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Synthesis and pharmacological evaluation of some novel 2-(5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazoles

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ABSTRACT

A series of novel 2-(5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazoles (**6**) were synthesized by condensing 3-(2-bromoacetyl)coumarins (**4**) with various 5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-thiocarboxamides (**5**), obtained by the reaction of thiosemicarbazide with trifluoromethyl-β-diketones. All the tested compounds displayed significant to moderate *in vivo* anti-inflammatory activity when compared to the standard drug indomethacin, and good broad spectrum *in vitro* antibacterial activity against three Gram-positive and four Gram-negative bacteria when compared with cefixime.

1. Introduction

Thiazole moiety is a prevalent scaffold in a number of naturally occurring and synthetic molecules with attractive biological activities such as antiviral, anticancer, antibacterial, antifungal, anticonvulsant, antiparkinsonian and anti-inflammatory activities that is well illustrated by the large number of drugs in the market containing this heterocyclic moiety [1–8]. The presence of pyrazole and pyrazoline nuclei is a common feature in the chemical structure of several COX-2 inhibitors. The most common COX-2 selective inhibitors-celecoxib and SC-558 contain a pyrazole moiety. Many pyrazolines like kebuzone, mefobutazone, ramifenazone and phenylbutazone etc. are already reported in literature having potent anti-inflammatory activity. Similarly, antibiotics containing coumarin ring exhibit antibacterial activity against Gram-positive bacteria especially towards strain of *Staphylococcus aureus* [9,10]. Warfarin, a naturally occurring coumarin derivative and dicoumarol, a synthetic analogue, are used as anticoagulant to prevent clotting of blood in veins, lungs and heart [11,12]. Phenprocoumon shows high activity against the protease enzyme HIV-PR with an inhibition potential of 1 μM [13].

Recently, synthesis of many substituted coumarin derivatives having heterocyclic ring e.g. thiazole, pyrazole/pyrazoline moiety (i–iii, Fig. 1) has been reported in the literature to possess antimicrobial, analgesic and anti-inflammatory activities [14–16]. Maddi et al. have described the synthesis of 5-aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines (iv, Fig. 1), as anti-inflammatory agents [17]. Therefore, it was thought that the introduction of pyrazole/pyrazoline and coumarin moieties in thiazole derivatives may render them as useful substances in drug research. Also, the high lipophilicity of the trifluoromethyl group improves transport characteristics of the molecule *in vivo* and thus allows the avoidance of undesirable metabolic transformations [18,19]. In view of these observations and in continuation of our research program on the synthesis of fluorinated heterocyclic compounds [20–25], we herein report the syntheses of some novel 2,4-disubstituted thiazoles containing 5-trifluoromethylpyrazoline and coumarin rings at positions 2 and 4, respectively, which have

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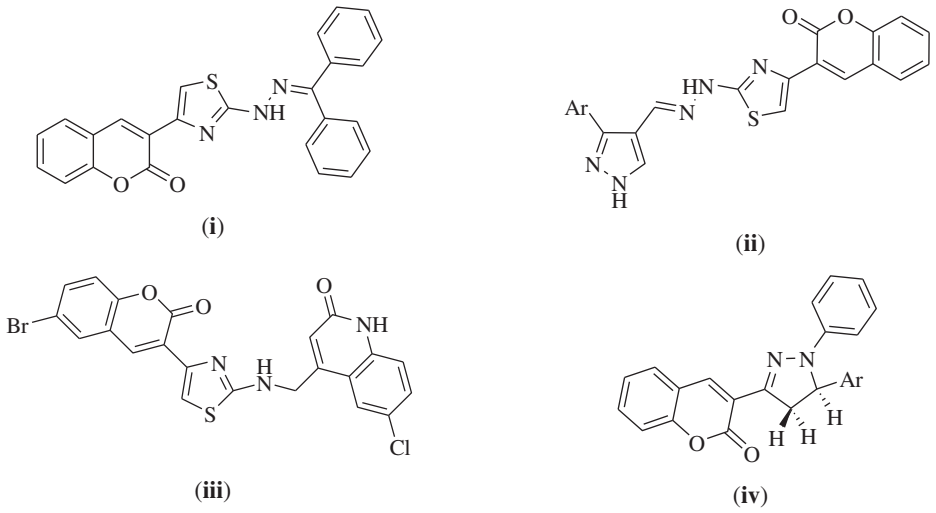


Fig. 1. Some bioactive coumarin containing heterocycles.

been found to possess an interesting profile of anti-inflammatory and antibacterial activities.

2. Results and discussion

2.1. Chemistry

3-Acetylcoumarins (**3**), obtained by the reaction of salicylaldehydes (**1**) with ethyl acetoacetate (**2**) in presence of catalytic amount of piperidine, were brominated in chloroform to obtain corresponding 3-(2-bromoacetyl)coumarins (**4**) [26]. Synthesis of the other precursors 5-hydroxy-5-trifluoromethylpyrazol-1-thiocarboxamides (**5**) was accomplished by the condensation of various trifluoromethyl-β-diketones with thiosemicarbazide according to literature procedure [20]. The synthetic reactions are outlined in Scheme 1.

Synthesis of the title compounds 2-(5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazoles (**6**) was accomplished following the well known Hantzsch's thiazole synthesis involving the reaction of synthons **5** and **4**. Thiazole formation in ethanol was indicated by the appearance of yellow colour of the reaction mixture and was found to be completed in 6 h (observed by TLC).

Recently, we have reported the synthesis of some fluorinated pyrazolylthiazoles in which the synthesis of thiazole ring is accompanied by simultaneous dehydration of 4-phenyl-2-(3-phenyl-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)thiazole to 4-phenyl-2-(3-phenyl-5-trifluoromethylpyrazol-1-yl)thiazole under the reaction conditions [20]. However, in the present work presence of a strong electron withdrawing coumarin ring in **6** makes them resistant to undergo dehydration under neutral conditions. Structure of all the newly synthesized compounds was established by spectral data such as IR, mass, NMR (^{1}H, ^{13}C and ^{19}F) and elemental analyses. These data are given in the Experimental part. The complete assignment of carbon signals of the compounds **6** and NMR spectra are given in Supplementary information.

The ^{1}H NMR spectra of **6** displayed two doublets appearing at about δ 3.85 (J_{HA-HB} = 18–21 Hz) and δ 3.70 ppm (J_{HA-HB} = 18–21 Hz) (about δ 3.73 and 3.55 ppm for **6a**–**c**) due to geminal coupling of H_B with H_A at position-4 of hydroxypyrazoline in **6**. Also, two singlets of one proton intensity each appearing at about δ 8.10 and δ 8.35 ppm correspond to thiazole-5H and coumarin-4H, respectively. Further, the ^{13}C NMR spectra of **6** confirmed the presence of pyrazoline ring by exhibiting signals at about δ 44 and 93 ppm as a quartet ($^2J_{C-F}$ = 30–35 Hz) attached to sp^3 carbons due to C_4 and C_5 carbons, respectively. Finally, ^{19}F NMR spectra of **6** showed a signal in the range δ −81 to −80 ppm, which is characteristic for CF_3 bound to saturated carbon. In case of **6a**–**c**, second CF_3 resonated at δ −67 to −66 ppm, which is a typical position of CF_3 located at position-3 of hydroxypyrazoline. These values are in accordance with the literature value [20–22,27–29].

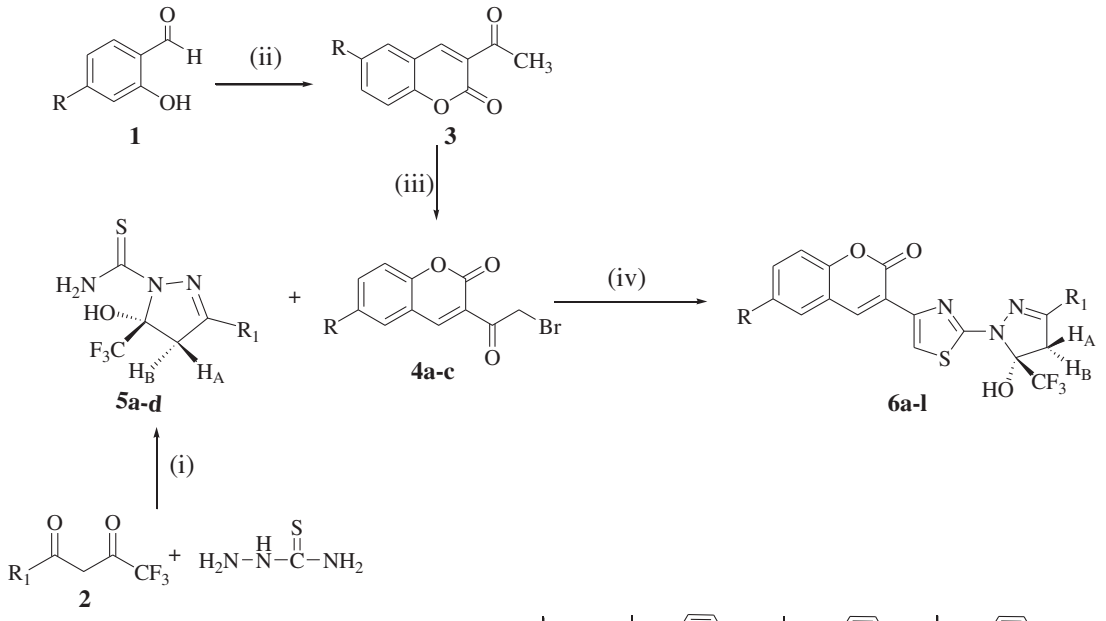
2.2. Biological evaluation

2.2.1. In vivo anti-inflammatory activity

Six synthesized compounds **6a**, **6b**, **6d**, **6e**, **6g** and **6j** were evaluated for their *in vivo* anti-inflammatory activity using carrageenan-induced paw oedema method described by Winter et al. [30]. The protocol of animal experiments has been approved by the Institutional Animal Ethics Committee (IAEC). Each test compound was dosed orally (50 mg/kg body weight) 30 min prior to induction of inflammation by carrageenan injection. Indomethacin was utilized as a reference anti-inflammatory drug at a dose of 10 mg/kg, i.p. The results of anti-inflammatory activity of the tested compounds and the reference non-steroidal anti-inflammatory drug indomethacin are listed in Table 1.

All the tested compounds exhibited potent anti-inflammatory activity (73–86% reduction in inflammation after 1 h), significantly $p < 0.01$. Activity table shows that the synthesized compounds act as fast acting anti-inflammatory agents and also remain active for a long duration of 4 h. Compounds **6a** ($R_1 = CF_3$, R = H) and **6b** ($R_1 = CF_3$, R = Cl) showed the highest (83 and 86%, respectively) anti-inflammatory activity, when compared to standard anti-inflammatory drug indomethacin (94%).

There are in fact a large number of enzymes/receptors involved in inflammatory process. Without specific tests it is quite difficult to hypothesize the mechanism of action of active compounds. Probably, the active compounds exert their action *via* inhibition of the cyclooxygenase enzymes like other non-steroidal anti-inflammatory agents. Carrageenan-induced inflammation is a non-specific inflammation resulting from a complex of diverse mediators [31]. This model is conventional, sensitive and accepted for screening of newer anti-inflammatory agents [32]. Further, this model reliably predicts the anti-inflammatory efficacy based on the inhibition of prostaglandin amplification [33].



R	H	Cl	Br
1, 3, 4	a	b	c

R_1	$-CF_3$	Cl	Br	F
2, 5	a	b	c	d

Comp.	R_1	R
6a	CF_3	H
6b	CF_3	Cl
6c	CF_3	Br
6d	4-Cl-C_6H_4	H

Comp.	R_1	R
6e	4-Cl-C_6H_4	Cl
6f	4-Cl-C_6H_4	Br
6g	4-Br-C_6H_4	H
6h	4-Br-C_6H_4	Cl

Comp.	R_1	R
6i	4-Br-C_6H_4	Br
6k	4-F-C_6H_4	H
6k	4-F-C_6H_4	Cl
6l	4-F-C_6H_4	Br

Reagents and reaction conditions: (i) ethanol, reflux; (ii) ethyl acetoacetate, piperidine, 0-5 °C, (iii) liquid Br_2, chloroform; (iv) ethanol, reflux.

Scheme 1. Synthesis of 2-(5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazoles (**6a**–**l**).

2.2.2. *In vitro antibacterial activity*

Nine newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against pathogenic *S. aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis* representing Gram-positive bacteria and *Klebsiella aerogenes*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* representing Gram-negative bacteria by agar well diffusion method using cefixime as the reference drug. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth surrounding the well (in millimetres) (Table 2). The Minimum Inhibitory Concentration (MIC) measurements were performed using a macrodilution tube method [34,35] (Table 3).

Results revealed that in general, all the tested compounds possessed moderate to good antibacterial activity against Gram-

Table 1
Anti-inflammatory activity of test compounds (carrageenan-induced paw oedema test in rats).

S. No.	Compound	Mean value of oedema volume[a] (% inhibition)[b]			
		1 h	2 h	3 h	4 h
1.	**6a**	0.12 ± 0.07** (83.10)	0.25 ± 0.09** (79.17)	0.48 ± 0.15** (64.81)	0.78 ± 0.18** (37.90)
2.	**6b**	0.10 ± 0.01** (85.91)	0.44 ± 0.07** (63.33)	0.62 ± 0.13** (54.54)	0.74 ± 0.12** (41.08)
3.	**6d**	0.16 ± 0.05** (77.46)	0.21 ± 0.07** (82.50)	0.71 ± 0.06** (47.95)	0.56 ± 0.20** (55.41)
4.	**6e**	0.19 ± 0.03** (73.24)	0.45 ± 0.01** (62.5)	0.79 ± 0.21* (42.08)	0.86 ± 0.10** (31.52)
5.	**6g**	0.17 ± 0.04** (76.06)	0.35 ± 0.08** (70.83)	0.88 ± 0.09 (35.48)	0.94 ± 0.16** (25.16)
6.	**6j**	0.18 ± 0.03** (74.65)	0.52 ± 0.05** (56.67)	1.06 ± 0.4 (22.29)	1.03 ± 0.08** (17.99)
7.	Indomethacin	0.04 ± 0.02** (94.37)	0.19 ± 0.04** (84.17)	0.26 ± 0.02** (80.94)	0.17 ± 0.03** (86.46)
	Control (DMSO)	0.71 ± 0.10	1.2 ± 0.23	1.364 ± 0.22	1.256 ± 0.09

Significantly different compared to respective control values, $^{**}p < 0.01$, $^{*}p < 0.05$.
[a] All values are expressed as mean ± SEM of five rats in each group analysed by Anova followed by Dunnett's '*t*' test.
[b] Values in parenthesis represents % inhibition.

Table 2
Antibacterial activity of synthesized compounds using agar well diffusion method.

S. No.	Compound[a]	Diameter of growth of inhibition zone (mm)[b]						
		S. aureus	*B. subtilis*	*S. epidermidis*	*K. aerogenes*	*E. coli*	*P. mirabilis*	*P. aeruginosa*
1.	**6c**	3.13	25.00	20.10	10.97	11.93	17.23	9.13
2.	**6d**	4.50	15.74	15.07	10.10	13.10	15.17	8.10
3.	**6e**	4.13	–	11.23	–	–	–	–
4.	**6f**	2.00	10.30	12.50	13.00	–	15.06	8.10
5.	**6h**	2.07	–	11.23	–	–	–	–
6.	**6i**	2.03	–	13.10	10.47	10.03	–	–
7.	**6j**	3.96	12.00	14.00	12.07	13.03	17.03	7.87
8.	**6k**	4.93	–	14.00	–	–	–	–
9.	**6l**	1.87	12.39	14.00	10.96	10.96	–	–
10.	Cefixime	3.90	26.00	22.00	15.00	14.10	20.00	10.00

– No activity.
[a] Concentration 2.0 mg/ml.
[b] Values, including diameter of the well (8 mm), are means of three replicates.

positive bacteria (*S. aureus*, *B. subtilis*, *S. epidermidis*) as well as Gram-negative bacteria (*K. aerogenes*, *E. coli*, *P. mirabilis* and *P. aeruginosa*). On the basis of zone of inhibition against the test bacterium, compound **6c** was found to be most effective against *B. subtilis*, *S. epidermidis*; compounds **6d**, **6e**, **6j** and **6k** were found to be most effective against *S. aureus* (Gram-positive bacteria) and compounds **6c**, **6d** and **6j** were found to be most active against *E. coli* and *P. aeruginosa* (Gram-negative bacteria), as compared with the standard drug cefixime. The zone of inhibition of compounds **6d**, **6e**, **6j** and **6k** against *S. aureus* was 4.50 mm, 4.13 mm, 3.96 mm and 4.93 mm, respectively and the zone of inhibition of standard antibiotic cefixime was 3.90 mm. However, in terms of MIC compounds **6d** and **6j** were found to be most effective against *E. coli* and *P. mirabilis* showing a MIC of 2 and 4 μg/ml, respectively, when compared to the standard antibiotic cefixime.

3. Conclusions

In conclusion, we have described an efficient and simple protocol for the preparation of triheterocyclic compounds 2-(5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazoles **6** by the condensation of 5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-thiocarboxamides **5** and 3-(2-bromoacetyl) coumarins **4** in excellent yields. The newly synthesized compounds **6** displayed significant anti-inflammatory and antibacterial activities when compared to well known reference drugs (indomethacin and cefixime). The preliminary studies of these compounds evidenced that coumarin ring and trifluoromethyl group at position-3 and 5 of pyrazoline ring enhance the antibacterial as well as anti-inflammatory activities of thiazoles, which might serve as new templates in the synthesis and development of potent therapeutics.

4. Experimental section

4.1. General

Melting points were determined in open capillaries in electrical apparatus and are uncorrected. IR spectra were recorded on a Buck Scientific IR M500 instrument. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument at 300 MHz and 75 MHz, respectively. D_2O exchange was applied to confirm the assignment of the signals of OH protons. ^{19}F NMR spectra were run on DRX 300 and DPX 400 at 282 and 376 MHz, respectively, (at SAIF, CDRI, Lucknow, India) using trichlorofluoromethane as a standard, setting the $CFCl_3$ signal at δ 0.0 ppm. Mass spectra were measured in EI mode on a Kratos MS-50 spectrometer at MS Facilities at SAIF, Panjab University, Chandigarh, India. Elemental analyses were performed at NIPER (Mohali), Chandigarh, India. All the compounds gave C, H and N analysis within ±0.5 of the theoretical values.

1,1,1,6,6,6-Hexafluoroacetylacetone **2a** was commercially available and other fluorinated diketones **2b**–**d** were prepared according to the literature procedure [36,37]. 5-Hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-thiocarboxamides **5a**–**d** and 3-(2-bromoacetyl) coumarins **4a**–**c** were also synthesized according to literature procedure [20,25,26].

4.2. General procedure for the preparation of 2-(3-alkyl/aryl-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazo-1-yl)-4-(coumarin-3-yl)thiazoles *6a–l*

An ethanolic solution (30 ml) of 3-(2-bromoacetyl)coumarin (**4**) (2 mmol) and 5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-thiocarboxamide (**5**) (2 mmol) was refluxed on a water bath for 6 h. On completion of the reaction (observed by TLC), solvent was

Table 3
Minimum inhibitory concentration (MIC) determination using agar well diffusion method.

S. No.	Compound	Minimum inhibitory concentration (MIC) (μg/ml)						
		S. aureus	*B. subtilis*	*S. epidermidis*	*K. aerogenes*	*E. coli*	*P. mirabilis*	*P. aeruginosa*
1.	**6c**	64	8	16	512	128	16	128
2.	**6d**	16	512	64	8	2	4	32
3.	**6e**	64	–	512	–	–	–	–
4.	**6f**	128	512	512	8	–	16	128
5.	**6h**	64	–	512	–	–	–	–
6.	**6i**	128	–	512	512	256	–	–
7.	**6j**	32	512	64	8	2	4	32
8.	**6k**	32	–	64	–	–	–	–
9.	**6l**	128	512	64	256	512	–	–
10.	Cefixime	2	2	2	2	2	2	2

evaporated. The solid thus obtained was dissolved in chloroform (25 ml) and neutralized with saturated solution of sodium bicarbonate (50 ml). Organic layer was extracted and excess solvent was distilled off. It was recrystallized from aqueous ethanol to afford the target compounds **6**.

4.3. Characterization data of synthesized compounds

4.3.1. 2-(5-Hydroxy-3,5-bis-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazole (**6a**)

Yield 87%; mp 253–254 °C; IR (KBr, cm^{-1}): 2957 (OH), 1720 (C=O), 1535, 1273, 1180, 1142, 1095, 756; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.34 (s, 1H, coumarin 4-H), 8.13 (s, 1H, thiazole 5-H), 7.67 (d, 1H, J = 7.5 Hz, coumarin 5-H), 7.61–7.41 (m, 3H, coumarin 6,7,8-H), 6.86 (bs, 1H, OH, exchangeable with D_2O), 3.73 (d, 1H, J = 19.5 Hz, pyrazoline 4-H_A), 3.55 (d, 1H, J = 20.1 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.33 (5-CF_3), −67.02 (3-CF_3); MS (*m*/*z*): 452, 450 ($[M + H]^+$), 412, 403, 355, 267; Anal. Calcd. for $C_{17}H_9F_6N_3O_3S$: C, 45.44; H, 2.02; N, 9.35. Found: C, 45.23; H, 2.10; N, 9.32.

4.3.2. 2-(5-Hydroxy-3,5-bis-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(6-chloro-coumarin-3-yl)thiazole (**6b**)

Yield 91%; mp 239–40 °C; IR (KBr, cm^{-1}): 2948 (OH), 1720 (C=O), 1493, 1273, 1170, 1121, 1092, 767; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.26 (s, 1H, coumarin 4-H), 8.14 (s, 1H, thiazole 5-H), 7.65 (d, 1H, J = 2.4 Hz, coumarin 5-H), 7.53 (dd, 1H, J = 8.7 Hz, 2.4 Hz, coumarin 7-H), 7.35 (d, 1H, J = 8.7 Hz, coumarin 8-H), 6.73 (bs, 1H, OH, exchangeable with D_2O), 3.73 (d, 1H, J = 19.2 Hz, pyrazoline 4-H_A), 3.55 (d, 1H, J = 19.2 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.26 (5-CF_3), −66.94 (3-CF_3); MS (*m*/*z*): 486, 484 ($[M + H]^+$), 448, 355, 281. Anal. Calcd. for $C_{17}H_8ClF_6N_3O_3S$: C, 42.21; H, 1.67; N, 8.69. Found: C, 42.20; H, 1.61; N, 8.65.

4.3.3. 2-(5-Hydroxy-3,5-bis-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(6-bromo-coumarin-3-yl)thiazole (**6c**)

Yield 85%; mp 250–251 °C; IR (KBr, cm^{-1}): 2993 (OH), 1728 (C=O), 1535, 1180, 1157, 1103, 1080, 1003, 787; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.24 (s, 1H, coumarin 4-H), 8.13 (s, 1H, thiazole 5-H), 7.79 (d, 1H, J = 2.1 Hz, coumarin 5-H), 7.66 (dd, 1H, J = 8.7 Hz, 2.4 Hz, coumarin 7-H), 7.28 (d, 1H, J = 7.5 Hz, coumarin 8-H), 6.72 (bs, 1H, OH, exchangeable with D_2O), 3.73 (d, 1H, J = 19.2 Hz, pyrazoline 4-H_B), 3.55 (d, 1H, J = 19.5 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −78.75 (5-CF_3), −62.42 (3-CF_3); MS (*m*/*z*): 529, 528 ($[M + H]^+$), 526, 512, 492, 475, 355, 281; Anal. Calcd. for $C_{17}H_8BrF_6N_3O_3S$: C 38.65; H 1.53; N 7.95. Found: C, 38.74; H, 1.14; N, 7.80.

4.3.4. 2-(3-(4-Chlorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazole (**6d**)

Yield 87%; mp 207–208 °C; IR (KBr, cm^{-1}): 2994 (OH), 1720 (C=O), 1532, 1284, 1183, 1092, 765; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.39 (s, 1H, coumarin 4-H), 8.07 (s, 1H, thiazole 5-H), 7.71 (d, 2H, J = 8.4 Hz, *p*-chlorophenyl 2, 6-H), 7.65 (s, 1H, coumarin 5-H), 7.59–7.54 (m, 1H, coumarin 7-H), 7.46 (d, 2H, J = 8.4 Hz, *p*-chlorophenyl 3,5-H), 7.41 (m, 2H, coumarin 6,8-H), 6.99 (bs, 1H, OH, exchangeable with D_2O), 3.86 (d, 1H, J = 18.6 Hz, pyrazoline 4-H_A), 3.72 (d, 1H, J = 18.6 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.12 (5-CF_3); MS (*m*/*z*): 494, 492 ($[M + H]^+$), 355, 281, 267; Anal. Calcd. for $C_{22}H_{13}ClF_3N_3O_3S$: C, 53.72; H, 2.66; N, 8.54. Found: C, 53.85; H, 2.35; N, 8.76.

4.3.5. 2-(3-(4-Chlorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(6-chloro-coumarin-3-yl)thiazole (**6e**)

Yield 84%; mp 222–223 °C; IR (KBr, cm^{-1}): 2965 (OH), 1716 (C=O), 1503, 1312, 1107, 1024, 787; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.29 (s, 1H, coumarin 4-H), 8.07 (s, 1H, thiazole 5-H), 7.70 (d, 2H, J = 8.4 Hz, *p*-chlorophenyl 2,6-H), 7.65 (d, 1H, J = 2.1 Hz, coumarin 5-H), 7.51 (dd, 1H, J = 8.7 Hz, 2.4 Hz, coumarin 7-H), 7.46 (d, 2H, J = 8.7 Hz, *p*-chlorophenyl 3,5-H), 7.34 (d, 1H, J = 8.7 Hz, coumarin 8-H), 6.85 (bs, 1H, OH, exchangeable with D_2O), 3.86 (d, 1H, J = 18.2 Hz, pyrazoline 4-H_A), 3.71 (d, 1H, J = 18.9 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.40 (5-CF_3); MS (*m*/*z*): 527, 526 ($[M + H]^+$), 525, 393, 355, 281; Anal. Calcd. for $C_{22}H_{12}Cl_2F_3N_3O_3S$: C, 50.20; H, 2.30; N, 7.98. Found: C, 49.87; H, 2.28; N, 7.95.

4.3.6. 2-(3-(4-Chlorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(6-bromo-coumarin-3-yl)thiazole (**6f**)

Yield 86%; mp 230–232 °C; IR (KBr, cm^{-1}): 2974 (OH), 1720 (C=O), 1543, 1304, 1173, 1095, 1003, 825, 787; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.29 (s, 1H, coumarin 4-H), 8.08 (s, 1H, thiazole 5-H), 7.81 (d, 1H, J = 2.1 Hz, coumarin 5-H), 7.70 (d, 2H, J = 8.4 Hz, *p*-chlorophenyl 3,5-H), 7.65 (dd, 1H, J = 8.7 Hz, 2.1 Hz, coumarin 7-H), 7.46 (d, 2H, J = 8.4 Hz, *p*-chlorophenyl 2,6-H), 7.28 (d, 1H, J = 6.9 Hz, coumarin 8-H), 6.84 (bs, 1H, OH, exchangeable with D_2O), 3.86 (d, 1H, J = 18.6 Hz, pyrazoline 4-H_A), 3.71 (d, 1H, J = 18.9 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.28 (5-CF_3); MS (*m*/*z*): 572, 570 ($[M + H]^+$), 473, 355, 299, 281; Anal. Calcd. for $C_{22}H_{12}BrClF_3N_3O_3S$: C, 46.29; H, 2.12; N, 7.36. Found: C, 46.12; H, 2.12; N, 7.35.

4.3.7. 2-(3-(4-Bromophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazole (**6g**)

Yield 89%; mp 232–234 °C; IR (KBr, cm^{-1}): 2997 (OH), 1718 (C=O), 1543, 1281, 1108, 949, 769; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.39 (s, 1H, coumarin 4-H), 8.07 (s, 1H, thiazole 5-H), 7.67 (d, 1H, J = 7.5 Hz, coumarin 5-H), 7.62 (m, 4H, *p*-bromophenyl), 7.57 (d, 1H, J = 7.2 Hz, coumarin 7-H), 7.41 (m, 2H, coumarin 6,8-H), 6.99 (bs, 1H, OH, exchangeable with D_2O), 3.86 (d, 1H, J = 18.3 Hz, pyrazoline 4-H_A), 3.72 (d, 1H, J = 18.3 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.50 (5-CF_3); MS (*m*/*z*): 537 ($[M + H]^+$), 535, 497, 438, 355, 281; Anal. Calcd. for $C_{22}H_{13}BrF_3N_3O_3S$: C, 49.27; H, 2.44; N, 7.83. Found: C, 49.22; H, 2.41; N, 7.85.

4.3.8. 2-(3-(4-Bromophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(6-chloro-coumarin-3-yl)thiazole (**6h**)

Yield 87%; mp 237–238 °C; IR (KBr, cm^{-1}): 3032 (OH), 1715 (C=O), 1548, 1287, 1149, 1018, 818, 787; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.29 (s, 1H, coumarin 4-H), 8.08 (s, 1H, thiazole 5-H), 7.65 (s, 1H, J = 2.4 Hz, coumarin 5-H), 7.61 (m, 4H, *p*-bromophenyl), 7.51 (dd, 1H, J = 8.7 Hz, 2.4 Hz, coumarin 7-H), 7.34 (d, 1H, J = 9.0 Hz, coumarin 8-H), 6.86 (bs, 1H, OH, exchangeable with D_2O), 3.86 (d, 1H, J = 18.3 Hz, pyrazoline 4-H_A), 3.71 (d, 1H, J = 18.3 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): -80.65 (5-CF_3); MS (*m*/*z*): 572, 570 ($[M + H]^+$), 355, 281, 299, 267; Anal. Calcd. for $C_{22}H_{12}BrClF_3N_3O_3S$: C, 46.29; H, 2.12; N, 7.36. Found: C, 46.22; H, 2.03; N, 7.34.

4.3.9. 2-(3-(4-Bromophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(6-bromo-coumarin-3-yl)thiazole (**6i**)

Yield 88%; mp 255–256 °C; IR (KBr, cm^{-1}): 2952 (OH), 1720 (C=O), 1528, 1381, 1173, 1103, 1072, 1018, 787; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.29 (s, 1H, coumarin 4-H), 8.07 (s, 1H, thiazole 5-H), 7.80 (d, 1H, J = 2.4 Hz, coumarin 5-H), 7.65 (m, 5H, p-bromophenyl 4H and coumarin 6-H), 7.28 (d, 1H, J = 8.1 Hz, coumarin 8-H), 6.84 (bs, 1H, OH, exchangeable with D_2O), 3.85 (d, 1H, J = 18.6 Hz, pyrazoline 4-H_A), 3.71 (d, 1H, J = 18.6 Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.32 (5-CF_3); MS (*m*/*z*): 614 ($[M + H]^+$), 612, 518, 355, 267; Anal. Calcd. for $C_{22}H_{12}Br_2F_3N_3O_3S$: C, 42.95; H, 1.97; N, 6.83. Found: C, 42.68; H, 1.95; N, 6.80.

4.3.10. 2-(3-(4-Fluorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(coumarin-3-yl)thiazole (**6j**)

Yield 78%; mp 207–208 °C; IR (KBr, cm^{-1}): 2924 (OH), 1720 (C=O), 1605, 1535, 1311, 1180, 1095, 1018, 841, 756; ^{1}H NMR (300 MHz,

$CDCl_3$, δ ppm): 8.39 (s, 1H, coumarin 4-H), 8.07 (s, 1H, thiazole 5-H), 7.79 (m, 2H, *p*-fluorophenyl 2,6-H), 7.68 (d, 1H, $J = 7.2$ Hz, coumarin 8-H), 7.59 (s, 1H, coumarin 5-H), 7.41 (m, 2H, coumarin 6,7-H), 7.20 (m, 2H, *p*-fluorophenyl 3,5-H), 7.01 (bs, 1H, OH, exchangeable with D_2O), 3.87 (d, 1H, $J = 18.3$ Hz, pyrazoline 4-H_A), 3.73 (d, 1H, $J = 18.6$ Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.55 (5-CF_3); MS (*m*/*z*): 476 ($[M + H]^+$), 429, 357, 355, 281; Anal. Calcd. for $C_{22}H_{13}F_4N_3O_3S$: C, 55.58; H, 2.76; N, 8.84. Found: C, 55.63; H, 2.41; N, 8.40.

4.3.11. 2-(3-(4-Fluorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(6-chloro-coumarin-3-yl))thiazole (**6k**)

Yield 80%; mp 241–242 °C; IR (KBr, cm^{-1}): 2974 (OH), 1724 (C=O), 1528, 1295, 1161, 1088, 958, 756; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.31 (s, 1H, coumarin 4-H), 8.08 (s, 1H, thiazole 5-H), 7.78 (m, 2H, *p*-fluorophenyl 2,6-H), 7.66 (d, 1H, $J = 2.1$ Hz, coumarin 5-H), 7.52 (dd, 1H, $J = 2.1$ Hz, 8.7 Hz, coumarin 7-H), 7.36 (m, 1H, coumarin 8-H), 7.20 (m, 2H, *p*-fluorophenyl 3,5-H), 6.93 (bs, 1H, OH, exchangeable with D_2O), 3.87 (d, 1H, $J = 18.6$ Hz, pyrazoline 4-H_A), 3.72 (d, 1H, $J = 18.6$ Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.76 (5-CF_3); MS (*m*/*z*): 512, 510 ($[M + H]^+$), 488, 464, 390, 281; Anal. Calcd. for $C_{22}H_{12}ClF_4N_3O_3S$: C, 51.83; H, 2.37; N, 8.24. Found: C, 51.69; H, 2.46; N, 8.40.

4.3.12. 2-(3-(4-Fluorophenyl)-5-hydroxy-5-trifluoromethyl-4,5-dihydropyrazol-1-yl)-4-(6-bromo-coumarin-3-yl)thiazole (**6l**)

Yield 75%; mp 249–251 °C; IR (KBr, cm^{-1}): 2942 (OH), 1715 (C=O), 1609, 1535, 1165, 1107, 1018, 756; ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.29 (s, 1H, coumarin 4-H), 8.07 (s, 1H, thiazole 5-H), 7.80 (d, 1H, $J = 2.1$ Hz, coumarin 5-H), 7.78 (m, 2H, *p*-fluorophenyl 2,6-H), 7.64 (dd, 1H, $J = 2.1$ Hz, 8.7 Hz, coumarin 7-H), 7.25 (m, 1H, coumarin 8-H), 7.17 (d, 2H, $J = 8.7$ Hz, *p*-fluorophenyl 3,5-H), 6.86 (bs, 1H, OH, exchangeable with D_2O), 3.86 (d, 1H, $J = 18.6$ Hz, pyrazoline 4-H_A), 3.71 (d, 1H, $J = 18.9$ Hz, pyrazoline 4-H_B); ^{19}F NMR ($CDCl_3$, δ ppm): −80.52 (5-CF_3); MS (*m*/*z*): 554 ($[M + H]^+$), 552, 533, 435, 355, 267; Anal. Calcd. for $C_{22}H_{12}BrF_4N_3O_3S$: C 47.67; H 2.18; N 7.58. Found: C, 47.53; H, 2.28; N, 7.92.

5. Pharmacological assay

5.1. In vivo anti-inflammatory assay

5.1.1. Carrageenan-induced rat paw oedema assay

Male Wistar 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 rats were divided into three groups (control, standard drug and drugs treated) of five animals each. One group (control group) of five rats was kept as control and received tween 80 (95%). Standard group received indomethacin at a dose of 10 mg/kg body weight orally. Test group received test compounds at a dose of 50 mg/kg body weight. 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. A freshly prepared suspension of carrageenan (1% in 0.9% saline), 0.1 ml was injected under the planter region of the left hind paw of each rat. Test compounds and standard drug were administered orally to the animals, respectively 30 min before the carrageenan injection. The paw volume (up to the tibiotarsal articulation) of each rat was measured at 0 h (before carrageenan injection) and after 1 h, 2 h, 3 h and 4 h of carrageenan treatment with the help of a Plethysmometer (model 7140, Ugo Basile, Italy). The percentage inhibition of anti-inflammatory activity was calculated according to the following equation

$$\text{Anti} - \text{inflammatory activity (\% inhibtion)} = \{(V_c - V_t/V_c)\} \times 100$$

where V_t and V_c are the volume of oedema in drug treated/standard drug and control group, respectively.

5.2. In vitro antibacterial assay

5.2.1. Test microorganisms

Seven pathogenic bacteria, *S. aureus*, *B. subtilis*, *S. epidermidis* (Gram-positive), *K. aerogenes*, *E. coli*, *P. mirabilis*, and *P. aeruginosa* (Gram-negative) isolated from the patients in Maharishi Markandeshwar Medical College, M. M. University, Mullana, Haryana, were used in the present study.

5.2.2. In-vitro antibacterial activity

The antibacterial activity of nine newly synthesized compounds was evaluated by agar well diffusion method [38]. 25 ml of nutrient agar medium was poured into each petri plate and the agar plates were swabbed with 100 μL inocula of each test bacterium and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into seeded agar plates and these were loaded with a 50 μL volume. Solutions of the test compounds and standard were prepared in dimethylsulphoxide (DMSO) at concentration of 2.0 mg/ml. From this stock solution, two-fold dilutions of the compounds (2, 4, 8,…512 μg/ml) were inoculated to the corresponding wells. All the plates were incubated at 37 °C for 24 h. Antibacterial activity of compounds was evaluated by measuring the zone of growth inhibition and MIC against the test organisms with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas cefixime was used as a reference drug. Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of the compound tested that was able to inhibit visible growth of a microorganism after overnight incubation. The experiments were performed in triplicates and the results averaged.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.11.046.

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