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Development of Nickel(II) Thiosemicarbazone Complexes as Potential Antibacterial agents: Microwave Assisted Synthesis, Spectral Characterization and Antibacterial studies

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ABSTRACT

Some novel Nickel(II) aryl thiosemicarbazone complexes with the formula [Ni(LIGAND-1)$_2$ $(NO_3)_2$], [Ni(LIGAND-2)$_2$$(NO_3)_2$], [Ni(LIGAND-3)$_2$$(NO_3)_2$], and [Ni(LIGAND-4)$_2$$(NO_3)_2$] have been successfully synthesized. LIGAND-1 corresponds to 4-Phenyl-3-buten-2-one thiosemicarbazone (PBTSC), LIGAND-2 is 4-Hydroxy-3-methylbenzaldehyde thiosemicarbazone (HMBTSC), LIGAND-3 refers to 4-Methoxybenzaldehyde thiosemicarbazone (MBTSC), and LIGAND-4 represents Propiophenone thiosemicarbazone (PTSC). The Ligands were synthesized by reacting thiosemicarbazide with substituted aromatic aldehydes and ketones using microwave irradiation. Nickel(II) ions were subsequently complexed with the Ligands to produce the final complexes. FTIR, UV-Visible spectroscopy, along with elemental analysis were used to characterize produced compounds. Additionally, antibacterial activity of ligands and their analogous nickel (II) complexes was established.

Keywords: Microwave irradiation, Nickel(II) complexes, Spectral characterization, Aryl thiosemicarbazones, Antimicrobial activity etc.

INTRODUCTION

Because of its numerous uses in a wide range of industries, including electrochemistry, medicine, and catalysis, nickel (Ni) complexes have garnered a lot of interest.[1] Among the numerous classes of Ni-complexes, those that involve thiosemicarbazones as ligands are particularly interesting due to their chelating ability and biological activity.[2] Thiosemicarbazones, organic compounds containing a thiosemicarbazide moiety, are renowned for their capacity to build stable complexes with metal ions, including transition metals such as Ni.[3] These complexes have been investigated for their activeness as antimicrobial agents, anticancer drugs, and in other therapeutic applications, owing to their ability to interact with biological systems, particularly enzymes and DNA.[4] This study concentrates on the production and antibacterial screening of novel Nickel(II) aryl thiosemicarbazone complexes, which may provide new insights into the design of metal-based therapeutics and industrial applications.[5-6]

Nickel is a transition metal which have

crucial part in various biological mechanisms. It is involved in catalysis of several organic reactions.[7] Nickel complexes, particularly those with coordination sites available for Ligands, are commonly studied for their stability, reactivity, and biological properties.[8] Nickel(II) complexes, in particular, are of interest due to their d^8 electronic configuration, which allows them to form stable complexes with a variety of Ligands, including thiosemicarbazones.[9] The coordination chemistry of nickel has been widely explored, and the versatility of nickel complexes in forming both square planar and octahedral geometries with Ligands contributes to their diverse biological as well as chemical activities.[10]

In current years, the use of metal complexes, especially those containing nickel, in medicinal chemistry has garnered increasing attention.[11] Metal-based drugs are being explored as alternatives to traditional organic drugs for treating infectious illnesses, cancer, besides other conditions.[12] The capacity of metal complexes to engage with biomolecules such as proteins, DNA, RNA as well as their potential for drug delivery, makes them promising candidates for pharmaceutical development.[13] Nickel(II) complexes, in particular, have demonstrated antimicrobial, anticancer, and antidiabetic activities, among others, which has led to increased interest in their potential therapeutic applications.[14] Thiosemicarbazones (TSCs) are a class of compounds that contain a group i.e. -NH-NH-C=S attached to an aromatic or aliphatic carbonyl group.[15] These Ligands are known for their strong chelation ability, which enables them to form stable metal complexes, particularly with transition metals like nickel.[16] The chelation occurs via nitrogen and sulphur atoms of thiosemicarbazone group, which can effectively coordinate with metal ion.[17] This results in formation of metal–Ligand complexes with enhanced stability and bioactivity compared to the free Ligand.[18]

Thiosemicarbazones are widely studied for their biological properties, including their antimicrobial, anticancer and other activities.[19] These properties are attributed to their capability to interact with metal ions, which can facilitate inhibition of various enzymes and disrupt cellular processes.[20] In particular, thiosemicarbazones have shown promise as antimicrobial agents, capable of inhibiting the growth of pathoges.[21] When complexed with metal ions like Ni^{2+}, the biological activity of thiosemicarbazones can be enhanced due to the metal's ability to interact with biological targets, such as bacterial enzymes and cell membranes.[22]

Antimicrobial action of thiosemicarbazone's metal complexes is well-documented in the literature. Numerous studies have shown that thiosemicarbazone-metal complexes exhibit strong antibacterial properties against both *Gram-ve* and *Gram+ve* microbes. This makes them attractive contenders for developing innovative antibiotics, especially in light of the growing resistance to antibiotics.[23-25]

The synthesis of nickel(II) thiosemicarbazone complexes generally involves the reaction of a nickel(II) salt, such as nickel nitrate, with a thiosemicarbazone Ligand in the presence of a suitable solvent.[26] The thiosemicarbazone Ligands, typically produced by condensation of semicarbazide or thiosemicarbazide with carbonyl compounds (ketones or aldehydes), form stable complexes with nickel due to their capability to chelate metal ion concluded sulphur and nitrogen atoms of the thiosemicarbazone moeity.[27-28] In this study, four novel Nickel(II) thiosemicarbazone complexes are synthesized by reacting nickel nitrate with four different thiosemicarbazone Ligands, namely 4-Phenyl-3-buten-2-one thiosemicarbazone (PBTSC), 4-Hydroxy-3-methylbenzaldehyde thiosemicarbazone (HMBTSC), 4-Methoxybenzaldehyde thiosemicarbazone (MBTSC), and Propiophenone thiosemicarbazone (PTSC).

In current work, the antibacterial action of synthesized Nickel(II) complexed thiosemicarbazones was evaluated against two bacterial pathogens: *Escherichia coli*, a *Gram-negative* microbe, and *Staphylococcus aureus*, a *Gram-positive* microbe. Outcomes of our antimicrobial testing will offer insightful information about potential of these complexes as beneficial agents in battle against bacterial illnesses.

MATERIAL AND METHODS

All solvents as well as chemicals used in this study were of AR class sourced from *E. Merck*, besides Sigma-Aldrich. These were used as gathered without-decontamination. Purity of produced molecules was tested through thin-layer chromatography (TLC). Infrared (IR) spectra was

logged using a Bruker FT-IR spectrometer, covering the range from $4x10^3$ to $5x10^2$ cm^{-1} with KBr discs. Measurements of magnetic susceptibility, were completed with a VSM 155, at a magnetic field strength of 5500 Gauss.

For microwave-assisted synthesis, a household microwave (2450 MHz, 800 W) and a Green Microwave Biochemical Reactor (GMBR) were used. UV-Visible absorption spectra were recorded using a Double-beam Ultraviolet-Visible Spectrophotometer (ECIL), endowed with a 10 mm cuvette (quartz cell) for light path measurements. All biological activity assays were performed in an aseptic environment using a horizontal laminar airflow system at the Biotechnology and Infection Research Facility (BIFR), Bikaner.

Microwave irradiation synthesis of Ligands

Four Ligands were synthesized via microwave-assisted methodology: LIGAND-1 (4-Phenyl-3-buten-2-one thiosemicarbazone, PBTSC), LIGAND-2 (4-Hydroxy-3-methylbenzaldehyde thiosemicarbazone, HMBTSC), LIGAND-3 (4-Methoxybenzaldehyde thiosemicarbazone, MBTSC), and LIGAND-4 (Propiophenone thiosemicarbazone, PTSC). In a typical synthesis, a mixture containing thiosemicarbazide (0.01 mol), aldehyde or ketone (0.01 mol), and glacial acetic acid (2 mL) in H_2O or alcohol-H_2O mixture was placed in an Erlenmeyer flask capped with a funnel. This setup was irradiated in a microwave oven at 200 W for 2-6 minutes.

Advancement of reaction was observed through the application of thin-layer chromatography (TLC). When reaction was complete, this blend was permitted to cool to ambient temperature, then resulting solid was carefully filtered away. Crude product underwent recrystallization using redistilled ethanol, resulting in the acquisition of purified ligands[29-30].

Microwave irradiation synthesis of the complexes

In the pursuit of novel synthesis of Ni(II) thiosemicarbazone complexes, a slurry comprising ligands (namely PBTSC, HMBTSC, MBTSC, PTSC) at a concentration of 0.02 mol was meticulously formulated in either H_2O or alcohol-H_2O mixture. A solution of Nickel nitrate hexahydrate (0.01 mol in 30 mL of ethanol) was incorporated into this mixture. The resultant amalgamation underwent irradiation within a microwave oven for a duration ranging from 2 to 10 min, set at a medium power (600Watt), while ensuring intermittent agitation. This solution was permitted to reach ambient temperature before being introduced into ice-chilled methanol, followed by drying under vacuum conditions over P_2O_5[31].

Antimicrobial action

A saturated sol. of Nutrient agar (75 g) was meticulously made using double distilled H_2O, followed by autoclaving for 15 minutes. Subsequently, the solution was carefully poured into Petri plates within the laminar flow hood. Following the application of a solidification loan of bacteria, specifically *Staphylococcus aureus* and *Escherichia coli*, the investigation into antimicrobial activity was initiated. Preparations were made for all four ligands and their corresponding complexes with Ni(II). A distinct paper-disc was immersed in each solution for a duration of 10 minutes. Consequently, the prepared paper disc was positioned within a Petri-plate, and the finalized Petri-plates were then placed in an incubator for 24 h at 37°C. After this, these Petri-plates were retrieved and examined to assess the zone of inhibition measured in millimetre.

RESULTS AND DISCUSSION

Complexes of Ni(II) ion with all the four Ligands demonstrated stability at ambient temperature for long time. Nickel complexes under research are colored crystals. They are solvable in DMSO, DMF, partly solvable in ethyl alcohol, methyl alcohol and insoluble in water and other solvents. The outcomes of the elemental estimates were excellent.

Ligands as well as complexes were discerned through elemental analysis and spectral investigations. The data pertaining to yield, colour, besides elemental analysis is presented in Table 1.

Infrared Spectra

Infrared spectral figures concerning Ni(II) thiosemicarbazone complexes are meticulously compiled in Table 2. An examination and analysis of the IR spectra of the free ligand besides its metal complexes suggest that ligand functions as a bidentate agent, with metal ion coordinated via thione sulphur and azomethine nitrogen[32]. The IR reflects the shifts in vibrational frequencies upon complexation of Ni(II) with various thiosemicarbazone ligands (PBTSC, HMBTSC, MBTSC, PTSC). By

relating the IR frequencies of free ligands with their corresponding complexes, insights can be drawn about the bonding and coordination nature of these ligands with Ni(II). Key vibrations are examined for each compound and its Ni(II) complex, highlighting shifts in thiocarbonyl (C=S) as well as azomethine (C=N) groups, along with newly observed metal-Ligand (M-N and M-S) bands that confirm coordination[33]. Infrared spectrum data for all ligands and complexes each row illustrates the synthesis of a chemical and its manganese complex. The band at ν(C=N) for the Ligand is 1692 cm^{-1}, whereas for the complex it is 1583 cm^{-1}, indicating a drop in the C=N stretching frequency upon complex formation. The IR frequency range of 1569-1692 cm^{-1} for ν(C=N) in all the ligand and complexes suggests coordination of the thiosemicarbazone C=N formation in each complex[34]. This sharp peak indicates the presence of the azomethine group, a common feature in thiosemicarbazones. This significant move to a reduced frequency signals coordination through nitrogen of azomethine group, as bonding weakens C=N bond. The infrared frequency range of 1070-1153 cm^{-1} for ν(N-N) in all complexes indicates the coordination of thiosemicarbazone N-N formation with each complex[35]. For the ν(N-N) bond complex, the ligand exhibits a stretching frequency of 1070 cm^{-1}, whereas the complex shows a stretching frequency of 1100 cm^{-1}. Typical of the N-N single bond in thiosemicarbazones, this increase indicates structural changes upon complexation.

For the ν(C=S) bond in the Ligand, a stretching frequency of 1338 cm^{-1} is observed, while in the complex, the stretching frequency shifts to 1246 cm^{-1}. Ligand associated with the thiocarbonyl (C=S) group. Complex indicates coordination via the sulphur atom, as the C=S bond is weakened. The IR frequency range of 1234-1349 cm^{-1} for ν(C=S) in all complexes indicates coordination through the thiosemicarbazone C=S group in each complex[36]. For the δ(C=S) bond in the Ligand, a bending frequency of 853 cm^{-1} is observed, which shifts to 821 cm^{-1} in the complex. This slight decrease in the C=S bending frequency further supports the involvement of sulphur in metal coordination[37]. The IR frequency range of 815-887 cm^{-1} for δ(C=S) in all Ligands and complexes indicates coordination through the thiosemicarbazone C=S bending vibration[38]. For the $\nu(^2$N-H) and $\nu(^4$N-H) bond in the Ligand, a stretching frequency of 3193 cm^{-1} and 3476, 3351 cm^{-1} is observed, the frequency remains the same (3193 cm^{-1}), indicating no involvement of this group in complexation[39]. These values remain largely unchanged, indicating that the N-H bonds are not significantly affected by complexation[40]. The IR frequency range of 3153-3212 cm^{-1} for ν(2N-H) and 3377-3476, 3246-3366 cm^{-1} for ν(4N-H) in all Ligands and complexes.[89] For the ν(Ni-N) and (Ni-S) bond in the complex, a stretching frequency of 448 cm^{-1} (Ni-N) and 437 cm^{-1} (Ni-S) is observed, in all the complexes. complex confirm the formation of Ni-N and Ni-S bonds, indicating coordination through nitrogen and sulphur[41]. The IR frequency range of 431-478 cm^{-1} (Ni-N) and 418-455 cm^{-1} (Ni-S) is observed in all complexes indicates formation of Ni-N and Ni-S bonds in each complex.[42]

Magnetic Moments and Electronic Spectra

Table 3 presents results of magnetic susceptibility experiments made at room temperature in a polycrystalline state. Both geometry of the complexes and properties of ligand affect the magnetic behaviour of bivalent nickel complexes. Ni(II) complexes have magnetic moment values between 2.71 and 2.25 BM, which indicates that there are two unpaired electrons present and that the environment around Ni(II) is octahedral. Each of the Ni(II) thiosemicarbazone complexes demonstrates paramagnetic characteristics, attributable to the existence of two unpaired electrons. Nickel(II) complexes exhibit Para magnetism owing to existence of unpaired electrons in $3d^8$ configuration. Magnetic moments (μ_{eff}) for four nickel(II)-thiosemicarbazone complexes fall in range expected for Ni(II) complexes with a distorted octahedral geometry.[43] Observed values for μ_{eff} for complexes are as follows: 2.71 BM, 3.25 BM, 3.08 BM, 2.94 BM. Where the expected spin-only value ranges from 2.8 to 3.2 BM due to 2 unpaired electrons[44].

These figures align with high-spin Ni(II) complexes in an octahedral or distorted octahedral field[45]. Complex $[Ni\text{-}(HMBTSC)_2(NO_3)_2]$ shows the highest magnetic moment of 3.25 BM, suggesting a greater degree of orbital contribution or spin-orbit coupling effects in this specific complex, possibly due to the ligand environment around Ni(II) centre.

Electronic spectral assignments of four Nickel(II) thiosemicarbazone complexes are specified in Table 3. All Ni(II) thiosemicarbazone

complexes display two $\pi \rightarrow \pi^*$ bands in 40000-41100 cm^{-1} range (in aromatic ring) and 33100-35300 cm^{-1} respectively with one $n \rightarrow \pi^*$ band in 31000- 33500 cm^{-1} range due to transition involved in thiosemicarbazone moiety.

The ground state term for Ni(II) (d^8 electronic configuration) in a high spin octahedral coordination is $^3A_{2g}$.[46] Electronic spectra of nickel(II) thiosemicarbazone complexes shows 3 bands due to spin allowed transitions in range 9500-11000 cm^{-1}, 16700-17500 cm^{-1}, 24800-27700 cm^{-1} which correspond to $^3A_{2g}$ (F)$\rightarrow ^3T_{2g}$ (F) (ν_1), $^3A_{2g}$(F)$\rightarrow ^3T_{1g}$ (F) ($\nu 2$), $^3A_{2g}$(F)$\rightarrow ^3T_{1g}$(P) (ν_3) correspondingly. A fourth band near 10750 cm^{-1} also appears in spectrum which is probably spin forbidden and might be ascribed as $^2B_{1g} \rightarrow ^2E_g$ transition, expected for distorted octahedral geometry around Ni(II) ion[47].

The electronic spectral bands for Ni(II)-thiosemicarbazone complexes provide valuable insight into the geometry and nature of d-d transitions. The electronic spectral bands and their tentative assignments for each complex are listed below. These spectra were analysed, taking into account typical transitions observed for Ni(II) in octahedral fields[48]:

Tentative Assignments:

$^3A_{2g}$ (F) to $^3T_{2g}$ (F) (Transitions 1)
$^3A_{2g}$(F) to $^3T_{1g}$ (F) (Transitions 2)
$^3A_{2g}$(F) to $^3T_{1g}$ (P) (Transitions 3)

These transitions are detected in ranges typical for nickel(II) complexes, which further confirms the distorted octahedral geometry[49]. Key bands include those in the lower energy region (10,000–20,000 cm^{-1}), accredited to spin-allowed d-d transitions characteristic of Ni(II) in such coordination environments[50-51].

Table 1: Ni(II)-thiosemicarbazone Complexes' Physico-chemical Information

Sr. No	Complexes	Melting point (°C)	Colour	Yield%	Elemental evaluation Calculated (Discovered)%		
					C	N	H
1	PBTSC	190	Pale yellow	67	60.24(59.90)	19.16(19.03)	5.97(5.92)
2	HMBTSC	198	Light brown	64	51.65(51.47)	20.07(19.81)	5.29(5.23)
3	MBTSC	180	Orange	61	51.65(51.58)	20.07(19.91)	5.29(5.24)
4	PTSC	202	Dark red	69	57.94(57.88)	20.27(20.22)	6.32(6.27)
5	$[Ni\text{-}(PBTSC)_2(NO_3)_2]$	190	Yellow	66	42.52(42.32)	18.03(17.83)	4.21(4.17)
6	$[Ni\text{-}(HMBTSC)_2(NO_3)_2]$	196	Redish Brown	62	35.95(35.82)	18.63(18.46)	3.68(3.58)
7	$[Ni\text{-}(MBTSC)_2(NO_3)_2]$	201	Dark Brown	71	35.95(35.83)	18.63(18.47)	3.68(3.61)
8	$[Ni\text{-}(PTSC)_2(NO_3)_2]$	210	Creamy white	61	40.21(40.12)	18.76(18.67)	4.38(4.33)

Table 2: Vibrational Spectral Assignments (cm^{-1}) of Ni(II)-thiosemicarbazone complexes

Sr. No	Compound	ν							
		C=N	N-N	C=S	C=S	2N-H	4N-H	M-N	M-S
1	PBTSC	1692	1074	1338	853	3193	3476, 3351		
	$[Ni\text{-}(PBTSC)_2(NO_3)_2]$	1583	1100	1246	821	3193	3476, 3351	448	437
2	HMBTSC	1655	1070	1347	846	3159	3391, 3246		
	$[Ni\text{-}(HMBTSC)_2(NO_3)_2]$	1614	1109	1293	823	3159	3391, 3246	431	455
3	MBTSC	1620	1102	1349	887	3153	3377, 3366		
	$[Ni\text{-}(MBTSC)_2(NO_3)_2]$	1571	1153	1240	817	3153	3377, 3366	478	418
4	PTSC	1618	1098	1320	843	3212	3390, 3311		
	$[Ni\text{-}(PTSC)_2(NO_3)_2]$	1569	1153	1234	815	3212	3391, 3310	466	439

Table 3: Ni(II)-thiosemicarbazone Complexes' Magnetic Moments and Electronic Spectral information

Sr. No	Complex	μ_{eff}(BM)	Spectral Bands λ_{max} (Electronic spectra) (cm^{-1})	Expected Geometry
1	$[Ni\text{-}(PBTSC)_2(NO_3)_2]$	2.71	40981, 33237, 31541, 24842, 24036, 22548, 21954, 18553, 17467, 10380	Distorted
2	$[Ni\text{-}(HMBTSC)_2(NO_3)_2]$	3.25	40090, 33114, 31053, 27244, 25883, 23453, 19033, 18795, 17464, 10976	Octahedral
3	$[Ni\text{-}(MBTSC)_2(NO_3)_2]$	3.08	40567, 35275, 33463, 27249, 24910, 24244, 19016, 18574, 17477, 10103	
4	$[Ni\text{-}(PTSC)_2(NO_3)_2]$	2.94	41052, 33367, 32540, 27253, 24975, 23848, 19034, 18577, 17488, 10117	

Table 4: Antibacterial action of produced compounds

Sr. No	Ligands & their Complexes	Inhibition Zone in mm	
		S. aureus	*E. coli*
1	PBTSC	15	8
2	HMBTSC	21	10
3	MBTSC	15	0
4	PTSC	12	0
5	$[Ni(PBTSC)_2(NO_3)_2]$	0	0
6	$[Ni(HMBTSC)_2(NO_3)_2]$	12	8
7	$[Ni\text{-}(MBTSC)_2(NO_3)_2]$	14	0
8	$[Ni(PTSC)_2(NO_3)_2]$	0	0

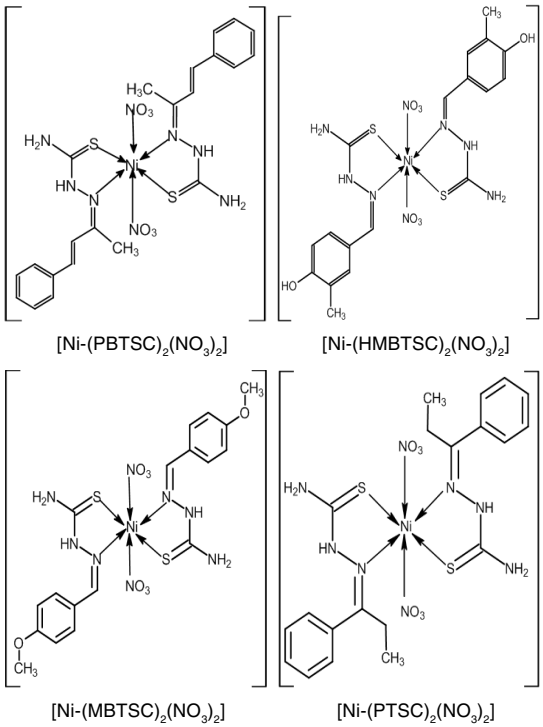

Fig. 1. Tentative Structures of Ni(II) Complexes

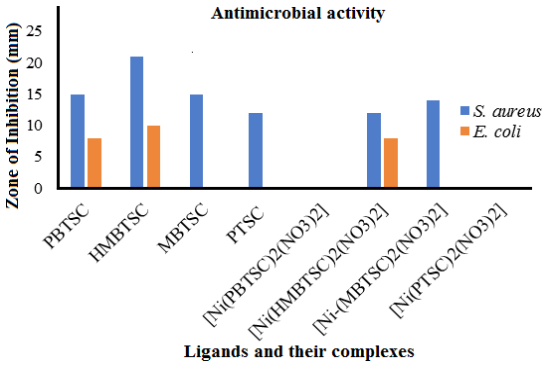

Fig. 2. Graphical Presentation of Antimicrobial Activity

CONCLUSION

Magnetic moment measurements, spectral analyses, along with elemental analysis were used to analyze thiosemicarbazone Ligands as well as their Ni(II) complexes. Thiosemicarbazone ligands show bidentate coordination, binding through azomethine nitrogen along with thione sulphur atoms, according to data that was acquired. Among the ligands, HMBTSC showed highest antibacterial action against both *S. aureus* as well as *E. coli*, indicating its potential as a standalone antimicrobial agent. Similar to the previous nickel complex, this compound has a 14 mm inhibition zone against *S. aureus* but no activity against *E. coli*, implying that the nickel complex still retains some activity against *S. aureus* but lacks efficacy against *E. coli.* The results suggest that the free compounds like HMBTSC have better antibacterial potential, especially against *S. aureus*, while the nickel complexes generally show reduced or no activity, particularly against *E. coli*. The antibacterial effect of these compounds is likely influenced by their chemical structure, and the formation of nickel complexes appears to diminish the overall antimicrobial effectiveness.

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Conflict of Interest Statement

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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