Spectroscopic Characterization and Biological Potential of Palladium(II) Complexes of Benzylidenehydrazinecarboxamide or -carbothioamide

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In this article a brief account of the synthesis, stereochemistry, and biochemical behavior of palladium(II) complexes of benzylidenehydrazinecarboxamide or -carbothioamide is presented. The bimolar addition and substitution products have been characterized by elemental analyses, conductance measurements, molecular-weight determinations, magnetic susceptibilities, and spectral studies viz., IR, ^{1}H NMR, ^{19}F NMR, and UV. The data support the binding of sulfur or oxygen and nitrogen to the palladium in $[Pd(RN^{\frown}XH)_2]Cl_2$ and $[Pd(RN^{\frown}X)_2]$ (X is O or S) types of complexes. The square-planar geometry has been proposed. The representative free ligands ($RN^{\frown}XH$) and their respective metal complexes were tested in vitro against a number of microorganisms to assess their antimicrobial properties and in vivo to test their sterilizing potential. The results are indeed positive.

The patent literature abounds with examples of the application of transition-metal complexes to improve the performance of biological processes. The major recent stimulus for investigating the palladium(II) complexes of benzylidenehydrazinecarboxamide (BHCO) or -carbothioamide (BHCS) arose from knowledge of metal complexes having N and O/S binding sites, which have prominent roles in biochemical processes along with their inherent biological properties.[1—4] It was observed that metal chelation apparently plays a definite role in the cause and cure of malignancy.[5] Various palladium(II) complexes were tested in animals bearing transplanted tumours, and were found to be quite active.[6,7] The cytostatic activity of palladium complexes was observed in the human epidermoid carcinoma of the nasopharynx.[8] Transition-metal complexes of azomethine ligands were screened for their antitumour activity in the P388 lymphocytic leukaemia test system in mice; it was interesting to note that most of the complexes that exhibited this property contained palladium.[9]

The used BHCO or BHCS are strong π-donors, a property which induces interesting reactivity patterns in palladium(II) complexes. Minor changes in the structures of BHCO or BHCS markedly affected the activity of the compounds. The fungicidal and bactericidal activities of the ligands, along with their metal complexes, have also been studied using a conventional fungicide, Bavistin, and a conventional bactericide, Streptomycin, as the standards for the respective activities. The sterilizing ability of a representative ligand, along with its addition and substitution products, has also been tested on male albino rats.

Experimental

Preparation of BHCO or BHCS. 2-(2-Fluorobenzylidene)hydrazinecarbothioamide ($MN^{\frown}SH$), white crystals, mp 190 °C; 2-[1-(2-fluorophenyl)ethylidene]hydrazinecarbothioamide ($EN^{\frown}SH$), white crystals, mp 122 °C; 2-(2-fluorobenzylidene) hydrazinecarboxamide ($MN^{\frown}OH$), off-white crystals, mp 218 °C; and 2-[1-(2-fluorophenyl)ethylidene]hydrazinecarboxamide ($EN^{\frown}OH$), white crystals, mp 194 °C were prepared by condensing 2-fluorobenzaldehyde and 1-(2-fluorophenyl)ethanone with thiosemicarbazide and semicarbazide, respectively, in an ethanol medium. The ligands were recrystallized from the same solvent. They exist in the following tautomeric forms (Chart 1):

Synthesis of $[Pd(RN^{\frown}XH)_2]Cl_2$ Complexes. A solution of $PdCl_2$ in ethanol was mixed with an ethanolic solution of the ligand in 1 : 2 molar ratios. A few drops of HCl were added, and the reaction mixture was stirred for 2 h at room temperature. The obtained precipitate was collected by filtration, washed with ethanol and dried in vacuo.

Synthesis of $[Pd(RN^{\frown}X)_2]$ Complexes. Reactions of $PdCl_2$ with BHCO or BHCS were carried out in alcohol in bimolar ratios (Metal : Ligand). Aqueous NH_3 was added to the reaction mixture dropwise until the solution was weakly alkaline; it was then refluxed for 1 h. The so-obtained solid derivative was filtered off, washed with ethanol and dried in vacuo. The physical properties and analyses of these complexes are recorded in Table 1.

Analytical Methods and Physical Measurements. The analytical methods and procedures for the physical measurements were the same as that reported elsewhere.[10] Conductivity measurements in dry DMF were made with a conductivity bridge (type-305 Systronics model). The molecular weights were determined by the Rast Camphor method. IR spectra were recorded on a Perkin–Elmer 577 grating spectrophotometer as Nujol mulls using KBr optics.

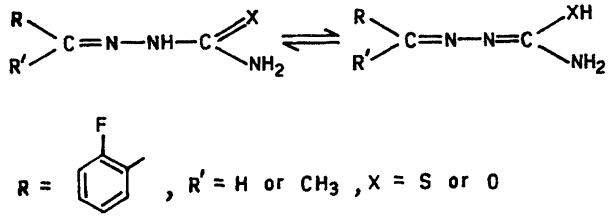

Chart 1.

Table 1. Analytical Data and Physical Properties of Pd(II) Complexes

Compound[a)]	Color	Mp	Analyses/%			Molecular
		°C	Pd Found (Calcd)	N Found (Calcd)	S Found (Calcd)	weight Found (Calcd)
$[Pd(MN⌒SH)_2]Cl_2$	Orange	170	18.50 (18.61)	14.21 (14.69)	11.01 (11.21)	544 (571)
$[Pd(MN⌒S)_2]$	Brown	235	21.55 (21.32)	16.67 (16.83)	12.62 (12.85)	458 (499)
$[Pd(EN⌒SH)_2]Cl_2$	Yellow	140d	17.91 (17.73)	14.21 (14.00)	10.82 (10.69)	621 (600)
$[Pd(EN⌒S)_2]$	Chocolate	210	20.04 (20.19)	15.36 (15.94)	11.82 (12.17)	506 (527)
$[Pd(MN⌒OH)_2]Cl_2$	Off white	240	19.52 (19.71)	15.21 (15.56)	—	530 (539)
$[Pd(MN⌒O)_2]$	Dark green	190	22.41 (22.79)	17.52 (17.99)	—	480 (466)
$[Pd(EN⌒OH)_2]Cl_2$	Yellow	200d	18.83 (18.74)	14.98 (14.79)	—	540 (567)
$[Pd(EN⌒O)_2]$	Gray	>300	21.03 (21.50)	16.44 (16.97)	—	510 (494)

a) MN⌒SH = $C_8H_8N_3SF$, EN⌒SH = $C_9H_{10}N_3SF$, MN⌒OH = $C_8H_8N_3OF$, FN⌒OH = $C_9H_{10}N_3OF$.

The electronic spectra of the powdered sample were recorded on a Varian-Cary/2390 spectrophotometer at R.S.I.C., I.I.T. (Madras). ^{1}H NMR and ^{19}F NMR spectra were recorded on a JEOL FX 90Q spectrometer in DMSO-d_6 using TMS as the internal standard and C_6F_6 as the external standard, respectively.

Fungicidal and Bactericidal Screening. The fungicidal activity was evaluated against Fusarium oxysporum, Alternaria alternata, and Sclerotium rolfsi by the agar-plate technique.[11] The antibacterial activity was tested by the paper-disc plate method.[12] The bacteria used during the present course were Escherichia coli, Staphylococcus aureus, and Xanthomonas compestris.

Sterilizing Activity. Thirty two male albino rats of an inbred colony were housed in an air-conditioned room at 24±2 °C with 14 h of light each day. Water and food was given *ad libitum*. They were divided into four groups containing eight animals each. The first group served as a vehicle (olive oil) treated control. In the second group ligand (EN⌒XH) (50 mg/kg body weight suspended in 0.2 ml olive oil) was given orally for 60 d. The animals of the third and fourth groups received the same dose of $[Pd(EN⌒X)_2]$ and $[Pd(EN⌒XH)_2]Cl_2$ products, respectively, for a similar period. The animals were screened for fertility tests and autopsized for the determination of detailed biochemical studies. The reproductive organs were excised, blotted free from blood, weighed and then frozen for biochemical estimations. The sperm motility and density of cauda epididymal spermatozoa, the total cholesterol, protein, sialic acid, and fructose were determined by standard laboratory techniques.

Results and Discussion

Palladium(II) chloride reacts with BHCS or BHCO having MN⌒S, EN⌒S or MN⌒O, EN⌒O donor set in 1 : 2 molar ratios in the presence of few drops of concentrated HCl. The refluxing medium used was ethanol.

$$PdCl_2 + 2RN⌒XH \xrightarrow{1:2} [Pd(RN⌒XH_2]Cl_2$$

However, the replacement of both chloride ions is possible in the presence of aqueous ammonia. The reaction can be depicted as follows:

$$PdCl_2 + 2RN⌒XH + 2NH_3 \xrightarrow{1:2} [Pd(RN⌒X)_2] + 2NH_4Cl$$
(R = M or E)

These reactions are quite facile and require only 1—2 h of refluxing for their completion. All of the derivatives are colored solids with high melting/decomposition temperature and are insoluble in common organic solvents. Their solubility is appreciable in DMSO and DMF. The molar conductance of 10^{-3} M solutions (1 M = 1 mol dm^{-3}) of $[Pd(RN⌒XH)_2]Cl_2$ in DMF lie in the range of 210—230 ohm^{-1} mol^{-1} cm^2, indicating that they behave as 1 : 2 electrolytes. However, the $[Pd(RN⌒X)_2]$ complexes are nonelectrolytes (molar conductance values of 10—15 ohm^{-1} mol^{-1} cm^2) in dry DMF at 28±1.0 °C. All of the complexes are monomers, as revealed by their molecular-weight determinations.

These derivatives are diamagnetic, as expected for square-planar d^8 complexes. Their magnetic susceptibilities lie in the range (0.3—0.8×10^{-6}) c.g.s. units.

Spectral Studies. The newly synthesized compounds were characterized by different spectral techniques. The electronic spectra of Pd(II) complexes were recorded so as to confirm their square-planar geometry. These d–d spin-allowed transitions are expected, corresponding to the transitions from the three lower lying d levels to the empty $d_{x^2-y^2}$ orbitals; the ground state is $^1A_{1g}$ and the excited states corresponding to the above transitions are $^1A_{2g}$, $^1B_{1g}$, and ^1E_g in order of increasing energy. By assuming a value of F_2=10F_4=600 cm^{-1} for the Slater–Condon interelectronic repulsion parameters (F_2 and F_4), and subsequently the equations suggested by Gray and Ballhausen,[11] it is possible to calculate the single-electron parameters: Δ_1, Δ_2, and Δ_3. The ν_2/ν_1 values (Table 2) lie in the 1.13 to 1.17 range,

Table 2. Electronic Spectral Data of Pd(II) Complexes

Complex	Spectra bands	Transitions	Δ_1	Δ_2	Δ_3	ν_2/ν_1
	cm^{-1}		cm^{-1}	cm^{-1}	cm^{-1}	
$[Pd(EN^{\frown}SH)_2]Cl_2$	19200	$^1A_{1g}\rightarrow{}^1A_{2g}$	21300	4400	2000	1.16
	22400	$^1A_{1g}\rightarrow{}^1B_{1g}$				
	24700	$^1A_{1g}\rightarrow{}^1E_{2g}$				
$[Pd(EN^{\frown}S)_2]$	19600	$^1A_{1g}\rightarrow{}^1A_{2g}$	21700	3800	1900	1.13
	22200	$^1A_{1g}\rightarrow{}^1B_{1g}$				
	24400	$^1A_{1g}\rightarrow{}^1E_{1g}$				
$[Pd(MN^{\frown}OH)_2]Cl_2$	19150	$^1A_{1g}\rightarrow{}^1A_{2g}$	21250	4450	2600	1.17
	22400	$^1A_{1g}\rightarrow{}^1B_{1g}$				
	25300	$^1A_{1g}\rightarrow{}^1E_{1g}$				
$[Pd(MN^{\frown}O)_2]$	19400	$^1A_{1g}\rightarrow{}^1A_{2g}$	21500	3900	3250	1.14
	22100	$^1A_{1g}\rightarrow{}^1B_{1g}$				
	25650	$^1A_{1g}\rightarrow{}^1E_{1g}$				

which are in close agreement with those reported earlier for square-planar complexes.[13,14]

In the IR spectra of free ligands, broad and strong bands at ca. 3280 cm^{-1} assigned to ν_{NH} vibrations, disappear in the corresponding $[Pd(N^{\frown}X)_2]$ type of complexes, indicating a possible deprotonation of the functional group upon complexation. The non-involvement of the NH_2 group in chelation has been confirmed by the appearance of its bands due to asymmetric and symmetric modes at ca. 3440 and 3320 cm^{-1}, respectively, in the same positions as in the spectra of the complexes.[10]

The use of absorption due to the >C=N stretching frequency in identifying the bonding site is somewhat-limited because of the complex nature of absorption in the 1550—1630 cm^{-1} region. However, a strong peak of the ligands at ca. 1610 cm^{-1} registers a substantial increase in the intensity ($\Delta\nu$=10—20 cm^{-1}) after complexation. This band may be assigned to complex vibration involving $\nu_{C=N}$, δ_{NH_2} and the aromatic ring.[15] Its shifting to the higher side is due to an increase in the bond order, showing the coordination of the azomethine nitrogen to the metal atom. The $\nu_{C=S}$ and $\nu_{C=O}$ bands in BHCO or BHCS appear at ca. 820 and ca. 1690 cm^{-1}, respectively. These bands suffer a negative shift upon chelation.[16] The appearance of a new sharp band, in $[Pd(RN^{\frown}X)_2]$ simultaneously in the region 700—600 cm^{-1} due to ν_{C-S} is evidence of the ligands coordinating via the thioenol structure.[15]

Non-ligand bands present in the far-IR spectra of complexes in the ca. 360, ca. 310, and ca. 410 cm^{-1} regions were assigned to ν(Pd←N), ν(Pd–S), and ν(Pd–O), respectively.[10]

We can thus say that the IR spectra of ligands and their metal chelates indicate that the BHCO or BHCS act as a neutral bidentate ligand as well as an uninegative bidentate ligand, the binding sites being sulfur or oxygen and azomethine nitrogen.

The ^1H NMR spectra of the $MN^{\frown}SH$, $EN^{\frown}SH$, $MN^{\frown}OH$, and $EN^{\frown}OH$ along with their metal complexes were also recorded in DMSO-d_6, in order to confirm the above-discussed bonding pattern. The free ligands exhibit –NH proton resonance signals at δ=10.24—11.82, which is shifted downfield in complexes of the $[Pd(RN^{\frown}XH)_2]Cl_2$ type, due to the involvement of carbonyl oxygen or thiolo sulfur in bonding with the metal atom, resulting in a shielding of the –NH proton, whilst the –NH proton signal disappears completely in $[Pd(RN^{\frown}X)_2]$-type complexes, thereby showing its deprotonation. The signals due to methyl protons are positioned downfield in the case of metal complexes, as compared with the corresponding signals of the ligands, thus confirming the chelation of BHCO or BHCS to the metal atom via azomethine nitrogen (Table 3).

The ^{19}F NMR spectra of ligands $EN^{\frown}SH$ and $EN^{\frown}OH$ give sharp singlets at δ=109.00 and 103.36, respectively. The metal complexes of these ligands also show a sharp singlet in the δ=109.061—110.798 range, which indicates that fluorine does not participate in the coordination.[17]

Suitable square-planar structures have been proposed for the complexes based on the preceding discussions (Figs. 1 and 2).

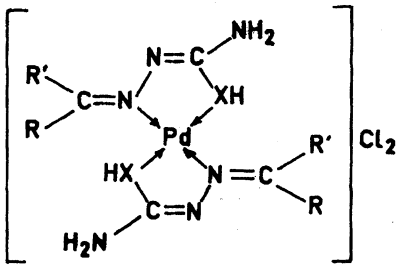

Fig. 1.

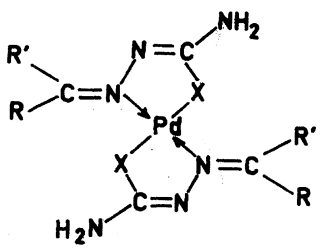

Fig. 2.

Table 3. ^{1}H NMR Spectral Data (δ/ppm) of the Ligands and Their Pd(II) Derivatives

Compound	–NH bs	–NH$_2$ bs	–CH$_3$ s	–CH=N s	Aromatic M
MN⌒SH	11.24	2.16	—	8.33	7.78—6.70
[Pd(MN⌒SH)$_2$]Cl$_2$	11.48	2.12	—	8.44	7.90—6.84
[Pd(MN⌒S)$_2$]	—	2.18	—	8.48	7.88—7.24
EN⌒SH	10.24	3.16	2.12	—	8.28—6.92
[Pd(EN⌒SH)$_2$]Cl$_2$	11.20	3.12	2.36	—	8.24—7.40
[Pd(EN⌒S)$_2$]	—	3.20	2.38	—	8.38—7.12
MN⌒OH	11.67	2.35	—	8.42	7.68—6.65
[Pd(MN⌒OH)$_2$]Cl$_2$	11.82	2.32	—	8.48	8.16—7.04
[Pd(MN⌒O)$_2$]	—	2.36	—	8.54	8.00—7.20

Antimicrobial Screening. The BHCO or BHCS and their respective metal complexes were tested against selected pathogenic fungi and bacteria to examine their growth inhibitory potential towards test organisms. The results are indicative of the fact that these compounds exhibit antimicrobial properties. It was important to note that the metal chelates show more inhibitory effects than do the parent BHCO or BHCS[18] (Tables 4 and 5). In some cases they inhibit the growth of fungi completely. The enhanced activities of the metal complexes compared to free ligands may be due to inherent properties of the metal ion of the precipitating or denaturing proteins. Since enzymes are proteins, it would be expected that a heavy metal would inactivate these catalysts. The mode of action of antimicrobials may involve various targets in microorganisms e.g. interference with cell-wall synthesis, damage to the cytoplasmic membrane, causing an alteration of the cell permeability or a disorganization of the lipoproteins, leading to cell death. Ribosomes are concerned with protein synthesis, the process of which is directed by m.RNA, which carries the code for such synthesis from

Table 4. Fungicidal Screening Data of Ligands and Their Pd(II) Complexes

Compound	Inhibition after 96 h Concn in ppm								
	F. oxysporum			*S. rolfsi*			*A. alternata*		
	50	100	200	50	100	200	50	10	200
MN⌒SH	74	80	84	68	77	82	88	98	100
[Pd(MN⌒SH)$_2$]Cl$_2$	76	82	88	74	81	85	92	100	100
[Pd(MN⌒S)$_2$]	77	83	87	73	80	86	93	100	100
EN⌒SH	83	88	92	71	78	83	89	100	100
[Pd(EN⌒SH)$_2$]Cl$_2$	85	90	94	76	84	87	92	100	100
[Pd(EN⌒S)$_2$]	86	92	95	78	85	87	94	100	100
EN⌒OH	74	79	86	69	76	82	85	92	100
[Pd(EN⌒OH)$_2$]Cl$_2$	77	82	89	72	81	84	90	96	100
[Pd(EN⌒O)$_2$]	78	81	88	74	80	85	89	97	100
Standard	86	100	100	98	100	100	100	100	100

Statistically analysed data using CR Design.

	p=0.05	SEM	p=0.05	SEM	p=0.05	SEM
Compound	1.67	0.59	1.84	0.65	1.05	0.2
Concentration	0.91	0.32	1.01	0.36	0.57	0.37
Interaction	2.98	1.02	3.20	1.13	1.81	0.64

Table 5. Bactericidal Screening Data of the Ligands and Their Pd(II) Complexes

Compound	Diameter of inhibition zone (mm) Concn in ppm					
	E. coli		*S. aureus*		*X. compestris*	
	500	1000	500	1000	500	1000
MN⌒SH	+	++	++	++	+	++
[Pd(MN⌒SH)$_2$]Cl$_2$	++	++	++	+++	++	++
[Pd(MN⌒S)$_2$]	++	+++	+++	+++	++	++
EN⌒OH	+	+	+	++	+	+
[Pd(EN⌒OH)$_2$]Cl$_2$	++	++	++	++	++	++
[Pd(EN⌒O)$_2$]	++	++	++	+++	++	++
Standard	+++	++++	+++++	+++++	++++	+++++

Table 6. Changes in the Body Weight and Organs Weight of Reproductive Organs after Treatment with Ligands and Their Pd(II) Complexes

Treatment[d)]	Body Weight/g		mg/100 g Body weight			
	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
Control*	190.0±12.0	220.0±9.50	1050.0±70.5	400.0±28.5	340.0±27.8	250.7±30.5
EN⌒SH**	200.0±17.0	225.0±17.50[c)]	810.0±50.0[b)]	310.0±30.0[b)]	290.0±20.0[a)]	190.5±30.0[b)]
$Pd(EN⌒S)_2]^+$	188.0±20.0	202.0±17.5[c)]	730.0±70.0[a)]	260.0±30.0[a)]	250.0±20.0[a)]	180.0±35.0[c)]
$[Pd(EN⌒SH)_2]Cl_2^{++}$	210.0±25.0	225.0±10.3[c)]	710.0±50.7[b)]	250.0±25.0[a)]	230.0±10.5[b)]	152.3±27.5[a)]

Level of significance: a) $P<0.05$, b) $P<0.001$, c) P=Non significant. d) Group A* compared with Group B**. Group B compared with Group C+ & D++.

Table 7. Altered Sperm Dynamics and Fertility Test after Treatment with Ligand and Their Pd(II) Complexes

Treatment[d)]	Sperm density (million/ml)		Sperm motility	Fertility test
	Testes	Epididymis	Cauda epididymis	%
Control	1.75±0.09	45.52±1.50	72.0±5.21	95 (+ve)
EN⌒SH	0.91±0.15[b)]	32.5±0.9[b)]	53.0±3.1[b)]	72 (−ve)
$[Pd(EN⌒S)_2$	0.82±0.21[a)]	24.0±0.3[b)]	40.0±2.5[b)]	85 (−ve)
$Pd(EN⌒SH)_2]Cl_2$	0.56±0.13[b)]	20.0±0.5[b)]	42.1±1.7[b)]	90 (−ve)

Level of significance: a) $P<0.05$, b) $P<0.001$, c) P=Non significant. d) Group A compared with Gr. B. Group C & D compared with Gr. B.

the DNA. Messenger RNA becomes attached to ribosomes where the transcribed code is translated into appropriate protein synthesis. Antimicrobials can bind to ribosomes, and may interfere with peptide chain formation in microorganisms or with the transcription mechanisms.[19] It has also been proposed that the ultimate action of a toxicant is to inactivate one or more proteins of the cell as a result of which normal cellular processes are impaired.[20] For a toxicant to be effective two requirements must be met. First, there must be one or more reactive atomic configuration(s) that can combine with and inactivate essential materials of the cell. Second, the total structure of the toxicant molecule must allow a concentration at the site of action, which would tend to stabilize the enzyme-inhibitor complex.[21] This also explains why the activity is associated with the chemical potential; A relatively high proportional concentration is necessary if weak bonds are to occupy receptor sites sufficient amount of time for perceptible inhibition to occur. It may also be postulated that these compounds might act as uncoupling agents of oxidative phosphorylation, since they possess the properties of uncoupling agents. These agents are mostly lipid soluble containing an acidic group and an aromatic ring. They allow electron transport to continue, but prevent the phosphorylation of ADP to ATP, i.e., they uncouple the energy-yielding from the energy-conserving reactions.[22]

The antifungal activity of these compounds may also be explained in the light of modern electronic theory, since resonating rings also exert effects on the fungitoxicity. Resonating structures, such as benzene rings and other conjugated systems, may serve as power houses to activate potentially reactive groupings. If the fungitoxicity is dependent on one or more chemical reactions, as it must undoubtedly be in most cases, then any property of the fungitoxic molecule which would increase the rate of chemical reactions must, perforce, enhance the fungitoxicity.[23] The ability of the antimicrobials to permeate through the semipermeable defences of the cell along with their stability and molecular architecture are the main factors which govern their antimicrobial properties.

The results of Table 4 reveal that the palladium(II) complexes are most active against *Alternaria alternata*. Many of the chelates were able to completely inhibit fungal growth. A closer look to the results also show that the toxicity had a direct relation with the sulfur content as well as the bulkiness of the groups present in the variety of ligands. However, it is a well-known fact that the palladium(II) chelates are recognized more for their antitumor and anticancerous properties than their antimicrobial assay; still, to assess the growth-inhibiting potential of the synthesized palladium(II) complexes, the results of bioactivity were compared with the conventional fungicide bavistin and bactericide, streptomycin taken as standards in either case. The complexes can be rated to exhibit from moderate-to-good activity.

Sterilizing Activity. Body and Organ Weight: No significant change in the body weight was observed in any experimental group when compared with their initial body weights. The weight of testes EN⌒XH $P<0.001$, $[Pd(EN⌒X)_2]$, $P<0.05$, $[Pd(EN⌒XH)_2]Cl_2$ $P<0.001$, epididymis EN⌒XH $P<0.001$, $[Pd(EN⌒X)_2]$ $P<0.05$, $[Pd(EN⌒XH)_2]Cl_2$ $P<0.05$, seminal vesicle EN⌒SH $P0<.05$, $[Pd(EN⌒X)_2]$ $P<0.001$, $[Pd(EN⌒XH_2]Cl_2$ $P<0.05$, and ventral prostate EN⌒XH $P<0.001$, $[Pd(EN⌒SH)_2]Cl_2$ $P<0.05$, were significantly decreased when the animals of group B, compared with Gr. A as well as Gr. C and Gr. D, respectively (Table 6).

Sperm Motility and Sperm Density: The sperm density in testes and cauda epididymis were significantly reduced ($P<0.001$). A significant decline in the sperm motility was observed in rats treated with ligands and their complexes (Table 7).

Biochemical Parameters of Reproductive Organs (Table 8). Sialic Acid: The ligands and their complexes resulted in a significant reduction in the sialic acid contents of the testes, epididymis, seminal vesicle, and ventral prostate ($P<0.001$).

Total Protein: The total protein contents of the testes, epididymis, ventral prostate, and seminal vesicle were significantly decreased ($P<0.001$).

Total Cholesterol: Although the total cholesterol contents of the testes were increased in all experimental groups ($P<0.001$) when compared with controls, no significant change were observed when the complex-treated animals (C and D) were compared with ligand-treated animals.

Fructose: The fructose contents of seminal vesicle were reduced significantly ($P<0.001$) in all experimental groups.

The present study revealed that a treatment with a ligand and its complexes resulted in a significant reduction in the weight of the testes and other sex accessories. The reduced testicular weights reflect wide-spread cellular damage.[24] Exposure of the ligands and their complexes reduced the sperm density, the sperm motility and the sperm counts. Takihara et al.[25] documented a strong correlation between the testicular size, total sperm count, sperm motility, and sperm density. A decrease in the sperm density and motility in cauda epididymis is of importance in the end result of fertilization.[26] In the present study, the various androgen dependent parameters viz. protein, sialic acid, and fructose were decreased. That the reduced protein contents of the testes impaired the onset of spermatogenesis supports the finding of Wright et al.,[27] who showed that changes in the protein synthetic and secretory activity of seminiferous tubules affect spermatogenesis.

The increase in the cholesterol contents suggests an inhibition of steroidogenesis in the testes of rats treated with ligand and its complexe(s). A reduction in the fructose concentration of seminal vesicle after ligand and its complexe(s) treatment further support the antiandrogenic nature of these compounds. The complexes of this ligand were found to be a more-effective fertility inhibitor in male albino rats; this activity is due to a synergistic action, including the chloro and fluoro moiety.

The present results amply demonstrate the antifertility effects of ligands and their complexes on the male gonad, i.e. the testes and its important endocrine function involving the biosynthesis and secretion of testosterone, the principle androgen.

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Table 8. Effects of Ligands and Their Complexes on Various Biochemical Parameters of Reproductive Organ

Treatment[d]	Sialic acid (mg/g)				Total protein (mg/g)				Total cholesterol (mg/g)	Fructose (mg/g)
	Testes	Epididymis	Seminal vesicle	Ventral prostate	Testes	Epididymis	Seminal vesicle	Ventral prostate		Seminal vesicle
Control	7.30±0.9	6.30±1.2	6.80±1.3	6.90±0.5	225.0±17.0	205.0±19.3	205.0±10.8	230±20.5	7.30±0.52	450.0±30.0
EN⌒SH	5.30±0.4[b]	4.80±0.9[b]	5.4±0.7	4.9±0.1[b]	147.0±11.5[b]	155.0±20.5[a]	170.0±10.5[a]	175.0±10.6[a]	8.9±0.10[b]	370.0±27.0[b]
[Pd(EN⌒S)$_2$]	4.5±0.2[b]	4.2±0.5[b]	4.3±0.5	4.1±0.2[b]	120.0±15.0[b]	110.0±13.7[b]	115.0±12.0[b]	128.0±15.0[b]	9.2±0.50[c]	310.0±15.0[b]
Pd(EN⌒SH)$_2$]Cl$_2$	4.0±0.3[b]	4.0±0.2[b]	4.1±0.2	3.9±0.5[b]	118±14.2[b]	108.7±12.2[a]	110.0±11.3[b]	120.0±14.7[b]	9.4±0.50[a]	305.0±12.0[b]

Level of significance: a) $P<0.05$, b) $P<0.001$, c) P=Non significant. d) Group A compared with Group B. Group B compared with Group C & D.

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