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Synthesis, characterization and *in-vitro* anti-inflammatory, antimicrobial activities of some novel2,4-disubstituted-1,5-benzothiazepine derivatives

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ABSTRACT

In the present work somenew series of 2,4-disubstituted-1,5-benzodiazepine derivatives were synthesized by the condensation of 2-aminothiophenol and Piperidine in presence of ethanol and various 1-(4-substituted phenyl)-3-(6-methoxynapthaline)-2-propene-1-one. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, mass and elemental analysis. All the synthesized compounds were evaluated for anti-inflammatory (in-vitro) and antimicrobial activity.

Keywords: Chalcone, benzothiazepines, anti-inflammatory (*in-vitro*), antibacterial, antifungal activity.

INTRODUCTION

Heterocyclic chemistry is now a fast growing research field in chemistry. The chemistry of heterocyclic compounds has been an interesting field of study from long time. Because heterocyclic compounds have been associated with various biologically activities. Due to bioactivity connected with heterocycle and ease of preparation, a number of researchers are takings keen interested into the study of heterocyclic compounds.

Many heterocyclic compounds due to their specific activity are employed in the treatment of many infectious diseases. Their use in the treatment is attributed to their inherent toxicity to various pathogens. One very interesting and promising class of heterocycle is 2,4-disubstituted-1,5-benzodiazepine. This type of heterocycle constitute an interesting class, due to their synthetic versatility and effective significant pharmacological and biological activity such as antifeedent[1], coronary vasodilatory[2], tranquilizer[3], antidepressant[4], CNS stimulant[5], calcium channel blocker[6], antiulcer[7], anti-HIV[8], antifungal[9], antimicrobial[10]and for treatment of smallpox[11]. Benzothiazepine derivatives have been reported to be more potent selective inhibitors ofthe mitochondrial Na+–Ca2+ exchangers[12].

Literature survey reveals several synthetic protocols for the synthesis of these compounds, and the presence of this core in any molecule plays a key role in enhancing the activity. Phenyl ring containing halogen[13] and methoxy groups have shown significant biological activities or enhance the biological activities of 2,4-disubstituted-1,5-benzodiazepinederivatives drastically.

Owing to the biological significance of these classes of compounds and in continuation of our ongoing study on heterocyclic compounds and anti-inflammatory, antimicrobial agents[14], we planned to synthesize a series of some novel 2,4-disubstituted-1,5-benzodiazepine derivatives. In this present study various 1-(4-substituted phenyl)-3-(6-methoxynapthaline)-2-propene-1-one (Chalcones) **(3a-f),** were prepared by Claisen-Schmidt condensation of 4-substituted acetophenones with 6-methoxy-1-naphthaldehyde in presence of ethanol and KOH. The Chalcones were subjected to condensation reaction with 2-aminothiophenol in ethanol under the influence of Piperidine and acetic acid to produce 2-(6-methoxynaphthalen-1-yl)-4-phenyl-2,3-dihydrobenzothiazepine

derivatives **(4a-f)(Scheme-I).** The structures of all synthesized compounds were assigned on the basis of IR, Mass, ^{1}H NMR spectral data and elemental analysis. Further these compounds were subjected for anti-inflammatory (*in-vitro*), antifungal and antibacterial activity.

MATERIALS AND METHODS

Experimental

Melting points were determined in open capillaries and are uncorrected.IR spectra were recorded using Perkin – Elmer spectrometer.^{1}H NMR spectra were recorded on Brucker Advance II 400 spectrometer in DMSO by using TMS as internal standard. Thin layer chromatography was performed with E. Merk precoated TLC plates, silica gel $60F_{254}$ with thickness of 0.25mm and spots were visualized by irradiation with ultraviolet light (254 nm).

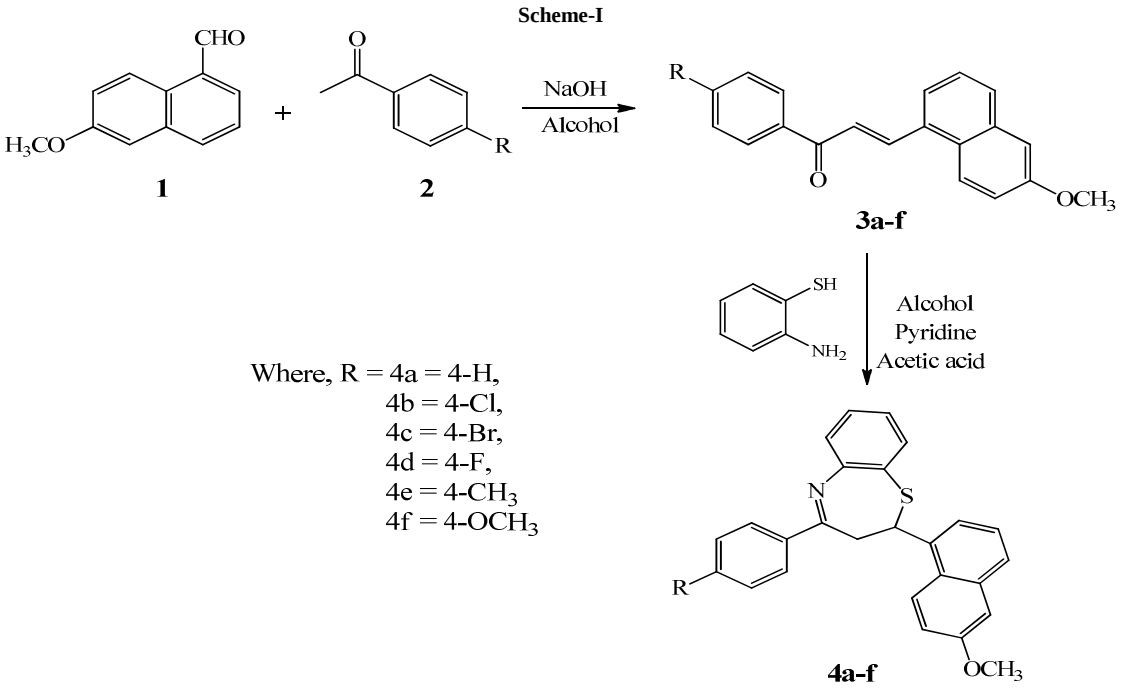
Synthesis of 1-(4-substituted phenyl)-3-(6-methoxynapthaline)-2-propene-1-one (Chalcone)[15](3a-f).

A mixture of 4-substituted acetophenones (0.01 mole) and 6-methoxy-1-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 overnight at room temperature and then it was poured into crushed ice and acidified with dil. HCl. The chalcone derivatives precipitate out as solid.The obtained solid was filtered, washed with water, dried and purified by recrystallization from acetic acid.

Synthesis of 2,4-disubstituted-1,5-benzodiazepine derivatives[16] (4a-f).

To a mixture of Chalcone (0.01 mole) and *o*-aminothiophenol (0.11 mole) in 50 ml ethanol, further 2-3 ml of Piperidine were added. The mixture was refluxed for 5-6 hours. Then this reaction mixture was acidified by 10 ml glacial acetic acid and further refluxed for 2 hours. On cooling solid compound obtained was recrystallized from aq. acetic acid purity of the compound was monitored by TLC.

Scheme-I



RESULTS AND DISCUSSION

Literature survey reveals that synthesis of2-(6-methoxynaphthalen-1-yl)-4-phenyl-2,3-dihydrobenzothiazepine derivatives **(4a-f)** was not reported. Hence it was thought worthwhile to synthesize these compounds. The structures of the synthesized compounds (**4a-f**) were confirmed on the basis of spectral and elemental analysis. Selected diagnostic band of the IR spectra of **(3a-f)** showed useful information about the structure of the compounds. It showed 1622 Cm^{-1} (-C=N str. in benzothiazepine) and 858 Cm^{-1} (C-S-C str.), because of ring closure.

The ^{1}H NMR spectra of **(4a-f)** showed similar patterns of signals, they displayed doublet of doublet (dd) for two protons and triplet (t) for one proton. The methine proton of thiazepine shows a triplet at δ 2.50 with coupling constant nearly 7.85Hz. the two methylene protons shows two signals, one doublet of doublet at around δ 3.85 with coupling constants of nearly 13Hz & 4.8Hz, similarly another doublet of doublet at around δ 5.40 with coupling

constant of nearly 10.4Hz & 5Hz. The synthetic pathway followed for the synthesis of the title compounds is described in **Scheme-I**.

Spectral data of compounds

3-(-6-methoxynaphthalen5yl)-1-phenylprop-2-en-1-one (3a):
Yield 82%; m.p. 143°C: Elemental analysis Calcd 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 (KBrpellets 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):
Yield 80%; m.p. 202°C: Elemental analysis Calcd 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 (KBrpellets 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):
Yield 85%; m.p. 221°C: Elemental analysis Calcd 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 (KBrpellets 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):
Yield 70%; m.p. 158 °C: Elemental analysis Calcd 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):
Yield 80%; m.p. 161°C: Elemental analysis Calcd 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):
Yield 82%; m.p. 191°C: Elemental analysis Calcd 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).

2-(6-methoxynaphthalen-1-yl)-4-phenyl-2,3-dihydrobenzothiazepine(4a):
Yield 80%; m.p. 141°C: Elemental analysis Calcd for($C_{26}H_{21}NOS$); C, 78.95; H, 5.35; N, 3.54; found:C, 78.85; H, 5.32; N, 3.50%; ^{1}H NMR (DMSO, 400 MHz), δ 2.52-2.50(t,1H, CH-Hx, J_{XA} = 2.60Hz, J_{XB}= 7.85Hz), 3.35 (s, 3H, OCH_3),3.85(dd, 1H, CH_2-H_A, J_{AX}= 4.8Hz, J_{AB}= 12.10 Hz), 5.40(dd, 1H, CH_2-H_B, J_{BX}= 5.04 Hz, J_{BA}= 10.40Hz), 8.58-6.40 (m, 15H, Ar-H), IR (KBr pellets Cm^{-1}): 3009 cm^{-1} (Ar-C-H str.), 1622 cm^{-1} (-C=N str.in benzothiazepine),1596cm^{-1} (C=Cstr. in Ar), 1478 & 1459 cm^{-1} (CH_2bend.), 1198 (OCH_3).

4-(4-chlorophenyl)-2-(6-methoxynaphthalen-1-yl)-2,3-;dihydrobenzothiazepine(4b):
Yield 85%; m.p. 152°C: Elemental analysis Calcd for ($C_{26}H_{20}ClNOS$);C, 72.63; H, 4.69; N, 3.26; found:C, 72.58; H, 4.65; N, 3.25%; ^{1}H NMR (DMSO, 400 MHz),), δ 2.50-2.47(t,1H, CH-Hx, J_{XA}= 2.60Hz, J_{XB} = 7.85Hz), 3.30 (s, 3H, OCH_3), 3.80(dd, 1H, CH_2-H_A, J_{AX}= 4.8Hz, J_{AB}= 12.10 Hz), 5.35(dd, 1H, CH_2-H_B, J_{BX}= 5.04 Hz, J_{BA}= 10.40Hz), 8.55-6.35 (m, 14H, Ar-H),IR (KBr pellets Cm^{-1}): 3025 cm^{-1} (Ar-C-H str.), 1620 cm^{-1} (-C=N str.in benzothiazepine),1595cm^{-1} (C=Cstr. in Ar), 1475 & 1455 cm^{-1} (CH_2bend.), 1190 (OCH_3).

4-(4-bromophenyl)-2-(6-methoxynaphthalen-1-yl)-2,3-dihydrobenzothiazepine (4c):
Yield 84%; m.p. 161°C: Elemental analysis Calcd for ($C_{26}H_{20}BrNOS$);C, 65.82; H, 4.25; N, 2.95; found:C, 65.80; H, 4.22; N, 2.88%; ^{1}H NMR (DMSO, 400 MHz),), δ 2.54-2.50(t,1H, CH-Hx, J_{XA} = 2.60Hz, J_{XB} = 7.85Hz), 3.32 (s, 3H, OCH_3), 3.75(dd, 1H, CH_2-H_A, J_{AX}= 4.8Hz, J_{AB}= 12.10 Hz), 5.30(dd, 1H, CH_2-H_B, J_{BX}= 5.04 Hz, J_{BA}= 10.40Hz), 8.58-6.40 (m, 14H, Ar-H),IR (KBr pellets Cm^{-1}): 3061 cm^{-1} (Ar-C-H str.), 1608 cm^{-1} (-C=N str.in benzothiazepine),1582cm^{-1} (C=Cstr. in Ar), 1470 & 1443 cm^{-1} (CH_2bend.), 1192 (OCH_3).

4-(4-fluorophenyl)-2-(6-methoxynaphthalen-1-yl)-2,3-dihydrobenzothiazepine (4d):
Yield 75%; m.p. 165°C: Elemental analysis Calcd for ($C_{26}H_{20}FNOS$);C, 75.52; H, 4.88; N, 3.39; found:C, 75.48; H, 4.85; N, 3.32%; ^{1}H NMR (DMSO, 400 MHz),), δ 2.52-2.50(t,1H, CH-Hx, J_{XA} = 2.60Hz, J_{XB} = 7.85Hz), 3.30 (s, 3H, OCH_3), 3.82(dd, 1H, CH_2-H_A, J_{AX}= 4.8Hz, J_{AB}= 12.10 Hz), 5.42(dd, 1H, CH_2-H_B, J_{BX}= 5.04 Hz, J_{BA}= 10.40Hz),

8.58-6.45 (m, 14H, Ar-H), IR (KBr pellets Cm^{-1}): 3035 cm^{-1} (Ar-C-H str.), 1635 cm^{-1} (-C=N str.in benzothiazepine),1580cm^{-1} (C=Cstr. in Ar), 1470 & 1453 cm^{-1} (CH_2bend.), 1190 (OCH_3).

4-(4-methylphenyl)-2-(6-methoxynaphthalen-1-yl)-2,3-dihydrobenzothiazepine (4e):
Yield 78%; m.p. 89^oC: Elemental analysis Calcd for ($C_{27}H_{23}NOS$);C, 79.18; H, 5.66; N, 3.42; found:C, 79.12; H, 5.62; N, 3.35%; ^1H NMR (DMSO, 400 MHz),), δ 2.35 (s, 3H, Ar-CH_3), 2.52-2.50(t,1H, CH-Hx, J_{XA} = 2.60Hz, J_{XB} = 7.85Hz), 3.35 (s, 3H, OCH_3), 3.85(dd, 1H, CH_2-H_A, J_{AX}= 4.8Hz, J_{AB}= 12.10 Hz), 5.40(dd, 1H, CH_2-H_B, J_{BX}= 5.04 Hz, J_{BA}= 10.40Hz), 8.55-6.42 (m, 14H, Ar-H), IR (KBr pellets Cm^{-1}): 3051 cm^{-1} (Ar-C-H str.), 1618 cm^{-1} (-C=N str.in benzothiazepine),1592cm^{-1} (C=Cstr. in Ar), 1480 & 1453 cm^{-1} (CH_2bend.), 1195 (OCH_3).

4-(4-methoxyphenyl)-2-(6-methoxynaphthalen-1-yl)-2,3-dihydrobenzothiazepine (4f):
Yield 84%; m.p. 154^oC: Elemental analysis Calcd for ($C_{27}H_{23}NO_2S$);C, 76.21; H, 5.45; N, 3.29; found:C, 76.15; H, 5.40; N, 3.25%; ^1H NMR (DMSO, 400 MHz),), δ 2.52-2.50(t,1H, CH-Hx, J_{XA} = 2.60Hz, J_{XB} = 7.85Hz), 3.45 (s, 3H, 2xOCH_3), 3.90(dd, 1H, CH_2-H_A, J_{AX}= 4.8Hz, J_{AB}= 12.10 Hz), 5.48(dd, 1H, CH_2-H_B, J_{BX}= 5.04 Hz, J_{BA}= 10.40Hz), 8.51-6.42 (m, 14H, Ar-H),IR (KBr pellets Cm^{-1}): 3060 cm^{-1} (Ar-C-H str.), 1610 cm^{-1} (-C=N str.in benzothiazepine),1580cm^{-1} (C=Cstr. in Ar), 1462 & 1442 cm^{-1} (CH_2bend.), 1170 (OCH_3).

Biological activity:

In-vitro anti-inflammatory activity[17,18]

The standard drug and synthesized compounds **(4a-h)** were dissolve in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different concentration of drugs was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at 27±1^{0}C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60±1 ^{0}C in water bath for 10 min. After cooling, the turbidity was measured at 660nm (UV-Visible Shimadzu Spectrophotometer). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The ibuprofen was used as standard drug. Results are tabulated in **table no. 1**

$$\% \text{ of inhibition} = \left(\frac{Vt}{Vc} - 1\right) X\ 100$$

Where, Vt = Mean absorbance value of test group.
Vc = Mean absorbance value of control group

Table 1 Anti-inflammatory activity of synthesized compounds (4a-h)

Sr. No.	Compounds	Mean absorbance value ± SEM	Inhibition of denaturation (in %)
1	Control	0.0850	-
2	Ibuprofen	0.162 ± 0.008	90.58
3	4a	0.120 ± 0.005	41.17
4	4b	0.142 ± 0.002	67.05
5	4c	0.136 ± 0.007	60.00
6	4d	0.146 ± 0.001	71.76
7	4e	0.105 ± 0.003	23.52
8	4f	0.112 ± 0.007	31.76

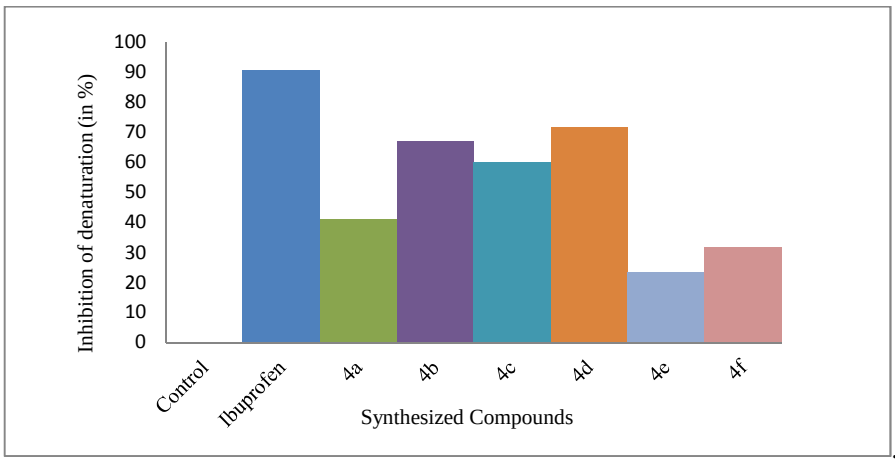


Fig. 1 Anti-inflammatory activity of the synthesized compounds

Antimicrobial activity

The newly synthesized compounds were screened for their antibacterial activity against*E.coli*, *Salmonella typhi*, *Staphylococcusaureus*and *Bacillus subtilis*by disc diffusion method[19,20]using penicillin as standard and antifungal activity against *Aspergillusniger*, *Aspergillusflavuspenicilliumchrysogenum*, *Fusariummoneliforme*, by poison plate method[21]using Griseofulvin as reference standard and DMSO as control solvent.The investigation of antibacterial screening results indicates that few of the compounds shows significant property and some of the compounds are moderately active. The investigation of antifungal activity data revealed that some compounds have promising and some showed no antifungal activity. The results are shown in **Table 2 and 3** respectively.

Table 2-Antibacterial screening results of the compounds 4a-h

Sr. No.	Compounds	*E.coli*	*Salmonella typhi*	*Staphylococcus aureus*	*Bacillus subtilis*
1	4a	14	09	11	12
2	4b	14	13	16	18
3	4c	16	16	23	18
4	4d	20	19	22	20
5	4e	10	14	13	11
6	4f	11	12	11	15
7	Penicillium	22	25	35	38
8	DMSO	-ve	-ve	-ve	-ve
	-ve no antibacterial activity				

Table 3 Antifungal screening results of the compounds 3a-h

Sr. No.	Compounds	*Aspergillus niger*	*Aspergillusflavus*	*Penicillumchrysogenum*	*Fusariummoneliforme*
1	4a	-ve	+ve	-ve	-ve
2	4b	-ve	-ve	RG	-ve
3	4c	RG	-ve	+ve	-ve
4	4d	-ve	-ve	-ve	-ve
5	4e	-ve	+ve	-ve	RG
6	4f	-ve	RG	-ve	-ve
9	Griseofulvin	-ve	-ve	-ve	-ve
10	DMSO	+ve	+ve	+ve	+ve
-ve	*No growth Antifungal activity present*				
+ve	*Growth Antifungal activity absent*				
RG	*Reduced growth*				

CONCLUSION

In summary it describes the synthesis of Chalcones and their 2,4-disubstituted-1,5-benzodiazepine derivatives along with their anti-inflammatory (*in-Vitro*) and antimicrobial activity (antibacterial and antifungal). The reaction completion was confirmed by TLC and the synthesized compounds were purified by recrystallization. The structures of the synthesized compounds were assigned on the basis of the spectral data (IR, ^{1}H NMR and MS). The antimicrobial activity of these compounds was evaluated against various Gram-positive, Gram-negative bacteria and fungi. Most of the compounds showed a moderate degree of antimicrobial activity. Compounds bearing pharmacophors such as fluoro, bromo and chloro exhibited significant anti-inflammatory and antimicrobial activity, similar methoxyl group substituent responsible for activities among the synthesized compounds. Among them, compounds **4b**, **4c**and **4d**were found to be significant anti-inflammatory and most active against all the microorganisms employed both for antibacterial and antifungal activity. The data reported in this article may be helpful guide for the medicinal chemist who is working in this area.

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REFERENCES

[1]Reddy J R, Ashok D, Sharma P N, *Indian Journal ofChem B*,**1993**, 32, 404-406.
[2]T Hashiyama, H Inoue, M Takeda, S Murtates, T Nagao, *Chem Pharm Bull*,**1985**,33,2348.
[3]Kugita H, Inoue H, Ikezaki M,*Jap. Pat.***1971**, 46016749; *Chem. Abstr*.**1971**, 75, 63848.
[4]H M Geyer, N Watzman, J P Buckeley, *J Pharmacol Sci*.**1970**, 59, 964-968.
[5]Kawashima co. Lt., *Jap. Pat*., **1985**,60056972 *chem. Abstr*.**1985**, 103, 105014.
[6]Masafumi Hagiwara,Satomi Adachi-Akahane, Taku Nagao, *Ind. J.Pharmacology*, **1997**, 281(1), 173-179.

[7]Ohno S, Izumi K,Mizukoshi K, Kato K,Hori M,*Chem. Pharm. Bull*. **1983**, 31,1780-1783.
[8]Yang X,Buzon L, Hamanaka E,Liu. K K-C, *Tetrahedron*, **2000**, 11, 4447-4450.
[9]DandiaA, SinghaR, KhaturiaS, *Cheminform*,**2007**, 38(36), 236.
[10] Nikalje A G, Mane R A, Ingle D B, *Indian Journal Heterocyclic Chemistry*, **2006**, 15(3), 309.
[11] Raju N K, Seniappan T S, Thangaraju S, Palathurai S M, *Heterocycles*,**2000**,52(2),357.
[12] Chiesi M,Schwaller R, Eichenberger K,*Biochem. Pharmacol.***1988**, 37, 4399-4403.
[13] Ghotekar D S, Joshi R S, Mandhane P G, Bhagat S S, Gill C H, *Indian Journal of Chemistry*, **2010**, 49B, 1267-1270.
[14] (a) SatishBabulalJadhav and Shantilal D. Rathod, *World Journal Of Chemistry And Pharmaceutical Science*, **2015**,4(09),1288-1297 ,
(b) Kishor V Gaikwad, Sandip V Gaikwad, Satish B Jadhav and Shantilal D Rathod, *Indian Journal of Chemistry*, **2010**, 49B, 131-136.
[15] Jadhav S B, Shastri R A, Gaikwad K V, Gaikwad S V, *E-journal of chemistry*, **2009**; 6 (S1), 183.
[16] SanjeevanGaikwad, VenkatSuryawanshi, KishanLohar**,** *Journal of Chemical, Biological and Physical Sciences*, **2013**, 3(2), 936-940.
[17] Gelias, M NRao, *Indian J. Exp. Biol*,**1988**, vol.26.
[18] Gajraj Sharma, Jayachandran E, Sreenivasa G M, *Ultra Chemistry*, **2008**, 4(1), 103, 144.
[19] Cruickshank R, Duguid J P, Marion B P, Swain R H A,*Twelfth ed. MedicinalMicrobiology, vol. II Churchill Livingstone*, London, **1975**, 196-202.
[20] Collins A H, (Ed), *Microbiological Method*, Second ed. Butterworth, London **1976**.
[21] Cruickshank R J, Duguid P, Swain R R,*Medicinal Microbiology* Vol. 1. PublishersChurchill Livingstone.