J Chem Biol (2017) 10:91–104
DOI 10.1007/s12154-017-0167-y

ORIGINAL ARTICLE

Molecular docking, PASS analysis, bioactivity score prediction, synthesis, characterization and biological activity evaluation of a functionalized 2-butanone thiosemicarbazone ligand and its complexes

Tahmeena Khan[1,2] · Shalini Dixit[3] · Rumana Ahmad[4] · Saman Raza[2] · Iqbal Azad[1] · Seema Joshi[2] · Abdul Rahman Khan[1]

Received: 10 December 2016 / Accepted: 9 March 2017 / Published online: 4 April 2017

Abstract 2-Butanone thiosemicarbazone ligand was prepared by condensation reaction between thiosemicarbazide and butanone. The ligand was characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectrometry and UV spectroscopic studies. Docking studies were performed to study inhibitory action against topoisomerase II (Topo II) and ribonucleoside diphosphate reductase (RR) enzymes. Inhibition constants (K_i) of the ligand were 437.87 and 327.4 μM for the two enzymes, respectively. The ligand was tested for its potential anticancer activity against two cancer cell lines MDA-MB-231 and A549 using MTT assay and was found to exhibit good activity at higher doses with an IC_{50} = 80 μM against human breast cancer cell line MDA-MB-231. On the other hand, no significant activity was obtained against the lung carcinoma cell line A549. Antibacterial activity of the ligand was tested against *Staphylococcus aureus* and *E. coli* using the disc diffusion method. Ligand did not exhibit any significant antibacterial activity. Four complexes of Co(III), Fe(II), Cu(II), and Zn(II) were prepared with the ligand and characterized by various spectroscopic studies. Low molar conductance values were obtained for all complexes displaying non-electrolyte nature except in Co(III) complex. As expected, complexation with metal ions significantly increased the cytotoxicity of the ligand against the tested cell lines viz. IC_{50} values of <20 μM for Co, Fe, and Zn complexes and approx. 80 μM against MDA cells versus IC_{50} value of <20 μM for Co and Cu complexes and that of 30 and 50 μM for Fe and Zn complexes, respectively, against A549 cells. The Cu complex was found to be active against *E. coli* and *S. aureus* with MIC values in the range of 6–10 mg/mL. Other than Cu, only Co complex was found to possess antibacterial activity with MIC values of 5–10 mg/mL when tested against *S. aureus*. Bioactivity score and Prediction of Activity Spectra for Substances (PASS) analysis also depicted the drug-like nature of ligand and complexes.

Keywords Thiosemicarbazone · Topoisomerase II · Ribonucleotide reductase · Docking · PASS · Anticancer · Antibacterial

Introduction

Metal complexes provide a rich platform for being used as therapeutic agents. Transition metals show variable oxidation states and an array of coordination geometries with different ligands. The ligands can alter the reactivity of the metal and also play a significant role in determining the nature of secondary coordination sphere interactions which involve recognition of biological targets such as enzymes, protein receptors, and DNA [25]. Schiff-based thiosemicarbazones are a versatile class of ligands, and their complexes have been used as therapeutic agents since long [30, 36]. Thiosemicarbazone-based metal complexes have

Electronic supplementary material The online version of this article (doi:10.1007/s12154-017-0167-y) contains supplementary material, which is available to authorized users.

✉ Abdul Rahman Khan
arahman@iul.ac.in

[1] Department of Chemistry, Integral University, Dasauli, P.O. Bas-ha, Kursi Road, Lucknow, UP 226026, India

[2] Department of Chemistry, Isabella Thoburn College, 7, Faizabad Road, Lucknow, UP 226007, India

[3] Department of Analytical Chemistry, CSIR-Central Institute of Medicinal and Aromatic Plants, P.O.-CIMAP, Near Kukrail Picnic Spot, Lucknow, UP 226015, India

[4] Department of Biochemistry, Era's Lucknow Medical College & Hospital, Sarfarazganj, Hardoi Road, Lucknow, UP 226003, India

been used as antineoplastic, antibacterial, antiviral, and antifungal agents [12, 20]. They are usually prepared by the condensation reaction between aldehydes or ketones with thiosemicarbazide [5]. Thiosemicarbazones as ligands show polydentate nature giving rise to a great variety of coordination modes [4]. The coordination ability of the basic ligand moiety can be enhanced by adding additional functional groups making them suitable for chelation [24]. Medical applications of thiosemicarbazones began to appear in the 1950s against tuberculosis and leprosy. In the 1960s, their antiviral properties were discovered, and after a considerable amount of research, methisazone and Marboran® were commercialized to treat smallpox [3, 23, 27]. Enhancement in activity can be obtained by optimization of the structure, which can result in groundbreaking discovery of new drug compounds [22]. It has been observed that coordination to a metal ion almost systematically increases the activity or contributes to mitigate the side effects of the organic parent compounds [14]. The major known effects related to the anticancer activity of thiosemicarbazone complexes include inhibition of ribonucleotide reductase (RR) [6], reactive oxygen species (ROS) production [38], topoisomerase II inhibition [17], mitochondrial disruption [43], and more recently, a multidrug resistance protein (MDR1) inhibition [31, 41]. The gray zone between chemistry and molecular biology further needs to be elucidated.

Materials and methods

Molecular docking was performed using Auto Dock Tools (ADT) version 1.5.6 and Auto Dock version 2.0. The structures of receptors topoisomerase II (PDB ID: 5IWI) and ribonucleoside diphosphate reductase (PDB ID: 5CI3) were downloaded from protein data bank (www.rcsb.org/pdb). IR spectra were recorded on a Bruker vertex 70 IR spectrophotometer in the frequency range 4000–500 cm^{-1}. The samples were run as their KBr pellets. ^1H NMR spectra were recorded on a Bruker spectrophotometer on 500.1 MHz at 295 K in the range of 0–10 δ using tetramethylsilane (TMS) as an internal standard. The samples were run in $CDCl_3$ and dimethyl sulfoxide (DMSO)-d_6, and chemical shifts were given relative to TMS (Merck) in ppm. UV spectra were recorded on a SpectraMax-5 spectrophotometer (Molecular Devices) in the range 200–400 nm. Mass spectrum of ligand was recorded on FAB-MS, JEOL SX-102. The conditions applied were as follows: ion source: ESI, positive; temperature 350 °C; nebulizer pressure 45 psi (N2); fragmenter voltage 130 V; capillary voltage 4000 V, and scan range *m/z* 100–700 (cycle time 800 ms). For chemical syntheses, all chemicals and solvents were of analytical grade and purchased from commercial sources. Hydrated salts of metals, i.e., $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$ and $FeSO_4.7H_2O$, were used for complex formation. Melting points were assessed in Ambassador electrical melting point apparatus up to 400 °C by the open glass capillary method. Conductivity measurements were made on an EI Deluxe conductivity meter, Model-601, in DMSO (1.0×10^{-3} mol). For biological activity evaluation, 0.4% Trypan blue, PBS (pH = 7.2, 1×), 0.25% trypsin-EDTA (1×), DMEM/F-12 (1×) (Dulbecco's modified Eagle's medium), and antibiotic/antimycotic solution (100×) were obtained from Gibco, Life Technologies, whereas fetal bovine serum (FBS) and MTT were from HiMedia. DMSO was purchased from Calbiochem. MDA-MB-231 (human breast carcinoma, ER^-, tumorigenic and invasive), abbreviated as MDA cells, and A549 (lung carcinoma epithelial cell line) were obtained from the National Centre for Cell Science (NCCS), Pune, India. All other chemicals used in the biological activity study were of analytical grade.

Molecular docking studies

The synthesized ligand and metal complexes were subjected to molecular docking studies using the Auto Dock Tools (ADT) version 1.5.6 and Auto Dock version 2 docking programs (interactive molecular graphics programs) to understand the drug molecule interaction with topoisomerase II and ribonucleotide diphosphate reductase (RR) enzymes to investigate the potential binding mode and energy.

Toxicity potential assessment

Toxicity risk assessment gives an idea about the probable side effects of synthesized compounds that may be used for further processing in drug development and discovery. The mutagenic, tumorigenic, irritant, and reproductive toxicities were measured by means of precomputed set of structural fragments. The prediction of different properties of molecules in the early stage is a vital step in the drug discovery and development process. Toxic parameters of the ligand and complexes were generated by OSIRIS Data Warrior Software.

Bioactivity score prediction

Drug score values indicate overall potential of a compound to be a drug candidate. Mol inspiration is a web-based tool used to predict the bioactivity score of the synthesized compounds against regular human receptors such as GPCRs, ion channels, kinases, nuclear receptors, proteases and enzymes [34].

Evaluation of drug likeliness based on Lipinski's rule of five

Lipinski's rule of five is helpful in describing molecular properties of drug compounds required for estimation of important pharmacokinetic parameters such as absorption, distribution, metabolism, and excretion. The rule is helpful in drug design and development [13, 28, 40].

Chemistry

Synthesis of 2-butanone thiosemicarbazone

The ligand was prepared using a previously reported procedure with some modifications [26]. Reaction was monitored by TLC in $CHCl_3$, Rf value (0.514). Mass spectrometry data confirmed the structure of ligand as indicated by molecular ion peak (M^{+1}) corresponding to its molecular formula $C_5H_{11}N_3S$. Yield 90%; MW 145; MP (°C) 99 °C; FTIR (KBr) (cm^{-1}) 1609 (C=N), 761, 1077 (C=S); ^{1}H NMR (MeOD) (MHz) 1.1 (t-3H), 1.9 (s, 3H), 2.3 (q, 2H), 3.3 (s, 1H,NH), 4.9 (s, 2H,NH_2); ^{13}C NMR (DMSO) (δ) 10.70 (CH_3), 18.72 (CH_3), 31.62 (CH_2), 155.5 (C=N) and 178.72 (C=S); ESI MS (*m*/*z*) 146.1 M^+; solubility: ethanol, DMSO, DMF; λ_{max} (nm) 267.

Synthesis of metal complexes

Hydrated AR-grade metal salts were used for the preparation of metal adducts. The complexes were synthesized in 1:2 (metal-ligand) molar ratios through a general preparatory route. After refluxing the ethanolic solution of ligand with the aqueous solution of metal salts, colored precipitate was obtained which was filtered under vacuum. The precipitate was washed with ethanol and dried in a desiccator. The Co(II) salt was first oxidized to (III) state by using a few drops of conc. HNO_3 as an oxidizing agent.

$[Cu(C_5H_{11}N_3S)_2SO_4]$

Yield 38%; MW 450; MP (°C) 195; FTIR (KBr) (cm^{-1}) 1562 (C=N), 705, 1058 (C=S); ^{1}H NMR (DMSO) (ppm) 0.31 (t, 3H), 2.3 (s, 3H), 2.5 (q, 2H), 6.6 (br s, 1H,NH), 7.8 (br s, 2H,NH_2); Solubility DMSO, DMF; λ_{max} (nm) 299; molar conductance 12.78 Ω^{-1} cm^{-1} mol^{-1}.

$[Fe(C_5H_{11}N_3S)_2SO_4]$

Yield 23%; MW 442; MP (°C) 190; FTIR (KBr) (cm^{-1}) 1562 (C=N), 705, 1050 (C=S); ^{1}H NMR (DMSO) (ppm) not obtained as 1H NMR spectrum of the Fe(II)-thiosemicarbazone complex system is difficult to detect due to high spin state of Fe(II) in its complex [9, 15, 37]; solubility: DMSO, DMF; λ_{max} (nm) 300; molar conductance 19.68 Ω^{-1} cm^{-1} mol^{-1}.

$[Zn(C_5H_{11}N_3S)_2SO_4]$

Yield 62%; MW 452; MP (°C) 250; (KBr) (cm^{-1}) 1570 (C=N), 705, 1050 (C=S); ^{1}H NMR (DMSO) (ppm) 1.1 (t, 3H), 1.9 (s, 3H), 2.5 (q, 2H), 7.3 (br s, 1H,NH), 7.5 (br s, 2H,NH_2); solubility: DMSO, DMF; λ_{max} (nm) 300; molar conductance: 8.08 Ω^{-1} cm^{-1} mol^{-1}.

$[Co(C_5H_{11}N_3S)_2Cl_2]^+$

Yield 46%; MW 420; MP (°C) 210; FTIR (KBr) (cm^{-1}) 1570 (C=N), 700, 1050 (C=S); ^{1}H NMR (DMSO) (ppm) 1.1 (t-3H), 2.2 (s,3H), 2.5 (q, 2H), 7.6 (br s, 1H,NH), 8.3 (br s,2H,NH_2); solubility DMSO, DMF; λ_{max} (nm) 320; molar conductance 105.04 Ω^{-1} cm^{-1} mol^{-1}.

Biological activity evaluation

MDA-MB-231 and A549 cell lines were maintained by subculturing and passaging as monolayers in 25- and 75-cm^2 cell culture flasks (Nest, Tarsons) at 37 °C in Tissue and Cell Culture Lab, Era's Medical College, Lucknow, in a 5% CO_2 incubator at 95% humidity for producing HCO_3 buffering capacity as reported earlier [21]. The cells were maintained at pH 7.4 in DMEM containing phenol red as a pH indicator and supplemented with 5% FBS [21]. The medium, prior to being used in cell culture experiments, was vacuum filtered using a Corning filtration system (Corning®, Sigma-Aldrich).

Anticancer activity

Solutions (0.1 M) of the ligand and its four complexes were prepared in 1.0 mL 50% DMSO solution. The solutions were diluted ten times in DMEM to give 0.01 M (10^4 μM) solutions. In separate experiments, cells were trypsinized and cultured in a six-well plates (0.5×10^5 cells/well) initially for 24 h, so as to allow the cells to adhere. After 24 h of incubation, the cells were exposed to 20–100 μM of the ligand and its complexes for the next 48 h. Suitable untreated controls (containing 50% DMSO as vehicle) were also concomitantly employed. Each dose was tested in at least three replicate wells. For morphological analysis, cells in six-well plates were observed under a phase contrast microscope (Nikon Eclipse Ti, Japan) and photographed.

Cell viability/cytotoxicity analysis

1. Cell viability analysis using Trypan blue dye exclusion assay

To determine the cell viability, cells exposed to varying concentrations of the ligand and its synthesized complexes were subjected to Trypan blue assay. Briefly, cell suspensions were made at a suitable dilution (10^5cells/mL) in PBS. Respective cell suspensions (50 μL) were taken and mixed with an equal volume of 0.4% Trypan blue, mixed thoroughly and allowed to stand for 5 min at room temperature. Solution (50 μL)was transferred to a hemocytometer and viable cells were counted as clear cells and dead cells as blue ones. The number of live cells in both treated (ligand and its complexes) and control (cells and vehicle)

Table 1 Docking results with Topo II (PDB ID: 5IWI)

S. No.	Compound	Binding energy (kcal/mol)	Inhibition constant (K_i)	Interacting amino acid(s)
1	$C_5H_{11}N_3S$	−4.58	437.87μM	G41, L42, K43, H46, Q95, Y99, L103, N166, N170, G171, A172
2	$[Fe(C_5H_{11}N_3S)_2SO_4]$	−5.08	188.07μM	P36, G41, L42, K43, H46, R92, M93, Y99, L103, N166, N170, G171, A172, S173
3	$[Zn(C_5H_{11}N_3S)_2SO_4]$	−3.37	3.38mM	P36, G41, L42, H46, S98, Y99, L103, N166, L167, N170, G171, A172, S173, F266

wells was used for calculating the percentage cytotoxicity as % Cytotoxicity = Live Cell No. in Treated Wells − Live Cell No. in Control Wells/Live Cell No. in Control Wells × 100).

2. Methyl tetrazolium-MTT assay

MTT was performed as per published protocol [33] in 96-well microtiter tissue culture plates (Linbro, MP Biomedicals). For MTT assay, 10^4 MDA cells were seeded in 200 μL of medium in a 96-well microtiter tissue culture plate and cultured in a humidified 5% CO_2 incubator at 37 °C for 24 h. Defined concentrations (20–100 μM) of the ligand and its complexes in 50% DMSO were freshly prepared in culture media by serial dilution. Serial dilution was carried out in cell culture media in such a way that the final concentration of DMSO in the well did not exceed 0.5% (*v*/*v*). Three control wells containing the medium alone to serve as blanks were also included. After 24 h of incubation, in separate

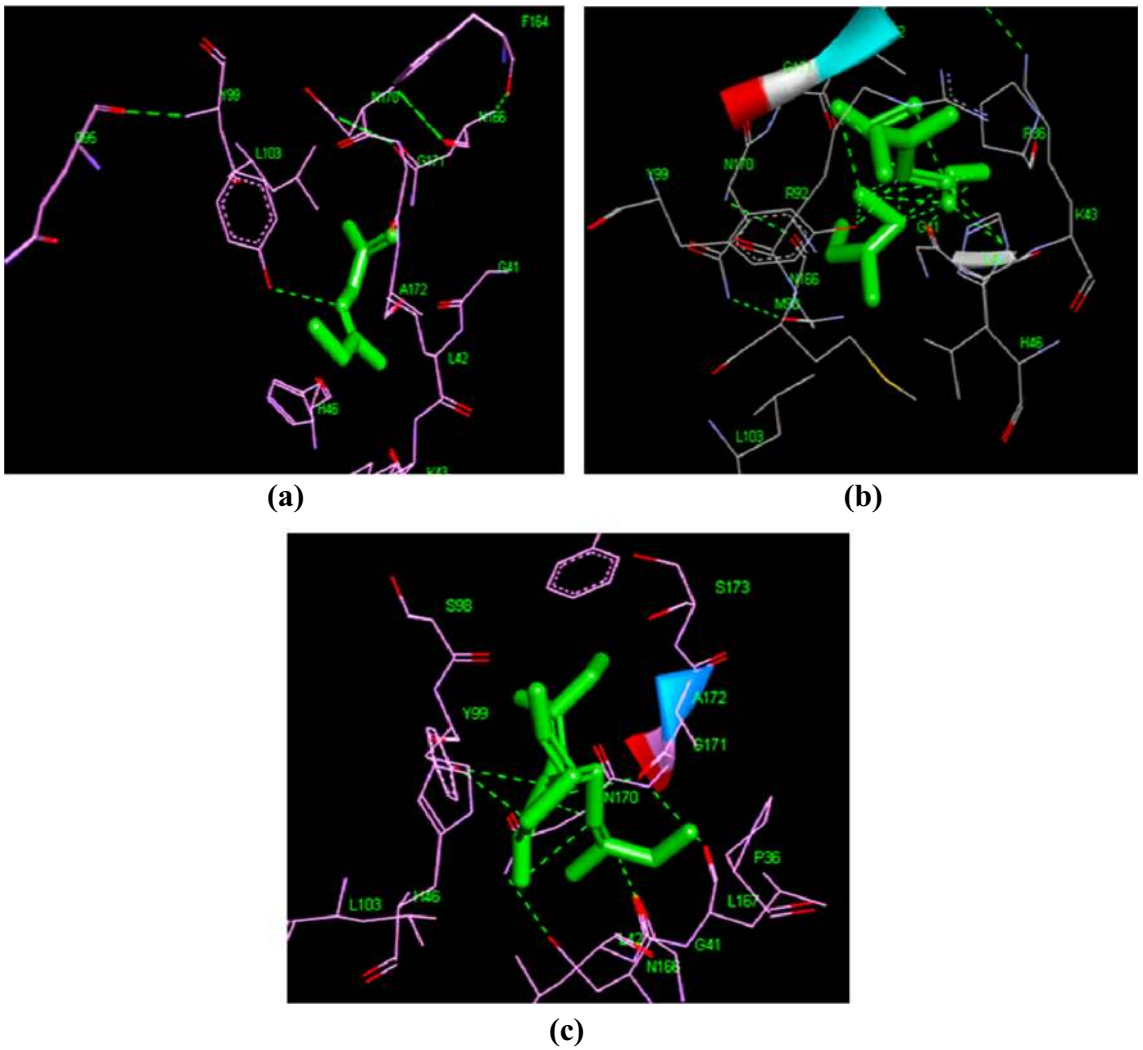


Fig. 1 Docking pose of ligand (**a**), its Fe complex (**b**), and Zn complex (**c**) in the active site of Topo II

Table 2 Docking results with RR (PDB ID: 5 CI3)

S. No.	Compound	Binding energy (kcal/mol)	Inhibition constant (K_i)	Interacting amino acid(s)
1	$C_5H_{11}N_3S$	−4.75	327.4μM	T81, Q80, D84, H118, S119, S121, T123, I125, F208, I234
2	$[Fe(C_5H_{11}N_3S)_2SO_4]$	−3.43	3.08mM	R89, S90, V93, A94, I96, I153, S154, Y157, D158, I161
3	$[Zn(C_5H_{11}N_3S)_2SO_4]$	−4.42	571.48μM	I86, R89, S90, V93, I153, S154, Y157, D158, I161

experiments, cells were treated with the previously mentioned concentrations of the ligand and its four complexes in triplicates for 48 h. Equal volumes of 50% DMSO (in cell culture media) were used as vehicle controls. At the end of treatment, cell culture medium containing varying amounts of ligand/complexes was removed and 20 μL of MTT (stock made in PSS at 5.0 mg/mL) reagent was added to each well and incubated for 4 h. Thereafter, MTT was removed and formazan crystals were dissolved in 200 μL of DMSO. The plates were read in a Bio-Rad PW41 ELISA plate reader at a wavelength of 570 nm with a reference wavelength of 630 nm. Percentage cell viability (*Y*-axis) was calculated from absorbance and plotted against concentration in micromolar (*X*-axis).

% Cell survival was calculated as = $\{(A_T - A_B)/(A_C - A_B)\} \times 100$ where

A_T = Absorbance of treatment well.

A_B = Absorbance of blank.

A_c = Absorbance of control well.

% cell inhibition = 100 − Cell Survival.

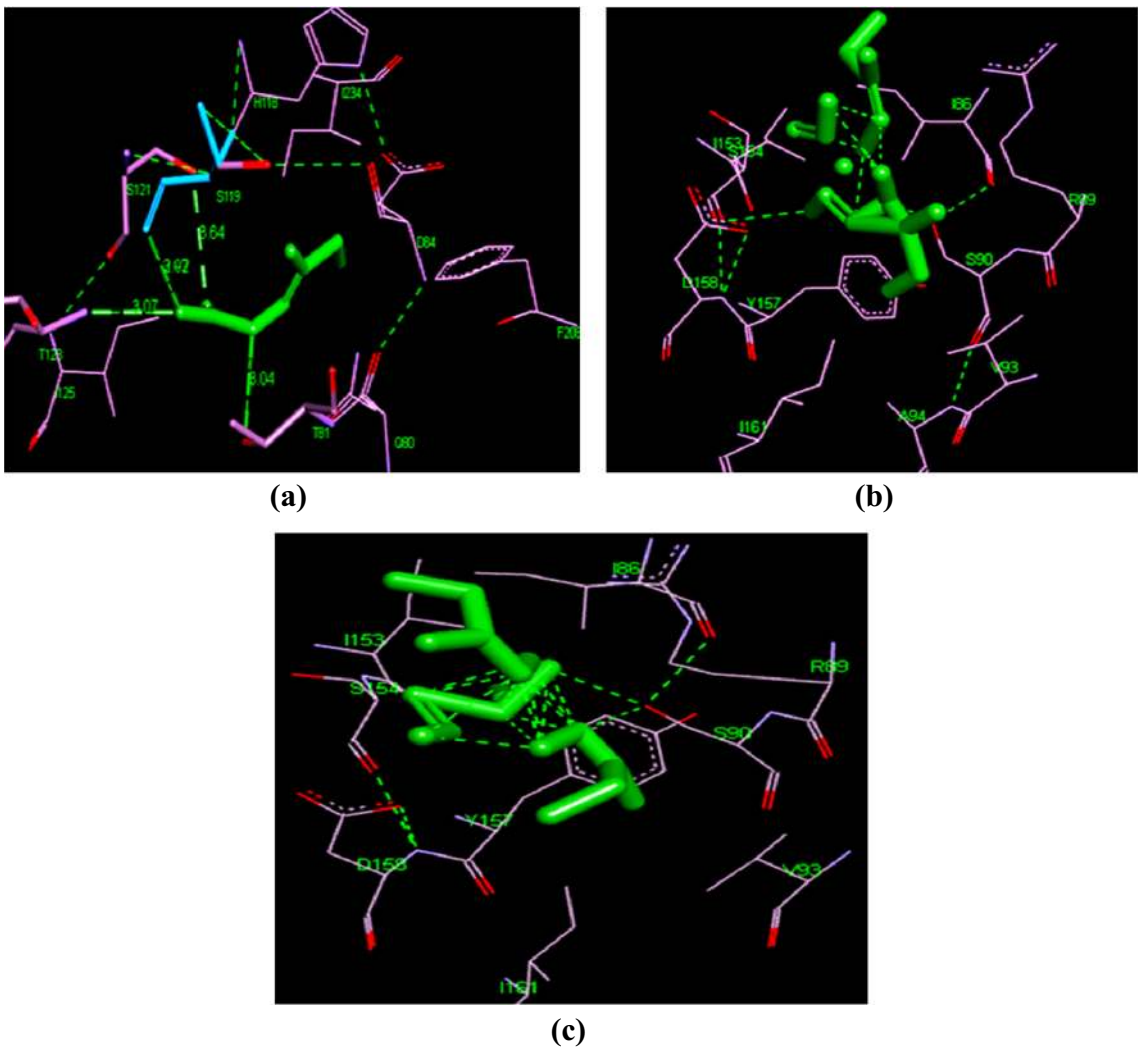


Fig. 2 Docking pose of ligand (**a**), its Fe complex (**b**), and Zn complex (**c**) in the active site of RR

IC_{50} values were obtained from the graph as the concentration, which decreased cell by viability 50%.

Antibacterial activity

The in vitro antibacterial activity of the ligand and its complexes was evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria by the disc diffusion method [1] using Mueller-Hinton agar (MHA) medium. The bacteria were subcultured in the agar medium and were incubated for 24 h at 37 °C. The discs (sterile filter paper discs, Whatman No. 1.0), having a diameter of 5 mm, were then soaked in the test solutions with the appropriate equivalent amounts of the ligand and its four complexes dissolved in sterile 50% DMSO at concentrations of 2–10 mg/disc and placed on lawn culture of the respective microbial organism and stored in an incubator for the previously mentioned period of time. Formation of inhibition zone (if any) around each disc was measured, and the results were recorded in the form of inhibition zones as a function of diameter (mm). To clarify any effect of DMSO (used as a vehicle for the dissolution of the ligand and its complexes) on biological screening, 50% DMSO was used as negative control where it showed no activity against any bacterial strains. Tetracycline was used as a positive control.

Data interpretation and statistical analysis

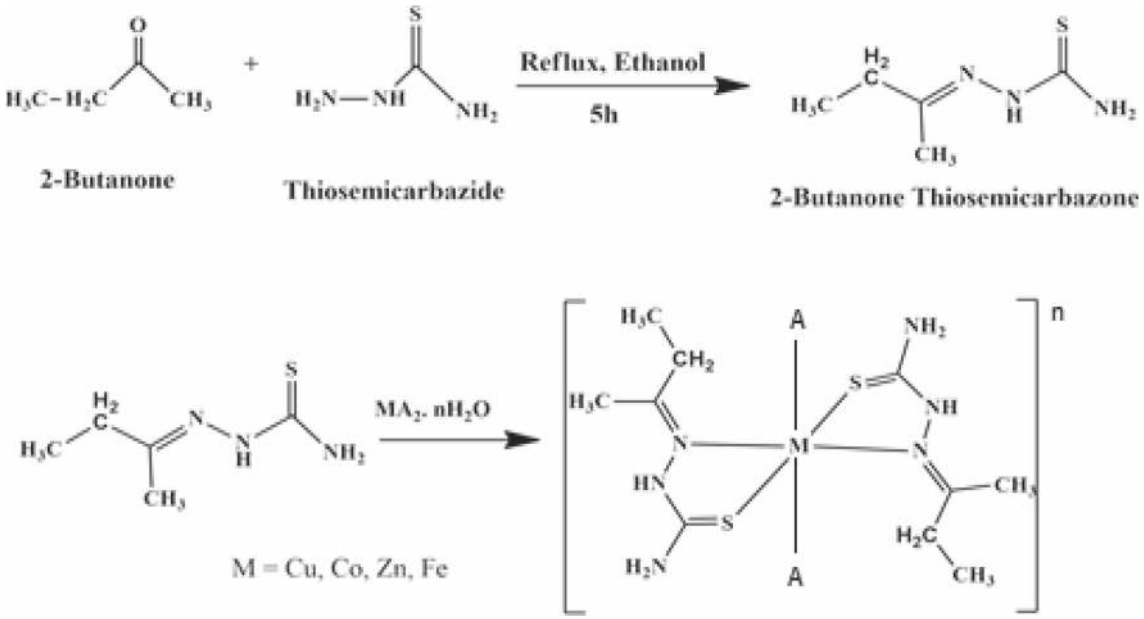
Absorbance values that were lower than the control wells indicated a reduction in the rate of cell proliferation. Conversely, a higher absorbance value indicated an increase in cell proliferation. Rarely, an increase in proliferation might be offset by cell death; evidence of cell death was inferred from morphological analysis. Results were expressed as mean ± SD of experiments done in duplicates.

Results and discussion

Computational docking is an extremely useful tool to gain an understanding about synthesized compounds and their interactions with biological drug targets, which is very important in drug discovery research. The Molecular Docking software predicted the amino acids in active site region of the studied target proteins.

Docking with topoisomerase II

Calculated binding energies and inhibition constants (K_i) of 2-Butanone Thiosemicarbazone and its Fe(II) and Zn(II) complexes with respect to the enzyme and the interacting amino acids in its active site have been summarized in Table 1. Fe (II) complex was found to have minimum binding energy (-5.08 kcal/mol) released on interaction with target protein (Topo II) and also the lowest K_i



n= +1 in case of Co (III) complex, in case of sulfate as anion the donor atoms would occupy two adjacent coordination site or Cl^-

Fig. 3 Synthesis scheme

Table 3 Toxicity potential of the ligand and its complexes

S. No.	Compound	Toxicity potential (K_i or IC_{50} in nmol/L)					
		Mutagenic	Tumorigenic	Reproductive	Irritant	Molecular shape index Spherical < 0.5 < linear	Molecular flexibility Low < 0.5 < High
1	$C_5H_{11}N_3S$	Low	None	None	None	0.77778	0.46893
2	$[Fe(C_5H_{11}N_3S)_2SO_4]$	None	None	None	None	0.47368	0.84037
3	$[Zn(C_5H_{11}N_3S)_2SO_4]$	None	None	None	None	0.47368	0.84037
4	$[Cu(C_5H_{11}N_3S)_2SO_4]$	None	None	None	None	0.47368	0.84037
5	$[Co(C_5H_{11}N_3S)_2Cl_2]^+$	None	None	None	None	0.47368	0.84037

value (188.07 μM) as compared to the Zn (II) complex and ligand. The docking interactions of the ligand and its Fe(II) and Zn(II) complexes with Topo II have been shown in Fig. 1a–c.

Docking with ribonucleotide diphosphate reductase

Calculated binding energies and inhibition constants (K_i) of the ligand and its Fe(II) and Zn(II) complexes with respect to the enzyme RR and the interacting amino acids in its active site have been summarized in Table 2. The interactions between the drug compound and the active sites on the target receptors may be through non-covalent interactions like hydrogen bonding, hydrophobic, and van der Waals interaction [10]. Zn (II) complex had a lower binding energy (-4.42 kcal/mol) and lower K_i value (571.48 μM) with respect to RR as compared to Fe (II) complex.The docking interactions of the ligand and its Fe(II) and Zn(II) complexes with RR have been shown in Fig. 2a–c.

Characterization and complex formation

Preparatory scheme of the ligand and metal complexes is given in Fig. 3. Spectral analysis confirmed complex formation. ^{1}H NMR spectrum of the ligand revealed that NH and NH_2 protons were observed downfield due to higher electronegativity of nitrogen making the attached protons de-shielded. On complexation, the frequencies further got down field (7–8 ppm) due to electronegativity difference between the metal ion and nitrogen. In the ^{13}C spectrum of ligand, the thiol carbon peak (C=S, 178.72) and the carbon attached to nitrogen (C=N, 155.5) were found to be the most downfield because of electronegativity difference. The 1H NMR spectrum of the Fe(II) thiosemicarbazone complex (t_2g^4, e_g^2 system) was difficult to detect due high spin state of the Fe(II) complex. In order to characterize d^6 Fe-thiosemicarbazone complex, the signal of protons centered at 0 to 10 ppm was found to be very broad and difficult to distinguish from the baseline, thereby making it not useful for monitoring the existence of the Fe(II) complex [9, 15, 37]. Mass spectrum data gave a molecular ion peak (M^{+1}) at 146 corresponding to its molecular weight of 145. The bonding mode of the ligand to metal ion was elucidated by analysis of the IR data. By comparing the frequencies of the ligand with the metal complexes, it was concluded that ligand coordinates with the metal centre through the azomethene nitrogen [11, 19] and the thione sulfur [7, 42], hence indicating the bidentate nature of the ligand because the C=N and C=S frequencies decrease in case of metal complex formation. The ligand may show thione-thiol tautomerization because it has a thiomide functional group. However, the absence of v (S–H) stretching in 2500–2600-cm^{-1} region of the IR spectrum of the ligand indicated that the ligand retained its thione form in the solid state. In addition, the band at 1119 cm^{-1} found in ligand was found to be missing in complexes. Appearance of new bands at 1058 cm^{-1} (Cu (II) complex), 1050 cm^{-1} (Co (III) complex, 1050 cm^{-1} (Fe (II) complex), and 1050 cm^{-1} (Zn (II) complex) confirmed the fact

Table 4 Bioactivity score of the ligand and its complexes

S. No.	Compound	Parameters of Bioactivity score					
		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	$C_5H_{11}N_3S$	−3.65	−3.31	−3.77	−3.80	−3.32	−2.34
2	$[Fe(C_5H_{11}N_3S)_2SO_4]$	−0.39	−0.17	−0.15	−0.54	−0.29	−0.11
3	$[Zn(C_5H_{11}N_3S)_2SO_4]$	−0.34	−0.09	−0.15	−0.54	−0.29	−0.11
4	$[Cu(C_5H_{11}N_3S)_2SO_4]$	−0.39	−0.17	−0.15	−0.54	−0.29	−0.11
5	$[Co(C_5H_{11}N_3S)_2Cl_2]^+$	−0.39	−0.17	−0.15	−0.54	−0.29	−0.11

that there was a contribution of C=S vibration in this region. Bands higher than 1080 cm^{-1} disappeared in complexes [16]. ^1H NMR data provided information about the different positions of protons. In the ^1HNMR spectra of the complexes, a high-frequency shift of the methyl hydrogen atoms (C–CH_3), vis-à-vis ligand spectrum, gave evidence that coordination occurred through the azomethine nitrogen atom [39]. High melting points of the complexes were another indication of coordination. λ_{max} of the ligand (267 nm) corresponded to π–π* transition [2], whereas in complexes, the absorption maximum between 285 and 385 nm was assigned to *n*–π* transition [8, 18]. Increase in absorbance (hyperchromic shift) in all the complexes was attributed to complexation behavior of ligand towards metal ions confirming the coordination of the ligands to the metal ions.

Conductivity measurements can predict the mode of coordination of counter ions to metal complexes. They provide a method of testing degree of ionization of the complexes. The larger the number of ions outside the coordination sphere, the greater would be the molar conductance [35]. Low molar conductivities of the Cu(II), Zn(II), and Fe(II) butanone thiosemicarbazone complexes were indicative of non-electrolytic nature indicating the bonding of sulfate ions to the metal ions [32]. Co(III) complex showed higher molar conductance of 105.04 Ω^{-1} cm^{-1} mol^{-1}, which may be due to positive charge over the coordination sphere. The Fe, Cu, and Zn complexes dissolved in DMSO and DMF with time whereas Co complex dissolved readily further confirming its electrolytic nature. The solubility difference can be explained on the basis of different salts used for the synthesis having sulfate and chloride as anions.

$n = +1$ in case of Co (III) complex, A = SO_4^{2-} or Cl^-

Toxic parameters of the complexes

The parameters predict the probable side effects of the compounds. The synthesized ligand and complexes were predicted to have no side effects as shown in Table 3.

Bioactivity score prediction

The pharmacological activity describes the beneficial effects of drugs in living beings. The drug is supposed to bind with a biological target. Biological targets are the most common proteins such as enzymes, ion channels, and receptors. The biological target is also referred to as drug target. The bioactivity scores of the synthesized complexes were calculated for different parameters such as binding to G protein-coupled receptor (GPCR) ligand and nuclear receptor ligand, ion channel modulation, kinase inhibition, protease inhibition, and enzyme activity inhibition. All the parameters were calculated with the help of online software Molinspiration (www.molinspiration.com) , which predicted moderate biological activity for the synthesized complexes. The bioactivity score is given in Table 4. It is known that for metal complexes, if the bioactivity score is more than 0.0, then the complex is active; if it is between −5.0 and 0.0, then the complex is moderately active, and if the bioactivity score is less than −5.0, then it is inactive. As seen in Table 4, the bioactivity scores of the ligand as well as all the four complexes were between −5.0 and 0.0, which clearly indicate that they possess such properties as are required for the complexes to act as potential drugs with some modifications in chemical structure [10].

Table 5 Physicochemical properties of the synthesized ligand and its complexes

Physicochemical Properties									
S. No.	Compound	Lipinski's Parameters							
		% Absorption[a]	Topological polar surface area (Å)^2 (TPSA)[b]	MW	M log P[c]	Hydrogen bond donors (nOHNH)	Hydrogen bond acceptors (nON)	Number of rotatable bonds	Lipinski's violations
1	$C_5H_{11}N_3S$	91.6085	50.41	<500	1.08	3	3	3	0
2	$[Fe(C_5H_{11}N_3S)_2SO_4]$	80.6720	82.11	<500	−6.02	6	6	2	1
3	$[Zn(C_5H_{11}N_3S)_2SO_4]$	80.6720	82.11	<500	−5.88	6	6	2	1
4	$[Cu(C_5H_{11}N_3S)_2SO_4]$	80.6720	82.11	<500	−5.99	6	6	2	1
5	$[Co(C_5H_{11}N_3S)_2Cl_2]^+$	80.6720	82.11	<500	−5.93	6	6	2	1

[a] Percentage absorption (% absorption) was calculated by % Absorption = 109 − [0.345 × Topological Polar Surface Area]

[b] Topological polar surface area (defined as a sum of surfaces of polar atoms in a molecule)

[c] Logarithm of compound partition coefficient between *n*-octanol and water

PASS Analysis: Lipinski's parameters

Lipinski's rule of five (RO5) is used to evaluate drug likeliness of a chemical compound possessing properties that would make it a likely or potential drug in humans [29]. The oral activity of a drug compound is predicted by calculating certain molecular parameters like log P (partition coefficient), polar surface area, number of hydrogen bond donors, number of hydrogen bond acceptors, and molecular weight. The rule states that most metal complexes with good membrane permeability have log $P \leq 5$, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors ≤5 [10]. In general, an orally active drug has no more than one violation of the given criteria. In the present study, the synthesized ligand and its complexes were found to be in good agreement with the given criteria and can be said to possess good oral bioavailability (Table 5).

Biological evaluation of ligand and complexes

1. Morphological analysis

Suppl. Figs. 1-10 (a-l) respectively depict the dose-dependent effect of the ligand and its four complexes on MDA and A549 cells using phase contrast microscopy. Ligand was found to be active against MDA cells in the tested range. No

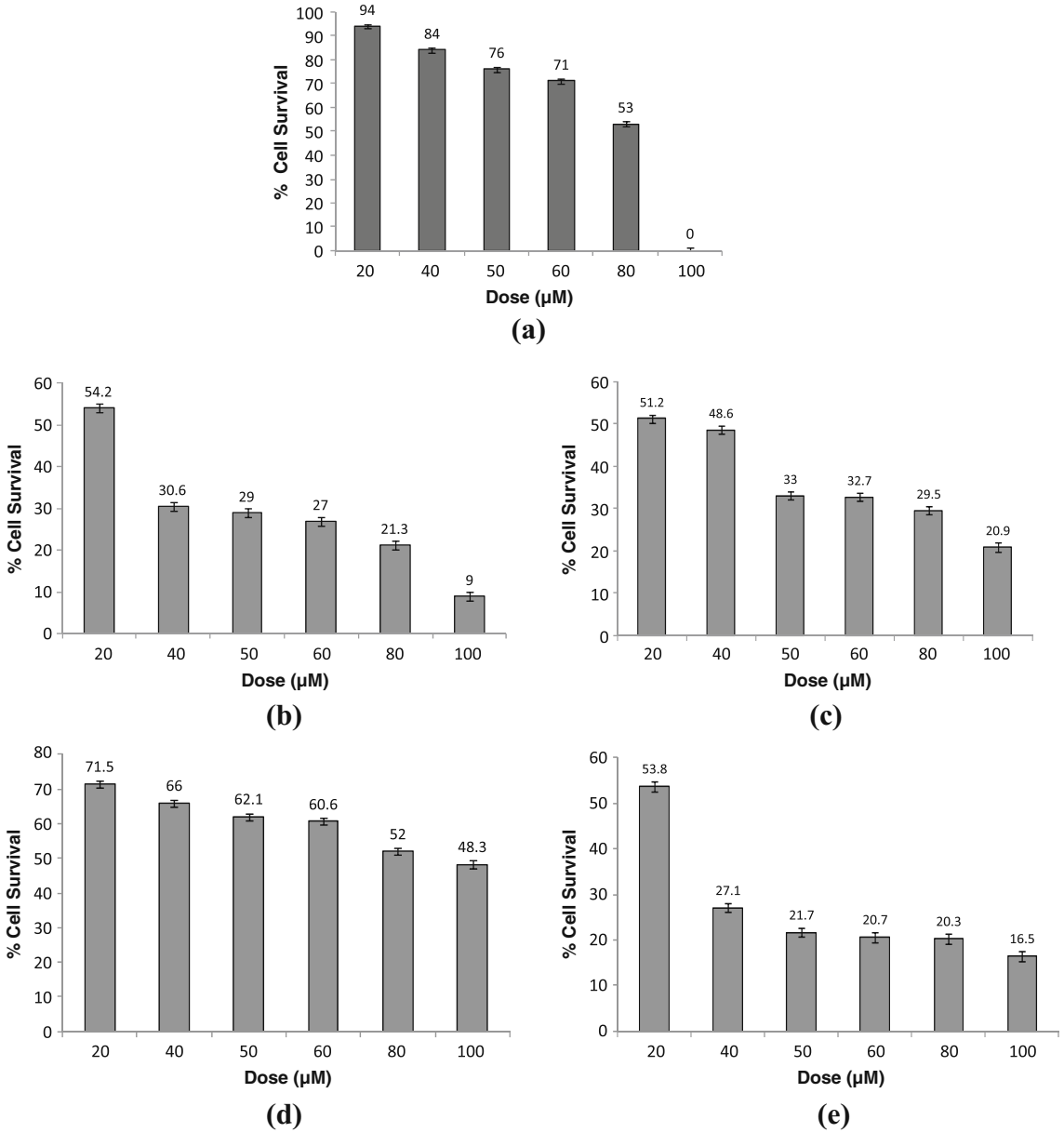


Fig. 4 **a**–**e** Dose-dependent effect of ligand (**a**), its Co (**b**), Fe (**c**), Cu (**d**), and Zn (**e**) complexes on viability of MDA cells in vitro using MTT assay. Final concentration of DMSO in each well did not exceed 0.5% (*v*/*v*). Results were expressed as mean ± SD of experiments done in triplicates.

significant activity of the ligand was detected in the tested range against A549 cells. However, all complexes were found to possess significant activity against both cell lines with IC_{50} values of <50 μM (refer to Suppl. data).

2. Cell viability analysis

Suppl. Tables 1-5 present the dose-dependent cytotoxicity of the ligand and its complexes when tested in the range of 20–100 μM against breast cancer cells MDA-MB-231 and lung carcinoma cells A549 cells using the Trypan blue dye exclusion assay. As mentioned before, ligand did not exhibit any activity against A549 cells whereas its complexes displayed significant cytotoxicity against both the tested cell lines. Results were expressed as mean ± SD of experiments done in duplicates (refer to Suppl. data).

3. MTT assay

Figures 4 and 5 respectively depict the dose-dependent effect of the ligand (Figs. 4a and 5a), its Co (Figs. 4b and 5b), Fe (Figs. 4c and 5c),Cu (Figs. 4d and 5d) and Zn (Figs. 4e and 5e) on MDA and A549 cells using the MTT assay. Ligand showed 50% cytotoxicity against MDA at

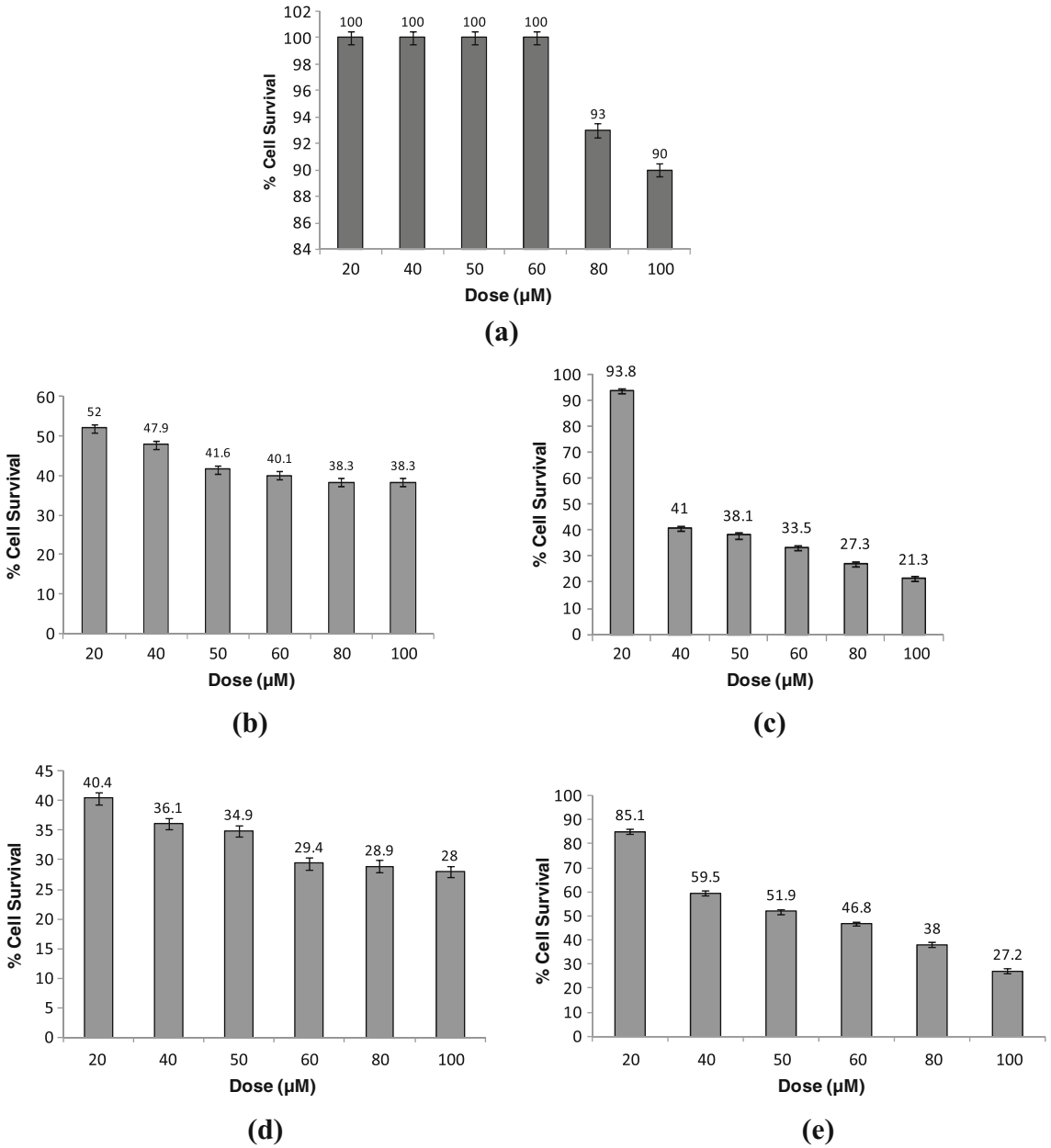


Fig. 5 **a**–**e** Dose-dependent effect of ligand (**a**), its Co (**b**), Fe (**c**), Cu (**d**), and Zn (**e**) complexes on viability of A549 cells in vitro using MTT assay. Final concentration of DMSO in each well did not exceed 0.5% (*v*/*v*). Results were expressed as mean ± SD of experiments done in triplicates.

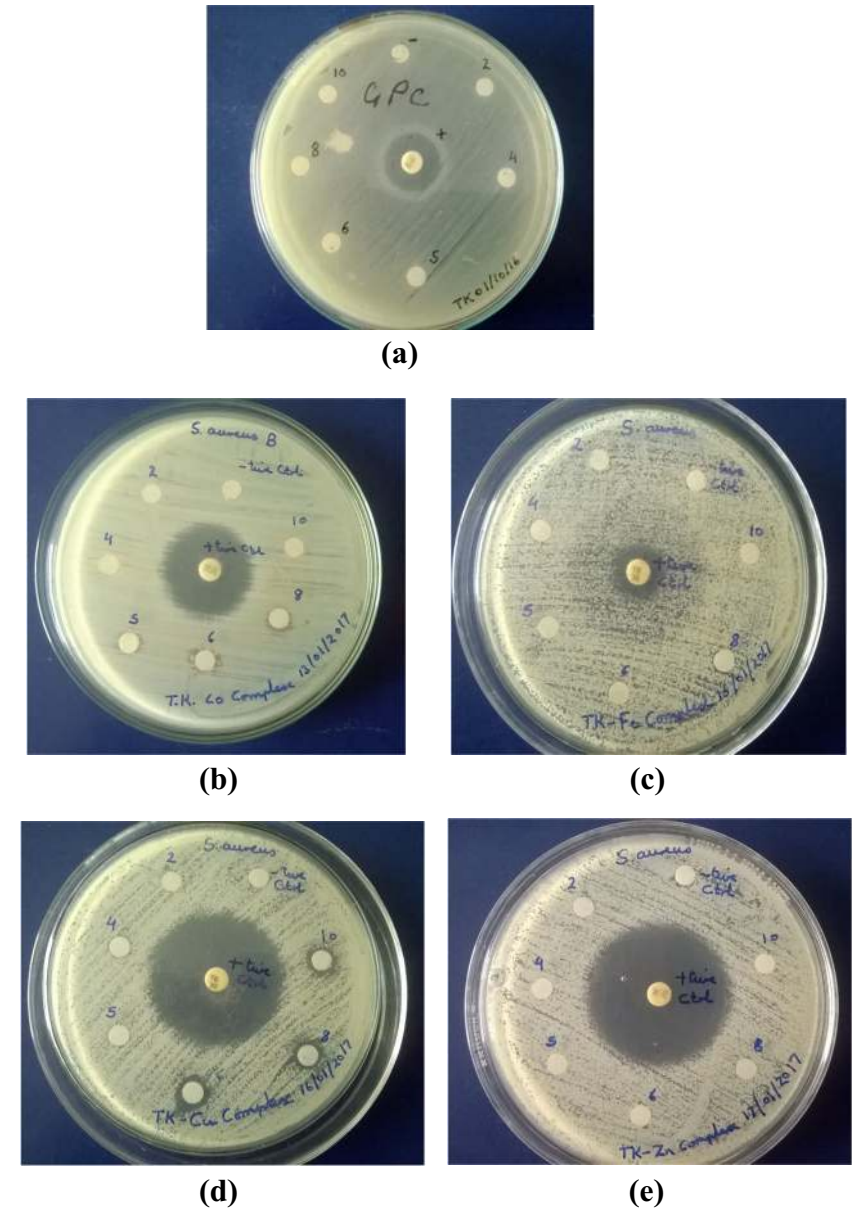


Fig. 6 Antibacterial activity of ligand (**a**), its Co (**b**), Fe (**b**), Cu (**c**), and Zn (**d**) complexes against *S. aureus* using disc diffusion method

Table 6 Comparison of MIC values (in mg/mL) of ligand and its complexes and standard antibiotic tetracycline against *S. aureus*

Ring diameter (mm)								
Compound	Tetracycline (30 mg/mL)	DMSO	Compound dose (mg/mL)					
			2	4	5	6	8	10
$C_5H_{11}N_3S$	12	ND	ND	ND	ND	ND	ND	ND
$[Co(C_5H_{11}N_3S)_2Cl_2]^+$	14	ND	ND	ND	6	8	8	ND
$[Fe(C_5H_{11}N_3S)_2SO_4]$	10	ND	ND	ND	ND	ND	ND	ND
$[Cu(C_5H_{11}N_3S)_2SO_4]$	21	ND	ND	ND	ND	8	9	9
$[Zn(C_5H_{11}N_3S)_2SO_4]$	21	ND	ND	ND	ND	ND	ND	ND

ND not detected

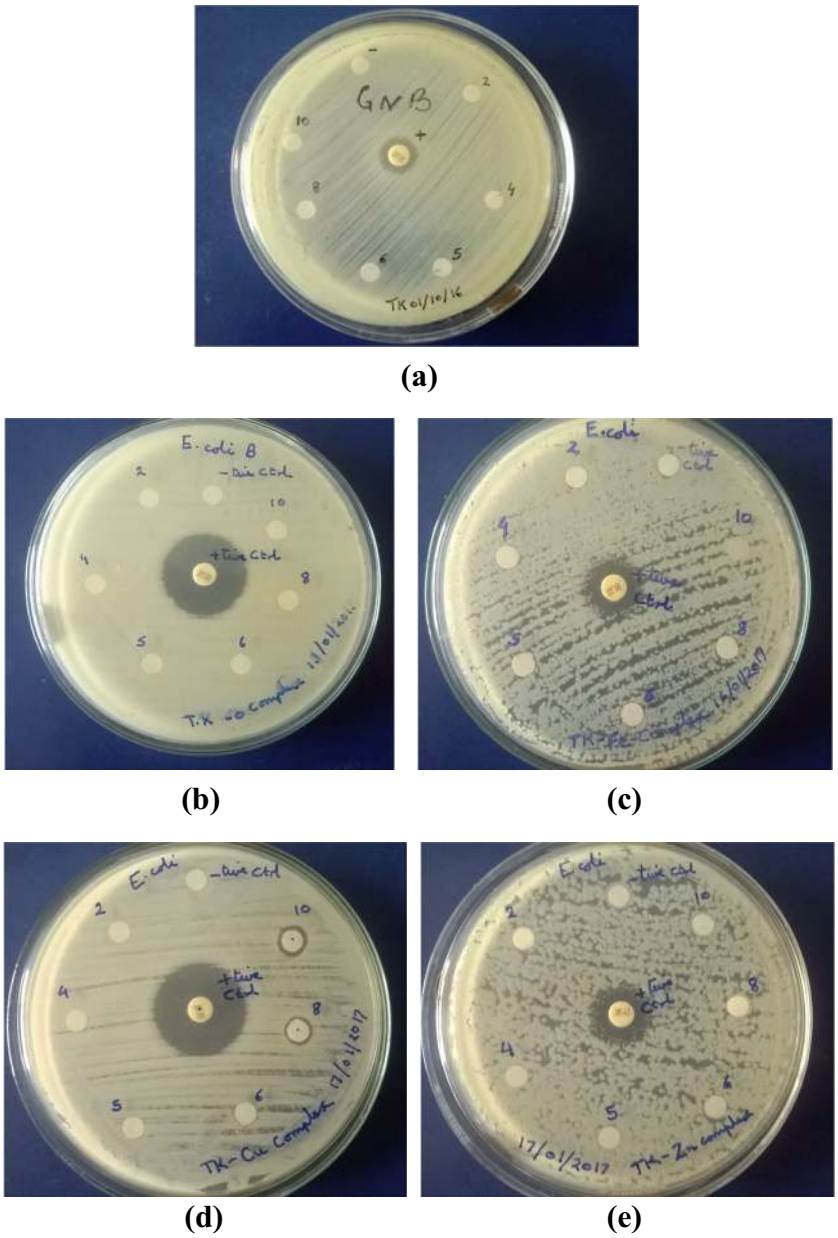


Fig. 7 Antibacterial activity of ligand (**a**), its Co (**b**), Fe (**b**), Cu (**c**), and Zn (**d**) complexes against *E. coli* using disc diffusion method

Table 7 Comparison of MIC values (in mg/mL) of ligand and its Co and Fe complexes and standard antibiotic tetracycline against *E. coli*

Ring diameter (mm)								
Compound	Tetracycline (30 mg/mL)	DMSO	Compound dose (mg/mL)					
			2	4	5	6	8	10
$C_5H_{11}N_3S$	5	ND	ND	ND	ND	ND	ND	ND
$[Co(C_5H_{11}N_3S)_2Cl_2]^+$	13	ND	ND	ND	ND	ND	ND	ND
$[Fe(C_5H_{11}N_3S)_2SO_4]$	10	ND	ND	ND	ND	ND	ND	ND
$[Cu(C_5H_{11}N_3S)_2SO_4]$	14	ND	ND	ND	ND	ND	4	5
$[Zn(C_5H_{11}N_3S)_2SO_4]$	8	ND	ND	ND	ND	ND	ND	ND

ND not detected

80 μM (IC_{50} value approx. 80 μM). As mentioned earlier, the ligand did not show any significant activity against A549 cells within the tested range. However, complexation with metals significantly increased the cytotoxicity of the ligand against both cell lines.

Antibacterial activity

The ligand together with its complexes was also evaluated for their potential antibacterial activity against *S. aureus* and *E. coli*. The ligand did not exhibit any significant activity against both species. However, Co and Cu complexes exhibited mild antibacterial activities against *S. aureus* (Fig. 6, Table 6), whereas Cu complex alone showed moderate activity against *E. coli* (Fig. 7, Table 7).

Conclusion

The overall activity spectrum of biologically active compounds can be predicted by computational analyses like molecular docking with probable receptors or by prediction of bioactivity score and physicochemical properties. In this paper, anticancer and antibacterial activities of ligand 2-butanaone thiosemicarbazone and its four metal complexes have been reported against two cancer cell lines and two species of bacteria. While the ligand displayed mild anticancer activity against MDA cell line (80 μM) and none against A549 cell line, its four complexes displayed significant anticancer activities against both the tested cell lines with IC_{50} <50 μM for Co, Fe, and Zn complexes. However, only Cu complex exhibited appreciable activity against *S. aureus* and *E. coli* while Co complex was found to be mildly active against *S. aureus* only. Molecular docking studies showed significant binding of the ligand with selected targets. Bioactivity score and PASS analysis showed that the metal complexes can have potential inhibitory action against the selected cell lines. The study has shed light on the role of different anions on the overall solubility of the complexes, which can also affect their conductivity. Likewise, the oxidation state of metal significantly influences its affinity for a particular ligand working on the hard-soft acid-base concept. This phenomenon would be explored in more detail in future. In future, the complexes of ligand would be tested against other cancer as well as normal cell lines and would also be evaluated for their probable anticancer and antibacterial activities in vivo. The complexes would also be analyzed for their potential antioxidant and DNA cleavage activity.

Acknowledgements The authors gratefully acknowledge Dr. E.S. Charles, President and Dr. M. Harrison, Head, Department of Chemistry,Isabella Thoburn College, Lucknow, for providing necessary facilities to carry out the lab work. The authors are also thankful to Mr. Mohsin Ali Khan, Secretary, EET and Chairman Research, Era's Lucknow Medical College & Hospital, Lucknow, for providing the necessary support and cooperation for carrying out the biological activity evaluation studies and to the R&D wing of Integral University, Lucknow, for providing support and communication number (IU/R&D/2016-MCN008IU/R&D/2016-MCN008) for the manuscript.

Author's contributions Tahmeena Khan carried out the chemical syntheses, analyzed and interpreted data, and drafted the manuscript. Shalini Dixit carried out spectroscopic studies and analyzed data. Rumana Ahmad carried out biological activity evaluation experiments, analyzed and interpreted the data, and edited and revised the manuscript. Saman Raza coanalyzed and interpreted the chemical data and revised the manuscript. Iqbal Azad carried out the computational analyses, docking studies, and data interpretation. Seema Joshi and Abdul Rahman Khan conceived and conceptualized the overall study design and coordination and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards No permission was sought from the Institutional Ethics Committee, Era's Lucknow Medical College, Lucknow, as the work did not involve human or animal subjects.

Conflict of interest The authors declare that they have no competing interests.

References

1. Al-Amiery AA, Mohammed A, Ibrahim H, Abbas A (2010) J Biotechnol Res Cent 4:5
2. Barnum W (1961) J Inorg Nucl Chem 21:221
3. Bavin EM, Rees RJW, Robson JM, Seiler M, Seymour DE, Suddaby D (1950) J Pharm Pharmacol 2:764
4. Beraldo H, Gambino D (2004) Med Chem 4:31
5. Bjelogrlic S, Todorovic T, Bacchi A, Zec M, Sladic D, Srdic-Rajic T (2010) J Inorg Biochem 104(673):12–1339
6. Brockman RW, Sidwell RW, Arnett G, Shaddix S (1970) Heterocyclic thiosemicarbazones: correlation between structure, inhibition of ribonucleotide reductase, and inhibition of DNA viruses. Proc Soc Exp Biol Med 133:609–614
7. Chandra S, Sangeetika RAJ (2001) Saudi Chem Soc 5:175
8. Cotton FA, Wilkinson CW (1972) Advanced inorganic chemistry, 3rd edn. Interscience Publisher, New York
9. Crans DC, Yang L, Khan EGR, Jin W, Simonis U (2003) Applications of Paramagnetic NMR Spectroscopy for Monitoring Transition Metal Complex Stoichiometry and Speciation. Ameriacan Chemica Society: 304–326
10. Dar AM, Khan MA, Mir S, Gatoo MA (2016) DNA binding, cleavage activity, molecular docking, cytotoxicity and genotoxicity studies of newly synthesized copper based metal complexes. Pharm Anal Acta 7:464
11. Deepa KP, Aravindakshan KK (2000) Synth React Inorg Met Org Chem: 3
12. Dhar DN, Taploo CL (1982) J Sci Ind Res 41:501
13. Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment- based contributions and its application to the prediction of drug transport properties. J Med Chem 43(20):3714–3717
14. French FA, Blanz EJJ (1965) The carcinostatic activity of alpha-(N) heterocyclic carboxaldehyde thiosemicarbazones. I. Isoquinoline-1-carboxaldehyde thiosemicarbazone. Cancer Res 25:1454–1458

15. Fryzuk MD, Leznoff DB, Ma ESF, Rettig SJ, Young VG (1998) Synthesis, structure, and reactivity of paramagnetic iron(II) and iron(III) amidodiphosphine complexes. Organometallics 17:2313–2323
16. Gingras BA, Sdomorjai RL, Bayley CH (1961) Can J Chem 39:973
17. Hall IH, Lackey CB, Kistler TD (2000) Cytotoxicity of copper and cobalt complexes of furfural semicarbazone and thiosemicarbazone derivatives in murine and human tumor cell lines. Pharmazie 55: 937–941
18. Holm RH, Cotton FA (1958) J Am Chem Soc 80:5658
19. Joseph M, Sreekanth A, Suni V and Kurup MRP (2006) Spectrochim Acta A: 637–641
20. Jutten P, Schumann W, Hartl A, Heinisch L, Grafe U, Warner W, Uldrich T (2002) BIoorg Med Chem Lett 12:1339
21. Khajah MA, Almorhi I, Mathew PM, Lugmani YA (2013) PLoS One 8:e76327
22. Khan T, Ahmad R, Joshi S, Khan AR (2015) Anticancer potential of metal thiosemicarbazone complexes: a review. Der Chemica Sinica 6(12):1–11
23. Koch O, Stuttgen G, Schmiedebergs N (1950) Arch Exp Pathol Pharmakol 210:409
24. Kowal CR, Eichinger R, Jakupec MA (2007) Effect of metal ion complexation and chalcogen donor identity on the antiproliferative activity of 2-acetylpyridine N, N-dimethyl(chalcogen)semicarbazones. J Inorg Biochem 101:1946–1957
25. Kumar D, Singh VK (2014) Application of metal complexes of Schiff base with special reference to thiosemicarbazone: a review. Journal of Drug Discovery and Therapeutics 2(13):24–32
26. Kumar S, Kumar N (2013) Int Research Journal of Pharmacy 4(1): 177–181
27. Kune GA (1964) Br Med J 2:621
28. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Adv Drug Deliv Rev 23(1–3):3–25
29. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26
30. Lovejoy DB, Richardson DR (2002) Blood 100:666
31. Ludwig JA, Szakács G, Martin SE (2006) Selective toxicity of NSC73306 in MDR1-positive cells as a new strategy to circumvent multidrug resistance in cancer. Cancer Res 66:4808–4815
32. Mohamed GG, Ibrahim NA, Attia HAE (2009) Spectrochim. Acta 72:610
33. Mosmann T (1983) J Immunol Meth 65:55
34. Proudfoot JR (2002) Drugs, leads, and drug-likeness: an analysis of some recently launched drugs. Bioorg Med Chem Lett 12:1647–1650
35. Rafat MS (2007) J Mol Struct 842:24
36. Richardson DR (2002) Crit Rev Oncol/Hematol 42:267
37. Roger C, Hamon P, Toupet L, Raba H, Saillard JY, Hamon J, Lapinte C (1991) Halo- and alkyl(pentamethylcyclopentadienyl) (1,2-bis(diphenylphosphino)-ethane)iron(III) 17-electron complexes: synthesis, NMR and magnetic properties, and EHMO calculations. Organometallics 10:1045–1054
38. Shao J, Zhou B, Di Bilio AJ (2006) A ferrous-triapine complex mediates formation of reactive oxygen species that inactivate human ribonucleotide reductase. Mol Cancer Ther 5:586–592
39. Sharma B, Kothari R (2015) Int J Pharm Bio Sci 6 (1), (B): 1154–1169
40. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD (2002) Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 45(12):2615–2623
41. Wu C, Shukla S, Calcagno AM, Hall MD, Gottesman MM, Ambudkar SV (2007) Evidence for dual mode of action of a thiosemicarbazone, NSC73306: a potent substrate of the multidrug resistance linked ABCG2 transporter. Mol Cancer Ther 6:3287–3296
42. Youssef NS, Hegab KH (2005) Synth React Inor Met Org Chem 35: 391–399
43. Yuan J, Lovejoy DB, Richardson DR (2004) Novel di-2-pyridyl-derived iron chelators with marked and selective antitumor activity: *in vitro* and *in vivo* assessment. Blood 104:1450–1458