SYNTHESIS AND BIOLOGICAL ACTIVITY OF PLATINUM GROUP MET OF o-VANILLIN THIOSEMICARBAZONES

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Summary — o-Vanillin-(4-methylthiosemicarbazone), o-Vanillin-(4-phenylthiosemicarbazone) and some of their metal complexes of the platinum group have been synthesized, characterized by chemical and spectral methods and studied for their antibacterial, antifungal and amoebicidal activity *in vitro*. The platinum group metal chelates exibited significant activity against a wide spectrum of microorganisms at different concentrations. The Pt(II) and Ru(III) chelates derived from o-vanillin-(4-phenylthiosemicarbazone) seem to be the most efficient inhibitors. Evaluation of the antimalarial activity of the compounds in mice infected with *Plasmodium berghei* indicated that cures were attainable at dose levels of 40-160 mg/kg but with toxic death prevalence at higher dose levels.

INTRODUCTION

Considerable attention has been recently devoted to thiosemicarbazones and to their metal complexes, due to their biological activity and analytical importance[1-14]. The pharmacological activity of thiosemicarbazones is generally attributed to their ability to chelate some essential metals in biological systems[15-19]. Moreover, transition metal complexes of thiosemicarbazones have been found, in some instances, to have enhanced or modified activity in comparison with the uncomplexed ligand[18,20,21]. Although some o-vanillin thiosemicarbazones have been synthesized[22,23], the investigation of their platinum group metal complexes and of their biological activity has hardly been done. The interesting biochemical applications of platinum group metal complexes with nitrogen and sulphur ligands[24-26] led us to undertake the present systematic study of some substituted o-vanillin thiosemicarbazone complexes with platinum group metal ions.

As further development of our previous work on biological activity of thiosemicarbazones and of their metal complexes[10-13], we report here the synthesis and biological activity of o-vanillin-(4-methylthiosemicarbazone) (**1**), o-vanillin-(4-phenylthiosemicarbazone) (**2**) and of their Pd(II), Pt(II), Ru(III), Rh(III) and Ir(III) complexes (**3**).

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EXPERIMENTAL SECTION

A) CHEMICAL SYNTHESIS

IR spectra were measured with a FT-IR Perkin-Elmer 1600 spectrophotometer. ^{1}H-NMR spectra of the ligands were recorded on a Varian VXR 300 instrument. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane. Elemental analyses were carried out using a Perkin-Elmer model 240 C,H,N analyzer. Analyses indicated by the symbols were within $\pm 0.4\%$ of the theoretical values. The molar conductance measurements in DMF were carried out using a systronic direct reading conductivity bridge with a conventional dip-type black electrode.

o-VANILLIN-(4-METHYLTHIOSEMICARBAZONE) (o-VMtsc) (1)

o-Vanillin (30.43 g; 0.2 mol) dissolved in 100 ml of ethanol was added to 150 ml of an ethanolic solution of 4-methylthiosemicarbazide (21.03 g; 0.2 mol) and treated with glacial acetic acid (6 ml). The resulting solution was refluxed for 30 min., when a milky white precipitate of the ligand separated out. The precipitate was filtered, repeatedly recrystallized from ethanol and dried on P_2O_5 *under vacuo* to give the title compound, 32.6 g., 68% yield. M.p. 250°C.

Anal. ($C_{10}H_{13}N_3O_2S$) C,H,N.

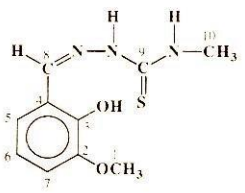
IR ν max (nujol, cm^{-1}) 3420 br (-OH), 3296 s (-NH), 2923 s (-NH), 1556 s (C=N), 1110 (C=S), 1066 (N-N).

^{1}H-NMR (d_6 DMSO) 11.5 (broad s, 1H, OH), 8.4 (d, 2H, NH), 7.6 (d, 1H, Ar), 7.0 (d, 1H, Ar), 6.8 (m, 1H, Ar), 3.8 (s, 3H, $-OCH_3$), 3.0 (s, 3H, CH_3), 2.5 (s, 1H, H-C=N).

^{13}C-NMR (d_6 DMSO) 177.8 (C-9), 148.2 (C-3), 146.1 (C-8), 139.0 (C-2), 121.2 (C-4), 119.2 (C-7), 118.3 (C-5), 113.0 (C-6), 56.2 (C-1), 31.1 (C-10).

o-VANILLIN-(4-PHENYLTHIOSEMICARBAZONE) (o-VPtsc) (2)

o-Vanillin (30.43 g; 0.2 mol) was dissolved in ethanol (100 ml) and treated with glacial acetic acid (7 ml). To this silightly warmed solution were added 150 ml of a hot ethanolic solution of 4-phenylthiosemicarbazide (33.45 g; 0.2 mol). The resulting mixture was refluxed for 20 min., when a light yellow precipitate



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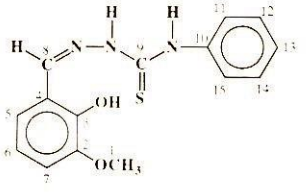

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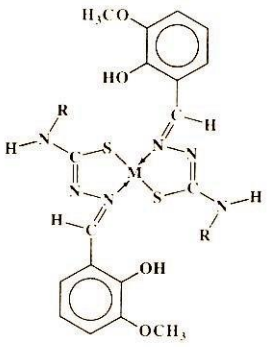
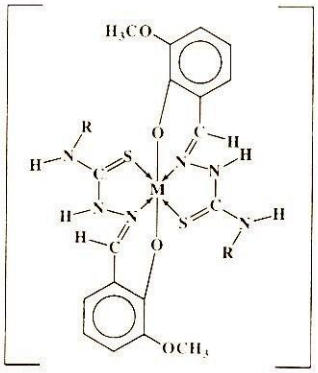


3a M = Pd (II), Pt (II)
R = CH_3, Ph



3b M = Ru (III), Rh (III), Ir (III)
R = CH_3, Ph

Fig. 1

separated out. The precipitate was filtered, repeatedly recrystallized from ethanol and dried on P_2O_5 *under vacuo* to give the title compound, 43.5 g., 72% yield. M.p. 210 °C.

Anal. ($C_{15}H_{15}N_3O_2S$) C,H,N.

IR ν max (nujol, cm^{-1}) 3440 br (-OH), 3298 s (-NH), 2910 s (-NH), 1539 s (C=N), 1185 (C=S), 1066 (N-N).

^{1}H-NMR (d_6 DMSO) 11.8 (broad s, 1H, OH), 10.05 (s, 1H, NH), 8.5 (broad, s, 1H, NH), 7.7 (d, 1H, Ar), 7.6 (d, 2H, Ar), 7.4 (m, 2H, Ar), 7.3 (m, 1H, Ar), 7.0 (d, 1H, Ar), 6.8 (m, 1H, Ar), 3.8 (s, 3H, $-OCH_3$), 3.0 (s, 3H, CH_3), 2.5 (s, 1H, H-C=N).

^{13}C-NMR (d_6 DMSO) 176.0 (C-9), 148.2 (C-3), 146.4 (C-8), 140.2 (C-2), 139.4 (C-4), 128.2 (C-11 and C-15), 126.0 (C-12 and C-14), 125.4 (C-10), 122.0 (C-13), 119.2 (C-7), 118.7 (C-5), 113.3 (C-6), 56.2 (C-1).

Synthesis of the metal complexes (3) (I-X)

The complexes Pd(o-VMtsc)$_2$ (I), Pt)(o-VMtsc)$_2$ (II), Ru(o-VMtsc)$_2$Cl (III), Rh(o-VMtsc)$_2$Cl (IV), Ir(o-VMtsc)$_2$Cl (V), Pd(o-VPtsc)$_2$(VI), Pt(o-VPtsc)$_2$ (VII), Ru(o-VPtsc)$_2$Cl (VIII), Rh(o-VPtsc)$_2$Cl (IX), Ir(o-VPtsc)$_2$Cl (X), were prepared by reacting an ethanolic solution of the appropriate metal ion salt (0.01 M) with an ethanolic solution of the respective ligands (0.02 M). The reaction mixture was stirred under reflux for 2-4 hr and the solution was concentrated and left in the refrigerator until the complexes separated out. The precipitates were filtered, washed several times with ethanol, then with ether and dried on P_2O_5 *under vacuo*. The yields, m.p. and other properties of the complexes are reported in Table I and II. The Ir spectra of the ligands and of their metal complexes reveal that the ligands are monobasic tridentate molecules in their Ru(III), Rh(III) and Ir(III) complexes. On the contrary, the ligands behave as monobasic bidentate molecules in Pd(II) and Pt(II) complexes.

The complexes are stable in air and slightly soluble in the most common organic solvents except DMF and DMSO. The molar

Table I - Physical properties of the ligands and of the metal complexes

Ligand	Complex	Formula	M.W.	Yield%	M.P.(°C)	Conductance $ohm^{-1}cm^2mol^{-1}$
o-VMtsc		$C_{10}H_{13}N_3O_2S$	239.3	65	250	-
	I	$Pd(C_{20}H_{26}N_6O_4S_2)$	585.0	60	130	5.5
	II	$Pt(C_{20}H_{26}N_6O_4S_2)$	673.7	60	185	4.9
	III	$Ru(C_{20}H_{26}N_6O_4S_2)Cl$	615.1	40	265	75.5
	IV	$Rh(C_{20}H_{26}N_6O_4S_2)Cl$	617.0	36	295	71.8
	V	$Ir(C_{20}H_{26}N_6O_4S_2)Cl$	706.3	31	300	68.9
o-VPtsc		$C_{15}H_{15}N_3O_2S$	301.4	74	210	-
	VI	$Pd(C_{30}H_{30}N_6O_4S_2)$	709.2	58	195	10.0
	VII	$Pt(C_{30}H_{30}N_6O_4S_2)$	797.9	53	185	7.5
	VIII	$Ru(C_{30}H_{30}N_6O_4S_2)Cl$	739.3	59	240	74.0
	IX	$Rh(C_{30}H_{30}N_6O_4S_2)Cl$	741.2	42	300	71.2
	X	$Ir(C_{30}H_{30}N_6O_4S_2)Cl$	830.5	40	295	67.0

TABLE II - IR spectra in nujol and elemental analysis of the metal complexes

Complex	IR, cm-1			Elemental analysis	
	ν (C=N)	ν (N-N)	ν (C=S)		
I	1458s	1061m	1108m	($C_{20}H_{26}N_6O_4S_2Pd$)	C,H,N,S,Pd
II	1458s	1064m	1112m	($C_{20}H_{26}N_6O_4S_2Pt$)	C,H,N,S,Pt
III	1460s	1060m	1110m	($C_{20}H_{26}N_6O_4S_2ClRu$)	C,H,N,S,Ru
IV	1461s	1081m	1109m	($C_{20}H_{26}N_6O_4S_2ClRh$)	C,H,N,S,Rh
V	1459s	1065m	1167m	($C_{20}H_{26}N_6O_4S_2ClIr$)	C,H,N,S,Ir
VI	1520s	1081m	1108m	($C_{20}H_{26}N_6O_4S_2Pd$)	C,H,N,S,Pd
VII	1522s	1078m	1168m	($C_{20}H_{26}N_6O_4S_2Pt$)	C,H,N,S,Pt
VIII	1500s	1080m	1110m	($C_{20}H_{26}N_6O_4S_2ClRu$)	C,H,N,S,Ru
IX	1495s	1081m	1110m	($C_{20}H_{26}N_6O_4S_2ClRh$)	C,H,N,S,Rh
X	1495s	1079m	1109m	($C_{20}H_{26}N_6O_4S_2ClIr$)	C,H,N,S,Ir

conductivity of their 1.10^{-3} M solutions are in the range 4.9-10.0 ohm^{-1} $cm^2 \cdot mole^{-1}$ for Pd(II) and Pt(II) complexes, indicating a non-electrolytic nature of the complexes. In the cases of Ru(III), Rh(III) and Ir(III) complexes, the relatively hygher values of molar conductivity (67.0-75.5 $ohm^{-1} \cdot cm^2 \cdot mole^{-1}$) suggest a 1/1 electrolytic nature[27]. The results of the elemental analyses, shown in Table II are consistent with a 1/2 metal to ligand stoichiometry for the complexes. Details on the stereochemical studies are reported elsewere.

B) ANTIBACTERIAL, ANTIFUNGAL, AMOEBICIDAL AND ANTIMALARIAL ACTIVITY

ANTIBACTERIAL ACTIVITY

The preliminary screening on antibacterial activity of o-vanillin-(4-methylthiosemicarbazone), o-vanillin-(4-phenylthiosemicarbazone) and of their metal complexes (I-X), in DMF solution, was performed *in vitro* by the paper disc (7.0 mm diameter) method. The microorganisms were obtained from stock cultures and were maintained separately on solid medium containing agar (2% Difco 15/1), Bushnell and Hass salt mixture, and glucose (1% w/v). All the materials used were sterilized and the inocolum was prepared by treating the nutrient agar media with 3ml of suspension of the respective cells. The colony of each of the tested microorganisms were subcultured and first incubated for about 6-8 hours before being poured into agar plates (Difco Laboratories, Detroit, Michigan, USA). The discs (7.0 mm diameter) were soaked with different test samples (concentration 1000 μg/ml), drained and then placed on the agar plate using sterilized forceps. The plates were incubated at 37°C for 24 h. At the end of the incubation period, the zones of inhibition around the discs were measured in mm. On the basis of this preliminary antibacterial screening, ligands and complexes affecting significant zones of inhibition (10 mm and above) were then selected and used for the minimum inhibitory concentration (MIC) determination. The minimum inhibitory concentrations of the ligands and complexes (I-X) were examined by double serial dilution containing 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 μg/ml of the test compounds. Ampicillin was used as reference standard. The results are reported in Table III.

ANTIFUNGAL ACTIVITY

The antifungal activity of o-vanillin-(4-methylthiosemicarbazone), o-vanillin-(4-phenylthiosemicarbazone) and of their metal complexes (I-X) was determined by the dilution method for *Candida albicans* and by the agar diffusion method for *Aspergillus fumigatus, Aspergillus niger, Penicillium islandicum* and *Gliocladium roseum*. *Candida albicans* was grown in Sabouraud Dextrose Broth (SDB) at 37°C. The inoculum was pepared by suspending the cells in SDB medium so as to obtain a final concentration of 8.8×10^6 CFU/ml as determined by spectrophotometric method (OD = 0.2 at 530 nm). A stock solution (5.000 μg/ml) of the ligand was used, whereas that of the metal complexes (I-X) was prepared by dissolving 25 mg of each compound in 1 ml of DMF and diluting the solution to 5 ml with SDB medium of pH 6.8.

SUSCEPTIBILITY TESTS

Six different concentrations of each compound (100, 200, 400, 600, 800 and 1000 μg/ml) were prepared in SDB medium from stock solution. The inoculum (0.05 ml) was added in each test tube containing 4.95 ml of the medium with the compound. The tubes were incubated at 37°C for 48 h. The lowest concentration of the compound at which there was no visible growth was considered as the minimum inhibitory concentration (MIC). In few cases it was necessary to further twofold dilute the medium containing the compound in order to determine the actual MIC.

For testing the susceptibility of *Aspergillus niger, Aspergillus fumigatus, Penicillium islandicum* and *Gliocladium roseum*, the inoculum was prepared on Czepel's medium. Three to four ml of spore suspension of *Aspergillus fumigatus* or *Aspergillus niger* or *Penicillium islandicum* or *Gliocladium roseum* (95% T at 530 nm) was uniformly spread on a Sabouraud Dextrose Agar medium in plates and the excess of fluid was drained. After drying the plates, wells of 6 mm size were cut in the medium.

TABLE III - Antibacterial activity of the ligands and of their metal complexes I-X (MIC in μg/ml)

Compd	a	b	c	d	e	f	g	h	i
L1	62.50	62.50	62.50	--	--	125.0	31.25	62.50	250.0
I	62.50	62.50	--	62.50	--	31.25	15.62	31.25	125.0
II	62.50	31.25	62.50	15.62	--	62.50	15.60	31.25	62.50
III	--	--	62.50	31.25	--	--	--	31.25	62.50
IV	62.50	62.50	--	62.50	--	62.50	62.50	--	--
V	62.50	31.25	--	31.25	--	62.50	125.0	62.50	--
L2	31.25	62.50	62.50	15.62	125.0	125.0	62.50	31.25	125.0
VI	62.50	62.50	62.50	15.62	62.50	--	15.62	15.62	--
VII	62.50	31.25	31.25	7.81	--	--	--	7.81	15.62
VIII	31.25	15.62	31.25	7.81	--	--	31.25	7.81	31.25
IX	62.50	--	--	15.62	--	62.50	62.50	--	62.50
X	31.25	31.25	--	--	125.0	31.25	62.50	--	--
A	3.91	31.25	15.62	15.62	7.8	62.50	3.91	7.81	62.50

L1: o-Vanillin-(4-methylthiosemicarbazone); L2: o-Vanillin-(4-phenylthiosemicarbazone); a: *Staphylococcus aureus:* b: *Escherichia coli;* c: *Pseudomonas;* d: *Streptococcus pyogenes;* e: *Salmonella typhi;* f: *Klebsiella-Enterobacter;* g: *Proteus vulgaris;* h: *Shigella flexneri;* i: *Serratia marcescens. A: Ampicillin.* The lines in place of numbers in the table indicate that the compound is not active at a conc of 250 μg/ml or lower.

TABLE IV - Antifungal activity of the ligands and of their metal complexes I-X (MIC in μg/ml)

Compd	j	k	l	m	n
L1	--	800	800	400	600
I	--	--	400	200	400
II	200	200	600	200	400
III	400	400	--	100	200
IV	--	600	--	--	400
V	--	200	400	--	200
L2	400	400	600	200	400
VI	400	--	600	200	200
VII	200	600	400	100	200
VIII	200	200	400	100	200
IX	400	400	--	--	--
X	800	400	400	--	200

L1: o-Vanillin-(4-methylthiosemicarbazone); L2: o-Vanillin-(4-phenylthiosemicarbazone); j: *Candida albicans;* k: *Aspergillus fumigatus;* l: *Aspergillus niger;* m: *Penicillium islandicum;* n: *Gliocladium roseum.* The lines in place of numbers in the table indicate that the compound is not active at a conc of 1000 μg/ml or lower.

TABLE V - Amoebicidal end-point against *E. Histolytica* (μg/ml)

Compd	End-point (μg/ml)	Compd	End-point (μg/ml)
L1	--	L2	500.0
I	250.0	VI	62.50
II	125.0	VII	31.35
III	125.0	VIII	31.25
IV	--	IX	62.50
V	500.0	X	--

L1: o-Vanillin-(4-methylthiosemicarbazone); L2: o-Vanillin-(4-phenylthiosemicarbazone). The lines in place of numbers in the table indicate that the compound is not active at a conc of 1000 μg/ml or lower.

To each one of these wells, 0.2 ml of a solution of different concentration of the compound was added. The zone of inhibition of the growth of *Aspergillus fumigatus, Aspergillus niger, Penicillium islandicum* and *Cliocladium roseum* in the presence of the prepared compounds with different concentrations was measured after 48 h and 72 h of incubation at 37°C. Nistatine was used as reference standard.

AMOEBICIDAL ACTIVITY

The amoebicidal activity against *Entamoeba hystolytica* was evaluated using the following techniques. *E. hystolytica trophozoites* were maintained in culture with TYI-S-33 (Biosate, iron and serum) with penicillin (100 U/ml) and streptomycin sulfate (100 μg/ml) (all from GIBCO Laboratories, Grand Island, N.Y., U.S.A.) as described by Diamond et al[28]. At 48 to 72 h following subculture, trophozoites were harvested by chilling the tube and adjusted to a concentration of 1.25×10^4 amoeba per ml in culture medium containing 10% serum. 0.2 ml of inoculum containing about 2500 amoebae was put into cavity slide filled with 0.8 ml of fresh medium having the

TABLE VI - Antimalarial activity of the ligands and of their metal complexes

	Increase in the mean survival time (days) and number of cure at the dosage indicated (mg/kg)					
Comp.	20	40	80	160	320	640
L1		0.1		0.1		0.1
I		5.2	4.6	7.1A	C(1/5) T(1/5)	0.3 T(3/5)
II		5.9	6.7	C (2/5)	C(3/5)	C(3/5) T(2/5)
III	3.5	6.2A	C(3/5)	C(2/5)	C(2/5) T(2/5)	C(2/5) T(3/5)
IV		0.3	0.1	2.5	6.1	C(1/5)
V		2.0	4.1	5.5	8.5A	C(2/5)
L2		1.1	4.3	4.8	C(2/5)	C(3/5)
VI		0.5	1.0	4.9	7.2A T(2/5)	C(2/5) T(3/5)
VII	2.9	6.0	C(2/5)	C(3/5)	C(4/5)	C(3/5)
VIII	2.1	5.0	C(3/5)	C(4/5)	C(3/5)	T(4/5)
IX	1.1	2.0	5.2	C(1/5) T(3/5)		T(5/5)
X	1.5	3.9	7.1A	9.7A	C(2/5)	C(2/5) T(1/5)

T = toxic; A = active; C = cure. See experimental section for definitions.
L1: o-Vanillin-(4-methylthiosemicarbazone); L2: o-Vanillin-(4-phenylthiosemicarbazone)

requisite drug concentration, the cavity was covered, sealed with paraffin wax and put in moist chamber at 37 °C. Mortality was observed after 18, 24 and 48 h with the aid of aqueous trypan blue solution (4%, 20 μl) added in the cavity, under inverted microscope. The viable trophozoites cells would normally exclude trypan blue and the result is confirmed by using a hemacytometer chamber. Flagyl (1 μg/ml) was used as a reference standard. Amoebicidal end point of the ligands and of their metal complexes (I-X) are listed in Table V.

Antimalarial activity

o-Vanillin-(4-methylthiosemicarbazone), o-vanillin-(4-phenylthiosemicarbazone) and their metal complexes (I-X) were tested for antimalarial activity against a drug-sensitive strain of *Plasmodium berghei* in mice following the method of Osdene et al[29]. Mice of one sex weighing 15-18 g were housed in metal cages with plastic tops and given standard laboratory diet and water ad lib. The animals were infected with an intraperitoneal infection of 0.5 ml of heparinized heart blood containing a minimum of 90% parasitized cells, drawn from donor mice infected one week earlier with *Plasmodium berghei*. The test compounds were suspended in peanut oil and a single dose was administered subcutaneously 72 h after infection. In the primary test the drug was administered in three dilutions: 640, 160 and 40 mg/kg. A minimum of five animals per dilution was used. If the drug proved to be toxic, lower dilutions were used. If the primary test gave a positive result, a confirmatory test was performed using five animals at six dilutions (640, 320, 160, 80, 40 and 20 mg/kg). A group of infected animals treated with pyrimethane was included in every experiment as a positive control. The compounds were judged to be "Toxic" (T) if the treated mice die before the 6th day, i.e. before the time the untreated mice begin to die, "Active" (A) if the mean survival time of the mice is at least double and "Curative" (C) if the mice survive 60 days post infection.

C) Toxicity of selected active compounds

Some of the tested compounds which, on the basis of their antibacterial, antifungal, amoebicidal and antimalarial activity, resulted to be active, were selected and used for determination of their LD_{50}. The selected active compounds Ru(o-VMtsc)$_2$Cl, Pt(o-VMtsc)$_2$, o-VPtsc, Pt(o-VPtsc)$_2$2 and Ru(o-VPtsc)$_2$Cl were administered orally to six groups of ten mice weighing 25-30g in dosages of 100, 250, 500, 1000, 2000 and 4000 mg/kg of body weight. The LD_{50} of the tested compounds were then calculated and the results were presented as means and evaluated statistically using Student's t-test or exact Fischer's test.

RESULTS AND DISCUSSION

Antibacterial activity

All the synthesized compounds were evaluated for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas, Streptococcus pyogenes, Salmonella typhi, Klebsiella-Enterobacter, Proteus vulgaris, Shigella flexneri* and *Serratia marcescens* at a concentration of 1000 μg/ml. The active compounds, effecting a minimum of 10 mm zone of inhibition, were then employed for the minimum inhibitory concentration (MIC) determination. The results are reported in Table III.

The results show that the compounds are able to inhibit several bacteria at low and high concentrations. *E. coli, Shigella flexneri, Streptococcus* and *Serratia marcescens* are sensitive to the tested compounds. Their sensitivity toward the compounds can be compared very favourably with that obtained with conventional antibiotics.

The metal chelates are relatively more active than their corresponding ligands. The Pt(o-VPtsc)$_2$Cl (VII) and Ru (o-VPtsc)$_2$Cl (VIII) chelates possess a MIC of 7.81 μg/ml against *S. flexneri* and *S. pyogenes*. A MIC of 15.62 μg/ml is recorded for o-VPtsc, Pt(o-VMtsc)$_2$ (II), Pd(o-VPtsc)$_2$ (VI) and Rh(o-VPtsc)$_2$Cl (IX) against the most sensitive *S. pyogenes*. In the present study *Salmonella typhi* appears to be the relatively most resistant to the tested compounds. The lowest MIC of 62.5 μg/ml against *S. typhi* is effected by Pd(o-VPtsc)$_2$ (VI). From the results it can be concluded that the order of activity, of the ligands is o-VMtsc < o-VPtsc. The order of activity of the metal ions in the chelates is: Pt(II) = Ru(III) < Pd(II) < Ir(III) < Ru(III).

Antifungal activity

The antifungal activity of o-VMtsc, o-VPtsc and of their metal complexes I-X are presented in Table IV. The results show that *Candida albicans, Aspergillus fumigatus, Aspergillus niger, Penicillium islandicum* and *Gliocladium roseum* are relatively sensitive to the tested compounds.

Candida albicans is inhibited only by o-VPtsc, whereas o-VMtsc is inactive towards this fungus. However, the presence of metal ions in the chelates significantly improves the growth inhibition of *Candida albicans*. The lowest MIC against this fungus (200 μg/ml) is exibited by Pt(o-VPtsc)$_2$ (VII), Ru(o-VPtsc)$_2$Cl (VIII) and Pt(o-VMtsc)$_2$ (II).

Aspergillus fumigatus is very sligtly inhibited by o-VMtsc with a MIC of 800 μg/ml, whereas o-VPtsc is more active against this fungus (MIC of 400 μg/ml). The most active compounds are the metal chelates Pt(o-VMtsc)$_2$ (II), Ir(o-VMtsc)$_2$Cl (V) and Ru(o-VPtsc)$_2$Cl (VIII) exibiting a MIC of 200 μg/ml against *Aspergillus fumigatus*. The Pd(II) chelates of both ligands are found rather inactive toward the fungus.

Aspergillus niger and *Penicillium islandicum* are sensitive to both ligands at relatively high concentrations. The metal chelates Pd(o-VMtsc)$_2$ (I), Ir(o-VMtsc)$_2$Cl (V), Pt(o-VPtsc)$_2$ (VII), Ru(o-VPtsc)$_2$Cl (VIII) and Ir(o-VPtsc)$_2$Cl (X) effect the lowest MIC (400 μg/ml) against *Aspergillus niger*.

However, *Penicillium islandicum* is the most sensitive among the other fungi to the tested compounds. Ru(o-VMtsc)$_2$Cl (III), Pt(o-VPtsc)$_2$ (VII) and Ru(o-VPtsc)$_2$Cl (VIII) inhibit the growth of *Penicillium islandicum* with a MIC of 100 μg/ml.

In the inhibition of *Gliocladium roseum* the ligands are scarcely active, but their activity is further enhanced by all their metal chelates except in the case of Rh(o-Ptsc)$_2$Cl (IX) which is inactive even at a concentration of 1000 μg/ml. The other chelates effect a MIC o 200 μg/ml except in the case of Pd(o-VMtsc)$_2$ (I) and

$Rh(o\text{-}VMtsc)_2Cl$ (IV) exibiting a MIC of 400 μg/ml.

Amoebicidal activity

Entamoeba hystolytica is a pathogenic amoeba causing an infection known as Amoebiasis. This endemic disease is prevalent in the developing countries where poverty and poor sanitation encourage its incidence. The amoebicidal end-point against *Entamoeba hystolytica* is given in Table V. The ligands are scarcely active: o-VPtsc records an end-point of 500 μg/ml., whereas o-VMtsc is inactive even at a concentration of 1000 μg/ml. The metal chelates $Pt(o\text{-}VPtsc)_2$ (VII) and $Ru(o\text{-}VPtsc)_2Cl$ (VIII) are the most active compounds with an end-point of 31.25 μg/ml. Only few metal chelates of o-VMtsc are moderately active with end points of 125 μg/ml.

These results support the fact that the metal chelates are more active than their parent ligands against the microorganisms.

Antimalarial activity

The antimalarial activity of o-VMtsc, o-VPtsc and of their metal complexes (I-X) against *Plasmodium berghei* are given in Table VII. The ligand o-VMtsc appears to be devoid of antimalarial activity, whereas o-VPtsc effects low level of curative activity at 320 mg/kg and moderate activity at 640 mg/kg. Metal chelates of o-VMtsc demonstrate some level of curative activity only at dosages of 160 mg/kg and above. However, $Ru(o\text{-}VMtsc)_2Cl$ (III) appears capable of curing three out of five animals at a dose level of 80 mg/kg and a diminishing cure is observed at 160 mg/kg (two animals out of five). Toxicity increases correspondingly for $Ru(o\text{-}VMtsc)_2Cl$ (III) with two out of five animals cured at 320 mg/kg and toxic deaths of three out of five at 640 mg/kg.

The metal chelates of o-VPtsc display curative activity against Plasmodium berghei at dosage levels up to 640 mg/kg. In this study the most active compounds are $Pt(o\text{-}VPtsc)_2$ (VII) and $Ru(o\text{-}VPtsc)_2Cl$ (VIII). This last cured four out of five mice at 160 mg/kg but with diminishing cure of three out of five mice at 320 mg/kg and caused toxic deaths to four out of five. The chelate $Pt(o\text{-}VPtsc)_2$ (VII) shows no evidence of toxicity at higher dosages, but the highest curative dose is 320 mg/kg curing four out of five mice.

The activity of chelates seems to vary with the metal ions complexed and appears to increase in the order $Rh(III) < Pd(II) < Ir(III) < Pt(II) = Ru(III)$.

Toxicology

The compounds chosen on the basis of the results obtained in antibacterial, antifungal, amoebicidal and antimalarial activity were: o-VPtsc, II, III, VII and VIII. The LD_{50} of the above compounds falls in the range 1650-3000 mg/kg.

CONCLUSIONS

The present investigation indicates that o-vanillin-(4-phenylthiosemicarbazone) is more active than o-vanillin-(4-methylthiosemicarbazone). The metal chelates are relatively more active than their corresponding ligands against a broad spectrum of microorganisms, the metal chelates of o-vanillin-(4-phenylthiosemicarbazone) being the most active. However, their activity remains generally low.

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