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Synthesis, characterization, antimicrobial, pesticidal and DNA cleavage activity of germanium(IV) derivatives of 3-(2-methyl-2,3-dihydro-benzthiazo-2-yl)-chromen-2-one and N′-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester ligands

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Abstract

The new ligands 3-(2-methyl-2,3-dihydro-benzthiazo-2-yl)-chromen-2one (AcBzH) and N′-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester (AcBDTZH) were prepared by the reaction of 3-acetyl-*2H*-chromen-2-one with 2-aminothiophenol and S-benzyl dithiocarbazate, respectively. The germanium(IV) complexes have been prepared by reacting Ph_3GeCl and Me_3GeCl in 1:1 molar ratio with these monofunctional bidentate AcBzH and AcBDTZH ligands by using microwave as well as conventional heating methods for comparison purposes. All the synthesized compounds were characterized by elemental analyses, melting point, IR, ^{1}H-NMR ^{13}C-NMR, mass and X-ray powder diffraction techniques. These studies showed that the ligands coordinated to the germanium atom in a monobasic bidentate manner and trigonal bipyramidal environment around the germanium atom have been established for the complexes. To evaluate the effect of metal ion upon chelation, both ligands and their complexes have been screened for their antimicrobial activity against the various pathogenic bacterial and fungal strains. The metal complexes have shown antimicrobial activity as compared to the free ligands. The pesticidal activity and DNA cleavage activity of both ligands and their metal complexes have been tested and discussed.

Keywords: antimicrobial; germanium(IV) complexes; pesticidal and DNA cleavage activity; S-benzyl dithiocarbazate.

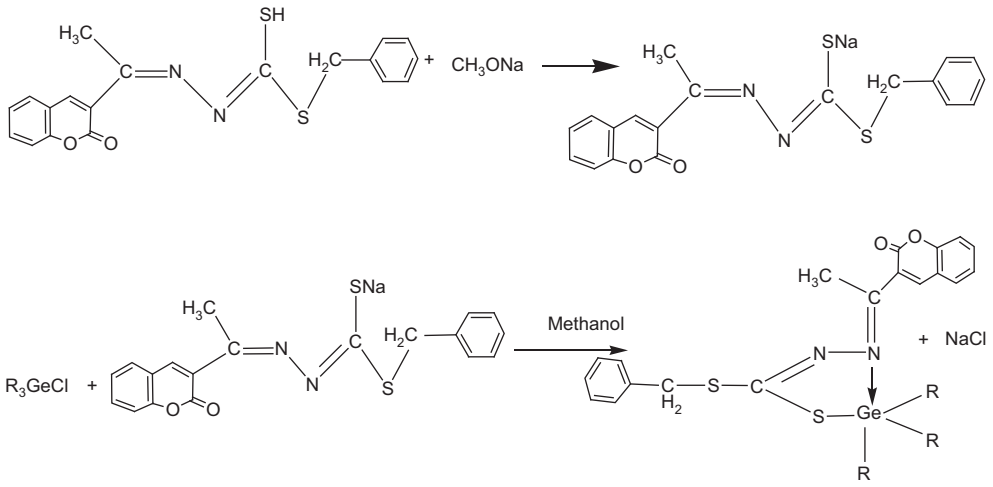
Introduction

Dithiocarbazate and its substituted compounds remain of interest to researchers because of their wide variation in structure and properties. The structural nature of the metal ion complexes of S-alkyl dithiocarbazates has been correlated to their biological activity (Mitra et al., 1997). Metal chelates of dithiocarbazic acid, its S-alkyl/aryl esters and their Schiff bases have been studied, mainly due to their potential anticancer (Tarafdar et al., 2000), antibacterial (Crouse et al., 2004), antifungal (Bi and Li, 1999), antiamoebic (Shailendra et al., 2002) and insecticidal (Tampouris et al., 2007) activities. Various methods which generally involved condensation reactions have been used to yield substituted dithiocarbazate compounds, which behave as bidentate (Zhou et al., 2007), tridentate (Wang et al., 2002) or multidentate (Ali et al., 2001) chelating agents with hetero donor atoms. Benzothiazolines have been the focus of much attention with regard to their vast potentiality as versatile coordinating agents, variety of biological activities and numerous applications in different fields of chemistry. The group N-C-S of benzothiazolines is of considerable chemotherapeutic interest and is responsible for pharmacological activity. Benzothiazolines generate strong interest stemming from their great physiological and biological activities (Sharma et al., 2006). Advances in organogermanium chemistry particularly, as regards organogermanium derivatives with extended pentacoordinaion spheres, most of which are biologically active compounds (Seifullina et al., 2004), have prompted our systematic studies in this area. The organic germanium has been clinically used in many parts of the world to treat a wide spectrum of illnesses and has been the subject of extensive research in many disciplines: pathology, biochemistry, pharmacology, immunology, oncology and neurochemistry. Organic germanium has been used in a broad spectrum of regimes – on its own, with diet and stress counseling – and as a drug in clinical trials of cancer therapy, in conjunction with chemotherapy, radiation therapy and surgery (Swami et al., 2008).

Our attempt to synthesize benzothioazoline and carbodithioic acid ligands and new substituted germanium(IV) complexes, along with the relationship between the structure and antimicrobial activities, is presented in this paper.

Results and discussion

The triphenylgermanium chloride and trimethylgermanium chloride react with sodium salt of ligands AcBzH and AcBDZH in unimolar ratio and led to the isolation of Ph_3Ge(AcBz), Me_3Ge(AcBz), Ph_3Ge(AcBDZ) and Me_3Ge(AcBDZ) solids. These reactions can be represented by the following general equation:



where R=Ph or Me.

These are soluble in DMF and DMSO. The reactions were carried out in perfectly dry methanolic medium and proceeded smoothly with the precipitation of NaCl. All the resulting complexes have been obtained as monomers and non-electrolytes as revealed by their molecular weight determinations and conductance measurements, respectively.

Electronic spectra

The electronic spectrum of 3-(2-methyl-2,3-dihydro-benzthiazo-2-yl)-chromen-2one consists of two bands at 266 nm and 320 nm attributable to ϕ-ϕ* and π-π* transitions which remain unchanged in the complexes. An additional band is also observed around 386 nm due to n-π* electronic transitions of the azomethine, which indicates isomerization of the ligand on complexation. The bands in the electronic spectrum of the ligand N′-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester and its complexes appear at 266 and 325 nm, assigned to π-π* electronic transitions within the benzene ring. Another band observed at 365 nm in the spectrum of the ligand is due to the n-π* transitions of the azomethine (>C=N) group. However, in the spectra of the complexes, this band appears in the lower wavelength region. This is due to the polarization within the >C=N chromophore resulting after chelation.

IR spectra

The IR spectral data of the synthesized ligands and their compounds are shown in Table 1. In the IR spectrum of the ligand (AcBzH), absence of the ν(SH) mode at 2610–2540 cm^{-1} and the presence of ν(NH) mode at 3310 cm^{-1} indicates the presence of the benzothiazoline ring structure in the ligand. In the spectra of metal complexes the disappearance of ν(NH) band shows its deprotonation and chelation of nitrogen of the ligand to the central metal atom. The IR spectrum of the ligand (AcBDZH) displays two sharp bands around 3350–3300 cm^{-1} and 3450–3350 cm^{-1} assignable to ν_{sym} and ν_{asym} vibrations of the NH_2 group, respectively. These bands remain unchanged in the germanium(IV) complexes. Furthermore, strong bands at 3250 cm^{-1} due to ν(NH) vibrations are observed. These ν(N-H) absorption bands are absent in the complexes. A sharp and strong band at 1622 cm^{-1} is due to the azomethine group of the ligand. In the IR spectra of the complexes this showed a lower shift of the order 15–25 cm^{-1}. One strong band located at 1050 cm^{-1} in the ligand was attributed to ν(C=S) moiety, which disappears in the case of complexes. These data on comparison with the spectrum of the ligand suggested that the azomethine nitrogen and thiolic sulfur atom of the ligand are involved in coordination with the metal ion. A doublet at ~2960 and ~2900 cm^{-1} is assigned to symmetric and asymmetric vibrations of S-CH_2-C_6H_5 grouping. The far IR spectra of these metal complexes exhibited new bands, which are not present in the spectrum of the ligand. These bands are located at 680–660 cm^{-1} and 410–415 cm^{-1} and are due to the Ge←N and Ge-S stretching vibrations, respectively, and this further lends support to the proposed coordination.

^{1}H NMR spectra

The ^{1}H NMR spectra further support the bonding pattern as discussed above. The ^{1}H NMR spectral data of the ligands and their corresponding organogermanium(IV) complexes were recorded in DMSO-d_6 with TMS as an internal standard. The -NH proton signal at δ 4.60 ppm in the spectrum of benzothiazoline disappears in the corresponding germanium complexes indicating deprotonation of this functional group. The free ligand shows a complex multiplet at δ 6.20–8.00 ppm for the aromatic protons and this remains more or less at the same position in the spectrum of the complexes. The ^{1}H NMR spectrum of the ligand (AcBDZH) exhibits -CH_2-proton signals at δ 4.15–4.16 ppm and aromatic proton signals at δ 6.38–7.50 ppm, and these remain at the same position in the spectra of the metal complexes. The proton of the -NH group of the ligand (AcBDZH) gives a signal at δ 10.05–10.10 ppm, which is absent in the spectra of metal complexes indicating the chelation of the ligand

Table 1 IR (cm^{-1}) and ^1H NMR (δ, ppm) spectral data of the ligands and their corresponding complexes.

Compound	IR spectral data (cm^{-1})					^1H NMR spectral data (δ, ppm)			
	(>C=N)	(Ge←N)	ν (-NH)	ν (C=S)	ν (Ge-S)	-NH	Aromatic	-S-CH_2	-CH_3
(AcBzH)	–	–	3310	–	–	4.60	8.00–6.20	–	1.08
Ph_3Ge(AcBz)	1600	680	–	–	412	–	8.15–6.80	–	1.10
Me_3Ge(AcBz)	1605	665	–	–	410	–	8.10–6.80	–	1.10
(AcBDTZH)	1622	–	3250	1050	–	8.30	7.50–6.38	4.16	1.09
Ph_3Ge(AcBDTZ)	1609	675	–	945	415	–	8.10–6.85	4.15	1.11
Me_3Ge(AcBDTZ)	1605	660	–	956	413	–	7.30–6.40	4.16	1.12

moiety to germanium with the sulfur atom. The signals at δ 10.52–10.65 ppm observed in the ligands, AcBzH and AcBDZH, were assigned to Ar-NH protons. The proton signals of the methyl groups appear at δ 1.08–1.12 ppm in the organogermanium(IV) complexes. The ^1H NMR values for all the compounds are given in Table 1.

^{13}C NMR spectra

^{13}C NMR spectral data have been recorded for the two ligands and their complexes and these spectra also support the authenticity of the proposed structures. The considerable shifts in the positions of carbons of the germanium complexes attached to N and S, respectively, clearly indicate that the nitrogen and sulfur of the ligands group participate in the complexation reaction. The signals due to the carbon atoms attached to the thionic and azomethine groups in ligands appear at 180.10–178.24 ppm and 162.90–160.75 ppm, respectively. However, in the spectra of the corresponding germanium(IV) complexes, these appear at ~163 ppm (thionic group) and at ~164 ppm (azomethine group), respectively. The considerable shifts in carbons attached to S and N indicate the involvement of sulfur and nitrogen atoms in coordination.

Mass spectra

The EI mass spectrum of the [Ph_3Ge(AcBDZ)] complex was studied as a representative case. The molecular ion peak for the complex [Ph_3Ge(AcBDZ)] was observed at *m/z* 671.01 and this is in good agreement with its molecular weight, which suggests the monomeric nature of the complex.

X-ray structure determination

The possible lattice dynamics of the finely powdered product, [Ph_3Ge(AcBDZ)], has been deduced on the basis of X-ray powder diffraction studies. The observed interplanar spacing values ('d' in Å) have been measured from the diffractogram of the compound and the Miller indices h, k and l have been assigned to each d-value and 2-θ angles are reported. The results show that the compound belongs to 'monoclinic' crystal system with unit cell parameters as a=9.2356, b=9.1593, c=6.2398, maximum deviation of 2-θ=0.044 and α=90, β=90, γ=120 at the wavelength of 1.540598 (Table 2 and Figure 1).

Bioassay

Antimicrobial assay The antimicrobial screening data show that both the ligands and their complexes individually exhibited varying degrees of inhibitory effects on the growth of the tested bacterial and fungal species. The antibacterial (Figure 2) and antifungal (Figure 3) results evidently show that the activity of the ligands became more pronounced when coordinated to the metal. The increased activity of the metal chelates can be explained on the basis of the chelation theory (Geeta et al., 2010), according to which the polarities of the ligand and the central metal atom are reduced through charge equilibration over the whole chelate ring. This increases the lipophilic character of the metal chelate and favors its permeation through the lipoid layer of the bacterial membranes. The variation in the effectiveness of different compounds against different organisms depends either on the permeability of the cells of the microbes or the difference in ribosomes of microbial cells. It has also been proposed that concentration plays a vital role in increasing the degree of inhibition: as the concentration increases, the activity increases.

Pesticidal activity Both the ligands and their germanium complexes were also evaluated for pesticidal activity and they

Table 2 X-Ray diffraction data of the complex Ph_3Ge(AcBDZ).

h	k	l	2-θ (exp.)	2-θ (calc.)	2-θ (diff.)	d (exp.)	d (calc.)	Intensity (exp.)
-1	3	3	53.638	53.594	0.044	1.70733	1.70862	3.02
-1	3	2	41.781	41.764	0.018	2.16021	2.16108	5.84
1	2	1	33.027	33.058	-0.031	2.71002	2.70752	5.15
-1	2	3	48.023	48.002	0.021	1.89300	1.89377	7.39
1	0	2	30.728	30.736	-0.008	2.90729	2.90660	27.26
-4	3	2	50.554	50.507	0.047	1.80400	1.80557	4.80
0	2	2	36.618	36.617	0.001	2.45206	2.45213	8.37
0	4	2	54.822	54.809	0.014	1.67320	1.67359	2.60
-3	2	0	44.698	44.673	0.024	2.02580	2.02685	6.37
-3	2	0	44.707	44673	0.034	2.02538	2.02685	7.81
2	2	2	49.188	49.185	0.002	1.85087	1.85095	17.25
-5	2	1	51.867	51.876	-0.009	1.76137	1.76109	4.75
-3	3	3	56.084	56.129	-0.045	1.63852	1.63732	6.92
-5	0	1	59.649	59.618	0.031	1.54882	1.54954	1.61
-3	6	1	62.617	62.596	0.022	1.48234	1.48280	10.63
0	5	2	66.025	66.011	0.014	1.41386	1.41412	7.65

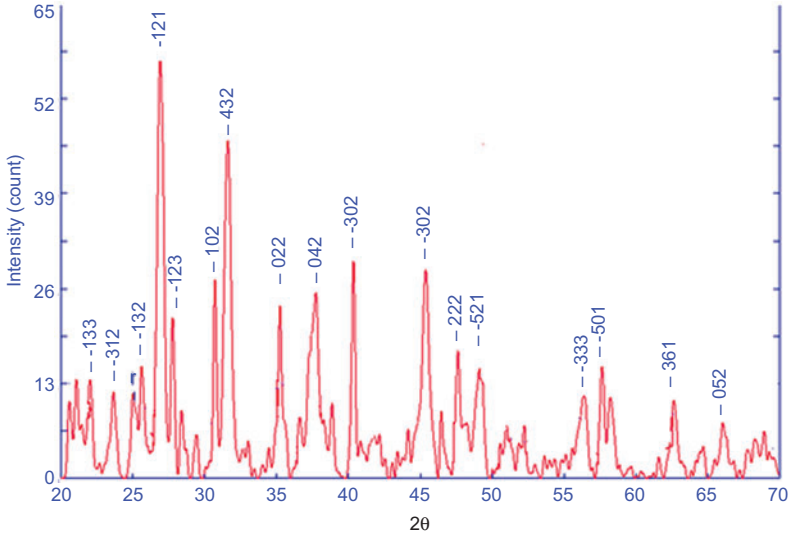


Figure 1 X-Ray diffraction screening of the complex [Ph_3Ge(AcBDZ)].

have a potent inhibitory effect on growth and development of *Corcyra cephalonica* larva. The LC_{50} values in mg/l are shown in Table 3. The data indicate that all germanium(IV) complexes exhibit greater pesticidal activity than the respective ligands but compound Ph_3Ge(AcBDZ) was highly effective as a pesticide with a LC_{50} value of 210 mg/l against *C. cephalonica*. A possible explanation is that the compound inhibits the molting hormone of pest larva, i.e., ecdysis disruption.

Electrophoresis analysis result The ligand AcBDZH and its germanium(IV) complexes were studied for their DNA cleavage activity by the agarose gel electrophoresis method and is presented in Figure 4. The gel after electrophoresis clearly revealed that the ligand AcBDZH and its complexes acted on DNA as there was a molecular weight difference between the control and the treated DNA samples. The difference was observed in the bands (Figure 4, lanes A–C) compared to the control DNA of *Escherichia coli*. This shows that the control DNA alone does not show any apparent cleavage where its complexes are shown. The results indicated the important role of metal in these isolated DNA cleavage reactions. As the compound was observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome.

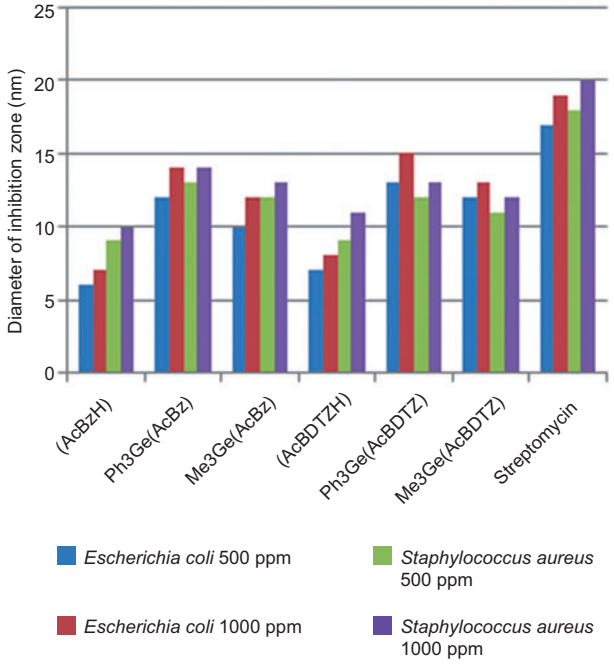


Figure 2 Antibacterial screening of the ligands and their complexes.

Experimental

The reagents Ph_3GeCl, Me_3GeCl and o-aminothiophenol as well as 3-acetylcoumarin were purchased from Alfa Aesar (MA, USA) and used as such. Solvents of analytical grade were distilled from appropriate drying agents immediately prior to use. Molecular weights were determined by the Rast Camphor method (Vogel, 2004). Germanium was determined gravimetrically as GeO_2. Nitrogen was estimated by the Kjeldahl's method and sulfur was estimated by the Messenger's method (Makode and Aswar, 2004). Infrared spectra of the ligands and their complexes were recorded with the aid of a Nicolet Megna FTIR-550 spectrophotometer (Shimadzu, USA) on KBr pellets. ^1H and ^{13}C NMR spectra were recorded on a JEOL-AL-300 FT NMR spectrometer (Japan) in DMSO-d_6 using TMS as the internal standard. A mass spectrum of the complex was carried out at IIT Chennai. X-Ray powder diffraction was recorded with the aid of a PANalytical system with CuK_α as a radiation source of wavelength 1.542 Å with 2-θ 10–70°.

Preparation of the ligands

Two different methods, microwave-assisted synthesis and a conventional method, were employed for the synthesis of the ligands.

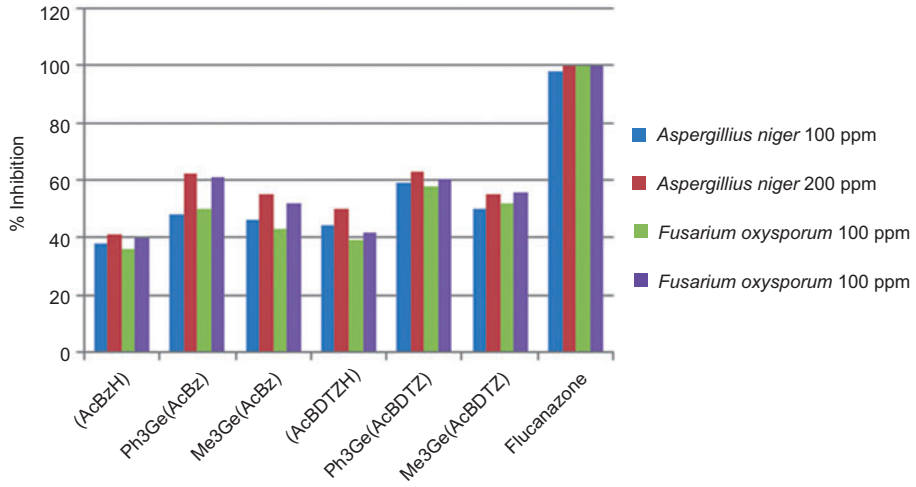


Figure 3 Antifungal screening of the ligands and their complexes.

Preparation of 3-(2-methyl-2,3-dihydro-benzothiazo-2-yl)-chromen-2-one (AcBzH) 3-(2-Methyl-2,3-dihydro-benzothiazo-2-yl)-chromen-2-one (AcBzH) was prepared by the condensation of 3-acetylcoumarin (0.01 mol) with 2-mercaptoaniline in 1:1 molar ratio in ethanol. The reaction mixture was stirred for 3–4 h and the resulting product was filtered off, recrystallized from ethanol and dried in vacuum. The analytical results yielded good consistence with the proposed formula as follows (Scheme 1):

Preparation of N′-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester (AcBDZH) N′-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester (AcBDZH) was prepared by the condensation of 3-acetylcoumarin (0.01 mol) with, S-benzyldithiocarbazate (0.01 mol) in 1:1 molar ratio. The reaction mixture was irradiated in the microwave oven by taking 2–3 ml solvent. The reactions were completed in a short period (5–7 min). The resulting precipitate was then recrystallized with alcohol and dried under vacuum. These were characterized and analyzed before use. Elemental analyses (N and S) were conducted using the methods mentioned above and their results were found to be in good agreement with the calculated values.

The above ligand was also synthesized by a thermal method, where instead of a few drops of alcohol the starting materials of the ligand was dissolved in ~100 ml of alcohol and the contents were refluxed for nearly 4–5 h. The solution was then concentrated under reduced pressure, which on cooling gave reddish brown crystalline precipitates. These were recrystallized twice in alcohol. A comparison between the thermal method and the microwave method is given in Table 4. Synthesis of AcBDZH is shown in Scheme 2.

Table 3 Pesticidal data of ligands and their metal complexes.

Compound	Correct motility (%)	χ^2-test	LC_{50} (mg/l)
(AcBzH)	50	0.156	520
Ph_3Ge(AcBz)	66.66	0.285	230
Me_3Ge(AcBz)	61.11	0.452	285
(AcBDTZH)	55.55	0.236	460
Ph_3Ge(AcBDTZ)	88.88	0.540	210
Me_3Ge(AcBDTZ)	72.22	0.456	296
Control	–	1.142	–

Preparation of the metal complexes

For the synthesis of the complexes, Ph_3GeCl and Me_3GeCl and sodium salt of the ligands (prepared by adding the corresponding weight of sodium metal to the ligands) in 5 ml of dry methanol in 1:1 molar ratio were irradiated inside a microwave oven for approximately 5–8 min. The products were recovered from the microwave oven and dissolved in few milliliters of dry methanol. The white precipitate of sodium chloride formed during the course of the reaction was removed by filtration, and the filtrate was dried under reduced pressure. The resulting product was repeatedly washed with petroleum ether and then finally dried at 40–60° C/0.5 mm Hg for 3–4 h. The purity was further checked by thin layer chromatography using silica gel-G.

Thermal method

These organogermanium(IV) complexes were also synthesized by the thermal method. The reaction mixtures were heated under reflux for 13–17 h and filtered to remove NaCl and the solvent was removed

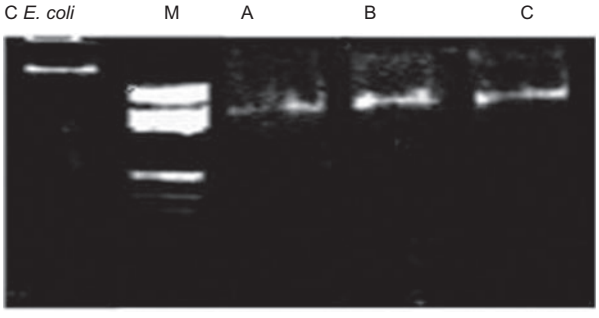
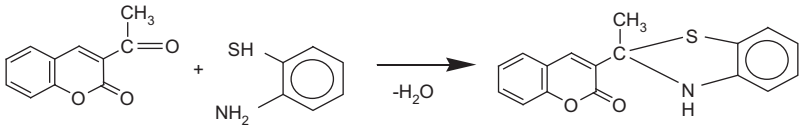


Figure 4 DNA cleavage gel diagram of synthesized compounds. M, standard molecular weight marker; (lane C) *E. coli*, control DNA of *E. coli*; (lanes A–C), *E. coli* DNA treated with the ligand AcBDZH and its Ge(IV) complexes, respectively.



Scheme 1 Synthesis of 3-(2-methyl-2,3-dihydro-benzothiazo-2-yl)-chromen-2-one (AcBzH).

Table 4 Comparison between microwave and thermal methods.

Compound	Yield (%)		Solvent (ml)		Time	
	Thermal	Microwave	Thermal	Microwave	Thermal (h)	Microwave (min)
(AcBzH)	75	83	100	5	4	–
Ph_3Ge(AcBz)	72	80	45	3	16	7
Me_3Ge(AcBz)	74	82	40	3	17	6
(AcBDTZH)	77	86	100	3	4	6
Ph_3Ge(AcBDTZ)	69	82	40	3	15	7
Me_3Ge(AcBDTZ)	70	85	50	2	13	6

by the same procedure mentioned above, which was adopted to obtain the complexes. The physicochemical properties and analytical data of these complexes are listed in Table 5.

Microbiological studies

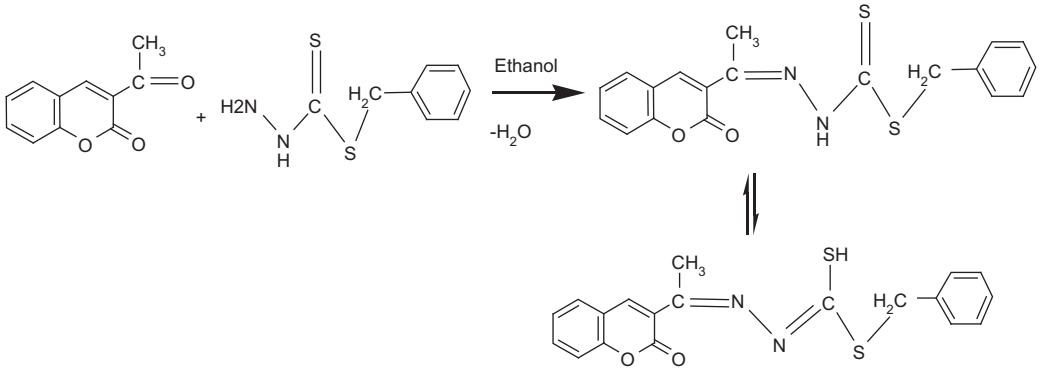
***In vitro* antifungal activity** The antifungal activity of the standard fungicide (Flucanazone), ligands and complexes were tested for their effect on the growth of microbial cultures and studied for their interaction with *Aspergillus niger* and *Fusarium oxysporum* using Czapek's agar medium with the composition: glucose 20 g, starch 20 g, agar-agar 20 g and distilled water 1000 ml. To this medium was added requisite amount of the compounds after being dissolved in methanol to obtain certain concentrations (50, 100 and 200 ppm). The medium was then poured into Petri plates and the spores of fungi were placed on the medium with the aid of an inoculation needle. These Petri plates were wrapped in polythene bags containing a few drops of alcohol and were placed in an incubator at 30±2°C. The controls were also run and three replicates were used in each case. The linear growth of the fungus was recorded by measuring the diameter of the fungal colony after 96 h and the percentage inhibition was calculated by the equation:

$$\% \text{ of inhibition} = \frac{C\text{-}T}{C} \times 100$$

where *C* and *T* are the diameters of the fungal colony in the control and the test plates, respectively (Chaudhary and Singh, 2004).

***In vitro* antibacterial activity** Antibacterial activity was tested against *E. coli* and *Staphylococcus aureus* using the paper disc plate method (Gaur et al., 2007). Each of the compounds was dissolved in DMSO and solutions of the concentrations (500 and 1000 ppm) were prepared separately. Paper discs of Whatman filter paper (No. 42) of uniform diameter (2 cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solutions were placed aseptically in the Petri dishes containing nutrient agar media (agar 20 g+beef extract 3 g+peptone 5 g) seeded with *E. coli* and *S. aureus* bacteria separately. The Petri dishes were incubated at 37°C and the inhibition zones were recorded after 24 h of incubation. The antibacterial activity of common standard antibiotic *Streptomycin* was also recorded using the same procedure as above at the same concentrations and solvent.

Pesticidal activity Larvae of *Corcyra cephalonica* were obtained from stock culture maintained at the storage section of the Division



Scheme 2 Synthesis of N′-[1-2-oxo-2H-chrome-3yl-ethylidene]-hydrazinecarbodithionic acid benzyl ester (AcBDZH).

Table 5 Analytical data and physical properties of ligands and their metal complexes.

Compound	Color	Melting point (°C)	Found (calcd.) (%)			Mol. wt. found (calcd.)
			N	S	M	
(AcBzH)	Yellow	77	4.74	10.86	–	293.12 (295.36)
$Ph_3Ge(AcBz)$	Dark brown	105	2.34	5.36	12.14	596.56 (598.3)
$Me_3Ge(AcBz)$	Light brown	130	3.40	7.78	17.63	410.86 (412.09)
(AcBDTZH)	Red	155	7.60	17.40	–	366.85 (368.47)
$Ph_3Ge(AcBDTZ)$	Maroon	125	4.17	9.55	10.82	670.25 (671.42)
$Me_3Ge(AcBDTZ)$	Orange	148	5.77	13.22	14.97	481.96 (485.21)

of Entomology, Agricultural Research Institute (Durgapura, Jaipur, India). Insects were reared on grains of wheat at 27±1°C and 70% relative humidity. Glass jars containing 500 g of wheat grains were labeled to indicate the date of introduction of larvae and new emergence. At alternate days, larvae were shifted to fresh jars so that successive rearing jars could be maintained and insects of known age could be obtained regularly. Insecticidal activity of the synthesized compounds was tested by dipping and spray methods. The synthetic compounds were weighed and dissolved in methanol to prepare 1000 mg/l stock solution. Further concentrations, viz., 900, 800, 700, 600, 500, 400, 300, 200 and 100 mg/l were prepared by serial dilution. Then, 1 ml of each concentration of various compounds was directly poured in each Petri plate (90 mm) with the aid of a micropipette. Petri plates with test solution were rotated vigorously to prepare uniformly and were allowed to dry for 3–5 min. Each concentrations as well as control in methanol were replicated thrice. In total, 20 adults (2–5 days old) were released in each Petri plate and were kept at 27±1°C and 70% relative humidity. Mortality was observed after 96 h. Adults were considered dead if they failed to respond to stimulus by touch. Control mortality was corrected by using Abbott's formula (Shaki et al., 2009) and LC_{50} was obtained by a graphical method. The χ^2-test was calculated by statistical analysis.

$$\text{Corrected \% mortality} = \frac{\text{\% mortality observed - \% mortality in control}}{\text{100 - \% mortality in control}} \times 100$$

DNA cleavage activity

Preparation of culture media Nutrient broth (peptone, 10; yeast extract, 5; NaCl, 10 in g/l) was used for culturing of *E. coli*. The 50-ml medium was prepared and autoclaved for 15 min at 121°C under 15 lb pressures. The autoclaved media was inoculated with the seed culture and *E. coli* was incubated for 24 h.

Isolation of DNA The fresh bacterial culture (1.5 ml) was centrifuged to obtain the pellet, which was then dissolved in 0.5 ml of lysis buffer (100 mM Tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 ml of saturated phenol was added and incubated at 55°C for 10 min. Then, it was centrifuged at 10 000 rpm for 10 min and equal volume of chloroform:isoamyl alcohol (24:1) and 1/20 volume of 3 M sodium acetate (pH 4.8) was added to this supernatant and centrifuged at 10 000 rpm for 10 min. To this supernatant three volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation. The pellet was dried and dissolved in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) and stored in cold conditions.

Agarose gel electrophoresis (Kapoor et al., 2011) Cleavage products were analyzed by the agarose gel electrophoresis method. Test samples (1 mg/ml) were prepared in DMF. The samples (25 µg) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 h at 37°C and then 20 µl of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/l) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. The gel was removed and stained with 10.0 g/ml ethidium bromide for 10–15 min, and the bands were observed under a UV transilluminator and photographed to determine the extent of DNA cleavage, and the results were compared with standard DNA marker.

Conclusions

Comparison between conventional and microwave synthesis revealed that microwave irradiation is an efficient and environmentally-benign method to accomplish various inorganic syntheses to afford products in higher yields in shorter reaction periods. On the basis of analytical data it has been observed that the ligands coordinated to the metal atoms in a monobasic bidentate manner and thus possess octahedral geometry. Antimicrobial and pesticidal activity of the complexes and the ligands showed that the former are more active than the parent ligands. Furthermore, DNA cleavage studies revealed that metal complexes cleave DNA more efficiently in comparison to the ligands. The germanium complexes possess better biological activities than the ligand because of the chelating effects between the ligand and metal ions. Results obtained from this work would be useful in the development of potential applications in the biological, pharmaceutical, and physiological fields in the future.

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