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Synthesis and characterization of halocobaloximes and its derivatives for antimicrobial activity

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Abstract: Cobaloximes of the types Trans-$BH_2[Co(dmgH)_2X]$, where $dmgH^-$ = Dimethylglyoximate, X^- = Cl^- or Br^- and Trans-$[Co(dmgH)_2(H_2O)I]$.B where, B = Piperazine (Pp) or 2-(N-Tert-butylamido)piperazine (t-Bu-PpAM) were prepared and characterized by TG/DTA, FABMS UV-visible, IR, NMR spectra and XRD studies. All the cobaloximes were screened against microorganisms for their antimicrobial activity and compared with streptomycin as standard. The free ligands viz., Pp & t-Bu-PpAM, showed antimicrobial activity in the order: Pp < t-Bu-PpAM. Most of the cobaloximes were found to be more active than the corresponding ligands such that the antimicrobial activity order for the axial halides was found to be Cl^- > Br^- > I^- for cobaloximes having piperazine where as in the case of cobaloximes having 2-(N-Tert-butylamido)piperazine the antimicrobial activity order was found to be reversed as Br^- > Cl^- >I^- .

Keywords: Cobalt(III) complexes, cobaloximes, antimicrobial activity of cobalt (III) complexes.

Introduction

Cobaloximes have been used as chemical models for vitamin-B_{12}. The urge to explore modification in such vitamin-B_{12} models leads way to the designing of simple models containing cobalamins (Pratt, 1972; Dodson *et al.*, 1981). Metal complexes containing nitrogen and sulphur donor atoms have been proved to be potential antibacterials as well as good fungicides. A large number of piperazine compounds have been tested for their antimicrobial and antibacterial activities. The piperazine motif appears in many drugs displaying anti-allergenic (Schering Corp), antibacterial (Laboratoire Roger Bellon), anti-anxiety (Taisho Pharmaceutical Co), anti-emetic (Schering Corp), antimigraine (Folia Pharmacol & Merck) and platelet anti-aggregatory (GlaxoSmithKline) activities. Derivatives of heterocyclic compounds such as pyridazine , pyrimidine and pyrazine (Acheson,1967) are valuable chemotherapeutic agents. Schiff's base complexes of nickel (Sllema& Ramesh Babu, 2005) and thio semicarbazone complexes of copper (Rai *et al.*, 2005) showed moderate to good antibacterial activity against several bacteria. Our earlier studies on pyrazine (Pz) and its derivatives showed that they are effective donors for cobaloximes with considerable antibacterial activity (Martin *et al.*, 2008).The present study aims at the use of piperazine (Pp) and 2-(N-Tert-butylamido)piperazine (t-Bu-PpAM) as effective axial donors for cobaloximes and as possible antimicrobially active cobalt(III) complexes.

Materials and Methods

Cobalt(II) carbonate obtained from Loba Chemie, Mumbai was treated with calculated amount of aq.HBr and evaporated to get crystals of cobalt(II) bromide. Cobalt(II) chloride(NICE Chemical Pvt Ltd. Cochin), SISCO sample of dimethylglyoxime (E.Merck) and Sigma-Aldrich samples of piperazine and 2-(N-Tert-butylamido)piperazine were used for the preparation of the complexes. Dimethyl sulphoxide (SD Research laboratory) was dried over calcium hydride and distilled fractionally at reduced pressure. The distilled solvent was stored under molecular sieves and used.

Preparation of the cobaloximes

Preparation of chlorocobaloximes: The green colored complex *Trans*-Hydrogen dichlorobis (dimethylgloximato) cobaltate (III), $H[Co(dmgH)_2Cl_2]$ and the other cobaloximes derived from it viz., $PpH_2[Co(dmgH)_2Cl_2]_2$ and (t-Bu-$PpAMH_2$) $[Co(dmgH)Cl_2]_2$ were prepared by adopting the method reported earlier (Pahor *et al.*, 1985). Hydrogen dichlorobis (dimethylglyoximato) cobaltate(III) (0.01mole) and the corresponding 0.01 mole of the piperazine or 2-(N-Tert-butylamido) piperazine were taken in about 60 mL of absolute alcohol and stirred for 60 min with warming, over water bath for 1hr until the green colour due to dichloro complex was discharged giving the required brown colored complexes.

Preparation of bromocobaloximes: Using cobalt(II) bromide and dimethylglyoxime , the green colored *Trans*-Hydrogen dibromobis(dimethylgloximato)cobaltate(III), $H[Co(dmgH)_2Br_2]$ and the other complexes derived from it viz.,$PpH_2[Co(dmgH)_2Br_2]_2$, (t-Bu-$PpAMH_2$) $[Co(dmgH)_2Br]_2$ were prepared by the same method as explained above.

Preparation of iodocobaloximes: Hydrogen dichlorobis(dimethylglyoximato)cobaltate(III), (0.01mole) was mixed with 30 mL of water and exposed to microwave irradiation for 3 min at regular intervals of 30 s each time after cooling down to room temperature. The completion of the reaction was indicated by the color change from green to light brown indicating the formation of aquochlorobis(dimethylglyoximato) cobalt(III), $[Co(dmgH)_2(H_2O)Cl]$. The aquochloro complex, viz., $[Co(dmgH)_2(H_2O)Cl]$ thus obtained, was filtered and treated with 0.01mole of KI in 30 mL of water and subjected to microwave irradiation as above till the solution turned dark brown(Vijayraghavan & Dayalan,1992,2001). The brown colored product, aquoiodobis(dimethylglyoximato)cobalt(III) viz.,$[Co(dmgH)_2 (H_2O)I]$ crystalised when allowed to stand over night. The aquoiodocobaloxime thus obtained was mixed separately with the equimolar amounts of Pp or t-Bu-PpAM and subjected to microwave irradiation for 3 min as above until the light brown color turned dark brown. Such iodocobaloximes, viz., $[Co(dmgH)_2(H_2O)I]$.Pp and $[Co(dmgH)_2(H_2O)I]$.t-Bu-PpAM were obtained as dark brown crystals on standing overnight.

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Table 1. Antimicrobial activity of cobaloximes containing piperazine screened against human pathogens (- =No activity)

Microbes	Cobaloximes(µg/disc), Zone of inhibition in mm									*Streptomycin* 10µg/disc
	$PpH_2[Co(dmgH)_2Cl_2]_2$			$PpH_2[Co(dmgH)_2Br_2]_2$			$[Co(dmgH)_2(H_2O)I].Pp$			
	250	500	1000	250	500	1000	250	500	1000	
Staphylococcus aureus	12	12	14	-	-	10	-	-	-	13
Yersinia enterocolitica	13	14	17	8	8	10	-	-	-	24
Xanthomonas pv.oryzae (Erwinia amylovora)	-	-	10	-	-	10	-	-	-	18
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-
Vibrio parahaemolyticus	-	-	10	-	-	-	-	-	-	24
Vibrio fischeri	7	7	10	-	-	10	7	7	10	13
Enterobacter aerogens	-	-	-	8	12	14	-	-	-	19
Bacillus subtilis	-	-	-	-	-	-	-	-	-	24
Escherichia coli	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	-	--
Candida albicans	-	--	-	-	--	-	-	-	-	-
Salmonella typhi	11	12	12	8	8	8	-	-	-	12
Staphylococcus epidermidis Epidermics	8	8	12	8	11	12	-	-	-	22
Enterococcus faeculis	18	18	21	-	-	-	-	-	10	25
Klebsiella pmermia	16	17	19	-	-	12	-	-	-	20

Table 2. Antimicrobial activity of cobaloximes containing 2-(N-Tert-butylamido)piperazine screened against human pathogens

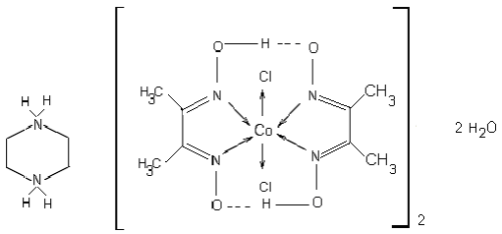
Microbes	Cobaloximes (µg/disc), Zone of inhibition in mm (- *= no activity*)									*Streptomycin* 10µg/disc
	(t-BuPpAMH$_2$) $[Co(dmgH)_2Cl_2]_2$			(t-BuPpAMH$_2$) $[Co(dmgH)_2Br_2]_2$			$[Co(dmgH)_2$ $(H_2O)I]$.t-BuPpAM			
	250	500	1000	250	500	1000	250	500	1000	
Staphylococcus aureus	-	10	10	8	11	15		10	14	18
Yersinia enterocolitica	-	-	-	8	9	13	13	17	21	16
Pseudomonas aerugenosa	11	11	13	8	10	14	-	12	14	14
Vibrio fischeri	-	-	10	-	9	12	-	-	-	11
Enterobacter aerogens	8	8	11	8	9	9	-	-	-	21
Bacillus subtilis	-	-	10	8	9	11	-	-	-	25
Escherichia coli	-	-	-	-	-	-	-	-	-	10
Proteus vulgaris	12	16	19	-	-	-	-	-	-	10
Candida albicans	-	-	-	-	-	-	-	-	-	18
Salmonella typhi	10	10	12	8	9	11	-	-	-	25
Epidermics	-	-	16	12	8	13	10	-	12	22
Enterococcus faeculis	-	10	14	10	9	10	-	12	-	24
Klebsiella Pmermia	-	-	15	10	10	12	-	12	-	16

Characterization of the complexes

The complexes were characterized by elemental, thermal analysis, FABMS (CDRI, Lucknow) , UV-visible, IR and NMR spectra. The cobalt in the complexes was estimated by Kitson method (Kitson,1950).The UV-visible spectra were recorded on LAMBDA-125 spectrophotometer in ethanol using matched quartz cells of path length 1 cm, IR spectra were obtained on KBr pellet on PERKIN ELMER IR Spectrum-1 spectrophotometer and the NMR spectra were recorded in DMSO-d_6 using JOEL 400 MHz NMR spectrophotometer. The thermograms (TG/ DTA) were recorded on Perkin Elmer Model Pyris Diamond at a heating rate of 10°C min^{-1} with argon gas purged at the rate of 12 L hr^{-1}. The cyclic voltagrams of the cobaloximes, on the surface of a Pt disk electrode in acetonitrile solution, were recorded in the potential range + 1.6 to -1.6 V using CH instrument model No-620 B Electrochemical analyzer.

The single crystal XRD data collection was done using Bruker axis (Kappa apex2) diffractometer 4985 independent reflections

Fig. 1. N,N'-Dihydrogenpiperazonium dichloridobis(dimethylglyoximato-k^2N,N')cobaltate(III) dihydrate, $PpH_2[Co(dmgH)_2Cl_2].2H_2O$



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Radiation source: fine-focus sealed tube 4156 reflections with I > 2s(I) Monochromator: graphite Rint = 0.038 T = 293(2) K .max = 29.9º and f scan .min = 2.1º .Absorption correction: multi-scan h = -11.11 Tmin = 0.752, T_{max} = 0.940, k = -14.14, 20840 measured reflections l = -15.15.

Antimicrobial studies

The following test organisms were commonly used to test the antimicrobial activity using disc diffusion method : *Staphylococcus aureus(*ATCC 25923), *Pseudomonas aeruginosa (*ATCC 27853), *Staphylococcus epidermidis* (MTCC 3615), *Vibrio fischeri* (MTCC 1738), *Enterococcus.faecalis* (ATCC 29212) Gram-positive, *Yersinia enterocolitica* (MTCC 840), *Vibrio parahaemolyticus*(MTCC 451), *Salmonella Typhi* (MTCC 733), *Enterobacter aerogens* (MTCC 111), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (ATCC 25922), *Proteus vulgaris* (MTCC 1771), *Erwinia* sp. (MTCC 2760) and *Candida albicans* (MTCC 227) with *Streptomycin* (*S*) as positive control. The complexes were also tested against clinical isolates, obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamilnadu, India. Each bacterial strain was inoculated in 3mL of Mueller Hinton broth and incubated at 37ºC for 24hrs. After incubation period, the culture was diluted. Antimicrobial activity studies were carried out using disc-diffusion method (Murray,1995). Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test cultures (100 µL of suspension containing 10^8 CFU/mL bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations of the crude extract (1000 µg, 500 µg and 250 µg per disc). The loaded discs were placed on the surface of the medium and left for 30 min at 37°C for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10µg /disc) was used as positive control. The plates were incubated for 24 hrs at 37°C. Zone of inhibition were recorded in millimeters and the experiments were repeated twice. The results are included in Tables 1 & 2

Results and discussion

Dichloro- and dibromocobaloximes are intense green crystalline compounds (Nayak, 2003). They turn brown on treatment with one mole of heterocyclic donors like piperazine (Pp) or 2-(N-Tert-butylamido) piperazine (t-Bu-PpAM).

Elemental analysis

The elemental analysis data, obtained by analytical methods agree well with the theoretical data proposed for cobaloximes (Table 3).

Thermal data

The thermal studies of the cobaloximes suggest that the axial halogen dissociates at 230° C, followed by the dissociation of piperazine (Nayak, 2003), based axial ligand around 250° C .The remaining residual complex viz; $[Co(dmgH)_2]$ was found to be stable up to 340°C, after which it decomposed to Co_2O_3 as a final residue (Brown,1997).

FAB Mass spectra

The molecular ion peak of the fast atom bombardment mass spectra (FABMS) of the cobaloximes confirm their molecular weights :$PpH_2[Co(dmgH)_2Cl_2]_2$; m/z= 410.5 and (t-Bu-$PpAMH_2$) $[Co(dmgH)_2\ Cl_2]_2$;m/z= 492.5.

UV-Visible spectra

The UV Spectrum of $PpH_2[Co(dmgH)_2Cl_2]_2$ showed a shoulder around 330 nm which may by due to the ligand to metal charge transfer (LMCT).This kind of LMCT peak was found to be disappearing upon reduction of similar cobaloximes by Fe(II) or Cr(III). The electronic spectrum of the complexes showed almost similar absorption pattern. For example the electronic spectrum Cr(II) (Dayalan & Vijayaraghavan,1993; 2001).The weak transition around 570 nm for the cobaloximes may be attributed to d^6 low spin cobalt(III) spin allowed d-d transitions viz., $^1A_{1g} \Rightarrow {}^1T_{1g}$ other high energy spin allowed d-d transition viz., $^1A_{1g} \Rightarrow {}^2T_{1g}$ occurring in the range 370-385 nm.

IR spectra

The C=N stretching of oxime in its complexes was observed in the range 1550-1600 cm^{-1} and the intra molecular hydrogen bonded -OH around 3400 cm^{-1}. A moderate intense peak around 1240 cm^{-1}, observed for all the complexes, may be assigned to the =N-O- stretching of the oxime in the complexes. The peak around 520 cm^{-1} noted for all the cobaloximes could be ascribed to cobalt (III)-nitrogen stretching.

NMR spectra

The methyl protons of dimethylglyoxime, in all the coboloximes, appear as a sharp singlet at at δ= 2.3 ppm (12H, s) (Silverstein & Bassler, 1984) whereas the hydrogen bonded hydroxyl protons of the oxime resonate at δ= 8.7 ppm (2H, s). The piperazine protons in the complexes of the type: $PpH_2[Co(dmgH)_2X_2]$ appear as doublet at δ = 8.0 ppm (d,4H)

Cyclic Voltametry

The cyclic voltagrams of the cobaloximes exhibited two well defined cyclic responses at -0.9 V and -1.4 V corresponding to Co(III)/Co(II) and Co(II)/Co(I) couples,

Table 3. Elemental data for the cobaloximes

Code	Complex	C		H		N		Co	
		Exp	Cal	Exp	Cal	Exp	Cal	Exp	Cal
1	$PpH_2[Co(dmgH)_2Cl_2]_2$	35.6	35.12	5.7	5.85	20.73	20.48	14.25	14.39
2	$PpH_2[Co(dmgH)_2Br_2]_2$	31.35	31.64	5.21	5.27	18.94	18.46	12.76	12.96
3	$[Co(dmgH)_2(H_2O)I]$.Pp	28.38	28.68	4.74	4.78	16.81	16.9	13.59	13.68
4	(t-Bu-$PpAMH_2$)$[Co(dmgH)_2Cl_2]_2$	41.81	41.04	6.12	6.4	19.88	19.9	11.20	11.87
5	(t-bu-$PpAMH_2$)$[Co(dmgH)_2Br_2]_2$	37.65	37.60	5.72	5.9	19.79	18.01	10.82	10.8
6	$[Co(dmgH)_2(H_2O)I]$.(t-Bu-PpAM)	34.8	34.64	5.0	5.43	16.03	16.63	10.76	10.84

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respectively (Shamsipur, 2001)

XRD studies

The single crystal XRD studies were done for a representative complex viz., $PpH_2[Co(dmgH)_2Cl_2]$. The study reveled that piperazine lies outside in the form of diprotonated cation as PpH_2^{2+} (Fig.1). Hence, a similar pattern was expected for the corresponding dibromo complexes whereas for the neutral iodocobaloximes it was proposed that they may act as molecular complexes with piperazine or 2-(N-Tert-butylamido)piperazine lying outside coordination sphere.

Antimicrobial studies

The zones of inhibition greater than 10 mm/disc are considered to be active. In general, the cobaloximes showed more activity than the respective free ligands. The antimicrobial activities of the cobaloximes were found to be dose dependent and were active at higher concentrations. However, majority of cobaloximes exhibited antimicrobial activity less than the commercial antibiotic, streptomycin. The cobaloxime $PpH_2[Co(dmgH)_2Br_2]$ showed moderate activity; whereas, $PpH_2[Co(dmgH)_2Cl_2]$ were more effective towards tested microbes.

But, $[Co(dmgH)_2(H_2O)(I)]$.Pp showed high inactivity except for (MTCC 111) and (ATCC 29212) at 1000 µg/disc.Maximum inhibition zone was observed against (ATCC 29212) and (ATCC 15380). Chlorocobaloxime are found to be more active than bromo and iodo counterpart with antibacterial order of axial halide $Cl^- > Br^- > I^-$ for the complexes having piperazine (Table 1). A slightly different trend viz., $Br^- > Cl^- > I^-$ was observed for halocobaloximes containing 2-(N-Tert-butylamido) piperazine (Table 2).

Conclusion

The halogenocobaloximes with piperazine (Pp), 2-(N-Tert-butylamido)piperazine piperazine carboxamide (t-Bu-PpAM), as their diprotonated counter cation or as neutral lattice molecules, were prepared and characterized. The free ligands: Pp and t-bu-PpAM showed antibacterial activity in the order : Pp < t-Bu-PpAM ; whereas, the free equatorial ligand $dmgH_2$ was inactive against all of the pathogen tested. Chlorocobaloximes are found to be more active than bromo- and iodocobaloximes in the order for the axial halides as $Cl^- > Br^- > I^-$ having piperazonium ion, PpH_2^{2+} as counter cation ion or piperazine as molecular complex; whereas, in the case of cobaloximes 2-(N-Tert-butylamido) piperazine the antibacterial activity order observed was $Br^- > Cl^- > I^-$. The over all analysis on the antimicrobial activity revealed that among all the tested cobaloximes, bromocobaloximes have superior activity than the corresponding chloro- and iodo-cobaloximes.

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