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Synthesis of novel pyrazoline derivatives and evaluation of their antimicrobial activity

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ABSTRACT: In this study, new nine pyrazoline derivatives were synthesized from chalcone derivatives. The antimicrobial activity of nine pyrazoline derivatives to four standard bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*) and four standard yeast strains (*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*) was investigated by the microdilution method for in accordance with the Clinical and Laboratory Standards Institute (CLSI) standard, and the Minimal Inhibitory Concentration (MIC) values of these derivatives were determined. Among these derivatives; compounds **7** and **8** against bacterial strains and compounds **2** and **3** against yeast strains exhibited good antimicrobial activity.

KEYWORDS: Pyrazoline; bacteria; yeast; MIC.

1. INTRODUCTION

Antimicrobial resistance has become a growing global public health problem [1]. It is estimated that there are more than 250 million cases of bacterial infections per year, resulting in approximately $1.6 billion in economic losses each year. There are difficulties in infection management due to the emergence of antimicrobial resistance and the transmission of resistance among the strains. As a result, the rates of hospitalization and mortality enhance significantly [2]. In the last decades, complications related to resistance and therapeutic difficulties have been expressed by gram-positive and gram-negative bacteria microorganisms such as *Staphylococcus aureus*, *Enterococcus faecium*, *Streptococcus pneumonia*, *Mycobacterium tuberculosis* [3]. Resistance to antimicrobial drugs used in the treatment of some bacterial, viral and fungal infectious diseases causes difficulties in the treatments [4]. While antibiotic resistance can develop naturally, misuse of drugs accelerates this process. Treating infections such as tuberculosis, pneumonia, salmonella and gonorrhea becomes more difficult as the effect of antibiotics decreases [5,6]. Therefore, the importance of the discovery and development of novel antimicrobial molecules increases day by day [7].

Chalcones and their pyrazoline derivatives are very important compounds in pharmaceutical chemistry because they can have many biological activities like antibacterial, antiviral, antifungal, anti-inflammatory, antihypertensive and antitumor [8-10]. Pharmaceuticals with many different activities in the pyrazole and pyrazoline structure are currently on the market (e.g., celecoxib, lonazolac, tepoxalin, rimonabant, pyrazofurin, epirizole) [11].

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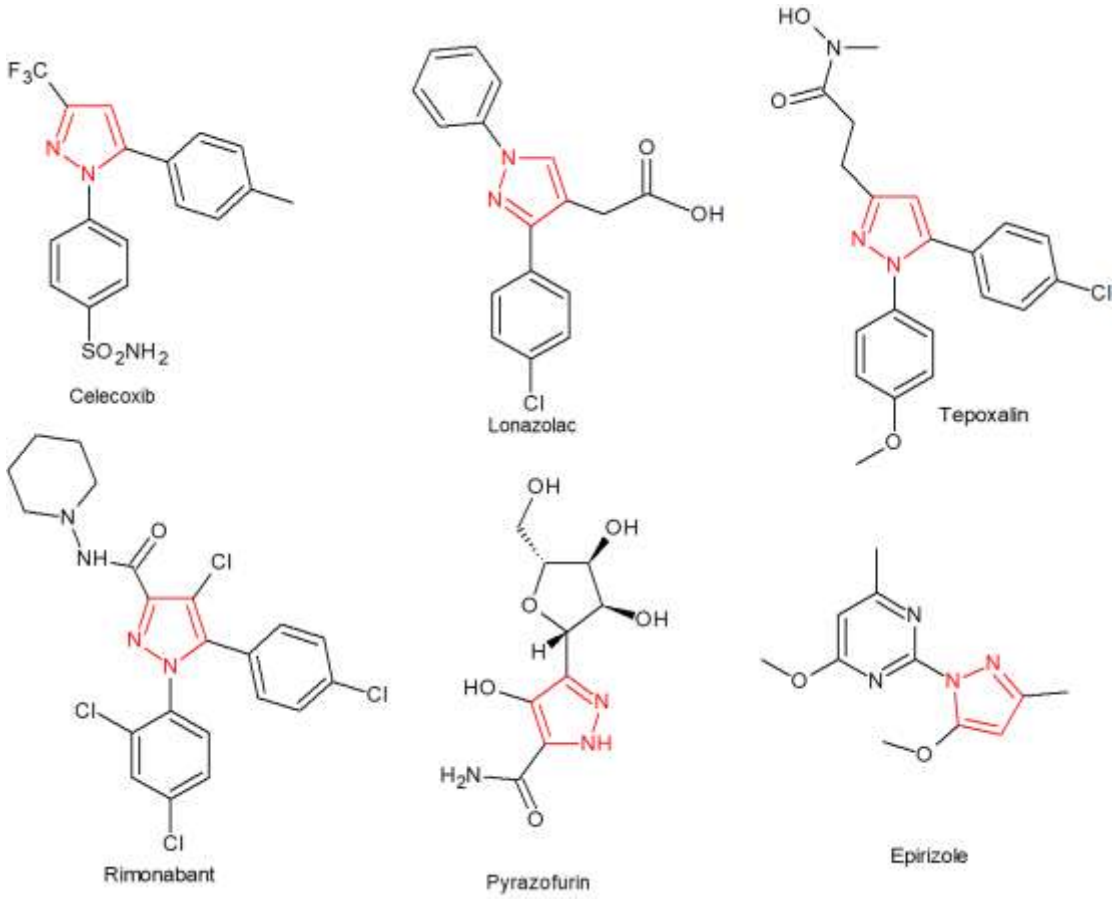

Figure 1. The chemical structures of the drugs carrying pyrazoline ring.

In this article, we synthesized chalcone derivatives in the first step and then pyrazoline derivatives were obtained from chalcone derivatives in the second step. In addition, the antimicrobial activity of pyrazoline derivatives; they were evaluated on four standard bacterial strains (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212) and four standard yeast strains (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida parapsilosis* ATCC 90018, *Candida tropicalis* KUEN 1021).

2. RESULTS

2.1. Synthesis

Various new pyrazoline derivatives were synthesized in two steps. In the first step, chalcone compounds were synthesized according to the Claisen-Schmidt condensation reaction from 4´-(methylsulfonyl)acetophenone and various substituted aromatic aldehydes an alkaline medium. In the second step, the targeted pyrazoline compounds were obtained with the cyclization reaction of chalcones and hydrazine monohydrate in acetic acid (Scheme 1). The structures of the obtained pyrazoline derivatives (**1-9**) were determined by IR, ^{1}H-NMR and elemental analysis.

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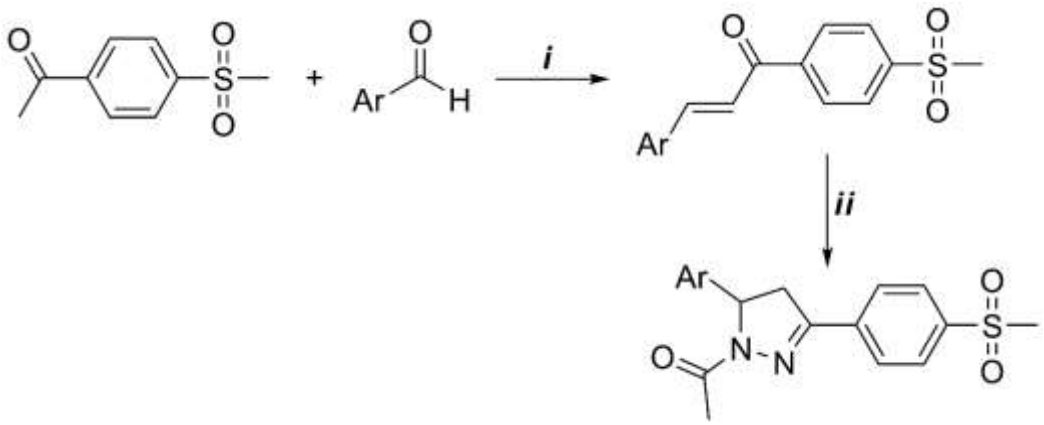

Scheme 1. The protocol for the synthesis of pyrazoline derivatives. Reagents and conditions: (*i*) Methanol, NaOH, 2h; (*ii*) Hydrazine hydrate, acetic acid, reflux, 6h. Ar: Substituted aromatic ring.

In the IR spectrum of pyrazoline derivatives, the characteristic imine (C=N) bond was detected at 1550-1600 cm^{-1}. The carbonyl (CO) bond in the acetyl group attached to the pyrazoline ring was recorded at 1650-1700 cm^{-1}. The other bonds gave different bands consistent with the structures. In the ^{1}H-NMR spectrum of pyrazoline derivatives, the most important indicator of the synthesis of the pyrazoline ring was the detection of three double-doublet peaks. These three double-doublet peaks represented the hydrogen atoms in the 4th and 5th positions of the pyrazoline structure. The Ha proton in the 4th position of the pyrazoline ring was detected in the range of 3.10-3.12 ppm, and the Hb proton in the range of 3.80-3.90 ppm. The Hx proton in the 5th position of the pyrazoline ring resonated in the range of 5.50-5.60 ppm. In addition, methyl protons in the acetyl group attached to the pyrazoline ring were detected at 2.30-2.35 ppm. Elemental analysis data was fully compatible with the chemical structures.

When the ^{1}H-NMR spectrum of compound **1** was examined in detail, the Ha, Hb and Hx protons of the pyrazoline structure gave double doublet peaks at 3.13, 3.88 and 5.52 ppm, respectively. The methyl protons on the aromatic ring, in the acetyl group and on the methylsulfonyl moiety resonated as singlet peaks at 2.25, 2.31 and 3.22 ppm, respectively. Aromatic protons to which methyl was attached from the phenyl ring were detected in the upfield area (Figure 2).

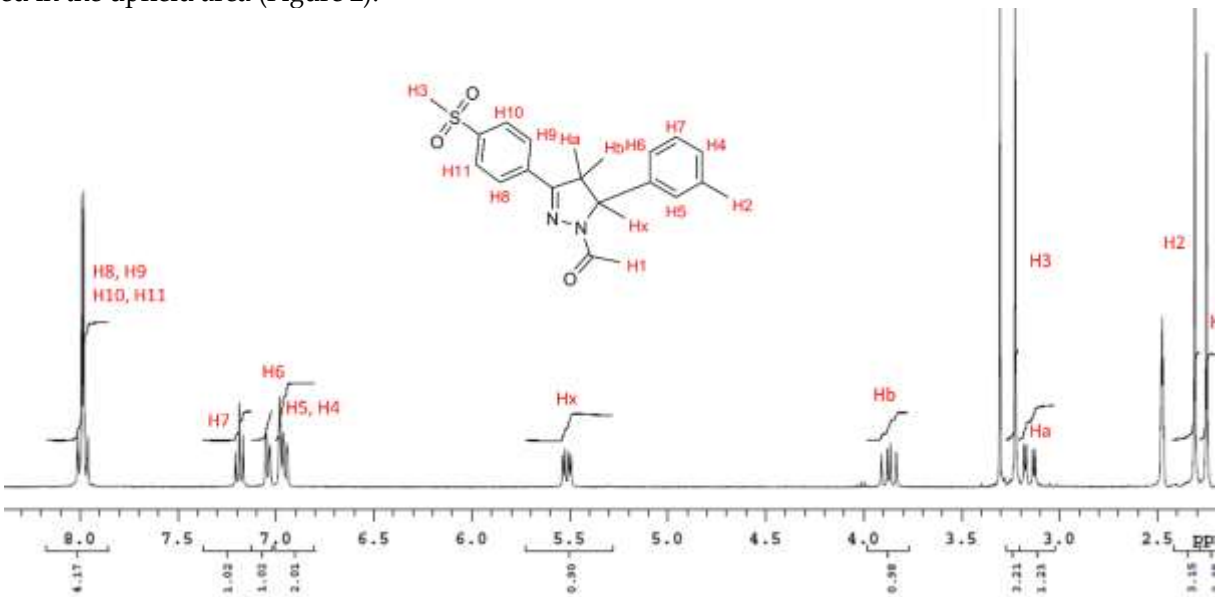

Figure 2. The ^{1}H-NMR spectra of compound **1**.

2.2. Antimicrobial activity

The synthesized compounds which have pyrazoline rings were investigated for their *in vitro* antibacterial activity against *S. aureus* (ATCC 25922), *E. coli* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *E. faecalis* (ATCC 29212) bacterial strains. Ciprofloxacin was used as a standard antimicrobial drug for

comparison. The results of antibacterial tests are depicted in Table 1. The antifungal activity of synthesized compounds was investigated against *C. albicans* (ATCC 90028), *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 90018), *C. tropicalis* (KUEN 1021). Fluconazole was used as standard drug. The results of antifungal tests are depicted in Table 2.

The minimum inhibitory concentration (MIC) was determined at the concentrations of the pyrazolines ranging from 0.39 to 800 µg/mL. According to activity results, compounds **7** and **8** showed higher antibacterial activity against all bacterial strains and their MIC values range from 25 to 100 µg/mL. Compounds **2** and **3** displayed higher antifungal activity against all fungal strains and their MIC values range from 50 to 100 µg/mL. On the other hand, all pyrazoline derivatives demonstrated antibacterial activity against *E. faecalis* ATCC 29212 and *C. glabrata* (ATCC 90030). Among the compounds, **2** and **7** have the smallest MIC values against all microbial strains.

Table 1. The MIC values of pyrazolines against bacterial strains.

Compounds (800-0.39 µg/ml)	**MIC (µg/ml)**			
	Staphylococcus aureus **(ATCC 25922)**	***Escherichia coli*** **(ATCC 25923)**	***Pseudomonas aeruginosa*** **(ATCC 27853)**	***Enterococcus faecalis*** **(ATCC 29212)**
1	>800	>800	>800	200
2	100	>800	>800	25
3	200	>800	>800	50
4	>800	>800	>800	200
5	>800	>800	100	200
6	200	>800	100	100
7	100	100	100	25
8	100	100	100	50
9	100	>800	>800	100
Ciprofloxacine (256-0.0039 µg/ml)	0.0625	0.0078	0.5	0.5

Table 2. The MIC values of pyrazolines against fungal strains.

Compounds (800-0.39 µg/ml)	**MIC (µg/ml)**			
	Candida albicans **(ATCC 90028)**	***Candida glabrata*** **(ATCC 90030)**	***Candida parapsilosis*** **(ATCC 90018)**	***Candida tropicalis*** **(KUEN 1021)**
1	>800	100	100	100
2	100	50	100	100
3	100	50	100	100
4	100	100	>800	100
5	>800	100	100	>800
6	100	100	>800	>800
7	>800	100	100	100
8	>800	100	100	100
9	100	100	100	>800
Fluconazole (1600-0.39µg/ml)	6.25	3.125	0.78125	0.78125

2.3. The prediction of ADME properties

A promising compound must pass *in silico* analysis before it can be taken up for further studies [12]. Therefore, we evaluated the pharmacokinetics, druglikeness and physicochemical properties of pyrazoline derivatives. The druglikeness such as Lipinski and Veber of all compounds were within the accepted range. The bioavailability score is 0.55 for all compounds, meaning good bioavailability. All data for the calculation were shown in Table 3.

Table 3. The calculation data of synthesized compounds.

Comp.	Lipinski				Veber		Pharmacokinetics		Bio. score
	MW	n-ON	n-OHNH	cLog P	n-ROTB	TPSA	GI abs.	BBB per.	
1	356,44	4	0	2,67	4	75,19	High	Yes	0,55
2	376,86	4	0	2,94	4	75,19	High	Yes	0,55
3	421,31	4	0	3,05	4	75,19	High	Yes	0,55
4	387,41	6	0	1,52	5	121,01	High	No	0,55
5	411,30	4	0	3,43	4	75,19	High	Yes	0,55
6	332,37	5	0	1,18	4	88,33	High	No	0,55
7	367,42	5	0	1,78	4	98,98	High	No	0,55
8	385,48	4	0	2,35	5	78,43	High	Yes	0,55
9	384,49	5	0	3,12	5	75,19	High	Yes	0,55

MW: Molecular weight, n-ON: number of hydrogen bond acceptors, n-OHNH: number of hydrogen bond donors, cLog P: n-ROTB: number of rotatable bonds, TPSA: Topological polar surface area, GI abs.: GI absorption, BBB per.: BBB permeant, Bio. Score: Bioavailability Score

3. CONCLUSION

The rapidly increasing resistance to antibiotics and antifungal drugs in recent years has prompted researchers to discover new molecules against microorganisms. In this study, new pyrazoline derivatives were synthesized, characterized by spectral data (IR and ^{1}H-NMR) and elemental analysis. Their antibacterial activities against *S. aureus*, *E. coli*, *P. aeruginosa*, *E. faecalis* and their antifungal activities against *C. albicans, C. glabrata, C. parapsilosis*, *C. tropicalis* were investigated. Among the compounds, **7** and **8** having cyano and dimethylamino groups, respectively, exhibited the highest antibacterial activity. Compounds **2** ve **3** having halogens such as chloro and bromo substituents on aromatic rings exhibited the most antifungal activity. The antimicrobial results displayed that the pyrazoline ring is a promising structure for antibacterial and antifungal activities.

4. MATERIALS AND METHODS

4.1. General

The chemicals and solvents used were purchased from Sigma Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Reactions were monitored using thin-layer chromatography (TLC) on silica gel coated aluminum plates (Merck TLC Silica gel 60) F254). Petroleum ether:ethyl acetate (10:90) was used as the solvent system. Thin-layer chromatography was terminated after the plates were observed under ultraviolet (UV) light (λ= 254 nm). Infrared spectra were recorded on a Shimadzu FT-IR 8400 S Spectrometer. ^{1}H-NMR spectra were obtained on a Bruker Avance DPX 400 spectrometer (Bruker Corp., Billerica, MA, USA). Elemental analysis was determined with CHNS-932 (LECO).

4.1.1. General procedure of chalcone synthesis

Firstly, 1 mmol 4´-(methylsulfonyl)acetophenone was dissolved in 15 mL methanol and 1 mmol substituted aldehyde derivatives were added to the reaction mixture. Then, 2 mL 40% NaOH (w/v) was added to the reaction mixture and stirred on a magnetic stirrer for 3 h at room temperature. The reaction process was monitored by thin-layer chromatography. After the reaction was completed, the chalcone mixture was poured onto ice, washed with distilled water, filtered, then crystallized from methanol [13].

4.1.1. General procedure of pyrazoline synthesis

2 mmol of Hydrazine monohydrate was added to a solution of 1 mmol chalcone derivative in 15 mL of acetic acid and refluxed for 6 hours. The reaction mixture was followed by thin layer chromatography, then poured onto ice, washed with distilled water, filtered and crystallized from ethanol [14].

1-{3-[4-(methanesulfonyl)phenyl]-5-(3-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**1**)

Yield: 80%, white crystals, m.p.: 265-266 °C. IR (vmax, cm^{-1}): 3012 (=C–H, aromatic), 2953 (CH_3), 1666 (C=O), 1587 (C=N), 1307, 1138 (SO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.25 (s, 3H, $COCH_3$), 2.31 (s, 3H, Ar-CH_3), 3.13 (dd, *Jax* 4.80 Hz, *Jab* 18.00 Hz, 1H, Ha), 3.22 (s, 3H, SO_2CH_3), 3.88 (dd, *Jbx* 12.00 Hz, *Jab* 18.00 Hz, 1H, Hb), 5.52 (dd, *Jax* 4.80 Hz, *Jbx* 12.00 Hz, 1H, Hx), 6.87–7.10 (m, 3H, Ar-H), 7.19 (t, 1H, Ar-H), 7.81-8.16 (m, 4H, Ar-H). Anal. Calcd for $C_{19}H_{20}N_2O_3S$: C, 64.02; H, 5.66; N, 7.86; S, 9.00. Found: C, 64.46; H, 5.71; N, 7.76; S, 8.91.

1-{3-[4-(methanesulfonyl)phenyl]-5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**2**)

Yield: 75%, of white crystals, m.p.: 257-258 °C. IR (vmax, cm^{-1}): 3022 (=C–H, aromatic), 2929 (-CH_3), 1676 (C=O), 1595 (C=N), 1311, 1147 (SO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.24 (s, 3H, $COCH_3$), 3.18 (dd, *Jax* 4.80 Hz, *Jab* 18.00 Hz, 1H, Ha), 3.25 (s, 3H, SO_2CH_3), 3.89 (dd, *Jbx* 12.00 Hz, *Jab* 18.00 Hz, 1H, Hb), 5.52 (dd, *Jax* 4.80 Hz, *Jbx* 12.00 Hz, 1H, Hx), 6.85–8.12 (m, 8H, Ar-H). Anal. Calcd for $C_{18}H_{17}ClN_2O_3S$: C, 57.37; H, 4.55; N, 7.43; S, 8.51. Found: C, 57.27; H, 4.47; N, 7.32; S, 8.61.

1-{3-[4-(methanesulfonyl)phenyl]-5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**3**)

Yield: 80%, of gray crystals, m.p.: 273-274 °C. IR (vmax, cm^{-1}): 3018 (=C–H, aromatic), 2928 (-CH_3), 1680 (C=O), 1595 (C=N), 1317, 1149 (SO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.27 (s, 3H, $COCH_3$), 3.13 (dd, *Jax* 4.80 Hz, *Jab* 16.40 Hz, 1H, Ha), 3.22 (s, 3H, SO_2CH_3), 3.77 (dd, *Jbx* 10.40 Hz, *Jab* 16.00 Hz, 1H, Hb), 5.58 (dd, *Jax* 4.80 Hz, *Jbx* 10.40 Hz, 1H, Hx), 7.14–7.26 (m, 4H, Ar-H), 7.81-7.99 (m, 4H, Ar-H). Anal. Calcd for $C_{18}H_{17}BrN_2O_3S$: C, 51.31; H, 4.07; N, 6.65; S, 7.61. Found: C, 51.64; H, 4.03; N, 6.74; S, 7.52.

1-{3-[4-(methanesulfonyl)phenyl]-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**4**)

Yield: 80%, yellow crystals, m.p.: 224-225 °C. IR (vmax, cm^{-1}): 3074 (=C–H, aromatic), 2926 (-CH_3), 1672 (C=O), 1593 (C=N), 1311, 1143 (SO_2). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.27 (s, 3H, $COCH_3$), 3.27 (s, 3H, SO_2CH_3), 3.28 (dd, 1H, Ha), 3.92 (dd, *Jbx* 13.20 Hz, *Jab* 18.20 Hz, 1H, Hb), 6.16 (dd, *Jax* 4.80 Hz, *Jbx* 8.25 Hz, 1H, Hx), 7.32 (d, *J* 8.05 Hz, 2H, Ar-H), 7.42 (d, *J* 8.05 Hz, 1H, Ar-H), 7.53 (d, *J* 8.05 Hz, 1H, Ar-H), 8.01-8.04 (m, 4H, Ar-H). Anal. Calcd for $C_{18}H_{17}N_3O_5S$: C, 55.80; H, 4.42; N, 10.85; S, 8.28. Found: C, 55.56; H, 4.37; N, 10.74; S, 8.38.

1-{5-(2,6-dichlorophenyl)-3-[4-(methanesulfonyl)phenyl]-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**5**)

Yield: 70%, gray crystals, m.p.: 218-219 °C. IR (vmax, cm^{-1}): 3076 (=C–H, aromatic), 2926 (-CH_3), 1672 (C=O), 1593 (C=N), 1311, 1144 (SO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.24 (s, 3H, $COCH_3$), 3.23-3.28 (m, 4H, Ha and SO_2CH_3), 3.90 (dd, *Jbx* 13.20 Hz, *Jab* 18.40 Hz, 1H, Hb), 6.14 (dd, *Jax* 8.40 Hz, *Jbx* 13.20 Hz, 1H, Hx), 7.31 (t, 1H, Ar-H), 7.41 (d, *J* 8.40 Hz, 1H, Ar-H), 7.48 (d, *J* 8.40 Hz, 1H, Ar-H), 7.97-8.02 (m, 4H, Ar-H). Anal. Calcd for $C_{18}H_{16}Cl_2N_2O_3S$: C, 52.56; H, 3.92; N, 6.81; S, 7.80. Found: C, 52.75; H, 3.98; N, 6.87, S, 7.88.

1-{5-(furan-2-yl)-3-[4-(methanesulfonyl)phenyl]-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**6**)

Yield: 70%, brown crystals, m.p.: 212-213 °C. IR (vmax, cm^{-1}): 3020 (=C–H, aromatic), 2920 (-CH_3), 1666 (C=O), 1587 (C=N), 1319, 1147 (SO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.27 (s, 3H, $COCH_3$), 3.24 (s, 3H, SO_2CH_3), 3.38 (dd, *Jax* 4.80 Hz, *Jab* 18.00 Hz, 1H, Ha), 3.77 (dd, *Jbx* 12.00 Hz, *Jab* 18.00 Hz, 1H, Hb), 5.68 (dd, *Jax* 4.40 Hz, *Jbx* 12.00 Hz, 1H, Hx), 6.33 (d, *J* 3.20 Hz, 1H, Ar-H), 6.38 (m, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 7.98-8.03 (m, 4H, Ar-H). Anal. Calcd for $C_{16}H_{16}N_2O_4S$: C, 57.82; H, 4.85; N, 8.43; S, 9.65. Found: C, 57.87; H, 4.84; N, 8.35, S, 9.57.

1-{3-[4-(methanesulfonyl)phenyl]-5-(4-cyanophenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**7**)

Yield: 70%, white crystals, m.p.: 243-244 °C. IR (vmax, cm^{-1}): 3005 (=C–H, aromatic), 2227 (CN), 2953 (-CH_3), 1680 (C=O), 1606 (C=N), 1300, 1147 (SO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.25 (s, 3H, $COCH_3$), 3.13 (dd, *Jax* 4.80 Hz, *Jab* 18.00 Hz, 1H, Ha), 3.22 (s, 3H, SO_2CH_3), 3.88 (dd, *Jbx* 12.00 Hz, *Jab* 18.00 Hz, 1H, Hb), 5.92 (dd, *Jax* 4.80 Hz, *Jbx* 12.00 Hz, 1H, Hx), 7.25–8.11 (m, 8H, Ar-H). Anal. Calcd for $C_{19}H_{17}N_3O_3S$: C, 62.11; H, 4.66; N, 11.44; S, 8.73. Found: C, 62.07; H, 4.71; N, 4.60, S, 8.85.

1-{3-[4-(methanesulfonyl)phenyl]-5-[4-(dimethylamino)phenyl]-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**8**)

Yield: 80%, white crystals, m.p.: 231-232 °C. IR (vmax, cm^{-1}): 3012 (=C–H, aromatic), 2974, 2920 (-CH_3), 1681 (C=O), 1604 (C=N), 1313, 1149 (SO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.31 (s, 3H, $COCH_3$), 2.70-3.02 (m, 6H, $2CH_3$), 3.21-3.28 (m, 4H, Ha and SO_2CH_3), 3.78 (dd, *Jbx* 12.00 Hz, *Jab* 18.00 Hz, 1H, Hb), 5.44 (dd, *Jax* 4.80 Hz, *Jbx* 12.00 Hz, 1H, Hx), 6.27-7.22 (m, 4H, Ar-H), 7.27–8.40 (m, 4H, Ar-H). Anal. Calcd for $C_{20}H_{23}N_3O_3S$: C, 62.32; H, 6.01; N, 10.90; S, 8.32. Found: C, 62.80; H, 5.98; N, 10.98; S, 8.54.

1-{3-[4-(methanesulfonyl)phenyl]-5-(4-isopropylphenyl)-4,5-dihydro-1H-pyrazol-1-yl}ethan-1-one (**9**)

Yield: 80%, white crystals, m.p.: 276-277 °C. IR (vmax, cm^{-1}): 3012 (=C–H, aromatic), 2968, 2929 (-CH_3), 1685 (C=O), 1587 (C=N), 1313, 1149 (SO_2). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.84-1.24 (m, 6H, $2CH_3$), 2.33 (s, 3H, $COCH_3$), 2.54 (m, 1H, Ar-CH), 3.01-3.22 (m, 4H, Ha and SO_2CH_3), 3.98 (dd, *Jbx* 11.90 Hz, *Jab* 18.00 Hz, 1H, Hb), 5.24 (dd, *Jax* 4.80 Hz, *Jbx* 11.90 Hz, 1H, Hx), 6.73–7.27 (m, 4H, Ar-H), 7.45-7.88 (m, 4H, Ar-H). Anal. Calcd for $C_{21}H_{24}N_2O_3S$: C, 65.60; H, 6.29; N, 7.29; S, 8.34. Found: C, 65.27; H, 6.19; N, 7.35; S, 8.48.

4.2. Antimicrobial activity

The antimicrobial activity of all synthesized compounds was investigated against standard bacterial strains such as *Staphylococcus aureus* ATCC 25922, *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and against standard yeast strains such as *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida parapsilosis* ATCC 90018, *Candida tropicalis* KUEN 1021. The minimum inhibitory concentration (MIC) test was performed in the concentration range of 800-0,39 µg/ml of compounds.

The antimicrobial activity of ten new pyrazoline derivatives against bacteria and fungi was investigated by the microdilution method following the Clinical and Laboratory Standards Institute (CLSI) standard [15]. Sterile 96-well microplates were used for this method.

Preparation of inoculum for MIC evaluation for bacteria: The suspensions of bacteria, prepared from 18-24 hours agar cultures with physiological saline according to Mac Farland 0.5 standard, were diluted to 0.5×10^3 CFU (colony forming units)/mL and used in the experiment.

Preparation of inoculum for MIC evaluation for yeasts: The suspensions of yeasts, prepared from 18-24 hour agar cultures with physiological saline according to Mac Farland 1 standard, were diluted to 2×10^3 CFU/mL and used in the experiment.

Cation-adjusted Mueller Hinton Broth (CAMHB) (Sigma) was used as a test medium for MIC determination of bacteria, and RPMI 1640 broth without sodium bicarbonate, with added L-glutamine, was buffered to a pH of 7.0 with 0.165 molars morpholinopropanesulfonic acid (MOPS) was used for yeasts. Sterile 96-well microplates were used in the experiment. First, 100 µl of CAMHB for bacteria and 100 µl of RPMI 1640 for yeasts were added to all wells of each microplate. Then, 100µl of pyrazoline derivatives was placed in the first wells of the plates and 12 serial dilutions were made. Finally, the prepared bacteria and yeast suspensions were added to all wells, and all microplates were incubated at 35^{0}C for 18-24 hours for bacteria and 24-48 hours for yeasts [16,17]. At the end of the period, the lowest concentration at which growth was inhibited was determined as the MIC value. Likewise, MIC values of Ciprofloxacin and Fluconazole tested as controls were determined.

4.3. The prediction of ADME properties

The physicochemical, pharmacokinetic and druglikeness properties of all compounds were predicted through SwissAdme online server (http://www.swissadme.ch/).

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