Synthesis and Biological Evaluation of some Novel Pyrazolines

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Chalcones (2a and 2b) were prepared from 2-acetyl benzofuran (1) and condensed with different aromatic acid hydrazides (3a-o) to get the corresponding pyrazolines (4a-o and 5a-o). The structures of all these compounds have been established on the basis of analytical and spectral data. Compounds have been screened for antiinflammatory, antioxidant and antibacterial studies. Among the 7 compounds that were screened for antiinflammatory activity, compounds 4g and 5m showed 83.4% and 80.5% inhibition of oedema volume, while the standard drug (ibuprofen) showed inhibition of 91.9%. Compounds 4k and 5h showed moderate activity of 72.8% and 59.6% respectively. All the 30 compounds were tested for antioxidant activity at 1000, 500, 250, 100, 50, 25 and 10 mg/ml concentrations against standard drug ascorbic acid. Compounds 4g, 4h, 4k, 4m, 5g, 5h, 5k and 5m showed excellent antioxidant activity as compared with ascorbic acid. Among the 30 compounds that were screened against two Gram +ve (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram –ve (*Escherichia coli* and *Pseudomonas aeruginosa*) organisms, compounds possessing p-chloro, p-fluoro, 2-amino-5-bromo, 2-hydroxy-5-nitro and 3,5-dichloro substitutions on the phenyl ring showed good activity against *Escherichia coli* and *Bacillus subtilis*. The activity is comparable with that of the standard drug ciprofloxacin.

Key words: Benzofuran, pyrazoline, antioxidant, antiinflammatory, antibacterial

In view of the various activities reported for compounds possessing benzofuran[1-2] and pyrazoline moiety[3-5], in the present study we attempted to synthesize benzofuran pyrazolines, as they appeared to be highly promising. Since the compounds are mainly targeted for antiinflammatory activity, an acidic group is introduced at the 5th position on the pyrazoline ring in the form of m (or) p-phenoxyacetic acid. Benzofuran chalcones (2a and 2b) were synthesized by reacting 2-acetyl benzofuran (1) with m (or) p-formyl phenoxyacetic acid in the presence of aqueous sodium hydroxide (10%). The chalcones on refluxing with substituted acid hydrazides (3a-o) in the presence of glacial acetic acid at above 130° for a period of 10 h afforded different pyrazolines (4a-o and 5a-o) and were recrystallized from boiling aqueous ethanol. The structure of chalcones and pyrazolines were confirmed by mp, tlc and spectral data.

The melting points of the compounds were determined on a Toshniwal electric melting point apparatus and the values were uncorrected. IR spectra of the compounds were recorded on a Shimadzu-FTIR 8300 using the KBr disc method. ^{1}H NMR spectra were recorded on a Joel-GSX 400, (IIT Chennai) using DMSO-d_6 as solvent. Mass spectra were recorded on a Shimadzu-GCMS 50508. All the solvents used were of analytical grade.

2-acetylbenzofuran (1) was prepared following the literature method[6]. Chalcone (2a/2b) was prepared by adding a solution of sodium hydroxide (8 ml, 10% w/v solution of NaOH in water) to a well-stirred solution of 2-acetyl benzofuran (0.01 mol) and m/p-formyl phenoxy acetic acid (0.01 mol) in 20 ml of ethanol at room temperature. The solution was stirred at room temperature for 24h using a magnetic stirrer. The reaction mixture was diluted with ice-cold water and acidified with concentrated HCl. The product obtained was filtered and washed with ice-cold water and recrystallized from aqueous ethanol. Solvent system used for tlc was chloroform:acetone (1:1). Pyrazolines (4a-o and 5a-o) were prepared as shown in scheme 1 by taking chalcone (0.01 mol) and aromatic acid hydrazide (0.02 mol) in 20 ml glacial acetic acid and refluxing at 130° for a period of 10 h. The reaction mixture was concentrated, poured into 300 ml of ice-cold water and the product was recrystallized from aqueous ethanol. The solvent system used for tlc was a 1:1 mixture of acetone: methanol. The yield and melting points are given in Table 1. 1-benzoyl-3-(benzofuran-2-yl)-5-(4'-carboxymethyleneoxyphenyl)-2-pyrazoline (4a): IR

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TABLE 1: PHYSICAL DATA OF SYNTHESIZED COMPOUNDS

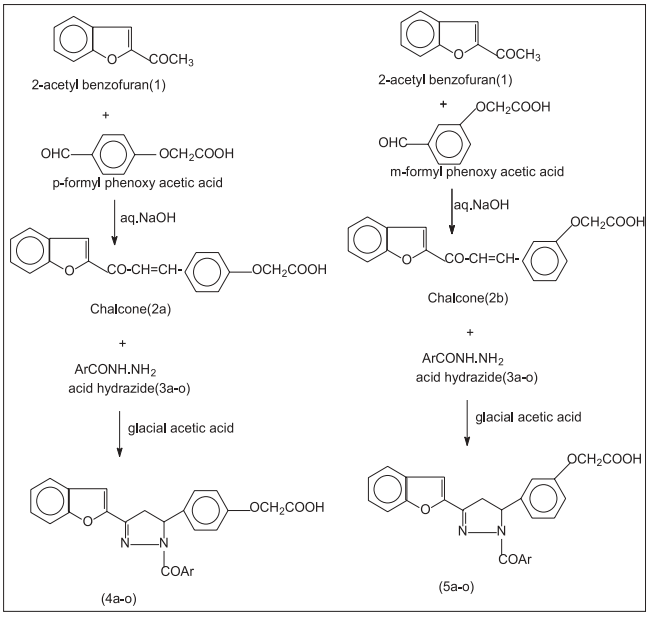
Comp.	Molecular formula	Ar	M.P. (O)	Yield (%)	Rf*
4a	$C_{26}H_{20}N_2O_5$	Phenyl	96-98	62	0.60
4b	$C_{26}H_{20}N_2O_6$	4-hydroxy phenyl	85-90	45	0.9
4c	$C_{26}H_{19}N_2O_5Cl$	4-chloro phenyl	80-85	75	0.66
4d	$C_{26}H_{19}N_2O_5Cl$	2-chloro phenyl	106-108	78	0.7
4e	$C_{26}H_{19}N_3O_7$	4-nitro phenyl	95-97	63	0.82
4f	$C_{26}H_{19}N_3O_7$	2-nitro phenyl	93-96	32	0.88
4g	$C_{26}H_{19}N_2O_5F$	4-fluoro phenyl	89-93	57	0.87
4h	$C_{27}H_{22}N_2O_5$	o-tolyl	120-122	67	0.8
4i	$C_{26}H_{20}N_2O_6$	2-hydroxy phenyl	80-83	68	0.89
4j	$C_{26}H_{21}N_3O_5$	4-amino phenyl	120-122	54	0.83
4k	$C_{25}H_{20}N_3O_5$	isonicotinyl	98-102	58	0.78
4l	$C_{27}H_{21}N_2O_6$	4- methoxy phenyl	89-92	72	0.85
4m	$C_{26}H_{20}N_3O_5Br$	2-amino-5-bromo phenyl	116-120	76	0.9
4n	$C_{26}H_{19}N_3O_8$	2-hydroxy- 5-nitro phenyl	123-125	66	0.89
4o	$C_{26}H_{18}N_2O_5Cl_2$	3,5 dichloro phenyl	100-112	62	0.75
5a	$C_{26}H_{20}N_2O_5$	Phenyl	100-103	60	0.62
5b	$C_{26}H_{20}N_2O_6$	4-hydroxy phenyl	90-93	42	0.89
5c	$C_{26}H_{19}N_2O_5Cl$	4-chloro phenyl	91-93	73	0.65
5d	$C_{26}H_{19}N_2O_5Cl$	2-chloro phenyl	100-105	73	0.7
5e	$C_{26}H_{19}N_3O_7$	4-nitro phenyl	90-94	35	0.82
5f	$C_{26}H_{19}N_3O_7$	2-nitro phenyl	89-91	52	0.87
5g	$C_{26}H_{19}N_2O_5F$	4-fluoro phenyl	115-120	67	0.86
5h	$C_{27}H_{22}N_2O_5$	o-tolyl	118-120	68	0.8
5i	$C_{26}H_{20}N_2O_6$	2-hydroxy phenyl	89-90	69	0.82
5j	$C_{26}H_{21}N_3O_5$	4-amino phenyl	116-118	72	0.83
5k	$C_{25}H_{20}N_3O_5$	isonicotinyl	113-115	70	0.78
5l	$C_{27}H_{21}N_2O_6$	4- methoxy phenyl	96-99	32	0.85
5m	$C_{26}H_{20}N_3O_5Br$	2-amino-5-bromo phenyl	112-116	35	0.9
5n	$C_{26}H_{19}N_3O_8$	2-hydroxy- 5-nitro phenyl	120-122	64	0.89
5o	$C_{26}H_{18}N_2O_5Cl_2$	3,5 dichloro phenyl	113-116	60	0.74

All the synthesized compounds were recrystallized in aqueous ethanol and *Solvent system used was acetone: methanol (1:1)

(KBr): 1735.8 (C=Ostr of COOH), 1662.5 (N-C=Ostr), 1608.5 (C=Nstr), 1548.7 (C=Cstr), 1139.9 (C-O-C Str), 713.6, 750.3 (mono substituted phenyl); ^{1}H NMR (DMSO) δppm: 3.1-3.5 (d, 2H, CH_2 of pyrazoline); 4.5-5.5 (t, 1H, N-CH-Ar); 10.5 (s, 1H of COOH); 6-9 (m, 14H, Ar-H); 4.6-4.9 (s, 2H of OCH2COOH); MS: (m/z): 440(M^+), 262.2 (100%), 248.1,186.

1-(2''-methylbenzoyl)-3-(benzofuran-2-yl)-5-(4'-carboxymethyleneoxyphenyl)-2-pyrazoline (5h): IR (KBr): 2923.9 (C-H str), 1666.4 (N-C=O str), 1610.5 (C=N str), 1548.7 (C=Cstr), 1139.9 (C-O-Cstr.); ^{1}H NMR(DMSO) δppm: 3.1-3.5 (d, 2H, CH_2 of pyrazoline); 4.5-5.5 (t, 1H, N-CH-Ar); 10.5 (s, 1H of OH), 10.1 (s, 1H of COOH); 6-9 (m, 13H, Ar-H); 4.6-4.9 (2s, 2H of OCH_2COOH); MS: (m/z): 454 (M^+), 336, 262, 248, 186, 158, 144.

All the compounds were screened for antioxidant activity using DPPH method[7]. Stock solutions of synthetic compounds have been diluted in 95% ethanol to obtain 1000, 500, 250, 100, 50, 25 and 10 mg concentrations. DPPH solution (2 mmol) was prepared in 95% ethanol. To 0.5 ml of drug solution, 0.5 ml of DPPH solution (freshly prepared) was added, mixed and the reaction was allowed for 20 min. UV absorbance was measured at 517 nm. Ascorbic acid was used as a standard drug. Percentage scavenging for each drug was calculated using the formula, (control absorbance-test absorbance/ control absorbance)×100. The results were presented graphically in fig. 1.



Scheme 1: Synthesis of pyrazolines bearing benzofuran moiety.

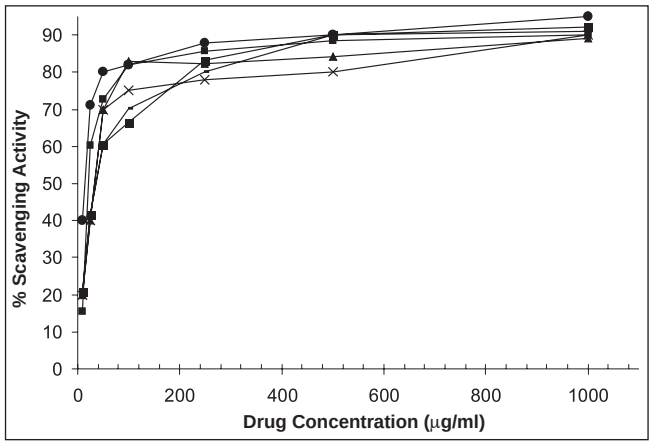


Fig. 1: Comparative antioxidant profiles of ascorbic acid (standard) and different concentrations of selected drugs.
Standard ascorbic acid (—◆—), 4h (—●—), 4k (—▲—), 4 m (—×—), 5 g (—■—) and 5k (—▪—).

Compounds with high antioxidant activity (7 compounds) were selected and screened for antiinflammatory activity using carrageenan-induced paw oedema method[8]. Ibuprofen was used as a standard drug and the results are shown in Table 2.

All the 30 synthesized compounds were screened against two gram+ve (*S. aureus* and *B. subtilis*) and two gram-ve (*E. coli* and *P. aeuruginosa)* organisms using cup-plate method[9] at a concentration of 10 mg and 25 mg of drug per cup. Ciprofloxacin was used as a standard drug at a concentration of 10 mg. The results are shown in Tables 3 and 4.

Among the 30 compounds synthesized, compounds 4g, 4h, 4k, 4m, 5g, 5h, 5k and 5m have shown good antioxidant activity, which was comparable with that of standard drug, ascorbic acid. Among the seven compounds screened for antiinflammatory activity, compounds 4g and 5m showed 83.9% and 80.5% inhibition of oedema volume, while the standard drug (ibuprofen) showed 91.9% inhibition. Compounds 4k and 5h showed moderate activity (72.8% and 59.6%). A good correlation was observed between antioxidant activity and antiinflamatory activity among the above compounds. The results clearly indicated that benzofuran pyrazolines with m/p-phenoxyacetic acid moiety at 5th position and with suitably substituted phenyl ring at 1st position on pyrazoline ring can exhibit good antioxidant and antiinflammatory activities. Among the 30 compounds that were screened against two gram +ve (*S. aureus* and *B. subtilis*) and two gram –ve (*E. coli* and *P. aeruginosa*) organisms, compounds possessing electron releasing groups (p-chloro, p-fluoro, 2-amino-5-bromo, 2-hydroxy-5-nitro and 3,5- dichloro) on the phenyl ring showed good activity against *E. coli* and *B. subtilis* and the activity is comparable with that of the standard drug ciprofloxacin.

TABLE 2: ANTIINFLAMMATORY STUDIES OF SYNTHESIZED COMPOUNDS

Synthetic compound code	Dose (mg/kg)	Mean oedema volume ± S.E. (0-3 hrs)	% reduction in oedema volume
Control	-	0.42±0.192	
Ibuprofen	200	0.0416±0.017	91.13
4c	200	0.26±0.19[a]	32.89
4e	200	0.28±0.171[a]	40.14
4g	200	0.075±0.03[a]	83.89
4k	200	0.19±0.077[a]	59.57
5b	200	0.27±0.17[a]	40.39
5h	200	0.12±0.049[a]	72.79
5m	200	0.09±0.037[a]	80.49

5% Allowance value is 0.239 (Scheffe's method), [a]$P<0.05$ Vs control note: Any two means showing a difference of 0.239 are statistically significant.

TABLE 3: ANTIBACTERIAL STUDIES OF SYNTHESIZED COMPOUNDS

Comp.	Amount of drug per cup	Zone of inhibition (mm)			
		Staph. aureus	*Bacillus subtilis*	*E. coli*	*P. aeruginosa*
4a	10 μg	12	-	-	-
	25 μg	14	-	-	-
4b	10 μg	13	-	14	11
	25 μg	14	-	15	12
4c	10 μg	14	16	12	-
	25 μg	15	18	13	-
4d	10 μg	-	-	-	-
	25 μg	-	-	-	-
4e	10 μg	14	14	14	-
	25 μg	15	16	15	-
4f	10 μg	-	-	-	-
	25 μg	-	-	-	-
4g	10 μg	14	16	12	12
	25 μg	15	18	16	13
4h	10 μg	-	-	-	-
	25 μg	-	-	-	-
4i	10 μg	-	-	-	-
	25 μg	-	-	-	-
4j	10 μg	-	-	-	-
	25 μg	-	-	-	-
4k	10 μg	11	10	10	-
	25 μg	12	12	11	-
4l	10 μg	-	-	-	-
	25 μg	-	-	-	-
4m	10 μg	13	15	13	-
	25 μg	14	17	15	-
4n	10 μg	12	16	14	-
	25 μg	13	17	16	-
40	10 μg	-	14	14	-
	25 μg	-	17	17	-

'-' indicates resistant and standard drug used was ciprofloxacin

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TABLE 4: ANTIBACTERIAL STUDIES OF SYNTHESIZED COMPOUNDS

Comp.	Amount of drug per cup	Zone of inhibition (mm)			
		Staph. aureus	*Bacillus subtilis*	*E. coli*	*P. aeruginosa*
5a	10 µg	10	-	-	-
	25 µg	11	-	-	-
5b	10 µg	12	-	13	10
	25 µg	13	-	14	11
5c	10 µg	12	14	10	-
	25 µg	14	15	12	-
5d	10 µg	-	-	-	-
	25 µg	-	-	-	-
5e	10 µg	14	14	14	-
	25 µg	15	16	15	-
5f	10 µg	-	-	-	-
	25 µg	-	-	-	-
5g	10 µg	14	16	12	12
	25 µg	15	18	16	13
5h	10 µg	-	-	-	-
	25 µg	-	-	-	-
5i	10 µg	-	-	-	-
	25 µg	-	-	-	-
5j	10 µg	-	-	-	-
	25 µg	-	-	-	-
5k	10 µg	11	10	10	-
	25 µg	12	12	11	-
5l	10 µg	-	-	-	-
	25 µg	-	-	-	-
5m	10 µg	11	12	14	-
	25 µg	12	13	16	-
5n	10 µg	10	14	13	-
	25 µg	11	16	17	-
5o	10 µg	-	14	13	-
	25 µg	-	16	16	-
Std.	10 µg	21	20	20	19

'-' indicates resistant and standard drug used was ciprofloxacin

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