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Synthesis and Biological Activities of Some Benzodiazepine Derivatives

Sharda Sharma*, Renuka Jain, Chetali Chawla

**Department of chemistry, Government College, Kota(Raj.)*

ABSTRACT

Some important chalcones have been prepared by condensing aryl ketones with aromatic aldehydes in presence of suitable condensing agents. The reactions have been carried out by conventional and green chemical techniques. These chalcones have been cyclised into benzodiazepines and their derivatives. The synthesized compounds were characterized by running TLC, elemental analysis, IR , ^{1}HNMR and Mass spectroscopy. The prepared compounds have been screened for their spermicidal activity in semen of Holstein friesian cattle and antibacterial activity against Staphylococcus aureus, Escherichia coli ,Pseudomonas aeruginosa.

Keywords: Benzodiazepines, antispermicidal activity, antibacterial activity,chalcone,heterocyclic compounds

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INTRODUCTION

Chalcones are an important class of naturally occurring compounds of interest to the pharmaceutical industry for their potential antitumor, antibacterial, antifungal and anti-inflammatory activities[1]. Chalcones form central core for a variety of important biological compounds.In addition chalcones have exhibited the property of skin lightening and cosmetic activity[2]. Hydrochalcones prevent the formation of melanin cultured by human melanocytes[3].

The synthesis of chalcones can be carried out by Aldol condensation. These reactions are widely used in synthetic organic chemistry as important C-C bond forming reactions[4-6].Many elegant synthetic procedures have been developed to facilitate this reaction is usually carried out using preformed enolates which are frequently themselves somewhat noxious and results in the generation of significant quantities of waste containing metals salts such as Li salts[7].

The increasing application of microwave irradiation source of thermal energy in organic reaction is due to the short reaction time and operational simplicity[8].In last few years microwave induced organic reaction enhancement [MORE] chemistry is gaining popularity as a non-conventional technique for rapid organic synthesis[9,10].

In Grindstone chemistry reactions are initiated by grinding with transfer of very small amounts of energy through friction, the reaction proceeds itself if it is exothermic[11] in nature. As the Aldol condensation reactions are exothermic in nature ,this method is also very useful for the synthesis. No heating and no organic solvent is utilized in the grinding procedure.

The chalcones on condensation with O-Phenylenediamine leads to the formation of benzodiazepines. This nucleus is a pharmacophoric scaffold and represent a class of heterocycles with a wide range of biological applications[12].Many of them are widely used as anticonvulsant,antianxiety, sedative, antidepressive, hypnotic and

neuroleptic agents[13-16]. Some heterocycles containing benzodiazepines moiety were reported to possess anti inflammatory[17], antiviral[18], anti-HIV-1[19], antimicrobial[20] and antitumor[21] activities. Other than their biological importance, benzodiazepines are valuable synthons for the preparation of fused ring compounds, such as triazolo[22], thiazolo[23], imidazo[24] and pyrimido-benzodiazepines[25]. It has been noticed that introduction of an additional ring to the benzodiazepine core tends to exert profound influence in conferring novel biological activities in these molecules.[22-26] Although many methods for synthesizing benzodiazepine ring systems have been reported, they continue to receive a great deal attention[20,27-29,37].

All the reactions are promoted using eco-friendlier conditions and the simplified protection-deprotection steps which ultimately results the reduction of waste generation. In addition, by the use of microwave irradiation[30]provided many advantages such as simple, rigid, rapid and efficient reactions, decreasing the use of energy in large scale transformations.

The biological essay of the compounds have been carried out by evaluating their spermicidal activity in semen of holstein friesian cattle and antibacterial activity against Staphylococcus *aureus*, Escherichia *coli*, Pseudomonas *aeruginosa*.

EXPERIMENTAL SECTION

General information-

Melting points were determined in open capillaries and are uncorrected. All solvents were then purified according to standard procedures. The ^{1}HNMR spectra were recorded at JEOL AL 300MHz FTNMR instrument, ν in cm^{-1}. Chemical shifts were calibrated to tetramethylsilane as an external reference. Coupling constants are given in hertz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; t, triplet; m, multiplet.IR spectra (cm^{-1}) were recorded on FTIR Nicolet Magna 550 and Shimadzu 8400s spectrophotometer in KBr disc and noteworthy absorptions levels (cm^{-1}) were listed. Mass spectra were determined by JEOL SX-102 spectrophotometer. TLC using silica gel G as adsorbent checked the purity of the compounds, UV light or iodine accomplished visualization. The reactions were carried out in a domestic microwave oven (Samsung M 1630 N/M 1610 W with maximum 600W Power).

General procedure for synthesis of 1,3-diaryl-2-propen-1-one by conventional method-

A mixture of substituted acetophenones (0.01mol) and aryl aldehydes (0.01mol) were dissolved in minimum amount of ethanol(30ml) and then stirred at room temperature for about 15 min. Sufficient 2N NaOH solution was added to it to make the solution with continuous stirring (about 30 min.) till yellow precipitate was formed. This was then neutralized with 2N HCl and dilutes with water and left overnight. The precipitate chalcones were filtered and washed well with water and recrystallised from ethanol.(Table-1)

General procedures for synthesis of (3a-j) by grindstone method-

A mixture of substituted acetophenones (0.01mol) and $Mg(HSO_4)_2$(.4g) was grinded together in a mortar using a pestle to generate different colour.Then aryl aldehydes was added to it and grinded continued for 10-25min. Reaction proceeds exothermically indicated by the rise in temperature (5-8^oC). After the reaction was completed (when no starting material was detected by TLC analysis), mixture was poured to ice cold water and acidified with dil. HCl and whole mixture was filtered. The filterate was concentrate and solid thus obtained was purified by recrystallization from ethyl alcohol to afford pure desired compound.(Table-1)

General procedures for prepration of (3a-j) by Microwave assisted method-

A mixture of substituted acetophenones(0.01mol) ,aryl aldehydes(0.01mol) and montmorillonite KSF(.4g) were thoroughly mixed in a pestle mortar. This mixture was then transferred into a 100ml conical flask and irradiated with microwaves for 50-190 s at 60 watts. After reaction was complete(indicated by absence of starting material in TLC) ethanol was added and mixture was filtered. The filterate was recrystallized from ethanol to afford dark yellow crystals of desired compounds.(Table-1)

2,3-Diaryl-2,3-dihydro-1H-1,5-benzodiazepines (5a-j)-

A mixture of chalcone (.001mol) and O-phenylenediamine(.001 mol) in abs. Alcohole (30 ml) and acetic acid (0.025 ml) was refluxed for 5 -7hrs. The excess solvent was removed under pressure to give solid product. It was washed with ethanol and crystallized from a suitable solvent.(Table-2)

1-Acetyl-2,3-Diaryl-2,3-dihydro-1H-1,5-benzodiazepines(6a-j)-
The benzodiazepines (.01 mol.)was refluxed in acetyl chloride (40 ml) for 6-8hrs. On cooling crystals separated out which were filtered, washed with little acetic acid and recrystallised from acetic acid.(Table-3)

2,3-Diaryl-2,3-dihydro-1H-1-morpholino-1,5-benzodiazepines(7a-j)-
The benzodiazepines (.01 mol), formaldehyde [(40%), 0.015 mol., 0.5gm],morpholine(.012 mol.,0.1gm) and abs. Alcohole (20 ml) was refluxed on a steam bath for 45-70 min. On cooling, crystals separated out which was filtered, dried and recrystallised from ethanol.(Table-4)

Scheme-

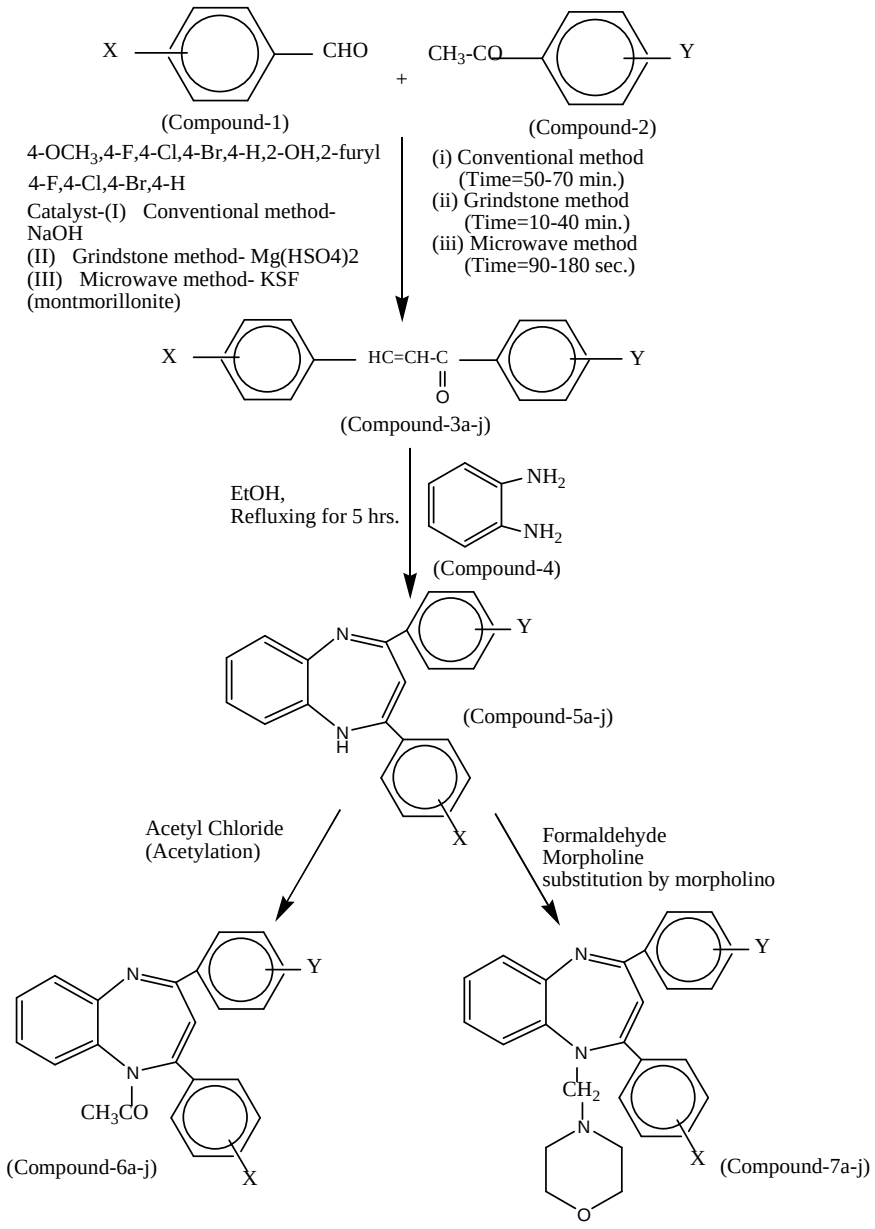


Table-1: Physical and analytical datas of substituted chalcones 3(a-j):-

Compounds	X	Y	Molecular Formula	Melting Point(^{0}C)	Molecular Weight	Yield (%)			Time required			Elemental Analysis		
						I	II	III	I(min.)	II(min.)	III(sec.)	C	H	O
3a	4-OCH_3	4-F	$C_{16}H_{13}O_2F$	95	256	70	78	88	55	30	120	75	5.07	12.5
3b	4-OCH_3	4-H	$C_{16}H_{14}O_2$	230	238	70	75	86	65	25	140	80.67	5.88	13.44
3c	4-F	4-H	$C_{15}H_{11}OF$	85	226	63	77	90	68	35	160	79.64	4.86	7.07
3d	4-OCH_3	4-Br	$C_{16}H_{13}O_2Br$	140	317	75	80	82	58	27	100	60.56	4.10	10.09
3e	4-OCH_3	4-Cl	$C_{16}H_{13}O_2Cl$	110	272.5	65	75	84	62	28	90	70.45	4.77	11.74
3f	4-F	4-F	$C_{15}H_{10}OF_2$	120	244	75	83	90	57	28	110	73.77	4.09	6.55
3g	4-Cl	4-F	$C_{15}H_{10}OClF$	130	260.5	68	75	85	60	28	150	69.09	3.83	6.14
3h	Furyl	4-Br	$C_{13}H_9O_2Br$	90	278	70	82	91	70	30	160	56.11	3.23	11.51
3i	Furyl	4-F	$C_{13}H_9O_2F$	70	217	65	74	90	75	32	165	71.88	4.14	14.74
3j	Furyl	4-Cl	$C_{13}H_9O_2Cl$	50	233.5	65	72	80	64	29	145	64.98	3.61	5.77

Table-2: Physical and analytical datas of 1,5-benzodiazepines 5(a-j):-

Compounds	X	Y	Molecular Formula	Melting Point (^{0}C)	Molecular Weight	Yield (%)	Time required (hrs.)	Elemental analysis		
								C	H	N
5a	4-OCH_3	4-F	$C_{21}H_{19}N_2OF$	95	334	79	6	75.44	5.68	8.38
5b	4-OCH_3	4-H	$C_{21}H_{20}N_2O$	80	316	70	5	79.74	6.32	8.86
5c	4-F	4-H	$C_{21}H_{19}N_2F$	100	304	75	7	78.94	5.59	9.21
5d	4-OCH_3	4-Br	$C_{21}H_{19}N_2OBr$	110	395	82	5	63.79	4.81	7.08
5e	4-OCH_3	4-Cl	$C_{21}H_{19}N_2OCl$	90	350.5	70	6	71.89	5.42	7.98
5f	4-F	4-F	$C_{20}H_{16}N_2F_2$	95	322	80	7	74.53	4.96	8.69
5g	4-Cl	4-F	$C_{20}H_{16}N_2FCl$	85	388.5	75	7	70.90	4.72	8.27
5h	Furyl	4-Br	$C_{19}H_{15}N_2OBr$	100	367	65	8	62.12	4.08	7.62
5i	Furyl	4-F	$C_{19}H_{15}N_2OF$	80	306	60	8	74.50	4.90	9.15
5j	Furyl	4-Cl	$C_{19}H_{15}N_2OCl$	75	322.5	55	7	70.69	4.65	8.68

Table-3: Physical and analytical datas of Acetyl derivative of 1,5-benzodiazepines 6(a-j):-

Compounds	X	Y	Molecular Formula	Melting Point (^{0}C)	Molecular Weight	Yield (%)	Time required (hrs.)	Elemental analysis		
								C	H	N
6a	4-OCH_3	4-F	$C_{23}H_{21}N_2O_2F$	105	376	76	7	73.04	5.58	7.44
6b	4-OCH_3	4-H	$C_{23}H_{22}N_2O_2$	85	358	70	8	77.09	6.14	7.82
6c	4-F	4-H	$C_{22}H_{19}N_2OF$	100	346	75	8	76.30	5.49	8.09
6d	4-OCH_3	4-Br	$C_{23}H_{21}N_2O_2Br$	120	437	80	6	63.15	4.80	6.40
6e	4-OCH_3	4-Cl	$C_{23}H_{21}N_2O_2Cl$	95	392.5	75	7	70.31	5.35	7.13
6f	4-F	4-F	$C_{22}H_{18}N_2OF_2$	120	364	85	7	72.52	4.94	7.69
6g	4-Cl	4-F	$C_{22}H_{18}N_2FOCl$	155	380.5	80	8	69.38	4.73	7.35
6h	Furyl	4-Br	$C_{21}H_{17}N_2O_2Br$	100	409	60	9	61.61	4.15	6.84
6i	Furyl	4-F	$C_{21}H_{17}N_2O_2F$	95	348	65	8	72.41	4.88	8.04
6j	Furyl	4-Cl	$C_{21}H_{17}N_2O_2Cl$	80	364.5	60	9	69.13	4.66	7.68

Table-4: Physical and analytical datas of Morpholine derivative of 1,5-benzodiazepines 7(a-j):-

Compounds	X	Y	Molecular Formula	Melting Point (^{0}C)	Molecular Weight	Yield (%)	Time required (min.)	Elemental analysis		
								C	H	N
7a	4-OCH_3	4-F	$C_{26}H_{28}N_3O_2F$	100	433	80	50	72.05	6.46	9.69
7b	4-OCH_3	4-H	$C_{26}H_{29}N_3O_2$	95	415	75	60	75.18	6.98	10.12
7c	4-F	4-H	$C_{25}H_{26}N_3OF$	120	403	75	55	74.44	6.45	10.42
7d	4-OCH_3	4-Br	$C_{26}H_{28}N_3O_2Br$	115	494	90	45	63.5	5.66	8.50
7e	4-OCH_3	4-Cl	$C_{26}H_{28}N_3O_2Cl$	100	449.5	80	60	69.41	6.22	9.34
7f	4-F	4-F	$C_{25}H_{25}N_3OF_2$	110	421	85	50	71.25	5.93	9.97
7g	4-Cl	4-F	$C_{25}H_{25}N_3FOCl$	140	437.5	80	55	68.57	5.71	9.60
7h	Furyl	4-Br	$C_{24}H_{24}N_3O_2Br$	110	466	72	65	61.80	5.15	9.01
7i	Furyl	4-F	$C_{24}H_{24}N_3O_2F$	100	405	68	60	71.11	5.92	10.37
7j	Furyl	4-Cl	$C_{24}H_{24}N_3O_2Cl$	90	421.5	70	65	68.32	5.69	9.96

Biological activities:-

Spermicidal activities :- Sperm motility scheme was followed to see the effect of chemicals. Compounds (6a-j) and (7a-j) were evaluated for spermicidal activities , briefly the motility of sperms was observed under 40x magnification of motic digital microscope with image processing software and CCD colored camera attached to a computer assembly in a cell counting chamber . While observing the motility, 25µl solution (compound + alcohol) was added to the 25 µl semen and it was observed that addition of compounds to semen produces a reduction of sperm motility in HF cattle spermatozoa; which was dose and time dependent. At 100 ppm concentration of compound 6f and 7f , HF cattle sperm motility was reduced to 25% in 2.5 min. Compound 6g and 7g at 100 ppm inhibited motility to 30% in 4 and 3.5 min respectively. After 3 and 4 min sperm motility was reduced to 35 % and 30% by compound 6c and 7c respectively where as 6a,7a,6b,7b,6d,7d,6e,7e,6h,7h,6i,7i,6j,7j compounds reduces the motility to 50% in 3.5-5 min at 100ppm concentration only. The results have been summarized in table and shown by graph.(Figure 1-3).

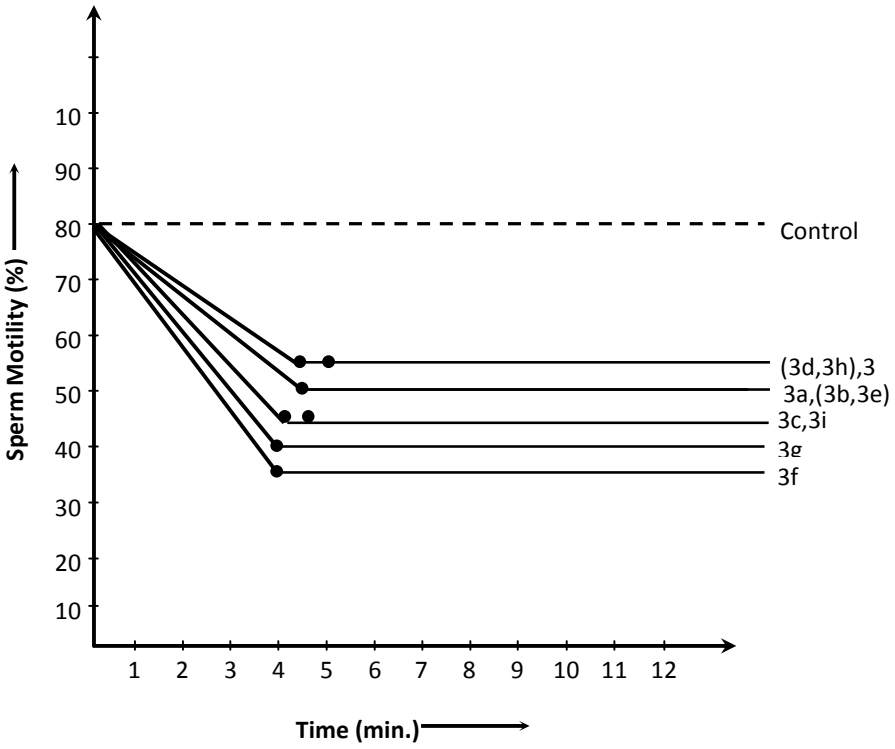


Figure:1 spermicidal activity of compound 3(a-j)

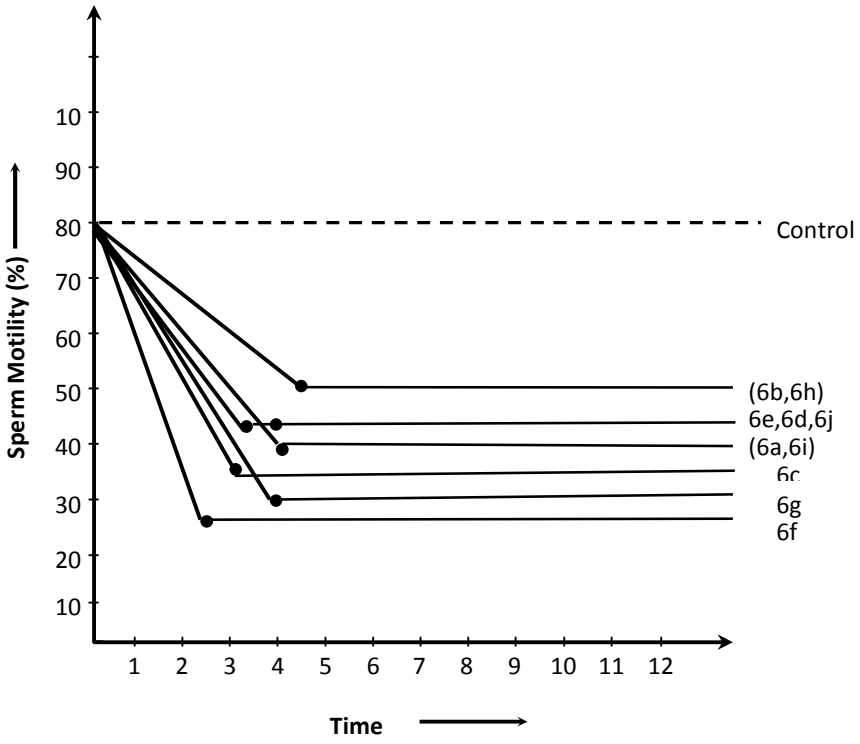


Figure:2 spermicidal activity of compound 6(a-j)

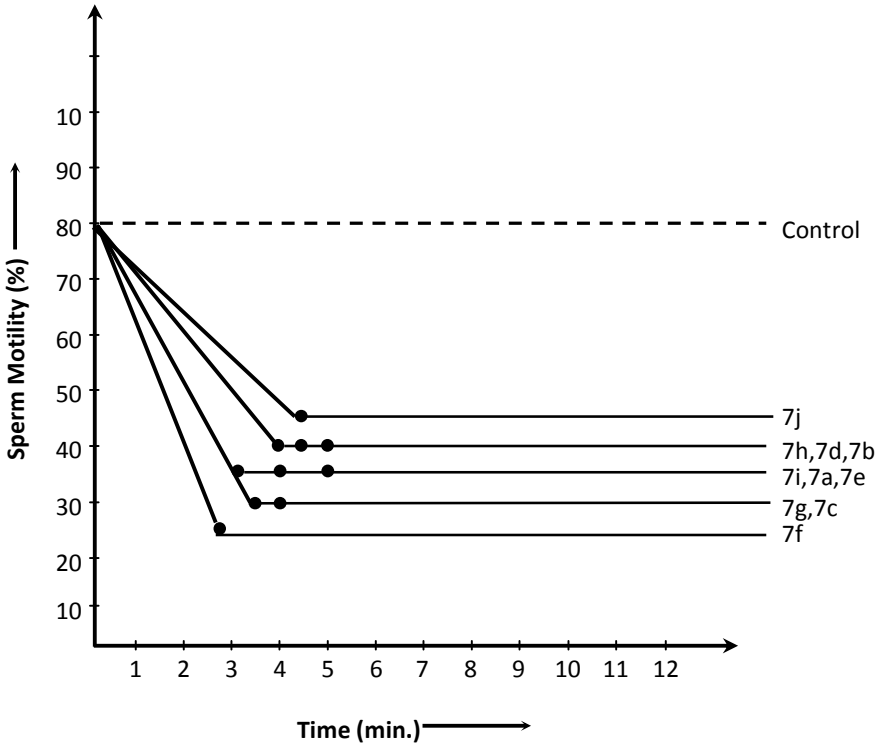


Figure:3 spermicidal activity of compound 7(a-j)

Antibacterial Activity-
All the synthesized compounds were evaluated in vitro for antibacterial activity by using filter paper disc method [31-38] against different strains of bacteria viz. staphylococcus *aureus*, Escherichia *coli*, Pseudomonas *aeruginosa*. All the compounds test along with standard antibacterial ampicillin were used at 100, 200,300,400 ppm concentrations.

Procedure: Solution of known concentrations(100,200,300,400ppm) of the test samples were made by dissolving in DMF. Dried and sterilized filter paper discs(6mm in diameter) soaked with known amount of test agents were placed on the nutrient agar media solidified in petridishes (120mm in diameter) and incubated with the test organisms. These plated were then kept at temperature(37^{o}C) for 18 hours to allow maximum growth of the organisms. The antibacterial activity was determined by measuring the average diameter of zone of inhibition in mm.(Figure 4-6)

Fig:4 Antibacterial activity for bacteria A

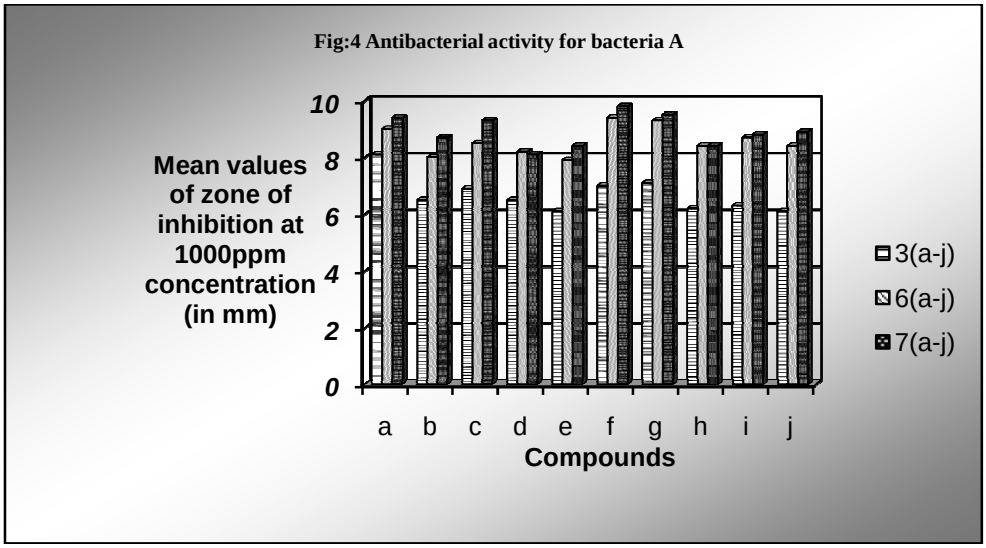
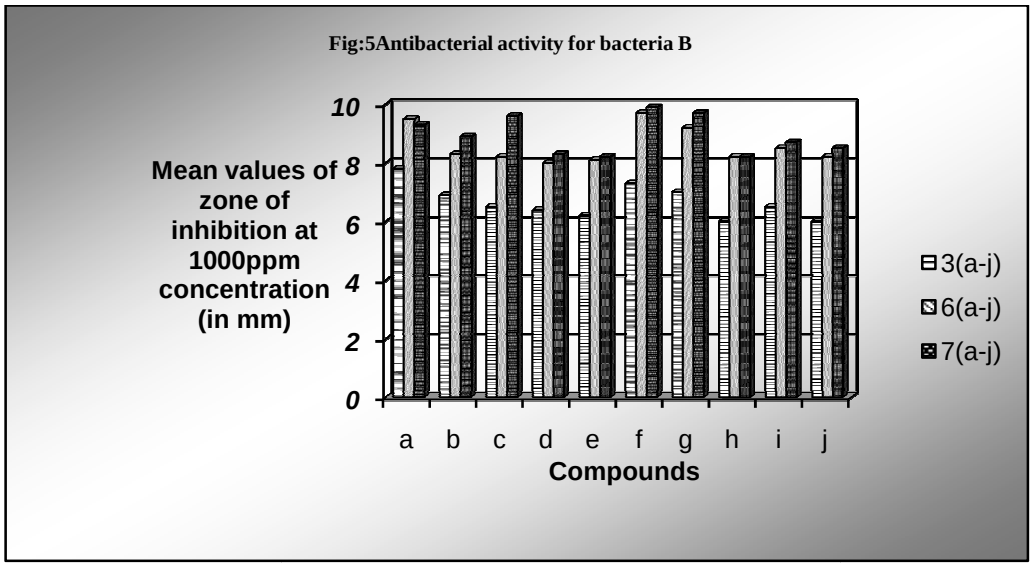
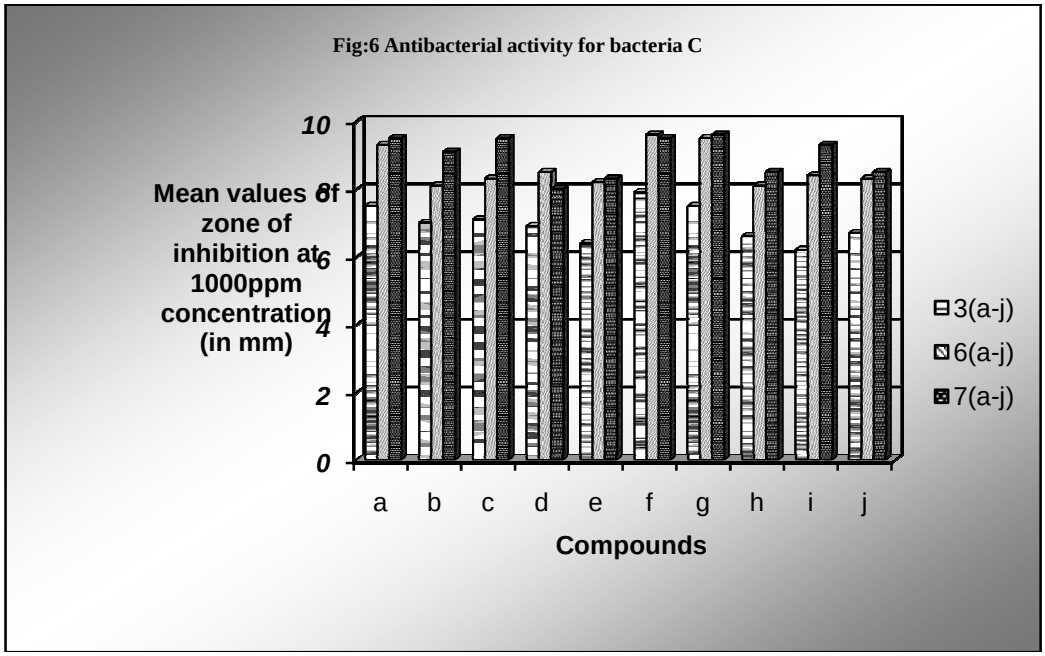


Fig:5Antibacterial activity for bacteria B





RESULTS AND DISCUSSION

All the chalcones/1,3-diaryl-2-propen-1-one were synthesized with conventional method by the reaction of appropriate substituted acetophenones and aryl aldehydes in the presence of catalysts ($Mg(HSO_4)_2$and KSF). In green chemical synthesis techniques we have used catalysts which have shown catalytic activity for chalcone and have many advantages over catalyst like NaOH, KOH such as ease of handling, low cost and elimination of metal wastes. The microwave procedure for the synthesis of compounds owes its important due to the fact that the reaction complete with in 1-3min. as compared to conventional method which required 6-8hr. and yield have been remarkably improved from 42-53% to 83-90%.

The reaction of compounds(3a-j) with O-phenylenediamine gave 2,3-diaryl-2,3-dihydro-1H-1,5-benzodiazepines(5a-j). The active hydrogen present on the nitrogen were substituted to its acetyl and morpholine derivatives.The synthesized compounds were further evaluated for biological activities.(Figure 1-6)

These acid catalysed reactions are understood to be initiated by the protonation of the carbonyl group of the propenones (3a-j). On protonation the methane carbon becomes more electrophilic and hence susceptible to nucleophilic attack by the electrons of the o-phenylenediamine to give Michael adduct intermediates which simultaneously undergo dehydrative cyclization to give final products 2,3-diaryl-2,3-dihydro-1H-1,5-benzodiazepine (5a-j).

The IR spectra exhibited peak in the region 1600-1650cm^{-1}(C=O, highly conjugated). In ^{1}HNMR ($CDCl_3$) doublets at δ 7.5 and δ 7.7 due to C_α-H and C_β-H respectively. All aromatic protons appears as multiplet at δ 6.5-8.0.

In the IR spectra of the final products (5a-j) a broad absorption band was observed at around 3140-3135 cm^{-1} which may be due to secondary amino group (N-H). However characterstics abs. In the range 3450-3350 and 1700-1640 were found to be absent due to absence of primary amino group and carbonyl group respectively. This indicated that the reaction had taken place in on step to form the final products 5(a-j) by the reaction 3(a-j) with compound-4.The ^{1}HNMR spectra of all the final products 5(a-j) showed a doublet at 6.8-6.95 (d,1H) integrating for one proton, which may be assigned to C_2-H. Another doublet at 7.93-8.03 (d,1H) may be assigned due to C-3 proton. The downfield abs. Of C-2 proton may be accounted due to deshielding zone of aryl ring and attachment of it with electronegative nitrogen atom. Multiplets at around 6.82-8.00 (m,12H/11H) may be assigned to aromatic protons. A broad singlet at around 4.08-4.13 (s,1H) may be assigned to secondary amino proton. All the compounds have also been

characterized by mass spectral studies by their molecular ion peaks respectively which corresponds to their molecular weights. Spectral data of compounds 5(a-j) ,6(a-j) and 7(a-j) are in harmony with proposed structures.

CONCLUSION

The aim of the present work was to synthesize derivatives of benzodiazepines with potential biological activity where the aryl groups(phenyl, 4-fluorophenyl, 4-fluoronaphthyl) were chosen on the basis of pharmacological considerations. We intended to prepare the target compounds by the reaction of the substituted acetophenones with aryl aldehydes.(cf Scheme-1)

Compounds with electron releasing groups such as methoxy, hydroxyl showed better antibacterial activity than the others not having such groups. Compounds having pharmacophores such as cholro, dichloro and fluoro groups have exhibited more activity on all the three bacteria than the others. The results suggests that the chalcone derivatives have excellent scope for further development as commercial anti microbial agents. Further experiments were needed to elucidate their mechanism of action.

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