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Design, synthesis, molecular docking, and antibacterial assessment of a new ciprofloxacin metal-complex derivative

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ABSTRACT

New azo - azo-containing and azo-metal complex derivatives of ciprofloxacin (D and E) were synthesized to increase its antibacterial activity and decrease the microbial resistance caused by different bacterial species. Many types of metal ions can be used, in our research we used Ag+2. Some of the physicochemical characteristics and FT-IR were used to confirm the chemical structure of the resulting compounds. In silico study was done by using a molecular operating environment program (MOE) and it was noticed that the synthesized compounds showed a high affinity to the target site in topoisomerase enzyme. These results were compatible with the in vitro biological study.

Keywords: Fluoroquinolone, Azo linkage, Metal ions, Metal- complexes.

I. INTRODUCTION

The Fluoroquinolones are a group of synthetic antibacterial agents, that have been available for clinical use for more than thirty years [1]. Their oral absorption and bioavailability are both good. The first quinolone to be used in therapy was nalidixic acid. Several types of modifications have been made to nalidixic acid to maximize the range of antibacterial activity and enhance pharmacokinetic characteristics.

The addition of a piperazine moiety or other N-heterocyclic ring types at the 7-position is one of the main changes, and the addition of a fluorine atom at the 6 position [2] Ciprofloxacin is a second-generation antibiotic which is one of the widely used agents in this group. The bacterial DNA gyrase is a major target for this type of antibiotic. However, currently used quinolones are less effective because of resistance [3,4]. Common mechanisms of resistance come from mutations in enzymatic target sites. Additional types of resistance include plasmid-mediated decreased uptake of the drug thereby decreasing cellular concentration and interaction with the target. Attempts were made to modify the quinolone structure to overcome the resistance. Because of the intriguing biological and chemical characteristics of quinolones, their interaction with metal ions has been extensively researched. Quinolones, which are often found in the form of 1 meta l: 2 ligands, will attach to the metal ions to create metal complexes in which they function as a bridging ligand, bidentate, or unidentate.

Most of the quinolones are zwitterionic due to the existence of a basic piperazinyl moiety at position -7 and a carboxyl group at position-3 so, they have good solubility in both acidic and basic media. It was found that metal-drug complexes possess greater antimicrobial activities when compared with the same un-complexed agents. [5]

II. METHODS AND MATERIAL

Central Drug House, Merck, and Sigma-Aldrich provided all chemicals and anhydrous solvents. The Thomas Hover device was used to record melting points. Using (Acetone: Methanol) (1:1) as a mobile phase, retention factor values were determined by TLC to verify the purity

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and reaction development[6]. Shimadzu spectrophotometer from Japan was used to capture FT-IR at the University of Kufa's faculty of pharmacy.

Synthesis of Methyl 1- cyclopropyl-6- fluoro-4- oxo- 7-(piperazin-1-yl)-1,4- dihydroquinoline-3- carboxylate Hydrochloride (A) [7]:

After cooling to a temperature of -15 °C, a suspension of ciprofloxacin (5g, 15.1 mmol) in absolute methanol (50 ml) was added dropwise, while the temperature remained at -15 °C. The reaction mixture was then refluxed for 45 hours (or until the HCl gas was stopped) at 40 °C, and then it was left to stand at room temperature for the entire night. The residue had been redissolved in methanol and evaporated after the solvent was vacuum-dried to a dryness. The procedure was carried out multiple times until all traces of thionyl chloride were eliminated. Methanol chloroform was used to crystallize the residue, which was gathered as a white-yellow powder. Rf value 0.4, m.p. 275 d, yield 94%. The stretching vibrations of the following compounds were measured using FT-IR (cm^{-1}): (3236) (N-H) secondary amine; 3024 (C-H) aromatic; 2951-2927 (C-H) alkane; 1732 (C=O) ester; 1627 (C=O) quinolone; and 1039 (C-F) stretching vibration.

Synthesis of Methyl 7-(4-(2-Chloroacetyl) piperazin-1-yl)-1-Cyclopropyl-6-Fluoro-4-Oxo-1,4-Dihydroquinoline-3-Carboxylate (B) [8,9] **:**

Chemical (A) was reacted with chloroacetyl chloride to create this chemical. Five grams (14.4 mmol) of compound (A) were dissolved in a combination of DMF: 50 ml of a 1:1:4 combination of chloroform was added first, followed by TEA (1.9 ml, 14.4 mmol) and chloroacetyl chloride (1.14 ml, 14.4 mmol in 10 ml of chloroform) added dropwise while stirring continuously for an hour. The reaction mixture was then refluxed for five hours. The precipitate was separated from the ethanol by filtering, drying, and re-crystallizing it, resulting in a white-yellow powder. 85% yield. m.p. 211-213 °C. R_f is equal to 0.6. The stretching vibrations of aromatic compounds are 3057 (C-H), alkanes are 2970–2937 (C-H), ester compounds are 1732 (C=O), amides are 1656 (C=O), quinolones are 1624 (C=O), and 658 (C-Cl) are examples of FT-IR (cm^{-1}) stretching vibrations.

Synthesis of Methyl 7-(4-(2-aminothiazol-4-yl) piperazine-1-yl)-1-Cyclopropyl-6-Fluoro-4-Oxo-1,4-Dihydroquinoline-3-Carboxylate (C) [10]**:**

After dissolving chemical B (5 g, 11.85 mmol) in 50 ml of 99% pure ethanol, thiourea (0.9 g, 11.85 mmol) was added and continuously shaken until it was dissolved. After four hours of refluxing the reaction mixture, the solvent was removed, diethyl ether was used to recrystallize the product, and the resultant white-yellow powder was collected. 85% yield, m.p. 126–128 °C, (R_f) is equal to 0.5. For FT-IR (cm^{-1}), the stretching vibrations of the primary amines are 3348, 3269 (N-H), aromatics are 3170 (C-H), alkanes are 2980 (C-H), esters are 1732 (C=O), and quinolones are 1608 (C=O).

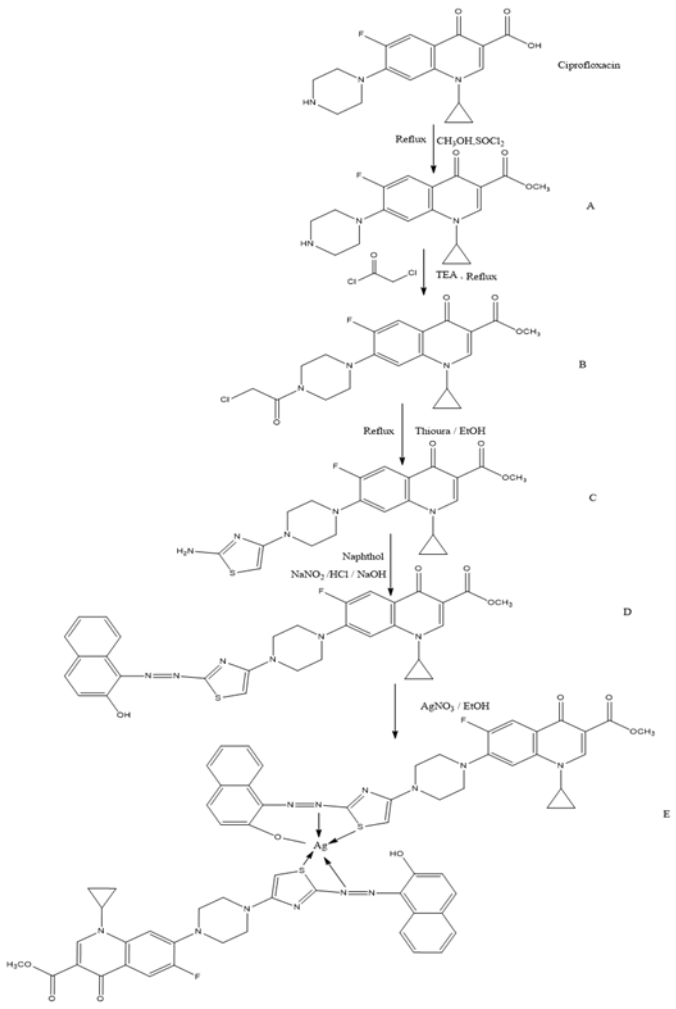
Synthesis of methyl 1-cyclopropyl-6-fluoro-7-(4-(2-((1-hydroxy naphthalene-2-yl) diazenyl) thiazol-4-yl) piperazine-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (D)[11]

The chemical (C) was dissolved into a solution containing 4 millilitres of strong hydrochloric acid and 25 milliliters of distilled water to create the organic reagent, as previously reported. Ten milliliters of distilled water were added together with 0.7 grams of sodium nitrite, which had been dissolved after the solution had been cooled to zero degrees Celsius. The mixture was left for fifteen minutes to finish the diazotization process. Then, while maintaining a temperature of 0 0C, drop by drop of diazonium salt was added to a solution containing (0.8g, 5 mmol) of 1- naphthol and 10% sodium hydroxide mixed in 150 ml of ethanol. After the contents were left for two hours, 150 millilitres of cool distilled water was added, and HCl was used to keep the solution's pH at six. After the solution was left for twenty-four hours, a dark brown product formed. After filtering off and repeatedly washing with a (ethanol: water) mixture, the precipitate was twice recrystallized from hot ethanol and dried in a vacuum desiccator. R_f value = 0.6, m.p. 224-227 d, yield 75%. FT-IR (cm^{-1}): 1147 (C-N) stretching vibration, 1627 (C=O) stretching vibration of quinolone, 1485 (N=N) stretching vibration of azo linkage, and 3383 (O-H) stretching vibration of the hydroxyl group of 1-naphthol.

Synthesis of ((3-((4-(4-(1-cyclopropyl-6-fluoro-3-(methoxycarbonyl)-1,4-dihydroquinolin-7-yl) piperazine-1-yl)thiazol-2-yl)diazenyl)naphthalene-2-yl)oxy)silver complex (E)[12]

$AgNO_3$ (0.942 g, 5 mmol) diluted in 10 ml of water was gradually added, stirring, to the ligand (2.99 g, 5 mmol) dissolved in 50 ml of 99% ethanol. As the two solutions are combined, the mixture turned dark brown, and no precipitate formed; but, as the solution evaporated, a

precipitate with a black colour formed. After filtering and washing with ethanol and water, the precipitate was recovered and allowed to dry for a few hours. 60% yield is achieved. Decomposition at 300 ºC. FT-IR (cm-1): aromatic stretching vibration 3107 (C-H), quinolone stretching vibration 1622 (C=O), azo linkage stretching vibration 1487 (N=N), aromatic stretching vibration 1145 (C-N), M-N stretching vibration 711cm-1, and M-O stretching vibration 613cm^{-1}.



Scheme 1: Target end compounds and intermediates preparation (A-E).

In The Study of Silico

2015.10, the Molecular Operating Environment (MOE) software, was used to conduct an in-silico investigation. Preparing the ligands and proteins is a step in the docking process. In the MOE software, protonation of a three-dimensional structure, partial charge addition, and energy minimization are steps in the ligand preparation process. To enable the interaction of only ligands and the chosen receptor, the protein 2xct was obtained from the PDB website (www.rcsb.org)[13]. It was then prepared by deleting any unwanted binding sites on topoisomerase enzyme II (DNA gyrase), adding deleted protons to enable the upload and download of the protein from PDB, and adding broken bonds and fixation of the protein molecule's potential. The final step involves a selection of the active site in which the interaction between the ligand and the amino acids occurs [14,15].

III. RESULTS AND DISCUSSION

Chemistry

A distinct band of C=O was shifted from 1708 cm-1 to 1732 cm-1, and the broadband over 3000 cm-1 vanished. This is thought to be proof that the carboxyl group in ciprofloxacin was converted to a methyl ester derivatives [16,17,18] . To obtain the 2-chloro-acetamide derivative, N-N-acetylation of the secondary amine of the piperazine ring of ciprofloxacin was accomplished by employing chloroacetyl chloride. Using the tetrahedral intermediate and a nucleophilic acyl substitution reaction, the chloroacetyl chloride was changed into an amide in this process[19]. At the α-α-carbon atom of chloroacetyl chloride, selectivity was produced through increased nucleophilic reactivity toward acid chlorides of nucleophilic substitution. This selectivity was caused by variations in the electrophilicity of the two carbon atoms in chloroacetyl chloride. This selection is influenced by variations in steric and electrical variables. The acyl derivative of ciprofloxacin ester reacted with thiourea to form the thiazole ring. The acetyl chloride's carbonyl oxygen and hydrogen link strengthen this group's electrophilicity, which causes the amino nitrogen of thiourea and the sulfur of chloromethyl carbon to attack each other, forming a thiazole ring and eventually removing an HCl molecule[20].

For the azo reagent, the disappearance of the primary amine peaks at 3348, 3269 cm^{-1} and the appearance of a new peak for the azo linkage at 1485 cm $^{-1}$ indicates that the compound is formed. In the azo-metal complexes, the infrared spectra are usually difficult to interpret because of many overlaps especially at areas between (1700-4000 cm^{-1}) because the referred area includes most of the effective peaks of groups such as these types of compounds containing heterogeneous rings as well as the aromatic ring and other types of substituents. There is a difference in the shape and intensity of the bands in the complex compared to the reagent bands also there is a

shifting of the bands either into a higher or a lower frequency. The (O-H) stretching vibration that occurred at 3383 cm^{-1} in the azo reagent disappeared in the complex because the oxygen atom was attached to the silver ion. The strong (N=N) band occurs at 1485 cm^{1-} and has a change in shape and intensity because of the metal ion binding with azo nitrogen.

Research on Antibacterial Agents

An in vitro antibacterial investigation was conducted against two different bacterial species: gram-negative E. coli and gram-positive S. aureus by applying the diffusion method of an agar well. We employed the Brain Heart Infusion Agar (BHIA) in this procedure. After dissolving the tested agents in dimethyl sulfoxide, 1 ml of each bacterium's spore solution was uniformly distributed using cotton swabs on the sterile solid medium.

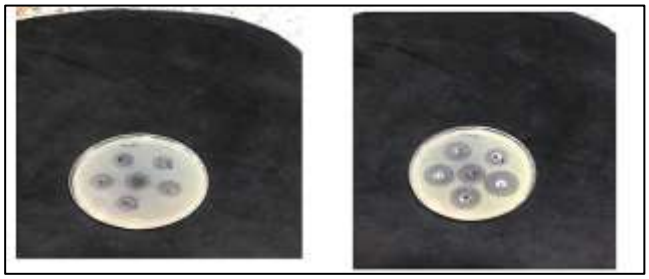


Fig 1: Ciprofloxacin's antibacterial action, B. gram-negative Bacteria (100D=ciprofloxacin), A. Bacteria that are Gram-positive (100D=ciprofloxacin).

Fig 2: Antibacterial activity of the synthesized compounds, A. Bacteria that are Gram-positive (PZ=D, PX=E), B. gram-negative Bacteria (PZ=D, PX=E).

Six-millimeter wells were created in the plates with 0.1 milliliters of each concentration. Following that, the plates were incubated for 24 hours at 37 °C. The antibacterial activity of the produced compounds was determined by measuring and observing the zones of inhibition [17,18] as in the tables (1) and (2).

TABLE I: Dosage calculations for derivatives of ciprofloxacin

Compound	Molecular weight (g/mol)	Dose (µg/ml)
D	584.2	584
E	1276.2	1276

TABLE II: Antimicrobial properties of the produced substance

Compound	Inhibition zone of bacterial growth (average in mm)	
	Staphylococcus aureus (G+ve)	*Escherichia coli* (G-ve)
Ciprofloxacin	19	15
D	15	14
E	17	15

Docking Analysis:

The target-produced compounds' docking process was studied utilizing the Molecular Operating Environment program (MOE 2015.10) with Topoisomerase II (DNA gyrase) 2xct, The Protein Data Bank (PDB) provided this. The synthesized compound demonstrated a good affinity for the enzyme, compound D displaying a good docking score and Rmsd that aligns with the findings of an antibacterial investigation conducted on *S. aureus.* and *E. coli.* The docking findings of ciprofloxacin and its derivative in 2D and 3D images are displayed in Table (3) and Figures (4 and 5). As its hydroxyl oxygen atom and naphthyl moiety will make hydrogen bonds with Arg, Lys, and Ser, and as its azo linkage formed a bond with His. amino acid residues, it demonstrated the ciprofloxacin derivative's affinity toward the DNA gyrase enzyme.

TABLE III: Ciprofloxacin and its derivative docking findings

Compound	Docking score	Rmsd
Ciprofloxacin	-5.08	2.19
D	-8.88	1.4

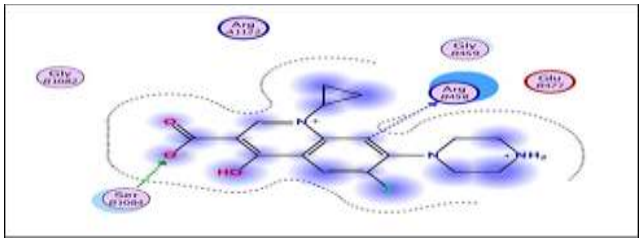


Fig. 3: Ciprofloxacin Docking result (2D).

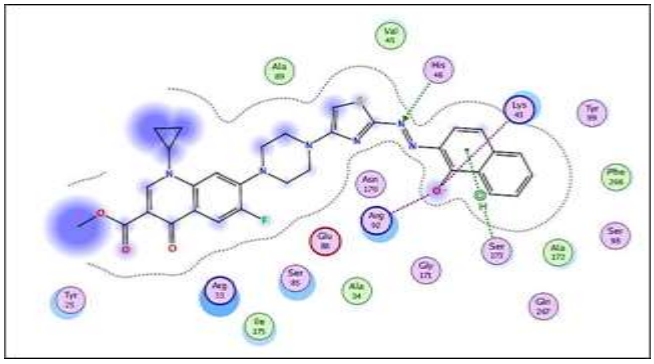


Fig 4: Ciprofloxacin Docking result (2D)

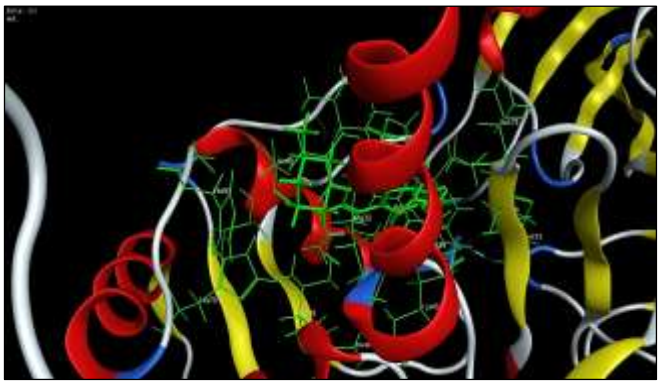


Fig 5: Compound D (3D) docking result.

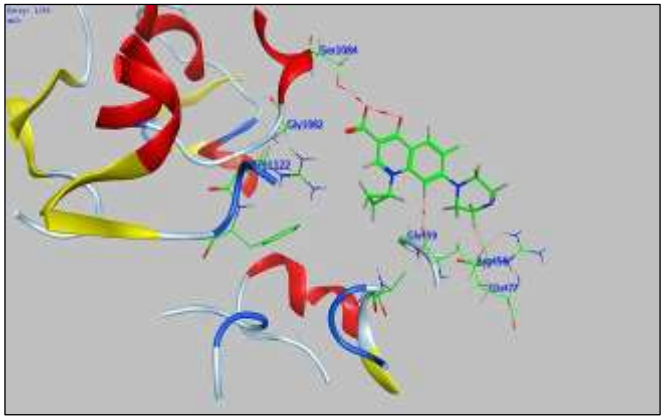


Fig. 6: Compound D (3D) docking result.

IV. CONCLUSION

The antibacterial investigation demonstrated that the synthesis of azo compounds with the α-naphthol moiety and their metal ion complexes at the piperazine ring's secondary amine enhances or preserves the antibacterial activity in ciprofloxacin, which agrees with the in-silico analysis outcomes of the synthesized substances.

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