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Synthesis and antioxidant properties of novel benzimidazoles containing substituted indole or 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene fragments

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Abstract
Some 6-fluoro-5-substituted-benzimidazole derivatives in which indole and 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene groups were attached to the 2-position of the benzimidazole ring were synthesized and tested for antioxidant properties *in vitro*. Almost all the synthesized compounds at the 10^{-3} M concentrations showed superoxide anion scavenging activity. Compounds **5, 3, 9, 4, 17** and **13** have strong inhibitory effects on superoxide anion formation (98%, 93%, 91%, 88%, 85% and 81%, respectively) at 10^{-3} M concentration and these results are better than 30 IU of superoxide dismutase (SOD) (76%). Compound **11** is the most effective scavenger of 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable free radical at 10^{-3} M (61%) concentration.

Keywords: *Benzimidazole, indole, retinoids, synthesis, free radical, antioxidant*

Introduction

Free radicals interfere with many diseases including inflammation, atherosclerosis, shock, ischemic disease, and cancer[1–3]. Hydroxyl ($OH^{\cdot}$), superoxide anion ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$) and peroxyl ($RO_2^{\cdot}$) radicals, reactive oxygen species (ROS), are involved in different physiological processes[4,5]. Protection of biological molecules such as lipids, carbohydrates, proteins and DNA from oxidative stress is very important to prevent inflammatory diseases, such as atherosclerosis, aging and cancer, accentuated by high levels of ROS[6]. Antioxidants scavenge and prevent the formation of free radicals so they are highly important for the potential treatment of these kinds of diseases, so that in recent years, there has been an increasing interest in finding new antioxidant compounds.

Melatonin (*N*-acetyl-5-methoxytryptamine) (Figure 1) is a free radical scavenger and antioxidant. Due to the stimulation of several antioxidative enzymes i.e., SOD, glutathione peroxidase (GPx), and glutathione reductase (GRd), melatonin increases antioxidant effectiveness of these enzymes[7]. *All trans*-retinoic acid (ATRA) and retinol (vitamin A) (Figure 1), which are natural retinoids, are used clinically for the treatment of proliferative dermatological diseases and for the prevention of some tumors[8]. A number of retinoic acid derivatives, termed retinoids as a biological general term[9], have been reported and their antioxidant potencies have been investigated[10–12]. However, some benzimidazole retinoids have been reported as retinoid antagonists[13].

Recently, many reports from us and others have revealed the antioxidant properties of some novel indole and benzimidazole derivatives[10–12,14–16]. In connection with these investigations our studies continue to search for new benzimidazole derivatives having potent antioxidant activities.

In this study we have aimed to connect both indole and tetrahydronaphthalene fragments to the benzimidazole ring. Synthesis of some new

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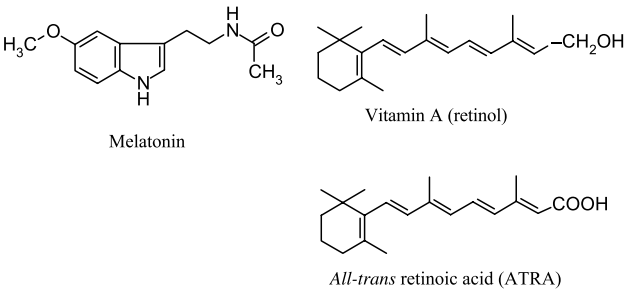


Figure 1. Structures of melatonin, retinol and retinoic acid.

5-substituted-6-fluoro-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1*H*-benzimidazoles (**1–5**) and 5-substituted-6-fluoro-2-(5-substituted-1*H*-indol-3-yl)-1*H*-benzimidazole derivatives (**6–17**) was performed and their free radical scavenging properties examined *in vitro* by determining their capacity to scavenge superoxide anion formation and to interact with the stable free radical DPPH.

Materials and methods

Chemistry

Uncorrected melting points were measured with an Electrotermal melting point apparatus. ^{1}H NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer in DMSO-d_6, Chemical shifts are expressed as δ (ppm) values with tetramethylsilane (TMS) as an internal standard and coupling constants (J) are reported in Hertz and multiplicity as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak. Mass spectra were determined on a Waters Micromass ZQ Ms spectrometer using the ESI technique. Elemental analyses (C, H, N) were determined on a Leco CHNS 932 instrument (St.Joseph, M1 USA), and were within ±0.4% of the theoretical values. All instrumental analyses were performed at Ankara University, Faculty of Pharmacy. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60F-254). Column chromatography was conducted on silica gel 60 (40–63 μm particle size) (Merck). All starting materials and reagents were high-grade commercial products purchased from Aldrich, Merck or Fluka.

For the synthesis of the final compounds (**1–17**) the reaction sequences are outlined in Figure 2 and the substituents of **1–17** are shown in Table II. The target compounds (**1–17**) were synthesized via condensation of related *o*-phenylenediamines with the sodium metabisulfite adduct of 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde in DMF (Method a) or with substituted indole and sodium metabisulfite in ethanol (method b). *o*-Phenylenediamines,[17,18] substituted indole carboxyaldehydes[19] and 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde sodium metabisulfite salt,[10,11] which are all required in this study, were synthesized by a few steps according to published procedures.

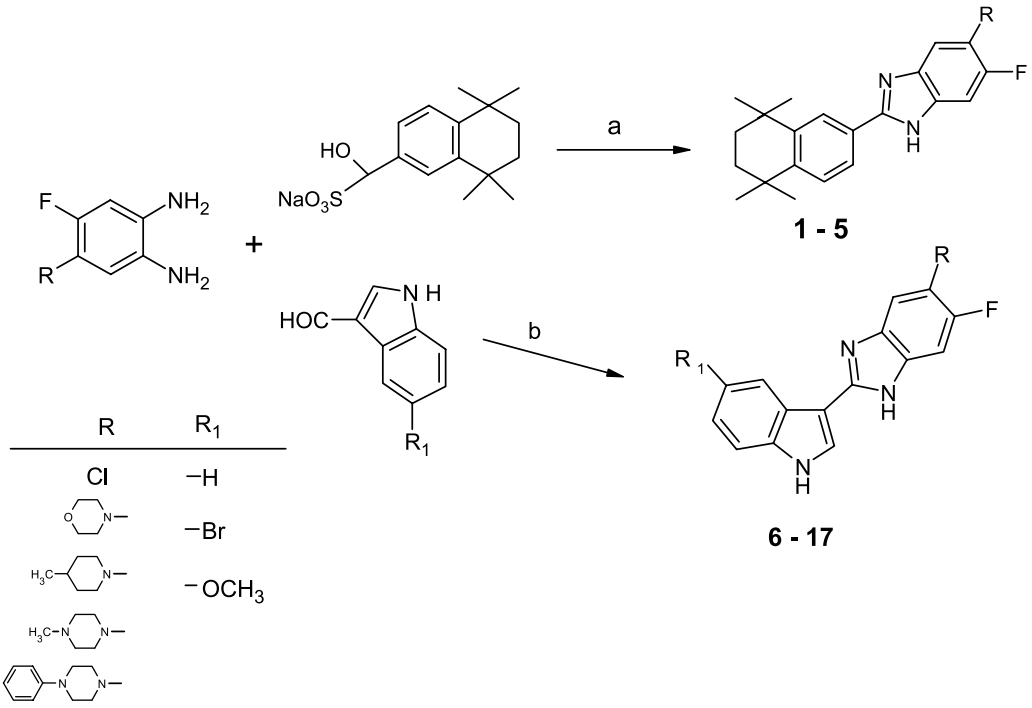


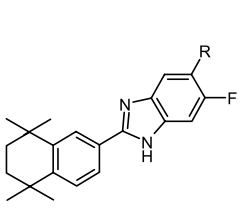
Figure 2. Synthetic scheme for the preparation of 5-substituted-6-fluoro-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1H-benzimidazole derivatives (**1–5**) and 5-substituted-6-fluoro-2-(5-substituted-1H-indol-3-yl)-1H-benzimidazole derivatives (**6–17**). see Table II for R, R′-substituents for respective compounds.

Table I. Physical and spectral data for compounds **1-17.**

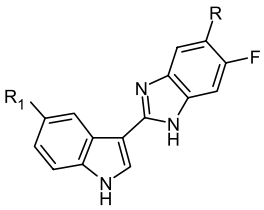
No	Mp (°C)	Yield (%)	Formulas	^{1}H NMR (DMSO-d_6)	Mass (ESI)	Isolation
1	116-118	68	$C_{21}H_{22}ClFN_2 \cdot 0.2\ C_6H_{14}$	1.25 (s, 6H), 1.31 (s, 6H), 1.65 (s, 4 H), 7.47 (d, 1H, $\mathcal{J}$ = 8), 7.52-7.8 (2H), 7.9 (dd, 1H, $\mathcal{J}$ = 8.4, $\mathcal{J}$ = 1.6), 8.1 (s,1H), 13.15 (br.s, 1H).	359 (M + 3, 32.9), 357 (M + 1, 100)	EtOAc: n-Hexane (1: 5) cc
2	170	71	$C_{25}H_{30}FN_3O \cdot H_2O$	1.30 (s, 6H), 1.32 (s, 6H), 1.65 (s, 4H), 3.0 (s, 4H), 3.75 (s, 4H), 7.04-7.42 (2H), 7.45 (d, 1H, $\mathcal{J}$ = 8.4), 7.83 (d, 1H, $\mathcal{J}$ = 8), 8.06 (s, 1H), 12.8 (s, 1H).	408 (M + 1, 100)	EtOAc: n-Hexane (3: 1) cc
3	158-159	62	$C_{27}H_{34}FN_3 \cdot H_2O$	0.9 (d, 3H, $\mathcal{J}$ = 6.4), 1.23 (s, 6H), 1.26 (s, 6H), 1.29(m, 2H), 1.45 (m, 1H), 1.62 (s, 4H), 1.64 (2H), 2.62 (t, 2H), 3.28 (2H), 7.0-7.35 (2H), 7.44 (d, 1H, $\mathcal{J}$ = 8.8), 7.82 (d, 1H, $\mathcal{J}$ = 7.6), 8.0 (s, 1H), 12.6 (br.s, 1H).	420 (M + 1, 100)	EtOAc: n-Hexane (1: 1) cc
4	176	55	$C_{26}H_{33}FN_4 \cdot H_2O$	1.30 (d, 12H, $\mathcal{J}$ = 18.8), 1.7 (s, 4 H), 2.3 (s, 3H), 3.07 (s, 4H), 3.35 (s, 4H), 7.2 (2H), 7.4 (s, 1H), 7.85 (s, 1H), 8.07 (s, 1H), 12.6 (br.s, 1H).	421 (M + 1, 100)	$CHCl_3$: isopropanol (4: 1) cc
5	175 bubl. 305-308	78	$C_{31}H_{35}FN_4 . 0.5\ H_2O$	1.28 (d, 12H, J = 21), 1.67 (s, 4H), 3.1 (4H), 3.3 (s, 4H), 6.8-7.5 (8H), 7.8 (d, 1H, J = 7.6), 8.1 (s, 1H), 12.75 (br.s, 1H).	483 (M + 1, 100)	EtOAc: n-Hexane (1: 3) cc
6	282	77	$C_{19}H_{17}FN_4O \cdot 0.25\ C_4H_8O_2$	3.0 (s, 4 H), 3.78 (s, 4 H), 7.01-7.4 (5 H), 8.06 (td, 1H), 8.44 (td, 1H), 11.6 (s, 1H), 12.4 (1H).	337 (M + 1, 100)	EtOAc: n-Hexane crystallization
7	165-167	73	$C_{21}H_{21}FN_4 . 0.2\ C_4H_8O_2$	0.9 (d, 3H, $\mathcal{J}$ = 6.9), 1.32 (m, 2H), 1.48 (m, 1H), 1.7 (2H), 2.65 (t, 2H), 3.25 (2H), 7.0-7.38 (4H), 7.47 (dd, 1H, $\mathcal{J}$ = 6.5, $\mathcal{J}$ = 1.2), 8.06 (s, 1H), 8.44 (s, 1H), 11.6 (s, 1H), 12.3 (1H).	349 (M + 1, 100)	EtOAc: n-Hexane (1: 1) cc
8	170-171	38	$C_{20}H_{20}FN_5 . C_3H_8O . 2\ H_2O$	2.2 (s, 3H), 2.99 (s, 4 H), 3.39-3.48 (m, 4 H), 7.0-7.4 (4 H), 7.49 (d, 1H, $\mathcal{J}$ = 7.2), 8.18 (s, 1H), 8.44 (td, 1H), 11.58 (s, 1H), 12.45 (1H).	350 (M + 1, 100)	$CHCl_3$: isopropanol: NH_3 (11: 4:1) cc
9	165-166	65	$C_{25}H_{22}FN_5 . 0.25\ C_4H_8O_2$	3.1 (s, 4H), 3.3 (s, 4H), 6.8- 7.5 (10 H), 8.1 (d, 1H, $\mathcal{J}$ = 2.8), 8.5 (s, 1H), 11.6 (s, 1H), 12.4 (br.s, 1H).	412 (M + 1, 100)	CH_2Cl_2: isopropanol (10: 1) cc EtOAc crystallization
10	199-202	69	$C_{19}H_{16}BrFN_4O .0.2\ H_2O$	2.99 (s, 4H), 3.77 (s, 4H), 7.02-7.44 (3H), 7.45 (d, 1H, $\mathcal{J}$ = 8.4), 8.1 (s, 1H), 8.63 (1H), 11.6 (s, 1H), 12.4 (1H).	417 (M + 3, 100), 415 (M + 1, 100)	EtOAc: isopropanol (10: 1) cc
11	150-152	88	$C_{21}H_{20}BrFN_4 .0.1\ H_2O$	0.95 (d, 3H, $\mathcal{J}$ = 6.4), 1.33 (m, 2H), 1.48 (br.s, 1H), 1.7 (2H), 2.65 (t, 2H), 3.28 (m, 2H), 7.01-7.4 (3H), 7.45 (d, 1H, $\mathcal{J}$ = 8.8), 8.1 (td, 1H), 8.63 (dd, 1H, $\mathcal{J}$ = 8.8, $\mathcal{J}$ = 2), 11.8 (s, 1H), 12.4 (1H).	429 (M + 3, 100), 427 (M + 1, 100)	EtOAc: n-Hexane (1: 1) cc
12	192-193	49	$C_{20}H_{19}BrFN_5 . 2\ H_2O$	2.25 (s, 3H), 3.0 (s, 4H), 3.4 (s, 4H), 7.01-7.47 (4H), 8.1 (s, 1H), 8.65 (1H), 11.8 (s, 1H), 12.45 (1H).	430 (M + 3, 100), 428 (M + 1, 100)	CH_2Cl_2: isopropanol: NH_3 (10: 5: 1) cc
13	190	53	$C_{25}H_{21}BrFN_5 . 0.2\ C_4H_8O_2$	3.14 (t, 4 H), 3.31 (4 H), 6.7 (td, 1H), 6.99 (d, 2H, $\mathcal{J}$ = 8.4), 7.09 - 7.48 (6H), 8.1 (s, 1H), 8.64 (dd, 1H, $\mathcal{J}$ = 8, $\mathcal{J}$ = 2), 11.78 (s, 1H), 12.43 (1H).	494 (M + 3, 100), 492 (M + 1, 100)	Crys. EtOAc: n-Hexane (50%)
14	152-155	67	$C_{20}H_{19}FN_4O_2 .1.7\ H_2O$	3.0 (s, 4H), 3.78 (t, 4H), 3.83 (s, 3H), 6.84 (dd, 1H, $\mathcal{J}$ = 8.4, $\mathcal{J}$ = 2), 7.01-7.4 (3 H), 7.9 (d, 1H, $\mathcal{J}$ = 14.8), 8.0 (s, 1H), 11.4 (s, 1H), 12.3 (1H).	367 (M + 1, 100)	EtOAc: n-Hexane (1: 1) cc
15	171-173	62	$C_{22}H_{23}FN_4O . 0.2\ C_4H_8O_2$	0.96 (d, 3H, $\mathcal{J}$ = 6.8), 1.34 (t, 2H), 1.48 (m, 1H), 1.7 (2H), 2.65 (t, 2H), 3.28 (2H), 3.8 (s, 3H), 6.8 (dd, 1H, $\mathcal{J}$ = 8.8, $\mathcal{J}$ = 2.4), 6.9-7.3 (2H), 7.35 (d, 1H, $\mathcal{J}$ = 8.8), 7.94 (dd, 1H, $\mathcal{J}$ = 2), 8.0 (1H), 11.4 (s, 1H), 12.2 (1H).	379 (M + 1, 100)	Crys. EtOAc: n-Hexane (50%)
16	192-193	73	$C_{21}H_{22}FN_5O . 0.5\ H_2O . 0.3\ C_6H_{14}$	2.2 (s, 3H), 2.43 (s, 4 H), 2.99 (s, 4 H), 3.8 (s, 3H), 6.8 (dd, 1 H, $\mathcal{J}$ = 8.8, $\mathcal{J}$ = 2.4), 6.9-7.4 (3H), 7.9 (d, 1H, $\mathcal{J}$ = 1.6), 8.0 (s, 1H), 11.4 (s, 1H), 12.3 (1H).	380 (M + 1, 100)	isopropanol: NH_3 (10: 0.5) cc EtOAc: n-Hexane crystallization
17	174-175	77	$C_{26}H_{24}FN_5O.H_2O$	3.15 (s, 4H), 3.3 (s, 4H), 3.8 (s, 3H), 6.78- 6.81 (td, 1H), 6.83 (dd, 1H, $\mathcal{J}$ = 8.8, $\mathcal{J}$ = 2.4), 6.9 (d, 2H, $\mathcal{J}$ = 8), 7.06-7.44 (5 H), 7.9 (dd, 1H, $\mathcal{J}$ = 1.6), 8.05 (s, 1H), 11.45 (s, 1H), 12.4 (1H).	442 (M + 1, 100)	EtOAc: n-Hexane (2: 1) cc

ESI: Electrospray ionization; cc: column chromatography; $C_4H_8O_2$: EtOAc; C_6H_{14}: *n*-hexane; C_3H_8O: isopropanol.

Table II. Structures and effects of the compounds (**1-17**) on DPPH free radical and superoxide anion radical scavenging activity.[a]



1 - 5



6 - 17

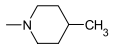
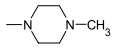
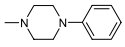
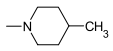
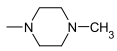
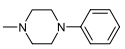
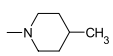
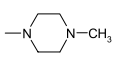
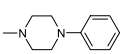
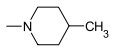
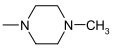
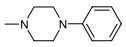
Comp.	R	R_1	Log P	Concentration in incubation medium (M)	DPPH free radical scavenger activity (percent of control)	Superoxide anion ($O_2^{\cdot-}$) scavenger activity percent of control
Control[b] DMSO					100 ± 7.2	100 ± 7.2
1	Cl	–	6.49	10^{-3}	68 ± 4.9	42 ± 9.9
				10^{-4}	93 ± 9.5	35 ± 9.5
2		–	5.82	10^{-3}	100 ± 4.9	100 ± 4.9
				10^{-4}	100 ± 3.5	100 ± 3.5
3		–	7.28	10^{-3}	87 ± 1.4	7.0 ± 1.4
				10^{-4}	86 ± 1.4	86 ± 1.4
4		–	5.97	10^{-3}	93 ± 0.7	12 ± 6.0
				10^{-4}	98 ± 2.1	72 ± 1.4
5		–	8.05	10^{-3}	85 ± 5.5	2.0 ± 5.5
				10^{-4}	90 ± 0.8	69 ± 0.8
6		—H	2.45	10^{-3}	82 ± 4.9	45 ± 4.2
				10^{-4}	96 ± 5.6	70 ± 5.6
7		—H	3.91	10^{-3}	78 ± 2.1	70 ± 2.2
				10^{-4}	83 ± 6.3	70 ± 11.2
8		—H	2.60	10^{-3}	84 ± 5.6	134 ± 5.6
				10^{-4}	90 ± 3.5	90 ± 3.5
9		—H	4.68	10^{-3}	59 ± 1.4	9 ± 2.1
				10^{-4}	89 ± 5.6	62 ± 5.6
10		—Br	3.28	10^{-3}	75 ± 1.4	44 ± 1.4
				10^{-4}	88 ± 2.8	14 ± 2.8
11		—Br	4.74	10^{-3}	39 ± 4.2	33 ± 5.6
				10^{-4}	90 ± 2.8	48 ± 3.5
12		—Br	3.43	10^{-3}	76 ± 28	33 ± 2.1
				10^{-4}	85 ± 4.2	85 ± 2.1
13		—Br	5.51	10^{-3}	47 ± 1.4	19 ± 0.7
				10^{-4}	87 ± 1.4	100 ± 1.4
14		OCH_3	2.32	10^{-3}	75 ± 4.9	68 ± 5.2
				10^{-4}	95 ± 2.8	72 ± 3.0

TABLE II – *continued*

Comp.	R	R_1	Log P	Concentration in incubation medium (M)	DPPH free radical scavenger activity (percent of control)	Superoxide anion ($O_2^{\cdot-}$) scavenger activity percent of control
15		OCH_3	3.78	10^{-3} 10^{-4}	54 ± 6.3 96 ± 4.2	70 ± 8.2 52 ± 4.4
16		OCH_3	2.48	10^{-3} 10^{-4}	76 ± 2.1 88 ± 2.8	70 ± 2.2 88 ± 2.2
17		OCH_3	4.55	10^{-3} 10^{-4}	75 ± 2.4 94 ± 5.6	15 ± 2.2 54 ± 13.4
Control[c]				Water		100 ± 2.1
BHT				10^{-3} 10^{-4}	10 ± 2.1 31 ± 0.7	
SOD				30 IU 45 IU		24 ± 2.1 11 ± 1.0

[a]Each value represents the mean ± S.D. of 3 independent experiments.
[b]DMSO only, control for compounds.
[c]Distilled water, control for SOD.

The structures of the synthesized compounds were consistent with their ^{1}H NMR spectra, and molecular weights of these compounds were determined by ESI mass spectra. Some physical and spectral data for **1-17** are summarized in Table I.

General procedure for the preparation of 5-substituted-6-fluoro-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1H-benzimidazole derivatives (1-5). A solution of 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde (6.6 g, 30 mmol) in 20 mL EtOH was added to a solution of $Na_2S_2O_5$ (3.12 g, 30 mmol) in 20 mL water and the mixture stirred in an ice-bath to give a white precipitate which was filtered and dried (m.p. decom. >300°C). The mixture of the sodium metabisulfite adduct of 5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde (2 mmol) and the appropriate 1,2-phenylenediamine (2 mmol) in DMF (5 mL) were heated at 130°C for 4 h. The reaction mixture was cooled, poured into the water, and the solid was filtered. The purification procedure and some spectral data for the synthesized compounds are given in Table I.

General procedure for the preparation of 5-substituted-6-fluoro-2-(5-substituted-1H-indol-3-yl)-1H-benzimidazole derivatives (6-17). A mixture of the appropriate *o*-phenylendiamine (1 mmol), related indole derivative (1 mmol) and $Na_2S_2O_5$ (40%) (2 mL) in EtOH (4 mL), was refluxed under nitrogen atmosphere for 4 h. The reaction mixture was poured into water, and the precipitate was filtered and washed with water. The purification procedure and some spectral data for the synthesized compounds are given in Table I.

Biological activity studies

Superoxide radical scavenging activity. The capacity of the benzimidazole compounds **1-17** to scavenge superoxide anion formation was determined spectrophotometrically on the basis of inhibition of cytochrome c reductase according to the modified method of McCord et al[20]. The results are shown in Table II. Superoxide anion was generated in the xanthine/xanthine oxidase system. The reaction mixture contained in a final volume of 1 mL, consisted of 0.05M phosphate buffer pH 7.8, 0.32 U xanthine oxidase, 50 μM xanthine, 60 mM ctytochrome c and different concentrations of synthesized compounds in 100 μL of DMSO. DMSO was used as the control solution. The absorbance was measured spectrophotometrically at 550 nm for cytochrome c reduction. Each experiment was performed in triplicate, and the results are expressed as a percentage of the control value.

DPPH free radical scavenging activity. The free radical scavenging activities of compounds (**1-17**) were tested by their ability to bleach the stable radical DPPH[21]. The results are shown in Table II. This assay has often been used to estimate the antiradical activity of antioxidants. Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy. The reaction mixture contained

100μM DPPH in methanol and different concentrations of compounds. Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity was calculated as a percentage of radical reduction. Each experiment was performed in triplicate. DMSO was used as a control solution and BHT as a reference compound.

Log P. Hydrophobicity, expressed as log P values, of compounds synthesized in this study was calculated with the programme CS Chem Draw Pro 5.0 CambridgeSoft Corporation, based on Crippen's fragmentation, Viswanadhan's fragmentation and Broto's method[22,23].

Results and discussion

Free radical scavenging properties of tetrahydronaphthalene (**1-5**) and indolobenzimidazole (**6-17**) derivatives were examined by determining their scavenging capability for superoxide anion and their interaction with the stable free radical DPPH.

In the present study our results on superoxide radical scavenging and DPPH free radical scavenging activity did not correlate. The compounds showed greater scavenger activity against superoxide radical than against DPPH free radical.

The results of the inhibitory effects of different concentrations of synthesized compounds on superoxide anion showed that almost all the synthesized compounds at 10^{-3} M concentration had superoxide radical scavenging activity, the scavenging extent being in the range 22–98%. Compounds **5, 3, 9, 4, 17** and **13** having methylpiperazine and phenylpiperazine substitutents on the benzimidazole ring had a strong scavenging effect on superoxide anion (98%, 93%, 91%, 88%, 85% and 81%, respectively) at 10^{-3} M concentration. Compounds **11, 1** and **6** also showed a scavenging effect on superoxide anion formation by about 67%, 58%, and 55%, respectively, at 10^{-3} M concentration. Compound **2** had no effect on superoxide anion formation. Because compound **8** increases the production of superoxide anion at the 10^{-3} M concentration but behaves like an antioxidant at the 10^{-4} M concentration, it is possible to conclude that it acts like an antioxidant or prooxidant depending on its concentration. Similar examples for this conclusion can be found in reports stating that some antioxidant compounds, i.e. ascorbic acid[24], may behave as an antioxidant or prooxidant depending on the concentration. Compound **7** had no effect on superoxide anion formation in a concentration-dependent manner. Interestingly, compounds **1** and **10** displayed better antioxidant activity at lower concentration (10^{-4} M) than at higher concentration (10^{-3} M). Compound **10** showed a strong scavenging effect on superoxide anion formation by about 86% at 10^{-4} M concentration.

The results in Table II show that the synthesized compounds have no significant effect on DPPH free radical scavenging activity. However, compound **11** had the most effective DPPH scavenging activity at 10^{-3} M concentration (61%), followed by compound **13** (53%). For compound **11**, a correlation existed between superoxide and DPPH free radical scavenging activities.

Because of the difference in the mechanism of free radical production in oxidative stress and radical scavenging activity of antioxidant compounds[25–27], it was not surprising to observe different effects of the compounds **1-17** on superoxide anion radical formation and DPPH free radical scavenging activity. Such contradictory results have previously been found in the literature[28,29]. In our previous study, we reported that benzimidazole derivatives carrying a triazole ring at the N_1 position of benzimidazole were found to interact with the stable free radical DPPH but not to have an effect on superoxide anion radical formation[15].

Lipophilicity has been reported[30,31] to have a positive influence on antioxidant activity so the log P values of **1-17** were calculated, in order to investigate if there was a correlation between lipophilicity and antioxidant activity. There was an interesting correlation between them. Compounds (**5, 9, 13** and **17**) having a 4-phenylpiperazine-1-yl substitutent at position-5 of the benzimidazole ring had higher lipophilicity and also displayed the best radical scavenging activities. It is well known that the contribution of the tetrahydronaphthalene fragment to lipophilicity proves to be greater than that of the indole ring. Similarly, compounds (**1-5**) having a retinoid fragment instead of indole had higher log P values and displayed better radical scavenging activity than **6-17**. Compound **5** having both tetrahydronaphthalene and 4-phenylpiperazine fragments (Log P = 8.05) displayed the best scavenging effect at 10^{-3} M concentration (98%) on superoxide anion radical, a result which is better than that achieved with 30 IU of SOD (76%).

The superoxide anion radical has been implicated in several pathophysiological processes, due to its transformation into more reactive species including hydroxyl radical that initiates lipid peroxidation. Superoxide has also been observed to directly initiate lipid peroxidation[32]. Some herbal plants display antioxidant properties via scavenging of superoxide anion radical[33–35]. Therefore, the scavenging of superoxide anion radical by **5, 3, 9, 4, 17** and **13** (98%, 93%, 91%, 88%, 85% and 81%, respectively, at 10^{-3} M concentration) is likely to make them promising antioxidants. The results observed here prompt us to further our studies in this way.

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