

Synthesis, Characterization, and Biological Evaluation of Heterocyclic Compounds with Potential Antimicrobial and Anticancer Activity

Ghufran Saadi Ekab^{1*}, Ibtisam Khalifa Jassim¹

¹ Department of Chemistry, College of Education for Pure Science ibn Al-Haitham, University of Baghdad, Baghdad, Iraq

ABSTRACT

Background: Heterocyclic compounds, including Schiff bases and tetrazoles, play a crucial role in medicinal chemistry due to their broad-spectrum biological activities. Tetrazole derivatives, in particular, have demonstrated significant antimicrobial, anti-inflammatory, antiviral, and anticancer properties, making them promising candidates for drug development. This study focuses on the synthesis, characterization, and biological evaluation of novel heterocyclic compounds derived from Schiff bases and tetrazole rings. **Methods:** Nine new heterocyclic compounds were synthesized, including Schiff bases (compounds 3–7) using aromatic aldehydes (p-chlorobenzaldehyde, N, N-dimethylaminobenzaldehyde, 3-nitrobenzaldehyde, and 4-nitrobenzaldehyde) in absolute ethanol. These Schiff bases were further converted into tetrazole derivatives (compounds 8–9). The synthesized compounds were characterized using melting point determination, Fourier-transform infrared (FT-IR) spectroscopy, and proton nuclear magnetic resonance (1H-NMR) spectroscopy. Thin-layer chromatography (TLC) was employed to confirm compound purity. **Results:** The synthesized compounds exhibited significant biological activity. Antimicrobial evaluation demonstrated potent inhibition zones at a concentration of 500 µg/mL, suggesting strong antibacterial and antifungal properties. Tetrazole derivatives, characterized by their five-membered ring structure containing four nitrogen atoms and one carbon, have been widely recognized for their therapeutic potential in treating inflammatory, viral, fungal, and metabolic disorders. **Conclusion:** This study successfully synthesized and characterized new heterocyclic compounds with promising biological activity. The findings suggest that Schiff base-derived tetrazoles have potential applications in the development of new antimicrobial and anti-inflammatory agents. Further investigations into their mechanism of action and pharmacological properties are warranted for future drug development.

Keywords: Heterocyclic compound, Schiff bases, tetrazole

INTRODUCTION

The heterocyclic compounds are considered a most important class because it has [1, 2] hetero atom for a unit at least. So, (N), (O), and (S) are considered the most employing hetero atoms. Also, heterocyclic compounds are very widely used in nature because it is essential to the life [1, 3-5]. A Schiff's base may be aldehyde or ketone that the carbonyl group (C=O) is substituted with imine group (C=N). It is made by condensation of an aldehyde or ketone with primary amines.

Most of Schiff's bases had antimicrobial, anti-inflammatory, analgesic and anticancer activities. M. Al-Kinani et. al., synthesized a new Schiff bases from amino thiadiazole and different aldehydes [6-9]. Tetrazoles are heterocyclic products which have a five-member ring including [5], and [1] carbon with [3] hydrogen atoms. Also, tetrazoles were used as anti-inflammatory, hypoglycemic, antiviral, antifungal and anticancer medicines [10]. Finnegan et.al., have improved the procedure for preparation of tetrazole from nitrile and DMF and using (NaN₃) and (NH₄Cl) [11-13].

Experimental Part:

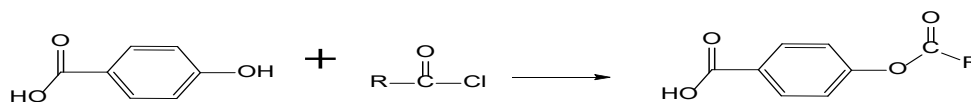
1 - The measuring of melting points is in degree centigrade and recorded by using Gallen kamp electric melting point type. 2-The

characterization of the prepared compounds was achieved through using FT-IR spectroscopy that determined on a SHIMADZU FT-IR 8300 spectrophotometer with the range of (4000 - 400) cm⁻¹, with KBr discs. 3-¹H-nmr spectra also, were used to identify the structures on fourier transformation bruker spectrometer, operating at (400MHz) with (DMSO-d₆), measurements were made Tahrn University. 4 - The reactions development was seen through using thin-layer chromatography (TLC) through Fertigfollen percolated sheets type Polygram Silg. 5-Antibacterial activity for prepared compounds was achieved in central service laboratory.

MATERIALS AND METHODS

Synthesis of Compound:

Naproxen (10 g, mole) and thionyl chloride (15 ml) was mixed and refluxed for 1.5 h with addition of drops of DMF. After 1.5 h the mixture cooled, p-hydroxy benzoic acid (g, mole) was dissolved in dry pyridine and DMF. then, acid chloride of naproxen mixed with the mix., and stirred about 24hrs, after that diluted with ice water. The precipitate then collected by filtration [14] ((Jaber and Jassim, 2019b).



R = naproxen

Figure 1: Synthesis of Compound

Synthesis for Product

Treating (0.029mole,10g) of compound 2-(6-methoxy naphthalene-2-yl) propionic acid with (0.032mole,3 g) thiosemicarbazide, (15ml) from POCl₃, after that refluxed it for 3hrs. then, diluted with (50 ml) from (H₂O) and after a period of time (1 hour).

Then cooled and filtered the result, and finally neutralized with sodium hydroxide and after that re-crystallized from ethanol and then filtered [15].

Synthesis for Schiff's Bases

A mixture of [2] (0.0019 mole) (0.75g) solved in ethanol (10ml) and some aromatic aldehydes(0.001 mole) solved in ethanol (10ml)

with (2) drops of (G.A.A) after that heated under reflux temperature about (5-6 hrs.) [16].

Synthesis of Tetrazole Compounds

For synthesis of tetrazole, Schiff base (4.07mol) was dissolved in tetrahydrofuran (THF) (25 ml) and (4.07mol) of (NaN₃) was refluxed at (60)^oC, about six (6) hrs. Then ,coold the mix., filtered and finally re-crystallized from ethanol [17].

Results and Discussion

Compound was synthesized from the reaction of naproxen after chlorinated it with *p*-hydroxy benzoic acid. The product was characterized from the presence of (C=O)[carbonyl]group of ester [18] in 1745cm⁻¹ .

Table 1: Some Characteristic Properties for Prepared Products.

Comp. No.	Molecular formula	Molecular Weight (gm/mole)	M.P. °C	Re-crystallized solvent	Color	Yield	RF
1	C ₂₀ H ₁₇ O ₃ CL	340.5	227-230	Ethanol	Brown	70	0.89
2	C ₂₂ H ₁₉ O ₃ N ₃	345	> 255	Ethyl Acetate	Yellow	65	0.88
3	C ₂₉ H ₂₂ O ₃ N ₃ CLS	527.5	127-130	Ethanol	Yellow	50	0.87
4	C ₂₃ H ₁₈ O ₅ N ₄ S	462	239-242	Benzene+Ethanol	Brown	70	0.9
5	C ₂₃ H ₁₈ O ₅ N ₄ S	462	207-209	Ethyl Acetate	Brown	80	0.88
6	C ₂₅ H ₂₄ O ₃ N ₄ S	436	172-174	Benzene+Ethanol	Brown	70	0.93
F	C ₂₉ H ₂₃ N ₇ O ₅ S	581.61	222-225	Methanol+Benzen	Yellow	4.6	0.65
G	C ₂₉ H ₂₃ N ₇ O ₅ S	581.61	227-229	Ethanol	Yellow	30	0.92

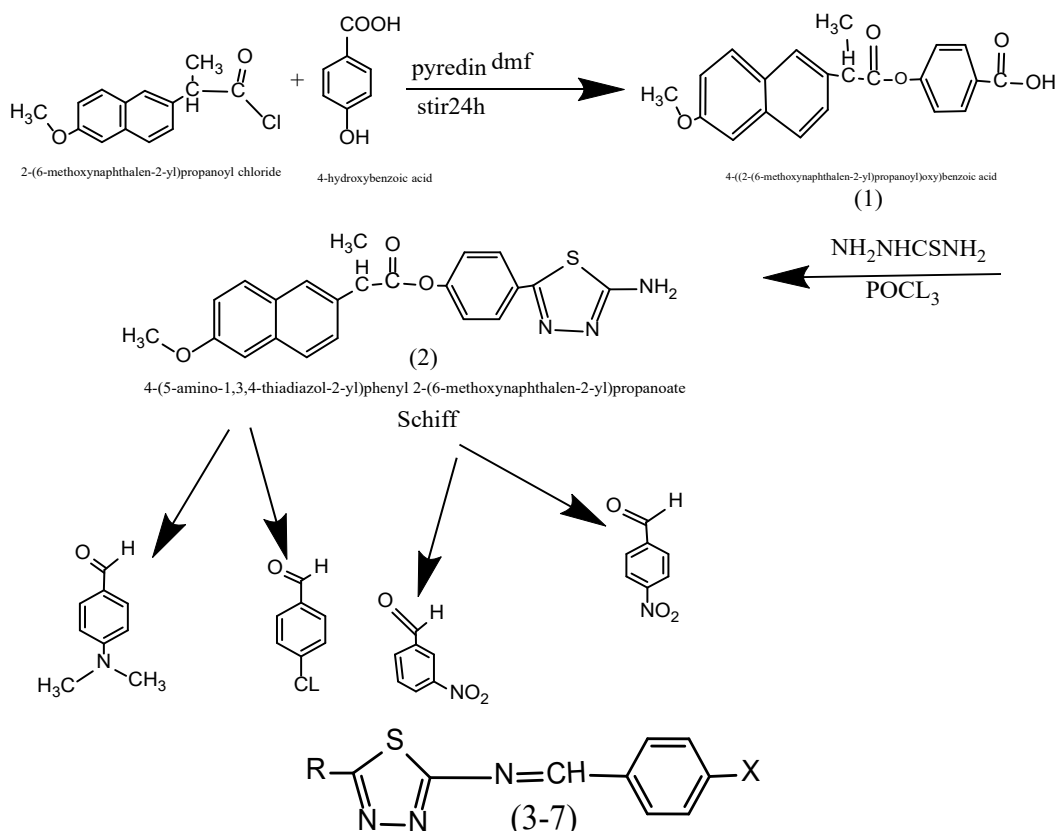


Figure 2: Some Characteristic Properties for Prepared Products

The structure of product was confirmed by recording the melting points and FT-IR, ¹H-NMR spectra. Thiadiazol compound was prepared from the reaction of carboxylic acid containing in Naproxen drug and thiosemicarbazide (NH₂NHCSNH₂) with POCl₃. The spectrum of FT-IR for aduct, which proved the presence of NH₂ stretching group

in (3566), band of (C=N) appeared at (1560,1602), band of (C-H) arom., appeared at (3074), band of (C-H) aliph. appeared at (2841,2935), and also, (C-S-C) band, asymmetrical and symmetrical was appeared at (1080) and (1112) cm⁻¹ respectively. Also, the ¹H-NMR was used to identified the resultants. So, the spectrum belongs to

product **2** shows an important shift, at (7.4-7.25) ppm attributed to the arom. protons appeared as multi signals. Also, the signal in 4.7 ppm is due to (O-CH₂-) group and at δ (1.9) attributed to the (-CH₃), besides the signal at δ (2.48) ppm for Methyl (CH₃) group, δ (9.5) ppm due to NH₂, and at δ (4.98) that for CHS.

Identification of New Schiff S Bases

The prepared compounds were synthesized through the reaction of compound and *p*-nitrobenzaldehyde, *p*-chlorobenzaldehyde, *m*-

methoxybenzaldehyde, *p*-N, N -dimethyl amino benzaldehyde, *m*-nitrobenzaldehyde in abs., ethanol to obtain imine. The reaction of these amines with the derivatives aromatic aldehydes considers the important method to get these targets of Schiff, s base. To characterize compound, the FT-IR spectrum showed the appearance of (C=N) group as a stretching band at (1593) cm⁻¹, besides the (C-H) for aliphatic protons at (2939,2972) cm⁻¹ and (C-H) for aromatic at (3096) cm⁻¹, also, for (C=C) in (1438,1463) cm⁻¹. Table (2)

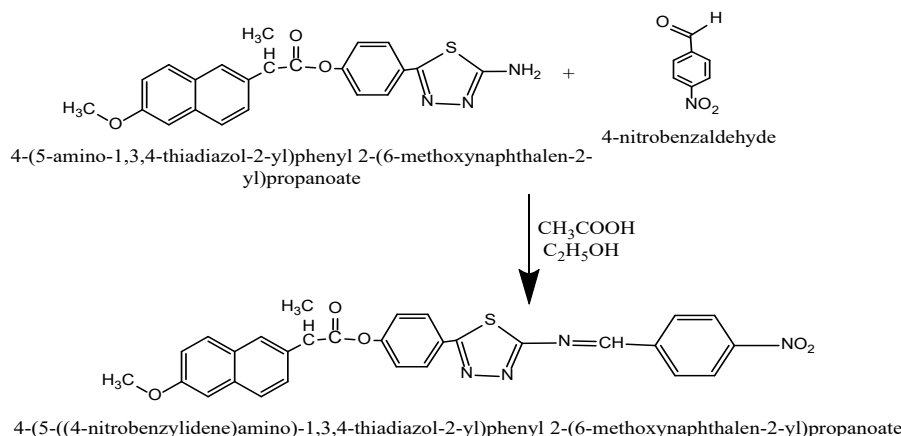


FIGURE 3: Identification of New Schiff, S Bases

Table 2: Characteristic (IR) Bands of Prepared Products (3-7):

Comp. No.	Changed part(x)	IR, KBr, ν , cm ⁻¹				
		(C-H) Ar.	(C-H) Aliph.	(C=N)	(C=C) Ar.	Others Bands
4	N(CH ₃) ₂	3065	2794,2918	1608	1508-1521	N-(CH ₃) ₂ 1166
5	NO ₂	3059	2937,2972	1598	1560-1598	C-NO ₂ 1344-1413
6	Cl	3057	2935,2972	1598	1622	690-715
7	OCH ₃	3053	2929,2975	1506	1602	1164

¹H-NMR spectrum of compound conformed [3] the appearance of the characteristic peaks;

Characterization of Tetrazole Compounds

The following scheme 2, including the reaction of Schiff's bases and sodium azide (NaN₃) to obtain new tetrazole rings. The characterization of Tetrazole [F, G] was confirmed through the FT-IR

and H-NMR spectra. Compounds [F, G] have prepared starting from condensation of Schiff bases [4,5] and (NaN₃) in (THF) as a solvent.

Also, for compound [F], the FT-IR spectrum has showed the stretching and at (1502) cm⁻¹ that due to (N=N) group for (tetrazole ring), also, band at (3201) cm⁻¹ which belonged to (NH) group. The mechanism for the new prepared tetrazole derivatives was shown below [19].

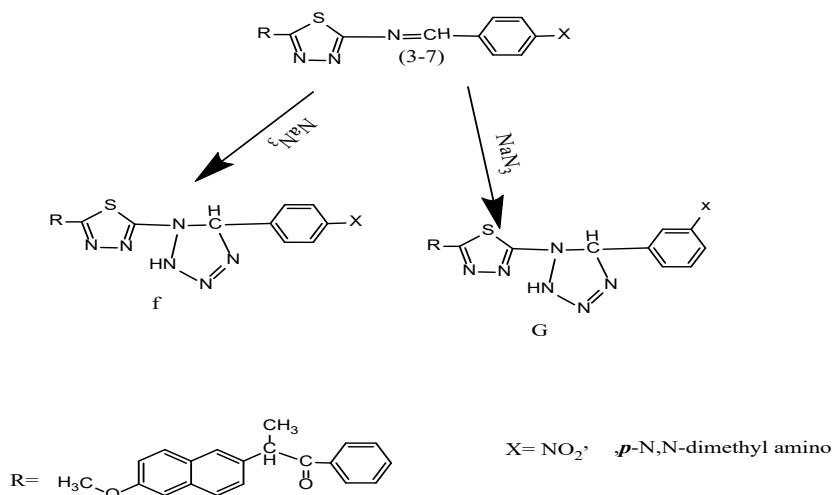


FIGURE 4: Characterization of Tetrazole Compounds

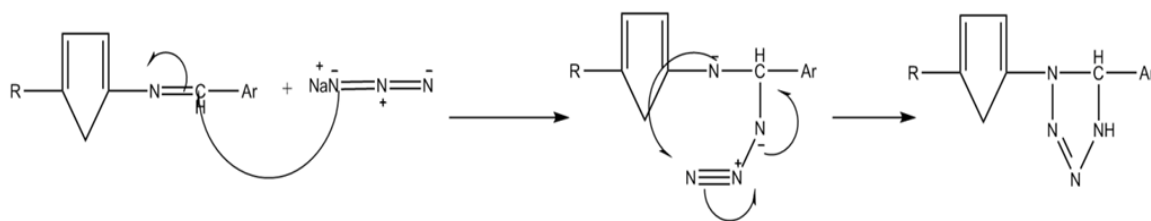


Figure 5: Compounds

Table 3: The characteristic (FTIR) bands of newly prepared compounds (F, G)

Comp. No	Changed part(x)	IR, KBr, ν , cm^{-1}					
		(C-H) Ar.	(C-H) Aliph.	(N-H)	(C=N)	(N=N)	Others Bands
F	NO_2	3070	2927, 2879	3201	1598	1502	C- NO_2 1460
G	$\text{N}(\text{CH}_3)_2$	3095	2929, 2875	3159	1598	1500	N-(CH_3) 1272

The ^1H -NMR spectrum due to product [F] has appeared the chemical shifts: at δ (7.54-7.31) which due to (aromatic protons), also, a signal at δ (4.97) ppm may be attributed to the ($\text{O}-\text{CH}_2$) group and besides the peak in δ (1.96) ppm belong to the ($-\text{CH}_3$) group, the single signal at δ (2.4) ppm due to CH_3 , at δ (9.55) ppm for NH, at δ (4.98) ppm for Ar-CH-NH at δ (5.05) ppm ($=\text{N}-\text{CH}$) and at δ (4.98) ppm for (CH_2) [20, 21].

Biological Activity

Antibacterial activity of prepared derivatives was tested (*in vitro*) against (20) four pathogenic strains: the chosen Bacteria are *S. aureus* (G+) and *E. coli* (G-), by using the appropriate diffusion method. So, the data showed that some of products have a good activity, appeared in Table 4, also, the imaging of the antibacterial activity (G+ and G-) that shown in (Figure 1).

 Table 4: Effects of (*S. aureus* G+), and (*E. coli* G-).

Sample no.	<i>S. aureus</i> (G+)	<i>E. coli</i> (G-)
17	8	12
18	8	12
1	8	12
12	16	12
19	12	12
23	8	12
15	10	14

Key: In active: (the inhibition zone < five mm) Highly active zone: (+++) (the inhibition zone > 20 mm) Moderately active: (++) (inhibition zone 11-20 mm).

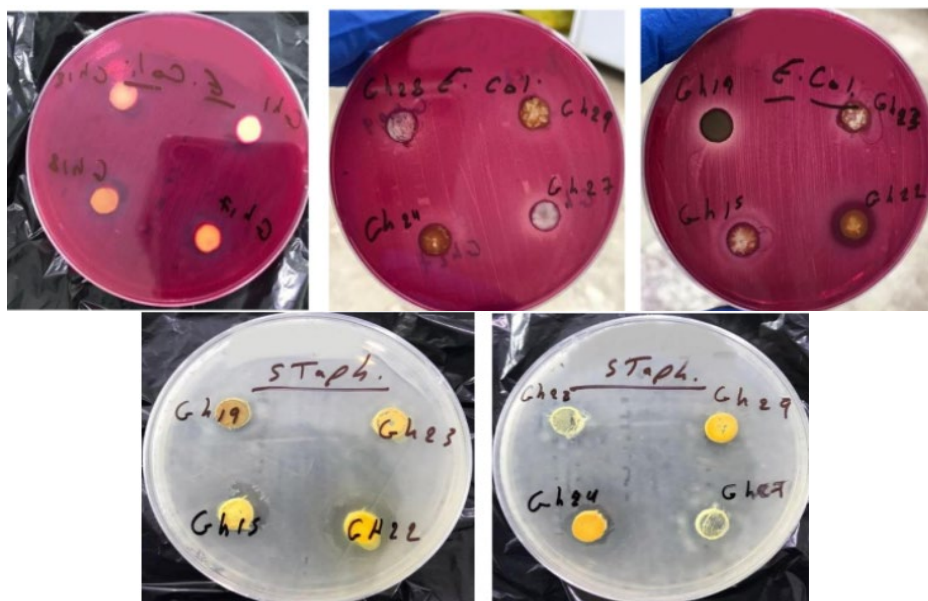


Figure 6: The Images of the Antibacterial Activity of (G+ and G-) Bacteria.

Conclusion

The prepared products were characterized using spectroscopic techniques (FT-IR and ^1H NMR and the melting points were

determined), also the structures also checked). Some of the products appeared a good results as antibacterial, so, the antibacterial studies showed that the new products caused activators effects.

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