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Synthesis, characterisation and antifungal activity of Cr(III), Mn(II), Fe(III), Co(II) and Ni(II) complexes of N-(2-nitro)-benzilidene-3-hydrazino benzoxazine-2-one

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Abstract: Cr(III), Mn(II), Fe(III), Co(II) and Ni(II) complexes of N-(2-nitro)-benzilidene-3-hydrazino benzoxazine-2-one (NBHBO) have been synthesized and characterized by elemental analysis, conductance, thermal, spectral and magnetic data. NBHBO acts as a bidentate NN donor towards trivalent and NO donor towards divalent metal ions. Octahedral geometries have been proposed for all the Complexes. The ligand, *NBHBO*, and its complexes were tested against Neurospora crassa, a non-pathogenic fungus. The Co(II) and Ni(II) complexes exhibited higher antifungal activities.

Keywords: Metal complexes; N-(2-nitro)-benzilidene-3-hydrazino benzoxazine-2-one; NN donor; ON donor.

INTRODUCTION

Benzoxazines are an important class of oxygen-nitrogen heterocyclic compounds with a variey of biological activities, specifically as antimicrobial(1), antifungal(2,3), antiangiogenic therapeutic agents(4), antiarrhythmics(5), neuropeptide y5 antagonists(6), neuroprotective agents(7), estrogen receptorβ agonists(8), antimycobacterial agents(9). Metal complexes of benzoxazine derivatives(10) have received less attention inspite of their potential metal binding properties and promising applicabilities. Importance was hence given to study these systems in our laboratories. In view of the importance of such benzoxazines, we describe here the synthesis and characterization of Cr(III), Mn(II), Fe(III), Co(II) and Ni(II) complexes of N-(2-nitro)-benzilidene-3-hydrazino benzoxazine-2-one (NBHBO).

EXPERIMENTAL

All the chemicals used were either of AR or chemically pure grade. Mass spectrum was recorded on a PerkinElmer Hitachi-RMU-61 spectrometer and ^{1}H NMR spectra was recorded in DMSO-d_6 at room temperature using TMS as internal standard on a Varian XL-200MHz. IR spectra were recorded using KBr discs in the 4000–400 cm^{-1} region on a Schimadzu IR-435 and in Nujol media in the 1000–200 cm^{-1} region on a PerkinElmer IR spectrometer Model No 7. Electronic spectra of solids were recorded on a Cary-2390 UV–Vis-

NIR spectrophotometer. The elemental analysis was carried out using Heraus-CHN-rapid analyzer. Chloride analysis was carried out by Mohr's method. Metal contents were estimated using Lab tam Plasma Scan-8410. Magnetic susceptibilities of complexes were measured on a Faraday balance CAHN-7550-03 USA at room temperature using $Hg[Co(NCS)_4]$ as calibrant. Diamagnetic correction using Pascal's constants and temperature independent paramagnetic correction were computed (11). The electrical conductance measurements were recorded using 10^{-3} M solutions in DMSO with an Elico digital conductivity bridge (Model CM-180) and dip type cell calibrated with KCl solutions. DTA was carried using a Lead and Northup-USA instrument and TGA on a PerkinElmer model TGS-2 instrument.

Synthesis of NBHBO

NBHBO was prepared by a three-step process involving the synthesis of benzoxazine-2,3-dione(12) and 3-hydrazinobenzoxazine-2-one (13). To 3-hydrazinobenzoxazine-2-one (0.200 gms) dissolved in hot distilled water (50 ml), 2-nitrobenzaldehyde (0.150 gms) was added. The reaction mixture was refluxed on a heating mantle for three hours. Dark yellow coloured compound was separated out. The compound was washed several times with distilled water and dried in vacuo. Yield : 55%; m.p. < 250^oC.

Synthesis of the complexes

A general method was adopted for the preparation of all the complexes. A methanolic solution of the ligand was added in small increments to a methanolic solution of the metal chloride. After complete addition the metal to ligand mole ratio was always kept as 1:2. The pH of the reaction mixture was then raised to 7 using a 1% alcoholic ammonia solution. The reaction mixture was refluxed for 3-4 h. The colored product obtained was filtered hot and washed successively with small increments of methanol, petroleum ether and ether and dried in vacuo. The purity of ligand and metal complexes was checked by TLC using 1:1 ethyl acetate and methanol mixtures. The analytical data and proposed formulae for the complexes are given in Table 1.

Pharmacology

A wild stain of Neurospora crassa (FGSC # 42000), obtained from Fungalenetics Stock Centre, Kansas City, U.S.A., was grown and maintained on a basal medium containing (G/100 ml) glucose, 2; Ammonium nitrate, 0.2; ammonium nitrate, 0.1; KH_2BO_4, 0.03; NaCl, 0.01; $CaCl_2$, 0.01; $MgSO_4 .7H_20$, 0.05, trace elements included as sulphates were (mg/100 ml) zinc, 20; manganese 20; iron 10; copper 8; ammonium molybdate, 2; biotin was added to final concentration of 0.5 (μ/100 ml). The organism was maintained by weekly subcultures on 2% agar slants, sterilization of media was carried out by autoclaving at 15 Lb/inch fnr 15 minutes. The organism was grown in 10 ml basal medium for 48 hours at 30^{o}"C, pH 4.8-5.0 in 50 ml conical flasks.

The metal salts, ligand, metal complexes and related compounds were aseptical1y added in solid form to the medium. A thick conical suspension of Neurospora crassa prepared from 7-10 days agar slant was inoculated into the medium (2-3 drops/flash). Cultures were incubated at 30°C for 2 days. At the end of incubation period the myceliia were harvested by filteration washed with distilled water and dried at 60°C overnight in hot air oven. The dry weight of the mycellium was recorded as the growth parameter and the inhibition of the growth by each of these compounds was calculated as percentage inhibition against the control culture (without added compounds) as follows :

(a) X =Dry weight of mycellium in control system in gms

(b) Y= Dry weight of mycellium in non-control system at different concentration in gms

$$\text{\% of growth inhibition by compound (IB)} = \left(\frac{Y}{X} X100\right)\text{-100}$$

All the growth experiments were repeated at least three times and only the average values were used to express the results.

RESULTS AND DISCUSSION

Characterization of the ligand

The analytical data of NBHBO suggests the molecular formula of $C_{15}H_{10}N_4O_4$. The analytical data also suggests the 1:1 condensation of o-nitro benzaldehyde (Table1) with 3-hydrazino-1, 4-benzoxazine-2-one (m/z 307).

^{1}H-NMR spectrum of ligand in DMSO-d_6 suggests the presence of two possible tautomeric forms (Figure 1) in solution(14). The spectrum has given two resonance signals with low intensity at δ11.45-11.06 can be attributed to ring NH and exocyclic NH groups. The integration of which put together equals to one proton which is shared by the two tautomers. This shows that two tautomeric forms of the ligand are at equilibrium in DMSO solution. The characteristic azomethine proton was identified at δ8.8 (singlet). The spectrum also indicated a broad multiplet band in the region δ 7.76-6.53 which can be assigned to phenyl protons. The integral strengths and ratio of the protons agree with the expected structure.

The IR spectrum of NBHBO presents several fundamental functional group bands corresponding to the expected structure. The spectrum has exhibited a sharp band at 3200 cm^{-1}, which can be assigned to ν N-H of ring. A broad band with a shoulder appearing at 1663 cm^{-1} can be attributed to the lactone carbonyl and free azomethine groups. The spectrum has shown a sharp band at 1604 cm^{-1} which can be assigned to exocyclic azomethine group. The spectrum has exhibited two bands at 1527 cm^{-1} and 1344 cm^{-1} which are due to the ν NO_2 asymmetric and symmetric stretching vibrations(15).

The IR data of the NBHBO ligand concludes that the exocyclic azomethine structure is favoured in solid state (Fig.1).

The electronic spectrum of NBHBO showed absorptions at 43478 cm^{-1}, 37037 cm^{-1}, 35714 cm^{-1}, 27277 cm^{-1}, 23255 cm^{-1} and 22222 cm^{-1}. The high energy band at 43478 cm^{-1} is due to the π-π* electronic transition corresponding to the benzene moiety. The band observed at 37037 cm^{-1} is a characteristic of nitro group to the benzene ring. The band observed at 35714 cm^{-1} can be assigned to benzoxazine moiety. The absorption at 27777 cm^{-1} is considered to be due to the lactone carbonyl group. The bands observed at 23255 and 22222 cm^{-1} are due to the exocyclic and free azomethine groups respectively.

Table 1: Analytical Data of Metal Complexes

No	Complexes with molecular formula	Calcd. (Found) %				
		Metal	Carbon	Hydrogen	Nitrogen	Chloride
1	NBHBO ($C_{15}H_{11}N_4O_4$)		58.06 (57.90)	3.22 (3.20)	18.06 (17.98)	-
2	[Cr(HBO)NH_3Cl_3] ($CrC_{15}H_{13}N_5O_4Cl_3$)	10.70 (10.40)	37.07 (36.98)	2.67 (2.79)	14.41 (14.20)	21.93 (21.50)
3	[Mn(NBHBO)H_2O NH_3Cl_2] (Mn $C_{15}H_{15}N_5O_5Cl_2$)	11.66 (11.00)	38.22 (37.00)	3.18 (2.95)	14.86 (14.50)	15.00 (14.85)
4	[Fe(NBHBO)NH_3Cl_3] (Fe $C_{15}H_{13}N_5O_4Cl_3$)	11.41 (11.20)	36.78 (36.00)	2.65 (2.71)	14.30 (14.20)	21.76 (21.00)
5	[Co(NBHBO)H_2O NH_3Cl_2] (Co $C_{15}H_{15}N_5O_5Cl_2$)	12.40 (12.60)	37.90 (36.50)	3.15 (3.05)	14.73 (14.00)	14.94 (14.50)
6	[Ni(NBHBO)$(H_2O)_2$ Cl_2] (Ni $C_{15}H_{14}N_4O_6Cl_2$)	12.33 (12.88)	37.83 (36.90)	2.94 (2.91)	11.77 (11.50)	14.92 (14.20)

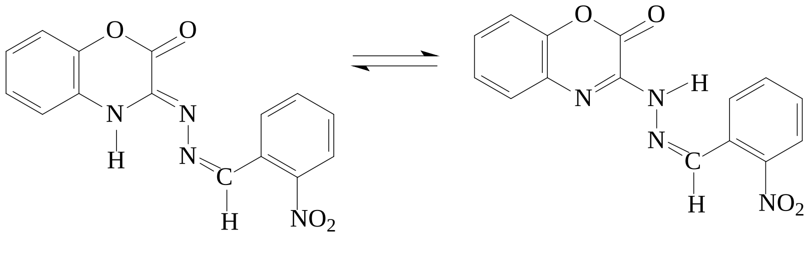


Fig 1. Tautomeric forms of NBHBO

Characterization of the complexes

All the complexes are dark in colour and quite stable to air and moisture. They neither melt nor decompose below 300°C. All the complexes are insoluble in most of the polar and non-polar solvents, but sparingly soluble in dimethyl sulphoxide (DMSO).

Elemental, conductance and thermal analysis of metal complexes

The analytical data of all the complexes is given in Table 1. The analysis of metal, carbon, hydrogen, nitrogen and chloride reveals the following formulae $CrC_{15}H_{13}N_5O_4C1_3$, $MnC_{15}H_{15}N_5O_5Cl_2$, $FeC_{15}H_{13}N_5O_4Cl_3$, $CoC_{15}H_{15}N_5O_5Cl_2$ and $NiC_{15}H_{14}N_4O_6Cl_2$. The data confirms that the metal to ligand ratio is 1: 1 in all the complexes. The data suggests the presence of two chlorides per divalent metal ion and three chlorides per trivalent metal ion in the complexes. This indicates that the ligand is neutral in nature. The data also suggests the presence of ammonia and water in complexes in addition to the expected ligand.

The conductivity mensurements were carried out in freshly prepared $1x10^{-3}$ M dimethyl sulphoxide (DMSO) solutions. The negligible molar conductance values in the range 2-8 mho cm^2 $mole^{-1}$ indicate the presence of chlorides within the coordination sphere in all the complexes(16).

TGA/DTA analysis is in support to establish the presence of coordinated water in Mn(II), Co(II) and Ni(II) complexes, wherein the endotherms are observed at $225-260^0C$. The elimination of water in Mn(II) and Co(Il) is in a single step while the same in Ni(II) is in two steps, at 250° and 260°C (17).

Infrared spectra

The characteristic IR bands of NBHBO and its metal complexes are presented in Table 2.

The IR spectra of metal complexes in comparison with the spectrum of free NBHBO suggest two types of binding modes differentiating between trivalent and divalent metal ions. It appears that the ligand tautomerises from exocyclic azomethine to ring azomethine systems in trivalent ions. The strong rather multiple band assigned to a combination of νC=O and νC=N (free) gets clearly differentiated into two bands in all the complexes. These two bands are located at $1700cm^{-1}$ and around 1563 cm^{-1}in Cr(III) and Fe(III) complexes, corresponding to νC=O and νC=N (free) respectively. These bands record a positive shift with respect to νC=O and negative shift with respect to νC=N (free), thus ruling out the possibility of complexation through lactone carbonyl and supporting metal binding through free azomethine nitrogen. The situation is different with divalent metal ions. In Mn(II), Co (II), Ni(II) complexes these bands are located around 1600 $cm-^1$ and 1560-1570 cm^{-1} indicating complexation through both the groups, involving lactone oxygen and free azomethine nitrogen in bonding. The νC=O band observed around $1600cm^{-1}$ is strong with multiple appearance, this may be due to the merging of νC=N (exo) and νC=C with νC=O, all of which can appear around the same region. Coordination through νC=N (exo) is ruled out on the basis of this observation and it is concluded that the ligand NBHBO is differentiating the trivalent metal ions, from the divalent ones with respect to lactone carbonyl oxygen binding, while νC=N (free) is involved in bonding in all the complexes the lactone carbonyl is preferring only divalent metal ions for coordination. There is no change in the asymmetric and symmetric stretchings of $–NO_2$ group, indicating non-involvement of $-NO_2$ group in coordination. The spectra of Cr(III) and Fe(III) complexes show a shoulder like structure in the range of $1500-1520cm^{-1}$ appended to these νNO_2 (asy) band, which can be understood to represent νC=N (ring) in these complexes. The position of this band at relatively lower frequency is attributed to its participation in coordination towards the trivalent metal ion. Close examination of the spectra of trivalent metal ion complexes shows the disappearance of νN-H (ring) corresponding to the free ligand at 3200 cm^{-1} and appearance of a sharp band at 3282 cm^{-1} assignable to νN-H of substituted hydrazine side chain. This observation supports the conversion of NBHBO from exocyclic azomethine system to ring azomethine system and subsequent involvement of ring azomethine nitrogen in coordination towards the trivalent Cr(III) and Fe(III) metal ions.

The spectra of complexes show strong bands in the region of $3396-3400cm^{-1}$ which can be attributed to the asymetric and symetric stretching modes of coordinated NH_3(19). This was also supported by the appearance of bending and rocking modes of the coordinated ammonia molecule in the region of $1152-1310cm^{-1}$ and $643-740cm^{-1}$. Mn(II), Co(II) complex spectra show multiple bands in the region of $3200-3418cm^{-1}$ (18) due to the coordinated ammonia and water. This was also supported by rocking mode of vibrations appeared in the region of $603-620cm^{-1}$ and $880-958cm^{-1}$ due to the coordinated ammonia and water molecules. In addition to this the spectra show low- medium intensity bands in the region of $550-650cm^{-1}$ due to the waging mode of coordinated water. Ni(II) complex spectrum shows a broad trough in the region of 3415cm^{-1} due to the coordinated water, this can also be substantiated by rocking and wagging mode of vibrations which appeared in the region of 650-980cm^{-1}. The Cr(III) and Fe(III) spectra show some new bands due to the M-N and M-Cl in the region of 460-323 cm-^{1} and 265-258 cm-^{1}. Mn(II), Fe(III) and Ni(II) complex spectra show non ligand bands due to the M-O, M-N and M-Cl stretching at 405-400cm^{-1} , 388-321cm^{-1}and 252-210 cm^{-1}(19).

The IR spectra of complexes suggest the ligand is neutral and showing ambidenticity involving NN donor sequency in trivalent ions and ON donor sequency in divalent ions.

Table 2: Characteristic Infra Red Frequencies Of Ligand & Complexes (cm^{-1})

No	Compound	ν_{NH} (ring)	ν_{NH} (exo)	$\nu_{C=O}$	$\nu_{C=N}$ (free)	$\nu_{C=N}$ (exo)	$\nu_{C=N}$ (ring)	ν_{NO2} (asym)	ν_{NO2} (sym)	New Bands
1	NBHBO	3200	_	1663		1604	_	1527	1344	_
2	[Cr(NBHBO)NH$_3$Cl$_3$]	_	3282	1700	1565	_	1510	1500-1520		3400, 1310, 740, 460, 265
3	[Mn(NBHBO)H$_2$ONH$_3$ Cl$_2$]	_	_	1600	1570			1526	1346	3400, 3200, 880, 620, 570, 400, 330, 280
4	[Fe(NBHBO)NH$_3$Cl$_3$]	_	3282	1700	1563	_		1500-1520		3396, 1152, 643, 323, 258
5	[Co(NBHBO)H$_2$O NH$_3$ Cl$_2$]	_	_	1607	1560			1526	1350	3418, 958, 603, 403, 321
6	[Ni(NBHBO)(H$_2$O)$_2$Cl$_2$]	_	_	1600	1570			1524	1346	3415, 980, 650, 405, 338

Table 3: Magnetic and Electronic Spectral Data of Complexes.

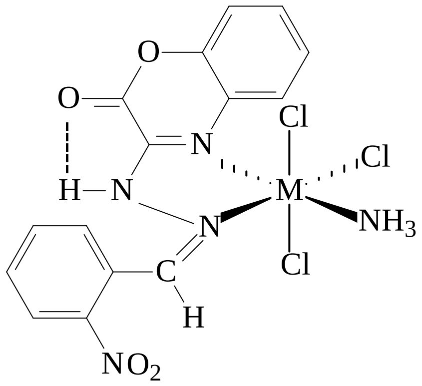
No	Complex	$_{eff}$ (BM)	Band position (cm^{-1})	Assignment
1	Cr(III)	3.80	24390-25641 16949 14285	${}^4A_{2g} \rightarrow {}^4T_{2g}$ ${}^4A_{2g} \rightarrow {}^4T_{1g}$ ${}^4A_{2g} \rightarrow {}^4T_{1g}(P)$
2	Mn(II)	6.10	26315-15384	-
3	Fe(III)	5.83	23255-11622	-
4	Co(II)	4.84	18818 11363-15267 6369-8130	${}^4T_{1g} \rightarrow {}^4T_{1g}(P)$ ${}^4T_{1g} \rightarrow {}^4A_{2g}$ ${}^4T_{1g} \rightarrow {}^4T_{2g}$
5	Ni(II)	3.19	20000-25641 14700-17857 11111	${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$ ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$ ${}^3A_{2g} \rightarrow {}^3T_{2g}$

Magnetic Moments and Electronic spectra

The room temperature magnetic moments and electronic spectral data are given in Table 3**.** The former data reflect the paramagnetic nature of all complexes. These values are consistent with a spin free con figuration. The absorption maxima for the complexes and the corresponding assignments of the electronic transitions are also included in Table 3. On the basis of the data octahedral geometries have been proposed for all complexes.

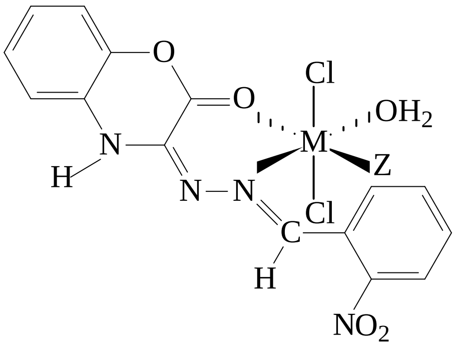
Antifungal activity

Though the ligand NBHBO has not shown inhibition at lower or higher concentrations, the Ni(II) complex has exhibited high activity. This is a supportive evidence to suggest the influence of complexation on the growth inhibition activity against Neurosporfl crassa. In the same way Co(II) complex also has shown 60% growth inhibition compared to free ligand at high concentration.



M = Cr(III), Fe(III)

Fig 2. Proposed structure of Cr(III) and Fe(III) complexes



M = Mn(II), Co(II) Z = NH_3

M = Ni(II), Z = H_2O

Fig 3. Proposed structure of Mn(II), Co(II) and Ni(II) complexes

CONCLUSIONS

Based on the analytical, thermal, conductance, spectral and magnetic data, octahedral geometries have been suggested to the complexes, wherein the ligand employs NN donor sequence towards the trivaient and NO donor sequence towards the divalent metal ions. The proposed structures are given in Fig. 2 & 3.

REFERENCES

1) S.Alper-Hayta , E.Aki-Sener, B.Tekiner-Gulbas,I.Yildiz,O.Temiz-Arpaci, I.Yalcin, N.Altanlar, Eur. J Med. Chem. 41 (2006) 1398-1404.
2) F. Renata, G.Nicola, M.Lara, C.Elio, M.Antonio, V.Anna, C.Gabriele, S.Fausto, Bioorg.Med. Chem. 17 (2009) 3838-3846.
3) S.Pavel, M.Milo, V.Marcela, K.Lenka, K.Jií, W.Karel, J. Heterocyclic Chem. 46 (2009) 873-880.
4) S.L.Daniel, B.Julie, V.B.James, C.Angela, D.Thomas, D.Nicholas, E.Juan, C.F.Julie, R.F.Shaun etal, J. Med. Chem. 51 (2008) 1695-1705.
5) N.K.Eftychia , P.Panagiota, V.Athanasios, K.Maria, H.Zolt, K.Istvn etal, J.Med. Chem.52 (2009)2328-2340.
6) T.Antoni, M.Josep, P.Adriana, Aurelio, S.Olga, G.Xavier, D.Alberto etal, J. Med. Chem. 48(2005) 2080-2092.
7) E. Blattes,B. Lockhart, P. Lestage, L.Schwendimann, P. Gressens, M.B.Fleury, M. Largeron,J.Med. Chem. 48 (2005) 1282-1286.
8) W.Yang, Y.Wang, Z.Ma, R.Golla, T.Stouch, R.Seethala, S. Johnson etal, Bioorg.Med.Chem. Lett. 14 (2004) 2327-2330.
9) K. Waisser, J. Gregor, L. Kubicova, V. Klimeova, J. Kune, M. Machaek, J.Kaustova, Eur. J. Med. Chem. 35 (2000) 733-741.

10) T.E.Apenysheva, K.S.Pushkareva, S.N.Bolotin, V.Y.Frolov, F.A. Kolokolov etal, Russ. J. Gen. Chem. 76 (2006) 641-644.
11) B.N. Figgis, J. Lewis, Modern Coordination Chemistry, Interscience Inc, New York, 1960.
12) B. Loev, H.Jones, R.E.Brown, F.C.Huang, A.Khandwala, M.J.Leibowitz, P. Sonnino-Goldman, J. Med Chem. 28 (1985) 24-27.
13) V.Haribabu, P.V. Anantha Lakshmi, V. Jayatyaga Raju, Der Pharma Chemica. 3 (2011) 413-421
14) B.S.Shyamala, P.V.Ananthalakshmi, V.Jayatyagaraju, International Journal of Pharmacy and Pharmaceutical Sciences. 2 (2010) 150-152.
15) P.V.Ananthalakshmi , P. Sarithareddy, V. Jayatyagaraju, Bull. Chem. Soc.Ethiop. 22 (2008) 385-390.
16) R.J. Angelici, Synthesis and Technique in Inorganic Chemistry, Saunders, Philadelphia, 1969.
17) A.V. Nikolaev, V.A. Logvinenko, L.I. Mayachina, Thermal Analysis, Academic Press, New York,1969.
18) P.V.Ananthalakshmi, P.Sarithareddy, V. Jayatyagaraju, Spect. Chim. Acta. PartA. 74 (2009) 52-57.
19) R.K.Agarwal , L.Singh, D.K.Sharma , R.Singh, Turk. J. Chem. 29 (2005) 309-316.

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