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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW MANNICH BASES OF 7-ACYL-5-CHLORO-2-OXO-3*H*-BENZOXAZOLE DERIVATIVES

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Abstract

A series of novel Mannich bases of 7-acyl-5-chloro-2-oxo-3H-benzoxazoles were synthesized by reacting them with formaldehyde and several seconder amine derivatives. Their chemical structures were elucidated by means of their IR, ^{1}H-NMR data and by elemental analysis. Investigation of in vitro antimicrobial activity of compounds was done by broth microdilution method against six pathogenic bacteria.

Key words: *Mannich bases, benzoxazole, antibacterial, antifungal*

7-Açil-5-Kloro-2-Okso-3*H*-Benzoksazol Türevlerinin Bazı Yeni Mannich Bazlarının Sentezi ve Antimikrobiyal Aktivitesi

7-Açil-5-Kloro-2-Okso-3H-Benzoksazollerin mannich bazları, bu türevlerin formaldehit ve birkaç sekonder amin türevleriyle tepkimeye sokulmasıyla sentezlenmiştir. Kimyasal yapıları, IR, ^{1}H-NMR ve elementel analiz verileriyle kanıtlanmıştır. Bileşiklerin in vitro antimikrobiyal aktivitelerinin incelenmesi, altı patojenik bakteriye karşı broth mikrodilisyon yöntemi kullanılarak yapılmıştır.

Anahtar kelimeler: *Mannich bazları, benzoksazol, antibakteriyel, antifungal*

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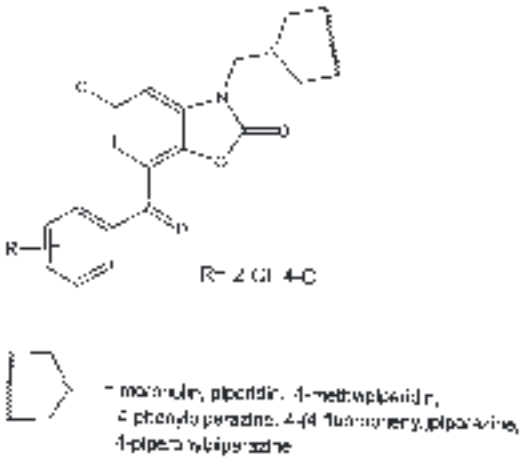
INTRODUCTION

After Lespagnol *et al*. (1) indicated that 2-(3*H*)-benzoxazolone and some of its derivatives have hypnotic activity, 2(3H)-benzoxazolone nucleus has attracted to researchers for many years. There have been many reports indicating that benzoxazolone derivatives carry various pharmacological activities including hypnotic, analgesic and antibacterial activity among any other biological activites (2-5). Meantime, the Mannich bases of 2-oxo-3*H*-benzoxazole derivatives bearing an acyl substituent at position 6 of the benzoxazole nucleus have been reported to have antibacterial and antifungal activities (6-9). Therefore, this work deals with the synthesis of the Mannich bases of 7-acyl-5-chloro-2-oxo-3*H*-benzoxazole derivatives and screening their antimicrobial activities to investigate the effect of the acyl group when placed in 7th position of the benzoxazole nucleus.



EXPERIMENTAL

Chemistry

Materials

All chemicals and reagents were obtained from Aldrich Chemical Co. (Steinheim, Germany) or Merck Chemical Co. (Darmstadt, Germany). Melting points were determined with "Electrothermal 9300 Melting Point Apparatus" and the values given are uncorrected. IR spectra were recorded on a Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrometer (KBr, □, cm^{-1}). Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker 400 FT-NMR spectrometer using TMS as an internal standard. All chemical shifts were reported as □ (ppm) values. Elemental analyses were performed with Leco-932 (C,H,N,S-O-Elemental Analyzer) at the Instrumental Analysis Center of the Scientific and Technical Research Council of Turkey (Ankara, Turkey), and were within the range of ±0.4% of theoretical value.

Synthesis of 7-acyl-5-chloro-2-oxo-3H-benzoxazole derivatives

7-acyl-5-chloro-2-oxo-3*H*-benzoxazole derivatives were prepared according to our previously published method (10).

General Method for the Preparation of the Mannich bases of 7-acyl-5-chloro-2-oxo-3H-benzoxazole derivatives

An appropriate seconder amine derivative (0.002 mol) and 7-acyl-5-chloro-2-oxo-3*H*-benzoxazole derivative (0.002 mol) were dissolved in methanol followed by the dropwise addition of formaline (0.0024 mol). The reaction mixture was stirred for 4h at room temperature. The mixture was concentrated in vacuum to yield the crude product which subsequently was washed with water, dried and recrystallized from the appropriate solvent.

*3-(morpholin-4-yl-methyl)-7-(2-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**1**)*

Recrystallized from 2-propanol to yield 24.6%. M.p.: 143.1 °C. FT-IR (KBr) cm^{-1}: 3093.7 (CH arom.), 2968, 2828 (CH aliph.), 1793 (lactam C=O), 1668.9 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.87 (d, 1H, benzoxazole-H^6), 7.54 (m, 4H, benzoyl H^3, H^4, H^5, H^6), 7.33 (d, 1H, benzoxazole-H^4), 4.67 (s, 2H, N-$\underline{CH_2}$-N), 3.55 (t, 4H, $(\underline{CH_2})_2O$), 2.61 (t, 4H, $(CH_2)_2N$). Anal. ($C_{19}H_{16}Cl_2N_2O_4$): C, H, N calc. 56.04, 3.96, 6.88 found. 56.36, 3.5, 6.62.

*3-(morpholin-4-yl-methyl)-7-(4-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**2**)*

Recrystallized from methanol to yield 44.26%. M.p.: 189.8 °C. FT-IR (KBr) cm^{-1}: 3078.1 (CH arom.), 2953.1 (CH aliph.), 1789 (lactam C=O), 1651.2 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.88 (d, 2H, benzoyl H^2, H^6), 7.84 (d, 1H, benzoxazole-H^6), 7.81 (d, 2H, benzoyl H^3, H^5), 7.76 (d, 1H, benzoxazole-H^4), 4.68 (s, 2H, N-$\underline{CH_2}$-N), 3.57 (t, 4H, $(\underline{CH_2})_2O$), 2.65 (t, 4H, $(\underline{CH_2})_2N$). Anal. ($C_{19}H_{16}Cl_2N_2O_4$): C, H, N calc. 56.04, 3.96, 6.88 found. 55.82, 3.57, 6.64.

*3-(piperidin-1-yl-methyl)-7-(2-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**3**)*

Recrystallized from n-hexane to yield 37.03%. M.p. : 109.4 °C. FT-IR (KBr) cm^{-1}: 3078 cm^{-1}(CH arom.), 2936.5, 2796 (CH aliph.), 1782.7 (lactam C=O), 1680.9 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.81 (d, 1H, benzoxazole-H^6), 7.57 (m, 4H, benzoyl H^3, H^4, H^5, H^6), 7.30 (s, 1H, benzoxazole-H^4), 4.67 (s, 2H, N-$\underline{CH_2}$-N), 2.58 (t, 4H, $(\underline{CH_2})_2N$), 1.46 (m, 4H, piperidine-3,piperidine-5), 1.32(m, 1H, piperidine-4). Anal. ($C_{20}H_{18}Cl_2N_2O_3$): C, H, N calc. 59.27, 4.48, 6.91 found. 59.41, 4.5, 6.79.

*3-(piperidin-1-yl-methyl)-7-(4-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**4**)*

Recrystallized from methanol to yield 33.08%. M.p.: 158 °C. FT-IR (KBr) cm^{-1}: 3109.3 (CH arom.), 2935.2 (CH aliph.), 1792.7 (lactam C=O), 1655.9 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.87 (d, 2H, benzoyl H^2, H^6), 7.76 (d, 1H, benzoxazole-H^6), 7.65 (d, 2H, benzoyl H^3, H^5), 7.35 (s, 1H, benzoxazole-H^4), 4.68 (s, 2H, N-$\underline{CH_2}$-N), 2.61 (t, 4H, piperidine-2, piperidine-6), 1.48 (m, 4H, piperidine-3, piperidine-5), 1.34 (m, 2H, piperidine-4). Anal. ($C_{20}H_{18}Cl_2N_2O_3$): C, H, N calc. 59.27, 4.48, 6.91 found. 59.34, 4.44, 6.84.

*3-(4-methylpiperidin-1-yl-methyl)-7-(2-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**5**)*

Recrystallized from methanol to yield 18.07%. M.p.: 131.3 °C. FT-IR (KBr) cm^{-1}: 3093 (CH arom.), 2953.7, 2929.4 (CH aliph.), 1780 (lactam C=O), 1678.5 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.88 (d, 1H, benzoxazole-H^6), 7.56 (m, 4H, benzoyl H^3, H^4, H^5, H^6), 7.29 (s, 1H, benzoxazole-H^4), 4.68 (s, 2H, N-$\underline{CH_2}$-N), 2.98 (m, 2H, piperidine-H^{2}e, piperidine-H^{6}e), 2.19 (m, 2H, piperidine H^{2}a, piperidine H^{6}a), 1.56 (m, 2H, piperidine H^{3}e, piperidine H^{5}e), 1.09 (m, 1H, piperidine H^4), 1.06 (m, 2H, piperidine H^{3}a, piperidine H^{5}a), 0.85 (m, 3H, -CH_3). Anal. ($C_{21}H_{20}Cl_2N_2O_3$): C, H, N calc. 60.15, 4.81, 6.68 found. 59.75, 4.68, 6.56.

*3-(4-(4-fluorophenyl)piperazine-1-yl-methyl)-7-(2-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**6**)*

Recrystallized from methanol to yield 47%. M.p.: 164.5 °C. FT-IR (KBr) cm^{-1}: 3093.7, 3046.8 (CH arom.), 2834 (CH aliph.), 1780.2 (lactam C=O), 1666.2 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.92 (s, 1H, benzoxazole-H^6), 7.59 (m, 4H, benzoyl H^3, H^4, H^5, H^6), 7.34 (s, 1H, benzoxazole-H^4), 6.98 (m, 4H, N-phenyl), 4.77 (s, 2H, N-$\underline{CH_2}$-N), 3.06 (t, 4H, C_6H_5-N($\underline{CH_2}$)$_2$), 2.77 (t, 4H, CH_2-N($\underline{CH_2}$)$_2$). Anal. ($C_{25}H_{20}Cl_2FN_3O_3$): C, H, N calc. 60.01, 4.03, 8.4 found. 60.21, 4.5, 8.4.

*3-(4-(4-fluorophenyl)piperazine-1-yl-methyl)-7-(4-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**7**)*

Recrystallized from methanol to yield 45.33%. M.p. : 146.7 °C. FT-IR (KBr) cm^{-1}: 3080.6 (CH arom.), 2829.1 (CH aliph.), 1786.8 (lactam C=O), 1667.1 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.86 (d, 2H, benzoyl H^2, H^6), 7.82 (d, 1H, benzoxazole-H^6), 7.61 (d, 2H, benzoyl H^3, H^5), 7.34 (d, 1H, benzoxazole-H^4), 6.94 (m, 4H, N-phenyl), 4.78 (s, 2H, N-$\underline{CH_2}$-N), 3.08 (t, 4H, C_6H_5-N($\underline{CH_2}$)$_2$), 2.83 (t, 4H, CH_2-N($\underline{CH_2}$)$_2$). Anal. ($C_{25}H_{20}Cl_2FN_3O_3$): C, H, N calc. 60.01, 4.03, 8.4 found. 59.99, 3.67, 8.37.

*3-(4-piperonylpiperazine-1-yl-methyl)-7-(2-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**8**)*

Recrystallized from n-hexane to yield 64.8%. M.p.: 129.1 °C. FT-IR (KBr) cm^{-1}: 3093.7 (CH arom.), 2968.7, 2828.1 (CH aliph.), 1794.3 (lactam C=O), 1672.6 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.84 (s, 1H, benzoxazole-H^6), 7.57 (m, 4H, benzoyl H^3, H^4, H^5, H^6), 7.32 (s, 1H, benzoxazole-H^4), 6.80 (m, 2H, piperonyl H^4, H^7), 6.70 (m, 1H, piperonyl H^6), 5.96 (s, 2H, O-$\underline{CH_2}$-O), 4.69 (s, 2H, N-$\underline{CH_2}$-N), 3.34 (s, 2H, piperonyl $\underline{CH_2}$), 2.62 (m, 4H, N-CH_2-N($\underline{CH_2}$)$_2$), 2.34 (m, 4H, N($\underline{CH_2}$)$_2$). Anal. ($C_{27}H_{23}Cl_2N_3O_5$): C, H, N calc. 60.01, 4.29, 7.78 found. 60.49, 4.38, 7.74.

*3-(4-piperonylpiperazine-1-yl-methyl)-7-(4-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**9**)*

Recrystallized from methanol to yield 60.49%. M.p.: 188.2 °C. FT-IR (KBr) cm^{-1}: 3078.1 (CH arom.), 2937.5, 2821.5 (CH aliph.), 1789.1 (lactam C=O), 1653.1 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.88 (d, 2H, benzoyl H^2, H^6), 7.77 (s, 1H, benzoxazole-H^6), 7.65 (d, 2H, benzoyl H^3, H^5), 7.37 (s, 1H, benzoxazole-H^4), 6.81 (m, 2H, piperonyl H^4, H^7), 6.71 (m, 1H, piperonyl H^6), 5.97 (s, 2H, O-$\underline{CH_2}$-O), 4.70 (s, 2H, N-CH_2-N), 3.37 (s, 2H, piperonyl CH_2), 2.67 (m, 4H, N-CH_2-N($\underline{CH_2}$)$_2$), 2.35 (m, 4H, N($\underline{CH_2}$)$_2$). Anal. ($C_{27}H_{23}Cl_2N_3O_5$): C, H, N calc. 60.01, 4.29, 7.78 found. 60.06, 3.08, 7.71.

*3-(4-phenylpiperazine-1-yl-methyl)-7-(2-chlorobenzoyl)-5-chloro-2-oxo-3H-benzoxazole (**10**)*

Recrystallized from methanol to yield 49.48%. M.p.: 170.5 °C. FT-IR (KBr) cm^{-1}: 3084.5, 3055.7 (CH arom.), 2831.9 (CH aliph.), 1781 (lactam C=O), 1663.7 (ketone C=O). ^{1}H-NMR (DMSO-d_6) □ 7.50 (d, 1H, benzoxazole-H^6), 7.45 (m, 4H, benzoyl H^3, H^4, H^5, H^6), 7.30 (s, 1H, benzoxazole-H^4), 7.13 (t, 2H, phenyl H^3,H^5), 6.81 (d, 2H, phenyl H^2,H^6), 6.73 (t, 1H, phenyl H^4), 4.67 (s, 2H, N-CH_2-N), 3.11 (m, 4H, C_6H_5-N(C$\underline{H_2}$)$_2$), 2.77 (m, 4H, CH_2-N(C$\underline{H_2}$)$_2$). Anal. ($C_{25}H_{21}Cl_2N_3O_3$): C, H, N calc. 62.25, 4.39, 8.71 found. 62.41, 4.55, 8.71.

Microbiology

Minimal inhibitory concentrations (MICs) were determined by broth microdilution method following the procedures reported by the National Committee for Clinical Laboratory Standards (11,12). Fluconazole and ampicillin were used as reference compounds for fungi and bacteria, respectively. Two Gram-positive (*S. aureus ATCC 29213* and *S. pyogenes ATCC 19615*) and two Gram-negative (*E. coli ATCC 35218* and *P. aeruginosa ATCC 27853*) bacteria were used as quality control strains (11). For determining anti-yeast activities of the compounds, the following reference strains were tested: *C. albicans ATCC 10231*, *C. tropicalis ATCC 13803*[L2]. The MIC values of the compounds are presented in the table. The reference compounds were dissolved in sterile distilled water. The stock solutions of the synthesized compounds were prepared in dimethylsufoxide (DMSO). The dilutions in the test medium were prepared at the required concentration of 250-3.25 µg/mL, and for the reference compounds at 64-0.0625 µg/mL. The final inoculum densities were $5x10^5$ cfu/mL for bacteria and $0.5\text{-}2.5x10^3$ cfu/mL for fungi. MIC was defined as the lowest concentration of the compound that inhibited visible growth. It was established that the dilution of DMSO lacked antimicrobial activity against any of the test micro-organisms.

Antibacterial activity

The cultures were grown on Mueller-Hinton Agar (Merck) for *P. aeroginosa ATCC 27853*, *E. coli ATCC 35218* and *S. aureus ATCC 29213,* and *S. pyogenes ATCC 19615* was grown on Todd-Hewitt Blood Agar after 18-24 h of incubation at 36 °C. Before the assay, Gram-negative bacteria and *S. aureus ATCC 29213* were grown in Müeller-Hinton Broth, and *S. pyogenes* was grown in Todd-Hewitt Broth for 2-6 h. Then, the bacterial suspensions were adjusted to 0.5 McFarland turbidity ($1x10^8$ cfu/mL). The microtiter plates were incubated at 36 °C, and inspected visually after 18-24 h for bacteria. The MIC values were recorded as the lowest concentrations of the substances which had no visible turbidity. The minimum bacteriostatic and bactericidal concentrations of the compounds were evaluated using colonies growing in solid medium. Inoculations were done from the wells of MIC on the Müeller-Hinton Agar and Todd-Hewitt Blood Agar. After 24 hours their growing was examined.

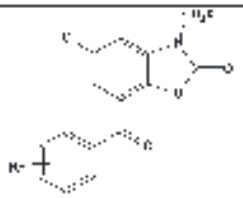
Antifungal activity

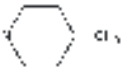
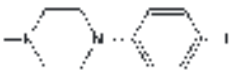
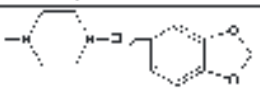
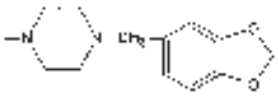
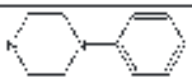
All fungi were cultivated in Sabouraud Dextrose Agar (Merck). RPMI-1640 medium (ICN-Flow, Aurora, OH, USA) with L-glutamin, buffered with 3-(*N*-morpholino)propanesulphonic acid (MOPS) (Buffer-ICN-Flow, Aurora, OH, USA) at pH=7.4 was used as the culture medium. The microtiter plates were incubated at 36 °C and evaluated visually after 48 h. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity. The minimum fungistatic and fungicidal concentrations of the compounds were evaluated using colonies growing in solid medium. Inoculations were done from the wells of MIC on the Sabouraud Dextrose Agar. After 24 hours, their growth was examined.

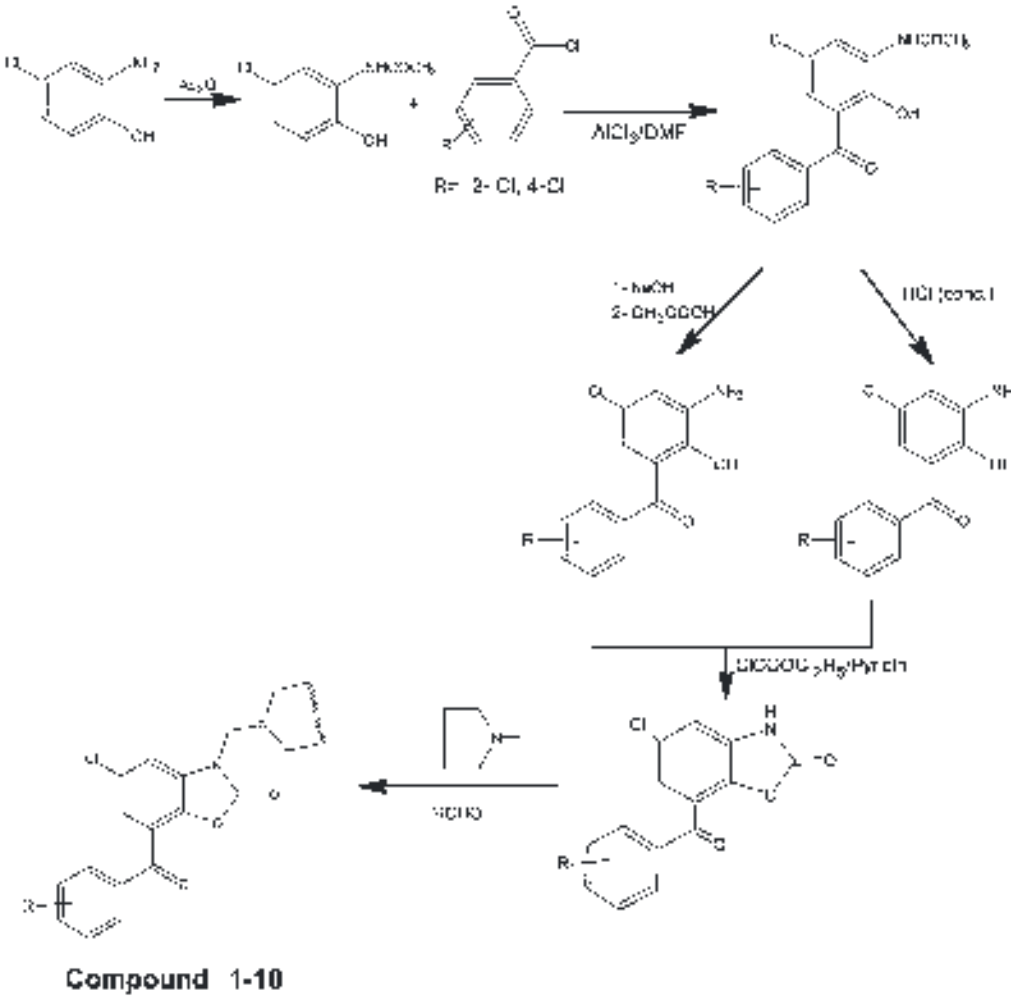
RESULTS AND DISCUSSION

The Mannich bases of 7-acyl-5-chloro-2-oxo-3*H*-benzoxazole derivatives (Table 1) were prepared by the modification of the previously published general reaction sequence (10) depicted in Scheme 1.

Table 1. Synthesized Mannich bases of 7-Acyl-5-chloro-2-oxo-3*H*-benzoxazole derivatives.



Compound	R	R'
1	2-Cl	
2	4-Cl	
3	2-Cl	
4	4-Cl	
5	2-Cl	
6	2-Cl	
7	4-Cl	
8	2-Cl	
9	4-Cl	
10	2-Cl	



Sheme 1. General Reaction Sequence of compound **1-10**

Compounds **1-10** were evaluated for their in vitro antibacterial and antifungal activity against four pathogenic bacteria and two pathogenic fungi by broth microdilution method and the results of these assays are summarized in Table 2. The data for ampicillin and fluconazole were included for comparison. The observation from the antimicrobial activity of the synthesized Mannich bases showed no considerable activity against tested bacteria and fungi showing the results less active than ampicillin and fluconazole against. Therefore, in the case of the substitution of the acyl group at position 7 of the benzoxazole nucleus resulted no significant contribution to the antimicrobial activity of these compounds.

Table 2. The Minimum Bactericidal-Bacteriostatic Concentrations and The Minimum Fungicidal-Fungistatic Concentrations (μg/mL) of Compounds **1-10**.

Compounds	Microorganisms					
	Candida albicans ATCC 10231	***Candida tropicalis ATCC 13803***	***Pseudomonas aeroginosa ATCC 27853***	***Escherichia coli ATCC 35218***	***Streptococcus pyogenes ATCC 19615***	***Staphylococcus aureus ATCC 29213***
1	125*	250	250*	250	15.6	15.6*
2	250	250	250*	250*	31.3	31.3*
3	250	250	250	250	15.6	15.6*
4	250	250	250*	250	31.3	31.3*
5	250	250	250*	250	31.3	31.3*
6	125*	250	250*	250	125*	125*
7	125*	250*	250*	250*	31.3*	62.5*
8	125*	250*	250*	250*	31.3	31.3*
9	125*	125*	250*	250*	62.5*	>250*
10	125*	125*	250	250*	62.5	62.5*
Ampicillin	-	-	16	16	0.12	0.12
Fluconazole	1	2	-	-	-	-

* The Minimum Bacteriostatic-Fungistatic Concentrations (μg/mL) while the others are the minimum Bactericidal-Fungicidal Concentrations (μg/mL)

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