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Original Article

IN VITRO ANTICANCER, ANTIOXIDANT AND MOLECULAR DOCKING STUDIES OF 4-AMINOBENZAMIDE METAL COMPLEXES

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ABSTRACT

Objective: Mixed ligand complexes of Cu(II) and Cd(II) with 4-aminobenzamide(4-ABZ) have been synthesized under microwave irradiation and they were characterized by elemental analysis, estimation of metal ion, molar conductivity, magnetic study, FT-IR, Far-IR, electronic spectra, NMR (1H and 13C) and EPR spectral studies.

Methods: The data of microanalysis and metal estimation showed that the complexes have the composition of $[M(L_1)_2(L_2)_2]$ for Cu(II) and $[M(L_1)(L_2)_2]$ for Cd(II) complex respectively. The conductivity data confirmed the neutral nature of the complexes. The tetrahedral geometry of the Cd(II) complex was confirmed using UV-Visible spectra. The tetragonally distorted octahedral stereochemistry of Cu(II) complex was further confirmed by UV and EPR spectral studies. The diamagnetic nature and probable geometry of Cd(II) complex were also confirmed using NMR spectral studies, which are also compared with free 4-aminobenzamide. The *in vitro* bio-potential activities of 4-ABA and Cd(II) complex were screened for *E. coli* and *C. Albicans* microorganisms by Agar disc diffusion methods.

Results: The cancer study of Cu(II) complex and antioxidant activity of DPPH free radical scavenging method of Cd(II) complex were measured and compared with 4-aminobenzamide and standard drugs. The breast cancer studies were also identified using the targeted proteins viz., BRCA1, PR and EGFR by molecular docking study.

Conclusion: In the present study, we synthesized new metal complexes of Cu(II) and Cd(II) with 4-ABA and nitrite ion by microwave irradiation method. The analytical data show that the metal-ligand stoichiometry they are non-electrolytes in acetonitrile solution. The spectral data show that the synthesized ligand binds with metal ions through the nitrogen atom of the 4-ABA and oxygen atoms of the nitrite ion.

Keywords: 4-aminobenzamide, Antibacterial, Antifungal, Anticancer, Antioxidant, Molecular docking

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INTRODUCTION

The biological importance of coordination chemistry is crucial in the present decades, especially in the designing of long-acting drugs in metabolism [1]. Coordination compounds are important in the catalysis and chemical industry. Derivatives of benzamide have heterocyclic moiety which is subject to greater interest due to a wide range of biological activities [2]. Benzimidazole derivatives were used in medicinal chemistry due to their microbiological, antimicrobial, analgesics, anti-inflammatory, anticancer, anticonvulsant, and antimalarial activities. The nitrogen and oxygen-containing heterocyclic compounds show this biological activity [3]. Metal complexes are also used as antiarthritic, antidepressant, and anti-hypertensive properties [4]. Metal-based anticancer drugs such as cisplatin were much attracted during past decades [5, 6]. The amide-containing metal complexes are very important due to their biological importance. The objective of the present investigation is to synthesize Cu(II) and Cd(II) complexes of 4-aminobenzamide and to provide structural data using spectral, thermal, and biological studies.

MATERIALS AND METHODS

Materials used for the preparation

4-aminobenzamide, copper nitrate, cadmium nitrate, sodium nitrate, ethanol, methanol, acetonitrile, DMSO, and DMF were of AR grade chemicals.

Synthesis of Cu(II) complex

About 1.127 g of (8.27 mmole) 4-aminobenzamide in ethanol, 0.571 g (8.27 mmole) of sodium nitrite in water were mixed with the 1g (4.13 mmol) of copper nitrate in methanol and the whole mixer was microwave-assisted using a microwave oven for a few seconds, the pale green-colored complex was filtered washed with ethanol: water (1:1) dried.

Synthesis of Cd(II) complex

By mixing 0.441 g of (3.24 mmole) 4-aminobenzamide in ethanol, 0.447 g (6.48 mmole) of sodium nitrite in water were mixed with the 1g (3.24 mmole) of copper nitrate in methanol and the whole mixer heated on a microwave oven for few seconds, the pale colorless complex was filtered washed with ethanol: water (1:1).

Methods used for characterization

Microanalytical data of the complexes were carried out by the elemental Vario instrument. The metal ions in the complexes were estimated by colorimetric and volumetric. 10^{-3} M complex solution in acetonitrile the conductivity was measured using a Systronic Conductometer. The magnetic property of the Cu(II) complex was measured using a VSM instrument at room temperature. The UV-Visible spectra of the complexes were recorded CARY-5000 model Spectrophotometer. FT-IR spectral data of 4-ABA and complexes were recorded Using Shimadzu, FT-IR, 8400 S Model IR spectrometer. The low-frequency IR spectra of the complexes using the Bruker-FT-IR instrument. The NMR spectra of ligand and its Cd(II) complex in DMSO-D6 were recorded using Bruker, AVANCE-III model, and 500 MHz NMR Spectrometer. X-band EPR spectra of Cu(II) complex were recorded on JEOL, Japan makes, JES-FA200 ESR spectrometer at LNT.

In vitro microbiological studies

The *in vitro* biological activity of ligand and their metal complexes were carried out by the Agar disc diffusion method at different concentrations with various microorganisms. The zone of inhibition of the antimicrobial potential of the test of ligand and complexes was

measured. The zone of inhibition in the disc was measured on a millimeters scale.

***In vitro* antioxidant activity**

The antioxidant activity of ligand and metal complexes was measured by the DPPH radical-scavenging method at different concentrations. The IC_{50} values were also measured.

***In vitro* anticancer activity**

In vitro anticancer activity of ligand and metal complex was predicted using human breast cancer cell line MCF 7. The Cytotoxic assay was evaluated by the reduction of MTT. The monolayer cells were detached and single-cell suspensions were produced with trypsin-ethylenediaminetetraacetic acid (EDTA). The maximum of four different concentrations, such as 12.5, 25, 50, 100, 200, and 400µg/ml of ligand and metal complex, dissolved in DMSO solvent. After dissolved, it was incubated for 48 h at 37 °C. The inhibition concentration (IC_{50}) was measured graphically and the absorbance was measured at 570 nm.

Molecular docking studies

Most docking of metal complexes was performed using PyRx 0.8 with the Auto dock Vina method with a Lamarckian genetic algorithm as the score feature was completed. Pose for the ligands found based on the highest binding energy. The PyMol molecular viewer (http:/www.pymol.org/) was used to analyze the docked structures. Using Lipinski's rule of five, the drug likeliness calculation was made with the software.

RESULTS AND DISCUSSION

The synthesized complexes are stable at room temperature and readily soluble in an organic solvent but insoluble in water. From the results of microanalytical data, the molecular formulae of the complexes were predicted [7]. It was concluded that the stoichiometry of 1:1:1 type of metal: ligand1: ligand2 of metal complexes confirmed $[M(L_1)_2(L_2)_2]$ for Cu(II) complex while $[M(L_1)(L_2)_2]$ for Cd(II) complex (table 1). The non-electrolytic (1:0 type) neutral nature of the metal complexes is confirmed by the molar conductivity of 10^{-3} M solution in acetonitrile [8].

Table 1: Physico-chemical and analytical data of the complex

S. No.	Complex	Color	% yield	Conductance $Ohm^{-1} cm^2 mol^{-1}$	Elemental analysis				
					%C	%H	%N	%O	%M
1	Cu(II) complex	Pale Green	80	40	39.26 (39.20)	03.73 (03.70)	19.63 (19.40)	22.43 (22.40)	14.85 (14.69)
2	Cd(II) Complex	Colorless	70	32	24.66 (24.20)	02.34 (02.10)	16.44 (16.35)	23.48 (23.38)	33.00 (33.10)

Electronic spectra and magnetic property

The UV-Visible spectrum of the copper complex shows an intense peak at 665 nm, 360 nm, and shoulder at 265 nm assigned to the $^2A_{1g} \leftarrow {}^2B_{1g}$, $^2B_{2g} \leftarrow {}^2B_{1g}$, and $^2A_{1g} \leftarrow {}^2B_{1g}$ transitions confirmed by the tetragonal geometry which is further confirmed by magnetic moment at 1.85 BM and Jahn Teller distortion. The Cd(II) complex gave only charge transfer transition at 275 nm showing tetrahedral geometry around Cd(II) ion. It shows only the d-d C-T band and no d-d transition [9].

IR spectra of complexes

IR spectrum of 4-aminobenzamide shows (C-H) at 3161 cm^{-1} and (C-C) stretching at 3075 cm^{-1}, asymmetric and symmetric stretching frequencies at 3443 cm^{-1} and 3198 cm^{-1}, respectively. The (C=O) stretching frequency at 1672 cm^{-1}. These frequencies are shifted to lower or higher frequencies upon coordination of ligand to the metal ions, which are exhibited at aromatic (C-H) and (C-C) stretching frequencies at 3002 cm^{-1} and 2790 cm^{-1} in Cu(II) complex, whereas in Cd(II) complex it is exhibited at 3009 cm^{-1} and 2762 cm^{-1}. The $\nu(NH_2)$ at 3448 cm^{-1} and 3199 cm^{-1} in Cu(II) complex but it exhibits 3364 cm^{-1} and 3193 cm^{-1} at respectively [10]. The carbonyl (C=O) stretching frequency at 1672 cm^{-1}. Similarly, the ν(C=O) group at 1662 and 1678 cm^{-1} in both complexes confirmed the 4-ABA coordination to the metal ions through the nitrogen atom of amine and oxygen atom of the carbonyl group. The mixed anionic ligand NO_2 exhibits asy(ONO), Sy(ONO) at 1250 cm^{-1} and 1335 cm^{-1} in the free ligand; after complex formation through its oxygen atoms, it was shifted to 1243 cm^{-1}, 1258 cm^{-1}, 1418 cm^{-1}, and 1384 cm^{-1} respectively in complexes [11]. This fact is further confirmed by δ(ONO) at 845, 851 cm^{-1}, and ρwNO_2 at 650 and 664 cm^{-1}. The M-N and M-O metal chelates stretching frequency was observed at 450 cm^{-1} and 350 cm^{-1}

NMR Spectra (^{1}H and ^{13}C)

The ^{1}H-NMR spectrum of 4-aminobenzamide gave four different chemical shift values for four different hydrogen atoms at 5.597 ppm (NH_2), 6.514-6.531 PPM(Ortho-H), 6.855 ppm (Amine NH_2), 7.529-7.595 ppm (meta-H) which are shifted to down or up-field in Cd(II) complex at 5.597 ppm, 6.518-6.200 ppm, 6.755 ppm, 7.532-7.335 ppm confirmed by the diamagnetic nature and tetrahedral geometry of the Cd(II) complex it was further confirmed by its ^{13}C-NMR spectra which shows five different carbon atoms 4-ABA at 152.12 ppm, 112.94 ppm, 129.59 ppm, 121.39 ppm and 168 ppm corresponding to C-NH_2, Ortho-C, Meta-C, C-C=O and C=O respectively which are shifted to downfield in C-NH_2, Ortho-C, Meta-C and up-field in C-C=O and C=O [12].

Biological activity

The antifungal activity of the 4-ABA and Cd(II) complex shows good activity due to the chelate effect of complexes. Pi electron delocalization on the whole chelate ring and lipophilicity of the complexes enhanced the bio-potential activity. The size, neutral nature, and conductivity of the complexes also enhanced the biological activity of the complexes [13].

Antioxidant activity of 4-ABA and Cd(II) complex

The antioxidant activities of the 4-ABA and Cd(II) complex are compared (table 2). The free radical scavenging ability of ligand and metal complexes is confirmed by its percentage inhibition (fig. 1 and 2). The scavenging activity of the metal complex is slightly higher than the free 4-ABA indicating that the complex is a much better free radical scavenger and antioxidant than standard. Radical scavenging activity of the metal complex is increased in a dose-dependent manner after chelation of transition metal ions and possesses higher antioxidant potential (IC_{50}) than the ligand and standard drugs [14]. The IC_{50} values as 4-ABA 42.28>44.64>50.41 μm.

Table 2: DPPH activity of your samples

Samples ID	Concentrations (µg/ml)				IC_{50} value
	20	40	60	80	
4-ABA	25.19±1.76	46.33±3.24	63.39±4.43	86.68±6.06	44.64
Cd(II) complex	21.05±1.47	41.62±2.91	55.66±3.89	80.06±5.60	50.41
Std. (Ascorbic acid)	27.51±1.92	47.68±3.33	64.75±4.53	92.98±6.50	42.28

Values expressed as mean±SD for triplicate

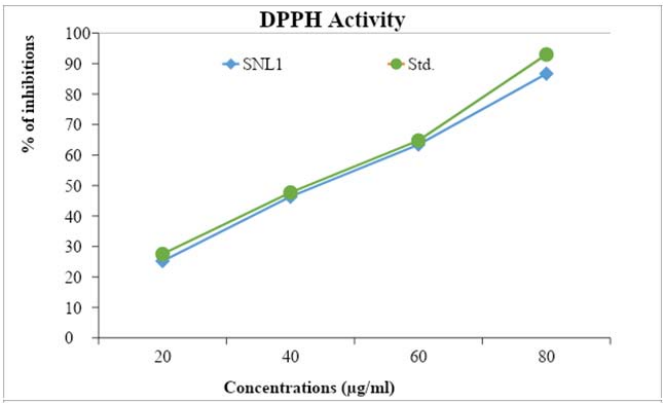

Fig. 1: Free radical scavenging activity of 4-Aminobenzamide

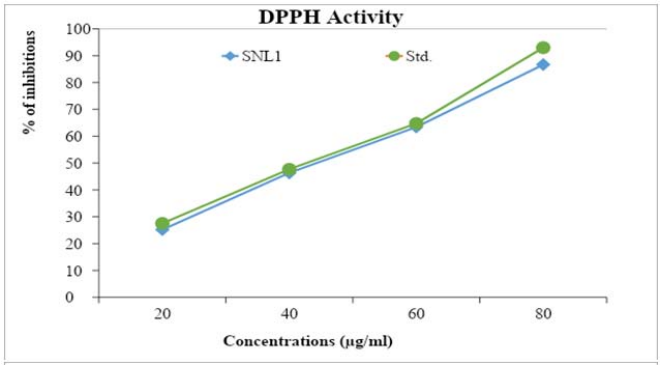

Fig. 2: Free radical scavenging activity of Cd(II) complex

In vitro anticancer activity of 4-ABA and Cu(II) complex

The *in vitro* breast cancer studies (fig. 3) of 4-ABA inhibition is 23.28% at 50µg/ml and the highest growth inhibition up to 88.21% observed at a higher concentration. The morphological change of the cancer cell line is apoptosis which was observed by inverted light microscopy in the 4-ABA treated cells. The Cu(II) should increase the cell growth inhibition, but it is found to be higher growth at 79.45 at higher concentrations. The IC_{50} value of Cu(II) was 99.54µg/ml. Among the Cu(II) and 4-ABA compounds, the 4-ABA possesses potential anticancer activity [15].

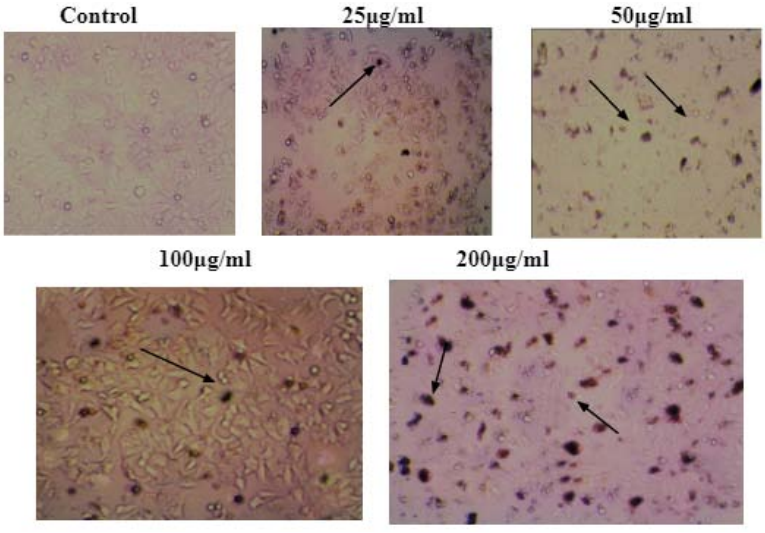

Fig. 3: Morphological change of MCF-7 cell line of 4-ABA

Molecular docking

The potential therapeutic targets for breast cancer were BRCA1, PR and EGFR. The three-dimensional structures of the following target breast cancer proteins were extracted from the BRCA1 protein database (PDB ID: 1T15), PR (PDB ID: 4OAR), EGFR (PDB ID: 2J6 M). The molecular docking has been used to investigate the binding mechanism and tests of the newly synthesized metal complex against

BRCA 1, PR and EGFR protein revealed that perhaps the free binding energy for the inhibitor was minimal, suggesting that the ligand binds favorably to the binding site [16]. The strongest confirmation shows that the free binding energies (ΔG bind kcal/mole) for the 4-ABA with target proteins BRCA1, PR and EGFR were 4.5 kcal/mole,-5.6 kcal/mole, and 6 kcal/mole. Few hydrogen bonding interactions and the best binding affinity were predicted by molecular docking studies. Analysis Docked complexes in PyMol showed that both compounds formed reasonable hydrogen bond formation with all the three target proteins (fig. 4 and 5). *In silico* investigation, it was reported that newly synthesized 4-ABA and Cu(II) compounds were produced as drug candidates which had target specificity for breast cancer receptors of well-defined physicochemical, pharmacokinetic, and toxicity properties. Each of these findings provided important information for any further development of these compounds as a possible inhibitor of breast cancer receptors [17, 18].

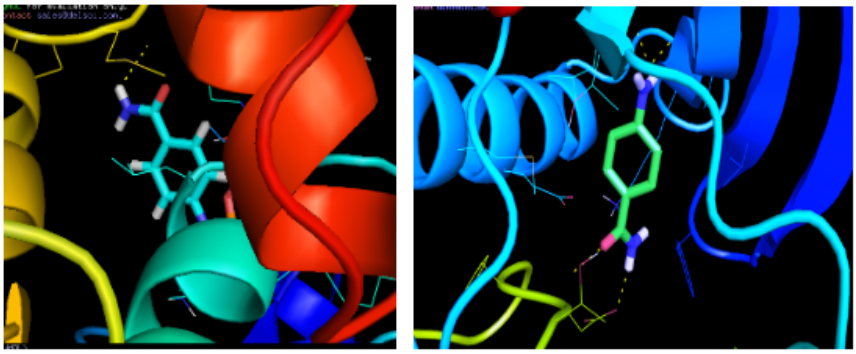

Fig. 4: Molecular docking of 4-ABA and Cu(II) complex of BRCA1

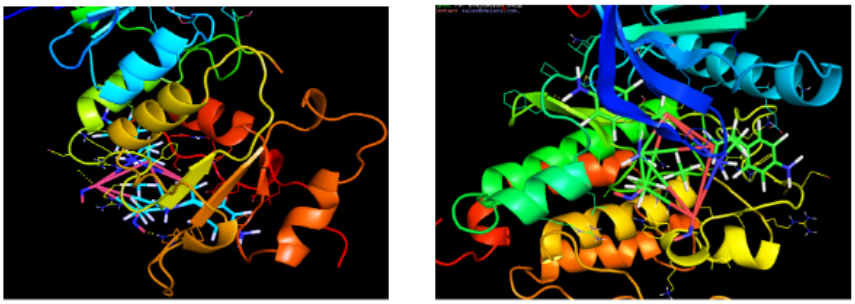

Fig. 5: Molecular docking of 4-ABA and Cu(II) complex of PR and EGF

Table 3: Calculated ADME properties

Compound name	Molecular mass	donors	Hydrogen bond donor	LOGP	Refractivity
4-ABA	136.0	4	3	0.367700	39.372299
Cu(II) complex	524.000000	1	2	0	0

CONCLUSION

In the present study, we synthesized new metal complexes of Cu(II) and Cd(II) with 4-ABA and nitrite ion by microwave irradiation method. The analytical data show that the metal-ligand stoichiometry they are non-electrolytes in acetonitrile solution. The spectral data show that the synthesized ligand binds with metal ions through the nitrogen atom of the 4-ABA and oxygen atoms of the nitrite ion. The *in vitro* biological activity, antioxidant activity confirming the bio-potential and radical scavenging property. The complex shows anticancer activity, which was further concluded by molecular docking.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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