Pyrazole and pyrimidine derivatives are potential bioactive molecules. Indisputably, pyrazolopyrimidine derivatives which are designed by heterocyclic fusion of this two heterocyclic compounds, have high impact in the field of medical sciences with an extensive array of biological activities. 

Nowadays, medicinal chemists are fascinated by fused pyrimidine derivatives due to their potential biological activities. It is well-known that several fused pyrimidines such as thienopyrimidines, pyrrolopyrimidines, thiazolopyrimidines, and imidazopyrimidines have shown good antimicrobial activity. Based on the concept of bioisosterism, our intention is to replace five member ring system such as thiophene, pyrrole, and imidazole by its bioisostere pyrazole ring system and to test the effect of this modification on its effectiveness as an antimicrobial agent.

Various synthetic approaches are well documented for the synthesis of pyrazolo[3,4-d]pyrimidines. However, conversion of 5-amino-1-substituted-1H-pyrazole-4-carbonitrile to pyrazolo[3,4-d]pyrimidine requires two steps with limitations of extreme reaction conditions, longer reaction times, and low yields.

At present, microwave assisted synthesis become the most popular as a contemporary technique for synthesis of medicinal compounds due to several advantages like higher yields, attainment to higher temperature, prompt synthesis of organic compounds, and better workup procedures. Therefore, present research has been considered worthy to synthesize some new pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives from ortho-amino esters of pyrazole by conventional and microwave irradiation method.

**ABSTRACT**

**Objectives:** The aim of the present work was to synthesize a novel series of pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives and evaluate their *in vitro* antimicrobial activity. **Methods:** Cyclization of an ortho-amino ester of 1-(2,4-dinitrophenyl)pyrazole with various aliphatic/aromatic nitriles under different reaction conditions such as conventional and microwave assisted synthesis, provided pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives. All the synthesized compounds were evaluated *in vitro* for their antimicrobial activity against selected bacteria and fungi by agar well diffusion method. **Results:** All newly synthesized compounds were characterized using spectral and elemental analysis. Compounds 2e, 2f, and 2g showed significant antimicrobial activity as compared to standard drugs used. **Conclusion:** The newly synthesized compounds could be useful templates for the design and optimization of more active analogs as a possible antimicrobial agent.

**KEY WORDS:** Antimicrobial activity, conventional synthesis, microwave assisted synthesis, pyrazolo[3,4-d]pyrimidin-4(5H)-one
Materials and Methods

Precoated silica gel 60G F254 plates (Merck, Mumbai, India) were utilized for preparative and analytical thin layer chromatography (TLC). Chemline CL.726 melting point apparatus was used for measurement of melting point in an open capillary tube and are uncorrected. The IR spectra (ν, cm⁻¹) were recorded in KBr on Shimadzu FT-IR 157 spectrophotometer. ¹H and ¹³C NMR (δ, ppm) spectra were recorded in DMSO-d₆ on Bruker advance III NMR spectrophotometer operating at 500 and 125 MHz respectively and chemical shifts are reported in ppm with respect to tetramethylsilane as internal standard; coupling constants (J values) are given in Hertz. Mass spectra were determined on Shimadzu GC-MSQP 2010 mass spectrometer. Elemental analysis was performed with Elementar Vario EL III analyzer for C, H, N. Commercially available reagents and solvents (LR grade) were used without purification. Compound 1 was prepared according to the reported method.¹¹⁴

General synthetic procedure for preparation of pyrazolo[3,4-d]pyrimidine-4(5H)-one derivatives (2a–2j)

Conventional method

Compound 1 (10 mM) and different aliphatic/aromatic nitriles (15 mM) in sufficient quantity of dioxane were thoroughly mixed in a round bottom flask. A flow of dry HCl gas was passed through the reaction mixture. After 6 h, reaction mixture was poured on crushed ice and basified with 5% sodium hydroxide solution. The crude precipitates thus obtained were filtered, dried, and recrystallized from suitable solvent to give targeted compounds in good to excellent yields.

Microwave assisted method

Compound 1 (1 mM), potassium tert-butoxide (0.1 mM) and different aliphatic/aromatic nitriles (1.5 mM) were taken in a 5 mL glass vial. The reaction mixture was capped and irradiated in microwave oven at the power of 960 W (CEM, discover microwave lab station operating at 2450 MHz under continuous internal temperature control) for 2.5–3.5 min. After completion of reaction (monitored using TLC), the mixture was poured on ice-cold water (50 mL). The reaction mixture was neutralized by dil. HCl. Precipitated crude product was filtered, dried, and recrystallized from suitable solvent to give targeted compounds in good to excellent yields.

6-methyl-1-(2,4-dinitrophenyl)-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-one (2a)

Yellow crystals, IR (KBr, cm⁻¹): 3284, 3071, 2969, 2910, 1649, 1578; ¹H NMR (DMSO-d₆, δ): 7.52-7.64 (m, 5H, aro. CH of phenyl), 7.73 (s, 1H, pyrazole CH), 8.38 (d, 1H, aro. CH at 6', J = 8.5 Hz), 8.75 (d, 1H, aro. CH at 5', J = 8.9 Hz), 8.96 (s, 1H, aro. CH at 3'), 12.5 (s, 1H, pyrimidine NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆, δ): 108.23 (C-3a), 120.56 (C-3’), 124.39 (C-6’), 128.1 (C-3’ and C-5’), 128.9 (C-3’ and C-5’), 130.1 (C-4’), 132.3 (C-1’), 137.57 (C-1’), 142.12 (C-3), 142.82 (C-7a), 146.26 (C-4’), 158.6 (C-6), 161.0 (C=O); MS: m/z 378 (M⁺).

1-(2,4-dinitrophenyl)-6-phenyl-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-one (2d)

Yellow crystals, IR (KBr, cm⁻¹): 3284, 3071, 2969, 2910, 1649, 1578; ¹H NMR (DMSO-d₆, δ): 7.44 (d, 1H, benzyl CH), 7.41-7.50 (m, 5H, aro. CH of phenyl), 7.74 (s, 1H, pyrazole CH), 8.39 (d, 1H, aro. CH at 6', J = 8.7 Hz), 8.74 (d, 1H, aro. CH at 5', J = 8.7 Hz), 8.96 (s, 1H, aro. CH at 3'), 12.5 (s, 1H, pyrimidine NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆, δ): 108.23 (C-3a), 120.56 (C-3’), 124.39 (C-6’), 128.1 (C-3’ and C-5’), 128.9 (C-3’ and C-5’), 130.1 (C-4’), 132.3 (C-1’), 137.57 (C-1’), 142.12 (C-3), 142.82 (C-7a), 146.26 (C-4’), 158.6 (C-6), 161.0 (C=O); MS: m/z 378 (M⁺).

1-(2,4-dinitrophenyl)-6-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-one (2e)

Yellow crystals, IR (KBr, cm⁻¹): 3284, 3071, 2969, 2910, 1649, 1575; ¹H NMR (DMSO-d₆, δ): 7.75 (s, 1H, pyrazole CH), 7.86 (d, 2H, aro. CH at 2’ and 6’), J = 9.1 Hz), 8.24 (d, 1H, aro. CH at 6’, J = 8.5 Hz), 8.34 (d, 2H, aro. CH at 3’ and 5’, J = 9.1 Hz), 8.75 (d, 1H, aro. CH at 5’, J = 8.6 Hz), 8.96 (s, 1H, aro. CH at 3’), 12.9 (s, 1H, pyrimidine NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆, δ): 108.23 (C-3a), 120.56 (C-3’), 124.1 (C-3’ and C-5’), 124.39 (C-6’), 128.3 (C-3’ and C-5’), 128.9 (C-3’ and C-5’), 130.1 (C-4’), 132.3 (C-1’), 137.57 (C-1’), 142.12 (C-3), 142.82 (C-7a), 146.26 (C-4’), 158.6 (C-6), 161.0 (C=O); MS: m/z 378 (M⁺).
Yellow crystals, IR (KBr, cm⁻¹): 3315, 3064, 2978, 2910, 1660, 1571; ¹H NMR (DMSO-d₆, δ): 7.70 (d, 2H, aro. CH at 2° and 6°), 7.72 (s, 1H, pyrazole CH), 8.14 (d, 2H, aro. CH at 3° and 5°), 8.35 (d, 1H, aro. CH at 6'), J = 9.5 Hz), 8.76 (s, 1H, aro. CH at 5'), J = 8.6 Hz), 8.95 (s, 1H, aro. CH at 3'), 12.86 (s, 1H, pyrimidine NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆, δ): 108.23 (C-3a), 120.56 (C-3), 124.65 (C-6), 128.30 (C-5), 128.90 (C-3° and C-5°), 129.3 (C-2° and C-6°), 130.5 (C-1°), 135.7 (C-4°), 137.57 (C-1'), 142.12 (C-3), 142.82 (C-7a), 142.96 (C-2'), 146.26 (C-4'), 158.6 (C-6), 161.0 (C=O); MS: m/z 412 (M⁺), 414 (M⁺²).

6-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (2g)

Yellow crystals, IR (KBr, cm⁻¹): 3322, 3064, 2978, 2910, 1660, 1571; ¹H NMR (DMSO-d₆, δ): 7.70 (d, 2H, aro. CH at 2° and 6°), J = 7.2 Hz), 7.74 (s, 1H, pyrazole CH), 8.20 (d, 2H, aro. CH at 3° and 5°), J = 7.2 Hz), 8.35 (d, 1H, aro. CH at 6'), J = 9.5 Hz), 8.75 (s, 1H, aro. CH at 5'), J = 8.6 Hz), 8.95 (s, 1H, aro. CH at 3'), 12.86 (s, 1H, pyrimidine NH, D₂O exchangeable); ¹³C NMR (DMSO-d₆, δ): 108.23 (C-3a), 120.56 (C-3), 124.65 (C-6), 128.30 (C-5), 128.90 (C-3° and C-5°), 129.3 (C-2° and C-6°), 131.8 (C-1°), 131.6 (C-4°), 137.57 (C-1'), 142.12 (C-3), 142.96 (C-2'), 146.26 (C-4'), 158.6 (C-6), 161.0 (C=O); MS: m/z 412 (M⁺), 414 (M⁺²).

Antimicrobial screening

The antibacterial and antifungal activities were tested by agar well diffusion method using Mueller-Hinton agar media and sterilized potato dextrose agar media, respectively. The antimicrobial activity of synthesized compounds was tested against several microorganisms: Gram (+) and Gram (-) bacteria (Staphylococcus aureus and Bacillus subtilis), Gram (-)ve bacteria (Escherichia coli and Pseudomonas aeruginosa), and Fungi (Aspergillus niger and Candida albicans). All test compounds were dissolved in DMSO and diluted to get final concentration 50 µg/mL. Under identical conditions, Streptomycin (50 µg/mL), and Fluconazole (50 µg/mL) were used as standard drug. DMSO was used as a control. Antimicrobial activity was determined by measuring the zone of inhibition.

Results

Compounds 1-6 substituted-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (2a-2j) were prepared according to the reaction sequence illustrated in Scheme 1 both by conventional and microwave assisted method. The physical and elemental data are tabulated in Table 1. Both the synthetic methods are compared in terms of % yield and reaction time. The results are presented in Table 2. The structures of the compounds were elucidated by their IR, NMR (¹H and ¹³C) and mass spectra, and their composition was determined through elemental analyses. The chemical shift and multiplicity patterns correlated well with the proposed structures. The elemental analysis results were found within ±0.4% of the calculated value. All the newly synthesized compounds were screened in vitro for preliminary antimicrobial activity against various microorganisms and results are summarized in Table 3.
Scheme 1: Synthetic route of compounds 2a–2j

Table 1: Physical and elemental data of newly synthesized pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives (2a–2j)

| Compound | Recrystallization solvent | Melting point (°C) | Molecular formula | Elemental analysis calculated (found) |
|----------|---------------------------|-------------------|-------------------|--------------------------------------|
| 2a       | Methanol                  | 227–228           | C_{12}H_{8}N_{6}O_{5} | 45.58 (45.45) 2.55 (2.50) 26.58 (26.46) |
| 2b       | Ethyl acetate             | 209–210           | C_{13}H_{10}N_{6}O_{5} | 47.28 (47.06) 3.05 (3.20) 25.45 (25.66) |
| 2c       | Ethanol                   | 218–219           | C_{18}H_{12}N_{6}O_{5} | 55.11 (55.06) 3.08 (3.12) 21.42 (21.36) |
| 2d       | Ethanol                   | 257–259           | C_{17}H_{10}N_{6}O_{5} | 53.97 (53.63) 2.66 (2.49) 22.22 (22.09) |
| 2e       | Ethanol                   | 348–349           | C_{17}H_{9}N_{7}O_{7} | 48.24 (48.08) 2.14 (2.11) 23.16 (23.09) |
| 2f       | Ethanol                   | 345–346           | C_{17}H_{9}ClN_{6}O_{5} | 49.47 (49.41) 2.20 (2.16) 20.36 (20.40) |
| 2g       | Ethanol                   | 298–300           | C_{16}H_{9}BrN_{6}O_{5} | 50.67 (50.49) 2.39 (2.32) 25.85 (25.54) |
| 2h       | Ethanol                   | 328–330           | C_{18}H_{12}N_{6}O_{6} | 52.95 (52.82) 2.96 (3.03) 20.58 (20.63) |
| 2i       | Ethanol                   | 323–324           | C_{18}H_{12}N_{6}O_{5} | 55.11 (55.03) 3.08 (2.98) 21.42 (21.29) |
| 2j       | Ethyl acetate             | 260–262           | C_{16}H_{9}ClN_{6}O_{5} | 50.67 (50.49) 2.39 (2.32) 25.85 (25.54) |

Table 2: Comparison of conventional and microwave assisted synthesis

| Compound | Conventional method | Microwave assisted method |
|----------|---------------------|---------------------------|
|          | Reaction time (h)   | Percentage of yield*      | Reaction time (min) | Percentage of yield* |
| 2a       | 6                   | 80                         | 2.5               | 86                  |
| 2b       | 6                   | 79                         | 2.5               | 84                  |
| 2c       | 6                   | 82                         | 3.0               | 88                  |
| 2d       | 6                   | 78                         | 3.0               | 84                  |
| 2e       | 6                   | 83                         | 3.0               | 89                  |
| 2f       | 6                   | 82                         | 3.0               | 87                  |
| 2g       | 6                   | 74                         | 3.5               | 81                  |
| 2h       | 6                   | 72                         | 3.5               | 78                  |
| 2i       | 6                   | 71                         | 3.5               | 77                  |
| 2j       | 6                   | 74                         | 3.0               | 81                  |

*Yield refers to pure isolated products

Discussion

Dave et al.\cite{16} have reported the reaction of various nitriles with ortho-amino carbonyl compounds in acidic condition to produce corresponding condensed pyrimidines in fair to good yields. The condensation reaction of compound 1 with various aliphatic/aromatic nitriles in dioxane using dry HCl gas as catalyst gave 71–83% yield of 1,6-substituted-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives (2a–2j). However, the usage of hydrochloric acid gas has certain limitations like major contributor in acid rain, depletion of protective ozone layer, etc. This impetus us to develop unique route to synthesize target molecule utilizing microwave irradiation that curtails the use of HCl gas as well as maintains good yield.

The reaction of compound 1 with various aliphatic/aromatic nitriles in the presence of basic potassium tert-butoxide under microwave irradiation offered 77–89% yield of pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives (2a–2j).

The structures of synthesized compounds were confirmed using spectral and microanalytical data. For instance, the disappearance of two peaks of the primary amino group and appearance of the secondary amino peak (3284 cm\(^{-1}\)) and shifting of carbonyl peak to 1645 cm\(^{-1}\) indicated the cyclization of an ortho-amino ester of pyrazole. The \(^1\)H NMR spectrum of compound 2a showed a singlet for NH proton of pyrimidine at \(\delta\) 12.3 ppm which is D\(_2\)O exchangeable, a singlet at \(\delta\) 7.74 is from the H-3 proton of pyrazole ring, two doublet at \(\delta\) 8.39 and 8.74 having J value 8.7 Hz and a singlet at \(\delta\) 8.95 belongs to 2,4-dinitrophenyl ring and a triplet at \(\delta\) 2.44 due to the methyl group (three protons). \(^13\)C NMR spectrum showed signals at 20.88 and 160.95 ppm which represents methyl group and carbonyl carbon of amide, respectively. The aromatic carbons
Table 3: Antimicrobial activity of newly synthesized pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives (2a–2j)

| Compound | Gram-positive bacteria | Gram-negative bacteria | Fungi |
|----------|------------------------|------------------------|-------|
|          | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeroginosa* | *Aspergillus niger* | *Candida albicans* |
|          | *Bacillus subtilis*    |                        |                  |                   |
| 2a       | 8.33±0.58              | 10.00±1.00             | 9.67±0.58       | 9.33±0.58         | 9.00±1.00         |
| 2b       | 8.00±1.00              | 10.67±0.58             | 10.00±1.00      | 9.67±0.58         | 9.33±0.58         |
| 2c       | 8.33±0.58              | 11.67±0.29             | 9.33±0.58       | 7.33±0.58         | 7.67±0.29         |
| 2d       | 8.67±0.76              | 13.33±0.58             | 9.67±0.29       | 7.50±0.50         | 8.33±0.29         |
| 2e       | 8.33±0.58              | 9.33±0.58              | 11.50±0.50      | 13.33±0.58        | 11.50±0.50        |
| 2f       | 8.67±0.76              | 10.00±0.00             | 8.50±0.50       | 9.50±0.50         | 8.33±0.29         |
| 2g       | 9.33±0.58              | 12.00±0.00             | 7.33±0.58       | 11.00±0.00        | 9.33±0.58         |
| 2h       | 10.33±0.58             | 10.50±0.50             | 9.50±0.50       | 11.00±0.00        | 9.33±0.58         |
| 2j       | 12.33±1.15             | 13.33±0.58             | 11.00±0.00      | 9.33±0.58         | 9.33±0.58         |
| Streptomycin | 15.00±0.00             | 15.67±0.58             | 12.00±1.00      | 13.67±0.58        | 15.00±0.00        |
| Fluconazole | -             | -                      | -               | 13.67±0.58        | 15.00±0.00        |
| Control  | -                      | -                      | -               | -                 | -                 |

*Average of triplicate reading (concentration 50 μg/mL); *SD*: Standard deviation

of 2,4-dinitrophenyl ring were appeared at δ 120.58 (C-3'), 124.46 (C-6'), 128.31 (C-5'), 137.60 (C-1'), 142.95 (C-2'), and 146.22 (C-4') ppm. Various carbons of pyrazolo[3,4-d]pyrimidine ring were found at δ 108.28 (C-3a), 124.07 (C-3), 142.86 (C-7a), and 154.58 (C-6) ppm. The molecular ion peak of compound 2a was observed at m/z 316 (M+), which was in accordance with its suggested molecular formula.

The advantages of microwave irradiation method over the conventional synthetic method for preparation of pyrazolo[3,4-d]pyrimidin-4(5H)-one includes the elimination of hydrochloric acid gas usage, curtails the use of solvents, easy work-up procedure, less time consuming, and more efficient.

The results of antimicrobial activity show entire series of compounds exhibiting weak to good activity against all tested microorganisms as compared to standard drugs. It is more conspicuous observation that the antimicrobial activity of various derivatives appeared to be related with the nature of substituents present at 6th position of pyrazolo[3,4-d]pyrimidin-4(5H)-one. The compound 2e bearing 4-NO2C6H4 group showed the highest sensitivity against *P. aeruginosa* and *A. niger* as compared to standard drugs. It is also highly sensitive against *C. albicans*. Compound 2i bearing 4-NH2C6H4 group exhibited the best activity against *S. aureus* while *B. subtilis* has been more sensitive against compound 2f having 4-ClC6H4 substituent. 4-BrC6H4H3 substituent containing compound 2g found to possess excellent activity against *E. coli* bacterial strain, while the compound 2j with substituent 4-C6H4N exhibited remarkable antimicrobial activity against all tested microorganisms. The remaining compounds showed weak to moderate activity against all tested microorganisms.

**Conclusion**

To summarize, the usage of potassium tert-butoxide under solvent free conditions is very convenient and more efficient than hydrogen chloride gas. Thus, we have developed an alternative and safer way to synthesize pyrazolo[3,4-d]pyrimidin-4(5H)-one derivatives, which restricts the use of hydrogen chloride gas. It is evident from the result that three compounds, namely, 2e, 2f, and 2g were found more potent having comparable activity with standard drugs, which indicates that pyrazolo[3,4-d]pyrimidin-4(5H)-one having an alkyl, aryl, or heterocyclic substitution at the 6th position may become a good lead for antimicrobial agent.

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**Conflicts of interest**

There are no conflicts of interest.

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