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Microwave-Assisted Synthesis of 3,5-Dibenzyl-4amino-1,2,4-triazole and its Diazo Ligand, Metal Complexes Along with Anticancer Activity

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Abstract: Synthesis of 3,5-dibenzyl-4-amino-1,2,4-triazole was accomplished via a conventional method as well as microwave irradiation method, followed by diazotization and coupling with 2,4-pentanedione. The dinucleating ligand was isolated and complexed with Ni(II), Cu(II) and Ru(III) chlorides. These complexes were screened on Jurkat, Raji & PBMC cell lines for anticancer activity. Ruthenium complexes showed potential anticancer activities.

Keywords: 3,5-Dibenzyl-4-amino-1,2,4-triazole, Diazo ligand, Ni(II), Cu(II) and Ru(II) complexes, Anticancer activity.

Introduction

Chemistry is critical to drug discovery, especially at the lead optimization phase, but methods for the synthesis of organic compounds have remained essentially unchanged for decades. Since optimization time is usually very long with a very high manpower requirement, new ways to improve the efficiency, output and quality in this phase are always needed. One feasible solution is microwave assisted synthesis, which is in many ways superior to traditional heating; reactions are completed in minutes, yields are generally higher than those achievable by traditional means¹. The application of microwave (MW) irradiation in organic synthesis has been the focus of considerable attention in recent years and is becoming an increasing popular technology^{2,3}. The salient features of the microwave approach are the rapid reaction rates, cleaner reaction conditions and the operational simplicity.

1,2,4-Triazole and its derivatives are found to be associated with various biological activities⁴. For example, fluconazole is used as an antimicrobial drug, while vorozole, letrozole and anastrozole are nonsteroidal used for treatment of cancer and loreclezole is used as an anticonvulsant⁵. Also 4-amino-1,2,4-triazoles are potentially good corrosion inhibitors⁶. These compounds can be prepared by the nucleophilic attack of hydrazine derivatives on nitriles^{7,8}. Additionally, diazonium salts of heterocycles coupled with 2,4-pentanedione have been used as precursor in the synthesis of antimicrobial triazines⁹.

Rosenberg's discovery¹⁰ of the important Pt-containing antitumor drug ciplatin led to the search for other metal ions that could complex with biomolecules and exhibit carcinostatic properties. Since the chemical nuclease activity of transition metal complexes was discovered in the 1980s¹¹ studying the interaction model and the mechanism of transition metal complexes with DNA and exploring the application of metal complexes in antineoplastic medication, molecular biology and bioengineering have become hotspots in recent years.

Based on the above literature precedents and importance of ligand-metal system, we chose to synthesize 3,5-disubstituted-4-amino-1,2,4-triazole by conventional means as well as Microwave irradiation method, followed by diazotization and coupled with 2,4-pentanedione. The ligand was further complexed with Ni(II), Cu(II) and Ru(III) chlorides and screened against cancerous cell lines.

Experimental

Melting points are uncorrected. IR spectra were recorded on Thermo Nicolet FT IR spectrophotometer at Andhra University, ¹H NMR spectra were taken on Perkin Elmer R-32, 90 MHz in DMSO-d₆ using TMS as internal reference at IICT, Hyderabad and ESR spectra were taken on Varian E 112 at room temperature and as well as liquid nitrogen temperature using DPPH as standard at IIT Chennai. Benzyl cyanide, hydrazine hydrate, ethylene glycol and 2,4-pentanedione were purchased from Across. All the solvents were of analytical grade and were distilled before use. Microwave irradiation was carried out using a domestic microwave oven. All the complexes were analyzed for the metal content by standard procedure. Elemental analyses were carried out at Micro Analytical Center at Andhra University. The HPLC was recorded using Shimadzu LC 6A with Shimpack silica gel column. Anticancer screening was done at department of Biotechnology, Mother Teresa Women's University, Kodaikanal, on Jurkat, Raji & PBMC cell lines.

Synthesis of 3,5-dibenzyl-4-amino-1,2,4-triazole(2)

In conventional method of synthesis a mixture of benzyl cyanide (2 mmol), hydrazine hydrate (1 mmol) and ethylene glycol (5 mL) were heated under reflux for 10 h. After cooling 5 mL of HCl was added and again refluxed for 4 h, 3,5-dibenzyl-4-amino-1,2,4-triazole(**2**) was isolated and characterized.

Two different MW reaction routes were adopted to synthesize 3,5-dibenzyl-4-amino-1,2,4-triazole. In first method¹² benzyl cyanide (2 mmol) and hydrazine hydrate (1 mmol) were irradiated under microwave irradiation in presence of ethylene glycol (5 mL) for 9 min at 300 W.The product was worked up and dark pink colored dihydro-1,2,4,5-tetrazine (1), (Scheme 1) formed, which rearranges on treatment under acidic conditions into the 3,5dibenzyl 4-amino-1,2,4-triazole (2). In the second method¹³ benzyl cyanide (2 mmol), hydrazine hydrate (1 mmol), hydrazine dihydro chloride (0.5 mmol) and ethylene glycol (5 mL) were mixed together and irradiated under microwave condition for 8 min at 250W. The addition of ethylene glycol led to form homogeneous solution and resulted the formation of (2) (Scheme 2) *via* intermediate (1)



Scheme 1. Method 1, where: (a) HOCH₂.CH₂OH/MW, 9 min; (b) 2mL HCl/MW, 5 min Scheme 2. Method 2, where: (a) NH₂NH₂. 2HCl, HOCH₂.CH₂OH/MW, 8 min; (b) Nil

Synthesis of ligand

Substituted amino triazole (2) of 0.1 mol was dissolved in 5 mL conc HCl and cooled below -5^{0} to -10^{0} C, diazotized⁹ by the addition of cold NaNO₂ (1.5 g in 10 mL H₂O) solution. In the resulting solution of diazonium salt, a cold solution of 2,4-pentanedione (0.1 mol) containing sodium acetate (5 g) in water (10 mL) was added. The mixture was stirred for 3 h at 20 0 C. The solid product formed was collected by filtration and crystallized from ethyl alcohol. The isolated ligand (Figure 1) was characterized by various spectral methods.

General procedure for the preparation of metal complexes

In the ethanolic solution of ligand (1 mmol), an ethanolic solution of respective metal salts (2 mmol each NiCl₂.6H₂O; CuCl₂.H₂O and RuCl₃.3H₂O) was added¹⁴ separately drop wise while stirring followed by the addition of 2-3 drops of $(C_2H_5)_3N$. The contents of the mixture were refluxed for 3-4 h. The crystalline substance thus obtained were filtered and washed successively with ethanol and ether and dried in vacuum.

Study of cytotoxicity

Anticancer activity testing was performed by dye exclusion method. Bioactivity assay was performed in a 96 well tissue culture plate (Greiner, Germany). Various concentrations of the compounds ranging from 25 ng-150 ng were made. A constant number of cancerous (Jurkat, Raji & PBMC) cell suspensions and a constant volume of the complete media were added into each well. Desired concentrations of the compounds were added into each well and the volume was made constant, using complete media. Negative (solvent used for dissolving the compound) and positive (known immunomodulator) controls were also maintained. The plate was incubated at 37 $^{\circ}$ C in CO₂ incubator with 5% CO₂. The numbers of live cells were counted after every 12 h by using inverted phase contrast microscope (Nikon TM) and the Newbauer's counting chamber by dye exclusion method.

Results and Discussion

Recently, organic transformation accelerated under solvent free microwave irradiation condition gained wide popularity due to many practical advantages associated with enhanced reaction rates, high yields; improve selectivity¹² and environment friendly reaction condition¹³ in tune with green chemistry. Keeping these facts in consideration, we adopted Microwave (MW) synthesis, for the first time. A symmetrically 3,5-dibenzyl-4-amino-1,2,4triazole was guickly prepared by the reaction of aromatic nitrile and hydrazine dihydrochloride in the presence of an excess of hydrazine hydrate in the ethylene glycol under microwave irradiation. In the first method¹² benzyl cyanide (2 mmol) and hydrazine hydrate (1 mmol) were irradiated in presence of ethylene glycol (5 mL) for 9 min at 300W. The product was worked up and dark pink coloured dihydro-1,2,4,5-tetrazine-3,5-dibenzyl (1), (Scheme 1) formed, which rearranges itself on treatment under acidic conditions into the 3,5-dibenzyl-4-amino-1,2,4-triazole (2). In the second method¹³ benzyl cyanide, hydrazine hydrate, hydrazine dihydro chloride and ethylene glycol were mixed together and irradiated under microwave condition for 8min at 250W. In conventional method, same ratio of reactants were mixed and heated under reflux for 10 h. The isolated product compound 2 from both the MW methods and conventional method was compared, identical TLC and melting point (142 °C) was found. The synthesized 3,5-dibenzyl-4-amino-1,2,4-triazole was subjected for diazotization and coupled with 2,4-pentanedione. The ligand Figure 1 were characterized by spectral methods. The analytical data of the ligand and their metal complexes are given in Table 1.

S No	Compound	Colour/MP ⁰ C	Found (Calcd)%				
5. 110.			С	Η	Ν	Μ	Cl
1	$C_{16}H_{16}N_4$	light pink/142	72.75	6.09	21.25		
			(72.72)	(6.06)	(21.21)	-	-
2	$C = H = N \cap (I)$	light yellow/>250	67.32	5.06	18.62	-	-
2	$C_{21}\Pi_{21}\Pi_{5}O_{2}(\mathbf{L})$		(67.20)	(5.61)	(18.66)		
3	$[Ni(L)Cl(H_2O)_3]$	leafy green/>250	48.20	5.19	13.40	11.31	6.82
			(48.16)	(5.16)	(13.37)	(11.22)	(6.78)
4	$[C_{\rm H}(I_{\rm c})C](I_{\rm c}O_{\rm c})$	Leafy green/>250	47.62	5.16	13.28	12.23	6.77
	$[Cu(L)Cl(H_2O)_3]$		(47.72)	(5.11)	(13.25)	(12.03)	(6.72)
5	$[Ru(L)Cl(H_2O)_3]$	Black />250	44.50	4.72	12.32		6.31
			(44.55)	(4.77)	(12.37)	(17.87)	(6.27)

Table 1. Physical and Analytical data of Synthesized Compound

The comparative IR spectral study of the ligands and their metal complexes reveal the interesting coordination mode of the ligand during complex formation. The disappearance of peaks in the region 3377 cm⁻¹ for ligand indicated that the NH₂ group of substituted triazole is changed to N=N in the formation of ligand¹⁵ which was further supported by comparing NMR spectra of substituted triazole and ligand The peaks 1708 and 1421 respectively were assigned to C=O and N=N, which were shifted to lower frequencies¹⁵ in the IR spectra of all metal complexes, indicating the coordination with ligand. The metal complexes show new bands in the regions 480-450 cm⁻¹ and 350-320 cm⁻¹, which are due to the formation of M-N and M-O bonds¹⁶ respectively.

The ¹H NMR spectra of 3,5-dibenzyl-4-amino-1,2,4-triazole(**2**) and ligand (Figure 1) were recorded in CDCl₃. The substituted triazole displayed multiplets in the region δ 7.4-7.2 due to aromatic protons, which remains same position in the ligand (Figure 1); δ 4.4 (NH₂) and 4.0 (CH₂) protons. The disappearance of NH₂ peak and presence CH₃ at 1.5 indicated that it

was diazotized and coupled with acetyl acetone¹⁴. In the ¹³C NMR of substituted triazole showed peaks at $31(\underline{CH}_2)$; 128,129,130,135 for aryl carbons; 155 for triazole ring carbon. However in ligand extra peaks were observed at 15, 55, 180 ppm were assigned to (\underline{CH}_3 , $\underline{CH} \& \underline{CO}$ of 2,4 pentanedione) Poor solubility of metal complexes restricted us to record NMR spectra.



ESR Spectra

The ESR spectra of Cu(II) complex of the ligand was recorded at room temperature (RT) and liquid nitrogen temperature (LNT). The spin Hamiltonian parameters, calculated from the spectra is given in Table 2. The powder state spectra of the complex $[Cu(L)Cl(H_2O)_3]$ at RT and LNT showed four equally spaced lines as expected for Cu(II) ion showing $g_{\parallel} > g_{\perp}$, however it is more clear in LNT spectra. The values are supportive of octahedral geometry around metal¹⁷. Basic spectral characteristics at both temperatures are the same with slightly better resolution¹⁸ at LNT. The half field signal was not observed in any spectra is indicated that there is no Cu-Cu interaction between the complex. On the basis of above spectral studies and metal estimation metal complexes were assigned to octahedral geometry Figure 2.

Table 2. Powder Spin-Hamiltonian parameters of Cu(II) complex



Figure 2. Structure of metal complexes

Study of cytotoxicity

Simultaneously, our objective of synthesis of metal ligand complexes and also to study their activity, the compound YLN-AJ-1, 2 & 3 (Table 3) were screened on Jurkat, Raji & PBMC cell lines for anticancer activity. The studies indicated that YLN-AJ-3 (Ru complex of Ligand) exhibited anti-cancer activity *in vitro*, in nanogram scale. The experimental results are enclosed below in Table 3. It is also indicated that Ni(II) and Cu(II) complexes of ligand were not active against Jurkat and Raji cell lines while it showed cytotoxicity at 50 & 100 ng against Jurkat and Raji cancer cell lines while it is non toxic even at 100 ng against PBMC cell line. Further sensitive studies are in process.

Table 3. Experimental results for samples screened on cancer cells

S.No.	Sample name	Jurkat	Raji	PBMC
1	YLN-AJ-1 [Ni(L)Cl(H ₂ O) ₃]	Not active	Not active	Cytotoxic (200 ng*)
2	YLN-AJ-2 [Cu(L)Cl(H ₂ O) ₃]	Not active	Not active	Cytotoxic (150, 200&250 ng)
3	YLN-AJ-3 [Ru(L)Cl(H ₂ O) ₃]	Toxic to cancer cell line (50 & 100 ng)	Toxic to cancer cell line (100 ng)	Nontoxic (100 ng)

*ng=nanogram

Conclusion

In conclusion present study provide a better route for synthesis of 3,5-dibenzyl-4-amino-1,2,4-triazole. Its ligand and metal complexes were synthesized to screen against cancer cell lines. On the basis of spectral studies metal complexes were assigned to octahedral geometry and ligand could form only mononuclear complexes due to bulky benzyl group. It is also observed that compound no. **3** in Table 3 *i.e.* Ru complex with ligand is a potential anticancer compound in nanogram scale while Ni(II) and Cu(II) complexes of ligand were not active against Jurkat and Raji cell lines however they showed cytotoxicity at 50 & 100 ng.

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