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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF SOME 4–SUBSTITUTED -1-(4-SUBSTITUTED PHENYL) PIPERAZINE DERIVATIVES

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ABSTRACT

Piperazines are the important group of compounds reported to have diverse biological activity like anthelmintic, antihistaminic, anticancer, antidepressant and hence the present study was undertaken in order to synthesize some piperazine derivatives and evaluate their biological properties. Experimental part involves the synthesis of Bis (β-chloroethyl) amine hydrochloride, then N-arylpiperazines hydrochloride which was then condensed with various alkyl bromides to form respective Arylpiperazine derivatives. The structures of the compounds were established on the basis of their IR and ^{1}HNMR spectral data. The compounds synthesized were studied for anti-bacterial activity using Ampicillin as standard drug respectively against four strains i.e. *S. aureus, S. epidermidis, P. aeruginosa* and *E. coli*. All the compounds show low to moderate activity while 1-(4-chlorophenyl)-1-propyl piperazine shows excellent activity against *S. aureus* and 1-(4-methylphenyl)-1-propyl piperazine shows excellent activity against *P. aeruginosa.*

Keywords: Piperazine derivatives, Antimicrobial activity, Synthetic derivative.

INTRODUCTION

The piperazines or cyclizines are generally considered as ethylenediamine derivatives or cyclic ethylenediamines (cyclizines); Piperazines are a broad class of chemical compounds with many important pharmacological properties. This dinitrogen moiety has been an inseparable component of plethora of drugs. Piperazines have the chemical similarity with piperidine, a constituent of piperazine in the black piper plant (Piper nigrum). Piperazine introduced into the medicine as a solvent for uric acid[1,2].

Piperazine which arrived in 1950s was the first modification with broad spectrum activity. Useful in the ascarides, small strongyles and pinworms. It still wasn't effective against the large strongyles (Merck index, 11th edition).

Piperazine is a saturated heterocyclic compound containing two nitrogen at 1 and 4 position (as called 1, 4-hexahydropyrazine). In this series, however, the connecting moiety is a CHN group, and the carbon chain, terminal amine functionality, and the nitrogen atom of the connecting group are all part of piperazine moiety[2]

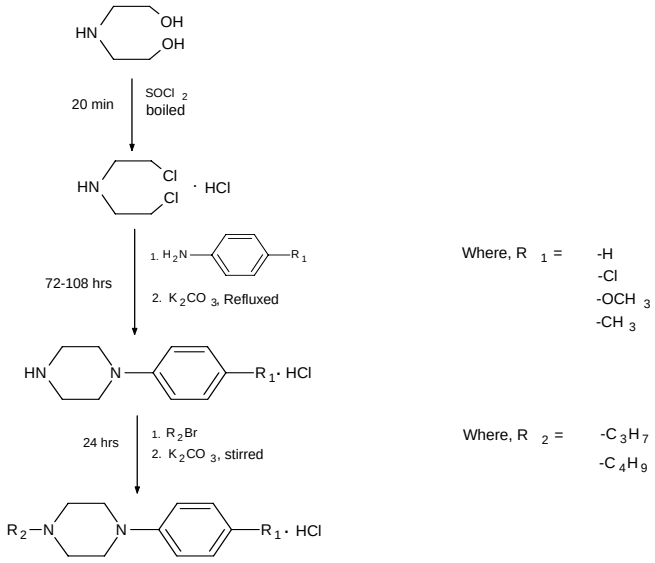


Fig. 1: Synthetic scheme

The antibiotic potency can be determined using the microbial assay, as conventional chemical methods are not suitable. It reflects the concentration and not the activity of the antibiotic. Therefore the reduction in antimicrobial action can be used in the determination of potency of antibiotics and their preparation. The basic principle of microbial assay lies in the comparison of the inhibition of growth of bacteria by measured concentration of antibiotics to be investigated with that produced by known concentration of the standard preparation of the antibiotics having a known activity.

MATERIALS AND METHODs

The chemicals used for the experimental work were commercially procured from various chemical units. The initial compound diethanolamine, p-substituted aniline, alkyl bromide and silica gel G were used of Central Drug House (CDH), New Delhi. Butanol and potassium carbonate were used of Rankem (New Delhi), and thionyl chloride was used of Merck (Mumbai), These solvents and reagent were of AR grade and purified before use, The commercially available grades of solvents and reagents were found to be of adequate purity[6,7,8].

Synthetic studies[6,7,8]:

STEP-I: Synthesis of bis(β-chloroethyl)amine hydrochloride

Thionyl chloride (130c.c.) in chloroform (130c.c.) is added to a solution of diethanolamine (50g.) in chloroform (150c.c.), at first cautiously and more than more rapidly, during about 10 minutes. Without delay the mixture is boiled vigorously and occasionally shaken until a clear solution is formed (about 20 minutes), within a few minutes thereafter, considerable crystallization rapidly occurs. The flask is at once chilled by immersion in ice water, and after standing for one hr., the semisolid product is filtered off, washed thrice with chloroform and then with ether, and dried. Bis (β-chloroethyl) amine hydrochloride is thus obtained as white crystals (15 g. or 59%). Recrystallization with the acetone containing a small quantity of alcohol (Fig.34).

STEP-II: Synthesis of 1-(4-chlorophenyl)piperazine hydrochloride :

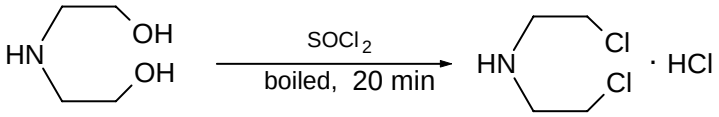
A mixture of the p-chloroaniline (0.3 mol) and bis (β-chloroethyl) amine hydrochloride (0.3 mol) in 1-butanol (200 ml) was refluxed for 24 hrs, the reaction mixture was cooled and powdered anhydrous K_2CO_3 (0.15 mol) was added and refluxing continues for another 48 hrs. The progress of reaction was 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 %.(Fig.35)

The procedure given above was for the p-chloroaniline. Apart from the p-chloroaniline, three p-substituted anilines as p-methoxy aniline (p-anisidine), p-methyl aniline (p-toludine) and aniline. The same procedure was adopted for these p–substituted anilines.

STEP-III: Synthesis of 4–ethyl -1-(4-chlorophenyl)piperazine :

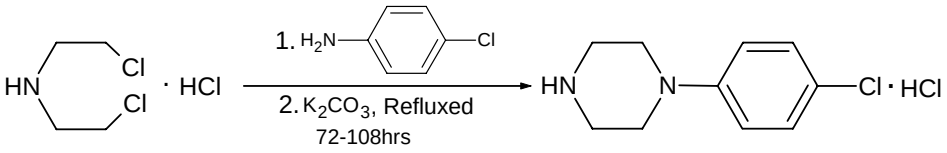
A mixture of the 1-(4-chlorophenyl) piperazine hydrochloride (3 mmol), anhydrous K_2CO_3 (1.2 g.), ethyl bromide (4 mmol), and acetone (20 ml) was stirred at room temperature until T.L.C. analysis reveals that the reaction is complete (usually about 24 hours). Free bases were dissolved in acetone, treated 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 (Fig.36).

The procedure given above was for the ethyl bromide, which reacted with 1-(4-chlorophenyl) piperazine hydrochloride. Instead of ethyl bromide we have taken two alkyl bromides as propyl bromide, butyl bromide. These alkyl bromides are used to treat with different N-aryl piperazines (formed in step II). The same procedure was adopted for these alkyl bromides.



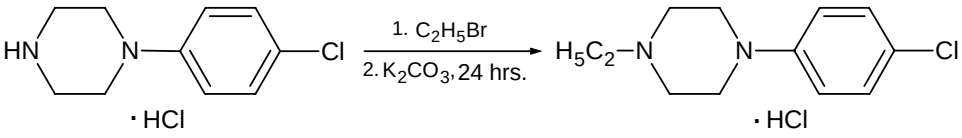
Diethanolamine — Bis (β-chloroethyl) amine Hydrochloride

Fig.2: Step I



Bis(β-chloroethyl)amine — 1-(4-chlorophenyl)piperazine Hydrochloride

Fig.3: Step II



1-(4-chlorophenyl)piperazine — 4-ethyl-(1-chlorophenyl)piperazine Hydrochloride

Fig. 4: Step III

Identification and characterization[9]

The compounds synthesis were identified and characterized by following methods such as:

A. Melting point determination.

B. Thin layer chromatography.

C. Infra red spectroscopy.

D. Nuclear magnetic resonance spectroscopy.

Melting point determination (M.P.)

The melting point of organic compound was determined by Thiel's melting point tube (capillary tube method). The determination of melting point is the most important and easy way of differentiating this physical constant of one compound from other.

Thin layer chromatography (TLC)

TLC is an important method for synthetic chemistry which helps to characterize the different properties of the compound based on the

R_f values since different compound will have different R_f values. It also helps in confirming the progress of the reaction.

The Ethylacetate:Acetone (3:7) was used as solvent system. Iodine chamber and U.V. lamps were used for visualization of spots.

Infra red spectroscopy (IR) [10,11]

IR is one of the most important tools for determining the various functional groups and the possible chemical structure. The important advantage of IR over the other techniques is that it gives fingerprints (1300-650 cm) information about the structure (functional group, bonding with each other) of molecules easily. No two compounds have identical fingerprint region.

This technique is based upon the molecular vibration of the compound such that each and every bond will vibrate at the different frequency and this vibration frequency corresponds to the IR frequency. Thus IR spectra of each and every bond will be formed. The infra red spectra of compounds were recorded in **JASCO FTIR** Spectrometer.

Nuclear magnetic resonance spectroscopy (NMR) [10,11]

The interaction between matter and electromagnetic forces can be observed by subjecting a substance simultaneously to 2 magnetic forces, one stationary and other varying at some radio frequency. At a particular combination of fields, energy is absorbed by the sample and absorption can be observed as a change in signal developed by a radio frequency detector and amplifier. This energy of absorption can be related to a magnetic dipolar nature of the spinning nuclei. This technique is known as Nuclear Magnetic Resonance. This technique is useful in assuming the structure of the molecule. The proton magnetic resonance spectra (^{1}HNMR) were recorded on Bruker Avance II 400 NMR spectrometer in DMSO using Tetramethyl Silane [$(CH_3)_4Si$] as internal standard.

Antibacterial activity[12,13]:

A Piperazine nucleus has the several important biological activities but in present work we test the antimicrobial activity of new synthesized compounds has been investigated against bacteria.

The antibacterial activities of the compounds were assayed by agar disc-diffusion method. The method was based on diffusion of antibacterial compound from reservoir disc to the microorganism was inhibited as circular zone around the disc.

Micro organisms tested

The antimicrobial activity was tested for four bacteria (two gram negative and two gram positive). Gram positive micro organisms were Staphylococcus aureus, and Staphylococcus epidermidis, and gram negative micro organisms were Escherichia coli, and Pseudomonas aeruginosa.

Antibacterial assay

Preparation of cultures

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. The nutrient broth for bacteria growth was prepared according to the following composition.

Composition of nutrient broth medium

Beef extract	1 gm
Yeast extract	2 gm
Peptone	5 gm
NaCl	5 gm
Distilled water	1 lit.

Agar disc diffusion method

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. The composition was as follows.

Composition of nutrient agar media

Agar	7.5 gm
Beef extract	500 mg
Yeast extract	1gm
Peptone	2.5 gm
Distilled water	500 ml

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.

synthesized compounds were checked for their anti-microbial activity. The inhibition of microorganism under standardized condition was utilized to demonstrate antimicrobial action of these compounds.

For present work efficacy of 12 compounds were detected against S. aureus, S. epidermidis, (G^+) P. aeruginosa, E. coli (G^-). The concentration of the test compound used was 1mg/ml. and Ampicillin was taken as the standard compound.

All the synthesized compounds were screened in vitro for anti-bacterial activity against S. aureus, S. epidermidis, P. aeruginosa, E. coli using disc diffusion method at 1mg/ml disc concentration, Ampicillin (19-21 mm, zone of inhibition) was taken as standard. DMSO (Dimethyl sulphoxide) is used as solvent control.

RESUTLS AND DISCUSSION

Physical characters

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

S. No	Compound Code	R_2	R_1	M.P (°C)	Rf value	% Yield	Mol. Weight
1.	D-1	$-CH_2CH_2CH_3$	-Cl	280	0.74	60	311.60
2.	D-2	$-CH_2CH_2CH_2CH_3$	-Cl	283	0.76	54	325.60
3.	D-3	$-CH_2CH_2CH_3$	$-OCH_3$	247	0.77	60	307
4.	D-4	$-CH_2CH_2CH_2CH_3$	$-OCH_3$	251	0.78	67	321
5.	D-5	$-CH_2CH_2CH_3$	$-CH_3$	109	0.73	60	218
6.	D-6	$-CH_2CH_2CH_2CH_3$	$-CH_3$	112	0.76	45	232
7.	D-7	$-CH_2CH_2CH_3$	- H	181	0.84	58	240
8.	D-8	$-CH_2CH_2CH_2CH_3$	- H	185	0.70	60	254

Table 1: Physical data for N 1-substituted piperazine

S. No.	Compound *Code*	R_1	M.P (°C)	Rf value	% Yield	Mol. Weight
1.	C-1	-Cl	275-278	0.33	72	269.60
2.	C-2	$-OCH_3$	240	0.30	57	265.18
3	C-3	$-CH_3$	103	0.27	62	176.26
4.	C-4	H	175	0..32	46	198.69

Spectral analysis

Table 3: Spectral characterization of compound (IR data)

S. No.	Compound Code	IR spectral data(Cm^{-1})
1.	C-1	3442(NHstr.),1253(ArN),3218(ArCHstr.),555(Clstr.)
2.	C-2	3446(NHstr.),1247(AssyC-O-Crstr.), 1029(SymC-O-Cstr.),1334(ArN).3125(ArCHstr.)
3.	C-3	3434(NHstr.),1309(ArNstr.),3050(ArCHstr.),1454,1398 (MethylC-Hstr.),
4.	C-4	3420(NHstr.),3065(ArCHstr.),1321(ArNstr.)
5.	D-1	3420(NHstr),3058(ArCHstr),1255(ArNstr.),535(Clstr.), 2981($\nu_{as.}CH_3$),2977($\nu_{sy}CH_3$),1455(δ_{as}CH3),1309(δ_{as}CH3), 817(ρCH2)
6.	D-2	3525(NHstr),3055(ArCHstr),1245(ArNstr.),534(Clstr.), 2957($\nu_{as.}CH_3$),2950($\nu_{sy}CH_3$),1455(δ_{as}CH3),1406(δ_{as}CH3), 740(ρCH2)
7.	D-3	3434(NHstr),3015(ArCHstr),1310(ArNstr.), 1261(AssC-O-C), 1071(SymC-O-C), 2976($\nu_{as.}CH_3$), 2979($\nu_{sy}CH_3$),1460(δ_{as}CH3),1376(δ_{as}CH3), 760 (ρCH2)
8.	D-4	3418(NHstr),3021(ArCHstr),1286(ArNstr.), 1258(AssC-O-C), 1060(SymC-O-C), 2976($\nu_{as.}CH_3$), 2979($\nu_{sy}CH_3$),1455(δ_{as}CH3),1381(δ_{as}CH3), 761 (ρCH2)
9.	D-5	1018(NHstr),1321(ArNstr.), 815(CH_2rock), 2918, 2856, 2835 (MethylCHstr.)
10.	D-6	3525(NHstr.),3022(ArCHstr),1245(ArNstr.),2957($\nu_{as.}CH_3$), 2950($\nu_{sy}CH_2$),1455(δ_{as}CH3),1406(δ_{as}CH3),741(ρCH2)
11.	D-7	3444(NHstr.),3018(ArCHstr),1267(ArNstr.),2930($\nu_{as.}CH_3$) 2924($\nu_{sy}CH_2$),1449(δ_{as}CH3),1372(δ_{as}CH3),759(ρCH2)
16	D-8	3331(NHstr.),3018(ArCHstr),1265(ArNstr.),2965($\nu_{as.}CH_3$)2961($\nu_{sy}CH_2$),1433(δ_{as}CH3),1364(δ_{as}CH3),760(ρCH2)

Table 4: Spectral characterization of compound (NMR data)

S. No.	Compound Code	NMR data (wave number)
4	D-3	1.08-1.19(H_t,CH_3), 2.22(H_s,CH_2), 3.25(H_s,OCH_3), 6.85-7.55(H_m,C_6H_4),3.66-3.87(H_m,$C_4H_8N_2$)
5	D-5	1.07-1.11(H_t,CH_3),1.65-1.80(H_m,CH_2),2.30-2.40 (H_m,CH_2),3.84-3.91(H_m,$C_4H_8N_2$),7.23-7.43 (H_m, C_6H_4)
6	D-4	0.88-0.92(H_t,CH_3),1.32-1.39(H_m,CH_2),1.72-1.80(H_m,CH_2),2.49(H_s,CH_2),3.68-3.71(H_m,OCH_3), 6.85-7.97(H_m,C_6H_4),3.08-3.12(H_m, $C_4H_8N_2$)
7	D-6	0.87-0.91(Hm,CH_3),1.30-1.39(Hm,CH2), 2.06(Hs, CH2),2.20-2.26(Hm,CH2),6.93-7.72 (Hm, C_6H_4), 3.53-3.67(Hm, $C_4H_8N_2$),
8	D-8	6.85-7.29(H_m,C_6H_4),3.11-3.22(H_m,$C_4H_8N_2$),0.87-0.91(H_m,CH_3),2.49(H_s,CH_2),3.30-3.05(H_m,CH_2),0.72-1.78(H_m,CH_2)

Antimicrobial studies

Table 5: Activity of the Synthesized compounds :

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

Diameter of zone of inhibition

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

Table 6: % 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	Std.
S.aureus	71%	67%	48%	43%	57%	--	43%	--	100%
S.epidermidis	47%	53%	57%	57%	63%	57%	53%	63%	100%
P.aeruginosa	53%	--	--	63%	73%	53%	57%	47%	100%
E.coli	60%	55%	60%	50%	45%	55%	50%	55%	100%

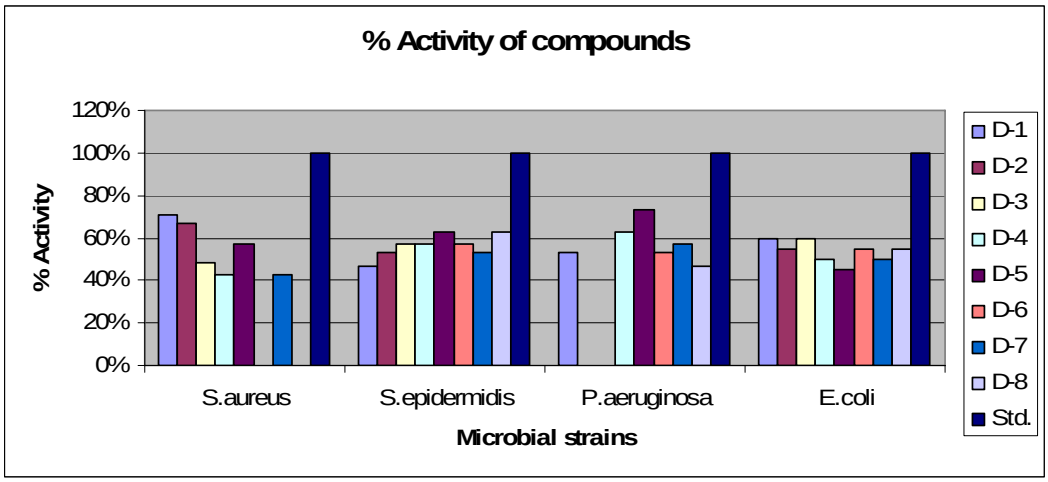


Fig. 5: % Activity

All the derivatives showed low to moderate activity while the compound D-5 showed excellent activity which was comparable to standard Ampicillin.

CONCLUSION

The piperazine derivatives were synthesized and characterized on the basis of IR and ^{1}H NMR spectra, established their structures.

Synthesized compounds were checked for their anti-microbial activity. The inhibition of microorganism under standardized condition was utilized to demonstrate microbial action to the compounds.

For present work efficacy of twelve compounds were detected against *S. aureus, S. epidermidis*, (G^+) *P. aeruginosa, E. coli* (G^-).The concentration of the test compound used was 1mg/ml. and Ampicillin was taken as the standard compound.

Synthesized derivatives were showed low to moderate activity and it observed that compound 1-(4-methylphenyl)-4-n-propylpiperazine hydrochloride show better activity against *P.aeruginosa* and 1-(4-chlorophenyl)-4-n-propylpiperazine hydrochloride show the better activity against S.aureus, while 4-n-butyl-1-(4-methoxyphenyl)piperazine hydrochloride show the poor activity against *S. aureus*, other compounds in between theses compounds have the moderate activity.

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