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Mononuclear indolyldithiocarbamates of $SnCl_4$ and R_2SnCl_2: Spectroscopic, thermal characterizations and cytotoxicity assays *in vitro*

Sadaf Khan, Shahab A.A. Nami, K.S. Siddiqi *

Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India

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Abstract

Several mononuclear Sn compounds ranging from mono to tetrakisindoline dithiocarbamates have been synthesized and characterized. Various spectroscopic techniques in combination with microanalytical data lead to the formation of 1:2 and 1:4 (Sn:Naindtc) type complexes depending on the molar ratio of the sodium salt of indolinedithiocarbamate employed. A symmetrical bidentate coordination of the indolinedithiocarbamate has been observed in all the cases as evident by a single sharp band at 1000 cm^{-1} in their IR spectra. The compounds were found to adopt an octahedral arrangement around the Sn atom as evident from their ^{119}Sn NMR data. The TGA/DSC profile of the ligand exhibits a two-stage thermogram while the complexes decompose in three steps leading to the formation of tin-sulfide as the eventual end product. The *in vitro* cytotoxicity of butyl and phenyl analogues has been studied against the standard human tumor cell lines. The compounds have also been screened for their antifungal and antibacterial activity against *E. coli*, *S. aureus*, *C. albicans* and *A. flavus*. The results indicated the compounds to be active.

Keywords: Indoline; Dithiocarbamates; ^{119}Sn NMR; FAB mass spectrometry; Cytotoxicity; TGA/DSC

1. Introduction

Dithiocarbamates owe special significance due to their wide application as vulcanization additives, stabilizers for PVC, fungicides, antibacterial and anticancer agents [1–3]. The recognition of the importance of Sn–S bond in biology has led to the study of organotin compounds with dithiocarbamates [4]. They are given importance due to their use as chemoprotectants in platinum based chemotherapy [5]. In particular, thiocarbonyl and thiol donors have shown promising properties for use in modulating *cis*-platin nephrotoxicity [6]. The antibacterial effect of dithiocarbamates was reported to arise by the reaction of HS-groups with physiologically important enzymes by transferring the alkyl group of the dithioester to the HS-function of the enzyme [7]. Investigations of various types of compounds possessing aminoalkylation ability showed that substituted aminoethyl *N*,*N*-dialkyldithiocarbamates have cytostatic features presumably *via* a similar mechanism proposed for antibacterial action [8].

The dithiocarbamate complexes of organotin have generated interest because of their dual structure and probable antitumor properties [9]. They are generally toxic even in minute quantities. Biological activity of such compounds is mainly dependant on the organic group bound to tin atom. $[Trialkyltin(IV)]^+$ and $[Triaryltin(IV)]^+$ are found to be highly toxic to central nervous system albeit the toxicity deceases with increasing size of the organic group [10]. Organotin compounds are also known to exert therapeutic effects on various tumor cells. Dialkyltin(IV) appear to be most effective probably due to the presence of $R_2Sn(IV)^{2+}$. Some of the organotin complexes possess antitumor activity although their mechanism of action is still unknown [11]. It has been shown that organotin compounds are active against two leukemia cell lines [12].

* Corresponding author. Tel.: +91 9837284930.
E-mail address: khwajas_siddiqi@yahoo.co.in (K.S. Siddiqi).

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Indoline and its derivatives are used in photoactive devices and as catalyst for asymmetric syntheses [13,14]. The protecting group on the nitrogen has a strong influence upon the regiochemistry of indoline reactions [15,16]. Over one thousand indole containing alkaloids are known, some of which are used in pharmacology [17].

During the recent years interest has been developed in evaluating the antimicrobial activity of many indoline derivatives and their dithiocarbamates. A cumulative study of the synthesis, characterization, antimicrobial and cytotoxic assays has seldom been done. Transition metal complexes of monothiocarbamates and dithiocarbamates of indoline have been synthesized and characterized [18], in no case, however, their organotin(IV) and $SnCl_4$ derivatives have been synthesized and antimicrobial properties screened.

In the present project synthesis and characterization of $(CH_3)_2SnCl_2$, $(C_4H_9)_2SnCl_2$, $(C_6H_5)_2SnCl_2$ and $SnCl_4$ complexes with indoline dithiocarbamate has been reported. They have been characterized by FT-IR, NMR, FAB-MS, TGA/DSC and elemental analysis. Also an attempt has been made to identify the symmetrical and unsymmetrical nature of bonding of the dithiocarbamate. Since the indoline and dialkyltin(IV) complexes are extensively used in clinical trials on humans, it was found worth studying their antitumor activity against the standard human tumor cell lines. These compounds have also been screened for their antifungal and antibacterial activity against *E. coli*, *S. aureus*, *C. albicans* and *A. flavus*.

2. Experimental

2.1. Materials and methods

Stannic chloride, Organotins (Merck, Across), Carbon disulfide (Merck) and Indoline (Koch Light) were used as received. Methanol was distilled and dried before use. Elemental analysis was carried out with a Flash EA-1112 Analyzer, CE Instrument. IR spectra (4000–200 cm^{-1}) were scanned with CsI on Nexus FT-IR Thermo Nicolet. The conductivity measurements were carried out with a CM-82T Elico conductivity bridge in DMSO. TGA/DSC was performed with a Universal V3.8 B TA SDT Q600 Build 51 Thermal Analyzer under nitrogen atmosphere using alumina powder as reference material. The heating rate was maintained at 10 °C/min. The FAB mass spectra were recorded on a JEOL SX 102/Da-6000 Mass Spectrometer/Data System using Argon/Xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature. *m*-Nitrobenzoyl alcohol (NBA) was used as the matrix.

2.2. Synthesis of ligand (Naindtc)

The ligand was prepared by a modified procedure given in the literature [17]. To a 40 mL methanolic solution of indoline (20.0 mmol, 2.19 mL) was added neat carbon disulfide (20.0 mmol, 1.2 mL) dropwise with continuous stirring in an ice bath followed by the addition of 10 mL NaOH (20.0 mmol, 0.8 g) dissolved in aqueous methanol. A shiny yellow product was obtained on standing the reaction mixture overnight. The product was, thoroughly washed with water, methanol and dried *in vacuo* over calcium chloride.

2.3. Synthesis of dimethyltin indolinebisdithiocarbamate, $[(CH_3)_2Sn(indtc)_2]$

To a stirred solution of the indoline dithiocarbamate (5 mmol, 1.1 g) in 20 mL of methanol was added (2.5 mmol, 0.55 g) dimethyltin dichloride in 10 mL of the same solvent to obtain an immediate precipitation. The reaction mixture was then stirred for 3 h at room temperature. The product was separated by filtration, washed with methanol and anhydrous diethyl ether and dried *in vacuo*.

2.4. Synthesis of dibutyltin indolinebisdithiocarbamate, $[(C_4H_9)_2Sn(indtc)_2]$

To a well stirred solution of the ligand (5 mmol, 1.1 g) in 20 mL of methanol was added (2.5 mmol, 0.76 g) dibutyltin dichloride in 10 mL of the same solvent to obtain an immediate precipitation. The reaction mixture was then stirred for 3 h at room temperature. The product was filtered, washed with methanol and anhydrous diethyl ether and dried *in vacuo*.

2.5. Synthesis of diphenyltin indolinebisdithiocarbamate, $[(C_6H_5)_2Sn(indtc)_2]$

To the solution of the ligand (5 mmol, 1.1 g) in 20 mL of methanol was added (2.5 mmol, 0.86 g) diphenyltin dichloride in 10 mL of the same solvent to obtain an immediate precipitation. The product was separated by filtration, washed with methanol and anhydrous diethyl ether and dried *in vacuo*.

2.6. Synthesis of dichlorotin indolinebisdithiocarbamate, $[(Cl)_2Sn(indtc)_2]$

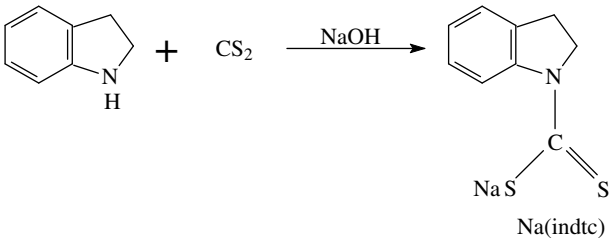
To a solution of the ligand (5 mmol, 1.1 g) in 20 mL of methanol was added (2.5 mmol, 4.49 mL) tin tetrachloride in 10 mL of the same solvent. This mixture was then stirred for 3 h at room temperature, which afforded the product. It was filtered, washed with methanol and anhydrous diethyl ether and dried *in vacuo*.

2.7. Synthesis of tin tetrakis indolinedithiocarbamate, $[Sn(indtc)_4]$

To a well stirred solution of the ligand (5 mmol, 1.1 g) in 20 mL of methanol was added (1.25 mmol, 2.24 mL) tin tetrachloride in 10 mL of the same solvent to obtain an immediate precipitation. The product was separated by filtration, washed with methanol and anhydrous diethyl ether and dried *in vacuo*.

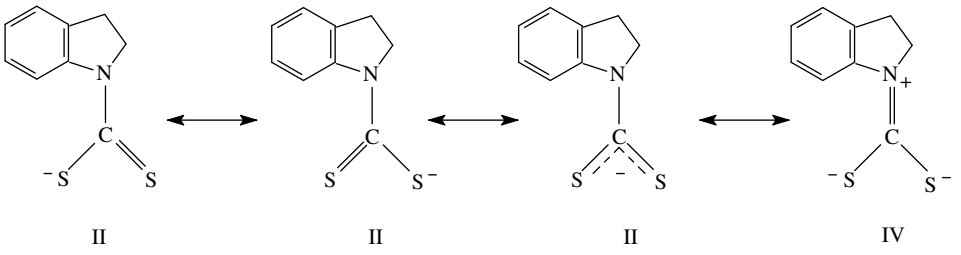
3. Results and discussion

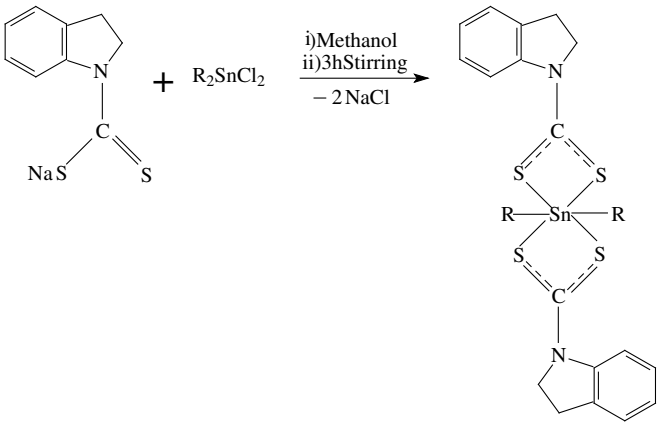
The ligand was obtained as sodium salt by the reaction of indoline with carbon disulfide and sodium hydroxide in methanol as shown below:



Na(indtc)

The organotin dithiocarbamates were obtained by the reaction of R_2SnCl_2 with Naindtc in 1:2, and $SnCl_4$ in 1:2 and 1:4 molar ratios, respectively. The general chemical reaction is given as:





The compounds are stable to moisture and heat, are non-conducting in DMSO [19] and give satisfactory elemental analysis (Table 1). They are soluble in chloroform and dichloromethane.

3.1. Infrared spectroscopy

The most significant IR bands are listed in Table 2. The ν(C–S) in the region $1000 \pm 70\ cm^{-1}$ is quite cardinal in deciding the nature of the dithiocarbamato group [20]. A singlet in the above region suggests bidentate symmetrical bonding while the splitting of this band into a doublet may be attributed to the unsymmetrical monodentate nature of the dithiocarbamato moiety [21]. We have observed a single sharp band at $1043\ cm^{-1}$ in the ligand, which was found to be shifted, by $\sim 25\ cm^{-1}$ in the compounds (**2**–**5**) indicating bidentate coordination of the ligand to tin atom. However, a doublet is observed at $998\ cm^{-1}$ in case of **6** implying the presence of both monodentate as well as bidentate dithiocarbamato moiety. This fact is further supported by ^{119}Sn NMR data. Furthermore, the C–N stretching frequency is also diagnostic in deciding the nature of dithiocarbamato group as evidenced by a large number of reports [1]. In our case the ν(C–N) at $1465\ cm^{-1}$ in the ligand was found to be shifted to higher wavenumber ($1477–1483\ cm^{-1}$) in complexes which is intermediate between ν(C–N) and ν(C=N) confirming a partial double bond character [22]. On the above findings the following resonance forms can be postulated for the ligand, Naindtc:

An interesting feature of these complexes is the concomitant strengthening of the C–N with C–S bond in the complexes which can be due to the coordination of the S–S chelate with Sn atom leading to a dispersal of electronic charge over the NCS_2 region [23]. The Sn–sulfur coordination is further supported by the presence of new medium to weak intensity bands in the $408–386\ cm^{-1}$ region, which are found to be absent in its precursor [24].

3.2. NMR spectroscopy

The ^1H NMR spectrum of the free ligand show signals for methylene proton bound to nitrogen at 3.80 ppm and are found to be unaffected by coordination (Table 3). The signals at 2.14 ppm belong to cyclic chain proton ($Ar–CH_2–C$), which are also insensitive to Sn coordination [25]. A multiplet was observed at 6.9 ppm corresponding to the presence of aromatic protons. The ^1H NMR spectra of compound **2** exhibits a sharp singlet at 1.52 ppm corresponding to the protons of methyl groups attached to the Sn atom [26]. However, in the case of compound **3**, three sets of signals are observed. As the butyl group is attached to electropositive Sn atom via carbon nuclei, a shielding effect is experienced through the carbon chain [27]. The methyl protons of the butyl group are observed as a triplet at 0.91 ppm while broad signals are observed at 1.65 and 1.38 ppm for the methylene protons. The aromatic protons

Table 1
Physicochemical properties of the ligand and its mononuclear compounds

Compounds (molecular formula)	Colour	Yield (%)	Molar conductance (ohm^{-1} mol^{-1} cm^2)	Anal. (%) Found (calc.)				
				C	H	N	S	Sn
Na(indtc) (**1**)	Yellow	55	12.3	49.6 (49.8)	3.8 (3.7)	6.5 (6.4)	29.8 (29.5)	
$Sn(indtc)_2(CH_3)_2$ (**2**)	Light-yellow	62	22.4	44.3 (44.5)	4.2 (4.5)	5.1 (5.2)	23.9 (23.7)	22.2 (22.0)
$Sn(indtc)_2(C_4H_9)_2$ (**3**)	Light-yellow	67	31.2	50.2 (50.3)	5.4 (5.5)	4.3 (4.5)	20.8 (20.6)	19.4 (19.1)
$Sn(indtc)_2(C_6H_5)_2$ (**4**)	Light-yellow	70	18.8	54.4 (54.5)	3.8 (4.0)	4.1 (4.2)	19.5 (19.3)	17.8 (17.9)
$Sn(indtc)_2Cl_2$ (**5**)	Yellow	68	35.2	37.6 (37.7)	2.2 (2.1)	4.8 (4.9)	22.5 (22.3)	20.9 (20.7)
$Sn(indtc)_4$ (**6**)	Yellow	55	22.7	48.1 (48.3)	3.4 (3.6)	6.4 (6.2)	28.8 (28.6)	31.3 (13.2)

$(CH_3)_2$ = dimethyl, $(C_4H_9)_2$ = dibutyl, $(C_6H_5)_2$ = diphenyl.

Table 2
Diagnostic IR bands of the ligand and its compounds

Compound	ν(Sn–S)	ν(Sn–C)	ν(C···S)	ν(C···N)	Ring vibrations
Naindtc (**1**)	–	–	1043 s	1465 s	1606 m
$Sn(indtc)_2(CH_3)_2$ (**2**)	404 w	582 m	1066 s	1477 s	1602 m
$Sn(indtc)_2(C_4H_9)_2$ (**3**)	408 w	597 m	1068 s	1479 s	1604 m
$Sn(indtc)_2(C_6H_5)_2$ (**4**)	401 w	604 w	1066 s	1477 s	1607 m
$Sn(indtc)_2Cl_2$ (**5**)	392 w	578 w	1070 s	1483 s	1601 m
$Sn(indtc)_4$ (**6**)	386 w	586 w	1074 s	1481 s	1600 m

Table 3
NMR chemical shifts of the compounds (in ppm)

Compound	NMR	CH_3	α-CH_2	β-CH_2	χ-CH_2	δ-CH_3	C_6H_5	$N(CH_2)$	(CH_2)ring	NCSS	(CH_2)aro
Naindtc (**1**)	^1H	–	–	–	–	–	–	3.80	2.14	–	6.91
	^{13}C	–	–	–	–	–	–	53.43	25.63	193.3	126.8
	^{119}Sn	–	–	–	–	–	–	–	–	–	–
$Sn(indtc)_2(CH_3)_2$ (**2**)	^1H	1.57	–	–	–	–	–	3.76	2.16	–	6.92
	^{13}C	–	–	–	–	–	–	53.42	25.42	196.2	128.9
	^{119}Sn	–	–	–	–	–	–	–	–	−274.5	–
$Sn(indtc)_2(C_4H_9)_2$ (**3**)	^1H	–	1.21	1.38	1.65	0.91	–	3.81	2.18	–	6.89
	^{13}C	–	28.42	29.92	26.12	13.45	–	53.28	25.63	198.5	129.6
	^{119}Sn	–	–	–	–	–	–	–	–	−296.3	–
$Sn(indtc)_2(C_6H_5)_2$ (**4**)	^1H	–	–	–	–	–	6.58	3.75	2.16	–	6.91
	^{13}C	–	–	–	–	–	123.5	52.61	25.62	199.5	128.7
	^{119}Sn	–	–	–	–	–	–	–	–	−301.2	–
$Sn(indtc)_2Cl_2$ (**5**)	^1H	–	–	–	–	–	–	3.77	2.17	–	6.90
	^{13}C	–	–	–	–	–	–	53.42	25.72	197.6	127.9
	^{119}Sn	–	–	–	–	–	–	–	–	−358.6	–
$Sn(indtc)_4$ (**6**)	^1H	–	–	–	–	–	–	3.79	2.16	–	6.91
	^{13}C	–	–	–	–	–	–	52.58	25.82	198.6	128.9
	^{119}Sn	–	–	–	–	–	–	–	–	−428.5	–

of the phenyl group directly attached to the Sn atom in **4** were observed at 6.58 ppm. The equivalence of the methyl, butyl and phenyl protons in compounds **2**–**4** indicates trans arrangement of the alkyl and aryl groups on Sn atom [28]. Highest downfield shift is observed for the methylene protons (1.21 ppm) directly attached to the Sn atom.

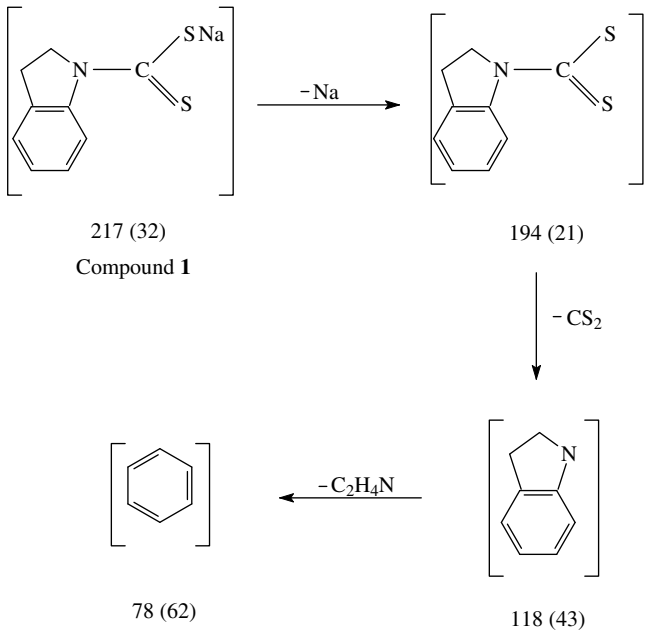
The ^{13}C NMR spectrum of the compounds exhibits two signals for $N(CH_2)$ carbons at 54.6 and 50.2 ppm while the ring methylene carbons were found to resonate at 25.8 and 24.0 ppm respectively. The CS_2 resonances for compound **1** were observed at 193.3 ppm while in complexes (**2**–**6**) it was shifted downfield to about 5–6 ppm indicative of the metal coordination [1]. The downfield shift is consistent with the dispersal of electronic cloud over the entire MS_2CN region. In order to provide further structural evidence, which establishes the structure of the compound in solution, we recorded ^{119}Sn NMR spectra.

It is well known that the high coordination number at the Sn center relates to high shielding effect. ^{119}Sn NMR chemical shift may be used to give tentative indications of the environment around tin atoms. It has been reported that the magnitude of the chemical shift in organotin(IV)

complexes is related to the coordination number of the metal center. Holecek et al. [29] have suggested values from +200 to −60 for four-coordinated, −90 to −190 for five-coordinated and −210 to −400 ppm for six-coordinated tin atoms in solution, respectively. The ^{119}Sn NMR spectra of compounds (**2**–**6**) exhibit chemical shift in the region −274.5 and −428.5 ppm, suggesting the presence of six-coordinated [30] Sn. An interesting feature is observed on examining the ^{119}Sn NMR chemical shifts. It was found that ^{119}Sn NMR signals shift to more positive frequencies on increasing the number of sulfur ions in the metal coordination sphere. This shift may be attributed to the loss of chloride ions, which facilitate the π back donation from p-electrons of the chloride to the empty 5d orbital of Sn center [31].

3.3. Mass spectrometry

The FAB mass spectral data and fragmentation pattern of compounds **1**, **2** and **4** are shown under Schemes 1–3 along with *m*/*z* and percent intensity. The mass spectrum of the ligand, Naindtc exhibits a low intensity molecular ion peak at *m*/*z* 217 while the base peak is observed at *m*/*z* 78 due to $[C_6H_6]^+$ fragment (Scheme 1). The disappearance of any peak above 217 implies the formation of anhydrous ligand, which is also evident from the TGA/DSC plot [32]. However, in case of compounds **2** and **4**, the reported mass spectral data is in accordance with the principal isotope ^{120}Sn. The molecular ion peak is observed only in case of methyl analogue, **2** at *m*/*z* 539. The base peak (100 %) are observed at 78 due to $[C_6H_6]^+$ and at 118 due to $[C_8H_8N]^+$ fragment for compounds **2** and **4**, respectively [33]. The absence of any higher peaks implies the formation of mononuclear compounds. The other possible fragments of the compounds are given in Schemes 2 and 3.



Scheme 1. Fragmentation pattern of ligand Naindtc (**1**).

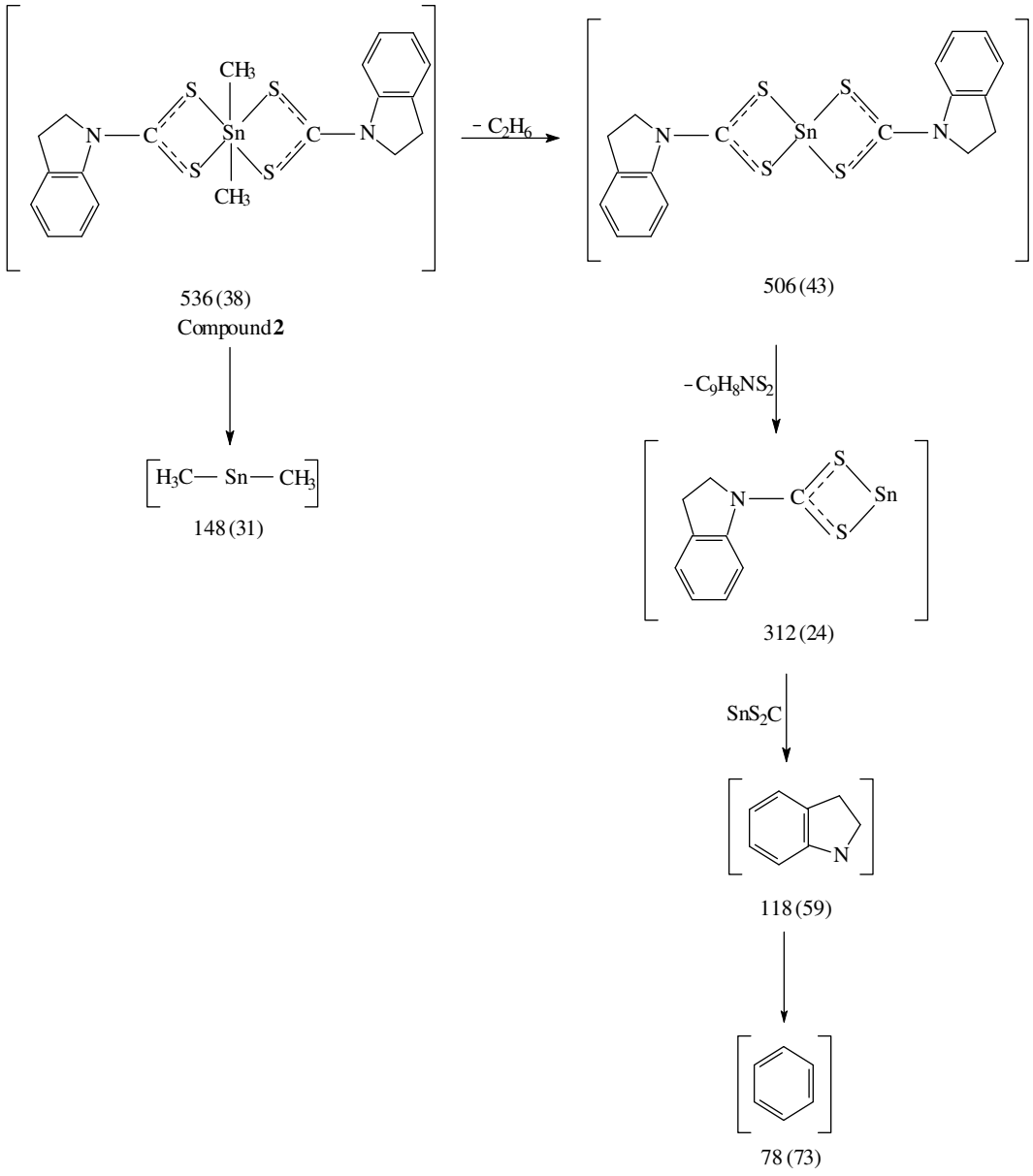
3.4. Thermal studies (TGA/DSC)

Thermogravimetric analysis of the ligand and its compounds has been carried out to study the pyrolysis pattern in the temperature range 25–580 °C. The thermogram of the ligand exhibits weight loss in two steps over the temperature range 145–180 and 205–320 °C. These are in agreement with the expulsion of CS_2 and indoline moieties, respectively [34]. The compounds start decomposing above 215 °C and the thermogram exhibits three distinct decomposition steps at 215, 354 and 447 °C. Since the TG curves of the compounds do not display noticeable weight loss up to ca. 215 °C it confirms the absence of water of hydration or water of coordination [35]. This behaviour also reveals greater thermal stability for compounds than its precursor. It is also supported by elemental analysis (Table 1) and infrared spectra (Table 2). A typical TGA plot of compound **2** is exhibited in Fig. 1. The first decomposition step of the compounds exhibits 58% weight loss corresponding to the decomposition of alkyl or aryl groups [36]. The second thermolytic stage accounts for the degradation of indoline nucleus which is a common feature of heterocyclic compounds while the third step at 450 °C, is accompanied by 20% weight loss corresponding to the expulsion of remaining part of the organic moiety. Finally the TG curve shows a plateau above 540 °C corresponding to the formation of tin-sulfide as the final product [37].

3.5. Antimicrobial assay

The antibacterial and antifungal activity of a solution of freshly synthesized compounds in dichloromethane was tested against some gram positive and gram negative bacteria such as, *E. coli*, *S. aureus*, *C. albicans* and *A. flavus*. The activity was then compared with some reference antibiotics that were purchased from the market. The results revealed that the dithiocarbamato compounds had activity comparable with the reference drug (Table 4).

All the compounds and the parent ligand were screened for their activity against the test organisms. The hole plate diffusion method was adopted for the activity measurements [38]. The bacterial strains were grown in nutrient agar slants and the fungal strains were grown in Sabouraud dextrose agar slants. The viable bacterial cells were swabbed onto Nutrient agar plates and the fungal spores onto Sabouraud dextrose agar plates. The compounds were dissolved in dichloromethane to a final concentration of 0.1%. A 0.5 cm diameter well was cut in a medium inoculated with the respective cultures, and the test solutions of the compounds in different concentrations (5 and 1 μg) for bacterial cultures and (15 and 50 μg) for fungal cultures were allowed to stay in the wells. The petri plates were incubated for 36 h for bacterial cultures and 72 h for fungal cultures. All the compounds were screened against

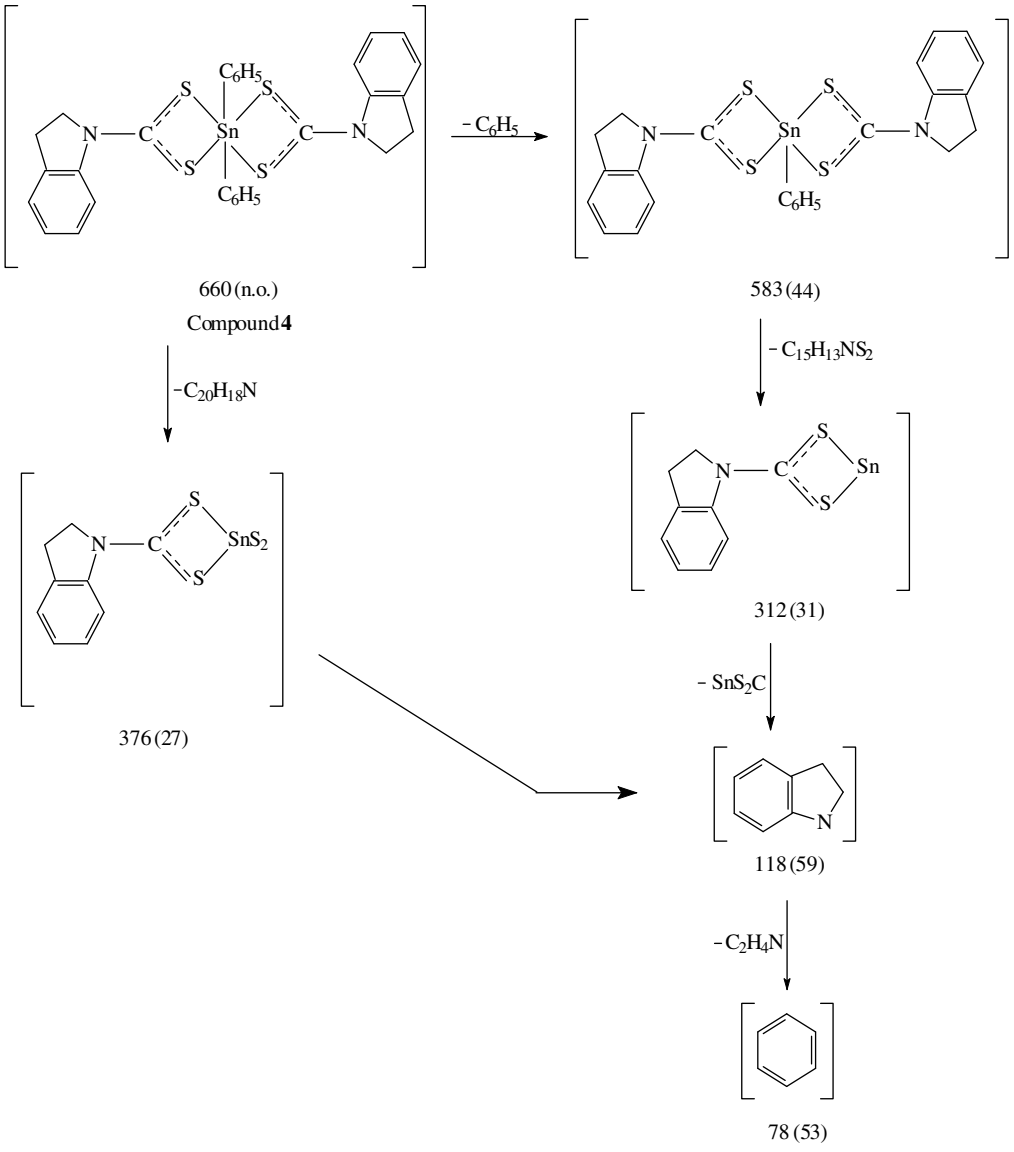


Scheme 2. Fragmentation pattern of compound, $Sn(indtc)_2(CH_3)_2$ (**2**).

flucanazole as reference for fungal cultures and gentamycin for bacterial cultures in their standard concentration of 200 μg/well. The activity of the compounds was evaluated by measuring the diameter of the inhibition zone around the respective wells. The results are presented in the Table 4. The compounds display stable inhibition zones and are more active than the ligand. They possess potential inhibitory activity in amounts as low as 1 μg/well for bacterial cultures and 15 μg/well for fungal cultures. The activity of the compounds is measured in terms of inhibition of the replication of DNA by interacting with the enzyme prosthetic group. It has been observed that the activity of dibutyltin dithiocarbamate is lower than that of tin(IV) complex, while that of methyl and phenyl analogues is quite comparable. Higher activity of $Cl_2Sn(indtc)_2$ is probably due to the chloride, which is more labile than the alkyl groups. Since it is relatively less hydrophobic compound it may easily penetrate the cell membrane as compared to the others [39]. The possible inhibitory action of the complexes can be due to their inhibition of the replication of DNA by interacting with the enzyme prosthetic group. These compounds are found to be quite active due to their membrane penetrating activity. However, the reduced activities in some cases can be attributed to their inability to form hydrogen bonds with the cell constituents [40].

3.6. Cytotoxicity screenings

The *in vitro* cytotoxicity test of the compounds **2**–**6** was performed using microculture sulforhodamine B (SRB) test for estimation of cell viability [41]. The human cancer cell lines used in the present study are WIDR (colon cancer),



Scheme 3. Fragmentation pattern of compound, $Sn(indtc)_2(C_6H_5)_2$ (**4**).

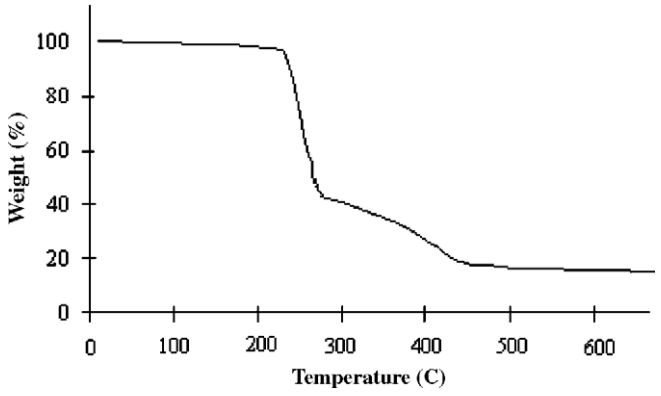


Fig. 1. TGA curve showing the degradation of compound **2**.

IGROV (ovarian cancer), M19 MEL (melanoma), A498 (renal cancer), MCF-7 (breast cancer) estrogen receptor (ER)/progesterone receptor (PgR)+, EVSA-T (breast cancer) (ER)-/ progesterone receptor (PgR)- and H226 (non small cell lung cancer). The test and reference compounds were dissolved to a concentration of 250 000 ng/mL in full medium, by 20-fold dilution of a stock solution, which contained 1 mg compound/200 μL. The compounds were dissolved in dichloromethane.

The experiment was started on day 0. On day 0, 10 000 cells per well were seeded into 96-wells flat-bottomed microtiter plates (falcon 3072, DB). The plates were incubated for 48 h at 37 °C, 8% CO_2 to allow the cells to adhere to the bottom. On day 2, a threefold dilution sequence of ten steps was made in full medium, starting with the 250 000 ng/mL stock solution. Every dilution was used in quadruplicate by adding 150 μL to a column of four wells. This procedure resulted in a highest concentration of 625 000 ng/mL present in column 12. Column 2 was used as a blank. After incubation of four days, the plates were washed with PBS twice. Subsequently 200 μL of Fluoresce-

Table 4
Inhibition zone in mm

Compounds	*E. coli*		*S. aureus*		*A. flavus*		*C. albicans*	
	5 μg	1 μg	5 μg	1 μg	5 μg	1 μg	5 μg	1 μg
Naindtc (**1**)	14.2	11.2	15.7	13.2	15.4	13.7	10.4	10.2
$Sn(indtc)_2(CH_3)_2$ (**2**)	15.6	13.6	21.5	20.3	11.0	10.0	16.9	14.8
$Sn(indtc)_2(C_4H_9)_2$ (**3**)	11.2	10.2	17.5	15.8	9.5	9.0	10.2	10.0
$Sn(indtc)_2(C_6H_5)_2$ (**4**)	21.9	20.2	21.9	20.5	16.4	15.9	15.3	14.8
$Sn(indtc)_2Cl_2$ (**5**)	25.0	22.6	28.7	25.5	19.8	18.6	20.3	20.6
$Sn(indtc)_4$ (**6**)	22.5	20.3	19.3	18.4	13.2	12.0	17.3	16.5
Gentamycin/Flucanazole	26.0	26.0	30.0	30.0	20.0	22.0	22.0	22.0

Table 5
Inhibition doses ID_{50} values (ng/mL) of test compounds *in vitro* using SRD as cell viability test

Test compound	Cell line 1 A498	Cell line 2 EVSA-T	Cell line 3 H226	Cell line 4 IGROV	Cell line 5 M19 MEL	Cell line 6 MCF-7	Cell line 7 WIDR
$Sn(indtc)_2(C_4H_9)_2$ (**3**)	2185	355	3105	165	485	670	950
$Sn(indtc)_2(C_6H_5)_2$ (**4**)	2205	375	3185	170	489	650	924
DOX	94	12	201	64	20	12	10
CPT	2256	422	3275	170	560	705	990
5FU	143	475	340	297	442	750	225
MTX	30	3	2225	7	27	20	<3.2
TAX	<3.3	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2

DOX: doxorubicin; CPT: *cis*-platin; 5-FU: 5-fluorouracil; MTX: methotrexate; TAX: taxol.

in Diacetate stock solution diluted to 2 μg/mL with PBS was added to each of the control, experimental and blank wells. The plates were then incubated for 30 min. at 37 °C. Fluorescence generated from each well was measured at an excitation wavelength of 485 nm and an emission wavelength of 540 nm using an automated microplate reader (Labsystems Multiskan MS). Data was used for the construction of concentration–response curves and the determination of ID_{50} values by use of Deltasoft 3 software.

The results of the *in vitro* cytoxicity test of compounds dibutyltin indoline bis(dithiocarbamate), $[(C_4H_9)_2Sn(indtc)_2]$ and diphenyltin indoline bis(dithiocarbamate), $[(C_6H_5)_2Sn(indtc)_2]$ are given in Table 5, as the inhibition doses ID_{50} observed against a panel of seven human tumor cell lines i.e. WIDR (colon cancer), IGROV (ovarian cancer), M19 MEL (melanoma), A498 (renal cancer), MCF-7 (breast cancer) estrogen receptor (ER)/progesterone receptor (PgR)+, EVSA-T (breast cancer) (ER)-/progesterone receptor (PgR)- and H226 (non small cell lung cancer). The cytotoxicity results were compared with those obtained for some clinically used reference compounds like doxorubicin DOX, cisplatin CPT, 5-fluorouracil 5-FU, methotrexate MTX and taxol TAX.

The Table 5 clearly shows that the compounds **3** and **4** are active *in vitro* and exhibit comparable activity with respect to cisplatin against all seven human tumor cell lines. Hence these compounds can be effectively tested as suitable candidates for improving cytotoxic and dissolution properties. This is in accordance with the findings of Pellerito et al. who reports that organotin(IV) complexes exert therapeutic effects on various tumor cells; dialkyltin(IV) complexes being the most effective [42,43]. It has been suggested that these behaviors are exhibited due to the interaction of organotin compounds with DNA at the level of phosphate group, which may be followed by the intercalation of the ligand into DNA [44,45]. Different active organotin compounds may still show slight variations in '*in vitro*' cytotoxicity due to different kinetic and mechanistic behavior [46].

4. Conclusion

The compounds reported here have been obtained as amorphous powders soluble in chloroform and dichloromethane. Spectroscopic techniques suggest and support the probable mode of coordination in all the compounds where coordination is predominantly bidentate with an exception in the case of $Sn(indtc)_4$. The '*in vitro*' antimicrobial assays of the synthesized complexes against the bacterial and fungal strains establish their inhibitory effect. Cytotoxicity assays of the compounds confirm the potency of these compounds, which can be used for clinical trials after further research.

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