TÜBİTAK

Turkish Journal of Chemistry

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

Research Article

Turk J Chem
(2017) 41: 40 – 47

doi:10.3906/kim-1601-1

Electrochemical detection of DNA interaction with Mannich base derivatives by disposable graphite electrodes

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Received: 03.01.2016 • **Accepted/Published Online:** 16.06.2016 • **Final Version:** 22.02.2017

Abstract: Four different Mannich base derivatives containing an aromatic/heteroaromatic propanone structure (C1 (3-(dimethylamino)-1-(thiophen-2-yl)propan-1-one hydrochloride); C2 (3-morpholino-1-(pyridin-3-yl)propan-1-one hydrochloride); C3 (3-(dimethylamino)-1-(*1H*-indol-3-yl)propan-1-one hydrochloride), C4 (3-(piperidin-1-yl)-1-(pyren-1-yl)propan-1-one hydrochloride)) were earlier synthesized and characterized with ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. The interaction of these compounds with fish sperm double stranded DNA (fs-dsDNA) was investigated by using differential pulse voltammetry (DPV) in connection with a disposable pencil graphite electrode (PGE). After the interaction procedure, there was a meaningful decrease in the oxidation signal of the electroactive DNA base guanine due to possible intercalation and/or alkylation mechanism between these Mannich base derivatives and DNA. The features of this electrochemical assay were discussed in comparison to previous reports related to DNA targeted agents/drug candidates in the literature.

Key words: Electrochemical DNA biosensor, Mannich base, pencil graphite electrode, differential pulse voltammetry

1. Introduction

Mannich bases are pharmacologically important molecules as they have been reported to show a wide range of bioactivities such as antineoplastic, diuretic, antipsychotic, anticonvulsant, central acting muscle relaxant, antibacterial, antimalarial, and antiviral activities.[1] The formations of activity of Mannich bases have different mechanisms; Mannich bases can turn into active unsaturated structures via a deamination reaction.[2] The resulting conjugated enone structures are very suitable centers for nucleophilic attack and are considered to be the Michael-type acceptor.[3] Because of these properties, Mannich bases may show cytotoxic activity by alkylating biological nucleophiles such as DNA guanine base.[4]

DNA is the pharmacological target of many of the drugs that are currently in clinical use or in advanced clinical trials as nucleophiles.[5] Small molecules bind to DNA either covalently (for example, alkylation-cisplatin) or noncovalently by the formation of hydrogen bonds, and electrostatic and/or hydrophobic interactions. The noncovalent binders are divided into two types: groove binders and intercalators.[6]

An important part of DNA-interacting compounds is intercalated compounds. Intercalator compounds are molecules that have a planar moiety usually including several fused rings, which interact with double-strand DNA by insertion of a planar moiety between adjacent base pairs of the double helix.[7] Alkylating agents can

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be defined as compounds capable of covalently binding an alkyl group to a biomolecule under physiological conditions. When the alkylating agents attack guanine bases in DNA, cross-linking DNA could be formed.[8] The interaction types of molecules with DNA have been studied by a variety of techniques: optical techniques (e.g., UV-Vis absorption spectroscopy, fluorescence spectroscopy, IR spectroscopy, NMR, circular and linear dichroism, viscosity measurement) and electrochemical techniques (e.g., electrochemical biosensors).[9−14]

Recently, there is growing interest in the design of biosensors for the detection of interactions between nucleic acids and target drugs for their rapid screening.[15−23] Electrochemical DNA biosensors (genosensors) play an important role in pharmaceutical, clinical, and environmental applications since electrochemistry provides rapid, simple, and low-cost point-of-care detection of specific nucleic acid sequences, and also the interaction between ligands and nucleic acids.[24−33]

In the study by Erdem et al.,[15] the interactions of *cis*-diaminedichloroplatinum(II)(*cis*-DDP) and *cis*-bis(3-aminoflavone) dichloroplatinum(II) (*cis*-BAFDP) with calf thymus double-stranded DNA (dsDNA) were explored electrochemically by using differential pulse voltammetry (DPV) in combination with a pencil graphite electrode (PGE). After DNA interaction with these drugs at the IC_{50} values, a decrease was obtained in DPV signals of both electroactive DNA bases, guanine and adenine. The voltammetric detection of the interaction between lycorine (LYC) and calf thymus dsDNA/calf thymus single-stranded DNA (ssDNA) was studied based on the oxidation signals of guanine and adenine by using DPV at a carbon paste electrode (CPE) and PGE.[22] Brett et al.[25] studied the electrochemical monitoring of the interaction between the anthraquinone drug mitoxantrone (MTX) and dsDNA or ssDNA by using a glassy carbon electrode (GCE) in connection with square-wave voltammetry (SWV) and differential pulse voltammetry (DPV). Fojta et al.[27] studied the conformational changes in DNA due to binding of DNA intercalators such as the anticancer agent doxorubicin (DXR) by using adsorptive transfer stripping alternative current voltammetry at a hanging mercury drop electrode (HMDE).

In addition, an important part of ongoing research in our pharmaceutical chemistry laboratory involves the investigation of the synthesis and cytotoxic activity of some Mannich bases and determination of their mechanism of this action. Within this framework, a series of Mannich bases with different ring sizes and different amines were synthesized, characterized (shown in Table 1), and investigated for their cytotoxic activity against HeLa, PC3, and MCF7 cell lines in our previous work.[34] Their alkylation capability with an in vitro incubation test and DNA-intercalating capability with an EtBr displacement assay were also reported.[34]

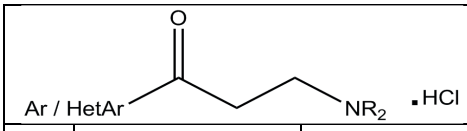
The aim of the present study was to investigate the electrochemical detection of the interaction capability of planar Mannich bases (C1/C2/C3/C4) with fs-dsDNA by using DPV in combination with disposable PGEs since these electrodes have crucial properties that have brought some important advantages, such as being single-use, easy to handle, and robust and providing a more sensitive and faster electrochemical detection protocol.

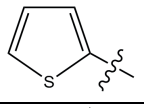
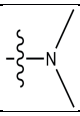
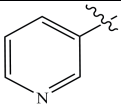
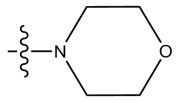
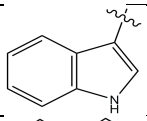
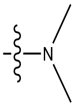
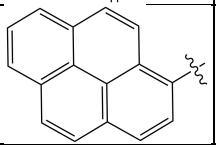
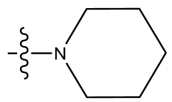
2. Results and discussion

The electrochemical detection of the interaction between fish sperm double-stranded DNA (fs-dsDNA) and Mannich bases was performed based on the changes in guanine oxidation signal, about +1.00 V, by using DPV. According to this procedure, 10 μg/mL concentration levels of compounds were used and the guanine oxidation signal before and after the interaction was measured after 30 min interaction time. After the DNA interaction with each compound, there was a meaningful decrease obtained in the guanine oxidation signal. Figure 1

shows the voltammograms obtained before and after the interaction between fs-dsDNA and each compound: C1/C2/C3/C4.

Table 1. Molecular formulae and chemical names of the compounds.



No	Ar / HetAr	NR_2	Chemical name
C1			3-(dimethylamino)-1-(thiophen-2-yl)propan-1-one hydrochloride
C2			3-morpholino-1-(pyridin-3-yl)propan-1-one hydrochloride
C3			3-(dimethylamino)-1-(*1H*-indol-3-yl)propan-1-one hydrochloride
C4			3-(piperidin-1-yl)-1-(pyren-1-yl)propan-1-one hydrochloride

A decrease was obtained in the guanine oxidation signals after the surface-confined interaction process between fs-dsDNA and each compound: C1/C2/C3/C4. The decrease ratio % was calculated and found respectively as 47.02%, 36.82%, 50.02%, and 35.80% (shown in Table 2). This decrease in electrode response obtained after the interaction process was similar to the results in earlier reports related to DNA interaction of some anticancer drugs and DNA-targeted compounds.[14,25,26,35,36] It may be attributed to the intercalation of these Mannich bases into the base pairs of DNA. This phenomenon could be explained by the shielding of oxidizable groups of electroactive base guanine,[14,16.22] while the compounds can interact with the double helix of DNA at the electrode surface due to possible intercalation and/or alkylation mechanism. Thus, we may explain this decrease in electrode response as the preferential binding of these compounds: Mannich bases to the electroactive DNA base guanine. The decrease in guanine signal can be explained by H-bond capability and/or reactivity of formed enone (Michael-type acceptor) structures. The studies are ongoing for establishing the structure–activity relationship.

A decrease was observed in the guanine signal (Figures 1 and 2b–2d) after the interaction of C1–C3 with dsDNA by possible alkylation. It could be a result of high alkylation capacity of enone structures, which occurred by deamination of Mannich base.[32] According to the results given in Figures 1 and 2e, a decrease was recorded in the guanine signal after a possible interaction of C4 with dsDNA due to its intercalation into DNA base pairs since C4 has a four-ring aromatic structure that is a compatible result with those of the EtBr displacement assay presented in an earlier study by our group[34] and other studies.[35,36]

In the presence of a surface-confined interaction between DNA and 10 μg/mL of each compound: C1/C2/C3/C4 individually, a series of three repetitive DPV measurements were performed by using an fs-dsDNA modified PGE. Thus, it resulted in reproducible results and they are shown in Table 2 with their

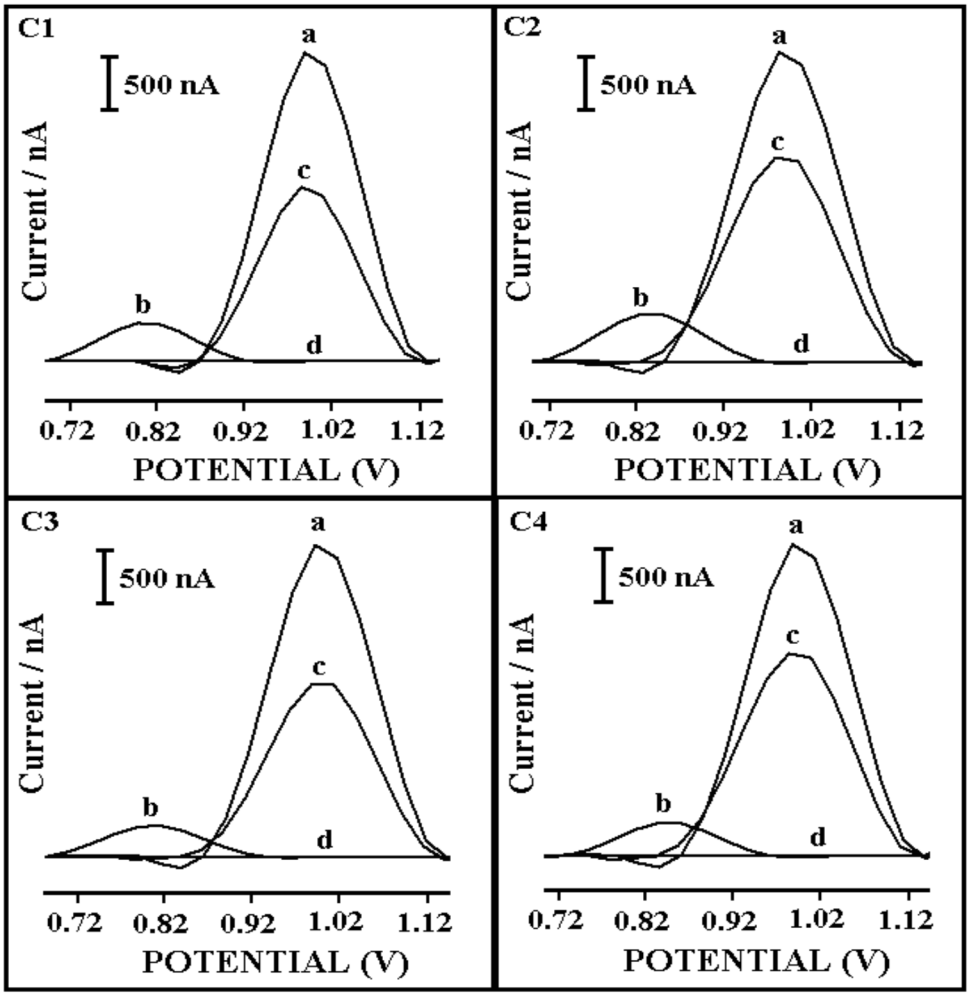


Figure 1. Voltammograms representing the oxidation signals of compound and guanine observed before and after the interaction of 10 μg/mL the compounds (C1/C2/C3/C4) with 16 μg/mL dsDNA for 30 min interaction time by using PGEs. The oxidation signals of dsDNA (a) and compound (b) before surface-confined interaction (control experiments), and the oxidation signal of guanine after surface-confined interaction of dsDNA with each compound (c) and blank ABS (d).

Table 2. The average guanine oxidation signal and the RSD % values obtained after interaction of dsDNA with each compound, and the decrease ratios (%) in the guanine signals.

	Average guanine signal (nA) with standard deviation	RSD % (n = 3)	Decrease ratio (%) in guanine signal after surface-confined interaction
DNA	2909 ± 303	10.4	—
DNA + C1	1541 ± 225	14.6	47.0
DNA + C2	1838 ± 250	13.6	36.8
DNA + C3	1454 ± 122	8.4	50.0
DNA + C4	1867 ± 134	7.2	35.8

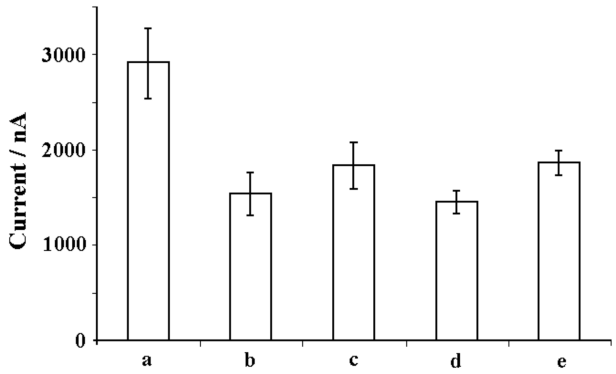


Figure 2. Histograms representing the average guanine oxidation signals (n = 3) obtained before (a) and after the interaction of dsDNA with each compound individually (b) C1, (c) C2, (d) C3, and (e) C4 by using PGEs.

relative standard deviations (n = 3). The detection limit estimated from $S/N = 3$ corresponds to 0.47, 0.41, 0.48, and 0.41 μg/mL, respectively, for C1/C2/C3/C4.

In conclusion, the benefit of the electrochemical recognition of the interaction between each Mannich base (C1/C2/C3/C4) and DNA has been shown for the first time herein using a faster, more sensitive, and less laborious voltammetric technique with the advantages of disposable PGE technology.[14,16,37] The success of the PGE over existing carbon electrodes is because it is commercially available, practical and cost-effective in use, and has better reproducibility. The electrochemical method presented here is experimentally convenient and sensitive, requiring only small amounts of DNA or compounds. The electrochemical detection of interactions between DNA-targeted molecules and nucleic acids would be valuable in the design of a molecule-specific electrochemical biosensor for applications in diagnosis tests and development of drugs for chemotherapy.

3. Experimental

3.1. Reagents and chemicals

Fish sperm double-stranded DNA (fs-dsDNA) was purchased from Sigma (Germany) and stock solutions (1000 mg/L) were prepared with 10 mM Tris–HCl containing 1 mM EDTA (TE; pH 8.00) and were kept frozen. The stock solutions of fs-dsDNA were then diluted using 0.5 M acetic acid/acetate buffer containing 20 mM NaCl (acetic acid/acetate buffer solution (ABS); pH 4.80) according to an earlier report.[38]

Other chemicals were of analytical reagent grade and were supplied by Sigma and Merck (Germany). All buffer solutions were prepared using deionized water.

3.2. Synthesis and characterization of compounds

Synthesis of compounds was carried out according to a conventional Mannich reaction (C3) and with a Mannich reagent (C1, C2, C4).[39,40] Detailed synthesis and characterization data were reported previously (shown in Table 1).[34] The solutions of compounds were prepared in 0.05 M sodium dihydrogen phosphate and sodium monohydrogen phosphate buffer solution (PBS; pH 7.40).[38]

According to the results of our earlier study, the IC_{50} values of each compound were calculated and found in micromolar concentration level for in vitro cytotoxic activity.[34] The IC_{50} values of C1 on HeLa, PC3, and MCF7 cell lines were 78.68, 65.98, and 35.59 μM, corresponding to 14.43, 12.10, and 6.53 μg/mL, respectively. The IC_{50} values of C2 on HeLa, PC3, and MCF7 cell lines were 90.90, 73.29, and 49.89 μM, corresponding to

20.05, 16.16, and 11.00 μg/mL, respectively. The IC_{50} values of C3 on HeLa, PC3, and MCF7 cell lines were >100 μM, corresponding to >21.63 μg/mL. IC_{50} values of C4 on HeLa, PC3, and MCF7 cell lines were 60.10, 34.48, and 25.50 μM, corresponding to 20.52, 11.77, and 8.70 μg/mL, respectively. Due to the critical amount of each synthesized compound, the approximate value to each concentration value of each compound as 10.00 μg/mL concentration level was chosen for the DNA interaction study.

3.3. Apparatus

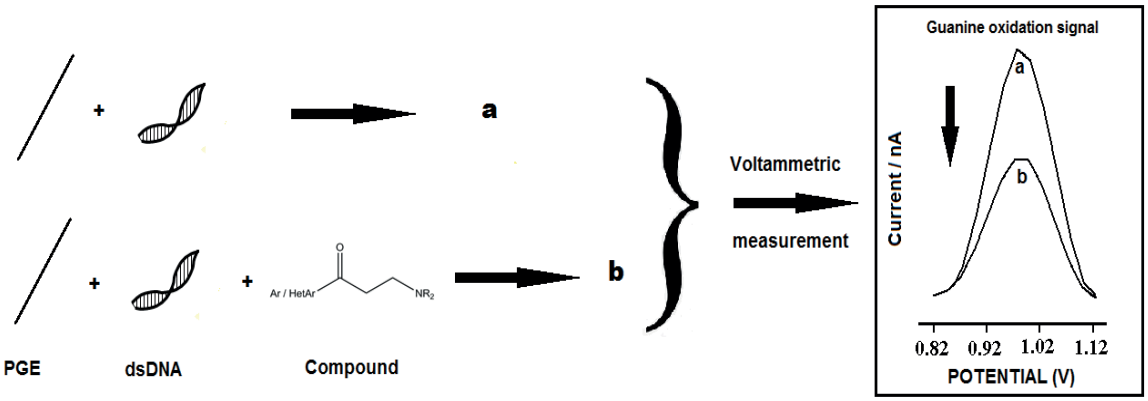
The guanine oxidation signal was measured using differential pulse voltammetry (DPV) with an AUTOLAB-PGSTAT 302 electrochemical analysis system and a GPES 4.9 software package (Eco Chemie, Utrecht, the Netherlands). The three-electrode system consisted of a pencil graphite electrode (PGE), a Ag/AgCl/KCl reference electrode (BAS, Model RE-5B, W. Lafayette, IN, USA), and a platinum wire as the auxiliary electrode. The raw data were also treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a peak width of 0.01.

3.4. Electrode preparation

The disposable PGE was used in DPV measurements for the electrochemical detection of interaction.[35] A Tombo pencil was used as a holder for the graphite lead (0.5 HB, Tombo, Japan). Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was held vertically with 14 mm of the lead extruded outside (10 mm of which was immersed in the solution). Then the PGE was pretreated by applying +1.40 V for 30 s in 0.05 M acetate buffer solution (pH 4.80) without stirring.[38]

3.5. Procedure

Each measurement involved the immobilization of the DNA, interaction of DNA with each of Mannich bases (C1/C2/C3/C4), and detection cycle at a new surface of the PGE. A representative experimental procedure is given in the Scheme. All the experiments were performed at room temperature (25.0 $\pm$ 0.5 °C). Repetitive measurements were carried out using freshly prepared PGEs in the absence/presence of the interaction process.



Scheme. Schematic presentation of electrochemical monitoring of interaction between DNA and a compound (representative Mannich base) using PGE.

3.6. Interaction of surface-confined DNA with drugs by PGE

First 16 μg/mL fs-dsDNA was immobilized onto the surface of the pretreated PGE by wet-adsorption procedure over 7.5 min as mentioned in our previous work.[38] The electrode was then rinsed with ABS for 10 s. The dsDNA-modified PGE was then immersed into the solution of 10 μg/mL each compound (C1/C2/C3/C4) prepared in PBS (pH 7.40). Each electrode was then rinsed with PBS for 10 s. The control experiments were done in the absence of compounds or DNA.

3.7. Voltammetric measurement

The guanine oxidation signal was measured in ABS by DPV in the potential range varying between +0.4 V and +1.3 V with 50-mV pulse amplitude and 50-mV/s scan rate according to the literature.[12,14,17,18,22]

Acknowledgment

This work has been partially supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) (project number: SBAG-110S082), and Ege University, Faculty of Pharmacy, Project coordination (project number: 09/ECZ/016).

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