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Synthesis and antioxidant properties of some new flavone derivatives on lipid peroxidation in the rat liver

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A series of 2′-, 3′-, 4′- and 6-substituted flavone derivatives was synthesized and their *in vitro* effects on rat liver microsomal NADPH-dependent lipid peroxidation (LP) levels were determined. Retinoic acid, at 10^{-4} M concentration, decreased the LP level by about 34%. A significant decrease in male liver microsomal LP level was noted for the compounds **3d**, **1a**, **3b** and **4b** at a concentration of 10^{-4} M (100%, 95%, 75% and 62%, respectively).

1. Introduction

The past three decades have seen increasing interest in the role played by oxygen derived free radicals such as superoxide, hydroxyl anions and hydrogen peroxide and antioxidants in cancer, cardiovascular disease and other degenerative diseases [1]. It is known that lipid peroxidation is a free radical chain reaction [2] which causes degeneration of cell membranes. On the other hand, the antioxidants and antioxidant enzyme systems are major protective systems of the organism. However, environmental chemicals can decrease the antioxidant level of the organism and thus could lead to cancer and various diseases. Hence, synthetic antioxidant compounds are required from external sources. Therefore, drugs possessing antioxidant and free radical scavenging properties are considered for preventing and/or treatment of diseases which are directly related to the lack of the antioxidant capacity of the organism [3].

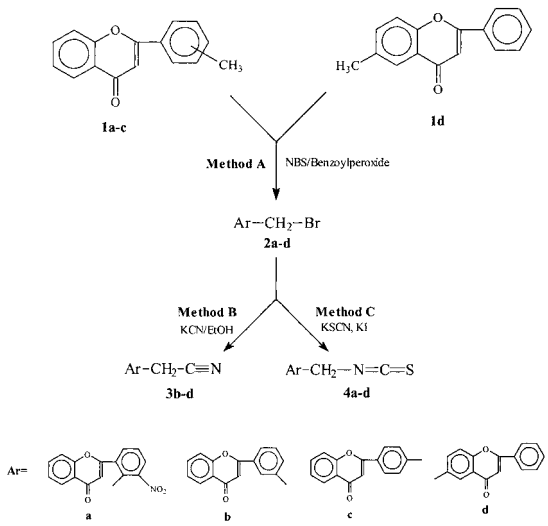
Flavonoids have been acknowledged as having interesting medicinal properties, such as antiinflammatory [4], antiallergic [5], antiviral [6], antibacterial [7] and antitumor [8, 9] activities. They have also been reported to possess antioxidant and antiradical properties [10–13].

The present work was carried out to assess the antioxidant capacity of some flavone derivatives.

2. Investigations, results and discussion

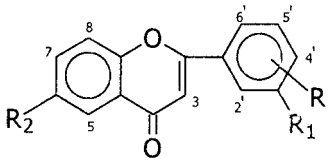
The synthesis of compounds **3b–d**, **4a–d** is illustrated in the Scheme.

Scheme



Compounds **1a** [14], **1b–c** [15], **1d**, **2d** [16], **2b–c** [17] were synthesized according to literature procedures. By reacting **1a–d** with NBS (*N*-bromosuccinimide)/benzoyl peroxide the corresponding **2a–d** were obtained. The bromides **2a–d** were then converted into the nitriles **3b–d** by reaction with KCN and to isothiocyanato derivatives **4a–d** by reaction with KSCN/KI. The physical data of the compounds are shown in Table 1. In the ^{1}H NMR spectra, characteristic protons belonging the flavone moiety can be seen at the various δ ppm values. The H-5 proton of flavone was observed at 8.13–8.29 ppm with the effect of the CO-group of the γ-pyron ring. All the compounds except **4d** have a molecular ion (M^+) peak. Ion peaks m/z = 92, m/z = 57 and m/z = 102 are base peaks for compounds **3b–c** and **4a–c**, **3d**, **4d**, respectively.

Table 1: Some physical properties of the synthesized compounds.



Compd.	R	R_1	R_2	M.p. (°C)	Solvent[a]	Yield (%)
2a	2′-CH_2Br	NO_2	H	149	A	75
3b	3′-CH_2CN	H	H	169	B	36
3c	4′-CH_2CN	H	H	146	B	57
3d	H	H	6-CH_2CN	265	B	20
4a	2′-CH_2NCS	NO_2	H	101	C	53
4b	3′-CH_2NCS	H	H	162	C	38
4c	4′-CH_2NCS	H	H	134	C	58
4d	H	H	6-CH_2NCS	155	C	66

[a] Recrystallization solvents: A = toluene, C = ethanol-water, Column chromatography solvent: B = EtOAc : n-hexane (1 : 1)

In the current study, a series of 2′, 3′, 4′ and 6 substituted flavon derivatives were observed to have *in vitro* effects on lipid peroxidation (LP) levels in rat liver microsomes. All compounds produced inhibition of LP at 10^{-4} and 10^{-5} M concentrations in the liver (Table 2). However, compounds **3d**, **1a**, **3b** and **4b** had stronger inhibitory effects on liver microsomal LP levels than the others. Moreover, these compounds seem to have greater antioxidant potentials than retinoic acid at 10^{-4} and 10^{-5} M concentrations. Thus, this feature of the compounds is likely to render them promising antioxidant compounds.

Table 2: Effects of the compounds on liver LP levels[a] *in vitro*

Compd.	Concentration in incubation medium (M)	LP (nmol MDA/mg) protein	%
Control[b]	–	67.50 ± 0.29	100
1a	10^{-4}	3.70 ± 0.29	5
	10^{-5}	6.59 ± 0.11	10
1c	10^{-4}	58.41 ± 0.54	86
	10^{-5}	55.74 ± 5.51	83
1d	10^{-4}	55.91 ± 0.17	81
	10^{-5}	53.13 ± 0.40	79
3b	10^{-4}	16.93 ± 0.13	25
	10^{-5}	56.76 ± 1.76	84
3c	10^{-4}	32.67 ± 3.01	48
	10^{-5}	58.75 ± 3.75	87
3d	10^{-4}	0.00 ± 0.00	0
	10^{-5}	62.67 ± 0.85	93
4a	10^{-4}	53.41 ± 6.01	79
	10^{-5}	59.20 ± 9.2	87
4b	10^{-4}	26.14 ± 2.16	38
	10^{-5}	51.87 ± 4.83	77
4c	10^{-4}	57.79 ± 3.36	86
	10^{-5}	59.95 ± 2.10	89
4d	10^{-4}	48.52 ± 11.25	71
	10^{-5}	61.99 ± 5.73	92
Retinoic acid	10^{-4}	44.43 ± 0.56	66
	10^{-5}	49.65 ± 0.27	74

[a] Each value represents the mean ± S.D. of 2–4 independent experiments
[b] Dimethylsulphoxide only, control for compounds

3. Experimental

3.1. Apparatus

M.p.'s were determined with a Buchi SMP-20 apparatus and are uncorrected. IR spectra were recorded on a Jasco FT/IR 420 spectrometer as potassium bromide discs. All the instrumental Analyses were performed by TUBITAK (Instrumental Analysis Lab., Ankara) with a VG Platform II mass spectrometer with ionization energy maintained at 70 eV and Bruker AC 400 NMR spectrophotometer using TMS internal standard and $CDCl_3$. All chemical shifts were reported as δ (ppm) values. Microanalyses were performed on a Leco CHNS 932 analyzer and satisfactory results ± 0.4% of calculated values (C, H, N) were obtained. For the chromatographic analyses Merck Silica Gel 60 (230–400 mesh ASTM) was used. The chemical reagents used in synthesis were purchased from E. Merck and Aldrich. D-Glucose-6-phosphate monosodium salt, yeast D-glucose-6-phosphate dehydrogenase ($NADP^+$) were purchased from Sigma. Crystalline bovine serum albumin was obtained from BDH Chemicals.

3.2. Synthesis

*3.2.1. Preparation of 2-(2′-bromomethyl-3′-nitro-phenyl)-4 H-1-benzopyran-4-one=(2′-bromomethyl-3′-nitro-flavone) (**2a**, method A)*

A mixture of 2-(2′-methyl-3′-nitro-phenyl)-4 *H*-1-benzopyran-4-one (1 g, 2.8 mmol), NBS 0.5 g (2.8 mmol) and a catalytic amount of benzoyl peroxide, in 25 ml of CCl_4 was refluxed for 9 h. Afterwards, it was concentrated to dryness and the residue on crystallizing from toluene gave 1.26 g of product. IR (KBr)cm^{-1}: 1644 (γ-Pyrone C=O). ^1H NMR ($CDCl_3$): δ = 4.89 (s, 2 H, CH_2Br), 6.49 (s, 1 H, 3-H), 7.47–7.59 (m, 2 H, 6, 8-H), 7.64–7.82 (m, 3 H, 4′,5′, 7-H), 7.99 (d, 1 H, $J_{6',5'}$ = 8.13 Hz, 6′-H), 8.29 (d, 1 H, $J_{5,6}$ = 8.10 Hz, $J_{5,7}$ = 1.63 Hz, 5-H). MS (EI): m/z (%) = 280 (0.4) [M^+-Br], 205 (1), 160 (1), 120 (24), 113 (20), 92 (100), 76 (37), 63 (77).
$C_{16}H_{10}BrNO_4$ (360)

*3.2.2. Preparation of cyanomethyl-flavones **3b–d** (method B)*

A suspension of **2a–d** (1.6 mmol) in boiling method ethanol (3 ml) was added in 3–4 portions to a stirred solution of KCN (1.38 g, 2.13 mmol) in water (3 ml) maintained at 70 °C. The mixture was heated under reflux for 12 h, the ethanol was removed evaporation and purified by column chromatography.

3.2.2.1. 2-(3′-Cyanomethyl-phenyl)-4 *H*-1-benzopyran-4-one (3′-cyanomethyl-flavone (**3b**)

IR (KBr) cm^{-1}: 2252 (C≡N), 1638 (γ-Pyrone C=O). ^1H NMR ($CDCl_3$): δ = 3.87 (s, 2 H, CH_2CN), 6.82 (s, 1 H, 3-H), 7.43 (ddd, 1 H, $J_{6,5} = J_{6,7}$ = 8.05 Hz, $J_{6,8}$ = 1.03 Hz, 6-H), 7.53–7.59 (m, 3 H, 4′,5′, 8-H), 7.72 (dtd, 1 H, $J_{7,6} = J_{7,8}$ = 7.79 Hz, $J_{7,5}$ = 1.70 Hz, 7-H), 7.80–7.90 (m, 2 H, 2′,6′-H). 8.23 (dd, 1 H, $J_{5,6}$ = 7.95 Hz, $J_{5,7}$ = 1.59 Hz, 5-H). MS (EI): m/z (%) = 261 (49) [M^+], 234 (7) [M^+-HCN], 233 (39), 221 (7), 140 (13), 121 (8), 120 (82), 92 (100), 76 (15).
$C_{17}H_{11}NO_2 \cdot 0.15\,H_2O$ (264.2)

3.2.2.2. 2-(4′-Cyanomethyl-phenyl)-4 *H*-1-benzopyran-4-one (4′-cyanomethyl-flavone) (**3c**)

IR (KBr) cm^{-1}: 2248 (C≡N), 1624 (γ-Pyrone C=O). ^1H NMR ($CDCl_3$): δ = 3.91 (s, 2 H, CH_2CN), 6.88 (s, 1 H, 3-H), 7.49 (ddd, 1 H, $J_{6,5} = J_{6,7}$ = 8.04 Hz, $J_{6,8}$ = 1.04 Hz, 6-H), 7.57 (d, 2 H, $J_{3',2'} = J_{5',6'}$ = 8.54 Hz, 3′,5′-H), 7.63 (d, 1 H, $J_{8,7}$ = 7.86 Hz, 8-H), 7.77 (dtd, 1 H, $J_{7,6} = J_{7,8}$ = 8.43 Hz, $J_{7,5}$ = 1.70 Hz, 7-H), 8.01 (d, 2 H, $J_{2',3'} = J_{6',5'}$ = 8.42 Hz, 2′,6′-H), 8.29 (dd, 1 H, $J_{5,6}$ = 7.97 Hz, $H_{5,7}$ = 1.60 Hz, 5-H). MS (EI): m/z (%) = 261 (21) [M^+], 234 (3) [M^+-HCN], 233 (17), 221 (4), 140 (11), 121 (4), 120 (46), 92 (100), 64 (41).
$C_{17}H_{11}NO_2$ (261)

3.2.2.3. 6-Cyanomethyl-2-phenyl-4 *H*-1-benzopyran-4-one (6-cyanomethyl-flavone) (**3d**)

IR (KBr) cm^{-1}: 2246 (C≡N), 1649 (γ-Pyrone C=O). ^1H NMR ($CDCl_3$): δ = 4.23 (s. 2 H, CH_2CN), 7.07 (s, 1 H, 3-H), 7.58–7.62 (m, 3 H, 3′,4′,5′-H), 7.80–7.86 (m, 2 H, 7, 8-H), 8.06–8.13 (m, 3 H, 2′,6′, 5-H). MS (EI): m/z (%) = 261 (3.9) [M^+], 234 (0.4) [M^+-HCN], 233 (2), 149 (42), 130 (2), 121 (4), 102 (14), 77 (14), 57 (100).
$C_{17}H_{11}NO_2$ (261)

*3.2.3. Preparation of isothiocyanatomethyl flavones **4a–d** (method C)*

A mixture of **2a–d** (1.62 mmol), potassium thiocyanate (0.18 g, 1.87 mmol) and potassium iodide (0.135 g, 0.82 mmol) in methanol (6 ml) was heated under reflux for 2 h. After cooling, water was added. The precipitate was collected and crystallized from ethanol-water (if necessary active carbon was used) and gave compounds **4a–d**.

3.2.3.1. 2-(2′-Isothiocyanatomethyl-3′-nitro-phenyl)-4 *H*-1-benzopyran-4-one (2′-isothiocyanatomethyl-3′-nitro-flavone) (**4a**)

IR (KBr) cm^{-1}: 2153 (N=C=), 1645 (γ-Pyrone C=O). ^1H NMR ($CDCl_3$): δ = 4.77 (s, 2 H, CH_2NCS), 6.54 (s, 1 H, 3-H), 7.50 (ddd, 1 H, $J_{6,5} = J_{6,7}$ = 7.78 Hz, 6-H), 7.61 (d, 1 H, $J_{8,7}$ = 8.41 Hz, 8-H), 7.70–7.77 (m, 3 H, 4′,5′,7-H), 7.85 (dd, 1 H, $J_{6',5'}$ = 7.69 Hz, $J_{6',4'}$ = 0.91 Hz, 6′-H), 8.27 (d, 1 H, $J_{5,6}$ = 7.88 Hz, 5-H. MS (EI): m/z (%) = 338 (0.5) [M^+], 280 (2) [M^+-NCS], 234 (2), 221 (2), 205 (4), 160 (16), 120 (24), 92 (100).
$C_{17}H_{10}N_2O_4S \cdot 0.5\,H_2O$ (347)

3.2.3.2. 2-(3′-Isothiocyanatomethyl-phenyl)-4 *H*-1-benzopyran-4-one (3′-isothiocyanatomethyl-3′-nitro-flavone) (**4b**)

IR (KBr) cm^{-1}: 2155 (N=C=), 1640 (γ-Pyrone C=O). ^1H NMR ($CDCl_3$): δ = 4.28 (s, 2 H, CH_2NCS), 6.86 (s, 1 H, 3-H), 7.46 (ddd, 1 H, $J_{6,5} = J_{6,7}$ = 8.03 Hz, $J_{6,8}$ = 1.03 Hz, 6-H), 7.53–7.66 (m, 3 H, 4′,5′,8-H), 7.74 (dtd, 1 H, $J_{7,6} = J_{7,8}$ = 6.95 Hz, $J_{7,5}$ = 1.70 Hz, 7-H), 7.91–7.96 (m, 2 H, 2′,6′-H), 8.26 (dd, 1 H, $J_{5,6}$ = 7.95 Hz, $J_{5,7}$ = 1.64 Hz, 5-H). MS (EI): m/z (%) 293 (33) [M^+], 235 (58) [M^+-NCS], 207 (8), 120 (16), 115 (56), 92 (100), 64 (45), 63 (87).
$C_{17}H_{11}NO_2S \cdot 0.15\,H_2O$ (295.7)

3.2.3.3. 2-(4′-Isothiocyanatomethyl-phenyl)-4 *H*-1-benzopyran-4-one (4′-isothiocyanatomethyl-flavone) (**4c**)

IR (KBr) cm^{-1}: 2143 (N=C=), 1643 (γ-Pyrone C=O). ^1H NMR ($CDCl_3$): δ = 4.21 (s, 2 H, CH_2NCS), 6.83 (s, 1 H, 3-H), 7.43 (ddd, 1 H, $J_{6,5} = J_{6,7}$ = 7.65 Hz, 6-H), 7.54 (d, 2 H, $J_{3',2'} = J_{5',6'}$ = 8.24 Hz, 3′,5′-H, 7.57 (d, 1 H, $J_{8,7}$ = 8.47 Hz, 8-H), 7.70 (ddd, 1 H, $J_{7,6} = J_{7,8}$ = 8.34 Hz, $J_{7,5}$ = 1.01 Hz, 7-H), 7.96 (d. 2 H, $J_{2',3'} = J_{6',5'}$ = 8.21 Hz, 2′,6′-H), 8.23 (d, 1 H, $J_{5,6}$ = 7.85 Hz, 5-H). MS (EI): m/z (%) = 293 (5) [M^+], 235 (26) [M^+-NCS], 207 (19), 120 (15), 115 (69), 92 (100), 63 (85), 64 (47).
$C_{17}H_{11}NO_2S \cdot 0.3\,CH_3OH \cdot 0.2\,H_2O$ (306.2)

3.2.3.4. 6-Isothiocyanatomethyl-2-phenyl-4 *H*-1-benzopyran-4-one (6-isothiocyanatomethyl-flavone) (**4d**)

IR (KBr) cm^{-1}: 2153 (N=C=), 1650 (γ-Pyrone C=O). ^1H NMR ($CDCl_3$): δ = 4.27 (s, 2 H, CH_2NCS), 6.84 (s, 1 H, 3-H), 7.55 (m, 3 H, 3′,4′,5′-H), 7.62 (d, 1 H, $J_{8,7}$ = 8.62 Hz, 8-H), 7.73 (dd, 1 H, $J_{7,8}$ = 8.61 Hz, $J_{7,5}$ = 2.25 Hz, 7-H), 7.93 (dd, 2 H, $J_{2',3'} = J_{6',5'}$ = 7.33 Hz, $J_{2',4'} = J_{6',4'}$ = 2.06 Hz, 2′,6′-H), 8.22 (d, 1 H, $J_{5,7}$ = 2.15 Hz, 5-H). MS (EI): m/z (%) = 235 (7) [M^+-NCS], 133 (15), 102 (100), 77(44), 76 (54).
$C_{17}H_{11}NO_2S \cdot 0.6\,H_2O$ (303.8)

3.3. Assay of lipid peroxidation

Male albino (local strain) rats (200–225 g) were used in the experiments. Animals were fed with standard laboratory rat chow and tab water ad libi-

tum. The animals were starved for 24 h pirior to sacrifice. They were killed by decapitation. The livers were removed immediately and washed in ice-cold distilled water and the microsomes were prepared as described previously [18].

NADPH-dependent LP was determined using the optimum conditions determined and described previously [18]. NADPH-dependent LP was measured spectrophotometrically by estimation of thiobarbituric acid reactant substances (TBARS). Amounts of TBARS were expressed in terms of nmol malondialdehyde (MDA)/mg protein. The assay was essentially derived from the methods of Wills [19, 20] as modified by Bishayee [21]. A typical optimized assay mixture contained 0.2 mM Fe^{++}, 90 mM KCl, 62.5 mM potassium phosphate buffer, pH 7.4, NADPH generating system consisting of 0.25 mM $NADP^{+}$, 2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 1.0 U glucose-6-phosphate dehydrogenase and 14.2 mM potassium phosphate buffer pH 7.8 and 0.2 mg microsomal protein in a final volume of 1.0 ml.

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