International Journal of Chemical Science
www.chemicaljournals.com
Online ISSN: 2523-2843
Received: 27-04-2026, Accepted: 28-05-2026, Published: 12-06-2026
Volume 10, Issue 2, 2026, Page No. 79-82

Synthesis and antifungal activity of 1,3-Benzoxazole-5-carbonyl-pyridazine and phthalazine diones

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DOI: https://doi.org/10.66856/chemical.2026.10.2.10030

Abstract
A series of novel 1,3-benzoxazole derivatives incorporating pyridazine and phthalazine dione moieties were synthesized and evaluated for their antifungal potential. The target compounds, 1-((2-substituted-1, 3-benzoxazol-5-yl)carbonyl)-1,2-dihydropyridazine-3,6-diones (6a-c), 1-((2-substituted-1, 3-benzoxazol-5-yl)carbonyl)tetrahydropyridazine-3,6-diones (7a-c), and 2-((2-substituted-1, 3-benzoxazol-5-yl)carbonyl)-2,3-dihydrophthalazine-1,4-diones (8a-c), were obtained through the condensation of 2-substituted benzoxazole-5-carboxylic acid hydrazides with various cyclic anhydrides. The antifungal activity was assessed against four pathogenic fungi: *Candida albicans, Fusarium oxysporium, Drechslera halodes, and Colletotrichum falcatum* using the cup-plate method. Among the screened compounds, 8b and 8c exhibited significant antifungal activity, nearly equal to the standard drug Itrazole. These results suggest that phthalazine-fused benzoxazole derivatives are promising leads for the development of new antifungal agents.

Keywords: Benzoxazole, pyridazine, phthalazine, antifungal activity, zone of inhibition

Introduction

The global burden of life-threatening fungal infections has surged, causing over 1.5 million deaths annually. This crisis is primarily driven by an expanding population of immunocompromised individuals, including those with HIV/AIDS, cancer patients on chemotherapy, and organ transplant recipients. Furthermore, the clinical utility of existing antifungals such as azoles, polyenes, and echinocandins is severely compromised by the rapid emergence of multidrug-resistant (MDR) strains like *Candida auris* and triazole-resistant *Aspergillus fumigatus*. These challenges have necessitated the urgent development of novel chemotypes with distinct mechanisms of action.

Benzoxazoles have emerged as a privileged scaffold in medicinal chemistry due to their ability to act as structural isosteres of the purine bases, adenine and guanine [1]. This bioisosteric relationship facilitates high-affinity interactions with essential biological polymers and enzymes, such as topoisomerases and fungal cell wall biosynthetic pathways [2, 3]. Recent studies highlight that 2-substituted benzoxazoles can be specifically tailored to inhibit the ergosterol synthesis pathway or disrupt mitochondrial function in fungal cells [4, 5]. By modifying the benzoxazole core with other bioactive heterocycles such as pyridazines or thiazoles, researchers have successfully produced hybrids that demonstrate enhanced cell membrane permeability and reduced host toxicity [2, 6]. Their chemical versatility and stability make benzoxazoles ideal candidates for overcoming metabolic degradation and bypass-resistance mechanisms found in clinical isolates [7].

Pyridazine and phthalazine scaffolds are essential in modern medicinal chemistry due to their unique electronic properties and ability to form stable hydrogen bonds with biological targets [8, 9]. The clinical success of drugs like *Levosimendan*, a pyridazinone used as a calcium sensitizer in heart failure [10], and *Azelastine*, a phthalazinone-based antihistamine [11], underscores the pharmacological drug ability of these nitrogen-rich heterocycles. These cores are often referred to as privileged scaffolds because they can be easily modified to interact with a variety of enzymes and receptors [8, 12].

Recent research highlights molecular hybridization as a primary strategy to address the crisis of antifungal resistance. By covalently linking the benzoxazole nucleus a known purine isosterewith pyridazine or phthalazine diones, researchers can create multi-structure entities [13, 14]. This approach aims to achieve synergistic effects, where the hybrid molecule targets multiple fungal pathways simultaneously, such as inhibiting ergosterol biosynthesis while disrupting mitochondrial respiration [4]. The dione functionality is particularly significant as it provides a rigid framework that optimizes the orientation of the molecule within the active site of fungal enzymes [15]. Preliminary evaluations of these hybrids suggest they possess enhanced lipophilicity and membrane permeability, which are critical for overcoming the protective cell walls of pathogenic fungi. These benzoxazole-linked leads represent a robust starting point for developing high-potency, broad-spectrum antifungal agents that can bypass established resistance mechanisms. Combining these pharmacophores into a single molecular entity (multi-structure heterocycles) is a recognized strategy to enhance potency and broaden the spectrum of activity [16, 17]. In this study, we have synthesized the benzoxazole-linked pyridazine/phthalazine diones by established procedure [18] and done preliminary antifungal evaluation to identify potential leads for drug development.

Experimental

Materials and Methods

Laboratory-grade reagents and solvents were used throughout the study. The Melting points of the synthesized compounds were determined using the open capillary method and are reported without correction. Reaction

progress and product purity were monitored via Thin Layer Chromatography (TLC) on Merck silica gel-G plates. The IR spectra were recorded using KBr pellets on a Perkin Elmer 337 spectrometer [1].H NMR spectra were obtained in $CDCl_3$ solvents on a Bruker Avance 300 MHz spectrometer, using TMS as the internal standard. Additionally, Mass Spectra of all the synthesized compounds were recorded on Liquid Chromatography Mass Spectrometer.

Preparation of Methyl 4-Hydroxy-3-nitrobenzoate [2]: The synthesis involves the electrophilic aromatic nitration of methyl *p*-hydroxybenzoate [1] using a mixture of mineral acids. This transformation introduces a nitro group ortho to the hydroxyl moiety. The resulting nitro derivative is typically isolated and purified via recrystallization from an ethanol-water solvent.

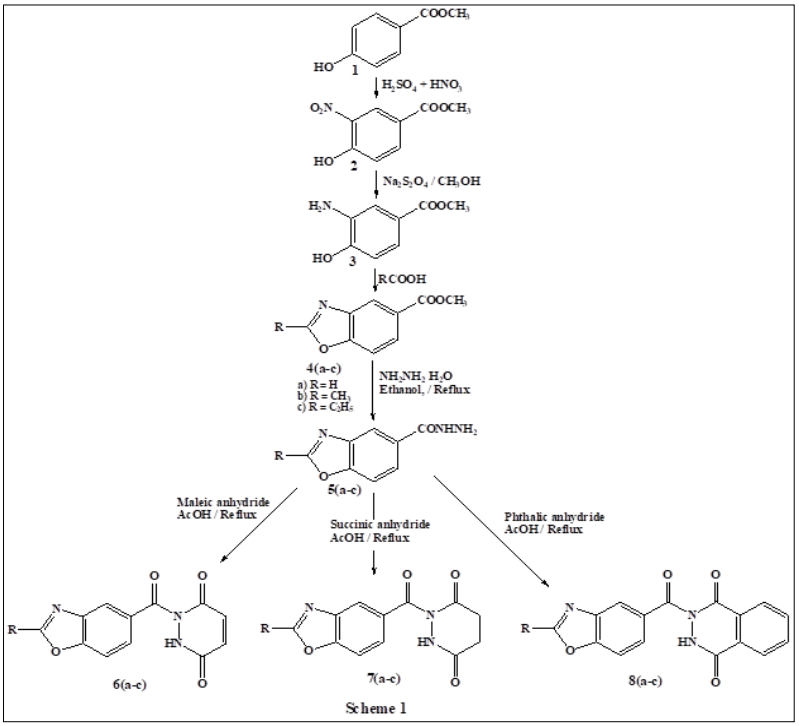
Preparation Methyl 3-Amino-4-hydroxybenzoate [3]: This compound is prepared through the chemical reduction of the nitro group in compound 2, using $Na_2S_2O_4$ as reducing agent in CH_3OH solvent. The nitro substituent is converted to a primary amine. Purification is achieved through an ethanol/water mixture by recrystallization.

General Procedure for the Synthesis of Methyl 2-Substituted Benzoxazole-5-carboxylates (4a–4c): The formation of the benzoxazole ring system proceeds via the condensation of the ortho-aminophenol derivative [3] with various aliphatic carboxylic acids. This cyclization reaction yields the corresponding ester-functionalized benzoxazoles, which are purified by recrystallization using ethanol/water.

General Procedure for the Synthesis of 2-Substituted Benzoxazole-5-carboxylic Acid Hydrazides (5a–5c): To a stirred solution of the methyl 2-substituted benzoxazole-5-carboxylate (4a–4c, 1.0 equiv.) in absolute ethanol (0.2 M) was added hydrazine hydrate (5.0–10.0 equiv., 50–64% aq. solution). The reaction mixture was heated at reflux under a nitrogen atmosphere for 4–8 h until thin-layer chromatography (hexanes/ethyl acetate) indicated complete consumption of the starting ester. Upon completion, the mixture was cooled to ambient temperature, during which a heavy precipitate formed. The flask was subsequently chilled in an ice-water bath for 30 min to ensure maximum precipitation. The resulting solid was collected via vacuum filtration using a Büchner funnel and washed sequentially with ice-cold ethanol and diethyl ether. The crude product was recrystallized from ethanol. The target carboxylic acid hydrazides (5a–5c) were isolated as stable, crystalline solids, which were dried under high vacuum at 50 °C for 12 h.

Synthesis of 1-[(2-substituted-1,3-benzoxazol-5-yl) carbonyl]-1,2-dihydropyridazine-3,6-dione (6a–6c): To a stirred solution of 2-substituted-1,3-benzoxazole-5-carbohydrazide (11.29 mmol) in glacial acetic acid (20 mL), maleic anhydride (11.29 mmol) was added. The reaction mixture was heated under reflux for 6 hours, with progress monitored by thin-layer chromatography (methanol/ethyl acetate, 6:4 v/v). Upon completion, the mixture was quenched by pouring into crushed ice with vigorous stirring. The resulting precipitate was collected by filtration, washed thoroughly with chilled water, and recrystallized from a minimum volume of methanol to yield compounds (6a–6c) as white crystalline.

Synthesis of 1-[(2-substituted-1,3-benzoxazol-5-yl) carbonyl] tetrahydropyridazine-3,6-dione (7a–7c): To a stirred solution of 2-substituted-1,3-benzoxazole-5-carbohydrazide (11.29 mmol) in glacial acetic acid (20 mL), oxolane-2,5-dione (succinic anhydride, 11.29 mmol) was added. The reaction mixture was heated under reflux for 8 hours, and the progress was monitored by thin-layer chromatography (methanol/ethyl acetate, 6:4 v/v). Upon completion, the mixture was quenched by pouring into crushed ice with vigorous stirring. The resulting solid was collected by filtration, washed thoroughly with chilled water, and recrystallized from a minimum volume of methanol to afford compounds (7a–7c) as white powders.



Scheme 1

Synthesis of 2-[(2-substituted-1,3-benzoxazol-5-yl) carbonyl]-2,3-dihydrophthalazine-1,4-dione (8a–8c): To a stirred solution of 2-substituted-1,3-benzoxazole-5-carbohydrazide (11.29 mmol) in glacial acetic acid (20 mL), phthalic anhydride (11.29 mmol) was added. The reaction mixture was heated under reflux for 8 hours, with progress monitored by thin-layer chromatography (methanol/ethyl acetate, 6:4 v/v). Upon completion, the mixture was quenched by pouring into crushed ice with vigorous stirring. The resulting precipitate was collected by filtration, washed thoroughly with chilled water, and recrystallized from a minimum volume of methanol to yield compounds (8a–8c) as white crystalline powders.

Antifungal Activity

The *in vitro* antifungal activity of the synthesized compounds 6(a–c), 7(a–c), and 8(a–c) was evaluated using the agar cup plate method. The evaluation was carried out against four pathogenic fungal strains: *Candida albicans*, *Fusarium oxysporum*, *Drechslera halodes*, and *Colletotrichum falcatum*. The test organisms were sub-cultured on potato dextrose agar (PDA) medium. Sterilized agar media were inoculated with the respective fungal cultures and incubated at 37 °C for 48 h, after which they were maintained at 4°C for preservation.

The test compounds were dissolved in Dimethyl sulfoxide (DMSO) and subsequently diluted using a sterile growth medium Sabouraud Dextrose Broth (SDB) to reach the final test concentrations and evaluated at two distinct concentrations: 600 and 900 µg/mL. Itrazole served as the positive reference standard. Following incubation, the antifungal efficacy was determined by measuring the diameter of the zone of inhibition (DIZ) in millimeters (mm).

Table 1: Antifungal activity of the compounds 6(a-c), 7(a-c), 8(a-c)

S. No	Compound	Zone of inhibition (in mm)							
		Candida albicans		***Fusarium oxysporum***		***Drechslera halodes***		***Colletotrichum Falcatum***	
		A	B	A	B	A	B	A	B
1	6a	NA	NA	1.27	3.69	2.22	4.10	2.21	5.25
2	6b	NA	NA	2.21	5.47	1.51	3.90	2.41	4.39
3	6c	NA	NA	1.61	3.85	2.29	4.21	3.21	6.21
4	7a	NA	NA	2.21	5.22	1.51	3.80	3.89	4.10
5	7b	NA	NA	3.21	6.25	2.15	4.39	2.31	5.21
6	7c	NA	NA	2.18	4.89	2.61	5.12	4.27	8.58
7	8a	NA	NA	2.29	5.29	3.21	6.71	3.51	7.21
8	8b	NA	NA	6.25	12.37	6.41	12.10	6.11	12.63
9	8c	NA	NA	7.14	15.47	5.21	10.22	7.28	13.15
10	Itrazole	5.35	12.01	10.80	20.03	7.56	14.76	9.34	18.26
Test solution and standard solution; A: 600 µg/ml; B: 900 µg/ml NA – No Activity									

Results and discussion

Antifungal Activity Evaluation

The *in vitro* antifungal data for the synthesized compounds 6(a–c), 7(a–c), and 8(a–c) against the select fungal strains are summarized in Table 1. All tested derivatives exhibited a concentration-dependent inhibitory profile, with significantly higher zones of inhibition observed at 900 µg/mL compared to 600 µg/mL. However, none of the newly synthesized derivatives surpassed the broad-spectrum potency of the reference standard, Itrazole.

Against *Candida albicans*, all synthesized compounds (6–8) failed to exhibit any observable zone of inhibition at both tested concentrations. In contrast, the standard drug Itrazole produced clear zones of inhibition measuring 5.35 mm and 12.01 mm at 600 and 900 µg/mL, respectively. This indicates that the current structural modifications do not satisfy the pharmacophoric requirements necessary to combat *Candida albicans*.

Conversely, the synthesized compounds displayed weak to promising inhibitory activity against the filamentous fungi *Fusarium oxysporum*, *Drechslera halodes*, and *Colletotrichum falcatum*. Structure-activity relationship (SAR) analysis revealed that the core scaffold and the nature of the substituent (R) significantly influenced the antifungal profile, with the 8(a–c) series emerging as the most potent among the groups tested.

Activity against *Fusarium oxysporum*: The reference drug Itrazole exhibited strong inhibition (10.80 mm at 600 µg/mL; 20.03 mm at 900 µg/mL). Among the synthesized analogs, compound 8c demonstrated the highest efficacy, producing inhibition zones of 7.14 mm and 15.47 mm at 600 and 900 µg/mL, respectively. This was closely followed by 8b, which reached a DIZ of 12.37 mm at 900 µg/mL. Derivatives within the 6 and 7 series demonstrated low to moderate activity, with DIZ values remaining below 6.25 mm even at the higher concentration.

Activity against *Drechslera halodes*: Itrazole showed a DIZ of 14.76 mm at 900 µg/mL. Compound 8b showed exceptional sensitivity against this strain, displaying a zone of 6.41 mm at 600 µg/mL and 12.10 mm at 900 µg/mL, which closely approached the performance of the standard drug. Compound 8c also performed moderately well with a zone of 10.22 mm at 900 µg/mL. Derivatives 6b and 7a showed minimal effectiveness.

Activity against *Colletotrichum falcatum*: The standard drug achieved an 18.26 mm zone of inhibition at 900 µg/mL. Derivative 8c yielded the highest inhibition zone among the test candidates, measuring 13.15 mm at 900 µg/mL, followed by 8b at 12.63 mm. Within the other series, compound 7c showed moderate inhibition (8.58 mm at 900 µg/mL), highlighting that the ethyl group often enhances the activity profile against *Colletotrichum falcatum*.

Structure-Activity Relationships (SAR)

Based on the experimental observations, the following insights can be derived regarding the chemical modifications:

Core Scaffold Influence: The overarching order of antifungal potency among the series was found to be 8 > 7 > 6. This suggests that the core system of series 8 offers a superior electronic or spatial fit for fungal target interaction.

Substituent (R) Influence: Within each series, the presence of alkyl groups (R = CH_3 or C_2H_5) generally led to a significant increase in antifungal activity compared to the unsubstituted (R = H) analogs. Specifically, the ethyl-substituted derivatives (6c, 7c, 8c) showed maximum inhibition against *Fusarium oxysporum* and *Colletotrichum falcatum*, pointing toward a favorable contribution from increased lipophilicity. For *Drechslera halodes*, the methyl substitution (8b) provided the optimal steric profile.

Conclusion

In conclusion, a novel series of 1,3-benzoxazole derivatives hybridized with pyridazine and phthalazine diones was successfully synthesized using known procedure and evaluated for their antifungal efficacy. While none of the synthesized analogs displayed inhibitory action against *Candida albicans* or outperformed the reference standard, Itrazole, notable concentration-dependent potency was observed against filamentous fungi. Specifically, the phthalazine-dione series compounds 8b and 8c emerged as the most effective candidates, demonstrating prominent activity against *Fusarium oxysporum*, *Drechslera halodes*, and *Colletotrichum falcatum*. These results highlight that phthalazine-fused benzoxazole scaffolds are promising, lipophilic leads that warrant further structural optimization to develop potent, broad-spectrum antifungal agents.

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