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Synthesis, anticancer activity and molecular docking study of Schiff base complexes containing thiazole moiety

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ABSTRACT

A Schiff base ligand **1** was prepared from condensation of salicyaldehyde with 2-amino-4-phenyl-5-methyl thiazole. The ligand forms complexes with Co^{II}, Ni^{II}, Cu^{II}, and Zn^{II} in good yield. The synthesized compounds were characterized by elemental analysis, magnetic susceptibility, molar conductance, infrared spectra, ^{1}H and ^{13}C NMR, mass, electronic absorption and ESR spectroscopy. The anticancer activity of the synthesized compounds was studied against different human tumor cell lines: breast cancer MCF-7, liver cancer HepG2, lung carcinoma A549 and colorectal cancer HCT116 in comparison with the activity of doxorubicin as a reference drug. The study showed that Zn^{II} complex showed potent inhibition against human TRK in the four cell lines (HepG2, MCF7, A549, HCT116) by the ratio 80, 70, 61 and 64% respectively as compared to the inhibition in the untreated cells. Moreover, the molecular docking into TRK (PDB: 1t46) was done for the optimization of the aforementioned compounds as potential TRK inhibitors.

1. Introduction

The chemistry of the Schiff base ligands and their metal complexes has expanded enormously and encompasses a vast area of organometallic compounds and various aspects of bioinorganic chemistry (Abu-Diefa and Mohamed, 2015). Schiff base ligands are considered "privileged ligands" because they are mainly prepared by condensation between aldehydes and primary amines (Chang et al., 2016). These ligands are able to coordinate many different metals and to stabilize them in various oxidation states (Ejidike and Ajibade, 2015). They are used also as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers (Menati et al.,

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2013). Large numbers of Schiff bases have also been shown to exhibit a broad range of biological activities, including anti-tumor, anti-bacterial, fungicidal and anticarcinogenic properties (Nagesh et al., 2015; Salehi et al., 2015; Shukla et al., 2013; Zaltariov et al., 2015; Zayed and Zayed, 2015). Metal complexes of Schiff bases with heterocyclic compounds also find applications as potential drugs, (Andersen, 1999; Konstantinović et al., 2003) due to the presence of multifunctional groups (Chohan et al., 2004; Joseyphus et al., 2006; Vashi and Naik, 2004; Venugopala and Jayashree, 2003). The excessive attention of synthesizing determined broad range of N and S chelating ligands as thiazole molecule have attracted significant interest. This is because thiazoles have a great pharmacological activity. Besides these atoms play an important role in the coordination of metals at the active sites of various metal biomolecules that have a therapeutic activity or serving as study models for metallo-enzymes (Chen et al., 2012; Venkatraman et al., 2010; Yenilmez et al., 2013). Thiazoles are very important building blocks in medicinal chemistry and can be found in numerous natural products (e.g. epothilone) and biologically important compounds including the anticancer drug dasatinib, antiviral clinical candidate TMC435350 and anti-diabetic drug candidate MB06322 (Dang et al., 2008; Doggrell, 2005; Erion et al., 2005; Lin et al., 2009). Recently, thiazoles found application in drug development for the treatment of allergies (Brzezińska et al., 2003), hypertension (Mishra et al., 2015), inflammation (Sharma et al., 1998), schizophrenia (Jaen et al., 1990), bacterial (Suzuki et al., 1994), HIV infections (Bell et al., 1995), hypnotics (Ergenc et al., 1999), as fibrinogen receptor antagonist with antithrombotic activity (Badorc et al., 1997) and as new inhibitors of bacterial DNA gyrase B (Rudolph et al., 2001).

Following all these observations and as a part of our continuing research on the coordination chemistry of multidentate ligands (Abd-Elzaher, 2004a, 2004b; Abd-Elzaher et al., 2005, 2006, 2010, 2012a, 2012b; Fouda et al., 2008a, 2008b), we report here the preparation and characterization of a Schiff base ligand derived from condensation of salicyaldehyde with 2-amino-4-phenyl-5-methyl thiazole. The study has been extended to synthesize Cu^{II}, Co^{II}, Ni^{II} and Zn^{II} complexes with the prepared ligand. All the prepared complexes have been characterized by IR, ^{1}H and ^{13}C NMR, mass spectra, ESR, UV–Vis, in addition to elemental analysis, molar conductivity and magnetic susceptibility.

In the same direction and in continuing effort to find more potent and selective anticancer compounds, herein, anticancer activity of the prepared compounds was carried out against four different human tumor cell lines including breast cancer cell line MCF-7, liver cancer cell line HepG2, lung carcinoma A549 and colorectal cancer HCT116 that may act through tyrosine kinase (TRK) inhibition. Molecular Docking has been done to evaluate the binding affinity of the Ni and Zn complexes to TRK.

2. Experimental

2.1. Materials and methods

All chemicals were obtained from Merck. Elemental analyses were determined at the micro analytical center, Cairo University. IR spectra were recorded in the 4000-400 cm^{-1} on a spectrometer (Jasco FTIR- 6100 Japan), using KBr pellets. ^{1}H and ^{13}C NMR were recorded on a Bruker DPX 300, δ values relative to the deuterated DMSO. Mass spectra: Jeol JMS-700 using FAB technique with a 3-nitrobenzyl alcohol NBA matrix. Magnetic susceptibilities were measured at 20 °C by the Gouy method at the Faculty of Science, Cairo University. The molar conductance measurements were measured in solution of the metal complexes in DMF (10^{-3}) using Metrohem 660 conductivity meter. Electronic absorptions were recorded on a automatic spectrophotometer (PG Instruments ltd., +80 + UV–Vis) in DMSO. ESR measurements were made at approximately 298K with a Bruker E500, X-band spectrometers operating at a frequency of 9.5 GHz at National Institute for Standards, Giza.

2.2. Synthesis of the Schiff base ligand $C_{17}H_{14}N_2OS$ (1) and metal complexes (2–5)

2-amino-4-phenyl-5-methyl thiazole (1 mmol, 2.95 g) dissolved in about 20 mL absolute ethanol was added slowly to a magnetically stirred solution of salicyladehyde (1 mmol, 1.22 g), in the presence of few drops of glacial acetic acid. The mixture was refluxed for four hours. Then the solution was concentrated to its half volume then cooled; n-hexane was added to the reaction mixture drop wise until a product began to precipitate. The formed product was filtered off, washed several times with n-hexane, and recrystallized from ethanol. The different complexes were prepared by addition of 1 mmol of $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$ and $ZnCl_2$, dissolved in about 20 mL ethanol, into a solution of the ligand (2 mmol in 20 mL ethanol). The mixture was refluxed for three hours, then the solution was concentrated. The obtained solid products were filtered, washed twice with cold n-hexane and dried.

2.3. Anticancer activity

2.3.1. Chemicals

Fetal bovine serum (FBS) and L-glutamine were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's medium (DMEM) was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, and streptomycin were obtained from Sigma Chemical Company (Saint Louis, MO, USA). Human tyrosine kinase (TRK) ELISA kit was purchase from Glory Science Co., Ltd (Del Rio, TX 78840, USA).

2.3.2. Cell lines and culturing

Anticancer activity screening for the tested compounds utilizing 4 different human tumor cell lines including breast cancer cell line MCF-7, liver cancer cell line HepG2, lung carcinoma A549 and colorectal cancer HCT116 were obtained from the American Type Culture Collection (Rockville, MD, USA) through LGC Standards GmbH, Wesel, Germany. The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 UmL^{-1}) and streptomycin (100 μgmL^{-1}) at 37 °C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50×10^6 were grown in a 25 cm^2 flask in 5 mL of complete culture medium.

2.3.3. *In vitro cytotoxicity assay*

The antiproliferative activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previously reported standard procedure (Skehan et al., 1990). Cells were inoculated in 96-well microtiter plate (10^4 cells *per* well) for 24 hours before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO at 1 mg mL^{-1} immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of tested compounds and doxorubicin (2, 5, 10 or 20 μg mL^{-1}) were added to the cells. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the prepared compounds for 48 hours at 37 °C and in atmosphere of 5% CO_2. After 48 hours, cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and the attached stain was recovered by Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between both the surviving fraction and the drug concentration was plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1. The results were compared to the antiproliferative effects of the reference control doxorubicin.

2.3.4. *In vitro human tyrosine kinase (TRK) concentration assay*

The effect of tested compounds on the level of human tyrosine kinase (TRK) was determined in the four different human tumor cell lines (MCF-7, HepG2, A549 and HCT116). The cells at a concentration of 0.50×10^6 were grown in a 25 cm^2 flask in 5 mL of DMEM culture medium and were treated with 20 μl of IC_{50} values of the compounds or the standard reference drug, Doxorubicin dissolved in DMSO, then incubated for 24 h at 37 °C, in a humidified 5% CO_2 atmosphere. The cells were harvested and homogenates were prepared in saline using a tight pestle homogenizer until complete cell disruption.

To determine the level of TRK in samples, a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used. In brief, samples containing TRK were added to monoclonal antibody enzyme well which is pre-coated with human TRK monoclonal antibody, incubation; then, add TRK antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and wash again to remove the uncombined enzyme. Then add Chromogen solution A, B, the color of the liquid changes into blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human TRK of sample were positively correlated and the optical density was determined at 450 nm. The level of TRK in samples was calculated (pmol mL^{-1}) as duplicate determinations from the standard curve.

2.4. *Molecular docking study*

Molecular Operating Environment (MOE) 2008.10 (Moe source: Chemical Computing Group Inc., Quebec, Canada, 2008) program was used in molecular docking studies. Firstly, a Gaussian Contact surface around the binding site was sketched, then the surface enclosed the van Waals surface (we made filling in solvent in accessible gaps). Finally docking studies were done to assess the binding free energy of the inhibitor inside the macromolecule. The Dock scoring in MOE software was done utilizing London dG scoring function and has been upgrading by using two unrelated refinement methods, the Grid-Min pose and the Force-filed have been improved to check that refined poses meet the specified conformations. Auto rotatable bonds were allowed; the best ten binding poses were directed to analyze for achieving the best score. To compare the docking poses to the ligand in the co-crystallized structure and to obtain RMSD of the docking pose database browser was used.

2.4.1. *Preparation of ligands and target protein-tyrosine kinase*

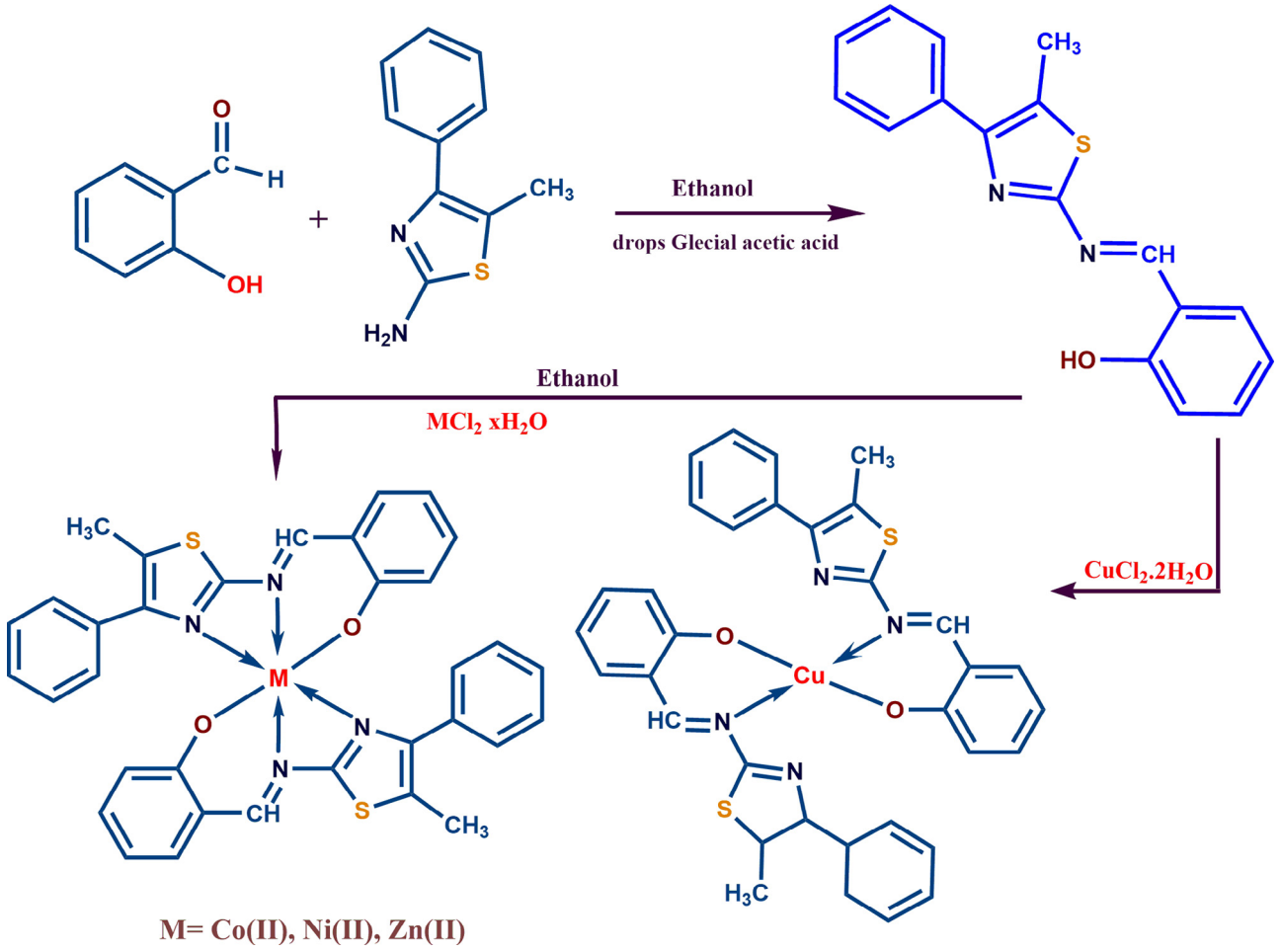
The compound contributed in this study as ligands was studied for their binding affinity into protein-tyrosin kinase (TRK). To build a three dimensional model of the structures, molecular builder tool in MOE was used. Energy minimization was carried out through Force-Filed MMFF94X. Optimization was carried out using gradient of 0.001 for determining the lowest energy confirmation with most favorable geometry. The crystal structures of c-kit receptor protein-tyrosine kinase in complex with STI-571 (Imatinib or Gleevec) were picked up from the Protein Date Bank (PDB) (http://www.rcsb.org/pdb/explore/explore.do?structureId=1T46) (PDB code: 1t46) (Mol et al., 2004). Partial charges and Hydrogen atom were put on to the protein with the protonation 3d application in MOE. This application is carried out to assign position hydrogen atom in the macromolecule structures and ionization states.

2.4.2. *Molecular modeling and analysis of the docked results*

To rank the binding affinity of the synthesized compounds to TRK protein the binding free energy and hydrogen bonds between the ligand and amino acid in TRK were used. Evaluation of the hydrogen bonds were done by measuring the hydrogen bond length, which does not exceed 3.7 A°. In addition, RMSD of the co-crystal ligand position compared to the

Table 1 – Analytical and some physical characteristics for the prepared compounds.

No.	Ligand/complexes	Color	M. wt.	Calcd. (found) (%)			A_M	Yield (%)
				C	H	N		
1	HL ($C_{17}H_{15}N_2SO$)	Yellow	295	69.13 (68.91)	5.12 (5.26)	9.48 (9.21)	–	82
2	($C_{34}H_{28}CoN_4O_2S_2$)	Greenish blue	647.6	63.05 (62.85)	4.36 (4.43)	8.65 (8.41)	16	65
3	($C_{34}H_{28}N_4NiO_2S_2$)	Light brown	647.4	63.07 (62.85)	4.36 (4.32)	8.65 (8.52)	18	63
4	($C_{34}H_{28}CuN_4O_2S_2$)	Brown	652.2	62.61 (62.26)	4.33 (4.2)	8.59 (8.48)	14	70
5	($C_{34}H_{28}N_4O_2S_2Zn$)	Light yellow	654.1	62.43 (62.19)	4.31 (4.45)	8.57 (8.42)	11	64



Scheme 1 – Preparation of the ligand and its complexes.

docking pose was used in ranking. Both RMSD as well as the mode of interaction of the native ligand within the crystal structure of c-kite tyrosin kinase receptor were used as standard docked model.

3. Results and discussion

The Schiff base ligand **1** was prepared from condensation of salicyaldehyde with 2-amino-4-phenyl -5-methyl thiazole (Scheme 1). The ligand and its metal complexes **(1–5)** were found to be stable in air and light. All compounds **1–5** were soluble in common organic solvents such as ethanol, methanol, acetone, and chloroform. The elemental analysis confirmed that all complexes **2–5** have 2:1 molar ratio between the ligand and the metal. The elemental analyses of the compounds **1–5** were consistent with the calculated results from the empirical formula of each compound (Table 1).

3.1. *IR spectra*

The IR spectra of the compounds **1–5** were represented in Table 2. The IR spectra of the prepared ligand **1**, which showed a strong band at 1632 cm^{-1}, were assigned to the formation of the azomethine (C═N) group (Abd-Elzaher, 2004a, 2004b; Abd-Elzaher et al., 2005). The band at 1567 cm^{-1} was attributed to υ_{cyclic}(C═N) of thiazole ring (Venkatraman et al., 2010). Phenolic C—O stretch band is observed at 1278 cm^{-1}; another medium band appeared at 753 cm^{-1}, which was assigned to C—S—C (ring) stretching vibration (Abd-Elzaher, 2004a, 2004b). Another band was observed centered at 2987 cm^{-1}, which

Table 2 – The IR spectral (cm^{-1}) assignment for the ligand and its complexes.

No.	Ligand/complexes	$(OH)_{hydrogen\text{-}bond}$	C═N	$(C═N)_{thiazole}$	C—O	C—S—C	M—O	M—N
1	$HL(C_{17}H_{15}N_2OS)$	2987	1632	1567	1278	753	–	–
2	$(C_{34}H_{28}CoN_4O_2S_2)$	–	1611	1517	1261	758	502	424, 443
3	$(C_{34}H_{28}N_4NiO_2S_2)$	–	1615	1522	1256	754	500	421, 454
4	$(C_{34}H_{28}CuN_4O_2S_2)$	–	1606	1562	1257	749	492	428
5	$(C_{34}H_{28}N_4O_2S_2Zn)$	–	1612	1523	1263	757	529	422, 449

Table 3 – ^{1}H and ^{13}C NMR data of the ligand and its Zn^{II} complex.

Ligand/ complex	Ligand	Zn^{II} complex
^{1}H NMR (DMSO-d_6), δ (ppm)	2.38(s, CH_3 in thiazole ring), 6.93–7.80 (m,9 H,2Ph), 9.19(s, 1H, CH═N), 11.51(s, 1H, OH)	2.25 (s, CH_3 in thiazole), 6.58–7.78 (m,18H, 4Ph), 9.08 (s, 2H, 2CH═N)
^{13}C NMR (DMSO-d_6), δ (ppm)	16.3 (CH_3 attached to thiazole rings), 120.2, 141.6, 153.7 (thiazole rings), 148.2 (C═N), 117.4–164 (aromatic carbons)	16.5 (CH_3 attached to thiazole rings), 120.3, 141.6, 153.9 (thiazole rings), 149.1 (C═N), 118.4–164.3 (aromatic carbons)

indicate the presence of an intramolecular hydrogen bond interaction between phenolic group with imine-nitrogen atom (Nazır et al., 2000; Yıldız et al., 1998).

By comparing the IR spectra of complexes **2–5** with the spectrum of the free ligand, the disappearance of the intramolecular hydrogen bond signal of the free ligand was observed, which indicates the removal of the proton of hydroxyl group during the chelation. This is further supported by the shift of C—O band from 1278 cm^{-1} (in ligand) to 1256–1263 cm^{-1} (in complexes) (Abd-Elzaher et al., 2005). However, the band due to the azomethine (C═N) group in the free ligand (at 1632 cm^{-1}) was shifted to a lower frequency (1606–1615 cm^{-1}) in the complexes. This shift indicates coordination of the azomethine nitrogen to the metal ions in the complexes. In addition, the band due to υ_{cyclic}(C═N) of thiazole ring (in ligand) was shifted in all complexes except Cu-complex; this shift indicates the involvement of the thiazole nitrogen atom in the complex formation (**2,3,5**) and not coordinated in the case of complex **4** (Bolos et al., 1999). The C—S—C band of the free ligand, which appears at 753 cm^{-1}, showed a very little effect by complexation indicating that the sulfur atom is not involved in the chelation (Nakamoto, 1998; Neelakantan et al., 2008; Omar and Mohamed, 2005). Two new bands in the range 484–502 and 421–454 cm^{-1} were also observed (Table 2). These two bands were observed in the complexes and not found in the free ligand and they are attributed to M—O and M—N bonds in the complexes respectively (Rana et al., 1982).

3.2. *NMR spectra*

The ^{1}H and ^{13}C NMR spectra of the ligand and its Zn^{II} complex were recorded at room temperature using DMSO-d_6 as a solvent (Table 3). The ^{1}H NMR spectra of the ligand showed a singlet at δ2.38 ppm attributed to methyl group attached to the thiazole ring, and multiplet at δ 7.80–6.93 ppm due to aromatic protons. The spectra showed also azomethine proton (CH═N) as singlet at δ 9.19 ppm. The OH proton is observed as singlet at δ 11.51 ppm. A comparison of the ^{1}H NMR spectra of the Zn^{II} complex and the free ligand indicates that the proton signal corresponding to OH group of ligand has disappeared in Zn^{II} complex, which may be due to deprotonation. The CH═N proton in the ligand is shifted to 9.08 ppm in the Zn^{II} complex, which suggested that the azomethine nitrogen is involved in the coordination (Maurya et al., 2005).

The ^{13}C NMR of the ligand showed a signal at 16.3 ppm which was assigned to the methyl group attached to the thiazole rings, and three signals at 120.2, 141.4 and 159.4 ppm were assigned to the thiazole rings (Abd-Elzaher et al., 2012a, 2012b). The signal that appeared at 152.2 ppm was assigned to the carbon of the azomethine group. Signals due to phenyl carbons appeared in the range of 117.4–164 ppm (Yıldız et al., 2010). The ^{13}C NMR signals of the Zn^{II} complex were shifted slightly downfield compared with that of the ligand, which may be due to coordination of the ligand with the metal ions (Abd-Elzaher et al., 2012a, 2012b).

3.3. *Molar conductivity measurements*

The molar conductivities of 10^{-3} M of the complexes (dissolved in DMF) at room temperature were measured (Table 1). The results were in the range 11–18 $\Omega^{-1}.mol^{-1}.cm^{2}$ for Co^{II}, Ni^{II}, Cu^{II} and Zn^{II} complexes. Conductivity measurements reveal that all the metal complexes have conductivity values in the range characteristic for non-electrolytic nature (Andersen, 1999; Chohan et al., 2004; Golcu et al., 2005; Joseyphus et al., 2006; Vashi and Naik, 2004; Venugopala and Jayashree, 2003).

3.4. *Electronic spectra and magnetic moment*

The electronic absorption spectrum of the ligand showed three bands at 272, 338 and 453 nm (Table 4). The first one may be assigned to intra-ligand π–π* transition, which is nearly unchanged on complexation, whereas the second and third bands may be assigned to the n–π* and charge transfer transition of the azomethine group and nitrogen atom of the thiazole ring (Sreeja et al., 2004).

The electronic spectra of the Co^{II} complex showed two bands at 572 and 484 nm. These bands are assigned to the transitions $^{4}T_{1g}(F) \rightarrow {}^{4}A_{2g}(F)$ and $^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(P)$, respectively; the Co^{II} complex also showed magnetic moment at 4.6 B.M., which indicates the presence of Co^{II} complex in octahedral geometry (Abd-Elzaher et al., 2012a, 2012b; Fouda et al., 2008a, 2008b). The electronic absorption of Ni^{II} complex showed three bands at 595, 387 and 841 nm (Table 4). These bands are assigned to the transitions $^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(F)$, $^{3}A_{2g} \rightarrow {}^{3}T_{1g}(p)$ and $^{3}A_{2g} \rightarrow {}^{3}T_{2g}$ respectively (Abd-Elzaher et al., 2006; El-Shiekh et al., 2006; Lever, 1984); the magnetic moment of Ni^{II} complex was 3.2 B.M. These results suggested the presence of octahedral geometry for Ni^{II} complex. The electronic spectra of the Cu^{II} complex showed two bands at 642,

Table 4 – The electronic spectra and magnetic moments for the ligand and its complexes.

No.	Ligand/complexes	Bands in DMSO	μ_{eff} (BM)
1	HL($C_{17}H_{15}N_2OS$)	453, 338, 272	–
2	($C_{34}H_{28}CoN_4O_2S_2$)	572, 484, 453, 340, 270	4.6
3	($C_{34}H_{28}N_4NiO_2S_2$)	841, 595, 450, 387, 271	3.2
4	($C_{34}H_{28}CuN_4O_2S_2$)	624, 508, 452, 356, 270	1.81
5	($C_{34}H_{28}N_4O_2S_2Zn$)	450, 350, 270	Dia.

508 nm, which are assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions respectively (Abd-Elzaher, 2004a, 2004b; Fouda et al., 2008a, 2008b), and the magnetic moment of Cu^{II} complex was 1.81 B.M. Both the electronic spectra and the magnetic values proposed the presence of the square planar geometry for Cu^{II} complex. On the basis of the above observation and spectral data, it is suggested that the Co^{II}, Ni^{II} and Zn^{II} complexes show octahedral geometry structures; however, the Cu^{II} complex shows square-planar geometry structure (Lever, 1984).

3.5. *Mass spectra*

The mass spectrum supports the proposed empirical formula of the ligand. It reveals the molecular ion peak m/z at 294 consistent with the molecular weight of the ligand. While the fragments at m/z = 214, 201, 188, and 83 correspond to $C_{12}H_{12}N_3O$, $C_{12}H_{12}N_2O$, $C_{11}H_{11}N_2O$, and C_4H_3S, respectively. The mass spectra of the complexes 2–5 show molecular ion peaks m/z at 648, 647, 652 and 654, consistent with the molecular weight of these complexes, respectively.

3.6. *Electron spin resonance spectra*

The solid state ESR spectra of 4 exhibit axially symmetric g-tensor parameters with $g\| <= g\perp > 2.0023$ indicating that the copper site has a d_{x2-y2} ground-state characteristic of square planar stereochemistry (El-Sonbati et al., 2011; Speie et al., 1996). The spin Hamiltonian parameters of the complex at $g\|$, $g_\perp$, and $A\|$ have the values 2.157, 2.064, 2.095 and 207×10^{-4} cm^{-1}, with exchange interaction parameter (G) 4.6. A good indication of square planar coordination geometry for the copper complex moiety according to the $g\|$ and $|A\|$ values has been shown.

In addition, in axial symmetry, the g-values are related by the expression $G = (g\| - 2)/(g\perp - 2) = 4$, where G is the exchange interaction parameter. According to Hathaway and Billing (Diab et al., 2010; El-Sonbati et al., 2011; Hathaway and Billing, 1970), if the value of G is greater than 4, the exchange interaction between Cu^{II} centers in the solid state is negligible, whereas when it is less than **4**, a considerable exchange interaction is indicated in the solid complex. The calculated G values for **4** is 4.6 suggesting that there are no copper–copper exchange interactions (Diab et al., 2010; El-Sonbati et al., 2011; Hathaway and Billing, 1970; Speie et al., 1996).

3.7. *Anticancer activity*

Chemotherapy is the major approach for both localized and metastasized cancer. Therefore, the synthesized compounds were screened for their *in vitro* cytotoxicity and growth inhibitory activities against 4 different human tumor cell lines including breast cancer cell line MCF-7, liver cancer cell line HepG2, lung carcinoma A549 and colorectal cancer HCT116. The results were compared with the activity of the known anticancer doxorubicin as a reference drug. The cytotoxicity of the tested compounds was expressed by median growth inhibitory concentration (IC_{50}) which required producing 50% cytotoxic effect against cancer cells after 48 hours exposure to tested compounds. The screening results are given in Table 1.

It is evident that for the HepG2 and MCF-7 cells, all the tested compounds showed anticancer activity with IC_{50} values that ranged from 6.20 to 9.22 and from 6.00 to 10.00 $\mu g\ mL^{-1}$ respectively (Table 5). It is clear from the data that the order of cytotoxicity activity against the two cancer cell lines of the tested compounds was Zn^{II} complex, Ni^{II} complex, Cu^{II} complex, Co^{II} complex and (L)**1** in descending order. While in case of A549 cells, only Zn^{II} complex and Ni^{II} complex showed anticancer activity at IC_{50} of 5.30 and 9.10 $\mu g\ mL^{-1}$ respectively. In addition, HCT116 revealed anticancer activity for L, Cu^{II} complex and Zn^{II} complex with IC_{50} of 9.50, 6.70 and 6.20 μgmL^{-1} respectively. It is clear from the results that in all four cell lines the cytotoxicity of Zn^{II} complex showed a strong activity compared with doxorubicin. Furthermore, to elucidate the mechanism by which the prepared compounds exert their antitumor activities, we estimated the level of human TRK in the cancer cells treated with the prepared compounds.

Tyrosive kinases (TRKs) are indispensable for numerous processes in the cell. These enzymes catalyze phosphorylation of different cellular substrates. Phosphorylation in turn regulates various cellular functions. Normally, their activity is stringently regulated. However, under pathological conditions, TRKs can be deregulated, leading to alterations in the phosphorylation and resulting in uncontrolled cell division, inhibition of apoptosis, and other abnormalities and consequently to diseases (Shchemelinin et al., 2006a, 2006b). Various cancers and other diseases are known to be caused or accompanied by deregulation of the phosphorylation. Inhibition of TRKs has

Table 5 – The IC_{50} values and the percent of TRK of the tested compounds in 4 different human cancer cell lines.

Compound	IC_{50} (μgmL^{-1})				% of TRK inhibition*			
	HepG2	MCF-7	A549	HCT116	HepG2	MCF-7	A549	HCT116
L (ligand)	9.22	10.00	–	9.50	19	6.5	–	18
Co^{II} complex	8.16	9.11	–	–	33	19	–	–
Ni^{II} complex	7.20	7.30	9.10	–	73	55	38.5	–
Cu^{II} complex	7.50	8.70	–	6.70	67	33	–	43
Zn^{II} complex	6.20	6.00	5.30	6.20	80	70	61	64
DMSO	–	–	–	–	–	–	–	–
Doxorubicin	4.20	4.40	4.70	5.25	81	84	83.5	83

Data were expressed as average of three independent experiments.
The percentage changes as compared with control untreated cells (DMSO treated).

been shown to be a promising therapeutic strategy. Many TRK inhibitors (TRKs) have been produced and tested in clinic by now. These molecules have a low molecular weight and most of them bind to protein kinases competing with ATP for the ATP-binding site (Bogoyevitch et al., 2005). The availability of newer inhibitors will be the main target of many researchers in the future.

The results in Table 5 showed that most of the tested compounds exhibited inhibitory potential against human TRK. In case of human liver cancer HepG2 Ni^{II}, Cu^{II} and Zn^{II} complexes revealed inhibition to TRK by 73, 67 and 80% as compared with the untreated cancer cells, while doxorubicin showed 81% inhibition. In case of human breast cancer MCF7 Ni^{II} and Zn^{II} complexes were found to be potent inhibition against human TRK by 55% and 70% respectively, while doxorubicin showed 84% inhibition. Moreover, in case of human lung cancer A549, only Zn^{II} complex was found to be potent inhibition against human TRK by 61% while doxorubicin showed 83.5% inhibition. Furthermore, in case of colorectal cancer HCT116 Cu^{II} and Zn^{II} complexes were found to be potent inhibition against human TRK by 43 and 64% respectively, while doxorubicin showed 83% inhibition. It is clear that Zn^{II} complex showed potent inhibition against human TRK in the four cell lines (HepG2, MCF7, A549, HCT116) by the ratio 80, 70, 61 and 64% respectively as compared to the inhibition in the untreated cells as listed in Table 5.

The highest activity of the Zn^{II} complex than the others may be attributed to the function of the Zn^{II} complex as a competitive inhibitor of hemeoxygenase (HMOX1), which is produced in large amounts in solid tumors (Huang et al., 2005), in humans and animal tumor models. HMOX1 has been adapted to defend against oxidative and other cellular stresses (Huang et al., 2005).

3.8. *Molecular modeling: docking study*

3.8.1. *Molecular modeling: docking study*

The prepared compounds were analyzed for the binding affinity of tyrosine kinases receptor (PDB 1t46) for the purpose of both find out the interaction between studied compounds and c-kit receptor and for lead optimization. Molecular modeling calculation was carried out to investigate the binding free energies of these inhibitor inside the target c-kit kinase receptor.

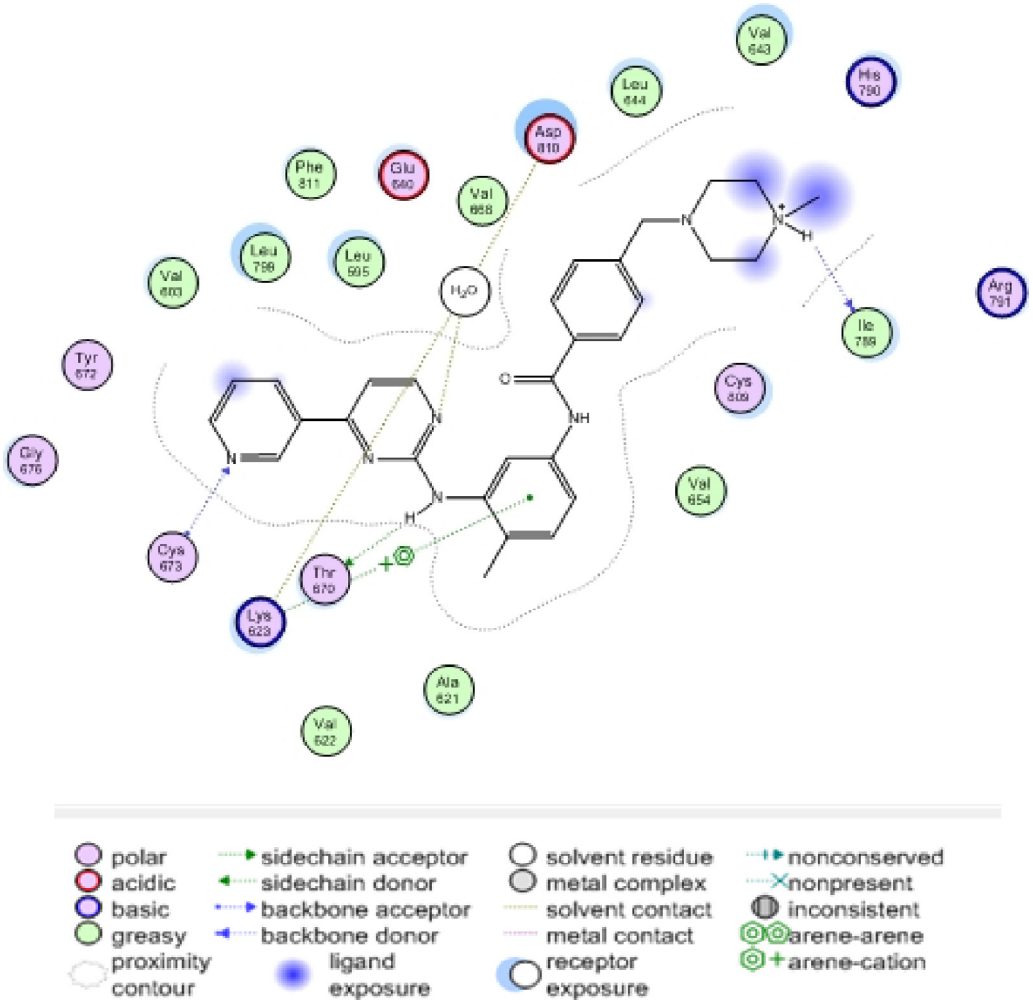


Fig. 1 – The binding mode of the native ligand STI with C-kit exhibited one H-bond donor with THR 670 at distance 2.08 and one H-bond donor with ILE 789 at distance 2.19 and one H-bond donor with CYS 673 at distance 2.85 and one H-bond acceptor with HOH 1105 at distance 2.82 its score was –20.04 k.calmol^{-1}.

3.8.2. *Validation of the docking performance and accuracy*

Docking of the native co-crystallized STI-571 ligand (Imatinib or Gleevec) was used to validate the docking accuracy of the program. The docked ligand was exactly superimposed on the native co-crystallized one with RMSD being 0.40 Å and binding free energies of –20.04 kcal.mol^{-1}. The hydrogen bonds between the amino acids and the docked ligand were the same as those between the amino acid and the native ligand.

3.8.3. *The binding affinities of the synthesized compounds into c-kit kinase receptor*

To compare affinity and to find out the interaction between ligand and receptor, molecular docking study was done (Fig. 1). For the docking calculation, firstly, the protein structure (PDB code: 1t46) was detached from the inhibitor and hydrogen atoms were added. The binding free energy, hydrogen bond and RMSD were used to determine the binding affinity. All the prepared

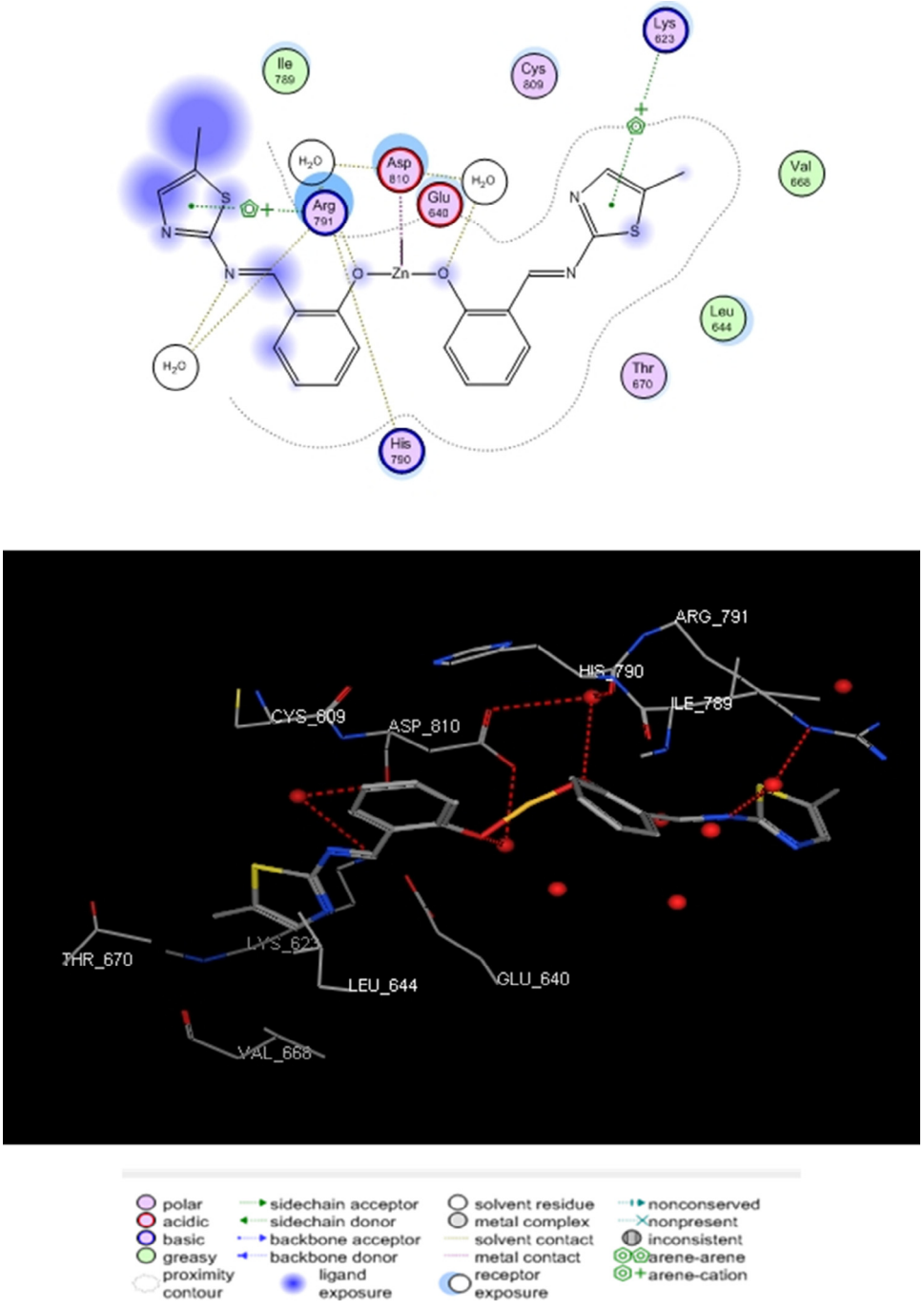


Fig. 2 – The binding mode of the Zn-complex with C-kit receptor exhibited one H-bond acceptor with HOH 1105 at distance 2.64; and one H-bond acceptor with HOH 1105 at distance 2.63 and one H-bond acceptor with HOH 1106 at distance 3.32 and its score was –15.77 k.cal mol^{-1}.

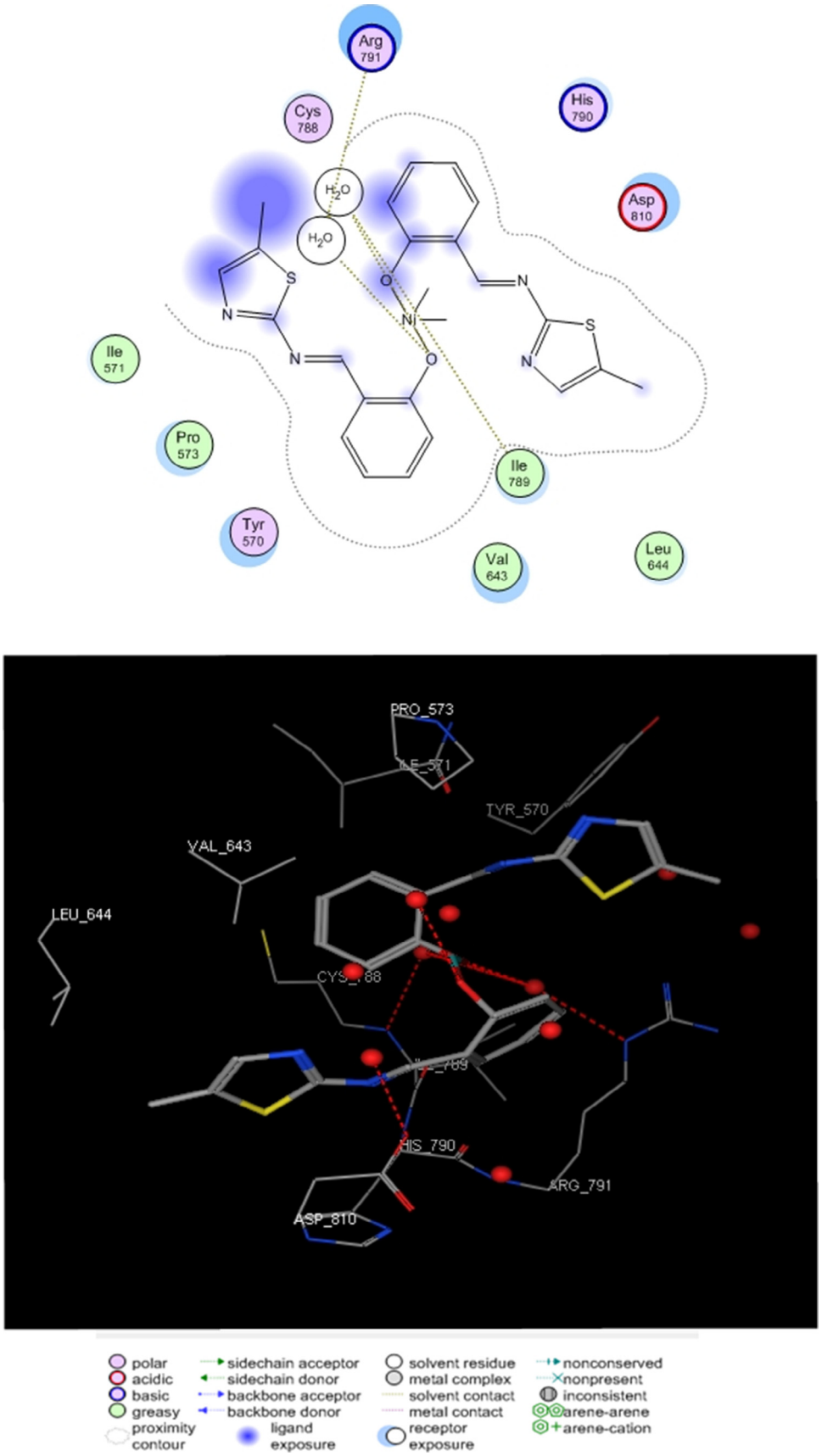


Fig. 3 – The binding mode of the Ni-complex with C-kit exhibited one H-bond acceptor with HOH 1033 at distance 3.16 and its score was –11.98 kcalmol^{-1}.

compounds were docked into the same binding site of the native co-crystallized ligand. Both Zn^{II} and Ni^{II} 5,3 respectively, gave the best docking score (Figs. 1–3).

4. Conclusion

The Schiff-base ligand **1** (E)-2-(((5-methyl-4-phenylthiazol-2-yl)imino)methyl)phenol was synthesized and determined with different spectroscopic techniques, followed by synthesis of four novel cobalt, nickel, copper and zinc complexes. The new compounds were investigated for inhibition against human TRK in vitro cytotoxicity for four human tumor cell lines. The Zn^{II} complex showed potent inhibition against human TRK in the four cell lines (HepG2, MCF7, A549, HCT116) by the ratio 80, 70, 61 and 64% respectively as compared to the inhibition in the untreated cells in comparison with the reference drug (doxorubicin); also most of the tested compounds exhibited inhibitory potential against human protein tyrosine kinase (PTK). The synthesized compounds were investigated for the binding affinity of tyrosine kinases receptor (PDB1t46). MOE (molecular modeling environment) evaluated the binding free energies of these inhibitors into the target c-kit kinase receptor. It was found that the MOE were typically concise with the experimental data.

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