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Antibacterial activity estimation of new pyrazole compounds

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Abstract

A series of modern compounds N,N'-(1,4-phenylene) bis (1-(1,3- diphenyl-1H-pyrazol-4-yl) methanimine from 5a to 5e were synthesized and characterized through methods were applied to enhance the structures for the new compounds Mass spectrometry, infrared and nuclear magnetic resonance. These modern compounds have antimicrobial activity estimated against bacteria and fungi, involved both Gram-positive and Gram-negative. Specific substitutions of the pyrazole rings were conclusive in enhancing the antibacterial activity. According to these results, hybrid compounds of pyrazole showed promising potential as potential candidates for the creation of new antimicrobial drugs.

Keywords: Antibacterial Activity; Characterization, pyrazole; Schiff`s bases.

Introduction

The modern pyrazole compounds play a necessary role in different pharmacological activity[1]. Some drugs involving patented ones are evolved from the pyrazole derivatives of five-membered ring[2]. The unique chemical action of pyrazoles is attached to the pharmacological properties of their nuclei; pyrazoles contain a nitrogen atom 1 (N_1), which is called "pyrrole-like" because its unshared electrons are paired with the aromatic system, and a nitrogen atom 2 (N_2), which is called "pyridine-like" because its unshared electrons are not affected by resonance, just like pyridine systems[3] as shown in Fig. 1. Thus, pyrazoles can react with both bases and acid. Pharmacological activity involving anti-inflammatory[4], anticancer[5], analgesic[6], antiviral[7], antibacterial[8] and antifungal[8]. Pyrazole derivatives provide a special chance to create drugs with improved microbial activity[9]. Microbial infections reason widespread and dangerous diseases[10]. To inhibit these dangerous medical issues, the detection of new kinds of antibacterial and antifungal agents is a very significant function[11]. In recent years, the researchers have important on assists modern antimicrobial agents[12], which may be work during prepare modern structures and targets, and controlling on the problem of obtained resistance. Drug-resistant strains of bacteria have become a serious global health concern[13]. Activation and overcoming resistance mechanisms is a viable approach to creating drugs[14]. In this study, we synthesized a novel heterocyclic

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derivative containing pyrazole molecules, which may lead to more effective biological actions in order to help create new therapeutic agents for the treatment of microbial infections. The following work provides comprehensive explanations of the synthetic procedures, spectroscopic characterization, and antimicrobial evaluation of the recently synthesized pyrazole derivatives.

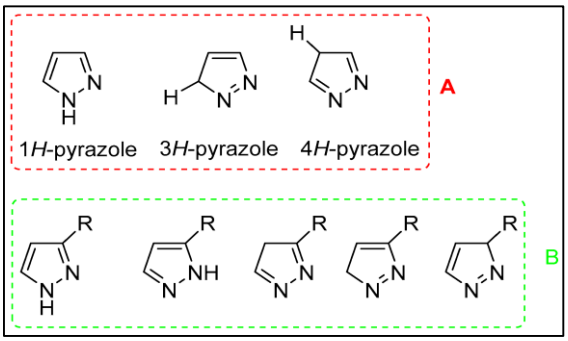
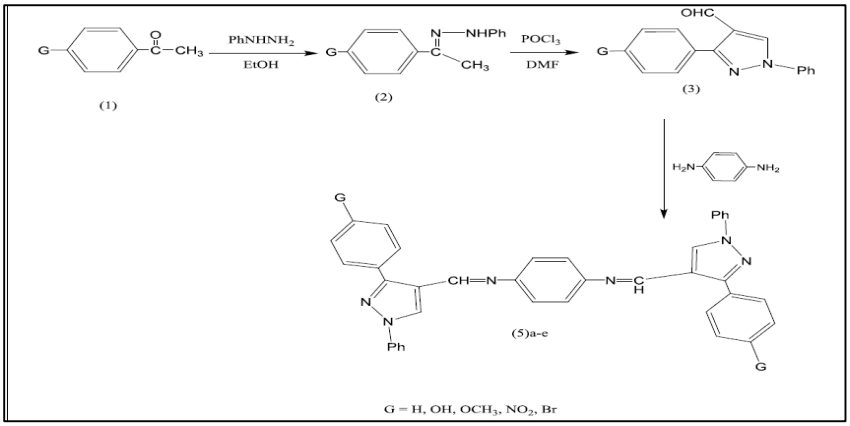


Figure (1). (A) Unsubstituted pyrazoles tautomerism. (B) Mono-substituted pyrazoles tautomerism.



Scheme 1. Synthetic path of N,N'-(1,4-phenylene)bis(1-(1,3- diphenyl-1H-pyrazol-4-yl)methanimine 5a – 5e.

Materials and Methods

All substance utilizing in the study was origin from diverse companies, involving Merck and Fluka.

General Procedure for the Synthesis of Phenyl Hydrazone Derivatives 2a–e[15].

A combination of substituted acetophenones 1a-e (1 mol), phenyl hydrazine (1 mol), and acetic acid (1 mL) was refluxed in ethanol (20 mL) for Half an hour. The mixture was cooled to room temperature and through TLC determined complete the reaction. In final, recrystallized utilizing absolute ethanol. Table 1 offers the physical information for compound 2a-e.

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Table (1). Physical information of compounds 2a–e.

Compound	Color	m.p. (◦C)	*Rf* Value/Solvent System (Hexanes: Ethyl Acetate)	Yield (%)
2a	reddish orange	133-134	0.1/6:4	90
2b	Yellow	146-148	0.15/6:4	75
2c	Yellowish green	170-172	0.18/6:4	80
2d	Yellowish green	98-100	0.1/6:4	80
2e	Yellowish green	210-212	0.16 / 6:4	70

General Procedure for the Synthesis of 1-Phenyl-3-(substituted -phenyl)-1H-pyrazole-4-carbaldehydes 3a–e[16].

The product of 2a-2e combination with cooled to (0 ˚C) DMF solution (12 mL), add 6 mL of $POCl_3$ dropwise for One hour. After that the reaction was heated at 65-70 ˚C for two hours. In finally, after heated the product is solidify and then filtrated washed with Na_2CO_3 (5%, 30 mL) and water, then recrystallized from the DMF-ethanol combination. Table 2 contains physical information for compound 3a-e.

Table (2). Physical information of compounds 3a–e.

Compound	Color	m.p. (˚C)	*Rf* Value/Solvent System (Hexanes: Ethyl Acetate)	Yield (%)
3a	Orange	136-138	0.27/7:3	75
3b	Yellow	148-150	0.31/7:3	77
3c	Yellow	161-163	0.2/7:3	82
3d	Yellow	168-170	0.24/7:3	74
3e	Pale green	189-191	0.25/ 7:3	69

General Procedure for the Synthesis of *N,N'-(1,4-phenylene)bis(1-(1,3-diphenyl-1H-pyrazol-4-yl)methanimine)5a-e*[17].

The combination between 3a-e (0.01 mole, 2.64 gm) and *p*-phenelyendiamine (0.02 mole, 0.54 gm) respectively was dissolved in 10 ml absolute ethanol to give mixture of dark brown. Then the mixture was added Three drops glacial acetic acid. The consequence mixture of components was warming under temperature reflux Five hours. The components were reacted then enable to coldish

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minimum to room temperature. The product was purified through filtered and washed through water, recrystallized from ethanol to give the product as reddish orange. The product was filtered and washed with water, recrystallized from ethanol to give the product. The physical information of synthesized compounds is listed in Table 3.

Table (3). Physical information of compounds 5a–e.

Compound	Color	m.p. (°C)	*Rf* Value/Solvent System (Hexanes: Ethyl Acetate)	Yield (%)
5a	Reddish Orange	182-184	0.1 / 6:4	56
5b	Yellow	140-143	0.15 / 6:4	74
5c	Yellow	182-185	0.16 / 6:4	81
5d	Orange	100-112	0.14 / 6:4	70
5e	Yellow	230-232	0.15 / 6:4	65

Results and Discussion

The synthesis procedure involves the reaction of two moles of pyrazole-4-carbaldehydes derivatives with 4-phenylene diamine. The synthesized compounds 5a-e were characterized by FT-IR spectroscopy. Fig. 2 offers the FT-IR spectrum of compound (5a); which shows the appearance of the (CH = N) in 1624 cm^{-1}. Bands at 3029, 1594, 835 cm^{-1} that attributed to (C – H) aromatic, (C = C) stretching and out of plane bending of *para*-disubstituted benzene ring. The other spectral data for series 5a-e show in table 4.

Table (4): FT-IR spectral data of compounds 5a-e.

Comp. No.	**Alkyl Group**	**FT-IR Spectral data, cm^{-1}**				
		ν CH = N	**ν C-H (Aromatic)**	**ν C = C**	**γ-disubstituted**	**Others**
5a	H	1624	3029	1594	835	-
5b	OH	1622	3026	1602	829	3376 (-OH)
5c	OCH_3	1622	3043	1602	824	1149 (C – O)
5d	NO_2	1618	3048	1604	830	1535, 1368 (NO_2)
5e	Br	1616	3069	1598	841	-

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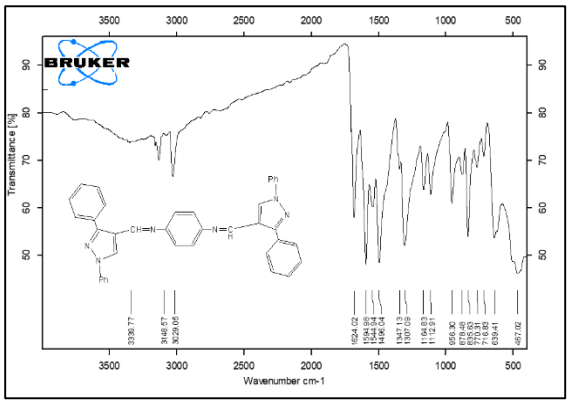

Figure (2). FT-IR spectrum of (5a).

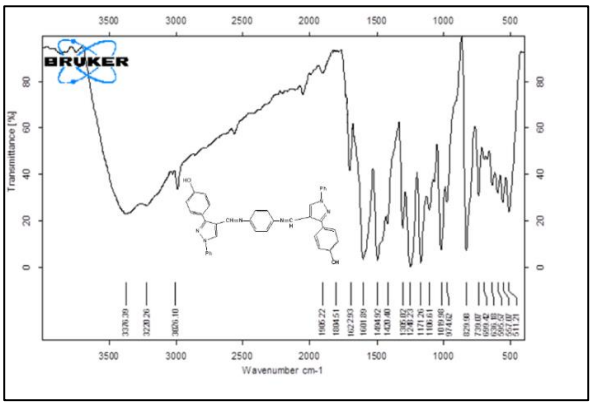

Figure (3). FT-IR spectrum of (5b).

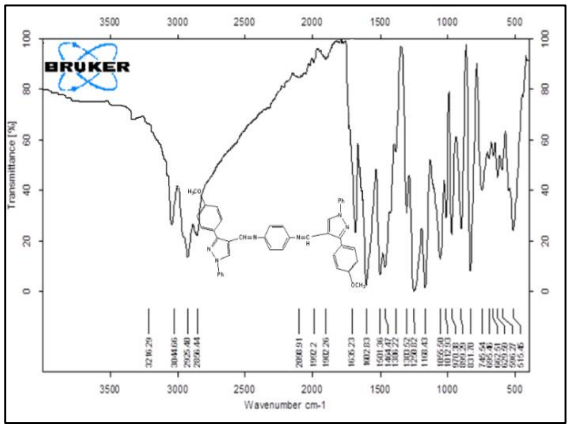

Figure (4). FT-IR spectrum of (5c)

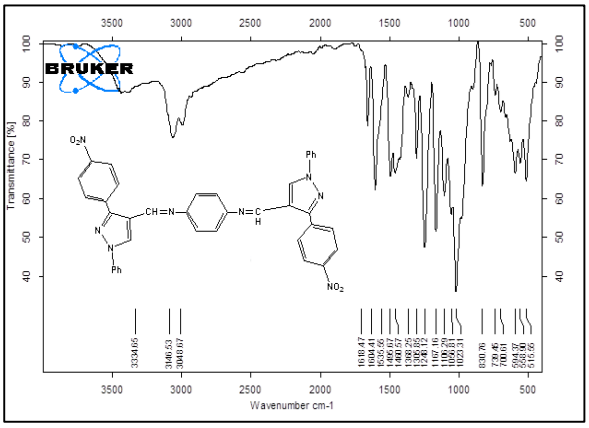

Figure (5). FT-IR spectrum of (5d).

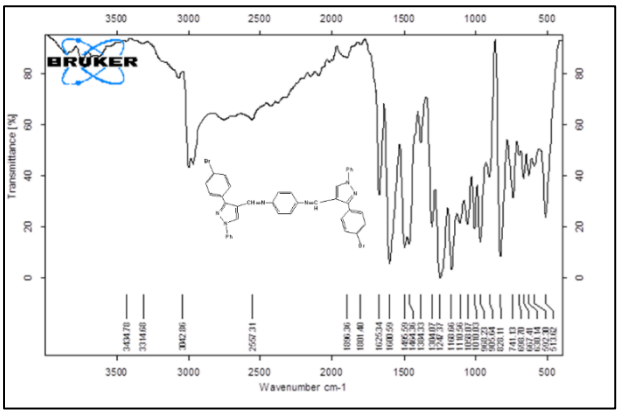

Figure (6). FT-IR spectrum of (5e).

The ^{1}H-NMR spectrum of chemical 5a in Fig. 7 displays the following characteristics (DMSO-*d6*, ppm): 8.3 (s, 2H, CH=N), 9.4 (s, 2H, pyrazolyl-H), 6.98–7.10 (m, 10H, Ar-H, Phenyl ring), 7.3-7.31 (d, 10H), 7.48- 7.34 (d, 4H)[18]. Table 5 provides an explanation of the ^{1}H-NMR spectra for compounds 5a-5e.

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Table (5): ^{1}HNMR spectral data (δ ppm) for compounds 5a-e.

Com. No.	Formula	^{1}H-NMR spectral data (δ ppm)
5a	$C_{38}H_{28}N_4$	9.4 (s, 2H, pyrazolyl-H), 8.3 (s, 2H, CH=N), 6.98-7.10 (m, 10H, Ar-H, Phenyl ring), 7.48-7.34 (s, 4H, Ar-H), 7.13-7.32 (s, 10H Ar-H), (2.5) singlet for DMSD-*d6* solvent.
5b	$C_{38}H_{28}N_4O$	10.71 (s, 2H, phenolic OH), 8.8 (s, 2H, pyrazolyl-H), 8.37 (s, 2H, CH=N), 7.99-8.17–8.17 (m, 10H, Ar-H, Phenyl ring), 7.59-7.61 (s, 4H, Ar-H), 7.36-7.38 (s, 10H Ar-H), (2.5) singlet for DMSD-*d6* solvent.
5c	$C_{39}H_{32}N_4O$	8.87 (s, 2H, pyrazolyl-H), 8.46 (s, 2H, CH=N), 7.94–8.00 (m, 10H, Ar-H, Phenyl ring), 7.82-7.89 (s, 4H, Ar-H), 7.72-7.78 (s, 10H Ar-H), (2.5) singlet for DMSD-*d6* solvent.
5d	$C_{38}H_{26}N_6O_4$	9.10-9.13 (s, 2H, pyrazolyl-H), 8.37 (s, 2H, CH=N), 8.01–8.17 (m, 10H, Ar-H, Phenyl ring), 7.36-7.99 (s, 4H, Ar-H), 6.52-6.54 (s, 10H Ar-H), (2.5) singlet for DMSD-*d6* solvent.
5e	$C_{38}H_{28}Br_2N_4$	9.05-9.10 (s, 2H, pyrazolyl-H), 8.30-8.37 (s, 2H, CH=N), 8.01–8.17 (m, 10H, Ar-H, Phenyl ring), 7.59-7.99 (s, 4H, Ar-H), 6.52-7.38 (s, 10H Ar-H), (2.5) singlet for DMSD-*d6* solvent.

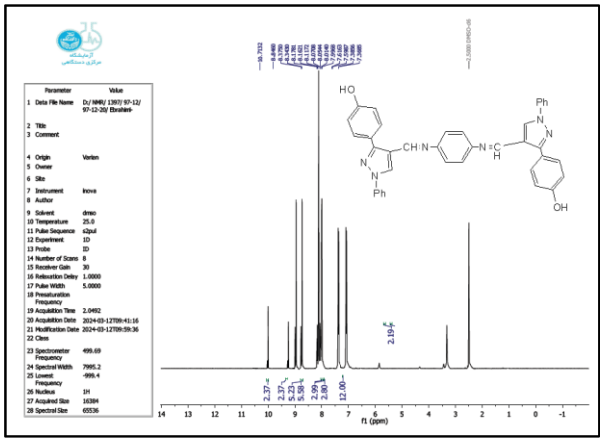

Figure (7). 1HNMR spectrum of compound 5a.

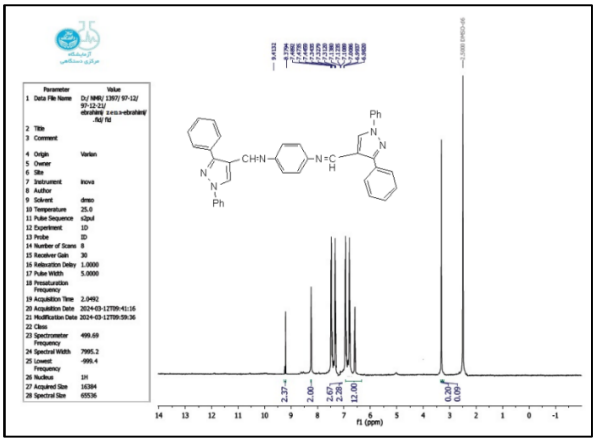

Figure (8). 1HNMR spectrum of compound 5b.

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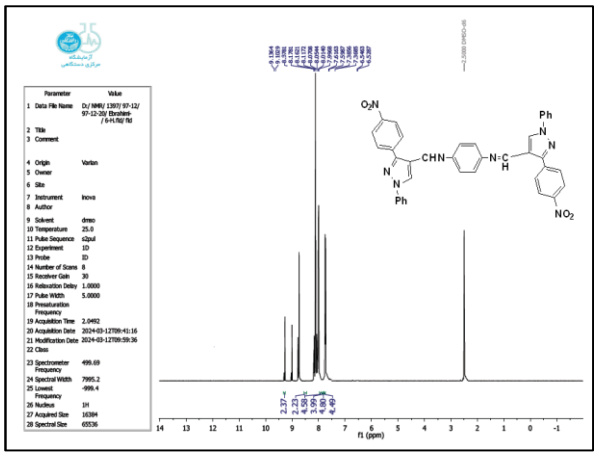

Figure (9). ^{1}HNMR spectrum of compound 5c

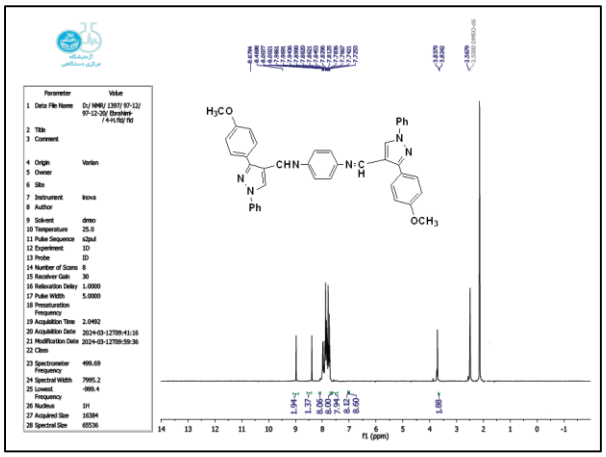

Figure (10). ^{1}HNMR spectrum of compound 5d.

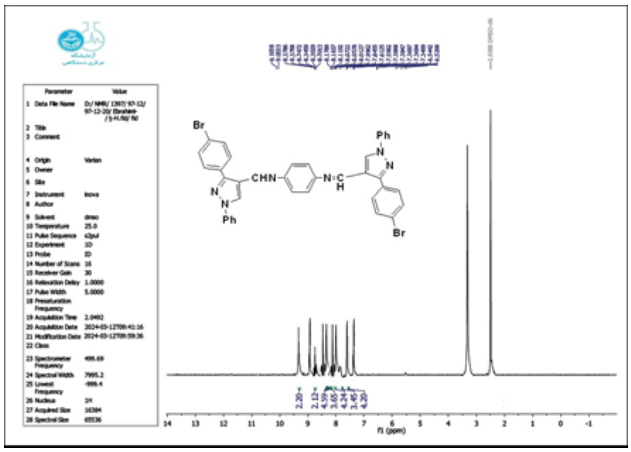

Figure (11). ^{1}HNMR spectrum of compound 5e.

Further chemical proof for pyrazole derivatives was obtained from its mass spectroscopy. The mass spectrum of compound 5a, Fig. 12, gives a strong intense molecular ion at m/z 540, which corresponds to the molecular weight of the structure assigned to 5a. the most informative bands, due to fragmentation as below:

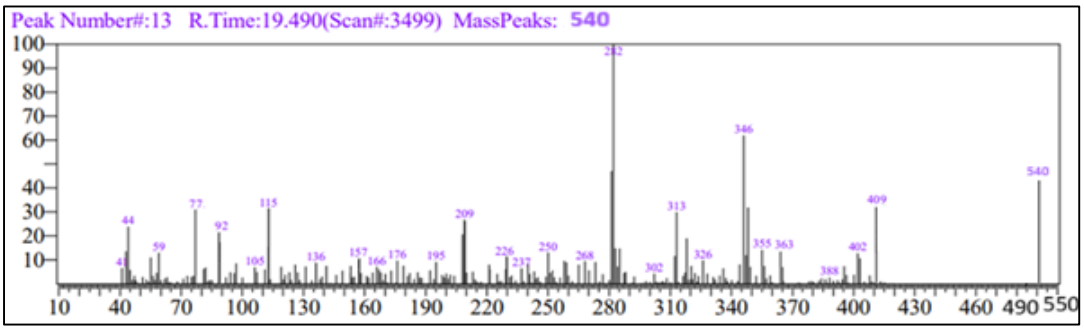

Figure (12). Mass spectrum of compound 5e.

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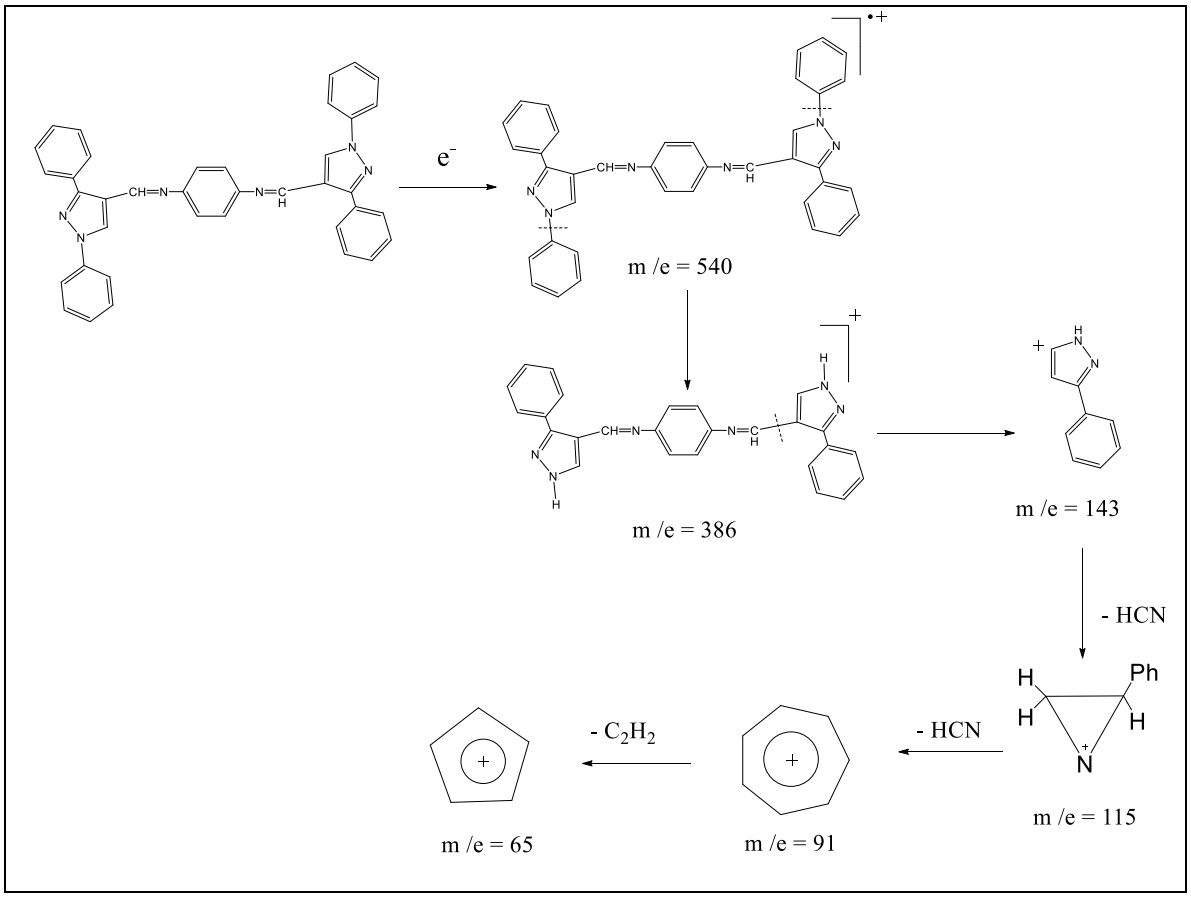
The ion peak of molecular gives m/z= 540, the fragments with m/z 409 was created on loss of ammonia (NH_3) from the molecular ion, while in the next step the fragment with m/z 364 on cleavage of methyl but-2-enoate moiety resulted in the formation of m/z 282. There onwards a parallel pathway was initiated from the fragment with m/z 295, here a direct loss of methoxy ethane entity led to the formation of a daughter ion with m/z 221, while loss of 1-chloro-2-methyl benzene and propionic acid moieties resulted in the created of the last daughter ion with m/z 105. Finally, the fragment with m/z 221 underwent cleavage of hydrogen chloride entity and resulted in the formation of ion with m/z 112 and 77[19]. The mass fragmentation pattern of 5a is shown in Scheme 2.



Scheme (2): Fragmentation pattern of 5a

Biological activity:

The disk diffusion method was used to test the antibacterial activity of most of the targeted compounds against one gram-negative bacteria *(E. coli)* and one gram-positive bacteria *(S. aureus).* DMSO was used to dissolve the test substances. As a comparative benchmark, two conventional antibiotics (*STREPTOMTCIN*) were utilized. The compounds' zones of inhibition were measured in

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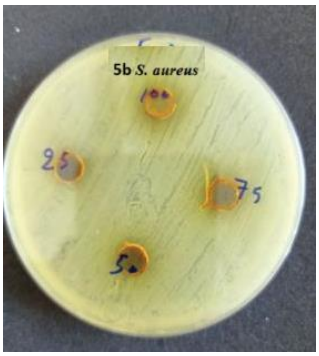
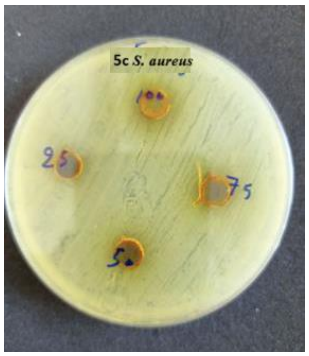
Fig. 13. Table 6 lists the antibacterial activity data of the produced compounds. The test chemicals' antibacterial properties are briefly displayed here.
The result indicated that the activity against *S. aureus* bacteria was high for (5b, 5c), low for compounds (5d, 5e) and no activity has appeared for (5a), The results it is also confirmed the activity against (E. coli) bacteria of the compounds (5b-5e), whereas the compound (5a) showed no activity against this type of bacteria, Fig. 12.

Table (6). The inhibition zones of the compounds (5a-5e)

Sample code	Conc. (µg/ml)	Zone of inhibition (mm)		Sample code	Conc. (µg/ml)	Zone of inhibition (mm)	
		Gram positive	Gram negative			Gram positive	Gram negative
		S. aureus	*E. coli*			*S. aureus*	*E. coli*
5a	100	20	13	**5c**	50	22	18
	75	28	19		25	17	19
	50	12	10	**5d**	100	30	19
	25	10	8		75	44	17
5b	100	44	22		50	19	14
	75	38	25		25	12	10
	50	28	15	**5e**	100	24	20
	25	18	14		75	21	18
5c	100	44	22		50	17	15
	75	30	25		25	14	11





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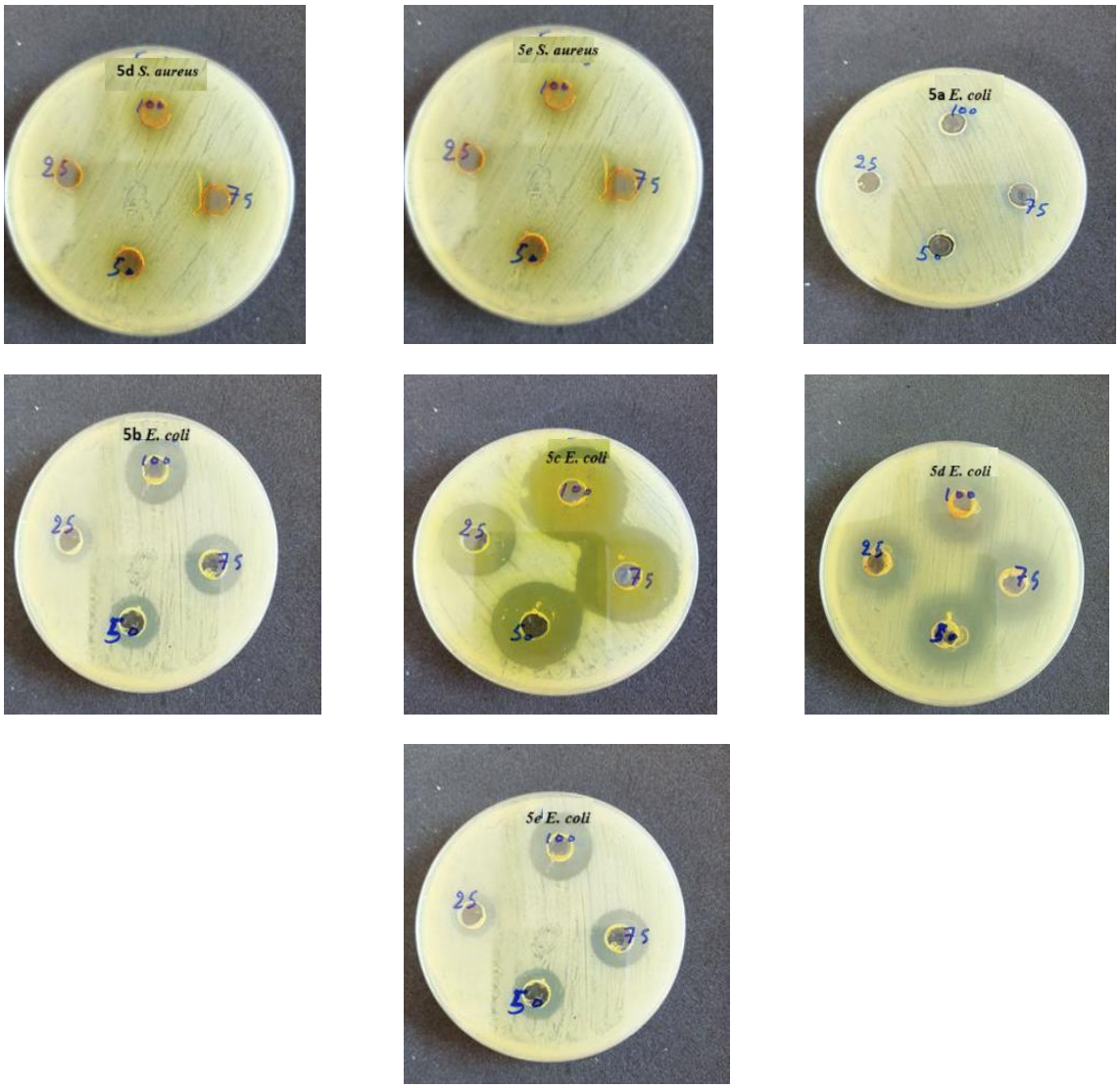

Figure (12). The compounds' zones of inhibition were measured.

Acknowledgments

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Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Chemistry]. This approval underscores our commitment to ethical research practices and the well-being of our participants.

- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Chemistry], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

Noor F. Abbas: Conducted some experiments, collection a part of literature review and conducted some characteristics of the products

Nasreen R. Jber: Contributed to the conception and design of the study, conducted some experiments, data rearrangement and drafted the initial manuscript.

References

1. El Shehry, M., Ghorab, M., Abbas, S., Fayed, E., Shedid, S., Ammar, Y. Quinoline derivatives bearing pyrazole moiety: Synthesis and biological evaluation as possible antibacterial and antifungal agents. EJMECH, 143:1463-73, 2018.

2. El-Sawy, E., Abdelwahab, A., Kirsch, G. Synthetic routes to coumarin (benzopyrone)-fused five-membered aromatic heterocycles built on the α-pyrone moiety. Part II: five-membered aromatic rings with multi heteroatoms. Molecules, 26(11): 3409, 2021.

3. Küçükgüzel, Ş., Şenkardeş, S. Recent advances in bioactive pyrazoles. EJMECH. 97:786-815, 2015.

4. Szabó, G., Fischer, J., Kis-Varga, Á., Gyires, K. New celecoxib derivatives as anti-inflammatory agents. Journal of medicinal chemistry. 51(1): 142-7, 2018.

5. Marengo, B., Meta, E., Brullo, C., De Ciucis, C., Colla, R., Speciale, A. Biological evaluation of pyrazolyl-urea and dihydro-imidazo-pyrazolyl-urea derivatives as potential anti-angiogenetic agents in the treatment of neuroblastoma. Oncotarget. 11(37): 3459, 2020.

6. Taher, A., Sarg, M., Ali, N., Elnagdi, NH. Design, synthesis, modeling studies and biological screening of novel pyrazole derivatives as potential analgesic and anti-inflammatory agents. Bioorganic chemistry. 89: 103023, 2019.

7. Genin, M., Biles, C., Keiser, B., Poppe, S., Swaney, S., Tarpley, W. Novel 1, 5-diphenylpyrazole nonnucleoside HIV-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant P236L mutant: lead identification and SAR of 3-and 4-substituted derivatives. Journal of medicinal chemistry. 43(5): 1034-40, 2000.

8. Brullo, C., Caviglia, D., Spallarossa, A., Alfei, S., Franzblau, S., Tasso, B., Schito, A.. Microbiological Screening of 5-Functionalized Pyrazoles for the Future Development of Optimized Pyrazole-Based Delivery Systems. Pharmaceutics. 14(9): 1770, 2022.

9. Sunitha, T., Dixit, P., Naaz, A., Chitrapu, P., Panda, K., Kumar, P. Pyrazole Scaffolde: A Promising Frontier in Drug Discovery. Biochemical & Cellular Archives. 24(1), 2024.

10. Soni, J., Sinha, S., Pandey, R,. Understanding bacterial pathogenicity: a closer look at the journey of harmful microbes. Frontiers in Microbiology. 15:1370818, 2024.

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

11. Vanzolini, T., Bruschi, M., Rinaldi, A., Magnani, M., Fraternale, A. Multitalented synthetic antimicrobial peptides and their antibacterial, antifungal and antiviral mechanisms. International Journal of Molecular Sciences. 23(1): 545, 2022.

12. Abu-Hashem, A., Al-Hussain, S.A. Design, Synthesis, Antimicrobial Activity, and Molecular Docking of Novel Thiazoles, Pyrazoles, 1, 3-Thiazepinones, and 1, 2, 4-Triazolopyrimidines Derived from Quinoline-Pyrido [2, 3-d] Pyrimidinones. Pharmaceuticals. 17(12): 1632, 2024.

13. Mortada, S., Karrouchi, K., Hamza, E., Oulmidi, A., Bhat, M., Mamad, H. Synthesis, structural characterizations, in vitro biological evaluation and computational investigations of pyrazole derivatives as potential antidiabetic and antioxidant agents. Scientific Reports. 14(1):1312, 2024.

14. Zhang, F., Cheng, W. The mechanism of bacterial resistance and potential bacteriostatic strategies. Antibiotics. 11(9): 1215, 2022.

15. Kumar, N., Sreenivasa, S., Kumar, V., Mohan, N. 3-(3, 5-Difluorophenyl)-1-phenyl-1 H-pyrazole-4-carbaldehyde. Molbank. 1(3): M1011, 2018.

16. Bono, N., Nyella, S., Manchala, M. Synthesis of 1-benzoyl-3-phenyl-1H-pyrazolw-4-carbaldehyde and evaluation of their antioxidant and anti-inflammatory activity. Asian J Pharm Clin Res, 14(10): 48-52, 2021.

17. Mohamed, A., Samir, B., Hossam, E., Ez-Eldin, M. Thiosemicarbazides: Synthesis and Reactions, Journal of Sulfur Chemistry. 32(5): 489–519, 2011.

18. Anita, R., Banpurkar, S., Wazalwar, S., Franc, P. Copper(II) ions Mediated Crystal Formation of 3-(3-Hydroxy Phyenyl)-1-Phenyl-1H-Pyrazole-4-Carbaldehyde, Bull. Chem. Soc. Ethiop. 34(3): 589-596, 2020.

19. Thuijl, J., Klebe, K., Houte, J. The mass spectra of some pyrazole compounds. 3(12): 1549-1559, 1970.