Full Paper

Identification of a Novel Series of *N*-Phenyl-5-[(2-phenylbenzimidazol-1-yl)methyl]-1,3,4-oxadiazol-2-amines as Potent Antioxidants and Radical Scavengers

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In this study, some novel 5-[[2-(phenyl/*p*-chlorophenyl)-benzimidazol-1-yl]-methyl]-*N*-substituted phenyl-1,3,4-oxadiazol-2-amine derivatives (**28**–**45**) with an oxadiazole ring were synthesized. The antioxidant properties and radical scavenging activities of the compounds were investigated employing various *in vitro* systems: hepatic microsomal NADPH-dependent inhibition of lipid peroxidation levels, scavenging of DPPH free radicals, and inhibition of microsomal ethoxyresorufin *O*-deethylase activity (EROD). Compounds **34** and **41** were found to be good scavengers of DPPH radicals (76% and 84%) when compared to BHT (90%). Almost all of the compounds examined were found to possess a good inhibitor effect on the microsomal EROD activity. Moreover, **32** and **41** were more active analogs (97% and 98%) on the microsomal EROD activity than caffeine (85%).

Keywords: Benzimidazoles / DPPH / EROD / Lipid peroxidation / Oxadiazoles

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Introduction

Nowadays, plenty of benzimidazole derivatives are in clinical usage, e.g., as antiparasitic, antihistaminic, angiotensin II antagonist, proton pump inhibitor, and antipsychotic. The position and the type of the substituents on the benzimidazole ring are responsible for the variety of biological activities, including antimicrobial, antifungal, anticancer, anthelmintic, antiallergic, antioxidant, etc. Previously, antimicrobial [1, 2], antiparasitic [3], antihistaminic [4], and antioxidant [5–11] activity evaluations of some benzimidazole derivatives have been studied in our faculty.

Antioxidant systems, including superoxide dismutase, catalase, and glutathione, should keep the oxidative processes in balance. However, in the case of the deficiency of nutritional antioxidants (vitamin A, C, E, the minerals selenium and zinc, coenzyme Q10, lipoic acid, etc.), these systems could be affected. Antioxidants can exert their effects by scavenging radicals, binding metal ions, and inhibiting enzymatic systems. The biological activities of antioxidants have been reviewed many times and some have been declared to be involved in cancer, heart diseases, brain dysfunction, atherosclerosis, and immune system problems [12, 13].

We have already reported the synthesis, characterization, and antioxidant properties of some benzimidazole derivatives that have thiadiazole, triazole, and oxadiazole [5–10]. This report deals with the antioxidant properties of some novel benzimidazole derivatives, 5-[[2-(phenyl/*p*-chlorophenyl)benzimidazol-1-yl]methyl]-*N*-substituted phenyl-1,3,4-oxadiazol-2-amines (**28**–**45**), having an oxadiazole ring instead of thiadiazole and triazole rings.

Results and discussion

In this study, a series of new compounds **28**–**45** having 2-(substituted phenylamino)oxadiazole as a substituent at 1st position of benzimidazole were synthesized and evaluated for their effects on the rat liver microsomal NADPH-dependent lipid peroxidation levels by measuring the formation of 2-thiobarbituric acid reactive substances. The interaction with

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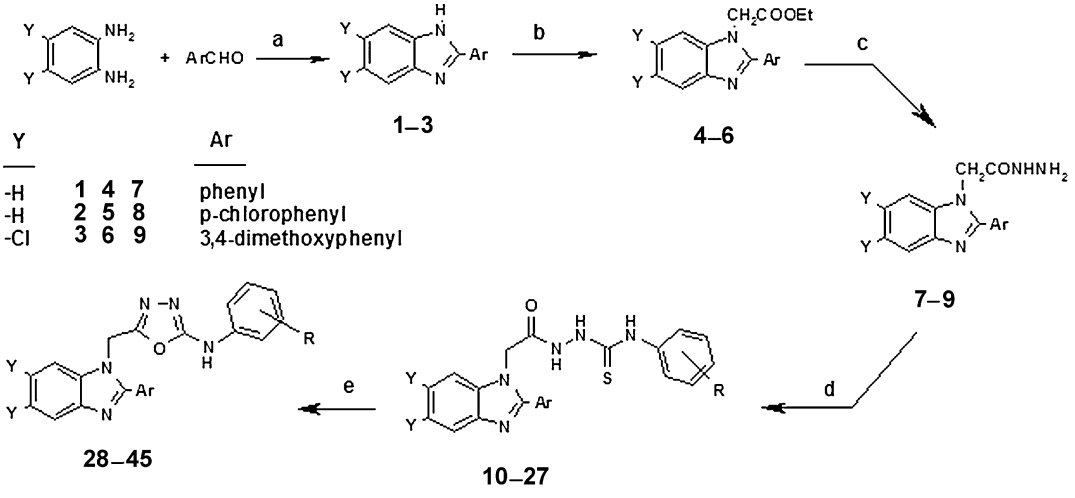
the stable free radical DPPH and inhibition on microsomal ethoxyresorufin *O*-deethylase (EROD) activity was also examined. For the synthesis of the desired compounds, the reaction sequences outlined in Scheme 1 were followed.

The final compounds (**28–45**) having oxadiazole ring instead of triazole or thiadiazole were synthesized according to our previous studies [5–10]. Compounds **1–3** having benzimidazole ring were prepared via oxidative condensation of *o*-phenylenediamine/4,5-dichloro-1,2-phenylenediamine, non-substituted/*p*-chloro/3,4-dimethoxy benzaldehyde, and sodium metabisulfite [14]. Treatment of compounds **1–3** with ethyl chloroacetate in KOH/dimethylsulfoxide (DMSO) gave the *N*-alkylated products (**4–6**) [15]. Hydrazine hydrate and the ester (**4–6**) in ethanol were refluxed for 4 h to give the desired hydrazide compounds, (2-aryl-benzimidazol-1-yl)-acetic acid hydrazides **7–9** [16]. The thiosemicarbazides (**10–27**) were obtained upon the reaction of the acid hydrazide with aryl isothiocyanates in ethanol [17]. Cyclization of **10–27** with KI and I_2 [18] resulted in the formation of *N*-substituted phenyl-5-[(2-substituted phenyl benzimidazol-1-yl)-methyl]-1,3,4-oxadiazol-2-amine (**28–45**) derivatives (Scheme 1). For the synthesis of compound **45**, appropriate thiosemicarbazide **27** (2-[(5,6-dichloro-2-(4-chloro-phenyl)-1*H*-benzimidazol-1-yl)acetyl]-*N*-(3,4-dimethoxyphenyl)hydrazine carbothioamide) was used directly without further purifications.

The structures of the synthesized compounds were consistent with the ^{1}H and ^{13}C NMR spectra. In the ^{13}C NMR spectra, signal of methylene carbon of compound **29** appears at 40.28 ppm. At the same time, the signals of methylene carbon of compounds **28**, **38**, and **43** could not seen due to being overshadowed by DMSO-d_6. Thus, further verification was obtained from the HSQC spectrum, which clearly showed the ^{1}H–^{13}C connections of **43**, so this methylene carbon was very easily designated at 40.08 Hz. In addition to this, it was not possible for the compounds **28** and **38**. Because of the fluorine atom at the ^{13}C NMR spectrum in DMSO-d_6 and at 100 MHz of **38** and **43**, highly characteristic ^{13}C–^{19}F couplings were observed. Three doublet resonances centered at 116.20 and 116.42 ($J = 22.24$), 119.29 and 119.36 ($J = 7.65$), 156.08 and 153.74 ($J = 235.36$) with 115.42 and 115.65 ($J = 23.0$), 118.47 and 118.54 ($J = 7.0$), 156.03 and 158.39 ($J = 236.0$ Hz), respectively, **38** and **43**, arising from the aromatic carbons of *p*-fluorophenyl coupled by the fluorine atom. Resembling ^{13}C–^{19}F couplings were informed in our former articles too [2]. There were similar ^{1}H–^{19}F couplings in the ^{1}H NMR spectra of **31**, **35**, **38**, **40**, and **43**. As expected, in the mass spectra of **30**, **32**, **34**, **36**, **39**, **41–44**, and **45**, chlorine and bromine isotope signals were seen. Some physico-chemical properties and spectral findings of final products are given in Table 1.

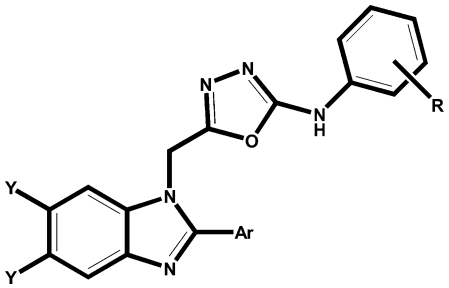
Compounds **28–45** were evaluated by their effects on the rat liver microsomal NADPH-dependent lipid peroxidation levels by measuring the formation of 2-thiobarbituric acid reactive substances, and also examined to interact with the stable free radical DPPH and to inhibit on microsomal EROD activity (Table 2). The results seem variable and fairly similar to our former published results [5–10].

The inhibition of NADPH-dependent lipid peroxidation produced by all new compounds in the rat liver microsomes was examined by measuring the formation of the 2-thiobarbituric acid reactive substances for their antioxidant capacity. Compound **42** is the most active one that caused 42% inhibition on LP level in rat liver microsomes at 10^{-3} M concentration while BHT showed 65% inhibition at the same concentration. Compounds **33** (26%), **37** (16%),



Scheme 1. Synthesis of derivatives **28–45**.

Table 1. Physical and spectral data of compounds **28**–**45**.



No.	Formula	Ar	Y	R	% Yield	Mp (°C)	^{1}H and ^{13}C NMR δ (ppm) DMSO-d_6	MS (M+H) (%)
28	$C_{22}H_{17}N_5O$		–H	–H	37	277–279	5.74 (s, 2H, $-CH_2-$), 6.95–7.05 (m, 1H, Ar–H), 7.28–7.36 (m, 4H, Ar–H), 7.48 (d, 2H, $J_o = 7.60$ Hz, Ar–H), 7.58–7.62 (m, 3H, Ar–H), 7.69 (d, 1H, $J_o = 6.40$ Hz, Ar–H), 7.74 (d, 1H, $J_o = 6.80$ Hz, Ar–H), 7.84–7.88 (m, 2H, Ar–H), 10.48 (s, 1H, NH); ^{13}C NMR: 111.64, 117.68, 120.03, 122.63, 123.30, 123.72, 129.57, 129.72, 130.01, 130.14, 130.77, 136.43, 139.09, 143.20, 153.75, 156.07, 160.90	368 (100)
29	$C_{23}H_{19}N_5O$		–H	*o*-CH_3	72	241–243	2.19 (s, 3H, $-CH_3$), 5.71 (s, 2H, $-CH_2-$), 7.00–7.04 (m, 1H, Ar–H), 7.16–7.33 (m, 4H, Ar–H), 7.47–7.60 (m, 4H, Ar–H), 7.67 (d, 1H, $J_o = 7.20$ Hz, Ar–H), 7.74 (d, 1H, $J_o = 7.20$ Hz, Ar–H), 7.84–7.86 (m, 2H, Ar–H), 9.53 (s, 1H, NH); ^{13}C NMR: 18.50 ($-CH_3$), 40.28 ($-CH_2-$), 111.65, 120.01, 121.93, 123.29, 123.69, 124.72, 127.11, 129.53, 129.96, 130.02, 130.15, 130.74, 131.29, 136.41, 137.13, 143.19, 153.76, 156.22, 162.19	382 (100)
30	$C_{22}H_{16}ClN_5O$		–H	*o*-Cl	45	239–241	5.68 (s, 2H, $-CH_2-$), 7.08–7.13 (td, 1H, Ar–H), 7.27–7.34 (m, 3H, Ar–H), 7.46 (dd, 1H, $J_o = 8.40$ Hz, $J_m = 1.60$ Hz, Ar–H), 7.56–7.58 (m, 3H, Ar–H), 7.65 (dd, 1H, $J_o = 6.80$ Hz, $J_m = 1.60$ Hz, Ar–H), 7.71 (dd, 1H, $J_o = 6.80$ Hz, $J_m = 1.60$ Hz, Ar–H), 7.82–7.86 (m, 3H, Ar–H), 9.94 (s, 1H, NH)	402 (100), 404 (30.3)
31	$C_{22}H_{16}FN_5O$		–H	*o*-F	56	281–283	5.72 (s, 2H, $-CH_2-$), 7.06–7.10 (m, 1H, Ar–H), 7.17–7.35 (m, 4H, Ar–H), 7.59–7.61 (m, 3H, Ar–H), 7.67 (d, 1H, $J_o = 8.00$ Hz, Ar–H), 7.74 (d, 1H, $J_o = 7.20$ Hz, Ar–H), 7.86–7.88 (m, 2H, Ar–H), 7.94–7.98 (m, 1H, Ar–H), 10.38 (s, 1H, NH)	386 (100)
32	$C_{22}H_{16}BrN_5O$		–H	*o*-Br	48	239–242	5.70 (s, 2H, $-CH_2-$), 7.07–7.11 (m, 1H, Ar–H), 7.28–7.41 (m, 3H, Ar–H), 7.58–7.76 (m, 7H, Ar–H), 7.80 (dd, 2H, $J_o = 7.20$ Hz, $J_m = 2.00$ Hz, Ar–H), 9.84 (s, 1H, NH)	446 (100), 448 (96.1)
33	$C_{23}H_{19}N_5O$		–H	*m*-CH_3	79	258–260	2.27 (s, 3H, $-CH_3$), 5.75 (s, 2H, $-CH_2-$), 6.81 (d, 1H, $J_o = 7.82$ Hz, Ar–H), 7.17–7.21 (t, 1H, $J_o = 7.6$, Ar–H), 7.26–7.35 (m, 4H, Ar–H), 7.58–7.64 (m, 3H, Ar–H), 7.70 (d, 1H, $J_o = 7.42$ Hz, Ar–H), 7.75 (d, 1H, $J_o = 7.81$ Hz, Ar–H), 8.34 (d, 2H, $J_m = 1.95$ Hz, Ar–H), 10.43 (s, 1H, NH)	382 (100)
34	$C_{22}H_{16}ClN_5O$		–H	*m*-Cl	38	247–250	5.74 (s, 2H, $-CH_2-$), 7.03 (d, 1H, $J_o = 7.42$ Hz, Ar–H), 7.27–7.37 (m, 4H, Ar–H), 7.58–7.73 (m, 6H, Ar–H), 7.85 (dd, 2H, $J_o = 7.03$ Hz, $J_m = 2.35$ Hz, Ar–H), 10.76 (s, 1H, NH)	402 (100), 404 (37.3)
35	$C_{22}H_{16}FN_5O$		–H	*m*-F	45	274–276	5.77 (s, 2H, $-CH_2-$), 6.82 (m, 1H, Ar–H), 7.24 (d, 1H, Ar–H), 7.31–7.36 (m, 4H, Ar–H), 7.44 (d, 1H, Ar–H), 7.61 (d, 2H, $J_m = 2.4$ Hz, Ar–H), 7.70 (d, 1H, $J_o = 7.2$ Hz, Ar–H), 7.75 (d, 1H, $J_o = 7.6$ Hz, Ar–H), 7.86–7.87 (m, 2H, Ar–H), 10.79 (s, 1H, NH)	386 (100)
36	$C_{22}H_{16}BrN_5O$		–H	*m*-Br	51	265–269	5.76 (s, 2H, $-CH_2-$), 7.17 (d, 1H, $J_o = 7.82$ Hz, Ar–H), 7.27–7.42 (m, 4H, Ar–H), 7.59–7.61 (m, 3H, Ar–H), 7.69 (d, 1H, $J_o = 8.21$ Hz, Ar–H), 7.74 (d, 1H, $J_o = 7.81$ Hz, Ar–H), 7.81 (s, 1H, Ar–H), 7.85–7.87 (m, 2H, Ar–H), 10.75 (s, 1H, NH)	446 (79), 448 (100)
37	$C_{23}H_{19}N_5O$		–H	*p*-CH_3	62	287–289	2.23 (s, 3H, $-CH_3$), 5.73 (s, 2H, $-CH_2-$), 7.11 (d, 2H, $J_o = 8.59$ Hz, Ar–H), 7.30–7.38 (m, 4H, Ar–H), 7.59–7.61 (m, 3H, Ar–H), 7.68 (d, 1H, $J_o = 7.42$ Hz, Ar–H), 7.74 (d, 1H, $J_o = 7.42$ Hz, Ar–H), 7.85–7.87 (m, 2H, Ar–H), 10.37 (s, 1H, NH)	382 (100)

(*Continued*)

Table 1. (*Continued*)

No.	Formula	Ar	Y	R	% Yield	Mp (°C)	^{1}H and ^{13}C NMR δ (ppm) DMSO-d_6	MS (M+H) (%)
38	$C_{22}H_{16}FN_5O$		−H	*p*-F	54	284–286	5.75 (s, 2H, $-CH_2-$), 7.16–7.20 (m, 2H, Ar–H), 7.29–7.37 (m, 2H, Ar–H), 7.50–7.53 (m, 2H, Ar–H), 7.60–7.62 (m, 3H, Ar–H), 7.69 (d, 1H, $J_o = 7.81$ Hz, Ar–H), 7.74–7.87 (m, 3H, Ar–H), 10.54 (s, 1H, NH); ^{13}C NMR: 111.63 [116.20 and 116.42 (d, $J = 22.24$)] [119.29 and 119.36 (d, $J = 7.65$)], 120.03, 123.29, 123.72, 129.56, 130.01, 130.13, 130.77, 135.60, 136.43, 143.19, 153.74 [156.08 and 153.74 ($J = 235.36$ Hz)], 160	386 (100)
39	$C_{22}H_{16}ClN_5O$		−H	−H	42	286–288	5.76 (s, 2H, $-CH_2-$), 6.98 (td, 1H, $J_o = 7.04$, 7.42 Hz, Ar–H), 7.29–7.37 (m, 4H, Ar–H), 7.48 (d, 2H, $J_o = 6.81$ Hz, Ar–H), 7.66–7.75 (m, 4H, Ar–H), 7.89 (d, 2H, $J_o = 8.60$ Hz, Ar–H), 10.47 (s, 1H, NH)	402.1 (100), 404.1 (35.4)
40	$C_{22}H_{15}ClFN_5O$		−H	*o*-F	48	270–272	5.73 (s, 2H, $-CH_2-$), 7.18–7.34 (m, 5H, Ar–H), 7.65–7.69 (m, 3H, Ar–H), 7.73 (d, 1H, $J_o = 8.20$ Hz, Ar–H), 7.88–7.94 (m, 3H, Ar–H), 10.35 (s, 1H, NH)	420 (M+H)
41	$C_{22}H_{15}BrClN_5O$		−H	*o*-Br	69	280–283	5.73 (s, 2H, $-CH_2-$), 7.10–7.14 (m, 1H, Ar–H), 7.29–7.36 (m, 3H, Ar–H), 7.48 (dd, 1H, $J_o = 7.60$ Hz, $J_m = 1.20$ Hz, Ar–H), 7.65–7.75 (m, 4H, Ar–H), 7.85–7.89 (m, 3H, Ar–H), 9.95 (s, 1H, NH)	479.9 (65.7), 480.1 (87), 482.1 (100), 482.3 (91.6)
42	$C_{23}H_{18}ClN_5O$		−H	*p*-CH_3	63	268–270	2.23 (s, 3H, $-CH_3$), 5.74 (s, 2H, $-CH_2-$), 7.11 (d, 2H, $J_o = 8.21$ Hz, Ar–H), 7.31–7.37 (m, 4H, Ar–H), 7.65–7.75 (m, 4H, Ar–H), 7.89 (d, 2H, $J_o = 8.60$ Hz, Ar–H), 10.35 (s, 1H, NH)	416 (100), 418 (34.8)
43	$C_{22}H_{15}ClFN_5O$		−H	*p*-F	43	279–281	5.75 (s, 2H, $-CH_2-$), 7.15 (td, 2H, $J_o = 8.98$, 8.99 Hz, Ar–H), 7.18–7.35 (m, 2H, Ar–H), 7.48–7.51 (m, 2H, Ar–H), 7.51–7.70 (m, 3H, Ar–H), 7.74 (d, 1H, $J_o = 7.43$ Hz, Ar–H), 7.87–7.89 (m, 2H, Ar–H), 10.51 (s, 1H, NH); ^{13}C NMR: 40.08, 110.85 [115.65 and 115.42 ($J = 23.0$ Hz)] [118.54 and 118.47 ($J = 7.0$ Hz)], 119.24, 122.51, 122.93, 128.77, 129.22, 129.35, 129.98, 134.75, 135.64, 142.41, 152.94, 155.32 [158.39 and 156.03 ($J = 236.0$ Hz)], 160.11	420 (100), 422 (39.2)
44	$C_{23}H_{18}ClN_5O_2$		−H	*p*-OCH_3	45	267–269	3.7 (s, 3H, $-OCH_3$), 5.73 (s, 2H, $-CH_2-$), 6.89 (d, 2H, $J_o = 8.98$ Hz, Ar–H), 7.31–7.40 (m, 4H, Ar–H), 7.65–7.75 (m, 4H, Ar–H), 7.88 (d, 2H, $J_o = 8.21$ Hz, Ar–H), 10.25 (s, 1H, NH)	432 (100), 434 (37.8)
45	$C_{24}H_{18}Cl_3N_5O_3$		−Cl	*p*-Cl	48	271–272	3.82 (s, 3H, $-OCH_3$), 3.84 (s, 3H, $-OCH_3$), 5.78 (s, 2H, $-CH_2-$), 7.15 (d, 1H, $J_o = 8.20$ Hz, Ar–H), 7.36–7.40 (m, 4H, Ar–H), 7.52 (d, 2H, $J_o = 8.99$ Hz, Ar–H), 8.01 (s, 1H, Ar–H), 8.11 (s, 1H, Ar–H), 10.67 (s, 1H, NH)	530 (100), 532 (99.8), 534 (31.9), 536 (3.5)

41 (28%), and **45** (12%) displayed highly limited inhibitory effects on LP and the rest of the compounds enhanced LP levels.

Compound **44**, being an oxadiazole isostere of triazole [7] and thiadiazole [7], enhanced LP level but the other isosteres inhibited lipid peroxidation by 55% and 85%, respectively. Similar results, oxadiazole derivatives enhancing lipid peroxidation levels, were obtained in another study on liver LP levels too [8]. These results clearly indicate that triazole and thiadiazole isosteres are more appropriate for LP inhibition.

Nearly all of the tested compounds showed significant inhibition of EROD activity. Compounds **30**, **32**, **41**, and **45** decreased liver EROD activities by 89%, 97%, 98%, and 89%, respectively, better than the specific inhibitor caffeine (85%). Compounds **34** and **42** caused 85% inhibition being equal to caffeine. Significant inhibitory activities were also observed for compounds **28** (77%), **29** (84%), **33** (74%), **35** (65%), **36** (81%), **43** (66%), and **44** (71%). Compounds **32** and **41** have *o*-Br substituent at aniline moiety and they are the most active analogs of this series (97% and 98%, respectively) on the microsomal EROD activity. Both of the compounds **30** and **45** inhibited the microsomal EROD activity (89%) being similarly better than that of the specific inhibitor caffeine at 10^{-3} M concentration. The rest of the compounds inhibited EROD activity in the range of 52–48%.

Compound **45** containing *p*-chlorophenylamino substituent at the second position of oxadiazole ring has allowed us to obtain a very good EROD profile (89%). When it is compared to mercapto substituted analog (60%), 5-[5,6-dichloro-2-(3,4-dimethoxy)phenyl-benzimidazol-1-yl-methyl]-2-mercapto-[1,3,4]-oxadiazol, reported before [8], it can be

Table 2. Effects of compounds on the liver LP levels, EROD enzyme, and DPPH free radical scavenging activities *in vitro*.[a)]

Comp.[b)]	LP nmol/mg/min	Percent of control %	EROD pmol/mg/min	Percent of control %	DPPH % inhibition	DPPH IC_{50} (mM)
28	64.18 ± 0.26	396	9.66 ± 0.89	23	28 ± 3.9	
29	20.98 ± 2.58	129	6.71 ± 0.25	16	39 ± 4.4	
30	20.28 ± 1.60	125	4.64 ± 1.13	11	30 ± 2.2	
31	24.31 ± 0.37	150	22.13 ± 4.23	53	15 ± 2.0	
32	22.36 ± 0.63	138	1.05 ± 0.25	3	28 ± 0.6	
33	11.97 ± 0.42	74	10.83 ± 0.96	26	12 ± 2.2	
34	57.69 ± 0.42	355	6.12 ± 0.33	15	76 ± 3.8	0.35
35	26.52 ± 0.98	163	14.60 ± 1.07	35	9 ± 0.6	
36	17.63 ± 1.43	108	7.75 ± 1.13	19	16 ± 0.8	
37	13.61 ± 2.31	84	21.43 ± 1.29	52	8 ± 1.0	
38	24.75 ± 4.72	152	19.96 ± 1.22	48	12 ± 1.7	
39	20.03 ± 2.67	123	19.90 ± 2.45	48	10 ± 0.6	
40	24.31 ± 2.57	149	17.89 ± 0.51	49	20 ± 0.8	
41	11.66 ± 1.69	72	0.91 ± 0.05	2	84 ± 3.2	0.2
42	9.37 ± 1.67	58	6.35 ± 2.65	15	30 ± 3.8	
43	17.83 ± 3.65	110	14.13 ± 2.58	34	14 ± 3.0	
44	20.53 ± 3.56	126	12.07 ± 2.70	29	25 ± ± 4.4	
45	14.30 ± 0.63	88	4.66 ± 0.87	11	25 ± 3.5	
BHT	5.68 ± 0.22	35			90 ± 0.6	
Caffeine			6.41 ± 0.36	15		
Control[c)]	16.25 ± 1.45	100	41.53 ± 0.99	100		

[a)] Each value represents mean ± SD of two to four independent experiments.
[b)] Concentration in incubation medium (10^{-3} M).
[c)] DMSO only, control for compounds.

concluded that the substituted-phenylamino moiety is more important than mercapto substituent for EROD activity.

In terms of inhibition on EROD activity, it can be said that oxadiazole derivatives displayed better activity than thiadiazole and triazole derivatives. One of our studies demonstrated that in these series of compounds, to have cyano group is very important to improve EROD activity. Namely, some of the 2-(*p*-cyanophenyl)benzimidazole derivatives [8] have better activities (98% and 100%) than compounds **34** (85%) and **36** (81%) because they have phenyl substituent at 2nd position of benzimidazole.

Almost all of the tested compounds displayed significant inhibition on EROD activity in the range of 98–47%. In these series of compounds, the most active analogs on the microsomal EROD activity are **32** (97%) and **41** (98%).

In this study, almost all of the compounds' DPPH free radical scavenging activities were not enough, except for compounds **34** and **41**. IC_{50} values of *N*-(*m*-chlorophenyl)-5-[(2-phenyl-1*H*-benzimidazol-1-yl)methyl]-2,5-dihydro-1,3,4-oxadiazol-2-amine (**34**) (76%) and *N*-(*o*-bromophenyl)-5-[(2-(*p*-chlorophenyl)-1*H*-benzimidazol-1-yl)methyl]-2,5-dihydro-1,3,4-oxadiazol-2-amine (**41**) (84%) are similar, 0.35 and 0.2 mM, respectively, so it can be said that there is a little bit difference between their scavenger effects on DPPH radical.

Our other published results [5] clearly indicate that both *N*-aryl and *N*-methyl thiadiazole derivatives were found to have no interaction with DPPH-like oxadiazole derivatives except **34** and **41**. If we compare with *N*-methyl and *N*-aryl series reported before [5, 7], in all the series, triazole derivatives have moderate activity and thiosemicarbazides [10] display the best effect on DPPH.

Compounds that have pyridinyl or *p*-cholorophenyl substituent at second position of benzimidazole derivatives and thiosemicarbazides showed highest interaction with DPPH radical, even better than BHT [7]. Compound **41** has a *p*-chlorophenyl substituent at second position of benzimidazole ring like the compounds published in Ayhan-Kilcigil et al. [7].

It is well known that there exist two mechanisms for an antioxidant to scavenge DPPH, which we reported before. The first one is a direct H-atom abstraction process (Eq. 1), and the second one is a proton concerted electron-transfer process (Eq. 2) [19]

$$DPPH^{\bullet} + RXH \rightarrow DPPHH + RXH^{\bullet} \quad (1)$$

$$DPPH^{\bullet} + RXH \rightarrow DPPH^{-} + RXH^{+} \rightarrow DPPHH + RX^{\bullet} \quad (2)$$

DPPH-scavenging mechanism of our compounds could not have been clarified yet but our efforts in this direction are continuing [20].

Because of the diversity of the methods, it is quite difficult to explain the observed variant effects of synthesized

compounds. Separate effects of compounds in these systems have been noticed previously [5–10]. Therefore, the observation of distinct effects of synthetic compounds on DPPH radical, superoxide radical, LP levels, and EROD is not surprising since the mechanisms of production of oxidative stress using these methods are different [12, 13, 21]. The differences in the kinetic behavior of the radicals and substrates should also be considered when comparing the results of different free radical scavenging methods to determine antioxidant capacity [22]. Therefore, it is extremely difficult to compare the results from different assays.

As a result, data obtained from all our researches associated with this field guide us for the development of novel antioxidant compounds.

Experimental

Chemistry

Melting points were determined with an electrothermal melting point apparatus and are uncorrected. ^{1}H, ^{13}C NMR, and HSQC spectra were measured with a Varian Mercury 400, 100 MHz instrument using TMS internal standard and DMSO-d_6; coupling constants (J) are reported in Hertz. All chemical shifts were reported as δ (ppm) values. ES-MS were obtained with a Waters ZQ Micromass LC–MS spectrometer with positive electrospray ionization method. All instrumental analyses were performed at Ankara University, Faculty of Pharmacy. The chemical reagents used in synthesis were purchased from E. Merck and Aldrich. BHT and caffeine were obtained from Sigma. Analytical thin-layer chromatography (TLC) was performed with Merck precoated TLC plates and spots were visualized with ultraviolet light. Compounds **1**–**27** were previously prepared in our laboratory [5–8].

*General procedure for the preparation of N-(substituted phenyl)-5-[(2-substituted phenyl-1H-benzimidazol-1-yl)-methyl]-2,5-dihydro-1,3,4-oxadiazol-2-amines (**28**–**45**)*

Appropriate thiosemicarbazides (1 mmol) **10**–**27** were dissolved in 5 mL of EtOH and 0.5 mL of 5 N NaOH. I_2 solution was dropped into the solution at room temperature until brown color appeared. After reflux for 1–3 h, the reaction mixture was cooled and poured into ice water. The crude product was filtered off and recrystallized from ethanol to yield the desired oxadiazole derivatives. All physical, spectral data, and structure of **28**–**45** are seen in Table 1.

Antioxidant activity

Lipid peroxidation level

Male albino Wistar rats (200–225 g) used in the experiments were fed with standard laboratory rat chow and tab water *ad libitum*. The animals were starved for 24 h prior to sacrifice and then killed by decapitation under anesthesia. The livers were removed immediately and washed in icecold distilled water and the microsomes were prepared as described previously [23]. NADPH-dependent LP was determined using the optimum conditions determined and described previously [23]. 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) per mg protein. The assay was essentially derived from the methods of Wills [24, 25] as modified by Bishayee and Balasubramanian [26]. Lipid peroxidation was determined spectrophotometrically at 532 nm as the thiobarbituric acid reactive material. Compounds inhibit the production of MDA, and therefore the produced color after addition of thiobarbituric acid is less intensive. A typical optimized assay mixture contained 0.2 nM 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.

EROD enzyme activity

EROD activity was measured by the spectrofluorometric method of Burke et al. [27]. A typical optimized assay mixture contained 1.0 mM ethoxyresorufin, 100 mM Tris–HCl buffer pH 7.8, 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 liver microsomal protein in a final volume of 1.0 mL.

DPPH free radical scavenging activity

The free radical scavenging activities of these compounds were tested by their ability to bleach the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Blois [28]. 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. DPPH was dissolved in methanol to give a 100 mM solution. To 1.0 mL of the methanolic solution of DPPH, 0.1 mL of the test compounds was added and BHT dissolved in DMSO. The 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. The radical scavenging activity was expressed as IC_{50}, which was determined from a calibration curve for compounds **34** and **41**.

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