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Investigation of biological activities of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol Schiff Base

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Abstract

Schiff bases are the most widely used compounds in pharmaceutical chemistry as therapeutic agents. DNA is the main target of drugs in various diseases, especially cancer, allowing the research of new molecules that interact with DNA. In our study, it was targeted to determine the potential of newly synthesized 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol Schiff base as an antimicrobial, antimutagenic and anticancer agent by *in vitro* methods. Therefore, in our study, the antimicrobial activity of Schiff base was tested against bacteria and yeasts with a minimum inhibitory concentration (MIC) method. The antimutagenic effect of Schiff base was determined by the Ames/Salmonella test. The interaction with DNA was investigated *in vitro* by UV titration method and agarose gel electrophoresis method. In our study, it showed antimicrobial activity at concentrations of 32-128 µg/mL against bacteria and 32-64 µg/mL against yeasts. It was determined that Schiff base showed an antimutagenic effect depending on increasing concentration, and as a result, it prevented both base pair exchange and frameshift mutation ($p<0.05$). *In vitro* results showed that the Schiff base intercalates and electrostatically binds to CT-DNA (Calf thymus DNA) and cleaves pBR322 plasmid DNA both hydrolytically and oxidatively. As a result, it can be suggested that 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol interacts with DNA according to *in vitro* findings and can be used as an antimicrobial and anticancer agent.

Keywords: Antimicrobial activity; Antimutagenic effect; DNA binding; DNA cleavage

1. Introduction

Schiff bases are the most extensively used compounds in pharmaceutical chemistry. These compounds are among the important drugs used in the medical field. Schiff bases and complexes, which have been used in the treatment of cancer, have an important position in the medical world due to their anticancer properties. Moreover, Schiff bases have antimicrobial [1], antimalarial [2], antiviral [3], antitumor [4], and antioxidative [5] effects. In addition, the need for the synthesis of Schiff bases is increasing day by day due to their wide range of pharmacological and biological applications.

Antimicrobial drugs play a critical role in the treatment of infections caused by pathogenic microorganisms. These drugs work by inhibiting the growth or reproduction of various microorganisms, such as bacteria, viruses, fungi, and parasites. Antibiotics are one of the most common classes of antimicrobial drugs and are widely used to treat bacterial infections. However, the rapid increase in antibiotic resistance in recent years poses a serious threat to public health and reduces the effectiveness of these drugs.

Antibiotic resistance occurs when microorganisms undergo genetic changes that neutralize the effects of antibiotics or develop tolerance to these drugs. This may cause patients not to be treated, infections to last longer, and mortality rates to increase. The World Health Organization (WHO) and other health authorities emphasize that antibiotic resistance is a global health crisis and state that urgent measures must be taken to solve this problem [6].

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To overcome antimicrobial resistance, researchers are conducting intensive studies to develop broader-spectrum and more powerful antimicrobial agents. These studies include new molecules obtained from natural products, as well as peptide-based agents produced by biotechnological methods. Antimicrobial peptides show promising results in overcoming resistance mechanisms [7-8]. Therefore, researchers tend to search for antimicrobial agents with stronger efficacy and broad-spectrum [9-11]

Cancer which ranks second after cardiovascular diseases in the world, is the most important social problem and cause of death [12]. Cancer occurs as a result of mutations in genes related to oncogenes and tumor suppressors. For this reason, it is known that chemical agents that cause mutations generally have carcinogenic potential. In our daily lives, people are in constant contact with many chemicals. Each of these chemicals must be tested for their carcinogenic potential. Scientists investigate various antimutagen and anticarcinogenic substances that can be used for the treatment of cancer. The development of new and effective anticancer drugs that can bind to DNA in chemotherapy is one of the most studied topics recently. Research has focused on new drug molecules that can be effective against cancer and also have no adverse effects on human health. DNA, which is of primary importance in the maintenance of cell viability; ensures the growth and proliferation of the cell by providing the synthesis of proteins and enzymes by replication and transcription [13]. The way to stop cancerous cells from multiplying uncontrollably will be possible with the development of molecules targeting DNA.

Today, drugs used for treatment as antitumor, antiviral and antibiotics show their effects by binding to DNA [14]. When antitumor drugs bind to DNA, they inhibit the replication process and cell growth, causing cell death [15]. Therefore, it is very important to search for new molecules for the development of more effective anticancer and antibiotic drugs.

DNA-binding properties have become the focus of attention in studies with molecules with small structures such as Schiff bases. This is because most of the intracellular targets of anticancer and antibiotic drugs are DNA [16-18]. Binding mode of small molecules to DNA; Binding to grooves occurs in different ways, including intercalation and electrostatic interactions. Of these binding modes, intercalation and groove binding are the most important as they lead to cell disruption [19,20]. Compounds that can cut or bind DNA from a specific region will undoubtedly contribute greatly to the development of new antibiotic drugs and chemotherapeutic agents.

In the literature review, there is no study on the antimicrobial and anticancer activity of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol Schiff base. In this study, antimicrobial activity and interactions with DNA were tried to be determined for the first time using *in vitro* methods to investigate the potential of newly synthesized Schiff base to be a drug for use in the treatment of both anticancer and antimicrobial agents.

2. Material and Methods

2.1. Materials

Mueller Hinton Broth, CT-DNA, RPMI 1640, ethidium bromide, 4-nitro-*o*-phenylenediamine (NPD) L-histidine, glucose, D-biotin, and agar (Difco) were purchased from Sigma Aldrich. DNA marker and pBR322 Plasmid DNA were purchased from Thermo Fisher Scientific. Sodium hydroxide (NaOH), tris base, hydrochloric acid (HCI), Ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl) Magnesium sulfate ($MgSO_4.7H_2O$), potassium phosphate (K_2HPO_4), disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$), citric acid monohydrate ($C_6H_8O_7.H_2O$), sodium dihydrogen phosphate monohydrate ($Na_2HPO_4.H_2O$), nutrient broth (Oxoid) and sodium azide (SA) were purchased from Merck Millipore. The structure of the Schiff base used in the study is shown in Figure 1.

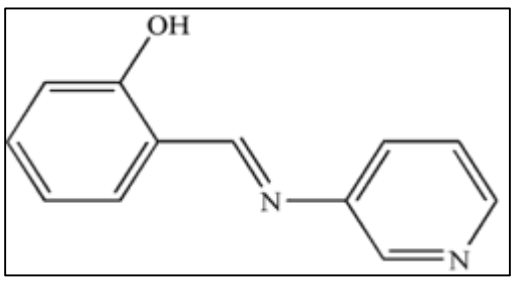

Figure 1 Chemical formula of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol Schiff base

2.2. Methods

2.2.1. Antimicrobial activity

For the antimicrobial activity of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* bacteria; *Candida albicans* and *Candida tropicalis* yeasts were used. Gentamicin, ampicillin and fluconazole were used as standard antibiotics. The antimicrobial activity of the Schiff base was determined by the microdilution minimum inhibition concentration (MIC) method according to the protocol in Essential Procedures for Clinical Microbiology [21]. The MIC test was performed as specified in the Clinical and Laboratory Standards Institute (CLSI) guidelines [22].

2.2.2. Antimutagenic effects

The antimutagenic activity of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol was performed with the Ames/*Salmonella* assay using the TA98 and TA100 mutant strains of *Salmonella typhimurium* [23]. In the experiment, 0.1 mL of bacterial culture was added to 2 mL of top agar containing 0.5 mL of sodium phosphate buffer (PBS) and different concentrations of Schiff base. It was then poured onto minimal glucose agar (MGA) plates. Positive, negative, and spontaneous controls were used in parallel during the study. SA for strain TA100 and NPD for strain TA98 were used as a positive control, while dimethyl sulfoxide (DMSO) was used as a negative control. Each sample was evaluated with 3 replicate plates.

2.2.3. DNA binding

UV-Vis absorption titration method was used for the DNA binding activity of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol. The study was performed at room temperature using CT-DNA and Tris-HCl/NaCl (pH 7.2) buffer. The UV-Vis absorbance of CT-DNA in Tris buffer should be between 1.8-1.9 at 260-280 nm, thus indicating the absence of protein in the DNA [24]. The Schiff base and DNA were prepared with DMSO and double distilled water, respectively. In the study, the concentration of the Schiff base (30 μM) was kept constant and the CT-DNA concentration was increased gradually.

2.2.4. DNA cleavage

The DNA cleavage activity of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol was determined using agarose gel electrophoresis with pBR322 DNA [25]. The DNA cleavage activity of the Schiff base was performed in the presence of an oxidizing agent (H_2O_2, oxidative cleavage) and in the absence of an oxidizing agent (hydrolytic cleavage). The experiment was carried out using pBR322 plasmid DNA (0.1 mg/mL) in Tris-HCl buffer (pH: 7.2) with different concentrations of Schiff base (25, 50, 100, 200, 400 μM) and incubated at 37°C for 3 hours. Then, the samples were run at 60 V for 60 minutes and UV light was used to visualize the resulting bands.

2.2.5. Statistical analysis

Statistical analysis for antimutagenic activity of Shiff base, differences between application groups and positive control groups were determined by one-way analysis of variance. The Tukey test was used to determine the significance level; $p<0.05$ was considered significant.

3. Result and discussion

3.1. Antimicrobial Activity

The antimicrobial activities of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol were determined with broth microdilution method [22]. The MIC values obtained from the antimicrobial activity assay are given in Table 1. Antibacterial activity was determined as 32-128 μg/mL concentrations range, and antifungal activity was determined as 32-64 μg/mL concentrations. As a result, the Schiff base has antimicrobial activity. Also studies in the literature indicate that Schiff bases have antimicrobial activity [26-28].

The results showed that Schiff base was effective on the bacteria *B. subtilis* and the yeast *C. albicans*. Both microorganisms that cause serious infections in humans [29,30]. In particular, 75% of the infections caused by the Candida genus, which has 200 species, are *C. albicans* [31]. The fact that the newly synthesized Schiff base is effective against *B. subtilis* and *C. albicans* species is promising for treatment.

Table 1 MIC values (µg/mL) of the 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol

Microorganisms	**Schiff base**	**Gentamicin**	**Ampicillin**	**Fluconazole**
Staphylococcus aureus ATCC 25923	64	1	0.016	-
Enterococcus faecalis ATCC 29212	64	1	0.016	-
Bacillus cereus NRRL B-3711	64	0.125	0.125	
Bacillus subtilis ATCC 6633	32	0.08	0.06	-
Escherichia coli ATCC 25922	64	0.125	32	-
Escherichia coli ATCC 35218	64	0.03	32	-
Pseudomonas aeruginosa ATCC 27853	64	0.08	2	-
Proteus vulgaris ATCC 13315	128	0.125	0.06	-
Candida albicans ATCC 60198	32	-	-	0.0625
Candida tropicalis ATCC 13803	64	-	-	0.5

3.2. Antimutagenic Effects

The antimutagenic effect of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol was performed using the TA98 and TA100 mutant strains of *S. typhimurium* by the Ames/*Salmonella* test. The evaluation the Schiff base is given in Table 2 by comparing different concentrations with positive control and spontaneous control. The antimutagenicity to inhibition ratio was calculated using the following equation 1.

$$\text{Inhibition rate (\%)} = (A\text{-}B/A\text{-}C) \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

A in the formula is the number of revertant colonies in the presence of mutagen/plate; B in the formula is the number of revertant colonies in the presence of Schiff base/plate; C in the formula is the number of spontaneous colonies/plate. An inhibition rate of 25-40% is considered moderate, and an inhibition rate above 40% is considered a strong antimutagenic effect. If the inhibition rate is below 25%, it is considered to have no antimutagenic effect [32-34].

It was determined that all concentrations of the Schiff base (0.5, 5, 50 and 500 ppm) had an antimutagenic effect. Our experimental results with the TA98 strain of *S. typhimurium*, the Schiff base had a moderate antimutagenic effect (39.63%) only at a concentration of 0.5 ppm ($p<0.05$), while it had a strong effect at other concentrations. It was found to have inhibition (5 ppm; 41.49%, 50 ppm; 45.86%, 500 ppm; 51.73%) and as a result, it had a strong antimutagenic effect ($p<0.05$). Experimental result with the TA100 strain of *S. typhimurium*, the Schiff base was determined to have a strong antimutagenic effect at all concentrations ($p<0.05$).

In previous studies, the antimutagenic effects of Schiff bases were examined using TA98 and TA100 strains of *S. typhimurium* and it was found that they prevented both mutations [35, 36]. Schiff base aminoguanidine compounds were tested using mutant strains of *S. typhimurium* and it was determined that Schiff bases have strong antimutagenic and antibacteriostatic effects. Researchers have considered aminoguanidine compounds as possible pharmacological agents for the prevention of many diseases [37]. According to the data, it was determined that 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol Schiff base had an antimutagenic effect due to increasing concentration, and as a result, it prevented both base pair change and frameshift mutation.

Table 2 Antimutagenic effects of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol on TA98 and TA100 strains of *S. typhimurium* according to different concentrations

Treatment		**Concentration (ppm)**	**His⁺ Revertant Colony Number/Plate**			
			TA98	**% Inhibition**	**TA100**	**% Inhibition**
			Means±SE		**Means±SE**	
Schiff base		0.5	469.33±25.32*	39.63	555.00±09.16*	64.80
		5	455.66±30.33*	41.49	599.66±10.50*	65.52
		50	423.33±21.54*	45.86	675.33±24.50*	72.59
		500	380.00±18.00*	51.73	699.66±11.01*	77.35
Positive Control	NPD	10^{-2}	762.00±71.04			
	SA	10^{-3}			793.00±39.61	
Negative DMSO Control			33.00±06.24		110.66±10.50	
Spontaneous Control			23.66±02.51		99.66±01.52	

An asterisk (*) indicates that the difference between treatment groups and the positive control is significant at the $p<0.05$.

3.3. DNA Binding

The primary target of drugs used in the treatment of microbial diseases and cancer is DNA [38-40]. UV-Vis absorption spectroscopy is widely utilized in the determination of drug-DNA interactions [41, 42]. The differentiation of the absorption properties of the drug or DNA is followed by UV-Vis absorption spectroscopy. UV-Vis spectroscopy is a common technique used to determine different modes of binding of drug molecules to DNA [43-44]. When the UV-Vis spectrum of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol is examined, the change in absorbance intensity with the increase of CT-DNA concentration is hyperchromism at 589 nm and hypochromism at 268 nm (Figure 2). 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol showed 57% hyperchromism (an increase in absorption intensity), 501% hypochromism (a decrease in absorption intensity), and a 6-8 nm bathochromic (red) shift in absorptions at 589 and 268 nm.

The presence of hyperchromism indicates that the interaction of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol with DNA is via hydrogen bonding [45]. Hypochromism indicates that compounds interact with nucleic acids through intercalation, which involves a strong π-π* stacking interaction between the compound and the DNA base pairs [46, 47]. The intrinsic binding constant K_b (binding constant) was calculated utilizing equation 2 below.

$$[DNA]/(\varepsilon A - \varepsilon f) = [DNA]/(\varepsilon B - \varepsilon f) + 1/Kb(\varepsilon B - \varepsilon f) \quad \text{.....} (2)$$

In the equation, [DNA] represents the concentration of DNA base pairs. εa, εf and εB show the apparent absorption coefficient of the compound that interacts with DNA, free and fully DNA, respectively.

The Kb value is calculated from the slope/intercept ratio. From here, K_b is calculated from the slope/intercept ratio. The calculated K_b values of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol were $7x10^4$ M^{-1} for hypochromism and $1.3x10^5$ M^{-1} for hyperchromism. When the K_b values of the Schiff base are compared with the commonly reported ethidium bromide intercalating agent ($K_b=7x10^7$ M^{-1}), it appears to show reasonable binding [48].

As a result, it was determined that 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol interacts with DNA intercalatively and electrostatically. It has been shown that Schiff base and its derivatives synthesized in previous studies can bind to DNA both intercalatively and electrostatically [27, 49]. Molecules whose mode of binding to DNA is intercalation inhibit DNA replication and are therefore used in cancer therapy [18, 50, 51]. The binding of the Schiff base, which interacts with DNA, via its intercalative mode shows its potential as an agent in cancer therapy. Extensive evaluations have been made on the effectiveness and clinical applications of Schiff bases in anticancer treatments. Schiff bases are known to interact with DNA in cancer cells, causing apoptotic cell death and effectively inhibiting tumor growth. At the same time, the side effects of these compounds on healthy cells were examined and they were reported to have low or high toxicity depending on the side groups added to Schiff bases [52]. In order to ensure the safe use of Schiff bases in clinical applications, it is very important to examine dose adjustments and long-term side effects in detail [53].

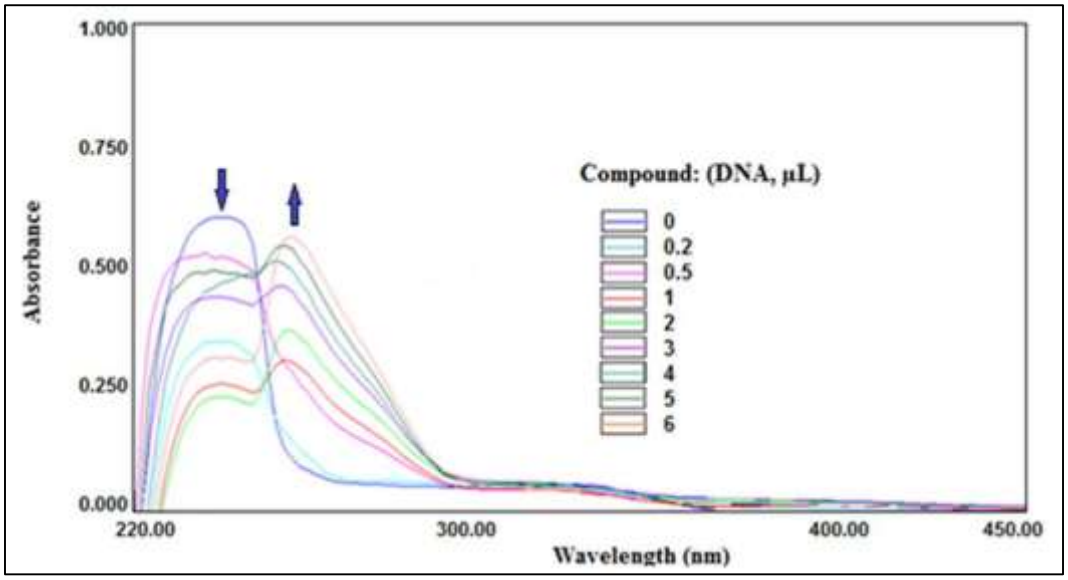

Figure 2 Absorption spectra of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol in the absence (blue colored peak) and with the addition of rising amounts of CT-DNA. Arrows (↑↓) shows the change in absorbance with rising DNA concentration

3.4. DNA Cleavage

The main target in DNA cleavage is to act on the deoxyribose sugar, nucleobases, and phosphodiester bonds in the structure of DNA by hydrolytic or oxidative pathways. The interaction of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol with plasmid pBR322 was studied using gel electrophoresis as oxidative and hydrolytic cleavage. DNA cleavage is determined by three different forms of DNA in the gel in gel electrophoresis with the transition of the closed circular form (Form I), which is the natural structure of plasmid DNA, into notched circular (Form II) and linear forms (Form III). Form I is the fastest progressing form on the gel. When cleavage come about in a single strand of plasmid DNA, the strand of the plasmid unwinds and Form II structure is formed. The Form II structure is the slowest in gel electrophoresis. Gel electrophoresis images of the Schiff base are shown in Figure 3. The band forms seen on the gel at five different concentrations (25, 50, 100, 200, and 400 µM) were examined as plasmid DNA cleavage, both hydrolytic and oxidative. The hydrolytic cleavage come about as a consequence of the hydrolysis of phosphodiester bonds in DNA, while oxidative cleavage occurs by oxidation of deoxyribose sugars or nucleobases [54, 55] In the literature, there are studies in which Schiff bases can cleave DNA both hydrolytically and oxidatively [56, 57].

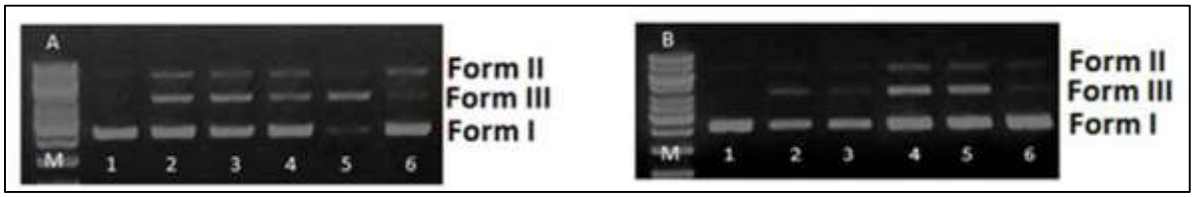

Figure 3 DNA cleavage activity of 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol; A. Oxidative cleavage: M marker,1. Plasmid DNA control, 2. DNA+25µM+H_2O_2, 3.DNA+50µM+H_2O_2, 4.DNA+100µM+H_2O_2, 5.DNA+200µM +H_2O_2, 6.DNA+ 400 µM+H_2O_2, B. Hydrolytic cleavage: M marker,1. Plasmid DNA control, 2. DNA+25µM, 3.DNA+50µM 4.DNA+100 µM, 5.DNA+ 200µM, 6.DNA+ 400 µM

4. Conclusion

Today, with rapidly increasing technological developments, many environmental factors, including chemical and physical agents, affect living things negatively. These negative effects cause permanent damage to the hereditary material in the cell. Uncontrolled proliferation of cells can occur due to mutations in DNA, and this leads to cancer. Although many drugs are used in cancer treatment, their use is limited due to their toxicity. However, despite a large amount of antimicrobial-effective drugs, the fact that microorganisms gain resistance to these drugs very quickly has made the research of new effective antimicrobial agents important. The main mechanisms of action of anticancer and

antibiotic drugs are on DNA interactions. Therefore, small molecules that can interact with DNA are needed. In our study, it was determined that 2-{(*E*)-[(pyridin-3-yl)imino]methyl}phenol has antimicrobial activity and interacts with DNA. It was determined that pBR322 cleave plasmid DNA hydrolytically and oxidatively and binds to CT-DNA intercalatively and electrostatically. As a result, it is thought that molecules that can cleave or bind DNA from a specific region will undoubtedly contribute greatly to the development of new antibiotic drugs and chemotherapeutic agents.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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