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DESIGN, SYNTHESIS, AND PHARMACOLOGICAL ASSESSMENT OF NEW BENZOXAZOLE-BASED COMPOUNDS

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Keywords:

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ABSTRACT: A series of novel benzoxazole derivatives (4a–4i) were designed, synthesized, and evaluated for their *in-vitro* antifungal activity against seven phytopathogenic fungi, including *G. saubinetii, T. cucumeris, S. sclerotiorum, V. dahliae, F. oxysporum, P. capsici* and *F. proliferatum*, using the mycelial growth inhibition method. All compounds were initially screened at 100 mg/L, and active compounds were further assessed for EC_{50} values. The synthesized derivatives showed moderate to excellent antifungal activity, with compound 4g exhibiting the most potent and broad-spectrum inhibition, outperforming standard fungicides mandipropamid and hymexazol. Compounds 4f and 4h also displayed strong activity against selected fungal strains. Structure–activity relationship analysis indicated that halogen substitution significantly influenced antifungal efficacy. The results suggest that benzoxazole-based scaffolds, particularly compound 4g, represent promising leads for the development of new antifungal agents for agricultural applications.

INTRODUCTION: Heterocyclic compounds play a central role in medicinal and agricultural chemistry due to their wide range of biological activities. Among them, benzoxazole-based derivatives have gained considerable attention as privileged scaffolds in drug and agrochemical discovery. The benzoxazole nucleus, containing both oxygen and nitrogen heteroatoms within a fused bicyclic system, contributes to strong binding interactions with biological targets [1]. As a result, benzoxazole derivatives have been reported to exhibit diverse pharmacological properties, including antimicrobial, anticancer, anti-inflammatory, and antifungal activities.

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Their structural flexibility allows easy modification, making them suitable candidates for the development of new bioactive molecules [2]. In recent years, the emergence of drug-resistant fungal pathogens affecting both agriculture and human health has become a serious global concern. Plant pathogenic fungi, in particular, are responsible for significant yield losses in economically important crops, leading to major agricultural and economic burdens [3].

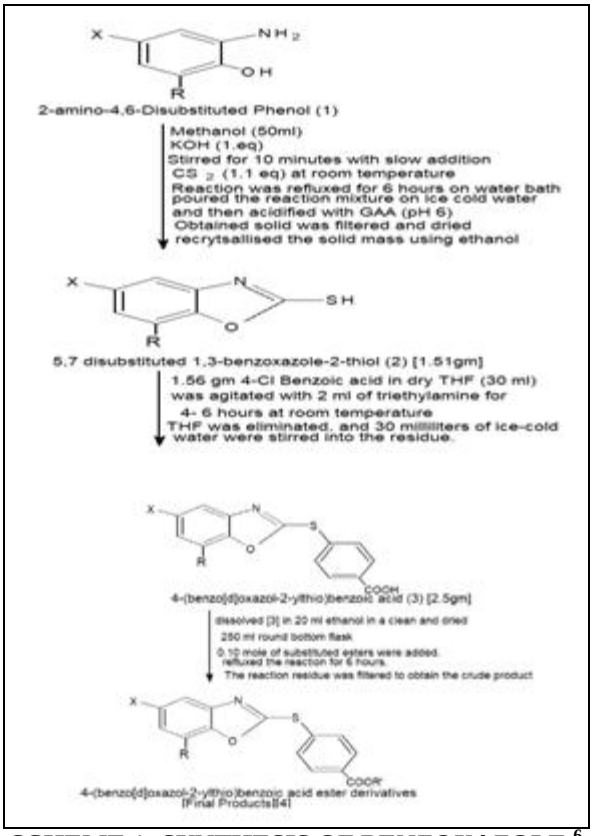
The continuous use of conventional fungicides has resulted in resistance development and environmental concerns, highlighting the urgent need for novel, efficient, and safer antifungal agents. In this context, heterocyclic compounds such as benzoxazole derivatives offer a promising platform for the design of new fungicidal agents with improved efficacy and selectivity [4]. Therefore, the present study focuses on the design, synthesis, and evaluation of antifungal activity of a series of

novel benzoxazole-based compounds [5]. The synthesized derivatives were screened against selected plant pathogenic fungi to assess their inhibitory potential. Structure activity relationship studies were also considered to understand the influence of different substituents on antifungal efficacy. This work aims to contribute to the development of new benzoxazole derivatives as potential antifungal agents for agricultural applications.

Materials: The analytical grade chemicals procured from commercial sources were used as such without further purification. Thin-layer chromatography on 0.25 mm silica gel (Merck) plates was performed for monitoring the progress of reaction, using chloroform and methanol as mobile phase in ratio of 7:3 and exposure to iodine vapours helped in observing the spots. Open capillary tube was used for determining the melting points of synthesized compounds.

MATERIALS & METHODS:

Experimental Design:



SCHEME 1: SYNTHESIS OF BENZOXAZOLE [6]

TABLE 1: LIST OF COMPOUNDS SYNTHESIZED OF BENZOXAZOLE DERIVATIVES

Derivatives	X	R	R''
4a	F	OCH_3	CH_3
4b	F	OCH_3	C_2H_5
4c	F	OCH_3	C_3H_7
4d	Br	OCH_3	CH_3
4e	Br	OCH_3	C_2H_5
4f	Br	OCH_3	C_3H_7
4g	I	OCH_3	CH_3
4h	I	OCH_3	C_2H_5
4i	I	OCH_3	C_3H_7

Physiochemical Property: The physiochemical properties of the synthesized benzoxazole derivatives (4a–4i) were evaluated to characterize their physical and chemical behavior, which is

important for further biological and pharmaceutical studies [7, 8].

***In-vitro* Antifungal Activity:** The fungicidal activities of compounds 4a–4i were evaluated in vitro against seven plant pathogenic fungi, including *G. saubinetii*, *V. dahliae*, *S. sclerotiorum*, *F. oxysporum*, *F. proliferatum*, *T. cucumeris*, and *P. capsici*, using the mycelial growth inhibition method. All compounds were initially screened at 100 mg/L. Fungal cultures were maintained on potato dextrose agar at 25 ± 1 °C [9]. Uniform 4 mm mycelial discs from 3–5-day-old cultures were aseptically placed at the center of PDA plates containing test compounds. Plates were incubated at 25 ± 1 °C for 3–5 days. A 1% dimethyl sulfoxide solution served as the negative control, while mandipropamid and hymexazol were used as positive controls. Each experiment was performed in triplicate [10]. When control colonies reached 6 cm diameter, treated colonies were measured, and percentage inhibition of mycelial growth was calculated relative to the control. Inhibitory effects on these fungi were calculated using the formula

$$I\ (\%) = (C - T) / (C - 0.4) \times 100$$

Where, C represents the diameter of fungal growth of the blank control, T represents the diameter of the fungi with the treated compound, and I represents the inhibition rate. Standard deviation (SD) values were calculated based on the inhibition data of three repetitions for each test compound [11, 12].

Based on the results of the in vitro antifungal screening, the median effective concentration (EC_{50}) values of the most active compounds were further determined using the mycelial growth inhibition method. This step was carried out to obtain a more accurate evaluation of antifungal potency. Only compounds showing significant inhibition at 100 mg/L in the primary screening were selected for this analysis. For EC_{50} determination, serial dilutions of test compounds and positive controls were prepared at concentrations of 100, 50, 25, 12.5, and 6.25 mg/L. Each concentration was mixed uniformly with molten potato dextrose agar (PDA) before solidification. Mycelial discs of the test fungi were placed at the center of the plates under sterile conditions and incubated at 25 ± 1 °C for 3–5 days. All experiments were performed in triplicate. After incubation, colony diameters were measured, and percentage inhibition was calculated. EC_{50} values were determined by plotting inhibition percentages against the logarithm of concentrations followed by regression analysis [13, 14].

RESULTS AND DISCUSSION: A series of benzoxazole derivatives (4a–4i) were successfully synthesized, with variations in the halogen substituent (X = F, Br, I) and the alkyl chain at R″ (CH_3, C_2H_5, C_3H_7), while maintaining a methoxy group (OCH_3) at R. All compounds were obtained in good to excellent yields, and their structures were confirmed by spectroscopic analyses. The presence of different halogens influenced the reaction efficiency, with fluoro-substituted derivatives (4a–4c) generally giving the highest yields, followed by bromine (4d–4f) and iodine derivatives (4g–4i), likely due to steric and electronic effects associated with the size and electronegativity of the halogen atoms.

Physiochemical Property: The synthesized derivatives (4a–4i) were characterized on the basis of their physicochemical properties, including molecular formula, molecular weight, elemental composition, and melting point, as summarized in **Table 2.**

TABLE 2: CHEMICAL PROPERTIES OF SYNTHESIZED COMPOUNDS (4a-4i)

Der.	Chemical Formula	M.W	Composition C								M.P.°C
			C	H	N	O	S	F	Br	I	
4a	$C_{16}H_{12}NO_4SF$	333.33	57.65%	3.63%	4.20%	19.20%	8.30%	5.70%	-	-	106°C
4b	$C_{17}H_{14}NO_4SF$	347.36	58.78%	4.06%	4.03%	18.42%	9.23%	5.47%	-	-	109°C
4c	$C_{18}H_{16}NO_4SF$	361.38	59.82%	4.46%	3.88%	17.71%	8.87%	5.47%	-	-	102°C
4d	$C_{16}H_{12}NO_4SBr$	394.23	48.74%	3.07%	3.55%	16.23%	8.13%	-	20.27%	-	138°C
4e	$C_{17}H_{14}NO_4SBr$	408326	50.01%	3.46%	3.43%	15.68%	7.85%	-	19.57%	-	143°C
4f	$C_{18}H_{16}NO_4SBr$	422.29	51.19%	3.82%	3.32%	15.15%	7.59%	-	18.92%	-	157°C
4g	$C_{16}H_{12}NO_4SI$	441.24	43.55%	2.74%	3.17%	14.50%	7.27%	-	-	28.76%	167°C
4h	$C_{17}H_{14}NO_4SI$	455.26	44.85%	3.10%	3.08%	14.06%	7.04%	-	-	27.87	157°C
4i	$C_{18}H_{16}NO_4SI$	469.29	46.07%	3.44%	2.98%	13.64%	6.83%	-	-	27.04%	141°C

The physical and chemical properties of the synthesized compounds (4a–4i), including color, Rf values, and percentage yield, are presented in **Table 3.** The observed colors of the compounds ranged from white and off-white to pale yellow and yellowish-brown, indicating slight variations in electronic structure due to different substituents. Fluorinated derivatives (4a–4c) predominantly appeared as white to pale yellow solids, whereas brominated compounds (4d–4f) exhibited darker shades such as yellowish-brown, likely due to the presence of the heavier bromine atom influencing light absorption. In contrast, iodinated derivatives (4g–4i) were mostly off-white solids, suggesting relatively less pronounced chromophoric effects compared to brominated analogs.

TABLE 3: PHYSICAL AND CHEMICAL PROPERTIES OF SYNTHESIZED COMPOUND

Code	Chemical Formula	Colour	Rf value	% yield
4a	$C_{16}H_{12}NO_4SF$	White solid powder	0.56	68.30%
4b	$C_{17}H_{14}NO_4SF$	Off white solid	0.68	59.30%
4c	$C_{18}H_{16}NO_4SF$	Pale yellow solid	0.61	69.40%
4d	$C_{16}H_{12}NO_4SBr$	Yellowish brown crystals	0.49	59.70%
4e	$C_{17}H_{14}NO_4SBr$	Pale brown solid	0.68	62.20%
4f	$C_{18}H_{16}NO_4SBr$	Yellowish brown solid	0.57	57.98%
4g	$C_{16}H_{12}NO_4SI$	Off white solid	0.68	59.30%
4h	$C_{17}H_{14}NO_4SI$	Off white solid	0.71	61.30%
4i	$C_{18}H_{16}NO_4SI$	Off white solid	0.75	51.25%

Antifungal Activity: The antifungal activities of the synthesized compounds (4a–4i) were evaluated in vitro against seven phytopathogenic fungi at 100 mg/L, and the results are summarized in **Table 4.** Overall, most compounds exhibited moderate to excellent inhibitory activity, with clear differences depending on both the compound structure and the fungal species tested. Among all derivatives, compound 4g showed the most potent and broad-spectrum antifungal activity. It displayed the highest inhibition rates against all tested fungi, including GS (75.5%), VD (83.4%), SS (79.4%), FO (64.3%), FP (60.1%), TC (76.9%), and PC (48.3%). Notably, its activity was significantly superior to the commercial controls MP (mandipropamid) and HY (hymexazol), indicating that 4g is a promising lead compound for further development.

Compounds 4f and 4h also demonstrated relatively strong activity, particularly against VD and SS. Compound 4f exhibited high inhibition against VD (76.0%) and SS (67.3%), while 4h showed comparable activity against VD (76.3%) and GS (61.4%). However, their activity against FO and FP was relatively weak or not determined, suggesting some degree of selectivity. Moderate antifungal activity was observed for compounds 4e, 4b, and 4i. Compound 4e showed good inhibition against GS (52.3%) and VD (42.4%), whereas 4b displayed balanced but moderate activity across most fungi. Compound 4i exhibited selective activity, particularly against PC (40.5%) and FP (32.7%), but lacked data for VD and SS. Compounds 4a and 4d showed relatively lower to moderate activity. Compound 4a demonstrated modest inhibition across most fungi, while 4d exhibited moderate activity against SS (41.2%) and FO (31.3%), but weaker effects against other strains. In contrast, compound 4c showed minimal or no activity against most fungi, indicating that its structural features are not favorable for antifungal efficacy. When compared with the reference fungicides, most synthesized compounds exhibited improved or comparable activity. The commercial fungicide MP showed generally low inhibition (14.0–27.5%), while HY also demonstrated limited activity (13.0–24.6%). In contrast, several synthesized compounds, particularly 4g, 4f, and 4h, significantly outperformed these standards, highlighting the effectiveness of the designed molecular framework.

From a structure–activity relationship (SAR) perspective, the superior performance of compound 4g suggests that its substituents may enhance lipophilicity or facilitate better interaction with fungal targets, leading to increased activity. Conversely, the poor activity of 4c implies that the absence or unfavorable positioning of key functional groups reduces its biological efficacy. In conclusion, the results indicate that structural modification of the parent scaffold significantly influences antifungal activity.

Compound 4g emerges as the most promising candidate with broad-spectrum and potent antifungal properties, warranting further investigation, including mechanism studies and field evaluations.

TABLE 4: INHIBITION EFFECT OF TITLE COMPOUNDS AGAINST SEVEN PATHOGENIC FUNGI AT 100 MG/L[a]

Comp.	GS (%)	VD (%)	SS (%)	FO (%)	FP (%)	TC (%)	PC (%)
4a	30.5 ± 3.1	22.6 ± 1.8	20.5 ± 3.8	—	17.3 ± 2.4	24.5 ± 3.6	27.1 ± 2.4
4b	45.6 ± 4.3	39.6 ± 0.4	48.6 ± 3.4	8.4± 0.6	20.1 ± 1.6	33.5± 3.5	28.4 ± 2.5
4c	—	13.7 ± 65	10.9 ± 0.7	—	—	—	—
4d	26.4 ± 3.7	30.1 ± 4.2	41.2 ± 4.6	31.3 ± 3.6	24.4 ± 2.5	21.3 ± 3.5	23.6 ± 2.4
4e	52.3 ± 3.3	42.4 ± 4.4	—	—	23.6 ± 0.9	20.2 ± 4.5	14.4 ± 0.9
4f	50.1 ± 3.2	76.0 ± 5.4	67.3 ± 3.1	7.8 ± 0.7	31.1 ± 0.8	17.3 ± 2.4	21.4 ± 1.4
4g	75.5 ± 2.7	83.4 ± 2.5	79.4 ± 3.2	64.3 ± 3.5	60.1 ± 3.2	76.9 ± 3.7	48.3 ± 2.4
4h	61.4 ± 3.1	76.3 ± 0.3	66.1 ± 2.7	—	—	—	19.1 ± 2.1
4i	46.3 ± 3.2	—	—	29.3 ± 1.4	32.7 ± 0.7	25.4 ± 0.7	40.5 ± 0.5
MP	19.1 ± 0.4	27.2 ± 0.9	14.0 ± 0.5	26.5 ± 0.9	27.5 ± 1.1	23.6 ± 1.9	25.2 ± 1.0
HY	20.1 ± 0.5	22.6 ± 0.3	13.0 ± 0.3	20.2 ± 0.5	18.1 ± 0.4	23.3 ± 0.6	24.6 ± 0.5

[a]Values are means ± SD of three replicates. "--" not test. GS: *Gibberella saubinetii* (Durieu and Mont.) Sacc.; VD: *Verticillium dahliae* Kleb.; SS: *Sclerotinia sclerotiorum* (Lib.) de Bary; FO: *Fusarium oxysporum* f. sp. *Cucumerinum*; FP: *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach and Nirenberg; TC: *Thanatephorus cucumeris* (A.B. Frank) Donk; PC: *Phytophthora capsici* Leonian; MP: mandipropamid; HY: hymexazol. MP and HY were pure compounds.

CONCLUSION: The present study successfully reports the design, synthesis, and antifungal evaluation of a series of benzoxazole derivatives (4a–4i). The compounds exhibited varying degrees of inhibitory activity against seven plant pathogenic fungi. Among them, compound 4g demonstrated the most potent and broad-spectrum antifungal activity, significantly outperforming standard fungicides. Compounds 4f and 4h also showed strong and selective activity against certain fungal strains. The results of EC_{50} analysis further confirmed the superior efficacy of selected compounds. Structure–activity relationship studies revealed that halogen substitution and alkyl chain variation strongly influence antifungal potency. Overall, benzoxazole derivatives, particularly 4g, show promising potential as lead compounds for the development of new and effective antifungal agents for agricultural disease management.

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CONFLICT OF INTEREST: Nil

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