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In Vitro Antimicrobial Activities of 6-Substituted-3(2H)-pyridazinone-2-acetyl-2- (substituted/nonsubstitutedbenzal/acetophenone) Hydrazone Derivatives

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Abstract

***Aim**: In vitro antibacterial activity of 6-substituted-3(2H)-pyridazinone-2-acetyl-2-(substituted/nonsubstitutedbenzal/acetophenone) hydrazone derivatives were tested in common species causing hospital-acquired infections.*

***Material and Method**: Antimicrobial activities of the compounds were performed by determining minimum inhibitory concentration (MIC) value against four Gram-positive, five Gram-negative and four Candida species fungi. Modified serial microdilution method was carried out. Reference strains of American Type Culture Collection (ATCC) were used.*

***Results**: In general, eleven compounds exhibited considerable activity. Comparatively, compound 3 exhibited strong activity against Enterobacter hormaechei and 5, 11 were the most active against Acinetobacter baumannii at 31.25 µg/mL. Compounds 1,2,3,4,8 and 10 were found to be as active as positive control ampicillin trihidrate against Stenotrophomonas maltophilia. On the other hand, compounds 1,2,3,4,7,8,9,10 and 11 showed strong antifungal activitiy as much as fluconazole against Candida tropicalis. Compound 1 was mostly active against Candida albicans, Candida glabrata, Candida parapsilosis and Candida tropicalis. It was also revealed that the antifungal activity of compounds 1, 6, 7, 8 and 9 were higher than the others. Compound 1 and 8 exhibited the best activity against Candida glabrata and Candida parapsilosis respectively.*

***Conclusions**: All tested compounds showed better activity against Gram-negative bacteria and yeast than Gram-positive bacteria. These compounds may be considered as alternative antimicrobial agents in the treatment of multiple drug resistant Gram-negative, Gram-positive bacteria and fungal pathogens. Especially, we suggested that Compound 1 and 8 might be a promising candidate of new antifungal agents.*

***Keywords**: antimicrobial, chemotherapeutic, microdilution, 3(2H)-pyridazinone*

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Introduction

Antimicrobial drugs still maintain their place as indispensable in combating infectious diseases in the world. However, increasing resistance to these drugs in recent years has reached a point of concern in the treatment of infectious diseases, especially in immunocompromised patients. Nowadays, there are various antibiotic

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resistance mechanisms and multidrug-resistant microorganisms (1). Multidrug antimicrobial resistance among clinical strains is increasing at an alarming rate to public health. There is a strong need for new antimicrobials against Gram-negative, Gram-positive bacterial and fungal pathogens, causing community acquired and nosocomial infections (2). Today, there are many researches on various chemotherapeutic compounds to develop new antimicrobials (3,4). Pyridazinone and its derivatives are one of these compounds commonly tested to overcome antimicrobial resistance (5). Recent researches have revealed that substituted pyridazinones have various chemical and biological activities such as anti-inflammatory and analgesic, antidepressant, anti-hypertensive, anticonvulsant, cardiotonic, antibacterial, antifungal, antitubercular, diuretics, anti-HIV, antimalarial and anti-cancer effects (6-9). To this effect, 3(2*H*)-pyridazinones first prepared by Fischer have a unique structure and display variety of pharmacological and therapeutic properties (10-13). It was reported that this special skeleton could be functional at various ring positions. Therefore, this structure attracts many scientists to synthesize and design new drugs. There are various studies reporting antibacterial, antitubercular and anti-fungal effects of these compounds (11-13). Anti-fungal activity of pyridazinone derivatives was detected against *Candida albicans* and *Cryptococcus neoformans* which cause secondary infections in immunosuppressive patients (14,15).

Aim of this study is to investigate antibacterial (*Bacillus subtilis subsp. subtilis*, *Enterococcus faecium, Staphylococcus aureus, Enterobacter hormaechei*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli O157H7, Stenotrophomonas maltophilia*) and anti-fungal (*Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis*) activity of 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(substituted/nonsubstitutedbenzal/acetophenone) hydrazone derivatives by modified microdilution method. These hydrazone derivatives were synthesized and tested for their analgesic and anti-inflammatory or antimycobacterial activities previously by us (16,17).

Material and Method

Antimicrobial Activity Test

Reference strains of American Type Culture Collection (ATCC, USA) were used. Reference microbial strains were *B. subtilis subsp. subtilis* (ATCC 6051), *E. faecium* (VanR QC set ATCC 700221), *S. aureus* (ATCC 29213), *E. hormaechei* (ATCC 700323), *K. pneumoniae* (ATCC 27736), *A. baumannii* (ATCC 49139), *P. aeruginosa* (ATCC 27853), *E. coli O157:H7* (ATCC 35150), *S. maltophilia* (ATCC 17666), *C. albicans* (ATCC 14053), *C. glabrata* (ATCC 15126), *C. parapsilosis* (ATCC 22019), *C. tropicalis* (ATCC 1969). While lyophilized bacterial strains were inoculated to Mueller-Hinton agar (MHA) at 37°C for 24 h, fungal strains inoculated to sabouraud dextrose agar (SDA) at 28°C for 24 h. Microdilution method was applied by modification of the literature methods (18-20). Experiments were run in duplicate independently according to literature. Stock solutions of each compound were diluted in DMSO/H_2O (50%) at 1000 µg/mL. The suspension of the bacteria and fungi strains were prepared in normal saline and the turbidity adjusted to 0.5 McFarland with absorbance of 0.08-0.13 at 625 nm. For antibacterial activity test, 100 mL Mueller-Hinton Broth (MHB) was added to each of the 11 wells (in a 96-well plate). A 100 mL of tested compound solution was added to the first well, and two-fold dilutions were performed. Then, 10 mL bacterial suspension (1×10^6 CFU/mL) was added to each well, except the last control well. Ampicillin trihydrate was used as reference drug. Plates were incubated at 37°C for 24 h.

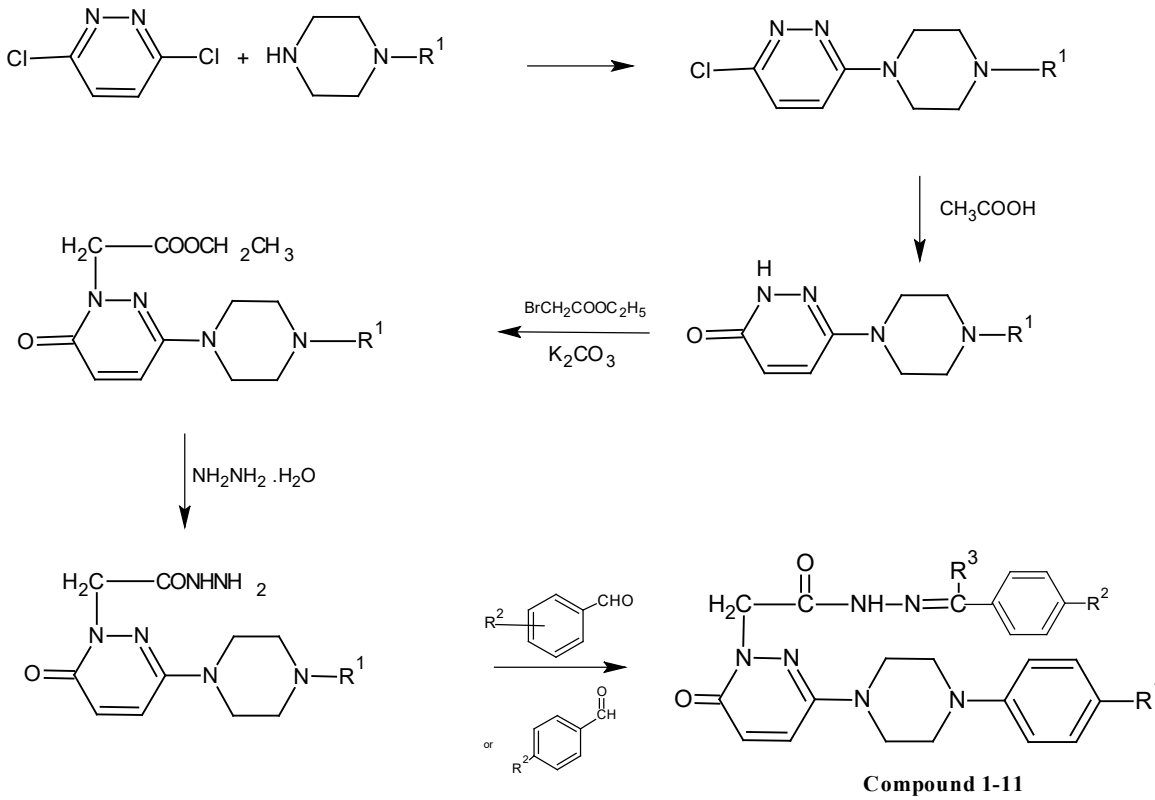
For antifungal activity test, 100 mL Tryptic Soy broth (TSB) was added to each of the 11 wells. A 100 mL of tested compound solution was added to the first well, and two fold dilutions were performed. Then, 1 mL fungal suspension was added to each well, except the last one, acting as control well. Fluconazole was used as reference drug. Plates were incubated at 28°C for 24 h. After incubation of all plates, the MICs were evaluated by spectrophotometer. The concentration resulting in a 50% reduction in the optical density (OD) values was compared to a reproduction control at 450 nm by spectrophotometric evaluation and defined as the MIC value.

In this study, descriptive analysis statistical method was used to evaluate the antibacterial activity of compounds. IBM SPSS free trial version was used as statistical package software.

Clinical isolates weren't tested in this study. Therefore, ethical approval was not required.

Synthesis of Pyridazinone Compounds

General procedure for synthesis of 6-substituted-3(*2H*)-pyridazinone-2-acetyl-2-(substituted-/ nonsubstitutedacetophenone) hydrazone derivatives 1-11 was shown in **Scheme 1**. Reaction of 3,6-dichloropyridazine with arylpiperazines afforded 3-chloro-6-substitutedpyridazine derivatives. Hydrolysis of 3-chloro-6-substitutedpyridazines were carried out upon heating in glacial acetic acid to afford 6-substituted-3(2*H*)-pyridazinone derivatives. The formation of these compounds were confirmed by IR spectra of a C=O signal at about 1660 cm^{-1}. Ethyl 6-substituted-3(2*H*)-pyridazinone-2-ylacetate derivatives were obtained by the reaction of 6-substituted-3(2H)-pyridazinone deriva-



Scheme 1. Synthesis of compounds 1-11.

tives with ethyl bromoacetate in the presence of K_2CO_3 in acetone. 6-Substituted-3(2*H*)-pyridazinone-2-yl-acetohydrazide derivatives were synthesized by the condensation reaction of ethyl 6-substituted-3(2*H*)-pyridazinone-2-ylacetate derivatives with hydrazine hydrate. A mixture of 6-substituted-3(2*H*)-pyridazinone-2-yl-acetohydrazide derivatives and appropriately substituted p-substituted acetophenone or benzaldehydes was refluxed in 15 mL of ethanol for 6 h. The mixture was then poured into ice water. The formed precipitate was recrystallized from ethanol. Chemical structures of compound 1-11 are given in **Table 1**. 6-substituted-3(2*H*)-pyridazinone-2-yl-acetohydrazide derivatives 1-11 were synthesized as described previously by us as stated above (16-17). Elemental analysis, IR and ^{1}H-NMR spectral data of the 1-11 were in accordance with the data reported previously (**Table 2**).

Results

Antimicrobial Activity Assay

The antimicrobial activities of target com-

Table 1. Chemical structures of the 6-Substituted-3(2H)-pyridazinone-2-acetyl-2-(substituted/ nonsubstitutedbenzal/acetophenone) hydrazone derivatives

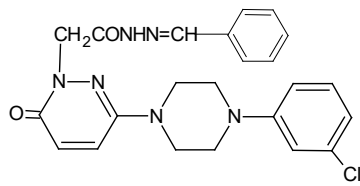
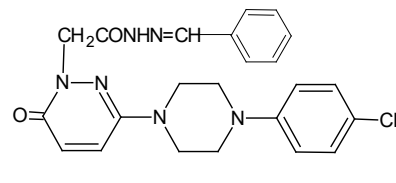
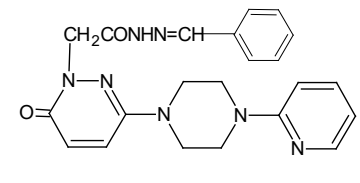
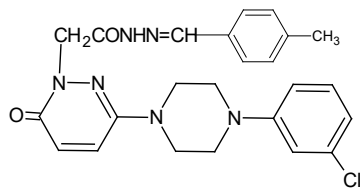
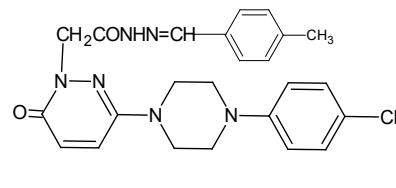
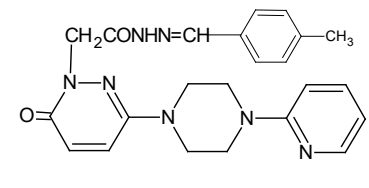
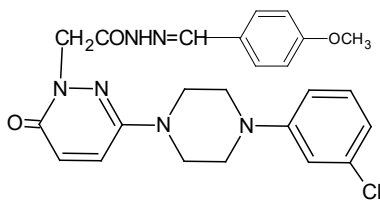
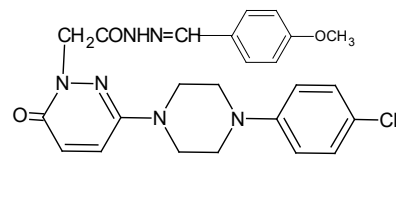
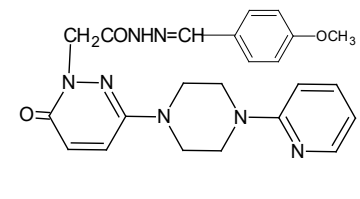
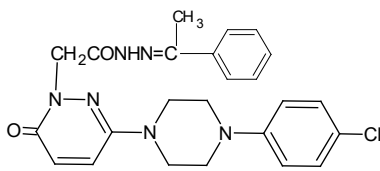
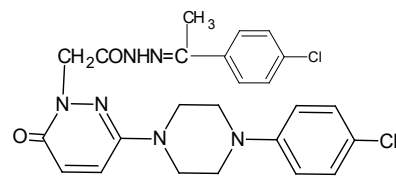
		
Compound 1	Compound 2	Compound 3
		
Compound 4	Compound 5	Compound 6
		
Compound 7	Compound 8	Compound 9
		
Compound 10	Compound 11	

Table 2. Elemental analysis IR and 1H NMR spectral data of 1-11 derivatives

1	Anal. Calc. For $C_{23}H_{23}ClN_6O_2$: C, 61.26; H, 5.14; N, 18.64. Found: C, 61.38; H, 5.21; N,18.83 IR (KBr) υ (cm^{-1}): 3185, 1681, 1652. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 11.64 (s, 1H, NH), 7.98 and 8.18 (1H, s, s, N=CH), 7.65-7.62 (d, 1H, pyridazinone H_5), 7.66-6.90 (m, 9H, ArH), 6.83-6.80 (d, 1H, pyridazinone H_4), 5.05 and 4.66 (s, s, 2H, CH_2), 3.38-3.36 (m, 4H, piperazinea+a'), 3.28-3.26 (m, 4H, piperazineb+b').
2	Anal. Calcd. For $C_{23}H_{23}ClN_6O_2$: C, 61.26; H, 5.14; N, 18.64. Found: C, 61.46; H, 5.10; N, 18.43. IR (KBr) υ (cm^{-1}): 3182, 1683, 1653. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 11.61 (s, 1H, NH), 8.18 and 7.99 (1H, s, s, N=CH), 7.70-6.91 (m, 10H, ArH and pyridazinone H_5), 6.86-6.78 (d, 1H, pyridazinone H_4), 5.01 and 4.63 (s, s, 2H, CH_2), 3.40-3.34 (m, 4H, piperazinea+a'), 3.33-3.26 (m, 4H, piperazineb+b').
3	Anal. Calc. For $C_{22}H_2N_7O_2$: C, 63.30; H, 5.55; N, 23.49. Found: C, 63.42; H, 5.62; N, 23.83. IR (KBr) υ (cm^{-1}): 3185, 1682, 1658. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 11.60 (s, 1H, NH), 8.17 and 7.98 (1H, s, s, N=CH), 7.70-6.96 (m, 10H, ArH and pyridazinone H_5), 6.80-6.78 (d, 1H, pyridazinone H_4), 5.02 and 4.68 (s, s, 2H, CH_2), 3.40-3.34 (m, 4H, piperazinea+a'), 3.33-3.25 (m, 4H, piperazineb+b').
4	Anal. Calc. for $C_{24}H_{25}ClN_6O_2$: C, 62.00, H, 5.42, N, 18.08. Found: C, 62.13, H, 5.51, N, 18.33.IR (KBr) υ (cm^{-1}): 3189, 1678, 1661.^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 11.57 (s, 1H, NH), 7.97 and 8.12 (1H, s, s, N=CH), 6.97–7.63 (m, 9H, ArH and pyridazinone H_5), 6.85–6.91 (d, 1H, pyridazinone H_4), 4.60 and 5.00 (2H, s, s, CH_2), 3.42–3.49 (m, 4H, piperazinea+a'), 3.18–3.35 (m, 4H, piperazineb+b'), 2.28 (s, 3H, CH_3).
5	Anal. Calc. for $C_{24}H_{25}ClN_6O_2$: C, 62.00, H, 5.42, N, 18.08. Found: C, 61.96, H, 5.30, N, 18.24.IR (KBr) υ (cm^{-1}): 3186, 1680, 1660.^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 11.56 (1H, s, NH), 7.93 and 8.13 (1H, s, s, N=CH), 6.96–7.62 (m, 9H, ArH and pyridazinone H_5), 6.76–6.89 (d, 1H, pyridazinone H_4), 4.61 and 5.03 (2H, s, s, CH_2), 3.41–3.47 (m, 4H, piperazinea+a'), 3.23–3.36 (m, 4H, piperazineb+b'), 2.29 (s, 3H, CH_3).
6	Anal. Calc. for $C_{24}H_{25}ClN_6O_2$: C, 62.00, H, 5.42, N, 18.08.Found: C, 61.96, H, 5.30, N, 18.24.IR (KBr) υ (cm^{-1}): 3182, 1677, 1658.^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm):11.58 (1H, s, NH), 7.93 and 8.13 (1H, s, s, N=CH), 6.96–7.62 (m, 9H, ArH and pyridazinone H_5), 6.76–6.89 (d, 1H, pyridazinone H_4), 4.61 and 5.03 (2H, s, s, CH_2), 3.42–3.48 (m, 4H, piperazinea+a'), 3.24–3.35 (m, 4H, piperazineb+b'), 2.30 (s, 3H, CH_3),
7	Anal. Calc. for $C_{23}H_{25}N_7O_2$: C, 64.02, H, 5.84, N, 22.72.Found: C, 64.21, H, 5.60, N, 22.31.IR (KBr) υ (cm^{-1}): 3181, 1679, 1661.^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 11.61(1H, s, NH), 7.95 and 8.14 (1H, s, s, N=CH), 6.93–7.62 (m, 9H, ArH and pyridazinone H_5), 6.77–6.87 (d, 1H, pyridazinone H_4), 4.61 and 5.01 (2H, s, s, CH_2), 3.41–3.47 (m, 4H, piperazinea+a'), 3.24–3.35 (m, 4H, piperazineb+b'), 2.30 (s, 3H, CH_3),
8	Anal. Calc. for $C_{24}H_{25}ClN_6O_3$: C, 59.94, H, 5.24, N, 17.47.Found: C, 59.63, H, 5.43, N, 17.13.IR (KBr) υ (cm^{-1}): 3180, 1679, 1659.^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm):11.50 (1H, s, NH), 7.91 and 8.10 (1H, s, s, N=CH), 6.97–7.62 (m, 9H, ArH and pyridazinone H_5), 6.86–6.94 (d, 1H, pyridazinone H_4), 4.59 and 4.99 (2H, s, s, CH_2), 3.75 (s, 3H, OCH_3), 3.36–3.43 (m, 4H, piperazinea+a'), 3.18–3.35 (m, 4H, piperazineb+b').
9	Anal. Calc. for $C_{24}H_{25}ClN_6O_3$: C, 59.94, H, 5.24, N, 17.47.Found: C,59.47, H, 5.18, N, 17.24. IR (KBr) υ (cm^{-1}): 3181, 1680, 1660.^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm):11.51 (1H, s, NH), 7.92 and 8.11 (1H, s, s, N=CH), 6.95–7.63 (m, 9H, ArH and pyridazinone H_5), 6.76–6.90 (d, 1H, pyridazinone H_4), 4.60 and 4.99 (2H, s, s, CH_2), 3.76 (s, 3H, OCH_3), 3.38–3.48 (m, 4H, piperazinea+a'), 3.24–3.34 (m, 4H, piperazineb+b').
10	Anal. Calc. for $C_{24}H_{25}ClN_6O_2$: C, 62.00; H, 5.42; N, 18.08. Found: C, 62.17; H, 5.56; N, 17.98. IR (KBr) υ (cm^{-1}): 3218, 1706, 1664. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm):11.72 (s, 1H, NH), 7.73-7.70 (d, 1H,pyridazinone H_5), 7.67-6.90 (m, 9H, aromatic protons), 6.82-6.80 (d, 1H, pyridazinone H_4), 5.05 and 4.70 (s, s,2H, CH_2), 3.37-3.35 (t, 4H, piperazinea+a'), 3.27-3.25 (t, 4H, piperazineb+b'), 2.22 (s, 3H,CH_3).
11	Anal. Calc. for $C_{24}H_{24}Cl_2N_6O_2$: C, 57.72; H, 4.84; N, 16.83. Found: C, 57.96; H, 5.01; N, 16.59. IR (KBr) υ (cm^{-1}): 3214, 1707, 1666. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 11.72 (s, 1H, NH), 7.75-7.73 (d, 1H, pyridazinone H_5), 7.69-6.92 (m, 8H, aromatic protons), 6.82-6.80 (d, 1H, pyridazinone H_4), 5.09 and 4.72 (s, s,2H, CH_2), 3.37-3.35 (t, 4H, piperazinea+a'), 3.29-3.27 (t, 4H, piperazineb+b'), 2.23 (s, 3H,CH_3).

pounds 1-11 were tested against common pathogenic five Gram-positive, four Gram-negative and four Candida spp. by modified microdilution method. The antibacterial activity results of the target compounds were given in **Table 3**. Ampicillin trihydrate for bacteria and fluconazole for fungi were used as reference drugs. It was found that compound 3 exhibited the strongest activity against *E. hormaechei* at a MIC of 31.25 µg/mL and other compounds were detected moderately effective against this strain. The compounds that showed intermediate activity against *B. subtilis subsp. subtilis* and *E. faecium*, were found to have low activity against *S. aureus* at a MIC of 125 µg/mL. All the tested concentrations of target compounds 1,2,3,4,8 and 10 showed high activity against *S. maltophilia*. Compounds 5 and 11 exhibited high activities against *A. baumannii*. All of the target compounds were detected to have moderate activity against *E. faecium* (VanR), *K. pneumoniae, P. aeruginosa* and *E. coli O157:H7.*

Compounds 1-11 exhibited good antifungal activity. Compound 1 showed high activity against *C. glabrata* at a MIC of 15.625 µg/mL. Compound 9 was found less active against *C. glabrata* at a MIC of 31.25 µg/mL. Compound 8 exhibited more significant activity against *C. parapsilosis* at a MIC of 3.9 µg/mL than compounds 1,5 and 9 at MICs of 7.81 µg/mL.

Antifungal activities of compounds 1,5,6,7,8 and 9 were detected higher than the others. Especially, compounds 1,2,3,4,7,8,9,10 and 11 showed excellent inhibition activity against *C. tropicalis* except for 5 and 6. The MIC values of the synthesized compounds obtained against *Candida spp.* were given in **Table 4**.

When compared to ampicillin used for positive control, all of the compounds showed good activity against Gram-positive and Gram-negative strains according to statistical analysis (**Scheme 2**). No statistical difference was observed between compounds.

Table 3. In vitro antibacterial activity of compounds 1-11

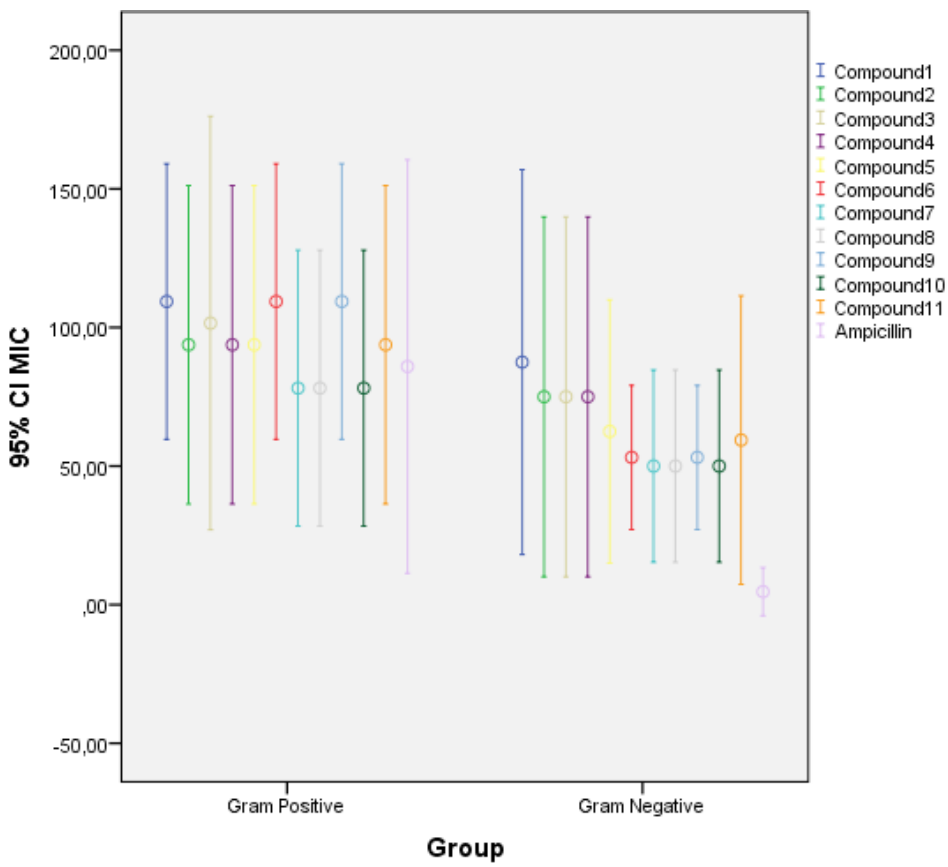
Minimal InhibitoryConcentrations (µg/mL)									
	Gram (+)					Gram (-)			
Comp.	A	B	C	D	E	F	G	H	I
1	125	125	125	62.5	62.5	125	125	125	*
2	62.5	125	125	62.5	62.5	125	125	62.5	*
3	125	125	125	31.25	62.5	125	125	62.5	*
4	125	62.5	125	62.5	62.5	125	125	62.5	*
5	125	62.5	125	62.5	62.5	31.25	125	62.5	31.25
6	125	125	125	62.5	62.5	62.5	62.5	62.5	15.625
7	62.5	62.5	125	62.5	62.5	62.5	62.5	62.5	3.9
8	62.5	62.5	125	62.5	62.5	62.5	62.5	62.5	*
9	125	125	125	62.5	62.5	62.5	62.5	62.5	15.625
10	62.5	62.5	125	62.5	62.5	62.5	62.5	62.5	*
11	62.5	125	125	62.5	62.5	31.25	125	62.5	15.625
Amp.	125	31.25	125	62.5	7.81	15.625	7.81	*	*

A: *Bacillus subtilis subsp. subtilis;* B:*Enterococcus faecium;* C:*Staphylococcus aureus*; D:*Enterobacterhormaechei*; E: *Klebsiella pneumonia;* F:*Acinetobacter baumannii*; G: *Pseudomonas aeruginosa*; H: *Escherichia coli*; I:*Stenotrophomonas maltophilia. Amp.: Ampicilline .3H_2O.*: No growth*

Table 4. In vitro antifungal activity of compounds 1-11

	Minimal InhibitoryConcentrations (µg/mL)			
Comp.	***Candida albicans***	***Candida glabrata***	***Candida parapsilosis***	***Candida tropicalis***
1	62.5	15.625	7.81	*
2	125	62.5	15.625	*
3	62.5	62.5	15.625	*
4	125	62.5	31.25	*
5	125	62.5	7.81	7.81
6	62.5	62.5	15.625	31.25
7	62.5	62.5	15.625	*
8	125	62.5	3.9	*
9	62.5	31.25	7.81	*
10	62.5	62.5	31.25	*
11	125	62.5	31.25	*
Fluconazole	*	125	*	15.625

*: No growth



Scheme 2. Antibacterial statistical analysis of compounds

Discussions

Due to the increasing resistance to antimicrobials, most of the antimicrobials clinically used have become ineffective in recent years (1). Therefore, many antimicrobials are thought to be inadequate in the prevention and treatment of infections. This situation could result in an increased mortality and morbidity rates of infectious diseases (2). Accordingly, the need for the treatment of multiple drug resistant (MDR) microorganisms is increasing in both community-borne and hospital-acquired infections. Centers for Disease Control and Prevention has declared that antimicrobial resistance causes failure in the treatment of MDR microorganisms such as carbapenem-resistant Enterobacteriaceae, MDR tuberculosis, MDR *Acinetobacter*, fluconazole-resistant *Candida*, vancomycin-resistant *Enterococcus* (VRE), MDR *Pseudomonas aeruginosa* and methicillin-resistant *S. aureus* (MRSA) (21).

The number of antimicrobials used in the treatment of these microorganisms are limited. *S. aureus* and coagulase-negative staphylococci are Gram-positive bacteria causing nosocomial and community acquired infections. Though MRSA infections still problem for developing countries, the rates of MRSA have declined due to the strict infection control measures taken in the West in the last decade. It is reported that MRSA rate is between 25% and 50% in blood, brain spinal fluid and other clinic samples in European Union Countries. (22).

Another life-threatening Gram-positive bacteria is *E. faecium*. It was declared that *E. faecium* is the second most frequently isolated microorganism in catheter-associated infections in the United States. High aminoglycoside and aminopenicillin resistance were reported in both *E. faecalis* and *E. faecium* isolates (23).

Gram negative-ESCAPE pathogens (*K. pneumoniae, A. baumannii, P. aeruginosa, and Enterobacter spp*.) resistant to the majority of broad-spectrum antimicrobials are important etiologic agents in nosocomial infections. Karlowsky et al. conducted a study for monitoring antimicrobial resistance trends (SMART) under the global surveillance program (24). They collected samples (2113 isolate intra-abdominal and 970 isolate urinary tract infections) from 11 Latin American countries between 2013-2015. Antibiotic susceptibilities of the isolates were (2113 isolate intra-abdominal and 970 isolate urinary tract infections); amikacin 92.2%, *Enterobacter* spp. 97.5%, *P. aeruginosa* 85.3% and the sensitivity of *A. baumannii* to all tested antimicrobials was ≤30.9% (24). Koksal et al. reported that *E. coli* was the major pathogenic bacteria in community based urinary system infections, intensive care and intra abdominal infections in SMART study (25). Similarly, Antibiotic resistance rates of Gram-negative bacteria (*E. coli, Klebsiella spp., Pseudomonas spp.*) were determined to reach high levels in a meta-analysis study at West Africa (26). Studies from West Africa declared that antibiotic resistance is increasing between *E. coli* and *Klebsiella* spp. in blood and urinary system infections. Resistance to ampicillin was reported 75% and 97% in *E. coli* and *Klebsiella spp.* isolates, respectively (26). Furthermore, carbapenemase-producing *Enterobacteriaceae are being reported* 0.04-29.5% *worldwide (*27). *The threat of infections due to* carbapenemase-producing *Enterobacteriaceae reveal new challenges in the treatment of inpatients and immunocompromised patients. Most of the studies emphasized that antibiotic resistance rates were higher especially in developing countries (27).*

*In recent years, incidence of fungal infections has increased along with anti-bacterial resistance (*1,2). *Fungal infections are related with AIDS,* cystic fibrosis, cancers, immunocompromised or suppressed patient groups *commonly*. Especially, opportunistic pathogenic fungi such

as *Coccidioides immitis, Histoplasma capsulatum*, *Candida spp., Trichosporon spp., Cryptococcus spp., Aspergillus spp., Fusarium spp., Scedosporium spp., Pneumocystis jirovecii* were detected in these patient groups (28). Among these opportunistic pathogens, *Candida spp.* and *Aspergillus spp.* are the most frequently isolated fungi from patients in intensive care units (28). *Candida spp.* was reported as the most seen infection agent with mortality of 38% in immunocompromised patients. *C. albicans* was isolated as a primary factor in 50-60% of nosocomial candidiasis. Similarly, echinocandin and azole resistance start to cause challenges in the treatment of *Candida spp.* in intensive care unit patients (29). It was determined that frequent use of fluconazole results in an increase of resistance in *Candida spp*. and *C. neoformans* (30). CYP5IA and CYP5IB mutations cause azole resistance in *Aspergillus spp.* Increase of azole resistance has been reported by many European countries such as Austria, Belgium, Denmark, Germany, France, Holland, Norway, Spain, Switzerland, UK (31).

Over the last decade, chemical structure of pyridazinones have become a striking field of study for developing new antimicrobials (5,6). The synthesis of novel pyridazinone derivatives is an important point to evaluate their chemical and biological activities. In particular, the cardiovascular effects of these compounds have been studied extensively (10,11). Various compounds synthesized in this area are being investigated as phosphodiesterase-III inhibitors, new antiplatelet and cardiotonic agents (8). Besides cardiovascular effects, these compounds were reported to have anti-depressant, antihypertensive, anticonvulsant, cardiotonic, antibacterial, diuretic, anti-HIV and anti-cancer effects (5-10). In particular, 3(2*H*)-pyridazinones are becoming remarkable therapeutic agent. Purohit et al. found that 3(2*H*)-pyridazinone derivatives were effective against *B. megaterium*, *B. subtilis*, *E. coli*, *P. fluorescens* and *Aspergillus* species (11). Sonmez et al. detected that 5-benzoyl-4-hydroxy-2-methyl-6-phenyl-2H-pyridazin-3-one compound showed good inhibition activity against Gram-positive, Gram-negative bacteria and fungi isolates with MICs in the range of 0.16-0.005 mg / ml (14). Akbas et al. reported that these compounds had the highest antimicrobial activities against Gram-positive and Gram-negative bacteria with MICs in the range of 0.31 to <0.0024 mg/ml^{-1} (15).

Generally, in our study, antibacterial evaluation show that all compounds have moderate activity against the bacterial strains. Compound 3 shows high activity against *E. hormaechei.* All tested concentrations of compounds 1,2,3,4,8 and 10 exhibit high inhibition activity against *S. maltophilia*. All compounds show higher activity against Gram-negative bacteria and fungi than Gram-positive bacteria. In addition to this, antifungal activity of compounds 1,2,3,4,7,8,9,10 and 11 exhibit the strongest activity against *C. tropicalis.* The synthesized pyridazinone derivatives show moderate activity against *C. albicans*. It is remarkable that the antifungal activities of target compounds are high. However, benzalhydrazone derivatives (1-9) and acetophenone (10 and 11) derivatives were found to exhibit similar antimicrobial activity in this study.

Conclusions

In conclusion, our study revealed that some of the prepared 6-Substituted-3(2*H*)-pyridazinone-2-acetyl-2-(substituted/nonsubstituted-benzal/acetophenone) hydrazone derivatives displayed higher MIC values than the above mentioned standard drugs. Therefore, we suggested that the compounds 1,8 and 9 might be a promising candidate of new antimicrobial agents and must be taken into consideration in the future studies.

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