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Synthesis, Characterization and Antimicrobial, Antioxidant Properties of Some Benzopyrone Derivatives

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The purpose of present study is to develop potential antimicrobial and antioxidant agents. In this attempt three series of the substituted flavones such as 3-hydroxy flavones (**4a-j**), 3-methoxy flavones (**5a-e**) and 3-acetyloxy flavones (**6a-g**) were synthesized and characterized by IR, ^{1}H NMR and mass spectral analysis. Test compounds were subjected for the determination of lipophillic parameter and pharmacological activities such as quantitative antibacterial activity and antioxidant activity by MIC (ELISA) and DPPH* method, respectively.

Key Words: Hydroxy flavones, Methoxy flavones, Acetyloxy flavones, Antimicrobial activity, Antioxidant and Partition coefficient.

INTRODUCTION

Compounds having chromone (γ-benzopyrone) moiety are associated with interesting pharmacological activities such as antibacterial, antifungal, antidiabetic, antiallergic, diuretic[1], antiinflammatory, analgesic and antipyretic[2], bioflavonoids are also reported as strong inhibitors of HIV reverse transcriptase[3], antioxidant[4], anticancer[5] and against cardiovascular diseases and disorder of liver[6]. Flavonoids, the derivatives of chromones are polyhydroxylated compounds and they are capable of selectively reacting with free radicals[7]. Even though natural flavonoids are highly potent, attempts have been made to improve their stability, solubility, efficacy and kinetics. Hence, with the knowledge of semisynthetic and synthetic science, flavonoids were synthesized in the laboratory by convenient and cost effective methods from past few decades. Based on their significant biological activities, it was planned to synthesize substituted flavones for the determination of antimicrobial, antioxidant activity. Some of their physical properties are determined such as λ_{max} and partition coefficient (log P).

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EXPERIMENTAL

Melting points were determined in open capillary tubes and are found uncorrected. IR spectra were recorded on Fourier transform IR spectrophotometer (Shimadzu 8700) using KBr disc method. ^{1}H NMR spectra were recorded on AMX-400 liquid state NMR spectrometer in $CDCl_3$ and CD_3COCD_3 using TMS as an internal reference standard. Mass spectra were recorded on LC-MS Triple Quadruple Mass Spectrometer (Sciex 3000, Applied Biosystems) using electrospray ionization-positive ion mode. The purity of the test compounds was determined by thin layer chromatography. The λ_{max} of the synthesized compounds for determination of log P was recorded on UV-visible spectrophotometer (Shimadzu 1601, 200 to 400 ηm). Physical data of the intermediate chalcones were recorded in Table-1 and final test compounds in Table-2. Quantitative antimicrobial and antioxidant studies were done by two fold dilution method and DPPH* method, respectively and shown in Table-3.

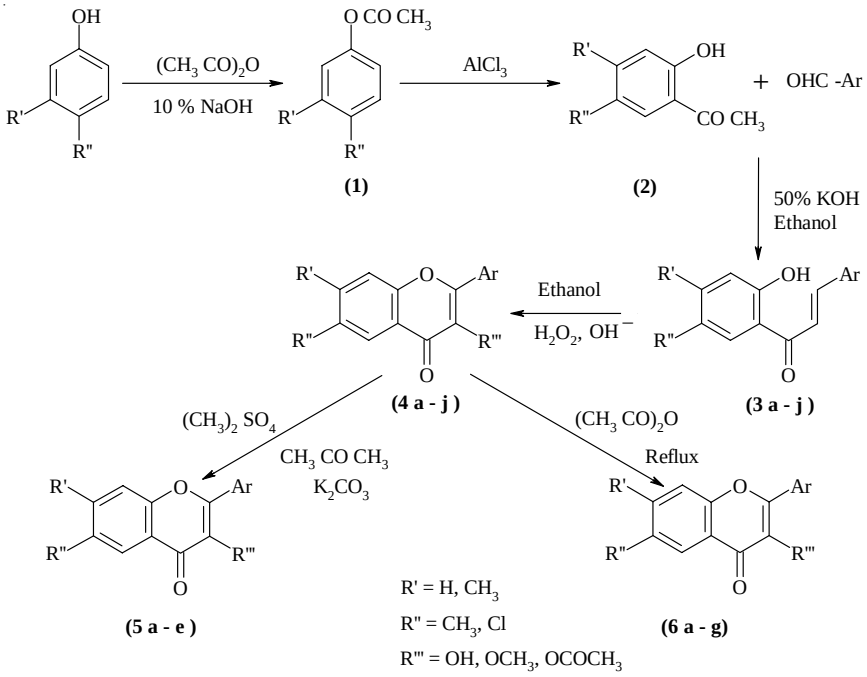
TABLE-1
PHYSICAL PROPERTIES OF VARIOUS SUBSTITUTED CHALCONES (**3a-j**)

Comp	R'	R"	Ar	m.f.	m.w.	Yield (%)	m.p. (°C)
3a	CH_3	Cl	5-Chloro-2-hydroxy phenyl	$C_{16}H_{12}O_3Cl$	322	88	181-183
3b	CH_3	Cl	4-Methoxy phenyl	$C_{17}H_{15}O_3Cl$	302	89	177
3c	CH_3	Cl	4-Dimethyl amino phenyl	$C_{18}H_{18}O_2NCl$	315	85	174
3d	CH_3	Cl	2-Chloro phenyl	$C_{16}H_{13}O_2Cl$	307	78	132-133
3e	CH_3	Cl	3,4-Dimethoxy phenyl	$C_{18}H_{17}O_4Cl$	332	75	178-180
3f	CH_3	Cl	4-Methyl phenyl	$C_{17}H_{15}O_2Cl$	286	78	132-134
3g	H	Cl	4-Chloro phenyl	$C_{15}H_{10}O_2Cl_2$	292	68	172-174
3h	H	Cl	4,5-Dimethoxy phenyl	$C_{17}H_{15}O_4Cl$	318	67	163-165
3i	H	Cl	4-Methyl phenyl	$C_{16}H_{13}O_2Cl$	272	65	123-124
3j	H	CH_3	4-Dimethyl amino phenyl	$C_{18}H_{19}NO_2$	281	71	118

5-Chloro-2-hydroxy-4-methyl acetophenone (**2**) was obtained by the reaction of 4-chloro-3-methyl phenol with acetic anhydride in presence of 10% NaOH to form phenolic ester (**1**), which on subjecting to Fries reaction[8,9] gave compound (**2**). The chalcones[10] were prepared by the reaction of substituted aromatic aldehydes with intermediate (**2**) in presence of 50% KOH in ethanol medium, finally the substituted 3-hydroxy flavones (**4a-j**) were prepared from 2-hydroxy chalcones (**3a-j**) to flavonols with hot alkaline hydrogen peroxide (**Scheme-1**).

6-Chloro-3-hydroxy-7-methyl-2-(4'-methoxy phenyl)-benzopyran 4-one (4b): To a suspension of 0.01 mol of chalcone (**3b**), in 85 mL ethanol was added to 10 mL of 20% aqueous sodium hydroxide followed by the careful addition of 18 mL of 30% hydrogen peroxide over a period of 0.5 h. The reaction mixture was stirred for 3.0 to 3.5 h at 30ºC and

poured into crushed ice containing 5N hydrochloric acid. The precipitate was filtered, washed, dried and recrystallized from ethyl acetate. Compounds (**4a-j**) showed IR (KBr, cm^{-1}) range at 3300-3200 ν(-OH), 3100-3000 ν(ArC-H), 2985-1875 ν(-C-H), 1650-1600 ν(lactone C=O), 1600, 1450 ν(ArC=C), 1360-1250 ν(C-N), 1100-1000 ν(C-Cl), 965-675 ν(ArC-H).



Scheme-1

6-Chloro-3-methoxy-7-methyl-2-(4'-methoxy phenyl)-benzopyran 4-one (5a): 0.01 mol of **4b** was suspended in dry acetone containing powdered anhydrous potassium carbonate (0.03 mol) and dimethyl sulphate (0.02 mol). The suspension was refluxed for 5.0 h. The solvent was evaporated under pressure and the residue diluted with water. The precipitate obtained was filtered, washed, dried and recrystallized from alcohol.

The absence of -OH stretching and presence of C-O stretching in methoxy derivative confirmed the completion of reaction. IR spectra showed the characteristic bands for substituted 3-methoxy flavones (**5a-e**) at the range IR (KBr, cm^{-1}) 3100-3000 ν(ArC-H), 2985-1875 ν(-C-H), 1650-1640 ν(lactone C=O), 1600, 1450 ν(ArC=C), 1360-1250 ν(C-N), 1150-1300 ν(C-O), 1100-1000 ν(C-Cl), 965-675 ν(ArC-H).

3-Acetyloxy-6-chloro-7-methyl-2-(4'-methoxy phenyl)-benzopyran 4-one (6a): To 0.01 mol of substituted 3-hydroxy flavone (**4b**) in 100 mL

TABLE-2
PHYSICO-CHEMICAL PROPERTIES OF SUBSTITUTED 3-HYDROXY (**4a-j**), 3-METHOXY (**5a-e**) AND 3-ACETYLOXY FLAVONE (**6a-g**)

Comp.	R'	R''	R'''	Ar	m.w.	Yield (%)	m.p. (°C)	λ_{max}	log P
4a	CH_3	Cl	OH	5-Chloro-2-hydroxy phenyl	338	68	232	376	1.056
4b	CH_3	Cl	OH	4-Methoxy phenyl	316	71	205-206	388	0.578
4c	CH_3	Cl	OH	4-Dimethyl amino phenyl	329	69	230	453	0.880
4d	CH_3	Cl	OH	2-Chloro phenyl	320	75	235	407	0.707
4e	CH_3	Cl	OH	3,4-Dimethoxy phenyl	346	64	178-181	404	0.783
4f	CH_3	Cl	OH	4-Methyl phenyl	300	69	199-201	389	0.524
4g	H	Cl	OH	4-Chloro phenyl	306	63	212	375	0.998
4h	H	Cl	OH	4,5-Dimethoxy phenyl	332	70	238-240	402	0.520
4i	H	Cl	OH	4-Methyl phenyl	286	65	189-190	388	0.468
4j	H	CH_3	OH	4-Dimethyl amino phenyl	295	71	215-216	448	0.623
5a	CH_3	Cl	OCH_3	4-Methoxy phenyl	330	69	148-153	340	1.160
5b	CH_3	Cl	OCH_3	4-Dimethyl amino phenyl	343	65	150	416	1.200
5c	CH_3	Cl	OCH_3	3,4-Dimethoxy phenyl	360	64	150	325	1.221
5d	CH_3	Cl	OCH_3	4-Methyl phenyl	314	68	122-125	308	1.128
5e	H	Cl	OCH_3	4-Methyl phenyl	300	63	127	340	0.968
6a	CH_3	Cl	$OCOCH_3$	4-Methoxy phenyl	358	69	176-178	334	0.986
6b	CH_3	Cl	$OCOCH_3$	4-Dimethyl amino phenyl	371	65	203-204	409	1.060
6c	CH_3	Cl	$OCOCH_3$	4-Methyl phenyl	342	67	170-171	331	1.080
6d	H	Cl	$OCOCH_3$	4-Chloro phenyl	348	68	139	317	0.946
6e	H	Cl	$OCOCH_3$	4,5-Dimethoxy phenyl	374	67	108-110	339	1.090
6f	H	Cl	$OCOCH_3$	4-Methyl phenyl	328	65	178-180	326	0.952
6g	H	CH_3	$OCOCH_3$	4-Dimethyl amino phenyl	337	69	134	405	0.845

TABLE-3
ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF SUBSTITUTED 3-HYDROXY, 3-METHOXY, 3-ACETYLOXY FLAVONE (**4a-6g**)

Comp.	*Bacillus subtilis*			*Staphyloccus aureus*			*Escherichia coli*			Antioxidant activity (EC_{50}) (μg/mL)
	MIC (μg/50μL)	% Inhibition compared to Gentamycin	% Inhibition compared to Amoxicillin	MIC (μg/50μL)	% Inhibition compared to Gentamycin	% Inhibition compared to Amoxicillin	MIC (μg/50μL)	% Inhibition compared to Gentamycin	% Inhibition compared to Amoxicillin	
4a	32.00	25.00	50.00	67.20	47.76	23.88	64.80	25.00	12.50	350
4b	33.00	24.24	48.48	70.40	45.71	22.85	76.80	21.05	10.52	280
4c	64.00	12.50	25.00	128.00	25.00	12.50	64.00	25.00	12.50	280
4d	32.00	25.00	50.00	67.20	47.76	23.88	65.00	24.61	12.30	320
4e	64.00	12.50	25.00	32.00	100.00	50.00	64.00	25.00	12.50	400
4f	89.00	08.98	17.97	115.20	27.82	13.91	89.00	17.97	08.98	330
4g	35.00	22.85	45.71	44.00	72.72	36.36	38.00	42.10	21.05	320
4h	28.00	28.57	57.14	33.00	96.96	48.48	67.00	23.88	11.94	340
4i	44.00	18.18	36.36	89.00	35.95	17.97	64.00	25.00	12.50	350
4j	38.00	21.05	42.10	64.00	50.00	25.00	44.00	36.36	18.18	550
5a	22.00	36.36	72.72	65.00	49.23	24.61	57.00	28.07	14.03	480
5b	28.00	06.25	12.50	32.00	100.00	50.00	32.00	50.00	25.00	480
5c	28.00	28.57	57.14	35.00	91.42	45.71	44.00	36.36	18.18	440
5d	32.00	25.00	50.00	64.00	50.00	25.00	35.00	45.71	22.85	580
5e	57.00	14.03	28.07	120.00	26.26	12.33	65.00	24.61	12.30	620
6a	28.00	28.57	57.14	70.00	45.71	22.85	57.00	28.07	14.03	550
6b	32.00	25.00	50.00	64.00	50.00	25.00	16.00	100.00	50.00	610
6c	22.00	36.36	72.72	38.00	84.21	42.10	32.00	50.00	25.00	660
6d	32.00	25.00	50.00	32.00	100.00	50.00	32.00	50.00	25.00	510
6e	64.00	12.50	25.00	64.00	50.00	25.00	64.00	25.00	12.50	430
6f	35.00	22.85	45.71	115.00	27.82	13.91	67.00	23.88	11.94	460
6g	64.00	12.50	25.00	32.00	100.00	50.00	32.00	50.00	25.00	540
Gentamycin	08.00	–	–	32.00	–	–	16.00	–	–	–
Amoxycillin	16.00	–	–	16.00	–	–	08.00	–	–	–
Ascorbic acid	–	–	–	–	–	–	–	–	–	5.1

round bottom flask was added 10-15 mL of acetic anhydride and the mixture was refluxed for 2 h. The resulting solution was cooled at room temperature followed by addition of ice cold water. The solid separated was filtered, washed with cold water and recrystallized from alcohol.

The absence of -OH stretching and presence of C=O stretching above 700 cm^{-1} confirmed the completion of reaction. IR spectra showed the characteristic bands for 3-acetyloxy flavones (**6a-g)** at the range IR (KBr, cm^{-1}) 3100-3000 ν(ArC-H), 2985-1875 ν(-C-H), 1750-1700 ν(C=O), 1650-1640 ν(lactone C=O), 1600, 1450 ν(ArC=C), 1360-1250 ν(C-N), 1150-1300 ν(C-O), 1100-1000 ν(C-Cl), 965-675 ν(ArC-H).

^{1}H NMR **4b:** = 8.19-8.21 (d, 2H, Ar-H, J = 9.10 Hz), 8.18 (s, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 7.03-7.05 (d, 2H, Ar-H, J = 9.08 Hz), 6.89 (s, 1H, OH), 3.89 (s, 3H, OCH_3), 2.52 (s, 3H, CH_3). **4c:** = 8.16 (s, 1H, Ar-H), 8.13-8.15 (d, 2H, Ar-H, J = 9.2 Hz), 7.42 (s, 1H, Ar-H), 6.85 (s, 1H, OH), 6.77-6.79 (d, 2H, Ar-H, , J = 9.18 Hz), 3.06 (s, 6H, NMe_2), 2.50 (s, 3H, CH_3). **5a:** = 8.11-8.13 (d, 2H, Ar-H, J = 9.06 Hz), 8.05 (s, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 7.11-7.13 (d, 2H, Ar-H, J = 9.05 Hz), 3.91 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 2.52 (s, 3H, CH_3). **6b:** = 8.16 (s, 1H, Ar-H), 7.79-7.82 (d, 2H, Ar-H, J = 9.13 Hz), 7.40 (s, 1H, Ar-H), 6.72-6.75 (d, 2H, Ar-H, J = 9.13 Hz), 3.07 (s, 6H, NMe_2), 2.50 (s, 3H, CH_3), 2.37 (s, 3H, CH_3).

Mass Spectra **4b:** = m/e 318 (M+2), 316 (base peak). Other peaks were observed at m/e: 301, 273, 245, 181, 158, 152, 135, 133, 119, 105, 92 and 77. **4c:** = m/e 331 (M+2), 329 (base peak). Other peaks were observed at m/e: 301, 272, 229, 164, 132, 118, 105 and 90. **5a:** = m/e 332 (M+2), 329 (base peak). Other peaks were observed at m/e: 315, 299, 287, 259, 244, 209, 181, 165, 136, 132, 107 and 92.

Partition coefficient

Hydrophobicity is generally parameterized by partition coefficient or some. It was determined by using the Classical Shake Method[11] *n*-octanol and phosphate buffer (pH 7.4). Partition coefficient is defined as the ratio of the amount of the drug present in organic phase to that present in the aqueous phase.

Partition coefficient = $P = C_{org} / C_{aq}$

$P = B_E / B_E - A_E$

where, B_E = Absorbance before extraction, A_E = Absorbance after extraction, log P was then calculated gives an idea about the solubility and lipid profile of test sample.

Evaluation of antioxidant activity

Newly synthesized test compounds (**4a-j, 5a-e** and **6a-g**) were subjected for *in vitro* free radical scavenging activity[12] using a method based on the reduction of a methanolic solution of the colored DPPH (1,1-

diphenyl-2-picryl hydrazyl) radical. The activity was expressed as effective concentration at 50% reduction (EC_{50}) or the concentration of the test solution required to give a 50% decrease in absorbance compared to that of blank solution as shown in Table-3.

Evaluation of antimicrobial activity

Antibacterial screening of the synthesized compounds was carried out by cup-plate method[13] using three strains *i.e.*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. Gentamycin and Ampicillin were used as reference samples and antibacterial activity of the test compounds (**4a-6g**) is presented in Table-3. The MIC of the test compounds showing promising antimicrobial activity was determined using 96-well plate (Two fold dilution technique) using an ELISA Reader[14] and percentage of inhibition compared to gentamycin and amoxycillin was recorded for the test compounds.

RESULTS AND DISCUSSION

All the twenty two test compounds synthesized, purified and characterized were screened for their qualitative antimicrobial activity. They were tested against three species of bacteria namely *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. The technique used was Agar diffusion method using 100 mg/0.1 mL of amoxycillin and gentamycin as standard. The test compounds such as **4h**, **5a**, **6a** and **6c** showed activity with MIC values at 28, 22, 28 and 22, respectively for *Bacillus subtilis*. Test compounds such as **6g**, **6d** and **5b** showed activity with MIC values at 32, 32 and 32, respectively for *Staphylococcus aureus*. The percentage inhibition were found to be 100, 100 and 100%, respectively with that of gentamycin and 50, 50 and 50%, respectively with that of amoxicillin as shown in Table-3. Thus, it was speculated that the minimum structural requirements to show optimal antibacterial activity was the presence of 4'-dimethylamino phenyl group at 2-position and presence of 4'-chloro phenyl group at 2-position. The test compounds such as **6g**, **6d**, **5b** and **6b** showed activity with MIC values at 32, 32, 32 and 16, respectively for *Escherichia coli* as shown in Table-3. it was found that test compounds such as **6g**, **6b** and **5b** with 4'-dimethylamino phenyl group at 2-position showed significant activity against *Staphylococcus aureus* than that of test compounds such as **6d** and **6e** substituted with 4'-chloro phenyl and 4', 5'-dimethoxy phenyl group at 2-position, respectively.

The EC_{50} values for the 3-hydroxy flavones (**4a-j**), 3-methoxy flavones (**5a-e**) and 3-acetyloxy flavones (**6a-g**) were found to be in the range of 280-550, 440-620 and 430-660, respectively. Significant activity was observed in molecules with -OH functional groups.

The presence of 3-hydroxyl group in the flavones had a significance influence on the partition coefficient of the test compounds. Out of twenty two test compounds (**4a-6g**) synthesized, ten test compounds having -OH group at position 3 were found to have lower log P values than those test compounds having $-OCH_3$ and $-COCH_3$ group at 3-position. This might be due to the presence of polar -OH group at 3-position compared to the methoxy and acetyloxy group.

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