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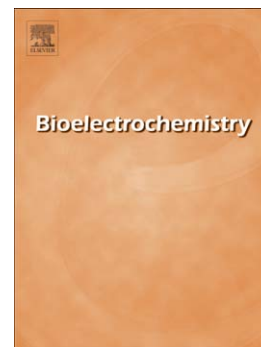
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PII: S1567-5394(15)30024-4
DOI: doi: [10.1016/j.bioelechem.2015.10.004](https://doi.org/10.1016/j.bioelechem.2015.10.004)
Reference: BIOJEC 6890

To appear in: *Bioelectrochemistry*

Received date: 3 August 2015
Revised date: 22 September 2015
Accepted date: 23 October 2015



Please cite this article as: Victor Constantin Diculescu, Ana Maria Oliveira-Brett, *In situ* electrochemical evaluation of dsDNA interaction with anticancer drug danusertibnitrenium radical metabolite using the DNA-electrochemical biosensor, *Bioelectrochemistry* (2015), doi: [10.1016/j.bioelechem.2015.10.004](https://doi.org/10.1016/j.bioelechem.2015.10.004)

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***In situ* electrochemical evaluation of dsDNA interaction
with anticancer drug danusertibnitrenium radical
metabolite using the DNA-electrochemical biosensor**

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Abstract

Danuserib is a kinase inhibitor and anti-cancer drug. The evaluation of the interaction between danuserib and dsDNA was investigated in bulk solution and using the dsDNA-electrochemical biosensor. The dsDNA-danuserib interaction occurs in two sequential steps. First, danuserib binds electrostatically to dsDNA phosphate backbone through the positively charged piperazine moiety. The second step involved the pyrrolo-pyrazole moiety and led to small morphological modifications in the dsDNA double helix which were electrochemically characterised through the changes of guanine and adenine residues oxidation peaks and confirmed by electrophoretic and spectrophotometric measurements. The nitrenium cation radical product of danuserib amino group oxidation was electrochemically generated *in situ* on the dsDNA-electrochemical biosensor surface. The danuserib nitrenium cation radical redox metabolite was covalently attached to the C8 of guanine residues preventing their oxidation. An interaction mechanism of dsDNA-danuserib is proposed and the formation of the danuserib redox nitrenium radical metabolite-guanine adduct explained.

Keywords: danuserib; nitrenium radical; DNA interaction; guanine adduct; electrochemical oxidation

1 Introduction

Protein kinases are enzymes responsible for phosphorylation processes [1] and play an important role in the control of the cell cycle and signal transduction pathways [2]. Overexpression of kinases have been reported in a number of cancers accounting for their role in tumour genesis and progression [3,4]. Several classes of kinases have been recognised as targets for the development of small inhibiting molecules for anticancer therapy [5,6].

Danuserib, (*R*)-*N*-(5-(2-methoxy-2-phenylacetyl)-1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazol-3-yl)-4-(4-methylpiperazin-1-yl)benzamide[7,8], Scheme 1, is a recent inhibitor of Aurora kinases [9] which also binds and inhibits with high affinity ($IC_{50} < 0.50 \mu M$) other tyrosine kinases [10]. The cross-reactivity of danuserib with different kinases has great anticancer therapeutic potential, making danuserib an effective drug for the treatment of multiple tumours [11,12].

Here Scheme 1

The occurrence of point mutations into the target proteins as well as promotion of genomic instability due to DNA damage accounted for toxicity and resistance to treatment with small molecule kinase inhibitors [13,14]. *In vitro* studies demonstrated that kinase inhibitors and/or their metabolites can increase the amount of DNA damages [15] and showed that some of these compounds retained a genotoxic activity either through intercalation into the DNA, formation of alkali-labile sites and/or DNA strand breaks [15-17]. In these conditions, methods that enable screening for toxicity are crucial.

Electrochemical methods, such as pulse techniques, are suitable for investigating biological systems since they present high sensitivity and allow the *in situ* electrochemical generation, on the electrode surface, of reactive radicals and metabolites arising from a compound redox reaction that interact with the biological material immobilised on the electrode surface [18].

A dsDNA-electrochemical biosensor consists of an electrochemical transducer with dsDNA immobilised on the surface [18,19]. Among the electrochemical transducers, carbon electrodes demonstrated several unique properties. The extensive potential window in the positive direction allowed sensitive electrochemical detection of both dsDNA conformational changes and oxidative damage caused to DNA. The occurrence of the DNA components oxidation peaks, nucleotides, nucleosides, purine and pyrimidine bases, and of guanine (Gua) oxidation product, 8-oxoguanine (8-oxoGua), and adenine (Ade) oxidation product, 2,8-dihydroxyadenine (2,8-oxoAde), biomarkers of DNA base oxidative damage [18,19], was monitored. On the other hand, the surface characteristics of dsDNA-electrochemical biosensors visualised by *ex situ* MAC mode AFM showed that the complete coverage of the electrode surface is crucial for the robustness of the dsDNA film and reduction of non-specific adsorption [18].

The dsDNA-electrochemical biosensor has been applied to investigate the interaction of DNA with chemical compounds [20] such as antidepressant [21], antibiotics [22] and anticancer drugs [23-26] including kinase inhibitor imatinib mesylate [27], heavy metals [28,29] and radical species [30]. Comparing with other methods, the dsDNA-electrochemical biosensor shows greater sensitivity towards detecting small perturbations of the DNA double-helical structure and detection of oxidative damage caused to DNA.

The voltammetric behaviour of danusertib was previously studied and compared to similar compounds such as imatinib mesylate providing insights into the redox reactions of this class of molecules [31-33]. Danusertib is oxidised in a cascade mechanism resulting in a nitrenium radical [31]. In general, the electrophilic nitrenium radicals produced *in vivo* after conversion of amine groups are genotoxically active [34]. Nitrenium radicals covalently bind to DNA bases, especially to guanine residues producing DNA damage [35].

In this context and since danusertib may participate in several redox reactions in the intracellular milieu, the aim of the present study is the electrochemical investigation of the DNA interaction with danusertib and its nitrenium radical redox metabolite using the dsDNA-electrochemical biosensor.

2 Experimental

2.1 Materials and reagents

Danusertib was obtained from Selleck Chemicals. Sodium salt double stranded DNA (dsDNA) from calf thymus, polyadenylic (poly[A]) and polyguanylic (poly[G]) acids were obtained from Sigma-Aldrich and used without further purification.

A stock solution of 100 μM danusertib was prepared in DMSO/deionised water mixture (10% v/v) and kept at 4 °C. Solutions of different concentrations of danusertib were prepared by dilution of the appropriate quantity in 0.1 M acetate buffer pH = 4.5 supporting electrolyte. Stock solutions of 300 $\mu\text{g mL}^{-1}$ dsDNA, poly[A] and poly[G] were prepared in deionised water and diluted to the desired concentrations prior to use.

The pH measurements were carried out using a Crison micropH 2001 pH-meter with Ingold combined glass electrode. All experiments were done at room temperature ($25 \pm 1^\circ\text{C}$) and microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Raining Instrument Co. Inc., Woburn, USA).

2.2 Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using an Ivium-n-Stat, running Ivium software version 2.425. Measurements were carried out using a three-electrode system, a glassy carbon working electrode (GCE, $d = 1.0\text{ mm}$), a Pt wire counter electrode and an Ag/AgCl (3M KCl) reference electrode, in a 3 mL one compartment electrochemical cell (eDAQ Europe).

The experimental conditions for differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 100 ms, potential increment of 5 mV and scan rate 5 mV s⁻¹.

The GCE was polished using diamond spray (UK Kemet, particle size 1 µm) before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q water. Following the mechanical treatment, the GCE was placed in supporting electrolyte and voltammograms were recorded until a steady state baseline voltammogram was obtained.

2.3 UV spectrophotometry

The UV-VIS measurements were performed using a spectrophotometer U-2810 from Digilab Hitachi. The experimental conditions for absorption spectra were: slit width 1.5 nm, sampling interval 0.5 nm and a scan speed 400 nm min⁻¹. All UV-Vis spectra were measured from 230 nm to 400 nm, in a quartz glass cuvette with an optic path of 1 cm. UV-Vis spectra were recorded for different incubation times in 0.1 M acetate buffer pH 4.5 of 50 µg mL⁻¹ dsDNA with different concentrations of danusertib. Control solutions of 50 µg mL⁻¹ dsDNA or danusertib were also prepared and UV-Vis spectra and recorded for the same time periods.

2.4 Gel electrophoresis

Nondenaturing agarose (1.0 %, ultrapure DNA grade from Sigma) gel was prepared in TAE buffer (10 mM Tris base, 4.4 mM acetic acid and 0.5 mM EDTA, pH 8.0). 25 µL of dsDNA control solution and danusertib-dsDNA samples aliquots (with 0.25 % bromophenol blue in water) were loaded into wells, and electrophoresis was carried out in TAE buffer for 2 h at ~ 100 V. After 0.5 % ethidium bromide (EtBr) stained DNA was visualised and photographed under UV (312 nm) transillumination to visualise DNA mobility.

2.5 Incubation procedures

2.5.1 Procedure 1- incubated solutions

Mixed solutions of 1 μM danusertib and 50 $\mu\text{g mL}^{-1}$ dsDNA were incubated in 0.1 M acetate buffer pH = 4.5 during different time periods. Control solutions of 50 $\mu\text{g mL}^{-1}$ dsDNA and of 1 μM danusertib were prepared 0.1 M acetate buffer pH = 4.5 and stored in similar conditions and during the same time periods. DP voltammograms were recorded in solution after different incubation times always using a clean GCE surface.

2.5.2 Procedure 2- dsDNA-electrochemical biosensor

The dsDNA-electrochemical biosensors were prepared covering the GCE surface with one drop of 2.5 μL from a 35 mg mL^{-1} dsDNA solution diluted in deionised water. After placing the drop on the electrode surface the biosensor was dried under a constant flux of N_2 .

For poly[A] and poly[G]-electrochemical biosensors, the surface of the GCE was covered with one drop of 5 μL from a 150 $\mu\text{g mL}^{-1}$ poly[A] or poly[G] solution.

The dsDNA-, poly[A]- or poly[G]-electrochemical biosensors were immersed in solutions of different concentrations of danusertib, in 0.1 M acetate buffer pH = 4.5, and allowed to incubate during different periods of time as indicated in the text.

Afterwards, the dsDNA-, poly[A]- or poly[G]-electrochemical biosensors were removed from the solution, washed with deionised water in order to remove the unbounded danusertib molecules and placed in the electrochemical cell, containing only the 0.1 M acetate buffer pH = 4.5 supporting electrolyte, where the transduction was performed by DP voltammetry.

For control experiments, the dsDNA-electrochemical biosensor was incubated in 0.1 M acetate buffer pH = 4.5 supporting electrolyte during the same time periods and in similar conditions as the solutions with danusertib.

2.6 Acquisition and presentation of data

Each experiment was carried out always with a freshly prepared biosensor in order to reduce the electrode fouling effects with oxidation products. Experiments were performed in triplicate and the standard deviation for danusertib, desoxiguanosine and desoxyadenosineoxidation peakswere 0.27, 1.80 and 1.92 nA, respectively.

All the voltammograms presented were baseline corrected using the automatic function included in the software. The mathematical treatment of the original voltammograms was used in the presentation of all experimental data for a better and clearer identification of the peaks. The values for peak currents were determined from the original untreated voltammograms after subtraction of the baseline.

3 Results and discussion

3.1 Voltammetric behaviour of danusertib and dsDNA revisited

The electrochemical behaviour, in 0.1 M acetate buffer pH = 4.5, of danusertib and dsDNA was revisited for control, Fig. 1.

The DP voltammogram in 0.1 M acetate buffer pH = 4.5, after adsorption during 5 min in a solution of 1 μ M danusertib, showed three consecutive charge transfer reactions: peak D1, at $E_{D1} = +0.83$ V, peak D2, at $E_{D2} = +0.88$ V, and peak D3, at $E_{D3} = +1.24$ V, Fig. 1.

Here Fig. 1

The danusertib electroactive centres were previously identified [31]. The oxidation peak D1 corresponded to the transfer of two electrons and one proton from the amine moiety involving the formation of a nitrenium radical cation. The oxidation peak D2 was due to the

two electrons and two protons reversible oxidation of the pyrrolo-pyrazol moiety, while oxidation peak D3 corresponded to the oxidation of the piperazine group.

In a new experiment, the GCE was held at + 0.85 V during 5 min in a solution of 1 μM danusertib, in 0.1 M acetate buffer pH = 4.5, in order to allow the formation of danusertibnitrenium radical cation that occurred at peak D1. Then, the electrode was washed with a jet of deionised water and transferred to the electrochemical cell that contained only buffer electrolyte, where DP voltammetry was performed. In these conditions only peaks D2 and D3 occurred, Fig. 1.

The DP voltammograms in 0.1 M acetate buffer pH = 4.5 of 50 $\mu\text{g mL}^{-1}$ dsDNA showed two small peaks corresponding to the DNA purine bases nucleosides oxidation, dGuo of guanine (G) residues, at $E_G = +1.01$ V, and dAdo of adenine (A) residues, at $E_A = +1.28$ V, Fig. 1.

3.2 Evaluation of danusertib-dsDNA interaction in incubated solutions

3.2.1 Electrochemistry

The electrochemical investigation of the danusertib-dsDNA interaction was carried out in incubated solutions containing 1 μM danusertib and 50 $\mu\text{g mL}^{-1}$ dsDNA, in 0.1 M acetate buffer pH = 4.5. DP voltammograms were recorded after different incubation periods, Fig. 2. After each measurement, the GCE surface was cleaned and rinsed with deionised water to remove all oxidation products adsorbed at the electrode surface.

Here Fig. 2

The DP voltammogram recorded immediately after the addition of danusertib to the dsDNA solution showed, similarly to the control experiments, Fig. 1, the occurrence of danusertib oxidation peaks D1 and D2 at the same potentials as in the danusertib solution, Figs. 1 and 2, followed by dsDNA oxidation peaks dGuo and dAdo, Figs. 1 and 2.

For increased incubation times, Fig. 2, peak D1 current remained constant but peak D2 current progressively decreased in a time-dependent manner. At the same time, dGuo and dAdo oxidation peaks increased in agreement with conformational modification of the dsDNA double helix, Fig. 2.

3.2.2 UV spectrophotometry

UV spectra were recorded in incubated solutions of 25 μM danusertib and 50 $\mu\text{g mL}^{-1}$ dsDNA, in 0.1 M acetate buffer pH = 4.5, after different incubation time periods, Fig. 3. Control solutions of 50 $\mu\text{g mL}^{-1}$ dsDNA and 25 μM danusertib were also prepared and the UV spectra recorded for the same incubation times.

Here Fig. 3

The UV spectra of danusertib showed an absorption band at $\lambda = 300.0$ nm, and the dsDNA absorption band at $\lambda = 258.5$ nm, Fig. 3. Upon incubation, the dsDNA absorption band showed a time-dependent hyperchromic effect during 3 hours incubation, in agreement with the relaxation and/or unwinding of the double helix structure. At the same time, the bathochromic shift of dsDNA adsorption band to $\lambda = 261.5$ nm as well as the presence of an isosbestic point at $\lambda = 248.0$ nm were indicative of a binding process between danusertib and DNA.

3.2.3 Electrophoresis

Agarose gel electrophoresis was performed to evaluate the relative migration profile of dsDNA samples before and after interaction with different concentrations of danusertib, Fig. 4. The dsDNA sample (lane 1) showed a large band due to different-sized long and three-dimensional fragments present in the sample. Increasing the danusertib concentration (lanes 2 to 4), the DNA migrated more relative to the control sample (lane 1). At the same time, the

corresponding bands became narrower while the intensity located at the loaded wells diminished with increasing danusertib concentration.

Here Fig. 4

The increased mobility observed after interaction with danusertib is in agreement with the relaxation and/or unwinding of the dsDNA double helix. In general, the migration distances for dsDNA and ssDNA fragments of the same length are expected to be different due to the accelerated mobility of ssDNA relative to dsDNA, although dsDNA senses a higher electrophoretic force due to the higher charge. Danusertib binding to dsDNA reduces the negative charge which is in agreement with the band narrowing observed for higher danusertib concentrations.

3.3 Evaluation of danusertib-DNA interaction using the dsDNA-electrochemical biosensor

During the electrochemical evaluation of the danusertib-DNA interaction in incubated solutions, an incomplete network film of co-adsorbed free dsDNA, free danusertib and dsDNA-danusertib complexes occurred at the GCE surface. Complete coverage of the electrode surface is important to avoid the undesired danusertib adsorption to the electrode surface, and it can be achieved using the GCE with immobilised dsDNA.

The dsDNA-electrochemical biosensor showed the changes that occurred to the dsDNA immobilised on the electrode surface during the interaction with danusertib, *in situ* and in real time. The DP voltammogram recorded with the dsDNA-electrochemical biosensor in buffer showed both dGuo and dAdo oxidation peaks, Fig. 5A, in agreement with the control dsDNA-electrochemical biosensor DP voltammogram, Fig. 1.

Here Fig. 5

In a new experiment, a newly prepared dsDNA-electrochemical biosensor was incubated in 10 μ M danusertib solution with no conditioning potential, **Fig. 5A**. Then, the

dsDNA-electrochemical biosensor was washed with deionised water to remove unbound danusertib molecules and transferred to the electrochemical cell containing only 0.1 M acetate buffer pH = 4.5. The DP voltammograms recorded in these conditions showed the main danusertib oxidation peak D1 followed by the dsDNA oxidation peaks dGuo and dAdo. The increase of danusertib oxidation peak D1 current after interaction with DNA shows that danusertib bound to the DNA double helix and was pre-concentrated on the modified electrode surface.

The binding of danusertib leads to small modifications on the dsDNA morphological conformation which were responsible for the small changes in dGuo and dAdo oxidation peak currents of the dsDNA immobilised at the GCE surface, Fig. 5A, when compared with the dsDNA control DP voltammogram, Fig. 1. The experiment was repeated, always with a new dsDNA-electrochemical biosensor, for different incubation times up to 60 min incubation, Fig. 5A, and all peaks progressively increased. This experiment showed that the interaction between danusertib and dsDNA induces time-dependent conformational modifications of the double helix structure.

The effect of danusertib concentration was also studied. The dsDNA-electrochemical biosensor was incubated during 30 min in danusertib solutions with different concentrations, Fig. 5B. Danusertib oxidation peaks increased with concentration in agreement with more danusertib molecules binding to the immobilised dsDNA film. At the same time, dGuo and dAdo peaks of the immobilised dsDNA increased in agreement with the morphological changes taking place in the dsDNA conformation. This experiment also showed that danusertib pre-concentrates in the dsDNA film, and that after interaction danusertib still undergoes oxidation.

3.4 *In situ* evaluation of interaction of danusertib redox metabolites with dsDNA

The interaction of oxidised danusertib products with dsDNA immobilised at the GCE surface was studied. When the potential corresponding to the peak D1 oxidation, at +0.85 V, is applied to the dsDNA-electrochemical biosensor, during incubation in the danusertib solution, the danusertib molecules diffusing towards the electrode surface are immediately oxidised.

The danusertib oxidation product formed at peak D1 was a nitrenium radical [31]. Nitrenium radicals covalently bind to DNA bases, especially to guanine residues, which is the purine base with the lowest oxidation potential, causing damage to DNA.

A newly prepared dsDNA-electrochemical biosensor was incubated at +0.85 V in 10 μ M danusertib solution during different time periods. Then, the dsDNA-electrochemical biosensor was washed with deionised water and the DP voltammogram recorded in 0.1 M acetate buffer pH = 4.5, Fig. 6A. Increasing the incubation time, a progressive time-dependent decrease of the dGuo oxidation peak was observed. The application of the +0.85 V conditioning potential will not oxidise the immobilised dsDNA since the guanine oxidation peak only occurs at higher potentials. Therefore, the decrease of dGuo oxidation peak is due to the interaction between guanine residues in the dsDNA and the *in situ* electrogenerated nitrenium radical redox product of danusertib oxidation. At the same time a small decrease of dAdo oxidation peak was observed.

Here Fig. 6

A newly prepared dsDNA-electrochemical biosensor was used in a similar experiment performed after 30 min incubation time at +0.85 V conditioning potential and the danusertib concentration was changed, Fig. 6B. As expected, the dGuo oxidation peak decreased with increasing danusertib concentration and a decrease of dAdo oxidation peak current was also observed for high danusertib concentration.

These experiments indicated the time-dependent interaction between the *in situ* electrogenerated nitrenium radical redox product of danusertib oxidation and the guanine residues.

In order to verify these interactions experiments using purine homo-polynucleotide single stranded poly[A]- and poly[G]-electrochemical biosensors were also performed, Fig. 7.

Here Fig. 7

The DP voltammogram, in 0.1 M acetate buffer pH = 4.5, of the poly[A]-electrochemical biosensor showed one anodic peak, at $E_A = +1.28$ V, corresponding to the oxidation of dAdo, Fig. 7A. Newly prepared poly[A]-electrochemical biosensors were incubated for 15 min in 10 μ M danusertib without conditioning potential and after applying +0.85 V conditioning potential. Without applying condition potential the danusertib oxidation peak D1 occurred and no changes on adenine residues oxidation peak were observed. However, after incubation at +0.85V conditioning potential for 15 min, Fig. 7A, the adenine residues oxidation peak remained constant but the danusertib oxidation peak D1 disappeared because the nitrenium radical, the redox product of danusertib oxidation, was *in situ* electrogenerated.

The DP voltammogram, in 0.1 M acetate buffer pH = 4.5, of the poly[G]-electrochemical biosensor showed one anodic peak, at $E_G = +1.01$ V, corresponding to the oxidation of dGuo residues, Fig. 7B. Newly prepared poly[G]-electrochemical biosensors were incubated for 15 min in 10 μ M danusertib without conditioning potential and after applying +0.85 V conditioning potential. On the voltammogram recorded without applying the conditioning potential danusertib oxidation peaks D1, D2 and D3 were all observed, as well as the guanine residues oxidation peak dGuo. After incubation at +0.85 V conditioning potential during 15 min, the disappearance of D1 and the decreasing of D2 danusertib oxidation peaks occurred, due to the *in situ* electrogenerated nitrenium radical formation, the redox product of

danusertib oxidation. The decrease of the guanine oxidation peak due to the reaction with the nitrenium radical, Fig. 7B, confirmed the results obtained with the dsDNA-electrochemical biosensor, Figs. 5 and 6.

3.5 Interaction mechanism

The experiments carried out in incubated solutions and with the dsDNA-electrochemical biosensor have shown that danusertib binding to dsDNA involved morphological conformational modifications of the double helix that can be detected through the changes of dGuo and dAdo oxidation peaks, Figs. 1 and 2.

The DP voltammograms recorded after 30 min dsDNA incubation with danusertib, showed both D1 and D2 danusertib oxidation peaks. The D3 danusertib oxidation peak was not seen because it was always superimposed with dAdo residues oxidation peak, Fig. 2. For longer incubation times, the D2 oxidation peak also disappeared. These experimental data were consistent with a two-step interaction mechanism. First, the formation of the danusertib-dsDNA complex involved the electrostatic attraction between the positively charged piperazine moiety, the electroactive centre responsible for D3 danusertib oxidation peak with $pK_a \sim 10.0$ [31], Scheme 1, and the negatively charged dsDNA phosphate backbone.

In the second step, for longer incubation times, a more stable danusertib-DNA complex involved the danusertib pyrrolo-pyrazole moiety, the electroactive centre responsible for D2 danusertib oxidation peak. This interaction caused the disappearance of D2 danusertib oxidation peak, Fig. 2. At the same time, the amino group responsible for D1 danusertib oxidation peak was still available for oxidation, Fig. 2. The interaction danusertib-dsDNA induces the distortion and opening of the dsDNA phosphate backbone enabling a better contact between the dsDNA bases and the electrode surface, and consequently the increase of

the dGuo and dAdooxidation peak currents, Figs. 2 and 5. This effect was confirmed by spectrophotometry, Fig. 3, and electrophoresis, Fig. 4.

The mechanism described was also confirmed by the experiments carried out with the poly[A]- and poly[G]-electrochemical biosensors, Fig. 7. At pH = 4.5, poly[A] occurs as a double-helical, parallel-stranded duplex held together by AH^+-H^+A base pairs [36]. In agreement with the proposed interaction mechanism, the DP voltammogram recorded after incubation in danusertib showed only D1 danusertib oxidation peak, Fig. 7A, since the electroactive centres responsible for the D2 and D3 danusertib oxidation peaks are involved in the interaction with the dsDNA structure. Whereas, at pH = 4.5, poly[G] occurs as single stranded, and for this reason the D2 and D3 danusertib oxidation peaks were also observed on the DP voltammograms recorded after incubation of the poly[G]-electrochemical biosensors in the danusertib solution, Fig. 7B.

Danusertib D1 oxidation peak involved the transfer of two electrons and one proton from the amine group resulting in a nitrenium cation radical [31], Scheme 2. Nitrenium radicals are well known carcinogens derived from the amine-containing compound that interacts with and covalently attaches to dsDNA leading to the formation of different DNA base-adducts [34,35].

Here Scheme 2

When the +0.85 V conditioning potential was applied to the dsDNA-electrochemical biosensor in the danusertib solution during different incubation periods, the nitrenium cation radical, product of D1 danusertib oxidation peak, was formed at the dsDNA-electrochemical biosensor surface and immediately interacted with dsDNA guanine residues, Scheme 2. The danusertib redox metabolite-guanine adduct formation involved the guanine electroactive centre at position C8, Scheme 2, explaining the dGua oxidation peak decrease with increasing incubation time at +0.85 V conditioning potential, Fig. 6. On the other hand, the *in*

in situ electrogenerated nitrenium cation radical, danusertib redox metabolite-guanine adduct alters the dsDNA morphological conformation and was also responsible for the small changes of dAdo oxidation peaks, Fig. 6.

4 Conclusion

The interaction between kinase inhibitor and anti-cancer drug danusertib and dsDNA in bulk incubated solutions and at the dsDNA-electrochemical biosensor surface was investigated.

The dsDNA-danusertib interaction occurred in two sequential steps. First, the positively charged piperazine danusertib moiety was bound electrostatically to the dsDNA phosphate backbone. In the second step, a more stable danusertib-DNA complex formation involved the danusertib pyrrolo-pyrazole moiety, leading to small morphological modifications in the DNA double helix which were electrochemically characterised through the changes of the guanine and adenine residues oxidation peaks and confirmed by electrophoretic and spectrophotometric measurements.

Controlling the applied potential to the dsDNA-electrochemical biosensor surface, the oxidation of the danusertib amino group enabled the *in situ* electrochemical generation of nitrenium cation radicals and the study of their interaction with dsDNA. The decrease of guanine residues oxidation peak was in agreement with the covalent attachment of the danusertib nitrenium cation radical redox metabolite to the C8 of guanine residues, protecting them and preventing their oxidation. Using polynucleotides of known sequence it was confirmed that the interaction between danusertib and dsDNA takes place preferentially at guanine enriched segments.

An interaction mechanism was proposed and the formation of danusertib redox metabolite-guanine adduct formation explained.

5 Acknowledgements

Financial support from: Fundação para a Ciência e Tecnologia (FCT), projects PTDC/QEQ-MED/0586/2012, PTDC/DTP-FTO/0191/2012, and CENTRO-07-0224-FEDER-002001 (MT4MOBI) (co-financed by the European Community Fund FEDER), FEDER funds through the program COMPETE – Programa Operacional Factores de Competitividade is gratefully acknowledged.

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Schemes and Figures

Scheme 1 - Chemical structure of danusertib at pH = 4.5.

Scheme 2 - Proposed electrochemical mechanism of danusertib redox metabolite-guanine adduct formation.

Fig. 1 - DP voltammograms control, in 0.1 M acetate buffer pH = 4.5 supporting electrolyte in: (—) 50 $\mu\text{g mL}^{-1}$ dsDNA; and in 1 μM danusertib after adsorption during 5 min onto GCE: (—) without conditioning potential and (•••) with + 0.85 V conditioning potential.

Fig. 2 - DP voltammograms, in 0.1 M acetate buffer pH = 4.5, of 50 $\mu\text{g mL}^{-1}$ dsDNA incubated with 1 μM danusertib during different time periods: (—) 30 min, (•••) 180 min and (—) 48h.

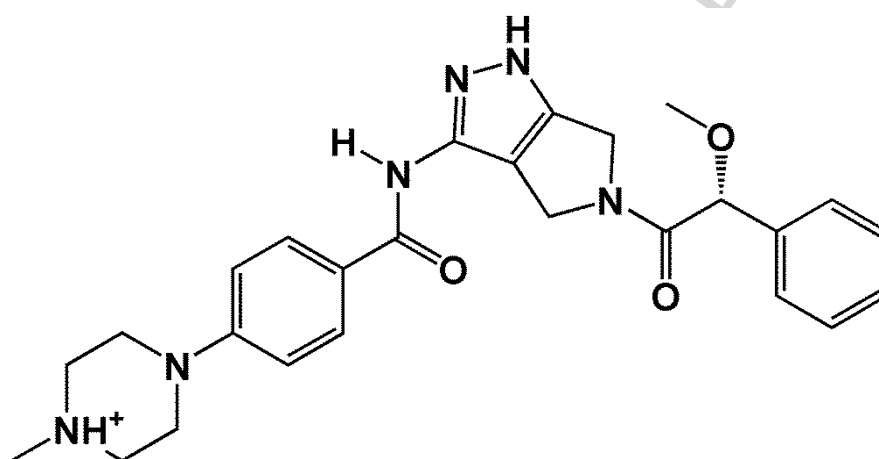
Fig. 3 - UV spectra of (---) 50 $\mu\text{g mL}^{-1}$ dsDNA, (•••) 25 μM danusertib and incubated solutions of 50 $\mu\text{g mL}^{-1}$ dsDNA and 25 μM danusertib during different time periods of 0, 1 and 3 hours.

Fig. 4 - Gel electrophoresis of 50 $\mu\text{g mL}^{-1}$ dsDNA control (lane 1) and 50 $\mu\text{g mL}^{-1}$ dsDNA incubated with danusertib: 10 μM (lane 2), 25 μM (lane 3) and 100 μM (lane 4) during 6 hours.

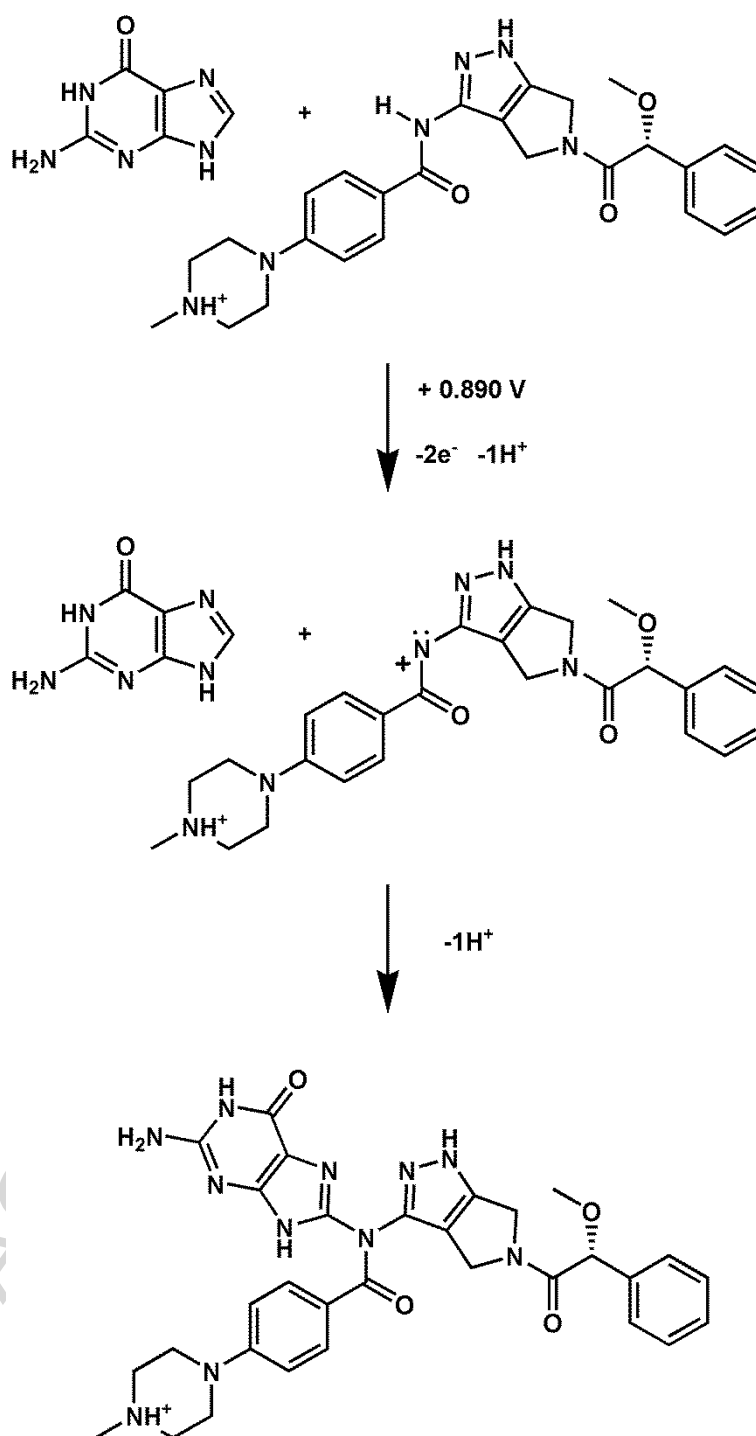
Fig. 5 DP voltammograms, with no conditioning potential, in 0.1 M acetate buffer pH = 4.5, with the dsDNA-electrochemical biosensor after: **(A)** (—) 30 min in buffer control experiment, and incubation in: 10 μ M danusertib during (•••) 15, (---) 30 and (—) 60 min, and **(B)** incubation in (—) 5 μ M and (—) 25 μ M danusertib during 30 min.

Fig. 6 - DP voltammograms, in 0.1 M acetate buffer pH = 4.5, with the dsDNA-electrochemical biosensor, after incubation at + 0.85 V conditioning potential: **(A)** (—) 30 min in buffer control experiment, and incubation in: 10 μ M danusertib during (---) 5, (•••) 15 and (—) 30 min, and **(B)** incubation in (—) 1 and (—) 25 μ M danusertib during 30 min.

Fig. 7 - DP voltammograms, in 0.1 M acetate buffer pH = 4.5, after 15 min incubation with 10 μ M danusertib: (—) without conditioning potential and (•••) with + 0.85 V conditioning potential of: **(A)** poly[A]-electrochemical biosensor (—) control; and **(B)** poly[G]-electrochemical biosensor (—) control.



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Scheme 2 - Proposed electrochemical mechanism of danusertib redox metabolite-guanine adduct formation.

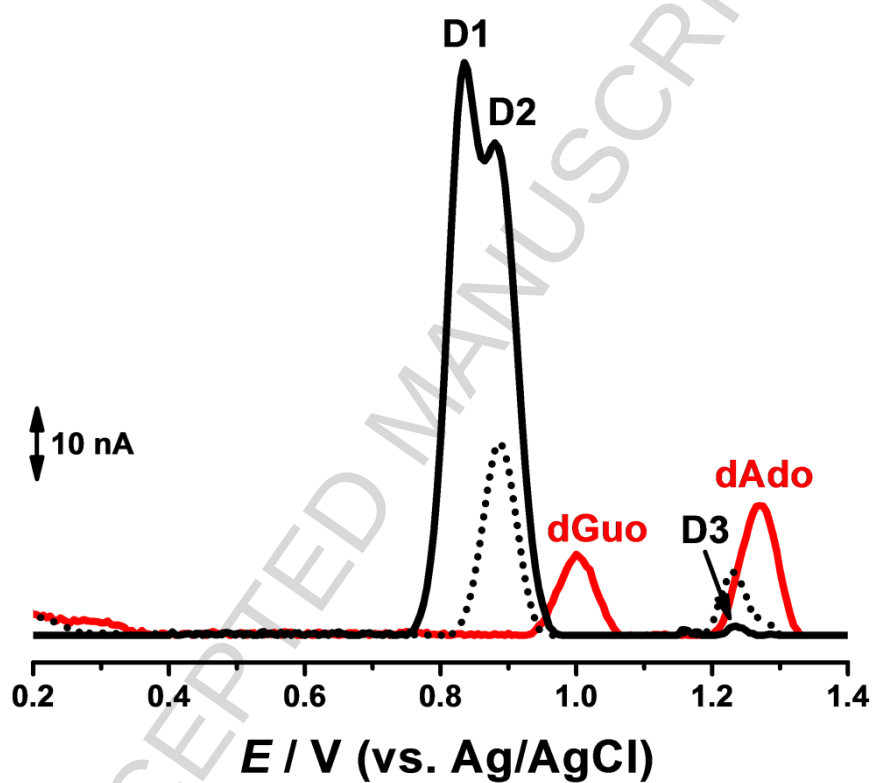


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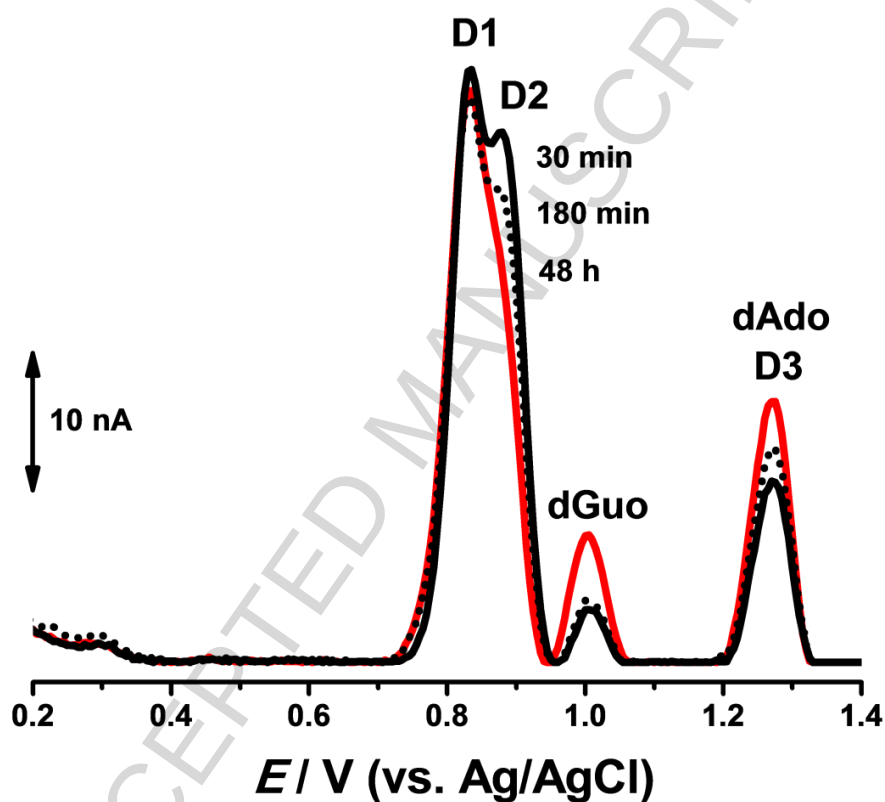


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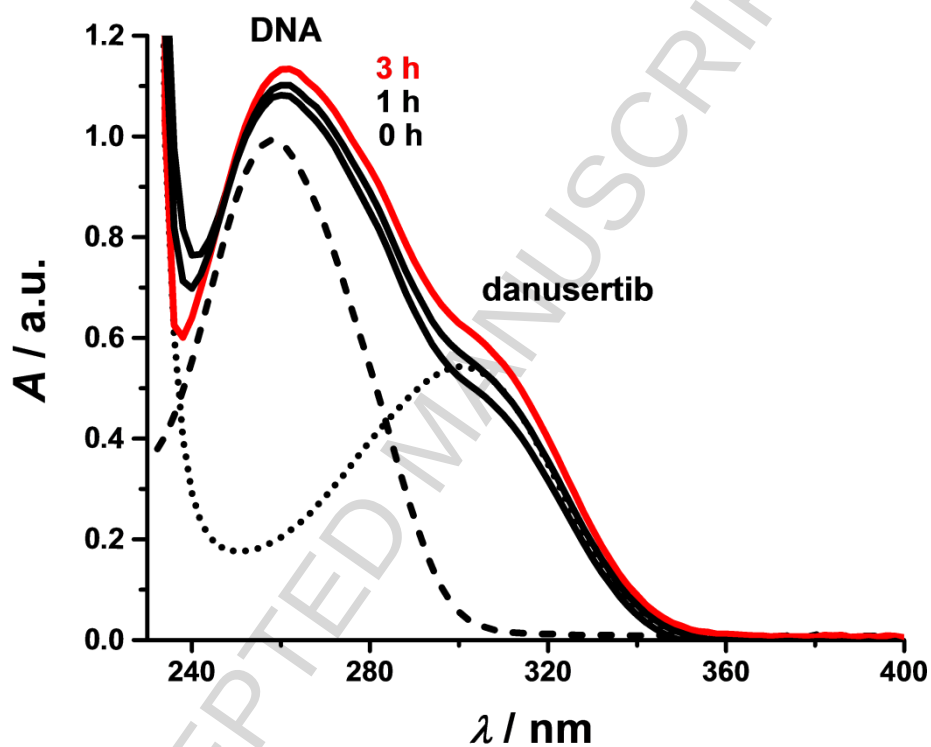


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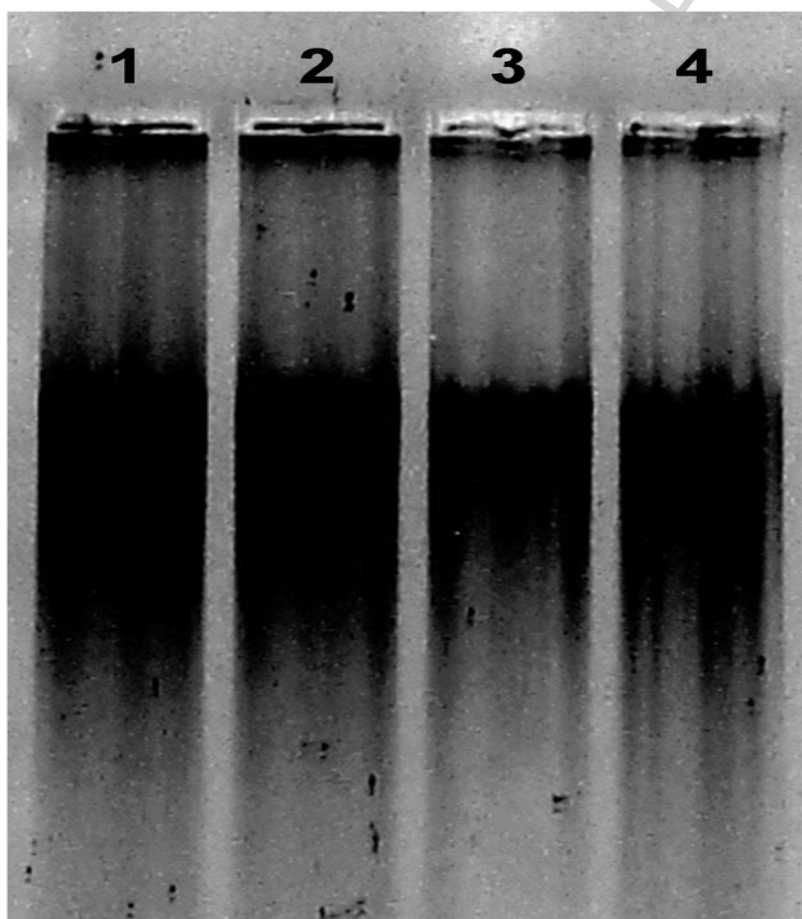


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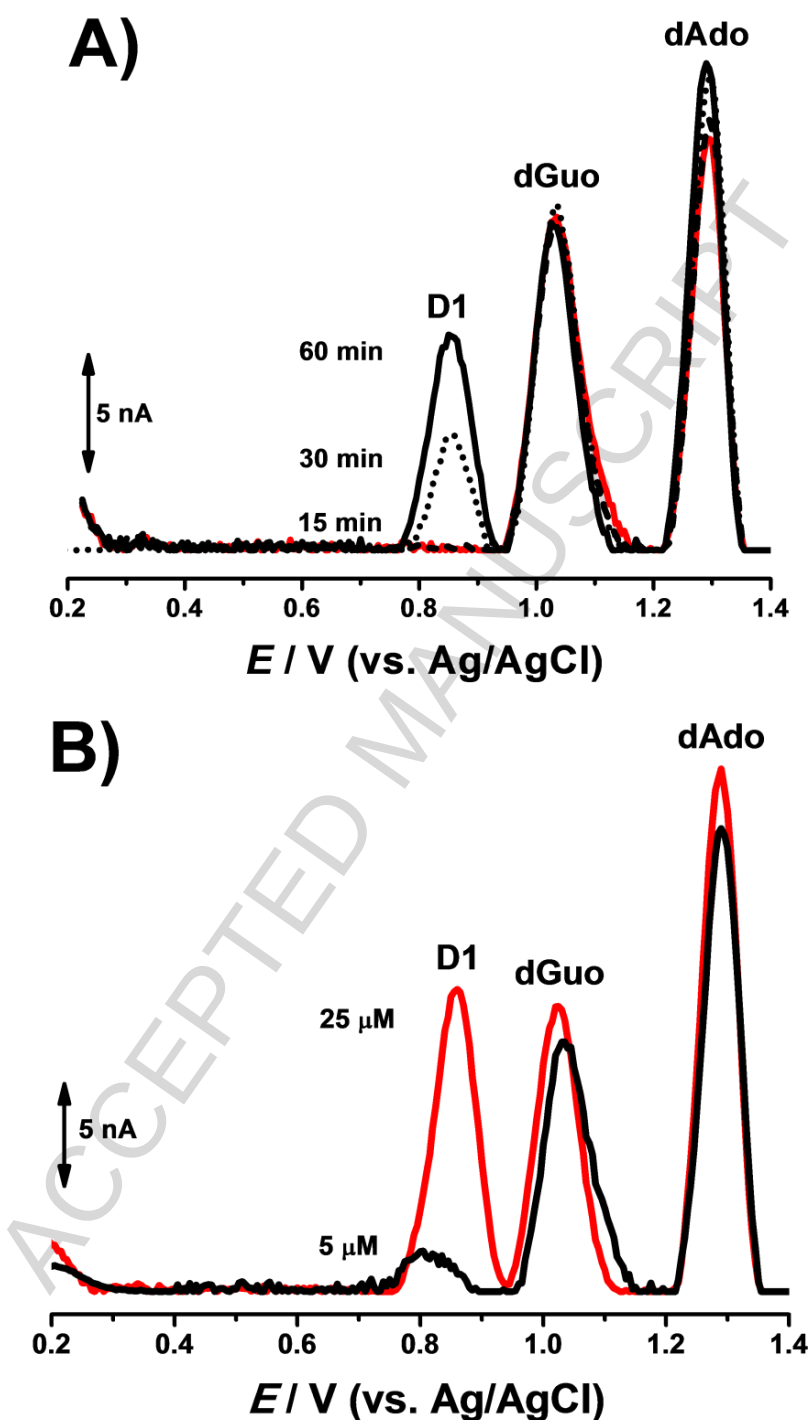


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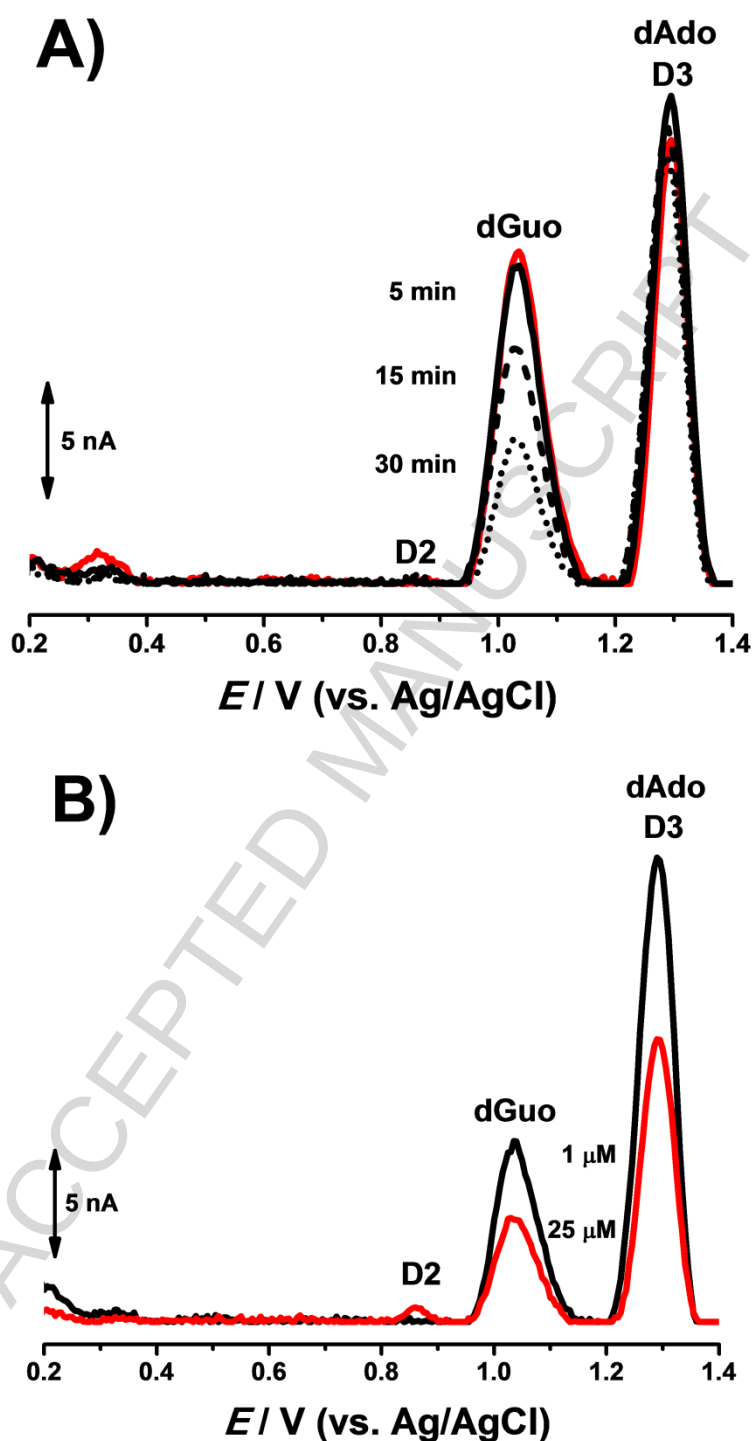


Fig. 6 - DP voltammograms, in 0.1 M acetate buffer pH = 4.5, with the dsDNA-electrochemical biosensor, after incubation at + 0.85 V conditioning potential:

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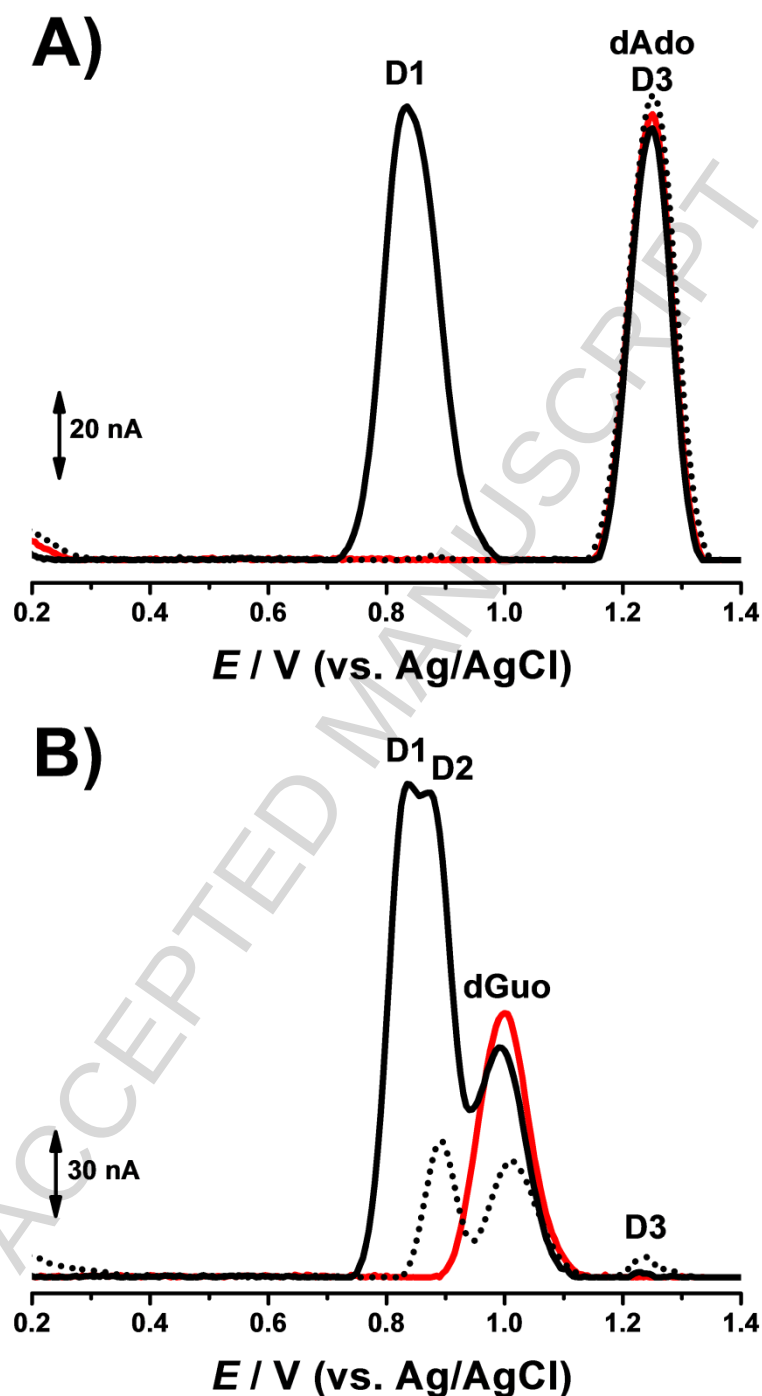
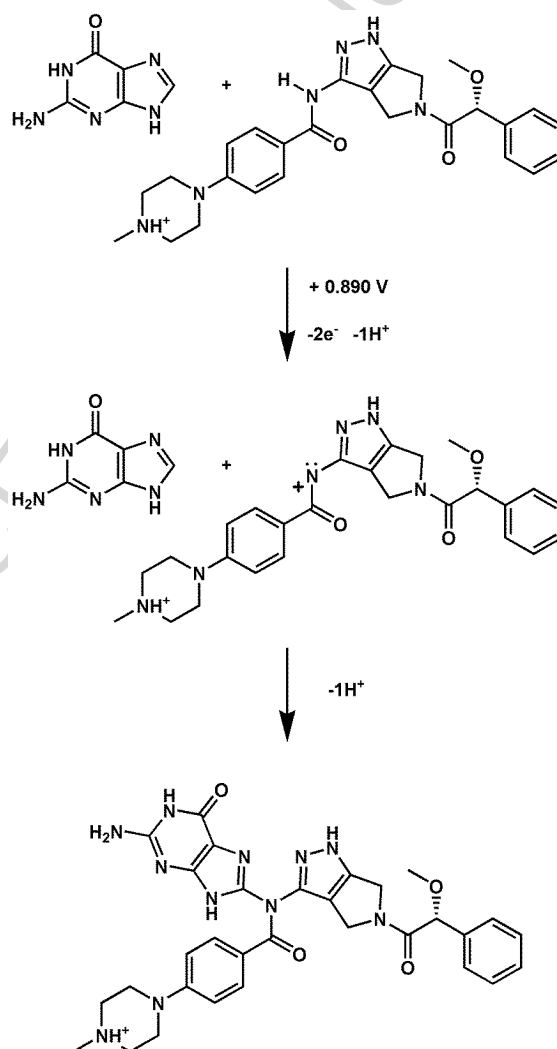


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***In situ* electrochemical evaluation of DNA interaction with anticancer drug danusertib nitrenium radical metabolite using the DNA-electrochemical biosensor**

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Graphical Abstract



Electrochemical mechanism of danusertib redox metabolite-guanine adduct formation.

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Highlights

- evaluation of danusertib-dsDNA interaction using a dsDNA-electrochemical biosensor
- morphological modifications in the DNA double helix
- *in situ* electrochemically generated nitrenium radical danusertib oxidation product
- danusertib redox metabolite covalently attached to guanine residues
- nitrenium radical metabolite-guanine adduct explained