INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

SYNTHESIS OF NOVEL 2,5-DIHYDRO-1*H*-1,5-BENZODIAZEPINES ENCOMPASSING NAPHTHO[2,1-*B*]FURAN AND EVALUATION OF THEIR PHARMACOLOGICAL ACTIVITIES

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ABSTRACT

The reaction of 2-acetylnaphtho[2,1-*b*]furan **1** with different aromatic aldehydes afforded corresponding chalcones **2(a-f)**. These chalcones **2(a-f)** on reacting with o-phenylenediamine in presence of a base produced 4-(naphtho[2,1-*b*]furan-2-yl)-2-(substituted)phenyl-2,5-dihydro-1*H*-1,5-benzodiazepines **3(a-f)**. The structures of newly synthesized compounds have been established by elemental analysis and spectral studies. Some of the newly synthesized compounds **3a-f** exhibited potent antibacterial, antifungal, antiinflammtory, diuretic, anthelmintic and antipyretic activities.

Keywords: Naphtho[2,1-*b*]furan; chalcones; 2,5-dihydro-1*H*-1,5-benzodiazepines.

INTRODUCTION

The chemistry of the compounds containing the condensed diazepine play significant role in pharmaceutical industry. Amongst various isomers of benzodiazepine, 1,5-benzodiazepines and its derivatives attracted attention due to their wide spectrum of biological and pharmacological activities such as anxiolytic[1], cytotoxic[2], neuroleptic[3] and other biological activities[4-8]. These compounds are also known to act as HIV reverse transcriptase inhibitors[9], and modulators for γ-aminobutyric acid receptors[10-11]. Benzodiazepines also serve as excellent starting material for the synthesis of some fused heterocycles involving triazole, oxadiazole etc[12-14]. Several derivatives of naphtho[2,1-b]furan synthesized in our laboratory have been reported to possess many biological and pharmacological activities such as antimicrobial, analgesic, anti-inflammatory, diuretic, anthelmintic, antipyretic etc [15-18]. Survey of literature revealed that, similar type of work involving 1,5-benzodiazepines and naphtho[2,1-*b*]furan either in condensed form or in coupled form has not been reported. Hence it is thought of interest to synthesize 4-naphtho[2,1-*b*]furan-2-yl-2-(substituted)phenyl-2,5-dihydro-1*H*-1,5-benzodiazepines and evaluate them for antibacterial, antifungal, antiinflammtory, diuretic, anthelmintic and antipyretic activities.

MATERIALS AND METHOD

All the reagents were A. R. grade and used with further purification. Melting points were determined with the open capillary and are uncorrected. IR spectra recorded in KBr pellets by using JASCO FT-IR 300E spectrophotometer.

^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on Bruker Supercon FT-NMR 400 MHz instrument. Chemical shifts are reported in δ (ppm) relative to TMS as internal standard. Mass spectral data were obtained on a Jeol JMS-D 300 Mass spectrometer operating at 70 eV. Elemental analyses were performed using a Vario-EL elemental analyzer. All the reactions were monitored by TLC.

EXPERIMENTAL

Synthesis of 3-(4-methoxyphenyl)-1-naphtho[2,1-*b*]furan-2-yl-prop-2-en-1-one 2a;

To a solution of 2-acetylnaphtho[2,1-*b*]furan **1** (2.10 g, 0.01 mol) in ethanol (50 ml) containing sodium hydroxide (0.4 g, 0.01 mol), 4-methoxybenzaldehyde (1.06 g, 0.01 mol) was added and the mixture was refluxed on water bath for 2 h, and poured into ice-cold water. The solid that separated was filtered, dried and recrystallised from ethanol to give **2a**.

Similarly compounds **2b-f** were synthesized by using 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, benzaldehyde, 2-hydroxy benzaldehyde, and 2-chlorobenzaldehyde in place of 4-methoxybenzaldehyde.

Synthesis of 4-naphtho[2,1-*b*]furan-2-yl-2-(4-methoxyphenyl)-2,5-dihydro-1*H*-1,5-benzodiazepine 3a

A mixture of 3-(4-methoxyphenyl)-1-naphtho[2,1-*b*]furan-2-yl-prop-2-en-1-one **2a** (1.09 g, 0.0033 mol), *o*-phenylenediamine (0.33 g, 0.0033 mol), sodium hydroxide (0.01 mol) in absolute ethanol (50 ml) was refluxed on a water bath for 6 h. The contents were cooled and poured on to crushed ice. The solid product thus obtained was filtered, dried and recrystallised from aqueous DMF. (1.00 g, 72 %) m.p. 238 ^{0}C.

The compounds **3b-f** were synthesized similarly by using compounds **2b-f**. The sequence of reactions is outlined in scheme-1. The physical and analytical data of the synthesized compounds is presented in Table-1.

Evaluation of pharmacological activities

The compounds encompassing naphthofuran, and benzodiazepines are known to exhibit wide spectrum of biological and pharmacological activities. Hence, it was intrigued to evaluate newly synthesized compounds for antimicrobial, antiinflammtory, analgesic, diuretic, anthelmintic, and antipyretic activities by adopting literature procedure. In animal experiments, ethical guidelines have been followed.

Antimicrobial activity

The *in vitro* antimicrobial activity was carried out against 24 h old cultures of two bacteria and two fungi by cup-plate method[19]. The compounds **3a-f** have been investigated for their antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* and *Curvularia lunata*. Chloramphenicol and fluconazole were used as standards for antibacterial and antifungal activity respectively. The compounds were tested at a concentration of 0.001 mol/ml in DMF against all organisms. The zone of inhibition was compared with the standard drug after 24 h of incubation at 25 ^{0}C for antibacterial activity and 48 h at 30 ^{0}C for antifungal activity. The results are presented in Table 2.

Anti-inflammatory activity

The anti-inflammatory activity was evaluated by a rat paw edema method. This method is based on plethysmographic measurement of carrageenan-induced acute rat paw edema produced by sub plantar injection of carrageenan in hind paw of the rat[20-21]. Ibuprofen was used as standard and tween-80 (0.1%, 1 ml) solution as control for this study. The percentage inhibition of paw volume was calculated by using the formula

% Inhibition= (1 - Vt/Vc) x 100.

Where, Vt = Mean increase in the paw volume in test animals group.

Vc = Mean increase in the paw volume in control group.

Analgesic activity

Analgesic activity was determined by the method based on acetic acid induced writhing in mice[22-23]. Acetyl salicylic acid (aspirin) was used as standard and Tween-80 (0.1%) solution as control. The percentage inhibition of writhing was calculated by using the formula

% Inhibition = (1-Nt/Nc) x 100,

Where, Nt = Mean number of writhing in test animals

Nc = Mean number of writhing in control.

The results of anti-inflammatory activity and analgesic activities are given in Table 3.

Diuretic activity

The diuretic activity was evaluated on albino rats (Wistar strain) by literature method[24]. For this study aqueous solution of tween-80 (0.1%, 5 ml) served as control and Frusamide as standard.

Anthelmintic activity

Anthelmintic activity was evaluated by using *Pheritima posthuma* (class-Annelida and order-Oligichaeta). The technique adopted was that described by Giand et al[25-26]. For this study 25 ml of 0.1% Tween-80 prepared in 6% dextrose solution was served as control. Albendazole suspended in 6% dextrose solution served as standard.

Antipyretic activity

The antipyretic activity was carried out on colony bred albino male rats as by a modified yeast induced hyperpyrexia method[27]. Tween–80 used as control and paracetamol as standard drug. All values are expressed as mean ± SEM.

The results of diuretic, anthelmintic and antipyretic activities are presented in Table 4.

RESULT AND DISCUSSION

The key starting material 2-acetylnaphtho[2,1-*b*]furan **2** was synthesized by the reaction of 2-hydroxy-1-naphthaldehyde **1** with chloroacetone in presence of potassium carbonate[28]. 2-Acetylnaphtho[2,1-*b*]furan on treatment with different aromatic aldehydes in presence of sodium hydroxide produced appropriate chalcones **3(a-f).** The selection of aromatic aldehydes was based upon presence of electron withdrawing and electron donating groups which could enable to study structure activity relationship during the evaluation of pharmacological activities. These chalcones **2(a-f)** on reaction with *o*-phenylenediamine in presence of sodium hydroxide in ethanol afforded 4-naphtho[2,1-*b*]furan-2-yl-2-(substituted)phenyl-2,5-dihydro-1*H*-1,5-benzodiazepines **3(a-f)**. The structure assigned to **3a** has been established by spectral studies: IR (KBr): 3387 cm^{-1} (NH): ^1H NMR (DMSO-d_6): δ 3.8 (s, 3H, OCH_3); δ 6.4 (d, 1H, C=CH); δ 6.7 (d, 1H, CCHPh); δ 7.2- 8.6 (m, 17H, 15ArH + 2NH): ^{13}C NMR (DMSO-d_6): δ 54.75 (OCH_3); δ 112.56, 112.69, 112.77, 113.08, 113.63, 113.74, 113.93, 123.22, 123.52, 123.59, 125.35, 125.65, 127.24, 127.36, 127.60, 127.83, 128.42, 128.50, 128.65, 128.73, 128.92, 129.18, 129.47, 129.54, 129.82, 129.94 and 130.21 (27 carbon atoms): M^+; 418 (m/z); 404, 298, 179, 139 and 101 fragmentation pattern.

The same method was employed to yield compounds **3b-f** from **2b-f**. The IR and ^1H NMR spectral data of these compounds is summarized in Table 5.

The newly synthesized compounds were evaluated for antimicrobial activity. The compounds **3c, 3e-f,** displayed significant antibacterial activity against both the organisms. Rest of the compounds exhibited substantial activity against both the organisms. It is observed that electron withdrawing groups resulted in enhancement of activity. The compounds **3c-f** exhibited promising antifungal activity, whereas remaining compounds are found to be considerably active. In this case also electron withdrawing groups have much more pronounced effect on antifungal activity.

Anti-inflammatory activity of the synthesized compounds was investigated by carrageenan induced rat paw edema method on albino rats (Wistar strain) using ibuprofen as standard drug. The compounds **3a-b** and **3d** exhibited promising activity, having percentage inhibition of 57.66, 59.67, 61.69 comparable with that of standard drug having percentage inhibition of 79.59, while rest of the compounds were found to be moderately active. The presence of electron donating groups resulted in increase of activity to greater extent.

Acetic acid induced writing method was adopted to evaluate analgesic activity of the synthesized compounds. The experiment was carried out on albino mice (Swiss strain) using aspirin as standard and % protection was calculated for each compound as well as standard. The results indicated that compounds **3a-d** possess substantial analgesic activity and remaining compounds exhibited significant activity. The activity is independent of the substituent present in the molecule. The mechanism of action of all the tested compounds at present could not be ascertained and needs detailed investigation.

Diuretic activity was evaluated on albino rats (Wistar strain) using Frusamide as standard drug. Lipschitz values were calculated. The compounds **3a**, **3c**, **3e**, were found to display promising activity having T/S value 0.55, 0.58, 0.58 compared with that of standard and remaining compounds possessed moderate activity. Diuretics are drugs that increase the rate of urine flow. However, clinically useful diuretics also increase the rate of excretion of Na^+ and accompanying anion, usually Cl^-. The standard drug Frusamide used in this case, contains furan ring in its structure, hence the diuretic effect of the test compounds may due to the presence of naphthofuran moiety in their structures.

The synthesized compounds were screened for anthelmintic activity and the time required paralysis and death of the worm were noted in each case. It was observed that none of the compounds exhibited considerable anthelmintic activity. Outer layer of the earthworm is a mucilaginous layer and composed of polysaccharides. This layer, being slimy, enables the earthworms to move freely. Any damage to

the muco polysaccharide membrane will expose the outer layers, and this restricts its movement and can cause paralysis. This action may lead to death of the worm and will be expelled out from the body. None of the compounds seems to have such an effect on earthworms.

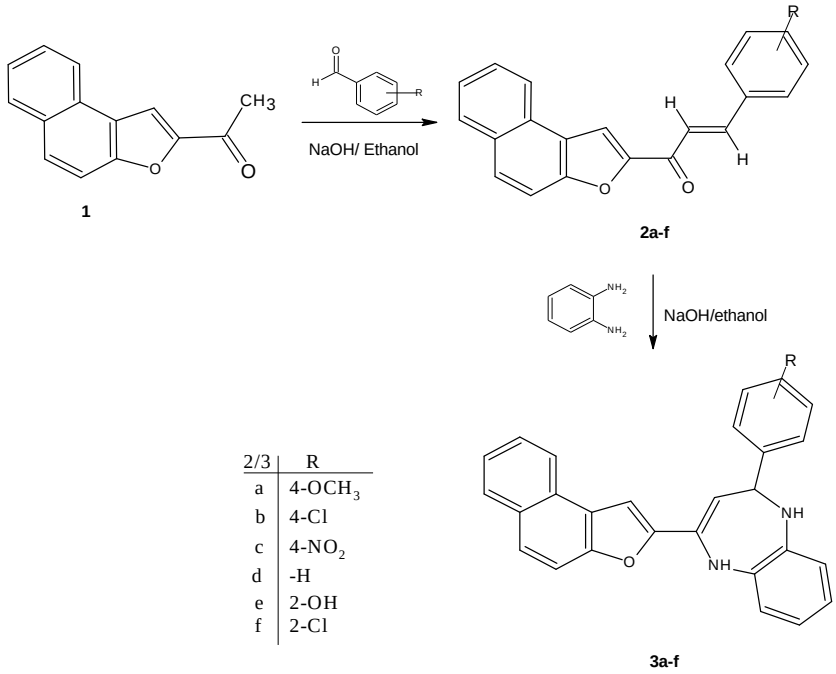
Antipyretic activity of the synthesized compounds was determined by yeast induced hyperpyrexia method on albino rats (Wistar strain) using paracetamol as standard drug. Decrease in rectal temperature was recorded in each case. The results indicated that compound **3a-d** exhibited excellent antipyretic activity showing the decrease in temperature to the extent of 0.5 ^{0}C. Rest of the compounds were either moderately active or less active. Chlorine atom which is present in position 4 increased the activity to considerable activity. The test compounds possess a significant antipyretic effect in yeast-induced elevation of body temperature in rats and this may be due to combined anti-inflammatory and analgesic effects.

CONCLUSION

A number of 4-naphtho[2,1-*b*]furan-2-yl-2-(substituted)phenyl-2,5-dihydro-1*H*-1,5-benzodiazepines **3a-f** were synthesized and characterized by analytical and spectral studies. The newly synthesized compounds were evaluated for antibacterial, antifungal, anti-inflammatory, analgesic, diuretic, anthelmintic, and antipyretic activities. The results obtained hitherto indicated, that introduction of benzodiazepine moiety enhances the activity to considerable extent. In many cases, presence of electron withdrawing groups results in increase of activity and in few cases electron donating methoxy group has marked influence in enhancing activity.

ACKNOWLEDGEMENTS

The authors are thankful to The Chairman, Department of Chemistry, Kuvempu University for providing laboratory facilities. The authors are also thankful to Convener, Sophisticated Instruments Facility, IISc, Bangalore for providing spectral data. Finally the authors are thankful to Principal, SCS College of Pharmacy for providing laboratory facilities and animals to conduct pharmacological investigations. One of the authors (MNK) is thankful to CSIR-UGC for awarding Senior Research fellowship.



2/3	R
a	4-OCH_3
b	4-Cl
c	4-NO_2
d	-H
e	2-OH
f	2-Cl

Scheme-1

Table 1: Physical data of synthesized compounds 3a-f

Comp.	R	M.p.^{0}C	Yield (%)	Mol. formula	Found (Calcd) %		
					C	H	N
3a	4-OCH_3	238	72	$C_{28}H_{22}N_2O_2$	80.25 (80.36)	5.20 (5.30)	6.59 (6.69)
3b	4-Cl	225	68	$C_{27}H_{19}N_2OCl$	76.59 (76.68)	4.41 (4.53)	6.51 (6.62)
3c	4-NO_2	>250	65	$C_{27}H_{19}N_3O_3$	74.70 (74.81)	4.32 (4.42)	9.58 (9.69)
3d	H	208	70	$C_{27}H_{20}N_2O$	83.37 (83.48)	5.08 (5.19)	7.13 (7.21)
3e	2-OH	>250	69	$C_{27}H_{20}N_2O_2$	80.06 (80.18)	4.86 (4.98)	6.82 (6.93)
3f	2-Cl	231	62	$C_{27}H_{19}N_2OCl$	76.59 (76.68)	4.41 (4.53)	6.49 (6.62)

Table 2: Antimicrobial activity data of the compounds 3a-f

Compd.	Zone of Inhibition in mm			
	Antibacterial activity		Antifungal activity	
	P. aerugenosa	S. aureus	A. niger	C. lunata
Standard	24	26	22	24
DMF	Nil	Nil	Nil	Nil
3a	16	16	15	15
3b	17	16	16	16
3c	18	17	18	18
3d	17	16	17	17
3e	18	17	18	17
3f	18	16	18	16

Table 3: Anti-inflammatory and Analgesic activity of the compounds 3a-f

Compd.	Anti-inflammatory activity		Analgesic activity
	Group	Inhibition (%) of edema after 3 hrs	% Protection
Control	I	----	----
Standard	II	75.80	71.00
3a	III	57.66	62.98
3b	IV	59.67	55.63
3c	V	53.62	60.00
3d	VI	61.69	58.67
3e	VII	53.62	54.42
3f	VIII	58.72	54.76

Table 4: Diuretic, Anthelmintic and Antipyretic activities of the compounds 3a-f

Compd.	Group	Diuretic activity	Anthelmintic activity		Antipyretic activity		
		T/S	Time in minutes		Mean rectal temperature (^{0}C)		Decrease in temperature (^{0}C)
			Mean time of paralysis	Mean death time	0 hr	3 hr	
Control	I	0.27	------	-----	38.7	38.5	0.2
Standard	II	1.00	33	46	38.4	37.7	0.7
3a	III	0.55	135	155	38.1	37.8	0.4
3b	IV	0.65	126	149	38.4	38.1	0.4
3c	V	0.58	137	159	38.0	37.7	0.3
3d	VI	0.62	131	152	38.3	38.0	0.3
3e	VII	0.62	120	140	37.8	37.5	0.2
3f	VIII	0.58	126	146	38.0	37.7	0.3

T/S: Lipschitz value

Table 5: IR and NMR Spectral data of 4-(naphtho[2,1-*b*]furan-2-yl)-2-(substituted)- phenyl-2,5-dihydro-1*H*-1,5-benzodiazepines 3b-f

Comp.	R	IR (KBr) cm^{-1} (NH)	^{1}H NMR ppm
3b	4-Cl	3375	δ 6.6 (d, 1H, C=CH), δ 6.8 (d, 1H, CCHPh), δ 7.4-8.6 (m, 17H, 15ArH + 2NH)
3c	4-NO_2	3362	δ 6.5 (d, 1H, C=CH), δ 6.9 (d, 1H, CCHPh), δ 7.4-8.5 (m, 17H, 15ArH + 2NH)
3d	H	3378	δ 6.4 (d, 1H, C=CH), δ 6.6 (d, 1H, CCHPh), δ 7.3-8.3 (m, 18H, 16ArH + 2NH)
3e	2-OH	3369	δ 4.5 (b, 1H, OH), δ 6.7 (d, 1H, C=CH), δ 6.9 (d, 1H, CCHPh), δ 7.5-8.7 (m, 17H, 15ArH + 2NH)
3f	2-Cl	3381	δ 6.5 (d, 1H, C=CH), δ 6.8 (d, 1H, CCHPh), δ 7.6-8.5 (m, 17H, 15ArH + 2NH)

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