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SYNTHESIS AND EVALUATION OF SOME NEW 1,2,4-OXADAZOLE DERIVATIVES AS CATALYSTS IN BARLEY CULTIVATION

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Abstract. In this research, a series of new 1,2,4-oxadazole derivatives(3a-e). The new S-(benzo[d]thiazol-2-yl) 2-cyanoethanethioate (1) has been synthesized was afforded via the reaction of 2-mercaptothiol with ethyl 2-cyanoacetate. As shown, thioester (1) has a cyano group in its structure, which was then used to make the amidoxime compound S-(benzo[d]thiazol-2-yl) (Z)-3-amino-3-(hydroxyimino) propanethioate (2), by the addition reaction of hydroxylamine hydrochloride with pyridine as a base. The new 1,2,4-oxadazole derivatives(3a-e) were obtained by reaction of amidoxime compound (2) with several carboxylic acid chlorides/ The purity of all synthesized compounds was determined using thin-layer chromatography (TLC). Additionally, the newly synthesized compounds were confirmed through physical and spectroscopic analysis methods..." (Fixing comma splice and repetition (FT-IR, 1H NMR, and 13C NMR).

Keywords: *2-mercapto benzothiazole, thioester, amidoxime, 1,2,4-oxadiazole, agriculture, Barley.*

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1. Introduction

1,2,4-oxadiazole is one of the most important heterocyclic compounds in organic chemistry due to contains two nitrogen and one oxygen atom in addition to carbon atoms in its molecular structure contributes to use in chemical reactions, therefore it has been used in the pharmaceutical industry (Wang *et al*., 2015), recently utilized in drug discovery being bio isosteric with ester and amide groups (Basak *et al*., 2021), 1,2,4-oxadiazole show a wide range of biological activities (Tawfik & Mohammed, 2022) including antifungal (Liu *et al*., 2022) antibacterial (Cunha *et al*., 2018) anti tubercular (Upare *et al*., 2019) anticancer (Mahmoud *et al*., 2024) anti-Alzheimer (Zhang *et al*., 2019) antioxidant (Gobec *et al*., 2015; Atiyah *et al*., 2023) and have good activity in agriculture, especially in the growth of the plant (Zhu *et al*., 2020; Luo *et al*., 2022). 1,2,4-oxadiazole was synthesized by reaction amidoxime with acid chloride derivatives. amidoxime has bifunctional groups in its structure oxime and amino group due to utilized as an intermediate in the organic synthetic (Rahman *et al*., 2018) and can be obtained from thioester that holds cyano group in their composition by addition of hydroxyl amine hydrochloride and base, thioester is an organic compound that contains functional group characterized by present of sulfur atom bonded to the carbonyl group serve as good intermediate in an organic synthetic (Kazemi & Shiri, 2015). Research builds upon some chemical derivative findings to further investigate their structural variations and

biological activity (Ozliman *et al*., 2021). Therefore, numerous studies have been conducted to synthesize, characterize and evaluate the biological potential of different chamisal derivatives (Saleh *et. al*., 2022; Saleh & Saleh, 2022).

Barley, one of the oldest cultivated grains worldwide, Barley (*Hordeum distichum*) is an important vital crop grown in most parts of the world, ranking fourth in terms of area and productivity after wheat, rice and yellow corn. The cultivated area in Iraq for the year 2020 was estimated at 4,528 thousand dunums (Central Bureau of Statistics, 2020). Barley grains are characterized by containing 55.8% carbohydrates, 3.4% fats, protein and 11% vitamins (Kumari, 2019). Barley is used as animal feed, either as grains or green fodder and is also used in some industries to produce many medical products, understanding the biological mechanisms behind these products provides insight into designing synthetic compounds with enhanced efficacy, such as imidazolone derivatives (Hamdoon *et al.*, 2022). In addition, barley grains are important in human nutrition because of their role in reducing the risk of some diseases, as they contain phytochemicals that help treat chronic heart diseases, high blood pressure, colon cancer and gallstones (Ware, 2018). Sustainability is becoming a cornerstone of agricultural practices and barley farming is no exception. There is a growing emphasis on regenerative agriculture techniques that enhance soil health and promote biodiversity. Farmers are increasingly cropping rotation, covering cropping and reducing tillage to improve soil fertility and water retention, which are crucial for sustainable barley production. They are using some compounds that lessen pollution in the environment and making efforts to improve the wastewater treatment (Benouis *et al*., 2022) and to reduce salinity which affects the crop (Saleh *et al*., 2024). Air pollution significantly impacts plant growth and morphology, as demonstrated by (Fadhil *et al*., 2023), these findings highlight the need for assessing external environmental factors when evaluating plant growth responses to new chemical treatments. Scientists become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components, possessing valuable properties. The wide occurrence of heterocycles in bioactive natural products, pharmaceuticals and agrochemicals has made them important synthetic targets, oxadiazole is important as it constitutes the structural features of many bioactive compounds (Barbuceanu *et al*., 2012). The goal of this study was to explore the bioactivity of new 1,2,4-oxadiazole compounds and study their influence on barley division and growth.

2. Materials and methods

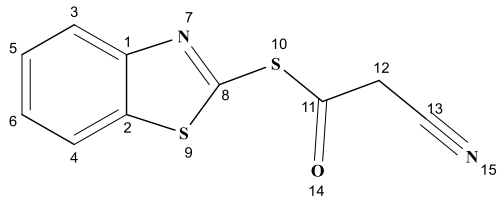
All compounds used in the research were purchased from Fluka, BDH and Aldrich companies, the solvent system of TLC benzene: methanol with ratio (9:1) and FT-IR spectra were used the ATR-FTIR, ^{1}HNMR and ^{13}CNMR were recorded by DMSO-d_6 at 400 MHz and 100 MHz respectively with tetramethyl silane as the internal standards, melting point apparatus was recorded by Stuart SPM30 and were not corrected.

3. Experimental

3.1. Synthesis of thioester (1) (Ali et al., 2023)

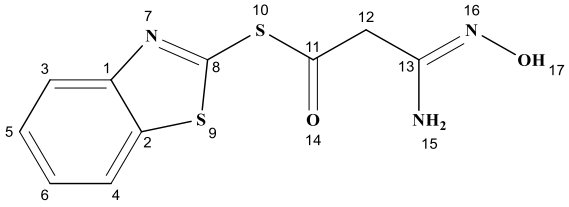
A mixture of 2-mercaptobenzothiazole (0.0239 mol) and K_2CO_3 (0.0239 mol) was stirred in (40 ml) of dry acetone and then followed by adding dropwise of ethyl cyanoacetate, followed by reflux for (6hrs) the reaction was monitored by TLC, then

filtration the crude product. The solvent was evaporated under reduced pressure and the residue was poured into crushed ice to afford the product a yellow color in 85% yield, m.p. 311°C; FT-IR:2194 (C≡N), 1660 (S-C=O), 1595(C=N) cm^{-1},
^{1}H NMR (400 MHz, DMSO) (ppm) 7.39 (d, *J* = 7.6 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 1H), 7.07 (s, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 3.97 (s,2H).
^{13}C NMR (101 MHz, DMSO) δ 185.38 C11, 159.88 C8, 156.32 C1, 137.48 C2, 124.55 C5, 123.85 C6, 120.70 C4, 119.40 C3, 118.97, 117.34 C13, 27.84 C12



3.2. Synthesis of amidoxime (2) (Samsonowicz-Górski *et al*., 2021)

Hydroxylamine hydrochloride (0.00427 mol) and 0.3 ml of pyridine were added to stirred solution of thioester (0.00427 mol) in 70 ml of methanol. The mixture was stirred at 70°C for 12 hours (monitored by TLC). The resulting mixture was poured into water and then extracted with ethyl acetate (2×25ml). Dried with $MgSO_4$ and the solvent removal by rotatory to afford amidoxime as orange color in 70% yield, m.p. 160°C; FT-IR:3400 (O-H) 3333-3300(NH_2), 1665 (S-C=O), 1596(C=N) cm^{-1}.
^{1}H NMR (400 MHz, DMSO) δ 9.35 (s, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.30 (dd, *J* = 15.6, 7.8 Hz, 4H), 6.35 (s, 2H), 4.49 (s, 2H).
^{13}C NMR (101 MHz, DMSO) δ 190.27 C11, 166.93 C8, 152.20 C1, 151.82 C13, 132.36 C2, 125.22 C5, 124.67 C6, 122.24 C4, 122.04 C3, 39.09 C12.



3.3. Synthesis of 1,2,4-oxadiazole(3a-e) (Allam *et al*., 2024)

A solution of amidoxime (0.001 mol) in dioxane was added to acid chlorides (0.0018 mol) and 1 ml of pyridine. The reaction mixture was refluxed for 12 hours (monitored by TLC). Then poured into ice water and filtrated and dried to afford the 1,2,4-oxadiazole derivatives. The physical and chemical properties were listed in the Tables 1, 2 and 3.

Effect of 1,2,4-oxadiazole in Barley germination and growth

1. Laboratory experiment

A. **Biological test**

This research was carried out in 2024 at Mosul University, Faculty of Science and the barley seeds cultivar (Local black variety) was used for the experiments. The seeds were initially washed with hypochlorite 3% for 1 min; afterward, they were washed with distilled water. Then 10 seeds were placed in 9 cm Petri dishes (on filter paper) and wetted

with 8 ml of distilled water (control sample) or a solution of 1,2,4-oxadiazole derivative. 3b were examined at concentrations: of 0.1 mg. Seeds were germinated in the dark for 7 days at 25 ± 2 °C in a Gallenkhamp incubator (Hussain & Shihab, 2024). The germination rate was measured after 14 days of cultivation. The lengths of each Plumule and radical of the seedlings were measured and the dry weight of the seedlings was taken after drying them in the oven at a temperature of 60 °C for 72 hours (Al-Jehaishy, 2017). Germination percentage (Gr%) was measured:

Gr% = Natural seedlings No. / total seeds No. x 100 (Saied, 1984).

B. Study of cell division coefficient:

According to (Taher & Hussain, 2021). The division coefficient and phase coefficient were calculated according to the equations:

Division Coefficient = (NO of Dividing cells/cells total number) ×100

Phase coefficient= (No dividing cells for a given phase/Total number of dividing cells) ×100

2. Greenhouse experiment

To study the effect of 1,2,4-oxadiazole on barley seeds cultivar (Local black variety) was studied in a glasshouse. For this, 10 seeds were planted in Plastic pots of diameter (20 cm) and height (25 cm), at 0.5 cm depth from the surface of the soil and then irrigated with water and put in a glass house at a temperature of 20 ± 2 and used distilled water as the control. Studied Traits: 1. Germination: Germination% = number of germinated seeds/ number of seeds cultver×100. Leaf area (cm^2). Germination Efficiency., Number of leaf Chlorophyll content.

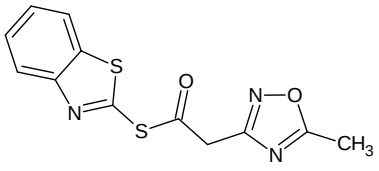
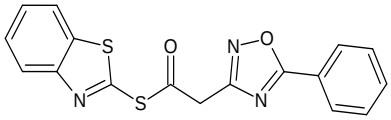
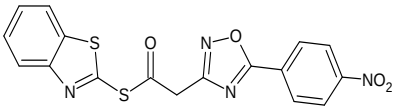
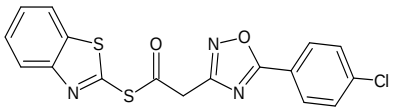
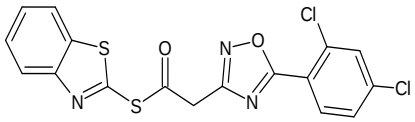
Table 1. The physical properties of (3a-e)

Comp	R	M.P	Yield%	Color
3a	**-CH_3**	**173-175**	**70**	**Yellow**
3b	**-Ph**	**170-171**	**72**	**Yellow**
3c	**-4-NO_2-C_6H_5**	**170-171**	**75**	**Yellow**
3d	**-4-Cl-C_6H_5**	**165-167**	**80**	**Yellow**
3e	**-2,4-diCl-C_6H_4**	**170-173**	**75**	**Yellow**

Table 2. Spectral data of FT-IR of (3a-e)

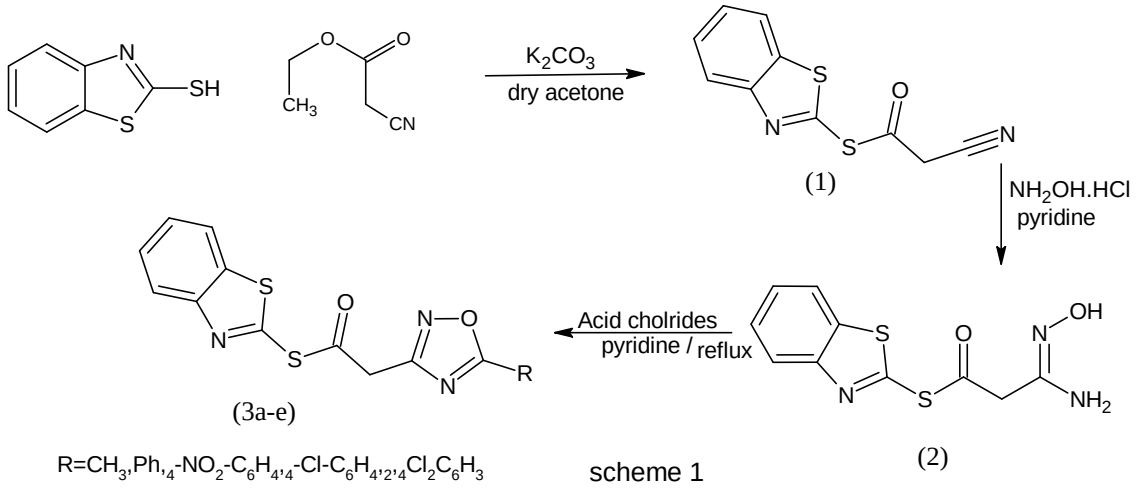
Comp.	ATR-FTIR υ(cm^{-1})					
	R	C-H	C=O	C=N	C-S	Other
3a	**-CH_3**	**2957 2922**	**1666**	**1582**	**757**	**---**
3b	**-Ph**	**2990 2959**	**1664**	**1596**	**756**	**---**
3c	**-4-NO_2-C_6H_5**	**2958 2921**	**1622**	**1586**	**756**	**Asym NO2 1559 Sym NO2 1346**
3d	**-4-Cl-C_6H_5**	**2968 2910**	**1679**	**1591**	**759**	**C-Cl 809**
3e	**-2,4-diCl-C_6H_4**	**2956 2922**	**1622**	**1585**	**756**	**C-Cl 872**

Table 3. Spectral data of 1H-NMR of (3a-e)

Comp.	Structure	^{1}HNMR
3a		^{1}H NMR (400 MHz, DMSO) δ 8.08 (d, *J* = 7.9 Hz, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.58 – 7.50 (m, 1H), 7.46 (d, *J* = 7.9 Hz, 1H), 3.57 (s, 2H), 2.51 (s, 3H).
3b		^{1}H NMR (400 MHz, DMSO) δ 8.08 (d, *J* = 7.3 Hz, 1H), 7.96 (d, 1H), 7.54 (m, 1H), 7.45 (d, 4H), 3.57 (s, 2H).
3c		^{1}H NMR (400 MHz, DMSO) δ 8.58 (s, 1H), 8.32 (s, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 8.07 (d, *J* = 7.7 Hz, 1H), 7.57 (dd, *J* = 7.1 Hz, 4H), 3.57 (s, 2H).
3d		^{1}H NMR (400 MHz, DMSO) δ 8.73 (d, *J* = 2.9 Hz, 2H), 7.91 (dd, *J* = 8.5, 2.8 Hz, 3H), 7.63 – 7.56 (m, 3H), 4.50 (s, 2H).
3e		^{1}H NMR (400 MHz, DMSO) δ 8.07 (d, *J* = 7.5 Hz, 1H), 7.95 (s, 2H), 7.61 – 7.50 (m, 2H), 7.47 – 7.41 (m, 3H), 3.57 (s, 2H).

3. Result and Discussion

In this study, the thioester was initially prepared by reaction of the mercapto benzothiazole with ethyl cyanoacetate and acetone as a solvent as shown below in scheme 1, the structure was confirmed by using TLC and spectroscopic methods (IR, H-NMR and C-NMR) where the IR showed a clear band for cyano group at 2194 cm^{-1} and a band at 1660 cm^{-1} belong to the carbonyl of thioester in addition to the disappearance of ester band, whereas H-NMR showed a clear signal at 3.97 ppm for methylene group and C-NMR showed clear signal for at 185.38 ppm for carbonyl of thioester and signal at 117.38 ppm for cyano group and 27.84 for methylene group. After confirming the validity of the prepared compound, it was introduced into a reaction with hydroxylamine hydrochloride in presence pyridine as base to afford the amidoxime where confirmed the structure through the TLC and spectroscopic methods (IR, H-NMR and C-NMR) where the IR showed broad band at 3400 for hydroxyl group and two band at 3333 and 3300 for primary amine in addition to absent band of cyano group, whereas H-NMR showed signal at 11.11 ppm for hydroxyl group and signal at 9.35 ppm for amino group and C-NMR give good evidence to validity prepared compound through absent cyano group, in the last step, to afford of the 1,2,4-oxadiazole derivatives, intercyclization between amidoxime and acid chlorides in presence pyridine as base, where confirmed the structure through the TLC and spectroscopic methods (IR, H-NMR and C-NMR) where the IR showed absent bands of hydroxyl, primary amine and carbonyl group of acid chlorides also the H-NMR showed absent signals of hydroxyl (Al-Jubori *et al*., 2024), primary amine in addition to appear another's signal as showed in Table 3.



scheme 1

In the first experiment (in the laboratory) (Table 4), the Germination percentage, Plumule length, Radical length, Plumule Dry weight and Radical Dry weight were the highest when barley seeds were treated with 3b (0.1 mg l-1), in comparison with the control sample. When seeds were treated with 0.1 mg l-1 solutions of the tested compounds, the best results were obtained for 1,2,4-oxadiazole derivative A (12.5, 90.29, 53.53 and 31.41 %, respectively). In conclusion, the results presented in Table 4 demonstrated, that the traits of barley in vitro were most influenced by compounds containing oxadiazole moiety. That may be due to the founded of N atoms in the structure

Table 4. Effect of 3b in some laboratory traits of Barley

Treatment	Gr%	PL	RL	R DW	P DW
Control	80	4.12	4.67	0.206	0.145
3b	90	7.84	7.17	0.204	0.175

Gr% = Germination percentage, Pl = Plumule length, Rl = Radical length, RDw = Radical Dry weight; PDw = Plumule Dry weight,

The result in Table 5 shows the effect of 1,2,4-oxadiazole division coefficient and phase index at the top of the roots of Barley Figure 1, that clear cure increased in division coefficient by effect of 1,2,4-oxadiazole that commanded with increased in coefficient of Metaphase, Anaphase and Telophase. It may have affected the cell life cycle and chromosome division in the anaphase and telophase or it may have affected the growth hormones responsible for division such as IAA (Taher & Hussain, 2021).

Table 5. Effect of 1,2,4-oxadiazole on the division coefficient and phase index at the top of the roots of Barley

Treatment	Division Coefficient	Coefficient phase P	coefficient phase M	Coefficient phase A	coefficient phase T
Control	73	34	17	30	17
3b	84	26	31	31	36

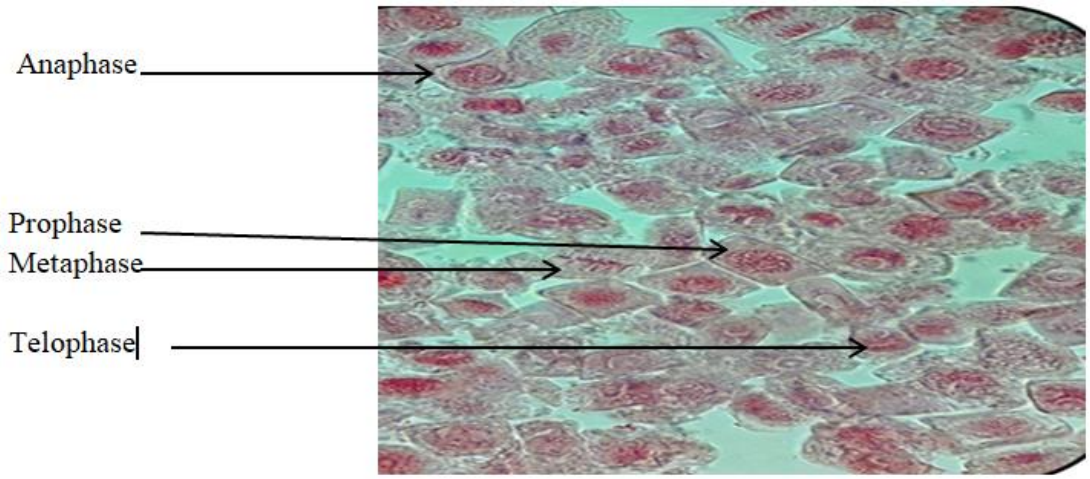


Figure 1. Effect of 1,2,4-oxadiazole on the division of the root tip cells of the barley plant

As seen from the results of (Cunha *et al*., 2018), the Gr%, Shoot height, Leaf area cm^2 and Ch of barley seedlings were the biggest when seeds were exposed to azole (0.1 mg l-1). Were bigger in comparison with the control sample. 3b gave the best results (50, 14.32 and 3.6 %, respectively) traits of the barley seedlings. That may be due to 1,2,4-oxadiazole having good plant growth-regulating properties (Hussain & Shihab, 2024).

Table 6. Effect of 1,2,4-oxadiazole on germination and growth of Barley

Treatment	Gr%	Shoot height cm	Leaf area cm^2	Ch
Control	60	16.2	12.47	30
3b	90	18.52	20.04	32.8

4. Conclusion

We were able to obtain a new intermediate material using a suggested method and using different functional groups, where we based on the thiol group in the 2-mercapto benzothiazole compound as a good nucleophile and its reaction with the ethyl cyanoacetate compound, where the thioester was obtained and then the cyano group was reduced to amidoxime in the traditional methods and then cyclized to obtain 1,2,4-oxadiazole, where the validity of the compounds was confirmed by diagnosing them using the available spectral methods and also one of the 1,2,4-oxadiazole substitutes (3b) was introduced as a catalyst in the germination and stem elongation process of barley plants, which in turn gave good results.

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