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Synthesis of pyrimidine linked pyrazole heterocyclics by microwave irradiative cyclocondensation and evaluation of their insecticidal and antibacterial potential

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The compounds (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-substituted phenyl/*H*-pyrazol-3-yl)-amines and (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-substituted benzoyl/isonicotinoyl-pyrazol-3-yl)-amines have been synthesized by microwave irradiative cyclocondensation of N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide with substituted hydrazines and acid hydrazides respectively. The required butyramide has been synthesized by the condensation of 2-amino-4,6-dimethyl pyrimidine with ethyl acetoacetate under microwave. The pyrimidine linked pyrazol-3-yl amines on acylation afforded mono/di-acetyl derivatives. Structural elucidation of synthesized compounds has been performed by IR, ^{1}H NMR and mass spectral studies besides chemical transformation and elemental analysis. Title compounds have been screened for their insecticidal activity against Pseudococcidae insects and evaluated for antibacterial potential against some selected microorganisms to establish the structure activity relationship.

Keywords: Pyrimidine-pyrazole, microwave, insecticidal, antimicrobial activity

The microwave irradiation technique has number of advantages over the conventional heating in synthesis of organic compounds[1]. High density microwave irradiation technology has emerged as a reliable and useful technique for accelerating the time consuming reactions[2] and it can be used for high speed parallel synthesis of number of biologically active molecules[3,4]. Several pyrazole derivatives are well established in the literature. The activity of pyrazoles covers domains such as antimicrobial, antiviral, anticonvulsant, antidepressant, antitubercular and antihistaminic[5-8]. Literature also reveals excellent analgesic and anti-inflammatory activity associated with pyrazole nucleus[9,10]. It is observed that pyrazoles linked with different heterocyclics are known to contribute to various chemotherapeutic effects. In addition, some pyrazole derivatives were reported to induce various antileukemic, antitumor and antiproliferative activities[11-14]. Investigations in chemistry and pharmacology of pyrazoles have been highly intensified with the recognition that they constitute essential pharmacophore. Research also reveals good about insecticidal property associated with pyrazole chromophore[15,16]. Wide range of chemotherapeutic activities has been ascribed to pyrimidine ring as well[7].

On perusal of literature, it was observed that position N-1, C-3, C-4 are much important for the studies of structure activity relationship and C-3 should be linked to different heterocyclics for better chemotherapeutic activities[17]. With relevance to all these observations our efforts are directed towards the synthesis and study of pyrimidine linked pyrazoles at C-3 position. We report herein, the synthesis of pyrimidine linked pyrazol-3yl amines and their mono/di-acetyl derivatives by microwave irradiation. Title compounds have been screened for their insecticidal activity and evaluated for antibacterial potential.

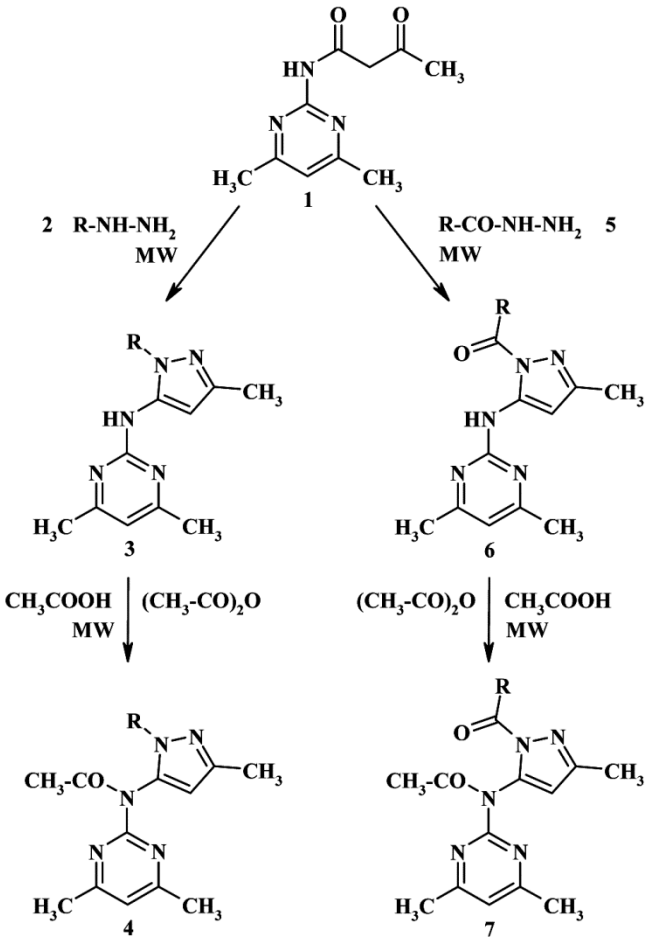
Results and Discussion

The compound N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide **1** was prepared by the condensation of 2-amino-4,6-dimethyl pyrimidine (0.01 mol) with ethyl acetoacetate (0.01 mol) under microwave irradiation in solvent free condition. The compound **1** was then treated with substituted hydrazines **2a-f** (0.01 mol) under microwave in ethanolic medium to afford (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-substituted phenyl/*H*-pyrazol-3-yl)-amines **3a-f**. The amines **3a-f** were acylated using acetic anhydride and acetic acid to afford mono/di-acetyl derivatives **4a-f**. The reaction of 2-amino-4,6-dimethyl pyrimidine (0.01 mol) with ethyl acetoacetate (0.01 mol) was further extended by reacting the product N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide **1** with substituted acid hydrazides **5a-d** (0.01 mol) under microwave in ethanolic medium

to afford (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-substituted benzoyl/isonicotinoyl-pyrazol-3-yl)-amines **6a-d**. The amines **6a-d** were acylated using acetic anhydride and acetic acid to afford mono/di-acetyl derivatives **7a-d** (Scheme I). It was observed that the microwave irradiative reactions received high product yield, enhanced reaction rates and high purity than conventional heating. IR, ^{1}H NMR and mass spectral studies of synthesized compounds fully supported the structures and showed single spots in TLC.

Insecticidal activity

To access the insecticidal property of compounds **3a-f** and **6a-d**, the insect affected plant surface with insect species Pseudococcidae[16] (Mealy bug) was selected. The insecticidal activity was determined by direct contact application[15,16]. The heavy infested plant part affected from the insect pests was selected for application. The aqueous solutions (2,4,6 ppm) of the test compounds **3a-f** and **6a-d** were prepared and applied by direct spray method under same conditions of temperature and sunlight on differently labeled affected plant parts. The amount of solution sprayed was about 2 mL at the time of single application[16]. The results for mortality of insects were monitored from time to time for about 1 to 48 hours. The simple microscope was used to check any movement of body parts of insects. The results observed were recorded and in most of the cases it was found that the aqueous solutions of 2 ppm were sufficiently active against



Where,

2a = Phenyl hydrazine
2b = 2,4-Dinitro-phenyl hydrazine
2c = Hydrazine hydrate
2d = 2-Methyl-phenyl hydrazine hydrochloride
2e = 4-Methyl-phenyl hydrazine hydrochloride
2f = 4-Methoxy-phenyl hydrazine
5a = Benzoic acid hydrazide
5b = 2-Hydroxy-benzoic acid hydrazide
5c = Isonicotinic hydrazide
5d = 4-Amino-benzoic acid hydrazide

Scheme I

insect pests and no plant parts were affected due to the toxicity of compounds. The activity of test solutions was compared with activity of ethanol and hexane solutions of same concentrations and it was found to be good enough.

Antibacterial activity

The evaluation of antibacterial potential of synthesized compounds **3a-f** and **6a-d** was performed by using cup plate diffusion method (Kirby-Baur method)[18,19]. The bacterial organisms having both gram-positive and gram-negative strains *i.e*. *E. coli*, *S. aureus*, *S. typhi*, *B. subtilis* and *P. vulgaris* were used. Sensitivity plates were seeded with a bacterial inoculums of 1×10^6 CIU/mL and each well of diameter 10 mm was loaded with 0.1 mL of test compound solution (1000 µg/mL) in DMF, so that concentration of each test compound was 100 µg/mL. The zones of inhibition were recorded after incubation for 24 hr at 37°C using vernier caliper. It was observed that the compounds **3a**, **3c** and **6b**, **6c** were highly active against *E. coli* and *B. subtilis* and moderately active against *S. aureus*. Majority of the compounds were found to be inactive against *P. vulgaris* (Table I). To determine the minimum inhibitory concentration (MIC), serial dilution technique[20] was used using nutrient broth medium. MIC values of compounds **3a**, **3c** and **6b**, **6c** against *E. coli* and *B. subtilis* were found to be 68, 72 and 76, 70 µg/mL respectively.

Experimental Section

The MW assisted reactions were carried out using commercially available microwave oven (1200 W). Melting points of all synthesized compounds were recorded using Veego VMP-D digital melting point apparatus and are uncorrected. Chemicals used were of AR grade. ^{1}H NMR spectra were recorded with TMS as internal standard on a Bruker Avance-II 400 NMR spectrometer using $CDCl_3$ and DMSO-d_6 as solvents. IR spectra were recorded on Perkin-Elmer spectrophotometer in the range 4000-400 cm^{-1} in Nujol mull and as KBr pellets. Mass spectral measurements were carried out by EI method on Jeol-JMC 300 spectrometer at 70 eV. Homogeneity of the compounds was checked on silica gel-G plates by TLC and spots were visualized by the iodine vapours.

Table I — Antibacterial activity

Compd	Microorganisms				
	E. coli	*S. aureus*	*S. typhi*	*B. subtilis*	*P. vulgaris*
3a	+++	++	+	+++	+
3b	++	+	++	+	–
3c	+++	++	–	+++	+
3d	+	++	++	+	–
3e	+	–	++	–	–
3f	+	++	+	+	+
6a	+	+	–	–	–
6b	+++	++	+	+++	+
6c	+++	++	+	+++	+
6d	++	+	++	+	–
Streptomycin	+++	+++	++	+++	++

(–) : Inactive (10 mm and less) (+) : Weakly active (11-15 mm) (++): Moderately active (16-20 mm) (+++): Highly active (21 mm and above)

Synthesis of N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide 1.

The compound N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide **1** was prepared by irradiating the equimolar mixture of 2-amino-4,6-dimethyl pyrimidine (0.01 mol) and ethyl acetoacetate (0.01 mol) for about 3 min. Completion of the reaction was monitored with TLC. The resulting solid was crystallized from cold acetone.

1: Yield 78%. m.p.128°C. IR: 3406 (NH), 1712 (C=O), 1645 (C=N), 1338 cm^{-1} (C-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 6.36 (1H, s, Pyrm-H), 5.67 (1H, s, Pyrm-NH), 2.38 (2H, s, CO-CH_2), 2.28 (9H, s, Pyrm-CH_3, CO-CH_3)[21,22]. Anal. Found: C, 55.13; H, 5.02; N, 18.92. Calcd for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32; N, 20.28%.

Synthesis of (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-phenyl-pyrazol-3-yl)-amine 3a.

The compound (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-phenyl-pyrazol-3-yl)-amine **3a** was prepared by microwave irradiative cyclocondensation of N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide **1** (0.01 mol) with phenyl hydrazine **2a** (0.01 mol) for 3 min 30 sec using few drops of absolute ethanol. The crude solid residue obtained was crystallized from ethanol.

3a: Yield 85%. m.p.118°C. IR: 3408 (NH), 1649 (C=N), 1309 (C-N), 1176 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 7.39-7.41 (5H, m, Ar-H), 6.32 (1H, s, Pyrz-H), 6.12 (1H, s, Pyrm-H), 3.87 (1H, s, Pyrm-NH), 2.26 (9H, s, Pyrm-CH_3, Pyrz-CH_3); MS: *m/z* 279 (M^+), 264 (M^+-CH_3), 202 (M^+-C_6H_5), 172 (M^+-$(CH_3)_2.C_4HN_2$), 122 (M^+-$CH_3.C_6H_5.C_3HN_2$), 107 $(CH_3)_2.C_4HN_2^+$). Anal. Found: C, 66.66; H, 5.63; N, 22.87. Calcd for $C_{16}H_{17}N_5$: C, 68.80; H, 6.13; N, 25.07%.

This reaction was extended to synthesize other compounds **3b-f** using different substituted hydrazines **2b-f**.

3b: Yield 76%. m.p.132°C. IR: 3404 (NH), 1649 (C=N), 1517 (N=O), 1309 (C-N), 1172 cm^{-1} (N-N); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 7.81-9.11 (3H, m, Ar-H), 6.37 (2H, s, Pyrm-H, Pyrz-H), 4.09 (1H, s, Pyrm-NH), 2.28 (3H, s, Pyrz-CH_3), 2.17 (3H, s, Pyrm-CH_3), 2.08 (3H, s, Pyrm-CH_3); MS: *m/z* 368 (M^+-H), 354 (M^+-CH_3), 262 (M^+-$(CH_3)_2.C_4HN_2$), 247 (M^+-$(CH_3)_2.C_4HN_2.NH$), 122 (M^+-$CH_3.C_6H_3N_2O_4.C_3HN_2$), 107 $(CH_3)_2.C_4HN_2^+$). Anal. Found: C, 49.06; H, 2.56; N, 21.35. Calcd for $C_{16}H_{15}N_7O_4$: C, 52.03; H, 4.06; N, 26.55%.

3c: Yield 70%. m.p.138°C. Anal. Found: C, 57.07; H, 4.93; N, 32.29. Calcd for $C_{10}H_{13}N_5$: C, 59.10; H, 6.45; N, 34.46%.

3d: Yield 80%. m.p.121°C. Anal. Found: C, 68.55; H, 6.44; N, 23.38. Calcd for $C_{17}H_{19}N_5$: C, 69.60; H, 6.53; N, 23.87%.

3e: Yield 90%. m.p.138°C. Anal. Found: C, 67.31; H, 6.51; N, 23.83. Calcd for $C_{17}H_{19}N_5$: C, 69.60; H, 6.53; N, 23.87%.

3f: Yield 79%. m.p.141°C. Anal. Found: C, 65.88; H, 6.13; N, 22.60. Calcd for $C_{17}H_{19}N_5O$: C, 66.00; H, 6.19; N, 22.64%. The reactions were monitored on silica gel-G plates by TLC.

Synthesis of N-(4,6-dimethyl-pyrimidin-2-yl)-N-(5-methyl-2-phenyl-pyrazol-3-yl)- acetamide 4a

The mixture of (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-phenyl-pyrazol-3-yl)-amine **3a** (0.01 mol) and acetic anhydride (0.01 mol) in glacial acetic acid (2 mL) was irradiated under microwave conditions for 30 sec. The reaction mixture was cooled and poured on a little crushed ice to afford N-(4,6-dimethyl-pyrimidin-2-yl)-N-(5-methyl-2-phenyl-pyrazol-3-yl)-acetamide, it was crystallized from ethanol, **4a** (93%), m.p.94°C. IR: 1720 (C=O), 1651 (C=N), 1309 (C-N), 1176 cm^{-1} (N-N); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 7.15-8.15 (5H, m, Ar-H), 6.31 (1H, s, Pyrz-H), 6.27 (1H, s, Pyrm-H), 2.50 (3H, s, CO-CH_3), 2.16 (9H, s, Pyrm-CH_3, Pyrz-CH_3). Anal. Found: C, 65.21; H, 5.94; N, 21.22. Calcd for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79%.

This reaction was extended to synthesize other compounds **4b-f**.

4b: Yield 92%. m.p.98°C. IR: 1714 (C=O), 1649 (C=N), 1514 (N=O), 1300 (C-N), 1172 cm^{-1} (N-N); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 7.20-8.90 (3H, m, Ar-H), 6.27 (1H, s, Pyrz-H), 6.24 (1H, s, Pyrm-H), 2.50 (3H, s, CO-CH_3), 2.16 (9H, s, Pyrm-CH_3, Pyrz-CH_3). Anal. Found: C, 51.53; H, 3.97; N, 22.99. Calcd for $C_{18}H_{17}N_5O_5$: C, 52.55; H, 4.13; N, 23.84%.

4c: Yield 89%. m.p.96°C. Anal. Found: C, 58.11; H, 5.67; N, 24.02. Calcd for $C_{14}H_{17}N_5O_2$: C, 58.52; H, 5.96; N, 24.37%.

4d: Yield 88%. m.p.78°C. Anal. Found: C, 66.84; H, 5.95; N, 20.75. Calcd for $C_{19}H_{21}N_5O$: C, 68.04; H, 6.31; N, 20.88%.

4e: Yield 91%. m.p.99°C. Anal. Found: C, 67.81; H, 6.15; N, 20.69. Calcd for $C_{18}H_{21}N_5O$: C, 68.04; H, 6.31; N, 20.88%.

4f: Yield 78%. m.p.110°C. Anal. Found: C, 64.08; H, 5.84; N, 19.88. Calcd for $C_{19}H_{21}N_5O_2$: C, 65.00; H, 6.02; N, 19.93%. The reactions were monitored on silica gel-G plates by TLC.

Synthesis of (2-benzoyl-5-methyl-pyrazol-3-yl)-(4,6-dimethyl-pyrimidin-2-yl)-amine 6a

The compound (2-benzoyl-5-methyl-pyrazol-3-yl)-(4,6-dimethyl-pyrimidin-2-yl)-amine **6a** was prepared by microwave irradiative cyclocondensation of N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide **1** (0.01 mol) with benzoic acid hydrazide **5a** (0.01 mol) for 4 min using few drops of absolute ethanol. The crude solid residue obtained was crystallized from ethanol.

6a: Yield 93%. m.p.174°C. IR: 3406 (NH), 1703 (C=O), 1651 (C=N), 1338 (C-N), 1186 cm^{-1} (N-N); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 6.78-8.14 (5H, m, Ar-H), 6.42 (1H, s, Pyrz-H), 6.26 (1H, s, Pyrm-H), 5.22 (1H, s, Pyrm-NH), 2.16 (9H, s, Pyrm-CH_3, Pyrz-CH_3); MS: *m/z* 292 (M^+-CH_3), 230 (M^+-C_6H_5), 202 (M^+-$C_6H_5.CO$), 200 (M^+-$(CH_3)_2.C_4HN_2$), 122 $(CH_3)_2.C_4HN_2.NH^+$), 77 ($C_6H_5^+$). Anal. Found: C, 65.75; H, 5.14; N, 22.68. Calcd for $C_{17}H_{17}N_5O$: C, 66.43; H, 5.58; N, 22.70%.

This reaction was extended to synthesize other compounds **6b-d** using different substituted acid hydrazides **5b-d**.

6b: Yield 92%. m.p.123°C. IR: 3400 (NH), 3331 (OH), 1714 (C=O), 1651 (C=N), 1332 (C-N), 1319 (C-O), 1193 cm^{-1} (N-N); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 11.66 (1H, bs, Ar-OH), 7.30-7.38 (4H, m, Ar-H), 6.30 (1H, s, Pyrz-H), 6.24 (1H, s, Pyrm-H), 5.28 (1H, s, Pyrm-NH), 2.20 (9H, s, Pyrm-CH_3, Pyrz-CH_3); MS: *m/z* 322 (M^+-H), 308 (M^+-CH_3), 230 (M^+-$C_6H_4.OH$), 216 (M^+-$(CH_3)_2.C_4HN_2$), 122 $(CH_3)_2.C_4HN_2.NH^+$), 107 $(CH_3)_2.C_4HN_2^+$). Anal. Found: C, 61.95; H, 5.16; N, 20.86. Calcd for $C_{17}H_{17}N_5O_2$: C, 63.15; H, 5.30; N, 21.66%.

6c: Yield 92%. m.p.183°C. Anal. Found: C, 62.05; H, 5.07; N, 27.28. Calcd for $C_{16}H_{16}N_6O$: C, 62.33; H, 5.23; N, 27.26%.

6d: Yield 85%. m.p.171°C. Anal. Found: C, 61.68; H, 5.11; N, 25.37. Calcd for $C_{17}H_{18}N_6O$: C, 63.34; H, 5.63; N, 26.07%. The reactions were monitored on silica gel-G plates by TLC.

Synthesis of N-(2-benzoyl-5-methyl-pyrazol-3-yl)-N-(4,6-dimethyl-pyrimidin-2-yl)- acetamide 7a

The mixture of (2-benzoyl-5-methyl-pyrazol-3-yl)-(4,6-dimethyl-pyrimidin-2-yl)-amine **6a** (0.01 mol) and acetic anhydride (0.01 mol) in glacial acetic acid (2 mL) was irradiated under microwave conditions for 45 sec. The reaction mixture was cooled and poured on a little crushed ice to afford N-(2-benzoyl-5-methyl-pyrazol-3-yl)-N-(4,6-dimethyl-pyrimidin-2-yl)- acetamide, it was crystallized from ethanol.

7a: Yield 88%. m.p.76°C. IR: 1702 (C=O), 1645 (C=N), 1340 (C-N), 1186 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 6.88-7.99 (5H, m, Ar-H), 6.47 (1H, s, Pyrz-H), 6.27 (1H, s, Pyrm-H), 2.53 (3H, s, CO-CH_3), 2.19 (9H, s, Pyrm-CH_3, Pyrz-CH_3). Anal. Found: C, 63.18; H, 4.97; N, 19.63. Calcd for $C_{19}H_{19}N_5O_2$: C, 65.32; H, 5.48; N, 20.04%.

This reaction was extended to synthesize other compounds **7b-d**.

7b: Yield 89%. m.p.139°C. IR: 1716 (C=O), 1628 (C=N), 1334 (C-N), 1187 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 6.80-7.38 (4H, m, Ar-H), 6.32 (1H, s, Pyrz-H), 6.29 (1H, s, Pyrm-H), 3.50 (3H, s, O-CO-CH_3), 2.53 (3H, s, N-CO-CH_3), 2.19 (9H, s, Pyrm-CH_3, Pyrz-CH_3). Anal. Found: C, 60.16; H, 4.96; N, 16.82. Calcd for $C_{21}H_{21}N_5O_4$: C, 61.91; H, 5.20; N, 17.19%.

7c: Yield 90%. m.p.176°C. Anal. Found: C, 61.04; H, 5.01; N, 23.6. Calcd for $C_{18}H_{18}N_6O_2$: C, 61.70; H, 5.18; N, 23.99%.

7d: Yield 82%. m.p.198°C. Anal. Found: C, 60.78; H, 5.31; N, 20.72. Calcd for $C_{21}H_{22}N_6O_3$: C, 62.06; H, 5.46; N, 20.68%. The reactions were monitored on silica gel-G plates by TLC.

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