International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 2, 2011

Research Article

SYNTHESIS AND ANTIMICROBIAL STUDY OF SOME NOVEL 2,4-DISUBSTITUTED-1,5-BENZODIAZEPINE DERIVATIVES

SATISH B. JADHAV*

P. G. Department of Chemistry, Milind College of Science, Aurangabad-431001, Maharashtra, India. Email: orgchem.jadhav@yahoo.co.in

Received: 01 Jan 2011, Revised and Accepted: 02 Feb 2011

ABSTRACT

A new 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''-methoxynapthaline)-2-propene-1-one under microwave irradiation. The structures of newly synthesized compounds were confirmed by IR, ^{1}H 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, Antifungal activities

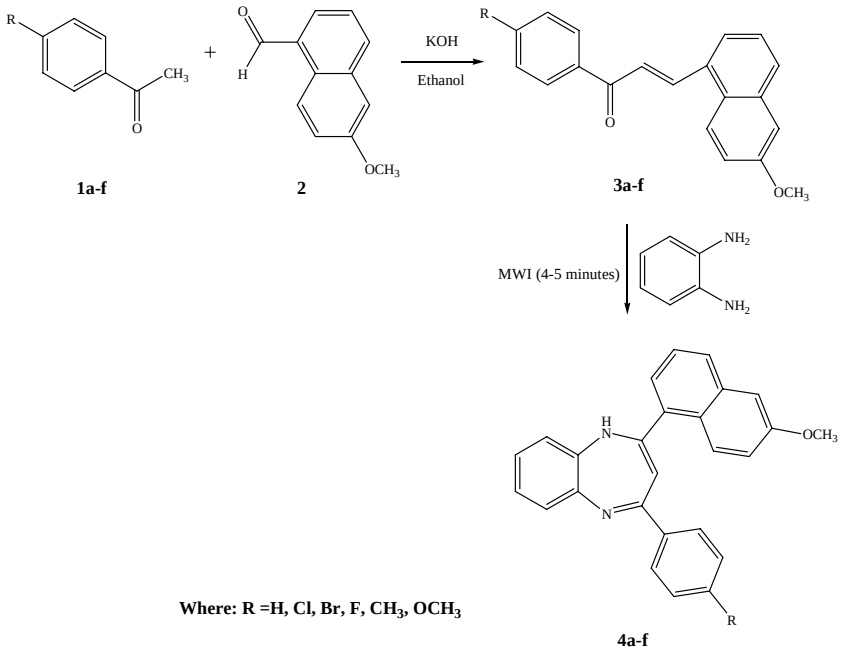
INTRODUCTION

Heterocyclic chemistry is a branch which is inseparable from mankind because human are totally dependent on the drugs derives from heterocyclic rings. Much attention has paid to the synthesis of nitrogen containing heterocyclic compounds like benzodiazepines, mainly due to their broad spectrum biological and pharmacological activities.

1,5-benzodiazepines have recently attracted attention as an important class of heterocyclic compounds in the field of drugs and pharmaceuticals. These compounds are widely used as anticonvulsant, antianxiety, analgesic, sedative, antidepressive, hypnotic agents[1] as well as anti-inflammatory agents[2]. Other than their biological importance, benzodiazepine derivatives are also commercially used as dyes for acrylic fibres[3]. Moreover, 1,5-benzodiazepine derivatives are valuable synthons that can be used in the preparation of other fused ring compounds such as triazolo, oxadiazolo, oxazino or furano-benzodiazepines[4]. As a result, research in this area is still very active and is directed towards the synthesis of compounds with enhanced pharmacological activity.

Generally, benzodiazepines were synthesized by the condensation of *o*-phenylenediamines with α,β-unsaturated carbonyl compounds[5], β-haloketones, or ketones[6]. A variety of reagents, such as BF_3-etherate[7], $NaBH_4$[8], polyphosporic acid[9], SiO_2, $MgO/POCl_3$[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[13,14], are utilized for condensation reactions. Most recently, this condensation has also been reported to proceed in the presence of CAN, (bromodimethyl)-sulfonium bromide, organic acids and $AgNO_3$. However, all of these methods have the common disadvantage of employing drastic reaction conditions and also producing several side-products. The exploitation of microwaves **(MW)** for assisting different organic solvents has blossomed into an important tool in the synthetic organic chemistry with large horizon of application. Due to the timeless ease of workability and eco-friendliness, MW provides an alternative to environmentally unacceptable procedures, which may be time-consuming or use toxic and expensive reagents. Considering the scope for further studies on diazepine derivatives and timeless ease of workability and eco-friendliness of MW, we have synthesized some new 2,4-disubstituted-1,5-benzodiazepin derivatives by microwave irradiation.

SCHEME-1



1a-f **2** **3a-f**

Where: R =H, Cl, Br, F, CH_3, OCH_3

4a-f

MATERIALS AND METHOD

Experimental

Melting points (m.p.) were determined in open capillary tube and are uncorrected. IR spectra were recorded using a Perkin-Elmer 1600FT spectrometer at a ca. 5-15% solution in DMSO or $CDCl_3$ (TMS as internal standard), ^{1}H-NMR spectra were recorded in $CDCl_3$ and in DMSO on a BRUKER (400 MHz) spectrometer using TMS as internal standard. Microwave reaction were carried out using LG555f multipower microwave oven operating at 2450 MHz frequency. Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapors or by irradiation with ultraviolet lights (254nm).

General procedure for the synthesis of chalcones[15] (3a-f)

A mixture of substituted acetophenones (0.01 mole) and 6-methoxy napthaldehyde (0.01mole) was stirred in methanol (50 mL) and then a solution of 15 mL potassium hydroxide (0.02 mole) was added to it. The mixture was kept over night at room temperature and then it was poured into crushed ice and acidified with dil. HCl. The chalcone derivatives precipitate out as solid (Scheme-I). The obtained solid was filtered, washed with water, dried and purified by recrystalization from acetic acid. The physical data listed in Table-1.

3-(6''-methoxynaphthalen-5-yl)-1-phenylprop-2-en-1-one (3a):

Anal. Calc. for ($C_{20}H_{16}O_2$); C, 83.31; H, 5.59; found: C, 83.00; H, 5.54 %; ^{1}H NMR (DMSO, 400 MHz), δ 8.0-7.3 (m, 11H, Ar-H), 7.2-7.0 (dd, 1H, >C=CH_B), 6.90-6.85 (dd, 1H, CH_A=C<), 3.80 (s, 3H, OCH_3), IR (KBr pellets Cm^{-1}): 1650 (>C=O),1620 (CH=CH), 1150 (OCH_3).

1-(4'-chlorophenyl)-3-(6''-methoxynaphthalen-5-yl)prop-2-en-1-one (3b):

Anal. Calc. for ($C_{20}H_{15}ClO_2$); C, 74.42; H, 4.68; found: C, 74.40; H, 4.65 %; ^{1}H NMR (DMSO, 400 MHz), δ 7.8-7.2 (m, 10H, Ar-H), 7.0-6.8 (dd, 1H, >C=CH_B), 6.70-6.60 (dd, 1H, CH_A=C<), 3.85 (s, 3H, OCH_3), IR (KBr pellets Cm^{-1}): 1665 (>C=O), 1620 (CH=CH), 1160 (OCH_3), 840 (C-Cl).

1-(4'-bromophenyl)-3-(6''-methoxynaphthalen-5-yl)prop-2-en-1-one (3c):

Anal. Calc. for ($C_{20}H_{15}BrO_2$); C, 65.41; H, 4.12; found: C, 65.35; H, 4.08 %; ^{1}H NMR (DMSO, 400 MHz), δ 7.9-7.2 (m, 10H, Ar-H), 7.0-6.85 (dd, 1H, >C=CH_B), 6.75-6.65 (dd, 1H, CH_A=C<), 3.82 (s, 3H, OCH_3), IR (KBr pellets Cm^{-1}): 1660 (>C=O),1635 (CH=CH), 1155 (OCH_3), 586 (C-Br).

1-(4'-fluorophenyl)-3-(6''-methoxynaphthalen-5-yl)prop-2-en-1-one (3d):

Anal. Calc. for ($C_{20}H_{15}FO_2$); C, 78.42; H, 4.94; found: C, 78.40; H, 4.85 %; ^{1}H NMR (DMSO, 400 MHz), δ 7.8-7.0 (m, 10H, Ar-H), 6.95-6.85 (dd, 1H, >C=CH_B), 6.70-6.60 (dd, 1H, CH_A=C<), 3.80 (s, 3H, OCH_3), IR (KBr pellets Cm^{-1}): 1650 (>C=O),1630 (CH=CH), 1150 (OCH_3), 1236 (C-F).

1-(4'-methylphenyl)-3-(6''-methoxynaphthalen-5-yl)prop-2-en-1-one (3e):

Anal. Calc. for ($C_{21}H_{18}O_2$); C, 83.42; H, 6.00; found: C, 83.39; H, 5.85 %; ^{1}H NMR (DMSO, 400 MHz), δ 8.0-7.2 (m, 10H, Ar-H), 7.1-6.95 (dd, 1H, >C=CH_B), 6.75-6.60 (dd, 1H, CH_A=C<), 3.85 (s, 3H, OCH_3), 2.35 (s, 3H, Ar-CH_3), IR (KBr pellets Cm^{-1}): 1665 (>C=O), 1635 (CH=CH), 1160 (OCH_3).

1-(4'-methoxyphenyl)-3-(6''-methoxynaphthalen-5-yl)prop-2-en-1-one (3f):

Anal. Calc. for ($C_{21}H_{18}O_3$); C, 79.22; H, 5.70; found: C, 79.17; H, 5.65 %; ^{1}H NMR (DMSO, 400 MHz), δ 8.2-7.0 (m, 10H, Ar-H), 7.1-6.90 (dd, 1H, >C=CH_B), 6.75-6.65 (dd, 1H, CH_A=C<), 3.90 (s, 3H, 2xOCH_3), IR (KBr pellets Cm^{-1}): 1670 (>C=O),1640 (CH=CH), 1170 (OCH_3).

General procedure for the synthesis of 2,4-disubstituted-1,5-benzodiazepines[16] (4a-f)

A mixture of chalcone (0.01 mole), *o*-phenylenediamine (0.01 mole) and glacial acetic acid 5 ml in DMF (15 ml) was added in a conical flask and placed in a microwave oven and irradiated for 4-5 min. with intermittent cooling at every half minute interval. The cooling was necessary to avoid the loss of product by evaporation. The reaction mixture was allowed to attain room temperature and treated with the cold water. The solid separated was filtered, washed with water and purified by recrystallised from alcohol. The physical data listed in Table-1.

2-(6''-methoxynaphthalen-5-yl)-4-phenyl-1H-benzodiazepine (4a):

Anal. Calc. for ($C_{26}H_{20}N_2O$); C, 82.95; H, 5.35; found: C, 82.90; H, 5.30 %; ^{1}H NMR (DMSO, 400 MHz), δ 7.93-7.76 (m, 5H, Ar-H), 7.75-7.65 (m, 4H, Ar-H), 7.50-7.47 (dd, 1H, J= 1.6 Hz, Ar-H), 7.40-7.25 (m, 4H, Ar-H), 7.16-7.14 (dd, 1H, J= 2.0 Hz, Ar-H), 4.2 (s, 1H, HC=C-Ar), 3.80 (s, 3H, OCH_3). IR (KBr pellets Cm^{-1}): 3316 (NH), 1660 (C=N, benzodiazepine), 1613 (CH=CH), 1150 (OCH_3).

4-(4'-chlorophenyl)-2-(6''-methoxynaphthalen-5-yl)-1H-benzodiazepine (4b):

Anal. Calc. for ($C_{26}H_{19}ClN_2O$); C, 76.00; H, 4.66; found: C, 75.96; H, 4.62; %; ^{1}H NMR (DMSO, 400 MHz), δ 7.87-7.70 (m, 4H, Ar-H), 7.68-7.58 (m, 4H, Ar-H), 7.54-7.51 (dd, 1H, J= 1.8 Hz, Ar-H), 7.45-7.30 (m, 4H, Ar-H), 7.25-7.26 (dd, 1H, J= 2.8 Hz, Ar-H), 4.3 (s, 1H, HC=C-Ar), 3.85 (s, 3H, OCH_3). IR (KBr pellets Cm^{-1}): 3310 (NH), 1655 (C=N, benzodiazepine), 1610 (CH=CH), 1140 (OCH_3), 845 (C-Cl).

4-(4'-bromophenyl)-2-(6''-methoxynaphthalen-5-yl)-1H-benzodiazepine (4c):

Anal. Calc. for ($C_{26}H_{19}BrN_2O$); C, 68.58; H, 4.21; found: C, 68.55; H, 4.15 %; ^{1}H NMR (DMSO, 400 MHz), δ 7.85-7.71 (m, 4H, Ar-H), 7.70-7.60 (m, 4H, Ar-H), 7.54-7.51 (dd, 1H, J= 1.6 Hz, Ar-H), 7.40-7.25 (m, 4H, Ar-H), 7.22-7.25 (dd, 1H, J= 2.6 Hz, Ar-H), 4.3 (s, 1H, HC=C-Ar), 3.82 (s, 3H, OCH_3). IR (KBr pellets Cm^{-1}): 3316 (NH), 1665 (C=N, benzodiazepine), 1620 (CH=CH), 1155 (OCH_3), 582 (C-Br).

4-(4'-fluorophenyl)-2-(6''-methoxynaphthalen-5-yl)-1H-benzodiazepine (4d):

Anal. Calc. for ($C_{26}H_{19}FN_2O$); C, 79.17; H, 4.86; found: C, 79.12; H, 4.84 %; ^{1}H NMR (DMSO, 400 MHz), δ 7.86-7.69 (m, 4H, Ar-H), 7.68-7.57 (m, 4H, Ar-H), 7.52-7.49 (dd, 1H, J= 1.8 Hz, Ar-H), 7.45-7.30 (m, 4H, Ar-H), 7.24-7.22 (dd, 1H, J= 2.4 Hz, Ar-H), 4.4 (s, 1H, HC=C-Ar), 3.80 (s, 3H, OCH_3). IR (KBr pellets Cm^{-1}): 3300 (NH), 1655 (C=N, benzodiazepine), 1615 (CH=CH), 1150 (OCH_3), 1230 (C-F).

4-(4'-methylphenyl)-2-(6''-methoxynaphthalen-5-yl)-1H-benzodiazepine (4e):

Anal. Calc. for ($C_{27}H_{22}N_2O$); C, 83.05; H, 5.68; found: C, 82.97; H, 5.63 %; ^{1}H NMR (DMSO, 400 MHz), δ 7.80-7.75 (m, 4H, Ar-H), 7.65-7.54 (m, 4H, Ar-H), 7.49-7.47 (dd, 1H, J= 1.6 Hz, Ar-H), 7.37-7.27 (m, 4H, Ar-H), 7.18-7.17 (dd, 1H, J= 2.8 Hz, Ar-H), 4.4 (s, 1H, HC=C-Ar), 3.82 (s, 3H, OCH_3), 2.40 (s, 3H, Ar-CH_3). IR (KBr pellets Cm^{-1}): 3310 (NH), 1660 (C=N, benzodiazepine), 1613 (CH=CH), 1150 (OCH_3).

4-(4'-methoxyphenyl)-2-(6''-methoxynaphthalen-5-yl)-1H-benzodiazepine (4f):

Anal. Calc. for ($C_{27}H_{22}N_2O_2$); C, 79.78; H, 5.46; found: C, 79.75; H, 5.42 %; ^{1}H NMR (DMSO, 400 MHz), δ 7.76-7.71 (m, 4H, Ar-H), 7.64-7.53 (m, 4H, Ar-H), 7.52-7.49 (dd, 1H, J= 1.8 Hz, Ar-H), 7.37-7.27 (m, 4H, Ar-H), 7.14-7.13 (dd, 1H, J= 2.0 Hz, Ar-H), 4.6 (s, 1H, HC=C-Ar), 3.90 (s, 6H, 2xOCH_3). IR (KBr pellets Cm^{-1}): 3330 (NH), 1665 (C=N, benzodiazepine), 1620 (CH=CH), 1165 (OCH_3).

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[17, 18]. 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° C 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 2.

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 37° 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° 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 3.

Table 1: Melting points, yield percentage, molecular formulae, and molecular weights of compounds (3a-f) & (4a-f)

Compound	R	M.p (°C)	Yield (%)	Mol. formula (Mol. wt)
3a	H	143	82	$C_{20}H_{16}O_2$ (288.34)
3b	Cl	202	80	$C_{20}H_{15}ClO_2$ (367.24)
3c	Br	221	85	$C_{20}H_{15}BrO_2$ (367.24)
3d	F	158	70	$C_{20}H_{15}FO_2$ (306.33)
3e	CH_3	161	80	$C_{21}H_{18}O_2$ (302.37)
3f	OCH_3	191	82	$C_{21}H_{18}O_3$ (318.37)
4a	H	150	60	$C_{26}H_{20}N_2O$ (376.16)
4b	Cl	175	62	$C_{26}H_{19}ClN_2O$ (455.35)
4c	Br	182	65	$C_{26}H_{19}BrN_2O$ (455.35)
4d	F	168	62	$C_{26}H_{19}FN_2O$ (394.44)
4e	CH_3	145	65	$C_{27}H_{22}N_2O$ (390.48)
4f	OCH_3	160	58	$C_{27}H_{22}N_2O_2$ (406.48)

Table 2: Antibacterial activities of pyrazolines (4a-f)

Compd. no.	Diameter of growth inhibition zone (mm)			
	Escherichia coli	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Streptococcus pyogenes***
4a	10	12	08	09
4b	12	13	13	12
4c	13	12	13	15
4d	14	13	14	16
4e	11	14	11	07
4f	09	11	10	09
Standard	20	19	25	20

Table 3: Antifungal activities of pyrazolines (4a-f)

Compd no.	Diameter of growth inhibition zone (mm)			
	Aspergillus fumigatus	***Aspergillus flavus***	***Penicillium marneffei***	***Candida albicans***
4a	11	08	10	08
4b	08	13	07	09
4c	10	09	11	08
4d	13	12	13	14
4e	12	11	11	12
4f	07	09	11	12
Standard	20	16	18	20

RESULTS AND DISCUSSION

Literature survey reveals that synthesis of 2-(6''-methoxynaphthalen-5-yl)-4-phenyl-1H-benzodiazepine was not reported. Hence it was thought worthwhile to synthesize these compounds. The structures of the synthesized compounds (**4a-e**) were confirmed on the basis of spectral and elemental analysis. The formulas, melting point, yield of the compounds are listed in Table 1. Selected diagnostic bands of the IR spectra of **4a** showed useful information about the structure of the compound. It showed 1150 Cm^{-1} (-OCH_3) and C=N stretching band at 1660 cm^{-1} because of ring closure. The compound **4a** showed additional band appearing in the region 3316 cm^{-1} due to NH stretch. Further evidence for the formation of benzodiazepines (**4a**) was obtained by recording the mass spectra. The mass spectrum of compound **4a** showed a molecular ion peak at *m/z* 376.16 which is in conformity with the molecular formula $C_{26}H_{20}N_2O$. 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.

ACKNOWLEDGMENT

The author is thankful to the Head, Department of Chemistry, Milind College of Science, Aurangabad, for providing laboratory facilities.

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