SYNTHESIS, CHARACTERIZATION, ENZYMATIC ACTIVITY OF SOME PYRAZOLINE DERIVATIVES, AZO DYES, AND CHALCONES DERIVED FROM ETHYL 4-(TRIFLUOROMETHYL)BENZOATE

Sh.H. Abdullah[1], A.B. Saber[1], K.A. Al-Badrany[1], J.N. Saleh[2], M.J. Saleh[2]

[1] Tikrit University, College of Education for Pure Science, Chemistry Department, Iraq
[2] Salah al-Din Education Directorate, Ministry of Education, Iraq
e-mail: shimaahatem1980@gmail.com, asmaa.b.sabr@tu.edu.iq, khalidaalbadrany@gmail.com, Mohammedjwhersaleh96@gmail.com, jamelnadhemsaleh@gmail.com

Received 12.05.2025
Accepted 08.07.2025

Abstract: *This study reports the use of 4-(trifluoromethyl)benzoate as a key synthone for preparing a series of heterocyclic derivatives. Reaction completion was verified by thin-layer chromatography (TLC), and the products were characterized by physical properties (color, yield, and melting point), Fourier-transform infrared spectroscopy (FT-IR), and nuclear magnetic resonance spectroscopy (^{1}H- and ^{13}C-NMR). The corresponding hydrazide was obtained by reacting 4-(trifluoromethyl)benzoate with aqueous hydrazine, confirmed by the NH_2 stretching bands at 3359 and 3326 cm^{-1} (FT-IR) and a signal at 4.56 ppm (^{1}H-NMR). Subsequent condensation with ethyl acetoacetate produced a pyrazoline intermediate, exhibiting an absorption band at 1606 cm^{-1} (FT-IR) and a carbon signal at 161.13 ppm (^{13}C-NMR). This intermediate was then employed in the synthesis of azo dyes by coupling pyrazolines with substituted anilines in pyridine, confirmed by characteristic FT-IR bands at 1446–1440 cm^{-1} and ^{1}H-NMR signals at 2.23–2.39 ppm (CH). Further reactions with benzaldehyde derivatives in dioxane yielded chalcones, where ^{1}H-NMR signals at 8.14–8.43 ppm corresponded to olefinic protons of the chalcone moiety. Finally, the enzymatic activity of selected compounds (SH_1, SH_2, SH_3, SH_6) was evaluated against alkaline phosphatase (ALP) using a phosphinate substrate, revealing significant bioactivity.*
Keywords: *chalcone, azo dyes, pyrazine, acid hydrazide, enzymatic activity*

Introduction

Chalcone is one of the carbonyl compounds and a common chemical compound in biological and organic systems [1]. It serves as a source for synthesizing significant chemicals used in industry and medicine [2]. A carbonyl group in α-β unsaturated compounds is substituted with an unsaturated group (C=C) [3]. One bond separates the double bond (C=C) from the bond (C=O) [4], and the two bonds alternate between each other. Because of resonance, the chain system is stable because of the coupling between the two groups, which stands for the potential for charges to be dispersed among the four atoms [5]. Chalcones and their derivatives are valued due to the keto ethylenic moiety, CO-CH=CH-. Moistures in the field of synthetic and heterocyclic organic chemistry [6] were produced by the condensation of acetophenones with benzaldehyde replacements [7] using Claisen-Schmidt synthesis [8]. α-β unsaturated compounds that dissolve in organic solvents but not in water are referred to as chalcones. When Kastanek carried out his initial investigations in 1899 to create naturally colored chemicals, he coined the name "chalcone" for the first time [9, 10]. Many plant-based goods, including fruits, tea, and vegetables, include chalcone, a fundamental chemical scaffold [11]. Due to its anti-inflammatory properties [12], chalcone holds a significant position in biochemistry and medicine and possesses an extensive range of biological action. It showed good bacterial activity [13]. Azo dyes have been used for a very long time as both dyes and pigments because of their intense colors, which can be yellow, red, orange, blue, or green depending on molecular structure and delocalization [14]. The dye-fiber interaction and color intensity are determined by the chemical formula and structure of the textile and the dye [15]. The structural element of Azo dyes that gives them their color is the Azo (–N=N–) group. The reason the dye sticks to the fiber is because of the salt that is created between the ionic groups of the dye and the fiber. Due

to the fact that most Azo dyes have acid-base characteristics, such as fixed isosbestic points, which indicate the number of equilibriums in such Azo dyes, these dyes are used to serve as markers of acid-base [16]. Azo-functionalized dyes containing aromatic compounds have attracted increasing interest in recent years because of their broad color spectrum, brightness, simplicity of production, and superior dyeing capabilities [17]. Pyrazoline is a heterocyclic compound containing five atoms, two of which are nitrogen and three of which are carbon, containing a double bond between nitrogen and carbon called azomethine [18].

Experimental part

Chemical used. Chemicals prepared by Aldrich, BDH Thomas, Fluka, and Merck were used.

2.1. Preparation of 4-(trifluoromethyl)benzo hydrazide (SH): Equal moles (0.001 mol) of ethyl 4-(trifluoromethyl)benzoate were mixed with aqueous hydrazine in 10 ml of ethanol. The mixture was stirred for 12 h, and the solution was concentrated, filtered, and recrystallized in ethanol [19]. TLC plates were used to confirm the completion of the reaction (Scheme 1).

Pearly white (76%); mp =110-112 °C; elemental analysis $C_8H_7F_3N_2O$; IR (KBr) v(cm^{-1})= 3359, 3326 (NH_2), 3222 (NH), 3060 (Ar-CH), 1631 (C=O), 1577, 1504 (C=C), 929 (C-F) cm^{-1}; ^{1}H-NMR (DMSO-d^6, 400 MHz): δ(ppm) = 9.96 (1H, s, NH), 7.94-7.67 (4H, d,d, Ar–H), 4.56 (2H, s, NH_2); ^{13}C-NMR (DMSO-d_6, *101* MHz): δ(ppm) = *162.73* (C=O), 140.86-128.61 (Ar-C=C), 126.0 (C-F), as in Fig. s 1-3.

2.2. Preparation of 5-methyl-2-(4-(trifluoromethyl)benzoyl)-2,4-dihydro-3H-pyrazole-3-one (SH_1): Mix (0.001 mol) of 4-(trifluoromethyl)benzo hydrazide was mixed with ethyl acetoacetate in 15 ml of ethanol, and the mixture was raised in a water bath for 3 h. The solution was then filtered and recrystallized from ethanol [20]. TLC confirmed the reaction.

white (72%); mp =131-133 °C; elemental analysis $C_{12}H_9F_3N_2O_2$; IR (KBr) v(cm^{-1})=3091 (Ar-CH), 2981, 2900 ($CH_{ALiphatic}$), 1656 (C=O), 1606 (C=N), 1550, 1485 (C=C), 1274 (C-N), 1058 (N-N), 900 (C-F) cm^{-1}; ^{1}H-NMR (DMSO-d^6, 400 MHz): δ(ppm) = 7.79-7.52 (4H, d, d, Ar–H), 3.33 (2H, s, CH_2), 2.19 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6, *101* MHz): δ(ppm) = 168.68, 165.65 (C=O), 161.13 (C=N), 135.21-129.29 (Ar-C=C), 125.97 (C-F), 51.87 (CH_2), 15,51 (CH_3) (Fig. s 4-6).

2.3. Preparation of Azo dyes (SH_2-SH_3): On crushed ice, a cold aqueous solution of $NaNO_2$ (0.0069 g) was added dropwise with stirring to the nitrate solution of the amine derivative (0.001 mol) previously dissolved in aqueous hydrochloric acid (10 mL). This process produced a clear solution of the diazonium salt of the amine. The temperature was maintained between 0 and 5°C. A solution of 5-methyl-2-(4-(trifluoromethyl)benzoyl)-2,4-dihydro-3H-pyrazole-3-one (0.001 mol) dissolved in pyridine (5 mL) was then filled with this mixture. The temperature was maintained between 0 and 5 °C while stirring the reaction mixture for 2 h. and then the mixture was added to water with continuous stirring. Afterwards, it was cleaned, filtered, and recrystallized from ethanol [21].

SH_2: Yellow (75%); mp = 148-150°C; elemental analysis $C_{18}H_{11}Cl_2F_3N_4O_2$; IR (KBr) v($cm^{-1}$)= *3089* (Ar-CH), 298*5*, 29*35* ($CH_{ALiphatic}$), 1664 (C=O), 16*18* (C=N), 155*4*,1*508* (C=C),*1446* (N=N), 1267 (C-N), 1072(N-N), 997 (C-F), 767 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ(ppm) = 7.98-7.27 (7H, m, Ar–H), 2.23 (1H, s, CH), 1.76 (3H, s, CH_3); ^{13}C-NMR (DMSO-d^6, *101* MHz): δ(ppm) = 168.86,165.00 (C=O), 159.34 (C=N), 137.54-125.74 (Ar-C=C), 123.03 (C-F), 59.38 (CH), 22.45 (CH_3).

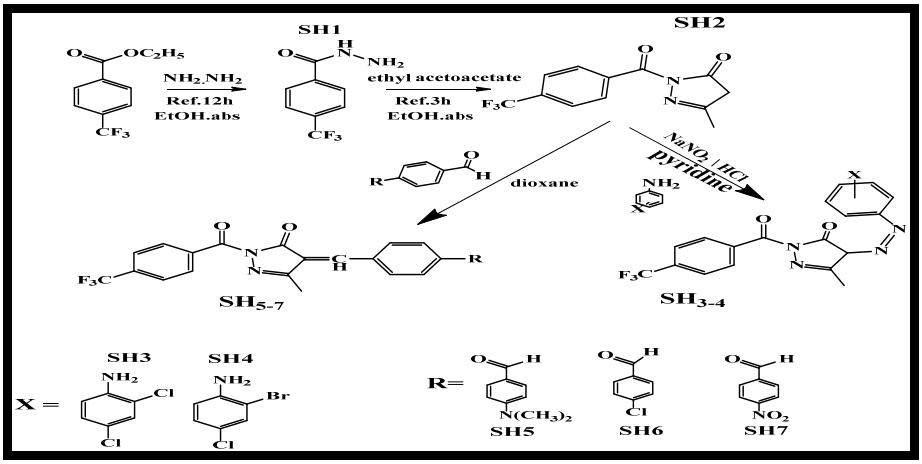
SH_3: Off-White (68%); mp = 167°C; elemental analysis $C_{18}H_{11}BrClF_3N_4O_2$; IR (KBr) v(cm^{-1})= 3055 (Ar-CH), 2902, 2848 ($CH_{ALiphatic}$), 1662 (C=O), 1598 (C=N), 1548,1*525* (C=C), 1440 (N=N), 1284 (C-N), 1062 (N-N), 945 (C-F), 813 (C-Cl), 617 (C-Br) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ(ppm) = 7.80-7.07 (7H, m, Ar–H), 2.39 (1H, s, CH), 2.03 (3H, s, CH_3); ^{13}C-NMR (DMSO-d^6, *101* MHz): δ(ppm) = 164.94,162.48 (C=O), 160.92 (C=N), 132.18-125.93 (Ar-C=C), 125.70 (C-F), 54.69 (CH), 38.37 (CH_3) (see Figures 7-12).

2.4. Preparation of Chalcone (SH_4-SH_6): Mix (0.001 mol) of each of 5-methyl-2-(4-(trifluoromethyl)benzoyl)-2,4-dihydro-3H-pyrazole-3-one and benzaldehyde substitutes with the addition of 5 mL of 10% NaOH in 20 mL of 1,4-dioxane and stir the mixture in a water bath for 3 hours at 25-40°C, then neutralize the medium in the presence of HCl [22, 23], filter, and recrystallize from ethanol.

SH_4: Yellow (65%); mp = 220-222°C; elemental analysis $C_{21}H_{18}F_3N_3O_2$; IR (KBr) v(cm^{-1})= 3043 (Ar-CH), 2916, 2833 ($CH_{ALiphatic}$), 1650 (C=O), 1604 (C=N), 1560, 1*508* (C=C), 1234 (C-N), 1062(N-N), 918 (C-F) cm^{-1}; ^{1}H-NMR (DMSO-d^6, 400 MHz): δ(ppm) = 8.43(1H, s, =CH), 7.84-7.32 (8H, m, Ar–H), 3.06 (6H, s, $(CH_3)_2$), 2.42 (3H, s, CH_3); ^{13}C-NMR (DMSO-d^6, *101* MHz): δ(ppm) = 162.50, 161.25 (C=O), 147.28 (C=N), 139.43 (=CH), 133.67-111.17 (Ar-C=C), 125.96 (C-F), 52.46 (CH_3), 19.01 (CH_3).

SH_5: Light Yellow (63%); mp = 207-209 °C; elemental analysis $C_{19}H_{12}ClF_3N_2O_2$; IR (KBr) v(cm^{-1})= 3024 (Ar-CH), 2918, 2856 ($CH_{ALiphatic}$), 1647 (C=O), 1608 (C=N), 1541, 1*515* (C=C), 1282 (C-N), 1062(N-N), 914 (C-F), 748 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO-d^6, 400 MHz): δ(ppm) = 8.14 (1H, s, =CH), 7.80-7.17 (8H, m, Ar–H), 2.36 (3H, s, CH_3); ^{13}C-NMR (DMSO-d^6, *101* MHz): δ(ppm) = 169.34, 166.79 (C=O), 152.77 (C=N), 147.71 (=CH), 137.56-123.00 (Ar-C=C), 125.98 (C-F), 23.42 (CH_3).

SH_6: Light Yellow (70%); mp = 213-215 °C; elemental analysis $C_{19}H_{12}ClF_3N_3O_4$; IR (KBr) v(cm^{-1})= 3033 (Ar-CH), 2956, 2837 ($CH_{ALiphatic}$), 1645 (C=O), 1606 (C=N), 1558, 1467 (C=C), 1288 (C-N), 1064 (N-N), 916 (C-F), 1506, 1326 (NO_2) cm^{-1}; ^{1}H-NMR (DMSO-d^6, 400 MHz): δ(ppm) = 8.33 (1H, s, =CH), 7.58-6.76 (8H, m, Ar–H), 2.16 (3H, s, CH_3); ^{13}C-NMR (DMSO-d^6, *101* MHz): δ(ppm) = 164.26, 161.92 (C=O), 149.87 (C=N), 138.07 (=CH), 131.88-121.77 (Ar-C=C), 123.05 (C-F), 30.00 (CH_3) (see Fig. s 13-21).



Scheme 1. Prepared compounds (SH-SH_6)

2.5. Evaluation of enzymatic activity. The effect of the compounds (SH_1, SH_2, SH_3, SH_6) on the alkaline phosphate enzyme ALP in human blood serum (normal, non-diseased cases) was reported. Solutions of these compounds were prepared at 7 different concentrations (mg/10 ml), and were dissolved using DMSO in preparing these solutions. The concentration of the base material was 0.3 mol. The enzyme activity was measured using the method described in the ready-made kit for measuring the enzyme activity GISSE DIAGNOSTICS [24] by adding 1 ml of the prepared compound solution to the base material solution. The inhibition is calculated by comparing the activity with and without the prepared compound under the same conditions according to the following equation [25]:

$$\%\text{inhibition} = 100 - \left[\frac{activity\ with\ inhibition}{activity\ without\ inhibition}\right] - 100$$

Results and discussions

3.1. Characterization of prepared compounds (SH-SH$_6$). In the first step, hydrazide was prepared by reacting ethyl 4-(trifluoromethyl) benzoate with hydrated hydrazine. The disappearance of the aliphatic (CH) bond was observed in the Fourier transform infrared (FT-IR) spectrum with a clear decrease in the (C=O) bond, due to its association with the amine group (NH) that appeared at (3222) cm^{-1} and the appearance of a double band for (NH_2) at 3359 and 3326 cm^{-1}, as evidenced by the hydrogen nuclear magnetic resonance (^{1}H-NMR) spectrum, which showed a signal at 9.96 ppm for (NH_2) and a signal at 4.56 ppm for (NH_2). As for the ^{13}C-NMR nuclear magnetic resonance spectrum, a decrease in the value of (C=O) was observed for the same reason mentioned above [26]. In the second step, pyrazoline was synthesized by condensing equimolar amounts of the hydrazide (prepared in step one) with ethyl acetoacetate. FT-IR analysis confirmed the reaction by showing the disappearance of NH and NH_2 absorption bands and the emergence of an azomethine (C=N) stretching band at 1606 cm^{-1}, indicating the formation of a five-membered pyrazoline ring. The ^{1}H-NMR spectrum further supported this transformation, revealing the absence of amine proton signals and the appearance of a signal at 3.33 ppm corresponding to the CH_2 group and a signal at 2.19 ppm corresponding to the CH_3 group. In addition, the ^{13}C-NMR spectrum displayed a new carbonyl (C=O) resonance at 165.65 ppm and an azomethine (C=N) resonance at 161.13 ppm, providing further evidence of pyrazoline ring formation [27].

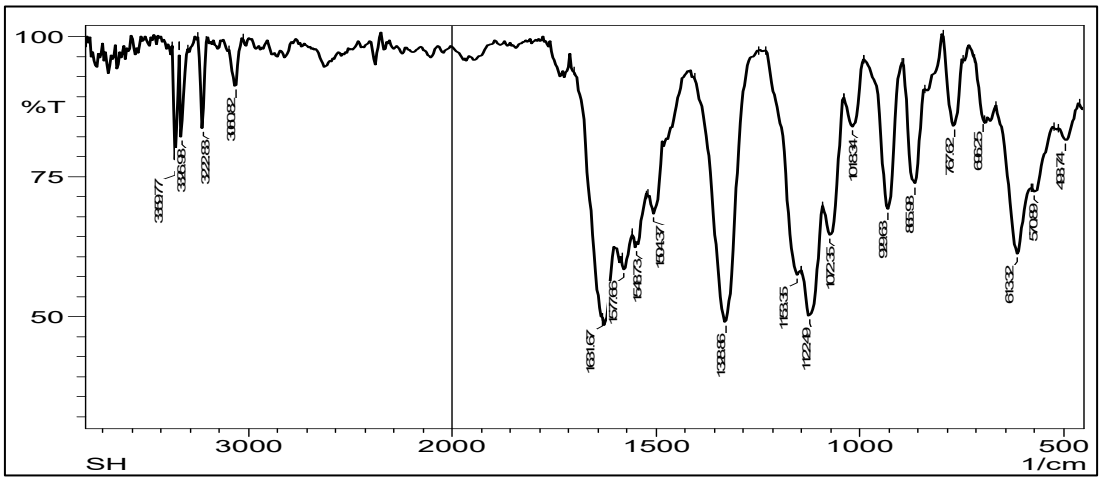


Fig. 1. FT-IR spectrum for the compound (SH)

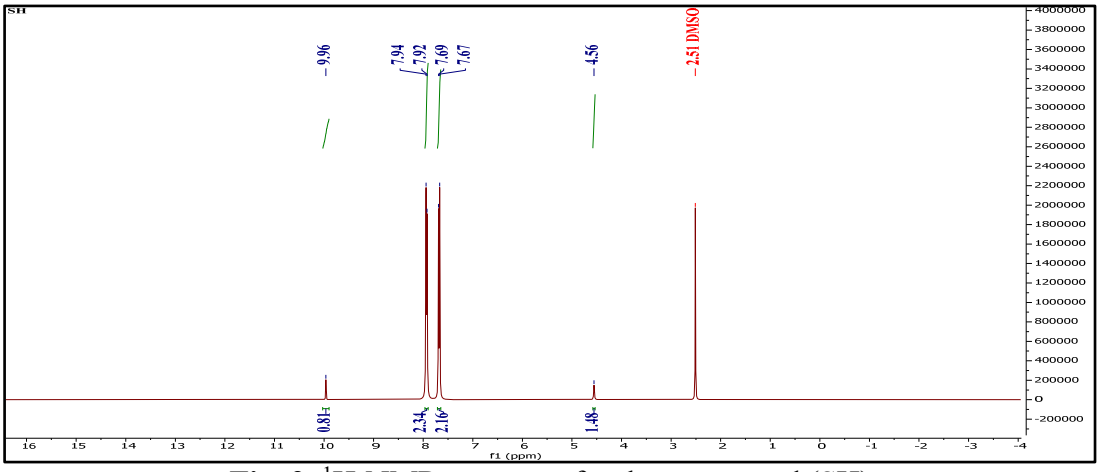


Fig. 2. ^{1}H-NMR spectrum for the compound (SH)

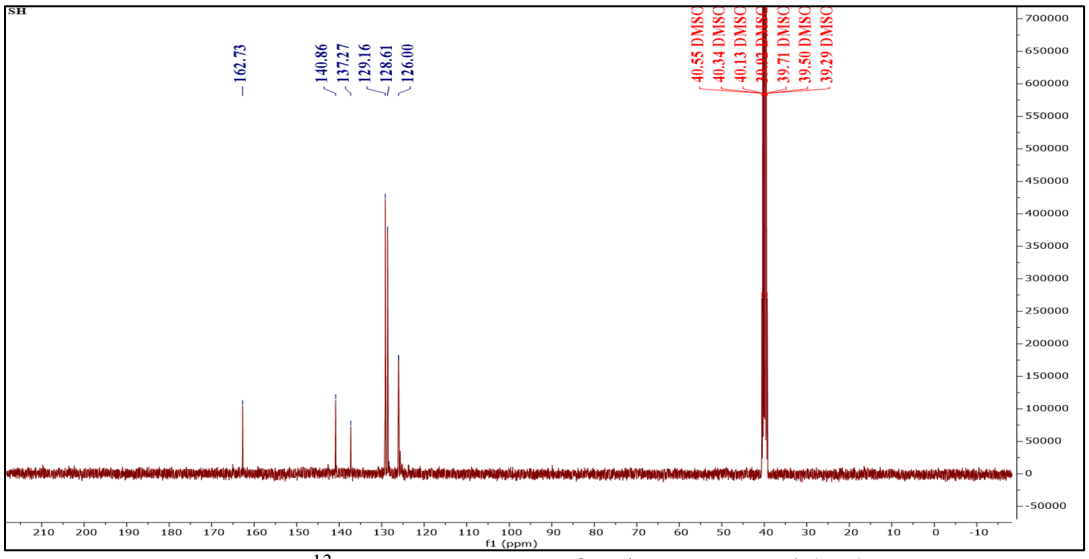


Fig. 3. ^{13}C-NMR spectrum for the compound (SH)

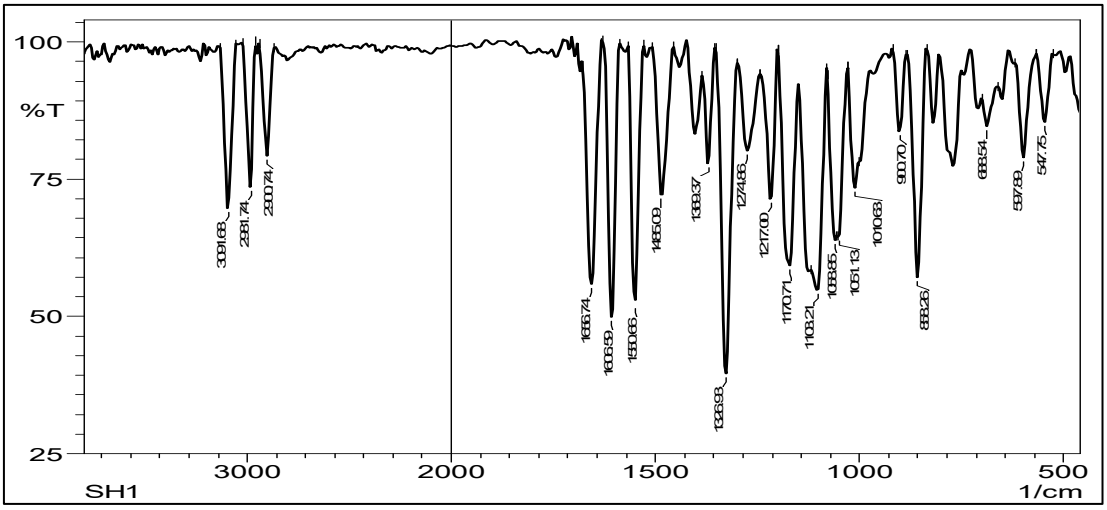


Fig. 4. FT-IR spectrum for the compound (SH_1)

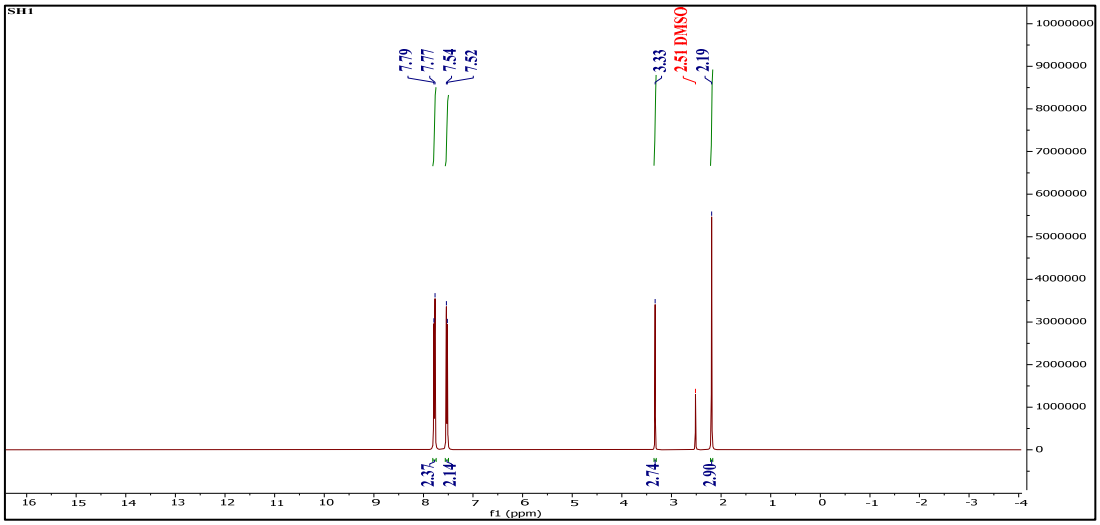


Fig. 5. ^{1}H-NMR spectrum for the compound (SH_1)

In the third step, azo dyes were prepared by reacting the resulting pyrazole SH1 with the diazonium salt prepared from aniline substituents. The Fourier transform infrared spectrum confirmed the reaction, as a signal appeared at (1446, 1440) cm^{-1} due to (N=N), which is a clear indication of the completion of the reaction. The hydrogen nuclear magnetic resonance (H-NMR) spectrum showed the disappearance of the CH_2 group and the appearance of a signal at (2.23, 2.39) ppm due to (CH), in addition to an increase in the number of aromatic ring signals. The same applies to the ^{13}NMR spectrum of carbon [28].

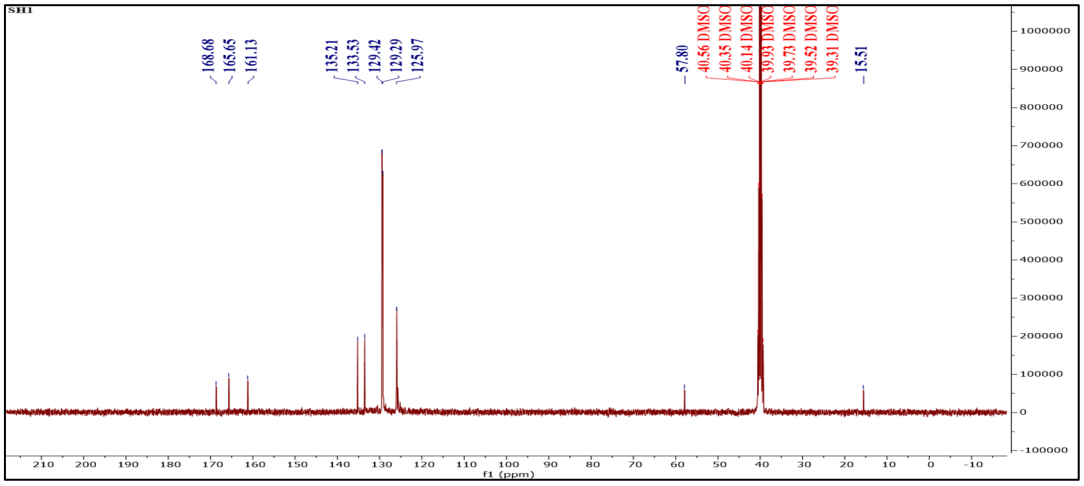


Fig. 6. ^{13}C-NMR spectrum for the compound (SH_1)

In the fourth step, chalcones were prepared by reacting the pyrazoline prepared in the second step with benzaldehyde substituents. This reaction was confirmed by spectroscopic measurements. The FT-IR spectrum showed a decrease in the (C=O) group, but its association with the double bond that appeared at the same position as azomethine in the measurements at (1604-1608) cm^{-1}. The hydrogen nuclear magnetic resonance (^{1}H-NMR) spectrum showed a signal in the range (8.14-8.43) ppm that returned (=CH) consecutively with the carbonyl, indicating the completion of the reaction with the disappearance of the (CH_2) group. Similarly, the ^{13}C-NMR spectrum showed the disappearance of this group, revealing a group adjacent to the azomethine attributed to (=CH) in the range (138.07-147.71) ppm, evidence of the localization of the chalcones [29-35].

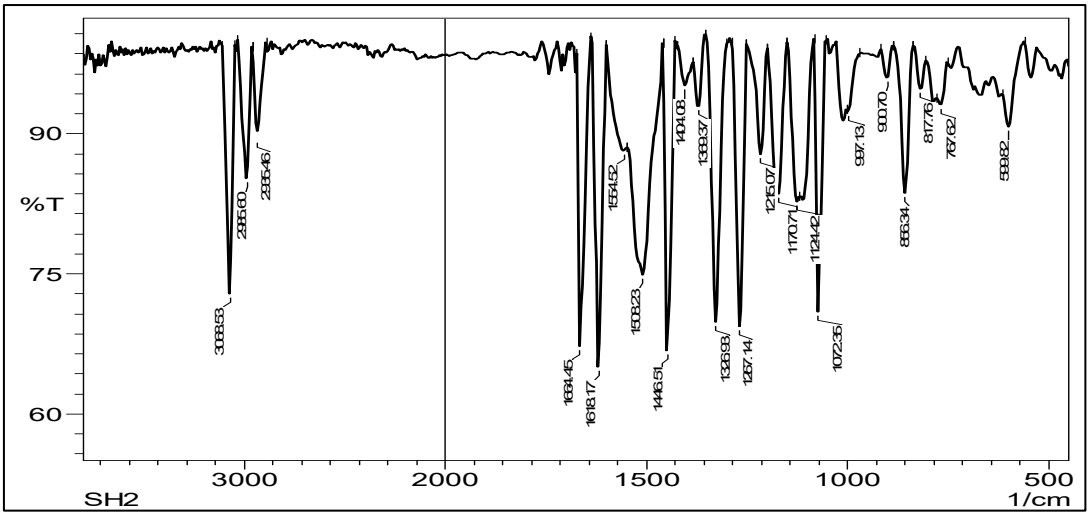


Fig. 7. FT-IR spectrum for the compound (SH_2)

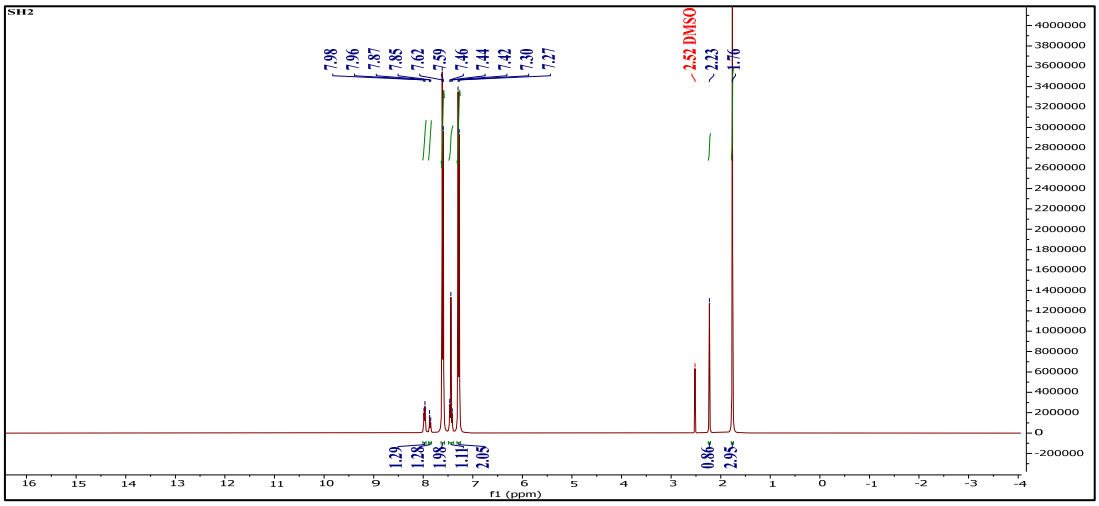


Fig. 8. ^{1}H-NMR spectrum for the substance (SH_2)

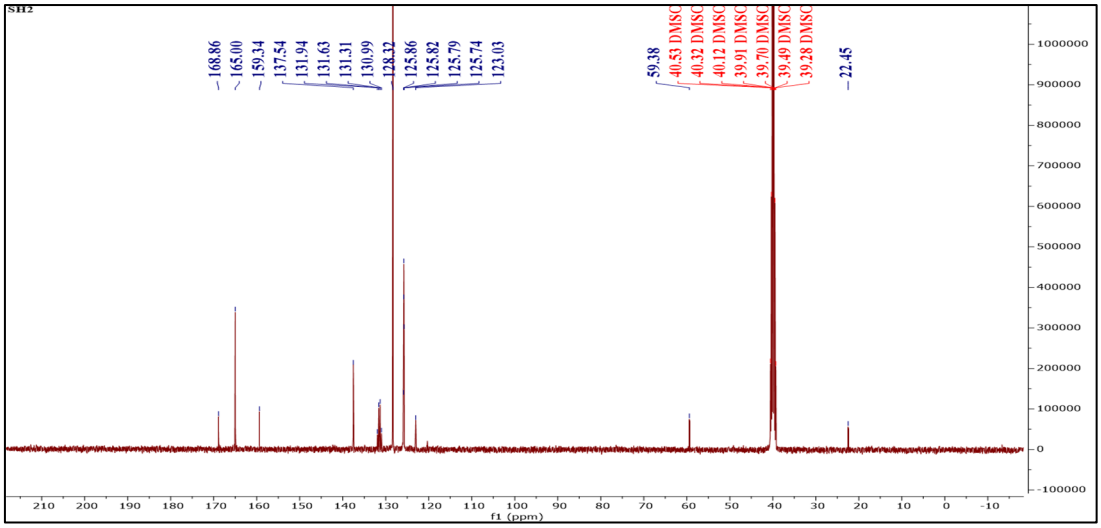


Fig. 9. ^{13}C-NMR spectrum for the compound (SH_2)

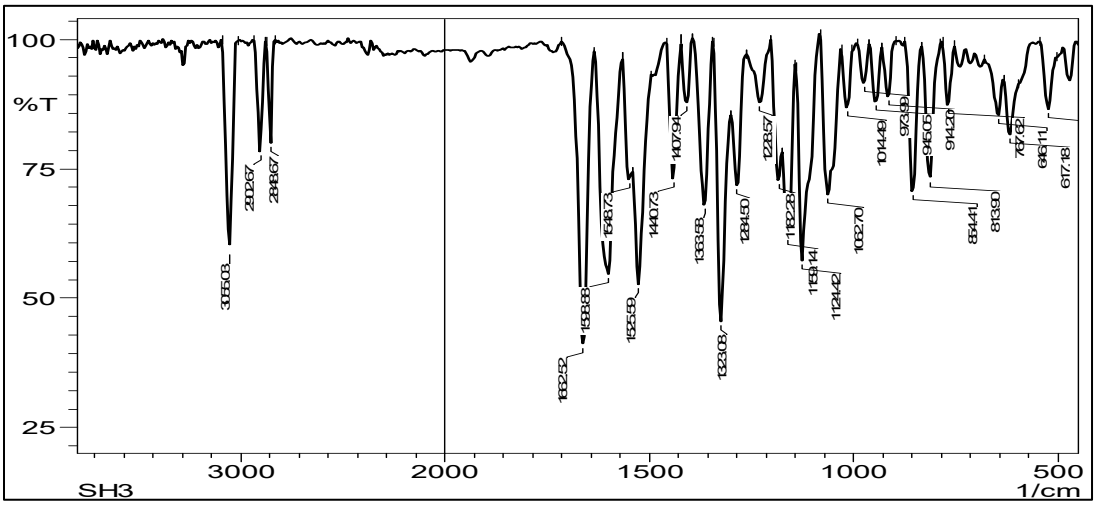


Fig. 10. FT-IR spectrum for the compound (SH_3)

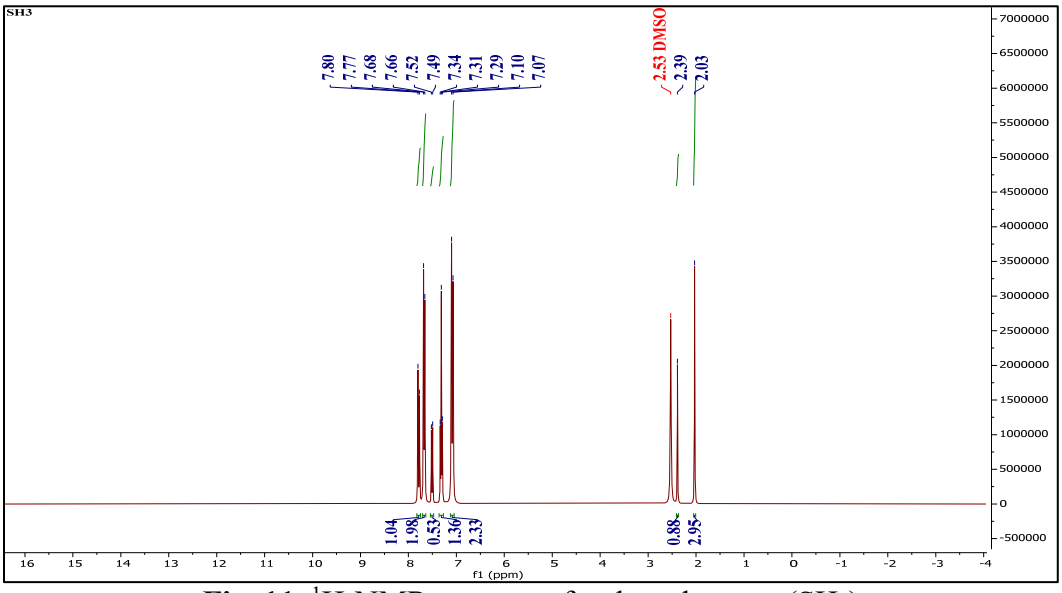


Fig. 11. ^{1}H-NMR spectrum for the substance (SH_3)

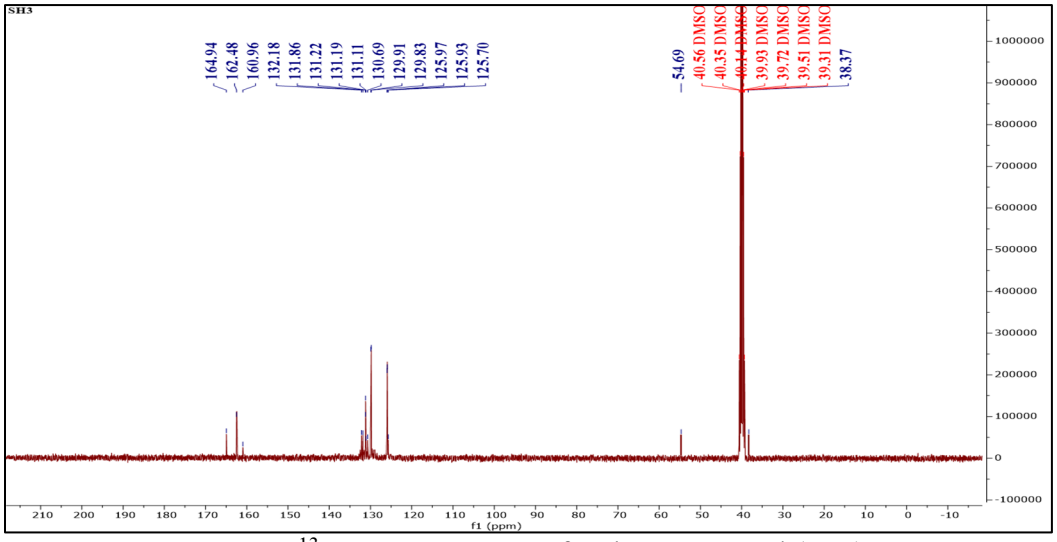


Fig. 12. ^{13}C-NMR spectrum for the compound (SH_3)

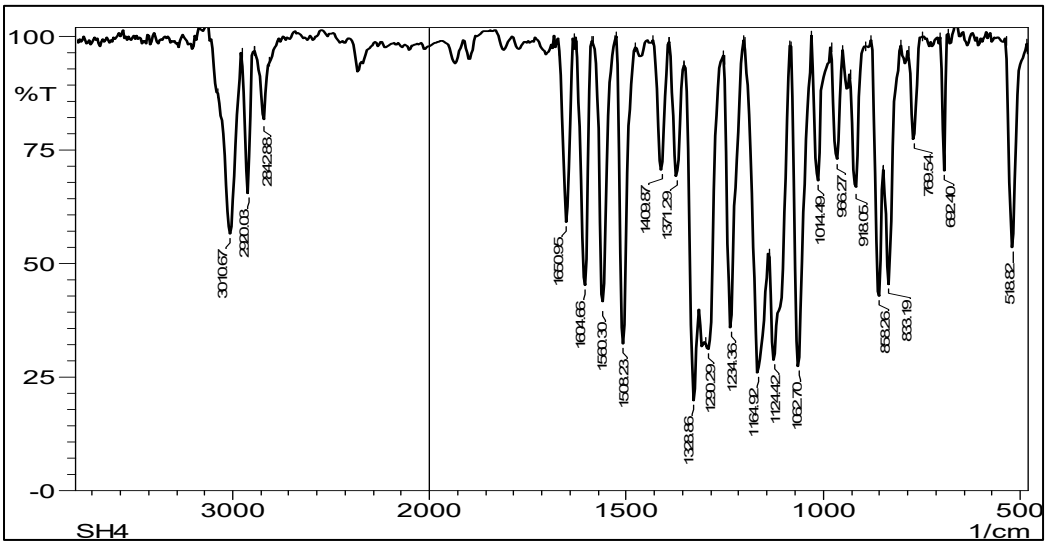


Fig. 13. FT-IR spectrum for the compound (SH_4)

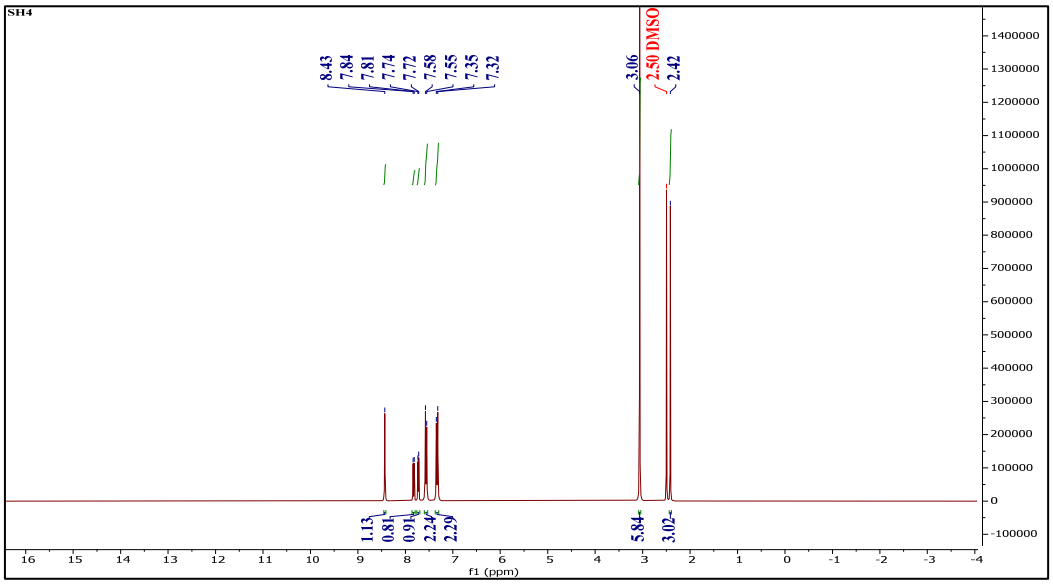


Fig. 14. ^{1}H-NMR spectrum for the substance (SH_4)

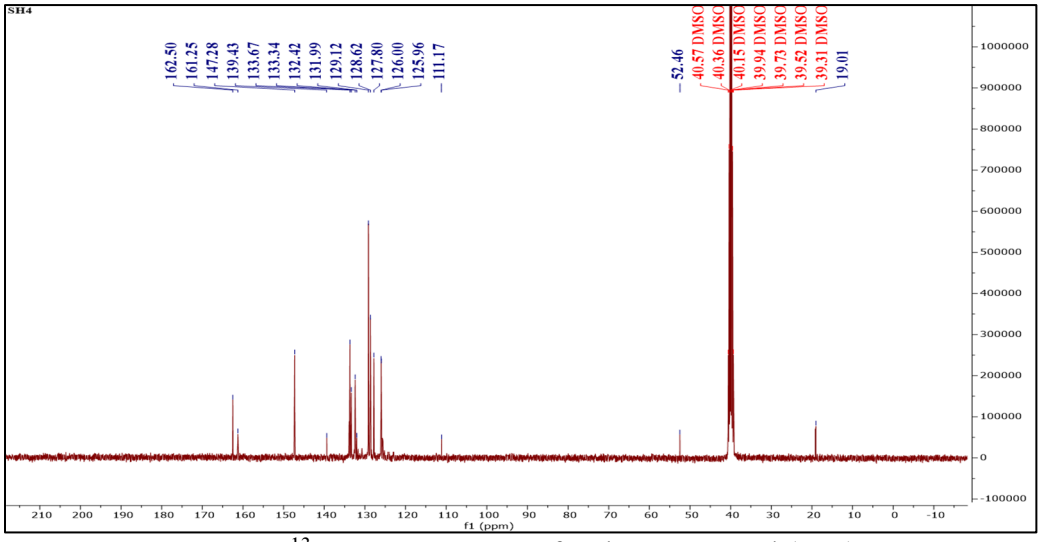


Fig. 15. ^{13}C-NMR spectrum for the compound (SH_4)

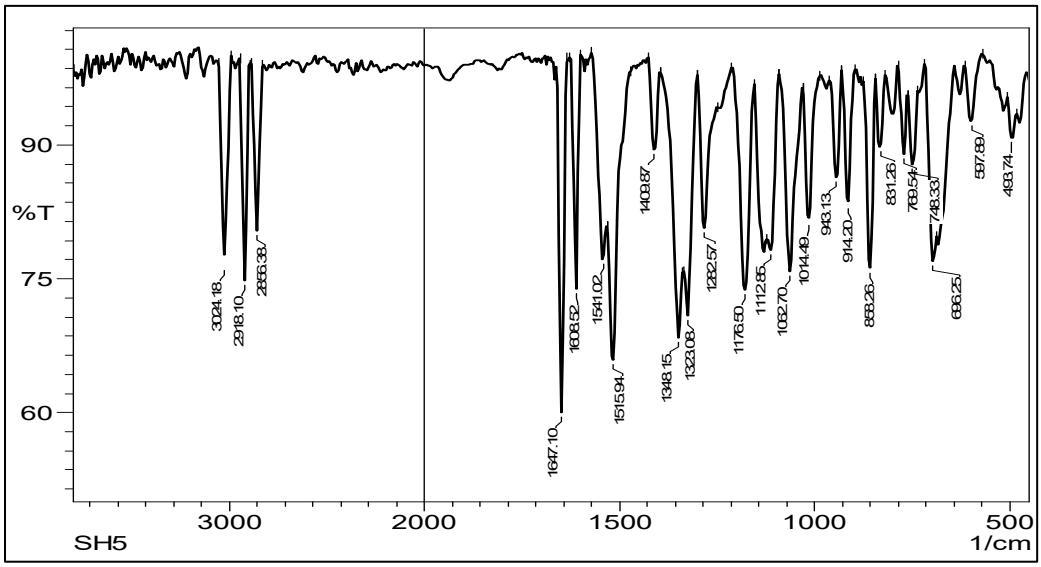


Fig. 16. FT-IR spectrum for the compound (SH_5)

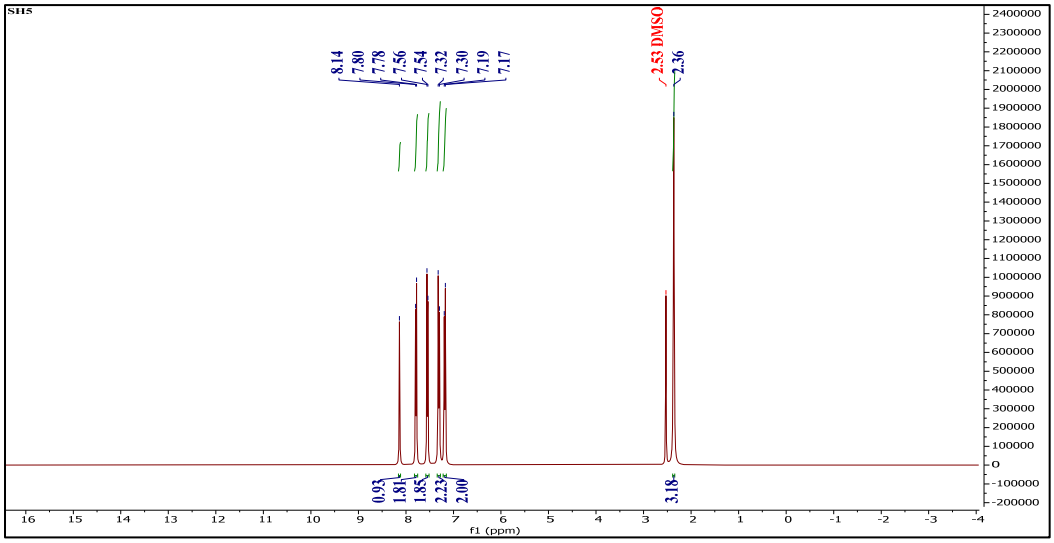


Fig. 17. ^{1}H-NMR spectrum for the substance (SH_5)

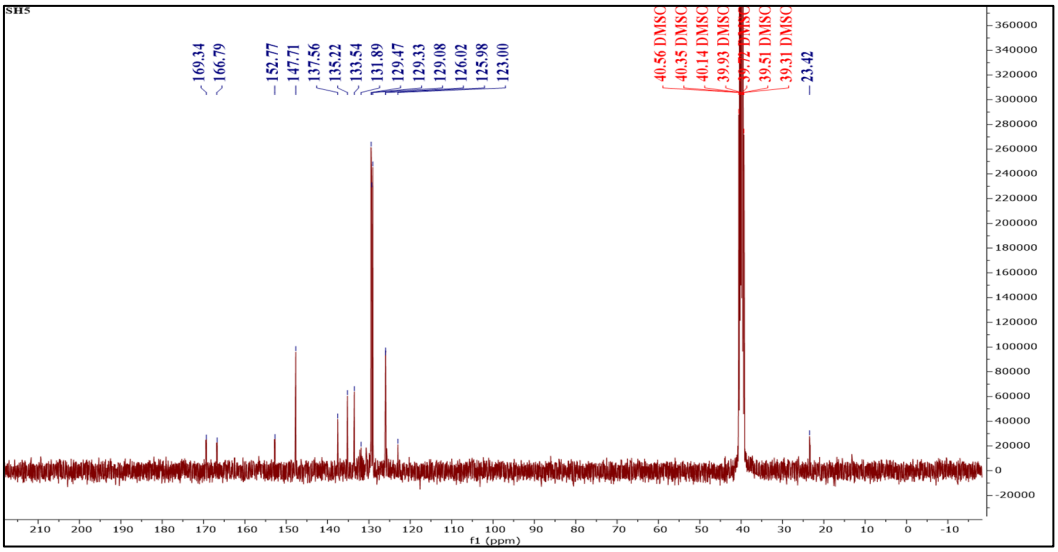


Fig. 18. ^{13}C-NMR spectrum for the compound (SH_5)

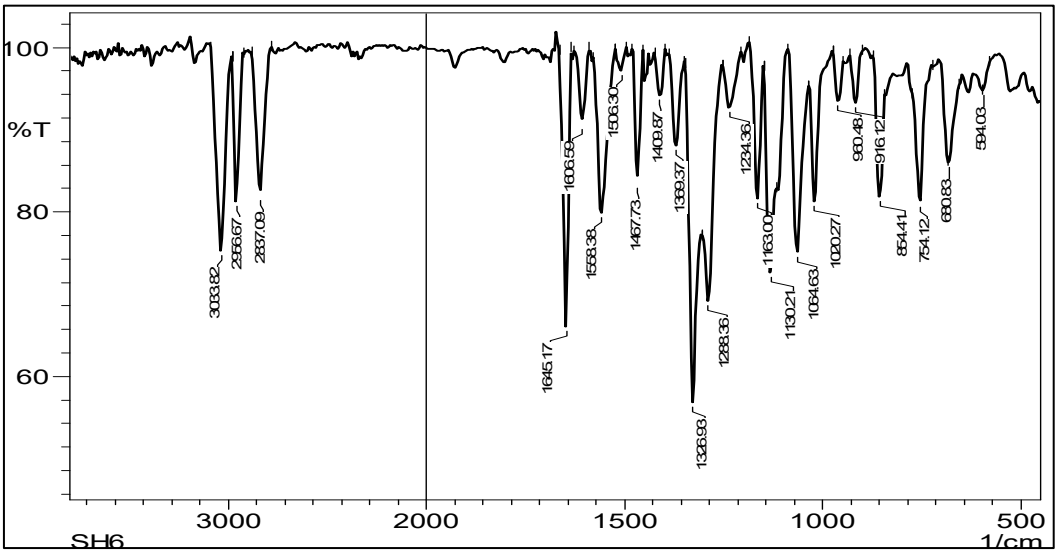


Fig. 19. FT-IR spectrum for the compound (SH_6)

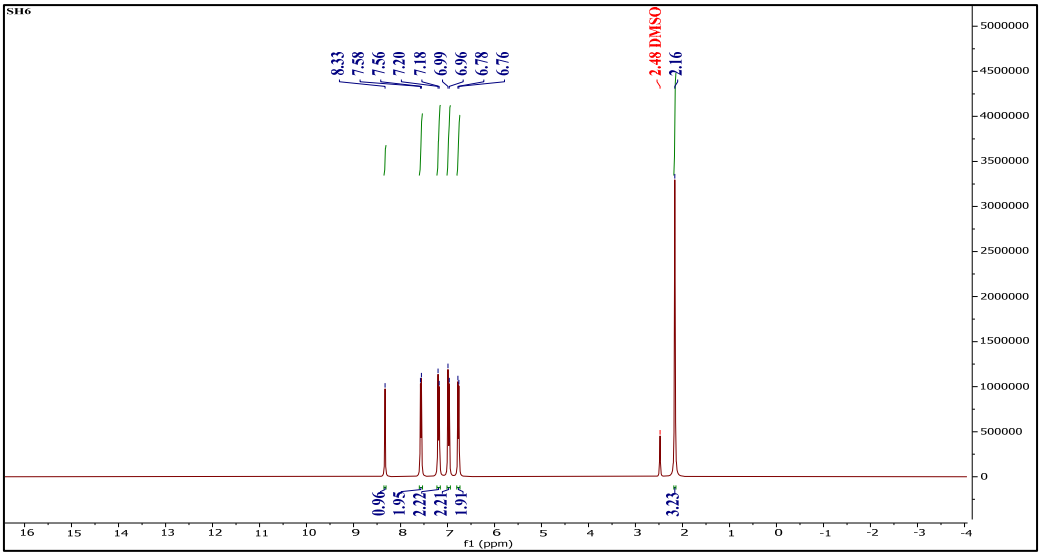


Fig. 20. ^{1}H-NMR spectrum for the substance (SH_6)

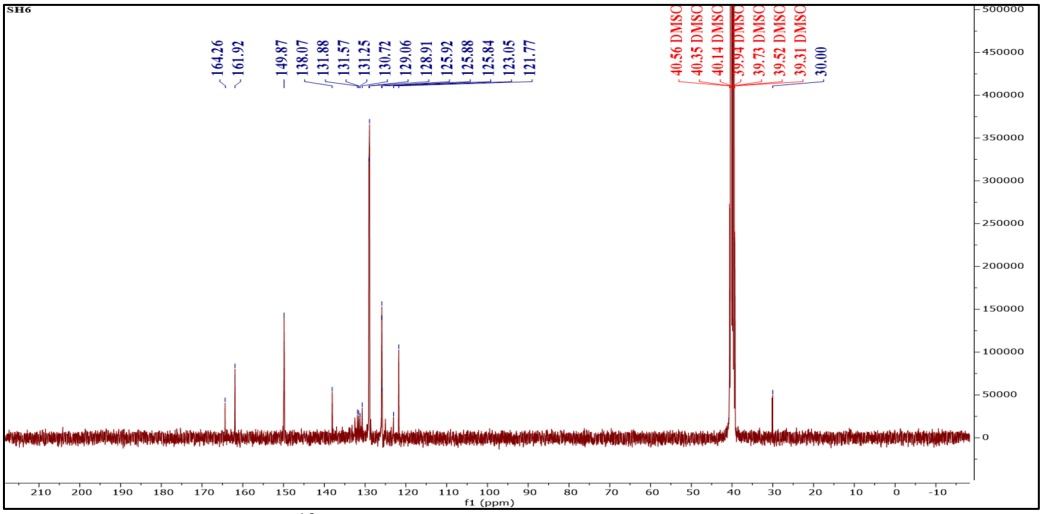


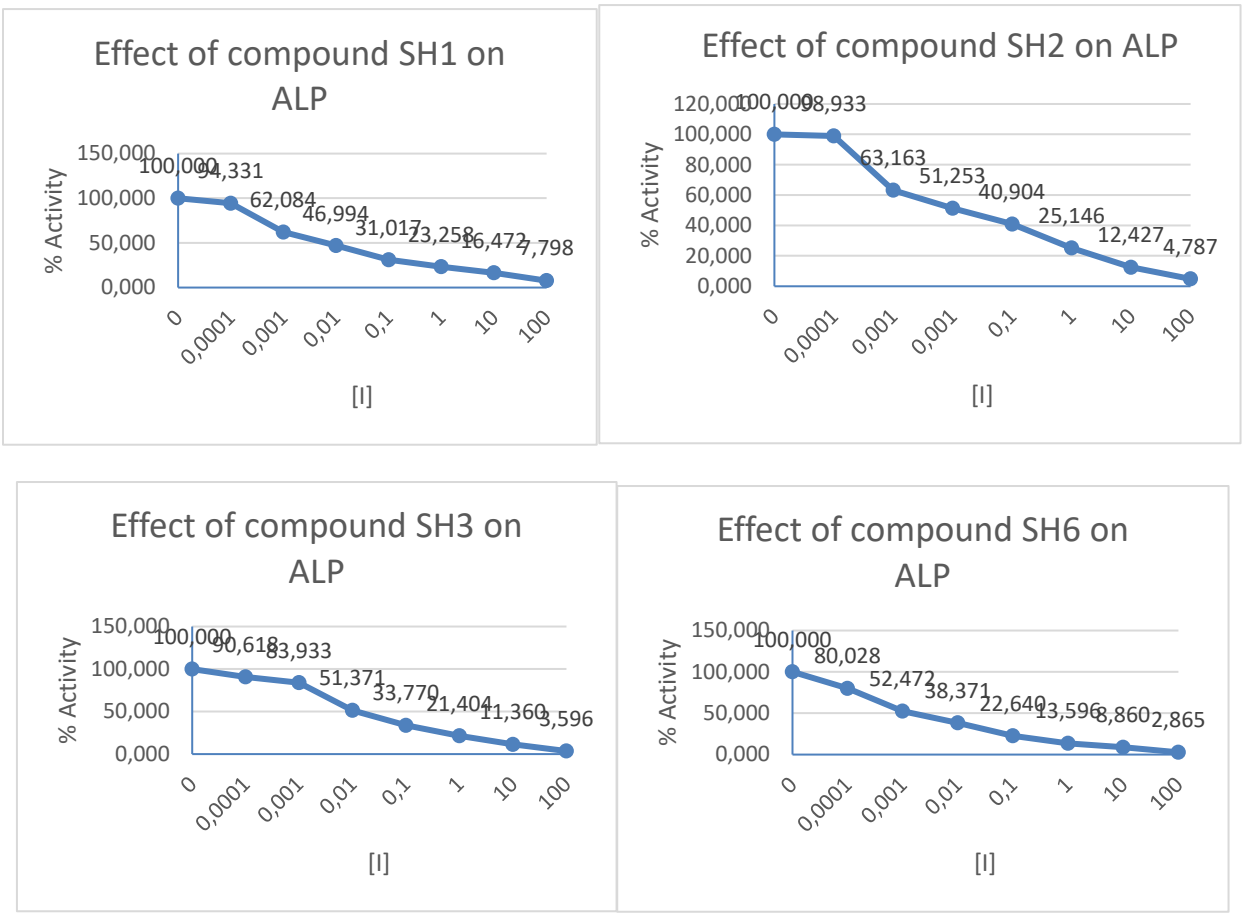
Fig. 21. ^{13}C-NMR spectrum for the substance (SH_6)

3.2. Enzymatic activity study. The compounds (SH_1, SH_2, SH_3, SH_6) showed that they have inhibitory activity against the basic ALP enzyme, as the study showed that the higher the concentration of the inhibitor, the lower the enzyme activity. This type of inhibition is competitive, as it competes with the substrate to bind to the active site of the enzyme. This inhibition can be removed by increasing the concentration of the substrate, which leads to the inhibitor binding to the active site of the enzyme and facilitates the binding of the substrate to the enzyme, which is the basic phosphines, the active site of the enzyme located on the surface of the enzyme [31-32] (Table 2 and Scheme 2).

Table 2. Efficacy of ALP enzyme against compounds (SH_1, SH_2, SH_3, SH_6)

Inhibitor Conce mg\10mL	SH_1		SH_2		SH_3		SH_6	
	Activity	% Activity	Activity	% Activity	Activity	% Activity	Activity	% Activity
0	178.0000	100.000	178.0000	100.000	178.0000	100.000	178.0000	100.000

0.0001	167.9100	94.331	176.1000	98.933	161.3000	90.618	142.4500	80.028
0.001	110.5100	62.084	112.4300	63.163	149.4000	83.933	93.4000	52.472
0.01	83.6500	46.994	91.2300	51.253	91.4400	51.371	68.3000	38.371
0.1	55.2100	31.017	72.8100	40.904	60.1100	33.770	40.3000	22.640
1	41.4000	23.258	44.7600	25.146	38.1000	21.404	24.2000	13.596
10	29.3200	16.472	22.1200	12.427	20.2200	11.360	15.7700	8.860
100	13.8800	7.798	8.5200	4.787	6.4000	3.596	5.1000	2.865



Scheme 2. Effect of compounds (SH_1, SH_2, SH_3, SH_6) on ALP

Conclusions

This study successfully synthesized a series of pyrazoline derivatives, azo dyes, and chalcones using ethyl 4-(trifluoromethyl)benzoate as a core structure. The characterization of these compounds was confirmed through FT-IR, 1H-NMR, and 13C-NMR spectroscopy, along with Thin Layer Chromatography (TLC) for reaction monitoring. The synthesized compounds exhibited high purity and good product yields, as demonstrated by their spectral and physical analyses.

According to the study's findings on biological activity, the Alkaline Phosphatase (ALP) enzyme was significantly inhibited by a few produced chemicals (SH_1, SH_2, SH_3, and SH_6). SH_2 had the strongest activity, and the inhibition was concentration-dependent, suggesting possible uses in pharmacological and biochemical research. The produced azo dyes also showed excellent color stability, which makes them viable options for industrial and textile dyeing applications.

According to the results, chalcones and pyrazoline derivatives may be useful starting points for more research on drug development and enzyme inhibition. To fully investigate the therapeutic

potential of these substances, future studies should concentrate on extending biological assessments, such as antibacterial and anti-inflammatory evaluations. Furthermore, to maximize the potential for enzyme inhibition in pharmaceutical applications, structure-activity relationship (SAR) investigations are advised. All things considered, this study advances the domains of organic synthesis, enzymatic inhibition, and dye chemistry, laying a solid basis for upcoming developments in industrial and medical chemistry.

References

1. Sekar P., Kumar S., Raju S.K. Chemistry and synthetic methodologies of chalcones and their derivatives: A review. *International Journal of Biological and Pharmaceutical Sciences Archive*, 2023, **Vol. 1**, p. 051-072. DOI: 10.53771/ijbpsa.2023.5.1.0020
2. Al-Tufah M. Synthesis and characterization of some new cyclohexenone compounds derived from chalcones and evaluation of bacterial activity. *College Of Basic Education Research Journal*, 2022, **Vol. 4**, p. 784-790. DOI:10.22034/CHEMM.2023.388126.1654
3. Bandeira P.N., Lemos, T.L.G, Santos H.S., de Carvalho M.C.S., Pinheiro D.P., de Moraes O., Filho M., Pessoa C., Barros-Nepomuceno F.W.A., Rodrigues T.H.S., Ribeiro P.R.V., Magalhães H.S., Teixeira A.M.R. Synthesis, structural characterization, and cytotoxic evaluation of chalcone derivatives. *Medicinal Chemistry Research*, 2019, **Vol. 28**, p. 2037-2049. DOI:10.1007/s00044-019-02434-1
4. da Silva L., Donato I.A., Bezerra S.R., Dos Santos H.S., Bandeira P.N., do Nascimento M.T.R., Guedes J.M., Freitas P.R., de Araújo A.C.J., de Freitas T.S., Coutinho H.D.M., de Matos Y.M.L.S., de Oliveira L.C.C., da Cunha F.A.B. Synthesis, spectroscopic characterization, and antibacterial activity of chalcone (2E)-1-(3′-aminophenyl)-3-(4-dimethylaminophenyl)-prop-2-en-1-one against multiresistant Staphylococcus aureus carrier of efflux pump mechanisms and β-lactamase. *Fundamental & Clinical Pharmacology*, 2024, **Vol. 1,** p. 60-71. DOI: 10.1111/fcp.12938
5. Hamad A.A., Omer R.A., Kaka K.N., Amin A.A., Ahmed K.M., Rashid R.F., Aggarwal T. A Review: New Synthesis of Chalcone Derivatives and Their Applications. *Chemical Review and Letters*. 2025, **Vol. 8**, p. 329-351. DOI: 10.22034/crl.2025.480229.1427
6. Chowdhary S., Preeti S., Gupta N., Kumar R., Kumar V. Advances in chalcone-based anticancer therapy: mechanisms, preclinical advances, and future perspectives. *Expert Opinion on Drug Discovery*, 2024, **Vol. 12**, p. 1417-1437. DOI: 10.1080/17460441.2024.2436908
7. Zhang N., Zeng W., Zhou Q., Sun Z., Meng K., Qin Y., Hu Y., Xue, W. Design, synthesis, antibacterial and antiviral evaluation of chalcone derivatives containing benzoxazole. *Arabian Journal of Chemistry*, 2024, **Vol. 1**, 105368. DOI: 10.1016/j.arabjc.2023.105368
8. Shalaby M.A., Rizk S.A., Fahim A.M. Synthesis, reactions and application of chalcones: A systematic review. *Organic & Biomolecular Chemistry*, 2023, **Vol. 26**, p. 5317-5346. DOI: 10.1039/D3OB00792H
9. El-Naggar M., Rashdan H.R., Abdelmonsef A.H. Cyclization of chalcone derivatives: design, synthesis, in silico docking study, and biological evaluation of new quinazolin-2, 4-diones incorporating five-, six-, and seven-membered ring moieties as potent antibacterial inhibitors. *ACS omega*, 2023,**Vol. 30**, p. 27216-27230. DOI: 10.1021/acsomega.3c02478
10. Kudličková Z., Michalková R., Salayová A., Ksiažek M., Vilková M., Bekešová S., Mojžiš J. Design, synthesis, and evaluation of novel indole hybrid chalcones and their antiproliferative and antioxidant activity. *Molecules*, 2023, **Vol. 18**, 6583. DOI:10.3390/molecules28186583
11. Cáceres M., Kesternich V., Pérez-Fehrmann M., Castroagudin M., Nelson R., Quezada V., Christen P., Castro-Alvarez A., Cárcamo J.G. Ultrasound-Assisted Synthesis of Substituted Chalcone-Linked 1, 2, 3-Triazole Derivatives as Antiproliferative Agents: In Vitro Antitumor

Activity and Molecular Docking Studies. *International Journal of Molecular Sciences*, 2025, **Vol. 7**, 3389. DOI: 10.3390/ijms26073389

12. Guazelli C.F., Fattori V., Ferraz C.R., Borghi S.M., Casagrande R., Baracat M.M., Verri Jr W.A. Antioxidant and anti-inflammatory effects of hesperidin methyl chalcone in experimental ulcerative colitis. *Chemico-biological interactions*, 2021, **Vol.** 333, 109315. DOI: 10.1016/j.cbi.2020.109315
13. Reda N.A., Khalid A.A.B. The use of 2-acetyl pyrazine as a basic nucleophile for the preparation of new derivatives of the 5, 6-dihydropyridine-2 (1h)-ylidene) cyanamide ring, their diagnosis, and evaluation of their bacterial efficacy. *European Journal of Modern Medicine and Practice*, 2024, **Vol. 5,** p. 508-518.
14. Raghav M.H., Patel V.S. Synthesis and application of 2-aminothiadiazole disperse dyes for nylon fabrics. *Journal of the Serbian Chemical Society*, 2001, **Vol. 2**, p. 87-93. DOI: 10.2298/JSC0102087R
15. Saini R., Choudhary K. Toxic potential of azo dyes: A broader understanding. In *Hazardous Chemicals.* 2025, **Vol. 8,** p. 469-481. DOI: 0.1016/B978-0-323-95235-4.00039-6
16. Kurda D.D. Synthesis, characterization, and dye performance investigations of azo-coumarin bearing chalcone moieties. *Zanco Journal of Pure and Applied Sciences*, 2023, **Vol. 6**, p. 85-105. DOI: 10.2298/JSC190123049A
17. Ali A.A. Synthesis and Spectroanalytical Studies of a New Azodye Derived From 2-Amino-6-ethoxybenzothiazole and 4Chloro-3, 5-dimethylphenol and its Complexes With Fe (III) Ion. *Ibn AL-Haitham Journal For Pure and Applied Sciences*, 2014, **Vol. 1**, p. 196-211.
18. Sihag M., Manuja A., Rani S., Soni R., Rani N., Malik S., Bhardwaj K., Kumar B., Kinger M., Miglani M., Aneja D.K. Synthesis of pyrazole and pyrazoline derivatives of β-ionone: Exploring anti-inflammatory potential, cytotoxicity, and molecular docking insights. *European Journal of Medicinal Chemistry Reports*, 2024, **Vol. 12**, 100204. DOI: 10.1016/j.ejmcr.2024.100204
19. Yeap G.Y., Mohammad A.T., Osman H. Synthesis, spectroscopic and mesomorphic studies on heterocyclic liquid crystals with 1, 3-oxazepine-4, 7-dione, 1, 3-oxazepane-4, 7-dione and 1, 3-oxazepine-1, 5-dione cores. *Journal of Molecular Structure*, 2010, **Vol. 3**, p. 33-44. DOI: 10.1016/j.molstruc.2010.07.043
20. Al Rashidy A.A.M., Al Badrany K.A., Al Garagoly G.M. Spectrophotometric determination of sulphamethoxazole drug by new pyrazoline derived from 2, 4-dinitro phenyl hydrazine. *In Materials Science Forum,* 2020, **Vol. 1002**, p. 350-359. Trans Tech Publications Ltd. DOI: 10.4028/www.scientific.net/MSF.1002.350
21. Al-Joboury W.M., Al-Badrany K.A., Asli N.J. Synthesis of new azo dye compounds derived from 2-aminobenzothiazole and study their biological activity. *Materials Today: Proceedings*, 2021, **Vol. 47**, p. 5977-5982. DOI: 10.1016/j.matpr.2021.04.538
22. Al-Kaabi M.M., Al-Hazam H.A., Al-Maliki L., Al-Masoudi N.A., Saeed B.A., Shtaiwi A., Al-Soud Y.A. Microwave-assisted synthesis, antiproliferative, antibacterial activities, in silico, computational studies and molecular dynamics simulation of new bis-(aryl-based chalcone) derivatives. *Journal of Molecular Structure*, 2025, **Vol.** 1321, 139992. DOI: 10.1016/j.molstruc.2024.139992
23. Al-Ghamdi A.R., Ahmed W.U., Al-Wabli R.I., Al-Mutairi M.S., Rahman A.F.M.M. Synthesis and Anticancer Evaluation of *O*-Alkylated (*E)*-Chalcone Derivatives: A Focus on Estrogen Receptor Inhibition. *International Journal of Molecular Sciences*, 2025, **Vol. 2**, 833. DOI: 10.3390/ijms26020833
24. Dik G., Bakar B., Ulu A., Ateş B. Propelling of enzyme activity by using different triggering strategies: Applications and perspectives. *Industrial & Engineering Chemistry Research*, 2023, **Vol. 36**, p. 14111-14129. DOI: 10.1021/acs.iecr.3c01678

25. Wen L., Peng Y., Zhou Y., Cai G., Lin Y., Li B. Effects of conservation tillage on soil enzyme activities of global cultivated land: A meta-analysis. *Journal of Environmental Management*, 2023, **Vol. 345**, 118904. DOI: 10.1016/j.jenvman.2023.118904
26. Kwiecien H., Smist M., Wrzesniewska A. Synthesis of aryl-fused 1, 4-oxazepines and their oxo derivatives: a review. *Current Organic Synthesis*, 2012, **Vol. 6**, p. 828-850. DOI: 10.2174/157017912803901664
27. AL-Garagoly G.M., AL-Rashidy A.A., AL-Badrany K.A. Spectrophotometric Determination of Sulfanilamide in Pure and Synthetic Sample by New Derivative from Pyrazoline Derived from 2, 4-Dinitro Phenyl Hydrazine. *Diyala Journal for Pure Science*, 2019, **Vol. 4**, DOI: 10.24237/djps.15.04.493C
28. Aftan M.M., Talloh A.A., Dalaf A.H., Salih H.K. Impact para position on rho value and rate constant and study of liquid crystalline behavior of azo compounds. *Materials Today: Proceedings*, 2021, **Vol. 45**, p. 5529-5534. DOI: 10.1016/j.matpr.2021.02.298
29. Havrylyuk D., Zimenkovsky B., Karpenko O., Grellier P., Lesyk R. Synthesis of pyrazoline–thiazolidinone hybrids with trypanocidal activity. *European Journal of Medicinal Chemistry*, 2014, **Vol. 85**, p. 245-254. DOI: 10.1016/j.ejmech.2014.07.103
30. Lévai A. Synthesis of pyrazolines by the reactions of α, β-enones with diazomethane and hydrazines. *Chemistry of Heterocyclic Compounds*, 1997, **Vol. 6**, p. 647-659. DOI: 10.1007/BF02291794
31. Wang Z., Yun S., An Y., Shu L., Li S., Sun K., Zhang W. Effect of fungicides on soil respiration, microbial community, and enzyme activity: A global meta-analysis *Ecotoxicology and Environmental Safety*, 2024, **Vol.** 289, 117433. DOI: 10.1016/j.ecoenv.2024.117433
32. Qiao Q., Liu Z., Hu F., Xu Z., Kuang Y., Li C. A Novel Ce─ Mn Heterojunction-Based Multi-Enzymatic Nanozyme with Cancer-Specific Enzymatic Activity and Photothermal Capacity for Efficient Tumor Combination Therapy. *Advanced Functional Materials*, 2025, **Vol. 6**, 2414837. DOI: 10.1002/adfm.202414837
33. Al-Badrani H., Ezzat N.S., Al-Jawaheri Y.S. Synthesis of oxazepino compound via electrophilic cyclization and evaluation of their biological activity. *Chemical Problems*, 2025, **Vol**. **23(3)**, p. 343-355. DOI: 10.32737/2221-8688-2025-3-343-355
34. Murad Z.A., Hamad A.S. Preparation and diagnosis of new derivatives of the tetrazole ring derived from 2-bromoisophthaladehyde and evaluation of their biological effectiveness. *Chemical Problems*, 2025, **Vol**. **23(1)**, p. 116-124. DOI: 10.32737/2221-8688-2025-1-116-124
35. Muhammad F.M., Khairallah B.A., Albadrany K.A. Synthesis, characterization and Antibacterial Evaluation of Novel 1,3-Oxazepine Derivatives Using A Cycloaddition Approach, *Journal of Angiotherapy*, 2024, **Vol**. **3**, 9506, 1-9 pages,
36. Khalil S.L., Saleem N.H. Synthesis and characterization of five-membered heterocyclic compounds of tetrazole derivatives and their biological activity. *Chemical Problems*, 2025, **Vol**. **23(3),** p. 365-374. DOI: 10.32737/2221-8688-2025-3-365-374
37. Hassan B.A., Mekky A.H. Synthesis, characterization and antibacterial activity of [1, 2, 4] triazolo [4, 3-b][1, 2, 4, 5] tetrazine derivatives. *Chemical Problems*, 2025, **Vol**. **23(1),** p. 78-94. DOI: 10.32737/2221-8688-2025-1-78-94