Synthesis and Antibacterial Evaluation of Some New 4-Substituted-3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazoles

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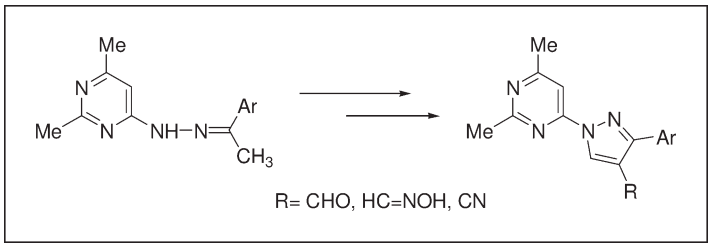

Synthesis of a series of new 4-substituted-3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazoles (**2a–g**, **3a–g**, and **4a–g)** is described. All the synthesized compounds were evaluated *in vitro* for their antibacterial activity against two gram-positive and two gram-negative bacteria, namely, *Bacillus subtilis* (MTCC 8509), *Bacillus stearothermophilus* (MTCC 8508), *Escherichia coli* (MTCC 51), and *Pseudomonas putida* (MTCC 121), and their activity was compared with two commercial antibiotics, streptomycin and chloramphenicol. Two compounds, namely, 3-(4-anisyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehyde **(2b)** and 3-(2-thienyl)-1-(2,6-dimethyl pyrimidin-4-yl)pyrazole-4-carboxaldehyde **(2g)** were found to be equipotent to streptomycin and chloramphenicol against gram-negative bacteria, *E. coli* having minimum inhibitory concentration (MIC) value = 4 μg/mL. Compounds **4b** and **4d** also displayed good activity against *E. coli* with MIC = 8 μg/mL.

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INTRODUCTION

Pyrazole ring is a prominent structural motif found in numerous pharmaceutically active compounds. This is mainly due to the easy preparation and important biological activity associated with pyrazoles. Pyrazole framework plays an essential role in biologically active compounds and, therefore, represents an interesting template for combinatorial as well as medicinal chemistry. Pyrazole derivatives have shown several biological activities such as antibacterial [1,2], GABA receptor antagonists and insecticidal [3], anxioyltic [4], anti-inflammatory [5], potent PET ligand for CBI receptors [6], and growth inhibition activity [7]. In addition to theses observations, several reports have shown that pyrimidine derivatives have diverse pharmacological properties, such as they act as antibacterial agents [8,9], anti-inflammatory agents [10–13], antihypertensive agents [14], anti-HIV agents [15], and H_1-antihistamines [16].

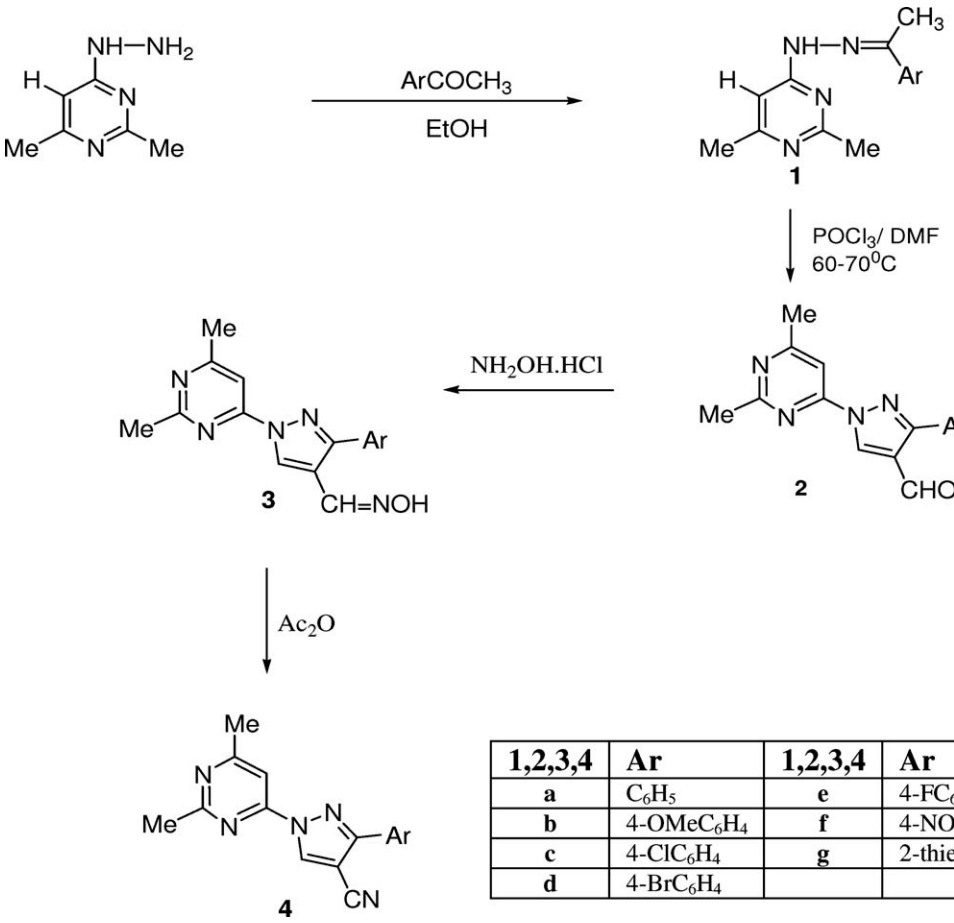
In view of these observations, we have now synthesized a series of 4-substituted-3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazoles (**2a–g, 3a–g**, and **4a–g**) and evaluated their antibacterial activity against two gram-positive and two gram-negative bacteria. In this study, an attempt has been made to explore the effect of various substituents in the azole ring at C(4)-position.

RESULTS AND DISCUSSION

Chemistry. Synthesis of 21 new 4-substituted-3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazoles (**2a–g**, **3a–g**, and **4a–g**) is outlined in Scheme 1.

A series of new 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehydes (**2a–g**) was synthesized using Vilsmeier–Haack reaction. Thus, pyrimidinyl hydrazones **1a–g** were treated with a mixture of phosphorous oxychloride ($POCl_3$) in DMF to give 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehydes (**2a–g**). The structure of all the compounds **2a–g** was characterized by analyzing their spectral (IR, ^{1}H NMR, ^{13}C NMR, and mass) and elemental data. The ^{1}H NMR spectra of the compounds **2a–g** displayed the carboxaldehyde proton as a sharp singlet between δ 10.05–10.16 ppm, whereas in the ^{13}C NMR spectra of these compounds, peak in the region δ 184.00–184.59 ppm further

Scheme 1



1,2,3,4	Ar	1,2,3,4	Ar
a	C_6H_5	e	4-FC_6H_4
b	4-$OMeC_6H_4$	f	4-$NO_2C_6H_4$
c	4-ClC_6H_4	g	2-thienyl
d	4-BrC_6H_4		

confirmed the presence of CHO group. In the IR spectra, the stretching frequency for aldehyde group (C=O) showed characteristic strong band between 1675–1694 cm^{-1}.

Furthermore, it has been reported in literature that placement of a cyano moiety on the azole often generates analogs with interesting antibacterial activities *in vivo* and *in vitro*, in particular 3-cyano and 4-cyano pyrazoles [17,18]. Keeping this in view, 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehydes (**2**) were transformed to 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazoles (**4a–g**) in good yields by making use of inexpensive and commercially available reagent, acetic anhydride (Scheme 1). 3-Aryl-1-(2,6-dimethyl pyrimidin-4-yl)-4-cyanopyrazoles (**4a–g**) were prepared by a two-step procedure. 3-Aryl-1-(2,6-dimethylpyrimidin-4-yl)-4-formylpyrazoles (**2a–g**) on treatment with $NH_2OH.HCl$ in refluxing ethanol gave 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoximes (**3a–g**) in excellent yields which on further reaction with acetic anhydride afforded 4-cyanopyrazoles (**4a–g**) (Scheme 1). The structural assignments of the compounds **3a–g** and **4a–g** were based on their spectral as well as elemental analyses. The complete characterization data of all the compounds is summarized in experimental section.

Biological investigation. All the synthesized compounds **2a–g**, **3a–g**, and **4a–g** were evaluated for their antibacterial activity against *Bacillus subtilis* (MTCC 8509), *Bacillus stearothermophilus* (MTCC 8508), *Escherichia coli* (MTCC 51), and *Pseudomonas putida* (MTCC 121), and their antibacterial activity was compared with two well-known drugs ''streptomycin'' and ''chlroamphenicol'' (Table 1). Some of the test compounds showed moderate to good antibacterial activity. A careful investigation of minimum inhibitory concentration (MIC) data showed that two compounds, in particular, **2b** and **2g** were found to be equipotent to streptomycin and chlroamphenicol against gram-negative bacteria, *E. coli* having MIC value = 4 μg/mL. Compounds **4b** and **4d** also displayed good activity against *E. coli* with MIC= 8 μg/mL. Moreover, compounds **2b**, **2g**, and **4d** were found active against *P. putida*, whereas **2g** and **4a** possess good antibacterial activity against *B. stearothermophilus* with MIC value= 8 μg/mL. Oximes **3a–g** were found to possess very modest to poor activity against gram-positive and gram-negative bacteria (MIC

Table 1

MIC of Test Compounds (**2a–h**, **3a–h**, and **4a–h**) against four bacteria.

Compounds	*B. subtilis*	*B. stearothermophilus*	*P. putida*	*E. coli*
2a	>64	>64	32	>64
2b	32	16	08	04
2c	>64	>64	>64	32
2d	>64	>64	>64	>64
2e	>64	32	>64	>64
2f	>64	>64	>64	64
2g	16	08	08	04
3a	>64	>64	>64	>64
3b	>64	>64	>64	>64
3c	>64	>64	>64	>64
3d	>64	>64	32	16
3e	32	32	16	32
3f	>64	>64	>64	64
3g	32	32	32	16
4a	16	08	16	16
4b	32	16	16	08
4c	64	64	32	32
4d	32	64	08	08
4e	16	32	16	16
4f	16	16	16	16
4g	32	16	16	32
Streptomycin	02	02	04	04
Chloramphenicol	02	02	02	04

B. subtilis: Bacillus subtilis (MTCC 8509); *B. stearothermophilus: Bacillus stearothermophilus* (MTCC 8508); *P. putida*: *Pseudomonas putida* (MTCC 121); *E. coli*: *Escherichia coli* (MTCC 51).

value ranges from 16–64 μg/mL and higher). The results of biological evaluation are given in Table 1.

EXPERIMENTAL

Chemical synthesis. Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on Perkin-Elmer IR spectrophotometer. The ^{1}H NMR spectra were recorded on BRUKER 300 MHz instrument (in some cases 400-MHz instrument was used). The chemical shifts are expressed in ppm units downfield from an internal TMS standard. Elemental analyses were performed on Perkin-Elmer 2400 instrument, and mass spectra were recorded on Kratos MS-3 mass spectrometer.

General procedure for the preparation of 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehydes (2a–g). To the Vilsmeier–Haack reagent prepared by dissolving $POCl_3$ (0.03 mol) in 20-mL DMF, hydrazone (**1,** 0.01 mol) was added, and the reaction mixture was stirred at 60–70°C for 3 h. The reaction mixture was then poured over crushed ice. The resulting solution was neutralized with sodium bicarbonate solution. Solid, thus obtained, was filtered, washed with water, and recrystallized from ethanol to afford pure 3-aryl-1-(2,6-dimethyl-4-pyrimidinyl) pyrazole-4-carboxaldehydes (**2a–g**).

Characterization data of 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehydes (2a–g). *3-Phenyl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehyde (2a).* Mp 116°C; Yield 75%; IR (ν_{max}, KBr): 1677.6 (CO stretch), 2914.5 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ, ppm): 2.735 (s, 6H, —2CH_3), 7.463–7.536 (m, 4H), 7.881–7.901 (m, 2H), 9.249 (s, 1H), 10.124 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 184.74 (CHO), 166.65, 154.20, 122.84–134.83 (aromatic carbons), 26.21 (2CH_3); ms: *m/z* 278 (M^+); *Anal. Calcd.* for $C_{16}H_{14}N_4O$: C, 69.05; H, 5.07; N, 20.13. Found: C, 68.98; H, 5.00; N, 20.10

3-(4-Anisyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehyde (2b). Mp 207–208°C; Yield 77%; IR (ν_{max}, KBr): 1693, 2884 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.526 (s, 3H, —CH_3), 2.538 (s, 3H, —CH_3), 3.869 (s, 3H, —OCH_3), 6.940–6.968 (d, 2H, J = 8.4 Hz), 7.287 (s, 1H), 7.750–7.778 (d, 2H, J =8.4 Hz), 9.20 (s, 1H), 10.07 (s, 1H); ^{13}C NMR: 184.58 (CHO), 160.85, 155.01, 122.91–139.28 (rest aromatic carbons), 55.75 (OCH_3), 26.21 (2CH_3); ms: *m/z* 308 (M^+); *Anal. Calcd.* for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.71. Found: C, 66.18; H, 5.21; N, 18.67.

3-(4-Chlorophenyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehyde (2c). Mp 250°C; Yield 78%; IR (ν_{max}, KBr): 1692, 2853 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.791 (s, 3H, —CH_3), 2.841 (s, 3H, —CH_3), 7.312–7.548 (m, 3H), 7.923–7.952(d, 2H, J = 8.7 Hz), 9.51 (s, 1H), 10.1 (s, 1H); ^{13}C NMR: 184.69 (CHO), 166.63, 155.39, 152.62, 122.89-136.07 (aromatic carbons), 100.00, 26.21 (2CH_3); ms: *m/z* 312 (M^+), 314 (M^++2). *Anal. Calcd.* for $C_{16}H_{13}N_4OCl$: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.42; H, 4.15; N, 17.89.

3-(4-Bromophenyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehyde (2d). Mp 177–179°C; Yield 78%; IR (ν_{max}, KBr): 1692, 2918 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.730 (s, 3H, —CH_3), 2.773 (s, 3H, —CH_3), 7.599–7.685 (m, 3H), 7.882–7.7.910 (d, 2H, J =8.4 Hz), 9.256 (s, 1H), 10.093 (s, 1H); ^{13}C NMR: 184.46 (CHO), 166.62, 155.44, 152.84, 122.86–135.77 (aromatic carbons), 99.99, 26.12 (2CH_3); ms: *m/z* 357 (M^+); *Anal. Calcd.* for $C_{16}H_{13}N_4OBr$: C, 53.80; H, 3.67; N, 15.68. Found: C, 53.78; H, 3.64; N, 15.66.

3-(4-Fluorophenyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehyde (2e). Mp 194–195°C; Yield 75%; IR (ν_{max}, KBr): 1692, 2775 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.622 (s, 6H, —CH_3), 7.173–7.231 (m, 3H), 7.958–7.986 (d, 2H, J = 8.4 Hz), 9.251 (s, 1H), 10.088 (s, 1H); ^{13}C NMR: 184.06 (CHO), 166.14, 154.95, 152.48, 122.26–135.11 (aromatic carbons), 115.16, 99.48, 25.66 (2CH_3); ms: *m/z* 296 (M^+); *Anal. Calcd.* for $C_{16}H_{13}N_4OF$: C, 64.86; H, 4.42; N, 18.91. Found: C, 64.82; H, 4.41; N, 18.89.

3-(4-Nitrophenyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehyde (2f). Mp 196°C; Yield 80%; IR (ν_{max}, KBr): 1694, 110, 2763, 2859 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.649 (s, 3H, —CH_3), 2.732 (s, 3H, —CH_3), 7.171 (s, 1H), 8.188–8.214 (d, 2H, J = 7.8 Hz), 8.507–8.570 (d, 2H, J =7.8 Hz), 9.538 (s, 1H), 10.05 (s, 1H); ^{13}C NMR: 184.16 (CHO), 166.14, 154.48, 123.68–137.79 (rest aromatic carbons), 25.66 (2 CH_3); ms: *m/z* 323 (M^+); *Anal. Calcd.* for $C_{16}H_{13}N_5O_3$: C, 59.44; H, 4.05; N, 21.66. Found: C, 59.39; H, 4.03; N, 21.65.

3-(2-Thienyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-carboxaldehyde (2g). Mp 178°C; Yield 74%; IR (ν_{max}, KBr): 1689.5, 2924 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ): 2.271 (s, 3H, —CH_3), 2.28 (s, 3H, —CH_3), 7.1509–7.1729 (m, 2H), 7.4398–7.4553 (dd, 1H, J =1.04 Hz, J' =5.12 Hz), 8.0768–8.0886 (dd, 1H, J' = 5.12 Hz, J'' = 3.68 Hz), 9.2169 (s, 1H), 10.1640 (s, 1H); ^{13}C NMR: 184 (CHO), 166.74, 155.27, 148.20, 122.21–136.05, 26.16 (2CH_3); ms: *m/z* 284 (M^+); *Anal. Calcd.* for $C_{14}H_{12}N_4OS$: C, 59.14; H, 4.25; N, 19.70. Found: C, 59.11; H, 4.23; N, 19.68.

General procedure for the preparation of 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoximes (3a–g). To the solution of pyrazole-4-carboxaldehyde (**2**, 0.01 mol) in ethanol were added hydroxylamine hydrochloride (0.02 mol) and sodium acetate (0.03 mol). The resulting mixture was refluxed for 4 h and cooled. Solid, thus obtained, on cooling was filtered and dried to get **3** (mixture of E/Z isomers was obtained).

Characterization data of 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoximes (3). *3-Phenyl-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoxime (E/Z) (3a).* Mp 190°C; Yield 76%; IR (ν_{max}, KBr): 1607, 3233 cm^{-1}; ^1H NMR (DMSO, 400 MHZ, δ): 2.734 (s, 6H), 2.7628 (s, 3H), 7.2129–8.0368 (m, rest aromatic protons), 8.9428 (s, 1H), 9.00 (s,1H), 9.3867 (s, 1H), 9.4897 (s, 1H); 11.0 (s, 1H), 11.5010 (s, 1H); ms: 293 (M^+); *Anal. Calcd.* for $C_{16}H_{15}N_5O$: C 65.52, H 5.15, N 23.88. Found: C 65.2, H 5.12, N 23.84.

3-(4-Anisyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoxime (E/Z) (3b). Mp 149°C; Yield 77%; IR (ν_{max}, KBr): 1608, 3243 cm^{-1}; ^1H NMR (DMSO, 400 MHZ, δ): 2.5819 (s, 3H), 2.73 (s, 3H), 2.7623 (s, 3H), 3.7979 (s, 3H), 3.8314 (s, 3H), 6.8423–7.0296 (m, 4H), 7.1589 (s, 1H), 7.3291–7.4642 (m, 3H), 7.7514–8.1307 (m, 2H), 8.2081 (s, 1H), 8.2493 (s,1H), 8.4933 (s, 1H), 8.5333 (s, 1H); 9.9952 (s, 1H), 10.7006 (s, 1H); ms: *m/z* 323 (M^+); *Anal. Calcd.* for $C_{17}H_{17}N_5O_2$: C, 63.15; H, 5.30; N, 21.66. Found: C, 63.12; H, 5.26; N, 21.63.

3-(4-Chlorophenyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoxime (E/Z) (3c). Mp 230°C; Yield 78%; IR (ν_{max}, KBr): 1604, 3239 cm^{-1}; ^1H NMR (DMSO, 400 MHZ, δ): 2.5819 (s, 3H), 2.7284 (s, 3H), 2.7523 (s, 3H), 7.4985–7.5294 (m, 4H), 7.6314–7.7400 (m, 2H), 7.6899–7.7252 (m, 2H), 7.8161 (s, 1H), 8.0594 (s,1H), 8.1456 (s, 1H), 9.4456 (s, 1H), 9.5362 (s, 1H), 11.0314 (s, 1H), 11.7091 (s, 1H); ms: *m/z* 328 (M^+), 330 (M^++2); *Anal. Calcd.* for $C_{16}H_{14}N_5OCl$: C, 58.63; H, 4.31; N, 21.37. Found: C, 58.61; H, 4.28; N, 21.35.

3-(4-Bromophenyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoxime (E/Z) (3d). Mp 244°C; Yield 77%; IR (ν_{max}, KBr): 1607, 3243 cm^{-1}; ^1H NMR (DMSO, 400 MHZ, δ): 2.70 (s, 3H), 2.71 (s, 3H), 6.996 (s, 1H), 7.4963–7.9288 (m, 8H), 8.1902 (s,1H), 8.999 (s,1H), 8.9944 (s,1H), 9.1662 (s, 1H), 9.5573 (s, 1H); ms: *m/z* 372 (M^+); *Anal. Calcd.* for $C_{16}H_{14}N_5OBr$: Found C, 51.63; H, 3.79; N, 18.82. Found: C, 51.60; H, 3.75; N, 18.81.

3-(4-Fluorophenyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoxime (E/Z) (3e). Mp 176°C; Yield 79%; IR (ν_{max}, KBr): 1604, 3248 cm^{-1}; ^1H NMR (DMSO, 400 MHZ, δ): 2.5919 (s, 3H), 2.7484 (s, 3H), 2.7623 (s, 3H), 7.2013–7.2621 (m, 4H), 7.4291 (s, 1H), 7.6899–7.7252 (m, 3H), 8.0220–8.1383 (m, 2H), 8.9523 (s, 1H), 9.0012 (s,1H), 9.4072 (s, 1H), 9.5794 (s, 1H); 10.9965 (s, 1H), 11.7006 (s, 1H); ms: *m/z* 311 (M^+); *Anal. Calcd.* for $C_{16}H_{14}N_5OF$: C, 61.73; H, 4.53; N, 22.3. Found: C, 61.70; H, 4.4; N, 22.48.

3-(4-Nitrophenyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoxime (E/Z) (3f). Mp 216°C; Yield 76%; IR (ν_{max}, KBr): 1588, 3452 cm^{-1}; ^1H NMR (DMSO, 400 MHZ, δ): 2.5955 (s, 3H), 2.5996 (s, 3H), 7.8268–8.4053 (m, 11H), 8.9618 (s, 1H), 9.0069 (s, 1H), 9.2988–9.6585 (m, 2H), 10.9721 (s, 1H), 11.0294 (s,1H); ms: *m/z* 338 (M^+); *Anal. Calcd.* for $C_{16}H_{14}N_6O_3$: C, 56.80; H, 4.17; N, 24.84. Found: C, 56.79; H, 4.14; N, 24.81.

3-(2-Thienyl)-1-(2,6-dimethylpyrimidin-4-yl)pyrazole-4-aldoxime (E/Z) (3g). Mp 170°C; Yield 78%; IR (ν_{max}, KBr): 1599, 3432 cm^{-1}; ^1H NMR (DMSO, 400 MHZ, δ): 2.7230 (s, 3H), 2.7319 (s, 3H), 2.7426 (s, 3H), 7.4689–7.5305 (m, 3H), 7.5793–7.9745 (m, rest aromatic protons), 8.9012–8.9668 (m, 2H), 9.1847 (s,1H), 9.3188 (s, 1H), 9.5500–9.5946 (m, 2H), 10.9963 (s, 1H), 11.6802 (s, 1H); ms: *m/z* 299 (M^+); *Anal. Calcd.* for $C_{14}H_{13}N_5OS$: C, 56.17; H, 4.38; N, 23.40. Found: C, 56.14; H, 4.36; N, 23.39.

General procedure for the preparation of 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazoles (4a–g). A mixture of aldoxime (**3**, 0.01 mol) in acetic anhydride (2 mL) was refluxed for 30 min. The reaction was quenched by pouring into crushed ice with stirring and neutralized with sodium bicarbonate. The solid, separated after standing overnight, was filtered with water, dried, and recrystallized from methanol to afford cyanopyrazoles **4**.

Characterization data of 3-aryl-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazoles (4a–g). *3-Phenyl-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazole (4a).* Mp 164°C; Yield 67%; IR (ν_{max}, KBr): 2232 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHZ, δ): 2.723 (s, 3H, —CH_3), 2.773 (s, 3H, —CH_3), 7.430–7.634 (m, 3H), 7.961–8.085 (m, 2H), 9.155 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 114.32 (CN); ms: *m/z* 275 (M^+); *Anal. Calcd.* for $C_{16}H_{13}N_5$ C, 69.80; H, 4.76; N, 25.44. Found: C, 69.78; H, 4.76; N, 25.43.

3-(4-Anisyl)-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazole (4b). Mp 153°C; Yield 69%; IR (ν_{max}, KBr): 2223 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHZ, δ): 2.6728 (s, 3H, —CH_3), 2.68 (s, 3H, —CH_3), 3.8528 (s, 3H, OCH_3), 7.5034–7.5255 (d, 2H, J =8.84 Hz), 7.7980 (s, 1H), 8.0083–8.0253 (d, 2H, J = 8.84 Hz), 9.15 (s,1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 114.59 (CN); ms: *m/z* 305 (M^+); *Anal. Calcd.* for $C_{17}H_{15}N_5O$ C, 66.86; H, 4.95; N, 22.94. Found (C, 66.83; H, 4.92; N, 22.91.

3-(4-Chlorophenyl)-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazole (4c). Mp 178°C; Yield 69 %; IR (ν_{max}, KBr): 2214 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHZ, δ): 2.7465 (s, 6H, —CH_3), 7.5019–7.5228 (d, 2H, J = 8.36 Hz), 7.6363 (s, 1H), 8.0682–8.0891 (d, 2H, J = 8.36 Hz), 9.2060 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 114.89 (CN); ms: *m/z* 310 (M^+), 312 (M^++2); *Anal. Calcd.* for $C_{16}H_{12}N_5Cl$ C, 62.04; H, 3.90; N, 22.61. Found: C, 62.03; H, 3.88; N, 22.57.

3-(4-Bromophenyl)-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazole (4d). Mp 198°C; Yield 71%; IR (ν_{max}, KBr): 2232 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHZ, δ): 2.7382 (s, 3H, —CH_3), 2. 7443 (s, 3H, —CH_3), 7.6210–7.6985 (m, 3H), 8.0072–8.0283 (d, 2H, J = 8.44 Hz), 9.1470 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 116.52 (CN); ms: *m/z* 354; *Anal. Calcd.* for $C_{16}H_{12}N_5Br$ C, 54.25; H, 3.41; N, 19.77. Found: C, 54.21; H, 3.38; N, 19.74.

3-(4-Fluorophenyl)-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazole (4e). Mp 157°C; Yield 68%; IR (ν_{max}, KBr): 2230 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHZ, δ): 2.7456 (s, 3H, —CH_3), 2.7672 (s, 3H, —CH_3), 7.2003–7.2222 (d, 2H, J = 8.76 Hz), 7.6346 (s, 1H), 8.0910–8.1129 (d, 2H, J = 8.76 Hz), 9.2051 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 116.54 (CN); ms: *m/z* 293 (M^+); *Anal. Calcd.* for $C_{16}H_{12}N_5F$ C, 65.52; H, 4.12; N, 23.88. Found: C, 65.48; H, 4.09; N, 23.85.

3-(4-Nitrophenyl)-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazole (4f). Mp 195°C; Yield 71%; IR (ν_{max}, KBr): 2238 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHZ, δ): 2.7521 (s, 3H, —CH_3), 2.90

(s, 3H, —CH_3), 7.6769 (s, 1H), 8.3201–8.3959 (m, 4H), 9.2091 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 115.89 (CN); ms: *m/z* 320 (M^+); *Anal. Calcd.* for $C_{16}H_{12}N_6O_2$. C, 60.00; H, 3.78; N, 26.24. Found: C, 59.98; H, 3.75; N, 26.22.

3-(2-Thienyl)-1-(2,6-dimethylpyrimidin-4-yl)-4-cyanopyrazole (4g). Mp 167°C; Yield 67%; IR (ν_{max}, KBr): 2218 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHZ, δ): 2.7651 (s, 3H, —CH_3), 2.7761 (s, 3H, —CH_3), 7.1692–7.1936 (dd, 1H, J =3.6 Hz, J' = 5.12 Hz), 7.4850–7.5867 (m, 2H), 7.8874–7.9479 (dd, 1H, J =3.6 Hz, J'' =1.04 Hz), 9.2125 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 116.32 (CN); ms: *m/z* 281(M^+); *Anal. Calcd.* for $C_{14}H_{11}N_5S$. C, 59.77; H, 3.94; N, 24.89. Found C, 59.75; H, 3.91; N, 24.85.

Biological investigation. ***Minimum inhibitory concentration.*** MIC is the lowest concentration of the antimicrobial agents that prevents the development of visible growth of microorganism after overnight incubation. MIC of chemically synthesized compounds against two Gram-positive and two Gram-negative bacteria, namely, *B. subtilis* (MTCC 8509), *B. stearothermophilus* (MTCC 8508), *E. coli* (MTCC 51), and *P. putida* (MTCC 121) was determined by reported method [19].

Nutrient broth was adjusted to pH 7.0 used for the determination of MIC. The inoculum of the test microorganisms were prepared by using 16-h-old cultures adjusted by reference to the 0.5 McFarland standards (10^8 cells/mL) [20]. These cultures were further diluted upto 10 folds with nutrient broth to get the inoculum size of 1.2×10^7 CFU/mL. A positive control (containing inoculum but no compound) and a negative control (containing compound but no inoculum) were also prepared. A stock solution of 4 mg/mL of each compound was prepared in DMSO and further appropriately diluted to get final concentration ranging from 23 to 0.03 μg/mL [21]. Requisite quantity of antifungal compound (cyclohexamide) was added to the broth to get its desirable final concentration of 100 μg/mL. Separate flasks were taken for each test dilution. To each flask was added the 100 μL of inoculum. Then appropriately diluted test sample was added to each flask having broth and microbial inoculum. The contents of the flask were mixed and incubated for 24–48 h at 37°C. The test bacterial culture were spotted in a predefined pattern by aseptically transferring 5 μL of each bacterial culture on the surface of solidified agar-agar plates and incubated at 37°C for 24 h for determining the MIC value.

CONCLUSIONS

All the chemically synthesized 21 compounds were characterized using their spectral and elemental analyses. These compounds were evaluated for their antibacterial activity against *B. subtilis* (MTCC 8509), *B. stearothermophilus* (MTCC 8508), *E. coli* (MTCC 51), and *P. putida* (MTCC 121), and their activity was compared with that of commercially known antibiotics, streptomycin and chlroamphenicol. Two compounds, in particular, **2b** and **2g** were found to be equipotent to streptomycin and chlroamphenicol against gram-negative bacteria, *E. coli*, having MIC value = 4 μg/mL. Compounds **4b** and **4d** also displayed good activity against *E. coli* (MTCC 51) with MIC= 8 μg/mL. Moreover, compounds **2b**, **2g**, and **4d** were found active against *P. putida*, whereas **2g** and **4a** possess good antibacterial activity against *B. stearothermophilus* with MIC value= 8 μg/mL.

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