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Research Article

Molecular docking, ADMET, synthesis and evaluation of new indomethacin hydrazide derivatives as antibacterial agents

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Abstract

Bacterial infections pose an ongoing challenge due to resistance developed by infectious bacteria. So much research targeting designing new antibacterials is published annually. Our goal is to synthesize compounds that have given antibacterial activity according to molecular docking against the chosen target protein and that have acceptable ADMET properties that can be synthesized and used in the future. New 2-(5-methoxy-1-(4-chlorobenzene)-2-methyl-1H-indol-3-yl)acetohydrazide derivatives' antibacterial efficacy against two common strains of Gram-negative and Gram-positive microorganisms has been developed, produced, and investigated. Sophisticated, modern analytical methods, including ATR-FTIR and 1H NMR spectroscopy, were used to determine their spectral and physicochemical features. Compound YA3N is more effective than ciprofloxacin against *K. pneumonia* (MIC = 125 µg/mL) and shows good suppression of isolated tests of *E. coli* (MIC = 125 µg/mL). While compound YA4C demonstrated comparable suppression of *S. pyogenes* strains (MIC = 250 µg/mL), compounds YA3S and YA4B exhibit lesser activity towards the tested strain of bacteria.

Keywords

Docking study, organic synthesis, antimicrobial activity

Introduction

Drug-resistant bacterial pathogen strains are becoming more prevalent, reducing current therapies' efficacy and increasing the risk of developing new bacterial infections. The relevance of this situation for modern pharmaceutical science is that it compels scientists worldwide to look for novel, low-toxic medications that are both effective and have unique mechanisms of action.

The nitrogen indole ring is a component of several chemical structures, both synthetic and natural, that can have a wide variety of biological functions in organic molecules. Relevant in this context are 2-(2-methyl-5-methoxy--1H-indol-3-yl)-1-(4-chlorobenzoyl) aceto hydrazide derivatives. The antibacterial activity of the indole ring is increased when it is attached to other simple aromatic rings such as phenyl, pyrazole, or isoxazole. (Shirinzadeh et al. 2011). In general, NSAIDs show a wide range of anti-microbial trends. However, they also exert a potent decrease in adherence, biofilm formation, and other pathogenicity factors, in addition to the capacity to elevate or reduce antibiotic susceptibility (Leme and da Silva 2021). Indomethacin could be a potential inhibitor of the QS (Quorum sensing) and could suppress the QS-related virulence factors of Acinobacter Baumannii (Elshaer et al. 2022).

Changes in an NSAID structure cause an increment in its antibacterial action, and further investigations could lead to efficient anti-microbial compounds (Hersh et al. 1991).

Chemicals of the hydrazone type with an azomethine group also constitute an important class of chemicals for novel drug discovery. The hydrazone group of these molecules is crucial to their antibacterial activity (Abdel-Fattah et al. 2000). Indole and its derivatives are a significant family of compounds in the continuous hunt for new antibacterial medications. Additionally, it has been demonstrated that azoles and their derivatives possess several biological properties, including antifungal and antibacterial ones. Antibacterial activities were examined to assess the effectiveness of several indole derivatives, 1,2,4-triazole, 1,3,4-thiadiazole, and hydrazine carbothioamide (Shirinzadeh et al. 2018).

Sulfonamides are a class of drugs that include the sulfonamide chemical functional group (R-SO2-NH2) (Ahmad and Akhyar 2012; Abdul Qadir et al. 2015). They are a member of the earliest anti-microbial class of chemicals with broad anti-microbial activity and are efficient against pathogenic strains of gram-negative and gram-positive bacteria. They treat many bacterial infections, like respiratory tract infections, skin infections, malaria, etc. (Siddique et al. 2013). Sulfonamides also have a wide range of pharmacological activities, like antiviral, antioxidant, anticarbonic anhydrase, diuretic, hypoglycemic, antithyroid, anti-inflammatory, antiglaucoma, antineoplastic, etc. (Kolaczek et al. 2014; Bagul et al. 2016).

Purpose

This work's objective was to create novel chemicals based on 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl) acetohydrazide (Fig. 1) in which aromatic aldehyde and sulfonyl chloride derivatives were used as hydrazone and sulfonamides agents (Fig. 6), and study the reaction of hydrazide that previously synthesized from carboxylic acid, examine their physicochemical characteristics and verify these derivatives' structures using a variety of contemporary identification techniques. We also attempted to look into the antibacterial activity of compounds to evaluate their pharmacological potential on a variety of strains of both Gram-positive and Gram-negative microorganisms. The main goal was to compare the connections between these compounds' functional groups and antibacterial activity.

Materials and methods

Generalities

The Stuart SMP30 Electronic Melting Point device, which also allowed for temperature measurements with a resolution of 0.1 °C and a maximum temperature of 400 °C, was utilized to test the melting points of new compounds. A Varian MR-400 spectrometer acquired 1H NMR spectra at 400 MHz using DMSO-d6 as the solvent. Chemical shifts are represented as ppm (δ scale) downfield, utilizing a common internal standard and protons of the solvent (DMSO-d6) present at δ = 2.52 ppm. Molecular docking was carried out using MOE2022, and the screening procedure utilized the crystallographic structure of *E. coli* gyrase B (24 kDa) (PDB: 6YD9) (Skok et al. 2020).

The subject of the research was represented 2-(5-methoxy-2-methyl-1H-indol-3-yl)-1-(4-chlorobenzoyl)-N'-(4-hydroxy-3,5-dimethoxybenzylidene)acetohydrazide **YA3S**, N'-(3-hydroxy-4-nitrobenzylidene)acetohydrazide; 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl) **YA3N**, N'-(2-(1-(4-chlorobenzene)-5-methoxy-2-methyl-1H-indol-3-yl)acetyl)-4-methylbenzenesulfonohydrazide **YA4B** Benzenesulfonohydrazide 4-chloro-N'-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetyl) **YA4C.** Since ciprofloxacin had good efficacy against several bacteria in earlier research, it was also selected as a possible antimicrobial agent.

Chemistry

Synthesis of 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide; comp. YA2

3 gm of indomethacin (0.0083 mol) dissolved in 30 ml of DMF with an excess of HOBt(1.694 gm, 0.0125 mol) and EDC(2.3989 gm, 0.0125) the resulting mixture was stirring at room temp for 30 min leading to form a ppt indicates formation of ester, this product added dropwise to solution of hydrazine 99% and 10 ml of DMF previously prepared in temp. (0–10 °C). Usually, the reaction was finished when the addition was finished. Water (40 mL) was then added. After extracting the aqueous DMF combination with EtOAc, the organic layer was washed with carbonate to get rid of HOBt. R_f 0.82, yield 83%. Diluting the reaction mixture with water allowed for the easy separation of water-insoluble hydrazides through filtration (Teitel and Brossi 1968; Montalbetti and Falque 2005; Chan and Cox 2007**).**

(YA2) m.p. = 176–178 °C. 1HNMR (400 MHz , DMSO-d_6) δ ppm 9.27(s, 1H, NHCO), 3.46(s, 2H, CH2), 4.27 (s, 2H, NH2),7.70 (s, 1H, ArH), 6.69–7.49 (d, 6H, ArH),2.26 (s, 3H, CH3), 3.80(s, 3H, OCH3),

IR Spectroscopy: ATR-FTIR 3305 cm^{-1} sym. primary amine (N-H) str, 3221 cm^{-1} amide (N-H) str, 3035 cm^{-1} Ar(C-H) str, 2927,2843 cm^{-1} asym. & sym. Of CH3, 1643 cm^{-1} amide (C=O) str. 1481 - 1523 & 1604 cm^{-1} aromatic (C=C) str.

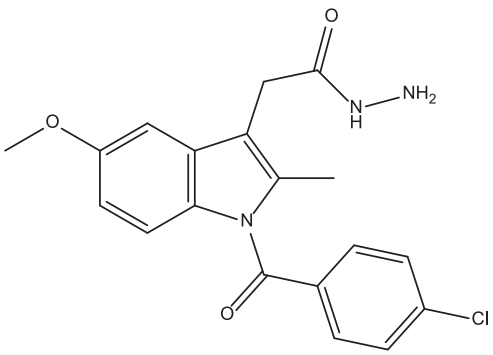

Figure 1. Synthesized hydrazide YA2.

Synthesis 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N′-(4-hydroxy-3,5-dimethoxybenzylidene) acetohydrazide YA3S

(Sulaiman and Sarsam 2020; Sadia et al. 2021; Shah et al. 2022; Shivhare et al. 2022; Kamms and Hadi 2023; Saeed and Al-Hamashi 2023)

1 gm of hydrazide (5-methoxy-2-methyl-2-(1-(4-chlorobenzoyl)-1H-indol-3-yl) acetohydrazide) (0.0028 mol) is suspended in 30 ml ethanol and is added to 5 drops of glacial acetic acid and equimolar of syringaldehyde in the round bottom flask with reflex overnight the reaction monitored by TLC (1.5 Ethyl acetate : 3.5 Chloroform) will form light yellow powder ppt washed and recrystallized in ethanol (Han et al. 2018) (Fig. 2), R_f 0.6, yield 60%, recrystallized with absolute ethanol

(YA3S) m.p. = 234–236 °C. 1HNMR (400 MHz, DMSO-d_6) δ ppm 11.35 (s, 1H, NHCO), 8.9 (s, 1H, phenolic OH), 7.95 (s, 2H, ArH), 6.71–7.69 (d, 6H, ArH), 3.64 (s, 3H, CH3), 3.77(s, 9H, OCH3),

IR Spectroscopy: ATR-FTIR 3514 cm^{-1} phenolic (O-H) Str, 3159.40 cm^{-1} (N-H) amide Str, 3016.67 cm^{-1} Ar. (C-H) Str , 2924 cm^{-1} methyl (CH3) str, 1678 cm^{-1} amide carbonyl (C=O) Str, 1651 cm^{-1} of C=N Str, 1589,1512 & 1454 cm^{-1} aromatic (C=C) Str.

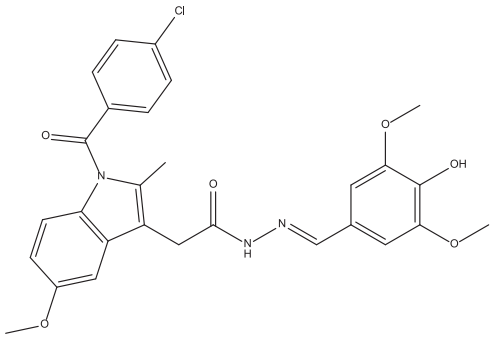

Figure 2. Synthsized hydrazone YA3S.

Synthesis 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N′-(3-hydroxy-4-nitrobenzylidene)acetohydrazide YA3N

(Sulaiman and Sarsam 2020; Sadia et al. 2021; Shah et al. 2022; Shivhare et al. 2022; Kamms and Hadi 2023; Saeed and Al-Hamashi 2023)

(0.0028 mol) (1 gm) of hydrazide (5-methoxy-2-(1-(4-chlorobenzoyl)-2-methyl-1H-indol-3-yl)acetohydrazide) YA2 suspended in 30 ml ethanol and added to 5 drops of glacial acetic acid with an equimolar of 3-hydroxy-4-nitro benzaldehyde) in a round-bottom flask with reflex. Throughout the reaction, TLC (0.5 Ammonia:3.5 Ethyl acetate :1 Methanol) is used to monitor the production of a yellow solid precipitate (ppt), which is then washed, recrystallized in ethanol with an 82% yield, and then recrystallized with pure ethanol (Fig. 3).

(YA3N) m. p. = 213–215 °C. 1HNMR (400 MHz, DMSO-d_6) δ ppm 11.48(1H, s, NHCO), 7.93(s, H, ArH) 7.91 (s, 2H, ArH), 6.69–79(d, 8H, ArH), 8.19 (s,1H, CH=N), 4.07(s, 2H, CH2), 3.58(s, 3H, OCH3), 3.77(s, 3H, CH3), 10.12(s, 1H, phenolic OH).

IR Spectroscopy: ATR-FTIR 3201 cm^{-1} phenolic (O-H) Str, 3159.40 cm^{-1} amide (N-H) Str, 3070 Ar. (C-H) str. 2924,2827 cm^{-1} (CH3) Str , 1670 cm^{-1} amide carbonyl (C=O) Str , 1593 cm^{-1} of C=N Str, 1543, 1469 & 1435 cm^{-1} aromatic (C=C) Str.

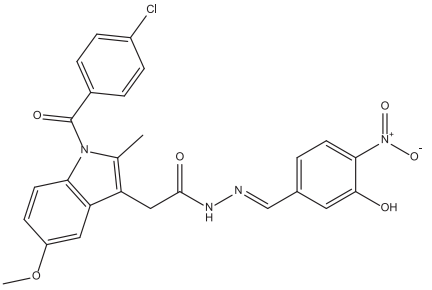

Figure 3. Synthsized hydrazone YA3N.

Synthesis N′-(5-methoxy-2-methyl-2-(1-(4-chlorobenzoyl)-1H-indol-3-yl)acetyl) benzenesulfonohydrazide derivatives

(Siddique et al. 2013; Sulaiman and Sarsam 2020; Hadi MK et al. 2021; Shah et al. 2022; Shivhare et al. 2022; Kamms and Hadi 2023; Saeed and Al-Hamashi 2023)

Solution of (2-(1-(4-chlorobenzene)-5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide) **(YA2)** (1.85 g, 5 mmol) in 30 mL dichloromethane (DCM) was mixed with different sulfonyl chloride para-toluene-sulfonyl chloride (0.98 g, 5 mmol) which gives YA4B Product, 4-chlorobenzene-sulfonyl chloride (1.05 g, 5 mmol) which gives YA4C Product in the presence of trimethylamine TEA (0.012 mol, 1.6 mL) and stirring for 24 hr at room temperature the reaction monitored by TLC (0.5 Ammonia:3.5 Ethyl acetate :1 Methanol). The components were poured into a funnel separator and rinsed with 30 milliliters of purified water. Over anhydrous sodium sulfate, the organic layer was dried, and the desired products were achieved by removing the solvent via a rotary evaporator (Hadi et al. 2021), both products were yellow in colour. R_f of YA4B 0.69, yield 59%, recrystallized with absolute ethanol, and R_f of YA4C 0.79, yield 50%, recrystallized with absolute ethanol also (Figs 4, 5).

(YA4B) m.p. = 260–262 °C. 1HNMR (400 MHz, DMSO-d_6) δ ppm 10.21 (s, 1H, NHCO), 10.13 (s, 1H, NHSO2) 6.68–7.69 (d, 10, ArH), 7.76 (s,1H, ArH).

IR Spectroscopy: ATR-FTIR 3217 cm^{-1} (NH) Str, 3020.53 cm^{-1} Ar. (C-H) Str , 3035 cm^{-1} Ar. (C-H) Str, 2924 cm^{-1} Sym of (CH3) Str, 2835 cm^{-1} Sym of (CH2) Str, 1678 cm^{-1} amide carbonyl (C=O) Str, 1658 cm^{-1} aromatic amide carbonyl (C=O) , 1593 & 1481 cm^{-1} Ar. C=C str.

(YA4C) m.p. = 274–277 °C. 1HNMR (400 MHz, DMSO-d_6) δ ppm 10.14 (s,2 H,NHCO,NHSO2),7.69 (s,1H, ArH), 6.68–7.76 (s,10H, ArH), 3.76(s,2H,CH2)

IR Spectroscopy: ATR-FTIR 3217 cm^{-1} (NH) Str, 3105 & 3032 cm^{-1} Ar. (C-H) Str , 1674 cm^{-1} amide carbonyl (C=O) Str , 1593,1562&1435 cm^{-1} Ar. C=C Str, 1357 & 1149 cm^{-1} asym. & sym. (S=O) Str.

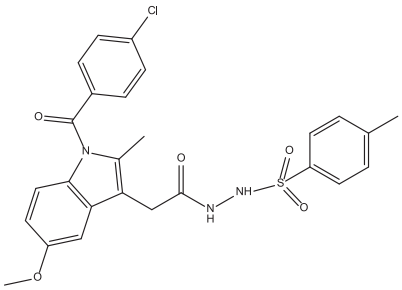

Figure 4. Synthesized sulfonhydrazide YA4B.

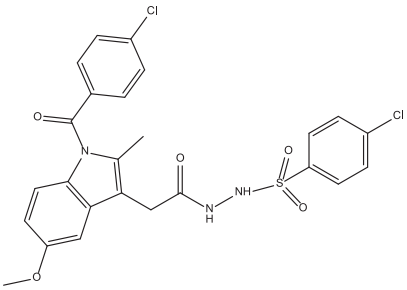

Figure 5. Synthesized sulfonhydrazide YA4C.

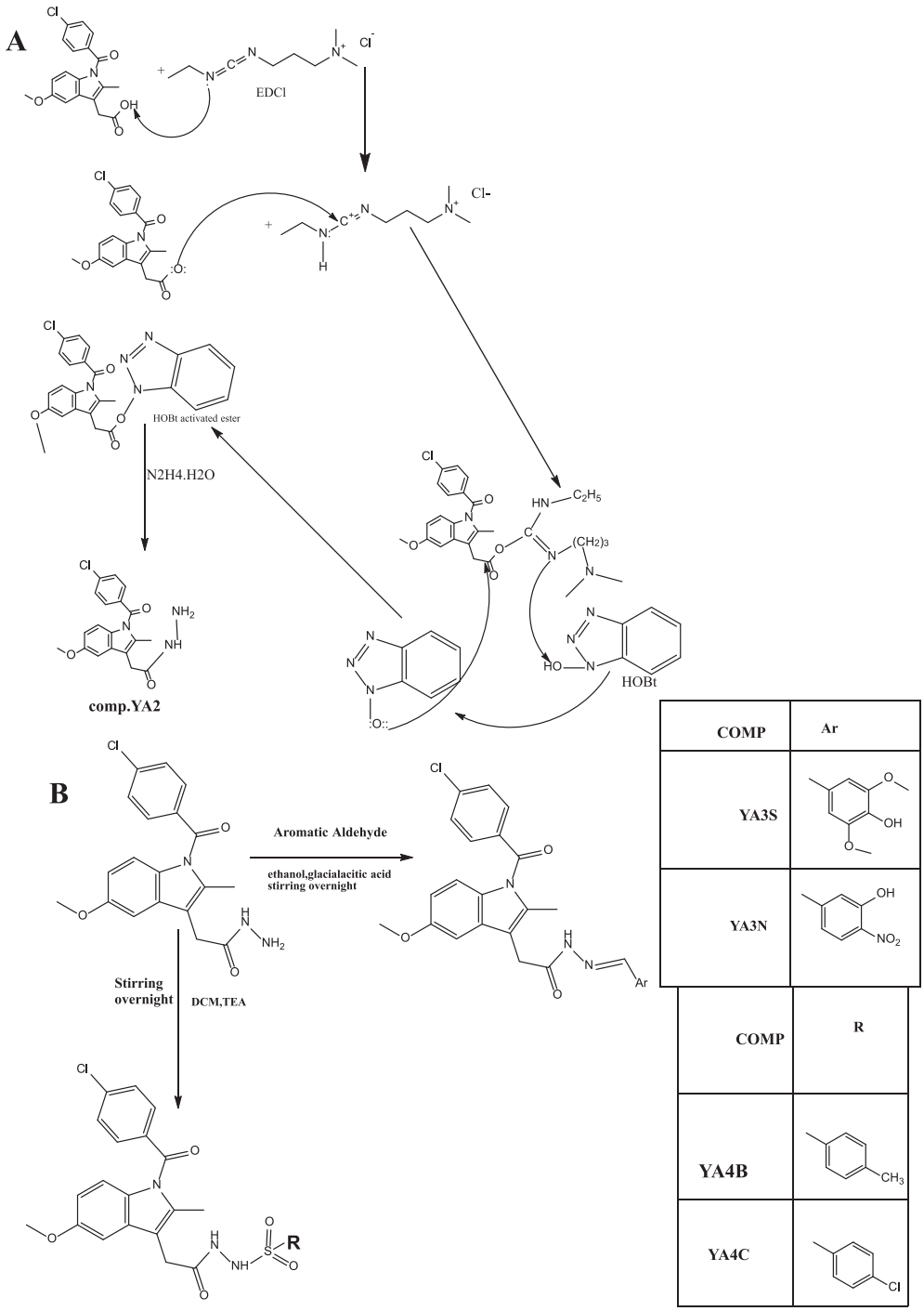

Figure 6. Scheme of synthesizing the 2-(5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl; (**B**) 2.4.2 N'-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3 yl)acetyl)benzene sulfonohydrazide derivatives and Formulation for the New Hydrazone .

Biological analysis

Minimum inhibitory concentration (MIC) determination

A fast resazurin microtiter assay method was used to determine bacterial susceptibility to antibiotics. Double serial dilutions of the compounds (YA3S, YA3N, YA4B, YA4C) and Ciprofloxacin were prepared in a microtiter plate with Mueller-Hinton broth. Concentrations tested were 1000, 500, 250, and 125 µg/ml. Each well, except for the negative control, was inoculated with 20 µl of bacterial suspension (1.5×10^8 CFU/ml). Plates were incubated at 37 °C for 18–20 hours. After incubation, 20 µl of resazurin dye was added to each well and incubated for 2 hours to observe color changes. The sub-MIC concentrations were identified as the lowest concentrations where the color changed from blue to pink, indicating bacterial growth inhibition (Table 1) (Ohikhena et al. 2017).

Inhibition zone determination

The agar well diffusion method was employed to assess the antibacterial activity of the compounds (YA3S, YA3N, YA4B, YA4C) against *E. coli*, *K. pneumoniae*, *S. aureus*, and *S. pyogenes*. Bacterial isolates were grown in nutrient broth and incubated at 37 °C for 18–24 hours. A bacterial suspension with moderate turbidity (1.5×10^8 CFU/ml) was prepared, and 0.1 ml was spread on nutrient agar plates. After drying for 10 minutes, 5 mm diameter wells were created in the agar. Each well was filled with 50 µl of the test materials at concentrations equal to the MIC values obtained from the Minimum Inhibitory Concentration (MIC) Determination, and distilled water was used as a negative control. Plates were incubated at 37 °C for 18 hours.

Post-incubation, the diameter of inhibition zones around each well was measured. A clear zone indicated antibacterial activity, with larger diameters representing higher activity (Table 1) (Lewus et al. 1991).

Statistical analysis

The MIC values follow an abnormal distribution. Therefore, we reverted to the Mann-Whitney U test. Based on the results of the Mann-Whitney U test, we cannot reject the null hypothesis, meaning there are no significant statistical differences in MIC values between the control (ciprofloxacin) and treatment compounds. This is because the p-value (0.138) is greater than the conventional significance level of 0.05. These results indicate that the treatment is comparable in potency to the strong antibiotic ciprofloxacin (Fig. 7).

Molecular induced fit (flexible) docking study

Molecular docking was performed on the protein (6YD9), a DNA-binding protein in *E.coli* (DNA Gyrase). This protein was identified using the x-crystallography technique, and its resolution is equal to 1.60 angstroms, and it does not contain mutations. This protein consists of one chain, chain A, which contains a co-crystal ligand **(ON2)** binding site. We made docking on chain A after removing water, determining the binding site, and adding hydrogen; the AMBER10 force field lowered the protein energy. the binding site contained the following amino acids**:** (VAL43 ASN46 ALA47 ASP49 GLU50 ALA53 VAL71 GLN72 ASP73 GLY75 ARG76 GLY77 ILE78 PRO79 VAL93 ILE94 VAL97 LEU98

Table 1. Results of the antibacterial activities of synthesized compound Minimum inhibitory concentration (MIC), µg/cm^3 and Inhibition Zone (I.Z), (mm).

Microorganism culture	Ciprofloxacin		YA3S		YA3N		YA4B		YA4C	
	MIC	I.Z	MIC	I.Z	MIC	I.Z	MIC	I.Z	MIC	I.Z
S. aureus	125	20	500	11	125	25	500	18	250	19
S. pyogenes	250	25	1000	16	250	24	1000	14	250	20
E. coli	125	22	500	14	125	20	500	14	500	18
K. pneumonia	500	25	1000	11	125	22	500	14	500	16

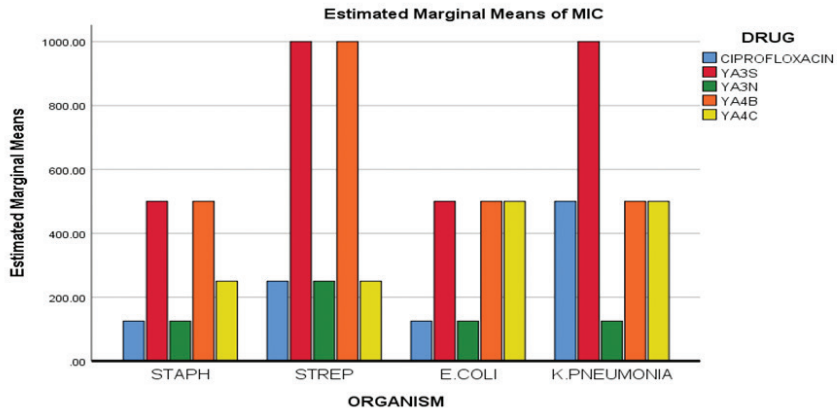

Figure 7. Minimum inhibitory concentrations (MICs) of Ciprofloxacin, YA3S,N, and YA4B,C against *Staph. aureus*, *Strep. pyogenes*, *E. coli*, and *K. pneumonia*.

HIS99 GLY119 VAL120 SER121 ARG136 GLY164 THR165 MET166 VAL167) . The molecular docking was done by using the MOE 2022 software. The SMILE structures of the studied compounds were created using Chem-Bio Draw Ultra13.0. The structures were then produced in 3D form using MOE 2022 software. The 3D structures were protonated, and energy was reduced by using a 0.1 Å RMSD. AMBER10 force field (Abbas et al. 2021). Numerous techniques for validating scoring functions and docking programs have been documented. Pose selection is a frequently employed technique involving docking algorithms to re-dock a chemical with a known conformation and orientation—typically from a co-crystal. The efficiency of the software was proven in the dock of co-crystallized ligand (NO2) with the same Target protein and calculated RMSD Dock through the DISCOVERY STUDIO VISHULIZER software and by VMD, and it was equal to 1.181 Ang, which is regarded as good. This indicates efficiency of the MOE program for docking of our new ligands (Kukol 2014; Prieto-Martínez et al. 2019).

Molecular docking analysis

The molecular docking process was completed using MOE software. The largest binding site found was for *E. coli* gyrase B (24 kDa) (PDB: 6YD9), according to SITE FINDER. The ligand and interacting amino acids were maintained throughout refining to exhibit flexibility during docking (induced fit-flexible-docking). Five distinct interactions with the protein were allowed for each molecule. Docking scores of the best-fitting pose with the active pocket were noted, and the binding site was identified and recorded in Table 2. This information anticipated the recommended binding mode (pose) and affinity. The expected affinity of the substances evaluated with (6YD9) represented the binding free energy (ΔG). The interaction between the ligand and protein is illustrated in (Figs 8–13).

Table 2. Scores for docking of tested compounds against target site of co-crystallized ligand are represented in ΔG values measured in kcal/mol.

Comp	Value of RMSD	Docking value (Kcal/mol)	Interactions	
			n.H.B	VDW
Cocrystal (ON2)	1.18	-6.28	1	15
ciprofloxacin	1.24	-5.98	4	8
Comp. YA3S	2.75	-8.31	0	11
Comp. YA3N	1.87	-7.78	3	7
Comp. YA4B	2.04	-8.41	3	12
Comp. YA4C	1.93	-7.23	2	10

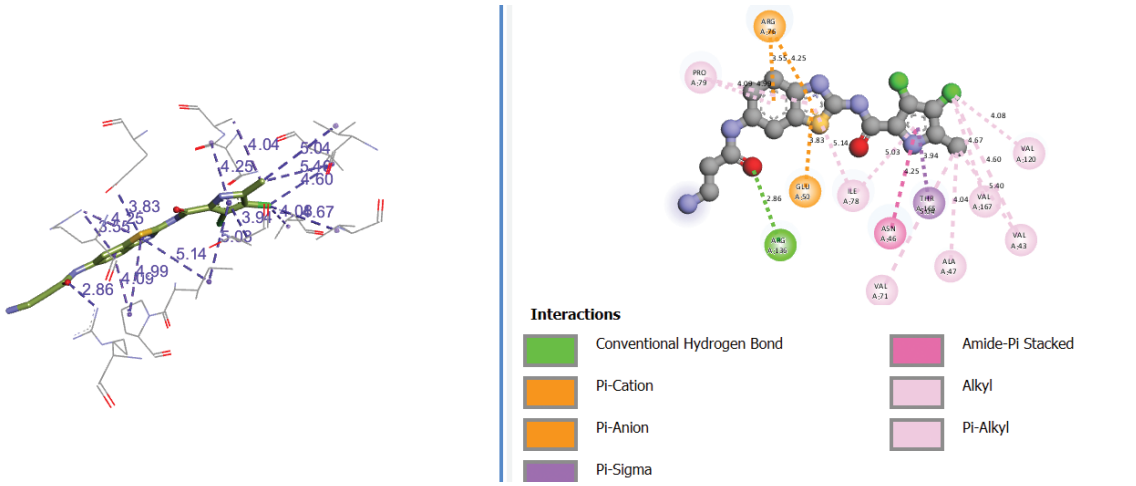

Figure 8. Ligand cocrystal (ON2) interaction.

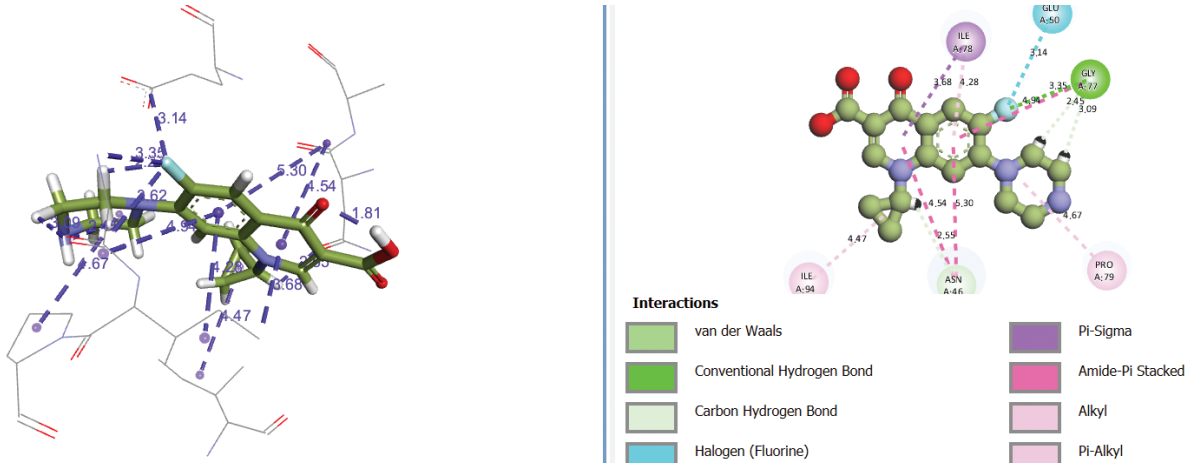

Figure 9. Ligand (ciprofloxacin) interaction.

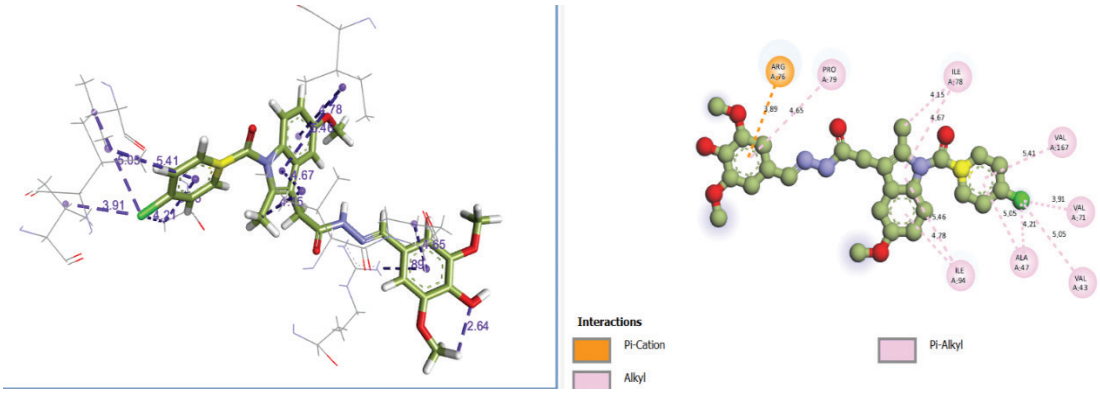

Figure 10. Ligand (YA3S) interactio.

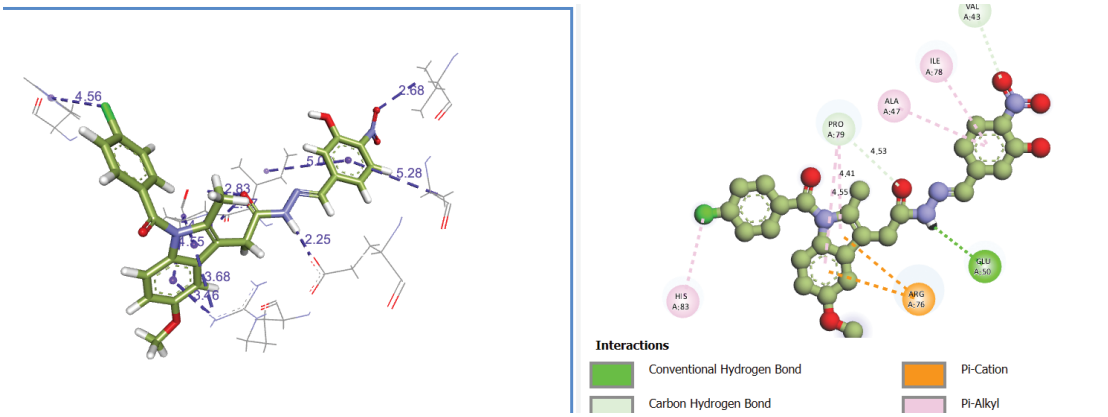

Figure 11. Ligand (YA3N) interaction.

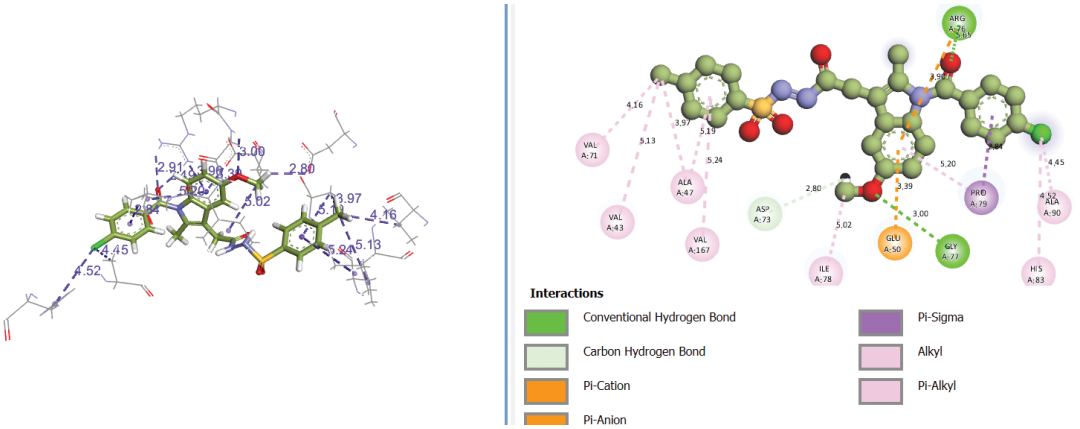

Figure 12. Ligand (YA4B) interaction.

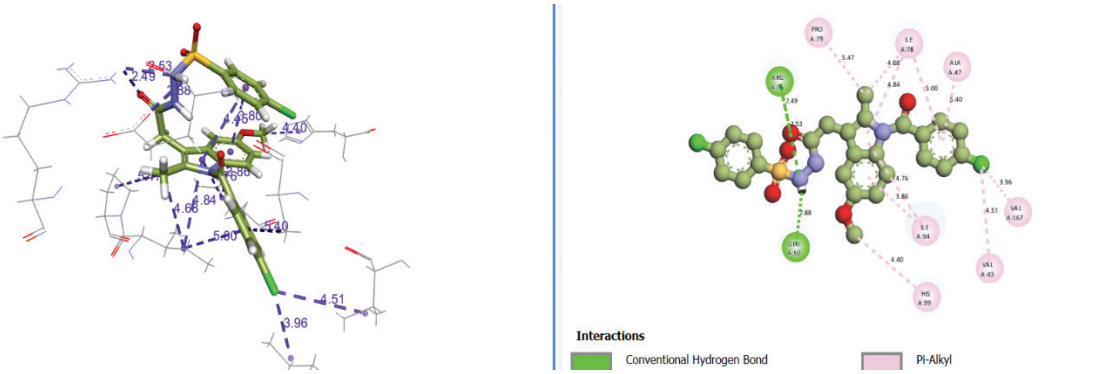

Figure 13. Ligand (YA4C) interaction.

Table 3. The properties related to the physical and chemical characteristics of the compounds that were synthesized (Daina and Zoete 2017).

Comp.	Clog p	Class of solubility	n. H-B acceptors	n. H-B donors	n. rotatable bonds	TPSA / °A
Cocrystal(ON2)	2.97	Moderate sol.	4	4	7	141.14
ciprofloxacin	1.10	Very sol.	5	2	3	74.57
YA3S	4.78	Poorly sol.	7	2	10	111.38
YA3N	4.74	Poorly sol.	7	2	9	138.74
YA4B	4.08	Poorly sol.	6	2	9	114.88
YA4C	4.35	Poorly sol.	6	2	9	114.88

Physicochemical properties

Understanding the physical characteristics of drugs is essential for improving their molecular activity. One parameter that plays a key role in this aspect is the partition coefficient (clogP), which predicts how drugs move within the human body. However, it is worth noting that all target compounds have a clogP value of less than 5, which violates the widely accepted rule of five Lipinski's rule. (TPSA) The total polar surface area is another important parameter that defines the surface area occupied by polar atoms in a compound. Lower TPSA values are much more desirable for drug-like properties since they are associated with higher membrane permeability. Therefore, by carefully analyzing these parameters, it's possible to enhance the therapeutic properties of drugs and achieve better treatment outcomes for patients (Table 3).

In-silico ADMET and toxicity properties

The ADMET properties of the synthesized compounds were analyzed using Swiss ADME cheminformatics software to identify safer drug candidates and exclude compounds with adverse ADMET properties for further drug development stages. Table 4 presents the expected ADMET analysis of the target compounds, while the in-silico toxicities of the new compounds are provided in Table 5. The admetSAR provided all the data (Hongbin et al. 2018).

Table 4. Analysis of ADMET properties has been predicted for the mentioned compounds.

Comp.	CYP2C9	CYP2D6	CYP3A4	BBB	GI absorption	P-gp subset.
Cocrystal (NO2)	YES	YES	YES	NO	Low	NO
ciprofloxacin	NO	NO	NO	NO	High	YES
YA3S	Yes	No	Yes	No	High	No
YA3N	Yes	No	Yes	No	Low	No
YA4B	Yes	No	No	No	Low	No
YA4C	Yes	No	Yes	No	Low	No

Discussion

Even though the delta G of 4-hydroxy-3,5-dimethoxybenzylidene is (-8.31 kcal/mol) greater than that of 3-hydroxy-4-nitrobenzylidene (-7.78 kcal/mol), the hydrazone derivative compound containing 3-hydroxy-4-nitrobenzylidene is more activgram-positive gram positive and has more negative bacteria than hydrazone that contains 4-hydroxy-3,5-dimethoxybenzylidene. Additionally, even though the delta G of toluene sulfonamide derivatives (-8.41 kcal/mol) is larger than the delta G of para chlorobenzene (-7.23 kcal/mol), the molecules containing para chlorobenzene in sulfonamide derivatives are more active than the molecules containing para toluene. To explain why the compounds containing para chlorobenzene in sulfonamide derivatives and 3-hydroxy-4-nitrobenzylidene in hydrazone derivatives may be more active against bacteria than the compounds containing toluene in sulfonamide derivatives and 4-hydroxy-3,5-dimethoxybenzylidene in hydrazone derivatives, it should be noted that Chlorine (Cl) and nitro (-NO2) are powerful electron-withdrawing groups. This indicates that it increases the molecule's positive charge by attracting electrons away from the other molecules. The molecule's interaction with the negative electrostatic charge of the bacterial cell surface may be improved by this positive attribute. However, the methyl group (CH3) in 4-hydroxy-3,5-dimethoxybenzylidene and the methoxy group (-OCH3) in 4-hydroxy-3,5-dimethoxybenzylidene are electron-donating groups, meaning they contribute electrons to the remainder of the molecule and increase its negative charge. This could impede and hinder the communication between, and the interaction of, the molecule with the bacterial cell surface. It's crucial to remember that delta G is only one element that can affect a compound's activity. Additionally, there are other elements like interactions and the compound's activity and properties can be greatly influenced by its capacity to create hydrogen bonds, ionic contacts, or other particular interactions with the target protein. Finally, a chemical's capacity to permeate the bacterial cell wall and membrane can also have an impact on its activity. A component's ability to exert

Table 5. The toxicity prediction for the titled compounds.

Comp.	Hepatotoxicity	Renal toxicity	Carcinogenicity	Rat Acute Toxicity LD50, mol/kg
ON2	YES	NO	NO	2.091
CIPROFLOXACIN	YES	NO	NO	2.163
YA3S	YES	No	No	2.8571
YA3N	YES	No	No	2.7294
YA4B	YES	No	No	2.5820
YA4C	YES	No	No	2.5820

its antibacterial action will be limited if bacteria quickly assimilate it. All things considered, the connection between delta G and biological activity is intricate and varies based on the particular system under investigation. Regarding our compounds, the variations in electrical. In the case of our compounds, the differences in electronic factors and permeability may outweigh the differences in delta G **(ΔG).**

Conclusion

Four novel compounds (YA3S, YA3N, YA4B, YA4C) were synthesized, and their structures were confirmed by ATR-FTIR and 1H NMR spectroscopic analysis. The results of the antimicrobial activity of these N-acyl hydrazones and N'- (2-(1-methoxy-2-methyl-1H-indol-3-yl)-5-(4-chlorobenzoyl)acetyl)benzenesulfonohydrazide compounds demonstrate their potential as antibacterial agents. The antibacterial activities were assessed using the agar well diffusion technique and compared to ciprofloxacin, a gold standard antibacterial agent, against both gram-positive and gram-negative microorganisms.

Based on the findings of the initial microbiological screening. The inhibition zones of the compounds ranged from moderate to potent antibacterial activity.

But YA3N showed good effectiveness and comparable activity to ciprofloxacin against *S. aureus*, *E. coli*, and *S. pyogenes*. It was more potent than ciprofloxacin towards *K. pneumonia* with an MIC concentration of 125 μg/cm³.

The main problem in bacterial treatment is bacterial resistance, so the search for new bioactive compounds that induce bacterial cell killing through new and atypical mechanisms that avoid bacterial resistance is of great importance. The current work demonstrated that indomethacin hydrazide derivatives, particularly YA3N, show comparable or superior activity to ciprofloxacin against various bacterial strains, indicating their potential applicability in bacterial treatment.

Authors Contribution

The following contributions to the work are confirmed by both authors Yaseen S. Hamdoon, Mohammed K. Hadi : importing of indomethacin, research methodology, supervision on the progress of the reactions, synthesis of the compounds and performing ATR-FTIR analysis, interpretation of ATR-FTIR and 1HNMR , 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.

Data availability

All data analysis reported in this paper are deposited on the Zenodo website (https://zenodo.org/records/12594146) Contained the following data:

- Infrared (IR) spectra of the synthesized compounds
- Proton nuclear magnetic resonance (H NMR) spectra of the synthesized compounds
- Minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) data for the synthesized compounds against tested microorganisms

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Supplementary material 1

Supplementary images

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Data type: docx

Explanation note: This supplementary data file contains additional information supporting the findings presented in the research article "Molecular Docking, ADMET, Synthesis and Evaluation of New Indomethacin Hydrazide Derivatives as Antibacterial Agents". The data includes: Infrared (IR) spectra of the synthesized compounds Proton nuclear magnetic resonance (H NMR) spectra of the synthesized compounds Minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) data for the synthesized compounds against tested microorganisms.

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