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ORIGINAL ARTICLE

Antimicrobial Activities of Some Pyrazoline and Hydrazone Derivatives

Bazı Pirazolin ve Hidrazon Türevlerinin Antimikrobiyal Aktiviteleri

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ABSTRACT

Objectives: Resistance to antibiotics is recognized as one of the biggest threats to human health worldwide. Frequent and unnecessary use of antibiotics has caused infectious agents to adapt to antibiotics and thus drugs have become less effective. The resistance to many antibiotics necessitates the discovery of new antibiotics. In this study, two new and 23 previously reported 2-pyrazoline derivatives and one hydrazone derivative were evaluated for their in vitro antibacterial and antifungal activities.

Materials and Methods: For the determination of the minimum inhibitory concentration (MIC) values of compounds, microbroth dilution was used.

Results: The antimicrobial activities of the compounds were found in a wide range with MIC values of 32-512 µg/mL.

Conclusion: The synthesized compounds showed moderate antimicrobial activity compared with the standards. They can be used as lead molecules for the synthesis of more effective compounds.

Key words: Synthesis, antimicrobial activity, pyrazoline derivatives, hydrazone derivatives

ÖZ

Amaç: Antibiyotik direnci, dünya çapında insan sağlığına yönelik en büyük tehditlerden biri olarak kabul edilmektedir. Sık ve gereksiz antibiyotik kullanımı, bulaşıcı organizmaların antibiyotiklere adapte olmasına neden olarak ilaçların daha az etkili hale gelmesine yol açmıştır. Birçok antibiyotiğe karşı gelişen direnç, yeni antibiyotiklerin keşfini gerektirmektedir. Bu çalışmada, daha önce başka etkileri nedeni ile yayınlanmış 23 2-pirazolin ve bir hidrazon türevi ile iki yeni 2-pirazolin türevi bileşiğin, *in vitro* antibakteriyel ve antifungal aktiviteleri incelenmiştir.

Gereç ve Yöntemler: Bileşiklerin minimum inhibitör konsantrasyon (MİK) değerlerinin belirlenmesi için mikrobroth dilüsyon yöntemi kullanıldı.

Bulgular: Bileşiklerin antimikrobiyal aktiviteleri, 32-512 µg/mL MİK değerleri ile geniş bir aralıkta bulunmuştur.

Sonuç: Sentezlenen bileşikler, standartlarla karşılaştırıldıklarında orta düzeyde antimikrobiyal aktivite göstermiştir ve daha etkili bileşiklerin sentezi için öncü moleküller olarak kullanılabilecekleri sonucuna varılmıştır.

Anahtar kelimeler: Sentez, antimikrobiyal aktivite, pirazolin türevleri, hidrazon türevleri

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INTRODUCTION

Antimicrobials are drugs that kill or inhibit the growth of microorganisms. Resistance to antimicrobials occurs when microorganisms change in a way that reduces the effectiveness of drugs. Antibiotic resistance has become a major clinical and public health problem worldwide today. Resistance rates are rising dangerously in the world. New resistance mechanisms are emerging, making it difficult to treat infectious diseases.[1-4] In order to control this global problem, all government sectors and societies should take the necessary precautions and should support investigations on developing new antimicrobial drugs.

Hydrazones are formed as intermediates in the reaction of hydrazine and its derivatives with β-unsaturated carbonyl compounds but they are often not isolated due to their low stability and give pyrazolines with ring closure.[5,6] These compounds have interesting biological properties, such as antimicrobial, antituberculous, antidepressant, analgesic, anticonvulsant, antitumor, antiviral, and antiinflammatory activities.[7] Pyrazolines are five-membered and two neighboring nitrogen-containing heterocyclic compounds. They can be synthesized by the reaction of chalcones and hydrazines/hydrazides.[8-10] Pyrazoline derivatives are electron-rich compounds that are thought to cause a wide variety of biological activities. Pyrazolines are important compounds because of their antimicrobial, analgesic, antiinflammatory, and antidepressant activities.[11-13] According to the literature above, both pyrazoline and hydrazone compounds have antimicrobial activity. Therefore, we tested our compounds for their antimicrobial activity. In the present study, two new and 23 previously reported 2-pyrazoline derivatives and one hydrazone derivative were tested for their antibacterial and antifungal activity.

MATERIALS AND METHODS

Antimicrobial activity tests

In the antibacterial activity tests, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used as test bacteria. For antifungal activity testing, *Candida albicans* ATCC 10231 was used. The cultures were prepared in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA). For determination of minimum inhibitory concentration (MIC) values, microbroth dilution was used.[14,15] Serial two-fold dilutions ranging from 1024 µg/mL to 8 µg/mL were made in the medium. The incubation conditions for the bacteria were 18-24 h at 35±1 °C and for the fungi were 48 h at 35±1 °C; the last well with no microbial growth was noted as the MIC value (mg/mL). Ampicillin, ofloxacin, and fluconazole were used as the positive control and 10% dimethyl sulfoxide (DMSO) was used as the negative control. All experiments were repeated three times. There was no statistical data analysis.

Chemistry

All compounds except compounds 20 and 24 have been reported earlier.[10,13]

Synthesis of chalcone derivatives (A, B)

2'-Hydroxy-4'-methoxy acetophenone/5'-chloro-2'-hydroxy acetophenone (4.99 mmol) and 4-bromobenzaldehyde/4-benzyloxybenzaldehyde (4.99 mmol) were reacted in ethanol (20 mL) using KOH solution (50% w/v) in water (5 mL) as catalyzer at room temperature overnight. Ice was added to the mixture and pH was set to 3-4 with 1 M HCl. Then the mixture was filtered and crystallized from ethanol.[16-18]

(E)-3-(4-bromophenyl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (A): Yellow product. 61.14% yield. M.p. 141.0 °C. [lit. 138.0-140.0 °C].[19] $C_{16}H_{13}BrO_3$.

(E)-3-(4-(benzyloxy)phenyl)-1-(5-chloro-2-hydroxyphenyl) prop-2-en-1-one (B): Orange product. 93.70% yield. M.p. 138.0 °C. [lit. 100.0-102.0 °C].[20] $C_{22}H_{17}ClO_3$.

Synthesis of compounds 20 and 24

First, 1 equiv of compound A/compound B and 1 equiv of isoniazid were heated and stirred in ethanol (20 mL) for 4-25 h. Then the filtered products recrystallized from ethanol to give 20 and 24.[21-23]

(5-(4-(benzyloxy)phenyl)-3-(5-chloro-2-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)(pyridin-4-yl)methanone (20): Beige product. Yield: 24.8%. M.p. 237.1 °C. IR (ν, cm^{-1}): 3167 (OH), 1641 (amide C=O), 1585 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz): 2.91 (dd, 1H, J_1=16.4 Hz, J_2=12.4 Hz, H_A), 3.46 (dd, 1H, J_1=3.2 Hz, J_2=3.2 Hz H_B), 5.14 (s, 2H, -OCH_2Ph), 5.22 (dd, 1H, J_1=2.4 Hz, J_2=2.8 Hz, H_x), 7.02-8.74 (16H, aromatic-H), 11.11 (s, 1H, OH). MS (ESI): m/z=484 [M+H] (100%). $C_{28}H_{22}ClN_3O_3$. 1.25 H_2O: C 66.36, H 4.44, N 8.05; calcd. C 66.14, H 4.72, N 8.26.

(5-(4-bromophenyl)-3-(2-hydroxy-4-methoxyphenyl)-4,5-dihydropyrazol-1-yl) (pyridin-4-yl)methanone (24): Cream colored product. Yield 29.5%. M.p. 225.5 °C. IR (ν, cm^{-1}): 3174 (OH), 1641 (amide C=O), 1576 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz): 2.82 (dd, 1H, J_1=12.4 Hz, J_2=12.8 Hz, H_A), 3.41 (dd, 1H, J_1=2.8 Hz, J_2=3.2 Hz, H_B), 3.79 (s, 3H, -OCH_3), 5.29 (dd, 1H, J_1=2.8 Hz, J_2=2.4 Hz, H_x), 6.59-8.73 (11H, aromatic-H), 11.02 (s, 1H, OH). MS (ESI): m/z=452 [M+H], 454 [M+H+2] (100%). $C_{22}H_{18}BrN_3O_3$. 0.5 H_2O: C 57.15, H 4.38, N 9.36; calcd. C 57.23, H 4.12, N 9.10.

RESULTS AND DISCUSSION

A number of pyrazoline derivatives (compounds 2-26) and one hydrazone derivative (compound 1) were prepared. The structures of the target compounds are outlined in Figure 1.

Twenty-six compounds were tested for their antibacterial and antifungal activities. Antimicrobial activity was screened against two Gram-negative (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853*)* and three Gram-positive (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *B. subtilis* ATCC 6633*)* bacteria and a fungus (*C. albicans* ATCC 10231) using ampicillin, ofloxacin, and fluconazole as the standard drugs. The results are given in Table 1.

Compound 1, the hydrazone, showed moderate activity against all the bacteria and the fungus. Pyrazoline derivatives were found to possess moderate activity against the bacteria and fungus. Whether the ring was open (hydrazone) or closed

Table 1. ***In vitro*** **antimicrobial activities of hydrazone (1) and 2-pyrazoline (2-26) derivatives**

	MIC values of test microorganisms (µg/mL)					
Compound	Gram-negative bacteria		Gram-positive bacteria			Fungus
	Escherichia coli ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 29213	*Enterococcus faecalis* ATCC 29212	*Bacillus subtilis* ATCC 6633	*Candida albicans* ATCC 10231
1	256	128	128	128	128	128
2	256	128	256	128	128	128
3	256	128	128	64	128	128
4	512	256	256	256	256	256
5	256	128	64	64	128	64
6	256	128	256	128	128	128
7	256	128	128	64	128	128
8	256	128	128	128	128	128
9	256	128	256	256	256	128
10	256	128	128	128	128	128
11	256	128	512	256	256	128
12	512	128	256	128	128	128
13	512	128	256	256	128	128
14	512	256	512	256	256	256
15	512	256	512	256	128	256
16	512	256	512	256	256	128
17	512	128	512	256	256	256
18	256	128	256	256	128	128
19	256	64	64	256	128	128
20	256	128	128	128	128	128
21	256	128	256	256	128	128
22	512	64	128	32	64	128
23	512	256	256	64	256	128
24	512	256	64	32	256	128
25	-	512	-	256	-	256
26	-	512	512	64	64	128
Ampicillin	NT	NT	0.3	1	6	NT
Ofloxacin	1	8	NT	NT	NT	NT
Fluconazole	NT	NT	NT	NT	NT	1

NT: Not tested, MIC: Minimum inhibitory concentration, -: Represents no activity

(pyrazolines) generally did not appear to make a large difference in antimicrobial effect. Compounds 5, 19, and 24 exhibited the highest antibacterial activity against *S. aureus*, with a MIC value of 64 µg/mL among the tested bacteria. Compounds 19 and 22 were found to have the best activity against *P. aeruginosa.* Compounds 22 and 26 showed the best activity against *B. subtilis*, with a MIC value of 64 µg/mL. Compounds 22 and 24 exhibited the highest antimicrobial activity against *E. faecalis*, with a MIC value of 32 µg/mL. Compound 5 was found the most active compound against *C. albicans*, with a MIC value of 64 µg/mL.

Karad et al.[24] synthesized (2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl) derivatives and studied their antibacterial activity. They found that the existence of -OCH_3 substituent at position-4 in the phenyl ring at the C-3 position in the pyrazoline scaffold enhanced the antibacterial activity and antimalarial potency. For our compounds, a methoxy substituent in this position increased the antibacterial activity against *S. aureus* and *E. faecalis*, when it had bromo at the R^7 position and pyridin-4-yl at the R^8 position (compound 24).

Replacement of 4-methyl with 4-bromo substitution on the B ring in the pyrazoline nucleus enhanced the activity against *S. aureus* and *E. faecalis* (compounds 23 and 24).

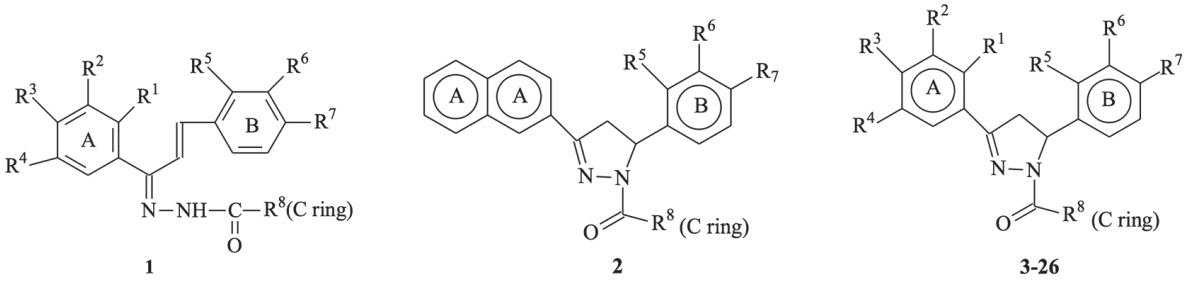

Figure 1. Structures of compounds 1-26

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8 (C ring)
1	-H	-H	-F	-H	-H	-H	-CH_3	Furan-2-yl
2	-	-	-	-	-H	-H	-OCH_3	Pyridin-4-yl
3	-OH	-H	-H	-CH_3	-H	-H	-OCH_3	Phenyl
4	-OH	-Cl	-H	-Cl	-OCH_3	-H	-H	Pyridin-4-yl
5	-OH	-Cl	-H	-Cl	-H	-H	-CH_3	Furan-2-yl
6	-OH	-H	-H	-CH_3	-H	-H	-CH_3	Phenyl
7	-OH	-H	-H	-Cl	-OCH_3	-H	-H	Phenyl
8	-OH	-H	-H	-Br	-OCH_3	-H	-H	Pyridin-4-yl
9	-OH	-H	-H	-CH_3	-H	-H	-CH_3	Furan-2-yl
10	-H	-OH	-H	-H	-OCH_3	-H	-H	Phenyl
11	-H	-OH	-H	-H	-OCH_3	-H	-H	Furan-2-yl
12	-OH	-H	-H	-Br	-OCH_3	-H	-H	Phenyl
13	-OH	-H	-H	-Cl	-H	-OCH_3	-H	Phenyl
14	-OH	-H	-H	-H	-OCH_3	-H	-H	Phenyl
15	-OH	-H	-OCH_3	-H	-H	-H	- CH_3	Phenyl
16	-OH	-H	-H	-Cl	-OCH_3	-H	-H	Furan-2-yl
17	-OH	-H	-H	-Cl	-H	-OCH_3	-H	Furan-2-yl
18	-OH	-H	-H	-Br	-H	-H	-OCH_3	Phenyl
19	-OH	-H	-H	-Cl	-H	-OCH_3	-H	Pyridin-4-yl
20	-OH	-H	-H	-Cl	-H	-H	-OCH_2Ph	Pyridin-4-yl
21	-OH	-H	-H	-Cl	-OCH_3	-H	-H	Pyridin-4-yl
22	-OH	-H	-H	-Br	-H	-H	-OCH_3	Pyridin-4-yl
23	-OH	-H	-OCH_3	-H	-H	-H	-CH_3	Pyridin-4-yl
24	-OH	-H	-OCH_3	-H	-H	-H	-Br	Pyridin-4-yl
25	-OH	-H	-H	-H	-H	-CH_3	-H	Pyridin-4-yl
26	-OH	-H	-H	-Cl	-H	-H	-OCH_3	Furan-2-yl

According to Hamada and Abdo,[9] the addition of pharmacophores such as chloro and bromo substituents with lipophilic properties increased the antimicrobial activity. For our compounds 7, 12, and 14, the substitution of chloro and bromo atoms at the 5-position of the A ring tended to increase the biological activity.

When the C ring had a phenyl scaffold, replacement of the 2-hydroxy-5-bromo phenyl (A ring) by 2-hydroxy-5-chloro phenyl increased the antibacterial activity against *E. coli*, *S. aureus*, and *E. faecalis* (compounds 7 and 12). When the compound carried a pyridine as the C ring, the substitution of 2-hydroxy-3,5-dichloro phenyl decreased the antimicrobial and antifungal activity. Replacement of this group by 2-hydroxy-5-bromo phenyl enhanced the antimicrobial activity against all bacteria and the fungus (compounds 4 and 8). Replacement of 2-hydroxy-3,5-dichloro phenyl by 2-hydroxy-5-chloro phenyl increased the activity against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *C. albicans* (compounds 4 and 21).

The addition of the naphthyl group instead of phenyl on the A ring in compound 2 resulted in increased efficacy against *E. coli*. It reduced the activity against *S. aureus*, *P. aeruginosa*, *E. faecalis*, and *B. subtilis.* Compound 25 showed no antimicrobial activity against *E. coli*, *S. aureus*, or *B. subtilis.* Compound 26 showed no antimicrobial activity against *E. coli*.

Addition of phenyl instead of 2-furyl as the C ring increased the activity (compounds 6 and 9; compounds 10 and 11; compounds 13 and 17; compounds 7 and 16). The presence of phenyl instead of pyridine as the C ring increased the antimicrobial activity against *E. coli*. However, addition of pyridine instead of phenyl as the C ring enhanced the antibacterial activity against *S. aureus*, *P. aeruginosa*, *E. faecalis*, and *B. subtilis* (compounds 18 and 22). The substitution by a methoxy group at the fourth position on the B ring produced comparable antimicrobial activity against *S. aureus* and *E. faecalis* to the substitution by a methyl group (compounds 3 and 9).

Meta methoxy substitution on the B ring increased the activity against *P. aeruginosa* and *S. aureus* in the presence of pyridine as the C ring (compounds 19 and 21). Para methoxy substitution on the B ring enhanced the antimicrobial activity against *P. aeruginosa*, *E. faecalis*, and *B. subtilis* in the case of a pyridine substituent at the R^8 position (compounds 8 and 22). Orto methoxy substitution on the B ring is not preferable, especially when the C ring is pyridine. According to Manna and Agrawal[25] ortho substitution in the phenyl ring with a methoxy group at the 5th position of the pyrazoline ring caused less or inactive antibacterial activity against Gram-negative bacteria.

Replacement of 5-bromo with 5-methyl substitution on the A ring enhanced the activity against *S. aureus* and *E. faecalis* (compounds 3 and 18*)*.

The presence of a methyl group at the fifth position of the A ring instead of a methoxy group at the fourth position of the A ring increased the antifungal activity and antimicrobial activity against *E. coli*, *S. aureus*, *E. faecalis, P. aeruginosa*, and *C. albicans* (compounds 6 and 15).

CONCLUSION

In this work, several pyrazoline derivatives and one hydrazone derivative were synthesized and screened for their antibacterial and antifungal activities. We noted that the pyridine ring as the C ring and methoxy and bromo substitutions on the B ring are preferable for a good antibacterial effect. 2-hydroxy-5-chloro substitution and 2-hydroxy-4-methoxy substitution substituents are favorable as the A ring. Further studies are necessary in order to understand the relation between the substitutions and activity, which could guide the design of more potent antimicrobial agents for therapeutic use.

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