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SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL ACTIVITY OF 2, 4-DISUBSTITUTED- 1, 5 -BENZODIAZEPINES DERIVATIVES

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ABSTRACT

A series of 2, 4-disubstituted-1,5-benzodiazepine derivatives were synthesized by the condensation of *o*-phenylendiamine and various 1-(4'-substituted phenyl)- 3- (6''- methoxy napthaline)-2-propene-1-one. The structures of newly synthesized compounds were confirmed by IR, 1H NMR, mass and elemental analysis. All the compounds were tested for in *vitro* activities against a panel of Gram-positive and Gram-negative bacteria. All the compounds exhibited mild to moderate antimicrobial activity.

KEYWORDS

Chalcones, 1,5-benzodiazepine, Antibacterial and Antifungal activities

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INTRODUCTION

Diazepines constitute a very important class of heterocyclic compounds having significant biological activities, characterized by a 7-membered ring structure composed of five carbon atoms and two nitrogen atoms. There are number of reports containing the benzodiazepine moiety, which show additional biological activities[1-3]. Benzodiazepine derivatives also find commercial use in photograph[4] and also as anti-inflammatory agents[5]. A number of 1, 5- benzodiazepine such as: 2,4-diphenyl-1, 5-benzodiazepine, 7-amino -2,4- dimethyl -1, 5-benzodiazepine, 7- nitro - 2, 4- dimethyl -1, 5 benzodiazepine, and 7-bis-(2-chloro-ethyl)-amino-

2,4- dimethyl-1,5-benzodiazepine have been reported to possess cancerostatic activity[6]. Generally, benzodiazepines were synthesized by the condensation of *o*-phenylene diamines with α,β-unsaturated carbonyl compound, β- haloketones, or ketones. A variety of reagents, such as BF3-etherate[7], $NaBH_4$[8] polyphosporic acid[9], SiO2, $MgO/POCl_3$[10], Yb(OTf)3[11], $Sc(OTf)_3$, Al_2O_3/P_2O_5, or AcOH under microwave irradiation[12] and even in the presence of ionic liquids[13-15] are utilized for condensation reactions. In the present work we have synthesize 2, 4-disubstituted-1, 5-benzodiazepine derivatives.

MATERIALS AND METHOD

EXPERIMENTAL

The melting points were determined by open capillary method and are uncorrected.IR (KBr) spectra were recorded on a Shimadzu 8201PC infrared spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker DRX-300 spectrophotometer in DMSO using TMS as internal standard (Chemical shift are expressed in ppm). The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G coated plates and the spots were visualized by exposure to iodine vapors (Table No.1-4).

Synthesis of chalcones

Potassium Hydroxide (0.1mol) was dissolved in 20 ml of methanol and stirred in ice cold conditions. 2-acetyl naphthalene (0.1mol) was dissolved in 20 ml of 95% v/v methanol and the solution was added drop wise with constant stirring under ice cold conditions. Pure benzaldehyde or substituted benzaldehyde (0.1 mol) was dissolved in 20 ml of 95% v/v methanol and added drop wise to the previous solution with constant stirring under ice cold conditions (Figure No.1).

The stirring was continued till the TLC (Petether: Ethylacetate) had shown the disappearance of aldehyde spot and pH of the reaction mixture was made neutral by addition of dil.Hcl. The product was filtered under vacuum, washed with excess distilled water.

Synthesis of 2,4-disubstituted-1,5benzpdiazepines (1a-1g)

A mixture of *o*-phenylenediamine (0.01 mol), chalcone (0.01 mol) were dissolved in methanol (15 ml) and two drops of acetic acid was added. Reaction mixture was refluxed on boiling water bath for 15-35 min. Half of the solvent was evaporated and cooled the solution to room temperature. Solid separated out. Solid was filtered washed with water and crystallized from ethanol.

Synthesis of 2,4-disubstituted-1,5-benzpdiazepines (2a-2g)

A mixture of ethylenediamine (0.01 mol), chalcone (0.01 mol) were dissolved in methanol (15 ml) and two drops of acetic acid was added. Reaction mixture was refluxed on boiling water bath for 1-2 hrs. Half of the solvent was evaporated and cooled the solution to room temperature. Solid separated out. Solid was filtered washed with water and crystallized from ethanol.

Antimicrobial Screening of the Compounds

Biological Evaluation

All synthesized compounds were evaluated for antimicrobial activity.

Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeuroginosa* and *Streptococcus pyogenes* (recultured) bacterial strains by disc diffusion method. Discs measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. The test compounds were prepared with different concentrations using dimethylformamide. One milliliter containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37^0C for 24 h. Ciprofloxacin was used as a standard drug. Solvent and growth controls were prepared and kept. Zones of inhibition and minimum inhibition concentrations (MICs) were noted. The results of antibacterial studies are given in Table No.5.

Antifungal studies

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Penicillium marneffei* (recultured) in DMSO by serial plate dilution method 19, 20. Sabourands agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7.

Normal saline was used to make a suspension of spore of fungal strain for lowning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Agar media (20 mL) were poured into each petri dish.Excess of suspensions was decanted and the plates were dried by placing in an incubator at 37o C for 1 h using an agar punch, wells were made and each well were labeled. A control was also prepared in triplicate and maintained at 37^{o}C for 3-4 days. Zone of inhibition and minimum inhibitory concentration (MIC) were noted. The activity of each compound was compared with flucanazole as the standard drug. The results of antifungal studies are given in Table No.6.

RESULTS AND DISCUSSION

In the present work we synthesize 14 compounds by the reaction of chalcone with o-phenyldiamine (1a-1g) and ehylenediamine (2a-2g). The melting points were determined by open capillary method and are uncorrected.IR (KBr) spectra were recorded on a Shimadzu 8201PC infrared spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker DRX-300 spectrophotometer in DMSO using TMS as internal standard (Chemical shift are expressed in ppm). The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G coated plates and the spots were visualized by exposure to iodine vapors the purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G coated plates and the spots were visualized by exposure to iodine vapors. The structures of the synthesized compounds were confirmed on the basis of spectral and elemental analysis.

The results of antimicrobial studies have shown that five compounds out of fourteen were found to possess antimicrobial activity against all tested micro-organisms. Results have shown that all the compounds possess mild antifungal activity against all four tested strains. The structures of the synthesized compounds were confirmed on the basis of spectral and elemental analysis. The formulas, melting point, yield of the compounds are listed in Table No.1. Compounds with electron releasing groups such as methoxy and compounds having pharmacophores such as chloro, fluoro, bromo groups and both these groups are present in one moiety exhibited mild to moderate antimicrobial activity. Hence, it is concluded that there is ample scope for further study in developing these as good lead compounds.

Table No.1: Molecular formulae, molecular weights, percentage yield, meltingpoints of compounds (1a-1g)

S.No	Compound No.	R	Mol. Formula	Mol. Wt.	Yield (%)	m.pt. (^{O}C)	Analytical Calculation (%)
1	1a	H	$C_{25}H_{20}N_2$	348.43	72	142-144	C,86.17;H,5.79;N,8.04 found C,83.6, H,4.9 N,7.8
2	1b	4-Cl	$C_{25}H_{19}ClN_2$	382.88	75	140-142	C,78.42, H,5.0; N,7.32, Cl;9.26 found C,77.8, H,4.5 N,7.0, Cl;8.9
3	1c	4-Br	$C_{25}H_{19}BrN_2$	427.41	78	165-167	C,70.24; H,4.48; N,6.56, Br,18.9 found C,69.2,H,4.0 N,6.1; Br,17.0
4	1d	4-F	$C_{25}H_{19}FN_2$	366.430	70	132-134	C,81.94; H,5.23; N,7.64; F,5.18 Found C,79.5,H,5.0;N,7.0 F,4.9
5	1e	4-CH_3	$C_{26}H_{22}N_2$	362.46	81	190-192	C,86.15; H,6.12;N,7.73 found C,85.0, H,6.1; N,6.9,
6	1f	4-OCH_3	$C_{26}H_{24}N_2O$	380.50	79	168-170	C,82.07; H,6.36; N,7.6; O 4.21 found C,80.9; H,5.9; N,6.8; O,3.9
7	1g	4-NO_2	$C_{25}H_{21}N_3O_2$	395.45	72	187-189	C,75.93; H,5.36; N,10.60; O,8.09 found C,74.12,H,4.9; N,9.9; O,7.9

Table No.2: Spectral data of compounds (1a-1g)

S.No	Compound No.	I.R. data(cm^{-1})	1H NMR data(ppm)
1	1a	3316 (N-H), 1669 (C=N)(str) 1613 (CH=CH), 1637 (N-H)(bend)	7.93-7.76 (m,5H,Ar) 7.75-7.65(m,4H,Ar) 7.5-7.47 (dd,1H,ArH) 7.40-7.25(m,4H,ArH) 7.15-7.14(dd,1H,ArH) 7.6 (s,1H,NH)
2	1b	3319 (N-H), 1675 (C=N)(str) 1620 (CH=CH), 1647 (N-H)(bend),840 (C-Cl)	7.83-7.66 (m,4H,Ar) 7.75-7.65(m,4H,Ar) 7.4-7.37 (dd,1H,ArH) 7.30-7.15(m,4H,ArH) 7.25-7.24(dd,1H,ArH) 7.8 (s,1H,NH)
3	1c	3321 (N-H), 1680 (C=N)(str) 1622 (CH=CH), 1657 (N-H)(bend) 580 (C-Br)	8.03-8.00 (m,5H,Ar) 7.55-7.45(m,4H,Ar) 8.5-8.47 (dd,1H,ArH) 7.60-7.45(m,4H,ArH) 7.25-7.14(dd,1H,ArH) 7.6 (s,1H,NH)
4	1d	3360 (N-H), 1659 (C=N)(str) 1630 (CH=CH), 1627 (N-H)(bend)1236 (C-F)	8.13-8.06 (m,5H,Ar) 8.75-8.65(m,4H,Ar) 7.9-7.8 (dd,1H,ArH) 7.40-7.25(m,4H,ArH) 7.19-7.14(dd,1H,ArH) 8.0 (s,1H,NH)
5	1e	3359 (N-H), 1669 (C=N)(str) 1600 (CH=CH), 1625 (N-H)(bend)1100 (C-C)	7.83-7.76 (m,5H,Ar) 7.15-7.05(m,4H,Ar) 7.5-7.47 (dd,1H,ArH) 7.80-7.275(m,4H,ArH) 7.95-7.84(dd,1H,ArH) 7.2 (s,1H,NH)
6	1f	3318 (N-H), 1675 (C=N)(str) 1633 (CH=CH), 1677 (N-H)(bend)260 (C-O-C), 1170 (C-C)	8.53-8.46 (m,5H,Ar) 7.75-7.65(m,4H,Ar) 8.5-8.47 (dd,1H,ArH) 8.40-8.25(m,4H,ArH) 7.15-7.14(dd,1H,ArH) 8.6 (s,1H,NH)
7	1g	3390 (N-H), 1695 (C=N)(str) 1680 (CH=CH), 1689 (N-H)(bend) 1165 (C-C) 870 (C-N), 610 (C-N-O)	7.93-7.76 (m,5H,Ar) 8.75-8.65(m,4H,Ar) 7.5-7.47 (dd,1H,ArH) 7.40-7.25(m,4H,ArH) 8.15-8.14(dd,1H,ArH) 8.4 (s,1H,NH)

Table No.3: Molecular formulae, molecular weights, percentage yield, melting points of compounds (2a-2g)

S.No	Compound No.	R	Mol. Formula	Mol. Wt.	Yield (%)	m.pt. (^{O}C)	Analytical Calculation (%)
1	2a	H	$C_{21}H_{18}N_2$	298.40	76	135-137	C,84.53;H,6.0;N,9.39 found C,83.6, H,5.9 N,8.8
2	2b	4-Cl	$C_{21}H_{17}ClN_2$	332.88	79	145-147	C,75.42, H,5.0; N,8.32, Cl;10.26 found C,74.8, H,4.5 N,7.5, Cl;9.9
3	2c	4-Br	$C_{21}H_{17}BrN_2$	377.28	70	152-154	C,66.24; H,4.54; N,7.56, Br,21.10 found C,64.2,H,4.0 N,6.9; Br,19.9
4	2d	4-F	$C_{21}H_{17}FN_2$	316.430	82	130-132	C,79.94; H,5.43; N,8.64; F,6.18 Found C,79.5,H,5.0;N,8.0 F,5.9
5	2e	4-CH_3	$C_{22}H_{20}N_2$	312.46	85	184-186	C,84.15; H,6.12;N,8.73 found C,83.0, H,6.1; N,7.9,
6	2f	4-OCH_3	$C_{22}H_{20}N_2O$	328.40	89	162-164	C,80.07; H,6.16; N,8.6; O 4.81 found C,70.9; H,5.9; N,7.8; O,3.9
7	2g	4-NO_2	$C_{21}H_{17}N_3O_2$	343.45	69	134-136	C,73.43; H,4.96; N,12.60; O,9.09 found C,72.12,H,4.0; N,10.9; O,8.9

Table No.4: Spectral data of compounds (2a-2g)

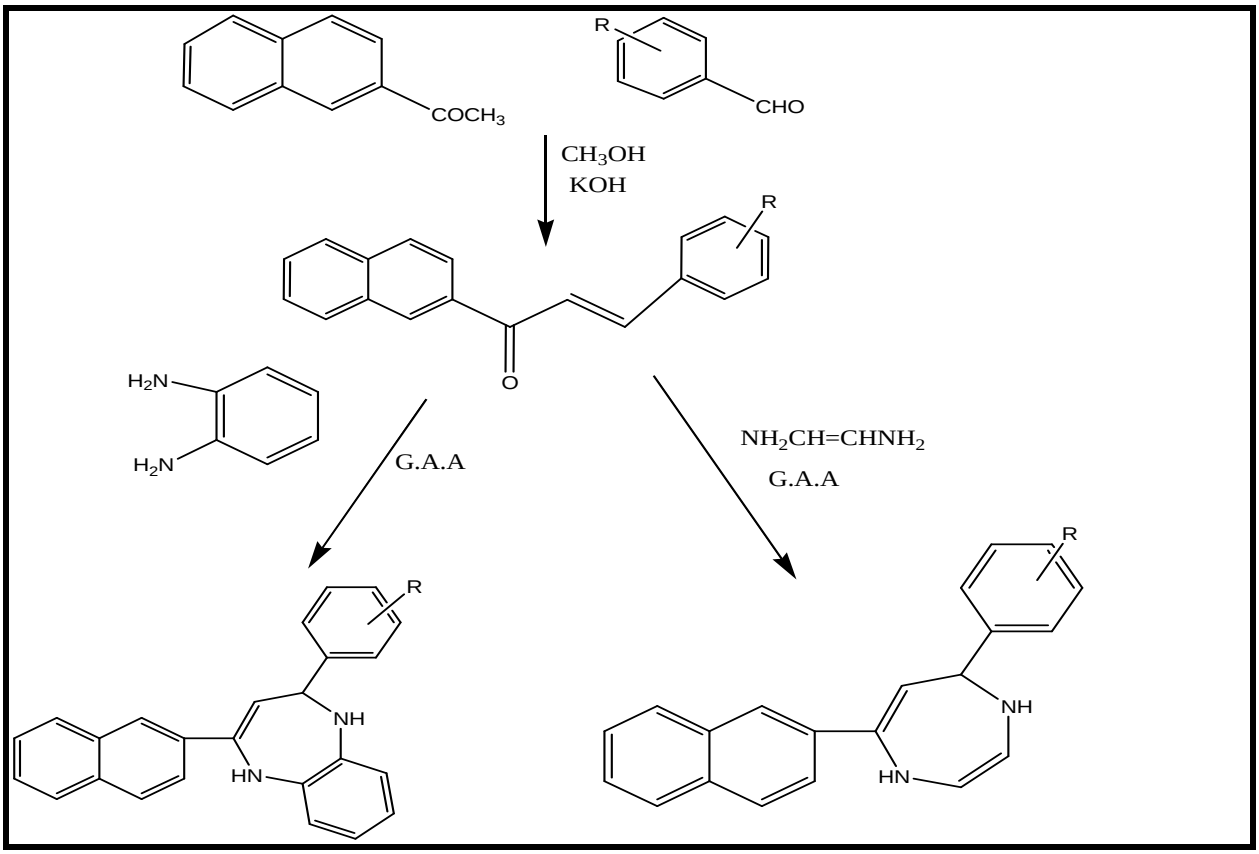
S.No	Compound No.	I.R. data(cm^{-1})	1H NMR data(ppm)
1	2a	3316 (N-H), 1669 (C=N)(str) 1613 (CH=CH), 1637 (N-H)(bend)	2.86(dd,1H,CH_2) 3.00(m,2H,CH_2) 3.37(dd,1H,CH_2) 3.85(m,2H,CH_2) 7.6 (s,1H,NH)
2	2b	3314 (N-H), 1675 (C=N)(str) 1620 (CH=CH), 1640 (N-H)(bend),840 (C-Cl)	2.26(dd,1H,CH_2) 3.50(m,2H,CH_2) 3.87(dd,1H,CH_2) 3.25(m,2H,CH_2) 7.9 (s,1H,NH)
3	2c	3400 (N-H), 1700 (C=N)(str) 1713 (CH=CH), 1737 (N-H)(bend) 580 (C-Br)	3.86(dd,1H,CH_2) 4.00(m,2H,CH_2) 4.37(dd,1H,CH_2) 4.85(m,2H,CH_2) 7.9 (s,1H,NH)
4	2d	3408 (N-H), 1650 (C=N)(str) 1700 (CH=CH), 1647 (N-H)(bend)1236 (C-F)	2.96(dd,1H,CH_2) 3.90(m,2H,CH_2) 4.35(dd,1H,CH_2) 4.25(m,2H,CH_2) 8.0 (s,1H,NH)
5	2e	3379 (N-H), 1625 (C=N)(str) 1690 (CH=CH), 1700 (N-H)(bend)1100 (C-C)	3.36(dd,1H,CH_2) 3.20(m,2H,CH_2) 3.97(dd,1H,CH_2) 3.85(m,2H,CH_2) 8.6 (s,1H,NH)
6	2f	3396 (N-H), 1769 (C=N)(str) 1613 (CH=CH), 1701 (N-H)(bend)260 (C-O-C), 1170 (C-C)	4.06(dd,1H,CH_2) 4.00(m,2H,CH_2) 3.57(dd,1H,CH_2) 3.85(m,2H,CH_2) 7.9 (s,1H,NH
7	2g	3410 (N-H), 1630 (C=N)(str) 1619 (CH=CH), 1637 (N-H)(bend) 1165 (C-C) 870 (C-N), 610 (C-N-O)	2.46(dd,1H,CH_2) 2.70(m,2H,CH_2) 3.17(dd,1H,CH_2) 3.25(m,2H,CH_2) 7.0 (s,1H,NH)

Table No.5: Antibacterial activities of compounds (1a-2g)

S.No	Compound No.	Diameter of growth inhibition zone (mm)			
		Escherichia Coli	***Staphylococcus Aureus***	***Pseudomonas Aeruginosa***	***Streptococcus Pyogenes***
1	1a	11	15	09	10
2	1b	13	13	13	12
3	1c	12	14	16	13
4	1d	14	13	12	16
5	1e	11	14	11	10
6	1f	09	16	17	06
7	1g	16	10	13	08
8	2a	09	12	12	13
9	2b	12	11	13	16
10	2c	15	11	17	14
11	2d	17	15	15	18
12	2e	10	16	17	07
13	2f	12	13	20	09
14	2g	16	09	19	15
15	Standard	20	19	25	20

Table No.6: Antifungal activities of compounds (1a-2g)

S.No	Compound No.	Diameter of growth inhibition zone (mm)			
		Aspergillus Fumigates	**Aspergillus Flavus**	**Penicillium Marneffei**	**Candida Albicans**
1	1a	13	09	12	15
2	1b	08	13	09	10
3	1c	10	09	13	08
4	1d	14	12	11	09
5	1e	11	15	12	12
6	1f	12	11	15	16
7	1g	07	09	10	10
8	2a	12	14	13	16
9	2b	17	15	12	18
10	2c	08	11	15	16
11	2d	18	16	14	09
12	2e	13	12	16	17
13	2f	11	11	13	16
14	2g	16	17	14	09
15	Standard	20	19	18	20



(1a-1g) **(2a-2g)**

Figure No.1: Synthesis of chalcones

Where R= H, Cl, F, Br, NO_2, CH_3, OCH_3.

CONCLUSION

In present study, a series of 2, 4-disubstituted-1,5-benzodiazepine derivatives were synthesized and evaluated for antimicrobial and antifungal properties The results of antimicrobial studies have shown that all compounds were found to possess antimicrobial activity against all tested micro-organisms. Results have shown that all the compounds possess mild antifungal activity against all tested strains.

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