Synthesis, Characterization and Biological Activities of a Schiff Base Derived from 2-[(1,3-benzothiazol-2-yl)sulfanyl]-*N*-[4-(hydrazinecarbonyl)phenyl]acetamide and its Complexes with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) Ions

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A new Schiff base ligand from 2-[(1,3-benzothiazol-2-yl)sulfanyl]-N-[4-(hydrazinecarbonyl)phenyl]acetamide (BHA) and its five metal complexes with transition metal ions [Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)] have been synthesized. The resulting compounds were subsequently characterized by FTIR, UV-Vis, AAS, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The proposed geometries of the Schiff base metal complexes were established on the basis of metal / ligand ratio, through AAS/ICP, electronic spectroscopy and magnetic data. The synthesized Schiff base and its complexes were further investigated for their anti-inflammatory and anticancer activity which exhibited relatively pronounced activity for metal complexes than Schiff base.

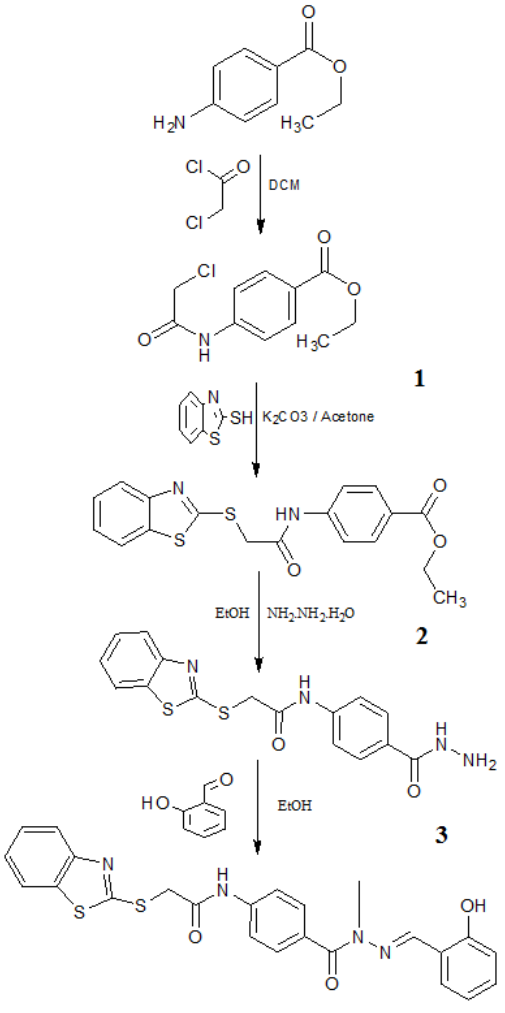
Keywords: Schiff base, metal complexes, anti-inflammatory activity

Heterocyclic compounds are oftenly distributed in nature and are considered to be vital for many biochemical processes [1]. These compounds have gained much attraction over the most recent couple of years as these demonstrated tremendous biological activities [2-4]. Today, the pattern in antimicrobial drug design [5, 6] is to join a few heterocyclic atoms having distinctive sites or mechanism of activity. In perspective of this information, we aimed the synthesis of Schiff base derived from the hydrazide containing the substituted benzothiazole moiety. Schiff base having an azomethine –NH-N=CH- functional group, presents an important class of compounds for new drug discovery. Schiff bases are of extraordinary interest for chemists since they are used as beginning materials in the synthesis of industrial items [7]. These are known to be versatile candidate for complexation with transition metal ions [8-15]. These complexes have been reported to posses several biological activities including antifungal, antibacterial, anticancer and herbicidal, etc [16-24]. Keeping in view, very interesting coordination behavior of Schiff bases and in particular their fascinating biological activities, in this paper, we report the synthesis, characterization, anti-inflammatory and anticancer activities of a new Schiff base ligand and its complexes with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) ions.

Experimental part

Materials and Methods

Chemicals (solvents and reagents) used were of analytical grade and were procured from Sigma Chemical Co. (USA) and E. Merck (Germany) and were utilized as such without further purification and distillation. All the glass apparatus was made up of Pyrex and was dried in oven before using. All the reactions were examined with pre-coated aluminum TLC cards. Melting and decomposition points were taken by using melting point apparatus



Scheme 1. Synthesis of Schiff base ligand

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(Gallenkamp). FTIR spectra were obtained using nujol mull technique on VENUS PHARMA/Agilent technologies Cary 630 FTIR spectrophotometer. NMR spectra were obtained in DMSO-*d6* on Brucker/Avance NMR (300 MHz) and internal standard used was TMS. Chemical shifts are obtained in δ(ppm). FAB-Mass spectra were recorded on a Jeol-600H-2 instrument. Conductivity measurements were made with the InoLab Cond 720 conductometer using DMSO as a solvent at room temperature. To evaluate the changes in biological activities of the ligand after complexation, anti-inflammatory and anticancer activities were carried out of the ligand and its complexes and compared their IC_{50} values, by using Ibuprofen and Doxorubicin as a standard drugs, respectively.

Methods of synthesis

Synthesis of Ethyl-4-(2-chloroacetamido)benzoate **(1)**

This compound **(1)** was synthesized according to a reported procedure [25]. Briefly, benzocain (5.0 mmol, 0.825 g) was added in dicloromethane (10 mL) and stirred till its dissolution, then added potassium carbonate (6.0 mmol, 0.828 g) and stirred the mixture using ice bath. Chloroacetyl chloride (6.0 mmol, 0.9 mL) was then added dropwise in the above mixture and later on the resulting mixture was restirred at room temperature for 18h. Completion of reaction was monitored by TLC. After completion, dicholoromethane was evaporated, and the precipitates were filtered, washed with plenty of water and then dried.

Synthesis of Ethyl-4-{2-[(1,3-benzothiazol-2-yl)sulfanyl] acetamido}benzoate **(2)**

Synthesis of **(2)** was also carried out as reported earlier [25] by mixing 2-mercaptobenzo-thiazole (1.0 mmol) in dry acetone (20 mL), anhydrous K_2CO_3 (1.1 mmol), and the compound **(1)** (1.0 mmol).

Synthesis of 2-[(1,3-benzothiazol-2-yl)sulfanyl]-*N*-[4-(hydrazinecarbonyl)phenyl]acetamide **(3)**

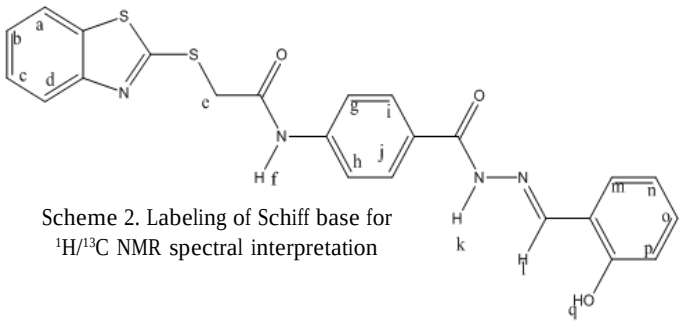
S-alkylated-2-mercaptobenzothiazole **(2)** (1.0 mmol) was dissolved in absolute ethanol (20 mL). Then hydrazine hydrate (80 %, 1.2 mmol) was added and subsequently the reaction mixture was refluxed with continuous stirring for 11-12 h. After completion of the reaction, monitored by TLC, the reaction mixture was poured in cold distilled water and re-stirred for 10-15 min. The resulting white colored precipitates formed were filtered washed with water, dried, and re-crystallized from ethanol (Yield, 58-60 %).

Synthesis of Schiff base ligand **(4)**

To the equimolar mixture of hydrazide **(3)** (1.0 mmol) and salicylaldehyde (1.0 mmol) in absolute ethanol (10 mL), 1-2 drops of glacial acetic acid were added and then refluxed the mixture with continuous stirring for 4-5 h. The precipitates formed during the reaction were filtered while hot and washing was also done by using hot ethanol. The product obtained was dried and re-crystallized from ethanol (Yield, 70-72 %).

General method for the synthesis of Schiff base complexes with various transition metal ions

To the hot ethanolic solution of the ligand (1 mmole, 0.462 g), was added drop wise, the warm ethanolic solution of metal acetates (1.0 mmol) and the contents were refluxed with continuous stirring 4–5 h [26]. The resulting solids were isolated by filtration, washed with hot ethanol, and then dried. These complexes were stored in desiccator for further use.



Scheme 2. Labeling of Schiff base for $^1H/^{13}C$ NMR spectral interpretation

SCHIFF BASE: Molecular formula; $C_{23}H_{18}N_4O_3S_2$, White solid, Yield: 71 %, m.p.: 233-235°C, Molecular weight: 462.54 g/mole.

FTIR (cm^{-1}): 1147, ν(C-N-C); 1639, ν(C=N); 1655, ν(C=O) Amide; 2850, ν(CH_2); 3320, ν(OH).

^{1}H NMR (300 MHz, DMSO-δ): (scheme 2) 4.46 (2H, s, He); 6.90-6.95 (2H, m, Hp and Hn); 7.28-7.55 (4H, m, Hb, Hc, Hm and Ho); 7.75 (2H, d, J = 8.7 Hz, Hi and Hj); 7.83 (1H, d, J = 8.1 Hz, Ha); 7.94 (2H, d, J = 8.4 Hz, Hg and Hh); 8.04 (1H, d, J = 7.8 Hz, Hd); 8.63 (1H, s, Hl); 10.77 (1H, s, Hf); 11.33 (1H, s, Hq); 12.05 (1H, s, Hk).

^{13}C NMR (75 MHz, DMSO-δ): 36.58 (-CH_2); 116.89-142.69 (All aromatic carbons); 148.39 (C=N); 158.02 (C-OH); 162.71 (C=O); 163.89 (C-S) and 168.46 (-C=O).

Mass Spectra: m/z 461 $[M-H]^+$

SCHIFF BASE-Mn(II) complex: Molecular formula; $C_{46}H_{44}MnN_8O_6S_4$, Dark brown solid, Yield: 73%, Decomposed above 300°C, Molecular weight: 978.01 g/mole, % metal for $[M(L)_2]$: Theoretical/Experimental: 5.62/4.99. **FTIR (cm^{-1}):** 1150, ν(C-N-C); 1598, ν(C=N); 1650, ν(C=O) Amide; 2800, ν(CH_2); 501, ν(M-N); 489, ν(M-O).

^{1}H NMR (300 MHz, DMSO-δ): 4.30 (2H, s, He); 6.95-7.95 (12H, m, Ha, Hb, Hc, Hd, Hg, Hh, Hi, Hj, Hm, Hn, Ho and Hp); 8.33 (1H, s, Hl); 10.53 (1H, s, Hf); 11.95 (1H, s, Hk).

SCHIFF BASE–Co(II) complex: Molecular formula; $C_{46}H_{34}CoN_8O_6S_4$, Green solid, Yield: 65 %, Decomposed above 300°C, Molecular weight: 982.01 g/mole, % metal for $[M(L)_2]$: Theoretical/Experimental: 6.00/5.56.

FTIR (cm^{-1}): 1148, ν(C-N-C); 1600, ν(C=N); 1649, ν(C=O) Amide; 2812, ν(CH_2); 467, ν(M-N); 409, ν(M-O).

^{1}H NMR (300 MHz, DMSO-δ): 4.47 (2H, s, Hc); 7.12-8.05 (12H, m, Ha, Hb, Hc, Hd, Hg, Hh, Hi, Hj, Hm, Hn, Ho and Hp); 8.55 (1H, s, Hl); 10.38 (1H, s, Hf); 11.95 (1H, s, Hk).

SCHIFF BASE–Ni(II) complex: Molecular formula; $C_{46}H_{34}NiN_8O_6S_4$, Green solid, Yield: 62 %, Decomposed above 300°C, Molecular weight: 981.76 g/mole, % metal for $[M(L)_2]$: Theoretical/Experimental: 5.98/5.45. **FTIR (cm^{-1}):** 1152, ν(C-N-C); 1603, ν(C=N); 1650, ν(C=O) Amide; 2800, ν(CH_2); 460, ν(M-N); 409, ν(M-O).

^{1}H NMR (300 MHz, DMSO-δ): 4.44 (2H, s, Hc); 6.92-8.01 (12H, m, Ha, Hb, Hc, Hd, Hg, Hh, Hi, Hj, Hm, Hn, Ho and Hp); 8.46 (1H, s, Hl); 10.77 (1H, s, Hf); 12.02 (1H, s, Hk).

SCHIFF BASE–Cu(II) complex: Molecular formula; $C_{46}H_{34}CuN_8O_6S_4$, Dark green solid, Yield: 51 %, m.p.: Decomposed above 300°C, Molecular weight: 985.08 g/mole, % metal for $[M(L)_2]$: Theoretical/Experimental: 6.44/6.40. **FTIR (cm^{-1}):** 1149, ν(C-N-C); 1599, ν(C=N); 1645, ν(C=O) Amide; 2827, ν(CH_2); 497, ν(M-N); 441, ν(M-O). **^{1}H NMR (300 MHz, DMSO-δ):** 4.40 (2H, s, He); 6.95-7.95 (12H, m, Ha, Hb, Hc, Hd, Hg, Hh, Hi, Hj, Hm, Hn, Ho and Hp); 8.53 (1H, s, Hl); 10.43 (1H, s, Hf); 11.99 (1H, s, Hk).

SCHIFF BASE-Zn(II) complex: Molecular formula; $C_{25}H_{20}ZnN_4O_5S_2$, Yellow solid, Yield: 68 %, Decomposed above 300°C, Molecular weight: 586.05 g/mole, % metal for

[M(L)(OAc)]: Theoretical/Experimental: 11.16/10.89. **FTIR (cm^{-1}):** 1153, ν(C-N-C); 1597, ν(C=N); 1651, ν(C=O) Amide; 2912, ν(CH_2); 452, ν(M-N); 415, ν(M-O).

^{1}H NMR (300 MHz, DMSO-δ): 1.90 (3H, s, CH_3COO); 4.30 (2H, s, He); 6.38-8.14 (12H, m, Ha, Hb, Hc, Hd, Hg, Hh, Hi, Hj, Hm, Hn, Ho and Hp); 8.53 (1H, s, Hl); 10.43 (1H, s, Hf); 12.01 (1H, s, Hk).

Antiinflammatory assay

Luminol-improved chemiluminescence measure was performed, as described in literature [27]. Precisely, 25 µL of sample of blood Hanks Balanced Salt Solution, and 25 µL of 3 different concentrations of the tested compounds (1, 10 and 100 µg/mL), was incubated. Control wells got Hanks Balanced Salt Solution without any compound. Test was performed in half zone (white) 96-well plates [Costar, NY, USA], which was hatched for 15 min at 37°C, in the indoor regulator chamber of luminometer. When incubation has done, 25 µL of serum opsonized zymosan (SOZ) and 25 µL of intracellular responsive oxygen species identifying test, luminol were included into each well, away from clear wells (having just HBSS++). The level of the ROS was noted in luminometer in term of light units (RLU). The reference drug utilized for comparison is Ibuprofen having IC_{50} = 11.2 ± 1.9.

Anticancerous assay

Anticancer activity of the compounds having different concentration (1-30 µM) was assessed in 96 well level bottomed micro plates utilizing the reported standard MTT (3-[4,5-dimethyl-2-yl]-2,5-diphenyltetrazoliumbromide) colorimetric assay [28].

Molecular Docking studies

Molecular docking studies have been carried out to anatomize the interactions between DNA-Ligand using AutoDock v4.2 and AutoDock tools v1.5.6 [29]. To determine best possible conformation of all synthesized metal complexes in DNA, molecular docking was carried out. DNA crystal structure was downloaded from RCSB Protein Data Bank having PDB ID1BNA. Prior to docking target metal complexes were prepared 3D optimized. Hydrogen atoms were added to DNA structure and a grid box of 70×70×110 dimensions was applied. Moreover, Lamarckian Genetic Algorithm was used which is a type of stochastic genetic algorithm and provides a greater degree of freedom for ligands in docking. A set of 100 different poses were retrieved and the best possible was then subjected to visualization for analysis of interactions between the DNA and metal ion complexes. To explore the most favorable interactions between metal complexes and cyclooxygenase isoenzymes (COX-1 and 2) for the determination of anti-inflammatory potential, molecular docking studies was carried out following the same procedure as mentioned above except the specified dimensions. PDB ID used for COX-1 and 2 was 1CQE and 3LN1 respectively [30, 31]. In this case blind docking was performed by considering the whole protein inside grid box for docking purpose.

Results and discussions

The ligand was synthesized as outlined in scheme 1. The analytical data of the ligand and its complexes is shown in the experimental section. The complexes thus obtained were synthesized by refluxing the acetates of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) with ligand in ethanol-DMSO mixture. The obtained complexes are soluble in DMSO and completely insoluble in all other organic solvents. All of these complexes are stable solid at room temperature. The molar conductance values of the metal complexes in the range 2.50-9.20 µS/cm indicates that these are non-electrolytic in nature.

FT-IR

The coordination of the ligand to the metal was studied by comparing the FTIR spectra of the complexes with that of the ligand. FTIR spectrum of the Schiff base showed the absence of bands at 1736 cm^{-1} and 3424 cm^{-1} due to carbonyl and ν(NH_2) stretching vibrations, instead it exhibited a new band at 1639 cm^{-1} that can be attributed to azomethine ν(HC=N) linkage. The comparison of the IR spectrum of the Schiff base and its metal complexes confirmed the coordination of the ligand to the metal ions in a tridentate manner. The peak at 1639 cm^{-1} in the Schiff base spectrum, shifted slightly to the lower value in all the ligand-complexes proposing the involvement of C=N moiety in coordination with metal ions [32]. Secondly, the absorption due to O=C–NH changed in the spectra of metal complexes indicating that carbonyl group of amide portion is also involved in coordination with metal ions. Absence of stretching frequency for OH group in all metal complexes reveals the deprotonation phenomenon and involvement in coordination. Moreover, some new strong absorption bands appeared in the range 460-497 cm^{-1} and 409-489 cm^{-1} which may be assigned to ν(M-N) and ν(M-O) respectively.

^{1}H-NMR

The ^{1}H NMR spectrum of the ligand exhibited a singlet at 12.05 ppm due –OH group, another singlet at 8.63 ppm attributed to azomethine proton CH=N. The multiplet in the region 6.90-8.04 ppm can be attributed to aromatic protons. The appearance of these chemical shifts are in agreement with the proposed structure of the ligand. In the ^{1}HNMR spectra of the ligand-complexes, the aromatic protons have been resonated in the region 6.34–8.82 ppm as multiplets which is slightly different than that of the ligand. The disappearance of the signal due to OH group in all the metal complexes indicates coordination of -OH group after deprotonation with the metal ions [33-35]. The HC=N proton signals have also been shifted to downfield region in the metal complexes thus confirming the involvement of HC=N moiety in coordination with metal ions.

^{13}C-NMR

^{13}C NMR of the ligand was recorded and the chemical shifts appeared are given in experimental section. The significant chemical shifts at 36.58 ppm (-**C**H_2), 116.89-142.69 ppm (All aromatic carbons), 148.39 ppm (**C**=N), 158.02 ppm (**C**-OH), 162.71 ppm (Ph-**C**=O), 163.89 ppm (**C**-S) and 168.46 ppm (CH_2-**C**=O) are in well agreement with the suggested structure of the ligand.

UV/Vis data and Magnetic moment

The electronic absorption spectrum of the ligand and its complexes in DMSO were obtained in UV-visible region. It was observed that all of the complexes show typical metal-ligand charge transfer absorption bands between 365-449 nm, which are ascribed to metal (dπ-M) to ligand (dπ*) charge transfer [36]. For the Cu(II) complex a broad band is observed at 15241 cm^{-1} (656 nm); this band is specific to the Cu(II) ion in octahedral stereochemistry and corresponds to the $^2E_g \rightarrow {}^2T_{2g}$ transition [37]; the magnetic moment value (2.20 B.M.) of Cu(II) complex support octahedral geometry [36]. In the spectrum of the Ni(II)

complex, two bands of absorption at 10374 cm^{-1} (964 nm) and 17385 cm^{-1} (575 nm), are assignable to the transitions $^3A_{2g} \rightarrow {}^3T_{2g}(F)(\nu 1)$ and $^3A_{2g} \rightarrow {}^3T_{1g}(F)(\nu 2)$ respectively; these transitions are characteristic of the Ni(II) ion in an octahedral coordination and the magnetic moment value (3.07 B.M.) for Ni(II) complex supports octahedral environment around it [38]. The Co(II) complex exhibits two bands at 10428 cm^{-1} (959 nm) and 17675 cm^{-1} (565 nm), which correspond to the $^4T_{1g} \rightarrow {}^4T_{2g}(F)(\rightarrow n1)$ and $^4T_{1g} \rightarrow {}^4T_{1g}(P)(\nu 2)$ transitions, respectively; these bands are specific to the Co(II) ion in octahedral stereochemistry; the magnetic moment value of Co(II) complex was found to be (4.21 B.M.) which also reveals that the Co(II) complex has typically octahedral geometry [36]. In the case of the Mn(II) complex, the *d-d* transitions, doubly forbidden from the fundamental term $^6A_{1g}$ towards the quartet terms $^4A_{1g}(G)$; $^4E_g(G)$; $^4T_{2g}(G)$; $^4T_{1g}(G)$; exhibit a very small intensity and are concealed by the intraligand transitions [39]; the geometry of Mn(II) complex is further confirmed by the high μeff value (5.81 B.M.) [40]. The Zn(II) complex did not show any *d-d* transition and its spectrum showed only charge transfer band [38]. As expected Zn(II) complex is diamagnetic; the complex is suggested to be tetracoordinated probably having tetrahedral geometry based on analytical, IR and conductance data [41].

Antiinflammatory activity

The Schiff base ligand and its metal complexes were screened for their antiinflammatory activity and their IC_{50} values were recorded using *Ibuprofen* as reference drug with IC_{50} value 11.2 ± 1.9 µg/mL. The activity was observed in the following order: Complex–Ni(II) > Complex–Mn(II) > Complex–Cu(II) > Complex–Zn(II) > Complex–Co(II) > Ligand. It reveals that all the metal complexes have relatively higher anti-inflammatory activity compared to uncomplexed ligand (table 1).

Anticancerous assay

The activity of the Schiff base ligand and its complexes were also tested in order to know their cytotoxic nature. The anticancer activity was recorded as their IC_{50} (concentration causing 50 % growth inhibition) values for HeLa. The results, obtained for the evaluated compounds are shown in table 2. Results of the ligand and its metal complexes were compared with the cytotoxic activity of *Doxorubicin* used as a standard drug. As shown in table 2, out of the five complexes, three were totally inactive. Interestingly, it is evident that the activity of the ligand decreases after its complexation with Zn(II), Mn(II) and Co(II) ions, while after coordination of the ligand with Cu(II) and Ni(II) ions, activity of the ligand enhanced greatly.

Molecular Docking Simulations

Among several drug targets of anticancer therapy, chemical agents targeting DNA have been known to produce significant effects against cancer and have been very interesting area for medicinal chemists since long. Chemical agents may bind with DNA structure in three possible ways, they may bind to the major groove or to the minor groove of DNA or they may bind across the DNA helix by intercalation as in case of *Doxorubicin*. To rationalize the anticancer potential of our synthesized metal complexes molecular docking was carried out. All metal complexes were found either major groove binders or minor groove binders. However, none of them was observed to intercalate. Binding affinities were calculated for each metal complex and is reported in table 3. Among the metal complexes, Ni(II) containing complex poses highest binding affinity of -11.70 $Kcal.mol^{-1}$ and from visualization it is evident that it binds to the narrow minor groove of the DNA double helix. Docking study of Ni(II) complex also revealed specific types of binding interactions such as the formation of a hydrogen bond between carbonyl oxygen and DC-20 nucleotide of DNA double helix, while sulphur interactions were found to form between the Ni(II) complex and DT-7, DT-8 and DT-20 nucleotide. Additionally, several non-polar interactions were also observed. Selected pose view of the Ni(II) complex inside DNA double helix can be found in figure 1.

Anti-inflammatory effect of the available NSAIDs are primarily mediated by inhibition of cyclooxygenase isoenzymes (COX-1 and 2) involved in synthesis of several mediatory prostaglandins that leads to pain and inflammation. Thus, through molecular docking study antiinflammatory potential of the synthesized metal complexes were invested by studying their binding inside cyclooxygenase isoenzymes. Binding affinities of metal complexes were determined inside COX-1 and 2 crystal

Table 1
ANTIINFLAMMATORY ACTIVITY OF THE LIGAND **(4)** AND ITS COMPLEXES
(Standard drug *Ibuprofen* having IC_{50} value = 11.2 ± 1.9 µg/mL)

Compound	Conc.(µg/mL)	IC_{50} ± SD
Ligand	100,10,1.0 µg/mL	> 100
Mn(II)-complex	100,10,1.0 µg/mL	1.7 ± 0.3
Co(II)-complex	100,10,1.0 µg/mL	23.8 ± 2.3
Ni(II)-complex	100,10,1.0 µg/mL	1.6 ± 0.6
Cu(II)-complex	100,10,1.0 µg/mL	17.2 ± 1.2
Zn(II)-complex	100,10,1.0 µg/mL	20 ± 1.3

Table 2
ANTICANCER ACTIVITY OF THE LIGAND **(4)** AND ITS COMPLEXES
(Standard drug *Doxorubicin* having IC_{50} value = 1.2 ± 0.4 µg/mL)

Compound	Conc.(µg/mL)	% Inhibition	IC_{50} ± SD
Doxorubicin	30 µM	71 %	1.2 ± 0.4
Ligand	30 µg/mL	70 %	22.3 ± 1.9
Mn(II)-complex	-	13 %	Inactive
Co(II)-complex	-	40 %	Inactive
Ni(II)-complex	-	91 %	2.8 ± 0.5
Cu(II)-complex	-	81 %	3.2 ± 1.9
Zn(II)-complex	-	21 %	Inactive

Metal complexes	Binding Free Energy ($Kcal.mol^{-1}$)		
	DNA Binding	COX-1	COX-2
Mn(II)-complex	-10.16	-11.17	-8.84
Co(II)-complex	-9.57	-10.31	-10.89
Ni(II)-complex	-11.70	-11.07	-11.04
Cu(II)-complex	-9.56	-9.78	-10.32
Zn(II)-complex	-5.47	-9.14	

Table 3
BINDING AFFINITIES OF METAL COMPLEXES

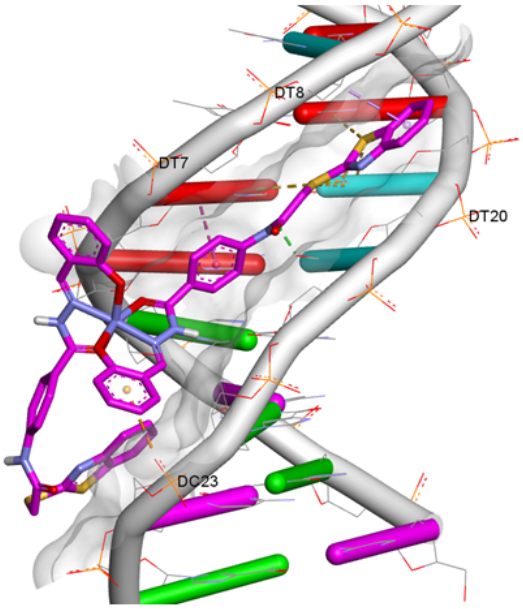


Fig. 1. Selected binding pose of Ni(II) complex with DNA double helix

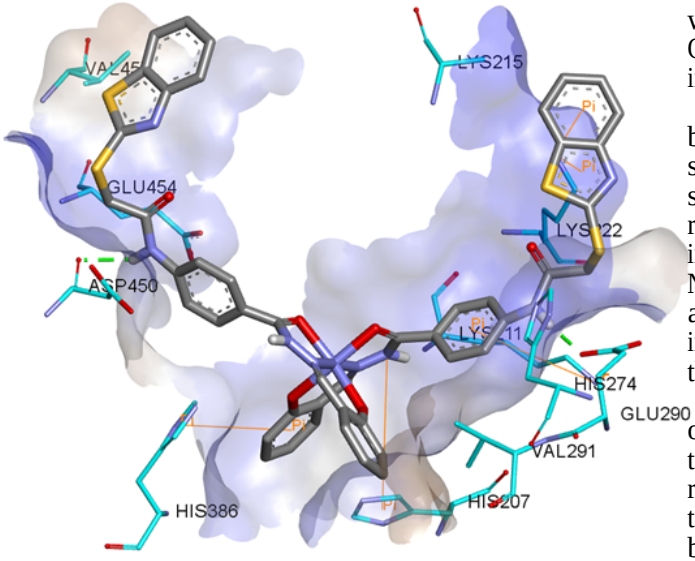


Fig. 2. Selected binding pose of Ni(II) complex with COX-1. Hydrogen bonding and π-charge interactions are shown in green dotted and narrow yellow lines, respectivel

structure. The synthesized complexes were found to be very large and could not be accommodated inside the active pocket defined by the presence reference ligands *Ibuprofen* and *Celecoxib* inside COX-1 and COX-2 respectively. Therefore, blind docking was carried in both cases and binding free energies were calculated. Similar to the in-vitro assay results, Ni(II)-complex and Mn(II)-complex poses highest binding affinity against COX-1 enzyme. However, in case of COX-2, Mn(II)-complex poses

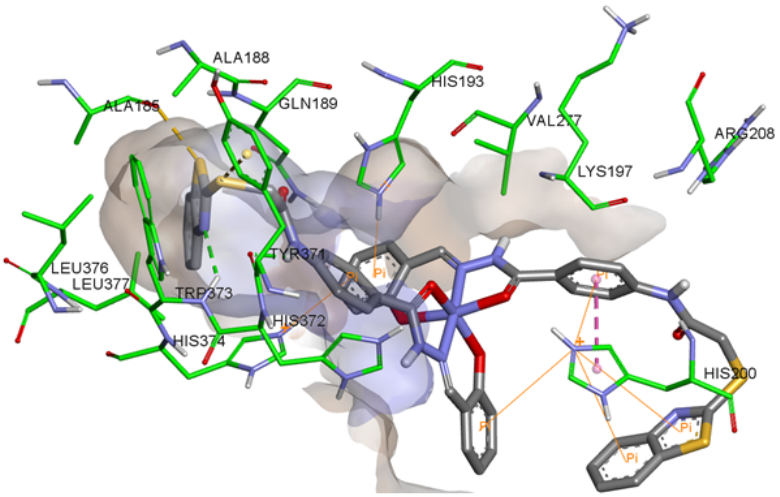


Fig. 3. Selected binding pose of Ni(II) complex with COX-2. Hydrogen bonding, sulphur, π-stacking and π-charge interactions are shown in dotted green, yellow, pink and narrow yellow lines, respectively

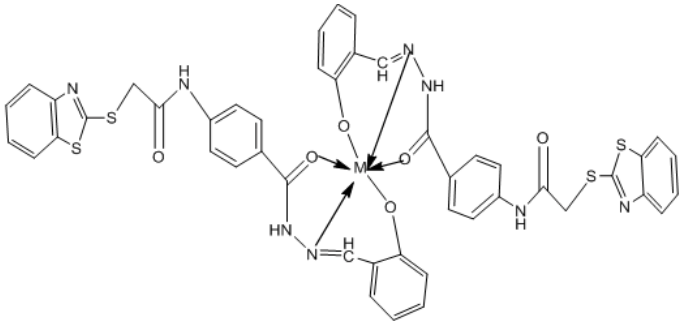
relatively lower binding affinity while Ni(II)-complex binds with approximately same potential as it does in case of COX-1 enzyme. Binding affinities of metal complexes is included in table 3.

Inside COX-1, the Ni(II) complex forms hydrogen bonding interactions with residue Asp450, while on other side it form same interaction with residue Glu290. π-π staking interaction where observed between one benzene ring of Ni(II) complex with residue His386 while π-charge interactions where observed between ring structures of Ni(II) complex and residues Lys211 and Lys222. Amino acid residue of His207 was also observed to form π-charge interaction –NH group of Ni(II) complex. Binding pose of the Ni(II) complex can be found in figure 2.

In case of COX-2, the Ni(II) complex was found to dock opposite to the site of *Celecoxib* binding site and was found to form hydrogen bonding interactions with -NH group of residue Trp373. Sulphur atom of Ni(II) complex was found to form interactions with residue Ala185 and Tyr371, while benzene groups of the complex forms several π-charge interactions with residue His200 and His374. Binding pose of the Ni(II) complex inside COX-2 can be found in figure 3.

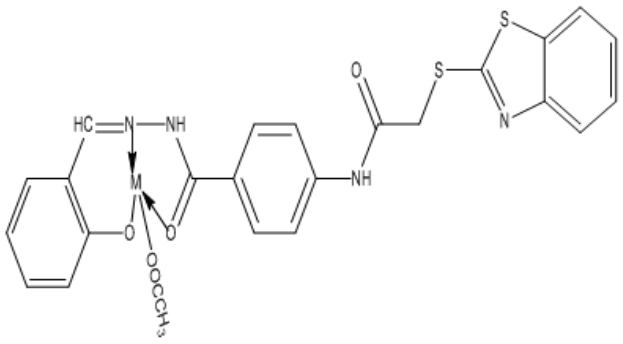
Conclusions

On the basis of structural investigations for Cu(II), Mn(II), Ni(II) and Co(II) octahedral geometry and for Zn(II) complex tetrahedral geometry is proposed in this study (fig. 4, 5). The complexes of the Schiff base ligand were found to be moderate to highly potent anti-inflammatory agents. Similarly, the ligand itself was found to be highly active anticancer agent, moreover its activity increased to a large extent by coordination with Cu(II) and Ni(II) ions.



$M = Cu^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}$

Fig. 4. Proposed structure of metal complexes with the Schiff base ligand



$M = Zn^{2+}$

Fig. 5. Proposed structure of Zn(II) complex with the Schiff base ligand

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