ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 4, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF SOME NOVEL 1,5 BENZODIAZEPINE DERIVATIVES

ISHWAR BHAT K, ABHISHEK KUMAR*

Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte University, Paneer, Deralakatte - 575 018, Mangalore, Karnataka, India. Email: abhi12bunty@gmail.com

Received: 14 September 2015, Revised and Accepted: 23 October 2015

ABSTRACT

Objective: The objective of the study is to synthesize some novel 1,5-benzodiazepine derivatives from chalcones. The structures of the newly synthesized compounds were characterized by elemental analysis, infrared, ^{1}H nuclear magnetic resonance, and mass spectroscopic studies. All titled compounds were screened for their anti-inflammatory activity.

Methods: In this study, a series of novel 2-(substituted phenyl)-3-styryl-2,3-dihydro-1*H*-benzo [b] [1,4] diazepine (1-12) has been synthesized from 1,5-(disubstituted phenyl)-2,4-pentadien-1-one (1a-12a). 1,5-(disubstituted phenyl)-2,4-pentadien-1-one was prepared by condensing cinnamaldehyde with various aromatic ketones in the presence of 20% NaOH as base. Different 1,5-(disubstituted phenyl)-2,4-pentadien-1-one on cyclisation with o-phenylene diamine in the presence of NaOH as base resulted in 2-(substituted phenyl)-3-styryl-2,3-dihydro-1*H*-benzo [b] [1,4] diazepine derivatives. The final synthesized benzodiazepine derivatives were screened for their anti-inflammatory activity using carrageenan-induced rat paw edema method.

Results: The compounds 4, 5, 7, 9, 10, 11, and 12 containing 4-nitrophenyl, 4-chlorophenyl, 3-nitro phenyl, 4-fluorophenyl, 4-bromophenyl, and 3-chlorophenyl benzodiazepine derivatives exhibited significant anti-inflammatory activity compared with the standard diclofenac sodium The presence of electron withdrawing groups such as nitro, chloro, fluoro, and bromo resulted in increased anti-inflammatory activity.

Conclusion: This study reports the successful synthesis of 1,5-benzodiazepine derivatives with moderate yields and most of the synthesized compounds showed significant anti-inflammatory activity.

Keywords: Chalcones, 1,5-benzodiazepines, Anti-inflammatory activity.

INTRODUCTION

Various derivatives of benzodiazepine constitute an important class of medicinal compound with diverse biological activities such as antimicrobial [1], hypnotic [2], anticonvulsant [3], analgesic [4], anti-inflammatory [5], anticancer [6], and neuroleptic [7]. Apart from their biological importance, benzodiazepines are valuable intermediates for the synthesis of fused ring compounds such as triazolo, thiazolo, imidazo, oxodiazolo, oxazino, furano, and pyrimido benzodiazepines. The intermediate compounds used for the synthesis of benzodiazepines are substituted chalcones known for their antimicrobial [8], anti-tubercular [9], anticancer [10], antimalarial, and anti-inflammatory [11] activities. Prompted by this literature herein is reported the synthesis of some novel derivatives of benzodiazepine derived from various substituted chalcones to improve the biological spectrum of benzodiazepines. All the novel compounds were evaluated for their anti-inflammatory activities.

METHODS

Melting points were determined in open capillary tubes and are uncorrected. The infrared (IR) spectra were recorded on Tensor 27 spectrophotometer, Bruker Optik (Germany) using a thin film support on KBr pellets. All ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker spectrophotometer AMX-400 (400 MHz) using $CDCl_3$ as solvent and tetramethylsilane as an internal standard. The mass spectra were recorded using a Jeol-D-300 mass spectrometer (70 eV), Shimadzu, Japan, by fast atom bombardment. The homogeneity of the compounds was checked on silica gel-G coated plate using petroleum ether:methanol (1:1) as solvent and iodine vapors as visualizing agent. All the synthesized compounds gave satisfactory elemental analysis and are recorded in Table 3.

General procedure

Synthesis of chalcone [12]

A mixture of cinnamaldehyde (0.01 mol) and substituted acetophenones (0.01 mol) in absolute ethanol (20 ml) was stirred together for 24 hrs in the presence of 40% NaOH (4-5 ml). The reaction mixture was poured onto crushed ice and acidified with HCl. The separated solid was filtered, washed with water, and recrystallized from ethanol.

2-(substituted phenyl)-3-styryl-2,3-dihydro-1H-benzo [b] [1,4] diazepine (1-12) [13]

A mixture of substituted 1,5-(disubstituted phenyl)-2,4-pentadien-1-one (1a-12a) (0.01 mol) and o-phenylene diamine (0.01 mol) was dissolved in absolute ethanol (30 ml) in the presence of 20% NaOH, and the reaction mixture was refluxed for about 10 hrs. After completion of the reaction, the reaction mixture was poured into crushed ice. The product obtained was filtered, washed with cold water. The compounds were obtained as yellow, brown, and dark brown crystals and it was recrystallized from ethanol (Fig. 1).

Spectral data

1a: IR (cm^{-1}): 1510 (Ar C=C str), 3115 (Ar C-H bending), 1694 (C=O str), 2905(aliphatic C-H str), 1651 (aliphatic C=C str);^{1}H NMR ($CDCl_3$): 7.4-7.9 (m, 10H, Ar-H), 7.0 (1H, d, J=14.9 Hz, =CH), 6.9 (1H, d, J=14.8 Hz, =CH), 7.1 (1H, d, J=15.0 Hz, =CH), 7.2 (1H, d, J=15.1 Hz, =CH); Mass: m/z 234 (M^+).

1: IR (cm^{-1}): 1652 (Ar C=C str), 3080 (Ar C-H str), 3352 (N-H str of benzodiazepine), 720(Ar C-H bending), 1569 (C=N str), 1237 (C-N str), 1560 (aliphatic C=C str), 2917 (aliphatic C-H str); ^{1}H NMR ($CDCl_3$):

7.2-7.4 (m, 14H, Ar-H), 3.0, 3.4, 4.9 (1H, d, J=14.5, 14.9, 15.4 Hz, 3H of benzodiazepine), 7.7 (1H, s, NH), 7.1 (1H, d, J=15.4 Hz, =CH), 7.3 (1H, d, J=15.1 Hz, =CH); Mass: m/z 324 (M^+).

2: IR (cm^{-1}): 1649 (Ar C=C str), 3075 (Ar C-H str), 3349 (N-H str of benzodiazepine), 720 (Ar C-H bending), 1561 (C=N str), 1232(C-N str), 3308 (N-H str), 2910(aliphatic C-H str), 1560 (aliphatic C=C str); ^{1}H NMR ($CDCl_3$): 7.1-7.6 (m, 13H, Ar-H), 3.1, 3.5, 4.8 (1H, d, J=14.2, 14.5, 15.2 Hz, 3H of benzodiazepine), 7.6 (1H, s, NH), 7.1 (1H, d, J=15.3 Hz, =CH), 7.2 (1H, d, J=15.4 Hz, =CH); Mass: m/z 358 (M^+).

7: IR (cm^{-1}): 1672 (Ar C=C str), 3059 (Ar C-H str), 3328 (N-H str of benzodiazepine), 720 (Ar C-H bending), 1560 (C=N str), 1228 (C-N str), 1548 (aliphatic C=C str), 2902 (aliphatic C-H str), 1540, 1345 (N=O str of NO_2); ^{1}H NMR ($CDCl_3$): 7.4-7.8 (m, 13H, Ar-H), 2.9, 3.2, 4.9 (1H, d, J=14.3, 14.6, 15.2 Hz, 3H of benzodiazepine), 7.6 (1H, s, NH), 7.1 (1H, d, J=15.2 Hz, =CH), 7.3 (1H, d, J=15.1Hz, =CH); Mass: m/z 369 (M^+).

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Table 1: Physical data of the newly synthesized benzodiazepine derivatives (1-12)

Compound No.	R (Ar-$COCH_3$)	Molecular formula	Melting point (°C)	R_f value	Yield (%)
1	H	$C_{23}H_{20}N_2$	92-94	0.52	78
2	4-OCH_3	$C_{24}H_{22}N_2O$	130-132	0.57	86
3	4-CH_3	$C_{24}H_{22}N_2$	152-154	0.68	74
4	4-NO_2	$C_{24}H_{19}N_3O_2$	143-145	0.62	84
5	4- Cl	$C_{23}H_{19}ClN_2$	110-112	0.60	83
6	4-NH_2	$C_{23}H_{21}N_3$	127-129	0.70	64
7	3-NO_2	$C_{24}H_{19}N_3O_2$	129-131	0.56	76
8	3-NH_2	$C_{23}H_{21}N_3$	137-139	0.58	61
9	4-F	$C_{23}H_{19}FN_2$	108-110	0.72	82
10	2-Br	$C_{23}H_{19}BrN_2$	115-117	0.48	73
11	2,4-Cl	$C_{23}H_{18}Cl_2N_2$	144-146	0.66	73
12	3,4-Cl	$C_{23}H_{18}Cl_2N_2$	149-152	0.74	85

12: IR (cm^{-1}): 1655(Ar C=C str), 3028 (Ar C-H str), 3286 (N-H str of benzodiazepine), 725 (Ar C-H bending), 1538 (C=N str), 1220 (C-N str), 1532 (aliphatic C=C str), 2878 (aliphatic C-H str), 763 (C-Cl str); ^{1}H NMR ($CDCl_3$): 7.6-7.9 (m, 12H, Ar-H), 3.1, 3.4, 5.4 (1H, d, J=14.8, 15.3, 15.6 Hz, 3H of benzodiazepine), 8.1 (1H, s, NH), 7.2 (1H, d, J=15.3 Hz, =CH), 7.3 (1H, d, J=15.6 Hz, =CH); Mass: m/z 394 (M^+).

Anti-inflammatory activity

The anti-inflammatory activities of the test compounds were carried out using carrageenan induced rat paw edema method [14] according to Winter *et al*. by employing 1% carrageenan solution as a phlogistic agent. All the experiments were carried out as per the rules and regulations of Institutional Animal Ethics Committee. (Animal Ethics Committee, K.S. Hedge Medical Academy, Deralakatte, Mangalore - 575 018). Edema was induced in the left hind paw of Wistar rats (150-200 g) of either sex by the subplantar injection of 0.1 ml of 1% carrageenan in distilled water. Each group composed of six animals. The animals which were bred in our laboratory were housed under standard conditions and received a diet of commercial food pellets and water ad libitum during the maintenance, but they entirely fasted during the experiment period. Our studies were conducted in accordance with recognized guidelines on animal experimentation. The test compounds were given intraperitoneally 30 minutes after carrageenan injection. Diclofenac sodium was taken as the standard at a dose of 10 mg/kg body weight. The rat paw volume was measured after 1, 2, 3 and 4 hrs, respectively, after carrageenan injection by plethysmometer. The difference between the paw volume at 4 hrs and 0 hr measurement was calculated and taken as edema volume. Percentage inhibition of the paw edema was calculated by using the formula:

% Edema inhibition = 100 $(1-V_t/V_c)$, where V_t represents mean increase in paw volume of test and V_c represents mean increase in paw volume of control.

Statistical analysis

All the experimental groups were composed of six animals. Data obtained from animal experiments were expressed as mean ±

Table 2: Anti-inflammatory activity of compounds (1-12) by Carrageenan-induced paw edema in rats

Treatment	Dose mg/kg	Increase in the paw edema volume (ml)			
		1 hr	2 hrs	3 hrs	4 hrs
Control	Vehicle	0.44±0.01	0.74±0.01	0.81±0.01	0.92±0.03
Diclofenac sodium	10	0.17±0.01** (58.1)	0.31±0.02** (56.15)	0.42±0.01** (50.62)	0.51±0.05** (46.81)
1	50	0.32±0.01 (39.4)	0.35±0.03 (35.23)	0.51±0.01 (33.28)	0.59±0.01 (31.93)
2	50	0.28±0.01 (39.12)	0.48±0.03 (35.62)	0.48±0.02 (33.82)	0.61±0.02 (32.61)
3	50	0.30±0.01 (38.4)	0.35±0.03 (35.23)	0.49±0.01 (34.28)	0.54±0.02 (40.91)
4	50	0.22±0.01** (50)	0.38±0.01** (46.5)	0.49±0.02** (40.72)	0.56±0.03** (41.49)
5	50	0.27±0.02** (41.2)	0.45±0.01** (38.36)	0.52±0.02** (37.10)	0.55±0.01** (42.52)
6	50	0.28±0.01 (39.41)	0.47±0.03 (36.61)	0.47±0.02 (33.56)	0.60±0.02 (32.21)
7	50	0.23±0.01** (50.1)	0.40±0.01** (46.6)	0.48±0.02** (40.72)	0.56±0.03** (41.43)
8	50	0.31±0.01 (38.5)	0.36±0.03 (35.41)	0.52±0.01 (33.26)	0.57±0.01 (31.65)
9	50	0.26±0.02** (46.9)	0.41±0.01** (43.9)	0.47±0.01** (43.6)	0.53±0.04** (43.21)
10	50	0.21±0.02** (54.32)	0.45±0.01* (39.98)	0.49±0.01* (38.51)	0.57±0.01* (38.18)
11	50	0.26±0.02** (42.3)	0.44±0.01** (38.46)	0.51±0.02** (37.41)	0.53±0.01** (42.42)
12	50	0.22±0.01** (50.2)	0.41±0.01** (46.5)	0.47±0.02** (40.61)	0.54±0.03** (41.52)

All values are expressed as mean±SEM (n=6). *p<0.05 significant compared to control, **p<0.01 significant compared to control. SEM: Standard error of mean

Table 3: Elemental analysis of benzodiazepine derivatives (1-12)

Compound	Molecular formula	Elemental analysis found (calculated) %		
		C	H	N
1	$C_{23}H_{20}N_2$	85.12 (85.18)	6.18 (6.17)	8.62 (8.64)
2	$C_{24}H_{22}N_{20}$	81.36 (81.35)	6.20 (6.21)	7.92 (7.91)
3	$C_{24}H_{22}N_2$	85.22 (85.21)	6.50 (6.51)	8.27 (8.28)
4	$C_{24}H_{19}N_3O_2$	74.77 (74.79)	5.10 (5.14)	11.39 (11.38)
5	$C_{23}H_{19}ClN_2$	77.02 (77.1)	5.32 (5.31)	7.81 (7.82)
6	$C_{23}H_{21}N_3$	81.43 (81.42)	6.18 (6.19)	8.25 (8.26)
7	$C_{24}H_{19}N_3O_2$	74.81 (74.80)	5.14 (5.15)	11.39 (11.38)
8	$C_{23}H_{21}N_3$	81.43 (81.42)	6.20 (6.19)	12.40 (12.39)
9	$C_{23}H_{19}FN_2$	80.71 (80.70)	5.57 (5.56)	8.20 (8.19)
10	$C_{23}H_{19}BrN_2$	68.65 (68.66)	4.72 (4.73)	6.98 (6.97)
11	$C_{23}H_{18}Cl_2N_2$	70.21 (70.23)	4.59 (4.58)	7.11 (7.12)
12	$C_{23}H_{18}Cl_2N_2$	70.21 (70.23)	4.59 (4.58)	7.11 (7.12)

Elemental analysis was carried out using Vario Elementar Model, C, H, N analyzer Instrument at the Department of Chemistry, Mangalore University, Karnataka

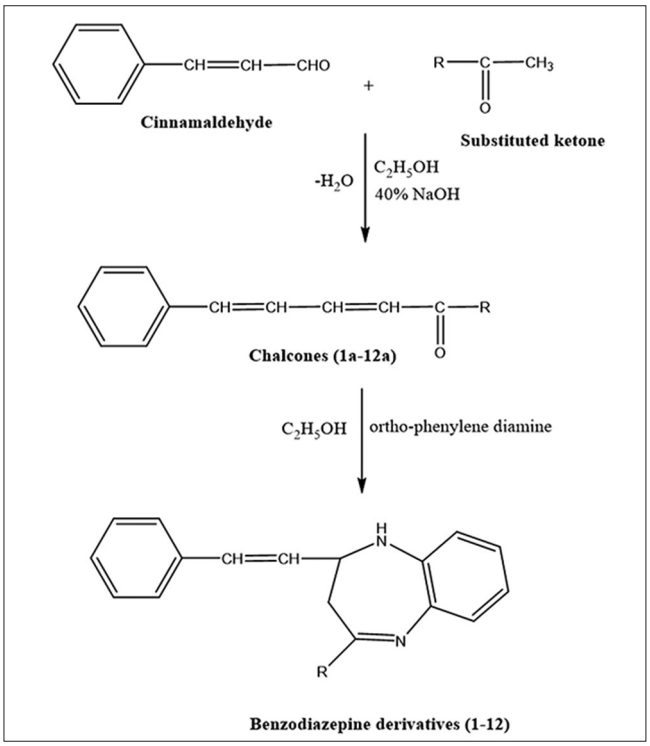

Fig. 1: Scheme for benzodiazepine derivatives

standard error of mean (SEM). The statistical significance of difference between groups was assessed using analysis of variance followed by Dunnett's test. The results of the anti-inflammatory studies are given in Table 2.

RESULTS AND DISCUSSION

Chemistry

Different substituted 1,5-(disubstituted phenyl)-2,4-pentadien-1-one were prepared by the reaction of cinnamaldehyde and substituted acetophenones in the presence of 20% NaOH as base. These compounds were characterized by TLC, melting point, and IR spectra that showed characteristic absorption band at 1650/cm of CH=CH group. The title compounds 2-(substituted phenyl)-3-styryl-2,3-dihydro-1*H*-benzo [b] [1,4] diazepine (1-12) derivatives were prepared by cyclization of chalcones with o-phenylene diamine in the presence of 20% NaOH as base. The IR spectra of substituted benzodiazepines (1-12) showed characteristic absorption bands at 1569 and 3370/cm corresponding to C=N and NH group, which was absent in the intermediate chalcones. Similarly, the ^{1}H NMR of the synthesized benzodiazepines showed one characteristic signal at δ 3.0, 3.4, 5.1 (d, 3H of benzodiazepine) which was absent in the ^{1}H NMR spectra of substituted chalcones. Hence, the formation of the title compounds benzodiazepines was confirmed and further established by mass spectra which are in accordance with molecular formula.

Anti-inflammatory activity

All the synthesized compounds were tested for their anti-inflammatory activity using carrageenan-induced rat hind paw edema method. Data of anti-inflammatory activity were expressed as mean ± SEM, and the Student's *t*-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds. The anti-inflammatory activity of the newly synthesized compounds was compared with the standard Diclofenac sodium 10 mg/kg body weight, showing 46.8% inhibition of rat paw edema whereas tested compounds showed inhibition ranging from 32.9% to 43.61% after 240 min. The compounds 4, 5, 7, 9, 10, 11, and 12 containing 4-nitrophenyl, 4-chlorophenyl, 3-nitrophenyl, 4-fluorophenyl, 4-bromophenyl, and 3-chlorophenyl benzodiazepine derivatives exhibited significant anti-inflammatory activities compared with the standard diclofenac sodium as given in Table 2. The presence of electron withdrawing groups such as nitro, chloro, fluoro, and bromo accounted for the anti-inflammatory activity.

CONCLUSION

Several 2-(substituted phenyl)-3-styryl-2,3-dihydro-1*H*-benzo [b] [1,4] diazepine were successfully synthesized in 61-86 % yields and are characterized by elemental analysis, ^{1}H NMR,mass spectrometry, and IR studies. Anti-inflammatory activity was evaluated by carrageenan induced paw edema method. Compounds 4, 5, 7, 9, 10, 11, and 12 were found to be biologically active whereas remaining compounds showed poor anti-inflammatory activity.

ACKNOWLEDGMENT

The authors are thankful to Nitte University for providing all the research facilities and financial assistance and Mangalore University for elemental analysis. The authors are also thankful to Head, SAIF, Punjab University, Chandigarh, for NMR and mass spectroscopic analysis.

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