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Synthesis and Pharmacological Activities of Biheterocycles 7-substitutedphenyl-5-(5'-substituted-2'-phenylindol-3'-yl)-1,4-benzo[b]diazepines.

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5-Substituted-2-phenylindol-3-carboxaldehydes (1a-c) were reacted with substituted acetophenones in ethyleneglycol in presence of piperidine to yield the respective chalcones (3a-r). When these chalcones were reacted in absolute ethanol with orthophenylene diamine produced 7-substitutedphenyl-5-(5'-substituted-2'-phenylindol-3'-yl)-1,4-benzo[b]diazepines (4a-r). The structures of the newly synthesised compounds were elucidated on the basis of analytical and spectral data. These compounds have been screened for analgesic, antiinflammatory and locomotor activities.

In continuation of our work on the synthesis of indole derivatives[1], we report here the synthesis and pharmacological activities of indole linked benzodiazepines. In view of the remarkable biological activity found with benzodiazepines, a wide variety of their derivatives have been synthesised and reported in the literature[2-7]. Benzodiazepines fused with other heterocyclic systems are reported as potential compounds exhibiting sedative, tranquilising, hypnotic, anticonvulsant, antispasmodic and prenarcotic properties[8-11]. Considering the pharmacological significance of diazepines, benzodiazepines and their derivatives many other heterocyclic systems are fused either with diazepine or with benzodiazepine to get the compounds possessing high degree of pharmacological activity with minimum toxic effect. Amongst the fused heterocyclic systems, there are very few compounds possessing indole and diazepine moieties. The synthesis and screening of such compounds has gained importance in recent years[12-17].

The reaction of chalcones with orthophenylenediamines represents a convenient and versatile method for the preparation of 1,4-diazepines[18-22]. 5-Substituted-2-phenylindol-3-carboxaldehydes[23] (1a-c) were reacted with substituted acetophenones (2a-f) in ethyleneglycol and piperidine to yield the respective chalcones[24] (3a-r). When these chalcones were reacted in absolute ethanol with orthophenylenediamine produced 7-substitutedphenyl-5-(5'-substituted-2'-phenylindol-3'-yl)-1,4-benzo[b]diazepines (4a-r) (Scheme 1). Newly synthesised compounds were characterised by IR, NMR, mass spectral data and elemental analysis. These compounds were screened for their analgesic, antiinflammatoy and locomotor activities.

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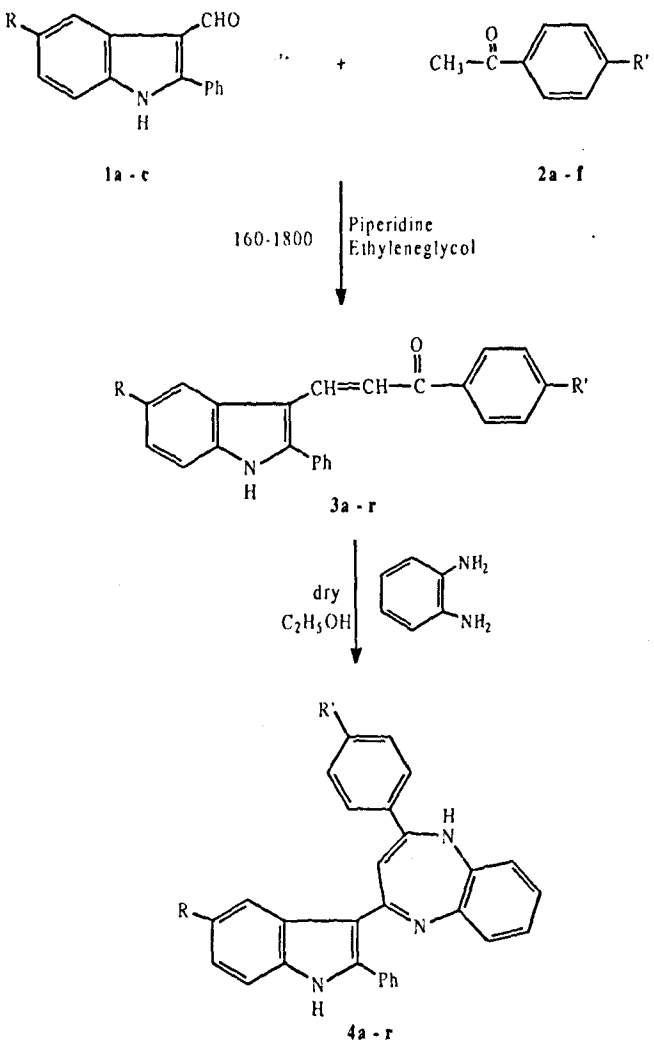
MATERIALS AND METHODS

Purity of the compounds was checked by TLC. Melting points were determined in open capillaries and are uncorrected. IR spectra (KBr) are recorded on a Perkin-Elmer 283 spectrophotometer. NMR spectra are recorded on a Varian A-90, 90MHz spectrometer using TMS as internal reference and mass spectra are recorded on 5970 It instrument. For analgesic and locomotor activity studies adult, healthy Swiss albino mice of either sex weighing 20-25 g were used. For antiinflammatory activity adult healthy wistar rats of either sex weighing between 150-200 g were used. All the animals were maintained under standard conditions and had access to pelleted animal feed and water. The study protocols were approved by the Institutional Animal Ethics Committee (CPCSEA Regd. No. 341).



1a - c 2a - f

160-180 Piperidine Ethyleneglycol

3a - r

dry C_2H_5OH

4a - r

1, 3 & 4 R = Cl, CH_3 and H

R' = CH_3, Br, NH_2, NO_2, OH and H

Scheme 1: Synthetic route.

5-Substituted-2-phenylindol-3-chalcones (3a-r):

5-Substituted-2-phenylindol-3-carboxaldehydes (1a-c) (0.01 mol) and substituted acetophenones (2a-f) (0.01 mol) were taken in ethyleneglycol (15 ml). To the reaction mixture piperidine (1 ml) was added and resulting mixture was heated at 160-180° for 4 h. The contents were cooled and decomposed in ice cold water containing 1ml of acetic acid. The solid thus separated was filtered washed thoroughly with water, dried and recrystallised from suitable solvents. The IR spectrum of 3f exhibited peaks at 3287 cm^{-1} (NH) and 1624 cm^{-1} (CO) indicating the condensation of substituted acetophenones with indol-3-carboxaldehydes to give α, β-unsaturated compounds (chalcones). The NMR spectrum of 3f exhibited signals at 8.7 δ (s, 1H, indole NH) and 7.0-8.2 δ (m, 15H, Ar-H). The two protons of the methine group have deshielded and appeared along with aromatic protons. Physical and elemental analysis data of the compounds are given in Table 1.

7-Substitutedphenyl-5-(5'-substituted-2'-phenylindol-3'-yl)-1,4-benzo[b]diazepines (4a-r):

5-Substituted-2-phenylindol-3-chalcones (3a-r) (0.01 mol) and orthophenylenediamine (0.01 mol) in dry ethanol (10 ml) was refluxed on a water bath for 6 h. The contents were cooled and poured on to crushed ice. The solid separated was filtered, washed with water, dried and recrystallised from suitable solvents. The IR spectrum of 4f has shown peak at 1548 cm^{-1} (C=N) stretching. The NMR spectrum of 4f has exhibited signals at 8.6δ (s, 1H, indole NH), 8.2δ (s, 1H, diazepine NH) and at 7.2-8.1δ (m, 18H, Ar-H). The methine proton of diazepine ring has appeared along with aromatic protons. Analytical data of the compounds support the proposed structure. (Table 1)

Analgesic activity:

Test for the analgesic activity was performed using the tail flick method[25]. Swiss mice of either sex (20-25 g) were distributed into control, standard and test groups each of four mice. The control groups were treated with 2% gum acacia suspension. The test animals were treated orally at a dose of 100 mg/kg body weight. The standard group was administrated with analgin at a dose of 100 mg/kg body weight orally. The reaction time was recorded at 0, 30, 60 and 120 min after the drug administration. The results are listed in Table 2.

Antiinflammatory activity:

Antiinflammatory activity was measured using the formalin-induced paw oedema test in rats[25]. Only selected compounds were screened for antiinflammatory activity. Wistar rats of either sex (150-200 g) were divided into control, standard and test groups each consisting of six rats. A group of rats was treated with Tween 80 (1%) suspension i.p (control). Another group was administered a dose of 100 mg/kg of suspension of phenylbutazone (standard) p.o and the third group was treated with 100 mg/kg of the suspension of the test compounds. After 30 min the animals were injected with 0.1 ml of formalin (1%, w/v) in the sub-plantar region of left hind paw of the rats. The volume of the paw was measured using the mercury displacement technique with the help of plethysmograph both in control as well as in standard animals including the test animals at 2 and 4 h

TABLE 1 : CHARACTERISATION DATA OF THE SYNTHESISED COMPOUNDS 3a-r AND 4a-r

Compd.	Substituent		M.P.	Yield	Molecular
	R	R'	°	%	formula
3a	Cl	CH_3	205-06	58	$C_{24}H_{18}NOCl$
3b	Cl	Br	215-16	60	$C_{23}H_{15}NOClBr$
3c	Cl	NH_2	198-99	61	$C_{23}H_{17}N_2OCl$
3d	Cl	NO_2	221-22	58	$C_{23}H_{15}N_2O_3Cl$
3e	Cl	OH	217-18	61	$C_{23}H_{16}NO_2Cl$
3f	Cl	H	250-51	65	$C_{23}H_{16}NOCl$
3g	CH_3	CH_3	191-92	58	$C_{25}H_{21}NO$
3h	CH_3	Br	164-65	44	$C_{24}H_{18}NOBr$
3i	CH_3	NH_2	188-89	61	$C_{24}H_{20}N_2O$
3j	CH_3	NO_2	173-74	66	$C_{24}H_{18}N_2O_3$
3k	CH_3	OH	151-52	45	$C_{24}H_{19}NO_2$
3l	CH_3	H	178-79	62	$C_{24}H_{19}NO$
3m	H	CH_3	181-82	56	$C_{24}H_{19}NO$
3n	H	Br	113-14	60	$C_{23}H_{16}NOBr$
3o	H	NH_2	118-19	58	$C_{23}H_{18}N_2O$
3p	H	NO_2	131-32	45	$C_{23}H_{16}N_2O_3$
3q	H	OH	185-86	61	$C_{23}H_{17}NO_2$
3r	H	H	170-71	40	$C_{23}H_{17}NO$
4a	Cl	CH_3	188-89	65	$C_{30}H_{22}N_3Cl$
4b	Cl	Br	191-92	55	$C_{29}H_{19}N_3ClBr$
4c	Cl	NH_2	172-73	62	$C_{29}H_{21}N_4Cl$
4d	Cl	NO_2	181-82	65	$C_{29}H_{19}N_4O_2Cl$
4e	Cl	OH	180-81	58	$C_{29}H_{20}N_3OCl$
4f	Cl	H	230-31	65	$C_{29}H_{20}N_3Cl$
4g	CH_3	CH_3	162-63	63	$C_{31}H_{25}N_3$
4h	CH_3	Br	212-13	67	$C_{30}H_{22}N_3Br$
4i	CH_3	NH_2	155-56	69	$C_{30}H_{24}N_4$
4j	CH_3	NO_2	162-63	69	$C_{30}H_{22}N_4O_2$
4k	CH_3	OH	180-81	61	$C_{30}H_{23}N_3O$
4l	CH_3	H	168-69	68	$C_{30}H_{23}N_3$
4m	H	CH_3	189-90	60	$C_{30}H_{23}N_3$
4n	H	Br	128-29	61	$C_{29}H_{20}N_3Br$
4o	H	NH_2	122-23	68	$C_{29}H_{22}N_4$
4p	H	NO_2	140-41	69	$C_{29}H_{20}N_4O_2$
4q	H	OH	120-21	62	$C_{29}H_{21}N_3O$
4r	H	H	216-17	65	$C_{29}H_{21}N_3$

Recrystallisation solvent:3a-f, 3k in benzene-chloroform ; 3g-i, 3l, 3o, 3q-r, 4a-r in ethanol; 3j, 3p in acetone-ethanol; 3m and 3n in benzene-ethanol. All the compounds showed satisfactory C, H and N analysis.

after injection. The initial volume of the paw was measured within 30 s of the injection. The percent inhibition of the inflammation after 2 and 4 h was calculated by using the formula. % Inhibition=$(1-V_t/V_c)100$. Where V_c and V_t are the mean relative changes in the volume of paw oedema in the control and test respectively. The results are summarized in Table 3.

Locomotor activity:

Locomotor activity was measured using actophotometer[25]. Swiss mice of either sex (20-25 g) were distributed into control, standard and test groups each of four mice. The control group received only 2% gum acacia suspension. The standard group was administrated with chlorpromazinehydrochloride intraperitonially at a dose of 3 mg/kg body weight and the test group was treated with

TABLE 2 : RESULTS OF ANALGESIC ACTIVITY OF THE COMPOUNDS 3a, 3d, 3j, 3n, 4a, 4b, 4d, 4e, 4h, 4j-l, 4p AND 4r.

Compd. No.	Dose mg/kg	Time taken to remove the tail at different time interval (mean±SE)			
		0	30	60	120
3a	100	4.00 (± 0.40)	4.00 (± 0.70)	3.00 (± 0.40)	3.00 (± 0.40)
3d	100	3.00 (± 0.00)	5.25 (± 0.25)	7.25 (± 0.40)	11.23 (± 0.25)
3j	100	2.75 (± 0.25)	3.50 (± 0.28)	4.25 (± 0.25)	8.00 (± 0.40)
3n	100	3.75 (± 0.47)	3.75 (± 0.25)	5.25 (± 0.47)	11.75 (± 0.25)
4a	100	3.00 (± 0.00)	3.25 (± 0.25)	4.00 (± 0.40)	5.00 (± 0.00)
4b	100	3.75 (± 0.47)	5.75 (± 0.25)	9.25 (± 0.00)	11.75 (± 0.25)
4d	100	3.25 (± 0.40)	4.25 (± 0.25)	4.00 (± 0.40)	4.25 (± 0.25)
4e	100	3.00 (± 0.00)	4.00 (± 0.40)	5.25 (± 0.00)	5.50 (± 0.40)
4h	100	3.00 (± 0.40)	5.75 (± 0.25)	10.25 (± 0.00)	11.00 (± 0.25)
4j	100	2.75 (± 0.25)	6.25 (± 0.25)	10.50 (± 0.48)	11.75 (± 0.48)
4k	100	3.00 (± 0.00)	3.25 (± 0.15)	4.00 (± 0.30)	5.25 (± 0.25)
4l	100	2.75 (± 0.25)	4.25 (± 0.25)	5.00 (± 0.47)	6.25 (± 0.47)
4p	100	3.25 (± 0.25)	4.75 (± 0.47)	8.75 (± 0.40)	11.75 (± 0.25)
4r	100	2.25 (± 0.25)	3.00 (± 0.48)	4.75 (± 0.47)	6.25 (± 0.25)
Standard (Analgin)	100	3.00 (± 0.25)	6.25 (± 0.25)	10.50 (± 0.40)	10.50 (± 0.25)
Control (2% gum acacia)	-	3.00 (± 0.00)	3.00 (± 0.05)	3.00 (± 0.00)	3.00 (± 0.05)

All the values are mean±SE of 4 samples.

200 mg/kg of the suspension of the test compounds orally. The equipment was turned on and each group of mice were placed in the activity cage for 10 min. The difference in the activity, before and after administration of drug and test compounds was noted. The readings were taken after half an hour of drug and test compounds administration. The percentage decrease or increase in motor activity was calculated and presented in Table 4.

RESULTS AND DISCUSSION

Among the compounds tested for analgesic activity, only compounds 3d, 3n, 4b, 4h, 4j and 4p showed good analgesic activity compared to analgin. The common feature among these compounds is they contain halogens, nitro or methoxy groups as one of the substituents and or two. Highest activity is found with 3n, 4b and 4j. Compound 3n contains bromo, 4b contain chloro and bromo, 4j contain methyl and nitro substituents.

Compounds (3a-r) and (4a-r) have been tested for locomotor activity. Amongst the compounds tested compounds 3a and 3c showed significant increase in locomotor activity by 56.3% and 71.3% respectively. Compounds (4a-r) have shown decrease in locomotor activity from 32.5% to 73.4%. Selected six compounds have been tested for antiinflammatory activity. Compounds 3j, 4h and 4j showed 33 to 38 % activity. Common feature amongst active compounds is, all the compounds contain methyl substituent and 3j and 4j contains nitro groups and 4h contains bromine in it. Based on these results we can't make any conclusion and they need further detailed investigation.

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TABLE 3 : RESULTS OF ANTIINFLAMMATORY ACTIVITY OF THE COMPOUNDS 3d, 3j, 4b, 4h, 4j AND 4p

Compd. No.	Dose mg / kg	Mean values (± SE) of oedema volume at different intervals.		Percentage of antiinflammatory at different intervals	
		2h	4h	2h	4h
3d	100	0.223 (± 0.006)	1.77 (± 0.008)	12.2	20.2
3j	100	0.198 (± 0.032)	0.137 (± 0.002)	22.0	38.2
4b	100	0.208 (± 0.002)	0.162 (± 0.001)	18.1	27.0
4h	100	0.195 (± 0.007)	0.148 (± 0.003)	23.2	33.3
4j	100	0.193 (± 0.001)	0.147 (± 0.000)	24.0	33.7
4p	100	0.228 (± 0.007)	0.175 (± 0.004)	10.2	21.1
Standard (Phenyl butazone)	100	0.110 (± 0.013)	0.030 (± 0.003)	56.6	86.4
Control (2% gum acacia)	-	0.254 (± 0.016)	0.222 (± 0.002)	-	-

All the values are mean±SE of 6 samples.

TABLE 4 : RESULTS OF LOCOMOTOR ACTIVITY OF THE COMPOUNDS 3a-c, 3f, 3k, 4a-f AND 4h-r

Compd. No.	Dose mg / kg	Locomotor acitivty (scores) in 10 min.		
		Before Treatment	After Treatment	%Change in activity
3a	200	215	336	56(↑)
3b	200	372	295	21(↓)
3c	200	202	346	71(↑)
3f	200	297	341	15(↑)
3k	200	297	341	15(↑)
4a	200	317	214	32(↓)
4b	200	540	221	40(↓)
4c	200	376	212	44(↓)
4d	200	570	383	33(↓)
4e	200	208	174	16(↓)
4f	200	600	340	43(↓)
4h	200	465	287	38(↓)
4i	200	550	490	11(↓)
4j	200	317	214	32(↓)
4k	200	208	174	16(↓)
4l	200	540	321	40(↓)
4m	200	477	211	56(↓)
4n	200	316	118	63(↓)
4o	200	402	116	71(↓)
4p	200	421	112	73(↓)
4q	200	307	117	62(↓)
4r	200	412	172	58(↓)
Standard (Chloropromazine)	3	420	18	96(↓)
Control (2% gum acacia)	-	441	438	0.68(↓)

All the values are mean of 4 samples. (↓) Indicates decrease in locomotor activity and (↑) indicates increase in locomotor activity.

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