SYNTHESIS, ANTIOXIDANT, ANTI-INFLAMMATORY ANTI-MICROBIAL ACTIVITY OF 2-[(5-SUBSTITUTED ARYL-1'N-SUBSTITUTED ARYL AMINO METHYL) PYRAZOL-3-YL] BENZOFURAN

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ABSTRACT

2-[(5-substituted aryl-1'N-substituted aryl amino methyl) pyrazol-3-yl] benzofuran (4_{a-l}) have been synthesized by treating 2-acetyl benzofuran (1) with different aromatic aldehyde in the presence of a strong base to give 2-substituted arylidene acetyl benzofuran (2_{a-c}) which on condensation with hydrazine hydrate in ethanol and glacial acetic acid yielded corresponding 2-(5-substituted aryl-4,5-dihydro pyrazolyl) benzofuran (3_{a-c}) followed by its reaction with aromatic amine and formaldehyde in glacial acetic acid. The structures of the synthesized compound have been established on the basis of IR, ^{1}H-NMR and MASS spectra data Compounds have been screened for anti-inflammatory, antioxidant and antibacterial studies. Among 12 compounds 4b, 4d, 4e, 4g, 4h, 4j, and 4l have shown good antioxidant activity, which were comparable with that of standard drug ascorbic acid. Among 7 compounds screened for anti-inflammatory activity, compounds 4b, 4d, 4h and 4l showed 81.02%, 84.89%, 85.89% and 82.02% inhibition of oedema volume, while the standard drug (Ibuprofen) showed inhibition of 91.15%. Among 12 compounds that were screened against three gram +ve (S.aureus , B.pumilus and B.subtilis) and two gram –ve (E.coli and P.aeruginosa) organisms, compounds 4c, 4e and4f have shown good activity as antibacterial antifungal agents comparable with that of standard drugs Amipicillin and Griseofulvin respectively.

Keywords: *Benzofuran, pyrazol, antioxidant, anti-inflammatory, antibacterial, antifungal.*

INTRODUCTION

Benzofuran[1-6] derivatives have been reported to possess sedative and hypnotic, anticonvulsant, CNS stimulant, antibacterial and antifungal activities. Pyrazole nucleus also has wide application in medicinal chemistry. It is reported that pyrazolines exhibit anti-inflammatory, anticarcinogenic, antidiabetic, anticonvulcant,, antibacterial, antifungal, antiviral, analgesic and antioxidant activities. These observations suggested that it would be of interest to study the activity of some new pyrazole derivatives. A series of these compounds have been synthesized and tested for their antibacterial, anti-inflammatory and antioxidant studies.

For the present investigation we have prepared 2-[(5-substituted aryl-1'N-substituted aryl amino methyl) pyrazol-3-yl] benzofuran **(4_{a-l})** starting from 2-acetyl benzofuran **(1)** which was

converted to corresponding 2-substituted arylidene acetyl benzofuran **(2_{a-c})** using different aromatic aldehydes in the presence of a strong base, which on condensation with hydrazine hydrate in ethanol and glacial acetic acid yielded corresponding 2-(5-substituted aryl-4,5-dihydro pyrazolyl) benzofuran **(3_{a-c})** followed by its reaction with aromatic amine and formaldehyde in glacial acetic acid.

EXPERIMENTAL

Melting points were determined by using Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using chloroform:carbon disulphide (1:1) solvent system and U.V lamp used as a visualizing agent. IR spectra's were recorded in cm^{-1} using KBr pellets on a Thermo Niolet Nexus 670 spectrophotometer. ^{1}H-NMR spectra on a Varian EM-200, advance 300 MHz spectrophotometer using $CDCl_3$ solvent and TMS as internal standard (chemical shift values expressed in δ ppm). Mass spectra recorded by ESI technique on an LC-MS spectrometer.

General method for the preparation of 2-substituted arylidene acetyl benzofuran (2_{a-c})[7-9] :2–acetyl benzofuran (0.02mole; 3.2g) **(1)** and substituted aromatic aldehydes (0.02mole) were taken in ethanol (25ml) and cooled. Aqueous sodium hydroxide solution (20N; 2.5ml) was added to the above solution with constant stirring, until the turbidity appears. The reaction mixture was further stirred for 2 hr which was left over night. The mixture was carefully acidified using dilute hydrochloric acid to get deep coloured (yellow–orange) solid. The course of the reaction was monitered by TLC plate using chloroform:carbon disulphide (1:1). The product obtained was filtered, washed with water and recrystallized from ethanol.

2b: IR (KBr) cm^{-1}: 1650 (C=O), 1620 (C=C str in aromatic nuclei), 1080 (C-O-C) of benzofuran and 1525 (C-NO_2) MS m/z 293 (M^+), 248, 235, 156, 129, 106, 91, 77, 65.

2c: IR (KBr) cm^{-1}: 1650 (C=O), 1620 (C=C str in aromatic nuclei), 1080 (C-O-C) of benzofuran and 2830 (C-CH_3 str). ^{1}H-NMR ($CDCl_3$) δppm: 6.68-7.00(m, 9H, Ar-H), 6.60(d, 1H, -COCH=), 8.60(d, 1H, =CH-Ar), 1.7(s, 3H, Ar-CH_3).

General method for the preparation of 2-(5-substituted aryl -4,5-dihydro pyrazol-3-yl) benzofuran (3_{a-c}): To a solution of the chalcone (0.015 mole) **(2_{a-c})** in ethanol (60ml) and glacial acetic acid (15ml) was added hydrazine hydrate (0.027mole; 1.30ml). The mixture was refluxed gently on a water bath for 8 hr. After cooling the separated solid was filtered and washed with a little ethanol followed by the water. The course of the reaction was monitored by TLC plate using chloroform:carbon disulphide (1:1). The compound obtained was recrystallized from ethanol.

3b: IR (KBr) cm^{-1}: 3353.60 (NH str), 3070.12 (C-H str), 1600.63 (C=C str in aromatic nuclei), 1517.70 (C-NO_2), 1344.01 (C-N), 1257.03 (C-O-C), 854.03 (C-N str).

General method for preparation of 2-[(5-substituted aryl-1'N-substituted aryl amino methyl) pyrazol-3-yl] benzofuran (4_{a-l}): A mixture of 2-(5-substituted aryl-4,5–dihydro pyrazol–3yl) benzofuran (0.002mole) **(3_{a-c})** aromatic amine (0.002mole) and formaldehyde (0.002mole;0.06ml) in glacial acetic acid (2ml) was refluxed for a period of 6 hr and cooled. The mixture was kept overnight and poured on ice cold solution of saturated sodium carbonate. The course of the reaction was monitered by TLC plate using chloroform:carbon disulphide (1:1). The product obtained was washed with water and recrystallized from ethanol and mixture of ethanol and benzene.

4c: IR (KBr) cm^{-1}: 3410.75cm^{-1} (NH str), 2922.76cm^{-1} (C-H str), 1661.91 cm^{-1} (C=N str), 1602.50cm^{-1} (C=C str in aromatic), 1312.60cm^{-1} (C-N str), 1258.03 cm^{-1} (C-O-C).

4g: IR (KBr) cm^{-1}: 3429.81cm^{-1} (NH str), 2922.43cm^{-1} (C-H str), 1661.53 cm^{-1} (C=N str), 1602.61cm^{-1} (C=C str in aromatic), 1454.33cm^{-1} (C-N str), 1261.63 cm^{-1}(C-O-C). ^{1}H-NMR ($CDCl_3$) δppm: 6.3–8.1 (m, 16H Ar-H), 4.3 (d 2H, CH_2), 4.0 (s, 1H, NH), 1.7 (s, 3H, Ar-CH3). MS m/z 426 (M^+), 304,214, 200, 185,144,116,91,77.

BIOLOGICAL EVALUATION [10-13]

Antioxidant activity: All the compounds were screened for antioxidant activity using DPPH method[10]. Drug have been diluted in 95% ethanol to get 1000 mg, 500 mg, 250 mg, 100 mg, 50 mg, 25
mg and 10 mg concentrations. DPPH solution (2mmol) was prepared in 95% ethanol. To0.5ml of drug solution, 0.5ml of DPPH solution (freshly prepared) was added mixed andthe reaction was allowed for 20 mins. UV absorbance was measured at 517nm. Ascorbicacid was used as a standard drug.

Anti-inflammatory activity: Male albino rats weighing between 100 – 200g were used for the experiment. They were procured from animal house, KMC, Manipal and individually housed, provided with adequate food and water. They were divided into various groups. These animals were used for antiinflammatory studies. Six pyrazoline derivatives were screened for antiinflammatory activity.The animals were dosed orally at 500, 1000 and 2000mg/kg body weight with all the test drugs. No visible toxic symptoms were observed for the first two hours and no death was reported after 24 hours. Since 2000 mg/kg body weight was observed as safe dose, the $1/10^{th}$ of 2000 mg/kg body weight i.e., 200mg/kg body weight was fixed as the dose for acute antiinflammatory screening.

Compounds with high antioxidant activity (7compounds) were selected and screened for antinflammatory activity using carrageenan induced paw oedema method. Ibuprofen was used as a standard drug. The results were presented in table.

Antibacterial activity: All the 12 synthesized compounds were screened against two gram+ve (*S.aureus* and *B.subtilis, B.pumilus*) and two gram-ve (*E.coli and P.aeuruginosa)* organisms using cup-plate method[12] at a concentration 100 µg/ml of drug per cup. Ampicillin was used as a standard drug at a concentration of 20 µg/ml.

Antifungal activity: All the 12 compounds were screened against *A.niger, C.utillis* organisms using cup-plate method[12] at a concentration 100µg/ml of drug per cup. Griseofulvin was used as standard drug at a concentration of 20 µg/ml.

RESULTS AND DISCUSSION

The structures of the compounds were confirmed by analytical and spectral data. Among 12 compounds 4b, 4d, 4e, 4g, 4h, 4j, and 4l have shown good antioxidant activity, which were comparable with that of standard drug ascorbic acid. Among 7 compounds screened for anti-inflammatory activity, compounds 4b, 4d, 4h and 4l showed 81.02%, 84.89%, 85.89% and 82.02% inhibition of oedema volume, while the standard drug (Ibuprofen) showed inhibition of 91.15%. Among 12 compounds that were screened against three gram +ve (*S.aureus , B.pumilus and B.subtilis*) and two gram –ve (*E.coli and P.aeruginosa*) organisms, compounds 4c, 4e and4f have shown good activity as antibacterial antifungal agents comparable with that of standard drugs Amipicillin and Griseofulvin respectively.

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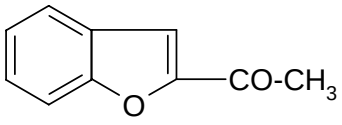
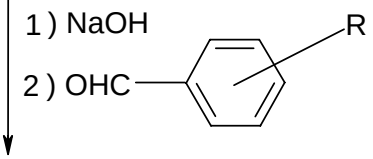
TABLE-1: PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS (4_{a-l})

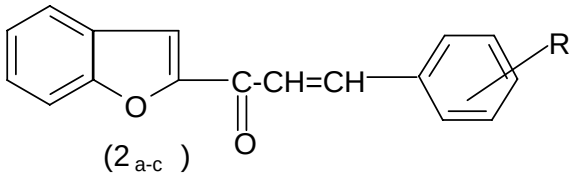
Compound Code	R	R'	Melting Point ^{0}C	Yield %	Molecular formula	Molecular Weight	R_f* Value
4_a	4–chloro	4–chloro	130	85	$C_{24}H_{19}N_3OCl_2$	435	0.80
4_b	4–chloro	4–nitro	148	80	$C_{24}H_{19}N_4O_3Cl$	446	0.60
4_c	4–chloro	4-methyl	108	78	$C_{25}H_{22}N_3OCl$	415	0.87
4_d	4–chloro	4-methoxy	135	81	$C_{25}H_{22}N_3O_2Cl$	431	0.77
4_e	4–nitro	4–chloro	165	80	$C_{24}H_{19}N_4O_3Cl$	446	0.67
4_f	4–nitro	4–nitro	152	78	$C_{24}H_{19}N_5O_5$	457	0.83
4_g	4–nitro	4-methyl	135	75	$C_{25}H_{22}N_4O_3$	426	0.76
4_h	4–nitro	4–methoxy	138	72	$C_{25}H_{22}N_4O_4$	442	0.71
4_i	4–methyl	4-chloro	136	84	$C_{25}H_{22}N_3OCl$	415	0.91
4_j	4–methyl	4–nitro	128	80	$C_{25}H_{22}N_4O_3$	426	0.81
4_k	4–methyl	4-methyl	118	76	$C_{26}H_{25}N_3O$	395	0.75
4_l	4–methyl	4-methoxy	125	78	$C_{26}H_{22}N_3O_2$	411	0.83



(1)

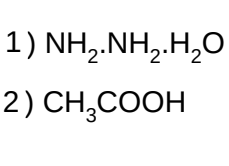
2 - Acetyl benzofuran

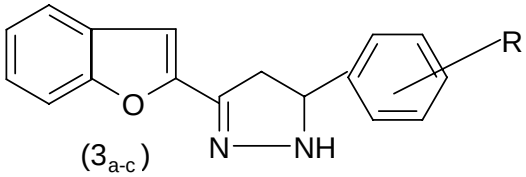




(2_{a-c})

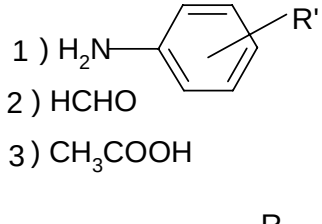
2 - Substituted arylidene acetyl benzofuran

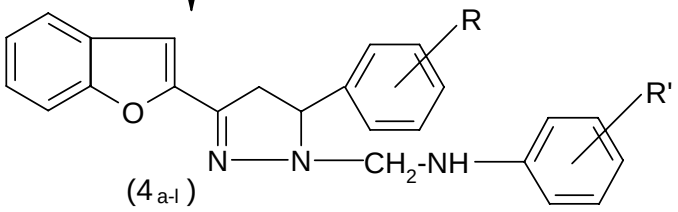




(3_{a-c})

2 (5 - substituted aryl - 4,5 - dihydro pyrazol - 3 yl) benzofuran





(4_{a-l})

2 - [(5 - substituted aryl - 1'N - substituted aryl amino methyl) pyrazol - 3 - yl] benzofuran.

R= 4-Cl, 4-NO_2 , 4-CH_3

R' = 4-Cl, 4-NO_2 , 4-OCH_3

TABLE- 2: ANTIOXIDANT ACTIVITY

	Control	10µg	50µg	100µg	250µg
Std.	0.100±0.1920	0.06±0.017(40.00)	0.020±0.171(80.00)	0.018±0.19(82.00)	0.012±0.03(88.00)
4a	0.130±0.0920	0.12±0.117(7.69)	0.01±0.071(23.00)	0.09±0.019(30.00)	0.088±0.03(32.00)
4b	0.100±0.0920	0.08±0.0117(20.00)	0.030±0.071(70.00)	0.025±0.19(75.00)	0.022±0.03(78.00)
4c	0.101±0.1920	0.06±0.017(39.62)	0.04±0.171(60.39)	0.03±0.19(40.59)	0.0162±0.03(83.96)
4d	0.101±0.1920	0.089±0.017(39.62)	0.069±0.171(31.68)	0.06±0.19(40.59	0.061±0.03(49.50)
4e	0.110±0.0192	0.093±0.01(15.45)	0.03±0.0171(72.72)	0.02±0.19(81.81)	0.158±0.03(85.63)
4f	0.101±0.1120	0.075±0.117(28.21)	0.071±0.0171(30.69)	0.0571±0.029(43.46)	0.042±0.04(58.41)
4g	0.100±0.0192	0.08±0.0017(20.00)	0.031±0.0171(70.00)	0.0171±0.019(82.82)	0.0176±0.013(82.36)
4h	0.110±0.1520	0.09±0.02(18.18)	0.06±0.0171(45.45)	0.052±0.19(52.27)	0.05±0.022(54.54)
4i	0.140±0.0190	0.012±0.01(14.28)	0.101±0.0171(27.85)	0.099±0.19(29.28)	0.09±0.03(35.71)
4j	0.101±0.1620	0.08±0.011(20.79)	0.04±0.0171(60.39)	0.028±0.19(66.36)	0.017±0.03(8316)
4k	0.130±0.1920	0.0142±0.01(6.15)	0.10±0.0171(23.07)	0.09±0.0191(30.76)	0.088±0.031(82.00)
4l	0.120±0.0190	0.098±0.01(19.00)	0.075±0.017(38.01)	0.05±0.19(58.67)	0.04±0.03(82.00)

TABLE-3: ANTI-INFLAMMATORY STUDIES

Sl. No.	Drug code	Dose (mg/kg)	Mean oedema volume ± S.E. (0-3 hrs)	% reduction in oedema volume
1.	Control		0.43 ± 0.182	
2.	Ibuprofen	200	0.0412 ± 0.017	91.15
3	4b	200	0.084 ± 00.11[a]	81.02
4.	4d	200	0.073±0.02[a]	84.89
5.	4e	200	0.19 ± 0.171[a]	59.57
6.	4g	200	0.25 ± 0.129[a]	40.40
7	4h	200	0.07 ± 0.047[a]	85.89
8.	4j	200	0 .175 ± 0.03[a]	60.27
9.	4l	200	0.089 ± 0.03[a]	82.02

One-way ANOVA followed by schiffe's post hoc test. Allowance value = 0.239.

a = $P<0.05$ (Vs) control.

Note: Any two means showing difference of 0.239 are statistically significant

TABLE-4:ANTIMICROBIAL ACTIVITY

Sample Code	*Inhibition zone diameter in mm						
	SA	***BS***	***BP***	***EC***	***PA***	***AN***	***CU***
4_a	16	15	16	15	16	13	12
4_b	13	16	14	13	15	14	11
4_c	17	20	18	18	20	13	10
4_d	13	15	14	15	14	10	10
4_e	18	20	18	19	21	15	12
4_f	17	20	19	17	20	17	12
4_g	14	14	15	16	15	12	13
4_h	12	13	11	13	16	12	17
4_i	13	16	12	14	13	14	10
4_j	16	14	16	14	16	15	11
4_k	15	13	15	12	17	15	11
4_l	13	15	13	14	15	14	13
Amp (20 µg/ml)	20	22	20	20	22		
Gri.vin (20µg/ml)	-	-	-	-	18	17	

***Average of triplicate ± Standard deviation**

Note: Concentration of standard compound is 20µg/ml.

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