SYNTHESIS OF PYRIMIDINE LINKED PYRAZOLE HETEROCYCLICS BY MICROWAVE IRRADIATIVE CYCLOCONDENSATION AND EVALUATION OF THEIR INSECTICIDAL AND ANTIBACTERIAL POTENTIAL

Pradip P. Deohate* and Kalpana A. Palaspagar

Department of Chemistry, Shri Radhakisan Laxminarayan Toshniwal College of Science, Akola-444001, India.

Abstract : The (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-substituted benzoyl/isonicotinoyl-pyrazol-3-yl)-amines have been prepared by cyclocondensation of N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide with substituted acid hydrazides under microwave. Synthesis of required 3-oxo butyramide was carried out by microwave irradiative condensation of 2-amino-4,6-dimethyl pyrimidine with ethyl acetoacetate. On acylation, the pyrimidine linked pyrazol-3-yl amines gave mono/di-acetyl derivatives. Structures of synthesized compounds were determined by IR, ^{1}H-NMR, mass spectroscopic studies and elemental analysis as well as chemical transformation. So as to establish the relation between structure and biological activity, synthesized compounds have been evaluated against Pseudococcidae insects for their insecticidal activity and also against some selected microorganisms for antibacterial potential.

Index Terms **- Pyrimidine, pyrazole, microwave, insecticidal, antimicrobial.**

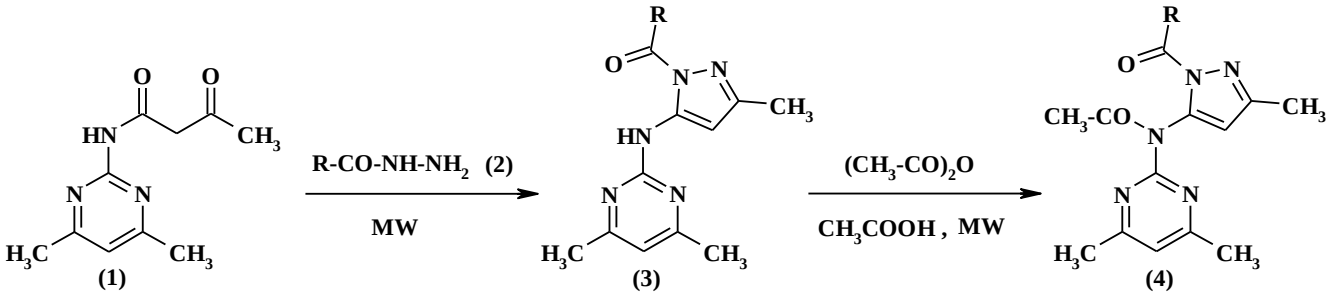
I. INTRODUCTION

In synthetic organic chemistry, the technique of microwave irradiation has so many advantages over the heating by conventional method[1]. For accelerating time consuming reactions as well as for high speed parallel synthesis of biologically active molecules, the technology of high density microwave irradiation has emerged as a most useful technique in organic synthesis[2,3]. In literature, pyrazole derivatives are well established and their activity covers the areas like antimicrobial, antiviral, antitubercular, antihistaminic, anticonvulsant and antidepressant[5-8] along with excellent analgesic and anti-inflammatory activities[9,10]. The pyrazoles linked with various other heterocyclics are found to contribute to different chemotherapeutic effects. Pyrazole derivatives were also reported to induce activities like antitumor, antileukemic and antiproliferative[11-14]. Investigations in chemistry revealed good about insecticidal activities related to pyrazole chromophore[15,16]. Different chemotherapeutic activities have been also ascribed to pyrimidine ring[7].

From the literature, it was found that N-1, C-3, C-4 positions are so much important for structure activity relationship. For better chemotherapeutic activities, C-3 should be linked to various heterocyclics[17]. With relevance to these findings, we reported herein synthesis of pyrimidine linked pyrazole heterocyclics by microwave irradiative cyclocondensation and evaluation of their insecticidal and antibacterial potential.

II. RESULTS AND DISCUSSION

Synthesis of N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide **(1)** was carried out by condensation of 2-amino-4,6-dimethyl pyrimidine (0.01 mole) with ethyl acetoacetate (0.01 mole) in solvent free condition using microwave. Then under microwave conditions, the compound **(1)** was reacted with substituted acid hydrazides **(2a-d)** (0.01 mole) in ethanolic medium to yield (4,6-dimethyl-pyrimidin-2-yl)-(5-methyl-2-substituted benzoyl/isonicotinoyl-pyrazol-3-yl)-amines **(3a-d)**. On acylation using acetic anhydride and acetic acid, the amines **(3a-d)** gave mono/di-acetyl derivatives **(4a-d) (Scheme I)**. It was found that, the microwave induced synthesis received high product yield with more purity and enhanced rate of reaction than conventional heating. Structures of synthesized compounds are fully supported by IR, ^{1}H-NMR, mass spectroscopy studies and showed single spots in TLC.



Where, 2a = Benzoic acid hydrazide, 2b = 2-Hydroxy-benzoic acid hydrazide
2c = Isonicotinic hydrazide, 2d = 4-Amino-benzoic acid hydrazide

Scheme I

2.1 Insecticidal Activity

For accessing the insecticidal properties of title compounds **(3a-d)**, the plant surface affected with insect species Pseudococcidae[16] (Mealy bug) was selected. Direct contact application method was used to determine the insecticidal activity[15,16]. The heavy infested plant parts affected with the insect pests were selected. Aqueous solutions of the test compounds **(3a-d)** of 2, 4, 6 ppm concentrations were prepared and applied by direct spray on differently labeled affected plant parts under similar conditions of temperature and sunlight. The solutions sprayed were about 2 ml at the time of single application[16]. From time to time, the results for mortality of insects were monitored for about 1 to 48 hours. To check any movement of body parts of insects, the simple microscope was used. The results showed that, in most of the cases, aqueous solutions of 2 ppm were sufficiently active against insect pests and no plant parts were affected due to the toxicity of compounds. The activities of test solutions were found to be good enough when compared with the activities of ethanol and hexane solutions.

2.2 Antibacterial Activity

Antibacterial potential evaluation of title compounds **(3a-d)** have been done using cup plate diffusion method (Kirby-Baur method)[18,19] using both gram-positive as well as gram-negative bacterial organisms strains i.e. *S. aureus, E. coli, B. subtilis, S. typhi* and *P. vulgaris*. Sensitivity plates have been seeded with a bacterial inoculums of $1x10^6$ CIU/mL. Wells of 10 mm diameter were loaded with 0.1 mL of test compound solution (1000 µg/mL) in DMF. Concentration of each test compound was 100 µg/mL. Inhibition zones were recorded using vernier caliper after incubation for 24 hr at 37^0C. It was found that the compounds **(3b)** and **(3c)** were highly active against *E. coli* and *B. subtilis* and moderately active against *S. aureus*. Most of the compounds were found inactive against *P. vulgaris* **(Table I)**. Minimum inhibitory concentration (MIC) was determined by the serial dilution technique[20] using nutrient broth medium. MIC values of compounds **(3b)** and **(3c)** against *B. subtilis* were found 76 and 70 µg/mL respectively.

Table I : Antibacterial activity

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

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

III. EXPERIMENTAL

Microwave induced syntheses were carried by using commercial microwave oven (1200 W). Melting points were checked using Veego, VMP-D digital melting point apparatus. Chemicals of AR grade were used. Recording of ^{1}H NMR spectra was done on a Bruker Avance-II 400 NMR spectrometer using the solvents $CDCl_3$, DMSO-d_6 and IR spectra on Perkin-Elmer spectrophotometer in the range 4000-400 cm^{-1}. Mass spectra were recorded on Jeol-JMC 300 spectrometer at 70 eV. Purity of synthesized compounds was checked by TLC on silica gel-G plates by visualizing the spots using iodine vapours.

3.1 Preparation of N-(4,6-Dimethyl-pyrimidin-2-yl)-3-oxo butyramide (1).

Synthesis of N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide **(1)** was done by irradiating the mixture of 2-amino-4,6-dimethyl pyrimidine (0.01 mole) and ethyl acetoacetate (0.01 mole) under microwave for 3 min. The crude solid residue obtained was crystallized from acetone, **(1)** (78%), m.p. 128^0C (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%); 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]. The progress of reaction was monitored by TLC.

3.2 Preparation of (2-Benzoyl-5-methyl-pyrazol-3-yl)-(4,6-dimethyl-pyrimidin-2-yl)-amine (3a).

Preparation of (2-benzoyl-5-methyl-pyrazol-3-yl)-(4,6-dimethyl-pyrimidin-2-yl)-amine **(3a)** was carried out by microwave induced cyclocondensation of N-(4,6-dimethyl-pyrimidin-2-yl)-3-oxo butyramide **(1)** (0.01 mole) with benzoic acid hydrazide **(2a)** (0.01 mole) using few drops of absolute ethanol for 4 min. The crude product obtained was crystallized from ethanol, **(3a)** (93%), m.p. 174^0C (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%); IR: 3406 (NH), 1703 (C=O), 1651 (C=N), 1338 (C-N), 1186 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 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 ($C_6H_5^+$). The reaction was further extended to prepare compounds **(3b-d)** using ubstituted acid hydrazides **(2b-d)**: **(3b)** (92%), m.p. 123^0C (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%); IR: 3400 (NH), 3331 (OH), 1714 (C=O), 1651 (C=N), 1332 (C-N), 1349 (C-O), 1193 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 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 $(CH_3)_2.C_4HN_2^+$); **(3c)** (92%), m.p. 183^0C (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%); **(3d)** (85%), m.p. 171^0C (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 progress of reaction was monitored by TLC.

3.3 Preparation of N-(2-Benzoyl-5-methyl-pyrazol-3-yl)-N-(4,6-dimethyl-pyrimidin-2-yl)-acetamide (4a).

Under microwave conditions, the mixture of (2-benzoyl-5-methyl-pyrazol-3-yl)-(4,6-dimethyl-pyrimidin-2-yl)-amine **(3a)** (0.01 mole) and acetic anhydride (0.01 mole) in glacial acetic acid (2 ml) was irradiated for 45 sec. It was cooled and poured on a little crushed ice to give N-(2-benzoyl-5-methyl-pyrazol-3-yl)-N-(4,6-dimethyl-pyrimidin-2-yl)-acetamide. It was then crystallized from ethanol, **(4a)** (88%), m.p. 76^0C (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%); IR: 1702 (C=O), 1645 (C=N), 1340 (C-N), 1186 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 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). The reaction was further extended to prepare compounds **(4b-d)**: **(4b)** (89%), m.p. 139^0C (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%); IR: 1716 (C=O), 1628 (C=N), 1334 (C-N), 1187 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 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); **(4c)** (90%), m.p. 176^0C (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%); **(4d)** (82%), m.p. 198^0C (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 progress of reaction was monitored by TLC.

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