Med Chem Res (2013) 22:2654–2664
DOI 10.1007/s00044-012-0267-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new 3-aryl-4-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole derivatives as potential antimicrobial agents

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Received: 16 April 2012 / Accepted: 28 September 2012 / Published online: 9 October 2012

Abstract New (E)-1-aryl-3-(3-aryl-1-phenyl-1*H*-pyrazol-4-yl)prop-2-en-1-ones **6** (pyrazolic chalcones) were synthesized from Claisen–Schmidt reaction of 3-aryl-1-phenylpyrazol-4-carboxaldehydes **4** with several acetophenone derivatives **5**. Subsequently, the cyclocondensation reaction of chalcones **6** with phenylhydrazine in acetic acid medium afforded the new 3-aryl-4-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazoles **7**. The synthesized compounds were characterized by spectral studies and evaluated for their in vitro antibacterial activity against three pathogenic bacterial strains, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and in vitro antifungal activity against three pathogenic fungal strains *Aspergillus flavus*, *Chrysosporium keratinophilum*, and *Candida albicans.*

Keywords Claisen–Schmidt reaction · Pyrazolic chalcones · Pyrazolines · Antimicrobial

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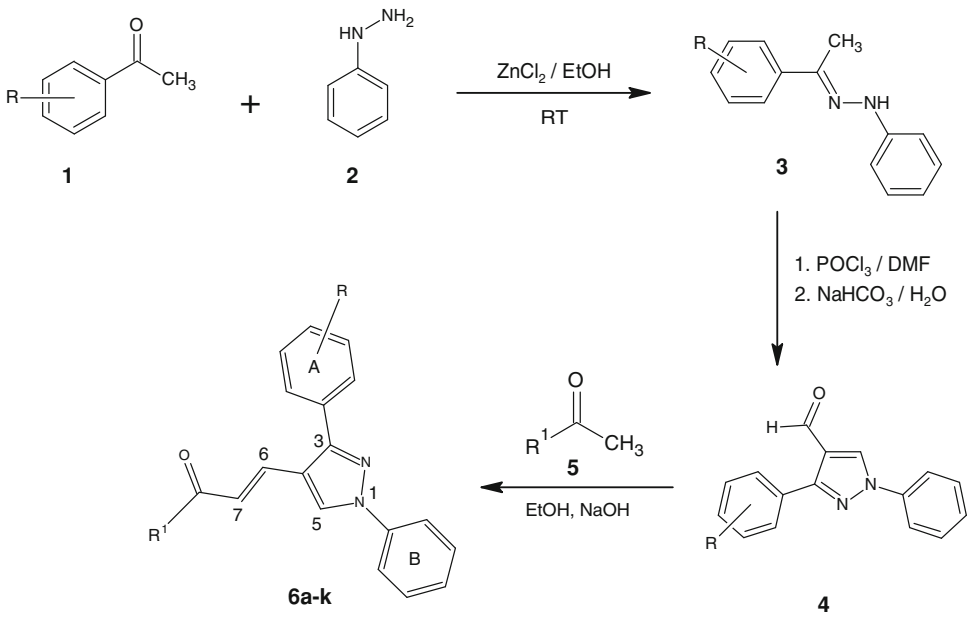
Introduction

Antimicrobial resistance is a global public health concern that is impacted by both human and non-human antimicrobial use. The consequences of antimicrobial resistance are particularly important when pathogens are resistant to antimicrobials that are critically important in the treatment of human disease. The treatment of microbial infections still remains an important and challenging therapeutic problem because of factors that include emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. In spite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistant bacterial strains in the last decades constitutes a substantial need for new classes of antibacterial agents (Chopra *et al*., 2008). It has been postulated that the development of resistance to known antibiotics could be overcome by identifying new drug targets via genomics, improving the existing nature of antibiotics, and identifying new antibacterial agents with novel mode of action and structure (Walsh, 2000; Ritter and Wong, 2001; Wijkmans and Beckett, 2002).

1,2-Diaryl substituted heterocyclic system occurs in so many diverse classes of biologically interesting low molecular-weight compounds that it would be an understatement to link it to the ease of synthesis of the vicinal diaryl system (Kubinyi and Muller, 2004). Drugs based on a pyrazole ring bearing two adjacent aryl groups in a vicinal relation have often been occupying a position in the list of best selling pharmaceutical products since the beginning of this decade (Kubinyi and Muller, 2004). Recently, there has been considerable amount of progress in 1,3-diarylpyrazole chemistry because of the recognition of importance of the pyrazole structure in biological

Scheme 1 Synthesis of the novel pyrazolic chalcones **6a–k**



6	R	R^1
a	Cl	biphenyl
b	Br	biphenyl
c	OCH_3	biphenyl
d	H	4-SCH_3-C_6H_4
e	Cl	4-SCH_3-C_6H_4
f	Br	4-SCH_3-C_6H_4
g	OCH_3	4-SCH_3-C_6H_4
h	H	2,4-dichlorophenyl
i	Cl	2,4-dichlorophenyl
j	Br	2,4-dichlorophenyl
k	OCH_3	2,4-dichlorophenyl

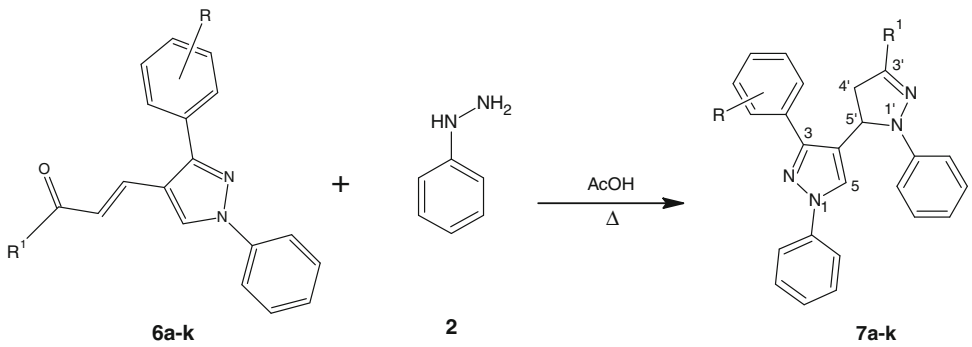
processes such as antimicrobial (Thumar and Patel, 2011), anti-inflammatory (Kumar *et al.,* 2011), antitubercular (Chovatia *et al.,* 2007), antitumor (Joksovic *et al.,* 2009; Insuasty *et al.,* 2010), antiangiogenesis (Abadi *et al.,* 2003), antiparasitic (Rathelot *et al.,* 2002), antiviral (Hashem *et al.,* 2007) and also possesses analegesic and anxiolytic activity (Shetty and Bhagat, 2008). Motivated by these findings, and in continuation of our interest in the synthesis of a variety of heterocyclic systems for biological and pharmacological evaluations (Vijesh *et al.,* 2011a, b), we set out to synthesize some novel 3-aryl-4-(3-aryl-4,5-dihydro-1*H*-pyrazol-5-yl)-1-phenyl-1*H*-pyrazole derivatives and evaluate their antimicrobial activities. It must be noted that this scaffold provides vicinal diaryl substitution pattern on both the pyrazole as well as pyrazoline nucleus.

Results and discussion

Chemistry

In order to obtain the pyrazolic chalcones, the corresponding 4-formylpyrazolic precursors **4** were initially synthesized from the phenylhydrazones **3** (prepared by condensation of the respective acetophenones **1** and phenylhydrazine **2**); following a reported procedure (Kira *et al.*, 1969; Insuasty *et al.,* 2010). In this sense, the Vilsmeier–Haack reagent ($POCl_3$/DMF) was employed affording compounds **4**. Subsequently, the Claisen–Schmidt condensation of the obtained aldehydes **4** with the acetophenones **5** afforded the corresponding pyrazolic chalcones **6** (Scheme 1). Continuing with the synthetic approach, the cyclocondensation of the chalcones **6a–k** with

Scheme 2 Synthesis of the new pyrazoles **7a–k**



7	R	R^1
a	Cl	biphenyl
b	Br	biphenyl
c	OCH_3	biphenyl
d	H	4-SCH_3-C_6H_4
e	Cl	4-SCH_3-C_6H_4
f	Br	4-SCH_3-C_6H_4
g	OCH_3	4-SCH_3-C_6H_4
h	H	2,4-dichlorophenyl
i	Cl	2,4-dichlorophenyl
j	Br	2,4-dichlorophenyl
k	OCH_3	2,4-dichlorophenyl

phenylhydrazine in the presence of acetic acid as solvent, afforded the desired products **7a–k** (Scheme 2). The structures of the synthesized compounds **(7a–k)** were confirmed by spectral data (IR, ^{1}H NMR, Mass and elemental analysis).

The structure elucidation of compounds **6a–k** was based on the spectral data (IR, mass spectrometry, and Elemental analysis). The IR spectrum of compound **6a** showed absorption bands at 3,122–3,055, 1,659, and 1,593 which were due to the presence of C–H, C=O, and C=N, respectively. In the ^{1}H NMR spectrum of compound **6a** the H-6 and H-7 each one appears as a doublet at $\delta = 7.61$ and $\delta =$ 7.52 ppm, respectively, with coupling constant between them of $^3J = 15.2$ and 14.6 Hz, which agrees with a trans configuration. The mass spectrum of **6a** showed a molecular ion peak at $m/z = 461$ (M+1), which is in agreement with the molecular formula $C_{30}H_{21}ClN_2O$. Furthermore, the compounds **6h** and **6j** were confirmed by single crystal X-ray analysis (Fig. 1).

Similarly, the IR spectrum of compound **7a** showed absorption bands at 3,042–2,923, 1,590, and 1,492 which were due to C–H, C=N, and C=C, respectively. In the ^{1}H NMR spectrum of compounds **7a**, the two methylenic 4′-H protons and the stereogenic 5′-H proton of the pyrazolinic moiety displayed a typical ABX type pattern of doublet of doublet. Thus, the two methylene protons (4′-H) displayed two signals; doublet of doublet at δ 4.08–4.01 ppm with coupling constants of 12 and 17.2 Hz and a doublet of doublet at δ 3.31–3.25 ppm with coupling constants of 7.6 and 17.6 Hz. Methine proton (5′-H) resonated as doublet of doublet at δ 5.54–5.49 ppm with coupling constants of 7.6 and 12.2 Hz. The pyrazole 5-H resonated as singlet at δ 8.36. In some cases, the doublet of doublet of methylene proton around δ 3.31 has not been observed clearly because of merging of this particular proton with the water signal from DMSO-d_6. The main signals in the ^{13}C NMR for **7a** corresponds to C-4′ at δ 42.8, C-5′ δ 56.4 ppm, and the quaternary C-3′ at δ 148.9 ppm. The mass spectrum of **7a** showed a molecular ion peak at m/z = 551(M+1), which is in agreement with the molecular formula $C_{36}H_{27}ClN_4$. Furthermore, the structure of compound **7c** was confirmed by single crystal X-ray analysis. 1,4-Dioxane was used as crystallization solvent for **7c**, which is in association with the crystal (Fig. 2). The crystallographic data of compounds **6h**, **6j**, and **7c** have been summarized in Table 1.

Antimicrobial activity

The newly synthesized compounds **7a–k** were tested for their antibacterial activity (in vitro) against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and their activity was compared to a well-known commercial

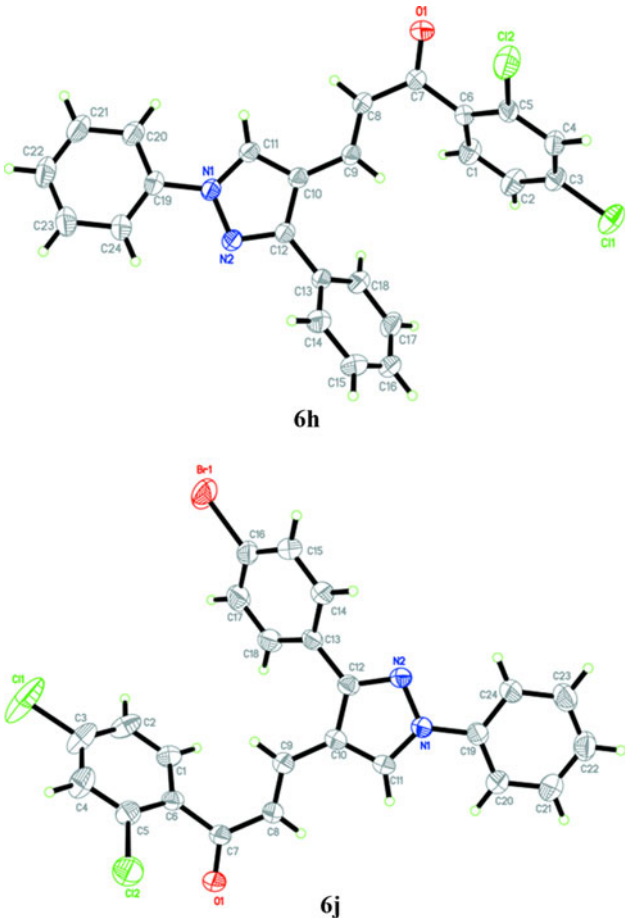


Fig. 1 Single crystal X-ray structures of compounds **6h** and **6j**

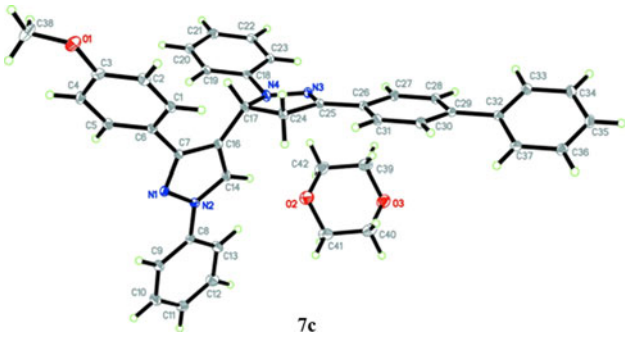


Fig. 2 The single crystal X-ray structure of compound **7c**

antibiotic, streptomycin. Antibacterial activity was carried out by well plate method by measuring its zone of inhibition. The compounds **7a–k** were screened for their antibacterial activity in triplicate against the above-mentioned bacterial strains at two different concentrations of 1,000 and 500 μg/mL as shown in (Table 2). Results revealed that in general, most of the tested compounds showed good activity against all the three bacterial strains. Six compounds **7a**, **7b**, **7e**, **7f**, **7i**, and **7j** showed good broad spectrum activity against all the tested bacterial strains. Compounds **7c**, **7g**, and **7k** were also relatively active against tested bacterial strains. All the synthesized compounds were also tested for their antifungal activity (in vitro) against *Aspergillus flavus*, *Chrysosporium keratinophilum*, and *Candida albicans* by measuring its average zone of inhibition (Table 3). Fluconazole was used as standard for antifungal activity. Among the tested compounds, **7a**, **7b**, **7e**, **7f**, **7i**, and **7j** were found to be moderately active against *Aspergillus flavus* and *Chrysosporium keratinophilum.* These compounds are also found to be slightly active against *Candida albicans.*

Based on these preliminary results, it can be seen that all the six compounds **7a**, **7b**, **7e**, **7f**, **7i**, and **7j** showing good antimicrobial activity have a halogen, Cl or Br as one of the substituents. However, in general, compounds containing a halogen substituents showed better antibacterial activity than the compounds with other substituent's which is also supported by the previous report (Sharma *et al.*, 2011). From the antimicrobial results we can conclude that synthesized compounds are specific antibacterial agents. Hence they are ideally suited for further modifications to obtain more efficacious antibacterial compounds.

Table 1 Crystallographic data for compounds **6h**, **6j** and **7c**

Crystal data	**6h**	**6j**	**7c**
Empirical formula	$C_{24}H_{16}Cl_2N_2O$	$C_{24}H_{15}BrCl_2N_2O$	$C_{37}H_{30}N_4O.C_4H_8O_2$
Formula weight	419.29	498.19	634.75
Temperature (K)	296	296	100
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	Triclinic	Monoclinic	Triclinic
Space group	$\overline{P1}$	$P2_1/c$	$\overline{P1}$
Cell dimensions			
a (Å)	9.6185 (8)	11.4203 (14)	11.1189 (2)
b (Å)	10.6596 (9)	9.9357 (13)	13.0541 (2)
c (Å)	11.8537 (10)	19.656 (3)	13.0852 (2)
Volume ($Å^3$)	1,044.64	2,222.9	1,662.48
Z	2	4	2
Density (Mg/m^3)	1.333	1.489	1.268
F (000)	432	1,000	672
Θ range for data collection (°)	2.4–28.8	2.9–22.2	2.3–29.9

Table 2 Antibacterial activity of compounds **7a–k**

Compound code	Zone of inhibition (mm)					
	Escherichia coli		*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
Concentration (μg/mL)	1,000	500	1,000	500	1,000	500
7a	07 ± 0.1	05 ± 0.2	08 ± 0.2	06 ± 0.2	04 ± 0.2	06 ± 0.1
7b	09 ± 0.2	06 ± 0.2	06 ± 0.1	04 ± 0.2	07 ± 0.2	05 ± 0.2
7c	04 ± 0.2	02 ± 0.1	04 ± 0.1	02 ± 0.2	05 ± 0.1	02 ± 0.1
7d	03 ± 0.1	01 ± 0.2	02 ± 0.2	00	03 ± 0.1	02 ± 0.1
7e	05 ± 0.1	04 ± 0.2	08 ± 0.2	05 ± 0.1	07 ± 0.1	04 ± 0.1
7f	07 ± 0.1	06 ± 0.1	09 ± 0.1	07 ± 0.2	08 ± 0.2	06 ± 0.1
7g	02 ± 0.2	01 ± 0.1	04 ± 0.2	03 ± 0.2	04 ± 0.1	02 ± 0.2
7h	00	00	00	00	00	00
7i	06 ± 0.2	04 ± 0.2	07 ± 0.2	04 ± 0.1	08 ± 0.1	06 ± 0.1
7j	08 ± 0.1	05 ± 0.1	08 ± 0.1	06 ± 0.1	05 ± 0.1	05 ± 0.1
7k	03 ± 0.2	02 ± 0.1	04 ± 0.1	03 ± 0.1	04 ± 0.2	01 ± 0.1
Streptomycin (Std.)	16 ± 0.2	10 ± 0.1	15 ± 0.2	10 ± 0.2	16 ± 0.2	13 ± 0.2

Table 3 Antifungal activity of compounds **7a–k**

Compound code	Zone of inhibition (mm)					
	Aspergillus flavus		*Chrysosporium keratinophilum*		*Candida albicans*	
Concentration (μg/mL)	1,000	500	1,000	500	1,000	500
7a	05 ± 0.1	03 ± 0.1	04 ± 0.2	03 ± 0.1	05 ± 0.1	02 ± 0.1
7b	06 ± 0.1	05 ± 0.2	04 ± 0.1	03 ± 0.1	05 ± 0.1	04 ± 0.1
7c	00	00	00	00	00	00
7d	00	00	00	00	00	00
7e	03 ± 0.1	02 ± 0.1	03 ± 0.1	01 ± 0.1	03 ± 0.1	01 ± 0.1
7f	04 ± 0.1	03 ± 0.2	06 ± 0.1	05 ± 0.2	04 ± 0.1	02 ± 0.1
7g	00	00	00	00	00	00
7h	00	00	00	00	00	00
7i	04 ± 0.1	02 ± 0.1	05 ± 0.1	02 ± 0.1	04 ± 0.1	02 ± 0.1
7j	04 ± 0.2	01 ± 0.1	04 ± 0.1	02 ± 0.1	03 ± 0.2	01 ± 0.2
7k	00	00	00	00	00	00
Fluconazole (Std.)	13 ± 0.2	10 ± 0.1	17 ± 0.2	15 ± 0.2	22 ± 0.2	20 ± 0.2

Conclusion

The present study highlights the synthesis and investigation of antimicrobial properties of a new series of pyrazolylpyrazolines with the hope of discovering new structure leads are served as antimicrobial agents. The structures of intermediate compounds **6h** and **6j** and the final compound **7c** hve been solved by single crystal X-ray analysis. Antimicrobial results showed that the compounds **7a**, **7b**, **7e**, **7f**, **7i**, and **7j** showed good broad spectrum activity against all the tested bacterial strains. Antifungal results showed that among the tested compounds, **7a**, **7b**, **7e**, **7f**, **7i**, and **7j** were found to be moderately active against *Aspergillus flavus* and *Chrysosporium keratinophilum* whereas slightly active against *Candida albicans.*

Experimental procedure

Chemistry

Melting points were determined by open capillary method and are uncorrected. The IR spectra were recorded on a Thermo Nicolet avatar 330-FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded (DMSO-d_6) on a Bruker (400 MHz) spectrometer. Chemical shift values are given in δ scales. The mass

spectra were recorded on LC–MS-Agilent 1100 series. Elemental analyses were performed on a Flash EA 1112 series CHNS-O analyzer. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminum sheets (silica gel 60 F254). Single crystal X-ray analysis was performed using Bruker APEXII CCD diffractometer. Commercial grade solvents and reagents were used without further purification.

General procedure for the preparation of chalcones **(6a–k)**

To a cold, stirred mixture of methanol (20 mL) and sodium hydroxide (12.09 mmol), appropriate acetophenone (**5**, 4.03 mmol) was added. The reaction mixture was stirred for 10 min. To this appropriate formyl pyrazole (**4**, 4.03 mmol) was added followed by tetrahydrofuran (30 mL). The solution was further stirred for 2 h at 0 °C and then at room temperature for 5 h. It was then poured into ice cold water. The resulting solution was neutralized with dil. HCl. The solid that separated out was filtered, washed with water, dried and crystallized from ethanol to afford product **6a–k**.

*(2E)-3-[3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-[4-biphenyl]prop-2-en-1-one (**6a**)*

Yield 79.3 %; mp 158–160 °C; IR (KBr, υ_{max} cm^{-1}): 3,122–3,055 (C–H), 1,659 (C=O), 1,593 (C=N); ^{1}H-NMR (DMSO-d_6): δ 8.32 (s, 1H, pyrazole-5H), 7.87 (d, 2H, $J = 7.5$ Hz, H_o biphenyl), 7.67 (d, 2H, $J = 7.8$ Hz, H_m biphenyl), 7.61 (d, 1H, $J = 15.2$ Hz, H-6), 7.52 (d, 1H, $J = 14.6$ Hz, H-7), 7.48 (m, 2H, H_o biphenyl), 7.42 (d, 2H, $J = 7.3$ Hz, H_oA), 7.34 (d, 2H, $J = 7.4$ Hz, H_mA), 7.32 (dd, 2H, $J = 7.8$ Hz, H_m biphenyl), 7.3 (m, 5H, Ar–H of B), 7.22 (m, 1H, H_p biphenyl). MS: m/z = 461 (M+1). Anal. calcd. for $C_{30}H_{21}ClN_2O$: C, 78.17; H, 4.59; N, 6.08. Found: C, 78.11; H, 4.54; N, 6.01 %.

*(2E)-3-[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-[4-biphenyl]prop-2-en-1-one (**6b**)*

Yield 77.7 %; mp 165–167 °C; IR (KBr, υ_{max} cm^{-1}): 3,116–3,052 (C–H), 1,656 (C=O), 1,596 (C=N); ^{1}H-NMR (DMSO-d_6): δ 8.37 (s, 1H, pyrazole-5H), 7.86 (d, 2H, $J = 7.51$ Hz, H_o biphenyl), 7.63 (d, 1H, $J = 15.2$ Hz, H-6), 7.65 (d, 2H, $J = 7.82$ Hz, H_m biphenyl), 7.55 (d, 1H, $J = 14.8$ Hz, H-7), 7.49 (d, 2H, $J = 7.7$ Hz, H_mA), 7.46 (m, 2H, H_o biphenyl), 7.36 (d, 2H, $J = 7.6$ Hz, H_oA), 7.34 (dd, 2H, $J = 7.8$ Hz, H_m biphenyl), 7.31 (m, 5H, Ar–H of B), 7.23 (m, 1H, H_p biphenyl). MS: m/z = 507 (M+2). Anal. calcd. for $C_{30}H_{21}BrN_2O$: C, 71.29; H, 4.19; N, 5.54. Found: C, 71.23; H, 4.14; N, 5.48 %.

*(2E)-3-[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-1-[4-biphenyl]prop-2-en-1-one (**6c**)*

Yield 80.8 %; mp 183–185 °C; IR (KBr, υ_{max} cm^{-1}): 3,118–3,047 (C–H), 1,654 (C=O), 1,580 (C=N); ^{1}H-NMR (DMSO-d_6): δ 8.34 (s, 1H, pyrazole-5H), 7.82 (d, 2H, $J = 7.5$ Hz, H_o biphenyl), 7.69 (d, 1H, $J = 15.6$ Hz, H-6), 7.64 (d, 2H, $J = 7.79$ Hz, H_m biphenyl), 7.54 (d, 1H, $J = 15.2$ Hz, H-7), 7.44 (m, 2H, H_o biphenyl), 7.37 (d, 2H, $J = 7.2$ Hz, H_oA), 7.34 (m, 5H, Ar–H of B), 7.32 (dd, 2H, $J = 7.5$ Hz, H_m biphenyl), 7.25 (m, 1H, H_p biphenyl), 6.89 (d, 2H, $J = 7.6$ Hz, H_mA). MS: m/z = 457 (M+1). Anal. calcd. for $C_{31}H_{24}N_2O_2$: C, 81.56; H, 5.30; N, 6.14. Found: C, 81.49; H, 5.26; N, 6.11 %.

*(2E)-3-(1,3-diphenyl-1H-pyrazol-4-yl)-1-[4-(methylsulfanyl)phenyl]prop-2-en-1-one (**6d**)*

Yield 74.6 %; mp 136–138 °C; IR (KBr, υ_{max} cm^{-1}): 3,122–3,051 (C–H), 1,662 (C=O), 1,586 (C=N); ^{1}H-NMR (DMSO-d_6): δ 8.33 (s, 1H, pyrazole-5H), 7.71 (d, 2H, $J = 7.65$ Hz, H_m SCH_3-phenyl), 7.65 (d, 1H, $J = 15.7$ Hz, H-6), 7.56 (d, 1H, $J = 15.4$ Hz, H-7), 7.48 (m, 2H, H_oA), 7.37 (d, 2H, $J = 7.6$ Hz, H_o SCH_3-phenyl),7.34 (m, 5H, Ar–H of B), 7.32 (dd, 2H, $J = 7.8$ Hz, H_mA), 7.22 (m, 1H, H_pA), 2.49 (s, 3H, SCH_3). MS: m/z = 397 (M+1). Anal. calcd. for $C_{25}H_{20}N_2OS$: C, 75.73; H, 5.08; N, 7.07. Found: C, 75.68; H, 5.01; N, 7.02 %.

*(2E)-3-[3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-[4-(methylsulfanyl)phenyl]prop-2-en-1-one (**6e**)*

Yield 73.3 %; mp 160–162 °C; IR (KBr, υ_{max} cm^{-1}): 3,118–3,057 (C–H), 1,647 (C=O), 1,583 (C=N); ^{1}H-NMR (DMSO-d_6): δ 8.35 (s, 1H, pyrazole-5H), 7.72 (d, 2H, $J = 7.6$ Hz, H_m SCH_3-phenyl), 7.68 (d, 1H, $J = 15.4$ Hz, H-6), 7.58 (d, 1H, $J = 15.6$ Hz, H-7), 7.42 (d, 2H, $J = 7.4$ Hz H_o A), 7.38 (d, 2H, $J = 7.6$ Hz, H_o SCH_3-phenyl), 7.35 (m, 5H, Ar–H of B), 7.33 (d, 2H, $J = 7.7$ Hz, H_mA), 2.49 (s, 3H, SCH_3). MS: m/z = 431 (M+1). Anal. calcd. for $C_{25}H_{19}ClN_2OS$: C, 69.68; H, 4.44; N, 6.50. Found: C, 69.62; H, 4.38; N, 6.46 %.

*(2E)-3-[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-[4-(methylsulfanyl)phenyl] prop-2-en-1-one (**6f**)*

Yield 73.5 %; mp 156–158 °C; IR (KBr, υ_{max} cm^{-1}): 3,116–3,060 (C–H), 1,648 (C=O), 1,583 (C=N); ^{1}H-NMR (DMSO-d_6): δ 8.34 (s, 1H, pyrazole-5H), 7.75 (d, 2H, $J = 7.7$ Hz, H_m SCH_3-phenyl), 7.64 (d, 1H, $J = 15.7$ Hz, H-6), 7.54 (d, 1H, $J = 15.4$ Hz, H-7), 7.49 (d, 2H, $J = 7.6$ Hz, H_mA), 7.39 (d, 2H, $J = 7.5$ Hz H_oA), 7.36 (m, 5H, Ar–H of B), 7.33 (d, 2H, $J = 7.8$ Hz, H_o SCH_3-

phenyl), 2.49 (s, 3H, SCH_3). MS: m/z = 477 (M+2). Anal. calcd. for $C_{25}H_{19}BrN_2OS$: C, 63.16; H, 4.03; N, 5.89. Found: C, 63.11; H, 3.98; N, 5.80 %.

*(2E)-3-[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]-1-[4-(methylsulfanyl)phenyl]prop-2-en-1-one (**6g**)*

Yield 74.1 %; mp 140–142 °C; IR (KBr, υ_{max} cm^{-1}): 3,114–3,053 (C–H), 1,657 (C=O), 1,592 (C=N); ^{1}H-NMR (DMSO-d_6): δ 8.31 (s, 1H, pyrazole-5H), 7.78 (d, 2H, J = 7.6 Hz, H_m SCH_3-phenyl), 7.62 (d, 1H, J = 15.8 Hz, H-6), 7.52 (d, 1H, J = 15.6 Hz, H-7), 7.38 (d, 2H, J = 7.5 Hz H_oA), 7.36 (m, 5H, Ar–H of B), 7.34 (d, 2H, J = 7.6 Hz, H_o SCH_3-phenyl), 6.83 (d, 2H, J = 7.4 Hz, H_mA), 3.82 (s, 3H, OCH_3), 2.5 (s, 3H, SCH_3). MS: m/z = 427 (M+1). Anal. calcd. for $C_{26}H_{22}N_2O_2S$: C, 73.21; H, 5.20; N, 6.57. Found: C, 73.17; H, 5.15; N, 6.51 %.

*(2E)-1-(2,4-dichlorophenyl)-3-(1,3-diphenyl-1H-pyrazol-4-yl)prop-2-en-1-one (**6h**)*

Yield 76.19 %; mp 133–135 °C; IR (KBr, υ_{max} cm^{-1}): 3,121–3,066 (C–H), 1,687 (C=O), 1,586 (C=N); MS: m/z = 419 (M+). Anal. calcd. for $C_{24}H_{16}Cl_2N_2O$: C, 68.75; H, 3.85; N, 6.68. Found: C, 68.71; H, 3.79; N, 6.60 %. Crystal structure of **6h** has been presented in Fig. 1 (Fun *et al*., 2011a).

*(2E)-3-[3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-(2,4-dichlorophenyl)prop-2-en-1-one (**6i**)*

Yield 70.83 %; mp 165–167 °C; IR (KBr, υ_{max} cm^{-1}): 3,118–3,088 (C–H), 1,658 (C=O), 1,585 (C=N). ^{1}H-NMR (DMSO-d_6): δ 8.45 (s, 1H, pyrazole-5H), 7.7 (d, 1H, J = 7.6 Hz, H_o 2,4-Cl_2-phenyl), 7.68 (d, 1H, J = 14.9 Hz, H-6), 7.53 (d, 1H, J = 15.6 Hz, H-7), 7.47 (s, 1H, J = 7.5 Hz, H_m 2,4-Cl_2-phenyl), 7.43 (d, 2H, J = 7.6 Hz H_oA), 7.38 (d, 1H, J = 7.2 Hz, H_m 2,4-Cl_2-phenyl), 7.35 (d, 2H, J = 7.8 Hz, H_mA), 7.3 (m, 5H, Ar–H of B). MS: m/z = 454 (M+1). Anal. calcd. for $C_{24}H_{15}Cl_3N_2O$: C, 63.53; H, 3.33; N, 6.17. Found: C, 63.47; H, 3.29; N, 6.11 %.

*(2E)-3-[3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-(2,4-dichlorophenyl)prop-2-en-1-one (**6j**)*

Yield 80.0 %; mp 184–186 °C; IR (KBr, υ_{max} cm^{-1}): 3,122–3,060 (C–H), 1,683 (C=O), 1,584 (C=N). MS: m/z = 499 (M+1). Anal. calcd. for $C_{24}H_{15}BrCl_2N_2O$: C, 57.86; H, 3.03; N, 5.62. Found: C, 57.81; H, 2.97; N, 5.58 %. Crystal structure of **6j** has been presented in Fig. 1 (Fun *et al*., 2011b).

*(2E)-1-(2,4-dichlorophenyl)-3-[3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl]prop-2-en-1-one (**6k**).*

Yield 80.55 %; mp 174–176 °C; IR (KBr, υ_{max} cm^{-1}): 3,118–3,071 (C–H), 1,687 (C=O), 1,610 (C=N); ^{1}H-NMR (DMSO-d_6): δ 8.35 (s, 1H, pyrazole-5H), 7.67 (d, 1H, J = 7.4 Hz, H_o 2,4-Cl_2-phenyl), 7.63 (d, 1H, J = 15.5 Hz, H-6), 7.59 (d, 1H, J = 15.2 Hz, H-7), 7.45 (s, 1H, H_m 2,4-Cl_2-phenyl), 7.39 (d, 1H, H_m J = 7.4 2,4-Cl_2-phenyl), 7.36 (m, 5H, Ar–H of B), 7.32 (d, 2H, J = 7.6 Hz, H_oA), 6.83 (d, 2H, J = 7.3 Hz, H_mA). MS: m/z = 449 (M+). Anal. calcd. for $C_{25}H_{18}Cl_2N_2O$: C, 66.83; H, 4.04; N, 6.23. Found: C, 66.78; H, 3.97; N, 6.18 %.

General procedure for the synthesis of pyrazolines **(7a–k)**

A mixture of chalcone **6a–k** (1.0 mmol) and phenylhydrazine **2** (1.5 mmol) was refluxed in glacial acetic acid (8 mL) for 4–5 h. Reaction progress was monitored by TLC and the precipitate formed was filtered off and recrystallized from ethanol, affording compounds **7a–k**.

*3-(4-chlorophenyl)-1-phenyl-4-(1-phenyl-3-biphenyl-4,5-dihydro-1H-pyrazol-5-yl)-1H-pyrazole (**7a**)*

Yield 76.0 %; mp 211–213 °C; IR (KBr, υ_{max} cm^{-1}): 3,042 (Ar–C–H), 2,923 (C–H), 1,590 (C=N), 1,492 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.36 (s, 1H, pyrazole-5H), 7.83–7.69 (m, 10H, Ar–H), 7.55 (d, 2H, J = 8.4 Hz, Ar–H), 7.48–7.41 (m, 5H, Ar–H), 7.27 (dd, 1H, J = 7.2 Hz, Ar–H), 7.15 (dd, 2H, J = 8 Hz, Ar–H), 7.03 (d, 2H, J = 7.6 Hz, Ar–H), 6.73 (dd, 1H, J = 7.2 Hz, Ar–H), 5.58–5.53 (dd, 1H, J = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.08–4.01 (dd, 1H, J = 12 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.31-3.25 (dd, 1H, J = 7.6 Hz, 17.6 Hz, 4′-H of pyrazoline). ^{13}C-NMR (DMSO-d_6): δ 148.9, 147.8, 145.0, 140.6, 139.9, 139.5, 130.1, 129.9, 129.4, 129.3, 129.2, 127.0, 126.8, 119.5, 118.7, 113.8, 56.4, 42.8. MS: m/z = 551 (M+1). Anal. calcd. for $C_{36}H_{27}ClN_4$: C, 78.46; H, 4.94; N, 10.17. Found: C, 78.39; H, 4.88; N, 10.13 %.

*3-(4-bromophenyl)-1-phenyl-4-(1-phenyl-3-biphenyl-4,5-dihydro-1H-pyrazol-5-yl)-1H-pyrazole (**7b**)*

Yield 85.0 %; mp 224–226 °C; IR (KBr, υ_{max} cm^{-1}): 3,041 (Ar–C–H), 2,920 (C–H), 1,590 (C=N), 1,492 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.38 (s, 1H, pyrazole-5H), 7.85–7.69 (m, 12H, Ar–H), 7.51–7.37 (m, 5H, Ar–H), 7.29 (dd, 1H, J = 7.4 Hz, Ar–H), 7.17 (dd, 2H, J = 8 Hz, Ar–H), 7.05 (d, 2H, J = 7.6 Hz), 6.76 (dd, 1H, J = 7.2 Hz, Ar–H), 5.61–5.56 (dd, 1H, J = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.11–4.03 (dd, 1H, J = 12 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.33–3.27

(m, 1H, 4′-H of pyrazoline merged with peak of HOD). ^{13}C-NMR (DMSO-d_6): δ 148.9, 147.8, 145.0, 140.6, 139.9, 139.5, 132.1, 130.4, 129.9, 129.4, 129.3, 128.1, 127.8, 127.2, 127.0, 126.8, 119.5, 118.7, 113.8, 56.4, 42.8. MS: m/z = 595 (M+1). Anal. calcd. for $C_{36}H_{27}BrN_4$: C, 72.61; H, 4.57; N, 9.41. Found: C, 72.57; H, 4.52; N, 9.37 %.

*3-(4-methoxyphenyl)-1-phenyl-4-(1-phenyl-3-biphenyl-4,5-dihydro-1H-pyrazol-5-yl)-1H-pyrazole (**7c**)*

Yield 87.0 %; mp 163–165 °C; IR (KBr, υ_{max} cm^{-1}): 3,031 (Ar–C–H), 2,939 (C–H), 1,593 (C=N), 1,491 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.31 (s, 1H, pyrazole-5H), 7.83–7.80 (m, 4H, Ar–H), 7.74–7.70 (m, 6H, Ar–H), 7.49–7.35 (m, 5H, Ar–H), 7.25 (dd, 1H, J = 7.4 Hz, Ar–H), 7.14 (dd, 2H, J = 8 Hz, Ar–H), 7.07–7.0 (m, 4H, Ar–H), 6.73 (dd, 1H, J = 7.2 Hz, Ar–H), 5.54–5.49 (dd, 1H, J = 7.6 Hz, 12.4 Hz, 5′-H of pyrazoline), 4.08–4.01 (dd, 1H, J = 12.4 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.80 (s, 3H, –OCH_3), 3.32–3.26 (m, 1H, 4′-H of pyrazoline merged with peak of HOD). ^{13}C-NMR (DMSO-d_6): δ 159.7, 150.0, 147.7, 145.0, 140.5, 139.9, 139.7, 131.9, 129.9, 129.7, 129.4, 129.3, 128.1, 127.4, 127.2, 127.0, 126.8, 126.6, 125.5, 122.9, 119.4, 118.5, 114.6, 113.8, 56.5, 55.6, 42.8. MS: m/z = 547 (M+1). Anal. calcd. for $C_{37}H_{30}N_4O$: C, 81.29; H, 5.53; N, 10.25. Found: C, 81.24; H, 5.49; N, 10.20 %. Crystal structure of **7c** has been presented in Fig. 2 (Fun *et al*., 2012).

*4-(3-(4-(methylthio)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole (**7d**)*

Yield 81.0 %; mp 208–210 °C; IR (KBr, υ_{max} cm^{-1}): 3,042 (Ar–C–H), 2,917 (C–H), 1,590 (C=N), 1,492 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.33 (s, 1H, pyrazole-5H), 7.83–7.77 (2d, 4H, J = 8 Hz, J = 7.2 Hz, Ar–H), 7.67 (d, 2H, J = 8.4, Ar–H), 7.52–7.41 (m, 5H, Ar–H), 7.30–7.24 (m, 3H, Ar–H), 7.12 (dd, 2H, J = 7.8 Hz, Ar–H), 6.98 (d, 2H, J = 7.6 Hz, Ar–H), 6.71 (dd, 1H, J = 7.2 Hz, Ar–H), 5.51–5.46 (dd, 1H, J = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.05–3.97 (dd, 1H, J = 12.4 Hz, 17.0 Hz, 4′-H of pyrazoline), 3.27–3.21 (dd, 1H, J = 8.0 Hz, 17.0 Hz, 4′-H of pyrazoline), 2.49 (s, 3H, –SCH_3, merged with DMSO-d_6 peak). ^{13}C-NMR (DMSO-d_6): δ 150.1, 147.8, 145.1, 139.6, 139.5, 129.9, 129.3, 129.2, 128.6, 128.4, 126.8, 126.6, 126.1, 119.3, 118.6, 113.7, 56.49, 42.9, 14.9. MS: m/z = 487 (M+1). Anal. calcd. for $C_{31}H_{26}N_4S$: C, 76.51; H, 5.39; N, 11.51. Found: C, 76.47; H, 5.34; N, 11.47 %.

*3-(4-chlorophenyl)-4-(3-(4-(methylthio)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole (**7e**)*

Yield 78.0 %; mp 219–221 °C; IR (KBr, υ_{max} cm^{-1}): 3,038 (Ar–C–H), 2,916 (C–H), 1,586 (C=N), 1,488 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.35 (s, 1H, pyrazole-5H), 7.83–7.78 (m, 4H, Ar–H), 7.67 (d, 2H, J = 8.8, Ar–H), 7.54 (d, 2H, J = 8.8 Hz, Ar–H), 7.43 (dd, 2H, J = 8.0 Hz, Ar–H), 7.30–7.27 (m, 3H, Ar–H), 7.13 (dd, 2H, J = 8.0 Hz, Ar–H), 6.99 (d, 2H, J = 7.6 Hz, Ar–H), 6.72 (d, 1H, J = 7.4 Hz, Ar–H), 5.53–5.48 (dd, 1H, J = 7.6 Hz, 12.4 Hz, 5′-H of pyrazoline), 4.03–3.96 (dd, 1H, J = 12.0 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.25–3.19 (dd, 1H, J = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline), 2.49 (s, 3H, –SCH_3, merged with DMSO-d_6 peak). ^{13}C-NMR (DMSO-d_6): δ 148.9, 147.8, 145.1, 139.5, 130.1, 129.9, 129.3, 129.2, 127.8, 126.9, 126.6, 126.1, 119.4, 118.7, 113.8, 56.4, 42.8, 14.9. MS: m/z = 521 (M+1). Anal. calcd. for $C_{31}H_{25}ClN_4S$: C, 71.45; H, 4.84; N, 10.75. Found: C, 71.39; H, 4.80; N, 10.71 %.

*3-(4-bromophenyl)-4-(3-(4-(methylthio)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole (**7f**)*

Yield 76.0 %; mp 206–208 °C; IR (KBr, υ_{max} cm^{-1}): 3,034 (Ar–C–H), 2,916 (C–H), 1,586 (C=N), 1,490 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.34 (s, 1H, pyrazole-5H), 8.03 (d, 1H, J = 8.4 Hz, Ar–H), 7.94 (d, 1H, J = 7.6 Hz, Ar–H), 7.87–7.56 (m, 5H, Ar–H), 7.45–7.41 (m, 3H, Ar–H), 7.30–7.25 (m, 3H, Ar–H), 7.13 (dd, 2H, J = 8.0 Hz, Ar–H), 6.99 (d, 2H, J = 7.6 Hz, Ar–H), 6.72 (dd, 1H, J = 7.2 Hz, Ar–H), 5.53–5.48 (dd, 1H, J = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.03–3.96 (dd, 1H, J = 12.0 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.25–3.19 (dd, 1H, J = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline), 2.49 (s, 3H, –SCH_3). MS: m/z = 565 (M+). Anal. calcd. for $C_{31}H_{25}BrN_4S$: C, 65.84; H, 4.46; N, 9.91. Found: C, 65.79; H, 4.41; N, 9.88 %.

*3-(4-methoxyphenyl)-4-(3-(4-(methylthio)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole (**7g**)*

Yield 72.0 %; mp 210–212 °C; IR (KBr, υ_{max} cm^{-1}): 3,011 (Ar–C–H), 2,917 (C–H), 1,591 (C=N), 1,491 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.30 (s, 1H, pyrazole-5H), 7.81 (d, 2H, J = 7.6 Hz, Ar–H), 7.71 (d, 2H, J = 8.8 Hz, Ar–H), 7.65 (d, 2H, J = 8.4 Hz, Ar–H), 7.42 (dd, 2H, J = 8.0 Hz, Ar–H), 7.30–7.23 (m, 4H, Ar–H), 7.12 (dd, 2H, J = 8.0 Hz, Ar–H), 7.06 (d, 2H, J = 8.8 Hz, Ar–H), 6.98 (d, 2H, J = 7.6 Hz, Ar–H), 6.71 (dd, 1H, J = 7.2 Hz, Ar–H), 5.48–5.43 (dd, 1H, J = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.03–3.95 (dd, 1H, J = 12.0 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.8 (s, 3H, –OCH_3), 3.25–3.19 (dd, 1H, J = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline), 2.49 (s, 3H, –SCH_3). MS: m/z = 517 (M+1). Anal. calcd. for $C_{32}H_{28}N_4OS$: C, 74.39; H, 5.46; N, 10.84. Found: C, 74.34; H, 5.41; N, 10.80 %.

*4-(3-(2,4-dichlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole (**7h**)*

Yield 71.0 %; mp 196–198 °C; IR (KBr, υ_{max} cm^{-1}): 3,058 (Ar–C–H), 2,871 (C–H), 1,590 (C=N), 1,494 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.39 (s, 1H, pyrazole-5H), 7.83 (d, 2H, J = 7.6 Hz, Ar–H), 7.78–7.72 (m, 3H, Ar–H), 7.67 (d, 1H, J = 2.4 Hz, Ar–H), 7.51–7.4 (m, 6H, Ar–H), 7.28 (dd, 1H, J = 7.4 Hz, Ar–H), 7.14 (dd, 2H, J = 8.0 Hz, Ar–H), 7.0 (d, 2H, J = 8.0 Hz, Ar–H), 6.75 (dd, 1H, J = 7.4 Hz, Ar–H), 5.59–5.54 (dd, 1H, J = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.15–4.08 (dd, 1H, J = 12.0 Hz, 17.4 Hz, 4′-H of pyrazoline), 3.42–3.36 (dd, 1H, J = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline). MS: m/z = 508 (M+). Anal. calcd. for $C_{30}H_{22}Cl_2N_4$: C, 70.73; H, 4.35; N, 11.00. Found: C, 70.69; H, 4.31; N, 10.97 %.

*3-(4-chlorophenyl)-4-(3-(2,4-dichlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole (**7i**)*

Yield 69.0 %; mp 185–187 °C; IR (KBr, υ_{max} cm^{-1}): 3,047 (Ar–C–H), 2,904 (C–H), 1,590 (C=N), 1,496 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.5 (s, 1H, pyrazole-5H), 7.83 (d, 2H, J = 7.6 Hz, Ar–H), 7.77–7.72 (m, 3H, Ar–H), 7.67 (d, 1H, J = 2.4 Hz, Ar–H), 7.53–7.43 (m, 5H, Ar–H), 7.28 (dd, 1H, J = 7.4 Hz, Ar–H), 7.16 (dd, 2H, J = 8.0 Hz, Ar–H), 7.02 (d, 2H, J = 8.0 Hz, Ar–H), 6.76 (dd, 1H, J = 7.4 Hz, Ar–H), 5.61–5.56 (dd, 1H, J = 7.6 Hz, 12.0 Hz, 5′-H of pyrazoline), 4.13–4.06 (dd, 1H, J = 12.4 Hz, 17.2 Hz, 4′-H of pyrazoline), 3.40–3.33 (dd, 1H, J = 7.6 Hz, 17.6 Hz, 4′-H of pyrazoline). MS: m/z = 543 (M+1). Anal. calcd. for $C_{30}H_{21}Cl_3N_4$: C, 66.25; H, 3.89; N, 10.30. Found: C, 66.20; H, 3.83; N, 10.25 %.

*3-(4-bromophenyl)-4-(3-(2,4-dichlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole (**7j**)*

Yield 70.0 %; mp 163–165 °C; IR (KBr, υ_{max} cm^{-1}): 3,035 (Ar–C–H), 2,922 (C–H), 1,589 (C=N), 1,495 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.4 (s, 1H, pyrazole-5H), 7.83 (d, 2H, J = 7.6 Hz, Ar–H), 7.77 (d, 1H, J = 8.4 Hz, Ar–H), 7.69–7.64 (m, 4H, Ar–H), 7.48–7.43 (m, 4H, Ar–H), 7.28 (dd, 1H, J = 7.4 Hz, Ar–H), 7.16 (dd, 2H, J = 8.0 Hz, Ar–H), 7.02 (d, 2H, J = 8.0 Hz, Ar–H), 6.76 (dd, 1H, J = 7.2 Hz, Ar–H), 5.61–5.56 (dd, 1H, J = 7.6 Hz, 12.2 Hz, 5′-H of pyrazoline), 4.13–4.05 (dd, 1H, J = 12.4 Hz, 17.4 Hz, 4′-H of pyrazoline), 3.39–3.33 (dd, 1H, J = 7.6 Hz, 17.2 Hz, 4′-H of pyrazoline). MS: m/z = 587 (M+1). Anal. calcd. for $C_{30}H_{21}BrCl_2N_4$: C, 61.25; H, 3.60; N, 9.52. Found: C, 61.21; H, 3.57; N, 9.48 %.

*4-(3-(2,4-dichlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazole (**7k**)*

Yield 68.0 %; mp 160–162 °C; IR (KBr, υ_{max} cm^{-1}): 3,064 (Ar–C–H), 2,926 (C–H), 1,594 (C=N), 1,489 (C=C); ^{1}H-NMR (DMSO-d_6): δ 8.36 (s, 1H, pyrazole-5H), 7.82, 7.80, 7.78 (d, 3H, J = 8 Hz, Ar–H), 7.67–7.64 (d, 3H, J = 12 Hz, Ar–H), 7.48–7.42 (m, 3H, Ar–H), 7.26 (dd, 1H, J = 6 Hz, Ar–H), 7.15 (t, 2H, J = 6 Hz, Ar–H), 7.05–7.0 (m, 4H, Ar–H), 6.76 (dd, 1H, J = 8 Hz, Ar–H), 5.57–5.52 (dd, 1H, J = 8 Hz, 12 Hz, 5′-H of pyrazoline), 4.13-4.06 (dd, 1H, J = 12 Hz, 16 Hz, 4′-H of pyrazoline), 3.80 (s, 3H, –OCH_3), 3.40–3.34 (dd, 1H, J = 8 Hz, 16 Hz, 4′-H of pyrazoline). Anal. calcd. for $C_{31}H_{24}Cl_2N_4O$: C, 69.02; H, 4.48; N, 10.39. Found: C, 68.97; H, 4.41; N, 10.34 %.

Antimicrobial activity

The following bacteria and fungi were used for the experiment. Bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. All bacterial strains were maintained on nutrient agar medium at ±37 °C. Fungi: *Aspergillus flavus, Chrysosporium keratinophilum*, and *Candida albicans MTCC 227* are used in this study. These cultures are obtained from the Department of Microbiology, Kuvempu University, Shimoga. All fungi strains were maintained on potato dextrose agar (PDA) at ±25 °C.

Antibacterial activity

The antibacterial activity of newly synthesized compounds (**7a–k**) were determined by well plate method in Mueller–Hinton Agar (Arthington-Skaggs *et al*., 2000; Rocha *et al*., 1995). The compounds were tested against a panel of pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Microorganism strains were maintained on nutrient agar medium at 37 °C. The cultures were inoculated in fresh 10 mL nutrient broth to yield an initial suspension of approximately 10-100 cfu/mL. All broths were then incubated statically at the aforementioned temperatures for microorganisms, for 18–24 h so that all cells were in the stationary phase. Susceptibility of the test organism to the compounds was determined by employing in the well plate technique. The bacterial suspensions were diluted tenfold in distilled water, and 0.1 mL from the appropriate dilution was spread plated on nutrient agar to give a population of approximately 10^6 cfu/plate. Six millimeter diameter well was then punched carefully using a sterile cork borer and 30 μL of test solutions of different concentrations (1 and 0.5 mg/mL) were added into each labeled well. The same procedure was repeated

for different microorganisms. Each experiment was carried out in triplicate. After the inoculation of the organism and compound, the Petri plates were incubated for 24 h at 37 °C. After the incubation, the inhibition zone was measured and the values for Dimethylsulfoxide (DMSO) were subtracted to get the actual values. Streptomycin was used as standard drug.

Antifungal activity

Antifungal studies of newly synthesized compounds **(7a–k)** were carried out against *Aspergillus flavus*, *Chrysosporium keratinophilum*, and *Candida albicans*. Sabourands agar media were prepared by dissolving peptone (10 g), D-glucose (40 g), and agar (20 g) in distilled water (1,000 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in incubator at 37 °C for 1 h. Wells were made on these seeded agar plates using sterile cork borer punched carefully, and different concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 25 °C for 72 h. Antifungal activity was determined by measuring the diameter of inhibition zone. The activity of each compound was compared with Fluconazole as standard (MacLowry *et al*., 1970; Portillo *et al*., 2001).

Acknowledgments The Authors extend their appreciation to The Deanship of Scientific Research at King Saud University for the funding the work through the research group project No. RGP-VPP-207.

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