Chem Sci Trans., 2013, **2(2),** 379-384
DOI:10.7598/cst2013.335

Chemical Science Transactions
ISSN/E-ISSN: 2278-3458/2278-3318

RESEARCH ARTICLE

Synthesis, Characterization, Antibacterial, Antifungal and Anticancer Studies of a New Antimetabolite: *N*-[(Diphenylamino)methyl]acetamide and Some of its Inner Transition Metal Chelates

L. MURUGANANDAM[a*], K. KRISHNA KUMAR[b] and K. BALASUBRAMANIAN[a]

[a]Department of Chemistry, Saranathan College of Engineering, Tiruchirapalli-12, India
[b]Department of Chemistry, National Institute of Technology, Tiruchirapalli-15, India
lmuruganandam@yahoo.co.in

Received 2 August 2012 / Accepted 4 September 2012

Abstract: *N*-[(Diphenylamino)methyl]acetamide was synthesized using Mannich reaction and its complexes of cerium(IV), thorium(IV) and dioxouranium(VI) were prepared and characterized by elemental analysis and various spectral studies. The bidendate chelation of ligand, bonding through carbonyl oxygen and azomethine nitrogen is suggested. Based on spectral and magnetic studies, hexa coordinate geometry is assigned for all the complexes. The complexes were screened for their antibacterial, antifungal and anticancer activities. They show positive results.

Keywords: Physicochemical, Tuberculosis, Monosubstituted, Antimetabolite, Lipophilic

Introduction

With their versatile structures, redox behavior and physicochemical properties, inner transition metal complexes are often useful as chemical nucleases[1]. The interaction of these complexes with DNA has gained much attention due to their possible applications as new therapeutic agents. The manipulation of the ligands greatly facilitates the interaction between the complexes and DNA[2]. Metal based drugs represent a novel group of antimicrobial agents with potential applications for the control of bacterial and fungal infections[3,4]. This inspires synthetic chemists to search for new metal complexes for bioactive compounds. This work presents the synthesis, characterization, antibacterial, antifungal and anticancer studies of a *N*-[(diphenylamino)methyl]acetamide and their metal complexes of cerium(IV), thorium(IV) and dioxouranium(VI).

Experimental

All the reagents used for synthesizing the ligand and its complexes were of A.R. grade. The solvents used were purified by distillation.

Synthesis of ligand and its metal ion complexes

N-[(Diphenylamino)methyl]acetamide (DPAMAce) was synthesized by Mannich[5] condensation reaction between acetamide, formaldehyde and diphenylamine in 1:1:0.5 mol ratio. Ethanolic solution of acetamide (5.90 g) was mixed with formaldehyde (10 mL) and diphenylamine (8.5 g) in acetone and stirred in an ice bath. After 10 days a colourless solid formed, was filtered, washed with water, acetone and dried. The ligand melts at 110 ^{0}C. The hot methanolic solution of the metal salts 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 ethanol and methanol to remove the unreacted metal and ligand. They were dried in an air oven.

Antibacterial and antifungal screening

Antibacterial activities of the ligand and its metal complexes such as cerium(IV) sulphato, and thorium(IV) & dioxouranium(VI) nitrato complexes were tested *in vitro* against six bacterial species *(E. coli, P. aeruginosa, S. typhi, B. subtilis, S. pyogenes, S. aureus*) and two fungal species *(A. niger* and *A. flavus)* by disc diffusion method using agar nutrient as medium and gentamycin as control.

***In-vitro* bioactivity test for immunomodulatory and cytotoxicity assessment**

Separation of human peripheral blood mononuclear cells

Human venous blood was drawn and defibrinated. It was diluted with RPMI 1640 media and layered over histopaque. After centrifugation, the whole mononuclear cell layer was transferred, thoroughly mixed with the medium and washed by centrifugation. The final suspension was made in 1 to 5 mL RPMI 1640 medium with 10% serum. Equal amounts of cell suspension and tryphan blue solution were mixed, fed into a haemocytometer. Live and dead cells were counted under phase contrast objective[6].

Tryphan blue dye exclusion method

A known concentration of the complexes was reconstituted with a known volume of PBS at pH 7.2. This was centrifuged, membrane filtered. The supernatant solution was used for preparing various concentrations of complexes. They were subjected to the *in-vitro* analysis on human PBMC. The assay was performed in well tissue culture plates, using various negative and positive controls. The addition of cell, media and complexes, the cultures were incubated at 37 ^{o}C. The cell suspension mixed with an equal volume of tryphan blue was used, followed by incubation. After 5 minutes, the stained cells were placed in a haemocytometer. The number of viable and dead cells was counted.

Cancer screening

Cell lines

Raji and Jukart cell lines[7] were used and massively expanded in a minimal number of passages. The cells were cryopreserved to provide a consistent, long-term frozen stock for future use. They were later grown in antibiotic-free growth medium to ensure the absence of microbial contaminants.

Preparation and inoculation of cells

Cells were separated into single cell suspensions and counted using tryphan-blue exclusion on a haemocytometer. After counting, dilutions were done to give the appropriate cell densities for inoculation onto the microtiter plates. Cells were inoculated and medium is added to cell free wells.

MTT assay

The assay was performed in a well tissue culture plate, using various negative and positive controls. After the addition of cell, media and complexes, the cultures were incubated at 37 ^{o}C for 72 hours. The assay was monitored on a spectrophotometer. MTT was added and incubated. After incubation period, the MTT was reduced by mitochondrial dehydrogenase as a result of which the colour changed. Detergent SDS was added to the wells to solubilize the formazan crystals. The absorbance was read by making use of an ELISA reader at 570 nm. The rate of tetrazolium reduction was directly proportional to the rate of cell proliferation[8].

Results and Discussion

The elemental analysis of the ligand indicates the molecular formula as $C_{15}H_{16}N_2O$. The IR spectrum[9] of the ligand shows a sharp band at 3258 cm^{-1} is due to ν_{N-H} stretching vibration. The $\nu_{C=O}$ band at 1637 cm^{-1} and that observed at 751 cm^{-1} can be attributed to monosubstituted aromatic ring. The medium bands at 1241 and 1067 cm^{-1} pertain to ν_{C-N-C} of diphenylamine moiety.

The UV-visible spectrum in DMF exhibited an absorption band at 301 nm is due to $n\rightarrow\pi^*$ transition of carbonyl group. The absorption band at 274 nm can be assigned to $\pi\rightarrow\pi^*$ transition of carbonyl and aromatic groups.

The ^{1}H NMR spectrum[10] in DMSO-d_6 contains four signals of protons. The multiplet in the range δ7.30 to 6.96 ppm centered at δ7.07 ppm is attributed to the protons of benzene ring. The NH proton chemical shift occurs at δ8.46 ppm, which gives a broad band. The CH_3 protons exhibit a signal at δ1.84 ppm and the CH_2 protons appear as a doublet at δ5.06 ppm and 5.05 ppm.

The ^{13}C NMR signal shows the presence of carbonyl carbon at δ169.60 ppm. The chemical shifts of aromatic carbons appear at δ146.61, 129.25, 121.72 and 120.91 ppm. The methylene carbon which is bonded to N exhibits a signal at δ56.12 ppm. The methyl carbon resonates at δ22.57 ppm.

The mass spectrum[11] 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 DPAMAce is shown in Figure 1.

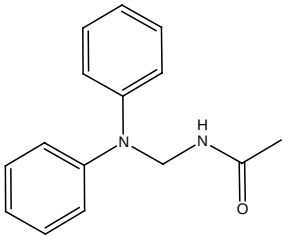

Figure 1. DPAMAce

Micro elemental analysis and conductance measurements

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

Table 1. Analytical data of Ce^{IV}, Th^{IV} and U^{VI} Complexes of DPAMAce

Complex	% C Obs. (Cal.)	% H Obs. (Cal.)	% N Obs. (Cal.)	%Metal Obs. (Cal.)	%Anion Obs. (Cal.)	Λm ohm^{-1} cm^2 mol^{-1}
L	73.01 (75.00)	6.19 (6.60)	11.58 (11.60)	-	-	-
$Ce(SO_4)_2$ .L	38.02 (37.80)	3.19 (3.36)	6.05 (5.88)	30.21 (29.43)	19.71 (20.17)	14.52
$Th(NO_3)_4$. L	25.10 (24.99)	2.64 (2.22)	11.13 (11.66)	32.83 (32.26)	34.09 (34.43)	09.86
$UO_2(NO_3)_2$. L	28.76 (28.37)	2.90 (2.52)	8.06 (8.83)	37.47 (37.54)	20.21 (19.16)	17.03

(L- DPAMAce)

Infrared spectra

Comparison of the IR spectrum of ligand with those of its complexes suggests the following: Perceptible shifts by about 32 to 40 and 65 to 108 cm^{-1} are observed in the ν_{CO} and ν_{CNC} respectively in the case of cerium(IV) sulphato and thorium(IV) & dioxouranium(VI) nitrato complexes.

The diagnostic bands of the nitrato group in thorium(IV) and dioxouranium(VI) complexes show a separation of $(\nu_5-\nu_1)$=74 cm^{-1} which indicates the monodentate mode of coordination. The band at 582 cm^{-1} represents the formation of ν_{Th-N} bond[9] in the thorium(IV) complex.. The new bands formed at 506 & 472 cm^{-1} indicates the presence of ν_{Th-O} bond. The bands at 914 & 940 cm^{-1} are evidences for O=U=O bond. The medium bands found at 500–584 cm^{-1} are assigned to ν_{M-N} and ν_{M-O} vibrations in uranium(VI) complex. The cerium(IV) sulphato complex registers new strong bands in the range of 1108, 996, 914(ν_3), 877(ν_1), 693, 654, 609(ν_4), 502 cm^{-1} (ν_2) which point to a bidentate coordination of the sulphato group.

Electronic spectra

In Ce(IV) sulphato complex, a broad band observed at 27800 cm^{-1} is due to charge transfer transition. The magnetic moment of this complex: 2.57 B.M., which indicates the absence of metal-metal interaction and spin exchange. 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 a band at 21934 cm^{-1} assignable to the $^1Eg^+ \rightarrow {}^3\Pi u$ transition. Also the presence of a band at 18579 cm^{-1} suggests that the uranyl ion is surrounded by four equatorial[12] groups. The complex exhibits a magnetic moment of 0.12 B.M, suggesting the six coordinated geometry.

Based on micro elemental analysis, conductance measurements, IR, UV-VIS and magnetic moment measurements, the following are the tentative structures proposed for the complexes (Figures 2 to 4).

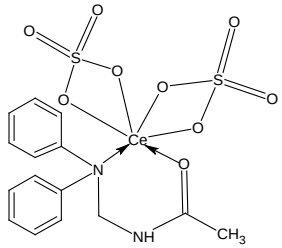

Figure 2. $Ce(SO_4)_2$. DPAMAce

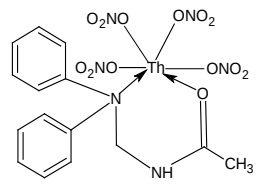

Figure 3. $Th(NO_3)_4$. DPAMAce

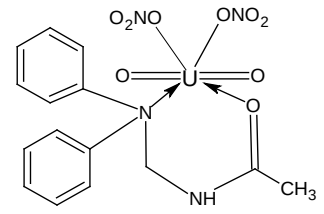

Figure 4. $UO_2(NO_3)_2$. DPAMAce

Biological studies

Antibacterial activity

The order of activity was found to be thorium(IV) nitrato > dioxouranium(VI) nitrato > cerium(IV) sulphato > standard > ligand. Among the compounds tested, the thorium(IV) nitrato complex was found to be the most active against all the species. The increase in activity may be due to its interaction with RNA[13]. It was found that the metal ion complexes were more biologically active than ligand in all cases. It was also observed[14] that there existed some relationship between the liability of M-O, M-N and M-X and the biological activities: *viz*. The activity increased with increasing lability of the metal complexes.

Antifungal studies

A possible mechanism of toxicity may be speculated in the light of chelation theory. Chelation reduces considerably the polarity of the metal ion mainly 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[14].

Anticancer screening

The results obtained for the ligand and its complexes on the growth of Raji and Jukart cells are given in Tables 2&3. In general, an increase in growth inhibition is observed as the concentration of test compound increases. Here also metal ion complexes show more activity than the ligand. The increase of activity on complexation is considerable in the case of the Th(IV) complex whereas the other complexes show moderate activity but higher than for free L.

Table 2. *In-vitro* bioactivity test for immunomodulatory and cytotoxicity assessment(MTT method**)**

S.No	Sample	Dye Exclusion	MTT
1	L	Non-toxic	Immunopotentiator at 100 ng
2	$CeSO_4$.L	Non-toxic	Cytotoxic at 400 ng
3	Th $(NO_3)_4$.L	Non-toxic	Immunopotentiator 100 ng
4	$UO_2(NO_3)_2$.L	Non-toxic	Immunopotentiator

(L- DPAMAce)

Table 3. Anticancer activities against Raji and Jukart Cell Lines(MTT Method)

S.No	Sample	Cell Lines	
		Raji	Jukart
1	L	Non toxic	Non toxic
2	$Ce(SO_4)_2$.L	Down regulation at 50 ng	Non toxic
3	$Th(NO_3)_4$.L	Up regulation at 50 ng	Up regulation at 50 ng
4	$UO_2(NO_3)_2$.L	Up regulation at 50 ng	Up regulation at 25 ng

(L- DPAMAce)

The Th(IV) nitrato complex is found to be most active among the complexes. The free ligand does not effectively reach the cancer cells and is consequently less active. However, among the complexes screened, the enhanced activity of the Th(IV) chelate can be explained as follows. A considerable amount of thorium is needed for rapidly dividing cancer cells. This thorium uptake tendency by cancerous cells leads to an easy uptake of the compound by cancerous cells[8]. After reaching the receptor site, the labile bonds in the complexes undergo dissociation and produce a free active antimetabolite, which is active, in its own right. From the present study it is seen that there is a positive relationship between the lability of M-O and M-N linkages and biological activity.

References

1 Lukyanenko N G, Pastushok V N and Bordunov AV, *Synthesis,* 1991, 241.
2 Wysor M S and Zollinhofer R E, *Chemotherapy (Basel),* 1972, **17(3),** 188-199.
3 Eichhorn G L, *Metal ions in Biological Systems,* 1980, **10,** 1-21.
4 Filion G J P, Zhenilo S, Salozhin S, Yamada D, Prokhortchouk E and Defossez P A, *Mol Cell Biol.,* 2006, **26**, 169-181.
5 Tramontini M and Angiolini L, Mannich Bases- Chemistry and Uses, CRC Press Inc.; USA, 1994.
6 Lambert D M, Scriba G K E, Poupaert J H and Dumont P, *Eur J Pharm Sci.,* 1996, **4,** 159-166.
7 Dewar M J S, Ford G, Mckee M, Rzepa H S and Yamaguchi Y, *J Mol Struct.,* 1978, **43,** 135.
8 Bailer B C, Keppler B K, Metal Complexes in Cancer Chemotheraphy*;* VCH; Weinheim, 1993.
9 Nakamoto K, Infrared and Raman Spectra of Inorganic and Coordination Compounds, 5th Edn., John Wiley & Sons, Toronto, 1997, 60, Part B.
10 Parkin G, *Chem Rev.,* 2004, **104**, 699-767.
11 Yongsheng Hu, Zhaoxiang Wang, Hong Li, Xuejie Huang and Liquan Chen, *Int J Mass Spectr.,* 2006, **255**, 251-264.
12 Szajna-Fuller E, Ingle G K, Watkins R W, Arif A M and Berreau L M, *Inorg Chem.,* 2007, **46**, 2353-2355.
13 Freifelder D, Molecular Biology, Jones and Bartlett Publishers Inc.; Boston, 1987.
14 Cronin C F and Walton P H, *Chem Commun.,* 2003, 1572.