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Synthesis and characterization of heterocyclic compounds incorporating 2-pyrazoline and 1,3-thiazol-4-one moieties with antimicrobial significance

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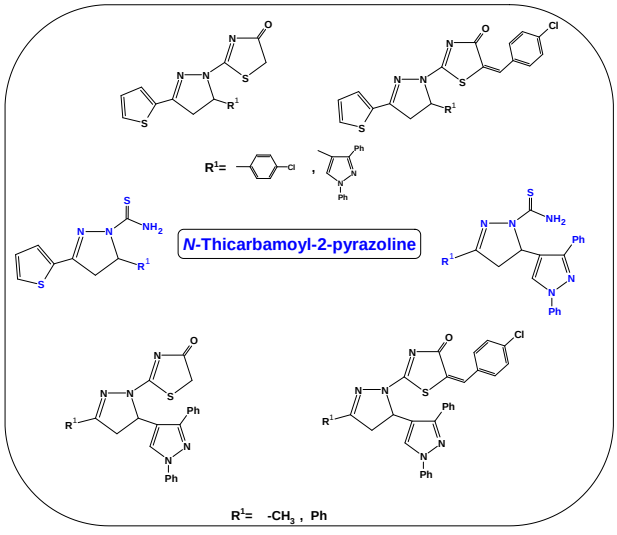
Abstract

The current study describes the synthesis of heterocycles incorporating 2-pyrazoline and 1,3-thiazol-4-one moieties. The cycloaddition of thiosemicarbazide with diverse chalcones afforded *N*-thiocarbamoyl pyrazolines, which then reacted with chloroacetic acid to yield 2-pyrazolinyl-1,3-thiazol-4(5*H*)-ones. The active methylene group at position 5 of the synthesized thiazolones increases its reactivity with aromatic aldehydes, forming the arylidene derivatives. The chemical structures of these compounds were determined through spectral and elemental analyses. Additionally, the compounds were assessed for the antibacterial effectiveness against both *Gram*-positive and *Gram*-negative bacteria, as well as against specific species of fungi.



Keywords: Thiosemicarbazide, 2-pyrazolines, thiazol-4(5*H*)-one, thiophene, biological activity.

Introduction

Thiosemicarbazide is an essential precursor in organic chemistry, particularly in the synthesis of heterocyclic compounds. [1-12] Thiosemicarbazides are polyfunctional compounds exhibiting nucleophilic characteristics. This nucleophilic tendency has facilitated the synthesis of various heterocycles, including imidazole, [11, 13, 14] oxadiazole, [15–20] thiadiazole, [19, 20, 21] triazole, [17, 22, 23] and triazine. [23–27]

The interaction between thiosemicarbazide and chalcone is noteworthy, leading to the synthesis of a 2-pyrazoline moiety. [28-33] 2-Pyrazoline analogues play an essential role in organic and pharmaceutical chemistry. They have extensive use in the treatment of leukemia, [34] and as antitumor, [35] and anticancer agents. [36] Research findings indicated that 1-thiocarbamoyl-2-pyrazoline analogs **Aa-d**, **B,** and **C** exhibited significant biological efficacy. [37] They effectively scavenge DPPH and hydroxyl radicals, and compounds **B** and also **C** have exceptional antibacterial action (Figure 1).

Conversely, 1,3-thiazol-4-one has been actively researched for over a century owing to its remarkable pharmacological properties. 1,3-Thiazol-4-one exhibits a wide array of synthesized organic compounds having biological activity, encompassing anticancer, [38] and antibacterial properties. [29, 39, 40] Among the most important analogs of 1,3-thiazol-4-one is 2-(5-aryl-4,5-dihydropyrazol-1-yl)thiazol-4-ones **D** and **E** which function as inhibitors of the epidermal growth factor receptor (EGFR), [41] a transmembrane protein that acts as a receptor for epidermal growth factor members.

Informed by the previously mentioned findings and the ongoing attempts in the design of biologically active heterocycles derived from 2-pyrazoline and thiazole nuclei, [29, 42-46] we investigated the potential of 2-pyrazoline and 1,3-thiazol-4-one as essential scaffolds for the development of potent antimicrobial heterocycles (Figure 2).

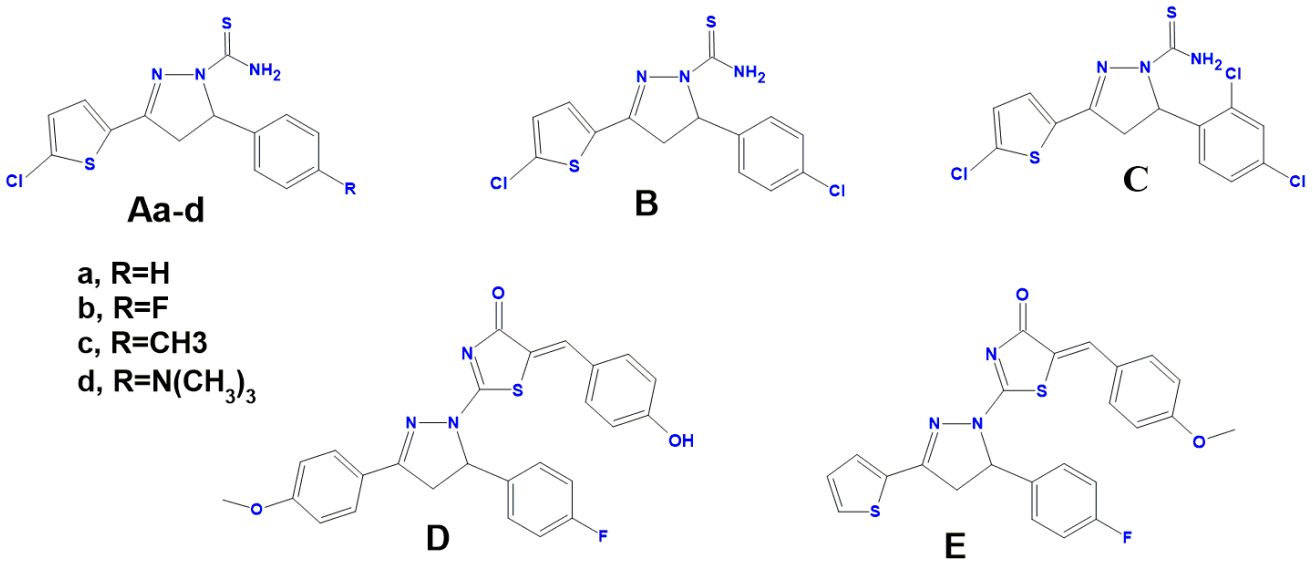


Figure 1. Some of the published 2-pyrazoline and 1,3-thiazol-4-one analogs with biological interest.

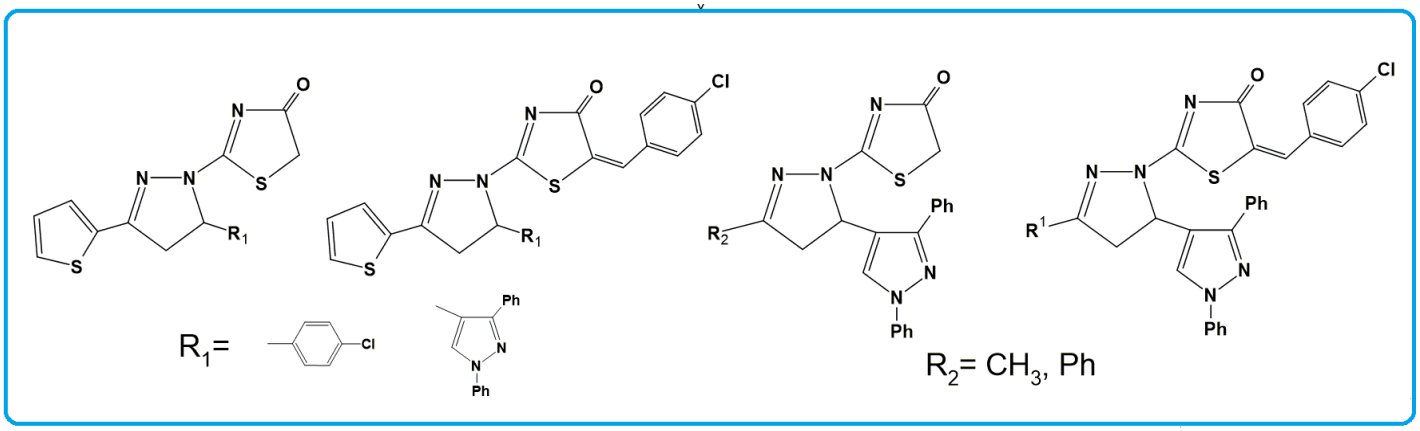
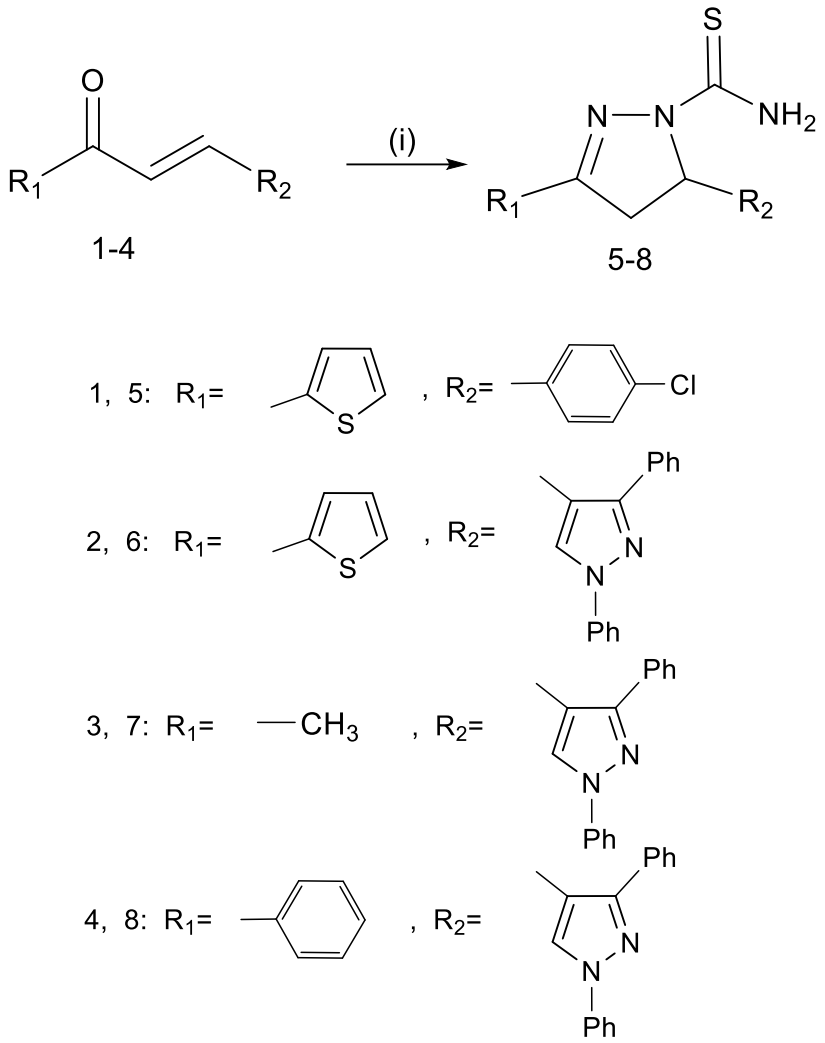


Figure 2. The novel synthesized compounds.

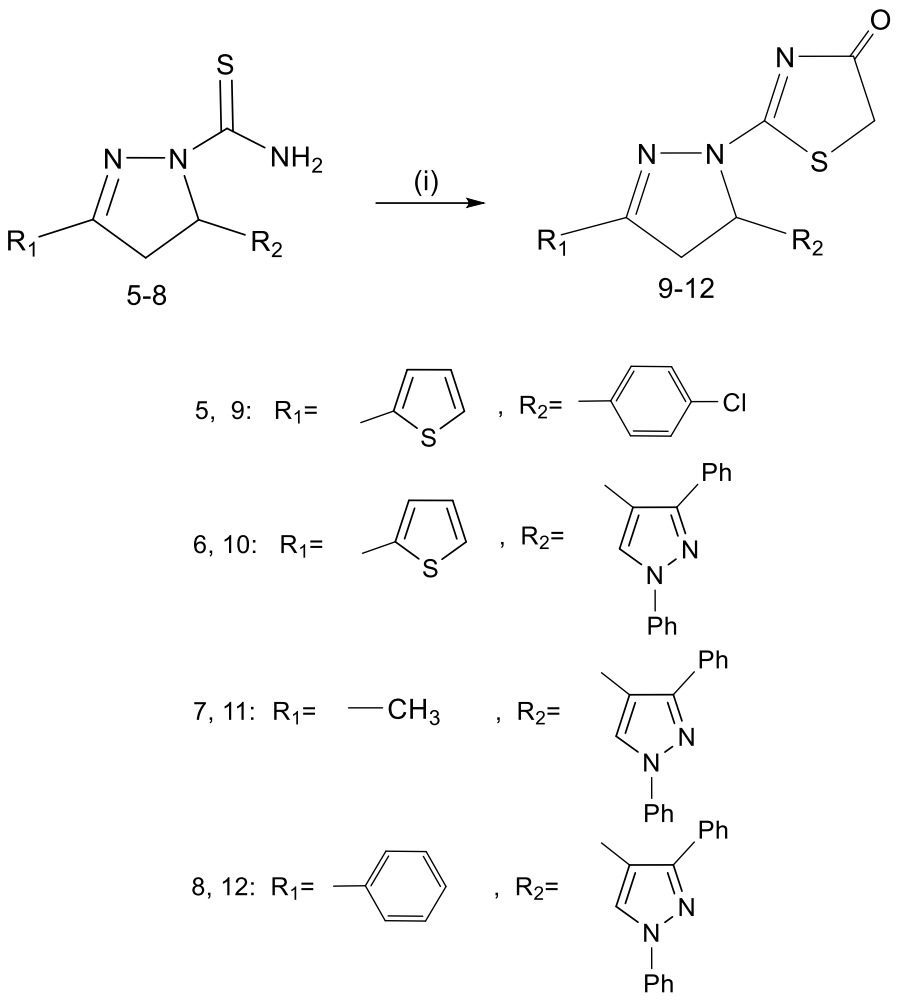
Results and Discussion

The 2-propen-1-one derivatives have a ketoethylenic group, -CO-CH=CH-, which provides significant reactivity with thiosemicarbazide, a polyfunctional reagent. The chalcones **1-4** [47-49] were treated with thiosemicarbazide in an alkaline ethanol setting, resulting in the formation of *N*-thiocarbamoyl 2-pyrazolines **5-8**, respectively (Schemes 1). The structures of these compounds were confirmed using spectral analyses (FTIR, NMR, and mass spectrometry). The Fourier Transform Infrared (FTIR) spectra revealed the disappearance of absorbance peaks associated with carbonyl groups in the chalcones and the appearance of new peaks for NH_2 functional groups. The NMR spectra validated the formation of 2-pyrazoline rings and the existence of NH_2 groups. The ^{1}H-NMR spectrum of *N*-thiocarbamoyl-3-(thien-2-yl)-5-(4-chlorophenyl)-2-pyrazoline (**5**) exhibited a singlet signal for the amino group at δ 9.10 ppm, with aromatic protons signals at δ 7.19-7.60 ppm. The presence of thiocarbonyl carbon was validated by a signal in the ^{13}C NMR spectrum at δ 176.2 ppm. In addition, two signals were observed at δ 48.0 ppm of pyrazoline-CH_2, and δ 66.5 ppm of pyrazoline-CH.



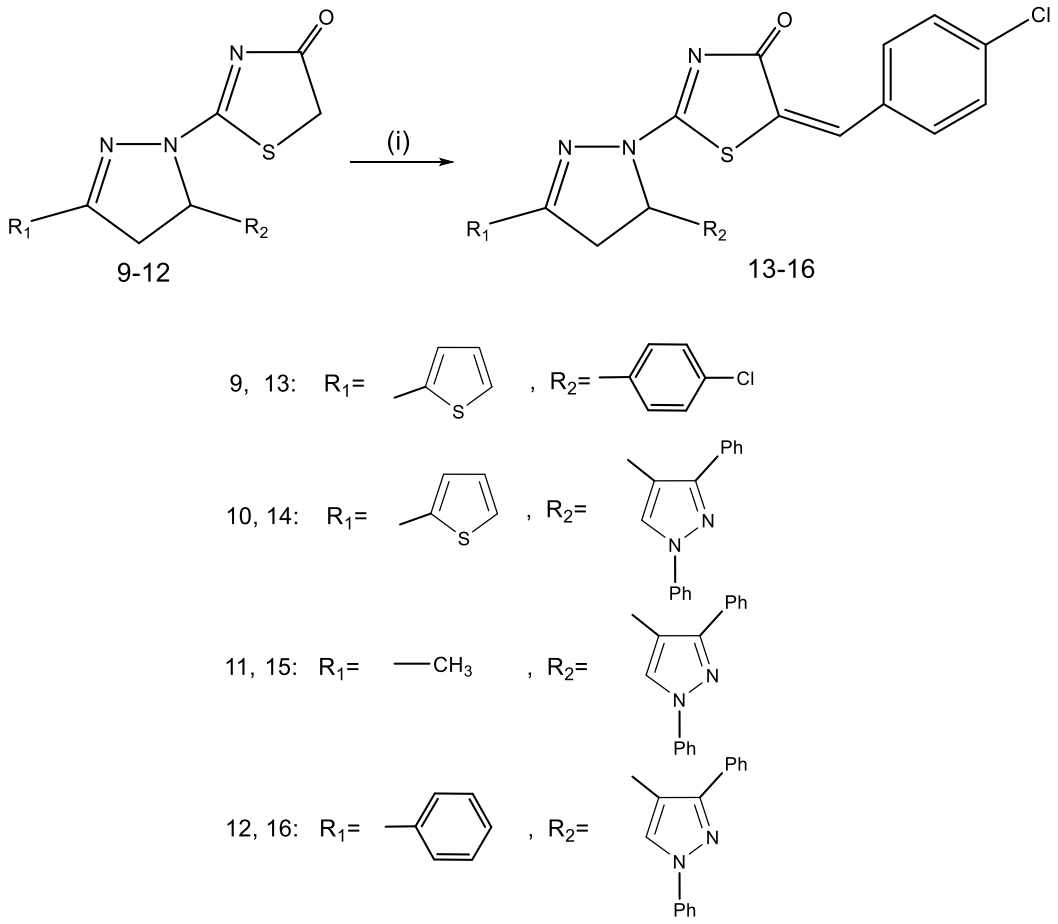
Scheme 1. The synthesis of *N*-thiocarbamoyl-2-pyrazolines (**5-8**): (i) $NH_2NHCSNH_2$, NaOH, 10 h.

1,3-Thiazol-4(5*H*)-ones **9-12** were obtained by heating *N*-thicarbamoyl-2-pyrazolines **5-8** with chloroacetic acid in glacial acetic acid containing anhydrous sodium acetate (Schemes 2). The FTIR spectra of the later compounds demonstrated the absence of thiocarbonyl and amino functional groups. Furthermore, the presence of absorption bands in the region of *v* 1690 to 1704 cm^{-1} indicated the carbonyl groups of thiazole-4(5*H*)-one rings. The ^1H- and ^{13}C-NMR spectra align with the FTIR spectra about the development of a novel thiazo-4(5*H*)-one ring. The FTIR spectrum of 2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(thien-2-yl)-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (**10**) exhibited a peak at *v* 1662 cm^{-1}, indicating the presence of the thiazole-CO functional group. The ^1H-NMR spectrum showed a singlet signal at δ 4.09 ppm, which due to the two methylene protons of the thiazolone ring. Additionally, the ^{13}C-NMR spectrum supportef these finding by revealing two distinctive signals at δ 62.3 and 169.0 ppm, which are attributed to (thiazole-CH_2) and thiazole-CO, respectively.



Scheme 2. The synthesis of 1,3-thiazol-4(5*H*)-one (**9-12**): (i) $ClCH_2COOH$, AcOH, AcONa, 8 h.

Ultimately, the active methylene in the thiazol-4(5*H*)-one analogs (**9-12**) exhibited reactivity with aromatic aldehydes, as *p*-chlorobenzaldehyde, resulting in the formation of 5-arylidene derivatives (**13-16**), respectively (Schemes 3). The synthesis of the arylidene derivatives was confirmed using elemental analysis and other spectral data, including FTIR, NMR, and MS spectrometry. confirmed with FTIR, ^{1}H NMR, and ^{13}C NMR.



Scheme 3. The synthesis of the arylidene derivatives (**13-16**): (i) 4-Chlorobenzaldehyde, AcOH, AcONa, 6 h.

Antimicrobial screening

In vitro antibacterial activity

Using the agar well-diffusion method, [50] compounds **1-16** were tested for antibacterial activity against *Gram*-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) as well as *Gram*-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). *Chloramphenicol* was used as a control at a concentration of 20 mg/ml (see Tables 1 and 2). The findings indicated the mean diameter of the bacterial growth inhibition zone for the investigated drugs, measured in millimeters. The minimal inhibitory concentrations (*MICs*) were performed, showing efficacy in the primary screening. The 5-arylidene derivatives **13-16** demonstrated noteworthy antibacterial efficacy, with *MIC* values ranging from 1.4 to 0.2 mg/ml. Specifically, the compounds 5-(4-chlorobenzylidene)-2-[5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(thien-1,3-thiazol-4(5*H*)-one] (**14**) and 5-(4-chlorobenzylidene)-2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-phenyl-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (**16**) exhibited significant efficacy against both *E. coli* and *P. aeruginosa*, with *MICs* of 0.2 mg/ml for each of these pathogens. The thiazolone derivatives **9-12** exhibited reasonable antibacterial efficacy, with *MICs* ranging from 1.9 to 0.3 mg/ml. The representative thiazolone is 2-(5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(thien-2-yl)-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (**10**), which has significant activity against *E. coli* and *B. cereus*, with *MIC*s of 0.3 mg/ml for both. Conversely, the thiocarbamoyl derivatives **5-8** had antibacterial efficacy, while the initial chalcones displayed low efficacy (Table 2).

In vitro antifungal activity

The compounds **1-16** were evaluated against four fungal strains: *Candida albicans*, *Trichophyton rubrum*, *Aspergillus flavus*, and *Fusarium oxysporum*. *Clotrimazole* served as a control at a concentration of 20 mg/ml (Table 1). The results were in accordance with that in the antibacterial activity. 5-(4-Chlorobenzylidene)-2-[5-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3-(thien-2-yl)]-2-pyrazolin-1-yl-1,3-thiazol-4(5*H*)-one **14** displayed substantial antifungal efficacy against all fungal species, with *MICs* ranging from 0.09 to 0.12 mg/ml. *N*-Thiocarbamoyl 2-pyrazolines **5-8**, containing a 2-pyrazoline structure, displayed greater efficacy compared to the chalcone derivatives **1-4** [Table 2]. The outcomes of the antimicrobial efficacy indicate that all the compounds exhibit antibacterial and antifungal properties. However, the activity levels vary due to the five-membered aromatic ring containing one or two heteroatoms. The 2-pyrazolinyl thiazol-4(5*H*)-ones **9-12** are more active than the *N*-thiocarbamoyl-2-pyrazolines **5-8**. The pyrazoline moiety, which has two nitrogen atoms, and the five-membered ring, which has one nitrogen atom and one sulfur atom, give this compound its higher activity. Additionally, it is noteworthy that the *N*-thiocarbamoyl-2-pyrazolines **5-8** demonstrate more activity than the chalcones **1-4**. That is likely because chalcones lack the pyrazoline moiety. Furthermore, incorporating an aromatic ring at position 5 in the thiazolone compounds enhances their activity compared to the thiazolone compounds alone.

Table 1 *In vitro* antimicrobial activity of the compounds **1-16** (Diameter of growth of inhibition zone mm)

Compd.	Bacterial species				Fungal species			
	E. coli	***P. aeruginosa***	***B. cereus***	***S. aureus***	***F. oxysporum***	***T. rubrum***	***C. albicans***	***A. flavus***
1	3	2	3	2	1	2	2	4
2	1	3	4	3	2	1	3	5
3	1	4	2	1	3	3	4	4
4	2	1	3	2	3	3	3	4
5	8	6	5	6	9	5	10	11
6	7	7	1	8	10	6	12	12
7	9	8	5	1	9	7	15	11
8	1	6	6	8	11	8	16	10
9	9	7	8	9	12	13	18	13
10	18	16	20	14	22	19	19	19
11	13	15	12	10	14	12	18	18
12	19	16	20	13	21	23	20	21
13	10	12	18	9	15	11	18	16
14	20	18	18	14	21	20	23	22
15	10	16	10	10	14	10	18	18
16	20	18	18	16	22	18	21	19
Reference*	21	19	23	18	20	35	25	25

*Chloramphenicol was used as antibacterial standard and Clotrimazole as antifungal standard.

Table 2 The minimum inhibitory concentration of compounds **1-16** (*MIC* in mg/ml)

Compd.	Bacterial species				Fungal species			
	E. coli	*P. aeruginosa*	*B. cereus*	*S. aureus*	*F. oxysporum*	*T. rubrum*	*C. albicans*	*A. flavus*
1	2.5	2.44	2	2.11	1.7	1.6	1.55	1.61
2	2.8	2.3	1.92	1.98	1.56	1.62	1.39	1.49
3	2.8	2.28	2.2	2.3	1.32	1.41	1.38	1.61
4	2.4	2,53	2	2.11	1.3	1.48	1.39	1.68
5	2	2	1.8	1.8	0.8	0.79	0.8	0.7
6	2.1	1.9	2.3	1.7	0.76	0.72	0.69	0.61
7	1.7	1.9	1.8	2.33	0.8	0.63	0.66	0.71
8	3	2	1.7	1.7	0.59	0.57	0.6	0.9
9	1.7	1.9	1.5	1.6	0.4	0.41	0.5	0.47
10	0.3	1.1	0.3	1.2	0.09	0.21	0.32	0.24
11	1.2	1	1.2	1.5	0.51	0.52	0.47	0.6
12	0.3	1.1	0.3	1.3	0.1	0.09	0.31	0.17
13	1.2	1.2	1.1	1.4	0.49	0.48	0.51	0.57
14	0.2	0.2	1.1	1.2	0.1	0.12	0.09	0.09
15	1.2	1.1	1.2	1.4	0.5	0.39	0.54	0.48
16	0.2	0.2	1.1	1.1	0.09	0.22	0.14	0.29
Reference*	0.07	0.3	1.25	0.08	0.15	0.08	0.08	0.15

*Chloramphenicol was used as antibacterial standard and Clotrimazole as antifungal standard.

Conclusions

A novel group of 2-pyrazoline and thiazol-4(5*H*)-one derivatives, based on thienyl, phenyl, and/or pyrazole rings, has been described. The reactivity of the ketoethylenic moiety (-CO-CH=CH-) in chalcones **1-4** was investigated by its reaction with thiosemicarbazide, resulting in the formation of *N*-thiocarbamoyl 2-pyrazolines **5-8**. These products were then subjected to cycloaddition with chloroacetic acid, yielding 1,3-thiazol-4(5*H*)-ones **9-12**. The arylidene derivatives **13-16** were obtained from the condensation of thiazol-4(5*H*)-ones **9-12** with *p*-chlorobenzaldehyde. The novel compounds were structurally verified using spectroscopy information (FTIR, NMR, as well as MS spectrometry) alongside elemental studies. The compounds **1-16** have been assessed for the antimicrobial activities. The findings showed that the 5-arylidenes of thiazol-4(5*H)*-one **13–16** were the most potent antibacterial and antifungal agents. In addition, the thiazol-4(5*H*)-ones **9-12** are more active than 2-pyrazolines **5-8**.

Experimental Section

General. In the current study, the analytical-grade compounds were utilized. The melting points were established utilizing the APP Digital ST 15 melting point instrument. FTIR spectroscopy were acquired using the Shimadzu-408 infrared spectrophotometer and are presented in cm-1 mode. NMR spectroscopy were collected utilizing a Bruker AV-400 spectrometer. The chemical changes are expressed in parts per million, with TMS serving as the standard reference. Mass spectrometry was performed using a Varian MAT 312 instrument in electron impact mode at a scan energy of 70 eV. The System GmbH vario EL V2.3 1998 CHNS Mode was employed for elemental analysis.

The preparation of chalcones (1-4). The compounds were produced earlier.[47-49]

General process for the synthesis of *N*-thiocarbamoyl-2-pyrazolines 5-8.

The mixture of chalcones **1-4** (2 mmol), thiosemicarbazide (0.27 g, 3 mmol), and KOH (0.11 g, 2 mmol) was allowed to reflux in ethanol (30 ml) for 10h. After cooling, the solution was transferred into crushed ice and stirred. The resulting precipitate was filtered and crystallized using dioxane to obtain products **5-8**.

***N*-Thiocarbamoyl-3-(thien-2-yl)-5-(4-chlorophenyl)-2-pyrazoline** (**5**). Yield: 79%, white crystals: mp 189-191◦C. IR (ν/cm^{-1}): 3440, 3334 (NH_2), 3061, 3012 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.60-3.68 (dd, J = 20.0, 12.0 Hz, H_A), 3.92-3.98 (dd, J = 16.0, 8.0 Hz, H_B), 6.18-6.23 (dd, J = 12.0, 8.0 Hz, H_X), 7.19-7.60 (m, 7 H, 3 thienyl-H and 4 Ar-H), 9.10 (s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 48.0 (pyrazoline-CH_2), 66.5 (pyrazoline-CH), 119.0, 122.6, 128.0, 129.6, 130.8, 131.8, 135.8, 138.2, 176.2 (C=S) ppm. ESIMS *m/z* (%) 321.6 (M^+, 72%), 323.5 (M^++2, 23%). Anal. Calcd. for $C_{14}H_{12}N_3S_2Cl$ (321.84): C, 52.25; H, 3.76; N, 13.06; S, 19.92; Cl, 11.01%. Found: C, 52.20; H, 3.70; N, 13.14; S, 19.84; Cl, 11.17%.

***N*-Thiocarbamoyl-3-(thien-2-yl)-5-(1,3-diphenyl-*1H*-pyrazole-4-yl)-2-pyrazoline** (**6**). Yield 81%, white powder: mp 232-234°C. IR (ν/cm^{-1}): 3447, 3312 (NH_2), 3039 (Ar-H); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.39-3.48 (dd, J = 24.0, 12.0 Hz, H_A), 3.82-3.88 (dd, J = 16.0, 8.0 Hz, H_B), 6.12-6.34 (dd, J = 14.2, 12.0 Hz, H_X), 7.00-7.51 (m, 3 H, thienyl-H), 7.60-8.10 (m, 11 H, 10 Ar-H and pyrazole-H), 8.90 (s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 45.0 (pyrazoline-CH_2), 68.0 (pyrazoline-CH), 119.0, 121.5, 122.4, 124.6, 125.8, 126.4, 127.1, 127.9, 128.0, 128.4, 128.5, 129.2, 130.3, 131.8, 133.7, 140.1, 174.8 (C=S) ppm. ESIMS *m/z* (%) 429.10 (M^+, 76%). Anal. Calcd. for $C_{23}H_{19}N_5S_2$ (429.56): C, 64.31; H, 4.46; N, 16.30; S, 14.93%. Found: C, 64.26; H, 4.40; N, 16.21; S, 14.85%.

5-(1,3-Diphenyl-*1H*-pyrazole-4-yl)-3-methyl-*N*-thiocarbamoyl-2-pyrazoline (**7**). Yield: 84%, red crystals: mp 191-193°C. IR (ν/cm^{-1}): 3447, 3212 (NH_2), 3062 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.20 (s, 3H, CH_3), 3.20-3.29 (dd, J = 24.0, 12.0 Hz, H_A), 3.52-3.61 (dd, J = 20.0, 16.0 Hz, H_B), 5.90-5.97 (dd, J = 16.0, 12.0 Hz, H_X), 7.30-7.98 (m, 11 H, 10 Ar-H and pyrazole-H), 8.94 (s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.2 (CH_3), 47.3 (pyrazoline-CH_2), 65.1 (pyrazoline-CH), 114.8, 118.4, 118.7, 123.4, 124.4, 126.6, 128.1, 128.5, 129.9, 133.7, 134.2, 139.8, 140.1, 176.6 (C=S) ppm. ESIMS *m/z* (%) 361.13 (M^+, 75%). Anal. Calcd. for $C_{20}H_{19}N_5S$ (361.46): C, 66.46; H, 5.30; N, 19.38; S, 8.87%. Found: C, 66.41; H, 5.25; N, 19.31; S, 8.82%.

5-(1,3-Diphenyl-*1H*-pyrazole-4-yl)-3-phenyl-*N*-thiocarbamoyl-2-pyrazoline (**8**). Yield: 77%, orange powder: mp 250-251°C. IR (ν/cm^{-1}): 3446, 3239 (NH_2), 3098 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.94-4.04 (dd, J = 24.0, 16.0 Hz, H_A), 4.62-4.70 (dd, J = 20.0, 12.0 Hz, H_B), 6.14-6.21 (dd, J = 16.0, 12.0 Hz, H_X), 7.30-7.88 (m, 16 H, 15 Ar-H and pyrazole-H), 8.87 (s, 2H, NH_2) ppm. ESIMS *m/z* (%) 423.15 (M^+, 83%). Anal. Calcd. for $C_{25}H_{21}N_5S$ (423.53): C, 70.90; H, 5.00; N, 16.54; S, 7.57%. Found: C, 70.83; H, 4.92; N, 16.48; S, 7.50%.

The general method for the synthesis of 1,3-thiazol-4(5*H*)-ones 9-12.

Compounds **5-8** (2 mmol), chloroacetic acid (0.23 g, 2.4 mmol), acetic anhydride (0.38 ml, 4 mmol), and sodium acetate (0.33 g, 4 mmol) were heated in acetic acid (30 ml) for 8 h. After cooling, the solution was placed on crushed ice. The precipitate formed was then filtered and crystallized from ethanol to yield compounds **9-12**.

2-(5-(4-Chlorophenyl)-3-(thien-2-yl)-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (**9**). Yield: 66%, yellow crystals: mp 200-202◦C. IR (*v*/cm^{-1}): 3055 (Ar-H), 1659 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.50-3.59 (dd, *J* = 24.0, 12.0 Hz, H_A), 3.90-3.99 (dd, *J* = 20.0, 16.0 Hz, H_B), 4.28 (s, 2H, CH_2), 5.72-5.80 (dd, *J* = 16.0, 12.0 Hz, H_X), 7.10-7.87 (m, 7 H, 3 thienyl-H and 4 Ar-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 42.3 (pyrazoline-CH_2), 62.9 (thiazole-CH_2), 66.8 (pyrazoline-CH), 118.7, 123.1, 126.3, 127.1, 128.5, 128.9, 129.8, 130.8, 131.1, 133.4, 139.7, 138.2, 149.7, 168.0 (C=O) ppm. ESIMS *m/z* (%) 361.6 (M^+, 59%), 363.4 (M^++2, 19%). Anal. Calcd. for $C_{16}H_{12}N_3OS_2Cl$ (361.86): C, 53.11; H, 3.34; N, 11.61; S, 17.72; Cl, 9.80 %. Found: C, 53.05; H, 3.27; N, 11.52; S, 17.92; Cl, 9.69 %.

2-(5-(1,3-Diphenyl-*1H*-pyrazol-4-yl)-3-(thien-2-yl)-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (**10**). Yield: 66%, orange crystals: mp 210-212◦C. IR (*v*/cm^{-1}): 3060 (Ar-H), 1662 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.91-4.00 (dd, *J* = 24.0, 12 Hz, H_A), 4.55-4.64 (dd, *J* = 20.0, 16.0 Hz, H_B), 4.09 (s, 2H, CH_2), 5.91-5.98 (dd, *J* = 16.0, 12.0 Hz, H_X), 7.29-7.50 (m, 4 H, 3 thienyl-H), 7.66-7.85 (m, 11 H, Ar-H and pyrazole-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 42.0 (pyrazoline-CH_2), 62.3 (thiazole-CH_2), 68.0 (pyrazoline-CH), 118.7, 119.2, 121.7, 122.5, 124.2, 126.7, 127.4, 128.5, 129.0, 129.3, 130.1, 132.2, 132.4, 133.2, 135.2, 137.2, 139.5, 139.8, 169.0 (C=O) ppm. ESIMS *m/z* (%) 469.10 (M^+, 59%). Anal. Calcd. for $C_{25}H_{19}N_5OS_2$ (469.58): C, 63.95; H, 4.08; N, 14.91; S, 13.65%. Found: C, 63.90; H, 4.02; N, 14.83; S, 13.60%.

2-(5-(1,3-Diphenyl-*1H*-pyrazol-4-yl)-3-methyl-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (**11**). Yield: 60%, white crystals: mp 190-192◦C. IR (*v*/cm^{-1}): 3009 (Ar-H), 1669 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.42 (s, 3H, CH_3), 3.20-3.26 (dd, *J* =16.0, 8.0 Hz, H_A), 3.62-3.66 (dd, *J* = 12.0, 4.0 Hz, H_B), 3.90 (s, 2H, CH_2), 5.70-5.73 (dd, *J* = 8.0, 4.0 Hz, H_X), 7.29-7.90 (m, 11 H, 10 Ar-H and pyrazole-H) ppm. ESIMS *m/z* (%) 401.13 (M^+, 70%). Anal. Calcd. for $C_{22}H_{19}N_5OS$ (401.48): C, 65.82; H, 4.77; N, 17.44; S, 7.99%. Found: C, 65.75; H, 4.71; N, 17.39; S, 7.93%.

2-(5-(1,3-Diphenyl-*1H*-pyrazol-4-yl)-3-phenyl-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (12). Yield: 69%, yellow crystals: mp 194-195◦C. IR (*v*/cm^{-1}): 3049, 3019 (Ar-H), 1687 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.19-3.27 (dd, *J* = 20.0, 12.0 Hz, H_A), 3.71-3.80 (dd, *J* = 20.0, 16.0 Hz, H_B), 4.29 (s, 2H, CH_2), 6.00-6.07 (dd, *J* = 16.0, 12.0 Hz, H_X), 7.40-7.68 (m, 16 H, 15 Ar-H and pyrazole-H) ppm. ESIMS *m/z* (%) 463.14 (M^+, 80%). Anal. Calcd. for $C_{27}H_{21}N_5OS$ (463.55): C, 69.96; H, 4.57; N, 15.11; S, 6.92%. Found: C, 69.91; H, 4.52; N, 15.03; S, 6.85%.

The synthesis of target 5-arylidene-1,3-thiazol-4(5*H*)-ones 13-16.

Sodium acetate (0.83 g, 10 mmol) and 4-chlorobenzaldehyde (0.71 g, 5 mmol) were added to a hot stirring solution of 1,3-thiazol-4(5*H*)-one intermediates **9-12** (5 mmol) in glacial acetic acid (20 ml). The solution was heated for 3h, then cooled and mixed with crushed ice. The resulting precipitate was collected by filtration and crystallized from dioxane to yield products **13-16**.

5-(4-Chlorobenzylidene)-2-[5-(4-chlorophenyl)-3-(thien-2-yl)-2-pyrazolin-1-yl]-1,3-thiazol-4(5*H*)-one (**13**). Yield: 73%, brown crystals: mp 222-224 ◦C. IR (*v*/cm^{-1}): 3051 (Ar-H), 1703 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.70-3.78 (dd, *J* = 24.0, 12.0 Hz, H_A), 4.18-4.27 (dd, *J* = 20.0, 16.0 Hz, H_B), 6.01-6.08 (dd, *J* = 16.0, 12.0 Hz, H_X), 7.22-7.51 (m, 3 H, thienyl-H), 7.90-8.30 (m, 9 H, 8 Ar-H, and =CH-) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 22.3 (=CH-), 46.2 (pyrazoline-CH_2), 66.8 (pyrazoline-CH), 118.7, 123.1, 123.7, 123.9, 126.6, 127.0, 128.5, 128.9, 129.3, 129.8, 131.1, 132.1, 133.4, 139.8, 141.4, 168.1 ppm. ESIMS *m/z* (%) 484.2 (M^+, 59%), 486.1 (M^++2, 21%).

Anal. Calcd. for $C_{23}H_{15}N_3OS_2Cl_2$ (484.41): C, 57.03; H, 3.12; N, 8.67; S, 13.24; Cl, 14.64 %. Found: C, 57.97; H, 3.08; N, 8.60; S, 13.16; Cl, 14.55 %.

5-(4-Chlorobenzylidene)-2-[5-(1,3-diphenyl-*1H*-pyrazol-4-yl)-3-(thien-2-yl)-2-pyrazolin-1-yl]-1,3-thiazol-4(5*H*)-one (**14**). Yield: 74%, pale yellow crystals: mp 210-212 ◦C. IR (ν/cm^{-1}): 3058 (Ar-H), 1701 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.78-3.85 (dd, J = 20.0, 8.0 Hz, H_A), 4.76-4.83 (dd, J = 16.0, 12.0 Hz, H_B), 6.11-6.17 (dd, J = 12.0, 8.0 Hz, H_X), 7.29-7.69 (m, 3 H, thienyl-H), 7.79-8.12 (m, 16 H, 14 Ar-H, pyrazole-H, and =CH-) ppm. ESIMS *m/z* (%) 591.8 (M^+, 73%), 593.4 (M^++2, 25%). Anal. Calcd. for $C_{32}H_{22}N_5OS_2Cl$ (592.13): C, 64.91; H, 3.75; N, 11.83; S, 10.83; Cl, 5.99 %. Found: C, 64.84; H, 3.70; N, 11.75; S, 10.89; Cl, 5.88 %.

5-(4-Chlorobenzylidene)-2-(5-(1,3-diphenyl-*1H*-pyrazol-4-yl)-3-methyl-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (**15**). Yield: 79%, orange powder: mp 229-231°C. IR (ν/cm^{-1}): 3055 (Ar-H), 1703 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.51 (s, 3H, CH_3), 3.98-4.07 (dd, J = 24.0, 12.0 Hz, H_A), 4.80-4.89 (dd, J = 20.0, 16.0 Hz, H_B), 5.69-5.76 (dd, J = 16.0, 12.0 Hz, H_X), 7.30-8.05 (m, 16 H, 14 Ar-H, pyrazole-H and =CH-) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 19.9 (=CH-), 24.2 (CH_3), 47.7 (pyrazoline-CH_2), 67.8 (pyrazoline-CH), 114.8, 118.4, 118.7, 123.4, 124.4, 126.2, 126.6, 127.9, 128.1, 129.0, 129.9, 132.0, 133.7, 134.2, 139.8, 140.1, 170.0 ppm. ESIMS *m/z* (%) 523.4 (M^+, 80%), 525.2 (M^++2, 27%). Anal. Calcd. for $C_{29}H_{22}N_5OSCl$ (524.03): C, 66.47; H, 4.23; N, 13.36; S, 6.12; Cl, 6.76 %. Found: C, 66.42; H, 4.16; N, 13.30; S, 6.20; Cl, 6.69 %.

5-(4-Chlorobenzylidene)-2-(5-(1,3-diphenyl-*1H*-pyrazol-4-yl)-3-phenyl-2-pyrazolin-1-yl)-1,3-thiazol-4(5*H*)-one (16). Yield: 74%, orange powder: mp 233-235°C. IR (ν/cm^{-1}): 3047 (Ar-H), 1705 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.19-3.26 (dd, J = 20.0, 8.0 Hz, H_A), 3.79-3.86 (dd, J = 16.0, 12.0 Hz, H_B), 6.20-6.28 (dd, J = 12.0, 8.0 Hz, H_X), 7.40-8.34 (m, 21 H, 19 Ar-H, pyrazole-H, and =CH-) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 19.2 (=CH-), 46.1 (pyrazoline-CH_2), 66.0 (pyrazoline-CH), 118.6, 123.1, 124.3, 126.4, 127.8, 128.5, 129.1, 129.4, 129.8, 130.8, 132.4, 133.3, 139.7, 148.6, 149.0, 149.9, 154.5, 169.0 ppm. ESIMS *m/z* (%) 585.3 (M^+, 67%), 587.10 (M^++2, 22%). Anal. Calcd. for $C_{34}H_{24}N_5OSCl$ (586.11): C, 69.68; H, 4.13; N, 11.95; S, 5.47; Cl, 6.05%. Found: C, 69.63; H, 4.06; N, 11.90; S, 5.41; Cl, 6.14%.

Biological activity

In vitro antimicrobial screening method

The compounds being studied (**2-16**) were dissolved in DMSO to form a 5% solution. The solution was employed to fill filter paper discs (Whatman No. 3, 5 mm in diameter). To evaluate antibacterial activity, the discs were set on solidified nutrient agar plates that were previously inoculated with bacteria beforehand. The discs were positioned on Czapek Dox agar plates that were already inoculated with fungi to assess antifungal activity. The incubation period extended from 24 to 48 hours at 37°C for bacteria and from 4 to 7 days at 28°C for fungi.[50] Following the incubation time, the diameters of the inhibitory zones were obtained in millimeters. Clotrimazole and chloramphenicol were used as standards on control discs that had been treated with DMSO. To determine the minimal inhibitory concentration (*MIC*), DMSO was added to the solutions to create a range of concentrations. The *MIC* values for antibacterial and antifungal activity are presented in µg/ml and are detailed in Tables 1 and 2.

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Supplementary Material

All data generated or analyzed during this study is included in this published paper and its supplementary files.

References

1. Kozyra, P.; Kaczor, A.; Karczmarzyk, Z.; Wysocki, W.; Pitucha, M. *Struct. Chem.* **2023**, *34*, 1973. https://doi.org/10.1007/s11224-023-02152-w
2. Kozyra, P.; Korga-Plewko, A.; Karczmarzyk, Z.; Hawrył, A.; Wysocki, W.; Człapski, M.; Iwan, M.; Ostrowska-Leśko, M.; Fornal, E.; Pitucha, M. *Biomolecules* **2022**, *12*, 151. https://doi.org/10.3390/biom12020151
3. Janowska, S.; Stefańska, J.; Khylyuk, D.; Wujec, M. *Molecules* **2024,** *29*, 1333. https://doi.org/10.3390/molecules29061333
4. Narkhede, H. I.; Dhake, A. Sh.; Surana, A. R. *Bioorg. Chem*. **2022,** *124***,** 105832. https://doi.org/10.1016/j.bioorg.2022.105832
5. Mangood, A. H.; El-Saied, F. A.; El-Shinawy, F. H.; Abo-Elenan, S. A. *Polycycl. Arom. Comp.* **2023**, *44*, 1760-1780. https://doi.org/10.1080/10406638.2023.2206669
6. Aslan, E. K.; Sağlık, B. N.; Özkay, Y.; Palaska, E. *Chem*. *Select* **2023,** *8***,** e202302069. https://doi.org/10.1002/slct.202302069
7. Boroujeni, Sh. Y.; Haghighijoo, Z.; Mohammadi-Khanaposhtani, M.; Mosadeghkhah, A.; Moazzam, A.; Yavari, A.; Hajimahmoodi, M.; Sabourian, R.; Hosseini, S.; Larijani, B.; Hamedifar, H.; Ansari, S.; Mahdavi, M.; *Chem. Biodiversity* **2022**, *19*, e202100666. https://doi.org/10.1002/cbdv.202100666
8. Alagöz, T.; Çalişkan, F. G.; Bilgiçli, H. G.; Zengin, M.; Sadeghi, M.; Taslimi, P.; Gulçin, I. *Arch. Pharm*. **2023**, *356*, e2300370. https://doi.org/10.1002/ardp.202300370
9. Antonov, D. I.; Dmitriev, M. V.; Kourova, O. A.; Maslivets, A. N. *Russ. J. Org. Chem.* **2021**, *57*, 2063. https://doi.org/10.1134/S1070428021120241
10. Liang, B.; Xiao, D.; Wang, Sh.; Xu, X. *Eur. J. Med. Chem*. **2024,** *275*, 116595. https://doi.org/10.1016/j.ejmech.2024.116595
11. Bekier, A.; Kawka, M.; Lach, J.; Dziadek, J.; Paneth, A.; Gatkowska, J.; Dzitko, K.; Dziadek, B. *Cells* **2021**, *10*, 3476. https://doi.org/10.3390/cells10123476
12. Han, M. İ.; İnce, U. *Phosphorus, Sulfur Silicon Relat. Elem.* **2022**, *197*, 981. https://doi.org/10.1080/10426507.2022.2052880
13. Ishak, N. N. M.; Jamsari, J.; Ismail, A. Z.; Tahir, M. I. M.; Tiekink, E. R. T.; Veerakumarasivam, A.; Ravoof, Th. B. S. A. *J. Mol. Struct.* **2019**, *1198,* 126888. https://doi.org/10.1016/j.molstruc.2019.126888
14. Dziduch, K.; Janowska, S.; Andrzejczuk, S.; Strzyga-Łach, P.; Struga, M.; Feldo, M.; Demchuk, O.; Wujec, M. *Molecules* **2024**, *29*, 3023. https://doi.org/10.3390/molecules29133023
15. Ullah, H.; Fayyaz, F.; Hussain, A.; Rahim, F.; Hayat, Sh.; Uddin, I.; Khan, F.; Zada, H.; Rehman, A. U.; Abdul Wadood, Khan. Kh. M. *Chem. Data Collect.* **2022**, *41*, 100915.

https://doi.org/10.1016/j.cdc.2022.100915
16. Yang, S. J.; Choe, J. H.; Abdildinova, A.; Gong, Y. D. *ACS Comb. Sci.* **2015**, *17*, 732. https://doi.org/10.1021/acscombsci.5b00140
17. Keshk, E. M.; El-Desoky, S. I.; Hammouda, M. A. A.; Abdel-Rahman, A. H.; Hegazi, A. G. *Phosphorus, Sulfur Silicon Relat. Elem.* **2008**, *183*, 1323. https://doi.org/10.1080/10426500701641304
18. Taha, M.; Ismail, N. H.; Imran, S.; Wadood, A.; Ali, M.; Rahim, F.; Khane, A. A.; Riaz, M. *RSC Adv*. **2016**, *6*, 33733. http://dx.doi.org/10.1039/C5RA28012E
19. Yang, S. J.; Lee, S. H.; Kwak, H. J.; Gong, Y. D. *J. Org. Chem.* **2013**, *78*, 438. https://doi.org/10.1021/jo302324r
20. Dilipkumar, P.; Rohit, T.; Deepak, P. D.; Preety, M. *J. Adv. Pharmaceut. Technol. Res.* **2014**, *5*, 196. https://doi.org/10.4103/2231-4040.143040
21. Bharti, A.; Bharati, P.; Singh, N. K.; Bharty, M. K. *J. Coord. Chem.* **2016,** *69*, 1258. https://doi.org/10.1080/00958972.2016.1160074
22. Sicak, Y. *Med. Chem. Res.* **2021**, *30*, 1557. https://doi.org/10.1007/s00044-021-02756-z
23. Hashem, H. E.; Amr, A. E.; Nossier, E. S.; Anwar, M. A.; Azmy, E. M. *ACS Omega* **2022**, *7*, 7155. https://doi.org/10.1021/acsomega.1c06836
24. Badrey, M. G.; Gomha, S. M.; Abdelmonsef, A. H.; El-Reedy, A. A. M. *Polycycl. Arom. Comp.* **2023**, *44*, 275. https://doi.org/10.1080/10406638.2023.2173617
25. Papadopoulou, M. V.; Taylor, E. C. *Tetrahedron* **2021**, *89*, 132158. https://doi.org/10.1016/j.tet.2021.132158
26. Izmest'ev, A. N.; Baranov, V. V.; Kolotyrkina, N. G.; Kravchenko, A. N.; Gazieva, G. A. *J. Heterocycl. Chem*. **2025**, *62*, 58. https://doi.org/10.1002/jhet.4925
27. Patel, J. J.; Modh, R. P.; Asamdi, M.; Chikhalia, K. H. *Mol. Divers.* **2021**, *25*, 2271. https://doi.org/10.1007/s11030-020-10117-y
28. Chinnaraja, D.; Rajalakshmi, R.; Latha, V.; Manikandan, H. *J. Saudi Chem. Soc*. **2016**, *20*, S599. https://doi.org/10.1016/j.jscs.2013.04.006
29. Abeed, A. A. O.; Mohany, M.; Djurasevic, S.; Al-Rejaie, S. S.; El-Emary, T. I. *Arkivoc* **2024**, *8*, 202412289. https://doi.org/10.24820/ark.5550190.p012.289
30. Sahu, B.; Mondal, S.; Mondal, S.; Patra, C.; Singha, T.; Maity, T. K. *Asian J. Pharm. Pharmacol*. **2019**, *5*, 1010. https://doi.org/10.31024/ajpp.2019.5.5.22
31. Ragab, F.A.; Eissa, A.A.M.; Fahim, S.H.; Salem, M.A.; Gamal, M.A.; Nissan, Y.M. *New J. Chem*. **2021**, *45*, 19043. https://doi.org/10.1039/d1nj02862f
32. Rana, M.; Faizan, M.; Dar, S.; Ahmad, T.; Rahisuddin. *ACS Omega* **2022**, *7*, 22639. https://doi.org/10.1021/acsomega.2c02033
33. Abid, M. N.; Fahad, A. M.; Musa, T. M.; Ismail, A. H. *J. Physics: Conf. Series* **2021**, 1853. https://doi.org/10.1088/1742-6596/1853/1/012017
34. Altıntop, M. D.; Cantürk, Z.; Özdemir, A. *ACS Omega* **2023**, *8*, 42867. https://doi.org/10.1021/acsomega.3c05860

35. Alex, J. M.; Kumar, J. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 427. https://doi.org/10.3109/14756366.2013.795956
36. Li, Q. S.; Shen, B. N.; Zhang, Z.; Luo, S.; Ruan, B. F. *Curr. Med. Chem*. **2021**, *28*, 940. https://doi.org/10.2174/0929867327666200306120151
37. Achutha, D. K.; Vagish, C. B.; Renuka, N.; Lokeshwari, D. M.; Kariyappa, A. K. *Chem. Data Collect*. **2020**, *28*, 100445. https://doi.org/10.1016/J.CDC.2020.100445
38. Matiichuk, Y.E.; Horak, Y.I.; Chaban, T. I.; Horishny, V. Ya.; Tymoshuk, O. S.; Matiychuk, V. S. *Russ. J. Org. Chem.* **2020**, *56,* 1720. https://doi.org/10.1134/S1070428020100085
39. Insuasty, B.; Insuasty, A.; Tigreros, A.; Quiroga, J.; Abonia, R.; Nogueras, M.; Cobo, J.; Derita, M.; Zacchino, S. *J. Heterocycl. Chem*., **2011**, *48*, 347. https://doi.org/10.1002/jhet.565.
40. Insuasty, B.; Gutiérrez, A.; Quiroga, J.; Abonia, R.; Nogueras, M.; Cobo, J.; Svetaz, L.; Raimondi, M.; Zacchino, S. *Arch. Pharm. Pharm. Med. Chem*., **2010**, *343*, 48. https://doi.org/10.1002/ardp.200900187
41. Al-Warhi, T.; El Kerdawy, A. M.; Said, M. A.; Albohy, A.; Elsayed, Z. M.; Aljaeed, N.; Elkaeed, E. B.; Eldehna, W. M.; Abdel-Aziz, H. A.; Abdelmoaz, M. A. *Drug Des. Devel. Ther.* **2022**, *16*, 1457. https://doi.org/10.2147/DDDT.S356988
42. Abeed, A. A. O.; El Shamy, E. A.; Abd El-Wahed, S. I. A.; Mohany, M.; Milošević, M.; Al-Rejaie, S. S.; Ibrahim, H. A. M.; Farag, A. E. A. *J. King Saud Univ. Sci.* **2024**, *36*, 103416. https://doi.org/10.1016/j.jksus.2024.103416
43. Abeed, A. A. O.; El-Emary, T. I.; Youssef, M. S. K.; Hefzy, I.; Ibrahim, H. A. M. *Chem. Biol. Technol. Agric.* **2024,** *11*, 83. https://doi.org/10.1186/s40538-024-00602-z
44. Abeed, A. A. O.; Jaleel, G. A. A.; Youssef, M. S. K. *Curr. Org. Synth*. **2019**, *16*, 921. https://doi.org/10.2174/1570179416666190703115133
45. Abeed, A. A. O.; El-Emary, T. I.; Youssef, M. S. K. *Curr. Org. Synth*. **2019**, *16*, 405. https://doi.org/10.2174/1570179416666181210160908
46. Abeed, A. A. O. *J. Heterocycl. Chem.* **2015**, *52*, 1175. https://doi.org/10.1002/jhet.2225
47. Baddepuri, S.; Gamidi, R. K.; Kumari, J.; Sriram, D.; Basavoju, S. *New J. Chem.* **2024**, *48*, 9970. https://doi.org/10.1039/D4NJ00563E
48. Rajalakshmi, R.; Santhi, R.; Elakkiya, T. *Asian J. Chem.* **2020**, *32*, 2125. https://doi.org/10.14233/ajchem.2020.22710
49. Bratenko, M. K.; Kadeinik, Y. V.; Chornous, V. A.; Vovk, M. V. *Russ. J. Org. Chem*. **2008**, *44*, 256. https://doi.org/10.1134/S1070428008020103
50. Kwon-Chung, K. J.; Bennett J. E. *Medical Mycology* Lea & Febiger, Philadelphia, PA **1992**, 866.