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Short communication

Synthesis and biological evaluation of some pyrazolylpyrazolines as anti-inflammatory–antimicrobial agents

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ABSTRACT

A new series of pyrazolylpyrazolines (**5a–k**) was synthesized by the reaction of appropriate chalcones (**3a–k**) with 4-hydrazinobenzenesulfonamide hydrochloride (**4**) in ethanol. All the newly synthesized target compounds (**5a–k**) were screened for their anti-inflammatory activity using carrageenan-induced rat paw edema assay. Compounds **5g** and **5j** showed pronounced anti-inflammatory activity comparable to the reference standard nimesulide, whereas, compounds **5b**, **5d** and **5h** displayed good anti-inflammatory activity. Additionally, the synthesized compounds were evaluated for their in vitro antimicrobial activity against two Gram-positive bacteria and two Gram-negative bacteria. Four compounds **5c**, **5h–5j** showed good broad spectrum activity against all the tested Gram-positive and Gram-negative bacterial strains. Compound **5j** could be identified as the most biologically active member within this study with an interesting dual anti-inflammatory and antibacterial profile.

1. Introduction

Five-membered heterocycles with a conserved vicinal 1,2-diaryl substitution pattern are ideal representatives of a recurring core structure that is found in numerous biologically active compounds, including cyclooxygenase inhibitors, kinase inhibitors, GPCR antagonists and even agonists, phosphatase inhibitors, and dopamine transporter inhibitors [1]. 1,2-Diaryl substituted heterocycle 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 [2]. 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 [2]. Many pyrazole derivatives have been reported to possess diverse pharmacological activities such as anti-inflammatory [3–5], antimicrobial [6–8], antihypertensive [9], etc. However studies investigating the potential of pyrazole derivatives as dual antimicrobial–anti-inflammatory agents have only recently been initiated [10–13]. Appreciation of these findings motivated us to synthesize a novel series of pyrazolylpyrazolines (**5a–5n**) as a potential template for dual antimicrobial–anti-inflammatory agents. It must be noted that this scaffold provides vicinal diaryl substitution pattern on both the pyrazole as well as pyrazoline nucleus.

2. Results and discussion

2.1. Chemistry

The synthetic route used to synthesize the target 4-[5-(1-phenyl-3-aryl-1*H*-pyrazol-4-yl)-3-aryl-4,5-dihydro-1*H*-pyrazol-1-yl]benzenesulfonamides (**5a–k**) is outlined in Scheme 1. 1-Phenyl-3-aryl-1*H*-pyrazole-4-carbaldehydes (**1a–d**), prepared by the Vilsemeier–Haack reaction of the corresponding hydrazones [14,15], were subjected to base catalyzed Claisen–Schmidt condensation reaction with appropriate acetophenones (**2a–2d**) generating the required 1-aryl-3-(1-phenyl-3-aryl-1*H*-pyrazol-4-yl)prop-2-en-1-ones (**3a–k**). Finally pyrazolylpyrazolines (**5a–5k**) with a conserved vicinal diaryl substitution pattern bearing benzenesulfonamide moiety were obtained by the condensation of appropriate chalcones (**3a–3k**) and 4-hydrazinobenzenesulfonamide hydrochloride (**4**) in ethanol containing catalytic amount of glacial acetic acid. 4-Hydrazinobenzenesulfonamide (**4**) was prepared via diazotization of sulfanilamide followed by reduction of the corresponding diazonium salt with stannous chloride [16]. The purity of compounds was

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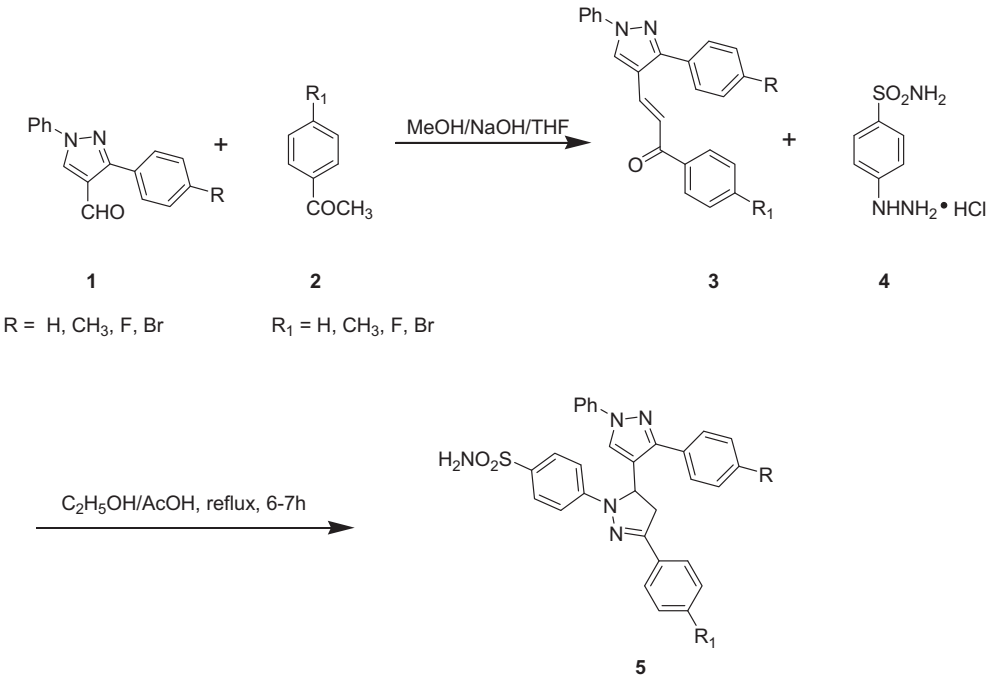

Compound **3,5**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**
R	H	H	H	H	CH_3	CH_3	CH_3	F	F	Br	Br
R_1	H	CH_3	F	Br	H	CH_3	F	H	F	H	F

Scheme 1. Synthesis of **5a–5k** from formylpyrazoles (**1**).

checked by TLC and elemental analysis. Analytical and spectral data (^{1}H NMR, ^{13}C NMR, IR and mass) of the newly synthesized compounds were in full agreement with the proposed structures. In general, the characteristic signals in ^{1}H NMR of target pyrazolylpyrazolines are the three pyrazoline protons which displayed a typical ABX type pattern of doublet of doublet. Methine proton of pyrazolines resonates at around δ 5.65 as a doublet of doublet with coupling constants of nearly 12 Hz and 6 Hz. The two methylene protons displayed two signals; a doublet of doublet at around δ 4.00 with coupling constants of nearly 17 Hz and 12 Hz and a doublet of doublet at around δ 3.30 merging with the water signal from DMSO-d_6 in most of the cases.

2.2. Biological evaluation

2.2.1. In vivo anti-inflammatory activity

All the newly synthesized pyrazolylpyrazolines (**5a–k**) were evaluated for their in vivo anti-inflammatory activity by carrageenan-induced paw edema method [17]. The protocol of animal experiments has been approved by the Institutional Animal

Table 1
Anti-inflammatory activity (AI) of the compounds **5a–5k** in carrageenan-induced rat paw edema assay (acute inflammatory model).

Compound[a]	Volume of edema (mL)[b]				
	0 min	30 min	60 min	90 min	120 min
Control	0.38 ± 0.03	0.53 ± 0.04	0.67 ± 0.09	0.84 ± 0.04	0.93 ± 0.01
5a	0.32 ± 0.01	0.51 ± 0.01 (5)[c]	0.64 ± 0.02 (4)	0.75 ± 0.02 (10)	0.92 ± 0.01 (1)
5b	0.30 ± 0.01	0.50 ± 0.06 (5)	0.55 ± 0.02* (17)	0.65 ± 0.01**(27)	0.68 ± 0.01**(26)
5c	0.37 ± 0.02	0.41 ± 0.01* (22)	0.50 ± 0.06** (25)	0.64 ± 0.02** (23)	0.79 ± 0.01* (15)
5d	0.34 ± 0.03	0.49 ± 0.06 (7)	0.51 ± 0.01** (23)	0.65 ± 0.01** (22)	0.71 ± 0.04** (23)
5e	0.32 ± 0.02	0.42 ± 0.08 (18)	0.57 ± 0.15 (14)	0.64 ± 0.04** (22)	0.78 ± 0.03* (16)
5f	0.35 ± 0.01	0.44 ± 0.01 (18)	0.54 ± 0.01** (19)	0.70 ± 0.03 (16)	0.84 ± 0.01 (9)
5g	0.36 ± 0.04	0.37 ± 0.04** (30)	0.38 ± 0.10** (43)	0.55 ± 0.01** (34)	0.63 ± 0.01** (32)
5h	0.30 ± 0.01	0.45 ± 0.05 (18)	0.58 ± 0.02 (14)	0.66 ± 0.01** (22)	0.71 ± 0.03** (23)
5i	0.29 ± 0.02	0.43 ± 0.07 (18)	0.61 ± 0.02 (8)	0.70 ± 0.03 (16)	0.84 ± 0.02 (9)
5j	0.34 ± 0.01	0.44 ± 0.01 (16)	0.48 ± 0.06** (26)	0.52 ± 0.01** (38)	0.64 ± 0.01** (32)
5k	0.36 ± 0.04 (5)	0.46 ± 0.04 (13)	0.49 ± 0.09** (26)	0.69 ± 0.03* (17)	0.79 ± 0.01* (15)
Nimesulide	0.27 ± 0.10	0.33 ± 0.01** (37)	0.38 ± 0.10** (43)	0.48 ± 0.08** (45)	0.59 ± 0.06** (36)

*Significantly different compared to respective control values, $P < 0.05$.
**Significantly different compared to respective control values, $P < 0.01$.
[a] Dose levels: test compounds (50 mg/kg body wt), nimesulide (4 mg/kg body wt).
[b] Values are expressed as mean ± SEM and analyzed by ANOVA.
[c] Values in parentheses (percentage anti-inflammatory activity, AI%).

Table 2
In vitro antibacterial activity of compounds **5a–5k** by using agar well diffusion method.

Compound	Diameter of zone of inhibition in mm[a]			
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
5a	17.66 ± 0.57 (32)	18.66 ± 0.57 (32)	10.66 ± 0.57 (64)	11.66 ± 0.57 (64)
5b	16.33 ± 0.57 (32)	17.00 (32)	11.66 ± 0.57 (64)	11.66 ± 0.57 (64)
5c	23.66 ± 0.57 (8)	23.33 ± 0.57 (8)	17.66 ± 0.57 (32)	21.66 ± 0.57 (16)
5d	17.00 (32)	11.33 ± 1.15 (64)	9.66 ± 0.57 (128)	11.66 ± 0.57 (64)
5e	16.00 (32)	11.00 (64)	9.33 ± 0.57 (128)	11.66 ± 0.57 (64)
5f	16.66 ± 0.57 (32)	10.66 ± 0.57 (64)	9.33 ± 0.57 (128)	10.66 ± 0.57 (64)
5g	16.66 ± 0.57 (32)	16.33 ± 0.57 (32)	11.00 (64)	11.66 ± 0.57 (64)
5h	23.33 ± 0.57 (16)	11.66 ± 0.57 (16)	17.33 ± 1.15 (32)	17.33 ± 0.57 (32)
5i	21.00 ± 1.00 (16)	10.66 ± 0.57 (16)	19.33 ± 0.57 (32)	10.66 ± 0.57 (32)
5j	24.00 (8)	23.66 ± 0.57 (8)	17.33 ± 1.15 (32)	17.33 ± 1.15 (32)
5k	10.00 (64)	11.00 ± 1.00 (64)	9.33 ± 0.57 (128)	9.33 ± 0.57 (128)
Ciprofloxacin	32.6 (2)	29.2 (2)	28.0 (4)	28.6 (4)

[a] Values in parentheses; MIC (μg/mL) = minimum inhibitory concentration.

Ethics Committee (IAEC). Each test compound was dosed orally (50 mg/kg body weight) 30 min prior to induction of inflammation by carrageenan injection. Nimesulide was utilized as a reference anti-inflammatory drug at a dose of 4 mg/kg, i.p. The anti-inflammatory activity was then calculated 30–120 min after induction and presented in Table 1 as the mean paw volume (mL) in addition to the percentage anti-inflammatory activity (AI%).

A comparative study of the anti-inflammatory activity of test compounds relative to the reference drug at different time intervals indicated the following: after 1 h, compound **5g** was nearly as effective in inhibiting the paw edema with percentage activity of 43% when compared with that of nimesulide (43%). Five other compounds **5c**, **5d**, **5f**, **5j** and **5k** showed distinctive pharmacokinetic profiles as revealed from their potent and rapid onset of action with percentage activity of 19–26%. After 90 min, seven compounds, **5b**–**5e**, **5g**–**5j** showed significant anti-inflammatory activity ranging from 22 to 38% inhibition as compared to nimesulide (45%). Taking the anti-inflammatory activity after 2 h time interval as a criterion for comparison, it can be concluded that compounds **5g** and **5j** showed potent anti-inflammatory activity (32%) comparable with nimesulide (36%), whereas, compounds **5b**, **5d** and **5h** displayed a good anti-inflammatory activity (23–26%), however, none of them was found to be superior over the reference drug.

2.2.2. In vitro antimicrobial activity

All the target compounds were evaluated for their in vitro antimicrobial activity against *Staphylococcus aureus* (MTCC 3160) and *Bacillus subtilis* (MTCC 121) representing Gram-positive bacteria, and *Pseudomonas aeruginosa* (MTCC 741) and *Escherichia coli* (MTCC 51) representing Gram-negative bacteria (Table 2). The microdilution susceptibility test in Müller–Hinton broth was used for the determination of antibacterial activity. Ciprofloxacin was used as the reference drug. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was determined using a microplate dilution method [18,19] (Table 2).

Results revealed that in general, most of the tested compounds showed better activity against the Gram-positive rather than the Gram-negative bacteria. Four compounds **5c**, **5h**–**5j** showed good broad spectrum activity against all the tested Gram-positive and Gram-negative bacterial strains. Based on these preliminary results, it can be seen that all the four compounds **5c**, **5h**–**5j** showing good antimicrobial activity have a halogen, F or Br, as one of the substituents. It can further be noted that the presence of halogenated benzene ring on the pyrazole moiety of the pyrazolylpyrazolines is better for activity as compared to the presence of halogenated benzene ring on the pyrazoline moiety.

3. Conclusion

The objective of the present study was to synthesize and investigate the anti-inflammatory and antimicrobial activities of a new series of pyrazolylpyrazolines with the hope of discovering new structure leads serving as dual anti-inflammatory–antimicrobial agents. The substitution pattern of the pyrazole ring as well as the pyrazoline ring was rationalized so as to be correlated to the vicinal diaryl heterocycles template. Amongst the tested compounds, **5g** and **5j** showed pronounced anti-inflammatory activity (32%) that was comparable to nimesulide (36%), whereas, compounds **5b**, **5d** and **5h** displayed good anti-inflammatory activity (23–26%). However, none of the newly synthesized compounds was found to be superior over the reference drug. On the other hand, some of the newly synthesized pyrazolylpyrazolines containing F or Br as a substituent (**5c**, **5h**–**5j**) were able to inhibit the growth of both the Gram-positive as well as Gram-negative bacteria. Finally compound **5j** could be identified as the most biologically active member within this study with an interesting dual anti-inflammatory and antibacterial profile. Consequently pyrazolylpyrazolines represent a class that needs further investigation with the hope of finding new dual anti-inflammatory–antimicrobial agents.

4. Experimental protocols

Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu-21 FT-IR or Perkin–Elmer IR Spectrophotometer using the KBr pellet technique. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Bruker NMR spectrometer at 300 MHz and 75.5 MHz respectively using tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in δ, ppm. Mass spectra were recorded on a Waters Micromass Q-Tof Micro instrument in ES^{+} mode. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate. Iodine was used as a visualizing agent.

*4.1. General procedure for the preparation of chalcones **3a–k***

To a cold, stirred mixture of methanol (20 mL) and sodium hydroxide (12.09 mmol) was added appropriate acetophenone (**2**, 4.03 mmol). The reaction mixture was stirred for 10 min. To this was added appropriate formyl pyrazole (**1**, 4.03 mmol) 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 so separated was filtered, washed with water, dried and crystallized from ethanol to afford product **3a**–**3k** (60–80% yield).

*4.1.1. (E)-3-(1,3-Diphenyl-1H-pyrazol-4-yl)-1-phenyl-2-propen-1-one (**3a**)*

m.p. 133–134 °C; lit. [20] m.p. 134 °C.

*4.1.2. (E)-1-(4-Methylphenyl)-3-(1,3-diphenyl-1H-pyrazol-4-yl)-2-propen-1-one (**3b**)*

m.p. 158–160 °C, yield 70%; IR (KBr) 1659 (s, C=O stretch), 1292 (m, CH=CH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.36 (s, 1H, Pyrazole-H), 8.03–7.27 (m, 16H, Ar, 2× =CH), 2.45 (s, 3H, CH_3).

*4.1.3. (E)-1-(4-Fluorophenyl)-3-(1,3-diphenyl-1H-pyrazol-4-yl)-2-propen-1-one (**3c**)*

m.p. 175–176 °C; lit. [18] m.p. 177–178 °C.

*4.1.4. (E)-1-(4-Bromophenyl)-3-(1,3-diphenyl-1H-pyrazol-4-yl)-2-propen-1-one (**3d**)*

m.p. 157–158 °C; lit. [18] m.p. 158–160 °C.

*4.1.5. (E)-3-[3-(4-Methylphenyl)-1-phenyl-1H-pyrazol-4-yl]-1-phenyl-2-propen-1-one (**3e**)*

m.p. 130–134 °C, yield 60%; IR (KBr) 1659 (s, C=O stretch), 1292 (m, CH=CH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.35 (s, 1H, Pyrazole-H), 8.00–7.29 (m, 16H, Ar, 2× =CH), 2.42 (s, 3H, CH_3).

*4.1.6. (E)-1-(4-Methylphenyl)-3-[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-yl]-2-propen-1-one (**3f**)*

m.p. 101–103 °C, yield 62%; IR (KBr) 1659 (s, C=O stretch), 1291 (m, CH=CH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.33 (s, 1H, Pyrazole-H), 8.00–7.27 (m, 15H, Ar, 2× =CH), 2.43 (s, 3H, CH_3), 2.40 (s, 3H, CH_3).

*4.1.7. (E)-1-(4-Fluorophenyl)-3-[3-(4-methylphenyl)-1-phenyl-1H-pyrazol-4-yl]-2-propen-1-one (**3g**)*

m.p. 120–122 °C, yield 70%; IR (KBr) 1659 (s, C=O stretch), 1293 (m, CH=CH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.36 (s, 1H, Pyrazole-H), 8.03–7.98 (m, 2H, Ar), 7.91 (d, 1H, J = 15.3 Hz, =CH), 7.82 (d, 2H, J = 8.1 Hz, Ar), 7.62 (d, 2H, J = 8.1 Hz, Ar), 7.54–7.49 (m, 3H, Ar), 7.39–7.27 (m, 3H, Ar, =CH), 7.17 (t, 2H, J = 8.4 Hz, Ar), 2.45 (s, 3H, CH_3).

*4.1.8. (E)-3-[3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-phenyl-2-propen-1-one (**3h**)*

m.p. 110–112 °C, yield 83%; IR (KBr) 1659 (s, C=O stretch), 1290 (m, CH=CH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz):.δ 8.35 (s, 1H, Pyrazole-H), 8.00–7.30 (m, 14H, Ar, 2× =CH), 7.12–7.10 (m, 2H, Ar)

*4.1.9. (E)-1-(4-Fluorophenyl)-3-[3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-2-propen-1-one (**3i**)*

m.p. 168–170 °C, yield 58%; IR (KBr) 1661 (s, C=O stretch), 1291 (m, CH=CH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.35 (s, 1H, Pyrazole-H), 7.99 (dd, 2H, J = 9.0 Hz, J = 5.4 Hz, Ar), 7.86 (d, 1H, J = 15.6 Hz, =CH), 7.81–7.78 (m, 2H, Ar), 7.69 (dd, 2H, J = 9.0 Hz, J = 5.4 Hz, Ar), 7.53–7.48 (m, 3H, Ar), 7.33 (d, 1H, J = 15.6 Hz, =CH), 7.13–7.19 (m, 4H, Ar).

*4.1.10. (E)-3-[3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-phenyl-2-propen-1-one (**3j**)*

m.p. 162–164 °C, yield 76%; IR (KBr) 1662 (C=O stretch), 1291 (m, CH=CH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.36 (s, 1H, pyrazole-H), 7.98–7.78 (m, 5H, Ar, =CH), 7.64–7.35 (m, 11H, Ar, =CH).

*4.1.11. (E)-3-[3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-(4-fluorophenyl)-2-propen-1-one (**3k**)*

m.p. 134–136 °C, yield 65%; IR (KBr) 1661 (s, C=O stretch), 1290 (m, CH=CH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.36 (s, 1H, pyrazole-H), 8.10–7.92 (m, 2H, Ar), 7.87 (d, 1H, J = 15.6 Hz, =CH), 7.80 (d, 2H, J = 8.7 Hz, Ar), 7.66–7.62 (m, 3H, Ar), 7.57 (d, 2H, J = 8.7 Hz, Ar), 7.36 (d, 1H, J = 15.6 Hz, =CH), 7.28–7.16 (m, 4H, Ar).

*4.2. General procedure for the preparation of pyrazolylpyrazolines **5a**–**k***

To an acidic solution of appropriate chalcone (**3**, 2.57 mmol) in ethanol was added 4-hydrazinobenzenesulfonamide hydrochloride (**4**, 2.57 mmol). The reaction mixture was refluxed for 5–6 h, cooled to room temperature. The solid separated was filtered, dried, and crystallized from ethanol.

*4.2.1. 1-(4-Aminosulfonylphenyl)-3-phenyl-5-(1,3-diphenylpyrazol-4-yl)-2-pyrazoline (**5a**)*

m.p. 184–188 °C, yield 720 mg (72%); IR (KBr) 3395 and 3267 (m, N–H stretch), 1591 (s, N–H bend), 1337 and 1153 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.34 (s, 1H, C5′-H pyrazole), 7.83 (d, 2H, J = 8.4 Hz, Ar), 7.77–7.79 (m, 4H, Ar), 7.58 (d, 2H, J = 8.4 Hz, Ar), 7.54 (d, 2H, J = 7.2 Hz, Ar), 7.52–7.44 (m, 6H, Ar), 7.29–7.24 (m, 1H, Ar), 7.04 (d, 2H, J = 8.4 Hz, Ar), 7.00 (s, 2H, ex, SO_2NH_2), 5.68 (dd, 1H, J = 6.6 Hz, 12.3 Hz, C_5-H pyrazoline), 4.09 (dd, 1H, J = 12.3 Hz, 17.4 Hz, C_4-H pyrazoline), 3.35 (m, 1H, C_4-H pyrazoline merged with peak of HOD); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 149.9, 149.8, 146.1, 139.2, 133.3, 132.6, 131.9, 129.5, 129.3, 128.8, 128.7, 128.1, 127.1, 126.4, 122.1, 118.3, 118.2, 112.2, 55.2, 42.6; Elemental analysis Found: C, 69.04; H, 4.52; N, 13.20%: $C_{30}H_{25}N_5O_2S$ requires: C, 69.34; H, 4.85; N, 13.49%; LRMS: m/z, 519 (M+); $C_{30}H_{25}N_5O_2S$ requires: m/z, 519.

*4.2.2. 1-(4-Aminosulfonylphenyl)-3-(4-methylphenyl)-5-(1,3-diphenylpyrazol-4-yl)-2-pyrazoline (**5b**)*

m.p. 180–182 °C, yield 57%; IR (KBr) 3372 and 3271 (m, N–H stretch), 1593 (s, N–H bend), 1338 and 1153 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 7.88 (s, 1H, C5′-H pyrazole), 7.75 (d, 2H, J = 7.2 Hz, Ar–H), 7.68 (d, 2H, J = 8.1 Hz, Ar), 7.64–7.61 (m, 4H, Ar), 7.53–7.48 (m, 2H, Ar), 7.45–7.35 (m, 3H, Ar), 7.25–7.18 (m, 3H, Ar, SO_2NH_2), 6.82 (d, 2H, J = 8.7 Hz, Ar), 5.58 (dd, 1H, J = 5.7 Hz, 11.7 Hz, C_5-H pyrazoline), 3.92 (dd, 1H, J = 12 Hz, 16.8 Hz, C_4-H pyrazoline), 3.37–3.20 (m, 1H, C_4-H pyrazoline merged with peak of HOD), 2.17 (s, 3H, CH_3); Elemental analysis Found: C, 69.61; H, 5.04; N, 13.01%; $C_{31}H_{27}N_5O_2S$ requires: C, 69.77; H, 5.10; N, 13.12%; LRMS: m/z, 533 (M+); $C_{31}H_{27}N_5O_2S$ requires: m/z, 533.

*4.2.3. 1-(4-Aminosulfonylphenyl)-3-(4-fluorophenyl)-5-(1,3-diphenylpyrazol-4-yl)-2-pyrazoline (**5c**)*

m.p. 196–198 °C, yield 81%; IR (KBr) 3393 and 3271 (m, N–H stretch), 1593 (s, N–H bend), 1337 and 1153 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.36 (s, 1H, C5′-H pyrazole), 7.84 (d, 4H, J = 7.5 Hz, Ar), 7.77 (d, 2H, J = 8.1 Hz, Ar), 7.60 (d, 2H, J = 8.7 Hz, Ar), 7.55–7.42 (m, 5H, Ar), 7.31–7.28 (m, 3H, Ar), 7.05 (d, 2H, J = 8.7 Hz, Ar), 7.01 (s, 2H, ex, SO_2NH_2), 5.70 (dd, 1H, J = 6.3 Hz, 12.0 Hz, C_5-H pyrazoline), 4.08 (dd, 1H, J = 12.0 Hz, 17.4 Hz, C_4-H pyrazoline), 3.37–3.24 (m, 1H, C_4-H pyrazoline merged with peak of HOD); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 162.6 (d, $^1J_{CF}$ = 246 Hz), 149.8, 148.9, 146.1, 139.1, 133.3, 129.5, 128.7, 128.5, 128.2, 128.1 (d, $^3J_{CF}$ = 8.5 Hz), 127.1, 126.4, 122.0, 118.2, 115.7 (d, $^2J_{CF}$ = 21.9 Hz), 112.2, 55.2, 42.6. Elemental analysis Found: C, 67.12; H, 4.63; N, 13.22%; $C_{30}H_{24}FN_5O_2S$ requires: C, 67.02; H, 4.50; N, 13.03%; LRMS: m/z, 537 (M+); $C_{30}H_{24}FN_5O_2S$ requires: m/z, 537.

*4.2.4. 1-(4-Aminosulfonylphenyl)-3-(4-bromophenyl)-5-(1,3-diphenylpyrazol-4-yl)-2-pyrazoline (**5d**)*

m.p. 195–198 °C, yield 62%; IR (KBr) 3368 and 3258 (m, N–H stretch), 1595 (s, N–H bend), 1336 and 1152 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.36 (s, 1H, C5′-H pyrazole), 7.83 (d, 2H, J = 8.1 Hz, Ar), 7.76–7.70 (m, 4H, Ar), 7.65–7.59 (m, 2H, Ar), 7.56–7.42 (m, 8H, Ar), 7.04 (d, 2H, J = 9.0 Hz, Ar), 7.00 (s, 2H, ex, SO_2NH_2), 5.70 (dd, 1H, J = 6.3 Hz, 12.0 Hz, C_5-H pyrazoline), 4.07 (dd, 1H, J = 12.0 Hz, 17.4 Hz, C_4-H pyrazoline), 3.42–3.34 (m, 1H, C_4-H pyrazoline merged with HOD peak); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 149.9, 148.8, 145.9, 139.1, 133.5, 131.6, 131.2, 129.5, 129.4, 128.7, 128.1, 127.2, 127.1, 126.5, 122.4, 121.9, 118.2, 112.3, 55.3, 42.4. Elemental analysis Found: C, 60.02; H, 4.00; N, 11.54%; $C_{30}H_{24}BrN_5O_2S$ requires: C, 60.20; H, 4.04; N, 11.70%; LRMS: m/z, 597, 599 (M+); $C_{30}H_{24}BrN_5O_2S$ requires: m/z, 597, 599

*4.2.5. 1-(4-Aminosulfonylphenyl)-3-phenyl-5-[3-(4-methylphenyl)-1-phenylpyrazol-4-yl]-2-pyrazoline (**5e**)*

m.p. 196–198 °C, yield 64%; IR (KBr) 3374 and 3273 (m, N–H stretch), 1593 (s, N–H bend), 1337 and 1151 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.31 (s, 1H, C5′-H pyrazole), 7.82 (d, 2H, J = 8.4 Hz, Ar), 7.79–7.76 (m, 2H, Ar), 7.66 (d, 2H, J = 8.1 Hz, Ar), 7.57 (d, 2H, J = 9.0 Hz, Ar), 7.47–7.23 (m, 8H, Ar), 7.03 (d, 2H, J = 8.7 Hz, Ar), 6.99 (s, 2H, ex, SO_2NH_2), 5.67 (dd, 1H, J = 6.3 Hz, 12.0 Hz, C_5-H pyrazoline), 4.08 (dd, 1H, J = 12.0 Hz, 17.4 Hz, C_4-H pyrazoline), 3.41–3.34 (m, 1H, C_4-H pyrazoline merged with HOD peak), 2.37 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 149.8, 149.7, 146.0, 139.1, 137.7, 133.2, 131.9, 129.7, 129.5, 129.3, 129.2, 128.6, 127.1, 126.3, 122.0, 121.7, 118.2, 112.2, 55.1, 42.5, 20.8. Elemental analysis Found: C, 69.41; H, 4.89; N, 12.75%; $C_{31}H_{27}N_5O_2S$ requires: C, 69.77; H, 5.10; N, 13.12%; LRMS: m/z, 533 (M+); $C_{31}H_{27}N_5O_2S$ requires: m/z, 533.

*4.2.6. 1-(4-Aminosulfonylphenyl)-3-(4-methylphenyl)-5-[3-(4-methylphenyl)-1-phenylpyrazol-4-yl]-2-pyrazoline (**5f**)*

m.p. 206–208 °C, yield 55%; IR (KBr) 3372 and 3260 (m, N–H stretch), 1597 (s, N–H bend), 1337 and 1157 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.29 (s, 1H, C5′-H pyrazole), 7.82 (d, 2H, J = 7.8 Hz, Ar), 7.67 (d, 4H, J = 8.1 Hz, Ar), 7.57 (d, 2H, J = 9.0 Hz, Ar), 7.45–7.40 (m, 2H, Ar), 7.32 (d, 2H, J = 7.8 Hz, Ar), 7.28–7.24 (m, 3H, Ar), 7.02 (d, 2H, J = 9.0 Hz, Ar), 6.98 (s, 2H, ex, SO_2NH_2), 5.63 (dd, 1H, J = 6.6 Hz, 12.0 Hz, C_5-H pyrazoline), 4.05 (dd, 1H, J = 12.0 Hz, 17.1 Hz, C_4-H pyrazoline), 3.40–3.26 (m, 1H, C_4-H pyrazoline merged with HOD peak), 2.38 (s, 3H, CH_3), 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 149.8, 149.8, 146.1, 139.1, 138.9, 137.7, 133.7, 129.7, 129.4, 129.3, 129.2, 129.1, 127.9, 127.1, 126.3, 122.0, 118.2, 112.1, 55.1, 42.6, 20.9, 20.8. Elemental analysis Found: C, 70.43; H, 5.45; N, 13.01%; $C_{32}H_{29}N_5O_2S$ requires: C, 70.18; H, 5.34; N, 12.79%; LRMS: m/z, 547 (M+); $C_{32}H_{29}N_5O_2S$ requires: m/z, 547.

*4.2.7. 1-(4-Aminosulfonylphenyl)-3-(4-fluorophenyl)-5-[3-(4-methylphenyl)-1-phenylpyrazol-4-yl]-2-pyrazoline (**5g**)*

m.p. 196–200 °C, yield 65%; IR (KBr) 3372 and 3273 (m, N–H stretch), 1593 (s, N–H bend), 1337 and 1153 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.29 (s, 1H, C5′-H pyrazole), 7.79 (d, 4H, J = 7.8 Hz, Ar), 7.68 (d, 2H, J = 8.7 Hz, Ar), 7.62 (d, 2H, J = 7.8 Hz, Ar), 7.40 (t, 2H, J = 7.5 Hz, Ar), 7.25 (m, 5H, Ar), 7.01 (d, 2H, J = 9.0 Hz, Ar), 6.96 (s, 2H, ex, SO_2NH_2), 5.63 (dd, 1H, J = 6.0 Hz, 12.0 Hz, C_5-H pyrazoline), 4.02 (dd, 1H, J = 12.0 Hz, 17.1 Hz, C_4-H pyrazoline), 3.43–3.32 (m, 1H, C_4-H pyrazoline merged with HOD peak), 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 161.4 (d, $^1J_{CF}$ = 245 Hz), 149.9, 148.9, 146.0, 139.1, 137.7, 129.8, 129.7, 129.4 (d, $^3J_{CF}$ = 11.1 Hz), 128.6, 128.3, 127.1, 126.3, 122.0, 118.2, 118.1, 115.7 (d, $^2J_{CF}$ = 22.1 Hz), 114.1, 55.2, 42.6, 21.9. Elemental analysis Found: C, 67.78; H, 4.99; N, 13.08%; $C_{31}H_{26}FN_5O_2S$ requires: C, 67.50; H, 4.75; N, 12.70%; LRMS: m/z, 551 (M+); $C_{31}H_{26}FN_5O_2S$ requires: m/z, 551.

*4.2.8. 1-(4-Aminosulfonylphenyl)-3-phenyl-5-(3-(4-fluorophenyl)-1-phenylpyrazol-4-yl)-2-pyrazoline (**5h**)*

m.p. 216–218 C, yield 69%; IR (KBr) 3370 and 3269 (m, N–H stretch), 1593 (s, N–H bend), 1337 and 1153 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.36 (s, 1H, C5′-H pyrazole), 7.82 (d, 2H, J = 8.4 Hz, Ar), 7.80–7.75 (m, 4H, Ar), 7.59 (d, 2H, J = 8.7 Hz, Ar), 7.46–7.40 (m, 6H, Ar), 7.35–7.25 (m, 2H, Ar), 7.07–7.04 (d, 2H, J = 9.0 Hz, Ar), 7.00 (s, 2H, ex, SO_2NH_2), 5.68 (dd, 1H, J = 6.0 Hz, 17.1 Hz, C_5-H pyrazoline), 4.07 (dd, 1H, J = 12.0 Hz, 17.1 Hz, C_4-H pyrazoline), 3.42–3.31 (m, 1H, C_4-H pyrazoline merged with HOD peak); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 162.3 (d, $^1J_{CF}$ = 246 Hz), 149.7, 148.9, 146.0, 139.0, 133.2, 131.8, 130.2 (d, $^3J_{CF}$ = 8.6 Hz), 129.5, 129.1, 129.0, 128.6, 127.2, 126.5, 122.0, 118.2, 118.1, 115.6 (d, $^2J_{CF}$ = 21.7 Hz), 112.2, 55.0, 42.4. Elemental analysis Found: C, 66.74; H, 4.14; N, 12.67%; $C_{30}H_{24}FN_5O_2S$ requires: C, 67.02; H, 4.50; N, 13.03%; LRMS: m/z, 537 (M+); $C_{30}H_{24}FN_5O_2S$ requires: m/z, 537.

*4.2.9. 1-(4-Aminosulfonylphenyl)-3-(4-fluorophenyl)-5-[3-(4-fluorophenyl)-1-phenylpyrazol-4-yl]-2-pyrazoline (**5i**)*

m.p. 212–214 °C, yield 58%; IR (KBr) 3371 and 3259 (m, N–H stretch), 1598 (s, N–H bend), 1337 and 1155 (s, SO_2 stretch) cm^{-1}; ^1H NMR ($CDCl_3$/DMSO-d_6, 300 MHz): δ 7.80 (s, 1H, C5′-H pyrazole), 7.70–7.66 (m, 4H, Ar), 7.62–7.58 (m, 4H, Ar), 7.36–7.31 (m, 2H, Ar), 7.20–7.17 (m, 3H, Ar), 7.13 (d, 2H, J = 8.7 Hz, Ar), 7.07 (s, 2H, ex, SO_2NH_2), 7.03 (d, 2H, J = 8.7 Hz, Ar), 6.97 (d, 2H, J = 9.0 Hz, Ar), 5.13 (dd, 1H, J = 5.7 Hz, 12.0 Hz, C_5-H pyrazoline), 3.50 (dd, 1H, J = 12.0 Hz, 17.1 Hz, C_4-H pyrazoline), 2.92–2.82 (m, 1H, C_4-H pyrazoline merged with HOD peak); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 162.3 (d, $^1J_{CF}$ = 246 Hz), 162.0 (d, $^1J_{CF}$ = 245 Hz), 149.0, 148.3, 146.2, 130.2, (d, $^3J_{CF}$ = 8.4 Hz), 130.1 (d, $^3J_{CF}$ = 8.3 Hz), 129.5, 129.2, 129.0, 127.2, 126.4, 122.1, 118.2, 115.7, (d, $^2J_{CF}$ = 22.3 Hz), 115.6 (d, $^2J_{CF}$ = 22.3 Hz), 112.1, 55.4, 42.8. Elemental analysis Found: C, 65.17; H, 4.34; N, 12.69%; $C_{30}H_{23}F_2N_5O_2S$ requires: C, 64.85; H, 4.17; N, 12.61%; LRMS: m/z, 555 (M+); $C_{30}H_{23}F_2N_5O_2S$ requires: m/z, 555.

*4.2.10. 1-(4-Aminosulfonylphenyl)-3-phenyl-5-[3-(4-bromophenyl)-1-phenylpyrazol-4-yl]-2-pyrazoline (**5j**)*

m.p. 224–226 °C, yield 58%; IR (KBr) 3372 and 3269 (m, N–H stretch), 1592 (s, N–H bend), 1336 and 1151 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.34 (s, 1H, C5′-H pyrazole), 7.82 (d, 2H, J = 7.8 Hz, Ar), 7.76 (d, 2H, J = 8.4 Hz, Ar), 7.72 (d, 4H, J = 8.7 Hz, Ar), 7.59 (d, 2H, J = 8.7 Hz, Ar), 7.46–7.40 (m, 6H, Ar), 7.05 (d, 2H, J = 9.0 Hz, Ar), 7.00 (s, 2H, ex, SO_2NH_2), 5.70 (dd, 1H, J = 6.3 Hz, 12.0 Hz, C_5-H pyrazoline), 4.07 (dd, 1H, J = 12.0 Hz, 17.4 Hz, C_4-H pyrazoline), 3.40–3.31 (m, 1H, C_4-H pyrazoline merged with HOD peak); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 149.8, 148.6, 146.1, 139.1133.3, 131.8, 131.7, 130.0, 129.5, 129.3, 128.7, 127.4, 126.1, 122.2, 121.7, 118.3, 112.3, 55.0, 42.5. Elemental analysis Found: C, 60.02; H, 3.71; N, 11.32%; $C_{30}H_{24}BrN_5O_2S$ requires: C, 60.20; H, 4.04; N, 11.70%; LRMS: m/z, 597, 599 (M+); $C_{30}H_{24}BrN_5O_2S$ requires: m/z, 597, 599.

*4.2.11. 1-(4-Aminosulfonylphenyl)-3-(4-fluorophenyl)-5-[3-(4-bromophenyl)-1-phenylpyrazol-4-yl]-2-pyrazoline (**5k**)*

m.p. 230–234 °C, yield 65%; IR (KBr) 3373 and 3261 (m, N–H stretch), 1595 (s, N–H bend), 1337 and 1153 (s, SO_2 stretch) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 8.35 (s, 1H, C5′-H pyrazole), 7.81–7.65 (m, 8H, Ar), 7.59 (d, 2H, J = 9.0 Hz, Ar), 7.47–7.41 (m, 2H, Ar), 7.31–7.25 (m, 3H, Ar), 7.05 (d, 2H, J = 9.0 Hz, Ar), 7.00 (s, 2H, ex, SO_2NH_2), 5.70 (dd, 1H, J = 6.3 Hz, 12.3 Hz, C_5-H pyrazoline), 4.07 (dd, 1H, J = 12.3 Hz, 18.0 Hz, C_4-H pyrazoline), 3.43–3.32 (m, 1H, C_4-H pyrazoline merged with HOD peak); ^{13}C NMR (DMSO-d_6, 75.5 MHz); δ 162.7 (d, $^1J_{CF}$ = 247 Hz), 149.0, 148.7, 146.1, 139.0, 133.3, 131.7 (d, $^3J_{CF}$ = 9.5 Hz), 130.1, 129.6, 129.5, 128.2, 126.5, 122.2, 121.7, 118.3, 115.7 (d, $^2J_{CF}$ = 22.1 Hz), 112.3, 55.2, 42.5. Elemental analysis

Found: C, 58.12; H, 3.47; N, 11.72%; $C_{30}H_{23}BrFN_5O_2S$ requires: C, 58.45; H, 3.76; N, 11.36%; LRMS: *m*/*z*, 615, 617 (M+); $C_{30}H_{23}BrFN_5O_2S$ requires: *m*/*z*, 615, 617.

4.3. Pharmacological assay

4.3.1. Carrageenan-induced rat paw edema assay

Male Wister albino rats weighing 200–250 g were used throughout the study. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. Food was withdrawn 12 h before and during experimental hours. The animals were randomly divided into groups each consisting of six rats. One group of six rats was kept as control and received tween 80 (95:5). Another group received the standard drug nimesulide at a dose of 4 mg/kg body weight, i.p. Other groups of rats were administered the test compounds at a dose of 50 mg/kg body weight orally. A mark was made on the left hind paw just beyond the tibiotarsal articulation, so that every time the paw was dipped up to fixed mark and constant paw volume was ensured. Paw volumes were measured using a plethysmometer (model 7140, Ugo Basile, Italy). Thirty minutes after administration of test and standard drugs, 0.1 mL of 1% w/v of carrageenan suspension in normal saline was injected into subplanter region of the left hind paw of all the animals. The initial paw volume was measured within 30 s of the injection and remeasured again 30, 60, 90, and 120 min after administration of carrageenan. The edema was expressed as an increase in the volume of paw, and the percentage of edema inhibition for each rat and each group was obtained as follows:

$$\%\ \text{inhibition} = (V_t - V_0)\text{control} - (V_t - V_0)\text{tested compound}/(V_t - V_0)\text{control} \times 100$$

where V_t = volume of edema at specific time interval and V_0 = volume of edema at zero time interval.

4.3.2. In vitro antimicrobial assay

The antibacterial activity of newly synthesized compounds was evaluated in vitro by agar well diffusion method [21]. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 CFU/mL [22,23]. 20 mL of Mueller Hinton agar media was poured into each Petri plate and plates were swabbed with 100 μL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 μL volume with concentration of 4 mg/mL of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antimicrobial activity of 11 synthetic compounds was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). The medium with DMSO as solvent was used as a negative control whereas media with Ciprofloxacin was used as positive control. The experiments were performed in triplicates. The antimicrobial activities of the compounds were compared with Ciprofloxacin as standard. MIC of newly synthesized compounds against tested bacteria was determined using a microplate dilution method [16,17].

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