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Anthelmintic activity of transition metal complexes of some benzamides

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ABSTRACT

Copper and cobalt complexes of four benzamides, [N-(3'-nitrophenyl) (piperidin-1"-yl) methyl] benzamide, [N (piperidin1"-yl) (p-tolyl) methyl] benzamide, [N-(4' – chloro - phenyl) (piperidin-1"-yl) methyl] benzamide and [N-(4'-methoxy- phenyl) (piperidin-1"-yl] benzamide have been synthesized. Anthelmintic activity of benzamides and their complexes have been evaluated against Indian Earth worm species Eicinia foetida. The results reveal a dose dependent increase in activity of the compounds at 5, 10 and 20 mg/ml concentration. All the metal complexes exhibit significant activity in dose dependant manner. Both cobalt and copper complexes are found to be more active as compared to ligand and standard drug albendazole

Key words: Anthelmintic activity**,** benzamides, *Eicinia foetida*, albendazole.

INTRODUCTION

Helminthiasis or worm infection is a common infection in human, affecting a large population of the world. Helminthiasis is one of the major cause of gastrointestinal disorder[1]. It also affects the productivity loss of small ruminant livestock and pets [1]. Parasitic worm also affect livestock crops, affecting food production with a resultant economic impact. The infection due to worm is a great threat to health and contribute to the prevalence of malnutrition, anemia, pneumonia etc [2]. Despite this prevalence of parasitic infections the research on anthelmintic drug is limited [3]. The gastro-intestinal helminthes become resistant to currently available drugs. Chemotherapy by developing new anthelmintic compounds which can circumvent the problems of drug resistance remains an essential weapon.

Benzamide molecules are widely used drugs in the therapeutic practice [4]. They possess diverse types of biological activities viz. antibacterial, analgesic, anthelmintic, antifungal, antimalarial etc [5-11]. Benzamides are also reported for antiallergic, antiemetic, antipsychotic, antihistaminic, anti-leukotriene activities [12-14]. N-phenyl benzamides are potent antibacterial agents. Bio-activity of N-(1- morpholinobenzyl) semi carbazide and its transition metal complexes have been reported in literature [7]. Antibacterial properties of lanthanide (III) complexes of N-(pyrrolidinobenzyl) benzamide and transition metal complexes of N-(piperidinobenzyl) benzamide have been studied [15,16]. Earlier work reported that some drugs showed increased activity, when administered as metal complexes rather than as organic compounds [17]. Several antibiotics and therapeutic agents are known to produce their antibacterial effect after the formation of metal complexes [18]. Considering the bioactivity of various benzamide derivatives and their metal complexes, it was considered worth synthesizing and studying the antihelmintic activity of cobalt and copper complexes of [N - (3'-nitrophenyl) (piperidin-1"-yl) methyl]

benzamide, [N -(piperidin-1"-yl) (p-tolyl) methyl] benzamide, [N-(4' – chloro - phenyl) (piperidin-1"-yl) methyl] benzamide and [N-(4'-methoxy- phenyl) (piperidin-1"-yl] benzamide. The work is carried out for the first time.

Experimental

MATERIALS AND METHODS

All the reagents used for the preparation of the ligand and the complexes were products of Merck, all AR grade. The IR spectra (4000 -350 cm-1) was recorded on Shimadzu FTIR-84005 spectrophotometer. NMR spectra were recorded on Bruker Armce III (400 MHz) machine. UV-VIS spectra were recorded on Shimadzu UV-1700 Thermaspec spectrophotometer. Mass spectra were recorded on Sciex API 3000 (ESI) spectrometer. The magnetic susceptibilities were measured on powdered samples using Guy balance..

Synthesis of Mannich Base

1.21 g (0.1 mol) benzamide was mixed with 0.98 ml (0.1 mol) of piperidine with constant stirring at room temperature. To this solution, ethanolic solution of either 1.51g (0.1 mol) of m-nitro benzaldehyde or 1.4 g (0.1 mol) of P- chloro benzaldehyde or 1.19 ml of p-methyl benzaldehyde and 1.01 ml p- methoxy benzaldehyde was added drop wise under same conditions to prepare the corresponding benzamide derivatives. The resulting reaction mixture was refluxed for 8 hr. The solution produced was kept at room temperature for four days. The crystalline product formed was washed with ethanol and recrystallized from acetone-hexane mixed solvent system [16].

Synthesis of Metal complexes

For the synthesis of metal complexes, the ligand was dissolved in chloroform and mixed with an ethanolic solution of metal chloride, MCl_2 [M= Cu (II) and Co (II)] in 1:1 mole ratio. The resulting mixture was refluxed for 1 hr. Then the reaction mixture was kept overnight at room temperature. The supernatant liquid was removed and the solid product was washed with hexane and diethyl ether and dried in vacuo[16].

Anthelmintic Assay

Chemicals: Albendazole, normal saline were purched from authorized phermaceuticals. The solvents and other chemicals used during experimental protocol were of analytical grade.

Animal: Indian Earth worm species *Eicinia foetida* was collected from Mahatma Phule Agriculture University, Pune, Maharashtra, India. All earthworms were of approximately equal size (9 – 10 cm).

The anthelmintic assay was carried out as per the method reported by Ajaiyeoba *et al* with minor modification [19]. The assay was performed on adult Indian Earth species *Eicinia foetida* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. Albendazole with normal saline was used as standard (5, 10 and 20 mg/ml). Compounds in same concentration (5, 10 and 20 mg/ml) in normal saline solution were used for the assay and normal saline was served as control. The time taken for complete paralysis and death was recorded. External stimuli were applied to ascertain the paralysis time.The time taken by worm to become motionless was considered as paralysis time and lethal time was ascertained by death of motionless worm followed by fading of their body colour.

RESULTS AND DISCUSSION

The benzamides were prepared as described in the experimental part, crystallized and dried in air and subjected to physical and spectral analysis. Analytical and physical data of benzamides and their complexes are presented (**Table 1&2**). The structure of benzamides has been characterized by using IR, NMR and mass spectral data. The coordination behaviour of benzamides towards transition metal ions was investigated via the IR spectra, molar conductance, magnetic moment and UV spectra. The elemental analysis of the complexes show 1:1 (metal: ligand) stoichiometry for all the complexes. The magnetic moment of the complexes were measured at room temperature. Both copper and cobalt complexes has paramagnetic character. The low molar conductance values of the complexes reveal their non-electrolytic nature. The results are in good agreement with the suggested formula: $[CuL(H2O)_2Cl_2]$ and[CoLCl2].$(H_2O)_2$ .Proposed structure of complexes are:

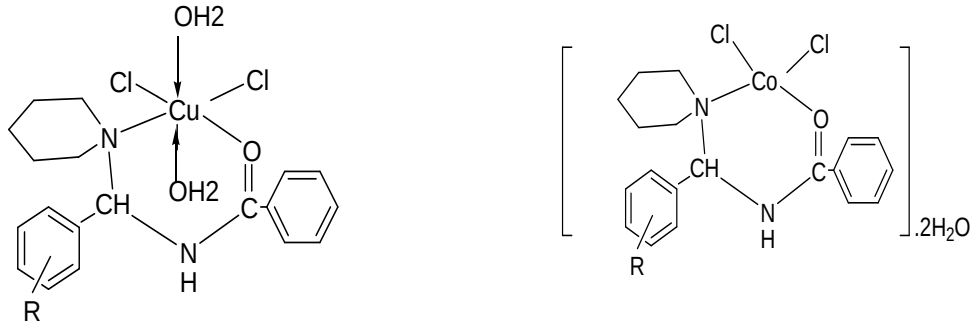

Fig.-1: structure of complexes (X= -NO_2, -CH_3, -Cl, -OCH_3)

Table-1: Ligands and complexes prepared

Ligand/complex	Numbered in Text as	Molecular formula	Yield (%)
L1	1	$C_{19}H_{21}O_3N_3$	74
L2	2	$C_{20}H_{24}ON_2$	79
L3	3	$C_{19}H_{21}O\ N_2Cl$	76
L4	4	$C_{20}H_{24}N_2O_2$	72
[L1$CuCl_2$.2(H_2O)]	1a	$C_{19}H_{21}O_3N_3$. $CuCl_2.2H_2O$	57
[L2$CuCl_2$.2(H_2O)]	2a	$C_{20}H_{24}ON_2$. $CuCl_2.2H_2O$	56
[L3$CuCl_2$.2(H_2O)]	3a	$C_{19}H_{21}ON_2Cl.CuCl_2.2H_2O$	58
[L4$CuCl_2$.2(H_2O)]	4a	$C_{20}H_{24}N_2O_2$. $CuCl_2.2H_2O$	50
[L1$CoCl_2$].2(H_2O)	1b	$C_{19}H_{21}O_3N_3$. $CoCl_2.2H_2O$	50
[L2$CoCl_2$].2(H_2O)	2b	$C_{20}H_{24}ON_2$. $CoCl_2.2H_2O$	56
[L3$CoCl_2$].2(H_2O)	3b	$C_{19}H_{21}O\ N_2Cl.\ CoCl_2.2H_2O$	54
[L4$CoCl_2$].2(H_2O)	4b	$C_{20}H_{24}N_2O_2.CoCl_2.2H_2O$	51

Table 2 Analytical and physical data

Product	Colour	MP (ºC)	Analytical data % found (Cal)					M Wt	μ_{eff} (BM)	Λ_M ($\Omega^{-1}cm^2$ mol^{-1})
			M	C	H	N	O			
1	White	175	-	-	-	-	-	339	-	-
2	White	150	-	-	-	-	-	308	-	-
3	White	148	-	-	-	-	-	328	-	-
4	White	140	-	-	-	-	-	324	-	-
1a	Green	200 (Dec)	12.1 (12.7)	43.9 (44.7)	4.2 (4.9)	7.9 (8.2)	15.0 (15.6)	509	1.90	10.8
2a	Green	180 (Dec)	13.0 (13.5)	49.8 (50.1)	5.1 (5.8)	5.6 (5.8)	9.9 (10.0)	479	1.89	10.5
3a	Green	175 (Dec)	12.7 (13.0)	44.8 (45.6)	4.5 (5.0)	4.9 (5.6)	9.5 (9.6)	499	1.91	11.0
4a	Green	190 (Dec)	12.2 (12.7)	48.0 (48.6)	5.1 (5.6)	5.0 (5.6)	12.1 (12.9)	493	1.88	10.8
1b	Blue	140 (Dec)	10.9 (11.5)	45.0 (45.2)	4.5 (4.9)	7.9 (8.3)	15.2 (15.8)	504	4.52	4.2
2b	Blue	170 (Dec)	11.8 (12.2)	49.9 (50.6)	6.0 (5.9)	5.5 (5.9)	10.0 (10.1)	474	4.45	3.8
3b	Blue	195 (Dec)	11.1 (11.7)	45.2 (46.2)	4.8 (5.1)	5.0 (5.6)	9.5 (9.7)	494	4.50	3.2
4b	Blue	175 (Dec)	11.1 (11.8)	48.8 (49.1	5.2 (5.7)	5.01 (5.7)	12.7 (13.1)	488	4.6	3.0

^{1}H-NMR of the ligands was recorded in $CDCl_3$ solution . ^{1}H-NMR Spectrum of the ligands indicates characteristic integration pattern of aromatic moieties at between δ 8.38- 7.31 . A doublet at δ 6.65 – 6.57 is observed due to amide nitrogen proton. A doublet is detected at δ 6.12- 5.90 as a consequence of methine proton .Two merged broad peaks of piperidine ring are observed at δ 2.6 - 2.5 methylene protons. Multipletes are detected at δ 1.59 and δ 1.45 for methylene protons of piperidine ring. The δ shift values support the proposed structure of the ligands.

The important IR frequency of ligand and complexes are presented which supports the formation of complexes(Table 3)

Table 3 Important IR frequencies (cm^{-1}) of the ligands and their Complexes

Compound	Peak (v in cm^{-1})					
	(OH)/H_2O	(C=O) Amide-1	(C-N) alicyclic	M-Cl	M-O	M-N
Compound **1**	-	1643	1205-1099	-	-	-
1a	3348-3448 New 850	1610	1165- 1080	389	550	453
1b	3561-3234	1620	1128-1076	381	547	432
Compound 2	-	1640	1269-1101	-	-	-
2a	3348-3450 New 854	1620	1161-1078	399	549	472
2b	3390-3557	1624	1143-1080	393	565	462
Compound 3	-	1642	1321-1161	-	-	-
3a	3350-3454(w) New 860	1610	1159-1078	389	543	432
3b	3150-3545	1601	1172-1024	374	528	449
Compound 4	-	1636	1250-1105			
4a	3354-3446 New 862	1604	1161-1026		547	
4b	3557	1620	1180- 1031		524	459

The mass spectra of the ligands and their complexes were recorded and their stoichiometric composition compared. Ligand **1** (C19H21N3O3: Mol. Wt.= 339) gives the molecular ion peak at m/z =340 in (+) ve mode whereas its copper complex,1a ($C_{19}H_{21}N_3O_3CuCl_2.H_2O$) shows the molecular ion peak at m/z = 531 in (-) ve mode which is associated with one sodium ion The data confirm the stoichiometry of the metal complex as being of the [$CuLCl_2(H_2O)_2$].

Similarly cobalt complex, 2b ($C_{19}H_{25}O_5N_3Cl_2Co$, MW: 474) shows molecular ion peak at m/z = 496 in in (-) ve mode which is associated with one sodium ion . The data confirm the stoichiometry of the cobalt complexes as being of [$LCoCl_2$].2(H_2O).

The electronic absorption spectra of the ligand and its Cu and Co complexes were recorded at room temperature in DMF solution. The complexes 1a, 2a, 3a and 4a showed band at 14727 cm^{-1}, 14836 cm^{-1}, 14836 cm^{-1} and 14858 $cm^{-1}cm^{-1}$ which is assigned to ^2Eg - ^2T2g transition. This conforms the octahedral geometry of the complexes. The Co complexes 1b, 2b, 3b and 4b exhibited band at 14,836 cm^{-1}, 14992 cm^{-1} , 14858 cm^{-1} and, 14836 cm^{-1} assigned as $^4A_2$ – $^4T_1$ transition. This supports the tetrahedral geometry of the complexes. No peak was observed for the ligands in this region.

Anthelmintic activity

Benzamides and newly synthesized complexes are evaluated for anthelmintic activity against Earthworm, *Eicinia foeitida.*The compounds are screened for activity by time taken for complete paralysis and death of worms. The results reveal a dose dependent increase in activity of the compounds at 5, 10 and 20 mg/ml concentration. All the metal complexes exhibit significant activity in dose dependant manner. Both cobalt and copper complexes are found to be more active as compared to ligand and standard drug albendazole. The biochemical mechanism of anthelmintic action of the compounds may be due to interfering with metabolic processes, interfering with neuromuscular physiology of parasites. They may

Table Anthelmintic activity data of compounds

Compound	Concentration (mg/ml)	*Eicinia foeitida* (Earthworm)	
		Time for Paralysis in min. (mean &SD)	Time for Death in min. (mean &SD)
1	5	332±1.30	353±0.88
	10	310±2.20	332±1.30
	15	260±1.25	290±0.56
2	5	342±1.08	363±0.53
	10	318±1.09	348±0.51
	15	262±0.760	295±0.48
3	5	331±0.72	365±0.76
	10	312±0.70	342±1.08
	15	255±0.88	301±0.57
4	5	220±0.63	270±0.73
	10	190±0.81	220±0.63
	15	160±0.95	190±0.81
1a	5	39±0.66	116±0.44
	10	23±2	61±0.74
	20	18±0.58	33±0.47
1b	5	68±0.86	122±0.51
	10	52±0.61	105±0.42
	20	45±0.55	85±0.47
2a	5	37±0.59	58±0.59
	10	22±0.78	42±0.45
	20	15±0.57	28±0.44
2b	5	73±0.56	131±0.37
	10	62±0.74	101±0.36
	20	50±0.55	78±0.36
3a	5	63±0.53	115±0.44
	10	36±0.69	58±0.59
	20	25±0.52	29±0.37
3b	5	60±0.51	119±0.36
	10	48±0.50	107±0.44
	20	32±0.47	75±1.10
4a	5	37±0.66	60±0.47
	10	20±0.58	41±0.45
	20	14±0.52	27±0.44
4b	5	75±1.10	128±0.44
	10	61±0.47	98±0.36
	20	46±0.50	78±0.37
Albendazole	5	142±0.80	210±0.74
	10	135±1.40	185±0.44
	20	112±0.79	153±0.47
Control (Saline)	-------	--------	-------

Values are mean± SD from five observations , ------- no paralysis, no death inhibit the glucose uptake and depleted the glycogen content in the presence of glucose and affect the energy generating mechanism of the parasite. In general, the possible mechanism of anthelmintic action of complexes may be related to either inhibition of energy metabolism and/ or alteration in the motor activity of the parasite. The results are presented (**Table 4**).

CONCLUSION

In conclusion it can be point out that the benzamides (compound 1, 2, 3 and 4) were found to be less active as anthelmentic agent compared to albendazol whereas the results of their cobalt and copper complexes are quite encouraging. They are found to be superior anthelmintic agents than the standard. These observations provide some predictions in order to design further anthelmintic compounds. Both cobalt and copper complexes can also be promising candidates for the development of new anthelmintic agents .Further studies are required to explore these complexes as drugs.

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