Synthesis and Biological Evaluation of Fused Pyrrolo–
Pyran– Pyrimidine and Pyrrolo– Pyran– Pyridine
Derivatives

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Abstract
New Fused Pyrrolo – Pyran – Pyrimidine, and Pyrrolo – Pyran – Pyridine Derivatives were synthesized and characterized
by FT-IR and 1HNMR, to obtain these cycles, 2-amino-4-(4-(dimethylamino)phenyl)-7-methyl-4,5,6,7-tetrahydropyrano[2,3-
b]pyrrole - 3-carbonitrile [1]. which was synthesized by cyclization of compound 4-(dimethylamino)benzaldehyde with
malononitrile and N-methyl-2-pyrrolidinone in ethanol in the presence of tetraethylammonium bromide as a catalyst. Reaction
[1] with triethylorthoformate in acetic anhydride gave ethyl(E)-N-(3-cyano-4-(4-(dimethylamino) phenyl)-7-methyl-4,5,6,7-tetra
hydropryro-[2,3-b]pyrolyl-2-yl)formimidate [2].
Compounds [3], [4] and [5] were synthesized by cyclization of compound [2] with hydrazine hydrate, ethylenediamine,
and phenylhydrazine respectively in methanol. Other cycles [7,8] were synthesized by hydrolysis of [1] with alcoholic solution
of KOH in dry DMF containing concentrated HCl to 5-amino-1-phenyl-1H-pyrazole-4-carboxamide [6], then cyclization with
4(dimethylamino) benzaldehyde and 3,5-dinitrobenzaldehyde, and gave compounds [7] [ 8] respectively, cyclization of (1) with
benzoyl chloride in presence of pyridine gave [9].
Pyrrolo – Pyran – Pyridine cycles [10-13] were synthesized by cyclization [1] with some of the aliphatic ketones namely;
acetone, 2-butaneone and 4-methyl-2-pentanone and cyclohexanone, respectively in presence of FeCl3 as catalysis and NaOH.
These compounds were evaluated biological against (gram +ve) bacteria (Staphylococcus aureus and Staphylococcus
epidermidis), and (gram – ve) bacteria (Escherichia coli and Klebsiella pneumonia); and antifungal activity against (Candida
albicans) and the data obtained show that all compounds show activity against all bacteria and candida.

Key words: pyrmidine, cyclic fused, biological activity

INTRODUCTION
Pyrimidine derivatives and heterocyclic annealed pyrimidines continue to attract great interest due to the
wide variety of interesting biological activities observed for
these compounds, such as antiviral[1] anticance[2]
antitumor[3], anti-inflammatory[4], antimicrobial[5],
antifungal[6], antihistaminic[7], anti-HIV[8], antitubercular[9],
anti-neoplastic[10], antihypertensive[11], antibacterial[12],
antidiabetic[13], antioxidants[14], antileishmanial[15],
diuretic[16], analgesic[17] activities, and cardio-protective
effects[18], and many of pyrimidines derivatives are reported
to possess potential central nervous system (CNS)
depressant properties[19] and also act as calcium channel
blockers[20].
Pyrrolopyrimidines is a class of fused pyrimidine scaffolds
which was found to exhibit good pharmacological
properties. So, one can improve the activity of heterocyclic
compounds by fusing the pyrimidine analogs with different
heterocyclic moieties[21].
Pyridopyrimidine derivatives were recognized for their
anticonvulsant and antidepressant activities but showed no
neurotoxicity[22]. Some novel 2-heterocycle-substituted
phenothiazines having a pyrazolo[3,4-d]pyrimidine (I) nucleus
were achieved by using the biginelli multi-component
cyclocondensation reaction. The products were evaluated
for their antitubercular activity against Mycobacterium tuberculosis H37 Rv[23]. Hamid H.M. and cowokers[24] synthesized 9-amino-8-imino-7-(naphthalene-
1-yl)-8,9-dihydrochremeno[3,4:5,6]pyrano[2,3-d]
pyrimidine-6-one (II) and methyl-7-(naphthalene-1-yl)
chremeno[3,4:5,6]pyrano[2,3-d]pyrimidin-6,8-dione (111)
and tested for their in vitro anti-HIV-1 (strain IIIB) and
HIV-2 (strain ROD) activity in human (MT-4) cells.
The aim of this work synthesis new fused cyclic compounds, fused pyrrolo – pyrano – pyrimidine and pyrrolo – pyrano – pyridine derivatives and study the biological activity.

2. EXPERIMENTAL

2.1. Chemical materials
All reactants and solvents used in this study were reagents grade and they are available from Sigma-Aldrich and Fluka companies. Melting points are determined in open capillary tubes in a German, Stuart, SMP30 Melting points apparatus and are uncorrected. Infrared spectra (FT-IR) were recorded using a SHIMADZU FT-IR8400S spectrophotometer at the Department of Chemistry/College of Science/University of Mustansiriyah. 1HNMR spectra were recorded on a Bruker, Ultra Shield 400Mhz, spectrometer (Switzerland) using DMSO-d$_6$ and CDCl$_3$ as a solvent with a tetramethylsilane (TMS) as an internal standard, at the Iran polymer & Petrochemical institute. All progress of the reactions and checking the purity were performed with thin layer chromatography (TLC) technique and revealed by a mixture of n-hexane and ethyl acetate (3: 2) as eluent in the mobile phase.

2.2. Synthesis of compounds

2.2.1. Synthesis of 2-amino-4-(4-(dimethylamino)phenyl)-7-methyl-4,5,6,7-tetrahydropyranro[2,3-b]pyrrrole-3-carbonitrile [1]
In a typical procedure, equimolar amounts of 4-(dimethylamino) benzaldehyde (1.49g, 0.01mol), malononitrile (0.66g, 0.01mol) and N-methyl-2-pyrrolidinone (0.99g, 0.01mol) were mixed with tetrachloromonomium bromide (10mol %) in (15ml, 90%) of ethanol and refluxed with stirring for 95min. After the completion of the reaction, the mixture was cooled to room temperature and poured into the ice to get the crude products. The crude products were purified by recrystallization from 1,4-dioxane to give [1].

The physical properties of compound [1] are listed in table (1).

2.2.2. Synthesis of ethyl(E)-N-(3-cyano-4-(4-(dimethylamino) phenyl) -7-methyl-4,5,6,7-tetrahydropyranro[2,3-b]pyrrrole-2-yl) formimidate [2]
A mixture of compound [1] (2.96g, 0.01mol) and triethylorthoformate (1.48g, 0.01mol) and acetic anhydride (16ml) was refluxed for 5hrs. The solvent was removed under reduced pressure and the resulting solid product is recrystallized from benzene to give [2].

The physical properties of compound [2] are listed in table (1).

2.2.3. Synthesis of 5-(4-(dimethylamino)phenyl)-4-imino-8-methyl-5,6,7,8-tetrahydropyrrrolo[3',2':5,6]pyrano[2,3-b]pyrrrole-3-carboxamide[6]
To (100ml) of an alcoholic solution of KOH (5%) (2.96g, 0.01mol) of [1] was added and the reaction mixture was refluxed for 30min. After cooling the reaction mixture was diluted with water and the formed solid was filtered off, washed with water and recrystallized from DMF to give [6].

The physical properties of compound [6] are listed in table (1).

2.2.4. Synthesis of 2-amino-4-(4-(dimethylamino)phenyl)-7-methyl-5,6,7-tetrahydropyrrrolo[2,3-b]pyrrrole-3-carboxamide[6]
To (100ml) of an alcoholic solution of KOH (5%) (2.96g, 0.01mol) of [1] was added and the reaction mixture was refluxed for 30min. After cooling the reaction mixture was diluted with water and the formed solid was filtered off, washed with water and recrystallized from DMF to give [6].

The physical properties of compound [6] are listed in table (1).

2.2.5. Synthesis of 2,5-bis(4-(dimethylamino)phenyl)-8-methyl-5,6,7,8-tetrahydropyrrrolo[3',2':5,6]pyrano[2,3-d]pyrimidin-4(3H)-one [7];  and 5-(4-(dimethylamino)phenyl)-2-(3,5-dinitrophenyl)-8-methyl-5,6,7,8-tetrahydro pyrrrolo[3',2':5,6]pyrano[2,3-d]pyrimidin-4(3H)-one [8]
A mixture of [6] (3.14g, 0.01mol) and 4-(dimethylamino) benzaldehyde (1.49g, 0.01mol) or 3,5-dinitrobenzaldehyde (1.96g, 0.01mol) in dry DMF (25ml) containing concentrated HCl (0.2ml) was refluxed for 24hrs. The reaction mixture was cooled filtered and the precipitate was crystallized from ethanol to give compounds [7], and [8] respectively. The physical properties of compounds [7], and [8] are listed in the table (1).

A mixture of compound [1] (2.96g, 0.01mol) and benzoyl chloride (1.40g, 0.01mol) in pyridine (20ml) was refluxed for 18hrs. The solid product formed upon pouring onto iced-water is collected by filtration and recrystallized from benzene to give [9].

The physical properties of compound [9] are listed in the table (1).

2.2.7. Synthesis of 4-(4-(dimethylamino)phenyl)-1,7-dimethyl-1,2,3,4-tetrahydropyrrrolo[3',2':5,6]pyrano[2,3-b]pyrridine-5-amine [15]; 4-(4-(dimethylamino)phenyl)-1,6,7-trimethyl-1,2,3,4-tetrahydropyrrrolo[3',2':5,6]pyrano[2,3-b]pyrridine-5-amine [16]; 4-(4-(dimethylamino)phenyl)-6-isopropyl-1,7-dimethyl-1,2,3,4-tetrahydropyrrrolo[3',2':5,6]pyrano[2,3-b]pyrridin-5-amine [17]; and 4-(4-(dimethylamino)phenyl)-1-methyl-1,2,3,4,5,6,7,8-octahydropyrrolo[3',2':5,6]pyrano[2,3-b]quinolin-5-amine [18]
A solution of [2] (3.52g, 0.01mol) in methanol (25ml), a solution of hydrazine hydrate (0.5g, 0.01mol), or ethylendiamine (0.6g, 0.01mol), or phenyldihydrazine (1.08g, 0.01mol) was added and the mixture stirred for 1hr. Then it is allowed to stand overnight. The precipitate formed is filtered, dried and crystallized from 1,4-dioxane to give compounds [3], [4] and [5] respectively.

The physical properties of compounds [3], [4] and [5] are listed in the table (1).
with CHCl₃ (3x8ml), the organic layers were combined and dried over Na₂SO₄. The solvent was evaporated under reduced pressure and recrystallized from ethanol/water to give compounds [10], [11], [12], and [13] respectively. The physical properties of compounds [10], [11], [12], and [13] are listed in the table (1).

2.3. Biological activity

Applying the agar plate diffusion technique[25] some of the synthesized compounds were screened in vitro for antibacterial activity against (gram +ve) bacteria (Staphylococcus aureus and Staphylococcus epidermidis), and (gram –ve) bacteria (Escherichia coli and Klebsiella pneumiae); and antifungal activity against (Candida albicans). Prepared agar and petri-dishes were sterilized by autoclaving for (15 min) at 121°C. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all (6 mm) in diameter, were filled with (100 µl) of the prepared compounds. The synthesized compounds [1], [3], [5], [6], [10], [11], [12] and [13] were dissolved in DMSO in concentration (10⁻³ mol.L⁻¹).

These plates were incubated at (37°C) for (24hrs.). The inhibition zones caused by the various compounds on the bacteria were examined as in the table (7).

<table>
<thead>
<tr>
<th>Comp.</th>
<th>M.F</th>
<th>M.W gm/mole</th>
<th>Rec. solvent</th>
<th>Rf</th>
<th>Yield(%)</th>
<th>Color</th>
<th>m.p/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>C₁₇H₂₃N₂O₆</td>
<td>296.37</td>
<td>1,4-dioxane</td>
<td>0.72</td>
<td>92</td>
<td>orange</td>
<td>178-180</td>
</tr>
<tr>
<td>[2]</td>
<td>C₂₀H₂₆N₆O₄</td>
<td>352.43</td>
<td>benzene</td>
<td>0.64</td>
<td>77</td>
<td>brown</td>
<td>162-164</td>
</tr>
<tr>
<td>[3]</td>
<td>C₁₈H₂₄N₄O₄</td>
<td>338.41</td>
<td>1,4-dioxane</td>
<td>0.91</td>
<td>80</td>
<td>yellow</td>
<td>261-263</td>
</tr>
<tr>
<td>[4]</td>
<td>C₂₀H₂₈N₆O₆</td>
<td>366.46</td>
<td>1,4-dioxane</td>
<td>0.89</td>
<td>76</td>
<td>reddish brown</td>
<td>232-234</td>
</tr>
<tr>
<td>[5]</td>
<td>C₂₀H₂₈N₆O₆</td>
<td>414.51</td>
<td>1,4-dioxane</td>
<td>0.51</td>
<td>84</td>
<td>light brown</td>
<td>140-142</td>
</tr>
<tr>
<td>[6]</td>
<td>C₁₇H₂₃N₂O₆</td>
<td>314.38</td>
<td>DMF</td>
<td>0.20</td>
<td>81</td>
<td>light brown</td>
<td>211-213</td>
</tr>
<tr>
<td>[7]</td>
<td>C₂₀H₂₄N₄O₄</td>
<td>443.54</td>
<td>ethanol</td>
<td>0.63</td>
<td>59</td>
<td>brown</td>
<td>121-123</td>
</tr>
<tr>
<td>[8]</td>
<td>C₂₀H₂₄N₄O₄</td>
<td>490.47</td>
<td>ethanol</td>
<td>0.68</td>
<td>67</td>
<td>dark brown</td>
<td>187-189</td>
</tr>
<tr>
<td>[9]</td>
<td>C₂₀H₂₄N₄O₄</td>
<td>400.48</td>
<td>benzene</td>
<td>0.57</td>
<td>77</td>
<td>dark brown</td>
<td>88-90</td>
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<tr>
<td>[10]</td>
<td>C₂₀H₂₄N₄O₄</td>
<td>336.43</td>
<td>ethanol /water</td>
<td>0.23</td>
<td>78</td>
<td>light brown</td>
<td>72-74</td>
</tr>
<tr>
<td>[12]</td>
<td>C₂₀H₂₄N₄O₄</td>
<td>376.50</td>
<td>ethanol /water</td>
<td>0.82</td>
<td>62</td>
<td>brown</td>
<td>104-106</td>
</tr>
<tr>
<td>[13]</td>
<td>C₂₀H₂₄N₄O₄</td>
<td>376.50</td>
<td>ethanol /water</td>
<td>0.82</td>
<td>62</td>
<td>brown</td>
<td>104-106</td>
</tr>
</tbody>
</table>

Table (1) : The physical properties of compounds

Scheme (1): Synthesis of compounds (1-9)
NH₂

N

compound [1] was reacted with triethyl orthoformate in tetraethylammonium bromide as phase transfer catalysis, phenylhydrazine to give fused pyrimidine derivatives [3-5].

To synthesise these compounds, compound (1) was reacted with triethyl orthoformate in acetic anhydride to give imidoformate derivative [2], the compound [2] was reacted for one hour at room temperature with hydrazine hydrate, ethylenediamine and acetic anhydride to gave imidoformate derivative [2], the compound [2] was reacted for one hour at room temperature with hydrazine hydrate, ethylenediamine and phenylhydrazine to give fused pyrimidine derivatives [3-5].

These compounds were characterized by FT-IR and ¹H NMR, the FTIR of compound [1], shows disappearing stretching band of (NH₂) at 3335, 3433 cm⁻¹ for symmetrical (C=O), 1612 cm⁻¹ for (C=N), 1564 cm⁻¹ for (C=C) groups gave a good indication to successful all cyclization reactions.

3. RESULTS AND DISCUSSION

All derivatives in this work were prepared by cyclization reaction between NH₂ and C≡N with different reagent through nucleophilic substitution and disappearance of stretching bands of (NH₂ and C≡N) groups gave a good indication for successful all cyclization reactions.

3.1. Synthesize and characterization of fused pyrrolo-pyrano-pyrimidine derivatives

3.1.1. Synthesize and characterization of compounds [1-9]

To synthesise these compounds, compound (1) was selected as starting compound and it synthesized from reaction malononitrile, N,N-dimethylbenzaldehyde and 1-methyl pyrrolidine-2-one in presence of FeCl₃ and NaOH, then it react with substituted aromatic aldehyde, such as N,N-dimethylaldehyde, and 3,5-dinitroaldehyde to give [7] and [8] respectively, while the reaction [1] with benzyl chloride in presence of pyridine gave [9]. Compounds [6-9] were characterized by FT-IR and ¹H NMR, the FTIR of compound [6], shows disappearance of stretching bands (C≡N) at 2208 cm⁻¹, appearance stretching bands at 3325, 3427 cm⁻¹ for symmetrical and unsymmetrical (NH₂), 3205 cm⁻¹ for (C=C-H) aromatic, 2854-2935 cm⁻¹ for (C-H) aliphatic, 1699 cm⁻¹ for (C=O amid), 1608 cm⁻¹ for (C=N), 1560 cm⁻¹ for (C=C). The FTIR of compounds [7-9], shows disappearance stretching band of (NH₂), and the other characteristic bands show in the table (3).

The ¹H NMR spectrum of compound [9], shows signals at δ = 2.28 ppm (s, 3H, NCH₃), δ = 2.39-2.49 ppm (t, 2H, CH₂), δ = 2.66-2.75 ppm (t, 2H, NCH₂), δ = 2.98 ppm (s, 6H, N(CH₃)₂), δ = 4.38 ppm (s, 1H, CH), δ = 6.69-7.25 ppm (m, 4H, ArH) (N, N-dimethyl benz. ring), δ = 7.33-7.98 ppm (m, 5H, ArH) (benz. ring) and δ = 11.28 ppm (s, 1H, NH).

3.2. Synthesize and characterization of fused pyrrolo-pyrano-pyridine [10-13]

Table (2) : The bands of compounds [3-5]

Table (3) : The bands of compounds [7 – 9]

![Scheme (2): Synthesis of compounds (10-13)](image-url)
Compound [10-13] were synthesized by treatment (1) with some aliphatic ketones such as acetone, 2-butanoine, 4-methyl-2-pentanone and cyclohexanone respectively in presence FeCl₃. Compounds [10-13] were characterized by FT-IR and ¹H-NMR, the FTIR of compound [10-13], shows disappearance stretching band of (NH₂) at 3335, 3433 cm⁻¹, (C≡N) at 2208 cm⁻¹, and the other characteristic bands show in the table (4).

### Table (4) : The bands of compounds [10-13]

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Stretching bands (cm⁻¹)</th>
<th>NH₂</th>
<th>C-H aром.</th>
<th>C-H aliph.</th>
<th>C=N</th>
<th>C=C</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3363, 3400</td>
<td>3111</td>
<td>2841-2955</td>
<td>1594</td>
<td>1579</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3363, 3462</td>
<td>3203</td>
<td>2850-2960</td>
<td>1654</td>
<td>1595</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3319, 3416</td>
<td>3192</td>
<td>2850-3072</td>
<td>1593</td>
<td>1558</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>3331, 3423</td>
<td>3213</td>
<td>2852-3080</td>
<td>1641</td>
<td>1610</td>
<td></td>
</tr>
</tbody>
</table>

The ¹H-NMR spectrum of compound [13], shows signals at δ = 1.42-1.55 ppm (m, 2H, CH₂a), δ = 1.59-1.74 ppm (m, 2H, CH₂b), δ = 2.27 ppm (s, 3H, NCH₃), δ = 2.39 ppm (t, 2H, CH₂c), δ = 2.52 ppm (t, 2H, CH₂CH₂N), δ = 2.63 ppm (t, 2H, NCH₂), δ = 2.75 ppm (t, 2H, CH₂d), δ = 2.87 ppm (s, 6H, N(CH₃)₂), δ = 3.98 ppm (s, 2H, NH₂), δ = 4.35 ppm (s, 1H, CH), δ = 6.62-7.24 ppm (m, 4H, ArH).

### 3.4. Biological activity

The results of the preliminary screening test are listed in (table 5) and the data obtained showed all compounds have biological activity against bacteria and candida and compounds [3], [5], [11] and [12] have highest activity than other compounds against all bacteria and candida, and other compounds gave slight activity against some bacteria and candida. It's found that compounds [5] and [6] have the highest activity against Staphylococcus aureus, compounds [12] are found to have the highest activity against Staphylococcus epidermidis, compounds [13] are found to have the highest activity against Escherichia coli, while the other compounds show either slight or no activity at all. This high activity due to the presence of poly heterocyclic which contains heteroatoms such as nitrogen, oxygen, and sulfur.

### Table (5) : Antibacterial activities of some of synthesized compounds

<table>
<thead>
<tr>
<th>Comp. symbol</th>
<th>Gram positive</th>
<th>Gram negative</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>S. epidermidis</td>
<td>E. coli</td>
</tr>
<tr>
<td>[1]</td>
<td>12</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>[3]</td>
<td>17</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>[5]</td>
<td>19</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>[6]</td>
<td>20</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>[10]</td>
<td>12</td>
<td>10</td>
<td>16</td>
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<tr>
<td>[12]</td>
<td>18</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>[13]</td>
<td>14</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

*Zone diameter of growth inhibition (mm) after 24 hours, at the conc. 1x10⁻⁷ mol.L⁻¹ in DMSO.

### REFERENCES

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