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Research Article

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Synthesis and Anticandidosic Activities of Some 2-Benzylidene Benzimidazo Thiazolone Derivatives

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Abstract Candida infections present a significant health challenge, especially with increasing drug resistance and the emergence of less common pathogenic species. In response, novel antifungal agents are urgently needed. The benzimidazole derivatives, known for diverse bioactivities, including antifungal properties, offer a promising avenue for new therapies. This study focuses on the design, synthesis, and evaluation of thiazolobenzimidazolyl-benzylidene derivatives as potential antifungal agents.

These compounds were synthesized through aldol condensation of benzimidazo-thiazol-3-ones with benzaldehydes and characterized using spectroscopic methods, including NMR and mass spectrometry. Their antifungal activity was assessed using bioautography and microdilution techniques against clinical strains of Candida albicans, C. tropicalis, and C. glabrata, with ketoconazole and miconazole as the reference.

Six 2-benzylidene derivatives were synthesized with yields ranging from 54% to 81%. Spectral analyses confirmed expected structures. Three derivatives demonstrated significant antifungal activity at a Minimum Inhibitory Quantity (MIQ) of 10 µg, comparable to ketoconazole. MIC assays revealed compound-specific efficacy, with some derivatives outperforming ketoconazole against certain Candida strains. Structural variations influenced activity, underscoring the role of functional groups. Among the synthesized derivatives, several demonstrated significant antifungal activity, with MIC values in the micromolar range. A pyridine derivative emerged as particularly potent, showing broad-spectrum efficacy with MICs between 0.7 and 372,73 µM across all tested strains.

These findings highlight the potential of thiazolobenzimidazolyl-benzylidene derivatives as promising candidates for developing new antifungal therapies to address resistance and biofilm-associated challenges.

Keywords: Thiazolobenzimidazole, Candida strains, Antifungal agents.

1. Introduction

In recent decades, fungal infections, particularly those caused by Candida species, have become a significant global health concern, particularly for immunocompromised individuals [1]. Systemic Candida infections can lead to high mortality rates, reaching up to 40% even with antifungal treatments [2,3]. Current antifungal therapies are limited to five main drug classes: allylamines, azoles, echinocandins, polyenes, and pyrimidine analogs [4]. However, the effectiveness of these drugs is increasingly compromised by issues such as drug resistance, adverse side effects, and the inability to treat biofilm-associated infections effectively [5,6]. Biofilms, often formed on medical devices, exacerbate therapeutic failures as conventional antifungals struggle to penetrate these protective layers [7]. Resistance to antifungal agents like fluconazole and echinocandins is particularly concerning. Fluconazole, a widely used antifungal, has shown declining efficacy, especially in AIDS patients [8]. Furthermore, inherent resistance in certain fungal species highlights the urgent need for innovative antifungal agents. Recent research has explored alternative strategies, such as combination therapies and novel compounds targeting both fungal biofilm and the fungal germ, to address these challenges [9].

The study of benzimidazole derivatives, a well-established class of bioactive compounds, offers promising avenues for antifungal drug development [10]. Benzimidazole-based compounds are widely recognized in medicinal chemistry for their diverse therapeutic applications, including antifungal, antibacterial, antiviral, and anticancer activities [11,12,13]. The benzimidazole core structure, as seen in drugs like albendazole and omeprazole, demonstrates significant bioactive potential, particularly when functionalized with specific chemical groups [11,12,13]. The research described in this paper focuses on the synthesis and evaluation of 2-benzylidene benzimidazo-thiazolone derivatives as potential antifungal agents. These compounds are designed as hybrid molecules combining the bioactive features of 2-mercaptobenzimidazole and the phenylpropenone chain of chalcones [13,14]. The 2-mercaptobenzimidazole moiety, characterized by a thiol group at position 2, has shown notable therapeutic potential, particularly in anti-infective applications [14]. Similarly, the phenylpropenone chain is recognized for its ability to interact with pathogenic enzyme systems, enhancing its anti-infective properties [15].

The structural hybridization of these entities into a benzimidazo-thiazolone framework capitalizes on the biological versatility of this tricyclic heterocycle. Recent studies have reported benzimidazo-thiazolones to exhibit a wide range of pharmacological activities, including antiviral, antibacterial, anticancer, and antifungal effects [16,17,18]. For example, these derivatives have shown promise as non-nucleoside reverse transcriptase inhibitors active against HIV-1, as well as potent antifungal agents effective against resistant strains [19,20].

This research aims to assess the antifungal activity of 2-benzylidene benzimidazo-thiazolone derivatives against clinical strains of Candida. By exploring these novel compounds, the study seeks to address the urgent need for safer and more effective antifungal therapies capable of overcoming current limitations in treating fungal infections.

2. Materials and Methods

2.1. Chemistry

The progress of reactions and product purity were monitored via thin-layer chromatography (TLC) on silica gel plates with UV fluorescence at 254 nm. Analytical data, including ^{1}H-NMR and mass spectra, were obtained at the CEISAM Laboratory, University of Nantes. NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ at 300 MHz using a BRUKER ADVANCE 300 device, with TMS as the reference. Mass spectra were measured using an HP 5889A spectrometer, and melting points were determined with a KOFLER bench.

The approach required the stepwise synthesis of key intermediates, starting with 2-mercaptobenzimidazole from orthophenylenediamine, followed by the preparation of 2-thiobenzimidazol-2-yl acetic acid, which was cyclized to yield benzimidazo-thiazol-3-one. Finally, this compound was reacted with benzaldehyde derivatives to obtain the target 2-benzylidene benzimidazo-thiazol-3-ones.

2.1.1 Synthesis of 2-Mercaptobenzimidazole

The synthesis of 2-mercaptobenzimidazole (1) followed the procedure described by Van Allan and Deagon [21]. Orthophenylenediamine (a) was condensed with carbon disulfide (b) in the presence of potassium hydroxide (KOH)

under reflux in ethanol for 3 hours (Figure 1). The reaction mixture was then cooled and neutralized with a 20% aqueous acetic acid solution to isolate 2-mercapto-1H-benzimidazole as the product.

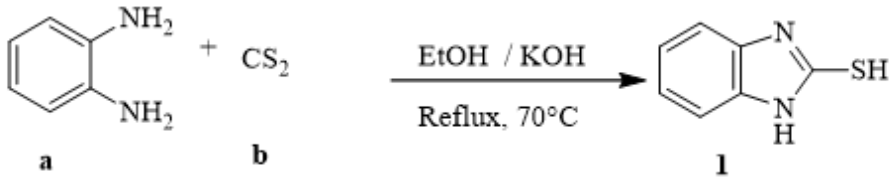


Figure 1: Synthesis of 2-mercaptobenzimidazole following the Van Allan method

- ✓ **Procedure for the synthesis of 2-mercaptobenzimidazole**

To 5g of O-phenylenediamine are added carbon disulfide (46.2 mmol) and potassium hydroxide (46.2 mmol in 7 ml of water). The reaction mixture is heated under reflux of ethanol for 3 hours. 1.5 g of activated carbon is added to the reaction medium, then heated for an additional 10 minutes.

After hot filtration, hot water is added to the filtrate and allowed to cool. 2-Mercaptobenzimidazole is isolated with a yield of 70%, after neutralization with 20% acetic acid and then recrystallization from a water / ethanol mixture (1: 1).

2.1.2. Synthesis of 2-Thiobenzimidazol-2-yl Acetic Acid

To synthesize 2-thiobenzimidazol-2-yl acetic acid, we used the method reported by Omprakash G. [22]. This involved a nucleophilic substitution reaction between 2-mercaptobenzimidazole (1) and 2-chloroacetic acid (c) in the presence of potassium hydroxide as a base. The reaction mixture was refluxed in ethanol for 4 hours. Subsequent acidification yielded the desired product (2) (Figure 2).

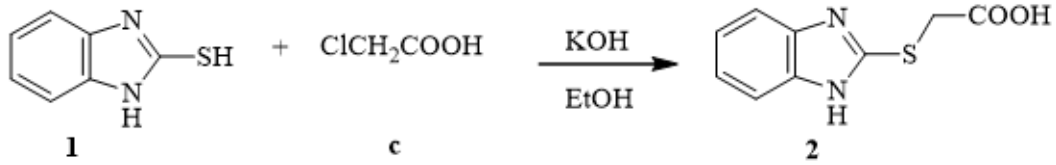


Figure 2: Synthesis of 2-thiobenzimidazol-2-yl acetic acid as described by Omprakash G. et al.

- ✓ **Procedure for the synthesis of 2-thiobenzimidazol-2-yl acetic acid**

A mixture of 2-mercaptobenzimidazole (2g, 13.3 mmol), chloroacetic acid (13.3 mmol) and potassium hydroxide (47.9 mmol) is heated under reflux of ethanol for 4 hours. After filtration and cooling, the reaction medium is acidified. The precipitate formed is recrystallized from water to give 2-(Thiobenzimidazol-2-yl) acetic acid as a white solid.

2-(Thiobenzimidazol-2-yl) acetic acid as a white solid was isolated and purified by recrystallization in a yield of 81%.

The mechanism of this reaction involves nucleophilic substitution in a basic medium. The base enhances the nucleophilicity of the thiol group, facilitating its attack on the electrophilic methylene carbon of 2-chloroacetic acid (Figure 3).

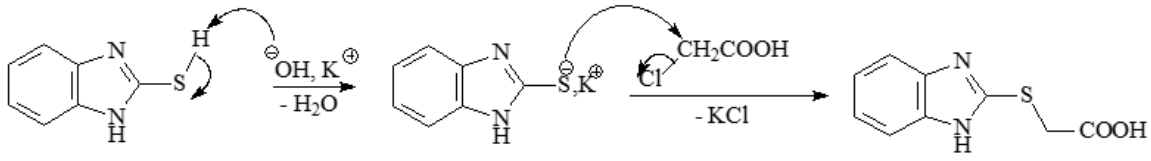


Figure 3: Mechanism of the nucleophilic substitution reaction for synthesizing 2-thiobenzimidazol-2-yl acetic acid

2.1.3. Synthesis of 2-Benzylidene Benzimidazo-Thiazol-3-ones

The final step involved the synthesis of 2-benzylidene benzimidazo-thiazol-3-ones (3a–3h) following the condensation methodology outlined by Omprakash et al. [22]. Initially, an intramolecular cyclization of 2-thiobenzimidazol-2-yl acetic acid (2) forms benzimidazo-thiazol-3-one. This intermediate undergoes an aldol condensation reaction with benzaldehyde or its derivatives in the presence of sodium acetate and refluxing acetic acid, resulting in the formation of the target compounds (Figure 4).

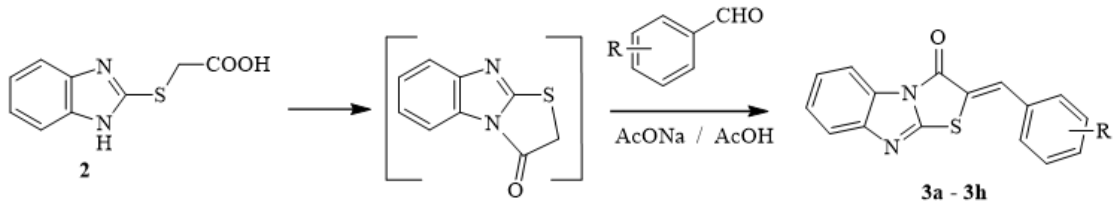


Figure 4: Synthesis of 2-benzylidene benzimidazo-thiazol-3-ones according to the method of Omprakash G. et al.

- ✓ **Procedure for the synthesis of -benzylidene benzimidazo-thiazol-3-ones**

A suspension of 1 g of 2-(Thiobenzimidazol-2-yl) acetic acid; 1.2 eq of aldehyde and 1 eq of anhydrous sodium acetate in glacial acetic acid was performed. The reaction mixture is heated at reflux for 3 hours. After cooling, the precipitate obtained is filtered and washed with cold water.

These derivatives were isolated and purified by recrystallization with yields ranging between 54% and 73%.

The reaction mechanism involves classical aldol condensation in a basic medium, followed by dehydration (crotonization). A base abstracts an α-proton from the carbonyl group of the intermediate, forming a carbanion. This carbanion attacks the carbonyl carbon of benzaldehyde, leading to the formation of a β-hydroxyketone. Subsequent dehydration yields the final product (Figure 5).

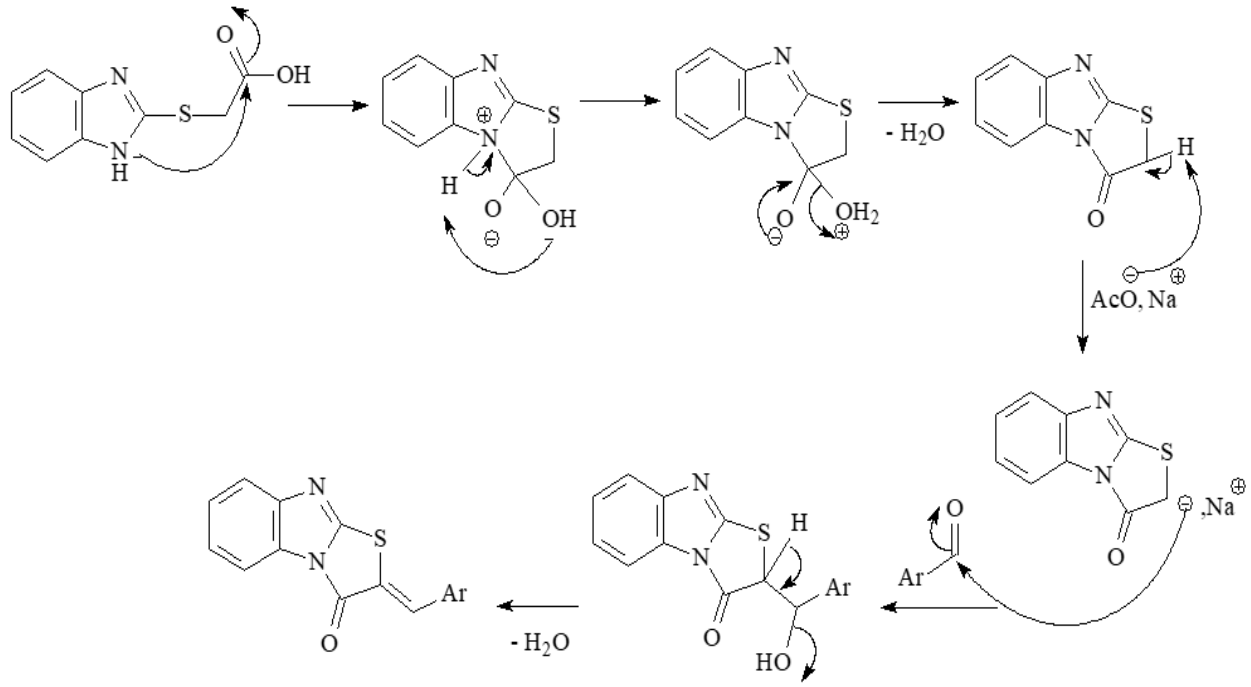


Figure 5: Mechanism of aldol condensation and dehydration in the synthesis of 2-benzylidene benzimidazo-thiazol-3-ones

2.2. Biological Evaluation

2.2.1 Materials

Synthetic Compounds

The synthesized 2-benzylidene benzimidazolo-thiazolone derivatives were evaluated for antifungal activity in their pure powder form. Stock solutions of these compounds were prepared by dissolving 1 mg in 1 mL of methanol or DMSO.

Reference Compound

Ketoconazole, a well-established antifungal agent, was used as the reference drug for comparative evaluation.

Microbiological Materials

The antifungal activity was tested against clinical strains of Candida albicans (strain 1812454), Candida tropicalis (strain 1902145), and Candida glabrata (strain 1903956) provided by CeDReS. Susceptibility testing of these strains to ketoconazole and miconazole revealed varying sensitivity levels. Notably, Candida albicans strain was sensitive to ketoconazole and miconazole. In addition, C. tropicalis exhibited intermediate sensitivity to ketoconazole, while C. glabrata showed intermediate sensitivity to miconazole.

2.2.2 Method of Antifungal Evaluation

The antifungal activity of the compounds was assessed through bioautography and Minimum Inhibitory Concentration (MIC) determination.

Screening via Bioautography

This approach identified compounds active against Candida species at a Minimum Inhibitory Quantity (MIQ) threshold of 10 µg.

Preparation of Inoculum

Candida cultures were grown on Sabouraud dextrose agar for 24–48 hours at 30°C, transferred to Tryptone Soy broth, and incubated to reach exponential growth. This culture was mixed with molten agar to achieve an inoculum density of approximately 10^5 cells/mL.

Detection of Activity

Methanolic solutions of the synthesized compounds (1 mg/mL) and ketoconazole were spotted (10 µL, 10 µg/spot) on silica gel plates. Plates were overlaid with inoculated agar and incubated overnight at 30°C. Zones of inhibition, visualized by MTT staining, indicated antifungal activity.

Determination of Minimum Inhibitory Concentrations (MIC)

The MIC assay determined the lowest concentration of each compound that completely inhibited fungal growth.

Preparation of Inoculum

The inoculum was prepared as described for bioautography. Cultures were adjusted to ~10^5 cells/mL for the assay.

MIC Assay

Stock solutions of the compounds (1 mg/mL in DMSO) were serially diluted in broth to obtain working concentrations. In 96-well plates, 50 µL of inoculum and 50 µL of each dilution were combined. Ketoconazole served as the positive control, and sterile water as the negative control. Plates were incubated at 30°C for 48 hours, followed by MTT addition. The MIC was defined as the lowest concentration showing no purple coloration, indicating inhibition of fungal growth.

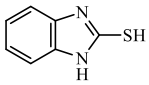
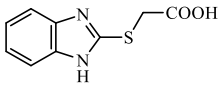
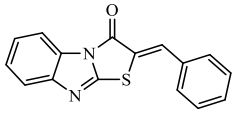
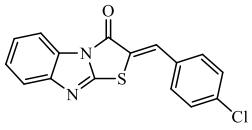
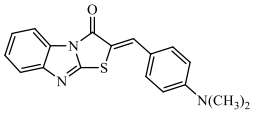
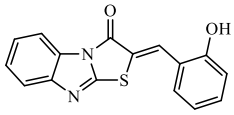
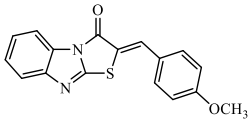
All tests were performed in duplicate and repeated twice to ensure reliability.

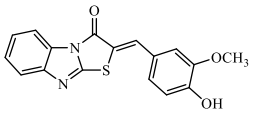
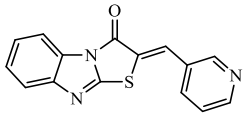
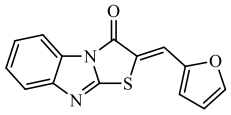
3. Results

3.1. Chemistry

The synthesis of 2-benzylidene benzimidazo-thiazol-3-one derivatives was carried out using established methods, yielding two intermediates and eight final compounds. These derivatives were characterized and evaluated for antifungal activity. The synthetic pathway demonstrated good efficiency, with yields ranging from 54% to 81%, as summarized in Table 1.

Table 1: Characterization data of synthesized 2-mercaptobenzimidazole derivatives

Comp.	Structure	Chemical name	Appearance / Purification method	% yield (w/w) / Melting point
1		1H-benzo[d]imidazole-2-thiol	White powder Recrystallization from a water/ethanol mixture (1:1)	Yield = 70 %; MP = 298 - 302°C
2		2-((1H-benzo[d]imidazol-2-yl)thio)acetic acid	White powder Recrystallization in water	Yield = 81 %; MP = 205-209°C;
3a		(Z)-2-benzylidenebenzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one	Yellow crystalline powder Recrystallization from ethanol	Yield = 73 %; Mp = 210 - 214°C
3b		(Z)-2-(4-chlorobenzylidene)benzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one	Yellow crystalline powder Recrystallization from ethanol	Yield = 77 %; Mp = 198 - 202°C
3c		(Z)-2-(4-(dimethylamino)benzylidene)benzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one	Orange solid Recrystallization from ethanol	Yield = 56 %; Mp = 242 - 246°C
3d		(Z)-2-(2-hydroxybenzylidene)benzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one	Yellow crystalline powder Recrystallization from ethanol	Yield = 65 %; Mp = 210 - 214°C
3e		(Z)-2-(4-methoxybenzylidene)benzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one	Orange powder Recrystallization from	Yield = 67 %; Mp = 190 - 194°C

			ethanol	
3f		(Z)-2-(4-hydroxy-3-methoxybenzylidene)benzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one	Pale Yellow powder Recrystallization from ethanol	Yield = 67 %; Mp = 230 - 344°C
3g		(Z)-2-(pyridin-3-ylmethylene)benzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one	Yellow solid Recrystallization from ethanol	Yield = 54% Mp = 258 -262°C
3h		(Z)-2-(furan-2-ylmethylene)benzo[4,5]imidazo[2,1-b]thiazol-3(2H)-one	Orange solid Recrystallization from ethanol	Yield = 56% Mp = 242 -246°C

Spectral Analysis

• 2-Mercaptobenzimidazole (Intermediate 1):

The 1H NMR spectrum showed a singlet at 12.52 ppm for pyrrolic protons and a multiplet at 7.16–7.08 ppm for aromatic protons.

The mass spectrum confirmed the molecular ion peak, consistent with the proposed structure.

The compound exhibited thiol-thione tautomerism, enhancing its reactivity. The NMR analysis data of synthesized compounds are summarized in Table 2.

Table 2: NMR analysis data of synthesized 2-mercaptobenzimidazole derivatives

Compounds	**^{1}H and ^{13}C NMR (DMSO-d6) (300 MHz, DMSO-d_6, δ ppm)**	**SM $[M]^+$(%) [EI, 70 eV, m/z (rel. Int)**
1	12.52 (s, 2H, 2 NH) 7.16-7.08 (m, 4H, H_{Ar})	150 ($[M]^+$, 100) ; 91 ($[M-59]^+$, 21) ; 80 ($[M-70]^+$, 34) ; 70 ($[M-80]^+$, 68) ; 65($[M-85]^+$, 21) ; 64 ($[M-86]^+$, 29) ; 63 ($[M-87]^+$, 38) ; 59 ($[M-91]^+$, 90). **[CI, NH_3, m/z]:** 151 $[M+1]^+$
2	12.79 (s, 1H, N-H) 7.43 (dd, *J* = 3 Hz, *J* = 6 Hz, 2H, H_{Ar}) 7.11 (dd, *J* = 3 Hz, *J* = 6 Hz, 2H, H_{Ar}) 4.13 (s, 2H, CH_2) 3.34 (s, 1H, CO_2H)	164 ($[M-44]^+$, 23) ; 163 ($[M-45]^+$, 16) ; 149 ($[M-59]^+$, 9) ; 131 ($[M-77]^+$, 23) ; 118 ($[M-90]^+$, 23) ; 91 ($[M-117]^+$, 15) ; 45 ($[M-163]^+$, 100) **[CI, NH_3, m/z]:** 209 $[M+1]^+$
3a	8.13 (s, 1H, C=CH) 7.98-7.95 (m, 1H, H_{Ar}) 7.78-7.36 (m, 8H, H_{Ar})	278 ($[M]^+$, 88) ; 144 ($[M-134]^+$, 32) ; 134 ($[M-144]^+$, 100) ; 129 ($[M-149]^+$, 63) ; 116 ($[M-162]^+$, 60) ; 102 ($[M-176]^+$, 90) ; 90 ($[M-188]^+$, 85).
3b	8.44 (s, 1H, C=CH) 8.06-8.03 (m, 1H, H_{Ar}) 7.69-7.66 (m, 2H, H_{Ar}) 7.55-7.52 (m, 1H, H_{Ar}) 7.45-7.33 (m, 4H, H_{Ar})	314 ($[M+2]^+$, 37); 313 ($[M+1]^+$, 21); 312 ($[M]^+$, 100); 284 ($[M-28]^+$, 8) 283 ($[M-29]^+$, 11); 249 ($[M-63]^+$, 11); 248 ($[M-64]^+$, 9)

3c	9.67 (s, 1H, C=CH) 7.70-7.67 (m, 2H, H_{Ar}) 7.62-7.59 (m, 2H, H_{Ar}) 6.91-6.87 (m, 2H, H_{Ar}) 6.81-6.78 (m, 2H, H_{Ar}) 3.04 (s, 6H, $2H_3$)	323 ($[M+2]^+$, 6); 322 ($[M+1]^+$, 23); 321 ($[M]^+$, 100) ; 320 ($[M-1]^+$, 22) ; 319 ($[M-2]^+$, 4) ; 304 ($[M-17]^+$, 3) ; 277 ($[M-44]^+$, 3)
3d	10.23 (s, 1H, OH) 8.27 (s, 1H, C=CH) 7.59 – 7.47 (m, 3H, H_{Ar}) 7.29 – 7.23 (m, 2H, H_{Ar}) 7.08 – 6.73 (m, 3H, H_{Ar})	294 ($[M]^+$, 78) ; 160 ($[M-134]^+$, 37) ; 150 ($[M-144]^+$, 100) ; 145 ($[M-149]^+$, 53) ; 132 ($[M-162]^+$, 63) ; 118 ($[M-176]^+$, 84) ; 106 ($[M-188]^+$, 88).
3e	7.87 (s, 1H, C=CH) 7.64 – 7.53 (m, 4H, H_{Ar}) 7.29 – 7.21 (m, 2H, H_{Ar}) 7.16 – 7.11 (m, 2H, H_{Ar}) 3.84 (s, 3H, CH_3)	310 ($[M+2]^+$, 36); 309 ($[M+1]^+$, 33); 308 ($[M]^+$, 100) ; 307 ($[M-1]^+$, 12) ; 306 ($[M-2]^+$, 24) ; 294 ($[M-17]^+$, 13) ; 264 ($[M-44]^+$, 3)
3f	9.43 (s, 1H, OH) 7.97 (s, 1H, C=CH) 7.60 – 7.52 (m, 2H, H_{Ar}) 7.32 – 7.19 (m, 3H, H_{Ar}) 7.18 – 6.94 (m, 2H, H_{Ar}) 3.84 (s, 3H, CH_3)	326 ($[M+2]^+$, 42); 325 ($[M+1]^+$, 33); 324 ($[M]^+$, 100); 296 ($[M-28]^+$, 8); 295 ($[M-29]^+$, 11); 286 ($[M-63]^+$, 21); 285 ($[M-64]^+$, 17)
3g	8.98 ppm (s, 1H, H_{Ar}) 8.70 ppm (d, *J* = 6 Hz, 1H, H_{Ar}) 8.17 ppm (s, 1H, C=CH) 8.16-8.12 ppm (m, 1H, H_{Ar}) 7.97 ppm (d, *J* = 6 Hz, 1H, H_{Ar}) 7.72-7.69 ppm (m, 1H, H_{Ar}) 7.66-7.63 ppm (m, 1H, H_{Ar}) 7.44-7.40 ppm (m, 2H, H_{Ar})	279 ($[M]^+$, 88) ; 145 ($[M-134]^+$, 32) ; 135 ($[M-144]^+$, 100) ; 130 ($[M-149]^+$, 63) ; 117 ($[M-162]^+$, 60) ; 103 ($[M-176]^+$, 90) ; 91 ($[M-188]^+$, 85).
3h	8.17ppm (d, J = 5.1Hz 1H, HAr) 7.92 ppm (s, 1H, C = CH) 7.59 ppm (d, J = 3.3 Hz, 2H, HAr) 7.22 ppm (m, 3H, HAr) 6.87 ppm (t, 1H, HAr)	270 ([M + 2] +, 6); 269 ([M + 1] +, 16); 268 ([M] +, 100); 240 ([M-28] +, 18) 212 ([M-56] +, 9); 211 ([M-57] +, 16)

• 2-Thiobenzimidazol-2-yl Acetic Acid (Intermediate 2):

Key NMR signals included a singlet at 4.13 ppm for methylenic protons, doublets at 7.11 and 7.43 ppm for aromatic protons, and singlets at 3.34 and 12.79 ppm for carboxylic and pyrrolic nitrogen protons, respectively.

The mass spectrum exhibited a peak at m/z 209, confirming the structure (Table 2).

• 2-Benzylidene benzimidazo-thiazol-3-ones (Final Derivatives 3a–3h):

The 1H NMR spectra showed a singlet at 8–10 ppm for the ethylenic proton (-CH=) and multiplets between 7.36 and 8.06 ppm for aromatic protons.

The disappearance of peaks corresponding to methylenic and carboxylic acid protons confirmed successful cyclization and condensation (Table 2).

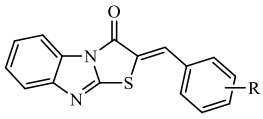
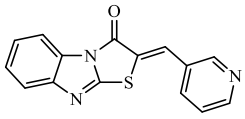
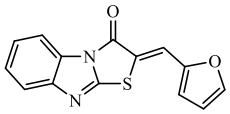
3.2 Antifungal Activity

The antifungal potential of the synthesized compounds was tested against Candida albicans, Candida tropicalis, and Candida glabrata, with ketoconazole and fluconazole as the reference drug.

Bioautography:

Compounds 3a, 3c, 3f, 3g, and 3h demonstrated significant activity against all Candida strains, comparable to ketoconazole. Compound 3e was active against C. albicans, while compound 3d was effective against C. tropicalis. Compound 3b showed no antifungal activity. Active or inactive antifungal status of compounds 3a to 3h and ketoconazole against Candida strains is summarized in Table 3.

Table 3: Results of Bioautography of compounds 3a to 3h and ketoconazole against *Candida* strains

Compounds	Structures	R	*Candida Albicans*	*Candida tropicalis*	*Candida Glabrata*
3a		**H**	+	+	+
3b		4-Cl	-	-	-
3c		4-$N(CH_3)_2$	+	+	+
3d		2-OH	-	+	-
3e		4-OCH_3	+	-	-
3f		4-OH 3-OCH_3	+	+	+
3g			+	+	+
3h			+	+	+
	Kétoconazole		+	+	+

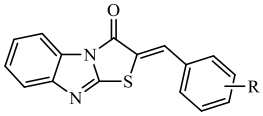
(+): Active (QMI = 10µg) / (-): Inactive (QMI >10µg)

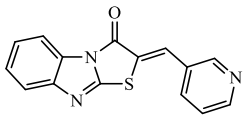
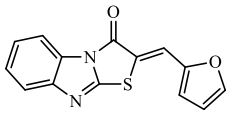
Minimum Inhibitory Concentrations (MICs):

MICs varied across strains and compounds. Fluconazole was most effective against C. tropicalis (MIC = 0.64 µM) but less so against C. albicans and C. glabrata.

ketoconazole showed consistent efficacy, especially against C. albicans (94.08 µM) (Table 4).

Table 4: In vitro antifungal activities of 2-benzylidene benzimidazolo-thiazolones against the three clinical strains of Candida

Compounds	Structures	R	Minimum Inhibitory Concentration (µM)		
			Candida qlbicans	***Candida tropicalis***	***Candida glabrata***
3a		H	89.82	0.70	359.28
3b		4-Cl	-	-	-
3c		4-$N(CH_3)_2$	311.13	4.86	1.22
3d		2-OH	-	1.33	-
3e		4-OCH_3	1.26	-	-
3f		4-OH 3-OCH_3	308.3	154.15	308.3

3g		5.59	0.7	358.01
3h		372.73	1.46	372.73
	Kétoconazole	94.08	188.17	23.52
	Fluconazole	326.5	0.64	20.41

• ***Summary of Key Findings****:*

C. albicans: Compound 3e was the most potent (MIC = 1.26 µM), surpassing fluconazole (326.5 µM) and ketoconazole (94.08 µM).

C. tropicalis: Compounds 3a and 3g exhibited strong activity (MIC = 0.7 µM), comparable to fluconazole.

C. glabrata: Compound 3c demonstrated the highest efficacy (MIC = 1.22 µM), outperforming both ketoconazole (23.52 µM) and fluconazole (20.41 µM).

4. Discussion

4.1. Spectroscopic Analysis

The spectral data confirmed the successful synthesis of the target compounds:

• The presence or absence of key peaks in the NMR spectra indicated successful transformations at each step.

• Mass spectrometry validated the molecular structures, ensuring the integrity of intermediates and final compounds.

4.2. Structure-Activity Relationship (SAR)

The antifungal activity was significantly influenced by substituent variations:

Against Candida albicans:

- Compound 3e: The methoxy group enhanced activity (MIC = 1.26 µM), suggesting that moderate electron-donating groups improve efficacy.
- Compounds 3b and 3d: Chlorine or hydroxyl groups resulted in a loss of activity, indicating the detrimental effects of strong electron-withdrawing or electron-donating groups.
- Compound 3g: The pyridine moiety improved activity (MIC = 5.59 µM), likely due to increased basicity.

Against Candida tropicalis:

- The unsubstituted Compound 3a matched fluconazole's activity.
- Substituents such as hydroxyl (3d) showed moderate activity, while methoxy (3e) derivatives lost efficacy.
- Pyridine (3g) and furan (3h) derivatives maintained activity similar to the parent compound.

Against Candida glabrata:

- Compound 3c (dimethylamino group) demonstrated the highest activity, suggesting that this substitution enhances interaction with C. glabrata targets.
- Other derivatives showed weak or negligible activity, highlighting the strain-specific nature of antifungal efficacy.

Summary of SAR Findings

- Best Overall Activity: Compound 3g (pyridine derivative) exhibited broad-spectrum efficacy.
- Best Against C. albicans: Compound 3e (methoxy derivative) was the most effective.
- Enhancing Activity: Moderately electron-donating groups (e.g., methoxy) or basic substitutions (e.g., pyridine) enhanced antifungal efficacy.
- Reducing Activity: Strongly electron-withdrawing (e.g., chlorine) or overly electron-donating groups diminished activity.
- The findings suggest that substituent modifications can tailor antifungal specificity and potency. In particular:

- Electron-donating groups enhance activity by potentially improving molecular interactions with fungal targets.
- Basic groups, such as pyridine, may enhance systemic properties, aligning with trends observed in azole antifungals.
- Strain-specific responses underscore the need for targeted modifications to optimize efficacy against particular fungal pathogens.

The synthesized 2-benzylidene benzimidazo-thiazol-3-one derivatives demonstrate promising antifungal activity, with select compounds outperforming reference drugs against specific Candida strains. Structure-activity relationship insights provide a foundation for designing derivatives with enhanced antifungal properties, paving the way for novel therapeutic agents.

5. Conclusion

The study successfully synthesized and characterized 2-benzylidene benzimidazo-thiazol-3-one derivatives and evaluated their antifungal efficacy against Candida albicans, Candida tropicalis, and Candida glabrata. Several derivatives exhibited promising activity, some comparable or superior to ketoconazole, suggesting their potential as candidates for antifungal therapy.

Efficient synthetic pathways yielded derivatives with well-defined structures, confirmed through NMR and mass spectrometry. Structural-functional analysis revealed that specific substituents, such as methoxy, hydroxyl, and pyridine, significantly influenced antifungal activity. Notably, compound 3e demonstrated the highest potency against C. albicans (MIC = 1.26 µM), while 3g showed broad efficacy across all tested strains. Compound 3a, lacking substituents, also displayed notable activity, indicating that steric and electronic factors critically affect antifungal properties.

The study emphasized the role of electron-donating groups, such as hydroxyl and methoxy, in enhancing efficacy, potentially due to improved interactions with fungal enzyme systems. Moreover, the derivatives exhibited competitive profiles against resistant strains like C. tropicalis and C. glabrata, highlighting their potential to address challenges associated with resistance and biofilm-associated infections.

These findings underscore the promise of 2-benzylidene benzimidazo-thiazol-3-one derivatives as alternative or adjunct antifungal therapies. Compounds 3a and 3g, in particular, stand out for further development. Future work involving in vivo studies and broader screenings will be essential to establish their therapeutic potential and safety, advancing efforts to combat the growing issue of antifungal resistance in Candida infections.

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