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Design, Molecular Docking, ADMET Studies, Synthesis, Characterization, and Antimicrobial of Nicotinamide Derivatives via Mannich reaction

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Abstract

In this study, a series of novel Mannich bases were synthesized by the conjugation of Vitamin B3 with the medicinal compound sulfamethoxazole. Five Schiff base compounds were prepared via a condensation reaction involving five different aldehydic precursors. Additionally, a compound incorporating an aromatic thiadiazine ring was synthesized. The prepared compounds were characterized using FT-IR, ^1H and ^{13}C NMR spectrometric methods, and melting point measurements. Molecular docking studies were performed to investigate the interactions of the synthesized compounds with three specific bacterial protein targets, namely: 4H2M from *Escherichia coli*, 3FYV from *Staphylococcus aureus*, and 6P4T from *Salmonella*. Besides, Docking simulations showed that all the prepared compounds exhibited hydrophilic interactions with the target proteins by forming hydrogen bonds. The formation of rings and arenes aromatics in molecular structure facilitated water softened interactions between drugs and bacterial proteins. The biological activity of these prepared compounds was investigated against five classes of bacteria, negative and positive grams. The obtained results showed a significant activity compared to sulfamethoxazole medication. On the other hand, absorbed and poisoning of the prepared compounds were examined using the Swiss ADME tool. The yielded results showed that all ligands did not fulfill Lipinski's rule, except compound M (Mannich compound). This could be attributed to the large area and the high molecular weight of the compounds which were more than 500g/mol.

Keywords: ADMET, Antibacterial, Mannich base, Molecular docking, Schiff base.

التصميم والالتحام الجزيئي ودراسات ADMET والتحضير والتشخيص ومضادات الميكروبات لمشتقات النيكوتيناميد عبر تفاعل مانخ.

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الخلاصة

في هذه الدراسة تم تصنيع سلسلة من قواعد مانخ الجديدة من خلال اقتران فيتامين B3 مع المركب الطبي سلفاميثوكسازول. حضرت خمسة مركبات من قاعدة شيف من خلال تفاعل تكثيف يتضمن خمسة مركبات ألدهايد مختلفة. بالإضافة إلى ذلك، تم تصنيع مركب يشتمل على حلقة ثيادايازين أروماتية. تم تشخيص المركبات المحضرة باستخدام الطرق الطيفية FT-IR، ^{13}C NMR، ^1H وقياس درجة الانصهار.

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إجريت دراسات الالتحام الجزيئي لدراسة تفاعلات المركبات المحضرة مع ثلاثة أهداف بروتينية بكتيرية محددة، وهي: H2M4 من الإشريكية القولونية، وFYV3 من المكورات العنقودية الذهبية، وP4T6 من السالمونيلا. أظهرت عمليات محاكاة الإرساء أن جميع المركبات المحضرة أظهرت تفاعلات محبة للماء مع البروتينات المستهدفة من خلال تكوين روابط هيدروجينية. أدى تكوين الحلقات الاروماتية والأرينات في التركيب الجزيئي إلى تسهيل التفاعلات الكارهة للماء بين الأدوية والبروتينات البكتيرية. تم دراسة النشاط البيولوجي للمركبات المحضرة ضد خمس سلاسل من البكتيريا سالبة الجرام وموجبة الجرام. أظهرت النتائج المتحصل عليها نشاطاً جيداً مقارنة بأدوية السلفاميثوكسازول. فحص امتصاص وسمية المركبات المحضرة باستخدام أداة Swiss ADME. أظهرت النتائج أن جميع الليكاندات لم تحقق قاعدة ليبينسكي، باستثناء المركب M (مركب مانخ). ويمكن أن يعزى ذلك إلى المساحة الكبيرة والوزن الجزيئي العالي للمركبات التي تزيد عن 500 غم/مول. في

1. Introduction

Sulfamethoxazole is one of the sulfa drugs that were discovered in 1968. It is used as an antibiotic antibacterial agent against (gram-positive) and (gram-negative) bacteria. It is frequently used in drugs as a dose with trimethoprim in a 1:5 ratio in co-trimoxazole medicinal compound [1, 2]. Nicotinamide, which is known as niacin amide is a form of vitamin B3 and is used as a dietary supplement [3, 4]. Niacin amide is the preferred medicine for pellagra which is caused by lack of niacin. In recent years, considerable research effort has been devoted to the synthesis of more potent derivatives of the antimicrobial agent sulfamethoxazole. Among the synthetic methods investigated, Schiff base derivatives have been born series has proved to be a promising method. Schiff bases are formed by condensation reactions between aldehydes and primary amines [5, 6]. Notably, the Schiff base from sulfamethoxazole exhibited enhanced antimicrobial activity against gram-positive and gram-negative bacteria compared to the parent sulfamethoxazole compound itself [7], [8]. The main objective of this research effort is to enhance the biological activity of sulfamethoxazole by Mannich reaction. Followed by addition of active groups by condensation of Schiff bases. Through couple of years ago, we designed some sulfonamide derivatives incorporating imine including (-C=N-), (N-NH), and azo (-C-C-N-) functional groups [9, 10]. The antimicrobial activity of these prepared compounds have been investigated. In addition to the characterization of functional groups of the compounds (Scheme 1), the antibacterial and antiviral activity of the compounds have been tested. The (C=N-NH) group is one of the functional groups that have π -electrons and acidic hydrogen. The presence of such a group is strengthening the electrochemical potential of the molecule. Hence, raising the compound binding capability with bacteria proteins. Consequently, inhibition the growth of bacteria [11]. ADMET method was exploited for measuring the drug activity and toxicity of the compounds. Molecular docking studies have been adopted with H2M4, 3FVY, and, 6P4T proteins (obtained from PDB source of protein data bank), and DNA to test their binding at the best pose condition of the compounds.

2. Experimental

2.1 Material and Methods

All chemicals were obtained from Sigma-Germany. All solvents were used without a further purification. Thin-layer chromatography (TLC) was exploited to follow up the reaction progress and the purity of compounds. The spots were visualized using a UV Cabinet. FT-IR measurements were carried out using Shimadzu, FT-IR 8400S spectrophotometer. ^1H - ^{13}C NMR spectra were recorded using a (400 MHz) NMR spectrophotometer at the College of Sciences, University of Basra.

2.2 Synthesis of Mannich base M

The reaction began by dissolving 10 mmol of sulfamethoxazole in 10 mL of anhydrous ethanol. To this solution was added 10 mmol of formalin, 37% aqueous formaldehyde. Nicotinamide was then added dropwise to the reaction mixture [12], which monitored the reaction periodically by thin-layer chromatography (TLC) kept under constant rotation for 2 hours at ambient temperature, where a 1:1 mixture of ethyl acetate and hexane The compound mobile phase was employed After completion of the reaction, the obtained solid phase was separated by recrystallization from ethanol, followed by , the desired compound is obtained by filtration and drying.

N-(((4-(N-(5-methylisoxazol-3-yl)sulfamoyl) phenyl) amino)methyl)nicotinamide M: The physical data were given in Table 2. %yield= 67 %, M.P=155-157 °C. FT-IR: 1381cm⁻¹(C-N). ¹H-NMR (DMSO, 400MHz)δ (ppm): 2.17 (s, 3H, CH₃), 4.67(s, 2H, CH₂NH), 6.34(s, 1H, NH), 8.81(d, 1H, NHCO), 7.32-8.26(m, 9H, Ar-H), ¹³C-NMR (DMSOd⁶) δ(ppm): 21.6(CH₃), 94.2, 163.5, 171.2, (oxazole),121.4-149.6(aromatic-carbon), 174(carbonyl). Elemental Analysis: Calc.; C,52.71; H, 4.42; N,18.08; found: C,51.73; H, 3.93; N, 17.91.

2.3 Synthesis of compound (M₁).

In this experimental procedure, a solution containing 1 mmol of dye (M) dissolved in 20 mL of dimethylformamide (DMF) was prepared. Then, 3 mL of chloroacetyl chloride (equivalent to 3 mmol) was added dropwise to the solution. The reaction mixture was then stirred again for 8 h. After refluxing, the reaction mixture was cooled, then filtered, dried, and recrystallized from ethanol. Characteristics of the resulting compounds are presented in Tables 1 and 2.

2.4 Synthesis of compound (M₂).

A mixture of (2 mL, 2 mmol), 99.5% hydrazine hydrates with 1 mmol of compound M was refluxed for 6 hours. The reaction was monitored with TLC. The precipitate was collected, washed by water, and then recrystallized from ethanol. The data of compound M is shown in Tables 1 and 2.

2.5 General procedure for the preparation of compounds Schiff bases. (S₁-S₅).

To a stirred solution of aldehyde (5mmol) in ethanol (10 mL), a few drops of GAA were added at room temperature. To the resulting mixture, (5 mmol) of compound M₂ in (10 mL) ethanol was added drop by drop with stirring for 4 hours [7]. The development of the reaction was followed up by TLC using an ethyl acetate: benzene(1:3) mixture. The produced mixture was then evaporated under reduced pressure to remove most of the solvent. The precipitate was then filtered and recrystallized. The yielded compounds S₁-S₅ compounds were dried at 70°C, then the melting point was measured. The data of compounds are shown in Tables (1 and 2).

2.6 Synthesis of a compound (M₃).

To a refluxed solution of compound M₂ (1 mmol in 20 mL ethanol), (2.73g, 1 mmol) of p-chloro phenyl isocyanate was added drop by drop for 6 hrs. The reaction was monitored with TLC, The reaction mixture was filtered, dried, and re-crystallized from ethanol. Tables 1 and 3 show the spectroscopic measurement data (FT-IR, ¹H- ¹³C-NMR) of compounds M₃.

2.7 Synthesis of a compound (M₅).

A mixture of compound M₄ (1 mmol) with ethyl acetoacetate (1 mmol) and ethanol (15 mL) was prepared. The mixture solution was and refluxed with stirring for 4 hours. The produced mixture was concentrated and cooled with crushed ice to form a precipitate. The precipitate was filtered, dried and the product was then re-crystallized from ethanol. Tables 1

and 3 show the spectroscopic measurements data (FT-IR, ^1H -NMR, and ^{13}C -NMR) of compounds M5

2.8 The Anti-Bacterial Activity.

The study focused on the biological activity of compounds S1-S5 and M5 on five bacterial species, namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella*, and *Proteus* performed this study using disk diffusion method, as described in references [13]. Muller Hinton agar medium was prepared following the manufacturer's instructions to facilitate the experiments [14]. Into this conical flask containing 1.0 L of distilled water, 38.0 g of agar was added. The mixture was then sterilized at 120°C under pressure (15 lb for 15 minutes) to ensure the insolubility of the compound and its effectiveness in supporting bacterial growth. After that, the solution was left to cool, and then poured into sterile dishes until use. The zone (mm) of inhibition was used to determine how effective the antibacterial activity after incubating the sample for 24 hours. On the other hand, one dose of the synthesized chemical was prepared by diluting them in dimethyl sulfoxide DMSO (100 μL). They compared to Sulfamethoxazole (as a standard compound for inhibiting bacteria growth). The effectiveness of each concentration was determined by measuring the diameter of the inhibition zone around each hole [15].

2.9 Computational Method

In silico screening of nicotinamide derivatives and prior to undertaking antibacterial activity assays, we decided to screen a representative set of compounds against 4H2M (*E.coli*), 3FYV (*S.aureus*), and 6P4T (*Salmonella*) to see their relative binding affinity with the target. From the protein database (<http://www.rcsb.org/pdb>), the protein preparation was carried out in (PyMOL Stereo 3D Quad-buffer) by removing water molecules and co-crystallized ligands. Docking calculations were carried out using the PyRx.lnk program implemented in discovery studio. The method utilized in this study aimed to identify the binding site spheres utilized by the target protein. This method offers valuable structural insights, including details on hydrogen bonding interactions, electrostatic interactions, molecular surface complementarity, and more. Furthermore, to anticipate the pharmacokinetic profile of the synthesized compounds, which includes aspects such as absorption, distribution, metabolism, and excretion (ADME), the Swiss ADME server was employed. This server provides valuable predictive information to aid in understanding the potential behavior of the compounds in biological systems [16]. All compounds (ligands) were drawn by Chem Sketch (v. 14), converted to SMILE and named using the Swiss ADME tool, which predicts the physicochemical descriptors and pharmacokinetic properties. BOILED-EGG was utilized to compute the lipophilicity and polarity of the small molecules.

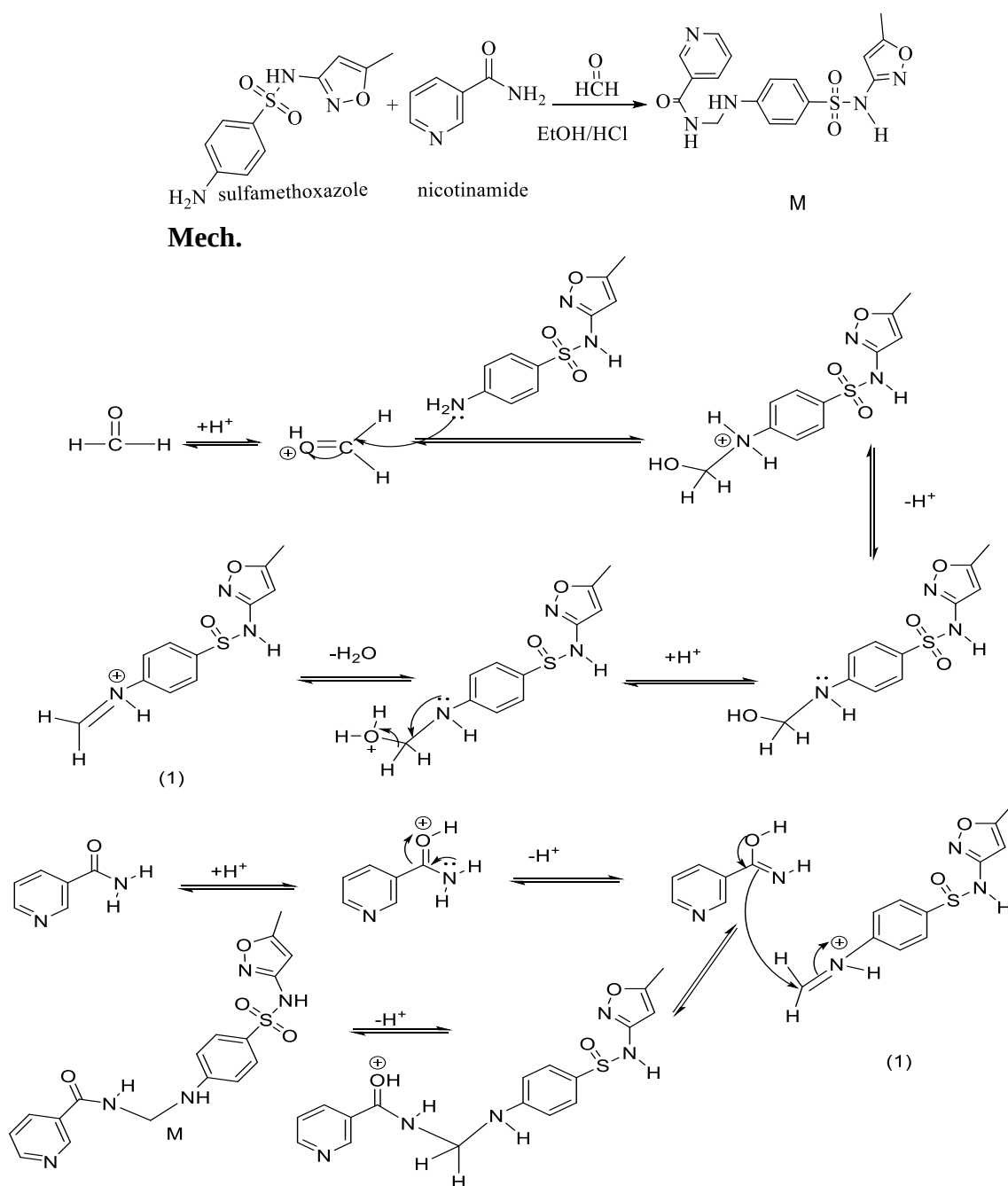
3. Results and Discussion

3.1 Synthesis and Characterization

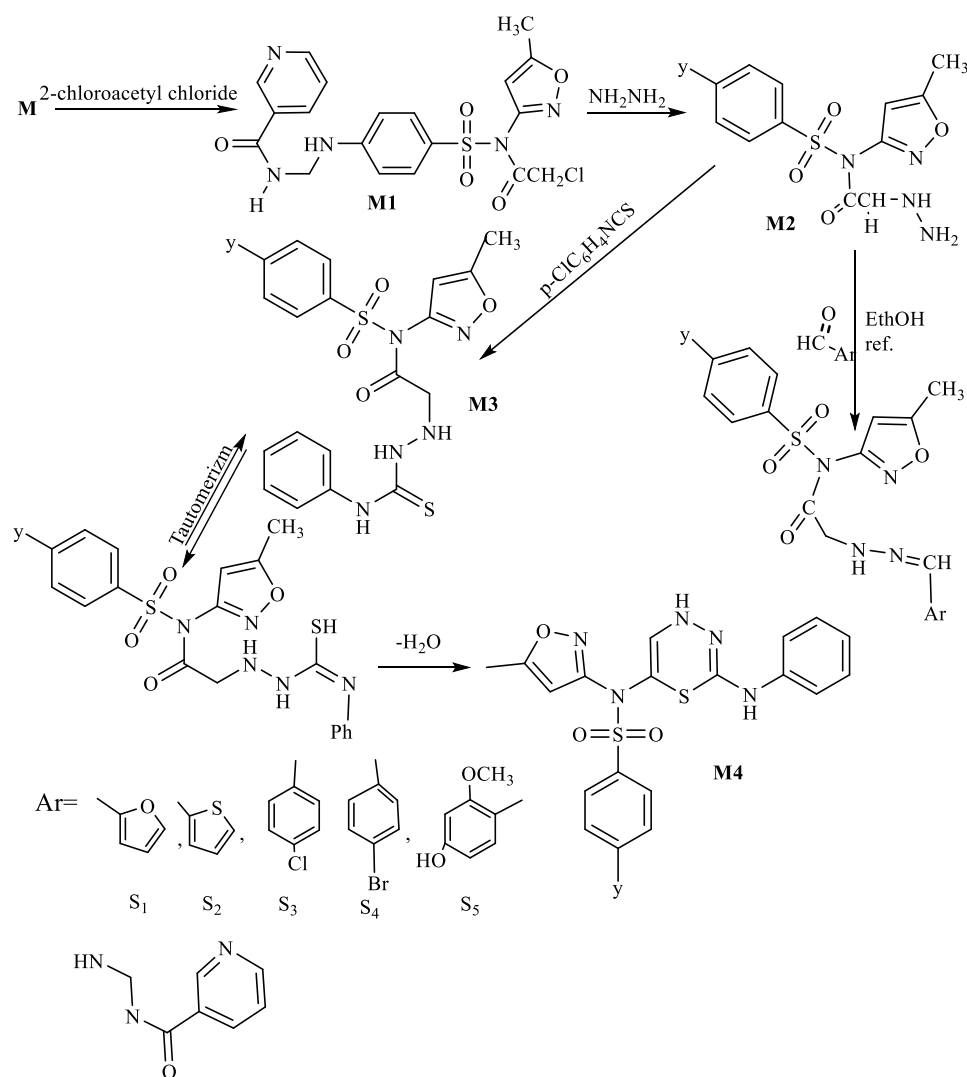
Newly synthesized derivatives of sulfamethoxazole are presented in Schemes (1 and 2). The FT-IR frequencies of Schiff bases, Mannich's base, and the new heterocyclic compound M5 are reported in Table 1. The FT-IR spectra illustrated the existence of (C-N) group at 1381 cm^{-1} with a sharp peak in compound M and appeared with one peak instead of two bonds in the area of 3401 cm^{-1} . However, the (C-H) aliphatic peaks are located at $2942\text{--}2823\text{ cm}^{-1}$, which refers to the formation of the $\text{CH}_2\text{--CH}_2\text{--NH}$ [17].

Table 1: A Comparison of experimental and theoretical vibrational frequencies data (M-S₅)

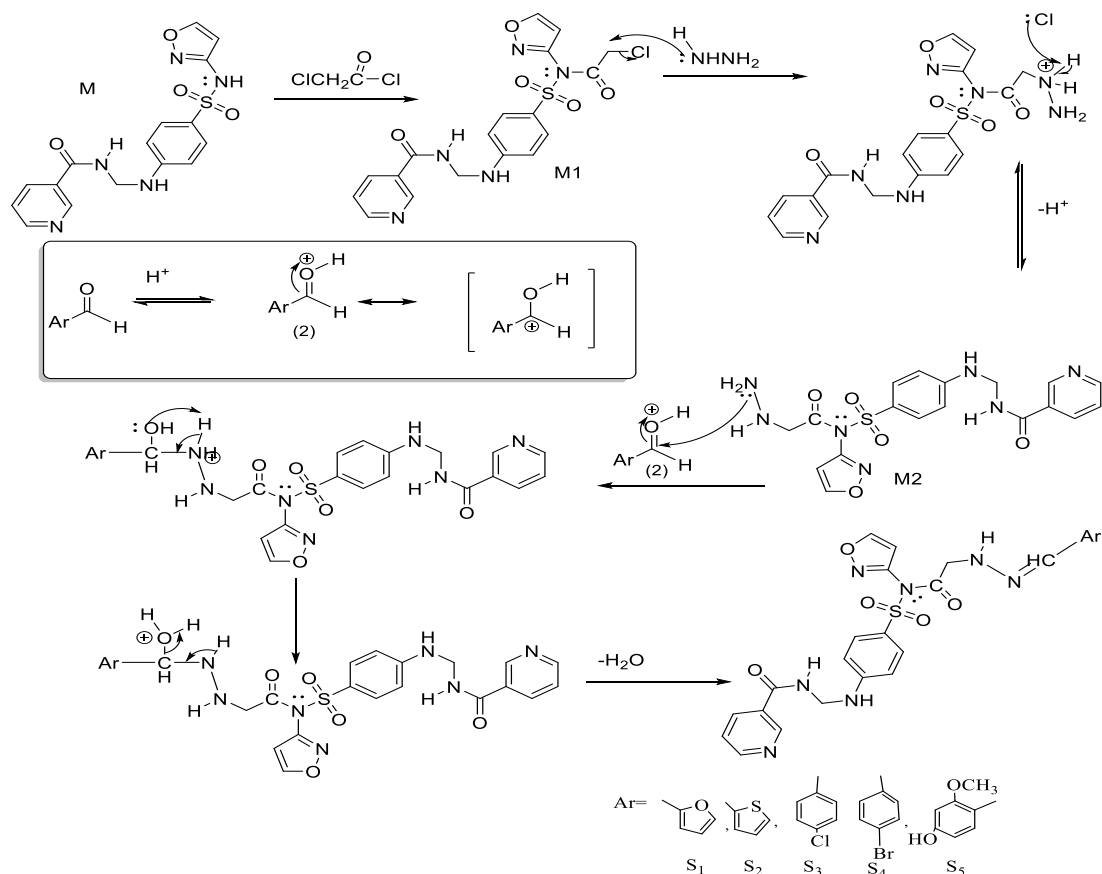
NO.	$\nu(\text{OH})$	$\nu(\text{NH})$	C-H aromatic	C-H Alph	$\nu(\text{C}=\text{N})$	others.
M	-	3268	3039	2951	-	$\nu \text{C}=\text{O}$ (1641), (1556) $\nu \text{N}-\text{H}$ def (amide), (1381) $\nu \text{N}-\text{C}$.
M ₅	-	3283	3022	2954	-	$\nu(\text{C}=\text{N})$ 16291thiadiazine
S ₁	-	3262	3076	2943	1623	$\nu(\text{C}=\text{O})$ 1711.
S ₂	-	3274	3051	2941	1642	$\nu(\text{C}=\text{O})$ 1675.
S ₃	-	3284	3082	2953	1618	$\nu(\text{C}=\text{O})$ 1712., $\nu(\text{SO}_2)$ sym.1182
S ₄	-	3281	3075	2972	1604	$\nu(\text{C}=\text{O})$ 1723., $\nu(\text{SO}_2)$ sym.1182
S ₅	3423	3243	3078	2981	1648	$\nu(\text{C}=\text{O})$ 1712., $\nu(\text{SO}_2)$ sym.1162

**Scheme 1:** Synthesis Mannich base M and mechanism reaction

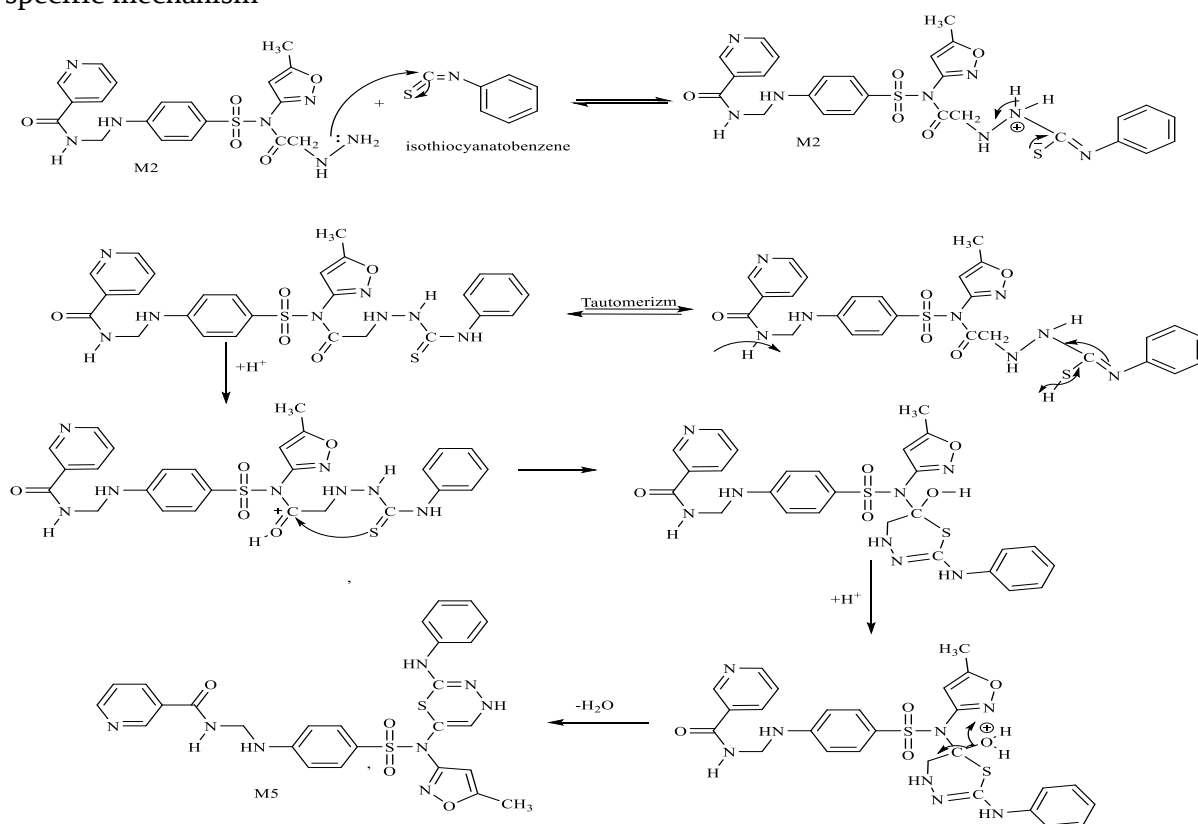
The formation of the Schiff bases (S1-S5) was confirmed by the presence of characteristic azomethine (CH=N) stretching vibrations in their FT-IR spectra, with the corresponding absorption bands listed in Table 1. Furthermore, the FT-IR spectrum of compound (M4) exhibited the absence of bands attributed to the ν (-NHNH₂), (SH), and (C=O) functional groups, providing strong evidence for the successful cyclization and formation of the thiadiazine ring in compounds (M5). Table 1 compiles the most significant IR absorption peaks observed for these newly synthesized compounds, highlighting the distinctive spectroscopic features that corroborate their anticipated molecular structures. ¹H-NMR spectra of compounds (S1-S5 and M5), show the main attributes of chemical shifts (DMSO, δ ppm) as tabulated in Table 3. ¹H NMR spectrum of compound M exhibits a signal at δ 2.17 ppm due to (3H) in methyl group (CH₃), chemical shift at δ 4.67 ppm due to (2H) in the CH₂NH group [18], chemical shift at δ 6.34 ppm due to (2H) in the Ar-H [19]. The ¹H NMR spectra of compound M is shown in Figure 1. The signals of ¹H NMR and ¹³C NMR of all the prepared compounds are shown in Table 3. It showed signals corresponding to compound M that connected to thiadiazine moiety, (CH₃) group connected to the isoxazole ring, for two-CH- groups of thiadiazine ring as shown in Table 3. The ¹H NMR and ¹³C NMR spectra of some compounds (M4-S5) are shown in Figures (2- 6) respectively.



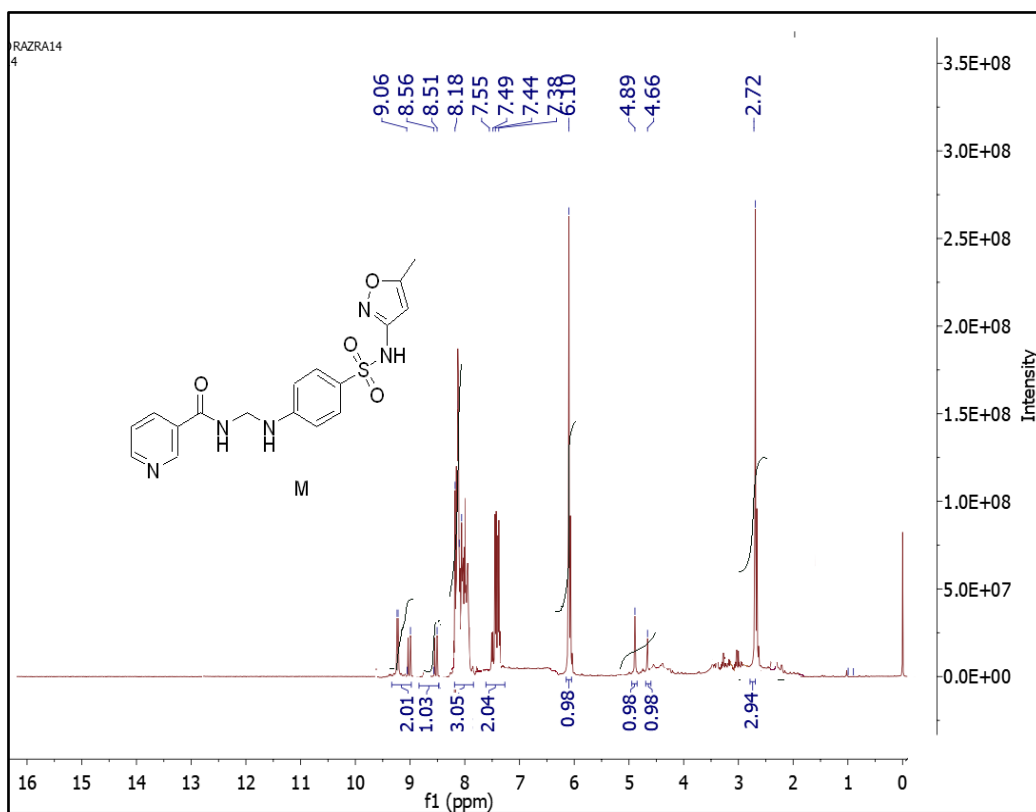
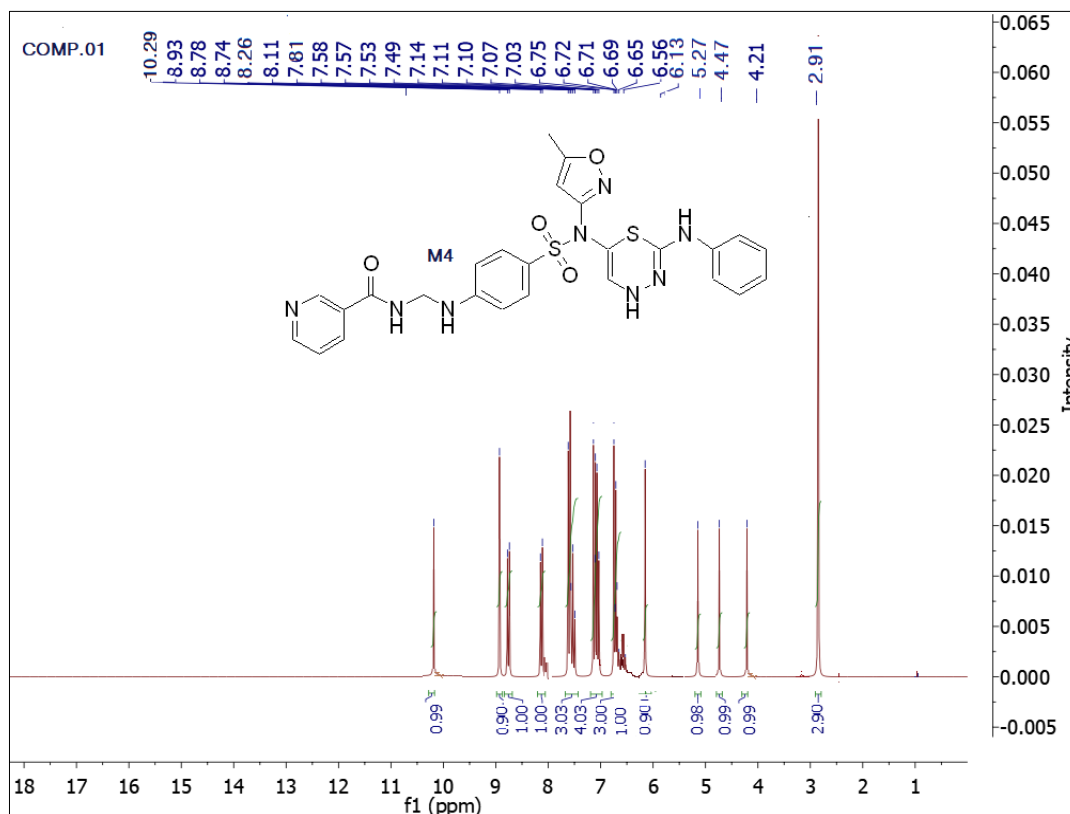
Scheme 2: Synthesis derivatives nicotinamide

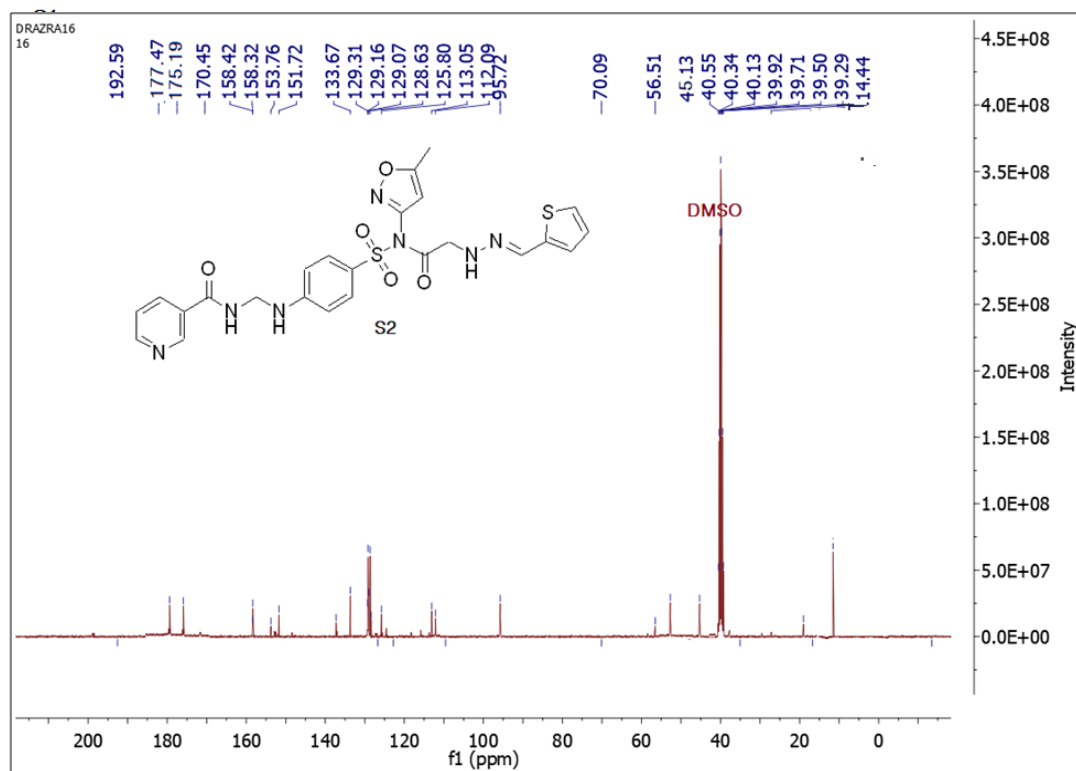
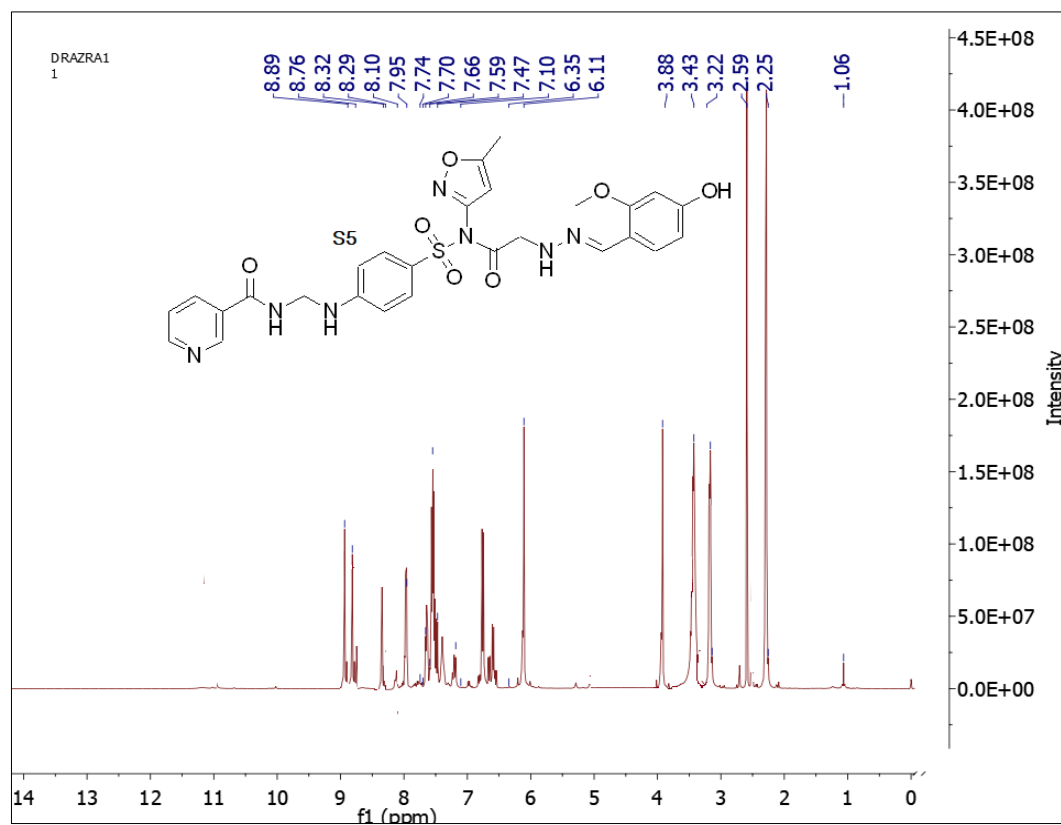


Scheme 3: The process of synthesizing Schiff bases derived from nicotinamide involves a specific mechanism



Scheme 4: The synthesis mechanism of compound M5 from nicotinamide

Figure 1: ¹H NMR of compound M.Figure 2: ¹H NMR of compound M4.

Figure 3: ¹³CNMR of compound S2.Figure4: ¹HNMR of compound S5.

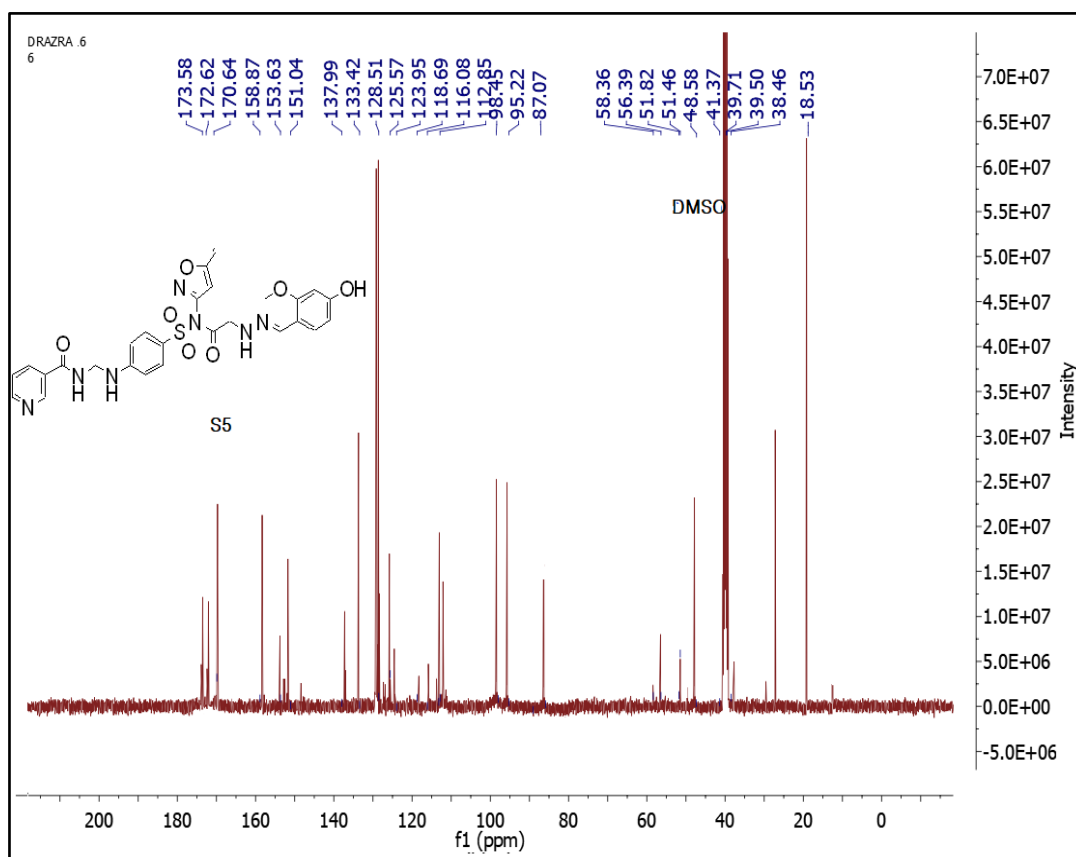
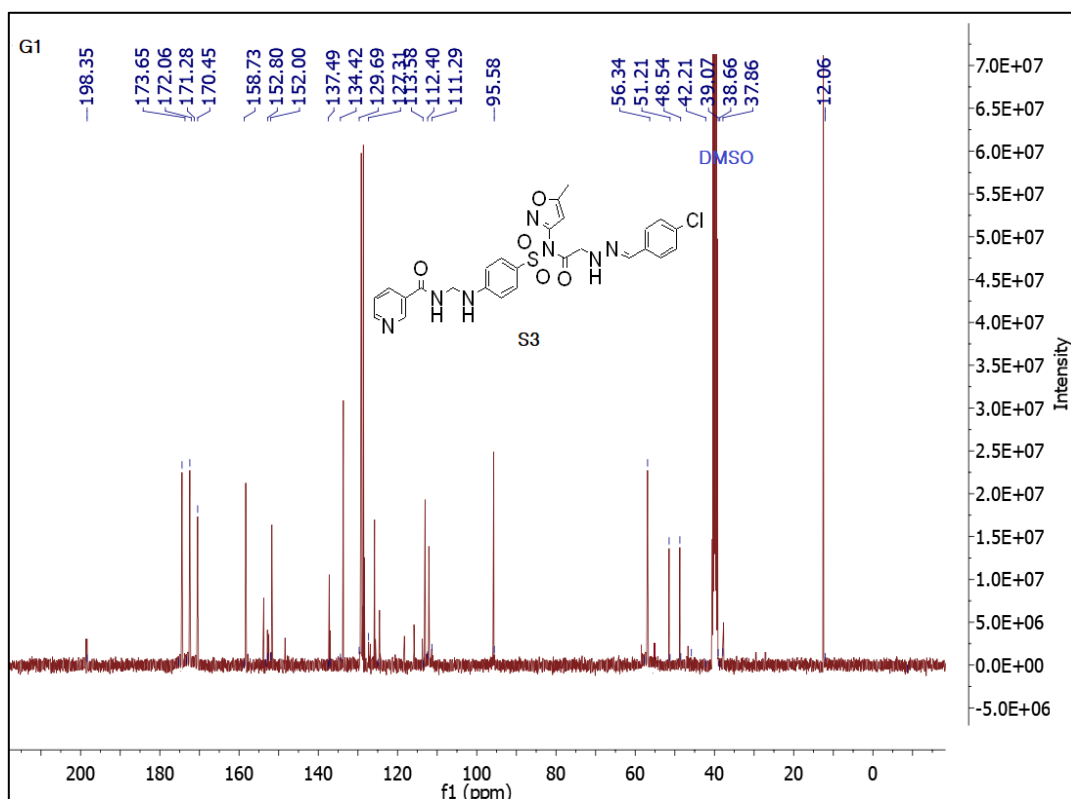


Table 2: Physical properties of selected prepared compounds.

No.	Mol. Formulas	Yield(%)	M.P.°C.	Color	Rf
S ₁	C ₂₄ H ₂₃ N ₇ O ₆ S	44	166	yellow	0.43
S ₂	C ₂₄ H ₂₃ N ₇ O ₅ S ₂	43	178	brown	0.35
S ₃	C ₂₆ H ₂₄ ClN ₇ O ₅ S	80	260	yellow	0.41
S ₄	C ₂₆ H ₂₄ BrN ₇ O ₅ S	81	253	Light pink	0.38
S ₅	C ₂₇ H ₂₇ N ₇ O ₇ S	80	203	Light yellow	0.50

Table 3: ¹H-¹³C NMR spectral data (δ ppm) for selected prepared compounds(M₄-S₅)

¹ H-NMR	¹³ C-NMR
N-(((4-(N-(5-methylisoxazol-3-yl)-N-(2-(phenylamino)-4H-1,3,4-thiadiazin-6-yl)sulfamoyl)phenyl) amino) methyl)nicotinamide. M ₄ 2.19 (s, 3H, CH ₃), 5.27 (s, 1H, C-H thiadiazine), 6.75-7.81 (m, 8H, Ar-H and 1H, pyridine), 8.26-8.93 (m, 3H, pyridine), 6.13(s, 1H,NH-Ph), 8.51(s,1H,NHCO pyridine). 4.21and 4.47(s, 2H, NH-CH ₂ -NH), 10.29(s, 1H, NH thiadiazine).	39.1 (-CH ₃), 51.2 (-CH ₂ NH), 113.2-141.8 (C _{aromatic}), 176.3(C=O).
N-(((4-(N-(((furan-2-ylmethylene) aminoglycyl)-N-(5-methyl isoxazol-3-yl)sulfamoyl) phenyl)amino)methyl) nicotinamide S ₁ 2.67 (s, 3H, CH ₃), 3.21 (s, 2H, OC-CH ₂ -NH), 6.11(s,1H, HN-Ar) 7.2-7.61 and 8.31-8.81(m, 4H, Ar-H, 4H, pyridine, and H, furan), (s, 1H, azomethine) in 8.13, 4.49(s, 2H, CH ₂ -NHPh)	14.5 (-CH ₃), 42.6,53.1 (2CH ₂), 115.6-149.2 (C _{aromatic}), 171 and 174.3 (two C=O groups).
N-(((4-(N-(5-methylisoxazol-3-yl)-N-(((thiophen-2-ylmethylene) amino)glycyl)sulfamoyl)phenyl) amino) methyl) nicotinamide S ₂ 2.52 (s, 3H, CH ₃), 3.52 (s, 2H, OC-CH ₂ -NH), 6.34(s,1H, HN-Ar) 6-91-7.4 and 8.43- 8.93(m, 4H, Ar-H, 4H, pyridine, and 3H, Thiophene), (s, 1H, azomethine) in 8.63, 4.49(s, 2H, CH ₂ -NHPh)	15.1 (-CH ₃), 45.1, 56.51 (2CH ₂), 112.1-138.9 (C _{aromatic}), 175.1,177.3 (two C=O).
N-(((4-(N-(((4-chlorobenzylidene) amino) glycyl)-N-(5-methyl isoxazol -3-yl)sulfamoyl)phenyl) amino)methyl) nicotinamide S ₃ 2.42 (s, 3H, CH ₃), 3.61 (s, 2H, OC-CH ₂ -NH), 6.31(s,1H, HN-Ar) 7.1-7.79 and 8.2-8.7(m, 7H, Ar-H and 4H, pyridine), (s, 1H, azomethine) in 8.51, 4.59(s, 2H, CH ₂ -NHPh)	12.06 (-CH ₃), 48.5, ,56.3(2CH ₂), 117.2 -143.1 (C _{aromatic}), 175 and 176.2 (two C=O).
N-(((4-(N-(((4-bromo benzylidene) amino)glycyl)-N-(5-methylisoxazol-3-yl)sulfamoyl) phenyl)amino) methyl)nicotinamide S ₄ 2.33 (s, 3H, CH ₃), 3.45 (s, 2H, OC-CH ₂ -NH), 6.01(s,1H, HN-Ar) 7.2-7.61 and 8.2-8.4(m, 7H, Ar-H and 4H, pyridine), (s, 1H, azomethine) in 8.32, 4.8(s, 2H, CH ₂ -NHPh)	12.7 (-CH ₃), 43.5,52.6 (2CH ₂), 117-139.4 (C aromatic),171and 173.2 (two C=O).
N-(((4-(N-(((4-hydroxy-2-methoxybenzylidene)amino)glycyl)-N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl) amino) methyl)nicotinamide.S ₅ 2.72 (s, 3H, CH ₃), 3.47 (s,3H, OCH ₃), 3.21(2H,CH ₂ C=O), two singl signals(s,2H, NH-CH ₂ -NH), 6.35(s,1H, NH-Ar) 7.1-7.79 (m, 6H, Ar-H and 1H, pyridine), 8.1-8.8 (m, 3H, pyridine) and (s,1H, azomethine) in 8.37, 6.11(s, 1H, NH-Ph)	18.5 (-CH ₃), 48.5,41 3. (2CH ₂ NH-), 95.2 (CH ₂ -Isoxazole), 112.8-137.8(C _{aromatic}), 170(NC=O), 173.5(NH-C=O).

3.2. Activity of compounds against microbes

Both Gram- *Escherichia coli* (*E. coli*), *Proteus*, and *Klebsiella pneumonia*). Gram⁺ bacteria, (*Staphylococcus aureus* and *Salmonella*) were used to determine the activity. Well diffusion and disc diffusion methods were utilized to measure the compounds activity as explained in the experimental part. Sulfamethoxazole was utilized as a standard compound. The obtaining results showed that compounds (M and S₃) were active against all bacteria, whereas the compounds (S₁ and S₂) were not active against *E. coli*.

Table 4: Antibacterial compounds against various bacterial strains

No.	<i>E-coli</i> (Gram-)	<i>Klebsiella pneumoniae</i> (Gram-)	<i>Salmonella</i> (Gram+)	<i>Staphylococcus aureus</i> (Gram+)	<i>Proteus</i> (Gram-)
Conc.	100 μ L				
M ₅	12	17	12	15	17
S ₁	-	12	-	15	15
S ₂	-	14	12	10	15
S ₃	15	15	11	11	12
S ₄	12	14	-	12	12
S ₅	14	13	-	11	11
Sulfamethoxazole	20	14	-	25	22

3.3 Molecular docking study.

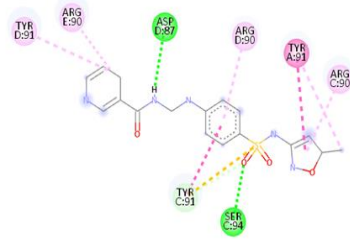
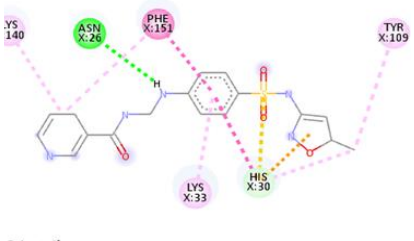
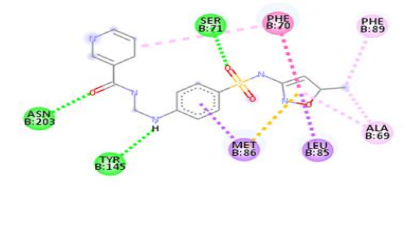
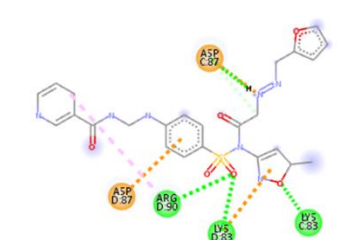
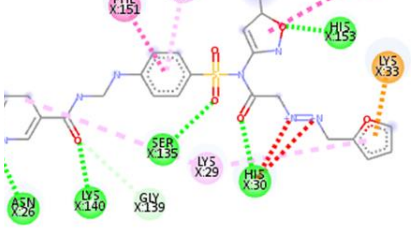
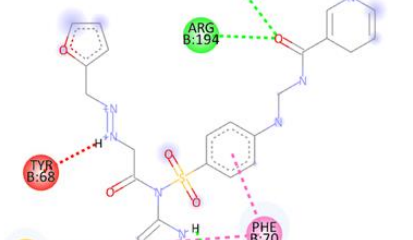
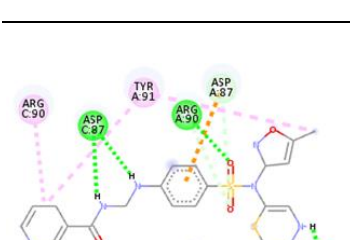
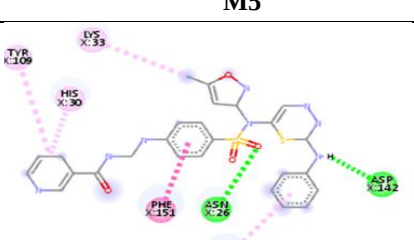
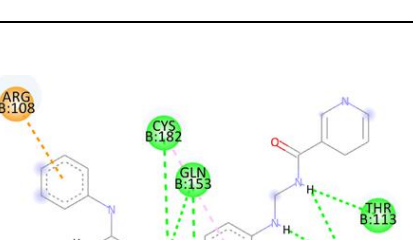
The molecular docking was carried out on synthesized compounds (M, M₅, S₁, S₂, S₃, and S₅) in order to anticipate the closeness to the targeted proteins, which obtained from three categories of bacteria. These proteins are 4H2M (*E. Coil*), 3FYV (*S. aureus*) and 6P4T (*Salmonella*) [20]. Analysis of the docking results demonstrated that all derivatives have the ability to inhabit the different sites of 4H2M, 3FYV, and 6P4T binding pockets with perfect docking interaction scores as shown in Table 5. Furthermore, docking studies of nicotinamide derivatives revealed their ability as antibacterial agents. All compounds (M- S5) consist of aromatic rings which show remarkable hydrophobic interactions with the amino acids in a protein. These compounds are ranked according to their binding energy, and a check of each molecule's total interactions with the binding site was effectively performed by counting the total number of conventional hydrogen bonds. The outcomes attained are listed in Tables 5 and 6, which revealed that the docked ligands are involved in many interesting hydrogen bonds and hydrophobic interactions. Hence, this demonstrates a good protein inhibition of prepared compounds. It can be noted that the molecular docking of compound M which consists of a sulfamethoxazole H-bond with amino acids in [4H2M (*E. coli*)] SER; B:71, ASN; B: 203 and TYR; B: 145 via the carbonyl group and sulfonyl group. Furthermore, it is important as an inhibitor is perceptible through many hydrophobic such as Alkyl and pi-Alkyl with ALA; B:69, Pi-Alkyl with PHE; B:89, Amide-Pi stacked with PHE; B: 70. Pi-Sigma bond with leuB:85 and MET; B: 86. in 4H2M (*E-coil*). On the other hand, compound M is linked mainly to protein 3FYV via one H-bonds with residues of ASN:26. Inhibitors are perceptible through hydro-phobic interactions such as Pi-Alkyl, Pi-Cation and Carbon hydrogen with HIS; X:30 also Pi-Pi-T-Shaped bond with PHE; X:151. Compound M₅ which consists of a thiadiazine ring added to M compound structure, exhibited docking results with 4H2M (nine hydrogen bonds). Tables 5 and 6 show amino acids and types of bonds on compound M5. Moreover, results showed that most of our compounds exhibit binding affinity = -7.0 to - 8.7 kcal/mol compared to the relocked sulfamethoxazole finding affinity = -6.8 kcal/mol). The values of the energy of the complexes (target-protein) resulting from these interactions are referred to as the lower energy, more stable complex, and a good activity.

Table 5: Binding affinity (kcal/mol) with bacteria protein and hydrophobic contacts (from molecular docking) in ligands S₁-S₅ and M₅ and Various interactions are involved between receptors and compounds.

code	Bindin g affinity	H- bond contacts(Bond length(A°)	Type of bond (Bond length(A°)
M/4H2M (E.Coil)	-7.7	SER;B:71.(2.00), ASN;B:203.(2.42) TYR;B:145.(2.39)	Pi-sigma(3.55), Alkyl(3.66) Amide-Pi-Stacked (4.00).
M/3FYV (S.aureus)	-7.0	ASN;X:26(2.78)	Pi-Alkyl(5.01), Pi-Cation;(2.88) Pi- Donor(2.88), Carbon(4.48), Alkyl(4.38) Pi-Pi-T- Shaped(5.01,4.87,4.84)
M/6P4T (Salmonella)	-7.6	ASP;D:87(2.28),SER;C:94(2.43)	Pi-Alkyl(3.75), Alkyl(4.94), Pi -Pi- Stacked(4.00)
S ₁ /4H2M(E. coli)	-8.4	SER;B:202(1.88),ARG;B:194 (2.75), SER;B:71(2.79)	Alkyl (4.7), Pi -Pi-Stacked(4.63), Donor-Donor(1.87).
S ₁ /3FYV (S.aureus)	-7.2	ASN;X:26(2.45), LYS;X:140(2.41) SER;X:135(2.18), HIS;X:30.(1.96), HIS;X:153(2.29).	Pi- Alkyl(4.18), Pi-Cation(4.07, 4.76,3.94.),Pi -Pi-Stacked(4.66, 3.85.), Positive –positive(4.43)
S ₁ /6P4T (Salmonella)	-7.0	LYS;D:83.(2.4), LYS;C:83.(2.71), ARG;D:90.(2.16)	Alkyl(4.87), Pi- anion(4.07), Pi- Cation(4.59), Donor- donor(2.04), Charge-charge(4.36).
S ₂ /4H2M (E. coli)	-8.3	ARG;B:194.(1.80),SER;B:202(2.61), TYR;B:145(2.46)SER;B:71(2.70)	Alkyl(4.68) , Pi-Sulfur(5.45), Donor- Donor(1.66) Pi--Pi-Stacked (4.78).
S ₂ /3FYV (S.aureus)	-7.2	ASN;X:26.(2.51), LYS;X:140(2.24), SER;X:135(2.20), HIS;X:153(2.14) HIS;X:30(2.02)	Pi-Alkyl(4.26), Donor –Donor(1.37), Pi-Sulfur(5.80), Pi--Pi-Stacked(5.44).
S ₂ /6P4T (Salmonella)	-7.6	SER;D:5(01.8) , LYS;D:74(2.40). THR;D:109.** (2.85, 2.79).	Pi-Alkyl(4.3), Pi- Alkyl(4.74), Pi- Sulfur(3.94),Pi -Pi-Stacked(3.85).
S ₃ /4H2M (E. coli)	-7.3	ILE;B:1092.67, THR;B:113**(2.20, 2.49))GLN;B:153(2.04),GLN;B:157(2.18)	Pi-Sulfur(5.37),Pi-Alkyl(4.79).
S ₃ /3FYV (S.aureus)	-7.2	ASN;X:26(2.7)LYS;X:140(2.84) HIS;X:30**(2.17, 2.04), SER;X:135(2.52)	Pi-Alkyl(4.09), Pi-Sulfur(5.41),Pi- Cation(4.85), Carbon(3.19), Pi-Pi-T- Stacked(3.94)
S ₃ /6P4T(Salmonella)	-7.8	SER;E:94(2.15), ARG;C:90(2.71) ARG;D:90(2.48), ASP;C:87(2.27) ARG;A:125(2.17), CYS;A:182(2.18)	Alkyl(3.99), Pi- Alkyl (4.43)Pi- Cation(4.74), Pi -Pi-Stacked4.35.
S ₅ /4H2M(E. coli)	-7.2	ILE;A:109(3.47), GLN;A:153.**(2.06, 2.49), GLY;A:109(2.17), GLU;A:185(2.42)	Pi-Sulfur(5.38),Charge-charge (5.08)
S ₅ /3FYV (S.aureus)	-7.6	ASN;X:26.*** (2.84, 2.48, 2.35) LYS;X:140(2.09), SER;X:13(2.13) HIS;X:149.(2.74), HIS;X:30(2.23) HIS;X:153(2.05)	Pi-Alkyl (4.47), Alkyl(4.88), Pi-Pi-T- Shaped (3.51,4.66)
S ₅ /6P4T (Salmonella)	-8.1	ASP;B:87(2.32),LYS;D:83(2.42),AR G;D:90(2.49)ARG;A:90(2.32) GLU;B:185**(2.6, 2.49),CYC;B:182 (2.18)GLN;B:153**(2.66, 2.26), GLN; B:157(2.22), GLY;B:111** (2.2, 2.25), THR;B:113(2.05)	Pi-Alkyl(5.21), Alkyl(4.71), Pi-Pi-T- Shaped(5.16). Pi-Alkyl (3.0)Pi- Alkyl(3.43),Pi- Cation(5.94), Pi -Pi-Stacked(4.35,4.3)
M ₅ /4H2M (E. coli)	-7.6		Donor-Donor(5.52)
M ₅ /3FYV(S.aureus)	-7.7	ASP; X:124(2.6), ASN;X:26(2.49)	Pi-Alkyl (5.94), Alkyl(4.35).
M ₅ /6P4T (Salmonella)	-8.7	ASP;B:87***((2.1, 2.04,2.91) ASP:87**(2.18,2.66), ARG;A:90(2.91)	Pi-Alkyl (5.33, 5.05) Pi -Pi-T-shaped(4.25), Pi -Pi-Stacked (4.49)

: two H-bonds, *: three H-bonds.

Table 6: Short contacts interaction profile for some synthesized compounds. The interaction between compounds and amino acid residues.

(6P4T)Salmonella	3FYV (S.aureus)	4H2M(E. coli)
M		
 Interactions - Conventional Hydrogen Bond - Carbon Hydrogen Bond - Pi-Cation - Pi-Sulfur - Pi-Pi T-shaped - Alkyl - Pi-Alkyl	 Interactions - Conventional Hydrogen Bond - Carbon Hydrogen Bond - Pi-Cation - Pi-Sulfur - Pi-Pi T-shaped - Alkyl - Pi-Alkyl	 Interactions - Conventional Hydrogen Bond - Pi-Sigma - Pi-Sulfur - Amide-Pi Stacked - Alkyl - Pi-Alkyl
S1		
 Interactions - Attractive Charge - Conventional Hydrogen Bond - Carbon Hydrogen Bond - Pi-Cation - Pi-Anion - Alkyl	 Interactions - Conventional Hydrogen Bond - Carbon Hydrogen Bond - Unfavorable Positive-Positive - Pi-Cation - Pi-Pi Stacked - Pi-Pi T-shaped - Alkyl - Pi-Alkyl	 Interactions - Conventional Hydrogen Bond - Unfavorable Donor-Donor - Pi-Sulfur - Pi-Pi Stacked - Pi-Pi T-shaped - Alkyl
M5		
 Interactions - Conventional Hydrogen Bond - Carbon Hydrogen Bond - Unfavorable Donor-Donor - Pi-Anion - Pi-Sigma - Alkyl - Pi-Alkyl	 Interactions - Conventional Hydrogen Bond - Pi-Pi Stacked - Alkyl - Pi-Alkyl	 Interactions - Conventional Hydrogen Bond - Unfavorable Donor-Donor - Pi-Cation - Pi-Pi T-shaped - Pi-Alkyl

3.4 ADME Studies

The ADME properties profile of our produced compounds (M, M2, M5, and S1-S5) were studied using the Swiss ADME server [21]. The aim was to identify the safer and potential drug to eliminate the compounds that feasibly will be failed at subsequent steps of drug development because the unfavourable ADME features. In this investigation, we assessed the absorption, distribution, metabolism, and excretion (ADME) properties of all synthesized compounds. Furthermore, we determined their topological polar surface area (TPSA),

another critical physicochemical parameter influencing drug bioavailability. The inertly soaked up molecules with a TPSA > 140 Å are believed to possess low oral bioavailability. Our findings indicated that all compounds have TPSA above 140, which is within the range of 167-196. This indicates that all ligands do not enter the systemic circulation, except compound M which has TSPA below 140. All compounds except compound (M) did not fulfil Lipinski's rule. Also, this study did not have good results because the molecular formula was above 500g/mol. The ADME properties profiles for the created compounds are shown in Table 7.

Table 7: ADME properties profile of the synthesized compounds M, M₂, M₅, and S₁-S₅

Comp.	M.Wt (g/mol)	H-bond acceptors	H-bond donors	MR	TPSA Å ²	GI Asb.	BBB parament	Lipinski violations
M	387.41	6	3	98.20	134.60	high	No	0
M ₂	459.48	9	4	113.62	180.93	Low	No	1
M ₅	576.65	7	4	160.27	187.53	Low	No	2
S ₁	537.55	9	3	136.16	180.41	Low	No	2
S ₂	553.61	8	3	141.77	195.51	Low	No	2
S ₃	582.03	8	3	148.,90	167.27	Low	no	2
S ₄	626.48	8	3	151.59	167.27	Low	no	2
S ₅	593.61	10	4	152.40	196.73	Low	no	2

3.5 Toxicity Prediction Results

The toxicity of synthesized compounds M-S₃ was calculated using the online ProTox-II soft-ware. Results of toxicological endpoints proposed that all compounds were predicted to be hepatotoxicity, non-mutagenicity [22], and non-cytotoxicity. Compounds (M to S₂) were predicted to be non- Immunotoxicity [23]. Compounds M and M₅ were non-carcinogenic and other compounds were carcinogenic. The predicted toxicity class for all compounds was 5 as follows: Class I: fatal if swallowed (LD₅₀ ≤ 5); Class II: fatal swallowed (5 < LD₅₀ ≤ 50); Class III: toxic if swallowed (50 < LD₅₀ ≤ 300); Class IV: harmful if swallowed (300 < LD₅₀ ≤ 2000); Class V: might be harmful if swallowed (2000 < LD₅₀ ≤ 5000); Class VI: non-toxic (LD₅₀ > 5000) [24]. The predicted LD₅₀ results of the produced compounds werunsafe if swallowed and belonged to class IV as shown in Table 8.

Table 8: In-silico toxicity evaluation of compounds(M-S₅).

Com P	Organ Toxicity	Toxicity - endpoints				Predicted LD50 (mg/kg)	Predict ed Toxicit y Class
	Hepatotoxicit y	Carcinogeni- city	Immunotoxi- city	Mutageni -city	S2		
M	active	Inactive	Inactive	Inactive	Inactive	3471mg/kg	5
M ₂	active	active	Inactive	Inactive	Inactive	3471mg/kg	5
M ₅	active	inactive	Inactive	inactive	inactive	3471mg/kg	5
S ₁	active	active	Inactive	Inactive	Inactive	3471mg/kg	5
S ₂	active	active	Inactive	Inactive	Inactive	3471mg/kg	5
S ₃	active	active	active	Inactive	Inactive	3471mg/kg	5
S ₄	active	active	active	Inactive	Inactive	3471mg/kg	5
S ₅	active	active	active	Inactive	Inactive	3471mg/kg	5

Conclusion

A series of compounds, designated M-S5, were synthesized and their structures elucidated using Fourier-transform infrared (FT-IR) spectroscopy, proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectroscopy, and melting point determination. The synthesized compounds exhibited broad-spectrum antibacterial activity against both Gram-negative bacteria (*Escherichia coli*, *Proteus*, and *Klebsiella pneumoniae*) and Gram-positive bacteria (*Staphylococcus aureus* and *Salmonella*). Molecular docking was employed to understand the binding process between targets and proteins. The ADMET studies disclosed that the prepared compounds (M₅-S₅) did not full-fill the Lipinski rule, except compound M.

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6. Conflict of interest

"The authors declare that they have no conflicts of interest."

Reference

- [1] S. A. Alsahib, "Characterization and Biological Activity of Some New Derivatives Derived from Sulfamethoxazole Compound", *Baghdad Science Journal*, vol. 17, no. 2, pp. 471-480, 2020.
- [2] Y. T. Lee, Y. J. Tan and C. E. Oon, "Benzimidazole and its derivatives as cancer therapeutics: The potential role from traditional to precision medicine", *Acta Pharmaceutica Sinica B*, vol. 13, no. 2, pp. 478-497, 2023.
- [3] J. Sławiński, C. T. Supuran, K. Szafrński and D. Vullo, "Carbonic anhydrase inhibitors. Synthesis of heterocyclic 4-substituted pyridine-3-sulfonamide derivatives and their inhibition of the human cytosolic isozymes I and II and transmembrane tumor-associated isozymes IX and XII", *European Journal of Medicinal Chemistry*, vol. 69, pp. 701-710, 2013.
- [4] S. Mondal, S. M. Mandal, T. K. Mondal and C. Sinha, "Structural characterization of new Schiff bases of sulfamethoxazole and sulfathiazole, their antibacterial activity and docking computation with DHPS protein structure", *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 150, pp. 268-279, 2015.
- [5] C. Yamali, H. Sakagami, Y. Uesawa, K. Kurosaki, K. Satoh, Y. Masuda, S. Yokose, A. Ece, S. Bua, A. Angeli, C. T. Supuran, H. I. Gul and C. Yamali, "Comprehensive study on potent and selective carbonic anhydrase inhibitors: Synthesis, bioactivities and molecular modeling studies of 4-(3-(2-arylidenehydrazine-1-carbonyl)-5-(thiophen-2-yl)-1H-pyrazole-1-yl)benzenesulfonamides", *Eur J Med Chem*, vol. 217, p. 113351, 2021.
- [6] H. S. Al-Atbi, B. K. Al-Salami and I. J. Al-Assadi, "New azo-azomethine derivative of sulfanilamide: Synthesis, Characterization, Spectroscopic, Antimicrobial and Antioxidant activity study," *Journal of Physics: Conference Series*, vol. 1294, no. 5, p. 052033, 2019.
- [7] K. Dhahagani, M. P. Kesavan, G. G. Vinoth Kumar, L. Ravi, G. Rajagopal and J. Rajesh, "Crystal structure, optical properties, DFT analysis of new morpholine based Schiff base ligands and their copper(II) complexes: DNA, protein docking analyses, antibacterial study and anticancer evaluation", *Materials Science and Engineering: C*, vol. 90, no. 1, pp. 119-130, 2018.
- [8] H. Hayun, I. Gavrilă, S. Silviana, A. E. Siahaan and M. I. Latifah, "Synthesis and antioxidant activity study of new mannich bases derived from vanillic acid", *Rasayan Journal of Chemistry*, vol. 13, no. 1, pp. 131-138, 2020.
- [9] N. Asemanipoor, M. Mohammadi-Khanaposhtani, S. Moradi, M. Vahidi, M. Asadi, M. A. Faramarzi, M. Mahdavi, M. Biglar, B. Larijani, H. Hamedifar and M. H. Hajimir, "Synthesis and biological evaluation of new benzimidazole-1,2,3-triazole hybrids as potential α -glucosidase inhibitors", *Bioorganic Chemistry*, vol. 95, no. 1, pp. 1-9, 2020.
- [10] W. J. Dunn and E. M. Hodnett, "Structure-antitumor activity correlation of some Schiff bases", *Journal of Medicinal Chemistry*, vol. 13, no. 4, pp. 768-770, 1970.

- [11] B. Shen, X. Shangguan, Z. Yin, S. Wu, Q. Zhang, W. Peng, J. Li, L. Zhang and J. Chen, "Inhibitory Effect of Fisetin on α -Glucosidase Activity: Kinetic and Molecular Docking Studies", *Molecules*, vol. 26, no. 17, pp. 5306-5322, 2021.
- [12] S. S. Raoof and A. S. Sadiq, "Mannich Bases: Synthesis, Pharmacological Activity, and Applications: A Review", *Iraqi Journal of Science*, vol. 63, no. 12, pp. 5086-5105, 2022.
- [13] F. W. Askar, Y. A. Aldheif, N. A. Jinzeel and O. A. Nief, "Synthesis and Biological Activity Evaluation of New Sulfonamid Derivatives", *Iraqi Journal of Science*, vol. 58, no. 4B, pp. 2012-2021, 2021.
- [14] S. M. H. Al-Majidi, H. A. Ibrahim and Y. A. H. Al-Issa, "Synthesis and Identification of Some New Derivatives of ([Benzyl Thio) Benzimidazole -N- (Methylene-5-Yl)] - 4,5- Di Substituted 1,2,4-Triazole and Evaluation of Their Activity as Antimicrobial and Anti-Inflammatory Agents", *Iraqi Journal of Science*, vol. 62, no. 4, pp. 1054-1065., 2021.
- [15] A. G. Abdul Maged and N. Al-Lami, "Synthesis of New Oxopyrimidine and Cyanopyridine Derivatives Containing the Pyrimidine Benzothiazole Moiety with the Evaluating their Colon Anticancer Bioactivity", *Iraqi Journal of Science*, vol. 64, no. 8, pp. 3725-3734, 2023.
- [16] A. G. Sager, J. K. Abais and Z. R. Katoof, "Molecular Docking, Synthesis and Evaluation for Antioxidant and Antibacterial Activity of New Oxazepane and Benzoxazepine Derivatives", *Baghdad Science Journal*, vol. 11, no. 1, pp. 1-17, 2023.
- [17] A. G. Sager, Z. R. Katoof and R. S. Radh, "Determination of Folic Acid in both Pure and Pharmaceutical Preparations via Oxidative Coupling Reaction", *Iraqi Journal of Science*, vol. 64, no. 8, pp. 3735-3747., 2023.
- [18] Y. M. Ibrahim and M. I. Khalaf, "Synthesis, Characterization, and Antioxidant Screening of Some New Azo Compounds Containing Pyrazole Moiety from Metoclopramide Drug", *Iraqi Journal of Science*, vol. 64, no. 11, pp. 5517-5524., 2023.
- [19] T. M. Yassen and A. Al-Azzawi, "Synthesis and Characterization of New Bis-Schiff Bases Linked to Various Imide Cycles", *Iraqi Journal of Science*, vol. 64, no. 3, pp. 1062-1070, 2023.
- [20] S. Akili, D. B. Hadda, A. Balash and M. F. Chehna, "Design, Synthesis and Characterization of Novel Sulfonamides Derivatives as Anticancer Agent Targeting EGFR TK, and Development of New Methods of Synthesis by Microwave Irradiation", *International Journal of Organic Chemistry*, vol. 11, no. 4, pp. 199-223, 2021.
- [21] F. Sangande , E. Julianti and D. H. Tjahjono , "Ligand-Based Pharmacophore Modelling, Molecular Docking, and Molecular Dynamic Studies of Dual Tyrosine Kinase Inhibitor of EGFR and VEGFR2", *International Journal of Cell Science & Molecular Biology*, vol. 21, no. 20, p. 7779, 2020.
- [22] Y. Mizushima and M. Kobayashi, "Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins", *Journal of Pharmacy and Pharmacology*, vol. 20, no. 3, p. 169–173, 1969.
- [23] K. A. Shadid, A. K. Shakya, R. R. Naik, T. S. Al-Qaisi , G. A. Origuat, A. . M. Atoom and H. Farah, "Exploring the Chemical Constituents, Antioxidant, Xanthine Oxidase and COX Inhibitory Activity of Commiphora gileadensis Commonly Grown Wild in Saudi Arabia", *Molecules*, vol. 28, no. 5, p. 2321, 2023.
- [24] Y. T. Hussein and Y. H. Azeez, "DFT analysis and in silico exploration of drug-likeness, toxicity prediction, bioactivity score, and chemical reactivity properties of the urolithins", *Journal of Biomolecular Structure and Dynamics*, vol. 41, no. 4, pp. 1168-1177, 2023.