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RESEARCH ARTICLE

BAKER'S YEAST CATALYZED AND ULTRASOUND ACCELERATED SYNTHESIS OF 2-AMINO-2-CHROMENES

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Abstract

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

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 area of interest in recent years due to their useful biological and pharmacological aspects [2]. 2-Amino-chromene derivatives also serves as the main components of cosmetics and pigments [3], biodegradable agrochemicals [4] and medicaments [5]. Particularly, they are endowed with wide spectrum of activities such as antitumor [6], sex pheromonal [7], central nervous system [8], antiproliferative [9], antiviral [10], mutagenicitical [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 core 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 of 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].

Vitamin E is an evident example for the naturally occurring chromane, which possess antioxidant activity [19]. Many of the natural compounds having chromene moiety are found to possess anticancer activity [20-23].

Owing to the medicinal and synthetic significance of 2-amino-2-chromenes, the development of novel, highly efficient and cost effective methods for their synthesis have immerged as the need of time.

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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 [24]. (**Scheme 1**).

Scheme 1

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 [25], cetyl trimethylammonium chloride [26], triethylbenzylammonium chloride [27] and tetra-nbutylammonium bromide [28]. γ-Alumina [29], methanesulfonic acid [30], nanostructured diphosphate [31], sodium hydroxide [32], triton B [33], sodium salt of ethylenediamine tetraacetic acid [34], magnessium oxide [35] and titanium tetrachloride [36], have also been used. There are also reports on the use of catalysts such as DBU [37], selectfluor [38], piperazine [39]. potassium phthalimide-N-oxyl [40], gel entrapped DABCO [41] and Poly 4-vinylpyridine has also been used as a catalyst [42] 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.

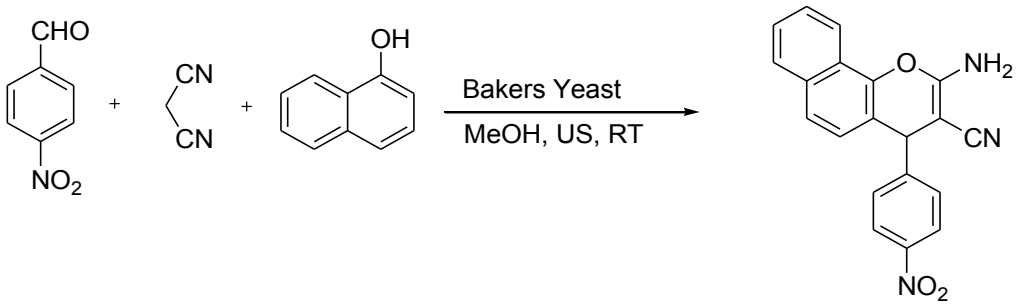
Literature reveals that there is no report on the use of biocatalyst/ enzyme for accelerating the one pot multicomponent condensation leading to 2-amino-2-chromenes. Considering the above observations and in continuation of our work [43-45] towards the acceleration of synthetic protocols leading to bioactive molecules 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 [46]. Its development in the past few years has been considerably increased to know its mechanism of action inside the reaction flask [47]. Several applications in organic synthesis have made sonochemistry an attractive technique [48] and hence increasingly used in organic synthesis [49]. It has proved to be a great tool for improving yields and decreasing the reaction time [50]

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 [28-42]. The objective was also set in mind to use non-conventional energy source, ultrasonication for assisting the cyclocondensation.

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 2)**. 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 2

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 1, entry 1)**. The model reaction was then separately performed in organic solvents viz. chloroform, 1,4-dioxane, ethanol and methanol **(Table 1).** Comparatively better results were obtained when methanol was used as a solvent for the reaction **(Table 1, entry 5)**. Hence methanol was selected as the reaction medium.

Table 1:-Optimization for appropriate solvent for the reaction[a] **(Scheme 1).**

Entry	Solvent	Condition	Time (h)	Yield (%)[b]
1	Water	RT	36	33
2	$CHCl_3$	RT	36	28
3	1,4-Dioxane	RT	36	37
4	Ethanol	RT	36	42
5	Methanol	RT	36	57

[a]Reaction conditions: 4-nitrobenzaldehyde (5 mmol), malononitrile (5 mmol), 1-naphthol (5 mmol), baker's yeast (2g) in the solvent. [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 [51]. Model reaction in methanol when performed under ultrasonication gave 87% yield within 2 h **(Table 2, entry 3**).

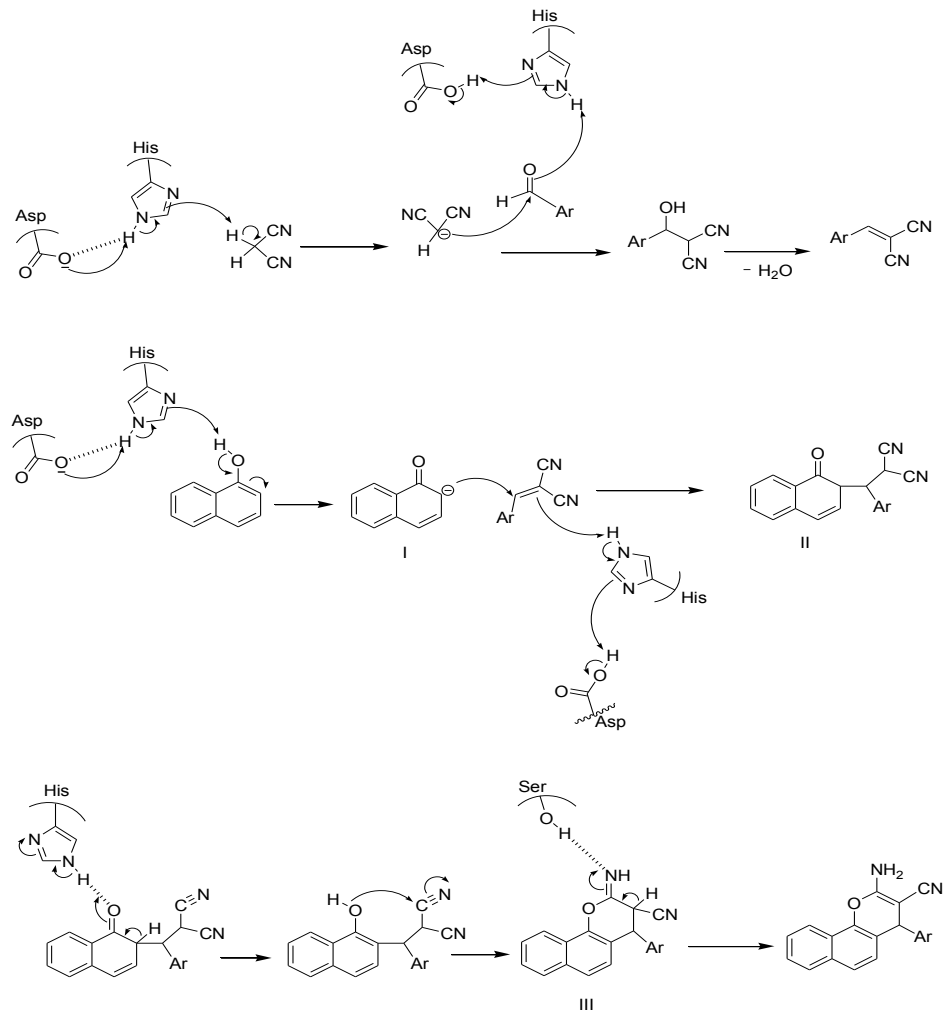
Table 2:-Optimization for appropriate reaction time **(Scheme 2)**.

Entry	Solvent	Condition	Time (hr)	Yield (%)[a]
1	Methanol	US	1	50
2	Methanol	US	1.5	76
3	Methanol	US	2	87
4	Methanol	US	2.5	89
5	Methanol	US	2.5	nd[b]

[a]Reaction conditions: 4-nitrobenzaldehyde (5 mmol), malononitrile (5 mmol), 1-naphthol (5 mmol), baker's yeast (2g) in methanol under ultrasonication. [a]Isolated yields. [b]Reaction 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 [52] the physical parameters of the obtained products are in good agreement with those reported in the literature [27,32,52]. The results are recorded in **Table 3 (Scheme 2)**. 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 2, 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:
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 malononirile formed in the first step and takes back proton from Asp-His dyad to form C-alkylated intermediate (II) **(Scheme 3)**

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)**.

Conclusion:-

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. The route is simple and user friendly.

3. 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) (**Table 3, 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, $DMSOd_6$): 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_2O **),** 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 $C_{20}H_{13}N_3O_3$ $[M+ K]^+$: 382.0588, found : 382.0560.

Table 3:- Baker's yeast catalyzed synthesis of 2-amino-2-chromenes accelerated by ultrasonication[a] **(Scheme 2).**

Entry	R	Product	Yield (%)[b]	M.P. (°C)[c]
1	H	**4a**	87	208-210
2	4-OCH_3	**4b**	90	183-184
3	4-CH_3	**4c**	85	207-209
4	4-Cl	**4d**	75	233-234
5	2-Cl	**4e**	65	233-235
6	4-Br	**4f**	91	241-242
7	4-NO_2	**4g**	88	240-242
8	3-NO_2	**4h**	75	212-213
9	3-Cl	**4i**	68	228-230
10	4-$N(CH_3)_2$	**4j**	77	180-181
11	4-OH	**4k**	84	248-250

[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. [27,32,52]

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