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Ecofriendly one-pot synthesis and antiviral evaluation of novel pyrazolyl pyrazolines of medicinal interest

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Abstract: Ethyl 3-acetyl-1,5-diphenyl-1*H*-pyrazole-4-carboxylate reacts with a variety of arylaldehydes by grinding method in the presence of a catalytic amount of sodium hydroxide at ambient temperature to give the respective chalcones. The latter compounds react also by grinding method with nitrogen nucleophiles such as hydrazine hydrate, phenylhydrazine, and thiosemicarbazide to afford the corresponding pyrazol-3-yl pyrazolines. A series of 6-pyrazolylpyrimidine-2-thione derivatives were prepared by reaction of the above chalcones with thiourea by grinding method in the presence of a catalytic amount of sodium hydroxide at room temperature. In addition, 7-pyrazolylpyridopyrimidine-3-thione was prepared by reaction of chalcone with 6-aminothiouracil. All the newly synthesized compounds were characterized on the basis of elemental analysis and spectral data (IR, ^{1}H and ^{13}C NMR, mass). Moreover, some of the products were evaluated for their antiviral activity against the herpes virus at different concentrations. The results obtained indicated that compounds **4a**, **4b**, **4f**, **5a**, **5b**, and **5c** have promising activity.

Key words: Chalcones, pyrazolines, pyrimidines, pyridopyrimidines, grinding, antiviral activity

1. Introduction

Chalcones occupy a prominent place among various classes of molecular targets due to their broad spectrum of biological and pharmacological activities such as antitubercular,[1] antimicrobial,[2] antiviral,[3] anxiolytic,[4] and anticancer activities.[5–8]

On the other hand, 2-pyrazolines have been reported to possess a variety of significant and diverse pharmacological activities such as antibacterial,[9] antifungal,[10] anticonvulsant,[11] antiviral,[12] antitubercular,[13] antidepressant,[14] anti-inflammatory,[15] antiamoebic,[16] analgesic,[17] and anticancer[18] activity.

Moreover, the grinding method is of interest in synthetic organic chemistry because it is carried out in the absence of solvent and under environmentally friendly conditions.[19–25] The grinding method is performed at room temperature and the reaction time ranges from 2 to 5 min. Therefore, it contributes to the development of a green strategy for the preparation of organic compounds in high yields with fewer waste products that is simple, efficient, economical, and environmentally benign compared to classical procedures. Thus, keeping in view the advantages of the grinding method and the immense biological importance of chalcones and pyrazolines, and in continuation of our previous work to discover new biologically active heterocyclic compounds,[26–35] we aimed in this context to synthesize new chalcones, pyrazolines, and pyrimidines via ecofriendly methods and evaluate their antiviral activity against the herpes virus.

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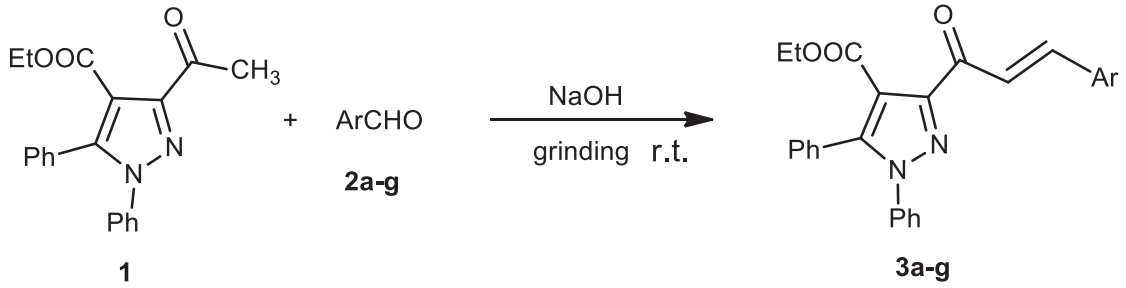
2. Results and discussion

2.1. Chemistry

Toda et al.[36] reported that many exothermic reactions can be accomplished in high yield by grinding solids together (or liquid/solid) using a mortar and pestle, a technique known as grindstone chemistry. Reactions are initiated by grinding, with the transfer of very small amounts of energy through friction. Based on this simple technique, we synthesized a series of chalcones containing pyrazole moiety and utilized these chalcones in the preparation of pyrazolylpyrazoles, pyrazolylpyrimidine thiones, and pyrazolylpyridopyrimidine thiones. Thus, reaction of ethyl 3-acetyl-1,5-diphenyl-1*H*-pyrazole-4-carboxylate **1**[37] with a number of substituted benzaldehydes **2a**–**g** in the presence of sodium hydroxide by grinding method at room temperature afforded the respective chalcones **3** in good yields (Scheme 1). The structure assigned for the products was established based on both elemental and spectral data (IR, ^{1}H NMR, and mass). For example, the IR spectra of products **3** revealed in each case the presence of two absorption bands at = 1723–1735 and 1660–1692 cm^{-1} attributed to the carbonyl groups of the ester and enone residue. The ^{1}H NMR spectra showed the absence of the signal of the methyl group and instead revealed the presence of two doublets at δ = 6.86–7.14 and 7.57–7.77 assigned for the olefinic protons of the enone residue. The mass spectra revealed in each case a molecular ion peak consistent with the proposed structure.



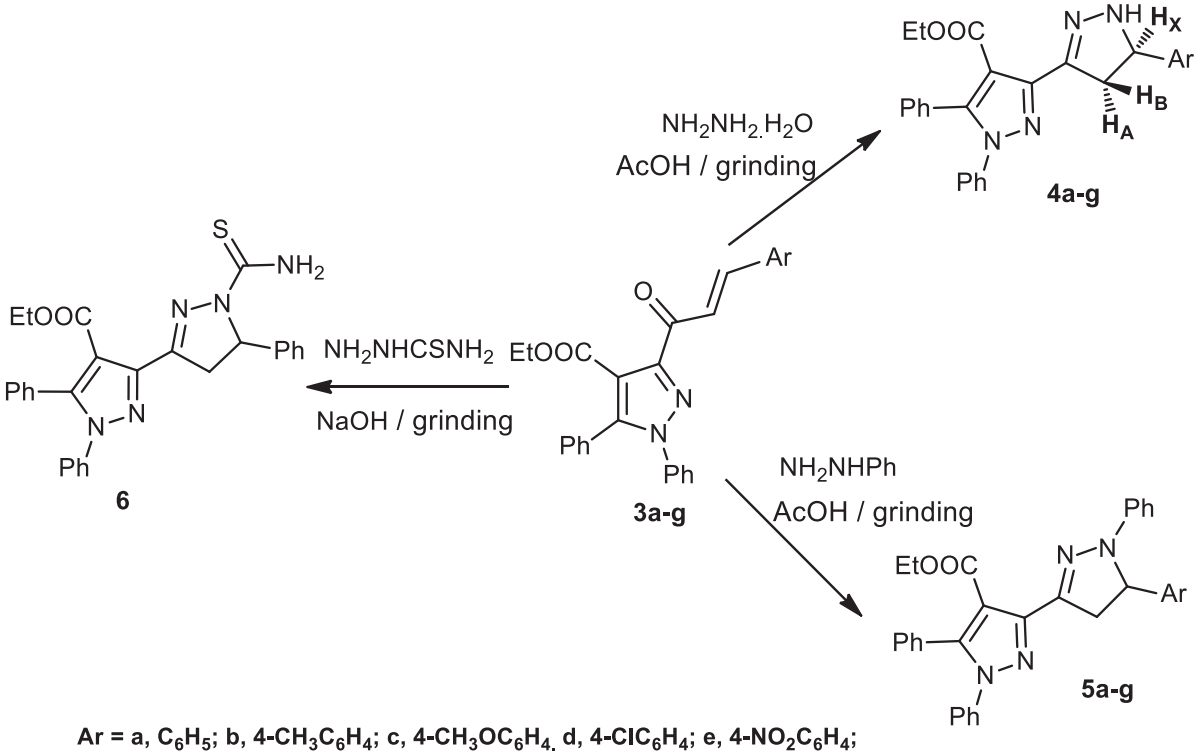
Ar = a, C_6H_5; b, 4-$CH_3C_6H_4$; c, 4-$CH_3OC_6H_4$. d, 4-ClC_6H_4; e, 4-$NO_2C_6H_4$; f, 4-$(CH_3)_2NC_6H_4$; g, 2,4-$Cl_2C_6H_3$

Scheme 1. Synthesis of chalcones **3a**–**g**.

The chalcones **3** were utilized as building blocks for construction of a pyrazole ring via their reactions with nitrogen nucleophiles such as hydrazine hydrate and phenyl hydrazine.

Thus, reaction of each of the chalcones **3** with hydrazine hydrate or phenyl hydrazine by grinding method in the presence of a catalytic amount of acetic acid at room temperature led to the formation of pyrazolyl pyrazolines **4** and **5**, respectively (Scheme 2). The structure assigned for each of products **4** and **5** was elucidated via elemental analysis and spectral data. The IR spectra of each of compounds **4** and **5** revealed the absence of the enone carbonyl group and showed only the presence of an ester carboyl group at = 1710–1735 cm^{-1}. The ^{1}H NMR spectra of compounds **4** and **5** revealed the absence of signals due to the olefinic protons, and instead indicated the presence of three doublet of doublet signals at δ = 2.84–2.93, 3.38–3.47, and 5.10–5.17 ppm assigned for the H_A, H_B, and H_x protons of the pyrazoline ring, in addition to a singlet signal for products **4** that appeared at δ 8.14–8.96 ppm assigned to the NH proton of the pyrazoline ring. On the other hand, chalcone **3a** reacted with thiosemicarbazide by grinding in the presence of a catalytic amount of sodium hydroxide at room temperature to give the respective ethyl 1'-carbamothioyl-1,5,5'-triphenyl-4',5'-

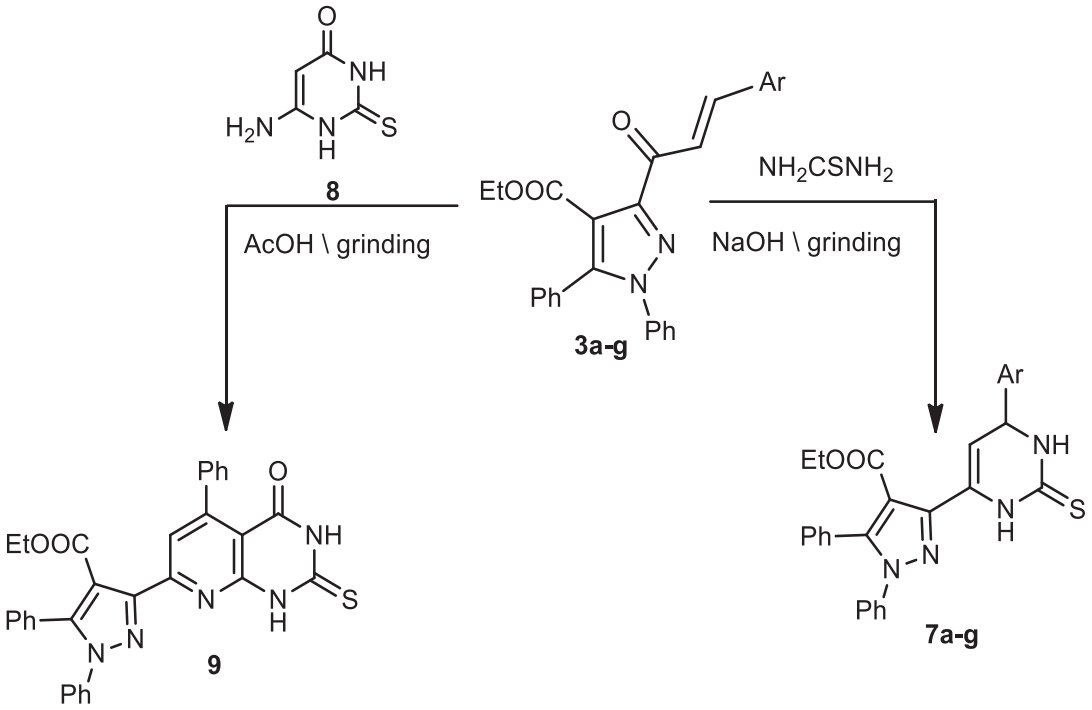
dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (**6**). The structure assigned for product **6** was confirmed by elemental and spectral data (IR, ^{1}H NMR, mass). The IR spectrum of **6** revealed the presence of two absorption bands at = 3433, 3289 cm^{-1} for the thioamide group and one absorption band at = 1699 cm^{-1} for the carbonyl of the ester group. The ^{1}H NMR spectrum of **6** revealed the two signals characteristic for the ester protons, and three doublet of doublet signals at δ = 3.01–3.08, 3.96–4.02, and 5.95–6.03 ppm assigned for the H_A, H_B, and H_X protons for the pyrazoline ring, in addition to a broad signal at δ 8.24 due to the NH_2 group.



Scheme 2. Synthesis of pyrazolines **4a**–**g**, **5a**–**g**, and **6**.

In an extension of our study on exploring the utility of chalcones **3** in the synthesis of heterocyclic compounds, we prepared a series of 6-pyrazolylpyrimidine-2-thiones using the grinding method at room temperature. Thus, reaction of each of the chalcones **3** with thiourea in the presence of a catalytic amount of sodium hydroxide by grinding at room temperature afforded the corresponding pyrazolylpyrimidine thiones **7** in high yield (Scheme 3). The structure assigned for the products **7** was established via elemental analysis and spectral data. For example, the IR spectra showed in each case the disappearance of the carbonyl absorption band of the enone group and instead revealed the presence of two absorption bands at = 3193–3297 and 3409–3428 cm^{-1} due to the two –NH groups of the pyrimidine ring. The ^{1}H NMR spectra of products **7** revealed in each case the presence of the characteristic signals of the ester protons, the multiplet of the aromatic protons, in addition to the two singlet signals attributed to the two –NH protons of the pyrimidine ring (see Experimental). The mass spectra of compounds **7** showed in each case a molecular ion peak at the correct calculated molecular weight for the respective structure. Similarly, grinding together equivalent amounts of chalcone **3a** and 6-aminothiourocil **8** in the presence of catalytic amounts of acetic acid without solvent using a mortar and pestle at room temperature gave product **9**, namely ethyl 3-(4-oxo-5-phenyl-2-thioxo-1,2,3,4-tetrahydro

pyrido[2,3-*d*]pyrimidin-7-yl)-1,5-diphenyl-1*H*-pyrazole-4-carboylate. The structure assigned for the product **9** was confirmed as usual by elemental analysis and spectral data (IR, ^{1}H NMR, and mass). The IR spectrum of **9** revealed two absorption bands at = 3424 and 3216 cm^{-1} (for the 2NH of pyrimidine ring, and one absorption band at = 1712 cm^{-1} for the ester carbonyl group. The ^{1}H NMR spectrum of **9** revealed the characteristic signals for the ester protons, the multiplet of the aromatic protons, in addition to two broad signals at $\delta = 4.35$ and 11.36 ppm assigned for the 2NH of the pyrimidine ring (see Experimental).



Ar = a, C_6H_5; b, 4-$CH_3C_6H_4$; c, 4-$CH_3OC_6H_4$, d, 4-ClC_6H_4; e, 4-$NO_2C_6H_4$; f, 4-$(CH_3)_2NC_6H_4$; g, 2,4-$Cl_2C_6H_3$

Scheme 3. Synthesis of pyrimidine and pyridopyrimidine thiones **7a**–**g** and **9**.

2.2. Antiviral activity

The results of the tested compounds showed different viral activity against the herpes virus as follows (Table; Figure):

A. The presence of a phenyl group at position 1 of the pyrazoline ring enhances the potency of these compounds as antiviral agents (pyrazoline 5 is more reactive than pyrazoline **4**).

B. Compounds **4b**,**f** and **5b,c** showing high antiviral activity due to the presence of electron-donating groups in the p-position of the phenyl group (CH_3, OCH_3, and NMe_2).

C. Compounds **4a**, **4c**, **4d**, **4g**, **5a**, **5g**, and **6** showing moderate antiviral activity.

D. Compounds **4e**, **5d**, and **5e** showing low antiviral activity.

E. Compound **5f** showing no antiviral activity.

Table. The antiviral activity of compounds **4a**–**g**, **5a**–**g**, and **6** against the herpes virus.

Compound no.	Antiviral			
	VTPrT	VTPoT	IFN	log diff
4a	7.2	5.20	2.5	2.00
4b		4.88		2.32
4c		5.75		1.45
4d		5.75		1.45
4e		6.66		0.54
4f		4.50		2.70
4g		6.00		1.20
5a		5.00		2.20
5b		4.20		3.00
5c		3.00		4.20
5d		6.60		0.54
5e		6.75		0.45
5f		7.20		0.00
5g		5.50		1.70
6		6.20		1.00

VTPrT = Virus titer pretreatment.
VTPoT = Virus titer posttreatment.
IFN = Interferon (positive control).

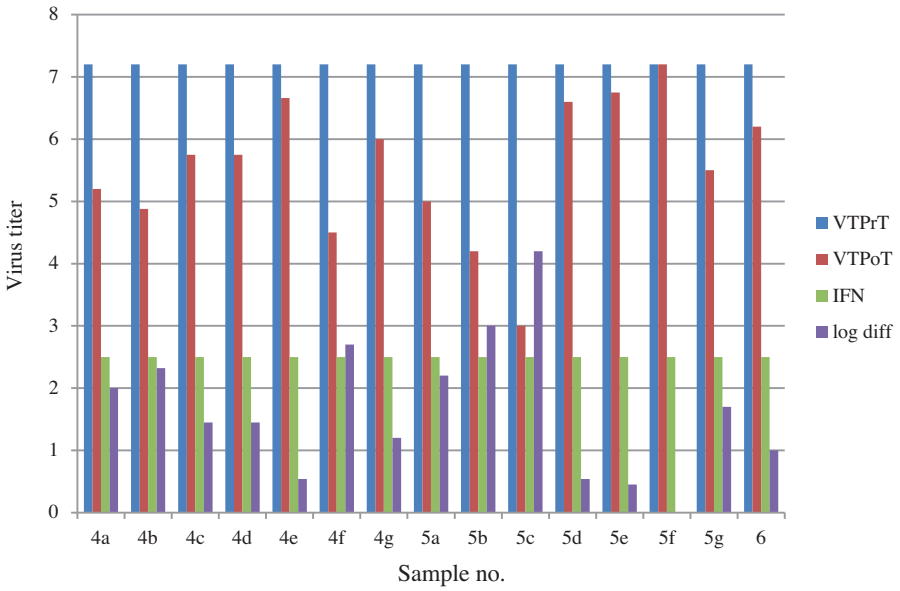


Figure. The antiviral activity of compounds **4a**–**g**, **5a**–**g**, and **6** against the herpes virus.

3. Experimental section

3.1. Chemistry

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus. IR spectra were recorded in potassium bromide discs on PyeUnicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers. ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating

at 300 MHz (^{1}H NMR) and run in deuterated dimethylsulfoxide (DMSO-*d6*). Chemical shifts were related to that of the solvent. ^{13}C NMR was recorded on a Bruker spectrometer at 75 MHz. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were measured by using a German made Elementarvario LIII CHNS analyzer. Antiviral activity of the products was determined at the Veterinary and Serum Research Institute, Giza, Egypt.

3.1.1. Synthesis of chalcones 3a–g

A mixture of ethyl 3-acetyl-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**1**) (3.34 g, 10 mmol) and the appropriate arylaldehyde (10 mmol) was taken in a mortar at room temperature. To a catalytic amount of solid sodium hydroxide was added a few drops of water. The reaction mixture was ground by the pestle, under the hood, for 15–20 min (monitored through TLC). The reaction mixture was poured into 2 N HCl, and the solid product was collected by filtration followed by washing with water and ethanol. The crude product was then recrystallized from the appropriate solvent. Compounds **3** with their physical constants and spectral data are shown below.

Ethyl 3-(3-phenylpropenoyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (3a*)*. White solid, (77% yield), mp 280–282 °C (EtOH); IR (KBr) ν = 3061 (CH=), 2960, 2928 (CH), 1723, 1667 (2C=O), 1601 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.09 (t, J = 7.2 Hz, 3H, CH_3), 4.11 (q, J = 7.2 Hz, 2H, CH_2), 7.27–7.82 (m, 17H, Ar–H and CH=CH) ppm; ^{13}C NMR (DMSO-d_6): δ13.2 (CH_3), 62.2 (CH_2), 115.8, 119.6, 121.2, 125.8, 127.7, 128.2, 128.8, 129.0, 129.2, 129.7, 132.2, 132.7, 138.4, 140.4, 143.9, 148.2 (Ar–C), 168.4, 193.5 (C=O); MS, m/z (%) 422 (M^+, 19). Anal. Calcd. For $C_{27}H_{22}N_2O_3$ (422.48): C, 76.76; H, 5.25; N, 6.63. Found: C, 76.58; H, 5.21; N, 6.50%.

Ethyl 1,5-diphenyl-3-(3-(p-tolyl)acryloyl)-1*H*-pyrazole-4-carboxylate (**3b**). White solid, (78% yield), mp 122–124 °C (EtOH); IR (KBr) ν = 3061 (CH=), 2968, 2920 (CH), 1725, 1660 (2C=O), 1595 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.08 (t, J = 7.2 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.13 (q, J = 7.2 Hz, 2H, CH_2), 7.24–7.71 (m, 16H, Ar–H and CH=CH) ppm; ^{13}C NMR (DMSO-d_6): δ13.2, 19.7 (CH_3), 62.0 (CH_2), 114.6, 119.2, 120.8, 122.4, 125.7, 127.3, 128.1, 128.6, 129.0, 129.9, 131.1, 132.8, 138.5, 140.1, 142.6, 148.7 (Ar–C), 169.3, 193.2 (C=O); MS, m/z (%) 436 (M^+, 32). Anal. Calcd. For $C_{28}H_{24}N_2O_3$ (436.50): C, 77.04; H, 5.54; N, 6.42. Found: C, 77.01; H, 5.48; N, 6.37%.

Ethyl 3-(3-(4-methoxyphenyl)acryloyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**3c**). White solid, (68% yield), mp 304–306 °C (EtOH/dioxane); IR (KBr) ν = 3057, 2998 (CH=), 2965, 2932 (CH), 1729, 1666 (2C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.09 (t, J = 7.2 Hz, 3H, CH_3), 3.81 (s, 3H, CH_3), 4.15 (q, J = 7.2 Hz, 2H, CH_2), 6.98 (d, J = 8.7 Hz, 1H, CH=CH), 7.27–7.41 (m, 14H, Ar-H), 7.68 (d, J = 8.7 Hz, 1H, CH=CH) ppm; ^{13}C NMR (DMSO-d_6): δ13.2, 53.6 (CH_3), 62.4 (CH_2), 114.9, 119.3, 120.2, 121.7, 123.1, 125.6, 127.5, 128.6, 129.2, 130.4, 131.4, 132.6, 138.3, 140.6, 142.1, 147.9 (Ar–C), 169.4, 193.6 (C=O); MS, m/z (%) 452 (M^+, 38). Anal. Calcd. For $C_{28}H_{24}N_2O_4$ (452.50): C, 74.32; H, 5.35; N, 6.19. Found: C, 74.39; H, 5.28; N, 6.08%.

Ethyl 3-(3-(4-chlorophenyl)acryloyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**3d**). White solid, (68% yield), mp 125–127 °C (EtOH); IR (KBr) ν = 3026 (CH=), 2926 (CH), 1730, 1641 (2C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.12 (t, J = 7.2 Hz, 3H, CH_3), 4.16 (q, J = 7.2 Hz, 2H, CH_2), 7.26–7.41 (m, 16H, Ar–H and CH=CH) ppm; MS, m/z (%) 458 (M^++2, 4), 456 (M^+, 14). Anal. Calcd. For $C_{27}H_{21}ClN_2O_3$ (456.92): C, 70.97; H, 4.63; N, 6.13. Found: C, 70.92; H, 4.59; N, 6.07%.

Ethyl 3-(3-(4-nitrophenyl)acryloyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**3e**). Brown solid, (71% yield), mp 185–187 °C (dioxane); IR (KBr) ν = 3057, 2998 (CH=), 2965, 2932 (CH), 1729, 1666 (2C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.05 (t, J = 7.2 Hz, 3H, CH_3), 4.03 (q, J = 7.2 Hz, 2H, CH_2), 6.86 (d, J = 8.7 Hz, 1H, CH=CH), 7.11–7.37 (m, 14H, Ar–H), 7.57 (d, J= 8.7 Hz, 1H, CH=CH) ppm; MS, m/z (%) 467 (M^+, 70). Anal. Calcd. For $C_{27}H_{21}N_3O_5$ (467.47): C, 69.37; H, 4.53; N, 8.99. Found: C, 69.30; H, 4.52; N, 8.78%.

Ethyl 3-(3-(4-(dimethylamino)phenyl)acryloyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**3f**). Yellow solid, (70% yield), mp 146–148 °C (EtOH); IR (KBr) ν = 3060 (CH=), 2921 (CH), 1726, 1660 (2C=O), 1607 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.05 (t, J = 7.2 Hz, 3H, CH_3), 3.00 (s, 6H, $2CH_3$), 4.03 (q, J = 7.2 Hz, 2H, CH_2), 6.73 (d, J = 9 Hz, 1H, CH=CH), 7.28–7.43 (m, 14H, Ar–H), 7.63 (d, J = 9 Hz, 1H, CH=CH) ppm; MS, m/z (%) 465 (M^+, 100). Anal. Calcd. For $C_{29}H_{27}N_3O_3$ (465.54): C, 74.82; H, 5.85; N, 9.03. Found: C, 74.76; H, 5.81; N, 8.93%.

Ethyl 3-(3-(2,4-dichlorophenyl)acryloyl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**3g**). Yellow solid, (70% yield), mp 100–102 °C (EtOH); IR (KBr) ν = 3063 (CH=), 2929 (CH), 1735, 1692 (2C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6)δ: 1.09 (t, J = 7.2 Hz, 3H, CH_3), 4.13 (q, J = 7.2 Hz, 2H, CH_2), 7.14 (d, J = 9 Hz, 1H, CH=CH), 7.34–7.57 (m, 13H, Ar–H), 7.77 (d, J = 9 Hz, 1H, CH=CH) ppm; MS, m/z (%) 491 (M^+, 12). Anal. Calcd. For $C_{27}H_{20}Cl_2N_2O_3$ (491.37): C, 66.00; H, 4.10; N, 5.70. Found: C, 65.92; H, 4.14; N, 5.62%.

3.1.2. Synthesis of pyrazoline derivatives 4a–g and 5a–g

A mixture of the appropriate chalcone **3** (1 mmol) and hydrazine hydrate or phenyl hydrazine (1 mmol) was ground in a mortar at room temperature, in the presence of catalytic drops of acetic acid, for 10–15 min. The reaction mixture was poured into water and the solid product was collected by filtration followed by washing with ethanol. The crude product was then recrystallized from the appropriate solvent to give pure product **4** or **5.** Compounds **4** and **5** with their physical constants and spectral data are depicted as shown below:

Ethyl 1,5,5'-triphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (4a). Beige solid, (73% yield), mp 305–307 °C (DMF); IR (KBr) ν = 3312 (NH), 3060 (CH=), 2949 (CH), 1735 (C=O), 1592 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 2.99–3.05 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.63–3.68 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.16 (q, J = 7.2 Hz, 2H, CH_2), 4.93–5.02 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 7.21–7.47 (m, 15H, Ar–H), 8.14 (s, br, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ13.2 (CH_3), 40.9, 62.8 (CH_2), 52.3 (CH), 112.4, 120.8, 125.7, 126.5, 127.3, 127.8, 128.3, 128.4, 128.8, 130.1, 132.4, 138.5, 142.1, 143.3, 145.0, 147.2 (Ar–C), 162.4 (C=O); MS, m/z (%) 436 (M^+, 83). Anal. Calcd. For $C_{27}H_{24}N_4O_2$ (436.51): C, 74.29; H, 5.54; N, 12.84. Found: C, 74.22; H, 5.49; N, 12.75%.

Ethyl 1,5-diphenyl-5'-(p-tolyl)-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (**4b**). Yellow solid, (73% yield), mp 200–202 °C (EtOH/DMF); IR (KBr) ν = 3318 (NH), 3055 (CH=), 2940 (CH), 1732 (C=O), 1590 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.90–2.99 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.48–3.57 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.14 (q, J = 7.2 Hz, 2H, CH_2), 4.81–4.93 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 7.01–7.78 (m, 14H, Ar–H), 8.83 (s, br, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ13.2, 22.8 (CH_3), 41.8, 62.7 (CH_2), 52.3 (CH), 112.3, 125.2, 126.3, 127.1, 127.4, 127.8, 127.9, 128.0, 129.3, 129.6, 129.8, 130.2, 130.5, 139.4, 140.8, 149.7 (Ar–C), 168.6 (C=O); MS, m/z (%) 450

(M^+, 18). Anal. Calcd. For $C_{28}H_{26}N_4O_2$ (450.53): C, 74.65; H, 5.82; N, 12.44. Found: C, 74.49; H, 5.75; N, 12.38%.

Ethyl 5'-(4-methoxyphenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (**4c**). Pale yellow solid, (70% yield), mp 208–210 °C (EtOH); IR (KBr) ν = 3335 (NH), 3062 (CH=), 2937 (CH), 1730 (C=O), 1590 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 2.92–2.97 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.44–3.53 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 3.74 (s, 3H, OCH_3), 4.14 (q, J = 7.2 Hz, 2H, CH_2), 4.74–4.81 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 6.89–7.37 (m, 14H, Ar–H), 8.82 (s, br, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ13.4, 53.1 (CH_3), 41.3, 62.8 (CH_2), 52.0 (CH), 114.7, 120.2, 124.3, 126.2, 127.3, 127.8, 128.0, 128.4, 129.3, 130.1, 135.6, 138.3, 140.8, 143.8, 145.4, 148.1 (Ar–C), 162.5 (C=O); MS, m/z (%) 466 (M^+, 18). Anal. Calcd. For $C_{28}H_{26}N_4O_3$ (466.53): C, 72.09; H, 5.62; N, 12.01. Found: C, 72.02; H, 5.53; N, 11.86%.

Ethyl 5'-(4-chlorophenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (**4d**). White solid, (71% yield), mp 260–262 °C (EtOH); IR (KBr) ν = 3305 (NH), 3093 (CH=), 2921 (CH), 1737 (C=O), 1598 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.08 (t, J = 7.2 Hz, 3H, CH_3), 2.95–2.99 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.46–3.58 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.18 (q, J = 7.2 Hz, 2H, CH_2), 4.76–4.84 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 7.36–7.47 (m, 14H, Ar–H), 8.96 (s, br, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ13.5 (CH_3), 41.8, 62.7 (CH_2), 52.3 (CH), 115.3, 119.5, 123.6, 126.2, 127.2, 127.8, 128.0, 129.1, 129.8, 130.1, 135.2, 137.4, 140.2, 144.4, 145.7, 147.3 (Ar–C), 162.7 (C=O); MS, m/z (%) 472 (M^++2, 12), 470 (M^+, 33). Anal. Calcd. For $C_{27}H_{23}ClN_4O_2$ (470.95): C, 68.86; H, 4.92; N, 11.90. Found: C, 68.81; H, 4.78; N, 11.79%.

Ethyl 5'-(4-nitrophenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (**4e**). Orange solid, (70% yield), mp 180–182 °C (EtOH); IR (KBr) ν = 3414 (NH), 3063 (CH=), 2926 (CH), 1729 (C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.04 (t, J = 7.2 Hz, 3H, CH_3), 2.92–2.96 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.42–3.53 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.11 (q, J = 7.2 Hz, 2H, CH_2), 4.74–4.82 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 6.90–7.82 (m, 14H, Ar–H), 8.93 (s, br, 1H, NH) ppm; MS, m/z (%) 481 (M^+, 64). Anal. Calcd. For $C_{27}H_{23}N_5O_4$ (481.50): C, 67.35; H, 4.81; N, 14.54. Found: C, 67.28; H, 4.75; N, 14.51%.

Ethyl 5'-(4-(dimethylamino)phenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (4f). Yellow solid, (73% yield), mp 280–282 °C (EtOH/dioxane); IR (KBr) ν = 3333 (NH), 3060 (CH=), 2931 (CH), 1733 (C=O), 1593 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 2.90 (s, 6H, $2CH_3$), 2.92–2.96 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.38–3.47 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.16 (q, J = 7.2 Hz, 2H, CH_2), 4.68–4.75 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 6.68–7.46 (m, 14H, Ar–H), 8.90 (s, br, 1H, NH) ppm; MS, m/z (%) 479 (M^+, 40). Anal. Calcd. For $C_{29}H_{29}N_5O_2$ (479.57): C, 72.63; H, 6.10; N, 14.60. Found: C, 72.53; H, 6.00; N, 14.52%.

Ethyl 5'-(2,4-dichlorophenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (4g). Yellow solid, (75% yield), mp 214–216 °C (EtOH); IR (KBr) ν = 3336 (NH), 3059 (CH=), 2926 (CH), 1736 (C=O), 1591 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.07 (t, J = 7.2 Hz, 3H, CH_3), 2.84–2.93 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.67–3.76 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.18 (q, J = 7.2 Hz, 2H, CH_2), 5.10–5.17 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 7.05–7.63 (m, 13H, Ar–H), 7.94 (s, br, 1H, NH) ppm; MS, m/z (%) 505 (M^+, 34). Anal. Calcd. For $C_{27}H_{22}Cl_2N_4O_2$ (505.40): C, 64.17; H, 4.39; N, 11.09. Found: C, 64.11; H, 4.26; N, 11.02%.

Ethyl 1,1',5,5'-tetraphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (5a). White solid, (70% yield), mp 280–282 °C (EtOH/dioxane); IR (KBr) ν = 3059 (CH=), 2926 (CH), 1716 (C=O), 1591 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.04 (t, J = 7.2 Hz, 3H, CH_3), 2.93–2.99 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.91–3.96 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.10 (q, J = 7.2 Hz, 2H, CH_2), 5.32–5.39 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 6.94–7.35 (m, 20H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ13.3 (CH_3), 43.2, 62.4 (CH_2), 51.2 (CH), 113.4, 116.3, 118.2, 121.5, 124.4, 127.4, 127.9, 128.2, 128.7, 129.3, 129.8, 134.1, 138.3, 140.1, 141.2, 143.8, 144.4, 147.0, 156.4 (Ar–C), 164.3 (C=O); MS, m/z (%) 512 (M^+, 47). Anal. Calcd. For $C_{33}H_{28}N_4O_2$ (512.60): C, 77.32; H, 5.51; N, 10.93. Found: C, 77.39; H, 5.41; N, 10.76%.

Ethyl 1,1',5-triphenyl-5'-(p-tolyl)-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (5b). White solid, (72% yield), mp 230–232 °C (EtOH); IR (KBr) ν = 3048 (CH=), 2925 (CH), 1715 (C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.04 (t, J = 7.2 Hz, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.90–2.97 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.87–3.92 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.13 (q, J = 7.2 Hz, 2H, CH_2), 5.39–5.42 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 7.15–7.49 (m, 19H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ13.3, 20.3 (CH_3), 43.0, 62.2 (CH_2), 51.6 (CH), 113.9, 116.2, 119.4, 121.3, 124.1, 126.3, 127.1, 128.5, 128.7, 129.3, 129.8, 130.3, 136.4, 140.1, 142.4, 143.8, 144.1, 147.6, 156.1 (Ar–C), 164.1 (C=O); MS, m/z (%) 526 (M^+, 73). Anal. Calcd. For $C_{34}H_{30}N_4O_2$ (526.63): C, 77.54; H, 5.74; N, 10.64. Found: C, 77.46; H, 5.71; N, 10.53%.

Ethyl 5'-(4-methoxyphenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (5c). Beige solid, (73% yield), mp 184–186 °C (EtOH); IR (KBr) ν = 3046, 3006 (CH=), 2948 (CH), 1723 (C=O), 1592 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.05 (t, J = 7.2 Hz, 3H, CH_3), 3.09–3.16 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.71 (s, 3H, OCH_3), 3.90–4.00 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.16 (q, J = 7.2 Hz, 2H, CH_2), 5.41–5.47 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 6.70–7.49 (m, 19H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6): δ13.0, 54.9 (CH_3), 43.9, 62.2 (CH_2), 51.6 (CH), 113.1, 114.2, 118.8, 122.9, 125.5, 127.1, 128.1, 128.6, 128.7, 128.8, 129.8, 134.1, 138.8, 141.8, 143.4, 143.8, 144.0, 147.0, 158.4 (Ar–C), 164.6 (C=O); MS, m/z (%) 542 (M^+, 55). Anal. Calcd. For $C_{34}H_{30}N_4O_3$ (542.63): C, 75.26; H, 5.57; N, 10.33. Found: C, 75.03; H, 5.52; N, 10.24%.

Ethyl 5'-(4-chlorophenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylateEthyl (5d). Beige solid, (73% yield), mp 246–248 °C (EtOH); IR (KBr) ν = 3050 (CH=), 2961 (CH), 1698 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 3.02–3.08 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.91–4.00 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.16 (q, J = 7.2 Hz, 2H, CH_2), 5.40–5.45 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 6.76–7.41 (m, 19H, Ar–H) ppm; MS, m/z (%) 549 (M^++2, 8), 547 (M^+, 27). Anal. Calcd. For $C_{33}H_{27}ClN_4O_2$ (547.05): C, 72.45; H, 4.97; N, 10.24. Found: C, 72.29; H, 4.83; N, 10.04%.

Ethyl 5'-(4-nitrophenyl)-1,1',5-triphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (5e). Brown solid, (71% yield), mp 146–148 °C (EtOH); IR (KBr) ν = 3058 (CH=), 2979, 2933 (CH), 1710 (C=O), 1598 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 3.00–3.07 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.89–3.97 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.13 (q, J = 7.2 Hz, 2H, CH_2), 5.41–5.47 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 6.78–7.40 (m, 19H, Ar–H) ppm; MS, m/z (%) 557 (M^+, 76). Anal. Calcd. For $C_{33}H_{27}N_5O_4$ (557.60): C, 71.08; H, 4.88; N, 12.56. Found: C, 71.00; H, 4.69; N, 12.48%.

Ethyl 5'-(4-(dimethylamino)phenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (5f). Yellow solid, (73% yield), mp 140–142 °C (EtOH); IR (KBr) ν = 3051 (CH=), 2963 (CH), 1698 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 2.85 (s, 6H, $2CH_3$),

2.99–3.09 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.39–3.45 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.11 (q, J = 7.2 Hz, 2H, CH_2), 5.38–5.43 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 6.68–7.37 (m, 19H, Ar–H) ppm; MS, m/z (%) 555 (M^+, 62). Anal. Calcd. For $C_{35}H_{33}N_5O_2$ (555.67): C, 75.65; H, 5.99; N, 12.60. Found: C, 75.58; H, 5.90; N, 12.48%.

Ethyl 5'-(2,4-dichlorophenyl)-1,5-diphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (5g). Pale brown solid, (70% yield), mp 138–140 °C (EtOH); IR (KBr) ν = 3057 (CH=), 2933 (CH), 1712 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.05 (t, J = 7.2 Hz, 3H, CH_3), 3.08–3.17 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.42–3.55 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.15 (q, J = 7.2 Hz, 2H, CH_2), 5.41–5.48 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 7.09–7.47 (m, 18H, Ar–H) ppm; MS, m/z (%) 581 (M^+, 76). Anal. Calcd. For $C_{33}H_{26}Cl_2N_4O_2$ (581.49): C, 68.16; H, 4.51; N, 9.64. Found: C, 68.03; H, 4.42; N, 9.56%.

3.1.3. Reaction of chalcones 3 with thiosemicarbazide and thiourea

A mixture of the appropriate chalcones **3a**–**g** (10 mmol) and thiosemicarbazide or thiourea (10 mmol each) was taken in a mortar at room temperature. To a catalytic amount of solid sodium hydroxide was added a few drops of water. The reaction mixture was ground by the pestle, under the hood, for 15–20 min (monitored through TLC). The reaction mixture was then poured into 2 N HCl, and the solid product was collected by filtration followed by washing with water and EtOH. The crude product was recrystallized from the appropriate solvent to give the respective pyrazoline **6** or pyrimidinethiones **7a**–**g**.

Ethyl 1'-carbamothioyl-1,5,5'-triphenyl-4',5'-dihydro-1*H*,1'*H*-[3,3'-bipyrazole]-4-carboxylate (6). Beige solid, (71% yield), mp 240–242 °C (EtOH/dioxane); IR (KBr) ν = 3433, 3298 (NH_2), 3059 (CH=), 2919 (CH), 1699 (C=O), 1591 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.03 (t, J = 7.2 Hz, 3H, CH_3), 3.01–3.08 (dd, 1H, H_A, J= 17.6, 6.6 Hz), 3.96–4.02 (dd, 1H, H_B, J = 17.6, 10.8 Hz), 4.25 (q, J = 7.2 Hz, 2H, CH_2), 5.95–6.03 (dd, 1H, H_X, J = 10.8, 6.6 Hz), 7.17–7.37 (m, 15H, Ar–H), 8.24 (s, br, 2H, NH_2) ppm; MS m/z(%): 495 (M^+, 61). Anal. Calcd. For $C_{28}H_{25}N_5O_2S$ (495.60): C, 67.86; H, 5.08; N, 14.13. Found: C, 67.79; H, 5.02; N, 14.07%.

Ethyl 1,5-diphenyl-3-(6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (7a). Yellow solid, (70% yield), mp 268–270 °C (dioxane); IR (KBr) ν = 3419, 3238 (2NH), 3057 (CH=), 2932 (CH), 1712 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 4.14 (q, J = 7.2 Hz, 2H, CH_2), 4.48 (s, 1H, pyrimidine-H4), 6.88 (s, 1H, pyrimidine–H5), 7.12–7.37 (m, 16H, Ar–H and NH), 8.52 (s, br, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ13.5 (CH_3), 48.5 (CH), 56.0 (CH_2), 108.2, 112.2, 114.3, 118.6, 123.5, 125.7, 125.8, 128.0, 128.2, 128.9, 129.3, 129.5, 130.1, 137.9, 141.5, 144.8, 148.0 (Ar–C), 164.9 (C=O); MS m/z(%): 480 (M^+, 51). Anal. Calcd. For $C_{28}H_{24}N_4O_2S$ (480.58): C, 69.98; H, 5.03; N, 11.66. Found: C, 69.80; H, 5.07; N, 11.52%.

Ethyl 1,5-diphenyl-3-(2-thioxo-6-(p-tolyl)-1,2,3,6-tetrahydropyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (7b). Yellow solid, (73% yield), mp 189–191 °C (EtOH); IR (KBr) ν = 3428, 3279 (2NH), 3050 (CH=), 2967 (CH), 1713 (C=O), 1594 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.05 (t, J = 7.2 Hz, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.15 (q, J = 7.2 Hz, 2H, CH_2), 4.53 (s, 1H, pyrimidine–H4), 6.92 (s, 1H, pyrimidine–H5), 7.18–7.39 (m, 15H, Ar–H and NH), 8.54 (s, br, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ13.5, 20.3 (CH_3), 48.2 (CH), 56.2 (CH_2), 112.6, 114.2, 116.3, 118.2, 121.7, 125.3, 125.9, 127.6, 128.0, 128.8, 129.3, 129.8, 131.8, 133.9, 140.4, 143.6, 148.3 (Ar–C), 164.6 (C=O); MS m/z(%): 494 (M^+, 100). Anal. Calcd. For $C_{29}H_{26}N_4O_2S$ (494.61): C, 70.42; H, 5.30; N, 11.33. Found: C, 70.36; H, 5.25; N, 11.27%.

Ethyl 3-(6-(4-methoxyphenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**7c**). Yellow solid, (68% yield), mp 280–282 °C (dioxane); IR (KBr) ν = 3419, 3193 (2NH), 3001 (CH=), 2959 (CH), 1713 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.05 (t, J = 7.2 Hz, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.17 (q, J = 7.2 Hz, 2H, CH_2), 4.52 (s, 1H, pyrimidine–H4), 6.92 (s, 1H, pyrimidine–H5), 7.10–7.27 (m, 15H, Ar–H and NH), 8.57 (s, br, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ13.5, 53.5 (CH_3), 48.2 (CH), 56.2 (CH_2), 112.8, 114.0, 116.9, 118.5, 120.6, 124.3, 125.4, 127.6, 128.2, 128.9, 129.3, 129.8, 130.6, 135.5, 141.3, 143.9, 147.2 (Ar–C), 164.7 (C=O); MS m/z(%): 510 (M^+, 100). Anal. Calcd. For $C_{29}H_{26}N_4O_3S$ (510.61): C, 68.21; H, 5.13; N, 10.97. Found: C, 68.06; H, 5.05; N, 10.81%.

Ethyl 3-(6-(4-chlorophenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7d). Yellow solid, (75% yield), mp 240–242 °C (EtOH/dioxane); IR (KBr) ν = 3419, 3198 (2NH), 3060 (CH=), 2959 (CH), 1715 (C=O), 1597 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.06 (t, J = 7.2 Hz, 3H, CH_3), 4.16 (q, J = 7.2 Hz, 2H, CH_2), 4.54 (s, 1H, pyrimidine–H4), 6.90 (s, 1H, pyrimidine–H5), 7.12–7.36 (m, 15H, Ar–H and NH), 8.68 (s, br, 1H, NH) ppm; MS, m/z (%) 517 (M^++2, 30), 515 (M^+, 95). Anal. Calcd. For $C_{28}H_{23}ClN_4O_2S$ (515.03): C, 65.30; H, 4.50; N, 10.88. Found: C, 65.21; H, 4.36; N, 10.69%.

Ethyl 3-(6-(4-nitrophenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**7e**). Yellow solid, (74% yield), mp 232–234 °C (EtOH); IR (KBr) ν = 3428, 3193 (2NH), 3059 (CH=), 2930 (CH), 1701 (C=O), 1598 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.05 (t, J = 7.2 Hz, 3H, CH_3), 4.14 (q, J = 7.2 Hz, 2H, CH_2), 4.52 (s, 1H, pyrimidine–H4), 6.83 (s, 1H, pyrimidine–H5), 7.01–7.52 (m, 15H, Ar–H and NH), 8.33 (s, br, 1H, NH) ppm; MS m/z(%): 525 (M^+, 70). Anal. Calcd. For $C_{28}H_{23}N_5O_4S$ (525.58): C, 63.99; H, 4.41; N, 13.33. Found: C, 63.74; H, 4.28; N, 13.18%.

Ethyl 3-(6-(4-(dimethylamino)phenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (7f). Yellow solid, (71% yield), mp 213–215 °C (EtOH); IR (KBr) ν = 3433, 3233 (2NH), 3041 (CH=), 2953 (CH), 1717 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.08 (t, J = 7.2 Hz, 3H, CH_3), 3.02 (s, 6H, $2CH_3$), 4.17 (q, J = 7.2 Hz, 2H, CH_2), 4.52 (s, 1H, pyrimidine–H4), 6.75 (s, 1H, pyrimidine–H5), 7.25–7.84 (m, 15H, Ar–H and NH), 8.53 (s, br, 1H, NH) ppm; MS m/z(%): 523 (M^+, 68). Anal. Calcd. For $C_{30}H_{29}N_5O_2S$ (523.65): C, 68.81; H, 5.58; N, 13.37. Found: C, 68.73; H, 5.46; N, 13.25%.

Ethyl 3-(6-(2,4-dichlorophenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**7g**). Yellow solid, (71% yield), mp 240–242 °C (EtOH/dioxane); IR (KBr) ν = 3409, 3212 (2NH), 3065 (CH=), 2969 (CH), 1713 (C=O), 1590 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.03 (t, J = 7.2 Hz, 3H, CH_3), 4.15 (q, J = 7.2 Hz, 2H, CH_2), 4.55 (s, 1H, pyrimidine–H4), 6.79 (s, 1H, pyrimidine–H5), 7.15–7.34 (m, 14H, Ar–H and NH), 8.63 (s, br, 1H, NH) ppm; MS m/z(%): 550 (M^+, 60). Anal. Calcd. For $C_{28}H_{22}Cl_2N_4O_2S$ (549.47): C, 61.20; H, 4.04; N, 10.20. Found: C, 61.05; H, 4.01; N, 10.12%.

Synthesis of ethyl 3-(4-oxo-5-phenyl-2-thioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidin-7-yl)-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (9). A mixture of ethyl 3-cinnamoyl-1,5-diphenyl-1*H*-pyrazole-4-carboxylate (**3a**) (4.22 g, 10 mmol) and 6-amino-2-thioxo-2,3,4-trihydro-1*H*-pyrimidin-4-one (**8**) (1.43 g, 10 mmol) was ground in a mortar at room temperature, in the presence of catalytic drops of acetic acid, for 10–15 min. The reaction mixture was poured into water and the solid product was collected by filtration followed by washing with ethanol. The crude product was then recrystallized from DMF to give thione **9** as yellow crystals,

79%, mp 260–262 °C; IR (KBr) ν = 3424, 3216 (2NH), 3054 (CH=), 2962 (CH), 1712 (C=O), 1593 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.04 (t, J = 7.2 Hz, 3H, CH_3), 4.14 (q, J = 7.2 Hz, 2H, CH_2), 4.35 (s, br, 1H, NH), 7.13–7.93 (m, 16H, Ar–H and pyridine–H), 11.36 (s, br, 1H, NH) ppm; MS m/z(%): 545 (M^+, 49). Anal. Calcd. For $C_{31}H_{23}N_5O_3S$ (545.61): C, 68.24; H, 4.25; N, 12.84. Found: C, 68.16; H, 4.20; N, 12.65%.

3.2. Pharmacology: antiviral activity

3.2.1. Maintenance of cell lines

African green monkey cells (Vero) was cultured in 75-cm^2 cell culture flasks using E-MEM supplemented with 10% fetal bovine serum (FBS) as culture medium.

Cell line was maintained in the following way according to McAteer and Davis,[38] where growth media were discarded from the cell culture flasks and the cell layer was washed gently with sterile PBS. The PBS was decanted and then the cell monolayer was washed with 5 mL of trypsin solution (prewarmed at 37 °C). The trypsin was decanted and the cell culture flasks were incubated with trace trypsin in the incubator at 37 °C until the cells detached from the surface. Growth media were added to the detached cells. The cells were resuspended in growth medium to the desired concentration according to cell count. The cell suspension was cultured in another cell culture flask or in 96-well cell culture plates and incubated at 37 °C until confluency.

3.2.2. Cell Counting

Accurate cell number in the suspension was calculated by counting the cells using the hemocytometer according to McAteer and Davis[38] as follows:

- Double-fold dilution of the original cell suspension was prepared by adding 0.5 mL of undiluted cell suspension to 0.5 mL of 0.4% trypan blue dye.
- The mixture was mixed well with a fine pipette and immediately aspirated to fill the hemocytometer counting chambers.
- All viable (unstained) cells in the 8 squares of 2 hemocytometer chambers were counted, omitting cells lying on the upper line and left line of each chamber.

The volume of each chamber = 0.1 mm^3 (1.0 × 1.0 × 0.1).

Note: To perform an accurate cell count, 75% of the cells in the suspension should be viable and the difference between the cell counts in the 2 hemocytometer chambers should be minimal.

- If cell clumping (aggregation) was observed, the clumps were disaggregated by vigorous aspiration through a pipette.
- The mean count of the cells in each chamber was calculated.
- The total number of cells in the suspension was calculated using the following formula:

  $\mathbf{N_1 = m \times tb \times V \times 10^4}$, where:

  $\mathbf{N_1}$ = number of cells in the cell suspension, $\mathbf{m}$ = mean of cell count per 0.1 mm^3

  $\mathbf{tb}$ = correction of the trypan blue dilution (2 in double-fold dilution with trypan blue)

  $\mathbf{V}$ = volume of the original cell suspension in mL.

$\mathbf{10^4}$ = conversion factor for counting chamber volume.

$\mathbf{N}$ (number of cells per mL) = $\mathbf{N_1/V}$

If a new suspension was required to be prepared with a new concentration (N_2), the new volume (V_2) could be calculated as follows.

$\mathbf{N \times V = N_2 \times V_2}$ $\mathbf{V_2 = N \times V/N_2}$

- The new cell suspension can be prepared by adding growth medium equal to the difference between the new volume ($\mathbf{V_2}$) and the original volume ($\mathbf{V}$).

- The hemocytometer and cover slip were cleaned immediately after use with 70% EtOH.

3.2.3. Cryopreservation of cell lines

a) Freezing

Healthy, viable cells and preferably low in passage number should be preserved according to McAteer and Davis[38] as follows:

A cell suspension was prepared by trypsinization as previously. The cell suspension was cold centrifuged at 1500 rpm for 10 min. The supernatant was discarded and the cell pellet was adjusted to a concentration of 5–10 $\times$ 10^6 cells per mL in preservation medium containing 10% dimethyl sulfoxide (DMSO) and 10% serum. Each 1 mL of the resuspended cells was dispensed in a cryotube clearly labeled with cell type, passage number, cell concentration, and date of preservation. The cryotubes were frozen in a cryobath for 30 min and then transferred to a deep-freeze at –70 °C overnight. The cryotubes were finally stored in liquid nitrogen (–196 °C).

b) Thawing

According to McAteer and Davis,[38] the cryotube was removed from liquid nitrogen and transferred immediately to a water bath at 37 °C. When the contents were completely thawed, the outside of the tube was wiped with alcohol to reduce bacterial load. The cell suspension in the cryotube was transferred to a culture flask containing growth medium (added very slowly, drop by drop as the viability of the thawed cells would be severely affected if the cells were added rapidly). The culture flask was incubated overnight at 37 °C. The growth medium was carefully decanted (to remove DMSO) and replaced with fresh growth medium. The flask was then incubated until confluency. Thawed cells were tested concerning viability and sterility.

3.2.4. Cytotoxicity of pyrazolyl pyrazolines of Vero cell line using MTT assay

MTT assay is a sensitive, quantitative, and reliable colorimetric method that measures the viability of cells. The assay is based on the ability of mitochondrial lactate dehydrogenase enzymes in living cells to convert the water soluble substrate 3-(4,5-dimethylthiazol-2-yl) 2^-,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan that is water insoluble. A solubilization solution (dimethyl sulfoxide) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring it using a spectrophotometer at a wavelength usually at 570 nm.[39]

Cytotoxic effects of the tested compounds were evaluated 24 h posttreatment by MTT assay, in which test pyrazolyl pyrazolines were culture media dissolved to contain 1 mg/mL, filtered through a 0.22-μm syringe filter. Double-fold dilutions were prepared by adding equal volumes of the dissolved pyrazolyl pyrazolines.

Ninety-six-well vero cells precultured plates were treated with descending double-fold serially diluted pyrazolyl pyrazolines at 37 °C for the required time. A negative cell culture control was included. Residual living cells were treated with 20 μL of filtered MTT (5 mg/mL) at 37 °C for 4 h. The MTT was discarded and plates were PBS washed three times. DMSO was added as 50 μL/well. Plates were shaken on plate shaker for 30 min to dissolve the produced intracellular blue formazan complex.

Optical densities (ODs) were measured at 570 nm using an ELISA plate reader (Biotec-8000 USA). Data were reported for three independent experiments.[40]

Viability percentage was calculated as follows: Cell viability percentage = (OD of treated cells /OD of untreated cells) × 100.[41]

3.2.5. Evaluation of antiviral activity in cells pretreated with test of pyrazolyl pyrazolines

Vero cells were pretreated with test of pyrazolyl pyrazolines for 24 h followed by viral inoculation to evaluate the effect of pyrazolyl pyrazolines on the initial stages of viral replication as follows. Vero cells were counted as 10^5 cells/mL and cultured in 96-well cell culture plates. On confluency, the growth media were discarded. The plates were incubated for 24 h at 37 °C with pyrazolyl pyrazolines. In the meantime, control plates were maintained and left untreated for viral control titration.

Herpes simplex type-1 virus was 10-fold serially diluted in E-MEM. The growth media were discarded and virus dilutions were inoculated as 0.1 mL/well in Vero cell cultured plates; either they were treated with pyrazolyl pyrazolines or not. Untreated and uninfected wells were included the control HUND (Germany). Plates were incubated at 37 °C and examined daily under the inverted microscope. Seven days postincubation, the virus titers in pyrazolyl pyrazolines treated and untreated cells were determined using the endpoint of cytopathic effect (CPE) assay.[42]

Virus infectivity titer was determined by endpoint method dependent on determining the highest dilution of the virus that produced CPE in 50% of the cell cultures. The 50% endpoint was calculated according to Reed and Muench[43] as follows:

50% endpoint ($CCID_{50}$) = (percentage of CPE > 50% − 50) /(percentage of CPE > 50% − percentage of CPE < 50%) × log dilution. All titrations where carried out in triplicate. The differences between the mean viral titers in treated and untreated plates refer to the antiviral activity.

4. Conclusions

In this context, we have developed a simple and ecofriendly synthetic protocol for the synthesis of a number of heterocyclic compounds incorporating pyrazole moiety starting with chalcones and using grindstone technology. The products were screened for their antiviral activity and the results obtained indicated that compounds **4a**, **4b**, **4f**, **5a**, **5b**, and **5c** showed high antiviral activity.

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