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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, $^{1}$H and $^{13}$CNMR 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 اروماتية. تم تشخيص المركبات المحضرة باستخدام الطرق الطيفية FT-IR، $^{13}$CNMR – $^{1}$H 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، و3FYV 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 جيدا مقارنة بأدوية السلفاميثوكسازول. فحص امتصاص وسمية المركبات المحضرة باستخدام أداة Swiss ADME . أظهرت النتائج أن جميع الليكاندات لم تحقق قاعدة ليبنسكي، 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. $^{1}$H-$^{13}$CNMR 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 $^{o}$C. FT-IR: 1381cm$^{-1}$(C-N). $^{1}$H-NMR (DMSO, 400MHz)δ (ppm): 2.17 (s, 3H, $CH_3$), 4.67(s, 2H, $CH_2NH$), 6.34(s, 1H, NH), 8.81(d, 1H, NHCO), 7.32-8.26(m, 9H, Ar-H), $^{13}$C-NMR (DMSOd$^{6}$) δ(ppm): 21.6($CH_3$), 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 ($M_1$).*

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 ($M_2$ ).*

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. ($S_1$-$S_5$).*

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 $M_2$ 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 $S_1$-$S_5$ compounds were dried at 70$^{o}$C, then the melting point was measured. The data of compounds are shown in Tables (1 and 2).

*2.6 Synthesis of a compound ($M_3$).*

To a refluxed solution of compound $M_2$ (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, $^{1}$H- $^{13}$C-NMR) of compounds $M_3$.

*2.7 Synthesis of a compound ($M_5$).*

A mixture of compound $M_4$ (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, $^{1}$H-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.

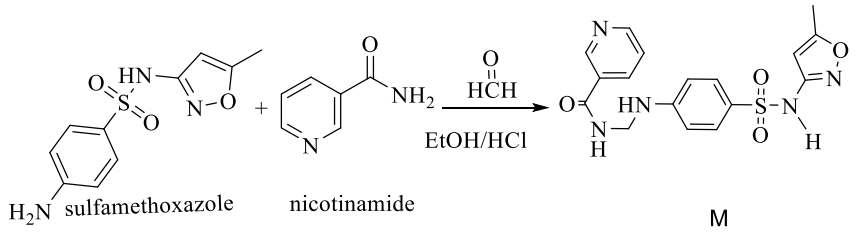
## 3. Results and Discussion

*3.1 Synthesis and Characterization*

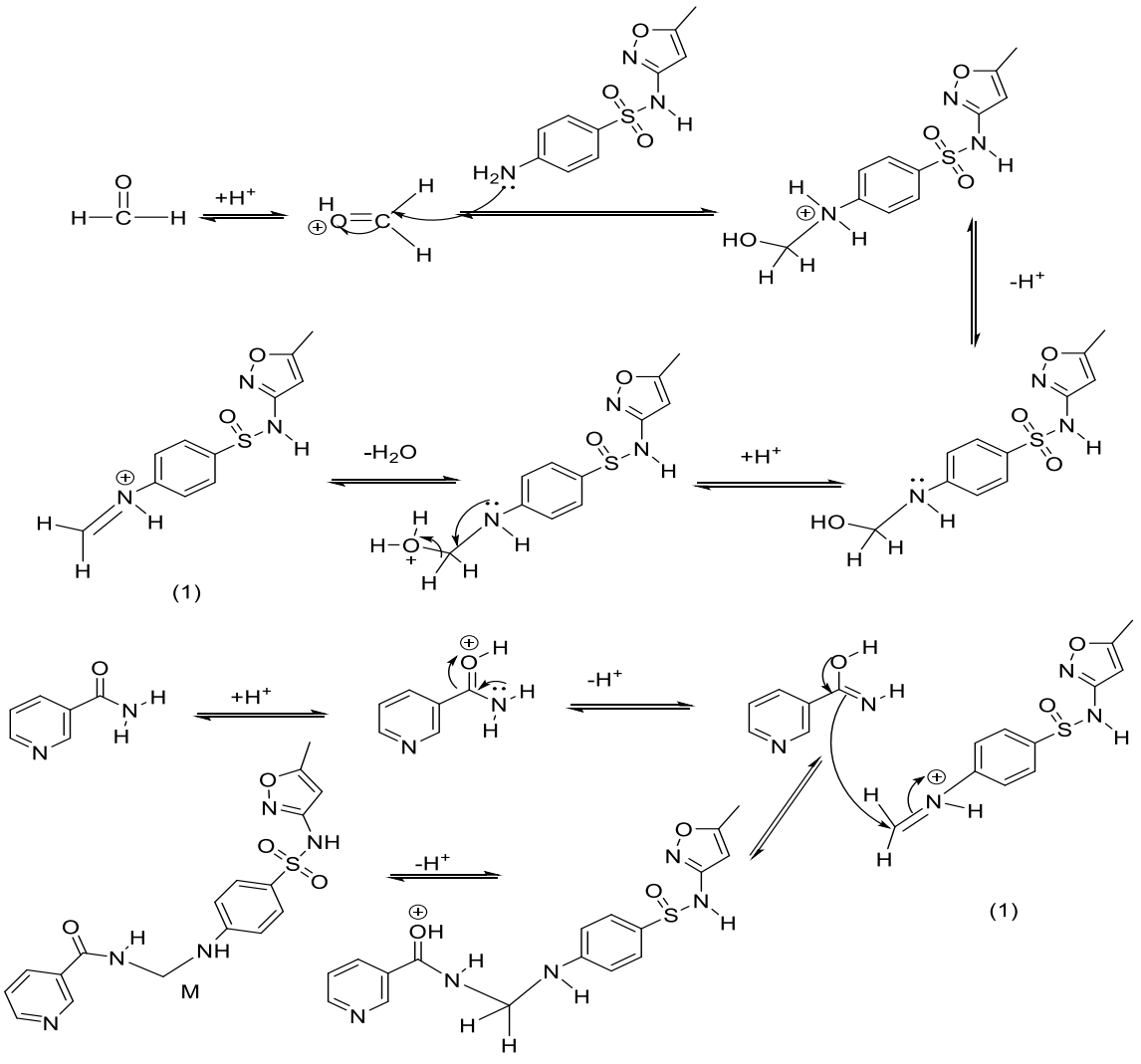
Newly synthesized derivatives of sulfamethoxazole are presented in Schemes (1 and 2). The FT-IR frequencies of Schiff bases, Mannich's base, and the new heterocyclic compound M5 are reported in Table 1. The FT-IR spectra illustrated the existence the of (C-N) group at 1381 $cm^{-1}$ with a sharp peak 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 2942-2823 $cm^{-1}$, which refers to the formation of the $CH_2$-$CH_2$-NH [17].

**Table 1:** A Comparison of experimental and theoretical vibrational frequencies data (M-$S_5$)

| *NO.* | υ(OH) | υ(NH) | C-H aromatic | C-H Alph | ν (C=N) | others. |
|---|---|---|---|---|---|---|
| *M* | - | 3268 | 3039 | 2951 | - | ν C=O(1641), (1556) ν N–H def (amide), (1381) ν N-C. |
| $M_5$ | - | 3283 | 3022 | 2954 | - | ν (C=N) 16291thiadiazine |
| $S_1$ | - | 3262 | 3076 | 2943 | 1623 | ν (C=O) 1711. |
| $S_2$ | - | 3274 | 3051 | 2941 | 1642 | ν (C=O) 1675. |
| $S_3$ | - | 3284 | 3082 | 2953 | 1618 | ν (C=O) 1712., ν($SO_2$) sym.1182 |
| $S_4$ | - | 3281 | 3075 | 2972 | 1604 | ν (C=O) 1723., ν($SO_2$) sym.1182 |
| $S_5$ | 3423 | 3243 | 3078 | 2981 | 1648 | ν (C=O) 1712., ν($SO_2$) sym.1162 |

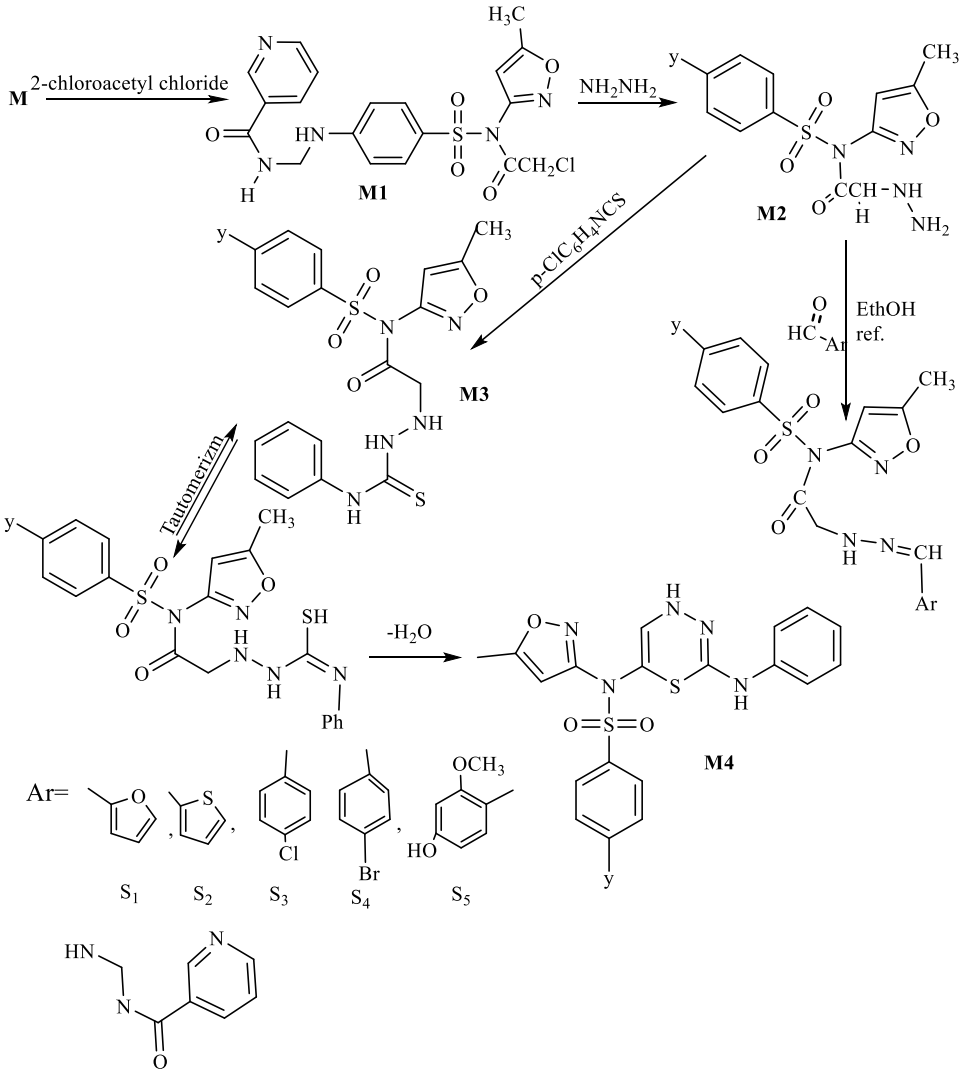



**Mech.**

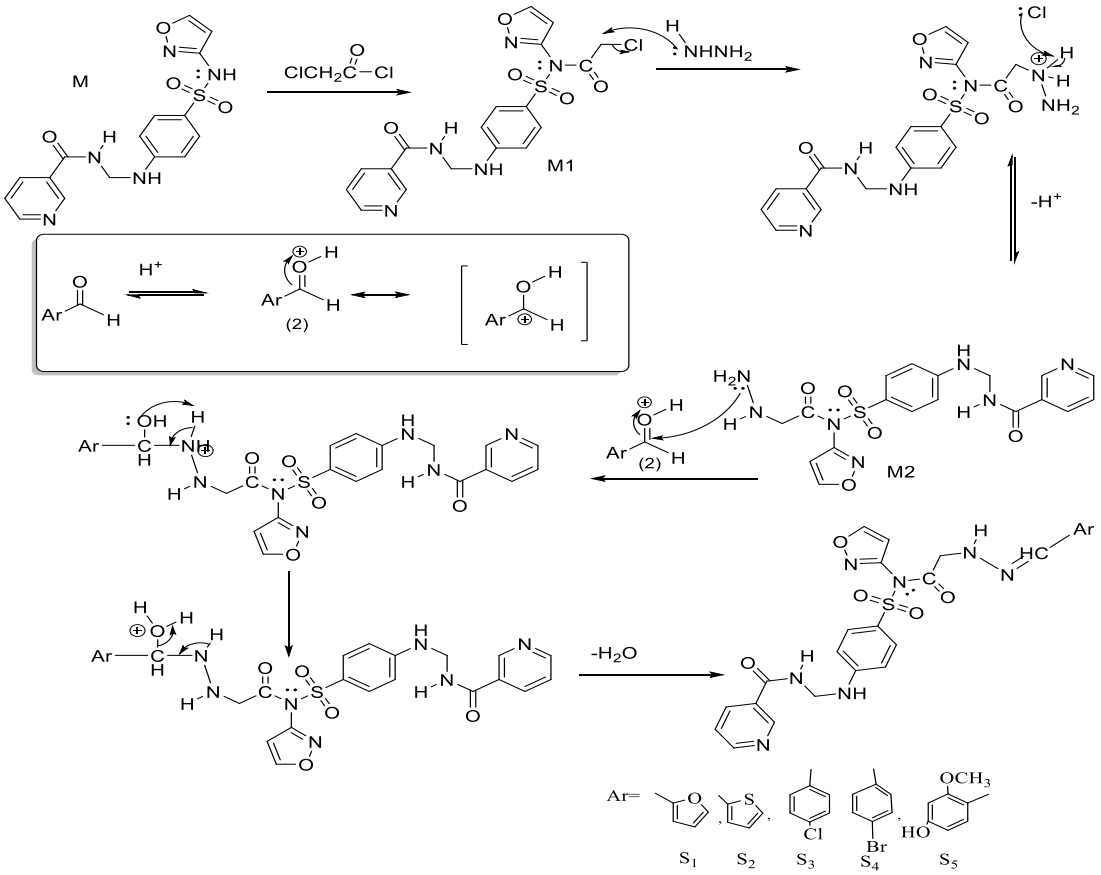



**Scheme 1:** Synthesis Mannich base M and mechanism reaction

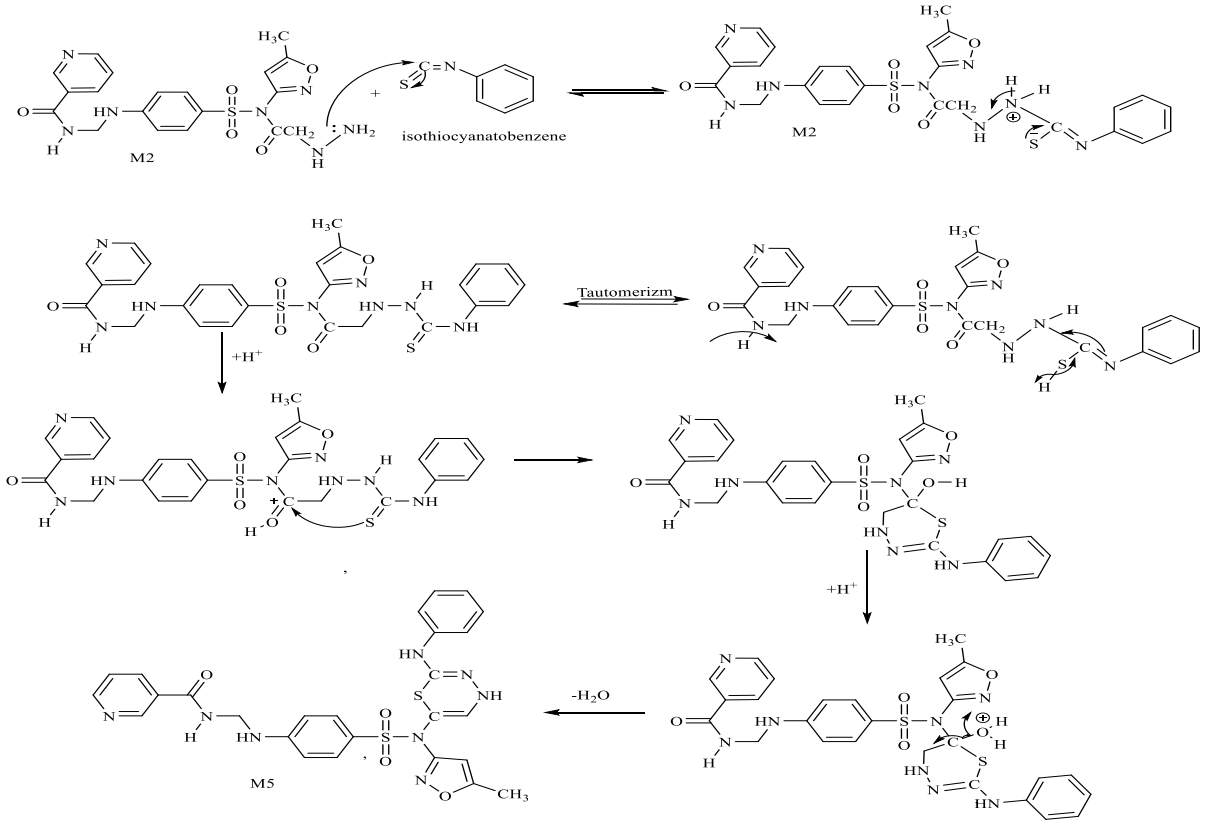
The formation of the Schiff bases (S1-S5) was confirmed by the presence of characteristic azomethine (CH=N) stretching vibrations in their FT-IR spectra, with the corresponding absorption bands listed in Table 1. Furthermore, the FT-IR spectrum of compound (M4) exhibited the absence of bands attributed to the ν (-$NHNH_2$), (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. $^{1}$H-NMR spectra of compounds (S1-S5 and M5), show the main attributes of chemical shifts (DMSO, δ ppm) as tabulated in Table 3. $^{1}$H NMR spectrum of compound M exhibits a signal at δ 2.17 ppm due to (3H) in methyl group ($CH_3$), chemical shift at δ 4.67 ppm due to (2H) in the $CH_2NH$ group [18], chemical shift at δ 6.34 ppm due to (2H) in the Ar-H [19]. The $^{1}$HNMR spectra of compound M is shown in Figure 1. The signals of $^{1}$HNMR and $^{13}$CNMR of all the prepared compounds are shown in Table 3. It showed signals corresponding to compound M that connected to thiadiazine moiety, ($CH_3$) group connected to the isoxazole ring, for two-CH- groups of thiadiazine ring as shown in Table 3. The $^{1}$HNMR and $^{13}$CNMR 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

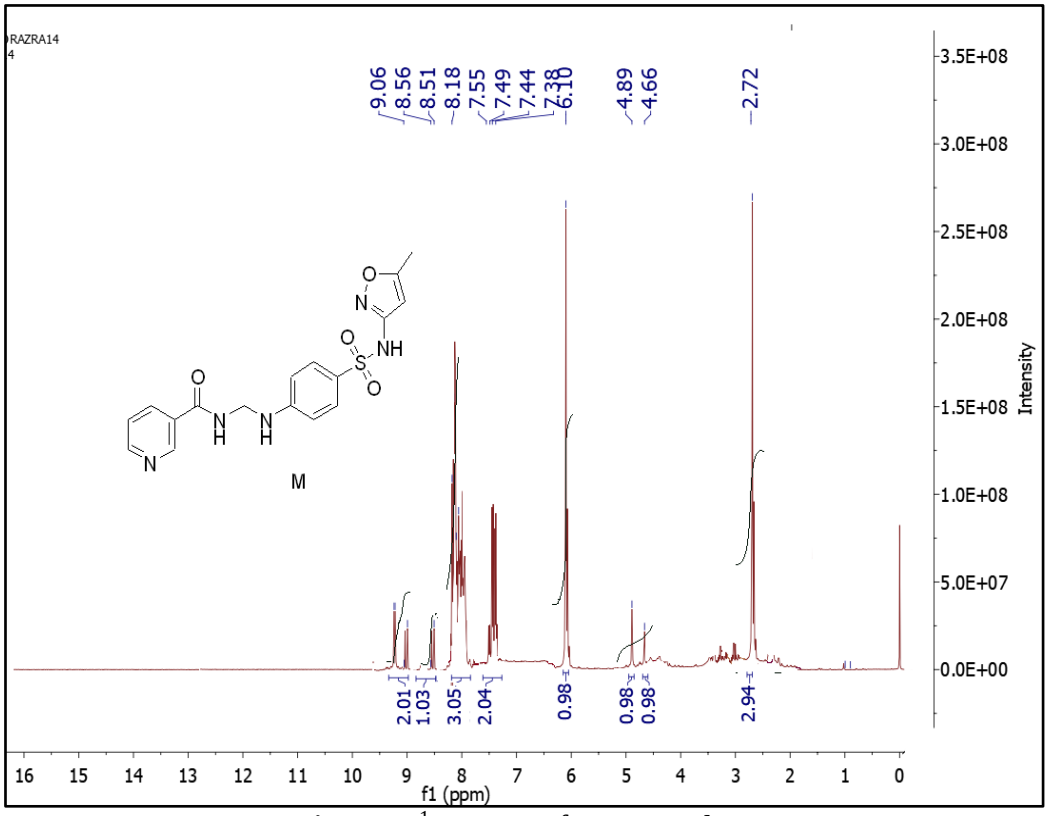



**Figure 1:**[1] HNMR of compound M.

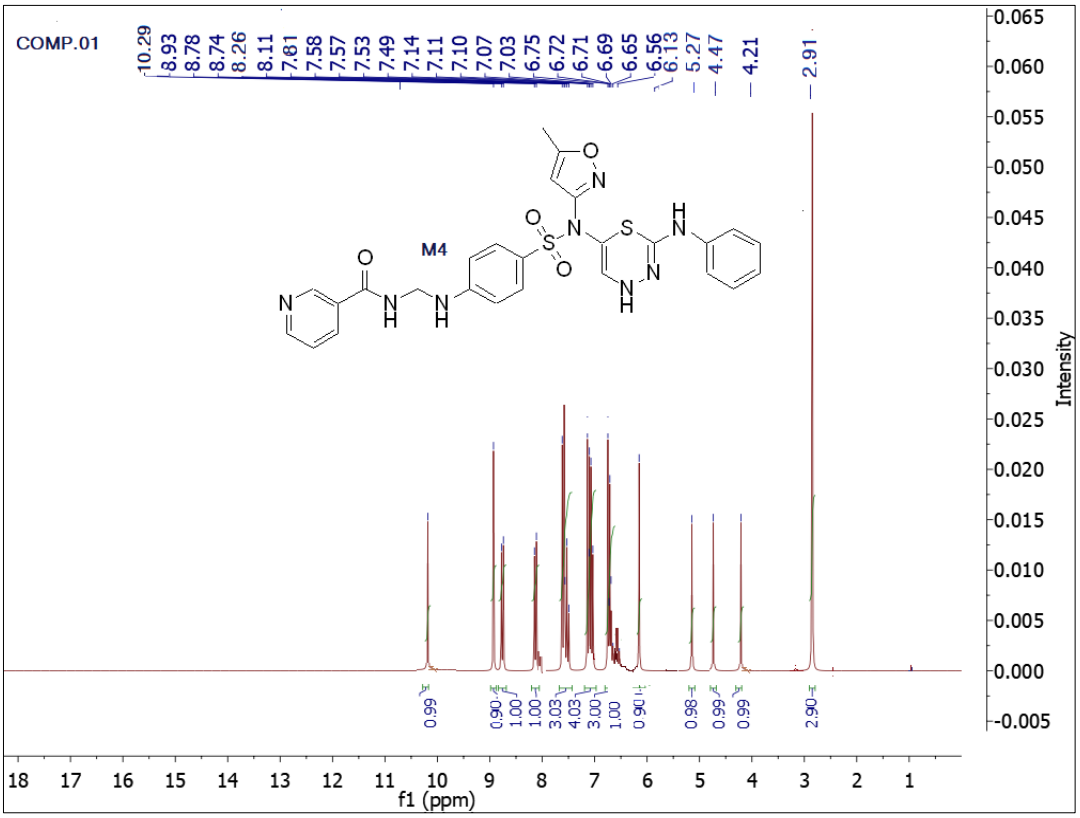



**Figure 2**:[1] HNMR of compound M4.

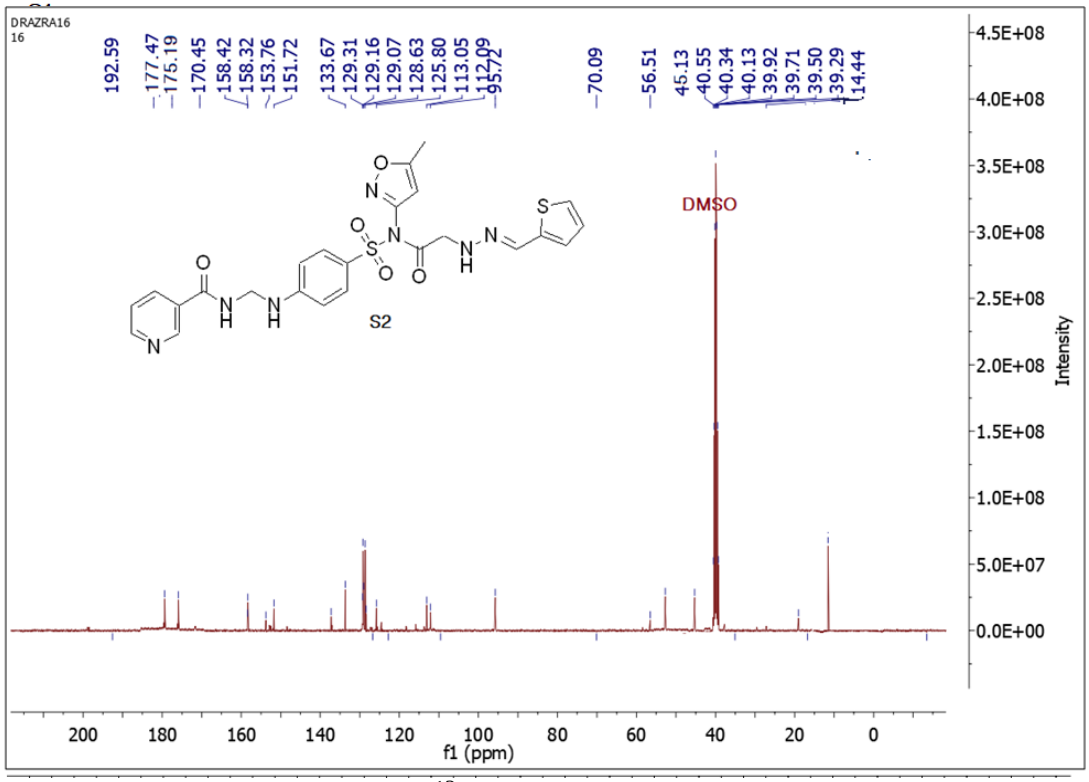



**Figure 3**:$^{13}$CNMR of compound S2.

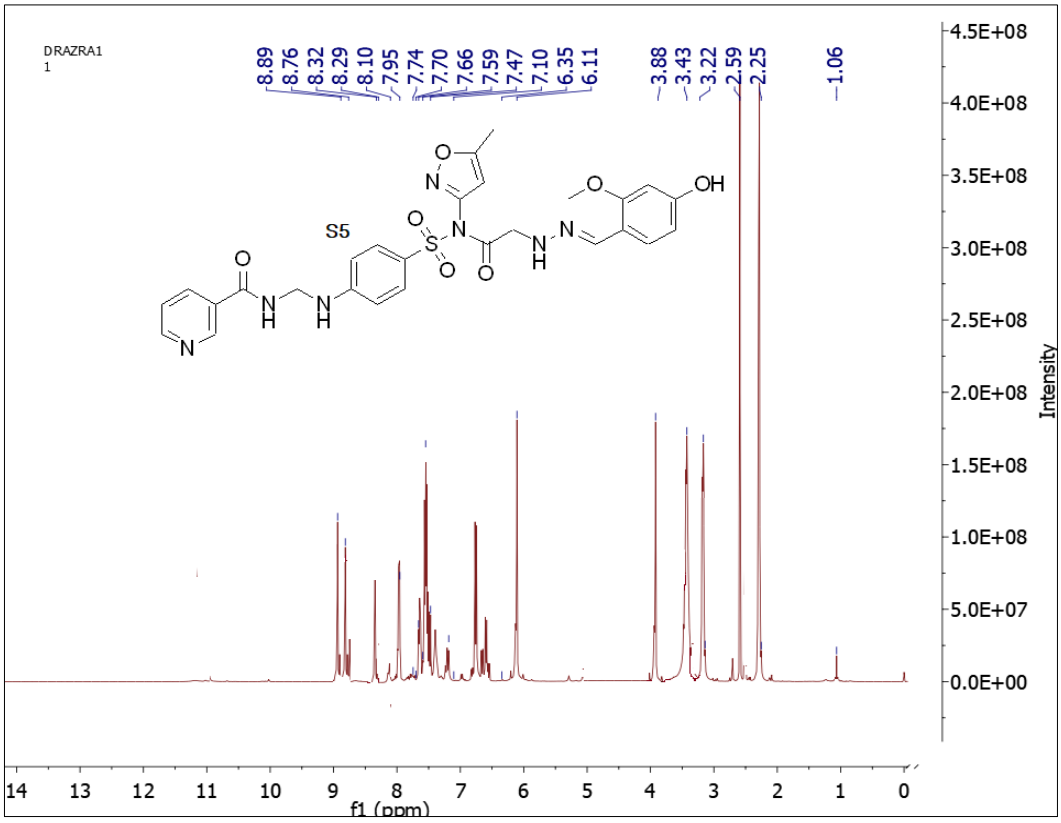



**Figure4:**$^{1}$ HNMR of compound S5.

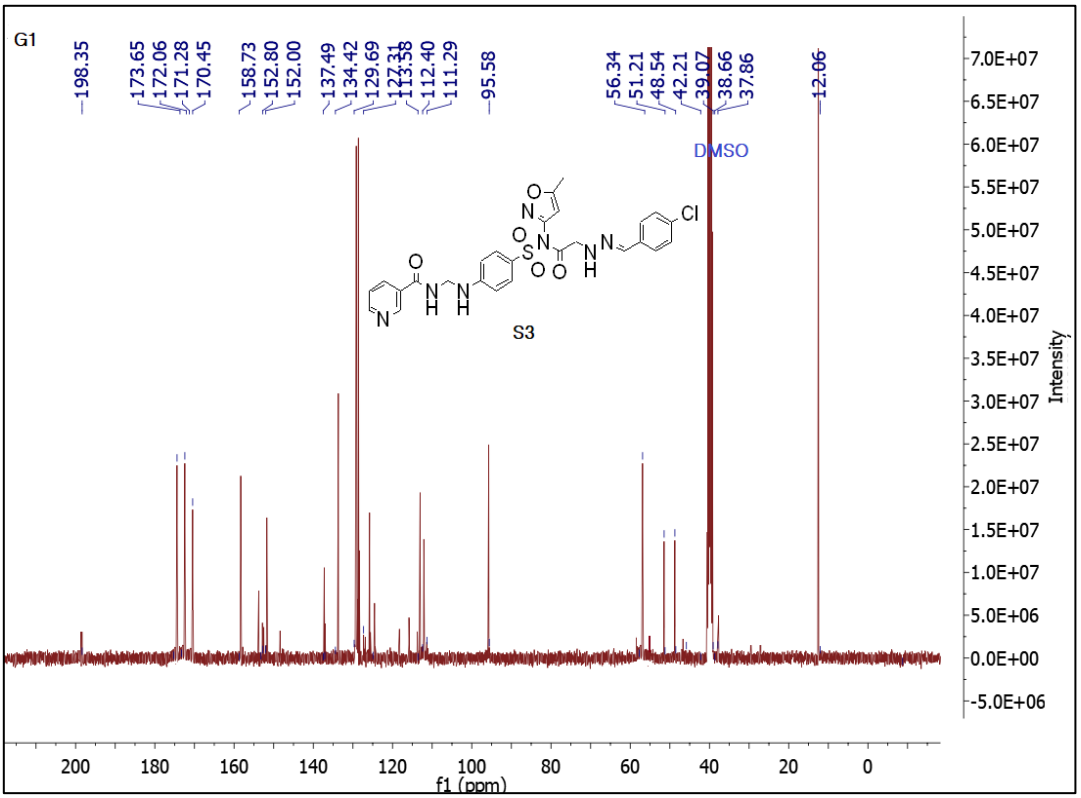



**Figure 5:** $^{13}$ CNMR of compound S3

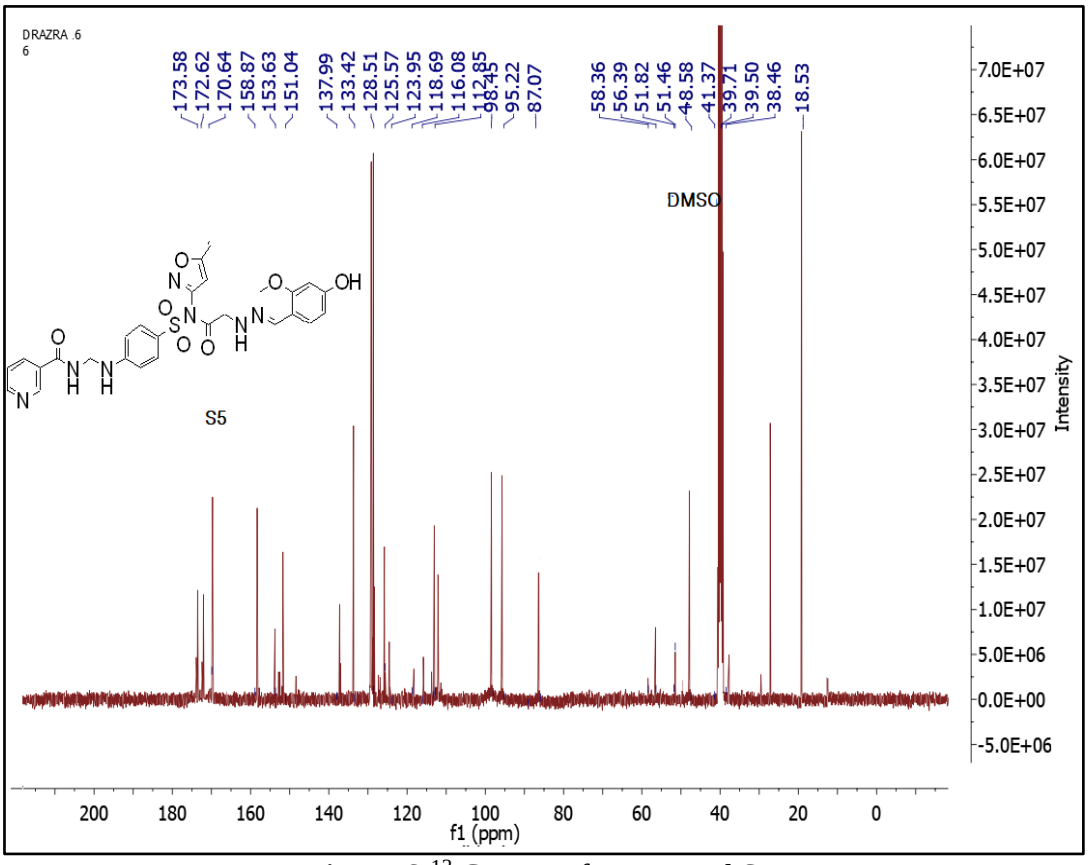



**Figure 6:**$^{13}$ CNMR of compound S5.

**Table 2:** Physical properties of selected prepared compounds.

| No. | Mol. Formulas | Yield(%) | M.P.°C. | Color | Rf |
|---|---|---|---|---|---|
| $S_1$ | $C_{24}H_{23}N_7O_6S$ | 44 | 166 | yellow | 0.43 |
| $S_2$ | $C_{24}H_{23}N_7O_5S_2$ | 43 | 178 | brown | 0.35 |
| $S_3$ | $C_{26}H_{24}ClN_7O_5S$ | 80 | 260 | yellow | 0.41 |
| $S_4$ | $C_{26}H_{24}BrN_7O_5S$ | 81 | 253 | Light pink | 0.38 |
| $S_5$ | $C_{27}H_{27}N_7O_7S$ | 80 | 203 | Light yellow | 0.50 |

**Table 3**: $^{1}H$-$^{13}C$ NMR spectral data (δ ppm) for selected prepared compounds($M_4$-$S_5$)

| $^{1}$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 (2$CH_2$), 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 (2$CH_2$), 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(2$CH_2$), 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 (2$CH_2$), 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. (2$CH_2$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 $S_3$) were active against all bacteria, whereas the compounds ($S_1$ and $S_2$) were not active against *E. coli.*

**Table 4**: Antibacterial compounds against various bacterial strains

| No. | *E-coli* (Gram-) | *Klebsiella pneumoniae (Gram-)* | *Salmonella* (Gram+) | *Staphylococcus aureus(*Gram+) | *Proteus* (Gram-) |
|---|---|---|---|---|---|
| *Conc.* | | | *100 µL* | | |
| $M_5$ | 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.*

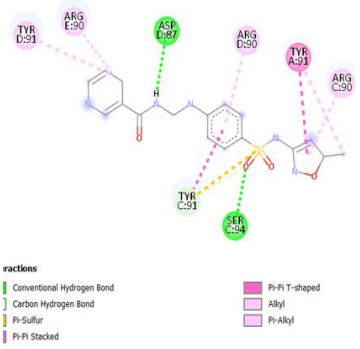
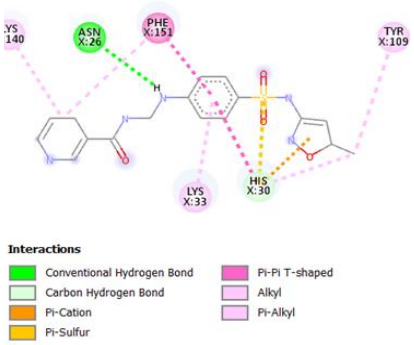
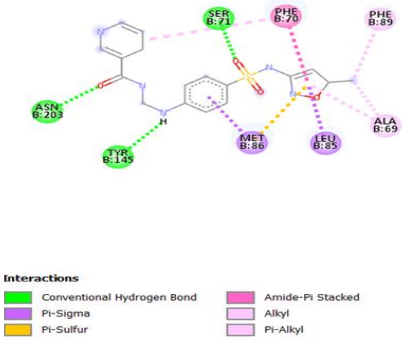
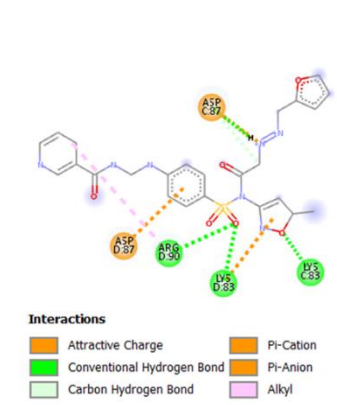
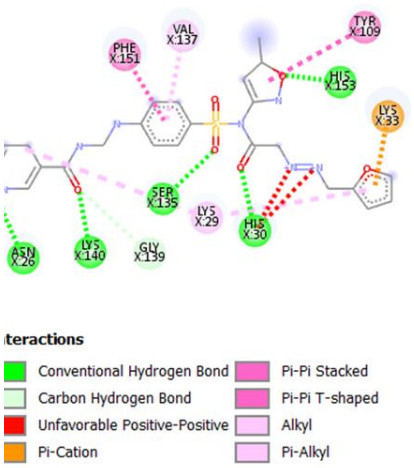
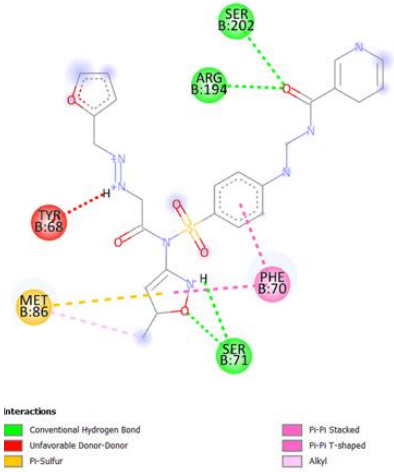
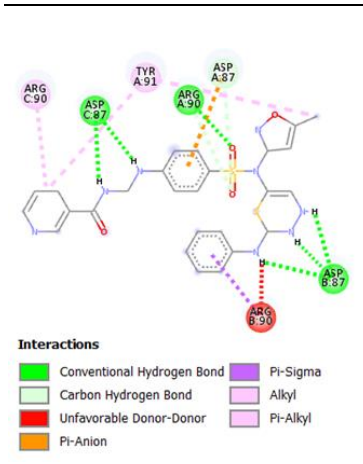
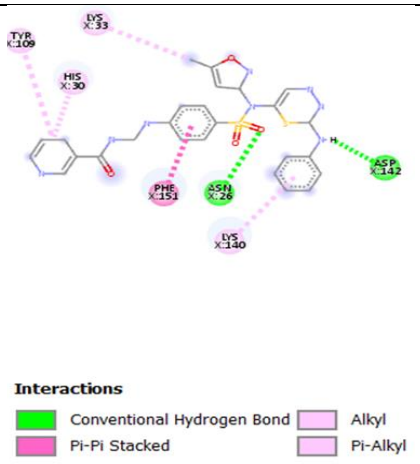
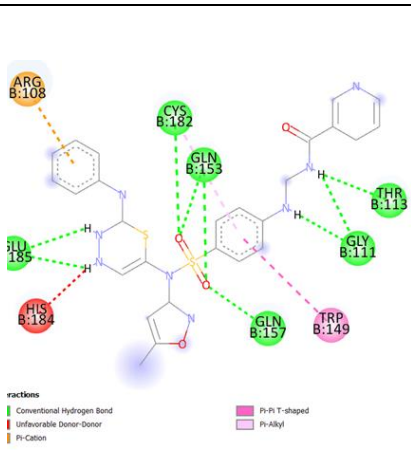
The molecular docking was carried out on synthesized compounds (M, $M_5$, $S_1$, $S_2$, $S_3$, and $S_5$) in order to anticipate the closeness to the targeted proteins, which obtained from three categories of bacteria. These proteins are 4H2M (*E. Coil*), 3FYV (*S. aureus*) and 6P4T (*Salmonella*) [20]. 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 $M_5$ 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 $S_1$-$S_5$ and $M_5$ and Various interactions are involved between receptors and compounds.

| *code* | **Binding 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). |
| *S2/3FYV (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(Salmonell)* | -7.8 | SER;E:94(2.15), ARG;C:90( 2.71) ARG;D:90(2.48), ASP;C:87(2.27) | Alkyl(3.99), Pi- Alkyl (4.43)Pi-Cation(4.74), Pi -Pi-Stacked4.35. |
| *$S_5$/4H2M(E. coli)* | -7.2 | ARG;A:125(2.17), CYS;A:182(2.18) 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),ARG;D:90(2.49)ARG;A:90(2.32) | Pi-Alkyl(5.21), Alkyl(4.71), Pi-Pi-T-Shaped( 5.16). |
| *$M_5$/4H2M (E. coli)* | -7.6 | 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 (3.0)Pi- Alkyl(3.43),Pi-Cation(5.94), Pi -Pi-Stacked(4.35,4.3) 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** | | |
|  |  |  |
| **S1** | | |
|  |  |  |
| **M5** | | |
|  |  |  |

*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, $M_2$, $M_5$, and $S_1$-$S_5$

| Comp. | M.Wt (g/mol) | H-bond acceptors | H-bond donors | MR | TPSA Å² | 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 $S_2$) 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-$S_5$).

| Comp | Organ Toxicity | Toxicity - endpoints | | | | Predicted LD50 (mg/kg) | Predicted Toxicity Class |
|---|---|---|---|---|---|---|---|
| | Hepatotoxicity | Carcinogenicity | Immunotoxicity | Mutagenicity | 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 |
| *S1* | active | active | Inactive | Inactive | Inactive | 3471mg/kg | 5 |
| *S2* | active | active | Inactive | Inactive | Inactive | 3471mg/kg | 5 |
| *S3* | active | active | active | Inactive | Inactive | 3471mg/kg | 5 |
| *S4* | active | active | active | Inactive | Inactive | 3471mg/kg | 5 |
| *S5* | 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 ($^1$H) 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 ($M_5$-$S_5$) 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."

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