TÜBİTAK

Turkish Journal of Chemistry

http://journals.tubitak.gov.tr/chem/

Research Article

Turk J Chem
(2015) 39: 42 – 53

doi:10.3906/kim-1403-44

Synthesis and evaluation of antioxidant activities of novel 1,3,4-oxadiazole and imine containing 1*H*-benzimidazoles

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Received: 17.03.2014 • **Accepted:** 21.06.2014 • **Published Online:** 23.01.2015 • **Printed:** 20.02.2015

Abstract: Some novel 2-(substitutedbenzylthio)-5-((2-(4-substitutedphenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazoles (**5**–**12**) and 2-(2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-(arylmethylene)acetohydrazide derivatives (**13**–**22**) were prepared and their in vitro antioxidant properties were investigated by determination of rat liver microsomal NADPH-dependent inhibition of lipid peroxidation (LP) levels and microsomal ethoxyresorufin O-deethylase (EROD) activity. Compound **18** was found to be the most active compound with 100% inhibition on LP level and 92% inhibition on EROD. Compounds **4b**, **17**, and **19** showed the strongest inhibitory effect (97%) on EROD. The free radical scavenging capacities of the compounds were also tested in vitro determining the interaction of the stable free radical 2,2,diphenyl-1-picrylhydrazyl (DPPH), and compounds **4a** and **4b** exhibited good antioxidant activities.

Key words: Antioxidant, lipid peroxidation, benzimidazole, oxadiazole, imine

1. Introduction

Antioxidant defense mechanisms are required to prevent cellular damage observed in various diseases. Impairment of the antioxidants and antioxidant systems could be related to increased oxidative stress.[1,2] Therefore, drugs possessing antioxidant and free radical scavenging properties are considered for preventing and/or treatment of diseases that are directly involved with the lack of antioxidant capacity of organisms. It is known that lipid peroxidation (LP) is a free radical-initiated reaction that causes the degeneration of the cell membranes[3] and is involved in the evaluation of the antioxidant properties of a compound. Most products of lipid peroxidation are known to have mutagenic and/or carcinogenic properties. Furthermore, reactive oxygen/nitrogen species are produced by different mechanisms such as cytochrome P450 (CYP)-dependent enzymes that metabolize chemicals and endogenous substances. In this system, CYP1A1/2 have an important role in NADPH-dependent LP. Therefore, it is important to evaluate the effects of synthesized compounds on NADPH-dependent LP and CYP systems.[4] On the other hand, DPPH assay is recommended as an accurate method for measuring the antioxidant capacity of various compounds.[5]

Previously, we have reported the synthesis, characterization, and antioxidant properties of some benzimidazole derivatives containing thiadiazole, triazole, oxadiazole, and thiazolidinone rings at the first position.[6–14] In the present study, the design and synthesis of some novel benzimidazole derivatives having an oxadiazole ring

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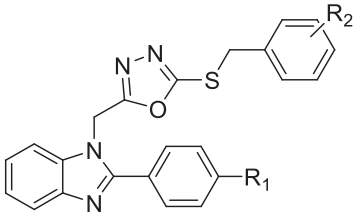
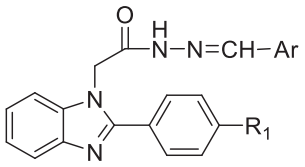
Table 1. Formula of compounds **5**–**22**.



5-12



13-22

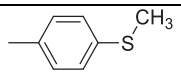
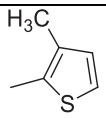
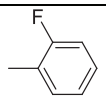
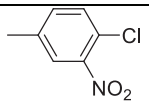
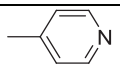
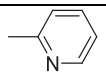
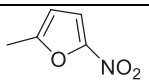
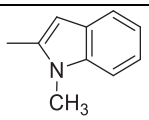
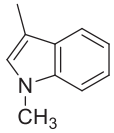
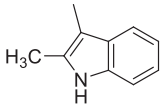
Compound	R_1	R_2	Ar
5	Cl	H	-
6	Cl	4-Br	-
7	Cl	2,4-diCl	-
8	Cl	4-F	-
9	Cl	4-NO_2	-
10	OCH_2Ph	4-Br	-
11	OCH_2Ph	2,4-diCl	-
12	OCH_2Ph	4-NO_2	-
13	Cl	-	
14	Cl	-	
15	Cl	-	
16	Cl	-	
17	Cl	-	
18	Cl	-	
19	Cl	-	
20	Cl	-	
21	Cl	-	
22	Cl	-	

Table 2. In vitro effects of compounds **4a**, **4b**, and **5**–**22** on liver LP levels, EROD enzyme, and DPPH free radical scavenging capacities*.

Compound**	DPPH (% inh.)	EROD (pmol/mg/min)	% inh.	LP (nmol/mg/min)	% inh.
4a[10]	88	25.05 ± 1.80 [10]	40	16.57 ± 0.07 [10]	NA
4b	88	1.10 ± 0.25	97	3.60 ± 0.36	78
5	5	2.18 ± 0.68	95	8.92 ± 0.44	46
6	3	6.35 ± 1.51	85	8.42 ± 0.78	49
7	5	8.70 ± 0.70	79	6.68 ± 0.44	59
8	NA	2.60 ± 0.16	94	6.76 ± 0.69	58
9	6	2.59 ± 0.41	94	6.32 ± 0.71	61
10	33	9.59 ± 1.46	77	7.45 ± 0.42	54
11	24	5.34 ± 0.72	87	8.10 ± 0.42	50
12	76	9.61 ± 2.30	77	7.51 ± 0.18	54
13	NA	6.07 ± 0.41	85	6.21 ± 0.92	62
14	6	4.01 ± 1.23	90	8.03 ± 0.33	51
15	5	3.29 ± 0.64	92	5.16 ± 0.35	68
16	13	2.29 ± 0.07	95	17.11 ± 3.14	NA
17	12	1.23 ± 0.22	97	5.95 ± 0.78	63
18	17	3.21 ± 0.48	92	0	100
19	20	1.37 ± 0.20	97	18.77 ± 4.25	NA
20	20	4.30 ± 0.58	90	18.09 ± 2.27	NA
21	35	3.44 ± 0.74	92	18.32 ± 2.09	NA
22	57	4.88 ± 0.22	88	9.33 ± 0.98	43
BHT	85			5.68 ± 022	65
Caffeine		6.41 ± 0.36	85		
Control (DMSO)		41.53 ± 0.99	-	16.25 ± 1.45	-

*Each value represents mean ± SD of 2–4 independent experiments. **Concentration in incubation medium (10^{-3} M). NA: No activity

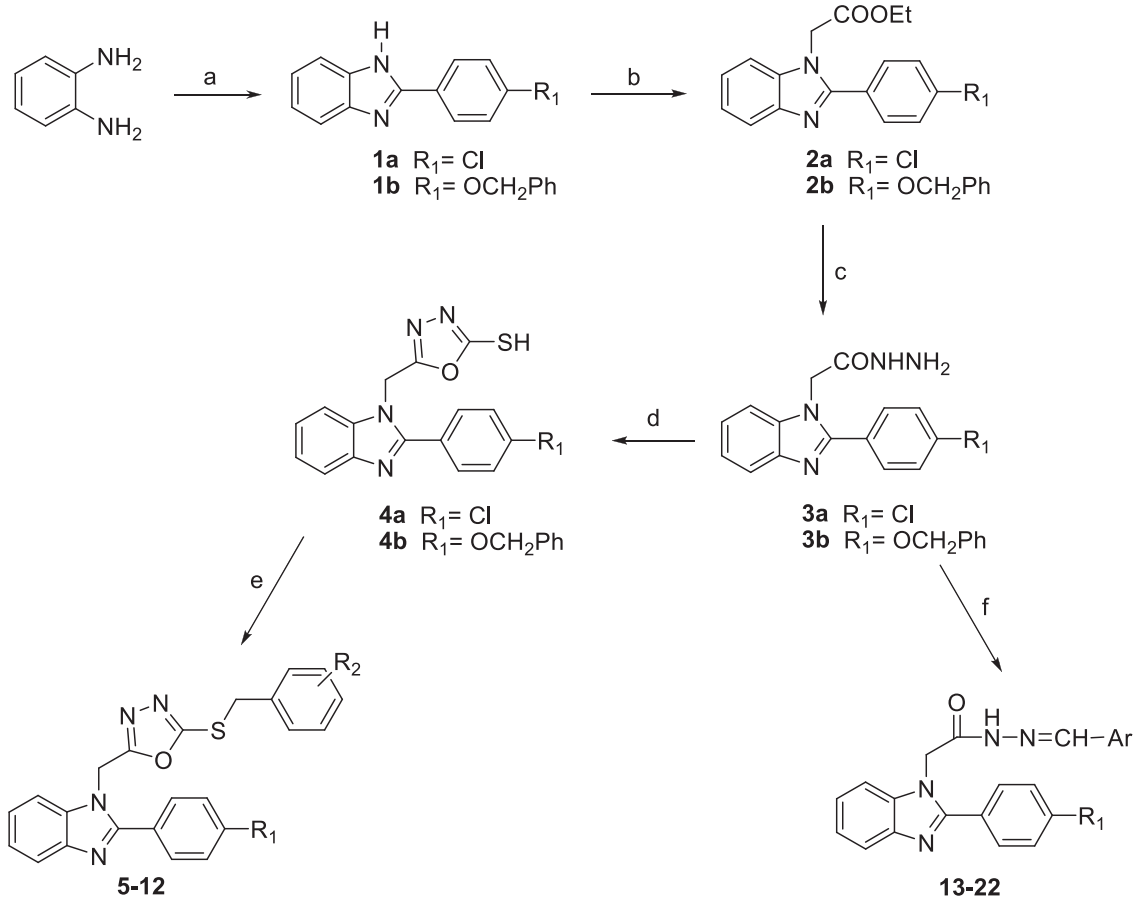
(**5**–**12**) and arylmethyleneamino acetamide (**13**–**22**) (Table 1) were performed and their antioxidant properties were investigated (Table 2).

2. Results and discussion

2.1. Chemistry

The desired benzimidazole derivatives were synthesized according to the Scheme. Firstly, 2-(4-chlorophenyl)-1*H*-benzo[d]imidazole (**1a**) and 2-(4-(benzyloxy)phenyl)-1*H*-benzo[d]imidazole (**1b**) were prepared via oxidative condensation of *o*-phenylenediamine, the corresponding aldehyde (*p*-chloro benzaldehyde or 4-benzyloxy benzaldehyde, respectively), and sodium metabisulfite.[10,12] Treatment of **1a** (or **1b**) with ethyl chloroacetate in KOH/DMSO gave the *N*-alkylated products ethyl 2-(2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)acetate (**2a**) or ethyl 2-(2-(4-(benzyloxy) phenyl)-1*H*-benzo[d]imidazol-1-yl)acetate (**2b**).[10,12] Hydrazine hydrate and the ester (**2a** or **2b**) in ethanol were refluxed for 4 h to give the desired hydrazide compounds, 2-(2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)acetohydrazide (**3a**) or 2-(2-(4-(benzyloxy) phenyl)-1*H*-benzo[d]imidazol-1-yl)acetohydrazide (**3b**).[12,15] Then 5-((2-(4-chloro phenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol

(**4a**)[10] and 5-((2-(4-(benzyloxy)phenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (**4b**) were synthesized by the reaction of the hydrazide compounds **3a** and **3b**, respectively, with carbon disulfide/KOH in ethanol.[16] Thiol compounds **4a** and **4b** were alkylated with related benzylbromide in the presence of potassium hydroxide to obtain 2-(substitutedbenzylthio)-5-((2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazole derivatives **5**–**9** and 2-(substitutedbenzylthio)-5-((2-(4-(benzyloxy) phenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazole derivatives **10**–**12**. Moreover, compounds **13**–**22** were obtained by condensing acyl hydrazide **3a** with the corresponding aromatic aldehyde derivatives in the presence of catalytic amounts of ceric ammonium nitrate (CAN) in ethanol (Scheme).[14] The chemical structures of the synthesized compounds were consistent with their mass and ^{1}H and ^{13}C NMR spectra. The spectral data are summarized in the Experimental section.



Scheme. Synthetic route to the compounds **5**–**22**. Reagents: (a) $Na_2S_2O_3$ Adduct of 4-chlorobenzaldehyde or 4-(benzyloxy)benzaldehyde/DMF; (b) Ethyl chloroacetate/KOH; (c) Hydrazine/EtOH; (d) CS_2/KOH; (e) Related benzylbromide/KOH; (f) Corresponding aromatic aldehyde/CAN/EtOH.

^{1}H NMR and ^{13}C NMR spectra were measured in chloroform-d for compounds **14** and **17** and in dimethyl sulfoxide-d_6 for the other imine-containing compounds (**13**, **15**, **16**, **18**–**22**) at ambient temperature. *N*-acylhydrazones can exist as 4 isomers due to the geometric isomerism with respect to the imino group (*E*, *Z* isomers) and conformers about the amide linkage (*syn/anti* amide conformers).[17] In the ^{1}H NMR spectra measured in the less polar solvent chloroform-d, distinguished proton signals belonging to aromatic hydrogens

were observed. Imino hydrogen (CH=N), methylene protons (CH_2CO), and amide hydrogen (NH) displayed 1 singlet signal about the single isomer in chloroform-d. However, in polar solvent dimethyl sulfoxide-d_6, 2 singlet signals expressing the imino hydrogen (CH=N) were observed at 8.20–8.79 and 8.01–8.62 ppm; the *syn/anti* methylene protons (CH_2CO) were also observed typically as 2 singlet signals at 5.02–5.15 and 5.32–5.64 ppm, respectively, for compounds **13**–**17** and **19**–**22** favoring 1 geometric isomer (*E* or *Z*). For compound **18**, 4 singlet signals were observed at 12.01, 12.19, 14.34, and 14.68 ppm for the NH proton; at 8.61, 8.62, 8.78, and 8.79 ppm for the CH=N proton; and at 5.10, 5.32, 5.57, and 5.58 for $-CH_2$ protons belonging to *syn* and *anti* conformers about both of the *E* and *Z* isomers.

For establishing the solvent effect in isomerism of the *N*-acylhydrazones, ^{1}H-NMR spectra for compound **14** were determined in 2 different (less polar and polar) solvents. According to the results, methylene, CH=N, and NH protons displayed 1 singlet signal belonging to 1 isomer at 5.34, 7.88, and 10.8 ppm in chloroform-d, while it was observed as 2 singlet signals belonging to *anti* and *syn* conformers at 5.02 and 5.40, 8.29 and 8.48, and 11.64 and 11.83 ppm in dimethyl sulfoxide-d_6, respectively.

The upfield lines of methylene protons have been assigned to *syn* amide conformers and the downfield lines of methylene protons to *anti* amide conformers.[17,18] The intensities of the ^{1}H NMR signals of methylene protons have allowed us to make measurements of the ratio of amide *syn/anti* conformers. *Syn* amide conformers were predominant over *anti* conformers when dissolved in dimethyl sulfoxide-d_6 at the ratio of 6/4 for compounds **13**, **14**, **17**, and **19**; at the ratio of 7/3 for compounds **15**, **16**, and **20**–**22**; and at the ratio of 6.5/3.5 for compound **18**.

2.2. In vitro antioxidant activity

The synthesized compounds were evaluated based on their antioxidant effects on the rat liver microsomal NADPH-dependent lipid peroxidation (LP) levels by measuring the formation of 2-thiobarbituric acid reactive substances (TBARS) (Table 2). The in vitro inhibitory effect of intermediate thiol compound **4b** bearing benzyloxyphenyl at the second position of the benzimidazole ring on LP levels was stronger (78%) than that of the corresponding *S*-substituted 1,3,4-oxadiazole derivatives. On the other hand, compound **4a** bearing chloro substituent showed no activity on LP levels.[10] 5-Mercapto-*S*-substituted 1,3,4-oxadiazole derivatives (**5**–**12**) had moderate inhibitory activity on LP levels in the range of 46%–61%, whereas imine containing compounds (**13**–**22**) exhibited diverse levels of activity. Compounds **15** (68%) and **18** (100%) displayed the highest activity among all of the synthesized compounds. The most active compound, **18**, led to 100% inhibition on LP level, while butylated hydroxy toluene (BHT) showed 65% inhibition at the same concentration.

The compounds were tested for their in vitro effects on liver microsomal EROD activity. The inhibitory effects of intermediate thiol compound **4b** on EROD activity were more powerful (97%) than those of the corresponding 5-mercapto-*S*-substituted 1,3,4-oxadiazole derivatives **10**–**12**, while compound **4a** displayed the lowest inhibitory activity (40%) on EROD[10] among all of the compounds. All the final 1,3,4-oxadiazole derivatives caused significant inhibition (77%–95%) of EROD activity. Typically, imine-containing compounds (**13**–**22**) showed better EROD inhibitory effects than 1,3,4-oxadiazoles (**5**–**12**). All the compounds except **7** (79%), **10** (77%), and **12** (77%) inhibited microsomal EROD activity better than (87%–97%) standard caffeine (85%), while compounds **6** and **13** possessed the same inhibitory effect as caffeine (85%) (Table 2).

The compounds' interaction with the stable free radical DPPH was also examined. It was observed that the final compounds did not show a significant inhibition in the DPPH scavenging assay, except compound

12, which exhibited the highest scavenger capacity on DPPH radical with 76%, which is close to that of BHT (85%). Furthermore, the DPPH radical scavenger capacities of the intermediate thiol compounds **4a** and **4b** were the same (88%) at 10^{-3} M concentration (Table 2). Compound **4b** (81%) was more active than compound **4a** (71%) at 10^{-4} M concentration, with IC_{50} values of 0.8×10^{-4} M and 0.65×10^{-4} M, respectively.

These findings indicate that some of the synthesized compounds possess beneficial effects on the human antioxidant defense system and have been suggested to act as antioxidants. Therefore, they could be thought as promising treatment candidate compounds for diseases related to excess of some reactive species.

3. Conclusion

The synthesized compounds had different effects on the systems examined. Compound **18**, which bears 2-pyridinyl, showed the best inhibitory activity on liver microsomal LP levels (100%) and EROD (92%). Intermediate thiol compound **4b** and final imine compounds **17** and **19**, bearing 4-pyridinyl and 5-nitrofuran-2-yl substituents, respectively, had the most powerful inhibitory effect (97%) on EROD. Among all of the final compounds, compound **12** displayed the highest inhibition (76%) in the DPPH scavenging assay. The substitution of intermediate **4b** with benzylic groups led to a reduction in the antioxidant activity for all systems tested, while the substitution of intermediate **4a** decreased the DPPH scavenging activity at the same concentration.

4. Experimental section

4.1. General synthesis

All starting materials and chemical reagents used in the synthesis were high-grade commercial products purchased from Aldrich or Merck (Germany). BHT and caffeine were obtained from Sigma. Analytical thin-layer chromatography was performed with Merck precoated TLC plates, and spots were visualized with ultraviolet light. Column chromatography was accomplished on silica gel 60 (40–63-μm particle size) (Merck, Germany). Melting points were determined with an Electrothermal 9100 digital melting point apparatus and were uncorrected. The structures of all synthesized compounds were assigned on the basis of NMR and mass spectral analyses. ^{1}H NMR and ^{13}C NMR spectra were measured with a Varian Mercury 400 MHz instrument (Varian Inc., Palo Alto, CA, USA) using TMS internal standard and $CDCl_3$ or DMSO-d_6; coupling constants (J) were reported in Hertz. All chemical shifts were reported as δ (ppm) values. ES–MS were obtained with a Waters ZQ Micromass LC–MS spectrometer (Waters Corporation, Milford, MA, USA) with positive electrospray ionization. All instrumental analyses were performed at the Central Instrumentation Laboratory of the Pharmacy Faculty of Ankara University, Ankara, Turkey.

General procedure for the preparation of 4b

2-(2-(4-(Benzyloxy)phenyl)-1*H*-benzo[d]imidazol-1-yl)acetohydrazide (**3b**) (0.4 mmol) and CS_2 (31 mg, 0.4 mmol) were added to a solution of KOH (22.4 mg, 0.4 mmol) in 1 mL of H_2O and 1 mL of ethanol. The reaction mixture was refluxed for 3 h. The solid obtained after evaporation under reduced pressure was dissolved in water and acidified with conc. HCl. The precipitate was filtered, washed with water, and recrystallized from ethanol.[10]

5-((2-(4-(Benzyloxy)phenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (4b)

White solid (yield 77%), mp 218–220 °C. MS (ESI+) M+H (%): 415 (100). ^{1}H NMR δ ppm (DMSO-d_6): 7.72 (d, 2H, J = 8.8 Hz), 7.63–7.70 (m, 2H), 7.46 (d, 2H, J = 6.8 Hz), 7.39 (t, 2H, J = 7.6 Hz), 7.26–7.34 (m, 3H), 7.19 (d, 2H, J = 8.8 Hz), 5.67 (s, 2H, –O–CH_2), 5.19 (s, 2H, –N–CH_2). ^{13}C NMR δ ppm (DMSO-d_6): 178.6, 160.6, 159.8, 153.6, 142.2, 137.4, 136.1, 131.6, 129.2, 128.7, 128.5, 123.8, 123.6, 121.8, 119.4, 115.9, 111.6, 70.2.

General procedure for the preparation of 2-(substitutedbenzylthio)-5-((2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazoles (5–9) and 2-(substitutedbenzylthio)-5-((2-(4-(benzyloxy)phenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazoles (10–12)

To a mixture of the thiol compound **4a** (or **4b**) (0.15 mmol) in 0.1 mL of 1 N KOH and 1 mL of H_2O was added corresponding benzylbromide (0.15 mmol). The mixture was stirred overnight at room temperature. The end of the reaction was monitored by TLC. The solid separated was collected and dried. The crude product was purified by column chromatography eluting with an appropriate solvent system or by recrystallization from ethanol to give **5**–**12**.

2-(Benzylthio)-5-((2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazole (5)

Crude **5** was purified by column chromatography (n-hexane/ethyl acetate (3:1)) to provide **5** (yield 40%) as a white solid, mp 112–114 °C. MS (ESI+) M+H (%): 433 (100), 435 (37). ^{1}H NMR δ ppm ($CDCl_3$): 7.83–7.86 (m, 1H), 7.78 (d, 2H, J = 8.4 Hz), 7.54 (d, 2H, J = 8.8 Hz), 7.50–7.52 (m, 1H), 7.31–7.37 (m, 4H), 7.25–7.28 (m, 3H), 5.49 (s, 2H, –N–CH_2), 4.42 (s, 2H, –S–CH_2). ^{13}C NMR δ ppm ($CDCl_3$): 165.9, 162.3, 152.5, 142.8, 136.8, 135.4, 134.9, 130.9, 129.4, 129.0, 128.8, 128.2, 127.6, 123.9, 123.5, 120.3, 110.1, 39.6, 36.9.

2-(4-Bromobenzylthio)-5-((2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-1,3,4-oxadiazole (6)

Crude **6** was recrystallized from ethanol to provide **6** (yield 44%) as a white solid, mp 159 °C. MS (ESI+) M+H (%): 511 (66), 513 (100), 515 (31). ^{1}H NMR δ ppm ($CDCl_3$): 7.82–7.85 (m, 1H), 7.78 (d, 2H, J = 8.4 Hz), 7.54 (d, 2H, J = 8.8 Hz), 7.48–7.51 (m, 1H), 7.39 (d, 2H, J = 8.0 Hz), 7.34–7.37 (m, 2H), 7.21 (d, 2H, J = 8.4 Hz), 5.49 (s, 2H, –N–CH_2), 4.35 (s, 2H, –S–CH_2). ^{13}C NMR δ ppm ($CDCl_3$): 165.5, 162.4, 152.5, 142.9, 136.8, 135.4, 134.2, 131.9, 130.9, 130.7, 129.4, 127.6, 123.9, 123.6, 122.3, 120.3, 110.1, 39.5, 36.1.

2-((2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-5-(2,4-dichlorobenzylthio)-1,3,4-oxadiazole (7)

Crude **7** was recrystallized from ethanol to provide **7** (yield 45%) as a white solid, mp 166–168 °C. MS (ESI+) M+H (%): 501 (95), 503 (100), 505 (40), 507 (10). ^{1}H NMR δ ppm ($CDCl_3$): 7.82–7.85 (m, 1H), 7.78 (d, 2H, J = 8.8 Hz), 7.54 (d, 2H, J = 8.8 Hz), 7.48–7.51 (m, 1H), 7.43 (d, 1H, J = 8.4 Hz), 7.32–7.37 (m, 3H), 7.12 (dd, 1H, J = 8.4 Hz, J = 2.0 Hz), 5.49 (s, 2H, –N–CH_2), 4.47 (s, 2H, –S–CH_2). ^{13}C NMR δ ppm ($CDCl_3$): 165.6, 162.5, 152.5, 142.9, 136.8, 135.4, 134.9, 132.2, 131.8, 130.9, 129.6, 129.4, 127.6, 127.4, 123.9, 123.5, 120.3, 110.1, 39.5, 33.9.

2-((2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-5-(4-fluorobenzylthio)-1,3,4-oxadiazole (8)

Crude **8** was recrystallized from ethanol to provide **8** (yield 38%) as a white solid, mp 127–128 °C. MS (ESI+) M+H (%): 451 (100), 453 (47). ^{1}H NMR δ ppm ($CDCl_3$): 7.83–7.86 (m, 1H), 7.79 (d, 2H, J = 8.8 Hz), 7.55 (d, 2H, J = 8.8 Hz), 7.50–7.52 (m, 1H), 7.30–7.39 (m, 4H), 6.96 (t, 2H, J = 8.8 Hz), 5.51 (s, 2H, –N–CH_2), 4.40 (s, 2H, –S–CH_2). ^{13}C NMR δ ppm ($CDCl_3$): 165.7, 163.7, 162.3, 152.5, 142.9, 136.8, 135.4, 130.9, 130.8, 129.4, 127.6, 123.9, 123.5, 120.4, 115.9, 115.7, 110.1, 39.6, 36.1.

2-((2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-5-(4-nitrobenzylthio)-1,3,4-oxadiazole (9)

Crude **9** was recrystallized from ethanol to provide **9** (yield 40%) as a white solid, mp 154–155 °C. MS (ESI+) M+H (%): 478 (100), 480 (43). ^{1}H NMR δ ppm ($CDCl_3$): 8.12 (d, 2H, J = 9.2 Hz), 7.82–7.85 (m, 1H), 7.78 (d, 2H, J = 8.4 Hz), 7.54 (d, 4H, J = 8.8 Hz), 7.47–7.50 (m, 1H), 7.33–7.37 (m, 2H), 5.51 (s, 2H, –N–CH_2), 4.47 (s, 2H, –S–CH_2). ^{13}C NMR δ ppm ($CDCl_3$): 164.9, 162.6, 152.5, 147.6, 142.9, 142.8, 136.8, 135.4, 130.9, 129.9, 129.4, 127.6, 123.9, 123.6, 120.4, 109.9, 39.5, 35.6.

2-((2-(4-(Benzyloxy)phenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-5-(4-bromobenzylthio)-1,3,4-oxadiazole (10)

Crude **10** was recrystallized from ethanol to provide **10** (yield 53%) as a white solid, mp 119–121 °C. MS (ESI+) M+H (%): 583 (100), 585 (97). ^{1}H NMR δ ppm ($CDCl_3$): 7.82 (d, 1H, J = 7.2 Hz), 7.76 (d, 2H, J = 8.0 Hz), 7.31–7.46 (m, 10H), 7.20 (d, 2H, J = 7.6 Hz), 7.14 (d, 2H, J = 8.4 Hz), 5.51 (s, 2H, –O–CH_2), 5.15 (s, 2H, –N–CH_2), 4.34 (s, 2H, –S–CH_2). ^{13}C NMR δ ppm ($CDCl_3$): 165.6, 162.9, 160.6, 153.9, 143.2, 136.6, 135.6, 134.5, 132.2, 131.3, 130.9, 128.9, 128.4, 127.7, 123.7, 123.5, 122.5, 121.8, 120.3, 115.7, 110.2, 70.4, 39.9, 36.3.

2-((2-(4-(Benzyloxy)phenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-5-(2,4-dichlorobenzylthio)-1,3,4-oxadiazole (11)

Crude **11** was purified by column chromatography (n-hexane/ethyl acetate (2:1)) to provide **11** (yield 36%) as a white solid, mp 124–125 °C. MS (ESI+) M+H (%): 573 (100), 575 (91), 577 (20). ^{1}H NMR δ ppm ($CDCl_3$): 7.81 (d, 1H, J = 6.8 Hz), 7.76 (d, 2H, J = 8.8 Hz), 7.28–7.48 (m, 10H), 7.14 (d, 2H, J = 9.2 Hz), 7.11 (dd, 1H, J = 8.0 Hz, J = 2.0 Hz), 5.51 (s, 2H, –O–CH_2), 5.15 (s, 2H, –N–CH_2), 4.46 (s, 2H, –S–CH_2). ^{13}C NMR δ ppm ($CDCl_3$): 165.5, 162.8, 160.4, 153.7, 142.9, 136.4, 135.4, 134.9, 134.9, 132.2, 131.9, 131.1, 129.6, 128.7, 128.2, 127.5, 127.4, 123.4, 123.3, 121.6, 120.1, 115.4, 109.9, 70.1, 39.6, 33.9.

2-((2-(4-(Benzyloxy)phenyl)-1*H*-benzo[d]imidazol-1-yl)methyl)-5-(4-nitrobenzylthio)-1,3,4-oxadiazole (12)

Crude **12** was purified by column chromatography (n-hexane/ethyl acetate (2:1)) to provide **12** (yield 37%) as a yellowish solid, mp 69–71 °C. MS (ESI+) M+H (%): 550 (100). ^{1}H NMR δ ppm ($CDCl_3$): 8.11 (d, 2H, J = 8.4 Hz), 7.82 (d, 1H, J = 7.2 Hz), 7.76 (d, 2H, J = 8.8 Hz), 7.52 (d, 2H, J = 8.4 Hz), 7.28–7.47 (m, 8H), 7.14 (d, 2H, J = 8.8 Hz), 5.52 (s, 2H, –O–CH_2), 5.15 (s, 2H, –N–CH_2), 4.45 (s, 2H, –S–CH_2). ^{13}C NMR

δ ppm ($CDCl_3$): 164.8, 162.9, 160.4, 153.6, 147.6, 142.9, 142.8, 136.3, 135.4, 131.1, 129.9, 128.7, 128.2, 127.5, 123.9, 123.5, 123.3, 121.6, 120.1, 115.5, 109.9, 70.2, 39.6, 35.6.

General procedure for the preparation of 2-(2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-(arylmethylene)acetohydrazide derivatives (13–22)

A mixture of acyl hydrazide **3a** (0.02 mol), related aromatic aldehyde derivative (0.02 mol), and ceric ammonium nitrate (0.05 mol) in ethanol (10 mL) was heated under reflux with stirring for 30 min. Water was added, and the precipitated product was filtered and crystallized from ethanol.[14]

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-(4-methylthio)benzylidene) acetohydrazide (13)

White solid (yield 93%), mp 247–249 °C. MS (ESI+) M+H (%): 435 (100), 437 (68). ^{1}H NMR δ ppm (DMSO-d_6): 11.78, 11.93 (2s, 1H, NH), 8.01, 8.20 (2s, 1H, –N=CH), 7.27–7.85 (m, 12H, Ar–H), 5.06, 5.53 (2s, 2H, $-CH_2$), 2.50 (s, 3H, $-CH_3$).

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-((3-methylthiophen-2-yl) methylene) acetohydrazide (14)

White solid (yield 74%), mp 200–202 °C. MS (ESI+) M+H (%): 409 (100), 411 (37). ^{1}H NMR δ ppm ($CDCl_3$): 10.8 (s, 1H, NH), 7.88 (s, 1H), 7.83 (d, 1H, J = 8.4 Hz), 7.73 (d, 2H, J = 8.0 Hz), 7.45 (d, 2H, J = 8.0 Hz), 7.25–7.31 (m, 4H), 6.81 (d, 1H, J = 5.2 Hz), 5.34 (s, 2H, $-CH_2$), 2.11 (s, 3H, CH_3).

^{1}H NMR δ ppm (DMSO-d_6): 11.64, 11.83 (2s, 1H, NH), 8.29, 8.48 (2s, 1H, –N=CH), 7.52–7.49 (m, 7H, Ar–H), 7.32–7.26 (m, 2H, Ar–H), 6.97 (d, 1H, Ar–H, J = 5.2 Hz), 5.02, 5.40 (2s, 2H, $-CH_2$), 2.31 (s, 3H, CH_3).

^{13}C NMR δ ppm ($CDCl_3$): 169.1, 153.3, 142.7, 141.3, 140.2, 136.3, 136.3, 131.2, 130.9, 130.7, 129.2, 128.4, 127.9, 123.4, 122.9, 119.9, 109.7, 45.6, 13.9.

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-(2-fluorobenzylidene) acetohydrazide (15)

White solid (yield 96%), mp 226–228 °C. MS (ESI+) M+H (%): 407 (100), 409 (39). ^{1}H NMR δ ppm (DMSO-d_6): 11.94, 12.12 (2s, 1H, NH), 8.28, 8.48 (2s, 1H, –N=CH), 7.47–7.99 (m, 8H, Ar–H), 7.27–7.33 (m, 4H, Ar–H), 5.09, 5.57 (2s, 2H, $-CH_2$).

***N*′-(4-Chloro-3-nitrobenzylidene)-2-(2-(4-chlorophenyl)-1*H*-benzo[d]imidazol-1-yl) acetohydrazide (16)**

White solid (yield 67%), mp 217 °C. (ESI+) M+H (%): 468 (100), 470 (66), 472 (12). ^{1}H NMR δ ppm (DMSO-d_6): 12.12, 12.29 (2s, 1H, NH), 8.33, 8.39, 8.45 (s, 2d, 1H, –N=CH), 8.12 (s, 1H, Ar–H), 8.02–8.06 (m, 1H, Ar–H), 7.59–7.86 (m, 7H, Ar–H), 7.30–7.36 (m, 2H, Ar–H), 5.15, 5.64 (2s, 2H, $-CH_2$).

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-(pyridin-4-yl-methylene) acetohydrazide (17)

White solid (yield 67%), mp 76–79 °C. MS (ESI+) M+H (%): 390 (100), 392 (35). ^{1}H NMR δ ppm ($CDCl_3$): 10.93 (s, 1H, NH), 8.65 (br s, 2H), 7.84 (d, 1H, J = 4.8 Hz), 7.72 (s, 1H), 7.70 (d, 2H, J = 9.2 Hz), 7.46 (d, 2H, J = 8.4 Hz), 7.41 (d, 2H, J = 3.6 Hz), 7.31–7.34 (m, 3H, Ar–H), 5.38 (s, 2H, –CH_2).

^{1}H NMR δ ppm (DMSO-d_6): 12.08, 12.23 (2s, 1H, NH), 8.66 (br s, 2H), 8.03, 8.25 (2s, 1H, –N=CH), 7.59–7.83 (m, 8H, Ar–H), 7.27–7.31 (m, 2H, Ar–H), 5.10, 5.59 (2s, 2H, –CH_2).

^{13}C NMR δ ppm ($CDCl_3$): 169.1, 153.2, 150.4, 143.5, 142.7, 140.2, 136.5, 136.3, 130.6, 129.3, 128.2, 123.5, 123.1, 121.1, 120.1, 109.6, 45.95.

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-(pyridin-2-yl-methylene) acetohydrazide (18)

White solid (yield 34%), mp 201–204 °C. MS (ESI+) M+H (%): 390 (100), 392 (34). ^{1}H NMR δ ppm (DMSO-d_6): 12.01, 12.19, 14.34, 14.68 (4s, 1H, NH), 8.61, 8.62, 8.78, 8.79 (4s, 1H, –N=CH), 7.27–8.25 (m, 12H, Ar–H), 5.10, 5.32, 5.57, 5.58 (4s, 2H, –CH_2).

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-((5-nitrofuran-2-yl)methylene) acetohydrazide (19)

White solid (yield 62%), mp 242–245 °C. MS (ESI+) M+H (%): 424 (100), 426 (38). ^{1}H NMR δ ppm (DMSO-d_6): 12.21, 12.33 (2s, 1H, NH), 8.01, 8.21 (2s, 1H, –N=CH), 7.56–7.82 (m, 7H, Ar–H), 7.26–7.34 (m, 3H, Ar–H), 5.13, 5.51 (2s, 2H, –CH_2).

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-((1-methyl-1*H*-indol-2-yl) methylene) acetohydrazide (20)

White solid (yield 82%), mp 268–269 °C. MS (ESI+) M+H (%): 442 (100), 444 (33). ^{1}H NMR δ ppm (DMSO-d_6): 11.51, 11.66 (2s, 1H, NH), 8.23, 8.39 (2s, 1H, –N=CH), 8.13–8.19 (m, 1H, Ar–H), 7.49–7.92 (m, 8H, Ar–H), 7.23–7.32 (m, 3H, Ar–H), 7.12–7.16 (m, 1H, Ar–H), 5.04, 5.55 (2s, 2H, –CH_2), 3.83 (s, 3H, –CH_3).

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-((1-methyl-1*H*-indol-3-yl) methylene) acetohydrazide (21)

White solid (yield 69%), mp 235 °C. MS (ESI+) M+H (%): 442 (100), 444 (35). ^{1}H NMR δ ppm (DMSO-d_6): 11.79, 11.97 (2s, 1H, NH), 8.19, 8.38 (2s, 1H, –N=CH), 7.50–7.88 (m, 8H, Ar–H), 7.23–7.31 (m, 3H, Ar–H), 7.08 (t, 1H, Ar–H, J = 7.6 Hz), 6.94 (d, 1H, Ar–H, J = 9.6 Hz), 5.08, 5.54 (2s, 2H, –CH_2), 4.01 (s, 3H, –CH_3).

2-(2-(4-Chlorophenyl)-1*H*-benzo[d]imidazol-1-yl)-*N*′-((2-methyl-1*H*-indol-3-yl) methylene) acetohydrazide (22)

White solid (yield 88%), mp 299 °C. MS (ESI+) M+H (%): 442 (100), 444 (48). ^{1}H NMR δ ppm (DMSO-d_6): 11.39, 11.52, 11.60 (3s, 2H, NH, indole NH), 8.34, 8.47 (2s, 1H, –N=CH), 7.56–8.12 (m, 7H, Ar–H), 7.27–7.34 (m, 3H, Ar–H), 7.02–7.12 (m, 2H, Ar–H), 5.03, 5.54 (2s, 2H, –CH_2), 2.50 (s, 3H, –CH_3).

4.2. In vitro antioxidant activity

4.2.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging activities of the compounds were tested based on their ability to bleach the stable radical 2,2,diphenyl-1-picrylhydrazyl (DPPH). The stock solutions of the compounds and BHT were prepared at 10^{-2} M in DMSO. A series of stock solutions in DMSO were diluted to varying concentrations in 96-well microplates. Methanolic DPPH solution (100 μM) was then added to each well. The plate was shaken and placed in darkness. After 30 min, the optical density of the solution was read at the wavelength 517 nm. The methanolic solution of DPPH served as a control. Percentage inhibition was calculated using the following formula:

Radical scavenging activity % = $[(A_{Control} - A_{Sample})/A_{Control}] \times 100$

($A_{control}$: absorption of blank sample; A_{sample}: absorption of tested solution)

All tests and analyses were run in triplicate and averaged. The standard used in this assay was BHT.[19]

4.2.2. 7-Ethoxyresorufin O-deethylase (EROD) assay

7-Ethoxyresorufin O-deethylase (EROD) enzyme activity was determined by the spectrofluorometric method described by Burke et al.[20] A typical optimized assay mixture contained 1.0 mM ethoxyresorufin; 10^{-3} M test compound; 100 mM Tris–HCl buffer pH 7.8; a 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 of liver microsomal protein in a final volume of 1.0 mL. The standard used in this assay was caffeine. DMSO was used as a control.

4.2.3. Lipid peroxidation (LP) assay

Male albino Wistar rats (200–225 g) were used in the experiments. The animals were fed with standard laboratory rat chow and tap water ad libitum. The animals were starved for 24 h prior to sacrifice and were killed by decapitation under anesthesia. The livers were removed immediately and washed in ice-cold water, and the microsomes were prepared as defined previously.[21] NADPH-dependent LP was determined using the optimum conditions as determined and described previously[21] and measured spectrophotometrically by estimation of thiobarbituric acid-reactive substances (TBARS). Amounts of TBARS were expressed in terms of nmol malondialdehyde (MDA)/mg protein. The assay was essentially obtained from the methods of Wills[22,23] as modified by Bishayee et al.[24] LP was measured spectrophotometrically at 532 nm as the thiobarbituric acid-reactive material. Compounds inhibit the production of MDA, and the color produced after addition of thiobarbituric acid is less intensive. A typical optimized assay mixture contained 10^{-3} M test compound; 0.2 nM Fe^{++}; 90 mM KCl; 62.5 mM potassium phosphate buffer, pH 7.4; a NADPH-generating system comprising 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 of microsomal protein in a final volume of 1.0 mL. The standard used in this assay was BHT. DMSO was used as a control.

Acknowledgment

The Central Instrumental Analysis Lab. at the Faculty of Pharmacy, Ankara University, provided the support for acquisition of the NMR and mass data used in this work.

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