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Antioxidant and Antibacterial Activity of Some Newer Flavone Derivatives

KEYWORDS: Anti-microbial, antioxidant, flavones, activity

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ABSTRACT *Synthesis of novel chalcone from the 2-hydroxy acetophenone and 5-nitro thiophene carboxyaldehyde with various substitutions are described. They were evaluated for their antioxidant and antibacterial activity against Gram positive and Gram negative bacteria. The test compound JPC-9 is one of the test compounds with highest IC50 values by DPPH and ABTS method and has shown log P value at 1.76 against standard ascorbic acid. Compounds such as JPC-3, JPC-4, JPC-5, JPC-6, JPC-9, JPC-10, and JPC-13 were found to have shown zone of inhibition against Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa and Escherichia coli at the range 31.25-250 µg/50µL when compared to that of the standard ciprofloxacin at 25µg/50µL.*

1. Introduction

In recent years, scientific and public interest in flavonoids has grown enormously as antioxidant agents due to their property and they play a potential role in maintaining human health (Middleton E & C. Kandaswami, 1994, Jurd L, 1962, Mabry T J, Markham K R, & Tomas M B, 1970, Harborne J B & Baxter H, 1999). Flavones are polyphenolic compounds exhibiting antioxidant and antimicrobial activity. In our earlier work we have reported several synthetic flavonoids and their biological activities (Jayashree B S, Alam A, Nayak Y, Vijay Kumar D, 2012, Jayashree B S, Kuppast B K, Venugopala K N, 2007, Jayashree B S, Thejaswini J C, Nayak Y, Vijay Kumar D, 2010). In the present work, 2-hydroxy acetophenone and 5-nitro thiophene carboxyaldehyde with various substitutions were synthesized and explored for their antioxidant and antimicrobial activities.

2. Materials and Methods

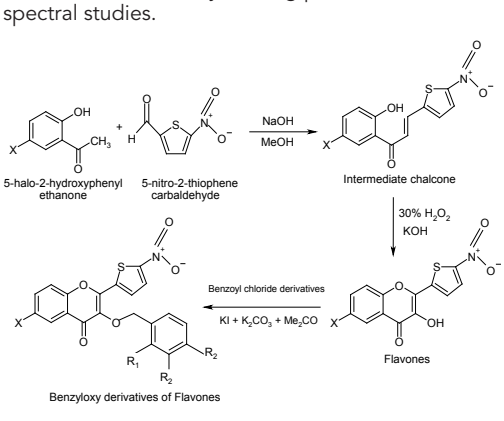
2.1 Chemicals and Instruments

Chemicals were purchased from Aldrich, Himedia, Merc and Rankem. All the chemicals were of AR and LR grade and solvents were of HPLC grade. Melting points were determined on a melting point apparatus (Shital Scientific Industries, Mumbai) and are uncorrected. The reactions were monitored by TLC and the Rf values were determined on pre-coated TLC plates. λ_{max} and ε_{max} for the test compounds were obtained on UV –visible spectrophotometer (Shimadzu UV-Visible spectrophotometer UV-1650 PC) in methanol (HPLC grade). The FTIR studies were performed on Shimadzu FTIR 8310. ^{1}H NMR spectra were taken on a NMR (AMX 400) and the mass spectra were recorded on Shimadzu GC-MS (QP5050, Japan).

2.2 General procedure for the synthesis

Equi-molar concentrations of 5-halo-2-hydroxyphenyl ethanone and 5-nitro-2-thiophene carbaldehyde were stirred in an ice-cold condition in the presence of NaOH for 5 hours and it was then poured into an ice cold HCl, which gave an intermediate chalcones. The intermediate chalcones were then purified by recrystallization and were further used for the preparation of the corresponding 3-hydroxyflavones. The intermediate chalcone was dissolved in methanol and NaOH. Further, resulting solution was cooled and stirred at ice-cold condition with drop-wise addition of 30% H_2O_2. The final solution was stirred for 5 hours and the mixture was then poured onto ice-cold HCl to get the corresponding 3-hydroxyflavone derivative (Fig. 1).

To a suspension of the 3-hydroxyflavone derivative, benzyl halides, KI, and freshly ignited anhydrous K_2CO_3, dry acetone was added and refluxed for 5 hours. The reaction mixture was then filtered, evaporated and was subjected to percolation by passing through column of silica-gel to obtain corresponding flavone derivatives (JPC-3 to JPC13). All the flavones and chalcones were then purified by recrystallization and they were obtained in high purity and the structure was later confirmed by melting point, UV, IR, Mass and NMR spectral studies.



Scheme of synthesis

Figure 1

Position of different functional groups on the final compound

S.N.	Compound Code	X	R_1	R_2	R_3
1	JPC-1	-Cl			
2	JPC-2	-Cl			
3	JPC-3	-Cl	-H	-H	$-NO_2$
4	JPC-4	-Cl	$-NO_2$	-H	-H
5	JPC-5	-Cl	-H	-H	-Cl
6	JPC-6	-Cl	-H	$-NO_2$	-H
7	JPC-7	-Cl	-Cl	-H	-H
8	JPC-8	-Cl	-H	-H	$-OCH_3$
9	JPC-9	-Cl	-H	$-OCH_3$	-H
10	JPC-10	-Cl	-H	-H	-CH3
11	JPC-11	-Cl	-H	-H	-F
12	JPC-12	-Cl	-F	-H	-H
13	JPC-13	-Br	-H	-H	$-NO_2$

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2.3 Biological Activity

2.3a Antioxidant activity

The synthesised test compounds were evaluated for their antioxidant activity by DPPH (Rajakumar D V & Rao M N A, 1995) radical scavenging method and ABTS (Gupta R., Sharma M, Lakshmy R.,et.al., 2009, Roberta R.E, Pellegrini N & Proteggente A, 1999), radical scavenging method. For DPPH radical scavenging method, compounds such as, JPC-3, JPC-4, JPC-6, JPC-8, JPC-9, JPC-10 and JPC-13 have IC_{50} values 640, 420, 182, 815, 147, 872, 378µg/mL respectively, as compared to Ascorbic acid with its IC_{50} value at 3.72µg/mL. None of the test compounds showed antioxidant activity where, ascorbic acid was used as standard with its IC_{50} value at 3.72µg/mL as shown in the Table 2.

For ABTS radical scavenging method, compounds such as, JPC-5, JPC-6, JPC-8, and JPC-9 have shown IC_{50} values at 14.46, 85.78, 75 and 87.7µg/mL respectively and were found to be less than 100µg/mL. The IC_{50} values of all other synthesized compounds were above 100µg/mL as shown in Table 3.

2.3b Antibacterial activity

The synthesized test compounds were evaluated for their antibacterial activity by agar diffusion method and tube dilution method. They were tested against four species of bacteria namely *Bacillus subtilis, Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* and *Escherichia coli* (Gram negative). Out of the 11 compounds tested for their antibacterial activity, JPC-3, JPC-4, JPC-5, JPC-6, JPC-9, JPC-10, and JPC-13 were found to have zone of inhibition against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* and *Escherichia coli* at 31.25-250 µg/50µL when compared to that of the standard ciprofloxacin at 25µg/50µL as shown in the Table 4.

3. Results and Discussion

3.1 Chemistry

The newer chalcones and 3-hydroxy flavones were synthesized with their yield, ranging from 25 to 55%. Their logP values were in the range between 1.5 and 3 as shown in the table 1.

Spectral data for the representative molecule 6-chloro-3-[(4-methoxybenyl)oxy]-2-(5-nitrothiophen-2-yl)-4H-chromen-4-one (JPC-8):

IR (KBr) (cm^{-1}): 1645.33 (C-O-CH_2), 1502.26 [Ar-NO_2 (N=O)], 752 (C-Cl); ^{1}HNMR (DMSO-d6): δ 3.72 (s 12), δ 0.0082 (s 3); MS: m/z 444 (M^+).

3.2 Biological Activity

The test compound, JPC-3 was found to have zone of inhibition at 19mm, 16mm, 14mm, 14mm against *Bacillus subtilis* at 250, 125, 62.5 and 31.25µg/50µL respectively, which was found to be more than the zone of inhibition at 13mm, 12mm, 11mm, 9mm against *Staphylococcus aureus* at 250, 125, 62.5, and 31.25 µg/50µL respectively and zone of inhibition at 12mm, 10mm, 9mm, 8mm against *Escherichia coli* at 250, 125, 62.5, and 31.25 µg/50µL respectively. However, the zone of inhibition was insignificant when tested against *Pseudomonas aeruginosa*.

Further, when tested against *Bacillus subtilis*, at concentrations 250, 125, 62.5 and 31.25µg/50µL respectively the test compound, JPC-4 was found to have zone of inhibition at 18mm, 17mm, 16mm, and 12mm respectively, whereas JPC-5 was found to have zone of inhibition at 29mm, 28mm, 26mm, and 25mm. The test compound JPC-6 was found to have zone of inhibition at 14mm, 13mm, 11mm, and 10mm against the same bacteria whereas the test compound JPC-13 was found to have zone of inhibition at 17mm, 14mm, 13mm, and 11mm respectively.

When tested against *Escherichia coli* at 250, 125 and 62.5µg/50µL respectively, the test compound JPC-9 was found to have zone of inhibition at 19mm, 16mm and 15mm and the test compound JPC-10 was found to have zone of inhibition 20mm, 17mm respectively. The test compounds, JPC-7, JPC-11and JPC-12 did not show any antibacterial activity against any of the four organisms, clearly suggesting that these derivatives did not possess any antibacterial activity.

Thus, the test compounds such as, JPC-3, JPC-4, JPC-5, JPC-6 JPC-10 and JPC-13 showed antibacterial activity comparable to that of the standard ciprofloxacin as shown in the Table 4.

3.3 Discussion

The effect of 'P' value on the antioxidant activity of 11 synthesized test compounds were studied by DPPH method and 8 synthesized compounds were studied by ABTS method. It was found that, test compound JPC-9 showed log P value at 1.76 with its IC_{50} value at 147µg/mL by DPPH method and 87.7µg/mL by ABTS method, when compared to that of the standard ascorbic acid, having IC_{50} value at 3.72µg/mL. The compounds showing antioxidant activity must have optimum log P value and there should be a balance between the solubility of the compound in hydrophilic and lipophillic phases. The test compound JPC-9 is one of the probable compounds with highest IC_{50} values by DPPH and ABTS method and has shown log P value at 1.76 as shown in Table 5.

Table 1: Physico-chemical properties of different substituted flavone

Sr No	Comp. Code	Recrystallization Solvent	MP (°C)	Rf	λ_{max} (nm)	λ_{max} (l mol^{-1} cm^{-1})	P	log P
1	JPC1	glacial acetic acid	68	0.46	372	23687.48	691.8	2.84
2	JPC2	glacial acetic acid	86	0.35	295	14852.44	239.9	2.38
3	JPC-3	Methanol	106	0.86	271	1191.61	60.3	1.78
4	JPC4	Methanol	116	0.87	264	11162.08	91.2	1.96
5	JPC5	Methanol	104	0.51	260	10190.28	19.4	1.29
6	JPC6	Methanol	112	0.89	289	13024.00	38.0	1.58
7	JPC7	Methanol	102	0.55	259	10816.42	15.4	1.19
8	JPC8	Methanol	106	0.43	280	12461.58	43.9	1.64
9	JPC9	Methanol	110	0.47	275	11046.31	58.3	1.76
10	JPC10	Methanol	108	0.59	262	10238.30	561.8	2.75
11	JPC11	Methanol	104	0.39	263	10270.06	79.3	1.9
12	JPC12	Methanol	104	0.37	264	10540.09	89.3	1.95
13	JPC13	Methanol	118	0.72	284	13721.64	60.3	1.78

Table 2: Percentage DPPH Scavenging Activity

Drugs Code	Concentration(µg/mL)								
	7.5	15.6	31.25	62.5	125	250	500	1000	IC 50
JPC 3	8.32	21.75	17.72	54.88	31.41	34.10	49.88	60.45	640
JPC 4	12.45	15.00	15.74	23.69	53.60	62.80	67.74	70.25	420
JPC 5	12.42	13.05	19.13	46.12	36.62	40.41	42.83	17.92	>1000
JPC 6	29.74	31.45	64.28	57.53	59.01	57.83	39.40	72.27	182
JPC 7	17.92	16.75	19.63	16.98	31.82	14.83	32.25	44.98	>1000
JPC 8	14.80	15.13	24.03	18.93	23.73	23.93	26.75	63.30	815
JPC 9	10.90	16.51	37.42	65.79	72.67	78.41	81.87	81.90	147
JPC10	14.63	16.91	22.39	25.04	34.84	40.91	33.97	53.67	872
JPC11	12.31	12.15	19.87	21.01	20.51	20.20	18.16	12.75	>1000
JPC12	13.46	19.16	22.22	21.71	21.55	22.55	19.97	15.50	>1000
JPC13	9.06	17.65	22.28	26.55	40.78	41.52	78.21	86.97	378

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Table 3: ABTS free radical scavenging

% Scavenging Activity								
Conc. (µg/ml)	JPC 3	JPC 4	JPC 5	JPC 6	JPC 7	JPC 8	JPC 9	JPC10
6.25	19.44	44.65	33.84	47.32	51.91	47.16	48.54	15.46
12.5	27.41	45.78	52.52	49.61	45.78	46.70	44.25	19.90
25	37.67	46.40	56.81	48.85	46.86	48.54	47.93	24.65
50	43.64	47.62	61.25	46.40	44.56	51.45	47.77	38.13
100	56.96	48.69	62.02	46.70	46.40	48.08	48.85	47.16
200	42.41	55.13	65.23	56.50	56.20	55.89	56.35	49.31
IC_{50} (µg/ml)	183	106.5	14.46	85.78	104.05	75	87.7	169

Table 4: Anti-Bacterial Activity - Agar Diffusion Method

Sr. No	Comp. code	B.subtilis		S.aureus		P. aerugi-nosaa		E.coli	
		Zone (mm)	Conc. (µg/50µl)	Zone (mm)	Conc. (µg/50 µl	Zone (mm)	Conc. (µg/50µl)	Zone (mm)	Conc. (µg/50µl)
1	JPC3	19 16 14 14	250 125 62.5 31.25	13 12 11 9	250 125 62.5 31.25	20 18 17 17	250 125 62.5 31.25	12 10 9 8	250 125 62.5 31.2
2	JPC4	18 17 16 12	250 125 62.5 31.25	13 12 12 10	250 125 62.5 31.25	11 10 9 7	250 125 62.5 31.25	15 14 12 11	250 125 62.5 31.2
3	JPC5	29 28 26 25	250 125 62.5 31.25	20 19 17 15	250 125 62.5 31.25	21 20 18 17	250 125 62.5 31.25	10 9 7 6	250 125 62.5 31.2
4	JPC6	14 13 11 10	250 125 62.5 31.25	19 17	250 125	16 15 13 11	250 125 62.5 31.25	12 11 9 8	250 125 62.5 31.2
5	JPC7	-	-	-	-	-	-	-	-
6	JPC8	-	-	-	-	-	-	-	-
7	JPC9	23 20 18 14	250 125 62.5 31.25	15 13	250 125	-	-	19 16 15	250 125 62.5
8	JPC10	28 24 20	250 125 62.5	15 14	250 125	14 13 12 11	250 125 62.5 31.25	20 17	250 125
9	JPC11	-		-		-		-	
10	JPC12	-		-		-		-	
11	JPC13	17 14 13 11	250 125 62.5 31.25	20 17 15 14	250 125 62.5 31.25	15 14 12 11	250 125 62.5 31.25	16 14 13 12	250 125 62.5 31.2
12	Std.	36	25	30	25	36	25	38	25

Table 5: The relation of IC_{50} of antioxidant activity of synthesized test compounds with their P and Log P values

Comp.	P	Log P	IC_{50} of DPPH Method	IC_{50} of ABTS Method
JPC-3	60.3	1.78	640	183
JPC-4	91.2	1.96	420	106.5
JPC-5	19.4	1.29	>1000	14.46
JPC-6	38.0	1.58	182	85.78
JPC-7	15.4	1.19	>1000	104.05
JPC-8	43.9	1.64	815	75
JPC-9	58.3	1.76	147	87.7
JPC-10	561.8	2.75	872	169
JPC-11	79.3	1.9	>1000	-
JPC-12	89.3	1.95	>1000	-
JPC-13	60.3	1.78	378	-

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