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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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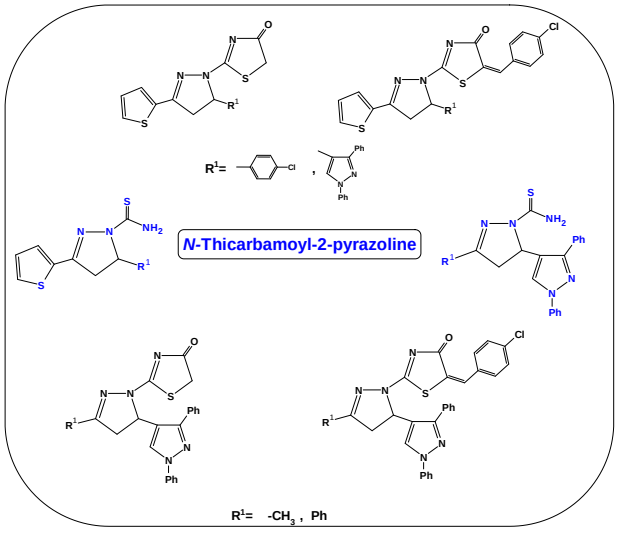
Abstract

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 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 compounds were structurally validated through spectral and elemental studies. Additionally, the substances were assessed for the antibacterial effectiveness effectiveness versus the two types of 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, specifically for the production of heterocyclic substances. [1-12] Thiosemicarbazides are polyfunctional substances 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 analogues of thiazol-4-one are 2-(5-aryl-4,5-dihydropyrazol-1-yl)thiazol-4-ones **D** and **E,** which act as blockers of receptor for epidermal growth factors (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).

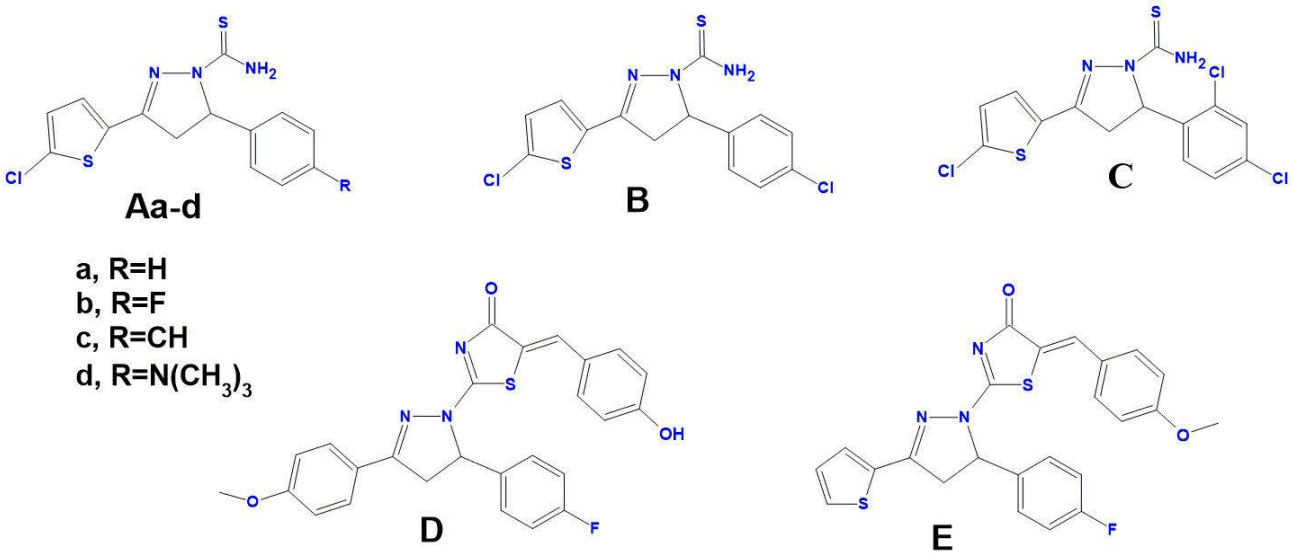


Figure 1. Some of the published 2-pyrazoline and 1,3-thiazol-4-one analogs with biological interest.

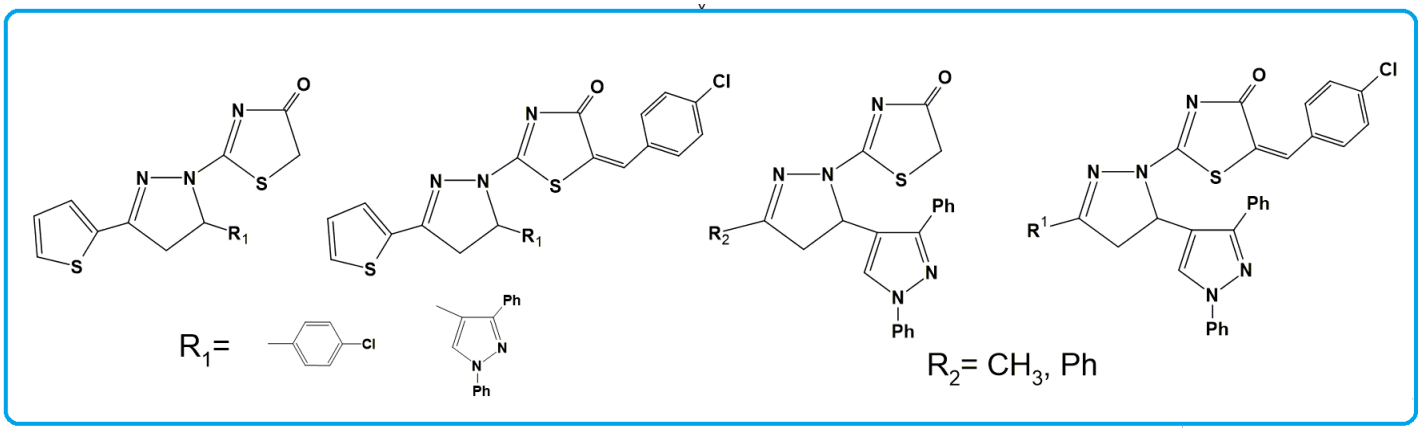
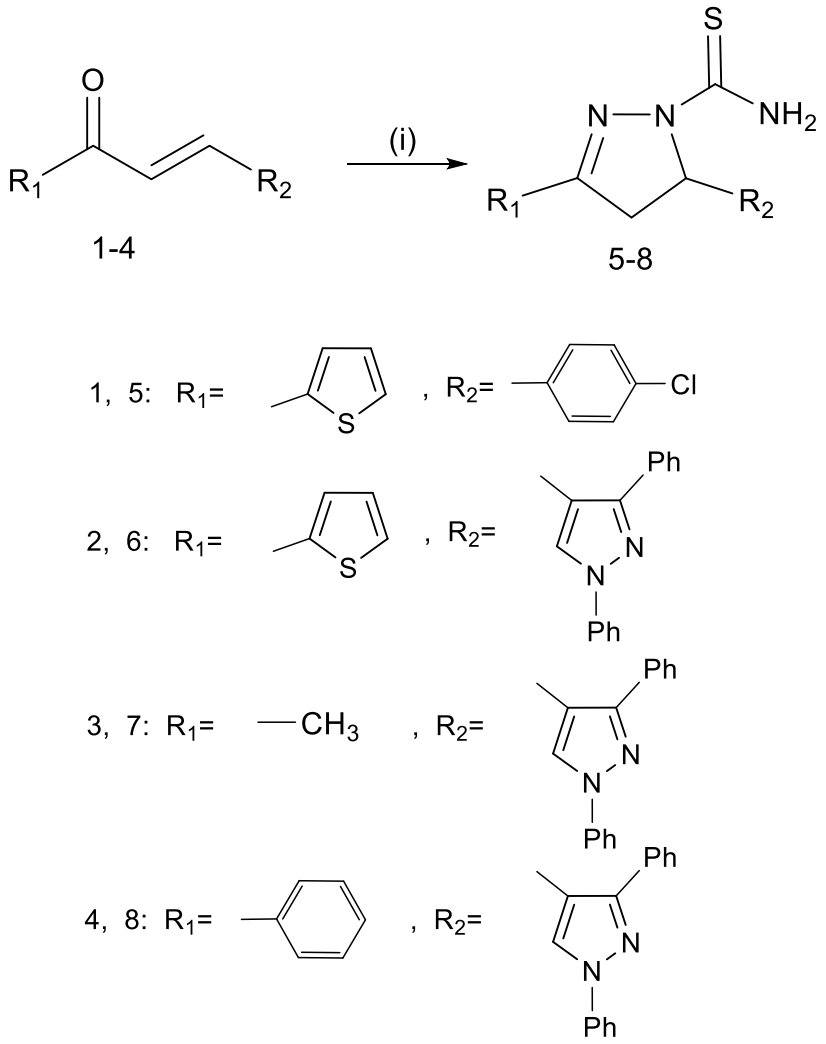


Figure 2. The novel synthesized compounds.

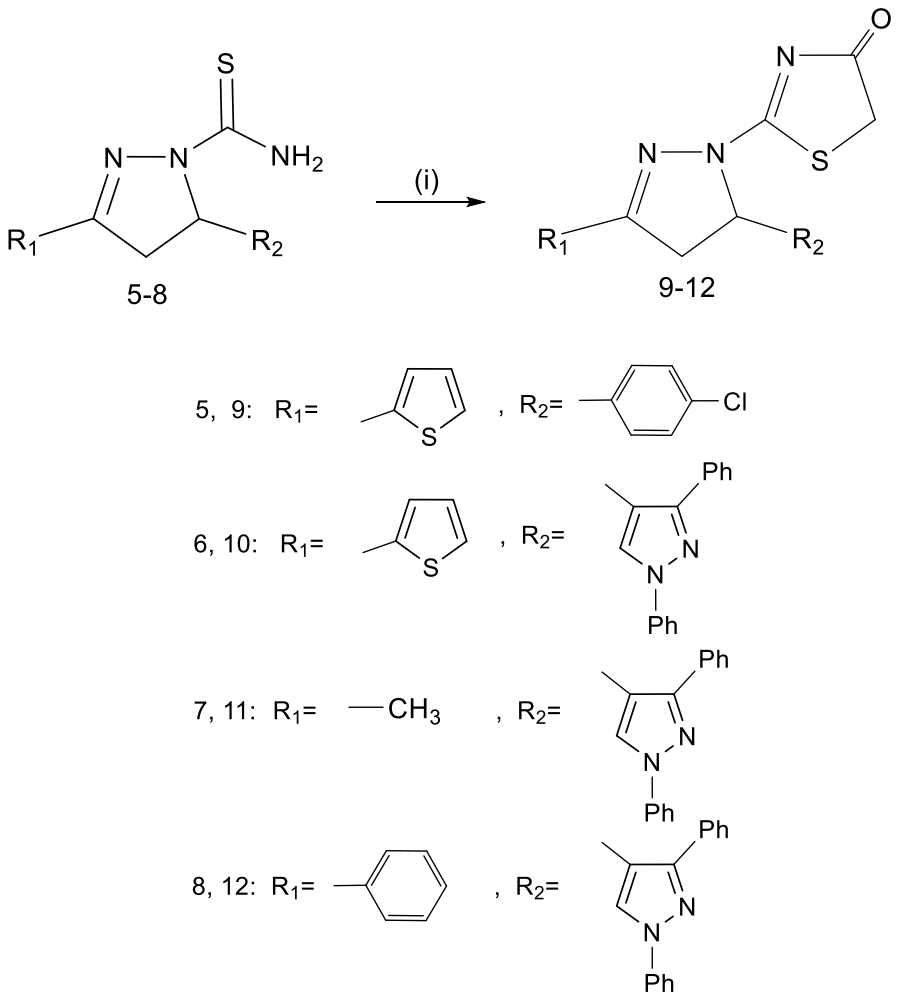
Results and Discussion

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). These compounds were structurally elucidated with 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 thiocarbonyl carbon presence was validated by a signal in the ^{13}C NMR figure 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.



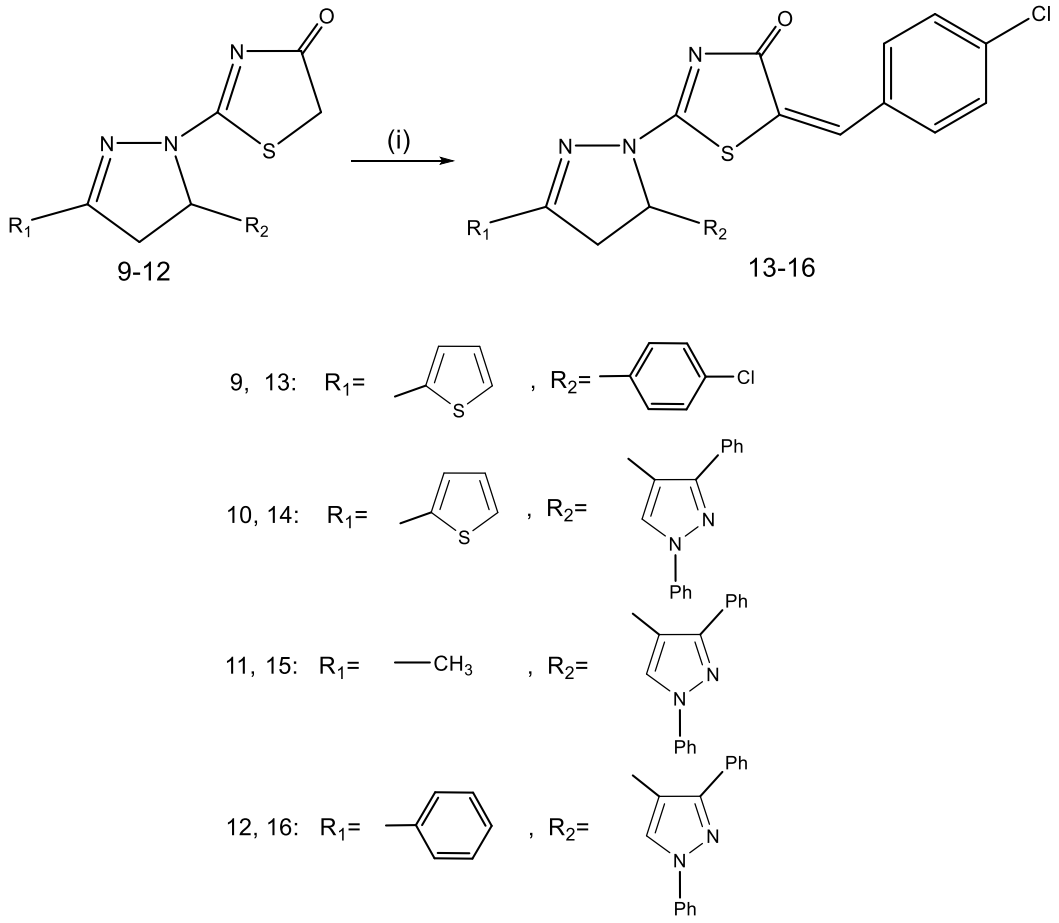
Scheme 1. The synthesis of *N*-thiocarbamoyl-2-pyrazolines (**5-8**): (i) $NH_2NHCSNH_2$, NaOH, 10 h.

Thiazol-4(5*H*)-ones **9-12** were obtained by heating *N*-thicarbamoyl-2-pyrazolines **5-8** with chloroacetic acid in ACOH 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 ^{1}H- 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 ^{1}H NMR figure appeared 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 verified 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

The agar well-diffusion method [50] worked to test the antibacterial activity of compounds **1-16** towards both *Gram*-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and *Gram*-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). As a reference, 20 mg/ml of chloramphenicol was utilized. The findings indicated the average 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 with *MICs* from a range of 1.9-0.3 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-chlorob- enzylidene)-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 efficacy versus *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 substances **1-16** were evaluated towards four fungal strains: *Trichophyton rubrum*, *Candida albicans*, *Aspergillus flavus*, and *Fusarium oxysporum*. *Clotrimazole* served as a control at a 20 mg/ml concentration (Table 1). The results were in accordance to 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* with a range of 0.09-0.12 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 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 for antibacterial standard and Clotrimazole for 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

* The same references apply to antibacterial efficacy.

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 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 compounds of the analytical quality were utilized. The APP Digital ST 15. The melting points were apparatus was used for measuring melting points. Applying the Shimadzu-408

infrared spectrometer, FTIR spectroscopy was carried out and displayed in cm^{-1} mode. a Bruker AV-400 spectrometer was used for ^{1}H and ^{13}C NMR spectra. Chemical changes (δ) were obtained in ppm, and *J* values were gained in Hz. a Varian MAT, 312 spectrometer was used for mass spectra (ESIMS). A GmbH vario EL V2.3 1998 CHNS system 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 heat in ethanol (30 ml) for 10h. Upon cooling, the solution was mixed with crushed ice and stirred. The resulting precipitate underwent filtering and crystallization with dioxane to obtain products **5-8**.

***N*-Thiocarbamoyl-3-(thien-2-yl)-5-(4-chlorophenyl)-2-pyrazoline** (**5**). White crystals; yield 79% mp mp 189-191◦C. FTIR (cm^{-1}): 3440, 3334 (NH_2), 3061, 3012 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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); ^{13}C NMR (100 MHz, DMSO-d_6): δ/ppm: 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). MS (ESI) *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**). White powder; yield 81% mp mp 232-234°C. FTIR (cm^{-1}): 3447, 3312 (NH_2), 3039 (Ar-H); ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 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/ppm: 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. MS (ESI) *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**). Red crystals; yield 84% mp mp 191-193 °C. FTIR (cm^{-1}): 3447, 3212 (NH_2), 3062 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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); ^{13}C NMR (100 MHz, DMSO-d_6): δ/ppm: 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). MS (ESI) *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**). Orange powder; yield 77% mp 250-251 °C. FTIR (cm^{-1}): 3446, 3239 (NH_2), 3098 (Ar-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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). MS (ESI) *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%.

A general approach for synthesizing 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 anhydrous AcONa (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 substance formed was then filtered, dried and purified using ethanol to afford 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**). Orange crystals; yield 66% mp 210-212◦C. FTIR (cm^{-1}): 3060 (Ar-H), 1662 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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); ^{13}C NMR (100 MHz, DMSO-d_6): δ/ppm: 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). MS (ESI) *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**). White crystals; yield 60% mp 190-192 ◦C. FTIR (cm^{-1}): 3009 (Ar-H), 1669 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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). MS (ESI) *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). Yellow crystals; yield 69% mp 194-195◦C. FTIR (cm^{-1}): 3049, 3019 (Ar-H), 1687 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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). MS (ESI) *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 thiazol-4(5*H*)-one intermediates **9-12** (5 mmol) in glacial ACOH (20 ml). After 3 hours of heating, the solution was cooled and mixed with crushed ice. The resultant precipitate was obtained through filtration and crystallized by dioxane, yielding 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**). Yellow crystals; yield 73% mp 222-224 ◦C. FTIR (cm^{-1}): 3051 (Ar-H), 1703 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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-); ^{13}C NMR (100 MHz, DMSO-d_6): δ/ppm: 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. MS (ESI) *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**). Pale yellow crystals; yield 74% mp 210-212 ◦C. FTIR (cm^{-1}): 3058 (Ar-H), 1701 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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-). MS (ESI) *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; yield 79% mp 229-231°C. FTIR (cm^{-1}): 3055 (Ar-H), 1703 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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-); ^{13}C NMR (100 MHz, DMSO-d_6): δ/ppm: 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. MS (ESI) *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). Orange powder; yield 74% mp 233-235°C. FTIR (cm^{-1}): 3047 (Ar-H), 1705 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm: 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-); ^{13}C NMR (100 MHz, DMSO-d_6): δ/ppm: 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. MS (ESI) *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 treated with DMSO to form a solution of 5%. The solution was utilized to saturate filtering paper discs (Whatman 3, 5 mm in size). To evaluate antibacterial activity, the discs were placed on hardened agar plates with nutrients 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. Bacteria were incubated for 24 to 48 hours at 37 °C, whereas fungi were incubated for 4 to 7 days at 28 °C.[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.

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