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Research Article

Synthesis and Biological Activities of Some 1, 3, 4-thiadiazine Derivatives

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Abstract : In this study, some 1,3,4-thiadiazine derivatives were synthesized by condensation of appropriate acetophenone derivatives and thiosemicarbazides because of their wide range of biological applications. Structures of synthesized compounds characterized by 1H NMR and FTIR techniques and evaluated as potential antibacterial or antioxidant agents. The activities of the compounds against Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Escherichia coli (E. coli), and Salmonella typhimurium (S. typhimurium) were evaluated by the Kirby-Bauer disk diffusion method. From the results, it is clear that the compounds (4) and (5) have stronger activity against Gram-positive bacteria but show no inhibition against Gram-negative bacteria. In addition, the DPPH radical scavenging activity of the compounds was determined by microplate assay with various concentrations of the test compounds. Among the synthesized compounds, (4), (5) and (2) showed the highest interactions. The result showed that the DPPH scavenging activity of compounds also appeared to depend on an increase in concentration. The findings of this work have significance in view of the possibility to design new 1, 3, 4-thiadiazine derivatives with improved potency and activity.

Keywords : 1, 3, 4-thiadiazine; antibacterial activity; DPPH radical; 1H NMR; FTIR.

1 Introduction

Human pathogens can cause a variety of serious infections, and the recent increase in multidrug resistance in pathogens has created an urgent and worrying need for new and potent antimicrobial agents. The World Health Organization (WHO) published a list of antibiotic-resistant bacteria urgently needed for new treatments in the mid-2000s. Strains to be developed in this process are the most effective strategy to counter the rise of multidrug-resistant (MDR) organisms [1]–[3]. Therefore, the new design and synthesis of heterocyclic compounds, including Nitrogen and sulfur, are very important as they have a wide range of biological applications [4], [5]. Among heterocyclic compounds, 1, 3, 4-thiadiazine scaffolds have been found to have good antimicrobial [6], anticancer [7], [8], analgesic, anticandidal [6], anti-inflammatory, antiaggregant, antitumor, and antioxidant activities [9]. In addition, thiadiazine-thione derivatives are also involved in the treatment of atherosclerosis and have antifibrinolytic, cytotoxic and antiepileptic activities. Furthermore, Thiadiazine-thione derivatives have been investigated as potential components of prodrugs for various biological activities [10]. Also, 3-Nitrobenzyl-5-aryl-1,3,4-thiadiazine-2-one and 1,3,4-thiadiazine-2-one derivatives are used for the treatment of tumors and AIDS because of their Phosphodiesterase 4 inhibitory abilities [11].
Thiazole and thiadiazine derivatives, in addition to their antimicrobial, antifungal, antihypertensive, cardiotonic, antiviral, anti-inflammatory, analgesic, and antioxidant activities, may be good candidates for antidiabetic drug development. Steroidal 1,3,4-thiadiazines exhibit anticancer, antitumor, antimicrobial, and antibacterial properties. Heterocyclic thiadiazole systems, which enhance interactions with biomolecules, show a natural mesoionic structure that offers low toxicity, high in vivo stability, and good cell and tissue permeability [12]–[14]. Since 1,3,4-thiadiazine derivatives have biological and pharmaceutical importance, they can be considered starting points for the production of pharmacologically active substances [15].
In this study, we designed and synthesized a series of 1, 3, and 4-thiadiazine derivatives whose structures were characterized by FTIR and 1H NMR. The in vitro susceptibilities of the Gram-positive and Gram-negative bacteria to the synthesized thiadiazine

derivatives were analysed using disk diffusion assays. The radical-scavenging capacity of the compounds was evaluated using the DPPH radical-scavenging assay.

2 Experimental Methods

2.1 Materials

All solvents and reagents were analytical grade and purchased from Sigma-Aldrich. An EZ-Melt Automated Melting Point Apparatus was used to determine melting points. Infrared spectra of the compounds were recorded using a Thermo Nicolet IS5 FTIR spectrometer. In addition, 1H NMR spectra of the compounds were determined using a Varian 400 MHz NMR Spectrometer in DMSO-d6 by expressing chemical shifts as δ ppm.

2.2 Biologic Materials And Apparatus

Lyophilized Gram-positive and Gram-negative bacteria cultures were purchased from Microbiologics Inc. (Saint Cloud, MN, USA). Bacterial strains were stored in Nutrient Broth with 20 % glycerol at -18 ˚C. The antioxidant capacity of the compounds was determined by the DPPH (1,1-diphenyl-2picrylhydrazil) radical-scavenging assay). The experiment was carried out on the 96-well microplate using the Herald method [16].

2.3 Chemistry

Substituted 1,3,4-thiadiazine derivatives synthesized according to the method described in the literature [17]. However, we determined with TLC that our reactions did not occur in 20 minutes at room temperature as stated in the literature. Therefore, unlike the literature, we observed by following our reactions with TLC that they were completed in 6 hours under reflux. Compounds N-ethyl-5-(4-phenoxyohenyl)-6H-1,3,4-thiadiazine-2-amine 1, N-ethyl-5-(3-nitrophenyl)-6H-1,3,4-thiadiazine-2-amine 3, and N-ethyl-5-(4-nitrophenyl)-6H-1,3,4-thiadiazine-2-amine 7 were first synthesized in this study.

2.3.1 General procedure for the synthesis of 1, 3, 4- thiadiazine derivatives

Acetophenone derivative (1 mmol) and thiosemicarbazide (1 mmol) in 5 mL ethanol with a few drops of dil. hydrochloric acid was refluxed for 6 h. The reaction was followed by TLC. After completion of reaction, pH adjusted to 8-9 by adding of Ammonia solution. The precipitate was filtered and crystallized from ethanol to give 1,3,4-thiadiazine derivatives.

2.4 Biological Assay

2.4.1 Antibacterial activity assay

Lyophilized cultures of *B. cereus* (ATCC 11778), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *S. typhimurium* (ATCC 14028) were obtained from Microbiologics Inc. (Saint Cloud, MN, USA). The antibacterial activity of the compounds was evaluated using the Kirby-Bauer disk diffusion method. Stock solutions of the compounds were prepared using dimethyl sulfoxide (DMSO). Each bacterial strain was separately inoculated into cation-adjusted Mueller Hinton broth (MHB; BD, Auckland, New Zealand) and incubated at 37˚C for 24 hours. The bacterial suspensions were then standardized to a 0.5 MacFarland opacity standard (1.5×10^8 colony-forming units (CFU)/mL) and spread onto cation-adjusted Mueller Hinton agar (MHA, Lab M, UK) plates before placing sterile paper disks (6 mm diameter; BD, Auckland, New Zealand) equidistantly on the plate. $10\mu L$ of the compounds from stock solutions were then added to the disks. The plates were incubated at $37^0 C$for $24h$, and the diameter of the zone of inhibition, which indicated the antimicrobial activity of the compounds, was calculated in millimeters using a ruler. Gentamicin ($10\mu g$discs) was used as a reference antibacterial agent. DMSO alone ($20\mu L$) was used as a negative control, and experiments were performed in duplicate.

2.4.2 *In vitro* antioxidant study

The DPPH radical-scavenging activity was assessed using a microplate assay based on a modified version of the Herald method. The scavenging potential of the compounds against the DPPH radical was tested at various concentrations (25, 50, 100, 200, and 400 μ g/mL). For the assay, 20 μL of the diluted sample was mixed with 180 μL of DPPH solution (150 μ) in a methanol-water mixture (80:20, v/v) and shaken for 60 seconds in a 96-well microtiter plate. Following a 40-minute incubation in the dark at room temperature, absorbance was recorded at 515 nm using a Thermo Scientific Multiskan GO spectrophotometer (ThermoFisher Scientific, MA, USA). Ascorbic acid served as the standard for comparison.

$$\%DPPHScavening = 100x\frac{[(AbsSample + DPPH) - (AbsBlank)]}{[(AbsControl) - (AbsBlank)]} \quad (1)$$

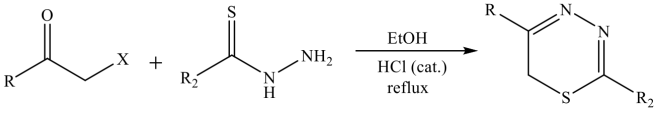
Abs (sample); Absorbance of $20\mu L$ sample or standard with$180\mu L$DPPH solution

Abs (blank); Absorbance of $180\mu L$ of methanol-water $(80:20, v/v)$ and $20\mu L$of water

3 Results and Discussion

3.1 Synthesis and Characterization

Substituted 1,3,4-thiadiazine derivatives were prepared via condensation of α-haloketones and thiosemicarbazides as shown in Scheme 1. Structures of synthesized compounds were confirmed by FTIR and 1H NMR spectroscopic techniques.



	R	X	R_2
(1)	Ph-O-Ph-	-Cl	-N-Ethyl
(2)	3-NO_2-Ph-	-Br	-NH_2
(3)	3-NO_2-Ph-	-Br	-N-Ethyl
(4)	4-Br-Ph-	-Br	-NH_2
(5)	4-Cl-Ph-	-Br	-NH_2
(6)	4-NO_2-Ph-	-Br	-NH_2
(7)	4-NO_2-Ph-	-Br	-N-Ethyl

	R	X	R_2
(1)	Ph-O-Ph-	-Cl	-N-Ethyl
(2)	3-NO_2-Ph-	-Br	-NH_2
(3)	3-NO_2-Ph-	-Br	-NH_2
(4)	4-Br-Ph-	-Br	-NH_2
(5)	4-Cl-Ph-	-Br	-NH_2
(6)	4-NO_2-Ph-	-Br	-NH_2
(7)	4-NO_2-Ph-	-Br	-N-Ethyl

Table 1: Synthetic pathway for synthesis of compounds

N-ethyl-5-(4-phenoxyohenyl)-6H-1,3,4-thiadiazine-2-amine **(1)***:* Yellow crystals. Yield 56 %. mp.: 156–158 °C; FTIR (cm^{-1}): 3171, 2968, 1734, 1700, 1636, 1590, 1470, 1412, 1255, 1152, 986, 855, 749, 988; 1H NMR (400 MHz, DMSO): δ = 7.90 (s, 1H, NH), 6.9-7.19 (m, 9H, Ar–H), 3.35-3.4 (m, 2H, NCH_2), 3.80 (s,2H, CH_2S), 1.15 (t, 3H, CH_3).

5-(3-nitrophenyl)-6H-1,3,4-thiadiazine-2-amine **(2)***:* Green crystals. Yield 89 %. mp.: 149- 153 °C; FTIR (cm^{-1}): 3386, 3366, 3129, 1617, 1507, 1340, 1168, 959, 800, 712, 669; 1H NMR (400 MHz, DMSO): δ= 9.44 (s, 2H, NH_2), 7.64-7.84 (m, 4H, Ar–H), 4.35 (s,2H, CH_2S).

N-ethyl-5-(3-nitrophenyl)-6H-1,3,4-thiadiazine-2-amine (3): Yellow crystals. Yield 66 %. mp.: 169- 172 °C; FTIR (cm^{-1}): 3180, 2971, 2862, 1733, 1700, 1653, 1517, 1345, 1258, 1058, 890,732, 605; 1H NMR (400 MHz, DMSO): δ= 8.75 (s, 1H, NH), 7.5-8.1 (m, 4H, Ar–H), 3.60 (q, 2H, NCH_2), 3.96 (s,2H, CH_2S), 1.3 (t, 3H, CH_3).

5-(4-bromophenyl)-6H-1,3,4-thiadiazine-2-amine (4): White crystals. Yield 68 %. mp.: 198- 200 °C; FTIR (cm^{-1}): 3321, 3050, 1660, 1601, 1568, 1478, 1397, 1341, 1283, 1189, 952, 900, 829, 731, 695; 1H NMR (400 MHz, DMSO): δ = 9.97 (s, 2H, NH_2), 7.61-7.86 (m, 4H, Ar–H), 4.26 (s, 2H, CH_2S).

5-(4-(chlorophenyl)-6H-1,3,4-thiadiazine-2-amine (5): Light brown crystals. Yield 60 %. mp.: 179- 182 °C; FTIR (cm°C): 3370, 3078, 1652, 1491, 1408, 1116, 1011, 830, 756, 595; 1H NMR (400 MHz, DMSO): δ= 7.52-7.55 (m, 4H, Ar–H), 3.94 (s,2H, CH_2S).

5-(4-nitrophenyl)-6H-1,3,4- thiadiazine-2-amine (6): Light green crystals. Yield 75 %. mp.: 209- 210 °C; FTIR (cm°C): 3380, 3165, 2888, 1672, 1595, 1513, 1325, 1106, 1005, 854, 750, 666; 1H NMR (400 MHz, DMSO): δ = 10.1 (s, 2H, NH2), 8.11-8.17 (m, 4H, Ar–H), 4.33 (s,2H, CH2S).

*N-ethyl-5-(4-nitrophenyl)-6H-1,3,4-thiadiazine-2-amine (*7): Orange crystals. Yield 66 %. mp.: 126- 128 °C; FTIR (cm°C): 3146, 2972, 2799, 1596,1499, 1465, 1336, 1275, 1218, 1147, 1107, 995, 750, 692, 566; 1H NMR (400 MHz, DMSO): δ= 8.38 (s, 1H, NH), 8.04 (d, 2H, Ar–H), 7.2-7.5 (m, 2H, Ar-H), 3.37-3.42 (m, 2H, NCH_2), 3.76 (s,2H, CH_2S), 1.14 (t, 3H, CH_3).

The FTIR spectrum of the thiadiazine derivatives showed peaks at 3050-3386 cm^{-1}; -NH or -NH_2 stretching, 1596-1672 cm^{-1}; -C=N stretching. The 1H NMR spectrum of synthesized compounds showed δ 3.76-4.35 (-CH_2S-) thiadiazine ring -CH- proton peak. All aromatic protons were assigned between δ6.9 and 8.17 ppm. The antimicrobial activity of the selected microorganisms against the samples was evaluated using the disk diffusion assay. The diameters of the inhibition zone (mm) around the disks containing the samples are presented in Figures 1 and 2. The findings indicated that samples (4) and (5) exhibited activity against *S. aureus* and *B. cereus* but failed to produce inhibition zones against the Gram-negative bacteria *E. coli* and *S. typhimurium*. Specifically, sample (4) demonstrated inhibition zones of 8.47 $\pm$ 0.45 mm and 8.33 $\pm$ 0.35 mm against *S. aureus* and *B. cereus*, respectively. In contrast, sample (5) exhibited the highest activity, forming inhibition zones of 9.77 $\pm$ 0.47 mm and 10.40 $\pm$ 0.40 mm against *S. aureus* and *B. cereus*, respectively. Other tested compounds showed no antibacterial activity against either bacterial group. The stronger activity of samples (4) and (5) against Gram-positive bacteria, while being ineffective against Gram-negative bacteria, can be attributed to structural differences, such as the presence of an additional outer membrane in

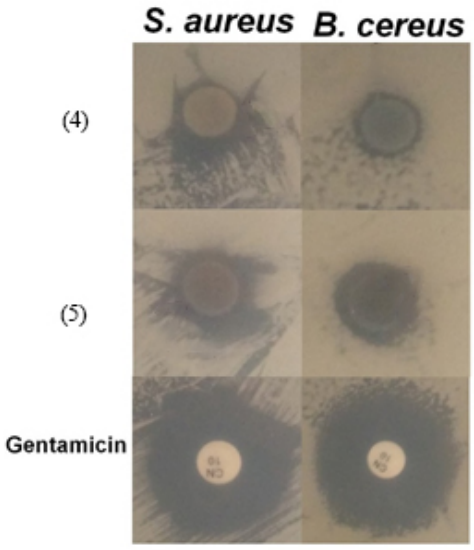


Figure 1: Inhibition zones of the compounds and Gentamicin against tested microorganisms on Muller Hinton agar

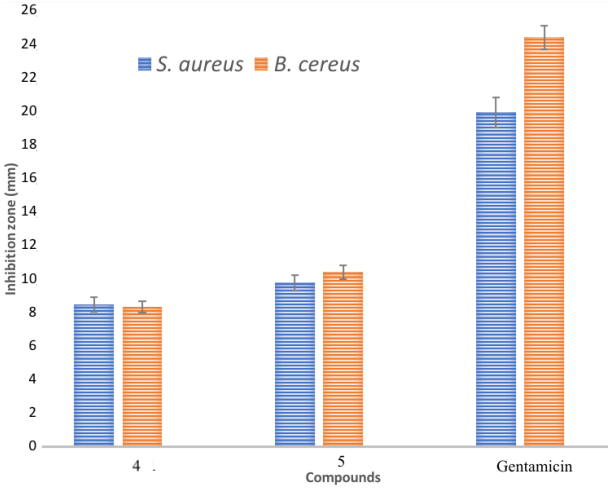


Figure 2: Inhibition zones of the compounds and Gentamicin against tested microorganisms by disc diffusion method

Gram-negative bacteria, which acts as a barrier to many antibacterial agents. [18], [19]. In the evaluation of antioxidant activities in a shorter time, the scavenging activity of the DPPH radical, which can accept an electron or hydrogen radical and thus be converted into a stable diamagnetic molecule, is widely used [20], [21]. We determined the reducing abilities of the synthesized compounds using the 1,1-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay compared to those of the free drug and ascorbic acid.

The DPPH radical scavenging model is a widely used method for assessing antioxidant activity in a shorter time compared to other techniques. DPPH is a stable free radical capable of accepting an electron or hydrogen atom and transforming into a stable, nonmagnetic molecule. The reducing potential of the complexes was determined using the 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay, which reflects their ability to act as reducing agents. The scavenging efficiency of the complexes was evaluated and compared with that of the free drug and ascorbic acid. [22].

According to the results, it was found that not all of the compounds tested interact with the stable free radical DPPH. Among the synthesized compounds, compound 4 showed the highest DPPH scavenging activity because it had fewer electron-withdrawing groups than other compounds. The DPPH radical scavenging activity of all samples was observed to be dependent on the concentration.

4 Conclusion

In this study, we synthesized, characterized, and determined the antibacterial and antioxidant activities of some 1,3,4-thiadiazine derivatives. Among the synthesized compounds, (4) and (5) showed stronger activity against Gram-positive bacteria *S. aureus* and *B. cereus* but did not show any antibacterial activity against Gram-negative bacteria *E. coli* and *S. typhimurium*. In addition,

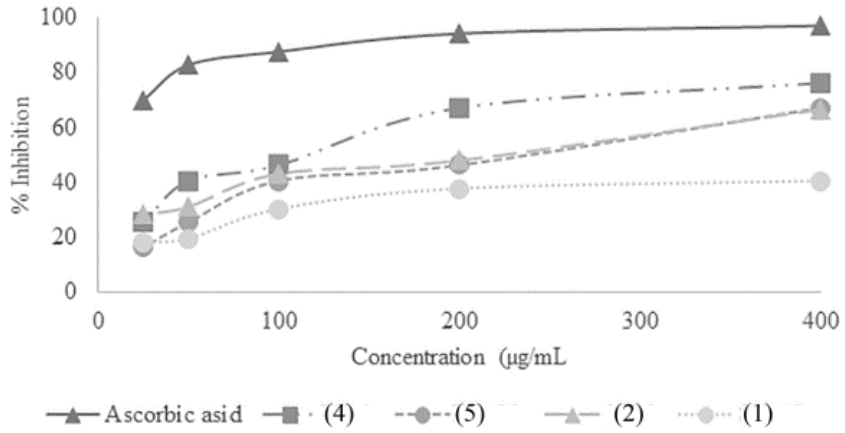


Figure 3: DPPH free radical scavenging activity of the compounds

the results showed that compounds (4), (5), and (2) had the strongest interactions with DPPH. These findings have obvious implications for the possibility of designing new 1,3,4-thiadiazine derivatives with improved potency and activity for use in different applications in various fields.

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EK and ŞKB designed the synthesis scheme. ŞKB performed the syntheses. AC and EK interpreted the structure characterization of the synthesized compounds. FE performed antimicrobial and antioxidant studies. DTA and ABS wrote the article in collaboration with EK. ABS, EK, and AC read and approved the final version of the manuscript. EK is the corresponding author of the article.

References

[1] M. Dinarvand and M. Spain, "Identification of bioactive compounds from marine natural products and exploration of structure-activity relationships (sar)," *Antibiotics*, vol. 10, no. 3, p. 337, 2021.

[2] E. Bentivegna, M. Luciani, L. Arcari, I. Santino, M. Simmaco, and P. Martelletti, "Reduction of multidrug-resistant (mdr) bacterial infections during the covid-19 pandemic: a retrospective study," *International journal of environmental research and public health*, vol. 18, no. 3, p. 1003, 2021.

[3] T. M. Terreni, Marco and M. Pregnolato, "New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives," *Molecules*, vol. 26, no. 9, p. 2671, 2021.

[4] P. C. Jilloju, L. Persoons, S. K. Kurapati, D. Schols, S. De Jonghe, D. Daelemans, and R. R. Vedula, "Discovery of (±)-3-(1 h-pyrazol-1-yl)-6, 7-dihydro-5 h-[1, 2, 4] triazolo [3, 4-b][1, 3, 4] thiadiazine derivatives with promising in vitro anticoronavirus and antitumoral activity," *Molecular Diversity*, vol. 26, no. 3, pp. 1357–1371, 2022.

[5] S. Chhabra and K. Shah, "The novel scaffold 1, 2, 4-benzothiadiazine-1, 1-dioxide: a review," *Medicinal Chemistry Research*, vol. 30, pp. 15–30, 2021.

[6] C. Kamoutsis, M. Fesatidou, A. Petrou, A. Geronikaki, V. Poroikov, M. Ivanov, M. Soković, A. Ćirić, A. Carazo, and P. Mladěnka, "Triazolo based-thiadiazole derivatives. synthesis, biological evaluation and molecular docking studies," *Antibiotics*, vol. 10, no. 7, p. 804, 2021.

[7] F. Hassanzadeh, E. Jafari, F. Shojaei, and H. Sadeghi-Aliabadi, "Synthesis and cytotoxic activity evaluation of some new 1, 3, 4-oxadiazole, 1, 3, 4-thiadiazole and 1, 2, 4-triazole derivatives attached to phthalimide," *Research in Pharmaceutical Sciences*, vol. 16, no. 6, pp. 634–642, 2021.

[8] A. M. A. Ouf, H. A. Allam, M. Kamel, F. A. Ragab, and S. A. Abdel-Aziz, "Design, synthesis, cytotoxic and enzyme inhibitory activities of 1, 3, 4-oxadiazole and 1, 3, 4-thiadiazine hybrids against non-small cell lung cancer," *Results in Chemistry*, vol. 4, p. 100373, 2022.

[9] F. Z. Mohammed, Y. W. Rizzk, I. M. El-Deen, E. M. Gad, M. El Behery, and A. R. Mahdy, "Discovery of 2-amino-4h-1, 3, 4-thiadiazine-5 (6h)-one derivatives and their in vitro antitumor investigation," *ChemistrySelect*, vol. 7, no. 7, p. e202104333, 2022.

[10] S. Qureshi, G. Ali, M. Idrees, T. Muhammad, I.-K. Kong, M. Abbas, M. I. A. Shah, S. Ahmad, R. D. Sewell, and S. Ullah, "Selected thiadiazine-thione derivatives attenuate neuroinflammation in chronic constriction injury induced neuropathy," *Frontiers in Molecular Neuroscience*, vol. 14, p. 728128, 2021.

[11] S. Alghamdia and M. Asifb, "phosphodiesterase-iii, iv, and v inhibitors."

[12] S. Baddi, A. Y. Dang-i, T. Huang, C. Xing, S. Lin, and C.-L. Feng, "Chirality-influenced antibacterial activity of methylthiazole-and thiadiazole-based supramolecular biocompatible hydrogels," *Acta Biomaterialia*, vol. 141, pp. 59–69, 2022.

[13] M. Alshubramy, M. Asem, and M. Abdel-Motaal, "Efficient synthesis of new fused thiadiazines and their spectroscopic, in silico drug likeness, and adme properties," *Russian Journal of Organic Chemistry*, vol. 58, no. 4, pp. 619–627, 2022.

[14] A. Maslat, B. Al-Trad, I. Alameen, M. Al-Talib, H. Tashtoush, and B. Ababneh, "The effect of two novel substituted thiazole and thiadiazine derivatives on experimental type-1 diabetes mellitus," *Jordan Journal of Chemistry (JJC)*, vol. 19, no. 1, pp. 41–47, 2024.

[15] A. De, S. Sarkar, and A. Majee, "Recent advances on heterocyclic compounds with antiviral properties," *Chemistry of Heterocyclic Compounds*, vol. 57, no. 4, pp. 410–416, 2021.

[16] T. J. Herald, P. Gadgil, and M. Tilley, "High-throughput micro plate assays for screening flavonoid content and dpph-scavenging activity in sorghum bran and flour," *Journal of the Science of Food and Agriculture*, vol. 92, no. 11, pp. 2326–2331, 2012.

[17] M. Čačić, V. Pavić, M. Molnar, B. Šarkanj, and E. Has-Schon, "Design and synthesis of some new 1, 3, 4-thiadiazines with coumarin moieties and their antioxidative and antifungal activity," *Molecules*, vol. 19, no. 1, pp. 1163–1177, 2014.

[18] M. T. Madigan, J. M. Martinko, J. Parker *et al.*, *Brock biology of microorganisms*. Prentice hall Upper Saddle River, NJ, 1997, vol. 11.

[19] G. Guzel Kaya, S. Medaglia, V. Candela-Noguera, M. Á. Tormo-Mas, M. D. Marcos, E. Aznar, H. Deveci, and R. Martínez-Máñez, "Antibacterial activity of linezolid against gram-negative bacteria: Utilization of ε-poly-l-lysine capped silica xerogel as an activating carrier," *Pharmaceutics*, vol. 12, no. 11, p. 1126, 2020.

[20] S. B. Kedare and R. Singh, "Genesis and development of dpph method of antioxidant assay," *Journal of food science and technology*, vol. 48, pp. 412–422, 2011.
[21] H. Amira, F. Benchikh, H. Benabdallah, W. Mamache, S. Amira *et al.*, "Evaluation of antioxidant activities and total phenolic content of hydro-ethanol extract from phlomis bovei de noé areal parts," *Journal of drug delivery and therapeutics*, vol. 10, no. 5, pp. 45–48, 2020.
[22] K. Jakovljević, M. D. Joksović, B. Botta, L. S. Jovanović, E. Avdović, Z. Marković, V. Mihailović, M. Andrić, S. Trifunović, and V. Marković, "Novel 1, 3, 4-thiadiazole conjugates derived from protocatechuic acid: Synthesis, antioxidant activity, and computational and electrochemical studies," *Comptes Rendus Chimie*, vol. 22, no. 8, pp. 585–598, 2019.