Monatsh Chem
DOI 10.1007/s00706-014-1371-x

ORIGINAL PAPER

Design, synthesis, and pharmacological evaluation of new pyrazoline derivatives

Majal Sapnakumari · Badiadka Narayana · Purawarga Matada Gurubasavarajswamy · Balladka Kunhanna Sarojini

Received: 1 October 2014 / Accepted: 24 November 2014

Abstract In the present investigation, a new series of pyrazoline derivatives has been synthesized by the reaction of chalcones with hydrazine derivatives like hydrazine hydrate and phenyl hydrazines in the presence of different aliphatic acids. The chemical structure of the newly prepared compounds was characterized by ^{1}H NMR, ^{13}C NMR, FTIR, LC–MS and some of them by single crystal XRD. The synthesized compounds were screened for their antimicrobial, antitubercular, DPPH scavenging, and anti-inflammatory properties. Some of the tested compounds exhibited promising antimicrobial, antitubercular, DPPH scavenging, and anti-inflammatory activities.

Keywords Chalcone · Antimicrobial activity · Anti-inflammatory activity · Antitubercular activity · DPPH scavenging activity

Introduction

Pyrazolines are well-known nitrogen-containing five-membered heterocyclic compounds and various methods have been reported for their synthesis [1]. Pyrazolines occupy a major place in medicinal and pesticide chemistry due to their capability to exhibit a wide range of bioactivities. 2-Pyrazolines seem to be the most frequently studied pyrazoline-type compounds. Compounds with pyrazoline moieties were known to possess antidepressant, antitubercular, antipyretic, analgesic, tranquilizing, muscle relaxant, psychoanaleptic, antiepileptic, antidepressant, anti-inflammatory, insecticidal, antimicrobial, and antihypotensive activities [2, 3]. Their derivatives were also found to exhibit cytotoxic activity, inhibitory activity of platelet aggregation, herbicidal activity, and cannabinoid CB1-receptor modulators. 1,3,5-Triaryl-2-pyrazolines were also used as scintillation solutes. Pyrazoline derivatives with a phenyl group at the 5-position found to possess good film-forming properties, exhibit excellent characteristics of blue photoluminescence, fluorescence, and electroluminescence [4]. Also, literature review revealed that phenyl ring with halo substituent enhances the antimicrobial, antitubercular, and anti-inflammatory activities [5–7]. Based on their utility in pharmaceutical and other fields, a new series of pyrazoline derivatives was synthesized and screened for their pharmacological activities.

Results and discussion

The synthetic route for pyrazoline derivatives **2a**–**2j** and **3a**–**3f** is depicted in Scheme 1. Accordingly, the chalcones **1a**–**1c** were reacted with hydrazine hydrate in the presence of various aliphatic acids, namely formic acid, acetic acid, propionic acid, butyric acid, and 2-chloropropionic acid to yield *N*-alkyloxypyrazoline derivatives **2a**–**2j**. Also, the reaction of chalcones with phenyl hydrazine and 4-nitrophenyl hydrazine yielded 1,3,5-triaryl-2-pyrazolines **3a**–**3f**.

M. Sapnakumari · B. Narayana (✉)
Department of Studies in Chemistry, Mangalore University, Mangalagangotri 574 199, Karnataka, India
e-mail: nbadiadka@yahoo.co.uk

P. M. Gurubasavarajswamy
Department of Pharmaceutical Chemistry, Acharya and B M Reddy College of Pharmacy, Soledevanahalli, Bangalore 560 090, Karnataka, India

B. K. Sarojini
Industrial Chemistry Section, Department of Studies in Chemistry, Mangalore University, Mangalagangotri 574 199, Karnataka, India

Published online: 20 December 2014

The chalcones **1a**–**1c** in turn were prepared by the Claisen–Schmidt condensation of 4-fluoroacetophenone with different aldehydes. The structure of the newly synthesized compounds was characterized by ^{1}H NMR, ^{13}C NMR, FTIR, LC–MS, elemental analysis, and also by single crystal XRD data. Characterization data of pyrazoline derivatives are given in Table 1.

IR spectra of all the compounds exhibited absorption band due to C=N stretching vibrations in the range of 1,598–1,608 cm^{-1} confirmed the formation of pyrazolines. Also, in the IR spectra of the newly synthesized *N*-alkyloxypyrazoline derivatives, appearance of a strong band in the region 1,651–1,664 cm^{-1} along with C=N stretching, corresponds to the carbonyl group suggested the formation of *N*-alkyloxy-substituted pyrazolines.

Scheme 1

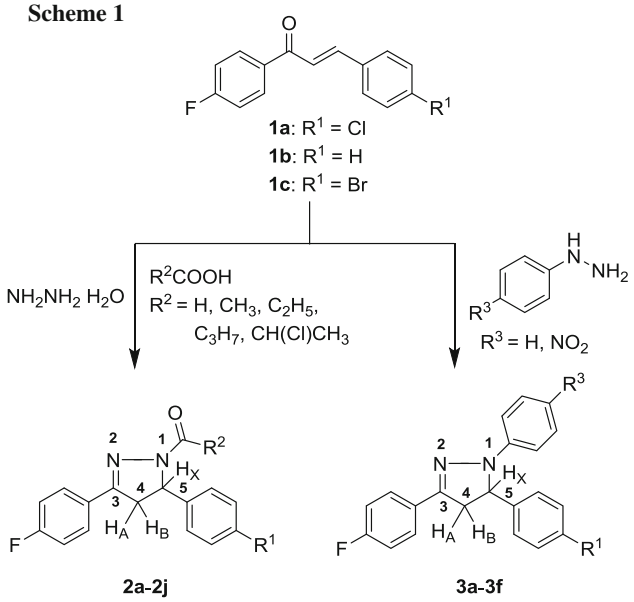

Table 1 Characterization data of pyrazoline derivatives **2a**–**2j** and **3a**–**3f**

Comp.	Molecular formula	R^1	R^2	R^3	Yield/%	M.p./°C
2a	$C_{16}H_{12}ClFN_2O$	Cl	H	–	62	129–131
2b	$C_{17}H_{15}FN_2O$	H	CH_3	–	79	119–121
2c	$C_{17}H_{14}BrFN_2O$	Br	CH_3	–	84	99–101
2d	$C_{18}H_{16}ClFN_2O$	Cl	C_2H_5	–	89	86–88
2e	$C_{18}H_{16}BrFN_2O$	Br	C_2H_5	–	73	88–90
2f	$C_{19}H_{18}ClFN_2O$	Cl	C_3H_7	–	61	98–100
2g	$C_{19}H_{19}FN_2O$	H	C_3H_7	–	33	83–85
2h	$C_{19}H_{18}BrFN_2O$	Br	C_3H_7	–	51	110–112
2i	$C_{18}H_{15}Cl_2FNO$	Cl	$CHClCH_3$	–	18	152–154
2j	$C_{18}H_{16}ClFN_2O$	H	$CHClCH_3$	–	34	144–146
3a	$C_{21}H_{16}ClFN_2$	Cl	–	H	89	119–121
3b	$C_{21}H_{17}FN_2$	H	–	H	54	128
3c	$C_{21}H_{16}BrFN_2$	Br	–	H	59	124–126
3d	$C_{21}H_{15}ClFN_3O_2$	Cl	–	NO_2	46	110
3e	$C_{21}H_{16}FN_3O_2$	H	–	NO_2	47	143
3f	$C_{21}H_{15}BrFN_3O_2$	Br	–	NO_2	39	134

In the proton NMR spectra, the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The proton NMR spectra of the newly prepared compounds exhibited an ABX pattern of signals attributed to methine (C-5) and methylene (C-4) protons. The protons H_A and H_B on C-4 are geminal protons and appeared in the region $\delta = 3.02$–3.29 and 3.43–3.98 ppm as doublet of doublets. The proton H_X at C-5 also appeared as a doublet of doublet in the region 5.12–5.77 ppm due to the vicinal coupling with two non-equivalent geminal protons of C-4 carbon. A similar pattern in spectrum for 2-pyrazolines was reported in the literature [8, 9]. The presence of a singlet at 8.87 ppm in compound **2a** corresponds to the aldehydic proton. The protons of aliphatic CH_3 and CH_2 of compounds **2d** and **2e** appeared as a triplet (1.10 and 1.11 ppm) and quartet (2.70 and 2.23 ppm). Similarly, the compounds **2f** and **2g** exhibited a triplet and two multiplets corresponding to the aliphatic protons. The appearance of a doublet and multiplet in proton NMR spectrum of compounds **2i** and **2j** was due to the protons of CH_3 and CHCl group, respectively. Similarly, the aromatic protons of new triaryl pyrazolines appeared as multiplets in the aromatic region (6.97–8.31 ppm). The proton NMR data confirmed the formation of new derivatives.

^{13}C NMR chemical shift values at $\delta = 53.41$–59.41 ppm (C-5) and 38.98–42.89 ppm (C-4) confirmed the formation of 2-pyrazolines. The presence of a peak at 160.12 ppm in the ^{13}C NMR spectrum of compound **2a** was accounted for the aldehydic carbon whereas the other newly synthesized *N*-alkyloxypyrazoline derivatives exhibited peaks in the range of 167.96–176.85 ppm, accounted for the carbonyl carbons. The ^{13}C NMR spectrum of the new compounds exhibited chemical shift values in the range of 151.79–164.73 ppm corresponding to C=N carbon, also confirmed the formation of 2-pyrazolines. LC–MS data were also in agreement with the formation of 2-pyrazolines. Elemental analysis also gave satisfactory results for all the compounds.

The pyrazoline derivatives **2b**, **2c**, **2h**, and **3c** were crystallized into neat single crystals by slow evaporation technique and the structures of those compounds were confirmed by single crystal XRD data (Fig. 1). The compounds **2b** and **2h** were crystallized in an orthorhombic, *Pbca* space group and triclinic $\overline{P}1$ space group, respectively, while the compounds **2c** and **3c** were crystallized in a monoclinic $P2_1/c$ space group [10–13].

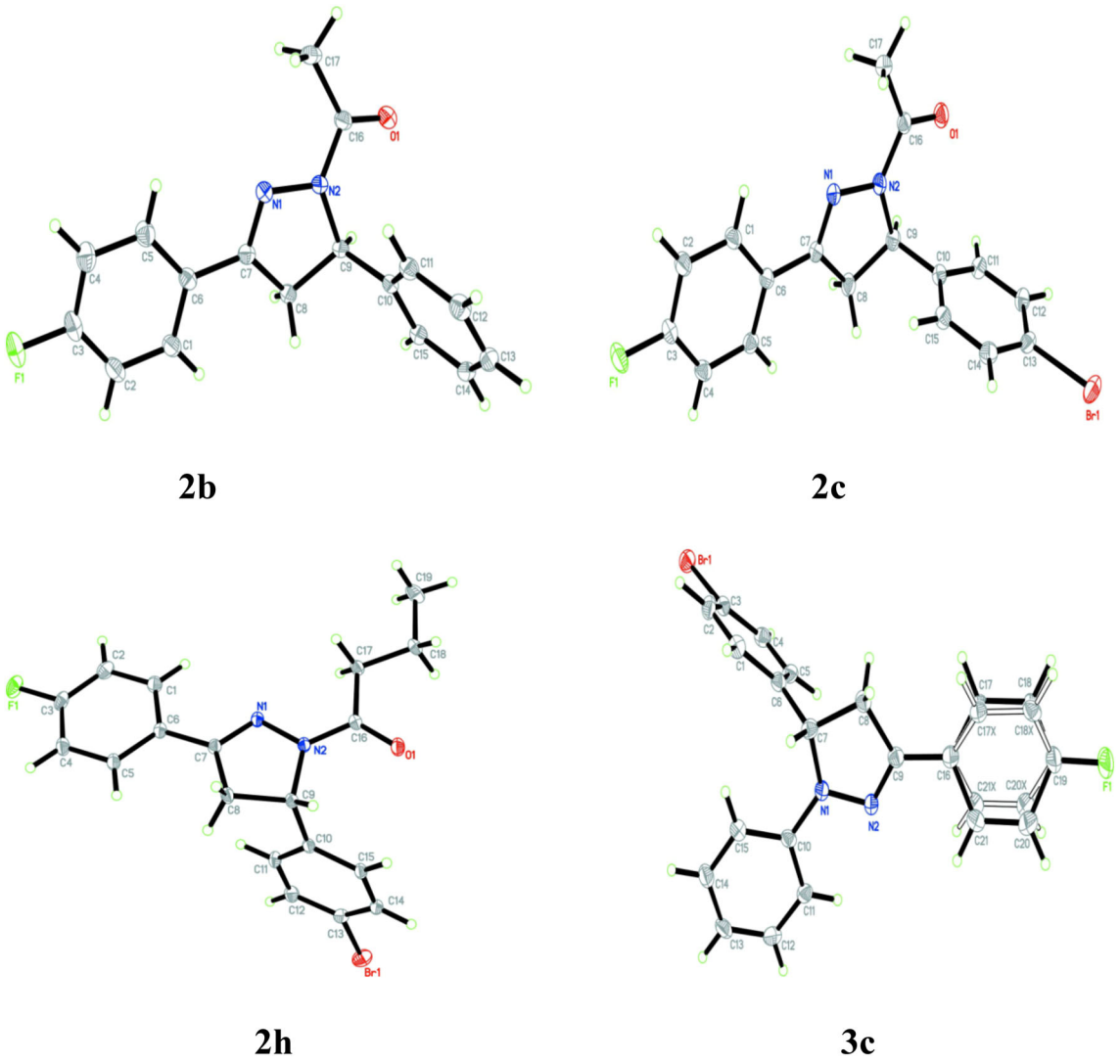

Fig. 1 The molecular structure and numbering scheme for the compounds **2b**, **2c**, **2h**, and **3c**, with displacement ellipsoids drawn at the 50 % probability level

Antibacterial activity

The in vitro antibacterial activities of pyrazoline derivatives **2a**–**2j** and **3a**–**3f** were carried out using Gram positive *Staphylococcus aureus* (ATTC-25923), Gram negative *Escherichia coli* (ATTC-25922), *Pseudomonas aeruginosa* (ATCC-27853), and *Klebsiella pneumoniae* (recultured) bacterial strains by serial plate dilution method. Activity of each compound was compared with ciprofloxacin as standard. The results are summarized in Table 2. Among the newly synthesized compounds, 2-chloro-1-[3-(4-fluorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (**2j**) and 5-(4-chlorophenyl)-3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (**3a**) were found to be active against all the tested microorganisms. Among these two compounds, compound **2j** exhibited the maximum zone of inhibition of 8 mm at MIC 6.25 μg/cm^3 against all the tested bacterial strains except *E. coli* while the compound **3a** against *S. aureus* and *E. coli*. The compounds 3-(4-fluorophenyl)-1,5-diphenyl-4,5-dihydro-1*H*-pyrazole (**3b**) and 5-(4-bromophenyl)-3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (**3c**) inhibited all the tested bacterial strains except *Klebsiella pneumoniae*. The compounds **2d** (with 4-chlorophenyl and propyl group), **2g** (with butyl group), and **2h** (with 4-bromophenyl and butyl group) also exhibited the zone of inhibition of 8 mm at MIC of 6.25 μg/cm^3 against *S. aureus*. The results of the antibacterial activity revealed that the majority of the synthesized pyrazoline derivatives showed varying degree of inhibition against the tested microorganisms.

Table 2 Antibacterial activity of the synthesized compounds: MIC/μg cm^{-3} (zone of inhibition/mm)

Compound	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*
2a	6.25 (6)	6.25 (7)	R	R
2b	R	R	R	R
2c	R	R	R	R
2d	6.25 (8)	R	R	R
2e	R	R	R	R
2f	12.5 (6)	12.5 (6)	R	R
2g	6.25 (8)	R	R	R
2 h	6.25 (8)	R	R	R
2i	6.25 (6)	12.5 (6)	R	R
2j	6.25 (8)	6.25 (7)	6.25 (8)	6.25 (8)
3a	6.25 (8)	6.25 (8)	12.5 (7)	12.5 (6)
3b	12.5 (6)	12.5 (6)	12.5 (7)	R
3c	12.5 (6)	12.5 (6)	12.5 (6)	R
3d	R	R	R	R
3e	6.25 (7)	12.5 (6)	R	R
3f	12.5 (6)	R	R	R
Ciprofloxacin	6.25 (20)	6.25 (19)	6.25 (17)	6.25 (18)

R indicates bacterial strains are resistant to the compounds >100 μg/cm^3

Antifungal activity

The in vitro antifungal activity of the synthesized compounds was examined against *Aspergillus fumigatus* (NCIM No. 902), *Penicillium marneffei* (recultured), *Trichophyton mentagrophytes* (recultured), and *Aspergillus flavus* (NCIM No. 524) by serial plate dilution method. Activity of each compound was compared with itraconazole as standard. The results are summarized in Table 3. The compounds 1-[3-(4-fluorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**2b**), 1-[5-(4-bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]butan-1-one (**2h**), and 3-(4-fluorophenyl)-1,5-diphenyl-4,5-dihydro-1*H*-pyrazole (**3b**) were found to be active against all the tested fungal strains. The compound **2g** with butyl substituent was active against all tested microorganisms except *A. Fumigatus*, whereas compounds **3a** with 4-chlorophenyl group and **3d** with 4-chlorophenyl and 4-nitrophenyl group were active against only three fungal stains *T. mentagrophytes, A. flavus*, and *A. fumigatus*. Among the tested compounds, the maximum zone of inhibition of 8 mm at MIC 6.25 μg/cm^3 was observed for the compound **2g** against *P. marneffei*, **2h** and **3a** against *A. flavus*, **3b** and **3d** against *T. mentagrophytes*, and compound **3b** against *A. fumigatus*. In general, the antifungal activity of the newly synthesized pyrazoline derivatives depends on different substituents present in the molecule.

Antitubercular activity

All the newly synthesized pyrazoline derivatives **2a**–**2j** and **3a**–**3f** were assessed for their in vitro antitubercular activity against *Mycobacterium tuberculosis* using the microplate Alamar Blue assay (MABA) method. Minimum inhibitory concentration (MIC) was determined and the results obtained were compared with standard drugs pyrazinamide and streptomycin (Table 4). The compounds 1-[5-(4-bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]butan-1-one (**2h**) and 1,3,5-triaryl-2-pyrazolines, 5-(4-chlorophenyl)-3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (**3a**), 3-(4-fluorophenyl)-1,5-diphenyl-4,5-dihydro-1*H*-pyrazole (**3b**), 5-(4-bromophenyl)-3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (**3c**) inhibited *M. tuberculosis* at a concentration of 0.8 μg/cm^3.

DPPH radical scavenging assay

A rapid, simple, accurate, and inexpensive method to measure antioxidant capacity of substances involves the use of organic nitrogen radical DPPH. It is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. Further, this simple test provides information on the ability of a compound to contribute electrons during antioxidant action [14]. The odd electron in the DPPH free radical gives strong absorption maximum at 517 nm and is purple in color. The color changes from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces upon the transfer of acidic H-atom from the compound to DPPH radical to form DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. The results are summarized in Table 5.

Among the tested compounds, compounds 5-(phenyl/4-chlorophenyl/4-bromophenyl)-3-(4-fluorophenyl)-1-phenyl-

Table 3 Antifungal activity of the synthesized compounds: MIC/μg cm^{-3} (zone of inhibition/mm)

Compound	*Penicillium marneffei*	*Trichophyton mentagrophytes*	*Aspergillus flavus*	*Aspergillus fumigatus*
2a	12.5 (6)	R	R	R
2b	6.25 (7)	12.5 (6)	12.5 (6)	6.25 (6)
2c	R	R	R	R
2d	R	R	R	R
2e	R	R	R	R
2f	12.5 (6)	R	R	R
2g	6.25 (8)	12.5 (6)	6.25 (7)	R
2h	12.5 (6)	12.5 (6)	6.25 (8)	6.25 (6)
2i	R	R	R	R
2j	R	R	R	R
3a	R	12.5 (6)	6.25 (8)	12.5 (6)
3b	6.25 (7)	6.25 (8)	12.5 (6)	6.25 (8)
3c	12.5 (6)	R	R	R
3d	R	6.25 (8)	12.5 (6)	6.25 (7)
3e	6.25 (7)	12.5 (6)	R	R
3f	12.5 (6)	R	R	R
Itraconazole	6.25 (17)	6.25 (19)	6.25 (18)	6.25 (17)

R indicates fungal strains are resistant to the compounds >100 μg/cm^3

Table 4 Antitubercular activity of synthesized compounds

Compound	MIC/μg cm^{-3}
2a	3.125
2b	–
2c	–
2d	50
2e	50
2f	50
2g	–
2h	0.8
2i	–
2j	–
3a	0.8
3b	0.8
3c	0.8
3d	6.25
3e	–
3f	–
Pyrazinamide	3.125
Streptomycin	6.25

4,5-dihydro-1*H*-pyrazole **3a–3c** and 5-(phenyl/4-chlorophenyl/4-bromophenyl)-3-(4-fluorophenyl)-1-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole **3d–3f** showed moderate radical scavenging capacity with concentration of 1 mg/cm^3 in comparison with the standard glutathione. The radical scavenging capacity of the compounds **3a–3c** was due to the presence of phenyl ring in the pyrazoline. The scavenging capacity of compounds **3d–3f** is decreased slightly because of the electron withdrawing nitro group. All other pyrazoline derivatives exhibited low scavenging capacity.

Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was assessed by carrageenan induced rat paw edema method. The tested compounds exhibited anti-inflammatory activity ranging from 27 to 59 %, whereas the standard diclofenac showed 65 % inhibition after 24 h. Among the tested compounds, compound 1-[5-(4-bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**2b**) showed 71 % inhibition of paw edema at 4th hour and this inhibition is of significant when compared to the standard diclofenac. The compounds, 1-[5-(4-bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone (**2c**) and 1-[5-(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (**2d**) exhibited 58 % inhibition of paw edema at 4th hour whereas 1-[5-(4-bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]propan-1-one (**2e**) showed 62 % inhibition of paw edema at 5th hour. The results are tabulated in Tables 6 and 7.

Conclusion

A new series of 2-pyrazoline derivatives was synthesized from chalcones and characterized by ^{1}H NMR, ^{13}C NMR, IR, mass spectral data, and some of them by single crystal XRD. The synthesized compounds were screened for their antimicrobial, antitubercular, DPPH scavenging, and anti-inflammatory properties. Some of the tested compounds exhibited promising antimicrobial, antitubercular, DPPH scavenging, and anti-inflammatory activities.

Experimental

Melting points were taken in open capillary tubes. The purity of the compounds was confirmed by thin layer chromatography using Merck silica gel 60 F_{254}-coated aluminum plates using ethyl acetate: *n*-hexane (1:3 v/v) as solvent system. IR spectra were recorded on Shimadzu-FTIR Infrared spectrometer in KBr. ^{1}H NMR spectra were recorded on a Bruker AMX 400 spectrometer (400 MHz) with 5 mm PABBO BB-1H TUBES and ^{13}C NMR spectra were recorded for approximately 0.03 M solutions in DMSO-d_6 at 75 MHz or 100 MHz with TMS as internal standard. LC–MS were obtained using Shimadzu LCMS-8030/Agilent 1200 series LC and Micromass zQ spectrometer. Elemental analysis was carried out using VARIO EL-III (Elementar Analysensysteme GmBH).

Table 5 DPPH scavenging assay of synthesized compounds

Compound	DPPH scavenging/ %
2a	19.23 ± 0.22
2b	14.93 ± 0.33
2c	32.41 ± 0.24
2d	23.11 ± 0.31
2e	15.15 ± 0.42
2f	16.12 ± 0.23
2g	17.34 ± 0.21
2h	22.12 ± 0.35
2i	15.98 ± 0.16
2j	27.36 ± 0.13
3a	52.43 ± 0.32
3b	49.35 ± 0.14
3c	53.40 ± 0.41
3d	43.83 ± 0.23
3e	42.54 ± 0.41
3f	45.32 ± 0.18
Glutathione	92.02 ± 0.34

*General procedure for the synthesis of pyrazoline derivatives **2a–2j***

A mixture of chalcone (0.01 mol) and 0.5 cm^3 hydrazine hydrate (0.01 mol) in 20 cm^3 formic acid/acetic acid/propionic acid/2-chloropropionic acid/butyric acid was refluxed for 8 h. The reaction mixture was cooled and poured into the 50 cm^3 ice-cold water. The precipitate formed was filtered and purified by recrystallization from ethanol.

5-(4-Chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (**2a**, $C_{16}H_{12}ClFN_2O$)
IR (KBr): $\bar{\nu}$ = 1,662 (CHO), 1,606 (pyrazoline C=N), 2,922 (CH), 1,219 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.19$ (dd, 1H, CH_2-H_A, $J_{AB} = 18$ Hz, $J_{AX} = 5.2$ Hz), 3.86 (dd, 1H, CH_2-H_B, $J_{BA} = 18.2$ Hz, $J_{BX} = 11.8$ Hz), 5.51 (dd, 1H, pyrazoline 5C–H, $J_{XA} = 5.2$ Hz, $J_{XB} = 11.6$ Hz), 7.25–8.87 (m, 8H, Ar–H), 8.87 (s, 1H, CHO) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 42.89$ (aliphatic CH_2, C-4), 59.10 (aliphatic CH, C-5), 116.28, 122.35, 126.18, 127.97, 129.21, 131.98, 141.67, 144.67, 155.72 (C=N of pyrazoline ring), 160.12 (C=O, C-6) ppm; LC–MS: $m/z = 303.6$ ($[M + 1]^+$).

*1-[3-(4-Fluorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**2b**)*
M.p.: 119–121 °C (Ref. [10] 119–121 °C).

*1-[5-(4-Bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]ethanone (**2c**)*
M.p.: 99–101 °C (Ref. [11] 99–101 °C).

1-[5-(4-Chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]propan-1-one (**2d**, $C_{18}H_{16}ClFN_2O$)
IR (KBr): $\bar{\nu}$ = 1,664 (C=O), 1,598 (pyrazoline C=N), 2,981 (CH), 1,224 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.10$ (t, 3H, CH_3, $J = 7.6$ Hz), 2.70 (q, 2H, CH_2), 3.10 (dd, 1H, CH_2-H_A, $J_{AB} = 18$ Hz, $J_{AX} = 4.8$ Hz), 3.79 (dd, 1H, CH_2-H_B, $J_{BA} = 18$ Hz,

Table 6 Anti-inflammatory activity data of the compounds **2a–2f**

Compounds	Paw edema volume/cm^3 (mean ± SEM)						
	0 h	1 h	2 h	3 h	4 h	5 h	24 h
Control	0.11 ± 0.03	0.216 ± 0.03	0.333 ± 0.01	0.333 ± 0.02	0.516 ± 0.02	0.433 ± 0.02	0.283 ± 0.02
Diclofenac	0.1 ± 0.01	0.2 ± 0.01	0.191 ± 0.02	0.183 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.1 ± 0.02
2a	0.11 ± 0.04	0.216 ± 0.02	0.216 ± 0.01	0.216 ± 0.02	0.35 ± 0.01	0.30 ± 0.01	0.2 ± 0.02
2b	0.1 ± 0.04	0.133 ± 0.01	0.133 ± 0.02	0.133 ± 0.03	0.15 ± 0.02	0.25 ± 0.02	0.128 ± 0.02
2c	0.11 ± 0.04	0.158 ± 0.02	0.15 ± 0.02	0.240 ± 0.03	0.262 ± 0.02	0.316 ± 0.02	0.271 ± 0.02
2d	0.1 ± 0.04	0.158 ± 0.01	0.216 ± 0.02	0.183 ± 0.02	0.216 ± 0.01	0.215 ± 0.02	0.116 ± 0.02
2e	0.11 ± 0.04	0.158 ± 0.02	0.15 ± 0.02	0.191 ± 0.03	0.216 ± 0.02	0.2 ± 0.02	0.141 ± 0.02
2f	0.11 ± 0.04	0.133 ± 0.02	0.233 ± 0.02	0.266 ± 0.02	0.281 ± 0.02	0.333 ± 0.02	0.208 ± 0.02

SEM standard error of mean

Table 7 Percentage inhibition of in vivo anti-inflammatory activity of the compounds **2a**–**2f**

Compounds	Inhibition/%					
	1 h	2 h	3 h	4 h	5 h	24 h
Control	–	–	–	–	–	–
Diclofenac	10**	43**	45**	62**	62**	65**
2a	0**	35**	35**	32**	31**	30**
2b	38**	60**	60**	71**	52**	27**
2c	27**	55**	43**	58**	52**	50**
2d	27**	35**	45**	58**	58**	59**
2e	27**	55**	43**	58**	62**	50**
2f	38**	33**	20**	52**	45**	27**

(ANOVA) followed by Dunnett's *t* test for multiple comparisons
* $P < 0.05$ and ** $P < 0.01$ were taken as significant

$J_{BX} = 12$ Hz), 5.50 (dd, 1H, pyrazoline 5C–H, $J_{XA} = 4.8$ Hz, $J_{XB} = 12$ Hz), 7.19–7.83 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 10.58$ (aliphatic CH_3), 29.43 (aliphatic CH_2), 39.13 (aliphatic CH_2, C-4), 57.18 (aliphatic CH, C-5), 115.61, 128.07, 128.71, 129.66, 130.43, 132.45, 141.62, 159.54, 164.73 (C=N of pyrazoline ring), 176.85 (C=O, C-6) ppm; LC–MS: *m/z* = 331.6 ($[M + 1]^+$).

1-[5-(4-Bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]propan-1-one (**2e**, $C_{18}H_{16}BrFN_2O$)
IR (KBr): $\bar{\nu} = 1{,}662$ (C=O), 1,602 (pyrazoline C=N), 2,989 (CH), 1,226 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.11$ (t, 3H, CH_3, $J = 7.8$ Hz), 2.23(q, 2H, CH_2), 3.02 (dd, 1H, CH_2-H_A, $J_{AB} = 18.2$ Hz, $J_{AX} = 4.8$ Hz), 3.43 (dd, 1H, CH_2-H_B, $J_{BA} = 18$ Hz, $J_{BX} = 12$ Hz), 5.12 (dd, 1H, pyrazoline 5C–H, $J_{XA} = 4.8$ Hz, $J_{XB} = 11.8$ Hz), 7.21–7.85 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 9.41$ (aliphatic CH_3), 24.63 (aliphatic CH_2), 39.60 (aliphatic CH_2, C-4), 55.16 (aliphatic CH, C-5), 115.28, 127.94, 128.85, 129.82, 130.29, 131.98, 141.91, 158.98, 160.34 (C=N of pyrazoline ring), 176.81 (C=O, C-6) ppm; LC–MS: *m/z* = 375.9 ($[M + 1]^+$).

1-[5-(4-Chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]butan-1-one (**2f**, $C_{19}H_{18}ClFN_2O$)
IR (KBr): $\bar{\nu} = 1{,}651$ (C=O), 1,602 (pyrazoline C=N), 2,870 (CH), 1,217 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 0.92$ (t, 3H, CH_3, $J = 7.4$ Hz), 1.53 (m, 2H, CH_2), 2.61 (m, 2H, CH_2), 3.09 (dd, 1H, CH_2-H_A, $J_{AB} = 18$ Hz, $J_{AX} = 4.8$ Hz), 3.79 (dd, 1H, CH_2-H_B, $J_{BA} = 18$ Hz, $J_{BX} = 12$ Hz), 5.51 (dd, 1H, pyrazoline 5C–H, $J_{XA} = 4.8$ Hz, $J_{XB} = 12$ Hz), 7.18–7.87 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 14.06$ (aliphatic CH_3), 19.63 (aliphatic CH_2), 38.80 (aliphatic CH_2), 39.92 (aliphatic CH_2, C-4), 59.26 (aliphatic CH, C-5), 115.81, 128.46, 128.70, 129.60, 130.82, 132.35, 141.67, 161.23, 151.83 (C=N of pyrazoline ring), 172.31 (C=O, C-6) ppm; LC–MS: *m/z* = 344.7 (M^+).

1-[3-(4-Fluorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]butan-1-one (**2g**, $C_{19}H_{19}FN_2O$)
IR (KBr): $\bar{\nu} = 1{,}653$ (C=O), 1,602 (pyrazoline C=N), 2,935 (CH), 1,222 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 0.94$ (t, 3H, CH_3, $J = 7.6$ Hz), 1.58 (m, 2H, CH_2), 2.66 (m, 2H, CH_2), 3.21 (dd, 1H, CH_2-H_A, $J_{AB} = 18$ Hz, $J_{AX} = 4.8$ Hz), 3.82 (dd, 1H, CH_2-H_B, $J_{BA} = 18$ Hz, $J_{BX} = 12$ Hz), 5.54 (dd, 1H, pyrazoline 5C–H, $J_{XA} = 4.8$ Hz, $J_{XB} = 12$ Hz), 7.19–7.88 (m, 8H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 14.21$ (aliphatic CH_3), 19.48 (aliphatic CH_2), 37.91 (aliphatic CH_2), 39.96 (aliphatic CH_2, C-4), 59.41 (aliphatic CH, C-5), 115.62, 128.31, 128.68, 129.51, 130.77, 132.51, 141.80, 160.34, 151.79 (C=N of pyrazoline ring), 172.34 (C=O, C-6) ppm; LC–MS: *m/z* = 310.2 (M^+).

1-[5-(4-Bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]butan-1-one (***2h***)
M.p.: 110–112 °C (Ref. [12] 110–112 °C).

2-Chloro-1-[5-(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]propan-1-one
(**2i**, $C_{18}H_{15}Cl_2FN_2O$)
IR (KBr): $\bar{\nu} = 1{,}658$ (C=O), 1,600 (pyrazoline C=N), 2,804 (CH), 1,220 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.50$ (d, 3H, CH_3, $J = 6.8$ Hz), 4.59 (q, 1H, CH–Cl), 3.10 (dd, 1H, CH_2-H_A, $J_{AB} = 18.2$ Hz, $J_{AX} = 4.8$ Hz), 3.79 (dd, 1H, CH_2-H_B, $J_{BA} = 18$ Hz, $J_{BX} = 12$ Hz), 5.47 (dd, 1H, pyrazoline 5C–H, $J_{XA} = 4.8$ Hz, $J_{XB} = 12$ Hz), 7.11–7.94 (m, 9H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 22.70$ (aliphatic CH_3), 39.91 (aliphatic CH_2, C-4), 58.26 (aliphatic CH), 58.78 (aliphatic CH, C-5), 115.61, 128.45, 128.76, 129.64, 130.68, 132.34, 141.65, 165.20, 152.61 (C=N of pyrazoline ring), 168.09 (C=O, C-6) ppm; LC–MS: *m/z* = 366.4 ($[M + 1]^+$).

2-Chloro-1-[3-(4-fluorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]propan-1-one (**2j**, $C_{18}H_{16}ClFN_2O$)
IR (KBr): $\bar{\nu} = 1{,}662$ (C=O), 1,608 (pyrazoline C=N), 2,823 (CH), 1,218 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.57$ (d, 3H, CH_3, $J = 6.8$ Hz), 5.48 (q, 1H, CH–Cl), 3.17 (dd, 1H, CH_2-H_A, $J_{AB} = 18$ Hz, $J_{AX} = 4.8$ Hz), 3.88 (dd, 1H, CH_2-H_B, $J_{BA} = 18.2$ Hz, $J_{BX} = 11.8$ Hz), 5.56 (dd, 1H, pyrazoline 5C–H, $J_{XA} = 4.8$ Hz, $J_{XB} = 11.6$ Hz), 7.14–7.90 (m, 9H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 22.64$ (aliphatic CH_3), 38.98 (aliphatic CH_2, C-4), 58.22 (aliphatic CH), 58.71 (aliphatic CH, C-5), 115.64, 128.41, 128.72, 129.60, 130.71, 131.94, 141.55, 165.23, 152.63 (C=N of pyrazoline ring), 167.96 (C=O, C-6) ppm; LC–MS: *m/z* = 331.6 ($[M + 1]^+$).

*General procedure for the synthesis of pyrazoline derivatives **3a–3f***

A mixture of chalcone (0.01 mol) and phenyl hydrazine or 4-nitrophenyl hydrazine (0.01 mol) in 20 cm^3 glacial acetic acid was refluxed for 8 h. The reaction mixture was cooled and poured into the 50 cm^3 ice-cold water. The precipitate formed was filtered and purified by recrystallization from ethanol.

5-(4-Chlorophenyl)-3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (**3a**, $C_{21}H_{16}ClFN_2$)
IR (KBr): $\bar{\nu}$ = 1,604 (pyrazoline C=N), 2,916 (CH), 1,496 (Ar–H), 1,218 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.07 (dd, 1H, CH_2-H_A, J_{AB} = 17.4 Hz, J_{AX} = 6.6 Hz), 3.87 (dd, 1H, CH_2-H_B, J_{BA} = 17.6 Hz, J_{BX} = 12.4 Hz), 5.43 (dd, 1H, pyrazoline 5C–H, J_{XA} = 6.6 Hz, J_{XB} = 12.2 Hz), 6.97–7.80 (m, 13H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 40.01 (aliphatic CH_2, C-4), 53.47 (aliphatic CH, C-5), 113.51, 115.64, 117.24, 128.45, 128.79, 129.63,129.67, 130.82, 132.32, 141.65, 143.83, 165.28, 151.82 (C=N of pyrazoline ring) ppm; LC–MS: *m/z* = 351.9 ($[M + 1]^+$).

3-(4-Fluorophenyl)-1,5-diphenyl-4,5-dihydro-1H-pyrazole (**3b**, $C_{21}H_{17}FN_2$)
IR (KBr): $\bar{\nu}$ = 1,598 (pyrazoline C=N), 2,908 (CH), 1,498 (Ar–H), 1,222 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.04 (dd, 1H, CH_2-H_A, J_{AB} = 18 Hz, J_{AX} = 6.2 Hz), 3.79 (dd, 1H, CH_2-H_B, J_{BA} = 18.2 Hz, J_{BX} = 12 Hz), 5.46 (dd, 1H, pyrazoline 5C–H, J_{XA} = 6.4 Hz, J_{XB} = 12.2 Hz), 7.03–7.84 (m, 13H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 40.09 (aliphatic CH_2, C-4), 53.41 (aliphatic CH, C-5), 113.52, 115.60, 117.32, 128.53, 128.81, 129.77,129.89, 130.83, 132.51, 141.76, 143.63, 165.31, 152.80 (C=N of pyrazoline ring) ppm; LC–MS: *m/z* = 317.7 ($[M + 1]^+$).

*5-(4-Bromophenyl)-3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (**3c**)*
M.p.: 124–126 °C (Ref. [13] 124–126 °C).

5-(4-Chlorophenyl)-3-(4-fluorophenyl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**3d**, $C_{21}H_{15}ClFN_3O_2$)
IR (KBr): $\bar{\nu}$ = 1,600 (pyrazoline C=N), 2,923 (CH), 1,502 (Ar–H), 1,220 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.29 (dd, 1H, CH_2-H_A, J_{AB} = 18 Hz, J_{AX} = 4.6 Hz), 3.95 (dd, 1H, CH_2-H_B, J_{BA} = 18 Hz, J_{BX} = 11.8 Hz), 5.72 (dd, 1H, pyrazoline 5C–H, J_{XA} = 4.6 Hz, J_{XB} = 12 Hz), 7.13–8.31 (m, 12H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 40.04 (aliphatic CH_2, C-4), 53.63 (aliphatic CH, C-5), 114.38, 115.47, 121.94, 128.52, 128.84, 129.72, 130.88, 132.34, 136.78, 141.64, 149.98, 165.33, 152.45 (C=N of pyrazoline ring) ppm; LC–MS: *m/z* = 395.9 ($[M + 1]^+$).

3-(4-Fluorophenyl)-1-(4-nitrophenyl)-5-phenyl-4,5-dihydro-1H-pyrazole (**3e**, $C_{21}H_{16}FN_3O_2$)
IR (KBr): $\bar{\nu}$ = 1,598 (pyrazoline C=N), 2,928 (CH), 1,496 (Ar–H), 1,218 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.21 (dd, 1H, CH_2-H_A, J_{AB} = 18.2 Hz, J_{AX} = 4.6 Hz), 3.91 (dd, 1H, CH_2-H_B, J_{BA} = 17.8 Hz, J_{BX} = 12 Hz), 5.77 (dd, 1H, pyrazoline 5C–H, J_{XA} = 4.6 Hz, J_{XB} = 11.8 Hz), 7.14–8.24 (m, 12H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 40.03 (aliphatic CH_2, C-4), 53.41 (aliphatic CH, C-5), 114.48, 115.58, 121.89, 128.28, 128.82, 129.72, 130.81, 132.43, 136.86, 141.54, 149.94, 165.72, 152.47 (C=N of pyrazoline ring) ppm; LC–MS: *m/z* = 362.1 ($[M + 1]^+$).

5-(4-Bromophenyl)-3-(4-fluorophenyl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**3f**, $C_{21}H_{15}BrFN_3O_2$)
IR (KBr): $\bar{\nu}$ = 1,597 (pyrazoline C=N), 2,924 (CH), 1,500 (Ar–H), 1,219 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.25 (dd, 1H, CH_2-H_A, J_{AB} = 18 Hz, J_{AX} = 4.4 Hz), 3.98 (dd, 1H, CH_2-H_B, J_{BA} = 17.8 Hz, J_{BX} = 11.8 Hz), 5.75 (dd, 1H, pyrazoline 5C–H, J_{XA} = 4.6 Hz, J_{XB} = 11.8 Hz), 7.06–8.27 (m, 12H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 40.01 (aliphatic CH_2, C-4), 53.47 (aliphatic CH, C-5), 114.46, 115.63, 121.92, 128.43, 128.80, 129.66, 130.85, 132.37, 136.82, 141.60, 149.92, 165.29, 152.43 (C=N of pyrazoline ring) ppm; LC–MS: *m/z* = 441.1 ($[M + 1]^+$).

Antibacterial activity

The antibacterial efficacy of the newly synthesized pyrazoline derivatives was tested against Gram positive *S. aureus* (ATTC-25923), Gram negative *E. coli* (ATTC-25922), *P. aeruginosa* (ATCC-27853), and *K. pneumoniae* (recultured) bacterial strains by serial plate dilution method. Serial dilutions of the drug in Mueller–Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16–18 h at 37 °C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. About 20 cm^3 of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. Using a punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (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 37 °C for 3–4 days

[15]. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard.

Antifungal activity

Antifungal studies of newly synthesized pyrazoline derivatives were determined by serial plate dilution method against *A. fumigates* (NCIM No. 902), *P. marneffei* (recultured), *T. mentagrophytes* (recultured), and *A. flavus* (NCIM No. 524). Sabouraud agar media were prepared by dissolving 4 g D-glucose, 1 g peptone, and 2 g agar in 100 cm^3 distilled water 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 cm^3 saline to get a suspension of corresponding species. Agar media (20 cm^3) 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. Using a punch, wells were made on these seeded agar plates. Minimum inhibitory 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 37 °C for 3–4 days [16, 17]. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with itraconazole as standard.

Antitubercular activity

The antitubercular activity of newly synthesized compounds was assessed against *M. tuberculosis* using Microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Sterile deionized water (0.2 cm^3) was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 plates received 0.1 cm^3 of the Middlebrook 7H9 broth and serial dilution of compounds was made directly on the plate. The final drug concentrations tested were 100–0.2 μg/cm^3. Plates were covered and sealed with parafilm and incubated at 37 °C for 5 days. After this, 0.025 cm^3 of freshly prepared 1:1 mixture of Alamar Blue reagent and 10 % tween 80 were added to the plate and incubated for 24 h [18]. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. The minimum inhibition concentration (MIC) was defined as lowest drug concentration which prevented the color change from blue to pink.

DPPH radical scavenging assay

Free radical scavenging activity of compounds was measured by DPPH using the reported method [19]. Briefly, 1 mM solution of DPPH in ethanol was prepared, and 1 cm^3 of this solution was added to sample solutions 1 mg/cm^3 of distilled water. The mixture was shaken vigorously and allowed to stand at room temperature for 20 min. Then, reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect}\,(\%) = (A_0 - A_1 / A_0) \times 100$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples or standards. Each sample was assayed at 1 mg/cm^3 and all experiments were carried out in triplicate.

Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was assessed by carrageenan induced rat paw edema method [20–22]. The wistar albino rats weighing between 150 and 250 g were selected and divided into different groups each containing six rats. The first group of rats was treated with normal saline (control), second group was administered with a dose of 20 mg/kg of diclofenac (standard) and the other groups were treated with 20 mg/kg of the suspension of test compounds. After 1 h, 0.1 cm^3 1 % carrageenan suspension in isosaline solution was injected into the subplantar tissue of the right hind paw. The paw volume of legs was noted for 0, 1, 2, 3, 4, 5, and 24 h after carrageenan challenge using plethysmometer. The mean paw edema value for the test group is compared with its mean value for the control group. Anti-inflammatory activity was measured as the percentage reduction in edema level when drug was present, relative to control and was calculated by the formula

$$\%\,\text{Inhibition} = (1 - V_t / V_c) \times 100$$

where V_t is edema volume in drug-treated group and V_c is edema volume in the control group.

Statistical analysis: the results were expressed in mean ± SEM. The data from experiments were analyzed separately using one-way ANOVA followed by Dunnett's *t* test to determine significant difference between the groups and $P < 0.05$ was considered significant.

Acknowledgments The authors are thankful to the Director, IISc, Bangalore for NMR data and DST-PURSE for LC–MS data. Authors are also thankful to Nalilu Suchetha Kumari, Department of Biochemistry, K. S. Hegde Medical Academy, Deralakatte for the microbial screening. BN thanks the UGC for financial assistance through BSR one time grant for the purchase of chemicals. MS thanks

DST for providing financial help for the research work through INSPIRE Fellowship.

References

1. Fustero S, Simon-Fuentes A, Sanz-Cervera JF (2009) Org Prep Proced Int 41:253
2. Li JT, Zhang XH, Lin ZP (2007) Beilstein J Org Chem 3:13
3. Rahman MA, Siddiqui AA (2010) Int J Pharm Sci Drug Res 2:165
4. Sarojini BK, Vidyagayatri M, Darshanraj CG, Bharath BR, Manjunatha H (2010) Lett Drug Des Disc 7:214
5. Alam S, Panda R, Kachroo M (2014) Indian J Chem 53B:440
6. Giles D, Roopa K, Sheeba FR, Gurubasavarajaswamy PM, Divakar G, Vidhya T (2012) Eur J Med Chem 58C:478
7. Kumar C, Reddy V, Fasiulla (2013) Int J Sci Res Publications 3:1
8. Levai A, Jeko J, Brahmbhatt DI (2005) J Heterocycl Chem 42:1231
9. Samshuddin S, Narayana B, Sarojini BK, Khan MTH, Yathirajan HS, Raj CGD, Raghavendra R (2012) Med Chem Res 21:2012
10. Fun HK, Chia TS, Sapnakumari M, Narayana B, Sarojini BK (2012) Acta Cryst E68:o2680
11. Fun HK, Loh WS, Sapnakumari M, Narayana B, Sarojini BK (2012) Acta Cryst E68:o2586
12. Fun HK, Loh WS, Sapnakumari M, Narayana B, Sarojini BK (2012) Acta Cryst E68:o2655
13. Fun HK, Ooi CW, Sapnakumari M, Narayana B, Sarojini BK (2012) Acta Cryst E68:o2634
14. Tiwari AK (2004) Current Sci 86:1092
15. Barry AL (1991) Procedure for testing antimicrobial agents in agar media. In: Corian VL (ed) Antibiotics in laboratory medicine. Williams and Wilkins, Baltimore
16. Arthington-Skaggs BA, Motley M, Warnock DW, Morrison CJ (2000) J Clin Microbiol 38:2254
17. Fenlon CH, Cynamon MH (1986) Antimicrob Agents Chemother 29:386
18. Maria CSL, Marcus VND, Alessandra CP, de Marcelle LF, Rasnisb BG, Thais Cristina MN, Monica AP (2007) Arkivoc 15:181
19. Blois MS (1958) Nature 181:1199
20. Alagarsamy V, Meena S, Ramseshu KV, Solomon VR, Thirumurugan K, Dhanabal K, Murugan M (2006) Eur J Med Chem 41:1293
21. Giles D, Roopa K, Sheeba FR, Venkatesh DP, Gurubasavarajaswamy PM (2011) J Pharm Sci Res 3:1253
22. Winter CA, Risley EA, Nuss CW (1962) Proc Soc Exp Biol Med 111:544