

## Green and Efficient Chemo-selective Synthesis of Chalcone Imines ( $\alpha,\beta$ -Unsaturated imine) using Baker's Yeast

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A simple, efficient, chemo-selective and sustainable biocatalytic method for the synthesis of  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) was developed by using Baker's yeast. When, chalcones (**1a-b**) were reacted with the substituted anilines (**2a-f**) in the presence of Baker's yeast act as a whole cell biocatalyst at ambient temperature in ethanol as a green solvent to afford the  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) in good to excellent yield. The reaction procedure was easy to follow and takes place at room temperature (25-28 °C). The commercially available Baker's yeast is very cheap and affordable catalyst, easy to use and facilitate this method as an effective and facile chemo-selective synthesis of  $\alpha,\beta$ -unsaturated imines (chalcone-imines) (**3a-l**). This is the first report about the biocatalytic reactions of  $\alpha,\beta$ -unsaturated imines (chalcone-imines) (**3a-l**) formation and it is assumed that this method will open an gateway for the organic chemists to design and synthesized chemo-selective chalcone imines.

**Keywords:**  $\alpha,\beta$ -Unsaturated imines, Chalcone-imines, Biocatalysis, Baker's yeast, Chemo-selective.

### INTRODUCTION

Catalysts are those chemical substances which alter the rate of a chemical reaction without any change in the composition and structure at the end of the reaction. In presence of catalysts, reactants molecules acquire a new path of low activation energy and helps to attain the equilibrium hasten. Catalysts are classified into two on the basis of physical state of reactants and catalyst *i.e.* homogenous and heterogeneous catalysts. On the other hand, catalysts may also be classified into two on the basis of whether it increases or decrease the rate of reaction. *i.e.* positive and negative catalyst. Enzymes are also an example of catalyst. However, the fundamental difference between enzymes and ordinary catalysts lies in their specificity. Enzymes are capable of catalyzing only one process at a time, while the conventional catalysts are non-specific and can catalyze multiple reactions simultaneously [1].

Organocatalyst is an example of homogenous catalyst and work through both formations of covalent bonds (enamine and iminium catalysis) as well as through non-covalent interactions (hydrogen bonding) [2]. Organocatalysts can be grouped into (i) biomolecules such as secondary amines, proline, phenylalanine, oligopeptides and cinchona alkaloids, (ii) hydrogen bonding catalysts like BINOL and TADDOLS and (iii) organocatalysts based on thioureas, Baker's yeast based organocatalyst, *etc.* Baker's yeast are generally preferred as organocatalyst due to some inherent properties such as non-toxic nature, easy to handle, cost effective, reaction rate is higher than ordinary catalyst and able to catalyze various organic reactions either in water or organic medium [3]. Baker's yeast plays an important role in various organic transformation like one-pot synthesis of cinnamyl acetate from cinnamaldehyde, reduction of ethyl acetoacetate and synthesis of optically active 2-oxazolines, *etc.* [4].

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However, the enzyme based biocatalysis has not yet explored its full potential, as their laboratory isolation has been only marginally explored. Another aspect of enzymatic catalysis is immobilization. It is well-known fact that the properties of biocatalyst can be altered by this method, it has only led to impoverished catalytic activity due to the distortions in the structure of enzyme upon interaction with the surface on which it is immobilized [5,6]. In addition to this, enzyme catalysis can also demand functional group protection [7-10], moreover, isolated enzymes require the addition of cofactors. Therefore, the processes of biocatalysis has shifted its attention towards employing whole cells instead of isolated enzymes [11]. Whole cells already contain all of the cofactors and under given conditions, they can continue with the metabolic pathways required for the regeneration of the cell, thereby aiding the process of reusability of the catalyst. The elimination of the process of addition of cofactors eventually eliminates the potential for generating products due to side reactions [12].

$\alpha,\beta$ -Unsaturated imines or conjugated imines are the key compounds or intermediates and are used in the synthesis of nitrogen containing heterocyclic compounds, agrochemicals and pharmaceuticals [13]. Such imines or conjugated imines provide a lot of significant cyclic as well as acyclic products through diverse reactivity with a versatile family of compounds [14,15]. The literature revealed that  $\alpha,\beta$ -unsaturated imines can be prepared the following reactions by (i) heat elimination of imino-alcohol skeleton; (ii) allyl cation-mediated Schmidt between azides and alcohols; (iii) aza-Wittig reaction of  $\alpha$ -ketophosphonates and trimethyl phosphazenes and (iv) dehydrogenative coupling between primary allylic alcohol and a primary amine [16]. Recently, Sc(OTf)<sub>3</sub>-catalyzed annulations reactions of vinyl diazoacetates with *in situ* formation of indole-derived,  $\alpha,\beta$ -unsaturated imines for the preparation of cyclopenta[*b*]indoles has been reported [17]. From the biological point of view, the  $\alpha,\beta$ -unsaturated imines exhibit the antimicrobial antioxidant activity and anti-corrosion activity [18-20]. Thus, in view of the importance of selective synthesis of  $\alpha,\beta$ -unsaturated imines or chalcone-imines, there is a immense requirement for the chemo-selective synthesis of  $\alpha,\beta$ -unsaturated imines or chalcone-imines. In continuation of our ongoing efforts for the successful application of Baker's yeast as biocatalysts in the synthesis of biologically active compounds by various functional group transformation [21] and multi-component reactions). Thus, we wish to report our efforts for the chemo-selective synthesis of the  $\alpha,\beta$ -unsaturated imines or chalcone-imines.

## EXPERIMENTAL

All the solvents were distilled and dried before use. The chemicals purchased from the commercial vendors were used without any further purification. The reactions were monitored by using TLC on silica gel 60 plates with a typical ratio of petroleum ether:ethyl acetate (7:3) as mobile phase. Melting points were determined with a hot-plate microscope apparatus and are uncorrected. The <sup>1</sup>H-NMR spectra were recorded on Varian-400NMR spectrometer at 400 MHz using CDCl<sub>3</sub> and

TMS as solvent and internal standard, respectively. DIP Mass Spectrum was recorded on Agilent- G6160 A infinity lab LC/MSD/IQ mass spectrometer.

**General procedure for the synthesis of imino-chalcones (3a-l):** A reaction mixture was prepared by mixing chalcone (1a-b) (1 mmol) and substituted anilines (2a-f) (1 mmol) in 10 mL of ethanol, then 500 mg Baker's yeast was added. The reaction mixture was stirred in an orbital shaker for 36 h. After completion of the reaction as indicated by TLC (ethyl acetate: hexane 30:70, v/v), the reaction mixtures were filtered through Celite to remove the Baker's yeast. The residue was washed thoroughly with absolute ethanol. To filtrate, 20 mL water was added and the contents were extracted with ethyl acetate (2 × 30 mL). The combined organic layers were collected and sodium sulfate was added to remove the moisture. The organic layer was evaporated to get the pure crude product. The obtained products were recrystallized with ethanol to get pure  $\alpha,\beta$ -unsaturated imines or chalcone-imines (4a-l) in good to excellent yields. All the compounds were characterized by their melting points, mass and <sup>1</sup>H NMR spectral analysis.

## Spectral data

**(1E,2E)-N-1,3-Triphenylprop-2-en-1-imine (3a):** Light yellow solid; m.p.: 189-191 °C; yield: 88%; FTIR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3049 (Ar, C-H), 1608 (C=N), 1530, 1492 (C=C); <sup>1</sup>H NMR (400 MHz)  $\delta$  ppm: 7.42-7.43 (dd, 3H), 7.49-7.59 (m, 7H), 7.64-7.67 (dd, 2H), 7.71-7.80 (tt, 2H), 7.80-7.84 (d, 1H), 8.01-8.04 (dt, 2H); Mass (m.f.: C<sub>21</sub>H<sub>17</sub>N): calculated: 283.14; found: 284.12 (M<sup>+</sup>).

**(1E,2E)-N-(4-Chlorophenyl)-1,3-diphenylprop-2-en-1-imine (3b):** Light yellow solid; m.p.: 195-197 °C; yield: 85%; FTIR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3037 (Ar, C-H), 1607 (C=N), 1527, 1478 (C=C); <sup>1</sup>H NMR (400 MHz)  $\delta$  ppm: 7.42-7.43 (dd, 3H), 7.49-7.59 (m, 5H), 7.64-7.67 (dd, 2H), 7.71-7.730 (tt, 2H), 7.84 (s, 1H), 8.01 (s, 1H), 8.02-8.03 (t, 2H); Mass (m.f.: C<sub>21</sub>H<sub>16</sub>ClN): calculated: 317.10; found: 317.18 (M<sup>+</sup>).

**(1E,2E)-N-(4-Fluorophenyl)-1,3-diphenylprop-2-en-1-imine (3c):** Off white solid; m.p.: 176-178 °C; yield: 86%; FTIR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3020 (Ar, C-H), 1594 (C=N), 1530, 1492 (C=C); <sup>1</sup>H NMR (400 MHz)  $\delta$  ppm: 7.40-7.43 (dd, 3H), 7.47-7.53 (m, 6H), 7.59-7.64 (dd, 2H), 7.70-7.73 (tt, 2H), 7.73-7.80 (d, 1H), 7.84-8.03 (dd, 2H); Mass (m.f.: C<sub>21</sub>H<sub>16</sub>FN): calculated: 301.13; found: 302.10 (M<sup>+</sup>).

**(1E,2E)-N-(4-Bromophenyl)-1,3-diphenylprop-2-en-1-imine (3d):** Yellow solid; m.p.: 206-208 °C; yield: 84%; FTIR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3038 (Ar, C-H), 1608 (C=N), 1320, 1512 (C=C); <sup>1</sup>H NMR (400 MHz)  $\delta$  ppm: 6.54-6.56 (d, 1H), 7.09-7.13 (t, 2H), 7.21-7.23 (d, 1H), 7.44-7.59 (m, 5H), 7.62-7.65 (dd, 3H), 7.71-7.73 (dd, 2H), 8.00-8.02 (dd, 2H); Mass (m.f.: C<sub>21</sub>H<sub>16</sub>BrN): calculated: 361.05; found: 380.07 (M<sup>+</sup> + H<sub>2</sub>O).

**(1E,2E)-1,3-Diphenyl-N-(*p*-tolyl)prop-2-en-1-imine (3e):** Orange solid; m.p.: 179-182 °C; yield: 90%; FTIR (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3021 (Ar, C-H), 1604 (C=N), 1508, 1465 (C=C); <sup>1</sup>H NMR (400 MHz)  $\delta$  ppm: 7.09-7.13 (t, 2H), 7.44-7.59 (m, 7H), 7.62-7.66 (tt, 4H), 7.71-7.29 (dd, 3H), 7.73-7.76 (d, 1H), 7.80-8.02 (t, 2H), 2.12 (s, 3H); Mass (m.f.: C<sub>22</sub>H<sub>19</sub>N): calculated: 297.15; found: 320.17 (M<sup>+</sup> + sodium salt).

**(1E,2E)-N-(4-Methoxyphenyl)-1,3-diphenylprop-2-en-1-imine (3f):** Orange solid; m.p.: 185-187 °C; yield: 92%; FTIR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3045 (Ar, C-H), 1610 (C=N), 1621, 1553 (C=C);  $^1\text{H NMR}$  (400 MHz)  $\delta$  ppm: 6.90-6.92(d, 1H), 7.06-7.09 (tt, 2H), 7.11-7.26 (tt, 1H), 7.44-7.60 (m, 12H), 7.62-7.74 (m, 4H), 7.78-8.01 (dd, 2H), 3.94 (s, 3H); Mass (m.f.:  $\text{C}_{22}\text{H}_{19}\text{NO}$ ): calculated: 313.15; found: 341.19 ( $\text{M}^{+2}$  + MeOH adduct).

**(1E,2E)-3-(4-Fluorophenyl)-N-1-diphenylprop-2-en-1-imine (3g):** Yellow solid; m.p.: 182-184 °C; yield: 90%; FTIR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3073 (Ar, C-H), 1600, (C=N), 1509, (C=C);  $^1\text{H NMR}$  (400 MHz)  $\delta$  ppm: 7.41-7.43(tt, 3H), 7.49-7.59 (m, 6H), 7.61-7.66 (dd, 2H), 7.70-7.73 (tt, 2H), 7.80-7.84 (d, 1H), 8.01-8.03 (dd, 2H); Mass (m.f.:  $\text{C}_{21}\text{H}_{16}\text{FN}$ ): calculated: 301.13; found: 302.16 ( $\text{M}^{+}$ ).

**(1E,2E)-N-(4-Chlorophenyl)-3-(4-fluorophenyl)-1-phenylprop-2-en-1-imine (3h):** Yellow solid; m.p.: 193-195 °C; yield: 88%; FTIR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3086 (Ar-C-H), 1589 (C=N), 1522 1432 (C=C);  $^1\text{H NMR}$  (400 MHz)  $\delta$  ppm: 7.42-7.44 (tt, 3H), 7.49-7.59 (m, 6H), 7.61-7.73 (tt, 3H), 7.80-7.84 (d, 1H), 8.01-8.03 (dd, 2H); Mass (m.f.:  $\text{C}_{21}\text{H}_{15}\text{ClFN}$ ): calculated: 335.09; found: 336.07 ( $\text{M}^{+}$ ).

**(1E,2E)-N-3-bis(4-Fluorophenyl)-1-phenylprop-2-en-1-imine (3i):** Off white solid; m.p.: 187-189 °C; yield: 89%; FTIR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3090 (Ar-C-H), 1585 (C=N), 1516 1428 (C=C);  $^1\text{H NMR}$  (400 MHz)  $\delta$  ppm: 7.09-7.13 (t, 2H), 7.48-7.59 (m, 5H), 7.62-7.65 (tt, 3H), 7.71-7.73 (dd, 2H), 7.76-7.80 (d, 1H), 8.00-8.02 (dd, 2H); Mass (m.f.:  $\text{C}_{21}\text{H}_{15}\text{F}_2\text{N}$ ): calculated: 319.12; found: 320.14 ( $\text{M}^{+}$ ).

**(1E,2E)-N-(4-Bromophenyl)-3-(4-fluorophenyl)-1-phenylprop-2-en-1-imine (3j):** Yellow solid; m.p.: 177-179 °C; yield: 88%; FTIR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3044 (Ar-C-H), 1592 (C=N), 1506 1434 (C=C);  $^1\text{H NMR}$  (400 MHz)  $\delta$  ppm: 7.09-7.13 (t, 2H), 7.44-7.59 (m, 6H), 7.62-7.65 (tt, 3H), 7.71-7.73 (dd, 1H), 7.76-7.80 (d, 1H), 8.00-8.02 (dd, 2H); Mass (m.f.:  $\text{C}_{21}\text{H}_{15}\text{BrFN}$ ): calculated: 379.04; found: 380.07 ( $\text{M}^{+}$ ).

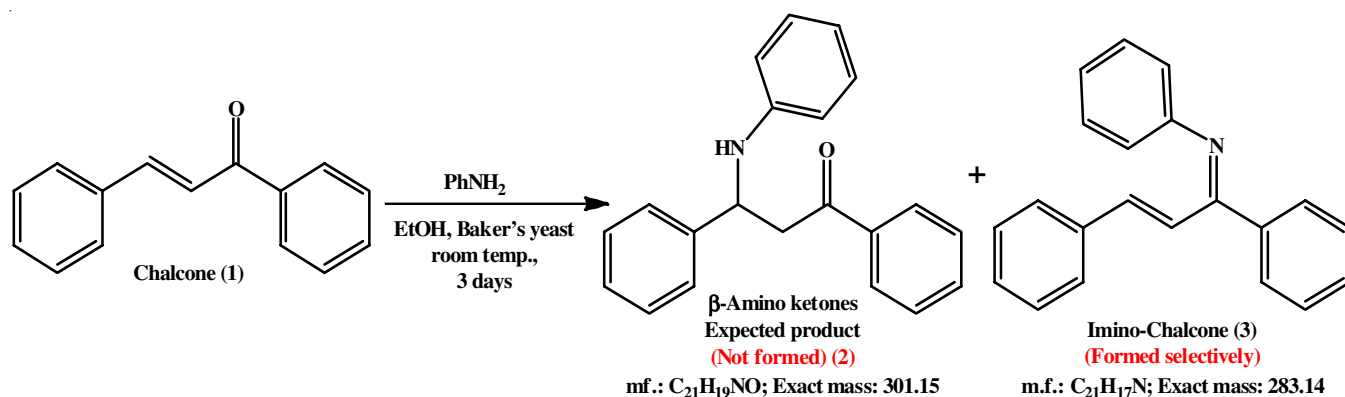
**(1E,2E)-3-(4-Fluorophenyl)-1-phenyl-N-(p-tolyl)prop-2-en-1-imine (3k):** Off white solid; m.p.: 184-186 °C; yield: 92%; FTIR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3012 (Ar-C-H), 1588 (C=N), 1516, 1438 (C=C);  $^1\text{H NMR}$  (400 MHz)  $\delta$  ppm: 7.09-7.14 (t, 2H), 7.44-7.54 (m, 7H), 7.58-7.66 (tt, 2H), 7.71-7.73 (dd, 1H), 7.76-7.80 (d, 1H), 8.00-8.02 (dd, 2H), 2.14 (s, 3H); Mass (m.f.:  $\text{C}_{22}\text{H}_{18}\text{FN}$ ): calculated: 315.14; found: 316.07 ( $\text{M}^{+}$ ).

**(1E,2E)-3-(4-Fluorophenyl)-N-(4-methoxyphenyl)-1-phenylprop-2-en-1-imine (3l):** Yellow solid; m.p.: 172-174 °C; yield: 94%; FTIR (KBr,  $\nu_{\max}$ ,  $\text{cm}^{-1}$ ): 3054 (Ar-C-H), 1592 (C=N), 1518, 1460 (C=C);  $^1\text{H NMR}$  (400 MHz)  $\delta$  ppm: 7.08-7.14 (t, 3H), 7.45-7.54 (m, 5H), 7.58-7.60 (d, 2H), 7.63-7.66 (tt, 4H), 7.76-7.80 (d, 1H), 8.00-8.03 (dd, 2H), 4.14 (s, 3H); Mass (m.f.:  $\text{C}_{22}\text{H}_{18}\text{FNO}$ ): calculated: 331.14; found: 354.12 ( $\text{M}^{+}$  + sodium salt).

## RESULTS AND DISCUSSION

While working on the Baker's yeast-mediated synthesis, we tried to synthesize  $\alpha,\beta$ -aminocarbonyl compounds *via* the addition of the arylamine to chalcone by employing Baker's yeast as biocatalyst in ethanol by following our earlier reported conditions [21]. During the monitoring of the reaction process, thin-layer chromatography (TLC) was used to detect the consumption of the starting material. Surprisingly, we did not obtain the desired product, however, upon purifying the reaction mixture and conducting mass analysis, we were fascinated to discover the absence of the mass peak corresponding to the desired compound, which should have been 301.15. Whereas a peak at 284.12 was observed, indicating the presence of the chalcone-imine  $\text{M}+1$  (283.14+1) peak (**Scheme-I**).

Thus, encourage from the initial results, the optimization of the reaction conditions like effect of solvent was investigated in order to synthesize the  $\alpha,\beta$ -unsaturated imines or chalcone-imines effectively from moderate to excellent yields. Table-1 revealed that the reaction in benzene (entry-1), toluene (entry-2), DCM (entry-3), EtOAc (entry-4) and THF (entry-5) did not proceed, as there was no product formation were observed after 12 h. Interestingly, when ethanol was used as a solvent, the reaction proceeds and the progress of the reaction was monitored at different intervals of times (entry-6-11). The best results were obtained when the reaction mixture was shaken on an orbital shake for 30 h in ethanol as green solvent and Baker's yeast as biocatalyst as the product **3a** was formed in 88% yield. Further, to check the effect of the Baker's yeast as a biocatalyst, the reaction was allowed to take place in the absence of Baker's yeast and it was observed that the reaction did not proceed even after 18 h of time. Therefore, after getting the optimized reaction conditions, the generality of the reaction was further checked by taking substituted chalcones (**1a-b**) and substituted

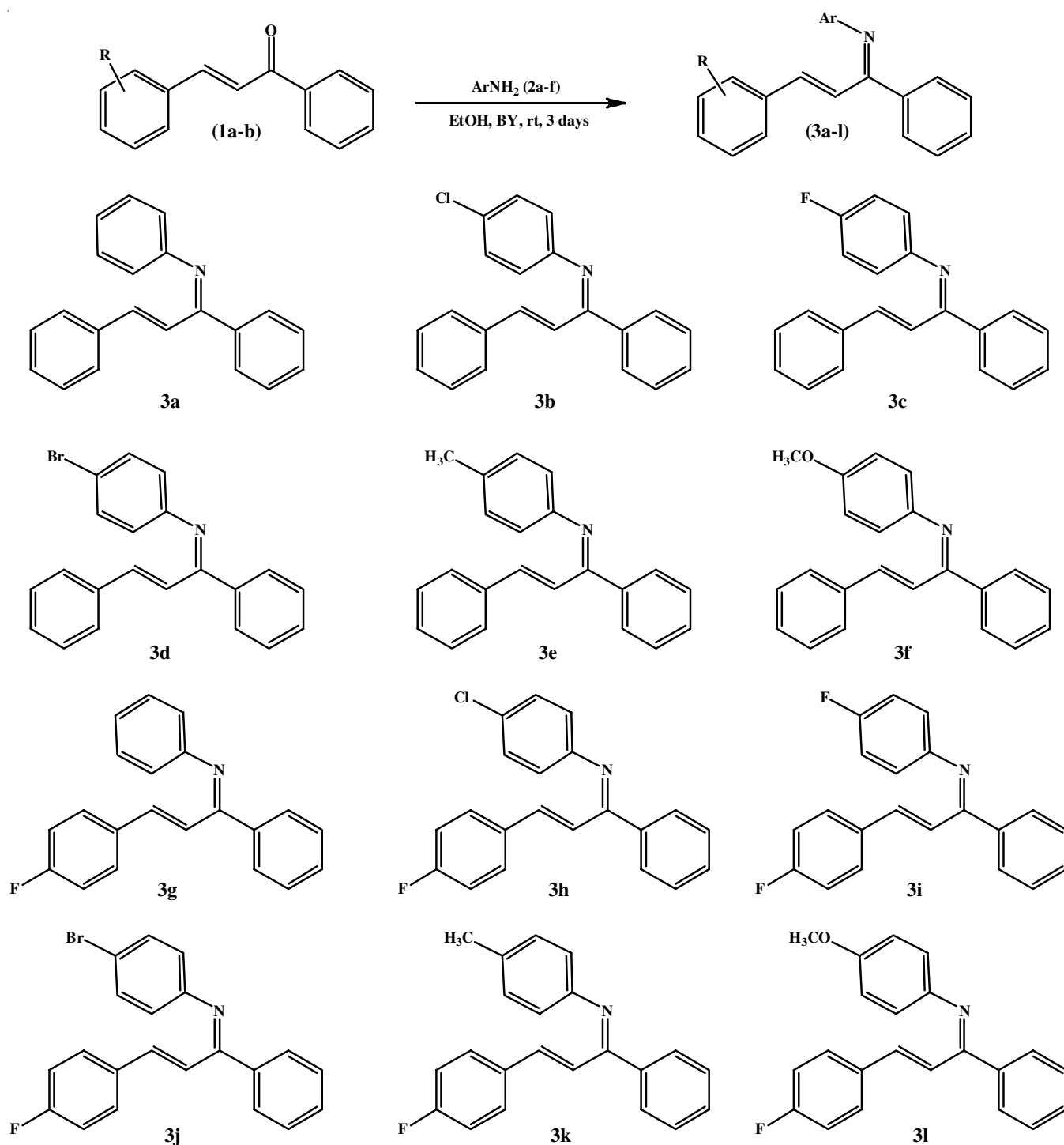


**Scheme-I:** Efforts for the reaction of chalcone with aniline for the formation of  $\beta$ -aminoketone by using BY as catalyzed

anilines (**2a-l**) to afford the desired  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) (**Scheme-II**).

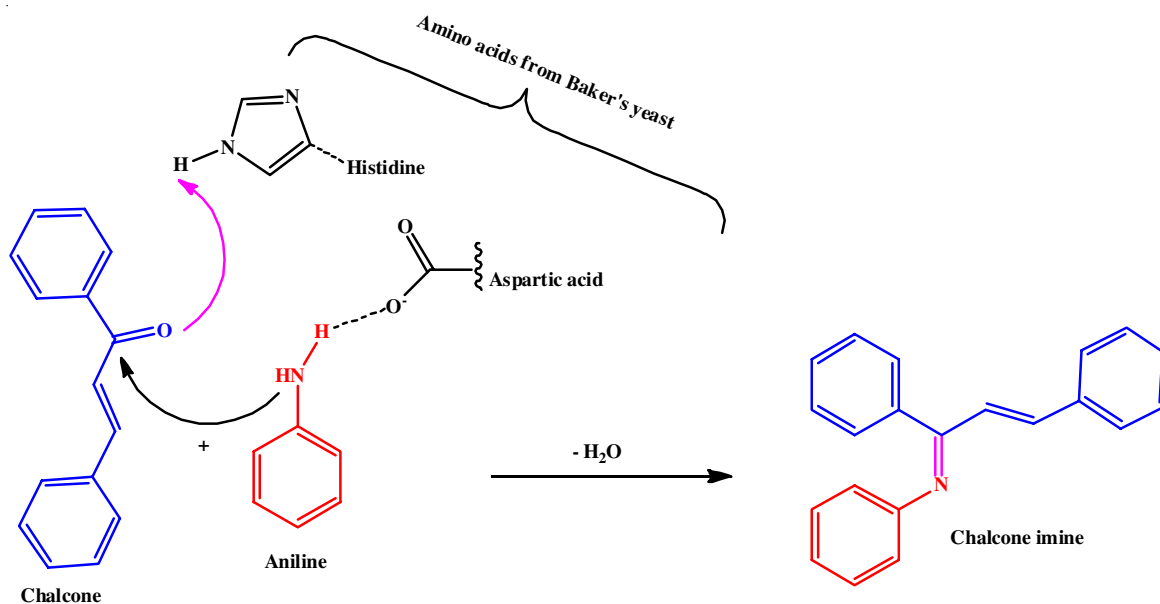
It is of great importance to emphasize that no aza-Michael addition product was formed in these reactions, as all of the mass spectra exhibit mass peaks corresponding to the  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) were synthesized. The mass peaks obtained were in the form of  $M^+$ ,  $M^{+1}$ ,  $M^{+1}+H_2O$ ,  $M^{+1}$ +sodium salt or  $M^+$ +Methanol adduct. These findings provided further evidence that no aza-Michael addition reaction was occurring during the procedure and that the imines were

being formed selectively. Furthermore, the  $^1H$  NMR spectra of the synthesized compounds, the peaks between  $\delta$  7.40-7.45 ppm appear as dd represent the olefinic protons of the  $\alpha,\beta$ -unsaturated imines C=C protons. The other protons appears at  $\delta$  7.49-8.03 ppm as singlets, doublets, triplets and multiples corresponds to the aromatic protons of the three aromatic rings. It has also been found that the reactions proceed more smoothly and produce higher yields when performed with electron-donating groups.



**Scheme-II:** BY catalyzed synthesis of  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**)





**Scheme-III:** Tentative mechanism for the BY catalyzed synthesis of  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) [11,24]

**TABLE-1**  
BAKER'S YEAST CATALYZED SYNTHESIS OF  
 $\alpha,\beta$ -UNSATURATED IMINES OR CHALCONE-IMINES:  
OPTIMIZATION FOR THE COMPOUND **3a**

Solvent	Catalyst Baker's yeast (BY)	Reaction condition (stirring)	Yield (%)
Benzene	BY	12 h	No reaction
Toluene	BY	12 h	No reaction
Dichloromethane	BY	12 h	No reaction
EtOAc	BY	12 h	No reaction
Tetrahydrofuran	BY	12 h	No reaction
Ethanol	BY	6 h	No reaction
Ethanol	BY	12 h	10
Ethanol	BY	18 h	30
Ethanol	BY	24 h	50
Ethanol	BY	30 h	70
Ethanol	BY	36 h	88
Ethanol	Without BY	18 h	No reaction

To understand the actual reaction pathway, a possible mechanism for the synthesis of  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) is depicted in **Scheme-III**. It is hypothesized that the carbonyl double bond in Baker's yeast is polarized by the binding of amino acid (histidine) from the yeast's active side to the oxygen atom of the carbonyl group of chalcone. Instead, the carbonyl carbon is attacked by aniline as a nucleophile due to its interaction with the aspartate ion of Baker's yeast, forming a C-C bond and finally, the elimination of water *via* dehydration process yield the required  $\alpha,\beta$ -unsaturated imines or chalcone-imines [22].

In the case of chalcone reactions, anilines undergo aza-Michael addition at the carbon-carbon double bond, resulting in the formation of  $\alpha,\beta$ -aminocarbonyl derivatives. However, the fact that the formation of  $\alpha,\beta$ -aminocarbonyl product came as a complete and utter surprise to us. In light of this, it should come as no surprise to assert that the aza-Michael addition reaction, also known as the 1,2-addition reaction, is not being

carried out by chalcones. The  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) was obtained which occurred due to the condensation reaction of carbonyl group to anilines (simple Schiff base formation). Thus, the chemo-selective reactions involving chalcones or other unsaturated compounds are attainable using the present approach [23].

## Conclusion

This developed method is simple, efficient, chemo-selective and sustainable biocatalytic method for the synthesis of  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) by using Baker's yeast. When chalcones (**1a-b**) reacts with substituted anilines (**2a-f**) in the presence of Baker's yeast act as a whole cell biocatalyst at ambient temperature in ethanol as a green solvent give  $\alpha,\beta$ -unsaturated imines or chalcone-imines (**3a-l**) in good to excellent yields. The reaction can be performed at room temperature (25-28 °C) and has the added benefit of being very simple to perform.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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