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A Novel Triazolopyrimidinone Derivative: A Portable Electrochemical Approach to Investigate DNA Interactions

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ABSTRACT

In this study, a novel triazolopyrimidinone derivative, 2-(2-chlorophenyl)-5-(morpholinomethyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7(3H)-one, abbreviated as CPD-1, was synthesized as a drug candidate. By employing electrochemical techniques, we analyzed the electrochemical behavior of this compound and its interactions with both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). Experimental parameters such as pH, concentration, scan rate, immobilization time were studied using Differential Pulse Voltammetry (DPV) and Cyclic Voltammetry (CV) to obtain the most precise analytical signals. We present an innovative approach to evaluate the toxicity effect of this drug candidate on DNA. We also propose a simplified equation to quantify toxicity effects based on changes in electrochemical signals, specifically peak current of guanine bases, before and after drug-DNA interactions. Our methodology contributes to the burgeoning field of electrochemical toxicity assessment and holds promise for advancing drug development and safety evaluation. Furthermore, stability tests for the drug candidate were conducted on different days. Notably, our investigation revealed significant alterations in guanine bases upon the interaction of CPD-1 with both ssDNA and dsDNA, underscoring the potential impact of these compounds on DNA structure. Based on our experimental data, we conclude that this molecule can be utilized as a drug due to its effects on DNA.

Keywords: Drug candidate, Pencil graphite electrodes, Drug-DNA interaction, Triazolopyrimidinone.

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Introduction

Heterocyclic compounds, which encompass nitrogen-containing aryl substituents with five- and six-membered rings, have garnered significant interest due to the discovery of their effects in chemistry and various applications [1]. Triazolopyrimidinone, a fused pyrimidinone-triazole heterocyclic compound, has been extensively studied in diverse fields. It consists of a fused structure with a triazole and pyrimidinone ring. The unique structure of this compound imbues it with intriguing and potentially valuable properties, rendering it a subject of keen interest across diverse research domains, such as medicinal chemistry and drug discovery. These compounds have demonstrated a wide range of activities, including antibacterial, antifungal, antiviral, anticancer, herbicidal, antitumor, and antioxidant properties [2,3]. Some triazolopyrimidinones derivatives have demonstrated promising antimicrobial activity against a range of bacterial and fungal strains. These compounds could potentially serve as leads for the development of new antibiotics [4]. Certain triazolopyrimidinones have shown cytotoxic effects against cancer cell lines. They may interfere with cellular processes and pathways that are crucial for cancer cell survival and proliferation. Triazolopyrimidinones have also exhibited antiviral activity against certain viruses [5]. They may inhibit viral replication and entry into host cells, making them potential candidates for antiviral drug development. Besides their antimicrobial, anticancer, and antiviral properties, triazolopyrimidinones have been investigated for other activities, including anti-inflammatory and antioxidant effects [6-8].

Deoxyribonucleic acid (DNA) is the most popular pharmacological target of many drugs. The interaction between drugs and DNA is crucial for modern medicine, impacting both therapy and adverse effects. Understanding this interplay is key to developing effective treatments and safer drugs. By comprehending how drugs interact with DNA at the molecular level, scientists can tailor medications to target specific genetic factors, optimize treatment outcomes, and minimize potential side effects. Studying the mechanisms underlying drug-DNA interactions is of paramount importance, offering insights into drug actions and paving the way for innovative DNA-targeted drug design. A variety of techniques, spanning optical and infrared spectroscopy, nuclear magnetic resonance (NMR), circular and linear dichroism, viscosity assays, mass spectrometry (MS), molecular docking, and electrochemical methods, are employed to unravel the intricate nature of DNA-drug interactions [9-12]. This multifaceted approach empowers researchers to decipher the complex interplay between drugs and DNA, fostering advancements across the

spectrum of biomedical and pharmaceutical research. Exploring drug-DNA interactions holds pivotal significance, especially in the realm of electrochemical methods. These techniques, ranging from CV to DPV, offer a unique window into the dynamic interrelationship between drugs and DNA molecules. By harnessing the power of electron transfer processes, electrochemical methods unveil valuable insights into binding affinities, structural alterations, and even potential toxicity. This sophisticated approach not only enhances our understanding of drug mechanisms but also propels the development of cutting-edge DNA-targeted therapeutics. They are commonly favored for their rapidity, simple operation, and portability. Their high selectivity ensures accurate identification of target analytes even in complex samples, while their sensitivity allows for the detection of trace concentrations.

Furthermore, the interplay between drugs and DNA holds significant importance in both the pharmaceutical industry and the realm of biomedicine. This interaction offers numerous critical advantages and applications, including drug screening, optimizing drug delivery, personalized medicine, and genetic research. This knowledge forms the bedrock for advancing our comprehension of diseases and enhancing patient outcomes. Comprehending the intricate dance of drugs with DNA can pave the way for the development of precisely targeted therapies. Specifically tailored drugs that interact with DNA can be engineered to target particular genes or sequences linked to diseases, such as cancer. Many chemotherapeutic agents operate by engaging with DNA, thereby inhibiting cell division or triggering programmed cell death. Profound insights into these interactions are essential for crafting chemotherapy drugs that are more effective and less toxic. Delving into the nuances of how drugs interact with DNA also yields insights into their potential toxic effects, which is vital for evaluating the safety of pharmaceutical compounds. For example, certain antibiotics like quinolones function by engaging with bacterial DNA, impeding replication, and ultimately causing bacterial cell demise. This understanding underpins the development of antibiotics and is indispensable for ensuring their efficacy and safety.

In our research, we explored the electrochemical characteristics of 2-(2-chlorophenyl)-5-(morpholinomethyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7(3H)-one (abbreviated as CPD-1) a triazolopyrimidinone derivative and its interactions with both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) through DPV and CV. Specifically, we directed our attention to the novel potential drug candidate CPD-1, distinguished by its triazolopyrimidinone structure, and conducted an examination of its electrochemical properties. Various experimental parameters such as pH, concentration, scan rate, and immobilization time were systematically investigated using DPV. Additionally, we determined the detection limit, reproducibility, precision, linearity, as well as the limits of detection (LOD) and quantification (LOQ). The binding constant (K) was quantified at 7.4 x 10^1 M^{-1}, indicating the weak strength of the interaction. In terms of toxicity, the values recorded were 31% for ssDNA and 26% for dsDNA, highlighting the varying impact on these DNA types. Moreover, the calculations involving Gibbs free energy (G°) resulted in a value of -10.66 kJ/mol. This insightful calculation offers additional insights into the thermodynamic aspects of the interaction, contributing to a deeper understanding of CPD-1's behavior and its potential applications. To assess the stability of CPD-1, comprehensive tests were conducted under optimal storage conditions (a dark room at 25°C) on specific days (day 0, day 1, day 3, day 7, and day 30). DPV was employed before and after the interaction process to uncover the mechanism of CPD-1's interaction with both ssDNA and ds DNA. Notably, CPD-1's interaction with ssDNA and dsDNA led to significant alterations in guanine bases, emphasizing the crucial nature of these findings.

Our study introduces CPD-1, an entirely novel compound characterized as a "tri-azolo-pyrimidinone derivative." This constitutes a substantial innovation, as the synthesis of novel compounds frequently yields unique properties and prospective applications within the domain of pharmaceutical development. Such compounds often possess distinctive structural attributes and latent pharmacological functionalities that remain relatively unexplored within the extant body of literature. The study elucidates "significant modifications in guanine bases" consequent to the interaction between CPD-1 and DNA, thereby accentuating the conceivable influence of this compound on the structural integrity of DNA. This discernment represents an unprecedented discovery, which may bear implications for comprehending the mechanistic underpinnings of CPD-1's mode of action. CPD-1 could potentially exhibit a distinctive mechanism of action or a heightened affinity for DNA binding, avenues worthy of further exploration for potential therapeutic applications [13-15].

Materials and Methods

Apparatus

The SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, Waltham, MA) was utilized to generate two distinct single-stranded DNA (ssDNA) strands. DPV and CV measurements were performed using a PalmSens4 potentiostat/galvanostat/impedance analyzer. The PalmSens4 was connected to a computer via a USB cable and operated using PSTrace 5.8 software. The three-electrode system included a pencil graphite electrode (PGE) as the working electrode, an Ag/AgCl reference electrode, and a platinum wire counter electrode. A Rotring T 0.5 mm pencil (Rotring, Germany) served as the holder for the graphite lead. HB pencil leads (Tombo,

Japan) measuring 60 mm in length and 0.5 mm in diameter were obtained from a local bookstore.

PGEs are frequently employed in electrochemical sensors and biosensors due to their excellent electrical conductivity, stability, and ease of functionalization for specific analytes. PGEs represent a category of carbon-based electrodes known for their affordability, simplicity, wide availability, adaptability, and disposability. Graphite is an excellent conductor of electricity, which makes pencil graphite electrodes suitable for various electrochemical processes. They can efficiently transfer electrical energy to the reaction medium, enabling precise control over the process. Graphite is chemically inert, which means it does not react with most chemicals or substances. This property makes PGEs compatible with a wide range of chemical reactions and environments. PGEs typically exhibit low polarization characteristics, meaning they can facilitate electrochemical reactions with minimal energy loss due to overpotential. This can result in more efficient and cost-effective processes. They are relatively strong and can endure mechanical stress and pressure, which is advantageous in industrial applications where electrodes may experience mechanical wear and tear. These characteristics make PGEs a desirable choice for electrochemical sensing applications, particularly due to their distinct advantage of being disposable, setting them apart from other commonly employed carbon-based electrodes. Additionally, GPEs, being mechanically robust, offer ease of customization and miniaturization [16,17].

The active surface area of a pencil graphite electrode can be estimated using the Randles-Sevcik equation, which relates the peak current of an electrochemical reaction to the diffusion coefficient, concentration, and active surface area of the electrode. By measuring the peak current of a well-characterized electroactive species, such as ferrocyanide, and using its known diffusion coefficient and concentration, the active surface area of the pencil graphite electrode was calculated as 0.255 cm^2, as demonstrated in our previous study [18].

Chemicals

Ethanol absolute (99.9%), and glacial acetic acid were purchased from Isolab Chemicals. Double-stranded salmon sperm DNA (dsDNA) was sourced from Sigma Chemical Co. (St. Louis, USA), and all other chemicals were of high purity, obtained from Merck (Darmstadt, Germany), Tokyo Chemical Industry C. LTD. (Tokyo, Japan). All solvents used were of high analytical grade and were employed without further purification. Buffers, including 0.5 M Acetate (ACB) at pH levels of 3.8, 4.8, and 5.6, 0.05 M Phosphate (PBS) at pH 7.4, 0.1 M sodium borate (BBS) at pH 8.1, along with 0.02 M NaCl and 0.05 M Tris-EDTA (TE) buffer at pH 8.0, were utilized in the experiments. Stock solution of CPD-1 was prepared in Dimethylformamide (DMF) and stored in a dark room at 4°C to prevent degradation.

The salmon sperm DNA used in this experiment (Sigma Aldrich, purity ≥ 98%) was dissolved in deionized water to prepare stock solutions. 3-amino-5-(methylthio)-4H-1,2,4-triazole (Merck, purity ≥ 97%) and chloroform (Alfa Aesar-Acros Organics, purity ≥ 99%) were used as received without further purification. The buffers were prepared using analytical grade chemicals form various from different companies as Carlo Erba, Alfa Thermo Fisher Scientific, and Isolab.

Experimental

Candidate Drug Molecules

Triazolopyrimidinones can be synthesized through various chemical reactions, including cycloaddition reactions, condensation reactions, and cyclization reactions. The compound 2-(2-chlorophenyl)-5-(morpholinomethyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7(3H)-one was synthesized following literature procedures at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Izmir Katip Celebi University, and generously provided [19].

Pre-treatment of PGE

Before activation, the pencil graphite electrodes (PGEs) were trimmed to a length of 3 cm. Subsequently, all PGEs underwent activation by applying a potential of +1.4 V for 30 seconds in acetate buffer (ACB) at pH 4.8, aiming to minimize background current. The electrochemically pre-treated PGEs were then utilized as working electrodes for subsequent experiments.

To cleanse and activate the electrode surface, we initially applied a predetermined voltage for a specific duration to activate the electrodes. Following the immobilization of DNA or drug molecules, we subsequently rinsed the electrodes with buffer solutions to eliminate any unbound substances.

Interaction

CPD-1's electrochemical behavior on PGEs was studied using DPV, considering both the presence and absence of ssDNA and dsDNA. The interaction of CPD-1 with ssDNA. After a 1-minute heating step of dsDNA at 95°C and cooling on ice of CPD-1 was added, and ssDNA was immobilized on PGEs. In our experiment, there was no drying step performed before the measurements. DPV measurements were conducted following this step. In the subsequent stage, CPD-1's interaction with dsDNA was examined. Solutions containing dsDNA and CPD-1 were agitated at 45°C and 600 rpm for 30 minutes. Interaction solutions were then analyzed using DPV.

Measurement

DPV and CV measurements were carried out in the range of + 0.4 to + 1.4 V at 50 mV/s of scan rate. The experimental procedure is shown in Figure 1.

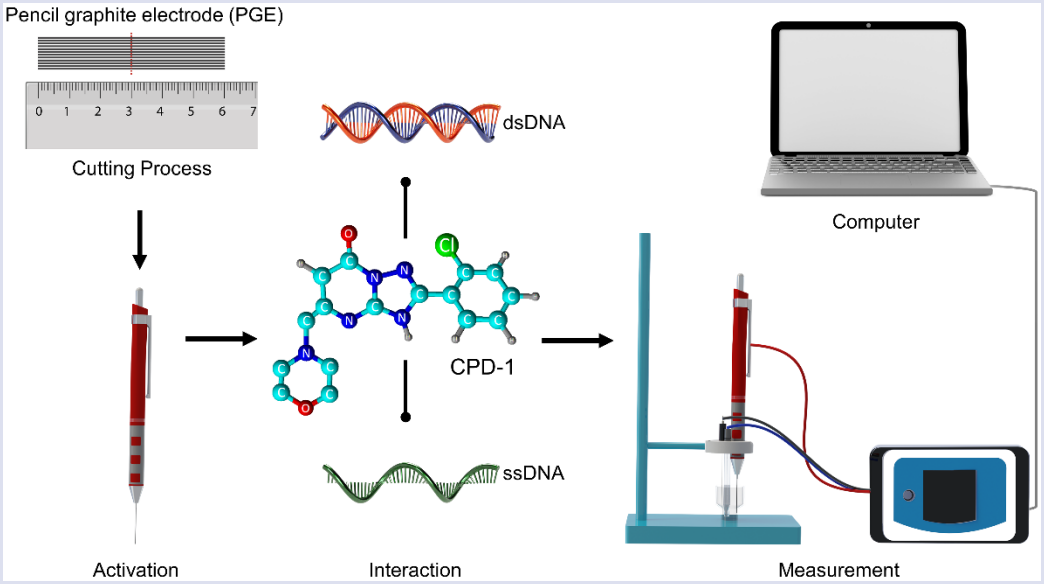

Figure 1. Experimental steps: activation of PGE with ACB; CPD-1 interaction with ssDNA and dsDNA; DPV measurements.

Results and Discussion

Investigation of Electrochemical Properties of Compounds

In this section, the electrochemical behaviors of the drug candidate molecules were investigated using DPV. To begin, the impact of pH on the electrochemical oxidation signals of the drug candidates was assessed, and the corresponding results are presented in Figure 2. Notably, pH plays a pivotal role in drug metabolism and therapeutic efficacy. For the pH study, a range spanning from 3.8 to 9.8 was examined using DPV. The investigation aimed to identify the optimal pH conditions that yield significant changes in peak current and peak potential for the drug candidates. The outcomes revealed that CPD-1 exhibited stable responses, with the most prominent peak observed at pH 5.6. Consequently, a pH of 5.6 was selected for the dilution buffer. This meticulous pH exploration provides essential insights into the electrochemical behavior of the drug candidates and informs their potential therapeutic applications [20]. As an additional point of clarification, in our study, we achieved consistent and reproducible current signals through rigorous optimization of experimental parameters. Therefore, we did not explore the influence of different supporting electrolytes by testing various electrodes.

The subsequent phase of the study focused on establishing the analytical concentration ranges of the drug candidates through DPV measurements conducted at a scan rate of 50 mV/s.

Calibration plots, depicted in Figure 3, were constructed across varying concentrations of the drug candidate. Notably, the resulting calibration graph exhibited a linear relationship between current response and concentrations. Notably, the resulting calibration graph showcased a linear relationship between the current response and the concentrations studied.

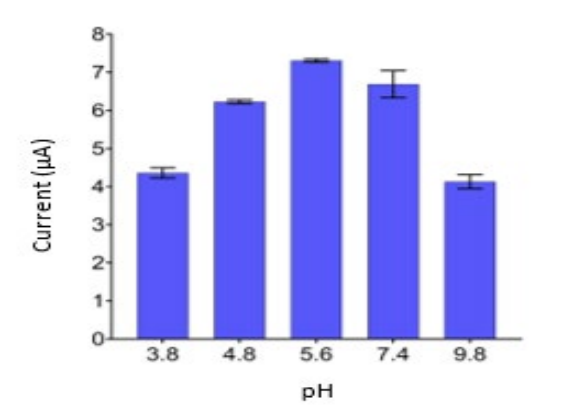

Figure 2. The effect of pH on peak current and peak potential. The bar graph showcases the behavior of the CPD-1 drug molecule across various pH values spanning from 3.8 to 9.8. Notably, the highest peak currents for CPD-1 were observed at pH 5.6.

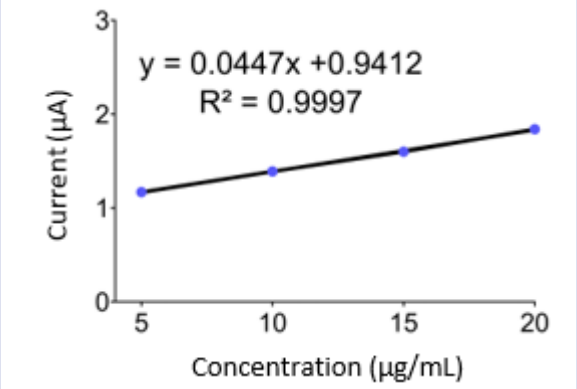

Figure 3. Calibration graph of CPD-1 was obtained by plotting drug concentrations against the current peaks. DPV measurements were performed at 50 mV/s of scan rate with different concentrations from 5 µg/mL to 20 µg/mL.

Specifically, the calibration plot for CPD-1 exhibited a slope of 0.0447 and a correlation coefficient of 0.9997 (as depicted in Figure 3).These outcomes distinctly emphasize the precision and reliability inherent in the developed methodology for quantifying CPD-1's concentrations. Such robust findings augment the analytical potential of the electrochemical approach utilized in this study.

Calibration graph played a pivotal role in calculating the Limit of Detection (LOD) and Limit of Quantification (LOQ) for the CPD-1 drug molecule. The formulas for calculating LOD and LOQ depend on the specific method used, but generally, LOD is calculated as 3 times the standard deviation of the response divided by the slope of the calibration curve ($LOD = 3s/m$), while LOQ is calculated as 10 times the standard deviation of the response divided by the slope of the calibration curve ($LOQ = 10s/m$) [21],[22]. Specifically, the concentrations 5, 10, 15, and 20 µg/mL were employed for the determination of LOD and LOQ for CPD-1. The calculated values for CPD-1 unveiled a LOD of 0.48 µg/mL and a LOQ of 1.59 µg/mL, respectively.

By varying the scan rate, valuable insights can be gained into the kinetics and mechanism of electrochemical reactions. Adjusting the scan rate allows researchers to probe different reaction pathways, investigate the reversibility of redox processes, and analyze the overall electrochemical behavior of compounds. Therefore, the study of scan rate provides a comprehensive understanding of the underlying processes and aids in the design and optimization of electrochemical detection methods and sensor devices. In the last part of the study the influence of scan rate (V) on peak current (Ip) values was explored utilizing CV, covering a scan rate range from 25 mV/s to 150 mV/s.

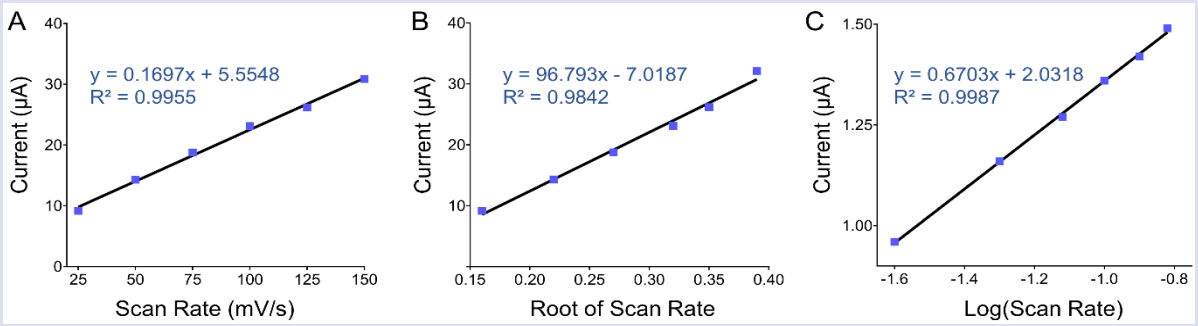

Figure 4. Effect of scan rate on peak current (A), effect of scan rate root on peak current (B), effect of scan rate on the log of peak current of drugs (C).

As shown in Figure 4A, the anodic peak currents (Ip_a) is well linear to scan rates (v) and the linear equation is expressed as:

$$Ip_a(\mu A) = 0.01697v + 5.5548 \quad (R^2 = 0.9955) \qquad (1)$$

The relationship between Ip_a and the root of the scan rate ($v^{1/2}$) also possesses a linear behavior (Figure 4B) and the linear equation is as follows:

$$Ip_a(\mu A) = 96.793v^{(1/2)} - 7.0187 \quad (R^2 = 0.9842) \quad (2)$$

Such linear behavior was also determined between log(Ip_a) and log(v) within the scan rate range between 25 mV/s and 150 mV/s (Figure 4C):

$$log\ Ip_a = 0.6703 log v + 2.30318 \quad (R^2 = 0.9987) \quad (3)$$

Considering the insights from the literature, these obtained slope values closely align with theoretical expectations, particularly in the case of a value close to 0.5. This alignment suggests the prevalence of diffusion-controlled processes. In contrast, a theoretical value of 1 indicates processes influenced by adsorption [23]. For Equation 3, the determined slopes were calculated as 0.6703. This result distinctly implies that the electrochemical oxidation of CPD-1 diffusion-controlled processes.

Interaction

The electrochemical behaviors of CPD-1 on PGEs were examined using DPV in the presence and absence of both ss-DNA and dsDNA. We begin by conducting a test to assess the interaction of CPD-1 with ssDNA. To facilitate this, a common method involving physical denaturation of dsDNA fragments was employed, as detailed in a referenced study [24]. In this process, a stock solution of dsDNA was initially prepared, and subsequently, 100 µL of dsDNA was aliquoted and sealed in centrifuge tubes. Following this, all samples were subjected to a 1-minute heating step at 95°C in a thermal cycler, followed by cooling on ice for 10 minutes. After adding 10 µg/mL of CPD-1, the resulting ssDNA was immobilized onto pre-treated PGEs for 30 minutes through adsorption. Subsequent to this immersion, DPV measurements were carried out to evaluate the outcomes of the interaction.

In the second stage, the interaction of CPD-1 with ssDNA was examined. Solutions were prepared by mixing 50 µg/mL of dsDNA and 10 µg/mL of the CPD-1 drug candidate in ACB. Following this, these solutions underwent thermal agitation at 45°C and 600 rpm using a thermal shaker for 30 minutes. Subsequently, 100 µL of the resultant interaction solution was carefully transferred into tubes. The Pencil Graphite Electrodes (PGEs) were then submerged in the interaction solutions for a duration of 30 minutes. After this immersion, DPV measurements were carried out to evaluate the outcomes of the interaction.

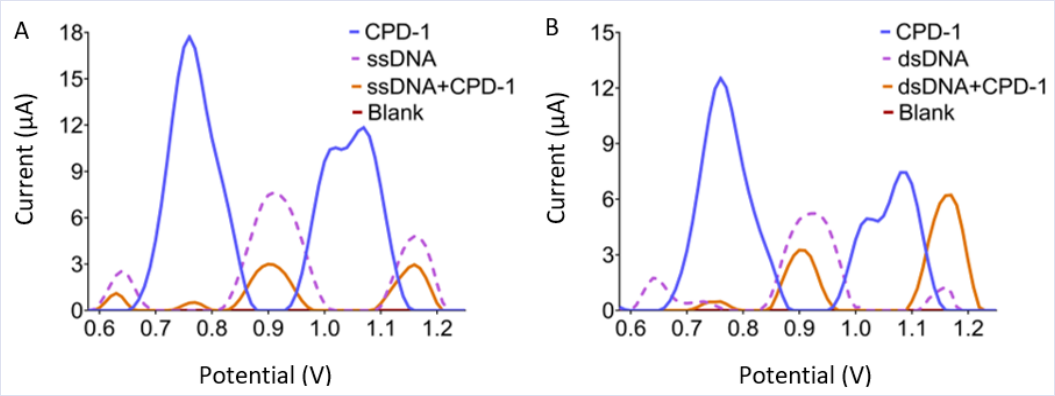

Figure 5. Impact of CPD-1 interaction on ssDNA and dsDNA. Differential pulse voltammograms presenting guanine oxidation currents for ssDNA (A) and dsDNA (B) following interaction with CPD-1.

In Figure 5A, two distinct oxidation signals associated with ssDNA in ACB (pH: 5,6) were obtained at +0.91 V and +1.15 V, respectively. After the interaction with CPD-1, the peak potential of dsDNA shifted to +0.90 V and +1.14 V, respectively. On the other hand, In the presence of CPD-1, the oxidation potentials of dsDNA exhibited a negative shift. (Figure 5B). One of the peak potentials of dsDNA underwent a negative shift, transitioning from +0.93 V to +0.90 V. Similarly, the other peak experienced a shift from +1.16 V to +1.15 V.

In this context, variations in peak potential—whether they are positive or negative—offer insights into the interaction mechanism between the drug candidate CPD-1 and DNA. Positive shifts in peak potential commonly signify intercalative binding, whereas negative shifts are indicative of electrostatic binding [25]. In the scope of our investigation, the observed negative shift in peak potential could be attributed to an irreversible electrode process. This phenomenon might be elucidated by electrostatic binding occurring between CPD-1 and DNA.

The value of the binding constant ($K_{dsDNA-CPD-1}$) was determined using the following Equation 4:

$$log(1/[DNA]) = log\ K + log\ (IH\text{–}G/IG - IH\text{–}G) \quad (4)$$

where K is the apparent binding constant, I_G and I_{H-G} are the peak current of the free guest (G; here free CPD-1) and the host-guest complex (H-G; CPD-1 intercalated into ss-DNA), respectively. In general, binding constants (K) can range from very low values (indicating weak or transient interactions) to high values (indicating strong, stable interactions). Typical binding constants for drug-DNA interactions can fall within the range of 10^2 to 10^7 M^{-1}, but these values can differ significantly based on the system being studied. A high K value indicates intercalation-based interactions, while a low value indicates electrostatic or groove interactions [26]. Based on changes in current response (I) intensity caused by DNA binding to CPD-1, the binding constant was calculated as 7,4 x 10^1 M^{-1}. In the literature, the binding of Doxorubicin (an anthracycline antibiotic) to DNA yields a K value of 3.1 x 10^5 M^{-1}, Ethidium bromide (a DNA intercalator) binding to DNA results in a K value of 1.0 x 10^6 M^{-1}, and Hoechst 33258 (a DNA minor groove binder) binding to DNA exhibits a K value of 2.0 x 10^7 M^{-1}.

The Gibbs free energy (G°) values provide insights into the thermodynamic favorability of a reaction or interaction. In the context of drug interactions, Gibbs free energy provides insights into the spontaneity and feasibility of a given reaction or binding process. Specifically, in the realm of drug design and development, Gibbs free energy is often utilized to assess the thermodynamic stability of drug molecules when binding to their target biomolecules, such as proteins or DNA. The binding affinity between a drug and its target molecule can be influenced by the change in Gibbs free energy during the binding process. Negative G° values indicate that the interaction is thermodynamically favorable, meaning that the binding process is spontaneous and releases energy [26]. Positive G° values suggest that the interaction is not favorable, requiring an input of energy to occur. A lower Gibbs free energy change (ΔG°) corresponds to a higher K value indicating a tighter binding between the drug and its target. The change in Gibbs free energy (G°) was calculated using Equation 5:

$$\Delta G^\circ = -RT\ ln\ K \quad (5)$$

where R is the universal gas constant (8.31 J K^{-1} mol^{-1}), T is the absolute temperature and K is the binding constant. The ΔG° for CPD-1 calculated as -10,66 kJ/mol.

In general, the calculation of toxicity effects involves quantifying the change in electrochemical response, such as peak current or potential, caused by the interaction of the drug with DNA. The exact equation will depend on the specific parameters being measured and the data analysis approach chosen. In order to investigate the toxicity effects of CPD-1 on ssDNA and dsDNA, guanine peak changes were calculated before and after interaction. Here's a simplified example of an equation that could be used to calculate the percentage change in peak current as an indicator of toxicity:

$$Toxicity\ (\%) = \frac{Peak\ Current\ (after\ interaction) - Peak\ Current\ (before\ interaction)}{Peak\ Current\ (before\ interaction)} \times 100\%$$

This equation provides a percentage value that indicates how much the electrochemical response has changed due to the drug-DNA interaction. A higher percentage change may suggest a higher degree of toxicity. In our study, this value was calculated as 31 % for ssDNA and 26 % for dsDNA.

Stability

Assessing the stability of a drug is of paramount importance in gauging its effectiveness over time. In our study, we scrutinized the stability of CPD-1 by subjecting freshly prepared solutions to controlled storage conditions within a light-protected environment at a constant temperature of 25°C. This scrutiny was carried out over distinct time intervals, specifically on day 0, day 1, day 3, day 7, and day 30. Temporal changes in the peak currents of the drug were meticulously observed. To delve into the stability aspect further, drug solutions were meticulously formulated using ACB (pH: 5.6) at specific concentrations. Swift DPV measurements were executed by immersing Pencil Graphite Electrodes (PGEs) into these solutions immediately post-preparation and at various intervals thereafter (0, 1, 3, 7, and 30 days). The investigation of drug stability occurred under identical molar concentrations using DPV, encompassing a potential range from 0 to +1.4 V, and a scan rate of 100 mV/s. This comprehensive approach allowed us to gain valuable insights into CPD-1's stability profile over the designated timeframes.

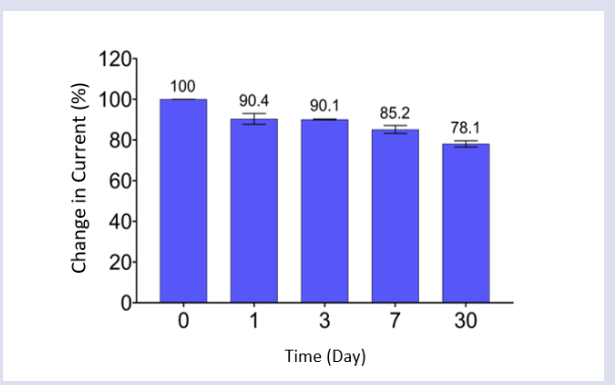

Figure 6. Change in current for CPD-1 examined at 25 °C for different days, e.g., 0, 1, 3, 7, and 30. The current value of the drug candidate was about 78 % by the end of 30 days.

Here, CPD-1 exhibited good stability for 30 days of storage without significant percentage changes in current values, e.g. At the end of day 30, the percentage of the current value for CPD-1 was determined as 78.1%. These findings provide substantial evidence of the stock solutions' stability for a duration of 30 days, with the notable exception of CPD-1. This observation underscores the potential advantages of prolonged utilization, as the majority of the drug candidates retained their vital pharmaceutical attributes over the extended timeframe.

Conclusion

In conclusion, our study delved into the electrochemical properties of triazolopyrimidinone derivative, CPD-1 and its intricate interaction with single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA), employing DPV and CV. This endeavor contributes to the ongoing pursuit of novel therapeutic agents. Our investigation centered on the newly identified drug candidate, CPD-1 characterized by its triazolopyrimidinone structure, and systematically probed its electrochemical behavior. Through meticulous DPV experimentation, we systematically assessed diverse parameters including pH, concentration, scan rate, and immobilization time. The ensuing determination of key parameters such as detection limits, reproducibility, precision, linearity, and limits of detection and quantification (LOD and LOQ) further enriched our understanding. In the quest for stability, comprehensive assessments were conducted under optimal storage conditions, shedding light on the compounds' resilience on specific days. Employing DPV before and after interaction revealed substantial modifications in guanine bases upon the interaction of CPD-1 with dsDNA, thereby highlighting the pivotal role of our research findings. Our endeavor underscores the intricate interplay between electrochemistry, drug candidate, and DNA, underscoring the potential for innovative drug development and therapeutic advancement in the field of biomedicine.

The synthesis and characterization of CPD-1 as a potential drug candidate represent a pivotal advancement in drug development. This innovative compound holds promise as a cornerstone for the formulation of medications engineered to establish specific interactions with DNA, thereby potentially yielding more precisely targeted and efficacious therapies across a spectrum of diseases. The proposed simplified equation for the quantitative assessment of toxicity effects, rooted in alterations in electrochemical signals, constitutes a pragmatic instrument for both researchers and pharmaceutical entities. It furnishes a systematic means of gauging the influence of nascent drug candidates on DNA, thereby rationalizing the drug development process and bolstering safety appraisals. The profound modifications observed in guanine bases within our study, consequent to the interaction of CPD-1 with both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA), constitute pivotal discoveries. These findings illuminate the potential ramifications of CPD-1 and analogous compounds on DNA architecture, providing indispensable insights for the comprehensive evaluation of the safety and efficacy of these prospective drug candidates.

Conflicts of interest

There are no conflicts of interest in this work.

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