Research Article

Ultrasonication-Induced Synthesis and Antimicrobial Evaluation of Some Multifluorinated Pyrazolone Derivatives

Anil Gadhave,¹ Shashikant Kuchekar,¹ and Bhausaheb Karale²

¹ P. G. Department of Chemistry and Research Centre, Padmashri Vikhe Patil College, Pravaranagar, Ahmednagar 413713, India

² Department of Chemistry, Radhabai Kale Mahila Mahavidyalaya, Ahmednagar 414001, India

Correspondence should be addressed to Bhausaheb Karale; bkkarale@yahoo.com

Received 6 June 2012; Revised 6 August 2012; Accepted 28 August 2012

Academic Editor: Qing Li

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A series of novel fluorine containing pyrazole-pyrazolone (**4a-j**) and chromone-pyrazolone (**5a-i**) was synthesized from multifluorinated pyrazolone by the Knoevenagel condensation reaction. All compounds were synthesized by conventional heating as well as ultrasound irradiation technique. It was found that ultrasonication method was more efficient than conventional heating method. The newly synthesized compounds were subjected for *in vitro* antimicrobial screening against four bacterial pathogens, namely, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* and three fungal pathogens *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*, using broth microdilution (MIC) method (CLSI guidelines). Among them, some compounds exhibited promising antibacterial activity against the tested strains. All synthesized compounds were characterized by IR, ¹H-NMR, mass, and elemental analysis.

1. Introduction

An alarming increment in pathogenic resistance to existing drugs is a serious problem with antimicrobial therapy, and therefore it is very important to search for new class of antimicrobial agents [1]. The introduction of fluorine in organic molecules may lead to significant influence on the biological and physical properties of compounds due to increase of membrane permeability, hydrophobic binding, and stability against metabolic oxidation [2]. As a consequence of key role of fluorine in medicinal chemistry, the interest to incorporate fluorine to prepare biologically active compounds is increased during the past decades. The compounds containing fluorine or trifluoromethyl substituents such as fluoxetine and Prozac are well-known antidepressant drugs (Figure 1). Fluoxetine as well as the related antidepressants functions as selective serotonin reuptake inhibitors (SSRIs).

Pyrazolone is a key heterocyclic moiety present in numerous organic compounds because they possess antifungal [3], antibacterial [4, 5], antimycobacterial [6, 7], anti-inflammatory [8, 9], antitumor [10], gastric secretion stimulatory [11], antidepressant [12], and antifilarial [13] activities. They inhibit the production of TNF- α and decrease levels

of proinflammatory cytokines and thereby reduce inflammation and prevent further tissue destruction in disease as rheumatoid arthritis, osteoarthritis, and crohns' disease [14–16]. Pyrazolone-containing drugs such as phenazone, propyphenazone, ampyrone, and metamizole are useful antipyretic and analgesic drugs [17], while edaravone (MCI-186) has been used for treating brain [18, 19] and myocardial ischemia [20] (Figure 1).

Pyrazole derivatives are known to possess wide spectrum of biological activities such as antibacterial [21–23], antifungal [24, 25], antidiabetic [26], herbicidal [27, 28], and antianxiety [29], and it is present as an active pharmacophore in celecoxib which is a COX-2 inhibitor [30] and sildenafil citrate as cGMP-specific phosphodiesterase type-5 inhibitors [31]. The chromone is an important oxygen containing heterocycle present in numerous naturally occurring compounds exhibiting interesting biological activities such as antiviral [32], anticancer [33], anti-inflammatory [34, 35], and antioxidant [36, 37] activities.

Knoevenagel condensation reaction [38] is an important tool for construction of new carbon-carbon bonds. It is used for synthesis of different pharmaceutical products

FIGURE 1

[39, 40] by using acidic and basic condition. Ultrasonication has increasingly been used for organic synthesis in the last three decades. It has been demonstrated as an alternative energy source for organic reactions ordinarily accomplished by heating. A large number of organic reactions have been carried out in higher yields, shorter reaction time, and milder conditions under ultrasound [41–44].

In view of broad spectrum of biological activities associated with pyrazolone, pyrazole, chromone nucleus, high potential of fluorine-containing compounds, and advantageous use of ultrasonication technique and in continuation to our ongoing efforts for search of biologically important heterocycles [45–47], it was envisaged to construct a system which combines all these pharmacophores in a single molecular framework to explore the additive effects towards their biological activities. Herein we wish to report green synthesis and antimicrobial evaluation of some multifluorinated pyrazolone derivatives containing pyrazole and chromone nucleus. The general route for synthesis of multifluorinated pyrazolone derivatives is outlined in Scheme 1.

2. Experimental

2.1. Chemistry. Melting points were taken in open capillaries and are uncorrected. The IR spectra were recorded in KBr on a Shimadzu FTIR 8400 spectrophotometer, and only characteristic peaks are reported in cm^{-1} . The ^1H -NMR spectra were recorded in $\text{DMSO}-d_6$ on a Bruker Avance spectrometer using TMS as an internal standard at 400 MHz, and chemical shifts are reported in parts per million (ppm). Mass spectra were scanned on a Finnigan mass spectrometer. Elemental analysis was performed on a Perkin-Elmer analyzer. Thin-layer chromatography (TLC, on aluminium plates coated with silica gel 60F254, 0.25 mm thickness, Merck) was used for monitoring the progress of reactions, purity, and homogeneity of the synthesized compounds. UV radiation and iodine were used as the visualizing agents. Experiment under ultrasound irradiation was carried out in ultrasonic cleaner model EN-20U-S manufactured by Energetech Electronics Pvt. Ltd., Mumbai, India, has maximum output of 100 W and 33 KHz operating frequency.

2.2. General Procedure for Synthesis of Pyrazole-Pyrazolone (4a–j) and Chromone-Pyrazolone (5a–i)

2.2.1. Method (A) Conventional Method. Fluorinated pyrazolone **3** (0.001 mole) and 4-formyl pyrazole or 3-formyl

chromone (0.001 mole) were taken in 10 mL glacial acetic acid in 50 mL single neck round bottom flask equipped with condenser. The reaction mixture was heated in an oil bath at 100°C till completion of reaction (checked by TLC). After completion of reaction, contents were allowed to cool and then poured into crushed ice. Solid obtained was separated by filtration and purified by recrystallization from acetic acid to get pure compounds. The formation of compounds was confirmed by spectral techniques. The data of synthesized compounds is given in Tables 1 and 2.

2.2.2. Method (B) Ultrasound Method. Fluorinated pyrazolone **3** (0.001 mole) and 4-formyl pyrazole or 3-formyl chromone (0.001 mole) were taken in 10 mL glacial acetic acid in 50 mL round bottom flask. Contents of flask were subjected for ultrasound irradiation for time as shown in Tables 1 and 2, till completion of reaction (checked by TLC). After completion of reaction, contents were poured into crushed ice. Solid obtained was separated by filtration and purified by recrystallization from acetic acid to get pure compounds. The formation of compounds was confirmed by mp, mixed mp, and spectral techniques. The physical data of synthesized compounds is given in Tables 1 and 2.

(4Z)-4-[(1,3-Diphenyl-1H-pyrazol-4-yl)methylene]-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**4a**). Yellow solid; IR (ν_{max} , cm^{-1}): 1688 (C=O lactum), 1595 (C=C), 1515 (C=N), 1121 (Ar-F); ^1H -NMR δ (400 MHz, $\text{DMSO}-d_6$): 1.01–1.04 (t, 3H, $J = 12$ Hz), 1.71–1.79 (m, 2H), 2.56–2.60 (t, 2H, $J = 16$ Hz), 7.13–7.18 (m, 1H, Ar-H), 7.35–7.39 (m, 1H, Ar-H), 7.46–7.51 (m, 2H, Ar-H), 7.54–7.60 (m, 4H, Ar-H), 7.66–7.69 (m, 2H, Ar-H), 7.87–7.90 (m, 2H, one proton Ar-H & one pyrazole proton), 10.16 (s, 1H, olefinic proton); MS: m/z 504 (M^+); Anal. Calcd for $\text{C}_{28}\text{H}_{20}\text{F}_4\text{N}_4\text{O}$: C, 66.66; H, 4.00; N, 11.11. Found: C, 66.71; H, 3.92; N, 11.04.

(4Z)-4-[[3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene]-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**4b**). Yellow solid; IR (ν_{max} , cm^{-1}): 1697 (C=O lactum), 1580 (C=C), 1521 (C=N), 1133 (Ar-F); ^1H -NMR δ (400 MHz, $\text{DMSO}-d_6$): 1.01–1.05 (t, 3H, $J = 16$ Hz), 1.73–1.79 (m, 2H), 2.56–2.60 (t, 2H, $J = 16$ Hz), 7.13–7.18 (m, 1H, Ar-H), 7.25–7.29 (m, 2H, Ar-H), 7.36–7.40 (m, 1H, Ar-H), 7.47–7.52 (m, 3H, Ar-H), 7.64–7.68 (m, 2H, Ar-H), 7.86–7.89 (m, 2H, one proton Ar-H & one pyrazole proton), 10.15 (s, 1H, olefinic proton);

TABLE 1: Physical data of synthesized pyrazole-pyrazolone (**4a-j**).

Compound	R	R ₁	M.P. (°C)	Conventional method		Ultrasound method	
				Time (min)	Yield (%)	Time (min)	Yield (%)
4a	Propyl	C ₆ H ₅	151-152	60	75	16	90
4b	Propyl	4-FC ₆ H ₄	179-180	60	71	15	91
4c	Propyl	4-MeC ₆ H ₄	192-193	60	69	18	88
4d	Propyl	4-ClC ₆ H ₄	159-160	60	73	16	85
4e	Propyl	2-Thienyl	149-150	60	66	19	90
4f	CF ₃	C ₆ H ₅	237-238	60	78	21	86
4g	CF ₃	4-FC ₆ H ₄	235-236	60	74	19	93
4h	CF ₃	4-MeC ₆ H ₄	253-254	60	71	18	89
4i	CF ₃	4-ClC ₆ H ₄	216-217	60	76	17	94
4j	CF ₃	2-Thienyl	215-216	60	69	16	90

TABLE 2: Physical data of synthesized chromone-pyrazolone (**5a-i**).

Compound	R	R ₁	R ₂	R ₃	M. P. (°C)	Conventional method		Ultrasound method	
						Time (min)	Yield (%)	Time (min)	Yield (%)
5a	Propyl	H	H	H	144-145	60	70	14	81
5b	Propyl	H	H	Me	146-147	60	68	16	80
5c	Propyl	Cl	H	Cl	194-195	60	69	13	82
5d	Propyl	H	H	Cl	166-167	60	72	17	78
5e	Propyl	H	Me	Cl	241-242	60	75	12	82
5f	CF ₃	H	H	H	222-223	60	71	14	85
5g	CF ₃	H	H	Me	238-239	60	66	15	81
5h	CF ₃	Cl	H	Cl	212-213	60	69	12	82
5i	CF ₃	H	H	Cl	208-209	60	71	16	84

SCHEME 1: Synthesis of multifluorinated pyrazolone containing pyrazole and chromone.

MS: m/z 522 (M^+); Anal. Calcd for $C_{28}H_{19}F_5N_4O$: C, 64.37; H, 3.67; N, 10.72. Found: C, 64.50; H, 3.61; N, 10.65.

(4Z)-4-([3-(4-Methylphenyl)-1-phenyl-1H-pyrazol-4-yl]methylene)-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**4c**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}):

1693 (C=O lactum), 1597 (C=C), 1505 (C=N), 1114 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO}-d_6$): 0.99–1.03 (t, 3H, J = 16 Hz), 1.72–1.77 (m, 2H), 2.46 (s, 3H, Ar-CH₃), 2.56–2.58 (t, 2H, J = 8 Hz), 7.09–7.17 (m, 1H, Ar-H), 7.35–7.38 (m, 3H, Ar-H), 7.44–7.48 (m, 2H, Ar-H), 7.54–7.56 (d, 2H, J = 8 Hz, Ar-H), 7.59 (s, 1H, pyrazole proton), 7.85–7.87 (d, 2H,

$J = 8$ Hz, Ar-H), 10.13 (s, 1H, olefinic proton); MS: m/z 518 (M^+); Anal. Calcd for $C_{29}H_{22}F_4N_4O$: C, 67.18; H, 4.28; N, 10.81. Found: C, 67.11; H, 4.37; N, 10.89.

(4Z)-4-([3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene)-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**4d**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1681 (C=O lactum), 1583 (C=C), 1526 (C=N), 1125 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 1.02–1.06 (t, 3H, $J = 16$ Hz), 1.74–1.79 (m, 2H), 2.57–2.60 (t, 2H, $J = 12$ Hz), 7.13–7.18 (m, 1H, Ar-H), 7.36–7.40 (m, 1H, Ar-H), 7.47–7.52 (m, 3H, Ar-H), 7.54–7.56 (m, 2H, Ar-H), 7.62–7.63 (m, 2H, one proton Ar-H & one pyrazole proton), 7.86–7.88 (m, 2H, Ar-H), 10.16 (s, 1H, olefinic proton); MS: m/z 538 (M^+); Anal. Calcd for $C_{28}H_{19}ClF_4N_4O$: C, 62.40; H, 3.55; N, 10.40. Found: C, 62.30; H, 3.61; N, 10.35.

(4Z)-4-([1-Phenyl-3-(2-thienyl)-1H-pyrazol-4-yl]methylene)-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**4e**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1688 (C=O lactum), 1603 (C=C), 1509 (C=N), 1101 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 1.05–1.08 (t, 3H, $J = 12$ Hz), 1.79–1.85 (m, 2H), 2.65–2.68 (t, 2H, $J = 12$ Hz), 7.13–7.18 (m, 1H, Ar-H), 7.24–7.26 (m, 1H, Ar-H), 7.35–7.38 (m, 1H, Ar-H), 7.45–7.51 (m, 3H, Ar-H), 7.54–7.55 (m, 1H, Ar-H), 7.83 (s, 1H, pyrazole proton), 7.86–7.88 (m, 2H, Ar-H), 10.16 (s, 1H, olefinic proton); MS: m/z 510 (M^+); Anal. Calcd for $C_{26}H_{18}F_4N_4OS$: C, 61.17; H, 3.55; N, 10.97. Found: C, 61.29; H, 3.49; N, 10.85.

(4Z)-4-([1,3-Diphenyl-1H-pyrazol-4-yl]methylene)-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**4f**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1701 (C=O lactum), 1591 (C=C), 1515 (C=N), 1112 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 7.20 (s, 1H, Ar-H), 7.41–7.44 (m, 1H, Ar-H), 7.46–7.57 (m, 5H, Ar-H), 7.76 (s, 2H, Ar-H), 7.81–7.92 (m, 2H, Ar-H), 7.99 (s, 1H, pyrazole proton), 10.23 (s, 1H, olefinic proton); MS: m/z 530 (M^+); Anal. Calcd for $C_{26}H_{13}F_7N_4O$: C, 58.88; H, 2.47; N, 10.56. Found: C, 58.80; H, 2.51; N, 10.50.

(4Z)-4-([3-(4-Fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene)-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**4g**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1691 (C=O lactum), 1609 (C=C), 1522 (C=N), 1130 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 7.27–7.31 (m, 3H, Ar-H), 7.42–7.52 (m, 3H, Ar-H), 7.66 (s, 2H, Ar-H), 7.88–7.91 (m, 3H, two proton Ar-H & one pyrazole proton), 10.22 (s, 1H, olefinic proton); MS: m/z 548 (M^+); Anal. Calcd for $C_{26}H_{12}F_8N_4O$: C, 56.94; H, 2.21; N, 10.22. Found: C, 56.90; H, 2.30; N, 10.28.

(4Z)-4-([3-(4-Methylphenyl)-1-phenyl-1H-pyrazol-4-yl]methylene)-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**4h**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1708 (C=O lactum), 1600 (C=C), 1510 (C=N), 1122 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 2.46 (s, 3H, Ar-CH₃), 7.19–7.21 (m, 1H, Ar-H), 7.35–7.40 (m, 3H,

Ar-H), 7.47–7.50 (m, 2H, Ar-H), 7.54–7.56 (d, 2H, $J = 8$ Hz, Ar-H), 7.87–7.89 (d, 2H, $J = 8$ Hz, Ar-H), 7.99 (s, 1H, pyrazole proton), 10.20 (s, 1H, olefinic proton); MS: m/z 544 (M^+); Anal. Calcd for $C_{27}H_{15}F_7N_4O$: C, 59.57; H, 2.78; N, 10.29. Found: C, 59.65; H, 2.70; N, 10.20.

(4Z)-4-([3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl]methylene)-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**4i**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1711 (C=O lactum), 1599 (C=C), 1501 (C=N), 1109 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 7.26 (s, 1H, Ar-H), 7.42–7.61 (m, 7H, Ar-H), 7.87–7.91 (m, 3H, two proton Ar-H & one pyrazole proton), 10.23 (s, 1H, olefinic proton); MS: m/z 564 (M^+); Anal. Calcd for $C_{26}H_{12}ClF_7N_4O$: C, 55.29; H, 2.14; N, 9.92. Found: C, 55.21; H, 2.20; N, 9.81.

(4Z)-4-([1-Phenyl-3-(2-thienyl)-1H-pyrazol-4-yl]methylene)-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**4j**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1701 (C=O lactum), 1605 (C=C), 1505 (C=N), 1116 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 7.22 (s, 1H, Ar-H), 7.41–7.56 (m, 6H, Ar-H), 7.85–7.89 (m, 2H, Ar-H), 8.22 (s, 1H, pyrazole proton), 10.20 (s, 1H, olefinic proton); MS: m/z 536 (M^+); Anal. Calcd for $C_{24}H_{11}F_7N_4OS$: C, 53.74; H, 2.07; N, 10.44. Found: C, 53.69; H, 2.10; N, 10.55.

(4Z)-4-[(4-Oxo-4H-chromen-3-yl)methylene]-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**5a**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1700 (C=O lactum), 1659 (C=O chromone), 1623 (C=C), 1599 (C=N), 1129 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 0.99–1.02 (t, 3H, $J = 12$ Hz), 1.69–1.75 (m, 2H), 2.71–2.74 (t, 2H, $J = 12$ Hz), 7.59–7.63 (m, 1H, Ar-H), 7.79–7.81 (d, 1H, $J = 8$ Hz, Ar-H), 7.90–7.94 (m, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 8.11–8.19 (m, 2H, Ar-H), 10.35 (s, 1H, olefinic proton); MS: m/z 430 (M^+); Anal. Calcd for $C_{22}H_{14}F_4N_2O_3$: C, 61.40; H, 3.28; N, 6.51. Found: C, 61.31; H, 3.22; N, 6.61.

(4Z)-4-[(6-Methyl-4-oxo-4H-chromen-3-yl)methylene]-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**5b**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1694 (C=O lactum), 1669 (C=O chromone), 1627 (C=C), 1588 (C=N), 1140 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 0.97–1.01 (t, 3H, $J = 16$ Hz), 1.68–1.73 (m, 2H), 2.45 (s, 3H, Ar-CH₃), 2.69–2.72 (t, 2H, $J = 12$ Hz), 7.66–7.72 (m, 2H, Ar-H), 7.95 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 8.07–8.11 (m, 1H, Ar-H), 10.35 (s, 1H, olefinic proton); MS: m/z 444 (M^+); Anal. Calcd for $C_{23}H_{16}F_4N_2O_3$: C, 62.16; H, 3.63; N, 6.30. Found: C, 62.24; H, 3.59; N, 6.23.

(4Z)-4-[(6,8-Dichloro-4-oxo-4H-chromen-3-yl)methylene]-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**5c**). Yellow solid; IR ($\nu_{\max}$, cm^{-1}): 1707 (C=O lactum), 1659 (C=O chromone), 1624 (C=C), 1577 (C=N), 1126 (Ar-F); $^1\text{H-NMR}$ δ (400 MHz, $\text{DMSO-}d_6$): 1.03–1.07 (t, 3H, $J = 12$ Hz), 1.77–1.82 (m, 2H), 2.68–2.71 (t, 2H, $J = 12$ Hz), 7.10–7.16 (m, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.99 (s,

1H, Ar-H), 8.13 (s, 1H, Ar-H), 10.66 (s, 1H, olefinic proton); MS: m/z 449 (M^+); Anal. Calcd for $C_{22}H_{12}Cl_2F_4N_2O_3$: C, 52.93; H, 2.42; N, 5.61. Found: C, 52.86; H, 2.47; N, 5.70.

(4Z)-4-[(6-Chloro-4-oxo-4H-chromen-3-yl)methylene]-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**5d**). Yellow solid; IR (ν_{max} , cm^{-1}): 1695 (C=O lactum), 1655 (C=O chromone), 1605 (C=C), 1580 (C=N), 1119 (Ar-F); 1H -NMR δ (400 MHz, DMSO- d_6): 1.06–1.09 (t, 3H, J = 12 Hz), 1.79–1.85 (m, 2H), 2.71–2.74 (t, 2H, J = 12 Hz), 7.14–7.19 (m, 1H, Ar-H), 7.52–7.54 (d, 1H, J = 8 Hz, Ar-H), 7.69–7.72 (m, 1H, Ar-H), 8.08 (s, 1H, Ar-H), 8.25–8.26 (d, 1H, J = 4 Hz, Ar-H), 10.65 (s, 1H, olefinic proton); MS: m/z 464 (M^+); Anal. Calcd for $C_{22}H_{13}ClF_4N_2O_3$: C, 56.85; H, 2.82; N, 6.03. Found: C, 56.78; H, 2.87; N, 6.11.

(4Z)-4-[(6-Chloro-7-methyl-4-oxo-4H-chromen-3-yl)methylene]-5-propyl-2-(2,3,5,6-tetrafluorophenyl)-2,4-dihydro-3H-pyrazol-3-one (**5e**). Yellow solid; IR (ν_{max} , cm^{-1}): 1711 (C=O lactum), 1649 (C=O chromone), 1630 (C=C), 1588 (C=N), 1135 (Ar-F); 1H -NMR δ (400 MHz, DMSO- d_6): 1.05–1.09 (t, 3H, J = 16 Hz), 1.79–1.84 (m, 2H), 2.53 (s, 3H, Ar-CH₃), 2.70–2.74 (t, 2H, J = 16 Hz), 7.14–7.18 (m, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 10.63 (s, 1H, olefinic proton); MS: m/z 478 (M^+); Anal. Calcd for $C_{23}H_{15}ClF_4N_2O_3$: C, 57.69; H, 3.16; N, 5.85. Found: C, 57.60; H, 3.29; N, 5.89.

(4Z)-4-[(4-Oxo-4H-chromen-3-yl)methylene]-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**5f**). Yellow solid; IR (ν_{max} , cm^{-1}): 1699 (C=O lactum), 1655 (C=O chromone), 1625 (C=C), 1596 (C=N), 1133 (Ar-F); 1H -NMR δ (400 MHz, DMSO- d_6): 7.52–7.60 (m, 3H, Ar-H), 7.77–7.82 (m, 1H, Ar-H), 8.30–8.33 (d, 1H, J = 8 Hz, Ar-H), 8.53 (s, 1H, Ar-H), 10.69 (s, 1H, olefinic proton); MS: m/z 456 (M^+); Anal. Calcd for $C_{20}H_7F_7N_2O_3$: C, 52.65; H, 1.55; N, 6.14. Found: C, 52.53; H, 1.46; N, 6.08.

(4Z)-4-[(6-Methyl-4-oxo-4H-chromen-3-yl)methylene]-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**5g**). Yellow solid; IR (ν_{max} , cm^{-1}): 1705 (C=O lactum), 1662 (C=O chromone), 1620 (C=C), 1593 (C=N), 1141 (Ar-F); 1H -NMR δ (400 MHz, DMSO- d_6): 2.48 (s, 3H, Ar-CH₃), 7.20–7.23 (m, 2H, Ar-H), 7.44–7.46 (d, 1H, J = 8 Hz, Ar-H), 8.06 (s, 1H, Ar-H), 8.51 (s, 1H, Ar-H), 10.64 (s, 1H, olefinic proton); MS: m/z 470 (M^+); Anal. Calcd for $C_{21}H_9F_7N_2O_3$: C, 53.63; H, 1.93; N, 5.96. Found: C, 53.53; H, 1.89; N, 5.90.

(4Z)-4-[(6,8-Dichloro-4-oxo-4H-chromen-3-yl)methylene]-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**5h**). Yellow solid; IR (ν_{max} , cm^{-1}): 1711 (C=O lactum), 1677 (C=O chromone), 1622 (C=C), 1599 (C=N), 1135 (Ar-F); 1H -NMR δ (400 MHz, DMSO- d_6): 7.52–7.57 (m, 1H, Ar-H), 7.69–7.71 (m, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 8.40 (s, 1H, Ar-H), 10.59 (s, 1H, olefinic proton);

MS: m/z 525 (M^+); Anal. Calcd for $C_{20}H_5Cl_2F_7N_2O_3$: C, 45.74; H, 0.96; N, 5.33. Found: C, 45.61; H, 0.88; N, 5.41.

(4Z)-4-[(6-Chloro-4-oxo-4H-chromen-3-yl)methylene]-2-(2,3,5,6-tetrafluorophenyl)-5-(trifluoromethyl)-2,4-dihydro-3H-pyrazol-3-one (**5i**). Yellow solid; IR (ν_{max} , cm^{-1}): 1695 (C=O lactum), 1671 (C=O chromone), 1611 (C=C), 1580 (C=N), 1109 (Ar-F); 1H -NMR δ (400 MHz, DMSO- d_6): 7.54–7.56 (m, 2H, Ar-H), 7.72–7.74 (d, 1H, J = 8 Hz, Ar-H), 8.26 (s, 1H, Ar-H), 8.46 (s, 1H, Ar-H), 10.66 (s, 1H, olefinic proton); MS: m/z 490 (M^+); Anal. Calcd for $C_{20}H_6ClF_7N_2O_3$: C, 48.95; H, 1.23; N, 5.71. Found: C, 48.90; H, 1.12; N, 5.64.

2.3. Antimicrobial Activity. The antimicrobial activity of all newly synthesized compounds was evaluated by broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI 2005, formerly known as NCCLS) [48]. The results were determined using minimum inhibitory concentration (MIC) values in $\mu g/mL$. Antibacterial activity was screened against two Gram-positive (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 443) and two Gram-negative (*Escherichia coli* MTCC 442 and *Pseudomonas aeruginosa* MTCC 441) bacterial strains using ampicillin as a standard antibacterial agent. Antifungal activity was screened against three fungal species (*Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282, and *Aspergillus clavatus* MTCC 1323) where griseofulvin was used as a standard antifungal agent. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh.

Sterile 96-well microtiter plates were used in this assay. All tests were performed in Mueller Hinton broth for bacterial strains and Sabouraud's dextrose broth for fungal strains. Overnight broth cultures of each strain were prepared, and the final inoculum concentration in each well was adjusted to 2×10^6 CFU mL^{-1} for the bacterial strains and 2×10^7 CFU mL^{-1} for fungal strains. The inoculum concentration was adjusted by diluting broth culture supernatant in sterile distilled water to a density of 0.5 McFarland standards using a nephelometer. Stock solutions of all compounds at 2000 $\mu g/mL$ were prepared by dissolving them in DMSO. Serial dilutions were prepared in primary and secondary screening. In primary screening, 500, 250, and 200 $\mu g/mL$ concentrations of the synthesized compounds were taken. The compounds found active in primary screening were further tested in a second set of dilutions against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 100, 62.5, 50, and 25 $\mu g/mL$ concentrations. In the tests, triphenyltetrazolium chloride (TTC) was also added to culture medium as growth indicator. The final concentration of TTC after inoculation was 0.05%. The microbial growth was determined after 24 h incubation at 37°C for the bacteria and at 25°C after 48 h for the fungi. The MIC is defined as the lowest concentration of a compound at which the microorganism does not demonstrate visible growth. All determinations were performed in duplicate to check the accuracy of results. The antimicrobial activity results (MIC) are presented in Table 3.

TABLE 3: Antibacterial and antifungal data of newly synthesized pyrazole-pyrazolone (4a–j) and chromone-pyrazolone (5a–i) indicated by MIC values ($\mu\text{g/mL}$).

Compound	Minimum inhibitory concentration (MIC, $\mu\text{g/mL}$)						
	Gram-positive bacteria		Gram-negative bacteria			Fungi	
	<i>S. aureus</i>	<i>S. pyogenes</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>	<i>A. clavatus</i>
4a	250	200	200	100	>1000	>1000	>1000
4b	125	100	62.5	100	500	1000	1000
4c	200	250	100	125	1000	1000	1000
4d	100	100	62.5	200	1000	>1000	>1000
4e	125	62.5	200	250	>1000	>1000	>1000
4f	200	250	250	250	1000	500	500
4g	250	200	100	200	>1000	250	500
4h	500	250	250	125	>1000	500	500
4i	500	500	200	200	500	>1000	>1000
4j	250	500	125	250	500	1000	1000
5a	200	250	250	125	500	1000	1000
5b	100	62.5	200	100	1000	>1000	>1000
5c	125	100	250	200	1000	>1000	>1000
5d	200	200	250	200	>1000	500	500
5e	100	100	500	200	250	1000	>1000
5f	62.5	200	500	500	250	>1000	>1000
5g	100	250	200	200	500	>1000	>1000
5h	250	200	100	250	1000	>1000	>1000
5i	250	200	125	62.5	1000	500	1000
Ampicillin	250	100	100	100	—	—	—
Griseofulvin	—	—	—	—	500	100	100

3. Result and Discussion

3.1. Chemistry. In the present investigation, our aim was to do the synthesis of multifluorinated pyrazole-pyrazolone (4) and chromone-pyrazolone (5) by using green technique such as ultrasonication and investigate their antibacterial and antifungal activities (Scheme 1). The starting material multifluorinated pyrazolone (3) was prepared by literature known procedure [49] using β -ketoester (1) and 2,3,5,6-tetra-fluoro phenyl hydrazine (2). The pyrazolone (3) was treated with 3-formyl chromone or 4-formyl pyrazole in acetic acid under conventional heating as well as ultrasound irradiation to give pyrazole-pyrazolone (4) and chromone-pyrazolone (5). The structures of all compounds were confirmed on the basis of spectral analysis. The IR spectra of compounds 4 and 5 showed a peak in the range $1680\text{--}1711\text{ cm}^{-1}$ for C=O stretching of five membered lactum ring. They also exhibited a peak in the range $1500\text{--}1600\text{ cm}^{-1}$ for C=N stretching. The $^1\text{H-NMR}$ spectra of compounds 4a–e and 5a–e clearly showed peaks for $\text{CH}_3\text{--CH}_2\text{--CH}_2\text{--}$ (propyl group) protons, while such peaks were absent in compounds 4f–j and 5f–i as they have no propyl group. The exocyclic methylene group proton appeared as a singlet in the range of $10.11\text{--}10.66\delta$. The aromatic protons present in all compounds were also confirmed by $^1\text{H-NMR}$ spectra. Further the structures of all compounds were also confirmed by molecular ion peak in

their mass spectra. For all the synthesized compounds, elemental analysis values are in good agreement with theoretical data.

It was observed that ultrasound irradiation method was practically more superior than conventional heating method in terms of higher yields, rapid, and environmentally benign process which makes this protocol more useful.

3.2. Antimicrobial Activity. Antibacterial activity was screened against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacterial strains using ampicillin as a standard antibacterial agent. Antifungal activity was screened against fungal species *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus* where griseofulvin was used as a standard antifungal agent.

Reviewing the antibacterial activity data (Table 1) of all newly synthesized pyrazole-pyrazolone (4a–j) and chromone-pyrazolone (5a–i), it was revealed that the compounds (4b, 4d), (5i), (5f), and (4e, 5b) showed highest activity, that is, $62.5\text{ }\mu\text{g/mL}$ against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes*, respectively. Among pyrazole-pyrazolone, the compounds 4b, 4c, 4d, 4e containing propyl group and compound 4f containing trifluoromethyl group, similarly among chromone-pyrazolone, the compounds 5a, 5b, 5c, 5d, 5e containing propyl group and compounds 5f, 5g containing trifluoromethyl group are found to possess

high potency against Gram-Positive *S. aureus* bacterial strain as compared with standard bactericidal ampicillin. So it is concluded that compounds with propyl group rather than CF_3 group exhibited high potency against gram positive *S. aureus* bacterial strain. It is also observed that compounds (**4c**, **4g**, **5h**), (**4a**, **4b**, **5b**), (**4a**, **4g**, **4j**, **5h**, **5i**), and (**4b**, **4d**, **5c**, **5e**) showed promising activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenes*, respectively, as compared with standard ampicillin drug.

Antifungal activity study of synthesized compounds reveals that compounds **5e** and **5f** exhibited more potency while compounds **4b**, **4i**, **4j**, **5a**, and **5g** showed good antifungal activity against *C. albicans* as compared with standard fungicidal griseofulvin. All the synthesized compounds showed moderate-to-poor antifungal activity profile against *A. niger* and *A. clavatus* fungal strains.

4. Conclusion

Present study describes synthesis of a series of novel multifluorinated pyrazolone containing pyrazole and chromone nucleus by conventional heating as well as ultrasonication technique. The ultrasonication method was more efficient in terms of short reaction time, high yields, and clean reaction process. The antimicrobial activity result reveals that some of the synthesized compounds exhibited promising activity against the tested bacterial and fungal strains. The insights gained in this study will be useful for further studies on potential anti-infective agents. A study regarding structure activity relationship is in progress.

Acknowledgments

The authors are thankful to Dr. S. R. Walunj (Principal, PVP, College), Dr. T. N. Gholap (Principal, RKMM, Ahmednagar), and Management, PRES, for providing necessary facilities and constant encouragement. The authors also thank SAIF, Chandigarh, for spectral analysis.

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