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Schiff base method characterization of the newly synthesized Modified Levofloxacin Complex (MOLVX) and its activity against the HRT-18 cell line isolated from a male patient with colorectal adenocarcinoma

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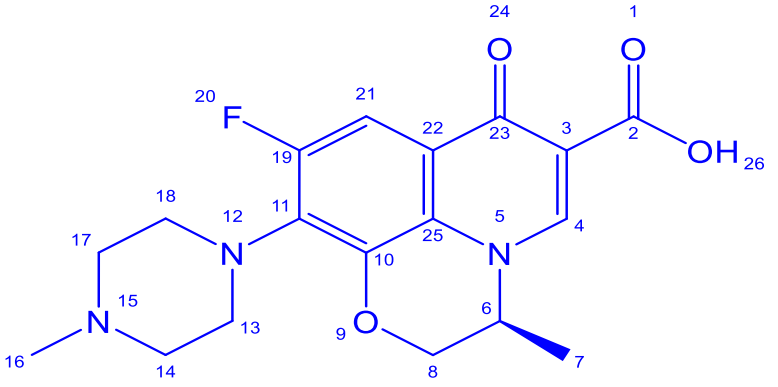
***Abstract*---**Complexes have been synthesized using 10mL methanolic solution of raw levofloxacin ($C_{18}H20FN_3O_4$) (0.0144g, 0.2mmol), 5mL methanolic solution of copper chloride dihydrate (II) ($CuCl_2\_2H_2O$) (0.068g, 0.17mmol,), and 20mL methanolic solution of 1,10-phenanthroline($C_{12}H_8N_2$) (132mg, 0.2mmol). The complex was characterized by the Schiff base method for detection of the modification of the structure of levofloxacin using Hydrogen-Nuclear Magnetic Resonance H-NMR, Fourier Transform Infrared Spectroscopy (FT-IR), UV–visible (UV–vis) spectrophotometry, and elemental analysis of the complex using CHN analysis. The dried precipitate appears as light green crystals which are ground well by a clean spatula and weighed. Yield: 340 mg; 99%. This complex and levofloxacin have been subjected to melting point checking which was 268-270, and 221℃ respectively. Elemental analyses were achieved and were calculated for [Cu (Levo.) (Phen)Cl]. Cl, ($C_{30}H_{33}Cl_2CuFN_5O_4$), molecular weight = 681.07 g/mol−1: C,53.29; H, 4.14; N, 10.36%. Found practically

analyzed: C, 52.554; H, 3.921; N, 9.55%. Furthermore, this newly synthesized modified levofloxacin complex MOLVX exhibits a highly cytotoxic effect on the colorectal cancer cell line HRT-18 with different concentrations (3.125, 6.25, 12.5, 25, 50, and 100 µg/mL for 72 hours of treatment, and the value of IC50 for the HRT18 cancer cell line was estimated at various MOLVX concentrations, with IC50 values ranging from (1.407–13.040µM) and the IC50 was 4.285µg which is the concentration of the compounds or drugs that inhibits cell viability or survival by fifty percent.

***Keywords*---**HRT-18 cell line, Modified Levofloxacin Complex MOLVX, Colorectal Adenocarcinoma.

Introduction

When compared to other cancers, colorectal carcinoma is regarded as having the second-highest death rate in the world(Eisenach et al., 2013). Quinolones are widely prescribed due to their broad spectrum of activity and safety. The mode of action is mediated by Inhibiting the critical DNA replication enzymes including DNA gyrase and topoisomerase IV(Andriole, 2005). Levofloxacin is the synthetic racemic isomer mixture of quinolone ofloxacin which is 3rd generation quinolone antibiotic that is used to treat bacterial infections Levofloxacin is significantly more active against Levofloxacin is a broad-spectrum antibiotic, available as the oral and intravenous dosage form. It is a chiral fluorinated carboxyquinolone, levofloxacin, that is pure ofloxacin, the racemic drug substance (-)-(S). The chemical name is (-) -(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl1-piperazinyl)-7-oxo-7Hpyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate. The structure is revealed in (figure 2.5) (Al-Soufi & Al-Rekabi, 2018).



levofloxacin

Chemical Formula: $C_{18}H_{20}FN_3O_4$

Molecular Weight: 361.37

Elemental Analysis: C, 59.83; H, 5.58; F, 5.26; N, 11.63; O, 17.71

Figure (2.5): chemical structure and formula of levofloxacin.

(Aldred et al., 2014; Takács-Novák et al., 1990). Lung cancer cell lines respond well to Levofloxacin, which induces apoptosis and inhibits proliferation (Song et al., 2016). When levofloxacin inhibits the prokaryotic type II topoisomerase and DNA gyrase enzymes, it may cause the cells to be destroyed by creating a large amount of double-strand DNA breaks, which may be useful in cancer treatment. Transient double-stranded breaks are generated in intact DNA helixes by the enzyme DNA gyrase. This results in the formation of a new DNA segment (Bax et al., 2010). Eukaryotic species also need a type II topoisomerase for viability, just like bacteria. “The sequences around activated tyrosine residues in type II topoisomerases of bacteria and mammals appear to share a common homology” (Rajabalian et al., 2007). Topoisomerase II is frequently affected by levofloxacin in mammalian eukaryotic cells. The mechanisms of levofloxacin's antitumor cell killing are similar to those of fluoroquinolone antibacterial agents. Fluoroquinolone antitumor drugs appear to have similar cell-killing mechanisms to quinolone antibacterial agents (Yamashita et al., 1992). As a result, it has been demonstrated that antibacterial Fluoroquinolones have a cytotoxic effect on cancer cells (Kloskowski et al., 2011). DNA-gyrase (topo II) is made up of two subunits that are encoded by the letters GyrA and GyrB, and its function is to induce negative supercoils into DNA, thereby accelerating the separation of daughter chromosomes from their parents. Two ParC subunits and two ParE subunits are found in DNA topoisomerase IV, which is responsible for the decatenation of DNA, to allow it to divide into two new daughter cells (Hooper & Jacoby, 2016). DNA Topo IV is made up of four subtypes; 2 ParC and two ParE. levofloxacin interacts with the DNA-enzyme complex, resulting in the formation of a levofloxacin-DNA-enzyme-complex that prevents further progression of the DNA-enzyme complex and the replication process (Hooper & Jacoby, 2016; Schaumann & Rodloff, 2007). A metallic enzyme known as topoisomerase II is responsible for regulating the topology of DNA. It does this by cleaving a double DNA strand and relegating them. These transitory actions are essential for the release of supercoil tension and the untying of DNA knots that occur during various processes of replication, transcription, recombination, and repair processes that involve the separating of DNA strands. As a consequence of this, topoisomerase II is an essential target for therapeutic antibiotics e.g., fluoroquinolones and anticancer drugs such as anthracyclines (Palermo et al., 2013). Even though Topoisomerase II enzymes are biologically important and have been identified as potential drug discovery targets, the cleavage and relegation of DNA by a metal-mediated process is still not completely understood (Sutormin et al., 2021). Several studies have been conducted to recognize the structural characteristics and features that permit fluoroquinolones to transition from antibacterial to anticancer agents (Sissi & Palumbo, 2003). It was found that altering the carboxylic acid at position 3 or the basic group at position 7, or both, was an efficient modification for reducing the zwitterionic feature of fluoroquinolones. Another modification was discovered at position 7 of the quinolone molecule, and this one involved increasing the number of condensed and aromatic rings. Other modifications have a little or greater effect on quinolone molecules. It has been demonstrated that a cyclopropyl group, as opposed to an ethyl moiety, results in a significant increase in the amount of activity that can be achieved by the ring nitrogen in position (Sissi & Palumbo, 2003). In the meantime, researchers found that substituting phenyl and thiazolyl rings in this

position resulted in positive outcomes. In position 3, there ought to be a substituent that is in the same plane as the main quinolone nucleus (Abdel-Aal et al., 2019). Furthermore, Other researchers have discovered that altering the structure of the molecule at position 4 by adding an alkoxy group or amino substituent can produce highly effective anticancer agents (Newmark et al., 2009). Substitution at position 5 has the potential to alter the overall steric configuration and geometries of the compound. This, in turn, has an impact on cell penetration and drug binding to its binding site (Llorente et al., 1996). Although fluorine is the most important substituent for anticancer activity at this position, the absence of any substituent at position Carbone 6 may enhance antitumor activity, according to a published study (Atanasova et al., 2007). An investigation into the cytotoxicity of simple fluoroquinolone" ethyl 6-fluoro-8-nitro-4-oxo-1,4 dihydroquinoline-3-carboxylate1", was carried out using two different cell lines, one of which was cancerous and normal cell line. After 48–72 hours of incubation, the higher concentrations applied (40–100 M) resulted in a complete cessation of cell growth in all of the cell lines that were examined. It has been demonstrated that inhibiting cell growth by inducing apoptosis through the activation of caspases "9 as well as 3" (Jantová et al., 2018). The use of aryl and alkyl carbamoyl methyl derivatives in place of ciprofloxacin at the piperazinyl N4 position resulted in powerful antiproliferative compounds against non-small cell lung cancer. In the A549 cell line, A cell cycle study revealed that over-expression of Cyclin-Dependent Kinase inhibitor 1 (p53/21) and down-expression of cyclin B1 "Cdc2/B1 which is a protein required for mitotic initiation" resulted in arresting of the cell cycle at the G2/M before mitosis (H. H. H. Mohammed et al., 2016). Numerous studies have examined the interactions of transitional metal ions with a variety of deprotonated fluoroquinolones (Efthimiadou et al., 2007). Many metal ions can form stable coordination compounds with levofloxacin, making it a promising drug for antibacterial use. This in vitro chelation potential is formed in the following order is "Al + 3 > Cu + 2 > Zn + 2 > Mg + 2 > Ca + 2." Metal ions can form complexes with these compounds for both biological and analytical purposes, which is a crucial feature (Măciucă et al., 2020). Copper(II) is an important pharmacological and physiological agent in biological systems (Ramakrishnan et al., 2009). Several Cu(II) complexes have been identified as potential anticancer drugs, with in vitro and in vivo activity(Wu et al., 2005). Phenanthroline was used to synthesize several mononuclear Cu (II) complexes, which were tested for their DNA-binding and cytotoxic properties (Cai et al., 2007; Goswami et al., 2011). The interaction of transitional metal ions with Deoxy-ribonucleic acid has been extensively studied because these ions can serve as independent models for biological systems (Patel et al., 2011). Among the copper complexes, the copper (II) complexes containing the 1,10-phenanthroline ligand have received the most attention due to their high nucleolytic efficiency, anti-tumor action, and antibacterial activity (Lakshmipraba et al., 2015). The current study aimed to synthesize a new modified levofloxacin complex and characterization of this complex. In addition to an assessment of their biological effect in vitro study on the colorectal cancer cell line.

Methodology

Materials and tools:

For the synthesis of a new modified levofloxacin complex, we used pure levofloxacin Hymihydrate 99.54% TaviPharma-Holland (batch. No.: DK21-1902202-II, CODE. No: W. S 041, done by: ENG. OLA), Copper chloride dihydrate (BDH, UK), 1,10-Phenanthroline (Phen), (332.40, Kanto chemical- No. 910E0162, Japan), Absolute Methanol (Sigma-Aldrich, UK), Potassium Chloride (KCL – BDH, UK), Potassium Hydroxide (KOH- BDH, UK). Dimethyl Sulfoxide (DMSO- BDH, UK), Diethyl ether (Sigma-Aldrich, UK), and Paraffin wax paper for covering classes (Himedia, India). PH meter (Toledo, Switzerland), Refrigerator (LG- S. Korea), Sensitive Balance (Sartorius, German), UV visible spectrophotometers-electronic absorption (Shimadzu 3600 UV–Vis-NIR spectrophotometer- German), FTIR spectrophotometers (Perkin-Elmer Spectrum BX, USA), Water Bath (Electrothermal-England), Water distillatory (Cotterman- Switzerland), spectrophotometers PerkinElmer Lambda-25 spectrophotometer, German), NMR spectrophotometers (BRUKER- VARIAN INOVA, USA), Nitrogen/Carbon/Hydrogen/Sulphur Analyzer (Flash Smart Elemental Analyzer-Thermo scientific, German), Milting point apparatus (UK), Magnetic Stirrer, Incubator (Memmert, German), Autoclave (Gallenkamp, German), Hotplate, Hettich, CHINA), Condenser GLASS (Bayer, Germany), Beaker (30mL, National, China), Atmos paper- (Chine-lab, China). Pasture Pipette (Slammed, German), Mortar glass (Chine-lab, China), Spatula (National, China), flask (5,10,25,100 Ml) (BYER, German).

Methods:

Synthesis of the complexes

This complex was produced by gradually adding 5mL of a methanolic solution of copper chloride dihydrated (CuCl2_2H_2O)(0.79 mmol, 0.068 g) (solution 1) into a methanolic pure levofloxacin solution of 10mL 0.0144 g, 0.2 mmol $C_{18}H_{20}FN_3O_4$ (solution 2), that underwent deprotonation by the wise drop (1-2.5 mL) of KOH by pasture pipette or until to be an alkaline solution with pH (7.4-7.8), and the solution mixture was continuously stirred by the magnetic bar 30 minutes at room temp. Thence, we prepared (solution 3) which may be completely let dissolving 0.0132 g (0.2 mmol) 1,10-phenanthroline (Phen) ($C_{12}H_8N_2$) (132 mg, 0.2 mmol) in 20 mL methanol, so, the final solution was prepared by adding solution 1 into solution 2, then, solution 3 was added to the mixed solution (solution1 and solution2) then the mixture was continuously stirred (refluxing) using a condenser linked with hotplate at approximately 55-60 0C for 2hrs. The complex was then left at room temperature until the next day. On the next day, the final mixture was washed with some drops of methanol at cold temperature (ice-containing container) with a glass stirrer. Then, the mixture is wrapped in paraffin paper and put in the fridge for 3 days. Then, washed with some drops of diethyl ether to precipitate the particles of the substances to the bottom of Beeker. Then, leave it at a cool temperature to dry. The dried precipitate appears as light green crystals which are ground well by a clean spatula and weighed. We

obtained the final yield of MOLVX which was dark green crystals, 340 mg; 99%. M.P., 268-270 °C. CHN-calculated analysis for [Cu(lvx)(phen)Cl]. Cl, ($C_{30}H_{33}Cl2CuFN_5O_4$) M.W = 681.07 g/mol−1: C,53.29; H, 4.14; N, 10.36%. CHN-practical analysis was: C, 52.554; H, 3.921; N, 9.55%.

The Elemental Analysis using the CHN test was carried out by using an "Elementary System GmbH Vario EL-III spectrometer instrument complexes" (Electronic Absorption-UV-visible spectra lex) were recorded in solution on a PerkinElmer Lambda spectroscope. The complex fluorescence spectral (FTIR) data were recorded in solution using a Perkin-Elmer Spectrum BX. Finally, the structures of the molecules of levofloxacin and copper-1,10-phenanthroline were built using the Perkin Elmer software ChemBio3D (B Cambridge Software).

Characterization of MOLVX

Melting point:

Place a small and appropriate amount of the complex in a capillary tube that is closed at one end placed in a melting point meter and record the temperature at which the mixture turns into a liquid.

FTIR Fourier Transform Infrared Spectroscopy:

FTIR is an acronym that stands for "Fourier Transform infrared," and it is the method of infrared spectroscopy identification (qualitative analysis) that is most commonly used for all of the various types of materials. FT-IR analysis of ligands and complexes provided information on coordination mode. Due to the numerous functional groups in fluoroquinolone molecules, their infrared spectra are complex, so their interpretation is based on typical vibrations(Turel, 2002). During the analysis of the complex and pure levofloxacin, Infrared spectra were recorded between 4000 and 400 cm−1 in a wavelength where we prepared the KBr disc for the levofloxacin and cesium iodide disc for MOLVX(Imran et al., 2010).

UV–visible (UV–vis) spectrophotometry:

Stock solutions (34 x 10-4M) of MOLVX were prepared in methanol(5X103M), and their UV-vis spectra were obtained in the range 225-400nm for indicating the presence of levofloxacin band and between 400-1000nm for indicating for copper particles. The maximum wavelengths of levofloxacin-based copper-phenanthroline ligand have shifted slightly bathochromically, a phenomenon that is indicative of complex formation. Spectra of solutions were acquired during 3-4h. The solution was left at room temperature and exposed to light. Furthermore, also the solution was irradiated for 30min. with UV radiation (250-280). All solutions were stored in the dark at 4 degrees Celsius and were tracked over time. Changes in the spectra, most notably in the 287 nm band, indicating that the complex is not stable in solution. Despite being a crystal form, the compound retained its stability(Vogel, 1961).

Hydrogen-Nuclear Magnetic Resonance:

Hydrogen-NMR spectroscopy determines a compound's molecular structure. NMR can provide quantitative and qualitative compositional information. This procedure can be used for quality control, research, or identifying an unknown. The instrument used to record the H NMR spectra was a Mercury300BB, and the frequency used was 300 MHz. As a solvent, Dimethylsulphoxide, also known as DMSO-d6, was utilized, while tetramethyl saline, often known as TMS, served as an internal reference (Mabbs & Machin, 2008).

Invitro study

MTT assay:

The cytotoxicity of modified levofloxacin (MOLVX) was tested against a colorectal cancer cell line. HRT-18 cells are colorectal cancer cells isolated from the large intestine of a 67-year-old male adenocarcinoma patient obtained from the Iraqi Center for Cancer and Medical Genetic Research of Al-Mustansiriyah University. These cells can be used for oncology and toxicological research benefit from these cells, and highly put screening can be performed on them.

Materials for MTT assay:

HRT-18 colorectal cell line, Trypsin and EDTA (CAPRICORN, GERMANY), RPMI (Roswell Park Memorial) 1640(CAPRICORN, GERMANY), PI (propidium iodide)-(SIGMA, USA), MTT(methylthiotetrazolium)stain (BIO WORLD, USA), FBS (Fetal bovine serum)-(CAPRICORN, German), Dimethyl sulfoxide (DMSO)-(SANTACRUZ BIOTECHNOLOGY, USA), AO (Acridine orange)-(SIGMA, USA), Microtiter (MT)reader (GennexLab, USA), Micropipette ((Cypress Diagnostic, Belgian), (LFH)Laminar flow hood (K and K Scientific Supp., Korean), (IM)Inverted microscope (OLYMPUS, USA), (FM)Fluorescent microscope (OLYMPUS, USA), Carbon dioxide incubator ((Cypress Diagnostic- Belgian), Culture plate ((Santacruz Biotechnology, USA).

Maintenance of cell cultures:

MEM was supplemented with 10 percent fetal bovine, 100 units/mL penicillin, and 100g/mL streptomycin, and was used to cultivate HRT18 cell lines. Trypsin/EDTA was used to pass the cells, and they were reseeded at 50 percent confluence twice a week while being maintained at 37 degrees Celsius (Attoub et al., 2018).

Assay of Cytotoxicity:

The modified levofloxacin (MOL) that was synthesized for this investigation was subjected to a cytotoxicity test using the MTT cell viability assay. The test was carried out on HRT18 Adenocarcinoma cells. The number of cell lines that were seeded in each well was one times 104. After twenty-four hours had passed or a confluent monolayer had been formed, the cells were subjected to treatment with

modified levofloxacin complex (MOLVX) at varying concentrations. After 72 hours of treatment, the cell viability was determined by removing the medium, adding 28 L of a solution of MTT stain with a concentration of 2 mg/mL, and then incubating the cells at 37 °C for three hours. Following the removal of the MethylTetraTiozolium solution, the crystals that were still present in the wells were solubilized by the addition of 130 L of DMSO, which was then followed by an action at 37 °C for 15 minutes while the wells were being shaken (Al-Ziaydi et al., 2020). The assay was performed in triplicate and the absorbency was measured using a microplate reader set to 492 nm. The percentage of cytotoxicity (inhibition of cell growth) was computed as follows:

- Cell growth or cell viability% (c.v) equal to (=) Absorbance in test wells(mean)/ Absorbance in control wells(mean) multiplying 100.

- Cell growth-inhibitory rate (cytotoxicity%) a:

Cytotoxicity (IR) = A-B divided by (A*100), where A= mean-optical density of control by DMSO, and B= mean-optical density of treated wells by newly synthesized Modified Levofloxacin (MOLVX).

Statistical analysis:

GraphPad Prism (GPP)9 was used to perform a statistical analysis of the collected data (M. S. Mohammed et al., 2019). Triplicate measurements were used to calculate each value's mean ± standard deviation (SD).

Result and discussion:

Synthesis and identification of the complexes [Cu(Levo.)(Phenan.) Cl]

Fluoroquinolones contain two ionizable functional groups: a carboxylic acid group and a piperazinyl group. A new ring is introduced between positions 1 and 8 of Levofloxacin, causing a change in nomenclature and adjusting the substituent positions to match the new given potential nomenclature. We successfully created a new copper II cationic complex with the antibiotic levofloxacin and the (2N heterocyclic) compound 1,10-phenanthroline (phen). The complexes' molecular structures were determined using proton Nuclear Magnetic Resonance (hydrogen-1 NMR), Fourier-transform infrared spectroscopy (FTIR), a Carbon-Hydrogen-Nitrogen elemental test, and spectroscopic examination (Ultraviolet-Visible). The coordinated form of the complex produced in the form that we observed was deduced based on the evidence that we worked on and obtained, where was the distorted square pyramidal shape, levofloxacin acts as the bidentate compound that coordinates the copper II cation through the carbonyl and carboxyl oxygen molecules, while phenanthroline acts as the coordinator for two nitrogen atoms, formation of the equatorial level. An(O-atom) from a levofloxacin molecule typically occupies the Penta coordinated Cu II center's fifth position axial direction. In the other words, the cupper plays a central role in the binding and coordination of two ligands used in the synthesis of the new complex by two nitrogen atoms of phenanthroline from one side, on the other side, with two

oxygen atoms of the ketone and carboxylic acid group after losing their hydroxyl group of the structure of levofloxacin drug. (Fig.

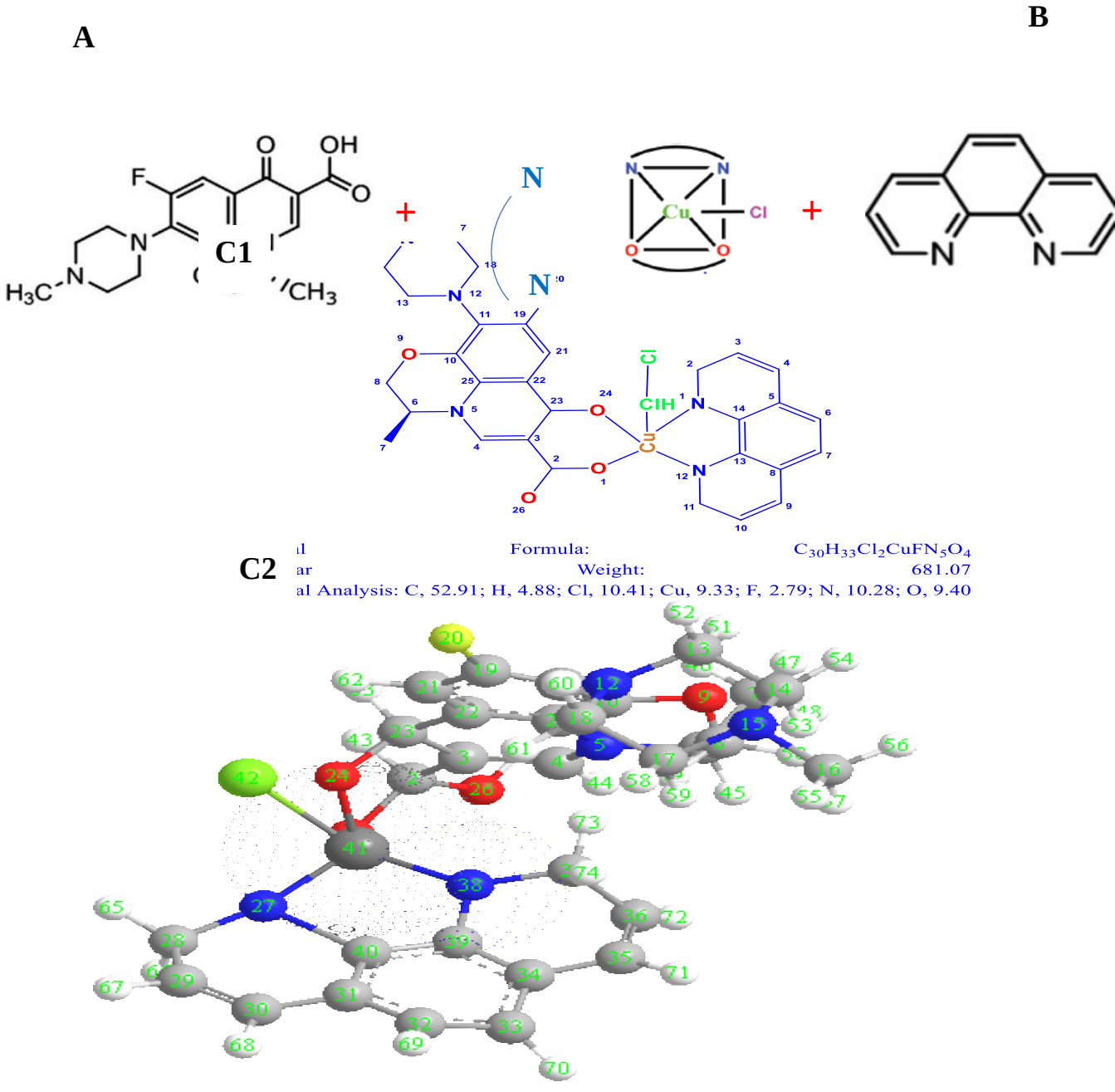


Figure (1.): (A)- Structure of levofloxacin, (2S)-7-fluoro-2-methyl-6- {4-methyl piperazin-1-yl}-10-oxo-4-oxa1-azatricyclo {7.3.1.05,13} trideca-5,7,9-(13),11 – tetraene -11- carboxylic-acid($C_{18}H_{20}FN_3O_4$), MW361.368 g.mol^{-}; and **(B)-** 1,10-phenanthroline**; (C1;** potential coordination of the mixed compound copper-II complexes with levofloxacin and (Phenanthroline), MOLVX, Proposed temporary name of the complex. ($C_{30}H_{33}Cl2CuFN_5O_4$) MW = 681.07 g/mol−1**;(C2)** 3D structure of MOLVX.

In our study, we have synthesized this complex by following Schiff bases substitution and characterized by Physiochemical that mentioned on the previous page, the study's results revealed that levofloxacin coordinates to metal ions to form stable complexes, transition metal complexes with levofloxacin and N-containing heterocyclic ligands [Cu(lvx)(Phen)Cl]. Cl. (MOLVX) [$C30H_{33}Cl2CuFN_5O_4$] and performed its anticancer activities of this complex in both *In Vivo* study in mice and studying their cytotoxic effect against colorectal adenocarcinoma cell line in *In Vitro*.

This complex was produced by gradually adding 5mL of a methanolic solution of copper chloride dihydrated (CuCl2_2H2O)(0.79 mmol, 0.068 g) (solution 1) into a methanolic pure levofloxacin solution 10mL 0.0144 g, 0.2 mmol $C_{18}H_{20}FN_3O_4$ (solution 2), that underwent deprotonation by the wise drop (1-2.5 mL) of KOH by pasture pipette or until to be an alkaline solution with pH (7.4-7.8), and the solution mixture was continuously stirred by the magnetic bar 30 minutes at room temp. Thence, we prepared (solution 3) which may be completely let dissolving 0.0132 g (0.79 mmol) 1,10-phenanthroline($C_{12}H_8N_2$) (132 mg, 0.2 mmol) in 20 mL methanol, so, the final solution was prepared by adding solution 1 into solution 2, then, solution 3 was added to the mixed solution (solution1 and solution2) then the mixture was continuously stirred (refluxing) using a condenser linked with hotplate at approximately 55-60 0C for 2hrs. The complex was then left at room temperature until the next day. On the next day, the final mixture was washed with some drops of methanol at cold temperature (ice-containing container) with a glass stirrer. Then, the mixture is wrapped in paraffin paper and put in the fridge for 3 days. Then, washed with some drops of diethyl ether to precipitate the particles of the substances to the bottom of Beeker. Then, leave it at a cool temperature to dry. The dried precipitate appears as light green crystals which are ground well by a clean spatula and weighed. Yield: 340 mg; 99%. M.P., 268-270℃. Anal. calcd for [Cu(levo.) (Phen)Cl]. Cl, (C30H33Cl2CuFN5O4) MW = 681.07 g/mol−1: C,53.29; H, 4.14; N, 10.36%. Found practically analyzed: C, 52.554; H, 3.921; N, 9.55% table (4-1) and Table (4-2).

Table (4-1) physicochemical properties of levofloxacin and newly synthesized Modified levofloxacin complex

Sample	% Yield	Color	Melting point	Form	Solubility
Levofloxacin		Pale yellow	221	Powder	Methanol
Copper-1,10-Phenanthroline-based levofloxacin complex (MOLVX)	344 mg 99%	Dark green	268-270	Crystal	Hot Methanol, DMSO, Diethyl ether, chloroform, water

Table (4-2)
Analytical properties of levofloxacin and newly synthesized Modified levofloxacin complex

Compound	Formula		C H N analytical test %					
		M.W g/mol^{-1}	calculated			Found		
			C%	H%	N%	C%	H%	N%
Levofloxacin	$C_{18}H_{20}FN_3O_4$	361.368 g/mol^{-1}	59.83	5.54	11.63	59.79	4.8	11.62
MOLVX	$C_{30}H_{33}Cl_2CuFN_5O_4$	681.07 g/mol^{-1}	52.29	3.92	9.55	53.29	4.14	10.36

The 1,10-phenanthroline and other nitrogen-donor heterocyclic ligands have been found to have a greater biological affinity than the parent drugs in most cases. (Chalkidou et al., 2012). Levofloxacin-metal ion coordination is extremely interesting from a biological and pharmacological point of view because it improves DNA and protein binding affinity and nuclease activity against genome sequencing, plasmid, and inter-nucleosomal DNA. In the last few decades, many different types of the copper-II compound complex with fluoroquinolone derivatives have been reported in the literature, including I-ionic compounds with (N)-propyl fluoroquinolones such as norfloxacin and ciprofloxacin, which have undergone extensive research into this interaction. (Drevenšek et al., 2005) Complexes of the (ii)-type with cinoxacin according to (Efthimiadou, Sanakis, Raptopoulou, et al., 2006; Ruiz et al., 1995) and with sparfloxacin based on (Efthimiadou et al., 2008) and with ciprofloxacin studied by (Overgaard et al., 2007)and neutral mono-nuclear complexes of ciprofloxacin, and enrofloxacin with mixed ligands depending on the study (Efthimiadou, Sanakis, Katsarou, et al., 2006) as well with nalidixic acid according to (Kumar et al., 2019).

H-NMR spectrum of both pure levofloxacin and chemically modified levofloxacin complex (MOLVX):

The ^{1}HNMR spectrum of ***levofloxacin***, figure (4.2), in DMSO-d_6 exhibits signals at 8.96ppm(*s*,1H,Ha), 7.56ppm(*s*,1H, Hb), 4.36ppm (*s*,1H, Hc), 4.60ppm(*d*,1H,Hd),4.93 ppm(*d*,1H,Hd),3.27ppm(*dd*,4H,He), 2.24 ppm(*dd*,4H,Hf), 2.45ppm(s,3H, N-CH_3), and 1.45 ppm (*s*,3H, CH-CH_3)(Drevenšek et al., 2006). The acidic proton of the carboxylic group (COOH) not appeared as a separate signal due to exchange with solvent (Ezugwu et al., 2013).

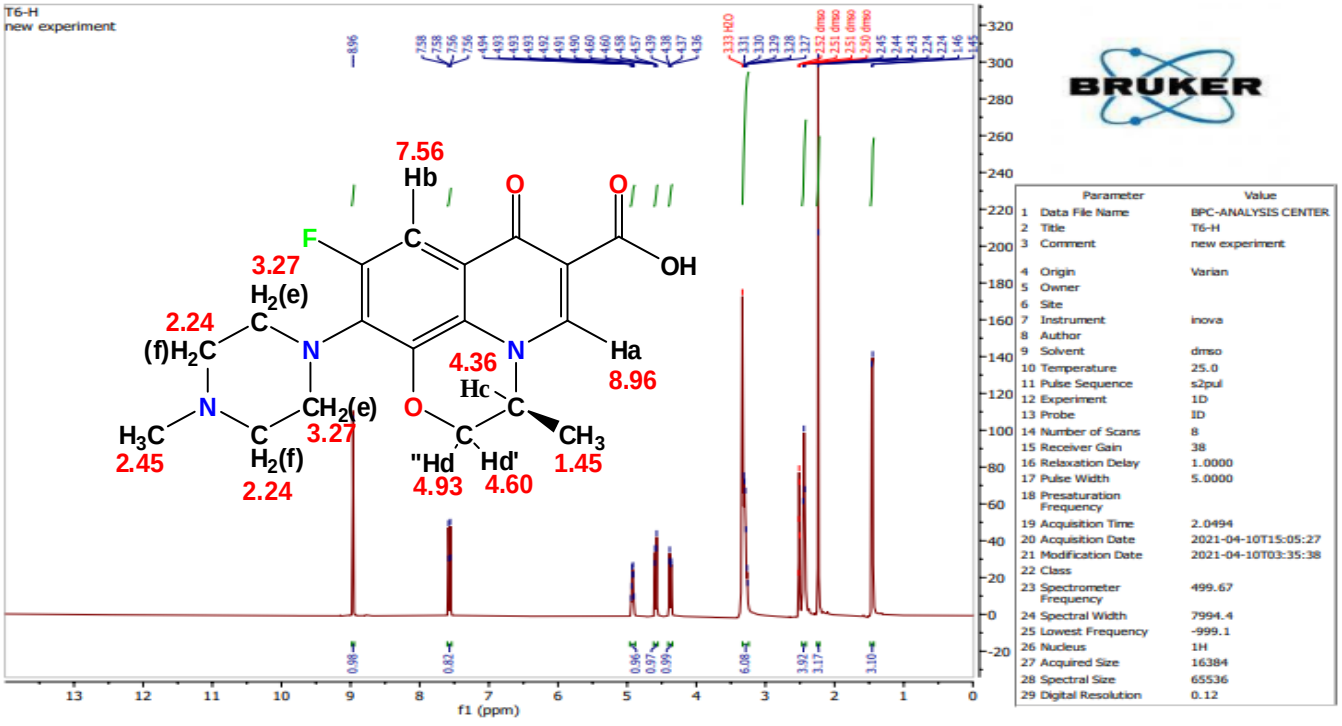
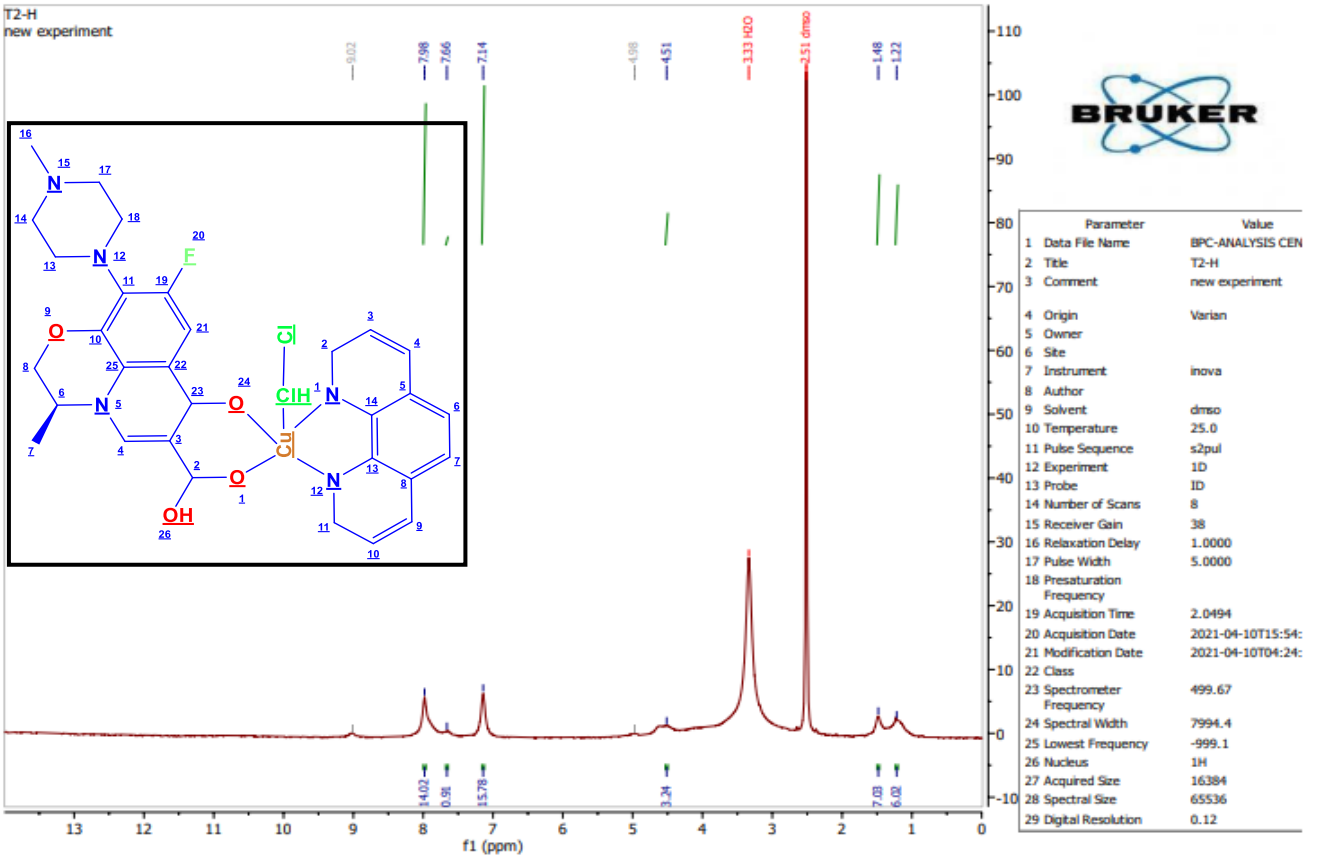


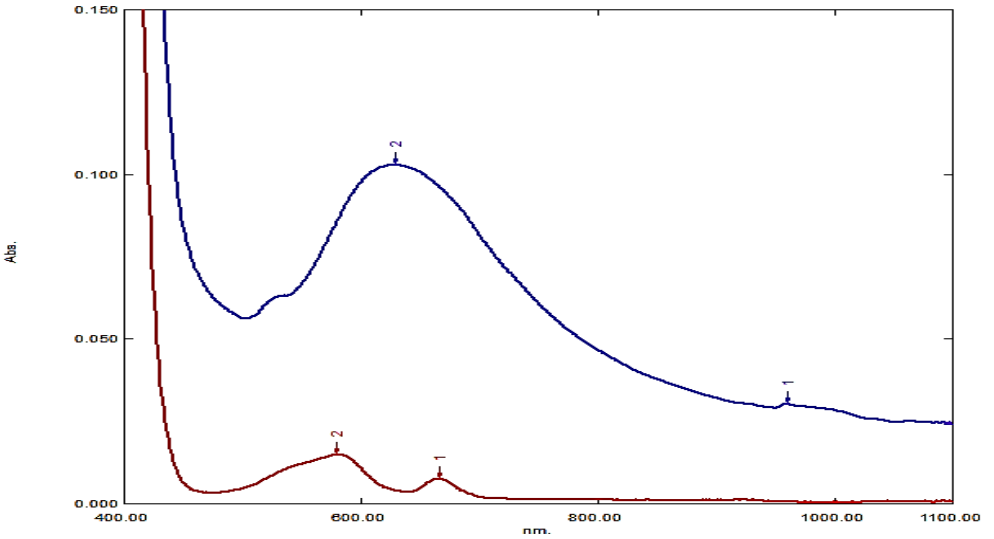
Figure (4.2): H-NMR spectrum of pure levofloxacin

In the ^{1}HNMR spectrum of [Cu(lvx)(Phen)Cl] complex, in figure (4.2) the signals appeared broad compared with those of free (*lvx*). The spectrum of Cu (^{II}) complex reveals noticeable shifting to the downfield region for most hydrogen atoms of (lvx) molecule, where (Ha) was shifted from (8.96ppm") to (9.02 ppm), (Hb) shifted from (7.56 ppm) to (7.66 ppm), (Hc) was shifted also from (4.36ppm) to (4.51ppm), (Hd") shifted from (4.93 ppm) to (4.98 ppm), (CH-CH_3) was shifted from (1.45ppm) to (1.48ppm). This shift was accompanied by a clear change in the shape and intensity of the signal for each proton. All these changes proved the corporation of the (lvx) molecule in the coordination sphere of the Cu (^{II}) ion. Protons far from levofloxacin binding sites leave their signals virtually untouched. The spectrum also showed another signal in the aromatic region related exactly at (7.98 ppm) and (7.14 ppm) assigned to aromatic protons of the 1,10-phenanthroline molecule(Hosseini et al., 2013; Sultana et al., 2013; Turel et al., 1999).



UV-VISIBILE of pure levofloxacin and (MOLVX):

It appears that the complexes in dimethyl sulfoxide DMSO are stable because the UV-Vis spectra show no changes over time. A UV-VIS quartz cuvette with a one-centimeter path length was used to study the optical properties of the prepared samples. Figures (4.4) and (4.5**)** show the relationship between wavelength and absorbance for pure Levofloxacin and Complex-Levofloxacin. From the figures, the results indicate the high ability of the samples to absorb electromagnetic radiation in the ultraviolet region, and this result agreed with the optical behavior of Levofloxacin(Roy & Roy, 2017). The results also show a red-shift in the absorption edge towards the visible region for Complex-Levofloxacin (MOLVX) with the presence of a broad peak within the wavelength range from 550 nm to 700 nm. This wide peak can be attributed to the presence of copper nanoparticles (CuPs) in the composition of Levofloxacin and the occurrence of the surface resonance plasmon phenomenon which occurs as a result of the collective movement of free electrons in the small particles when light falls on them(Kaur et al., 2015; Reather, 1988).



Wavelength λ (nm)

Figure (4.4): UV-visible absorption spectra of pure Levofloxacin (Red line) and Complex-Levofloxacin (Blue line).

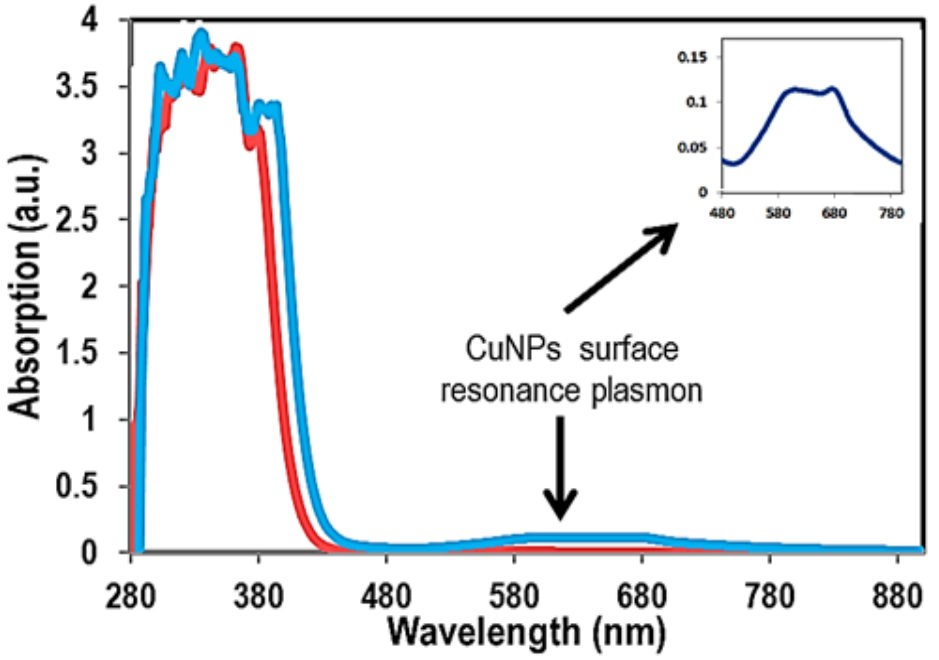


Figure (4.5): UV-visible absorption spectra of pure Levofloxacin (Red line) and Complex-Levofloxacin (Blue line). The input image is surface resonance Plasmon peak of CuNPs.

FTIR spectrum of pure levofloxacin and MOLVX complex:

Fourier transformation infrared spectra were used to analyze and determine the mechanism of coordination between levofloxacin, phenanthroline, and metal cations, Due to the presence of numerous functional groups in the levofloxacin molecules, levofloxacin infrared spectra are quite complex; therefore, their interpretation is based on the most common vibrations, with the most significant region of the levofloxacin FTIR spectrum found between 1800 and 1100cm^{-1}.

Figure (4.6) Levofloxacin demonstrated a particular quality. The strong absorption band for the carboxylic group is located at 1724.24cM^{-1}, and the pyridone stretch v(c=o") is located at 1620.09cM^{-1}. (Goyne et al., 2005; Neugebauer et al., 2005).

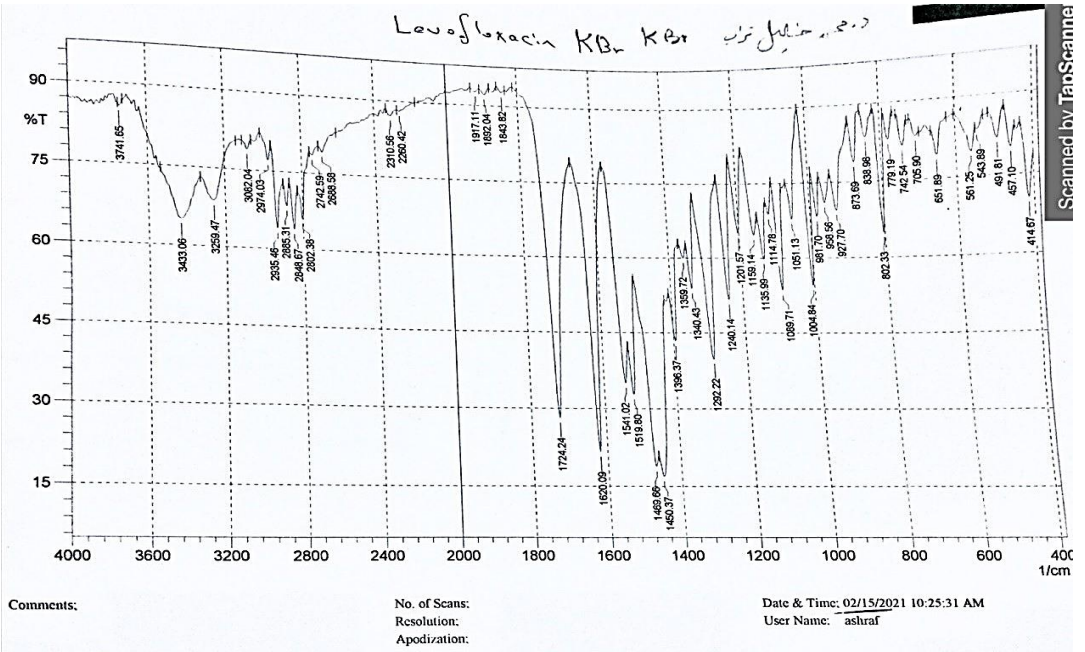


Figure (4.6): FT-IR spectra (1100–1800 cm−1 region) of levofloxacin using KBr pellet.

In the chemically synthesized modified levofloxacin complex (MOLVX), based on the attachment, *υ(c=o") p* is shifted to 1602.74cM^{-1}., and the stretching of hydroxyl of the carboxylic acid group disappears resulting in a broadband appearance in 1602.74cm^{-1} that doesn't present in the original structure of pure levofloxacin, being Replaced by two distinct and strong asymmetrical bands(1519.80 cm^{-1}")as well as symmetric (1469.66cm^{-1}") vibrations, where we found the disappearance of tow characteristic bands of the levofloxacin spectrum that are 1541.02 cm^{-1} and 1450.37 cm^{-1}**,** this shows that the carboxyl moiety has been deprotonated and is most likely coordinated with the copper ion Cu II. It is also possible that the carboxylate group has monodentate mechanism coordination based on these spectral changes. Changes in overall structure indicate levofloxacin is coordinated with Cu++ by the carbonyl group and oxygen of the carboxyl group as revealed in figure (4.7). The (N-H&O-H) stretching vibrations of the piperazinyl molecule &

water molecule, respectively, are responsible for the appearance of a split band between 3500 and 3000 cm1, which can also be observed. (Goyne et al., 2005; Neugebauer et al., 2005).

The band at 1620.09cm^{-1} of levofloxacin coincides with v(coo)asymmetry, and the band at 1292.22cm^{-1} of levofloxacin coincides with v(coo)symmetry. Both of these bands are present on the levofloxacin-complex with copper ion which shifts at about 1602.74cm^{-1} and 1272.93 cm$^{-1,}$ respectively. Levofloxacin's deprotonation was confirmed by the deduction of the stretching for band peak transmission, which moved from 34434.06 cm^{-1} of levofloxacin to 3463.92cm^{-1} of the complex due to hydrogen bonding. appearing of the band at 561.25cm^{-1} assure the ligand's N-N donating nature (Trivedi & Vasudevan, 2007).

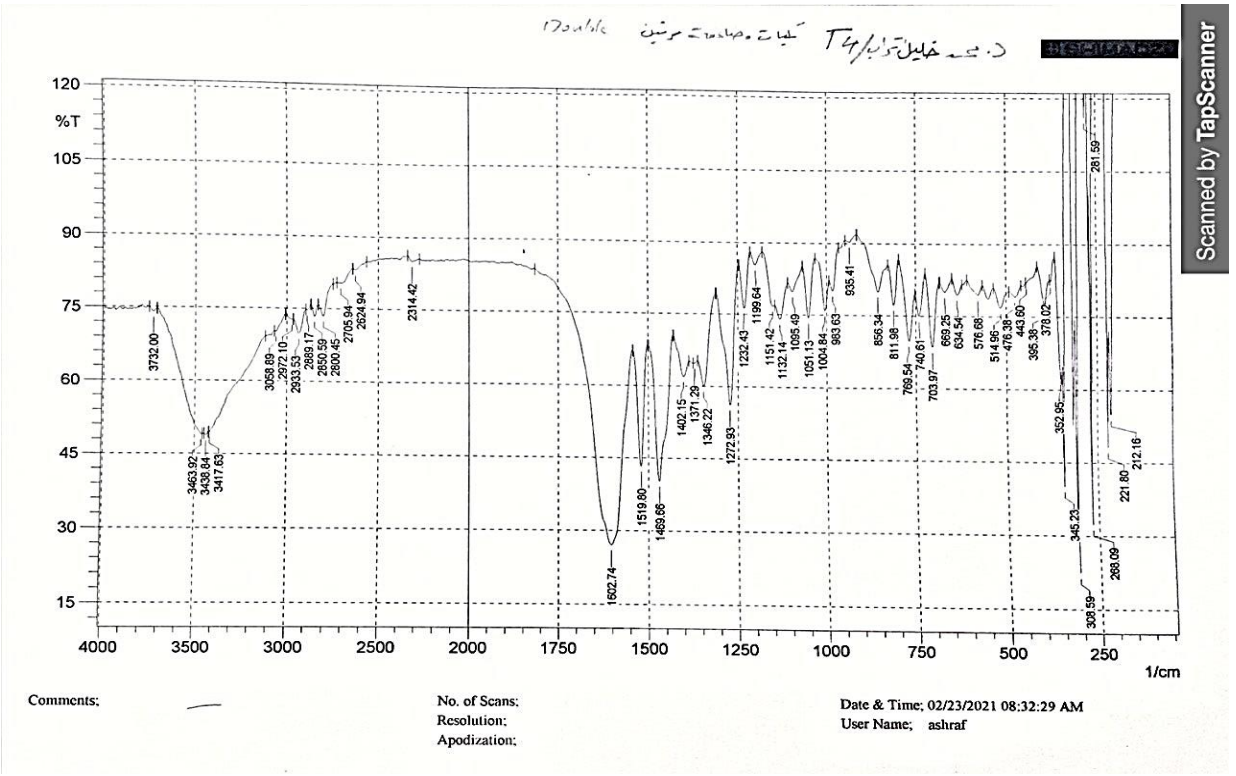


Figure (4.7): FT-IR of chemically modified levofloxacin complex (MOLVX) (250-4000 Cm^{-1}), CSI (cesium iodide)

In conclusion, in coordination chemistry, Schiff bases are frequently used as chelating ligands(Roth et al., 2006; Shibuya et al., 2008). It is possible to use the Schiff-bases method with donors (N, O, S") to better understand how racemization reaction transformation occurs in biological systems because these compounds share structural similarities with neutral biological systems (Gungor et al., 2012).

Due to their use as starting materials for industrial products, the Schiff bases method is an important way to explore and determination of different functional groups for each comparative compound (El-Sayed & Abd Allah, 2001).

Moreover, Schiff bases are privileged and highly valued ligands (Yoon & Jacobsen, 2003). It is because of their ability to form transition-metal complexes that they can be used as catalysts in a wide range of chemical processes (G. Kumar et al., 2010). Fluoroquinolone derivatives, like several anti-cancer medications like doxorubicin, can poison eukaryotic DNA topoisomerases II, resulting in antitumor action. Quinolones have been found to contain structural characteristics that are essential for anticancer action. The majority of the necessary chemical modifications took place at position 7, and with the carboxylic group at position 3, respectively, to convert fluoroquinolones from antibacterial effect into their anticancer analogs (Abdel-Aal et al., 2019).

Levofloxacin's complex formation with transition copper metals of biological interest was examined in this study. An analysis of the physical and chemical properties of levofloxacin, as well as its spectroscopic examination including the hydrogen-1NMR and FTIR, and elemental analyses, have shown that it forms stable complexes with copper II ions through oxygen moiety of the carboxylic acid group, and which serves as an effective bidentate ligand. The modified compound that was newly synthesized in this study increased the drug's anticancer activity and affinity to DNA gyrase in eukaryotic cancerous cells rather than intact cells, and the synthesized complex was more potent against HRT-18 in our in vitro study as well as the treatment of Azoxymethane-induced colorectal cancer in mice, as well as against MCF7 breast cancerous cell (M. Kumar et al., 2019).

Therefore, the current research was based on the structural changes of levofloxacin that occur during the synthesis of this compound to change the drug's efficacy from an antibacterial agent to an anticancer one. The remarkable ability of (quinolone-metal- based) complexes (MOLVX) to target several topoisomerase types (II)enzymes is undoubtedly one of their distinguishing features. during in vitro study, we observed that newly synthesized MOLVX are very toxic to the colorectal cancer cell line HRT-18 and exhibit high potency during MTT ASSAY against these cancer cell lines.

In the same way, depending on the mechanism of action of fluoroquinolone confirmed throughout published studies on the various pathogenic bacteria and their targeting of these enzymes, we suggest the MOLVX can act similarly against eukaryotic cells by targeting different types of topoisomerase enzymes.

As a result, these cytotoxic of the MOLVX represent a potentially useful source of new anticancer agents, which may also aid in the treatment of side effects and decrease the chance of emergence and development of resistance Additionally, levofloxacin's ability to bind to copper (II)ion cofactors and increase their affinity for the drug is one of the important methods of modulating their pharmacological responses.

***In vitro* study:**

Cytotoxicity and IC50 of MOLVX

The cytotoxicity of MOLVX against the HRT-18 cancerous cell line was determined using a Methylthiotetrazolium (MTT) assay for assessment of the cytotoxicity of drugs which is also known as a cell viability assay. The cells were treated with various concentrations of MOLVX for 72 hours in a medium containing the complexes at concentrations of 3.125, 6.25, 12.5, 25, 50, and 100µg/mL. The result is a good cytotoxic effect of MOLVX and was 66.4 % at 100 µg/mL. Cytotoxicity levels are expressed as the IC50 value, which is the concentration of the compounds or drugs that inhibits cell viability or survival by fifty percent. The result as shown in Figure (4.8) a significant decrease in viable cells based on the dose.

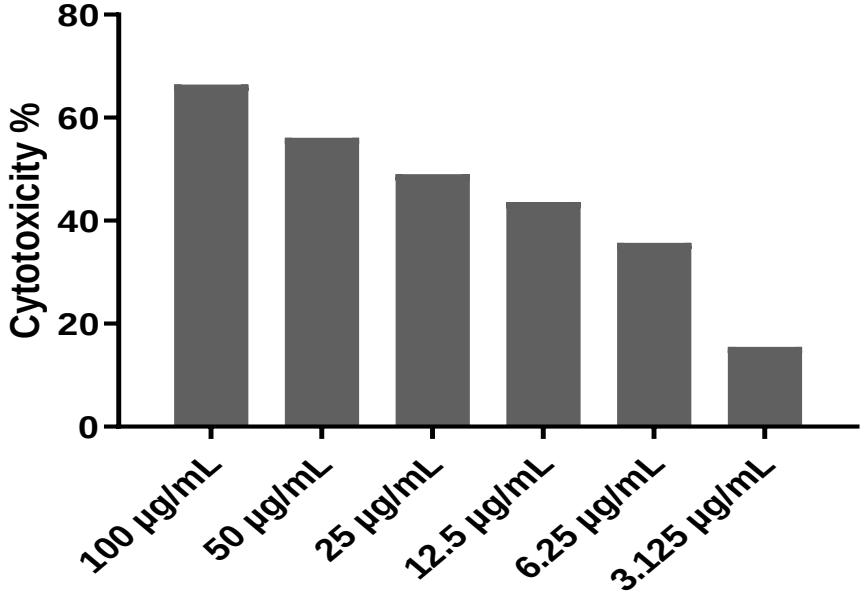


Figure. (4.8): Cytotoxicity effect of MOLVX in HRT cells.

The effect of the complex on the cells was also observed under the inverted microscope, as the HRT-18 cancer cells line that was exposed to different effective doses, as shown in the above figure (4.8) and the following table (4-3) and table (4-4), revealed remarkably good and selective cytotoxic activity towards HRT-18 colorectal cancer cells line was significantly killed compared to the control group figure (4.9).

For 72 hours of treatment with MOLVX, the value of IC50 for the HRT18 cancer cell line was estimated at various MOLVX concentrations, with IC50 values ranging from (1.407–13.040) µM to (4.285) µg, figure (4.10).

Table (4-3)
Growth inhibition effect of MOLVX in different concentrations in HRT colon cancer cell line after 72 hours of exposure

NO.	MOLVX concentrations per µg at 72 hours	Growth inhibition percentages of HRT-18 cells line %
1-	100µg/mL	66.4
2-	50µg/mL	56.1
3-	25µg/mL	49
4-	12.5µg/mL	43.6
5-	6.25µg/mL	35.7
6-	3.125µg/mL	15.5

Table (4-4)
Optical density O.D. of various concentrations of MOLVX against HRT-18 for 72 hrs.

Log concentration	Cell viability	
	Mean O.D.	SD
control	64.498	1.404
0.494	54.473	6.178
0.795	41.471	0.633
1.096	36.378	0.732
1.397	32.889	1.543
1.698	28.307	0.532
2.000	21.678	0.564

IC50= 1.407 — 13.05 µM

LogIC50= 0.1483 — 1.116 µM

IC50= 4.285 µM

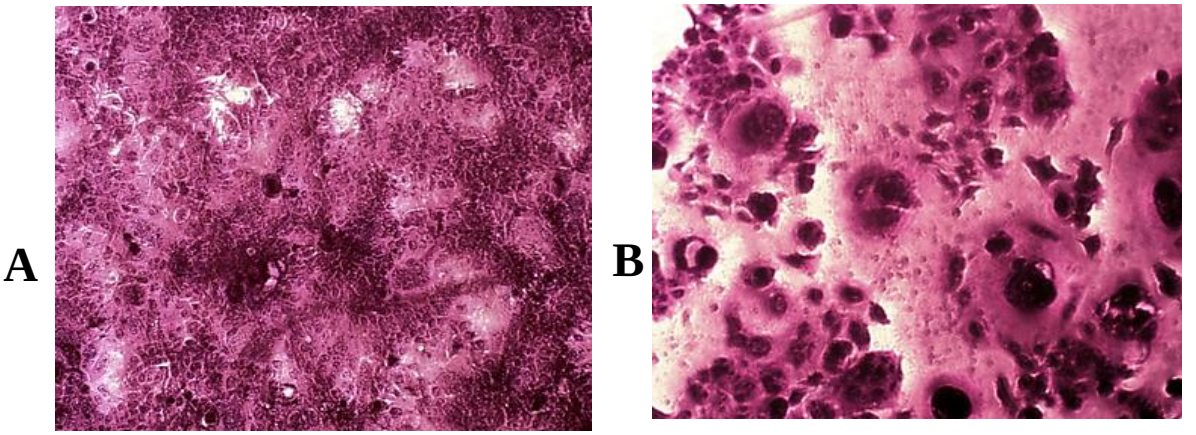


Figure (4.9): Morphological picture for HRT-18 cancer cell line in vitro, (A) Control cells treated with DMSO (B) Cytotoxic effect of MOLVX various concentrations, characteristic features of apoptosis including cell shrinkage, vascularization, and autophagy under an inverted

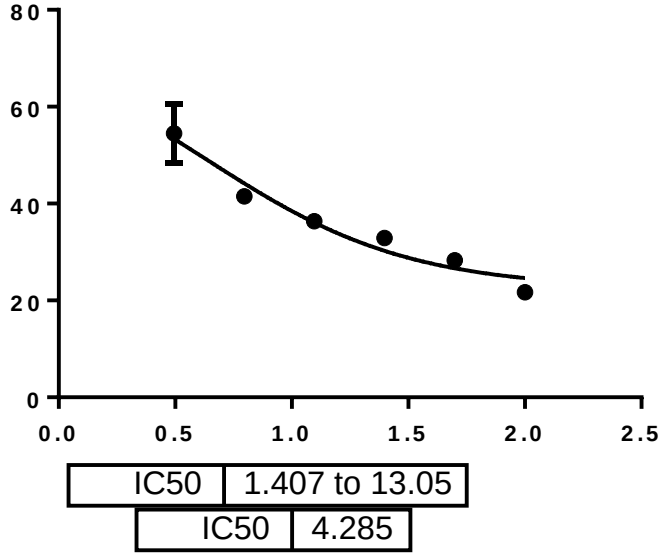


Figure (4.10): IC50 *(μM)* of sample MOLVX on HRT-18 cell line, Log concentration of MOLVX versus percentage of cell viability%.

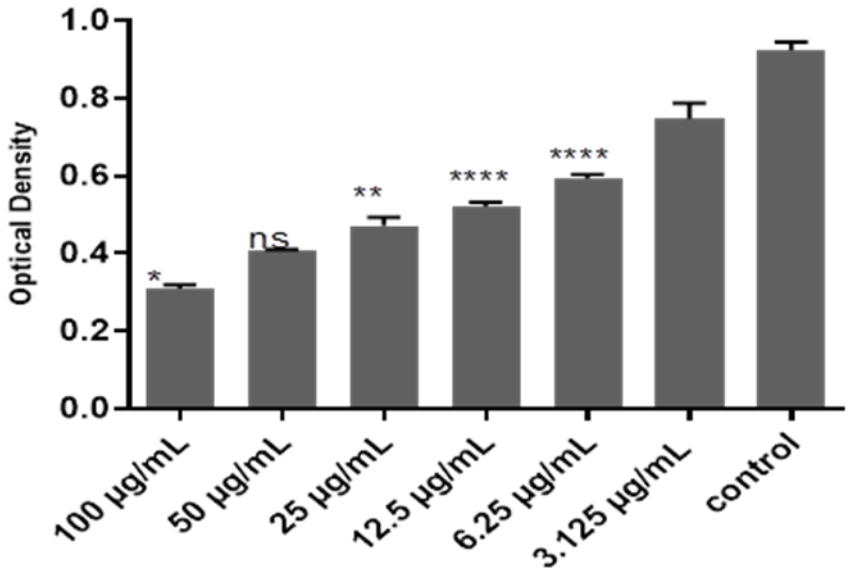


Figure (4.11): Optical density of the different concentrations of MOLVX on HRT-18 cancer cell line.

The obtained results in the present study reveal that the complex has a higher activity against the HRT18-cancerous cell line and this resulted in an effective IC50 value of 4.285µM which lessened than that of the pure levofloxacin which stated in previous studies which were 125.74 ±0.8 on MCF-7, >100 µM on HepG2, HeLa 71.1 ± 4.98 µM, and SW480 primary colon cancer cell line 160.4 ± 6.7 µM,(Wang et al., 2018), We conclude that the MOLVX complex has increased cytotoxicity (IC50 = 4.285 M), and we indicate that because it can induce a high incidence of apoptosis, this may be due to whether it severely inhibits cell proliferation, as many researchers have suggested. (Anderson & Osheroff, 2001; Herold et al., 2002), and/or it could be due to inhibiting DNA synthesis by preventing supercoiling and strand segregation, which is mediated by topoisomerase II inhibition and enhancing DNA cleavage in eukaryotic cells (Pommier et al., 2010; Robinson et al., 1992). This study confirmed that modified levofloxacin complex (MOLVX) can decrease cell growth and is a more effective broad-spectrum anticancer medication against colorectal carcinoma cells than levofloxacin (Herold et al., 2002). Fluoroquinolones like levofloxacin inhibit tubulin polymerization and selectively target cancer cells (Chen et al., 2007). Furthermore, these studies support what we suggested about the effectiveness of levofloxacin and the complex (MOLVX) synthesized in this study, as it was proven that the cytotoxic concentration of MOLVX is many times stronger than levofloxacin and that the IC50 of the MOLVX complex is much less than the drug itself, indicating its effectiveness against HRT-18 colorectal cancer cells and could also have an effective effect on other cancer cell lines, which need further study.

Fluoroquinolone chemicals induce apoptosis in, cancer cell lines and inhibit the proliferation of carcinoma cells (Mazandaran et al., 2019). Recent research reveals fluoroquinolone may suppress prostate and lung-adenocarcinoma cell

proliferation (Cao et al., 2017; Mondal et al., 2004), as well as, colorectal cancer cells (Melo et al., 2011). Some fluoroquinolones have antiproliferative characteristics in vitro by preventing prospective cancer cells from undergoing a biochemical transition, which increases chemotherapeutic absorption and/or mediates immunomodulatory responses (El-Rayes et al., 2002). Several novel tetra-cyclic fluoroquinolones such as levofloxacin have anticancer properties against a variety of human cells, including breast cancer cell line and non-small lung cancerous cell line(A549). Other types that do not contain tetracyclic groups in their structures are not toxic to normal human dermal fibroblasts.(HuDe") (Al-Trawneh et al., 2010).

Ciprofloxacin's anticancer action may be attributable to intrinsic apoptosis and cell cycle arrest, both of which are reversible once the quinolone is withdrawn (Bourikas et al., 2009). Fluoroquinolones, including Ciprofloxacin, decrease cell proliferation and induce apoptosis in a range of cancer cell lines, including leukemia, osteosarcoma, and carcinoma (Herold et al., 2002). Ciprofloxacin inhibits eukaryotic cell proliferation by inducing tumor growth factor (TGF-1) in colon epithelial cells at 10 g/mL; this is a clinically achievable concentration in human tissues (Bourikas et al., 2009).

Levofloxacin inhibits DNA helicase activity, preventing bacterial DNA duplication. Antibiotics that inhibit DNA duplication in prokaryotic cells may similarly influence cancer cell viability because mammalian cells have similar intracellular biology to prokaryotic cells. By arresting the cell cycle at G2-M and increasing apoptosis in drug-exposed cells, levofloxacin lowers cancer cell proliferation, clone formation, and xenograft tumorigenesis (He et al., 2022).

In our study, we have found the profound cytotoxic effect of MOLVX against the HRT-18 colorectal cancer cell line, this effect may attribute to pure levofloxacin which binds to and inhibits the DNA helicase activity thus preventing DNA duplication, as well as, contributes to 1,10-phenanthroline. The high cytotoxicity of the MOLVX complex may be due to the extensive structural-planer configuration induced by the copper ion's chelation with the 1,10-Phen compound. This effect may be due to the copper ion's significant intercalative chelation with DNA molecules. Thus, a more potent anticancer drug may be produced by incorporating 1,10-Phen into copper (II) complexes, increasing the activity. The review also briefly discusses metal chelates with fluoroquinolones and their involvement in topoisomerase toxicity and anticancer activity. This should interest researchers working on fluoroquinolone anticancer medication rational design and manufacture (Abdel-Aal et al., 2019). The cytotoxicity of a simple fluoroquinolone, ethyl 6fluoro8nitro4oxo1,4dihydroquinoline 3 carboxylates 1, has been studied using two carcinoma cell lines and noncancerous cell lines (Jantová et al., 2018).

In this study, we suggest that MOLVX is act as a more powerful anticancer and has superior effectiveness to enrofloxacin in the same manner, which acts on the adenocarcinoma cell line after 72 hours of incubation, the higher concentrations used (100 µg/mL) significantly inhibited cell growth in the tested HRT-18 cell lines, it's because the induction of apoptosis by caspase 9 and 3 is thought to be responsible for inhibiting cell growth (Jantová et al., 2018).

Conclusion

We successfully synthesized the modified levofloxacin complex by a modified Schiff base method mentioned in detail in the chapter on the methodology of the current study, the characterization of the new complex was achieved by different physicochemical studies including FT-IR to indicate the coordination of atoms between ligands, where we indicate the binding of the copper (Cu)++ to both levofloxacin from one side by two oxygen atoms that present on the carboxylic acid after the loss of a proton from a hydroxyl group, and the oxygen on the ketone in comparison of these groups on the FT-IR test of both the ligand" MOLVX" and the parent drug" levofloxacin", on the other side, binding of the copper on the two doner -N- atoms (bidentate) of 1,10-phenanthroline (Phen). Also, H-NMR is one of the reliable tests for indicating the Schiff base that indicates for coordination of copper with both levofloxacin and phenanthroline. Moreover, the elemental analysis (CHN-analysis) also was used to determine the correct chemical formula and molecular weight of the synthesized ligand. The final chemical structure was (C30H33Cl2CuFN5O4) MW = 681.07 g/mol−1.

In our in vitro study, we observed that the MOLVX in different concentrations exhibited high growth inhibition on the HRT-18 colorectal adenocarcinoma cell line by concentration-dependent growth inhibition manner for 72h incubation. Moreover, the IC50 of this complex was between 1.4 - 13.05 (µM), which is best than pure levofloxacin alone when we compared that based on other studies. The synthesized MOLVX has effectiveness against colorectal adenocarcinoma cell line HRT-18 and we suggest that ligand has a powerful cytotoxic effect against HRT-18 which is useful for the treatment of colorectal neoplasia.

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