITUTED PHENYL) PIPERAZINE DERIVATIVES

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ABSTRACT

A novel series of piperazine derivatives were synthesized and evaluated for potential antibacterial activities. Total twelve new 4–substituted -1- (4-substituted phenyl) piperazine derivatives were synthesized. Firstly bis (β-chloroethyl) amine hydrochloride was prepared by the reaction of Thionyl chloride and diethanolamine in chloroform. Then obtained intermediate was condensed with various *pera* substituted aniline give 1-(4-substituted phenyl) piperazine hydrochloride. Finally 1-(4-substitutedphenyl) piperazine hydrochloride intermediate was subjected to react with various substituted alkyl bromide to synthesize a series of 4– substituted -1-(4-substituted phenyl) piperazine with good yields. Newly synthesized piperazine derivatives were screened for their antibacterial activity against Gram+ve bacteria (*B. subtilis* & *S. aureus*) and Gram-ve bacteria (*E. Coli* & *K. pneumoniae*). Investigation of antimicrobial activity of the synthesized compounds was based on zone of inhibition produced against standard drug Ampicillin (100µg/ml) through Agar disc diffusion method.

Keywords: Piperazine derivatives, Antimicrobial activity, Synthetic derivative

INTRODUCTION

Piperazine is a common source for the development of new potential therapeutic agents. Piperazine (Cyclohexane) is the prototype of saturated six-memberedring molecules firstly modified in 1950s with broad-spectrum activity. Useful in the ascarides, small strongyles and pinworms.[1]

Piperazines (cyclizines) are ethylenediamine derivatives (cyclic ethylenediamines derivatives); Piperazines are potential therapeutic agents with various pharmacological activity. Piperazine is dinitrogen moiety have the chemical similarity with piperidine, a chemical constituent of piper nigram. Piperazine introduced into the medicine as a solvent for uric acid[2,3].

In this letter we report the synthesis of new

4– substituted 1(4substituted phenyl) piperazine derivatives and screened them for their antimicrobial activity. The primary structural differences within this series involve the nature of the p-aromatic ring substitutents (H or Cl) and more importantly, the nature of terminal piperazine nitrogen substitutents[4]. In the aryl piperazine derivatives it is found that a substitution on the aromatic ring of the arylpiperazines substructure with an electron withdrawing substitution (Cl, F, NO_2, CF_3) increase their action. X-ray data revealed that a free rotation ortho-hinderance effect imposed by the substitution and conjugation of the lone pair 4-N piperazine nitrogen with the π-electron system of the aromatic ring within the arylpiperazine moiety influence the biological action[5]. Nitrogen in piperazine ring plays an important role in biological research and drug manufacturing industry including the anthelmintic, antiallergic, antibacterial, antihistaminic, antiemetic and antimigrainic agents.

The compounds were evaluated for the antimicrobial activity against Gram+ve bacteria (*B. subtilis* & *S. aureus*) and Gram-ve bacteria (*E. Coli* & *K. pneumoniae*).based on their ability to inhibit the growth of microorganism through agar disc diffusion method, standard taken as Ampicillin (100µg/ml)

CHEMISTRY:

The structures of synthesized compounds are shown in the **table 1.** and their synthesis are illustrated in **scheme 1.**

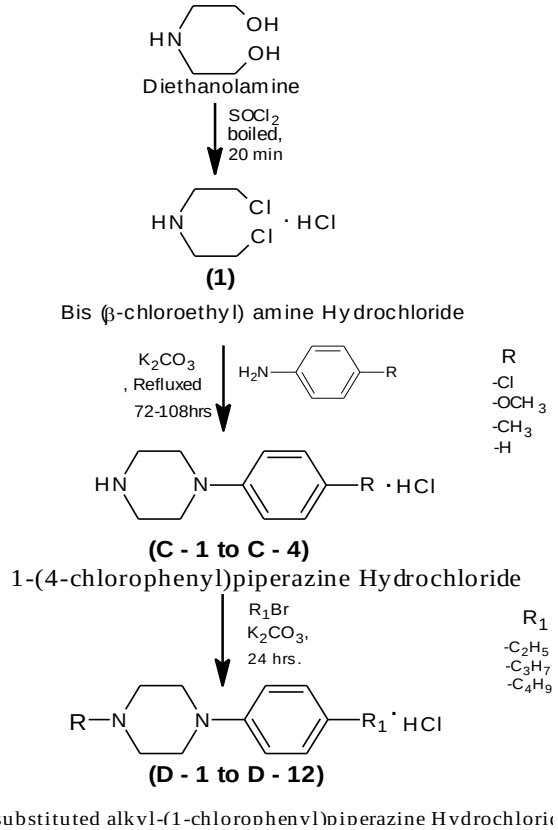
Literature survey brings forth the availability of a number of targets and pathways involved in the etiology of different diseases. We, therefore explore these uncharted pathways. This novel approach will be to reduce the deleterious side effects of high doses of piperazine. At the same time these molecules will retain the inherent activity of the piperazine moiety & also have in them another moiety to augment the overall desired pharmacological effect. Since the synthesized molecule will have available in it two moieties with independent mode of action, the propensity of inducing resistance will be reduced.

The modular molecules of piperazine were synthesized to accomplish the desired task, different piperazine derivatives will be synthesized using diethanolamine or its chloro- counterpart and the amine derivatives. The multi-step synthesis will require us to standardize the synthetic scheme at each step and characterize the thus obtained molecule.

The primary structural differences within this series involve the nature of the p-aromatic ring substitutents (H or Cl) and more importantly, the nature of terminal piperazine nitrogen substitutents[4]. In the aryl piperazine derivatives it is found that, a substitution on the aromatic ring of the arylpiperazines substructure with an electron withdrawing substitution (Cl, F, NO_2, CF_3) increase their action. X-ray data revealed that a free

rotation ortho-hinderance effect imposed by the substitution and conjugation of the lone pair 4-N piperazine nitrogen with the π-electron system of the aromatic ring within the arylpiperazine moiety influence the biological action[5]. Nitrogen in piperazine ring plays an important role in biological research and drug manufacturing industry including the anthelmintic, antiallergic, antibacterial, antihistaminic, antiemetic and antimigrainic agents.

The structures of newly synthesized compounds were confirmed by ^{1}HNMR spectra and elemental analysis.



Scheme 1: Synthetic scheme

PHARMACOLOGY:

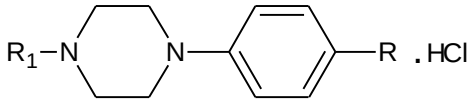
The compounds were tested for their antimicrobial activity. The inhibition of microorganism under standardized condition was utilized to demonstrate antimicrobial action of these compounds. All the synthesized compounds were screened in vitro for anti-bacterial activity against four micro-organism, two of Gram positive (*S. aureus & S. epidermidis*) and two of Gram negative (*P. aeruginosa & E. coli*) bacteria, using disc diffusion method at 1mg/ml disc concentration, Ampicillin (19-21 mm, zone of inhibition) was taken as standard. DMSO (Dimethyl sulphoxide) was used as solvent control.

Firstly, the bacteria used were obtained from slants. Loop full samples taken from the slants were grown in sterile nutrient broth medium, which had been autoclaved at 121°C under a

pressure of 15 atmospheres for 15 min, and left to grow for 48 hrs at 37°C in an incubator. After that the samples were prepared by dissolving 1 mg of each sample in 1 ml of DMSO (Dimethyl sulphoxide). Agar plates for the diffusion tests against bacteria were prepared by using agar solid medium.

After preparing the media, it was sterilized as for the nutrient broth media, and 25 ml of the media were poured into sterile petri dish. Petri dishes were allowed to cool and after solidification of media 0.8 ml of uniform mixture of an inoculate was introduced to each petri plate. Previously cut and sterilized paper disc of 5 mm diameter were loaded with samples of 1 mg/ml concentration. The plates were later incubated at 37°C. In positive reactions, clear zones of inhibition appeared around the discs. Measurement of the diameter of the zones extending from the edge of the discs was taken after 18, 19 and 20 hours[6,7].

Table 1. Synthesized compounds:



N 2substituted 1(4substitutedphenyl) piperazine hydrochloride

S.No.	Compound Code	R	R_1	Name of compound
1.	D-1	-Cl	-CH2CH3	**1-(4-chlorophenyl)-4-*N*-ethylpiperazine hydrochloride**
2.	D-2	-OCH3	-CH2CH3	**4- *N*-ethyl-1-(4-methoxyphenyl)piperazine hydrochloride**
3.	D-3	-CH3	-CH2CH3	**4- *N*-ethyl-1-(4-methylphenyl)piperazine hydrochloride**
4.	D-4	-H	-CH2CH3	**4-*N*-ethyl-1-phenylpiperazine hydrochloride**
5.	D-5	-Cl	-CH2CH2CH3	**1-(4-chlorophenyl)-4-*N*-propylpiperazine hydrochloride**
6.	D-6	-OCH3	-CH2CH2CH3	**1-(4-methoxyphenyl)-4-*N*-propylpiperazine hydrochloride**
7.	D-7	-CH3	-CH2CH2CH3	**1-(4-methylphenyl)-4-*N*-propylpiperazine hydrochloride**
8.	D-8	-H	-CH2CH2CH3	**1-phenyl-4-*N*-propylpiperazine hydrochloride**
9.	D-9	-Cl	$-CH2CH2CH_2CH_3$	**4-*N*-butyl-1-(4-chlorophenyl)piperazine hydrochloride**
10.	D-10	-OCH3	$-CH2CH2CH_2CH_3$	**4-*N*-butyl-1-(4-methoxyphenyl)piperazine hydrochloride**
11.	D-11	-CH3	$-CH2CH2CH_2CH_3$	**4-*N*-butyl-1-(4-methylphenyl)piperazine hydrochloride**
12.	D-12	-H	$-CH2CH2CH_2CH_3$	**4-*N*-butyl-1-phenylpiperazine hydrochloride**

Table 2: Activity of the Synthesized compounds:

Compounds	Antibacterial activity zone of inhibition (mm)

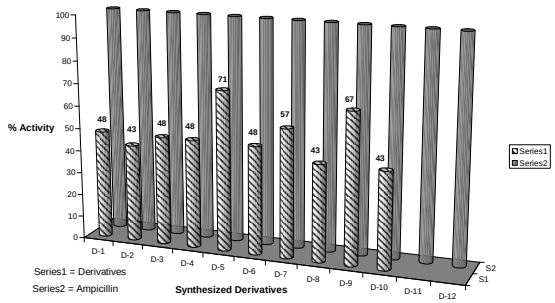
	S. aureus	*S. epidermidis*	*P. aeruginosa*	*E. coli*
Ampicillin(std)	21mm	19mm	19mm	20mm
D-1	10mm	11mm	9mm	10mm
D-2	9mm	10mm	--	10mm
D-3	10mm	--	11mm	9mm
D-4	10mm	--	9mm	12mm
D-5	15mm	9mm	10mm	12mm
D-6	10mm	11mm	--	12mm
D-7	12mm	12mm	14mm	9mm
D-8	9mm	10mm	11mm	10mm
D-9	14mm	10mm	--	11mm
D-10	9mm	11mm	12mm	10mm
D-11	--	11mm	10mm	11mm
D-12	--	12mm	9mm	11mm

Diameter of zone of inhibition

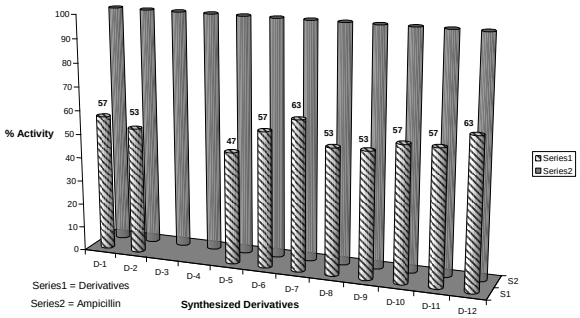
14-19mm (High activity), 8-13mm (moderate activity), 4-7mm (low activity)

Table 3: % activity of compounds compared with that of standard

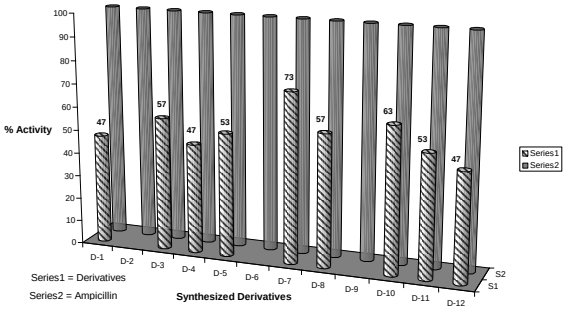
S.No.	D-1	D-2	D-3	D-4	D-5	D-6	D-7	D-8	D-9	D-10	D-11	D-12	Stnd.
S. aureus	48%	43%	48%	48%	71%	48%	57%	43%	67%	43%	0%	0%	100%
S. epidermidis	57%	53%	0%	0%	47%	57%	63%	53%	53%	57%	57%	63%	100%
P. aeruginosa	47%	0%	57%	47%	53%	0%	73%	57%	0%	63%	53%	47%	100%
E. coli	50%	50%	45%	60%	60%	60%	45%	50%	55%	50%	55%	55%	100%



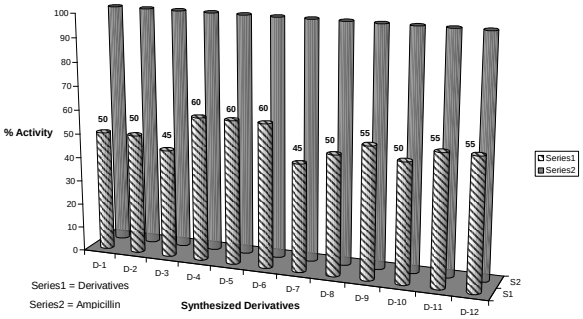
Graph 1.% Activity in case of *S. aureus*



Graph 2.% Activity in case of *S. epidermidis*



Graph 3.% Activity in case of *P. aeruginosa*



Graph 4.% Activity in case of *E.coli*

EXPERIMENTAL:

MATERIALS AND METHOD:

Melting points were determined in open capillary tubes on melting point apparatus

and are uncorrected[8]. The 1H NMR spectra were recorded on Bruker- NMR 500 mHZ using DMSO as solvent. The IR spectra of the synthesized compounds were recorded on Perkin-Elmer FT-IR spectrophotometer with KBr pellets. Structure confirmed by elemental analysis[9,10]. The purity of the compounds was checked by TLC on pre-coated silica gel G plates by using Ethyl acetate and acetone as a mobile phase and visualized in iodine vapour[8,9,10].

Synthetic studies[11,12,13]:

Total twelve new 4–substituted 1 (4-substituted phenyl)piperazine derivatives were synthesized. Firstly bis(β-chloroethyl)amine hydrochloride **(1)** was prepared by the reaction of thionyl chloride and diethanolamin in chloroform. Then obtained intermediate was codensed with various *pera* substituted aniline give 1-(4-substituted phenyl)piperazine hydrochloride (**C-1 to C-4**). Finally 1-(4-substituted phenyl)piperazine hydrochloride intermediate was subjected to react with various substituted alkyl bromide to synthesize a series of 4– substituted 1(4substituted phenyl) piperazine **(D-1 to D-12).**

Synthetic procedure:

General procedure for the synthesis of bis(β-chloroethyl)amine hydrochloride (1):

Thionyl chloride (130 ml) in chloroform (130 ml) is added to a solution of diethanolamine (0.48 mol) in chloroform (150 ml), cautiously and slowly. the mixture was boiled for 20 min. on electric water bath with occasionally shaken until a clear solution was obtained, the reaction mixture was chilled by immersion in ice water, After standing for one hr., the semisolid product was separated and filtered off, washed thrice with chloroform and then with ether. Bis (β-chloroethyl) amine hydrochloride was thus obtained as white crystals (15 g. or 59%). The compound was recrystallses, using acetone containing a small quantity of alcohol.

General procedure for the Synthesis of 1(4-substitutedphenyl)piperazine hydrochloride (C-1 to C-4):

A mixture of the p-substituted aniline (0.3 mol) and bis (β-chloroethyl) amine hydrochloride (0.3 mol) in 1-butanol (200 ml) was refluxed for 24 hrs, the reaction mixture was cooled and powdered anhydrous K_2CO_3 (0.15 mol) was added and refluxing continues for another 48 hrs. The progress of reaction was checked with help of TLC using silica gel G. After completion of reaction, reaction mixture was filtered while hot, the filtrate was cooled, and the 1-(4-chlorophenyl) piperazine hydrochloride which separated were filtered and washed successively with 1–butanol and ether; Yield 50-72 %.

The p-substituted anilines such as p-chloroaniline, p-methoxy aniline, p-methyl aniline and aniline was used for the synthesis of 1-(4-chlorophenyl)piperazine hydrochloride **(C-1),** 1-(4-methoxyphenyl) piperazine hydrochloride **(C-2),** 1-(4-methylhenyl) piperazine hydrochloride **(C-3)** and 1-phenyl

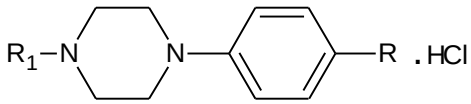
piperazine hydrochloride **(C-4)** respectively.

General procedure for the Synthesis of 4–substituted alkyl 1(4-substitutedphenyl)piperazine (D-1 to D-12):

The title compounds were synthesized by reaction of 1-(4-substitutedphenyl) piperazine hydrochloride (3 mmol) formed in 6.3.2, anhydrous K_2CO_3 (8.7 mmol), substituted alkyl bromide (4 mmol), and acetone (20 ml). The mixture was stirred at room temperature until thin layer chromatographic analysis reveals that the reaction is complete (usually about 24 hours). Free bases were dissolved in acetone, treated with an excess of diethyl ether saturated with dry, gaseous HCl, and kept in refrigerator to give colorless crystalline product. The product was recrystallized with the suitable solvent.

The procedure given above was used for the various substituted alkyl bromide such as ethyl bromide, propyl bromide, butyl bromide to produce the series of 4–substituted alkyl 1-(4sustitutedphenyl)piperazine **(D-1 to D-12).**

Table 4: Physical data for N 2substituted 1(4substitutedphenyl) piperazine:



N 2substituted 1(4substitutedphenyl) piperazine

S. No	Compound Code	R1	R	M.P (°C)	Rf value	% Yield	Mol. Weight
1.	D-1	-CH2CH3	-Cl	277	0.60	65	311
2.	D-2	-CH2CH3	-OCH3	243	0.62	65	325
3.	D-3	-CH2CH3	-CH3	104	0.61	53	307
4.	D-4	-CH2CH3	- H	178	0.59	46	321
5.	D-5	-CH2CH2CH3	-Cl	279	0.74	60	218
6.	D-6	-CH2CH2CH3	-OCH3	247	0.77	60	232
7.	D-7	-CH2CH2CH3	-CH3	108	0.73	60	240
8.	D-8	-CH2CH2CH3	- H	181	0.84	58	254
9.	D-9	$-CH_2CH_2CH_2CH_3$	-Cl	282	0.76	54	218
10.	D-10	$-CH_2CH_2CH_2CH_3$	-OCH3	251	0.78	68	232
11.	D-11	$-CH_2CH_2CH_2CH_3$	-CH3	111	0.76	45	240
12.	D-12	$-CH_2CH_2CH_2CH_3$	- H	185	0.70	60	254

1-(4-chlorophenyl) -4-N-ethyl piperazine hydrochloride (D-1):

IR (KBr) (ν, cm $^{-1}$); 1610 3420(NHstr), 3058(ArCHstr), 1284(ArNstr), 535(Clstr), 2980($\nu_{as}CH_3$, 2976($\nu_{sy}CH_2$), 815(CH_2 rock); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm),11.54-1.58 (t, CH_3), 2.18 (s, CH_2), 7.27-7.63 (m, ArH), 3.26-3.92 (m, Pip.H). Anal. Calc. for C12H18Cl2N2: C, 55.18; H, 6.95; Cl, 27.15; N, 10.73. Found: C, 55.20; H, 6.97; N, 10.74.

4- N-ethyl-1-(4-methoxyphenyl) piperazine

hydrochloride (D-2) **:**

IR (KBr) (ν, cm $^{-1}$); 1249 (Asymc-o-c), 1018 (Symc-o-c), 3099 (ArCHstr)**,** 1348(ArN), 2925(ν_{ss} CH_3), 825 (CH_2 rock); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 3.84 (s,OCH_3), 6.98-7.85(m, ArH), 2.17 (s, CH_2), 3.30-4.76(m, Pip.H), 1.54-1.57 (t, CH_3) . Anal. Calc. for C13H21ClN2O: C, 60.81; H, 8.24; Cl, 13.81; N, 10.91; O, 6.23. Found: C, 60.83; H, 8.25; N, 13.83; O, 6.24.

4- N-ethyl-1-(4-methylphenyl) piperazine hydrochloride (D-3):

IR (KBr) (ν, cm $^{-1}$); 3430 (NHstr.), 3018 (ArCHstr), 1271 (ArNstr.), 2977 ($\nu_{as.}CH_3$), 2980 ($\nu_{sy}CH_2$), 1455 (δ_{as}CH3), 1309 ($\delta_{as}CH_3$), 755 (ρCH_2); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), .39-1.42 (t, CH_3), 7.13-7.53 (m, ArH), 3.15-3.60 (m, Pip.H), 2.26(s, CH_2). Anal. Calc. for C13H21ClN2: C, 64.85; H, 8.79; Cl, 14.72; N, 11.63. Found: C, 64.86; H, 8.80; N, 14.75.

4-N-ethyl-1-phenyl piperazine hydrochloride (D-4):

IR (KBr) (ν, cm $^{-1}$); 3428(NHstr.), 3018(ArCHstr), 1266(ArNstr.), 2980($\nu_{as.}CH_3$), 2985($\nu_{sy}CH_2$), 1455($\delta_{as}CH_3$), 1390($\delta_{as}CH_3$), 710(ρCH_2); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 1.38-1.41 (t, CH_3), 7.02-7.63 (m, ArH), 3.13-3.83 (m, Pip.H), 2.49-2.50 (m, CH_2). Anal. Calc. for C12H19ClN2: C, 63.56; H, 8.45; Cl, 15.64; N, 12.35. Found: C, 63.57; H, 8.47; Cl, 15.66; N, 12.37.

1-(4-chlorophenyl) -4-N-propyl piperazine hydrochloride (D-5):

IR (KBr) (ν, cm $^{-1}$); 3420(NHstr), 3058(ArCHstr), 1255(ArNstr.), 535(Clstr.), 2981(vas.CH3), 2977(vsyCH3), 1455(δasCH3), 1309(δssCH3), 817(ρCH2); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 0.90-0.94 (t, CH_3), 6.88-7.82 (m, ArH), 2.99-3.65 (m, Pip.H), 2.49 (s, CH_2), 1.77-1.87 (m, CH_2). Anal. Calc. for C13H20Cl2N2: C, 56.73; H, 7.32; Cl, 25.76; N, 10.18. Found: C, 56.75; H, 7.33; Cl, 25.78; N, 10.20.

1-(4-methoxyphenyl)-4-N-propyl piperazine hydrochloride (D-6):

IR (KBr) (ν, cm $^{-1}$); 3434(NHstr),3015(ArCHstr), 1310 (Ar Nstr.),1261(AssC-O-C), 1071 (SymC-O-C), 2976($\nu_{as}.CH_3$), 2979 (ν_{sy} CH_3), 1460($\delta_{as}CH_3$), 1376 ($\delta_{ss}CH_3$), 760 (ρCH_2); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 0.88-0.92 (t, CH_3), 1.32-1.39 (m, CH_2), 1.72-1.80 (m, CH_2), 3.71(s, OCH_3), 6.85-7.97 (m, ArH), 3.08-3.81 (m, Pip.H). Anal. Calc. for C14H23ClN2O: C, 62.09; H, 8.56; Cl, 13.09; N, 10.34; O, 5.91. Found: C, 62.10; H, 8.58; Cl, 13.11; N, 10.35; O, 5.93.

1-(4-methylphenyl) -4-N-propyl piperazine hydrochloride (D-7):

IR (KBr) (ν, cm $^{-1}$); 1018(NHstr),1321(ArNstr.), 815(CH_2rock), 2918, 2856, 2835 (MethylCHstr.); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 1.07-1.11(t, CH_3), 1.95-2.01 (m, CH_2), 2.37-2.40 (Hm, CH_2) 3.13-4.73 (Hm, Pip.H), 7.23-7.43 (Hm, ArH). Anal. Calc. for C14H23ClN2: C, 65.99; H, 9.10; Cl, 13.91; N, 10.99. Found: C, 66.01; H, 9.11; Cl, 13.93; N, 11.02.

1-phenyl-4-N-propylpiperazine hydrochloride (D-8):

IR (KBr) (ν, cm $^{-1}$); 3444(NHstr.), 3018(ArCHstr), 1267(ArNstr.), 2930($\nu_{as}.CH_3$),

2924($\nu_{sy}CH_2$),1449($\delta_{as}CH_3$),1372($\delta_{ss}CH_3$),759(ρCH$_2$).; ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 1.87-0.91(t, CH_3), 1.31-1.38 (m, CH_2), 1.76-1.80 (m, CH_2), 2.49 (s, CH_2), 3.06-4.2 (m, Pip.H), 7.19-7.74 (m, ArH). Anal. Calc. for C13H21ClN2: C, 64.85; H, 8.79; Cl, 14.72; N, 11.63. Found: C, 64.86; H, 8.81; Cl, 14.73; N, 11.65.

4-N-butyl-1- (4-chlorophenyl) piperazine hydrochloride (D-9):

IR (KBr) (ν, cm $^{-1}$); 3525(NHstr), 3055(ArCHstr), 1245 (ArNstr.),534(Clstr.),2957($\nu_{as.}CH_3$2950($\nu_{sy}CH_3$),1455($\delta_{as}CH_3$), 1406($\delta_{ss}CH_3$), 740(ρCH$_2$); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 0.86-0.90 (t, CH_3), 1.28-1.37 (m,CH_2), 1.75-1.83 (m, CH_2), 2.49-2.50 (s, CH_2), 3.00-3.77 (m, Pip.H), 6.94-7.60 (m, ArH). Anal. Calc. for C14H22Cl2N2: C, 58.13; H, 7.67; Cl, 24.51; N, 9.69. Found: C, 58.15; H, 7.68; Cl, 24.52; N, 9.71.

4-N-butyl-1 -(4-methoxyphenyl) piperazine hydrochloride (D-10):

IR (KBr) (ν, cm $^{-1}$); 3418(NHstr), 3021(ArCHstr), 1286 (ArNstr.),1258(AssC-O-C), 1060 (SymC-O-C),2976($\nu_{as.}CH_3$), 2979 ($\nu_{sy}CH_3$), 761 (ρCH$_2$); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 0.86-0.90 (t, CH_3), 1.28-1.37 (m,CH_2), 1.75-1.83 (m, CH_2), 2.49-2.50 (s, CH_2), 3.00-3.77 (m, Pip.H), 6.94-7.60 (m, ArH). Anal. Calc. for C15H25ClN2O: C, 63.25; H, 8.85; Cl, 12.45; N, 9.84; O, 5.62. Found: C, 63.27; H, 8.86; Cl, 12.46; N, 9.86; O, 5.63.

4-N-butyl-1 -(4-methylphenyl) piperazine hydrochloride (D-11):

IR (KBr) (ν, cm $^{-1}$); 3525(NHstr.),3022(ArCHstr), 1245 (ArNstr.), 2957($\nu_{as.}CH_3$), 2950($\nu_{sy}CH_2$), 1455($\delta_{as}CH_3$), 1406($\delta_{ss}CH_3$),741(ρCH$_2$); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 0.87-0.91 (m, CH_3), 1.30-1.39(m,CH_2), 2.06 (s, CH_2), 1.74-1.82 (m, CH_2), 7.03-7.72 (m, ArH), 3.04-3.97 (m, Pip.H). Anal. Calc. for C15H25ClN2: C, 67.02; H, 9.37; Cl, 13.19; N, 10.42. Found: C, 67.04; H, 9.38; Cl, 13.20; N, 10.44.

4-N-butyl-1 -phenylpiperazine hydrochloride (D-12):

IR (KBr) (ν, cm $^{-1}$); 3331(NHstr.),3018(ArCHstr), 1265 (ArNstr.),2965($\nu_{as.}CH_3$)2961($\nu_{sy}CH_2$), 1433($\delta_{as}CH_3$),1364($\delta_{ss}CH_3$), 760(ρCH2); ^{1}H-NMR (DMSO, 200 MHz) δ(ppm), 0.87-0.91 (t, CH_3), 1.72-1.78 (m, CH_2), 2.49 (s, CH_2), 6.85-7.29 (m, ArH), 3.03-3.78 (m, Pip.H), 3.30- 3.05 (m, CH_2). Anal. Calc. for C14H23ClN2: C, 65.99; H, 9.10; Cl, 13.91; N, 10.99. Found: C, 66.01; H, 9.12; Cl, 13.92; N, 11.00.

RESULT AND DISCUSSION:

In the present work, an attempt was made to synthesize the ethylenediamine (piperazine) derivatives D1 to D12 (**Table 1**.).The primary structural differences within this series involve the nature of the p-aromatic ring substitutents (H, Cl, OCH_3 and CH_3) and more importantly, the nature of terminal piperazine nitrogen substitutents.

Synthesized derivatives were characterized with the help of analytical techniques such as Melting Point, Thin Layer Chromatography, IR, NMR, and elemental analysis. All compounds have been screened for their antibacterial activity.

Synthesized compounds were found to be

crystalline in nature and easily dissolved in the DMSO (Dimethyl sulphoxide), acetone and partially dissolved in the chloroform, ethyl acetate. Chromatographic analysis (TLC) of these compounds, used to check the completion of reaction. Ethyl acetate and acetone (3 : 7) solvent system was used for development of TLC plates. Melting points were determined in open capillary tubes on melting point apparatus and are uncorrected. The physical data of synthesized compounds are summarized in **table 4**.

The structures of the compounds were established based on their IR and 1H NMR spectra. The 1H NMR spectra were recorded on Bruker- NMR 500 mHZ using DMSO as solvent. The IR spectra of the synthesized compounds were recorded on Perkin-Elmer FT-IR spectrophotometer with KBr pellets.

All the synthesized compounds were screened in vitro for anti-bacterial activity against four micro-organism, two of Gram positive (*S. aureus & S. epidermidis*) and two of Gram negative (*P. aeruginosa & E. coli*) bacteria, using disc diffusion method at 1mg/ml disc concentration, Ampicillin (19-21 mm, zone of inhibition) was taken as standard. DMSO (Dimethyl sulphoxide) was used as solvent control **(Table 2).** The percentage growth of inhibition of microorganism of the synthesized compounds against the standard drug was summarized in **table 3** and shown through the graph-1,2,3 and 6.

The result show that compound 1-(4-methylphenyl) -4-n-propylpiperazine hydrochloride **(D-7)** show better activity against *P. aeruginosa* and 1-(4-chlorophenyl)-4-n-propylpiperazine hydrochloride **(D-5)** show the better activity against *S. aureus* while 4-ethyl-1-(4-methoxyphenyl)piperazine hydrochloride **(D-2)** and 4-n-butyl-1-(4-methoxyphenyl)piperazine hydrochloride **(D-10)** show the poor activity against *S. aureus*, other compounds in between these compounds have the moderate activity.

CONCLUSION:

All the synthesized compounds of piperazine were have low to moderate antibacterialactivity against the standard Amphicillin but the effect of substitution was observed on their activity due to the difference in the electronegativity of the substituted molecule. The activity dependent on the p-aromatic ring substitutents (H or Cl) and more importantly, the nature of terminal piperazine nitrogen substitutents. In the aryl piperazine derivatives it is found that a substitution on the aromatic ring of the arylpiperazines substructure with an electron withdrawing substitution (Cl, F, NO_2, CF_3) increase their action. The ortho-hinderance effect imposed by the substitution and conjugation of the lone pair 4-N piperazine nitrogen with the π-electron system of the aromatic ring within the arylpiperazine moiety influence the biological action.

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