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Research Article

Docking and Ultrasonic Synthesis of Schiff Base and Azo-Heterocyclic Compounds Derived from N-Aminophthalimide: Antibacterial Evaluation

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About Article

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ABSTRACT

In this study, a series of Schiff base derivatives derived from N-aminophthalimide were successfully synthesized through green chemistry protocols using ultrasonic techniques. The prepared compounds were further cyclized with chloroacetyl chloride and ethyl thioglycolate to obtain novel heterocyclic nitrogen-containing structures. The chemical structures of the synthesized compounds were confirmed via spectroscopic analyses, including FT-IR, ^{1}H-NMR, and ^{13}C-NMR techniques. Molecular docking studies were conducted using Discovery Studio 2024 to assess the interaction of the synthesized compounds with Coagulation Factor X (PDB ID: 1FJS). Among the evaluated compounds, S2 exhibited the most promising binding affinity, with a docking score of −10.4 kcal/mol, indicating strong interaction with the active site through multiple hydrogen bonds. Other compounds showed docking scores ranging between −7.7 and −9.3 kcal/mol. The antibacterial activities of the selected compounds were evaluated against Gram-negative and Gram-positive bacteria. The inhibitory zone diameters reached up to 19 mm for Klebsiella spp. (A1) and 17 mm for Staphylococcus aureus (A1). In contrast, several compounds exhibited moderate activity against Escherichia coli, with inhibition zones ranging from 10 to 12 mm. In conclusion, the synthesized compounds demonstrated significant potential as antibacterial agents and exhibited strong binding affinity towards Coagulation Factor X, suggesting their possible pharmaceutical applications.

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1. INTRODUCTION

Heterocyclic compounds containing nitrogen atoms are among the most significant classes of organic molecules due to their wide range of biological and pharmaceutical applications. These compounds play vital roles as building blocks in the synthesis of numerous bioactive molecules, including antimicrobial, antiviral, anti-inflammatory, and anticancer agents. The presence of nitrogen in the heterocyclic ring enhances the biological activity of these compounds by improving their interactions with biological targets.

Among the various nitrogen-containing heterocycles, Schiff bases and azo compounds have attracted considerable attention due to their notable biological properties. Schiff bases, which are characterized by the presence of an imine (-C=N-) functional group, have been extensively studied for their pharmacological potential. They exhibit a wide spectrum of biological activities such as antibacterial, antifungal, anticancer, and antioxidant properties. Similarly, azo compounds containing the (-N=N-) linkage are well known for their diverse biological activities and their utility in medicinal chemistry, especially in drug development and design.

N-aminophthalimide is an important intermediate in organic synthesis and has been widely utilized for the preparation of various nitrogen-containing heterocycles. This compound's structure offers multiple reactive sites, allowing the formation of Schiff bases and further cyclized products through reactions with suitable reagents such as chloroacetyl chloride and ethyl thioglycolate. The formation of such derivatives not only enhances structural diversity but also increases the potential for discovering new biologically active agents.

In recent years, green chemistry approaches have become increasingly important in the synthesis of pharmaceutical compounds. Ultrasonic-assisted synthesis is one such technique that offers several advantages, including reduced reaction times, higher yields, and environmentally friendly reaction conditions. Ultrasonication promotes chemical reactions through the generation of localized high temperatures and pressures, which enhance reaction rates and product purities.

Molecular docking has emerged as a powerful computational tool for predicting the interaction between small molecules and biological targets. It provides valuable insights into the binding affinity and potential biological activity of newly synthesized compounds. Coagulation Factor X (PDB ID: 1FJS) is a key enzyme in the blood coagulation cascade and has been identified as a valuable target in drug discovery efforts, particularly in the development of anticoagulant therapies. Docking studies targeting Factor X help predict the potential inhibitory effects of compounds on this enzyme.

In this study, new Schiff base and azo-heterocyclic derivatives derived from N-aminophthalimide were synthesized using ultrasonic techniques. Their structures were confirmed through spectroscopic analysis. Additionally, their biological activities were evaluated through molecular docking against Coagulation Factor X and through antibacterial assays against selected Gram-positive and Gram-negative bacteria. This work aims to contribute to the ongoing search for novel compounds with potential pharmaceutical applications.

2. LITERATURE REVIEW

Nitrogen-containing heterocyclic compounds, including Schiff bases and azo derivatives, have been extensively explored due to their broad spectrum of biological activities. Numerous studies have demonstrated that Schiff bases possess potent antibacterial, antifungal, anticancer, and antioxidant properties attributed to the presence of the imine (-C=N-) functional group, which facilitates interactions with various biological targets (Halliwell & Gutteridge, 2015; Kumar & Gupta, 2021). Likewise, azo compounds containing the diazo (-N=N-) group are known for their diverse pharmacological activities, including antibacterial and anticancer effects, and are frequently employed in medicinal chemistry for drug design (Ayala *et al.*, 2014; Sies, 2020).

Several research efforts have focused on the synthesis and biological evaluation of heterocyclic compounds derived from N-aminophthalimide. This intermediate is notable for its versatility in organic synthesis, allowing the formation of structurally diverse compounds through condensation and cyclization reactions (Dalle Donne *et al.*, 2006). Such compounds have been investigated for their pharmacological potential, with reports highlighting their antimicrobial, antioxidant, and enzyme inhibitory properties (Al Dleemy, 2009; Aziz, 2010).

In addition to conventional biological screening methods, molecular docking has emerged as a valuable tool in drug discovery for predicting the interactions between small molecules and target proteins. Several studies have emphasized the role of molecular docking in identifying potential inhibitors for key enzymes involved in disease pathways (Finkel & Holbrook, 2000; Kościuszko *et al.*, 2023). Among these targets, Coagulation Factor X (FXa) has gained significant attention. FXa is a serine protease that plays a pivotal role in the coagulation cascade, converting prothrombin to thrombin, a critical step in blood clot formation (Hanahan & Weinberg, 2011). Inhibitors of FXa are already used clinically as anticoagulants (e.g., rivaroxaban, apixaban), and its active site serves as a well-characterized model for docking studies in medicinal chemistry (Johnson *et al.*, 2014).

Furthermore, molecular docking studies targeting FXa provide insight into the binding affinities and potential inhibitory activities of novel compounds, particularly those containing nitrogen heterocycles which often interact favorably with serine protease active sites. The choice of FXa as a target in the current study is therefore justified based on its well-established role in thrombotic diseases and the abundance of structural data available for accurate computational modeling (Pita *et al.*, 2023).

3. METHODOLOGY

3.1. Chemicals and instruments

All reagents and solvents were of analytical grade and used without further purification. N-aminophthalimide and other chemicals were purchased from Sigma-Aldrich and BDH. Melting points were determined using a Stuart SMP30 apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer using KBr discs. ^{1}H-NMR and ^{13}C-NMR spectra were obtained in DMSO d_6 on a Bruker Avance 400 MHz spectrometer. Elemental analyses (CHNS)

were performed using a Euro EA3000 analyzer to confirm the purity of the synthesized compounds. Mass spectra (ESI-MS) were recorded on a Waters Micromass ZQ 4000 system.

3.2. Synthesis of schiff bases and azo-heterocyclic compounds

The Schiff base compounds were synthesized via condensation reactions of N-aminophthalimide with various aldehydes under ultrasonic irradiation in ethanol. Subsequent cyclization reactions with chloroacetyl chloride and ethyl thioglycolate afforded the desired heterocyclic derivatives. The synthetic route is outlined in Scheme 1.

3.3. Characterization of the synthesized compounds

The structures of the synthesized compounds were confirmed using spectroscopic methods, including FT-IR, ^{1}H-NMR, and ^{13}C-NMR. Purity was further verified by elemental analysis and mass spectrometry. The molecular structures were consistent with the proposed chemical formulas based on the analytical and spectral data.

3.4. Molecular docking studies

Molecular docking was performed using Discovery Studio 2024 against Coagulation Factor X (PDB ID: 1FJS). The docking protocol involved energy minimization of the ligands using the CHARMm force field and subsequent docking to the active site of Factor X. Binding energies and interaction profiles were analyzed to predict the potential inhibitory activity of the compounds.

3.5. Antibacterial activity

The antibacterial activity of the synthesized compounds was assessed by the agar well diffusion method against selected Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli, Klebsiella spp.) bacteria. Inhibition zones were measured after incubation at 37°C for 24 hours. Gentamicin was used as a positive control, and DMSO as a negative control. All experiments were performed in triplicate.

3.6. Green synthesis of schiff base compound ($M_1 - M_8$)

A mixture equimolar of N-aminophthalimide (0.003 mole, 0.30 g) and substituted benzaldehyde (0.0021 mole, o.34 g) and glacial acetic acid (3-5) drop in ethanol (10 ml). The reaction mixture was heated for (1) hours at (72 – 75 °C) in Ultrasonic technique with small amount of zirconyl chloride octahydrate $ZrOCl_2.8H_2O$ as a catalyst. The mixture cooled at room temperature to get the purest thorn crystals. The product was recrystallized from EtOH and dried at room temperature to give compounds (M_1. M_2. M_3. M_4. M_5. M_6. M_7. M_8) refrains. The physical properties of the compound are shown in the (Table 1).

Table 1. Some physical data of compounds M_1-M_8

Comp. No.	R	Molecular Formula	M.Wt	M.P °C	Yield%	Color
M_1	H	$C_{15}H_{10}N_2O_2$	250.26	150 – 155	65	White
M_2	$(C_6H_6)_2OH$	$C_{12}H_{19}N_2O_3$	316.23	240 – 245	81	Yellow
M_3	o-OH	$C_{15}H_{10}N_2O_3$	266.26	162 – 165	89	White
M_4	p-NO_2	$C_{15}H_9N_3O_4$	295.25	241 – 245	90	Orange
M5	o-OCH_3	$C_{16}H_{12}N_2O_3$	280.28	169 – 172	85	White – Yellow
M_6	m-Br	$C_{15}H_9N_2O_2Br$	325.19	179 – 183	80	white
M_7	m-OH	$C_{15}H_{10}N_2O_3$	266.26	230 – 237	93	Brown
M_8	o-NO_2	$C_{15}H_9N_3O_4$	295.25	183 – 197	88	white

3.7. Cyclization of the schiff base compounds with chloro acetyl chloride (A_1–A_8)

Stir cold solution from producing Schiff base in the previously step (0.00044 mole 0.130 g) dissolved in (20 ml) methanol Then add while cooling and stirring Acetyl chloride (0.0004 mole, 0.049 g) gradually and in the form of drops for (15) minutes Continue cooling and stirring for 20 minutes, then The reaction mixture was heated for (45) miniate at (72 – 75 °C) in Ultrasonic technique with small amount of zirconyl chloride octahydrate $ZrOCl_2.8H_2O$ as a catalyst Then pour it into a glass beaker containing ice water, separating it The precipitate formed by filtration to give compounds (A_1. A_2. A_3. A_4. A_5. A_6. A_7. A_8). refrains. The physical properties of the compound are shown in the (Table 2).

Table 2. Some physical data of compounds A_1-A_8

Comp. No	R	Molecular Formula	M.Wt	M.P °C	Yield%	Color
A_1	H	$C_{17}H_{11}CL_1N_2O_3$	326.74	190 – 195	73	white
A_2	$(C_6H_6)_2OH$	$C_{21}H_{13}CL_1N_2O_4$	392.80	220 – 225	79	light yellow
A_3	o-OH	$C_{17}H_{11}CL_1N_2O_4$	342.74	230 – 240	85	white
A_4	p-NO_2	$C_{17}H_{10}CL_1N_3O_5$	371.73	> 250	69	Orange

A_5	m-Br	$C_{17}H_{10}CL_1Br_1N_2O_3$	405.63	200 – 205	83	white
A_6	o-NO_2	$C_{17}H_{10}CL_1N_3O_5$	371.73	155 – 160	77	white
A_7	o-OCH_3	$C_{18}H_{13}CL_1N_2O_4$	357.76	181 – 186	85	White – Yellow
A_8	m-OH	$C_{17}H_{11}CL_1N_2O_4$	342.74	125 – 130	78	Brown

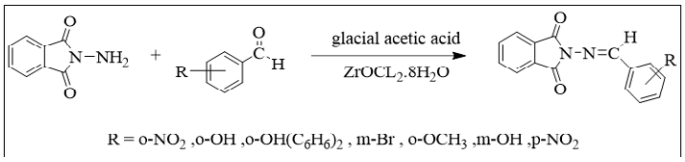
3.8. Cyclization of the Schiff base compound with ethyl thio glycolate (S_1 – S_8)

It has been dissolved (0.00027 mole, 0.072 g) of producing Schiff base in the ferst step in (15 ml) of methanol then add (0.00027 mole, 0.032 g) of ethyl thio glycolate was added under heat and stirring for (10mit) then add pipridine (0.5mL) then The reaction mixture was heated for (2 – 3 hrs) at (72 – 75 C°) in Ultrasonic technique with small amount of zirconyl chloride octahydrate $ZrOCl_2.8H_2O$ as a catalyst. Then, cooled at room temperature and The mixture was poured over ice Then filtered to get a precipitate. (S_1 – S_8). refrains. The physical properties of the compound are shown in the (Table 3)

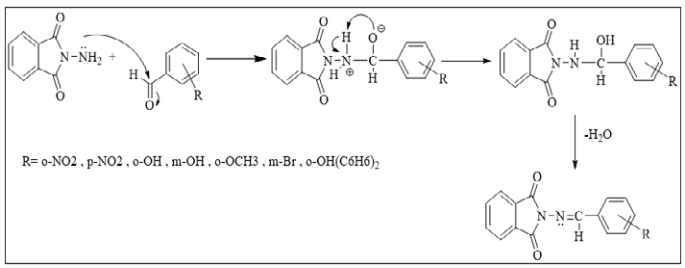
Table 3. Some physical data of compounds S_1-S_8

Comp. No.	R	Molecular Formula	M.Wt	M.P °C	Yield%	Color
S_1	o-OCH_3	$C_{18}H_{14}N_2O_4S_1$	354.38	165 – 167	77	white
S_2	$(C_6H_6)_2OH$	$C_{21}H_{14}N_2O_4S_1$	390.41	210 – 225	80	Yellow
S_3	m-Br	$C_{17}H_{11}N_2O_3S_1Br_1$	401.25	189 – 190	69	white
S_4	m-OH	$C_{17}H_{12}N_2O_4S_1$	340.35	200 – 203	72	Brown
S_5	H	$C_{17}H_{12}N_2O_3S_1$	324.35	203 – 205	64	White
S_6	o-OH	$C_{17}H_{12}N_2O_4S_1$	340.35	166 – 171	83	white
S_7	p-NO_2	$C_{17}H_{11}N_3O_5S_1$	369.35	210 – 215	71	Orange
S_8	o-NO_2	$C_{17}H_{11}N_3O_5S_1$	369.35	235 – 238	73	white

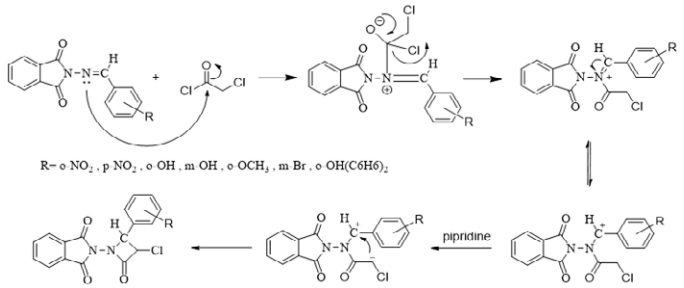
4. RESULTS AND DISCUSSION



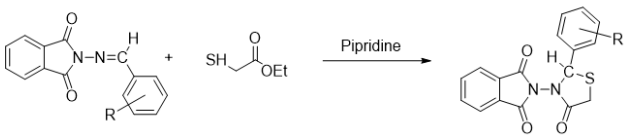
Scheme 1. Synthesis of Schiff bases derived from N-aminophthalimide and substituted aromatic aldehydes using $ZrOCl_2{\cdot}8H_2O$ as catalyst.



Scheme 2. Proposed reaction mechanism for the synthesis of Schiff bases and azo-heterocyclic compounds derived from N-aminophthalimide.



Scheme 3. Proposed reaction mechanism for the cyclization of Schiff bases with chloroacetyl chloride to form heterocyclic derivatives.



Scheme 4. Proposed reaction mechanism for the cyclization of Schiff bases with ethyl mercaptoacetate to form thiazolidinone derivatives.

Table 4. FT-IR spectral data of synthesized compounds

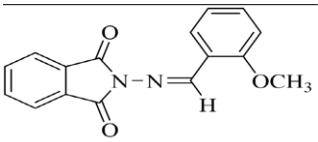
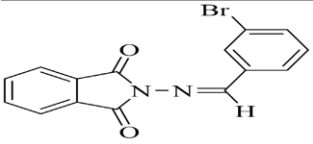
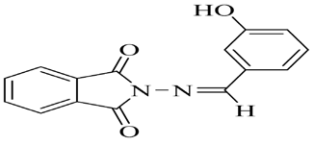
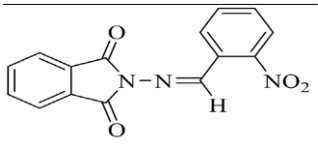
No.	1HNMR, DMSO-d6,δ (ppm)	13C-NMR, DMSO-d6,δ (ppm)	FT-IR I cm-1		
			C=O	C=N	Other
 M5	2.507 (1H, s,CH), 3.81(3H, OCH_3) 7.09-7.98 (8H, m, Aromatic protons)	112.56-121.28 -121.68 -123.39 -123.83-126.25-130.52-134.01-134.01 (C-aromatic rings); 154.96(C=N); 159.17-165.07(C=O amide); 56.30(O-CH_3);	1721	1601	
 M6	8.67 (1H, s, H1), 7.31-7.50-7.72-7.88 (4H, m) 7.90-8.24 (4H, m, Aromatic protons)	131.66-132.50-132.66-133.03.-135.49-136.31-136.85-136.96-137.18(C-aromatic rings);157.01(C=N); 164.89(C=O amide); 49.08,(CH)	1718	1661	Br, 644
 M7	9.15 (1H, s, OH),(4H, m) 6.96 (1H, s) 7.8-7.24 (4H, m, Aromatic protons)	119.65-120.43-123.89-130.49-130.60-135.05-135.35- (C- aromatic rings); 159.99(C=N); 164.97(C=O amide); 19.04(CH); 158.25,(C OH)	1711	1601	OH,3401
 M8	8.17 (1H, s) 7.82-8.15 (8H, m, Aromatic protons)	123.30-124.10-125.37-128.69-128.82-130.36-130.53-132.57-134.59-135.61-149.11(C- aromatic rings);167.40(C=O amide); 153.27(C=N);	1722	1605	NO_2, 1340

Table 5. [1]H-NMR spectral data of synthesized compounds

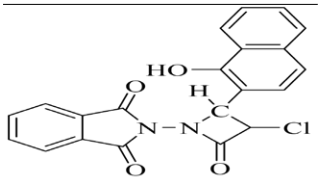
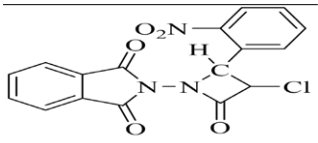
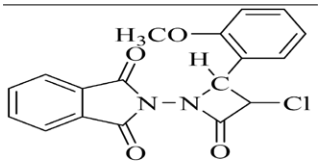
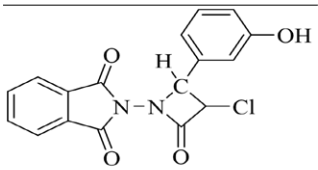
No.	1HNMR, DMSO-d6, δ (ppm)	13C-NMR, DMSO-d6, δ (ppm)	FT-IR I cm-1		
			C=O	C=N	Other
 A2	2.507 (1H, s,CH), 3.81(1H, OH) 7.550-7.788 (10H, m, Aromatic protons)	123.98-124.06-127.75-128.08-128.89-129.32-129.67-130.41-130.93-132.23-135.43-135.47 (C- aromatic rings); 164.78-164.15-167.76(C=O amide); 149.10(C-HO); 52.97(C-Cl)	1742		OH, 3063
 A6	8.69 (1H, s, H1), 7.00-7.71-7.80-7.91 (4H, m) 7.95-8.15 (8H, m, Aromatic protons)	131.19-132.52-132.58-132.63-132.76-133.94-134.26-134.41(C- aromatic rings);159.23(C=N); 167.11-166.78-165.09(C=O amide); 52.74,(CH)	1735		NO_2, 1564
 A7	6.87 (1H, s, OCH_3) 7.05 (1H, s, CH) 7.15-6.85 (8H, m, Aromatic protons)	122.39-128.09 -129.04-129.83-130.04-130.56-132.36-135.32- (C- aromatic rings); 157.97-159.20-165.11-(C=O amide); 56.16(CH); 56.09 (OCH_3)	1721		OCH_3, 1454
 A8	6.78 (1H, s, CH) 7.84 (1H, s, OH) 7.94-7.71 (8H, m, Aromatic protons)	116.52-117.04-119.28-120.07-123.91-127.64-129.84-129.87 (C- aromatic rings); 164.91-162.21-158.54 (C=O amide); 52.92 (C—H) 52.71 (C—CL)	1726		OH, 3065

Table 6. C-NMR spectral data of synthesized compounds

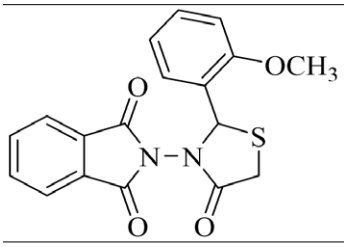
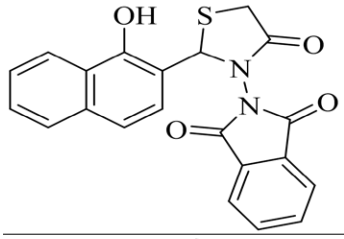
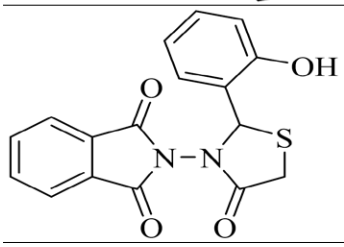
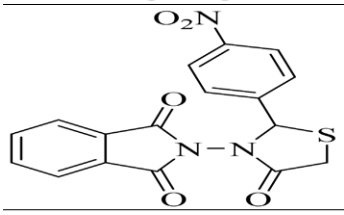
No.	1HNMR, DMSO-d6, δ (ppm)	13C-NMR, DMSO-d6, δ (ppm)	IR(KBr) V cm-1	
			C=O	Other
	6.83 (1H, s,CH), 3.75(3H, OCH_3) 3.80-3.78(2H, CH_2) 7.090-7.263 (4H, m, Aromatic protons) 7.848-7.908 (4H, m, Aromatic protons)	112.15-112.28-120.98-123.85-126.27-128.77-131.65-132.05-158.19-(C-aromatic rings); 167.28-164.36-(C=O amide); 56.09 (N—C—S) 56.16 (O—CH_3) 158.19 (C—O)	1720	OCH_3, 1463
	6.806 (1H, s, CH), 3.76-3.81 (2H, m, CH_2) 7.91-7.64 (6H, m, Aromatic protons) 7.41-7.47 (4H, m, Aromatic protons)	118.66-119.13-120.44-123.34-123.99-124.39-127.09-127.51-132.23-150.08(C- aromatic rings); 167.73-164.78-(C=O amide); 40.59,(S—C—C=O) 52.97(S—C—N) 150.08(C—OH)	1726	OH, 3056
	4.18 (1H, s, CH), 3.60-3.78 (2H, m, CH_2), 9.90 (1H, OH) 6.87-7.06 (4H, m, Aromatic protons) 7.80-7.86 (4H, m, Aromatic protons)	117.39-119.67-122.32-124.35-128.00-129.60-131.00-133.55-153.59 (C-aromatic rings); 170.61-168.36-162.56-(C=O amide); 153.59(C—OH) 49.49,(S—C—C=O) 52.96(N—C—S)	1732	OH, 3065
	7.52 (1H, s, CH), 3.79-3.63 (2H, m, CH_2) 8.16-8.27 (6H, m, Aromatic protons) 7.97-7.91 (4H, m, Aromatic protons)	124.13-129.09-130.76-131.24-132.58 (C- aromatic rings); 164.93-156.08(C=O amide); 148.76(C—NO_2) 39.33,(S—C—C=O) 52.93(N—C—S)	1723	NO_2, 1527

Table 7. Molecular docking binding scores of synthesized compounds

Compound No.	ΔG[Kcal/mol] 1FJS	Compound No.	ΔG[Kcal/mol] 1FJS
M1	-7.7	S2	-10.4
M2	-9.3	S3	-8.4
M4	-8.3	S4	-8.3
M5	-7.9	S7	-7.9
A2	-9.0	A5	-8.4
A4	-9.0	A7	-9.0

Table 8. Antibacterial activity (inhibition zones in mm) of synthesized compounds.

Compound No.	Concentration.Used	E. coli	klebsiella	Staph. Aureus	bacillus
M1	0.01	12	14	13	R
M2	0.01	R	R	10	R
M4	0.01	R	R	R	R
M5	0.01	R	R	R	R
M9	0.01	10	11	10	R
A1	0.01	9	19	17	R

A3	0.03	R	10	10	R
A4	0.02	R	10	12	R
A5	0.03	R	R	8	R
S1	0.03	10	R	R	R
S5	0.03	R	R	R	R
S8	0.03	R	R	8	R
S9	0.03	11	10	12	R

R : resistance

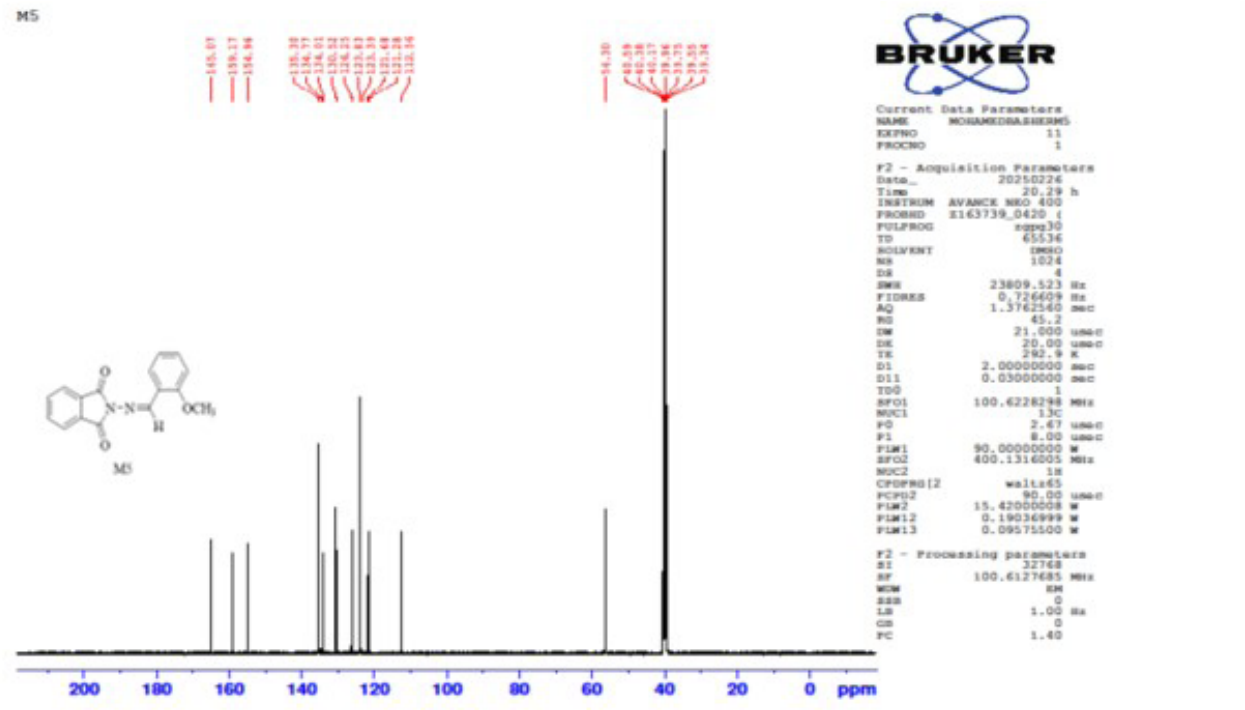


Figure 1. ^{13}C-NMR spectrum for M5

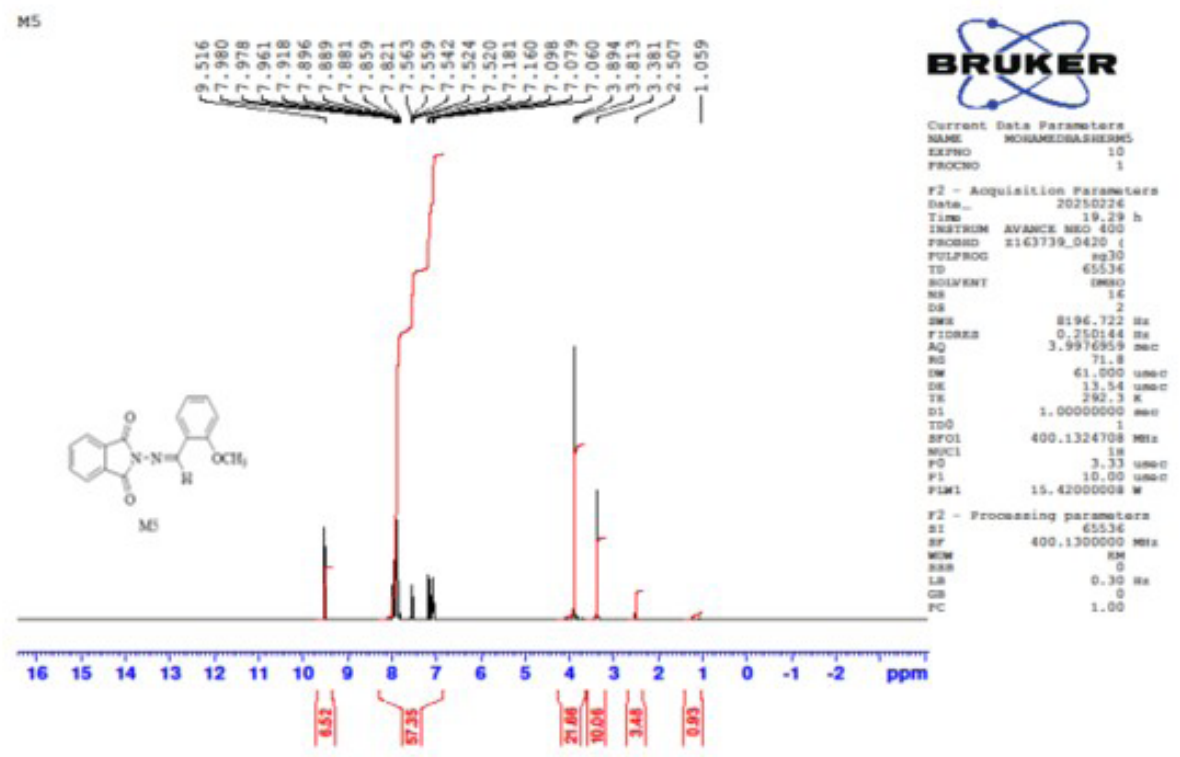


Figure 2. [1]H-NMR spectrum for M5

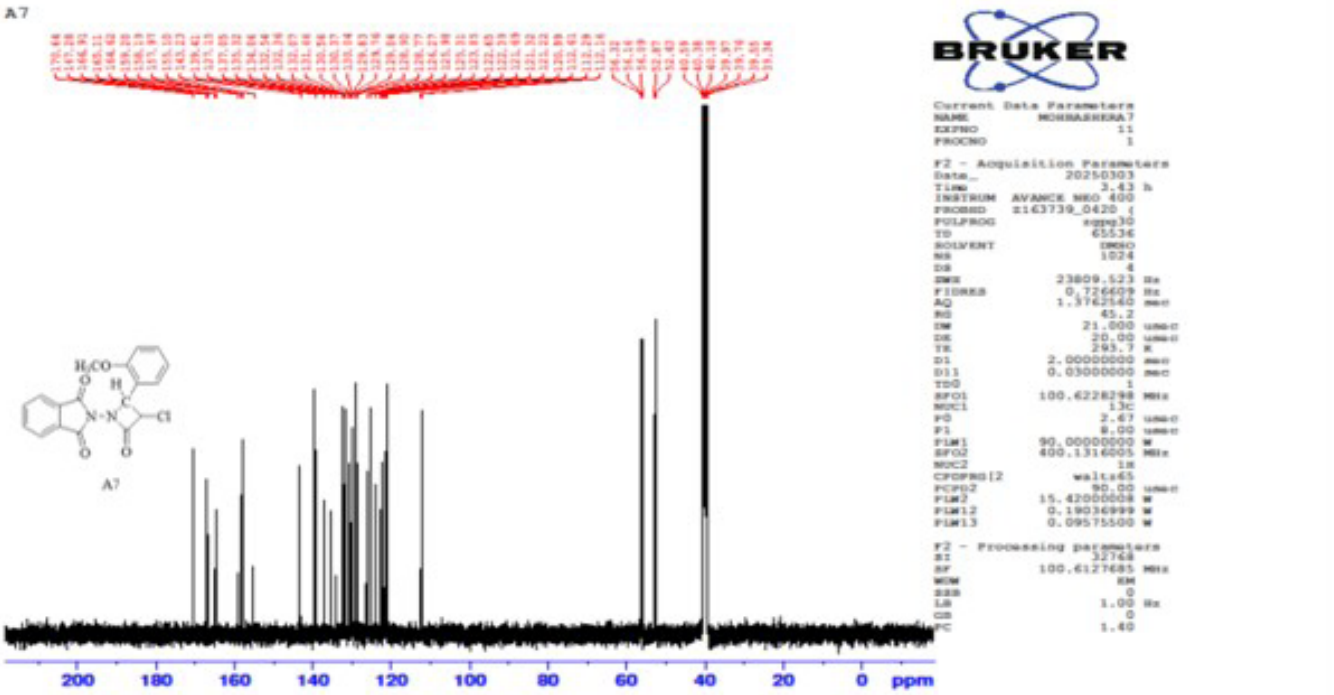


Figure 3. [13]C-NMR spectrum for A7

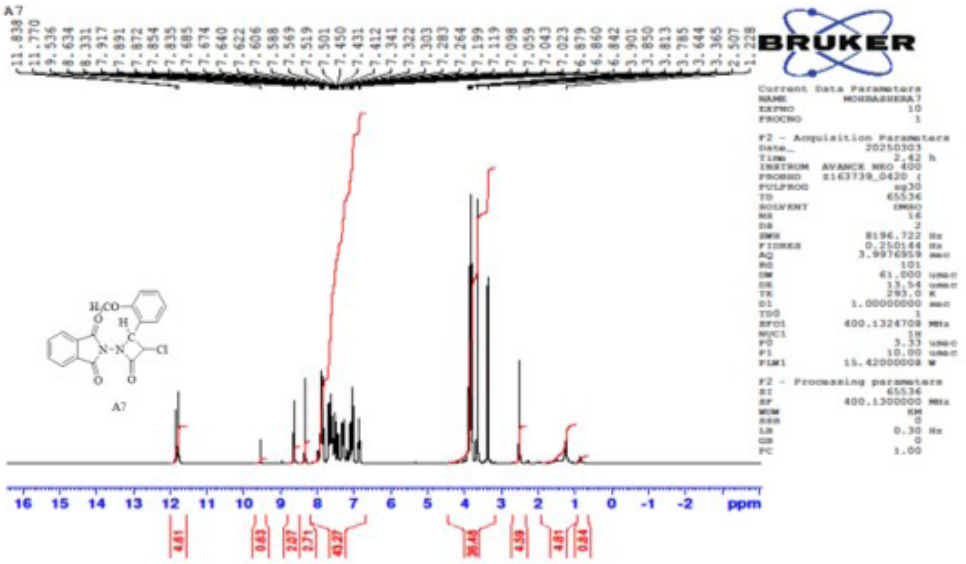


Figure 4. [1]H-NMR spectrum for A7

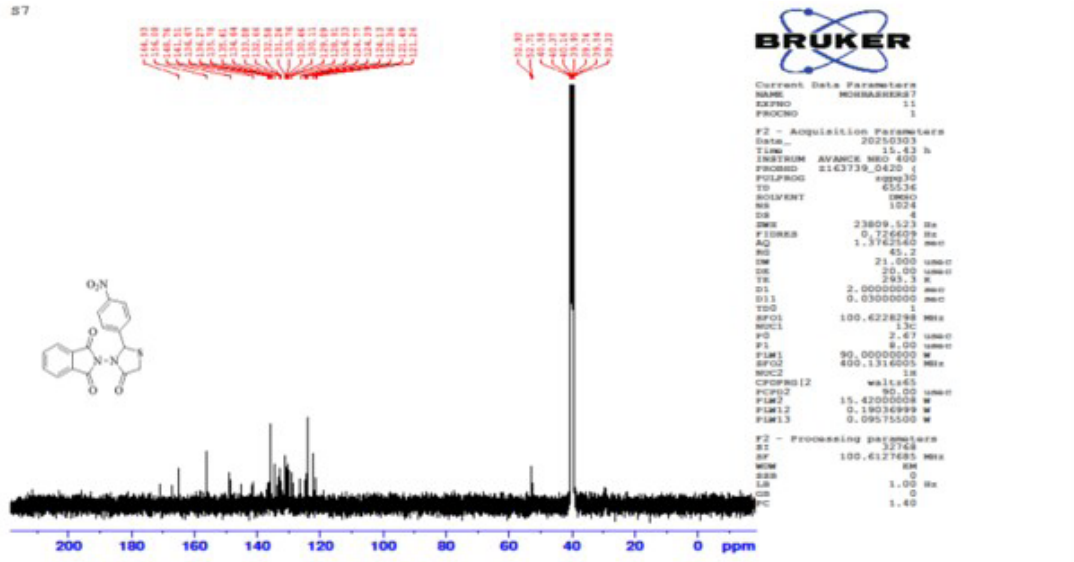


Figure 5. [13]C-NMR spectrum for S7

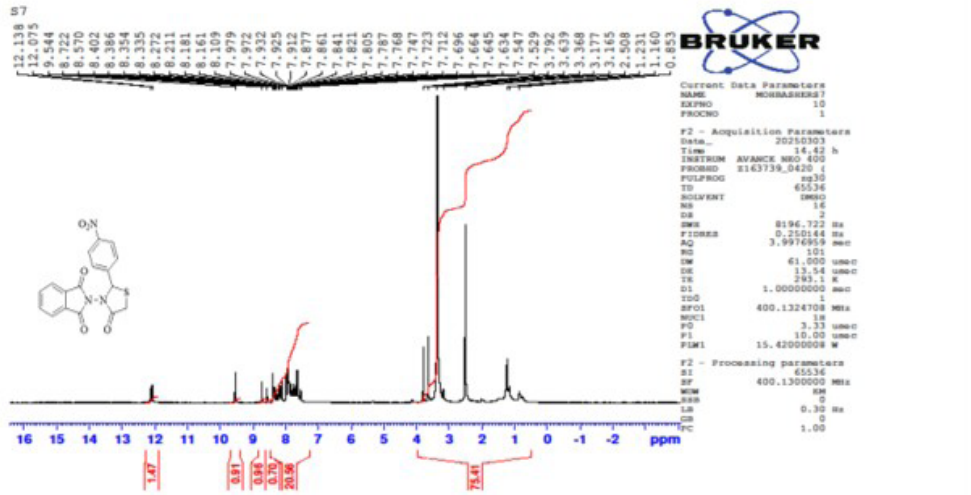


Figure 6. [1]H-NMR spectrum for S7

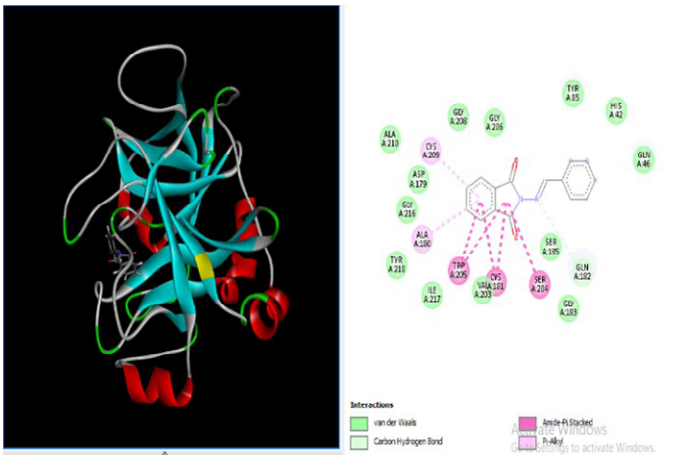


Figure 7. -2D and 3D compound M1, with Amino acids of (1FJS)

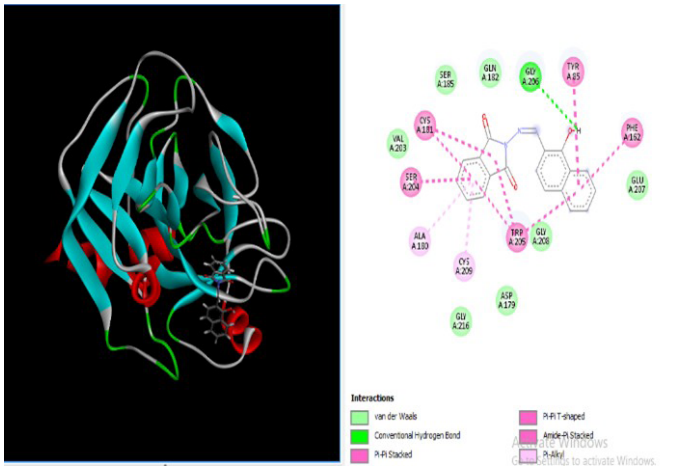


Figure 8. -2D and 3D compound M2, with Amino acids of (1FJS)

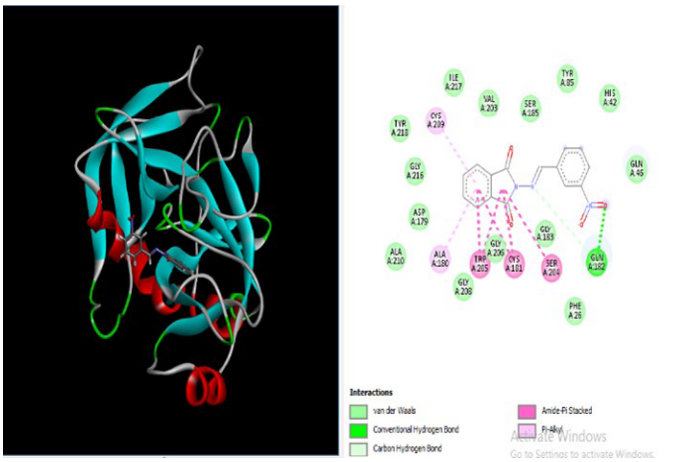


Figure 9. -2D and 3D compound M4, with Amino acids of (1FJS)

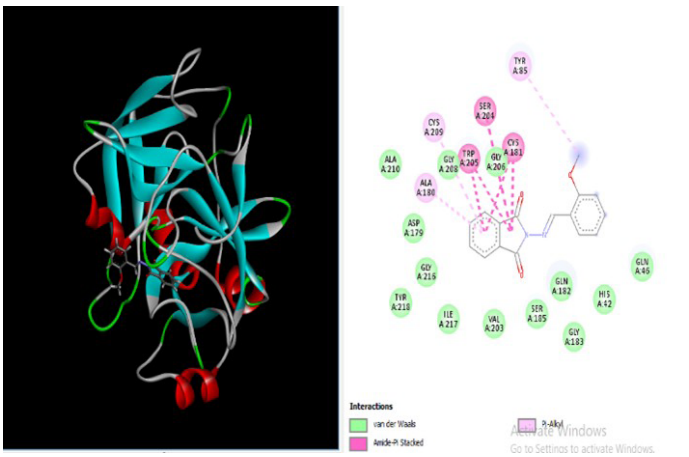


Figure 10. -2D and 3D compound M5, with Amino acids of (1FJS)

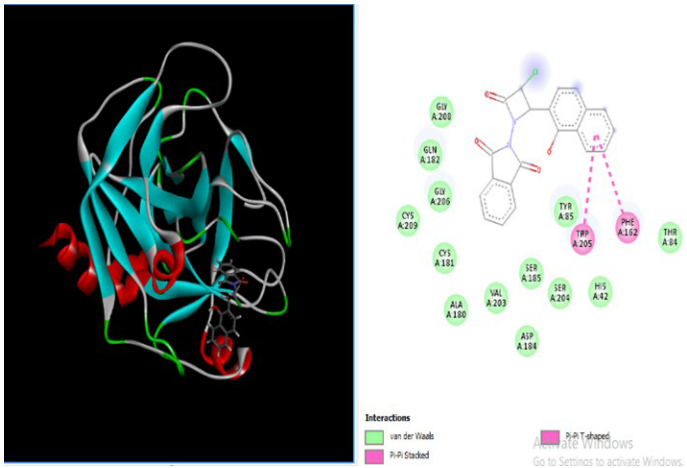


Figure 11. -2D and 3D compound A2, with Amino acids of (1FJS)

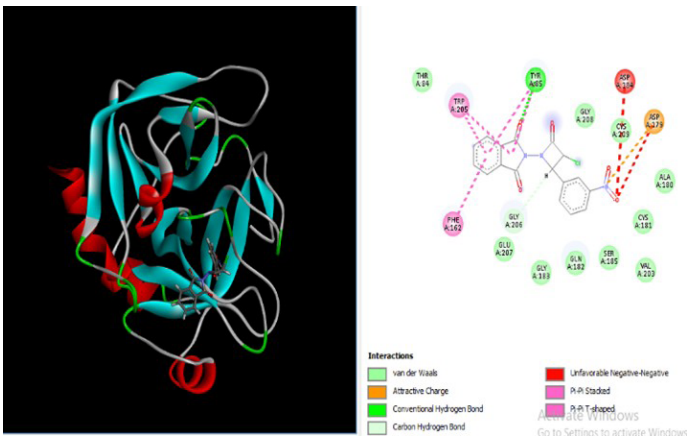


Figure 12. -2D and 3D compound A4, with Amino acids of (1FJS)

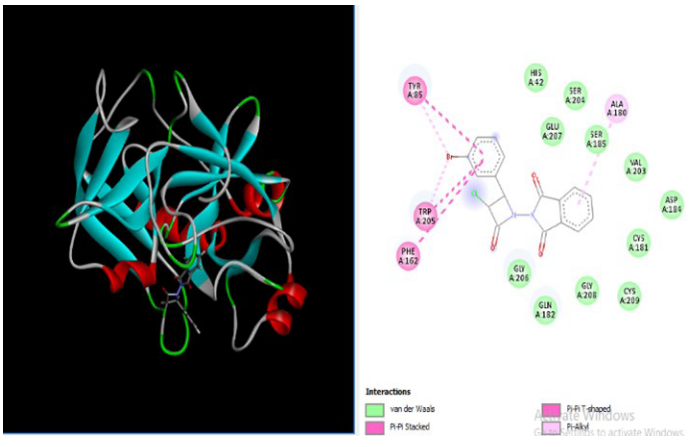


Figure 13. -2D and 3D compound A5, with Amino acids of (1FJS)

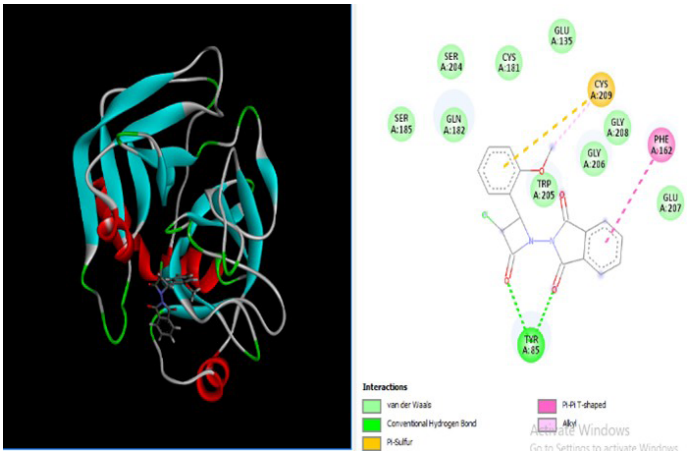


Figure 14. -2D and 3D compound A7, with Amino acids of (1FJS)

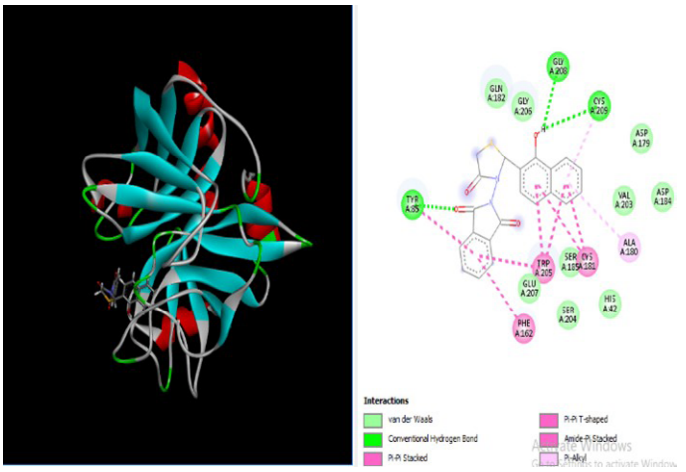


Figure 15. -2D and 3D compound S2, with Amino acids of (1FJS)

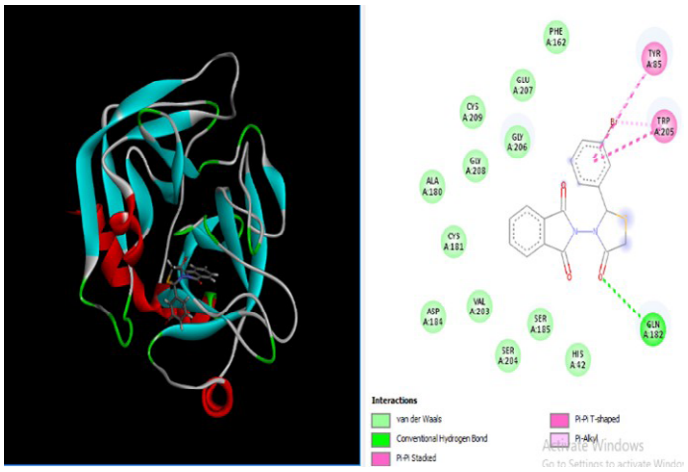


Figure 16. -2D and 3D compound S3, with Amino acids of (1FJS)

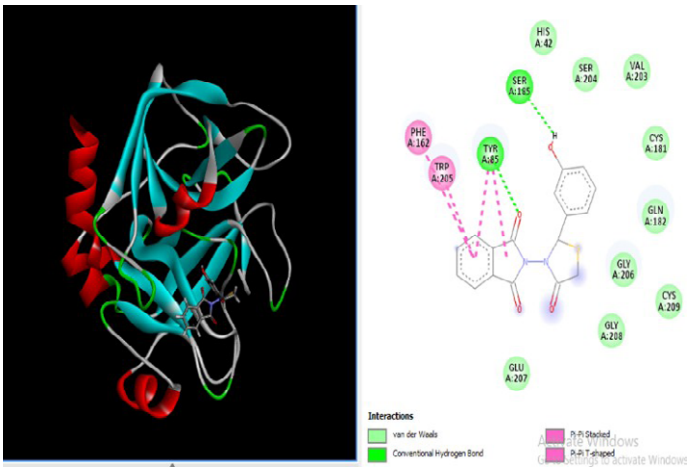


Figure 17. -2D and 3D compound S4, with Amino acids of (1FJS)

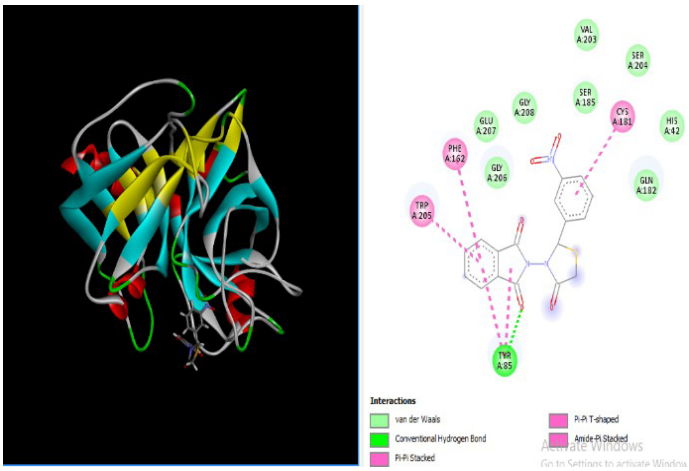


Figure 18. -2D and 3D compound S7, with Amino acids of (1FJS)

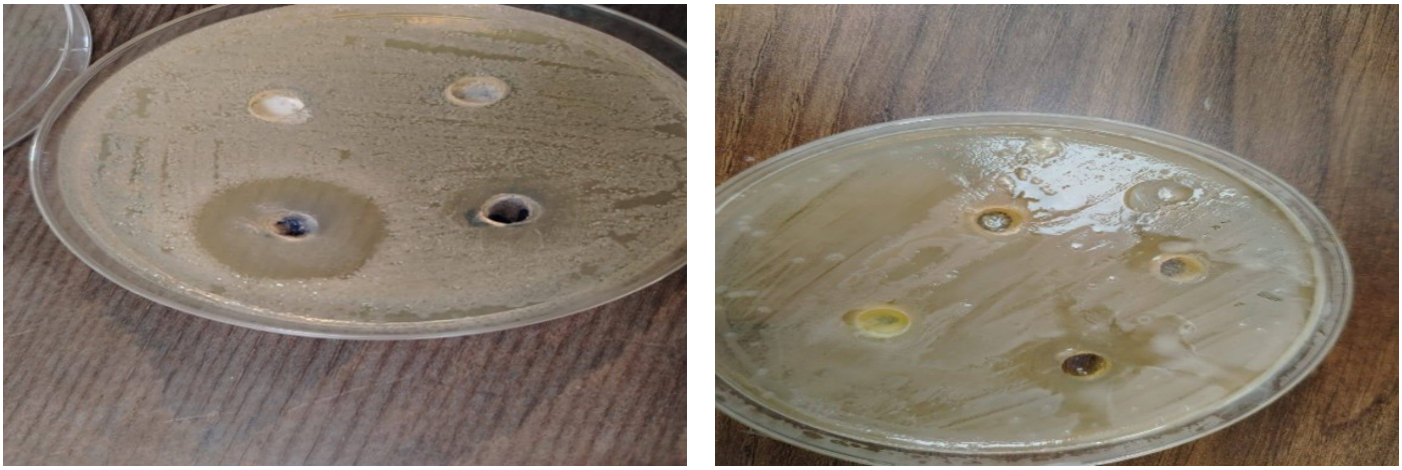


Figure 19. Antibacterial activity of compound A1 against Staphylococcus aureus and Klebsiella spp. using the agar well diffusion method.

4.1. Spectroscopic characterization

The proposed synthetic pathways for the preparation of Schiff bases and heterocyclic derivatives are illustrated in Scheme 1 to Scheme 4, which clearly depict the successive chemical reactions starting from N-aminophthalimide through condensation and subsequent cyclization reactions to afford the desired structures. These schemes support the synthetic strategy adopted in this study, providing a clear roadmap for compound generation.

The FT-IR spectra (Table 4) confirmed the presence of characteristic functional groups. Specifically, absorption bands attributed to the imine (C=N) groups appeared at 1601–1661 cm^{-1}, and those assigned to the carbonyl (C=O) groups appeared at 1711–1742 cm^{-1}, indicating successful formation of Schiff bases and heterocyclic derivatives.

^{1}H-NMR spectra (Table 5) displayed signals corresponding to aromatic and imine protons between δ 6.8–9.0 ppm. The ^{13}C-NMR spectra (Table 6) further confirmed the expected chemical environments, with carbon signals observed for C=N and C=O groups within δ 160–170 ppm. The ^{13}C-NMR spectrum of compound M5 is shown in Figure 1 as a representative example of the structural confirmation obtained for these compounds.

These spectroscopic findings corroborate the successful synthesis of the desired compounds, aligning well with previous literature on similar heterocyclic scaffolds (Ayala *et al.*, 2014; Halliwell & Gutteridge, 2015). The sequential appearance of these characteristic bands and chemical shifts validate the formation of the designed structures and support the synthetic claims presented in Schemes 1–4.

4.2. Molecular docking studies

Molecular docking was conducted to explore the interaction of the synthesized compounds with Coagulation Factor X (PDB ID: 1FJS). The binding affinities, summarized in Table 7, reveal that compound S2 exhibited the most favorable binding energy at −10.4 kcal/mol, whereas other compounds presented values between −7.7 and −9.3 kcal/mol. These results suggest reasonable potential for interaction with the active site of the enzyme.

The 2D and 3D docking interaction profiles are depicted in Figures 7–18, demonstrating hydrogen bonding and π-π stacking interactions within the Factor X active site. These molecular interactions are in good agreement with previous findings reporting the efficacy of nitrogen-containing heterocycles in targeting serine proteases (Kościuszko *et al.*, 2023; Pita *et al.*, 2023; Johnson *et al.*, 2014). The docking data provide a molecular rationale for the biological activities observed and further validate the pharmaceutical relevance of these structures.

4.3 Antibacterial activity

The synthesized compounds were evaluated for their antibacterial properties using the agar well diffusion method. Table 8 summarizes the inhibition zone diameters recorded against Gram-positive (Staphylococcus aureus) and Gram-negative (Klebsiella spp. and Escherichia coli) strains. Notably, compound A1 exhibited significant antibacterial activity, producing inhibition zones of 19 mm against Klebsiella spp. and 17 mm against Staphylococcus aureus. These results are visually documented in Figure 18, which clearly demonstrates the inhibition zones on agar plates.

The antibacterial activity observed correlates with literature findings where Schiff bases and heterocyclic compounds demonstrated membrane disruption and enzyme inhibition as mechanisms of action (Sies, 2020; Kumar & Gupta, 2021; Ayala *et al.*, 2014). The moderate activity against E. coli (inhibition zones of 10–12 mm) is consistent with the known resistance due to its robust outer membrane, while the higher efficacy against Gram-positive strains reflects better permeability and interaction potential.

4.4 Analytical perspective

The integration of synthetic, spectroscopic, molecular docking, and antibacterial data provides a comprehensive validation of the synthesized compounds. The combination of structural confirmation through IR, NMR, and elemental analyses (Tables 4–6; Figures 1, 3–6) with biological evaluations demonstrates the dual potential of these heterocycles as both enzyme inhibitors and antibacterial agents.

The proposed synthetic routes, as clearly illustrated in Schemes 1–4, facilitated the efficient preparation of structurally diverse heterocycles through environmentally friendly ultrasonic methods. These findings reinforce the significance of nitrogen-containing scaffolds in medicinal chemistry for the development of multifunctional bioactive agents (Pita *et al.*, 2023; Kościuszko *et al.*, 2023).

5. CONCLUSION

In this study, a series of Schiff bases and azo-heterocyclic derivatives were successfully synthesized from N-aminophthalimide using ultrasonic-assisted methods. The chemical structures were confirmed through FT-IR, ^{1}H-NMR, and ^{13}C-NMR analyses. Selected compounds, particularly S2, exhibited promising binding affinity toward Coagulation Factor X in molecular docking studies, suggesting potential anticoagulant properties. Additionally, compounds A1 and S2 demonstrated moderate to significant antibacterial activity against Gram-positive and Gram-negative bacteria. These findings highlight the potential of the synthesized compounds as dual-function agents for future pharmaceutical applications.

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