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Synthesis and Antioxidant Properties of Novel Benzimidazole Derivatives

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Some novel benzimidazole derivatives carrying thiosemicarbazide and triazole moieties at the N1 position were synthesized and their *in vitro* effects on rat liver microsomal NADPH-dependent lipid peroxidation (LP) levels determined by measuring the formation of 2-thiobarbituric acid reactive substance. The free radical scavenging properties of the compounds were also examined *in vitro* by determining the capacity to scavenge superoxide anion formation and the interaction with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The compounds showed a significant effect in the above tests except to scavenge superoxide anion formation.

Keywords: Thiosemicarbazides; Triazolylbenzimidazoles; Antioxidant

INTRODUCTION

Free radicals, including superoxide radical ($O_2^{\cdot -}$), nitric oxide ($NO^{\cdot}$), hydroxyl ($OH^{\cdot}$) and peroxyl ($RO_2^{\cdot}$) have been implicated in a number of disease processes, including atherosclerosis, rheumatoid arthritis and carcinogenesis.[1] It has also been reported that pathogenesis and symptoms of inflammatory processes are accompanied and/or initiated by the production of reactive oxygen species (ROS).[2] These ROS are produced as a normal consequence of biochemical processes in the body and as a result of increased exposure to environmental and dietary xenobiotics.

It is an imbalance in this oxidant versus antioxidant processes that is thought to cause subsequent cellular damage which leads to the disease processes named above.[3] The body's antioxidant systems, including enzymatic systems (superoxide dismutase, catalase) and both aqueous (glutathione-GSH and ascorbate) and non-aqueous scavengers (vitamin E) should control the oxidative processes

Drugs possessing antioxidant and free radical scavenging properties are considered for the prevention and/or treatment of such diseases which are directly related to the lack of the antioxidant capacity of the body.

Many reports indicate that benzimidazoles having a variety of biological activities such as antimicrobial,[4–9] antitubercular,[10] anticancer,[11,12] antihelmintic,[13] antiallergic[14–17] and antioxidant.[18] In addition, the triazoles display anti-inflammatory,[19] antimicrobial,[9,20,21] antiviral,[22] and antioxidant[23,24] activities. Incorporating the triazoles and their open-chain counterparts the thiosemicarbazides in position-1 of the benzimidazole ring might be expected to yield more potent antioxidant compounds. Thus, it was aimed to synthesize such compounds containing benzimidazoles and triazoles and their open-chain analogs, the thiosemicarbazides, in one structure and to determine their antioxidant properties.

MATERIALS AND METHODS

Melting points were determined with an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Jasco FT/IR 420 spectrometer as potassium bromide discs. ^{1}H NMR spectra were measured with a Bruker GmbH DPX-400, 400 MHz instrument using TMS

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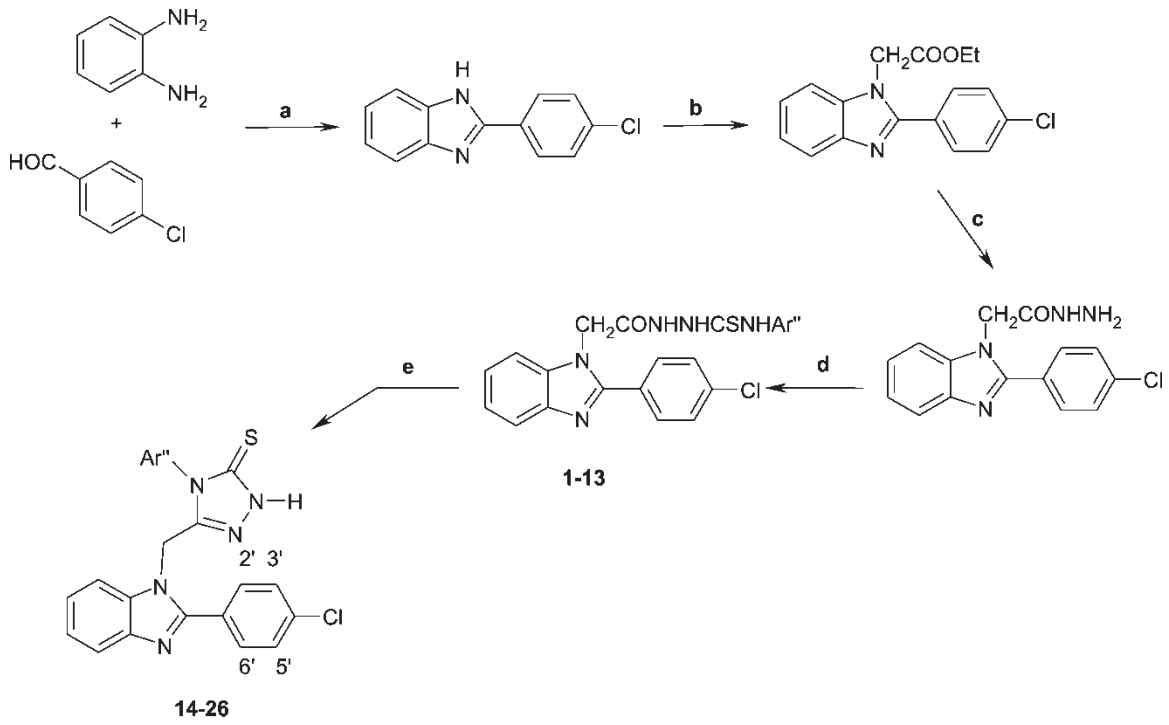

FIGURE 1 Synthetic route for the preparation of compounds **1–13** and **14–26**. Reagents **a**: $Na_2S_2O_5$ **b**: $ClCH_2COOEt$/KOH-DMSO **c**: $NH_2NH_2 \cdot H_2O$/EtOH **d**: appropriate phenylisothiocyanate/EtOH **e**: NaOH.

internal standard and DMSO-d_6. All chemical shifts were reported as δ (ppm) values. EIMS were obtained with a VG Platform II, Micromass spectrometer with ionization energy maintained at 70 eV using on Micromass ZSpec Mass Spectrometer. Elemental analyses (C, H, N, S) were determined on a Leco CHNS 932 instrument (St. Joseph, USA), and were within ± 0.4% of the theoretical values. All instrumental analysis was performed at the Scientific and Technical Research Council of Turkey. Xanthine oxidase, cytochrome c, DPPH and BHT were obtained from Sigma (Taufkirchen, Germany). The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, FRG) and Aldrich (Milwaukee, USA).

For the synthesis of the target compounds the reaction sequences outlined in Figure 1, were followed. 2-(*p*-Chlorophenyl)-1*H*-benzimidazole was prepared via oxidative condensation of *o*-phenylenediamine, *p*-chlorobenzaldehyde and sodium metabisulfite.[25] Treatment of 2-(*p*-chloro) phenyl-1*H*-benzimidazole with ethyl chloroacetate in KOH/DMSO gave the N-alkylated product, (2-(*p*-chlorophenyl)-benzimidazol-1-yl)-acetic acid ethyl ester.[26] Hydrazine hydrate and the ester in ethanol were refluxed for 4 h to give the desired hydrazide, (2-(*p*-chlorophenyl)-benzimidazol-1-yl)-acetic acid hydrazide, in 94% yield.[27] The thiosemicarbazides (**1–13**) (Figure 1) were obtained upon reaction of acid hydrazide with aryl isothiocyanates in ethanol.[28] Cyclization of **1–13** with sodium hydroxide[20,21,29] resulted in formation of 5-(2-(*p*-chlorophenylbenzimidazol-1-yl methyl)-4-substituted phenyl-2,4-dihydro-[1,2,4]-triazole-3-thiones (**14–26**). Some physico-chemical properties and spectral data of the compounds are given in Tables I and II.

General Procedure for the Preparation of the Thiosemicarbazides (1-(substituted thiocarbamoyl-hydrazine carbonyl) methyl- 2-(p-chlorophenyl)-1H-benzimidazoles) (1–13)

Acid hydrazide (0.54 g; 1.79 mmol) in absolute ethanol (20 ml) and the appropriate isothiocyanate (3.05 mmol) were heated under reflux for 30 min. The precipitate formed was cooled, filtered and recrystallized from ethanol. Because of the purification problem, compound **6** was used for further cyclization without recrystallization.

General Procedure for the Preparation of the 5-(2-(p-chlorophenylbenzimidazol-1-yl methyl)-4-substituted phenyl-2,4-dihydro-[1,2,4]-triazole-3-thiones (14–26)

The appropriate thiosemicarbazide (3.4 mmol) **1–13** in 10 ml 1 N sodium hydroxide were refluxed for 1 h. The reaction mixture was cooled and then acidified to pH 6 with 1 N hydrochloric acid. The precipitate was filtered, washed with water and recrystallized from ethanol.

TABLE I Physical and spectral data of compounds **1–5**, **7–13**

No	Formula	Yield	M.P. (°C)	^{1}H NMR data (δ ppm)	Mass data (70 eV)
1	$C_{22}H_{18}ClN_5OS$	75	206–208 (decomp)	5.02 (s, 2H, *CH*$_2$), 7.19–7.82 (13H, Ar − *H*), 10.33 (s, 2H, CO*NHNH*), 10.55 (s, 1H, CS*NH*)	242 (3.43), 227 (11.27), 162 (10.3), 137 (32), 90 (65.49), 64 (44.72), 43 (100)
2	$C_{23}H_{20}ClN_5OS$	80	217–219 (decomp)	2.29 (s, 3H, *CH*$_3$), 5.01 (s, 2H, *CH*$_2$), 7.15–7.80 (m, 12H, Ar − *H*), 9.68 (br s, 2H, CO*NHNH*), 10.70 (br s, 1H, CS*NH*)	338 (M − Ar″NCS), 268 (7.03), 242 (7.29), 228 (68.75), 164 (12.5), 138 (32.99), 128 (32.64), 90 (38.89), 63 (77.78), 50 (100)
3	$C_{23}H_{20}ClN_5OS$	72	205–207 (decomp)	2.32 (s, 3H, *CH*$_3$), 5.04 (s, 2H, *CH*$_2$), 7.02 (s, 1H, H-2″), 7.23-7.87 (m, 11H, Ar − *H*), 9.74 (br s, 2H, CO*NHNH*), 10.53 (br s, 1H, CS*NH*)	300 (M − Ar″NCS), 240 (2.48), 205 (6.96), 149 (46.67), 91 (22.25), 74 (46.67), 69 (78.82), 57 (100)
4	$C_{23}H_{20}ClN_5OS$	90	209–213 (decomp)	2.11 (s, 3H, *CH*$_3$), 5.02 (s, 2H, *CH*$_2$), 7.06–7.72 (m, 12H, Ar − *H*), 9.45 (s, 1H, *NH*), 9.62 (br s, 1H, *NH*), 10.50 (br s, 1H, CS*NH*)	434 (M − CH_3), 269 (2.63), 242 (3.74), 228 (22.93), 135 (26.59), 103 (20.38), 90 (32.32), 73 (85.35), 43 (100)
5	$C_{22}H_{17}ClFN_5OS$	58	204–206 (decomp)	5.01 (s, 2H, *CH*$_2$), 7.17–7.80 (m, 12H, Ar − *H*), 9.78 (s, 2H, CO*NHNH*), 10.55 (s, 1H, CS*NH*)	434 (M − F) (7.92), 339 (6.22), 296 (26.96), 265 (50.98), 227 (100), 205 (51.63), 134 (44.44), 129 (11.93), 95 (15.36), 72 (42.48), 49 (51.63)
7	$C_{22}H_{17}ClFN_5OS$	67	206–208 (decomp)	5.02 (s, 2H, *CH*$_2$), 7.22–7.81 (m, 12H, Ar − *H*), 9.60 (s, 1H, *NH*), 9.95 (s, 1H, *NH*), 10.50 (s, 1H, CS*NH*)	344(M − Ar″NH) (0.78), 301 (1.8), 264 (1.49), 238 (20.45), 203 (8.65), 134 (24.75), 99 (39.39), 73 (72.73), 43 (100)
8	$C_{22}H_{17}Cl_2N_5OS$	60	215–218 (decomp)	5.02 (s, 2H, *CH*$_2$), 7.26–7.82 (m, 12H, Ar − *H*), 9.63 (s, 1H, *NH*), 9.92 (br s, 1H, *NH*), 10.45 (br s, 1H, CS*NH*)	436 (M − Cl), 344(M − Ar″NH) (2.92), 300 (65.49), 238 (58.04), 205 (100), 134 (17.84), 102 (46.27), 76 (53.73), 58 (78.02)
9	$C_{22}H_{17}Cl_2N_5OS$	68	194–197 (decomp)	5.02 (s, 2H, *CH*$_2$), 7.23–7.87 (m, 12 H, Ar − *H*), 9.89 (br s, 2H, CO*NHNH*), 10.49 (br s, 1H, CS*NH*)	344 (3.28), 298 (100), 238 (58.04), 202 (83.14), 133 (25.49), 97 (36.86), 76 (68.63), 58 (100)
10	$C_{22}H_{17}Cl_2N_5OS$	85	217–218 (decomp)	5.01 (s, 2H, *CH*$_2$), 7.25–7.81 (m, 12 H, Ar − *H*), 9.61 (s, 1H, *NH*), 9.90 (s, 1H, *NH*), 10.60 (s, 1H, CS*NH*)	470 (M^{+}) (0.39), 343 (0.65), 300 (2.26), 241 (7.48), 206 (11.32), 137 (24.02), 99 (17.72), 76 (39.57), 70 (99.21), 44 (100)
11	$C_{22}H_{17}BrClN_5OS$	65	207–210 (decomp)	5.03 (s, 2H, *CH*$_2$), 7.25–7.94 (m, 12 H, Ar − *H*), 9.87 (s, 2H, CO*NHNH*), 10.44 (s, 1H, CS*NH*)	241 (14.42), 205 (7.77), 137 (65.71), 129 (29.81), 102 (26.92), 76 (61.22), 59 (93.59), 44 (100)
12	$C_{22}H_{17}BrClN_5OS$	88	193–195 (decomp)	5.02 (s, 2H, *CH*$_2$), 7.25–7.87 (m, 12 H, Ar − H), 9.89 (s, 2H, CO*NHNH*), 10.55 (s, 1H, CS*NH*)	342 (1.73), 300 (5.03), 240 (17.15), 205 (13.03), 137 (90.24), 129 (38.11), 102 (21.95), 76 (73.17), 59 (100)
13	$C_{22}H_{17}BrClN_5OS$	90	221–224 (decomp)	5.02 (s, 2H, *CH*$_2$), 7.24–7.81 (m, 12H, Ar − *H*), 9.60 (s, 1H, *NH*), 9.89 (s, 1H, *NH*), 10.55 (s, 1H, CS*NH*)	514 (M^{+}) (1.13), 241 (12.84), 205 (3.91), 137 (33.45), 129 (8.19), 102 (10.39), 70 (100), 76 (24.32), 59 (41.55)

Antioxidant Activity Studies

Assay of Lipid Peroxidation

Male albino Wistar rats (200–225 g) were used in the experiments. Animals were fed with standard laboratory rat chow and tap 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 ice-cold distilled water and the microsomes were prepared as described previously.[30]

NADPH-dependent LP was determined using the optimum conditions determined and described previously.[30] 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[31,32] as modified by Bishayee.[33] Lipid peroxidation was determined spectrophotometrically at 532 nm as the thiobarbituric acid reactive material. Compounds inhibit the production of malondialdehyde 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.

Superoxide Radical Scavenging Activity

The capacity of compounds to scavenge superoxide anion formation was determined spectrophotometrically on the basis of inhibition of cytochrome c reduction according to the modified method of McCord *et al.*[30]

TABLE II Physical and spectral data of compounds **14–26**

No	Formulas	Yield	M.P. (°C)	^{1}H NMR data (δ ppm)	Mass data (70 eV)
14	$C_{22}H_{16}ClN_5S$	62	290–292	5.44 (s, 2H, *CH*$_2$), 7.12–7.65 (m, 13H, Ar − *H*), 13.9 (s, 1H, *NH*)	419 (M+2) (2.0), 417 (M^+) (5.34), 306 (1.51), 227 (17.23), 213 (5.79), 189 (22. 10), 137 (12.04), 135 (31.10), 117 (21.04), 102 (26.98), 90 (45.12), 76 (73.78)
15	$C_{23}H_{18}ClN_5S$	68	236–238	2.31 (s, 3H, *CH*$_3$), 5.44 (s, 2H, *CH*$_2$), 6.95 (d, 2H, H-3″,5″, J_o = 8.15 Hz), 7.12 (d, 2H, H-2″,6″, J_o = 8.09 Hz), 7.23–7.66 (m, 8H,H-4,5,6,7,2′,3′,5′, 6′), 13.89 (s, 1H, NH)	432 (M^+) (5.06), 265 (7.92), 226 (34.55), 203 (32.96), 189 (10.83), 162 (12.26), 135 (27.55), 101 (35.39), 91 (74.52), 88 (100), 73 (46.5)
16	$C_{23}H_{18}ClN_5S$	70	296–298	2.17 (s, 3H, *CH*$_3$), 5.47 (s, 2H, *CH*$_2$), 6.72–7.65 (m, 12H, Ar − *H*), 13.90 (s, 1H, NH)	432 (M^+) (1.54), 265 (38.65), 230 (98.54), 226 (100), 201 (50.0), 189 (7.10), 164 (13.73), 135 (31.44), 101 (23.20), 88 (29.55), 74 (47.35)
17	$C_{23}H_{18}ClN_5S$	51	285–287	1.53 (s, 3H, *CH*$_3$), 5.34 (q, 2H, J = 17.20 Hz *CH*$_2$), 7.02–7.66 (m, 12H, Ar − *H*), 13.95 (s, 1H, NH)	432 (M^+) (17.42), 398 (20.98), 264 (11.58), 225 (21.26), 187 (12.38), 135 (21.50), 116 (17.04), 100 (33.64), 91 (44.39), 73 (100)
18	$C_{22}H_{15}ClFN_5S$	85	255–257	5.49 (s, 2H, *CH*$_2$), 7.17 (d, 4H, J_o = 6.98 Hz H-2′,3′,5′,6′), 7.24–7.26 (m, 2H, H-5,6), 7.41–7.65 (m, 6H, H-4,7,2″,3″,5″,6″), 13.93 (s, 1H, NH)	436 (M^+) (5.90), 340 (10.76), 294 (11.98), 264 (26.04), 226 (34.72), 205 (16.06), 163 (8.51), 134 (50.69), 125 (15.71), 107 (24.65), 93 (31.60), 74 (100)
19	$C_{22}H_{15}ClFN_5S$	40	261–263	5.51 (s, 2H, *CH*$_2$), 6.92–7.65 (m, 12 H, Ar − H), 13.98 (s, 1H, NH)	436 (M^+) (2.95), 341 (1.86), 298 (6.81), 268 (22.02), 226 (47.23), 204 (31.06), 163 (14.15), 137 (100), 134 (66.38), 125 (19.79), 111 (28.94), 93 (51.06), 76 (57.87)
20	$C_{22}H_{15}ClFN_5S$	58	276–279	5.47 (q, 2H, J = 17.40 Hz *CH*$_2$), 7.16–7.65 (m, 12 H, Ar − H), 14.02 (s, 1H, NH)	436 (M^+) (18.58), 294 (2.23), 264 (10.34), 205 (37.43), 154 (18.44), 134 (61.45), 126 (30.31), 108 (51.96), 94 (60.89), 74 (100)
21	$C_{22}H_{15}Cl_2N_5S$	63	310–313	5.38 (q, 2H, J = 17.53 Hz *CH*$_2$), 7.22–7.66 (m, 12 H, Ar − H), 13.99 (s, 1H, NH)	452 (M^+) (0.29), 417 (3.72), 264 (80.0), 240 (5.73), 229 (16.25), 186 (32.08), 146 (77.08), 133 (35.0), 76 (64.58), 72 (100)
22	$C_{22}H_{15}Cl_2N_5S$	71	281–284	5.44 (s, 2H, *CH*$_2$), 6.91–7.57 (m, 12 H, Ar − H), 13.49 (s, 1H, NH)	452 (M^+) (1.48), 240 (5.57), 228 (57.96), 137 (34.39), 90 (31.85), 76 (32.32)
23	$C_{22}H_{15}Cl_2N_5S$	60	304–306	5.22 (q, 2H, J = 17.53 Hz *CH*$_2$), 6.99–7.44 (m, 12 H, Ar − H), 13.77 (s, 1H, NH)	452 (M^+) (1.91), 380 (6.16), 269 (16.18), 267 (68.38), 265 (100), 238 (10.80), 137 (8.50), 90 (3.26), 76 (3.91)
24	$C_{22}H_{15}BrClN_5S$	65	226–228	5.51 (s, 2H, *CH*$_2$), 7.05 (d, 2H, J_o = 8.56 Hz H-3″,5″) 7.24–7.26 (m, 2H, H-5,6), 7.40–7.65 (m, 8H, H-4,7,2′,3′,5′,6′,2″,6″), 13.93 (br s, 1H, NH)	497 (M^+) (7.57), 267 (7.39), 228 (24.65), 188 (20.86), 164 (72.54), 137 (38.73), 129 (100), 96 (50.35), 76 (35.56), 63 (41,90)
25	$C_{22}H_{15}BrClN_5S$	50	284–286	5.51 (s, 2H, *CH*$_2$), 7.01–7.65 (m, 12 H, Ar − H), 13.98 (s, 1H, NH)	497 (M^+) (6.55), 267 (76.82), 228 (100), 164 (9.84), 137 (71.9), 129 (39.54), 111 (16.99), 76 (49.67), 63 (50.33), 44 (98.04)
26	$C_{22}H_{15}BrClN_5S$	30	292–295	5.35 (q, 2H, J = 17.59 Hz *CH*$_2$), 7.23–7.69 (m, 12H, Ar − H), 13.92 (br s, 1H, NH)	497 (M^+) (0.18), 418 (M − Br) (1.86), 266 (6.32), 228 (8.07), 188 (100), 137 (19.94), 116(41.67), 76 (23.36)

Superoxide anion was generated in the xanthine/xanthine oxidase system. The reaction mixture contained in a final volume of 1 ml, 0.05 M phosphate buffer pH 7.8, 0.32 U xanthine oxidase, 50 μM xanthine, 60 mM cytochrome c and different concentration of test compounds at 100 μl. The absorbance was measured spectrophotometrically at 550 nm for cytochrome c reduction.

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 Blosi.[34] This assay has often been used to estimate the anti-radical activity of antioxidants. Because of its odd electron DPPH gives a strong absorption bound at 517 nm in the visible part of the spectrum. To 1.0 ml of methanolic solution of DPPH (100 μM) was added 0.1 ml of the test compounds and BHT dissolved in dimethylsulfoxide (DMSO). Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity were calculated as a percentage of the 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 obtained

TABLE III Effects of the compounds on liver LP levels *in vitro*[a]

Compound[b]	Ar″	LP (nmol MDA/mg) protein	Inhibition %
Control[c]		18.48 ± 2.32	0
1	Phenyl	9.51 ± 2.33	70
2	4-tolyl	3.64 ± 0.27	80
3	3-tolyl	3.36 ± 0.88	82
4	2-tolyl	8.75 ± 0.10	53
5	4-fluorophenyl	8.01 ± 1.05	57
7	2-fluorophenyl	5.34 ± 0.81	71
8	4-chlorophenyl	8.01 ± 3.62	57
9	3-chlorophenyl	3.30 ± 0.49	82
10	2-chlorophenyl	4.60 ± 1.73	75
11	4-bromophenyl	4.26 ± 1.53	77
12	3-bromophenyl	2.96 ± 0.16	84
13	2-bromophenyl	4.66 ± 0.48	75
14	Phenyl	9.10 ± 0.64	51
15	4-tolyl	12.77 ± 5.04	31
16	3-tolyl	10.34 ± 0.13	44
17	2-tolyl	7.22 ± 0.56	61
18	4-fluorophenyl	8.35 ± 0.72	55
19	3-fluorophenyl	–	–
20	2-fluorophenyl	10.97 ± 4.26	41
21	4-chlorophenyl	12.27 ± 0.01	34
22	3-chlorophenyl	6.93 ± 2.57	62
23	2-chlorophenyl	4.15 ± 0.88	78
24	4-bromophenyl	6.84 ± 0.77	63
25	3-bromophenyl	5.11 ± 0	72
26	2-bromophenyl	3.01 ± 0.24	84
BHT		5.68 ± 0.22	65

[a] Each value represents the mean ± S.D. of 2–4 independent experiments. [b] Concentration in incubation medium (10^{-3} M). [c] Dimethylsulfoxide only, control for compounds. – Not tested.

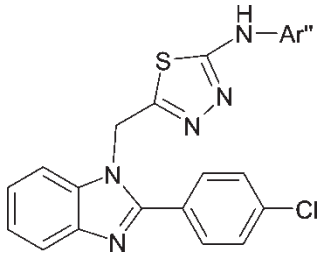

FIGURE 2 Thiadiazole benzimidazoles.

from the equation: Radical scavenging activity $\% = \{OD_{control} - OD_{sample})/OD_{control}\} \times 100$.

RESULTS AND DISCUSSION

All spectral data were in accordance with assigned structures. In the IR spectra of compounds **14**–**26** no absorption bands were detected at about 1675–1680 cm^{-1} indicating the absence of the CO group of acylthiosemicarbazides which is evidence for the conversion of thiosemicarbazides to triazoles. This cyclization was demonstrated by the presence of two absorption maxima at 1310–1322 cm^{-1} and 1265–1290 cm^{-1} belonging to the C = S group.[35]

TABLE IV Reduction of 2,2,diphenyl-1-picrylhydrazyl (DPPH) radical by the compounds

Compound	Concentration in incubation medium (M)	Inhibition %	Compound	Concentration in incubation medium (M)	Inhibition %
1	10^{-3}	96	**14**	10^{-3}	88
	10^{-4}	92		10^{-4}	80
2	10^{-3}	93	**15**	10^{-3}	83
	10^{-4}	94		10^{-4}	44
3	10^{-3}	91	**16**	10^{-3}	78
	10^{-4}	93		10^{-4}	43
5	10^{-3}	91	**17**	10^{-3}	76
	10^{-4}	92		10^{-4}	45
7	10^{-3}	90	**18**	10^{-3}	87
	10^{-4}	93		10^{-4}	49
8	10^{-3}	92	**19**	10^{-3}	87
	10^{-4}	93		10^{-4}	54
9	10^{-3}	92	**20**	10^{-3}	73
	10^{-4}	87		10^{-4}	48
10	10^{-3}	89	**21**	10^{-3}	83
	10^{-4}	89		10^{-4}	46
11	10^{-3}	92	**22**	10^{-3}	80
	10^{-4}	91		10^{-4}	50
12	10^{-3}	89	**23**	10^{-3}	84
	10^{-4}	90		10^{-4}	46
13	10^{-3}	90	**24**	10^{-3}	88
	10^{-4}	85		10^{-4}	61
			25	10^{-3}	89
				10^{-4}	57
			26	10^{-3}	88
				10^{-4}	49
			BHT	10^{-3}	91
				10^{-4}	63

It has been recognized for some time that the two protons of a methylene group adjacent to an asymmetrically substituted carbon or any dissymmetric moiety are magnetically non-equivalent and consequently split each other in the NMR spectra.[36] In accord with this expectation, in the triazole series some of the compounds which bear 2-CH_3, -F, -Cl, -Br and 4-Cl phenyl as Ar″ substituent have CH_2 as an AB quartet at 5.22–5.47 ppm and with a coupling constant 17.20–17.59 Hz. In the other compounds magnetically equivalent CH_2 protons were observed as a sharp singlet at 5.44–5.52 ppm.

All the new compounds were tested as *in vitro* NADPH-dependent lipid peroxidation inhibitors in rat liver microsomes by measurement of the formation of 2-thiobarbituric acid reactive substance and the results are summarized in Table III.

As seen in Table III, the thiosemicarbazides usually had stronger inhibitory effects on LP levels than those of their triazole counterparts **14**–**26**. All of the thiosemicarbazides produced more than 50% inhibition of LP levels at 10^{-3} M concentration (Table III). Moreover, some of them, **1**–**3**, **7**, **9**–**13**, bearing phenyl, *p*-tolyl, *m*-tolyl, *o*-fluoro, *m*- and *o*- chloro and *p*-, *m*- and *o*-bromo phenyl, respectively, as Ar″ substituents had a higher activity than that of butylated hydroxytoluene (BHT) used as a positive control. In the triazole series, the most active compounds were **23**, **25** and **26** which cause 72–84% inhibition of LP. These compounds appeared to have a stronger inhibitory effect than BHT (65% inhibition).

We also tested the superoxide and DPPH radical scavenging activity (Table IV) of the synthesized compounds. Almost all the compounds were found to interact with the stable free radical DPPH which indicates their radical scavenging activity in an iron-free system. The thiadiazole type compounds (Figure 2) were found to have no interaction with DPPH, (unpublished results) whereas all of the tested thiosemicarbazides (**1**–**13**) showed the highest interactions (**85**–**96**%) and the triazoles (**14**–**26**) exhibited dose-dependent inhibition at 10^{-3} M and 10^{-4} M concentrations. The most active compound **1** caused **96%** inhibition at 10^{-3} M concentration. All of the triazoles (**14**–**26**) were found to interact with DPPH strongly (**73**–**89%**) at 10^{-3} M and (**43**–**80%**) 10^{-4} M concentrations. In general this interaction expresses the reducing activity of the tested compounds and indicates their ability to scavenge free radicals. Thus, the response in both assay systems to all compounds occurred in a similar manner.

It is well known that there exists two mechanisms for an antioxidant to scavenge DPPH. The first one is a direct H-atom abstraction process (equation 1), and the second one is a proton concerted electron-transfer process (equation 2).[37]

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

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

Clarification of the DPPH-scavenging mechanism for these compounds, will be helpful in elucidating the structure–activity relationship (SAR) for the antioxidant and thus to design novel antioxidant compounds with a better pharmacological effect.

The superoxide anion radical scavenging activity was measured by the inhibition of cytochrome c reduction. The assay was also adapted to assess the capacity of antioxidants to react with superoxide anion radical. However, in the current study, none of the compounds at final concentrations of 10^{-3} M and 10^{-4} M showed any significant ability to scavenge $O_2^{\cdot -}$ (data not shown).

In conclusion, these results show that *in vitro* the newly synthesized benzimidazole derivatives possess highly potent antioxidant properties.

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