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Synthesis, characterization, antimicrobial and antimutagenic activities of *hydroxyphenylimino*ligands and their metal complexes of usnic acid isolated from *Usnea longissima*

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Abstract

Novel multifunctional *hydroxyphenylimino*ligands (L1, L2 and L3) weresynthesized bythecondensation of 2-aminophenol, 3-aminophenol and 4-aminophenol withusnic acid, a lichen metabolite. The synthesized ligands and their Cu(II), Co(II), Ni(II) and Mn(II) complexes were characterized using FT-IR, UV-Vis., ^{1}H-, ^{13}C-NMR, 1D- and 2D NMR (DEPT, COSY, HMQCand HMBC), LC-MS and TGA. Besides, the metal complexes of the novel ligands were prepared with high yields using Cu(II), Co(II), Ni(II) and Mn(II) salts and were characterized using FT-MIR/FAR, UV-Vis., elemental analysis, ICP-OES and TG/DTA techniques. The ligands and their complexes were tested against ten important pathogen microorganisms using disc diffusion method and the metal complexes of the ligands were more active against all microorganisms tested with a broad spectrum than the ligands exhibiting 11-32 mm inhibition zones. On the other hand, the broad spectrum of strongest antimicrobial activity was determined for Mn(II) and Cu(II) complexes of hydroxyphenyliminoligand of usnic acid (L3). In addition, the antimutagenic activities of all of the ligands and their metal complexes were determined using Ames/*Salmonella* and *E. coli* WP2 microbial assay systems and they showed various and strong antimutagenic effects. In general, it has been found that Co and Mn complexes of the ligands possess potent antimutagenic activity. In the view of these results, it can be concluded that some metal complexes can be used as antimicrobial and anticancer agent.

Keywords: Usnic acid, Lichen, *Usnea longissima*, Schiff Base, Imine, Antimicrobial, Antimutagenic.

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1. Introduction

Lichens are symbiotic organisms and include a fungal partner and an algae partner. Many lichen species are known to have therapeutic effects on various diseases and they are widely used in traditional medicine in many countries. Therefore, in recent years, many scientists have focused on the benefits from lichens in various biological activities [1-9]. Lichens have various biological characteristics through produced a great number of specific lichen metabolites known as "lichen substances"[1-4,6,7]. It is known about 800 different type lichen metabolites including dibenzofurans, depsides, depsidones, depsones, quinones, chromones, xanthones, terpenoids, steroids and carotenoids[1-3,6-10].

Usnic acid, a dibenzofuran derivative is a common lichen metabolite and biosynthesized at large amounts by many lichen species.It contains a chiral carbon atom in its chemical molecular structure and therefore it present *D*- or *L*-forms or as a racemic mixture in nature.It is widely used in many cosmetic and medical products such asmedical creams, deodorants, toothpaste, dental mouthwash and sunscreens[11]. In alternative medicine, it has also been used as analgesic, antipyretic, antiseptic, wound healing and expectorant[12,13]and its analgesic, antibacterial, antiprotozoal, anti-inflammatory, antiulcer, hepatotoxic, antioxidant, cytotoxic and antiproliferative properties were reported[6,8,9,11,14-22]. Natrium salt of usnic acid is added into slimming preparations in United States and some European countries [11]. It has also found to be inhibitors of breast, vulva and lungcancers[23-25]. In Europe, usnic acid is still used in the treatment of superficial infections either alone or together with other antibiotics[26].

In particular *Usnea* ve *Alectoria* species are rich species in terms of usnic acid content and these species synthesize up to approximately 6% usnic acid of their own weight. *Usnea longissima* contain high content of usnic acid [1,2,6].Other important aromatic metabolites in *U. longissima* are diffractaic acid, barbatic acid, evernic acid, fizot acid,salazinic acid, fumarprotocetraric acid, ramalinolicacid,fizodal acid, squamatic acid, orsinol andatronorin[2,3,27-30]. Recently, there were some reports on the use in nanotechnology of lichen and lichen metabolites. For instance, nonoparticules of usnic acid which have antifungal activity against some human pathogenic fungiwereproduced from mycobionts of *U. longissima* in the culture medium[31].

Reaction between a primary amines and aldehydes or ketones, forming with called as Schiff base ligands that have a wide area in inorganic and organic chemistry. Metal complexes of Schiff bases are used as catalysts for the catalytic synthesis reactions. Beside of this, some metal ions have an important role as enzyme cofactors in biological systems. Thus, metal complexes of organic compounds and ligands can exhibit important activities in biological systems and there are numerous reports on the various biological activities of ligands and metal complexes of Schiff bases [32-39].Recently, it has also been focused on the anticarcinogenic effect of metal salt complexes of some ligands (Schiff Bases) [36,40-42]. Antimutagenic substances can be of value in the treatment ofcanceror other diseases linked with mutation occurrence[43]. AMES-*Salmonella* and *Escherichia coli* WP2 test systems are two of the important in vitro bacterial test systems commonly used for determining the mutagenic and antimutagenic properties of various agents[43,44].

Usnic acid is an aromatic compound containing two carbonyl groups in the chemical structure. Hence,it's condensation Schiff bases derivatives with some amines, hydrazines and acyl hydrazines were reported[19,33,45].However, according to our literature survey, there was no any report on Schiff bases derivatives of usnic acid with 2-, 3- and 4-aminophenols, their metal complexes and their biological activities.Thus, in the current study,novel multifunctional *Schiff base (hydroxyphenylimino)* ligands were synthesized by condensation reaction of usnic acid with 2-, 3- and 4-aminophenols and their chemical structure were confirmed using FT-IR, UV-Vis., ^{1}H-, ^{13}C-NMR, 1D- and 2D NMR (DEPT, COSY, HMQC, HMBC), LC-MS and TGA. The metal complexes of these novel ligands were prepared using Cu(II), Co(II), Ni(II) and Mn(II) salts and their chemical structure were characterized using FT-MIR/FAR, UV-Vis., elemental analysis, ICP-OES and TG/DTA techniques. All ligands and their complexes were tested against ten important pathogen microorganisms using disc diffusion method. The mutagenic and antimutagenic properties of the multifunctional Schiff base ligands of the usnic acid and their metal complexes were also investigated in the current study.

2. Results and Discussion

2.1. Synthesis and chemical characterization of the ligands and their metal complexes

Usnic acid (yield 4.28%), which is a common lichen metabolite were isolated from the lichen sample, *U. longissima* as yellow crystals and its chemical structure was characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, DEPT ^{13}C-NMR, HMQC, HMBC spectroscopic methods.Its chemical structure was also confirmed by previous spectroscopic data previously reported[1,4,6,7,46]. The hydroxyphenyliminoligands (L1-L3) of usnic acid with aminophenol derivatives and their metal-complexes were synthesized and characterized successfully using spectroscopic and analytical techniques[12,19,32]. All the synthesized ligands (Fig.1) and their metal complexes were characterized by FT-MIR/FAR, ^{1}H, ^{13}C-NMR, 1D and 2D NMR (DEPT, ^{1}H, ^{1}H-COSY, HMQC, HMBC), LC-MS, UV-Vis., elemental analysis, ICP-OES and TG/DTA techniques.

The backbone and the functional groups of the synthesized ligands were assigned by infrared spectra. The important FT-IR signals of the synthesized ligands and their complexes are given in Table 1. In FT-MIR spectra, Ar-OH vibrations ofthe ligands L1, L2 and L3 and their complexes were observed at around 3565-3260 cm^{-1}[39,47]. Aromatic and aliphaticC-H stretches can be attributed at a range of 3080-2850cm^{-1}as broad and small peaks in the FT-IR spectra.Intra-molecular H-bonding vibrations were observed as small signals around of 2600 cm^{-1}. The signals of imine and carbonyl groupscan be separated clearly about 1620 cm^{-1} and about 1690 cm^{-1}. New M-O and M-N absorption bands for the metal complexes of L1, L2 and L3 were observed at around 420 cm^{-1} and 520 cm^{-1} respectively in the FT-FAR spectra (Table 1).

ESI-MS data of the ligands L1, L2 and L3 were collected using positive and negative ion modes simultaneously in MS analyses.In the wide range MS scanning, while molecular ion peaksweredisappeared, radicallic amin ions, positively charged aromatic radicallichidrazo ions and other aromatic degradation products were observed (Fig. 2). MS results confirmed the expected structures when evaluated the collected data with the positive ion mode in a narrow or wide range.

L1 was synthesized as a straw-yellow compound. Its molecular formula was determined to be $C_{24}H_{21}NO_7$ by the molecular ion peak at m/z 435 (100%) in the mass spectrum (negative

ion mode). The ^{1}H-NMR spectral data of the L1 were similar to those of usnic acid, except for the resonance peaks between δ=6.95-7.40 ppm. These data and the integration ratio of the signals between δ=6.95-7.40 ppm (represented the 4 hydrogen atoms) and that of the signals, δ=6.16 ppm (H-4) also indicated that 2-aminophenol successfully linked to the usnic acid over one carbonyl group. The hydrogen signals of 2-aminophenol parts of theL1 in this spectrum were observed at δ=6.97 ppm (*td*, J_1=7.38Hz, J_2=1.84 Hz, H-4'), δ=7.27 (*m*, H-3'), δ=7.29 (*m*, H-6') and δ=7.31 (*m*, H-5'). Furthermore, the -OH signals were atδ=15.26 ppm, δ=13.91 ppm ve δ=12.55 ppm.In accordance with the chemical structure, total 24 carbon signals (18 carbon signals from usnic acid and 6 carbon signals from 2-aminophenol) were observed in the ^{13}C-NMR spectrum. As compared with the ^{13}C-NMR spectrum of the usnic acid, the fact that the observation of the C-14 signalsatδ= 175.0 indicated that 2-aminophenol linked to usnic acid over the C-14 carbonyl carbons. Four –CH_3 and five –CHsignals observed in DEPT ^{13}C-NMR spectrum of the L1 is in accordance with the proposed chemical structure of L1. The chemical structure of the L1 was also confirmed the 2D NMR spectroscopic data, ^{1}H, ^{1}H-COSY, HMQC and HMBC. As shown in Fig.3, HMBC correlations corroborated the proposed structure of L1.

L2 was synthesized as a cream compound and its molecular formula was determined to be $C_{24}H_{21}NO_7$ by the molecular ion peak at m/z 435 (100%) in the mass spectrum. Based on the M^+ peak and molecular formula, 2-aminophenol linked to usnic acid over one carbonyl group (C-14). In the ^{1}H-NMR spectrum of the L2, additional four proton signals belonging to 2-amino phenol part of the L2 were observed at δ=7.20 ppm (*m*, H-4'), δ=6.81 ppm (*dd*, J_1=8.92 Hz, J_2=2.20 Hz, H-6'),δ=7.11 ppm (*t*, *J*=2.14 Hz)and δ=7,36 (*t*, *J*=8.02 Hz, H-5').The hydrogen signals of the three -OH groups were assigned atδ=15.25 ppm, δ=13.89 ppm and δ=12.42 ppm. Likewise, in accordance with the chemical structure of the L2, total 24 carbon signals (18 carbon signals from usnic acid and 6 carbon signals from 3-amino phenol) were assigned in the ^{13}C-NMR spectrum. As compared with the ^{13}C-NMR spectrum of the usnic acid, the fact that the observation of the C-14 signals at δ=175.0 indicated that 3-aminophenol linked to usnic acid over the C-14 carbonyl carbons. The fact that observations of four –CH_3 signals (C-16, C-15, C-18 and C-13) and five –CH signals in DEPT 135 and 90 spectra of the L2 is in completelly accordance with its chemical structure. The correlations in the ^{1}H, ^{1}H-COSY, HMQC and HMBC spectra of L2 also confirm the chemical structure of L2. HMBC correlations of L2 are given in Fig. 3.

As similar to L2, L3 was synthesized as a cream compound and in its mass spectrum, the molecular ion peak was observed at m/z 435 (100%) corresponding to $C_{24}H_{21}NO_7$ molecular formula. M^+ peak and the fact that observation of total 22 carbon signals in the ^{13}C-NMR spectrum of the L3 confirmed that 4-amino phenol linked to usnic acid over one carbonyl group (C-14). As expected, in the ^{13}C-NMR spectrum of L3,22 carbons signals instead of 24 carbons signals were observed due to conflicting of the resonance of C-2', C-6' (δ=127.1 ppm) and C-3', C-5' (δ=116.8 ppm). As similar to the L1 and L2, the resonance of C-14 were assigned at δ=174.3 ppm at ^{13}C-NMR spectrum.In the ^{1}H-NMR spectrum of L3, the protons of 4-aminophenol part of the L3 produced signals corresponding to an AA'BB' system (AA' part, δ=7.13 ppm, *dd*,J_1=8.76 Hz and J_2=2.28 Hz, H-2' and H-6'; BB' part, δ=6.95, *dd*,J_1=8.76 Hz and J_2=2.28 Hz, H-3' and H-5'). DEPT135 and DEPT90 ^{13}C-NMR spectral data and the correlations in the ^{1}H, ^{1}H-COSY, HMQC and HMBC spectraof L3 are also in accordance with the proposed chemical structure of L3. HMBC correlations of L3 are drawn in Fig. 3.

^{1}H, ^{13}C-NMR spectral data and the correlations in the^{1}H-COSY, HMQC and HMBC spectra of the metal complexes are similar with their ligand form. The proposed chemical structuresof the metal complexes are given in Fig. 4.

Thermal behavior of the ligands and their complexes were investigated by using thermogravimetry (TG) between the temperatures of 30-900 °C. L1, L2, L3 and their metal complexes showed similar TGA curves in general. L1 and L2 ligands does not have any physical and hydrated water up to 200-230 °C, however, L1 have an endothermic loss at 150 ºC (3.17%) possibly because of *para*-hydroxy degradation of L1. Major degradations of the ligands, L1, L2 and L3 are between of a range, 200-400 ºC as an exothermic peak. Aromatic back bond, azomethine and hydroxy groups degradated in this range with a loss of 40-55%. It can be said that the ligands are stable up to 200 °C. According to TG/DTA curves of the complexes of L1, there was no any physical adsorbed water or metal-coordinated water in the metal complexes. Based on TG/DTA and elemental analyses results, metal-ligand ratio in the complexes was determined as 1:2. Metal complexes of the ligands showed the similar TG curves, however, metal oxide forms ($MO/M_2O_3/M_3O_4$) occurred as a new band in the range from 400 to 800 °C [39,47,48].

The selected bond lengths and angles are given in Table 2, crystallographic data are listed in Table 3 and hydrogen bond data are given in Table 4.The molecular structure and the packing

diagram of L1 along with the atom-numbering scheme are depicted in Fig. 4 and 5, respectively. The rings A (C1-C6) and D (C14-C19) are, of course, planar, and they are oriented at a dihedral angle of 71.07(18)°. The rings B (C8-C13) and C (O5/C11/C12/C14/C19) adoptenvelopeconformationswith C12 atomsdisplacedby 0.388(5) and 0.311(5) Å fromtheplanes of theother ring atoms, respectively. The intra-molecular O-H…O and N-H…O hydrogen bonds result in the formation of six- and seven-membered rings E (O3/N5/H5A/C7-C9), G (O7/O6/H6/C17/C18/C20) and F (O2/O4/H4/C12-C15) (Fig. 4). The six-membered rings E and G are almost planar with maximum deviations of –0.042(5) Å (for atom C9) and –0.037(6) Å (for atom C20) from the mean planes of the rings, respectively, while the seven membered ring F is not planar having total puckering amplitude, $Q_{T,}$ of 1.484(11) Å. In the crystal, intermolecular O-H…O hydrogen bonds link the molecules into infinite chains along the b-axis (Fig. 5), in which they may be effective in the stabilization of the crystal structure. A weak C-H… π interaction also occurs in the crystal.

In the view of the characterization data, possible chemical structures of the coordination compounds were suggested as shown in Fig. 4. The results are also in accordance with the previous results[19,28,45]. It has been shown that the Pd(II) and Cu(II) complexes of usnic acid with hydrazides were appeared to be monomeric and a square-planar structure, with three binding sitesoccupied by the dianionic tridentate ligand[19]. Furthermore, the Cu(II) ve Pd(II) metal binding properties of usnic acid and its acetyl and enamine derivatives, 9-*O*-acetylusnic acid, 7,9-di-*O*-acetylusnic acid, $\Delta^{2,11}$–enaminousnic acid and N-substituted $\Delta^{2,11}$-enaminousnic acids havebeen reported [33]. In this report, it was documented that the Cu formed ternary complexes with usnic acid and 7,9-di-*O*-acetylusnic acid, whereas Pd formed binary complexes with usnic acid, 7,9-di-*O*-acetylusnic acid, $\Delta^{2,11}$–enaminousnic acid and N-substituted $\Delta^{2,11}$-enaminousnic acids according to X-Ray diffraction analyses results of the complexes[33].

2.2. Biological Activity

2.2.1. Antimicrobial Activity

It is well known that many lichen metabolites, in particular usnic acid have antimicrobial activities against various microorganisms[49-52]. There are numerous reports on antimicrobial activity of the hydroxyphenylimino ligands and their coordination compounds against

microorganisms[19,33,53]. In the literature, some reports has been found on the antimicrobial activity of ligands and their complexation products of usnic acid with some amines[19,33,45]. However, there was no record on the antimicrobial activity of the ligands and their metal complexes of usnic acid with 2-, 3-, and 4-aminophenols. Therefore, in the present study, in vitro antimicrobial activity of the ligands and their metal complexes at 0.25, 0.50, 1.00 and 2.00 mg/ml concentrations were tested against ten human pathogenic microorganisms (Tables 6-7). The current results indicated that the ligands and their metal complexes showed a broad spectrum of the antimicrobial activity producing 11-32 mm inhibition zones. As can be seen from the Table 6, L2 did not show any antimicrobial activity against the all microorganisms tested as compared with those of L1 and L3. On the other hand, when compared the antimicrobial activities of the ligands and their metal complexes, the metal complexes were more antimicrobial on the growth of the microorganisms than the ligands (Tables 5-7). These current results are in aggrement with the previus reports. For instance, it has been reported that Cu(II), Zn(II), Ni(II) and Co(II) coordination compounds of 4-hydroxy salisaldehyde with L-alanine were more antibacterial than the ligand[54]. The strong antimicrobial activity of the complexes as compared with the ligands can be attributed to inactivation of some enzymes in microorganisms[55].

Among the microorganisms, *E. cloacae* as well as *S. cerevisiae* were more resistant microorganisms against L1 and its metal complexes. As shown in Tables 5-7, *B. brevis* and *B. megaterium* among the microorganisms were more sensitive species against the ligands and their metal complexes. In particular, L1-Mn(II), L2-Mn(II), L3-Cu(II) and L3-Mn(II) were more toxic substances against all microorganisms and the highest inhibitiory effects on the growth of the microorganisms were exhibited by all doses of the L3-Cu and L3-Mn (Table 7). These results show that L3-Cu(II), L3-Mn(II) and L2-Mn(II) can be used as antimicrobial reagents. The differences of the antimicrobial activities of the tested ligands and their metal complexes (Tables 5-7) can be attributed to difference of active regions of the ligands and their coordination compounds, ribosomal differences and impermeability of the microorganism's cells[56]. It has also been shown that the ligands containing –OH and/or electron witdrawing groups, and in particular, their Cu complexes have strong antimicrobial activity[57]. In accordance with our results, Pd(II) and Cu(II) coordination compounds of acylhydrazones synthesized by the condensation reactions of usnic acid with some hydrazines have antimicrobial activity against *Aspergillus niger*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*[19].

2.2.2. Antimutagenic Activity

The lifestyle factors include cigarette smoking, diet (fried foods, red meat), alcohol, drug use, radiation, stress, infections and environmental pollutants may damage to the DNA in the organisms and this cause the uncontrolled cell profiration, known as the cancer. Coordination compounds have important roles in living organisms, therefore biologic and catalitic applications of the ligands and their coordination compounds has formed the basis of numerous scientific researches[33,39,48,53,54,58,59]. There are increasing interest on Schiff bases and their coordination compounds due to their anticancer, cytotoxic and apoptotic properties[33,35,60-62]. Some coordination compounds of the Schiff bases are used as oxygen carrier reagents to the substrates, which are important in the therapy of the cancer[63]. Reagents that cause the mutation are known as mutagenic substances and therefore, most of the mutagenic agents are carcinogenic and teratogenic substances[64-67]. In vitro tests are commonly preferred due to the ethical reasons and high cost inanticancer studies on the living organisms[67]. In the current study, the antimutagenic potentials of five different doses (20, 40, 60, 80 and 100 µg/plate) of the ligands and their complexes were examined against known mutagens, NaN_3, 9-AA and MNNG in *S. typhimurium* TA1535, TA1537 and *E. coli* WP2*uvr*A, respectively for the first time. The results were evaluated by using standard plate incorporation method and shown in Tables 8-12 and Figs.7-9. Antimutagenic activity results showed that the ligands and their coordination compounds exhibited various antimutagenic effects and their antimutagenic effects are also not dependent on the concentration (Tables 8-12 and Figs. 7-9). As shown in the Tables 8-12, the inhibition rates of the ligands and their metal complexes were between 82.53-25.17%. On the other hand, in particular, all ligands showed the a broad spectrum of the antimutagenic effects performed with *S. typhimurium* TA1535 (Table 8). It is clear that L3 is most effective ligand when compared with the antimutagenic activities of the L1 and L2.

The metal complexes of the ligands have various inhibition effects of the mutation depending on complexes and microorganisms species performed (Table 8-12 and Fig. 7-9). On the other hand, Co complexes of the ligands were found more effective at a broad spectrum in terms of inhibition of mutation as compared with other metal complexes (Table 10). For instance, Co complexes of all ligands had strong antimutagenic activity exhibiting 57.17-70.48% in the assays performed with *S. typhimurium* TA1537.Likewise, Co(II) complexes of the ligands were effective the

inhibition of the mutation in the assays performed with *S. typhimurium* TA1535. In the assays performed with *E. coli* WP2*uvrA*, some doses of the L1-Co(II) and L2-Co(II) complexes were found to be antimutagenic, whereas none of the doses of L3-Cohad antimutagenic effects. In particular, all doses of the L2-Mn(II) showed moderate antimutagenic activity in *E. coli* WP2*uvrA.* The similar results were also found for the Mn complexes of the ligands. In the assays performed with *S. typhimurium* TA1537, all doses of the L1, L2 and L3 were strongly inhibited the mutation with 53.01-70.63% (Table 11). However, in the assays performed with *E. coli* WP2*uvrA,* Mn(II) complexes of the ligands were more effective than Co(II)-complexes of the ligands. The antimutagenic properties of the Ni(II)-complexes of the ligands are shown in Table 12. In contrast to other complexes, Ni(II) complexes of the L1 and L2 were effective the inhibition of the mutation in *E. coli* WP2*uvrA*and *S. typhimurium* TA1535. However, as compared with other metal complexes of the ligands, Ni(II) complexes of the ligands have weak or no antimutagenic activity in the assays performed with *S. typhimurium* TA1537. Whereas L1-Ni(II) did not show any antimutagenic activity in *S. typhimurium* TA1537, 60 µg/plate concentration of L2-Ni(II), and 20 and 40 µg/plate concentrations of the L3-Ni(II) showed strong and moderate antimutagenic effects, respectively.The results of the antimutagenic potentials of the ligands were summarized in Table 9. As can be seen from this table, all doses of Cu(II) complexes showed various antimutagenic activities. In particular, L2-Cu(II) and L3-Cu(II) complexes were more effective than L1-Cu(II) complexes in all microorganisms. All doses except 20 µg/plate dose of L3-Cu(II) exhibited strong antimutagenic activity with 62.37-57.80% in *S. typhimurium* TA1537, whereas L3 did not showed any antimutagenic potential in *S. typhimurium* TA1537 (Table 8). Hence, the antimutagenic effect of L3-Cu(II) can be attributed to its Cu content.

Research on mutagenic and antimutagenic properties of various the natural and synthetic substances is a very important strategy to develop new anticancer agents. In the current study, our results showed that the ligands and their metal complexes have strong or moderate antimutagenic potentials in *E. coli* WP2*uvrA, S. typhimurium* TA1535 and TA1537. The ligands and their metal complexes inhibited the mutation with 82.53-25.17% inhibition rates (Tables 8-12) and also none of the ligands and their coordination compounds act as mutagen. These results show that the ligands and their coordination compounds can be used as potential anticancer drugs.

NaN_3 is one of the most powerful mutagens, which affects several organisms including bacteria, plants and animals[68]. Earlier studies showed that the mutagenicity of NaN_3 is related to the production of L-azidoalanine[68]. Observing antimutagenic effect against NaN_3 may be due to the fact that the ligands and their metal complexes have sufficient capability to inhibit formation of L-azidoalanine. The second mutagen used in the present study was 9-AA which is a member of Acridine family and known as a model frameshift mutagen[69]. In the frameshift mutagenesis mechanism, it binds to DNA non-covalently by intercalation. Through this way, 9-AA induces frameshift mutations at hot spots where a single base, especially guanine, is repeated[70]. The performed experiments to determine antimutagenic properties of the test materials, by using *S. typhimurium* TA1537 strain and 9-AA, depend on the inhibition of mentioned intercalation mechanism. Thus, the positive results for the ligands and their metal complexes may be explained by their inhibition capabilities by blocking 9-AA binding to DNA. MNNG is a well-known mutagen and carcinogen. It is known that its mutagenic and lethal effects take root from methylation of DNA. Previous reports showed that formation of O^6-methylguanine, which is one of its important products, appears to be responsible for its mutagenic activity[71]. In the curent study, test materials also showed a significantantimutagenicpotential at tested concentrations. This antimjutagenic activity may be explained with inhibitor activities of the ligands and the metal complexes on the formation of O^6-methylguanine.

It has been reported that the ligands and their various coordination compounds can show different biological activities[72,54,65,66]. Recently, there is a growing interest on the anticancer properties of some ligands and their coordination compounds[54,65,66,73-76]. However, there is only a report on the Schiff bases derivatives of usnic acid[19]. In accordance with our results, the Cu(II) and Pd(II) condensation products of usnic acid with hydrazides had significant cytotoxic activities (IC_{50}=1.8-86.0 µM) against *HeLa* cells *in vitro*. In particular, Cu(II) complexes were more cytotoxic than Pd(II) complexes, IC_{50} values of Cu(II) complexes of usnic acid 11-thiosemicarbazono was 1.8 µM. In the present study, Cu(II) complexes of the Sciff bases derived from usnic acid had various weak or moderate antimutagenic activities at different concentrations against in three microorganisms. Furthermore, it has been reported the antitumor activities against many tumor cells of Cu(II) complexes of salisaldehyde[77]. Likewise, Schiff bases derived from amine analoges of hydroxyl salisaldehyde and their metal complexes were most active against Ehrlich cancer[78]. Nevertheless, Zn(II), Ni(II) ve Cu(I) complexes of Schiff bases from the

hydration reacton of 2,4-dihydroxybenzaldehyde and *L*-arginineinhibited the cancer cells with 32.2%, 51.7% and 53.3%, respectively[79].

3. Conlusion

In the present study, the Schiff base ligands of usnic acid with aminophenol derivatives and their Cu(II), Co(II), Mn(II) and Ni(II) metalcomplexes were synthesized successfully and characterized using spectroscopic and analytical techniques for the first time.The present results also indicated that the ligands and their metal complexes were active at a broad spectrum against important human pathogenic microorganisms. These results showed that the metal complexes of the ligands can be used as new antimicrobial agents. Antimutagenic activity assays showed that the ligands and their metal complexes have various antimutagenic potential *in vitro*, in particular Co(II) and Mn(II) complexes of the ligands were very effective the inhibition of mutation. However, the anticancer activities of the ligands and their metal complexes should also be tested *in vivo* conditions.

4.Materials and Methods

4.1.Apparatus, materials and reagents

All reagents and organic solvents were purchased from Merck, Riedel de Haen, Fluka and Sigma and used as received, unless noted otherwise. The NMR spectra of the ligands and complexes were recorded at 25 ºC in DMSO-d_6 using a Bruker 400 MHz instrument. Mass spectra of the ligands were recorded on a Zivak Tandem Gold LC-MSMS spectrometer (ESI). Positive and negative ion modes were used simultaneously in MS analyses. Infrared spectra were collected using on a Perkin Elmer Spectrum 400 FT-IR (4000–400 cm^{-1}) fitted with Pike ATR and Spectrum 100 N FT-NIR (400-40 cm^{-1}).UV-Visible spectra in the 200–1000 nm range were obtained in DMF solvent on a Perkin Elmer Lambda 45 spectrophotometer. Elemental analyses were performed using LECO CHNS 932 instrument. The thermal analyses studies of the ligands were performed on a SII thermal system under nitrogen atmosphere at a heating rate of 10^{o}C/min in the range 30-1000 ºC. The amount of metal ions in adsorption tests were determined by using Perkin Elmer Optima 2100DV ICP-OES coupled with AS93 autosampler. Calibration standards

of all elements were prepared from Inorganic Ventures (USA) calibration stocks (about 1000 $mg.l^{-1}$). Ultrapure water obtained from a Milli-Q purifier system (Millipore Corp., Bedford, MA) was used throughout the work.

4.2. Lichen sample

Lichen sample, *U. longissima* Ach. was collected from the Artvin, Erzurum, Trabzon and Giresun regions (northern Anatolia)at different times of 2009-2010 years and identified by Dr. Ali Aslan[80]. A voucher specimen (KKEF-374) has been deposited in the herbarium of Kazim Karabekir Education Faculty, Ataturk University, Erzurum (Turkey).

4.3.Extraction of lichen sample and Isolation of Usnic acid

The lichen sample (1500g) was extracted refluxing with CH_2Cl_2 (50ºC, 1l x 6) using a water bath. The solvent was removed the extract using a rotary evaporator under reduced temperature (50ºC) and pressure and 110.5 gram of the extract was obtained (yield 7.40%). There was a yellow compound at the bottom of the flask when the extract was treated with diethylether (250 ml). This solid compound was retreated withdiethyl ether (250ml x 4) to remove other purities and controlled with thin layer chromatography. Thus, solid 43.6g of usnic acid was isolated.Usnic acid (20.7g) in the ether solution was isolated over silica gel column chromatography (200g, 70-230 mesh) using hexane: ethyl acetate (1:1) eluent system and thus total 64.30g of usnic acid (4.29%) were isolated from the *U. longissima*.

4.4. Synthesis of Hydroxyphenylimino Ligands (L1,L2,L3)

Appropriate amount of usnic acid (2 mmol, 0.6886 g) was dissolved in absolute ethanol by heating and the solution of 2-, 3- and 4-aminophenols (2 mmol, 0.2183 g) in absolute ethanol was added into this solution. The reaction mixtures were refluxed for 2 h. The final orange solutions were waited for 6 h in an ice bath. The obtained crystals of L1, L2 and L3 were filtered,

washed with cold water: methanol (1:1) and re-crystalized with absolute ethanol. The purity was checked by thin layer chromatography.

6-acetyl-7,9-dihydroxy-2-(1-(3-hydroxyphenyliminoo)ethyl)-8,9bdimethyldibenzo(b,d)furan-1,3(2H,9bH)-dione(L1). Color: yellowish,yield: 61%, mp: 259 ºC (decom.), elementalanalyses: Found (calcd.), C, 66.08 (66.20); H, 4.85 (4.86); N, 3.18 (3.22). ^{1}H NMR (Piridin-d_5, 25 ºC): δ (ppm)=6.14 (*s*, C4), 1.70 (*s*, C13), 2.74 (*s*, C15), 2.34 (*s*, C16), 2.65 (*s*, C18), 7.27 (*s*, C3'), 6.97 (*td*,C4' J_1=7.38Hz, J_2=1.84 Hz), 7.31 (*m*, C5'), 7.29 (*m*, C6'). ^{13}C NMR (Piridin-d_5, 25 ºC) : δ(ppm)= 198.5 (C1), 103.1(C2), 190.4 (C3), 102.7 (C4), 174.2 (C5), 156.2 (C6), 101.5 (C7), 163.6 (C8), 107.6 (C9), 158.5 (C10), 105.6 (C11), 59.7 (C12), 31.5 (C13), 175.0 (C14), 20.5 (C15), 7.8 (C16), 200.9 (C17), 30.9 (C18), 124.3 (C1'), 153.1 (C2'), 129.7 (C3'), 119.5 (C4'), 127.2 (C5'), 117.1 (C6') ppm. FTIR (KBr, cm^{-1}): 3374 (Ar-OH), 3076 (Ar-CH), 2985 (Aliphatic C-H); 1687 (C=O), 1622 (CH=N); 1551 (aromatic C=C); 1273cm^{-1} (C-OH). MS-ESI: m/z 433 ((M-2)$^+$, 8%), 434 ((M-1)$^+$, 23%), 435 ((M)$^+$, 100%), 436 ((M+1)$^+$, 59%), 408 ((M+2)$^+$, 36%).

6-acetyl-7,9-dihydroxy-2-(1-(3-hydroxyphenyliminoo)ethyl)-8,9b-dimethyldibenzo(b,d)furan-1,3(2H,9bH)-dione (L2). Color: cream, yield: 53%, mp: 220ºC (decom.), elemental analyses: C, 66.13 (66.20); H, 4.92 (4.86); N, 3.18 (3.22).^{1}H NMR (Piridin-d_5, 25 ºC) : δ (ppm)= 6.16 (*s*, C4), 1.74 (*s*, C13), 2.66 (*s*, C15), 2.35 (*s*, C16), 2.65 (*s*, C18), 7.11 (*dd*, C2', J_1=2,12 Hz, J_2=2,16 Hz), 7.20(*m*, C4', *J*=7.20Hz), 7.36 (*dd*, C5', *J*=8.02 Hz), 6.81 (*dd* , C6', J_1=8.92 Hz, J_2=2.20 Hz). ^{13}C NMR (Piridin-d_5, 25 °C) : δ(ppm)= 199.3 (C1), 103.6(C2), 191.0 (C3), 103.4 (C4), 174.9 (C5), 156.8 (C6), 102.2 (C7), 164.3 (C8), 108.3 (C9), 159.1 (C10), 106.2 (C11), 59.9(C12), 32.2 (C13), 175.0 (C14), 21.1 (C15), 8.4 (C16), 201.6 (C17), 31.6 (C18), 138.2 (C1'), 114.1 (C2'), 160,5 (C3'), 116.6 (C4'), 131.4 (C5'), 116.9 (C6'). FTIR (KBr, cm^{-1}): 3595 (Ar-OH), 2981 (Ar-CH), 2862 (Aliphatic C-H); 1694 (C=O), 1622 (CH=N);1575 (Aromatic C=C); 1275 cm^{-1} (C-OH). MS-ESI: m/z 433 (M-2$^+$, 5%), 434 (M-1$^+$, 10%), 435 (M$^+$, 100%), 436 (M+1$^+$, 90%), 408 (M+2$^+$, 20%). UV-Visible spectrum (nm): 394 (n-π^* transition), 334 (π-π^* transition), 288 (σ-σ^* transition).

6-acetyl-7,9-dihydroxy-2-(1-(4-hydroxyphenyliminoo)ethyl)-8,9b-dimethyldibenzo(b,d)furan-1,3(2H,9bH)-dione (L3). Color: cream, yield: 84%, mp: 255°C (decomp.), elemental analyses: Found (calcd.), C, 66.08 (66.20); H, 4.90 (4.86); N, 3.20 (3.22).^{1}H NMR (Piridin-d_5, 25 °C) : δ(ppm)= 6.19 (*s*, C4), 1.80 (*s*, C13), 2.68 (*s*, C15), 2.39 (*s*, C16), 2.69 (*s*, C18), 7.23 (*dd*, C2', *J*=8.76 Hz, *J*=2.30 Hz), 6.95(*dd*, C3', J_1=8.72Hz, J_2=2.28Hz), 6.95 (*dd*, C5', J_1=6.95 Hz, J_2=2.28 Hz), 7.23 (*dd*, C6', J_1=8,76 Hz, J_2=2,30 Hz). ^{13}C NMR (Piridin-d_5, 25 °C) : δ(ppm)= 198.5 (C1), 102.9 (C2), 190.2 (C3), 102.7 (C4), 174.2 (C5), 156.2(C6), 101.6 (C7), 163.7 (C8), 107.6 (C9), 158.5 (C10), 105.6 (C11), 57.2(C12), 31.6 (C13), 174.3 (C14), 20.4 (C15), 7.8 (C16), 200.9 (C17), 30.9 (C18), 141.3 (C1'), 127.1(C2'), 116.8 (C3'), 116.8 (C5'), 127.1 (C6'). FTIR (KBr, cm^{-1}): 3278 (Ar-OH), 3074 (Ar-CH), 2986 (Aliphatic C-H); 1690 (C=O), 1626 (CH=N); **1**538 (Aromatic C=C); 1269 cm^{-1} (C-OH). MS-ESI: m/z 433 (M-2^{+}, 8%), 434 (M-1^{+}, 12%), 435 (M^{+}, 100%), 436 (M+1^{+}, 100%), 408 (M+2^{+}, 25%). UV-Visible spectrum (nm): 491 (n-π* transition), 333 (π-π* transition), 278 (σ-σ* transition).

*4.5. Metal Complexes of **L1**, **L2** and **L3***

The ligands (3.44 mmol) was dissolved in dichloromethane:ethanol (1:2) and added 1.71 mmol Cu (II), Co (II), Ni (II) and Mn (II) cationcontaining metal salts to this solution. After stirring for 6hat 75ºC, the obtained product was washed with distilled water and dried in vacuum at 50 ºC. 1D and 2D NMR spectra of some complexes are presented in the supplementary file.

L1-Cu(II). Color: dark brown, yield: 37%, m.p.: 259ºC (decomp.), elemental analyses: Found (calcd.), C, 62.32 (62.40); H, 4.81 (4.82); N, 2.98 (2.91), Cu, 6.25 (6.60). FTIR (KBr, cm^{-1}): 3376 (Ar-OH), 3070 (Ar-CH), 2982 (Aliphatic C-H); 1688 (C=O), 1626 (CH=N); 1542 (Aromatic C=C); 1274 (C-OH), 417 (M-N), 521 cm^{-1} (M-O).

L1-Co(II). Color: cream, yield: 35%, m.p.: 254°C (decomp.), elemental analyses: Found (calcd.), C, 62.85 (62.70); H, 4.54 (4.84); N, 2.79 (2.92); Co, 6.01 (6.15). FTIR (KBr, cm^{-1}): 3374 (Ar-

OH), 3075 (Ar-CH), 2983 (Aliphatic C-H); 1687(C=O), 1625 (CH=N); 1538 (Aromatic C=C); 1274 (C-OH), 418 (M-N), 465 cm^{-1} (M-O).

L1-Mn(II). Color: orange, yield: 46%, m.p.: 254°C (decomp.), elemental analyses: Found (calcd.), C, 63.07 (62.96); H, 4.82 (4.86); N, 2.93 (2.94); Mn, 5.56 (5.76). FTIR (KBr, cm^{-1}): 3366 (Ar-OH), 3075 (Ar-CH), 2984 (Aliphatic C-H); 1688 (C=O), 1623 (CH=N); 1550 (Aromatic C=C); 1274 (C-OH), 469 (M-N), 526 cm^{-1} (M-O).

L1-Ni(II). Color: straw-colored, yield: 49%, m.p.: 255°C (decomp.), elemental analyses: Found (calcd.), C, 62.67 (62.71); H, 4.88 (4.84); N, 2.97 (2.93); Ni, 5.98 (6.13). FTIR (KBr, cm^{-1}): 3375 (Ar-OH), 3075 (Ar-CH), 2984 (Aliphatic C-H); 1687(C=O), 1625 (CH=N); 1538 (Aromatic C=C); 1274 (C-OH), 462 (M-N), 519 cm^{-1} (M-O).

L2-Cu(II). Color: dark brown, yield: 40%, m.p.: 257°C (decomp.), elemental analyses: Found (calcd.), C, 62.35 (62.40); H, 4.85 (4.82); N, 2.99 (2.91); Cu, 6.37 (6.60). FTIR (KBr, cm^{-1}): 3565 (Ar-OH), 2979 (Ar-CH), 2863 (Aliphatic C-H); 1695 (C=O), 1623 (CH=N); 1580 (Aromatic C=C); 1271 (C-OH), 422 (M-N), 518 cm^{-1} (M-O).

L2-Co(II). Color: dark brown, yield: 44%, m.p.: 255°C (decomp.), elemental analyses: Found (calcd.), C, 62.97 (62.70); H, 4.90 (4.84); N, 2.86 (2.92); Co, 6.10 (6.15). FTIR (KBr, cm^{-1}): 3475 (Ar-OH), 2956 (Ar-CH), 2863 (Aliphatic C-H); 1695(C=O), 1623 (CH=N); 1576 (Aromatic C=C); 1270 (C-OH), 424 (M-N), 492 cm^{-1} (M-O).

L2-Mn(II). Color: light brown, yield: 44%, m.p.: 250 °C (decomp.), elemental analyses: Found (calcd.), C, 63.09 (62.96); H, 4.78 (4.86); N, 3.01 (2.94); Mn, 5.62 (5.76). FTIR (KBr, cm^{-1}):

3278 (Ar-OH), 2956 (Ar-CH), 2860 (Aliphatic C-H); 1689(C=O), 1624 (CH=N); 1538 (Aromatic C=C); 1269 (C-OH), 474 (M-N), 525 cm^{-1} (M-O).

L2-Ni(II). Color: straw-colored, yield: 42%, m.p.: 252°C (decomp.), elemental analyses: Found (calcd.), C, 62.66 (62.71); H, 4.75 (4.84); N, 2.96 (2.93); Ni, 6.07 (6.13). FTIR (KBr, cm^{-1}): 3012 (Ar-OH), 2975 (Ar-CH), 2862 (Aliphatic C-H); 1694 (C=O), 1623 (CH=N); 1574 (Aromatic C=C); 1274 (C-OH), 473 (M-N), 521 cm^{-1} (M-O).

L3-Cu(II). Color: dark brown, yield: 48%, m.p.: 258°C (decomp.), elemental analyses: Found (calcd.), C, 62.34 (62.40); H, 4.78 (4.82); N, 3.00 (2.91); Cu, 6.44 (6.60). FTIR (KBr, cm^{-1}): 3273 (Ar-OH), 3073 (Ar-CH), 2985 (Aliphatic C-H); 1689(C=O), 1627 (CH=N); 1537 (Aromatic C=C); 1268 (C-OH), 418 (M-N), 516 cm^{-1} (M-O).

L3-Co(II). Color: straw-colored, yield: 44%, m.p.: 254°C (decomp.), elemental analyses: Found (calcd.), C, 62.89 (62.70); H, 4.96 (4.84); N, 2.98 (2.92); Co, 6.11 (6.15). FTIR (KBr, cm^{-1}): 3264 (Ar-OH), 3074 (Ar-CH), 2986 (Aliphatic C-H); 1689(C=O), 1628 (CH=N); 1538 (Aromatic C=C); 1268 (C-OH), 432 (M-N), 478 cm^{-1} (M-O).

L3-Mn(II). Color: metallic green, yield: 40%, m.p.: 254°C (decomp.), elemental analyses: Found (calcd.), C, 63.01 (62.96); H, 4.89 (4.86); N, 2.95 (2.94); Mn, 5.67 (5.76). FTIR (KBr, cm^{-1}): 3264 (Ar-OH), 3075 (Ar-CH), 2986 (Aliphatic C-H); 1689(C=O), 1627 (CH=N); 1537 (Aromatic C=C); 1267 (C-OH), 479 (M-N), 512 cm^{-1} (M-O).

L3-Ni(II). Color: straw-colored, yield: 41%, m.p.: 256°C (decomp.), elemental analyses: Found (calcd.), C, 62.93 (62.96); H, 4.87 (4.86); N, 2.92 (2.94); Ni, 6.06 (6.13). FTIR (KBr, cm^{-1}): 3263

(Ar-OH), 3075 (Ar-CH), 2985 (Aliphatic C-H); 1689 (C=O), 1627 (CH=N); 1538 (Aromatic C=C); 1269 (C-OH), 475 (M-N), 511 cm^{-1} (M-O).

4.6. X-Ray Crystallography

The ligand (L1) was crystallized from ethanol at room temperature. Crystallographic data of L1 were recorded on a Bruker Kappa APEXII CCD area-detector diffractometer using Mo $K_\alpha$radiation (λ=0.71073 Å) at T=100(2) K. Absorption correction by multi-scan[1] was applied. Structure was solved by direct methods and refined by full-matrix least squares against F^2 using all data.[2] All non-H atoms were refined anisotropically. Only the H atom of NH group was located in a difference synthesis and refined isotropically (N-H=0.88(6) Å, U_{iso}(H)=0.07(2) $Å^2$). The remaining H atom positions were calculated geometrically at distances of 0.93 Å (CH) and 0.96 Å (CH_3) from the parent C atoms; a riding model was used during the refinement process and the U_{iso}(H) values were constrained to be 1.2U_{eq}(for CH) and 1.5U_{eq}(for CH_3).

4.7. Antimicrobial Assay

Antimicrobial activities of the ligands and their metal complexes were determined using the Bauer Kirby disc diffusion method. At that time, inhibition zones appearing around the discs were measured and recorded in millimeter[35,38,39]. The ligands and their metal complexes were screened three times against each organism and inhibition zone data are expressed as mean values of three parallel experiments. Microorganisms, *Candida albicans*, *Saccharomyces cerevisiae* WET 136, *Enterobacter aerogenes* ATCC 27859, *Bacillus brevis* FMC3, *Micrococcus luteus* LA2971, *Staphylococcus aureus* 6538, *Bacillus megaterium* DSM 32, *Pseudomonas aeruginosa* 9027, *Enterobacter cloacae* ATCC 13047, *Eschericha coli* ATCC25922 were used in the antimicrobial tests.

4.8. Mutagenicity and AntimutagenicityTests

Chemicals. Direct acting mutagens 9-Aminoacridine (9-AA) and *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) were obtained from Merck and ABCR GmbH & Co. respectively. Other solvents and pure chemicals including magnesium sulfate ($MgSO_4$), sodium ammonium phosphate ($Na_2NH_2PO_4$), D-glucose, L-tryptophan, D-biotin, sodium chloride (NaCl), L-histidine HCl, sodium phosphate-dibasic (Na_2HPO_4), crystal violet, citric acid monohydrate, potassium phosphate-dibasic (K_2HPO_4), sodium phosphate-monobasic (NaH_2PO_4) were also obtained from Sigma, Merck, Difco and Fluka.

Bacterial Strains. *Salmonella typhimurium* TA1535 (ATCC® Number: 29629) and *S. typhimurium* TA1537 (ATCC® Number: 29630) strains was provided by The American Type Culture Collection – Bacteria Department of Georgetown University, Washington, USA, and *E. coli* WP2*uvrA* (ATCC® Number: 49979) strain was provided by LGC standarts Middlesex, UK. All strains were stored at -80 ºC. Working cultures were prepared by inoculating nutrient broth with the frozen cultures, followed by an overnight incubation at 37 ºC with gentle agitation.

Viability assays and determination of test concentrations. The toxicity of test materials toward *S. typhimurium* 1537 and *E. coli* WP2*uvrA* strains was determined as described in detail elsewhere[81,82]. These tests confirmed that there was normal growth of the background lawn, spontaneous colony numbers within the regular range, and no significant reduction in cell survival. Thus, for the concentrations and conditions reported here, no toxicity or other adverse effects were observed.

Mutagenicity and Antimutagenicity Tests. The bacterial mutagenicity and antimutagenicity assays were performed according to described before[44,83]. The known mutagens NaN_3(in distilled water -1 µg/plate) for *S. typhimurium* TA1535, 9-AA (in methanol-10 µg/plate) for *S. typhimurium* TA1537 and MNNG (in DMSO - 1 µg/plate) for *E. coli* WP2*uvrA* were used as positive controls and DMSO was used as negative control in these studies.

In the mutagenicity test performed with TA1537 strain of *S. typhimurium*,100µl of the overnight bacterial culture, 50µl test compounds at different concentrations, and 500µl phosphate buffer were added to 2 ml of the top agar containing 0.5 mM histidine/biotin. The mixture was

poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37ºC for 48 h.

In the antimutagenicity test performed with the same strains,100µl of the overnight bacterial culture, 50µl mutagen, 50µl of thetest compounds at different concentrations, and 500µl of phosphate buffer were added to 2 ml of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37 ºC for 48h.

The procedures of mutagenicity and antimutagenicity assays, which are described above [84-87]for the AMES/*Salmonella*assay, are all applicable to the *E. coli* WP2 reverse mutation assay. The only procedural difference is the addition of limited tryptophan (0.05 mM) instead of histidine to the top agar[44].

The plate incorporation method was used to assess the results of mutagenicity and antimutagenicity assays[83]. For the mutagenicity assays, the mutagenic index was calculated for each concentration, which is the average number of revertants per plate divided by the average number of revertants per plate with the negative (solvent) control. A sample was considered mutagenic when were observed a dose-response relationship and a two-fold increase in the number of mutants with at least one concentration was observed.

For the antimutagenicity assays, the inhibition of mutagenicity was calculated by using the following equation (M: number of revertants/plate induced by mutagen alone, S_0: number of spontaneous revertants, S_1: number of revertants/plate induced by the extract plus the mutagen): $\%\text{Inhibition} = ((M-S_1) / (M-S_0))\times 100$

25–40% inhibition was defined as moderate antimutagenicity; 40% or more inhibition as strong antimutagenicity; and 25% or less inhibition as no antimutagenicity[86-90].

Statistical Analysis. The results are presented as the average and standard error of three experiments with duplicate plates/dose experiment. The data was further analyzed for statistical significance using analysis of variance (ANOVA), and the difference among means was compared by high-range statistical domain using Tukey's test. A level of probability $p<0.05$ was taken as indicating statistical significance.

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Supplementary materials

Crystallographic data (excluding structure factors) has been deposited with the Cambridge Crystallographic data Centre as the supplementary publication Nos: CCDC 937189, Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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Table 1. ChemicalCompositionand FT-IR signals of the synthesized compounds

Compounds	Found (Calcd.) (%)			FT-MIR/FAR Signals (cm^{-1})									
	C	H	N	M[a]	*v*(OH)	*v*(Ar-C-H)	*v*(C-H)	*v*(CH=O)	*v*(CH=N)	*v*(C=C)	*v*(C- OH)	*v*(M-N)*	*v*(M-O)*
L1	66.08(66.20)	4.85(4.86)	3.18(3.22)	-	3374	3076	2985	1687	1622	1602	1273	-	-
L1-Cu	62.32(62.40)	4.81(4.82)	2.92(2.91)	6.25(6.60)	3376	3070	2982	1688	1626	1601	1274	417	521
L1-Co	62.85(62.70)	4.54(4.84)	2.79(2.92)	6.03(6.15)	3374	3075	2983	1687	1625	1601	1274	418	465
L1-Mn	63.07(62.96)	4.82(4.86)	2.93(2.94)	5.56(5.76)	3366	3075	2984	1688	1623	1601	1274	469	526
L1-Ni	62.97(62.71)	4.88(4.84)	2.97(2.93)	5.98(6.13)	3375	3075	2984	1687	1625	1600	1274	462	519
L2	66.13(63.20)	4.92(4.86)	3.18(3.22)	-	3595	2981	2862	1694	1622	1598	1275	-	-
L2-Cu	62.35(62.40)	4.85(4.82)	2.99(2.91)	6.37(6.60)	3565	2979	2863	1695	1623	1599	1271	422	518
L2-Co	62.97(62.70)	4.90(4.84)	2.86(2.92)	6.10(6.15)	3475	2956	2863	1695	1623	1600	1270	424	492
L2-Mn	63.09(62.96)	4.78(4.86)	3.01(2.94)	5.62(5.76)	3278	2956	2860	1689	1624	1600	1269	474	525
L2-Ni	62.66(62.71)	4.75(4.84)	2.96(2.93)	6.07(6.13)	3012	2975	2862	1694	1623	1599	1274	473	521
L3	66.08(66.20)	4.90(4.86)	3.20(3.22)	-	3278	3074	2986	1690	1626	1601	1269	-	-
L3-Cu	62.34(62.40)	4.78(4.82)	3.00(2.91)	6.44(6.60)	3273	3073	2985	1689	1627	1601	1268	418	516
L3-Co	62.89(62.70)	4.96(4.84)	2.98(2.92)	6.11(6.15)	3264	3074	2986	1689	1628	1600	1268	432	478
L3-Mn	63.01(62.96)	4.89(4.86)	2.95(2.94)	5.67(5.76)	3264	3075	2986	1689	1627	1601	1267	479	512
L3-Ni	62.93(62.96)	4.87(4.86)	2.92(2.94)	6.06(6.13)	3263	3075	2985	1689	1627	1601	1269	475	511

[a]Metal Analyses were performed by using ICP-OES after digestion using MWS3+ microwave digestion system.

* Measured and detected more sensitive by FT-FAR Spectrometer.

Table 2. The selected bond lengths (Aº) and angles of L-1

Atom no	Bond length	Atom no	Bond length	Atom no	Bond length
O1-H1	0.820	C3-H3	0.930	C12-C23	1.555 (6)
O2-C13	1.246 (6)	C3-C4	1.370 (8)	C14-C12	1.508 (6)
O3-C9	1.240 (6)	C4-H4A	0.930	C14-C15	1.378 (7)
O4-H4	0.820	C5-C4	1.376 (7)	C14-C19	1.369 (7)
O4-C15	1.359 (6)	C5-H5	0.930	C16-C15	1.99 (7)
O5-C11	1.376 (5)	C6-C1	1.375 (7)	C16-C17	1.389 (7)
O5-C19	1.397 (6)	C6-C5	1.368 (8)	C16-C22	1.491 (7)
O6-H6	0.820	C7-C8	1.429 (6)	C17-C18	1.430 (7)
O6-C17	1.343 (5)	C7-C24	1.483 (7)	C18-C19	1.407 (6)
N5-H5A	0.880 (6)	C8-C9	1.465 (7)	C18-C20	1.454 (7)
N5-C6	1.447 (6)	C8-C13	1.423 (7)	C20-O7	1.251 (6)
N5-C7	1.330 (6)	C9-C10	1.461 (6)	C20-C21	1.468 (8)
C1-O1	1.363 (6)	C10-H10	0.930	C21-H21A,B,C	0.960
C1-C2	1.395 (7)	C11-C10	1.323 (7)	C22-H22 A,B,C	0.960
C2-H2	0.930	C12-C11	1.483 (7)	C23-H23 A,B,C	0.960
C2-C3	1.386 (8)	C12-C13	1.521 (6)	C24-H24 A,B,C	0.960

Table 3. Crystallographic Details.

Empirical Formula	$C_{24}H_{21}NO_7$
Fw	435.42
Crystal System	Monoclinic
Space Group	P 2_1
a (Å)	7.782 (3)
b (Å)	9.277(3)
c (Å)	14.065(5)
α (°)	90.00
β (°)	100.13(2)
γ (°)	90.00
V (Å^3)	999.6 (6)
Z	2
μ (cm^{-1})	1.07 (Mo K_α)
ρ(calcd) (g cm^{-1})	1.447
Number of Reflections Total	9667
Number of Reflections Unique	4617
R_{int}	0.0842
$2\theta_{max}$ (°)	57.10
T_{min}/ T_{max}	0.9696 / 0.9925
Number of Parameters	298
R [$F^2 > 2\sigma(F^2)$]	0.0602
wR	0.1685

Table 4. Hydrogen-bond geometry (Å, °).

D-H···A	D-H	H···A	D···A	D-H···A
O1-H1···O3^{i}	0.82	2.00	2.692(6)	141
O4-H4···O2	0.82	1.85	2.614(5)	155
N5-H5A···O3	0.88(6)	1.83(6)	2.565(6)	139(5)
O6-H6···O7	0.82	1.76	2.504(6)	149
C21-H21B···Cg4^{ii}	0.96	2.95	3.815(6)	150

Symmetry codes: (i) 1 – x, y – 1/2, – z; (ii) – x, y + 1/2, 1 – z. Cg4 is the centroid of the ring
D (C14-C19).

Table5. Antimicrobial activities of L1and their metal complexes

Treatments	Concentration (mg/ml)	Microorganisms									
		E. aerogenes	*B. brevis*	*M. luteus*	*E. coli*	*B. megaterium*	*P. aeruginosa*	*E. cloacae*	*S. aureus*	*C. albicans*	*S. cerevisiae*
L1	0.25	12*	13	-	-	14	-	-	16	-	-
	0.50	12	17	-	-	14	-	-	16	-	-
	1.00	12	17	12	-	16	-	-	16	-	-
	2.00	13	17	13	-	16	-	-	16	-	-
L1-Co	0.25	15	15	14	14	14	-	-	15	-	-
	0.50	16	16	16	15	16	16	-	18	-	-
	1.00	17	19	15	15	16	16	-	16	-	-
	2.00	17	22	13	16	18	18	-	19	-	-
L1-Cu	0.25	12	12	12	-	13	-	-	14	14	-
	0.50	13	12	16	13	13	-	-	14	21	-
	1.00	15	17	14	15	17	16	-	14	26	-
	2.00	19	18	18	16	20	20	-	20	33	-
L1-Mn	0.25	22	21	22	20	22	20	-	22	-	-
	0.50	22	23	22	22	22	20	-	23	-	16
	1.00	25	26	23	22	24	23	-	23	-	16
	2.00	28	26	23	24	27	25	-	23	-	17
L1-Ni	0.25	13	16	13	14	15	16	-	16	-	-
	0.50	13	18	13	15	15	16	-	16	-	-
	1.00	15	18	15	14	17	16	-	18	-	-
	2.00	17	20	15	18	21	19	-	18	-	-

*Inhibition zone in diameter (mm) around the disks.

-: no inhibition.

Table 6. Antimicrobial activities of L2 and their metal complexes

Treatments	Concentration (mg/mL)	Microorganisms									
		E. aerogenes	*B. brevis*	*M. luteus*	*E. coli*	*B. megaterium*	*P. aeruginosa*	*E. cloacae*	*S. aureus*	*C. albicans*	*S. cerevisiae*
L2	0.25	-	-	-	-	-	-	-	-	-	-
	0.50	-	-	-	-	-	-	-	-	-	-
	1.00	-	-	-	-	-	-	-	-	-	-
	2.00	-	-	-	-	-	-	-	-	-	-
L2-Co	0.25	15*	15	18	12	16	20	13	15	16	-
	0.50	15	15	16	12	16	15	15	16	13	-
	1.00	16	19	17	13	17	15	13	16	14	-
	2.00	16	19	18	13	18	17	13	16	13	-
L2-Cu	0.25	11	11	-	-	-	-	-	-	-	-
	0.50	11	11	-	-	-	-	-	-	-	-
	1.00	11	15	11	-	13	-	-	-	-	-
	2.00	12	16	13	18	15	-	13	-	-	-
L2-Mn	0.25	17	15	18	14	16	16	17	18	15	-
	0.50	20	19	18	18	20	21	18	21	19	-
	1.00	17	21	18	18	18	18	16	18	16	-
	2.00	19	19	18	18	22	18	17	18	18	13
L2-Ni	0.25	18	17	15	15	18	15	18	16	17	-
	0.50	19	15	18	17	17	17	18	17	18	-
	1.00	17	18	18	16	17	18	16	17	15	-
	2.00	18	18	18	16	18	14	19	18	16	-

*Inhibition zone in diameter (mm) around the disks.

-: no inhibition.

Table 7. Antimicrobial activities of L3 and their metal complexes

Treatments	Concentration (mg/mL)	Microorganisms									
		E. aerogenes	*B. brevis*	*M. luteus*	*E. coli*	*B. megaterium*	*P. aeruginosa*	*E. cloacae*	*S. aureus*	*C. albicans*	*S. cerevisiae*
L3	0.25	-	-	-	-	-	-	-	-	-	-
	0.50	-	12*	12	-	-	-	-	-	-	-
	1.00	-	18	12	12	13	-	-	15	-	-
	2.00	12	16	20	12	20	20	17	16	13	18
L3-Co	0.25	14	-	-	-	-	-	-	-	12	-
	0.50	16	-	12	13	12	-	13	-	12	-
	1.00	13	12	12	15	17	-	13	13	14	-
	2.00	20	19	19	18	21	15	16	18	16	12
L3-Cu	0.25	25	25	23	23	23	25	17	25	20	15
	0.50	25	25	23	25	23	25	22	25	23	15
	1.00	27	28	28	21	32	30	27	30	27	20
	2.00	28	29	29	30	32	30	27	30	27	17
L3-Mn	0.25	16	15	16	16	17	22	18	16	16	-
	0.50	22	19	17	17	20	18	18	24	18	12
	1.00	23	22	21	18	20	20	20	22	19	13
	2.00	24	25	22	18	22	22	20	24	19	13
L3-Ni	0.25	-	-	-	-	-	-	-	-	-	-
	0.50	16	14	12	12	-	13	15	16	14	-
	1.00	18	19	18	17	17	18	17	20	19	12
	2.00	20	23	21	17	22	20	18	22	20	12

*Inhibition zome in diameter (mm) around the disks.

-: no inhibition.

Table 8. The antimutagenicity assays results of the ligands

Test Materials	Conc. (μg/plate)	Number of revertants					
		E. coli WP2*uvrA*		*S. typhimurium* TA1535		*S. typhimurium* TA1537	
		Mean ± SE	Inh.(%)	Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)
MNNG**	1	372.00±11.29	-				
NaN_3**	1			453.00±13.31	-		
9-AA**	40					240.50±5.86	-
DMSO**(μl/plate)	100	25.50±1.28	-	22.33±0.95	-	13.50±1.17	-
L1	20	288.00±6.25	22.58	357.00±10.43	21.19	121.50±6.25	**49.48***
	40	315.50±8.78	15.19	318.00±8.12	**29.80***	116.50±7.84	**51.56***
	60	343.50±11.33	7.66	262.50±9.05	**42.05***	221.00±7.37	8.11
	80	351.00±12.15	5.65	299.00±9.15	**34.00***	212.50±6.67	11.64
	100	316.50±8.31	14.92	360.50±10.98	20.42	205.00±12.41	14.76
L2	20	250.50±12.29	**32.66***	264.50±9.68	**41.61***	220.00±13.36	8.52
	40	337.00±12.18	9.41	258.00±7.97	**43.05***	215.00±10.47	10.60
	60	348.50±8.64	6.32	304.50±6.73	**32.78***	243.50±7.70	-
	80	324.50±9.64	12.77	328.00±7.22	**27.59**	248.00±8.23	-
	100	271.00±11.40	**27.15***	349.50±11.43	22.85	226.00±10.08	6.03
L3	20	241.00±7.26	**35.22***	328.50±6.64	**27.48***	248.00±10.85	-
	40	236.00±11.10	**36.56***	318.50±9.65	**29.69***	201.50±9.42	16.22
	60	242.50±13.17	**34.81***	228.50±6.68	**49.56***	190.50±14.22	20.79
	80	223.00±14.40	**40.05***	246.50±8.49	**45.58***	188.50±6.62	21.62
	100	241.50±8.95	**35.08***	269.00±10.17	**40.62***	199.50±9.84	17.05

Conc.: Concentraion; Inh.: Inhibition

*Statisticaly different (p<0.05)

** MNNG, $NaN_3$and 9-AA were used as positive controls for *E. coli* WP2*uvrA, S. typhimurium* TA1535 and 1537 strains, respectively.

DMSO was used as negative control.

Table 9. The Antimutagenicity assays results of the Cu(II) complexes of the ligands

Test Materials	Conc. (µg/plate)	Number of revertants					
		E. coli WP2*uvrA*		*S. typhimurium* TA1535		*S. typhimurium* TA1537	
		Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)
MNNG**	1	372.00±11.29	-				
NaN_3**	1			453.00±13.31	-		
9-AA**	40					240.50±5.86	-
DMSO** (µl/plate)	100	25.50±1.28	-	22.33±0.95	-	13.50±1.17	-
L1-Cu	20	285.50±10.82	23.25	332.00±8.33	**26.71***	202.50±3,27	15.80
	40	278.00±9.10	**25.27***	342.00±7.54	24.50	245.00±10.39	-
	60	283.00±9.53	23.92	326.00±9.17	**28.04***	235.00±8.74	2.29
	80	336.50±10.42	9.4	361.50±23.12	20.20	188.00±5.87	21.83
	100	351.50±10,92	5.51	320.00±8.95	**29.36***	221.00±7.16	8.11
L2- Cu	20	226.50±9.21	**39.11***	354.50±12.38	21.74	132.00±4.17	**45.11***
	40	250.50±11.24	**32.66***	255.00±11.05	**43.71***	162.00±6.80	**32.64***
	60	245.50±8.35	**34.01***	257.50±12.31	**43.16***	175.00±7.71	**27.23***
	80	246.50±8.37	**33.74***	256.50±14.44	**43.38***	200.00±4.85	16.84
	100	348.50±8.54	6.32	340.00±14.86	24.94	203.50±5.36	15.38
L3-Cu	20	288.50±5.77	22.45	395.00±7.94	12.80	189.50±9,00	21.21
	40	265.50±9.01	**28.63***	385.00±6.61	15.01	101.50±4.56	**57.80***
	60	255.00±7.30	**31.45***	257.00±13.32	**43.27***	99.00±4.03	**58.84***
	80	264.50±6.19	**28.90***	333.00±8.79	**26.49***	90.50±3.21	**62.37***
	100	285.50±12.81	23.25	327.00±8.19	**27.81***	99.00±2.85	**58.84***

Conc.: Concentraion; Inh.: Inhibition
*Statisticaly different (p<0.05)
** MNNG, NaN_3 and 9-AA were used as positive controls for *E. coli* WP2*uvrA*, *S. typhimurium* TA1535 and 1537 strains, respectively.
DMSO was used as negative control.

Table 10. The Antimutagenicity assays results of the Co(II) complexes of the ligands.

Test Materials	Conc. (µg/plate)	Number of revertants					
		E. coli WP2*uvrA*		*S. typhimurium*TA1535		*S. typhimurium* TA1537	
		Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)
MNNG**	1	372.00±11.29	-				
NaN_3**	1			453.00±13.31	-		
9-AA**	40					240.50±5.86	-
DMSO** (µl/plate)	100	25.50±1.28	-	22.33±0.95	-	13.50±1.17	-
L1-Co	20	270.50±10.45	**27.28***	299.00±9.70	**34.00***	90.00±3.17	**62.58***
	40	284.50±15.61	23.52	265.50±14.06	**41.39***	90.00±5.13	**62.58***
	60	348.00±11.50	6.45	276.00±11.51	**39.07***	77.00±5.73	**67.98***
	80	273.00±11.28	**26.61***	321.50±8.73	**29.03***	71.00±3.54	**70.48***
	100	308.50±8.92	17.07	332.50±8.83	**26.60***	88.00±5.24	**63.41***
L2-Co	20	332.50±11.90	10.62	390.00±8.41	13.91	82.50±2.72	**65.70***
	40	385.00±9.29	-	290.00±7.59	**35.98***	92.00±5.59	**61.75***
	60	326.50±12.85	12.23	282.00±12.57	**37.75***	82.00±3.03	**65.90***
	80	257.00±7.33	**30.91***	335.00±11.98	**26.05***	87.00±4.41	**63.83***
	100	248.00±6.14	**33.33***	248.50±8.99	**45.14***	85.00±5.04	**64.66***
L3-Co	20	344.50±14.45	7.39	312.00±8.94	**31.13***	103.00±2.60	**57.17***
	40	348.00±11.27	6.45	244.50±14.23	**46.03***	97.00±7.42	**59.67***
	60	401.50±9.34	-	284.00±10.61	**37.89***	100.00±2.59	**58.42***
	80	341.00±9.73	8.33	304.00±8.74	**32.89***	94.00±3.95	**60.91***
	100	351.00±6.87	5.65	298.50±6.47	**34.11***	102.00±3.26	**57.59***

Conc.: Concentraion; Inh.: Inhibition
*Statisticaly different (p<0.05)
** MNNG, NaN_3 and 9-AA were used as positive controls for *E. coli* WP2*uvrA*, *S. typhimurium* TA1535 and 1537 strains, respectively.
DMSO was used as negative control.

Table 11. The Antimutagenicity assay results of the Mn(II) complexes of the ligands

Test Materials	Conc. (µg/plate)	Number of revertants					
		E. coli WP2*uvrA*		*S. typhimurium* TA1535		*S. typhimurium* TA1537	
		Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)
MNNG**	1	372.00±11.29	-				
NaN_3**	1			453.00±13.31	-		
9-AA**	40					240.50±5.86	-
DMSO** (µl/plate)	100	25.50±1.28	-	22.33±0.95	-	13.50±1.17	-
L1-Mn	20	270.00±15.76	**27.42***	319.00±7.62	**29.58***	76.00±7.90	**68.40***
	40	267.00±11.61	**28.23***	335.00±10.74	**26.05***	86.00±7.86	**64.24***
	60	347.00±8.01	6.72	339.00±11.52	**25.17***	79.50±6.60	**66.94***
	80	318.00±12.16	14.52	311.50±8.85	**31.24***	78.00±6.66	**67.57***
	100	272.00±8.49	**26.88***	317.50±7.01	**29.91***	69.00±4.53	**71.31***
L2- Mn	20	199.50±10.71	**46.37***	301.00±8.41	**33.55***	86.50±3.59	**64.03***
	40	219.00±9.34	**41.13***	288.50±8.65	**36.31***	71.00±3.90	**70.48***
	60	227.50±10.80	**38.84***	382.00±9.24	15.67	63.50±2.59	**73.60***
	80	241.50±7.12	**35.08***	377.00±9.05	16.78	78.50±5.91	**67.36***
	100	233.00±8.08	**37.37***	368.00±12.25	18.76	112.00±4.57	**53.43***
L3- Mn	20	337.00±13.45	9.41	397.00±13.56	12.36	113.00±5.27	**53.01***
	40	314.00±6.98	15.59	332.50±10.98	**26.60***	95.50±2.32	**60.29***
	60	281.00±9.52	24.46	278.50±13.55	**38.52***	67.50±3.14	**71.93***
	80	102.00±4.50	**72.45***	344.50±14.31	23.95	75.00±6.05	**68.81***
	100	65.00±5.57	**82.53***	328.50±11.68	**27.48***	98.00±2.58	**59.25***

Conc.: Concentraion; Inh.: Inhibition
*Statisticaly different (p<0.05)
** MNNG, NaN_3 and 9-AA were used as positive controls for *E. coli* WP2*uvrA*, *S. typhimurium* TA1535 and 1537 strains, respectively.
DMSO was used as negative control.

Table 12. The Antimutagenicity assay results of the Ni(II) complexes of the ligands

Test Materials	Conc. (µg/plate)	Number of revertants					
		E. coli WP2*uvrA*		*S. typhimurium* TA1535		*S. typhimurium* TA1537	
		Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)	Mean ± SE	Inh. (%)
MNNG**	1	372.00±11.29	-				
NaN_3**	1			453.00±13.31	-		
9-AA**	40					240.50±5.86	-
DMSO** (µl/plate)	100	25.50±1.28	-	22.33±0.95	-	13.50±1.17	-
L1-Ni	20	247.50±10.14	**33.60***	295.00±6.83	**34.88***	280.00±5.65	-
	40	257.00±9.58	**30.91***	294.50±9.94	**34.99***	205.50±5.98	14.55
	60	261.00±8.16	**29.84***	241.00±9.14	**46.80***	237.50±7.81	1.25
	80	261.50±10.04	**29.70***	311.00±13.94	**31.35***	203.50±9.49	15.38
	100	250.00±8.88	**32.80***	348.50±13.20	23.07	299.00±5.47	-
L2- Ni	20	138.50±6.27	**62.77***	266.00±12.95	**41.28***	259.00±4.23	-
	40	99.00±6.12	**73.39***	250.00±14.11	**44.81***	256.50±7.86	-
	60	110.00±14.53	**70.43***	261.50±13.18	**42.27***	111.50±6.19	**53.64***
	80	275.00±13.18	**26.08***	259.50±6.88	**42.72***	252.00±7.34	-
	100	310.00±7.21	16.67	270.00±9.12	**40.40***	195.50±6.88	18.71
L3-Ni	20	276.00±7.40	**25.81***	323.00±8.58	**28.70***	165.00±8.53	**31.39***
	40	332.00±16.56	10.75	329.00±6.99	**27.37***	161.50±8.75	**32.35***
	60	394.50±10.26	-	295.50±9.64	**34.77***	190.00±7.77	21.00
	80	375.50±9.90	-	308.50±10.09	**31.90***	181.50±5.27	24.53
	100	383.00±8.78	-	327.00±7.52	**27.81***	195.50±5.62	18.71

Conc.: Concentraion; Inh.: Inhibition
*Statisticaly different (p<0.05)
** MNNG, NaN_3 and 9-AA were used as positive controls for *E. coli* WP2*uvrA*, *S. typhimurium* TA1535 and 1537 strains, respectively.
DMSO was used as negative control.

Figure Captions

Figure 1. Synthesis of theligandsfromusnicacidandaminophenols.

Figure 2. ESI-MS spectra of the ligands (positive ion mode).

Figure 3. The HMBC correlations of the ligands.

Figure 4. Metal complex structures of L1, L2 and L3.

Figure 5. An ORTEP-3[3]drawing of L1 with the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level. Intramolecular hydrogen bonds are shown as dashed lines.

Figure 6. The crystal packing diagram of L1. Intra- and inter molecular hydrogen bonds are shown as dashed lines.

Figure 7. Antimutagenic effects of the ligands and their metal complexes in *S. typhimurium* TA1535

Figure 8. Antimutagenic effects of the ligands and their metal complexes in *S. typhimurium* TA1537.

Figure 9. Antimutageniceffects of the ligands and their metal complexes in *E. Coli* WP2*uvrA*.

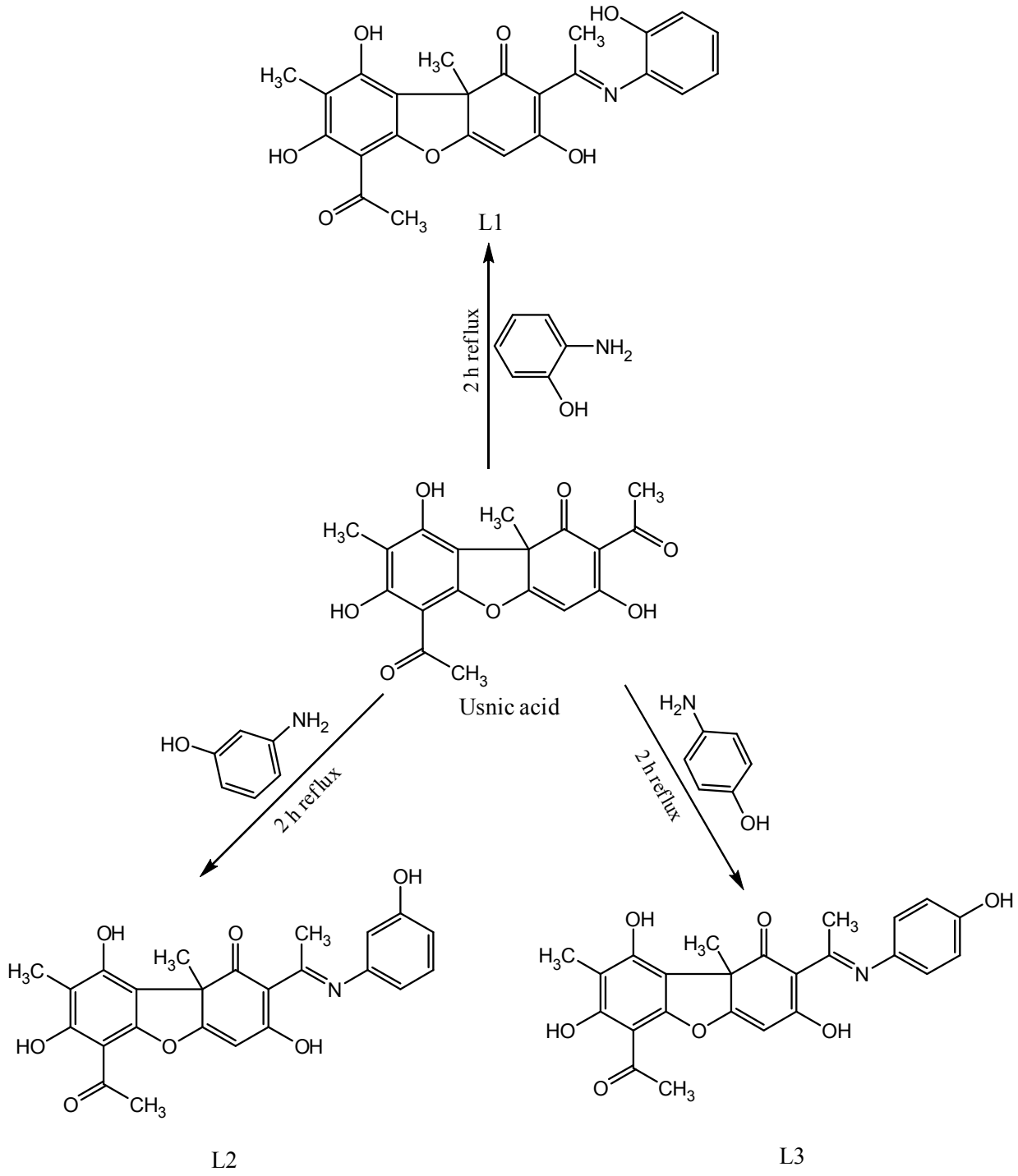
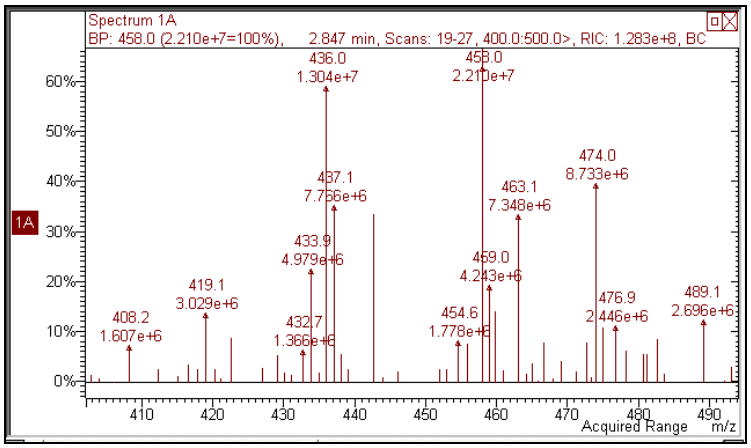
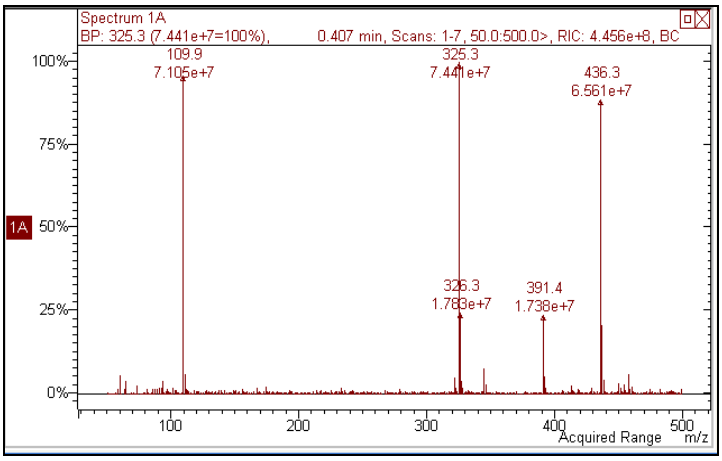


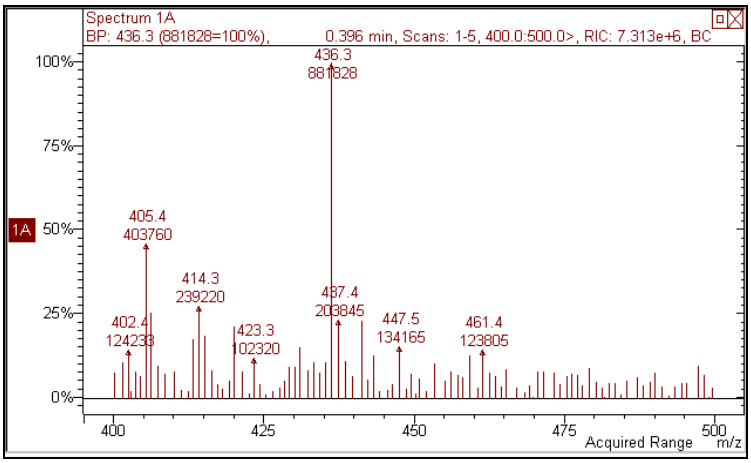
Figure 1. Synthesis of the ligands from usnic acid and amino phenols.



L1

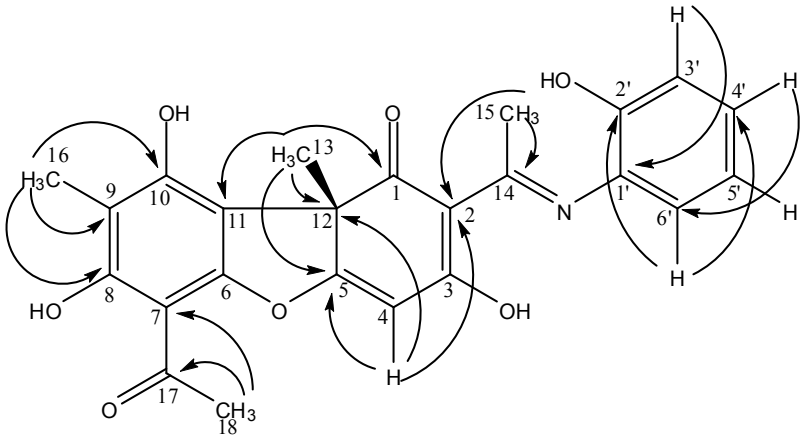


L2

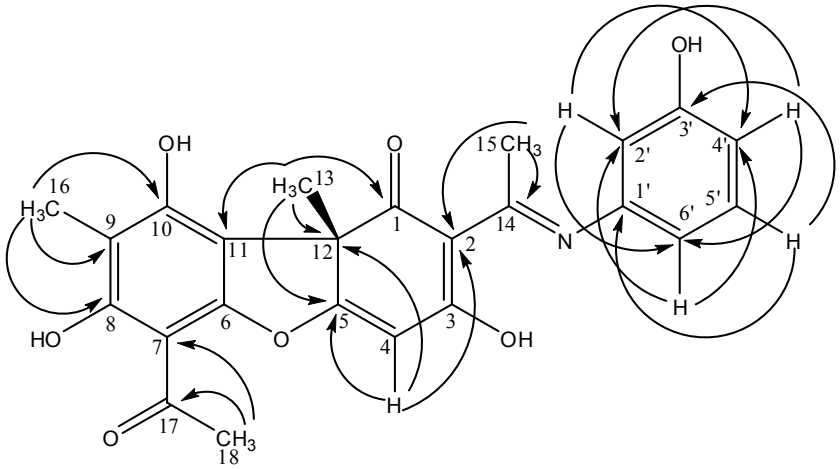


L3

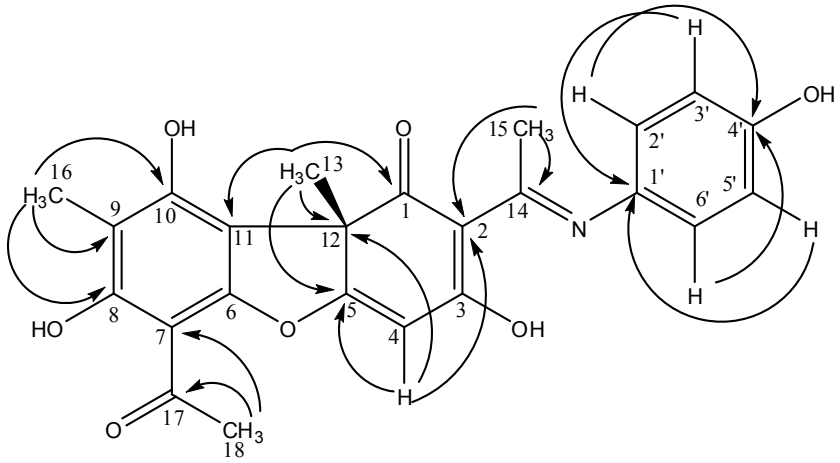
Figure 2. ESI-MS spectra of the ligands (positive ion mode).



L1

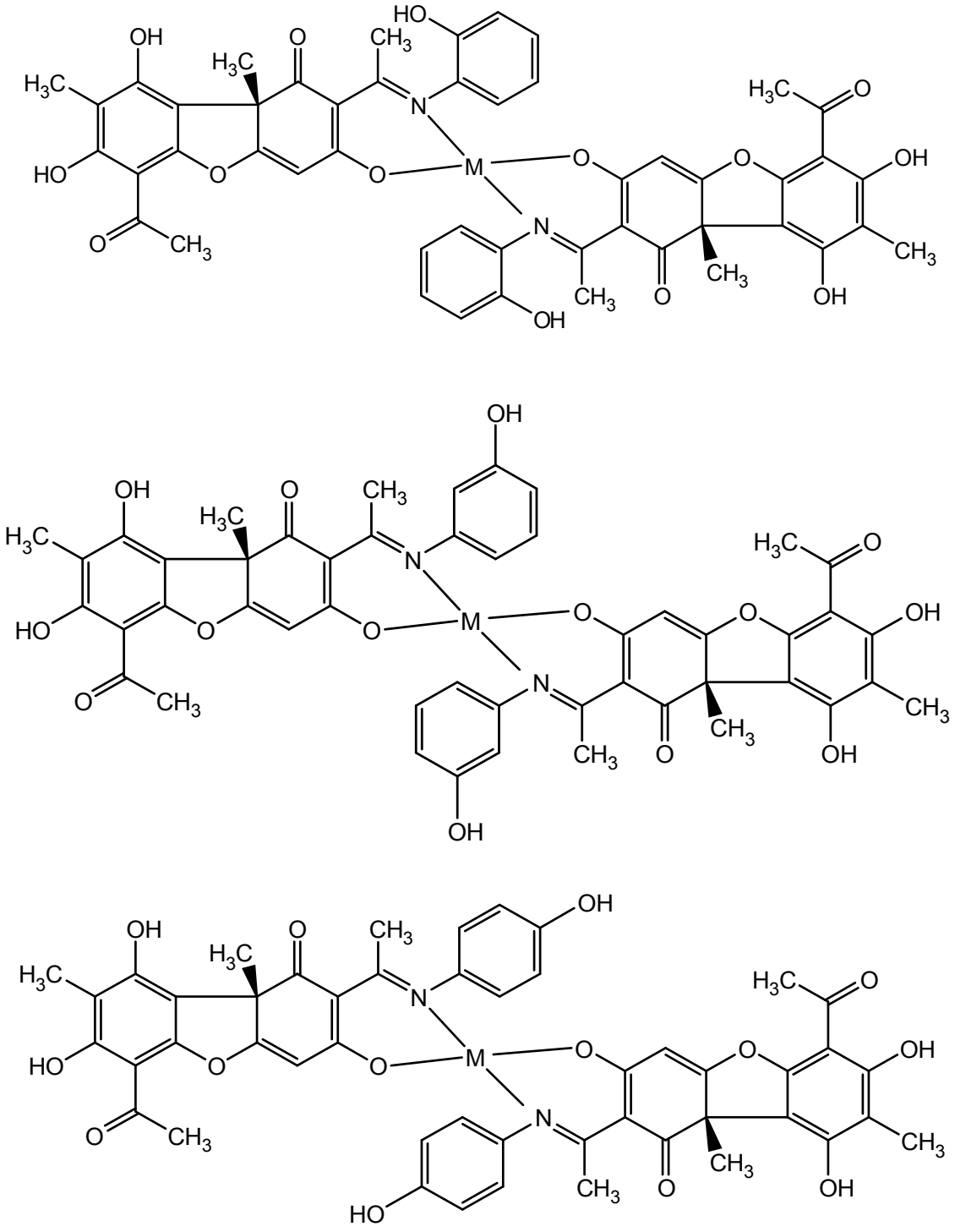


L2



L3

Figure 3. The HMBC correlations of the ligands.



M= Cu(II), Co(II), Mn(II), Ni(II).

Figure 4. Metal complexes of L1, L2 and L3.

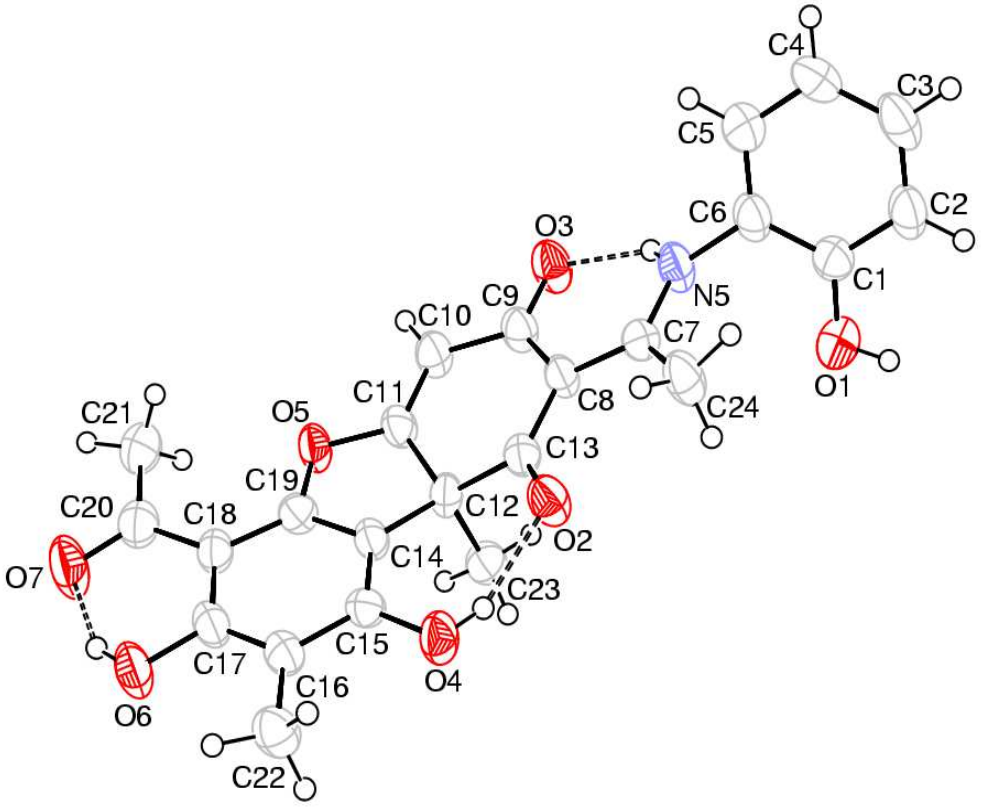


Figure 5. An ORTEP-3[3] drawing of L1 with the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probabilitylevel. Intra molecular hydrogen bonds are shown as dashed lines.

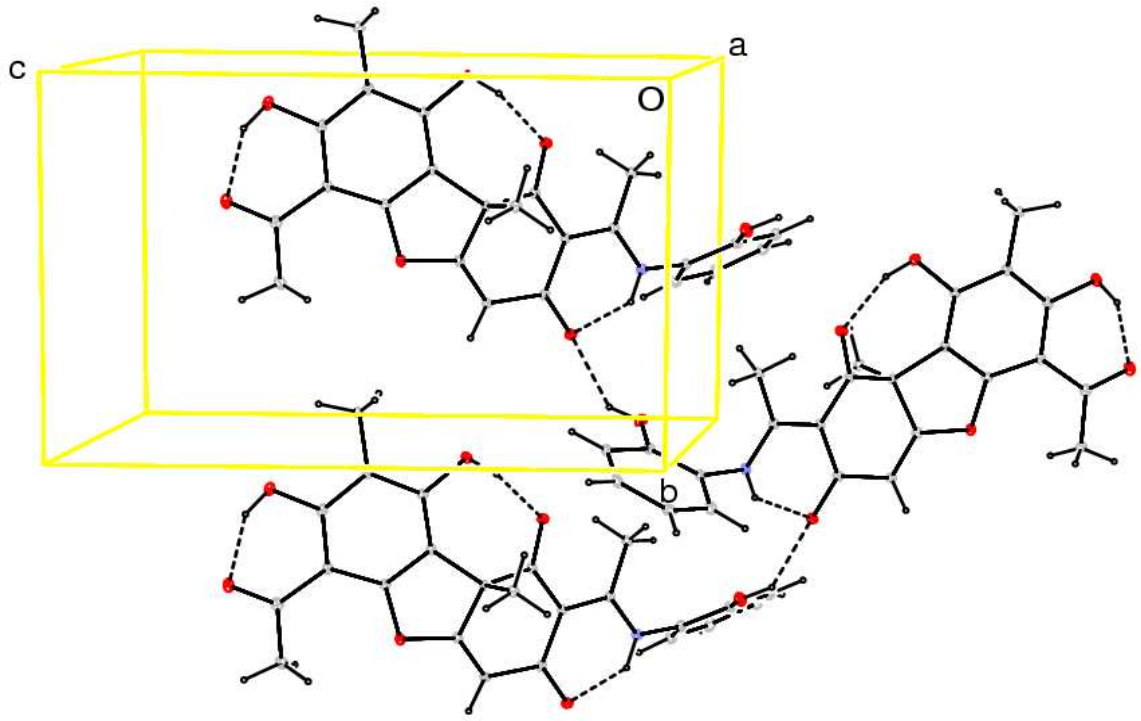


Figure 6. The crystal packing diagram of L1. Intra- and inter molecular hydrogen bonds are shown as dashed lines.

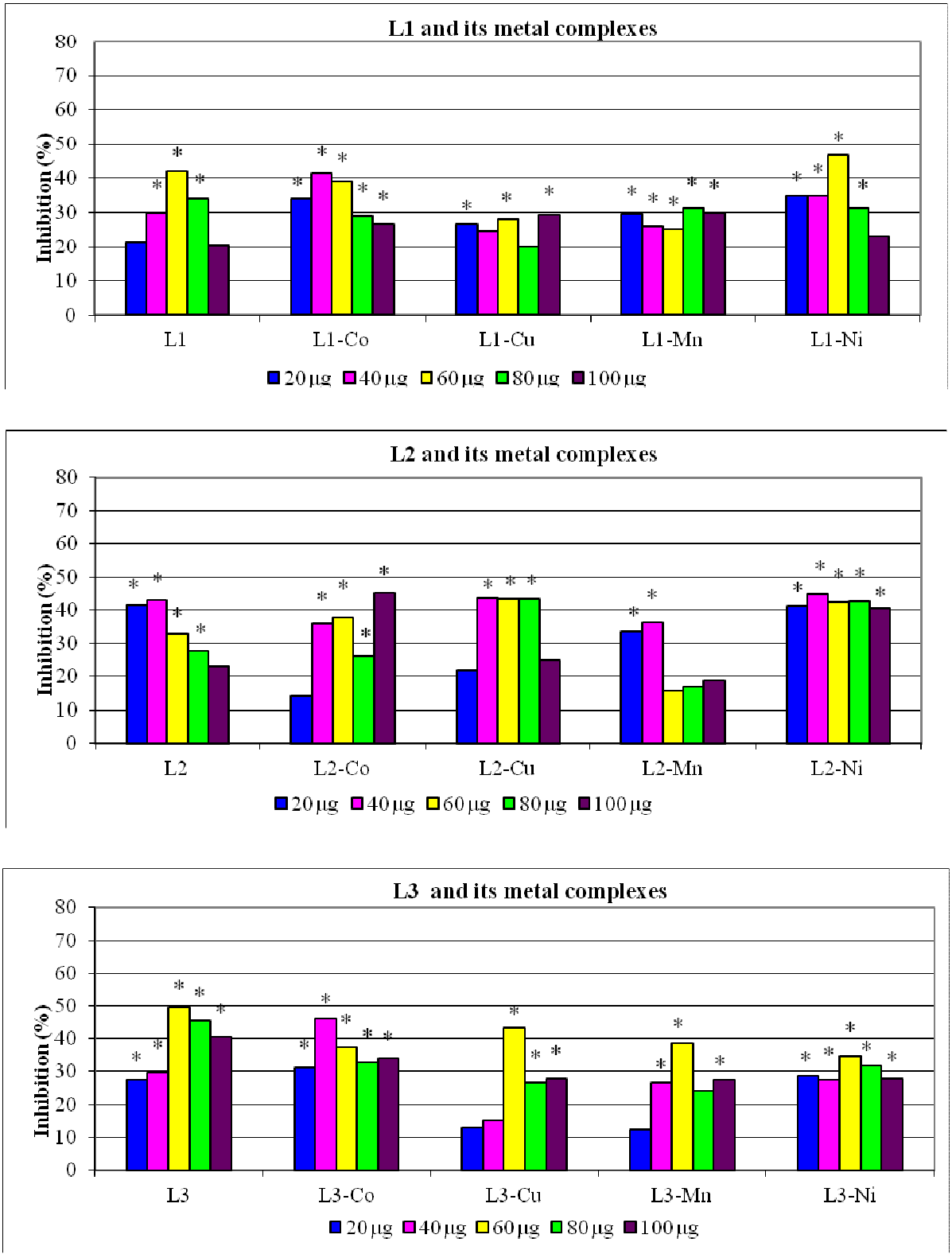
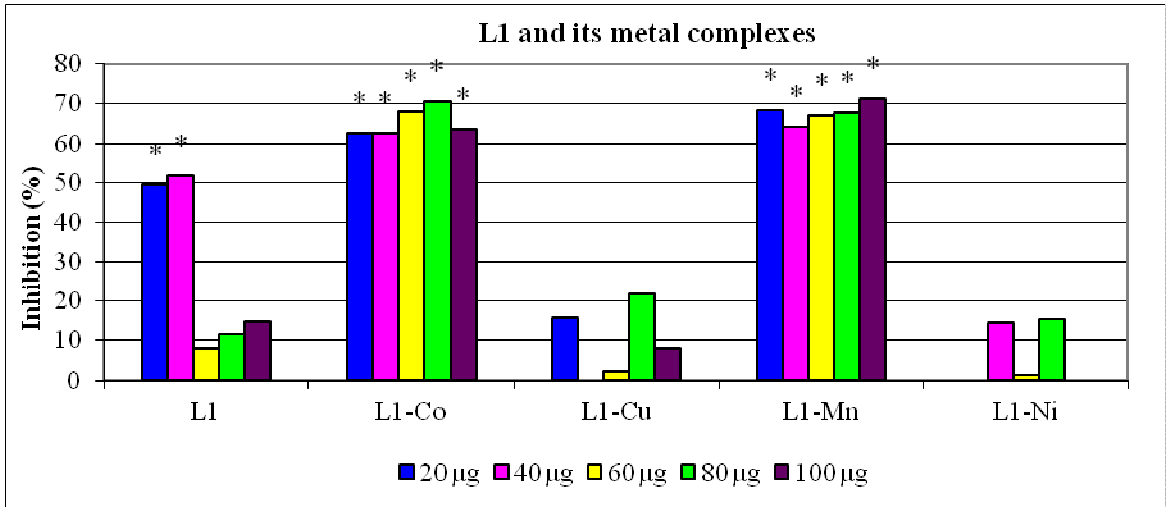
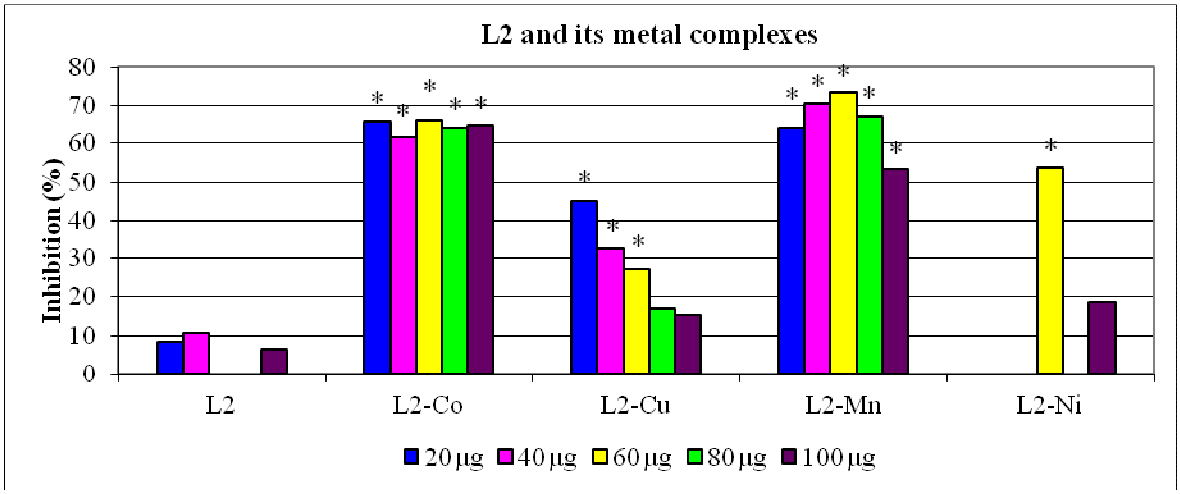


Figure 7. Antimutagenic effects of the ligands and their metal complexes in *S. typhimurium* TA1535





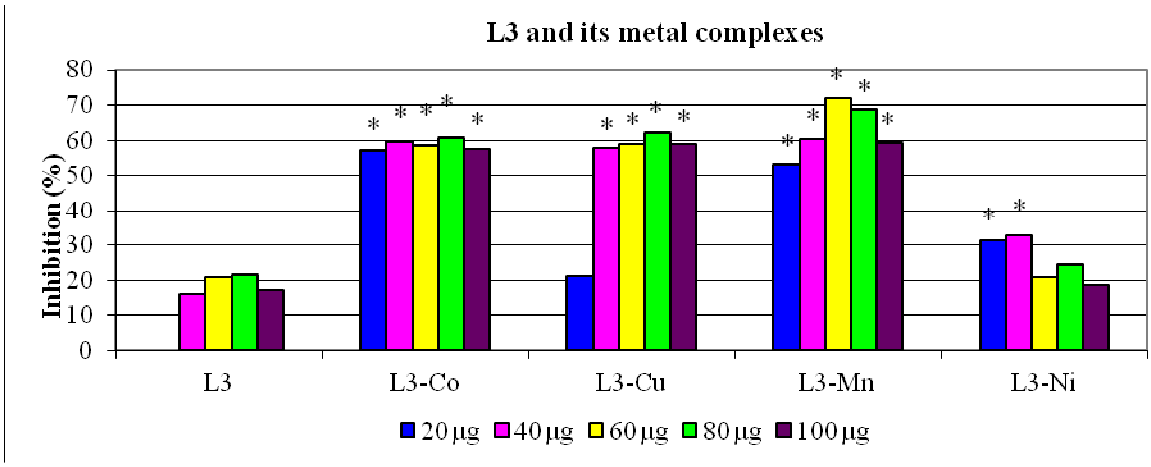


Figure 8. Antimutagenic effects of the ligands and their metal complexes in *S. typhimurium* TA1537.

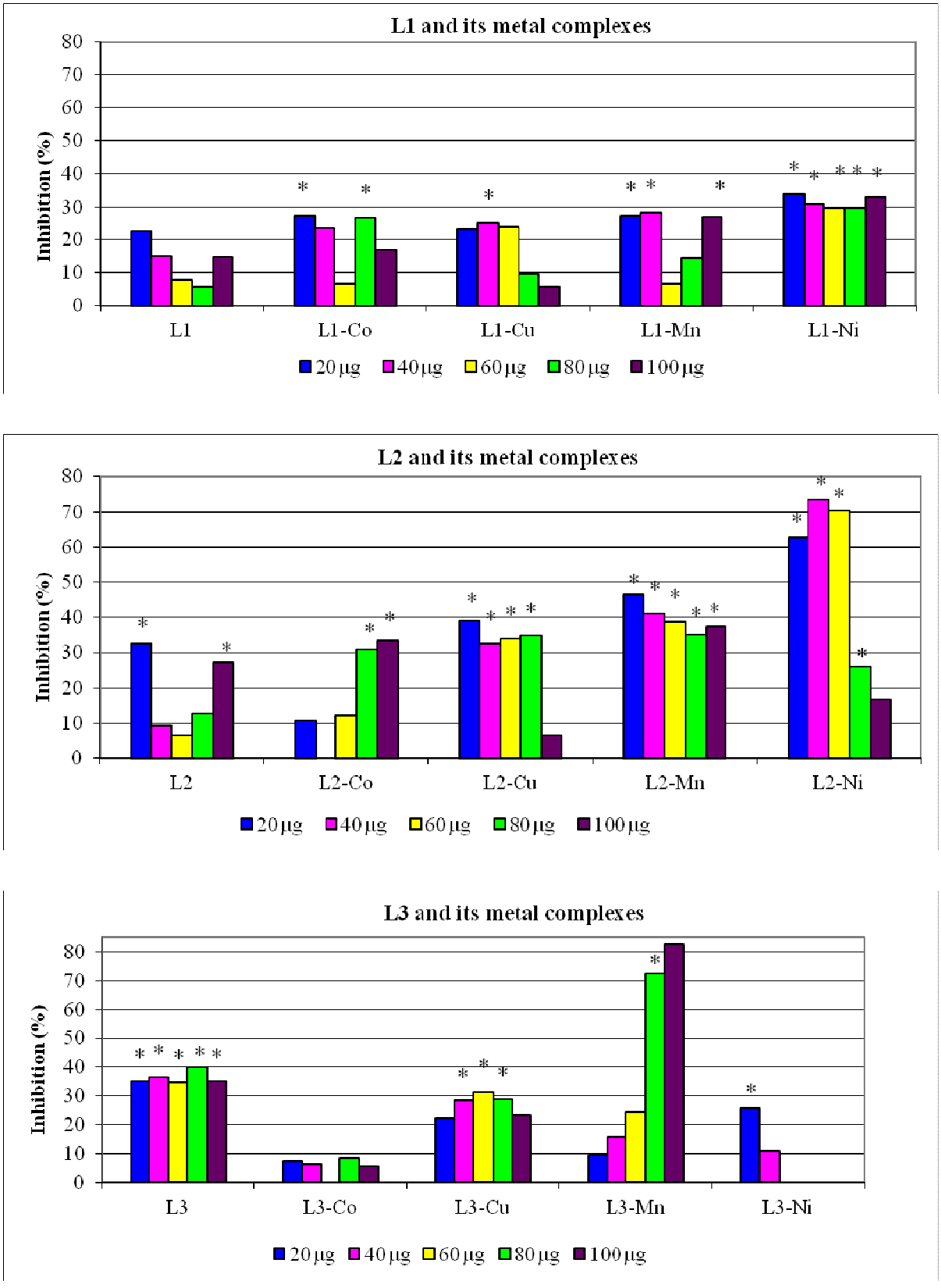
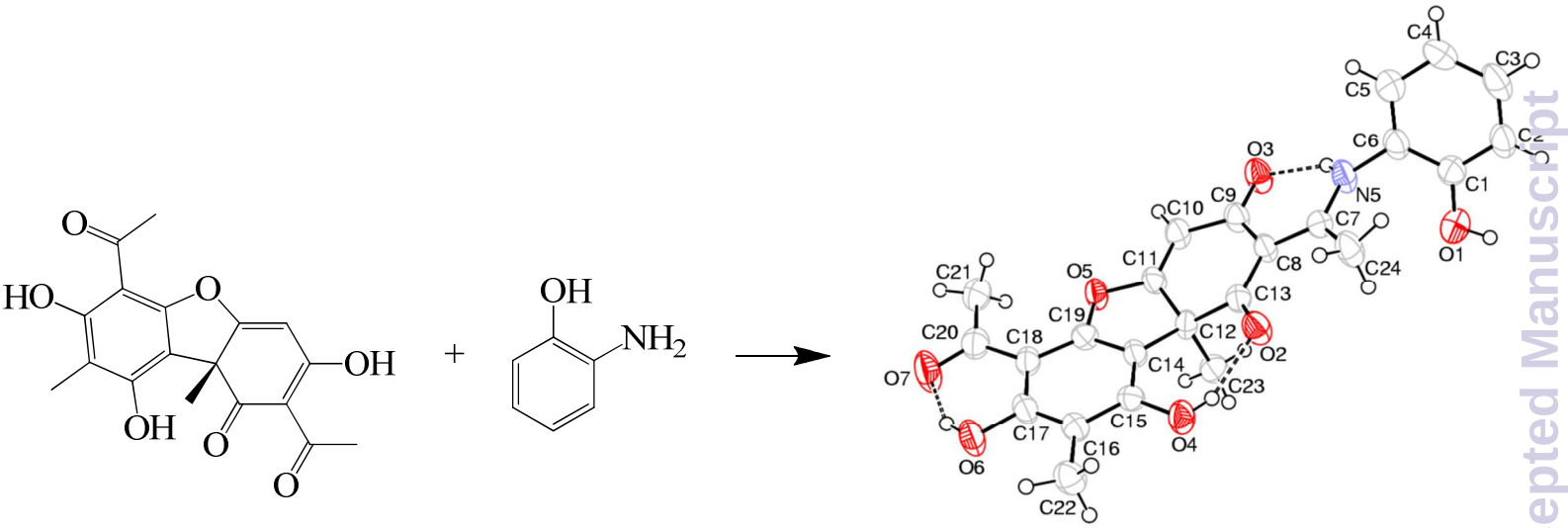


Figure 9. Antimutagenic effects of the ligands and their metal complexes in *E. coli* WP2*uvrA*.



ORTEP-3[3] drawing of novel multifunctional semi-synthetic *hydroxyphenylimino* ligand (L1).