SYNTHESIS AND CHARACTERIZATION OF TRIFLUOROMETHYL SUBSTITUTED PYRAZOLE DERIVATIVES

Jignesh K Sutariya,[a*] Jayesh L Gajera,[a] Jigar Soni,[a] Haresh Ram[b] and Pankaj K. Godhaviya[c]

Keywords: pyrazole; ethyl (2E)-2-(ethoxymethylidene)-4,4,4-trifluoro-3-oxobutanoate; arylhydrazine.

A series of functionally substituted pyrazole compounds have been synthesized using classical chemistry. Here we synthesized trifluoromethyl containing pyrazole compound by condensation of arylhydrazine derivative with ethyl (2E)-2-(ethoxymethylidene)-4,4,4-trifluoro-3-oxobutanoate. Pyrazole moieties have essential pharmaceutical and agrochemical activities. Our research-based on trifluoromethyl-containing pyrazole compounds synthesis also helps to improve biological activities due to fluorine binding affinity properties.

Corresponding author
E-mail: jignesh.0304@gmail.com
[a] Madhav University, Sirohi, Rajasthan, India
[b] Tolani College of Arts and Science, Adipur, India
[c] Navin Fluorination International Ltd, Bhestan, Surat, India.

INTRODUCTION

Pyrazoles and their derivatives, a class of well-known nitrogen heterocycles are one of the main groups of organic compounds possessing a wide range of applications in various areas of agrochemicals and pharmaceuticals. They have been known to exhibit reported to show a broad spectrum of biological activity, including antimicrobial, antiviral[1] anti-tumor,[2] anti-histaminic,[3] anti-depressant,[4] insecticides, and fungicides.[5]

In the current era, microorganisms cause infections to pose a serious challenge to the medical society, which needs effective therapy and searching for novel antibacterial agents. Literature suggests that pyrazole-containing compounds having pharmacological properties also possess antimicrobial activity. Such a heterocyclic moiety represents the core structure for several drugs such as Celebrex, Sildenafil (Viagra), Ionazlac, Rimonabant, and Difenamizole, etc. Motivated by all these facts, we aimed to synthesize a series of novel findings mentioned above and continue our research to develop a series of 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide derivatives.

MATERIALS AND METHODS

All chemicals were purchased from lab-scale reagent suppliers and used without further any other treatment. In-process check of the reaction was monitored by analytical TLC on pre-coated plates (purchased from Merc) and visualized with UV light or other TLC stain. Melting points were measured by an open capillary tube and are uncorrected. Flash column chromatography purification was carried out with the use of silica gel 60-120 mesh. NMR spectra were recorded on ^{1}H FT-NMR (Brucker AMX 400 MHz) spectrometer using DMSO-d_6 as a solvent, and chemical shifts are expressed in ppm, related to internal TMS. MASS spectra were analyzed by Water's LCMS.

Step 1: Synthesis of ethyl 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate

(3,5-Dichlorophenyl)hydrazine (17.7 g, 0.1 mol, 1 equiv.) and ethanol (350 mL, 20 vol.) were charged into a three-neck flask fitted with; stirrer bar, condenser, temp probe, N_2 inlet, and bubbler. (E)-1-ethyl-3-trifluoromethyl-2-(ethoxy-methylene)malonate (25.6 g, 0.1 mol, 1 equiv.) was added cautiously at 10 to 15 °C with stirring. After completion of the addition, the reaction mixture allows room temperature. After 30 min the reaction mixture heated to reflux (oil bath temp 80 °C).

The reaction was monitored by TLC and HPLC. After completion of the reaction by TLC, the solvent was removed completely by distillation to get a light-yellow solid crude product was obtained. Add water to the above solid and product extracted with ethyl acetate (3 X 50 mL) and separate the organic layer. Combine organic layer dried over sodium sulphate and distilled out completely to get the desired product ethyl 1-(3,5-dichlorophenyl)-5-(trifluoro-methyl)-1H-pyrazole-4-carboxylate (30 g, yield 85 %).

Step 2: Synthesis of ethyl 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxic acid

To a 2 L four neck flask, containing a mechanical stirrer bar and fitted with a condenser and bubbler, all placed within an oil bath was added ethyl 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate (30 g, 0.085 mol, 1.0 equiv.), and ethanol (300 mL, 10 vol.). The mixture

was stirred for 10 min to get a clear solution. Add mixture of ethanol (100 mL) and LiOH (10.2 g, 0.42 mol, 5.0 equiv.), at room temperature. The resulting stirred mixture was heated to reflux temperature.

HPLC analysis appeared to confirm the completion of the reaction, so forward it for workup. The reaction mixture was concentrated by distillation to get gummy solid. Water (90 mL, 3 vol.) was added to the above gummy solid to dissolve it. Wash the aqueous layer with ethyl acetate (50 mL). Acidify the aqueous layer's pH acidic by diluted hydrochloric acid, and the product is extracted with ethyl acetate. The organic layer was dried over sodium sulfate and distilled out solvent completely to get the desired product 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyr-azole-4-carboxylic acid as off white solid (23 g, yield 84 %)

Step 3: General procedure for the synthesis of ethyl 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic amide derivatives

1-(3,5-Dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid (3.2 g, 0.01 mol, 1 equiv.) tetrahydrofuran (15 mL, 5 vol.) was charged into an flask fitted with; a stirrer bar, temperature probe, and condenser. Add N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (1.91 g, 0.001 mol, 1 equiv.), and hydroxybenzotriazole (1.53g, 0.01 mol, 1 equiv.), followed by triethylamine (1.01 g, 0.01 mol, 1 equiv.). The reaction mixture was stirred for 30 min at room temperature. Then amine (0.011 mol, 1 equiv.) was added dropwise over 15 min at room temperature. After that reaction mixture was stirred for 5 h at room temperature.

The reaction was monitored by TLC and HPLC. Remove volatiles by distillation under vacuum to get solid. The solid was added to water, and the product was extracted by ethyl acetate (350 mL) and a separate organic layer. Combined organic layer was dried over sodium sulfate. The solvent was distilled out completely to get the crude product. Crude was purified by column chromatography over 60-120 mesh size silica and hexane-ethyl acetate gradient mobile phase to get the desired product amide derivatives as pure, and the average yield was 75-80 %.

1-(3,5-Dichlorophenyl)-N-(4-fluorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D1)

The synthesis process was the same as the general process step 3, isolated product was cream solid, Yield: 67 %, ^{1}H NMR (300 MHz, chloroform-d) δ 8.09 (s, 1H), 7.71 – 7.70 (d, *J* = 3.0 Hz, 2H), 7.60 – 7.57 (m, 2H), 7.47 – 7.46 (d, *J* = 3.0 Hz, 1H), 7.12 – 7.07 (m, 2H). MS: *m*/*z* 418.03 (M+1).

1-(3,5-Dichlorophenyl)-N-(3-fluorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D2)

The synthesis process was the same as the general process step 3, isolated product was light yellow solid, Yield: 69 %. ^{1}H NMR (300 MHz, chloroform-d) δ 8.01 (s, 1H), 7.79 – 7.76 (m, 1H), 7.71 (s, 2H), 7.62 – 7.59 (m, 1H), 7.47 – 7.47 (d, *J* = 3.0 Hz, 1H), 7.40 – 7.33 (m, 1H), 6.84 – 6.77 (m, 1H). MS: *m*/*z* 418.01 (M+1).

1-(3,5-Dichlorophenyl)-N-(2-fluorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D3)

The synthesis process was the same as the general process step 3, isolated product was white Solid, Yield: 65 %, ^{1}H NMR (300 MHz, chloroform-d) δ 8.04 (s, 2H), 7.83 – 7.78 (m, 1H), 7.72 (s, 2H), 7.47 – 7.45 (m, 1H), 7.17 – 7.05 (m, 2H). MS: *m*/*z* 418.06 (M+1).

N-(4-Bromophenyl)-1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D4)

The synthesis process was the same as the general process step 3, isolated product was white solid, Yield: 60 %, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.72 (s, 2H), 7.63 – 7.63 (m, 2H), 7.50 – 7.46 (m, 3H). MS: *m*/*z* 477.91 (M + 1).

N-(2-Bromophenyl)-1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D5)

The synthesis process was the same as the general process step 3, isolated product was light brown Solid, Yield: 58 %, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.72 (s, 2H), 7.62 – 7.55 (m, 2H), 7.46 – 7.45 (m, 1H), 7.37 – 7.32 (t, *J* = 9.0 Hz, 1H), 7.15 – 7.11 (m, 1H). MS: *m*/*z* 477.98 (M + 1).

N-(3-Chloro-2-methylphenyl)-1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D6)

The synthesis process was the same as the general process step 3, isolated product was white solid, Yield: 67 %, ^{1}H NMR (300 MHz, chloroform-d) δ 8.04 (s, 1H), 7.72 (s, 2H), 7.65 – 7.62 (m, 2H), 7.47 – 7.45 (m, 1H), 7.23 – 7.21 (m, 1H), 2.27 (s, 3H). MS: *m*/*z* 448.01 (M + 1).

1-(3,5-Dichlorophenyl)-N-(3-fluoro-2-methylphenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D7)

The synthesis process was the same as the general process step 3, isolated product was off-white solid, Yield: 66 %, ^{1}H NMR (300 MHz, chloroform-d) δ 8.04 (s, 1H), 7.72 (s, 2H), 7.56-7.53 (m, 1H), 7.47 - 7.46 (d, *J* = 3.0 Hz, 1H), 7.36 – 7.29 (m, 2H), 6.97 – 6.92 (m, 1H), 2.28 (s, 3H). MS: *m*/*z* 431.01 (M + 1).

N-(3-Bromo-2-methylphenyl)-1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D8)

The synthesis process was the same as the general process step 3, isolated product was off-white solid, Yield: 59 %, ^{1}H NMR (300 MHz, chloroform-d) δ 8.04 (s, 1H), 7.72 – 7.69 (m, 2H), 7.47 - 7.46 (d, *J* = 3.0 Hz, 1H), 7.33 – 7.33 (d, *J* = 9.0 Hz, 1H), 7.17 – 7.12 (t, *J* = 6 Hz, 1H), 2.29 (s, 3H). MS: *m*/*z* 490.91 (M + 1).

N-(2-Chloro-5-(trifluoromethyl)phenyl)-1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D9)

The synthesis process was the same as the general process step 3, isolated product was light brown solid, Yield: 60 %,

^{1}H NMR (300 MHz, chloroform-d) δ 8.04 (s, 1H), 7.69 – 7.65 (m, 2H), 7.45 – 7.44 (d, *J* = 3.0 Hz, 1H), 7.31 – 7.29 (m, 1H), 6.93 – 6.90 (d, *J* = 9.0 Hz, 1H). MS: *m*/*z* 502.03 (M + 1).

1-(3,5-Dichlorophenyl)-N-(pyridin-2-yl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (D10)

The synthesis process was the same as general process step 3, isolated product was off-white solid, Yield: 55 %, ^{1}H NMR (300 MHz, chloroform-d) δ 8.48 – 8.45 (d, *J* = 9.0 Hz, 1H), 8.14 – 8.04 (m, 2H), 7.74 – 7.68 (m, 2H), 7.71 (d, *J* = 16.5 Hz, 1H), 7.48 – 7.48 (m,1H), 7.20 – 7.15 (m, 1H). MS: *m*/*z* 432.02 (M + 1).

Antibacterial activity

To evaluation of the antimicrobial activity, we have used the broth dilution method. This method is non-automated in vitro microbial susceptibility tests. Synthesized compounds **D1** to **D10** molecule were investigated for their potency as antibacterial. Petri dish with bacterial lawn prepared by poured 20 mL agar media. Then disc is keeping in an incubator at 37 °C for an hour to dry. After drying, wells were prepared on these seeded agar plates for sampling. The solutions of synthesized molecules in dimethyl sulfoxide were added into each labeled well.

A control was also prepared using plain DMSO as a solvent for the plates in the same way. Prepared three sets of Petri dishes and maintained a 37 °C for 3-4 days.[6]

Antifungal activity

All the synthesized compounds were investigated for their antifungal activity. The compounds studied against fungi *Candida krusei* (ATCC 6258) *Candida albicans* (ATCC 90028), and Candida parapsilosis (ATCC 22018) using the Serial plate dilution method in DMSO.[6] Sabourauds agar media prepared by dissolving D-glucose (4 g), peptone (1 g), and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. To each Petri dish, 20 mL of agar media was poured. The excess suspension was removed, and plates are kept in an incubator at 37 °C for 1 h for drying. Wells were prepared on these seeded agar plates for sampling. The synthesized compounds solution in DMSO was poured into each identified well. A control was prepared using solvent DMSO in the same way. Three sets of the Petri dishes were prepared maintained at 37 °C for 3-4 days.

RESULTS AND DISCUSSION

The synthesis of 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide derivatives are summarized in Scheme 1. (3,5-dichlorophenyl)hydrazine is reacted with (E)-1-ethyl-3-trifluoromethyl-2-(ethoxymethylene)malonate at reflux temperature in ethanol, reaction undergo condensation followed by cyclisation to affords ethyl 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylate. This ester compound is hydrolyzed with lithium hydroxide in ethanol as a solvent to afford ethyl 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic acid derivatives.

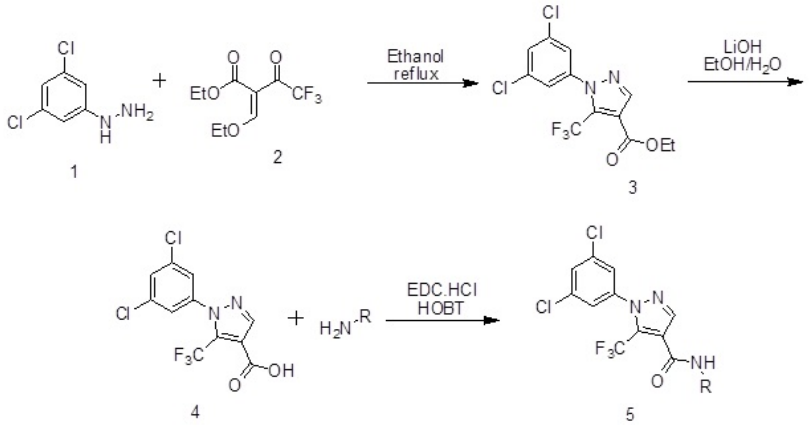


Figure 1. The synthesis of 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide derivatives

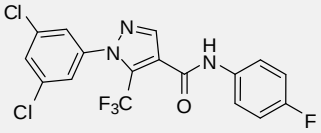
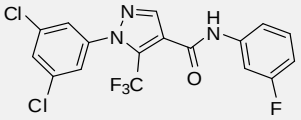
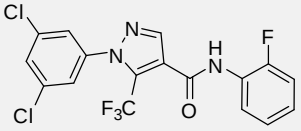
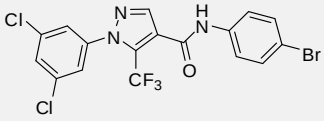
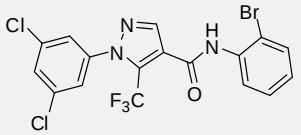
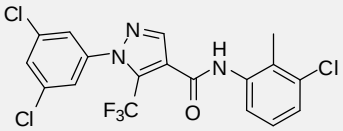
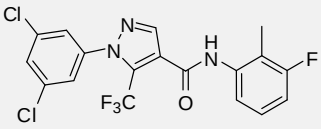
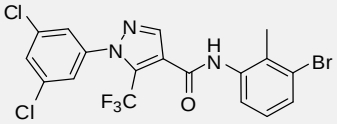
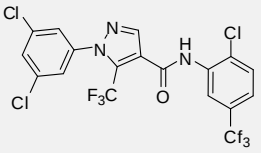
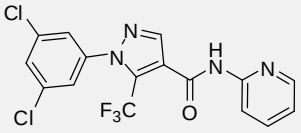
This acid compound undergoes amide formation with a different amine using-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), hydroxybenzotriazole, triethylamine as base and tetrahydrofuran as a solvent to get desired compounds ethyl 1-(3,5-dichlorophenyl)-5-(trifluoromethyl)-1H-pyrazole-4-carboxylic amide derivatives.Antibacterial activity was assessed by calculating the diameter of the inhibition area. Norfloxacin is used as a reference for comparing the activity of synthesized compounds.[7] The area of inhibition was determined for all synthesized compounds **D1** to **D10**. The results are recapitulated in (Table 2).

The following formula is used to calculate the total area of inhibition concerning the standard.

$$\text{Relative percentage inhibition} = 100 \times \frac{X - Y}{Z - Y}$$

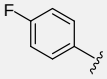
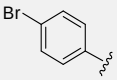
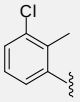
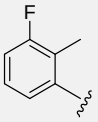
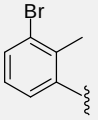
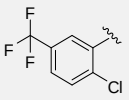
where: X = total area of inhibition (test plate), Y = total area of inhibition (solvent (DMSO) plate), Z = total area of inhibition (reference plate), area of inhibition=πr^2, where *r* = radius of inhibition zone in the test, solvent and reference plates.

Table 1. Molecular formula and yield of the synthesized trifluoromethylated pyrazole derivatives

No.	R	Mol. Formula	Mol. Wt.	Yield, %
D1		$C_{17}H_9Cl_2F_4N_3O$	418.17	67
D2		$C_{17}H_9Cl_2F4N_3O$	418.17	69
D3		$C_{17}H_9Cl_2F_4N_3O$	418.17	65
D4		$C_{17}H_9BrCl_2F_3N_3O$	479.08	60
D5		$C_{17}H_9BrCl_2F_3N_3O$	479.08	58
D6		$C_{18}H_{11}Cl_3F_3N_3O$	448.65	67
D7		$C_{18}H_{11}Cl_2F_4N_3O$	432.20	66
D8		$C_{18}H_{11}BrCl_2F_3N_3O$	493.10	59
D9		$C_{18}H_8Cl_3F_6N_3O$	502.63	60
D10		$C_{16}H_9Cl_2F_3N_4O$	401.17	55

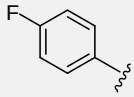
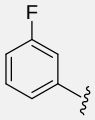
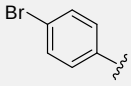
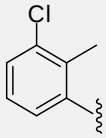
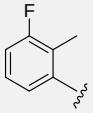
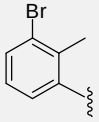
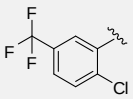
After that, antifungal activity was examined by assessing the inhibition area's diameter, activities of all synthesized compounds were compared with Fluconazole as a reference compound. Zones of inhibition were determined for **D1** and **D10.** The results are summarized in (Table 3). The variation in the antimicrobial activity of the test compounds was investigated by using different substituents. Among all the investigated molecules, compound **D1**, **D2**, and **D3** exhibited the highest antibacterial and antifungal activity against the given bacterial pathogens however, it is much less than the reference drug norfloxacin. The good activity was predictable to the presence of pharmacologically active fluorine group. Detailed investigation showed that fluorine group substitution to meta position gives comparatively more active. Also, compound D10 having trifluoromethyl and chloro-substitution exhibited moderate activity.

Table 1.Antibacterial activity of the synthesized compounds

No.	Compound	Bacillus cereus		Staphylococcus aureus		Pseudomonas pyocyanous	
		Area of inhibition, mm²	Relative % of inhibition	Area of inhibition, mm²	Relative % of inhibition	Area of inhibition, mm²	Relative % of inhibition
D1		10	11.0	8	8.6	2	2.2
D2		15	16.5	12	12.9	11	12.2
D3		9	9.9	8	8.6	10	11.1
D4		0	0.0	0	0.0	0	0.0
D5		0	0.0	0	0.0	0	0.0
D6		0	0.0	1	1.1	0	0.0
D7		2	2.2	0	0.0	0	0.0
D8		0	0.0	0	0.0	0	0.0
D9		5	5.5	2	2.2	1	1.1
D10		0	0.0	0	0.0	0	0.0

Comparative inhibition of reference standard drug norfloxacin is taken as 100%

Table 2. Anfigungal activity of the synthesized compounds

No.	R	Tested Fungus		
		C. Albicans	**C. krusei**	**C. parapsilosis**
D1		20	19	22
D2		18	17	21
D3		24	22	25
D4		10	10	10
D5		10.5	22	10
D6		9.1	9	9.2
D7		8.5	7	7.9
D8		10	11	10
D9		9.1	7	9.5
D10		10.5	8	9
	Fluconazole	100	100	100

Compound **D6** and *D7* having chloro, fluoro, or methyl groups attached to phenyl ring connected to base moiety exhibited less activity. Compounds D4, D5 D8, and D9 exhibited very less or inactivity compared to the standard drug.

ACKNOWLEDGMENT

The authors are thankful to Madhav University for support and guidance and Atmiya in-vitro Testing Laboratory, Yogidham, Rajkot for antimicrobial activity studies.

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This paper was presented at the International Conference "

CONFERENCE ON MOLECULAR STRUCTURE & INSTRUMENTAL APPROACHES"

at RK University, Rajkot (Gujarat-India) on 26-27th November 2020

Received: 14.12.2020.
Accepted: 05.01.2021.