doi.org/ 10.52568/001681/JCSP/47.04.2025 345

Interaction Activity of Metal-Free Phthalocyanine Compound Bearing Tetra -(2-(N-2´-Cyanoethyl)aminoethylsulfanyl) Units with DNA

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(Received on 8th October 2024, accepted in revised form 5th May 2025)

Summary: The phthalocyanine having 4-(2-(N-2´-cyanoethyl)aminoethylsulfanyl) group had been reported earlier in the literature. In this current studying, DNA binding activity of 1Pc phthalocyanine bearing 4-(2-(N-2´-cyanoethyl)aminoethylsulfanyl units was examined spectroscopically via elctronic absoption, fluorescence titration, melting point profile, electrophoresis and viscosity methods. The interaction activity of 1Pc compound was examined at differing concentrations. UV/Vis spectrometer, viscosity, fluorescence spectroscopy and thermal melting temperature confirmed that 1Pc binds to the DNA. The K_b of 1Pc is also estimated via UV/Vis titration and K_b of 1Pc was computed as 2.1394 x 10^6 M^{-1}. The K_b value demonstrated that 1Pc reacts with DNA by an intercalative mechanism. Alongside this research, the mechanism by which the compound binds to DNA was investigated by determining Tm. The Tm of DNA + 1Pc complex was identified as 74.31. This data confirmed that 1Pc binds to DNA intercalatively. All the results obtained from the used methods demonstrated that 1Pc phthalocyanine compound has an efficient DNA interaction activity and 1Pc phthalocyanine compound interacts with DNA via an intercalative mechanism. As a result, the compound may be a therapeutic agent due to its DNA interaction property.

Keywords: Phthalocyanines, DNA interaction, Absorption spectroscopy, Emission spectra, Melting temperature.

Introduction

Phthalocyanine compounds [1], which are commonly being used in various industrial and medical fields, are macrocyclic compounds that have a very wide range of applications and have recently received a great deal of attention [2]. In recent days, in a large number of scientists have been conducting researches on phthalocyanines to investigate the interactions of metal free and metal-containing complexes with DNA molecules and to discover more effective new chemotherapeutic drugs [3,4]. As the physicochemical characteristics of phthalocyanines are so easy to be altered through peripheral changes of the distribution of electron over aromatic ring, these compounds have been evaluated in areas such as dyes, diodes, sensors, semiconductors and photovoltaic materials [5-7]. As well as this, it can also be applied in the monitoring of the retina and the cardiovascular system, cancer treatment and photo-inactivation of bacteria [8-10]. Besides these knowledge, phthalocyanines possess a wide wavelength range with a long extinction coefficient, and most of them are of low toxicity [11].

Cancer is a deadly disease that is one of the leading health problems that deeply affects human life in the modern world. Although there have been many promising discoveries and techniques in the treatment of cancer in today's world, the drugs and methods developed in this field are still not sufficient to eradicate this health problem. The deoxyribose nucleic acid (DNA) molecule is a nucleic acid compound that contains vital genetic information and instructions needed to govern the vital activity and biological development of all living things and some types of viruses. Since the DNA molecule contains vital information necessary for the survival of living organisms, the interaction activities and reactions of metal-free and metal-containing phthalocyanine compounds with DNA continue to be an important subject of scientific research today [12]. Due to the unique structural properties of the DNA molecule, it has the ability to interact very efficiently with compounds with very different properties, such as phthalocyanines. Due to the effects of phthalocyanines and their derivatives on the human body structure, the ability of these compounds to bind to many cancer cells and their unique optical properties, the interactions of these compounds with DNA have been and will continue to be the center of attention for years. Scientific research on the interactions of phthalocyanine compounds and their derivatives with DNA has recently been of great interest [12]. Phthalocyanines and metal-containing phthalocyanine compounds and their high tendency to bind to DNA have accelerated the development of new

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DNA-oriented medicines [13-16]. DNA has been one of the leading targets of antibiotic, anticancer and antiviral therapeutics due to its fundamental role in replication and transcription [17]. In a number of diseases, including AIDS, cancer and bacterial infections, the strength of DNA-binding activity of therapeutics has been verified [18]. The substitution of functional groups and the formation of phthalocyanines with the correct central atom are as well of great significance in order to making them suitable to medication treatment [19].

In this study, the different methods were applied to scrutinize DNA binding activity of the phthalocyanine compound and the interacting mechanism of the compound was determined. From this perspective, the purpose of this research is to explore the potential use of DNA binding activity of previously prepared metal-free phthalocyanine containing 4-(2-(N-2'-cyanoethyl)aminoethylsulfanyl) group (1Pc). Electronic, fluorescence titration, melting temperature, viscosity and electrophoresis measurements were applied to elucidate the binding mechanism of 1Pc to CT DNA in a buffer solution at 7.15 of pH.

Experimental

Equipments and materials

The used chemical reagents and solvents in this present research were procured from Sigma-Aldrich commercial company. The cemical materials utilized in experiments such as DMF, THF and ethanol were of reagent grade quality. Tetra(2-(*N*-2´-cyanoethyl)aminoethylsulfanyl)phthalocyanine was previously synthesized and characterized and reported in literature [20]. ^{1}H NMR, FTIR and UV/Vis spectrometers were utilized to characterize of metal-free phthalocyanine. To examine the DNA binding activity, UV/Vis (Cary 60 UV/Vis spectroscopy), fluorescence spectra (Perkin Elmer LS Fluorescence Spectroscopy), viscosity (Ubbelohde viscometer), melting temperature and electrophoresis measurements (Thermo Scientific Owl Electrophoresis System) were implemented at 7.15 of pH.

Synthesis

The synthesis route for 1Pc

The tetra(2-(*N*-2´-cyanoethyl)aminoethylsulfanyl) phthalocyanine was previously prepared, synthesized and reported in literature [20]. Previously published paper in the literature, it was stated that 1Pc phthalocyanine compound dissolves like DMF and DMSO [20].

Describing 1Pc binding to DNA utilizing electronic titration

Ability to interact with DNA of 1Pc was evaluated at 7.15 of pH. Using the value of ε_{DNA} at 260 nm [21] and DNA concentrations per nucleotide phosphate were determined. The DNA samples were maintained in a refrigerator at 4 ^{o}C and the samples were utilized within a few days during the experiment. 15 µM CT-DNA solution was obtained in distilled water and the starting solution of 20 µM of 1Pc were produced in DMF solvent. Initially, the absorption spectra of 1Pc solution was taken and recorded. Following this, 15 µM of DNA from the previously prepared CT-DNA sample was injected sequentially using an automated pipette. Absorption spectra of 1Pc compound was measured in the range from 280 nm to 870 nm. Electronic titration was implemented until the Q band of the compound stayed on a constant wavelength as a result of the successive addition of the CT DNA.

The detection of binding activity of 1Pc by fluorescence assay

The DNA binding characteristic of quaternized 1Pc phthalocyanine compound soluble in organic solvents such as DMF were examined by emission titration method at ambient temperature. The solution of the EB-DNA was titrated by adding 1Pc (20 µM) respectively and after for each adding of DNA, the emission spectra of the 1Pc were captured. In the present study, fluorescence emission spectra were determined utilizing pre-prepared CT-DNA and 1Pc solution. A progressive with rising 1Pc concentration the fluorescence of the EB-DNA declines was detected [22].

The viscosity measurement assay

The viscosity data for 1Pc were collected via Ubbelohde viscometer at 25 ^{o}C. The solutions of 1Pc (0, 5, 10, 15 and 20 µM) were inserted sequentially to the DNA (20 µM) solution. The flowing times of solution of 1Pc was monitored by means of a precision stopwatch and the data obtained were recorded. The values of the relative viscosity $(\eta/\eta_0)^{1/3}$ of 1Pc compound obtained as a result of viscosity measurements were calculated and plotted against [1Pc]/[DNA] ratios. η and η_0 indicate the viscosity of the DNA in the absence and existing of 1Pc, sequentially.

The experiments on the melting temperature

Thermal DNA melting is an indicator for the durability of DNA temperature based. A variation at Tm (Thermal melting temperature) is interpreted as the important finding revealing an interacting between DNA

molecule and chemical agents [23, 24]. Furthermore, it is often possible to learn more about the degree of interactivity among the DNA and compounds. In a further effort to gather more evidence to validate the interaction between 1Pc and CT-DNA, the Tm experiments were conducted. The Tm of the complex of DNA + 1Pc was identified via monitoring the absorbance at 260 nm based on temperature, changing from 25 to 95 ^{o}C at room temperature and pH 7.15.

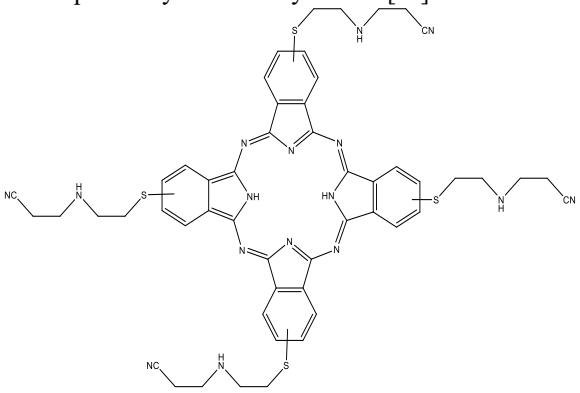
The assay of gel electrophoresis

DNA binding activity of 1Pc was analyzed via means of agarose gel electrophoresis. Reaction solutions consisting of different concentrations of 1Pc compound (10, 15 and 20 µM) and CT-DNA (20 µM) in the buffer solution were incubated at 37 ^{o}C for 80 min. The staining dye was then injected and electrophoresis test was conducted at 100 V for 3 h. Using TBE buffer at pH 7.15 by use of agarose gel [25]. The bands were photographed and imaged with the help of light of UV. The interacting activity was assessed via the intensity of bands.

Results and discussion

The synthesis and characterization of 1Pc

The structural formula of the compound used in this research is illustrated in Scheme 1. The synthesis of 1Pc compound, which is soluble in DMF, has already been synthesized, characterized and reported by Agirtas et al [20]. Sufficient yields of 44% for 1Pc was obtained [20]. It was found that the data generated is compatible with the given structure. The 1Pc compound that was generated in this study exhibit very good solubility in both DMF and DMSO. Elemental analysis was carried out for $C_{52}H_{50}N_{16}S_4$. The calculated (found) values for the compound were given as 60.79 (60.57), 4.90 (4.46) and 21.80 (21.59), respectively [20]. FT-IR spectra were determined to be 3285, 3029, 2927-2826, and 2263 cm^{-1}, accordingly [20]. It was seen that the FT-IR data obtained for the phthalocyanine is very similar [20].



Scheme-1: Chemical structural formula of 1Pc.

The strong absorption band at around 600 and 750 nm, is referred to as the Q-band, and UV band between 300 and 350 nm, is defined as the B-band, are detected in substituted metal-free 1Pc phthalocyanine. Q band around at 647-683 nm for 1Pc was observed in the characteristic electronic spectra of 1Pc in DMF as illustrated in Fig.1. In DMF solution, a B band region was detected about at 328 nm as presented in Fig. 1. The coplanar association of rings from monomers to dimers and higher order compounds is often defined as aggregation. It is depending on concentration, solvent character, peripheral substituents and temperature [26]. The aggregation perturbs electronic structure of a phthalocyanine molecule, which leads to the transformation of ground and excited states [27].

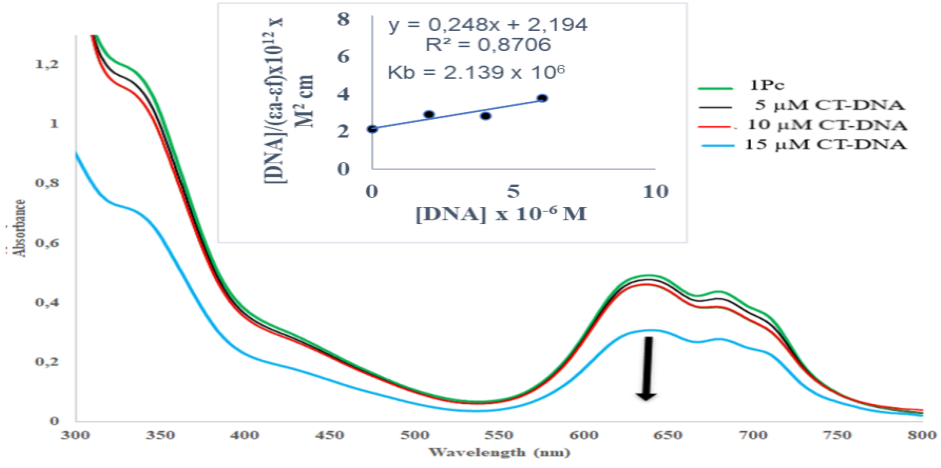


Fig. 1: Electronic absorbance spectrums of 20 µM 1Pc in the absance and presence varying amounts of (5 µM, 10 µM and 15 µM) CT-DNA) at ambient temperature.

The UV/Vis measurements of 1Pc binding to DNA

To study DNA interacting behavior of 1Pc and to obtain essential data for DNA binding modeling, the binding activity of 1Pc to DNA was investigated in the buffer at room temperature. Earlier studies published in the literature demonstrated that a compound that attaches to DNA by intercalating can produce hypochromism resulting from intercalation related the ~~p~~π-~~p~~π packing interacting between the aromatic chromophore and the bases of DNA [28]. In addition, the degree of intercalative interaction often determines the magnitude of hypochromism. Electronic spectra of 1Pc **(**20 µM**)** in the absence and in the presence of CT DNA **(**15 µM**)** were presented in Fig. 1. With rising on amount of DNA, the absorption peaks of 1Pc exhibited an apparent hypochromism, whereas red shift was not detected for the compound. For 1Pc, the reduction of the absorbance spectrums of the was observed as the amount of CT-DNA accumulates, and 1Pc exhibited hypochromism. The hypochromism caused by the binding of the DNA shows that the compound is interacting with the DNA. By observing the changes in absorbance for 1Pc, the intrinsic binding constant (K_b) of 1Pc to DNA was obtained [29]. The intrinsic binding constant K_b determined for the 1Pc was 2.139 x 10^6 M^{-1} as indicated in Fig. 1. The value was determined to be of the same order of magnitude as those of classical intercalators [29]. This revealed that 1Pc is most likely to be bound to the DNA by an intercalative model, involving strong stacking interactions between the compound and DNA. Furthermore, due to the various surface areas and hydrophobic feature of the ligand, the binding constant of 1Pc shows that the compound has strong DNA binding affinity. Unfortunately, because of surface aggregation produces similar results, the nature of DNA binding cannot be understood from a purely optical approach. Further studies will be necessary for a more detailed description of the DNA binding mode.

The viscosity measurement assay

A more detailed evaluation of the mechanism of DNA interaction of 1Pc was carried out by means of viscosity analysis. Viscosity assay can generate key data on the DNA binding type, which is highly delicate to variations in DNA size, and is recognized as an assay for the most critical mechanism of DNA bonding lacking crystallographic structural information [30, 31]. Basically, when a complex is incorporated into DNA, of the DNA helix stretches as the base pairs are pulled apart to hold of the bound ligand, resulting in increased the viscosity of DNA. On the other hand, 1Pc interacting by DNA via partial, non-classical intercalative linkage can diminish the efficient length and consequently the viscosity of DNA via the bending or the twisting of helix [32]. Additionally, DNA viscosity is not greatly affected by electrostatic or groove bonding of compounds. The viscosity values of the DNA were assessed in the presence of 1Pc. As is illustrated in Fig. 2, the relative viscosity of the CT-DNA was increased as progressively larger amounts of 1Pc was injected into the DNA sample. The enhanced degree of viscosity, which may be a function of DNA binding mechanism and affinity. The finding provided additional evidence that 1Pc has highly affinity intercalating between DNA bases. DNA binding affinity of 1Pc is in agreement with the above spectroscopic results and shows that compound 1Pc is able to penetrate deeper into the base pairs and therefore has a more predominant DNA linkage tendency due to the structure of the compound. From the result, it was concluded that1Pc intercalates among the base pairs of DNA.

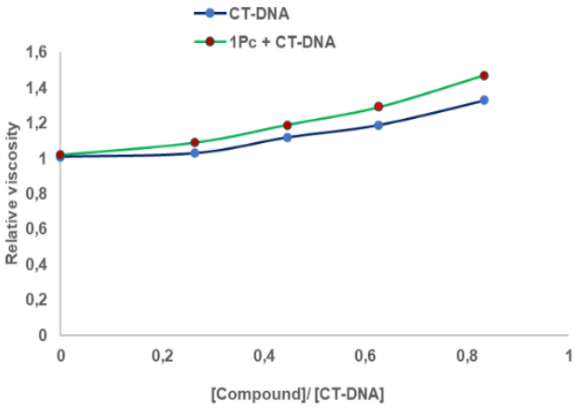


Fig. 2: The influence on the relative viscosity of CT-DNA, varying on amount of 1Pc compound.

The determination of binding activities of 1Pc using fluorescence measurement assay

Steady state emission titration is also utilized for verification of DNA-drug interactions as it is one of the most powerful and accurate procedures research on the binding of DNA and can offer a stronger the fundamental of the intercalative binding pattern of the compound. The 1Pc is able to produce emission in a buffer at room temperature. As can be displayed in Fig. 3, without DNA, 1Pc compound produces a powerful fluorescence that exhibits maxima at 503 nm in the buffer at a moderate temperature. For more analyzing the interacting of 1Pc via the DNA, the emission displacement assays of EB were conducted [33]. EB, an important DNA intercalator, penetrates into the DNA and induces an emission intensity that is higher

than the fluorescence intensity that it exhibits. Phthalocyanine compounds that are modified with functional groups can improve the affinity of the compound for DNA, leading to the dissociation of EB molecule from the CT DNA. A significant modification in the fluorescence intensity of the EB/DNA was detected. When adding increasing concentrations of 1Pc to EB-DNA in buffer solution as seen in Fig. 3. The native fluorescence band around at 503 nm (line 0) of the 1Pc compound was displaced and the fluorescence intensity was reduced (line 4). There may be two potential reasons for the decline the fluorescence intensity of DNA-EB when 1Pc are added. As a first possibility, there could be an interaction between the EB and the 1Pc, which would cause to reduce in emission intensity of the DNA-EB complex. The second is that 1Pc is more eager than the EB to interact with the DNA, thereby displacing the intercalated EB from the intercalation site and thus reducing on the amount of the EB bound to DNA. The type of competitive binding interaction is typically found to take place between two intercalative linkers. In this assay, it was seen that 1Pc does not undergo reaction with EB without DNA. Therefore, this experiment demonstrated that there is an intercalative bonding mechanism between 1Pc and the DNA [34, 35]. The result of this study demonstrated that the compound interacts with DNA.

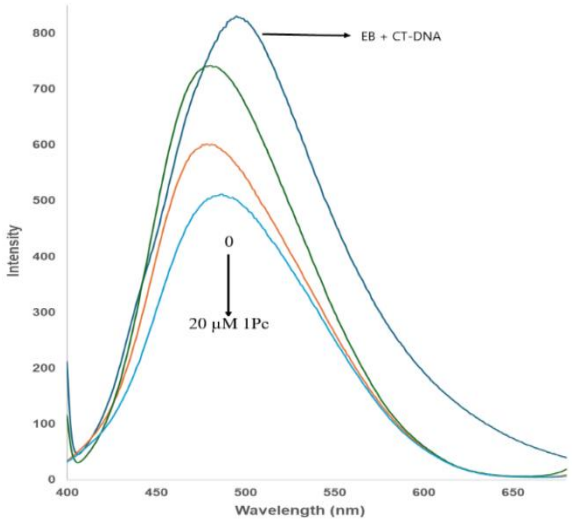


Fig. 3: Modification in fluorescence intensity of EB/DNA upon addition of 1Pc at room temperature. The concentrations of 1Pc compound were 10 µM, 15 µM and 20 µM, respectively. The arrow illustrates the intensity changing over increasing 1Pc.

The study of the gel electrophoresis for 1Pc

The capacity of 1Pc compound to attach with DNA was evaluated by agarose gel electrophoresis to detect the effect of 1Pc on the DNA at differing concentrations. The result of the experiment is illustrated in Fig. 4, where intensity of bands collected for 1Pc compound after attaching to CT-DNA is significantly diminished over the free DNA band. Decline in intensity of the bands produced after the linking of 1Pc compound to DNA is considered to be caused by break down DNA double helix. Earlier studies have proposed that DNA fragmentation is most likely to occur by backbone splitting as a result of nucleophilic attack on basic moieties [36]. The intensity of the bands was identified as ethidium bromide intercalation into bases of DNA, which depends on the length of the DNA as well as the number of molecules [37]. For this reason, blocking of the compound at interaction between stranded bases in the helix at nucleophilic surfaces in the DNA double helix may be attributed to reducing in intensity of electrophoretic bands of DNA on interacting with 1Pc compound.

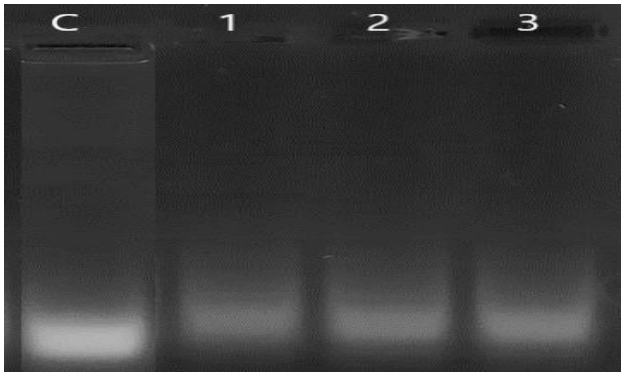


Fig. 4: The electrophoresis is illustrating the interaction between 1Pc and CT-DNA in the buffer. Lane C: untreated CT-DNA. Lanes 1–3: CT-DNA (20 µM) + 1Pc (10, 15 and 20 µM), respectively.

The study on the thermal denaturation

The thermal behaviour of DNA in the presence of chemical agents may provide about its conformational modification when temperature is allowed to rise and also it helps to understand how the interaction of compounds works with DNA. Tm of DNA solution is typically used to examine the linking of compounds to nucleic acids. Tm is given as the temperature at which in half of total base pairs are unbonded. In most cases, electrostatic attachment to phosphate backbone produces only a slight displacement in Tm, while the intercalation bond greatly elevates the thermal degradation temperature

of DNA owing to stabilization [38, 39]. Typically, the Tm of DNA rises when compounds attach to DNA via intercalative interacting mechanism, which occurs owing to the intercalation of compounds between DNA base pairs leads to stabilizing of base packing, which raises Tm of DNA. Melting temperature assay for 1Pc revealed a large shift in melting temperature (Tm). The Tm of the DNA in the absence and presence of 1Pc is shown in Table-1. In the absence of 1Pc compound, the Tm of CT-DNA was detected as 67.25 ^{o}C and in the presence of 1Pc compound, Tm of CT-DNA + 1Pc was identified as 74.31 ^{o}C in the specified experimental conditions. This significant elevation in the Tm of DNA with the 1Pc is comparable to that which has been recorded for the classical intercalators [40, 41]. This finding supports a strong intercalation correlation between 1Pc and CT DNA.

Table-1: The melting temperatures (T_m) *values* of CT-DNA in the absence and the presence of 1Pc compound.

Sample	The values of Tm
CT-DNA	67.25 ^{o}C
1Pc + CT-DNA	74.31 ^{o}C

Conclusion

In this research, the 1Pc phthalocyanine compound was previously synthesised and charaterized via elemental analysis, FT-IR, ^{1}H NMR and UV/Vis spectroscopy procedures. This phthalocyanine compound has a high degree of solubility in most organic solvents. UV/Vis, viscosity, fluorescence, gel electrophoresis and thermal denaturation experiments were performed to specify the DNA binding activity of the compound. On the basis of UV/Vis titrations of the compound tetra-4-(2-(N-2'-cyanoethyl)aminoethylsulfanyl)
phthalocyanine, it was found that the compound attaches to DNA via an intercalative binding mechanism. From the viscosity measurement data obtained in this work, the findings verified that the viscosity of the DNA was clearly enhanced when 1Pc binds to the DNA as compared to the partial viscosity of the DNA without the interacting. The linking of 1Pc to DNA displayed clear variation in the fluorescence intensity of EB/DNA. The data generated by this process demonstrated that the compound has high DNA binding activity. In addition to the above studies, the DNA binding activity of the compound was also examined using gel electrophoresis and thermal denaturation techniques. The results obtained from this study indicated that 1Pc interacts with DNA. These findings support that the compound may be suitable in photodynamic therapy. Although further studies are necessary for the verification of potential medicine for cancer therapy.

Conflicts of Interest

The authors have declared no conflicts of interest.

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