Full Paper

Synthesis and Biological Evaluation of Pyrazoline Derivatives Bearing an Indole Moiety as New Antimicrobial Agents

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1-(*p*-Methylphenyl)-3,5-diaryl-2-pyrazoline derivatives (**2a–f**) were synthesized via the treatment of 1-(1*H*-indol-3-yl)-3-aryl-2-propen-1-ones (**1a–f**) with *p*-methylphenylhydrazine hydrochloride in hot acetic acid. The structures of these compounds were elucidated by IR, ^{1}H NMR, and mass spectral data and elemental analysis. These compounds were investigated for their antimicrobial activity. Brine-Shrimp lethality assay was carried out to determine the toxicity of the compounds. Compound **2e**, which is the pyrazoline derivative bearing the 2,5-dichlorophenyl moiety, can be identified as the most promising agent against *Klebsiella pneumoniae* (ATCC 13883) and *Candida glabrata* (ATCC 36583) due to its inhibitory effects on *K. pneumoniae* and *C. glabrata* with a MIC value of 100 μg/mL as a non-toxic agent ($LC_{50} > 1000$ μg/mL).

Keywords: Antimicrobial activity / Brine-Shrimp lethality / Chalcone / Indole / Pyrazoline

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Introduction

The significant increase in resistance to conventional antimicrobial agents has become a major concern for public health. The high prevalence of multidrug-resistant pathogens has led to the failure of current treatments and deaths in immunocompromised patients. In order to cope with this serious problem, which is considered as the inevitable consequence of the widespread use of these agents, the development of new antimicrobial drugs has gained great importance [1–5].

Chalcones are considered as precursors of open chain flavonoids and isoflavonoids present in edible plants. Chalcones and their analogues are versatile and convenient starting materials or intermediates for the synthesis of naturally occurring flavonoids and various nitrogen-containing heterocyclic compounds [6–8]. Among chalcone-derived heterocycles, pyrazolines, which are synthesized by the ring closure reaction of chalcones with hydrazines, have attracted a great deal of interest due to their synthetic and biological importance in medicinal chemistry [9–13]. Chalcones and pyrazolines have been reported to exhibit a wide spectrum of biological effects including antimicrobial activity [12–18].

Medicinal chemists have also carried out considerable research on indole and its derivatives. One of the essential amino acids containing indole nucleus is tryptophan, which acts as a biochemical precursor for serotonin (5-hydroxytryptamine, 5-HT) [19–22]. Due to its crucial role in depression, bipolar disorder and anxiety, serotonin is one of important monoaminergic neurotransmitters as a drug target in several major neuropsychiatric diseases [23, 24]. In addition, a considerable number of currently available drugs carry an indole moiety [19–22]. Indolmycin is an example of antibacterial agents bearing an indole ring with anti-staphylococcal activity [25, 26]. Furthermore, some researchers synthesized indole derivatives and evaluated their antimicrobial activity against bacterial and fungal strains [27–30].

On the basis of these findings and in the continuation of our research on the synthesis and antimicrobial evaluation of pyrazoline derivatives [31–35], herein we report the synthesis and biological evaluation of chalcone and pyrazoline derivatives bearing an indole moiety as new antimicrobial agents. The compounds were also investigated for their toxicity.

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Results and discussion

As described in the Introduction, both indoles and 2-pyrazolines possess important biological activities which render them useful substances in drug research. On this basis, it appeared expedient to synthesize new heterocyclic compounds bearing both an indole moiety and a 2-pyrazoline unit. The reaction of (1*H*-indol-3-yl)chalcones, as easily available α,β-unsaturated ketones, with hydrazines seemed to be a convenient route to fulfil this aim.

The treatment of 1-(1*H*-indol-3-yl)-3-aryl-2-propen-1-one derivatives (**1a–f**) with *p*-methylphenylhydrazine hydrochloride in hot acetic acid afforded 1-(*p*-methylphenyl)-3,5-diaryl-2-pyrazolines (**2a–f**) in good yields (74–82%; Scheme 1). Some properties of the compounds are given in Table 1.

The structures of all new 1-(1*H*-indol-3-yl)-3-aryl-2-propen-1-ones (**1a–f**) and 1-(*p*-methylphenyl)-3,5-diaryl-2-pyrazolines (**2a–f**) were elucidated by IR, ^{1}H NMR, FAB$^+$-MS spectral data, and elemental analyses.

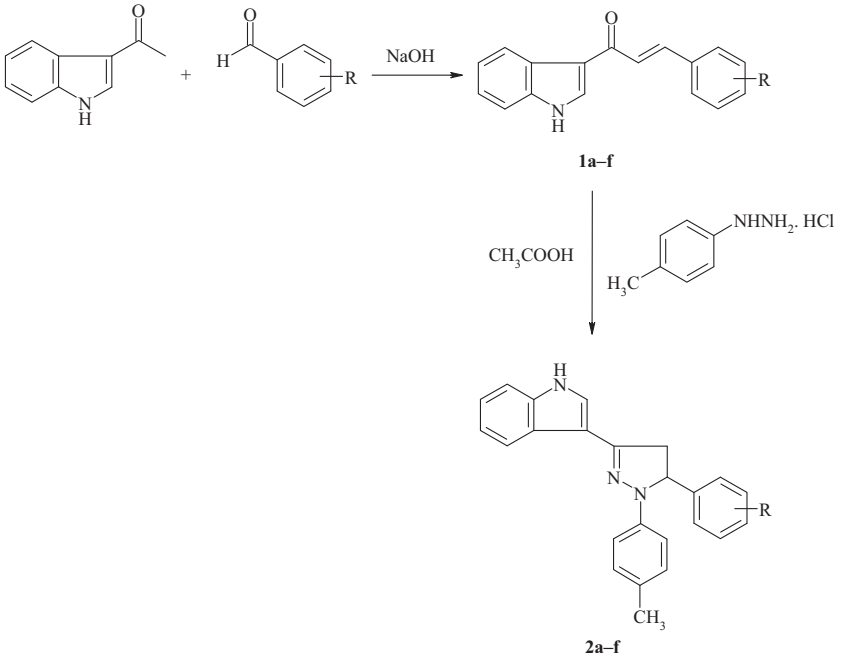
In the IR spectra of compounds **1a–f**, the characteristic band due to the C=O stretching vibration was observed in the region 1704–1640 cm^{-1}. On the other hand, N–H and C=C, C=N stretching bands of all compounds (**1a–2f**) appeared in the regions 3433–3153 cm^{-1} and 1614–1436 cm^{-1}, respectively.

In the ^{1}H NMR spectra of compounds **2a–f**, the three protons attached to the C-4 and C-5 carbon atoms of the 2-pyrazoline unit gave an ABX spin system. Both the chemical shifts and the coupling constant values (*cf*. Experimental) unequivocally prove the 2-pyrazoline structure. The protons belonging to the aromatic ring and the other aliphatic groups were observed with the expected chemical shift and integral values (*cf*. Experimental).

The mass spectra (MS-FAB$^+$) of all compounds showed [M+1] peaks, in agreement with their molecular formula. All compounds gave satisfactory elemental analysis.

The compounds were tested *in vitro* against various pathogenic bacteria and *Candida* species. Pyrazoline derivatives (**2a–f**) were more effective than chalcone derivatives (**1a–f**) against *E. faecalis* (ATCC 29212) and *E. faecalis* (ATCC 51299; Table 2). Among pyrazoline derivatives (**2a–f**), compounds **2b**, **2d**, and **2f** were found to be the most potent derivatives against *E. faecalis* (ATCC 29212). These compounds exhibited the inhibitory activity against *E. faecalis* (ATCC 29212) with a MIC value of 25 μg/mL, whereas chloramphenicol exhibited its inhibitory activity with a MIC value of 12.5 μg/mL. Compound **2d** was also the most effective derivative against *E. faecalis* (ATCC 51299). Compound **2d** showed the inhibitory activity against *E. faecalis* (ATCC 51299) with a MIC value of 12.5 μg/mL, whereas chloramphenicol showed its inhibitory activity with a MIC value of 6.25 μg/mL. This outcome confirms that the position of chloro substituent may have a considerable influence on antibacterial activity against *E. faecalis* (ATCC 29212) and *E. faecalis* (ATCC 51299).

Compounds **1a**, **2a**, **2b**, **2c**, **2d**, and **2e** exhibited the highest antibacterial activity against *K. pneumoniae* (ATCC 13883) with a MIC value of 100 μg/mL. Compound **1a** was the chalcone derivative bearing a 2,3-dichlorophenyl moiety, whereas



Scheme 1. Synthesis of the compounds **1a–2f**.

Table 1. Some properties of compounds **1a–2f**.

Compound	R	Yield (%)	m.p. (°C)	Molecular formula	Molecular weight
1a	2,3-di(Cl)	68	218–220	$C_{17}H_{11}Cl_2NO$	316.18
1b	2,4-di(Cl)	64	267–268	$C_{17}H_{11}Cl_2NO$	316.18
1c	2,6-di(Cl)	69	237–238	$C_{17}H_{11}Cl_2NO$	316.18
1d	3,4-di(Cl)	74	243–244	$C_{17}H_{11}Cl_2NO$	316.18
1e	2,5-di(Cl)	56	274–276	$C_{17}H_{11}Cl_2NO$	316.18
1f	3,5-di(Cl)	72	222–224	$C_{17}H_{11}Cl_2NO$	316.18
2a	2,3-di(Cl)	78	121–122	$C_{24}H_{19}Cl_2N_3$	420.33
2b	2,4-di(Cl)	82	124–125	$C_{24}H_{19}Cl_2N_3$	420.33
2c	2,6-di(Cl)	74	217–218	$C_{24}H_{19}Cl_2N_3$	420.33
2d	3,4-di(Cl)	76	117–118	$C_{24}H_{19}Cl_2N_3$	420.33
2e	2,5-di(Cl)	75	215–216	$C_{24}H_{19}Cl_2N_3$	420.33
2f	3,5-di(Cl)	79	110–111	$C_{24}H_{19}Cl_2N_3$	420.33

Table 2. Antibacterial activities of compounds **1a–2f** as MIC values (μg/mL).

Compound	A	B	C	D	E	F	G	H	I
1a	100	200	200	200	200	200	200	400	800
1b	400	400	400	400	400	400	400	200	100
1c	200	200	200	200	200	200	200	200	100
1d	200	200	200	200	200	200	100	100	100
1e	200	200	200	200	200	100	200	100	100
1f	200	200	200	200	200	200	200	50	100
2a	100	100	200	200	200	100	200	50	50
2b	100	200	200	200	200	100	200	25	25
2c	100	200	200	200	200	200	200	100	25
2d	100	100	200	200	200	200	200	25	12.5
2e	100	100	200	200	200	200	200	50	25
2f	200	100	200	200	200	200	200	25	25
Chloramphenicol	50	6.25	12.5	3.125	6.25	6.25	6.25	12.5	6.25

A: *K. pneumoniae* (ATCC 13883), B: *L. monocytogenes* (ATCC 7644), C: *E. coli* (ATCC 35218), D: *Y. enterocolitica* (clinical isolate, Osmangazi University, Faculty of Medicine, Department of Microbiology, Eskişehir, Turkey), E: *S. typhimurium* (NRRL B-4420), F: *S. aureus* (ATCC 25923), G: *E. coli* (ATCC 25922), H: *E. faecalis* (ATCC 29212), I: *E. faecalis* (ATCC 51299).

compounds **2a**, **2b**, **2c**, **2d**, and **2e** were the pyrazoline derivatives.

Compounds **2d**, **2e**, and **2f** showed their antifungal effects on *C. glabrata* (ATCC 36583) with a MIC value of 100 μg/mL, whilst ketoconazole showed its antifungal effect on *C. glabrata* (ATCC 36583) with a MIC value of 50 μg/mL (Table 3).

Brine-Shrimp toxicity test results were analyzed by the LC_{50} computer program (Trimmed Spearman-Karber Method, Version 1.5) so as to calculate LC_{50} values and 95% confidence intervals [36] (Table 4). According to LC_{50} values of the compounds (**1a–2f**), compounds **1a**, **1b**, **1c**, **1d**, **1e**, and **2e** ($LC_{50} > 1000$ μg/mL) were determined as non-toxic. Compounds **1f**, **2b**, **2c**, **2d**, and **2f** were found to be harmful with LC_{50} values of 178.18, 707.11, 428.71, 101.53, and 297.30 μg/mL, respectively. Compound **2a** ($LC_{50} =$ 56.12 μg/mL) was determined as toxic.

Table 3. Anticandidal activities of compounds **1a–2f** as MIC values (μg/mL).

Compound	A	B	C	D
1a	200	200	200	25
1b	400	200	200	25
1c	200	200	200	25
1d	200	400	200	25
1e	200	200	200	25
1f	200	200	200	25
2a	400	200	200	200
2b	200	200	200	200
2c	200	200	50	200
2d	200	100	50	100
2e	200	100	50	100
2f	200	100	50	100
Ketoconazole	12.5	50	1.56	1.56

A: *C. albicans* (ATCC 90028), B: *C. glabrata* (ATCC 36583), C: *C. krusei* (NRRL Y-7179), D: *C. parapsilosis* (NRRL Y-12696).

Table 4. Brine-Shrimp toxicity results of compounds **1a–2f**.

Compound	LC_{50} (μg/mL)	Lower 95% limit	Upper 95% limit	Toxicity
1a	>1000	–	–	Non-toxic
1b	>1000	–	–	Non-toxic
1c	>1000	–	–	Non-toxic
1d	>1000	–	–	Non-toxic
1e	>1000	–	–	Non-toxic
1f	178.18	77.96	407.25	Harmful
2a	56.12	25.55	123.28	Toxic
2b	707.11	176.78	2828.43	Harmful
2c	428.71	179.18	1025.72	Harmful
2d	101.53	57.97	177.81	Harmful
2e	>1000	–	–	Non-toxic
2f	297.30	127.66	692.38	Harmful

Conclusion

In the present paper, we synthesized 1-(1*H*-indol-3-yl)-3-aryl-2-propen-1-ones (**1a–f**) and 1-(*p*-methylphenyl)-3,5-diaryl-2-pyrazolines (**2a–f**), which were tested for their antimicrobial activity against pathogenic bacteria and *Candida* species. Brine-Shrimp lethality assay was carried out to determine the toxicity of the compounds. Among these compounds, compound **2e** can be identified as the most promising agent against *K. pneumoniae* (ATCC 13883) and *C. glabrata* (ATCC 36583) due to its inhibitory effects on *K. pneumoniae* and *C. glabrata* with a MIC value of 100 μg/mL as a non-toxic agent (LC_{50} > 1000 μg/mL). In the view of this study, further research can be carried out on the development of new effective antimicrobial agents bearing the pyrazoline moiety by the modification of compound **2e**.

Experimental

Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points were determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and were uncorrected. IR spectra were recorded on a Shimadzu 8400 FT-IR spectrophotometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer (Bruker, Billerica, USA). Mass spectra were recorded on a VG Quattro mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyzer (Perkin-Elmer, Norwalk, USA). The TLC was performed on Kieselgel 60 F_{254} (Merck) layer using toluene/ethyl acetate (4:1 v/v) as eluents.

General procedure for the synthesis of the compounds *1-(1H-Indol-3-yl)-3-aryl-2-propen-1-one derivatives (**1a–f**)*

A mixture of 3-acetylindole (0.04 mol), aromatic aldehyde (0.04 mol) and 10% aqueous sodium hydroxide (10 mL) in ethanol (50 mL) was stirred at room temperature for 16 h. The resulting solid was washed, dried, and crystallized from ethanol [37].

*1-(p-Methylphenyl)-3-(1H-indol-3-yl)-5-(disubstituted-phenyl)-2-pyrazoline derivatives (**2a–f**)*

A mixture of 1-(1*H*-indol-3-yl)-3-aryl-2-propen-1-one derivatives (**1a–f**) (10.0 mmol) and *p*-methylphenylhydrazine hydrochloride (30.0 mmol) in the presence of acetic acid (50 mL) was refluxed for 6 h, then poured onto crushed ice. The precipitate was separated by filtration, washed with water and crystallized from methanol to afford 2-pyrazolines [10].

*1-(1H-Indol-3-yl)-3-(2,3-dichlorophenyl)-2-propen-1-one (**1a**)*

IR (KBr) ν_{max} (cm^{-1}): 3421 (N–H), 1699 (C=O), 1521, 1444 (C=C), 1242, 1153, 1099 (C–N), 746 (1,2,3-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.19–7.23 (2H, m), 7.36–7.46 (1H, m), 7.54–7.57 (1H, m), 7.57–7.62 (2H, d, J = 8.02 Hz), 7.66–7.71 (2H, d, J = 8.03 Hz), 8.34–8.40 (1H, m), 8.53 (1H, s), 11.92 (1H, brs, NH).

For $C_{17}H_{11}Cl_2NO$, calculated: C, 64.58; H, 3.51; N, 4.43; found: C, 64.65; H, 3.55; N, 4.39.

MS (FAB) $[M+1]^+$: m/z 317.

*1-(1H-Indol-3-yl)-3-(2,4-dichlorophenyl)-2-propen-1-one (**1b**)*

IR (KBr) ν_{max} (cm^{-1}): 3176 (N–H), 1640 (C=O), 1515, 1506, 1471 (C=C), 1240, 1157 (C–N), 750 (1,2,4-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.25–7.28 (2H, m), 7.52–7.56 (2H, m), 7.71–7.72 (1H, d, J = 2.04 Hz), 7.93–7.92 (2H, m), 8.22–8.25 (1H, d, J = 8.83 Hz), 8.35–8.37 (1H, m), 8.79 (1H, s), 12.20 (1H, s, NH).

For $C_{17}H_{11}Cl_2NO$, calculated: C, 64.58; H, 3.51; N, 4.43; found: C, 64.62; H, 3.56; N, 4.41.

MS (FAB) $[M+1]^+$: m/z 317.

*1-(1H-Indol-3-yl)-3-(2,6-dichlorophenyl)-2-propen-1-one (**1c**)*

IR (KBr) ν_{max} (cm^{-1}): 3245 (N–H), 1704 (C=O), 1593, 1442 (C=C), 1242, 1157 (C–N), 736 (1,2,3-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.26–7.29 (2H, m), 7.38–7.44 (1H, t, J = 8.41 Hz), 7.52–7.56 (1H, m), 7.57–7.62 (2H, d, J = 8.02 Hz), 7.63–7.73 (2H, d, J = 8.80 Hz), 8.34–8.40 (1H, m), 8.60 (1H, s), 12.18 (1H, br, NH).

For $C_{17}H_{11}Cl_2NO$, calculated: C, 64.58; H, 3.51; N, 4.43; found: C, 64.59; H, 3.56; N, 4.42.

MS (FAB) $[M+1]^+$: m/z 317.

*1-(1H-Indol-3-yl)-3-(3,4-dichlorophenyl)-2-propen-1-one (**1d**)*

IR (KBr) ν_{max} (cm^{-1}): 3153 (N–H), 1643 (C=O), 1571, 1517, 1458 (C=C), 1157 (C–N), 752 (1,2,4-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.22–7.26 (2H, m), 7.51–7.62 (2H, m), 7.70–7.72 (1H, d, J = 8.80 Hz), 7.82–7.85 (1H, m), 7.91–7.95 (1H, d, J = 15.61 Hz), 8.23–8.24 (1H, m), 8.32–8.34 (1H, m), 8.78 (1H, s), 12.16 (1H, brs, NH).

For $C_{17}H_{11}Cl_2NO$, calculated: C, 64.58; H, 3.51; N, 4.43; found: C, 64.63; H, 3.56; N, 4.40.

MS (FAB) $[M+1]^+$: m/z 317.

*1-(1H-Indol-3-yl)-3-(2,5-dichlorophenyl)-2-propen-1-one (**1e**)*

IR (KBr) ν_{max} (cm^{-1}): 3429 (N–H), 1649 (C=O), 1624, 1442 (C=C), 1155, 1097 (C–N), 744 (1,2,4-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.23–7.29 (2H, m), 7.46–7.53 (2H, m), 7.60–7.61 (1H, d, J = 5.02 Hz), 7.87–7.90 (1H, d, J = 15.59 Hz), 7.97–8.00 (1H, d, J = 15.58 Hz), 8.32–8.34 (2H, m), 8.84 (1H, s), 12.23 (1H, brs, NH).

For $C_{17}H_{11}Cl_2NO$, calculated: C, 64.58; H, 3.51; N, 4.43; found: C, 64.54; H, 3.50; N, 4.45.

MS (FAB) $[M+1]^+$: m/z 317.

*1-(1H-Indol-3-yl)-3-(3,5-dichlorophenyl)-2-propen-1-one (**1f**)*

IR (KBr) ν_{max} (cm^{-1}): 3172 (N–H), 1689 (C=O), 1554, 1452 (C=C), 1153 (C–N), 746 (1,3,5-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.18–7.27 (2H, m), 7.51–7.65 (4H, m), 7.96–8.01 (2H, m), 8.31–8.33 (1H, m), 8.82 (1H, s), 12.20 (1H, s, NH).

For $C_{17}H_{11}Cl_2NO$, calculated: C, 64.58; H, 3.51; N, 4.43; found: C, 64.55; H, 3.43; N, 4.47.

MS (FAB) $[M+1]^+$: m/z 317.

*1-(p-Methylphenyl)-3-(1H-indol-3-yl)-5-(2,3-dichlorophenyl)-2-pyrazoline (**2a**)*

IR (KBr) ν_{max} (cm^{-1}): 3404 (N–H), 3062 (aromatic C–H), 2918 (aliphatic C–H), 1616, 1515, 1463 (C=N and C=C), 1245, 1099 (C–N), 746 (1,2,3-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.20 (3H, s, CH_3), 3.18 (1H, dd, J_{AM} = 16.87 Hz, J_{AX} = 5.58 Hz, C_4–H_A pyrazoline), 4.06 (1H, dd, J_{MA} = 16.88 Hz, J_{MX} = 11.57 Hz, C_4–H_M pyrazoline), 5.61 (1H, dd, J_{MX} = 11.59 Hz, J_{AX} = 5.59 Hz, C_5–H_X pyrazoline), 6.83 (2H, d, J = 8.81 Hz, phenyl $C_{2,6}$–H), 6.91 (2H, d, J = 8.83 Hz, phenyl $C_{3,5}$–H), 7.04 (1H, m, indole C_6–H), 7.21–7.58 (5H, m, phenyl and indole protons), 7.71 (1H, d, J = 2.53 Hz, indole C_4–H), 8.31–8.34 (1H, m, phenyl or indole proton), 11.53 (1H, s, NH).

For $C_{24}H_{19}Cl_2N_3$, calculated: C, 68.58; H, 4.56; N, 10.00; found: C, 68.54; H, 4.54; N, 9.96.

MS (FAB) $[M + 1]^+$: m/z 421.

*1-(p-Methylphenyl)-3-(1H-indol-3-yl)-5-(2,4-dichlorophenyl)-2-pyrazoline (**2b**)*

IR (KBr) ν_{max} (cm^{-1}): 3404 (N–H), 3031 (aromatic C–H), 2918 (aliphatic C–H), 1614, 1514, 1456, 1436 (C=N and C=C), 1245, 1099 (C–N), 808, 746 (1,2,4-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.13 (3H, s, CH_3), 3.05 (1H, dd, J_{AM} = 16.80 Hz, J_{AX} = 5.60 Hz, C_4–H_A pyrazoline), 3.96 (1H, dd, J_{MA} = 16.80 Hz, J_{MX} = 11.60 Hz, C_4–H_M pyrazoline), 5.43 (1H, dd, J_{MX} = 11.60 Hz, J_{AX} = 6.00 Hz, C_5–H_X pyrazoline), 6.80 (2H, d, J = 8.40 Hz, phenyl $C_{2,6}$–H), 6.96 (2H, d, J = 8.80 Hz, phenyl $C_{3,5}$–H), 7.07 (1H, d, J = 8.40 Hz, indol C_6–H), 7.16–7.48 (5H, m, phenyl and indole protons), 7.64 (1H, d, J = 2.42 Hz, indole C_4–H), 8.28–8.30 (1H, m, phenyl C_3–H), 11.45 (1H, s, NH).

For $C_{24}H_{19}Cl_2N_3$, calculated: C, 68.58; H, 4.56; N, 10.00; found: C, 68.54; H, 4.52; N, 9.95.

MS (FAB) $[M+1]^+$: m/z 421.

*1-(p-Methylphenyl)-3-(1H-indol-3-yl)-5-(2,6-dichlorophenyl)-2-pyrazoline (**2c**)*

IR (KBr) ν_{max} (cm^{-1}): 3433 (N–H), 1623, 1616, 1517, 1463 (C=N and C=C), 1245, 1126 (C–N), 786, 730 (1,2,3-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.15 (3H, s, CH_3), 3.29 (1H, dd, J_{AM} = 16.73 Hz, J_{AX} = 5.78 Hz, C_4–H_A pyrazoline), 3.93 (1H, dd, J_{MA} = 16.73 Hz, J_{MX} = 11.58 Hz, C_4–H_M pyrazoline), 5.83 (1H, dd, J_{MX} = 11.58 Hz, J_{AX} = 5.80 Hz, C_5–H_X pyrazoline), 6.78 (2H, d, J = 8.76 Hz, phenyl $C_{2,6}$–H), 6.95 (2H, d, J = 8.78 Hz, phenyl $C_{3,5}$–H), 7.16–7.46 (5H, m, phenyl and indole protons), 7.54–7.60 (1H, m, indole C_2–H), 7.63 (1H, d, J = 5.03 Hz, indole C_4–H), 8.26–8.28 (1H, m, phenyl or indole proton), 11.44 (1H, s, NH).

For $C_{24}H_{19}Cl_2N_3$, calculated: C, 68.58; H, 4.56; N, 10.00; found: C, 68.55; H, 4.57; N, 9.96.

MS (FAB) $[M + 1]^+$: m/z 421.

*1-(p-Methylphenyl)-3-(1H-indol-3-yl)-5-(3,4-dichlorophenyl)-2-pyrazoline (**2d**)*

IR (KBr) ν_{max} (cm^{-1}): 3396 (N–H), 3056 (aromatic C–H), 2916 (aliphatic C–H), 1622, 1616, 1515, 1456 (C=N and C=C), 1245, 1029 (C–N), 819 (1,2,4-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.17 (3H, s, CH_3), 3.22 (1H, dd, J_{AM} = 16.77 Hz, J_{AX} = 5.71 Hz, C_4–H_A pyrazoline), 3.89 (1H, dd, J_{MA} = 16.72 Hz, J_{MX} = 11.53 Hz, C_4–H_M pyrazoline), 5.91 (1H, dd, J_{MX} = 11.50 Hz, J_{AX} = 5.78 Hz, C_5–H_X pyrazoline), 6.76 (2H, d, J = 8.70 Hz, phenyl $C_{2,6}$–H), 6.98 (2H, d, J = 8.74 Hz, phenyl $C_{3,5}$–H), 7.19–7.44 (5H, m, phenyl and indole protons), 7.56–7.65 (1H, m, indole C_2–H), 7.69 (1H, d, J = 5.30 Hz, indole C_4–H), 8.27–8.29 (1H, m, phenyl or indole proton), 11.51 (1H, s, NH).

For $C_{24}H_{19}Cl_2N_3$, calculated: C, 68.58; H, 4.56; N, 10.00; found: C, 68.57; H, 4.55; N, 9.98.

MS (FAB) $[M+1]^+$: m/z 421.

*1-(p-Methylphenyl)-3-(1H-indol-3-yl)-5-(2,5-dichlorophenyl)-2-pyrazoline (**2e**)*

IR (KBr) ν_{max} (cm^{-1}): 3415 (N–H), 3029 (aromatic C–H), 2914 (aliphatic C–H), 1616, 1558, 1506, 1456 (C=N and C=C), 1245, 1087 (C–N), 810 (1,2,4-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.15 (3H, s, CH_3), 3.28 (1H, dd, J_{AM} = 16.77 Hz, J_{AX} = 5.68 Hz, C_4–H_A pyrazoline), 3.94 (1H, dd, J_{MA} = 16.79 Hz, J_{MX} = 11.56 Hz, C_4–H_M pyrazoline), 5.44 (1H, dd, J_{MX} = 11.58 Hz, J_{AX} = 5.65 Hz, C_5–H_X pyrazoline), 7.01 (2H, d, J = 8.73 Hz, phenyl $C_{2,6}$–H), 7.24 (2H, d, J = 8.70 Hz, phenyl $C_{3,5}$–H), 7.31–7.61 (5H, m, phenyl and indole protons), 7.70 (1H, m, indole C_2–H), 7.97 (1H, m, indole C_4–H), 8.29–8.35 (1H, m, phenyl or indole proton), 11.55 (1H, s, NH).

For $C_{24}H_{19}Cl_2N_3$, calculated: C, 68.58; H, 4.56; N, 10.00; found: C, 68.61; H, 4.58; N, 10.04.

MS (FAB) $[M+1]^+$: m/z 421.

*1-(p-Methylphenyl)-3-(1H-indol-3-yl)-5-(3,5-dichlorophenyl)-2-pyrazoline (**2f**)*

IR (KBr) ν_{max} (cm^{-1}): 3404 (N–H), 3060 (aromatic C–H), 2918 (aliphatic C–H), 1614, 1568, 1514, 1436 (C=N and C=C), 1245, 1099 (C–N), 796, 746 (1,3,5-trisubstituted benzene).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.19 (3H, s, CH_3), 3.20 (1H, dd, J_{AM} = 16.77 Hz, J_{AX} = 5.87 Hz, C_4–H_A pyrazoline), 3.90 (1H, dd, J_{MA} = 16.79 Hz, J_{MX} = 11.57 Hz, C_4–H_M pyrazoline), 5.37

(1H, dd, $J_{MX} = 11.56$ Hz, $J_{AX} = 5.86$ Hz, C_5–H_X pyrazoline), 6.93 (2H, d, $J = 8.79$ Hz, phenyl $C_{2,6}$–H), 7.03 (2H, d, $J = 8.79$ Hz, phenyl $C_{3,5}$–H), 7.22–7.34 (4H, m, phenyl and indole protons), 7.44–7.48 (1H, m, phenyl C_4–H), 7.51–7.53 (1H, m, indole C_2–H), 7.66 (1H, d, $J = 2.44$ Hz, indole C_4–H), 8.29–8.31 (1H, m, phenyl or indole proton), 11.49 (1H, brs, NH).

For $C_{24}H_{19}Cl_2N_3$, calculated: C, 68.58; H, 4.56; N, 10.00; found: C, 68.54; H, 4.52; N, 10.05.

MS (FAB) $[M+1]^+$: *m/z* 421.

Microbiology

The study was designed to compare MICs obtained by the CLSI reference M7-A7 broth microdilution method as described in the previous study [38]. MIC readings were performed twice for each chemical agent. For both the antibacterial and antifungal assays, the compounds were dissolved in DMSO. Further dilutions of the compounds and standard drugs in test medium were prepared at the required quantities of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 μg/mL concentrations with Mueller–Hinton broth and Sabouraud dextrose broth. In order to ensure that the solvent *per se* had no effect on bacteria or yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium.

Final products were tested for their *in vitro* growth inhibitory activity against *K. pneumoniae* (ATCC 13883), *L. monocytogenes* (ATCC 7644), *E. coli* (ATCC 35218), *Y. enterocolitica* (clinical isolate, Osmangazi University, Faculty of Medicine, Department of Microbiology, Eskişehir, Turkey), *S. typhimurium* (NRRL B-4420), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *E. faecalis* (ATCC 29212), *E. faecalis* (ATCC 51299), *C. albicans* (ATCC 90028), *C. glabrata* (ATCC 36583), *C. krusei* (NRRL Y-7179), *C. parapsilosis* (NRRL Y-12696).

Antimicrobial assay

The cultures were obtained from Mueller-Hinton broth (Difco) for the bacterial strains after overnight incubation at 35 ± 1°C. The yeasts were maintained in Sabouraud dextrose broth (Difco) after overnight incubation at 35 ± 1°C. The inocula of test microorganisms were adjusted to match the turbidity of a MacFarland 0.5 standard tube as determined with a spectrophotometer and the final inoculum size was 0.5–2.5 × 10^5 CFU/mL for antibacterial and antifungal assays. Testing was carried out in Mueller-Hinton broth and Sabouraud dextrose broth (Difco) at pH 7 and the twofold serial dilutions technique was applied. The last well on the microplates containing only inoculated broth was kept as control and the last well with no growth of microorganism was recorded to represent the MIC expressed in μg/mL. Each experiment in the antimicrobial assays was replicated twice in order to define the MIC values. Chloramphenicol and ketoconazole were used as control drugs.

Brine-Shrimp lethality assay

Brine-Shrimp toxicity assay was used for determination of toxicity levels of the synthesized compounds (**1a–2f**). Each test compound was dissolved in DMSO to obtain the stock concentration of 1000 mg/mL and then the stock solution was diluted to various concentrations (1000–7.8125 mg/mL). In order to prevent the toxicity results from possible false effects originated from DMSO's toxicity, stock solutions of the compounds (**1a–2f**) were prepared according to suggested volume range by dissolving 1 mg of test compound in 10 μL DMSO and completing to 1000 mL with artificial seawater [39]. Pure DMSO was used as a positive control for the toxicity assay. The eggs of Brine-Shrimp hatched in a conical flask containing 300 mL artificial seawater made by dissolving a commercial marine salt in deionized water. The flasks were well aerated with the aid of an air pump, and kept in a water bath at 25–30°C. The larvae hatched within 48 h. Ten larvae were transferred with pipetter into each vial containing test compound and artificial seawater. A check count was performed after 24 h of exposure at room temperature and the number of dead larvae, exhibiting no internal or external movement during several seconds of observation, was noted. Three independent experiments were performed for each concentration of compounds **1a–2f**.

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References

[1] M. A. Ghannoum, L. B. Rice, *Clin. Microbiol. Rev.* **1999**, *12* (*4*), 501–517.

[2] R. C. Moellering, *Int. J. Antimicrob. Agents* **2011**, *37*, 2–9.

[3] M. K. Kathiravan, A. B. Salake, A. S. Chothe, P. B. Dudhe, R. P. Watode, M. S. Mukta, S. Gadhwe, *Bioorg. Med. Chem.* **2012**, *20*, 5678–5698.

[4] M. A. Pfaller, D. J. Diekema, *Clin. Microbiol. Rev.* **2007**, *20* (*1*), 133–163.

[5] M. M. Canuto, F. G. Rodero, *Lancet Infect. Dis.* **2002**, *2*, 550–563.

[6] Z. Nowakowska, *Eur. J. Med. Chem.* **2007**, *42*, 125–137.

[7] A. Lévai, *ARKIVOC* **2004**, *7*, 15–33.

[8] Z. N. Siddiqui, M. Asad, S. Praveen, *Med. Chem. Res.* **2008**, *17*, 318–325.

[9] A. Lévai, J. Jekö, D. I. Brahmbhatt, *J. Heterocyclic Chem.* **2005**, *42*, 1231–1235.

[10] A. Lévai, J. Jekö, *J. Heterocyclic Chem.* **2006**, *43*, 111–115.

[11] B. Insuasty, A. Tigreros, F. Orozco, J. Quiroga, Rodrigo. Abonía, M. Nogueras, A. Sanchez, J. Cobo, *Bioorg. Med. Chem.* **2010**, *18*, 4965–4974.

[12] S. Kumar, S. Bawa, S. Drabu, R. Kumar, H. Gupta, *Drug Discov.* **2009**, *4*, 154–163.

[13] A. Rahman, A. A. Siddiqui, *Int. J. Pharm. Sci. Drug Res.* **2010**, *2*, 165–175.

[14] P. M. Sivakumar, S. Ganesan, P. Veluchamy, M. Doble, *Chem. Biol. Drug Des.* **2010**, *76*, 407–411.

[15] S. A. F. Rostom, M. H. Badr, H. A. Abd El Razik, H. M. A. Ashour, A. E. Abdel Wahab, *Arch. Pharm. Chem. Life Sci.* **2011**, *344*, 572–587.

[16] M. M. M. Ramiz, W. A. El-Sayed, A. I. El-Tantawy, A. A.-H. Abdel-Rahman, *Arch. Pharm. Res.* **2010**, *33* (*5*), 647–654.

[17] P. M. Sivakumar, S. Prabhu Seenivasan, V. Kumar, M. Doble, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3169–3172.

[18] P. K. Sharma, S. Kumar, P. Kumar, P. Kaushik, D. Kaushik, Y. Dhingra, K. R. Aneja, *Eur. J. Med. Chem.* **2010**, *45*, 2650–2655.

[19] V. Sharma, P. Kumar, D. Pathak, *J. Heterocyclic Chem.* **2010**, *47*, 491–502.

[20] S. Biswal, U. Sahoo, S. Sethy, H. K. S. Kumar, M. Banerjee, *Asian J. Pharm. Clin. Res.* **2012**, *5* (*1*), 1–6.

[21] R. B. Van Order, H. G. Lindwall, *Chem. Rev.* **1942**, *30* (*1*), 69–96.

[22] T. Eicher, S. Hauptmann, *The Chemistry of Heterocycles*, Wiley-VCH, Weinheim **2003**, chapter 5.

[23] G. P. Laddha, G. Vidyasagar, S. R. Bavaskar, S. B. Baile, S. S. Suralkar, *Der Pharmacia Lettre* **2012**, *4* (*2*), 443–455.

[24] A. M. Polter, X. Li, *Front Mol Neurosci.* **2011**, *4*, 1–14.

[25] J. G. Hurdle, A. J. O'Neill, I. Chopra, *J. Antimicrob. Chemother.* **2004**, *54*, 549–552.

[26] A. Hasuoka, Y. Nakayama, M. Adachi, H. Kamiguchi, K. Kamiyama, *Chem. Pharm. Bull.* **2001**, *49* (*12*), 1604–1608.

[27] D. Bhambi, V. K. Salvi, J. L. Jat, S. Ojha, G. L. Talesara, *J. Sulfur Chem.* **2007**, *28* (*2*), 155–163.

[28] V. N. Pathak, R. Gupta, N. Gupta, *Indian J. Chem.* **2008**, *47B*, 1303–1307.

[29] S. K. Sharma, P. Kumar, B. Narasimhan, K. Ramasamy, V. Mani, R. Kumar Mishra, A. B. Abdul Majeed, *Eur. J. Med. Chem.* **2012**, *48*, 16–25.

[30] S. M. Gomha, S. M. Riyadh, *Molecules* **2011**, *16*, 8244–8256.

[31] G. Turan-Zitouni, A. Özdemir, Z. A. Kaplancıklı, P. Chevallet, Y. Tunalı, *Phosphorus Sulfur Silicon Relat. Elem.* **2005**, *180* (*12*), 2717–2724.

[32] G. Turan-Zitouni, A. Özdemir, K. Güven, *Arch. Pharm. Chem. Life Sci.* **2005**, *338*, 96–104.

[33] Z. A. Kaplancıklı, G. Turan-Zitouni, A. Özdemir, G. Revial, K. Güven, *Phosphorus Sulfur Silicon Relat. Elem.* **2007**, *182* (*4*), 749–764.

[34] A. Özdemir, G. Turan-Zitouni, Z. A. Kaplancıklı, G. Revial, K. Güven, *Eur. J. Med. Chem.* **2007**, *42* (*3*), 403–409.

[35] A. Özdemir, G. Turan-Zitouni, Z. A. Kaplancıklı, G. Revial, F. Demirci, G. I˙şcan, *J. Enzyme Inhib. Med. Chem.* **2010**, *25* (*4*), 565–571.

[36] B. Ballantyne, T. C. Marrs, T. Syversen, *General and Applied Toxicology*, 2nd edition, Vol. *1*, Macmillan Reference Ltd, London, UK **2000**, p. 52.

[37] A. A. Khalaf, R. A. Kabli, M. T. Zimaity, A. M. Khalil, A. M. Kaddah, H. A. Al-Rifaie, *Indian J. Chem.* **1993**, *32B*, 1125–1129.

[38] A. Özdemir, G. Turan-Zitouni, Z. A. Kaplancıklı, Y. Tunali, *J. Enzym. Inhib. Med. Chem.* **2009**, *24*, 825–831.

[39] M. I. Choudhary, W. J. Thomsen, *Bioassay Techniques for Drug Development*, Harwood Academic Publishers, Amsterdam **2001**, pp. 9–10.

