

# Synthesis of Novel Furobenzopyrone Derivatives and Evaluation of their Antimicrobial and Antiinflammatory Activity

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Certain 4'-(4''-substituted phenyl)-4-methylfurobenzopyrones were synthesized and evaluated for antibacterial activity. Six of the synthesized compounds were also screened for their antiinflammatory activity. Substituted resorcinols were condensed with ethyl acetoacetate to afford different coumarins (2a-c). Various substituted phenacyl bromides (4a-g) were prepared by the bromination of para-substituted acetophenones. The coumarins (2a-c) and phenacyl bromides (4a-g) were condensed to give oxoethers (5a-s). These were cyclised by using 1 M sodium hydroxide to afford the desired furobenzopyrone derivatives (FCa-s). All the compounds have been evaluated for their antibacterial activity against different strains of gram positive and gram negative bacteria. All the compounds have shown good activity against *Pseudomonas aeruginosa*. Compounds, 3-(4-chlorophenyl)-5-methylfuro-[3,2-g][1]benzopyran-7-one, 3-(4-chlorophenyl)-5,9-dimethylfuro[3,2-g][1]benzopyran-7-one and 4,5-dimethyl-3-phenylfuro[3,2-g][1]benzopyran-7-one (FCe, FCi, FCn) were active against *E. coli*. A few compounds showed

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\*For correspondence

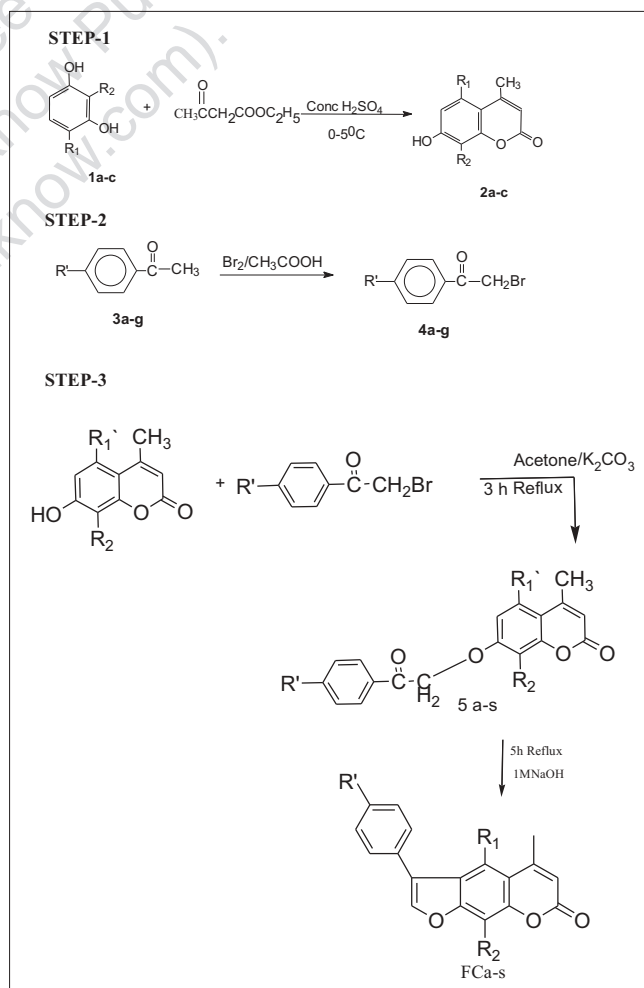
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moderate activity against *Bacillus subtilis* also. Antiinflammatory activity of six selected compounds was also tested using the carrageenan-induced rat paw oedema method. Among them, 5-methyl-3-p-tolylfuro[3,2-g][1]benzopyran-7-one (FCg) showed excellent activity. 5-Methyl-3-phenylfuro[3,2-g][1]benzopyran-7-one (FCa) and 4,5-dimethyl-3-(4-nitrophenyl)-furo[3,2-g][1]benzopyran-7-one (FCc) showed activity comparable to that of the standard drug ibuprofen.

Furobenzopyrone, also known as furocoumarin, the basic moiety in the present study occurs in many of the natural products. Members of the family Apiaceae (Umbelliferae) are well known producers of furobenzopyrones. They have several interesting biological activities such as analgesic, antiinflammatory, antioxidant, antifungal, antibacterial, antiviral and anticoagulant, in addition to their well known photosensitizing effect<sup>1-4</sup>. However there are only a few reports of their antibacterial action. Hence our study was aimed at the synthesis of novel 4'-para substituted phenyl-4-methylfurobenzopyrones and screening for antibacterial activity. Six of the synthesized compounds were also screened for their antiinflammatory activity.

Resorcinol (1a), 2-methylresorcinol (1b), 5-methylresorcinol (1c), were reacted with ethyl acetoacetate in the presence of concentrated H<sub>2</sub>SO<sub>4</sub> at 5-10<sup>0</sup> to give 7-hydroxy-4-methylcoumarin (2a), 4,5-dimethylcoumarin (2b), 4,8-dimethylcoumarin (2c), respectively, by Pechmann condensation. Acetophenone (3a), para substituted nitro (3b), fluoro (3c), chloro (3d), methyl (3e), methoxy (3f), and bromo (3g) acetophenones were brominated by using bromine in acetic acid to give phenacyl bromide (4a), p-nitro (4b), p-fluoro (4c), p-chloro (4d), p-methyl (4e), p-methoxy (4f) and p-bromo (4g) phenacyl bromides, respectively. The above prepared coumarins and phenacyl bromides were reacted by Williamson's synthesis to give oxoethers (5a-s). The oxoethers synthesized were cyclised by 1 M sodium hydroxide to afford the desired furobenzopyrone derivatives (FCa-s). The synthetic scheme leading to the target compounds is not shown any were in the manuscript. Scheme was given in the original manuscript send by us. here we are attaching the word file containing the scheme kindly include it in the manuscript for publication. The constitution of the products has been supported by IR, NMR, and mass spectral data. In the NMR spectrum of the compound 4a the singlet at  $\delta$  9.8 and  $\delta$  6.2 both integrating for one proton can be assigned to '7- OH' and 3-H hydrogen of benzopyrone, respectively while NMR spectrum of 2a showed a singlet at  $\delta$  4.5 integrating for two protons assignable to '-CH<sub>2</sub>Br' function of phenylacyl bromide. In the NMR spectrum of

the compound 5a, the fairly downfield singlet at  $\delta$  5.6 integrating for two protons assigned to 'CO-CH<sub>2</sub>-O-' function, proved the formation of -CH<sub>2</sub>O- grouping adjacent to carbonyl function. Other peaks include singlet at  $\delta$  6.1 and 7.0 both integrating for single proton assigned to the hydrogen in the third and fifth positions respectively. In the NMR spectrum of the compound FCa-s, the disappearance of peak at  $\delta$  5.6 and the appearance of a singlet at  $\delta$  7.2 corresponding to the aromatic 5' proton of furan ring confirmed that the oxoethers were successfully cyclised to the desired furobenzopyrone derivatives. The mass spectrum obtained for the compound FCa showed M<sup>+</sup> peak, which was also the base peak, at 276, exactly corresponding to



Scheme 1: Synthetic scheme of the title compounds.

its molecular weight. Thus the mass spectral data further corroborated the results. The compounds have been evaluated for antimicrobial activity against four different bacterial strains: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* at different concentrations of 25 and 50 µg/ml. Six of the synthesized compounds were screened for antiinflammatory activity using the carrageenan-induced rat paw oedema method and the results were subjected to one way ANOVA and the level of significance was tested by Scheffe's method. Melting points were determined in open capillary Toshniwal melting point apparatus and are uncorrected. UV absorbance was taken on Shimadzu UV/VIS spectrophotometer (UV-1601PC). Infrared spectra were recorded on Shimadzu FT-IR-8300/8700, Koyoto, Japan as KBr disc ( $\nu$   $\text{cm}^{-1}$ ). The  $^1\text{H-NMR}$  (300 MHz) spectra were obtained on a DPX-200 Bruker FT-NMR spectrometer using TMS as internal standard and are reported as parts per million ( $\delta$  ppm) downfield to TMS. Mass spectra were taken on GC-MS system at MCOPS, Manipal. Purity of the compounds were routinely assessed by TLC (solvent system: benzene: petroleum ether 6:4) on silica gel (Merck, Darmstadt, Germany). Visualization: Exposure to UV at 254 nm.

Preparation of substituted 7-hydroxy-4-methyl coumarins (2a-c)<sup>5</sup> was achieved by placing 5 g, (0.068 mol) of pure resorcinol (1a) in a 250 ml beaker and adding 6.5 ml of distilled ethyl acetoacetate and mixing to get a clear solution. This solution was cooled to 5-10<sup>o</sup>. Meanwhile, 25 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was cooled to about 5-10<sup>o</sup> in an ice bath. The above solution was added drop by drop into concentrated H<sub>2</sub>SO<sub>4</sub> with vigorous stirring. The resultant dark green solution was cooled and was stirred into crushed ice. The solid product thus separated out, was allowed to settle down. Crude product was filtered off, repeatedly washed with water and recrystallised from methanol as pale yellow flakes, mp: 185-186<sup>o</sup> (lit<sup>5</sup>.mp. 185-186<sup>o</sup>). NMR ( $\delta$  ppm): 9.8 (s, 1H, 7-OH), 6.2 (s, 1H, 3-H), 2.4 (s, 3H, 4-Me), 6.6 (s, 1H, 8-H), 6.7 (d, 1H, 6-H), 7.6 (d, 1H, 5-H). Similarly all the other derivatives were prepared. Yield ranged from 80-85% in all the cases.

Synthesis of substituted phenacyl bromides (4a-g)<sup>6</sup> was achieved using the following procedure. To 20 g (0.166 mol) of acetophenone (3a) dissolved in 30 g of glacial acetic acid, 28 g of bromine was added slowly and with constant shaking. If towards the end of the preparation the hydrobromic acid was not given off readily, the reaction flask was put in hot water for a few min. The colour of the solution lightened to straw yellow. After the

complete addition of bromine (heat was developed in the bromination) the solution was poured into crushed ice and was left to stand for an hour. The crude product precipitated was filtered by suction and recrystallised from hot methanol as white shining needles, mp: 50<sup>o</sup> (lit<sup>6</sup>.mp. 50<sup>o</sup>). NMR ( $\delta$  ppm): 4.5 (s, 2H, -CH<sub>2</sub>Br), 7.5-7.8 (m, 5H, Ar-H). Similarly all the other derivatives were prepared.

Synthesis of substituted 4-methyl-7- (21-oxophenacyloxy)-coumarin (5a-s)<sup>7,8</sup> was achieved by heating under reflux a mixture of 7-hydroxy-4-methylcoumarin (0.0088 mol) (2a), phenacyl bromide (0.019 mol) (4a) and potassium carbonate (1 g) in ethyl methyl ketone (50 ml) till 2a disappeared (TLC). The mixture was cooled; the solid was filtered off and washed with fresh acetone. The solvent was evaporated from combined filtrate and washings under reduced pressure and the residue collected was crystallized from glacial acetic acid to give 5a, mp: 126<sup>o</sup>. IR ( $\text{cm}^{-1}$ ): 1703 C=O Str, ( $\alpha$ ,  $\beta$  unsaturated  $\delta$  lactone) 1230.5 (Ar-O-C Str). NMR ( $\delta$  ppm): 5.6 (s, 2H, CO-CH<sub>2</sub>-O), 2.4 (s, 3H, 4-Me), 7.0 (s, 1H, 8-H), 6.1 (s, 1H, 3-H), 7.6 (m, 5H, 2'-Ar), 8.0 (d, 2H, 6-H & 8-H). Similarly all the other derivatives were prepared.

Synthesis of substituted-4-methylfurobenzopyrone derivatives (FCa-s)<sup>9,10</sup> was completed using the following method. Oxoethers 5 (a-s) (7.9 mmol) were refluxed in 1 M NaOH (500 ml) for 5 hr. The mixture was cooled and acidified with 1 M HCl, and the precipitate formed was collected, washed with water and crystallized from glacial acetic acid to give the desired furocoumarins (FCa-s). Physical data are given in Table 1. For FCa: IR ( $\text{cm}^{-1}$ ): 1716.5, C=O Str ( $\alpha$ ,  $\beta$  unsaturated  $\delta$ -lactone), 1141.8 (C-O-C Str). NMR ( $\delta$  ppm) : 2.4 (s, 3H, 4-Me), 6.2 (s, 1H, 3-H), 7.2 (s, 1H, 5'-H), 7.6 (m, 5H, 4'-Ar-H), 7.8 (s, 1H, 8-H), 7.9 (s, 1H, 5-H). Mass: 276 (M<sup>+</sup>).

All the synthesized compounds (FCa-s) were screened for antibacterial activity using cup-plate agar diffusion method by measuring the zone of inhibition in mm. Ciprofloxacin (25 µg/ml) was used as standard for antibacterial activity. The compounds were screened for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* in nutrient agar medium. This sterilized agar medium was poured into petri dishes and allowed to solidify. On the surface of the medium microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (Pre-sterilized) was used to bore the cavities. All the synthesized compounds (25 µg/ml) were

TABLE 1: THE PHYSICAL DATA OF SUBSTITUTED FUROBENZOPYRONES

Compound code	R <sub>1</sub>	R <sub>2</sub>	R <sup>1</sup>	Mol. Formula	Mol. Weight	m.p <sup>o</sup>	% Yield	R <sup>f</sup> value*
FCa	H	H	H	C <sub>18</sub> H <sub>12</sub> O <sub>3</sub>	276	187-188	68	0.72
FCb	H	H	Br	C <sub>18</sub> H <sub>11</sub> BrO <sub>3</sub>	354.9	185	42	0.64
FCc	H	H	NO <sub>2</sub>	C <sub>18</sub> H <sub>11</sub> NO <sub>5</sub>	321	267-269	70	0.58
FCd	H	H	OCH <sub>3</sub>	C <sub>19</sub> H <sub>14</sub> O <sub>4</sub>	306	191	65	0.69
FCe	H	H	Cl	C <sub>18</sub> H <sub>11</sub> ClO <sub>3</sub>	310.5	189-191	62	0.61
FCf	H	H	F	C <sub>18</sub> H <sub>11</sub> FO <sub>3</sub>	294	190-201	60	0.59
FCg	H	H	CH <sub>3</sub>	C <sub>19</sub> H <sub>14</sub> O <sub>3</sub>	290	176-179	69	0.79
FCh	H	CH <sub>3</sub>	H	C <sub>19</sub> H <sub>14</sub> O <sub>3</sub>	290	176-179	66	0.8
FCi	H	CH <sub>3</sub>	Cl	C <sub>19</sub> H <sub>13</sub> ClO <sub>3</sub>	324.5	183	60	0.69
FCj	H	CH <sub>3</sub>	H	C <sub>19</sub> H <sub>13</sub> NO <sub>5</sub>	335	220	69	0.62
FCk	H	CH <sub>3</sub>		C <sub>20</sub> H <sub>16</sub> O <sub>3</sub>	304	182	68	0.83
FCl	H	CH <sub>3</sub>	OCH <sub>3</sub>	C <sub>20</sub> H <sub>16</sub> O <sub>4</sub>	320	132-135	62	0.78
FCm	H	CH <sub>3</sub>	F	C <sub>19</sub> H <sub>13</sub> FO <sub>3</sub>	307	187	58	0.68
FCn	CH <sub>3</sub>	H		C <sub>19</sub> H <sub>14</sub> O <sub>3</sub>	290	178-181	68	0.8
FCo	CH <sub>3</sub>	H	OCH <sub>3</sub>	C <sub>20</sub> H <sub>16</sub> O <sub>4</sub>	320	210	63	0.78
FCp	CH <sub>3</sub>	H	Cl	C <sub>19</sub> H <sub>13</sub> ClO <sub>3</sub>	324.5	165	60	0.68
FCq	CH <sub>3</sub>	H	NO <sub>2</sub>	C <sub>19</sub> H <sub>13</sub> NO <sub>5</sub>	335	226	67	0.62
FCr	CH <sub>3</sub>	H	F	C <sub>19</sub> H <sub>13</sub> FO <sub>3</sub>	307	185	57	0.68
FCs	CH <sub>3</sub>	H	CH <sub>3</sub>	C <sub>20</sub> H <sub>16</sub> O <sub>3</sub>	304	132-135	69	0.83

All the compounds were recrystallized from glacial acetic acid,\*Solvent system used for TLC was (benzene:petroleum ether: 6:4)

placed serially in the cavities with the help of a micropipette and allowed to diffuse for 1 h. DMF was used as a solvent for all the compounds and as control. The plates were incubated at 37<sup>o</sup> for 24h. The zone of inhibition observed around the cups after incubation was measured. The results are presented in Table 2.

Carrageenan-induced rat hind paw edema method was used for antiinflammatory activity. Male Wistar rats weighing between 100-200 g were used for the experiment. They were procured from the animal house of KMC, Manipal and individually housed. The animals were dosed orally at 500, 1000 and 2000 mg/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 h. Since 2000 mg/kg body weight was observed as safe dose, 1/10th of 2000 mg/kg body weight i.e. 200 mg/kg body weight was fixed as the dose for acute antiinflammatory screening. The animals were divided into eight groups of six animals each. One group served as a standard (ibuprofen) and another group served as control (1% CMC) and rest of the groups were used for the test drugs. The rats were dosed with test drug orally at 200 mg/kg bodyweight. Ibuprofen was given at a dose of 18 mg/kg bodyweight. Test compounds and ibuprofen were suspended in 1% CMC which was used as a vehicle for the control group. A solution of 1% carrageenan was used to induce inflammation. The drugs were given orally. After 1h, a sub plantar injection of 0.05 ml of 1% carrageenan was administered. The volume of the injected paw was measured with a plethysmograph immediately. The paw volume was again measured after 3

hours. The average paw volume in a group of drug treated rats was compared with that of a group with vehicle (control group) and the percentage inhibition of edema was calculated using the formula: Percent inhibition =  $(1-Vt/Vc) \times 100$ , where Vt is the mean paw volume of the test drug, Vc is the mean paw volume of the control. The results are given in Table 3.

A total of nineteen compounds were screened for antibacterial activity; almost all the compounds have showed good activity against *Pseudomonas aeruginosa*. Compounds 3-(4-chlorophenyl)-5-methylfuro-[3,2-g][1]benzopyran-7-one, 3-(4-chlorophenyl)-5,9-dimethylfuro[3,2-g][1]benzopyran-7-one and 4,5-dimethyl-3-phenylfuro[3,2-g][1]benzopyran-7-one (FCe, FCi, FCn) were active against *E. coli*. A few compounds showed moderate activity against *Bacillus subtilis* also. Compounds FC<sub>a</sub>, FC<sub>c</sub>, FC<sub>d</sub>, FC<sub>f</sub>, FC<sub>g</sub>, and FCi were tested for antiinflammatory activity. Among them, 5-methyl-3-p-tolylfuro[3,2-g][1]benzopyran-7-one (FCg) showed excellent activity. 5-Methyl-3-phenylfuro[3,2-g][1]benzopyran-7-one (FCa) and 4,5-dimethyl-3-(4-nitrophenyl)-furo[3,2-g][1]benzopyran-7-one (FCc) showed activity comparable to that of standard drug ibuprofen. Others also showed moderate activity. The increased activity of FC<sub>g</sub> may be attributed to the introduction of methyl group at 4'' position of phenyl ring. Substitution of 4'' position of phenyl ring with electronegative groups resulted in diminished activity. Activity was not enhanced when methyl groups were introduced at 5 and 8 position of furobenzopyrones skeleton. These studies show that these compounds may prove to be good antiinflammatory agents

TABLE 2: ANTIBACTERIAL ACTIVITY OF THE TEST COMPOUNDS

Compound code	Zone of Inhibition (mm)			
	<i>E.coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>B. subtilis</i>
FCa*	—	12	—	—
FCa**	—	18	—	—
FCb*	—	12	—	10
FCb**	—	12	—	12
FCc*	—	16	—	11
FCc**	—	16	—	11
FCd*	—	19	—	—
FCd**	—	20	—	—
FCe*	13	12	—	10
FCe**	14	13	—	10
FCf*	—	10	—	—
FCf**	—	10	—	—
FCg*	—	11	—	—
FCg**	—	11	—	—
FCb*	—	15	—	—
FCb**	—	15	—	—
FCi*	9	8	—	—
FCi**	9	8	—	—
FCj*	—	10	—	—
FCj**	—	10	—	—
FCk*	—	12	—	—
FCk**	—	12	—	—
FCl*	—	15	—	—
FCl**	—	15	—	—
FCm*	—	12	—	—
FCm**	—	14	—	—
FCn*	10	10	—	—
FCn**	10	10	—	—
FCo*	—	22	—	—
FCo**	—	23	—	—
FCp*	—	12	—	—
FCp**	—	18	—	—
FCq*	—	17	—	—
FCq**	—	17	—	—
FCr*	—	15	—	—
FCr**	—	16	—	—
FCs*	13	16	—	—
FCs**	13	16	—	—
Standard*	20	22	25	21
Standard**	25	23	25	21

Standard used was ciprofloxacin. \* indicates resistance and \*\* indicate concentrations at 25 and 50 µg/ml

TABLE 3: ANTIINFLAMMATORY ACTIVITIES OF THE TEST COMPOUNDS

Drug	Dose (mg/kg)	Mean oedema volume±SE (0-3 h)	% Reduction in edema volume
Control	-	0.685±0.109	-
Ibuprofen	18	0.2417±0.014	70.5
FCa	200	0.1967±0.048	71.3
FC c	200	0.288±0.0419	64.7
FC d	200	0.5717±0.026	16.5
FC f	200	0.4483±0.043	34.6
FC g	200	0.07±0.0073	89.8
FCi	200	0.3967±0.1372	42.04

5% Allowance value is 0.39 (Scheffe's method),  $P < 0.05$  Vs Control. Any two means showing a difference of 0.39 are statistically significant.

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