INTRODUCTION
Recent years there is a big interest in the development and pharmacological evaluation of heteroaromatic organic compounds, such as benzimidazoles, because of their varied biological activities as antibacterial (1, 2), antifungal (3, 4), antimicrobial (5, 6), antiviral (7), anti-inflammatory (8), vasodilator (9), anticancer (10, 11). Substituted benzimidazole derivatives are also inhibitors of type I DNA topoisomerase (12-15).

The benzimidazole nucleus is found in a variety of naturally occurring compounds such as vitamin B12 and its derivatives, and it is structurally similar to purin bases. The substituents on position 1 and/or position 2 of the benzimidazole ligands can induce notable changes in the electronic, steri, and hydrophobic properties of the compounds. The benzimidazole ligands having hydroxyl and/or free N1-H moiety which would have hydrogen bound donor and/or acceptor properties, could facilitate novel types of lesions with cellular DNA, and might exhibit sequence selectivity.

Investigating the interactions between DNA and small molecules is an important field of molecular biology for understanding the biological roles of these molecules against biological components. The identification of interaction types between DNA and these molecules lead important biological studies in drug discovery and pharmaceutical development process. Several studies have been performed for detecting the interaction (16-18) and the mechanism (19, 20).

There are various types of interaction mechanisms between DNA and drug molecules including non-covalent groove binding, covalent binding / cross-linking, DNA cleaving and intercalation (20-23). Intercalator molecules accumulate in hydrogen bounds of double stranded DNA (dsDNA) due to their planar aromatic rings structure and are stacked themselves between bases (24).

Many biosensor investigations in the concerning interaction between DNA and drug molecules have been performed including various methods (such as NMR, raman, electrochemical, etc.) for the identification of interaction mechanisms. Electrochemical biosensors introduce a convenient, rapid and efficient way for determining, monitoring and diagnosing; using biological interactions (25, 26). Electrochemical DNA sensors (genosensors) provide a simple method for expecting changes in the DNA structure.

The DNA biosensor simply based on a recognition surface covered with DNA molecule, a signal transducer and amplifier that determines and
amplifies the biological interaction between two DNA strands and a user interface that shows the data from interaction (27-29).

In this work seven 1H-benzimidazole derivatives were investigated for potential DNA damage agents. The interactions between drug and DNA were analyzed by using drug’s signals with DPV. The voltammetric peak currents were obtained from ssDNA and dsDNA shows the mechanism of drug interacting with DNA.

RESULTS AND DISCUSSION

Chemistry

A synthetic route for the 1H-benzimidazole derivatives 1-7 is outlined in the Figure 1. In this work we synthesized seven benzimidazole derivatives bearing different substitutions at position 2- and 5/6-(Figure 1). In the initial step of the synthetic process, the reaction of 4-hydroxybenzaldehyde with sodium bisulfide solution afforded sodium hydroxy(4-hydroxyphenyl) methan sulphonate salt, while the condensation reaction of this salt with unsubstituted or 5/6-substituted o-phenylenediamine analogs were achieved in the second step (12). Following the reaction of 4-(1H-benzimidazol-2-yl)phenol (I) with substituted alkyl derivatives in the presence of sodium hydroxide, the reaction mixture were refluxed for two hours at 95°C after adding aminoaalkylhalogenates. The structures of the synthesized compounds were elucidated by melting point, IR, 1H NMR, 13C NMR and mass spectral data.

By the help of IR spectra data, the bands between 2400-3200 cm\(^{-1}\), are attributed to N-H-N type hydrogen bonds, which are characteristic for benzimidazole derivatives. Moreover, the N-H, C=C and C=N stretching bands were observed at 3390-3460 cm\(^{-1}\), 1650-1500 cm\(^{-1}\) and 1500-1400 cm\(^{-1}\), respectively (30). Also, asymmetric and symmetric C-O-C stretching were studied at 1200-1275 cm\(^{-1}\) and 1020-1075 cm\(^{-1}\) (31).

\(^{1}\)H NMR results were interpreted in experimental section. The aromatic proton signals of p-hydroxyphenyl substituent at position 2- of 1H-benzimidazole ring were observed within prospective chemical shift values and divisions as 1,4-disubstituted benzene system (as two protons doublets H-3', H-5' at \(\delta\) 6.87-6.95 and H-2', H-6' at \(\delta\) 7.90-7.98) while the hydrogen atoms at positions 4- and 7- were not detected at the prospective divisions (32).

The lack of the signal belonging to 3a, 4, 7 and 7a C in \(^{13}\)C NMR are noteworthy that may suggest a proton exchange due to 1, 3-tautomerization (32). All the other aliphatic and aromatic carbons were observed at expected regions.

Electrochemical Detection

The genosensor relied on the electrochemical transduction of interaction between DNA and 1H-benzimidazole derivatives for the detection of interaction mechanism. DGE surfaces were modified with ds and ssDNA by adsorption and accumulation was performed with various concentrations of drug solutions. The detection of interaction between DNA (dsDNA and ssDNA) and drugs were monitored by using the oxidation signals of 1, 2, 4, 5, 6, 7 and reduction signal of 3 where electrochemical responses had led to significant different voltammetric signals after interaction.

Typical DPV behaviors of 1H-benzimidazole derivatives in the absence and presence of ds / ss DNA were studied. The changes in the peak currents of 1H-benzimidazole derivatives such as (A) 1, (B) 3, (C) 6, (D) 2, (E) 7, (F) 5, (G) 4 at bare (a), drug modified (b) and after interaction with dsDNA (c) and single stranded DNA (ssDNA) (d) are shown in Figure 2.

Electrochemical responses of 1H-benzimidazole derivatives have been evaluated due to their accumulation magnitude. It was observed that compounds 1 (A), 3 (B) and 6 (C) have significant higher signals in the presence of dsDNA in the comparison with in the presence of ssDNA. Significantly decrease in the signals in the absence of DNA at bare electrodes shows the adsorption characteristic of these drugs, furthermore obtaining relatively higher decreases in the presence of ssDNA supports intercalation characteristic of compounds 1, 3 and 6 (33). Besides in the presence of ssDNA, significant higher responses obtained from compounds 2 (D), 5 (F) and 4 (G) when compared within the dsDNA due to their guanine interaction. On the other hand, the electrochemical responses of compound 7 (E), no significantly differences were observed between in the presence of ss and dsDNA.
For the detection of optimum drug concentration, the electrochemical responses of various concentrations of 1H-benzimidazole derivatives solutions were studied in Figure 3.

Optimum drug concentrations relied on their stable voltammetric signal, were found as, 2 μg/mL of compound 1, 10 μg/mL of 3, 2.4 μg/mL of 6, 10 μg/mL of 2, 10 μg/mL of 7, 18.20 μg/mL of 5, and 50 μg/mL of 4 at the detection limits of 0.62 nM, 1.26 nM, 0.69 nM, 1.23 nM, 0.42 nM, 1.13 nM, and 1.08 nM of respectively.

Figure 4 represents the histammograms of DPV signals of A) 1, B) 3, C) 6, D) 2, E) 7, F) 5, G) 4 at bare (a), drug modified (b) and after interaction with dsDNA (c) and ssDNA (d).

Optimum drug concentrations were accumulated at dsDNA and ssDNA modified surfaces. It was observed that before interaction, 1H-benzimidazole derivates were highly diffused at graphite surfaces due to their affinity. But this high diffusion could not be observed after DNA coated surfaces.

A resume of interaction types of 1H-benzimidazole derivatives with DNA was briefly presented in Table 1.

In conclusion, following the synthesis of 7 different 1H-benzimidazole derivatives, their interactions with DNA were monitored by using electrochemical genosensing technique. Detecting the voltammetric behavior of several drugs that interact with DNA would be valuable in the design of sequence-specific DNA binding molecules for application in chemotherapy and in the development of biotechnological tools for the point-of-care tests based on DNA. Determining the interaction mechanisms of new drugs with DNA lead new insight into rational drug design. These studies can play a key role in developing novel chemotherapeutic agents that could be pivotal in targeting specific genes and thereby provide selective control of gene expression.
EXPERIMENTAL
Chemistry
All melting points were determined with a capillary melting points apparatus (Buchi 510, BUCHI, Flawil, Switzerland). The IR spectra of compounds were monitored as potassium bromide pellets (FT/IR-430, JASCO, Tokyo, Japan). The NMR spectra (400 MHz for $^1$H and 100 MHz for $^{13}$C) were recorded in the deuterated solvent indicated with chemical shifts reported in parts per million (δ). δ units downfield from tetramethylsilane (TMS). Coupling constants (J) are reported in hertz (Hz) (AS 400 Mercury Plus NMR Varian, Palo Alto, USA). Mass spectra were measured in methanol (Merck) solution.

### TABLE 1. Interaction types of 1H-benzimidazole derivatives with DNA.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Electrochemical Behaviour</th>
<th>Hypothesis of Interaction Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxidation at ~ 0.5 V</td>
<td>intercalation</td>
</tr>
<tr>
<td>2</td>
<td>Oxidation at ~ 0.75V</td>
<td>guanine damage</td>
</tr>
<tr>
<td>3</td>
<td>Reduction at ~ 0.30 V</td>
<td>intercalation</td>
</tr>
<tr>
<td>4</td>
<td>Oxidation at ~ 0.5 V</td>
<td>guanine damage</td>
</tr>
<tr>
<td>5</td>
<td>Oxidation at ~ 0.95 V</td>
<td>guanine damage</td>
</tr>
<tr>
<td>6</td>
<td>Oxidation at ~ 0.5 V</td>
<td>intercalation</td>
</tr>
<tr>
<td>7</td>
<td>Oxidation at ~ 0.8 V</td>
<td>no interaction</td>
</tr>
</tbody>
</table>
LCMS-2010 High Performance Liquid Chromatograph Mass Spectrophotometer, Schimadzu Q-array (quadrupole) for 6 and HP 6890 Series GC System Mass spectrometer HP 6890 Mass Selective Detector, (Hewlett Packard, Palo Alto, USA) for the other derivatives. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60F254) with detection by UV light (254 nm). All starting materials and reagents were high-grade commercial products.

General procedure for the synthesis of N-2-[4-(1H-benzimidazole-2-yl)phenoxy]ethyl]substitutedamine derivatives;

4-(1H-benzimidazole-2-yl)phenol was synthesized with o-phenylenediamine as described (12). After reacting 4-(1H-benzimidazole-2-yl)phenol (0.005 mol) and A (Figure 1) (0.01 mol) with sodium hydroxide (0.015 mol) in 10 mL ethanol, the mixture was refluxed at 95 °C for 2 h in an oil bath. Ethanol was evaporated and the residue was extracted with ether. The compounds were obtained by preparative TLC (CHCl3/C6H6/CH3OH/25% NH4OH solution, 5:1:0.5:0.05) and crystallization with methanol/water.

4-(1H-Benzo[d]imidazol-2-yl)phenol (1).
C14H12N2O; Yield 77%; Mp: 271 °C; 1H NMR (CH3OD): 6.93 (2H, d, J= 9.0 Hz, H-3′, H-5′), 7.21 (2H, dd, J= 3.1, 6.2 Hz, H-5, H-6), 7.55 (2H, dd, J= 3.1, 6.3 Hz, H-4, H-7), 7.93 (2H, d, J= 9.0 Hz, H-2′, H-6′). 13C NMR (CH3OD): 115.7 (C-3′, C-5′), 120.9 (C-1′), 122.4 (C-5, C-6), 128.3 (C-2′, C-6′), 138.9 (C-3a and/or C-7a), 152.7 (C-2), 159.8 (C-4′). FT-IR (KBr), cm⁻¹: 3311, 2922, 1252. CI MS (m/z): 211 [M+1]+.

4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenol (2).
C13H9ClN2O; Yield 90%; Mp: 257 °C; 1H NMR (CH3OD): 6.93 (2H, d, J= 8.6 Hz, H-3′, H-5′), 7.18 (1H, dd, J= 2.0, 8.6 Hz, H-6), 7.48 (1H, d, J= 8.6 Hz, H-7), 7.52 (1H, d, J= 1.6 Hz, H-4), 7.91 (2H, d, J= 8.6 Hz, H-2′, H-6′). 13C NMR (CH3OD): 115.6 (C-3′, C-5′), 120.6 (C-1′), 122.6 (C-6), 127.8 (C-5), 128.4 (C-2′, C-6′), 154.1 (C-2), 160.1 (C-4′). FT-IR (KBr), cm⁻¹: 3631, 3201, 1492, 1268. CI MS (m/z): 246 [M+1]+.

FIGURE 4: Histograms based on DPV signals of A) 1, B) 3, C) 6, D) 2, E) 7, F) 5, G) 4 at bare (a), drug modified (b) and after interaction with dsDNA (c) and ssDNA (d).
2-(4-Hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (3).

C_{14}H_{16}N_{2}O_{2}; Yield 63%; Mp: >300 °C; 1H NMR (CD3OD): 6.95 (2H, d, J = 9.0 Hz, H-3', H-5'), 7.58 (1H, d, J = 8.6 Hz, H-7), 7.93 (1H, dd, J = 2.0, 8.4 Hz, H-6), 7.95 (2H, d, J = 8.6 Hz, H-2', H-6'), 8.26 (1H, d, J = 1.6 Hz, H-4). ^13C NMR (CD3OD): 113.8 (C-7), 115.8 (C-3', C-5'), 116.7 (C-4), 120.3 (C-1'), 124.2 (C-6), 125.1 (C-5), 128.7 (C-2', C-6'), 130.3 (C-3a), 142.3 (C-7a), 155.1 (C-2), 160.4 (C-4'), 169.4 (C=O), FT-IR (KBr), cm⁻¹: 3334, 2360, 1636, 1508, 1303. CI MS (m/z): 255 [M+1]^+.

4-(5,6-Dimethyl-1H-benzo[d]imidazol-2-yl)phenol (4).

C_{15}H_{18}N_{2}O; Yield 60%; Mp: >300 °C; 1H NMR (CD3OD): 2.29 (6H, s, 2xAr-CH3), 6.87 (2H, d, J = 9.0 Hz, H-3', H-5'), 7.27 (2H, s, H-4, H-7), 7.94 (2H, d, J = 8.6 Hz, H-2', H-6'). ^13C NMR (CD3OD): 20.7 (2xAr-CH3), 116.3 (C-3', C-5'), 122.2 (C-1'), 128.6 (C-2', C-6'), 130.5 (C-5, C-6), 151.6 (C-2), 159.5 (C-4'). FT-IR (KBr), cm⁻¹: 3254, 2967, 1610, 1253. CI MS (m/z): 239 [M+1]^+.

N,N-Dimethyl-2-(4-(5-methyl-1H-benzo[d]imidazol-2-yl)phenoxo)ethanamine (5).

C_{19}H_{25}N_{3}O; Yield 18%; Mp: 134 °C; 1H NMR (CDCl3) (δ/ ppm): 2.30 (6H, s, H-1'), 2.40 (3H, s, Ar-CH3), 2.72 (2H, t, J = 5.5 Hz, H-2'), 3.04 (2H, t, J = 5.5 Hz, H-1'), 4.00 (2H, t, J = 8.9 Hz, H-3', H-5'), 4.08 (2H, d, J = 8.9 Hz, H-2', H-6'). ^13C NMR (CDCl3) (δ/ ppm): 21.9 (Ar-CH3), 46.0 (C-1'), 58.3 (C-2'), 66.1 (C-1'), 115.1 (C-3', C-5'), 123.0 (C-1'), 124.3 (C-6), 128.3 (C-2', C-6'), 132.7 (C-5), 151.8 (C-2), 160.4 (C-4'). FT-IR (KBr), cm⁻¹: 3039, 2971, 2882, 1613, 1498, 1262, 1046, 837, 792. CI MS (m/z): 296 [M+1]^+.

2-(4-(5-Chloro-1H-benzo[d]imidazol-2-yl)phenoxy)-N,N-dimethylethanamine (7).

C_{17}H_{19}ClN_{2}O; Yield 25%; Mp: 115 °C; 1H NMR (CDCl3) (δ/ ppm): 2.37 (6H, s, H-1'), 2.78 (2H, t, J = 5.5 Hz, H-2'), 4.11 (2H, t, J = 5.5 Hz, H-1'), 5.09 (2H, d, J = 9.0 Hz, H-3', H-5'), 7.19 (1H, dd, J = 2.0, 8.6 Hz, H-6), 7.49 (1H, d, J = 8.6 Hz, H-7), 7.56 (1H, s, H-4), 7.93 (2H, d, J = 8.6 Hz, H-2', H-6'). ^13C NMR (CDCl3) (δ/ ppm): 49.6 (C-1'), 58.3 (C-2'), 66.2 (C-1'), 115.3 (C-3', C-5'), 122.4 (C-1'), 123.4 (C-6), 128.4 (C-2', C-6'), 151.3 (C-2), 160.8 (C-4'). FT-IR (KBr), cm⁻¹: 2974, 2830, 1612, 1499, 1263, 1046, 837, 742. CI MS (m/z): 317 [M+1]^+.

**Electrochemical Detection**

Calf Thymus dsDNA and ssDNA were purchased (as lyophilized powder) from Sigma. DNA stock solutions were prepared with Mili Q water and kept frozen.

To dilute the stock solutions 0.5 M acetic buffer (pH 4.80) is used. Other chemicals were of analytical reagent grade. All solutions are prepared with ultra pure water.

All of the electrochemical detections were carried out by an AUTOLAB PGSTAT 30 electrochemical analyzer (Eco Chemie, The Netherlands) using differential pulse voltammetry (DPV). Three electrode systems were utilized, which comprised the pencil graphite electrode (PGE), Ag/AgCl as the reference electrode and the indicator electrode, the Pt wire.

**General procedure for Electrochemical Detection**

Determination of the interaction mechanism of 1H-benzimidazole derivatives with DNA was performed at DNA modified DGE sensor surfaces. DGE's were pretreated by applying 1.4 V for 60 seconds in phosphate buffer solution (pH 7.4) for over-oxidation. Calf thymus ds and ssDNA's were immobilized onto DGE surfaces via adsorption (34). Accumulation of 1H-benzimidazole derivatives were performed at DNA modified DGE surfaces with various concentrations of their solutions by applying open circuit mode for 5 minutes (27, 28). Determination of interaction mechanisms of drugs's were obtained by applying DPV for transduction of benzimidazole derivatives' electrochemical responses. Figure 5 represents the schematic presentation of detection.
1H-Benzimidazol türevlerinin sentezi ve DNA ile etkileşimlerinin voltametrik tayini

ÖZET: Çalışma baza 1H-benzimidazol türevlerinin sentezi ve elektrokimyasal genosensör teknolojisi kullanılarak DNA ile etkileşimlerinin tayini gerçekleştirmiştir. DNA – ilaç etkileşiminin tayini ilacların tek kullanımlık grafet elektrot yüzeylerinde (DGE) diferansiyel puls voltametrisi (DPV) ile yükseltgenme sinyallerinin görüntülenmesine dayanılar. 7 farklı 1H-benzimidazol türevinin çift sarmal DNA ile etkileşimi sonucu artan voltametrik sinyallere dayalı olarak alınan sonuçlarda gözlenen tayin sınırları: 1. bileşik 0.62 nM, 2. bileşik, 1.23 nM, 3. bileşik, 1.26 nM, 4. bileşik, 1.08 nM, 5. bileşik 1.13 nM, 6. bileşik 0.69 nM ve 7. bileşik için 0.42 nM olarak gözlenmiştir.

ANAHTAR KELİMELER: 1H-benzimidazol, elektrokimyasal genosensör, DNA-ilaç etkileşimi, sentez.

REFERENCES


