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Synthesis and antimicrobial evaluation of 2-thioxoimidazolidinone derivatives

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Abstract: The infectious diseases caused by antimicrobial pathogens are often difficult to treat and thwarted by resistance to drugs. Therefore, designing new drugs to treat antimicrobial infections is a challenge in drug discovery research. A series of 2-thioxoimidazolidinone derivatives were synthesized in high yield from *N'*-arylideneamino-2-thioxoimidazolidin-4-ones using propionyl chloride and triethyl amine in toluene medium. The compounds were screened for the *in vitro* antifungal and antibacterial activities. The studies revealed that compounds (±)1,1'-(3-(((1*E*,2*E*)-3-(2-methoxyphenyl)allylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)-bis(propan-1-one) and (±)1,1'-(3-((2,4-dichlorobenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis-(propan-1-one) possess antifungal properties comparable to the reference drug ketoconazole. This study highlights the importance of substituted 2-thioxoimidazolidinone derivatives, and their potential as antimicrobial agents.

Keywords: 2-Thioxoimidazolidinone; *in vitro* studies; nitrogen heterocycles; antifungal agents.

1. Introduction

The rise of antibiotic-resistant bacteria and the growing prevalence of fungal infections present significant challenges in medicine. Addressing these threats necessitates the search for novel antimicrobial agents with potent activity and structural characteristics.[1] The unique structure of 2-thioxoimidazolidinones underlies their diverse biological activities. Their presence in numerous biologically active natural products has been linked to a wide range of therapeutic uses, including their potential as anticancer, anti-inflammatory, analgesic, anticonvulsant, and antimicrobial agents.[2-10] The 2-thioxoimidazolidin-4-one scaffolds are found in a large number of biologically active compounds.[11] A change in the substituents of the 2-thioxoimidazolidin-4-one nucleus often led to incredibly diverse biological activities.[12] For instance, the 5-[(2-phenyl-1*H*-indol-3-yl) methylidene]-2-thioxoimidazolidin-4-one **A** (Figure 1) is known for its anti-HIV properties.[13] Substituted 4-methylene-

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2-thiohydantoin **B** displayed cycline dependent kinases (CDK) inhibition [14] as well as antileishmanial activity.[15] Compound **C** was reported to be active for the treatment of hormone-refractory prostate cancer,[16] whereas 3-[(1-methyl-2,6-diphenyl piperidin-4-yliden)amino]-2-thiohydantoin **D** demonstrated remarkable antimicrobial activity.[17] Moreover, studies had revealed the highly potent antiviral effects of 3,5-disubstituted-2-thioxoimidazolidinones and their glycoside derivatives against HIV, herpes simplex virus (HSV), leukemia, and cancer.[18-24]

The 2-thioxoimidazolidinone scaffold shows a broad spectrum of biological properties.[25-30] Even though the derivatives of 2-thioxoimidazolidinone have attracted considerable attention towards antimicrobial research, attempts to modify the 2-thioxoimidazolidinone nucleus to enhance its antimicrobial activities have only met with limited success.[31-37] From the antibacterial and antifungal evaluations of a few 2-thioxo-4-imidazolidinone derivatives carried out, modest results were observed with *N*-(5-(2-((4-chlorophenyl)amino)-2-oxoethyl)-3-cyclohexyl-4-oxo-2-thioxoimidazolidin-1-yl) benzamide towards *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and *N*-(3-cyclohexyl-4-oxo-5-(2-oxo-2-(phenylamino)ethyl)-2-thioxoimidazolidin-1-yl)-4-methyl benzamide towards *Candida albicans* and *Aspergillus niger*.[25] Among the different heterocyclic systems synthesized and evaluated for antimicrobial activity, the 3-(2-phenylacetyl)-2-thioxoimidazolidin-4-one did not show any antibacterial activity.[27] Though the acetylation of substituted 2-thioxo-4-imidazolidinones with acetic anhydride in the presence of fused sodium acetate was attempted in an effort to obtain the corresponding acetyl derivatives, to our surprise, the antimicrobial activity of these compounds remains largely unexplored in the scientific literature.[38] Despite numerous studies on similar compounds, the effect of acyl side chains on the biological properties has not been thoroughly investigated. Driven by the limitations and gaps in current research, we sought to further explore this area by focusing on the synthesis and characterization of propionylated derivatives of substituted benzylideneamino analogues of 2-thioxo-4-imidazolidinones. This approach is intended to uncover the potential antimicrobial properties of these compounds, which could offer valuable insights into their therapeutic applications, especially in the context of antimicrobial drug discovery. The 1,1'-(3-((substituted benzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis (propan-1-one) compounds were synthesized with structural modifications on the 3-(benzylidene)amino group. Biological evaluations of these molecules against the different bacterial and fungal strains allowed us to understand the structure-activity relationships.

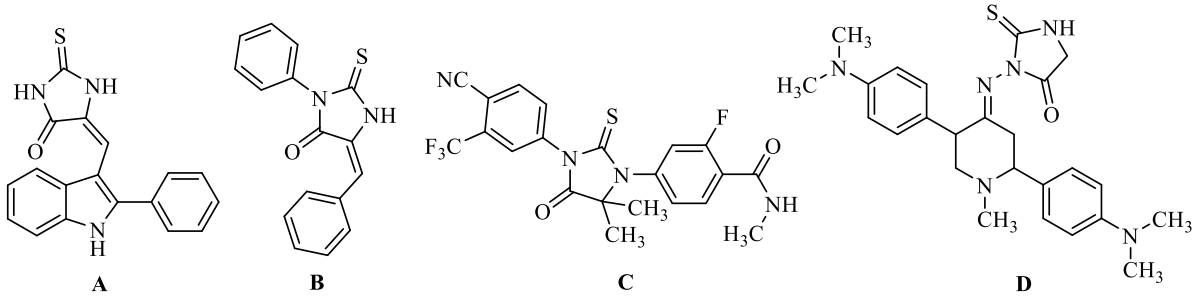

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Figure 1. Biologically active 2-thioxoimidazolidinones

2. Experimental

2.1. Synthesis

All the chemicals were obtained from commercial suppliers and used without further purification. The reactions were conducted in oven dried glassware and maintained under the appropriate atmospheric conditions. To monitor the progress of the reactions, thin-layer chromatography (TLC) was employed, specifically, 0.25 mm Merck Silica gel 60 F254 plates were used, and visualization was achieved using UV light. Column chromatography was performed using 100-200 mesh silica gel as the stationary phase. Elution was carried out using a mixture of hexane and ethyl acetate as the mobile phase. Nuclear magnetic resonance (NMR) spectra were recorded using a Jeol ECZ 400R spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. $CDCl_3$ was utilized as the solvent, and tetramethyl silane

(TMS) served as the internal standard. Chemical shifts (δ) were reported relative to residual solvent signals, specifically 7.25 ppm for ^{1}H NMR and a triplet centred at 77.00 ppm for ^{13}C NMR. Mass spectrometry analysis was conducted using an ESI (electrospray ionization) quadrupole time-of-flight Agilent mass spectrometer.

*General Procedure for the Synthesis of N'-Arylidene hydrazine carbothioamide Analogues (**1**)*

To a solution of substituted aldehyde (1.0 mmol) in EtOH (10 mL), HCl (12.1 N, 2.0 mmol) was added, and stirred at room temperature for 5 minutes. To this solution, thiosemicarbazide (1.0 mmol) was added and the reaction mixture was stirred at room temperature for 1 hour. The precipitate formed was filtered, washed with ice-cold water, hexane and dried. The resulting solid was filtered off, washed with hexane, and dried.

*General Procedure for the Synthesis of N'-Arylideneamino-2- thioxoimidazolidin-4-one Analogues (**2**)*

A mixture of substituted *N*'-arylidene hydrazine carbothioamide (1.0 mmol), ethyl chloroacetate (1.0 mmol) and anhydrous sodium acetate (3.0 mmol) in ethanol (50 mL) was refluxed for 4 hours. After cooling to room temperature, the reaction mixture was poured into ice-cold water. The resulting solid was filtered off, washed with water, and dried.

*General Procedure for the Synthesis of (±)1,1'-(3-((Substituted benzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) (**3**)*

A mixture containing substituted *N*'-arylideneamino-2-thioxoimidazolidin-4-one (0.01 mmol), propionyl chloride (0.02 mmol) and triethylamine (0.03 mmol) in toluene (20 mL) was heated at 110 °C with constant stirring for 20 minutes. After cooling to room temperature, the reaction mixture was concentrated, diluted with water, and extracted thrice with EtOAc. The combined organic extracts were concentrated under vacuum, and the resultant crude was subjected to purification by column chromatography on silica gel (60-120 mesh) using hexane-ethyl acetate mixture (10:1) as the mobile phase to obtain the desired product.

*(±)1,1'-(3-(benzylideneamino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) (**3a**)*: White colour solid (90% yield); M.p. = 72-74 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.91 (s, 1H, CH), 7.75 – 7.78 (m, 2H, Ar-H), 7.41 – 7.47 (m, 3H, Ar-H), 6.87 (s, 1H, CH), 2.92 – 2.98 (m, 2H, CH_2), 2.56 – 2.62 (m, 2H, CH_2), 1.22 – 1.27 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 175.54, 171.72, 155.06, 153.85, 150.10, 133.76, 131.32, 128.89, 128.22, 100.75, 28.25, 27.70, 8.89, 8.76 Mass (ESI): m/z calcd. for $C_{16}H_{17}N_3O_3S$ $[M+H]^+$: 332.09, found: 332.00.

*(±)1,1'-(3-((3,4-dimethoxybenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) (**3b**)*: Yellow colour solid (85% yield); M.p. = 105-107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H, CH), 7.43 (m, 1H, Ar-H), 7.21 – 7.25 (m, 1H, Ar-H), 6.87 – 6.91 (m, 1H, Ar-H), 6.79 (s, 1H, CH), 3.92 (s, 6H, OCH_3), 2.83 – 2.89 (m, 2H, CH_2), 2.50 – 2.59 (m, 2H, CH_2), 1.18 – 1.25 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.76, 171.68, 158.99, 154.24, 152.36, 149.90, 149.50, 126.29, 123.96, 110.78, 108.90, 99.85, 56.85, 28.21, 27.67, 8.86, 8.63; Mass (ESI): m/z calcd. for $C_{18}H_{21}N_3O_5S$ $[M+H]^+$: 392.12, found: 392.00.

*(±)1,1'-(3-((2-chlorobenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) (**3c**)*: White colour solid (87% yield); M.p. = 120-122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (s, 1H, CH), 8.01 – 8.07 (m, 1H, Ar-H), 7.29 – 7.41 (m, 3H, Ar-H), 6.89 (s, 1H, CH), 2.95 – 3.00 (m, 2H, CH_2), 2.55 – 2.61 (m, 2H, CH_2), 1.22 – 1.29 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.73, 171.65, 153.62, 150.33, 150.24, 135.60, 131.93, 131.63, 130.14, 127.44, 127.12, 101.18, 28.27, 27.70, 8.93, 8.75; Mass (ESI): m/z calcd. for $C_{16}H_{16}ClN_3O_3S$ $[M+H]^+$: 366.06, found: 366.00.

(±)1,1'-(3-((3-nitrobenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3d)***: Brown colour solid (88% yield); M.p. = 110-112 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H, CH), 8.56 – 8.57 (m, 1H, Ar-H), 8.25 – 8.29 (m, 1H, Ar-H), 8.03 – 8.09 (m, 1H, Ar-H), 7.62-7.64 (t, 1H, Ar-H), 6.94 (s, 1H, CH), 3.00 – 3.07 (m, 2H, CH_2), 2.59 – 2.65 (m, 2H, CH_2), 1.24 – 1.31 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.97, 171.71, 153.35, 150.26, 148.78, 148.31, 136.05, 133.34, 129.93, 125.18, 122.57, 101.65, 28.22, 27.70, 8.88, 8.74; Mass (ESI): m/z calcd. for $C_{16}H_{16}N_4O_5S$ $[M+H]^+$: 377.02, found: 377.00.

(±)1,1'-(3-((4-fluorobenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3e)***: White colour solid (86% yield); M.p. = 86-88 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H, CH), 7.74-7.78 (m, 2H, Ar-H), 7.09 – 7.15 (m, 2H, Ar-H), 6.86 (s, 1H, CH), 2.90 – 2.98 (m, 2H, CH_2), 2.55-2.63 (m, 2H, CH_2), 1.22 – 1.27 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.45, 171.72, 153.64, 150.06, 130.20, 130.11, 130.02, 116.22, 116.00, 100.73, 28.23, 27.69, 8.88, 8.70; Mass (ESI): m/z calcd. for $C_{16}H_{16}FN_3O_3S$ $[M+H]^+$: 350.08, found: 350.00.

(±)1,1'-(3-(((1E,2E)-3-(2-methoxyphenyl)allylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3f)***: Yellow colour solid (85% yield); M.p. = 108-110 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H, CH), 7.53 – 7.57 (m, 1H, Ar-H), 7.36 – 7.43 (m, 1H, Ar-H), 7.29 – 7.36 (m, 1H, Ar-H), 7.03 – 7.11 (m, 1H, Ar-H), 6.95 –7.00 (m, 1H, CH), 6.88 – 6.94 (m, 1H, CH), 6.84 (s, 1H, CH), 3.88 (s, 3H, OCH_3), 2.78 – 2.85 (m, 2H, CH_2), 2.55 – 2.62 (m, 2H, CH_2), 1.20 – 1.25 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.80, 171.68, 161.42, 157.66, 154.17, 150.12, 139.12, 131.00, 127.86, 124.96, 124.48, 120.92, 111.22, 100.12, 55.61, 28.10, 27.72, 8.88, 8.62; Mass (ESI): m/z calcd. for $C_{19}H_{21}N_3O_4S$ $[M+H]^+$: 388.12, found: 388.00.

(±)1,1'-(3-((2,4-dichlorobenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3g)***: White colour solid (85% yield); M.p. = 81-83 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (s, 1H, CH), 7.94 – 7.99 (m, 1H, Ar-H), 7.41– 7.44 (m, 1H, Ar-H), 7.28 – 7.32 (m, 1H, Ar-H), 6.89 (s, 1H, CH), 2.94 – 3.00 (m, 2H, CH_2), 2.56 – 2.62 (m, 2H, CH_2), 1.22– 1.27 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.77, 171.67, 153.49, 150.23, 140.37, 137.26, 136.03, 130.36, 129.94, 128.16, 127.71, 101.28, 28.26, 27.69, 8.93, 8.74; Mass (ESI): m/z calcd. for $C_{16}H_{15}Cl_2N_3O_3S$ $[M+H]^+$: 400.02, found: 400.00.

(±)1,1'-(3-((3-chlorobenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3h)***: White colour solid (90% yield); M.p. = 45-48 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H, CH), 7.73 – 7.76 (m, 1H, Ar-H), 7.57 – 7.60 (m, 1H, Ar-H), 7.38 – 7.41 (m, 1H, Ar-H), 7.33 – 7.37 (m, 1H, Ar-H), 6.89 (s, 1H, CH), 2.95 – 3.00 (m, 2H, CH_2), 2.57 – 2.62 (m, 2H, CH_2), 1.22 – 1.27 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.77, 171.69, 153.58, 151.27, 150.19, 135.82, 135.01, 130.98, 130.12, 127.53, 126.49, 101.22, 28.20, 27.68, 8.88, 8.71; Mass (ESI): m/z calcd. for $C_{16}H_{16}ClN_3O_3S$ $[M+H]^+$: 366.06, found: 366.00.

(±)1,1'-(3-((3-fluorobenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3i)***: White colour solid (89% yield); M.p. = 68-70 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H, CH), 7.45 – 7.52 (m, 2H, Ar), 7.36 – 7.42 (m, 1H, Ar), 7.10 – 7.16 (m, 1H, Ar), 6.90 (s, 1H, CH), 2.95 – 3.00 (m, 2H, CH_2), 2.57 – 2.63 (m, 2H, CH_2), 1.22 – 1.28 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.80, 171.72, 153.60, 151.47, 150.19, 136.31, 130.50, 130.41, 124.50, 118.16, 113.98, 101.22, 28.21, 27.69, 8.88, 8.73; Mass (ESI): m/z calcd. for $C_{16}H_{16}FN_3O_3S$ $[M+H]^+$: 350.08, found: 350.00.

(±)1,1'-(3-((2,6-dichlorobenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3j)***: White colour solid (88% yield); M.p. = 77-80 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H, CH), 7.36 – 7.38 (m, 1H, Ar-H), 7.32-7.35 (m, 1H, Ar-H), 7.20 – 7.24 (m, 1H, Ar-H), 6.94 (s, 1H, CH), 2.95 – 3.01 (m, 2H, CH_2), 2.56 – 2.61 (m, 2H, CH_2), 1.20 – 1.25 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.56, 171.64, 153.31, 150.48, 146.51, 135.29, 130.63, 130.40, 129.12, 101.91, 28.14, 27.68, 8.92, 8.78; Mass (ESI): m/z calcd. for $C_{16}H_{15}Cl_2N_3O_3S$ $[M+H]^+$: 400.02, found: 400.00.

(±)1,1'-(3-((4-methylbenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3k)***: Yellow colour solid (85% yield); M.p. = 91-93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H, CH), 7.64 – 7.68 (m, 2H, Ar-H), 7.22 – 7.25 (m, 2H, Ar-H), 6.84 (s, 1H, CH), 2.89 – 2.95 (m, 2H, CH_2), 2.54 – 2.61 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 1.21 – 1.25 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.32, 171.70, 156.48, 154.02, 150.04, 141.97, 130.95, 129.63, 128.28, 100.38, 28.23, 27.69, 21.70, 8.88, 8.70; Mass (ESI): m/z calcd. for $C_{17}H_{19}N_3O_3S$ $[M+H]^+$: 346.11, found: 346.00.

(±)2-(((5-oxo-3,4-dipropionyl-2-thioxoimidazolidin-1-yl)imino)methyl)phenyl propionate ***(3l)***: Brown colour solid (86% yield); M.p. = 74-76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H, CH), 8.01 – 8.06 (m, 1H, Ar-H), 7.42 – 7.50 (m, 1H, Ar-H), 7.26 – 7.35 (m, 1H, Ar-H), 7.12 – 7.17 (m, 1H, Ar-H), 6.86 (s, 1H, CH), 2.94 – 3.01 (m, 2H, CH_2), 2.53 – 2.66 (m, 4H, CH_2), 1.21 – 1.27 (m, 9H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.95, 172.69, 171.59, 153.75, 150.41, 150.03, 147.98, 131.95, 126.73, 126.36, 126.27, 123.02, 100.80, 28.27, 27.60, 27.54, 9.08, 8.86, 8.76; Mass (ESI): m/z calcd. for $C_{19}H_{21}N_3O_5S$ $[M+H]^+$: 404.12, found: 404.00.

(±)1,1'-(3-((4-methoxybenzylidene)amino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3m)***: White colour solid (87% yield); M.p. = 90-92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H, CH), 7.70 – 7.75 (m, 2H, Ar-H), 6.92 – 6.95 (m, 2H, Ar-H), 6.80 (s, 1H, CH), 3.84 (s, 3H, OCH_3), 2.84 – 2.91 (m, 2H, CH_2), 2.53 – 2.59 (m, 2H, CH_3), 1.19 – 1.25 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.00, 171.71, 162.47, 158.03, 154.20, 149.91, 130.12, 126.13, 114.36, 99.89, 55.51, 28.23, 27.67, 8.87, 8.65; Mass (ESI): m/z calcd. for $C_{17}H_{19}N_3O_4S$ $[M+H]^+$: 362.11, found: 362.00.

(±)1,1'-(4-oxo-3-(((1,2)-3-phenylallylidene)amino)-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) ***(3n)***: Yellow colour solid (88% yield); M.p. = 103-105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H, CH), 7.49 – 7.52 (m, 2H, CH), 7.33 – 7.40 (m, 3H, Ar-H), 6.96 – 7.07 (m, 2H, Ar-H), 6.85 (s, 1H, CH), 2.81 – 2.88 (m, 2H, CH_2), 2.56 – 2.62 (m, 2H, CH_2), 1.20 – 1.25 (m, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.06, 171.73, 158.82, 154.02, 150.05, 143.45, 135.58, 129.67, 128.99, 127.51, 124.76, 100.48, 28.08, 27.70, 8.87, 8.63; Mass (ESI): m/z calcd. for $C_{18}H_{19}N_3O_3S$ $[M+H]^+$: 358.11; found: 358.00.

2.2. Antimicrobial Activity

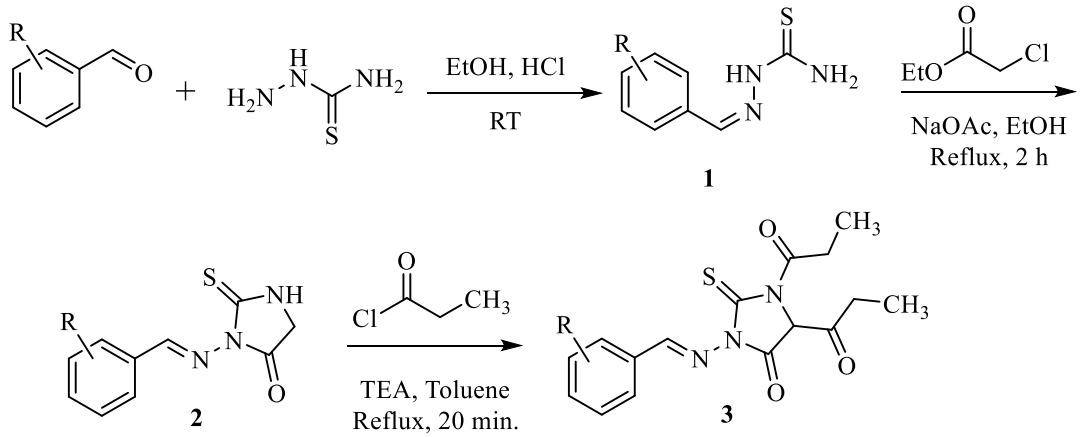
The compounds **3a-3n** were assayed for *in vitro* antimicrobial activity by agar well diffusion method against test organisms. Nutrient broth (NB) plates were swabbed with 24 h-old broth culture (100 µL) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petri plate. The compounds were dissolved in DMSO (5 mg/mL), and from this 10, 15, and 20 µL (50, 75, 100 µg/well) were added into the wells by using sterile pipettes. Simultaneously, the standard antibiotics, chloramphenicol for antibacterial activity, and ketoconazole for antifungal activity (as positive control) were tested against the pathogens. The samples were dissolved in DMSO, which showed no zone of inhibition and acts as negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After appropriate incubation, the diameter of the zone of inhibition of each well was measured. Duplicates were maintained, and the average values were calculated for eventual antimicrobial activity. Broth dilution test is used to determine the minimum inhibitory concentration (MIC) of the test samples. Freshly prepared nutrient broth was used as diluents. The 24 h old culture of the test bacteria and the test fungi were diluted 100-folds in nutrient broth (100 µL bacterial cultures in 10 mL NB). The stock solution of the synthesized compounds was prepared in DMSO by dissolving 5 mg of the compound in 1 mL of DMSO. Increasing concentrations of the test samples (2.5, 5, 10, 20 µL of stock solution contains 6.25, 12.5, 25, 50, 100 µg of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The tubes were examined for visible turbidity, and NB was used as a control. Control without test samples and with solvent was determined simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC. To determine the MBC and MFC for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated on sterile nutrient broth (for bacteria) and

PDA (for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37 °C for 24 h and at 28 °C for 48 h, respectively. After incubation, the lowest concentration at which no visible growth observed was noted for bacteria and fungi.

3. Results and Discussion

3.1. Chemistry

The synthetic route for the preparation of 4-oxo-2-thioxoimidazolidine derivatives is illustrated in Scheme 1. The benzylidene thiosemicarbazone analogues **1** were prepared by reacting substituted aromatic aldehydes and thiosemicarbazide in the presence of dilute HCl and ethanol. The addition of HCl to the aldehyde increases the electrophilicity of the carbonyl group facilitating the nucleophilic attack by the thiosemicarbazide upon introducing it to the reaction mixture. The reaction proceeds through a polar transition state which is stabilized by the protic solvent ethanol. Further, the protonation of the OH group facilitates its elimination by the assistance of the lone pair of electrons on the nitrogen atom to form thiosemicarbazone. The thiosemicarbazone intermediate **1** was refluxed with sodium acetate in ethanol medium wherein the nitrogen N2 of the thiosemicarbazone reacts with ethyl chloroacetate by a nucleophilic substitution mechanism. The resulting intermediate then proceeds to cyclization forming intermediate **2** by the intramolecular nucleophilic attack of the terminal amino nitrogen N1 of the thiosemicarbazone on the chloroalkyl chain, displacing the chloride. From these key precursors, the 1,1'-(3-(substituted benzylideneamino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) molecules **3** were obtained in high yields by acylation reaction using propionyl chloride in the presence of triethyl amine (TEA) in toluene. A plausible reaction mechanism for the synthetic transformations is depicted in Figure 2.



Scheme 1. Synthesis of 1,1'-(3-(substituted benzylideneamino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) derivatives **3**

Conditions were optimized for the formation of compound **3** by varying several factors such as solvent, temperature, nature and concentration of the bases. A typical reaction of 3-(benzylideneamino)-2-thioxoimidazolidin-4-one **2a** with propionyl chloride was chosen for optimizing the reaction conditions. Different solvents such as dimethyl sulfoxide, dimethyl formamide, dioxane, tetrahydrofuran, acetonitrile, and toluene were studied (Table 1) and among the solvents employed, toluene was found to be the most effective, completing the reaction within 20 minutes at the reflux temperature of 110 °C. Toluene provides an ideal environment for the reaction by being compatible with the reactants to interact efficiently, resulting in higher yield compared to the other solvents tested. Several bases (Table 2), were tried for the reaction, varying from potassium carbonate to potassium *tert*-butoxide. The results were discouraging in terms of time and product yield, except with triethyl amine wherein the reaction was complete in 20 minutes, yielding 90% of the desired product. Furthermore, using toluene as the solvent and TEA as the base, the reaction temperature was varied from room

temperature to 110 °C (Table 3) to study the reaction kinetics. It was observed that an increase in temperature led to an acceleration of the reaction rate. Reactions were also performed by varying the concentration of triethyl amine from 0.5 to 2.0 equivalents with respect to the reactant (Table 4). At a concentration of 0.5 equivalent, the reaction was completed in 4 hours with a yield of 50%, while at a concentration of 2.0 equivalents, the reaction time was reduced to 20 minutes, resulting in an optimal yield of 90%. Based on the optimized conditions a series of 1,1'-(3-(substituted benzylideneamino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) molecules **3** containing electron donating and withdrawing groups on the aromatic ring were synthesized, and are shown in Figure 3 with the yields.

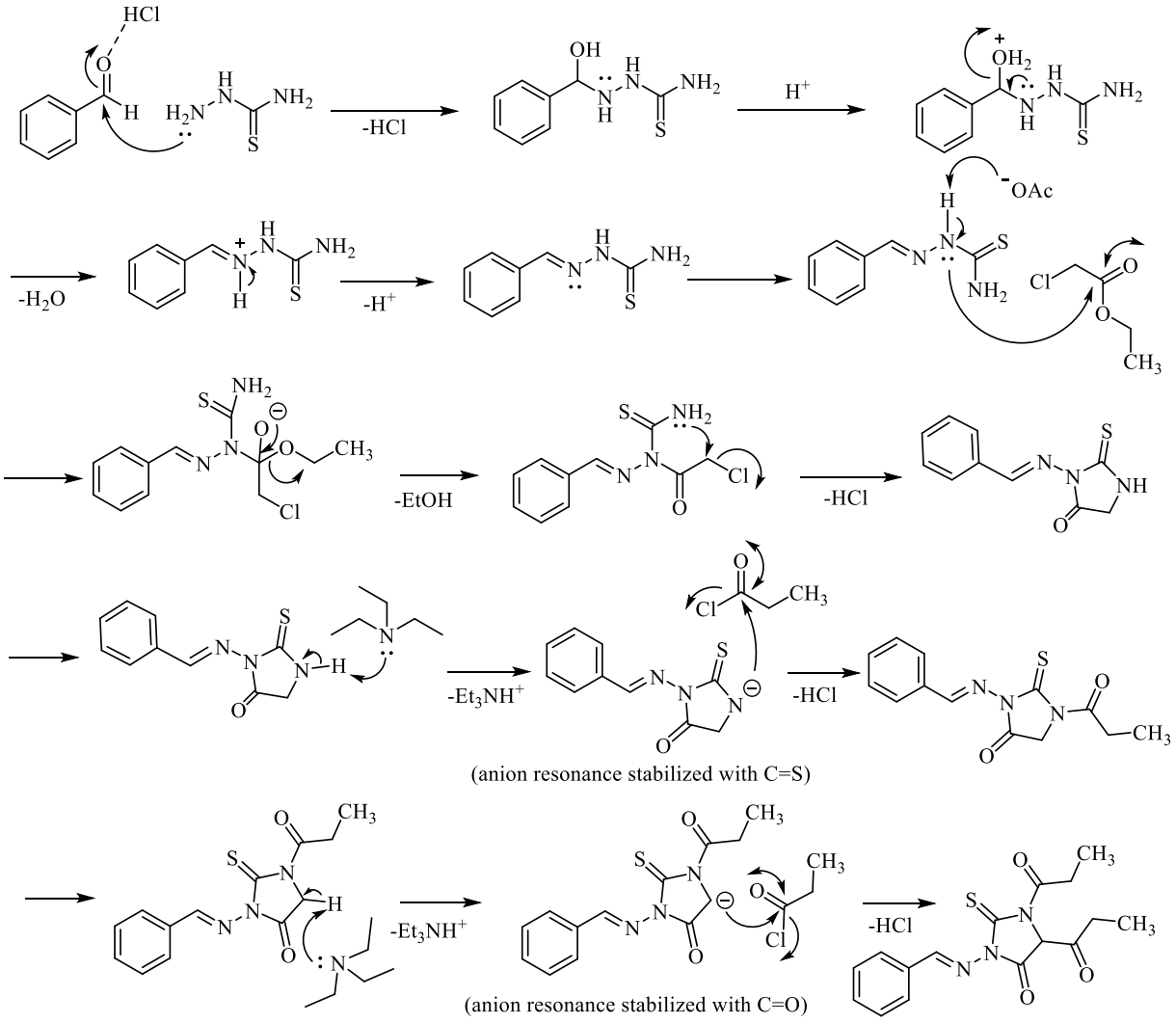


Figure 2. Plausible reaction mechanism for the formation of substituted 2-thioxoimidazolidinone derivatives

Table 1. Role of solvent

Entry	Solvent	Time	Yield (%)
01	DMSO	2 h	50
02	DMF	2 h	60
03	Dioxane	1 h	40
04	THF	1.5 h	30
05	Acetonitrile	1 h	70
06	Toluene	20 min.	90

Compound **2a** (0.01 mmol), Propionyl chloride (0.02 mmol), TEA (0.02 mmol), Solvent (10 mL), Temp. Reflux

Table 2. Influence of base

Entry	Base	Time	Yield (%)
01	K^{t}OBu	12 h	50
02	Na^{t}OBu	10 h	60
03	DIPEA	2 h	70
04	Na_2CO_3	8 h	30
05	K_2CO_3	8 h	30
06	NaH	6 h	40
07	TEA	20 min.	90

Compound **2a** (0.01 mmol), Propionyl chloride (0.02 mmol), Base (0.02 mmol), Toluene (10 mL), Temp. Reflux

Table 3. Effect of temperature

Entry	Temperature (°C)	Time	Yield (%)
01	RT (27°C)	24 h	Trace
02	40	24 h	Trace
03	60	12 h	60
04	80	2 h	70
06	110	20 min.	90

Compound **2a** (0.01 mmol), Propionyl chloride (0.02 mmol), TEA (0.02 mmol), Toluene (10 mL)

Table 4. Influence of triethyl amine concentration

Entry	TEA (Equiv.)	Time	Yield (%)
01	0.5	4 h	50
02	0.75	3 h	55
03	1.0	2 h	65
04	1.5	1 h	70
05	2.0	20 min.	90

Compound **2a** (0.01 mmol), Propionyl chloride (0.02 mmol), Toluene (10 mL), Temp. Reflux

3.2. Antimicrobial Activity

The antifungal activity of synthesized 2-thioxoimidazolidinone molecules was evaluated against two pathogenic strains, *Aspergillus niger* and *Candida albicans*. A comparison of the antifungal activity of the synthesized compounds to that of ketoconazole is presented in Figure 4. The corresponding data obtained at concentrations of 12.5, 25, 50 and 100 μg per well are provided in Table 5. Interestingly, all

tested compounds demonstrated inhibition of spore germination and displayed antifungal activity. Compounds **3f** and **3g** exhibited good activity against both strains, comparable to the standard ketoconazole. The presence of two chlorine substituents at the ortho and para positions of the aromatic ring in the compound **3g**, as well as the presence of an extended conjugation along with a methoxy substituent at the ortho position of the aromatic ring in the compound **3f**, demonstrated significant *in vitro* antifungal activity compared to other analogues of the series. These findings highlight the 2-thioxoimidazolidinone derivatives **3f** and **3g**, as valuable candidates for further structural exploration and development as antifungal agents.

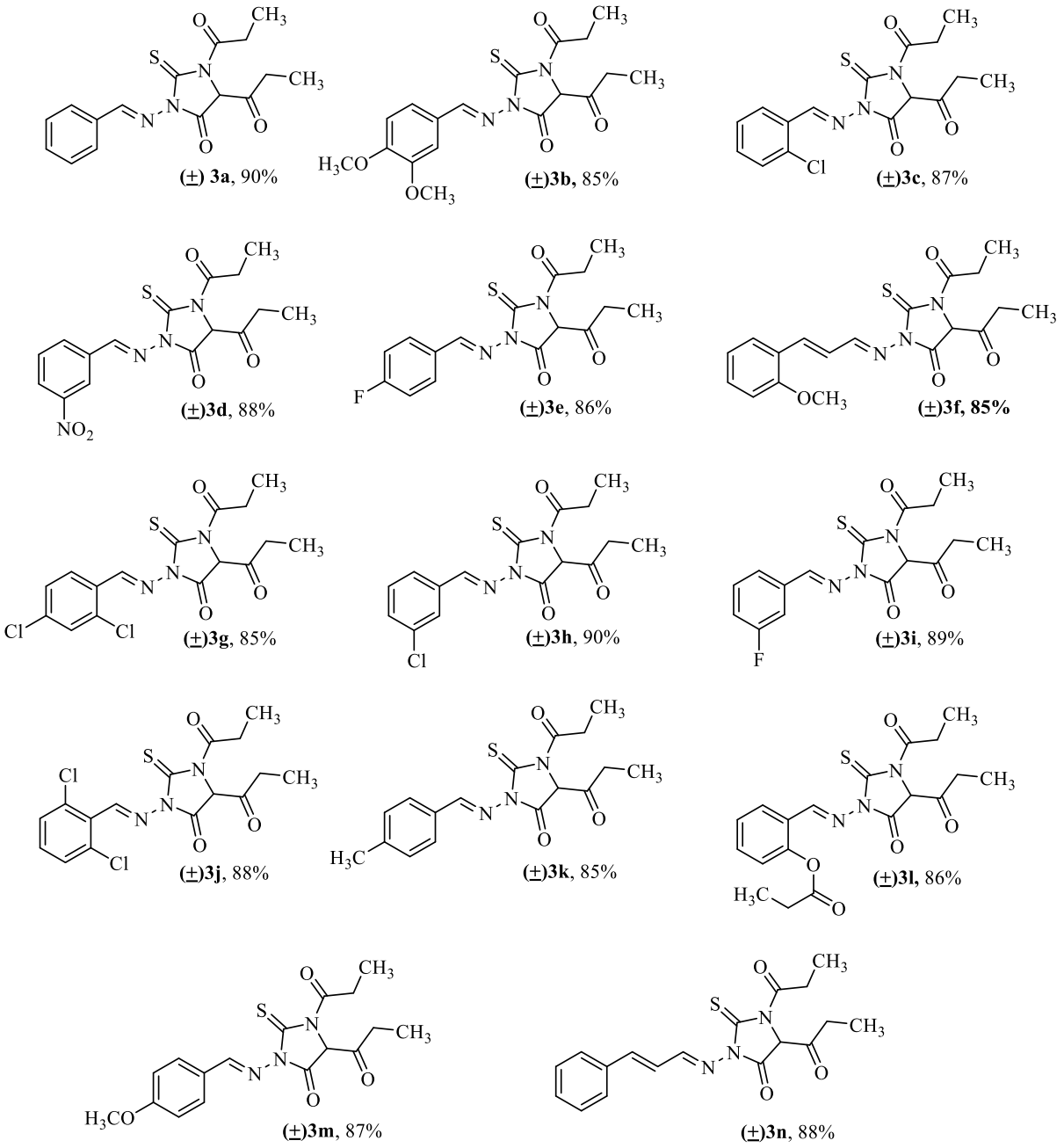


Figure 3. Synthesized substituted 2-thioxoimidazolidinone derivatives

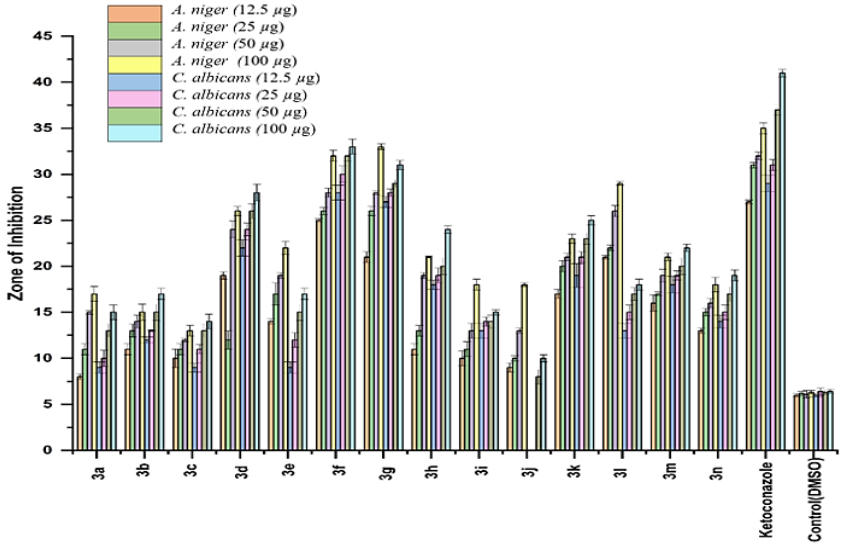


Figure 4. Antifungal activity against *A. niger* and *C. albicans*

Table 5. *In vitro* antifungal activity of substituted 2-thioxoimidazolidinone derivatives **3**

Compound	***A. niger***				***C. albicans***			
	12.5 µg/ well	**25 µg/ well**	**50 µg/ well**	**100 µg/ well**	**12.5 µg/ well**	**25 µg/ well**	**50 µg/ well**	**100 µg/ well**
3a	8±3	11±3	15±2	17±1	9±1	10±3	13±1	15±1
3b	11±2	13±1	14±1	15±2	12±2	13±1	15±1	17±2
3c	10±1	11±1	12±2	13±1.2	9±2.2	11±2.2	13±2.3	14±1.1
3d	19±2	22±1	24±1	26±2	22±3	24±2	26±2	28±3
3e	14±2	17±1	19±2	22±2	9±1	12±2	15±2	17±2.1
3f	**25±2**	**26±3**	**28±1**	**32±2**	**28±2**	**30±1**	**32±1**	**33±3**
3g	**21±1**	**26±2**	**28±1**	**33±1**	**27±3**	**28±1**	**29±2**	**31±2**
3h	11±1.6	13±1.3	19±2.1	21±2.3	18±2.4	19±1.6	20±2.6	24±2
3i	10±1	11±1	13±3	18±1	13±2	14±1	14±3	15±2
3j	9±3	10±1	13±2	18±2	6±0.2	6±0.3	8±1	10±2
3k	17±2	20±3	21±1	23±1	19±1	21±2	23±3	25±2
3l	21±3	22±2	26±1	29±2	13±2	15±1	17±3	18±1
3m	16±1	17±1	19±2	21±2	18±2	19±1	20±2	22±2
3n	13±2	15±1	16±1	18±2	14±1	15±1	17±1	19±1
Ketoconazole	27±2	31±1	32±3	35±1	29±1	31±3	37±1	41±2
Control (DMSO)	6.1±0.2	6.5±0.1	6.3±0.3	6.2±0.1	6.4±0.2	6.1±0.3	6.1±0.1	6.3±0.2

[-] No activity [±] Standard deviation. Values are Mean ± SD of three independent experiments. The zone was measured and represented in milli meters.

The antibacterial activity analysis of the substituted 2-thioxoimidazolidinone derivatives **3a-3n** against other pathogens is depicted in Figure 5(a-b). The derivatives were tested against Gram-positive bacteria, *E. faecalis* and *S. aureus*, and Gram-negative bacteria, *E. coli* and *P. aeruginosa*. The tested compounds exhibited moderate antibacterial activity against the bacterial strains (*E. faecalis* 51299, *E. coli* 35218, *P. aeruginosa ATCC15692* and *S. aureus* ATCC 25923). The derivatives were subjected to *in vitro* evaluation to assess their potential for inhibiting the growth of these selected pathogens. The antibacterial activity was determined by comparing the results to the standard antibiotic chloramphenicol (Table S1, see supporting information), and modest activity was observed. On the other

hand, the results underscore the selectivity of the molecules as potential antifungal agents. This also necessitates further modifications of the derivatives to enhance the antibacterial efficacy.

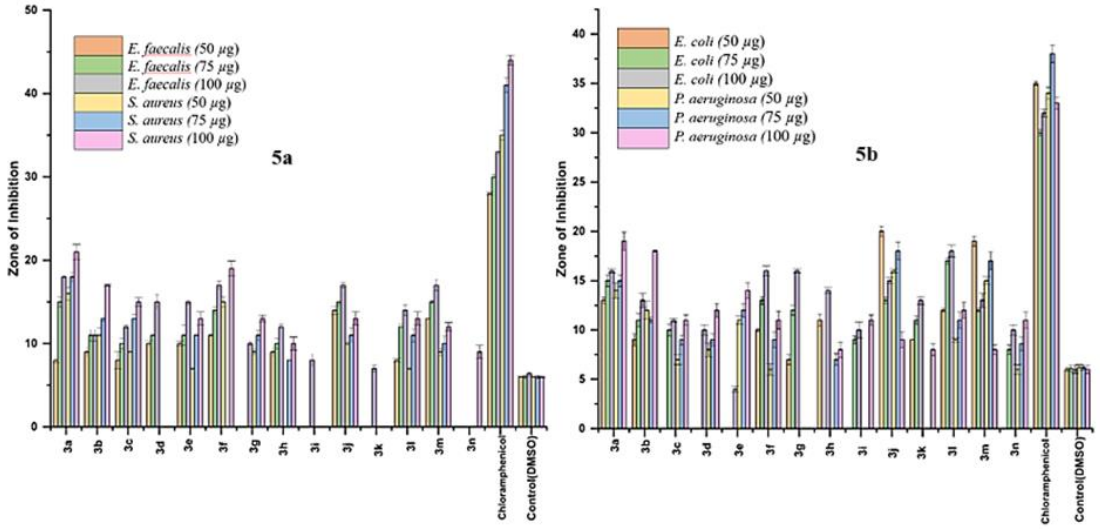


Figure 5(a-b). Antibacterial activity against (**a**) Gram-positive bacteria and (**b**) Gram-negative bacteria

In order to assess the antimicrobial potential, compounds **3a**-**3n** were further evaluated to determine their Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC) (Table 6). These values provide important insights into the concentration required to inhibit or kill microorganisms. It is determined by testing the compound against various microorganisms and observing the concentration at which growth is no longer visible. The MIC value indicates the effectiveness of the compound in inhibiting the growth of the microorganism.[39] The MBC, on the other hand, represents the minimum concentration required to kill the bacteria rather than just inhibiting its growth. It is determined by taking the samples from the wells with no visible growth from the MIC test and inoculating them onto agar plates without the compound. Similarly, the MFC, which is specific to fungi, represents the minimum concentration required to kill the fungal cells. It is determined using the same principle as the MBC but with fungal pathogens instead of bacterial pathogens.

All derived compounds evaluated in the study exhibited modest antimicrobial efficacy, as demonstrated by their relatively low minimum inhibitory concentration (MIC) values against the respective microorganisms compared to the reference drug. This denotes that further research and structural modifications of synthesized compounds are necessary to enhance the activity. By systematically modifying the chemical structures, it would be possible to improve the antimicrobial effects still further. Future studies are directed to explore the structural changes on this scaffold and to optimize the biological activity.

Table 6. MIC, MBC and MFC of substituted 2-thioxoimidazolidinone derivatives **3**

Compound	Minimum Inhibitory Concentration MIC (MBC / MFC) µg/well					
	E. faecalis	***E. coli***	***P. aeruginosa***	***S. aureus***	***A. niger***	***C. albicans***
3a	25 (100)	25 (100)	12.5 (50)	50 (>100)	50 (100)	25 (100)
3b	25 (50)	50 (200)	50 (200)	50 (100)	25 (50)	50 (200)
3c	100 (200)	50 (200)	50 (100)	50 (>100)	12.5 (50)	50 (100)
3d	25 (100)	50 (50)	25 (100)	25 (100)	25 (50)	25 (200)
3e	25 (50)	50 (>100)	100 (200)	50 (200)	100 (200)	50 (100)
3f	50 (100)	50 (>100)	25 (50)	50 (100)	50 (200)	100 (200)
3g	12.5 (50)	50 (100)	100 (200)	100 (200)	100 (>200)	50 (100)
3h	50 (200)	50 (>100)	25 (100)	50 (100)	50 (>100)	50 (>100)
3i	50 (100)	25 (100)	50 (200)	25 (50)	100 (200)	25 (100)
3j	25 (100)	12.5 (50)	25 (100)	25 (100)	100 (200)	100 (200)
3k	50 (>100)	25 (100)	50 (100)	25 (100)	50 (100)	100 (200)
3l	50 (100)	25 (100)	25 (100)	50 (200)	50 (>100)	25 (100)
3m	50 (200)	25 (50)	12.5 (50)	50 (100)	25 (100)	25 (100)
3n	25 (100)	50 (500)	100 (200)	50 (>100)	50 (200)	50 (200)
Chloramphenicol **3r**	6.25	6.25	6.25	6.25	-	-
Ketoconazole **3s**	-	-	-	-	12.5	12.5

4. Conclusion

In this study, 1,1'-(3-(substituted benzylideneamino)-4-oxo-2-thioxoimidazolidine-1,5-diyl)bis(propan-1-one) molecules **3** were synthesized by a three-step procedure. The reaction of aryl aldehydes with thiosemicarbazide gave substituted 2-benzylidene hydrazine-1-carbothioamide **1**, which was cyclized with ethyl chloroacetate to afford *N*'-arylideneamino-2-thioxoimidazolidin-4-one **2**. Treatment of the intermediate **2** with propionyl chloride afforded the product **3**. The antimicrobial activity of the synthesized compounds was evaluated using the agar well diffusion method. The results of the evaluation afforded comparable antimicrobial activity of the compounds **3f** and **3g** against the fungal strains, *Aspergillus niger* and *Candida albicans*. The presence of two chlorine substituents at the ortho and para positions of the aromatic ring in the compound **3g**, as well as the presence of an extended conjugation along with a methoxy substituent at the ortho position of the aromatic ring in the compound **3f**, resulted in improved *in vitro* antifungal activity. This also warrants that further structural modification of the scaffold is necessary to enhance the biological activity.

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/organic-communications

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