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In Silico Study, Synthesis and Evaluation of New Indole-Hydrazones as Potential Antibacterial Agents

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Abstract

Because pathogenic bacteria have gained resistance, bacterial illnesses are difficult to treat. Every year, a considerable number of studies aimed at creating novel antibacterials are released. Schiff-bases are an essential field in organic synthesis due to their biological activities. Schiff bases also possess a necessary range of pharmacological activities. Hybridizing two pharmacophores [heterocycle molecule and synthesized hydrazones] to form one molecule is important in designing new drugs. The new compounds' hydrazone derivatives were created using a multi-step process including synthesis of indomethacin hydrazide by coupling agent reaction (EDC.HCl, HOBt), then reaction with different aldehydes to form hydrazones, thin-layer chromatography, ATR-FTIR, and ^{1}HNMR spectroscopy were used to characterize them. We aim to use molecular docking to develop compounds with antibacterial activity and suitable ADMET properties that can be produced and used in the future.

The new compounds' antibacterial properties were assessed using the fast resazurin microtiter assay MIC (minimum inhibitory concentration) method. Some compounds (IIIC) showed comparable activities to ciprofloxacin against *S. aureus* and comparable activity of (IIIC, IIID) against *K. pneumonia*. Other (IIIB) showed more potent against *K. pneumonia*, and in general, all new compounds show good activities against *E. coli*, *K. pneumonia*, *S. pyogenes* and *S. aureus*.

Keywords: Antibacterial, Bacterial infections, Heterocycles, Hybridization, Molecular docking, Schiff-base.

Introduction

A major health problem worldwide is the resistance to commercially available antibacterial agents such as β-lactams, quinolones, and macrolides. [(1)] Infections produced by germs resistant to many drugs result in more significant morbidity and death, longer treatment times, and higher healthcare costs. [(2,3)] The challenge of microbial resistance can be addressed by the prudent use of already licensed antibiotics and the development of new anti-ineffective medicines with increased activity and a novel mode of action. [(4, 5)] Indomethacin is an NSAID and one of the indole acetic acid derivatives. Notably, the indole ring has superior antibacterial activity when joined to simple aromatic rings like phenyl. In the cup-plate agar diffusion assay, indole pyrimidine derivatives showed activity against various microorganisms at 5–10 µg/ml. [(6)] If the indole ring is joined to simple aromatic rings like phenyl, its antibacterial action is enhanced [(7)]. N-acyl hydrazone (NAH) is favored since it mimics the most minor necessary component and is present in several medications and lead compounds. It can interact with one or more receptor classes. Furthermore, because of its simplicity in synthesis, resistance to hydrolysis, capacity to modify H-bonding (protein donor and proton acceptor), and ability to change conformation, all of which provide unique molecular properties with various pharmacological effects [(8)]. On the other hand, Hydrazones are a significant class of pharmacologically active chemical compounds. One example of a hydrazone-containing medicinal molecule is nitrofurantoin. Medical scientists have become interested in hydrazones because of their wide range of biological activities, which include antiviral, anticancer, anti-inflammatory, anthelmintic, anticonvulsant, depressed, and antibacterial properties. [(9-12)] Molecular docking is a computational technique that predicts how two molecules interact. It can be used to identify new compounds that can bind to proteins or enzymes essential for bacterial survival[(13).]

The aim was to synthesize new molecules combining hydrazones with different aromatic rings as possible antibacterial agents, Figure (1):

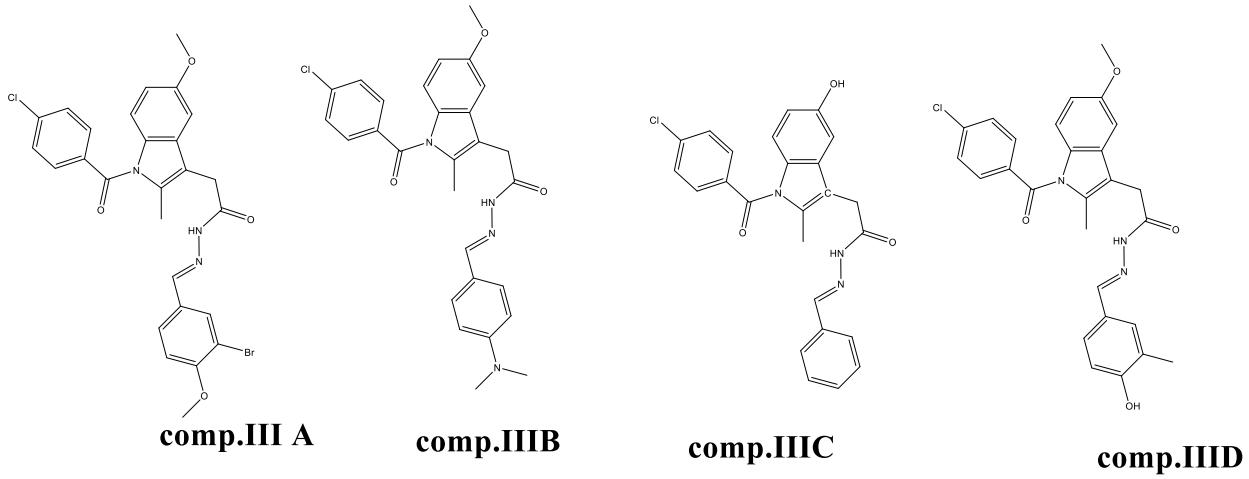


Figure 1. Structures of target compounds.

Materials and Methods

The necessary reagents were procured from reputable commercial suppliers, including Hyperchem (China). Using aluminum sheets pre-coated with silica gel GF254 (type 60) and subjected to UV-254 nm, thin-layer chromatography (TLC) was utilized to measure R_f values, assess the purity of the synthesized compounds, and record reaction completion; depending on **S_1**(chloroform:ethylacetate: methanol (**2.5:1.5:1**)) and **S_2**(ethylacetate:methanol: ammonia (**3.5:1:0.5**)) as eluents. Proton nuclear magnetic resonance (^{1}HNMR) at Basra University, (ATR-FTIR) at Baghdad University - College of Pharmacy were used to characterize all produced derivatives. All synthesized derivatives are characterized using the two spectroscopic techniques to ensure structural verification and purity assessment.

Chemical Synthesis

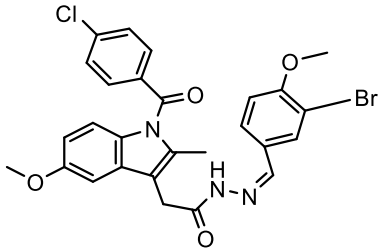
The synthesis of the target compounds (IIIA-D) proceeded as follows:

Synthesis of 5-methoxy-2-methyl-2-(1-(4-chlorobenzoyl)-1H-indol-3-yl)acetohydrazide ; comp. II[14]

Indomethacin (0.0083 mol, 3 g) was dissolved in 30 milliliters of DMF and a 50% excess of HOBt (1.694 grams, 0.0125 mol) and EDC (2.3989 grams, 0.0125mol) are added subsequently. The mixture was stirred at room temperature, and TLC monitored the reaction progress until all acid was converted to ester; this occurred after 30 minutes of stirring at room temperature, and the resultant mixture formed ppt. This product was then added dropwise to a solution of 99% hydrazine and 10 ml of DMF, previously produced at a temperature of 0–10°C. Usually, the completion of the addition marked the end of the reaction. Next, 40 mL of water was added. Following the extraction of the aqueous DMF mixture using EtOAc, followed by sodium bicarbonate wash of the organic layer to remove HOBt. Hydrazide was the end product in the organic layer. The organic layer was filtered, washed with 5% sodium bicarbonate solution, and then washed with distilled water several times to purify the product **ATR-FTIR spectrum in cm^{-1}:**NH_2 3305.99 and 3221.12 for NH, 1643.35str of the amide of hydrazide.

Synthesis of (E)-N'-(3-bromo-4-methoxybenzylidene)-2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide ; comp. IIIA[14-22]

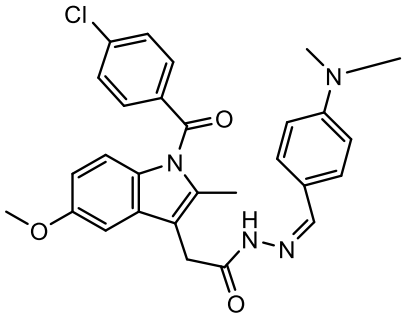
A quantity of 3-bromo-4-methoxybenzaldehyde (0.5806 g, 0.0027 mol) was dissolved in 30 mL of absolute ethanol. To this solution, five drops of glacial acetic acid were added. The mixture was continuously stirred in a round-bottom flask for 30 minutes to ensure the activation of aldehyde and facilitate the reaction process. Following this, an equimolar quantity of comp. II (0.0027mol) (1g) was added, and the combination was refluxed overnight. White ppt is formed at the end of the reaction; the precipitate was filtered and recrystallized from ethanol. R_f 0.80, yield 83%, M.P (197-199) ˚C. Spectrum ATR-FTIR in cm^{-1}: NH stretching of -CONH- group observed at 3201.83. Stretching band of carbonyl due to -CONH- group at 1674.21.^{1}HNMR (400 MHz, DMSO-d6) δ 11.36 (s, 1H) for proton -CONH- , 8.21 (s, 1H) for of imine proton -CH=N-, 7.79 – 6.71 (m, 10H) of 3 aromatic ring in structure, 3.80 – 3.71 (s, 3H) of OCH_3 in indole ring, 3.64 (s, 3H) of OCH_3 in other aromatic ring with bromine, 3.37 (s, 2H) of -CH_2-, 2.27 (s, 3H) of CH_3.



Compound IIIA

Synthesiso of 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N'-(4-(dimethylamino)benzylidene) acetohydrazide ; comp. IIIB *(14-22)*

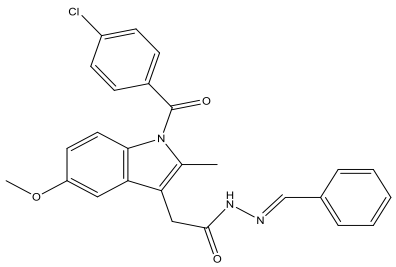
A quantity of 3-bromo-4-methoxybenzaldehyde (0.0027mol) (0.40g) was dissolved in 30 mL of absolute ethanol. To this solution, five drops of glacial acetic acid were added. The mixture was continuously stirred in a round-bottom flask for 30 minutes to ensure the activation of aldehyde and facilitate the reaction process. Then, an equimolar amount of compound II (0.0027 mol, 1 g) was added to the solution, and the mixture was heated under reflux overnight.. White ppt is formed at the end of the reaction; the precipitate was filtered and recrystallized from ethanol. R_f 0.88, yield 80% , M.P (210-212) ˚C. spectrum ATR-FTIR in cm^{-1}: NH stretching band of -CONH- group observed at 3201.83. Stretching band of carbonyl due to -CONH- group at 1654.92. ^{1}HNMR (400 MHz, DMSO-d6) δ 11.19 – 11.15 (s, 1H) for proton -CONH- , 8.18 – 8.10 (s, 1H) of imine proton -CH=N- , 7.70 – 6.72 (m, 11H) for three aromatic rings , 3.80 – 3.73 (s, 3H) of OCH_3, 3.65 (s, 2H) for $–CH_2$-, 2.99 – 2.93 (s, 6H) for $–N(CH_3)_2$, 2.31 – 2.27 (s, 3H) for -CH_3



Compound IIIB

Synthesis N'-benzylidene-2-(1 - (4 -chlorobenzoyl) – 5 – methoxy – 2 – methyl - 1H – indol – 3 - yl) acetohydrazide ; comp. IIIC *(14-22)*

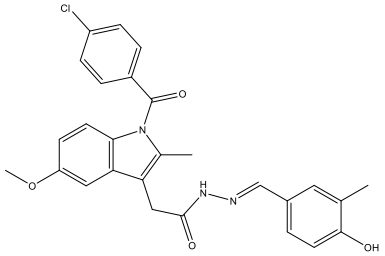
Benzaldehyde (0.0027mol) (0.286 g) was dissolved in 30 mL of absolute ethanol. To this solution, five drops of glacial acetic acid were added. The mixture was continuously stirred in a round-bottom flask for 30 minutes to ensure the activation of aldehyde and facilitate the reaction process. Then, an equimolar amount of compound II (0.0027 mol, 1 g) was added to the solution, and the mixture was heated under reflux overnight. White ppt is formed at the end of the reaction; the precipitate was filtered and recrystallized from ethanol. R_f 0.86, yield 85%, M.P (169-172) ˚C.spectrum ATR-FTIR in cm^{-1}: NH stretching band of -CONH- group observed at 3182.55. They were stretching the bands of carbonyl due to the -CONH- group at 1666.5. Stretching band of imine group -HC=N- at 1604.77. ^{1}HNMR (400 MHz, DMSO-d6) δ 11.64 – 11.48 (s, 1H) for proton -CONH-, 8.29 – 8.07 (s, 1H) of imine proton -CH=N-, 7.74 – 6.68 (m, 12H) for three aromatic rings, 3.76 – 3.64 (s, 3H) of OCH_3, 3.38-3.37 (s, 2H) for $–CH_2$-, 2.30 – 2.28 (s, 3H) for -CH_3 .



Compound IIIC

Synthesis of 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N'-(4-hydroxy-3-methyl-benzylidene) acetohydrazide ; comp. IIID *(14-22)*

A quantity of 3-4-hydroxy-3-methylbenzaldehyde (0.0027mol))(0.367g) was dissolved in 30 mL of absolute ethanol. To this solution, five drops of glacial acetic acid were added. The mixture was continuously stirred in a round-bottom flask for 30 minutes to ensure the activation of aldehyde and facilitate the reaction process. Then, an equimolar amount of compound II (0.0027 mol,1 g) was added to the solution, and the mixture was heated under reflux overnight. White ppt is formed at the end of the reaction; the ppt. Is washed and recrystallized from ethanol. R_f 0.90 yields 73%, M.P (208-210) ˚C. Spectrum ATR-FTIR in cm^{-1}: NH stretching band of -CONH- group observed at 3186.40. phenolic OH is stretching band at 3514.30. Stretching band of carbonyl due to -CONH- group at 1651.07.^{1}HNMR (400 MHz, DMSO-d6) δ 11.47-11.35 (s, 1H) for proton -CONH-, 8.88 (s, 1H) of -OH, 8.17 – 8.15 (s, 1H) of imine proton -CH=N-, 7.71 – 6.69 (m,10H) for three aromatic rings, 3.80 (s, 3H) for $–OCH_3$, 3.64 (s, 2H) for $–CH_2$-,2.51 (s, 3H) of $–CH_3$ in indole, 2.30 (m, 3H) of CH_3.



Compound IIID

Molecular docking study

The DNA binding protein 6YD9 (DNA Gyrase) in *E. coli* was subjected to molecular docking [23]. The X-ray crystallography method was used to identify this protein; it has a resolution of 1.60 angstroms and is mutation-free. This protein has just one chain, chain A, which has a binding site co-crystal ligand (ON2). After removing the water, identifying the binding site, and adding hydrogen, we perform docking on chain A. The AMBER10 force field is used to reduce the energy of the proteins. A fixed atom restriction was used to determine the stiff binding site of a protein.. the binding site contained the following amino acids: (SER121 ARG136 GLY164 THR165 VAL43 GLU50 ALA53 VAL71 GLN72 ASP73 GLY75 ARG76 GLY77 ILE78 PRO79 VAL93 ILE94 ASN46 ALA47 ASP49 VAL97 LEU98 HIS99 GLY119 VAL120 MET166 VAL167). The MOE 2022 program was used to complete the molecular docking. Chem-Bio Draw Ultra13.0 was utilized to generate the SMILE structures of the drugs under investigation. Then, utilizing MOE 2022 software, the structures were created in three dimensions. After protonating the three-dimensional structures, energy was decreased with a 0.1 Å RMSD. AMBER10 force field. A wealth of established methods for verifying docking algorithms and scoring systems exists. Pose selection is a widely used strategy that entails re-docking a chemical with a known conformation and orientation, usually from a co-crystal, utilizing docking algorithms. Re-docking the co-crystallized ligand (NO2) with the same target protein and calculating the RMSD Dock using the DISCOVERY STUDIO VISHULIZER software and VMD showed that the software was efficient; the result was 1.181Ang, which is considered good. This indicates that the MOE program is efficient in docking our new ligands.

Antibacterial Assay

The rapid resazurin microtiter test method is a colorimetric technique used to determine the antibacterial susceptibility of bacteria. Mueller-Hinton broth was used as a diluent to create double serial dilutions (1-1024 µg/ml) of exopolysaccharide and Ciprofloxacin in a microtiter plate starting from a stock (10 mg/1 ml). Except for the negative control wells, all wells received an injection of 20µl of bacterial suspension equivalent to McFarland standard no.0.5 (1.5×108CFU/ml). For 18 to 20 hours, microtiter plates were incubated at 37°C. Following incubation, each well received 20 µl of resazurin dye, which was applied and incubated for two hours to look for color changes.

The lower-MIC In the resazurin broth experiment, the lowest concentrations at which color changed from blue to pink were used to visually evaluate quantities in broth microdilutions [24]

(IIIA-D) was shown to have antibacterial activity against *K. pneumonia, S. aureus, E. coli,* and *S. pyogenes* at the concentration reported by Lewus et al. (1991) using the agar well diffusion technique.Every bacterial isolate that was the subject of the investigation—*K. pneumonia*, S. aureus, *E. coli*, and S. *pyogenes* were cultured in nutritional broth and incubated for 18 to 24 hours at 37^0 C. After incubation, 0.1 ml of each bacterial solution was applied to the nutrient agar surface and incubated for 24 hours at 37 º C. Then, a single colony was introduced to a test tube holding five milliliters of normal saline, producing a moderately turbid bacterial suspension comparable to the standard turbidity solution (about 1.5x108 CFU/mL).

A part of the bacterial suspension was gently and uniformly placed over the Mueller-Hinton agar medium using a sterile cotton swab. After that, the plate was allowed for ten minutes to dry. In the agar layer, three wells with a diameter of five millimeters were created on each plate. After removing the agar discs, 50 µl of each test substance (IIIA-D) was pipetted into a different well using a micropipette. Distilled water (D.W.) was added to the middle well as a negative control. After that, the plates were incubated for eighteen hours at 37º C. Following incubation, each well's surrounding inhibition zone diameter was measured.

Antibacterial action against the particular bacteria present in a well is shown by the existence of a clean zone surrounding the well that contains the test sample. The material's antibacterial activity increases with the diameter of the inhibitory zone [25] .

Statistical analysis

We cannot reject the null hypothesis, which states that there are no statistically significant changes in the mean concentration of the antibiotic (MIC) between the treatment and control groups, based on the findings of the Mann-Whitney U test. This is because the p-value (0.081) is higher than the standard significance level of 0.05, which is considered a positive outcome for obtaining a Compound that is on par with the effective antibiotic ciprofloxacin[26].

Results and Discussion

Chemistry

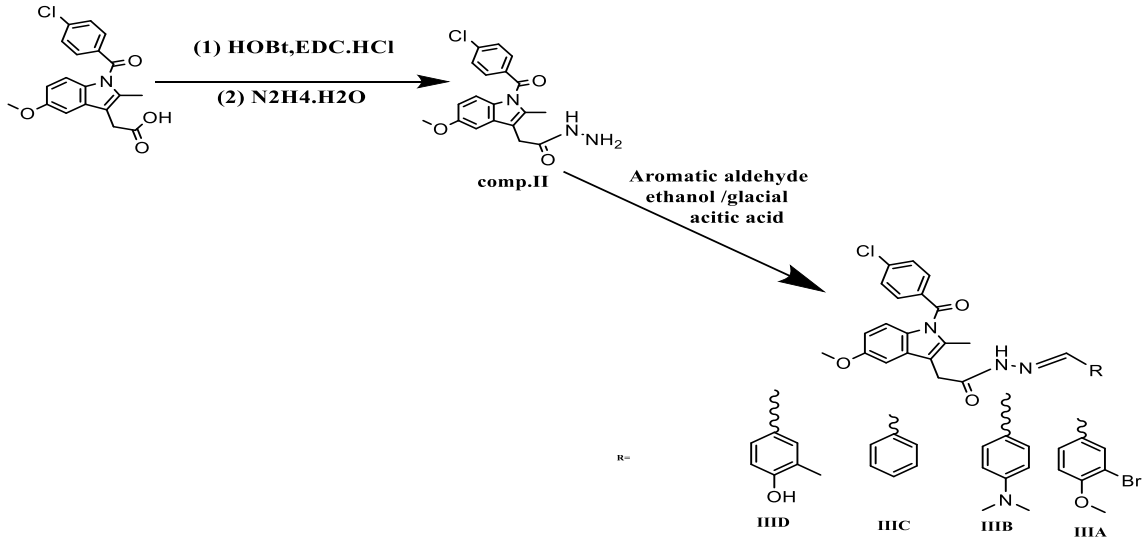
Stepwise synthesis of the targeted compounds is shown in scheme 1 at the end of this section. The synthesis of compound II by EDC.HCl/HOBt method includes the hydrazinolysis of HOBt ester that proceeds in the following steps as shown in the scheme1:

The reaction is a carboxy-addition reaction. The carboxylic acid 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetic acid (Indomethacin) addition to (EDC.HCl) ,(HOBt) formed HOBt ester then addition of (hydrazine hydrate) to HOBt ester result in (Comp. II) hydrazide [27].

Comp. II is characterized by asymmetric and symmetric stretching bands 3305.99 and 3221.12 of primary amine groups and ^{1}HNMR (400 MHz, DMSO-d6) δ 9.27 (s, 1H) for the proton of amide-CONH-, δ 4.27 (s,1H) of primary amine NH_2. [26, 27]

Comp. IIIA-D was Schiff-base products of the hydrazone type, produced when aldehydes and primary amines reacted in a somewhat acidic environment. . [18] Absence of hydrazide NH_2 asymmetric and symmetric stretching vibration bands was seen in the ATR-FTIR spectra. And the appearance of phenolic OH stretching bands at 3510.45 IIID and stretching bands of imine groups [-HC=N-] at [1612.49, 1604.77, and 1616.35] cm^{-1}; for Compounds IIIA, IIIC, and IIID respectively and overlapping with carbonyl in IIIB. [27, 28] ^{1}HNMR was characterized by the appearance of signal for OH proton at 9.35ppm for compound IIID ; peaks related to CONHN, which were [for compounds IIIC, IIID , IIIB, and IIIA] result from *E* and *Z* isomers showed *2signals* at [11.48 and 11.64]ppm,[11.35and 11.47]ppm, [11.17and 11.33]ppm,[11.36 and 11.53]ppm respectively and CONHN=CH which due to the conformers *syn/anti-syn* showed *2signals* at [8.29 and 8.21]ppm and [8.07 and 8.29]ppm. For compounds IIIA and IIIC, respectively. Signals for OH proton of at 8.90ppm due to free hydroxyl group; for compound IIID signal. And signals of imine protons at [8.15and 8.17]ppm and 8.12 ppm for compound IIID and compound IIIB, respectively. [27-37]

Scheme 1. Synthesis of target compounds



Molecular docking analysis

The molecular docking process was completed using MOE software, a grid-based technique for docking ligands into receptor binding sites. In plate number 1, SITE FINDER located the binding locations. In the refining process, the ligands were flexible, but the receptor stayed inflexible.

The binding mode of the comp. IIIA exhibited an affinity value of -8.67 kcal/mol formed (ten hydrophobic interactions by VDW force with ILE78(2), ILE94, PRO79, VAL167, VAL71(2), VAL120, VAL43, ASN46(2)) and (four hydrogen bonds with THR165(2), GLY77, VAL71). The binding mode of comp. IIIB exhibited an affinity value of -7.56 kcal/mol, formed nine hydrophobic interactions by VDW(ARG76, PRO79, VAL43, VAL167, VAL120(2), ILE78, ILE94(2)) and (three hydrogen bonds with Thr165, GLy77, HIS99).The binding mode of the comp. IIIC exhibited an affinity value of -7.43 kcal/mol. (The eight hydrophobic interactions by VDW forces with PRO79,ARG76,ILE78(2),VAL167,VAL43,ASN46 (2)) and (four hydrogen bonds with THR165,VAL71,GLY77(2)). The interacting method of the comp. IIID exhibited an affinity value of -^.76 kcal/mol formed eleven hydrophobic interactions by VDW forces with ASN46 (2), PRO79, ARG76, VAL43, VAL167, VAL71, ALA47, ILE78 (2), and VAL120, and six hydrogen bond with Gly77 , GLU50, Thr165 (2), VAL71, and ASP73 .

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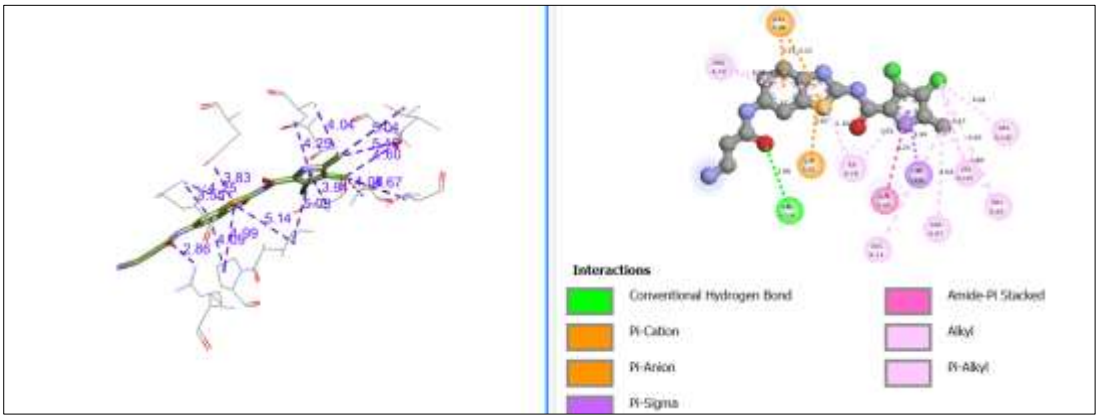

Figure 2. 2D and 3D Cocrystal (ON2) ligand interaction

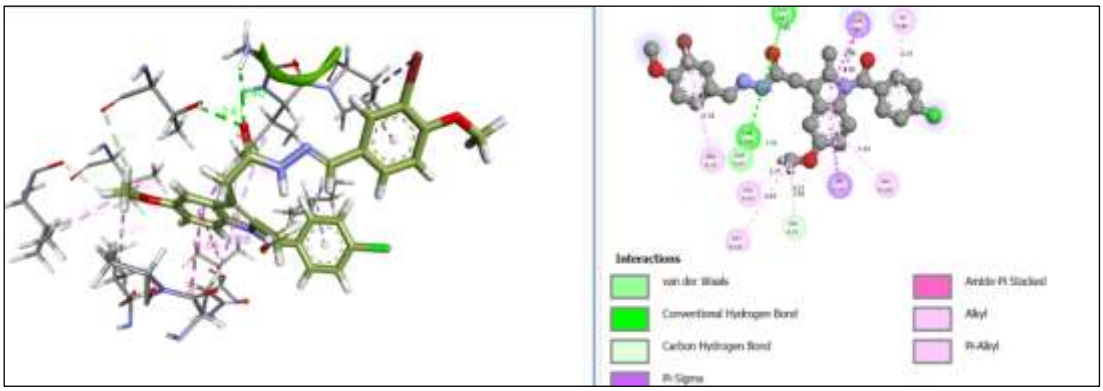

Figure 3. 2D and 3D ligand (IIIA) interaction

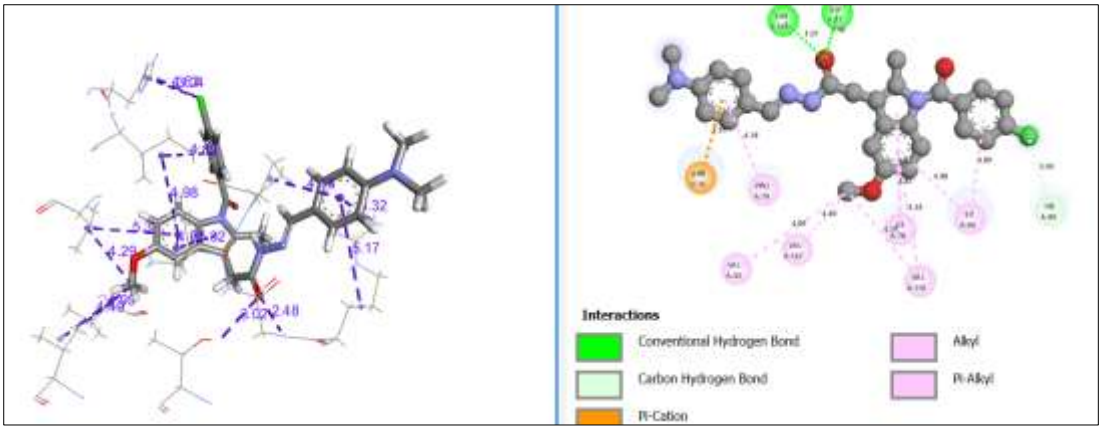

Figure 4. 2D and 3D ligand (IIIB) interaction

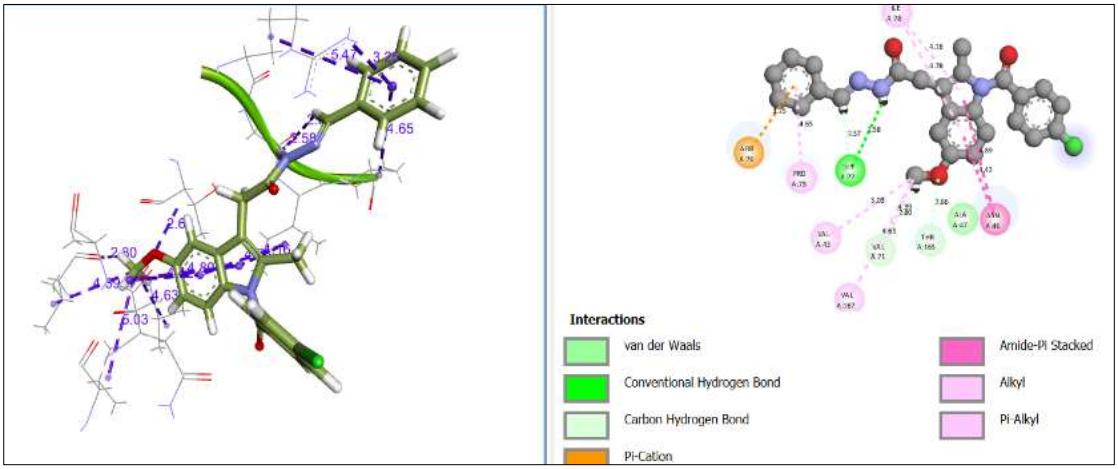

Figure 5. 2D and 3D ligand (IIIC) interaction

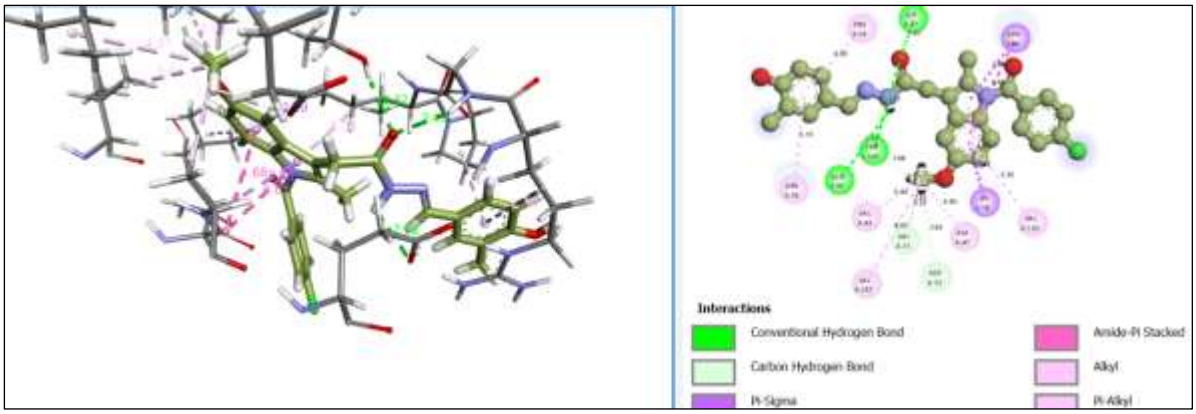

Figure 6. 2D and 3D ligand (IIID) interaction

Table 1. Information about the hydrogen bond interactions and binding energies (kcal Mol $^{-1}$) of four new compounds and the reference benzothiazole-based inhibitor and ciprofloxacin inhibitor with *E. coli* gyrase B (24 kDa) (PDB: 6YD9)

Ligand	RMSD(Ang)	Binding Energy (kcal mol-1)	Interacting Amino acid Residues	Number of Hydrogen Bonds	Number of hydrophobic bonds
ON2(cocrystal ligand)	1.18	-6.28	ARG76 (2),PRO79(2), ARG136,GLU50,ILE79(2) THR165,VAL43(2), VAL167,VAL120 ASN46,VAL71,ALA47	1	15
ciprofloxacin	1.24	-5.98	GLY77(3),GLU50,ILE78(2),ILE,ASN46(3),PRO79	4	8
IIIA	2.17	-8.67	ILE78(2),ILE94, THR165(2),GLY77,PRO79,VAL167,VAL71(2), VAL120,VAL43,ASN46(2)	4	10
IIIB	1.31	-7.56	GLY77,THR165,ARG76,PRO79,VAL43,VAL167,VAL120(2),ILE78,ILE94(2), HIS99	3	9
IIIC	1.50	-7.43	THR165,GLY77(2),PRO79,ARG76,ILE78(2),VAL167,VAL71,VAL43,ASN46(2)	4	8
IIID	2.5	-8.76	ASN46 (2), PRO79 ARG76,VAL43,VAL167, VAL71, ALA47 Gly77,GLU50, Thr165 (2) VAL71, ASP73 ILE78(2), VAL120	6	11

ADME studies

SWISS ADME server, admet SAR server, T.E.S.T. software, Crystal ligand (ON2), and my newly developed indomethacin hydrazide derivatives were used to evaluate the ADME parameters. Table 2 displays a summary of the findings. The findings showed that none of the derivatives could cross the blood-brain barrier (BBB), reducing the likelihood of CNS side effects. Furthermore, the solubility levels of all ligands vary from moderate to low. Additionally, every evaluated ligand had high absorption levels and didn't seem to inhibit cytochrome P450.

Table 2. ADME prediction for the synthesized indomethacin hydrazide derivatives

Comp	BBB absorption[38]	Solubility Level[39]	Absorption Level [38]	PPB prediction (100%) [38]	CYP2D6 [39]	Rule of five violation [39]
ON2	NO	moderate soluble	LOW	80.31	YES	0
Ciproflox	NO	Soluble	High	39.66	NO	0
IIIA	NO	Poorly soluble	High	97.28	NO	2
IIIB	NO	Poorly soluble	High	96.09	NO	1
IIIC	NO	Moderate Soluble	High	96.69	NO	0
IIID	NO	Poorly soluble	High	83.92	NO	0

Toxicity studies

AdmetSAR server has been used to conduct virtual toxicity studies against several toxicity models, as previously mentioned, with **ON2 and ciprofloxacin** serving as reference molecules. Regarding hepatotoxicity, all ligands were expected to be less harmful than **ciprofloxacin and cocrystal ON2** , which demonstrated a certain degree of in silico hepatotoxicity. Oral Rat LD_{50} exhibits a wide range of dosages and is generally safe. These findings suggested that all ligands had favorable pharmacodynamic characteristics and were recommended for more study. Table 3 provides a summary of the findings.

Table 3. In-silico toxicity properties of the newly synthesized indomethacin hydrazide derivatives [38]

Comp	Skin irritation	Eye irritation	Hepatotoxicity probability	Carcinogenicity	Oral Rat Toxicity (LD50) (mg/kg/Day)*
ON2	NO	NO	0.7500	NO	NA
Ciprofloxacin	NO	NO	0.9750	NO	4344.04
IIIA	NO	NO	0.7250	NO	481.47
IIIB	NO	NO	0.7301	NO	404.77
IIIC	NO	NO	0.8000	NO	366.91
IIID	NO	NO	0.5800	NO	431.61

T.E.S.T. Version 5.1.2 A program to estimate toxicity from molecular structure

Physicochemical properties

Improving a drug's molecular activity requires an understanding of its physical properties. The partition coefficient (clogP), which forecasts how medications will go through the human body, is one metric that is crucial to understanding this component. Nonetheless, it is essential to note that all target compounds coincide with the generally recognized Lipinski's rule of five, with clogP values of less than 5 except comp **IIIA** but still obey Lipinski's rule. Another crucial measure for determining the surface area filled by polar atoms in a compound is the topological polar surface area or TPSA. Since lower TPSA levels are linked to enhanced membrane permeability, they are significantly more desired for drug-like qualities. Thus, it is possible to improve the therapeutic attributes of medications and improve patient outcomes from treatment by closely examining these factors. [40-42]

Table 4. The properties related to physical and chemical characteristics of the compounds that were synthesized[39]

Comp	Clog p	No. H-bond acceptors	No. H-bond donors	No. rotatable bonds	TPSA / °A
Co crystal (NO2)	2.97	4	4	7	141.14
CIPROFLOXACIN	1.1	5	2	3	74.57
IIIA	5.32	5	1	9	81.92
IIIB	4.74	4	1	9	75.93
IIIC	4.45	4	1	8	83.69
IIID	4.68	5	2	8	92.92

Antibacterial evaluation

The synthesized compounds are structurally related to compounds synthesized by Saleh *et al*. [which were benzimidazole derivatives] and compounds synthesized by Kamat *et al*. [which were pyridine and thiazole-based hydrazones]. These compounds showed activities toward ***S. aureus, E. coli, K. pneumonia*** and ***P. aeruginosa.*** [43,44] .The antibacterial activities as shown in Table 4 of the targeted compounds **(comp. IIIA-D)** were evaluated by healthy diffusion technique and resazurin microtiter assay MIC (minimum inhibitory concentration) method, using gram-positive and gram-negative bacteria, compared with [ciprofloxacin] as standard antibacterial agents. DMSO was used as a solvent and as a control. **IIIA** effective against ***S. aureus*** with a concentration 250 **µg/cm3** and is less potent than ciprofloxacin. **IIIB** also shows effectiveness toward ***S. aureus*** and also show stronger towards ***K. Pneumonia*** than ciprofloxacin with a MIC concentration of 250 **µg/cm^3. IIIC** show comparable activity to ciprofloxacin towards ***S. aureus*. IIID** most effective toward ***S. aureus*** with a concentration of 250 **µg/cm3**. In general, resazurin microtiter assay MIC (minimum inhibitory concentration) method shows Compound **IIIB** exhibits superior efficacy against ***K. pneumonia***, with a lower MIC compared to ciprofloxacin.Compound **IIIC** demonstrates comparable efficacy to ciprofloxacin against both ***S. aureus*** and ***K. pneumonia***.The remaining synthesized compounds show lower efficacy in all cases.

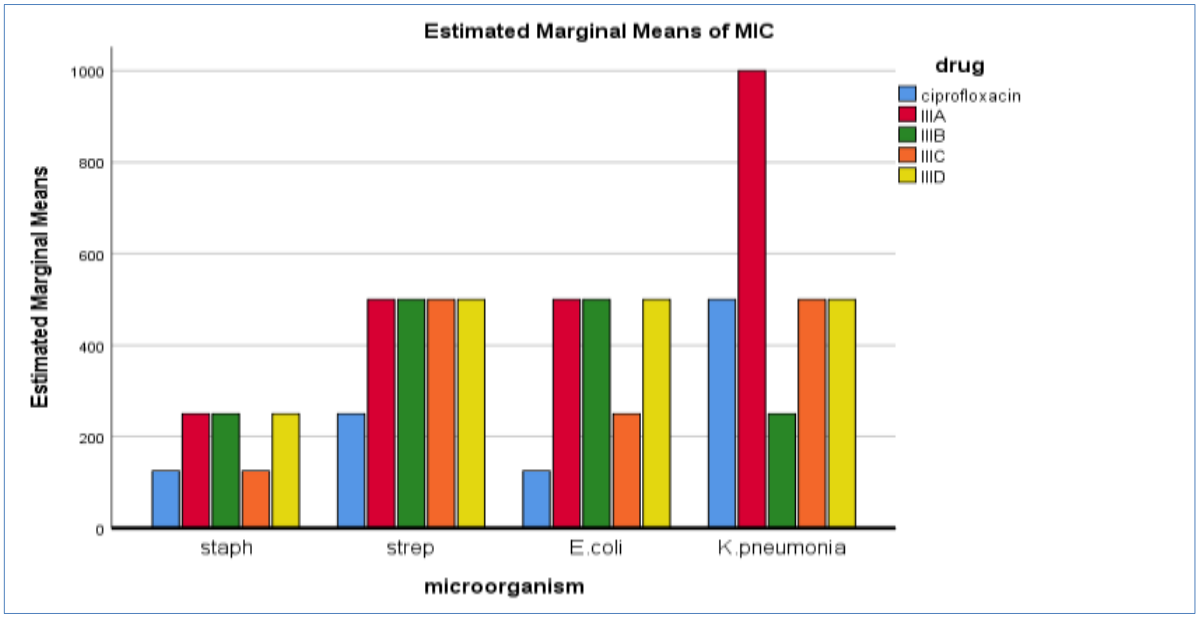

Figure 7. Comparative Evaluation of Novel Antimicrobial Compounds Using MIC Values against Diverse Microorganisms

Table 5. Results of the antibacterial activities of synthesized compound Minimum inhibitory oncentration (MIC), µg/cm3 and Inhibition Zone (I.Z), (mm)

Microorganism culture	Ciprofloxacin		IIIA		IIIB		IIIC		IIID	
	MIC	I.Z	MIC	I.Z	MIC	I.Z	MIC	I.Z	MIC	I.Z
S. aureus	125	20	250	14	250	20	125	14	250	18
S. pyogenes	250	25	500	17	500	18	500	15	500	16
E. coli	125	22	500	16	500	21	250	16	500	19
K. pneumonia	500	25	1000	15	250	20	500	14	500	13

Conclusion

ATR-FTIR and ^{1}HNMR spectroscopy was used to confirm the structures of four newly created compounds (IIIA-D). The antimicrobial activity data of these N-acyl hydrazone compounds show how effective they could be as antibacterial agents. The antibacterial activity against both gram-positive and gram-negative pathogens was assessed using the resazurin microtiter assay MIC (minimum inhibitory concentration) method and compared to ciprofloxacin, which is considered the gold-standard antibacterial agent, according to the results of the preliminary microbiological screening. The compounds' zones of inhibition encompassed moderate to vigorous antibacterial activity. However, **IIIC** had similar action to ciprofloxacin against *K. pneumonia* and *S. aureus* and IIIB is more potent than ciprofloxacin against *K. pneumonia*. Bacterial resistance is the primary issue with bacterial therapy. Hence, it is crucial to find new bioactive substances that cause bacterial cell death via novel and unusual methods while avoiding bacterial resistance. The present study showed that indomethacin hydrazide derivatives, especially **IIIC and IIIB,** have activity against different bacterial strains that are equivalent and potent to ciprofloxacin, suggesting their possible use in treating bacteria.

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Conflicts of Interest

There is no conflict of interest in this manuscript

Funding

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Ethics Statements

The study did not need ethical approval from an ethics committee.

Contribution of the Author

Both authors confirm the following contributions to the work, Yaseen S. Hamdoon and Mohammed K. Hadi: importing of indomethacin, research methodology, supervision of the progress of the reactions, synthesis of the compounds and performing **FTIR** analysis, interpretation of **FTIR and ^{1}HNMR**, and interpretation of antibacterial results, supplying of EDC.HCl, HOBt to form hydrazide and providing essential references: Both authors reviewed the results and approved the final version of the manuscript

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الدراسة الحاسوبية، تخليق وتقييم مركبات هيدرازونات الإندول الجديدة كعوامل محتملة مضادة للبكتيريا

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الخلاصة

بسبب اكتساب البكتيريا الممرضة لمقاومة المضادات الحيوية، أصبحت الأمراض البكتيرية صعبة العلاج. يتم نشر عدد كبير من الدراسات سنويًا.

بهدف تطوير مضادات بكتيرية جديدة. تُعد قواعد شف مجالًا مهمًا في الكيمياء العضوية نظرًا لأنشطتها البيولوجية، كما تتميز بمجموعة واسعة من الأنشطة الدوائية. يُعتبر دمج اثنين من الحوامل الدوائية [جزيء الحلقة غير المتجانسة والهيدرازونات المُصنَّعة] لتكوين جزيء واحد طريقةً هامة في تصميم الأدوية الجديدة. تم تصنيع المركبات الجديدة المشتقة من الهيدرازونات باستخدام عملية متعددة الخطوات تتضمن تكوين هيدرازيد الإندوميثاسين من خلال تفاعل عوامل الاقتران (EDC.HCl,HOBt) ثم التفاعل مع مختلف الألدهيدات لتكوين الهيدرازونات. تم استخدام كروماتوغرافيا الطبقة الرقيقة(TLC) ، وأطياف الأشعة تحت الحمراء (ATR-FTIR)، وأطياف الرنين المغناطيسي النووي للبروتون (HNMR^1)لتوصيف المركبات. نهدف إلى استخدام تقنية الالتحام الجزيئي لتطوير مركبات ذات نشاط مضاد للبكتيريا وخصائص ADMET (الامتصاص، التوزيع ،الأيض،الإخراج،والسمية) الملائمة، التي يمكن إنتاجها واستخدامها في المستقبل. تم تقييم الخصائص المضادة للبكتيريا للمركبات الجديدة باستخدام طريقة الميكروتايتر السريعة بمؤشر الريزازورين (MIC) لتحديد أقل تركيز مثبط (MIC). أظهرت بعض المركبات (IIIC) نشاطًا مقارنًا مع السيبروفلوكساسين ضد المكورات العنقودية الذهبية،كما أظهرت المركبات (IIIC، IIID) نشاطًا مشابهًا ضد الكليبسيلا الرئوية، في حين أظهر المركب (IIIB) فعالية أقوى ضد الكليبسيلا الرئوية. بشكل عام أظهرت جميع المركبات الجديدة أنشطة جيدة ضد الإشريكية القولونية، الكليبسيلا الرئوية ، المكورات العقدية المقيحة والمكورات العنقودية الذهبية.

الكلمات المفتاحية: مضادات البكتيريا، الالتهابات البكتيرية، الحلقات غير المتجانسة، التهجين، الالتحام الجزيئي، قاعدة شف.