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In vitro evaluation of antimicrobial properties of a new Mannich base N-[(Diphenylamino)methyl]acrylamide and its Oxovanadium(IV), Cerium(IV), Thorium(IV) and Dioxouranium(VI) metal chelates against human pathogenic microorganisms

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Abstract

N-[(Diphenylamino)methyl]acrylamide(DPAMAcry) was synthesized using Mannich reaction and its complexes of oxovanadium(IV), cerium(IV), thorium(IV) and dioxouranium(VI) were prepared and characterized by elemental analysis, UV, IR and EPR spectral studies. All the newly synthesized compounds have been screened for their antibacterial and antifungal activities. All of them show promising antibacterial and antifungal activity.

Keywords: Neumatocides, Heterocyclic, Polycrystalline, Chelating, Inhibition

INTRODUCTION

Acrylamides are one of the most important classes of heterocyclic compounds in the field of drugs and pharmaceuticals[1,2]. They are widely used as growth regulators, fungicides, bactericides, herbicides, insecticides, and neumatocides. Their derivatives known for their anti-inflammatory, immunomodulatory, antitumoural, antipsychotic and antiallergic activities are drugs used in medicine[3,4]. As a result, research in this area is still very active and is directed towards the synthesis of compounds with enhanced pharmacological activity[5].

The present work is focused on the synthesis of *N*-[(Diphenylamino)methyl]acrylamide due to the importance of this class of compounds, which has recently found application in medicinal chemistry. Chiral acrylamide derivatives of several substituted halopyridyl and thiazolyl compounds were used as non-nucleoside inhibitors of the reverse transcriptase enzyme of human deficiency virus (HIV-I)[6].

MATERIAL AND METHODS

All the reagents used for synthesizing ligand and its complexes were of A.R. grade and the solvents used were commercial product of the highest available purity and were further purified by distillation.

Micro elemental(C, H & N) data were obtained with Carlo Erba 1108 elemental analyzer. Metal contents were estimated by usual procedure, after digesting the complexes with con.HNO_3. Conductance data were obtained in ~10^{-3} M DMF solution of the complexes at room temperature using a systronics direct reading digital conductivity meter-304 with dip type conductivity cell. IR spectra were recorded using a spectrum-one Perkin Elmer FT-IR spectrometer by using KBr pellets. Absorbance in UV-Visible region was recorded in DMF solution using Double Beam UV-Visible spectrometer, Perkin EZ-301 of working range 1100-190 nm. The ^1H and ^{13}C NMR of the ligand were recorded on a bruker instrument and on a Jeol GSX-400 spectrometer employing TMS as internal reference and DMSO-d_6 as solvent at ambient temperature. The FAB mass recorded for the ligand was carried out using a Jeol-GC mate mass spectrometer. The room temperature magnetic susceptibility measurements of the complexes were made by using a Gouy Magnetic Balance calibrated using mercury(II)tetrathiocyanatocobaltate(II).

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EXPERIMENTAL

Synthesis of the ligand

N-[(Diphenylamino)methyl]acrylamide(L) was synthesized by employing the Mannich synthetic route[7]. Acrylamide (7.2 g, 0.1 mol) was dissolved in minimum quantity of ethanol. To this solution, formaldehyde (10 mL, 0.1 mol) followed by diphenylamine (8.5 g, 0.05 mol) dissolved in acetone was added in small quantities with constant stirring in an ice bath. After 15 days a colourless solid was obtained, which was washed with water and with acetone. It was dried at 60°C in an air oven. The percentage yield was 83.

Synthesis of metal complexes

The hot methanolic solution of the metal salt(s) was added slowly with constant stirring to the hot ethanolic solution of the ligand in 2:1 mol ratio. The insoluble complexes formed were filtered, washed with appropriate solvents to remove the unreacted metal and ligand, and then dried in an air oven at 80°C.

Antibacterial and Antifungal Screening

Antibacterial and antifungal activities of ligand and some of its

metal complexes were tested *in vitro* against six bacterial species viz *E.coli, P.aeruginosa*, *S.typhi, B.subtilis, S.pyogenes, S.aureus* and the fungal species *A.niger* and *A.flavus* by disc diffusion method[8] using agar nutrient as medium and gentamycin as control. The paper disc containing the compound (10, 20 and 30 µg/disc) was placed on the surface of the nutrient agar plate, previously spread with 0.1 mL of sterilized culture of microorganism. After incubating this at 37°C for 24 hrs, the diameter of inhibition zone around the paper disc was measured.

RESULTS AND DISCUSSION

Characterization of the ligand

The ligand crystallized from ethanol, registers a single R_f value on an HPLC run confirming its purity. The compound melts at 120^0 C. The C, H and N analytical results were found to be 74.35, 6.05 and 11.24% respectively, which are in close agreement with the calculated values of 76.19, 6.30 and 11.11%. Elemental analysis indicates the molecular formula as $C_{16}H_{16}N_2O$.

UV-Visible spectrum[9] of a 10^{-3} M ligand in DMF registered two bands at 302 and 250 nm which are assignable to the n→π* and π→π* transitions respectively of the carbonyl group. These bands occur at slightly longer wavelengths compared with those for diphenylamine, indicating the derivatisation of the compound.

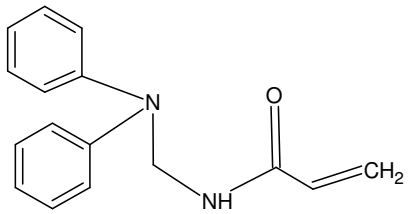
IR spectrum[10] of the ligand registers a sharp absorption band at 3295 cm^{-1} assigned to a symmetric stretch of NH. Other bands at 3042 and 2912 cm^{-1} arise due to ν_{CH} of CH and CH_2 groups, respectively. The sharp absorption bands at 1655 and 1534 cm^{-1} are due to ν_{CO} and δ_{NH} respectively. Compared with the IR spectrum of acrylamide, a new sharp and intense band at 1218 and 1088 cm^{-1} is due to $\nu_{C\text{-}N\text{-}C}$ of the diphenylamine moiety. This band confirms the insertion of the diphenylaminomethyl moiety into acrylamide. The strong absorption band observed at 775 cm^{-1} is due to monosubstituted aromatic ring.

^{1}H NMR spectrum[11] of ligand in DMSO-d_6 contained five signals, two singlets, one multiplet and two doublets. The chemical shift values of the signals are δ 8.66(s), 5.17&5.16(d), 6.31(s) and 6.17&5.63(d) which can be assigned to amide N-H, methylene CH_2, vinyl CH and vinyl CH_2 respectively. The multiplet in the range δ 7.3-6.92 ppm centered at 7.07 ppm is attributed to the protons of the benzene ring. These signals confirm the incorporation of a diphenylaminomethyl moiety at the N-position of acrylamide.

^{13}C NMR signals[12] at δ 164.78, 146.58, 129.26, 121.81, 120.95, 56.17, 125.91, 131.45 ppm are assigned to carbonyl C, phenyl C at 1, at 3&5 at 2&6 and at 4, methylene C, terminal C of vinyl group and vinyl C attached to CO group respectively. The observed carbon chemical shift values are in close agreement with the structure proposed on the basis of ^{1}H NMR data.

The mass spectrum[13] of the ligand exhibits a molecular ion peak at m/z = 252 which corresponds to the imposed molecular mass of the compound.

Based on the data obtained from various physical and chemical studies, the molecular structure of DPAMAcry is arrived as fig.1.



DPAMAcry

(Figure 1)

Characterization of metal complexes

Elemental analysis and conductance measurements

The Λ_M values for 10^{-3} M DMF solutions of all the complexes suggest being non-electrolytes[14]. The analytical data for C, H, N, metal and anion content in the complexes are given in Table-1.

Table 1 Analytical Data of V^{IV}, Ce^{IV}, Th^{IV} and U^{VI} Complexes of DPAMAcry

Complex	% C Obs. (Cal.)	% H Obs. (Cal.)	% N Obs. (Cal.)	%Metal Obs. (Cal.)	%Anion Obs. (Cal.)	Λm ohm^{-1} cm^2 mol^{-1}
$VOSO_4$.L	37.71 (38.02)	3.06 (3.17)	5.35 (5.54)	9.74 (10.09)	18.37 (19.02)	19.20
$Ce(SO_4)_2$.L	39.00 (39.33)	2.75 (3.28)	5.98 (5.77)	29.13 (28.70)	20.19 (19.68)	11.01
$Th(NO_3)_4$.L	25.68 (26.22)	1.94 (2.28)	12.61 (11.47)	32.48 (31.73)	34.21 (33.86)	23.46
$UO_2(NO_3)_2$. L	30.38 (29.72)	2.15 (2.48)	8.88 (8.67)	37.13 (36.84)	18.82 (19.20)	08.92

Infrared spectra

Comparison of the IR spectrum of the ligand with those of its complexes suggests the following: Perceptible shifts by about 57 to 58 and 42 to 82 cm^{-1} are observed in the ν_{CO} and ν_{CNC} respectively in the case of oxovanadium(IV) & cerium(IV) sulphato and thorium(IV) & dioxouranium(VI) nitrato complexes(Table 2).

Table 2 Important IR Absorption Bands (cm^{-1}) of DPAMAcry and V^{IV}, Ce^{IV}, Th^{IV} and U^{VI} Complexes of DPAMAcry

Compound	ν_{NH}	$\nu_{C=O}$	ν_{CNC}	ν_3	ν_4	ν_1	ν_2	ν_5	ν_6
DPAMAcry	3295	1655	1218, 1088	-	-	-	-	-	-
$VOSO_4$.L	3389	1597	1171	1125, 1077, 1016	694, 668, 649	810	505	-	-
$Ce(SO_4)_2$.L	3387	1598	1136	1136, 995, 878	693, 652, 608	809	505	-	-
$Th(NO_3)_4$.L	3391	1598	1176	-	-	1310	1027	1384	809
$UO_2(NO_3)_2$.L	3390	1597	1175	-	-	1310	1076	1384	809

The diagnostic bands of the nitrato group in the case of thorium(IV) and dioxouranium(VI) complexes show a separation of (ν_5-ν_1)=74 cm^{-1} which indicates the monodentate[15] mode of coordination. The oxovanadium(IV) & cerium(IV) sulphato complexes registers new strong bands in the range of 1136-878(ν_3), 810&809(ν_1), 694-608(ν_4), 505 cm^{-1} (ν_2) which point to a bidentate mode of coordination for the sulphato group. The new band at 582 cm^{-1} represents the formation of ν_{Th-N} bond[16] and the bands at 506 and 472 cm^{-1} indicate the presence of ν_{Th-O} bond in thorium(IV) complex. The bands at 914 and 940 cm^{-1} correspond to O=V=O bond. The medium bands at 500-584 cm^{-1} are assigned to ν_{M-N} and ν_{M-O} vibrations[17] respectively in oxovanadium(IV) sulphato complex.

Electronic spectra

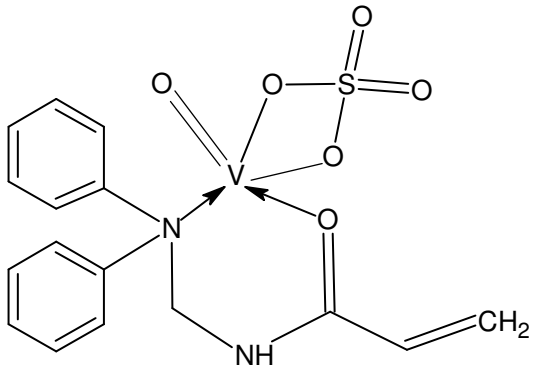
The UV-Visible spectrum of oxovanadium(IV) complex shows only one absorption band due to V=O. This indicates the absence of axial perturbation. In the present case the broad band observed at 27032 cm^{-1} is ascribed to the $^2B_2 \rightarrow {}^2A_1$ transition. The room temperature magnetic moment value is 1.83 B.M. which suggests a penta coordinate geometry[18] around VO^{IV} ion. In Ce(IV) sulphato complex, a broad band at 27800 cm^{-1} is due to charge transfer transition. There is no characteristic intense band in the visible region for Th(IV) nitrato complex, since it is a d_{10} system. The electronic spectrum of dioxouranium(VI) nitrato complex exhibits two bands at 20470 and 24620 cm^{-1} which may be assigned to the $^1Eg^+ \rightarrow {}^3\Pi u$ transition, typical of UO_2^{2+} moiety and charge transfer transitions respectively, considering an octahedral geometry.

EPR Spectrum

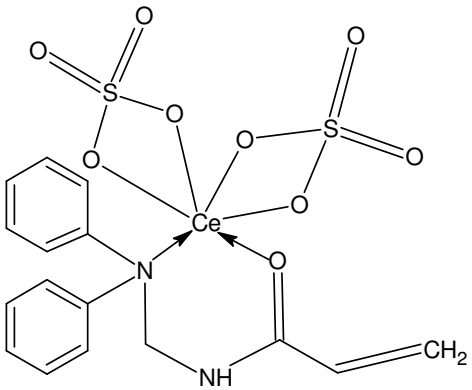
The EPR spectrum[19] of the oxovanadium(IV) complex does not show any resolution at g_{II} region. Hence it can be assumed that g_x and g_y are same (or) nearly the same. The g value of 1.91 indicates strong interaction between the ligand and the metal ion.

The room temperature magnetic moment value is 2.60, and 0.19 B.M. for cerium(IV) and dioxouranium(VI)complexes respectively.

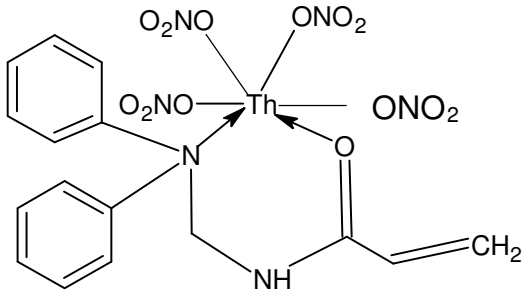
Based on micro elemental analysis, conductance measurements, IR, UV-VIS and magnetic moment measurements, the tentative structures proposed for the complexes are given in Figure 2 to 5.



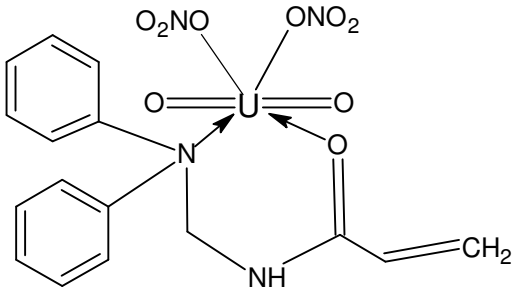
$VOSO_4$.L
(Figure 2)



$Ce(SO_4)_2$. L
(Figure 3)



$Th(NO_3)_4$. L
(Figure 4)



$UO_2(NO_3)_2$. L
(Figure 5)

L= DPAMAcry

Biological studies

Antibacterial activity

Table 3 Antibacterial Activities of DPAMAcry and its Complexes

Compound	*E. coli*			*P. aer.*			*S. typhi.*			*B. sub*			*S. pyo*			*S. aur*		
Conc.	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
L	08	11	14	06	09	10	08	10	16	07	07	12	10	13	14	09	12	15
Standard	12	15	18	10	13	16	11	13	18	12	14	16	14	14	17	13	15	19
$VOSO_4$.L	18	21	24	17	22	27	20	28	30	23	26	32	18	24	29	25	28	35
$CeSO_4$.L	16	18	20	14	16	18	14	16	20	15	18	21	15	17	20	16	18	24
$Th(NO_3)_4$.L	21	29	37	19	24	35	18	23	33	26	30	41	22	29	39	19	31	47
$UO_2(NO_3)_2$.L	16	20	22	14	18	20	15	18	22	19	20	26	18	18	24	14	20	26

(L=ligand)

Among the compounds tested, thorium(IV) nitrato complex was found to be the most active against all the species(Table 3). The activity of the thorium(IV) nitrato complex may be due to its interaction with RNA[20]. The order of activity was found to be thorium(IV)nitrato > oxovanadium(IV)sulphato > dioxouranium(VI)nitrato > cerium(IV)sulphato > standard > Ligand. It was found that the metal ion complexes were more biologically active than ligand in all the cases. It was also observed that there existed some relationship between the lability of M-O, M-N and M-X (X=anionic counter part of the metal ion) and the biological activities: viz. The activity increased with increasing lability of the metal complexes[21].

Antifungal studies

Table 4 Antifungal Activities of DPAMAcry and its Complexes

Compound	*A. niger*			*A. flavus*		
Conc.	10	20	30	10	20	30
L	07	10	12	10	14	16
Standard	09	11	14	12	16	17
$VOSO_4$.L	17	20	27	18	20	25
$CeSO_4$.L	11	14	15	13	18	20
$Th(NO_3)_4$.L	21	28	31	19	22	29
$UO_2(NO_3)_2$.L	18	22	25	15	19	23

The ligand(L) was found to be less active compared to its metal ion complex (Table 4) with $Th(NO_3)_2$. A possible mechanism of toxicity may be speculated in the light of Chelation Theory[22]. Chelation reduces the polarity of the metal ion considerably because of partial sharing of its positive charge with donor groups and possible π-delocalization of electron over the chelate ring. This increases the liphophilic character of the neutral chelate, which favours its permeation through lipoid layers of fungus membranes. Furthermore, the mechanism of action of the compounds may involve the formation of hydrogen bond through the uncoordinated

heteroatoms O, S and N with the active centers of the cell constituents resulting in the interference with the normal cell process. Presence of lipophilic and polar substituents like C=O, NH, etc., are expected to enhance the fungi toxicity[23].

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