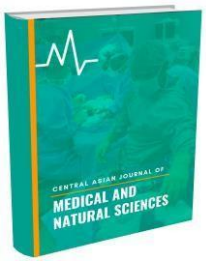

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Green Synthesis and Characterisation of New Hydroquinoline Derivatives by the Fusion Method, and Evaluation of Their Antibacterial Activity

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Abstract: In this study, A new solvent-free, and eco-friendly technique called the fusion method was used to create a number of compounds with a hydroquinazoline ring by reacting hydrazone with 2-aminobenzoic acid. Basic organic chemistry analyzes (FT-IR, ^{1}H, and ^{13}C-NMR) demonstrated a clear disappearance of azomethine, suggesting full consumption of the reactants, confirming the correctness of the synthesis. Additionally, antimicrobial activity was assessed against two common bacterial species: ***Escherichia coli*** and ***Staphylococcus aureus***. When compared to the antibiotic ***ampicillin***, which served as a control for inhibition, the molecule (M3) had the strongest antimicrobial activity against the bacterial strains employed in the investigation, and the results demonstrated that inhibition improved with increasing concentration. All things considered, these substances have encouraging potential for upcoming medicinal uses.

Keywords: Heterocyclic, Hydroquinazoline, Bacterial Bioactivity.

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1. Introduction

Small molecule synthesis has long piqued the curiosity of researchers, but a straightforward and effective way to construct the heterocyclic structures of hydroquinazole is still required [1]. Two nitrogen atoms and four carbon atoms, one of which is a carbonyl group, make up these six-membered rings [2]. These rings' highly electronegative carbonyl group, which has shown antibacterial [3], anticancer [4], and antifungal action [5], and their nitrogen atoms, which may form electron pairs, make them extremely active. Several traditional methods for creating these cyclic molecules in the lab have surfaced in recent decades. Green chemistry is a tactic that lessens chemical waste and environmental harm by using sustainable and eco-friendly reaction mechanisms [6]. These methods are predicated on a number of ideas, such as increasing reaction efficiency and reducing the usage of hazardous solvents. Green chemistry places a strong emphasis on using eco-friendly catalysts, safe solvents, and as little energy as possible during reactions. Microwave technology is one of the most sophisticated contemporary methods for synthesizing these chemicals [7]. Usually, this technique may increase efficiency and reduce reaction time. High temperatures are avoided and the reaction time is reduced by heating the compounds using the melting technique [8]. One of the numerous benefits of melting is that it speeds up the process and may be finished more quickly than traditional procedures [9]. Melting increases the process's chemical selectivity, which raises the degree

of selection and leads to higher output and better product purity. Additionally, melting drastically lowers energy use [10]. By lowering the production of hazardous chemical waste, waste reduction makes this strategy more ecologically friendly [11]. In conclusion, the aim of this study was to develop a new, cost-effective melting method for creating hydroquinazoline derivatives and to evaluate its bioavailability.

2. Materials and Methods

2.1. Materials

Reputable and industry-leading businesses including BDH, Aldrich, and FLUKA provided the research materials, which were used without further screening.

2.2. Preparation of hydroquinazoline derivatives (M_1-M_5) [12]

In a heat-resistant flask, 0.001 mol of neutral hydrazones (0.001 mol) was homogenized with 2-aminobenzoic acid and heated for 30 minutes with continuous stirring. As shown in Table 1, the flask containing the solution was dried, collected, and recrystallized with ethanol.

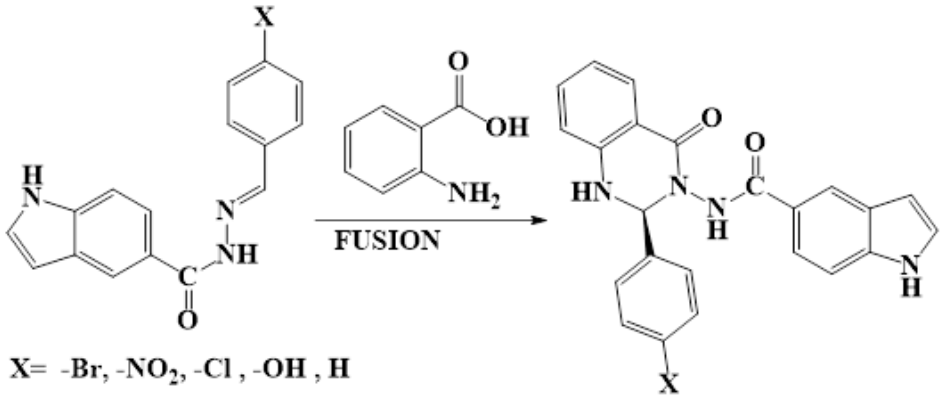

Figure 1. Synthesis of Hydroquinazoline Derivatives by Fusion.

Table 1. Information on the useful aspects of synthetic substances(M1-M5).

Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
M1	Br	$C_{23}H_{16}BrN_4O_2$	264-266	72	Yellow
M2	NO_2	$C_{23}H_{16}N_5O_4$	276-278	70	Light Brown
M3	Cl	$C_{23}H_{16}ClN_4O_2$	243-245	68	Red
M4	OH	$C_{23}H_{18}N_4O_3$	249-251	73	Light Yellow
M5	H	$C_{23}H_{18}N_4O_2$	235-237	75	White

2.3. Evaluation of bacterial bioactivity

Due to the fact that they are prevalent and easy to acquire via the Biology Laboratories of Tikrit University, we have taken two of the most common bacteria types, which grow mostly in soil and water, such as Staphylococcus aureus, which is a type of positive bacteria, and Escherichia coli, which is a type of negative bacteria. Firstly, the growth medium of these bacteria, Mueller-Hinton agar, was made by dissolving 40 grams of this solution in one liter of distilled water [13], [14], [15]. This medium was sterilized by heating it up to the temperature of 121°C under the pressure of 1.5 bar in an autoclave; after that, the solution was cooled down and poured in Petri dishes at normal temperature. These dishes were then contaminated with bacteria that we acquired in three different ways in order to make sure that the bacteria are evenly distributed, and then the dish was

pricked in three holes, which are 1mm in diameter [16], [17], [18].The chemical solutions were made by continuously adding DMSO solvent at three different concentrations (0.01, 0.0001, and 0.001) mg/ml. After that, each concentration was added to a well, and three distinct concentrations were added to one plate. After that, the plates were incubated for a whole day at 37°C in a dedicated incubator. The inhibition's diameter was measured in millimeters [19].

3. Results and Discussion

3.1. Characterization of hydroquinazoline derivatives (M1-M5)

When the tests were conducted, starting with FT-IR (Fourier transform infrared spectroscopy), it was clear that the azomethine bond had disappeared, meaning that the material had undergone reactions. New bands had been created in the spectrums showing the creation of a new material. This material created new bands similar to the (NH) bond, which was localized at (3341–3271) cm^{-1}. In addition, some new bonds that were absent before were created, such as the aliphatic bond, localized at (2950, 2852) cm^{-1}. Another characteristic of the new compound was the presence of the carbonyl bond of the hydroquinazoline compound at (1687–1656) cm^{-1}. Finally, the other remaining bonds retained their original positions without being affected [20], [21]. as shown in Table 2 and Figures 1 and 2.

Table 2. Metadata of the FT-IR spectrum for compounds (M1-M5).

Comp. No.	R	ν(NH) Hydroquinazol	ν(N-H)	ν(C-H) Arom	ν(C-H) Aliph.	ν(C=C) Arom.	ν(C=O)	Others
M1	Br	3290	3213	3050	2943,2871	1556,1493	1674	ν (C-Br)656
M2	NO_2	3341	3269	3064	2917,2874	1563,1482	1656	ν (NO)1342,1517
M3	Cl	3288	3191	3055	2950,2858	1564,1483	1687	ν (C-Cl)772
M4	OH	3271	3217	3039	2933,2852	1575,1479	1664	ν (OH)3429
M5	H	3312	3234	3028	2946,2879	1572,1486	1675	--

^{1}H-NMR also proved the absence of hydrogen, which is normally associated with azomethine group, together with signals that correspond to the formation of products after confirmation of the consumption of reactants. The signal that appeared was 6.46 ppm, which corresponds to the presence of NH group of the target ring. This is not where the information ends as another signal was detected at 5.90 ppm, which corresponded to CH group of the target ring. These signals correspond to triazole. The other groups, including the benzene ring, were still on their places at 7.17-8.39 ppm. Besides, NH groups were detected at 10.27 and 11.32 ppm [22]. As in Figure 4.

The use of Carbon-13 nuclear magnetic resonance spectroscopy further revealed that the azomethine has disappeared, while the signals showed the formation of the desired ring structure, hydroquinazoline. The signal at 84.35 ppmrepresented the carbon atom (CH) of the desired ring structure, while the signal at 166.05 ppm showed the formation of the carbonyl in the desired ring structure. The other atoms remained in the same position, with slight changes. The benzene signals were observed between 103.36 and 157.24 ppm, while the carbonyl hydrazide signal was observed at 168.98 ppm. As in Figure 5.

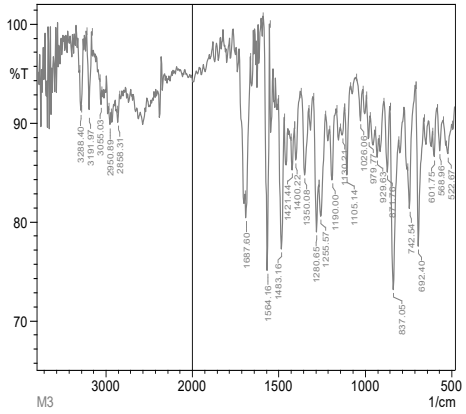

Figure 2. FT-IR OF (M3).

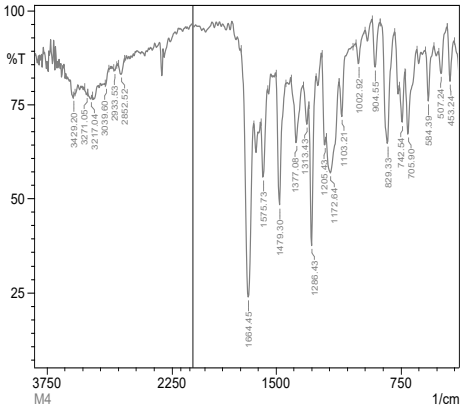

Figure 3. FT-IR OF (M4).

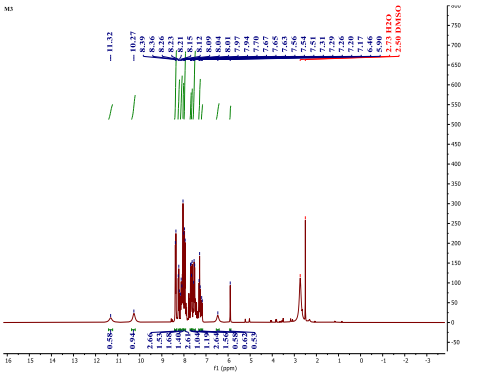

Figure 4. [1]H-NMR OF (M3).

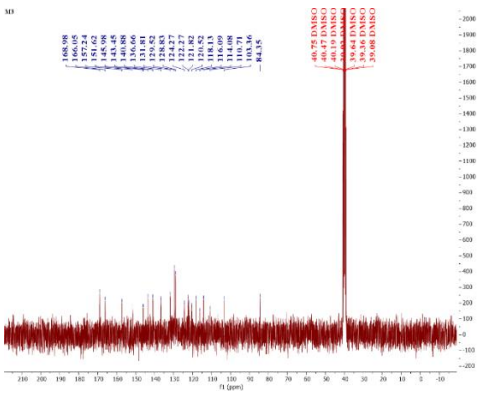

Figure 5. [13]C-NMR OF (M3).

3.2. Bacterial susceptibility to prepared compounds

At three distinct doses, the produced compounds showed different inhibitory effectiveness against the target microorganisms, according to the findings in Table 3. The capacity of the produced compounds to prevent the development of these microorganisms on bacterial culture plates improved with increasing concentration [23], [24]. Generally speaking, the substances worked better against Gram-positive bacteria than Gram-negative ones. This is explained by the fact that Gram-positive bacteria do not have an exterior barrier that prevents chemicals from diffusing into their cells, whereas Gram-negative bacteria do [25]. As far as the bacterial species are concerned, compound M3 exhibited maximum activity against Gram-positive bacteria up to 22mm diameter at the highest concentration. Its activity remained high at intermediate and low concentrations. Even though its activity was not on par with the activity of antibiotics, still it remained the most promising compound as a future antibiotic under different conditions. The same compound exhibited maximum activity at 15mm diameter [26]. As far as Gram-negative bacteria are concerned, activity is comparable for all concentrations and is almost non-existent for low concentration in some microorganisms. Compounds (M1, M2, and M3) exhibited higher activity than (M4, M5) compounds because of their higher pull groups, which increase the activity by penetrating into the cell membrane, compared to compounds with low activity [27].

Table 3. Data concerning the effectiveness of compounds (M1-M5) against microbes.

Comp	E.coli mg/ml			Staph. aureus mg/ml		
No.	0.01	0.001	0.0001	0.01	0.001	0.0001
M1	12	6	0	17	12	5
M2	8	5	5	20	15	10
M3	15	10	5	22	15	10
M4	7	4	0	10	5	0
M5	9	6	3	14	9	6
amepicillin	20	15	15	35	30	25

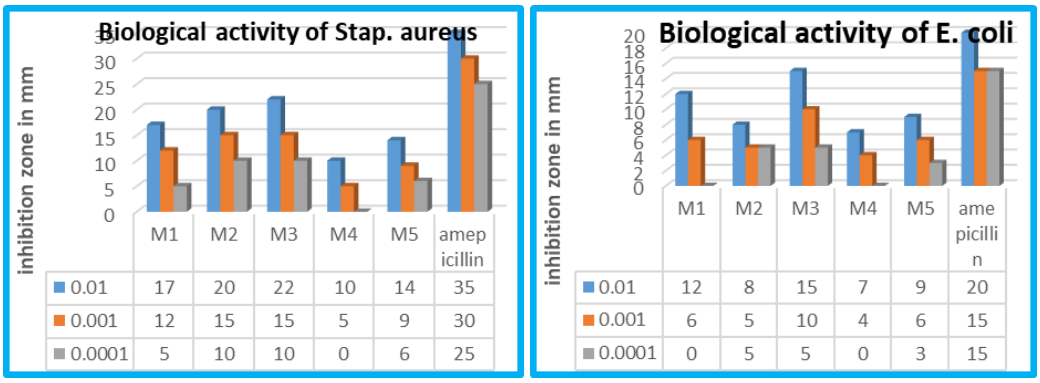

Figure 6. Information on how well substances (M1–M5) work against microorganisms.

4. Conclusion

In addition to being economical and solvent-free, using an eco-friendly process speeds up the synthesis of the chemicals, saving money. Along with excellent product purity, it also provides a simple and safe method. An azomethine molecule in hydrazone combines with 2-aminobenzoic acid to generate the hydroquinazole major rings. High purity was demonstrated by the resultant compounds, especially in nuclear magnetic resonance and infrared spectra, which showed full reactant absorption and the presence of distinctive groups suggestive of the target ring. Additionally, these substances showed

excellent action against *Staphylococcus aureus* and moderate activity against *Escherichia coli*, with activity rising with concentration. In particular, compound M3 shown encouraging action, suggesting that it may one day be used as an antibacterial agent.

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