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Synthesis and Antiinflammatory Activity of Aminomethylisoxazolinyl/Azopyrazolinyl Diphenylamine Derivatives

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Chalcones (2-5), isoxazolines (6-9), Mannich bases (10-25), pyrazolines (26-29) and azo derivatives (30-33) of diphenylamine were synthesized and screened against carrageenan-induced edema in rats at 50 mg/kg, orally. All the compounds of this series have shown promising antiinflammatory activity. Compound (29) [1-acetyl-5-(4-methoxyphenyl)-2-pyrazolinyl] diphenylamine was found to be the most potent, even more that the standard, phenylbutazone. This compound was also evaluated for ulcerogenicity and acute toxicity.

Nonsteroidal antiinflammatory drugs under current clinical usage are highly acidic in nature and suffer from a common drawback of gastrointestinal toxicity, thus indicating a clear need to develop a nonsteroidal antiinflammatory agent. Several isoxazolines[1-2], pyrazolines[3-5], Mannich compounds (i.e. aminomethyl derivatives)[6-7] and azo derivatives[8-9] exhibited antiinflammatory activity. Isoxazolines and pyrazolines have also exhibited antimicrobial activity[10]. Furthermore, diphenylamine derivatives[11-12] were also reported to possess potent antiinflammatory activity. In the light of these observation we synthesized aminomethylisoxazolinyl/azopyrazolinyl diphenylamines with the hope to obtain better anti-inflammatory agents with less ulcerogenic activity.

MATERIALS AND METHODS

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using silica gel-G (Qualigens Fine Chemicals, Mumbai) coated plates and spots were located by iodine. Analytical data of C, H and N were within ± 0.4% of the theoretical values and their structure were elucidated by IR in KBr on Perkin-Elmer-881, ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker DPX-300 MHz and mass spectra were determined on a Jeol-D-300 spectrometer.

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Synthesis of acetyldiphenylamine (1):

To a solution of diphenylamine (0.01 mol, Aldrich Fine Chemicals, Milwaukee, USA) in chloroform (100 ml, dry, Merck, Mumbai), acetyl chloride (0.02 mol, Thomas Baker Chemical Ltd., Mumbai) was added drop by drop with constant stirring at 0-5º. The reaction mixture was stirred on magnetic stirrer for 3 h. Excess of solvent was distilled off and solid was poured into ice water. The solid was filtered, washed with water and recrystallised from methanol (Qualigens Fine Chemicals, Mumbai). Physical and analytical data of compound **(1)** are given in table 1. Compound **(1)**: IR (ν_{max}): 1680 (C=O), 3030 (aromatic C-H), 1560 (C=C of aromatic ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.86 (s, 3H, $COCH_3$), 6.68-7.20 (m, 10H, Ar-H) (ppm); $[M]^+$ m/z 211.

Synthesis of chalconyldiphenylamines (2-5):

To a mixture of compound **(1)** (0.01 mol) in absolute ethanol (50 ml, Qualigens Fine Chemicals), various aromatic aldehydes (0.01 mol, Aldrich Fine Chemicals) and 2% NaOH solution (5 ml, BDH, Mumbai) were added with constant stirring at a temperature between 0-5°. The reaction mixtures were stirred for 10-12 h at room temperature, poured into cold water and the solids thus separated out were filtered and washed with water, dried and recrystallised from appropriate solvents separately. Physical and analytical data of compounds **(2-5)** are given in table 1. Compound **(3)**: IR

TABLE 1: CHARACTERISATION AND BIOLOGICAL DATA OF COMPOUNDS 1-33.

Compd. No.	R	R^1	m.p. (°)	Yield (%)	Solvent of Recrystallisation	Molecular formula##	% decrease in paw oedema#	UD_{50} (mg/kg, i.p.)
1	—	—	103	68	Methanol	$C_{14}H_{13}NO$	—	—
2	3-OCH_3, 4-OH	—	88	55	Ethanol	$C_{22}H_{19}NO_3$	18.6	—
3	H	—	90	45	Ethanol/water	$C_{21}H_{17}NO$	36.0*	—
4	4-$N(CH_3)_2$	—	70	50	Methanol	$C_{23}H_{22}N_2O$	15.5	—
5	4-OCH_3	—	97	60	Acetone	$C_{22}H_{19}NO_2$	20.0	—
6	3-OCH_3, 4-OH	—	94	45	Acetone/water	$C_{22}H_{20}N_2O_3$	26.66*	—
7	H	—	92	60	Ethanol	$C_{21}H_{18}N_2O$	22.70*	
							45.76*	166.6
							67.80	
8	4-$N(CH_3)_2$	—	80	40	Benzene/pet.ether	$C_{23}H_{23}N_3O$	21.29*	—
9	4-OCH_3	—	100	35	Benzene	$C_{22}H_{20}N_2O_2$	18.36	
							40.00*	199.6
							60.66	
10	3-OCH_3, 4-OH	H	80	40	Ethanol	$C_{29}H_{27}N_3O_3$	15.60	—
11	3-OCH_3, 4-OH	2-Cl	88	30	Pet.ether	$C_{29}H_{26}N_3O_3Cl$	20.83*	—
12	3-OCH_3, 4-OH	3-Cl	98	25	Pet.ether	$C_{29}H_{26}N_3O_3Cl$	10.00	—
13	3-OCH_3, 4-OH	2-OCH_3	94	45	DMF/water	$C_{30}H_{29}N_3O_4$	17.66	—
14	H	H	77	20	Ethanol	$C_{28}H_{25}N_3O$	22.80*	—
15	H	2-Cl	84	35	Dioxane	$C_{28}H_{24}N_3OCl$	18.25*	—
16	H	3-Cl	86	40	DMF	$C_{28}H_{24}N_3OCl$	9.00	—
17	H	2-OCH_3	95	30	Methanol/water	$C_{29}H_{27}N_3O_2$	12.50*	—
18	4-$N(CH_3)_2$	H	99	40	Methanol	$C_{30}H_{30}N_4O$	12.86*	—
19	4-$N(CH_3)_2$	2-Cl	82	35	Acetone	$C_{30}H_{29}N_4OCl$	11.20*	—
20	4-$N(CH_3)_2$	3-Cl	76	20	DMF/water	$C_{30}H_{29}N_4OCl$	8.33	—
21	4-$N(CH_3)_2$	2-OCH_3	72	30	Ethanol	$C_{31}H_{32}N_4O_2$	18.75*	—
22	4-OCH_3	H	92	50	Methanol	$C_{29}H_{27}N_3O_2$	26.10*	—
23	4-OCH_3	2-Cl	86	35	DMF/water	$C_{29}H_{26}N_3O_2Cl$	33.33*	—
24	4-OCH_3	3-Cl	98	40	Ethanol/water	$C_{29}H_{26}N_3O_2Cl$	11.11	—
25	4-OCH_3	2-OCH_3	90	25	Ethanol	$C_{30}H_{29}N_3O_3$	16.60	—
26	3-OCH_3, 4-OH	—	95	50	Methanol	$C_{24}H_{23}N_3O_3$	18.18*	—
27	H	—	102	30	DMF/water	$C_{23}H_{21}N_3O$	26.63*	—
28	4-$N(CH_3)_2$	—	220	55	Pet. ether	$C_{25}H_{26}N_4O$	35.45*	—
29	4-OCH_3	—	78	40	Methanol/water	$C_{24}H_{23}N_3O_2$	26.00	
							48.60*	138.6
							67.66	
30	3-OCH_3, 4-OH	—	82	30	Methanol	$C_{30}H_{26}N_5O_3Cl$	30.00	—
31	H	—	68	25	Ethanol	$C_{29}H_{24}N_5OCl$	15.00	—
32	4-$N(CH_3)_2$	—	210	40	DMF/water	$C_{31}H_{29}N_6O_2Cl$	20.00*	—
33	4-OCH_3	—	112	35	Ethanol	$C_{30}H_{26}N_5O_2Cl$	35.00	—
Phenyl							15.00	
butazone	—	—	—	—	—	—	38.90	66.6
							65.66	

##C, H, N analysis are within the limit (±0.4%), #All compounds were tested at a dose of 50 mg/kg p.o. except compounds 7, 9 and 29 and phenylbutazone, which were tested at three dose levels of 25, 50 and 100 mg/kg p.o, *$p < 0.001$.

(ν_{max}): 1630 (CH=CH), 1700 (C=O), 3010 (aromatic C-H), 1550 (C=C of aromatic ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 5.98 (d, 1H, COC<u>H</u>=), 6.82 (d, 1H, =C<u>H</u>-Ar), 7.11-7.90 (m, 15H, Ar-<u>H</u>) (ppm); $[M]^+$ m/z 299.

Synthesis of [5-(substitutedphenyl)-2-isoxazolinyl] diphenylamines (6-9):

To a solution of compounds **(2-5)** (0.01 mol) in ethanol (dry, 50 ml), hydroxylamine hydrochloride (0.01 mol, Sigma Chemicals Co., St. Louis, USA) and 0.4 g solid NaOH were added. The reaction mixtures were refluxed for 6-8 h, poured into ice water. The separated residues were filtered and recrystallised from appropriate solvents. Physical and analytical data of compounds **(6-9)** are given in Table 1. Compound **(7)**: IR (ν_{max}): 1660 (C=N), 1260 (C-O-N), 1510 (C-N), 3030 (aromatic C-H), 1540 (C=C of aromatic ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 5.68 (d, 2H, C<u>H</u>$_2$ of isoxazoline ring), 6.54 (t, 1H, C<u>H</u>-Ar), 6.98-7.93 (m, 15H, Ar-<u>H</u>) (ppm); $[M]^+$ m/z 314.

Synthesis of [4-(substitutedphenyl) aminomethyl-5-(substitutedphenyl)-2-isoxazolinyl]diphenyl amines (10-25):

A mixture of compounds **(6-9)** (0.01 mol) in methanol, formaldehyde (0.02 mol, Aldrich Fine Chemicals) and different aromatic anilines (0.02 mol, BDH) were added. The reaction mixtures were refluxed for 2-6 h. The solvent was distilled off and poured into ice water, resulting solids were filtered off, dried and finally recrystallised from appropriate solvents. Physical and analytical data of compounds **(10-25)** are given in Table 1. Compound **(23)**: IR (ν_{max}): 680 (C-Cl), 1680 (C=N), 1240 (C-O-N), 1500 (C-N), 3400 (NH), 3050 (aromatic C-H), 1560 (C=C of aromatic ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.01 (m, 1H, C<u>H</u>-CH$_2$), 3.68 (s, 3H, Ar-OC<u>H</u>$_3$), 5.42 (s, 1H, N<u>H</u>-Ar), 6.63 (t, 2H, CH-C<u>H</u>$_2$), 7.41-8.04 (m, 18H, Ar-<u>H</u>; 1H, C<u>H</u>-Ar) (ppm); $[M]^+$ m/z 483.

Synthesis of [1-acetyl-5-(substitutedphenyl)-2-pyrazolinyl]diphenylamines(26-29):

To a solution of compounds **(2-5)** (0.01 mol) in ethanol (dry, 50 ml), hydrazine hydrate (99%; 0.01 mol, Qualigens Fine Chemicals) and few drops of glacial acetic acid (Indian Drugs and Pharmaceuticals Ltd., Hyderabad) were added. The reaction mixtures were refluxed for 6-12 h. The excess of solvent distilled off and poured into cold water, solids thus obtained were filtered, washed with water and recrystallised from appropriate solvents. Physical and analytical data of compounds **(26-29)** are given in Table 1. Compound **(29)**: IR (ν_{max}): 1680 (C=N), 1600 (C-N), 1510 (N-N), 1730 (C=O), 3010 (aromatic C-H), 1570 (C=C of aromatic ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.64 (s, 3H, COC<u>H</u>$_3$), 3.68 (s, 3H, Ar-OC<u>H</u>$_3$), 6.32 (d, 2H, C<u>H</u>$_2$ of pyrazoline ring), 7.01 (t, 1H, C<u>H</u>-Ar), 7.66-8.24 (m, 14H, Ar-<u>H</u>) (ppm); $[M]^+$ m/z 385.

Synthesis of [1-acetyl-5-(substitutedphenyl)-4-(3-chlorophenylazo)-2-pyrazolinyl]diphenyl amines (30-33):

To a solution of 3-chloroaniline (0.01 mol) in glacial acetic acid (5 ml), concentrated HCl (3 ml. CDH, New Delhi) was added at 0-5°. A solution of sodium nitrite (1 g in 5 ml water, Sarabhai M. Chemicals, Vadodara) was then added dropwise. The diazonium salt solution thus prepared was added separately in each solution of compounds **(26-29)** (0.01 mol) in methanol dropwise below 0°. These mixtures were kept for 2-3 d at room temperature and then poured into cold water (150 ml) separately. The solids thus obtained were filtered and recrystallised from appropriate solvents. Physical and analytical data of compounds **(30-33)** are given in Table 1. Compound **(33)**: IR (ν_{max}): 680 (C-Cl), 1660 (C=N), 1600 (C-N), 1530 (N-N), 1710 (C=O), 1430 (N=N), 3030 (aromatic C-H), 1550 (C=C of aromatic ring) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.66 (s, 3H, -COC<u>H</u>$_3$), 3.66 (s, 3H, Ar-OC<u>H</u>$_3$), 6.31 (d, 1H, C<u>H</u>-N=N-Ar), 7.35-8.44 (m, 18H, Ar-<u>H</u>; 1H, C<u>H</u>-Ar) (ppm); $[M]^+$ m/z 523.

The compounds 2-33 were subjected to antiinflammatory, ulcerogenic and acute toxicity studies. These studies were conducted on adult Charles Foster rats of (80-100 g, Meerut, UP.) and mice (20-25 g, Meerut, UP) of either sex. The rats and mice were divided into groups. Phenylbutazone (from commercial sources) was used as reference drug. The study protocol was approved by the ethical committee of Lala Lajpat Rai Memorial Medical College, Meerut, U.P., India.

Antiinflammatory activity:

Antiinflammatory activity against carrageenan-induced hind paw edema in albino rats was determined by the method of Winter *et al.*[13]. A freshly prepared suspension of carrageenan solution (1% in 0.9% saline) in a volume of 0.05 ml was injected under the planter aponeurosis of right hind paw of rats. The animals were pretreated with test drugs orally 1 h before carrageenan. The volume of the foot was measured 3 h after carrageenan treatment by the method of Buttle *et al.*[14]. The % antiinflammatory activity was calculated by comparing test with the control.

Ulcerogenic activity:

Ulcerogenic liability was performed by the method of Djanhanjuiri[15] in Charles Foster rats of either sex (80-150 g)

were divided into groups of 6 animals each. The rats were fasted to 24 h prior to the administration of drugs. Water was allowed *ad lib.* to the animals. The most potent compounds and phenylbutazone were given by intraperitoneal route except the control group. All animals were killed 8 h after dosing and the stomach, duodenum and jejunum were removed and microscopically examined to assess (i) shedding of epithelium (ii) petechial and frank hemorrhages (iii) erosion or discrete ulceration with or without perforation. The presence of any one of these conditions taken as an evidence for ulcerogenic activity.

Acute toxicity:

Approximate lethal dose (ALD_{50}) of all the compounds in albino mice of either sex (20-30 g) were determined by following the procedure of Smith[16]. The test compounds were administered orally at various dose levels in one group and the same volume of normal saline in another group. The drug treated mice were observed continuously for 24 h. The percent mortality in each group was observed. From the data obtained ALD_{50} was determined.

RESULTS

All the compounds of this series have been evaluated for antiinflammatory activity using the carrageenan-induced rat paw edema test at a dose of 50 mg/kg, orally. All newly synthesized compounds (2-33) have shown remarkable antiinflammatory activity of varying degree from 8.33-48.60% (Table 1). In this series isoxazolines (7 and 9) and pyrazoline (29) were found to be more potent than the standard drug phenylbutazone with 45.67%, 40.00% and 48.60%, respectively.

Ulcerogenic activity and acute toxicity:

The compounds, which showed prominent inhibition of edema (7, 9 and 29) were evaluated for ulcerogenic potentiality and were also compared with phenylbutazone (UD_{50} of 7=166.6 mg/kg i.p., UD_{50} of 9=199.6 mg/kg i.p., and UD_{50} of 29=138.6 mg/kg i.p. and UD_{50} of phenylbutazone=66.6 mg/kg i.p.). These three compounds have shown less ulcerogenic potentiality than phenylbutazone. All the compounds were also subjected to acute toxicity testing. ALD_{50} values were found to be >800 mg/kg p.o.

DISCUSSION

In this series chalcones **(2-5)** exhibited mild to moderate degree of antiinflammatory activity ranging from (15.5-26.0%). In route **(1)**, the cyclisation of chalcones into their corresponding isoxazolines (6-9), in general, enhanced the

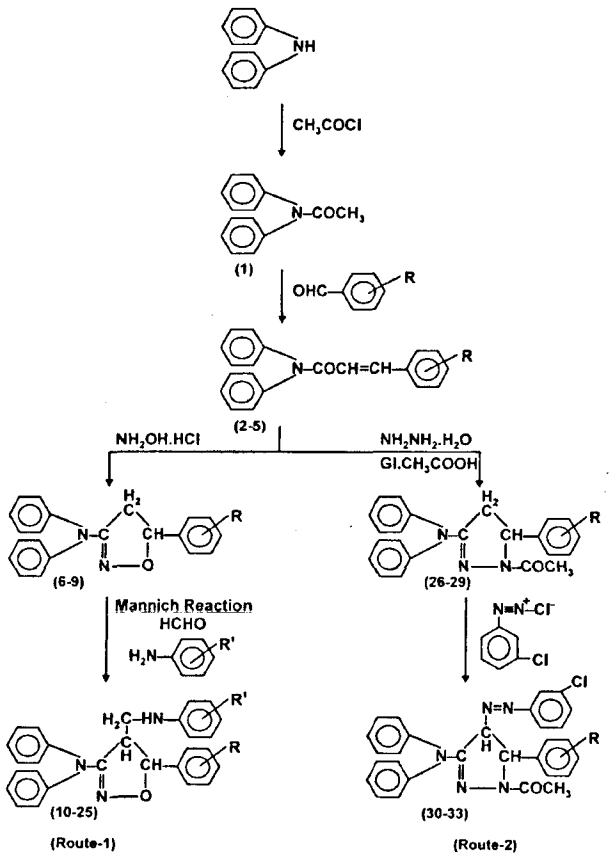

Fig. 1: Aminomethilisoxazolinyl/azopyrazolinyl diphenylamine derivatives.

activity (21.29-45.76%). Compound (7) showed 45.67% activity and was more potent than phenylbutazone. Mannich compounds (10-25) were found to possess mild to moderate degree of activity (8.33-33.33%).

Cyclisation of chalcones into pyrazolines (26-29), in general, enhanced the activity (18.18-48.60%). Compound (29), in which phenyl ring was substituted with methoxy group at para position elicited most potent activity (48.60%) at a dose of 50 mg/kg p.o. This compound was the most active member of this series. It was further studied at three dose levels (25, 50 and 100 mg/kg p.o.) and was found to be more potent than standard drug at all three dose levels. Moreover, the diazotisation of pyrazolines (26-29) yielded azo compounds (30-33), which exhibited promising antiinflammatory activity (15.00-35.00%). In this series, other two potent compounds 7 and 9 were also tested for antiinflammatory activity at three graded doses. By the observation of biological data of this series, it may be concluded that, 1. Isoxazolines (6-9) exhibited more potent activity than

chalcones (2-5) and aminomethyl derivatives (10-25), 2. Pyrazolines (26-29) showed higher degree of protection in inhibition of edema than their corresponding chalcones (2-5) and their azo derivatives (30-33) and 3. Compounds having an methoxy group at para position on phenyl ring as substitutent, elicited potent antiinflammatory activity.

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