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Original Research Paper

Synthesis, In Vitro Biological Activity and Anti-Breast Cancer of Diazepine Derivatives

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Abstract: Despite their discovery, diazepines have been important compounds that occur in nature or may be synthesized in labs. A novel diazepine (4-6) were synthesized, namely 2-[4-(4-chlorophenyl)-2,5-dihydro-1H-1,5-benzodiazepin-2-yl]phenol (4), 2-(4-{2-[(dioxo-l6-sulfanyl)oxy]phenyl}-2,5-dihydro-1H-1,5-benzodiazepin-2-yl)phenol (5), and 2-[4-(2-hydroxyphenyl)-4,5-dihydro-1H-1,5-benzodiazepin-2-yl]benzene-1,3-diol (6). These compounds are produced from 2-hydroxy acetophenone in conjunction with aldehyde derivatives, including 4-chlorobenzaldehyde, 2-sulfobenzaldehyde, and 2,6-dihydroxybenzaldehyde. The chalcone derivatives undergo a reaction with 2,4-diaminobenzene to get their final derivatives (4–6). Characterizations of these derivatives using FTIR and 1H-NMR. The diffusion approach assesses the interaction of these derivatives with different microorganisms, including Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. The sulphonyl group in derivative 5 enhances biological activity more than derivatives 4 and 6. The van der Waals (vdw) radii for hydrogen, oxygen, carbon, and sulphur atoms are 1.20, 1.52, 1.70, and 11.80, respectively, as calculated using PyMOL.

Keywords: Biological Activity; Diazepine; Five rings; Bacteria.

1.Introduction

The increasing resistance of bacteria and fungi to a broader range of antibiotics [1] has prompted research into discovering new and promising antimicrobial compounds [2]. The alteration of molecular structures to enhance the properties of lead compounds remains a prominent strategy in the pursuit of new pharmaceutical agents [3]. This process involves combining distinct pharmacophoric groups with comparable activity into a single drug (3), resulting in structural alterations in the biological activity [4]. Investigating novel and highly antimicrobial efficacious medicines represents the optimal approach for addressing microbial resistance and advancing the development of successful treatments [5].

The class of 1,5-benzodiazepines includes a diverse group of organic compounds that exhibit a broad range of biological activities [6] and possess various therapeutic effects [7, 8]. Anxiolytic and anticonvulsive medicines are frequently employed for medicinal purposes [9]. The utilization of 1,5-benzodiazepines in therapeutic contexts extends beyond addressing anxiety and stress-related disorders since slight modifications to their chemical structures can yield diverse biological effects [10], leading to the continual emergence of fresh applications for these molecules. Recent studies have

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shown that 1,5-benzodiazepines and their derivatives possess notable antibacterial and antifungal activities [11-13]. Furthermore, previous studies conducted on 1,5-benzodiazepines have demonstrated that the presence of a free ester group at various places within the molecular nuclei might augment the pharmacological characteristics of these compounds [14]. This enhancement is believed to result from their significant hydrophobic properties [15].

Also, it has been discovered that heterocyclic compounds with a seven-membered ring have critical structural features that make them very good at killing microbes [16, 17]. Furthermore, these molecules serve as essential pharmacodynamic heterocyclic nuclei [18]. The biological activity of 1,5-benzodiazepines, comprised of heterocyclic compounds with a five-membered ring structure, is notably enhanced [19].

In this study, we synthesized new diazepine derivatives via a chalcone reaction. We characterized them by different spectroscopic methods, such as FTIR and 1H-NMR, and their derivatives were tested as anti-bacterial and anti-fungal.

2. Materials and Methods

The materials used in this study were (99% purity, Sigma Aldrich (USA) and (97% purity Merck, Germany).

2.1 chalcone derivatives (1-3) Synthesized

A solution was prepared by using 0.01 mol of 2-hydroxy acetophenone with equimolar amounts of 4-chlorobenzaldehyde, 2-sulfobenzaldehyde, and 2,6-dihydroxybenzaldehyde, respectively. The mixture was dissolved in 30 ml of absolute ethanol and 10% NaOH. This solution was refluxed at 65°C and 7 hors, and filtered solution and solid remains were recrystallized via absolute ethanol and dried [20, 21].

2.2 Diazepine derivatives preparation (4-6)

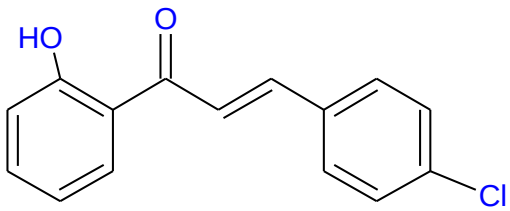
A 0.01 moles of the novel chalcone derivative (1-3) were mixed with 0.01 moles of O-phenylenediamine in EtOH. These solutions dissolved in 35 mL, 10% NaOH and underwent reflux for 7 hours. The resultant solution was added to 250 ml of cold distal water while being stirred for one hour. The resultant solid remains were then filtered, and recrystallized via absolute ethanol and dried [12, 22].

2.3 Antibacterial

The *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* bacterial cultures were obtained from the Baghdad laboratory in Baghdad, Iraq. The bacterial cultures were placed in nutritional agar and incubated at a precise temperature of 30 ± 0.1 °C for 24 hours. The diazepine (4-6) was stored in a dry and dissolved in dimethyl sulfonamide at 25 mg/ml concentration. The bactericidal efficacy of each drug was assessed using the agar disc-diffusion method. 9 cm Petri plates were inoculated with 50 µL of a standard saline solution containing a culture media with a concentration of microorganisms of 105–106 bacteria per ml. In a volume of 50 µL, the diazepine derivatives were injected into discs and pressed firmly into the solid agar substrate. The Petri plates were placed in an incubator and kept at 37 degrees Celsius for 24 hours. The inhibitory zones formed on the medium were quantified using a zone reader at the end of the specified period, and the measurements were documented in millimetres.

3. Results and Discussion

Chalcone derivative (1). Color: Reddish brown. Melting Point: 198 °C. FT-IR (cm^{-1}): 3418 υ(O-H), 3050 υ(C-H)$_{Aromatic}$, 2956 & 2868 υ(C-H of aliphatic), 1653 υ(carbonyl), 1611 υ(C=C alkene), 1575 υ(C=C of aromatic) [23]. As shown in Figure 1.



Chalcone derivative (2). Color: Light brown. Melting Point: 215 °C. FT-IR (cm^{-1}): 3433 υ(O-H), 3057 υ(C-H of aromatic), 2963 & 2875 υ(C-H aliphatic), 1650 υ(C=O), 1605 υ(C=C of alkene), 1597 υ(C=C Ar.) [24]. As shown in Figure 2.

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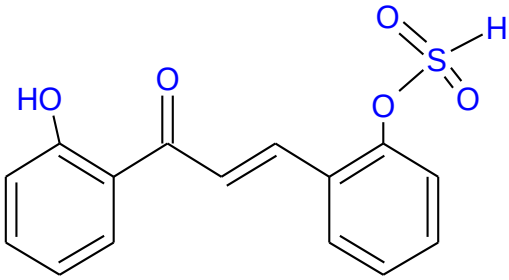
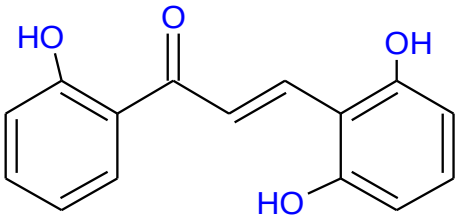
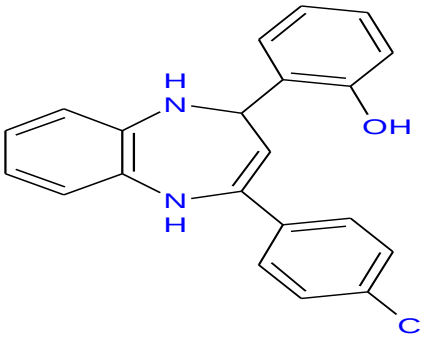


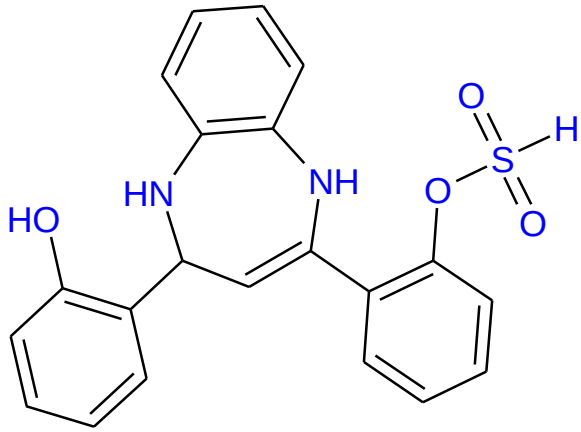
Chalcone derivative (3). Color: Dark yellow. Melting Point: 232 °C. FT-IR (cm^{-1}): 3355 υ(O-H), 3056 υ(C-H)$_{Aromatic}$, 2952 & 2865 υ(C-H Aliphatic), 1660 υ(C=O), 1628 υ(C=C)$_{Alkene}$, 1594 υ(C=C Ar.) [25]. As shown in Figure 3.



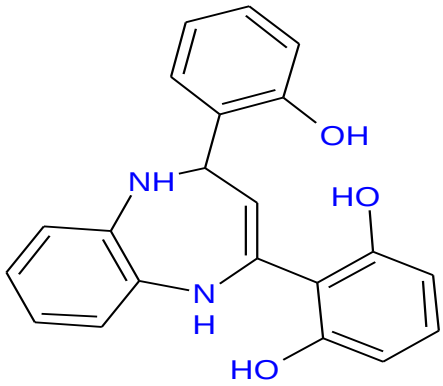
Diazepine derivatives (4): Color: Dark yellowish red. Melting Point: 177 °C. FT-IR (cm^{-1}): 3340 υ(primary amine), 3071 υ(C-H Ar.), 2979 & 2824 υ(C-H aliphatic), 1652 υ(C=C), 1593 υ(C=C Ar.). ^{1}H-NMR (ppm): 9.21 (2H, s, NH), 9.60 (1H, s, OH), 6.87-7.80 (12H, m, aromatic), 5.68 (1H, d, C_{β} of unsaturated) [26].



Diazepine derivatives (5): Color: Dark red. Yield: 71%. Melting Point: 186 °C. FT-IR (cm^{-1}): 3374 υ(primary amine), 3094 υ(C-H Ar.), 2921 & 2863 υ(C-H aliphatic), 1648 υ(C=C), 1601 υ(C=C Ar.). ^{1}H-NMR (ppm): 9.12 (2H, s, NH), 9.65 (1H, s, OH), 6.92-8.18 (12H, m, aromatic), 5.91 (1H, d, C_{β} of unsaturated).



Diazepine derivatives (6): Color: Light Brown. Yield: 71%. Melting Point: 201 °C. FT-IR (cm^{-1}): 3392 υ(primary amine), 3051 υ(C-H Ar.), 2931 & 2821 υ(C-H of aliphatic), 1657 υ(C=C), 1606 υ(C=C Ar). ^{1}H-NMR (ppm): 9.21 (2H, s, NH), 9.72 (1H, s, OH), 6.83-8.05 (11H, m, aromatic), 5.77 (1H, d, C_{β} of unsaturated).



Biological Activity

Three derivatives (4, 5, and 6) weighing 50 µg were synthesized and later evaluated for their ability to kill bacteria. These compounds were tested against a number of bacterial strains, including *Bacillus subtilis*, *S. aureus*, and *E. coli*, using the diffusion technique [27]. The diazepine derivative 5 showed efficacy against a wide range of currently known bacteria.

Table 1. Biological activities for derivatives (5, 6, 7 and 9).

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Derivative	Zone inhibition (mm)		
	Bacillus subtilis	*S. aureus*	*E. coli*
4	+	-	+
5	+	+	++
6	+	+	+

Note: + was (more 5 mm), ++ was (more 15 mm).

The present study focuses on the *in-silico* investigation of the inhibitory effects of diazepine derivatives on GP6 synthase. The formation of two conventional hydrogen bonds between the functional amine group of the diazepine and the carboxylate group is one notable observation that both derivatives displayed a notable binding affinity towards the receptor. Additionally, the interaction diagram reveals the presence of other interactions, such as van der Waals forces and π-alkyl interactions [28]. All derivatives 4, 5 and 6 have the same structure but differ in some groups, such as sulphonyl, hydroxy, and dihydroxyl. The sulphonyl group in derivative 5 increases biological activity more than other derivatives 4 and 6. Derivative 4 has less activity than derivative 6 because derivative 4 has one hydroxy group, while derivative 6 has two hydroxyl groups as active groups.

Viability cell of breast cancer by MTT assay

The effect of diazepine derivative (6) analyzed as shown in figure 10, after 48 h, increased concentration (PPM), decreased breast cancer viability % more than 24 h. All results that were obtained are shown in Table 1.

Table 2. The IC50 rates of derivative (A) induced breast cancer cells.

Concentration (PPM)	After 24 h		After 48 h	
	Mean	SD	Mean	SD
0	100	2.870042	100	3.895671
20	77.8648	3.127544	41.7643	3.221549
40	55.1532	2.8776257	26.7332	3.753113
80	35.9860	2.986058	11.0978	1.041876
160	17.0147	2.884173	8.8612	1.337521
320	7.7852	1.263410	1.2214	1.548773

The van der Waals (vdw radius) for each atom was 1.20 for the hydrogen atom, 1.52 for the oxygen atom, 1.70 for the carbon atom and 11.80 for the Sulphur atom that calculated by using pymol software with 3 dimension structures of derivative 5, as shown in figure 1.

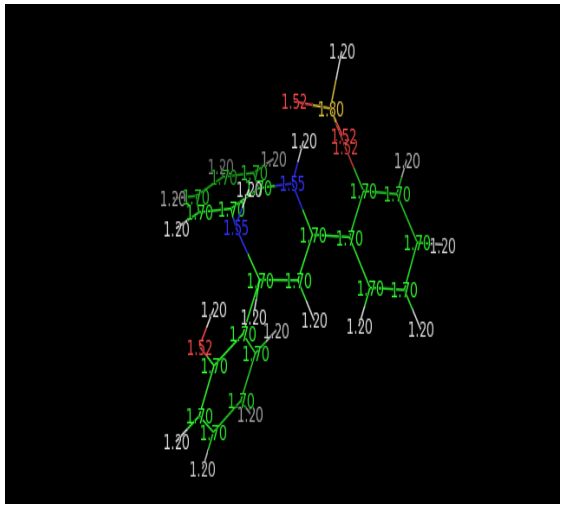

Fig. 1. Pymol test.

Conclusion

A novel diazepine derivatives (4-6) were synthesized using a simple and quick technique. Upon evaluating these derivatives using ^{1}H-NMR and FTIR spectroscopic techniques, it was observed that the FTIR results exhibited the presence of carbonyl and α, β-unsaturated functional groups in stage 1 for the synthesized chalcone derivatives. However, these peaks were no longer detected in stage 2 for the synthesized derivatives (4-5). The 4, 5, and 6 derivatives showed favourable outcomes as antibacterial agents against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. The sulphonyl group in derivative 5 increases biological activity more than other derivatives 4 and 6.

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Author's Contributions

Ban Ameen[a] and Baneen S. Rasoolb Conceived and designed the analysis, Asmaa A. Jawada, Baraa Watheqc, Abbas K. Abbasd are writing, and Evon Akrama and Waled Ahmed discuss the results.

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Ethics

This study was conducted under approval by the medical ethics committee at Al-Nahrain University, Ibn Sina University, and Thamar University (2024). Parents and agreement provided verbal and written consent for publication was obtained from participants and researchers.

References

1. 1. Salih, B. M., & Hadi, M. A. (2022). Synthesis, identification, and spectral studies of some new Schiff base ligand complexes containing 3, 4-diaminobenzophenone and pyridoxal hydrochloride compounds. *Journal of Kufa for Chemical Sciences*, *2*(9), 115-127.

2. Qadri, H., Khan, M. I., & Khedher, N. B. (2022). Natural products and their semi-synthetic derivatives against antimicrobial-resistant human pathogenic bacteria and fungi. *Saudi Journal of Biological Sciences*, *29*(9), 103376.

3. Church, N. A., & McKillip, J. L. (2021). Antibiotic resistance crisis: Challenges and imperatives. *Biologia*, *76*(5), 1535-1550.

4. Ramadan, H. H. E. D., & Al-Din, N. L. S. (2024). Preparation and characterization of chalcone compounds. *Journal of Kufa for Chemical Sciences*, *3*(2), 275-292.

5. Vanamala, K., Bhat, S., & Reddy, M. S. (2021). Novel approaches for the treatment of methicillin-resistant Staphylococcus aureus: Using nanoparticles to overcome multidrug resistance. *Drug Discovery Today*, *26*(1), 31-43.

6. Majeed, N. S. (2024). Synthesis, evaluation of the biological activity and anti-oxidant for some heterocyclic compounds seven-membered rings derived from Schiff bases. *Journal of Kufa for Chemical Sciences*, *3*(2), 415-434.

7. Mishra, R., Kumar, R., & Singh, S. (2022). Solid support based synthesis of 1, 5-benzodiazepines: A mini review. *Synthetic Communications*, *52*(4), 481-503.

8. Tayde, D., & Lande, M. (2021). Synthesis of 2, 4 disubstituted 1, 5 benzodiazepines promoted by efficient silica-alumina catalyst. *Chemical Review and Letters*, *4*(1), 30-36.

9. Diniz, T. C., Lima, F. A., & Silva, J. R. (2019). Anticonvulsant, sedative, anxiolytic and antidepressant activities of the essential oil of Annona vepretorum in mice: Involvement of GABAergic and serotonergic systems. *Biomedicine & Pharmacotherapy, 111*, 1074-1087.

10. Sarkar, F. K., Ghosh, S., & Karmakar, S. (2021). A green and sustainable approach for the synthesis of 1, 5-benzodiazepines and spirooxindoles in one-pot using a MIL-101 (Cr) metal–organic framework as a reusable catalyst. *New Journal of Chemistry*, *45*(41), 19553-19564.

11. Rasheed, M. K., Al-Rifaie, D. A., & Madab, D. I. (2021). Synthesis and evaluation of antibacterial and antifungal activity of new 1, 5-benzodiazepine derivatives containing cyclic imides and Mannich bases. *International Journal of Pharmaceutical Research, 13*(1).

DOI: 10.36320/ajb/v16.i3.17437

12. Bhathiwal, A. S., Bendi, A., & Tiwari, A. (2022). A study on synthesis of benzodiazepine scaffolds using biologically active chalcones as precursors. *Journal of Molecular Structure, 1258*, 132649.

13. Jaafar, Z., Khamis, M. F., & Al-Haj, M. (2019). Design and microwave-assisted synthesis of dimers of 1, 5-benzodiazepine-1, 2, 3-triazole hybrids bearing alkyl/aryl spacers and their biological assessment. *Journal of Molecular Structure, 1195*, 689-701.

14. Arora, N., Jain, S., & Gupta, R. (2020). Recent advances in synthesis and medicinal chemistry of benzodiazepines. *Bioorganic Chemistry, 97*, 103668.

15. Abbas, A. K., & Jber, N. R. (2020). Synthesis and estimation of biological activity of new oxazepine derivatives. *International Journal of Pharmaceutical Research, 12*(2), 3750-3758.

16. Li, B., Wang, Y., & Zhang, X. (2022). Research progress in antineoplastic, antibacterial, and anti-inflammatory activities of seven-member ed heterocyclic derivatives. *Current Medicinal Chemistry, 29*(30), 5076-5096.

17. Abbas, A. K., & Jber, N. R. (2020). Synthesis and characterization of new oxazepine compounds and estimation of its biological activity. *Al-Nahrain Journal of Science, 23*(3), 17-23.

18. Ali, A. A., & Ali, H. A. (2024). Analytical study of some azo dyes and its medical applications. *Journal of Kufa for Chemical Sciences, 3*(2), 16-30.

19. Kumar, N., & Goel, N. (2022). Heterocyclic compounds: Importance in anticancer drug discovery. *Anti-Cancer Agents in Medicinal Chemistry, 22*(19), 3196-3207.

20. Elkanzi, N. A., Al-Shahrani, M. A., & Al-Harbi, M. (2022). Synthesis of chalcone derivatives and their biological activities: A review. *ACS Omega, 7*(32), 27769-27786.

21. Okolo, E. N., Okwu, D. E., & Okwuosa, C. N. (2021). New chalcone derivatives as potential antimicrobial and antioxidant agents. *Scientific Reports, 11*(1), 21781.

22. Fahad, N. G., Alshahrani, S. M., & Alhassan, A. (2023). Synthesis, anticancer for prostate cancer cells and antibacterial activity of new diazepine derivatives. *Results in Chemistry, 6*, 101049.

23. Fu, Y., Zhang, Y., & Liu, X. (2020). New chalcone derivatives: Synthesis, antiviral activity and mechanism of action. *RSC Advances, 10*(41), 24483-24490.

24. Bandeira, N. P., Lima, J. R., & Santos, A. (2019). Synthesis, structural characterization, and cytotoxic evaluation of chalcone derivatives. *Medicinal Chemistry Research, 28*, 2037-2049.

25. Ngameni, B., Nguemo, M., & Tchouanguep, M. F. (2021). Design, synthesis, characterization, and anticancer activity of a novel series of O-substituted chalcone derivatives. *Bioorganic & Medicinal*

DOI: 10.36320/ajb/v16.i3.17437

Chemistry Letters, *35*, 127827.

26. Priyanka, N., Kumar, A., & Singh, R. (2022). Synthesis, characterization, anti-inflammatory and anti-oxidant activities of novel 2, 4-di substituted 1, 5-benzodiazepine derivatives. *Asian Journal of Research in Chemistry*, *15*(2), 109-114.

27. Al-Hamidawi, O. A., & Al-Rammahi, F. A. (2021). Synthesis, chalcone and study of the biological activity for different heterocyclic compounds from the chalcone derivative. *Journal of Kufa for Chemical Sciences*, *2*(7), 15-26.

28. Tiihonen, J., Kallio, M., & Kallio, H. (2021). Molecular signaling pathways underlying schizophrenia. *Schizophrenia Research*, *232*, 33-41.