Supplementary Information

Synthesis of some 2-(3-alkyl/aryl-5-trifluoromethylpyrazol-1-yl)-4-(coumarin-3-yl)thiazoles as novel antibacterial agents

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¹H NMR and ¹⁹F NMR spectra of some novel 2-(3'-alkyl/aryl-5'-trifluoromethylpyrazol-1'-yl)-4-(coumarin-3'''-yl)thiazoles are attached.

General

The ¹H and ¹³C NMR spectra were recorded on a Bruker instrument at 300 MHz and 75 MHz, respectively. ¹⁹F NMR spectra were run on DRX 300 and DPX 400 at 282 and 376 MHz, respectively. The internal standard for ¹⁹F spectra was fluorotrichloromethane, setting the CFCl₃ signal at δ 0.0 ppm.

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 ¹H and ¹³C NMR spectra were recorded on a Bruker instrument at 300 MHz and 75 MHz, respectively. ¹⁹F 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 CFCl₃ 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 analysis was performed at NIPER (Mohali), Chandigarh, India. High resolution mass spectra (HRMS) were measured in ESI mode

on Thermo LTQFT Spectrometer at MS facilities at UCSF, USA. All the compounds gave C, H and N analysis within ± 0.5 of the theoretical values.

Table 1. ^{13}C NMR data for 2-(3'-alkyl/aryl-5'-trifluoromethylpyrazol-1'-yl)-4-(coumarin-3'''-yl)thiazoles (**6a-g**) (chemical shifts in ppm).

Compd.	6a	6b	6c	6d	6e	6f	6g
Thiazole							
C-2	159.45	159.12	160.42	160.87	160.62	161.91	160.13
C-4	145.86	145.16	144.27	143.76	143.75	143.06	143.24
C-5	116.50	117.93	117.62	117.08	117.82	117.34	117.53
Pyrazole							
C-3'	132.11	131.57	133.54	133.66	133.51	133.92	132.94
C-4'	109.82	109.97	109.77	109.45	110.14	109.93	109.67
C-5'	133.35	131.98	132.92	134.02	134.02	134.50	134.36
Aryl							
C-1''	-	-	131.52	131.83	131.47	130.81	131.81
C-2'',6''	-	-	127.86	128.15	127.96	127.63	128.86
C-3'',5''	-	-	132.11	132.18	132.46	132.20	116.34
C-4''	-	-	120.58	122.76	121.79	123.90	126.47
Coumarin							
C-2'''	157.44	157.16	159.32	158.47	158.78	158.64	159.24
C-3'''	128.21	129.65	129.37	134.83	130.76	123.69	128.76
C-4'''	140.34	138.89	144.16	143.68	141.23	138.47	142.03
C-4a'''	120.28	119.91	120.76	120.62	126.27	121.00	120.47
C-5'''	124.79	121.28	119.18	121.49	128.76	125.42	119.93
C-6'''	123.42	130.16	125.46	124.35	119.86	118.15	124.48
C-7'''	128.79	127.84	129.62	130.79	127.68	124.63	127.36

C-8'''	119.27	117.75	116.38	117.35	117.36	118.78	116.40
C-8a'''	153.22	151.51	151.32	151.48	151.82	151.90	150.69
Others							
3'-CF ₃	126.49 (q, ¹ J _{C-F} = 266.41 Hz)	128.98 (q, ¹ J _{C-F} = 270.18 Hz)					
5'-CF ₃	120.08 (q, ¹ J _{C-F} = 267.92 Hz)	120.38 (q, ¹ J _{C-F} = 260.37 Hz)	122.68 (q, ¹ J _{C-F} = 267.75 Hz)	124.18 (q, ¹ J _{C-F} = 267.92 Hz)	123.42 (q, ¹ J _{C-F} = =265.65 Hz)	121.56 (q, ¹ J _{C-F} = =262.63 Hz)	122.35 (q, ¹ J _{C-F} = 273.20 Hz)

¹H NMR of 2-(3',5'-Bis-trifluoromethylpyrazol-1'-yl)-4-(coumarin-3''-yl)thiazole (6a)

F2 - Acquisition Parameters

Date_	20110211
Time	16.32
INSTRUM	spect
PROBHD	5 mm Multinucl
PULPROG	zg30
TD	65536
SOLVENT	CDC13

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===== CHANNEL f1 =====
NUC1      1H
P1        7.25 usec
PL1       0.00 dB
SFO1      300.1318534 MHz
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F2 - Processing parameters	
SI	32768
SF	300.130000 MHz
EM	
WDW	0
SSB	0.30 Hz
LB	0
GB	0
PC	1.00

¹H NMR of 2-(3'-(*p*-Chlorophenyl)-5'-trifluoromethylpyrazol-1'-yl)-4-(6'''-bromocoumarin-3'''-yl)thiazole (**6d**)

¹H NMR of 2-(3'-(*p*-Bromophenyl)-5'-trifluoromethylpyrazol-1'-yl)-4-(6'''-chlorocoumarin-3'''-yl)thiazole (**6e**)

^1H NMR of 2-(3'-(*p*-Bromophenyl)-5'-trifluoromethylpyrazol-1'-yl)-4-(6'''-bromocoumarin-3'''-yl)thiazole (**6f**)

¹⁹F NMR of 2-(3',5'-Bis-trifluoromethylpyrazol-1'-yl)-4-(coumarin-3''-yl)thiazole (**6a**)

^{19}F NMR of 2-(3'-(*p*-Chlorophenyl)-5'-trifluoromethylpyrazol-1'-yl)-4-(6'''-bromocoumarin-3'''-yl)thiazole (**6d**)

¹⁹F NMR of 2-(3'-(*p*-Bromophenyl)-5'-trifluoromethylpyrazol-1'-yl)-4-(6'''-bromocoumarin-3'''-yl)thiazole (**6f**)

***In vitro* antibacterial activity**

Test microorganisms

Total four microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121) and two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741) were screened for evaluation of antibacterial activity of the chemical compounds. All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were subcultured on Nutrient agar.

Antibacterial activity

The antibacterial activity of six chemical compounds was evaluated by the agar well diffusion method. All the bacterial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a bacterial suspension of approximately 1.5×10^8 cfu/ml. 20 ml of agar medium was poured into each petri plate and plates were swabbed with 100 μ l 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 μ l volume with concentration of 4.0 mg/ml of each compound reconstituted in the dimethyl sulphoxide (DMSO). All the plates were incubated at 37 °C for 24 hrs. Antibacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as positive control for bacteria. This procedure was performed in three replicate plates for each organism.

Determination of Minimum Inhibitory Concentration (MIC) of the synthesized compounds

MIC is the lowest concentration of an antibacterial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the various compounds against bacterial strains was tested through a modified agar well diffusion method [46]. In this method, a twofold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 512 to 1 μ g/ml. A 100 μ l volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 μ l of standardized inoculum (10^6 cfu/ml) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 hrs

and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that completely inhibited the growth of the microbe, showed by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin was used as positive control while DMSO as negative control.

Results

In the present study, six chemical compounds were screened for their antibacterial activity. Tested chemical compounds possessed variable antibacterial activity against the Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria whereas compounds don't exhibit any activity against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. Positive controls produced significantly sized inhibition zones against the tested bacteria and fungi, however, negative control produced no observable inhibitory effect against any of the test organism as shown in **Tables 2** and **3**.

Table 2. *In vitro* antibacterial activity of chemical compounds through agar well diffusion method

Compound	Diameter of growth of inhibition zone (mm)			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
6a	19.6	22.3	-	-
6b	20.4	19.0	-	-
6c	14.3	16.6	-	-
6d	15.0	16.3	-	-
6f	18.3	20.6	-	-
6g	15.6	16.3	-	-
Ciprofloxacin	24.6	23.0	24.0	21.0

- No activity; ^aValues, including diameter of the well (8mm), are means of three replicates

Table 3. Minimum inhibitory concentration (MIC) (in µg/ml) of compounds

Compound	<i>S. aureus</i>	<i>B. subtilis</i>
6a	32	32
6b	32	64
6c	128	64
6d	128	128
6f	64	32

6g	128	64
Ciprofloxacin	6.25	6.25

On the basis of maximum inhibitory activity shown against Gram-positive bacteria, compounds namely **6a**, **6b** and **6f** were found to be the most effective against *B. subtilis* and *S. aureus* with zone of inhibition of 19.6, 20.4, 18.3 mm, and 22.3, 19.2, 20.6 mm, respectively, whereas other tested compounds displayed moderate activity (**Table 2**). In the whole series, the MIC of various tested chemical compounds ranged between 32 and 256 µg/ml against Gram-positive bacteria. Compound **6a**, **6b** and **6f** were found to be best as they exhibit the lowest MIC of 32, 32, 64 µg/ml against *S. aureus* and MIC of 32, 64, 32 µg/ml against *B. subtilis* (**Table 3**). One compound could not be tested for antibacterial activity due to poor solubility in DMSO.