Synthesis and Molecular Docking Studies of N,N-Dimethyl Arylpyranopyrimidinedione Derivatives

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Received: 05.10.2021
Accepted after revision: 15.12.2021
Published online: 10.01.2022
DOI: 10.1055/s-0040-1719869; Art ID: so-2021-d0050-op

Abstract The synthesis of N,N-dimethyl arylpyranopyrimidinedione derivatives from aromatic aldehydes, N-methyl-1-(methylthio)-2-nitroethamine (NMSM) and 1,3-dimethyl barbituric acid, in the presence of piperidine as a catalyst, is reported. The reaction mechanism involves a Knoevenagel condensation, followed by Michael addition and intramolecular O-cyclization reaction sequence. The synthesized compounds were docked with human kinesin Eg5 protein to calculate binding energy, inhibition constant and H-bond interaction. All the compounds show good binding affinity towards the protein, with significant docking score.

Keywords N,N-dimethyl arylpyranopyrimidinedione, Knoevenagel condensation, Michael addition, O-cyclization, docking studies

Heterocyclic compounds are central to a wide range of bioactive molecules. Multicomponent reactions have attracted much attention from medicinal chemists due to the potential for rapid synthesis of complex molecules without isolation of intermediates. Furthermore, there is much interest in joining two privileged heterocyclic moieties into one molecular framework with the aim of obtaining a compound with combined bioresponses. This strategy has potential to lead to polyfunctional heterocycles in a green chemistry paradigm. The major challenge in such a synthetic approach is to construct complex molecules with diverse functionality in as few synthetic steps as possible.

The pyran heterocyclic moiety is present in natural products such as carbohydrates, alkaloids, antibiotics and pheromones. Based on these facts, and in continuation of our work on heterocyclic chemistry, it was decided to fuse a pyran moiety with a pyrimidine ring system with the aim of minimizing the number of synthetic and purification steps. N-Methyl-1-(methylthio)-2-nitroethamine (NMSM) is a versatile building block in organic synthesis as the presence of both electron-donating as well as electron-withdrawing groups leads to unique chemical properties that are highly useful for the preparation of a range of heterocyclic rings. For example, NMSM is used in the preparation of the pharmaceutically important drugs Nizatidine® and Ranitidine®.

Barbituric acid, which contains a pyrimidine ring system, acts as a key construction building block for the synthesis of drugs such as hypnotics, sedatives, and CNS depressants. It is proposed that the combination of a barbituric acid moiety with another heterocycle may yield new heterocyclic scaffolds with enhanced biological effects. Similarly, chromenes and related heterocycles are present in various natural as well as synthetic products with promising bioactivities.

Shehab et al. have reported nanoparticle-catalysed synthesis and biological application of pyranopyrimidine derivatives and their nucleoside analogues. Reddy et al. reported the synthesis, antiproliferative activity and docking studies of thiazole/benzothiazole-fused pyranopyrimidine derivatives. Ouf et al. have studied the anticanccer activity of novel pyrano[2,3-d][1,2,3]triazine derivatives using 1-(7-hydroxy-2,2-dimethylchroman-6-yl)ethenone. Inspired by these reports, we were encouraged to use NMSM to construct hybrid heterocyclic scaffolds with barbituric acid.

Compounds containing the pyranopyrimidine nucleus exhibit anticanccer, antioxidiant, antimicrobial, and antiviral properties. Hence, it was planned to explore their anticancer activities by molecular docking. Kinesin Eg5, also known as KIF 11, is a protein that plays a pivotal role in mitosis. These proteins act as nano-motors that move...
along microtubule tracks in the cell. To date, 70 kinesin 5 proteins have been identified to play key roles in mitotic spindle dynamics such as chromosome positioning, centrosome separation, and arrangement of the bipolar spindle during mitosis. At the cellular level, Eg5 is involved in stabilization of microtubule bundles, chromosome movement, and microtubule polymerization during cell division. The mitotic spindles are assembled and organized in a bipolar manner so that segregation of chromosomes can be achieved in a systematic way. In addition, kinesin 5 acts as a molecular brake that regulates microtubule motions.

Based on literature precedent on multicomponent one-pot reactions, we proposed that the NMSM scaffold could be a precursor to the target arylpyranopyrimidinediones by adopting a one-pot multicomponent reaction (MCR) using a mixture of aromatic aldehyde, 1,3-dimethyl barbituric acid and N-methyl-1-(methylthio)-2-nitroethenamine (NMSM) and piperidine as an organic base (Scheme 1).

Initially, the reaction was carried out under neat conditions in a mortar and pestle without any solvent or catalyst, but this resulted in no yield (Table 1, entry 1). Hence, we performed the next experiment using methanol as solvent, at reflux without base for 24 hours. Again, the reaction did not yield any product (entry 2). It was then decided to carry out the reaction with piperidine as base at room temperature (RT) stirring for 24 h, but the result was again disappointing, leading to no product (entry 3). We then modified the reaction conditions regarding temperature and time, using methanol as solvent and piperidine as base. This resulted in the product being obtained in 60% yield (entry 4).

The reaction conditions regarding temperature and time, using methanol as solvent and piperidine as base. This resulted in the product being obtained in 60% yield (entry 4). We further optimized the reactions by using ethanol as solvent and piperidine as base. This resulted in the product being obtained in 70–75% yield (entries 5 and 6). Using sodium carbonate as base under the same reaction conditions reduced the yield to 50% (entry 11). Similarly, when sodium carbonate and potassium carbonate were used as bases, no significant improvement in the yield was observed (entries 12 and 13). It was clearly observed that strong bases such as NaOH and KOH drastically reduced the yield of product to 20 and 30%, respectively (entries 14 and 15). Using water as solvent, the reaction did not proceed efficiently, presumably due to the poor solubility of the reactants (entry 16). With polar solvents such as DMF, DMSO, acetone, THF, and dioxane, poor, or no yield, of the desired product was observed (entries 18–22). In the absence of any base, the reaction did not proceed (entry 23).

Below are the details of the optimized reaction conditions for the synthesis of phenylpyranopyrimidinedione 4a in Table 1.

**Table 1 Optimization of Reaction Conditions for the Synthesis of Phenylpyranopyrimidinedione 4a**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Catalyst</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Isolated yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>no solvent</td>
<td>no catalyst</td>
<td>RT</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>MeOH</td>
<td>no catalyst</td>
<td>RT</td>
<td>24</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>MeOH</td>
<td>piperidine</td>
<td>RT</td>
<td>24</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>MeOH</td>
<td>piperidine</td>
<td>65</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>EtOH</td>
<td>piperidine</td>
<td>75</td>
<td>12</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>EtOH</td>
<td>piperidine</td>
<td>75</td>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>7</td>
<td>MeOH</td>
<td>H2O</td>
<td>piperidine</td>
<td>75</td>
<td>6</td>
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<tr>
<td>8</td>
<td>MeOH</td>
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<td>piperidine</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>EtOH</td>
<td>H2O</td>
<td>piperidine</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>EtOH</td>
<td>H2O</td>
<td>piperidine</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>EtOH</td>
<td>H2O</td>
<td>NaOEt</td>
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<td>6</td>
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<td>H2O</td>
<td>Na2CO3</td>
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<td>6</td>
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<tr>
<td>13</td>
<td>EtOH</td>
<td>H2O</td>
<td>K2CO3</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>EtOH</td>
<td>H2O</td>
<td>NaOH</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>EtOH</td>
<td>H2O</td>
<td>KOH</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>16</td>
<td>H2O</td>
<td>piperidine</td>
<td>90</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>17</td>
<td>no solvent</td>
<td>no catalyst</td>
<td>85</td>
<td>1</td>
<td>–</td>
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<tr>
<td>18</td>
<td>THF</td>
<td>piperidine</td>
<td>50</td>
<td>6</td>
<td>20</td>
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<tr>
<td>19</td>
<td>1,4-dioxane</td>
<td>piperidine</td>
<td>80</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>acetone</td>
<td>piperidine</td>
<td>50</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>21</td>
<td>DMF</td>
<td>piperidine</td>
<td>90</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>22</td>
<td>DMSO</td>
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<td>–</td>
</tr>
<tr>
<td>23</td>
<td>EtOH</td>
<td>H2O</td>
<td>no catalyst</td>
<td>80</td>
<td>6</td>
</tr>
</tbody>
</table>

**Scheme 1 Synthesis of arylpyranopyrimidinediones**

4a–k 60–85%
donating group at the para-position of the aldehyde improves the yield, presumably due to reduced steric hindrance. The reaction conditions were also applied to synthesize derivatives using aromatic aldehyde precursors with electron-withdrawing groups (F, Cl and NO2). Derivatives 4h and 4i were obtained in 75% yield, whereas 4j and 4k, derived from 2-nitrobenzaldehyde and 3-nitrobenzaldehyde, respectively, resulted in 60% yield of the desired product.

From these observations, it can be concluded that the use of aldehyde precursors possessing electron-donating groups result in better yields.

Eleven analogues of the arylpyranopyrimidinedione derivatives were subjected to docking studies to ascertain the binding affinity of the individual compounds. Human kinesin Eg5 was used as the macromolecular target, docking was performed using Autodock 4.2® software, and images were obtained using Pymol® software. The human kinesin Eg5 structure was obtained from the PDB data bank (PDB id 2X7D) with 2.3 Å resolution. The X-ray crystal structure of the protein was obtained using ADT. The three-dimensional structure of the protein 2X7D, downloaded from the Protein Data Bank, were optimized. Water molecules were removed from the 3D structure of the protein 2X7D. The ligand and protein optimizations include addition of Gasteiger charges, Kollman charges, and polar hydrogen bonds. The docked complexes resulting from the conformation with highest negative binding energy were converted into a 2D structure. Using this, interactions between the ligands and the binding site of 2X7D were analyzed.

The conformational changes for the library of 11 compounds were generated by exploring the torsional space of the ligand. A 3D grid was generated with spacing of 0.375 Å.

Then the docking was continued with a search algorithm to establish the best conformer. The docking results are shown in Table 2. Using these results, consideration of hydrogen bond generation between ligand and protein allowed identification of the binding pocket.

Almost all the compounds showed significant binding affinity towards the target protein (Table 2). However, compounds 4e, 4j and 4k showed very good binding affinity –7.28, –7.41, and –7.95 kcal M–1, respectively. Hence further exploitation of these derivatives may pave the way to design potent compounds with significant anticancer properties.

We have demonstrated the synthesis of a series of arylpyranopyrimidinedione derivatives by using a one-pot multicomponent procedure. The derivatives were submitted to docking score analysis and showed noticeable binding affinity with Human kinesin Eg5; compounds 4e, 4j and 4k resulted in most efficient binding scores on the target protein.

Melting points were determined in a capillary and are uncorrected. FTIR spectra were recorded with a Nicolet iS5 spectrometer using KBr pellets. NMR spectra were recorded with a Bruker (Avance) 500 MHz spectrometer. Tetramethylsilane (TMS) was used as internal standard, and CDCl3 or DMSO-d6 was used as solvent; chemical shifts are in parts per million (δ-scale) and coupling constants are given in Hertz. Mass spectra were recorded with a 6.450 XT Agilent spectrometer, using ESI. All the chemicals were purchased from Sigma-Aldrich, much used eluent for the purpose of TLC (silica-G plates-Merck) was a hexane/ethyl acetate (90:20) mixture.

**Figure 1** Arylpyranopyrimidinedione derivatives synthesized

**Figure 2** Molecular docking of active compounds (Binding energy –7.9 kcal M–1) with target protein

**Table 2**

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
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<tr>
<td>Me</td>
<td>H</td>
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</tr>
<tr>
<td>H</td>
<td>Me</td>
<td>70</td>
</tr>
<tr>
<td>H</td>
<td>Me</td>
<td>85</td>
</tr>
<tr>
<td>H</td>
<td>Et</td>
<td>85</td>
</tr>
<tr>
<td>OMe</td>
<td>H</td>
<td>70</td>
</tr>
<tr>
<td>H</td>
<td>OMe</td>
<td>75</td>
</tr>
<tr>
<td>F</td>
<td>H</td>
<td>75</td>
</tr>
<tr>
<td>Cl</td>
<td>H</td>
<td>75</td>
</tr>
<tr>
<td>NO2</td>
<td>H</td>
<td>60</td>
</tr>
<tr>
<td>NO2</td>
<td>H</td>
<td>60</td>
</tr>
</tbody>
</table>
(1:1.5 mL), piperidine (0.017 g, 0.2 mmol) was added and the mixture was heated to reflux for 6 h. The progress of the reaction was monitored by TLC and the resultant precipitate was filtered and washed with EtOH to give a white solid that was purified by recrystallization from EtOH to afford colorless crystals.

1,3-Dimethyl-7-(methylamino)-6-nitro-5-m-tolyl-1,5-dihydro-2H-pyrano[2,3-d]pyrimidine-2,4(3H)-dione (4d)
Yield: 0.251 g (70%); white solid; mp 160–162 °C.

\[ \text{HRMS (ESI): } m/z [M + H]^+ \text{ calcd for C}_{17}H_{18}N_4O_5: 345.1199; \text{ found: 345.1198.} \]

\[ \text{1}^1C \text{ NMR (500 MHz, CDC}_{13}\text{H}): \delta = 10.06 (d, J = 4.3 Hz, 1 H), 7.12 (dJ, J = 6.4 Hz, 1 H), 7.08–7.05 (m, 2 H), 6.88 (d, J = 7.7 Hz, 1 H), 5.38 (s, 1 H), 3.59 (s, 3 H), 3.26 (d, J = 2.6 Hz, 6 H), 2.82 (s, 3 H). \]

1,3-Dimethyl-7-(methylamino)-6-nitro-5-(p-tolyl)-1,5-dihydro-2H-pyrano[2,3-d]pyrimidine-2,4(3H)-dione (4e)
Yield: 0.289 g (85%); white solid; mp 164–166 °C.

\[ \text{1}^1C \text{ NMR (500 MHz, CDC}_{13}\text{H}): \delta = 10.01 (d, J = 4.9 Hz, 1 H), 7.24 (d, J = 8.1 Hz, 2 H), 7.08 (d, J = 7.8 Hz, 2 H), 5.20 (s, 1 H), 3.56 (s, 3 H), 3.26 (s, 3 H), 3.24 (d, J = 5.2 Hz, 3 H), 2.27 (s, 3 H). \]
13C NMR (125 MHz, CDCl3): δ = 160.37, 157.03, 150.13, 148.96, 138.09, 137.10, 129.04, 128.00, 109.86, 94.24, 36.08, 30.97, 29.51, 28.61, 28.48, 21.13.


13C NMR (125 MHz, CDCl3): δ = 160.53, 158.76, 156.97, 150.13, 148.89, 133.13, 129.17, 113.13, 109.93, 57.23, 55.24, 37.05, 30.98, 29.50, 28.61, 28.48.


5-(2-Chlorophenyl)-1,3-dimethyl-7-(methylamino)-6-nitro-1,5-dihydro-2H-pyra[2,3-d]pyrimidine-2,4(3H)-dione (4i)
Yield: 0.282 g (75%); white solid; mp 188–190 °C.

1H NMR (500 MHz, CDCl3): δ = 10.24 (s, 1 H), 7.83 (d, J = 8.1 Hz, 1 H), 7.66 (s, 1 H), 7.55 (d, J = 7.5 Hz, 1 H), 7.37 (t, J = 7.7 Hz, 1 H), 5.82 (s, 1 H), 3.60 (s, 3 H), 3.30 (s, 3 H), 3.26 (s, 3 H).

13C NMR (125 MHz, CDCl3): δ = 160.29, 157.77, 149.93, 148.34, 143.18, 136.32, 128.29, 122.64, 122.09, 108.71, 91.61, 36.79, 29.70, 28.80, 28.53.


1,3-Dimethyl-7-(methylamino)-6-nitro-5-(3-nitrophenyl)-1,5-dihydro-2H-pyra[2,3-d]pyrimidine-2,4(3H)-dione (4j)
Yield: 0.389 g (60%); white solid; mp 188–190 °C.

1H NMR (500 MHz, CDCl3): δ = 10.24 (s, 1 H), 7.83 (d, J = 8.1 Hz, 1 H), 7.66 (s, 1 H), 7.55 (d, J = 7.5 Hz, 1 H), 7.37 (t, J = 7.7 Hz, 1 H), 5.82 (s, 1 H), 3.60 (s, 3 H), 3.30 (s, 3 H), 3.26 (s, 3 H).

13C NMR (125 MHz, CDCl3): δ = 160.29, 157.77, 149.93, 148.34, 143.18, 136.24, 128.98, 122.64, 122.09, 108.71, 91.61, 36.79, 29.70, 28.80, 28.53.


1,3-Dimethyl-7-(methylamino)-6-nitro-5-(3-nitrophenyl)-1,5-dihydro-2H-pyra[2,3-d]pyrimidine-2,4(3H)-dione (4k)
Yield: 235 g (60%); white solid; mp 188–190 °C.

1H NMR (500 MHz, CDCl3): δ = 10.24 (s, 1 H), 7.83 (d, J = 8.1 Hz, 1 H), 7.66 (s, 1 H), 7.55 (d, J = 7.5 Hz, 1 H), 7.37 (t, J = 7.7 Hz, 1 H), 5.82 (s, 1 H), 3.60 (s, 3 H), 3.30 (s, 3 H), 3.26 (s, 3 H).

13C NMR (125 MHz, CDCl3): δ = 160.29, 157.77, 149.93, 148.34, 143.18, 136.24, 128.98, 122.64, 122.09, 108.71, 91.61, 36.79, 29.70, 28.80, 28.53.


Conflict of Interest
The authors declare no conflict of interest.

Funding Information
The authors acknowledge funding from the Department of Science and Technology through the Fund for Improvement of S&T Infrastructure in Universities and Higher Educational Institutions Program (DST-FIST; SR/FST/CS-II/2018/64).

Supporting Information
Supporting information for this article is available online at https://doi.org/10.1055/s-0040-1719869. 1H, 13C and Dept-135 NMR and HR-MS spectroscopic data of 4a–k and docking images are included.
References and Notes

(1) Muthusamy, S.; Ramkumar, R. Synlett 2015, 2156.
(3) Nagaraja, O.; Bodke, Y. D.; Pushpavathi, I.; Kumar, S. R. Helion 2020, 6, e04245.
(6) Kumar, A.; Shukla, R. D. Green Chem. 2015, 17, 848.
(7) Prabhakaran, S.; Velmathi, S. ChemistrySelect 2021, 6, 8696.