SYNTHESIS AND CHARACTERIZATION OF NOVEL N$_5$-(2-SUBSTITUTED BENZYLIDENE)-N$_2$, N$_2$-DIMETHYL/N$_4$-PHENYL PYRIMIDINE-2,4,5-TRIAMINE/ PYRIMIDINE-4,5-DIAMINE AS ANTIMICROBIAL AGENT

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ABSTRACT

In the present study, a novel series of N$_5$-(3-substituted benzylidene)-N$_4$-phenyl pyrimidine-4,5-diamine / N$_5$-(2-substituted benzylidene)-N$_2$,N$_2$-dimethyl-N$_4$-phenyl pyrimidine-2,4,5-triamine were synthesized by using the starting ingredient formimidamide/ 4-(dimethylamino) benzimidamide and it was characterized by IR, $^1$H NMR and Mass spectral analyses. The synthesized compounds aiming towards the development of potential antimicrobial agents in this connection the screening of structures of the synthesized scaffolds were possessing their antimicrobial activity against G$^+$ve bacteria Staph.aureus, Staph.epidermidis, Micr.luteus and Bac. cereus, G$^-$ve bacteria Ech.coli, Pseu.aeruginosa & Kleb.pneumoniae and fungi Asp.niger & Asp.fumigatus. Among all the synthesized compounds four derivatives were showed the highest significant zone of inhibition towards G$^+$ve, G$^-$ve bacteria and fungi microbial growth.

Keywords: Pyrimidine-4,5-diamine, Pyrimidine-2,4,5-triamine, Antimicrobial Activity.

INTRODUCTION

The microbial infection has become the foremost cause of endangering the health and life of humans. Most importantly, microbial resistance to the existing antimicrobial drugs also twisted to be the main anxiety for the treatment of microbial infection.$^1$ The current circumstances are highlights that, the urgent call for the search of development of new chemical scaffolds with greater efficacy and least side effect.$^2$ As a result of the identification of novel scaffolds is always a major confront for a medicinal chemist because it could be significant and reliable. In recent times, synthesis of pyrimidine derivatives has been considered of huge interest to medicinal chemists due to its various pharmacological and therapeutic behaviors like anticancer,$^{3,4}$ antimicrobial,$^5$ antifungal,$^3$ antiviral,$^6$ antiinflammatory,$^5$ antinociceptive,$^8$ $\beta$-blockers$^8$ and P38 MAP kinase inhibitors.$^{10}$ Many of the marketed drugs are containing pyrimidine scaffold as a core functioning unit like Taniplon, Fasiplon and Divaplon$^{11,12}$ (Fig.-1). Moreover, it was showing the potency of pyrimidine scaffolds and also it was evidence for its biological significance. From the literature survey, we observed that pyrimidine with different substitutions were possess a broad range of antimicrobial properties.$^8$ The substituted pyrimidine motifs have been widely studied for their ability and aiming to target multiple proteins, necessary at different stages of microbial infection.$^{13}$ On the other hand, The nitrogen atoms into the pyrimidine ring might confer bioactivity of molecules and improve the efficiency of the molecules. The survey reveals that the mixture of two or more bioactive pharmacophores into the same molecule is an important tool for scheming more active novel chemical entities.$^{14,15}$ Thus, based on aforesaid results and taking into account our purpose was to synthesize novel N$_5$-(3-substituted benzylidene)-N$_4$-phenyl pyrimidine-4,5-diamine [5A-5F]/ N$_5$-(2-substituted benzylidene)-N$_2$,N$_2$-dimethyl-N$_4$-phenyl pyrimidine-2,4,5-triamine
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EXPERIMENTAL

Materials and Methods

All solvents used were of laboratory grade and were obtained from SD fine chemicals (Mumbai, India), and Merck (Mumbai, India). Ciprofloxacin and Ketoconazole are received as gift samples from Dr. Reddy's Laboratories, Hyderabad, India. Melting points were determined in open glass capillary tubes and are uncorrected. Compounds were routinely checked for their purity on silica gel G (Merck) thin layer chromatography (TLC) plates; iodine chamber and UV lamp were used for visualization of TLC spots. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS) FT-IR spectrophotometer. 1H-NMR spectra were recorded on Bruker DPX-300 NMR spectrometer in CDCl3 using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. Elemental analyses were performed on a Perkine Elmer model 240C analyzer and were within ±0.4 % of the theoretical values.

General Procedure

The synthetic strategy to prepare the target compounds is depicted in the Scheme-1. To construct pyrimidine nucleus in the first step the equimolar quantity of formimidamide/ 4-(dimethylamino)benzimidamide [10mmol], sodium ethoxide, (0.5 g in 5mL water), in 25 ml of ethanol was stirred mechanically 05 min, then ethyl 3-(dimethylamino)-2-nitroacrylate (10mmol) was added and the mixture was subjected to heat for 1 hr at 40°C. The reaction was monitored by TLC to finalize the title compound. The crude product was purified by recrystallized by using ethyl alcohol to obtain pure product 5-nitropyrimidin-4-ol/2-(4-(dimethylamino)phenyl)-5-nitropyrimidin-4-ol (1).

A mixture of 5-nitropyrimidin-4-ol/2-(4-(dimethylamino)phenyl)-5-nitropyrimidin-4-ol (1) undergone under chlorination by using (10mmol) POCl3 (10mmol) and 10mL of N, N-Diisopropylethylamine [DIEA], further it was refluxed for 30 min on 80°C. End of the reaction was observed via TLC and the purified compound was obtained by recrystallized by using ethyl alcohol to get pure product 4-chloro-5-nitropyrimidine/4-(4-chloro-5-nitropyrimidin-2-yl)-N,N-dimethylaniline (2).

A mixture of 4-chloro-5-nitropyrimidine/4-(4-chloro-5-nitropyrimidin-2-yl)-N,N-dimethylaniline (2) (10mmol) reacted with aniline (10mmol) in 10mL of DMF at 80°C and quenched in ice-water to get the precipitate was filtered, washed with water and recrystallized from ethyl alcohol to give 5-nitro-N-phenylpyrimidine-4-amine/2-(4-(dimethylamino)phenyl)-5-nitro-N-phenyl pyrimidin-4-amine (3).

A mixture of 5-nitro-N-phenylpyrimidine-4-amine/2-(4-(dimethylamino)phenyl)-5-nitro-N-phenyl pyrimidin-4-amine (3) (10mmol) reacted with Zn (3 mmol), CuSO4 (3 mmol) in water on magnetic stirrer 3h under room temperature to give N4-phenylpyrimidine-4,5-diamine/ 2-(4-(dimethylamino)phenyl)-N4-phenylpyrimidine-4,5-diamine (4).

Spectral Analysis

N4-(4-methoxybenzylidene)-N4-phenylpyrimidine-4,5-diamine[5A]

IR: 3147 (NH), 3059 (Ar-CH), 1643 (C=N), 1591 (C=C), 1076 (C-O-C); 1H NMR: 3.12 (s, 3H, OCH3), 3.74 (s, 1H, =CH linkage), 5.15 (s, 1H, NH), 6.43-7.91 (m, 11H, Ar-H); Mass: C18H16N4O; calcd, 304 [M+], found, 304 [M+]; Elemental Analysis: calcd, C, 71.04; H, 5.30; N, 18.41; O, 5.26; found, C, 71.06; H, 5.32; N, 18.40; O, 5.23.

[5a-5f] scaffolds may display clinically significant antimicrobial agent and the derivatives with a selection of substitutions which might direct towards the improvement in bioactivity.
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**Scheme-1**

N_{2}-(4-methoxybenzylidene)-N_{2}-dimethyl-N_{4}-phenylpyrimidine-2,4,5-triamine [5a]

IR: 3119 (NH), 3081 (Ar-CH), 1642 (C=N), 1645 (C-O-C); \textsuperscript{1}H NMR: 2.03 (s, 6H, CH_{3}), 2.47 (s, 3H, OCH_{3}), 3.43 (s, 1H, =CH linkage), 5.05 (s, 1H, NH), 6.51-7.54 (m, 10H, Ar-H); Mass: C_{20}H_{21}N_{5}O; calcd, 347 [M+], found, 347 [M+]; Elemental Analysis: calcd, C, 69.14; H, 6.09; N, 20.16; O, 4.61; found, C, 68.43; H, 5.72; N, 21.07; O, 4.82

N_{2}-(4-methylbenzylidene)-N_{4}-phenylpyrimidine-4,5-diamine [5B]

IR: 3122 (NH), 3081 (Ar-CH), 1642 (C=N), 1623 (C=C); \textsuperscript{1}H NMR: 2.31 (s, 3H, CH_{3}), 2.72 (s, 3H, OCH_{3}), 3.82 (s, 1H, =CH linkage), 5.34 (s, 1H, NH), 6.51-7.13 (m, 11H, Ar-H); Mass: C_{18}H_{16}N_{4}; calcd, 288 [M+], found, 288 [M+]; Elemental Analysis: calcd, C, 74.98; H, 5.59; N, 19.43; found, C, 74.96; H, 5.57; N, 19.44

N_{2}-(4-methylbenzylidene)-N_{4}-phenylpyrimidine-2,4,5-triamine [5b]

IR: 3365 (NH), 3035 (Ar-CH), 1682 (C=N), 1611 (C-C); \textsuperscript{1}H NMR: 2.32 (s, 6H, CH_{3}), 2.72 (s, 3H, CH_{3}), 3.12 (s, 1H, =CH linkage), 5.42 (s, 1H, NH), 6.52-7.32 (m, 10H, Ar-H); Mass: C_{20}H_{21}N_{5}; calcd, 331[M+], found, 331[M+]; Elemental Analysis: calcd, C, 72.48; H, 6.39; N, 21.13; found, C, 72.47; H, 6.35; N, 21.12

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N\textsubscript{5}-(4-chloro benzylidene)-N\textsubscript{4}-phenylpyrimidine-4,5-diamine [5c]
IR: 3120 (NH), 3061 (Ar-CH), 1641 (C=N), 1595 (C=C), 726 (C-Cl);
1\textsuperscript{H} NMR: 3.11 (s, 1H, =CH linkage), 5.16 (s, 1H, NH), 7.19-7.82 (m, 11H, Ar-H) ; Mass: C\textsubscript{17}H\textsubscript{13}ClN\textsubscript{4}; calcd, 308 [M+], found, 310 [M+2]; Elemental Analysis: calcd, C, 66.13; H, 4.24; Cl, 11.48; N, 18.15; found, C, 66.16; H, 4.25; Cl, 11.47; N, 18.17

N\textsubscript{5}-(4-chlorobenzylidene)-N\textsubscript{2},N\textsubscript{2}-dimethyl-N\textsubscript{4}-phenylpyrimidine-2,4,5-triamine [5c]
IR: 3363 (NH), 3033 (Ar-CH), 1673 (C=N), 1594 (C=C), 873 (C-Cl);
1\textsuperscript{H} NMR: 2.23 (s, 6H, CH\textsubscript{3}), 3.23 (s, 1H, =CH linkage), 5.13 (s, 1H, NH), 6.63-7.83 (m, 10H, Ar-H); Mass: C\textsubscript{19}H\textsubscript{18}ClN\textsubscript{5}; calcd, 351 [M+], found, 353 [M+2]; Elemental Analysis: calcd, C, 64.86; H, 5.16; Cl, 10.08; N, 19.91; found, C, 64.84; H, 5.17; Cl, 10.12; N, 19.93

N\textsubscript{5}-(4-bromo benzylidene)-N\textsubscript{4}-phenylpyrimidine-4,5-diamine [5D]
IR: 3385 (NH), 3176 (Ar-CH), 1668 (C=N), 1548 (C=C), 668 (C-Br);
1\textsuperscript{H} NMR: 3.01 (s, 1H, =CH linkage), 5.41 (s, 1H, NH), 7.27-7.92 (m, 11H, Ar-H); Mass: C\textsubscript{17}H\textsubscript{13}BrN\textsubscript{4}; calcd, 352 [M+], found, 354 [M+2]; Elemental Analysis: calcd, C, 57.81; H, 3.71; Br, 22.62; N, 15.86; found, C, 57.84; H, 3.73; Br, 22.63; N, 15.85

N\textsubscript{5}-(4-bromobenzylidene)-N\textsubscript{2},N\textsubscript{2}-dimethyl-N\textsubscript{4}-phenylpyrimidine-2,4,5-triamine [5d]
IR: 3082 (NH), 2952 (Ar-CH), 1692 (C=N), 1604 (C=C), 794 (C-Br);
1\textsuperscript{H} NMR: 2.94 (s, 6H, CH\textsubscript{3}), 3.14 (s, 1H, =CH linkage), 5.14 (s, 1H, NH), 6.14-8.14 (m, 10H, Ar-H); Mass: C\textsubscript{19}H\textsubscript{18}BrN\textsubscript{5}; calcd, 396 [M+], found, 398 [M+2]; Elemental Analysis: calcd, C, 57.59; H, 4.58; Br, 20.16; N, 17.67; found, C, 57.61; H, 4.60; Br, 20.18; N, 17.69

N\textsubscript{5}-(4-aminobenzylidene)-N\textsubscript{4}-phenylpyrimidine-4,5-diamine [5E]
IR: 3351 & 3031(NH), 2961 (Ar-CH), 1664 (C=N), 1604 (C=C);
1\textsuperscript{H} NMR: 3.52 (s, 1H, =CH linkage), 4.72 (s, 2H, NH\textsubscript{2}), 5.52 (s, 1H, NH), 6.62-7.14 (m, 11H, Ar-H); Mass: C\textsubscript{17}H\textsubscript{15}N\textsubscript{5}; calcd, 289 [M+], found, 289 [M+]; Elemental Analysis: calcd, C, 70.57; H, 5.23; N, 24.21; found, C, 70.59; H, 5.22; N, 24.22

N\textsubscript{5}-(4-aminobenzylidene)-N\textsubscript{2},N\textsubscript{2}-dimethyl-N\textsubscript{4}-phenylpyrimidine-2,4,5-triamine [5e]
IR: 3354 (NH), 3024 (Ar-CH), 1664 (C=N), 1624 (C=C);
1\textsuperscript{H} NMR: 2.74 (s, 6H, CH\textsubscript{3}), 4.14 (s, 2H, NH\textsubscript{2}), 3.54 (s, 1H, =CH linkage), 5.44 (s, 1H, NH), 7.54-7.74 (m, 10H, Ar-H); Mass: C\textsubscript{19}H\textsubscript{20}N\textsubscript{6}; calcd, 332 [M+], found, 332 [M+]; Elemental Analysis: calcd, C, 68.65; H, 6.06; N, 25.28; found, C, 68.67; H, 6.10; N, 25.29

N\textsubscript{5}-(4-fluorobenzylidene)-N\textsubscript{4}-phenylpyrimidine-4,5-diamine [5F]
IR: 3126 (NH), 3086 (Ar-CH), 1656 (C=N), 1166 (C-F);
1\textsuperscript{H} NMR: 3.26 (s, 1H, =CH linkage), 5.26 (s, 1H, NH), 7.36-7.96 (m, 11H, Ar-H); Mass: C\textsubscript{17}H\textsubscript{13}FN\textsubscript{4}; calcd, 292 [M+], found, 292 [M+]; Elemental Analysis: calcd, C, 69.85; H, 4.48; F, 6.50; N, 19.17; found, C, 69.83; H, 4.46; F, 6.53; N, 19.14

N\textsubscript{5}-(4-fluorobenzylidene)-N\textsubscript{2},N\textsubscript{2}-dimethyl-N\textsubscript{4}-phenylpyrimidine-2,4,5-triamine [5f]
IR: 3354 (NH), 3024 (Ar-CH), 1664 (C=N), 1624 (C=C);
1\textsuperscript{H} NMR: 5.18 (s, 1H, NH), 6.61-7.83 (m, 10H, Ar-H); Mass: C\textsubscript{19}H\textsubscript{18}FN\textsubscript{5}; calcd, 335 [M+], found, 335 [M+]; Elemental Analysis: calcd, C, 68.05; H, 4.48; F, 6.50; N, 19.17; found, C, 68.08; H, 4.46; F, 6.53; N, 19.14

Antimicrobial Screening
All the N\textsubscript{5}-(3-substituted benzylidene)-N\textsubscript{4}-phenyl pyrimidine-4,5-diamine [5A-5F] / N\textsubscript{5}-(2-substituted benzylidene)-N\textsubscript{2},N\textsubscript{2}-dimethyl-N\textsubscript{4}-phenyl pyrimidine-2,4,5-triamine [5a-5f] compounds were evaluated by paper disc diffusion technique\textsuperscript{10,20} by using American Type Culture Collection [ATCC] antibacterial and fungus. The antibacterial action of the molecules was screened against four G+ve bacteria (Staph.aureus,
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Staphylococcus epidermidis, Micrococcus luteus and Bacillus cereus) & three G-ve bacteria (Escherichia coli, Pseudomonas aeruginosa & Klebsiella pneumoniae). The antifungal activity of the compounds was screened against two fungi (Aspergillus niger & Aspergillus fumigatus).

Paper Disc Diffusion Technique

The medium was sterilized as per the standard procedure and the impregnated paper with the different test compounds (µg mL⁻¹ in DMF) was placed on the medium. The pre-incubated plates for 1h at room temperature were incubated for 24-48h at 37°C for antibacterial and antifungal activities respectively. The Ciprofloxacin and Ketoconazole were used as a standard for antibacterial & antifungal activities, the samples are collected from Dr. Reddy’s Laboratories, India.

Minimum Inhibitory Concentration (MIC)

The MIC was conducted for the compounds based on the standard procedure, the nutrient agar medium used for antibacterial activity and Sabouraud dextrose agar medium used for antifungal activity. All the inoculated plates were incubated at 37°C for 24h and 48h for bacteria and fungi, respectively. The MIC was the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.

Statistical Analysis

The significant difference values determined between control with student’s t-test.

RESULTS AND DISCUSSION

Chemistry

The series of heterocycles, N₁-(3-substituted benzylidene)-N₄-phenyl pyrimidine-4,5-diamine [5A-5F]/ N₅-(2-substituted benzylidene)-N₂,N₂-dimethyl-N₄-phenyl pyrimidine-2,4,5-triamine [5a-5f] were synthesized by the reaction of formimidamide/ 4-(dimethylamino)benzimidamide with appropriate solution of sodium ethoxide as presented in Scheme-1. The novel compounds were characterized by FTIR, ¹H-NMR, mass spectroscopy. The IR spectrum of compounds [5A-5F]/ [5a-5f] showed bands of NH group at 3118-3385 cm⁻¹. In [5A-5F]/ [5a-5f], Ar-CH stretching bands appears at 2951-3089 cm⁻¹. The appearance of a strong intensity band in the IR spectra of compounds [5A-5F]/ [5a-5f] in the range of 3118-3385 cm⁻¹ attributable to -NH stretching and it provides strong evidence for the confirmation of the conversion chlorine to -NH. The proton magnetic resonance spectra of [5A-5F]/ [5a-5f] and their corresponding derivatives have been recorded in CDCl₃. In this [5A-5F]/ [5a-5f] has =CH linkage signals appear at 3.01-3.53 ppm respectively. The presence of =CH linkage proton signals in the ¹H-NMR spectra of final compounds confirms that, the formation benzylidene moiety Fig.-1. All these observed facts clearly envisages that the N₁-(3-substituted benzylidene)-N₄-phenyl pyrimidine-4,5-diamine [5A-5F]/ N₅-(2-substituted benzylidene)-N₂,N₂-dimethyl-N₄-phenyl pyrimidine-2,4,5-triamine [5a-5f] formation as indicated in Scheme-1 and confirms the proposed structure [5A-5F]/ [5a-5f].

Antimicrobial Screening

The antimicrobial screening results of novel N₁-(3-substituted benzylidene)-N₄-phenyl pyrimidine-4,5-diamine [5A-5F]/ N₅-(2-substituted benzylidene)-N₂,N₂-dimethyl-N₄-phenyl pyrimidine-2,4,5-triamine [5a-5f] derivatives were showed moderate to significant activity. Among these all, scaffolds 5A, 5C, 5c, and 5E showed significant activity in Tables-1 to 3 compared with the standard Ciprofloxacin and Ketoconazole, 5F, 5f, 5B, 5h, 5D, 5d, 5a and 5e showed moderate antimicrobial activity. The compounds 5A, 5C, 5c, and 5E were shown significant zone of inhibition to microbial growth against S.a (25mm, 25mm, and 25mm), E.c (26mm, 26mm, 25mm, and 26mm), P.a (23mm, 23mm, 23mm, and 23mm), K.p (25mm, 25mm, 24mm, and 26mm), A.n (21mm, 21mm, 21mm, and 23mm) and A.f (22mm, 22mm, 22mm, and 23mm) respectively.
Table 1: *In vitro* Antimicrobial Activities of the Synthesized Compounds (G⁺ve bacteria)

<table>
<thead>
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<th>Compound</th>
<th>Gram +ve Bacteria</th>
<th>S. aureus</th>
<th>S. epidermidis</th>
<th>M. luteus</th>
<th>B. cereus</th>
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</thead>
<tbody>
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<td>5A</td>
<td>4-Methoxy</td>
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<td>27(9.3)</td>
<td>25(9.7)</td>
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<td>21(10.2)</td>
<td>20(10.3)</td>
</tr>
<tr>
<td>5b</td>
<td>4-Methyl</td>
<td>22(10.2)</td>
<td>21(14.2)</td>
<td>22(11.3)</td>
<td>21(11.2)</td>
</tr>
<tr>
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<td></td>
<td>23(10.5)</td>
<td>21(14.3)</td>
<td>22(11.4)</td>
<td>20(11.5)</td>
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<tr>
<td>5d</td>
<td>4-Chloro</td>
<td>25(9.8)</td>
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<td>25(10.6)</td>
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<td>5e</td>
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<td>26(10.2)</td>
<td>25(10.3)</td>
<td>21(10.3)</td>
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<tr>
<td>5f</td>
<td>4-Bromo</td>
<td>20(10.2)</td>
<td>21(10.3)</td>
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<td>26(9.3)</td>
<td>23(9.3)</td>
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### Table-2: In *vitro* Antimicrobial Activities of the Synthesized Compounds (G^-ve bacteria)

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<th>Compound</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>K. pneumoniae</th>
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<tr>
<td>5A</td>
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<td>26(10.4)</td>
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<td>5a</td>
<td>21(12.1)</td>
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<td>21(11.4)</td>
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<tr>
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<td>4-Methyl</td>
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<td>21(11.3)</td>
</tr>
<tr>
<td>5b</td>
<td>20(10.4)</td>
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<td>4-Chloro</td>
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<td>Ciprofloxacin</td>
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<td>DMF</td>
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### Table-3: In-vitro Antimicrobial Activities of the Synthesized Compounds

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<th>A. n</th>
<th>A. f</th>
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<tbody>
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<td>4-Methoxy</td>
<td>21(10.2)</td>
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<tr>
<td>5a</td>
<td>19(11.1)</td>
<td>20(12.2)</td>
</tr>
<tr>
<td>5B</td>
<td>4-Methyl</td>
<td>18(11.3)</td>
</tr>
<tr>
<td>5b</td>
<td>19(11.2)</td>
<td>19(11.4)</td>
</tr>
<tr>
<td>5C</td>
<td>4-Chloro</td>
<td>21(11.3)</td>
</tr>
<tr>
<td>5c</td>
<td>21(11.5)</td>
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<tr>
<td>5D</td>
<td>4-Bromo</td>
<td>20(11.6)</td>
</tr>
<tr>
<td>5d</td>
<td>19(11.4)</td>
<td>19(12.4)</td>
</tr>
<tr>
<td>5E</td>
<td>4-Amino</td>
<td>23(9.4)</td>
</tr>
<tr>
<td>5e</td>
<td>19(11.1)</td>
<td>19(10.3)</td>
</tr>
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</table>
The MIC value was found between 9.3-14.3μg/mL to the tested compounds. The most sensitive range for G+ve bacteria against all the tested microorganisms with a MIC range for S.a (9.4-13.1μg/mL), S.e (9.3-13.2μg/mL), M.I (9.3-11.5μg/mL), B.c (9.4-12.3μg/mL), in G-ve, E.c (10.2-12.5μg/mL), P.a (9.2-14.2μg/mL), K.p (9.2-12.3μg/mL), and in fungi A.n (9.4-11.5μg/mL) and A.f (10.1-12.2μg/mL). The overall observation of antimicrobial data indicates that the improvement in microbial action was found with their electronic effects like electron-withdrawing or donating substitutions in pyrimidine analog. e.g., electron-donating groups (-OCH$_3$, -CH$_3$ and -NH$_2$), electron-withdrawing groups (-F, -Cl, and -Br) at -para positions on the phenyl ring. By considering the electronic effects on the biological actions, EWG was introduced 5$^\text{th}$-position on the pyrimidine ring. It was observed that N$_5$-(3-substituted benzylidene)-N$_4$-phenyl pyrimidine-4,5-diamine [5A-5F]/ N$_5$-(2-substituted benzylidene)-N$_2$N$_2$-dimethyl-N$_4$-phenyl pyrimidine-2,4,5-triamine [5a-5f] compounds showed an increase in antimicrobial activity.

**CONCLUSION**

The possible improvements of activity in the compounds N$_5$-(3-substituted benzylidene)-N$_2$-phenyl pyrimidine-4,5-diamine [5A-5F]/ N$_5$-(2-substituted benzylidene)-N$_2$N$_2$-dimethyl-N$_4$-phenyl pyrimidine-2,4,5-triamine [5a-5f] can be achieved by the substituents on the basic pyrimidine nucleus. The presence of the 4-Methoxy, 4-Chloro and amino groups on the aromatic ring has highly increased the activity of the compounds compared to other substituents of the compounds. In the view of the above findings and to identify new candidates that may value in designing novelty, selectivity, less toxicity antimicrobial agents who might serve as novel analog, we can promise here that, our synthesized analogs will generate a very good impact to the chemists and research scholars for further investigations in this field of pyrimidine and its selective influence of electronic effects as well as a change in the basic nucleus.

**REFERENCE**

6. L. Almirante, *Journal of Medicinal Chemistry* 9, 29(1966), DOI:10.1021/jm00319a007