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RESEARCH ARTICLE

Synthesis of 2-amino-4-(4-methoxyphenyl)-1,3-thiazole coated-magnetic nanoparticles: A potential antibacterial compound

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ABSTRACT

Objective(s): Schiff bases compounds are typically containing a nitrogen analogue of ketone or aldehyde, in which the group of carbonyl is replaced by an azomethine or imine group. These complexes are useful for various industrial goals, catalysis of chemical reactions, and have the capability of using as therapeutic compounds such as anti-microbial, anti-cancer, anti-inflammatory, and antiproliferative. Herein, we report the surface modification of Fe_3O_4 magnetic nanoparticles by a synthesized Schiff-base, named 2-amino-4-(4-methoxyphenyl)-1,3-thiazole.

Methods: Various characterization techniques including, XRD, 13C-NMR, 1H-NMR, SEM, and EDXRF were employed to survey the functional and structural features of the compound.

Results: Average particle size for the prepared Fe_3O_4 magnetic nanoparticles was calculated as about 12nm. SEM confirmed the spherical morphology of magnetic nanoparticles. Moreover, the coated Fe_3O_4 NPs with Schiff base displayed the desired anti-Escherichia coli and anti-Staphylococcus aureus activity.

Conclusions: The herein method is beneficial for the application in diverse fields such as drug delivery, industry, and water treatment.

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INTRODUCTION

Schiff bases complexes are diverse compounds that are generally constituted from a nitrogen analog of a ketone or aldehyde such that the group of carbonyl has been replaced by an azomethine or imine. Over the recent decade, the application of various Schiff bases complexes for disparate industrial purposes and catalysis of chemical reactions, and also in the extensive range of therapeutic functions including anti-angiogenic, anti-inflammatory, anti-cancer, anti-microbial, antiproliferative, and antiviral have been proposed (1-5). Despite many advantages of Schiff bases, they are encountered by some drawbacks such as the difficulty of separation and also recovery (5). Heterocyclic compounds bearing nitrogen, thiazole, and sulfur moieties have the core position in the structure of some interesting biological compounds. Likewise, 2-amino thiazole moiety was reported to possess antifungal, antitubercular, antimicrobial, and anti-oxidant activities (6-8).

Due to the unique properties of superparamagnetic nanoparticles, they have been widely employed in medical-biological investigations like drug delivery and biochemical sensing (9). Magnetic nanoparticles are very popular for antibacterial application, due to their high surface area and active sites (10). However, they are unstable since easily aggregate and oxidize limiting their applications. Therefore, for tackling

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this issue, surface coatings and functionalization could be utilized for improving the performance of these nanoparticles (11, 12). Schiff bases are a group of ligands that show a prominent binding tendency for metal ions (13). The surface modification of magnetic nanoparticles would incorporate the properties of both components and supply the proper compounds to develop high efficient compounds (14-16).

In this study, a novel Schiff base coated Fe_3O_4 with the capability of antibacterial activity is presented. The successful coating of the magnetic Fe_3O_4 nanoparticles (MNPs) by Schiff base leads to taking advantage of both Schiff base and MNPs properties in antimicrobial activity properties and preventing the aggregation of Fe_3O_4 MNP. Finally, the coated Fe_3O_4 MNP was successfully applied as an antibacterial against Staphylococcus aureus and Escherichia coli.

MATERIALS AND METHODS

Materials

All reagents including $FeCl_3{\cdot}4H_2O$, 99%, $FeCl_2{\cdot}6H_2O$, 98%, and NH_4OH, thiourea were provided from Sigma-Aldrich and then were utilized without subsequent purification. The aqueous solutions were prepared with deionized (DI) water.

Instruments

X-ray diffraction (XRD) patterns were determined by a PANalytical Empyrean with CuK radiation. Nuclear magnetic resonance spectroscopic analyses (1HNMR and 13C-NMR) were performed on Bruker Ultra Shield (300 MHz). Surface morphology analysis was conducted by scanning electron microscopy (SEM, NORAN at an electron acceleration voltage of 25kV).

Synthesis of 2-amino-4-(4-methoxyphenyl)-1,3-thiazole

To synthesize 2-amino-4-(4-methoxyphenyl)-1,3-thiazole, firstly, I_2 (5 gm, 0.02 mol) and thiourea (3gm, 0.04 mol) were triturated and then dissolved in acetophenone (0.02 mol). Next, it was warmed in a water bath at a temperature of 70 ^oC with occasional stirring for 8 h. The rest solid residue after evaporation of solvent by cooling was triturated with Et_2O to remove unreacted acetophenone. The precipitation was washed with aqueous thiosulphate (5%) followed by water to remove overplus iodine. The hot water was used to dissolve the resulting product. Then, it was filtered to remove the sulfone. Afterward, the ammonia solution was used to precipitate the substituted thiazole. Finally, the product was recrystallized with ethanol.

Synthesis of magnetic Fe_3O_4 nanoparticles

$FeCl_3{\cdot}4H_2O$, $FeCl_2{\cdot}6H_2O$, and NH_4OH were utilized (S. Wu et al., 2011). A mixture of ferric chloride (0.025M) and ferrous chloride (0.025M) solutions was added into a solution of 2 M of NH_4OH. 200 ml of double-distilled water and 50 ml of NH_4Cl (1M) were poured into a round flask. Then, mechanical agitation was employed to agitate the mixture (15 min, 800 rpm). Next, 15 ml of $FeCl_2{\cdot}4H_2O$ 0.025 M and 25 ml of $FeCl_3{\cdot}6H_2O$ 0.025 M were instantly surcharged. It led to appearing a black precipitate which was then separated using a magnetic. Eventually, double-distilled water was used to wash the yield and then dried at a temperature of 45°C for 6h.

Functionalization of MNPs with Schiff base compound

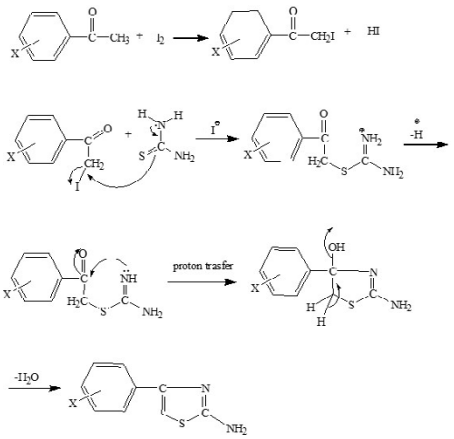
The magnetic Fe_3O_4 nanoparticles were coated by the Schiff base. To do this purpose, about 1.5 mg MNPs were dispersed in 50 ml ethanol via sonication for 50 minutes. Then, 20 ml of 2 M NaOH was appended to homogenize the solution for about 30 min. After that, 20 ml of 2-amino-4-(4-methoxyphenyl)-1,3-thiazole was augmented to the mixture under mechanical shaking at 50 ^oC during 7 h. The product was achieved via magnetic isolation. Finally, the product was washed several times using ethanol and double-distilled water until pH reached 7, then dried and kept for further use.

Antibacterial susceptibility

Fresh plates of S.aureus and E.coli isolates were prepared from the Koya University. The bacterial stock cultures were kept in nutrient agar slants at 4^oC. Gram staining technique was conducted to confirm the bacteria. A fresh bacterial sample was inoculated into 200ml of Nutrient broth and afterward incubated at 37^o C during 24 h. To obtain the biomass, the medium was centrifuged at 5000 rpm during 15 minutes. The antibacterial activity of coated MNPs was surveyed through reconciling the inhibition zones of bacteria generated by the synthesized compound.

Table1. Physical properties of synthesized 2-amino -4-(4-methoxyphenyl)-1,3-thiazole

M.F	Yield %	M_w	M.P °C	Color
$C_{10}H_{10}N_2OS$	76	206	207-209	Light yellow



Scheme 1. Typical mechanism of the formation of 2-amino -4-(4-methoxyphenyl)-1,3-thiazole.

Table 2. ^{1}H-NMR data of (2-amino -4-(4-methoxyphenyl)-1,3-thiazole).

δ / ppm	Multiplicity	Intensity	Assignments
3.77	s	3H	-OCH_3
6.81	s	2H	-NH_2
6.94	d	2H	Ar-H-$C_{7,11}$
7.13	s	1H	-CH(thiazole)
7.74	d	2H	Ar-H-$C_{8,10}$

RESULTS AND DISCUSSION

The physical properties of 2-amino-4-(4-methoxyphenyl)-1,3-thiazole are mentioned in Table 1. Moreover, the possible mechanism of the reaction is depicted in Scheme 1.

Characterization of 2-amino-4-(4-methoxy phenyl) 1,3-thiazole

^{1}H-NMR

The ^{1}H-NMR spectrum demonstrates two single peaks at δ 6.82 ppm and 3.77 ppm indicating the protons of (-NH_2) and (-OCH_3) groups, respectively. Another singlet peak at δ 7.13 ppm corresponds to the (-CH) group belonging to thiazole ring, while the peaks at δ 7.74 and 6.94 ppm are attributed to CHβ and CHα belonging to the phenyl group, respectively (Table 2 and Fig. 1).

^{13}C-NMR

The formation of amines was also confirmed through the observation of eight chemical shifts resulting from ^{13}C-NMR analysis. The appearance of three lines at δ (99.79, 150.17, and 168.55) ppm were assigned to C_5, C_4, and C_2 for thiazole rings, respectively. The attendance of a phenyl ring can be realized by the chemical shifts at δ C_9 (158.99), $C_{8,8'}$ (114.27), $C_{7,7'}$ (128.34), C_6 (127.30) ppm. Another line observed at δ 55.51 ppm is referred to as carbon of methoxy moiety (Table 3 and Fig. 2).

Characterization of MNPs coated by 2-amino -4-(4-methoxyphenyl)-1,3-thiazole

X-ray diffraction (XRD)

To identify the phase and crystalline structure of the synthesized Fe_3O_4 nanoparticles, high-resolution X-ray powder was employed. The XRD spectrum of Fe_3O_4 nanoparticles shows obvious peaks at 2θ angles of 30.1°, 35.4°, 43.1°, 53.5°, 57.2°, and 62.7° which attribute to (220), (311), (400), (42 2), (511), and (440) crystal planes, respectively (16). Moreover, the crystallite size of the synthesized specimen was determined by employing the Debye-Scherrer equation as below (17):

$$D = \frac{k\lambda}{\beta \cos\theta}$$

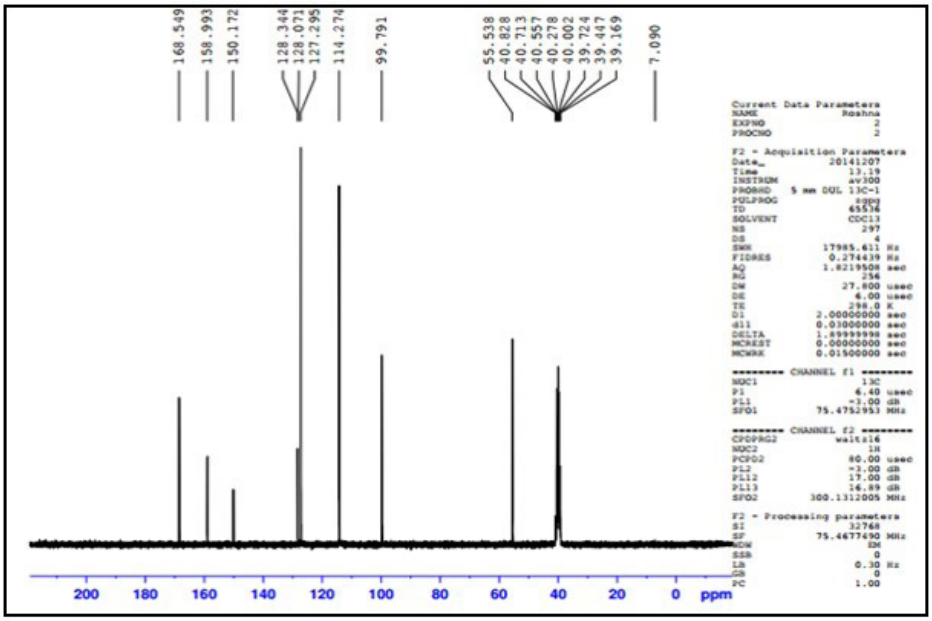


Fig. 1. [1]H-NMR spectrum of 2-amino -4-(4-methoxyphenyl)-1,3-thiazole.

Table 3. [13]C-NMR data of (2-amino -4-(4-methoxyphenyl)-1,3-thiazole).

δ / ppm	Assign.
55.54	-OCH_3
99.79	C_5
114.27	$C_{8,8'}$
127.30	C_6
128.34	$C_{7,7'}$
150.17	C_4
158.99	C_9
168.55	C_2

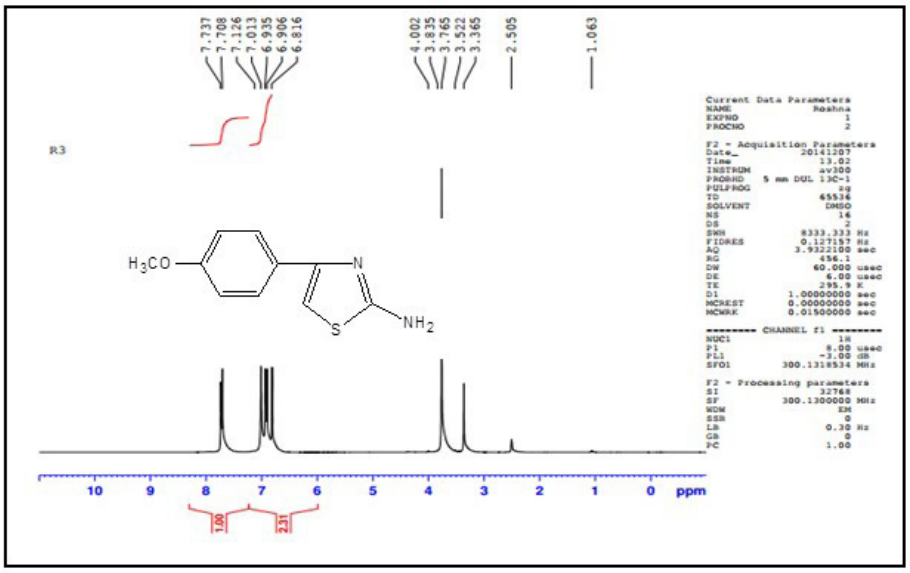


Fig. 2. [13]C-NMR spectrum of 2-amino -4-(4-methoxyphenyl)-1,3-thiazole.

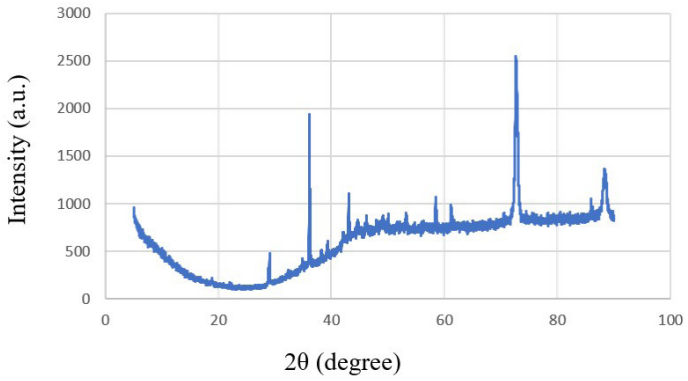


Fig. 3. XRD patterns of the synthesized Fe_3O_4 nanoparticles.

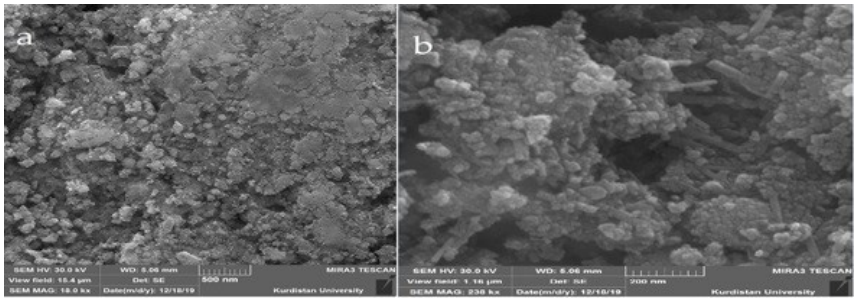


Fig. 4. The SEM images of (a) pure MNPs (b) decorated MNPs with 2-amino -4-(4-methoxyphenyl)-1,3-thiazole.

In the above equation, the wavelength is 0.1540 nm, β is the full-width at half-maximum amount of diffraction lines, and θ is the mid diffraction angle of 2θ. The average crystallite for the prepared samples was calculated as about 12 nm. The XRD indicates the no change in the crystal structure of Fe_3O_4 after coating.

Surface morphology analysis

The surface morphology of MNPs was surveyed using SEM. The SEM image (Fig. 4a) clearly shows the aggregation of pure MNPs which is related to the superparamagnetic property of Fe_3O_4, which is agreed with previous studies (15). The size of nanoparticles was calculated as 4.2-19.7 nm with a mean particle size of 10 nm. After the decoration of MNPs with 2-amino compounds, the sizes of nanoparticles were increased to about 30-50 nm with a mean particle size of 15 nm with porous shape (Fig. 4b). Due to the decoration of 2-amino compound on iron oxide, it seems that accumulation is prevented. Furthermore, the dispersion phase particles were improved which can also be explained by the electrostatic repulsion force between amino molecules landed on the surface of MNPs (18). The increment of the particle size approves the attendance of Schiff base on the MNPs.

The element composition of the synthesized compound is indicated by the peak height of the EDXRF spectra as shown in Fig. 5. The peaks around 0.4, 6.3, and 6.8 keV are correlated to the binding energies of Fe (19, 20). Moreover, the spectrum includes three peaks allocated to Fe, O, and C. The peak of C is due to the attachment of 2-amino -4-(4-methoxyphenyl)-1,3-thiazole on the MNP surface. As a result, the EDXRF confirms that Fe, O, and C are the major elements in the MNPs (21). The possible structure of the synthesized Schiff base coated on the surface of Fe_3O_4 nanoparticles is depicted in Scheme 2.

Antibacterial activity of coated Fe_3O_4 NPs

The antibacterial activity of coated Fe_3O_4 NPs was surveyed against the gram-positive (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli). The outcomes confirm that these compounds are efficacious antibacterial

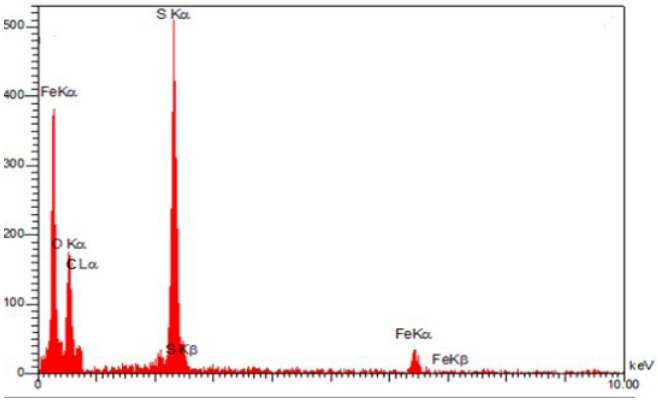
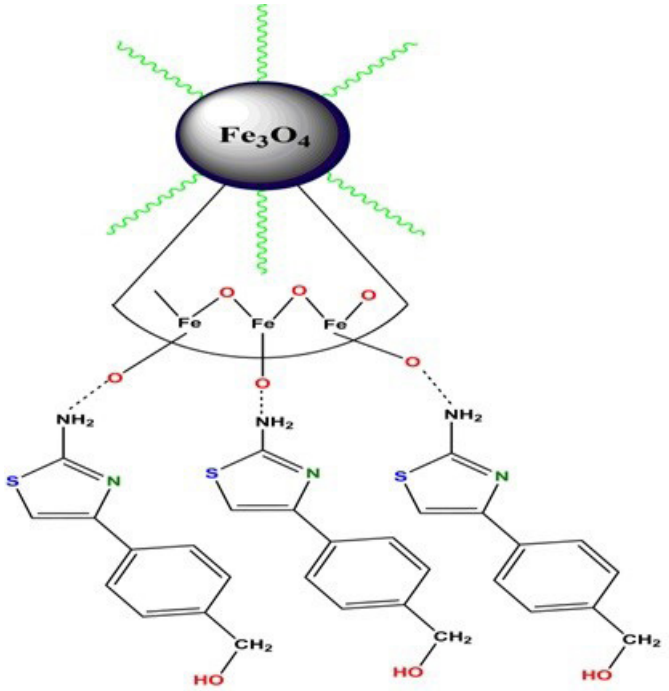


Fig. 5. EDXRF spectra of MNPs.



Scheme 2. Possible structure of 2-amino-4-(4-methoxyphenyle)-1,3-thiazole coated on the surface of magnetic Fe_3O_4 nanoparticles.

agents. These organisms were opted due to their functions in causing healthcare-associated infections and Bacteremia. Fig. 6 shows the antibacterial activity of Fe_3O_4 using the good diffusion method. The growth of Escherichia coli and Staphylococcus aureus were inhibited at a concentration of 10 mg/ml of Fe_3O_4@Schiff base. The control was the untreated culture (without Fe_3O_4@Schiff base). The antibacterial activity was corroborated through observation of clear zone around the discs (Table 4).

The bactericidal activity is due to the attendance of reactive oxygen species (ROS) produced by Fe_3O_4 NPs (22). ROS comprises singlet oxygen and radicals such as hydroxyl radicals, superoxide radicals, and hydrogen peroxide could be the cause of damaging the DNA and proteins in the bacteria (23). Actually, there is a chemical interaction between the substance generated in the attendance of MNPs and the exterior bacteria bilayer would be the cause of the MNP's antibacterial activity (24). It can be explained that iron oxide nanoparticles are positively charged while bacteria are negatively charged. Therefore, a strong electromagnetic

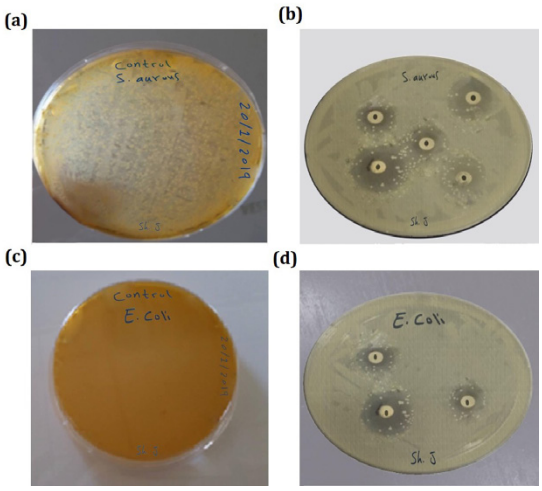


Fig. 6. Antibacterial activity of coated Fe3O4 NPs for (a) control for Staphylococcus aureus, (b) Staphylococcus aureus, (c) control for Escherichia coli, (d) Escherichia coli.

Table 4. The diameters of inhibition zones for two bacteria.

Escherichia coli	Staphylococcus aureus
Control: ----	**Control:** ----
Fe_3O_4@schiff base: 22 mm	**Fe_3O_4@schiff base:** 30 mm

force is established between them, which leads to the destruction of bacteria. Another mechanism that can be expressed is that MNPs can inactivate E. coli and Staphylococcus aureus through the diffusion of the tiny particles into E. coli and Staphylococcus aureus membranes (25). Therefore, the results indicate the strong antibacterial activity of coated Fe_3O_4 NPs against E. coli and S. aureus. Furthermore, it is obvious that the gram-negative bacteria are more affected in comparison with gram-positive bacteria which confirms the result of previous studies (26).

CONCLUSION

In conclusion, in this study, the Fe_3O_4 NPs have been successfully synthesized by the co-precipitation method and were functionalized by a thiazole Schiff base compound. The XRD and SEM outcomes affirmed that Fe_3O_4 has a size of around 12 nm. Moreover, the EDXRF spectra revealed that the composition elements of the compound including the Fe, O, and C. The Fe_3O_4 nanoparticles showed their antibacterial activity against bacterial species both gram-positive and gram-negative bacterial strains. It is suggested that this compound could be used in other bacterial strains. Also, it can be utilized for various fields such as biomedical, complex targeted delivery, MRI imaging, and water treatment.

FUNDING

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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