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Design, synthesis and anti-microbial activity of 1*H*-pyrazole carboxylates

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Abstract—In a SAR study, we have synthesized a few 1*H*-pyrazole carboxylate related microbicides using Vilsmeier reagent. The anti-microbial screening results of 1*H*-pyrazole-3-carboxylate are reported here for the first time. The effect of 1*H*-pyrazole carboxylates on the mycelial growth of plant pathogenic fungi is revealed. The first X-ray structure in the family of microbicidal 1*H*-pyrazole-4-carboxylates is presented.

1. Introduction

Pyrazole nucleus has pronounced pharmacological applications as anti-anxiety[1,2] antipyretic, analgesic and anti-inflammatory drugs.[3–5] Certain alkyl pyrazoles show significant bacteriostatic, bactericidal and fungicidal activities.[6] 1*H*-Pyrazole-3-carboxylic acid esters incorporated pyrazole nucleosides have shown potent and selective anti-viral/anti-tumour activity.[7] It was found that 1*H*-pyrazole-4-carboxylic acid esters act as intermediates for agricultural microbicides and herbicides.[8] But there was no explicit report on the plant pathogenic fungal inhibition by 1*H*-pyrazole-4-carboxylates. Besides it has been observed those 1*H*-pyrazole-4-carboxylic acids and their esters when subjected to in vitro anti-bacterial screening showed activity against some strains of Gram-positive bacteria.[9]

The present study was carried out to investigate the anti-bacterial and anti-fungal inhibitions of similar type of 1*H*-pyrazole-4-carboxylates whose synthesis in excellent yields has already been accomplished by us.[10] We targeted to study the structure–activity relationship by altering the alcoholic part of the ester moiety and substitutions at the 3-position of the pyrazole ring. Herein we report the screening results of the anti-bacterial and anti-fungal activities of both 1*H*-pyrazole-3-carboxylate and 1*H*-pyrazole-4-carboxylates. Herein we also report the X-ray crystal structure of ethyl-1(2,4-dinitrophenyl)-3(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate as a representative compound in this series of biologically active 1*H*-pyrazole-4-carboxylates.

2. Chemistry

Hydrazones and semicarbazones of ketones yield pyrazoles upon treatment with Vilsmeier reagent.[11] Substitution at α-methyl of acetophenone prevents formylation of the pyrazole ring.[12] 1*H*-Pyrazole-4-carboxylates were synthesized according to the reported procedures[10] from 2,4-dinitro phenyl hydrazones of β-ketoesters upon treatment with DMF/$POCl_3$ (Scheme 1). The ester group remains unaffected under the reaction condition and adds to the excellent yield of the pyrazole by increasing the acidity of active methylene proton (Table 1). No further formylation takes place even with excess of the reagent.

The structure of each product was identified from spectroscopic data.[13] The X-ray crystallographic structure of

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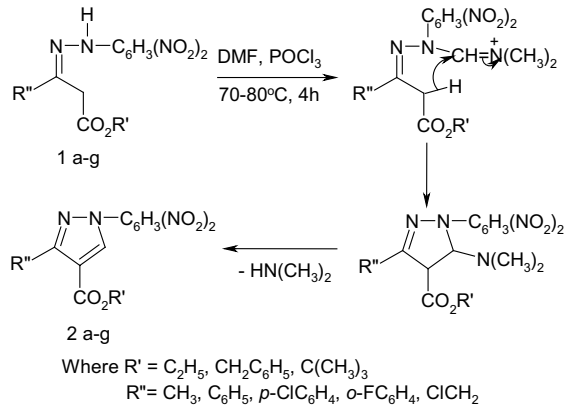


Scheme 1. Synthesis of 1*H*-pyrazole-4-carboxylates.

Table 1. Synthesis of 1*H*-pyrazole-4-carboxylic acid esters

Entry	R′	R″	Mp (°C)[a]	Conventional heating method	
				Time (h)	Yield (%)
2a	C_2H_5	*p*-ClC_6H_4	169	6	90
2b	C_2H_5	C_6H_5	138	6	85
2c	C_2H_5	$ClCH_2$	96	6	88
2d	C_2H_5	*o*-FC_6H_4	128	6	85
2e	$CH_2C_6H_5$	CH_3	140	6	88
2f	C_2H_5	CH_3	122	6	82
2g	$C(CH_3)_3$	CH_3	125	6	85

[a] Melting points recorded were uncorrected.

ethyl-1(2,4-dinitrophenyl)-3(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate as a representative compound in the series of 1*H*-pyrazole-4-carboxylates exhibiting antibacterial and anti-fungal activity is revealed here for the first time. The ORTEP III view of the molecule ethyl-1(2,4-dinitrophenyl)-3(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate is shown in Figure 1 and the crystal data in (Table 2) (**CCDC 222078**). Computation of the least-squares plane comprising atoms N1, N2, C1, C2 and C3 show that the pyrazole moiety is planar.

The planarity is further supported by the conformational angles [N1–N2–C3–C2] −0.8(3)°; [N2–C3–C2–C1] 0.1(3)°; [C3–C2–C1–N1] 0.6(3)°; [C2–C1–N1–N2] −1.1(3)° and [C1–N1–N2–C3] 1.2(3)°. The atom C12 of the ethyl moiety is slightly disordered; however, the ethyl carboxylate group attached to the pyrazole ring adopts an extended conformation. Bond lengths of the planar nitro groups at *ortho* and *para* positions of the phenyl ring suggest the resonance effect. Both the chloro and nitro phenyl rings orient at angles of 40.6(1)° and 27.7(1)°, respectively.

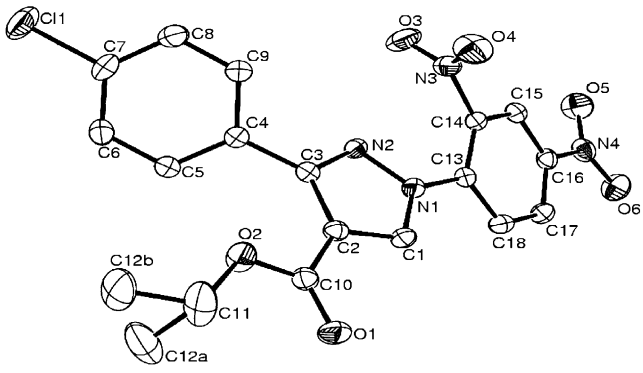
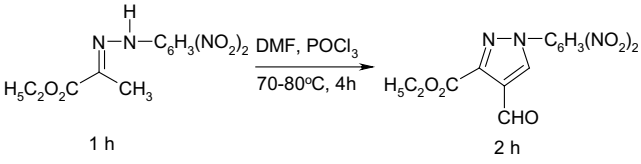


Figure 1. The ORTEP III view of ethyl-1(2,4-dinitrophenyl)-3(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate showing 30% probability displacement ellipsoids and the atom numbering scheme.[14]

Table 2. Crystal data and structure refinement for ethyl-1(2,4-dinitrophenyl)-3(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate (compound **2a**)

Identification code	Compound **2a**
Empirical formula	$C_{18}H_{13}ClN_4O_6$
Formula weight	411.73
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/c$
Unit cell dimensions	a = 11.536(1) Å b = 20.111(2) Å, β = 91.934(2)° c = 7.949(1) Å
Volume	1843.2(2) Å^3
Z, calculated density	4, 1.484 mg/m^3
Absorption coefficient	0.252 mm^{-1}
F(000)	836
Crystal size	0.20 × 0.25 × 0.40 mm
Theta range for data collection	1.77°–28.04°
Limiting indices	$-15 \leqslant h \leqslant 11$, $-26 \leqslant k \leqslant 23$, $-10 \leqslant l \leqslant 10$
Reflections collected/unique	11,620/4317 [R(int) = 0.0358]
Completeness to theta	25.00, 96.9%
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4317/0/303
Goodness-of-fit on F^2	1.056
Final R indices [I > 2sigma(I)]	$R1$ = 0.0721, $wR2$ = 0.1737
R indices (all data)	$R1$ = 0.1077, $wR2$ = 0.1948
Largest diff. peak and hole	0.512 and −0.270 e Å^{-3}



Scheme 2. Synthesis of ethyl-1(2,4-dinitrophenyl)-4-formyl-1*H*-pyrazole-3-carboxylate.

Ethyl-1*H*-pyrazole-3-carboxylate was synthesized in 80% yield from ethyl pyruvate 2,4-dinitrophenyl hydrazone following the same procedure with 8 equiv of $POCl_3$. The structure of ethyl-1(2,4-dinitrophenyl)-4-formyl-1*H*-pyrazole-3-carboxylate was confirmed by NMR spectral data (Scheme 2).[15]

3. Biological study

The anti-microbial activities of 1*H*-pyrazole carboxylates were evaluated[16] against four human pathogenic bacteria such as *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aerugi-*

nosa (ATCC 27853) and *Enterobacter faecalis* (ATCC 29212). Anti-fungal activity of these compounds was also tested against five plant pathogenic fungi viz., *Rhizoctonia solani*, *Fusarium oxysporum*, *Curvularia lunata*, *Bipolaris oryzae* and *Alternaria alternata* under in vitro condition. The biological screening results of 1*H*-pyrazole-4-carboxylates with ethyl 2,4-dinitrophenyl-4-formyl 1*H*-pyrazole-3-carboxylate as a control for microbial inhibition are tabulated below (Tables 3 and 4).

3.1. Effect of 1*H*-pyrazole-4-carboxylates on the growth of human pathogenic bacteria

All the eight 1*H*-pyrazole carboxylates exerted various degrees of inhibitory effects against four human pathogenic bacteria namely *E. coli*, *S. aureus*, *En. faecalis* and *P. aeruginosa*. The anti-bacterial activity of the test compounds was dose dependent and it was remarkable at 0.5 mg/mL concentration. Among the eight

Table 3. Effect of 1*H*-pyrazole-4-carboxylates on the growth of human pathogenic bacteria

Entry	Area of inhibition zone (cm)/concentration of compound (mg/mL)							
	E. coli		*P. aeruginosa*		*S. aureus*		*En. faecalis*	
	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5
2a	0.0 f (0.0)	4.7 e (52.2)	0.0 c (0.0)	0.0 c (0.0)	1.6 g (17.8)	3.3 e (36.7)	0.0 e (0.0)	4.9 c (54.4)
2b	3.9 c (43.3)	7.2 c (80.0)	1.8 a (20.0)	2.2 a (24.4)	3.6 f (40.0)	7.6 c (84.4)	4.3 c (47.8)	7.4 b (82.2)
2c	1.6 e (17.8)	4.6 e (51.1)	0.0 c (0.0)	0.0 c (0.0)	6.9 c (76.7)	8.3 b (92.2)	0.0 e (0.0)	0.0 e (0.0)
2d	1.8 d (20.0)	5.1 d (56.7)	0.0 c (0.0)	0.0 c (0.0)	4.2 e (46.7)	7.3 cd (81.1)	0.0 e (0.0)	2.3 d (25.6)
2e	1.6 e (17.8)	4.9 d (54.4)	1.7 b (18.9)	2.0 b (22.2)	4.1 e (45.6)	7.1 d (78.9)	5.1 b (56.7)	7.3 b (81.1)
2f	4.6 b (51.1)	7.8 b (86.7)	0.0 c (0.0)	0.0 c (0.0)	5.3 d (58.9)	8.2 b (91.1)	5.0 b (55.6)	7.6 b (84.4)
2g	1.5 e (16.7)	4.7 e (52.2)	0.0 c (0.0)	0.0 c (0.0)	7.3 b (81.1)	8.4 ab (93.3)	1.9 d (21.1)	4.8 c (53.3)
2h	7.3 a (81.1)	8.8 a (97.8)	0.0 c (0.0)	2.3 a (25.6)	7.7 a (85.6)	8.8 a (97.8)	7.5 a (83.3)	8.6 a (95.6)
Control	0.0 f (0.0)	0.0 g (0.0)	0.0 c (0.0)	0.0 c (0.0)	0.0 i (0.0)	0.0 f (0.0)	0.0 e (0.0)	0.0 e (0.0)
CD (5%)	0.26	0.26	0.09	0.11	0.31	0.42	0.27	0.37

In a column, means followed by the same letter do not differ significantly (LSD test; $P \leqslant 0.05$). Figures in parentheses are % inhibition of bacterial growth as compared to control.

Table 4. Effect of 1*H*-pyrazole-4-carboxylates on mycelial growth of plant pathogenic fungi

Entry	Mycelial growth (cm)/concentration of compound (mg/mL)									
	R. solani		*F. oxysporum*		*C. lunata*		*A. alternata*		*B. oryzae*	
	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5
2a	9.0 c (0.0)	7.2 c (20.0)	6.3 c (30.0)	4.0 c (55.6)	5.8 bc (35.6)	4.8 c (46.7)	5.4 cd (40.0)	4.2 d (53.3)	6.6 e (26.7)	3.9 b (56.7)
2b	8.0 b (11.1)	6.6 b (26.6)	5.3 b (41.1)	3.0 b (66.7)	5.6 b (37.8)	3.8 b (57.8)	5.5 d (38.9)	3.8 c (57.8)	5.6 b (37.8)	4.4 cd (51.1)
2c	9.0 c (0.0)	8.5 e (5.6)	6.8 d (24.4)	4.6 e (48.9)	6.0 c (33.3)	5.1 d (43.3)	6.0 e (33.3)	4.8 e (46.7)	5.9 cd (34.4)	4.6 de (48.9)
2d	9.0 c (0.0)	8.2 de (8.9)	6.0 c (33.3)	5.3 f (41.1)	5.0 a (44.4)	4.0 b (55.6)	4.2 b (53.3)	3.4 b (62.2)	5.7 bc (36.7)	4.3 c (52.2)
2e	7.9 b (12.2)	6.5 b (27.8)	6.3 c (30.0)	4.3 d (52.2)	6.4 d (28.9)	5.3 de (41.1)	5.2 c (42.2)	4.2 d (53.3)	6.0 d (33.3)	4.8 e (46.7)
2f	9.0 c (0.0)	8.0 d (11.1)	6.3 c (30.0)	5.4 f (40.0)	6.5 d (27.8)	5.3 de (41.1)	5.5 d (38.9)	4.8 e (46.7)	6.0 d (33.3)	4.5 cd (50.0)
2g	9.0 c (0.0)	8.5 e (5.6)	7.8 e (13.3)	5.7 g (36.7)	6.7 d (25.6)	5.4 e (40.0)	6.2 e (31.1)	5.1 f (43.3)	5.5 b (38.9)	4.3 c (52.2)
2h	6.8 a (24.4)	4.2 a (53.3)	4.3 a (52.2)	2.6 a (71.1)	5.6 b (37.8)	3.5 a (61.1)	3.8 a (57.8)	2.1 a (76.6)	3.5 a (61.1)	2.3 a (74.4)
Control	9.0 c (0.0)	9.0 f (0.0)	9.0 f (0.0)	9.0 h (0.0)	9.0 e (0.0)	9.0 f (0.0)	9.0 f (0.0)	9.0 g (0.0)	9.0 f (0.0)	9.0 f (0.0)
CD (5%)	0.46	0.41	0.34	0.23	0.32	0.27	0.29	0.23	0.27	0.23

In a column, means followed by the same letter do not differ significantly (LSD test; $P \leqslant 0.05$). Figures in parentheses are % inhibition of fungal growth as compared to control.

compounds tested, ethyl-1(2,4-dinitrophenyl)-4-formyl-1*H*-pyrazole-3-carboxylate **2h** significantly inhibited most of the bacterial growth ranged from 25.6% to 97.8% as compared to control at both 0.1 and 0.5 mg/mL concentrations. This was followed by ethyl-1(2,4-dinitrophenyl)-3-methyl-1*H*-pyrazole-4-carboxylate **2f**, which inhibited the bacterial growth from 51.1% to 91.1% (Table 3). However, most of the compounds failed to limit the growth of *P. aeruginosa* including these compounds **2h** and **2f**.

From the results obtained it is evident that among the 1*H*-pyrazole-4-carboxylates, ethyl-1(2,4-dinitrophenyl)-3-methyl-1*H*-pyrazole-4-carboxylate **2f** is found to exhibit enhanced bacterial inhibition as compared to others. No increase in activity was observed with increase of bulkiness of the ester group and other changes in 3-position produced almost no increased effect on bacterial inhibition. And it is inferred that 1*H*-pyrazole-3-carboxylate showed higher percentage of inhibition in comparison to all the 1*H*-pyrazole-4-carboxylates under study.

3.2. Effect of 1*H*-pyrazole-4-carboxylates on the growth of plant pathogenic fungi

All the 1*H*-pyrazole carboxylates inhibited the mycelial growth of all the five plant pathogenic fungi. However, the anti-fungal activity varied between the compounds and it was concentration dependent. Remarkable inhibition was observed at 0.5 mg/mL concentration than 0.1 mg/mL. Among the eight compounds, ethyl-1(2,4-dinitrophenyl)-4-formyl-1*H*-pyrazole-3-carboxylate **2h** exhibited highly significant anti-fungal activity against different fungi and inhibition of mycelial growth ranged between 24.4% and 76.6% as compared to control (Table 4).

The plant pathogenic fungal inhibition of 1*H*-pyrazole-4-carboxylates found to be better in the case of phenyl substitution at the 3-position of the pyrazole ring. Further substitutions in the phenyl ring do not have any marked effect on fungal inhibition. The benzyl ester of 1*H*-pyrazole-4-carboxylates showed better inhibition when compared to the ethyl ester. The 1*H*-pyrazole-3-carboxylate showed better activity than the 1*H*-pyrazole-4-carboxylates on fungal inhibition also.

4. Summary

The results obtained clearly indicate that the series of pyrazoles discussed here are active towards both plant pathogenic fungal growth inhibition and bacterial inhibition for the select group of strains under this investigation. Since 1*H*-pyrazole-4-carboxamides were already reported to be microbicides and herbicides[8] the corresponding amide derivatives of the 1*H*-pyrazole-4-carboxylates discussed here are also expected to exhibit similar activities. Further it was observed for the first time that 1*H*-pyrazole-3-carboxylate show better antimicrobial activity. This observation may promote the synthesis of more active 1*H*-pyrazole-3-carboxylates in future. The mechanism involved in the plant pathogenic fungal growth inhibition is under study. The X-ray structure of 1*H*-pyrazole-4-carboxylates is expected to throw light on the mechanistic pathway of microbial inhibition.

Acknowledgements

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13. General procedure for the preparation of 1*H*-pyrazole-4-carboxylates: 0.50 g of $POCl_3$ (0.003 mol) was added dropwise to an ice-cold stirred solution of hydrazone

(0.001 mol) in 4 mL dry DMF. The reaction mixture was allowed to attain room temperature and then refluxed at 70–80 °C for about 4 h. The resulting mixture was poured onto crushed ice, neutralized with dilute sodium hydroxide and left standing overnight. The pale yellow precipitate obtained was purified by silica gel (60–120 mesh) column chromatography with ethyl acetate–petroleum ether mixture (15:85) to yield the product.

Ethyl-1(2,4-dinitrophenyl)-3(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate, compound **2a**: yellow crystals (15% ethyl acetate–petroleum ether); mp 169 °C; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.76–8.77 (d, $J_1 = 2.3$ Hz, 1H), 8.55–8.57 (dd, $J_1 = 2.3$ and $J_2 = 8.9$ Hz, 1H), 8.39 (s, 1H, pyrazole –CH), 7.88–7.90 (d, $J_2 = 8.9$ Hz, 1H), 7.74–7.76 (d, $J = 8.0$ Hz, 2H, *p*-chlorophenyl), 7.39–7.41 (d, $J = 8.0$ Hz, 2H, *p*-chlorophenyl), 4.30–4.34 (q, $J = 7.4$ Hz, 2H), 1.32–1.35 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 162.0, 155.0, 146.5, 143.8, 136.6, 135.7, 135.5, 130.9, 129.2, 128.4, 127.8, 126.2, 121.5, 116.0, 61.1, 14.3; IR (KBr) cm^{-1}: 3136, 3091, 2926, 1691, 1603, 1536, 1349, 1285, 848, 774, 741; MS (*m*/*z*): 417 (M^+); Anal. Calcd for $C_{18}H_{13}ClN_4O_6$: C, 51.87; H, 3.14; N, 13.44. Found: C, 51.72; H, 3.10; N, 13.51.

Ethyl-1(2,4-dinitrophenyl)-3-phenyl-1*H*-pyrazole-4-carboxylate, compound **2b**: orange crystals (15% ethyl acetate–petroleum ether); mp 138 °C; ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 8.72–8.73 (d, $J_1 = 2.2$ Hz, 1H), 8.46–8.52 (dd, $J_1 = 2.2$ and $J_2 = 8.9$ Hz, 1H), 8.37 (s, 1H, pyrazole –CH), 7.79–7.84 (d, $J_2 = 8.9$ Hz, 1H), 7.72–7.77 (m, 2H), 7.40–7.44 (m, 3H), 4.26–4.37 (q, $J = 7.2$ Hz, 2H), 1.29–1.36 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$, ppm): δ 162.0, 155.8, 146.4, 143.5, 136.5, 135.3, 130.8, 129.4, 128.2, 128.0, 127.6, 126.2, 121.2, 116.1, 60.9, 14.2; IR (KBr) cm^{-1}: 3137, 3098, 2911, 1728, 1606, 1537, 1448, 1280, 832; MS (*m*/*z*): 382 (M^+); Anal. Calcd for $C_{18}H_{14}N_4O_6$: C, 56.53; H, 3.69; N, 14.66. Found: C, 56.47; H, 3.66; N, 14.59.

Ethyl-1(2,4-dinitrophenyl)-3-chloromethyl-1*H*-pyrazole-4-carboxylate, compound **2c**: pale yellow crystals (15% ethyl acetate–petroleum ether); mp 96 °C; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.75–8.76 (d, $J_1 = 2.3$ Hz, 1H), 8.55–8.58 (dd, $J_1 = 2.3$ and $J_2 = 8.6$ Hz, 1H), 8.29 (s, 1H, pyrazole –CH), 7.87–7.89 (d, $J_2 = 8.6$ Hz, 1H), 4.83 (s, 2H, chloromethyl), 4.34–4.38 (q, $J = 7.5$ Hz, 2H), 1.37–1.39 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 161.6, 153.3, 146.7, 143.8, 136.6, 134.9, 128.0, 127.1, 121.4, 116.2, 61.2, 36.6, 14.3; IR (KBr) cm^{-1}: 3100, 3091, 2984, 2900, 1715, 1608, 1548, 1349, 1264, 1106, 836, 780, 739; MS (*m*/*z*): 355 (M^+); Anal. Calcd for $C_{13}H_{11}ClN_4O_6$: C, 44.02; H, 3.13; N, 15.80. Found: C, 44.15; H, 3.18; N, 15.69.

Ethyl-1(2,4-dinitrophenyl)-3(2-fluorophenyl)-1*H*-pyrazole-4-carboxylate, compound **2d**: yellow crystals (15% ethyl acetate–petroleum ether); mp 128 °C; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.74–8.75 (d, $J_1 = 2.3$ Hz, 1H), 8.51–8.54 (dd, $J_1 = 2.3$ and $J_2 = 9.2$ Hz, 1H), 8.40 (s, 1H, pyrazole –CH), 7.86–7.88 (d, $J_2 = 9.2$ Hz, 1H), 7.45–7.48 (t, $J = 7.5$ Hz, 1H, *o*-fluorophenyl), 7.39–7.43 (m, 1H, *o*-fluorophenyl), 7.18–7.22 (t, $J = 7.5$ Hz, 1H, *o*-fluorophenyl), 7.10–7.13 (m, 1H, *o*-fluorophenyl), 4.21–4.26 (q, $J = 7.5$ Hz, 2H), 1.20–1.22 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 161.9, 159.5, 151.0, 146.5, 143.7, 136.6, 134.5, 131.4, 131.3, 127.9, 126.5, 124.07–124.09 (t, $J = 143$ Hz, fluorine substituted carbon), 121.4, 117.8, 115.6, 115.4, 61.0, 14.1; IR (KBr) cm^{-1}: 3143, 3090, 2992, 2775, 1721, 1608, 1548, 1350, 1294, 1132, 837, 767, 741; MS (*m*/*z*): 400 (M^+); Anal. Calcd for $C_{18}H_{13}FN_4O_6$: C, 54.01; H, 3.27; N, 14.00. Found: C, 53.89; H, 3.29; N, 13.88.

Benzyl-1(2,4-dinitrophenyl)-3-methyl-1*H*-pyrazole-4-carboxylate, compound **2e**: pale yellow crystals (15% ethyl acetate–petroleum ether); mp 140 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.70–8.71 (d, $J_1 = 2.9$ Hz, 1H), 8.49–8.52 (dd, $J_1 = 2.9$ and $J_2 = 8.6$ Hz, 1H), 8.25 (s, 1H, pyrazole –CH), 7.78–7.80 (d, $J_2 = 8.6$ Hz, 1H), 7.33–7.42 (m, 5H), 5.30 (s, 2H), 2.50 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 162.4, 154.9, 146.2, 143.5, 136.8, 135.7, 134.4, 128.8, 128.6, 128.5, 127.8, 126.3, 121.4, 116.4, 66.5, 13.7; IR (KBr) cm^{-1}: 3363, 3088, 2947, 2891, 1691, 1608, 1547, 1510, 1352, 1278, 1121, 745; MS (*m*/*z*): 382 (M^+); Anal. Calcd for $C_{18}H_{14}N_4O_6$: C, 56.55; H, 3.69; N, 14.65. Found: C, 56.42; H, 3.76; N, 14.51.

Ethyl-1(2,4-dinitrophenyl)-3-methyl-1*H*-pyrazole-4-carboxylate, compound **2f**: pale yellow crystals (15% ethyl acetate–petroleum ether); mp 122 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.70–8.71 (d, $J_1 = 2.4$ Hz, 1H), 8.48–8.50 (dd, $J_1 = 2.4$ Hz and $J_2 = 8.7$ Hz, 1H), 8.20 (s, 1H, pyrazole –CH), 7.83–7.85 (d, $J_2 = 8.7$ Hz, 1H), 4.28–4.35 (q, $J = 7.1$ Hz, 2H), 2.49 (s, 3H), 1.33–1.37 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.5, 154.6, 147.6, 144.7, 137.6, 134.6, 127.0, 125.7, 122.8, 116.8, 60.6, 14.4, 13.6; IR (KBr) cm^{-1}: 3074, 1722, 1693, 1550, 1349, 1276; MS (*m*/*z*): 320 (M^+); Anal. Calcd for $C_{13}H_{12}N_4O_6$: C, 48.75; H, 3.75; N, 17.50. Found: C, 48.82; H, 3.81; N, 17.46.

t-Butyl-1(2,4-dinitrophenyl)-3-methyl-1*H*-pyrazole-4-carboxylate, compound **2g**: pale yellow crystals (15% ethyl acetate–petroleum ether); mp 125 °C; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.82–8.83 (d, $J_1 = 2.3$ Hz, 1H), 8.59–8.61 (dd, $J_1 = 2.3$ and $J_2 = 9.2$ Hz, 1H), 8.21 (s, 1H, pyrazole –CH), 8.15–8.17 (d, $J_2 = 9.2$ Hz, 1H), 3.79 (s, 9H, *t*-butyl), 2.36 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 162.9, 154.6, 146.1, 143.3, 136.7, 134.2, 127.6, 126.3, 121.2, 116.4, 51.6, 29.7, 13.4; IR (KBr) cm^{-1}: 3100, 3091, 2984, 2900, 1715, 1608, 1548, 1349, 1264, 1106, 836, 780, 739; MS (*m*/*z*): 348 (M^+); Anal. Calcd for $C_{15}H_{16}N_4O_6$: C, 51.73; H, 4.63; N, 16.09. Found: C, 51.72; H, 4.70; N, 16.18.

14. Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565.
15. Typical procedure for the preparation ethyl-1(2,4-dinitrophenyl)-4-formyl-1*H*-pyrazole-3-carboxylate, compound **2h**: 1.4 g of $POCl_3$ (0.008 mol) was added dropwise to an ice-cold stirred solution of ethyl pyruvate 2,4-dinitrophenyl hydrazone (0.001 mol) in 10 mL dry DMF. The reaction mixture was allowed to attain room temperature and then refluxed at 70–80 °C for about 4 h. The resulting mixture was poured onto crushed ice, neutralized with sodium acetate and left standing overnight. The pale yellow precipitate obtained was purified by silica gel (60–120 mesh) column chromatography with ethyl acetate–petroleum ether mixture (15:85) to yield the product. Yellow crystals (15% ethyl acetate–petroleum ether); mp 130 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.45 (s, 1H, formyl –CH), 8.87–8.88 (d, $J_1 = 2.3$ Hz, 1H), 8.61–8.63 (dd, $J_1 = 2.3$ and $J_2 = 8.6$ Hz, 1H), 8.36 (s, 1H-pyrazole –CH), 7.91–7.93 (d, $J_2 = 8.6$ Hz, 1H), 4.47–4.51 (q, $J = 7.5$ Hz, 2H), 1.42–1.45 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 186.0, 160.6, 147.7, 146.1, 144.3, 136.6, 134.0, 128.7, 128.3, 126.5, 121.6, 62.6, 14.3; IR (KBr) cm^{-1}: 3330, 3122, 3079, 2999, 2890, 1734, 1686, 1610, 1551, 1351, 1268, 1242, 1109, 742, 629; MS (*m*/*z*): 334 (M^+); Anal. Calcd for $C_{13}H_{10}N_4O_7$: C, 46.70; H, 3.02; N, 16.77. Found: C, 46.75; H, 3.06; N, 16.58.
16. Materials and methods for anti-microbial activity: test organisms and their maintenance: The human pathogenic bacterial cultures such as *E. coli* (ATCC 25922), *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 27853) and *En. faecalis* (ATCC 29212) and plant pathogenic fungi viz., *R. solani*, *F. oxysporum*, *C. lunata*, *B. oryzae* and *A. alternata*

were obtained from the Centre for Advanced Studies in Botany, University of Madras, Chennai, India and the biological screening of 1*H*-pyrazole carboxylates was performed there itself. All the bacterial cultures were maintained on nutrient agar and that of fungal cultures on potato dextrose agar at 28 ± 2 °C. Nutrient agar (NA) medium was used for determining the bacterial growth and potato dextrose agar (PDA) was used for fungal growth. The media were sterilized at 15 psi for 20 min and the filter sterilized 1*H*-pyrazole carboxylate solution was amended in the media at 45 °C to obtain 0.1 and 0.5 mg/mL concentrations. Media devoid of 1*H*-pyrazole carboxylate served as control.

Effect of 1*H*-pyrazole carboxylates on the growth of human pathogenic bacteria: The anti-bacterial activity of 1*H*-pyrazole carboxylates was tested by the agar diffusion method.[17] Nutrient agar medium was prepared and 1 mL of bacterial inoculum of each test pathogen was added to the molten agar medium. It was poured into sterile Petriplates under aseptic conditions. After solidification, a 5 mm well was made in the centre of plate using a sterile cork borer. About 75 μL solution of each 1*H*-pyrazole carboxylate was added into the well and the plates were incubated at 28 ± 2 °C. Sterile distilled water was used for control. After 24 h, the appearance of inhibition zone around the well was observed. Effect of 1*H*-pyrazole carboxylates on the growth of plant pathogenic fungi: The anti-fungal activity of 1*H*-pyrazole carboxylates was evaluated by measuring the radial growth of fungal mycelium on agar dilution plate.[17] 1*H*-Pyrazole carboxylates amended and non-amended PDA was poured into 9 cm Petriplates and they were inoculated with 5 mm mycelial discs of the test fungi. The plates were incubated at 28 ± 2 °C for 6 days and the radial growth of fungal mycelium was measured.

17. Pandurangan, A.; Mathivanan, N.; Prabavathy, V. R.; Kabilan, V.; Murugesan, V.; Murugesan, K. *Indian J. Exp. Biol.* **1995**, *33*, 357.