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Research Article

Screening of Some Newly Synthesized Transition Metal Complexes of Salicylaldehyde Schiff Base of Isonicotinoyldithiocarbazic Acid against Some Pathogenic Microbial Strains

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ABSTRACT

Some new transition metal Schiff base dithiocarbazates, of the general formula $[M(IN\text{-}DtczH\text{-}Sal)_2]X_2$ (IN-DtczH-Sal = salicylaldehyde Schiff base of isonicotinoyldithiocarbazic acid; M = Co(II), Ni(II), Cu(II), Zn(II) when X = Cl; M = Co(II) when X = CH_3COO) have been synthesized. All the complexes have been screened for their antimicrobial activity against the pathogenic bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* and the pathogenic fungus *Candida albicans* by agar dilution method. The ligand as well as the metal complexes exhibited good antimicrobial activity, particularly against the pathogenic fungus *Candida albicans*. The nature of anion in the secondary coordination sphere also has a marked effect on the antimicrobial activity, as seen for two of the cobalt(II) complexes.

INTRODUCTION

The increasing interest in sulphur-nitrogen ligands and their complexes with metal ions, viz. Ni (II), Cu (II), Zn (II), Pd (II), Pt (II), silicon (IV), organotin etc[1-7]. is primarily because of their significant biological activity and practical applications in agriculture. Carcinostatic activities have also been found for metal complexes of dithiocarbazic acid and the Schiff bases derived from its S-methyl ester[8]. Infact, dithiocarbazates possess a broad spectrum of potentially useful chemotherapeutic properties. More recently, an *in vitro* insulin-mimetic potential of these compounds has been established[9]. The ruthenium (II), palladium (II) and dioxovanadium (V) complexes of Schiff bases derived from S-alkyldithiocarbazates show significant antiamoebic activity[10]. Large number of reports on antifungal[11], antibacterial[2,12], antiprotozoal[13], anticancer[2,14] and their role as uncouplers of oxidative phosphorylation in mitochondria[15], of the metal dithiocarbazate complexes have appeared in literature. Because large amount of data on antimicrobial activity studies on metal complexes of dithiocarbazates has been noticed in recent literature, it prompted us to undertake antimicrobial studies on the presently synthesized isonicotinoyldithiocarbazates, in which the dithiocarbazate group has been derived from isoniazid, a potent antitubercular drug compound.

MATERIALS AND METHODS

Metal(II) salts, isoniazid, CS_2 and salicylaldehyde, all from Merck used were of analytical grade and used as such.

Preparation of IN-DtczH-Sal ligand

To a solution of isonicotinic acid hydrazide (1 g; 7.29 mmol) in methanol (50 ml) was added with constant stirring (0.55 g; 7.29 mmol) carbon disulphide, when a light yellow liquid of isonicotinoyldithiocarbazic acid was obtained. To this liquid kept at 40-45 °C, a solution of salicyldehyde (0.89 g; 7.27 mmol) in methanol (20 ml) was added drop-wise with constant stirring. The resulting solution was further stirred for about half an hour when a creamish-white solid separated. It was filtered, washed with methanol and dried in air. Final drying was done by keeping the solid overnight in a $CaCl_2$ desiccator.

IN-DtczH-Sal: M.P.: 248 °C; yield: 95 %; IR(KBr)cm^{-1}: 3200-3100 (N-H), 1652 (C=O), 1408 (C-N), 1064 (N-N), 1032, 999 (CS); Anal.($C_{14}H_{11}N_3O_2S_2$) Found: C, 52.49; H, 3.87; N, 13.45; S, 19.86; Calculated: C, 52.95; H, 3.46; N, 13.23; S, 20.17.

Preparation of $[M(IN\text{-}DtczH\text{-}Sal)_2]X_2$ [M = Co(II), Ni(II), Cu(II), Zn(II) for X = Cl; M = Co(II) for X = CH_3COO]

To the continuously stirred ethanolic (20 ml) [when M = Co(II) with X = Cl, Ni(II), Zn(II)] or methanolic (15 ml) [when M = Co(II) with X = CH_3COO, Cu(II)] solution of IN-DtczH-Sal Schiff base (0.5 g, 1.57 mmol) was added the ethanolic solution (10 ml) of metal salt $MX_n.xH_2O$ (0.18 g, 0.78 mmol for M = Co(II), X = Cl and n = 2, x = 6; 0.18 g, 0.78 mmol for M = Ni(II), X = Cl and n = 2, x = 6; 0.13 g, 0.78 mmol for M = Cu(II), X = Cl and n = 2, x = 2; 0.11 g, 0.78 mmol for M = Zn(II), X = Cl and n = 2, x = 0) or methanolic solution (10 ml) of $Co(CH_3COO)_2.4H_2O$ (0.22 g, 0.78 mmol), in small portions after successive intervals of about 10-15 minutes in a total period of about 3 hours. The reaction was carried out at 40-45 °C. The contents of the reaction mixture were further stirred for another 1 hour. The solid product obtained (green when M = Co(II), X = Cl; brown when M = Co(II), X = CH_3COO; dirty brown when M = Ni(II); dirty green when M = Cu(II); yellow when M = Zn(II)) was filtered through Whatman filter paper no. 541, washed with methanol, ethanol and diethyl ether and then dried in air. Finally the sample was dried by keeping it overnight in a calcium chloride desiccator.

$[Co(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$: Dec. temp.: 230 °C; yield: 90 %; IR(KBr)cm^{-1}: 3600-3200 (N-H), 1656 (C=O), 1443 (C-N), 1065 (N-N), 1020, 995 (CS), 360 (Co-S), 340 (Co-N); Anal.($C_{28}H_{22}N_6O_4S_4Cl_2Co$) Found: C, 43.84; H, 2.69; N, 10.89; S, 16.65; Co, 7.59; Cl, 9.13 Calculated: C, 43.95; H, 2.87; N, 10.98; S, 16.74; Co, 7.70; Cl, 9.28. $[Co(IN\text{-}DtczH\text{-}Sal)_2](CH_3COO)_2$: Dec. temp.: 325 °C; yield: 80 %; IR(KBr)cm^{-1}: 3550-3250 (N-H), 1653 (C=O), 1444 (C-N), 1060 (N-N), 1030, 995 (CS), 345 (Co-S), 335 (Co-N); Anal.($C_{32}H_{28}N_6O_8S_4Co$) Found: C, 44.16; H, 3.19; N, 10.22; S, 15.68; Co, 7.00; CH_3COO, 14.38 Calculated: C, 47.32; H, 3.45; N, 10.35; S, 15.77; Co, 7.26; CH_3COO, 14.54. $[Ni(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$: Dec. temp.: 275 °C; yield: 85 %; IR(KBr)cm^{-1}: 3550-3250 (N-H), 1656 (C=O), 1443 (C-N), 1069 (N-N), 1021, 997 (CS), 370, 362 (Ni-S), 324, 332 (Ni-N); Anal.($C_{28}H_{22}N_6O_4S_4Cl_2Ni$) Found: C, 43.87; H, 2.77; N, 10.89; S, 16.62; Ni, 7.49; Cl, 9.12 Calculated: C, 43.96; H, 2.87; N, 10.99; S, 16.75; Ni, 7.68; Cl, 9.29. $[Cu(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$: Dec. temp.: 220 °C; yield: 90 %; IR(KBr)cm^{-1}: 3500-3200 (N-H), 1654 (C=O), 1445 (C-N), 1062 (N-N), 1028, 999 (CS), 355 (Cu-S), 310 (Cu-N); Anal.($C_{28}H_{22}N_6O_4S_4Cl_2Cu$) Found: C, 43.56; H, 2.68; N, 10.83; S, 16.52; Cu, 8.34; Cl, 9.18 Calculated: C, 43.68; H, 2.86; N, 10.92; S, 16.64; Cu, 8.26; Cl, 9.23. $[Zn(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$: M.P.: 280 °C; yield: 85 %; IR(KBr)cm^{-1}: 3550-3300 (N-H), 1658 (C=O), 1442 (C-N), 1066 (N-N), 1032, 999 (CS), 366 (Zn-S), 330 (Zn-N); Anal.($C_{28}H_{22}N_6O_4S_4Cl_2Zn$) Found: C, 43.44; H, 2.76; N, 10.76; S, 16.48; Zn, 8.51; Cl, 9.05 Calculated: C, 43.58; H, 2.85; N, 10.89; S, 16.60; Zn, 8.48; Cl, 9.20.

Elemental Analyses and Physical Measurements

Cobalt, nickel, copper and zinc in the complexes were determined volumetrically by EDTA titration using murexide as indicator in the case of cobalt and nickel, Pyrocatechol violet in the case of copper and Eriochrome Black T as indicator in the case of zinc metal ion. Chloride, acetate and sulphur content in the complexes was determined by methods as described earlier. Carbon, hydrogen and nitrogen analysis, molar conductance measurements (10^{-4} M DMSO solutions) and IR and solution electronic spectral (DMSO) and magnetic susceptibility measurements were made by the methods described elsewhere[16].

***In vitro* Antimicrobial Activity Screening**

As a preliminary screening for antimicrobial activity, compounds were tested against standard strains of gram(-) *Escherichia coli* (NCTC), *Pseudomonas aeruginosa* (NCTC), gram(+) *Staphylococcus aureus* (NCTC), *Enterococcus sp.* (ATCC) and the pathogenic fungus *Candida albicans*. Antimicrobial studies were performed according to agar dilution method. The MIC was expressed as the minimum compound concentration at which no growth of the pathogen takes place in 20-24 h (minimum inhibitory concentration). Various concentrations of ligands and metal complexes in the range of 200-1500 µg/ml were prepared in dimethylsulphoxide (DMSO Merck). A series of plates containing Mueller Hinton agar and solutions of metal complexes were prepared. The plates were dried at 36^0 for about 30 min in an incubator. To prepare the inoculum of the test strains, an inoculating loop was touched to four or five isolated colonies of the

pathogen growing on agar and then used to inoculate a tube of culture broth (peptone broth for bacterial strains and Sabourand's broth for fungal strain). The culture was incubated for two hours at 35^0 until it became slightly turbid and diluted to match 0.5 McFarland standard. The test strains were then placed on the dried solid Mueller Hinton agar + metal complex surface with the help of an inoculating loop (4 x 10^4 colony forming unit/ml). The plate was immediately placed in a 35^0 incubator, for 16-20 h. The lowest concentration of the compound resulting in no growth after 16-20 h of incubation is the MIC. The results were compared with that of chloramphenicol (MIC = 4 µg/ml)[17], a standard broad-spectrum antibiotic for prokaryotic microorganisms and nystatin (BDH) (MIC = 4-8 µg/ml)[18] for *Candida albicans* as positive control. Blank tests have shown that DMSO in the preparation of the test solution does not affect the test organisms.

RESULTS AND DISCUSSION

The ligand IN-DtczH-Sal and its metal complexes showed different antimicrobial activities against the test strains (Table1). The results of the MIC (minimum inhibitory concentration) values, the lowest compound concentration that inhibited microbial growth show that the ligand and all complexes are potential antimicrobial agents. The ligand IN-DtczH-Sal has been found to be most effective against the yeast *C. albicans* and bacterium *E. coli* with MIC values of 100 and 250 µg/ml respectively and moderately active against the *S. aureus* with MIC of 500 µg/ml.

All the transition metal isonicotinoyldithiocarbazates have been found to be most effective against *C. albicans* with MIC values in the range 75-1000 g/ml (Table 1), the best activity being shown by the $[Ni(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$ complex (MIC = 75 g/ml). Against the gram-negative pathogenic strain of *E. coli* the newly synthesized complexes have been found to inhibit the growth of bacteria with the MIC values in the range 188-750 µg/mL (Table 1). The complexes $[M(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$ (M = Ni(II) and Zn(II)) have been found to be remarkably active against this pathogen with MIC values of 188 µg/ml and 250 µg/ml respectively. MIC value of 500 µg/ml is observed for the $[M(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$ (M = Co(II) and Cu(II)) complexes. The isonicotinoyldithiocarbazate complexes under present investigation have shown antibacterial activity against *P. aeruginosa* with the MIC values ranging between 500-1000 µg/ml (Table). The $[M(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$ (M = Ni(II) and Zn(II)) complexes also show good antibacterial activity against *S. aureus* with MIC's of 375 and 500 µg/ml respectively.

A comparison of the MIC data of the present complexes under study suggests that the $[Ni(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$ complex is the most effective against all the microbes studied, except *Enterococcus faecalis*. The change of anion in the secondary coordination sphere in the $[Co(IN\text{-}DtczH\text{-}Sal)_2]X_2$ (X = Cl, CH_3COO) complexes leads to different antimicrobial activities. The $[Zn(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$ complex is also quite effective against *C. albicans* and *E. coli*.

The observed antimicrobial activity of the IN-DtczH-Sal and all of its the metal complexes finds support from the literature[12,19-22] because probably following factors can be operative. Chelation increases the liposolubility of the complexes which enhances the penetration of the complexes into the lipid membrane; the hydrocarbon tail functions as a lipophilic group to drive the compound through the semipermeable membrane of the cell; and blocks the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restrict further growth of the organisms. The presence of sulphur in the ligand molecule does apparently improve the activity of the ligand and its metal complexes. An additional important feature in the biologically active metal dithiocarbazate complexes of the present investigation may be imparted by the peculiar nature of the ligand in which heteroaroyl substitution and presence of salicylaldehyde group in IN-DtczH-Sal Schiff base may bring about a change in the coordinating ability through change in its hardness / softness tendency.

Table 1: Antimicrobial activity of the compounds

		Minimum Inhibitory Concentration (µg/ml)				
S.No.	Compounds	*E.coli*	*P.aeruginosa*	*S.aureus*	*E.sp.*	*C.albicans*
1.	$[Co(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$	500	500	1000	1000	1000
2.	$[Co(IN\text{-}DtczH\text{-}Sal)_2](CH_3COO)_2$	188	750	750	-	1000
3.	$[Ni(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$	75	188	750	-	375
4.	$[Cu(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$	1000	500	500	1000	1000
5.	$[Zn(IN\text{-}DtczH\text{-}Sal)_2]Cl_2$	250	250	1000	1000	500
6.	IN-DtczH-Sal	100	250	1000	1000	500
7.	DMSO	-	-	-	-	-
8.	Nystatin	04	-	-	-	-
9.	Chloramphenicol	-	08	16	16	08

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