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# Synthesis and Antimicrobial Activity of Some Novel Benzimidazole Hydrazides

Y. ÖZKAY[1,*], Y. TUNALI[2], H. KARACA[2] and I. ISIKDAG[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey

*Corresponding author: Fax: +90 222 3350750; Tel: +90 222 3350580/3779; E-mail: yozkay@anadolu.edu.tr

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The present study was undertaken to synthesize some 4-(5,6-dichloro-1*H*-benzimidazole-2-yl)phenoxyacetic acid-4-substituted benzylidene hydrazide derivatives and investigate their possible antimicrobial activity. Chemical structures of the synthesized compounds were elucidated by IR, $^1$H NMR and ES-MS spectral data and elemental analysis. Some of the compounds in the series exhibited notable antibacterial activity against various bacterial strains. Besides, most of them displayed significant antifungal activity against different *Candida* yeasts. Microbiological studies revealed that the most active compounds in the series were **6d** and **6n**, which were also evaluated in *Brine-Shrimp* lethality assay. The compound **6d** was found as non-toxic in addition to its significant antimicrobial activity, whereas the compound **6n** was determined as harmful.

**Key Words: Benzimidazole, Hydrazide, Antimicrobial, *Brine-Shrimp* lethality assay.**

## INTRODUCTION

The development of new antimicrobial agents to treat infections is one of the most important medical goal in 21st century since the resistance of fungi and bacteria to current antibiotic therapies is rapidly becoming a major public health threat throughout the world[1,2]. The incidence of multi-drug resistant gram-positive and gram-negative bacteria is rising and infections caused by them are becoming problematic nowadays[3]. Hence, there is a pressing need for the discovery of novel antimicrobial compounds that may have different mechanisms of action from those of well-known antimicrobial agents to which the pathogens indicate resistance[4].

Benzimidazole is a nitrogen containing heterocyclic ring which possesses biological and pharmaceutical importance. There are many biochemical and pharmacological studies[5-14] confirming that benzimidazole derivatives are effective against various strains of microorganisms. The reason for a special attention of researchers towards benzimidazole ring has been its structural similarity to purine. Antibacterial ability of benzimidazole derivatives is explained by their competition with purines resulting in inhibition of the synthesis of bacterial nucleic acids and proteins[15,16]. In addition to their antibacterial activity, the benzimidazole derivatives have also provided antifungal activity especially against the yeast *Candida albicans*[17,18]. Benzimidazoles have constituted the most important group of fungicides with systemic activity and are well-known for their pronounced ability to control a large number of fungal diseases. Benomyl, thiabendazole and thiophnate methyl are main examples of this fungicide class[8,19].

Hydrazone derivatives form another class of compounds possessing antimicrobial activity. Some widely used antibacterial drugs such as furacilin, furazolidone, ftivazide and nifuroxazide are well-known antibacterial agents bearing this pharmacophore group[20]. In the past decade, hydrazones have received a special interest and many studies[21-29] have been reported due to their chemotherapeutic value in the development of novel antimicrobial agents.

Looking at the antimicrobial importance of benzimidazole and hydrazone scaffolds, it was thought that it would be worthwhile to design and synthesize some new benzimidazole derivatives bearing hydrazone moiety and investigate their probable antibacterial and antifungal activity. Hence, antimicrobial activity screening of some novel benzimidazole-hydrazone compounds was undertaken in this study.

## EXPERIMENTAL

All of the chemicals used in syntheses were obtained from either Merck (Germany) or Acros (Belgium) companies. Melting points (m.p.) of target compounds were determined in open capillaries on an Electrothermal 9001 digital melting point apparatus and were uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck). IR spectra were recorded on a Shimadzu, 8400 FTIR spectrometer as KBr pellets. $^1$H NMR

spectra were recorded on a Bruker UltraShield 500 MHz spectrometer in deutoro dimethyl sulfoxide. ES-MS data were obtained on an Agilent 1100 Series LC/MSD Trap VL and SL spectrometer. Elemental analyses (C, H and N) were determined on a Perkin Elmer analyzer.

Reaction sequence outlined in the **Scheme-I** was followed to achieve target products. Previously described synthesis procedures were used to obtain the intermediate compounds (**1-5**). Briefly, 4-formylphenoxyacetic acid (**1**) was prepared according to Finkelstein reaction by heating 4-hydroxybenzaldehyde with chloroacetic acid in acetone with the presence of $K_2CO_3$ and KI[30]. Sodium disulfide adduct product of 4-formylphenoxyacetic acid (**2**) was reacted with 4,5-dichloro-1,2-phenylenediamine in DMF to give 4-(5,6-dichloro-1*H*-benzimidazole-2-yl)phenoxyacetic acid (**3**)[31]. Esterification of the **3** to 4-(5,6-dichloro-1*H*-benzimidazole-2-yl)phenoxyacetic acid ethyl ester (**4**) was performed in EtOH with the presence of $H_2SO_4$[32]. Treatment of the **4** with excess of hydrazine hydrate (80 %) in EtOH gave the 4-(5,6-dichloro-1*H*-benzimidazole-2-yl)phenoxyacetic acid hydrazide (**5**)[29].

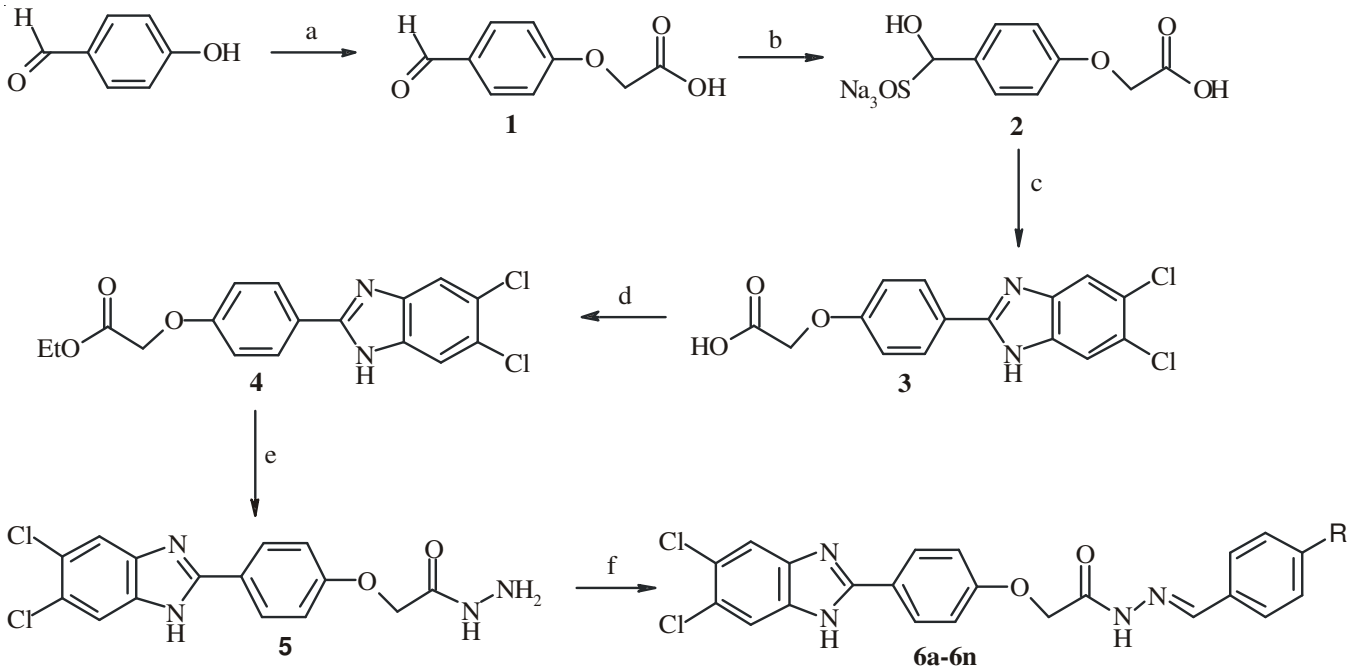
**General synthesis procedure for the target compounds (6a-6n):** Equimolar quantities (20 mmol) of the **5** and appropriate 4-substituted benzaldehyde derivative in 25 mL of butanol were refluxed for 4 h with the presence of catalytic amount of glacial acetic acid. The reaction mixture was allowed to cool and the resulting solid was filtered and recrystallized from EtOH.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-benzylidene hydrazide (6a):** Yield 74 %. m.p. 275 ºC. IR (KBr, $n_{max}$, $cm^{-1}$): 3293-3221 (N-H), 1667 (C=O), 1608-1402 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.11 (2H, s), 7.13 (2H, d, *J* = 8.67 Hz), 7.22-7.30 (3H, m), 7.76 (2H, s), 7.91 (2H, d, *J* = 8.24 Hz), 8.16 (2H, d, *J* = 8.68 Hz), 8.47 (H, s), 8.74 (H, s), 12.96 (H, br). ES-MS (m/z): M + 1: 440.4. Analysis for $C_{22}H_{16}N_4O_2Cl_2$: calcd. (%) (C, H and N): 60.15, 3.67 and 12.75; found (%) (C, H and N): 60.23, 3.66 and 12.79.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(dimethylamino)-benzylidene hydrazide (6b):** Yield 79 %. m.p. 235 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3285-3231 (N-H), 1672 (C=O), 1610-1423 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 3.05 (6H, s), 4.12 (2H, s), 6.91 (2H, d, *J* = 8.52 Hz), 7.13 (2H, d, *J* = 8.74 Hz), 7.77 (2H, s), 7.91 (2H, d, *J* = 8.49 Hz), 8.16 (2H, d, *J* = 8.77 Hz), 8.63 (H, s), 8.69 (H, s), 13.11 (H, br). ES-MS (m/z): M + 1: 483.4. Analysis for $C_{24}H_{21}Cl_2N_5O_2$: calcd. (%) (C, H and N): 59.76, 4.39 and 14.52; found. (%) (C, H and N): 59.65, 4.38 and 14.55.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(diethylamino)-benzylidene hydrazide (6c):** Yield 81 %. m.p. 181 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3279-3220 (N-H), 1673 (C=O), 1604-1403 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 1.08 (6H, t, *J* = 7.13 Hz and *J* = 7.19 Hz), 2.96 (4H, q, *J* = 7.17 Hz and *J* = 7.18 Hz), 4.11 (2H, s), 6.75 (2H, d, *J* = 8.34 Hz), 7.12 (2H, d, *J* = 8.61 Hz), 7.76 (2H, s), 7.93 (2H, d, *J* = 8.27 Hz), 8.17 (2H, d, *J* = 8.65 Hz), 8.54 (H, s), 8.67 (H, s), 13.04 (H, br). ES-MS (m/z): M + 1: 511.3. Analysis for $C_{26}H_{25}N_6O_2Cl_2$: calcd. (%) (C, H and N): 61.18, 4.94 and 13.72; found (%) (C, H and N): 60.97, 4.95 and 13.68.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(chloro)-benzylidene hydrazide (6d):** Yield 73 %. m.p. 208 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3282-3242 (N-H), 1666 (C=O), 1601-1421 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.09 (2H, s), 7.13 (2H, d, *J* = 8.61 Hz), 7.52 (2H, m), 7.74 (2H, s), 7.96 (2H, d, *J* = 8.18 Hz), 8.15




R: **6a**: -H; **6b**: -N(CH$_3$)$_2$; **6c**: -N(C$_2$H$_5$)$_2$; **6d**:-Cl; **6e**: -Br; **6f**: -F; **6g**: -CH$_3$; **6h**: -OCH$_3$; **6i**: -OC$_2$H$_5$; **6j**: -OH; **6k**: -NO$_2$; **6l**: -CF$_3$; **6m**: -COOH; **6n**: -CN

Reagents and conditions: **a**: (i) $ClCH_2COOH$, $K_2CO_3$, KI, acetone, reflux 12 h; (ii) treated with $CH_3COOH$; **b**: $Na_2S_2O_5$, 80 % EtOH, r.t. 0.5 h; **c**: 4,5-Dichloro-1,2-phenylenediamine, DMF, 130 ºC 4 h; **d**: EtOH, $H_2SO_4$, reflux 8 h, **e**: 80 % $NH_2NH_2{\cdot}H_2O$, EtOH, reflux 12 h; **f**: appropriate 4-substitutedbenzaldehyde, catalytic amount $CH_3COOH$, *n*-ButOH, reflux 4 h.

**Scheme-I:** Reaction sequence of 4-(5,6-dichloro-1*H*-benzimidazole-2-yl) phenoxyaceticacid-4-substitutedbenzylidene hydrazide derivatives (**6a-6n**)

(2H, d, *J* = 8.57 Hz), 8.51 (H, s), 8.72 (H, s), 13.08 (H, br). ES-MS (m/z): M + 1: 474.9. Analysis for $C_{22}H_{15}N_4O_2Cl_3$: calcd. (%) (C, H and N): 55.78, 3.19 and 11.83; found (%) (C, H and N): 55.94, 3.19 and 11.80.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(bromo)-benzylidene hydrazide (6e):** Yield 77 %. m.p. 256 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3279-3212 (N-H), 1670 (C=O), 1602-1428 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.11 (2H, s), 7.13 (2H, d, *J* = 8.65 Hz), 7.62 (2H, m), 7.74 (2H, s), 7.95 (2H, d, *J* = 8.42 Hz), 8.16 (2H, d, *J* = 8.66 Hz), 8.60 (H, s), 8.73 (H, s), 12.98 (H, br). ES-MS (m/z): M + 1: 519.1. Analysis for $C_{22}H_{15}BrN_4O_2Cl_2$: calcd. (%) (C, H and N): 50.69, 2.92 and 10.81; found (%) (C, H and N): 50.75, 2.91 and 10.83.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(fluoro)-benzylidene hydrazide (6f):** Yield 80 %. m.p. 212 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3282-3244 (N-H), 1681 (C=O), 1604-1406 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.08 (2H, s), 7.13 (2H, d, *J* = 8.52 Hz), 7.46 (2H, m), 7.78 (2H, s), 7.92 (2H, d, *J* = 8.23 Hz), 8.18 (2H, d, *J* = 8.57 Hz), 8.59 (H, s), 8.72 (H, s), 12.95 (H, br). ES-MS (m/z): M + 1: 458.3. Analysis for $C_{22}H_{15}FN_4O_2Cl_2$: calcd. (%) (C, H and N): 57.78, 3.31 and 12.25; found (%) (C, H and N): 57.70, 3.32 and 12.29.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(methyl)-benzylidene hydrazide (6g):** Yield 75 %. m.p. 306 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3293-3247 (N-H), 1671 (C=O), 1604-1411 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 2.28 (3H, s), 4.13 (2H, s), 7.14 (2H, d, *J* = 8.60 Hz), 7.25 (2H, d, *J* = 8.16 Hz), 7.74 (2H, s), 7.92 (2H, d, *J* = 8.19 Hz), 8.18 (2H, d, *J* = 8.63 Hz), 8.54 (H, s), 8.79 (H, s), 13.02 (H, br). ES-MS (m/z): M + 1: 454.2. Analysis for $C_{23}H_{18}N_4O_2Cl_2$: calcd. (%) (C, H and N): 60.94, 4.00 and 12.36; found (%) (C, H and N): 61.02, 3.99 and 12.38.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(methoxy)-benzylidene hydrazide (6h):** Yield 71 %. m.p. 298 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3274-3223 (N-H), 1676 (C=O), 1610-1413 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 3.94 (3H, s), 4.11 (2H, s), 7.02 (2H, d, *J* = 8.41 Hz), 7.13 (2H, d, *J* = 8.67 Hz), 7.77 (2H, s), 7.96 (2H, d, *J* = 8.37 Hz), 8.15 (2H, d, *J* = 8.48 Hz), 8.41 (H, s), 8.85 (H, s), 13.01 (H, br). ES-MS (m/z): M + 1: 470.3. Analysis for $C_{23}H_{18}N_4O_3Cl_2$: calcd. (%) (C, H and N): 58.86, 3.87 and 11.94; found. (%) (C, H and N): 58.74, 3.86 and 11.93.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(ethoxy)-benzylidene hydrazide (6i):** Yield 74 %. m.p. 277 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3278-3245 (N-H), 1664 (C=O), 1614-1460 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 1.17 (3H, t, *J* = 7.18 Hz and *J* = 7.20 Hz), 3.77 (2H, q, *J* = 7.16 Hz and *J* = 7.15 Hz), 4.13 (2H, s), 6.74 (2H, d, *J* = 8.31 Hz), 7.12 (2H, d, *J* = 8.62 Hz), 7.78 (2H, s), 7.91 (2H, d, *J* = 8.29 Hz), 8.18 (2H, d, *J* = 8.55 Hz), 8.67 (H, s), 8.80 (H, s), 12.97 (H, br). ES-MS (m/z): M + 1: 484.3. Analysis for $C_{24}H_{20}N_4O_3Cl_2$: calcd. (%) (C, H and N): 59.64, 4.17 and 11.59; found (%) (C, H and N): 59.68, 4.17 and 11.63.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(hydroxy)-benzylidene hydrazide (6j):** Yield 79 %. m.p. 289 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3286-3224 (N-H), 1661 (C=O), 1614-1460 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.13 (2H, s), 7.11 (2H, d, *J* = 8.64 Hz), 7.20 (2H, *J* = 8.25 Hz), 7.77 (2H, s), 7.96 (2H, d, *J* = 8.24 Hz), 8.17 (2H, d, *J* = 8.66 Hz), 8.39 (H, s), 8.72 (H, s), 9.96 (H, s), 13.02 (H, br). ES-MS (m/z): M + 1: 456.3. Analysis for $C_{22}H_{16}N_4O_3Cl_2$: calcd. (%) (C, H and N): 58.04, 3.54 and 12.31; found (%) (C, H and N): 57.96, 3.53 and 12.29.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(nitro)-benzylidene hydrazide (6k):** Yield 83 %. m.p. 328 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3283-3242 (N-H), 1669 (C=O), 1603-1421 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.13 (2H, s), 7.11 (2H, d, *J* = 8.54 Hz), 7.72 (2H, s), 8.07 (2H, d, *J* = 8.23 Hz), 8.19 (2H, d, *J* = 8.61 Hz), 8.44 (2H, d, *J* = 8.26 Hz), 8.56 (H, s), 8.70 (H, s), 13.04 (H, br). ES-MS (m/z): M + 1: 485.1. Analysis for $C_{22}H_{15}N_5O_4Cl_2$: calcd. (%) (C, H and N): 54.56, 3.12 and 14.46; found (%) (C, H and N): 54.64, 3.11 and 14.47.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(trifluoromethyl)-benzylidene hydrazide (6l):** Yield 79 %. m.p. 304 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3275-3226 (N-H), 1669 (C=O), 1601-1433 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.10 (2H, s), 7.09 (2H, d, *J* = 8.41 Hz), 7.76 (2H, s), 7.95 (2H, d, *J* = 8.32 Hz), 8.14 (2H, d, *J* = 8.48 Hz), 8.30 (2H, d, *J* = 8.34 Hz), 8.43 (H, s), 8.81 (H, s), 13.00 (H, br). ES-MS (m/z): M + 1: 508.3. Analysis for $C_{23}H_{15}F_3N_4O_2Cl_2$: calcd. (%) (C, H and N): 54.46, 2.98 and 11.04; found (%) (C, H and N): 54.51, 2.97 and 11.06.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)phenoxyacetic acid-4-(carboxy)-benzylidene hydrazide (6m):** Yield 78 %. m.p. 251 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3278-3227 (N-H), 1672 (C=O), 1607-1423 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.13 (2H, s), 7.12 (2H, d, *J* = 8.29 Hz), 7.76 (2H, s), 8.00 (2H, d, *J* = 8.41 Hz), 8.22 (2H, d, *J* = 8.27 Hz), 8.34 (2H, d, *J* = 8.45 Hz), 8.57 (H, s), 8.69 (H, s), 12.11 (H, s), 12.98 (H, br). ES-MS (m/z): M + 1: 484.4. Analysis for $C_{23}H_{16}N_4O_4Cl_2$: calcd. (%) (C, H and N): 57.16, 3.34 and 11.59; found (%) (C, H and N): 57.07, 3.34 and 11.56.

**4-(5,6-Dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-(cyano)-benzylidene hydrazide (6n):** Yield 81 %. m.p. 259 ºC. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3271-3244 (N-H), 1664 (C=O), 1605-1419 (C=N and C=C). $^1$H NMR (500 MHz) (DMSO-$d_6$) δ (ppm): 4.16 (2H, s), 7.13 (2H, d, *J* = 8.53 Hz), 7.76 (2H, s), 8.05 (2H, d, *J* = 8.42 Hz), 8.17 (2H, d, *J* = 8.53 Hz), 8.33 (2H, d, *J* = 8.46 Hz), 8.54 (H, s), 8.71 (H, s), 13.04 (H, br). ES-MS (m/z): M + 1: 465.2. Analysis for $C_{23}H_{15}N_5O_2Cl_2$: calcd. (%) (C, H and N): 59.50, 3.26 and 15.08; found (%) (C, H and N): 59.62, 3.66 and 15.10.

**Microbiology:** Final products were tested for their *in vitro* growth inhibitory activity against human pathogenic as gram-positive bacteria; *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* and *Listeria monocytogenes* (obtained from Faculty of Pharmacy Anadolu University, Eskisehir, Turkey), as gram-negative bacteria; *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Escerichia coli* ATCC 35218, *Escerichia coli* ATCC 25922, *Salmonella thyphimurium* NRRL B-4420 and *Proteus vulgaris* NRLL B-123 and yeast as *Candida albicans*, *Candida tropicalis* and *Candida globrata* ATCC 36583 (obtained from Faculty of Medicine Osmangazi University, Eskisehir, Turkey). Chloramphenicol and ketocanozole were used as control drugs.

Selected compounds were also tested for their toxicity by applying *Brine-Shrimp* (*Artemia salina*) lethality assay. Fresh eggs of *Brine-shrimp*, sold as a fish food, were purchased from the local pet shop, Eskisehir/Turkey.

**Antimicrobial assay:** Antimicrobial activity assay was performed according to CLSI reference M7-A7 broth microdilution method as described in previous study[29]. Twice MIC readings were carried out for each chemical agent. The compounds were dissolved in DMSO for antibacterial and antimycotic assays. Further dilutions of the compounds and standard drugs in test medium were prepared at the required quantities of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 µg/mL concentrations with Mueller-Hinton broth and Sabouroud dextrose broth. In order to ensure that the solvent *per se* had no effect on bacteria or yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium.

***Brine-Shrimp* lethality assay:** Selected test compounds were dissolved in DMSO to obtain the stock concentration of 1000 µg/mL and then stock solution was diluted to various concentrations (1000-31.25 µg/mL). In order to prevent the toxicity results from possible false effects originated from DMSO's toxicity, stock solutions of the compounds were prepared according to suggested volume range by dissolving 1 mg of test compound in 10 µL DMSO and completing to 1000 µL with artificial seawater[33]. Pure DMSO was used as a positive control for the toxicity assay. The eggs hatched in a conical flask containing 300 mL artificial seawater made by dissolving a commercial marine salt in deionised water. The flasks were well aerated with the aid of an air pump and kept in a water bath at 25-30 ºC. The larvae hatched within 48 h. Ten larvae were transferred with pipetter into each vial containing test compound and artificial sea water. A check count was performed after 24 h of exposure at room temperature and the number of dead larvae, exhibiting no internal or external movement during several seconds of observation, was noted. Three independent experiments were performed for each concentration of compounds.

## RESULTS AND DISCUSSION

In this study 4-(5,6-dichloro-1*H*-benzimidazole-2-yl)-phenoxyacetic acid-4-substituted benzylidene hydrazide derivatives were synthesized. Structure elucidations of the final compounds were performed with IR, $^1$H NMR and ES-MS spectroscopic methods and elemental analysis.

Characteristic stretching absorption of C=O groups were observed at 1681-1661 cm$^{-1}$. The stretching absorption at about 3393-3312 and 1612-1400 cm$^{-1}$ were recorded for N-H bonds and C=C and C=N double bonds, respectively. In the $^1$H NMR spectra, all of the aromatic and aliphatic protons were observed at estimated areas. N-H protons of benzimidazole and hydrazone gave peaks at 12.95-13.11 ppm as a broad and at 8.67-8.85 ppm as a singlet, respectively. C-H proton of azomethine group and 4th and 7th position protons of benzimidazole gave singlets at 8.39-8.67 ppm and 7.72-7.78 ppm, respectively. Four different doublets belonging to aromatic protons of 1,4-disubstituted phenyl rings were observed at 6.74-8.44 ppm. -$OCH_2$ protons gave peaks at 4.08-4.16 ppm as a singlet. M + 1 peaks in ES-MS spectra were in agreement with the calculated molecular weight of the target compounds. Elemental analysis results for C, H and N elements were satisfactory within ± 0.4 % calculated values of the compounds.

The substitution pattern of the benzimidazole-hydrazone derivatives, which may display an important role on the antimicrobial activity, was chosen carefully to confer different electronic environment to the molecules. Thus, electron donating groups to aromatic ring, such as halogens, methyl, methoxy, ethoxy, hydroxyl, dimethylamine and diethylamine and electron withdrawing groups from aromatic ring, such as nitro, trifluoromethyl, carboxyl and cyano were chosen as substituents on the chemical structure of target compounds.

All of the synthesized compounds exhibited insignificant antibacterial activity against gram-negative bacterial strains of *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883 and *Proteus vulgaris* NRLL B-123. On the other hand, some of the synthesized compounds showed important antibacterial activity against some other gram-negative bacterial strains. As follows, MIC values (12.5 µg/mL) of the **6a**, **6b**, **6c**, **6e** and **6f** against *Salmonella thyphimurium* NRRL B-4420 were equal to that of the reference drug chloramphenicol. Moreover, **6d**, **6g**, **6l** and **6n** indicated greater antibacterial activity than reference. The **6k** and **6m** exhibited lower antibacterial activity than chloramphenicol against *Pseudomonas aeruginosa* ATCC 27853. Nevertheless, antibacterial effect (MIC = 12.5 µg/mL) of the other compounds in the series were the same with that of reference. Among the tested compounds, the **6d** and **6n** showed notable antibacterial activity against *Escherichia coli* ATCC 35218 (Table-1).

TABLE-1
MIC VALUES (µg/mL) OF BENZIMIDAZOLE-HYDRAZONE DERIVATIVES AGAINST GRAM-NEGATIVE BACTERIAL STRAINS

| Compound | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| **6a** | 25 | 50 | 200 | 12.5* | 100 | 50* |
| **6b** | 25 | 50 | 200 | 12.5* | 50 | 50* |
| **6c** | 25 | 50 | 200 | 12.5* | 25 | 50* |
| **6d** | 12.5* | 50 | 100 | 6.25** | 25 | 50* |
| **6e** | 25 | 50 | 200 | 12.5* | 25 | 50* |
| **6f** | 25 | 50 | 400 | 12.5* | 25 | 50* |
| **6g** | 25 | 50 | 100 | 6.25** | 50 | 50* |
| **6h** | 25 | 50 | 200 | 25 | 50 | 50* |
| **6i** | 25 | 100 | 400 | 100 | 50 | 50* |
| **6j** | 25 | 100 | 400 | 100 | 50 | 50* |
| **6k** | 25 | 50 | 400 | 25 | 50 | 100 |
| **6l** | 25 | 50 | 200 | 6.25 | 50 | 50* |
| **6m** | 50 | 100 | 800 | 25 | 100 | 200 |
| **6n** | 12.5* | 50 | 100 | 6.25** | 25 | 50* |
| Chloramphenicol | 12.5 | 12.5 | 50 | 12.5 | 12.5 | 50 |

A: *Escherichia coli*, B: *Escherichia coli,* C: *Proteus vulgaris*, D: *Salmonella thyphimurium*, E: *Klebsiella pneumoniae*, F: *Pseudomonas aeruginosa*. *Equal MIC value to reference, **Lower MIC value than reference.

When compared with chloramphenicol, synthesized compounds exhibited poor antibacterial effect against gram-positive bacteria. However, the MIC values (12.5 µg/mL) of the **6d** and **6n** were equal to that of reference against *Staphylococcus aureus* ATCC 25923 (Table-2).

TABLE-2
MIC VALUES (μg/mL) OF BENZIMIDAZOLE-HYDRAZONE DERIVATIVES AGAINST GRAM-POSITIVE BACTERIAL AND FUNGAL STRAINS

| Compound | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| **6a** | 400 | 100 | 200 | 50 | 50* | 25* | 50* |
| **6b** | 400 | 50 | 400 | 50 | 50* | 25* | 50* |
| **6c** | 400 | 50 | 400 | 50 | 25** | 25* | 50* |
| **6d** | 400 | 12.5* | 25 | 25 | 25** | 25* | 50* |
| **6e** | 400 | 25 | 50 | 25 | 50* | 50 | 50* |
| **6f** | 400 | 50 | 100 | 50 | 50* | 25* | 50* |
| **6g** | 400 | 25 | 100 | 25 | 50* | 25* | 50* |
| **6h** | 400 | 100 | 50 | 50 | 50* | 25* | 50* |
| **6i** | 400 | 200 | 400 | 50 | 25** | 50 | 50* |
| **6j** | 400 | 100 | 400 | 50 | 50* | 50 | 50* |
| **6k** | 400 | 25 | 100 | 50 | 50* | 25* | 50* |
| **6l** | 400 | 25 | 50 | 50 | 50* | 25* | 50* |
| **6m** | 400 | 100 | 400 | 100 | 50* | 50 | 50* |
| **6n** | 200 | 12.5* | 25 | 25 | 25** | 25* | 50* |
| Chloramphenicol | 50 | 12.5 | 12.5 | 12.5 | – | – | – |
| Ketaconazole | – | – | – | – | 50 | 25 | 50 |

A: *Listeria monocytogenes*, B: *Staphylococcus aureus*, C: *Enterococcus faecalis*, D: *Bacillus subtilis*, E: *Candida albicans*, F: *Candida globrata*, G: *Candida tropicalis*. *Equal MIC value to reference, **Lower MIC value than reference.

Significant antifungal activity was displayed by the most of the compounds against *Candida* yeasts. All of the compounds were found to be as active as reference drug ketocanozole (MIC = 50 μg/mL) against *Candida tropicalis*. Only the **6e**, **6i** and **6j** exhibited lower antifungal activity than reference against *Candida globrata*. Antibacterial effect (MIC = 25 μg/mL) of the other compounds in the series against this fungal strain were the same with that of ketocanozole. The **6c**, **6d** and **6n** indicated greater antifungal activity (MIC = 25 μg/mL) than reference against *Candida albicans*. MIC values (50 μg/mL) of the other compounds in the series were equal to that of reference (Table-2).

General antimicrobial evaluation of the synthesized compounds demonstrates that the most active compounds **6d** and **6n** in the series bear chloro and cyano substituents. Observed results introduce that conferment of different electronic environment to the synthesized compounds plays no significant role on either antibacterial or antifungal activity. Since, both electron withdrawing and electron donating substituents bearing compounds showed similar antimicrobial activity on fungal and bacterial strains (Tables 1 and 2).

A chemical agent is valuable in medicinal field if only it possesses low toxicity with significant activity. Thus, toxicities of the compounds **6d** and **6n**, which have the best antimicrobial activity in the series, need to be revealed. For this purpose, *Artemia salina* lethality assay was performed. This assay is regarded as a useful method for preliminary evaluation of toxicity and it has been used for the establishing of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides and cytotoxicity testing of dental materials[34], natural and synthetic organic compounds[33]. Moreover, *Artemia salina* toxicity test results show a good correlation with rodent and human acute oral toxicity data. Likewise, the predictive screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *Artemia salina* toxicity test, was slightly better than the rat tests for the test compounds[35].

Toxicity test results were analyzed by the $LC_{50}$ computer program (Trimmed Spearman-Karber Method, Version 1.5) so as to calculate $LC_{50}$ values and 95 % confidence intervals[36]. The compounds **6d** ($LC_{50}$ > 1000 μg/mL) and **6n** ($LC_{50}$ = 252.07 μg/mL) were evaluated as non-toxic and harmful, respectively. This result indicates that the **6d** is the most noteworthy compound in the series due to its significant antifungal and non-toxic effects (Table-3).

TABLE-3
*Brine-shrimp* TOXICITY RESULTS OF THE COMPOUNDS **6d** AND **6n**

| Concentration (μg/mL) | Mortality* | |
|---|---|---|
| | **6d** | **6n** |
| 1000 | 4 | 10 |
| 500 | 3 | 10 |
| 250 | 3 | 4 |
| 125 | 1 | 3 |
| 62.5 | 1 | 2 |
| 31.25 | 1 | 2 |
| Control | 0 | 1 |
| $LC_{50}$ | >1000 μg/mL | 252.07 μg/mL |
| 95 % lower limit | – | 175.43 |
| 95% upper limit | – | 362.20 |
| Toxicity | Non-toxic | Harmful |

*Ten organisms (*Artemia salina*) tested for each concentration.

## Conclusion

The preliminary *in vitro* antibacterial, antifungal and toxicological screening results of novel benzimidazole-hydrazone derivatives reported herein have indicated the antimicrobial potent of the synthesized compounds. One of the most effective compounds **6d** which bear chloro substituent have found as non-toxic in *Artemia salina* toxicity test. Consequently, findings of the present study indicate the importance of chloro substituent on antimicrobial activity and in the future can have a good effect on medicinal chemists to achieve more effective and non-toxic compounds selectively bearing chloro substituent.

## REFERENCES


1. G. Turan-Zitouni, Z.A. Kaplancikli, M.T. Yildiz, P. Chevallet and D. Kaya, *Eur. J. Med. Chem*., **40**, 607 (2005).
2. J.C. Hegde, K.S. Girisha, A. Adhikari and B. Kalluraya, *Eur. J. Med. Chem*., **43**, 2831 (2008).
3. A. Khalafi-Nezhad, M.N. Soltani Rad, H. Mohabatkar, Z. Asrari and B. Hemmateenejad, *Bioorg. Med. Chem*., **13**, 1931 (2005).
4. M. Antolini, A. Bozzoli, C. Ghiron, G. Kennedy, T. Rossi and A. Ursini, *Bioorg. Med. Chem. Lett*., **9**, 1023 (1999).
5. H. Göker, C. Ku, D.W. Boykin, S. Yildiz and N. Altanlar, *Bioorg. Med. Chem*., **10**, 2589 (2002).
6. V. Klimesova, J. Koci, M. Pour, J. Stachel, K. Waisser and J. Kaustova, *Eur. J. Med. Chem*., **37**, 409 (2002).
7. G. Ayhan-Kilcigil and N. Altanlar, *Farmaco*, **58**, 1345 (2003).
8. N.S. Pawar, D.S. Dalal, S.R. Shimpi and P.P. Mahulikar, *Eur. J. Pharm. Sci*., **21**, 115 (2004).
9. M. Boiani and M. Gonzalez, *Mini Rev. Med. Chem*., **5**, 409 (2005).
10. K.G. Desai and K.R. Desai, *Bioorg. Med. Chem*., **14**, 8271 (2004).
11. B.G. Mohammad, M.A. Hussien, A.A. Abdel-Alim and M. Hashem, *Arch. Pharm. Res*., **29**, 26 (2006).
12. Ö.Ö. Güven, T. Erdogan, H. Göker and S. Yildiz, *Bioorg. Med. Chem. Lett*., **17**, 2233 (2007).
13. D. Sharma, B. Narasimhan, P. Kumar and A. Jalbout, *Eur. J. Med. Chem*., **44**, 1119 (2009).
14. M. Tuncbilek, T. Kiper and N. Altanlar, *Eur. J. Med. Chem*., **44**, 1024 (2009).

15. A.A. Spasov, I.N. Yozhitsa, L.I. Bugaeva and V.A. Anisimova, *Pharm. Chem. J.*, **33**, 232 (1999).
16. F. Arjmand, B. Mohani and S. Ahmad, *Eur. J. Med. Chem.*, **40**, 1103 (2005).
17. G. Ayhan-Kilcigil, M. Tuncbilek, N. Altanlar and H. Goker, *Farmaco*, **54**, 562 (1999).
18. C. Kus, H. Goker and N. Altanlar, *Arch. Pharm.*, **334**, 361 (2001).
19. B. Lopez-Garcia, A. Veyrat, E. Perez-Paya, L. Gonzalez-Candelas and J.F. Marcos, *Int. J. Food Microbiol.*, **89**, 163 (2003).
20. V.A. Chornous, M.K. Bratenko, M.V. Vovk and I.I. Sidorchuk, *Pharm. Chem. J.*, **35**, 203 (2001).
21. S. Papakonstantinou-Garoufalias, N. Pouli, P. Marakos and A. Chytyroglou-Ladas, *Farmaco*, **57**, 973 (2002).
22. S. Rollas, N. Gulerman and H. Erdeniz, *Farmaco*, **57**, 171 (2002).
23. P. Vicini, F. Zani, P. Cozzini and I. Doytchinova, *Eur. J. Med. Chem.*, **37**, 553 (2002).
24. C. Loncle, J.M. Brunel, N. Vidal, M. Dherbomez and Y. Letourneux, *Eur. J. Med. Chem.*, **39**, 1067 (2004).
25. A. Masunari and L.C. Tavares, *Bioorg. Med. Chem.*, **15**, 4229 (2007).
26. U. Salgin-Goksen, N. Gokhan-Kelekci, O. Goktas, Y. Koysal, E. Kilic, S. Isik, G. Aktay and M. Ozalp, *Bioorg. Med. Chem.*, **15**, 5738 (2007).
27. S.D. Joshi, H.M. Vagdevi, V.P. Vaidya and G.S. Gadaginamath, *Eur. J. Med. Chem.*, **43**, 1989 (2008).
28. P. Kumar, B. Narasimhan, D. Sharma, V. Judge and R. Narang, *Eur. J. Med. Chem.*, **44**, 1853 (2009).
29. A. Ozdemir, G. Turan-Zitouni, Z.A. Kaplancikli and Y. Tunali, *Enzym. Inhib. Med. Chem.*, **24**, 825 (2009).
30. C.K. Ingold, Structure and Mechanism in Organic Chemistry, London, Cornell University Press, edn. 2 (1969).
31. H.F. Ridley, R.G.W. Spickett and G.M. Timmis, *J. Heterocycl. Chem.*, **2**, 453 (1965).
32. J.R. Mohrig, C.N. Hammond and P.F. Schatz, Techniques in Organic Chemistry, New York, W.H. Freeman, edn. 2 (2006).
33. A. Rahman, M.I. Choudhar and W.J. Thomsen, Bioassay Techniques for Drug Development, Amsterdam, Harwood Academic Publishers (2001).
34. J.L. Carballo, Z.L. Hernandez-Inda, P. Perez and M.D. Garcia-Gravalos, *BMC Biotechnol.*, **2**, 17 (2002).
35. M.C. Calleja and G. Persoone, *Altern. Lab. Anim.*, **20**, 396 (2002).
36. B. Brayn, M. Timothy and S. Tore, General and Applied Toxicology, Michigan, Stockton Press, edn. 2, Vol. I (1993).