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Behavioural dynamics in the biological control of pests: role of silicon complexes

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The complexes of silicon (IV) with Schiff base ligands (L_1H and L_2H of isatin derivatives) having a sulfur and oxygen donor system were prepared by the reactions in methanol environment. These were isolated and characterized by elemental analysis, molecular weight determinations and conductance measurements. On the basis of electronic, infrared, ^1H, ^{13}C and ^{29}Si NMR spectral studies, trigonal bipyramidal geometry was suggested for the resulting complexes. These data support preferential binding of sulfur and oxygen atom to the silicon atom. The disease resistance activities of the ligands and their corresponding complexes were examined successfully in *in vitro* and *in vivo* experiments, against pathogenic fungi and bacteria. Results were quite encouraging and these were compared with the standard pesticides Bavistin and Streptomycin. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: organosilicon (IV) complexes; thio- and semi-ligands; spectral studies; biochemical studies; toxicity; *in vitro* and *in vivo* study

Introduction

In recent years, high academic, industrial and financial support has been extended to plant protection. The annual estimated losses through pests and pathogen of crops, soil nutrients robbed by weeds and ill health of animals run into several billion dollars.[1,2] The latest research proves that silicon compounds are beneficial for plants in many important ways. These include greater tolerance of environmental stresses and resistance to insects and fungi.[3] Schiff base metal chelates are important in biological processes, pre-concentration of metal ions and catalysis.[4] After suitable structural modifications, the derivatives of Schiff bases and metal coordinated drugs may be used as bioactive material for medicines as well as in industry.[5,6] Nowadays diuron (substituted phenyl urea herbicide) is widely used with other silicon pesticides in California as active pesticides both in crop sites and non-crop sites.[7] Generally, organosilicon compounds seem to owe their antitumour properties to the immuno defensive system of the organism.[8,9]

Recently, there have been considerable efforts to synthesize such organosilicon synthones containing hypervalent silicon atoms used for plant and animal growth stimulants and wound healing substances.[10] Owing to their outstanding chemical and physical properties, pure silicon nitride (Si_3N_4) ceramics and Si_3N_4/SiC ceramic composites have been considered as suitable materials for heat engine applications and are therefore of great economic interest. Since so many modern drugs, plant chemicals,[11,12] etc. are fluorine-containing materials, methods of introducing fluorine into molecules always have potential value. Several stable compounds of fluorine have been developed by scientists[13] using environmentally friendly CFC-replacements, such as HFCs. Prompted by favourable research work and our interest in the field of organosilicon complexes, two ligands and their silicon complexes were screened against several pathogens.

Experimental

Physical measurements

All the chemicals were dried and purified before use and the reactions were carried out with a distillation assembly, fitted with condensor and protected from moisture. Nitrogen and sulfur were estimated by Kjeldahl's and Messenger's methods, respectively. Silicon was determined gravimetrically as SiO_2. The conductance was measured using conductivity bridge type-304 Systronics model and the molecular weights were determined by the Rast Camphor method. IR spectra were recorded on an FTIR spectrophotometer, model IR-550 as nujol mulls using KBr optics. ^1H and ^{19}F NMR spectra were recorded in DMSO-D_6, and ^{13}C and ^{29}Si spectra were recorded in methanol, using TMS as the internal standard. C_6F_6 was used as the external reference for the ^{19}F NMR spectra.

Synthesis of the ligands, L_1H and L_2H

Ligand L_1H was prepared by the condensation of 1,3-dihydro-3 [2-(4-fluoro-3-methylphenyl)-2-oxo-ethylidene]2H-indol-2-one (5.5 g) with hydrazinecarboxamide (1.47 g) in the presence of sodium acetate in equimolar ratio (1 : 1) in absolute alcohol.

Ligand L_2H was prepared by the condensation of 1,3-dihydro-3-[2-(4-fluoro-3-methylphenyl)-2-oxo-ethylidene]-2H-indol-2-one (6.7 g) with hydrazinecarbothioamide (2.17 g) in 1 : 1 molar ratio in alcoholic medium. These mixtures were heated under reflux for 45 min. The solvent was then removed and the residue was dried *in vacuo*. The products were purified by re-crystallization from the same solvent. The analyses and physical properties of these ligands are enlisted in Table 1.

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Table 1. Physical properties and analytical data of the ligands

Ligand	Colour and state	M.p. (°C)	Yield (%)	Analysis (%) found/(calcd)				
				C	H	N	S	Molecular weight
L_1H $C_{18}H_{15}N_4O_2F$	Orange crystalline solid	159–160	90	63.60 (63.96)	4.31 (4.47)	16.48 (16.58)	–	332 (338)
L_2H $C_{18}H_{15}N_4OSF$	Orange solid	164–165	89	60.82 (61.97)	4.19 (4.27)	15.72 (15.83)	9.01 (9.06)	350 (354)

Table 2. Physical properties and analytical data of organosilicon (IV) complexes

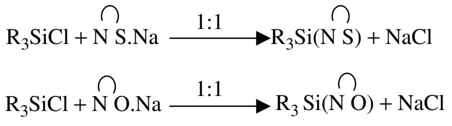
Reactants (g) R_3SiCl (R = Me or Ph)	Ligand (g)	Molar ratio	Yield (%)	Product formed (colour and state)	M.p. (°C)	Analysis (%) found/(calcd)					
						C	H	N	S	Si	Mol.wt
Me_3SiCl (1.18)	L_1H (3.67)	1 : 1	82	$Me_3Si(L_1)$ (orange solid) $C_{21}H_{23}N_4O_2FSi$	139–140	61.08 (61.37)	5.55 (5.64)	13.50 (13.63)	–	6.71 (6.83)	406 (411)
Ph_3SiCl (1.14)	L_1H (1.31)	1 : 1	83	$Ph_3Si(L_1)$ (orange solid) $C_{36}H_{29}N_4O_2FSi$	171–172	72.28 (72.43)	4.79 (4.90)	9.12 (9.39)	–	4.55 (4.72)	592 (597)
Me_3SiCl (1.29)	L_2H (4.21)	1 : 1	75	$Me_3Si(L_2)$ (brown solid) $C_{21}H_{23}N_4OSFSi$	181–182	59.01 (59.07)	5.52 (5.43)	13.05 (13.12)	7.50 (7.51)	6.50 (6.58)	420 (427)
Ph_3SiCl (0.98)	L_2H (1.18)	1 : 1	77	$Ph_3Si(L_2)$ (Orange solid) $C_{36}H_{29}N_4OSFSi$	184–185	70.32 (70.54)	4.59 (4.77)	8.93 (9.14)	5.10 (5.23)	4.48 (4.58)	607 (613)

Synthesis of the complexes

A calculated amount of the sodium salt of the ligands in dry methanol was added to the weighed amounts of Me_3SiCl and Ph_3SiCl in a round-bottom flask in a 1 : 1 molar ratio. The reaction was heated under reflux over a ratio-head for 16–18 h, the white precipitate of sodium chloride obtained was removed through the alkoxy funnel and excess solvent from mother liquor was removed under reduced pressure. Compounds were dried under vacuum for 3–4 h. These were purified by repeated washing with *n*-hexane and ether and again dried *in vacuo*. Their purities were further checked by TLC using silica gel-G. All the compounds were isolated as powdered solids. The details (analytical data, physical properties viz. melting points, quantities and yields) of these reactant and the resulting products are recorded in Table 2.

Results and Discussion

Reactions of organosilicon (IV) halides with monobasic bidentate ligands (Fig. 1) in 1 : 1 molar ratio in methanol may be represented by the following equations:



$$R_3SiCl + \overset{\frown}{N\,S}.Na \xrightarrow{1:1} R_3Si(\overset{\frown}{N\,S}) + NaCl$$

$$R_3SiCl + \overset{\frown}{N\,O}.Na \xrightarrow{1:1} R_3\,Si(\overset{\frown}{N\,O}) + NaCl$$

where R = Me or Ph; $\underset{N\,S}{\frown}$ and $\underset{N\,O}{\frown}$ = donar set of the ligands.

IR spectra

The infrared spectra of the ligands and their silicon complexes were recorded and the important features may be summarized as follows. The IR spectra of fluorohydrazines show two sharp bands around 3450 and 3350 cm^{-1} due to ν asym and ν sym NH_2 vibrations, respectively, which remain almost at the same positions in the metal complexes, showing non-involvement of this group in the complexation.

The bands of medium intensity appearing in the regions 3300 and 2700 cm^{-1} may be assigned to ν NH[14] and ν SH vibrations, respectively, which suggest that the ligands exist as in keto-enol tautomerism. These disappear in the corresponding silicon complexes.

The band due to >C=N in the free ligand gets shifted to the lower wave number ($\delta\nu = 15–20\ cm^{-1}$) in the silicon complexes, indicating coordination through azomethine nitrogen.[15] The ν C=O band in hydrazinecarboxamide and ν C=S in hydrazinecarbothioamide appear at 1690 and 1035 cm^{-1}, respectively. These bands disappear on complexation, which is due to the covalent bond formation of the ligand with the silicon atom through the oxygen or sulfur atoms.

Several new bands in the complexes at 770, 579 and 530 cm^{-1} are due to ν Si–C, ν Si ← N and ν Si–S, respectively, which are absent in the spectrum of the ligand, further supporting the

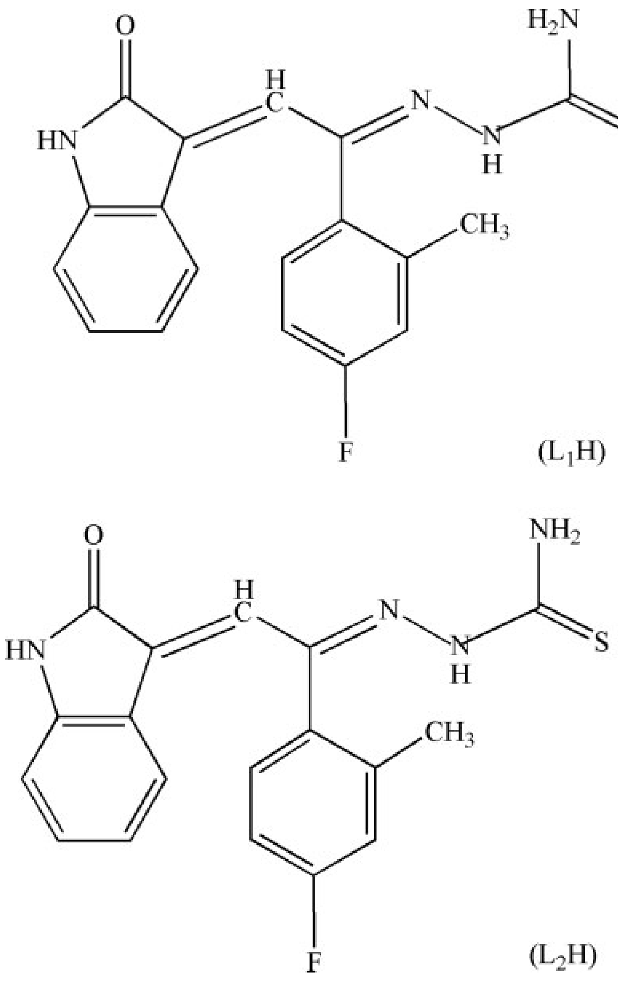


Figure 1. Structures of the ligands.

participation of the sulfur atom and the azomethine nitrogen in complexation.

UV spectra

A band due to the $>C{=}N$ chromophore in the spectrum of the ligand at 365 nm due to $n-\pi^*$ shifts to a lower wavelength in the silicon complexes and appears at 353, 348, 356 and 350 nm in the various 1 : 1 products. This clearly indicates the coordination of the azomethine nitrogen to the silicon atom. Such a shift in $n-\pi^*$ band is probably due to the donation of lone pair of electrons by the nitrogen of the fluoro-ligand to the central metal atom. Further, two bands at 252 nm and 283 nm are due to $\pi-\pi^*$ transitions; these are assigned to the benzenoid ring and $\rightarrow C{=}N$ band of the azomethine group, respectively. The K band $\pi-\pi^*$ showed a red shift due to the overlap of the central metal d-orbital with the p-orbital of the donor atom, which causes an increase in conjugation and the B-band undergoes a hypsochromic shift in the complexes.

^1H NMR spectra

The proton magnetic resonance spectra of the ligands and their corresponding silicon complexes were recorded in DMSO-d_6 using TMS as the internal standard. The chemical shift values (δ, ppm) of the different protons are given in Table 3. The ^1H NMR spectra of the ligands exhibit peaks around δ values 11.12–10.12 (1H) characteristic of −NH of the isatin ring. The peaks found around δ value 7.98–6.34 (7H) may be due to aromatic protons, while that observed at δ value 10.08–10.04 (1H) may be due to −NH of thiosemicarbazone/semicarbazone. The disappearance of signal which is due to −NH of thiosemicarbazone/semicarbazone in the silicon derivatives indicates the coordination of the azomethine nitrogen atom as well as covalent bond formation between silicon and sulfur/oxygen due to deprotonation of the ligands. Further, new signals at δ 0.64 (9H) and δ 0.78 (9H) ppm in the 1 : 1 complexes are due to the methyl protons of the Me_3Si group. In the spectra of the complexes, a downfield shift in the position of methyl and aromatic protons indicates deshielding, as well as the coordination of azomethine nitrogen to the silicon atom. This is probably due to the donation of the lone pair of electrons by the nitrogen to the central silicon atom, resulting in the formation of a coordinate linkage ($Si \leftarrow N$). A peak was observed at δ value 2.46 (3H) due to $-CH_3$ protons attached to the phenyl ring. The appearance of a signal around 2.98–2.56 δ value, due to $-NH_2$ group at the same positions in the ligand and its silicon complexes, shows the non-involvement of this group in coordination.

Table 3. ^1H NMR spectra data of the ligands and their organosilicon (IV) complexes

Compound	−NH ring (bs)	−NH free (bs)	$-NH_2$ (bs)	$\rightarrow C{=}CH-$ $C{=}N$ (s)	Aromatic (m)	Si−Me/Ph
L_1H	10.12	10.08	2.98	6.08	7.68–6.65	–
$Me_3Si(L_1)$	10.46	–	2.72	6.12	7.82–6.78	0.78
$Ph_3Si(L_1)$	10.36	–	2.64	6.16	7.90–6.80	6.72
L_2H	11.12	10.04	2.56	6.12	7.72–6.34	–
$Me_3Si(L_2)$	11.16	–	2.72	6.26	7.84–6.44	0.64
$Ph_3Si(L_2)$	11.24	–	2.78	6.34	7.98–6.54	6.06

^{13}C NMR spectra

The ^{13}C NMR spectra of the ligands and their corresponding silicon complexes were also recorded in dry MeOH. Considerable shifts in the values of the carbon atom attached to the azomethine nitrogen, thiolic sulfur or amido oxygen support the proposed coordination in these complexes. The heterocyclic moiety carbon signals, especially those of the carbon atoms directly bonded to the heteroatom, undergo slight upfield shifts relative to the other carbon atoms which remain almost undisturbed. The shift towards upfield in the signal of the thiolo carbon and azomethine carbon in the complexes suggests participation of these groups in coordination to the silicon atom (Table 4).

^{19}F NMR spectra

The ^{19}F NMR spectrum of the ligand L_2H displays a sharp singlet at δ 114.36 ppm. The organosilicon (IV) complexes of this ligand show no change in the position of the signal and thus support the non-involvement of fluorine in complexation.

^{29}Si NMR spectra

In the case of silicon complexes, signals were observed around δ 90.5 to δ 93 ppm, which stated coordination number five

Table 4. ^{13}C NMR spectra data of the ligands and their organosilicon (IV) complexes

	Chemical shift values (δ, ppm)				
Compound	Amido/thiolo	Azomethine	$-NH-C{=}O$	Aromatic	Si–Me/Ph
L_1H	170.20	160.24	159.98	143.66, 127.85, 126.54, 123.32, 122.36,120.66	–
$Me_3Si(L_1)$	166.42	157.38	158.91	143.82, 127.96, 126.68, 123.34, 122.12, 120.33	14.34
L_2H	172.52	155.12	164.58	147.24, 144.28, 136.92, 135.72, 130.22, 129.71	–
$Ph_3Si(L_2)$	165.39	152.23	162.96	147.94, 145.01, 137.42, 136.02, 131.18, 130.72	131.18, 134.18, 135.56, 138.86

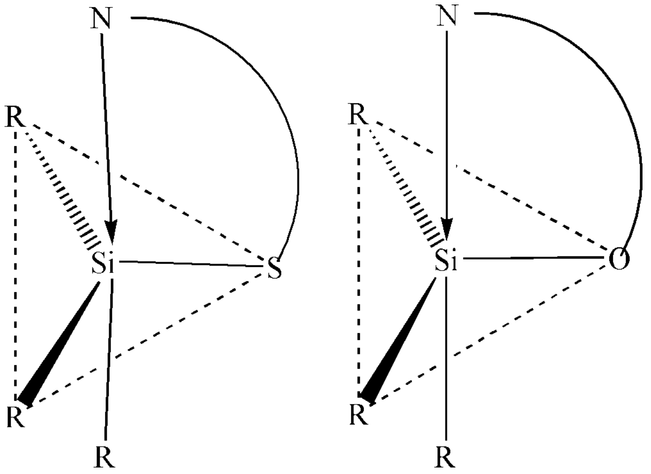


Figure 2. Suggested structures for the complexes; R = Me or Ph and $\overset{\frown}{\mathrm{N\ S}}$ and $\overset{\frown}{\mathrm{N\ O}}$ are the donor sites of the ligand molecule.

around the silicon atom. These data were supported by literature data.[16,17] On the basis of the above spectral studies, possible trigonal bipyramidal geometries have been suggested for the methyl and phenyl silicon complexes (Fig. 2).

Microbial Assay

Bioefficacies of the ligands and their compounds were tested in *in vitro* as well as in *in vivo* using the paper disc method[18] for antibacterial activity and the percentage disease incidence (PDI)[19,20] for antifungal screening.

Antibacterial activity *(in vitro)*

The organisms selected for this study were *Staphylococcus aureus* (+) and *Escherichia coli* (−) and the technique used was the paper disc method. In this technique sterilized hot nutrient agar and a 5 mm diameter paper disc of Whatman no. 1 were used. The agar medium was poured into the Petri plates. After solidification, the plates were stored in an inverted position so that there was condensation of water in the upper lid. Now bacterial suspension was uniformly spread on solidified nutrient agar. The solutions of test compounds were prepared in methanol (500 and 1000 ppm concentrations), in which discs were dipped and then placed on Petri plates. The Petri plates with these discs on the seeded agar were kept at low temperature for 2–4 h to allow for the diffusion of chemical before being incubated at a suitable optimum temperature (28+2 °C) for 24–30 h and the inhibition zone around each disc was measured and is reported in Table 5.

Table 5. Bactericidal screening data of the ligands and their silicon complexes

	Diameter of inhibition zone (mm)			
	Staphylococcus aureus (+) (concentration in ppm)		*Escherichia coli* (−) (concentration in ppm)	
Compound	500	1000	500	1000
L_1H	8	11	6	10
$Me_3Si(L_1)$	9	13	9	11
$Ph_3Si(L_1)$	11	14	10	12
L_2H	9	12	7	11
$Me_3Si(L_2)$	10	13	8	13
$Ph_3Si(L_2)$	12	15	10	14
Streptomycin	11	14	10	13

Antifungal activity *(in vivo)*

Plant diseases play an important role in determining the amount and cost of food. Diseases caused by fungi are a major threat to profitable production. Plant pathology must alleviate the food problem by devising new central measures and improve the older ones. The chemicals found most effective against fungal and bacterial strains which were tested in *in vitro* were also tested in the field for controlling Guar blight in Guar (*Cyamopsis tetragonoloba*), caused by *Alternaria cyamopsidae*.

Field experiments were laid out in randomized block design plots with three replications. The crops (20 plants) were raised in each plot. Compounds with a standard fungicide, Bavistin, [2-(methoxycarbamyl) benzimidazole] were tried. Forty-five days after sowing, the plants were inoculated artificially by spraying the conidial suspension. The suspension was prepared by crushing the infected leaves in water. The first spray of the respective fungicide was given when lesions were first seen, and repeated after ten days. Disease intensity was analysed for statistical significance and the percentage disease control of the test compounds was worked out.

$$\mathrm{PDI} = \frac{\text{Sum of score of infected plants} \times 100}{\text{Total number of plants observed} \times \text{maximum rating of score (10)}}$$

The effectiveness of the chemicals were calculated using the following formula:

$$\%\ \text{disease control} = \frac{\text{PDI in treated plants} - \text{PDI in untreated plants}}{\text{PDI in untreated plants}} \times 100$$

Table 6. Efficacy of the compounds against Guar blight was evaluated using the percentage disease incidence technique

Compound	PDI in treated plants	Disease control (%)
L_1H	12	57.1
$Me_3Si(L_1)$	10	64.2
$Ph_3Si(L_1)$	6	78.5
L_2H	11	60.7
$Me_3Si(L_2)$	8	71.4
$Ph_3Si(L_2)$	5	82.1
Bavistin	5	82.1

The results of these findings are given in Table 6.

Mode of action

Metal-based fungicides inhibit a wide range of enzymes involved in various metabolic pathways, ultimately causing cell death. Early work on the mode of action of fungicides showed that these compounds inhibit cell division. It was later[21] shown that the specific site of action is β-tubuline, a polymeric protein found in microtubules that is an essential component of the cytoskeleton. Phenyl and amine groups in the complexes affect nucleic acid synthesis and mitochondrial electron transport also.

Most of the fungi contain *chitin* (a homopolymer of β-1,4-linked *N*-acetyl-glucosamine) as a major component of their cell walls. All the plants have been shown to produce chitinases and another compound, β-1,3-glucanase associated with the defence system of the plants against chitin-containing pathogens.[22,23] Therefore we expect at least the following regulatory processes to be operative:

1. *Chelation theory*[24,25]: this theory accounts for the increased activity of the metal complexes. Chelation reduces the polarity of the metal atom, mainly because of partial sharing of its positive charge with the donor groups and possible π electron delocalization within the whole chelate ring. The chelation increases the lipophilic nature of the central atom, which subsequently favours its permeation through the lipid layer of the cell membrane.
2. *Penetration of cell wall*[26]: chitinases and a compound β-1,3-glucanase (defence system of the plants) hydrolyse fungal cell walls and inhibit the rapid growth of fungal pathogens.

The results of fungicidal and bactericidal screening of the silicon complexes against some pathogenic fungi and bacteria are recorded in Tables 5 and 6. The results show that the activity is enhanced on undergoing chelation. It is well known that the concentration plays a vital role in increasing the degree of inhibition. Hence as the concentration increases, the activity also increases.

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