Microwave-Assisted Synthesis and Antimicrobial Profiling of New Pyrrolo-Pyrimidine Analougues

HANDE BHUSAHEB* and GAVALI LAXMAN**

Chem Research Lab, Karmveer Bhausaheb Patil College, Vashi, Navi Mumbai-400703, India

*Corresponding author: E-mail: laxmanvg70@gmail.com

Received: 4 June 2023; Accepted: 25 July 2023; Published online: 31 August 2023; AIC-21366

INTRODUCTION

Emergence of resistance to conventional antimicrobials due to their massive use in the treatment of various infections makes investigators to continuously search for new antifungal and antibacterial entities [1]. Facts suggest that incorporation of heterocyclic groups into the organic moieties enhances their antimicrobial potential [2]. Fused pyrimidines are an interesting class of heterocycles, which are extensively explored by chemists due to their massive pharmacological profile [3]. Initial studies followed by exhaustive investigation with prime focus on the pharmacological spectrum of purines and its analogues, in particular deaza analogues, pyrrolo[2,3-d]pyrimidines and pyrrolo[3,2-d]pyrimidines [4]. Pyrrolo[2,3-d]pyrimidine analogues attributed to their strong antibacterial and antifungal properties always attracts the researchers attention [5]. The pyrrolopyrimidines development began in 1970s, such that initially synthesis of pyrrolopyrimidines involving monocyclic pyrimidines as the key substrate occurred in 1974 [6]. Later, based on pyrrole derivatives and modifications in skeleton of bicyclic pyrimidines, various synthetic strategies were developed. Likewise, simultaneously more simplified approaches involving substituted pyrimidines were developed that employed highly reactive reagents such as acetals of acid amides and amines [7].

Evidence suggest pyrrolopyrimidine antibiotics to inhibit microbial DNA topoisomerases GyrB and ParE and thymidylate monophosphate kinase (TMPK) the key enzymes essential for DNA synthesis, thereby act as bactericidal agents [8]. Recently, azomethine analogues attributed to their antimicrobial activities have become a subject of interest for many chemists [9,10]. Sindhu et al. [11] highlighted the synthesis of some new imines using pyrrolo-pyrimidine. Hence based on the literary evidences over microbial resistance offered by commercially available antibiotics, the high antimicrobial potential of pyrrolo-pyridines and azomethines, intended present study to perform the synthesis of new pyrrolo[2,3-d]pyrimidine analogues, followed by evaluation of their antibacterial (against S. aureus, B. subtilis, E. coli and P. aeruginosa) and antifungal (against C. albicans and S. cerevisiae) potential by determining the zone of inhibition using disk diffusion method.
Experimental

In current study, the chemicals to synthesize new pyrrolo-pyridine analogues (NPPAs) were obtained from Merck KGaA, Sigma-Aldrich, HmbG® Chemicals and Friedemann Schmidt Chemicals. The NPPAs 1H NMR spectral data were recorded by Bruker, DPX 300 spectrometer on δ value scale in ppm using tetramethylsilane and DMSO as solvent. In 1H NMR signal representation the terms ‘s’ for singlet and ‘brs’ for broad signal were used. The NPPAs IR spectral data were recorded using Alpha-P Bruker FT-IR Spectrometer at wavelength ranged between 4000 to 400 cm⁻¹. For the elemental analysis of NPPAs Perkin Elmer 240 B and 240 C instruments were used. The microwave synthesis was performed in an Anton Paar Monowave 300 microwave synthesizer. The melting points of the pyrrolo-pyrimidines analogues was recorded in open capillary using SMP11 Apparatus and are uncorrected. Reaction moni-toring was done by TLC using silica gel 60 F254 in Sprec-troline® CM-26 UV chamber and solvent system of methanol: chloroform (9.5:0.5).

General Procedure for synthesis of N-(substituted benzylidene)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (2a-h): The NPPAs 2a-h were synthesized as per the standard procedure given in the literature with some minor modification [12-15]. Briefly, a reaction mixture of equimolar concentration of 4-amine (2b):

\[
\text{4-amine (2b):}
\]

\[
\text{4-amine (2h):}
\]

\[
\text{White solid; yield: 80.79\%, m.p. 185 ºC; FTIR (KBr, v_{max} cm⁻¹): 3356 (NH), 3064 (═C-H), 1562 (C=N), 1492, 1461 (C=C); 1H NMR (DMSO-d₆, ppm) δ: 7.28-8.19 (m, 5H, aromatic-H), 8.72 (s, 1H, pyrimidine-H), 9.17 (s, 1H, N=CH), 9.96 (brs, 1H, NH, D₂O-exchangeable); Anal. calcd. (found) % for C₁₅H₁₅N₅O₂ (267.24): C, 70.26 (70.19); H, 4.54 (4.53); N, 26.21 (26.18).}

\[
\text{N-Benzylidene-7H-pyrrolo[2,3-d]pyrimidin-4-amine (2d):}
\]

\[
\text{N-(4-Dimethylamino)benzylidene)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (2e):}
\]

\[
\text{N-(2-Fluorobenzylidene)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (2g):}
\]

\[
\text{N-(4-Fluorobenzylidene)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (2h):}
\]

\[
\text{Antibacterial activity: The synthesized NPPAs 2a-h were evaluated for their antibacterial potential against the freshly cultured bacterial strains of S. aureus (MCC 2010), B. subtilis (MCC 2010), E. coli (MCC 2412) and P. aeruginosa (MCC 2080) by disk diffusion method over Mueller-Hinton plates using sterile nutrient agar media [16]. For the study NPPAs 2a-h were dissolved in 1 mL of 1% DMSO and bacterial strains were allowed to grow to log phase overnight with constant shaking at 37 ºC. Bacterial cultures were spread on the Mueller-Hinton (MH) plates agar media with 4 mm of depth. Next, discs with 1 mm thick and 6 mm diameters impregnated with 100 µg/mL solution of compounds 2a-h in 1% DMSO, 50 µg/mL of streptomycin (positive standard) and 1% DMSO (negative control) were placed on top of the plates and the plates were incubated at 37 ºC for 24 h. The diameter of inhibition zone was measured and recorded. The results were listed in the table below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Diameter of Inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>25.17</td>
</tr>
<tr>
<td>2b</td>
<td>23.18</td>
</tr>
<tr>
<td>2c</td>
<td>23.03</td>
</tr>
<tr>
<td>2d</td>
<td>26.11</td>
</tr>
<tr>
<td>2e</td>
<td>26.05</td>
</tr>
<tr>
<td>2f</td>
<td>25.89</td>
</tr>
<tr>
<td>2g</td>
<td>25.73</td>
</tr>
<tr>
<td>2h</td>
<td>25.67</td>
</tr>
</tbody>
</table>

Furthermore, the MIC values of these synthesized NPPAs were determined against S. aureus (MCC 2010), B. subtilis (MCC 2010), E. coli (MCC 2412) and P. aeruginosa (MCC 2080) and compared with the standard drug ciprofloxacin (MIC: 0.25 µg/mL). The MIC results for NPPAs 2a-h were listed in the table below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>0.5</td>
</tr>
<tr>
<td>2b</td>
<td>0.5</td>
</tr>
<tr>
<td>2c</td>
<td>0.5</td>
</tr>
<tr>
<td>2d</td>
<td>0.5</td>
</tr>
<tr>
<td>2e</td>
<td>0.5</td>
</tr>
<tr>
<td>2f</td>
<td>0.5</td>
</tr>
<tr>
<td>2g</td>
<td>0.5</td>
</tr>
<tr>
<td>2h</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Scheme-I: Synthesis of substituted benzaldehyde derivatives of the 4-aminopyrrolo[2,3-d]pyrimidine form
were placed firmly over MH plates agar surface. The plates were incubated for 24 h at 37 °C and same experimental protocol was repeated in triplicate. After 24 h incubation, the zone of inhibition of compound 2a-h was measured on mm scale.

**Antifungal activity:** The synthesized NPPAs 2a-h were also investigated for their antifungal potential against freshly cultured fungal strains of *Candida albicans* (MCC 1439) and *S. cerevisiae* (MCC1033) by disk diffusion method over Mueller-Hinton plates using sterile Sabouraud’s agar media [17]. For the study NPPAs 2a-h were dissolved in 1 mL of 1% DMSO and next discs with 1 mm thick and 6 mm diameters impregnated with 100 µg/mL solution of compounds 2a-h in 1% DMSO, 50 µg/mL of fluconazole (positive standard) and 1% DMSO (negative control) were placed over MH plates agar surface. Finally, the plates were incubated for 72 h at 37 °C and the same experimental protocol was repeated in triplicate. After 72 h incubation, the zone of inhibition of compounds 2a-h was measured in mm.

### RESULTS AND DISCUSSION

The facts over microbial resistance to commercially available antimicrobials, related side effects and high antimicrobial potential of azomethines and heterocycles especially pyrrolo pyrimidines emphasizes the need for new synthesis of new analogues of pyrrolo-pyrimidines [18-20]. The scheme for synthesis of NPPAs was based on the standard protocols given in the literature [16,21] and offered all NPPAs in good yield.

Microwave assisted synthesis (MAS) enhances the rate of organic reactions based on superheating of solvent [22]. In present study, various azomethine analogues of pyrrolo-pyrimidine (2a-h) were synthesized by treatment of 4-aminopyrrolo[2,3-d]pyrimidine with various aromatic aldehydes following Schiff’s reaction under microwave assisted conditions [23]. The purifying process of the resulting NPPAs involved recrystallization using methanol and activated charcoal. Purity of all NPPAs 2a-h was determined based on their melting point, pattern of single spot-on TLC plates and percentage of elemental analysis. The elemental analysis of NPPAs revealed that in all the synthesized compounds 2a-h, the elements C, H and N were within ±0.4% of theoretical range. Structures of NPPAs were elucidated and confirmed based on the FT-IR and ¹H NMR spectroscopic data and has also been supported with literary evidences [12,18,24]. The presence of characteristic FTIR bands ranging from 3364-3356 cm⁻¹ attributed to the N-H stretching and FTIR bands ranging from 1564-1562 cm⁻¹ ascribed to C=N stretching. The ¹H NMR signals at 8 value ranging from 8.57-9.80 ppm corresponding to N=CH protons confirmed the structure of newly synthesized NPPAs 2a-h. The characterization data of all the synthesized NPPAs compounds in the present study were also matched and found to be in agreement with the results of the other studies [25,26].

**Biological activity:** The synthesized NPPAs 2a-h were further evaluated for their *in vitro* their antibacterial potential against the freshly cultured bacterial strains of *S. aureus* (MCC 2010), *B. subtilis* (MCC 2010), *E. coli* (MCC 2412) and *P. aeruginosa* (MCC 2080) by disk diffusion method in triplicate following the standard protocol [16]. The zone of inhibition obtained as result of *in vitro* testing NPPAs 2a-h against different strains is presented in Table-1. It is revealed that all NPPAs exhibit significant antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* with zone of inhibition ranging from 13 to 21 mm, according to the antibacterial screening results. Additionally, all NPPAs were found to exhibit more antibacterial potential against *S. aureus* when compared with streptomycin (18-21 mm). Also, among all NPPAs, the NPPAs 2c, 2e and 2f (against *E. coli*), 2d (against *S. aureus*), 2f (against *B. subtilis*) and NPPA 2d (against *P. aeruginosa*) exhibited the maximum inhibitory activity.

The synthesized NPPAs 2a-h when investigated for their antifungal potential against *C. albicans* (MCC 1439) and *S. cerevisiae* (MCC1033) by disk diffusion method in triplicate [17], the results showed that all the synthesized compounds had nearly 2.5 times the potency (9-15 mm) compared to the common fluconazole and were active against the two fungi namely: *C. albicans* (MCC1439) and *S. cerevisiae* (MCC1033). The antifungal activity of NPPAs 2a-h revealed that NPPA 2g against *C. albicans* and NPPAs 2b and 2e against *S. cerevisiae* exhibited maximum activity. The data for zone of inhibition, obtained as result of *in vitro* testing NPPAs 2a-h against different strains is presented in Table-1.

The high antibacterial and antifungal potential of new synthesized NPPAs 2a-h supports their potential application as antibacterial and antifungal agents; however the synthesized

---

### TABLE-1

**ANTIBACTERIAL AND ANTIMICROBIAL STUDIES OF COMPOUNDS 2a-h**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antibacterial activity</th>
<th>Zone of inhibition (mm)</th>
<th>Antifungal activity</th>
<th>C. albicans</th>
<th>S. cerevisiae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td></td>
<td>19</td>
<td>16</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>2b</td>
<td></td>
<td>20</td>
<td>13</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>2c</td>
<td></td>
<td>18</td>
<td>15</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>2d</td>
<td></td>
<td>21</td>
<td>15</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>2e</td>
<td></td>
<td>20</td>
<td>16</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>2f</td>
<td></td>
<td>19</td>
<td>21</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>2g</td>
<td></td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>2h</td>
<td></td>
<td>19</td>
<td>20</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td>15</td>
<td>22</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
compounds must be additionally investigated for the in vivo and clinical studies.

**Conclusion**

The present study involved successful microwave assisted synthesis of \(N\)-(substituted benzylidene)-7H-pyrrolo[2,3-d]-pyrimidin-4-amine (2a-h) from 4-amino-pyrrolo[2,3-d]-pyrimidine, via Schiff’s reaction. The structures of synthesized NPPAs 2a-h were further confirmed based on the single spot TLC, sharp melting point, IR and NMR spectrometry data. Present study concludes that all synthesized NPPAs 2a-h exhibit significant antibacterial and antifungal activities and among all synthesized NPPAs, the NPPAs having nitro, fluoro and dimethyl amino groups at ortho, meta- and para-position of benzene ring impart strong antimicrobial activity. Current study recommends synthesized NPPAs as effective antimicrobial agents against. However, additional in vivo and clinical studies are required to further establish the safety and efficacy of the synthesized NPPAs to be established as an effective antimicrobial agents for the treatment of various bacterial and fungal infections.

**ACKNOWLEDGEMENTS**

The authors are thankful to the Karmveer Bhausaheb Patil College, Vashi, Navi Mumbai, India, for providing the necessary research facilities to successfully complete this research.

**CONFLICT OF INTEREST**

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

**REFERENCES**


17. N.K. Fuloria, V. Singh, M. Shaharyar and M. Ali, Molecules, 14, 1898 (2009); https://doi.org/10.3390/molecules14051898


20. M.S. El-Gaby, A.M. Gaber, A.A. Atalla and K.A.A.A. Wahab, Il Farmaco, 57, 613 (2002); https://doi.org/10.1007/S00142-001-0079-3


