ISOLATION, CHARACTERIZATION AND ANTI-TUMOUR PROPERTIES OF NOVEL CHIRAL ORGANOTIN(IV) COMPLEXES OF PHENANTHROLINES

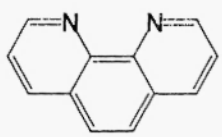
Ashu Chaudhary[a], Anita Phor[b], Shweta Gaur and R.V. Singh[a*]
[a]Department of Chemistry, University of Rajasthan, Jaipur – 302 004, India
[b]Hindu College, Sonepat – 131 001, India

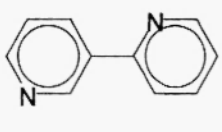
ABSTRACT

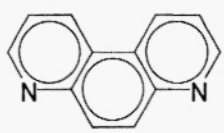
Novel chiral complexes of tin have been synthesized by using amino acids as chiral auxiliary and 1,10-phenanthroline, 4,7-phenanthroline or 1,7-phenanthroline as a secondary ligand. A series of di- and tri-organotin(IV) [$LSnR_nL'$) complexes where, L=amino acid (tyrosin and phenylalanine) and L′=1,10-phenanthroline, 4,7-phenanthroline or 1,7-phenanthroline and n = 2 or 3 have been prepared by the conventional methods. Structure elucidation has been done by IR, UV, ^1H, ^{13}C and ^{119}Sn NMR spectroscopy. All the complexes are air stable and non-electrolytic in nature. On the basis of spectral evidences, it has been concluded that the carboxylic acid of the amino acid is behaving as a monodentate ligand in all these complexes and the complexes are octahedral in shape with a coordination number six around the tin atom. An attempt has been made to correlate structural aspects of the compounds with the biological studies. The complexes have been screened against a number of fungi and bacteria to assess their growth inhibiting potential. The *in vitro* activity of the synthesized compounds has also examined against *Candida albicans* (commensal of the human body). Tin complexes incorporating the chelating 1,10-phenanthroline ligand showed a range of activities. The metal free non-chelating ligands 1,7-phenanthroline and 4,7-phenanthroline were inactive and the complexes derived from 1,7-phenanthroline displayed only marginal activity.

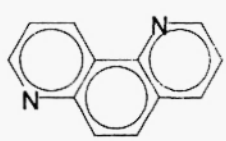
INTRODUCTION

Asymmetric synthesis has been a thrust area of organic chemistry[1,2]. Metal based chiral complexes have invoked interest to many researchers[3-5] primarily due to their use as catalysts[6-8] and has led to a challenging new subarea of inorganic asymmetric synthesis. Synthetic routes to asymmetric complexes are still very important and require a new approach which includes the choice of chiral auxiliary[9-11]. For chiral reagents or chiral pool we have chosen amino acids as a source of chiral stereogenic center firstly as they are cheap and home chiral and secondly they serve as excellent building blocks and lead to optically pure confirmation in good yield. There is an increased interest in the synthesis of the tin based antitumour drugs[12,13] and activity of these complexes closely related to their structure.[14] The chiral complexes have wide applications in the field of medicine as antitumour, anti HIV agents,[15] as catalysts[16,17] and also as enzyme model systems[18]. 1,10-Phenanthroline (1,10-phen), 2,2′-bipyridine (2,2′-bipy) and their substituted, both in the metal-free state and as ligands coordinated to transition metals, disturb the functioning of a wide variety of biological system.[19] When the metal-free N, N-chelating bases are found to be bioactive it is usually assumed that the sequestering of trace metals is involved, and that the resulting metal complexes are the actual active species[20,21].


(1,10-phen)


(2,2′-bipy)


(4,7-phen)


(1,7-phen)

The oxygen uptake of green leaves[22] and the endogeneous respiration of anabaema[23] are inhibited by the bases, both 1,10-phen and 2,2′-bipy accelerate the oxygen consumption of homogenised brain brei.[24] Highly potent anthelminatic action has been observed with both bases.[25] Furthermore, a solution of 1,10-phen suppresses the

* Author for correspondence : Professor R.V. Singh, Department of Chemistry, University of Rajasthan, Jaipur – 302 004 India. E-mail : kudiwal@datainfosys.net; Fax : +91-141-2708621

chemotactic power of "quineapig" leucocytes without destroying the cells[26]. The *in vitro* antibacterial action of 1,10-phen has been demonstrated on several species of bacteria,[27] whereas, phenanthroline metal complexes can be bacteriostatic[21] and bacteriocidal[28] towards many gram-positive bacteria. However, they are relatively ineffective against gram-negative organisms. On the other hand, m-and p-substituted phenanthrolines are less effective than 1,10-phen at preventing fungal growth and 2,9-dimethyl-1,10-phenanthroline (dmphen) was the most potent inhibitor.[22] Therefore, in view of the above facts it was considered as useful to synthesize such a type of compounds with an aim to characterize them structurally and biologically.

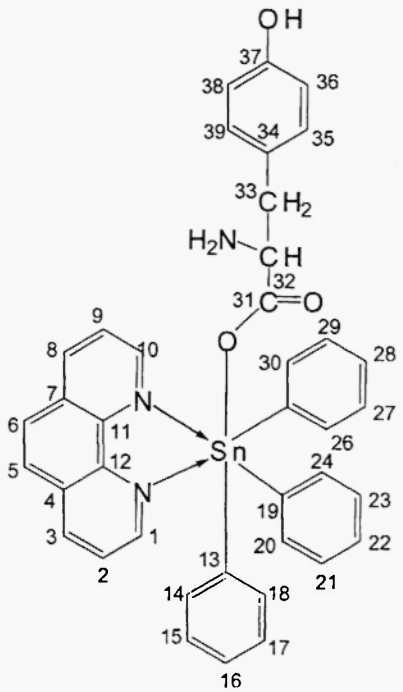
EXPERIMENTAL

All the glass apparatus with standard quick fit joints was used through out the work. Adequate precautions were taken to exclude moisture from the system. The chemicals and solvents used were dried and purified by the standard methods.

Synthesis of the Complexes

To a solution of tyrosin (0.45 g/2.47 mmol) in dry methanol (15 mL) was added a solution of 1,10-phenanthroline (0.44 g/2.44 mmol) in the same solvent. The resultant mixture was refluxed for 20 h after adding a solution of triphenyltinchloride (0.95 g/2.46 mmol) in hot methanol. The mixture was allowed to stand overnight in refrigerator. The solid product obtained was isolated by filtration, washed with ether and dried in vacuo (Fig.1).

Similarly, other complexes were performed by the reactions of 1, 10-phenanthroline, 1, 4-phenanthroline or 1, 7-phenanthroline with tyrosin or phenylalanine in 1:1:1 ratio.



(Fig.1)

$$L + R_nSnCl \xrightarrow{+L} [LR_nSnCl\,L']$$

Where, L = Tyrosin or phenylalanine
L′ = 1, 10-Phenanthroline, 4,7-phenanthroline or 1,7-phenanthroline
R = Phenyl, butyl or methyl; n = 3 or 2

Physical Measurements

The molecular weights were determined by the Rast Camphor method. Conductivity measurements in dry dimethylformamide were performed with a conductivity Bridge type 305. Infrared spectra were recorded on a Nicolet Magna FT-IR 550 spectrophotometer in KBr pellets. ^{1}H NMR spectra were recorded on a JEOL FX-90Q spectrometer in $CDCl_3$ using TMS as the internal standard. ^{13}C and ^{119}Sn NMR spectra were also recorded on the same spectrometer using MeOH as the solvent at 22.49 MHz and 33.35 MHz, respectively. Nitrogen and chlorine were estimated by the Kjeldahl's and Volhard's method, respectively. Tin was estimated as tin oxide gravimetrically. Carbon and hydrogen analyses were performed at Central Drugs Research Institute (CDRI) Lucknow. The physical properties and analytical data of the metal complexes are enlisted in Table 1.

RESULTS AND DISCUSSION

The complexes are coloured solids. These are characterized by the IR, UV, ^{1}H NMR, ^{13}C NMR and ^{119}Sn NMR spectroscopy.

The IR spectra of the starting materials and their complexes supported the formation of the complexes with the proposed coordination pattern. The amino acids exhibit ν(OH) band of the carboxylate group at ca. 3200 cm^{-1}. However, the IR spectra of the complexes do not show this band indicating the deprotonation of the carboxylic group. This is further supported by the appearance of a new medium intensity band in the far IR region 442-459 cm^{-1} attributed to Sn-O stretching vibrations indicating the coordination of the metal through oxygen. No splitting has been observed in the band at ca. 1650 cm^{-1} due to (COO) asym and (COO) sym vibrations.

Table 1. Physical properties and analytical data of the complexes.

Compound Formed	Yield (%)	Colour	Analysis Found (Calcd.)				Mol. Wt. Found (Calcd.)
			C	H	N	Sn	
$C_{39}H_{34}N_3O_3Sn$	56	Off white	65.6 (66.0)	4.6 (4.8)	5.1 (5.9)	16.2 (16.7)	686 (710)
$C_{39}H_{34}N_3O_3Sn$	64	White	65.8 (66.0)	4.7 (4.8)	5.2 (5.9)	16.1 (16.7)	677 (710)
$C_{39}H_{34}N_3O_3Sn$	60	White	65.6 (66.0)	4.7 (4.8)	5.1 (5.9)	16.1 (16.7)	687 (710)
$C_{39}H_{34}N_3O_3Sn$	59	Off white	67.1 (67.4)	4.5 (4.9)	5.9 (6.1)	16.5 (17.1)	667 (694)
$C_{39}H_{34}N_3O_3Sn$	52	White	67.0 (67.4)	4.7 (4.9)	5.3 (6.1)	16.5 (17.1)	661 (694)
$C_{39}H_{34}N_3O_3Sn$	68	Green	67.2 (67 4)	4.6 (4.9)	5.4 (6.1)	16.4 (17.1)	670 (694)
$C_{23}H_{26}N_3O_3SnCl$	47	Off white	50.1 (50.6)	4.5 (4.8)	2.5 (2.6)	21.1 (21.7)	517 (546)
$C_{29}H_{38}N_3O_3SnCl$	40	Off white	55.0 (55.4)	59 (6.1)	1.1 (2.2)	18.4 (18.9)	598 (629)

The electronic spectra of the complexes were recorded in dimethylsulphoxide. Two prominent peaks observed at 215-229 and 258-268 nm in the UV region and assigned to π-π^* transitions.

A comparative study of the ^{1}H NMR spectra of the starting materials and their complexes showed that the proposed skelton has been formed. A singlet was observed in the high field at δ 3.04-3.10 ppm due to $-CH_2$ protons of tyrosin or phenylalanine. A complex pattern in the region δ 3.67-3.84 ppm assigned as NH_2-CH. The phenyl proton signals appear in the range δ 6.95-7.30 ppm. Proton signals due to 1,10-phenanthroline and 1,7-phenanthroline appear at δ 8.40-9.26 ppm. Protons at δ 8.34 ppm overlap with R-CO-NH_2 group therefore exact position of $-NH_2$ remains unassigned. Since –COOH proton signal is absent in the ^{1}H NMR spectra, it confirms the coordination through the

carbonyl group which is already supported by the Sn-O peak in the far IR spectra.[23] ^{1}H NMR spectral data for these derivatives are also quite comparable with the results reported earlier[23,24].

^{13}C NMR spectra of the complexes recorded in MeOH, exhibit the carboxylic carbon signal at δ 4.19 ppm (showing unfield shift than the free amino acid) attributed to the monodentate nature of the carboxylic group. The bands due to the phenyl carbon attached to tin atom are observed at δ 126.7-128.2 ppm. The carbons of 1,10-phenanthroline, 1,7-phenanthroline and 4,7-phenanthroline (heterocylic carbons) are observed at δ 128.4-131.2, 142.5-144.6, 147.3-148.1 ppm, respectively. On the basis of spectral evidences, it may be inferred that the carboxylic acid of the amino acid (tyrosin or phenylalnine) is behaving as monodentate in these complexes and the complexes are octahedral in shape with a coordination number six around the tin atom.

^{119}Sn NMR spectra of the compounds give signals at δ 565.4-568.3 ppm indicating that tin is in an octahedral environment in the complexes,[25] while the value of tin in the complex $C_{23}H_{26}N_3O_2SnCl$ is in lower field δ 263.7 also indicates the hexa coordinate geometry around the central tin atom. The higher value for the triphenyl complex in comparison to other alkyltin complex may be accounted for the increased polarizability of the phenyl groups and chelate effects.

Biochemical Evaluation

The yeast *Candida albicans* is a commensal of the human body and is considered to be an important fungal pathogen. Opportunistic infection can lead to the development of vaginal candidosis, a condition from which over 75% of women suffer at some stage in their lifetime. Systematic candidosis is often fatal in immunocompromised patients[26]. The state of the art drugs currently sued to treat Candida infections are often ineffective because of problems with resistance or toxicity and subsequently the search for novel antifungal agents has gathered momentum[26]. A number of publications has appeared in the literature highlighting the fungicidal activity of the novel complexes[27-29]. As a results of these references, a great deal of attention is now being paid to these compounds after synthesizing them towards their bioinorganic chemistry because of the wide range of applications in living systems.

Antifungal and antibacterial activities were evaluated as reported earlier[30].

The results reveal that all the compounds are active against the organisms, *Alternatia alternata, Alternaria brassica, Fusarium oxysporum, Macrophomina phaseolina, Escherichia coli* and *Xanthomonas campastris,* even at low concentrations and the inhibition of the growth of microorganism was found to be dependent on the concentration of the compounds. The results of the biological screening indicated that the metal chelates are more active than starting materials.

Anti-Tumour Activity

Candida albicans (Clinical isolate) was obtained commercially from oxid culti-loops (ATCC 10231). The isolate was stored on Sabour and dextrose agar (SDA) plates at 4°C.

Suspensions of the test complexes were prepared by grinding them to a fine powder (0.02 g) followed by addition to sterile distilled water (100 cm^{-3}). This process yielded stock suspensions of 200 mg cm^{-3}. Those suspensions were furthered diluted to 100 μg cm^{-3}.

Susceptibillty Testing Method

RPMI-1640 broth medium (Sigma R 7755) was used for the anti-candida susceptibility testing. The medium (1 dm^3) was supplemented with L-glutamine (0.3 g) and morhalinepropanesulfonic acid (MOPS) (34.6 g) and was then adjusted to pH 7.0 using sterile NaOH (0.2 m). The broth microdilution reference method was used.[31] Prior to testing yeast cells were grown on sabouroud dextrose agar (SDA) at 37°C for 24 h. Cell suspensions were prepared in sterile phosphate buffered saline (5 cm^{-3}) to a density of 1 McFarland standard. A 1:100 dilution of these cell suspensions were made in RPMI-1640 medium so that the cell concentration of the find inoculum was $3.5 \times 10^4 - 5.0 \times 10^5$ cell

cm^{-3}. The prepared cells suspension (900/μd) was dispensed into sterile test tubes and to this was added the test complexes solutions (10 μl) to yield working solutions of the test complexes of concentration 2.0-0.0195 μg cm^{-3}. Minimum inhibitory concentration (MIC) determination on selected complexes was carried out for 24 hat 37°C with continuous shaking. Each complex was assessed in triplicate and three independent experiments were performed.

Oxidative Stress Studies

Yeast cells were grown on SDA at 37°C for 24 h. Cell suspensions were then prepared in sterile phosphate buffered saline (PBS, 5 cm^{-3}) to a density of 0.5 McFarland standard. A 1:100 dilution of these cell suspensions was made in RPM1-1640 medium (30 cm^3) to give a cell concentration in the final inoculum of $3.5 \times 10^4 - 5.0 \times 10^5$ cells cm^{-3}. The prepared cell suspension was dispensed into sterile Erylenmeyer flasks and the flasks then incubated in a shaking water bath for 24 h at 37°C with continuous shaking. Cell growth was assessed using an Neubauer haemocytometer chamber.

Cells growing in themed exponential phase (9 h) were collected by centrifugation (3, 000 rpm for 10 min), washed twice with sterile PBS and suspended in PBS (a cm^3). To this was added 1 cm^3 of the stock complex solution (0.2 g in 100 cm^3 of water) to yield working solutions of the test complexes of concentration 200 μg cm^{-3}. The cell suspension was then incubated at 37°C for 1 h with gentle shaking. Cells were collected by centrifugation (3, 000 rpm for 10 min), washed three times with PBS and then suspended in ice-cold PBS (5 cm^{-3}).

Cell viability studies were conducted by diluting the cells 1,000 fold with sterile distilled water. Serial dilutions were performed so that approximately 100 cells were planted onto individual SDA plates. The plates were incubated at 37°C for 24 h and the number of colonies counted. Cell viability is expressed as % cells surviving after treatment with drug compared with an untreated control.

Spheroplasts were prepared by adding glass beads to test and control cell suspensions and the cells disrupted using a whirlimixer (30 cycles each of 2 min duration). In between cycles cells were placed on ice. Cell wall removal was observed by light microscopy. The spheroplasts were separated from the glass beads by aspiration and collected by centrifugation (3,000 rpm for 15 min). The resulting spheroplast pellet was resuspended in PBS (10 cm^3) and samples of this were used separately for protein estimation, lipid peroxidation and glutathione assays.

Table 2. MIC of the selected starting materials and their corresponding complexes.

	Complexes	μg cm^{-3}
1.	1,10-Phenanthroline	1.25-2.5
2.	$C_{39}H_{34}N_3O_3$ [Sn(1,10-Phen) (tyrosin)]	1.25-2.5
3.	$C_{23}H_{26}N_3O_3SnCl$	720
4.	$C_{29}H_{38}N_3O_3SnCl$	720
5.	$C_{39}H_{34}N_3O_2SnCl$ [Sn(1,10-phen) (phenylalanine)]	72.5

Table 3. Anti-Candida activity of selected starting materials and their corresponding tin complexes.

	Complexes	% Cell growth
1.	1,10-Phenanthroline	0
2.	1,7-Phenanthroline	98±5
3.	4,7-Phenthroline	89.6±11
4.	[Sn(1,10-Phen) (tyrosin)]	0
5.	[Sn(1,7-Phen) (tyrosin)]	95±3
6.	[Sn(4,7-Phen) (tyrosin)]	88.3±13
7.	[Sn(1,10-Phen) (phenylalanine)]	2±0.1

RESULTS AND DISCUSSION

At a concentration of 20 μg cm^{-3} tyrosin and phenylalnine shows negligible inhibitory effect. 1, 10-Phenanthroline is highly active to inhibiting the growth of *Candida albicans* while 1, 7-phenanthroline and 4, 7-phenanthroline are inactive. The minimum inhibitory values for 1, 10-phenanthroline and [Sn(1, 10-phen) (tyrosin)] are in the range 1.25-2.5 μg cm^{-3} and substantially higher m/c values (>20 μg cm^{-3}) were found for the complexes [Sn(1, 10-phen) (tyrosin)] ($C_{29}H_{38}N_3O_3SnCl$) and [Sn(1, 10-phen) (tyrosin)] ($C_{32}H_{34}N_3O_2SnCl$). The complexes derived from 1, 7-phenanthroline and 4, 7-phenanthroline displayed only marginal activity.

All of the phenanthroline isomers can coordinate to metal centres 1, 10-phèn is the only ligand capable of actually chelating the metal and forming an extremely stable metalphen entity in solution. These observations would appear to substantiate the hypothesis that the bioactivity of N, N-chelating base is attributed to their ability and it is the resulting metal chelate complex that are the active species.

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