SECTION-B

Baker’s yeast catalyzed synthesis of 2-amino-2-chromenes, carried under ultrasonication
Introduction

2-Amino-2-chromenes represent an important class of organic compounds as they constitute the main components of many naturally occurring products.\(^1\) 2-amino-2-chromenes is an area of interest in recent years due to their useful biological and pharmacological aspects.\(^2\) 2-Amino-chromene derivatives are also served as the main components of cosmetics and pigments,\(^3\) biodegradable agrochemicals\(^4\) and medicaments.\(^5\) Particularly, they are endowed with a wide spectrum of activities such as antitumor,\(^6\) sex pheromonal,\(^7\) central nervous system,\(^8\) antiproliferative,\(^9\) antiviral,\(^10\) mutagenetic\(^{11}\) and antimicrobial.\(^{12}\) This heterocyclic structure also serves for generation of small-molecule ligands with highly pronounced spasmolytic, diuretic, anticoagulant and antianaphylactic activities.\(^{13}\)

Furthermore, several bio-active compounds such as enzyme inhibitors\(^{14}\) and antioxidants\(^{15}\) incorporate these key heterocycles as a part of their structures. The basic structural framework of chromene is a common feature of many tannins and polyphenols\(^{16}\) found in tea, fruits, vegetables and red wine. This class compounds have become more important as a result of their health-promoting effects.\(^{17}\)

These derivatives have shown their potential applications in the treatment of human inflammatory TNFα-mediated diseases, such as rheumatoid and psoriatic arthritis as well as in cancer therapy.\(^6\) Therefore, the interest of organic chemists in the synthesis and structure modifications of 2-amino-chromenes remains high.\(^2,18\) Following is a brief account of biological significance of chromene derivatives.

Vitamin E (3.10) is an evident example for the naturally occurring chromane, which possess antioxidant activity.\(^{19}\)
Anticancer activity of chromene derivatives

Many of the natural compounds having chromene moiety are found to possess anticancer activity. These compounds are isolated from plants, sea fish, etc. Naturally occurring anticancer compounds include tephrosin\(^{20}\) for lung cancer (3.11), calanone\(^{21}\) used for leukemia and cervical carcinoma (3.12), acronycine\(^{22}\) effective in lung, colon and ovary cancer (3.13) and seselin\(^{23}\) against skin cancer (3.14).

![3.11](image1.png)  ![3.12](image2.png)

![3.13](image3.png)  ![3.14](image4.png)

Anti-inflammatory activity of chromene derivatives

Chromene derivatives are potential anti-inflammatory agents. Inflammation is the first response of the immune system to infection, irritation or foreign substance.\(^{24}\) The chromene pharmacophore represents a novel class of COX-2 selective inhibitors (coxibs) in non-steroidal anti-inflammatory drugs (NSAIDs) which provide higher potency, efficacy and selectivity over the existing coxibs (eg. celecoxib, valdecoxib, rofecoxib and etoricoxib) for the treatment of inflammation.\(^{25}\)

Following are some of the examples of the chromene cyclooxygenase-2 selective inhibitors; 6-chloro-8-methyl-2-(trifluoromethyl)- 2\(H\)-chromene-3-carboxylic acid (3.15), 6-chloro-2-(trifluoromethyl)-4-phenyl-2\(H\)-chromene-3-carboxylic acid (3.16), 6-(4-
hydroxybenzoyl)-2-(trifluoromethyl)-2H-chromene-3-carboxylic acid (3.17) and 6-chloro-7-(4-nitrophenoxy)-2-(trifluoromethyl)-2H-chromene-3-carboxylic acid (3.18).

Dihydropyrano[3,2-c]chromene (3.19) is another important family of chromene heterocycles those have been used as cognitive enhancer, for the treatment of neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, Down’s syndrome, AIDS associated dementia, schizophrenia and Huntington’s disease.26

Substituted 2-amino-4H-benzochromenes have been proposed for the treatment of immune system diseases and diabetic complications resulted from an increase in permeability of blood vessels and change in blood pressure.27 2-Amino-chromene derivatives are also recognized as tubuline inhibitors (3.20, 3.21).18,28 Further, β-
enaminonitrile derivatives of $4\text{H}$-chromenes are useful synthetic intermediates to obtain heterocyclic systems having potential bioactivities.$^{29-32}$

![3.20](image1.png) ![3.21](image2.png)

Owing to the medicinal and synthetic significance of 2-amino-2-chromenes, the development of novel, highly efficient and cost effective methods for the synthesis of substituted-2-amino-2-chromenes have immerged as the thrust area of research in organic chemistry. Following is the brief review on methods reported for the synthesis of these significant molecules.

**Reported protocols for the synthesis of 2-amino-2-chromene**

2-Amino-2-chromenes are conventionally prepared by cyclocondensation of three components; aldehydes, malononitrile and activated phenols at reflux condition in the presence of organic bases like piperidine.$^{33}$ (Scheme 3.6).

![Scheme 3.6](image3.png)

It has been revealed that numerous modified procedures for this three component condensation have been reported. The modifications include the use of phase transfer catalysts viz. cetyl trimethylammonium bromide,$^{34}$ cetyl trimethylammonium chloride,$^{35}$ triethylbenzylammonium chloride$^{36}$ and tetra-$n$butylammonium bromide.$^{45}$ $\gamma$-Alumina,$^{37}$ methanesulfonic acid,$^{38}$ nanostructured diphosphate,$^{39}$ sodium hydroxide,$^{40}$ triton B,$^{41}$
sodium salt of ethylenediamine tetraacetic acid,\textsuperscript{42} magnesium oxide\textsuperscript{43} and titanium tetrachloride,\textsuperscript{44} have also been used. There are also reports on the use of catalysts such as DBU,\textsuperscript{46} selectfluor\textsuperscript{47} and piperazine.\textsuperscript{48}

Recently Dekamin et al. reported potassium phthalimide-N-oxyl as a novel organocatalyst for the one-pot three-component synthesis of various 2-amino-4H-chromene derivatives in water.\textsuperscript{49} Shinde et al. reported gel entrapped DABCO\textsuperscript{50} as a catalyst for the multi-component synthesis of 2-amino-4H-chromenes. Poly 4-vinylpyridine has also been used as a catalyst\textsuperscript{51} for the synthesis of the chromene derivatives.

These synthetic methodologies which have been developed to accomplish the cyclocondensation reaction have their own merits and demerits. Some of the significant disadvantages associated with many of the existing synthetic protocols involve; prolonged reaction times, formation of side products resulting in lower yields, harsh reaction conditions, tedious work-up procedures and the use of expensive/environmentally toxic catalysts.

**Scope and objectives**

As discussed above, most of the reported procedures for the synthesis of 2-amino-2-chromenes are associated with one or more drawbacks. Thus, organic chemists have challenge to overwhelm these shortcomings and develop an efficient, cost effective and sustainable approach for the synthesis of this valuable class of compounds and particularly, if possible using milder, non-hazardous and inexpensive catalysts.

Literature reveals that there is no report on the use of biocatalyst/ enzyme for accelerating the one pot multicomponent condensation leading to chromenes. Considering the above observations and in continuation of our work\textsuperscript{52} towards the acceleration of synthetic protocols by employing biocatalysts/ biomimetic catalysts, it was thought worthwhile to study the catalytic role of baker’s yeast for the synthesis of 2-amino-2-chromenes *via* three component reaction of aldehyde, malononitrile and 1-naphthol.
The application of ultrasonic waves is found to become more convenient to run organic synthesis.\textsuperscript{53} Its development in the past few years has been considerably increased to know its mechanism of action inside the reaction flask.\textsuperscript{54} Several applications in organic synthesis have made sonochemistry an attractive technique\textsuperscript{55} and hence increasingly used in organic synthesis.\textsuperscript{56} It has proved to be a great tool for improving yields and decreasing the reaction time.\textsuperscript{57}

Considering all the above facts here in the present work objective was set to develop an efficient, cost effective and sustainable route for one pot three component cyclocondensations of aryl aldehydes, malononitrile and 1-naphthol in non-aqueous media (organic solvents) under relatively mild reaction conditions using an easily available, cheaper whole cell biocatalyst, active dry baker’s yeast instead of the catalysts reported in the literature.\textsuperscript{34-51} The objective was also set in mind to use non-conventional energy source, ultrasonication for assisting the cyclocondensation.

**Present work**

In the present work, baker’s yeast catalyzed efficient synthesis of 2-amino-2-chromenes in methanol (Scheme 3.7) is presented. Here the ultrasonication has dual role i.e. as a source of energy for the reaction and disruption of the yeast cells. The cyclocondensation has been essentially carried under neutral conditions, thus reducing the possibility of unwanted side reactions.

![Scheme 3.7](image-url)
Results and discussion

The investigations were started with an optimization study of model reaction by allowing cyclocondensation of 4-nitrobenzaldehyde (1g) malononitrile (2) and 1-naphthol (3) in the presence of baker’s yeast (Scheme 3.7). To see the effect of reaction medium on the rate and yield of the reaction, the model reaction was carried in various solvents like water, chloroform, 1,4-dioxane, ethanol and methanol under stirring at room temperature (RT).

![Scheme 3.7](image)

Initially when the reaction was carried in water at room temperature (RT), it was found that the cyclocondensation gave low (33%) yield even after prolonged stirring at room temperature (36 h) (Table 3.4, entry 1). The model reaction was then separately performed in organic solvents viz. chloroform, 1,4-dioxane, ethanol and methanol (Table 3.4). Comparatively better results were obtained when methanol was used as a solvent for the reaction (Table 4, entry 5). Hence methanol was selected as the reaction medium.

**Table 3.4:** Optimization for appropriate solvent for the reaction\(^a\) (Scheme 3.7)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Condition</th>
<th>Time (h)</th>
<th>Yield (%)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>RT</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>CHCl(_3)</td>
<td>RT</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>1,4-Dioxane</td>
<td>RT</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol</td>
<td>RT</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>Methanol</td>
<td>RT</td>
<td>36</td>
<td>57</td>
</tr>
</tbody>
</table>

\(^a\)Reaction conditions: 4-nitrobenzaldehyde (5 mmol), malononitrile (5 mmol), 1-naphthol (5 mmol), baker’s yeast (2g) in methanol under ultrasonication.  
\(^b\)Isolated yields.
In the further attempts to reduce reaction time and enhancing the yield of the product, the model reaction was performed in methanol under ultrasonication. This attempt was made because ultrasonication has been one of the most widely used laboratory methods for the disruption of the cells of baker’s yeast for the fast release of enzymes. Model reaction in methanol when performed under ultrasonication gave 87% yield within 2 h (Table 3.5, entry 3).

**Table 3.5**: Optimization for appropriate reaction time (Scheme 3.7).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Condition</th>
<th>Time (hr)</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>US</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Methanol</td>
<td>US</td>
<td>1.5</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>Methanol</td>
<td>US</td>
<td>2</td>
<td>87</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>US</td>
<td>2.5</td>
<td>89</td>
</tr>
<tr>
<td>5</td>
<td>Methanol</td>
<td>US</td>
<td>2.5</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reaction conditions: 4-nitrobenzaldehyde (5 mmol), malononitrile (5 mmol), 1-naphthol (5 mmol), baker’s yeast (2g) in methanol under ultrasonication.

<sup>b</sup>Isolated yields. 5Reaction without baker’s yeast.

In view of these observations methanol was selected as the reaction medium to run baker’s yeast catalyzed synthesis of 2-amino-2-chromene under ultrasonication. Subsequently the cyclocondensations of other substituted aryl aldehydes, malononitrile and 1-naphthol have been carried under the optimized reaction conditions and obtained the respective 2-amino-2-chromenes. The chromenes synthesized by this optimized novel protocol are already reported in literature<sup>58</sup> the physical parameters of the obtained products are in good agreement with those reported in the literature<sup>36,40,58</sup>. The results are recorded in Table 3.6 (Scheme 3.7). From these results it seems that the baker’s yeast is capable of catalyzing efficiently the present cyclocondensation. To investigate the role of baker’s yeast in cyclocondensation the model reaction was run in the absence of baker’s yeast, as a control reaction and formation of desired product was not observed (Table 3.5, entry 5).
Baker’s yeast generates variety of enzymes which contains various amino acid residues. Among them, here in the present reaction it is proposed that Asp-His dyad present in the enzyme residues are active in catalyzing the cyclocondensation reaction.

Scheme 3.8: Plausible mechanistic path for biocatalytic cyclocondensation
In the first step, aspartate anion enhances the basicity of histidine which therefore easily abstracts the labile proton from active methylene, malononitrile. Thus formed carbanion attacks on the carbonyl carbon of aryl aldehydes and the oxyanion formed in this step would be abstracting proton from Asp-His dyad regenerating the Asp-His dyad in its initial form. The tetrahedral intermediate gives arylidinemalononitrile upon dehydration. In the second step of the mechanism, the Asp-His dyad abstracts hydroxyl proton from 1-naphthol and intermediate carbanion (I) would have generated after delocalization on the 2-position of naphthol. This further attacks on the arylidine malononitrile formed in the first step and takes back proton from Asp-His dyad to form C-alkylated intermediate (II) (Scheme 3.8).

Further hydrogen bonding between the carbonyl oxygen of intermediate II and Histidine NH, facilitate proton transfer which leads to aromatization of intermediate II giving imine intermediate (III). Serine might be activating the imine intermediate (III) by noncovalent interaction and hence be finally yielding the chromene derivative as a product (Scheme 3.8).

In summary, baker’s yeast has been used as whole cell biocatalyst to accelerate the one pot three component cyclocondensation of aryl aldehydes, malononitrile and 1-naphthol in methanol, to obtain 2-amino-2-chromenes. Ultrasonication as a non conventional energy source has been used for acceleration of synthetic route. The newly developed protocol has following advantages.

1) Baker’s yeast is inexpensive and readily available biocatalyst.
2) Reaction time has been reduced appreciably due to use of ultrasonication as energy source.
Experimental section

General procedure for the synthesis of 2-amino-2-chromenes (4a-k):

To the round bottom flask containing methanol (25 ml), active dry baker’s yeast (2 g) was added and sonicated for 10 min. After 10 min., aryl aldehyde (5 mmol) and malanonitrile (5 mmol) were added and further sonicated for 15 minutes. Then to this sonicated reaction mass 1-naphthol (5 mmol) was added and reaction was allowed to complete, under ultrasonication (20 KHz) at RT. The progress of the reaction was monitored by thin layer chromatography, using ethyl acetate: pet ether (2:8) as a solvent system. After two hours the reaction mass was filtered through the bed of celite (2 g). The solvent methanol was removed from filtrate under reduced pressure and the crude product obtained was crystallized from ethanol and obtained respective 2-amino-2-chromen (4a-k) (Table3.6, 4a-k).

Spectral data of a representative compound of the series

Compound (4g): 2-amino-3,4-dihydro-4-(4-nitrophenyl)-2H-benzo[h]chromene-3-carbonitrile

$^1$H-NMR (400 MHz, DMSO$_d_6$): δppm= 5.05 (s, 1H, CH), 7.03 (d, 1H, J=8.4 Hz, Ar-H), 7.27 (s, 2H, NH$_2$, exchangeble with D$_2$O ), 7.46 (d, 2H, J=8.4 Hz, Ar-H), 7.52-7.62 (m, 3H, Ar-H), 7.83 (d, 1H, J= 8 Hz, Ar-H), 8.12 (t, 2H, J=7.2 Hz and 8.4 Hz, Ar-H) and 8.24 (d, 1H, J=8.4 Hz, Ar-H)

$^{13}$C-NMR (100 MHz, CDCl$_3$): δppm= 38.9, 41.0, 55.7, 116.9, 120.8, 121.3, 123.2, 124.5, 126.3, 127.5, 128.2, 129.4, 133.4, 143.3, 146.9, 153.3 and 160.9.

HR-ESI-MS (m/z): Calculated for C$_{20}$H$_{13}$N$_3$O$_3$ [M+ K]$^+$ : 382.0588, found : 382.0560.
Table 3.6: Baker’s yeast catalyzed synthesis of 2-amino-2-chromenes accelerated by ultrasonication\(^a\) (Scheme 3.7)

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Product</th>
<th>Yield (%)(^b)</th>
<th>M.P. (°C)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>4a</td>
<td>87</td>
<td>208-210</td>
</tr>
<tr>
<td>2</td>
<td>4-OCH(_3)</td>
<td>4b</td>
<td>90</td>
<td>183-184</td>
</tr>
<tr>
<td>3</td>
<td>4-CH(_3)</td>
<td>4c</td>
<td>85</td>
<td>207-209</td>
</tr>
<tr>
<td>4</td>
<td>4-Cl</td>
<td>4d</td>
<td>75</td>
<td>233-234</td>
</tr>
<tr>
<td>5</td>
<td>2-Cl</td>
<td>4e</td>
<td>65</td>
<td>233-235</td>
</tr>
<tr>
<td>6</td>
<td>4-Br</td>
<td>4f</td>
<td>91</td>
<td>241-242</td>
</tr>
<tr>
<td>7</td>
<td>4-NO(_2)</td>
<td>4g</td>
<td>88</td>
<td>240-242</td>
</tr>
<tr>
<td>8</td>
<td>3-Cl</td>
<td>4i</td>
<td>68</td>
<td>228-230</td>
</tr>
<tr>
<td>10</td>
<td>4-N(CH(_3))(_2)</td>
<td>4j</td>
<td>77</td>
<td>180-181</td>
</tr>
<tr>
<td>11</td>
<td>4-OH</td>
<td>4k</td>
<td>84</td>
<td>248-250</td>
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</table>

\(^a\)Reaction conditions: aldehyde (5 mmol), malononitrile (5 mmol), 1-naphthol (5 mmol), baker’s yeast (2g) in methanol (25 ml) under ultrasonication.

\(^b\)Isolated yields.

\(^c\)The known 2-amino2-chromenes synthesized by this method are having their melting points in good agreement with those reported in the literature.\(^{36, 40, 58}\)
Syntheses of bioactive molecules, accelerated by biocatalysts/ biomimics

Balaji S. Londhe

$^1$H-NMR spectrum of 4g

$^{13}$C-NMR spectrum of 4g
HR-MS spectrum of 4g
References


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25. [http://www.inventi.in/Article/pmm/26/11.aspx](http://www.inventi.in/Article/pmm/26/11.aspx)


