Synthesis and antimicrobial evaluation of some novel 3,4-bis(substituted-phenyl)-7-(2,6-dichloro-4-(trifluoromethyl)phenyl)-5,7-dihydro-2H-pyrrolo[2,3-c:5,4-c']dipyrazole derivatives of 2,6-dichloro-4-trifluoro methyl aniline

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Abstract: Succinic anhydride was converted to 4-(2, 6-dichloro-4-(trifluoromethyl) Phenyl) amino)-4-oxobutanoic acid. It underwent cyclization in presence of acetyl chloride furnished 1-(2, 6-dichloro-4-trifluoromethyl-1-phenyl)-pyrrodine-2, 5-Dione. This pyrroline-2, 5-dione on condensation with substituted aromatic aldehydes in presence of acetic acid afforded (3E, 4E)-3, 4-bis (substituted benzylidene)-1-(2, 6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidione-2, 5- Dione. These derivative underwent ring closer with hydrazine hydrate afforded 3, 4-bis (substituted phenyl)-7-(2, 6-dichloro-4-(trifluoromethyl) phenyl)-5, 7-dihydro-2H-pyrole [2, 3-C; 5-4-C'] dipyrazole. All the synthesized compounds were analyzed by spectral and elemental analysis. Similarly these derivatives were screened for their microbial activity against S.aureus, E.coli, A. Alternaria and A.niger. Some of the derivatives showed potent activity against S.aureus and E.coli.

Keywords: Bis-chalcone, bis-pyrazole, anti-microbial activity.

Introduction:

Literature survey shows that the 2, 6-dichloro-4-trifluoro methyl aniline is the compound particularly used to synthesize pesticides such as fipronil1-3. Now a days the agrochemical research is at the peak of mountain. Researchers are much more attracted towards the project of pesticides synthesis. Here we have used this 2, 6-dichloro-4-trifluoro aniline for synthesis of some novel bis heterocyclic pyrazole via cyclic imide4 1-(2, 6-dichloro-4-trifluoromethyl-phenyl)-pyrrodine-2 and multifarious chalcones. Succinic anhydride was converted to 4-(2, 6-dichloro-4-(trifluoromethyl) Phenyl) amino)-4-oxobutanoic acid. It underwent cyclization in presence of acetyl chloride furnished 1-(2, 6-dichloro-4-trifluoromethyl-1-phenyl)-pyrrodine-2, 5-Dione. This pyrroline-2, 5-dione on condensation with substituted aromatic aldehydes in presence of acetic acid afforded (3E, 4E)-3, 4-bis (substituted benzylidene)-1-(2, 6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidione-2, 5- Dione. These derivative underwent ring closer with hydrazine hydrate afforded 3, 4-bis (substituted phenyl)-7-(2, 6-dichloro-4-(trifluoromethyl) phenyl)-5, 7-dihydro-2H-pyrole [2, 3-C; 5-4-C'] dipyrazole. All the synthesized compounds were analyzed by spectral and elemental analysis. Similarly these derivatives were
screened for their microbial activity against S.aureus, E.coli, A. Alternaria and A.niger. Some of the derivatives showed potent activity against S.aureus and E.coli.

Chalcones and their derivatives are known for their biological activities such as anti-bacterial\textsuperscript{5,6}, anti-ulcer\textsuperscript{7}, anti-fungal\textsuperscript{8}, anti-malarial\textsuperscript{9}, anti-cancer\textsuperscript{10} etc. They also act as precursor for many crucial heterocyclic compounds like bis heterocyclic pyrazole, isoxazole, flavones, pyrazole\textsuperscript{11-12} etc.

Now a days heterocyclic compounds are core heart in the research and development field and it covers the wide area of research especially in pharmaceutical sciences, by considering this advantage we have successfully synthesized series of novel bis heterocyclic pyrazole derivatives of 2, 6-dichloro-4-trifluoro methyl aniline.

Pyrazole is a heterocyclic organic compound having formula C\textsubscript{3}H\textsubscript{3}N\textsubscript{2}H. It is 5 membered ring which is classified by 3 carbon atoms and adjacent two nitrogen atoms.

It is reported that large number of pyrazole and their derivatives show significant biological activities. In the medicinal field pyrazole and their derivatives are utilized for various biological and pharmaceutical activities such as anti-inflammatory\textsuperscript{13}, antifungal\textsuperscript{14}, anti- analgesic\textsuperscript{15}, anti-viral\textsuperscript{16}, anti-tubercular\textsuperscript{17}, anti-convulsant\textsuperscript{18}, anti-bacterial\textsuperscript{19-20}, anti-oxidant\textsuperscript{21} etc.

Pyrazole moiety also plays valuable role in the field of cancer, Hitoshi et al synthesized Pyrimidinyl pyrazole derivatives as anti-tumor agent which is also acts as antiproliferative agent\textsuperscript{22}.

Ding et al too synthesized a series of novel 3 aryl-1—arylmethyl-1H- pyrazole-5-carboxamide derivatives which reduces A549 lung cancer cell growth\textsuperscript{23}.

The researchers utilized pyrazole moiety for synthesizing cancer drug and still broad research is continue against cancer growth cell\textsuperscript{24-27}.

**Material and Methods:**

All reagents and solvent utilized for synthesized compounds are of commercial grade, melting points are taken in open capillary method and were found uncorrected. FTIR spectra are recorded on Perkin-Elmer spectrum. H\textsuperscript{1}NMR spectra are recorded on Bruker DRX 500 MHz NMR spectrometer with DMSO-d\textsuperscript{6} as solvent and tetramethylsilane (TMS) used as internal reference (chemical shift in δ ppm) these all newly synthesized compounds were formed according to following scheme 1,2 and 4.
1. Synthesis of 1-(2, 6-dichloro-4-trifluoromethyl-phenyl)-pyrolidine-2, 5-Dione:

Succinic anhydride (0.01mol) was dissolved in 10mL benzene. Then 2, 6-dichloro-4-trifluoromethyl aniline (0.01mol) was added to it vigorously hence 4-((2, 6-dichloro-4-(trifluoromethyl) phenyl) amino)-4-oxobutanoic acid was formed. This acid was cyclized by using (0.09) mole of fresh acetyl chloride at reflux conditions. The product (4a) was obtained and recrystallized from methanol.

![Scheme I](image)

**1-(2, 6-dichloro-4-trifluoromethyl-phenyl)-pyrolidine-2, 5-Dione (4a)**: M.F: C_{11}H_{6}Cl_{2}F_{3}N, M.W: 312, Yield 90%, M.P. 165-167°C, C, H, N Elem. Anal: Calculated: C, 38.34; H, 1.94; N, 4.49. Obtained: C, 38.24; H, 1.83; N, 4.34.

**IR (KBr) cm\(^{-1}\)**: 2900-3000 cm\(^{-1}\) (CH\(_2\)), 1650-1700 cm\(^{-1}\) (C=O), 1470-1500 cm\(^{-1}\) (ArC=C), 1200-1220 cm\(^{-1}\) (C-N).

**H\(^1\) NMR (500 MHz, DMSO-d\(_6\) δ ppm)**: 2.5 (s, 4H), 7.7 (s, 2H, Ar-H).

2. Synthesis derivatives of chalcones (6a-e) : Cyclic imide 1-(2,6-dichloro-4-trifluoromethyl-phenyl)-pyrolidine-2,5-Dione (0.01 mol) and aromatic aldehyde (5a-e) (0.02 mol) was dissolved in glacial acetic acid (8 ml) then concentrated it on sand bath maintaining low flame. The colorless solid product was obtained (6a-e) and recrystallized from ethanol.

![Scheme 2](image)

IR (KBr) cm^{-1}: 3010-3050 cm^{-1} (C=C-H), 1520-1630 cm^{-1} (C=C), 1650-1700 cm^{-1} (C=O), 1425-1600 cm^{-1} (ArC=C), 1200-1220 cm^{-1} (N-C=O), 3200-3550 cm^{-1} (Ar-OH).

H{\textsuperscript{1}} NMR (500 MHz, DMSO-d{\textsuperscript{6}} \delta ppm): 8.08 (s, 2H, 2C=CH-Ar), 7.7 (s, 2H, Ar-H), 7-6.8 (8H dd 2Ar-H).

ii) (3E,4E)-3,4-bis(4-bromobenzylidene)-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)pyrrolidine-2,5-dione (6b): M.F: C_{28}H_{12}BrCl_{3}F_{3}NO_{2}, M.W: 646, Yield 90%, M.P. 191-193°C, C, H, N Elem. Anal. Calculated: C, 46.48; H, 1.87; N, 2.17. Obtained: C, 46.40; H, 1.83; N, 2.47.

IR (KBr) cm^{-1}: 3010-3050 cm^{-1} (C=C-H), 1520-1630 cm^{-1} (C=C), 1650-1700 cm^{-1} (C=O), 1425-1600 cm^{-1} (ArC=C), 1200-1220 cm^{-1} (N-C=O), 3200-3550 cm^{-1} (Ar-OH).

H{\textsuperscript{1}} NMR (500 MHz, DMSO-d{\textsuperscript{6}} \delta ppm): 8.01 (s, 2H, 2C=CH-Ar), 7.9 (s, 2H, Ar-H), 7.5-7.8 (8H dd, 8H, 2Ar-H).

iii) (3E,4E)-3, 4-bis (2-hydroxybenzylidene)-1-(2, 6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidine-2, 5-dione (6c): M.F: C_{28}H_{14}Cl_{2}F_{3}NO_{4}, M.W: 520, Yield 92%, M.P. 160-162°C, C, H, N Elem. Anal. Calculated: C, 57.71; H, 2.71; N, 2.69. Obtained: C, 57.68; H, 2.69; N, 2.67.

IR (KBr) cm^{-1}: 3010-3050 cm^{-1} (C=C-H), 1520-1630 cm^{-1} (C=C), 1650-1700 cm^{-1} (C=O), 1425-1600 cm^{-1} (ArC=C), 1200-1220 cm^{-1} (N-C=O), 3200-3550 cm^{-1} (Ar-OH).

H{\textsuperscript{1}} NMR (500 MHz, DMSO-d{\textsuperscript{6}} \delta ppm): 7.75 (s, 2H, 2C=CH-Ar), 7.77 (s, 2H, Ar-H), 6.9-8.08 (dd, 8H, 2Ar-H), 9.7 (s, 1H, Ar-O-H).

iv) (3E,4E)-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-3,4-bis(4-hydroxy-3-methoxybenzylidene)pyrrolidine-2,5-dione (6d): M.F: C_{27}H_{16}Cl_{2}F_{3}NO_{6}, M.W: 580, Yield 94%, M.P. 91-93°C, C, H, N Elem. Anal. Calculated: C, 55.88; H, 3.13; N, 2.41. Obtained: C, 58.86; H, 3.10; N, 2.43.

IR (KBr) cm^{-1}: 3010-3050 cm^{-1} (C=C-H), 1520-1630 cm^{-1} (C=C), 1650-1700 cm^{-1} (C=O), 1425-1600 cm^{-1} (ArC=C), 1200-1220 cm^{-1} (N-C=O), 3200-3550 cm^{-1} (Ar-OH), 1000-1300 cm^{-1} (O-CH), 3300 cm^{-1} (C-H).

H{\textsuperscript{1}} NMR (500 MHz, DMSO-d{\textsuperscript{6}} \delta ppm): 8.006 (s, 2H, 2C=CH-Ar), 7.4 (s, 2H, Ar-H), 6.9-7.4(s, 6H, 2Ar-H), 9.7 (s 1H, Ar-O-H), 3.85 (s, 3H, O-CH_{3}).

v) (3E, 4E)-3, 4-bis (4-nitrobenzylidene)-1-(2, 6-dichloro-4-(trifluoromethyl) phenyl) pyrrolidine-2, 5-dione (6e): M.F: C_{25}H_{12}Cl_{2}F_{3}N_{3}O_{6}, M.W: 574, Yield 89%, M.P. 141-143°C, C, H, N Elem. Anal. Calculated: C, 51.92; H, 2.09; N, 7.27. Obtained: C, 51.90; H, 2.1; N, 7.30.

IR (KBr) cm^{-1}: 3010-3050 cm^{-1} (C=C-H), 1520-1630 cm^{-1} (C=C), 1650-1700 cm^{-1} (C=O), 1425-1600 cm^{-1} (ArC=C), 1200-1220 cm^{-1} (N-C=O), 1550-1600 cm^{-1} (N=O)

H{\textsuperscript{1}} NMR (500 MHz, DMSO-d{\textsuperscript{6}} \delta ppm): 7.99 (s, 2H, 2C=CH-Ar), 8.17 (s, 2H, Ar-H), 8.1-8.4 (dd, 8H, 2Ar-H).

2. Synthesis of derivatives of Pyrolozap (4a-e): Dissolved chalcones derivatives (4a-e) (0.01 mol) in ethanol (8 ml) then add Hydrazine monohydrate (0.02 mol), reflux this mixture for next 16 hours with maintaining 80°C to 90°C temperature on water bath. The precipitated coloured solid compounds were obtained, recrystallized from benzene.
i) 3,4-bis(4-chlorophenyl)-7-(2,6-dichloro-4-(trifluoromethyl)phenyl)-5,7-dihydro-2H-pyrrolo[2,3-c:5,4-c']dipyrazole (8a): M.F: C_{25}H_{12}Cl_{2}F_{3}N_{5} O_{2}, M.W: 581, Yield 80%, M.P. 171-173°C, C, H, N Elem. Anal. Calculated: C, 55.16; H, 2.59; N, 12.87. Obtained: C, 51.68; H, 2.03; N, 12.01.

**IR (KBr) cm^{-1}:** 3300-3400 cm^{-1} (N-H), 1520-1630 cm^{-1} (C=C), 1000-1250 cm^{-1} (C-N), 1425-1600 cm^{-1} (ArC=C).

**H¹ NMR (500 MHz, DMSO-d⁶ δ ppm):** 10.16 (s, 2H, N-H), 8.06 (s, 2H, Ar-H), 7.4-7.9 (dd, 8H, 2Ar-H).

ii) 3,4-bis(4-bromophenyl)-7-(2,6-dichloro-4-(trifluoromethyl)phenyl)-5,7-dihydro-2H-pyrrolo[2,3-c:5,4-c']dipyrazole (8b): M.F: C_{25}H_{12}Br_{2}Cl_{2}F_{3}N_{5} O_{2}, M.W: 670, Yield 78%, M.P. 161-163°C, C, H, N Elem. Anal. Calculated: C, 49.88; H, 1.80; N, 10.25. Obtained: C, 49.84; H, 1.79; N, 10.27.

**IR (KBr) cm^{-1}:** 3300-3400 cm^{-1} (N-H), 1520-1630 cm^{-1} (C=C), 1000-1250 cm^{-1} (C-N), 1425-1600 cm^{-1} (ArC=C).

**H¹ NMR (500 MHz, DMSO-d⁶ δ ppm):** 10.16 (2H s, N-H), 8.1 (2H, Ar-H), 7.5-7.8 (dd, 8H, 2Ar-H).

iii) 2,2'-7-(2,6-dichloro-4-(trifluoromethyl)phenyl)-5,7-dihydro-2H-pyrrolo[2,3-c:5,4-c']dipyrazole-3,4-diyldiphenol (8c): M.F: C_{25}H_{12}Cl_{2}F_{3}N_{5} O_{2}, M.W: 543, Yield 80%, M.P. 233-235°C, C, H, N Elem. Anal. Calculated: C, 55.16; H, 2.59; N, 12.87. Obtained: C, 55.19; H, 2.69; N, 12.91.

**IR (KBr) cm^{-1}:** 3300-3400 cm^{-1} (N-H), 1520-1630 cm^{-1} (C=C), 1000-1250 cm^{-1} (C-N), 1425-1600 cm^{-1} (ArC=C).

**H¹ NMR (500 MHz, DMSO-d⁶ δ ppm):** 10.16 (2H s, N-H), 8.0 (2H, Ar-H), 7.5-7.9 (dd, 8H, 2Ar-H) 9.8 (s, 1H, Ar-O-H).


**IR (KBr) cm^{-1}:** 3300-3400 cm^{-1} (N-H), 1520-1630 cm^{-1} (C=C), 1000-1250 cm^{-1} (C-N), 1425-1600 cm^{-1} (ArC=C).
H¹ NMR (500 MHz, DMSO-d⁶ δ ppm): 10.16 (2H s, N-H), 8.0 (2H s, Ar-H), 7.5-7.9 (s, 2H, dd, 4H, 2Ar-H), 9.8 (s, 1H, Ar-O-H), 3.8 (s, 3H, O-CH₃).


IR (KBr) cm⁻¹: 3300-3400 cm⁻¹ (N-H), 1520-1630 cm⁻¹ (C=C), 1000-1250 cm⁻¹ (C-N), 1425-1600 cm⁻¹ (ArC=C).

H¹ NMR (500 MHz, DMSO-d⁶ δ ppm): 10.16 (s, 2H, N-H), 7.8 (s, 2H, Ar-H), 8.0-8.2 (dd, 8H, 2Ar-H).

Antimicrobial Activity (8a-e)

All the synthesized compounds (8a-e) were screened for their in vitro antimicrobial activity against bacteria and fungi such as Staphylococcus aureus, Escherichia coli, Alternaria alternata, and Aspergillus niger using paper disc diffusion method by aiding DMSO solvent.

Stock solution (100 microgram per ml) of each was compound prepared in DMSO solvent. Similarly stock solution of standard drug ciprofloxacin used for antibacterial activity and terbinafine used for antifungal activity had been prepared. Microbiological media used for bacteria is nutrient agar (Hi media) and potato dextrose agar (Hi-media) for fungi. Concentration 100µg/ml per well poured as per well diffusion method and incubated for 24 hours at 37°C after incubation the results were obtained, where the compounds showed activity there was zone of inhibition occurred, similarly for fungi stock solution 100µg/ml per well poured as per well diffusion method and incubated for next seven days at 29°C after seven days were results noted. The diameter of zone of inhibition were measured by Vernier Caliper in mm and tabulated in table I.

**Table 1 Antimicrobial activity of compounds 8a-e**

<table>
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<th>Sr.No.</th>
<th>Sample Code</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>A. alternata</th>
<th>A.niger</th>
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<td>1</td>
<td>8a</td>
<td>-</td>
<td>31</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>8b</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>3</td>
<td>8c</td>
<td>-</td>
<td>47</td>
<td>-</td>
<td>-</td>
</tr>
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<td>4</td>
<td>8d</td>
<td>46</td>
<td>-</td>
<td>-</td>
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<td>8e</td>
<td>10</td>
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<td>-</td>
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<tr>
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<tr>
<td>8</td>
<td>DMSO (control)</td>
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<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Keyword: ‘-‘ means no zone of inhibition, NA means not applicable Graphical comparison of antimicrobial activity 

Graph I: Antibacterial activity comparison with standard drugs

Graph II: Antifungal activity comparison with standard drug

Result and Discussion

Starting imide 1-(2, 6-dichloro-4-trifluoromethyl-phenyl)-pyrrolidine-2, 5-Dione (4a) was successfully synthesized and confirmed by $^1$H NMR and FTIR spectroscopy. This imide and different benzaldehydes (5a-e) were used to synthesize multifarious chalcones (6a-e), these multifarious chalcones were cyclized by hydrazine monohydrate and furnished various pyrazoles. All these compounds analyzed by spectral analysis and elemental analysis technique.
Antibacterial activity:

All synthesized compounds were screened for their in vitro antimicrobial activity against one Gram positive bacterial strains and one fungal strain i.e. *Staphylococcus aureus*, *Alternaria alternata* and one Gram negative bacterial strain and one fungal strain i.e. *Escherichia coli*, *Aspergillus niger* respectively. Some compounds were showed good activity and some showed moderate activity against these microorganism, Ciprofloxacin and terbinfine were used as standard drugs for bacteria and fungi respectively.

Conclusion:

All compounds have been successfully prepared and characterized by elemental analysis and spectroscopy techniques (H\(^1\)NMR and FTIR). Compounds 8a-e were screened for their in vitro antimicrobial activity, compound 8a and 8c showed potent activity against *Escherichia coli* and compounds 8b, 8d and 8e also showed good activity against *Staphylococcus aureus*.

In these compounds 8b, 8d and 8e, compound 8d exhibited good activity against *Aspergillus niger*.

Acknowledgment:

Authors thank to UGC-MANF, New Delhi (award letter: F1-17.1/2013-14 MUS-MAH-25825/ (SA-III/Website) dated 06-Feb-2014) for financial support, authors are thankful to university of Pune for providing spectral analysis facilities. Authors are also thankful to department of Chemistry and department of Microbiology of PSGVPM’S ASC College, Shahada for providing research laboratory facility and microbial screening facility.


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