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Microwave Irradiative Synthesis of Triazine Substituted Pyrazoles and Study of Antitubercular and Antimicrobial Activities

PRADIP P. DEOHATE* and ROSHANI S. MULANI

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

*Corresponding author: E-mail: pradip222091@yahoo.co.in

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Microwave irradiative synthesis of triazine substituted pyrazoles *i.e.* (4-benzylideneamino-6-methyl-[1,3,5]-triazin-2-yl)-(5-methyl-2-substituted benzoyl/isonicotinoyl/cinnamoyl-pyrazol-3-yl)-amines have been achieved by the cyclocondensation of N-(4-benzylideneamino-6-methyl-[1,3,5]-triazin-2-yl)-3-oxo butyramide with substituted acid hydrazides. Synthesis of required butyramide was done by reacting 2,4-diamino-6-methyl-[1,3,5]-triazine with benzaldehyde and then condensing the product with ethyl acetoacetate. Structural investigation of synthesized compounds has been done by chemical transformation, elemental analysis and IR, ^{1}H NMR, mass spectral studies. Study of antitubercular and antimicrobial activity of title compounds against some selected Gram-positive and Gram-negative microorganisms was performed to establish the relationship between structure and activity of compound.

Keywords: Microwave synthesis, Triazine, Pyrazole, Antitubercular, Antimicrobial study.

INTRODUCTION

Microwave irradiation technique has so many advantages over the conventional heating in synthesis of organic compounds [1]. High density microwave irradiation technique can be used for parallel high speed synthesis of number of bioactive compounds [2]. The pharmacophore pyrazole has different practical applications in synthetic organic chemistry [3]. Pyrazole fused heterocyclics have been widely used in pesticides and medicines [4]. Literature has been enriched with the progressive findings about the synthesis and activities of pyrazole which covers the domains like antitubercular [5,6], antitumor [7], antimicrobial [8], antipyretic [9], analgesic [10], ulcerogenic [11], antiinflammatory [12] and anticancer [13]. It was observed that positions N-1, C-3, C-4 are much more important for the study of relationship between structure and activity of compound and position C-3 should be linked to different heterocycles for better chemotherapeutic activities [14]. The presence of two bioactive molecules within a single compound increases the antimicrobial activity of that compound. The most common method used for synthesis of pyrazoles is the reaction of 1,3-dicarbonyl, oxo-amide, hydrazine hydrate, ester using suitable catalyst [15]. The double nucleophilic character of hydrazine for reaction with each carbonyl group of 1,3-diketone requires long period with high temperature [16].

With these observations, we report herein the synthesis of triazine substituted pyrazoles at C-3 position *i.e.* (4-benzylideneamino-6-methyl-[1,3,5]-triazin-2-yl)-(5-methyl-2-substituted benzoyl/isonicotinoyl/cinnamoyl-pyrazol-3-yl)-amines (**5a-f**) by microwave irradiation technique without the use of any catalyst. So as to establish the relationship between structure and activity of compound, all synthesized compounds were studied for their antitubercular and antimicrobial activity against selected microorganisms.

EXPERIMENTAL

All reactions were performed by the microwave irradiation using commercially available microwave oven. Chemicals used were of A.R. grade. Purity of the compounds was checked on silica gel-G plates by TLC and spots were visualized by iodine vapours. Melting points were recorded using Veego, VMP-D digital melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded using $CDCl_3$ and DMSO-d_6 as solvents and TMS as internal standard on Bruker Avance-II 400 NMR spectrometer. IR spectra were recorded in the range 4000-400

cm^{-1} on Perkin-Elmer spectrophotometer. Mass spectral measurements were carried out by EI method at 70 eV on Jeol-JMC 300 spectrometer.

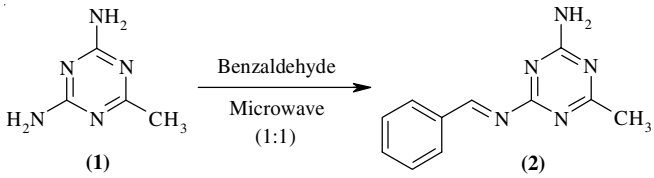
Preparation of 2-amino-4-benzylideneamino-6-methyl-[1,3,5]-triazine (2): The compound 2-amino-4-benzylideneamino-6-methyl-[1,3,5]-triazine (**2**) was prepared by irradiating the mixture of 2,4-diamino-6-methyl-[1,3,5]-triazine (**1**) (0.01 mol) and benzaldehyde (0.01 mol) for 3.5 min using microwave under solvent free conditions. The crude solid mass obtained was crystallized form hot ethanol, (95 %), m.p. 146 °C (Found: C, 60.02; H, 5.19; N, 32.81. Calcd. for $C_{11}H_{11}N_5$: C, 61.97; H, 5.16; N, 32.86 %); ^{1}H NMR: δ ($CDCl_3$ + DMSO-d_6) 7.94 (1H, s, Ar-CH=N), 7.90 (2H, s, Trz-NH_2), 6.90-7.58 (5H, m, Ar-H), 2.27 (3H, s, Trz-CH_3) [17,18]. Preparation of the compound was monitored by TLC using silica gel-G plates and the pure compound was separated using the technique of column chromatography.

Preparation of N-(4-Benzylideneamino-6-methyl-[1,3,5]-triazin-2-yl)-3-oxo butyramide (3): The compound N-(4-benzylideneamino-6-methyl-[1,3,5]-triazin-2-yl)-3-oxo butyramide (**3**) was prepared by treating 2-amino-4-benzylideneamino-6-methyl- [1,3,5]-triazine (**2**) (0.01 mol) with ethyl acetoacetate (0.01 mol) for 3 min by microwave irradiation, the resulting solid was crystallized from hot ethanol, (92 %), m.p. 128 °C (Found: C, 59.98; H, 5.08; N, 23.52. Calcd. for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.55 %); IR (KBr, ν_{max}, cm^{-1}): 3329 (NH), 1681 (C=O), 1546 (C=N), 1325 (C-N); ^{1}H NMR: δ ($CDCl_3$ + DMSO-d_6) 7.94 (1H, s, Ar-CH=N), 7.93 (1H, s, Trz-NH), 7.38-7.52 (5H, m, Ar-H), 3.46 (2H, s, CO-CH_2-CO), 2.21 (3H, s, Trz-CH_3), 2.19 (3H, s, CO-CH_3). Completion of the reaction was monitored with TLC.

Preparation of (2-benzoyl-5-methyl-pyrazol-3-yl)-(4-benzylideneamino-6-methyl-[1,3,5]-triazin-2-yl)-amine (5a): The compound (2-benzoyl-5-methyl-pyrazol-3-yl)-(4-benzylideneamino-6- methyl-[1,3,5]-triazin-2-yl)-amine (**5a**) was prepared by microwave irradiative cyclocondensation of mixture of N-(4-benzylideneamino-6-methyl-[1,3,5]-triazin-2-yl)- 3-oxo-butyramide (**3**) (0.01 mol) and benzoic acid hydrazide (**4a**) (0.01 mol) for 35 s. The crude solid residue obtained was crystallized from hot ethanol, **5a** (92 %), m.p. 86 °C (Found: C, 66.12; H, 4.77; N, 24.44. Calcd. for $C_{22}H_{19}N_7O$: C, 66.49; H, 4.82; N, 24.67 %); IR (KBr, ν_{max}, cm^{-1}): 3298 (NH), 1683 (C=O), 1544 (C=N), 1323 (C-N), 1176 (N-N); ^{1}H NMR: δ ($CDCl_3$ + DMSO-d_6) 7.95 (1H, s, Ar-CH=N), 7.28-7.94 (10H, m, Ar-H), 6.56 (1H, s, Pyrz-H), 4.24 (1H, s, Trz-NH), 2.06 (6H, s, Pyrz-CH_3, Trz-CH_3); ^{13}C NMR: δ ($CDCl_3$ + DMSO-d_6) 111.76-140.94 (19C, m, Ar-C, Pyrz-C, Trz-C, CH=N), 156.37 (1C, s, CO), 20.79, 20.56 (2C, s, Pyrz-CH_3, Trz-CH_3); MS: *m/z* 397 (M^+), 382 (M^+-CH_3), 293 (M^+-C_6H_5.CH=N), 292 (M^+-C_6H_5CO), 212 (M^+-$CH_3C_6H_5COC_3HN_2$), 200 ($CH_3C_6H_5CO$ $C_3HN_2NH^+$), 105 ($C_6H_5.CO^+$), 77 ($C_6H_5^+$). This reaction was extended to synthesize other compounds (**5b-f**) using different substituted acid hydrazides (**4b-f**): **5b** (90 %), m.p. 72 °C (Found: C, 66.81; H, 5.11; N, 23.69. Calcd. for $C_{23}H_{21}N_7O$: C, 67.14; H, 5.14; N, 23.83 %); IR (KBr, ν_{max}, cm^{-1}): 3296 (NH), 1681 (C=O), 1541 (C=N), 1325 (C-N), 1170 (N-N); ^{1}H NMR: δ ($CDCl_3$ + DMSO-d_6) 7.94 (1H, s, Ar-CH=N), 7.07-7.83 (9H, m, Ar-H), 6.57 (1H, s, Pyrz-H), 4.45 (1H, s, Trz-NH), 2.34 (3H, s, Ar-CH_3), 2.06 (6H, s, Pyrz-CH_3, Trz-CH_3); ^{13}C NMR: δ ($CDCl_3$ + DMSO-d_6) 111.76-140.29 (19C, m, Ar-C, Pyrz-C, Trz-C, CH=N), 156.91 (1C, s, CO), 28.05 (3C, s, Ar-CH_3, Pyrz-CH_3, Trz-CH_3); MS: *m/z* 410 (M^+-H), 396 (M^+-CH_3), 320 (M^+-$CH_3C_6H_4$), 307 (M^+-C_6H_5CH=N), 292 (M^+-$CH_3C_6H_4CO$), 199 ($CH_3CH_3C_6H_4COC_3HN_2^+$), 119 ($CH_3C_6H_4CO^+$), 91 ($CH_3C_6H_4^+$); **c** (95 %), m.p. 94 °C (Found: C, 63.62; H, 4.50; N, 23.65. Calcd. for $C_{22}H_{19}N_7O_2$: 63.91; H, 4.63; N, 23.71 %); IR (KBr, ν_{max}, cm^{-1}): 3500 (OH), 3379 (NH), 1680 (C=O), 1548 (C=N), 1323 (C-N), 1168 (N-N); ^{1}H NMR: δ ($CDCl_3$ + DMSO-d_6) 7.96 (1H, s, Ar-CH=N), 6.98-7.57 (9H, m, Ar-H), 6.76 (1H, s, Ar-OH), 6.73 (1H, s, Pyrz-H), 4.03 (1H, s, Trz-NH), 2.14 (6H, s, Pyrz-CH_3, Trz-CH_3); ^{13}C NMR: δ ($CDCl_3$ + DMSO-d_6) 121.04-134.07 (19C, m, Ar-C, Pyrz-C, Trz-C, CH=N), 156.41 (1C, s, CO), 13.72 (2C, s, Pyrz-CH_3, Trz-CH_3); **d** (90 %), m.p. 170 °C (Found: C, 63.74; H, 4.80; N, 27.07. Calcd. for $C_{22}H_{20}N_8O$: C, 64.07; H, 4.89; N, 27.17 %); IR (KBr, ν_{max}, cm^{-1}): 3327, 3097 (NH), 1680 (C=O), 1543 (C=N), 1323 (C-N), 1170 (N-N); ^{1}H NMR: δ ($CDCl_3$ + DMSO-d_6) 7.39-8.89 (10H, m, Ar-H, Ar-CH=N), 5.88 (1H, s, Pyrz-H), 3.43 (1H, s, Trz-NH), 2.50 (2H, s, Ar-NH_2), 2.20 (6H, s, Pyrz-CH_3, Trz-CH_3); ^{13}C NMR: δ ($CDCl_3$ + DMSO-d_6) 112.59-128.05 (19C, m, Ar-C, Pyrz-C, Trz-C, CH=N), 156.66 (1C, s, CO), 22.59 (2C, s, Pyrz-CH_3, Trz-CH_3); **e** (88 %), m.p. 118 °C (Found: C, 63.19; H, 4.52; N, 28.08. Calcd. for $C_{21}H_{18}N_8O$: C, 63.31; H, 4.55; N, 28.12 %); **f** (85 %), m.p. 104 °C (Found: C, 67.86; H, 5.01; N, 23.02. Calcd. for $C_{24}H_{21}N_7O$: C, 68.07; H, 5.00; N, 23.15 %). The reaction was monitored on silica gel-G plates by TLC.

RESULTS AND DISCUSSION

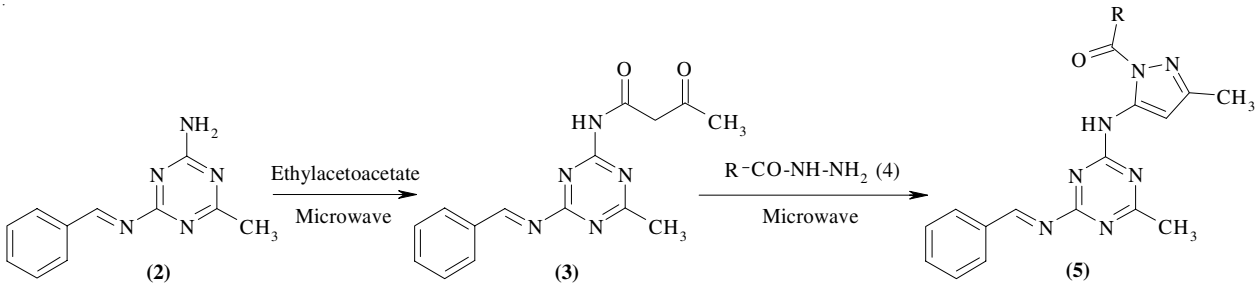
The compound 2-amino-4-benzylideneamino-6-methyl-[1,3,5]-triazine (**2**) was synthesized by reacting 2,4-diamino-6-methyl-[1,3,5]-triazine (**1**) (0.01 mol) with benzaldehyde (0.01 mol) by microwave irradiation for 3.5 min under solvent free conditions. Completion of the reaction was monitored with TLC and technique of column chromatography was used to separate the pure compound (**Scheme-I**).



(1) **(2)**

Scheme-I

Compound (**2**) was then treated with ethyl acetoacetate (0.01 mol) using microwave for 3 min to give the compound N-(4-benzylideneamino-6-methyl-[1,3,5]- triazin-2-yl)-3-oxo butyramide (**3**) which on microwave irradiative cyclocondensation with substituted acid hydrazides **(4a-f)** (0.01 mol) for 25 to 45 s afforded (4-benzylideneamino-6-methyl-[1,3,5]-triazin-2-yl)-(5-methyl-2-substituted benzoyl/isonicotinoyl/cinnamoyl-pyrazol-3-yl)-amines (**5a-f**) (**Scheme-II**). It was observed that microwave irradiative reactions have high product yield, purity and enhanced reaction rates. IR, ^{1}H NMR and mass spectral investigation of synthesized compounds fully supported the structures and showed single spots in TLC.



Where, 4a = Benzoic acid hydrazide 4c = 2-Hydroxy-benzoic acid hydrazide 4e = 4-Pyridine hydrazide
4b = 3-Methyl-benzoic acid hydrazide 4d = 4-Amino-benzoic acid hydrazide 4f = 3-Phenyl-acrylic acid hydrazide

Scheme-II

Antitubercular activity: The compounds (**5a-f**) have been studied for their *in vitro* antitubercular activity [19] by microplate alamar blue assay (MABA) method for direct determination of minimum inhibitory concentration (MIC) against *M. tuberculosis*. Test compounds were dissolved in 10 % (v/v) DMSO at a concentration of 10 mM. Two fold serial dilutions of compounds were made in Middle brook 7H9 medium supplemented with 10 % (v/v) ADC, in well plates (Nunc) in duplicate. An inoculums of 10^5 CFU mL^{-1} was prepared and 200 µL was added per well. For each assay, growth controls containing no drug and a sterile control without bacteria were also prepared. The plates were incubated at 37 °C for 5 days before adding 20 µL of sterile 0.01 % resazurin to the wells and then incubated further for 24 h at 37 °C. The growth of bacteria was indicated by a change in colour from blue to pink *i.e.* oxidized state to reduced state. The compounds showing MIC at 50 µM were further screened for CFU determination using agar dilution method. Serial dilutions of compounds prepared in 0.1 mL 10 % (v/v) DMSO were added to each well of well plates (Nunc). Then 1.9 mL MB7H10 agar medium supplemented with 10 % (v/v) OADC were poured to respective wells and allowed to solidify at room temperature. For positive control, streptomycin and rifampin were dissolved in water, filtered, sterilized and used in 6 and 2 µg mL^{-1} respectively. Solution 10 µL was inoculated in each well on solidified agar medium and incubated for 4 weeks at 37 °C to record the growth. The compounds (**5c**), (**5d**) and (**5e**) showed promising activity against *M. tuberculosis*. MIC values of compounds (**5d**) and (**5e**) have been found to be 6.25 µM and of compound (**5c**) was found to be 12.5 µM (Table-1).

TABLE-1
ANTITUBERCULAR ACTIVITY OF COMPOUNDS (**5a-f**)

Compd.	Concentration (µM)				
	3.125	6.25	12.5	25	50
5a	IA	IA	IA	IA	IA
5b	IA	IA	IA	IA	IA
5c	IA	IA	A	A	A
5d	IA	A	A	A	A
5e	IA	A	A	A	A
5f	IA	IA	IA	IA	IA

A = Active, IA = Inactive

Antimicrobial activity: The compounds (**5a-f**) have been studied for their antibacterial activity by using cup plate diffusion method [20,21]. The bacterial organisms having both Gram-positive and Gram-negative strains *i.e. S. aureus, E. coli, S. typhi, P. vulgaris* and *B. subtilis* were used. Sensitivity plates were seeded with a bacterial innoculum of 1×10^6 CIU mL^{-1} and each well of diameter 10 mm was loaded with 0.1 mL of test compound solution (1000 µg mL^{-1}) in DMF, so that concentration of each test compound was 100 µg mL^{-1}. After incubation for 24 h at 37 °C, the zones of inhibition were recorded using vernier caliper. It was found that the compounds (**5d**) and (**5e**) were highly active against *S. aureus* and *E. coli* and moderately active against *S. typhi* and *P. vulgaris*. Compounds (**5c**) was moderately active against *S. aureus* and *E. coli*. Majority of the compounds were found to be inactive against *B. subtilis* (Table-2). Serial dilution technique [22] using nutrient broth medium was used to determine the MIC values. For compounds (**5d**) and (**5e**) MIC values were found to be 50 and 65 µg mL^{-1} respectively against *S. aureus* and 60 and 45 µg mL^{-1} respectively against *E. coli*.

TABLE-2
ANTIMICROBIAL ACTIVITY OF COMPOUNDS (**5a-f**)

Compd.	Microorganisms				
	S. aureus	*E. coli*	*S. typhi*	*P. vulgaris*	*B. subtilis*
5a	+	+	–	–	–
5b	+	+	+	+	–
5c	++	++	+	+	+
5d	+++	+++	++	++	+
5e	+++	+++	++	++	+
5f	+	+	+	+	–

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

Conclusion

In present communication synthesis of triazine substituted pyrazoles (**5a-f**) have been reported by microwave irradiative cyclocondensation. This method was found to the simple, efficient and completed within a very short period of time with good yield. Study of antitubercular and antimicrobial activity of synthesized compounds showed that, compounds (**5c**), (**5d**) and (**5e**) have promising activity against *M. tuberculosis* and compounds (**5d**) and (**5e**) were highly active against *S. aureus* and *E. coli*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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