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Synthesis, cytotoxic and urease inhibitory activities of some novel isatin-derived *bis*-Schiff bases and their copper(II) complexes[1]

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Abstract

Several isatin-3-thiosemicarbazones (a class of Schiff bases) from our earlier studies have been validated as promising cytotoxicants and urease inhibitors. Also, a number of isatin-derived imines (Schiff bases) and their Cu(II) complexes have been reported in the literature to exhibit potential cytotoxic activity towards different cells. In view of this, a series of seven new 5-(un)-substituted isatin-derived *bis*-Schiff bases/ligands **3a-g** and their Cu (II) complexes **5a-g** were synthesized and evaluated for cytotoxic and urease inhibitory activities. All the Schiff base ligands **3a-g** proved to be active in sulforhodamine B (SRB) bioassay, displaying promising cytotoxic activity against lung carcinoma (H157) cells. Compound **3b** was found to be the most potent inhibitor of H157 cells, exhibiting IC_{50} value 2.32 ± 0.11 μM. Similarly, all the metal complexes 5**a-g** proved to be active in this assay, demonstrating enhanced cytotoxic activity in each case, occurring as a result of coordination of the Schiff base ligands to metal ion.

[1] The authors declare no competing interests.

Compound **5d** proved to be the most potent inhibitor of H157 cells, showing cytotoxic activity comparable to that of the standard drug, vincristine (VCN) (IC_{50} = 1.29 ± 0.06 vs. 1.03 ± 0.04 μM). In urease inhibition assay, all the synthesized Schiff base ligands except **3f** proved to be highly potent enzyme inhibitors, displaying inhibitory activity even better than the reference inhibitor, thiourea (IC_{50} = 0.04 ± 0.004 − 5.86 ± 0.09 vs. 22.3 ± 1.12 μM) and thus may act as promising lead molecules for further studies. Molecular docking studies were also carried out for the *bis*-Schiff bases **3a-g** to elucidate their relation with the binding pockets of the enzyme.

Keywords: Copper (II) complexes, Cytotoxicity, Isatin, Schiff bases, Urease inhibition

Introduction

Isatin and its derivatives have been reported to exhibit diverse biological activities.[1-14] Amongst isatin derivatives, isatin-imines (Schiff bases) have been found to display a variety of pharmacological properties, such as antimicrobial,[1,2,3,7,9,12,15-18] anticonvulsant,[2,5,7,8,10,12] anticancer,[1,10,19] anti-inflammatory,[1,7,9,12,20] antioxidant,[10,15,21] antiviral,[1,7,12] antiglycation,[12] antileishmanial,[12,22] analgesic[7,9,12] and enzyme inhibitory activities.[4,23,24] Isatin-derived Schiff bases have also been employed as ligands for complexation of metal ions.[1,3]In recent years, the study of the isatin-derived Schiff base metal complexes has received much attention.[25-39] Amongst these, copper(II) complexes of the Schiff base ligands derived from isatin and different amines have received much more attention, mainly because of their potential anticancer properties. Copper(II) complexes of the Schiff bases, obtained by the condensation of different amines and isatin, were prepared and characterized using spectroscopic means.[25] These complexes were found to catalyze the oxidation of common carbohydrates (glucose, fructose and galactose) by molecular oxygen.[25,26] Some of them were also found to exhibit potential antitumor

activity towards different cells, inducing apoptosis through a preferential attack at DNA and/or mitochondria.[27,28] Copper(II) complexes of the Schiff base ligands derived from 5-(un)-substituted isatin and 2,2-diphenylethanamine were also synthesized and characterized by different spectral techniques. Octahedral geometry was proposed around the metal ions for all the complexes.[36] The synthesized free ligands and their copper (II) complexes were evaluated for their cytotoxic and antibacterial activities. All the complexes were found to be cytotoxic to liver cancer cell line (HepG2). It was observed that the metal complexes of the 5-bromo- and 5-nitro-isatin derived imines exhibited excellent cytotoxicity to cancer cells without affecting the normal cells. Similarly, in antibacterial assay, copper (II) complex of the 5-bromoisatin-imine displayed excellent antibacterial activity against all the tested Gram-positive and Gram-negative bacteria. In view of these findings and in continuation of our earlier studies on the synthesis of bioactive isatin derivatives, including certain Schiff bases (thiosemicarbazones, hydrazones, imines) and metal complexes of some of them, exhibiting antibacterial, antifungal, antileishmanial and more importantly, cytotoxic and urease inhibitory activities,[40-50] we have synthesized a series of seven new *bis*-Schiff bases (derived from 5-(un)-substituted isatin and 4-methyl-*m*-phenylenediamine) and their copper(II) complexes, and examined them for their cytotoxic and urease inhibitory potential. This study provides some interesting and exciting results, which are reported herein.

Results and discussion

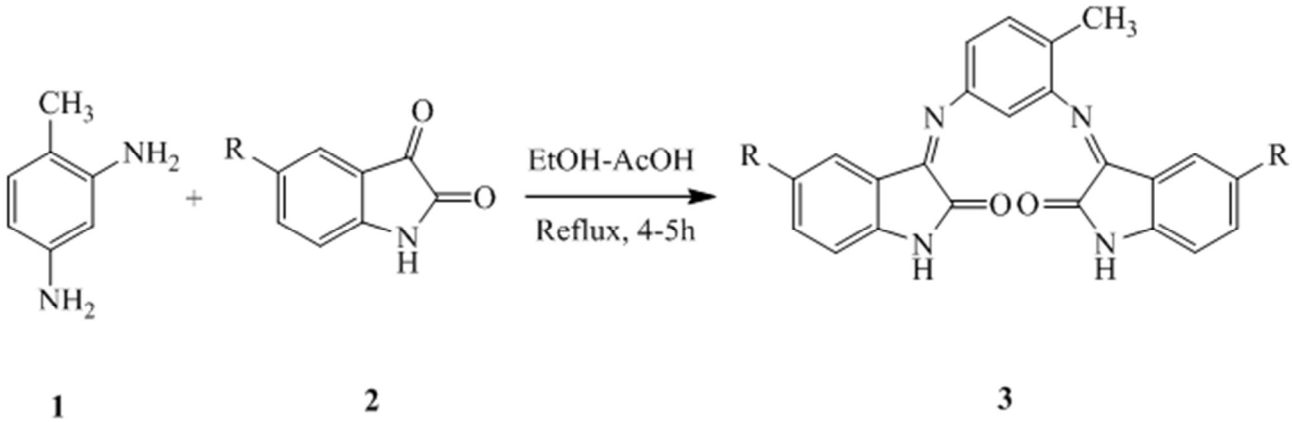
The present work describes the synthesis, characterization and *in vitro* evaluation of cytotoxic and urease inhibitory activities of a series of seven newly synthesized Schiff bases (ligands) **3a-3g** and their Cu(II) complexes **5a-5g**.

Chemistry

Chemistry of the *bis*-Schiff bases 3a-g

4-Methyl-*m*-phenylenediamine **1** and appropriate isatin **2** were dissolved in warm ethanol containing a catalytic amount of glacial acetic acid. The reaction mixture was then refluxed for 4-5 h. The refluxate upon standing at room temperature resulted into a solid, which was separated by suction filtration. Recrystallization from methanol gave the pure target compounds **3a-g** in good to excellent yields (62-93%; without work-up of mother liquors) **(Scheme 1)**.

The structures of the synthesized *bis*-Schiff bases **3a-g** were confirmed by means of their elemental (CHN) and spectral (IR, ^{1}H-NMR, MS) data. The IR spectra of **3a-g** showed bands of NH stretching in the 3027- 3008 cm^{-1} region. The lactam C=O and azomethine C=N stretchings were observed in the 1739-1734 and 1617-1609 cm^{-1} regions, respectively. The ^{1}H-NMR spectra of these compounds exhibited separate or shared singlets at δ 9.96-11.14 for indole NH; the rest protons of indole moieties and that of aryl rings in all the compounds appeared as multiplets in the region δ 6.16-8.12.[51-53] The EI mass spectra of **3a-e** and **3g** showed molecular ions of different intensities, which confirmed their molecular weights. The major fragmentation pattern involved the cleavage of exocyclic C-N and endocyclic NH-CO bonds. Compound **3f** did not show the molecular ion peak in its spectrum. However, the fragments corresponding to imine moiety, formed by C-N bond rupture, confirmed its structure. The proposed fragmentation pattern of **3e** is depicted in **Figure 1**.



1 **2** **3**

R:
3a = H
3b = Br
3c = F
3d = Cl
3e = CH_3
3f = SO_3H
3g = NO_2

Scheme 1. Synthesis of title Schiff bases **3a-g**

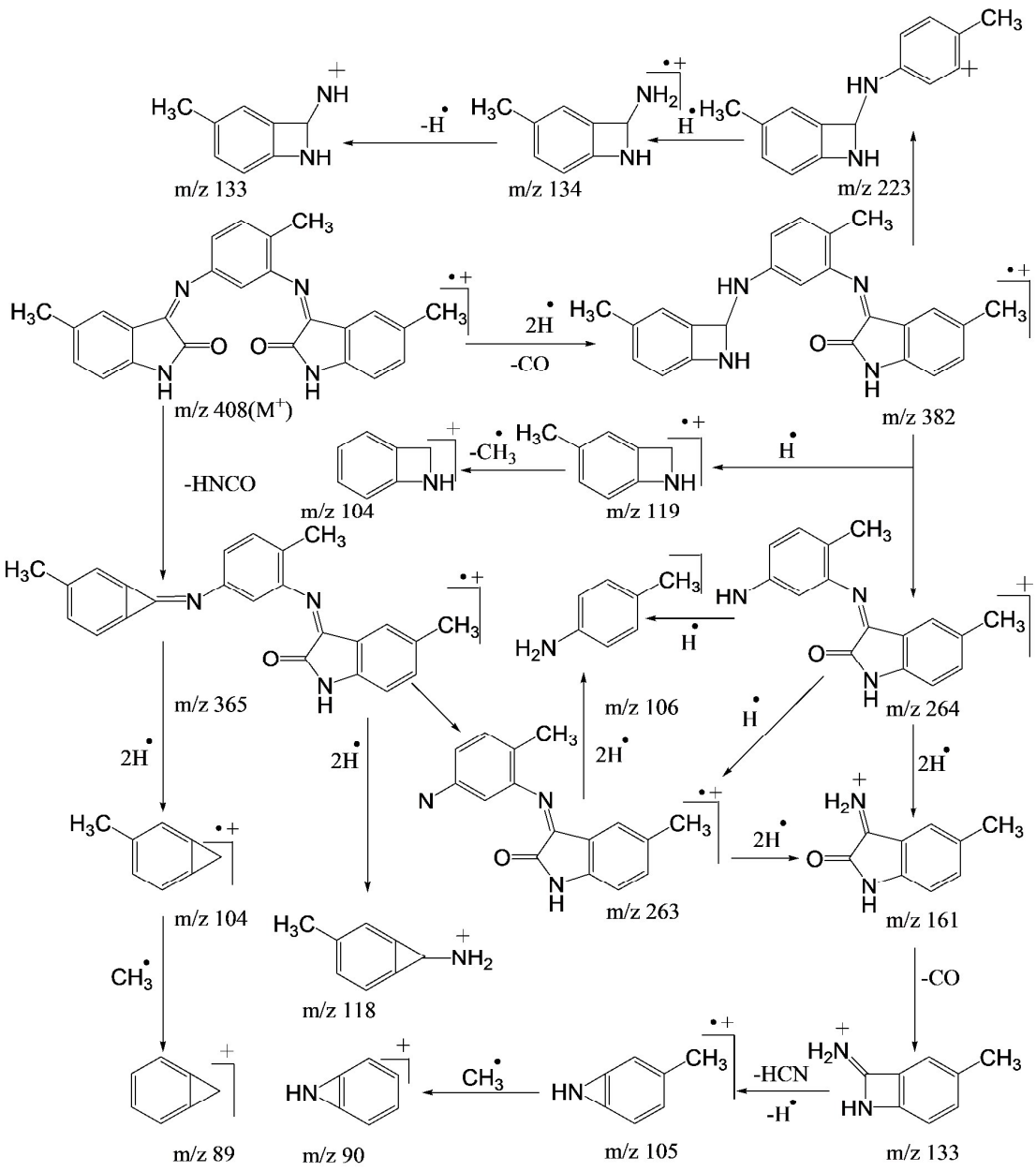


Figure 1. The proposed fragmentation pattern of compound **3e**

Chemistry of the copper (II) complexes 5a-g

Copper complexes of the Schiff base ligands **5a-g** were prepared by stoichiometric reaction of copper chloride salt with the ligands in a molar ratio of 1:2 (**Scheme S1,** see supporting information). All the prepared metal complexes are air stable and colored crystalline solids, and

decompose above 300°C without melting. They are insoluble in common organic solvents but soluble in DMSO and DMF. Their solubility behavior and analytical data proposed that they are all monomers. The molar conductance values (6.01-38.7 Ohm^{-1}, cm^2, mol^{-1}) of the complexes measured in DMSO (1×10^{-3} M solution at 23°C) showed that they are all non-electrolytic in nature.[54] The elemental analysis was also found to be in line with the proposed formulae of the complexes i.e. $[Cu(L)_2Cl_2]$ (**Figure S1**, see supporting information).

IR spectra

The IR spectra of the synthesized ligands **3a-g** showed two main bands in the 1739-1734 and 1617-1609 cm^{-1} regions, resulting from the stretchings of lactam ν(C=O) and azomethineν(C=N), respectively. A comparison of the IR spectra of the ligands and their metal (II) complexes **5a-g** showed that the ligands were principally co-ordinated to metal ion bidentately (**Figure S1**, see supporting information). The band appearing at ~ 1693-1718 cm^{-1} due to lactam ν(C=O) stretching vibration shifted to lower frequency by ~ 20- 41 cm^{-1}, indicating the participation of lactam–O in complexation. Similarly, shifting of the absorption band at ~ 1585-1614 cm^{-1} assigned to azomethine ν(C=N) to lower frequency by ~24-32cm^{-1} indicated the involvement of azomethine-N in complexation.[55,56] The conclusive evidence for the formation of metal complexes was obtained from the appearance of new bands at 515-571 and 418-448 cm^{-1} attributed to M-O and M-N, respectively. These bands were not found to be present in the spectra of the ligands. The expected new IR bands due to Cu-Cl bonds could not be observed because of instrumental limitations. This information was, however, made available by running Raman spectra of the complexes. In the Raman spectra, the frequency at 375-390 cm^{-1} was assigned to Cu-Cl,[57] which confirmed the presence of Cl^- in the coordination sphere (**Figures S2-S5,** See spectra of some representative examples in supporting information).

Magnetic moments

According to Figgis[58], the magnetic moment values give an idea about the geometry of the metal complexes. For Cu(II) complexes, a magnetic moment value greater than 1.9 BM suggests tetrahedral geometry, whereas less than 1.9 BM is indicative of octahedral one. The Cu(II) ion has one unpaired electron in the 3d shell; therefore, its compounds were expected to have magnetic moments close to the spin-only value 1.73 BM but because of spin orbit coupling, higher values are often observed.[59] The room temperature magnetic moments for the solid Cu(II) complexes **5a-g** were found to lie in the range 2.04-2.42 BM, which is slightly higher than the spin-only value, offering the possibility of octahedral environment around the metal ion.[60–62]

Electronic spectra

The electronic spectra of the Schiff base ligands **3a-g** and their Cu (II) complexes **5a-g** were recorded in DMSO. The spectra of Cu (II) complexes **5a-g** displayed three prominent bands; a low intensity broad band around 16026-14728 cm^{-1} and a strong high intensity one at 19083-16584 cm^{-1} were assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2B_2g$, $^2E_{2g}$ electronic transitions, respectively, whereas a medium intensity band at 22988-20492 cm^{-1} merged with that of ligand attributed to L → M charge transfer transition.[63] This pattern of transitions refers to Jahn-Teller distortion in the octahedral geometry.[60,64]

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Thermogravimetric analysis (TGA)

The thermal properties of the synthesized Cu(II) complexes **5a-g** were investigated by thermogravimetric (TG) and differential thermogravimetric (DTG) analyses. Thermograms of the Cu(II) complexes **5a-d** revealed two step degradations, while **5f** and **5e**, **5g** degraded in one and three steps, respectively. Overlaid TG and DTG curves of the Cu(II) complexes **5a-g** are presented in Figure S6 and S7 to elucidate their decomposition patterns. The initial, maximum

and final thermal degradation temperatures (Ti, Tm and Tf, respectively) for first degradation step of compound **5a** were found to be 185, 234 and 305°C, respectively, with 18.37% weight loss. For second degradation step, Ti, Tm and Tf values observed were 328, 395 and 482°C, respectively, with 51.44% weight loss. Char yield was found to be 8.11% at 720°C. All these thermal parameters for compounds **5b-g** were also evaluated for comparison purpose and are shown in Table S1. Significantly high degradation temperature values indicated the thermal stability of the synthesized complexes. Moreover, multistep degradation patterns may reveal the formation of stable intermediates, which too degraded at high temperatures.

Biology

In vitro cytotoxicity (SRB assay) of the *bis*-Schiff bases 3a-g

The anticancer drugs used in chemotherapy are systemic antiproliferative agents, which preferentially kill those cells that are dividing. In the present study, antiproliferative activity of the newly synthesized *bis*-Schiff bases **3a-g** was measured by the cell growth inhibition against lung carcinoma (H157) through SRB assay. Vincristine, a standard anticancer drug, was used as reference for comparison to the test compounds.

From the results presented in Table 1, it is evident that all of our trial compounds possess low cytotoxicity in Vero cells and good anticancer activity against lung carcinoma (H157) cells. Vero cells are normal epithelial cells extracted from African green monkey and used here as control to determine the safety of the anticancer agents. Further, all the compounds displayed activity with slightly different capacity due to their structural diversity in terms of the functional groups attached to C-5 of the isatin scaffold. At 1 mM end concentration in the experiment, the halo-substituted compounds **3b-d** were found to be relatively more potent inhibitors of H157 cells,

displaying IC_{50} values in the range 2.32 ± 0.11 – 2.99 ± 0.15 μM. Amongst these, compound **3b** having electro-withdrawing bromo function at position-5 of the isatin moiety was found to be the most potent one, exhibiting IC_{50} value of 2.32 ± 0.11 μM. This may be explained on the basis of its superior capability (because of having a higher degree of hydrophobicity/lipophilicity) to cross the lipid membrane and bind with the receptor sites. Next most potent inhibitor of H157 cells was compound **3d** with electron-attracting chloro substituent at position-5 of the isatin scaffold, demonstrating IC_{50} value of 2.56 ± 0.27 μM. These findings are not surprising, as similar substitution at position-5 of the isatin moiety has previously been associated with enhanced lipophilicity and cytotoxicity in such compounds.[65] The remainder compounds i.e. **3a**, **3c** and **3e-g** showed less potency towards antiproliferative activity (IC_{50} = 2.99 ± 0.15 – 3.88 ± 0.34 μM).

In conclusion, all the compounds under study exhibited promising cytotoxic activity against the lung carcinoma (H157) cells. Compounds **3b**-**d** as compared to the rest displayed higher activity and could be useful leads for anticancer drug development in the future.

Table 1. Cytotoxicity (SRB assay) of compounds **3a-g** on the H157 and Vero cell lines

Compounds	R	H157 IC_{50} ± SEM* (μM)	Vero (%age inhibition)
3a	H	3.76 ± 0.19	23.6 ± 3.2
3b	Br	2.32 ± 0.11	18.7 ± 2.5
3c	F	2.99 ± 0.15	22.1 ± 3.5
3d	Cl	2.56 ± 0.27	18.5 ± 2.2
3e	CH_3	3.01 ± 0.36	13.4 ± 1.9
3f	SO_3H	3.88 ± 0.34	11.7 ± 1.1
3g	NO_2	3.73 ± 0.49	10.9 ± 0.9
Vincristine (VCN)		1.03 ± 0.04	16.8 ± 1.15

*Values are the mean ±SEM of three experiments

***In vitro* urease inhibition of the *bis*-Schiff bases 3a-g**

The novel Schiff base compounds **3a-g** were evaluated against Jack bean urease *in vitro*. Initially, compounds were screened at a concentration of 10 mM. The compounds, which displayed more than 50% inhibition were selected for further characterization. Thiourea and compound **3a** served as reference points in this assay. All the compounds were found to be potent inhibitors of the enzyme, exhibiting good-excellent inhibitory activity with IC_{50} values ranging from 0.04 ± 0.004 to 25.2 ± 1.34 μM (Table 2). Compound **3b** bearing bromo function at position-5 of the isatin scaffold was the most potent derivative (IC_{50} = 0.04 ± 0.004 μM), while **3f** having sulphonic acid group at the same position of the isatin moiety was found to be the least potent one with an IC_{50} value 25.2 ± 1.34 μM. The results collected in the Table 2 revealed that

compared with the reference compound **3a** (with no substituent in the isatin scaffold), compounds **3b-g** having different inductively electron-withdrawing or –donating groups at position-5 of the isatin moiety exhibited either increased or decreased enzyme inhibitory activity. For example, compound **3b** bearing inductively electron-withdrawing bromo group at position-5 of the isatin scaffold displayed increased inhibitory activity (IC_{50} = 0.04 ± 0.004 μM) as compared to reference compound **3a**, which showed enzyme inhibition with IC_{50} value of 1.15 ± 0.01 μM. Similarly, as compared to **3a**, compounds **3d** and **3e** possessing inductively electron-attracting chloro and electron-donating methyl functions at position-5 of the isatin moiety, respectively, showed enhanced activity with IC_{50} values 0.11 ± 0.003 and 0.34 ± 0.07 μM. Relatively, much pronounced enhancement in enzymatic activity was observed in the cases of **3b** and **3d** (1.15 ± 0.01→ 0.04 ± 0.004 μM and 1.15 ± 0.01 → 0.11 ± 0.003 μM, respectively). On the contrary, as compared to reference point **3a**, compounds **3c**, **3f** and **3g** having fluoro, sulphonic acid and nitro groups at position-5 of the isatin scaffold displayed reduced activity (IC_{50} values 1.65 ± 0.05, 25.2 ± 1.34 and 5.86 ± 0.09 μM, respectively). Relatively, much pronounced reduction occurred in the cases of **3f** and **3g** (IC_{50} values 1.15 ± 0.01 → 25.2 ± 1.34 μM and 1.15 ± 0.01 → 5.86 ± 0.09 μM, respectively).This indicated that substitution of different inductively electron-attracting and electron-donating groups at position-5 of the isatin moiety caused the molecules to intermingle with the enzymatic activity differently, resulting into increment or decrement in their inhibitory potential. In general, the urease inhibitory activity of the Schiff base compounds **3a-g** was found to be dependent upon electronic effects of the substituents attached to C-5 of the isatin moiety.

On the whole, the synthesized novel Schiff bases **3a-g** displayed excellent urease inhibitory activity in the present assay. Of these, compounds **3a-e** and **3g** proved to be highly potent

inhibitors, exhibiting enzymatic activity (IC_{50} = 0.04 ± 0.004 − 5.86 ± 0.09 μM) even better than the standard inhibitor, thiourea (IC_{50} = 22.3 ± 1.12 μM) and may act as promising leads for further studies. Compound **3f** also proved to be potent inhibitor, showing enzymatic activity comparable to thiourea (IC_{50} = 25.2 ± 1.34 vs. 22.3 ± 1.12 μM). These compounds, having shown low or non-significant cytotoxicity, could be potential candidates for orally effective therapeutic agents to be used for the treatment of certain clinical conditions introduced by different microorganisms like *H. pylori*.

Table 2. Inhibition of Jack bean urease by compounds **3a-g**

Compounds	R	IC_{50} ± SEM (μM)
3a	H	1.15 ± 0.01
3b	Br	0.04 ± 0.004
3c	F	1.65 ± 0.05
3d	Cl	0.11 ± 0.003
3e	CH_3	0.34 ± 0.07
3f	SO_3H	25.2 ± 1.34
3g	NO_2	5.86 ± 0.09
Thiourea*		22.3 ± 1.12

*Reference inhibitor of urease enzyme

***In vitro* cytotoxicity (SRB assay) of the copper (II) complexes 5a-g**

All the synthesized Cu (II) complexes **5a-g** were also evaluated for their antiproliferative activity by the cell growth inhibition against lung carcinoma (H157). The results collected in Table 3 revealed that coordination of the Schiff base ligands **3a-g** to metal ion caused enhancement in

their activity (IC_{50} = 2.32 ± 0.11 − 3.88 ± 0.34 μM → IC_{50} = 1.29 ± 0.06 – 2.87 ± 0.18 μM). Much enhancement in the activity was observed in the cases of **5a-d** (IC_{50} = 3.76 ± 0.19→ 1.93 ± 0.07 μM, 2.32 ± 0.11→ 1.54 ± 0.04 μM, 2.99 ± 0.15→ 1.47 ± 0.03 μM and 2.56 ± 0.27 → 1.29 ± 0.06 μM, respectively). Of these, compound **5d** with electron-withdrawing chloro group at position-5 of the isatin scaffold, despite being less lipophilic / permeable than **5b**, exhibited a higher degree of cytotoxicity (IC_{50} = 1.29 ± 0.06 vs. 1.54 ± 0.04 μM) against lung carcinoma (H157) cells. Although, there is no ready explanation for this inconsistency in activity, it may be related to caspase-dpendent differential induction of apoptosis caused by the recently reported isatin-Schiff base copper(II) complexes, obtained from isatin and 1,3-diaminopropane or 2-(2-aminoethyl)pyridine: (Cu(isapn)) and ($Cu(isaepy)_2$), respectively, in human neuroblastoma SH-SY 5Y cells and in other tumor histotypes *via* the mitochondrial pathway and/or copper-dependent oxidative stress. Cu(isapn), although less lipophilic/permeable than $Cu(isaepy)_2$, induced a wide-spread oxidative stress, as demonstrated by analyses of reactive oxygen species concentration, and oxidation of proteins and lipids.[28] In the present study, compound **5d** in comparison to **5b** seems to interact with the enzyme activity differently and more competently, resulting into enhanced cytotoxic activity. Nevertheless, extensive mechanism-based studies are required to contribute to the better understanding of the mechanism of cytotoxic action of our trial compounds. Compounds **5a** and **5c** were the next potent inhibitors, displaying IC_{50} values 1.93 ± 0.07 and 1.47 ± 0.03 μM, respectively. The remaining compounds **5e-g** demonstrated anticancer activity with IC_{50} values 2.32 ± 0.11 − 2.87 ± 0.18 μM but still better than the corresponding ligands, which exhibited cytotoxicity with IC_{50} values ranging from 3.01 ± 0.36 to 3.88 ± 0.34 μM in the present assay.

In conclusion, coordination of the Schiff base ligands to metal ion significantly influenced their antiproliferative activity. This significant effect may be explained on the basis of superior capability of the metal complexes (because of their lipophilic nature) to cross the lipid membrane and bind with the receptor sites. Furthermore, regardless of several years of intensive research, the long-term outlook for patients with aggressive cancer remains discouraging, and there is a need for innovative approach towards the design of anticancer drugs with decreased toxicity and improved efficacy. The present investigations revealed some of the anticancer properties of the newly synthesized isatins-incorporated metal-based compounds **5a-g** with some promising results against lung carcinoma (H157).

Table 3. Cytotoxicity (SRB assay) of compounds **5a-g** on the H157 and Vero cell lines

Compounds	R	H157 IC_{50} ± SEM*(μM)	Vero (%age inhibition)
5a	H	1.93 ± 0.07	24.5 ± 2.1
5b	Br	1.54 ± 0.04	27.4 ± 2.4
5c	F	1.47 ± 0.03	28.9 ± 2.7
5d	Cl	1.29 ± 0.06	27.3 ± 2.3
5e	CH_3	2.87 ± 0.18	29.1 ± 3.5
5f	SO_3H	2.32 ± 0.05	25.8 ± 2.9
5g	NO_2	2.67 ± 0.23	27.7 ± 1.8
Vincristine(VCN)		1.03 ± 0.04	16.8 ± 1.15

*Values are the mean ± SEM of three experiments

***In vitro* urease inhibition of the copper (II) complexes 5a-g**

The synthesized Cu (II) complexes **5a-g** were evaluated for their urease inhibitory activity against Jack bean urease. In general, coordination of the novel *bis*-Schiff base ligands **3a-g** to metal ion was found to cause extensive decrement in the enzymatic activity possessed by them (Table 4). For example, metal complexes **5b-d**, **5f** and **5g** exhibited less than 50% enzymatic activity (36.4%, 33.5%, 26.3%, 21.2% and 8.61%, respectively), rendering them almost inactive, whereas the corresponding ligands **3b-d, 3f** and **3g** demonstrated high inhibitory activity with IC_{50} values 0.04 ± 0.004 μM, 1.65 ± 0.05 μM, 0.11 ± 0.003 μM, 25.2 ± 1.34 μM and 5.86 ± 0.09 μM, respectively. Similarly, coordination of the ligand **3a** with Cu (II) was found to decrease its activity (IC_{50} = 1.15 ± 0.01 → 26.1 ± 0.001 μM). In contrast, the metal complex **5e** showed enhanced inhibitory activity compared with that of the corresponding ligand **3e** (IC_{50} = 0.34 ± 0.07 → 0.03 ± 0.001 μM).

Overall, coordination of the Schiff base ligands to metal ion was found to reduce their enzymatic activity. Out of seven metal complexes tested in the present assay, only one i.e. **5e** displayed enhancement of enzymatic activity occurring as a result of coordination of the corresponding ligand with Cu (II).

Table 4. Inhibition of Jack bean urease by compounds **5a-g**

Compounds	R	IC_{50} ± SEM (μM)/ % Inhibition
5a	H	26.1 ± 0.001
5b	Br	36.4%
5c	F	33.5%
5d	Cl	26.3%
5e	CH_3	0.03 ± 0.001
5f	SO_3H	21.2%

5g	NO_2	8.61%
Thiourea*		22.3 ± 1.12

*Reference inhibitor of urease enzyme

Putative compound binding mode

The structures of the substituted Schiff base ligands were docked to the crystallographic structure of Jack bean urease. The structure with PDB ID: 4H9M was chosen because of its high resolution (1.5 Å). Figure 2 reports a prioritized binding mode model of the ligand **3b**. Compound **3b** was selected for docking, as it was the most potent ligand identified. The benzene ring with substituents attached was located towards the bottom of the binding pocket. The indolinone 2-carbonyl group was in hydrogen bond distance to the amino acid residue His593. Another possible hydrogen bond that could stabilize the binding mode was observed towards the backbone amino group of the residue Arg439. The benzene ring attached to N atoms of the imine (Schiff base) moiety was positioned near the flap region created by the methyl function of the amino acid residue Ala440.The indolinone benzene ring bearing a bromo substituent was flanked at one end of the molecule towards the amino acidsHis593, Leu589, Arg609, His492 and Met637, while at the other end, it was in close contact with the amino acid residues Ile411 and Gln635, as shown in Figure 2. The centre of the compound was found to be oriented towards the amino acid Arg439 and showed π-π interactions by the benzene ring.

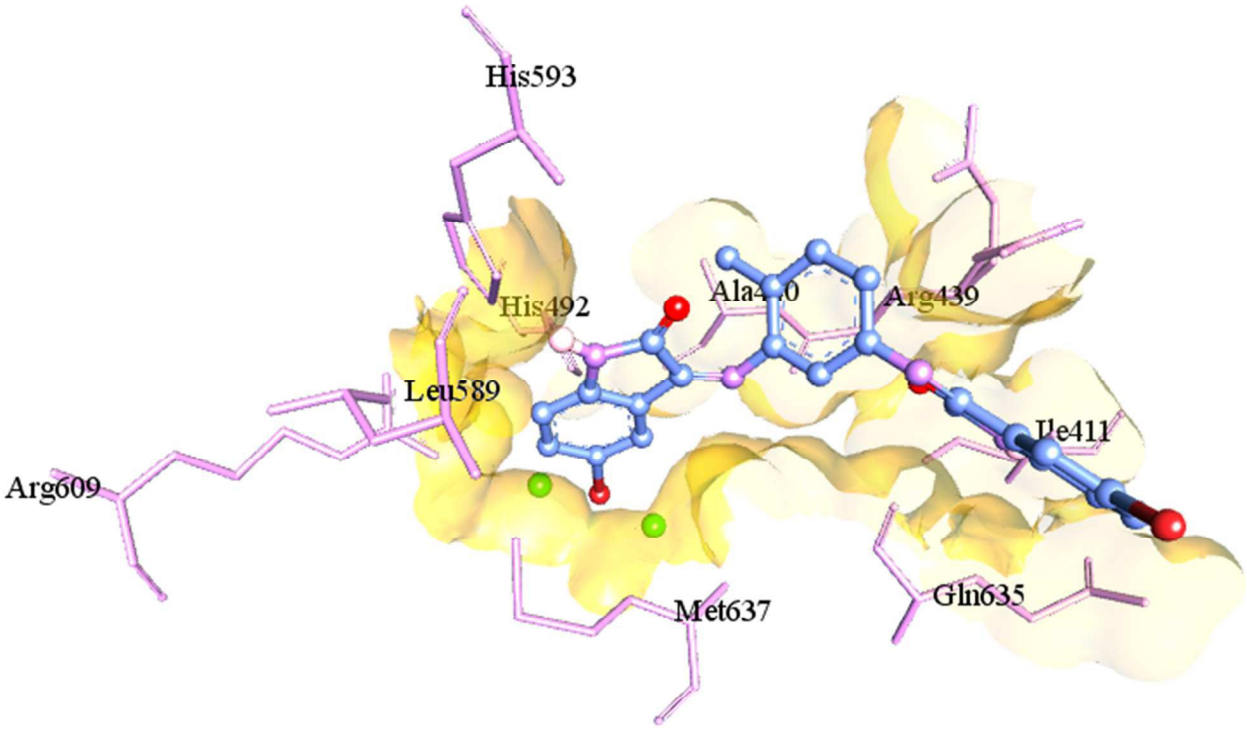

Figure 2. Putative binding mode: Compound **3b** (most active) bound to active site of Jack bean urease is shown. Carbon atoms of **3b** are colored grayish blue and that of protein light pink. The two nickel ions in the active site are represented as small green spheres. The solvent-accessible protein surface is displayed in golden color.

Conclusions

Conclusively, we have synthesized some novel 5-(un)-substituted isatin-derived *bis*-Schiff bases **3a-g** and their Cu (II) complexes **5a-g**.The newly synthesized Schiff bases act as bidentate ligands coordinating to metal ion through azomethine-N and lactam-O. Distorted octahedral geometries have been proposed for the complexes with the help of spectral and other data. In biological screening, all the Schiff base ligands and their metal complexes have been found to be active against lung carcinoma (H157) cells, showing good cytotoxic activity. The activity is

found to be significantly enhanced upon coordination in all the cases. Similarly, all the Schiff bases except **3f** exhibited much high antiurease activity, even better than the reference inhibitor, thiourea. These compounds may act as valid leads for further studies. In contrast to cytotoxicity assay, coordination of the Schiff base ligands to metal ion caused decrement in the urease inhibitory activity of all the complexes except **5e**. To our knowledge, such a group of Schiff bases and their metal complexes have been scarcely studied previously for antiurease activity. The structure-activity relationship (SAR) studies revealed the important role of the electronic effects of the substituents attached to position-5 of the isatin scaffolds of the Schiff base ligands and the metal ion in increasing or decreasing the biological activities of the trial compounds. To understand the binding mechanism, molecular docking studies were performed for ligands **3a-g**, which elucidated the relation of the synthesized series with the binding pockets of urease.

Experimental

General procedure

Chemical materials and solvents were obtained from Merck-Schuchatdt, Fluka and Sigma-Aldrich. Melting points were determined on a Fisher-Johns melting point apparatus and are not corrected. Elemental analyses were performed by using a Leco CHNS-9320 (USA) elemental analyzer. The data obtained from all the synthesized compounds were found to be satisfactory. Ultra-violet spectra were recorded on Spectro UV-Visible double beam Pc scanning spectrophotometer UVD-2950 LABOMED, INC. FT-IR spectra (KBr) were run on a Shimadzu 8400 or Thermo Scientific Nicolet 6700 FTIR spectrophotometer using ATR facility. Raman spectra were taken on Andor DV420-OE Raman spectrophotometer. ^{1}H-NMR spectra were recorded in C_2D_6SO on Bruker (Rhenistetten-Forchheim, Germany) AM 300 spectrometer,

operating at 300 MHz and using Si $(CH_3)_4$ as an internal standard. Mass spectra were obtained on JEOL JMS-600H, MASPEC system [msw/A091] and agilent 6310 ion trap LC/MS mass spectrometers. Molar conductance of the metal complexes was measured with conductivity meter 4510, Jenway (USA).Magnetic susceptibility measurements of the metal complexes in the solid state were determined with magnetic susceptibility balance Sherwood (Auto, 2005) at room temperature. Thermal decomposition of the synthesized metal complexes was carried out on SDT Q600 thermal analyzer (TA instruments, USA).Thermograms were recorded from ambient temperature to 1000 °C at a heating rate of 10 °C min^{-1} under nitrogen, flowing at a rate of 100 cm^3 min^{-1}. Initial, maximum and final thermal degradation temperatures (Ti, Tm and Tf, respectively) along with weight loss % and char yield % values of the samples were obtained from derivative thermogravimetric (DTG) curves using Universal Analysis 2000 software v 4.2E. Purity of all the synthesized compounds was checked by TLC using glass plates coated with Merck silica gel G-60 $GF_{254.}$ The spots were visualized under ultraviolet light at 254 and 366 nm and / or spraying with iodine vapors.

Synthesis

Typical procedure for the preparation of *bis*-Schiff bases

4-Methyl-*m*-phenylenediamine **1** (1.83g, 0.015 mol) and 5-methylisatin **2** (4.83g, 0.030 mol) were dissolved in warm ethanol (90 mL) containing a few drops of glacial acetic acid. The reaction mixture was then heated under reflux for 4-5 h. After standing at room temperature, the solid obtained was separated by suction filtration and recrystallized from methanol to furnish pure (3*Z*)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(5-methyl-1,3-dihydro-2*H*-indol-2-one) **3e** as dark purple crystals (5.32g, 87%); m.p. 313-315 °C; IR (KBr, cm^{-1}): 3027 (NH stretching), 1737 (C=O), 1616 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.51 (s, 9H, indole CH_3, phenyl $CH_{3,}$), 6.16-

7. 41 (m, 9H, indole C_4-H, C_6-H, C_7-H, phenyl C_2-H, C_5-H, C_6-H), 10.60, 10.91 (2s, 2H, indole NH); EIMS (70eV) *m/z* (%):410 (M^+ + 2, 5), 408 (M^+, 9), 390 (11), 383 (24), 382 (89), 381 (7), 366 (7), 365 (10), 161 (37), 134 (10), 133 (100), 106 (25), 105 (17), 104 (72), 78 (46), 77 (29).$C_{25}H_{20}N_4O_2$ (408).Calcd.: C 73.53, H 4.90, N 13.73. Found: C 73.54, H 4.94, N 13.70.

The following compounds were similarly prepared:

(3*Z*)-3, 3′-[(4-methyl-1, 3-phenylene)dinitrilo]*bis*(1,3-dihydro-2*H*-indol-2-one) 3a

Reddish brown crystals (4.73g, 83%); m.p. 318-320 °C; IR (KBr, cm^{-1}): 3008 (NH stretching), 1734 (C=O), 1613 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.50 (s, 3H, CH_3), 6.55-7.59 (m, 11H, indole C_4-H, C_5-H, C_6-H, C_7-H, phenyl C_2-H, C_5-H, C_6-H), 10.99, 11.03 (2s, 2H, indole NH); EIMS (70eV) *m/z* (%): 380 (M^+, 2), 355 (6), 354 (30), 147 (40), 119 (100), 92 (90), 91 (25), 90 (10), 76 (14), 64 (55).$C_{23}H_{16}N_4O_2$ (380).Calcd.: C 72.63, H 4.21, N 14.74.Found: C 72.60, H 4.23, N 14.73.

(3*Z*)-3,3′-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(5-bromo-1,3-dihydro-2*H*-indol-2-one) 3b

Brown crystals (6.62g, 82%); m.p. 326-328 °C; IR (KBr, cm^{-1}): 3008 (NH stretching), 1738 (C=O), 1609 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.51 (s, 3H, CH_3), 6.27-8.12 (m, 9H, indole C_4-H, C_6-H, C_7-H, phenyl C_2-H, C_5-H, C_6-H), 10.76, 11.14 (2s, 2H, indole NH); EIMS (70eV) *m/z* (%): 540 (M^+ + 2, 4), 538 (M^+, 6), 514 (33), 513 (17), 511 (100), 434 (7), 333 (13), 331 (16), 252 (19), 227 (24), 225 (22), 199 (67), 197 (73), 172 (9), 171 (14), 170 (14), 169 (14), 91 (8), 90 (33), 83 (9), 75 (15), 64 (54).$C_{23}H_{14}Br_2N_4O_2$ (538).Calcd.: C 51.30, H 2.40, N 10.41. Found: C 51.33, H 2.44, N 10.41.

(3*Z*)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(5-fluoro-1,3-dihydro-2*H*-indol-2-one) 3c

Reddish brown crystals (5.81g, 93%); m.p. 315-317 °C; IR (KBr, cm^{-1}): 3019 (NH stretching), 1737 (C=O), 1609 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.49 (s, 3H, CH_3), 6.63-7.78 (m, 9H,

indole C_4-H, C_6-H, C_7-H, phenyl C_2-H, C_5-H, C_6-H), 9.96, 10.52 (2s, 2H, indole NH); EIMS (70eV) *m/z* (%): 418 (M^+ +2, 100), 391 (2), 312 (2), 289 (5), 271 (98).$C_{23}H_{14}F_2N_4O_2$ (416).Calcd.: C 66.35, H 3.37, N 13.46. Found: C 66.30, H 3.34, N 13.50.

(3*Z*)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(5-chloro-1,3-dihydro-2*H*-indol-2-one) 3d

Brownish black crystals (5.44g, 81%); m.p. 320-322 °C; IR (KBr, cm^{-1}): 3020 (NH stretching), 1738 (C=O), 1609 (C=N); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.50 (s, 3H, CH_3), 6.26-7.62 (m, 9H, indole C_4-H, C_6-H, C_7-H, phenyl C_2-H, C_5-H, C_6-H), 10.60, 11.14 (2s, 2H, indole NH); EIMS (70eV) *m/z* (%): 450 (M^++2, 2), 422 (7), 283 (3), 252 (5), 243 (4), 181 (19), 155 (29), 153 (88), 126 (38), 125 (43), 90 (22), 85 (34), 83 (46), 78 (41), 75 (15), 63 (100), 44 (88).$C_{23}H_{14}Cl_2N_4O_2$ (448).Calcd.: C 61.61, H 3.13, N 12.50. Found: C 61.49, H 3.11, N 12.48.

(3*Z*)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(5-sulphonic acid-1,3-dihydro-2*H*-indol-2-one) 3f

Shiny black crystals (5.02g, 62%); m.p. 316-318 °C; IR (KBr, cm^{-1}): 3020 (NH stretching), 1739 (C=O), 1616 (C=N), 1365, 1024 (S=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.51(s, 3H, CH_3), 6.16 - 7. 41(m, 11H, indole C_4-H, C_6-H, C_7-H, SO_2-OH, phenyl C_2-H, C_5-H, C_6-H), 11.12 (s, 2H, indole NH); EIMS (70eV) *m/z* (%): 432 (2), 424 (7), 422 (13), 330 (12), 304 (15), 302 (16), 286 (20), 283 (9), 239 (8), 169 (36), 167(100), 153 (21), 152 (12), 148 (14), 147 (11), 141 (15), 140 (27), 139 (52), 138 (85), 133 (9), 127 (36), 126 (10), 125 (11), 122 (53), 121 (56), 112 (31), 111 (8), 106 (13), 105 (14), 104 (23), 94 (12), 90 (9), 89 (81), 78 (18), 77 (58), 75 (29).$C_{23}H_{16}N_4O_8S$ (540).Calcd.: C 51.11, H 2.96, N 10.37. Found: C 51.13, H 2.94, N 10.41.

(3*Z*)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(5-nitro-1,3-dihydro-2*H*-indol-2-one) 3g

Dark grey crystals (4.80g, 68%); m.p. 326-328 °C; IR (KBr, cm^{-1}): 3020 (NH stretching), 1739 (C=O), 1617 (C=N), 1456, 1365 (NO_2); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.50(s, 3H, CH_3), 6.30-

7.58 (m, 9H, indole C_4-H, C_6-H, C_7-H, phenyl C_2-H, C_5-H, C_6-H), 10.01, 11.05 (2s, 2H, indole NH); EIMS (70eV) *m/z* (%): 472 (M^+ +2, 60), 436 (20), 426 (25), 407 (58), 360 (90), 340 (80), 315 (100), 296 (38), 274 (40), 259 (18), 239 (18), 222 (17), 180 (18), 130 (10).$C_{23}H_{14}N_6O_6$ (470).Calcd.: C 58.72, H 2.98, N 17.87. Found: C 58.74, H 2.94, N 17.88.

Typical procedure for the preparation of Cu (II) complexes

To a solution of *bis*-Schiff base ligand **3e** (3.26g, 0.008 mol) in hot ethanol (80 mL) was added copper (II) chloride **4** (0.68g, 0.004 mol) dissolved in hot ethanol (80 mL). The reaction mixture was refluxed for 2-3h and then allowed to cool at room temperature. The solid product thus formed was separated by suction filtration, washed with ethanol and dried under vacuum to give pure *bis*-(3*Z*)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(5-methyl-1,3-dihydro-2*H*-indol-2-one)copper(II) chloride **5e** as dark grey crystals (2.58g, 68%); m.p. >300 °C; IR (KBr, cm^{-1}): 3184 (NH stretching), 1707 (C=O), 1605 (C=N), 536 (M-O), 430 (M-N); R (cm^{-1}): 375 (Cu-Cl); UV (DMSO): λ_{max}14881, 18939, 21978 cm^{-1}; Molar conductance (38.7 Ohm^{-1}, cm^2, mol^{-1}); μ_{eff} = 2.38.$C_{50}H_{40}N_8O_4Cl_2Cu$ (950.5).Calcd.: C 63.12, H 4.21, N 11.78. Found: C 63.21, H 4.34, N 11.88.

The following compounds were similarly prepared:

***Bis*-(3*Z*)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(1,3-dihydro-2*H*-indol-2-one)copper(II) chloride 5a**

Dirty green crystals (2.43g, 68%); m.p. >300 °C; IR (KBr, cm^{-1}): 3125 (NH stretching), 1710 (C=O), 1599 (C=N), 544 (M-O), 432 (M-N); R (cm^{-1}): 390 (Cu-Cl); UV (DMSO): λ_{max} 15337, 17699, 21277 cm^{-1}; Molar conductance (10.91Ohm^{-1}, cm^2, mol^{-1}); μ_{eff} = 2.04. $C_{46}H_{32}N_8O_4Cl_2Cu$ (894.5).Calcd.: C 61.71, H 3.58, N 12.52. Found: C 61.79, H 3.58, N 12.62.

***Bis*-(3*Z*)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]*bis*(5-bromo-1,3-dihydro-2*H*-indol-2-one)copper(II) chloride 5b**

Black crystals (3.78g, 78%); m.p. >300 °C; IR (KBr, cm^{-1}): 3008 (NH stretching), 1718 (C=O), 1599 (C=N), 518 (M-O), 418 (M-N); R (cm^{-1}): 390 (Cu-Cl); UV (DMSO): λ_{max} 15337, 18382, 21505 cm^{-1}; Molar conductance (8.23 Ohm^{-1}, cm^2, mol^{-1}); μ_{eff} = 2.32.$C_{46}H_{28}N_8O_4Br_4Cl_2Cu$ (1210.5).Calcd.: C 45.60, H 2.31, N 9.25. Found: C 45.66, H 2.23, N 9.34.

Bis-(3_Z_)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]_bis_(5-fluoro-1,3-dihydro-2_H_-indol-2-one) copper(II) chloride 5c

Dark grey crystals (3.02g, 78%); m.p. >300 °C; IR (KBr, cm^{-1}): 3224 (NH stretching), 1718 (C=O), 1614 (C=N), 545 (M-O), 446 (M-N); R (cm^{-1}): 380 (Cu-Cl); UV (DMSO): λ_{max}15798, 19083, 22727 cm^{-1}; Molar conductance (6.01 Ohm^{-1}, cm^2, mol^{-1}); μ_{eff} = 2.35.$C_{46}H_{28}N_8O_4F_4Cl_2Cu$ (966.5).Calcd.: C 57.11, H 2.90, N 11.59. Found: C 57.22, H 3.01, N 11.62.

Bis-(3_Z_)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]_bis_(5-chloro-1,3-dihydro-2_H_-indol-2-one) copper(II) chloride 5d

Dark brown crystals (3.14g, 76%); m.p. >300 °C; IR (KBr, cm^{-1}): 3176 (NH stretching), 1693 (C=O), 1607 (C=N), 571 (M-O), 436 (M-N); R (cm^{-1}): 375 (Cu-Cl); UV (DMSO): λ_{max}15649, 18832, 21739 cm^{-1}; Molar conductance (23.6 Ohm^{-1}, cm^2, mol^{-1}); μ_{eff} = 2.07.$C_{46}H_{28}N_8O_4Cl_6Cu$ (1030.5).Calcd.: C 53.57, H 2.72, N 10.87. Found: C 53.34, H 2.69, N 10.79.

Bis-(3_Z_)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]_bis_(5-sulphonicacid-1,3-dihydro-2_H_-indol-2-one) copper(II) chloride 5f

Black crystals (3.50g, 72%); m.p. >300 °C; IR (KBr, cm^{-1}): 3101 (NH stretching), 1703 (C=O), 1585 (C=N), 1350, 1151 (S=O), 534 (M-O), 436 (M-N); R (cm^{-1}): 385 (Cu-Cl); UV (DMSO): λ_{max}16026, 18727, 22988 cm^{-1}; Molar conductance (30.2 Ohm^{-1}, cm^2, mol^{-1}); μ_{eff} = 2.42.$C_{46}H_{32}N_8O_{16}S_4Cl_2Cu$ (1214.5).Calcd.: C 45.45, H 2.63, N 9.22. Found: C 45.47, H 2.66, N 9.29.

Bis-(3_Z_)-3,3'-[(4-methyl-1,3-phenylene)dinitrilo]_bis_(5-nitro-1,3-dihydro-2_H_-indol-2-one) copper(II) chloride 5g

Dark grey crystals (2.88g, 67%); m.p. >300 °C; IR (KBr, cm^{-1}): 3224 (NH stretching), 1697 (C=O), 1589 (C=N), 1522, 1448 (NO_2), 569 (M-O), 448 (M-N); R (cm^{-1}): 380 (Cu-Cl); UV (DMSO): λ_{max} 14728, 16584, 20492 cm^{-1}; Molar conductance (6.34 Ohm^{-1}, cm^2, mol^{-1}); μ_{ef} = 2.24.$C_{46}H_{32}N_{12}O_{12}Cl_2Cu$ (1074.5).Calcd.: C 51.37, H 2.61, N 15.64. Found: C 51.79, H 2.58, N 15.62.

Biological assays

(See supporting information)

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Graphical Abstract

Synthesis, cytotoxic and urease inhibitory activities of some novel isatin-derived bis-Schiff bases and their copper (II) complexes

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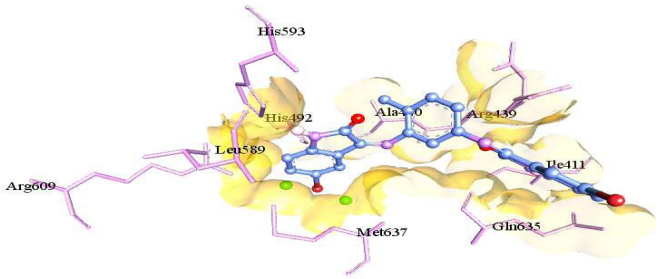

Putative binding mode of most active compound **3b** in the active site of Jack bean urease