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Synthesis and antioxidant properties of new benzimidazole derivatives

Yeni benzimidazol türevlerinin sentezi ve antioksidan özellikleri

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Synthesis and Antioxidant Properties of New Benzimidazole Derivatives

Highlights

- ❖ Synthesized compounds were evaluated for their *in vitro* antioxidant activity.
- ❖ The structures of synthesized compounds were established on the basis of spectral data.
- ❖ Enzyme activity was calculated by spectrofluorimetric measurement of the amount of resorufin formed.
- ❖ The reducing power activities of the compounds were compared with BHT.
- ❖ Values were the means of three replicates \pm Standard deviation.

Graphical Abstract

This study describes the synthesis and antioxidant properties of a novel benzimidazoles, benzimidazole-1-yl-acetic acid ethyl ester, benzimidazol-1-yl-acetic acid hydrazide and benzimidazol-1-yl-1,3,4-oxadiazole derivatives.

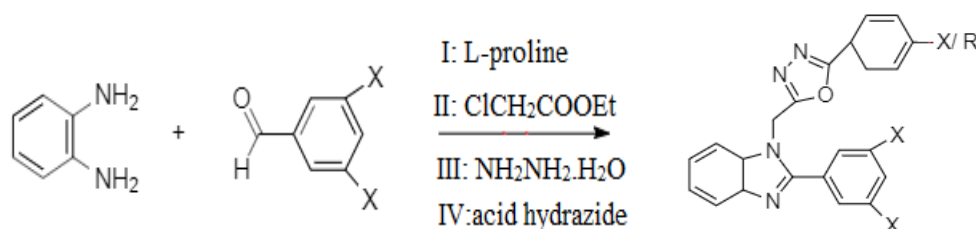


Figure. Synthetic route for the preparation of compounds

Aim

The aim of this study is to investigate the synthesis, structure and antioxidant properties of the benzimidazole compounds carrying 1,3,4-oxadiazole ring.

Design & Methodology

A series of benzimidazole derivatives were synthesized and antioxidant activities were investigated.

Originality

New benzimidazole derivatives were synthesized and their antioxidant properties were investigated.

Findings

Of all the synthesized compounds, the most active of all three activities is compound 2a.

Conclusion

The compounds were generally moderately active in terms of DPPH radical scavenging activity and lipid peroxidation inhibition compared to the standard compound and had a good effect on EROD enzyme activity.

Declaration of Ethical Standards

The author of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Synthesis and Antioxidant Properties of New Benzimidazole Derivatives

Araştırma Makalesi / Research Article

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ABSTRACT

In this study, new benzimidazole derivatives were synthesized. After purity checks were made with Thin Layer Chromatography (TLC) and melting points, their structures were proved by mass spectroscopy, ¹H, ¹³C NMR and elemental analysis technique. Antioxidant activities of the obtained materials were searched in terms of their effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, lipid peroxidation inhibition and ethoxyresorufin *O*-deethylase (EROD) enzyme activity. It was observed the compounds were generally moderately active in terms of DPPH radical scavenging activity and lipid peroxidation inhibition compared to the standard compound and had a good effect on EROD enzyme activity.

Keywords: DPPH, EROD enzyme activity, 1,3,4-oxadiazole.

Yeni Benzimidazol Türevlerinin Sentezi ve Antioksidan Özellikleri

ÖZ

Bu çalışmada yeni benzimidazol türevleri sentezlenmiştir. İnce Tabaka Kromatografisi (TLC) ve erime noktaları ile saflık kontrolleri yapıldıktan sonra yapıları kütle spektroskopisi, ¹H, ¹³C NMR ve element analizi teknikleri ile kanıtlanmıştır. Elde edilen bileşiklerin antioksidan aktiviteleri, 2,2-difenil-1-pikrilhidrazil (DPPH) radikal temizleme aktivitesi, lipid peroksidasyon inhibisyonu ve etoksresorufin *O*-deetilaz (EROD) enzim aktivitesi üzerindeki etkileri açısından araştırılmıştır. Bileşikler, standart bileşik ile karşılaştırıldığında DPPH radikal temizleme aktivitesi ve lipid peroksidasyon inhibisyonu açısından genellikle orta derecede ve EROD enzim aktivitesi üzerinde iyi bir etkiye sahip olduğu gözlenmiştir.

Anahtar Kelimeler: DPPH, EROD enzim aktivitesi, 1,3,4-oksadiazol.

1. INTRODUCTION

Free radicals are unstable oxygen molecules that can attack, destroy, or mutate normal cells. Cancer, heart disease and neurodegenerative diseases are the leading damages of free radicals in the body. Although our body has a system that recognizes and neutralizes free radicals, in some cases the balance between free radicals and the antioxidant defense system is disrupted. In this case, this balance is maintained by using antioxidant compounds. Highly reactive and highly destructive molecular free radicals have become very important in recent years due to their role in human health and diseases. The human body also has its own defense systems against damage that can be caused by oxidative reactions and is quite common. These antioxidant mechanisms protect cells against different forms of reactive oxygen species (ROS) [1]. However, these defense mechanisms in the living organism are inadequate in some cases and antioxidant intake. It is needed to protect our body against the damage of oxidative reactions. Because antioxidants sweep the ROS without damaging various biological molecules or prevent the spread of oxidative damage by

dividing the radical chain reaction of an oxidant system such as lipid peroxidation. Antioxidants sweep the ROS without damaging various biological molecules or prevent the spread of oxidative damage by dividing the radical chain reaction of an oxidant system such as lipid peroxidation.

The presence of highly active benzimidazoles as a drug active ingredient supports the tendency that new and more active species can also be synthesized. Therefore, studies on the synthesis of containing benzimidazole ring compounds and their biological activity are quite high. As a result of these studies, benzimidazole-derived compounds having biological activity in many areas, such as antimicrobial (antibacterial, antifungal, antihelmintic / antiparasitic, antiprotozoal, etc.) [2-5], antiviral (anti-HIV, anti-HBV and HCV (Hepatitis B and C, etc.) [6, 7], antiulcer [8, 9] anti-inflammatory/analgesic [10, 11], antihistaminic (antiallergic) [12, 13], antagonist [14], antitubercular [15], antidepressant [16], antidiabetic [17], antihypertensive [18], antioxidant [19-22] and antitumor/anticancer [23, 24], have been synthesized. Also, some benzimidazole derivatives have some optical applications such as photoluminescents [25], whitening agents [26] and dye laser [27].

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Benzimidazole-based compounds including oxadiazole, thiadiazole, triazole rings and Schiff base derivatives are also synthesized and studies are carried out on their activities. Studies have shown that these compounds also have a wide range of activity such as antibacterial, antitumor [28-31], antiviral [32], anticonvulsant [33], antidiabetic [34] and antidepressant [35]. Some examples of benzimidazole-centered compounds containing oxadiazole rings and their activities are shown in Figure 1[36].

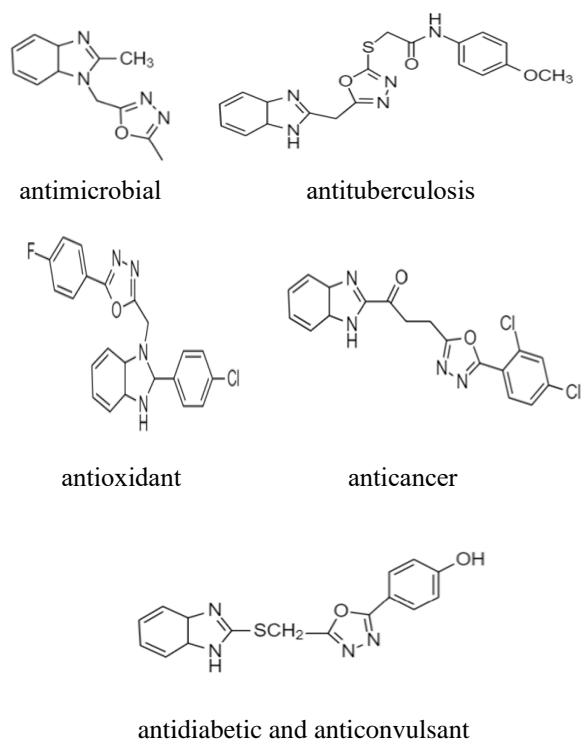


Figure 1. Some examples of benzimidazole-centered compounds and their activities

In addition, a series of thiosemicarbazide, thiadiazol-methylbenzimidazole and triazolmethylbenzimidazole compounds were synthesized and antioxidant properties, lipid peroxidation and EROD inhibition levels were investigated [37]. Lipid peroxidation inhibitory effects of thiosemicarbazide derivatives were found in the 80-100% range. In addition, thiosemicarbazide thiadiazol methyl benzimidazole derivatives inhibit EROD enzyme activity in the range of 98-100%, which is a better result than inhibition of caffeine (85%). Thiosemicarbazide, thiadiazole derivatives carrying m-chloro/bromo substituent in the aryl ring were found to be the most active compounds.

According to a study conducted in 2008, N-methyl-thiosemicarbazide compounds were synthesized and antioxidant properties, DPPH radical inhibition, superoxide anion inhibition ($O_2^{\cdot-}$), lipid peroxidation and EROD inhibition levels of these compounds were determined by defining [38]. IC_{50} values of bearing N-methyl-thiosemicarbazide derivatives showed good

activity in capturing DPPH radical in the range of (26-74 μM) compared to butylated hydroxytoluene (BHT) (54 μM); however, it has been reported that it does not show a good effect in capturing the superoxide radical.

The aim of this study is to investigate the synthesis, structure and antioxidant properties of the benzimidazole compounds carrying 1,3,4-oxadiazole ring (figure 2).

2. MATERIAL and METHOD

Chemistry

During the synthesis studies, Thin Layer Chromatography (TLC) was used to monitor the reaction and to check the purity of the obtained products. Silica Gel 60 F254 coated aluminum plates (Merck) used in the application and UV light at 254 nm wavelength was used to identify stains. The melting points of the synthesized compounds were determined with the Electrothermal 9100 instrument. 1H and ^{13}C NMR spectra of the compounds were taken by Varian Mercury 400 NMR spectrometer using dimethylsulfoxide- d_6 (DMSO- d_6) as the solvent. Mass spectra were taken on the Agilent LC / MS Spectrometer. Combustion analysis was performed on a Carlo Erba 1106 elemental analyzer.

synthesis of benzimidazole derivatives (1a, 1b)

A mixture of o-phenylenediamine (1mmole) and aldehydes (1 mmole) in water (5 ml) were mixed and refluxed on water bath in the presence of L-proline (10 mole %) and buffer tablet of pH 4.2 (0.1 g) for 5 h. The progress of the reaction was monitored by using TLC (petroleum ether: ethyl acetate, 8:2). Then the reaction mixture was cooled and filtered. Wash the residue with distilled water (5x5 ml) to recover the catalyst and dried it over vacuum. Recrystallize the crude product in ethanol to obtain the pure product. If essential crude product were purified by silica gel column chromatography using ethyl acetate: pet-ether (2:8) as an eluent [39].

2-(3,5-dichlorophenyl)-1H-benzimidazole (1a)

Yield, 91%, mp 185-187 °C; Proton Spectrum: Aromatic H; [7.10-7.14 (multiplet, 2H), 7.30-7.34 (multiplet, 2H), 7.73 (bold singlet, 1H), 7.89-7.97 (multiplet, 2H)]; Carbon Spectrum: ArC: [112.48 (CH), 115.51 (2CH), 115.72 (2CH), 120.23 (CH), 124.51 (C), 124.80 (C), 131.41 (CH), 132.56 (C), 133.88 (C), 144.22 (C), 157.32 (C=N)]; Liquid Chromatography-Mass Spectrometer (%): $[M]^+$ 263.12; Combustion Elemental Analysis Calculated (%) for $C_{13}H_8Cl_2N_2$: Carbon, 59.34; Hydrogen, 3.06; Nitrogen, 10.65. Found: Carbon, 59.28; Hydrogen, 3.11; Nitrogen, 10.71.

2-(3,5-dibromophenyl)-1H-benzimidazole (1b)

Yield, 94%; mp 231-233 °C; Proton Spectrum: Aromatic H; [7.11-7.21 (multiplet, 2H), 7.35-7.38 (multiplet, 3H), 7.40-7.45 (multiplet, 1H), 7.86-7.93 (multiplet, 1H)]; Carbon Spectrum: ArC: [114.00 (CH), 116.59 (2CH), 117.96 (2CH), 121.90 (CH), 124.30 (C), 125.15 (C), 133.24 (CH), 131.44 (C), 135.12 (C), 141.80 (C), 151.16 (C=N)]; Liquid Chromatography-Mass Spectrometer (%):

[M]⁺ 352.01; Combustion Elemental Analysis Calculated (%) for C₁₃H₈Br₂N₂: Carbon, 44.36; Hydrogen, 2.29; Nitrogen, 7.96. Found: Carbon, 44.39; Hydrogen, 2.25; Nitrogen, 7.92.

synthesis of benzimidazole-N-alkylated products (2a, 2b)

Treatment of 2-(3,5-dichloro/Bromo)-phenyl-1H-benzimidazole (0.01 mole) with ethyl chloroacetate (0.01 mole) and 20 ml of DMSO were refluxed for 5 hours. Then reaction was completed by checking with TLC. Water was added to the resulting product mixture, The precipitated materials were filtered and purified by crystallization from acetone-water mixture (1:1). They were dried in a desiccator and identified as compounds 2a, 2b [40].

2-(3,5-dichlorophenyl)-benzimidazol-1-yl-acetic acid ethyl ester (2a)

Yield, 89%; mp 242-244 °C; Proton Spectrum: 1.13 (triplet, 3H, CH₃), 4.21 (quartet, 2H, CH₂), 5.01 (singlet, 2H, N-CH₂), Aromatic H; [7.11-7.18 (multiplet, 2H), 7.32-7.35 (multiplet, 2H), 7.75- 7.88 (multiplet, 2H)], 8.10 (s, 1H); Carbon Spectrum: 14.87(CH₃), 45.17 (N-CH₂), 61.92 (CH₂), ArC: [112.64 (CH), 115.55 (CH), 115.76 (CH), 120.23 (2CH), 126,17 (C), 127.51 (C), 133.86 (CH), 134.52 (CH), 135.72 (C), 137.80 (C), 141.96 (C), 156.43 (C=N), 160.38 (C=O)]; Liquid Chromatography-Mass Spectrometer (%): [M]⁺ 349.22; Combustion Elemental Analysis Calculated (%) for C₁₇H₁₄Cl₂N₂O₂: Carbon, 58.47; Hydrogen, 4.04; Nitrogen, 8.02. Found: Carbon, 58.53; Hydrogen, 3.97; Nitrogen, 8.06.

2-(3,5-dibromophenyl)-benzimidazol-1-yl-acetic acid ethyl ester (2b)

Yield, 92%; mp 261-263 °C; Proton Spectrum: 1.13 (triplet, 3H, CH₃), 4.21 (quartet, 2H, CH₂), 4.99 (singlet, 2H, N-CH₂), Aromatic H; [7.14-7.18 (multiplet, 2H), 7.32-7.35 (multiplet, 2H), 7.76-7.85 (multiplet, 2H), 8.09 (singlet, 1H)]; Carbon Spectrum: 15.20 (CH₃), 45.17 (N-CH₂), 61.92 (CH₂), ArC: [112.61 (CH), 115.57 (CH), 115.78 (CH), 120.25 (2CH), 124.47 (C), 124.60 (C), 131.30 (CH), 132.00 (CH), 133.19 (C), 135.91 (C), 143.18 (C), 157.53 (C=N), 160.39 (C=O)]; Liquid Chromatography-Mass Spectrometer (%): [M]⁺ 438.12; Combustion Elemental Analysis Calculated (%) for C₁₇H₁₄Br₂N₂O₂: Carbon, 46.61; Hydrogen, 3.22; Nitrogen, 6.39. Found: Carbon, 46.58; Hydrogen, 3.27; Nitrogen, 6.32.

synthesis of benzimidazole-acetic acid hydrazides

0.03 mole Hydrazine hydrate and 0.01 mole the ester in ethanol were refluxed for 4 h. The reaction was completed by checking with TLC. Then they were kept in the refrigerator for one night, filtered after complete collapse. Purified by washing with alcohol. They were dried in a desiccator and identified as compounds 3a, 3b [20].

2-(3,5-Dichlorophenyl)-benzimidazol-1-yl-acetic acid hydrazide (3a)

Yield, 84%; mp 178-180 °C; Proton Spectrum: 4.88 (singlet, 2H, N-CH₂), 5.23 (singlet, 2H, NH₂), Aromatic

H; [7.12-7.17 (multiplet, 2H), 7.32-7.36 (multiplet, 2H), 7.84-7.85 (multiplet, 2H), 8.80 (singlet, 1H)], 9.49 (singlet, 1H, NH); Carbon Spectrum: 45.18 (NCH₂), ArC: [112.48 (CH), 115.51 (CH), 115.72 (CH) 120.23 (2CH), 124.51 (C), 124.80 (C), 131.41 (CH), 131.49 (CH), 134.40 (C), 141.99 (C), 157.32 (C=N), 164.91 (C=O)]; Liquid Chromatography-Mass Spectrometer (%): [M]⁺ 335.20; Combustion Elemental Analysis Calculated (%) for C₁₅H₁₂Cl₂N₄O: Carbon, 53.75; Hydrogen, 3.61; Nitrogen, 16.71. Found: Carbon, 53.81; Hydrogen, 3.57; Nitrogen, 16.75.

2-(3,5-Dibromophenyl)-benzimidazol-1-yl-acetic acid hydrazide (3b)

Yield, 88%; mp 217-219 °C; Proton Spectrum: 4.27 (singlet, 2H, N-CH₂), 5.42 (singlet, 2H, NH₂), Aromatic-H; [7.11-7.15 (multiplet, 2H), 7.31-7.34 (multiplet, 2H), 7.67 (bold singlet, 1H), 7.89-7.97 (multiplet, 2H)], 9.49 (singlet, 1H, NH); Carbon Spectrum: 42.48 (NCH₂), ArC: [111.88 (CH), 114.60 (CH), 114.99 (CH) 119.62 (2CH), 124.00 (C), 125.15 (C), 133.42 (CH), 133.95 (CH), 133.57 (C), 134.00 (C), 141.30 (C), 156.72 (C=N), 160.44 (C=O)]; Liquid Chromatography-Mass Spectrometer (%): [M]⁺ 424.10; Combustion Elemental Analysis Calculated (%) for C₁₅H₁₂Br₂N₄O: Carbon, 42.48; Hydrogen, 2.85; Nitrogen, 13.21. Found: Carbon, 42.54; Hydrogen, 2.91; Nitrogen, 13.16.

synthesis of 5-aryl-1,3,4-oxadiazole derivatives (4a-4d)

0.166 mmole of 2-(3,5-dichloro/Bromophenyl)-1H-benzimidazole acetic acid hydrazide and 0.166 mmole of arylcarboxylic acid derivative were reacted in the presence of 1 ml of POCl₃ with microwave process at 140 °C for 30 min at 300 W maximum power. Ice water was added to the residue obtained at the end of the reaction and the precipitate formed was filtered off. The residue which was washed with 10% NaHCO₃ solution was purified by column chromatography using hexane: ethylacetate (2:1) solvent system.

2-[(2-(3,5-dichlorophenyl)-1H-benzimidazol-1-yl)metil]-5-(4-chlorofenil)-1,3,4-oksadiazol (4a)

Yield, 43%; mp 204-206 °C; Proton Spectrum: 4.90 (singlet, 2H, NCH₂), Aromatic-H; [7.14-7.18 (multiplet, 3H), 7.30-7.35 (multiplet, 4H), 7.76 (singlet, 1H), 7.85 (bold singlet, 1H), 8.09 (bold singlet, 1H)]; Carbon Spectrum: 45.21 (NCH₂), ArC: [111.25 (CH), 113.50 (2CH), 114.70 (2CH), 121.43 (2CH), 125.05 (C), 125.15 (C), 130.36 (2CH), 131.44 (CH), 132.57 (CH), 135.90 (C), 141.50 (C), 151.09 (C), 156.72 (benzimidazole ring, C=N), 160.44, 162.85 (oxadiazole ring, 2C=N), 170.85 (2C)]; Liquid Chromatography-Mass Spectrometer (%): [M]⁺ 455.73; Combustion Elemental Analysis Calculated (%) for C₂₂H₁₃Cl₃N₄O: Carbon, 57.98; Hydrogen, 2.87; Nitrogen, 12.29. Found: Carbon, 57.93; Hydrogen, 2.81; Nitrogen, 12.35.

2-[(2-(3,5-dibromophenyl)-1H-benzimidazol-1-yl)metil]-5-(4-bromofenil)-1,3,4-oksadiazol (4b)

Yield, 47%; mp 228-229 °C; Proton Spectrum: 5.75 (singlet, 2H, NCH₂), Aromatic H; [7.07-7.13 (multiplet, 3H), 7.25-7.29 (multiplet, 3H), 7.31-7.38 (multiplet, 3H), 7.92 (multiplet, 1H), 7.92 (multiplet, 1H), 8.05 (singlet,

1H)]; Carbon Spectrum: 45.21 (NCH₂), ArC:[113.00 (CH), 114.60 (2CH), 114.89 (2CH), 119.31 (CH), 123.07 (C), 125.50 (C), 130.23 (2CH), 131.31 (2CH), 132.63 (CH), 134.20 (C), 141.94 (C), 147.70 (C), 157.26 (benzimidazole ring, C=N), 160.32, 162.74 (oxadiazole ring, 2C=N), 168.02 (2C)]; Liquid Chromatography-Mass Spectrometer (%): [M]⁺ 589.03; Combustion Elemental Analysis Calculated (%) for C₂₂H₁₃Br₃N₄O: Carbon, 44.85; Hydrogen, 2.22; Nitrogen, 9.51 Found: Carbon, 44.92; Hydrogen, 2.17; Nitrogen, 9.46.

2-[(2-(3,5-dichlorophenyl)-1H-benzimidazol-1-yl)metil, trichloromethylfenil]-1,3,4-oksadiazol (4c)

Yield, 39%; mp 233-235 °C; Proton Spectrum: 5.44 (singlet, 2H, N-CH₂), Aromatic-H; [6.67-6.97 (multiplet, 1H), 7.08-7.13 (multiplet, 2H), 7.28-7.32 (multiplet, 3H), 7.86-7.95 (multiplet, 5H)]; Carbon Spectrum: 59.71 (NCH₂), ArC: [112.71 (CH), 115.48 (2CH), 115.69 (2CH), 120.54 (CH), 125.12 (C), 125.35 (C), 131.20 (CH), 131.28 (2CH), 132.29 (2CH), 132.32 (C), 136.00 (C), 143.01 (C), 160.36 (C), 142.20 (C), 156.85 (benzimidazole ring, C=N), 162.77, 162.97 (oxadiazole ring, 2C=N)]; Liquid Chromatography-Mass Spectrometer (%): [M]⁺ 538.65; Combustion Elemental Analysis Calculated (%) for C₂₃H₁₃Cl₃Br₂N₄O: Carbon, 44.02; Hydrogen, 2.09; Nitrogen, 8.93 Found: Carbon, 44.09; Hydrogen, 2.14; Nitrogen, 8.86.

2-[(2-(3,5-dibromophenyl)-1H-benzimidazol-1-yl)metil, trichloro-methylfenil]-1,3,4-oksadiazol (4d)

Yield, 41%; mp 247-249 °C; Proton Spectrum: 5.07 (singlet, 2H, N-CH₂), Aromatic H; [7.11-7.21 (multiplet, 2H), 7.22-7.34 (multiplet, 2H), 7.36-7.45 (multiplet, 4H), 7.46-7.86 (multiplet, 2H), 7.93 (singlet, 1H)]; Carbon Spectrum: 45.25 (NCH₂), ArC: [111.00 (CH), 113.07 (2CH), 114.80 (2CH), 119.61 (CH), 123.22 (C), 123.19 (C), 127.65 (CH), 130.30 (2CH), 130.55 (2CH), 132.10 (C), 133.28 (C), 134.10 (C), 138.19 (C), 141.60 (C), 157.41 (benzimidazole ring, C=N), 160.39, 162.80 (oxadiazole ring, 2C=N)]; Liquid Chromatography-Mass Spectrometer (%): [M]⁺ 627.55; Combustion Elemental Analysis Calculated (%) for C₂₃H₁₃Cl₃Br₂N₄O: Carbon, 44.02; Hydrogen, 2.09; Nitrogen, 8.93 Found: Carbon, 44.09; Hydrogen, 2.14; Nitrogen, 8.86.

3. RESULTS AND DISCUSSION

In this study, a series of benzimidazole derivatives was synthesized and antioxidant activities were investigated. Compounds 2-(3,5-dichloro/bromobenzyl)-1H-benzimidazoles 1a, 1b were obtained by reaction of the o-phenylenediamine compound with the aldehyde derivatives. The reaction of compounds 1a, 1b with ethyl chloro acetate and KOH in DMSO medium, an ester of ethyl [-2-(3,5-dichloro/bromobenzyl)-1H-benzimidazole-1-yl] acetate 2a, 2b structures were obtained.

The peaks (-OCH₂CH₃), (-OCH₂CH₃) were observed at 1.13 and 4.21 ppm in the ¹H NMR spectrum. The carbon signals of these peaks were observed in the ¹³C NMR spectrum at 14.87, 15.20 and 61.92 ppm, respectively. The C=O signal from the carbonyl of the ester group is

also observed around 160.38 ppm. The proton signals of the N-CH₂ group are observed at 4.99, 5.01 and the carbon signals are observed at around 45.17 ppm. The carbon peak from the C=N group in the benzimidazole ring is also observed at 157.38 ppm as expected. The aromatic and benzimidazole ring protons resonate between 7.11-8.10 ppm. The carbon signals of these rings are also observed in the range of 112.61-142.19 ppm.

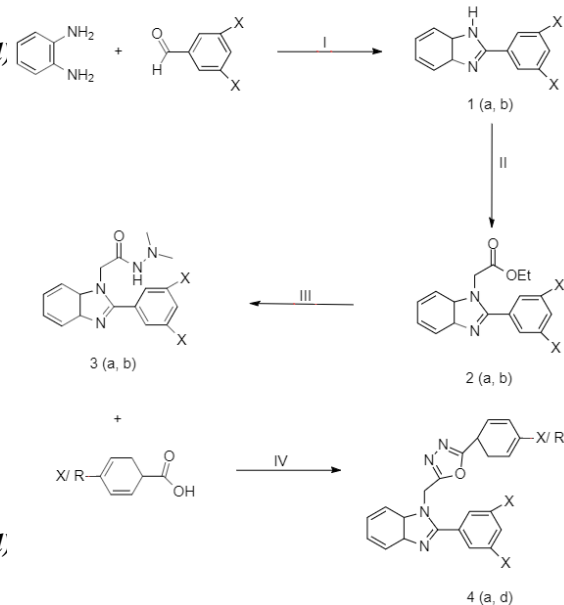


Figure 2. Synthetic route for the preparation of compounds 1-4. Reagents I: L-proline II: ClCH₂COOEt/DMSO III: NH₂NH₂·H₂O/EtOH IV: appropriate benzimidazole acetic acid hydrazide / POCl₃.

Compounds 3a, 3b which are hydrazide compounds were obtained by reaction of compounds 2a, 2b with hydrazine hydrate. Specific peaks of these groups were observed in proton NMR at 5.23, 5.76 ppm and 9.49 ppm (-NHNH₂) and their accuracy was confirmed by D₂O. Other specific peaks of compounds, C=O carbon signal 160.44, 165.97 ppm, proton signals of N-CH₂ group 4.35, 4.88 ppm, carbon signals 42.48, 45.18 ppm and benzimidazole ring C=N carbon peak was observed around 156.35 ppm. Benzimidazole ring, substituted aromatic ring protons and carbons are observed in the range of 7.11-8.80 ppm and 112.48-142.30 ppm.

1,3,4-oxadiazoles (4a-4d) were obtained by reaction of 2-(3,5-dichloro/bromophenyl)-1H-benzimidazole acetic acid hydrazide with aryl carboxylic acids in the presence of POCl₃. The reaction was synthesized using a microwave oven and the compounds were obtained in 39-47% yields. When the ¹H NMR and ¹³C NMR spectra of the compounds were examined, methylene protons were observed in the range of 4.99-5.75 ppm, carbon signals 45.21-59.71, aromatic protons were observed in the range of 6.67-8.09, and carbons in the range of 112.65-168.02 ppm. Benzimidazole ring C=N carbon peak 156.72-

157.41, oxadiazole ring C=N carbon peaks were observed between 160.30-162.97 ppm.

In addition to ^1H NMR spectra data, ^{13}C NMR spectra were taken as APT and clearly observing the orientation of peaks according to proton numbers in the spectra supports the structure of the compounds. $[\text{M}]^+$ ion peaks of the synthesized compounds were seen in accordance with the masses of the compounds; 263.12 (1a), 352.01 (1b), 349.22 (2a), 438.12 (2b), 335.20 (3a), 424.10 (3b), 455.73 (4a), 589.03 (4b), 538.65 (4c), 627.55 (4d).

3.1. Antioxidant Activity

3.1.1. Determination of DPPH radical sweeper activity

DPPH free radical scavenging effects of the synthesized products are measured by their ability to remove the violet / violet color of the 2,2-diphenyl-1-picrylhydrazil stable radical and are based on the measurement of the color produced by DPPH at 517 nm and comparison with the standard material and Based on the measurement of color produced by DPPH at 517 nm and comparison with standard material [41].

0.01 M solution of the synthesized compounds in DMSO was prepared 2×10^{-2} g/L DPPH solution was added and mixed in the vortex and allowed to stand at room temperature for 30 minutes. The color changes in the solutions were recorded by reading their absorbance at 517 nm. The results are given in Table 1. BHT, a potent antioxidant, was used as a reference compound and the radical scavenging effect was calculated according to the following equation as a percentage of radical reduction.

$$\% \text{ Radical Catch Capacity} = \frac{(A_{\text{solvent}} - A_{\text{sample}})}{A_{\text{solvent}}} \times 100$$

A: absorbance

Table 1. DPPH radical scavenging activities of synthesized compounds

Compound No	DPPH radical sweeper capacity (% control) (1 mM)
1a	79 ± 1.00
1b	73 ± 2.10
2a	42 ± 2.00
2b	59 ± 1.00
3a	47 ± 2.60
3b	61 ± 3.00
4a	84 ± 1.80
4b	71 ± 3.00
4c	68 ± 1.50
4d	76 ± 1.60
BHT	19 ± 2.00
DMSO	100

Footnote: Each value is shown with standard deviations averaging 2-3 experiments.

3.1.2. Determination of EROD enzyme activity

It was determined according to the spectrofluorimetric method described by Burke et al. [42] 7-Ethoxyresorufin O-deethylase is the enzyme that converts 7-ethoxyresorufin to resorufin. Enzyme activity was calculated by spectrofluorimetric measurement of the amount of resorufin formed. NADPH producing system was used as cofactor and the results are given in Table 2.

Resorufin solution was used as standard. This solution is very sensitive to light and is freshly prepared in a dark bottle. Five different concentrations (31.5, 62.5, 125, 250, 500 pmole) were added to the reaction medium. Values obtained from different concentrations of resorufin solutions were used in drawing the standard curve. The standard curve of resorufin was found to be linear in these studied conditions. The reaction medium contains 0.20 mg liver microsomal protein in a total volume of 1 mL, pH 7.8 Tris HCl buffer, 1 μM ethoxyresorufin and 2.5 μM glucose-6-phosphate as a cofactor producing NADPH, 0.25 μM NADP⁺, 1 U glucose-6-phosphate dehydrogenase and 2,5 μM MgCl₂.

The reaction was initiated by addition of NADPH producing system to the tubes containing other substances except the NADPH producing system from the reaction medium elements. The reaction was continued for 5 minutes at 37°C in a shaking water bath with open tubes. After the time, the reaction was stopped by the addition of 3 mL of methanol. The tubes were placed in an ice bath. The denatured protein was precipitated by spinning at 4000 rpm for 60 minutes. 3 mL of the supernatant was transferred to another tube with an automatic pipette. After measuring the FI values of this solution by spectrofluorimetric method (excitation 538 nm, emission 587 nm), EROD activity was calculated.

3.1.3. Determination of lipid peroxidation level

The level of lipid peroxidation, was determined according to the spectrophotometric method described by Wills [43, 44], NADPH producing system was used as cofactor and the results are given in Table 2. 0.2 mg liver microsomal protein in a total volume of 1.0, pH: 7.4 0.1 M Potassium phosphate buffer and 2,5 μM glucose-6-phosphate, 0.25 μM NADP⁺, 1U glucose-6-phosphate dehydrogenase and 2,5 μM MgCl₂ was used.

MDA solution was used as standard. In each experiment, 0.5 μM MDA solution was prepared. Four different levels (5.0, 12.5, 25.0, 50.0 nmole) were added in the incubation medium. The obtained absorbance values were used to plot the standard slope versus different MDA amounts.

The reaction was initiated by addition of NADPH-producing system from reaction medium elements. The reaction was continued by keeping the tubes open in a shaking water bath at 37 °C for 30 minutes. Then 0.5 ml of 25% trichloroacetic acid was added. The tubes were immediately placed in an ice bath.

The denatured protein was precipitated by spinning at 4000 rpm for 20 minutes. 1.0 ml of the supernatant was withdrawn with an automatic pipette and transferred to another tube. 0.5 ml of thiobarbituric acid (TBA) reagent /0.5 g TBA+ 1.65 ml 2 N NaOH/100 ml distilled water) has been added. The tubes were mixed with vortex. After the tubes were kept in a boiling water bath for 15 minutes, the absorbance of the pink color formed was measured on a spectrophotometer at 535 nm.

In vitro antioxidant activity determinations of synthesized compounds were tested by measuring their DPPH free radical capture capacities and their effects on lipid peroxidation inhibition and EROD enzyme activity. Capability of the obtained compounds to capture the DPPH radical was measured using BHT as standard material and compounds **2a**, **2b**, **3a**, **3b** which showed the most remarkable effect in the group, showed poor activity from BHT (81%) with inhibition values of 58, 41, 53 and 39%, respectively.

In terms of EROD enzyme activity, **2a** (69%), **4a** (64%), **4c** (68%) and **4d** (66%) compounds were found to be close to caffeine (79%), while other compounds were moderately (41-60%) were observed.

When the effects on lipid peroxidation were examined, it was found that compound **2a** (58%) had close to BHT (72%), and compounds **4b** (40%), **4c** (39%) and **1a** (34%) showed moderate effects.

Table 2. Effects of synthesized compounds on lipid peroxidation inhibition and EROD enzyme activity

Comp. No	EROD (pmole/mg/min)	% control	LP (nmole/mg/min)	% control
1a	15.96±0.08	38	17.60±0.85	66
1b	17.64±0.92	40	34.22±2.68	132
2a	11.87±0.34	31	8.16±0.90	42
2b	18.93±1.25	54	39.77±3.10	187
3a	21.13±0.41	59	27.64±1.20	125
3b	19.28±0.50	56	41.59±0.68	189
4a	14.90±0.48	36	34.60±2.53	163
4b	16.23±1,12	51	13.88±1.95	60
4c	12.44±0.56	32	14.71±0.45	61
4d	13.37±1.04	34	21.02±2.50	110
BHT	-	-	6.43±0.89	28
Cafein	5.86±0.24	21	-	-
DMSO	39.64±0.72	100	21.38±1.17	100

Footnote: ± (Standard deviation)

4. CONCLUSION

This study describes the synthesis of a novel benzimidazoles, benzimidazole-1-yl-acetic acid ethyl ester, benzimidazol-1-yl-acetic acid hydrazide and benzimidazol-1-yl-1,3,4-oxadiazole derivatives. All synthesized compounds were characterized by using spectroscopic techniques such as ¹H, ¹³C NMR, mass and elemental analysis.

After elucidating the structure of synthesized compounds, DPPH radical scavenging activity, inhibition of lipid peroxidation and their effects on EROD enzyme activity were investigated. The compounds were generally moderately active in terms of DPPH radical scavenging activity and lipid peroxidation inhibition compared to the standard compound and had a good effect on EROD enzyme activity. Of all the synthesized compounds, the most active of all three activities is compound **2a**.

DECLARATION OF ETHICAL STANDARDS

The author of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

AUTHORS' CONTRIBUTIONS

Nurhan GÜMRÜKÇÜOĞLU: Performed the experiments and analyse the results. Also wrote the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

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