A rapid and efficient route to synthesis of 2-amino-4H-pyran-3-carbonitrile derivatives in the presence of L-proline and their antimicrobial activity

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ABSTRACT

An efficient and mild method for the synthesis of 2-amino-4H-pyran-3-carbonitriles via coupling reaction of malononitrile and α,α′-Bis(benzylidene) cyclohexanones in the presence of L-proline as a catalyst and acetonitrile as a solvent has been described. This method is a good option to obtain the title compounds in quantitative yields in shorter reaction times and in a simple way. The synthesized compounds were bio-evaluated for their possible antimicrobial activity against a panel of gram-positive and gram-negative bacterial strains and fungal strains by the known methods.

Keywords: Antimicrobial activity; Cyclohexanones; L-proline; Malononitrile; Pyran derivatives.

INTRODUCTION

Development of organic reactions in presence of different catalysts has emerged as a frontier area of research in synthetic organic chemistry. These reactions are especially appealing as they have certain advantages such as shorter reaction times, high yield and easy processing. Recently, L-proline is found to be an efficient catalyst for effecting various organic transformations such as in catalysed aldol condensation [1], synthesis of coumarins in ionic liquid [2], Mannich [3a,b], Michael [4a,b,c], Diels–Alder [5], Biginelli reactions [6a,b] and density functional study of the L-proline-catalysed α-aminoxylation of aldehydes [7]. More recently, L-proline and its derivatives have been used in multicomponent reactions [8a,b]. The molecule and its derivatives are readily and commercially available. Fused heterocyclic scaffolds with oxygen atoms are fundamental to the medicinal chemistry. The derivatives of 4H-pyrans are found to exhibit various biological activities such as antianaphylactic, anticancer, anticoagulant and spasmodytic [9-11]. The synthesis of many pyranopyridine [12], polyanzapaphthalene [13], pyranopyrimidines [14] and pyridine-2-ones derivatives [15] involves 4H-pyran moiety as intermediates. Moreover, 4H-pyrans also represent the building blocks of natural products [16,17]. A number of 2-amino-4H-pyrans are used as photoactive materials [18], pigments [19] and potential biodegradable agrochemicals [20]. Synthesis of pyran derivatives via a three-component condensation of β-dicarbonyl compounds with aldehydes and malononitrile has been reported in the literature [21] and it was observed that only very few catalysts have been used for the synthesis of 2-amino-4H-pyran-3-carbonitriles [22-25] and there was no attempt to use L-proline as a catalyst for the synthesis of these derivatives. We, therefore, present an efficient and practical synthesis of 2-amino-4H-pyran-3-carbonitriles from α,α′-bis (benzylidene)cyclohexanones and malononitrile in acetonitrile under reflux conditions in inert atmosphere using L-proline as catalyst (Scheme 1).
Based upon the above findings and in continuation of our work on heterocycles, compounds 4 (a-i) were synthesized and screened against a panel of bacterial strains, viz: Bacillus subtilis (Bs), Bacillus pumilus (Bp), Staphylococcus aureus (Sa), Proteus vulgaris (Pv), Salmonella typhi (St), Pseudomonas aeruginosa (Pa), Escherichia coli (Ec) and unicalcellular and multicellular pathogenic fungi such as Candidantropicalis (Ct), Candida albicans (Ca), Aspergillus niger (An), Penicillium chrysogenum (Pe).

MATERIALS AND METHODS

All chemicals were purchased from Merck, Sdfine and Qualigens. Solvents and reagents were used without further purification, unless otherwise specified. Melting points were determined in open capillaries in an electrically heated block and are uncorrected. The progress of the reaction was monitored by TLC, using TLC grade silica gel (G) and were synthesized using 5 mol% L-proline with acetonitrile as solvent. The proposed mechanistic pathway has been illustrated in Scheme 1.

\[
\text{O} + \text{O} + \text{H} \rightarrow \text{R} \rightarrow \text{R} \rightarrow \text{N} \rightarrow \text{R} \rightarrow \text{CN} \rightarrow \text{CN} \rightarrow \text{CN}
\]

Scheme 1: Synthetic route of 2-amino-4H-pyran-3-carbonitrile derivatives 4(a-i)

a,a’-Bis(benzylidene)cyclohexanone (3a-i) have been synthesized through cross-aldol condensation of cyclohexanone 1 with substituted aromatic aldehyde 2 by the known literature procedure [26]. The compounds 4(a-i) were synthesized using 5 mol% L-proline with acetonitrile as solvent. The proposed mechanistic pathway has been illustrated in Scheme 2.

\[
\text{(3a-3)} \rightarrow \text{(I)} \rightarrow \text{(II)} \rightarrow \text{(III)} \rightarrow \text{(IV)} \rightarrow \text{(V)} \rightarrow \text{NH}_2 \rightarrow \text{CN}
\]

Scheme 2: Plausible Mechanism for synthesis of Compound 4(a-i)

**General Procedure for the synthesis of 2-amino-4H-pyran-3-carbonitrile derivatives 4 (a-i)**

a,a’-Bis(benzylidene)cyclohexanone 3 (0.274gm, 1 mmol), malononitrile (0.066 gm, 1 mmol) and L-proline (5 mol %) were taken in acetonitrile (10 mL) and refluxed for an appropriate time as indicated in Table 1 (5-30 min). The progress of the reaction was monitored by TLC (n-hexane/ethyl acetate:8.0/2.0). After completion, the reaction mixture was cooled and diluted with water. The precipitate thus formed was filtered and washed with n-hexane (10mL) to furnish the corresponding 2-amino-4H-pyran-3-carbonitriles.
The structure of the products was deduced from their IR, $^1$H and $^{13}$C NMR and Mass spectral analysis. The spectral data of synthesized compounds are given below.

(E)-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (4a). White solid, IR (KBr, cm$^{-1}$): 3428, 2359, 1648, 1215, 1154, 928; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 1.62-1.72 (m, 2H, CH$_2$), 1.97-2.12 (m, 2H, CH$_2$), 2.54-2.76 (m, 2H, CH$_2$), 2.79 (s, 6H, N(CH$_3$)$_2$), 2.90 (s, 6H, N(CH$_3$)$_2$), 3.30 (s, 2H, NH$_2$), 3.86-4.06 (m, 1H, CH), 5.95 (s, 1H, =CH), 6.95-7.12 (m, 2H, ArH), 7.22-7.46 (m, 4H, ArH), 7.77-7.91 (m, 4H, ArH); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 26.0, 30.0, 37.8, 57.7, 120.2, 127.4, 128.7, 128.8, 128.9, 129.2, 129.7, 131.9, 140.2, 145.2, 151.2, 159.1, 159.2; MS m/z: M$^+$ 340; Anal. Calcd for C$_{24}$H$_{23}$N$_2$O: C, 81.18; H, 5.88; N, 8.24; Found: C, 80.77; H, 5.86; N, 8.18.

(E)-2-amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (4c). Orange powder, IR (KBr, cm$^{-1}$): 3378, 2375, 1215, 1104, 945; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 1.62-1.72 (m, 2H, CH$_2$), 1.97-2.12 (m, 2H, CH$_2$), 2.54-2.76 (m, 2H, CH$_2$), 2.79 (s, 6H, N(CH$_3$)$_2$), 2.90 (s, 6H, N(CH$_3$)$_2$), 3.30 (s, 2H, NH$_2$), 3.86-4.06 (m, 1H, CH), 5.95 (s, 1H, =CH), 6.95-7.12 (m, 2H, ArH), 7.22-7.46 (m, 4H, ArH), 7.77-7.91 (m, 4H, ArH); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 26.0, 30.0, 37.8, 57.7, 111.9, 112.9, 120.2, 128.7, 129.3, 131.9, 132.3, 140.2, 145.1, 151.2, 159.1, 159.2; MS m/z: M$^+$ 426; Anal. Calcd for C$_{22}$H$_{19}$Cl$_2$N$_2$O: C, 76.02; H, 7.07; N, 13.13; Found: C, 77.56; H, 7.86; N, 14.18.

(E)-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (4e). Yellow crystals, IR (KBr, cm$^{-1}$): 3443, 3318, 2194, 1665, 1416, 821; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 1.62-1.79 (m, 2H, CH$_2$), 1.94-2.15 (m, 2H, CH$_2$), 2.44-2.86 (m, 2H, CH$_2$), 3.52 (s, 2H, NH$_2$), 3.86-4.06 (m, 1H, CH), 5.95 (s, 1H, =CH), 7.20-7.36 (m, 4H, ArH), 7.47-7.71 (m, 4H, ArH); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 26.0, 30.0, 37.8, 56.2, 57.8, 109.2, 113.9, 115.4, 115.7, 120.2, 124.0, 126.4, 126.7, 126.8, 129.2, 140.2, 141.8, 147.9, 148.1, 148.9, 151.2, 159.1, 159.2; MS m/z: M$^+$ 409; Anal. Calcd for C$_{22}$H$_{19}$Cl$_2$N$_2$O: C, 67.47; H, 4.44; N, 6.85; Found: C, 68.32; H, 5.28; N, 6.44.

(E)-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (4d). Dirty yellow powder, IR (KBr, cm$^{-1}$): 3493, 3323, 2210, 1102, 901; $^1$H NMR (75 MHz, CDCl$_3$) $\delta$: 1.60-1.78 (m, 2H, CH$_2$), 1.94-2.15 (m, 2H, CH$_2$), 2.44-2.86 (m, 2H, CH$_2$), 3.50 (s, 2H, NH$_2$), 3.86-4.06 (m, 1H, CH), 5.95 (s, 1H, =CH), 7.20-7.36 (m, 4H, ArH), 7.47-7.71 (m, 4H, ArH); $^{13}$C NMR (300 MHz, CDCl$_3$) $\delta$: 26.0, 30.0, 37.8, 56.2, 57.8, 109.2, 113.9, 115.4, 115.7, 120.2, 124.0, 126.4, 126.7, 126.8, 129.2, 140.2, 141.8, 147.9, 148.1, 148.9, 151.2, 159.1, 159.2; MS m/z: M$^+$ 432; Anal. Calcd for C$_{24}$H$_{23}$O$_3$N$_2$: C, 69.42; H, 5.60; N, 6.48; Found: C, 70.56; H, 6.23; N, 7.01.

(E)-2-amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (4e). Light yellow powder, IR (KBr, cm$^{-1}$): 3403, 3298, 2194, 1670, 1070, 701; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 1.62-1.79 (m, 2H, CH$_2$), 1.94-2.15 (m, 2H, CH$_2$), 2.44-2.86 (m, 2H, CH$_2$), 3.51 (s, 2H, NH$_2$), 3.86-4.06 (m, 1H, CH), 5.95 (s, 1H, =CH), 7.16-7.65 (m, 4H, ArH), 7.27-7.40 (m, 4H, ArH); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 26.0, 30.0, 37.8, 57.8, 120.2, 127.1, 128.3, 129.0, 129.2, 130.1, 130.6, 130.9, 132.9, 134.9, 140.4, 142.2, 151.2, 159.1, 159.2; MS m/z: M$^+$ 409; Anal. Calcd for C$_{22}$H$_{18}$Cl$_2$N$_2$O: C, 67.50; H, 4.43; N, 6.82; Found: C, 68.23; H, 5.33; N, 5.96.

(E)-2-amino-8-(3-nitrobenzylidene)-4-(3-nitropheno l)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (4d). Yellow powder, IR (KBr, cm$^{-1}$): 3479, 3298, 1813, 1530, 1340, 1280; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 1.62-1.79 (m, 2H,
Owing to the necessity for an improved methodology for the synthesis of pyran-3-carbonitrile scaffolds and prevalence of impressive biological properties of pyran derivatives, we were interested in developing a mild synthetic protocol for the synthesis of the title molecules. A literature survey showed that many different protocols have been developed for the synthesis of various pyran-3-carbonitrile derivatives. However, the general applicability of the reported methods are limited as the reactions require prolonged heating using the catalysts NaOH/Piperidine [22], NH$_4$OAc [23], K$_2$CO$_3$ and HTMAB [24] etc. Furthermore, purification of the product requires tedious workup. In order to circumvent these difficulties and to develop a facile chemical approach and in continuation of our work to synthesize heterocyclic molecules of biological importance, the authors envisaged L-proline mediated synthesis of the title molecules from α,ω-Bis(benzylidene) cyclohexanones using CH$_2$CN as solvent. The evaluation of various parameters such as catalyst, solvent and amount of the catalyst on the rate and the yields of 2-amino-4H-pyran-3-carbonitriles was our primary focus. To ascertain the efficiency of L-proline, initially, the reaction of 2,6-bis(benzylidene)cyclohexanone (3a) and malononitrile was studied using NH$_4$Cl and K$_2$CO$_3$ as catalyst (Table 2).

**RESULTS AND DISCUSSION**

Moderate to good yield of product 4a was obtained using NH$_4$Cl and K$_2$CO$_3$ as catalyst but the reaction took longer time for completion. The best result was obtained using L-proline, as seen in the yield and the reaction time (Table 2, entry 3). So L-proline was chosen as catalyst for this reaction. The significant effect of various reaction solvents on the yield was also observed in presence of L-proline (Table 3).
Aspergillus niger (An) and Penicillium chrysogenum (Pc) results are given in Table 3. Antimicrobial activities were evaluated by measuring the diameter of zone of inhibition against tested organism and their minimum inhibitory concentration values were examined only for the compounds having moderate zone of inhibition (Table 4).

Low yields were obtained in Ethanol, and surprisingly no product formation was seen even after 2 hrs, in H2O, as a solvent. CH3CN was found to be the best reaction solvent (Table 3, entry 3). So, acetonitrile was used as the solvent for further optimization of reaction conditions (amount of catalyst). When loading of L-proline was increased from 2 mol% to 5 mol% the reaction time was shortened with higher yield (Table 4, entry 3). It was also observed that when loading of L-proline was increased from 5 mol% to 10 mol% reaction required prolonged heating with the low yield (Table 4, entry 4). To check the reusability of catalyst after separation of the crude product, the filtrate including catalyst was concentrated to remove water and reused for further reaction but no product formation was observed after 2-3 hrs.

Table 3: Solvent effect on the synthesis of 4a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>Yield (%)</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>L-proline</td>
<td>H2O</td>
<td>b</td>
<td>2 hrs</td>
</tr>
<tr>
<td>2.</td>
<td>L-proline</td>
<td>EtOH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>L-proline</td>
<td>CH3CN</td>
<td>67-92</td>
<td>05-30 min</td>
</tr>
</tbody>
</table>

*Reaction conditions: α,α′-Bis(benzylidene)cyclohexanones 3a(l mmol), malononitrile (1 mmol), L-proline (5 mol%, 0.05 mmol) and solvent (10 mL) under reflux condition. b: reaction did not proceed.

Biological Activity

For evaluation of antibacterial properties of the synthesized compounds 4(a–i), clinically active Gram-positive [Bacillus subtilis (Bs)] MTCC 121, Bacillus subtilis (Bs) MTCC 441, Bacillus pumilus (Bp) MTCC 1607, Staphylococcus aureus (Sa) MTCC 96, Staphylococcus aureus (Sa) MTCC 902, Gram-negative [Proteus vulgaris (Py)] MTCC 426, Salmonella typhi (St) MTCC 537, Pseudomonas aeruginosa (Pa) MTCC 741, E. coli (Ec) MTCC 1304] bacterial and fungal [Candida tropicalis (Ct) MTCC 184, Candida albicans (Ca) MTCC 3017, Aspergillus niger (An) MTCC 1344, Penicillium chrysogenum (Pc) MTCC 2725] strains were selected. Antimicrobial activities were evaluated by measuring the diameter of zone of inhibition against tested organism and results are given in Table 5.

Table 5: Antibacterial and antifungal activities of synthesized compounds 4(a–i) against different Strains (diameter of zone of inhibition)

<table>
<thead>
<tr>
<th>Compds</th>
<th>Zone of Inhibition (mm) of Bacterial strains</th>
<th>Zone of Inhibition (mm) of Fungal strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bs′ Bp′</td>
<td>Bp′</td>
</tr>
<tr>
<td>4a</td>
<td>10′ 8′</td>
<td>4b</td>
</tr>
<tr>
<td>4c</td>
<td>10′ 0′</td>
<td>4d</td>
</tr>
<tr>
<td>4e</td>
<td>10′ 0′</td>
<td>4f</td>
</tr>
<tr>
<td>4g</td>
<td>10′ 0′</td>
<td>4h</td>
</tr>
<tr>
<td>4i</td>
<td>0′ 8′</td>
<td>10′ 0′</td>
</tr>
</tbody>
</table>

*No activity observed.

*Entries in bold font indicates moderate activity than reference drugs Erythromycin (E) and Vancomycin (V).
*Entry in bold font indicate moderate activity than reference drug Fluconazole.

The minimal inhibitory concentration values were examined only for the compounds having moderate zone of inhibition (4a, 4c, 4f, and 4h) and the results are illustrated in Table 6, 7 and Figure 1.
Erythromycin (E) and Vancomycin (V) were used as standard antibacterial and Fluconazole as standard antifungal drug. MIC of the synthesized compounds was determined by micro broth dilution method using 96 well plates according to the method described by Vipra et al., 2013. It was found that among the synthesized compounds, compound 4a and 4h possess pronounced antimicrobial activity against all tested bacterial and fungal strains.

Compound 4a has shown an MIC value of 1.56 against Bs\textsuperscript{b} MTCC 441 and Sa\textsuperscript{a} MTCC 96 which exceeds that of the reference drug Erythromycin (MIC=2.49 and 1.9 µg/mL).

Compound 4h has shown an MIC value of 1.56 against Sa\textsuperscript{a} MTCC 96 and 3.25 against Ca MTCC 3017 respectively which exceeds that of the reference drug Fluconazole (MIC=1.9 and 6.25 µg/mL).

CONCLUSION

A series of 2-amino-4H-pyran-3-carbonitriles and their structural analogs (4a-i) was designed and synthesized in excellent yields under mild reaction conditions and simple experimental work-up procedure. The economic
availability of the catalyst is the advantages of this method, which makes it a useful protocol for the synthesis of 2-amino-4H-pyran-3-carbonitrile derivatives. The use of L-proline as an eco-friendly catalyst is an interesting alternative that is quite simple, high yielding and time saving process. The possible use of such molecules can be of great benefit in many fields of action.

Supporting Information
Spectral data of compound 4a 1H, 13C-NMR, Mass and IR spectrum of product and 1H, 13C-NMR of intermediates 3b, 3d and 3g associated with this article.

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REFERENCES

Supplementary File

**$^1$H-NMR spectrum for compound 4a**

**$^{13}$C-NMR Spectrum for Compound 4a**
IR Spectrum for Compound 4a

Mass spectrum for Compound 4a
$^1$H-NMR spectrum for compound 3b

$^1$H-NMR spectrum for compound 3d
\(^1\)H-NMR spectrum for compound 3g

\(^{13}\)C-NMR Spectrum for Compound 3b
$^{13}$C-NMR Spectrum for Compound 3d

$^{13}$C-NMR Spectrum for Compound 3g