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Pesticidal and antifertility activities of triorganogermanium(IV) complexes synthesized using a green chemical approach

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Microwave chemistry is a green chemical method that improves reaction conditions and product yields while reducing solvent amounts and reaction times. This paper deals with the synthesis, spectral and biological studies of germanium(IV) complexes with chelating hydrazones derived from 1-(pyridine-2-yl)ethanone (F_1) and 1-(furan-2-yl)ethanone (F_2) with isonicotinohydrazide (INH). The complexes have been synthesized under a microwave–green chemical approach and investigated using a combination of microanalytical analysis, melting point, IR spectra, ^{1}H NMR spectra and ^{13}C NMR spectra. Trimethylgermanium (IV)chloride and triphenylgermanium(IV)chloride interact with the hydrazones in a 1:1 molar ratio (metal:ligand), resulting in the formation of coloured products. On the basis of conductance and spectral evidence, a pentacoordinated structure for germanium(IV) complexes has been assigned for these products. The ligand is coordinated to the germanium(IV) via the azomethine nitrogen atom and the enolic oxygen atom. The free ligands and their metal complexes have been tested in male rats in order to assess their antifertility properties. Ligands and their metal complexes have also been tested *in vitro* against a number of pathogenic microorganisms in order to assess their antimicrobial and pesticidal properties. Both the ligands and their complexes were found to possess appreciable antifertility activity and other activities, which have been discussed in brief.

Supporting information may be found in the online version of this article.

Keywords: 1-(pyridine-2-yl) ethanone; 1-(furan-2-yl)ethanone; isonicotinohydrazide; antimicrobial and pesticidal properties; antifertility activity

Introduction

Microwave dielectric effects are used increasingly in organometallic synthesis.[1] When we compare this unconventional energy source technology with classical heating we find that the conventional heating process usually starts at the wall of the reactor and, as a result, the core takes a much longer time to achieve the target temperature. Rapid and homogeneous heating by an unconventional method (microwave method) has many benefits, such as reaction rate acceleration,[2] milder reaction conditions and higher chemical yield;[3] also, it is a cost-effective technique and less equipment is required. The shorter reaction time and expanded reaction range offered by microwave-assisted synthesis are suited to increased demand in industry.

Hydrazones are a versatile class of ligands that have extensive applications in various fields, possessing pronounced biological and pharmaceutical activities such as antitumour,[4,5] antimicrobial,[6] antituberculosis[7] and antimalarial.[8] Hydrazones play an important role in improving the antitumour selectivity and toxicity profile of antitumour agents by forming drug carrier systems employing suitable carrier proteins.[9] They also have anti-inflammatory and analgesic activity equal or close to that of aspirin.[10] The remarkable biological activity of acid hydrazides R–CO–NH–NH_2 as well as their corresponding aroylhydrazones R–CO–NH–N=CH–R and the dependence of their mode of chelation with the metal ions present in the living system have been of great importance.[11,12] Enzymatic acetylation of the antitubercular isoniazid by *N*-acetyltransferase represents a major metabolic pathway for isoniazid in humans. Acetylation greatly reduces the therapeutic activity of the drug, resulting in underdosing, decreased bioavailability and acquired isoniazid resistance. Chemical modification of the hydrazine unit of isoniazid with a functional group that blocks acetylation while maintaining strong antimycobacterial action has the potential to improve clinical outcome and to reduce the emergence in patients of acquired isoniazid resistance.

Bioinorganic compounds and biomimetics containing a C=N bond are widely represented.[13] Schiff bases have been intensively investigated for their strong coordination capability and diverse biological activities.[14–16] Isoniazid Schiff bases have been found to show better anticancer, antitubercular and antimicrobial activity than isoniazid.[17,18]

The present article was directed to the preparation of germanium(IV) complexes with ligands *N′*-(1-(pyridine-2-yl)ethylidene) isonicotinohydrazone (F_1INH) and N-(1-(furan-2-yl)ethylidine)isonicotinohydrazone (F_2INH) through **a** green chemical approach and their biological aspects have been studied.

Experimental

Chemicals

Germanium(IV) compounds, Me_3GeCl and Ph_3GeCl, as well as 2-acetylpyridine and 2-acetylfuran, were purchased from Alfa Aesar

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and isoniazid was purchased from SD Fine Chemicals Ltd (Mumbai, India). Solvents of AR grade were distilled from appropriate drying agents prior to use.

Physical Measurements and Analytical Methods

Molecular weights of the synthesized ligands and their metal complexes were determined by the Rast camphor method.[19] Nitrogen was estimated by the Kjeldahl method.[20] Germanium (IV) was estimated gravimetrically as GeO_2. IR spectra of the ligands and their complexes were recorded with the help of a Nicolet Magna FTIR-550 spectrophotometer using KBr pellets as well as in Nujol mulls. ^1H and ^{13}C NMR spectra were recorded on a JEOL-AL-300 FT NMR spectrometer in DMSO-d_6 using tetramethylsilane (TMS) as the internal standard. Electronic spectra of the complexes were recorded in DMF on a UV-160A Shimadzu spectrophotometer in the range 200–600 nm.

Preparation of the Hydrazones F_1INH and F_2INH

Two different routes were employed for synthesis of the ligands for comparison purposes: eco-friendly microwave-assisted synthesis and the conventional thermal method. The progress of the reaction was monitored by thin-layer chromatography on silica gel-G.

Microwave-assisted synthesis of ligands F_1INH and F_2INH

F_1INH and F_2INH were prepared by the condensation of F_1 and F_2 with isonicotinohydrazide (INH) in the presence of a few drops of ethanol (~3 ml) and irradiated by microwave irradiation for ~4–5 min. The resulting coloured precipitates were recrystallized with alcohol and dried under vacuum.

Conventional thermal method for synthesis of ligands F_1INH and F_2INH. A similar procedure was followed for synthesis by the thermal method where instead of a few drops of alcohol ~100 ml was used and the contents were refluxed for nearly 5–6 h. The solution was then concentrated under reduced pressure, which on cooling gave a coloured crystalline precipitate. These were recrystallized twice in alcohol (Figs 1 and 2). Their physical properties and analytical data are given in Table 1.

Synthesis of the Complexes

Microwave method

For the preparation of [R_3Ge(FIN)] complexes (where, R = Me or Ph), methanol solutions of Me_3GeCl and Ph_3GeCl were mixed with the corresponding sodium salts of the ligands (F_1INH and F_2INH) in equimolar ratio using 2–3 ml methanol as a solvent in the microwave oven for 5–7 min. The products recovered from the microwave oven dissolved in a few millilitres of dry methanol.

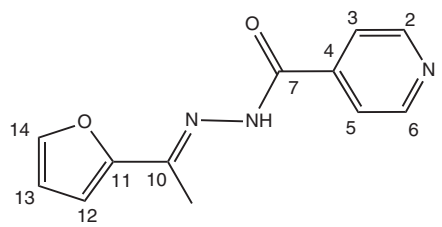

Figure 1. Structure of ligand (F_1INH).

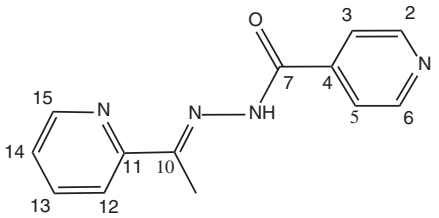

Figure 2. Structure of ligand (F_2INH).

The white precipitate of NaCl formed during the course of the reaction was recovered by filtration and the filtrate was dried under reduced pressure. The resulting products were repeatedly washed with *n*-hexane and petroleum ether and finally dried at 60°C/0.5 mmHg for 3–4 h (Figs 3 and 4).

Thermal method

In the thermal method, instead of 5–7 min reactions were completed in 7–8 h and the reaction mixture was refluxed over a distillation assembly fitted with quick-fit interchangeable joints and the white precipitation of sodium chloride obtained was removed by filtration. The mother liquor was concentrated by removing excess solvent. Compounds were dried under reduced pressure for 3–4 h. These were purified by the same process as described in the above method. Purity was further checked by TLC on silica gel-G using anhydrous methanol and tetrahydrofuran as a solvent. Physical properties and analytical data are given in Table 1.

Antifertility Activity

Male albino rats (*Rattus norvegicus*) weighing 170–200 g were used in the study. They were housed in separated polypropylene cases under controlled conditions of temperature (22 ± 3°C). humidity (50 ± 5%) and light (12 light:12 dark cycle), and fed with pellet diet (Ashirwad Industries Ltd, Punjab, India) and water *ad libitum*. The animals were divided into six groups containing six animals of each. Group A served as vehicle-treated control. In groups B and C ligands F_1INH and F_2INH and in groups D, E, F and G compounds [$Me_3Ge(F_1IN)$], [$Me_3Ge(F_2IN)$], [$Ph_3Ge(F_1IN)$] and [$Ph_3Ge(F_2IN)$] suspended in olive oil were given orally for a period of 60 days (30 mg kg^{-1} body weight per day). All the animals were screened for fertility and autopsied for biochemical examination. The sperm density in cauda epididymis and density of testicular and cauda epididymis, protein, sialic acid, glycogen, cholesterol, acid and alkaline phosphatase and serum testosterone were measured by standard laboratory techniques. The data were analysed statistically using Student's *t*-test and significance of differences was set at $P < 0.01$ and $P < 0.001$.

Pesticidal Activity

Pesticides can save farmers money by preventing crop losses to insects and other pests. With this in view the experiment was conducted to determine the pesticidal activities of the present compounds.

The pesticidal activity of the two ligands F_1INH and F_2INH and their germanium(IV) complexes were conducted on *Helicoverpa armigera* and *Spodoptera litura* pests. A bioactive experiment was conducted on their larvae. On the basis of the larval mortality rate the activity of the ligands and their metal complexes was

Table 1. Analytical data and physical properties of the ligands and their complexes

Compound	Formula and colour	m.p. (°C)	Analysis: found / (calcd) (%)				Mol. wt found / (calcd)	Time		Yield (%)	
			C	H	N	Ge		Th. (h)	MW (min)	Th.	MW
F_1INH	$C_{12}H_{11}N_3O_2$ Light brown	172–174	61.88 (62.87)	4.31 (4.84)	18.02 (18.33)	—	228 (229.24)	5	4	65	80
F_2INH	$C_{13}H_{12}N_4O$ Off white	165–168	65.12 (64.99)	5.56 (5.03)	23.56 (23.32)	—	238 (240.26)	6	5	68	86
$Me_3Ge(F_1IN)$	$C_{15}H_{19}N_3O_2Ge$ Light yellow	120–124	52.54 (52.08)	5.68 (5.54)	12.42 (12.15)	21.02 (20.99)	347 (345.94)	7	5	65	80
$Ph_3Ge(F_1IN)$	$C_{30}H_{25}N_3O_2Ge$ Light grey	180–184	67.98 (67.71)	4.98 (4.73)	8.05 (7.90)	13.22 (13.64)	534 (532.16)	8	6	68	82
$Me_3Ge(F_2IN)$	$C_{16}H_{20}N_4OGe$ Dark brown	122–125	53.25 (53.84)	5.71 (5.65)	15.18 (15.70)	20.10 (20.34)	362 (356.97)	7	5	62	76
$Ph_3Ge(F_2IN)$	$C_{31}H_{26}N_4OGe$ Dark grey	138–142	63.84 (68.55)	5.04 (4.83)	10.01 (10.32)	13.15 (13.37)	548 (543.18)	8	7	64	76

Th., thermal method; MW, microwave method.

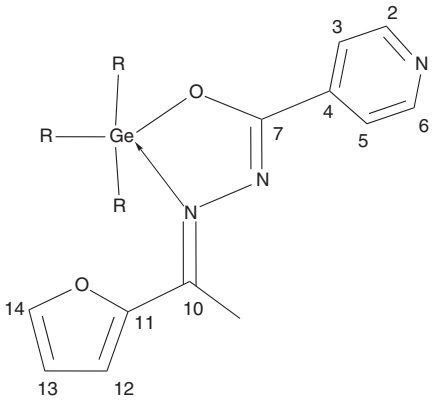

Figure 3. Structure of Ge complex ([$R_3Ge(F_1IN)$]).

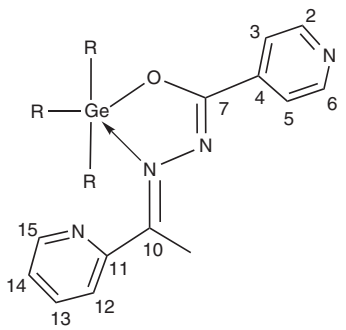

Figure 4. Structure of Ge complex ([$R_3Ge(F_2IN)$]), where R = CH_3 or C_6H_5.

determined. *Helicoverpa armigera* (cotton bollworm) is one of the key pests causing severe yield losses infesting several crops such as cereals, pulses, cotton, vegetables and fruit crops, as well as wild hosts.[21] This includes direct losses through shedding and reduced quality (Figs 5 and 6).

Spodoptera litura is a noctuid moth and is considered an agricultural pest. It is also known as the cluster caterpillar, cotton leafworm, tobacco cutworm and tropical armyworm. It is an international pest and feeds on many herbaceous plants, including lettuce, cabbage, beetroot, peanuts, geranium, cotton, banana, fuchsias, strawberry and tomato.

Materials and methods

Larvae of *Helicoverpa armigera* and *Spodoptera litura* were used for the experiments. The experiments were conducted during the months of August to October, i.e. during the reproductive period of the cotton crop. The equipment used in experiments, such as rearing trays, moth chambers, forceps and brushes, were regularly decontaminated in 5% sodium hypochlorite and rectified spirit.

Preparation of solutions

Solutions of different concentrations (0.02%, 0.05% and 0.1%) of compounds were prepared by adding the required quantity of the compounds to methanol. Distilled water was used as control.

Rearing technique of Helicoverpa armigera and Spodoptera litura

Field-collected larvae of both pests were reared separately in round galvanized trays (35 cm × 11.5 cm) containing fresh, tender leaves of cotton to raise the initial culture. Every day a sufficient amount of fresh leaves of cotton were provided to larvae

Figure 5. Cotton boll damage by *Helicoverpa armigera* larva.

Figure 6. Cottonleaf damage by *Spodoptera litura* larva.

of both pests, separately. The petiole of each leaf was wrapped with cotton wool and dipped in water to maintain leaf turgidity. Freshly formed pupae were transferred to glass jars (15 cm × 10 cm), having moist sponge and blotting paper at the bottom and covered with muslin, which was fastened with rubber bands. Two pairs were released in each earthen pot. The pots were placed in plastic trays containing water to maintain the desired level of humidity (>70%) inside the pot. The temperature of laboratory was maintained up to 27 ± 2°C. Eggs laid on the muslin were removed daily and kept in glass jars for hatching. The neonate larvae were reared in glass jars for hatching. After changing all the stages of life cycle of the pests, the neonate third instar larvae were reared and used for experimental purposes.

Bio-efficacy of the compounds

Ligands and their metal complexes were used at three concentrations, i.e. 0.02%, 0.05% and 0.1%, against both the pests in statistically designed experiments (Tables 2 and 3). 2 ml of each concentration of the suspension of the respective treatment was uniformly applied with the help of a Potter tower on leaves of cotton placed in Petri dishes (15 cm diameter). Petri dishes with treated leaves were kept under a ceiling fan to evaporate water from the leaves. After complete evaporation of water they were transferred to the glass jars (15 cm height × 10 cm diameter). Then third instar larvae of both pests were released into each glass jar separately and were allowed to feed on treated food materials for 24 h. Three replications of each treatment were maintained. Fresh untreated leaves of cotton were provided daily after 24 h treatment. Mortality counts were recorded at 72 h after treatment. The mortality percentage was calculated and the data thus obtained were statistically analysed.

Table 2. Pesticidal effect of ligands and their Ge(IV) complexes against *Helicoverpa armigera* at 72 h after treatment

Treatment	Concentration (%)		
	0.02	0.05	0.1
	Mortality (%)[a]	Mortality (%)	Mortality (%)
Control	4(2.40)	4 (2.40)	4 (2.40)
F_1INH	38.87[b] (56.73)[c]	53.90 (80.65)	59.34 (90.80)
F_2INH	34.98 (49.97)	45.87 (68.52)	52.47 (78.64)
$Me_3Ge(F_1IN)$	60.18 (88.50)	72.86 (98.85)	75.40 (99.54)
$Ph_3Ge(F_1IN)$	52.25 (78.93)	62.20 (90.52)	71.12 (97.60)
$Me_3Ge(F_2IN)$	69.05 (96.39)	73.22 (98.61)	75.30 (99.33)
$Ph_3Ge(F_2IN)$	61.88 (90.86)	69.05 (96.39)	72.46 (97.99)
SEM ± CD at 5%	1.20 3.52	1.42 4.17	1.12 3.16

[a]Mean of three replications.
[b]Figures outside parentheses are arcsine-transformed values.
[c]Figures in parentheses are retransformed values.

Table 3. Pesticidal effect of ligands and their Ge(IV) complexes against *Spodoptera litura* at 72 h after treatment

Treatment	Concentration (%)		
	0.02	0.05	0.10
	Mortality (%)[a]	Mortality (%)	Mortality (%)
Control	4(2.40)	4(2.40)	4(2.40)
F_1INH	26.29[b] (22.54)[c]	29.95(31.23)	52.76(69.66)
F_2INH	22.40(16.07)	20.28(20.42)	29.15(30.06)
$Me_3Ge(F_1IN)$	66.05(84.19)	69.05(86.39)	75.35(89.33)
$Ph_3Ge(F_1IN)$	58.75(72.00)	61.88(80.66)	72.45(87.99)
$Me_3Ge(F_2IN)$	55.35(72.60)	62.33(80.78)	73.12(88.61)
$Ph_3Ge(F_2IN)$	45.73(55.11)	54.95(70.69)	68.24(78.64)
SEM ± CD at 5%	1.82 4.78	1.83 4.29	2.17 5.32

[a]Mean of three replications.
[b]Figures outside parentheses are arcsine-transformed values.
[c]Figures in parentheses are retransformed values.

Statistical analysis

The mortality of *Helicoverpa armigera* and *Spodoptera litura* larvae in relation to the initial population was worked out in terms of percentage mortality in larvae. The percentage values were transformed to arcsine scale and statistically analysed following a completely randomized design (CRD).

Antifungal Activity

The complexes were screened against *Fusarium oxysporum* and *Macrophomia phaseolina* fungi. The antifungal activity of the synthesized compounds was evaluated by the 'radial growth method'.

Radial growth method

Then principle involved in this method is to 'poison' the nutrient medium with a fungi toxicant (compound) and then allowing a

test fungus to grow on such medium. The fungi were grown in potato dextrose agar (PDA) medium (glucose 20 g, starch 20 g, agar agar 20 g, 1000 ml water) and the complexes, after being dissolved in the requisite concentration in methanol, were mixed with this medium. Several series of concentrations were prepared. The medium was then poured into Petri plates and a small disc (0.7 cm) of the fungal culture was cut into a sterile cork borer and transferred aseptically to the centre of a Petri plate containing the medium with a certain amount of the compound. Suitable checks were kept of where the culture plates were grown under the same conditions on PDA without the compound. These Petri plates were wrapped in polythene bags containing a few drops of alcohol and were placed in an incubator at $25 \pm 2°C$. Two replicates were used in each case. The colony diameter, after 96 h, was compared with the check. The linear growth of the fungus was obtained by measuring the diameter of the colonies in the Petri plates, and the percentage inhibition was calculated using the following formula:

$$\%\,\text{inhibition} = 100(C - T)/C$$

where, C is diameter of the fungal colony in the check/control plate and T is the diameter of the fungal colony in the test plate.

Antibacterial Activity

Various methods are available for evaluation of the antibacterial activity of different types of compounds. In the present work, activities of synthesized compounds were evaluated by the 'paper disc plate method' using the inhibition zone technique. The complexes were screened against *Escherichia coli* and *Staphylococcus aureus* bacteria.

Inhibition zone technique

Activity against bacteria was evaluated by the paper disc plate method. For this purpose, pure cultures of the organism were dissolved in peptone–water and then uniformly seeded on nutrient agar plates having the following composition: peptone 5 g, beef extract 5 g, NaCl 5 g, agar agar 20 g and distilled water 1000 ml. The reference drug used was streptomycin. All compounds were dissolved in methanol in different concentrations. Paper discs of Whatman filter paper with a diameter of 5 mm were soaked in these solutions. These discs were placed on the medium previously seeded with organisms in Petri dishes at suitable distances. These discs were stored in an incubator at $35 \pm 2°C$. The inhibition zone around each disc was measured (in mm) after 24–30 h.

Spectroscopic Characterization

IR Spectra

A comparison of the IR spectra of the complexes and ligands F_1INH and F_2INH shows that ν(C=O) of the ligands at 1660–1670 cm^{-1} and ν(NH) at 3230–3235 cm^{-1} were absent in the spectra of the respective complexes . This is presumably due to amide–imidol tautomerism (Fig. 7) and their subsequent coordination through the imidol oxygen.[22] This is supported by the appearance of a new band in the spectra of the complexes at 1525–1545 cm^{-1} attributed to the azine group, >C=N–N=C<, which is absent in the spectra of the hydrazones. Bands at 1600–1610 cm^{-1} due to ν(C ;N) of the hydrazones shift to lower wavenumbers, indicating coordination of the azomethine nitrogen. Non-ligand bands at 880 ± 10 cm^{-1} and 670 ± 10 cm^{-1} have been assigned to

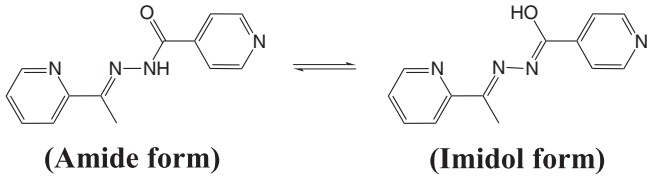

Figure 7. Equilibrium forms of the ligand: left, amide form; right, imidol form.

ν(Ge–O) and ν(Ge–N), respectively. One of the hydrazone bands at 1480–1500 cm^{-1} due to the pyridine ring nitrogen remain unchanged on complexation, indicating non-involvement of the ring nitrogen in complex formation.[23] The overall IR spectral evidence suggests that both the ligands are bidentate, coordinating through amide-oxygen and azomethine-nitrogen, forming a five-membered chelate ring.

^{1}H NMR Spectra

^{1}H NMR spectral data of the ligands and their corresponding germanium complexes were recorded in DMSO-d_6 with TMS as an internal standard . Disappearance of the –NH proton signal at δ 10.04–11.02 ppm in the spectra of the complexes is considered additional evidence of enolization of the ligands during complexation. In the ^{1}H NMR spectra of ligand F_1INH, the pyridine ring protons (i.e. H_2, H_6) appear as a doublet (δ 8.27ppm, $J = 5.8$Hz) and H_3, H_5 proton signal appears as a doublet at δ 8.10 ppm, $J = 5.8$Hz and furan ring proton (12-H to 14-H) signal as a multiplet at δ 7.68–6.54 (m) ppm. In the ^{1}H NMR spectra of the ligand F_2INH pyridine ring protons H_2, H_6 appear as a doublet at δ 8.23 ppm, $J = 5.6$ Hz and H_3, H_5 proton signal as a doublet at δ 7.12 ppm, $J = 5.6$ Hz and the proton of the other pyridine ring (12-H to 15-H) appeared as a multiplet at δ 8.80–7.87 (4H, m) ppm. In the spectra of germanium complexes the signal due to the pyridine ring and furan ring were shifted downfield. A sharp singlet at δ 2.32–2.49 ppm due to methyl protons attached to azomethine of the ligands underwent a downfield shift due to coordination of the azomethine nitrogen (Table 4).

^{13}C NMR Spectra

^{13}C NMR spectral data recorded for both the ligands and their complexes support the proposed structures (Figs 1–4). Signals due to carbons attached to the azomethine nitrogen and enolic oxygen appear at δ 158.54–162.72 and δ 167.60–174.98 ppm, respectively. However, in the spectra of corresponding metal complexes, a considerable downfield shift is observed in these signals. The shifts in the positions of carbons adjacent to the coordinating atoms clearly indicate bonding of the azomethine nitrogen and enolic oxygen to the metal atoms. Thus the ^{1}H and ^{13}C NMR spectra also confirm the monobasic bidentate nature of the ligands already established by the IR spectral studies.

Biological Activities

Body and Organ Weights

No significant change was observed in the body weights of male rats after treatment with ligands F_1INH and F_2INH and their complexes. However, the weights of testes, epididymis, seminal

Table 4. ^{1}H NMR spectral data of ligands and their complexes

Compound	^{1}H NMR spectral data (δ, ppm)						
	–;NH	–;CH_3	Aromatic protons				Ph–Ge/Me–Ge
			Pyridine ring protons			Furan ring protons 12-H to 14-H	
			2-H and 6-H	3-H and 5-H	12-H to 15-H		
F_1INH	10.04 (s)	2.49 (s)	8.27 (2H, d J = 5.8 Hz)	8.10 (2H, d J = 5.8 Hz)	—	7.68–6.54 (m)	—
F_2INH	11.02 (s)	2.32 (s)	8.23 (2H, d J = 5.6 Hz)	7.12 (2H, d J = 5.6 Hz)	8.80–7.87(4H, m)	—	—
$Me_3Ge(F_1IN)$	—	2.35 (s)	8.25 (2H, d J = 5.8 Hz)	8.06 (2H, d J = 5.8 Hz)	—	7.32–6.25 (m)	1.32 (9H, s)
$Ph_3Ge(F_1IN)$	—	2.16 (s)	8.15 (2H, d J = 5.8 Hz)	7.89 (2H, d J = 5.8 Hz)	—	7.12–6.12 (m)	7.32–7.47 (15H, m)
$Me_3Ge(F_2IN)$	—	2.28 (s)	8.18 (2H, d J = 5.6 Hz)	6.97 (2H, d J = 5.6 Hz)	8.78–7.69 (4H, m)	—	1.48 (9H, s)
$Ph_3Ge(F_2IN)$	—	2.10 (s)	8.08 (2H, d J = 5.6 Hz)	6.87 (2H, d J = 5.6 Hz)	8.68–7.43(4H , m)	—	7.50–7.59 (15H, m)

vesicle and ventral prostate were reduced after administration of ligands F_1INH and F_2INH and their germanium(IV) complexes (Table 5).

Sperm motility, density, fertility test and serum testosterone

Results showed a significant decrease ($P \leq 0.01$–0.001) in sperm motility in cauda epididymis after treatment with ligands F_1INH and F_2INH and their germanium(IV) complexes. Sperm density in testes and epididymis were also reduced after various treatments. A sharp decline in fertility of ligands F_1INH and F_2INH and their germanium(IV) complexes was observed. The test was 32–95% negative. The serum level of testosterone was also reduced in all treated groups (Table 6).

Biochemical change

A marked reduction in testicular **s**ialic acid and glycogen was observed in the ligands F_1INH and F_2INH and their germanium (IV) complexes, whereas testicular protein, cholesterol, acid and alkaline phosphatase activity were increased significantly (Table 7).

Results and discussion

The present study revealed that administration of ligands F_1INH and F_2INH and their germanium(IV) complexes [$Me_3Ge(F_1IN)$], [$Me_3Ge(F_2IN)$], [$Ph_3Ge(F_1IN)$] and [$Ph_3Ge(F_2IN)$] to male rats resulted in decreases in the weight of testis and accessory sex organs. The reduction in the weight of testis may be due to reduced tubular size, decreased number of germ cells and elongated spermatids.[24] The observed reduction in weight of accessory sex organs may be due to several factors, such as reduced bioavailability of oestrogen and/or anti-androgenic activities of these compounds.[25] The decreased testosterone level in the ligands F_1INH and F_2INH and their germanium(IV) complexes in treated animals supports this view. The decreased mortality of sperm in cauda epididymis indicates lowered ability of sperm to interact with the oocyte plasma membrane.[26] Reduction in sperm density may be due to alteration in androgen gonadotropin.[27] The observed negative fertility in the present study may be attributed to lack of forward progression, reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis.[28] A fall in testicular glycogen indicated inhibition of glycogen synthesis, eventually decreasing the spermatogenic process.[29] Reduction in testicular sialic acid content may be due to absence of spermatozoa or reduced androgen production.[30] Increased concentration of cholesterol in testes suggests that impairment of spermatogenesis is due to decreased androgen concentration [31]. Further, increase in testicular acid and alkaline phosphatase activity may be the result of labialization of lysosomal system[32] and releasing the enzyme that degenerates the tissue resulting in reduction of various micromolecules, including reduction in sperm count.[33] From the

Table 5. Body weight, testes and accessory sex organ weight analysis after administration of ligands and their germanium(IV) complexes

Group	Treatment	Body weight		mg 100 g^{-1} body weight			
		Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
A	Control	180.0 ± 15.4	190.0 ± 7.8	1220.0 ± 41.5	490.0 ± 20.5	470.0 ± 18.0	360.0 ± 27.4
B	F_1INH	190.0 ± 10.3	205.0 ± 9.6	1010.0 ± 40.2^a	410.0 ± 10.4^a	310.0 ± 14.5^b	290.0 ± 14.4^b
C	F_2INH	188.0 ± 9.2	200.0 ± 8.7	1022.0 ± 30.8^a	424.0 ± 11.3^a	340.0 ± 12.5^b	295.0 ± 14.8^b
D	$Me_3Ge(F_1IN)$	179.0 ± 8.7	198.0 ± 9.3	805.0 ± 31.3^a	325.0 ± 10.7^b	225.8 ± 11.7^b	210.0 ± 10.5^b
E	$Me_3Ge(F_2IN)$	178.0 ± 7.3	194.0 ± 8.4	825.0 ± 25.5^a	334.0 ± 9.6^b	230.0 ± 10.8^b	225.0 ± 10.2^b
F	$Ph_3Ge(F_1IN)$	185.0 ± 7.9	208.0 ± 10.3	750.0 ± 25.2^b	290.0 ± 8.7^b	210.0 ± 14.3^b	195.0 ± 11.4^b
G	$Ph_3Ge(F_2IN)$	184.0 ± 8.6	202.0 ± 9.5	764.0 ± 22.8^b	292.0 ± 7.8^b	218.0 ± 16.2^b	205.0 ± 10.9^b

Mean values of six animals: $^aP \leq 0.01$; $^bP \leq 0.001$.
Groups B and C compared with group A.
Groups D and E compared with group B.
Groups F and G compared with group C.

Table 6. Sperm dynamics, fertility test and serum testosterone level after administration of ligands and their germanium(IV) complexes

Group	Treatment	Sperm motility (%) cauda epididymis	Sperm density (million ml^{-1})		Fertility (%)	Sperm testosterone (mg ml^{-1})
			Testes	Cauda epididymis		
A	Control	70.0 ± 2.15	4.35 ± 0.75	49.8 ± 0.32	100% (+ve)	2.5 ± 0.1
B	F_1INH	48.0 ± 4.51^b	2.95 ± 0.71^a	36.0 ± 0.20^b	32% (−ve)	1.9 ± 0.1^a
C	F_2INH	52.0 ± 5.22^b	2.98 ± 0.95^a	38.0 ± 0.30^b	40% (−ve)	1.8 ± 0.2^b
D	$Me_3Ge(F_1IN)$	38.0 ± 4.30^b	1.15 ± 0.31^b	28.7 ± 0.15^a	70% (−ve)	1.0 ± 0.1^b
E	$Me_3Ge(F_2IN)$	41.0 ± 5.80^b	1.34 ± 0.42^b	30.0 ± 0.05^a	80% (−ve)	0.9 ± 0.8^b
F	$Ph_3Ge(F_1IN)$	31.0 ± 4.21^b	1.10 ± 0.22^b	26.0 ± 0.11^b	90% (−ve)	0.9 ± 0.2^b
G	$Ph_3Ge(F_2IN)$	34.0 ± 4.36^b	1.28 ± 0.51^b	29.3 ± 0.21^b	95% (−ve)	0.8 ± 0.4^b

Mean values of six animals: $^aP \leq 0.01$; $^bP \leq 0.001$.
Groups B and C compared with group A.
Groups D and E compared with group B.
Groups F and G compared with group C.

Table 7. Biochemical changes in testes of rats after administration of ligands F_1INH and F_2INH and their germanium(IV) complexes

Group	Treatment	Testicular glycogen (mg g^{-1})	Testicular sialic acid (mg g^{-1})	Testicular protein (mg g^{-1})	Testicular acid phosphatase (KA units)	Testicular alkaline phosphatase (KA units)	Testicular cholesterol (mg g^{-1})
A	Control	2.74 ± 0.08	4.90 ± 0.06	250.0 ± 10.5	4.71 ± 0.18	65.0 ± 2.15	6.91 ± 0.37
B	F_1INH	1.10 ± 0.07^b	3.60 ± 0.05^a	315.0 ± 8.7^a	6.71 ± 0.91^b	80.7 ± 3.17^b	9.10 ± 0.31^b
C	F_2INH	1.25 ± 0.11^b	3.75 ± 0.02^a	335.0 ± 6.4^a	7.62 ± 0.82^b	85.3 ± 3.54^b	10.11 ± 0.25^b
D	$Me_3Ge(F_1IN)$	0.30 ± 0.06^b	2.10 ± 0.03^b	350.0 ± 7.4^a	8.31 ± 0.62^b	93.0 ± 4.53^b	11.50 ± 0.23^b
E	$Me_3Ge(F_2IN)$	0.34 ± 0.02^b	2.12 ± 0.08^b	360.0 ± 8.5^a	8.98 ± 0.58^b	93.80 ± 6.02^b	12.17 ± 0.16^b
F	$Ph_3Ge(F_1IN)$	0.32 ± 0.07^b	2.15 ± 0.04^b	370.5 ± 9.4^a	9.15 ± 0.72^b	94.0 ± 5.41^b	12.80 ± 0.18^b
G	$Ph_3Ge(F_2IN)$	0.36 ± 0.10^b	2.21 ± 0.07^b	380.5 ± 2.5^a	9.85 ± 0.26^b	95.0 ± 4.45^b	13.22 ± 0.21^b

Mean values of six animals: $^aP \leq 0.01$; $^bP \leq 0.001$.
Groups B and C compared with group A.
Groups D and E compared with group B.
Groups F and G compared with group C.

above-mentioned findings, it has been concluded that ligands F_1INH and F_2INH and their germanium(IV) complexes are potent fertility inhibitors, and addition of Me_3 or Ph_3 moiety to the ligands enhances their activity in reducing fertility.

Pesticidal Results

After 72 h treatment the results of pesticidal activity revealed that there was a significant difference in percent mortality at different concentrations; it was noted that as concentration of ligands F_1INH and F_2INH and their germanium(IV) complexes increases the larval mortality rate also increases. The chemical-treated leaves were slowly or much less fed to the larvae of *Helicoverpa armigera* and *Spodoptera litura*, which led to starvation of the developing larvae.

Table 8. Antifungal screening data of the ligands and their organogermanium complexes

Compound	*Fusarium oxysporum* (concentration, ppm)			*Macrophomina phaseolina* (concentration, ppm)		
	50	100	200	50	100	200
F_1INH	52	64	72	48	60	75
F_2INH	54	66	74	50	62	78
$Me_3Ge(F_1IN)$	64	72	80	58	68	88
$Ph_3Ge(F_1IN)$	67	75	82	62	72	90
$Me_3Ge(F_2IN)$	72	78	84	68	78	92
$Ph_3Ge(F_2IN)$	76	80	86	72	82	94
Bavistin	92	100	100	84	94	100

Antifungal Screening

The results of antifungal activities of germanium complexes and their parent ligands against some pathogenic fungi are listed in Table 8. The inferences drawn from these observations clearly indicate that the ligands are active against various pathogenic fungi. The results also show that the metal complexes have more inhibitory effect than do the parental ligands, due to chelation[34] of the metal atom with the ligand moieties.

The enhanced activity of germanium complexes was ascribed to the increased lipophilic nature of these complexes arising due to chelation.[35] Further, the results of bioactivity were compared with the conventional fungicide Bavistin.

Table 9. Antibacterial screening data of the ligands and their organogermanium complexes

Compound	*Staphylococcus aureus* (concentration, ppm)		*Escherichia coli* (concentration, ppm)	
	500	1000	500	1000
F_1INH	12	13	7	10
F_2INH	14	16	10	14
$Me_3Ge(F_1IN)$	15	17	12	16
$Ph_3Ge(F_1IN)$	17	18	16	18
$Me_3Ge(F_2IN)$	16	17	16	17
$Ph_3Ge(F_2IN)$	17	19	17	18
Streptomycin	18	20	18	20

In the case of fungicidal activity, it has been observed that all of the organogermanium(IV) complexes were able to inhibit and kill the pathogen at 50 ppm concentration, whereas at the 100 ppm concentration only the standard proved invariably fatal.

Antibacterial Screening

The antibacterial activity of the ligands and their metal complexes was screened against two bacteria and the results are recorded in Table 9. The results clearly indicate that in the case of bacterial activity the germanium complexes exhibited remarkable potential in inhibiting the growth of pathogens. Thus it can be postulated that further intensive studies of these complexes in this direction as well as in agriculture could lead to interesting results in the future. The conventional bactericide streptomycin showed the highest activity.

Conclusions

All the compounds showed appreciable antifertility activity. The results of this study indicate that the test compounds are capable of suppressing the process of spermatogenesis by inhibiting serum testosterone levels. In conclusion, our study suggested that the addition of the germanium(IV) moiety to the ligands F_1INH and F_2INH enhances their fertility regulatory efficiency.

The results of pesticidal activities revealed that there was a significant difference in percent mortality at different concentrations and it was noted that as concentration increases the larval mortality rate also increases.

The complexes showed better antifungal and antibacterial activities than the parental ligands. The compounds also inhibit the growth of bacteria, dependent on concentration. In the present case we have used *Fusarium oxysporum* and *Macrophomina phaseolina* for antifungal activity, and *E. coli* and *Staphylococcus aureus* for antibacterial activity. The results showed that the compounds are more active than the ligands but less active than the standard drugs. However, there is sufficient scope for future studies.

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