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# Synthesis, spectral studies and *in vitro* antimicrobial activity of some new di-/tri-organotin(IV) complexes of Schiff bases derived from 2-benzoylpyridine

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*Abstract*: In the present work, a series of twenty-four organotin(IV) complexes of the type [$R_2SnClL$, $R_3SnL$] were synthesized by the condensation of 2-benzoylpyridine Schiff bases with $R_2SnCl_2$, $R_3SnCl$ (R = Me, *n*-Bu or Ph) in 1:1 mole ratio. These complexes were well characterized by IR, $^1$H-, and $^{13}$C-, $^{119}$Sn-NMR, XRD and mass spectral techniques. In the search for biologically more effective antimicrobial agents, all the synthesized ligands and organotin complexes were evaluated for their *in vitro* antimicrobial activities against two Gram-positive and two Gram-negative bacteria, and two fungal strains by the serial dilution method. The results of spectral data revealed that the formed complexes were hexacoordinated with tridentate ligands coordinated through azomethine N, pyridine N and carboxylate O ligation sites. The ligands on coordination with tin metal showed a discernible augmentation in biocidal activity; however, the Ph and Bu complexes were found to be more intoxicating. The results revealed that the synthesized complexes were more noxious towards Gram-positive strains as compared to Gram-negative strains, which may be attributed to the presence of the outer lipid membrane of lipopolysaccharides.

*Keywords*: Schiff bases; di-/tri-organotin complexes; antibacterial activity; antifungal activity.

## INTRODUCTION

There has been a tremendous growth in the synthesis of new safer antimicrobial drugs because of the resistance developed by microorganisms towards conventional drugs.[1,2] Therefore, extensive studies have been made on nitrogen-containing chelating Schiff bases in recent years owing to their pronounced pharmacological applications.[3,4] For instance, heterocyclic chelated Schiff bases

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with their metal complexes have shown remarkably enhanced biological activity.[5,6] Even lethal cancer-like disease was cured by the chelating complexes. Therefore, active and well predictable Schiff base ligands are considered ''privileged'' ligands. Schiff bases and their complexes have been widely used in catalysis,[3,7] organic light emitting diodes,[8] wood preservatives and pesticides,[9] non-linear optics,[10,11] *etc*. Consequently, they are regarded as potential scaffolds for chelating, which lead to the formation of organometallic complexes. Schiff bases derived hydrazones and their metal complexes are promising compounds that have numerous pharmacological applications, such as antimicrobial,[12,13] anticonvulsant,[14] anti-inflammatory,[15,16] anticancer,[17,18] and antituberculosis[19] agents. The bioactivity associated with metal complexes was increased as compared to their respective/parent ligands, while side effects may be decreased on complexation.[20] The thriving utilization of organometallic complexes is a dynamically escalating area in biomedical and inorganic chemistry for the treatment of several human diseases. In particular, some unique characteristics of organotin complexes, such as geometries, variation in coordination number, thermodynamic and kinetic characteristics, accessible redox states and the inherent properties of the tin metal ion recommend chemistry researchers to develop varied approaches for their utilization in different fields, such as biomedical, industrial[21,22] and agriculture.[21] Organotin complexes are potent motifs of organometallic compounds owing to their broad range of biocidal activities, in dependence on the nature and number of donor atoms of the ligands attached to the tin moiety. They are persistent moieties in a number of biocidal formulations in diverse areas, such as antifouling paints, fungicides, molluscicides, and they are found to be more effective against a large range of tumor lines than conventional metal anticancer drugs.[23,24] In order to search for new antimicrobial agents with better molecular diversity and increased biological potency, some organotin complexes with 2-benzoylpyridine Schiff bases were prepared that resulted in the formation of new drugs in which the ligand and metal might act synergistically. Of meticulous exigency, the exploration for new antimicrobial agent should be more biospecific and less lethal to the environment and to the host. Encouraged by these facts, herein, the synthesis of tri- and di-organotin complexes with different hydrazones of 2-benzoylpyridine is reported, which may lead to the formation of new pharmacophores with enhanced biological profiles.

## EXPERIMENTAL

### *Materials and methods*

All the chemicals were obtained from Sigma–Aldrich and the solvents used were dried by conventional methods. The reactions were performed under an inert atmosphere. Dimethyltin dichloride, di-*n*-butyltin dichloride, diphenyltin dichloride, tributyltin chloride, triphenyltin chloride, trimethyltin chloride and 2-benzoylpyridine were used as received without further purification. Elements (C, H and N) were analyzed on a Perkin–Elmer 2400 instrument and

the measured data corresponded to the calculated data. The Fourier transform infrared (FTIR) spectra (4000–400 $cm^{-1}$) were obtained in KBr pellets on a Perkin–Elmer spectrum RX1 instrument. The NMR spectra were recorded on a Bruker Avance II 400 MHz NMR spectrometer in $CDCl_3$ or DMSO-$d_6$ using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on an LCMS MS 6410 Agilent Technologies spectrometer with an electron impact quadropole analyzer. The X-ray powder diffraction measurements were obtained using a Rigaku Table Top X-ray diffractometer at a scan rate of 2° $min^{-1}$ in the $2\theta$ range 20–80°. Tin was estimated gravimetrically as $SnO_2$.[25]

Characterization data for the synthesized ligands are given in Supplementary material to this paper.

*Synthesis of di-/tri-organotin complexes with Schiff base hydrazones derived from 2- and 4-substituted benzoic acid hydrazide with 2-benzoylpyridine.*

2-Benzoylpyridine-derived hydrazones (*m*-$NO_2$, *p*-Cl, *p*-$NO_2$, *p*-$CH_3$) were prepared by reported methods.[19,26] The Schiff base ligands were prepared by dissolving (*m*-$NO_2$, *p*-Cl, *p*-$NO_2$, *p*-$CH_3$) benzoic acid hydrazides derivatives (5 mmol) in a methanolic solution of 2-benzoylpyridine (5 mmol, 1:1 mole ratio) and the reaction mixture was refluxed for 5–6 h. The mixture was left overnight and the obtained solid was separated, dried and washed with methanol. The Schiff base ligands were recrystallized from methanol and chloroform.

Initially, the sodium salts of the ligands were prepared by dissolving and refluxing a weighed amount (5 mmol) of sodium in a methanolic solution of the Schiff base ligands for 3 h. To this sodium salt solution, the starting material dialkyltin dichloride, diphenyltin dichloride ($R_2SnCl_2$)/trialkyltin chloride, triphenyltin chloride ($R_3SnCl$), (R = Ph, Bu, Me) were added dropwise in a 1:1 mole ratio. The mixture was refluxed for 9–10 h and filtered to remove the white-colored solid. The excess solvent was evaporated in vacuum to obtain the solid yellow complexes. The obtained complexes were washed with dry *n*-hexane. All the complexes were recrystallized from methanol and diethyl ether.

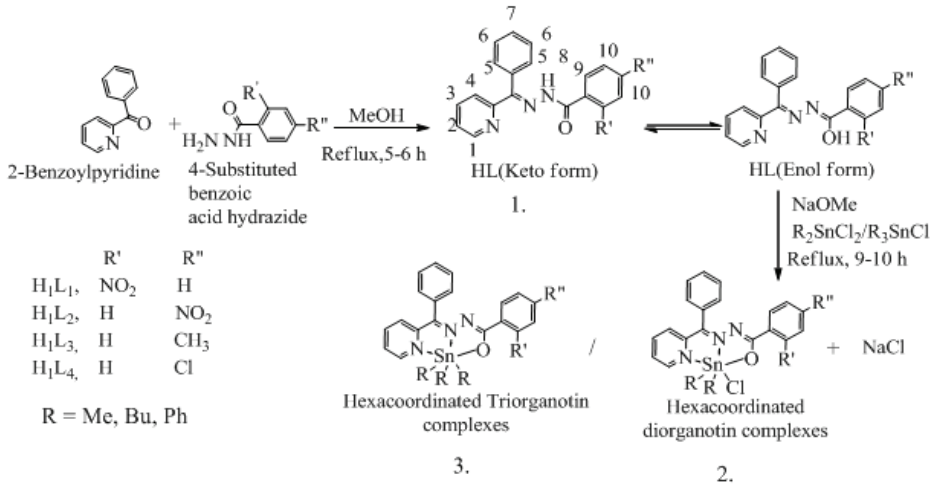
*Pharmacology*

Antimicrobial activity of all the synthesized ligands and organotin complexes were evaluated against four bacterial strains, *i.e.*, Gram-positive *Bacillus cereus* (MTCC 10072)*, Staphylococcus aureus* (NICM 2901) and Gram-negative *Escherichia coli* (MTCC 732)*, Pseudomonas aeruginosa* (MTCC 424)*,* and two fungal strains, *i.e.*, *Aspergillus flavus* (ITCC 76801), *Aspergillus niger* (MTCC 9933) by serial dilution method[27,28] and their *MIC* values were calculated. The bacteria and fungi were subcultured on nutrient agar and potato dextrose broth (PDB) from HIMEDIA, Mumbai, India, respectively. The stock solution was diluted to make concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.75 μg $mL^{-1}$. The bacteria and the fungi were inoculated to each solution and the solutions were then kept in incubator at 37 °C for 24 h in the case of the bacteria and for 7 days in the case of the fungi. Then, the minimum inhibitory concentration (*MIC*) was determined. The experimental values were compared with standard drugs, *i.e.*, ciprofloxacin for the antibacterial activity and fluconazole for the antifungal activity.

## RESULTS AND DISCUSSION

Organotin(IV) complexes were synthesized by reacting benzoylpyridine-derived hydrazones with dialkyltin dichloride, diphenyltin dichloride ($R_2SnCl_2$)/ /trialkyltin chloride, triphenyltin chloride ($R_3SnCl$, R= Ph, Bu, Me) in 1:1 mole ratio, Scheme 1. The purity of synthesized compounds was checked by thin layer

chromatography (TLC). All the metal complexes were colored solids, and were stable on wide exposure to air. The spectroscopic techniques (IR, NMR and mass) were used to determine the geometry of complexes, which was found to be distorted octahedral. The ligands were found to be tridentate (ONN) and chelated to the central tin atom with the replacement of one hydrogen atom through enolization and due to the chelation, the biocidal activities of the complexes were enhanced.




Scheme 1. Synthetic route for the synthesis of Schiff base ligands and their organotin complexes.

*IR spectra*

In the ligands, the sharp peaks that appeared due to ν(C=N) at 1603–1611 $cm^{-1}$, ν(C=O) at 1671–1686 $cm^{-1}$ and ν(NH) at 3190–3310 $cm^{-1}$ disappeared on complexation. In the IR spectra of the complexes, the appearance of 2 new bands in the region 1345 and 1210 $cm^{-1}$ due to ν(NCO) and ν(C–O), respectively, indicated coordination through oxygen and nitrogen to the tin atom after deprotonation. The peak due to C=N shifted to lower frequency by 15–20 $cm^{-1}$, showing the involvement of the electrons present on nitrogen of the azomethine group in bond formation to the central tin atom. The peak in the region 676–628 $cm^{-1}$ in the spectra of the hydrazones is due to the in-plane vibration mode of the pyridine ring, which is shifted to a higher frequency in the complexes, confirming the mode of coordination of the pyridine nitrogen.[12,29] New bands in appeared in the spectra of the complexes at 431–459 $cm^{-1}$, 523–566 $cm^{-1}$ and 608–731 $cm^{-1}$, corresponding to (Sn–N), (Sn–O) and (Sn–C) frequencies, respectively, which support the formation of complexes.[30] Based on these results, the coordination sites of the ligands with the tin atom were ascertained by comparing the fre-

quency shifts in the spectra of ligand and complexes. The disappearance of the bands assigned to the carbonyl group in IR spectra of complexes divulges that the ligands coordinate with tin metal in the enolic form.

*Electronic spectra*

Electronic spectra of the ligands and their metal complexes were recorded in DMF. In the electronic spectra of the ligands, a band due to the phenyl ring was observed at 218 nm. This band shifts nearly 20–30 nm to higher wavelengths on complexation. In addition, the band at 293 nm, ascribed to the C=N chromophore in the ligands, was shifted to a longer wavelength and was observed at 296 nm in the complexes. An absorption band found at 351 nm for the ligands is due to $n \rightarrow \pi^*$ transitions, and was decreased in the complexes. It is known that ligands containing nitrogen and oxygen as donor atoms are capable of forming $d\pi$–$p\pi$ bonds with the metal, due to which charge transfer and intense intraligand bonds were observed.[23]

*NMR spectral analysis*

The $^{1}$H- and $^{13}$C-NMR spectra of ligands and their organotin complexes were recorded in $CDCl_3$ or DMSO. By comparing the spectra of the ligands with their organotin complexes, the coordination sites (ONN) of the ligands were proposed. In the $^{1}$H-NMR spectra of the ligands, the main characteristic peak was a doublet obtained in the region at $\delta$ 8.63–8.82 ppm due to the proton present at the carbon adjacent to the N of the pyridine ring, which was further shifted downfield in the complexes as compared to the free ligands. This confirmed the coordination of pyridine N to the tin atom. The singlet due to azomethine proton observed in the region of $\delta$ 13.7–15.2 ppm was absent in the $^{1}$H-NMR spectra of the complexes,[12] which substantiates the coordination mode of the carbonyl oxygen through enolization. The peaks due to remaining aromatic and aliphatic protons appeared in the expected regions. In the complexes, new signals appeared in the spectra at $\delta$ 1.07–1.67 ppm, 0.70–1.75 ppm and 7.18–8.78 ppm due to the methyl, butyl and phenyl protons attached to the tin atom.

The $^{13}$C-NMR spectra also confirmed the proposed structures. In the spectra of the ligands, signals due to the carbonyl carbon and azomethine carbon appeared at $\delta$ 162.29–163.98 ppm and $\delta$ 153.11–158.66 ppm, respectively. On complexation, the signals due to the carbonyl carbon, azomethine carbon and the carbon adjacent to the coordinating atoms shifted downfield, which supported the coordination modes through the azomethine nitrogen and the carbonyl carbon. The aromatic carbons appeared in the $\delta$ range 153.94–122.50 ppm. In the complexes, new signals due to methyl and butyl groups appeared at $\delta$ 10.49–31.81 ppm and 13.57–35.43 ppm and the phenyl carbons appeared in their normal range.

The $^{119}$Sn-NMR spectra of all the synthesized organotin complexes showed one sharp singlet depending upon the coordination number and the R group attached to the centre atom, which inferred the formation of single tin species. A large upfield shift was observed in the spectra of complexes with increasing coordination number. The occurrence of chemical shift in $^{119}$Sn-NMR spectra in the ranges $\delta$ –208.18 to –228.23 ppm, –270.75 to –297.11 ppm and –331.45 to –376.12 ppm for the methyl, butyl and phenyl complexes, respectively, were in accordance with a hexacoordinated environment around the tin metal in the complexes.[31,32]

*Mass spectra*

The ESI-MS spectra of the diorganotin complexes exhibited different fragmentation patterns as expected and results were found to be in good agreement with their molecular formulae. In some cases, the fragments were observed as groups of peaks due to different isotopes of chlorine and tin.[24] The mass spectra of the compounds **1**–**28**, in each case displayed the $[M+H]^+$ peak, for instance, in the mass spectrum of $Bu_2SnClL_2$ (molecular mass 583.14), the peak due to $[M+H]^+$ was observed at *m*/*z* 584.50. The other peaks due to $[M–Cl]^+$, $[L]^+$ and $[Sn]^+$ fragments were observed at *m*/*z* 548.50 316.30 and 119.00, respectively. All these peaks are depicted in Fig. 1.

*X-Ray diffraction*

Analysis of the synthesized complexes by X-ray powder diffraction was in accordance with the crystalline nature of the complexes.[33] The patterns were recorded over the $2\theta$ range 20–80° and the average crystallite sizes $d_{XRD}$ were calculated to obtain information about the dynamics of the complexes. The X-ray diffraction pattern of the $Bu_2SnClL_2$ complex displayed a clear crystalline peak with maxima at $2\theta$ 31.680° and $d$ = 2.822Å, *FWHM* = 0.286 rad (Fig. 2).

The particle size of the complexes was calculated with the Debye–Scherrer formula and it was approximately found to be in the range 54–65 nm.

*Antimicrobial activities*

A detailed structure–activity relationship of hexacoordinated diorganotin(IV) complexes was made from the results of the antimicrobial evaluation presented in Table I, which revealed that the organotin complexes have comparable activity to those of standard drugs against Gram-positive bacteria and fungi rather than their respective ligands.

These results show that chelation of a ligand to tin leads to augmentation in activity due to delocalization of electrons, which increases the lipophilic character of the complexes and proficient dissemination of the metal complexes into bacterial cell walls. Furthermore, hexacoordinated complexes in which a chlorine atom is directly coordinated to the tin atom were found to be more active as com-

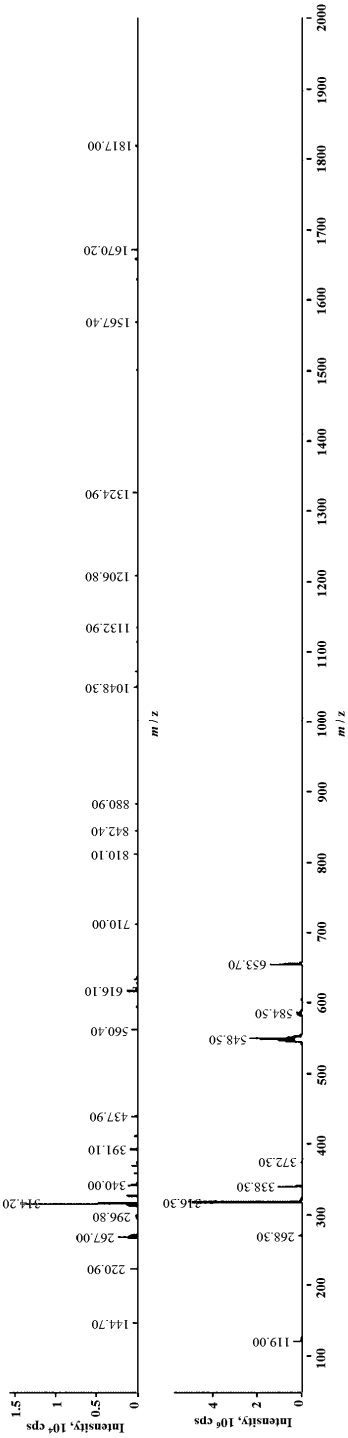



Fig. 1. Mass spectrum of $Bu_2SnClL_3$.

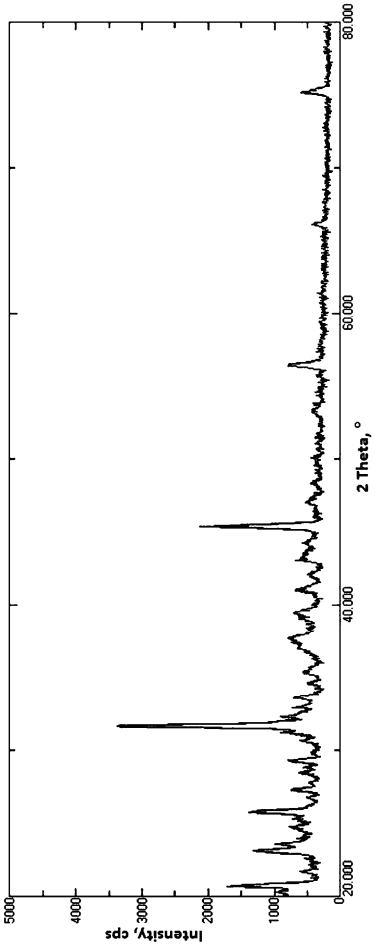



Fig. 2. XRD pattern for $Bu_2SnClL_2$.

TABLE I. The *in vitro* antibacterial activity (*MIC* / μmol $ml^{-1}$) of 2-benzoylpyridine-derived hydrazone Schiff base ligands, and their organotin(IV) complexes.

| Compound | Bacteria | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* | *P. aeruginosa* | *B. cereus* | *S. aureus* | *A. niger* | *A. flavus* |
| $HL_1$ | 0.0361 | 0.0361 | 0.0361 | 0.0180 | 0.0090 | 0.0180 |
| $HL_2$ | 0.0361 | 0.0361 | 0.0361 | 0.0180 | 0.0090 | 0.0180 |
| $HL_3$ | 0.0396 | 0.0396 | 0.0396 | 0.0198 | 0.0198 | 0.0198 |
| $HL_4$ | 0.0372 | 0.0186 | 0.0186 | 0.0186 | 0.0186 | 0.0093 |
| $Ph_2SnClL_1$ | 0.0096 | 0.0096 | 0.0191 | 0.0096 | 0.0048 | 0.0024 |
| $Bu_2SnClL_1$ | 0.0102 | 0.0051 | 0.0051 | 0.0025 | 0.0051 | 0.0025 |
| $Me_2SnClL_1$ | 0.0236 | 0.0059 | 0.0118 | 0.0118 | 0.0059 | 0.0059 |
| $Ph_3SnL_1$ | 0.0090 | 0.0045 | 0.0090 | 0.0090 | 0.0090 | 0.0045 |
| $Bu_3SnL_1$ | 0.0197 | 0.0098 | 0.0098 | 0.0098 | 0.0098 | 0.0049 |
| $Me_3SnL_1$ | 0.0246 | 0.0123 | 0.0246 | 0.0123 | 0.0123 | 0.0061 |
| $Ph_2SnClL_2$ | 0.0096 | 0.0048 | 0.0048 | 0.0024 | 0.0024 | 0.0048 |
| $Bu_2SnClL_2$ | 0.0204 | 0.0051 | 0.0102 | 0.0051 | 0.0025 | 0.0051 |
| $Me_2SnClL_2$ | 0.0236 | 0.0059 | 0.0118 | 0.0029 | 0.0059 | 0.0059 |
| $Ph_3SnL_2$ | 0.0090 | 0.0090 | 0.0090 | 0.0045 | 0.0045 | 0.0045 |
| $Bu_3SnL_2$ | 0.0197 | 0.0098 | 0.0098 | 0.0098 | 0.0025 | 0.0049 |
| $Me_3SnL_2$ | 0.0246 | 0.0123 | 0.0123 | 0.0123 | 0.0061 | 0.0061 |
| $Ph_2SnClL_3$ | 0.0100 | 0.0050 | 0.0100 | 0.0025 | 0.0025 | 0.0025 |
| $Bu_2SnClL_3$ | 0.0215 | 0.0054 | 0.0107 | 0.0107 | 0.0107 | 0.0054 |
| $Me_2SnClL_3$ | 0.0251 | 0.0125 | 0.0125 | 0.0031 | 0.0031 | 0.0031 |
| $Ph_3SnL_3$ | 0.0094 | 0.0094 | 0.0094 | 0.0049 | 0.0049 | 0.0049 |
| $Bu_3SnL_3$ | 0.0207 | 0.0103 | 0.0207 | 0.0103 | 0.0103 | 0.0052 |
| $Me_3SnL_3$ | 0.0261 | 0.0131 | 0.0131 | 0.0131 | 0.0131 | 0.0065 |
| $Ph_2SnClL_4$ | 0.0097 | 0.0097 | 0.0097 | 0.0024 | 0.0024 | 0.0024 |
| $Bu_2SnClL_4$ | 0.0104 | 0.0052 | 0.0104 | 0.0052 | 0.0104 | 0.0052 |
| $Me_2SnClL_4$ | 0.0241 | 0.0120 | 0.0120 | 0.0060 | 0.0120 | 0.006 |
| $Ph_3SnL_4$ | 0.0091 | 0.0091 | 0.0046 | 0.0046 | 0.0046 | 0.0046 |
| $Bu_3SnL_4$ | 0.0200 | 0.0100 | 0.0100 | 0.0100 | 0.0100 | 0.0100 |
| $Me_3SnL_4$ | 0.0251 | 0.0125 | 0.0125 | 0.0125 | 0.0125 | 0.0063 |
| Ciprofloxacin | 0.0047 | 0.0047 | 0.0047 | 0.0047 | – | – |
| Flucanazole | – | – | – | – | 0.0051 | 0.0051 |

pared to the complexes which lack a chlorine atom in the complexes directly attached to the tin atom. Therefore, chelation to the tin metal and the presence of a chlorine atom was assumed to impart a modification reaction in the biological system and it plays a significant role in the enhancement of biocidal activity, which may be due to a bacteriostatic or bactericidal effect. The order of biocidal activity for the complexes was Ph > Bu > Me.[34] This indicates that an R group directly attached to the tin atom also plays a significant role in the enhancement of activity, which is directly related to their electron donor ability. Hence, a phenyl group binds with biological molecules by π–π interactions and increases the electron density above azomethine nitrogen, which leads to the stronger interactions with the active centers of cell constituents.

## CONCLUSION

Diorganotin and triorganotin complexes were obtained by reacting sodium salts of benzoylpyridine-derived hydrazones with organotin(IV) chloride. The synthesized complexes were characterized by different spectroscopic ($^1$H-, $^{13}$C-, $^{119}$Sn-NMR, IR and mass) and other physical techniques. The Schiff base ligands were found to coordinate with tin metal in a tridentate manner (NNO) producing hexacoordinated tin(IV) complexes with distorted octahedral geometry. The compounds were further evaluated for their *in vitro* antimicrobial activity against different pathogenic bacteria and fungi. The tested complexes exhibited greater biocidal activity as compared to the free ligands due to the coordination with tin metal. In addition, the activity varied with the substitution on the tin atom, which increased in the order Me < *n*-Bu < Ph. A greater antimicrobial effect was observed in triphenyltin(IV) and diphenyltin(IV) complexes and the presence of a chlorine atom enhanced the biocidal activity against Gram-positive bacteria as compared to the Gram-negative bacteria, which may be due to difference in the nature of their cell wall and proficient diffusion of the complexes through the cell walls.

## SUPPLEMENTARY MATERIAL

Analytical and spectral data of the compounds, as well as copies of the corresponding $^1$H-NMR and $^{13}$C-NMR of the ligands $H_1L_2$ and $H_1L_4$ and $^1$H-NMR, $^{13}$C-NMR and $^{119}$Sn spectra of the complexes $Bu_2SnClL_2$ and $Bu_3SnL_4$ and mass spectra of all the complexes are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding authors on request.

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## ИЗВОД

## СИНТЕЗА, СПЕКТРАЛНА ИСПИТИВАЊА И *IN VITRO* АНТИМИКРОБНА АКТИВНОСТ НЕКИХ НОВИХ КОМПЛЕКСА ДИ/ТРИ-ОРГАНОКАЛАЈА(IV) СА ШИФОВИМ БАЗАМА ДОБИЈЕНИМ ИЗ 2-БЕНЗОИЛПИРИДИНА

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У овом раду су у реакцији кондензације Шифових база, које садрже 2-бензоилпиридин, са $R_2SnCl_2$ и $R_3SnCl$ (R = Me, *n*-Bu или Ph) у молском односу 1:1 синтетисана двадесет четири комплекса калаја(IV), опште формуле [$R_2SnLCl$, $R_3SnL$]. Комплекси су окарактерисани IR, $^1$H-, $^{13}$C- и $^{119}$Sn-NMR спектроскопијама, и техникама XRD и масене спектрометрије. У циљу проналажења најефекаснијег микробиолошког агенса, сви синтетисани лиганди и одговарајући комплекси калаја(IV) су испитивани на *in vitro* антимикробину активност на две грам-позитивне и две грам-негативне врсте бактерија, као и две врсте гљивица. Спектроскопским методама је нађено да је координациони број калаја(IV) у испитиваним комплексима шест и да су у овим комплексима тридентатни

лиганди координовани за калај(IV) преко азометинског и пиридинског атома азота, као и атома кисеоника. Лиганди координовани за калај(IV) су показали значајно већу биолошку активност, док су Ph и Bu комплекси показали већу токсичност. Резултати антимикробних испитивања су показали да синтетисани комплекси калаја(IV) имају веће токсично дејство према грам-позитивним сојевима, што се може приписати присуству спољашње липидне мембране липополисахарида.

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