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 heterocycles 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, o xo-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 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, 1H 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.
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 %). \[^{1}H\text{NMR}: \delta (\text{CDCl}_3 + \text{DMSO-d}_6) 7.94 (1\text{H}, \text{s, Ar-CH=N}), 7.90 (2\text{H}, \text{s, Trz-NH}), 6.90-7.58 (5\text{H}, \text{m, Ar-H}), 2.27 (3\text{H}, \text{s, Trz-CH}_3)[17,18]. \] 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\textsubscript{15}H\textsubscript{15}N\textsubscript{5}O\textsubscript{2}: C, 60.60; H, 5.09; N, 23.55 %); IR (KBr, \text{cm}–\text{1}): 3298 (NH), 1681 (C=O), 1541 (C=N), 1323 (C-N); 1\text{H} \text{NMR}: \delta (\text{CDCl}_3 + \text{DMSO-d}_6) 7.94 (1\text{H}, \text{s, Ar-CH=N}), 7.90 (2\text{H}, \text{s, Trz-NH}), 6.90-7.58 (5\text{H}, \text{m, Ar-H}), 2.27 (3\text{H}, \text{s, Trz-CH}_3)[17,18]. Preparation 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 cyclodehydration 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\textsubscript{22}H\textsubscript{21}N\textsubscript{7}O: C, 66.12; H, 4.77; N, 24.44. Calcd. for C\textsubscript{22}H\textsubscript{21}N\textsubscript{7}O\textsubscript{2}: 63.91; H, 4.63; N, 23.71 %); IR (KBr, \text{cm}–\text{1}): 3500 (OH), 3379 (NH), 1680 (C=O), 1548 (C=N), 1456 (C=O), 1417 (C=O), 1389 (C=N), 1324 (C-N), 119 (CH\textsubscript{3}C\textsubscript{6}H\textsubscript{4}CO+), 91 (CH\textsubscript{3}C\textsubscript{6}H\textsubscript{4}NH+), 77 (C\textsubscript{6}H\textsubscript{5}C\textsubscript{6}H\textsubscript{5}), 63 (C\textsubscript{6}H\textsubscript{5}), 58 (C\textsubscript{6}H\textsubscript{5}), 45 (C\textsubscript{6}H\textsubscript{5}), 36 (C\textsubscript{6}H\textsubscript{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\textsubscript{22}H\textsubscript{21}N\textsubscript{7}O\textsubscript{2}: C, 67.14; H, 5.14; N, 23.83 %); IR (KBr, \text{cm}–\text{1}): 3296 (NH), 1681 (C=O), (Found: C, 66.81; H, 5.11; N, 23.69. Calcd. for C\textsubscript{22}H\textsubscript{21}N\textsubscript{7}O\textsubscript{2}: C, 67.14; H, 5.14; N, 23.83 %); IR (KBr, \text{cm}–\text{1}): 3296 (NH), 1681 (C=O), 1541 (C=N), 1325 (C-N), 1170 (N-N); 1\text{H} \text{NMR}: \delta (\text{CDCl}_3 + \text{DMSO-d}_6) 7.94 (1\text{H}, \text{s, Ar-CH=N}), 7.70-7.83 (9\text{H}, \text{m, Ar-H}), 6.57 (1\text{H}, \text{s, Pyrz-H}), 4.45 (1\text{H}, \text{s, Trz-NH}), 2.34 (3\text{H}, \text{s, Ar-CH}_3), 2.06 (6\text{H}, \text{s, Pyrz-CH}_2, \text{Trz-CH}_2); 1\text{C} \text{NMR}: \delta (\text{CDCl}_3 + \text{DMSO-d}_6) 111.76-140.29 (19\text{C}, \text{m, Ar-C, Pyrz-C, Trz-C}, \text{CH}=\text{N}), 156.91 (1\text{C}, \text{s, CO}), 28.05 (3\text{C}, \text{s, Ar-CH}_3, \text{Pyrz-CH}_3, \text{Trz-CH}_3); \text{MS}: \text{m/z} (\text{M}^+): 397 (3\text{M}^+), 293 (2\text{M}^+), 292 (\text{M}^+, \text{C}_6\text{H}_4\text{CH}_3, \text{N}), 292 (\text{M}^+, \text{C}_6\text{H}_4\text{CO}), 212 (\text{M}^+, \text{C}_6\text{H}_4\text{CO}, \text{CH}_3\text{CO}), 200 (\text{CH}_3\text{CO}), 105 (\text{C}_6\text{H}_4\text{CO}_2), 77 (\text{C}_6\text{H}_4^\text{+}). This compound was monitored by TLC using silica gel-G plates and the pure compound was separated using the technique of column chromatography.

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 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).

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 cyclodehydration 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\text{H} \text{NMR} and mass spectral investigation of synthesized compounds fully supported the structures and showed single spots in TLC.
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 inoculum 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 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 sensitive plates, MB7H10 broth medium was used to determine 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-1

<table>
<thead>
<tr>
<th>Compd.</th>
<th>MIC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>1A</td>
</tr>
<tr>
<td>5b</td>
<td>1A</td>
</tr>
<tr>
<td>5c</td>
<td>1A</td>
</tr>
<tr>
<td>5d</td>
<td>1A</td>
</tr>
<tr>
<td>5e</td>
<td>1A</td>
</tr>
<tr>
<td>5f</td>
<td>1A</td>
</tr>
</tbody>
</table>

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 inoculum of 1 × 10^8 CFU 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.

Conclusion

In present communication synthesis of triazine substituted pyrazoles (5a-f) have been reported by microwave irradiative cyclocondensation. This method was found to be 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.

Acknowledgements

The authors are thankful to Director, S.A.I.F, Punjab University, Chandigarh and C.S.I.R. Central Drug Research
Institute, Lucknow, India for providing analytical, spectral data and antitubercular activity analysis.

CONFLICT OF INTEREST

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

REFERENCES

15. L. Knorr, *Chem. Ber.*, 17, 546 (1884); https://doi.org/10.1002/chb.188401701152.