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Synthesis and antimicrobial activity of nitrazepam (7- nitro- 1,3- dihydro-5- phenyl-2h-1,4- benzodiazepine -2-one) (drug) complexes of rare earth metals

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ABSTRACT

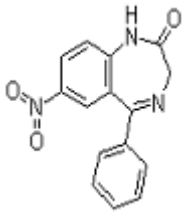
Metal complexes of Th (IV), Ce (IV) and UO_2 (IV) with drug nitrazepam (NZ) (7- nitro- 1,3- dihydro-5- phenyl-2H-1,4- benzodiazepine-2-one) have been synthesized and characterized with the help of elemental analysis and IR spectral data. On the basis of IR spectral data the band in the free ligand attributed to υ (C =N) & υ (N-H) undergoes the shifting to lower region in all the complexes, indicating the participation of azomethine nitrogen N (4) & N (1) in complexation. All the coloured complexes have been screened for their antimicrobial activity against fungi viz. *A. niger, A. flavous, P. triticena*, *Fusarium* species and bacteria viz. *E. coli, B. subtilis, S. typhi, S. aureus.* The results of antimicrobial activity show that Nz- metal complexes of tetravalent metals have more potency towards experimental fungi and bacteria. It also shows that Th (IV) complex is more toxic against bacteria *E. coli.*

Key words: Synthesis, Antimicrobial activity, nitrazepam.

INTRODUCTION

The drug nitrazepam (NZ), a benzodiazepine is commonly used as a sedative agent[1]. The benzodiazepine have wide applications in the pharmaceuticals industries [2] and in some cases they have carcinostatic activity [3-4] . The study of the stereochemistry and the chemical reactivity of the coordination compounds of benzodiazepines help to determine the relationship existing between chemical structure and biological activity of these drugs[5]. It is well known that the metal complexes of ligands are more biologically active than the free ligands[6-7].

The present communication deals with the synthesis of metal complexes of nitrazepam and screening of their biocidal activities.



Nitrazepam (NZ) 7- nitro- 1,3- dihydro-5-phenyl-2H-1,4- benzodiazepine-2-one

EXPERIMENTAL

All the chemicals used were of AR (BDH) grade and their solutions were prepared in double distilled water. The complexes were prepared by the mixing molar solutions of metal nitrates dissolved in double distilled water and ligand NZ dissolve in

ethanol. The pH of the reaction mixture was adjusted between 7.5 to 8.5. The resulting colored solids were dried in an oven at 100 ºC and stored in a desiccators over anhydrous $CaCl_2$.

Biological experimental:-

The biological experiments for determining antimicrobial activity of drug nitrazepam and their metal complexes have been done by serial dilution method[8]. In this technique the solutions of different concentrations (viz. 250 ppm, 500 ppm. 750 ppm and 1000 ppm) were prepared in dimethyl formamide. The graded dilution of the test compounds in a suitable nutrient (agar) and PDA medium were inoculated with the organisms under examination using aseptic techniques in an incubator at 37 ºC . The antibacterial and antifungal activity of ligand, nitrazepam and its metal complexes have been screened in vitro, against bacteria (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*) and fungi (*Aspergillus flavous, Aspergillus niger, Penicillium triticena* and Fusarium species).

The percentage of growth inhibition was calculated by measuring the diameter of the microbial colony in the control and test plates by the following expression-

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

Where

C = diameter of microbial colony in millimeter in control plate.

T = diameter of microbial colony in treated (test) plate.

Analytical and physical measurement

The purity of complexes were confirmed by running their T.L.C. on silica gel- G. The IR spectra in KBr matrix were recorded on Perkin- Elmer 842-spectro photometer at CDRI, Lucknow and elemental analysis of C, H, N, were carried out at NCL Pune.

RESULTS AND DISCUSSION

The molecular formula and molecular weight of the complexes were determined on the basis of elemental analysis. The colored complexes are stable at room temperature. These complexes are insoluble in common organic solvents while soluble in DMF. The physical and analytical data of complexes have been given in table 1.

On the basis of IR spectral data the band at 1611 cm^{-1} in the free ligand attributed to υ(C =N)[9,10] undergoes the shifting to lower region in all the complexes, indicating the participation of azomethine nitrogen N (4) in complexation . In all the complexes the band assigned to υ (N-H) in ligand (3214 cm^{-1}) shows lowering compared to the ligand indicating the involvement of N (1) atom in coordination.

Table 1: Analytical & physical data of NZ - Metal Complexes

Compounds	Colour	Mol.Wt.	M.P.ºC	pH	Elemental analysis found (cal.) %			
					C	H	N	Metal
Nitrazepam(NZ)	White	281	145		63.42 (64.05)	3.01 (3.91)	13.76 (14.94)	-
Th - NZ	Grey	761	>360	7.5	22.42 (23.65)	1.07 (1.45)	11.96 (12.88)	28.97 (30.48)
Ce - NZ	yellow	669	>360	7.5	25.85 (26.90)	1.03 (1.64)	13.42 (14.65)	19.67 (20.93)
UO_2 - NZ	Brown	675	>360	7.5	25.98 (26.67)	1.02 (1.63)	9.11 (10.37)	33.98 (35.25)

Table 2: Percentage of zone of inhibition of Rare earth metal Complexes of drug Nitrazepam against Bacteria

Compound	*E. coli* % Conc. In ppm				*S. typhi* % Conc. In ppm				*S. aureus* % Conc. In ppm				*B. subtilis* % Conc. In ppm			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Ligand (NZ)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Th- NZ	47.84	71.55	75	75	39.50	69.13	71.55	75	39.50	69.13	71.55	75	39.50	69.13	71.55	75
UO_2- NZ	39.50	69.13	71.55	75	39.50	69.13	71.55	71.55	39.50	64.00	69.13	71.55	39.50	69.13	71.55	75
Ce- NZ	39.50	64.00	69.13	71.55	39.50	64.00	69.13	71.55	47.84	69.13	71.55	75	39.50	64.00	69.13	71.55
DMF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Standard drug	39.50	69.13	71.55	75	39.50	69.13	71.55	75	39.50	69.13	71.55	75	39.50	69.13	71.55	75

Table 3: Percentage of zone of inhibition of Rare earth metal Complexes of drug Nitrazepam against Fungi

Compound	*A. flavous* % Conc. In ppm				*A. niger* % Conc. In ppm				*P. triticena* % Conc. In ppm				F. species % Conc. In ppm			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Ligand (NZ)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Th- NZ	39.50	69.13	71.55	75	39.50	71.55	75	75	39.50	69.13	71.55	75	39.50	69.13	71.55	75
UO2- NZ	39.50	62.66	69.13	71.55	39.50	69.13	71.55	75	39.50	62.66	64.00	69.13	39.50	64.00	69.13	71.55
Ce - NZ	39.50	62.66	64.00	69.13	39.50	62.66	69.13	71.55	39.50	62.66	69.13	71.55	39.50	64.00	69.13	71.55
DMF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Standard drug	39.50	62.66	69.13	71.55	39.50	62.66	69.13	71.55	39.50	62.66	69.13	71.55	39.50	62.66	69.13	71.55

The band attributed to the vibrational mode υ (C = O) appers at (1695 cm^{-1}) in the ligand shows a small positive shift in all the complexes. The presence of new bands in the region 515cm^{-1} & 313cm^{-1} in all the complexes is attributed to υ (M-N) linkage [11, 12].

The antibacterial and antifungal activity of ligand, diazepam and its metal complexes were screening in vitro, against bacteria (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*) and fungi (*Aspergillus flavous, Aspergillus niger, Penicillium triticena* and Fusarium species). The results are recorded in Table 2 and 3. The results of biocidal activities show that the percentage of zone of inhibition of 500 ppm concentration is the best. The percentage of growth inhibition capacities of metal complexes follows the following order against different bacteria and fungi- Bactericidal activities of metal complexes -

E. coli : Th(IV) > UO_2 (IV) > Ce (IV)
S. typhi : Th(IV) > UO_2 (IV) > Ce (IV)
S. aureus : UO_2 (IV) > Th(IV) > Ce (IV)
B. subtilis : Th(IV) > UO_2 (IV) > Ce (IV)

Fungicidal activities of metal complexes-

A. flavous : Th(IV) > UO_2 (IV) > Ce (IV)
A. niger : UO_2 (IV) > Th(IV) > Ce (IV)
P. triticena : Th(IV) > UO_2 (IV) > Ce (IV)
F. species : Th(IV) > Ce (IV) > UO_2 (IV)

The results indicate that the Th(IV) complex is more toxic compared to other complexes, as well as Th(IV) complex is more effective towards bacteria *E. coli* compared to other microbes. The over all results obtained from the above studies confirm that with increase in the concentration of the complexes the activity almost remains unchanged or is slightly increased.

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REFERENCES

1. H. Scultz, Benzodiazepines, springer verlag . Heidelberg (1982).
2. L. H. Sternbach, Angew, *Chem. Internat.* **10**: 34, (1971).
3. De Stevens , G., Topics in Heterocyclic chemistry (Edited by R.N. castle). Wiley – Interscience, New York (1969).
4. K. V. Levshina , E. I. Yumasheva, and T. A. Glazyrina, A. I. Kravchenko, *Puti Sin. Izyst. Prot. Prep.,* **3**: 257 (1970) .
5. C. Preti, and G. Those , *J. Inorg. Nucl. Chem.*, **41**: 263-266 (1997).
6. S. Kirschner, Y. K. Wei , D. Francis ,and J. G. Bergman, *J. Med. Chem.,* **9**: 369 (1966).
7. A. J. Thomson, R. J. P. Williams, and S. Reslova, *Structure and Bonding*, **11**: 1 (1972).
8. J. H. Quasted, *J. Gen. Microbial.*, **45**: 14 (1966).
9. N. B. Colthup, L. H. Daly, and S. E. Wiberley, "Introduction to IR and Raman spectroscopy" Academic press, New York (1964).
10. L. J. Bellamy, "The IR spectra of complex molecules" Wiley, New york (1966).
11. A. K. Narula, *J. of Indian chem. Soc.*, **68**: 313 (1999).
12. K. Nakamoto , J. Fujita, And H. Murata, *J. Amer. Chem. Soc.,* **80**: 4817 (1958).