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Synthesis, antioxidant and radical scavenging activities of novel benzimidazoles

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Abstract

The synthesis and antioxidant evaluation of some novel benzimidazole derivatives (**10**–**24**) are described. Antioxidant properties of the compounds were investigated employing various *in vitro* systems viz., microsomal NADPH-dependent inhibition of lipid peroxidation (LP), interaction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and scavenging of superoxide anion radical. Compounds **12** and **13** showed very good antioxidant capacity and were 17–18 -fold more potent than BHT (IC_{50} 2.3×10^{-4}M) with 1.3×10^{-5}M and 1.2×10^{-5}M IC_{50} values, respectively, by interaction of the stable DPPH free radical.

Keywords: *Thiosemicarbazides, thiadiazolylmethylbenzimidazoles, triazolylmethylbenzimidazoles, antioxidant activity, X-ray structure analysis, radical scavenging*

Introduction

Free radicals, oxidative stress and lipid peroxidation have been suggested as potentially important causative agent that led to uncontrolled reactions resulting in the cross-linking of DNA, proteins and lipids or oxidative damage of these important biological macromolecules. Damage to these moieties and DNA results in progression of a number of human diseases such as brain dysfunction, cancer, immune system decline, atherosclerosis [1] and Alzheimer's disease [2]. Hence, a number of studies have been performed to discover antioxidants for the prevention or treatment of these diseases.

In our antioxidative research program with benzimidazoles, we have already reported some antioxidant triazoyl- or thiadiazolyl benzimidazole derivatives (Figure 1) [3,4]. Furthermore, we have found that some compounds show potent antioxidant properties compared with the standard substance butylhydroxytoluen (BHT). In this study, we synthesized some novel compounds by modifying the structure of either the phenyl/*p*-chlorophenyl group at the 2- position of the benzimidazole ring to p-methoxyphenyl/pyridine ring or only the modifying Ar' group.

Materials and methods

Chemistry

Melting points were determined with an Electrothermal (Electrothermal Eng. Ltd., Essex, UK) and a Büchi SMP-20 (Büchi Laboratoriumstechnik, Flawil, Switzerland) melting point apparatus and are uncorrected. IR spectra (cm^{-1}) were recorded on a Jasco FT/IR 420 spectrometer (Jasco Corp., Tokyo, Japan) as potassium bromide discs. ^{1}H NMR spectra were measured with a Varian Mercury 400, 400 MHz

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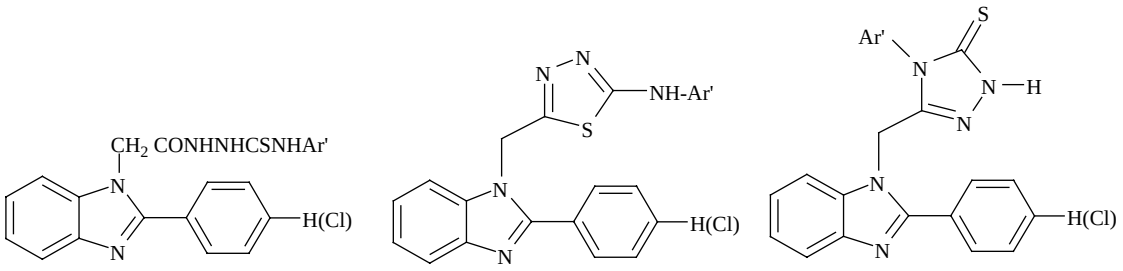


Figure 1. Structures of previously synthesized benzimidazole compounds.

instrument (California, USA) using TMS internal standard and DMSO-d_6. All chemical shifts are reported as δ (ppm) values. ESMS were obtained with a Waters ZQ Micromass LC-MS spectrometer (Milford, USA) with the Positive Electrospray Ionization method. Elemental analyses (C, H, N, S) 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. The chemical reagents used in synthesis were purchased from E.Merck (Darmstadt, Germany) and Aldrich (Milwaukee, M1, USA). Xanthine oxidase, Cytochrome c, DPPH and BHT were obtained from Sigma (Taufkirchen, Germany). Analytical thin layer chromatography was performed with Merck precoated TLC plates and spots were visualized with ultraviolet light. Log P values were calculated by the ChemDraw Pro version 5.0.

General method of preparation of 2-(p-methoxy)phenyl/(4-pyridinyl)-1H-benzimidazole acetic acid ethyl ester (5/6). Dimethylsulfoxide (15 ml) was added to crushed pellets of potassium hydroxide (1.5 g, 27 mmol) and the mixture was stirred for 15 min at room temperature. 2-(*p*-Methoxy)phenyl/(4-pyridinyl)-1*H*-benzimidazole (**2**–**3**)(6.7 mmol) was then added and the mixture was stirred for 2 h. Ethylbromoacetate (3 ml, 0.027 mol)was added and the mixture was cooled briefly and stirred for a further 2 h. Water was added and the mixture was extracted with ether. The ether layers were washed with water, dried and solvent and excess ethylbromoacetate were removed under reduced pressure. The residue was recrystallized from ethanol and gave compound **5** (Mp:95°C). Compound **6** was an oily residue and was used for further reaction without recrystallization.

*2-(p-Methoxy)phenyl)-1H-benzimidazole acetic acid ethyl ester(**5**)*. ES (+) (M + H) 281;^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, CH_2CH_3), 3.73 (s, 3H, OCH_3), 4.12 (q, 2H, CH_2CH_3), 4.87 (s, 2H, CH_2), 6.91 (d, 2H, Jo = 8.76 Hz, Jm = 1.99 Hz, H-3′, 5′), 7.13–7.21 (m, 3H, H-4,5,6), 7.56 (d, 2H, Jo = 8.76 Hz, Jm = 1.99 Hz, H-2′, 6′), 7.68 (d, 1H, H-7).

*2-(4-Pyridinyl)-1H-benzimidazole acetic acid ethyl ester (**6**)*. ES (+) (M + H): 268; ^{1}H NMR (DMSO-d_6) δ 1.29 (t, 3H, CH_2CH_3), 4.29 (q, 2H, CH_2CH_3), 4.94 (s, 2H, CH_2), 7.27-7.40 (m, 3H, H-4, 5, 6), 7.68 (d, 2H, H-2′,6′), 7.84 (d, 1H, H-7), 8.81 (d, 2H, H-3′,5′).

General method of preparation of 2-(p-methoxy) phenyl/(4-pyridinyl)-1H-benzimidazole acetic acid hydrazide (8–9). Hydrazine hydrate (4 ml) and 2-(*p*-methoxy) phenyl/(4-pyridinyl)-1*H*-benzimidazole acetic acid ethyl ester (1.5 mmol) in ethanol (5 ml) were refluxed for 4 h. The reaction mixture was cooled and poured into water. The crude product was filtered off and recrystallized from ethanol to give the desired hydrazide compounds **8**–**9**.

*2-(p-Methoxy) phenyl)-1H-benzimidazole acetic acid hydrazide (**8**)*. M.p:189–90°C;ES (+) (M + H) 297; ^{1}H NMR (DMSO-d_6) δ 3.71 (s, 3H, OCH_3), 4.31 (s, 2H, NH_2), 4.68 (s, 2H, CH_2), 6.97 (d, 2H, Jo = 8.84 Hz, Jm = 2.02 Hz, H-3′, 5′), 7.07–7.13 (m, 2H, H-5, 6), 7.29 (d, 1H, Jo = 8.87 Hz, H-4), 7.52 (d, 1H, Jo = 8.83 Hz, H-7), 7.64 (d, 2H, Jo = 8.83 Hz, Jm = 2.04 Hz, H-2′, 6′),9.42 (s, 1H, CONH)

*2-(4-Pyridinyl)-1H-benzimidazole acetic acid hydrazide (**9**)*. M.p:249–251°C;ES (+)(M + H) 268; ^{1}H NMR (DMSO-d_6) δ 4.38 (d, 2H, NH_2), 4.93 (s, 2H, *CH2*), 7.29–7.30 (m, 2H, H-5,6), 7.53 (d, 1H, Jo = 7.44 Hz, H-4), 7.74 (d, 1H, Jo = 7.44 Hz, H-7), 7.85 (d, 2H, Jo = 6.26 Hz, H-2′,6′), 8.76 (d, 2H, Jo = 6.26 Hz, H-3′,5′), 9.63 (s, 1H, *NH*).

General procedure for the preparation of the thiosemicarbazides (1-[(substituted thiocarbamoyl-hydrazinecarbonyl)methyl] 2-(p-methoxy/chloro)phenyl/(4-pyridinyl)-1H-benzimidazoles) (10–14). 0.54 g Acid hydrazide (2.03 mmol) in absolute ethanol (20 ml) and appropriate isothiocyanate (3.05 mmol) were heated under reflux for 30 min. The

precipitate formed was cooled, filtered and recrystallized from ethanol.

*1-[(p-Methoxyphenyl thiocarbamoyl hydrazine carbonyl] methyl(2-(p-chlorophenyl)-1H-benzimidazole (**10**)*. $C_{23}H_{20}ClN_5O_2S$ (C, H, N, S); M.p:206–208°C; ES (+) (M + H): 466; IR (KBr cm^{-1}) 1676 (C = O); ^{1}H NMR (DMSO-d_6) δ: 3.77 (s, 3H, OCH_3), 4.99 (s, 2H, CH_2), 6.91 (dd, 2H, Jo = 8.99 Hz, Jm = 2.34 Hz, H-2′, 6′), 7.25–7.31 (m, 4H, H-5,6,3′,5′), 7.55 (d, 2H, Jo = 8.22 Hz, H-3″,5″), 7.61 (d,1H, H-4), 7.71–7.81 (m, 3H, H-7,2″,6″), 9.65, 9.84, 10.52 (3H, CO*NHNH*CS*NH*).

*1-[(p-Methoxyphenyl thiocarbamoyl hydrazine carbonyl) methyl] 2-(p-methoxyphenyl)-1H-benzimidazole (**11**)*. $C_{24}H_{23}N_5O_3S$ (C, H, N, S); M.p:205°C decomp.: ES (+) (M + H): 462; IR (KBr cm^{-1}) 1679 (C = O); ^{1}H NMR (DMSO-d_6) δ: 3.75 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.98 (s, 2H, CH_2), 6.92 (d, 2H, Jo = 9.00 Hz, H-2′, 6′), 7.08 (d, 2H, Jo = 9.00 Hz, H-3′, 5′),7.22–7.26 (m, 4H, H-5, 6, 3″,5″), 7.51 (dd, 1H, H-4), 7.64–7.72 (m, 3H, H-7,2″,6″), 9.66, 9.83, 10.43 (3H, CO*NHNH*CS*NH*).

*1-[(Phenyl thiocarbamoyl hydrazine carbonyl) methyl] 2-(4-pyridinyl) 1H-benzimidazole (**12**)*. $C_{21}H_{18}N_6OS.1H_2O$ (C, H, N, S); M.p:220–21°C; ES (+) (M + H) 403; IR (KBr cm^{-1}) 1712 (C = O); ^{1}H NMR (DMSO-d_6)δ: 5.08 (s, 2H, CH_2), 7.17–7.74 (m, 11H, Ar-H), 8.73 (d, 2H, Jo = 6.26 Hz, H-3′, 5′), 9.74 (s, 2H, CO*NHNH*), 10.58 (s, 1H, CS*NH*).

*1-[(p-Methoxyphenyl thiocarbamoyl hydrazine carbonyl) methyl] 2-(4-pyridinyl) 1H-benzimidazole (**13**)*. $C_{22}H_{20}N_6O_2S.1H_2O$ (C, H, N, S); M.p:223–25°C; ES (+) (M + H) 433; IR (KBr cm^{-1}) 1709 (C = O); ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H, O*CH*$_3$), 5.07 (s, 2H, CH_2), 6.90 (d, 2H, Jo = 9.00 Hz, H-2″, 6″), 7.23–7.35 (m, 4H, H-5,6,3″,5″), 7.58 (d, 1H, Jo = 7.83 Hz, H-4), 7.71–7.78 (m, 3H, H-7, 2′,6′), 8.74 (d, 2H, Jo = 1.6 Hz, H-3′,5′), 9.64 (s, 2H, CO*NHNH*), 10.54 (s, 1H, CS*NH*).

*1-[(3,4-Dichlorophenyl thiocarbamoyl hydrazine carbonyl) methyl] 2-(4-pyridinyl) 1H-benzimidazole (**14**)*. $C_{21}H_{16}Cl_2N_6S.1.5H_2O$ (C, H, N, S); M.p:202–206°C decomp.; ES (+) (M + H) 471; IR (KBr cm^{-1}) 1687 (C = O); ^{1}H NMR (DMSO-d_6) δ 5.10 (s, 2H, CH_2), 7.29–7.88 (m, 9H, Ar-H), 8.76 (d, 2H, Jo = 5.87 Hz, H-3′, 5′), 10.02 (s, 2H, CO*NHNH*), 10.62 (s, 1H, CS*NH*).

General procedure for preparation of the [5-(2-(p-methoxy/chloro) phenyl / (4-pyridinyl) benzimidazol-1-yl methyl)-[1,3,4]-thiadiazole-2-yl(-substituted phenyl amines (15–19). Appropriate thiosemicarbazide **10**–**14** (3.4 mmol) in 10 ml ice-cold concentrated sulfuric acid was stirred for 10 min, and then left another 10 min at room temperature. The resulting solution was poured slowly into ice-cold water, made alkaline to pH 8 with aqueous ammonia and the precipitated product was filtered, washed with water and crystallized from ethanol.

*[5-(2-(p-Chlorophenyl) benzimidazol-1-yl methyl)-[1,3,4]-thiadiazole-2-yl(-4-methoxy phenyl amine (**15**)*. $C_{23}H_{18}ClN_5OS$ (C, H, N, S); M.p:213–15°C; ES (+) 447 (M + H); ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H, O*CH*$_3$), 5.82 (s, 2H, *CH*$_2$), 6.88 (d, 2H, Jo = 8.99 Hz, H-2′,6′), 7.26–7.34 (m, 2H, H-5,6), 7.39 (d, 2H, Jo = 9.39 Hz, H-3′,5′), 7.63–7.86 (m, 4H, H-4,7,3″,5″), 7.88 (d, 2H, Jo = 8.61 Hz, H-2″,6″), 10.10 (s, 1H, *NH*).

*[5-(2-(p-Methoxyphenyl) benzimidazol-1-yl methyl)-[1,3,4]-thiadiazole-2-yl]-4-methoxy phenyl amine (**16**)*. $C_{24}H_{21}N_5O_2S.\ 0.3H_2SO_4$-$0.3H_2O$ (C, H, N, S); M.p:162–64°C; ES (+) 444(M + H); ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H, O*CH*$_3$), 3.85 (s, 3H, O*CH*$_3$), 5.78 (s, 2H, *CH*$_2$), 6.88 (d, 2H, Jo = 9.00 Hz, H-2′,6′), 7.13 (d, 2H, Jo = 9.00 Hz, H-3′,5′), 7.22–7.30 (m, 2H, H-5,6), 7.42 (d, 2H, Jo = 9.00 Hz, H-3″,5″), 7.58–7.71(m, 2H, H-4,7), 7.79 (d, 2H, Jo = 9.00 Hz, H-2″,6″), 10.06 (s, 1H, *NH*).

*[5-(2-(4-Pyridinyl) benzimidazol-1-yl methyl)-[1,3,4]-thiadiazole-2-yl]-phenyl amine (**17**)*. $C_{21}H_{16}N_6S.\ 1.1H_2O$ (C, H, N, S); M.p:215–17°C; ES (+) 385 (M + H); ^{1}H NMR (DMSO-d_6) δ 5.82 (s, 2H, *CH*$_2$), 6.95 (td, 1H, Jo = 7.43, 7.44 Hz, H-4″), 7.17–7.75 (m, 8H, Ar-H) 7.86 (d, 2H, Jo = 5.87 Hz, H-2′,6′), 8.76 (d, 2H, Jo = 5.86 Hz, H-3′,5′), 10.31 (s, 1H, *NH*).

*[5-(2-(4-Pyridinyl) benzimidazol-1-yl methyl]-[1,3,4]-thiadiazole-2-yl]-4-methoxy phenyl amine (**18**)*. $C_{22}H_{18}N_6OS.\ 1.1H_2O$ (C, H, N, S); M.p:219°C; ES (+) 415(M + H); ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H, *OCH*$_3$), 5.90 (s, 2H, *CH*$_2$), 6.86 (dd, 2H, Jo = 9.00 Hz, Jm = 2.35 Hz, H- 2″,6″), 7.23–7.37 (m, 2H, H-5,6), 7.41 (d, 2H, Jo = 9.00 Hz, H-3″,5″), 7.76 (td, 2H, Jo = 8.61 Hz, 8.99 Hz, H-4,7), 7.86(dd, 2H, Jo = 6.26 Hz, Jm = 1.96 Hz, H-2′,6′), 8.77(dd, 2H, Jo = 6.26 Hz, H-3′,5′), 10.09(s, 1H, *NH*).

*[5-(2-(4-Pyridinyl) benzimidazol-1-yl methyl)-[1,3,4]-thiadiazole-2-yl]-3,4-dichloro phenyl amine (**19**)*. $C_{21}H_{14}Cl_2N_6S.\ 1.5H_2O$ (C, H, N, S); M.p:243–45°C; ES (+) 453(M + H); ^{1}H NMR (DMSO-d_6) δ 5.95(s, 2H, *CH*$_2$), 7.31–7.36 (m, 3H, H-5,6, 6″), 7.52 (d, 1H, Jo = 9.00 Hz, H-5″), 7.73–7.77 (m, 2H, H-4,7), 7.86 (d, 2H, Jo = 5.87 Hz, H-2′,6′), 7.98 (d, 1H, Jo = 2.35 Hz, H-2″), 8.77 (dd, 2H, Jo = 5.87 Hz, H-3″), 10.65 (s, 1H, *NH*).

General procedure for the preparation of 5-(2-(p-methoxy/p-chloro) phenyl/(4-pyridinyl) benzimidazol-1-yl methyl)-4-substitutedphenyl-2,4-dihydro-[1,2,4]-triazole-3-thiones (20-24). Appropriate thiosemi-carbazide (3.4 mmol)

10–**14** in 10 ml 1N sodium hydroxide was 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.

5-(2-(p-Chlorophenyl)benzimidazol-1-yl methyl)-4-methoxyphenyl-2,4-dihydro-[1,2,4]-triazole-3-thione (***20***). $C_{23}H_{18}ClN_5OS$ (C, H, N, S); M.p:230–31°C; ES (+) 447(M + H); IR (KBr cm^{-1}) 1604 (C = N), 1303 and 1285 (C = S); ^{1}H NMR (DMSO-d_6) δ 3.76 (s, 3H, OCH_3), 5.43 (s, 2H, CH_2), 6.83-769 (m, 12H, Ar-H).

5-(2-(p-Methoxyphenyl)benzimidazol-1-ylmethyl)-4-methoxyphenyl-2,4-dihydro-[1,2,4]-triazole-3-thione (***21***). $C_{24}H_{21}N_5O_2S$. $0.8H_2O$ (C, H, N, S); M.p:165–68°C; ES(+) 444 (M + H); IR 1610 (C = N), 1321 and 1300 (C = S); ^{1}H NMR (DMSO-d_6) δ 3.76 (s, 3H, *OCH₃*), 3.82 (s, 3H, *OCH₃*), 5.38 (s, 2H, *CH₂*), 6.86 (d, 2H, Jo = 9.00 Hz, H-3′,5′), 7.01 (d, 2H, Jo = 8.99 Hz, H-2′,6′), 7.03 (d, 2H, Jo = 8.61 Hz, H-3″,5″), 7.19–7.21 (m, 2H, H-5,6), 7.32 (m, 1H, H-4), 7.46 (d, 2H, Jo = 8.61 Hz, H-2″,6″), 7.54 (m, 1H, H-7), 13.84 (s, 1H, *NH*)

5-(2-(4-Pyridinyl)benzimidazol-1-yl methyl)-4-phenyl-2,4-dihydro-[1,2,4]-triazole-3-thione (***22***). $C_{21}H_{16}N_6S$. 0.1 HCl. $0.1H_2O$ (C, H, N, S); M.p:296°C; ES(+) 385 (M + H); IR 1608 (C = N), 1310 and 1291 (C = S); ^{1}H NMR (DMSO-d_6) δ 5.51 (s, 2H, *CH₂*), 7.14 (d, 2H, Jo = 7.04 Hz, H-2″,6″), 7.27–7.45 (m, 6H, H-4,5,6,3″,4″,5″), 7.54 (d, 2H, Jo = 5.87 Hz, H-2′,6′), 7.68 (d, 1H, Jo = 6.65 Hz, H-7), 8.69 (d, 1H, Jo = 6.26 Hz, H-3′,5′), 13.95 (s, 1H, *NH*).

5-(2-(4-Pyridinyl)benzimidazol-1-yl methyl)-4-methoxyphenyl-2,4-dihydro-[1,2,4]-triazole-3-thione (***23***). $C_{22}H_{18}N_6OS$ (C, H, N, S); M.p:271–73°C; ES(+) 415 (M + H); IR 1603 (C = N), 1317 and 1254 (C = S); ^{1}H NMR (DMSO-d_6) δ 3.75(s, 3H, *OCH₃*), 5.51 (s, 2H, *CH₂*), 6.83 (d, 2H, Jo = 8.99 Hz, H-3″,5″), 7.01 (d, 2H, Jo = 9.00 Hz, H-2″,6″), 7.26–7.29 (m, 2H, H-5,6), 7.46 (d, 1H, Jo = 6.65 Hz, H-4), 7.55 (d, 2H, Jo = 6.26 Hz, H-2′,6′), 7.69 (d, 1H, Jo = 6.58 Hz, H-7), 8.69 (d, 1H, Jo = 6.26 Hz, H-3′,5′), 13.88 (s, 1H, *NH*).

5-(2-(4-Pyridinyl)benzimidazol-1-ylmethyl)-3,4-dichlorophenyl-2,4-dihydro-[1,2,4]-triazole-3-thione (***24***). $C_{21}H_{14}Cl_2N_6S$ (C, H, N, S); M.p:309°C; ES(+) 453(M + H); IR 1606(C = N),1319 and 1295 (C = S); ^{1}H NMR (DMSO-d_6) δ 5.64 (s, 2H, *CH₂*), 7.03 (dd, 1H, Jo = 8.61 Hz, Jm = 2.35 Hz, H-6″), 7.24–7.30 (m, 2H, H-5,6), 7.40–7.45 (m, 2H, H-4,2″), 7.55 (d, 1H, Jo = 8.61 Hz, H-5″), 7.59 (dd, 2H, Jo = 6.26 Hz, Jm = 1.56 Hz, H-3′,5′), 7.66 (dd, 1H, Jo = 7.04 Hz, Jm = 1.96 Hz, H-7), 8.70 (dd, 2H, Jo = 5.87 Hz, Jm = 1.57 Hz, H-2′,6′).

Single crystal X-ray structure determinations

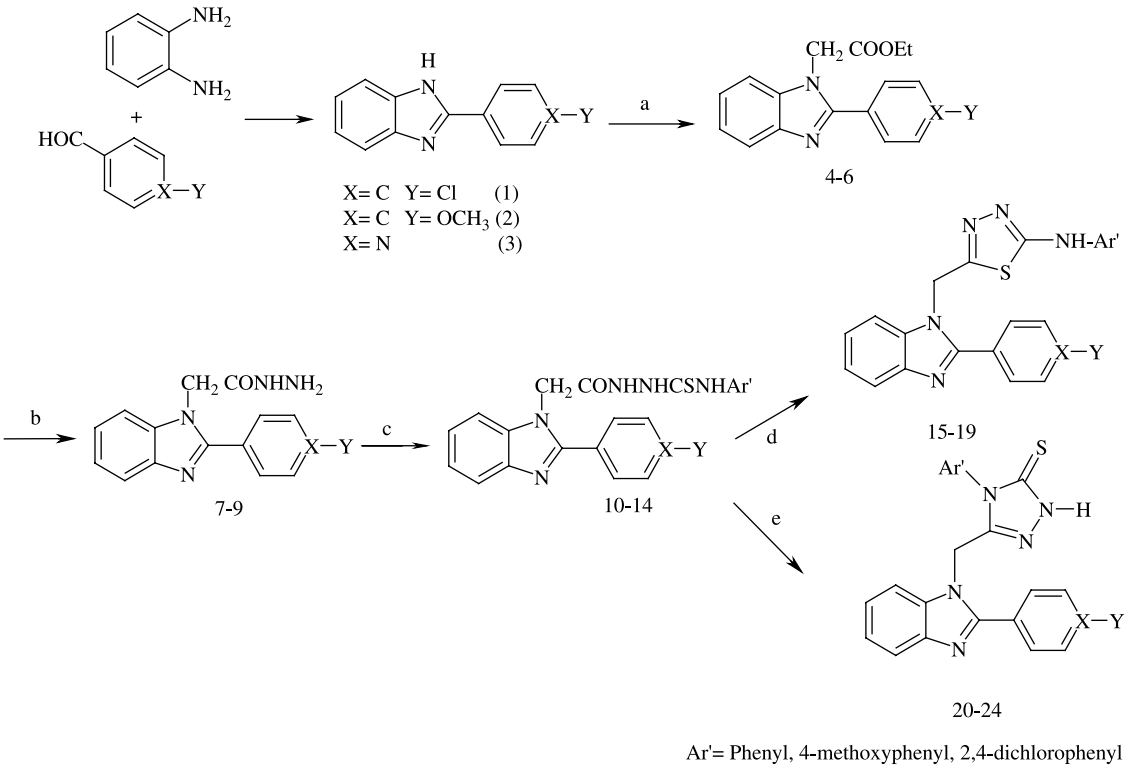
Single crystals suitable for X-ray diffraction analysis were obtained by crystallization from compound **20**. Measurements were performed on an Enraf-Nonius CAD4 four circle diffractometer equipped with graphite monochromator CuK_α radiation. Intensity of peaks was automatically corrected for standard reflections. Structure was solved by direct methods using SHELXS [5] and, refinement was performed with SHELXL [6]. Non-hydrogen atom parameters were refined anisotropically. All hydrogen atoms, except for N2, were placed in idealized positions and refined using a riding model with $U_{eq}(H) = 1.3U_{eq}(C)$, and fixed distances of C-H = 0.93 Å (aromatic) and C-H = 0.96 Å (methyl).

Hydrogen atom of N2 was found from a difference Fourier map and refined isotropically. The geometric calculations were performed using the program Platon [7].

Antioxidant activity studies

Assay of Lipid Peroxidation. 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 ice-cold distilled water and the microsomes were prepared as described previously [8].

NADPH-dependent LP was determined using the optimum conditions determined and described previously [8]. 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^{-1} protein. The assay was essentially derived from the methods of Wills [9,10] as modified by Bishayee [11]. 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.



Scheme 1. Reagents and conditions: (a) $BrCH_2COOEt$; (b) $H_2NNH_2.H_2O$; (c) Ar'NCS; (d) H_2SO_4; (e) NaOH

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. [12].

Superoxide anion was generated in the xanthine/xanthine oxidase system. The reaction mixture contained in a final volume of 1 ml, 0.05M phosphate buffer pH 7.8, 0.32 U xanthine oxidase, 50 μM xanthine, 60 mM ctytochrome c and different concentration of

Table I. Crystal data, intensity data collection parameters and final refinement results of compound **20**.

Chemical formula	C_{23} H_{18} Cl N^5 OS
Formula weight	447.94
Space group; Crystal system	P-1; Triclinic
Crystal dimensions (mm)	0.51 × 0.48 × 0.15
a (Å)	8.6039(16)
b (Å)	8.9864(10)
c (Å)	16.693(3)
α(°)	88.120(10)
β (°)	76.307(15)
γ (°)	64.9750(10)
Volume(Å^3); Z	1133.0(3); 2
D_c (g cm^{-3}); μ (mm^{-1})	1.313; 0.255
λ (Å); Scan type	1.5418; ω - 2θ
Absorption correction type	Empirical psi-scan
T_{min}; T_{max}	0.356; 0.7007
Range of θ (°)	2.73; 74.24
h, k, l range	0, 10; −10, 11; −20, 20
No. refls. measured/unique	4619/ 4330
No. reflns. [I > 2σ(I)]; R_{int}	3499; 0.0183
No. reflns. used in refinement	3499
No. param. refined	285
Weighting scheme	w = 1/[\s^2(Fo^2) + (0.0767P)^2 + 0.3893P] where P = (Fo^2 + 2Fc^2)/3
R and R_w values	0.0442; 0.1378
Goodness of fit	1.019
Extinction coef.	0.0041(6)
Max. and min. electron density (eÅ^{-3})	0.384, −0.286

synthesized 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 Blois [13]. This assay has often been used to estimate the antiradical activity of antioxidants. Because of its odd electra DPPH gives a strong absorption band at 517 nm in visible spectroscopy. DPPH was dissolved in methanol to give a 100μM solution. To 1.0 ml of the methanolic solution of DPPH 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 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 each compound.

Results

For the synthesis of the target compounds the reaction sequences outlined in Scheme 1 were followed. 1*H*-benzimidazoles (**1**–**3**) was prepared via oxidative condensation of *o*-phenylenediamine, *p*-chloro/*p*-methoxy benzaldehyde or pyridine-4-carboxyaldehyde and sodium metabisulfite[14]. Treatment of compounds **1**–**3** with ethyl chloroacetate in KOH/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 (7–**9**)[16]. The thiosemicarbazides (**10**–**14**) (Scheme 1) were obtained upon reaction of the acid hydrazide with aryl isothiocyanates in ethanol[3,4]. Cyclization of **10**–**14** with sodium hydroxide or concentrated sulfuric acid resulted in the formation of triazole (**15**–**19**) and thiadiazole (**20**–**24**) derivatives respectively. The crystal data, intensity data collection parameters and final refinement results for compound **20** are summarized in Table I. The molecular structure with atom numbering scheme and the packing arrangement of the molecules in the unit cell are presented in Figures 2 and 3. Selected bond lengths, bond angles and torsion angles are listed in Table II.

Compounds **10**–**24** 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 by determining their capacity to scavenge superoxide anions and interact with the stable free radical DPPH (Table III).

Discussion

Although two types of tautomers (compounds **20**–**24** and **25**–**29**) (Scheme 2) could be expected from the cyclisation of compounds **10**–**14** under alkaline conditions, compounds **20**–**24** were observed. This was demonstrated in the IR spectra by the presence of the two absorbtion maxima at 1303–1321 and 1254–1300 cm^{-1} belonging to the C = S group. The

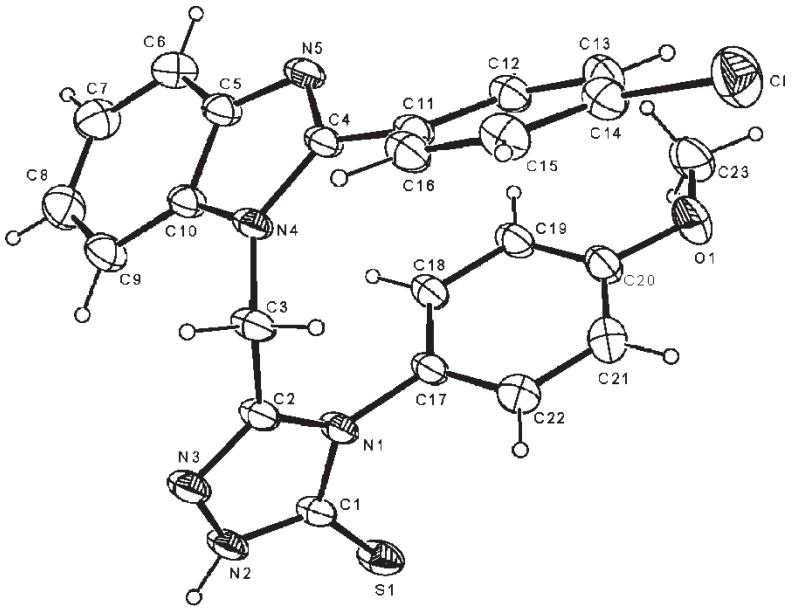


Figure 2. ORTEP diagram of the molecular structure of compound **20**showing the atom labeling scheme.

Table II. Selected bond distances (Å), bond angles (°) and torsion angles (°) of compound **20**.

Cl–C14	1.740(3)
S1–C1	1.663(3)
O1–C20	1.365(3)
O1–C23	1.431(3)
N1–C1	1.381(3)
N1–C2	1.378(3)
N1–C17	1.443(3)
N2–N3	1.373(4)
N2–C1	1.338(3)
N3–C2	1.297(3)
N4–C3	1.461(3)
N4–C4	1.378(2)
N4–C10	1.391(3)
N5–C4	1.316(3)
N5–C5	1.384(3)
C2–C3	1.488(4)
C4–C11	1.466(3)
C5–C10	1.400(3)
C20–O1–C23	117.5(2)
C1–N1–C2	107.7(2)
C1–N1–C17	125.1(2)
N3–N2–C1	114.0(2)
N2–N3–C2	103.9(2)
C3–N4–C4	127.9(2)
C4–N4–C10	106.9(2)
C4–N5–C5	106.4(2)
S1–C1–N1	128.3(2)
N1–C1–N2	103.1(2)
N1–C2–C3	124.4(2)
N1–C2–N3	111.4(2)
N4–C3–C2	112.1(2)
N4–C4–N5	111.8(2)
N5–C4–C11	123.0(2)
C6–C5–C10	120.1(2)
N5–C5–C10	109.6(2)
N4–C10–C5	105.3(2)
C5–C10–C9	122.2(2)
C4–C11–C12	117.4(2)
Cl–C14–C13	119.4(2)
N1–C17–C18	119.6(2)
O1–C20–C21	115.3(2)
O1–C20–C19	124.6(2)
C23–O1–C20–C21	−170.2(3)
C1–N1–C17–C18	−90.0(3)
C17–N1–C1–S1	2.1(3)
C17–N1–C2–C3	−3.6(3)
C4–N4–C3–C2	120.7(3)
C3–N4–C10–C9	−7.6(5)
C3–N4–C4–N5	−172.8(2)
N1–C2–C3–N4	−60.7(3)
N5–C4–C11–C12	40.7(4)

conversions were monitored by the disappearance of the strong C = O stretching bands of thiosemicarbazides at 1676–1712 cm^{-1} and the appearance of the medium bands at 1603–1610 cm^{-1} for the C = N stretching band in the IR spectra of the compounds **20**–**24**. The structural results also show that the compound **20** exists as the thione tautomeric form in the solid state. The location of the H-atom on atom N2 rather than atom S, and the C1-S1, N2-C1 and C1-N1 bond lengths support this idea.

As expected, the benzimidazole nucleus of compound **20** is planar, the dihedral angle between the imidazole and the benzene ring planes being 1.0(2)°. Planarity of the benzimidazole ring system is usually observed[17–21]. Overall, the molecule is not planar. The triazolylmethyl moiety at atom N4 is rotated around the N4 - C3 and C3 - C2 bonds, giving C4 - N4 - C3 - C2 and N4- C3 - C2 - N1 torsion angles of 120.7(3) and −60.7(3)° while the *p*-chlorophenyl moiety at atom C4 is twisted out of the plane of the benzimidazole ring with torsion angle of 40.7(4)° for N5-C4-C11-C12. In addition, the torsion angle C1-N1-C17-C18 is −90.0(3)° and the dihedral angle between the triazole and *p*-methoxyphenyl and benzimidazole rings are 89.5(1) and 84.9(1)°, respectively. Thus, the triazole part of the molecule is almost perpendicular to the phenyl and the benzimidazole parts of the molecule in order to avoid steric interactions between the methylene group and the *p*-methoxyphenyl group of the triazole.

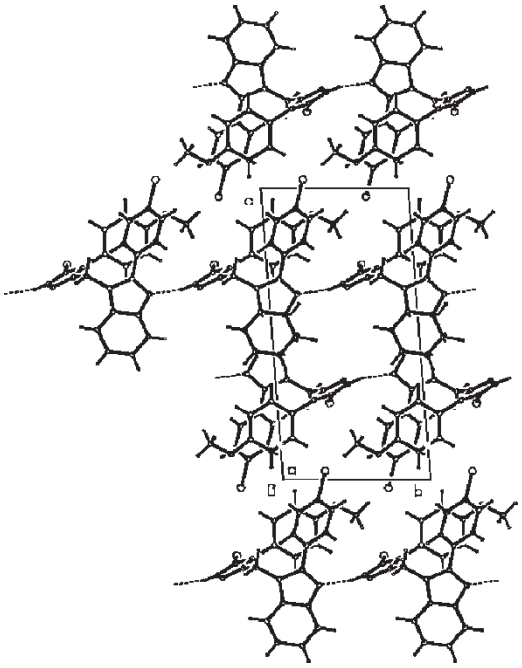


Figure 3. ORTEP packing diagram of compound **20** in the unit cell. Hydrogen bonds are shown by dotted lines.

The molecular and crystal structures are stabilized by an intermolecular hydrogen bond between the amino group of the triazole and the nitrogen atom of benzimidazole of a neighboring molecule [H2···N5^{i} = 1.92(3) Å, N2···N5^{i} = 2.794(3) Å, N2−H2···N5^{i} = 167(3)°; symmetry code (i) = x,− 1 + y,z]. The packing diagram (Figure 3) shows that the molecules are arranged into infinite one-dimensional chains along the *b* axis. A further, non-classical hydrogen bond is also observed [H16···S1^{ii} = 2.879 Å, C16···S1 = 3.782(3) Å, C16 − H16···S1 = 164°]; this is a presumably weak.

As seen in Table III, only compounds **10**–**12**, **15**, **16** and **20** inhibited lipid peroxidation (69%, 30%, 35%, 55%, 16% and 85%, respectively), the others having

Table III. Effects of the compounds on liver LP levels and superoxide anion and DPPH free radical scavenging activity *in vitro* ⋆.

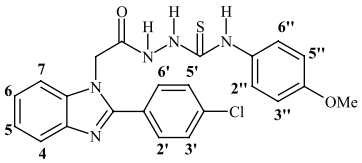
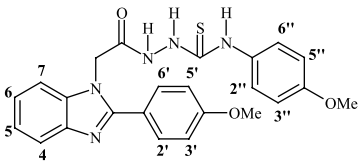
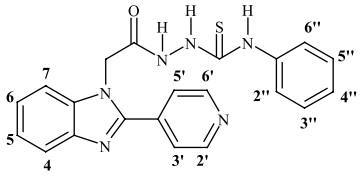
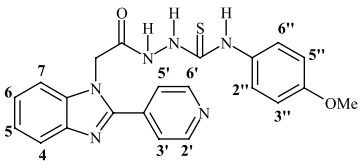
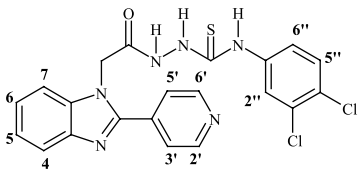
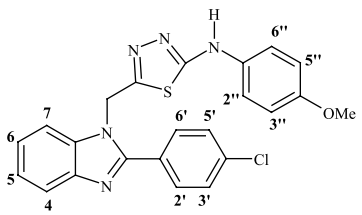
No	Formula	LP Percent of control†	Concentration in incubation medium (M)	Superoxide anion (O_2^-) production (Percent of control)	DPPH free radical scavenging activity IC_{50} (M)
10		31	5.0×10^{-4} 2.5×10^{-4}	187 ± 3 76 ± 3	1.6×10^{-5}
11		70	5.0×10^{-4} 2.5×10^{-4}	101 ± 4 96 ± 6	NE
12		65	5.0×10^{-4} 2.5×10^{-4}	97 ± 1 99 ± 3	1.3×10^{-5}
13		127	5.0×10^{-4} 2.5×10^{-4}	101 ± 3 93 ± 6	1.22×10^{-5}
14		238	5.0×10^{-4} 2.5×10^{-4}	102 ± 3 98 ± 4	1.76×10^{-5}
15		45	5.0×10^{-4} 2.5×10^{-4}	54 ± 6 95 ± 3	1.8×10^{-3}

Table III – *continued*

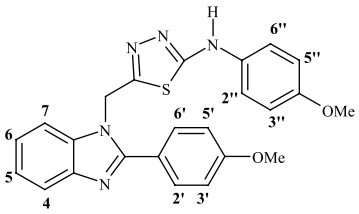
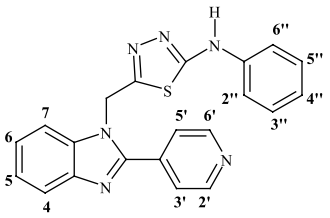
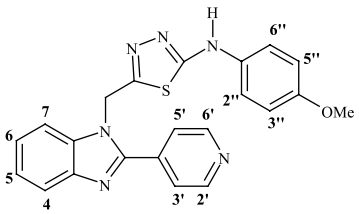
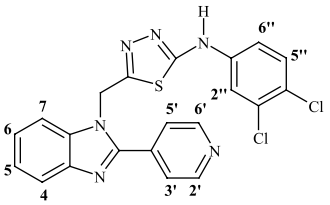
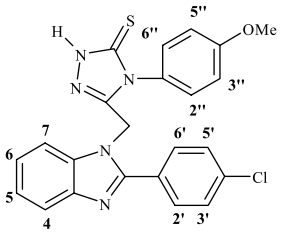
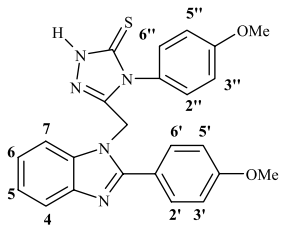
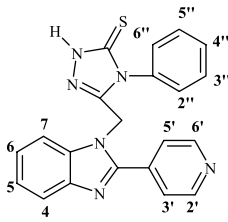
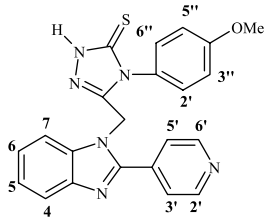
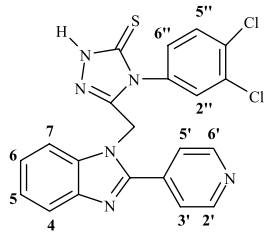
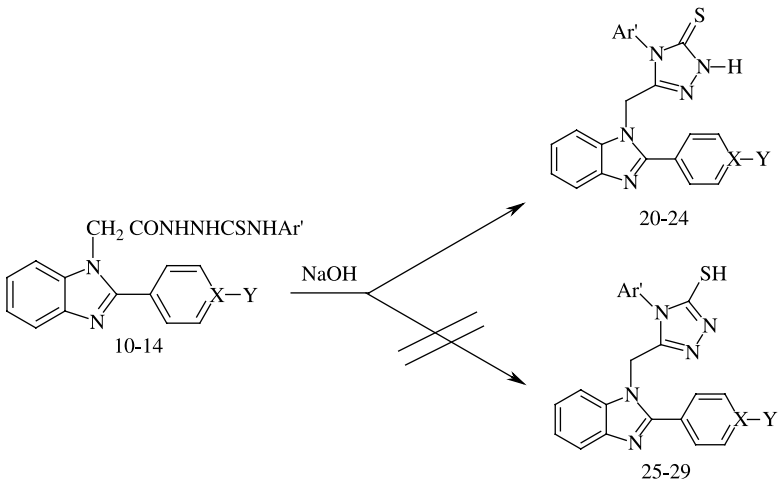
No	Formula	LP Percent of control†	Concentration in incubation medium (M)	Superoxide anion (O_2^-) production (Percent of control)	DPPH free radical scavenging activity IC_{50} (M)
16		84	5.0×10^{-4} 2.5×10^{-4}	122 ± 5 98 ± 6	6.5×10^{-5}
17		126	5.0×10^{-4} 2.5×10^{-4}	141 ± 6 100 ± 1	2.4×10^{-5}
18		118	5.0×10^{-4} 2.5×10^{-4}	191 ± 6 97 ± 6	1.7×10^{-5}
19		212	5.0×10^{-4} 2.5×10^{-4}	NE	NE
20		15	5.0×10^{-4} 2.5×10^{-4}	99 ± 3 101 ± 3	5.4×10^{-5}

Table III – *continued*

No	Formula	LP Percent of control[†]	Concentration in incubation medium (M)	Superoxide anion (O_2^-) production (Percent of control)	DPPH free radical scavenging activity IC_{50} (M)
21		112	5.0×10^{-4} 2.5×10^{-4}	108 ± 1 99 ± 4	3.3×10^{-4}
22		156	5.0×10^{-4} 2.5×10^{-4}	104 ± 1 102 ± 3	2.6×10^{-4}
23		174	5.0×10^{-4} 2.5×10^{-4}	117 ± 1 92 ± 6	2.9×10^{-4}
24		180	5.0×10^{-4} 2.5×10^{-4}	100 ± 1 98 ± 6	1.1×10^{-4}
BHT		37			2.3×10^{-4}
Control[‡]		100		100 ± 7	
SOD[‖]			30 IU	24 ± 2	
			45 IU	11 ± 1	

*Each value represents the mean ± S.D. of 2–4 independent experiments.
[†] Concentration in incubation medium (10^{-3} M).
[‡] DMSO only, control for compounds.
[‖]Distilled water, control for SOD. NE: No effect.

no effects on the level of LP. Compounds **10, 15** and **20** which bearing the *p*-chlorophenyl substituent at the 2-position of the benzimidazole ring were found to have stronger inhibitory effects than the other compounds and compound **20** also appeared to have a stronger inhibitory effect than BHT (63%). These and previous published results[3,4] clearly indicated that a lipophilic substituent is appropriate for the modification of the 2-position of the benzimidazole ring for LP inhibition. Consequently compounds



Scheme 2. Structures of **20**–**24** and **25**–**29**

which contain pyridine or a *p*-methoxyphenyl substituent at the same position had slightly or no inhibitory effects on LP levels.

Compounds **10, 12**–**14, 16**–**18, 20,** and **24** showed highest interaction with the DPPH radical, even better than BHT. The IC_{50} values for these compounds were 72–95% smaller than that for BHT, due to their hydrogen-donating ability with the presence of one or two *N-H* groups as a hydrogen-donating group necessary to react with the DPPH stable radical. Compounds **21**–**23** were slightly less potent than the standard BHT (12–30%). The thiosemicarbazides **10**–**14** (except compound **11**) had stronger interaction with the stable free radical DPPH. Compound **12** and **13** were the most active compounds with IC_{50} values of 1.3×10^{-5}M and 1.22×10^{-5}M, respectively.

The superoxide anion radical scavenging activities of the compounds were also examined. Only compound **15** which was the least active compound with DPPH exhibited significant ability to scavenge $O_2^{\cdot-}$, while others had no remarkable effects. We also calculated the Log P values of all the compounds and compound **15** was the most lipophilic compound with Log P = 6.86 (the others had values less than 6.20). So it can be said that a high Log P value is important for improving superoxide anion radical scavenging activity.

In this study it was observed that the effects of the compounds on superoxide radical-scavenging activity, lipid peroxidation, and DPPH free radical-scavenging activity were variable. Different effects of compounds in these systems have been noticed previously [22–25]. Therefore the observation of distinct effects of synthetic compounds on LP levels, superoxide anion radical formation, and DPPH free radical-scavenging activity is not surprising since the mechanisms of production of oxidative stress using these methods are different [26–28].

Acknowledgements

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