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RESEARCH ARTICLE

Synthesis, Characterization and Anti-Cancer Studies of Transition Metal Complexes of Tetrazole Derivative

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ABSTRACT

Tetrazole derivative have played a vital part in the development of heterocyclic compounds. During the last two decades, the study of the biological activities of tetrazole derivative has been the aim of many researchers. Based on these findings, a series of benzoic acid substituted tetrazole were synthesize and their metal complexes were synthesized by condensation of tetrazole and different transition metal chloride salt of Zn(II) and Cu(II) their characterization are done by different analytical techniques, such as elemental analysis, FT-IR, UV, ^{1}H-NMR, ^{13}C-NMR, and ES-Mass. All the compounds were tested for their anticancer activity against MCF-7 lung cancer cell line with MTT assay. Docking studies of the synthesized compounds was done with the help of HEX 6.1software using GRIP batch docking method to study their observed activity. Docking study was done and the compounds were found to fit well with the target PDB ID: 1NOW.

Keywords: Anticancer activity, Synthesis, Docking, MCF-7 cell line.

INTRODUCTION

The chemistry of heterocyclic compounds has been an attracting field of study of long time. The synthesis of novel tetrazole derivatives and investigation of their chemical and biological behaviour has gained more importance in recent decades for biological and pharmaceutical reasons (Sankari Kanakaraju et al, 2013). 1, 2, 3, 4-tetrazole represent an important class of heterocyclic compounds. Tetrazoles are class of synthetic organic heterocyclic compounds consisting of five-member ring of four nitrogen and one carbon atom (plus hydrogen). The simplest is tetrazole itself CN_4H_2. It is white to the pale yellow crystalline solid with the weak characteristic odour, soluble in water and alcohol. It is acidic in nature due to presence of four nitrogen atoms. Numbering of tetrazoles is as shown below. Usually, tetrazole is explosives. Nature of tetrazoles was unknown. It is used as gas generating agent for air bags (Mohite P.B and Bhaskar V.H, et al, 2011). A number of tetrazoles are used as pharmaceutical agents. They undergo electrophilic as well as nucleophilic substitution reactions. Tetrazoles can act as pharmacophore for the carboxylate group, raising their utility. Angiotensin II blocker often contain tetrazoles, as Losartan and candesartan. A well-known tetrazole is MTT, which is dimethyl thiazolyl diphenyl tetrazolium salt. This tetrazole is used in MTT assay to quantify the respiratory activity of live cells in cell culture, although it kills cells in the process (Dhayanithi Varadaraji et al,. 2010) (Diana Pintos et al,. 2007). Tetrazole and their derivatives possess broad spectrum of biological activity in both medicinal and pharmaceutical, such as anti-inflammatory (V. H. Bhaskar, P. B. Mohite et al, 2011), antibacterial (Helen P Kavitha et al, 2000), antifungal (Ismail Y, Ikay O et al, 2000), antitubercolous (Heffeter P., Jakupec M. A et al, 2006) antiviral (Lyakhov, S. Alexander et al, 2001), and anticancer activities (J.H. Toney, et al,. 1996)

MATERIALS AND METHODS

All the chemicals and solvents used were of AR-grade obtained from Sigma- Aldrich, Sisco Research Laboratories, Qualingens, Hi-media, nice chemicals, Spectrochem and were used without further purification. All melting points were taken in open capillaries and are uncorrected. Elemental analysis was performed on a Perkin-Elmer analyzer. IR spectral [12] were recorded in KBr on Shimadzu spectrometer, ^{1}H-NMR and ^{13}C-NMR in DMSO-d6 on a Bruker AC-400 spectrometer using TMS as an internal standard. The microorganisms were obtained from National Chemical Laboratory, Pune. Thin-layer chromatography (TLC) was performed on pre-coated aluminium plates (silica gel 60F254, Merck). Plates were visualized by UV light and iodine vapor.

Synthesis of Thiocyanate (TC1)

The substituted/unsubstituted benzoic acid (0.5 mole) (Maruthamuthu et al, 2016) was dissolved in acetic acid (125 ml) and the solution was added to the solution of ammonium thiocyanate (1.05mol, 80 g) in glacial acetic acid (250 ml). This solution was cooled to 10-20 ºC. To this well stirred solution, a solution of bromine (0.5 mol, 25.7 ml) in acetic acid (250 ml) was added drop wise for thirty minutes and the temperature was maintained below 20ºC. After the addition of bromine, it was kept at room temperature for ten minutes and then it was diluted with an equal amount of water. The solid material was filtered, washed, dried and recrystallized from ethanol.

Compound TC-1 2-acetoxy-4-thiocyanatobenzoicacid

Anal. Calcd. For $C_{10}H_7SNO_4$: C, 45.98; H, 2.81; N, 2.97; O 26.98; Found: C, 45.05; H, 2.94; N, 5.97; O, 27.05; Yield %(72), ES (+) 237.23 (M+H); M.p.: 242–243 °C; IR KBr (cm $^{-1}$): - $^{\nu}$C≡N: 2251.5cm-1.

Synthesis of Tetrazole (TT1)

A mixture of thiocyanate TC1 (0.01 mol), sodium azide (0.01 mol) and NH_4Cl), in DMF (10 mL) (Helen P Kavitha et al., 2000) was heated for 6 hours at 160ºC. The solvent was removed under reduced pressure and the residue was dissolved in (50mL) water and acidified with dil. HCl to pH. The solution was cooled in ice bath to give a precipitate which was recrystallized from aqueous ethanol.

Compound TT 1: 4-((H-tetrazol-5-yl) thio) 2-acetoxybenzoic acid

Anal. Calcd. For $C_{10}H_8SN_4O_4$: C, 52.16; H, 2.98; N, 08.16; O 11.50; Found: C, 52.25; H, 3.03; N, 08.21; O, 11.57; Yield % (79), ES (+) 280 (M+H); M.p.: 231–235 °C; ^{1}H NMR [DMSO-d6, ppm]: δ 7.90 (Ar-H, multiplet), δ 12.5 (Ar-OH, singlet)ppm; ^{13}C-NMR [DMSO-d6, ppm]: δ 169 (OH), δ 155 (C=N)ppm; IR KBr (cm $^{-}$1): 1607.19 (C=Nstr), 1412.38 (N=Nstr), 3513.80 (OH str).

Synthesis of metal complexes (1-5)

4-((H-tetrazol-5-yl) thio) 2-acetoxybenzoic acid (0.01M) was dissolved in ethanol than solid $M \cdot Cl_2 \cdot 6H_2O$ (0.02M) (where M= Zn, Cu) was added to reaction mixture. The resulting reaction mixture was refluxed for 24hours in the presence catalytic amount of NH_3 with continuous stirring. After completion of the reaction, the resulting solid was filtered and washed with cold methanol and dried at room temperature.

Zinc complex (3) [Zn $(L)_2$]

Anal. Calcd. For $C_{20}H_{16}N_8ZnS_2O_5$: C, 47.02; H, 2.31; N, 07.66; O,12.54; Found: C, 47.17; H, 2.39; N, 07.70 ;O,12.61; Yield % (68), ES (+) 625.83 (M+H); ^{1}H NMR (DMSO-d6) δ 7.2(Ar-H, multiplet), δ 12.5.0 (Ar-OH, singlet), ^{13}C-NMR: δ 178 (OH), δ 150.7(C=N), IR KBr (cm $^{-}$1): 1629.01(C=Nstr), 1547.28 (C=Cstr), 2919.18 (OH str) 3010.23 (C-Hstr) 505.72(N-Zn).

Copper Complex (4) [Cu $(L)_2$]

Anal. Calcd. For $C_{20}H_{16}N_8CuS_2O_8$: C, 44.02; H, 2.11; N, 03.67; O,12.57; Found: C, 45.12; H, 2.48; N, 07.73 ;O,12.49; Yield % (82), ES (+) 624.99 (M+H); ^{1}H NMR (DMSO-d6) δ 7.4 (Ar-H, multiplet), δ 12.7 (Ar-OH, singlet), ^{13}C-NMR: δ 168 (OH), δ 152.3(C=N), IR KBr (cm^{-}1): 1692.99 (C=Nstr), 1554.21 (C=Cstr), 2919.78 (OH str) 3012.89 (C-Hstr) 450.84(N-Cu).

BIOLOGICAL EVALUATION

ANTICANCER ACTIVITY

MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilisation of cells by the addition of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created. The color can be quantified using a multi-well plate reader. DMEM medium, Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plate and wash beaker were from Tarson (India).

Cell culture

A549 (human lung carcinoma) cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37°C.

MTT Assay

The SAMPLE 6 was tested for in vitro cytotoxicity, (V. H. Bhaskar, P. B. Mohite et al, 2011) using A549 cells by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured A549 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the SAMPLE 6 sample in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml)

was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value was calculated using GraphPad Prism 6.0 software (USA).

% Cell viability = [A] Test / [A]control x 100

Molecular Docking

Docking studies (Software Details)

Docking studies (R.K. Gundampati et al,. 2012) of ligand (L) and metal complexes have been performed using HEX 6.1 software which is an interactive molecular graphics program for the drug – protein binding interaction. The structure of the complexes were sketched by CHEMSKETCH (http://www.acdlabs.com). The crystal structure of EGFR kinase domain (PDB ID: CYP3A4 lung cancer) in complex with an irreversible inhibitors was obtained from the protein data bank. (http://www.rcsb.org.pdb). Visualization of the docked poses has been done by using PyMOL software.

Reaction Scheme

Scheme 1 Schematic route for the synthesis of ligand (L) and its metal complexes

RESULTS AND DISCUSSION

In the present work, novel seven Schiff bases and their metal complexes were synthesized as outlined in the Scheme 1. The substituted tetrazole and 4-((H-tetrazol-5-yl) thio) 2-acetoxybenzoic acid (L) and their metal complexes (2) were synthesized by reported procedures (S. Voitekhovich et al, 2006). Schiff bases and their metal complexes were obtained by condensation of both the moieties. The formation of compounds (L) and their metal complexes was evidenced by appearance of a band between 1631-1602 cm^{-1} for C=N, the infrared spectra of the tetrazole derivative TT1 reveal the presence of bands at 1450 cm^{-1} respectively, indicating the presence of N=N bond in all these tetrazole derivative. Similarly, the appearance of bands at 1272 cm^{-1} in the tetrazole derivative, TT 1 respectively reveals the presence of the cumulative bond, N-N=N, in all theses tetrazole compound in the IR spectra, presence of a peak in ^{13}C NMR spectra with a δ value between 164.4-141.1 for two carbons of C=N. The appearance of a band between 1735-1658 cm^{-1} for C-O of (L) and their metal complexes in the IR spectra; a peak in ^{13}C NMR spectra with a δ value between 148.5-131.0 for carbonyl carbon of (L) and their metal complexes. The presence of the OH group of (L) and their metal complexes was indicated at 3292-3057 cm^{-1} in the IR spectra; presence of a singlet in the ^{1}H NMR spectra at δ value 12.5-11.4. The OH of (L) and their metal complexes which undergoes tautomerisam was indicated by a band at 3178-2868 cm^{-1} and by a singlet peak at δ value 11.0-9.44 in ^{1}H NMR spectra. The presence of tautomeric form was also confirmed by a sharp band of C-S around 700-600 cm-1 and a peak at 172.2-180 in ^{13}C NMR spectra. Mass spectra of the ligand and its metal complex show molecular ion peaks, which are in good agreement with the expected values. The mass spectrum of ligand L gives a peak at 222.20 m/Z, which is assigned for [L+H] peak. Zn(II) > Cu(II)complexes gives molecular ion peak at 625.04 and 624.23 m/Z respectively and are assigned as [M+1] peak.

ANTI-CANCER ACTIVITY

All the synthesized compounds (1-3) were evaluated for their *in vitro* inhibitory activities against four Lung cancer cell lines (MCF-7) using MTT assay. The results as IC_{50} are mentioned in the Table - 1. The following table (Table - 1) shows the anti-cancer activity of the synthesized compounds (Ligand). All the synthesized compounds were screened for cytotoxicity on lung cancer cell line by MTT method. Cytotoxicity was checked at 24 hours and 48 hours duration. It was found that the activity of the compounds was increased after 48 hours as compared to 24 hours.

Among the tested compounds ligand and Cu-ligand showed potent activity and their % growth inhibition was 97.477 and 76.7878 at 100 μM/ml. Compounds ligand and Cu-ligand were showed IC_{50} 50 μM/ml and 47.1114 μM/ml (Table-1).

Docking Study

The interaction residues and energy values of the synthesised compounds with the target.

Molecular docking technique allows us to understand the interaction between a drug and protein at the molecular level (Schrodinger Release et al,. 2017). In the present study, molecular docking of the compounds (ligand, Zn-ligand and Cu-ligand) with protein the crystal structure of EGFR kinase domain (PDB ID: 1NOW) was performed in order to rationalize the mode of protein and most favourable binding conformations of the molecules. (Figure.3) shows the minimum energy docked pose of the compounds (ligand, Zn-ligand and Cu-ligand) from the results it is clear that, the compounds (ligand, Zn-ligand and Cu-ligand) interact with protein via intercalation mode of binding. This could be explained by the fact that, stacking interaction of compounds (ligand, Zn-ligand and Cu-ligand) with oxygen atom of the phosphate backbone leads to the formation of stable complex as reported in literature. The resulting relative binding energy of compounds (ligand, Zn-ligand and Cu-ligand) with protein were found to be -243.78, -273.1 and -281.12 KJ mol−1, respectively. The results of the docking view revealed the fact that the complexes bind with protein via intercalation and that the complexes stabilize the protein by van der Waal's and hydrophobic interaction. It is also to be noted that the complexes exhibit more binding affinity than compounds (ligand, Zn-ligand and Cu-ligand). The binding energy of the complexes follows the order Cu-ligand > Zn-ligand > ligand which is in good agreement with the binding constants obtained from absorption and emission spectral study (Table – 2).

CONCLUSION

The present investigation is focused on the synthesis, characterization and biological activities of a series of tetrazole compounds from 2-acetoxy-4-thiocyanatobenzoicacid and metal complex. The findings are furnished below:

- Compounds ligand, Zn-ligand and Cu-ligand are prepared using scheme 1.
- IR, Spectra are taken. The results are in good agreement with the reported results.
- The ^{1}H NMR and ^{13}C NMR spectra of all the three compounds provide the expected signals.
- The mass spectra of all the one compounds were recorded.
- The Anti-cancer activity is studied and the compound ligand has maximum activity.
- Docking study is done and the compound Cu-Ligand are found to fit well with the target protein.

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Table - 1: % Growth of inhibition of Ligand synthesized against MCF 7 Cell line

Compound	% Growth inhibition				
	0.25 µM	**2.5 µM**	**25 µM**	**50 µM**	**100 µM**
Ligand	0.5210	2.1774	20.2214	37.125	97.477
Zn-Ligand	0.4270	3.1400	25.2587	21.2570	65.1898
Cu-Ligand	0.1450	1.2047	18.8520	47.1147	76.7878

Table - 2: Energy values of the synthesized compound

S.No	Compound	Energy
1	Ligand	-243.78
2	Zn-Ligand	-273.10
3	Cu-Ligand	-281.11

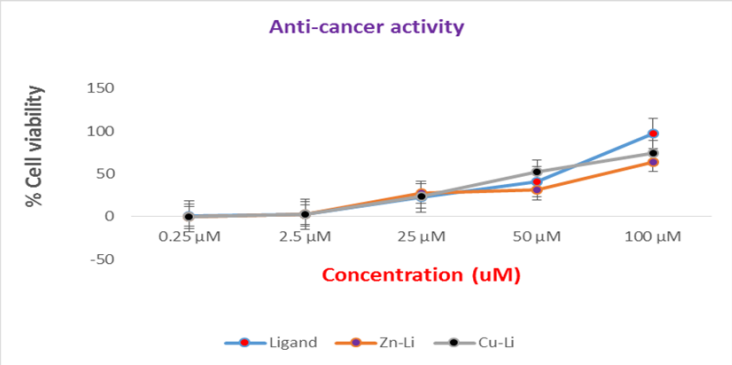

Fig.1 Anti-cancer activity of the synthesized compounds

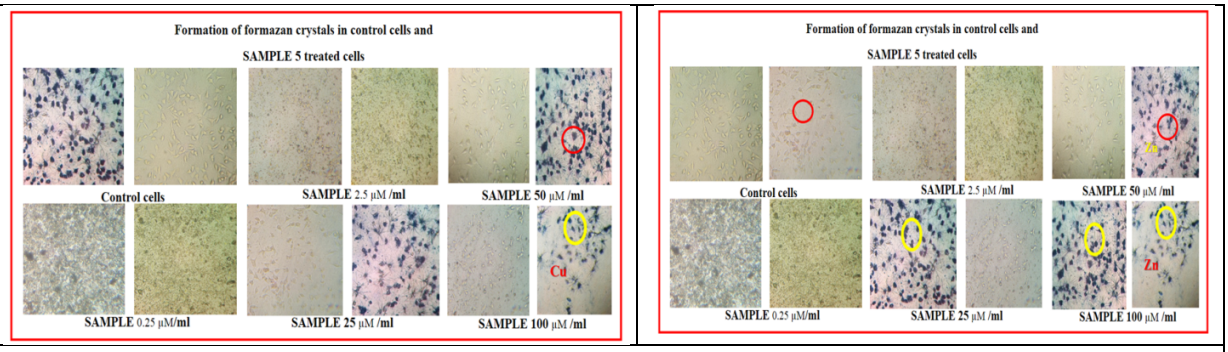

Fig.2.Formation of formazan crystals in control cells and sample treated cells

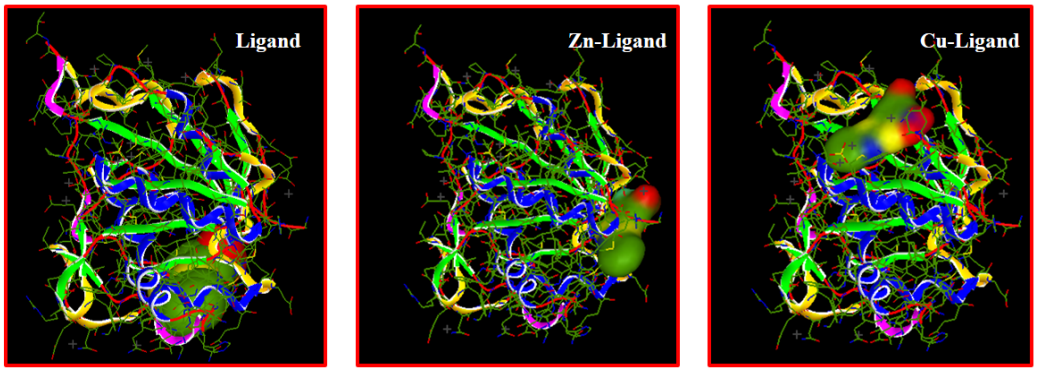

Fig .3 Molecular docked model of compounds (Ligand, Zn-Ligand and Cu-Ligand)