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ORIGINAL RESEARCH

Synthesis and voltammetric detection of 1*H*-benzimidazole derivatives on the interaction with DNA

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ABSTRACT: In this study, synthesis and determination of interaction between DNA and 1*H*-benzimidazole derivatives has been performed by using electrochemical genosensor technology. Detection of DNA – drug interaction mechanism relies on monitoring Differential pulse voltammetric (DPV) responses of drugs at disposable graphite electrodes (DGEs). Interaction of 7 different benzimidazole derivates between double-stranded (ds) and single-stranded (ss) DNA were observed by changing the voltammetric signals of drugs at the detection limits of 0.62 nM of compound 1, 1.23 nM of 2, 1.26 nM of 3, 1.08 nM of 4, 1.13 nM of 5, 0.69 nM of 6 and 0.42 nM of 7, respectively.

KEY WORDS: 1*H*-benzimidazole, electrochemical genosensor, DNA-drug interaction, synthesis

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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, steric, 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

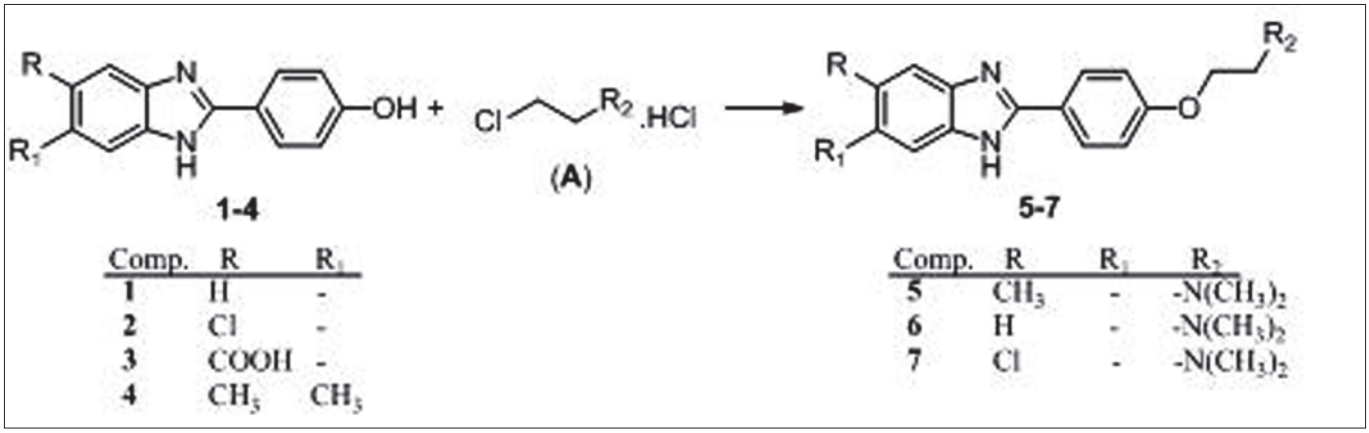

FIGURE 1: The preparation of substituted benzimidazoles.

amplifies the biological interaction between two DNA strands and a user interface that shows the data from interaction (27-29).

In this work seven 1*H*-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 1*H*-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 sulfonate salt, while the condensation reaction of this salt with nonsubstituted or 5/6-substituted *o*-phenylenediamine analogs were achieved in the second step (12). Following the reaction of 4-(1*H*-benzimidazol-2-yl)phenol (**1**) with substituted alkyl derivatives in the presence of sodium hydroxide, the reaction mixture were refluxed for two hours at 95 ^{0}C after adding aminoalkylhalogenates. The structures of the synthesized compounds were elucidated by melting point, IR, ^{1}H NMR, ^{13}C 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 1*H*-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 δ 6.87-6.95 and H-2′, H-6′ at δ 7.90-7.98) while the hydrogen atoms at positions 4- and 7- were not detected at the prospective divisions (32). The compounds substituted with the terminal of ethoxy chain at position 4- of phenyl group gave rise to similar chemical shift values and divisions at the ring of the benzimidazole nucleus as **1-3**.

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 1*H*-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 1*H*-beznimidazole derivatives in the absence and presence of ds / ss DNA were studied. The changes in the peak currents of 1*H*-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 1*H*-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.

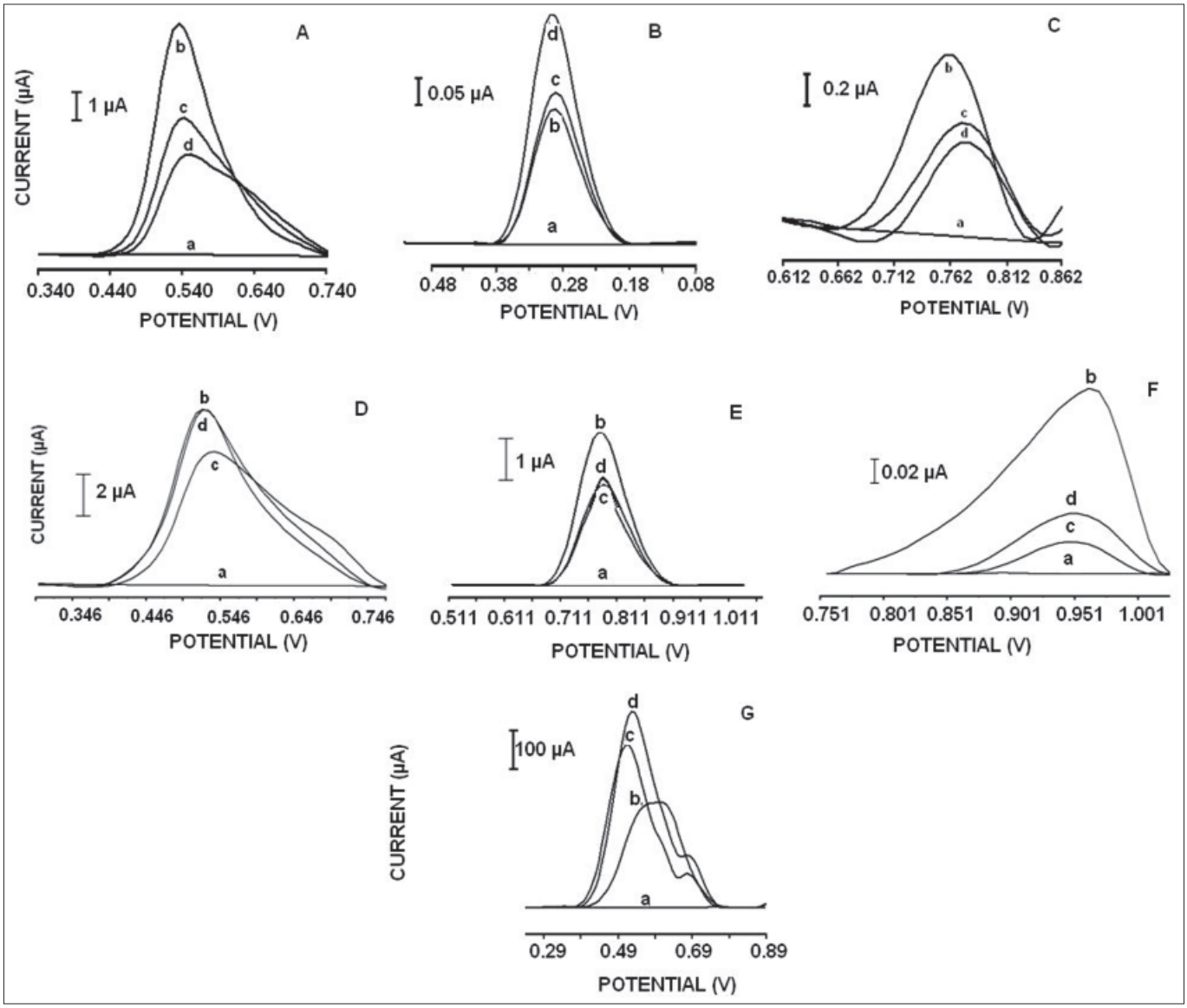

FIGURE 2: DPV signals of 1*H*-benzimidazole derivatives A) 1, B) 3, C) 6, D) 2, E) 7, F) 5, G) 4 at bare *(a)*, drug modified *(b)* and after interaction with dsDNA *(c)* and ssDNA *(d)*.

For the detection of optimum drug concentration, the electrochemical responses of various concentrations of 1*H*-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 30 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, 1*H*-benzimidazol derivates were highly difused 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 1*H*-benzimidazole derivatives with DNA was briefly presented in Table 1.

In conclusion, following the synthesis of 7 different 1*H*-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.

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

Compounds	Electrochemical Behaviour	Hypothesis of Interaction Mechanism
1	Oxidation at ~ 0.5 V	intercalation
2	Oxidation at ~ 0.75V	guanine damage
3	Reduction at ~ 0.30 V	intercalation
4	Oxidation at ~ 0.5 V	guanine damage
5	Oxidation at ~ 0.95 V	guanine damage
6	Oxidation at ~ 0.5 V	intercalation
7	Oxidation at ~ 0.8 V	no interaction

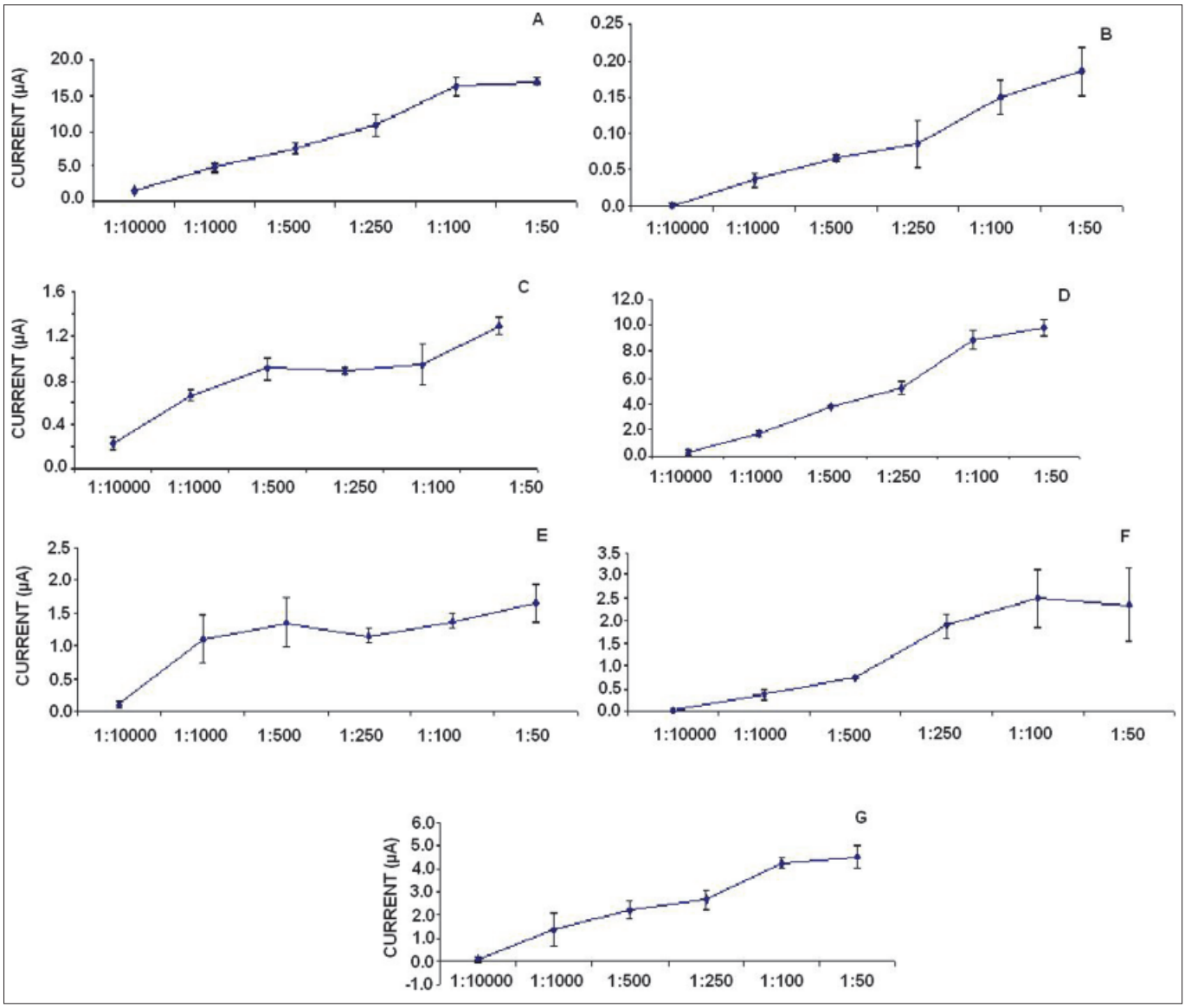

FIGURE 3: Calibration plot of various dilutions of 1*H*-benzimidazole derivatives based on drugs electrochemical signals.

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

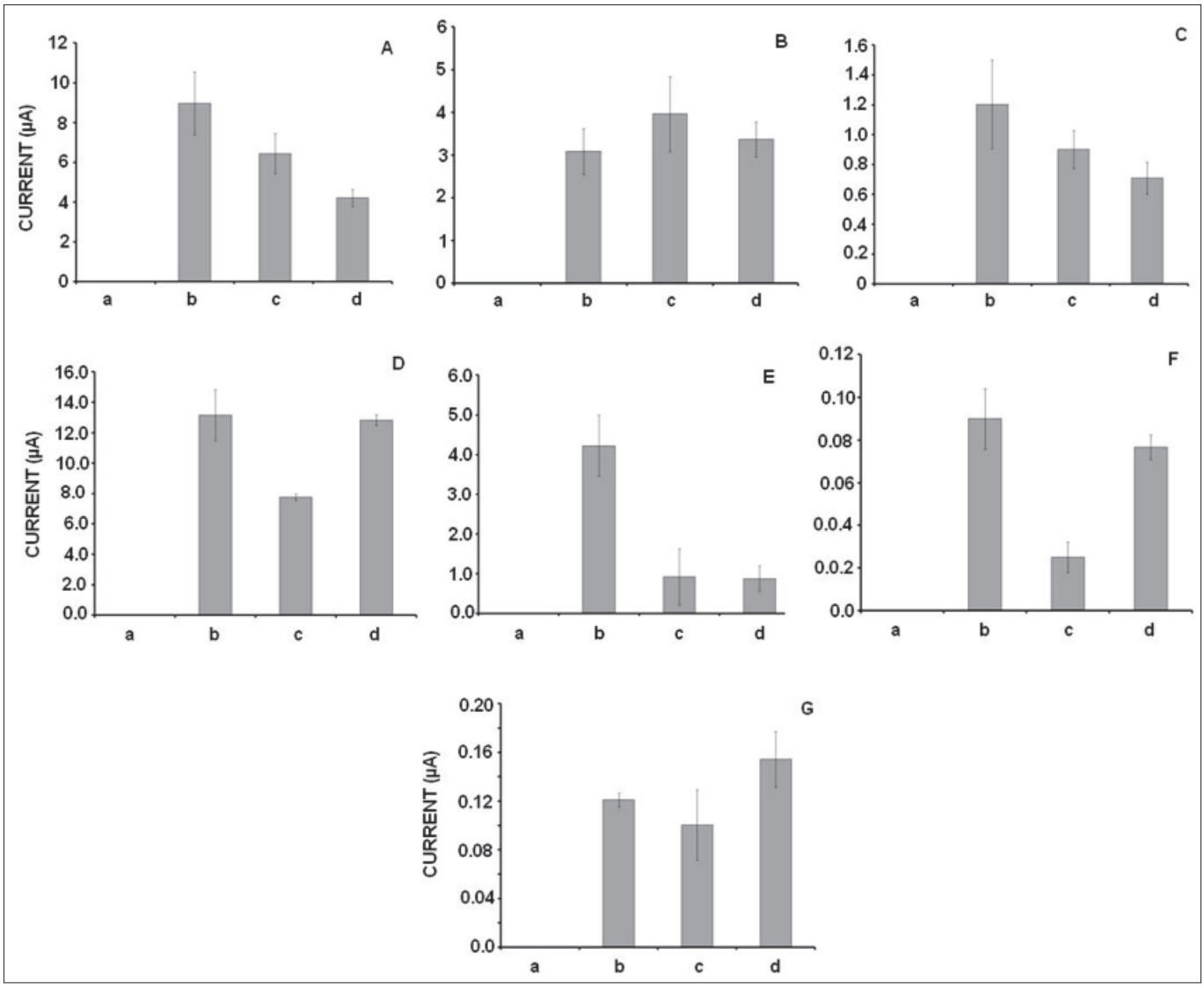

FIGURE 4: Histammograms based on DPV signals of A) 1, B) 3, C) 6, D) 2, E) 7, F) 5, G) 4 at bare *(a)*, drug modified *(b)* and after interaction with dsDNA *(c)* and ssDNA *(d)*.

LCMS-2010 High Performance Liquid Chromatograph Mass Spectrofotometer, 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-(1*H*-benzimidazole-2-yl)phenoxy]ethyl}substitutedamine derivatives;

4-(1*H*-benzimidazole-2-yl)phenol was synthesized with *o*-phenylenediamine as described (12). After reacting 4-(1*H*-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 ^{0}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 ($CHCl_3$/C_6H_6/CH_3OH/25% NH_4OH solution, 5:1:0.5:0.05) and crystallization with methanol/water.

4-(1*H*-Benzo[*d*]imidazol-2-yl)phenol (**1**).

$C_{14}H_{12}N_2O$; Yield 77%; Mp: 271 ^{0}C; ^{1}H NMR (CH_3OD): 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′). ^{13}C NMR (CH_3OD): 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, 1500, 1252. CI MS (m/z): 211 $[M+1]^+$.

4-(5-Chloro-1*H*-benzo[*d*]imidazol-2-yl)phenol (**2**).

$C_{13}H_9ClN_2O$; Yield 90%; Mp: 257 ^{0}C; ^{1}H NMR (CH_3OD): 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′). ^{13}C NMR (CH_3OD): 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 $[M+1]^+$.

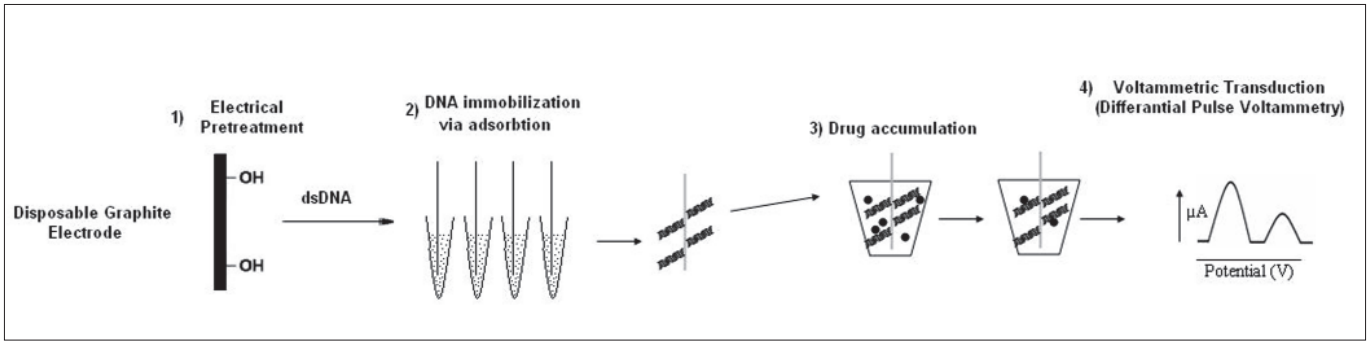

FIGURE 5: Voltammetric detection procedure of interactin between 1*H*-benzimidazole derivatives and DNA.

2-(4-Hydroxyphenyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid (**3**).

$C_{14}H_{10}N_2O_3$; Yield 63%; Mp: >300 ^{0}C; ^{1}H NMR (CH_3OD): 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). ^{13}C NMR (CH_3OD): 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′), 138.7 (C-3a), 142.3 (C-7a), 155.1 (C-2), 160.4 (C-4′), 169.4 (-COOH). FT-IR (KBr), cm^{-1}: 3334, 2360, 1636, 1508, 1303. CI MS (m/z): 255 $[M+1]^+$.

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

$C_{15}H_{14}N_2O$; Yield 60%; Mp: >300 ^{0}C; ^{1}H NMR (CH_3OD): 2.29 (6H, s, 2xAr-CH_3), 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′). ^{13}C NMR (CH_3OD): 20.7 (2xAr-CH_3), 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-1*H*-benzo[*d*]imidazol-2-yl)phenoxy)ethanamine (**5**).

$C_{18}H_{21}N_3O$; Yield 18%; Mp: 134 ^{0}C; ^{1}H NMR ($CDCl_3$) (δ/ppm): 2.30 (6H, s, H-1‴), 2.40 (3H, s, Ar-CH_3), 2.72 (2H, t, *J*= 5.5 Hz, H-2″), 4.03 (2H, t, *J*= 5.5 Hz, H-1″), 6.90 (2H, d, *J*= 8.9 Hz, H-3′, H-5′), 6.98 (1H, dd, *J*= 0.9, 8.2 Hz, H-6), 7.30 (1H, s, H-4), 7.41 (1H, d, *J*= 8.2 Hz, H-7), 7.90 (2H, d, *J*= 8.8 Hz, H-2′, H-6′). ^{13}C NMR ($CDCl_3$) (δ/ppm): 21.9 (Ar-CH_3), 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-(1*H*-Benzo[*d*]imidazol-2-yl)phenoxy)-*N,N*-dimethylethanamine (**6**).

$C_{17}H_{19}N_3O$; Yield 32%; Mp: 212 ^{0}C; ^{1}H NMR ($CDCl_3$): 2.36 (6H, s, H-1‴), 2.77 (2H, t, *J*= 5.2 Hz, H-2″), 4.09 (2H, t, *J*= 5.2 Hz, H-1″), 6.92 (2H, d, *J*= 8.8 Hz, H-3′, H-5′), 7.22 (2H,dd, *J*= 3.2, 6.4 Hz, H-5, H-6), 7.60 (2H, dd, *J*= 3.2, 6.0 Hz, H-4, H-7), 7.98 (2H, d, *J*= 9.2 Hz, H-2′, H-6′). ^{13}C NMR ($CDCl_3$) (δ/ppm): 46.0 (C-1‴), 58.4 (C-2″), 66.1 (C-1″), 115.2 (C-3′, C-5′), 122.9 (C-5, C-6), 128.4 (C-2′, C-6′), 152.1 (C-2), 160.5 (C-4′). FT-IR (KBr), cm^{-1}: 3061, 2973, 2851, 1611, 1497, 1247, 1042, 834, 745. CI MS (m/z): 282 $[M+1]^+$.

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

$C_{17}H_{18}ClN_3O$; Yield 25 %; Mp: 115 ^{0}C; ^{1}H NMR ($CDCl_3$) (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″), 6.94 (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′). ^{13}C NMR ($CDCl_3$) (δ/ppm): 46.0 (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′), 153.1 (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 0.50 M acetate 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 grahite 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 1*H*-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 overoxidation. Calf thymus ds and ssDNA's were immobilized onto DGE surfaces via adsorption (34). Accumulation of 1*H*-benzimdazole derivates 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 derivates' electrochemical responses. Figure 5 represents the schematic presentation of detection.

1*H*-Benzimidazol türevlerinin sentezi ve DNA ile etkileşimlerinin voltametrik tayini

ÖZET: Çalışmada bazı 1*H*-benzimidazol türevlerinin sentezi ve elektrokimyasal genosensör teknolojisi kullanılarak DNA ile etkileşimlerinin tayini gerçekleştirilmiştir. DNA – ilaç etkileşiminin tayini ilaçların tek kullanımlık grafit elektrot yüzeylerinde (DGE) diferansiyel puls voltametrisi (DPV) ile yükseltgenme sinyallerinin görüntülenmesine dayanır. 7 farklı 1*H*-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: 1*H*-benzimidazol, elektrokimyasal genosensör, DNA-ilaç etkileşmesi, sentez.

REFERENCES

1. Göker H, Özden S, Yıldız S, Boykin DW. Synthesis and potent antibacterial activity against MRSA of some novel 1,2-disubstituted-1H-benzimidazole-N-alkylated-5-carboxamidines. Eur J Med Chem 2005;40: 1062-9.
2. Coburn RA, Clark MT, Evans RT, Genco RJ. Substituted 2-(2-hydroxyphenyl)benzimidazoles as potential agents for the control of periodontal diseases. J Med Chem 1987;30: 205-8.
3. Göker H, Kus C, Boykin DW, Yıldız S, Altanlar N. Synthesis of some new 2-substituted-phenyl-1H-benzimidazole-5-carbonitriles and their potent activity against candida species. Bioorg Med Chem 2002;10: 2589-96.
4. Garcia-Domenech R, Rios-Santamarina I, Catala A, Calabuig C, Del Castillo L, Galvez J. Application of molecular topology to the prediction of antifungal activity for a set of dication-substituted carbazoles, furans and benzimidazoles. J Mol Struct (Theochem)2003;624: 97-107.
5. Güven OO, Erdoğan T, Göker H, Yildiz S. Synthesis and antimicrobial activity of some novel phenyl and benzimidazole substituted benzyl ethers. Bioorg Med Chem Lett 2007;17: 2233-6.
6. Ates-Alagoz Z, Yıldız S, Buyukbingol E. Antimicrobial activities of some tetrahydronaphthalene-benzimidazole derivatives. Chemotherapy 2007;53: 110-3.
7. Ramla MM, Omar MA, El-Khamry AM, El-Diwani HI. Synthesis and antitumor activity of 1-substituted-2-methyl-5-nitrobenzimidazoles. Bioorg Med Chem 2006;14: 7324-32.
8. Taniguchi K, Shigenaga S, Ogahara T, Fujitsu T, Matsuo M. Synthesis and antiinflammatory and analgesic properties of 2-amino-1H-benzimidazole and 1,2-dihydro-2-iminocycloheptimidazole derivatives. Chem Pharm Bull 1993;41: 301-9.
9. Estrada-Soto S, Villalobos-Molina R, Aguirre-Crespo F, Vergara-Galicia J, Oreno-Diaz H, Torres-Piedra M, Navarrete-Vazquez G. Relaxant activity of 2-(substituted phenyl)-1H-benzimidazoles on isolated rat aortic rings: design and synthesis of 5-nitro derivatives. Life Sci 2006;79: 430-5.
10. Shimai T, Islam MT, Fukushi Y, Hashidoko Y, Yokosawa R, Tahara SZ. Nicotinamide and structurally related compounds show halting activity against zoospores of the phytopathogenic fungus aphanomyces cochlioides. Naturforsch C 2002;57: 323-31.
11. Ray SD, Balasubramanian G, Bagchi D, Reddy CS. Ca2+-calmodulin antagonist chlorpromazine and poly(ADP-ribose) polymerase modulators 4-aminobenzamide and nicotinamide influence hepatic expression of BCL-XL and P53 and protect against acetaminophen-induced programmed and unprogrammed cell death in mice. Free Radic Biol Med 2001;31: 277-91.
12. Alpan AS, Günes HS, Topcu Z. 1H-Benzimidazole derivatives as mammalian DNA topoisomerase I inhibitors. Acta Biochim Pol 2007;54: 561-5.
13. Alpan AS, Zencir S, Zupkó I, Coban G, Réthy B, Gunes HS, Topcu Z. Biological activity of bis-benzimidazole derivatives on DNA topoisomerase I and HeLa, MCF7 and A431 cells. J Enzym Inhib Med Ch 2009;24: 844-9.
14. Jin S, Kim JS, Sim S, Liu A, Pilch DS, Liu LF, La Voie EJ. Heterocyclic bibenzimidazole derivatives as topoisomerase I inhibitors. Bioorg Med Chem Lett 2000;10: 719-23.
15. Seaton A, Higgins C, Mann J, Baron A, Bailly C, Neidle S, Van den Berg H. Mechanistic and anti-proliferative studies of two novel, biologically active bis-benzimidazoles. Eur J Cancer 2003;39: 2548-55.
16. Bagni G, Osella D, Sturchio E, Mascini M. Deoxyribonucleic acid (DNA) biosensors for environmental risk assessment and drug studies. Anal Chim Acta 2006;573-574: 81-9.
17. Rauf S, Gooding JJ, Akhtar K, Ghauri MA, Rahman M, Anwar MA, Khalid AM. Electrochemical approach of anticancer drugs-DNA interaction. J Pharmaceut Biomed 2005;37: 205-17.
18. Kara P, Kerman K, Ozkan D, Meric B, Erdem A, Ozkan Z, Ozsoz M. Electrochemical genosensor for the detection of interaction between methylene blue and DNA Electrochem Commun 2002;4: 705-9.
19. Haq I. Thermodynamics of drug-DNA interactions. Arch Biochem Biophys 2002;403: 1-15.
20. Shaikh SA, Ahmed SR, Jayaram B. A molecular thermodynamic view of DNA-drug interactions: a case study of 25 minor-groove binders. Arch Biochem Biophys 2004;429: 81-99.
21. Richards AD, Rodger A. Synthetic Metallomolecules as Agents for the Control of DNA Structure. Chem Soc Rev 2007;36: 471-83.
22. Eichhorn GL, Shin YA. Interaction of metal ions with polynucleotides and related compounds. XII. The relative effect of various metal ions on DNA helicity. J Am Chem Soc 1968;90: 7323-8.

23. Snow GA. The molecular basis of antibiotic action. Editors: Gale EF, Cundliffe E, Reynolds PE, Richmond MH, Waring Wiley MJ. London, New York, Sydney, Toronto 1982, pp.152.

24. Campbell PN. Nucleic acid biochemistry and molecular biology. Editors: Mainwaring WIP, Parish JH, Pickering JD, Mann NH. Blackwell Scientific Publications, Oxford, London 1982, pp. 57.

25. Wang J. Electrochemical biosensors: Towards point-of-care cancer diagnostics. Biosens Bioelectron 2006;21: 1887-92.

26. Bogdanovskaya VA, Tarasevich MR. Electrochemical biosensors for medicine and ecology. Biosens Bioelectron 1996;11: 853-61.

27. Meric B, Kerman K, Ozkan D, Kara P, Erdem A, Kucukoglu O, Erciyas E, Ozsoz M. Electrochemical biosensor for the interaction of DNA with the alkylating agent 4,4′-dihydroxy chalcone based on guanine and adenine signals. J Pharmaceut Biomed 2002;30: 1339-46.

28. Kerman K, Meric B, Ozkan D, Kara P, Erdem A, Ozsoz M. Electrochemical DNA biosensor for the determination of benzo[a]pyrene-DNA adducts. Anal Chim Acta 2001;450: 45-52.

29. Li N, Guo L, Jiang J, Yang X. Interaction of echinomycin with guanine: Electrochemistry and spectroscopy studies. Biophys Chem 2004; 111: 259-65.

30. Preaston PN. The Chemistry of Heretocyclic Compounds of Benzimidazoles and Congeneric Tricyclic Compounds. John Wiley & Sons, New York 198, pp 63-4.

31. Silverstein RM, Bassler GC, Morrill TC. Spectrometric Identification of Organic Compounds. John Wiley & Sons, New York 1981, chapter 3.

32. Sridharan V, Saravanan S, Muthusubramanian S, Sivasubramanian S. NMR investigation of hydrogen bonding and 1,3-tautomerism in 2-(2-hydroxy-5-substituted-aryl) benzimidazoles. Magn Reson Chem 2005;43: 551-6.

33. Kerman K, Ozkan D, Kara P, Karadeniz H, Ozkan Z, Erdem A, Jelen F, Ozsoz M. Electrochemical detection of specific DNA sequences from PCR amplicons on carbon and mercury electrodes using Meldola's Blue as an intercalator. Turk J Chem 2004;28: 523-33.

34. Ariksoysal DO, Karadeniz H, Erdem A, Sengonul A, Sayiner AA, Ozsoz M. Label-free electrochemical hybridization genosensor for the detection of hepatitis B virus genotype on the development of lamivudine resistance. Anal Chem 2005;77: 4908-17.